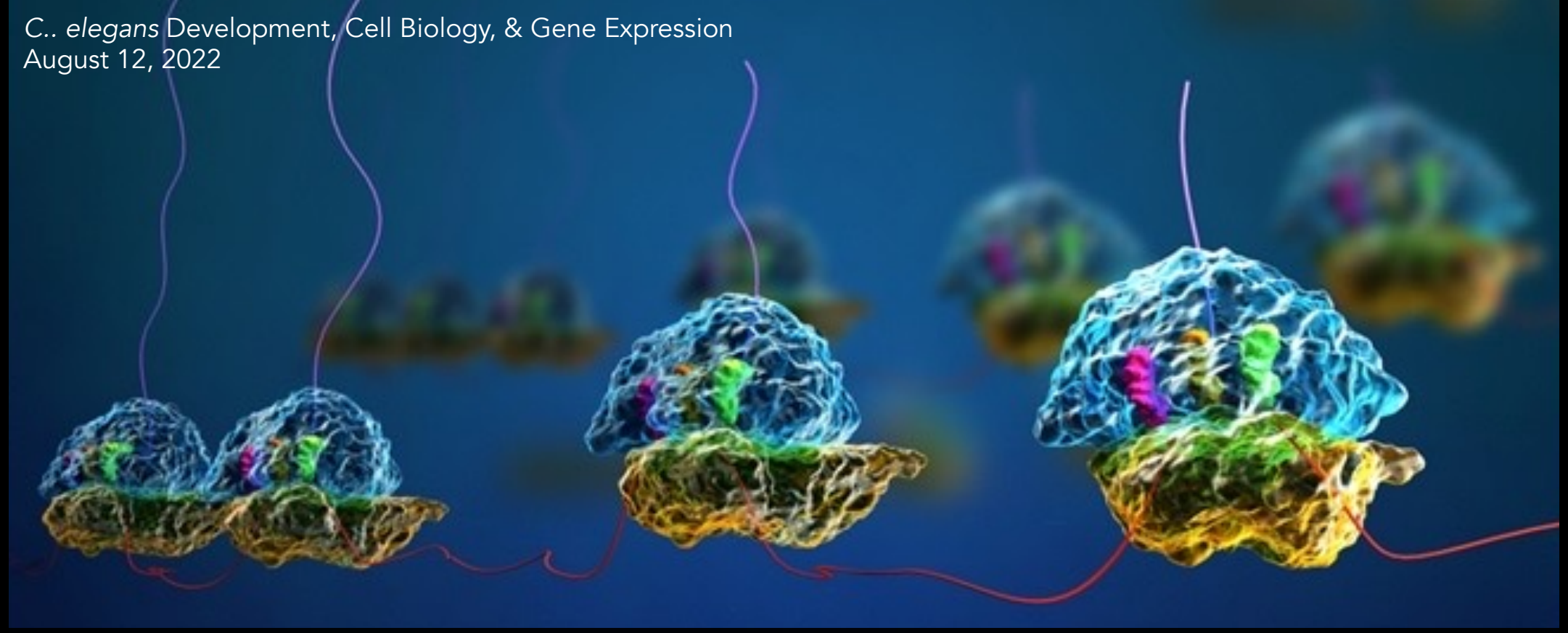
C.. elegans Development, Cell Biology, & Gene Expression
August 12, 2022



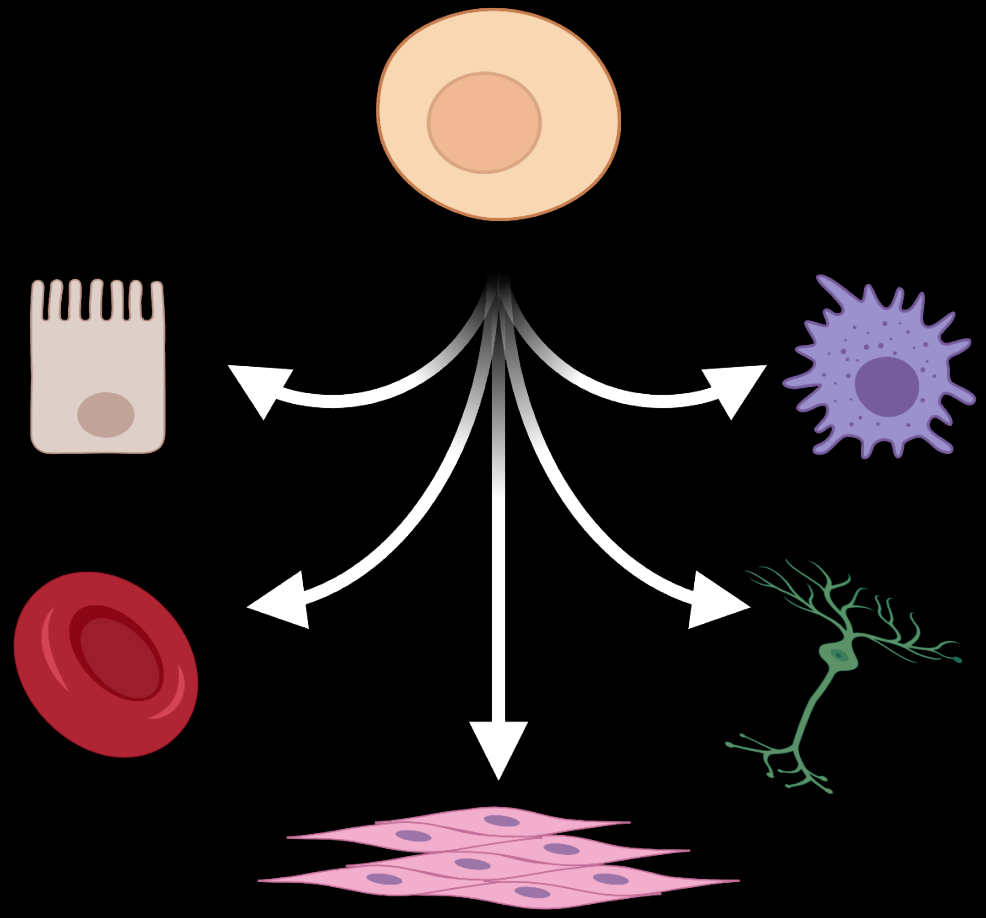
Improved and emerging methods for imaging single mRNA molecules

Erin Osborne Nishimura
Colorado State University

Online resources for today's talk

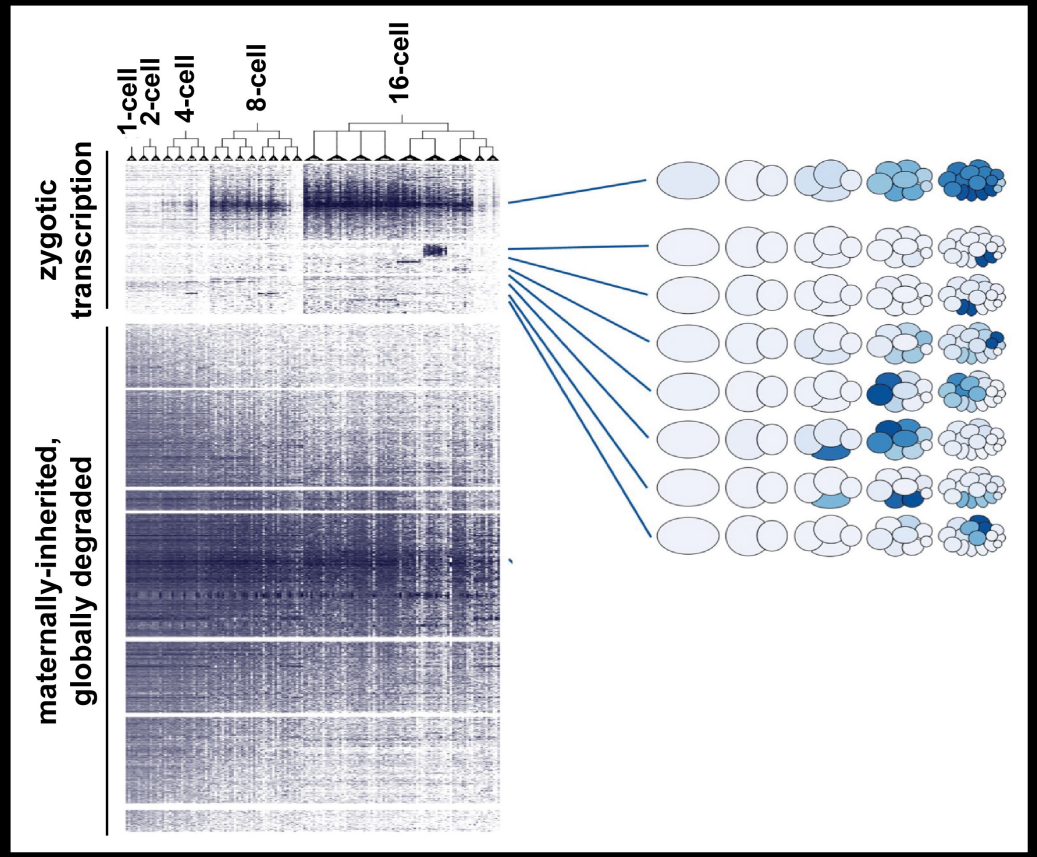
- onishlab.colostate.edu/mrna-workshop-2022
- Osborne Nishimura Lab Website
  - → Events → Single Molecule Imaging Workshop

How does gene expression promote cell diversity during embryogenesis?

