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Real-Time Imaging of Translation on Single mRNA Transcripts in Live Cells

Technical Journal Club

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Dynamics of Translation of Single mRNA Molecules In Vivo

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Real-Time Imaging of Translation on Single mRNA Transcripts in Live Cells

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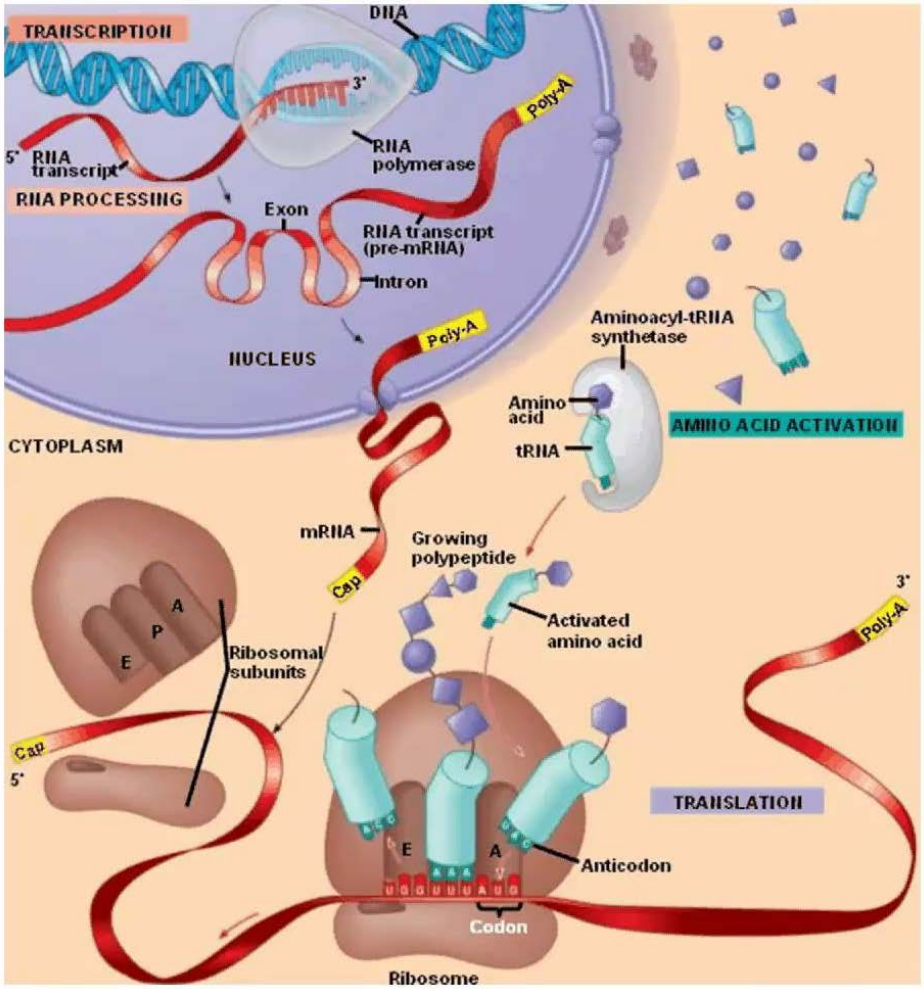
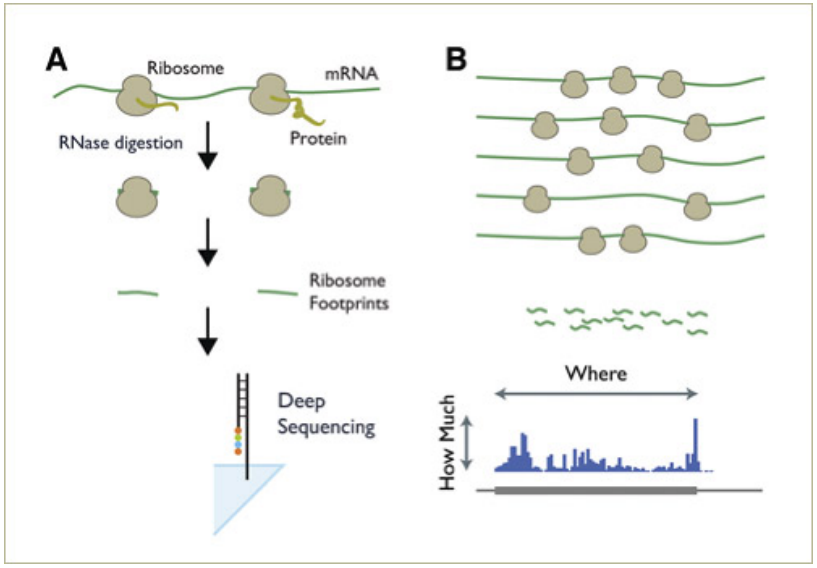
Introduction

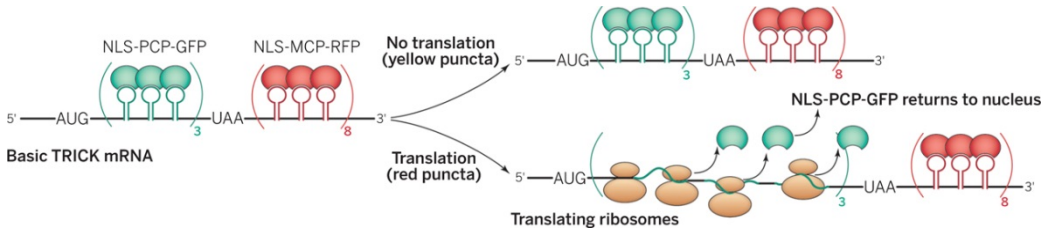


- Precise tuning of gene expression is critical for cell function
- Level of gene expression is regulated at multiple distinct steps (transcription, mRNA degradation and translation)
- The relative contribution of each regulatory step varies in different biological processes
- Measuring translation rate from individual mRNAs over time would provide valuable information on translation and regulation mechanisms in physiological and pathological states

Introduction



Ribosome profiling (Nature genetics)



