Best practices in mRNA live imaging

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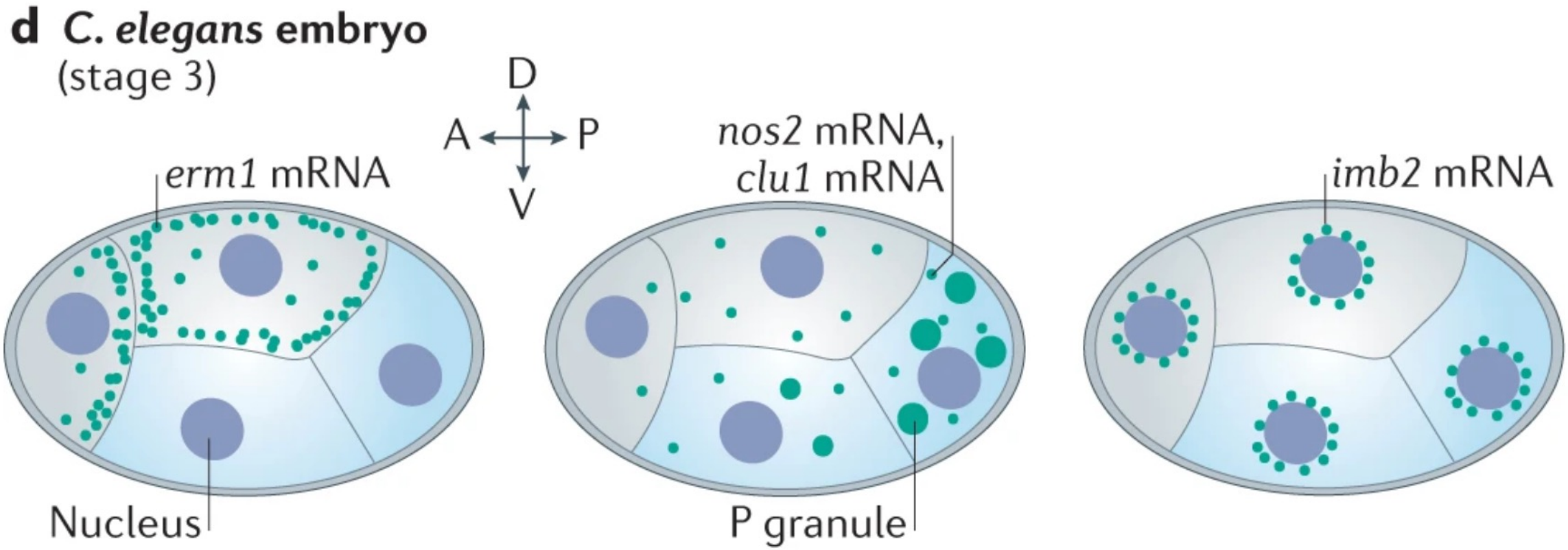
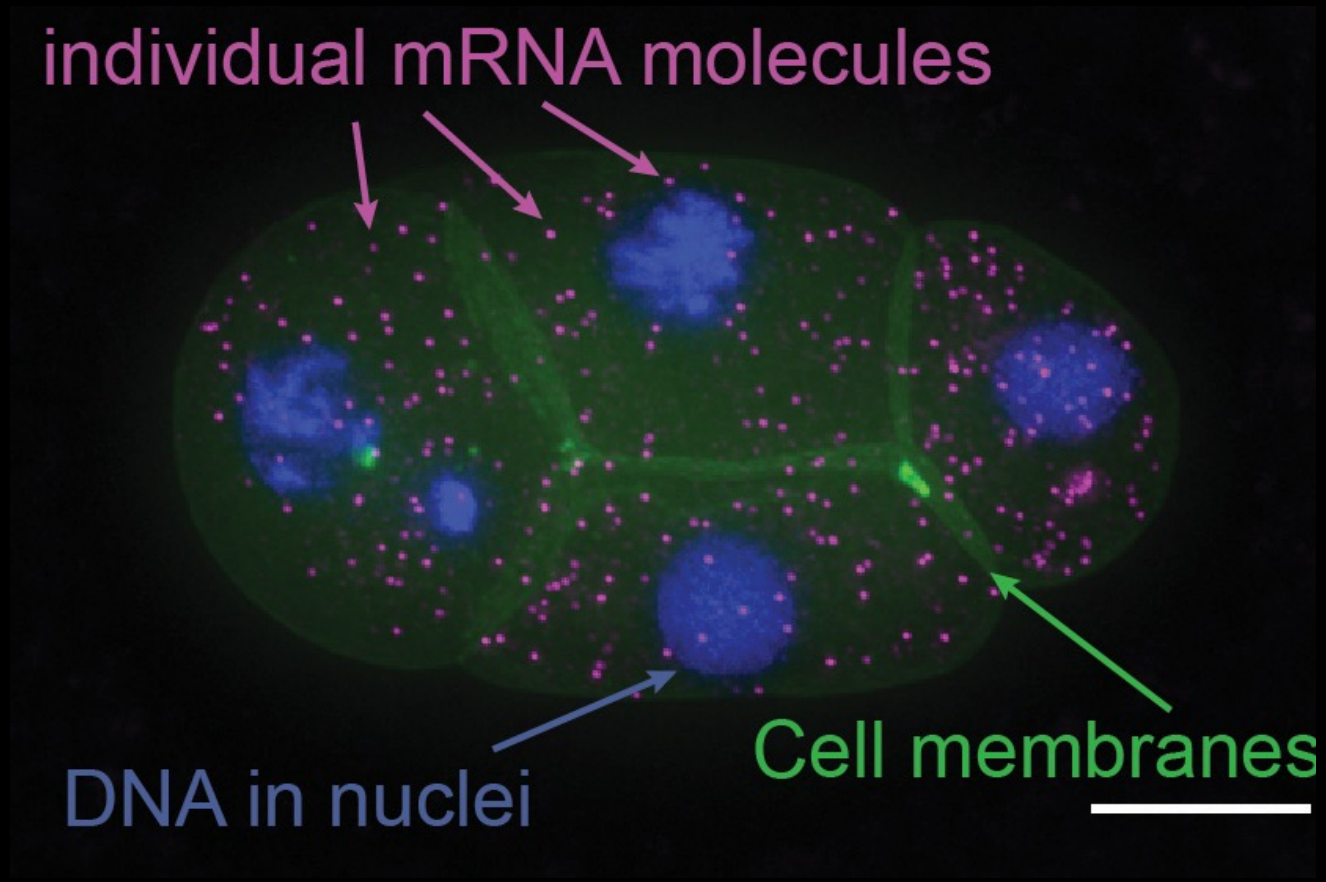
Colorado State University
mRNA Live Imaging Workshop #Worm21
June 23, 2021