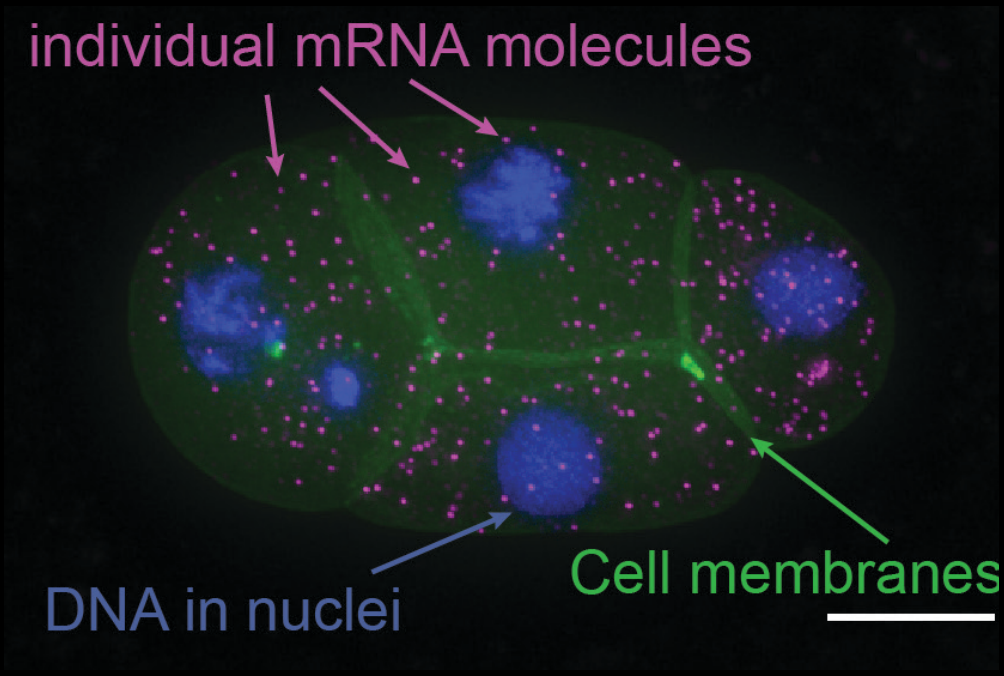


Two complementary approaches: global transcriptomics & single-molecule microscopy

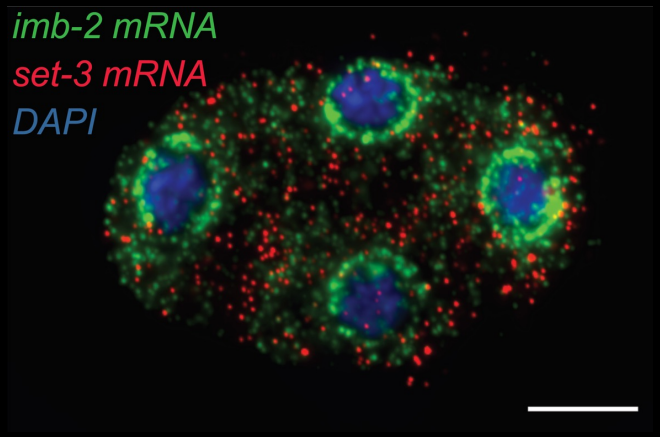
High-resolution RNA-seq

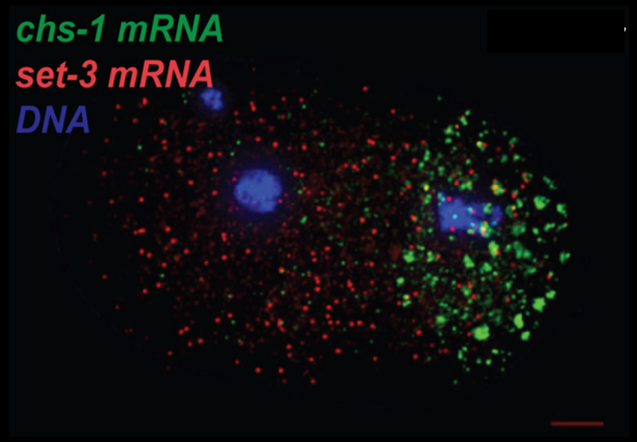


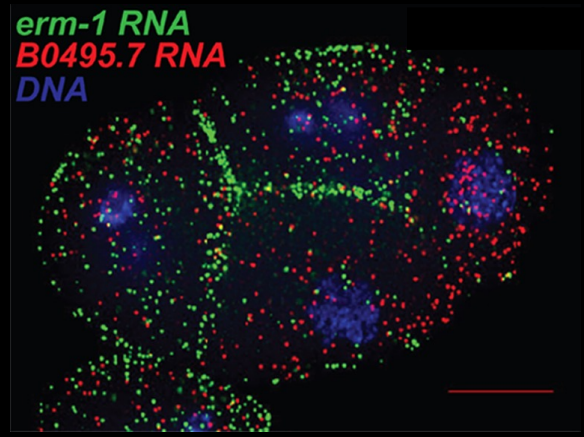
Single molecule Fluorescence In Situ Hybridization (smFISH)

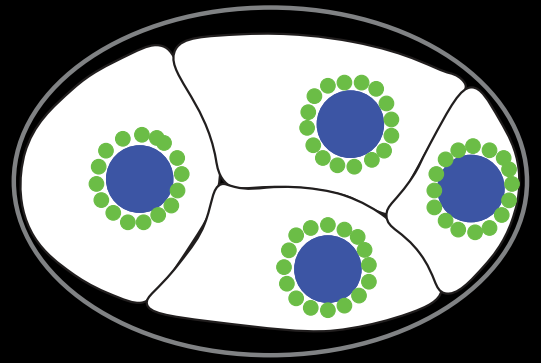


Many maternally-inherited transcripts have diverse subcellular localization patterns in early embryos

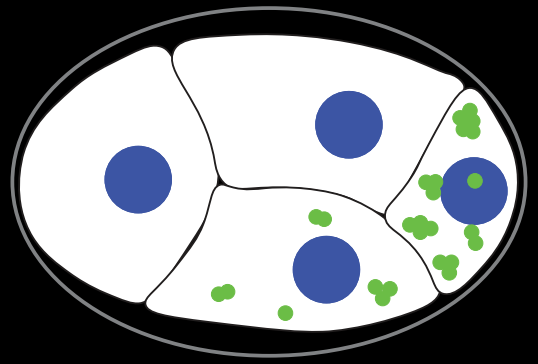




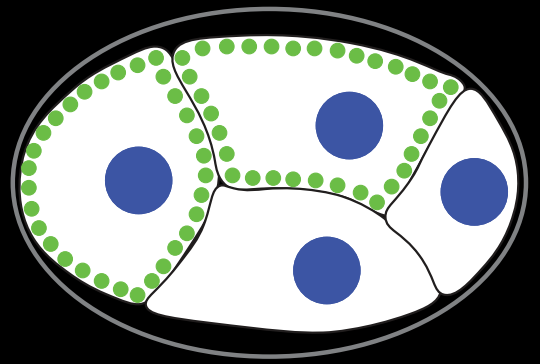




Nuclear periphery



Clustered



Cell cortex

[Parker et al., (2020) Development]

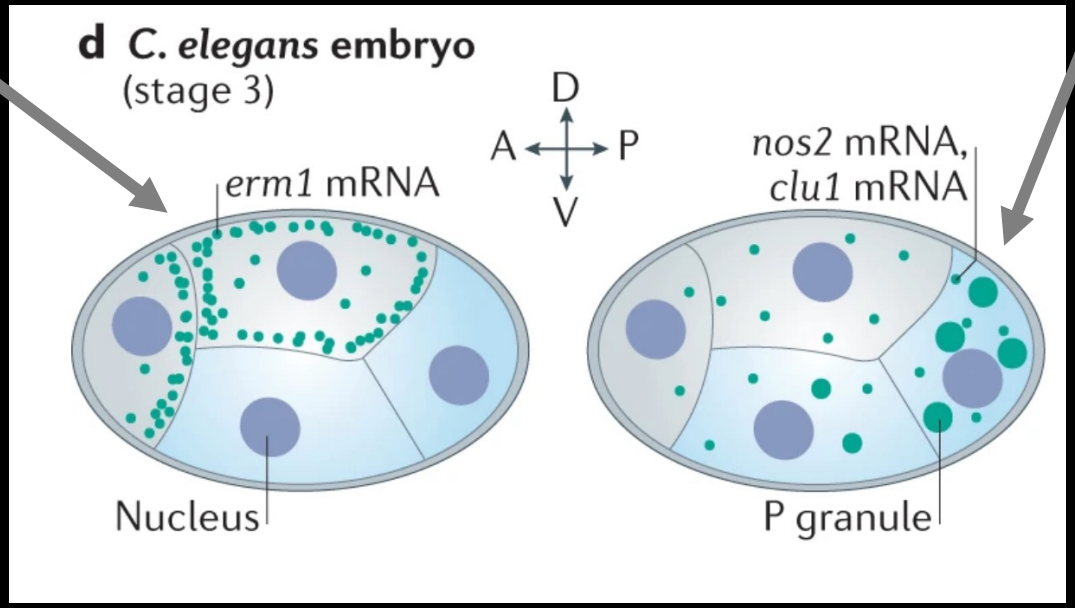
Emerging themes

Poster #P150
Saturday

erm-1 mRNA
(ezrin radixin moesin)

