# Drops in a regulator gradient:
# **P granule positioning in the C. elegans embryo**


Christoph A. Weber[1], Chiu Fan Lee[2], Omar Adame[1], and Frank Jülicher[1]

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Shambaditya Saha , Andres Diaz, Tony Hyman

MPICBG Dresden

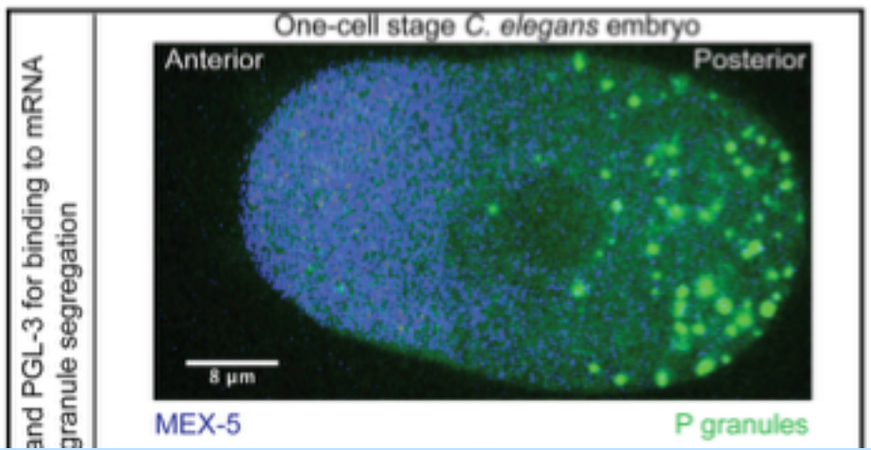


Cell

Theory


## Polar Positioning of Phase-Separated Liquid Compartments in Cells Regulated by an mRNA Competition Mechanism

### Graphical Abstract





### Authors

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### Correspondence

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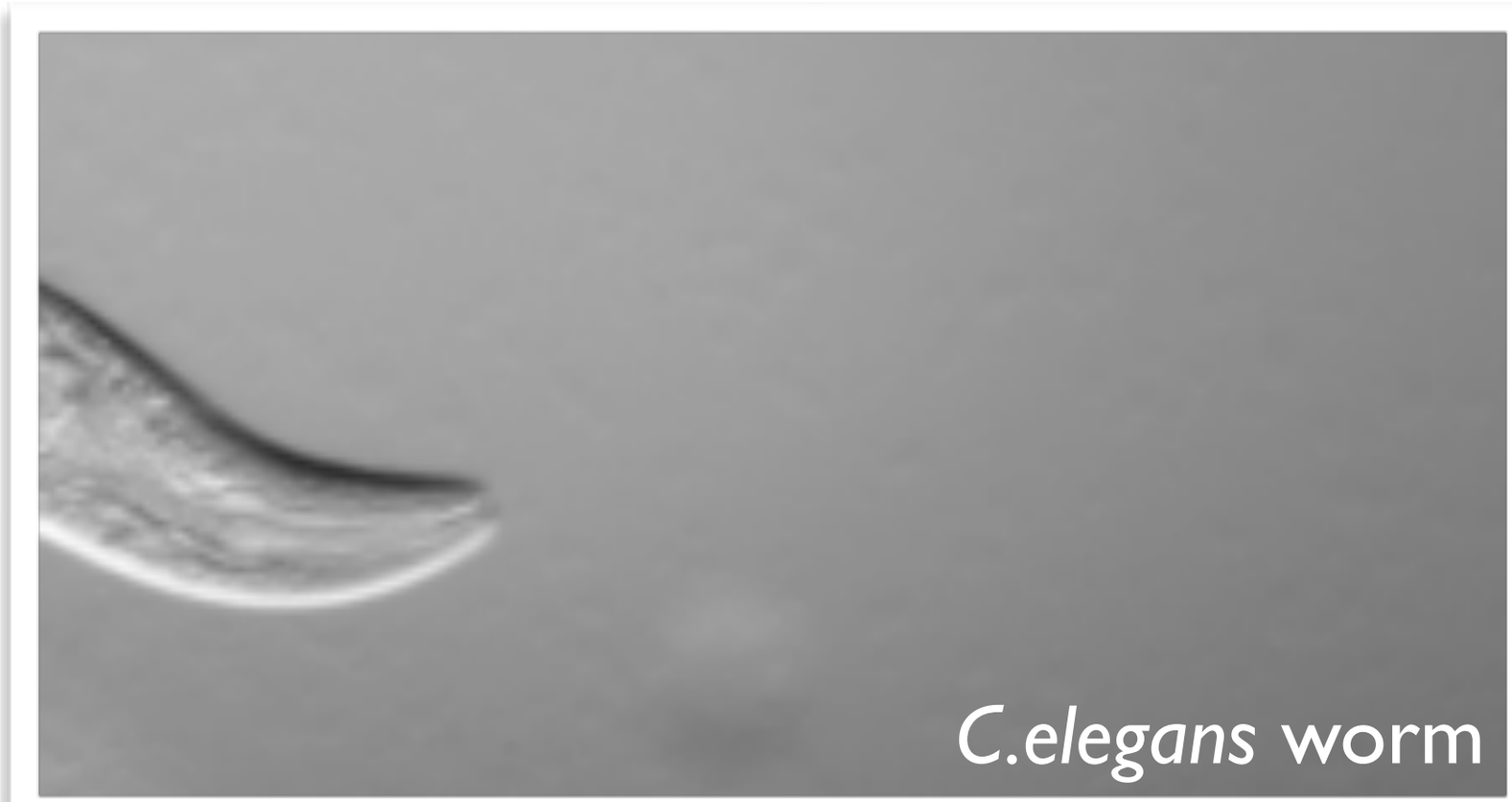
### In Brief

Asymmetric positioning of cellular compartments formed by phase

# Cell division of C.elegans embryo




http://labs.bio.unc.edu/Goldstein/movies.html

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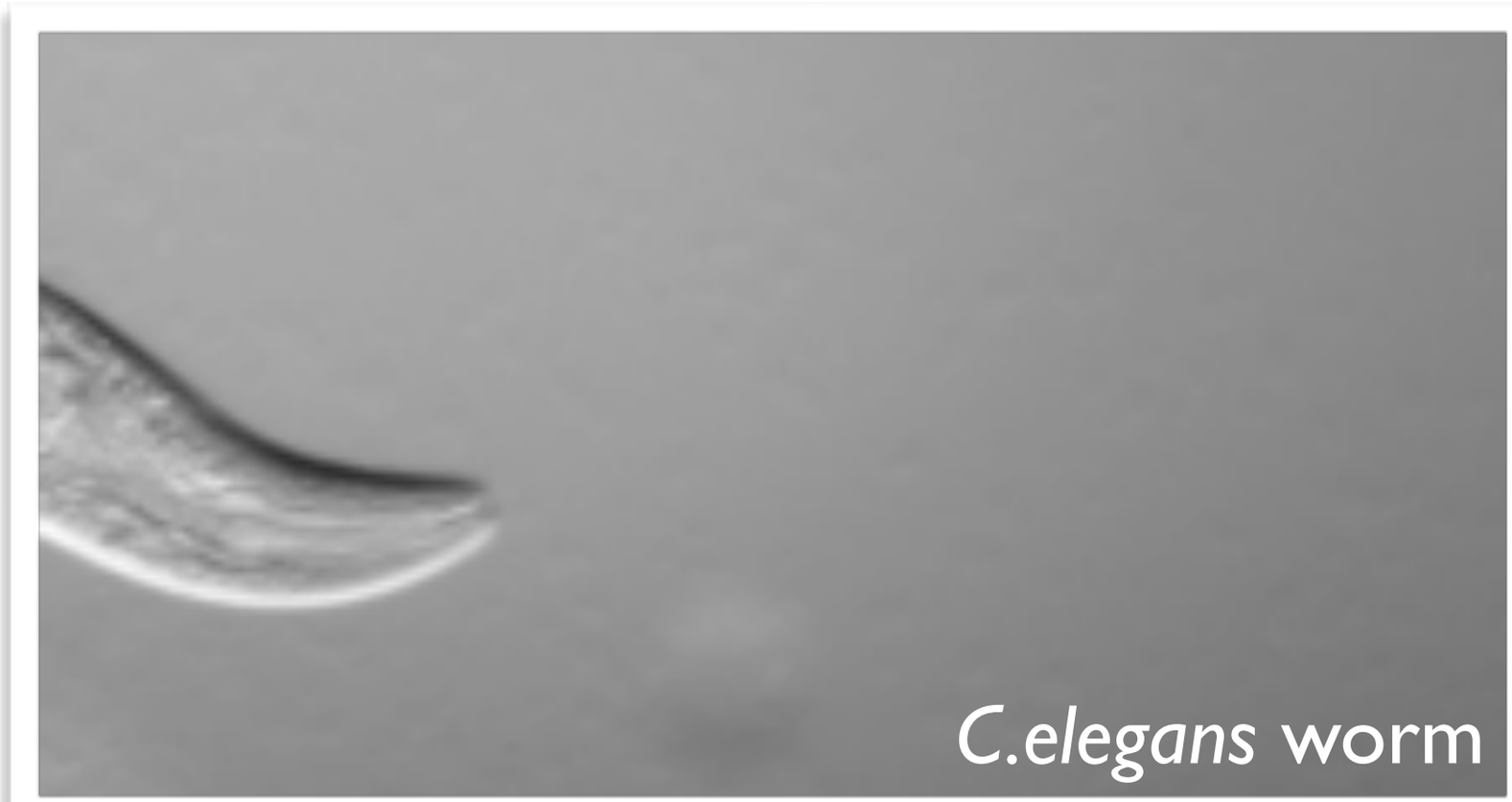


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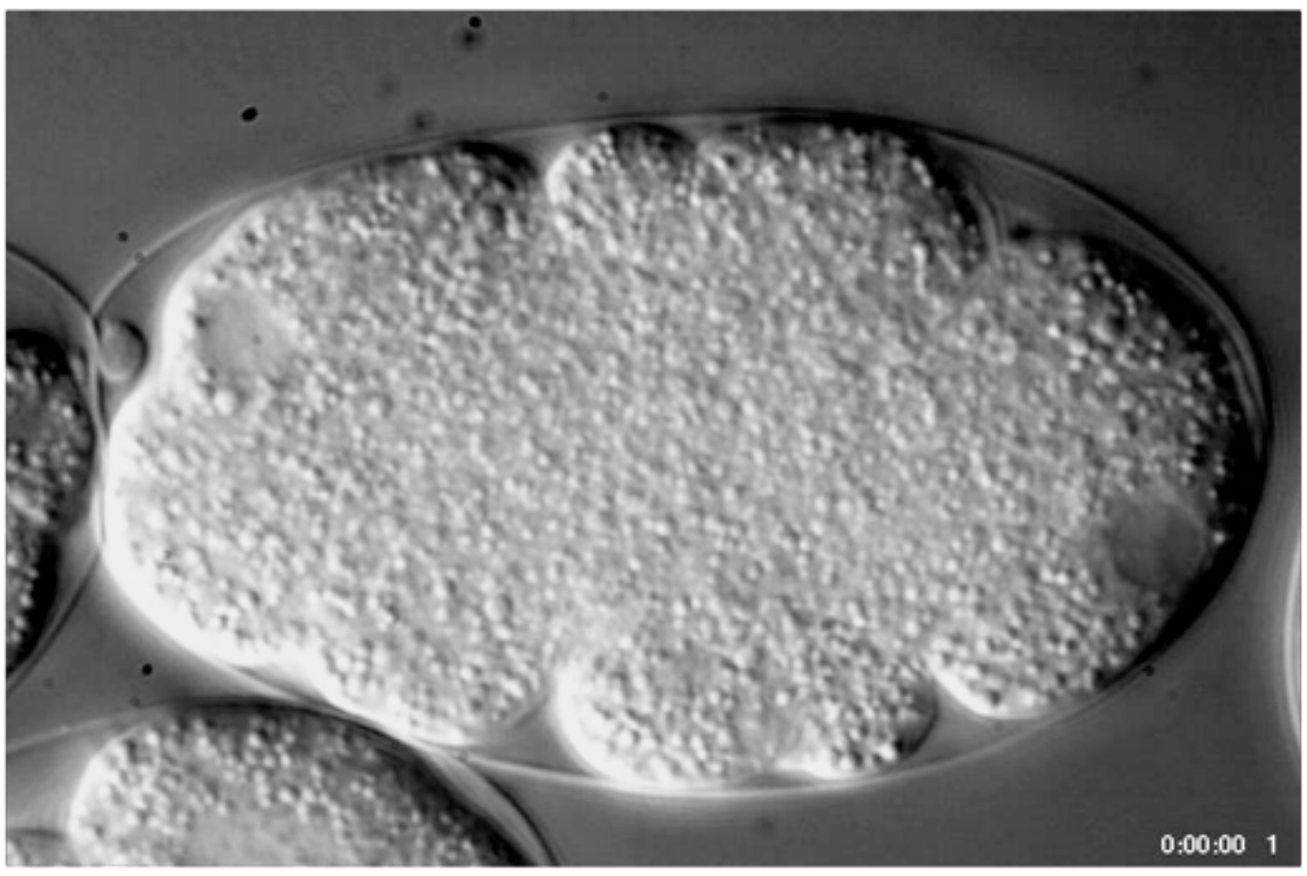

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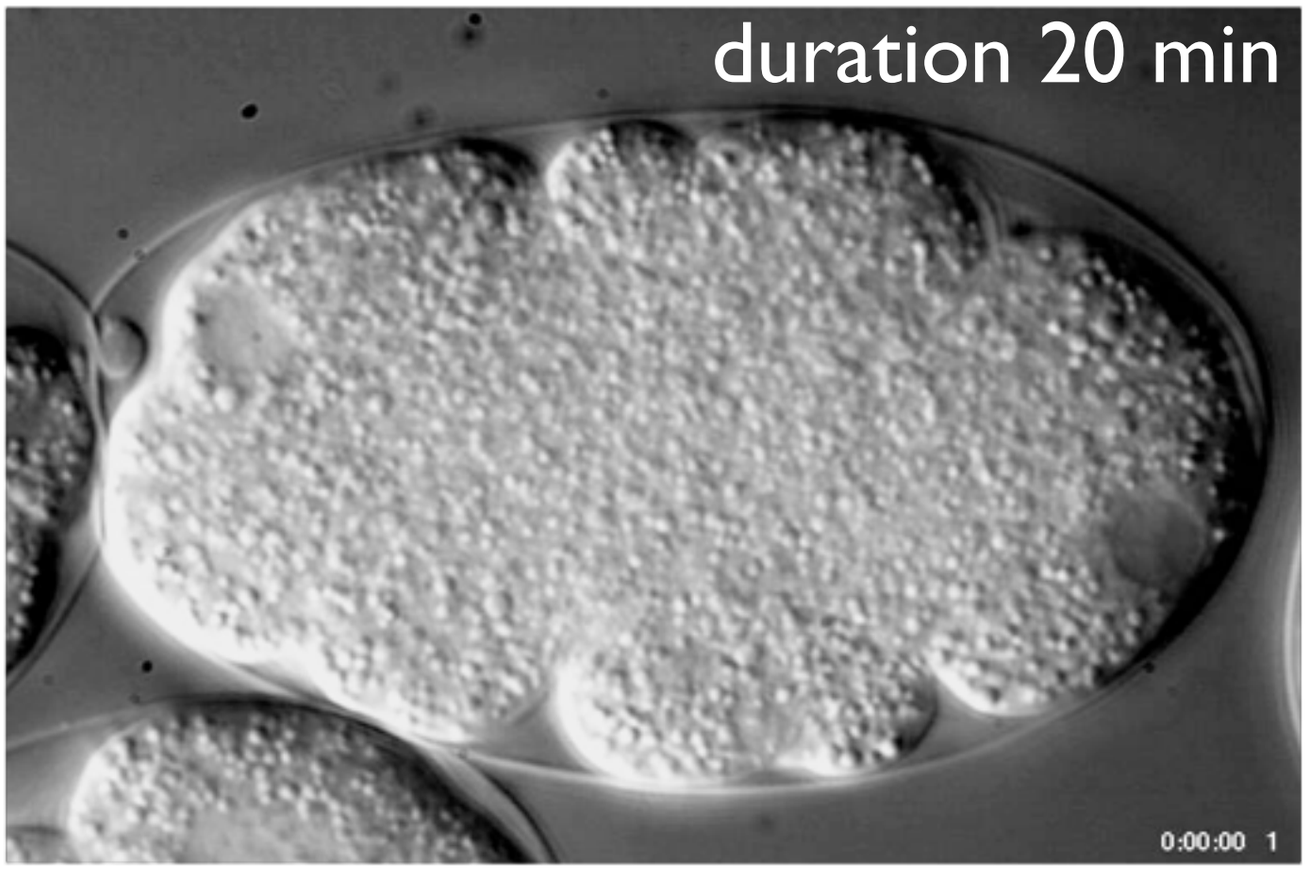


fertilized C.elegans egg (Hymanlab)

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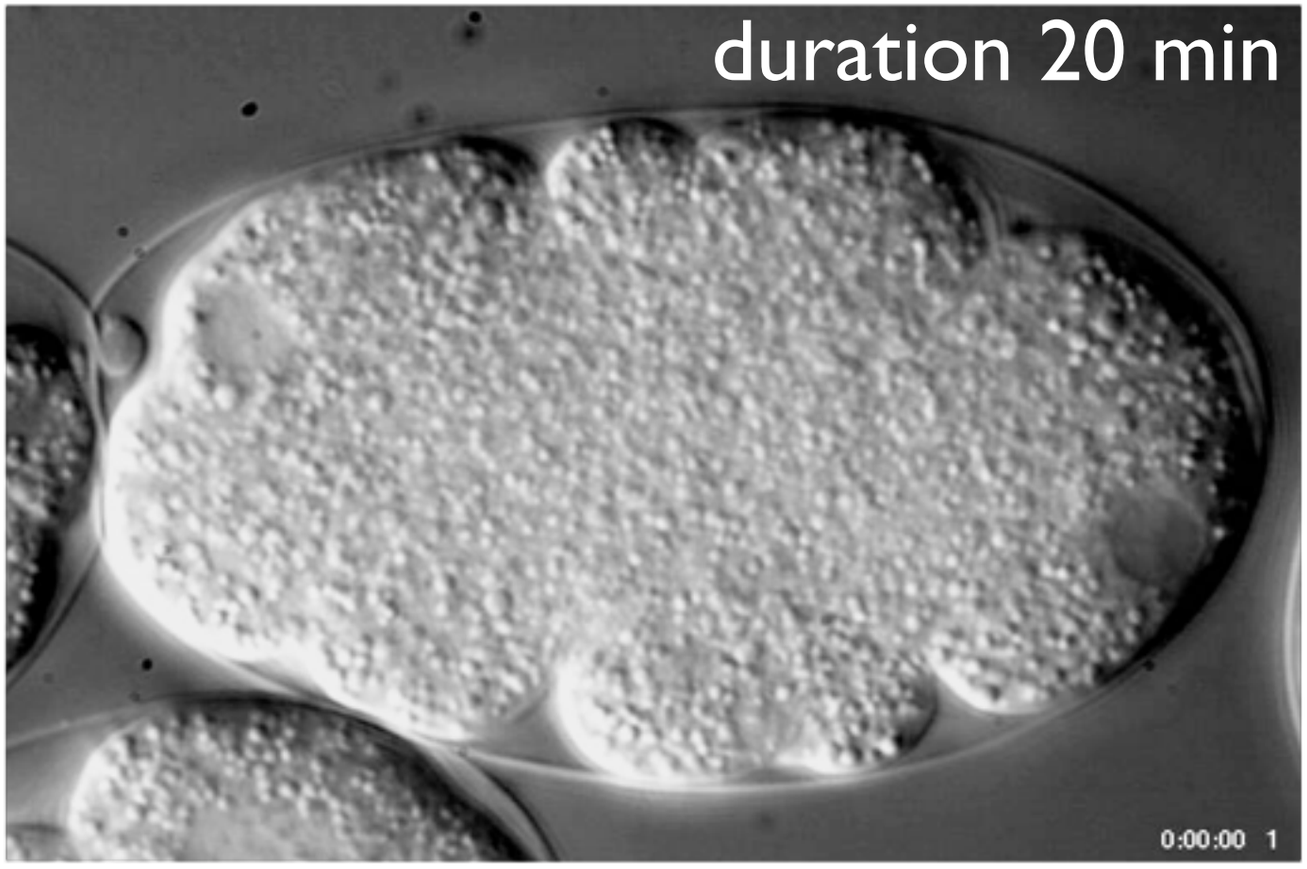


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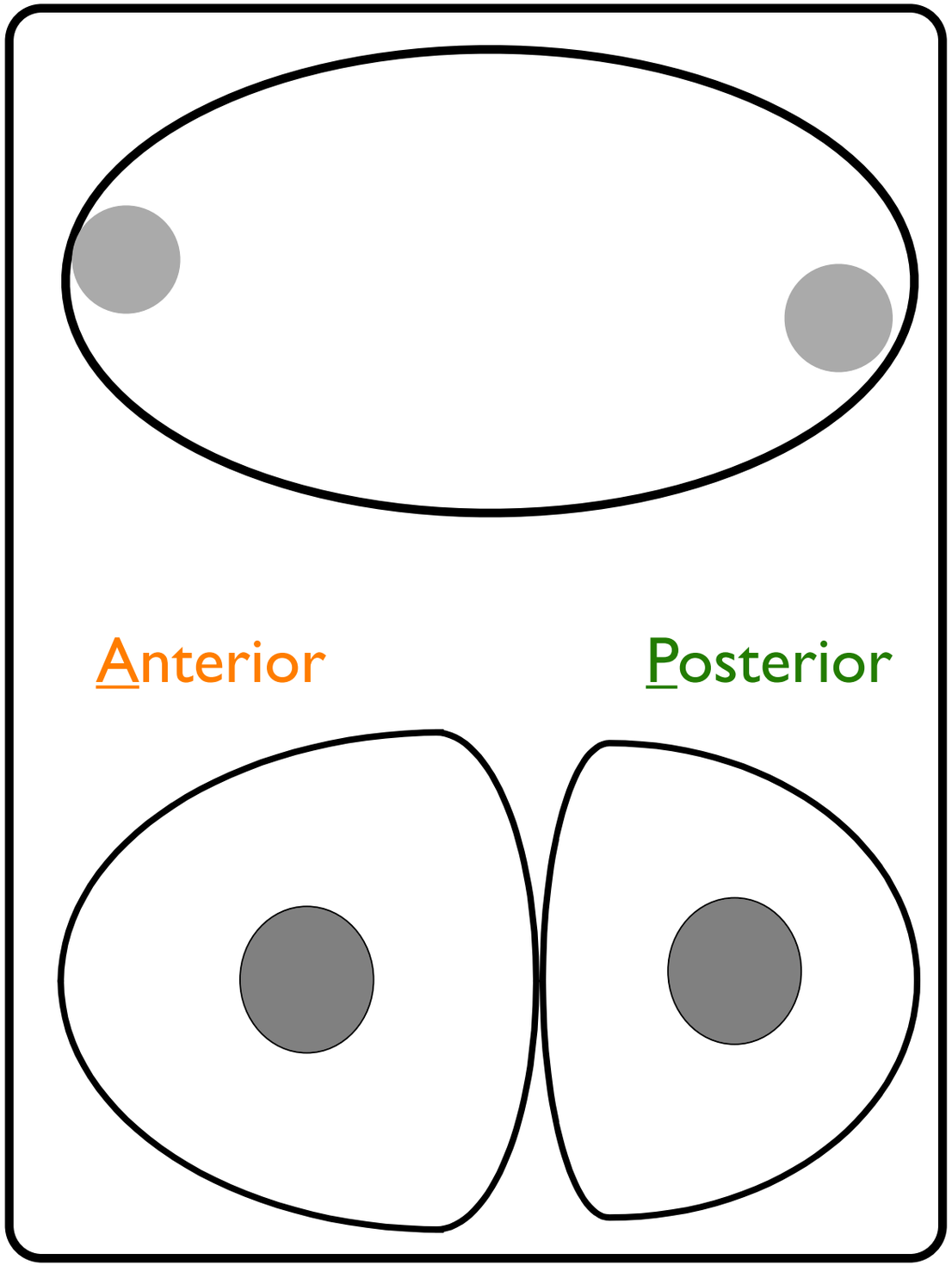


cytoplasm of the two cells after division is different

# Cell division is asymmetric

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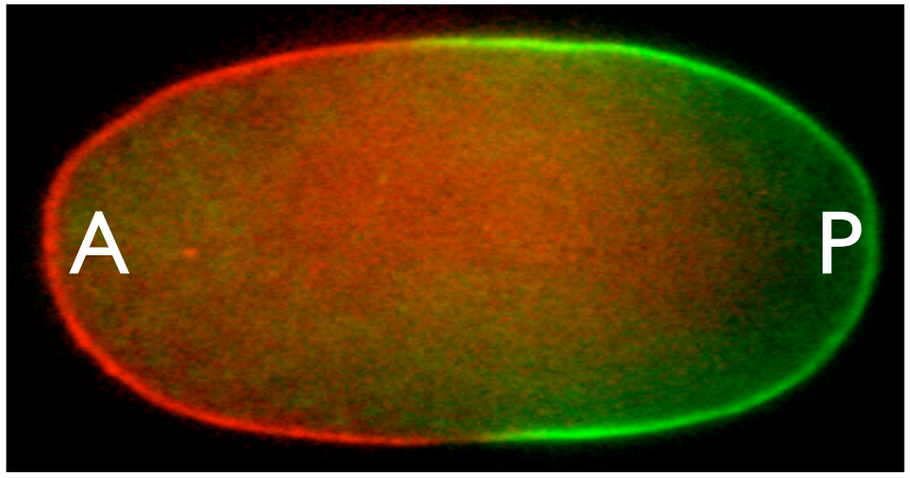
Polarity in cell membrane

