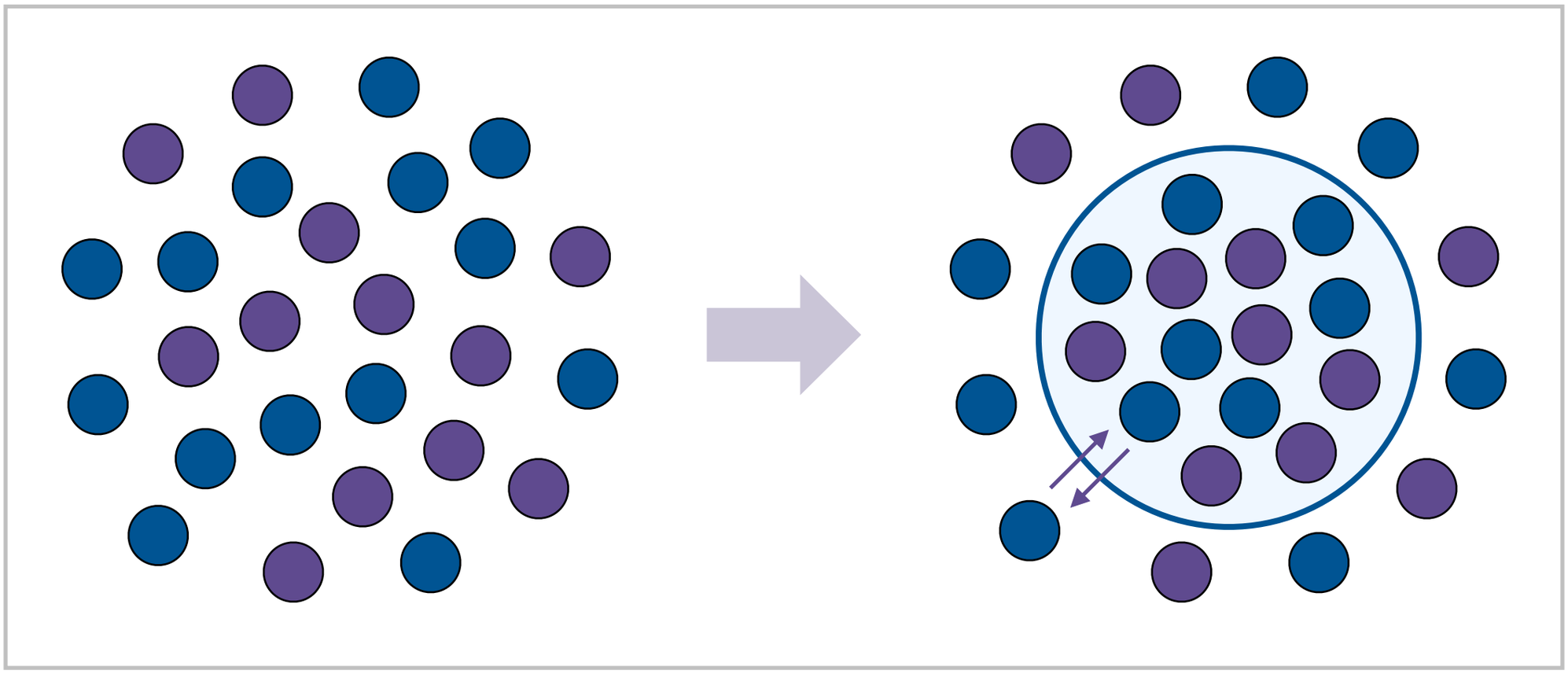
Liquid–Liquid Phase Separation in Biology



Benito Buksh

MacMillan Research Group

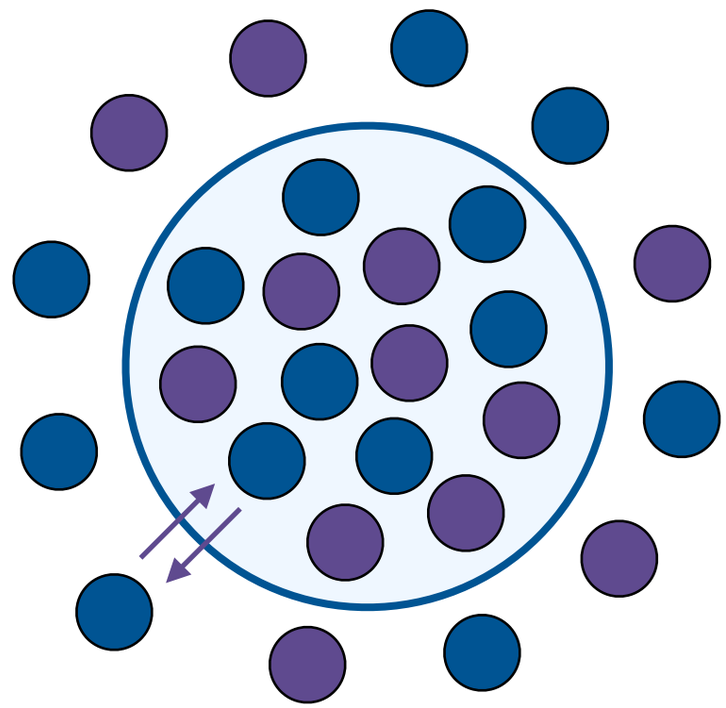
Group Meeting

December 2nd, 2020

Liquid–Liquid Phase Separation in Biology

outline

- Introduction to liquid–liquid phase separation (LLPS)
- The first observation of phase separation in cells
- Physiological functions of phase separation
  - Organization
  - Promote reactions
  - Sequestration
- Aberrant phase separation can cause disease
  - Amyotrophic lateral sclerosis (ALS)
- Conclusion and outlook



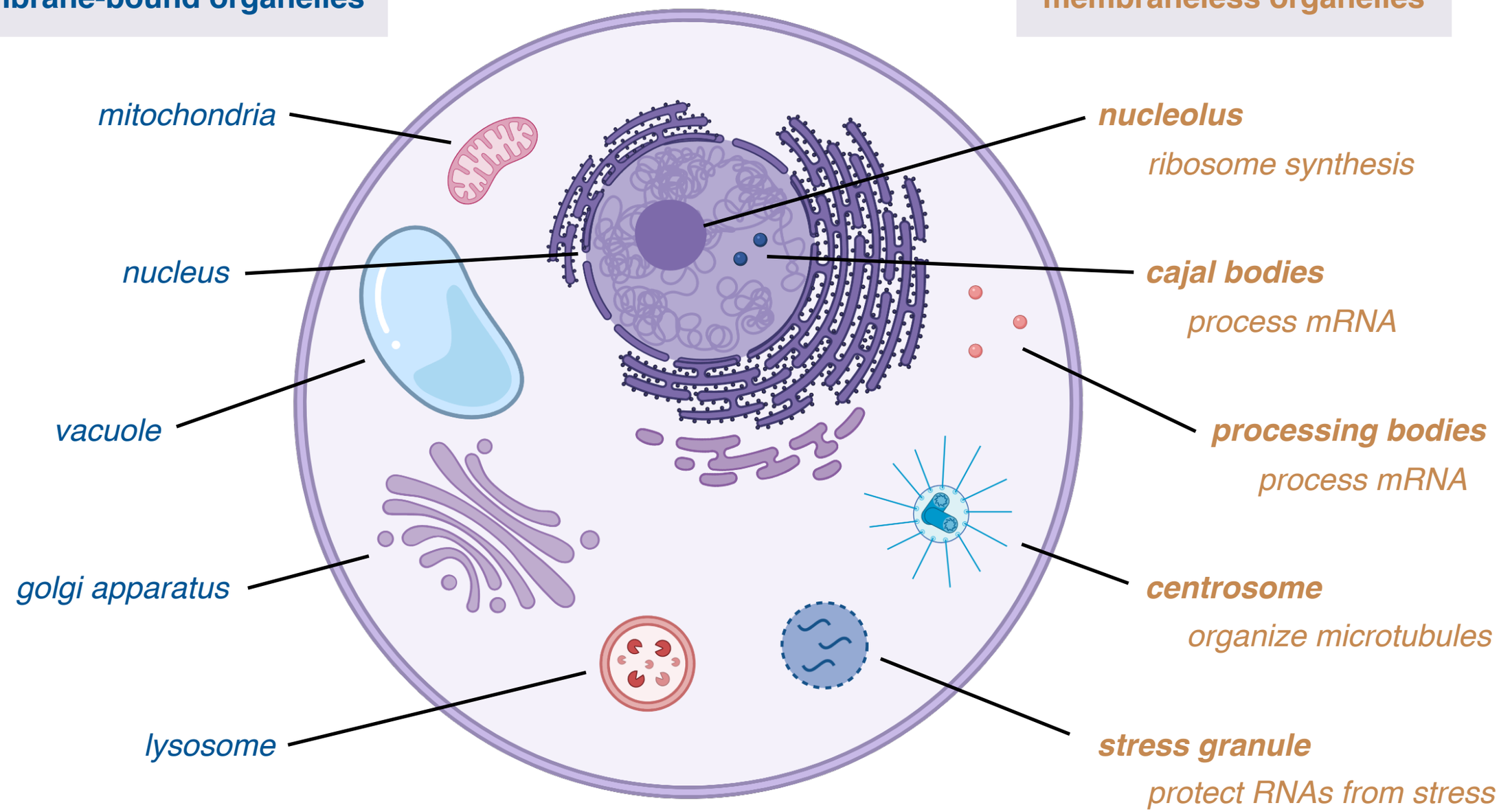
Liquid–Liquid Phase Separation in Biology

introduction

How do cells organize biochemical reactions?

membrane-bound organelles

membraneless organelles



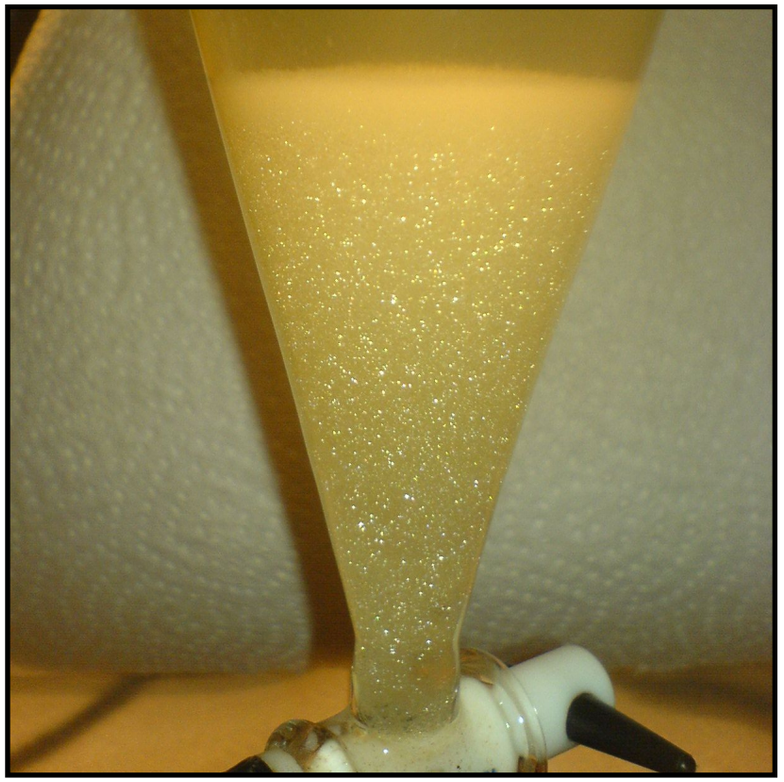
Hyman, A. A.; Weber, C. A.; Jülicher, F. *Annu. Rev. Cell Dev. Biol.* **2014**, *30*, 39.