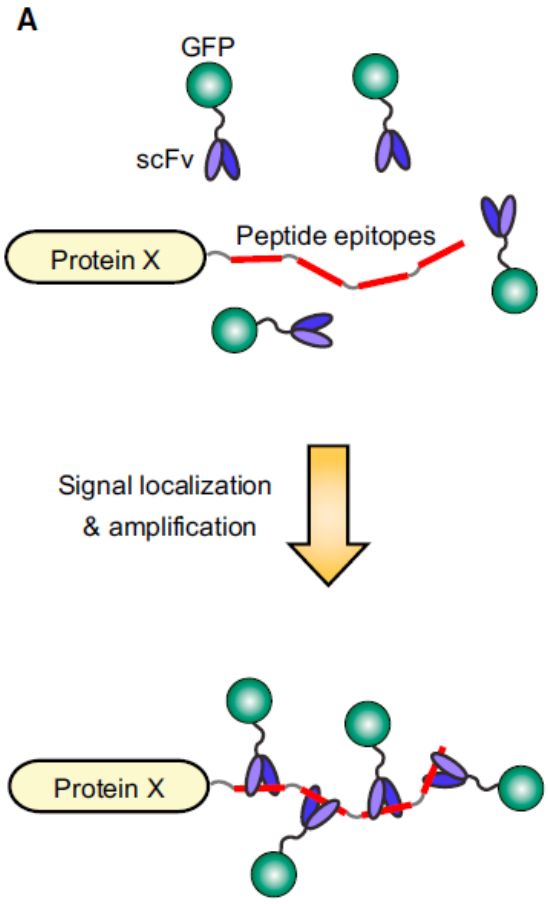
TRICK (Halstead et al, 2015)

- Ribosomal profiling: genome-wide snapshot of translation of endogenous mRNA in vivo
- Cons: averaging of many cells and limited temporal information
- TRICK mRNA imaging allows to distinguish between the translated and untranslated forms of an mRNA of interest in vivo
- mRNA is dual labeled in the coding region and the 3′ untranslated region using site-specific RNA-binding proteins fused to fluorescent proteins. Upon translation, proteins bound in the coding region are displaced by the ribosome → just the first translation event can be observed

Open questions

- Do different mRNAs produced in a single cell from the same gene behave similarly?
- What are the differences between individual mRNA molecules?
- Can mRNAs heterogeneity have an impact on the total amount of polipeptide produced?
- How does translation of single mRNA molecules vary over time?
- How does translation of single mRNAs vary with spatial location and transport?

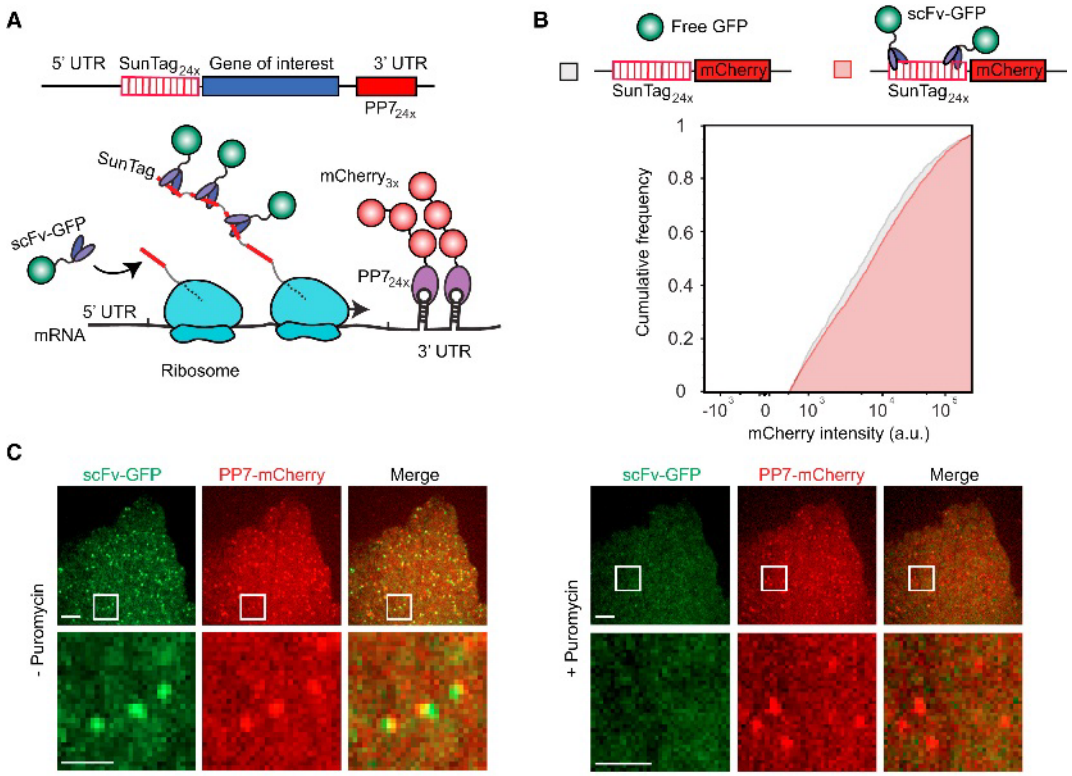
SunTag System



Tanenbaum et al, 2014

- Detecion of nascent polypeptides by the binding of fluorescent single-chain variable fragments antibody to an array of cognate peptides
- Pre-formed fluorophores avoid delayed readout of translation caused by the maturation time of fluorescent proteins
- Translation visible at translation sites
- Low expression levels of SunTag-proteins is sufficient for imaging
- Allows single-molecule imaging deep inside the cytoplasm and nucleus

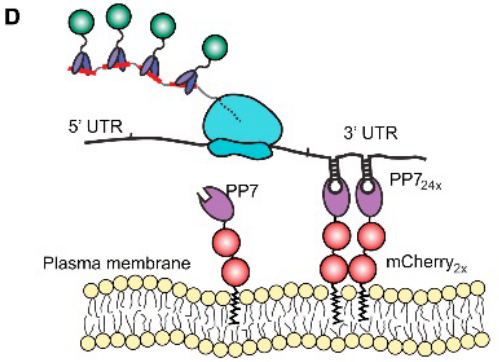
An assay for long-term observation of translation of individual mRNAs

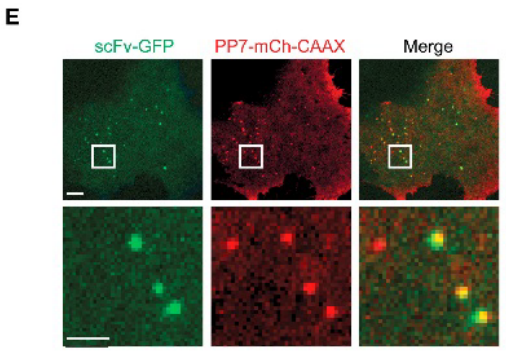


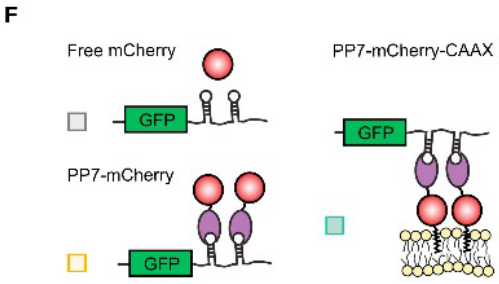
- Co-transfection with reporter transcript and scFv-GFP
- Labeling by SunTag ab did not alter protein synthesis rate of a reporter mRNA
- mRNA labeled with 24x short hairpin and co-expressing PP7 fused to 3 copies of mCheery (PP7-mCherry)
- Co-expression of reporter construct, scFv-GFP and PP7-mCherry resulted in bright red and bright or dim green spots