We study mRNA localization in early embryos



Parker et al, 2020; Das et al., 2021

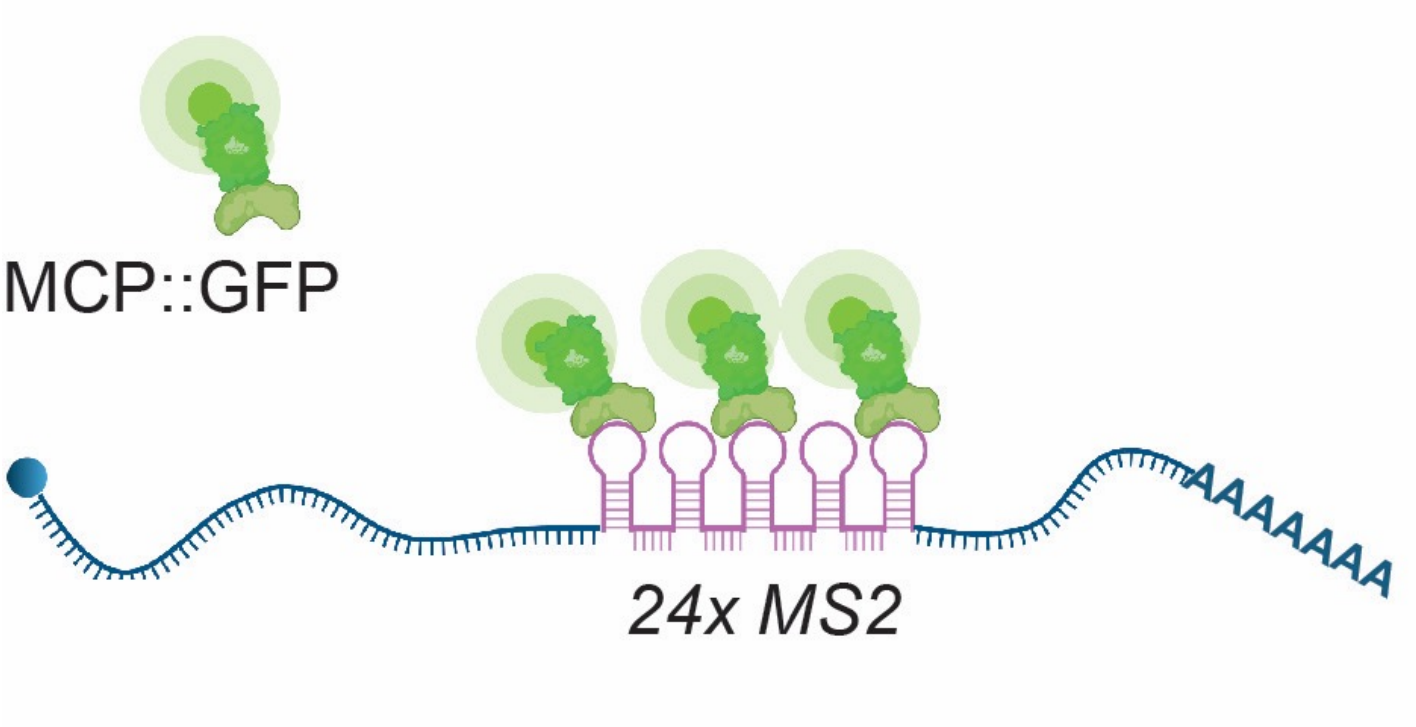
smFISH is a powerful tool



For new, updated protocols: Parker et al., BioRXiV, 2021

Lindsay Winkenbach
Dylan Parker

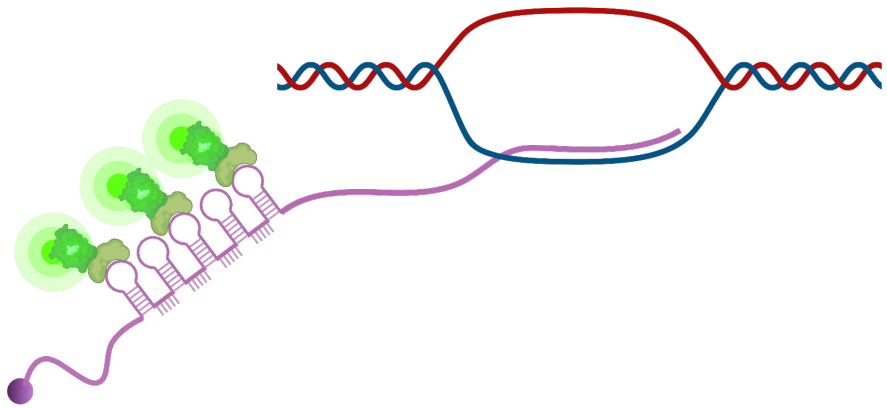
mRNA live imaging has the potential to address novel questions



- Dynamics
- Decay
- Trafficking
- Translation

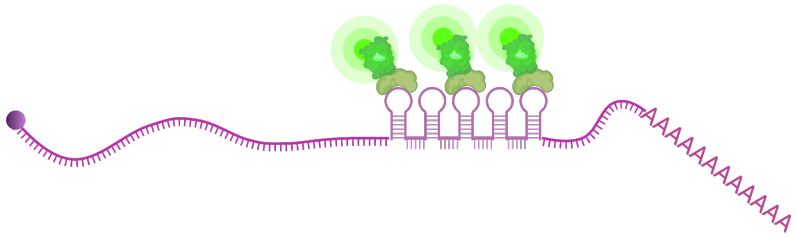
Meghan Costello
Nalysha Torres

Three major applications of mRNA live imaging



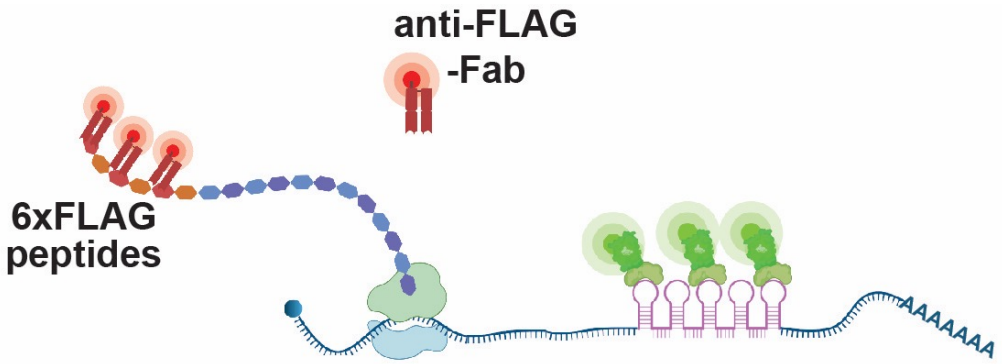
- As a reporter of transcriptional activation