Liquid–Liquid Phase Separation in Biology

introduction

How to think about membraneless organelles

vinaigrette



organic extraction

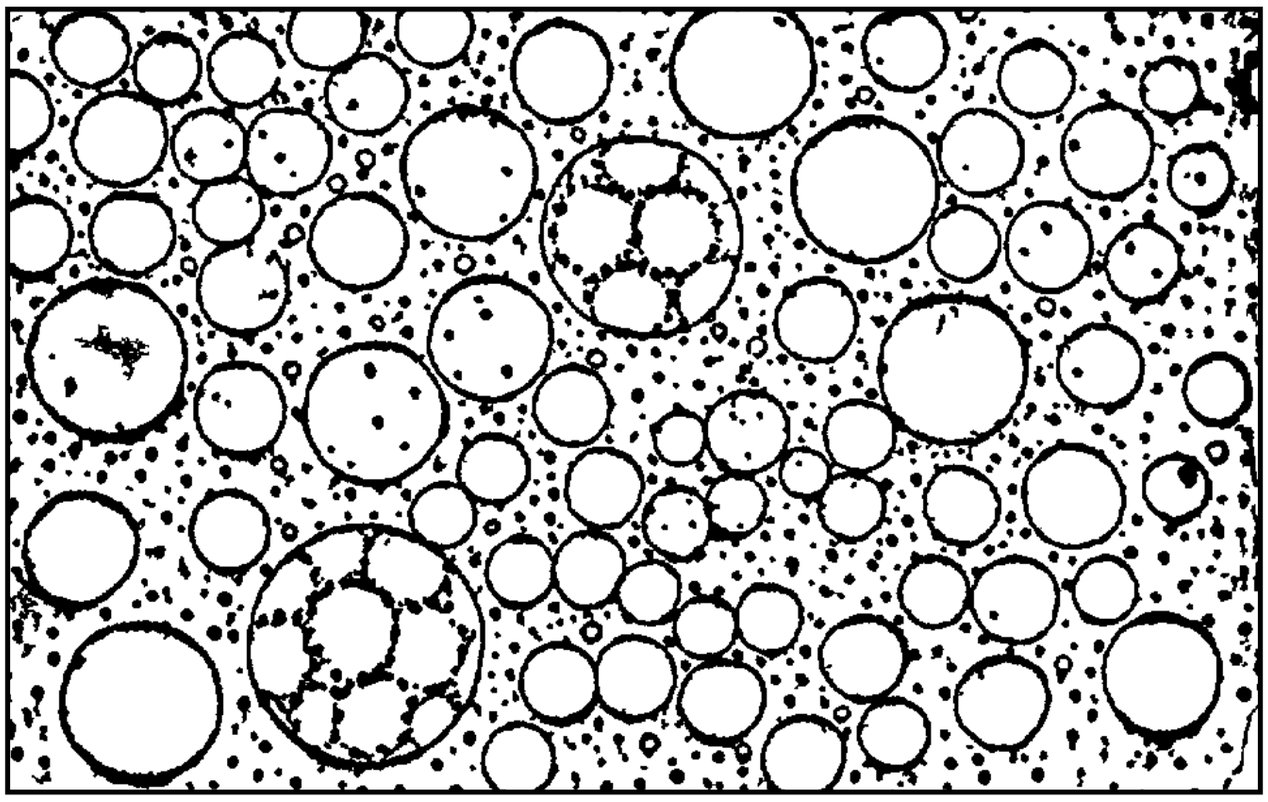
emulsions are driven by physical interactions between similar molecules

Hyman, A. A.; Weber, C. A.; Jülicher, F. *Annu. Rev. Cell Dev. Biol.* **2014**, *30*, 39.

Liquid–Liquid Phase Separation in Biology

introduction

The idea of the cell containing emulsions is over 100 years old



protoplasm from a starfish egg

> "The living protoplasm … is a liquid, or rather a mixture of liquids in the form of a fine emulsion consisting of a continuous substance in which are suspended drops … of different chemical nature."

Wilson, E. B. *Science* **1899**, *10*, 33.

Liquid–Liquid Phase Separation in Biology

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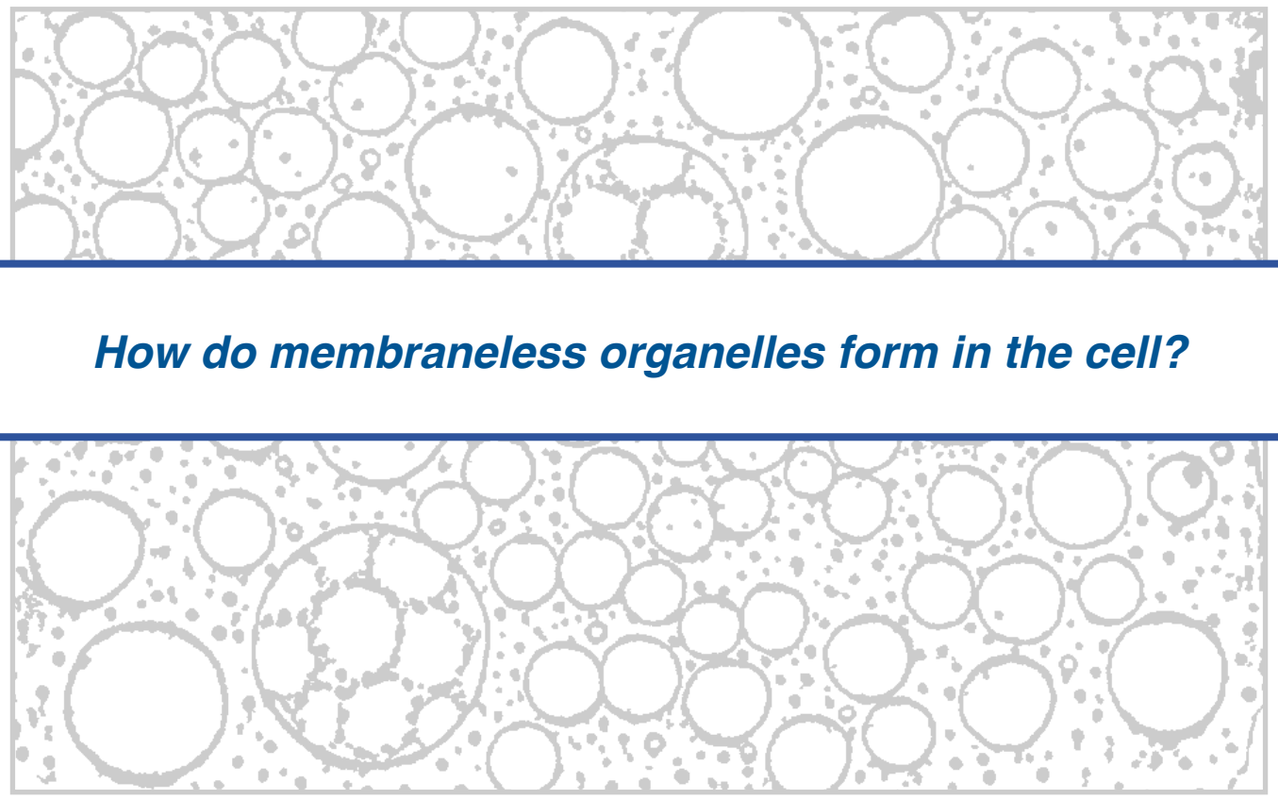

How do membraneless organelles form in the cell?

protoplasm from a starfish egg

"The living protoplasm … is a liquid, or rather a mixture of liquids in the form of a fine emulsion consisting of a continuous substance in which are suspended drops … of different chemical nature."

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Liquid–Liquid Phase Separation in Biology

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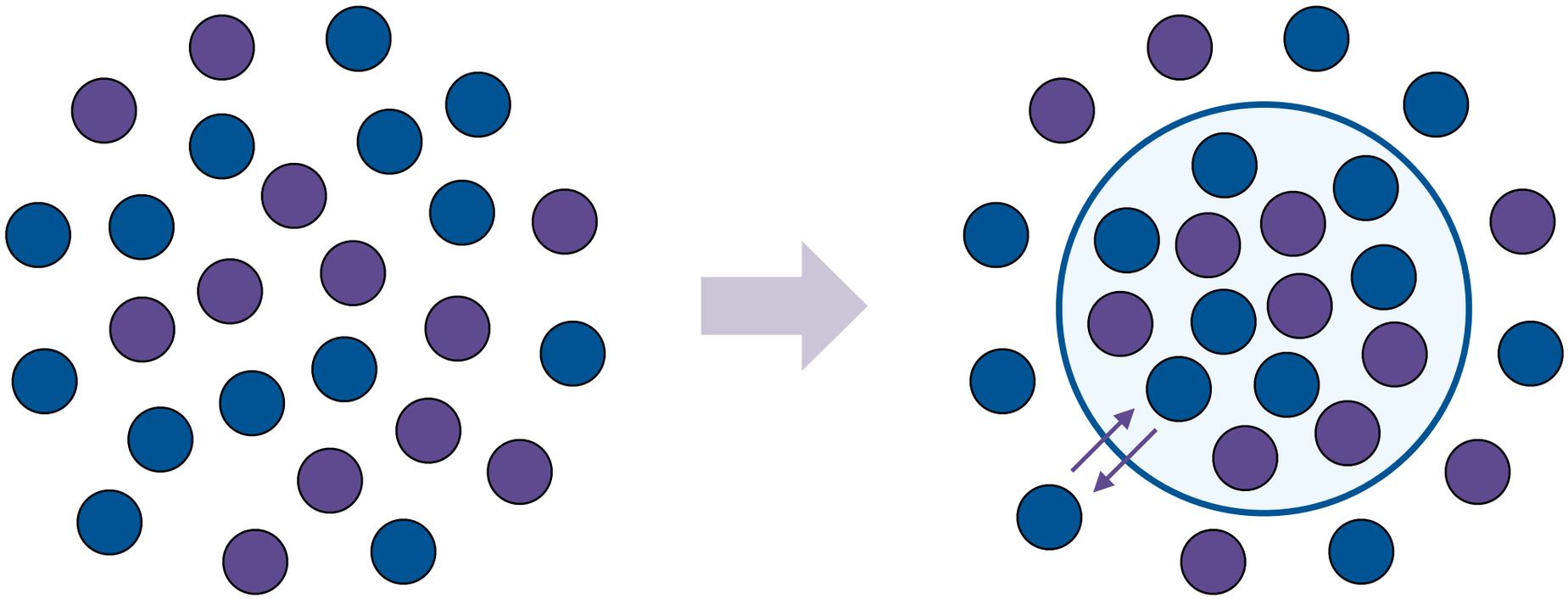
Liquid–Liquid Phase Separation (LLPS)



Definition: *spontaneous process of a homogeneous fluid de-mixing into two distinct liquid phases*

- In the cell, condensates are made up of RNA and protein
- Biomolecules diffuse between phase boundary

Hyman, A. A.; Weber, C. A.; Jülicher, F. *Annu. Rev. Cell Dev. Biol.* **2014**, *30*, 39.

