UniversitätsSpital Zürich

Spatial and Temporal Dynamics of mRNA Degradation

Technical Journal Club

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Simultaneous detection of mRNA transcription and decay intermediates by dual colour single mRNA FISH on subcellular resolution

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Nucleic Acids Research, Volume 45, Issue 7, 20 April 2017, Pages e49,

Technology

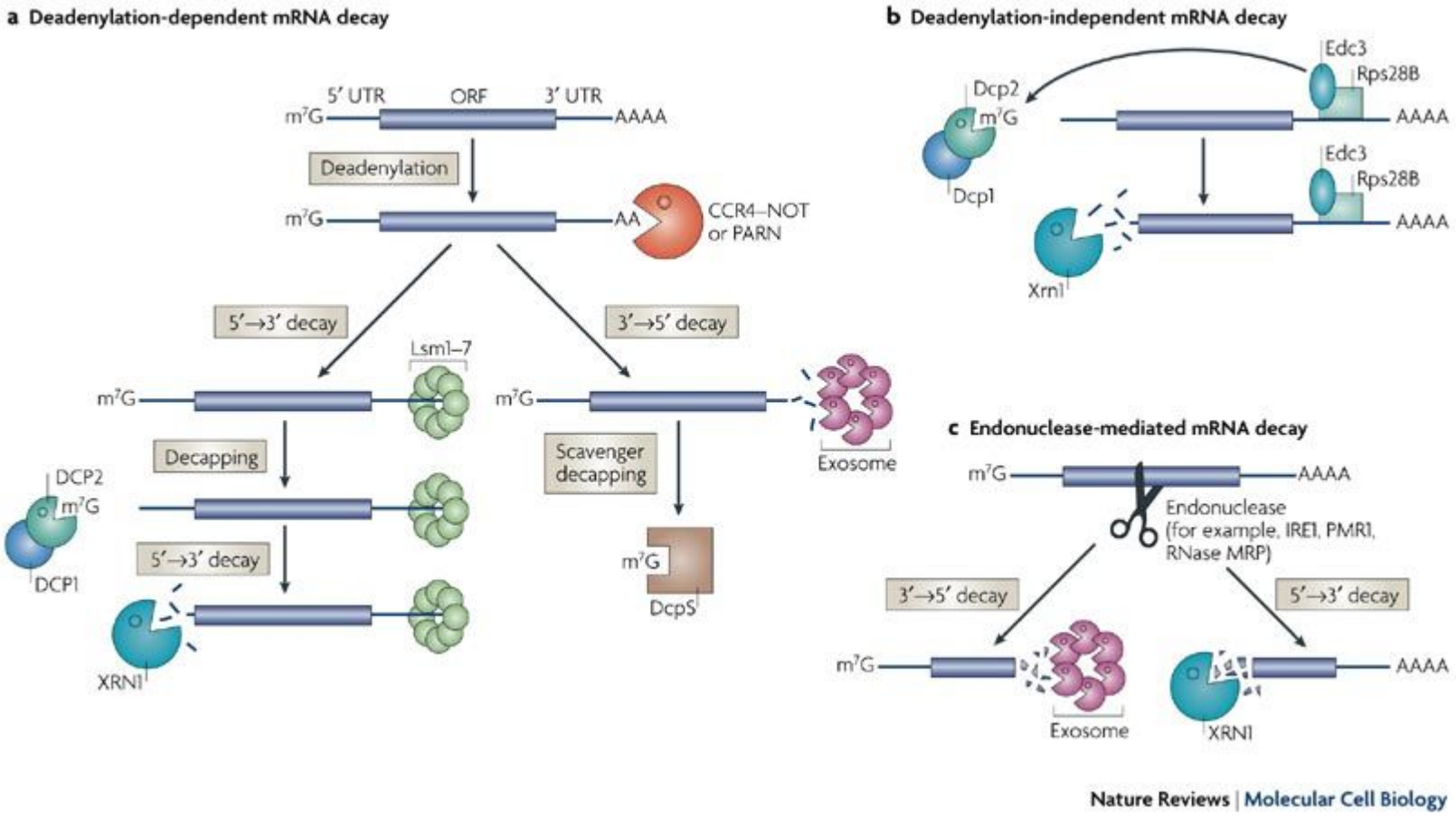
Molecular Cell

The Dynamics of mRNA Turnover Revealed by Single-Molecule Imaging in Single Cells

Ivana Horvathova [1, 2, 4], Franka Voigt [1, 4], Anna V. Kotrys [1], Yinxiu Zhan [1, 2], Caroline G. Artus-Revel [1], Jan Eglinger [1], Michael B. Stadler [1, 3], Luca Giorgetti [1], Jeffrey A. Chao [1, 5]

1 Friedrich Miescher Institute for Biomedical Research, 4058 Basel, Switzerland
2 University of Basel, 4003 Basel, Switzerland
3 Swiss Institute of Bioinformatics, 4058 Basel, Switzerland

Introduction



Challenges in the study of mRNA decay

- Degradation occupies only a small fraction of mRNAs lifetime
- High variability in decay rates between different mRNAs
- Limited understanding of temporal and spatial requirements for mRNA turnover

Open questions

- Which mRNA degradation pathways are affected in specific physiological and pathological conditions?
- Where does mRNA decay take place?