Three major applications of mRNA live imaging

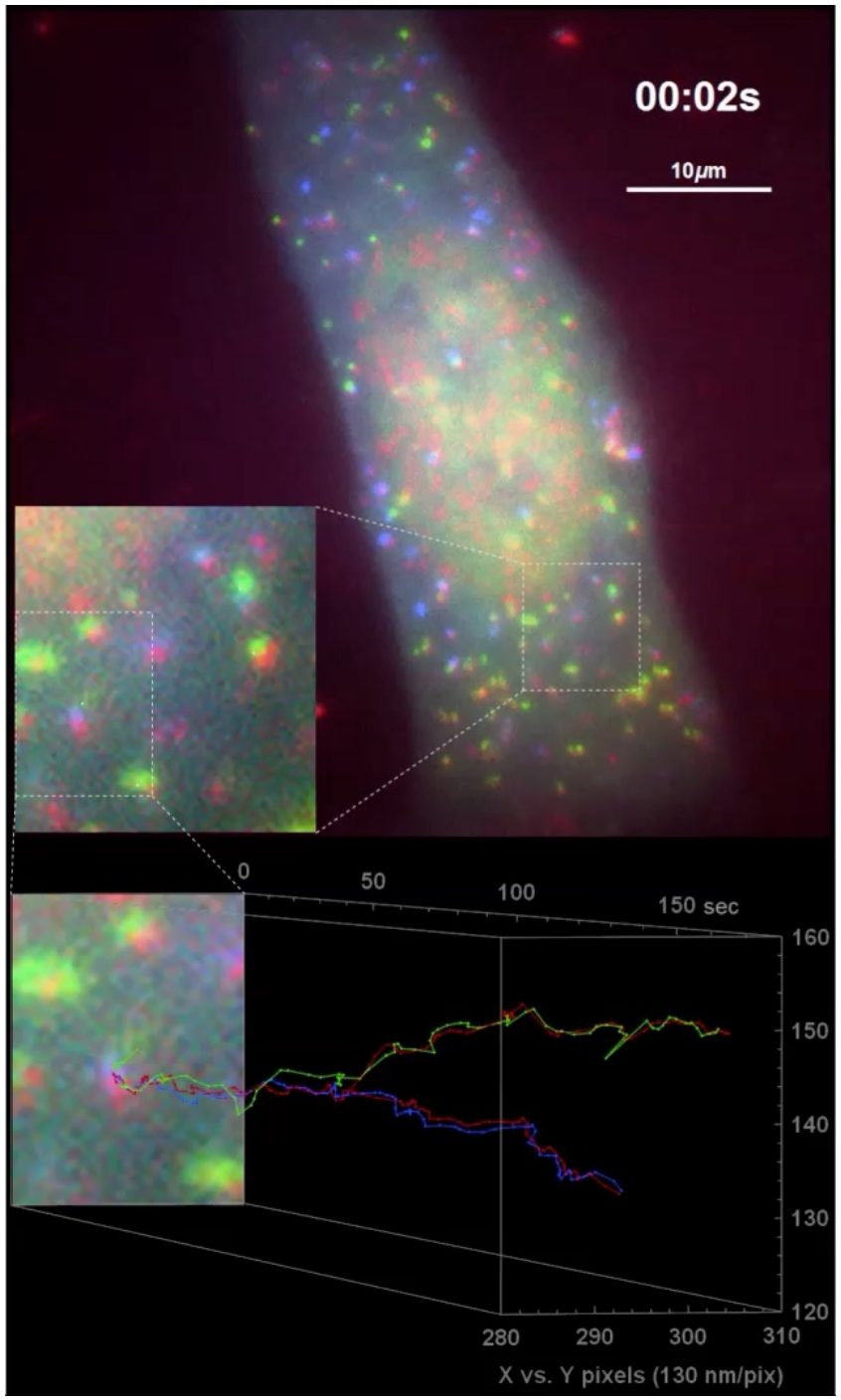


- As a reporter of transcriptional activation
- To track cytoplasmic mRNA movement and regulation

Three major applications of mRNA live imaging



- As a reporter of transcriptional activation
- To track cytoplasmic mRNA movement and regulation
- To report translational status in combination with other technologies



Three major applications of mRNA live imaging

- As a reporter of transcriptional activation
- To track cytoplasmic mRNA movement and regulation
- To report translational status in combination with other technologies

Tatsuya Morisaki, Stephanie Moon, Roy Parker, Tim Stasevich
U2OS cells: mRNA – red; peptide – green; stress granules – blue