Liquid–Liquid Phase Separation in Biology

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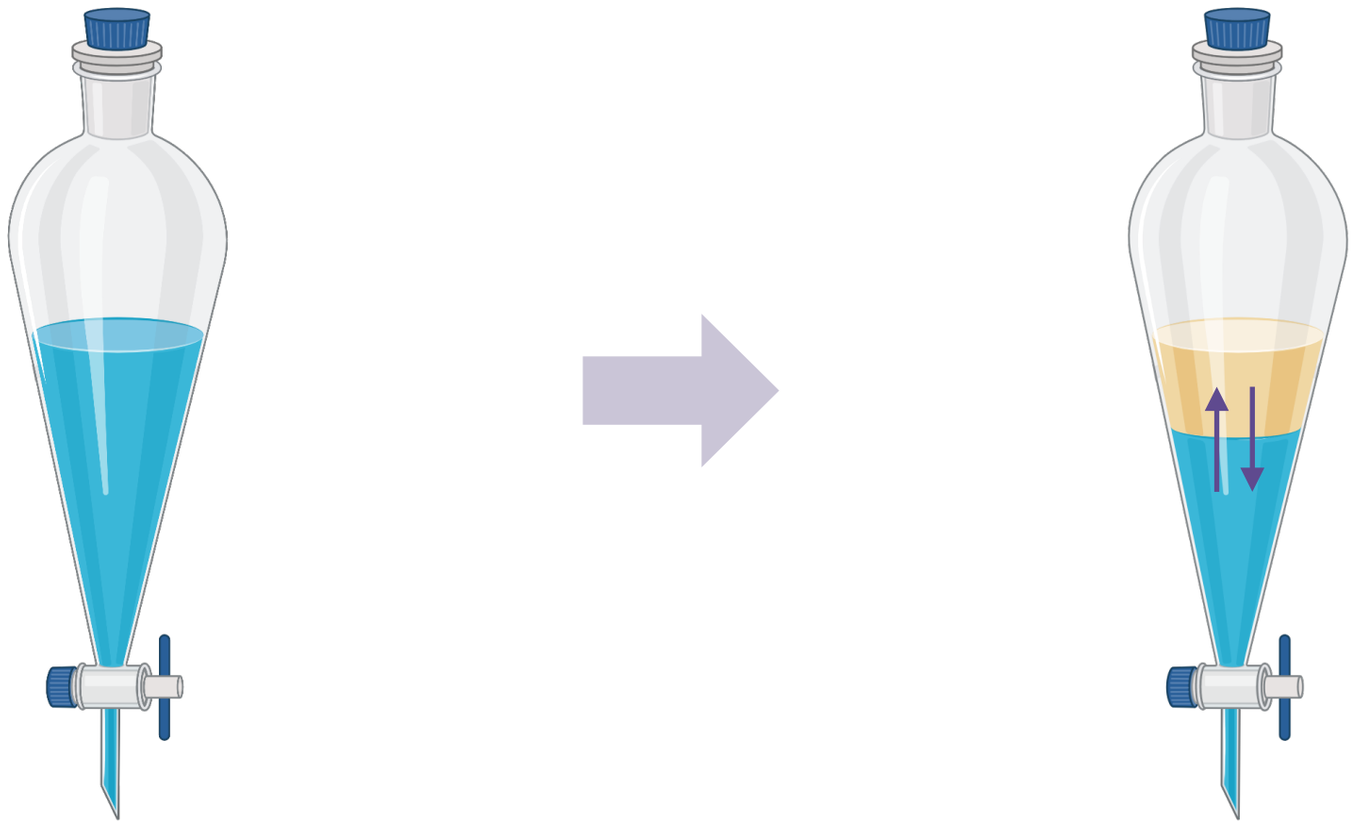

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Liquid–Liquid Phase Separation in Biology

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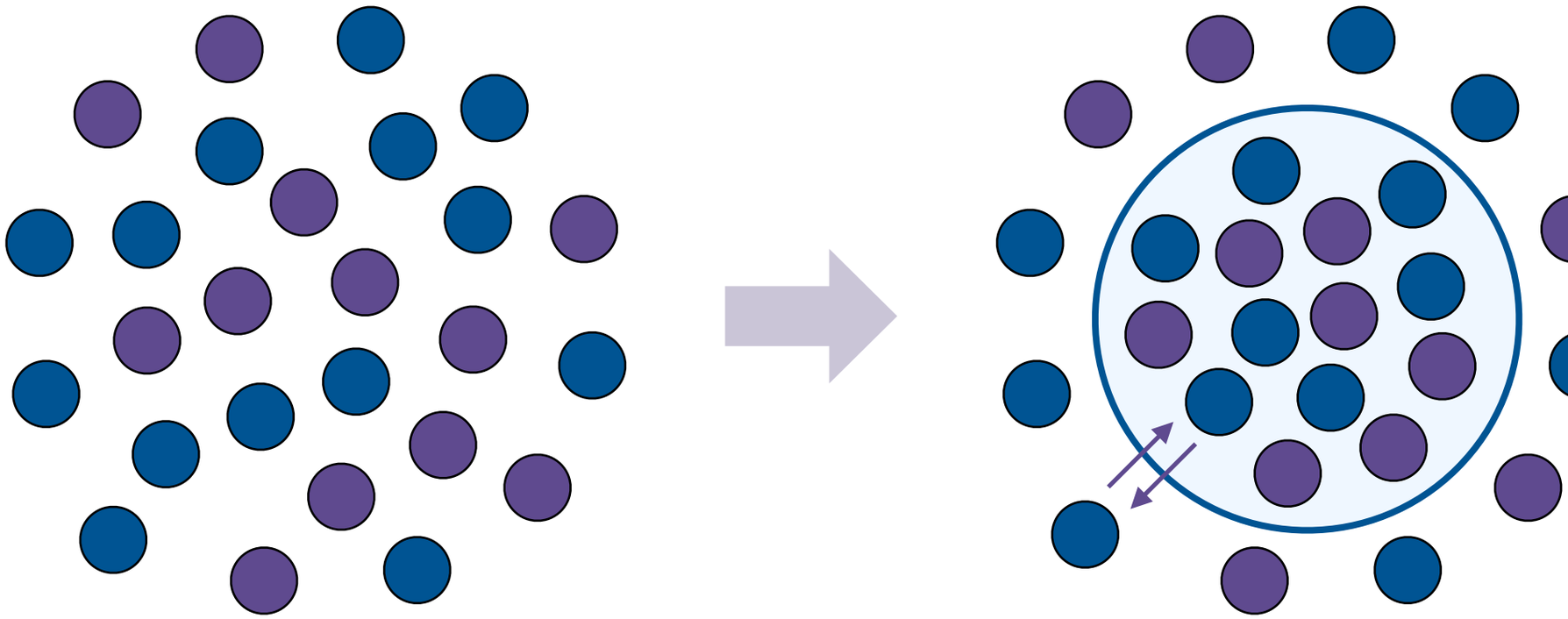
What drives liquid–liquid phase separation?



mixed phase

higher entropy

phase separated

lower entropy

entropy prefers a mixed state

Hyman, A. A.; Weber, C. A.; Jülicher, F. *Annu. Rev. Cell Dev. Biol.* **2014**, *30*, 39.

Liquid–Liquid Phase Separation in Biology

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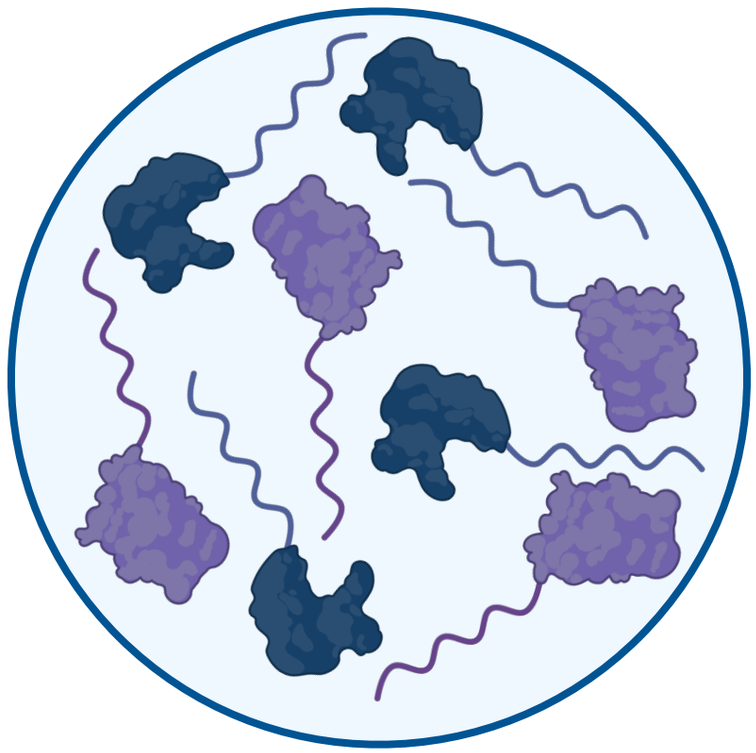
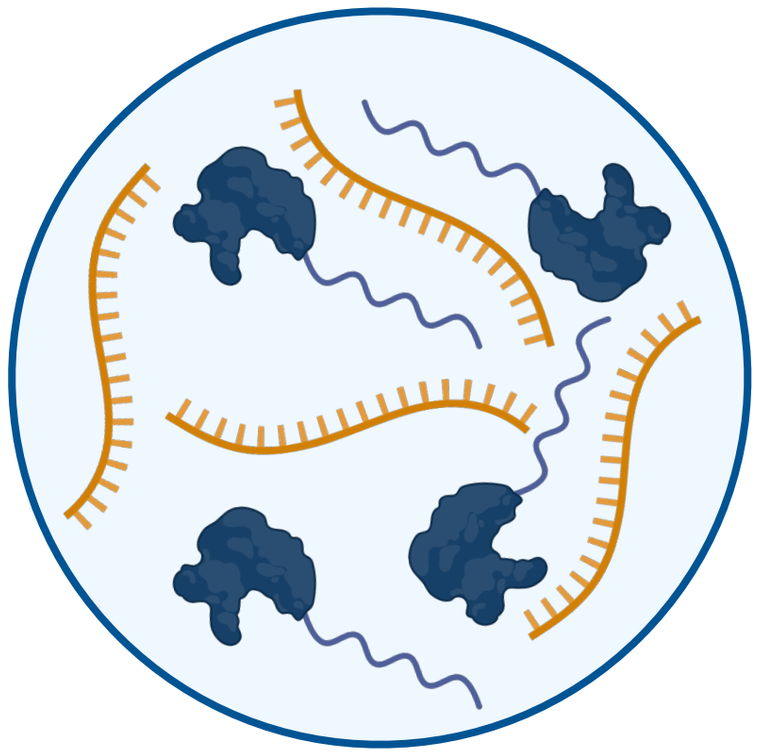
What drives liquid–liquid phase separation?

protein-protein interactions



RNA-protein interactions



enthalpic intermolecular interactions drive liquid–liquid phase separation

Hyman, A. A.; Weber, C. A.; Jülicher, F. *Annu. Rev. Cell Dev. Biol.* **2014**, *30*, 39.

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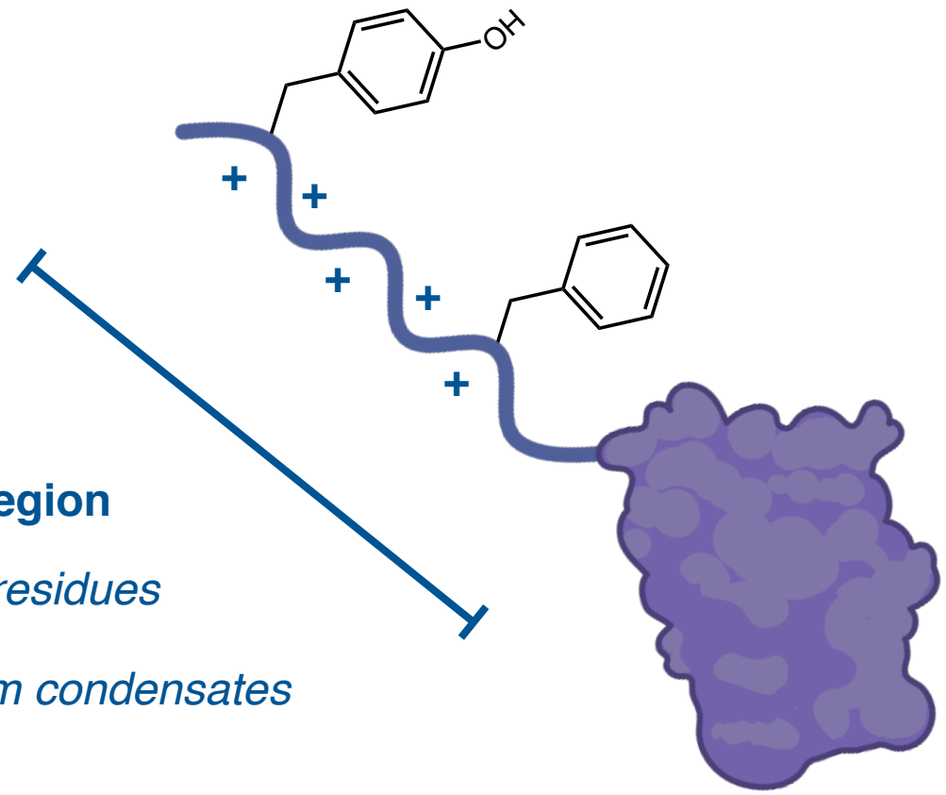

intrinsically disordered region

site of positive and aromatic residues

found frequently in proteins that form condensates

intrinsically disordered regions (IDRs) enable intermolecular charge-charge, charge-π, and π-π interactions

Hyman, A. A.; Weber, C. A.; Jülicher, F. *Annu. Rev. Cell Dev. Biol.* **2014**, *30*, 39.