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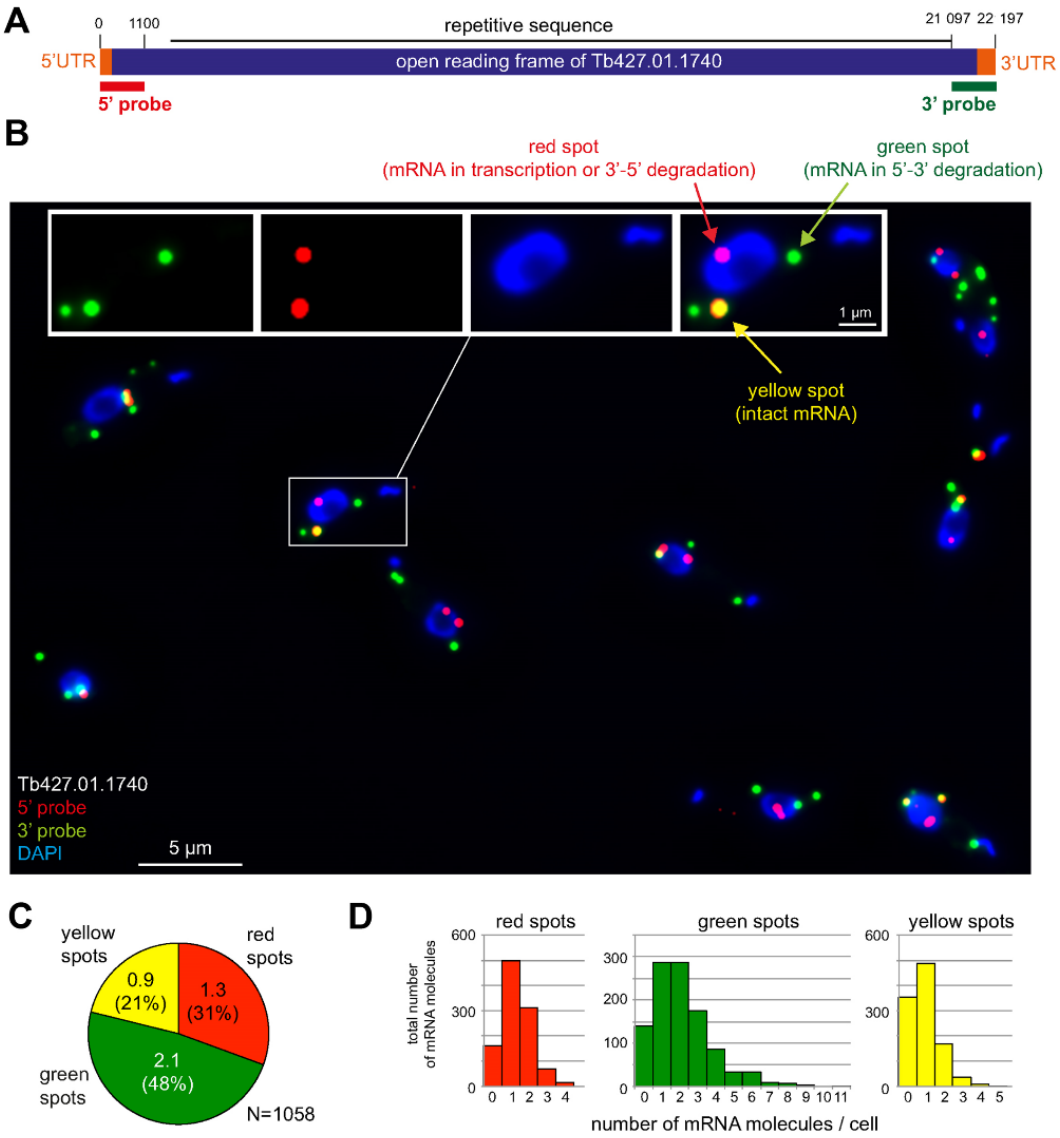
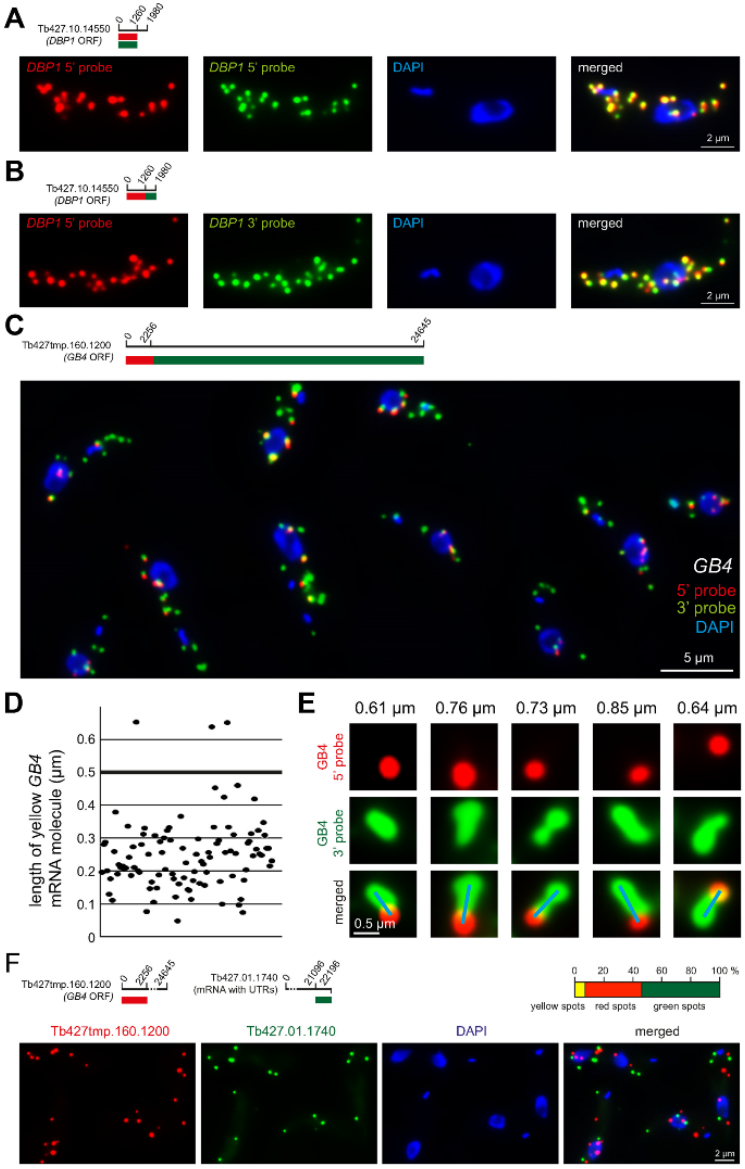
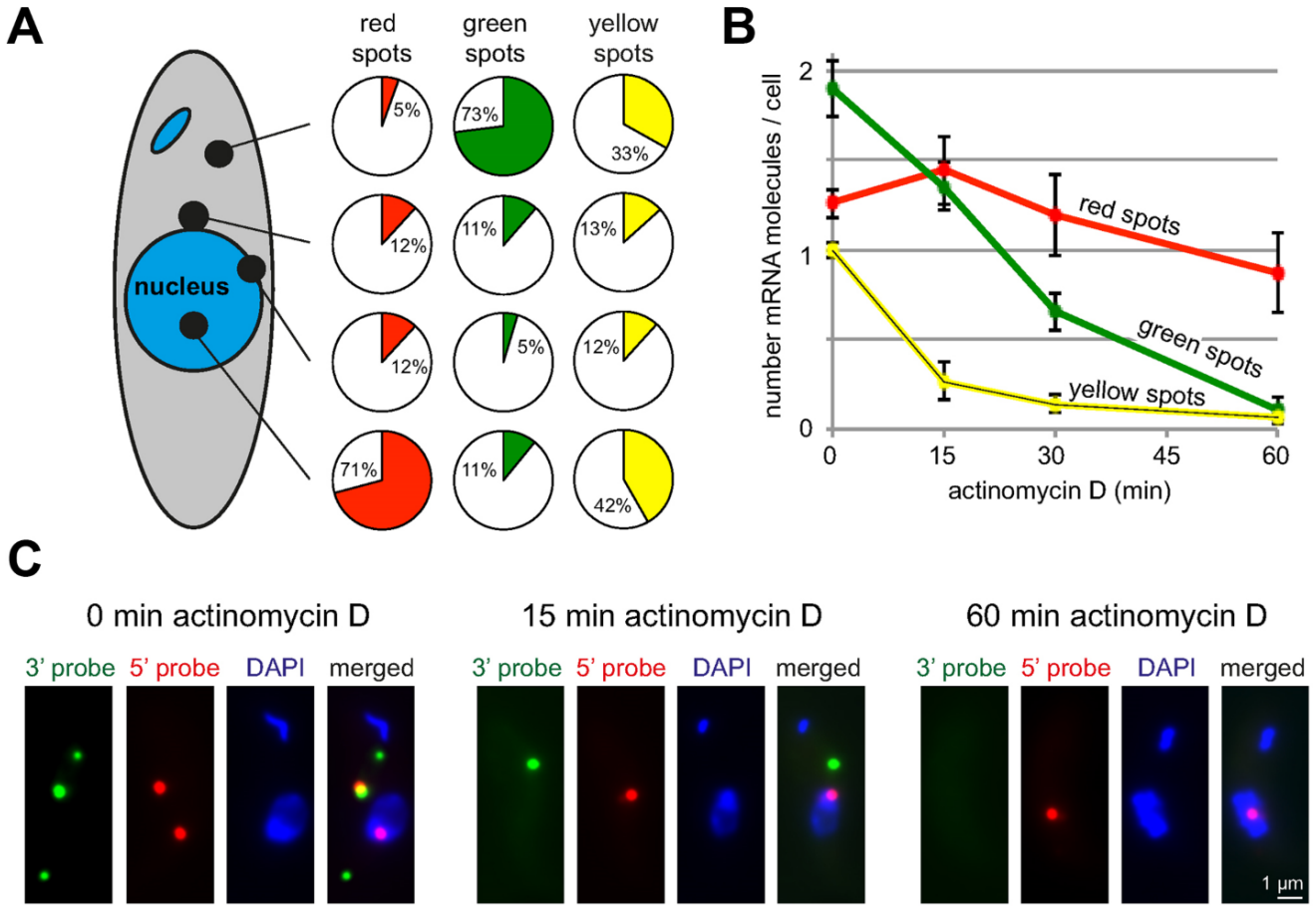
A long mRNA as a reporter for mRNA metabolism