PAR-6 / PAR-2




Cowan, Hyman, Development 2007 134: 1035-1043

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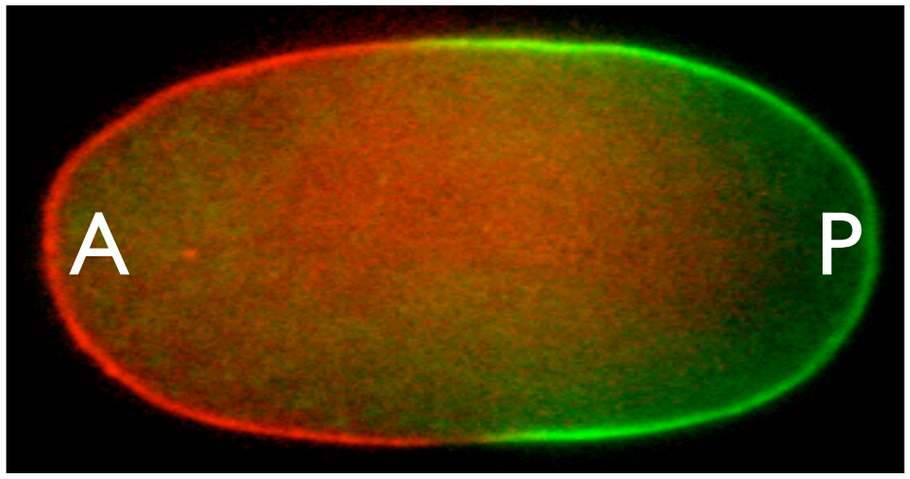


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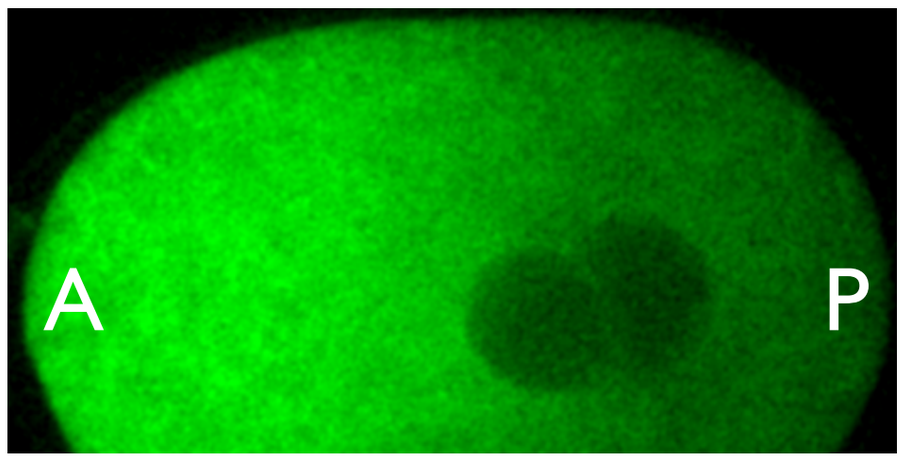
Cytoplasmic protein gradients

e.g. MEX-5

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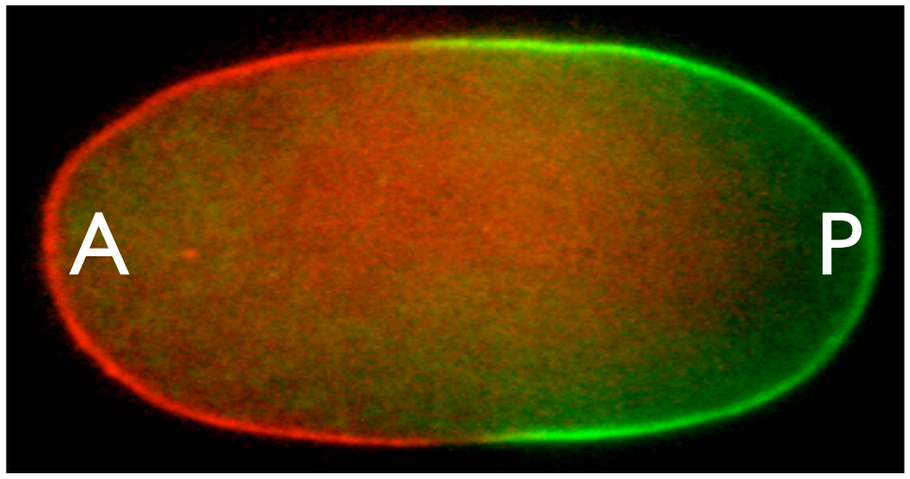


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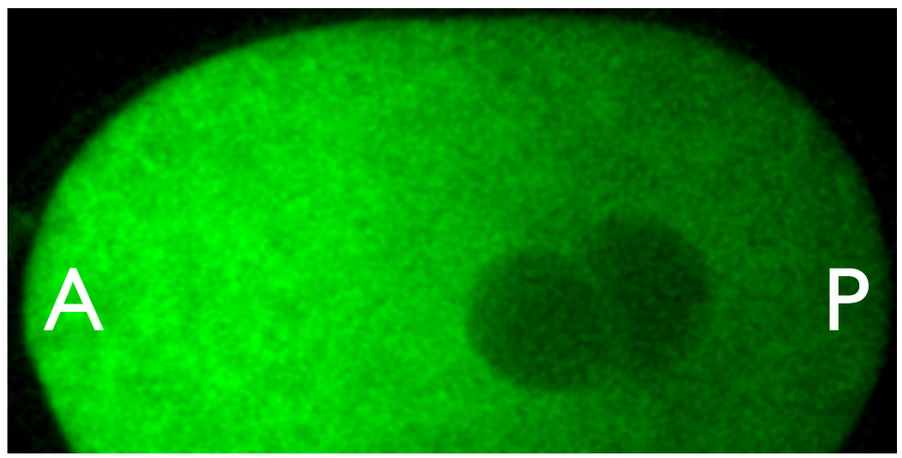


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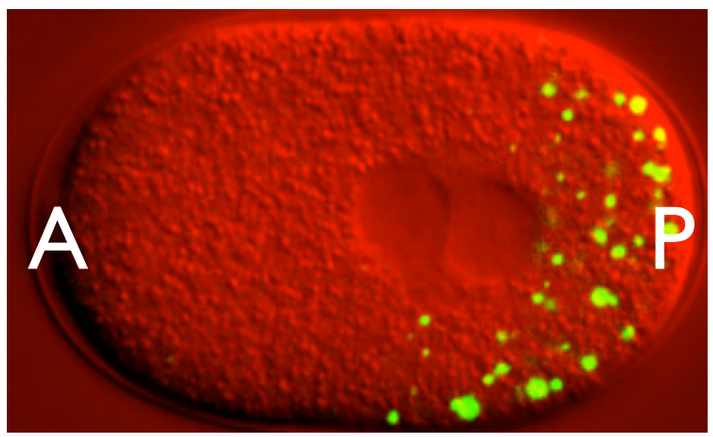
P granule segregation

PGL1-GFP

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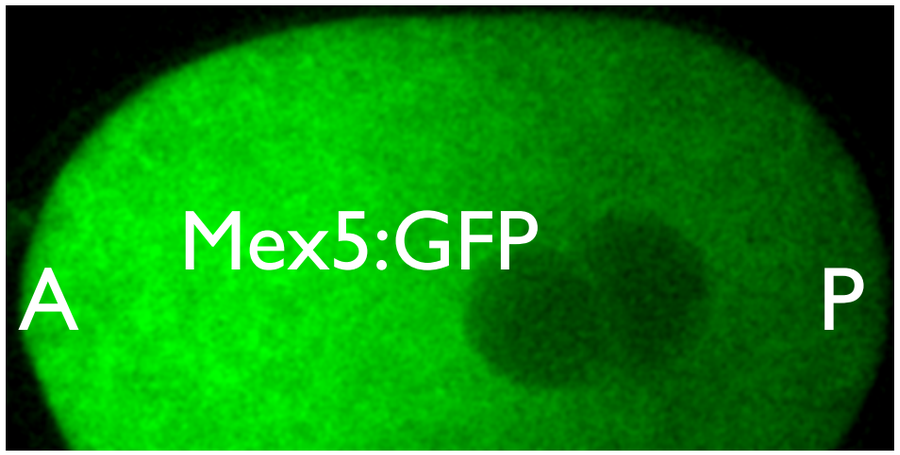
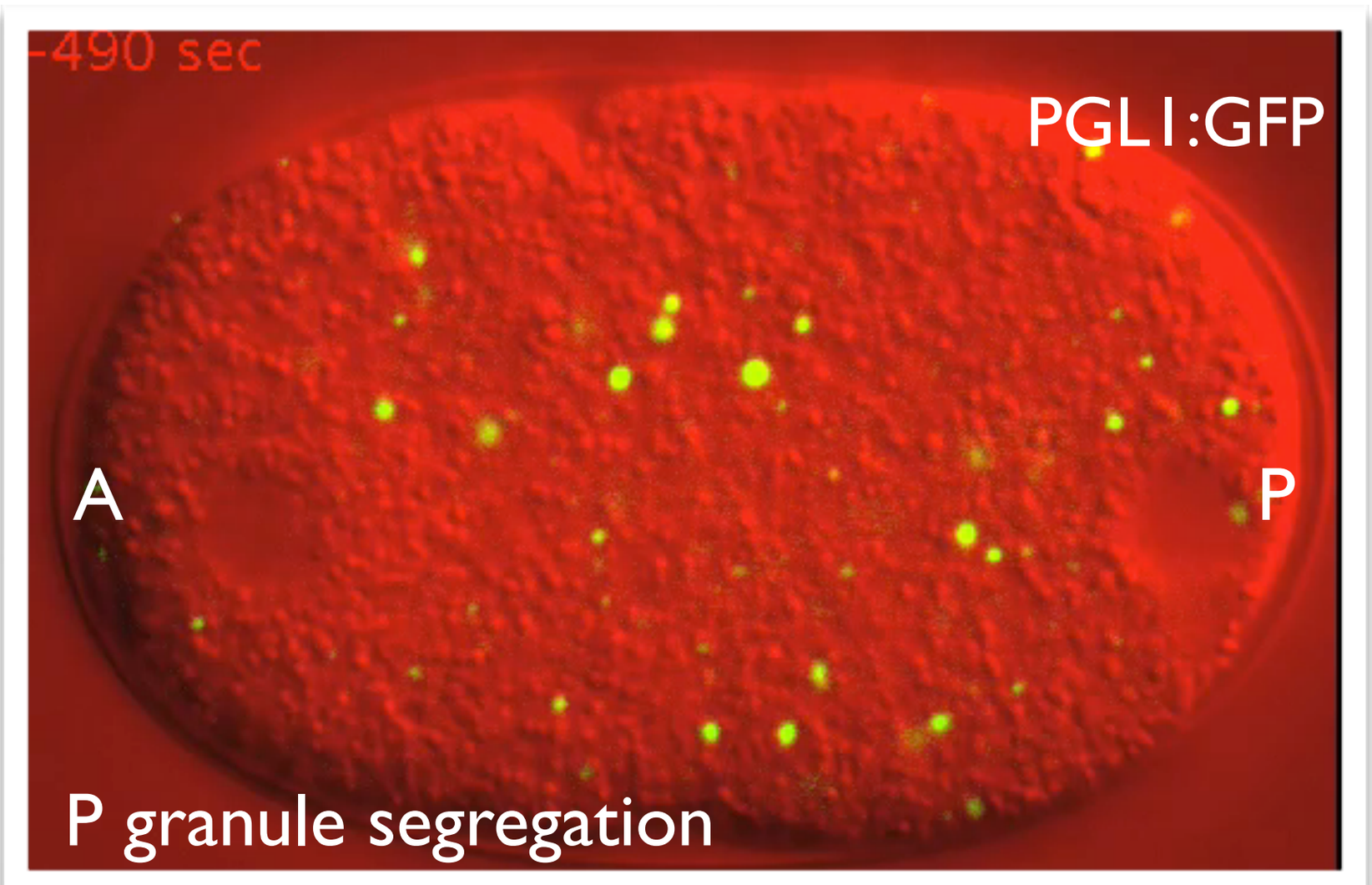
# Dynamics of asymmetric P granule segregation

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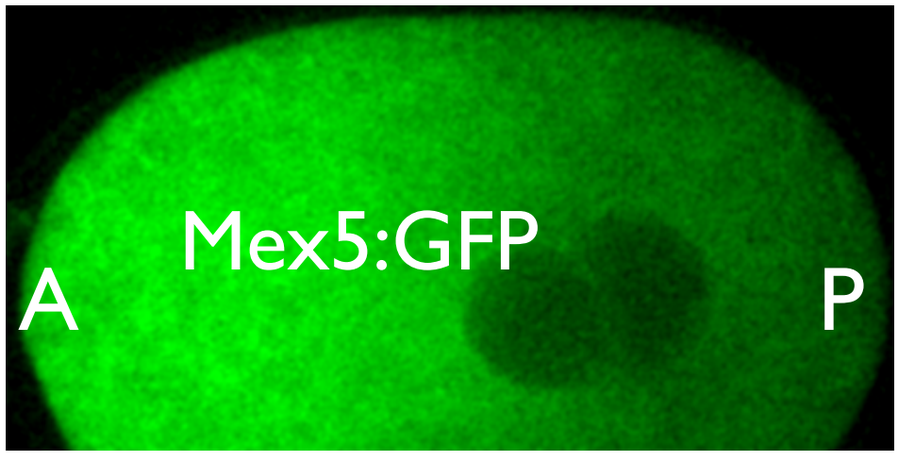


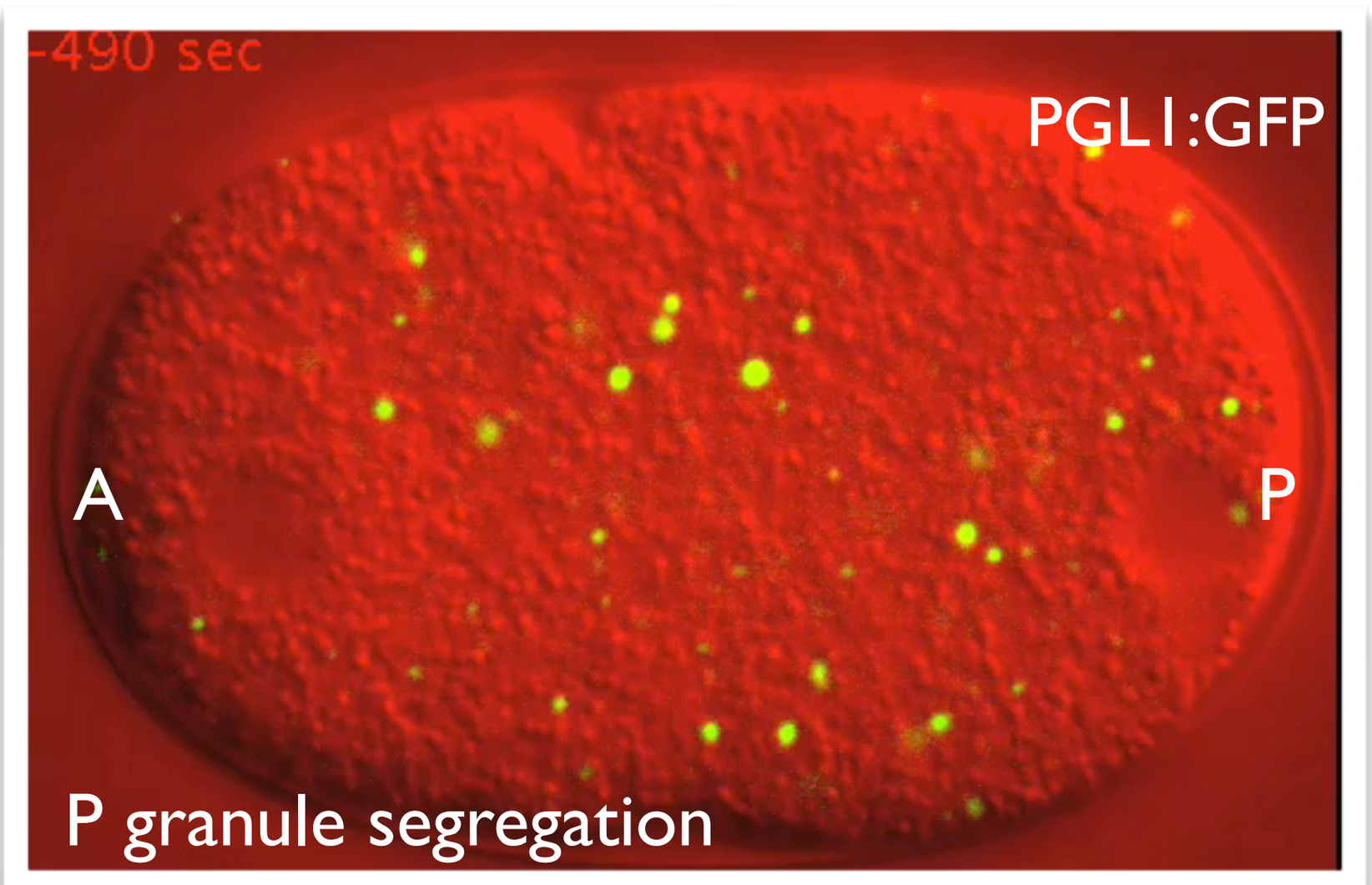


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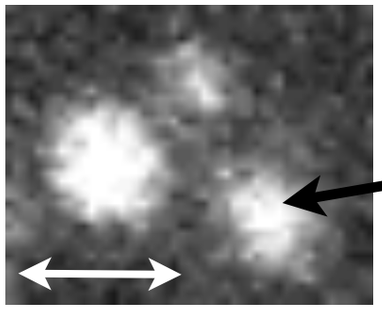


P-granule $\mathcal{O}(10)$ various different proteins

[PGL-1/3, GLH-1/3, OMA-1/3, ...]

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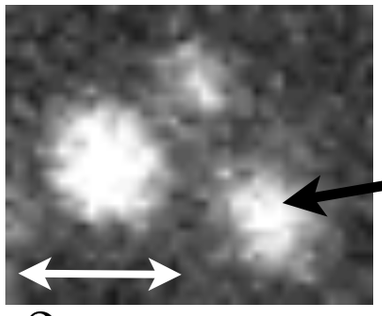

$2\mu m$

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P-granules are liquid drops
on cellular time scales:
fuse, shear, turn over in seconds and relax to a spherical shape

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$$\gamma \approx 0.1 \mu N/m$$

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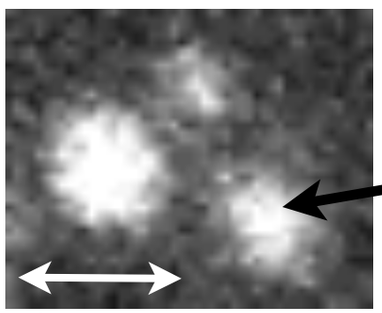

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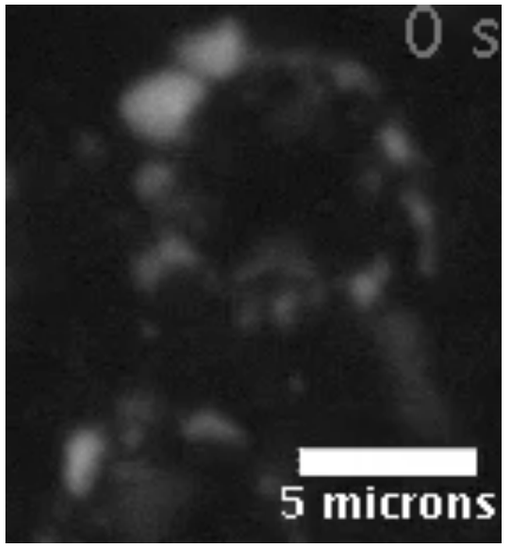


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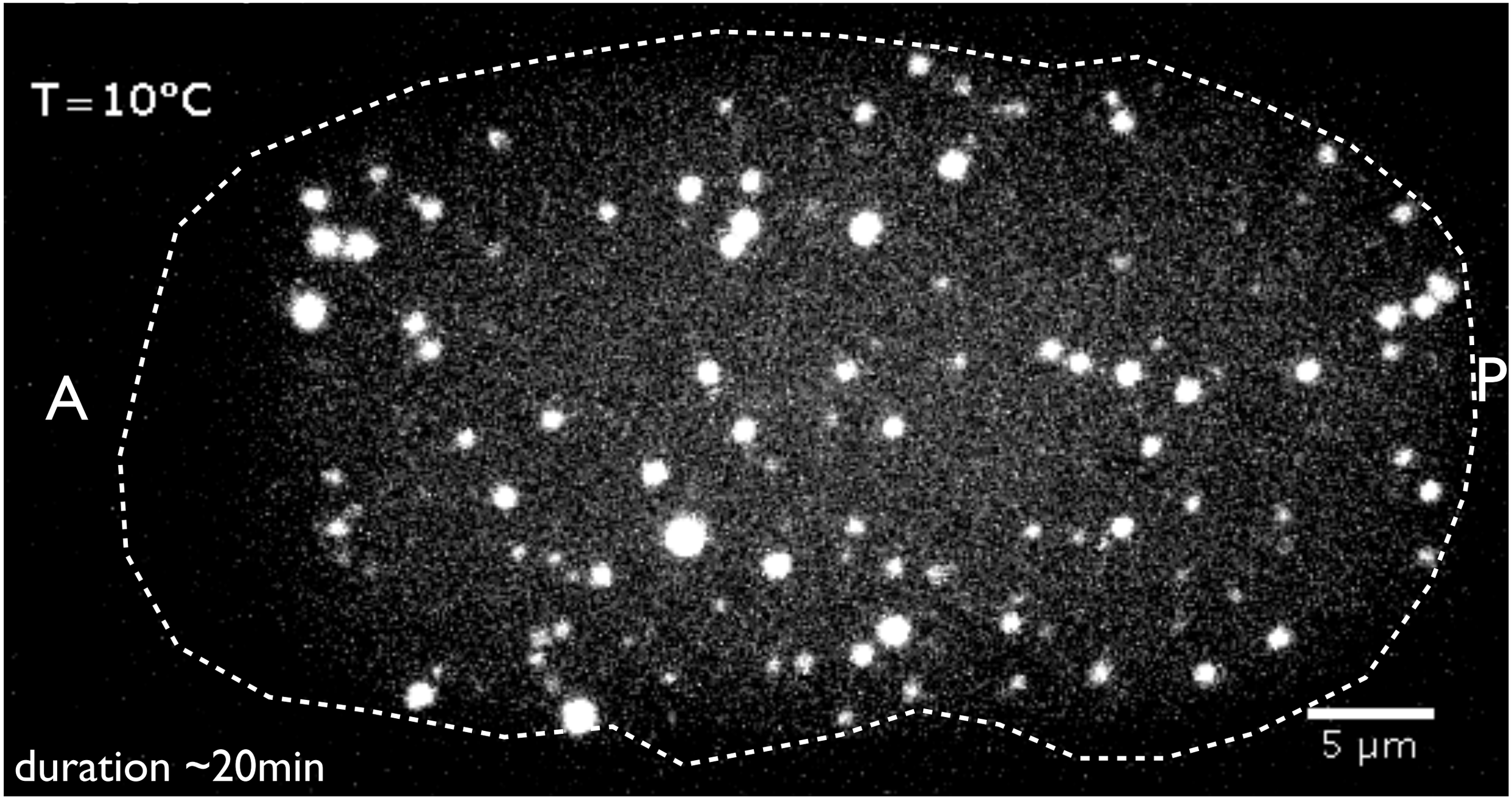


with Andres Diaz

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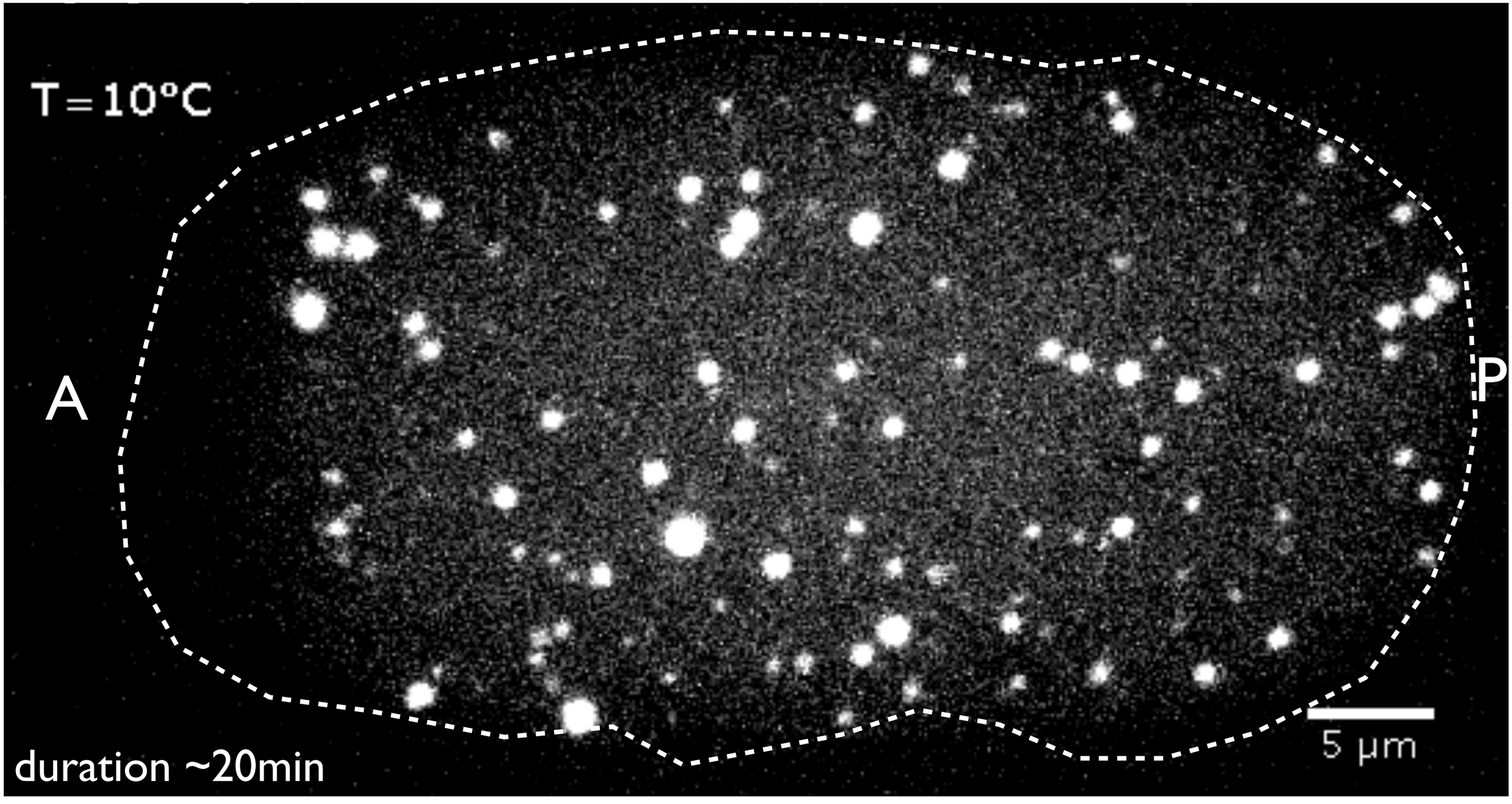