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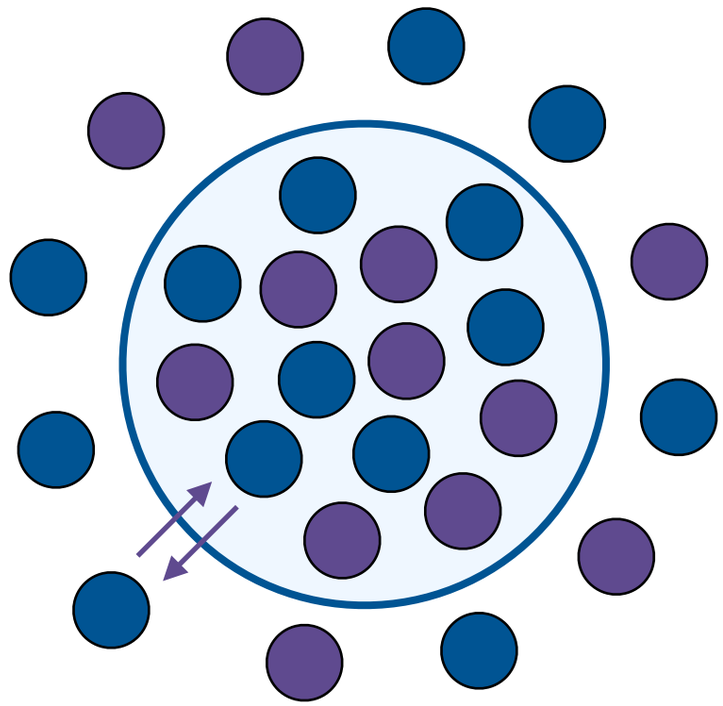
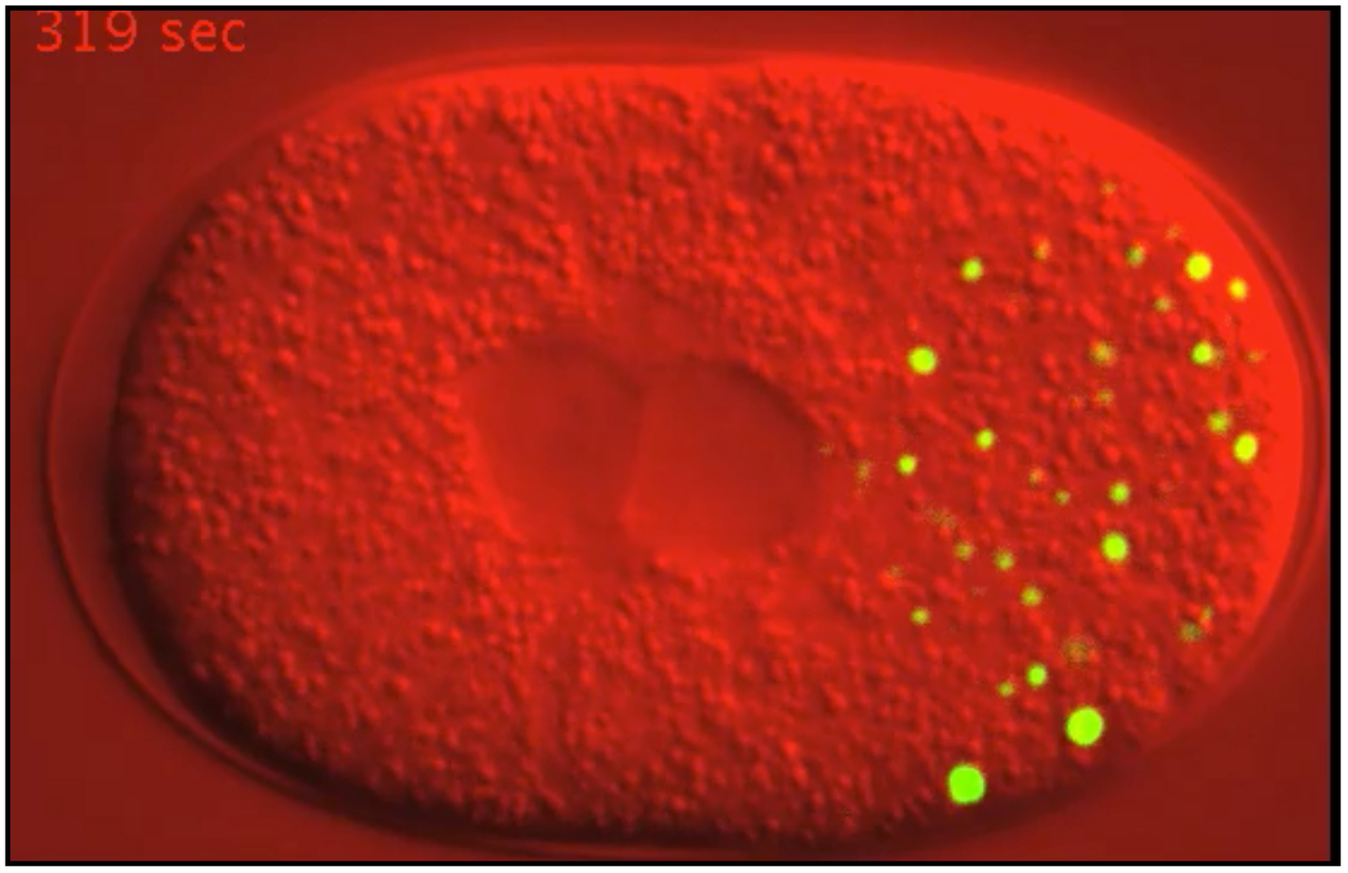


Liquid–Liquid Phase Separation in Biology

the first observation of phase separation in cells

P granules segregate to posterior end of dividing C. elegans embryo



Anterior (A)

Posterior (P)

● *P granules tagged with green fluorescent protein (GFP)*

Brangwynne, C. P. et al. *Science* **2009**, *324*, 1729.

Liquid–Liquid Phase Separation in Biology

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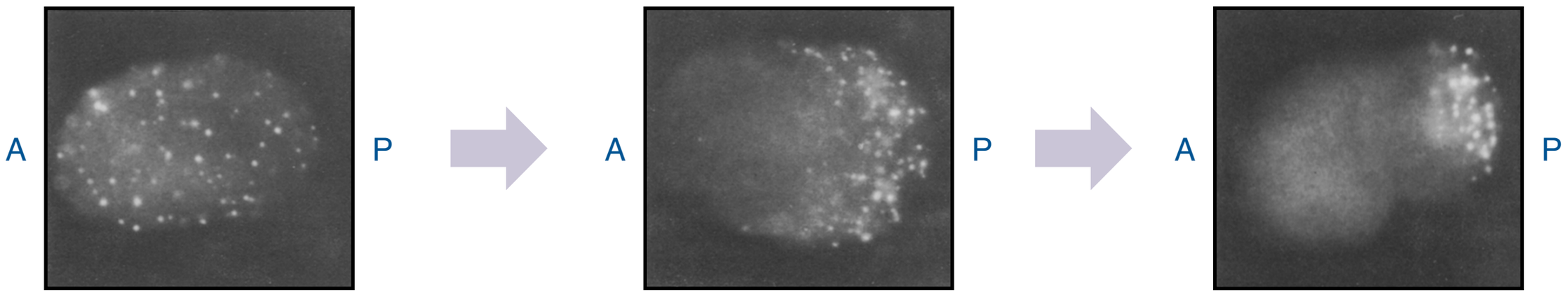


A = anterior P = posterior

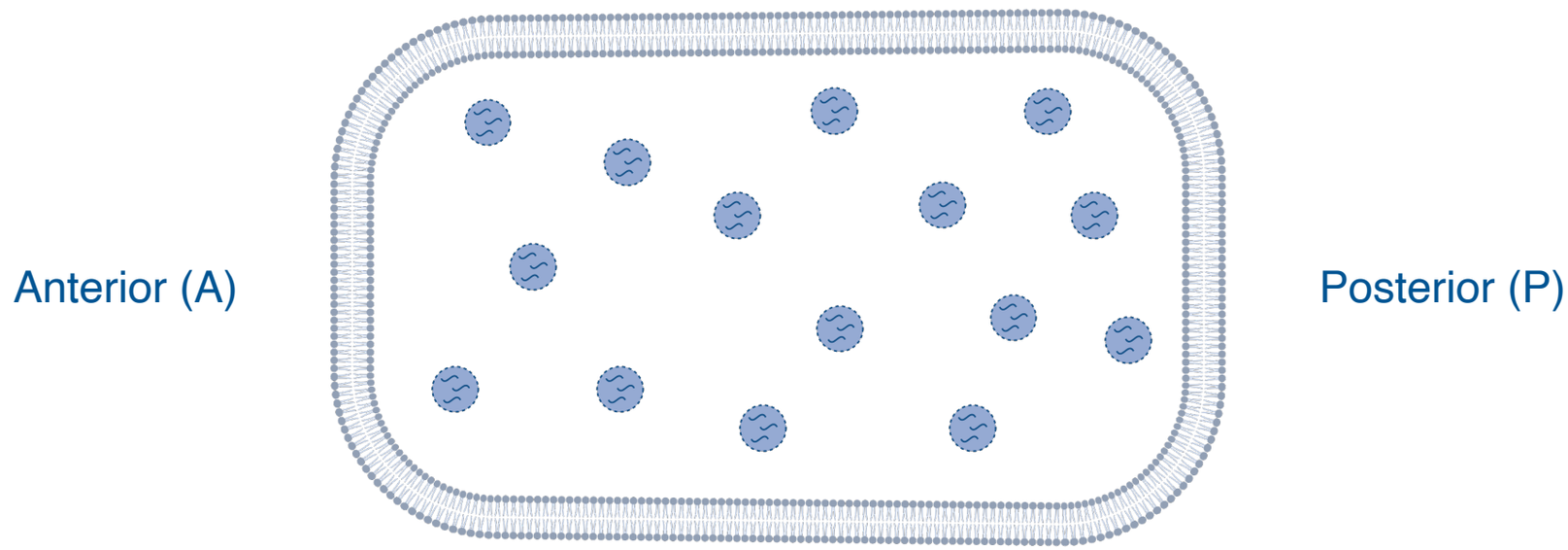
How do P granules localize to the posterior end during division?

Strome, S.; Wood, W. B. *Cell* **1983**, *35*, 15.

Liquid–Liquid Phase Separation in Biology

the first observation of phase separation in cells

P granules segregate to posterior end of dividing C. elegans embryo



P granules are evenly distributed

Brangwynne, C. P. et al. *Science* **2009**, *324*, 1729.