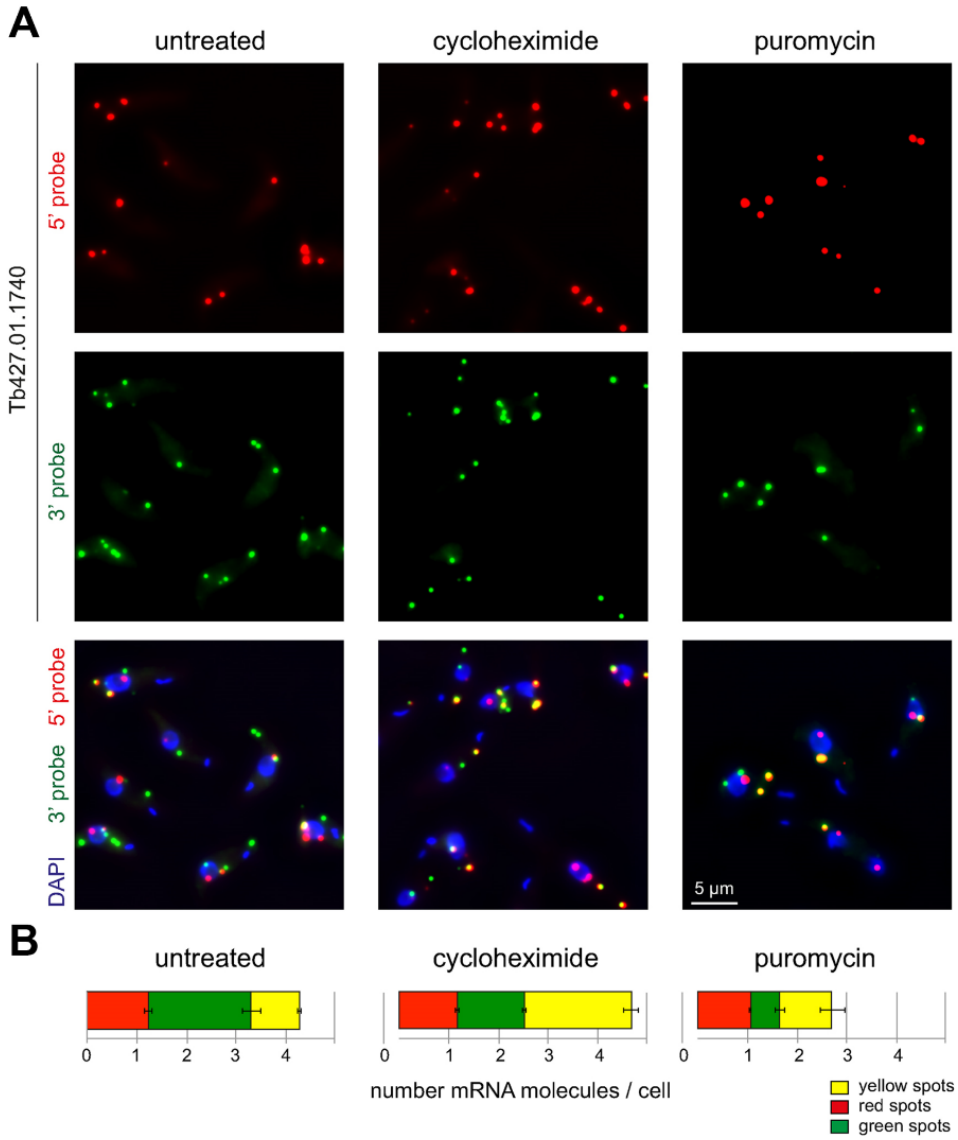
Technical controls and the definition of a yellow spot