plasma membrane

translation-dependent



Das et al. (2021) Nat Rev Mol Cell Bio. PMID: 33837370

nos-2, clu-1, cpg-2, chs-1

P granules

dependent on
translation repression

most transcripts degrade

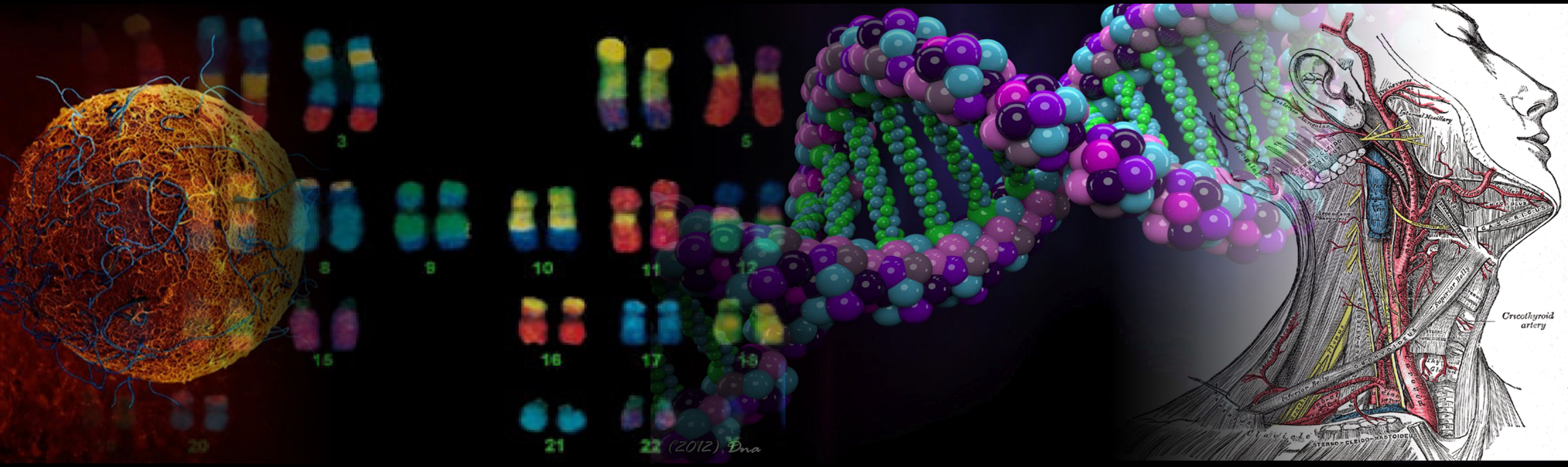
germline-specific *nos-2*
expression

Winkenbach et al., (2022) BioRXiV

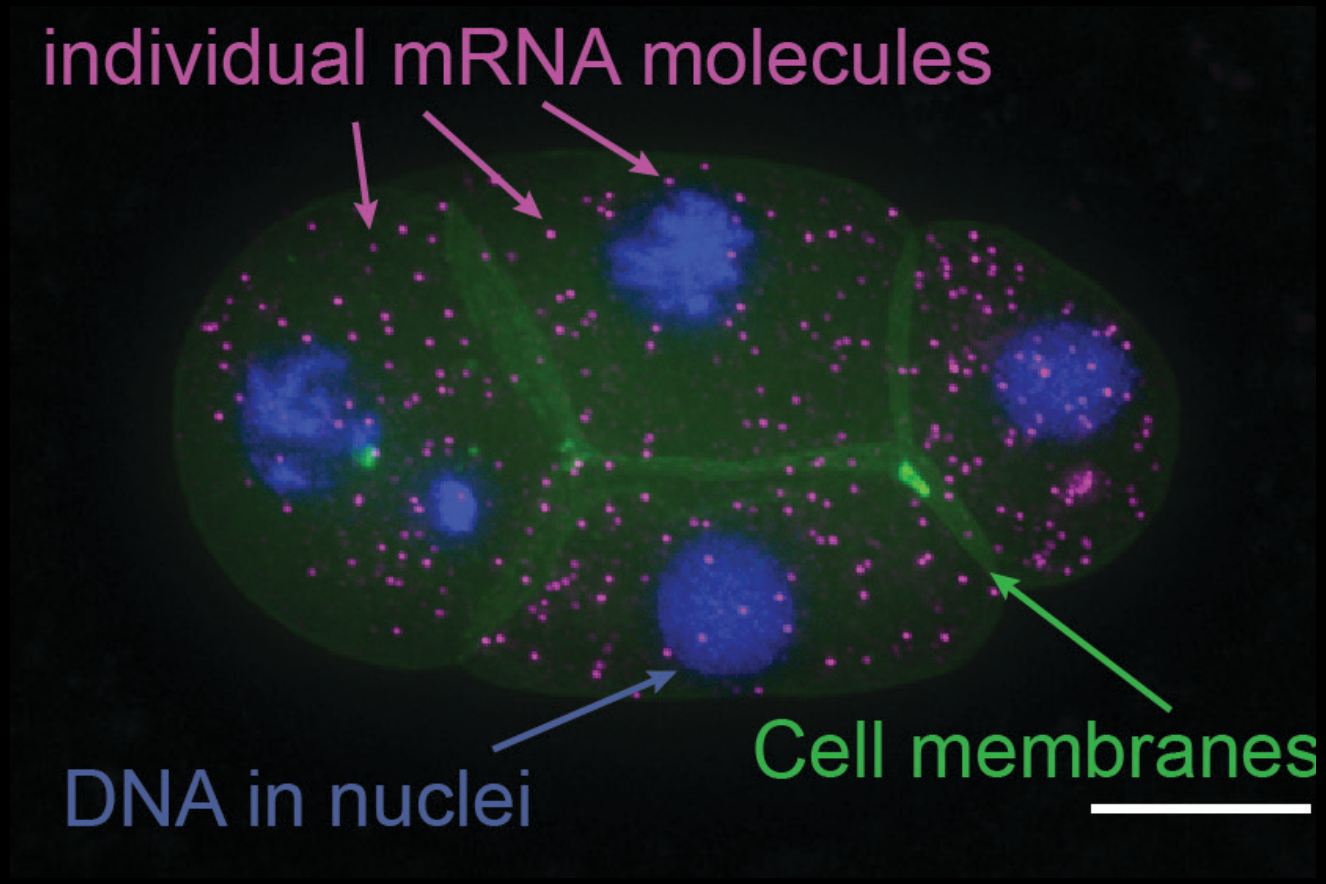
Parker et al., (2020) Development

Parker et al., (2021) Front Genet

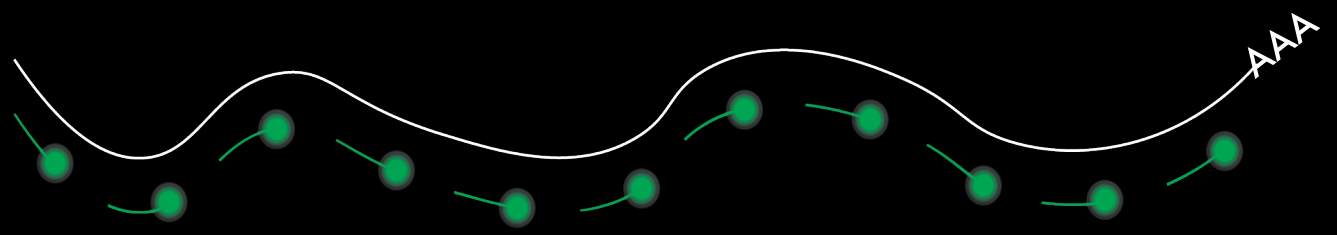
What could your research gain?



smFISH is a powerful tool for imaging individual mRNA molecules



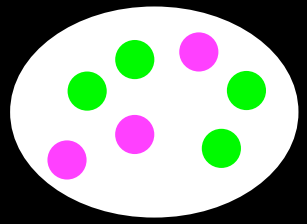
The smFISH approach



harvest embryos → methanol fix → freeze crack → acetone fix → wash steps → hybridize with probeset overnight → wash steps → mount & image

Download here: Parker et al., (2022) Current Protocols

Single mRNA imaging advanced techniques



Dual imaging

mRNA and proteins

Drugs

smFISH and drug treatment in embryos

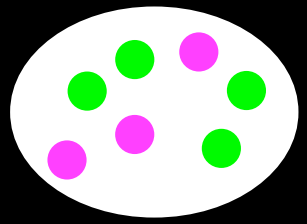
Saving $

smiFISH
i = inexpensive

Live imaging

MS2/MCP
PP7/PCP

Single mRNA imaging advanced techniques



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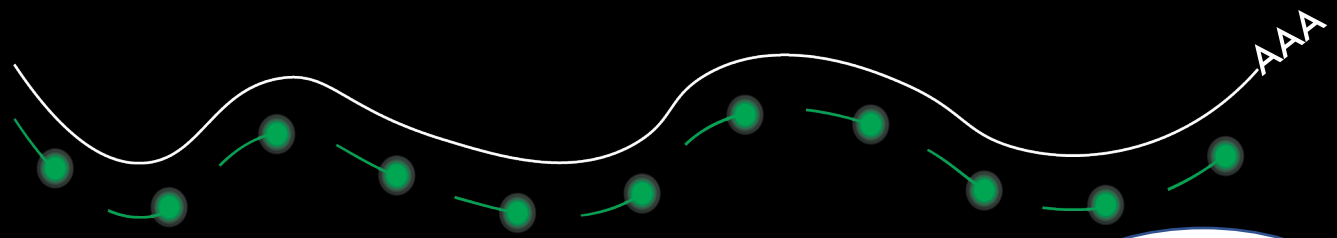
How can we dual image mRNA and proteins together?