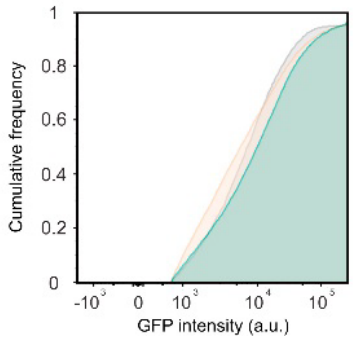
Dynamics of Translation of Single mRNA Molecules In Vivo (Yan et al., 2016)

An assay for long-term observation of translation of individual mRNAs



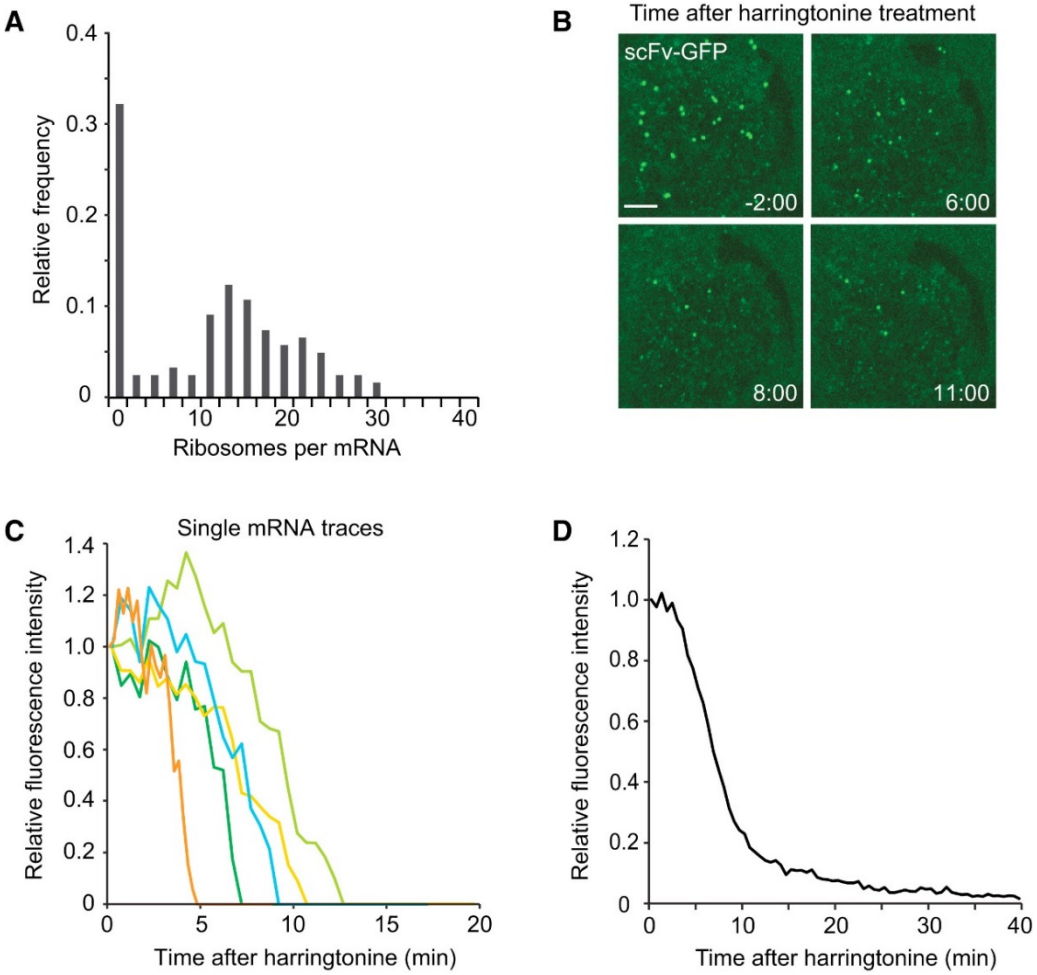






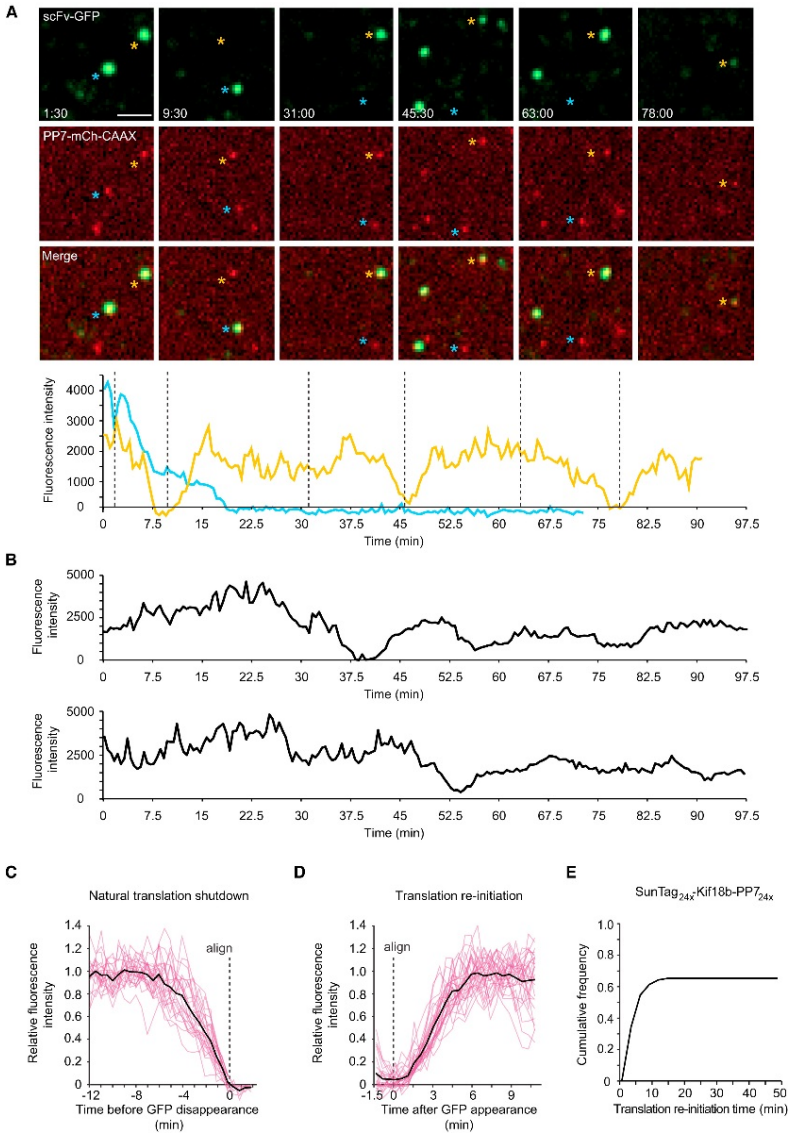
- Addition of CAAX sequence to track mRNAs for long periods of time
- Multiple red dots appeard on the plasma membrane, representing a tethered mRNA molecule
- Tethered mRNA co-migrated with sv-GFP foci, indicating that they are sites of active translation
- mRNA membrane tethering had minimal effects on the protein expression of a GFP reporter construct