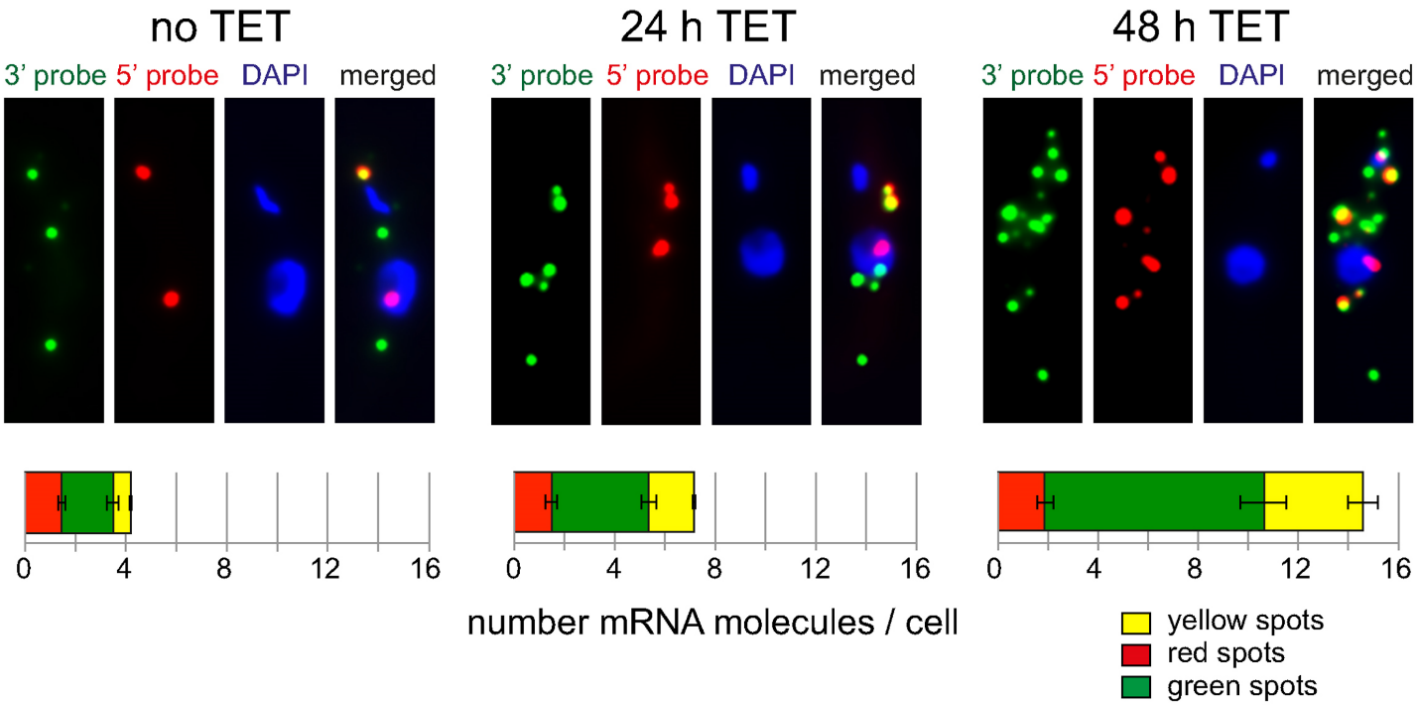
Subcellular localization and mRNA decay rates



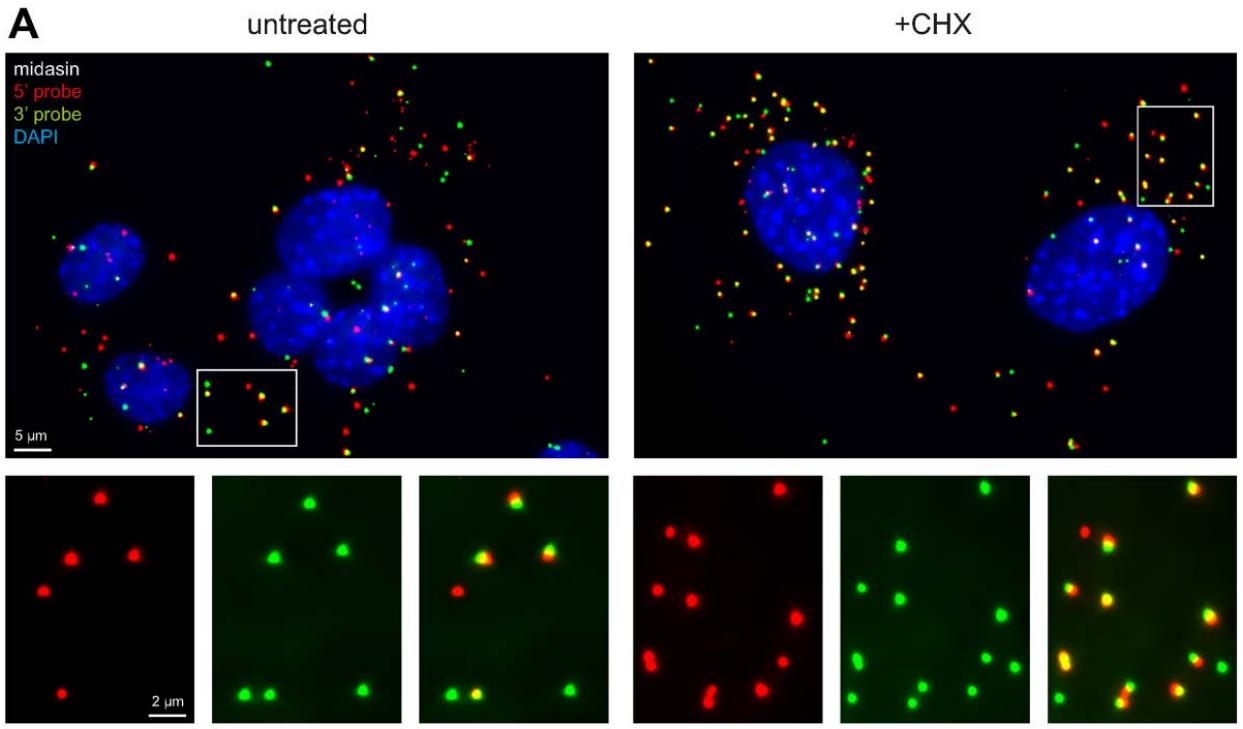
Translational inhibition by cycloheximide and puromycin