1
Use GFP Strain

3
Immuno-fluorescence before smFISH

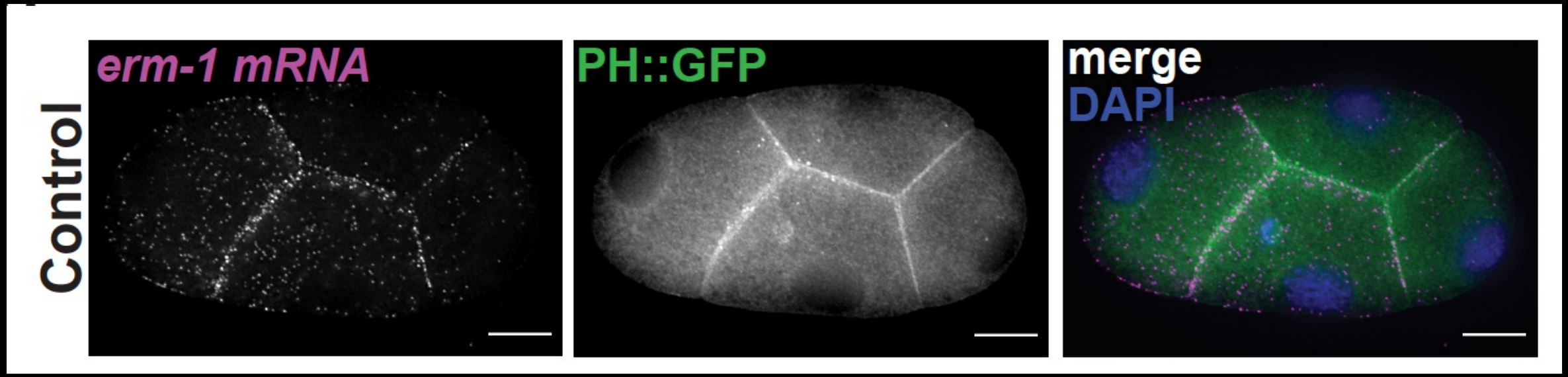
2
Hyb with probe + nanobody

harvest embryos → methanol fix → freeze crack → acetone fix → wash steps → hybridize with probeset overnight → wash steps → mount & image

Download here: Parker et al., (2022) Current Protocols

How can we dual image mRNA and proteins together?

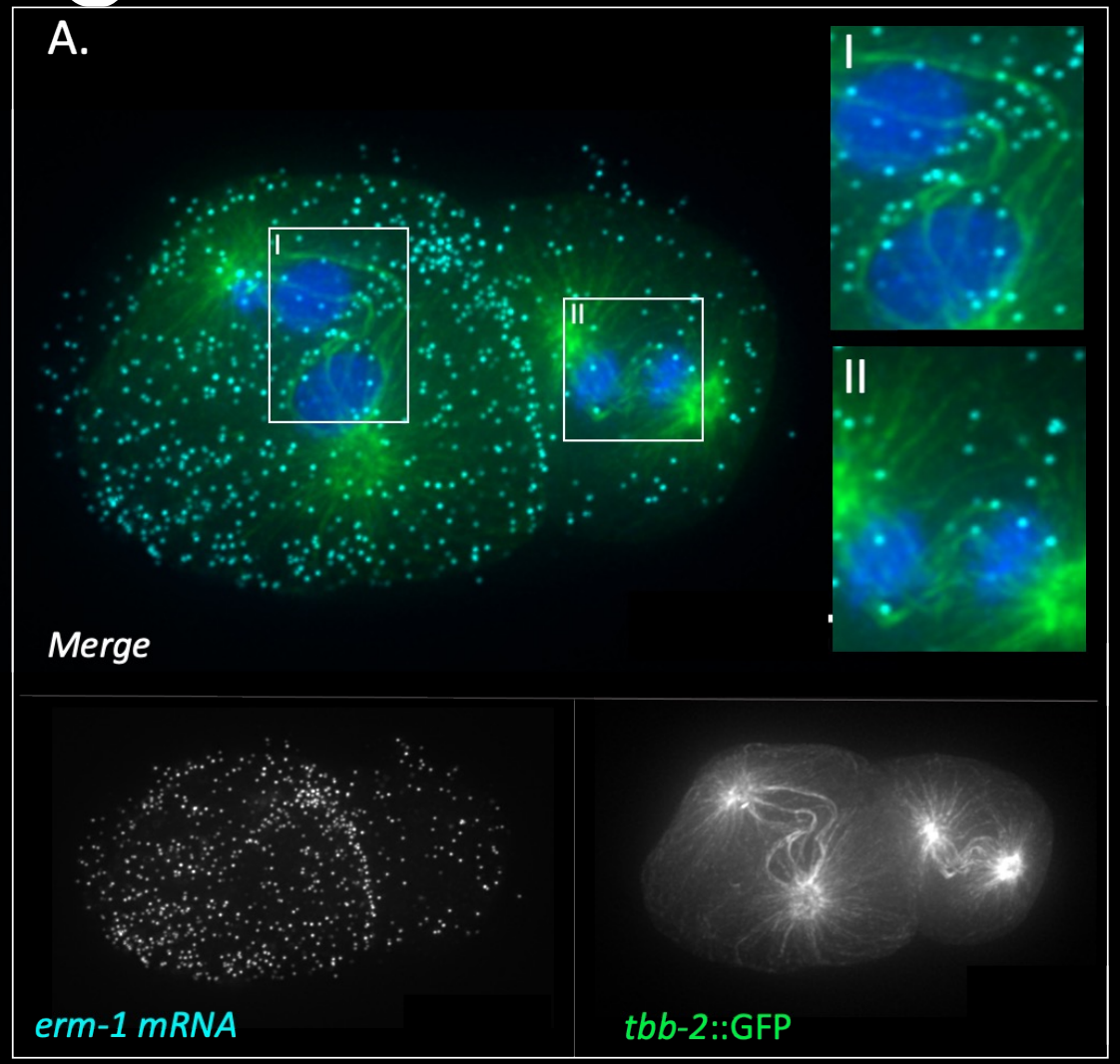
1) Try performing smFISH in a background GFP marker strain. This often works.



LP306 – PH::GFP
Membrane marker

Winkenbach et al., (2022) BioRXiV

How can we dual image mRNA and proteins together?