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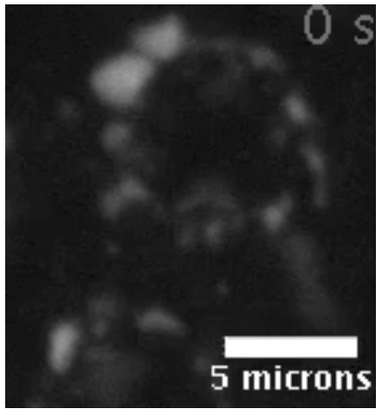

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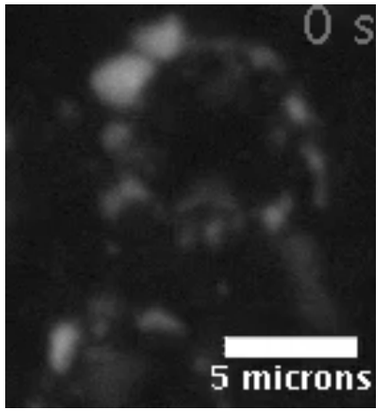
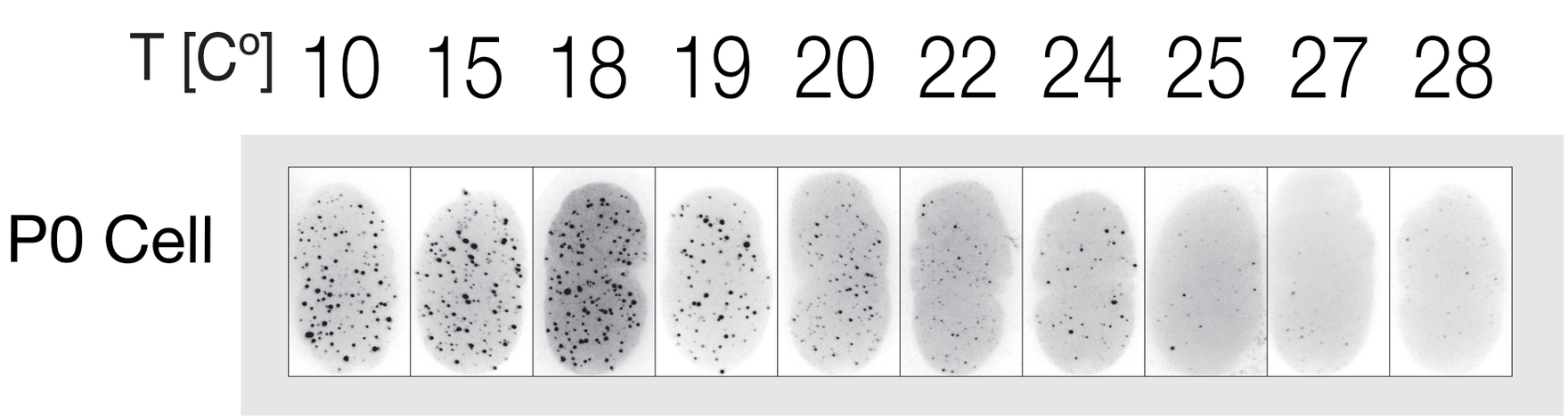



P granules resemble liquid-like drops

P granules form by liquid phase separation that can be reversibly affected by temperature



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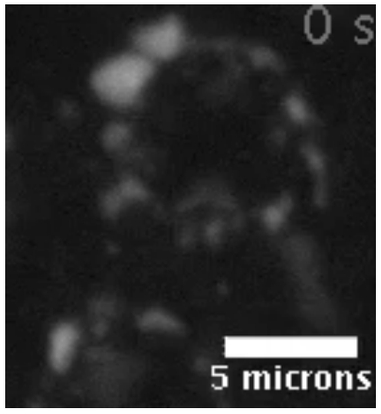


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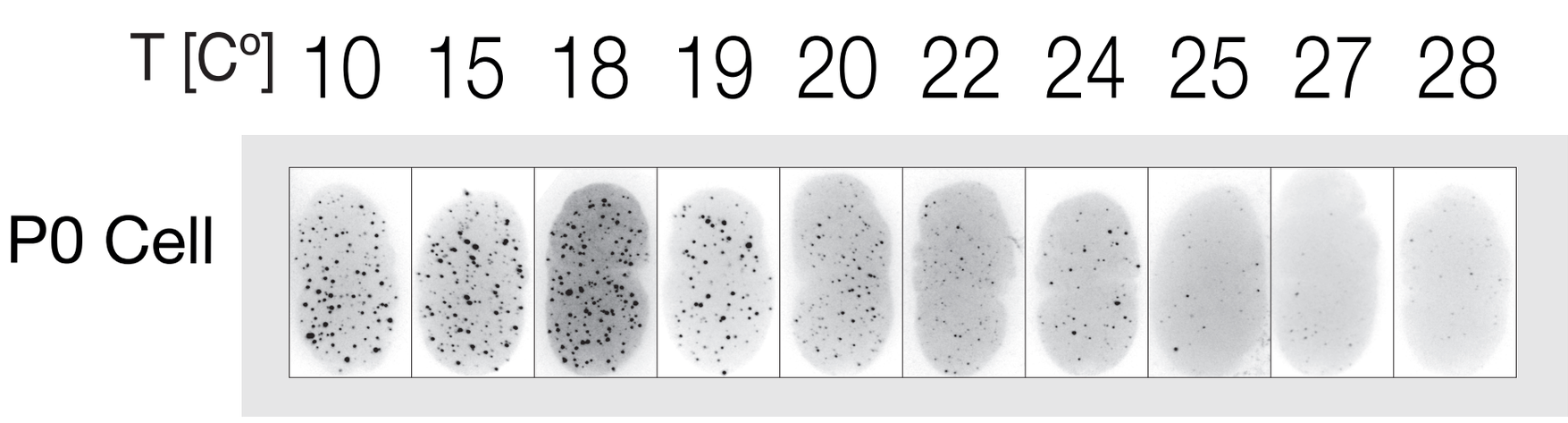
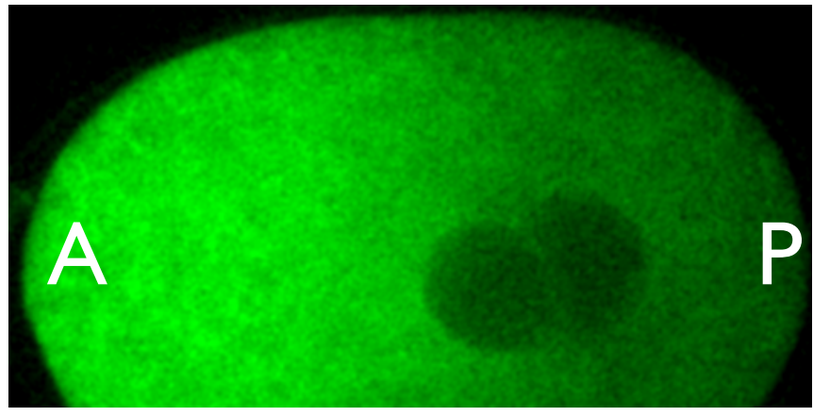



a robust regulator protein MEX-5 dissolves drops at the A side



**<u>Biology question:</u>**

**What is the mechanism**

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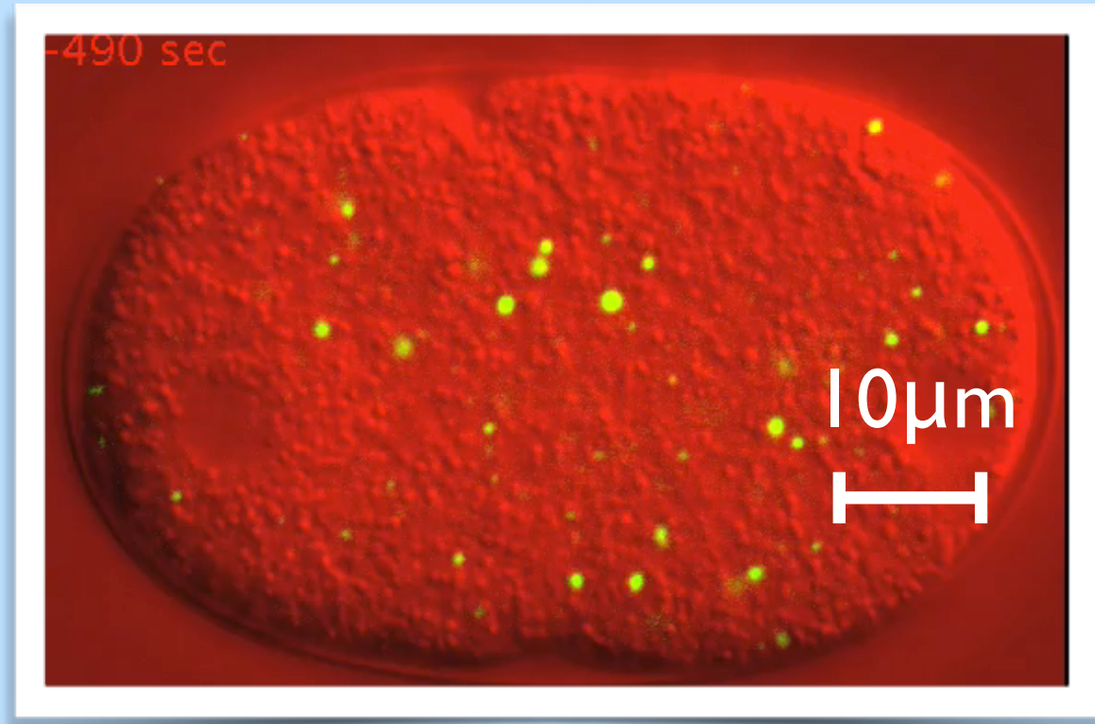


with Shamba Saha and Omar Adame

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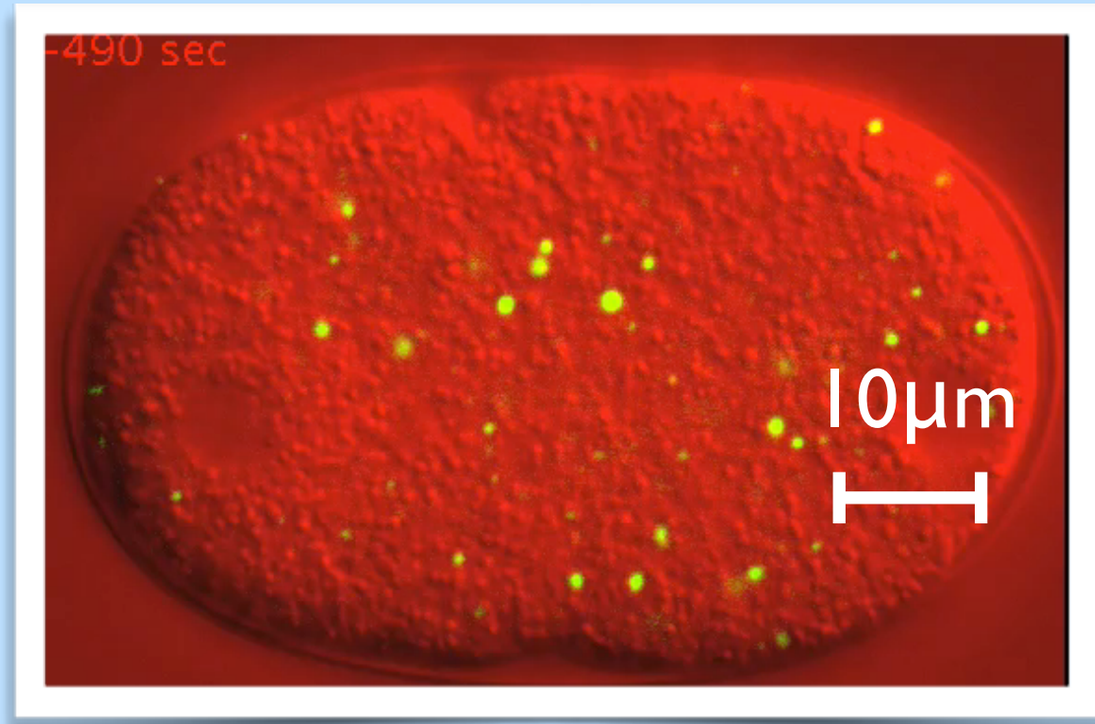


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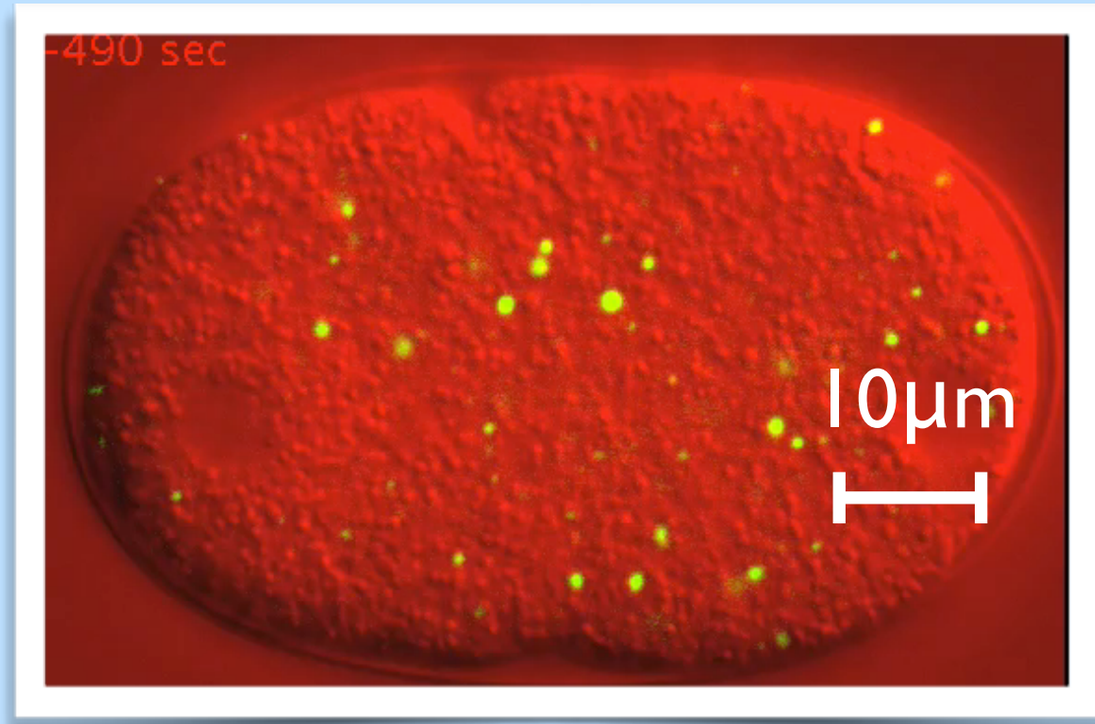


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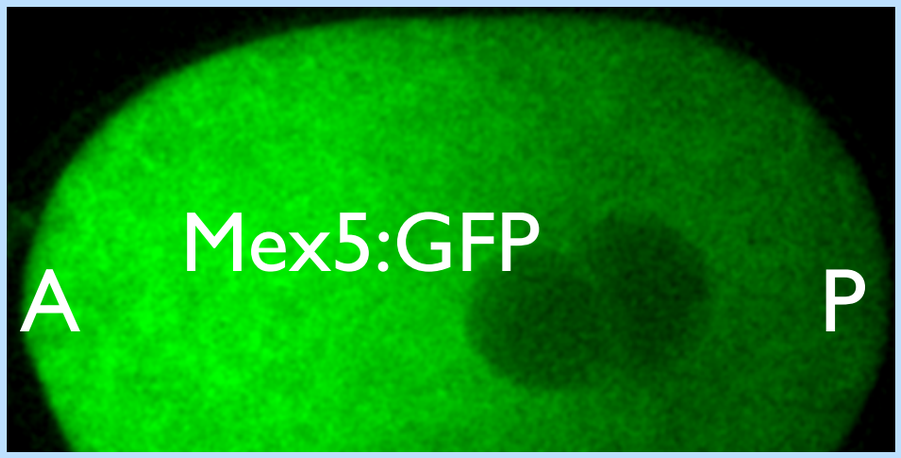
**Physics question:**

**What is the generic law of how droplets ripen in a gradient of regulating molecules?**

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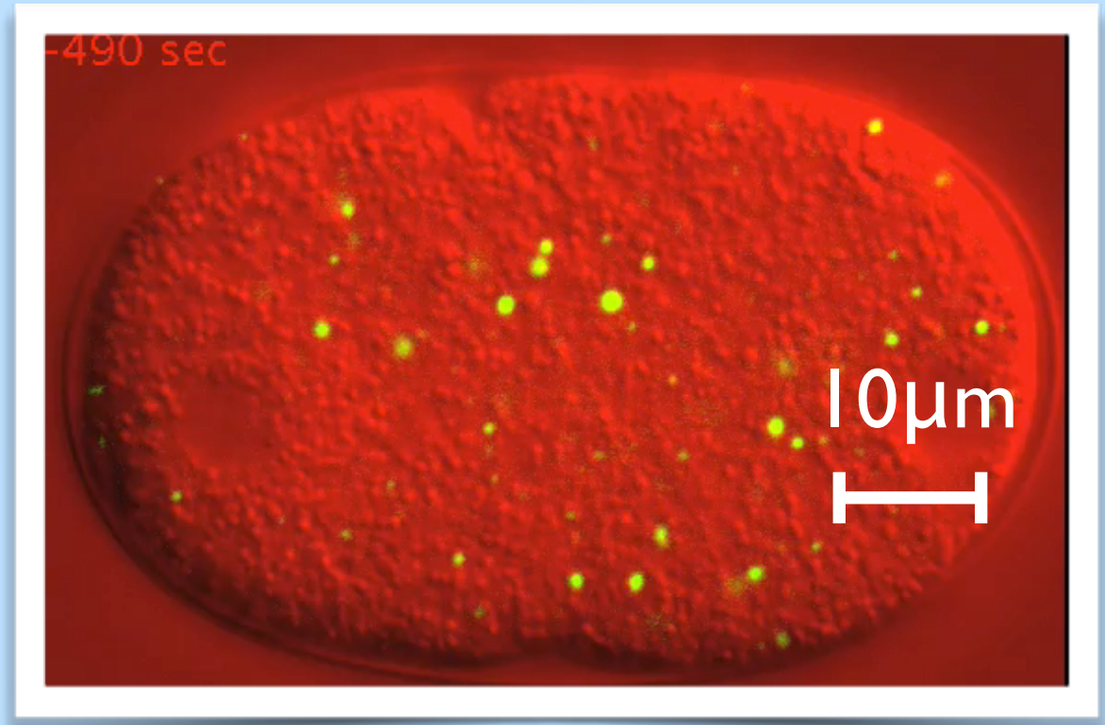


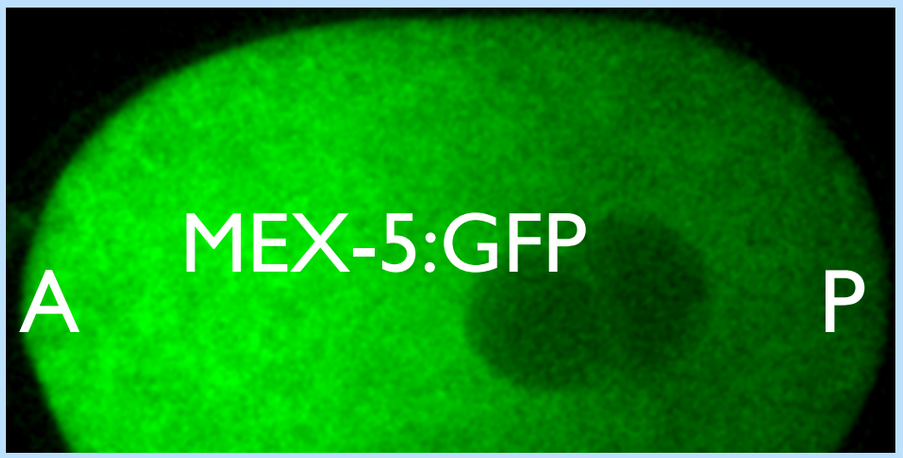


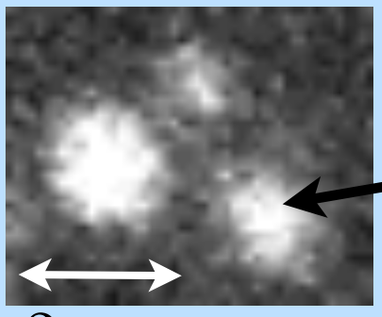


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$\mathcal{O}(10)$ <u>various different</u> proteins