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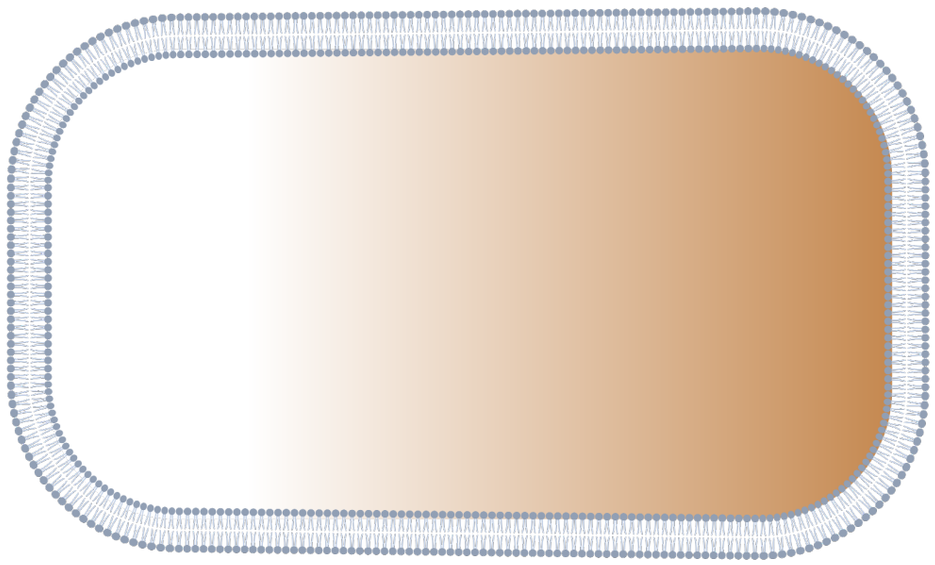
gradient of PAR-1 protein



Anterior (A)

Posterior (P)

increasing PAR-1

PAR-1 concentrates in the posterior of the cell

Brangwynne, C. P. et al. *Science* **2009**, *324*, 1729.

Liquid–Liquid Phase Separation in Biology

the first observation of phase separation in cells

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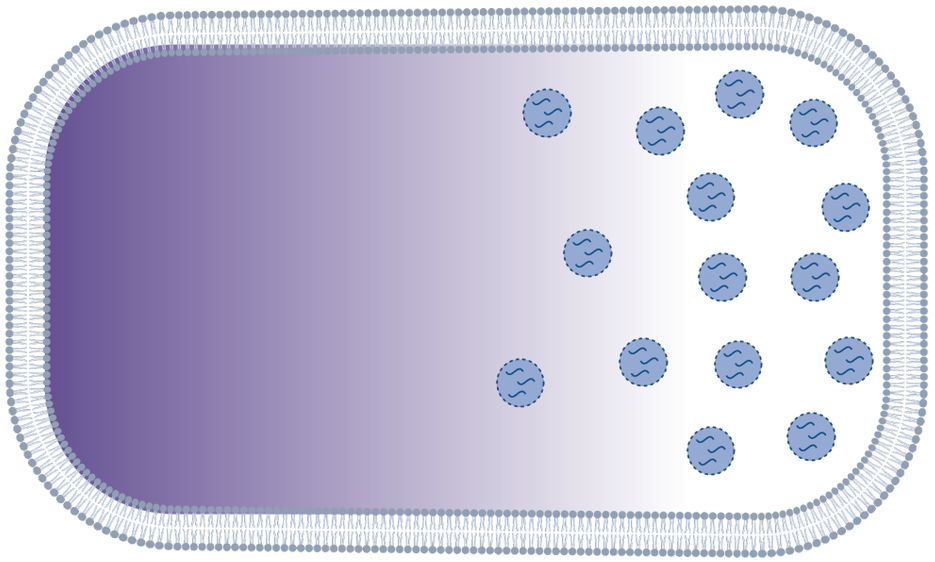
gradient of MEX-5 protein



Anterior (A)

Posterior (P)

increasing MEX-5

PAR-1 reduces MEX-5 in the posterior

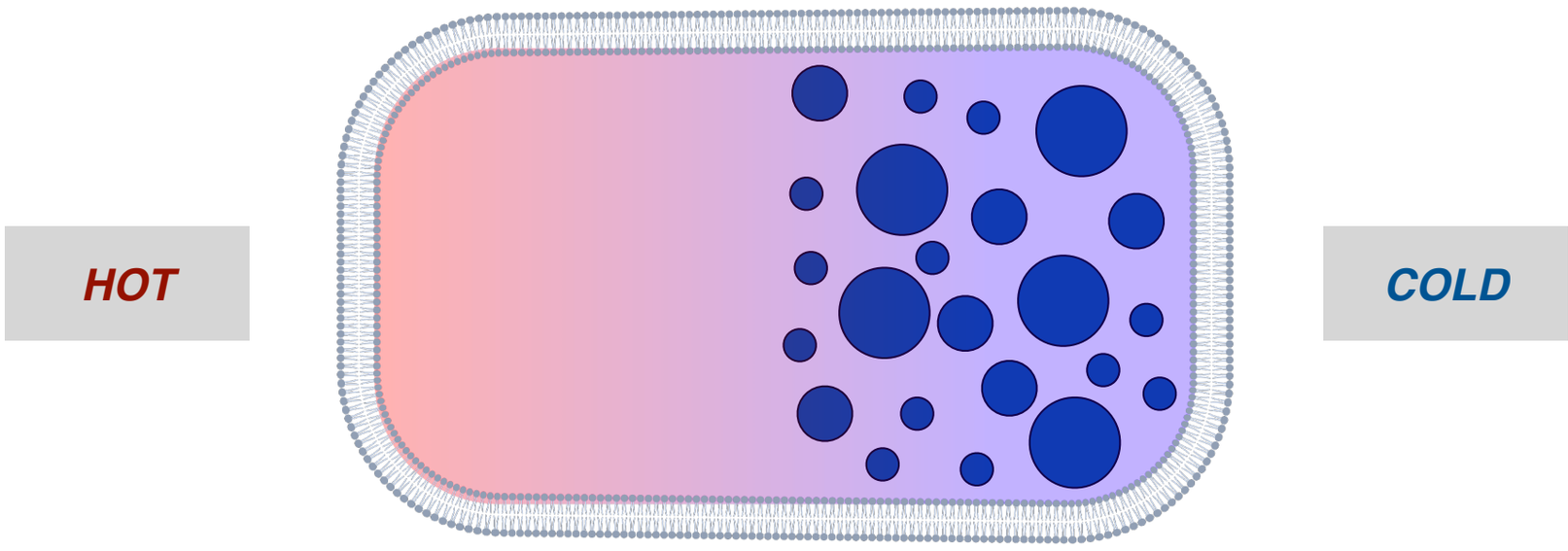
high MEX-5 levels correlate with P granule dissolution

Brangwynne, C. P. et al. *Science* **2009**, *324*, 1729.

Liquid–Liquid Phase Separation in Biology

the first observation of phase separation in cells

P granules segregate to posterior end of dividing C. elegans embryo



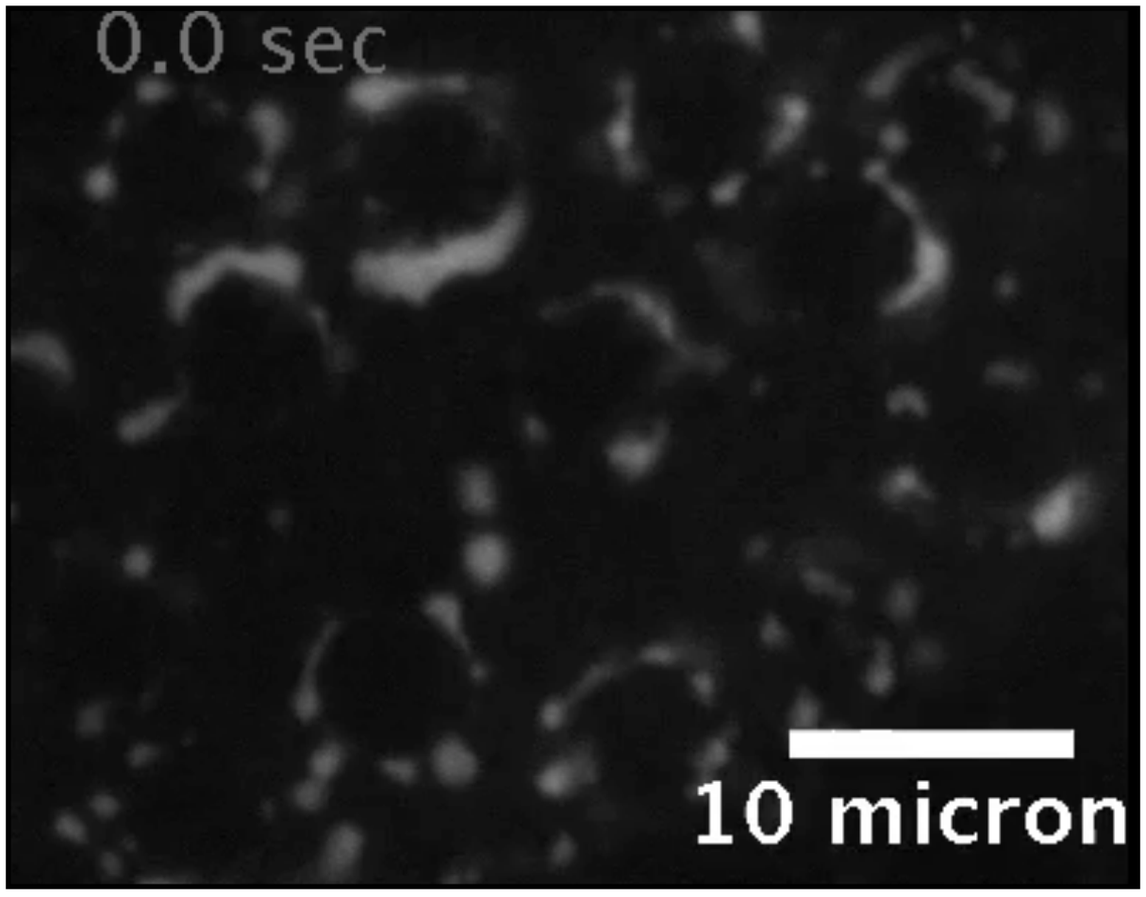
gradient of phase separation is analogous to condensing of water

Brangwynne, C. P. et al. *Science* **2009**, *324*, 1729.

Liquid–Liquid Phase Separation in Biology

the first observation of phase separation in cells

P granules behave like droplets



shear stress applied to nuclei

droplets flow off nuclei, drip, and often fuse into larger drops

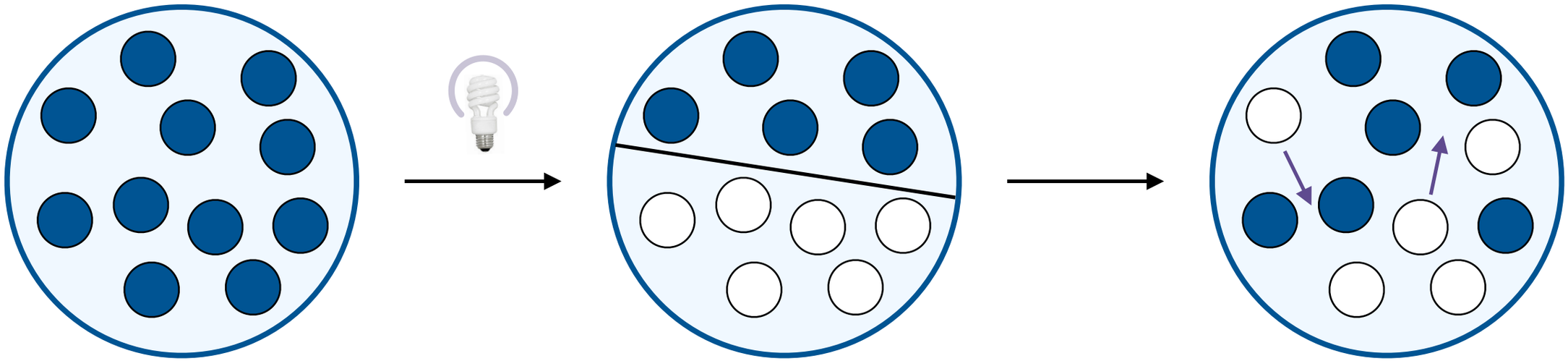
Brangwynne, C. P. et al. *Science* **2009**, *324*, 1729.