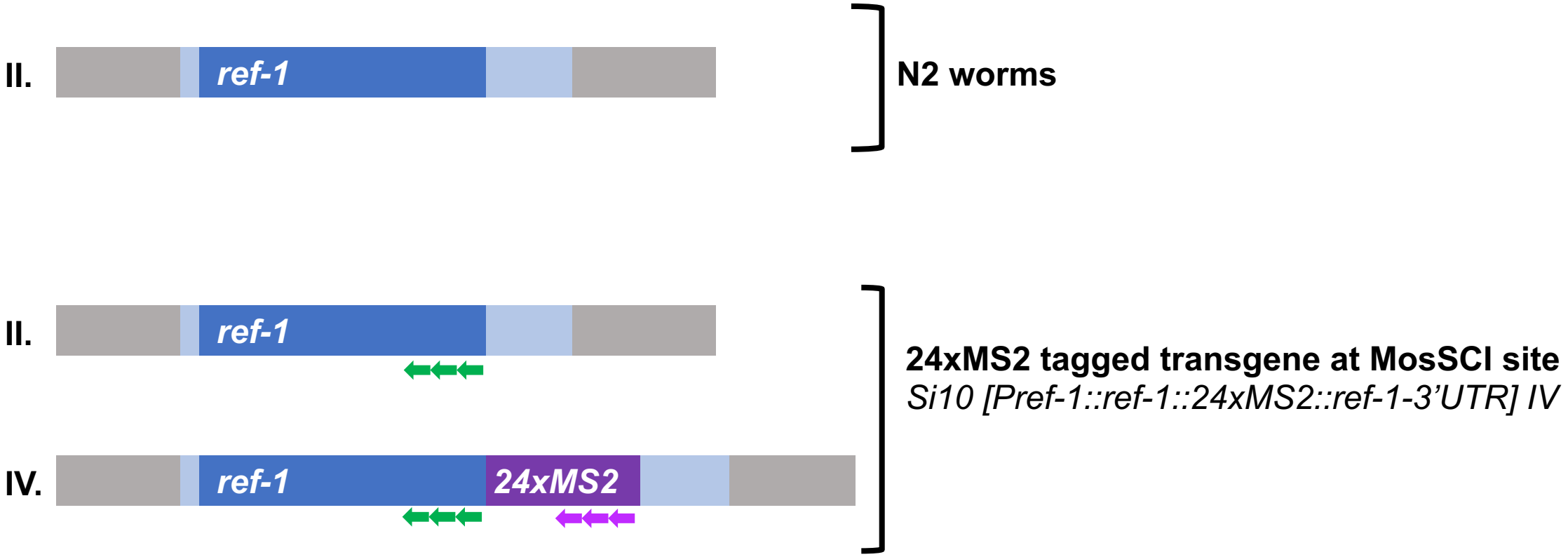
Questions and Concerns

- Does MS2/MCP alter mRNA behavior and function?
  - mRNA decay? transport? translation? small