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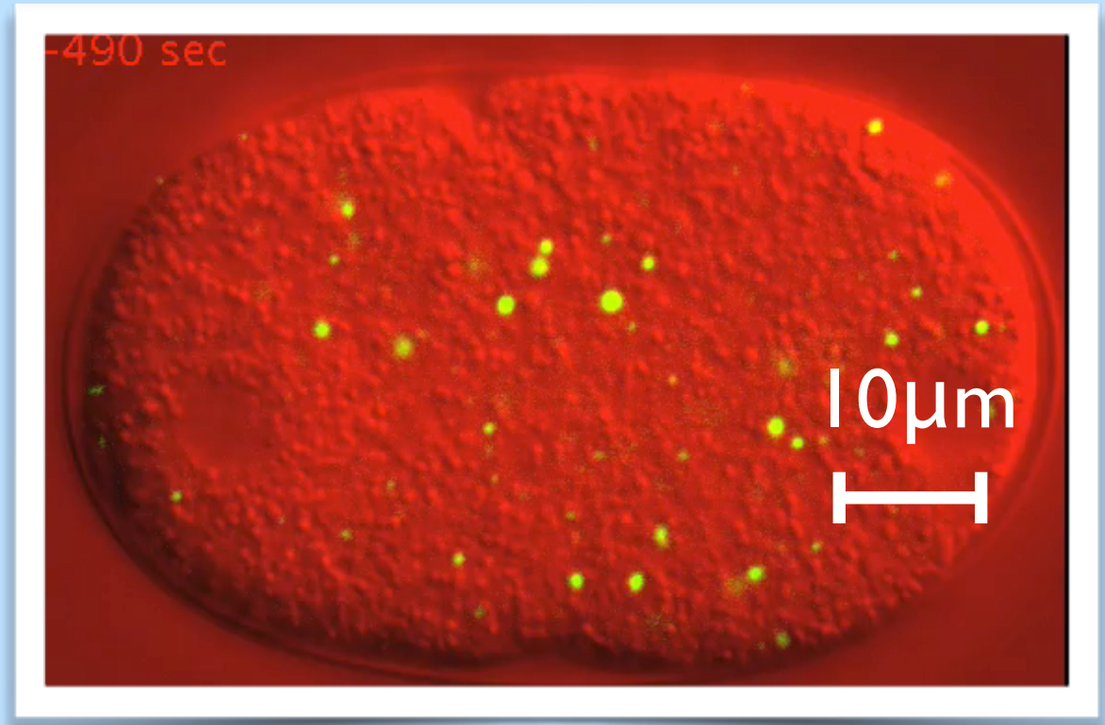


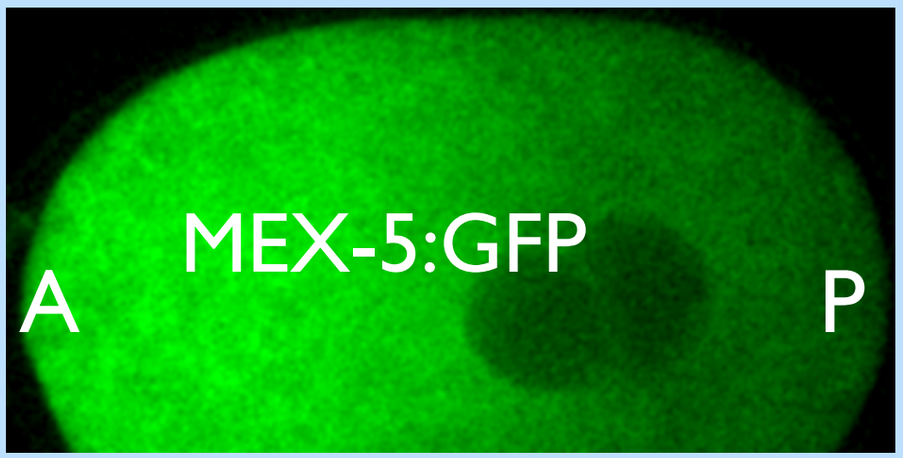


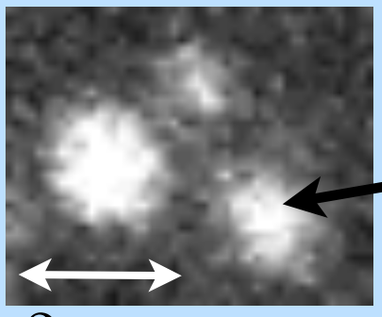


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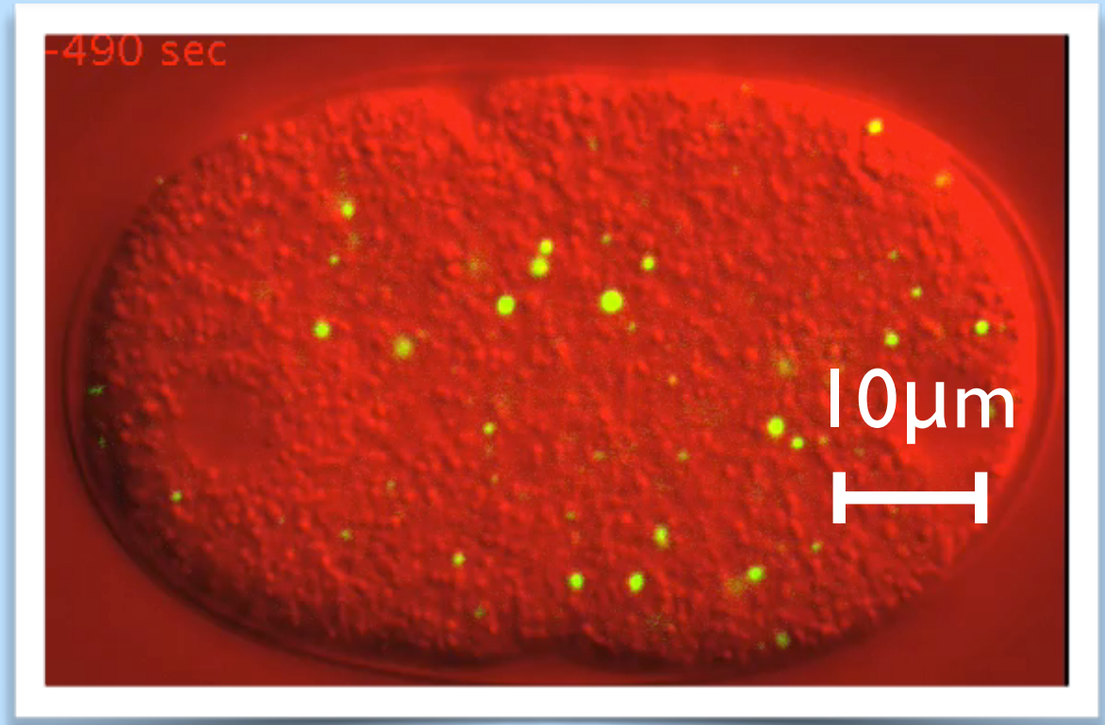


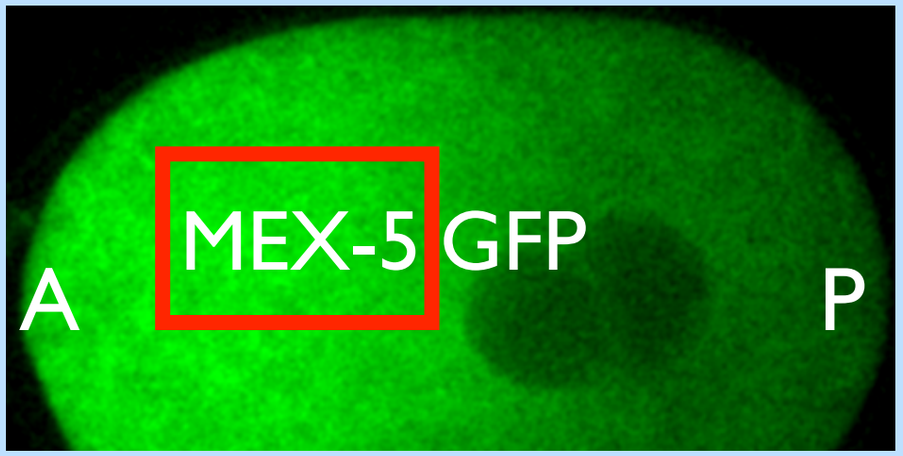


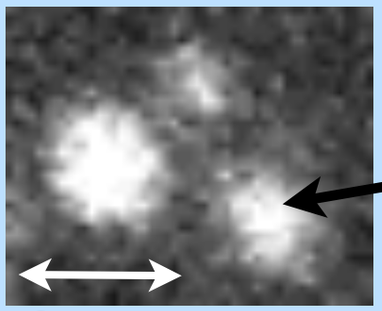


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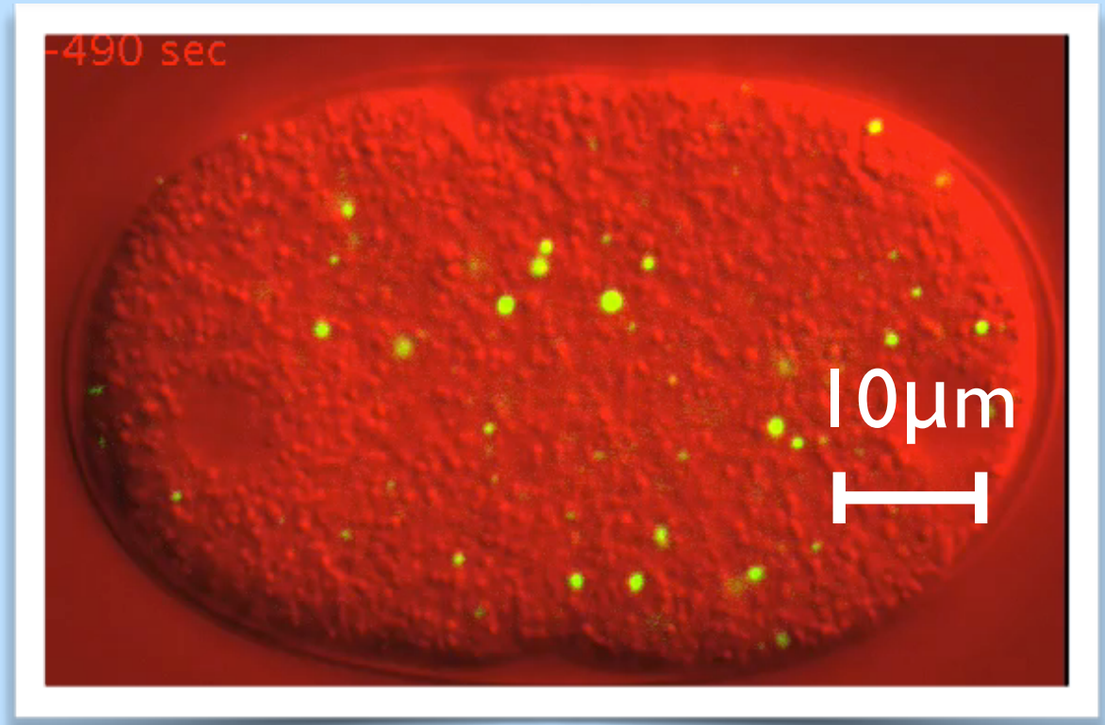


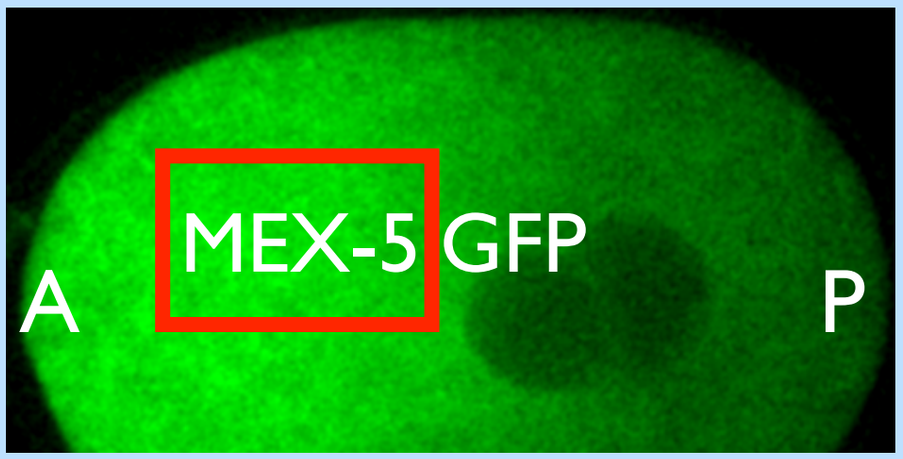


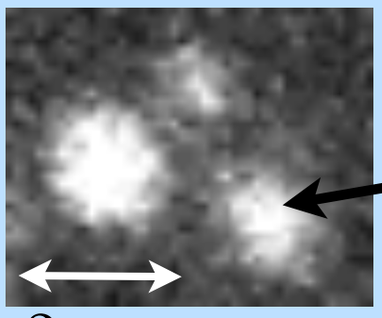


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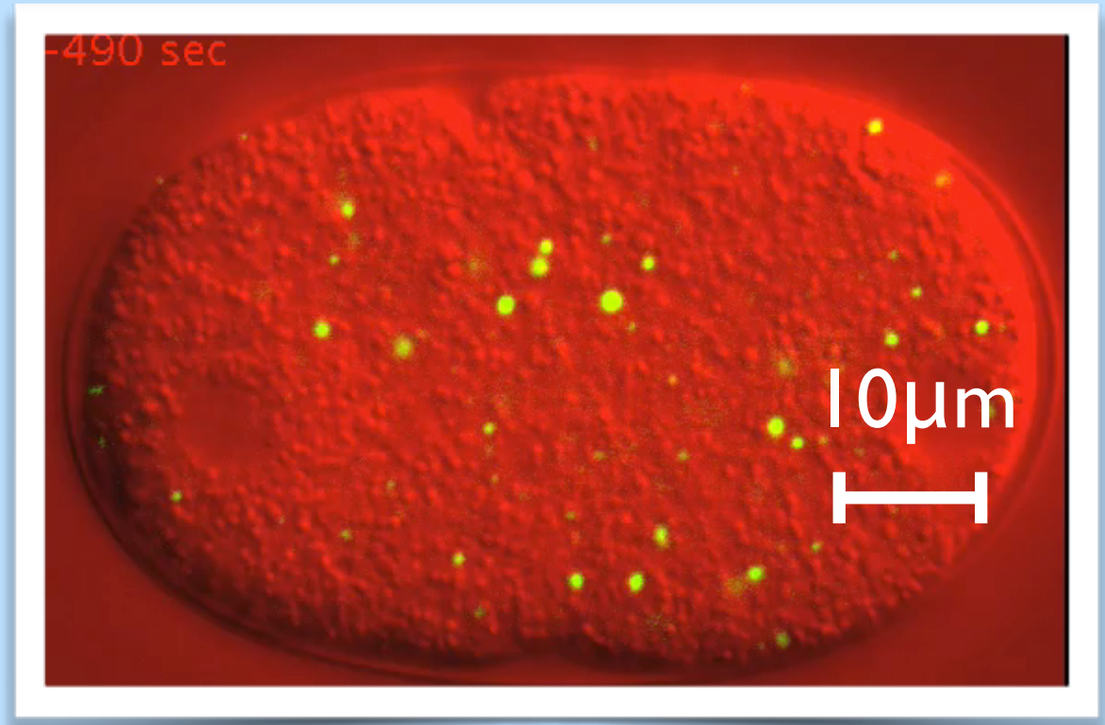
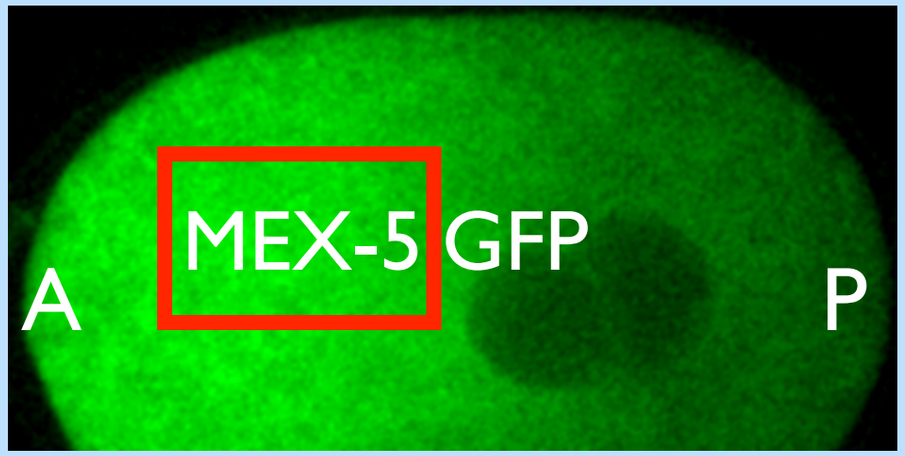
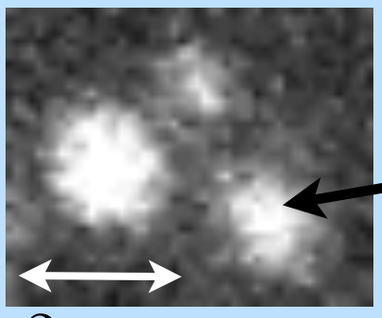



focus on **two key players**

---> in-vitro

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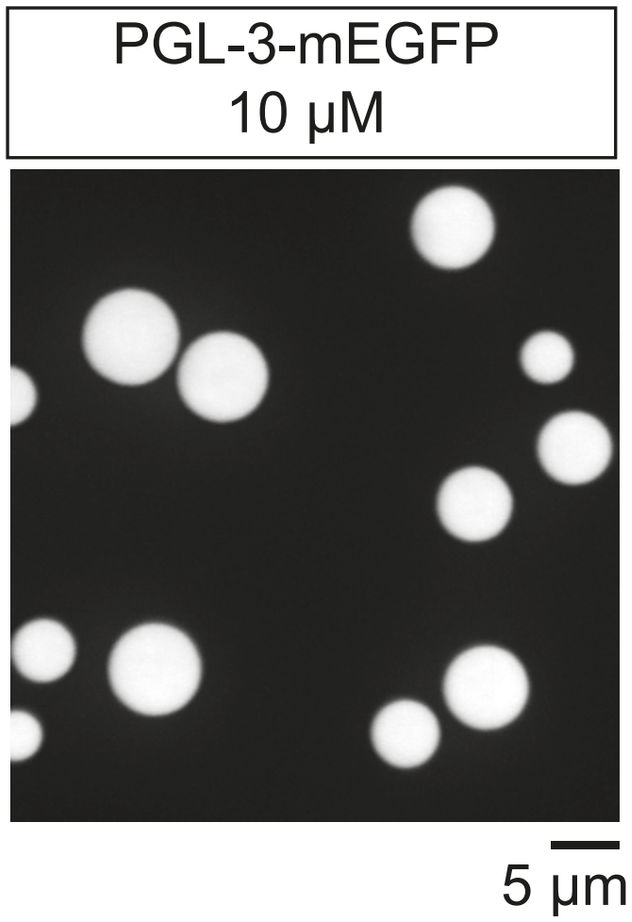


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# PGL-3 form liquid-like drops in-vitro

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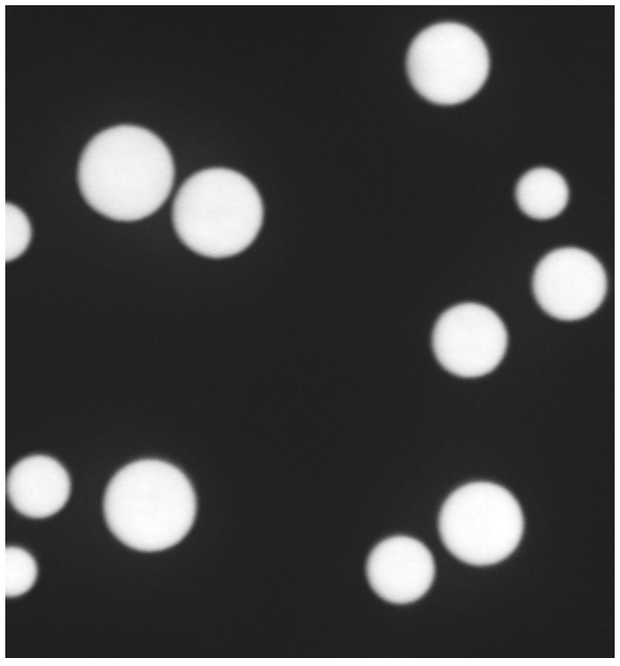
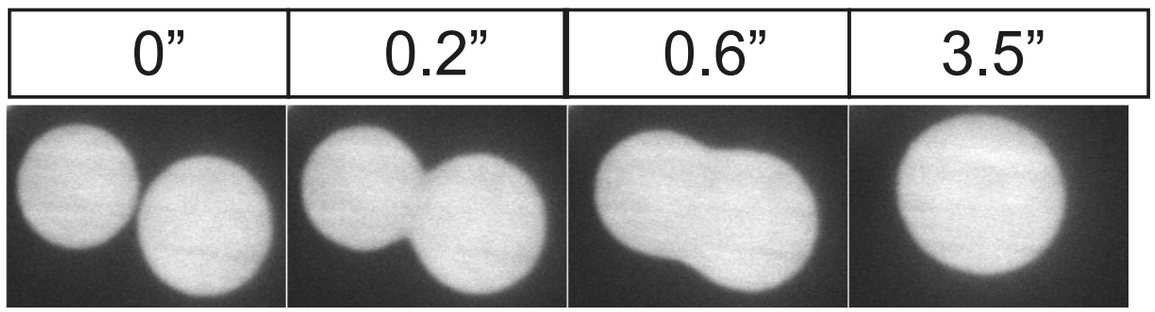
PGL-3-mEGFP
10 µM



5 µm

fusion within 5 seconds




5 µm

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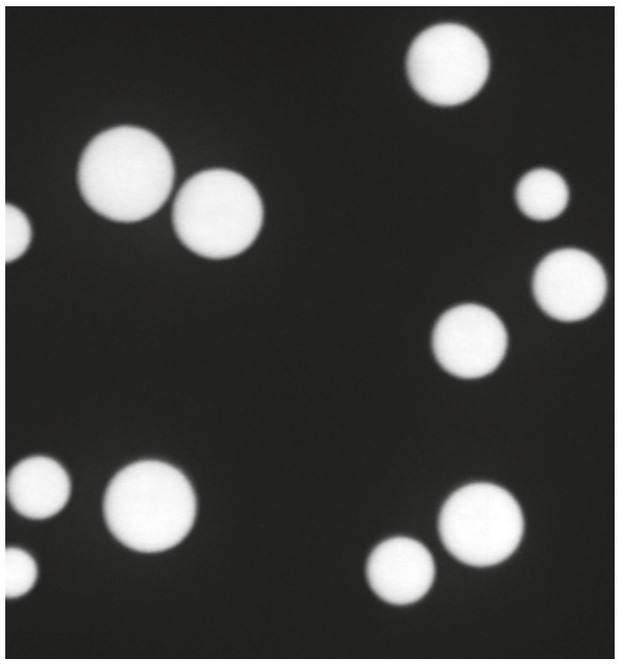
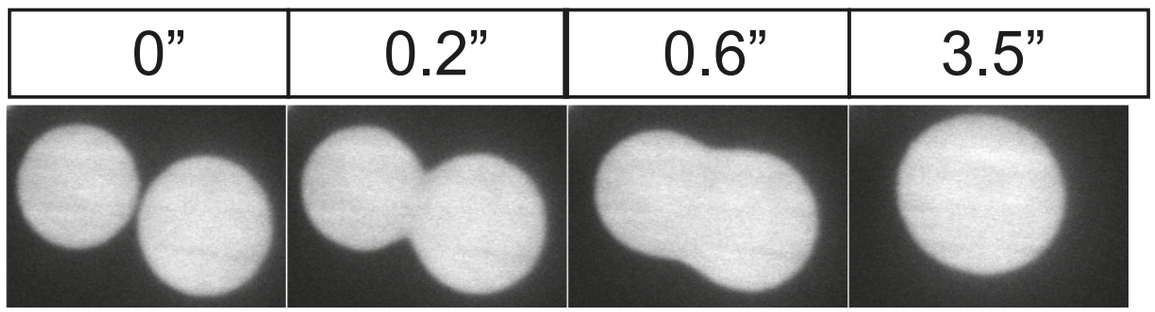


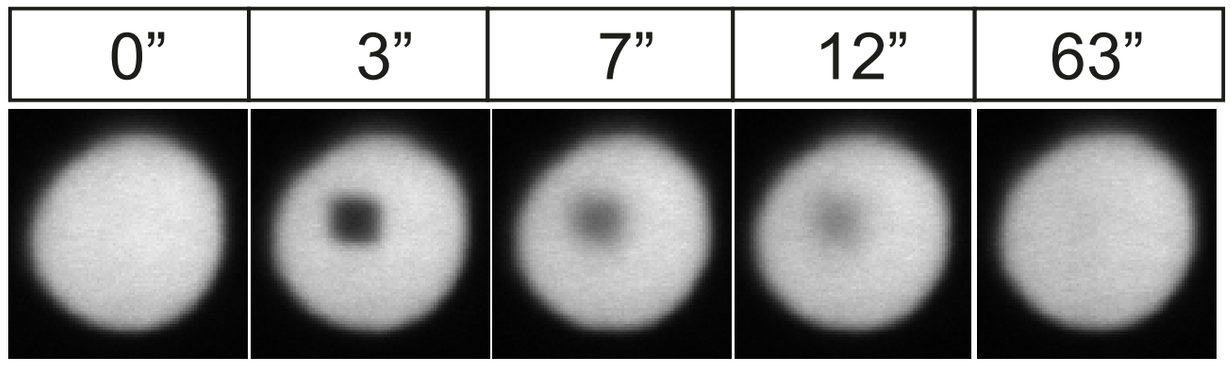
5 µm

fusion within 5 seconds




5 µm

FRAP within 30 seconds




5 µm

Shamba Saha

# What affects PGL-3 drop formation?

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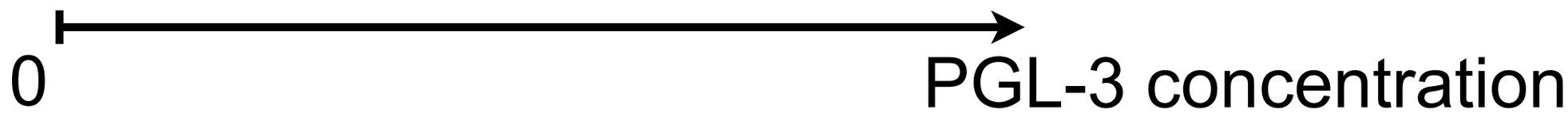