Liquid–Liquid Phase Separation in Biology

the first observation of phase separation in cells

P granules behave like droplets

Fluorescence Recovery After Photobleaching (FRAP)



= fluorescent

fraction of fluorophores bleached

fluorescence recovery (diffusion)

Brangwynne, C. P. et al. *Science* **2009**, *324*, 1729.

Liquid–Liquid Phase Separation in Biology

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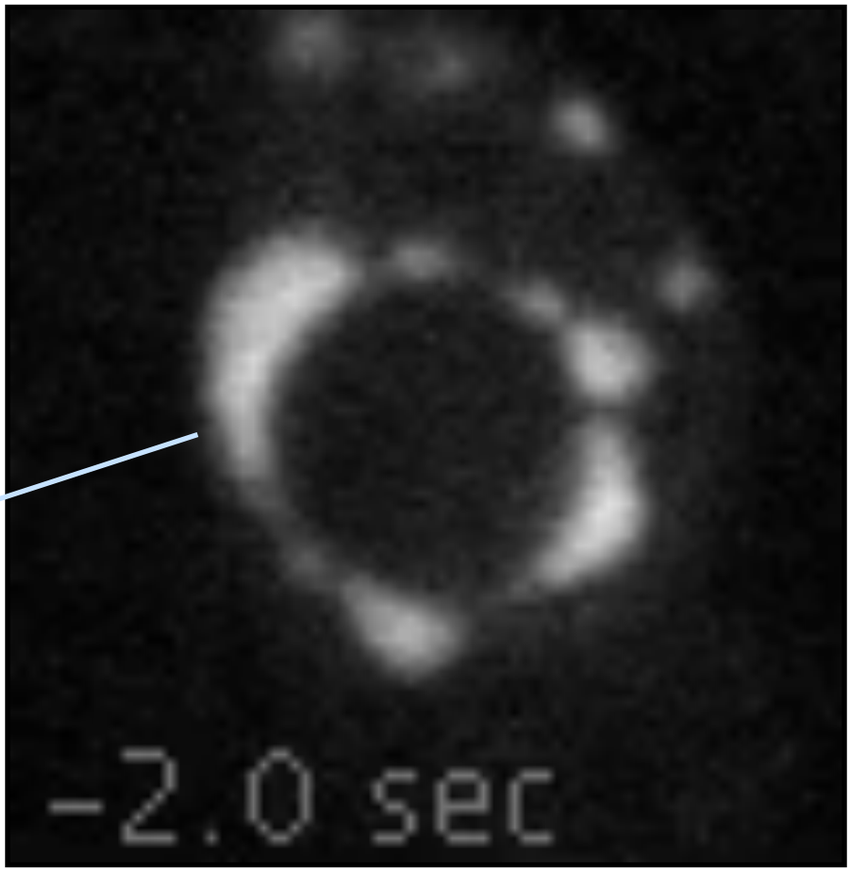

P granules possess a liquid like interior (diffusion occurs within granules)

η (viscosity) ≈ 1 Pa•s (similar to viscosity of glycerol)

Brangwynne, C. P. et al. *Science* **2009**, *324*, 1729.

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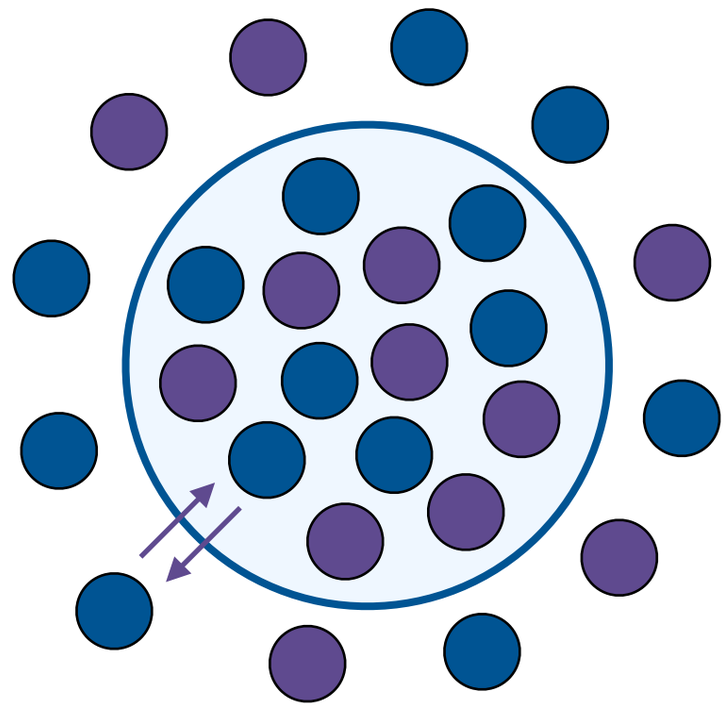
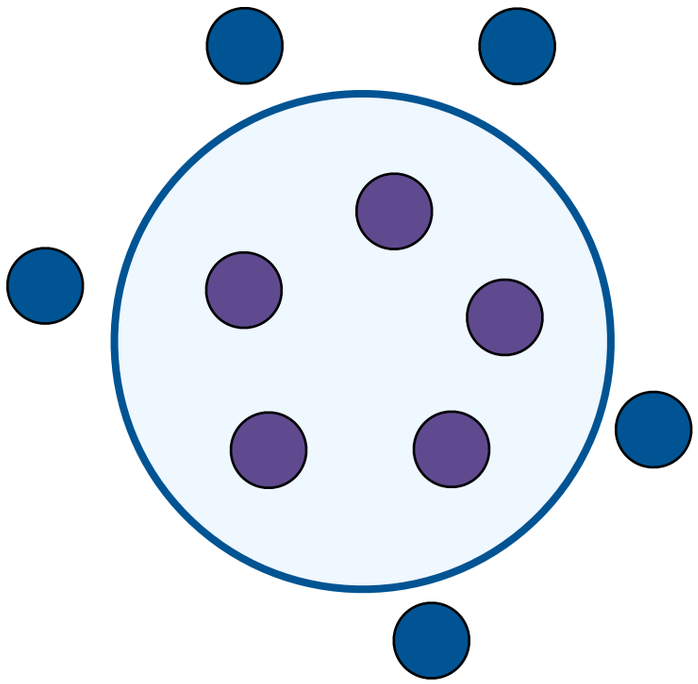


Liquid–Liquid Phase Separation in Biology

the physiological functions of membraneless organelles

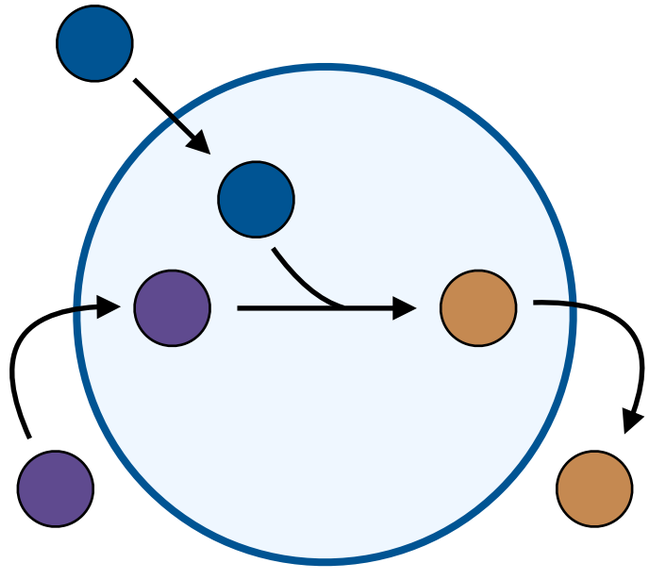
Functions of Intracellular Phase Separation

1. Organization



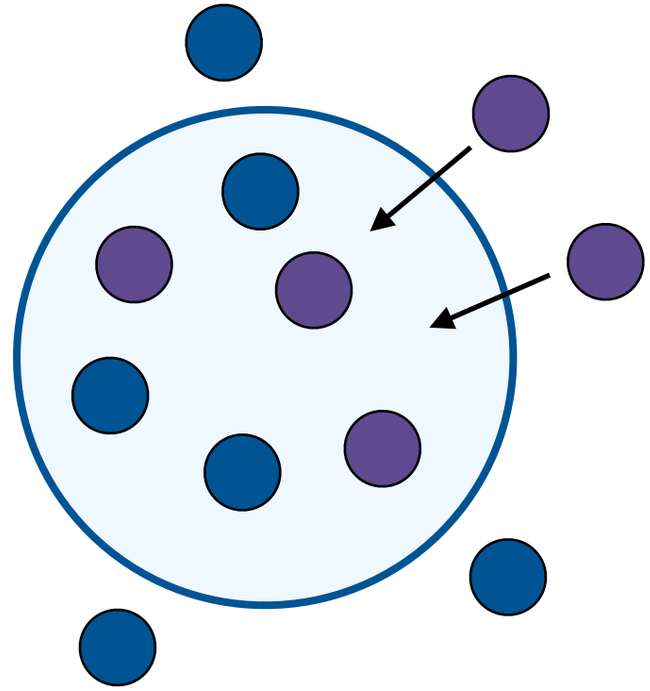
compartmentalize molecules

2. Reaction Crucible



promote reactions

3. Sequestration



prevent reactions

Shin, Y.; Brangwynne, C. P. *Science* **2017**, *357*, eaaf4382.