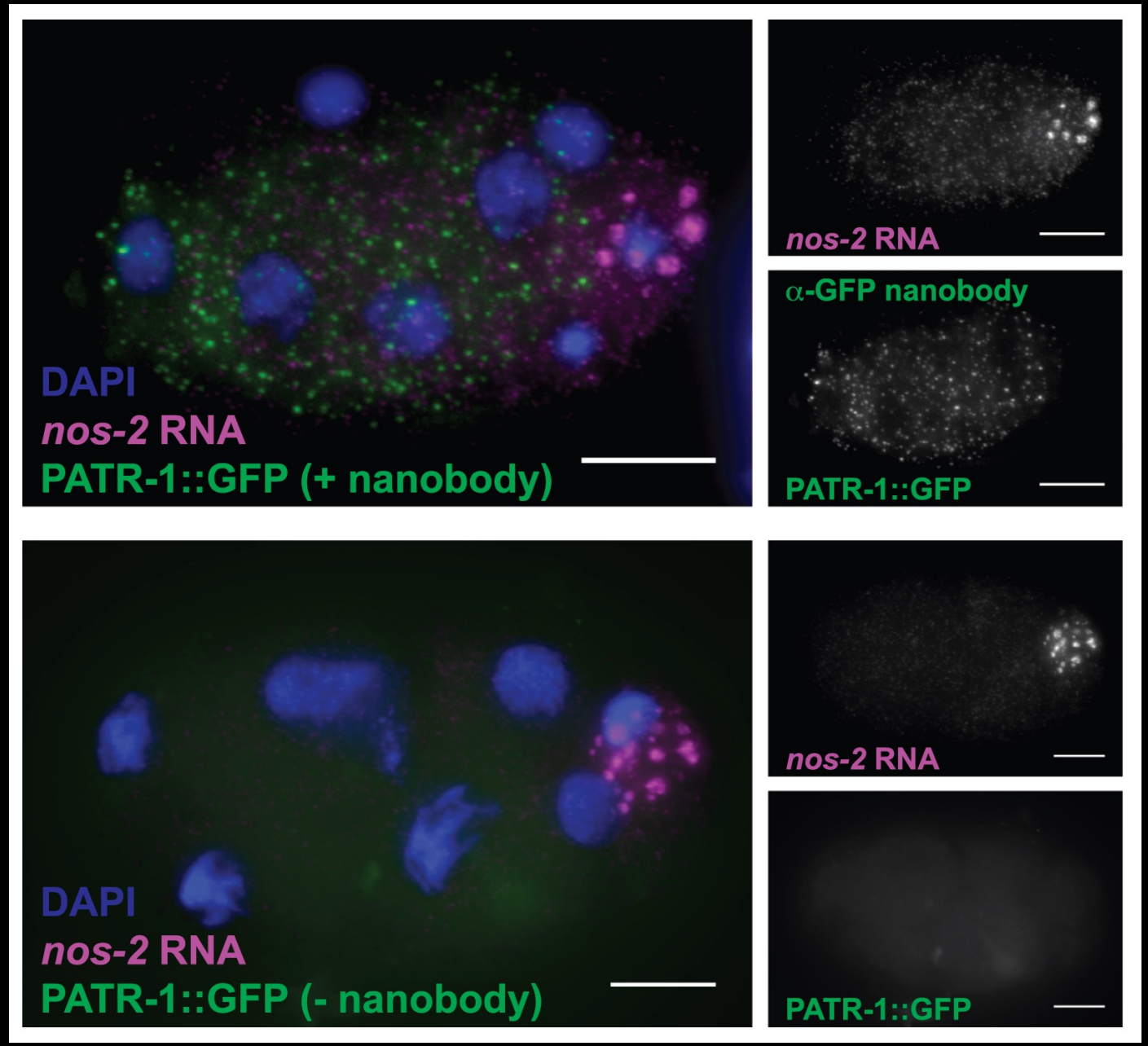


TBB-2::GFP
microtubule marker

Naly Torres, unpublished

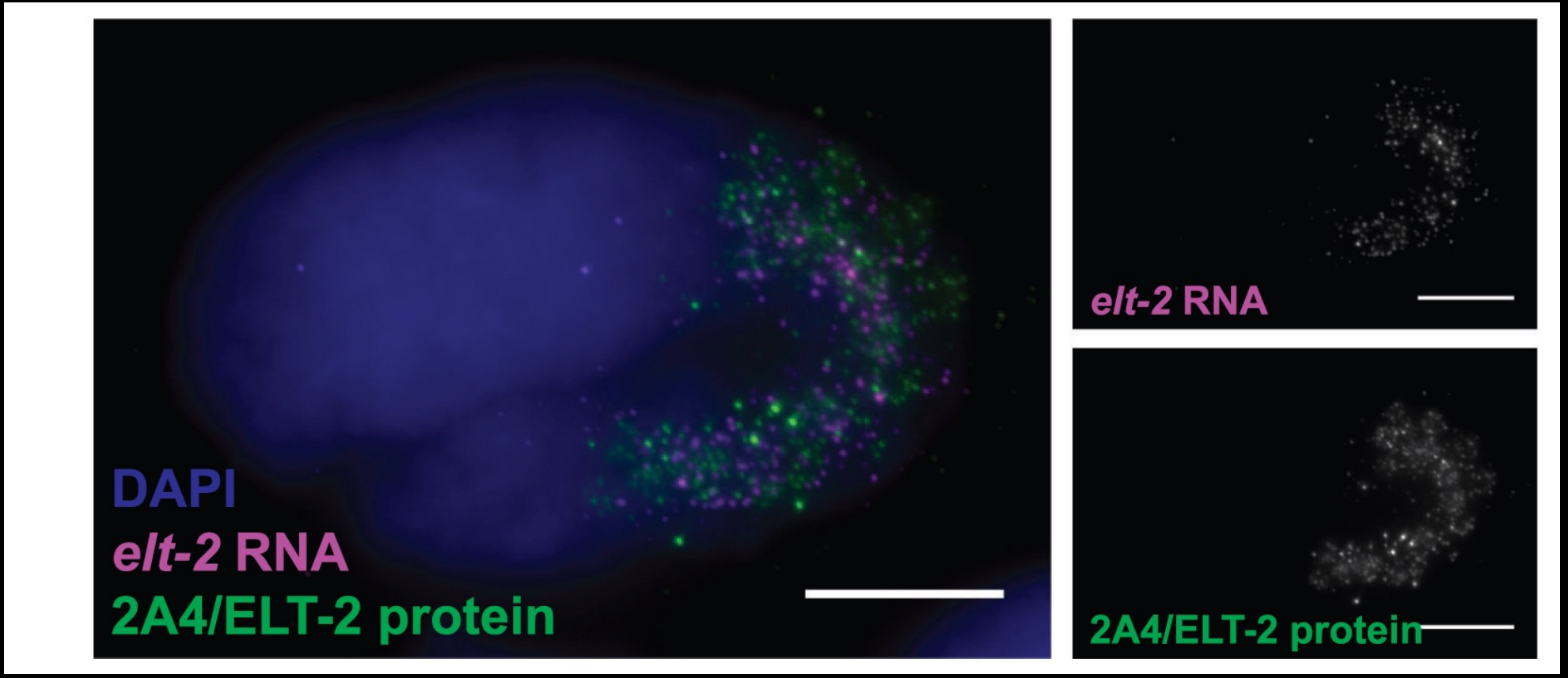
2) Nanobody and probeset dual hybridization

- This **has not** worked for us with polyclonal or monoclonal antibodies
- This **has** worked for us using nanobodies
  - Anti-GFP VHH single domain antibody fragment - Chromtek



Download here: Parker et al., (2022) Current Protocols

3) Tandem approach – immunofluorescence first followed by smFISH second



Also works well for K76/PGL-1

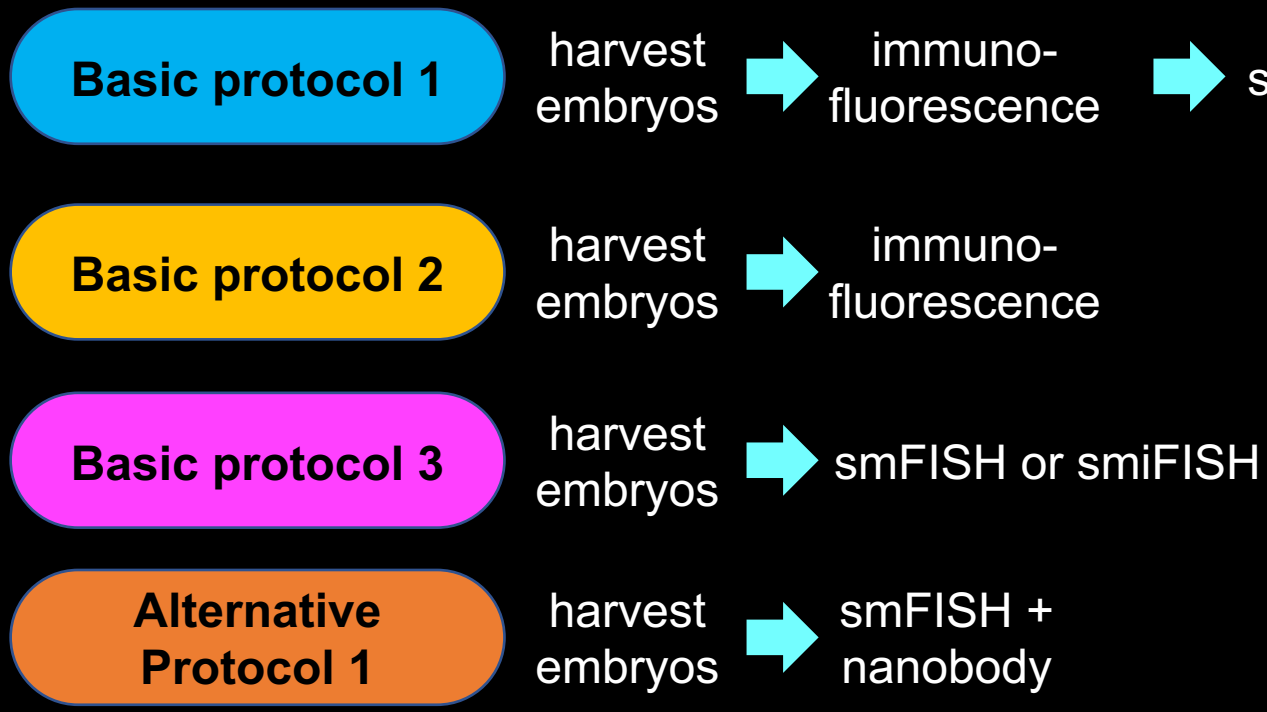
Download here: Parker et al., (2022) Current Protocols

A major breakthrough in dual imaging: fixative choice

	Methanol	Paraformaldehyde	Methanol followed by acetone
smFISH	✓	✗	✓
Immunofluorescence	✗	✓	✓

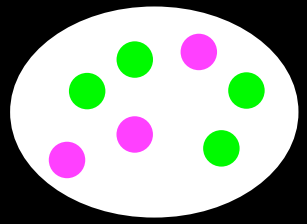
Our collection of protocols – Current Protocols

- Download here: Parker et al, (2021) Current Protocols



- Buffer comparisons
- Anti-fade comparisons
- Detailed troubleshooting guide

Single mRNA imaging advanced techniques



Dual imaging

smFISH and immunofluorescence

Drugs

smFISH and drug treatment in embryos

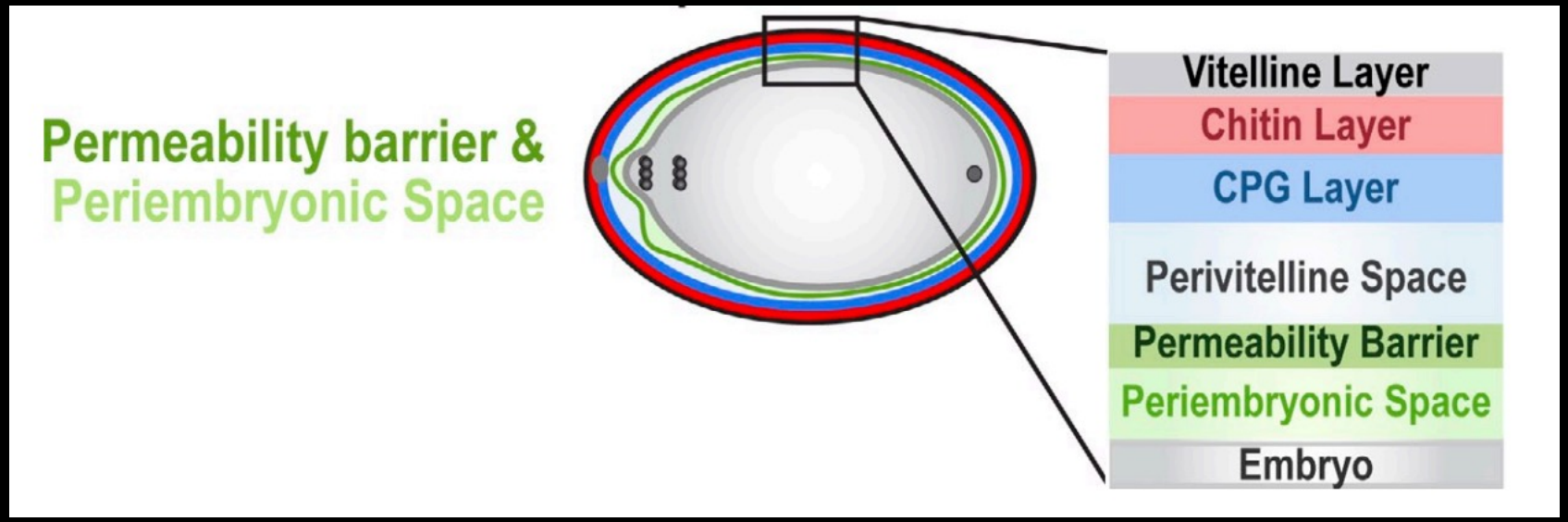
Saving $

smiFISH
i = inexpensive

Live imaging

MS2/MCP
PP7/PCP

The permeability barrier impedes drug treatment in embryos



Olson et al., (2012) JCB

perm-1 depletion by RNAi allows drugs to diffuse into embryos but makes them fragile