Measurement of ribosome number, initiation rate and elongation rate on single mRNAs



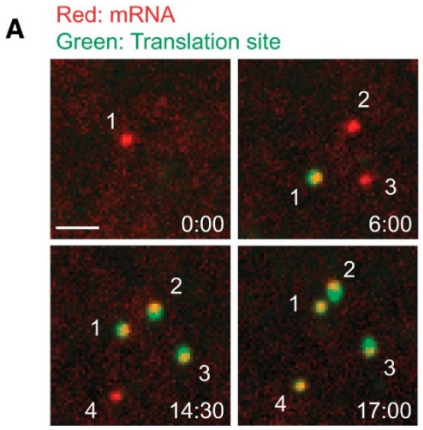
- Comparison of scFv-GFP fluorescence of translation sites with single, fully sinthesized peptides to estimate number of ribosomes per mRNA
- 30% mRNAs had no GFP signal
- 70% mRNAs were actively translating (10-25 ribosomes present)
- Treatment with harringtonine, causing stalling of new ribosomes, to measure translocation speed
- Mathematical model to fit decay in fluorescence estimated the translocation time in 3.5 codons/s

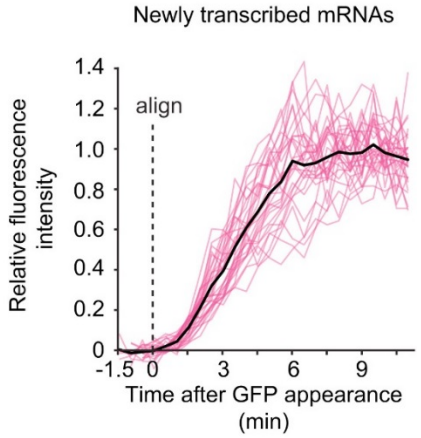
Temporal changes in translation of single mRNA molecules

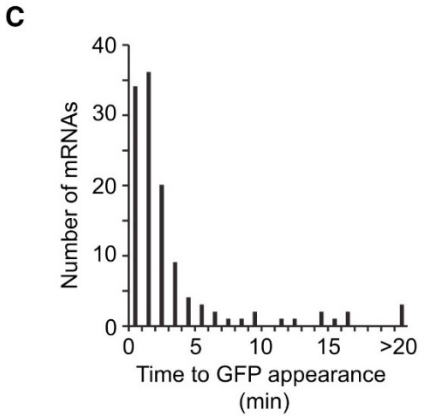


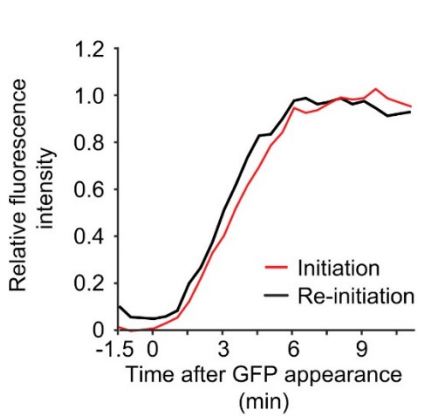
- Cells were imaged for 2 hr and the scFv-GFP signal was quantified from single mRNA that could be traced for >1 hr
- Heterogeneity of behaviour and fluctuations in the translational state of individual mRNAs over time
- Ribosome run-off rate of 3 codons/s
- A subset of shut-down mRNAs later reinitiated translation and recovered GFP signal
- Reversible switching between translational shutdown and polysome formation

Temporal changes in translation of single mRNA molecules



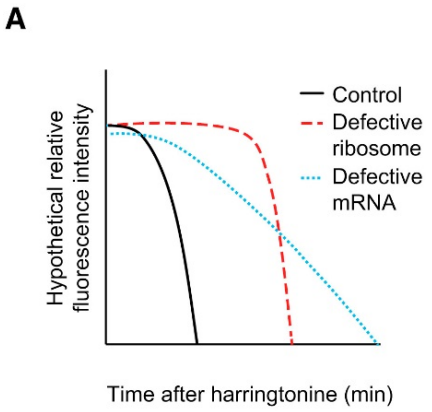


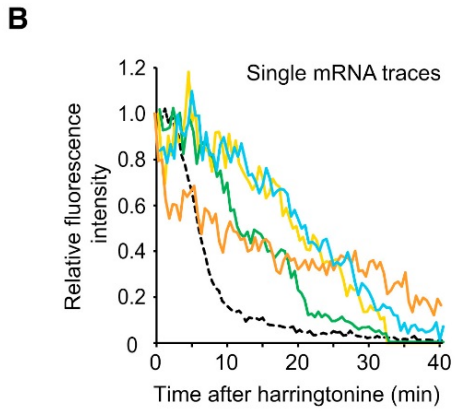




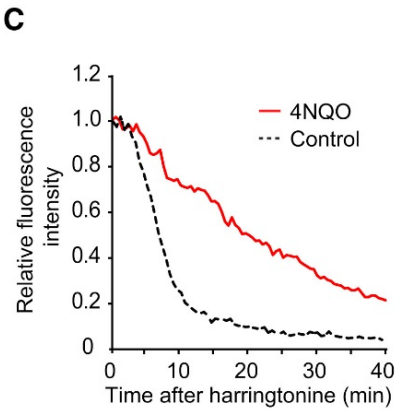
- Initial mRNA-membrane binding events visible after expression of constructs with inducible promoters
- The majority of mRNA initially appeared at the membrane in a non-translating state and converted to a translated state within 1-5 min
- These are likely newly transcribed mRNA that are undergoing translation for the first time.
- This assay additionally allows to analyze the polysome build-up and fluorescence recovery after shut-down