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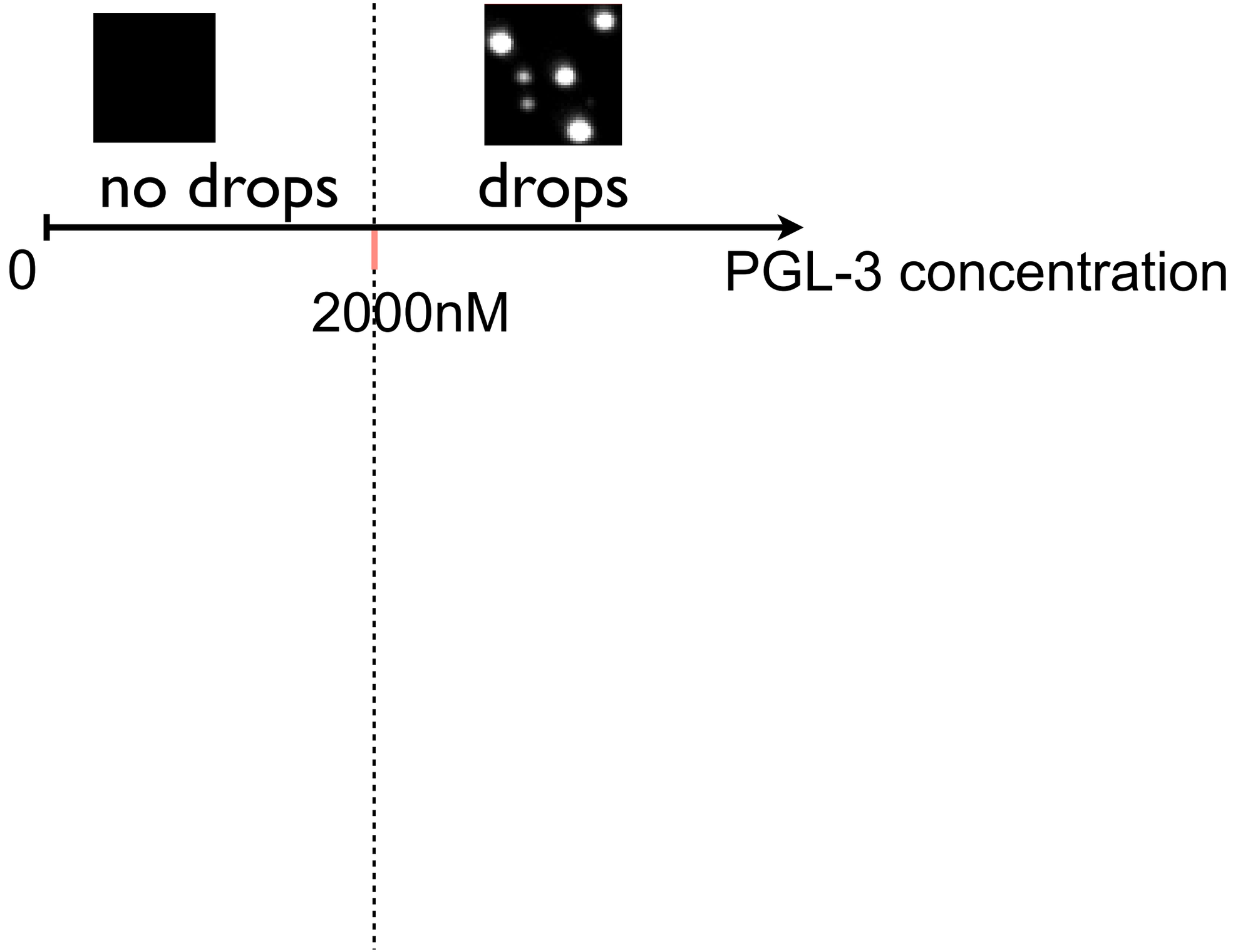

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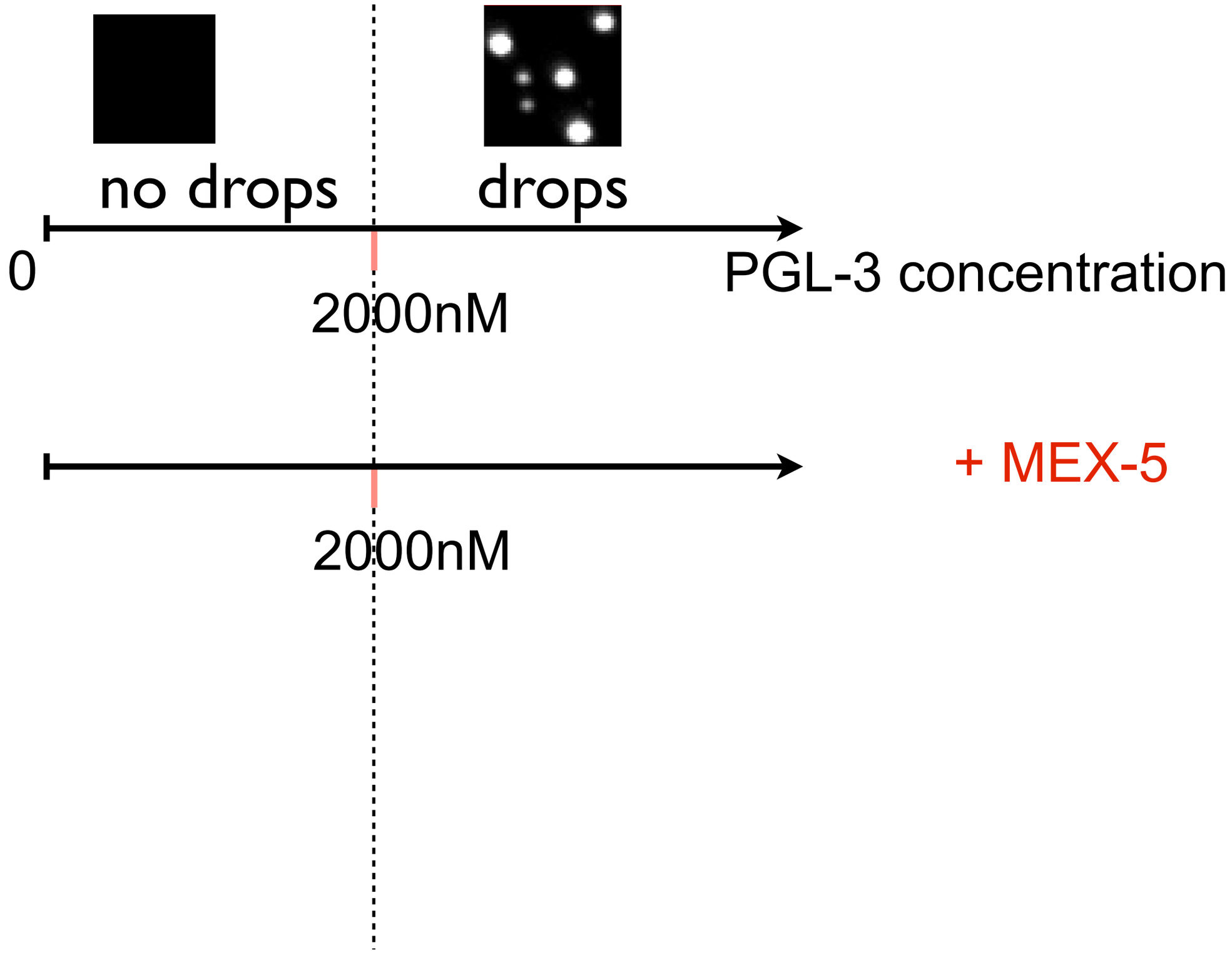

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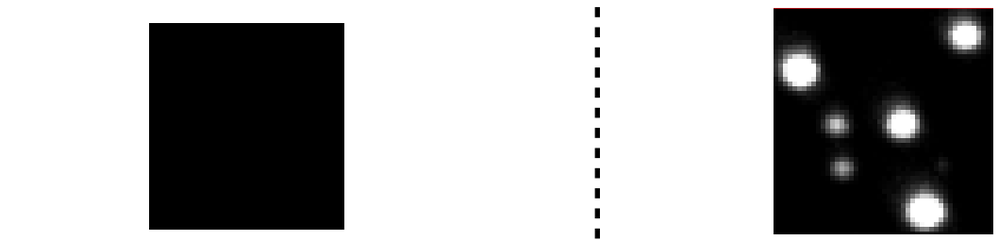


no drops



drops

0

2000nM

PGL-3 concentration

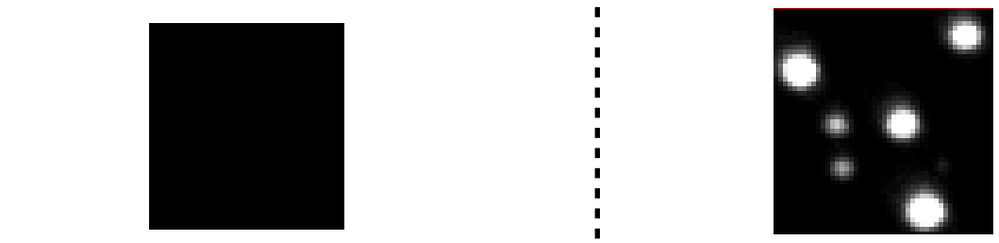
2000nM

+ MEX-5

200nM

+ mRNA

# What affects PGL-3 drop formation?





no drops

drops

0

2000nM

PGL-3 concentration

2000nM

+ MEX-5

200nM

+ mRNA

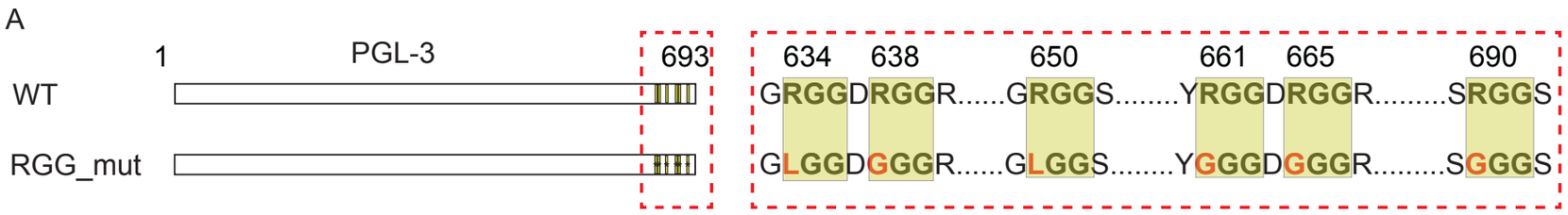
+ mRNA

+ MEX-5

for MEX-5↗

Shamba Saha

# mRNA promotes PGL-3 drops formation in-vitro through binding to PGL-3




arginines -> glycine or leucine

Shamba Saha

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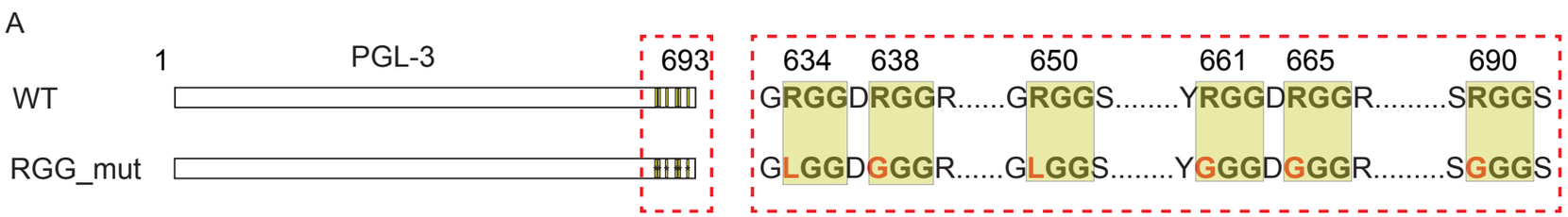


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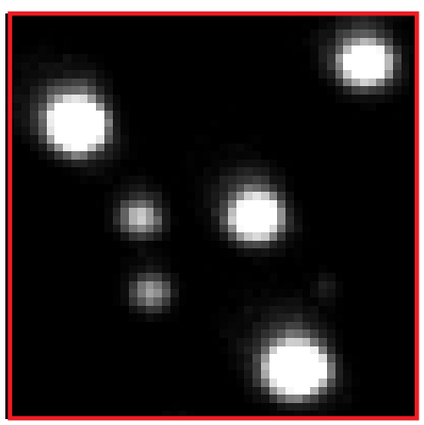

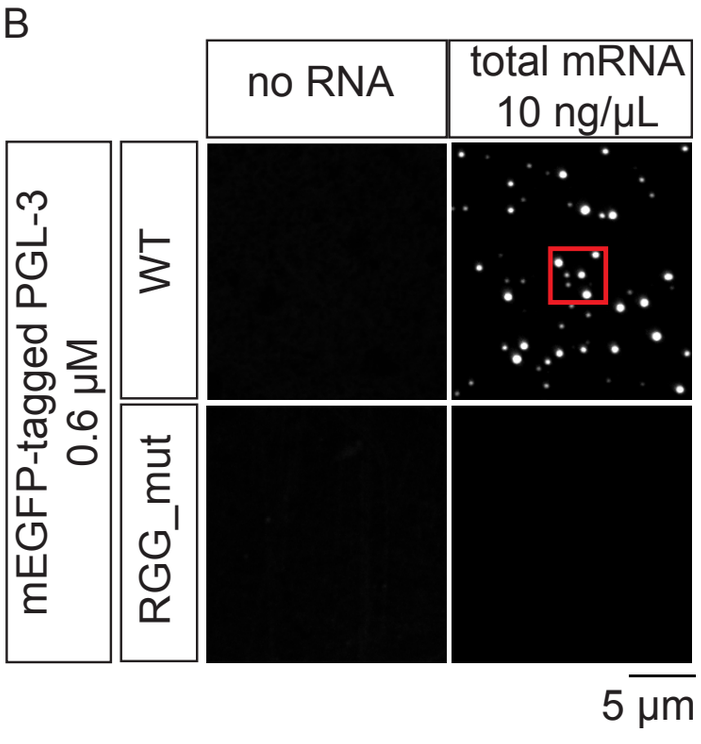


Shamba Saha

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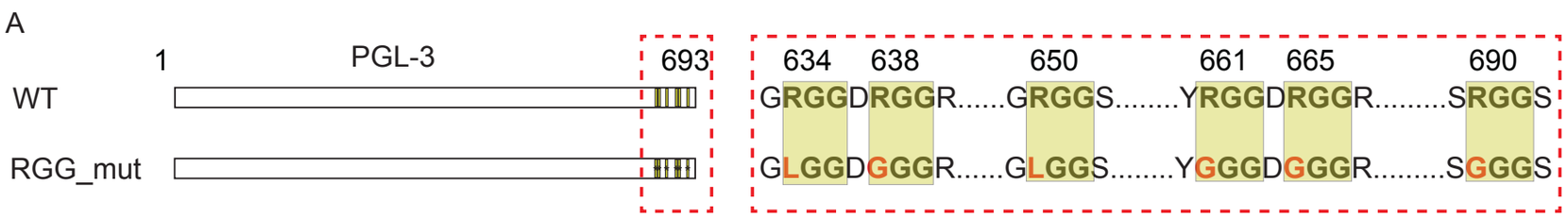


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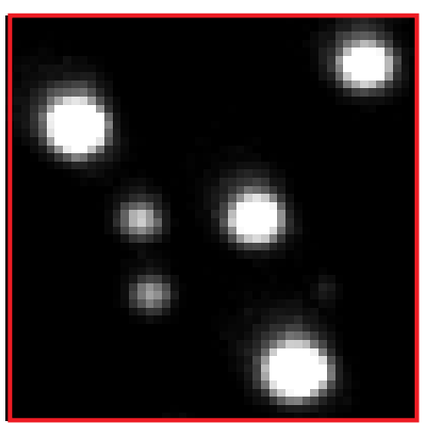
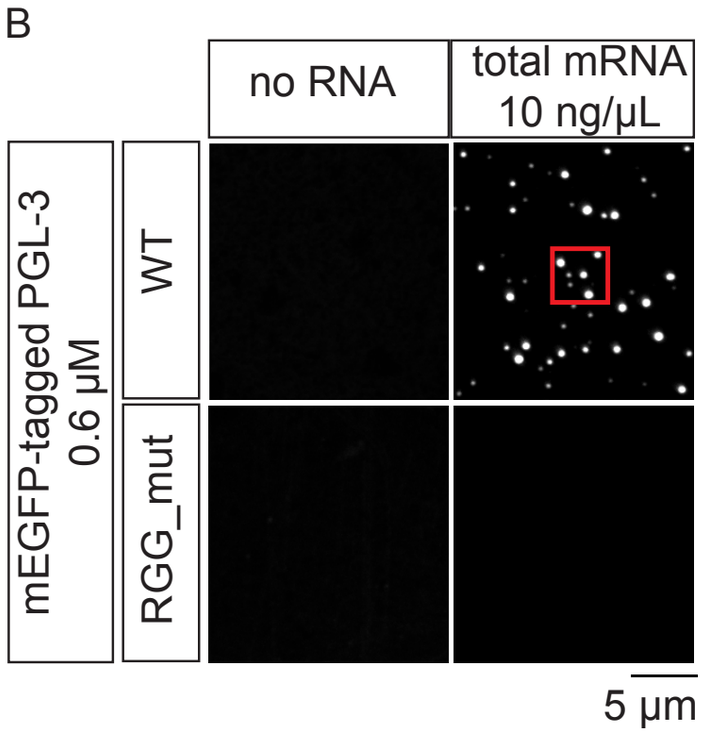
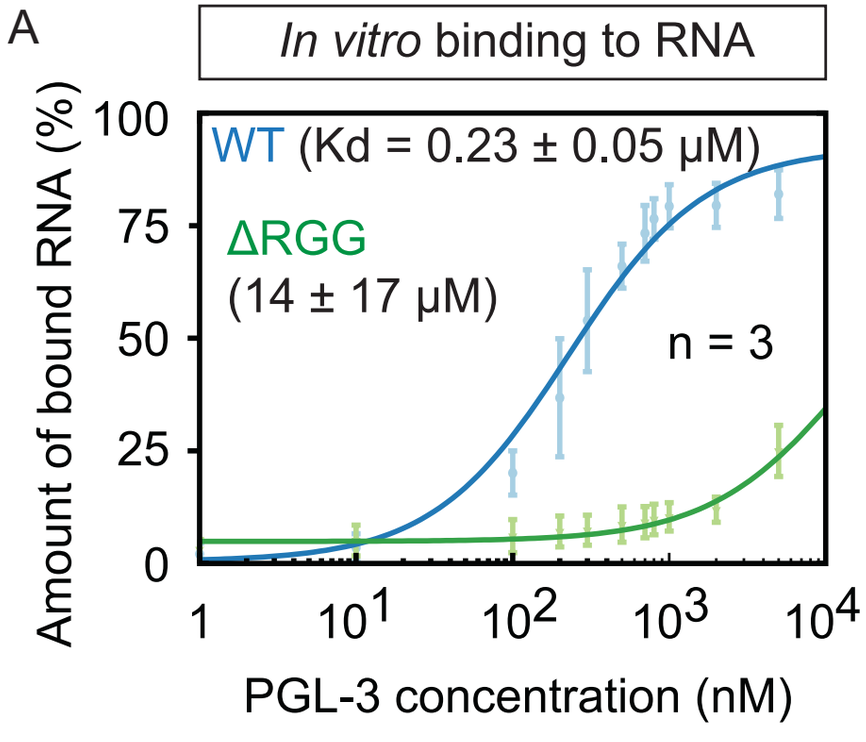
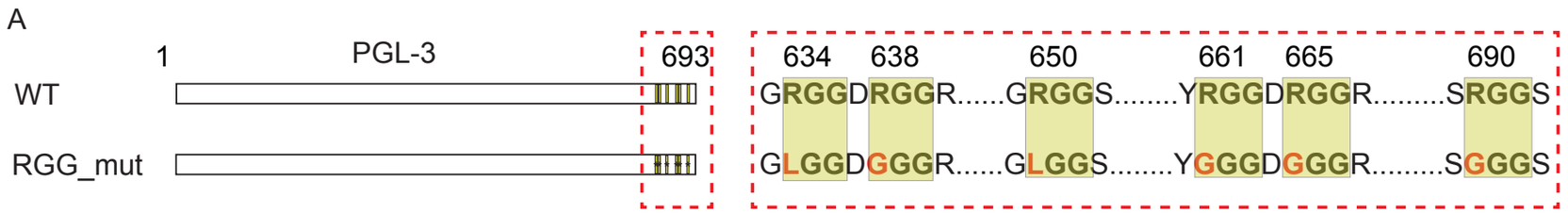




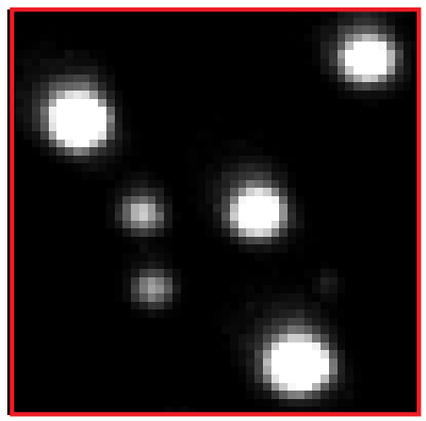



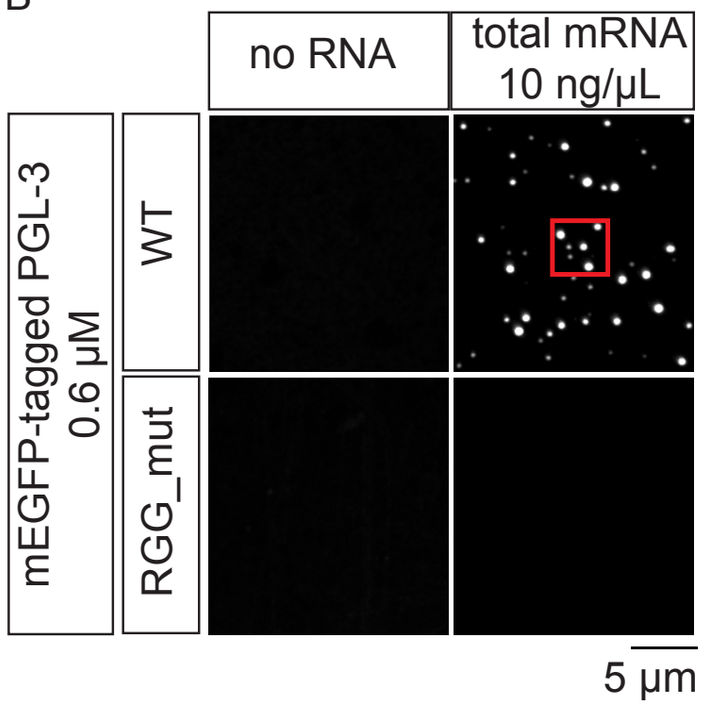

Shamba Saha

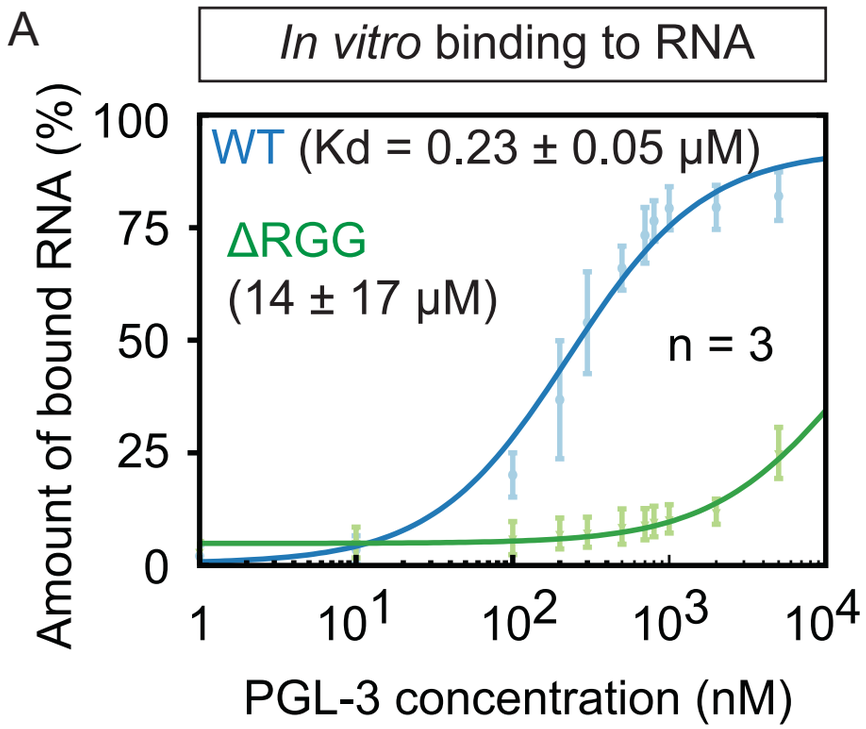
# mRNA promotes PGL-3 drops formation in-vitro through binding to PGL-3