- Embryos do not survive embryo prep

  Dounce homogenize instead of embryo prep

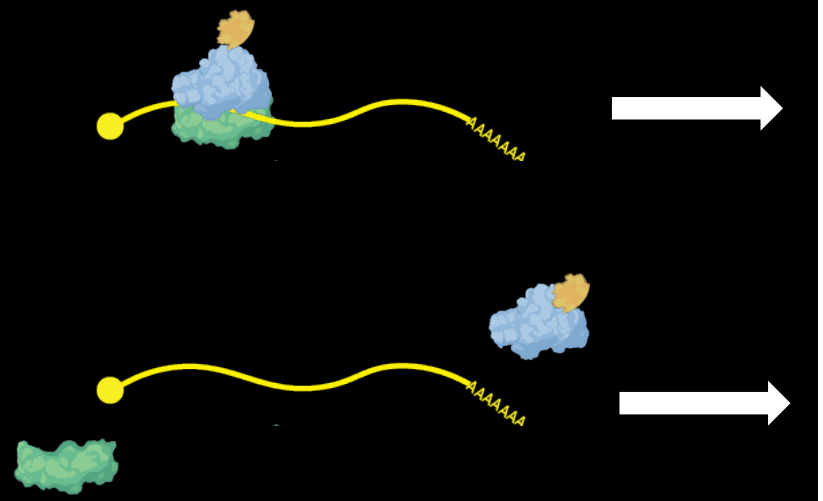
- Embryos do not survive centrifugation

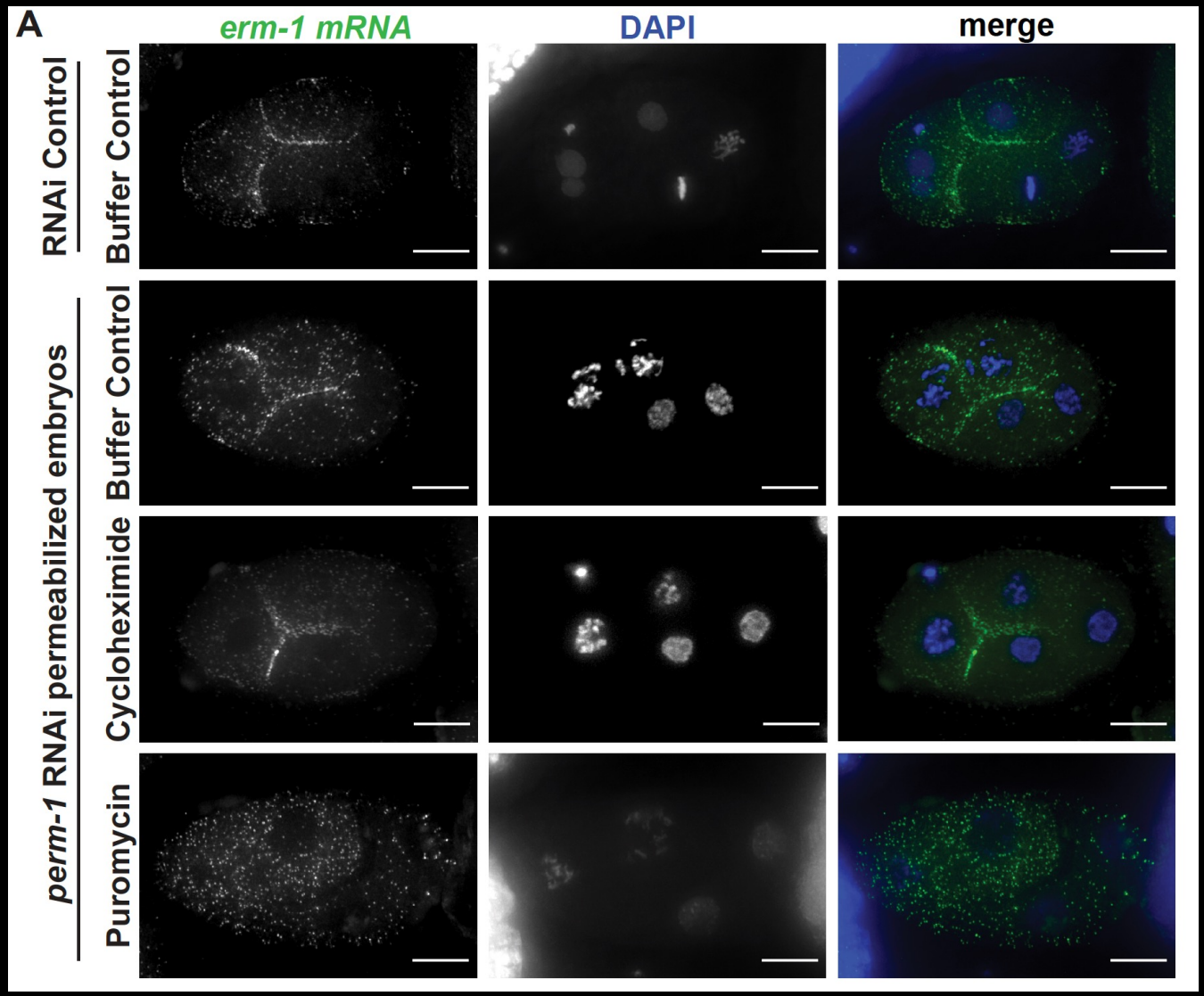
  Fix first, slower spins

- Slight variations in buffers can damage embryos

  Calibrate osmolarity of buffers using an osmometer

In the absence of active translation, *erm-1* mRNA localization at the plasma membrane requires an intact ribosome complex





Winkenbach et al., (2022) BioRXiV

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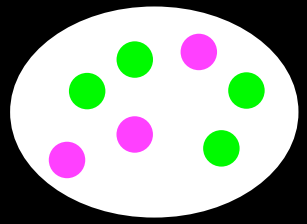

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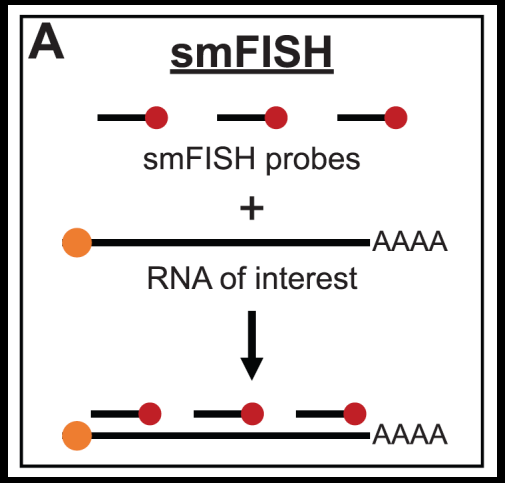
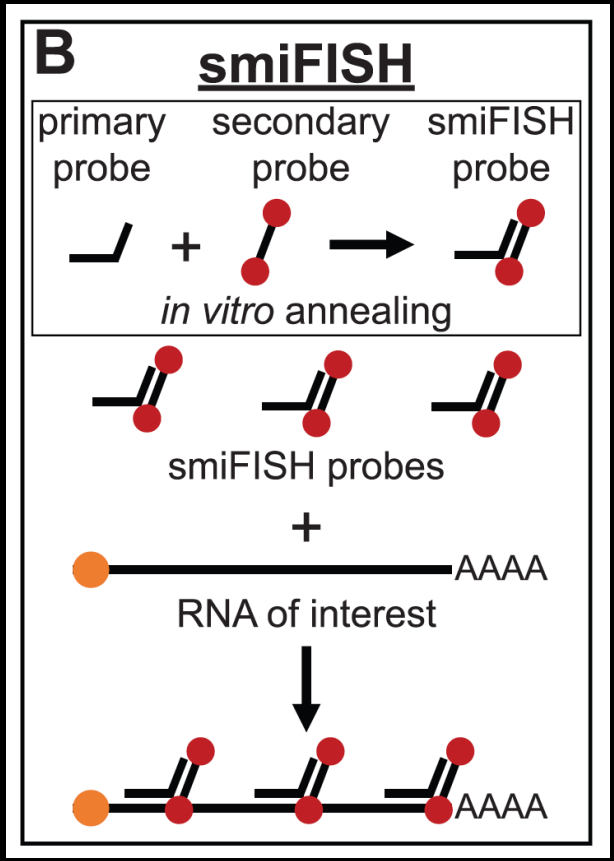
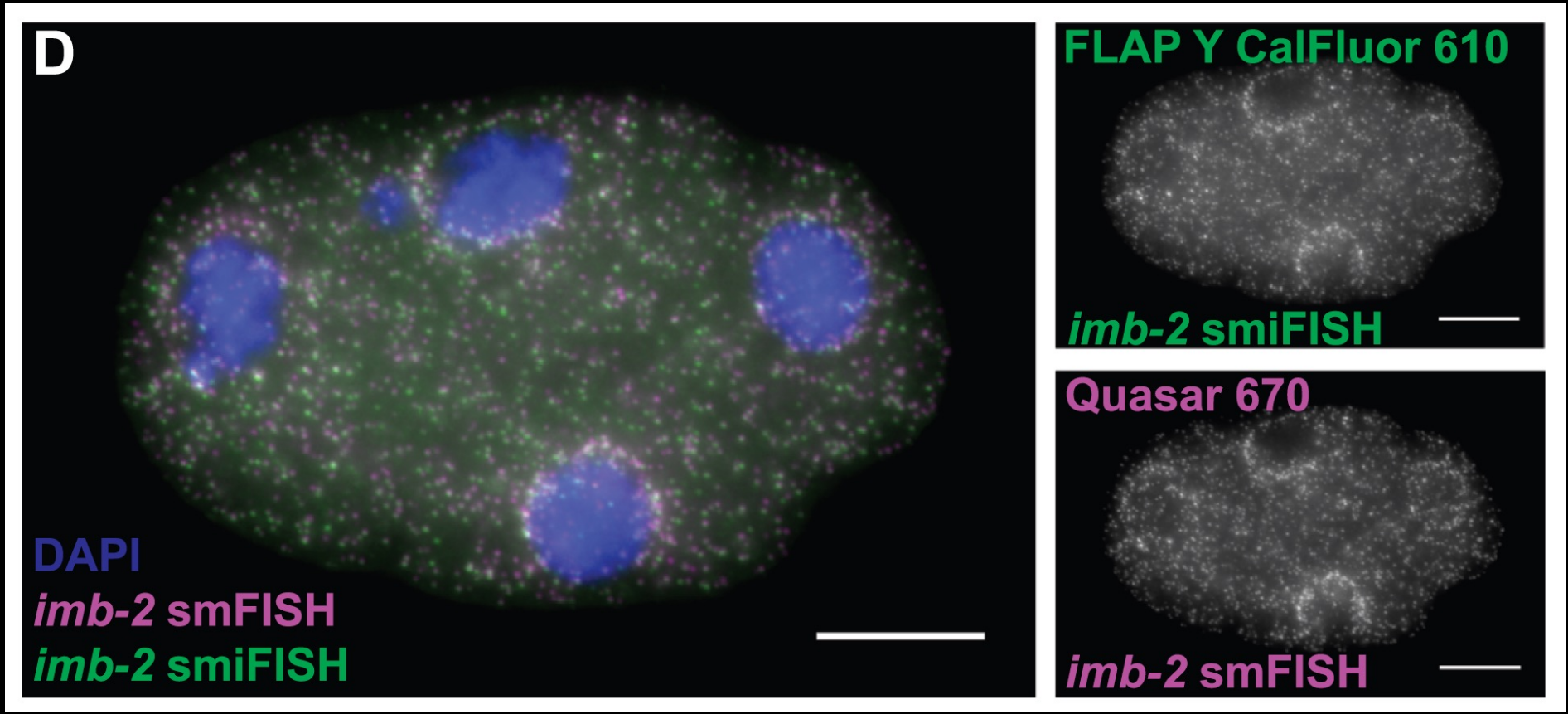
smFISH is expensive