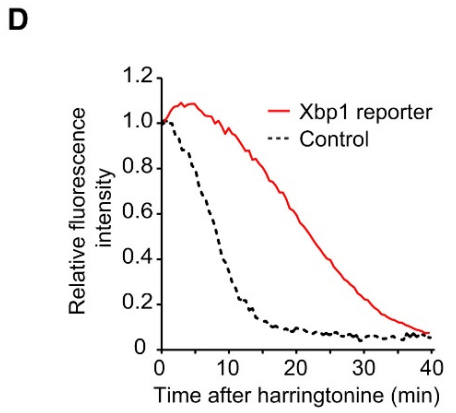
Ribosome stalling

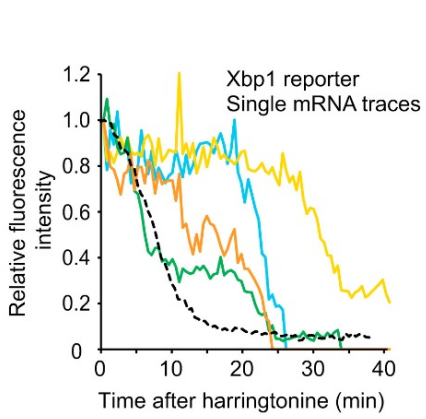




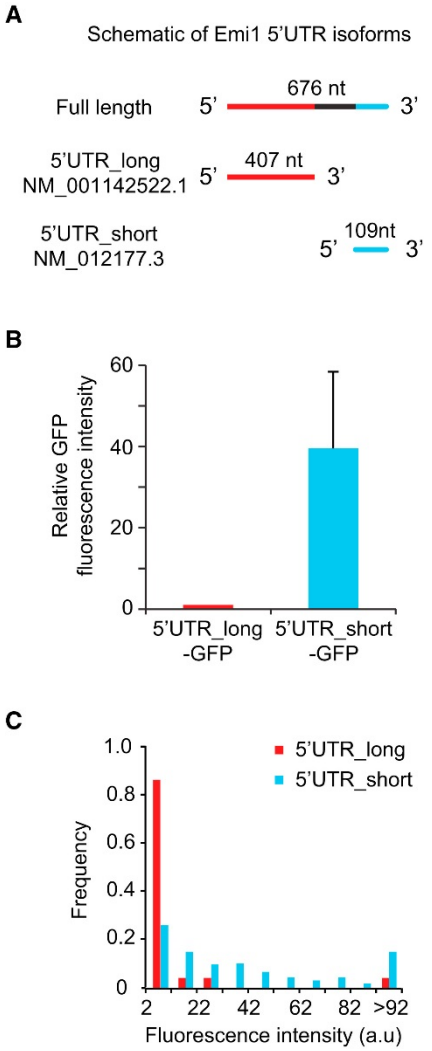
- Ribosomes can pause and stall at defined sequences with regulatory functions or at damaged nucleotides
- 5-10% of mRNA retained GFP signal 15 min after harringtonine, that disappeared after puromycine treatment
- Defective ribosome or defective mRNA?
- Heterogeneity of stalling behaviour