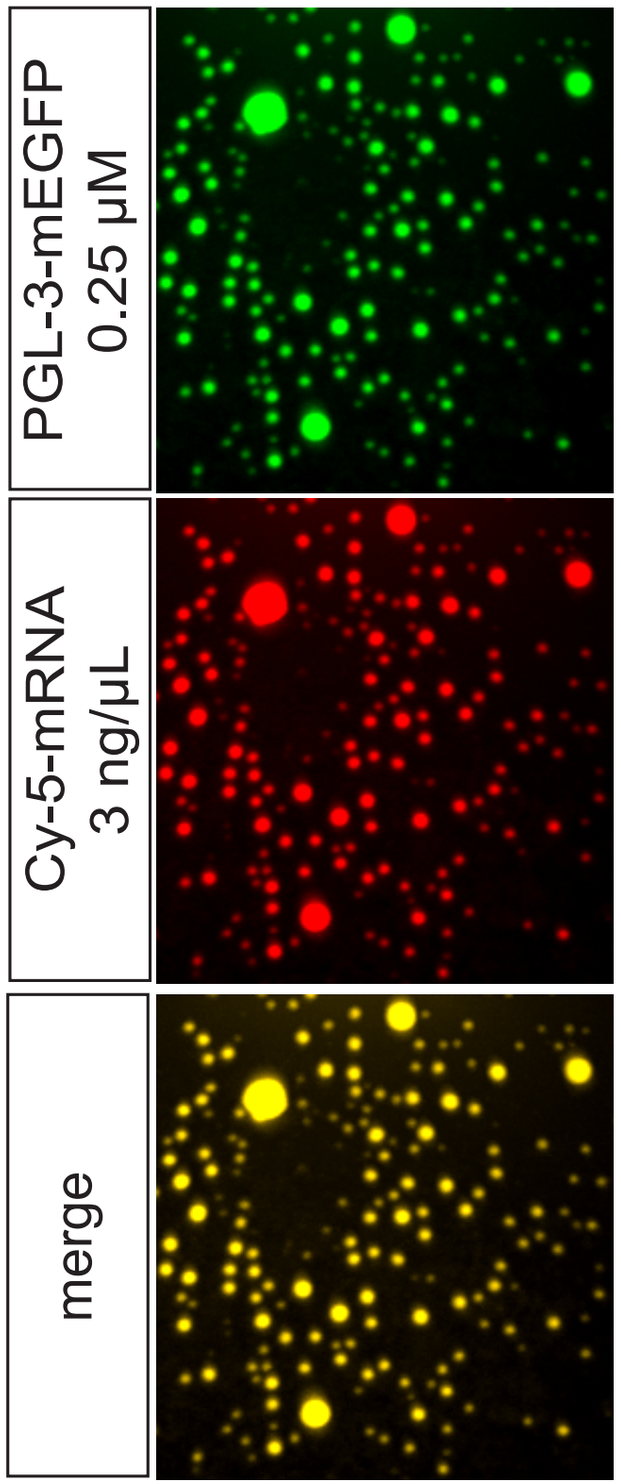



arginines -> glycine or leucine



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# MEX-5 also binds to mRNA

Shamba Saha

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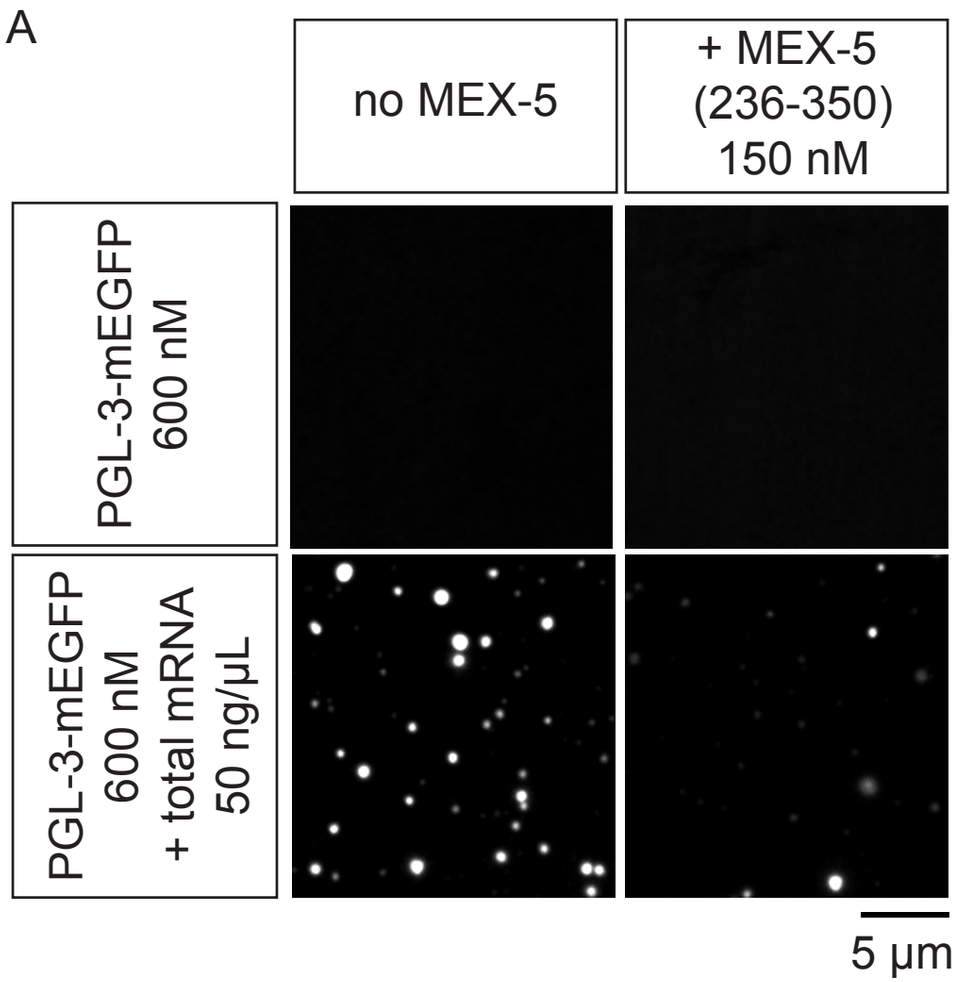

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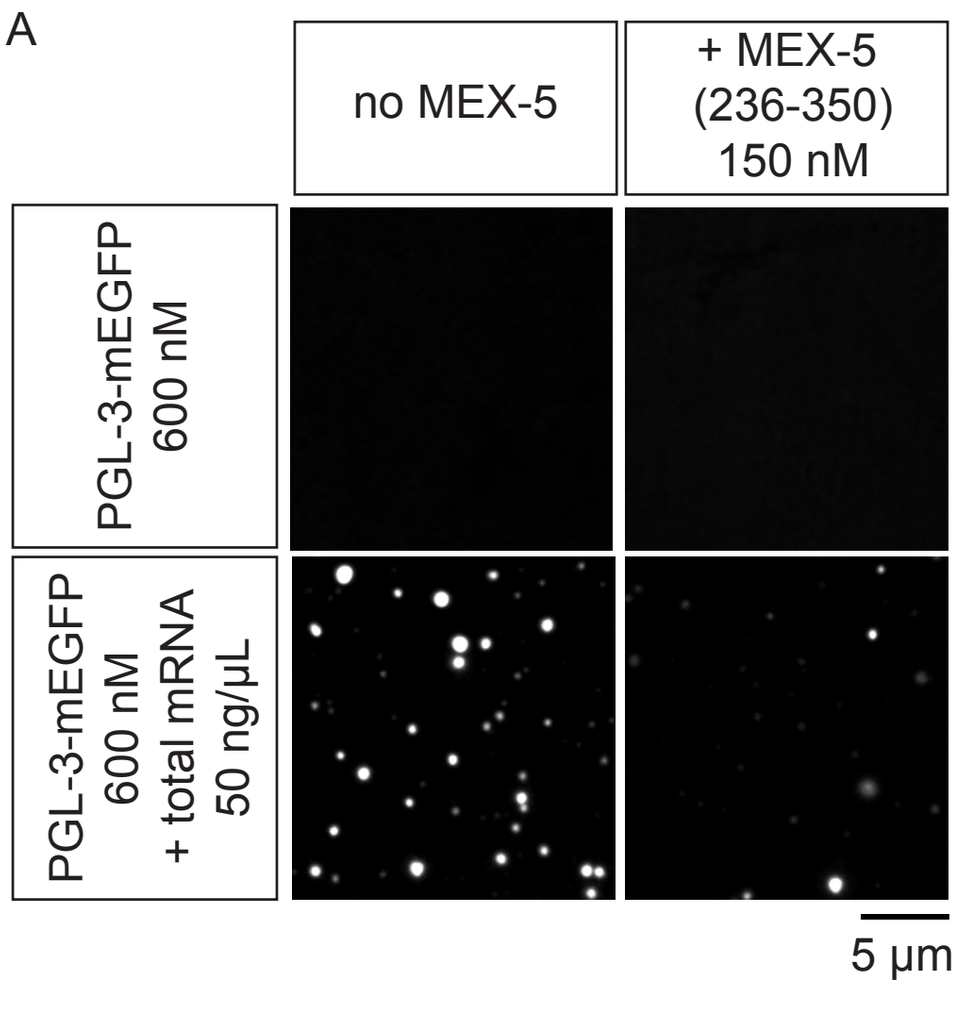


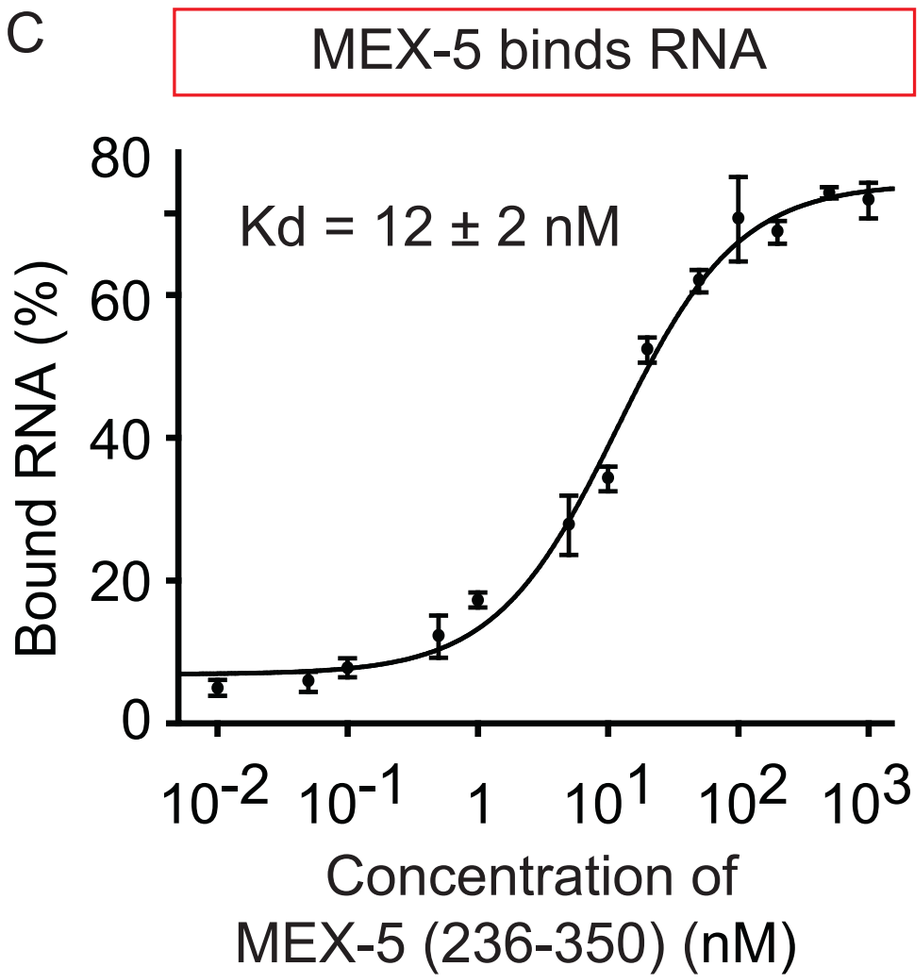


Shamba Saha

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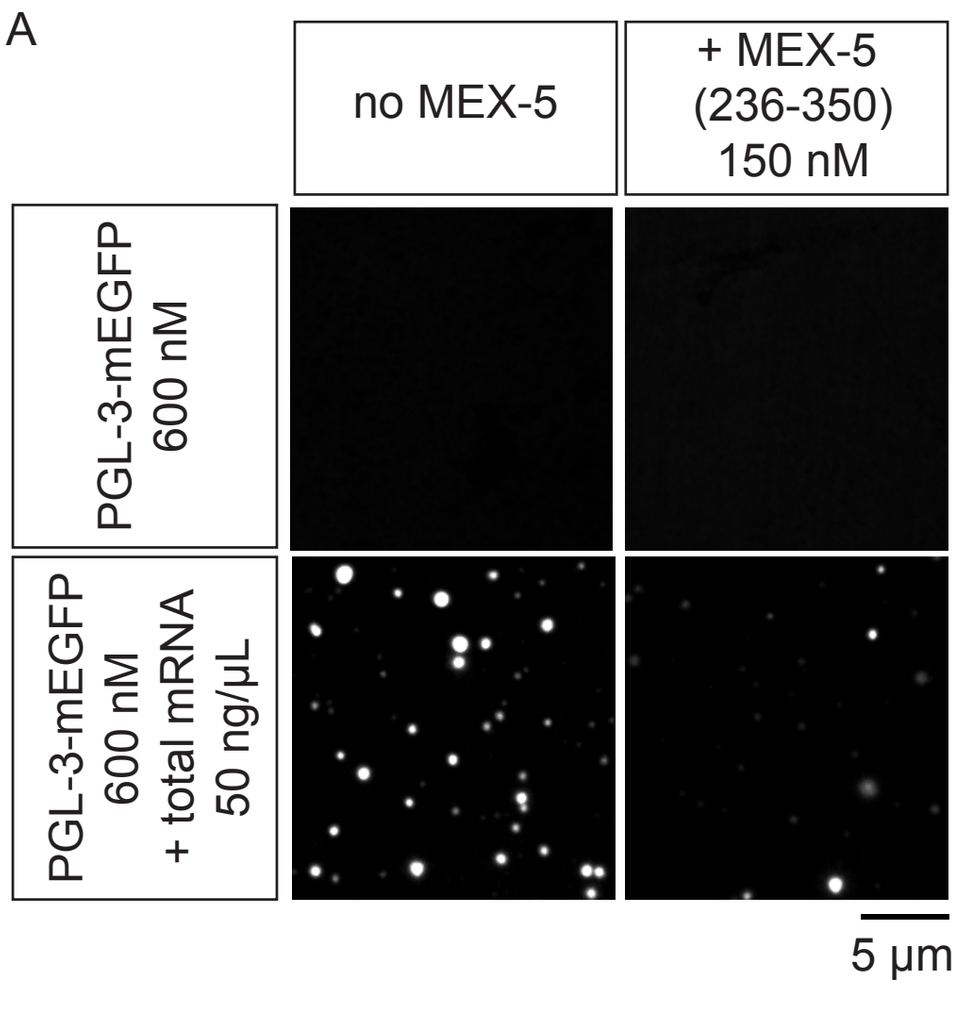


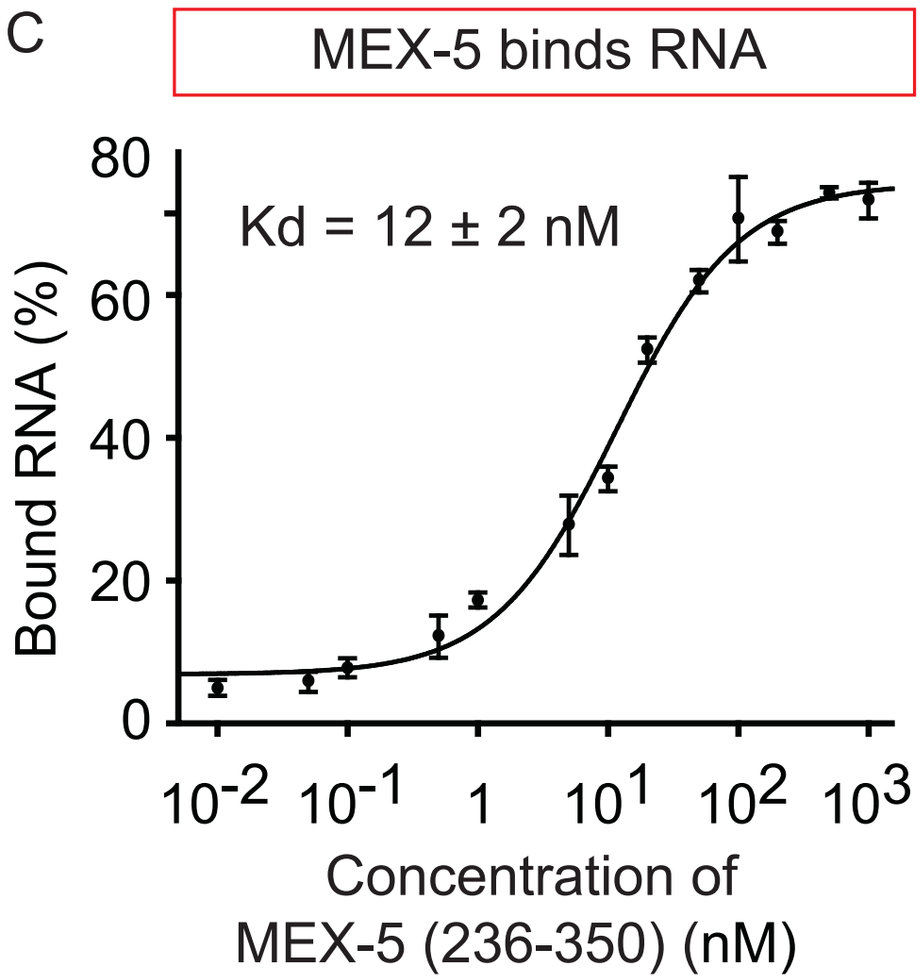


--> competition of MEX-5 and PGL-3 about mRNA

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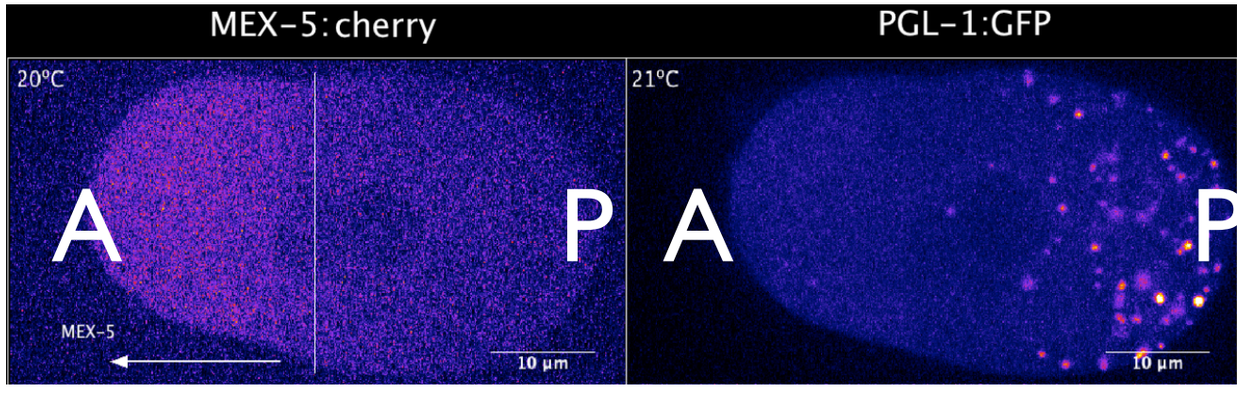
# hypothesis for P granules positioning in a MEX-5 gradient




**MEX-5 at the A side binds mRNA,**

**reducing locally the demixing affinity**

**--> droplet dissolution at A side**

**--> while at the P side,**

**PGL can bind mRNA leading to stable drops**

with Shamba Saha

# Model for PGL-3 phase separation in a MEX-5 gradient

# Model for PGL-3 phase separation in a MEX-5 gradient

$$f_{\mathrm{FH}}^{(N)} = \frac{k_b T}{a^3}\left[\sum_{i=1}^{N}\frac{\phi_i}{\mathcal{C}_i}\left(\ln\phi_i + \omega_i\right) + \sum_{i,j:i<j}\chi_{ij}\phi_i\phi_j\ ,\right]$$

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$\phi_N = 1 - \sum_{i=1}^{N-1}\phi_i$

6 components

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6 components

| | |
|---|---|
| $P$ | PGL-3 |
| $PR$ | |
| $W$ | water |
| $R$ | mRNA |
| $M$ | MEX-5 |
| $MR$ | |

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6 components

| | | |
|---|---|---|
| demixing components | $P$ | PGL-3 |
| | $PR$ | |
| | $W$ | water |
| regulating components | $R$ | mRNA |
| | $M$ | MEX-5 |
| | $MR$ | |

# Model for PGL-3 phase separation in a MEX-5 gradient

$$f_{\mathrm{FH}}^{(N)} = \frac{k_b T}{\nu}\left[\sum_{i=1}^{N}\frac{\phi_i}{n_i}\left(\ln\phi_i + \omega_i\right) + \sum_{i,j:i<j}\chi_{ij}\phi_i\phi_j\,,\right]$$

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regulators are dilute:

$$\phi_i \ll 1 \text{ for } i \in \{M, R, MR\} \qquad 1 - \phi_R - \phi_M - \phi_{MR} = \phi_P + \phi_{PR} + \phi_W \simeq 1$$

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$$\boxed{f_{\mathrm{FH}}^{(6)} \simeq f_{\mathrm{FH}}^{(3)} + f_{\mathrm{reg}} =: f}$$

$$f_{\mathrm{reg}} = k_b T \sum_{i=\{M,R,MR\}} c_i\,\left(\ln c_i\nu_i + \omega_i\right)$$

$$f_{\mathrm{FH}}^{(3)} = \frac{k_b T}{\nu}\left[\phi_W(\ln\phi_W + \omega_W) + \frac{\phi_P}{n_P}(\ln\phi_P + \omega_P) + \frac{\phi_{PR}}{n_{PR}}(\ln\phi_{PR} + \omega_{PR})\right.$$
$$\left. + \chi_{P,W}\,\phi_P\phi_W + \chi_{PR,W}\,\phi_{PR}\phi_W + \chi_{PR,P}\,\phi_{PR}\phi_P\right]\,.$$