- One probeset = 40 x 20mer probes (DNA oligos), each conjugated to a single fluorophore
- $600 – $800 per probeset
- LGC Biosearch Technologies

smiFISH reduces the number of probes required for purchase while retaining signal in embryos



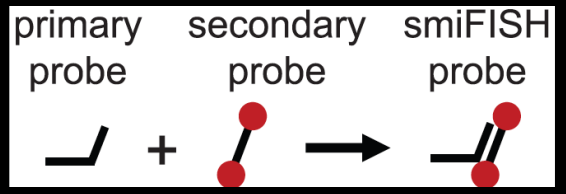


Tsanov et al., (2016) Nucl. Acids Research

Download here: Parker et al., (2022) Current Protocols

smiFISH is cost effective allowing for visual screens in embryos

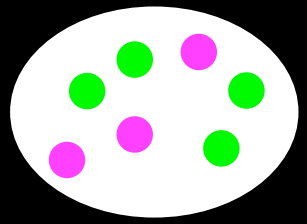
~40 labeled smFISH Probes = $600 - 800



~12 - 16 primary Probes = $100

1 dual-labeled secondary probe = $700 (reusable)

Single mRNA imaging advanced techniques



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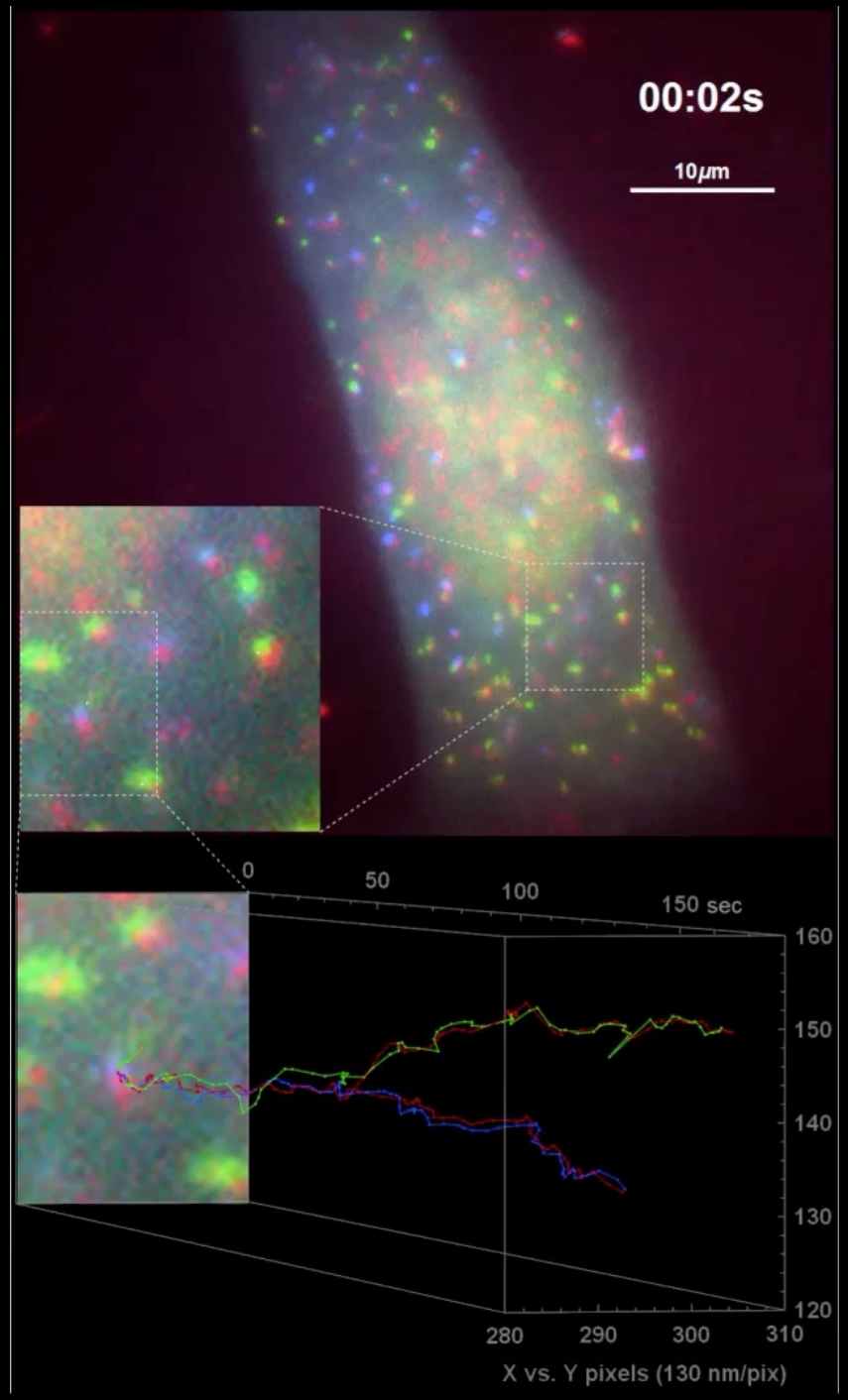
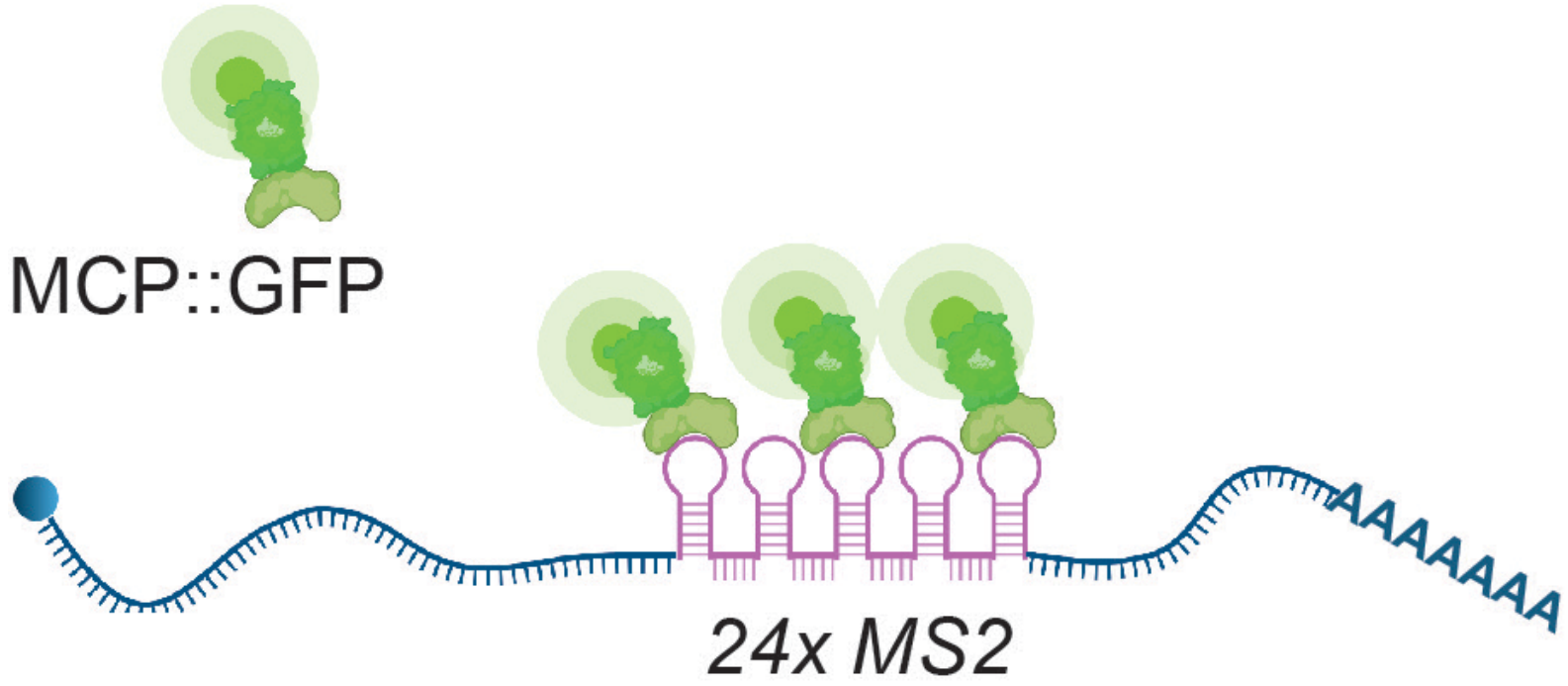