Inducible RNAi depletion of XRNA



Detection of mRNA decay intermediates in mammalian cells



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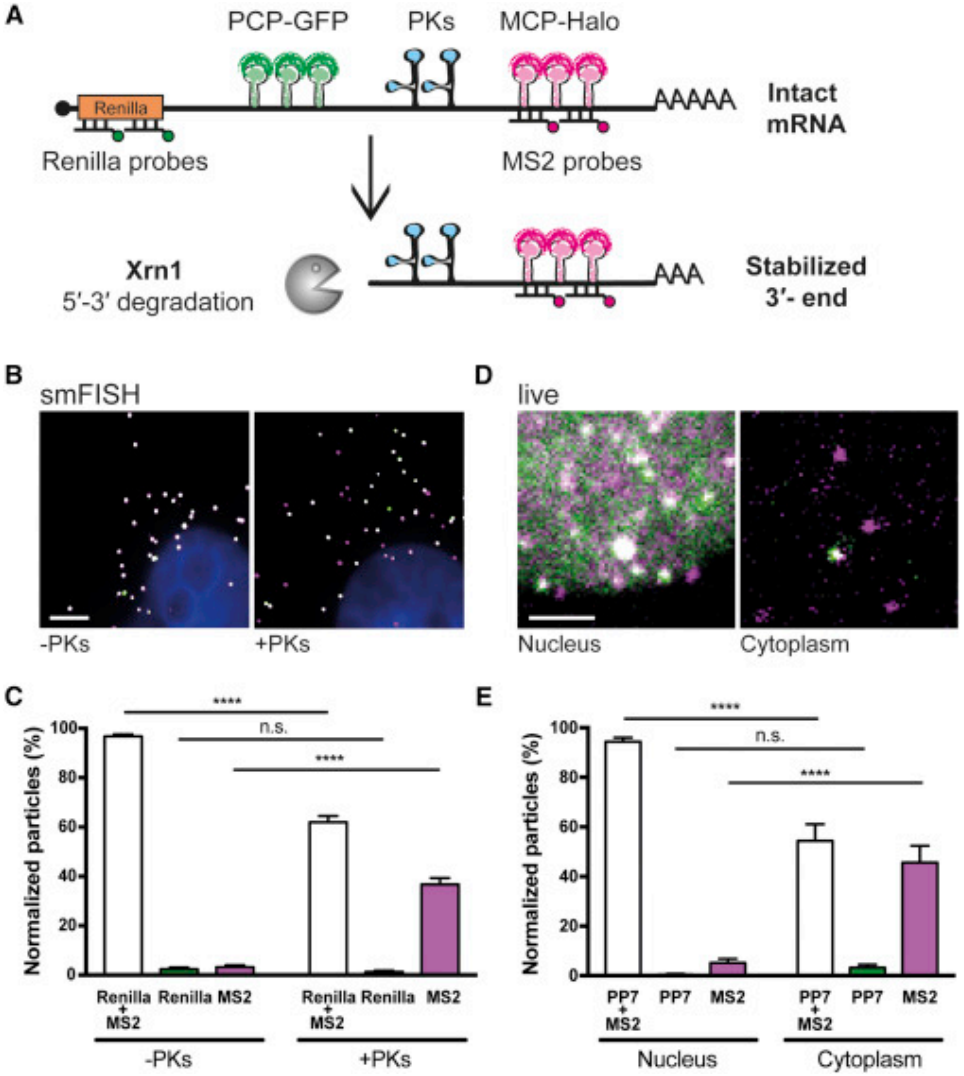
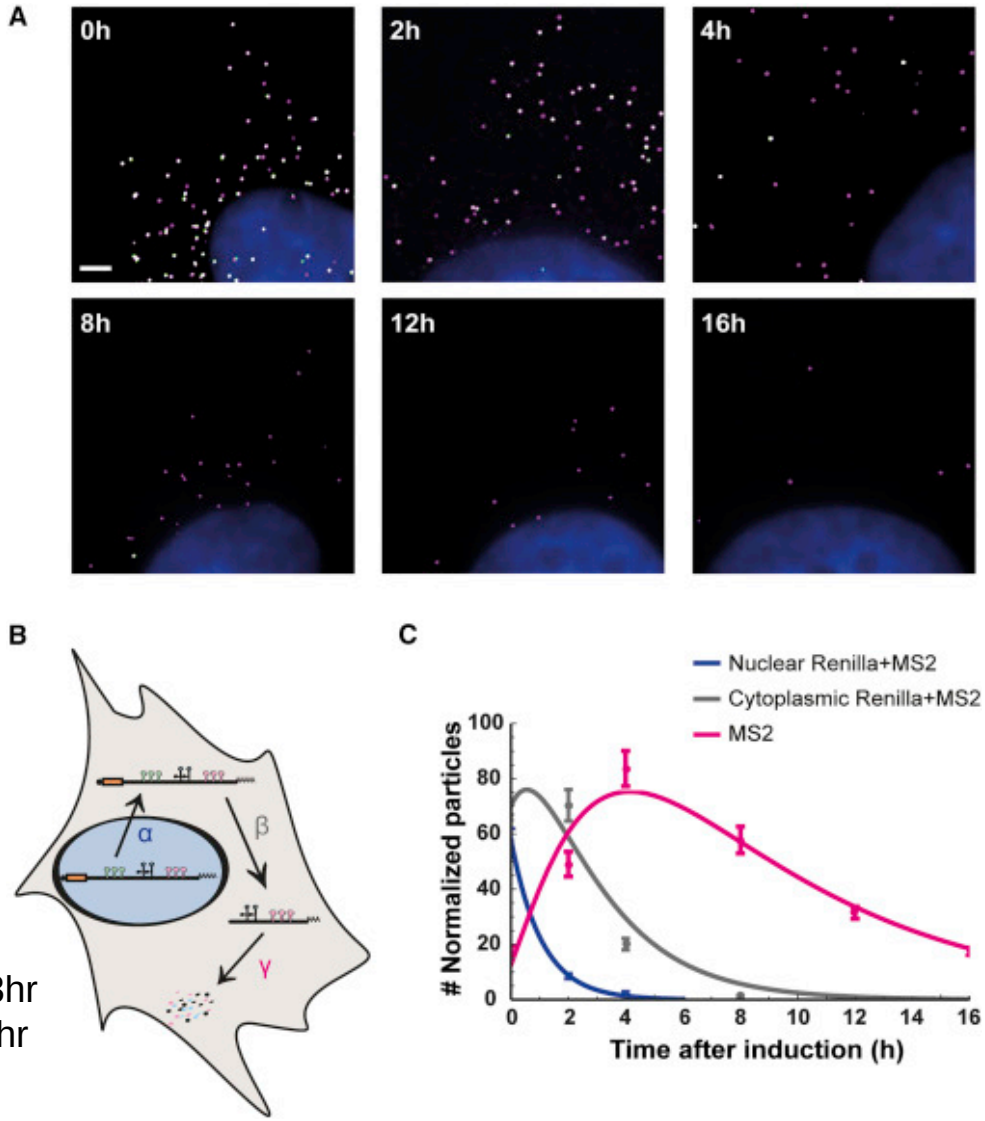
TREAT imaging of mRNA degradation in single cells