RNA-mediated regulation?
- What is the resolution?
- What further optimization is needed?

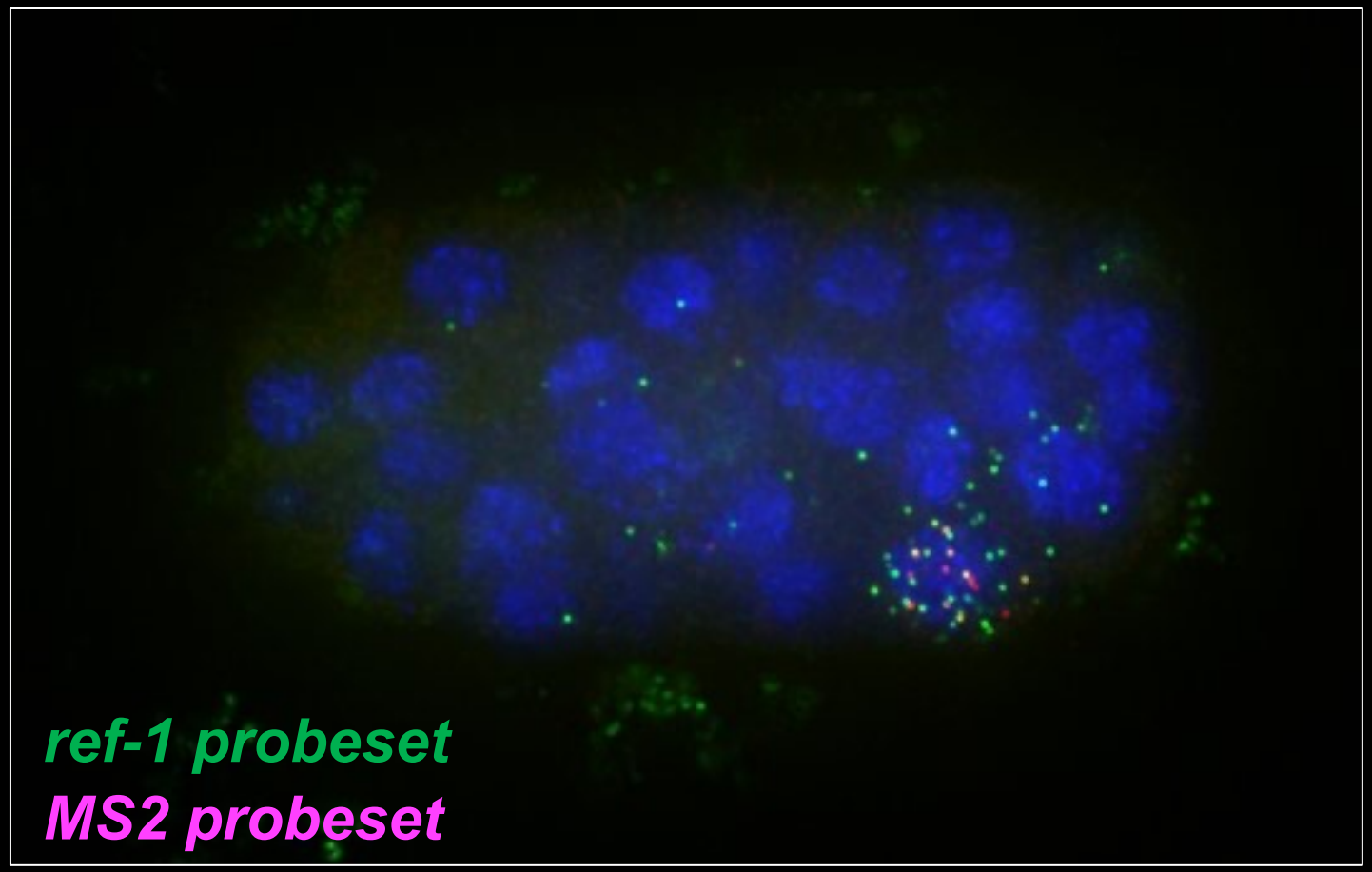
Does MS2/MCP alter mRNA?