# Model for PGL-3 phase separation in a MEX-5 gradient

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$$f_{\mathrm{reg}} = k_b T \sum_{i=\{M,R,MR\}} c_i\,(\ln c_i\nu_i + \omega_i)$$

regulation via binding:

$$P + R \rightleftharpoons PR$$

$$M + R \rightleftharpoons MR$$

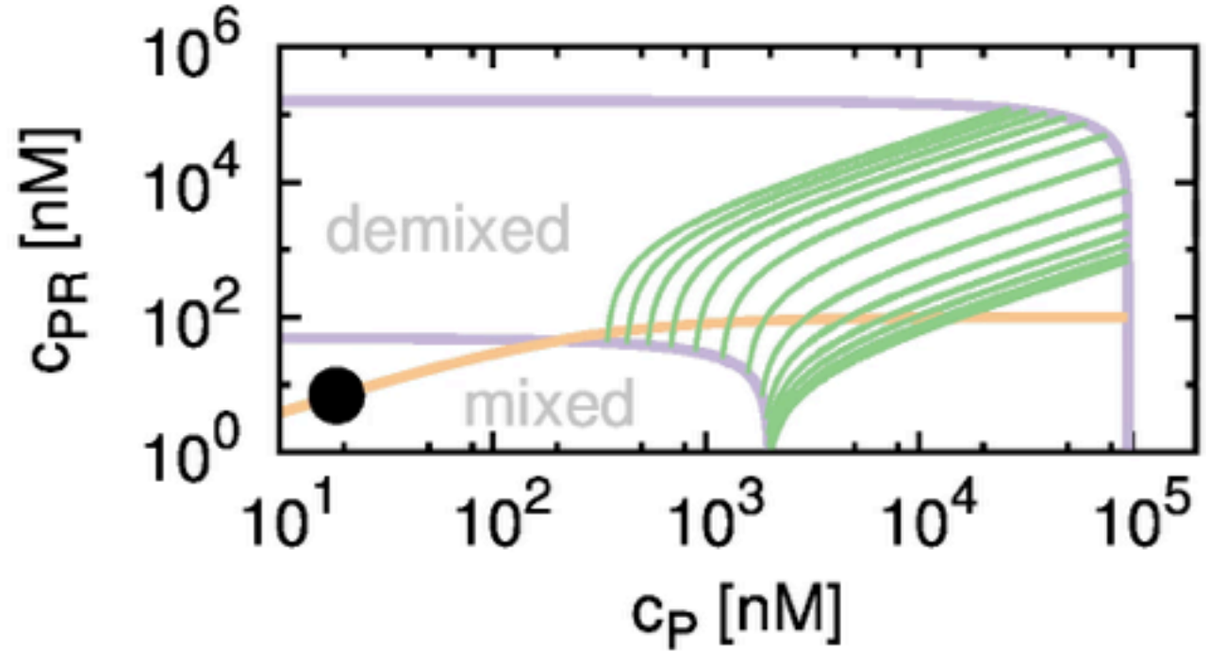
$$M + PR \rightleftharpoons MR + P$$

and binding constant are know from experiments

# determination of interaction parameters

consider PGL-3 and mRNA only

$$P + R \rightleftharpoons PR$$




with Omar Adame

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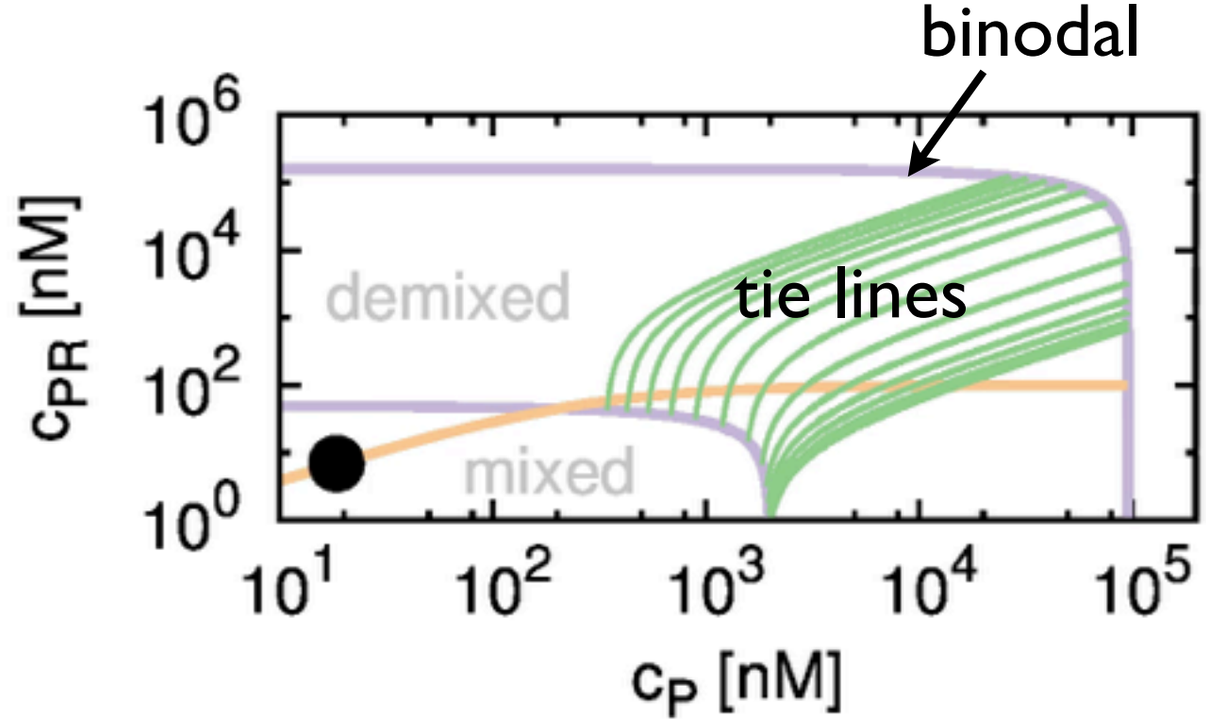


$$c_{PR} \simeq \frac{c_R^{\mathrm{T}}}{1 + \frac{K_{PR}}{c_P}}$$

with Omar Adame

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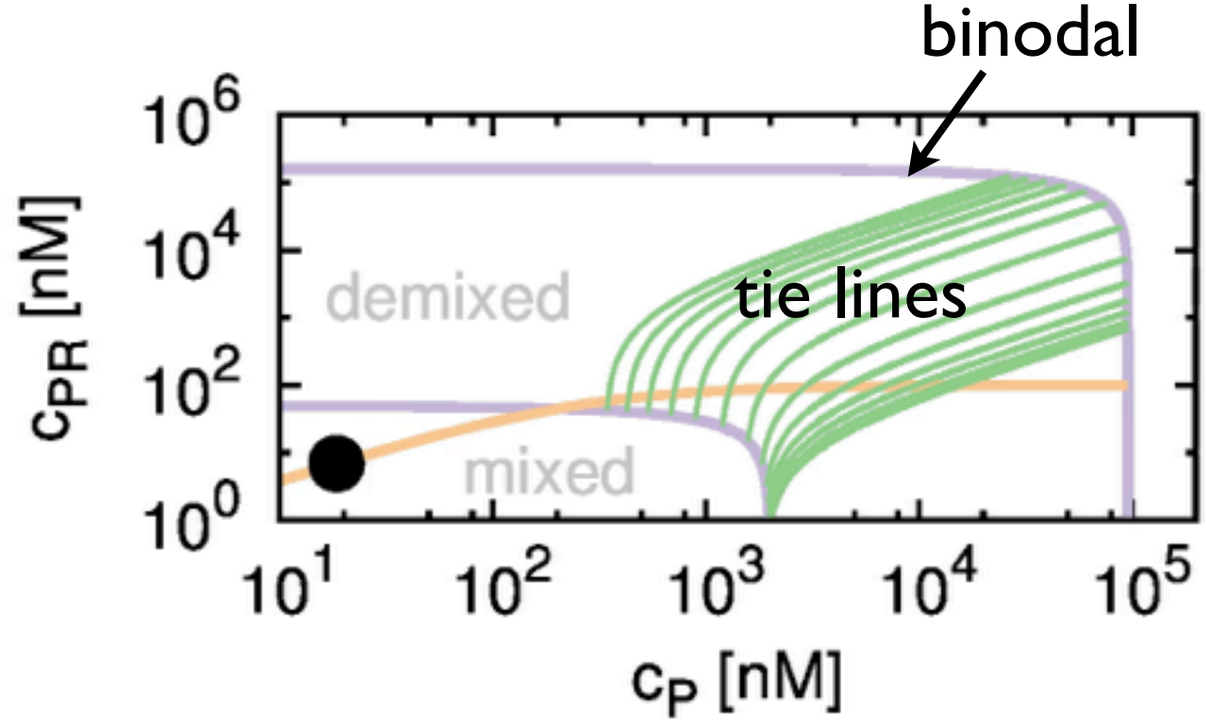


$$\Delta I = I_0(c_P^{in} - c_P^{out})$$


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with Omar Adame

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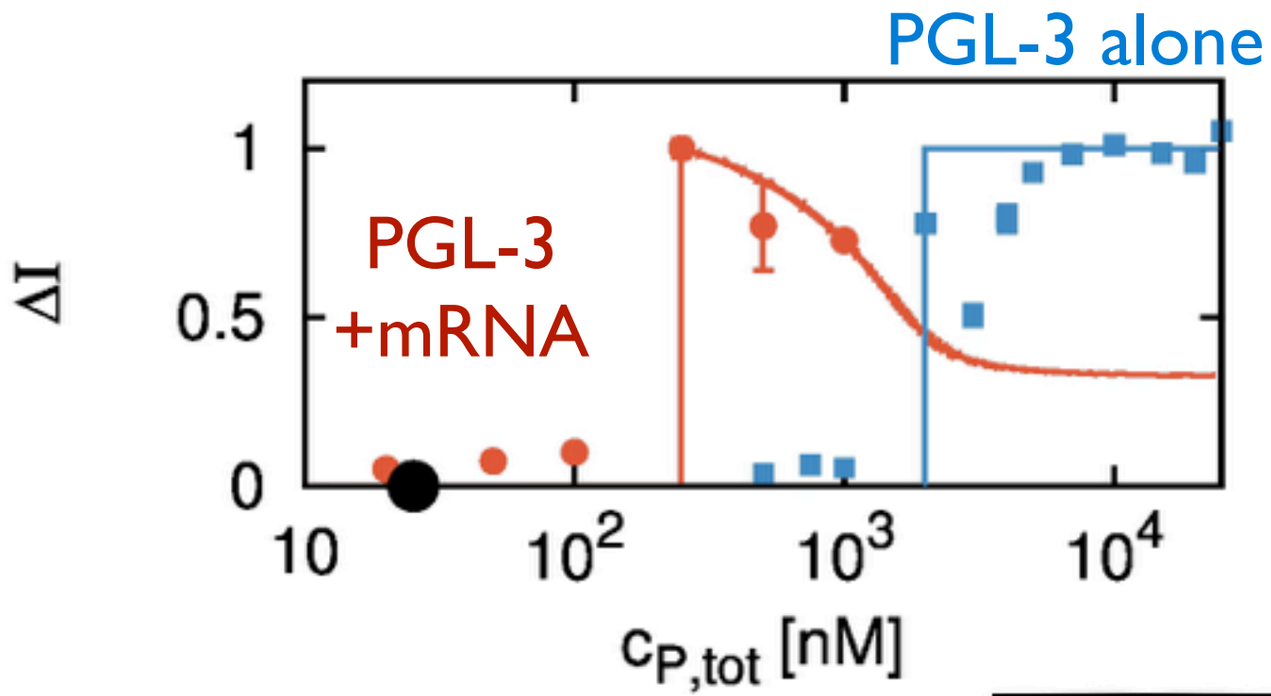


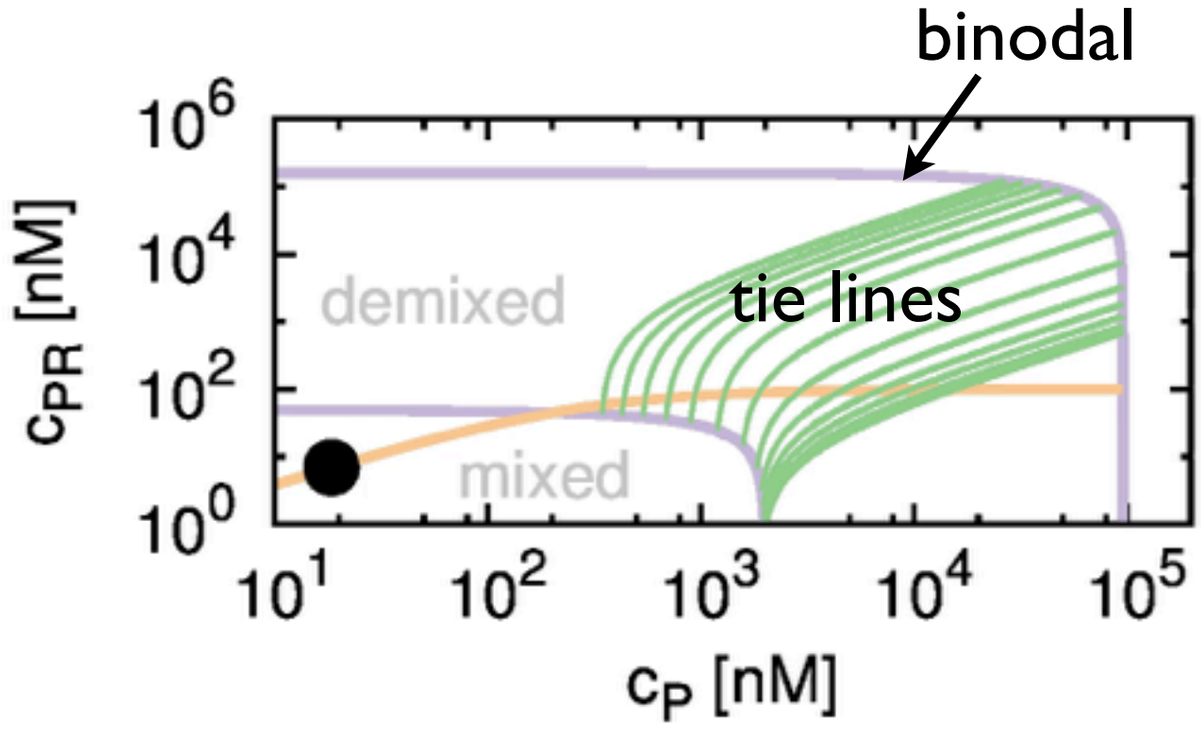


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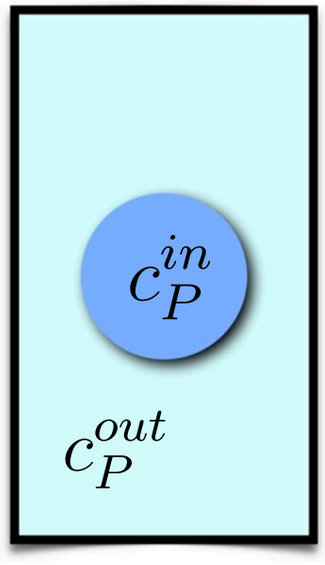


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with Omar Adame

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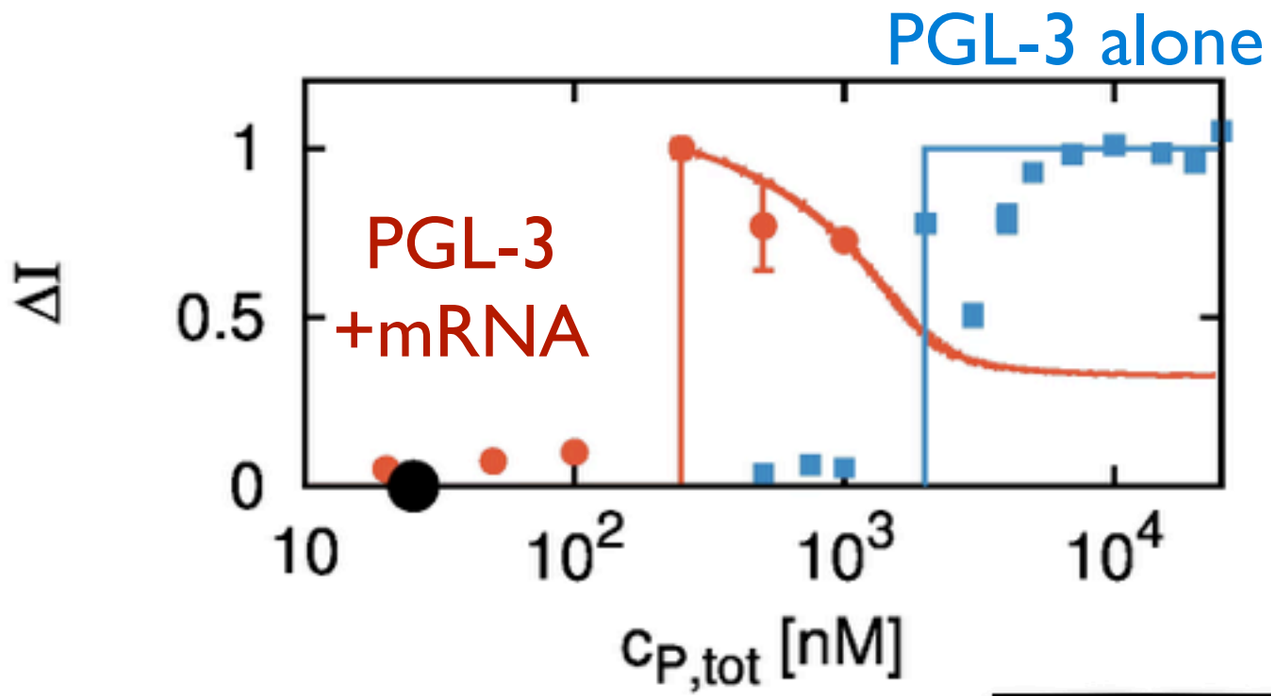


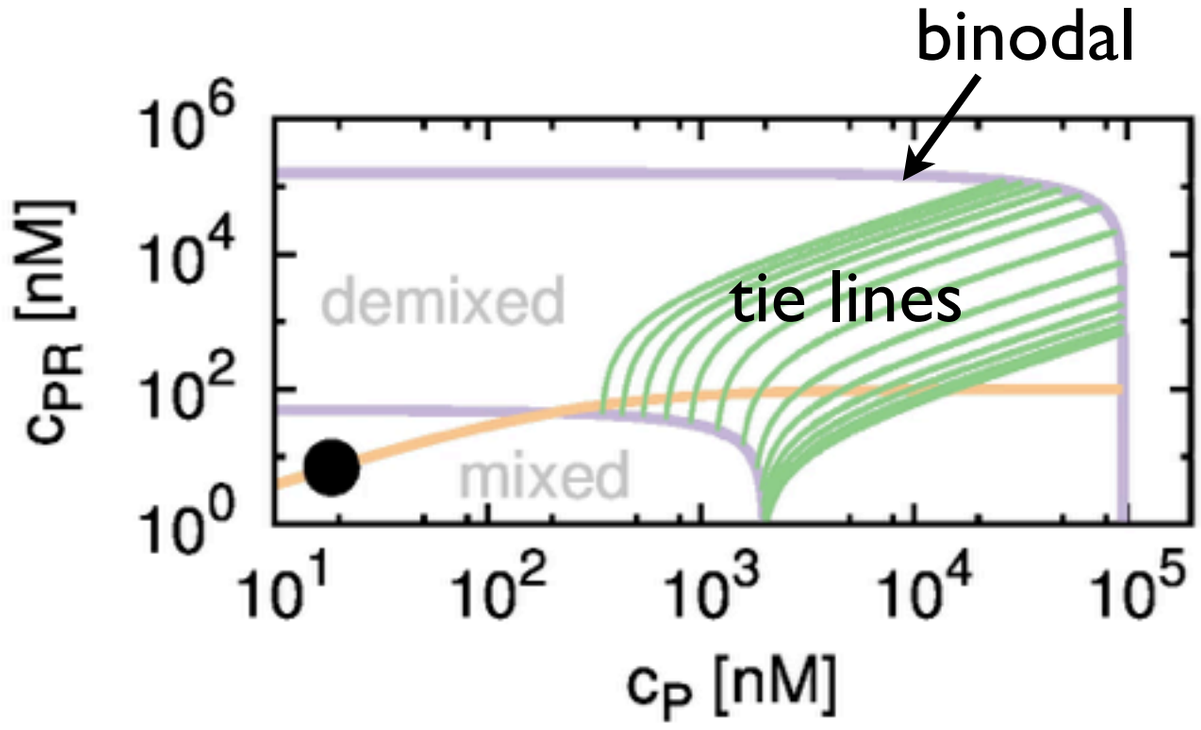


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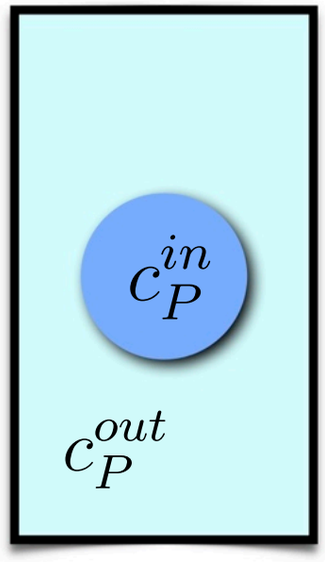


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with Omar Adame

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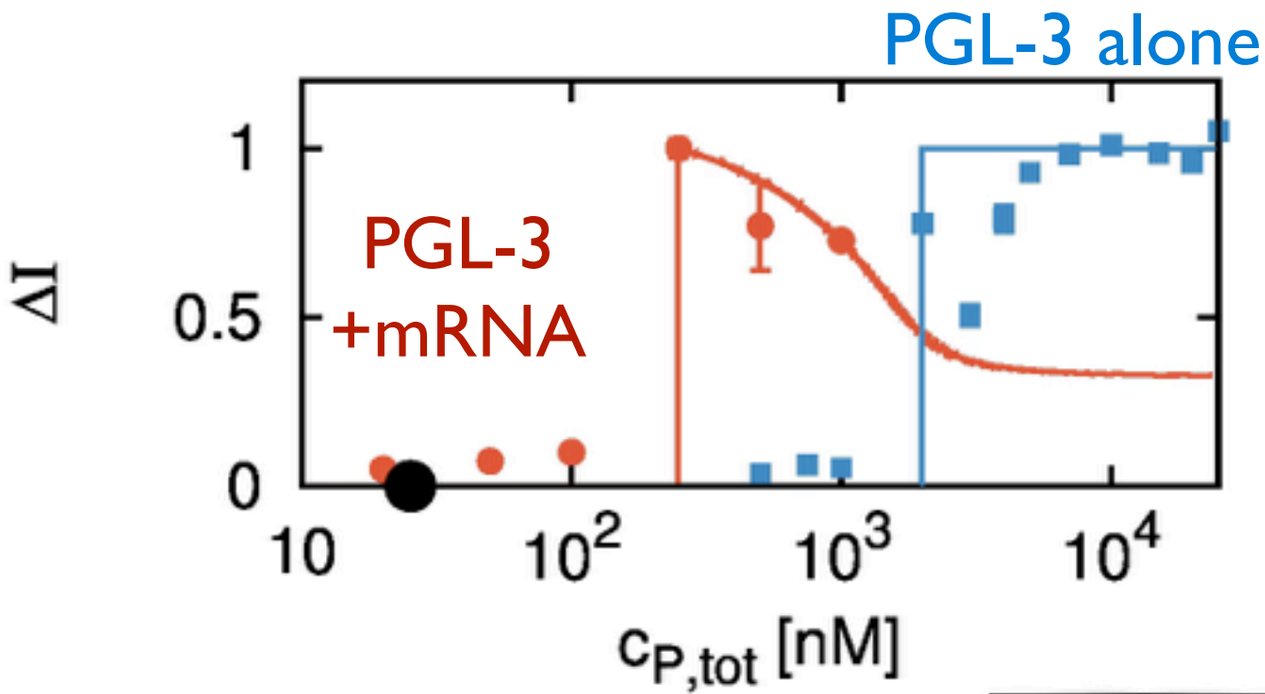


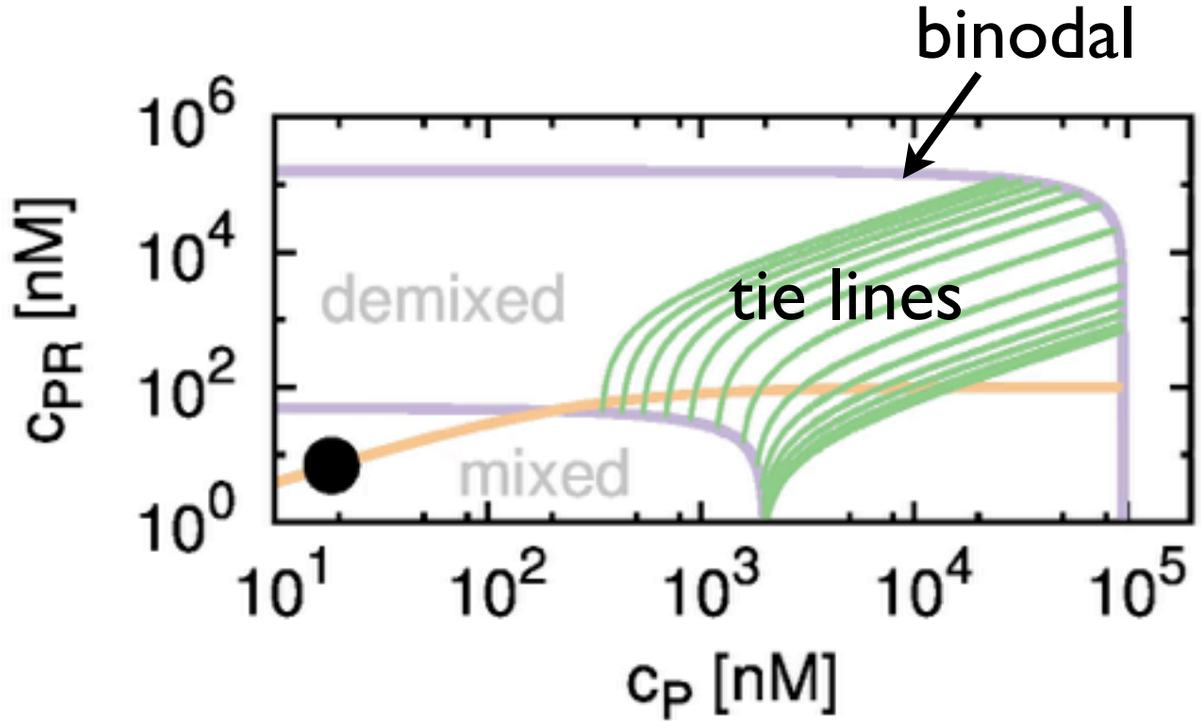


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$$c_{PR} \simeq \frac{c_R^{\mathrm{T}}}{1 + \frac{K_{PR}}{c_P}}$$