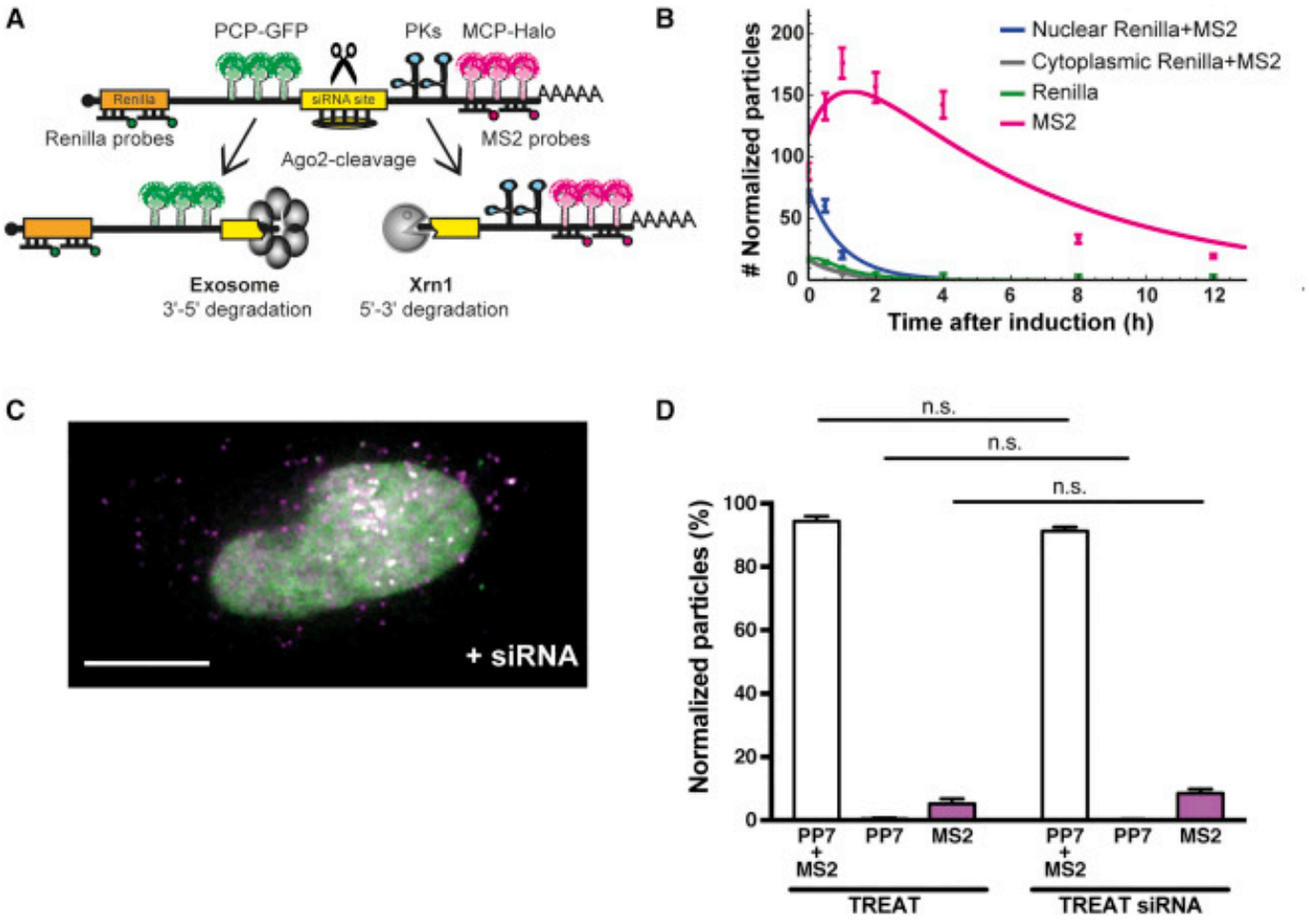


Half-life measurement of TREAT mRNA in single cells

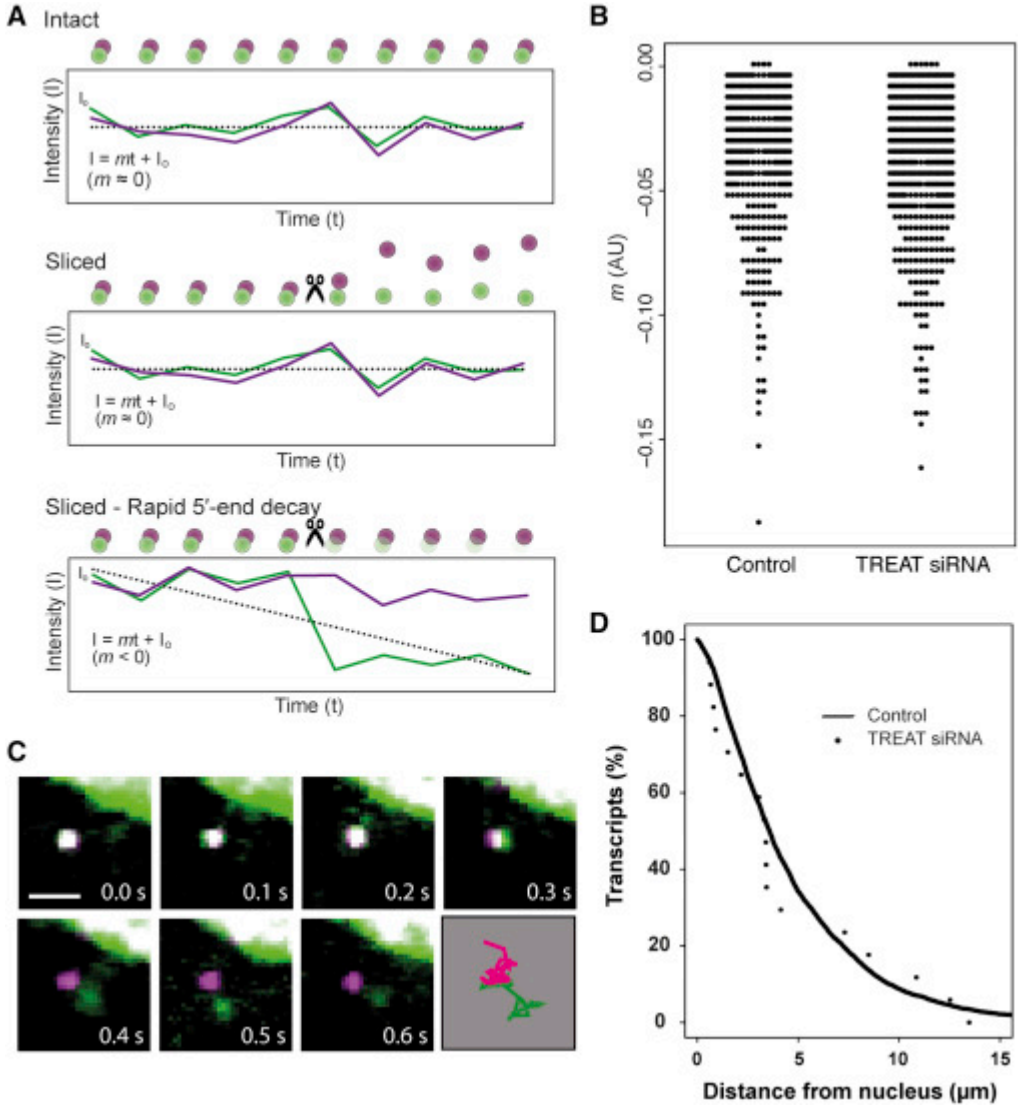


Nuclear export time: 0.74hr