1. Use smFISH to determine whether mRNA localization or abundance are changed
2. Determine whether translation is occurring normally
3. Assess worms for phenotypes

Using smFISH to compare endogenous and MS2 tagged transcripts

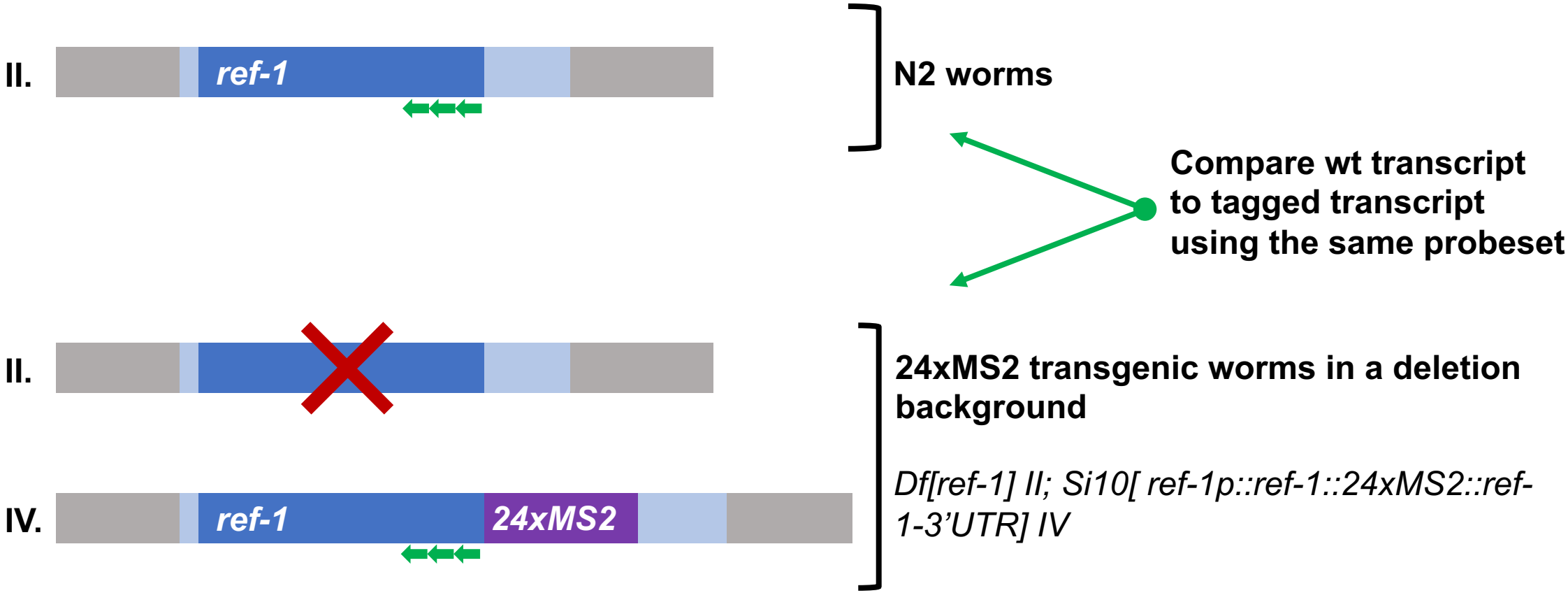


Endogenous and MS2 tagged *ref-1* mRNA can simultaneously imaged by smFISH

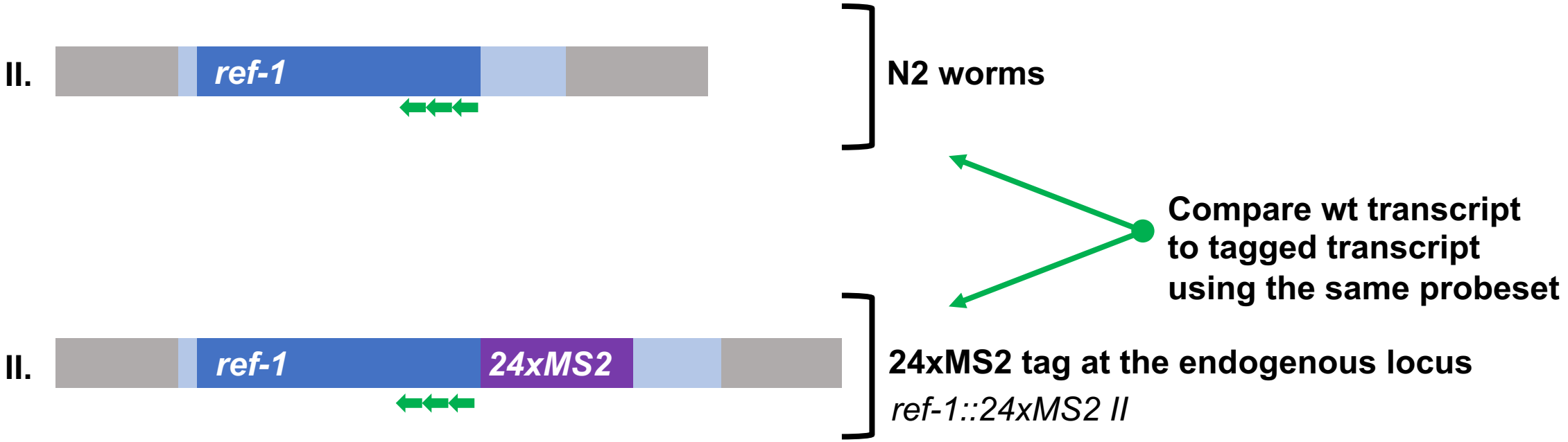


Nalysha Torres

A complementary approach:
use the same probeset and matched stoichiometry



Better yet:
tag the endogenous gene



Is translation perturbed by MS2/MCP?

1. Rescue approach
   - Does the MS2 tagged allele rescue a mutant?
2. Reporter approach