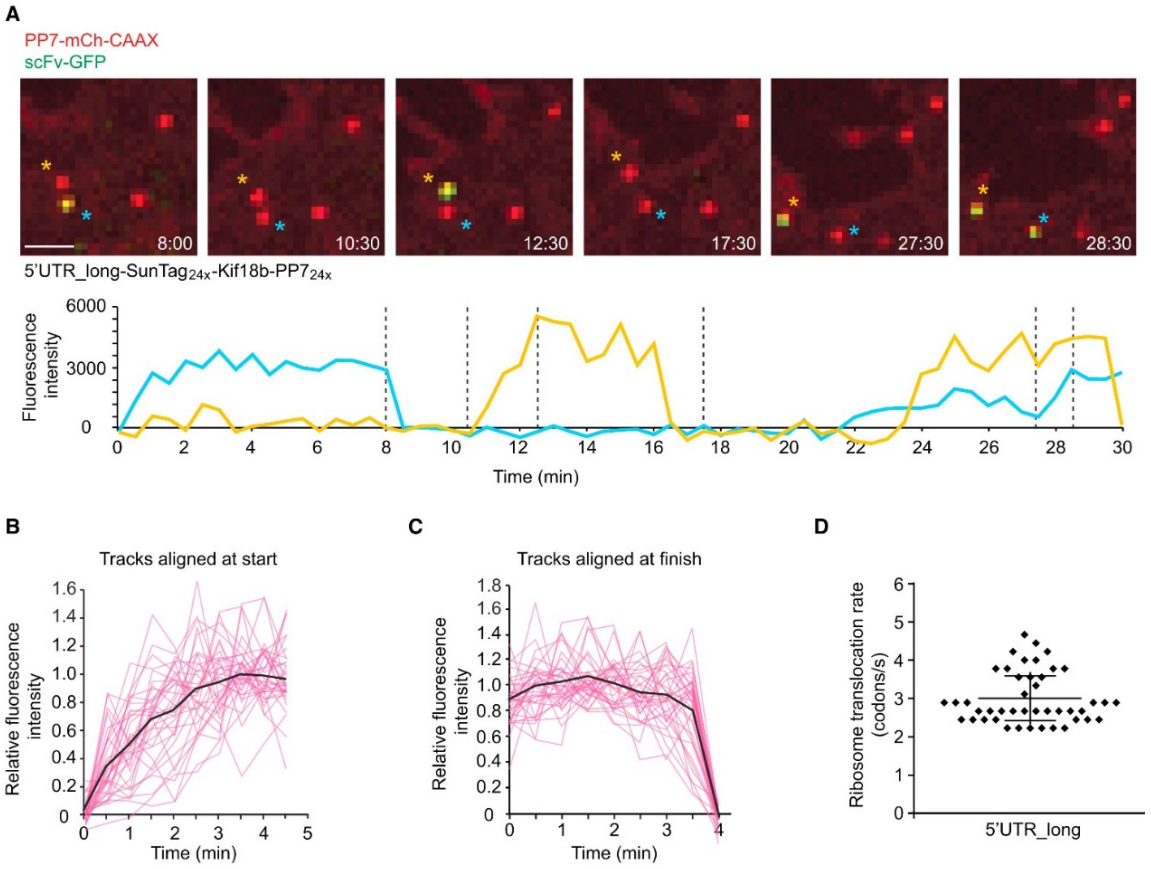


Translational regulation of the cell-cycle regulator Emi1



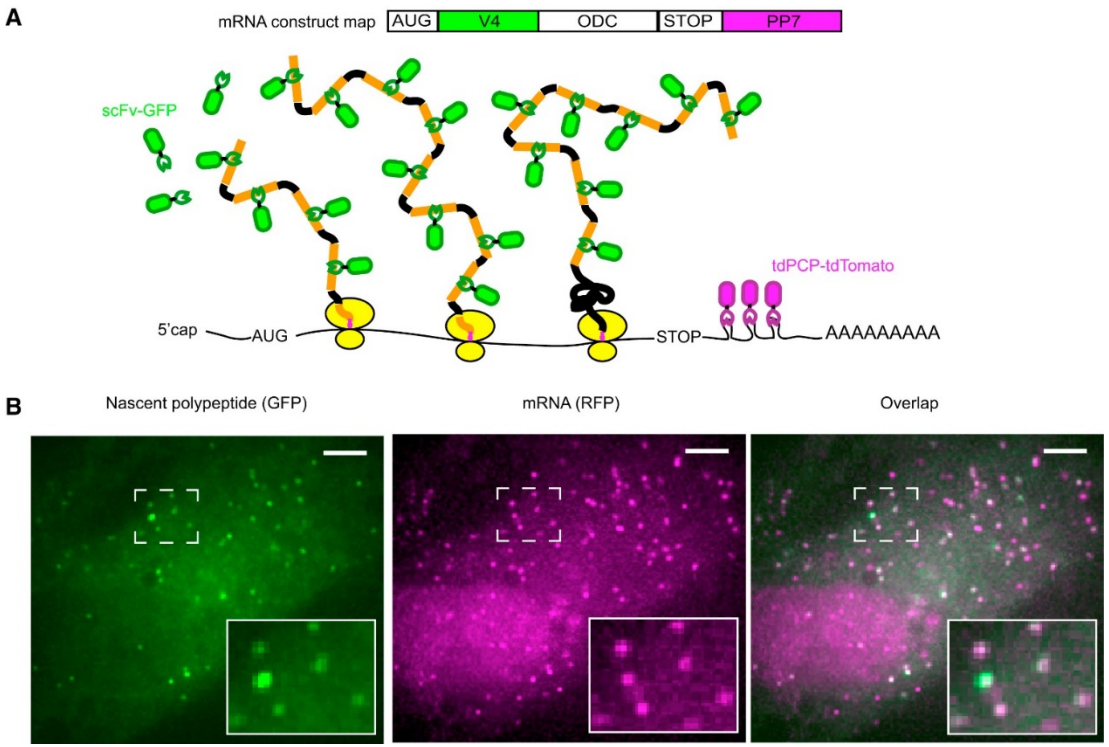
- 2 splicing forms of Emi1: 5‘UTR_long and 5‘UTR_short
- GFP protein fused downstream of 5‘UTR_long was expressed at 40-fold lower levels than a GFP fused to the 5‘UTR_short
- Robust translation in 5‘UTR_short reporter and weak scFv-GFP in 5‘UTR_long
- Heterogeneity in translation efficiency among different mRNA molecules within the same cell

Observation of translation by single ribosomes



- Emi1 5‘UTR_long shows appearance of weak GFP signal on a transcript that was initially silent.
- Heterogeneity in the decoding speed of individual ribosomes in vivo

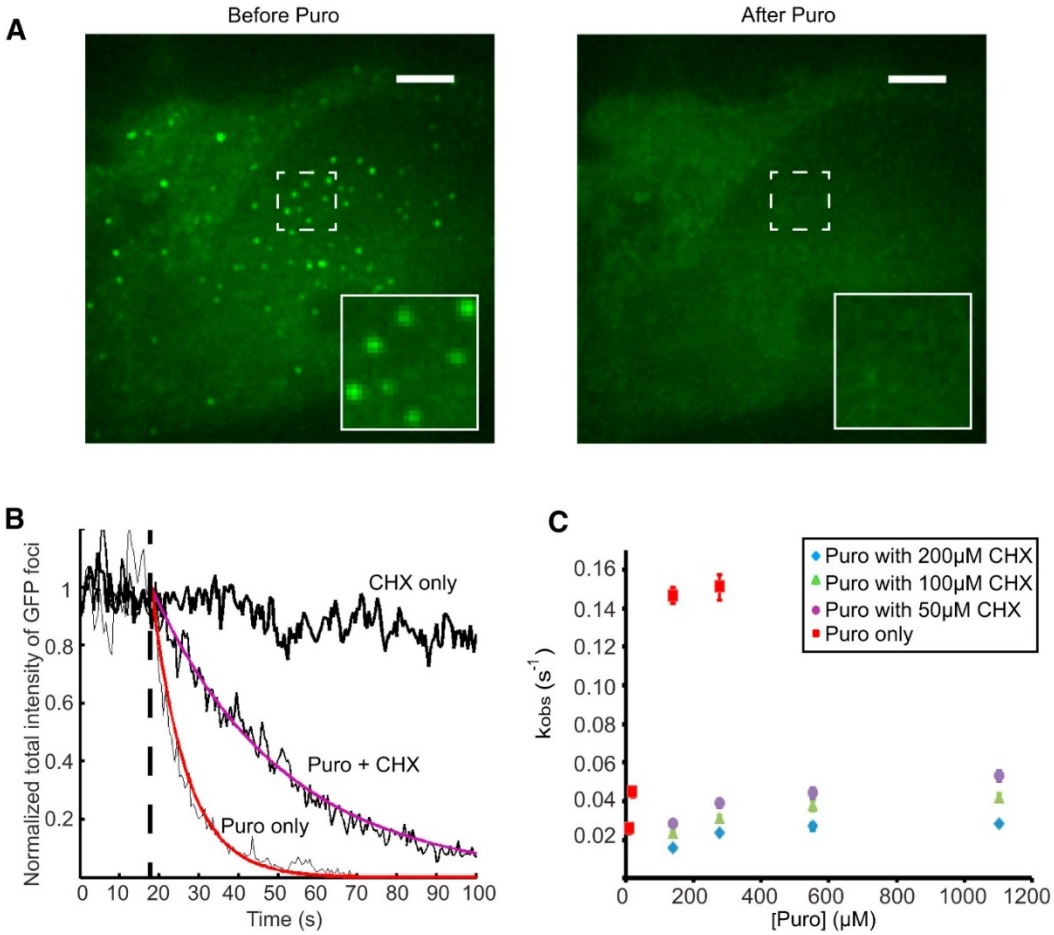
Visualizing translation on single mRNA molecules



- V4 peptides in a reporter mRNA expressed in HeLa cells stably expressing a GFP-labeled scFv
- ODC sequence fused to the C terminus of the V4 peptide array
- Tandem array of PP7 hairpins that can bind tdTomato-labeled PP7-coat proteins (tdPCP)
- Nuclear localization signal in tdPCP molecule