→ interaction parameters

with Omar Adame

## <u>test of hypothesis:</u>

## Can competition of MEX-5 and PGL-3 about mRNA explain P granule positioning in C.elegans?

**<u>test of hypothesis:</u>**

**Can competition of MEX-5 and PGL-3 about mRNA explain P granule positioning in C.elegans?**

**--> dynamic equations**

**<u>test of hypothesis:</u>**

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$$f \rightarrow f + \frac{1}{2}\kappa_P |\nabla \phi_P|^2 + \frac{1}{2}\kappa_{PR} |\nabla \phi_{PR}|^2$$

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$$\partial_t \phi_i = \nabla \cdot (\gamma_i \nabla \mu_i) + J_i$$

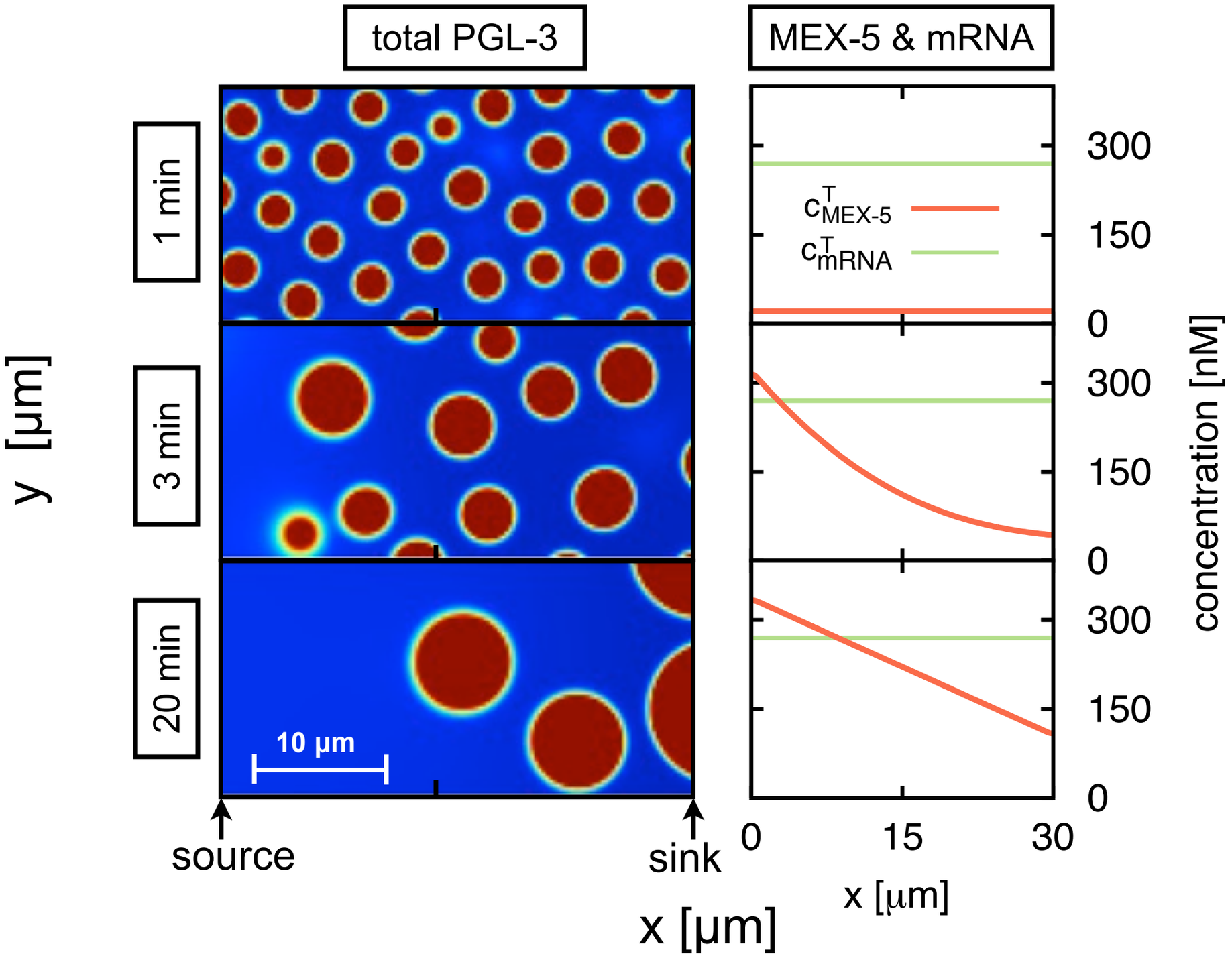
$$\mu_i / \nu_i = \frac{\partial f}{\partial \phi_i} - \partial_\alpha \frac{\partial f}{\partial \partial_\alpha \phi_i}$$

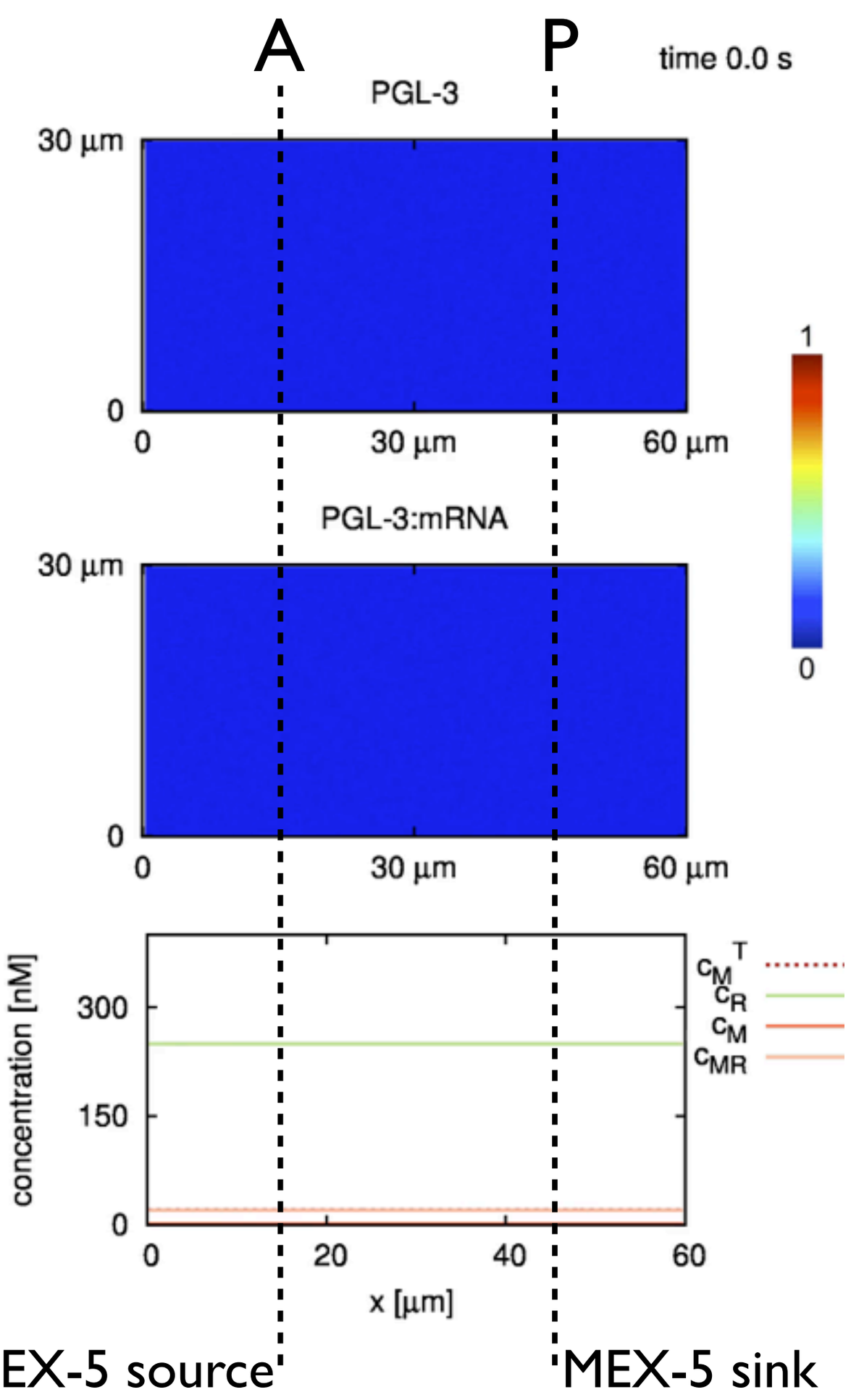
## <u>test of hypothesis:</u>

## Can competition of MEX-5 and PGL-3 about mRNA explain P granule positioning in C.elegans?

# test of hypothesis:

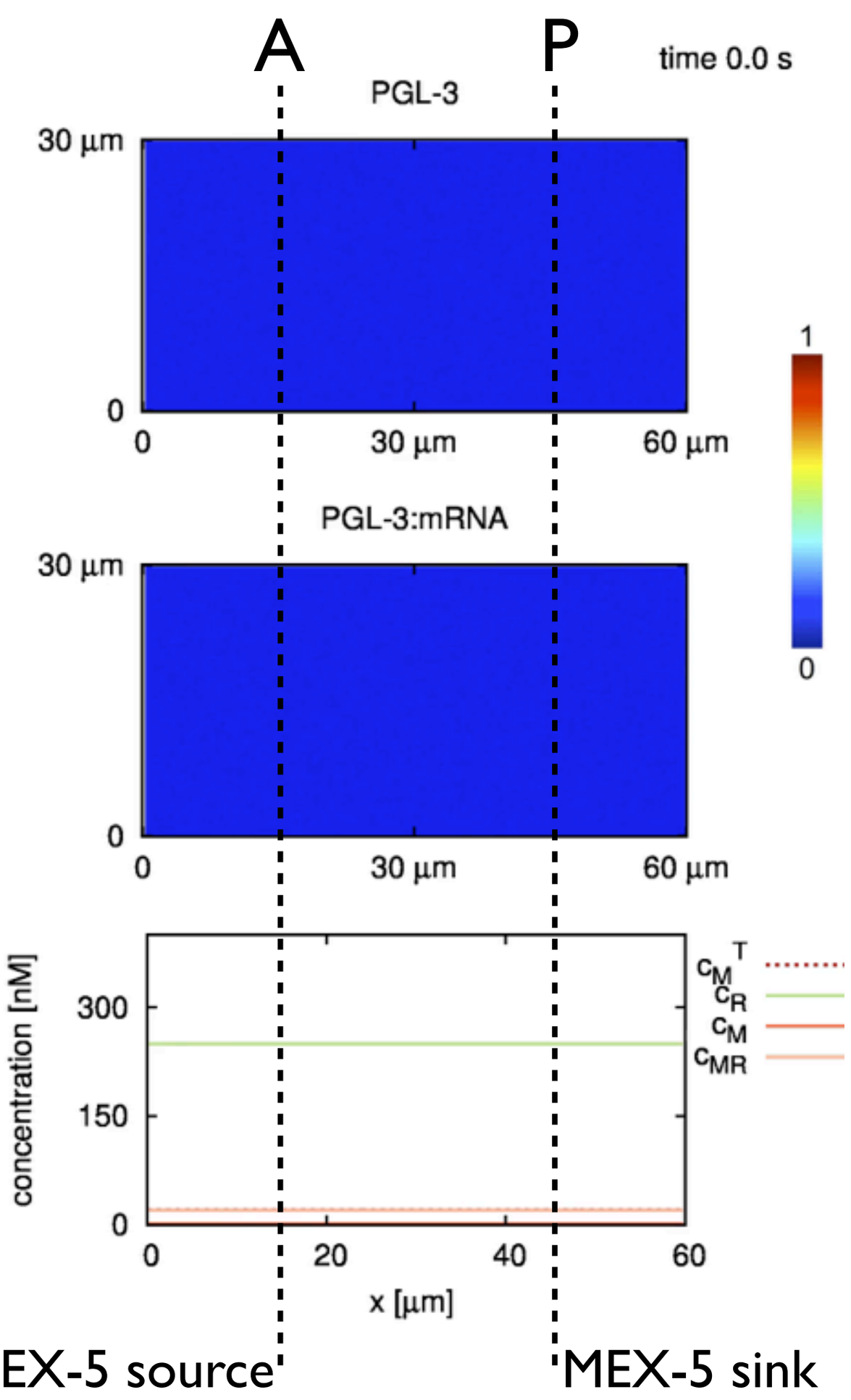
# Can competition of MEX-5 and PGL-3 about mRNA explain P granule positioning in C.elegans?





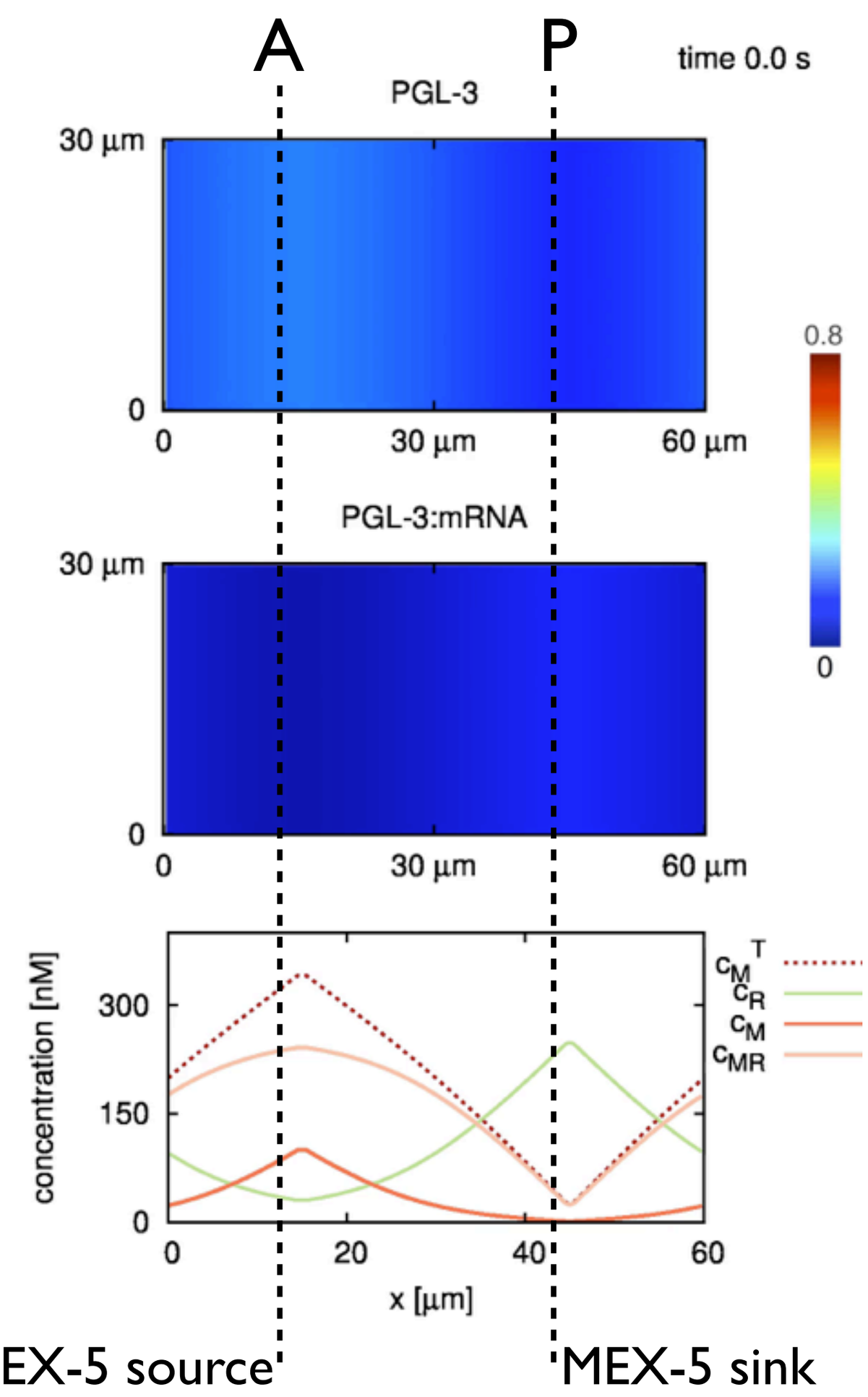

MEX-5 source

MEX-5 sink




MEX-5 source

MEX-5 sink


A
P
time 0.0 s
PGL-3
30 µm
0
0
30 µm
60 µm
0.8
0
PGL-3:mRNA
30 µm
0
0
30 µm
60 µm
concentration [nM]
300
150
0
0
20
40
60
x [µm]
$c_M^T$
$c_R$
$c_M$
$c_{MR}$
MEX-5 source
MEX-5 sink

# Summary:

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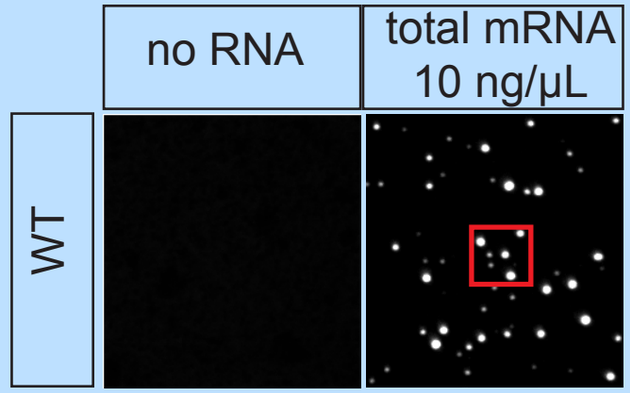
in-vitro: mRNA supports drop formation of PGL-3



# Summary:

in-vitro: mRNA supports drop formation of PGL-3

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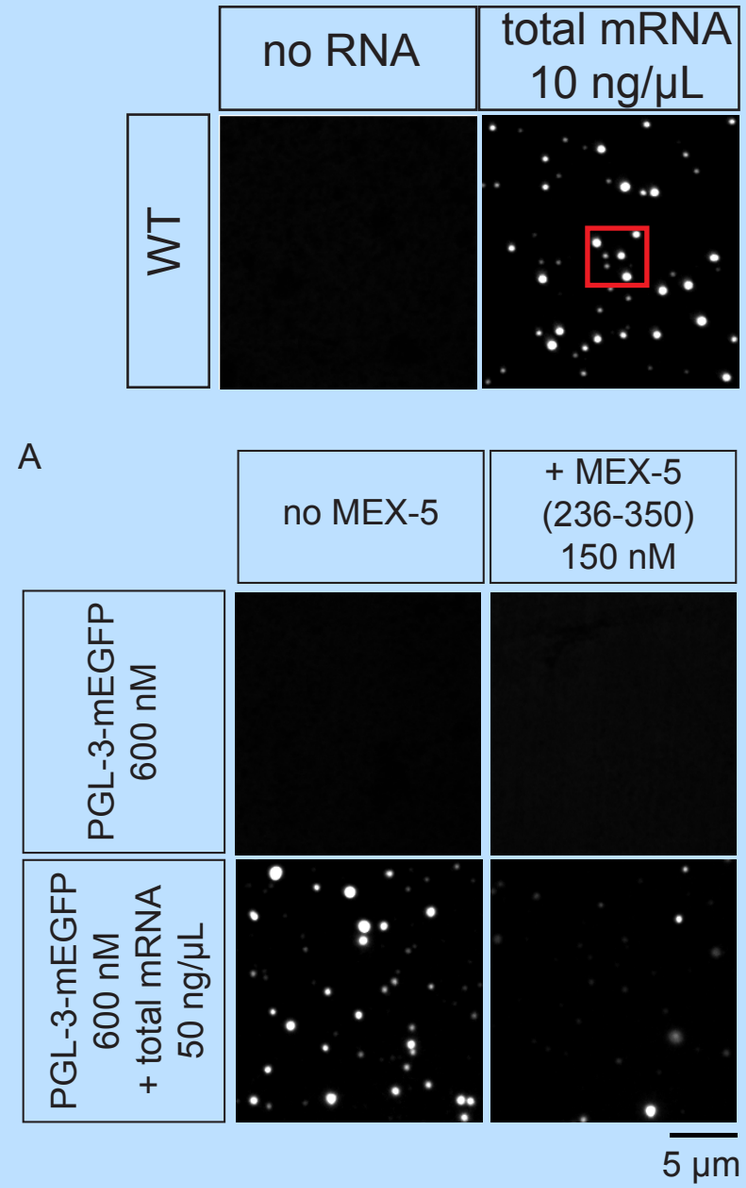
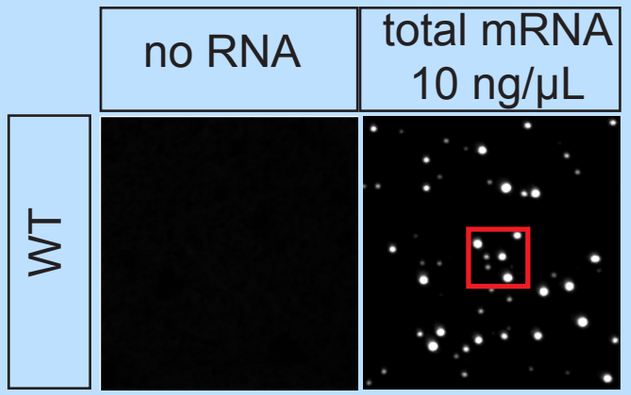


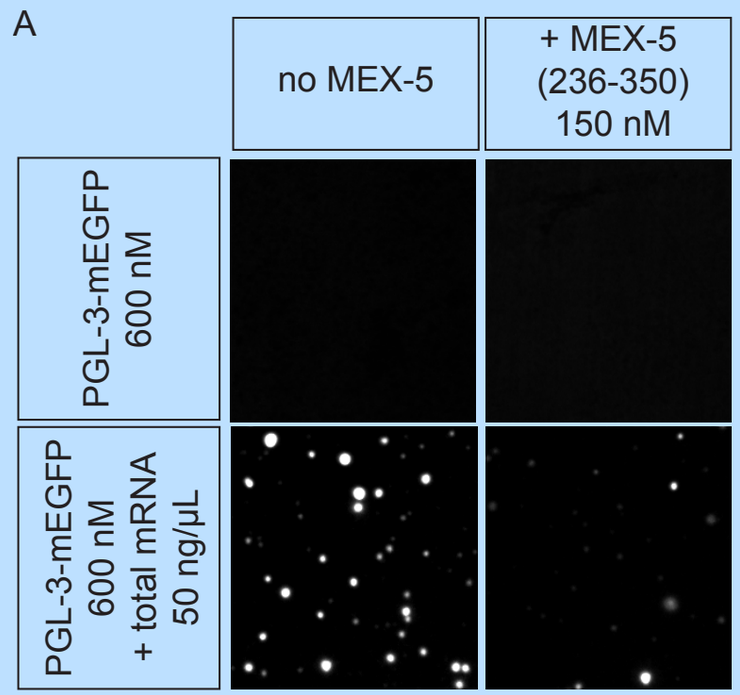
# Summary:

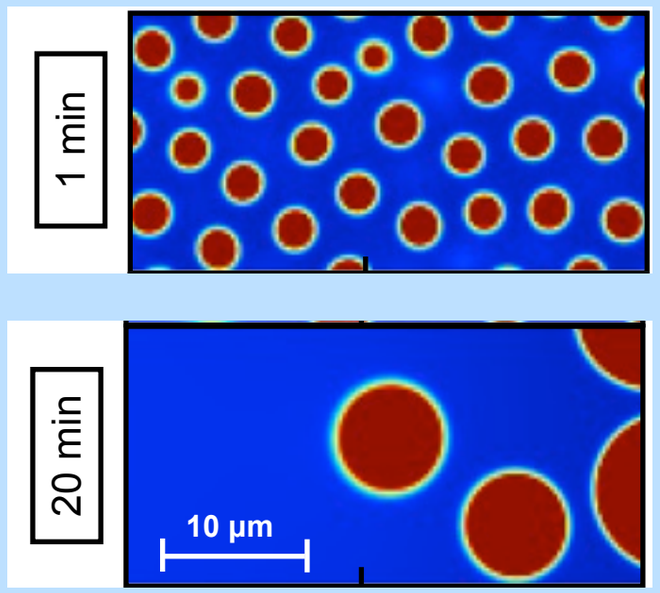
in-vitro: mRNA supports drop formation of PGL-3

in-vitro: Mex-5 and PGL-3 compete about mRNA and thereby MEX-5 can dissolve PGL-3 drops

model: competition about mRNA can explain the positioning of PGL-3 drops in a MEX-5 gradient, a candidate for P granule positioning in C. elegans?



# Thanks for listening.

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