   - Add GFP. Does it fluoresce?
3. Immunofluorescence
   - Is the protein produced? Is it properly localized?

Our preliminary results

- Nuclear export seems normal (*ref-1*, *nos-2*, *elt-2)*
- Localization seems normal (*ref-1*, *nos-2*, *elt-2)*
- Abundance – still unclear, still quantifying
- *elt-2::12xMS2* does not completely rescue the *elt-2* null
  - normal embryogenesis and brood size but slow larval growth

What if mRNA abundance or localization are perturbed?

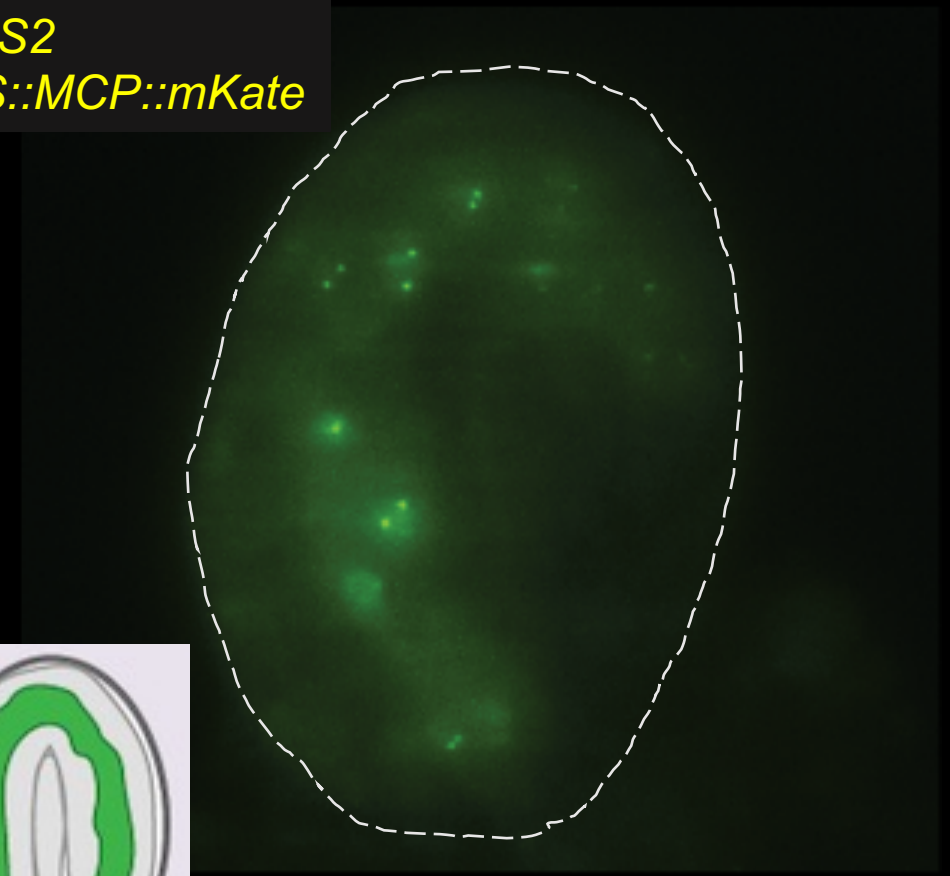
- Try another MS2 variant (V5, V6, Tutucci et al., 2018)
- Try to add spacers on either side of the MS2 loops