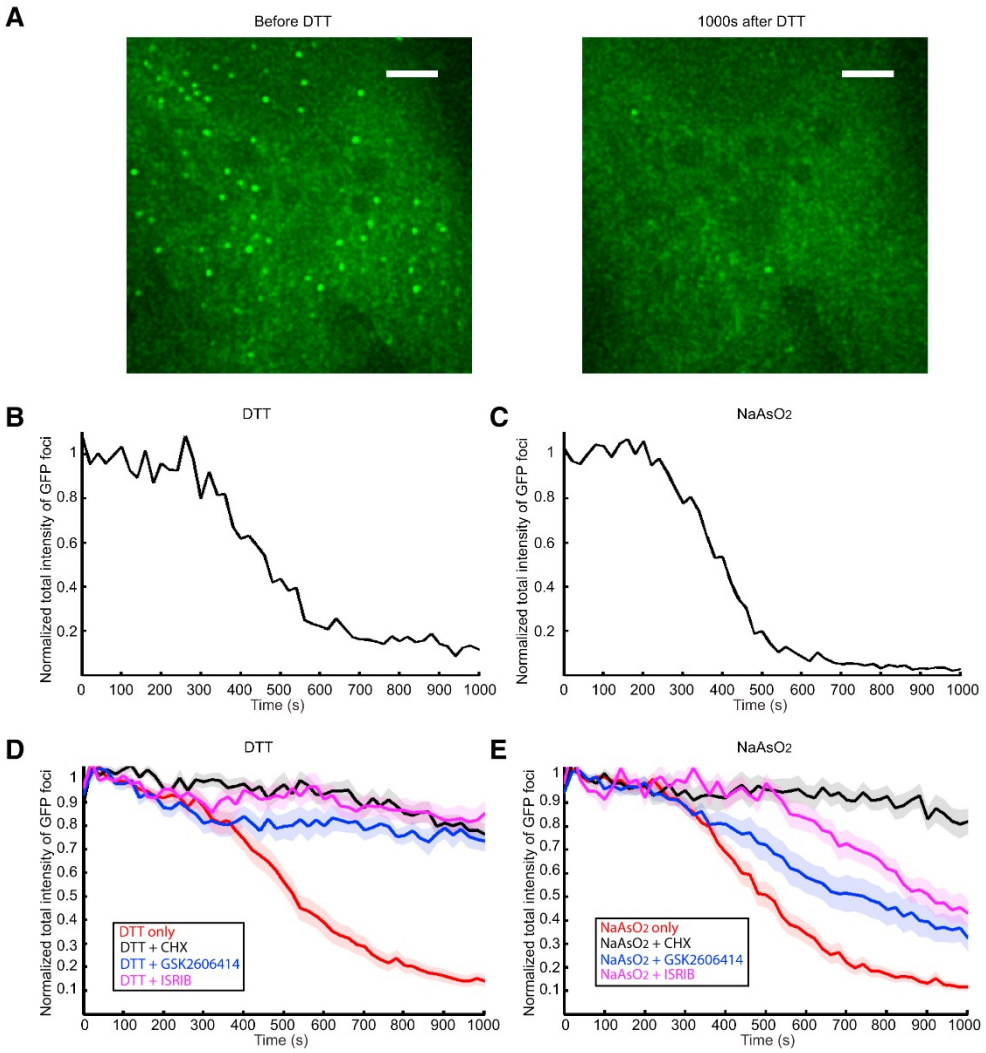
Real-Time Imaging of Translation on Single mRNA Transcripts in Live Cells (Wang et al., 2016)

Imaging changes in translation activity caused by translation inhibitors



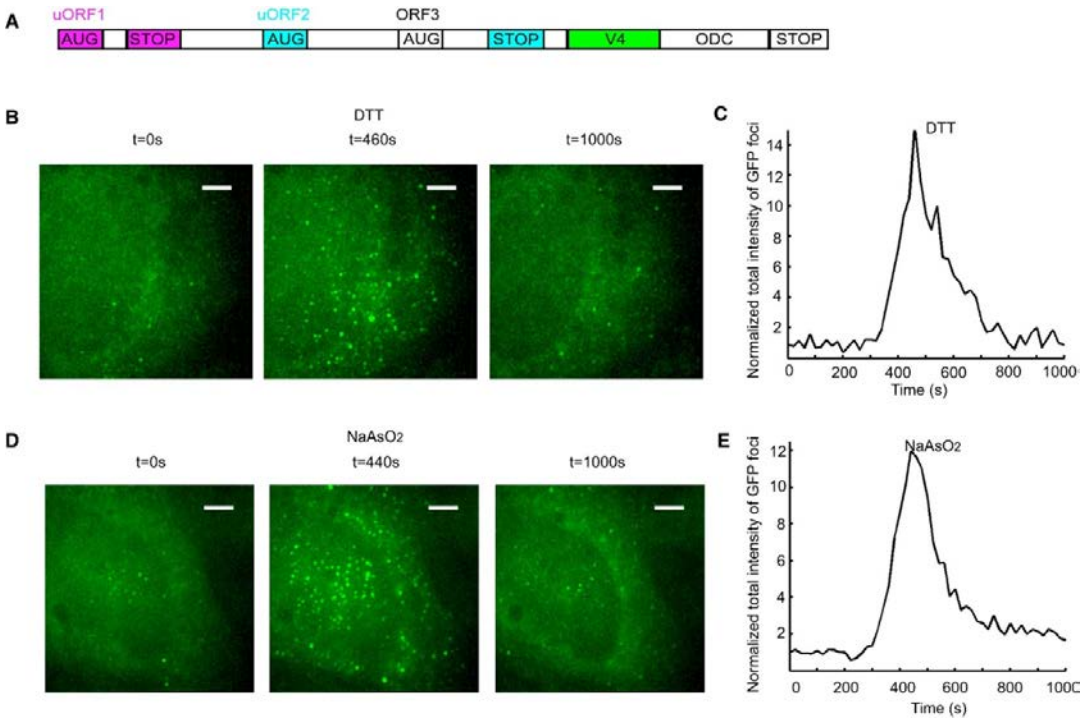
- GFP foci rapidly disappear after Puro treatment in a concentration dependent fashion
- GFP foci signal is stable after CHX treatment
- When combined, CHX delays the puro-induced GFP foci disappearance