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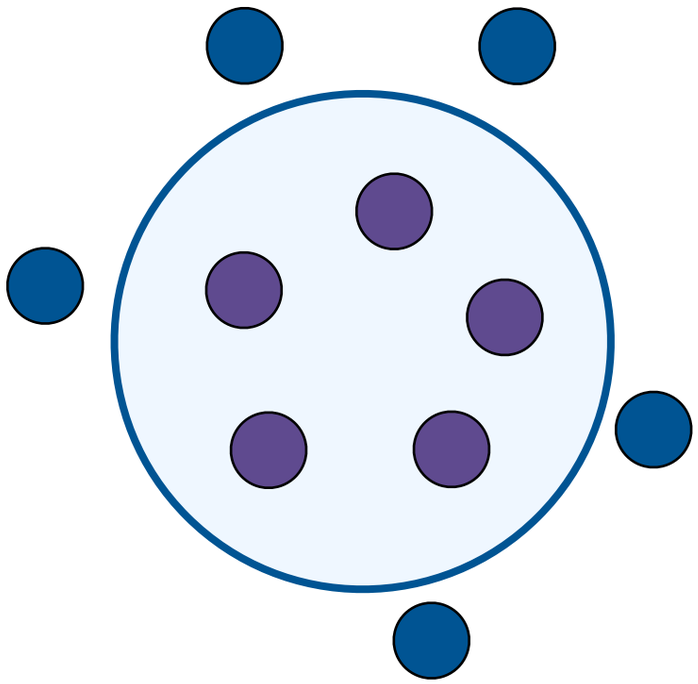

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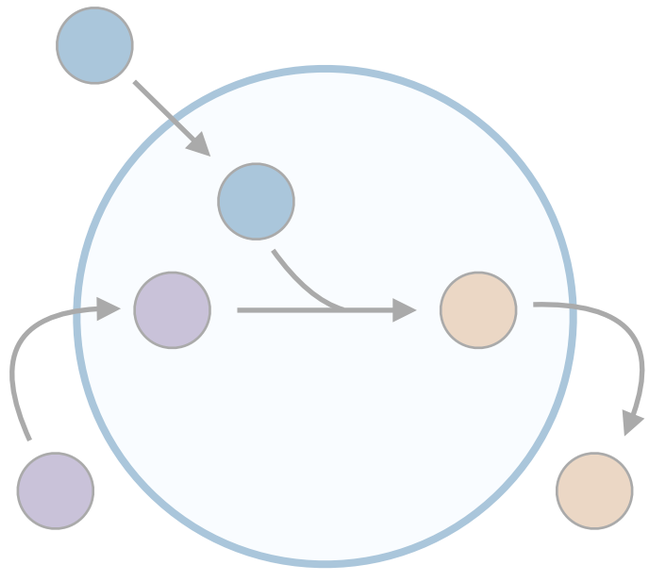

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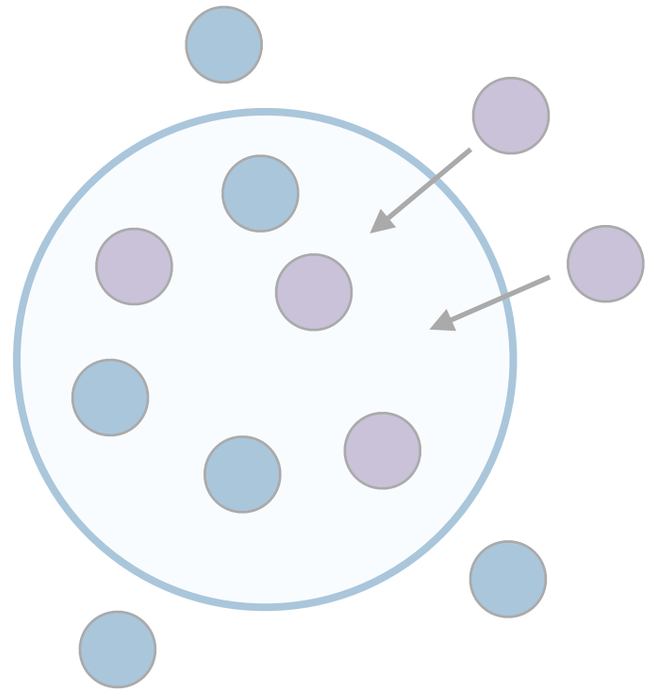

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Liquid–Liquid Phase Separation in Biology

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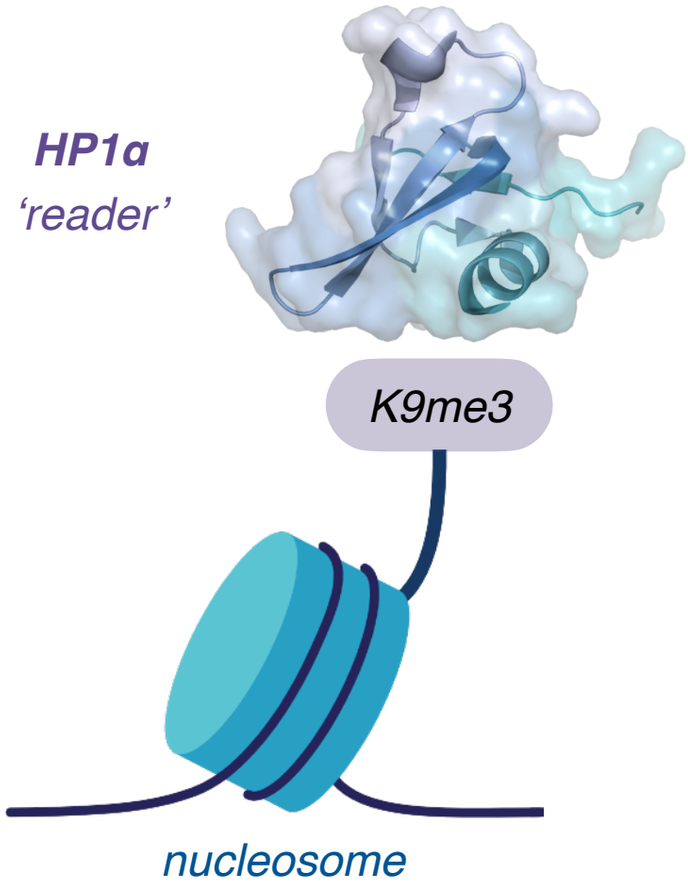

Gene silencing by heterochromatin, in part, occurs via heterochromatin protein 1 α (HP1α)

Larson, A. G. et al. *Nature* **2017**, *547*, 236.

Liquid–Liquid Phase Separation in Biology

the physiological functions of membraneless organelles

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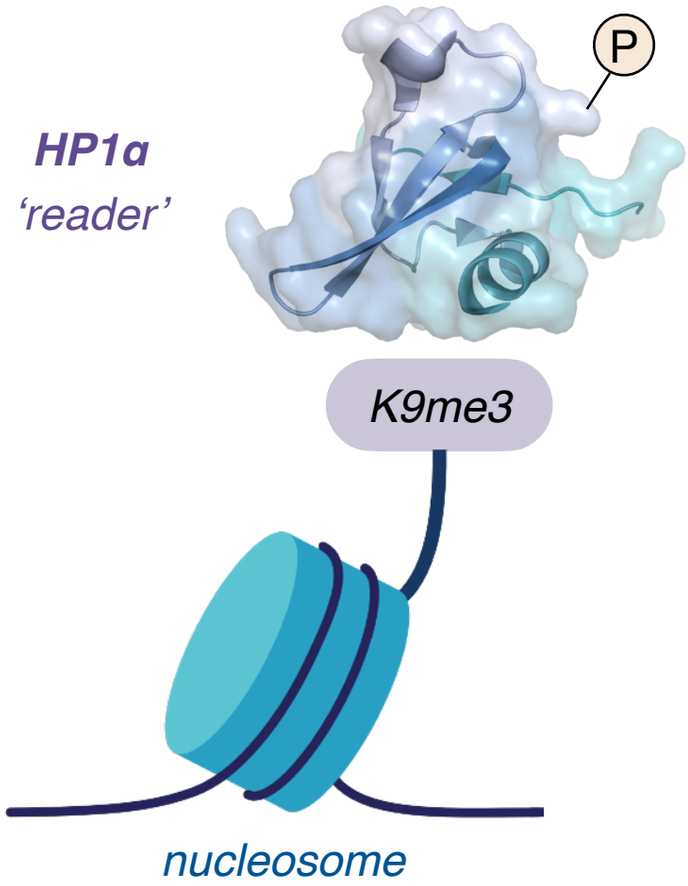

Phosphorylation of HP1α increases affinity for K9me3

Larson, A. G. et al. *Nature* **2017**, *547*, 236.

Liquid–Liquid Phase Separation in Biology

the physiological functions of membraneless organelles

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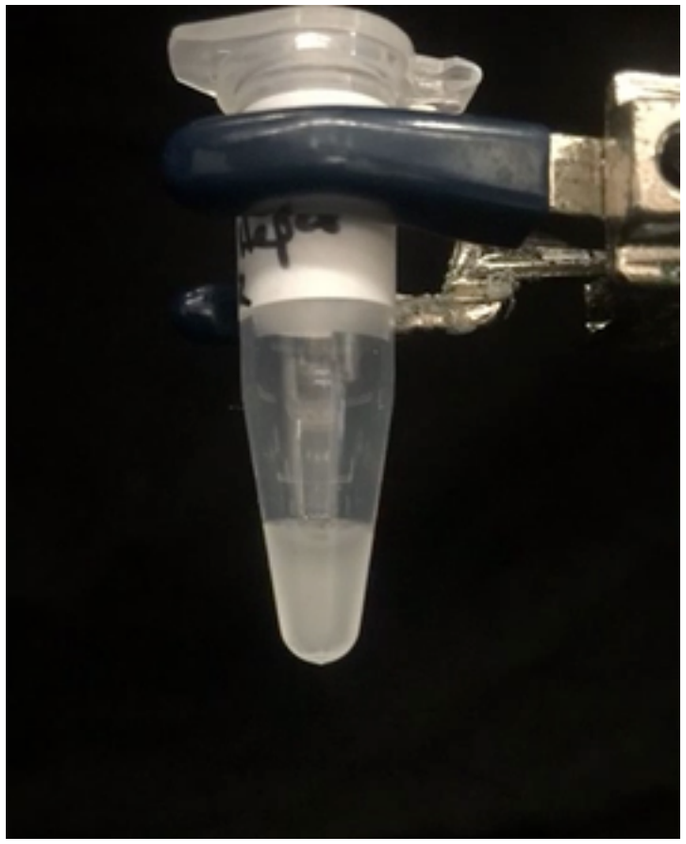

phosphorylated HP1α upon warming

phosphorylated HP1α forms a turbid solution 4 ºC

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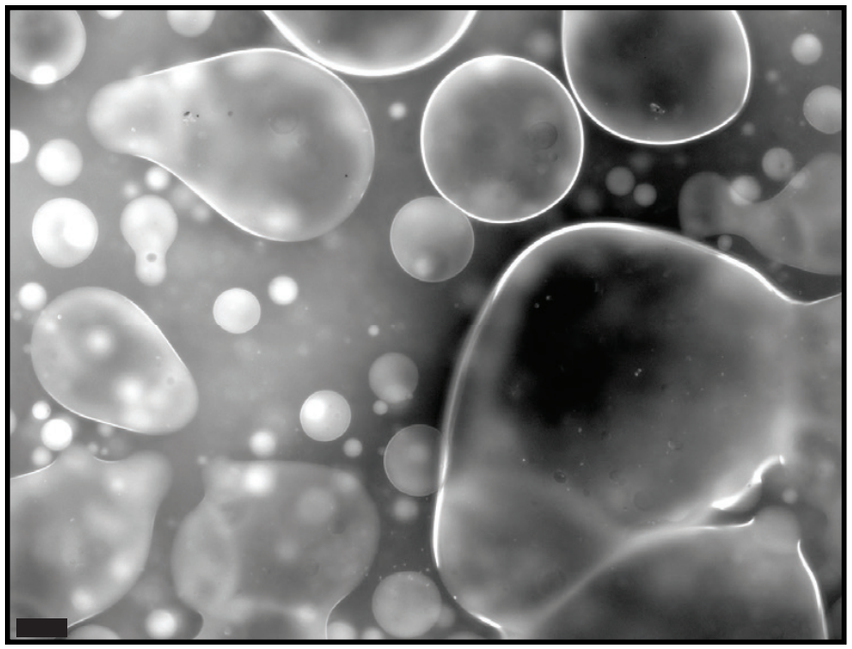
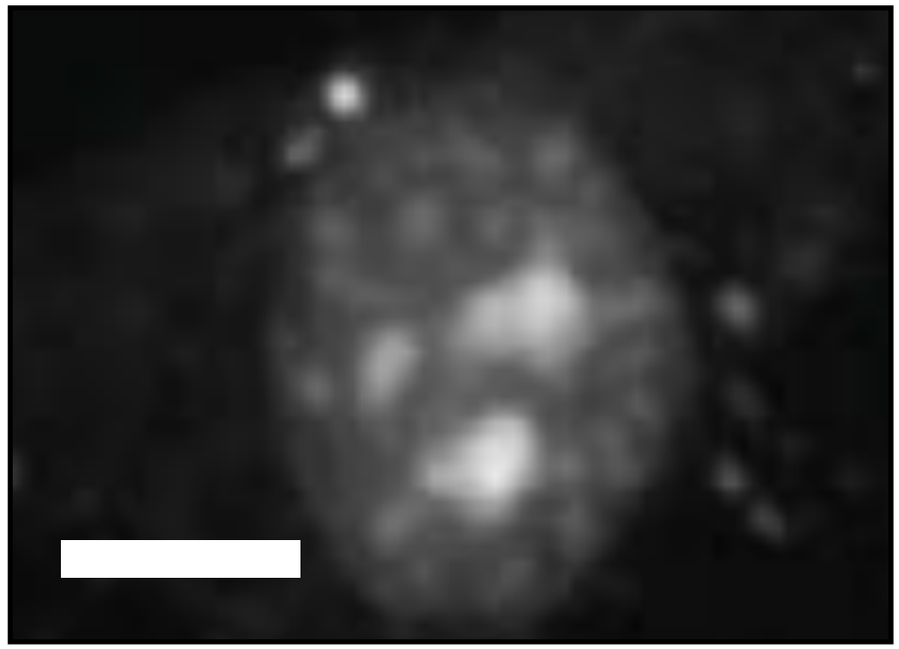


phosphorylated HP1α
forms phase-separated droplets



dye-conjugated HP1α phase separates
within the nucleus

Phosphorylated HP1α undergoes LLPS upon cooling in vitro

Larson, A. G. et al. *Nature* **2017**, *547*, 236.