Degradation of TREAT: 1.63hr
Degradation of 3´ end: 4.39hr

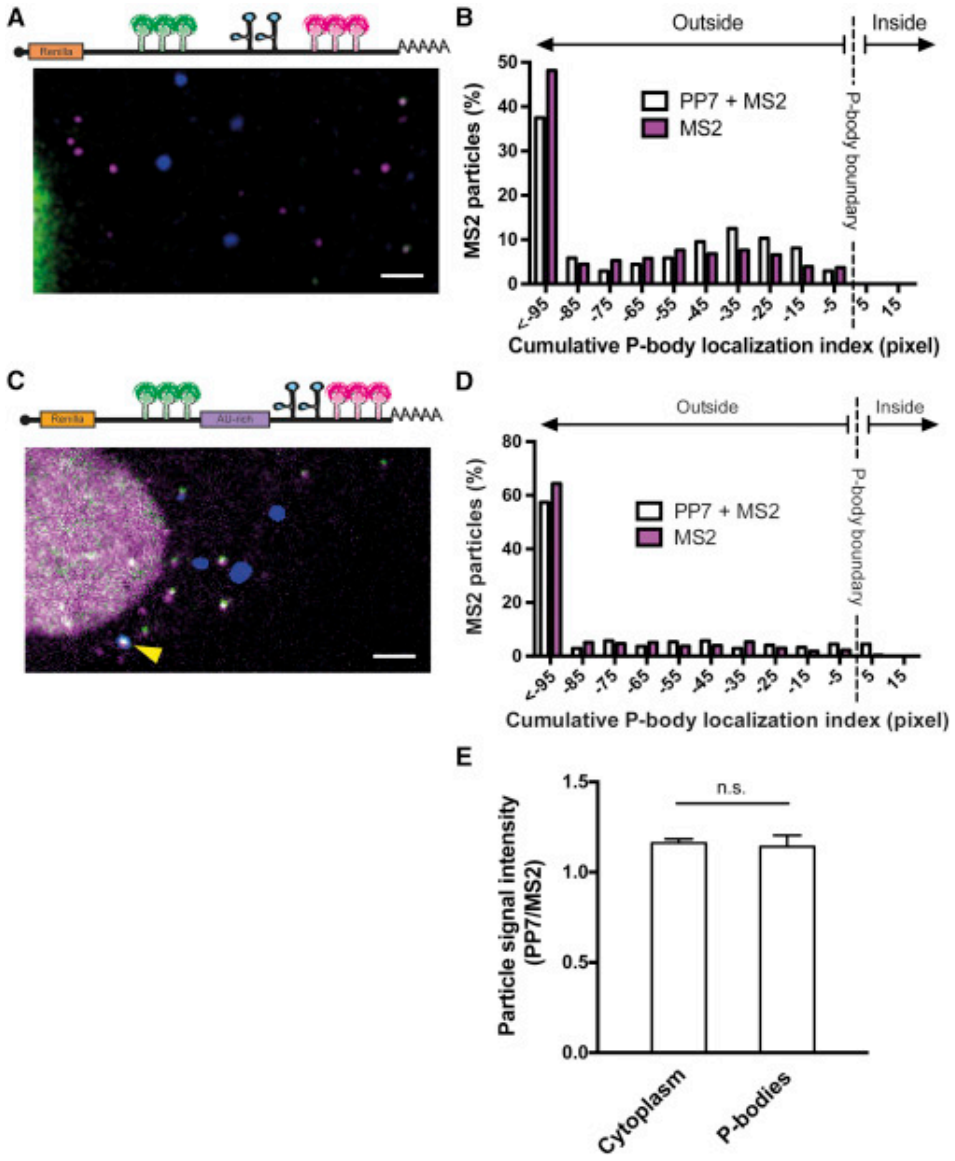
TREAT siRNA transcripts are rapidly degraded in the cytoplasm