Translational responses to unfolded protein stress and oxidative stress



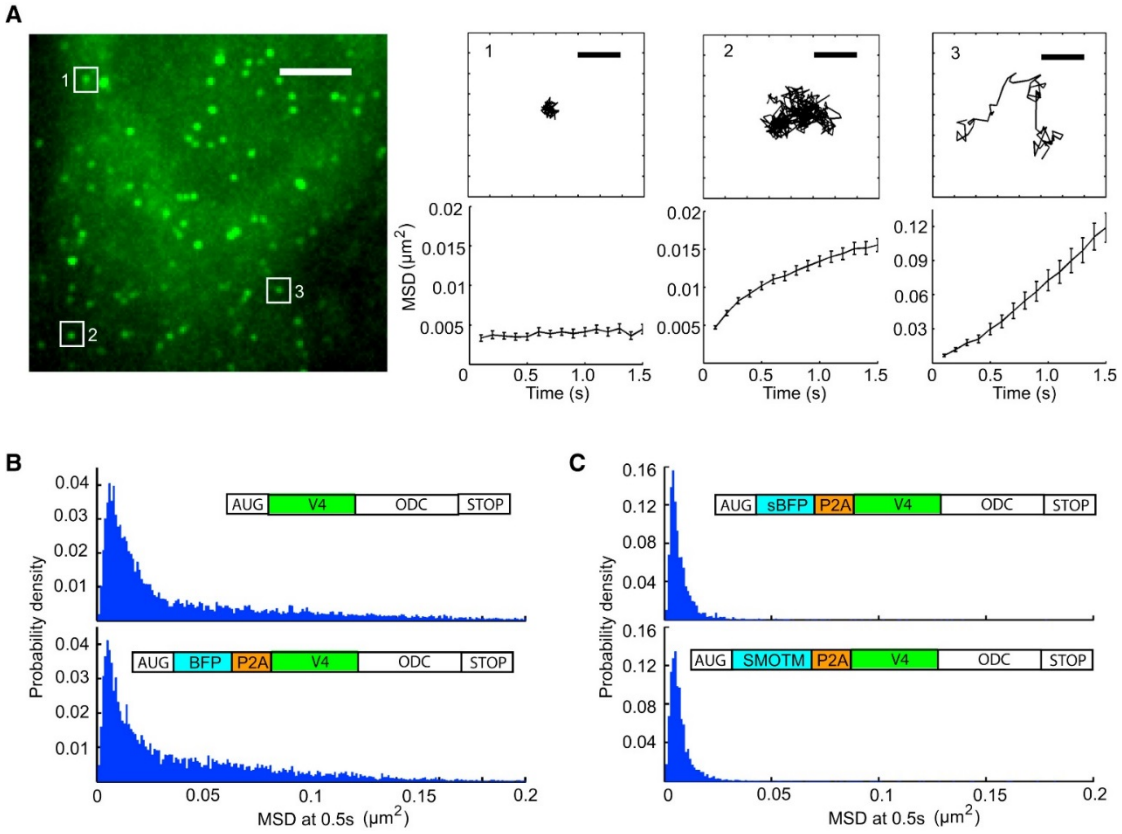
- Two stress conditions: 1) DDT-induced unfolded protein stress in ER and 2) $NaAsO_2$ – induced oxidative stress.
- CHX abrogated the reduction in signal under both treatments inhibited translational shut-down to a weaker extent
- Kinase inhibitors inhibited translational shut-down to a different extent

Translational responses to unfolded protein stress and oxidative stress



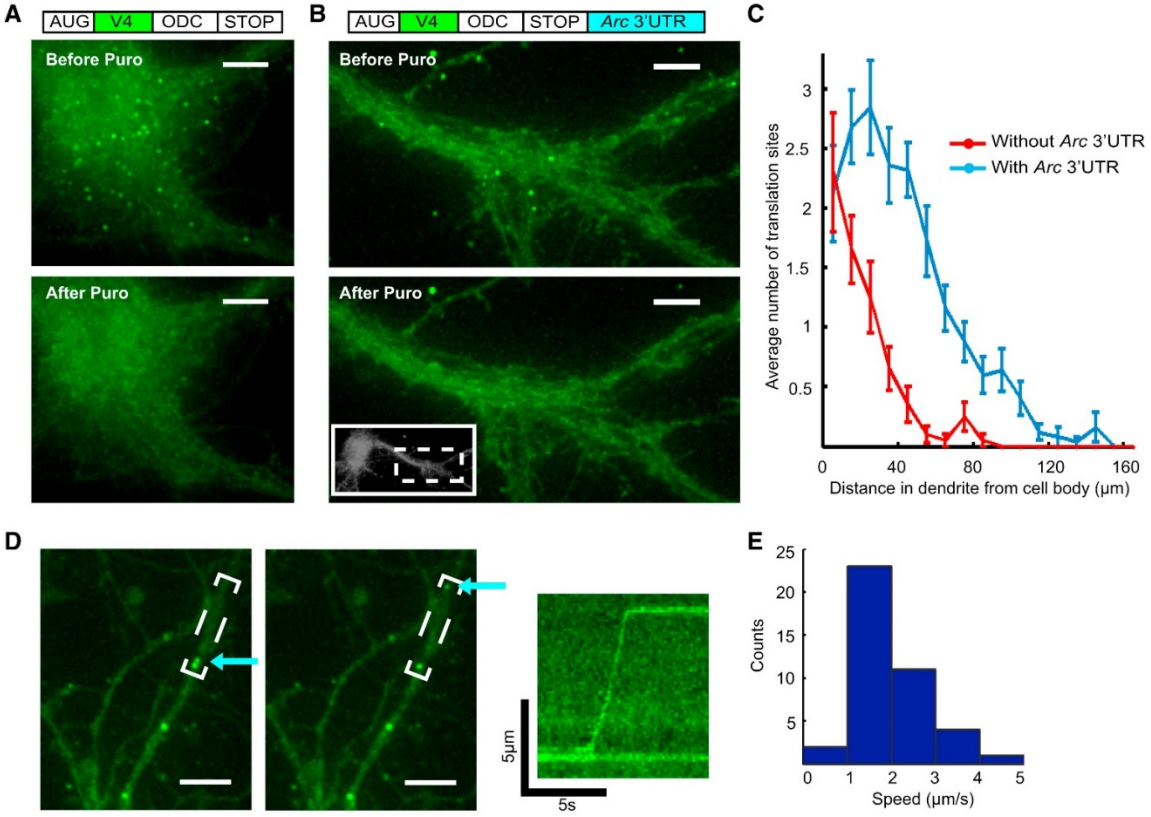
- ATF4 is upregulated upon unfolded protein and oxidative stress
- ORF3 activated during stress
- Similar transient increase upon DDT and $NaAsO_2$
- The imaging method efficiently captures transient changes in translational activity

Distinct mobilities of individual polysomes in different subcellular compartments



- mRNA transcripts movement can be tracked in real time
- Three categories of motion: stationary, sub-diffusive, diffusive
- Different mobility patterns in cytosolic, secreted and transmembrane proteins

Visualizing local translation in the dendrites of neurons



- Cultured primary hippocampal neurons expressing scFv-GFP and the reporter contruct
- 3‘UTR_Arc harbors zipcode sequences responsible for transport to dendrites for local translation
- Large fraction of translating polysomes exhibit rapid motion in the dendrites (anterograde and retrograde motion)
- Active transport of mRNA can occur after translation has already started

Conclusions

- SunTag approaches provide a real-time readout of translational activity
- Accurate spatial and temporal information of translation sites
- High detection sensitivity
- Low throughput, allowing the study of few mRNAs at a time
- Introduction of the reporter tag into the target mRNA could lead to perturbation
- Future work:
  - To incorporate the translation reporter tag into endogenous gene loci by Crispr-Cas9
  - To study protein factors regulators effects on translation dynamics