Live imaging single mRNA molecules while assessing translation

- MS2/MCP live imaging of mRNA - RED
- NCT tracking of translation - GREEN
- Stress granules – BLUE

Tatsuya Morisaki, Stephanie Moon, Roy Parker, Tim Stasevich
U2OS cells

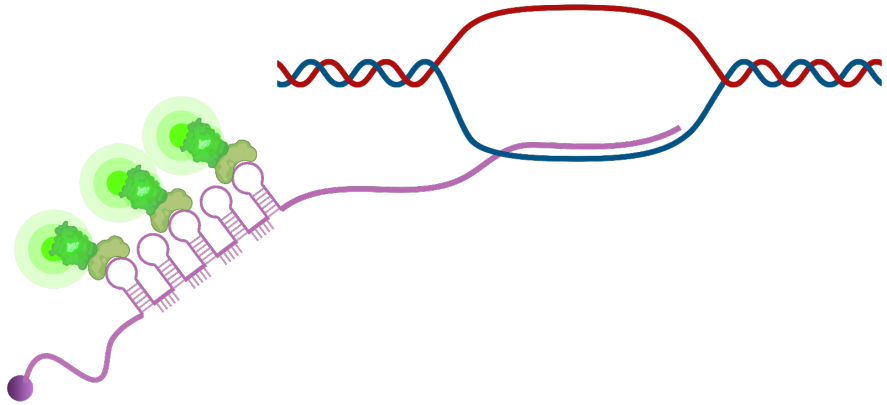
mRNA live imaging has the potential to address novel questions



MS2/MCP

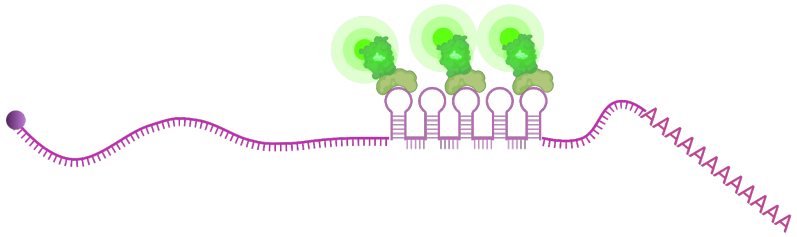
PP7/PCP

Three major applications of mRNA live imaging



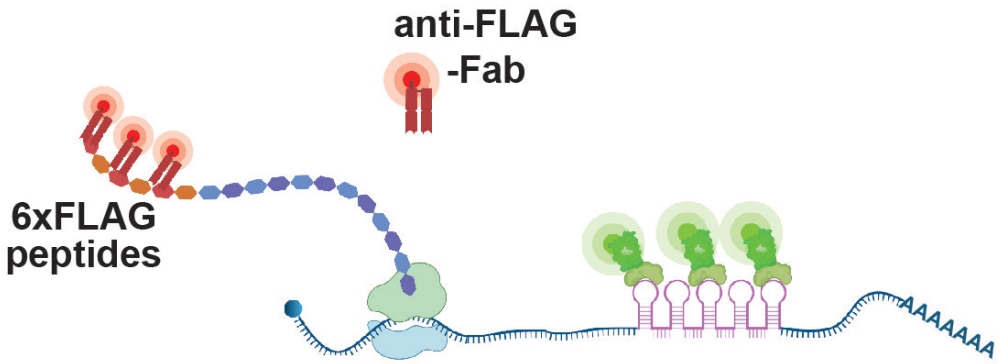
- As a reporter of transcriptional activation in the nucleus

Three major applications of mRNA live imaging



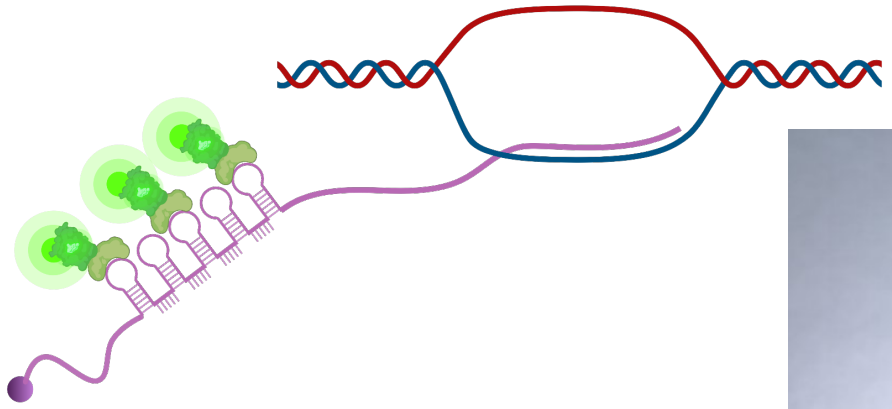
- As a reporter of transcriptional activation in the nucleus
- To track mRNA movement and regulation in the cytoplasm

Three major applications of mRNA live imaging



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

Tracking transcriptional activation works well in *C. elegans*



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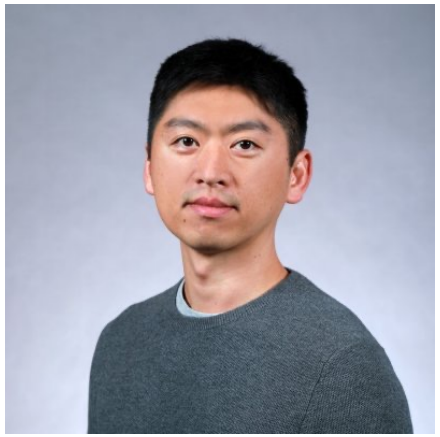


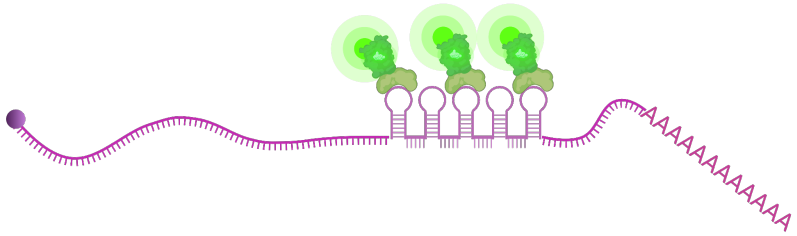
Changhwan Lee
University of Albany

Christopher Hammell
Shubham Sahu
Wolfgang Keil

Priya Sivaramakrishnan
John Murray
talk (21 – sat am)

Lee et al., (2019) Developmental Cell

Live imaging mRNA in the cytoplasm published for larvae



- To track mRNA movement and regulation in the cytoplasm

Hongjie Zhang
University of Macau

Li et al., (2021) Cell Reports

Questions, Optimization, and Potential of live imaging

- Does MS2/MCP alter mRNA behavior and function?
  - mRNA decay? transport? translation? RNAi?
  - Compare MS2-tagged v. untagged transcripts using smFISH
  - Rescue deletion strains with tagged versions

- Further optimization is needed
  - Achieving optimal spatial & temporal resolution
  - Comparing microscopy approaches

- 2021 mRNA live imaging Workshop slides - online content

Studies are ongoing but need more help!

- Phenotypic impact – sometimes yes, sometimes no
- Localization, export, and abundance impact – sometimes yes, sometimes no
- Reducing loops can improve mRNA behaviors but dims the signal
- Optimizing the number of loops – find a balance

Thanks: Rob Singer Lab, Evalina Tutucci, Carolyn Phillips, Hongjie Zhang, Changhwan Lee, Chris Hammell

Thank you!

- Dylan Parker
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- Izabella Mastroianni
- Zainab Al Mazaydeh
- Meghan Costello
- Romario Romain
- Lauren Billow
- Annemarie Parker
- Camryn Daidone
- Sam Boyson
- Florian Mueller
- Changhwan Lee
- Hongjie Zhang
- Sevinç Ercan
- Christopher Hammell
- Carolyn Phillips
- Tim Staseivch
- Wolfgang Keil
- Ari Pani
- Doug Shepherd
- Paul Maddox

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