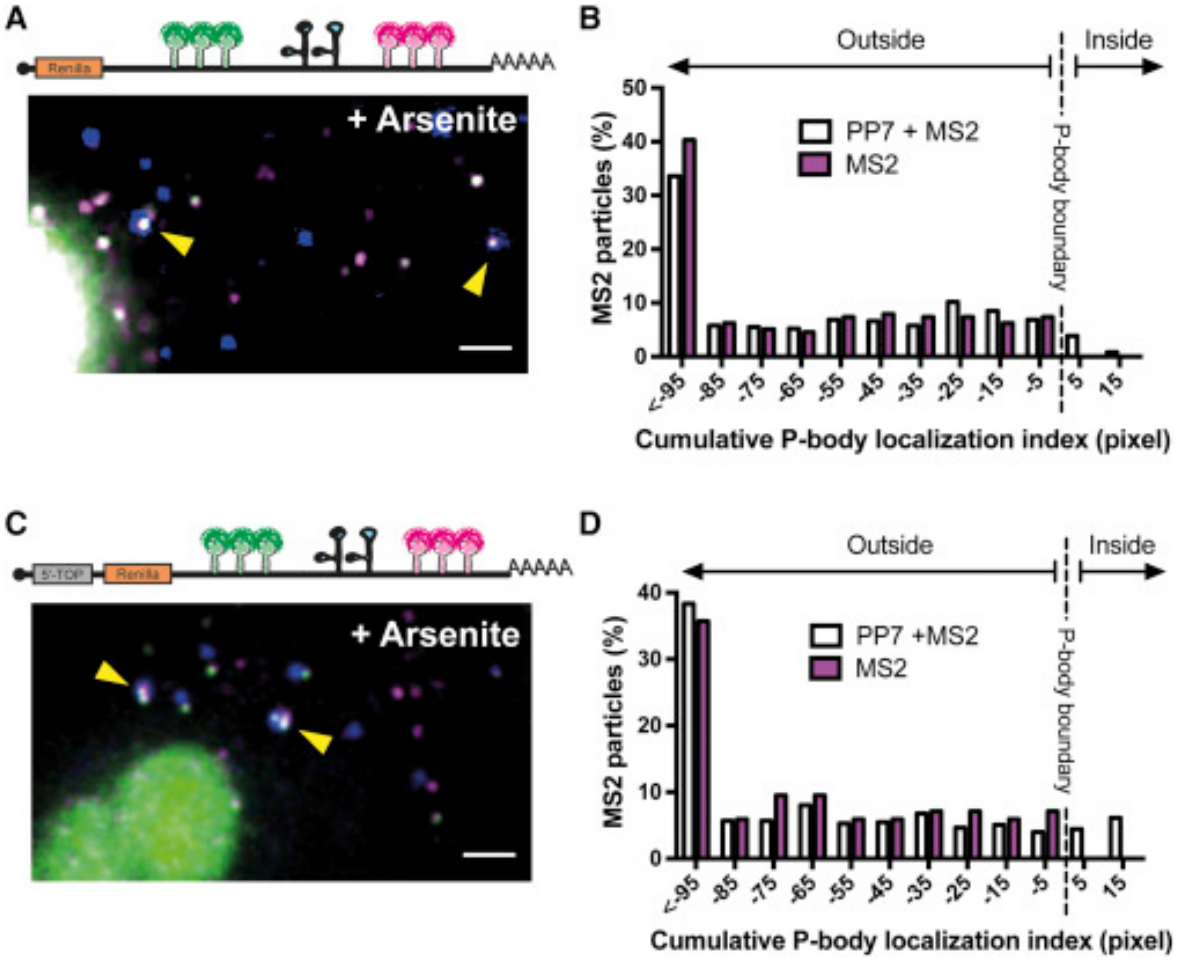
TREAT imaging of siRNA-mediated endonucleolytic cleavage in live cells



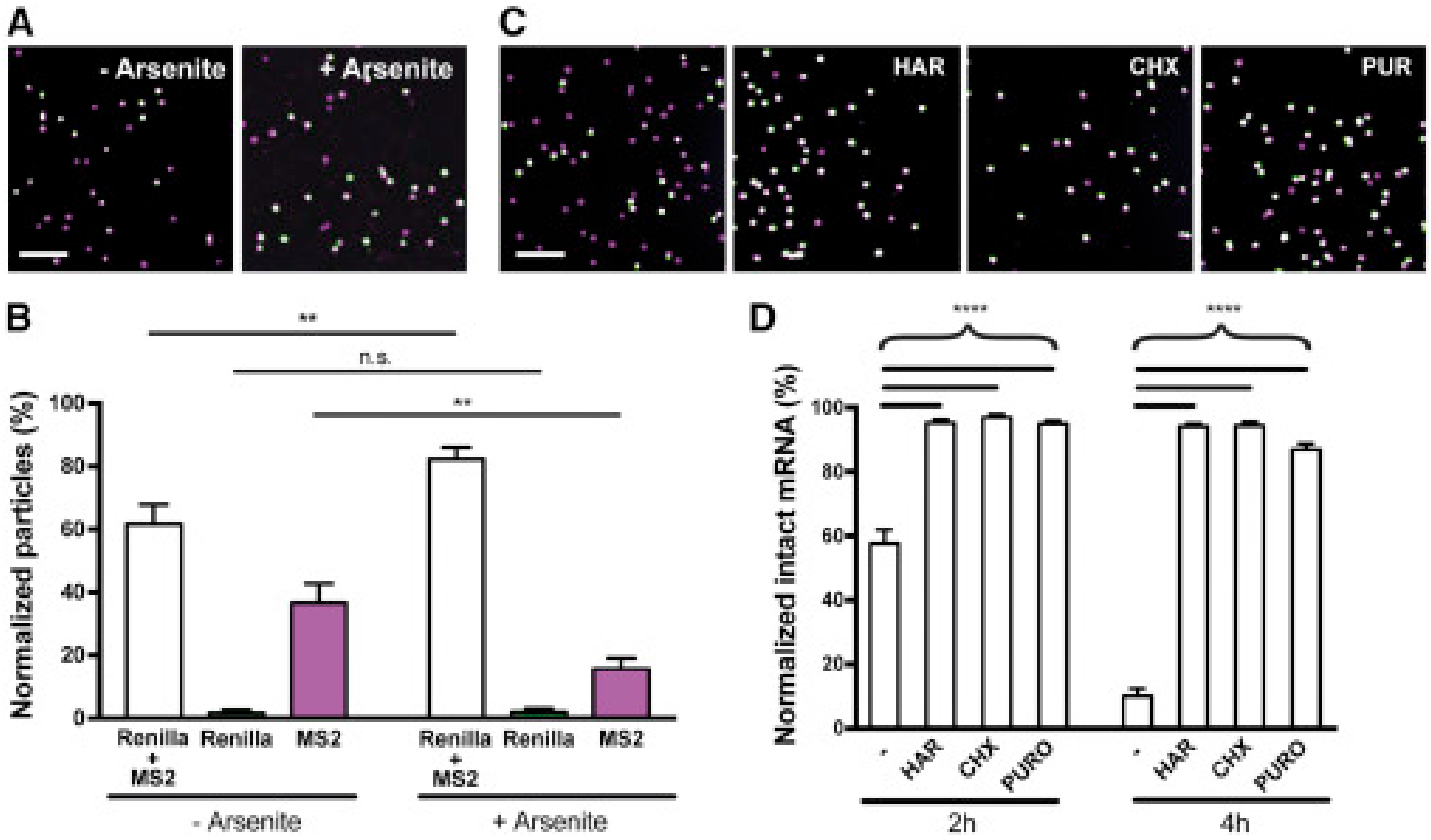
TREAT transcripts are not degraded in P-Bodies



TREAT transcripts are not degraded in P-Bodies during stress



Stabilization of TREAT transcripts during stress and translation inhibition



Potential Applications

- TREAT may uncover novel role for endonucleases in mRNAs decay
- Reveal novel aspects of the coordination between RNA localization and degradation
- Track mRNAs movements and connect it with their stability
- Elucidate aspects of the interplay between cytoplasmic mRNA turnover and RNA biogenesis in the nucleus

Limitations

- siRNA needed to shorten the half life of transcripts
- Difficulty in tracking decay during mRNA movements
- Need to test TREAT with endogenous transcripts