Thanks: Rob Singer Lab, Evalina Tutucci, Carolyn Phillips

What is the resolution of the system?

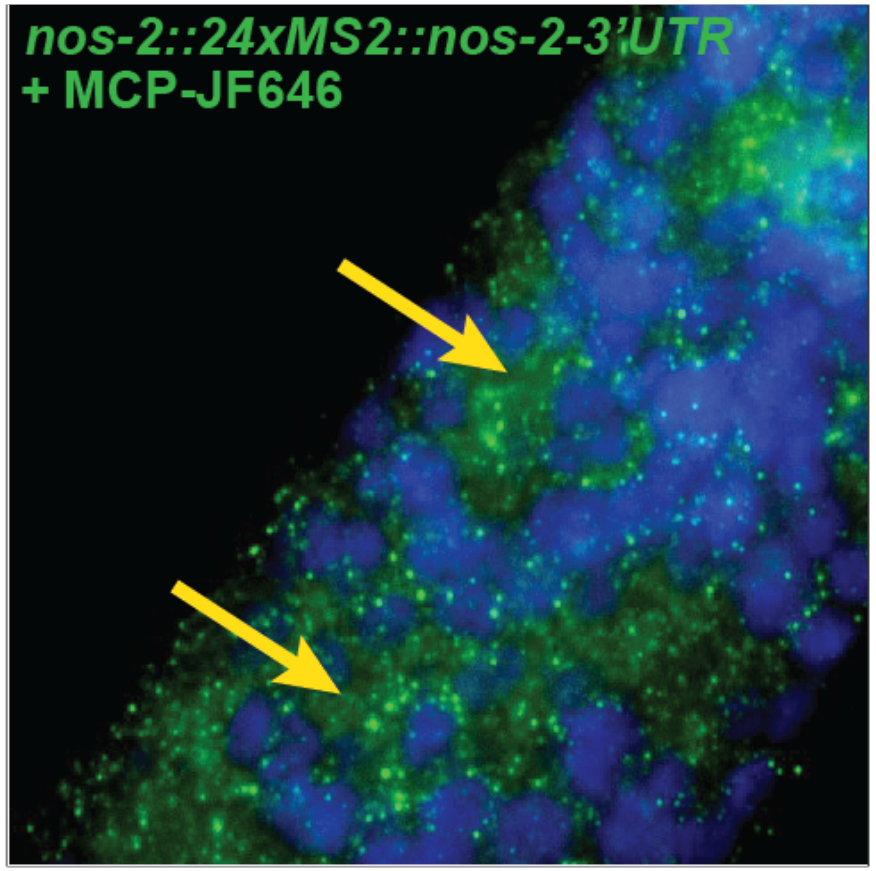
- Can we see single-molecules?
  - Currently we cannot
- Plan
  - Calibrate between smFISH and MS2/MCP using a transcript like *elt-2 mRNA*
  - *elt-2 mRNA* abundance increases through development, is robust and reproducible, and is well-characterized

elt-2::12xMS2
Peft-3::NLS::MCP::mKate



Meghan Costello

What are the major places where further optimization is needed?



Dylan Parker

1. MCP optimization
   - MCP-Janelia fluors yield higher signal to noise, greater photostability
   - MCP concentration is critical
2. Microscopy optimization
   - Speed
   - Sensitivity
   - Photobleaching
   - Depth in the sample
   - Light sheet microscopy (MIZAR, HiLO)

Thank you!

- Changhwan Lee
- Hongjie Zhang
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- Ari Pani
- Doug Shepherd
- Paul Maddox
- The Nishismura Lab
  - Dylan Parker
  - Robert Williams
  - Meghan Costello
  - Nalysha Torres

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