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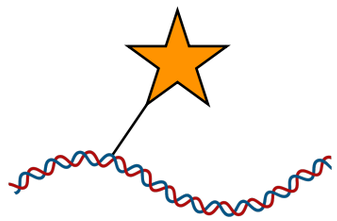
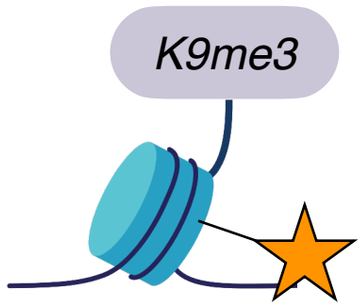
the physiological functions of membraneless organelles

1. Organization

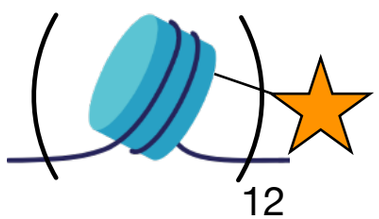
just dye



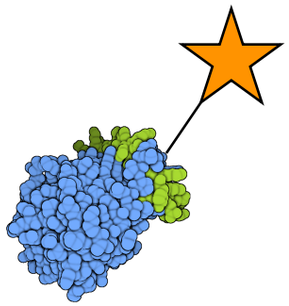
DNA - dye



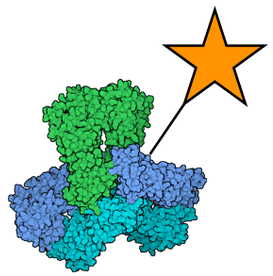
H3K9Me3 - dye



12-nucleosome array - dye



Aurora B kinase - dye



bHsp90 - dye

HP1a droplets selectively solvate or exclude nuclear components

Larson, A. G. et al. *Nature* **2017**, *547*, 236.

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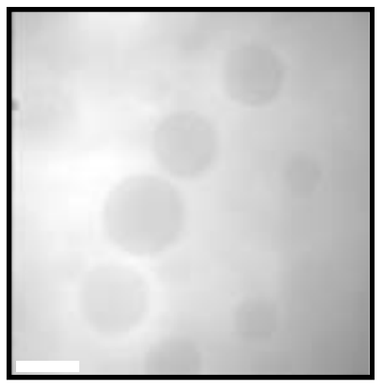
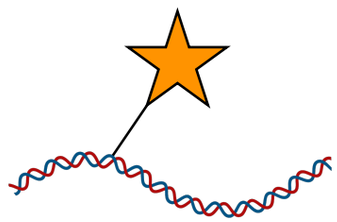
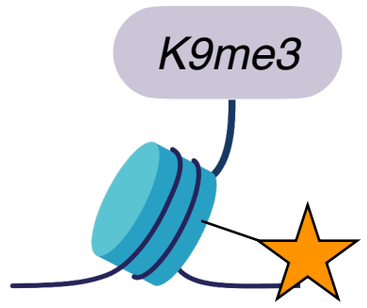


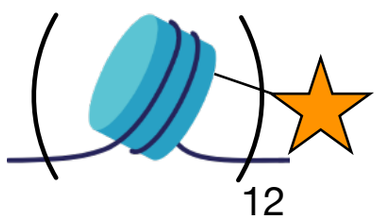
just dye



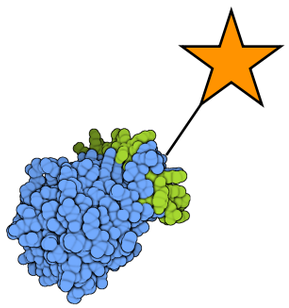
DNA - dye



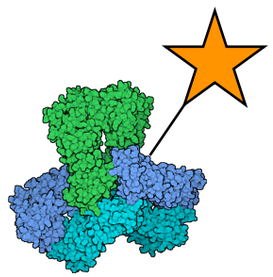
H3K9Me3 - dye



12-nucleosome array - dye



Aurora B kinase - dye



bHsp90 - dye

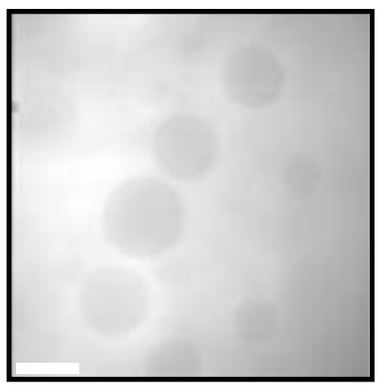
HP1a droplets selectively solvate or exclude nuclear components

Larson, A. G. et al. *Nature* **2017**, *547*, 236.

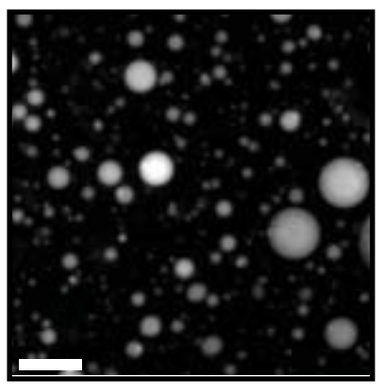
Liquid–Liquid Phase Separation in Biology

the physiological functions of membraneless organelles

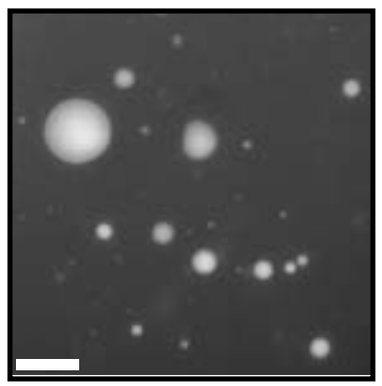
1. Organization



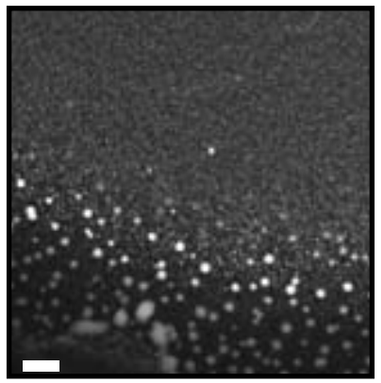
just dye



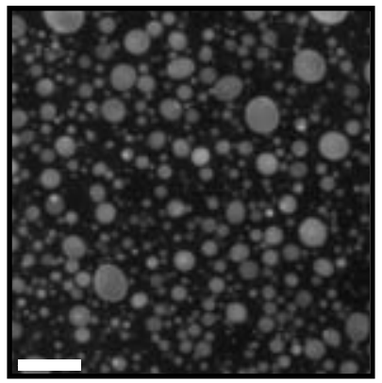
DNA - dye



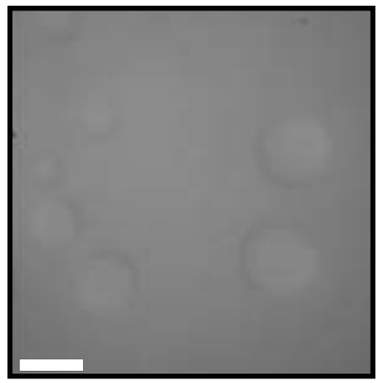
H3K9Me3 - dye



12-nucleosome array - dye



Aurora B kinase - dye



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Liquid–Liquid Phase Separation in Biology

the physiological functions of membraneless organelles

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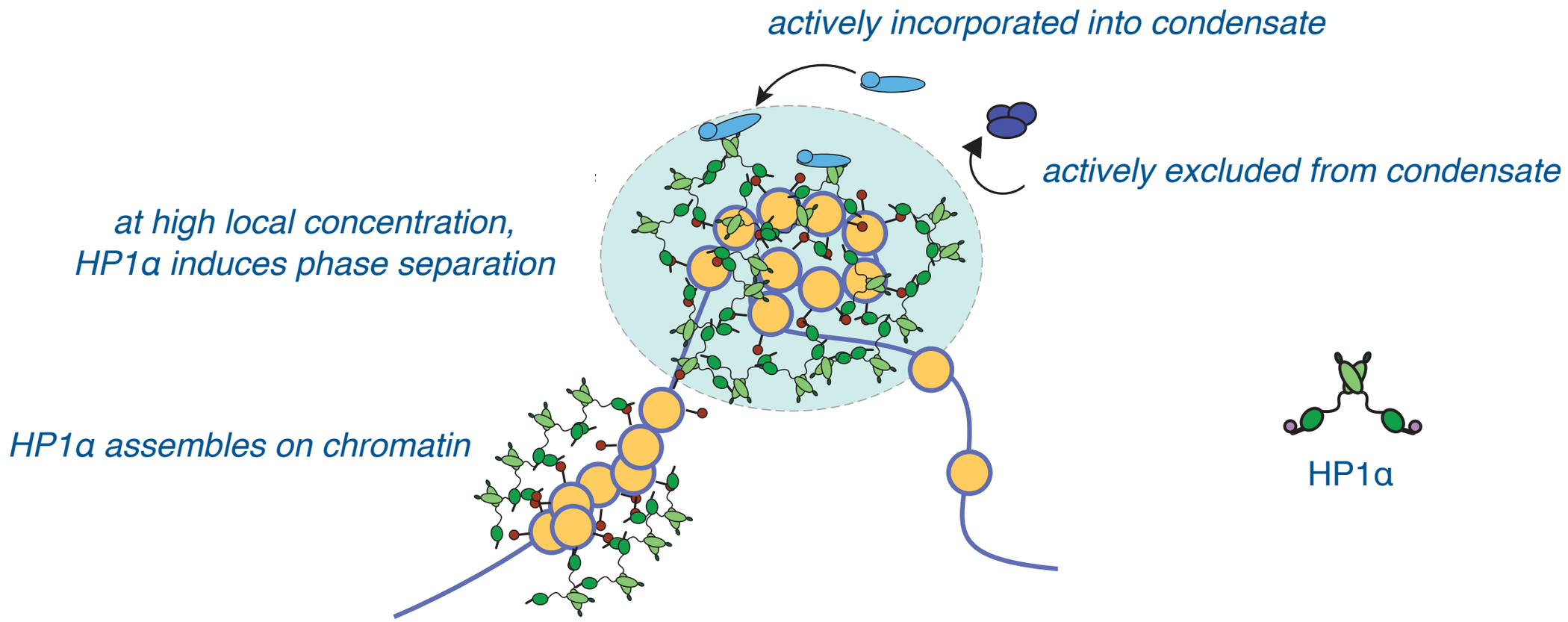


results suggest phase separation as a mechanism for chromatin organization

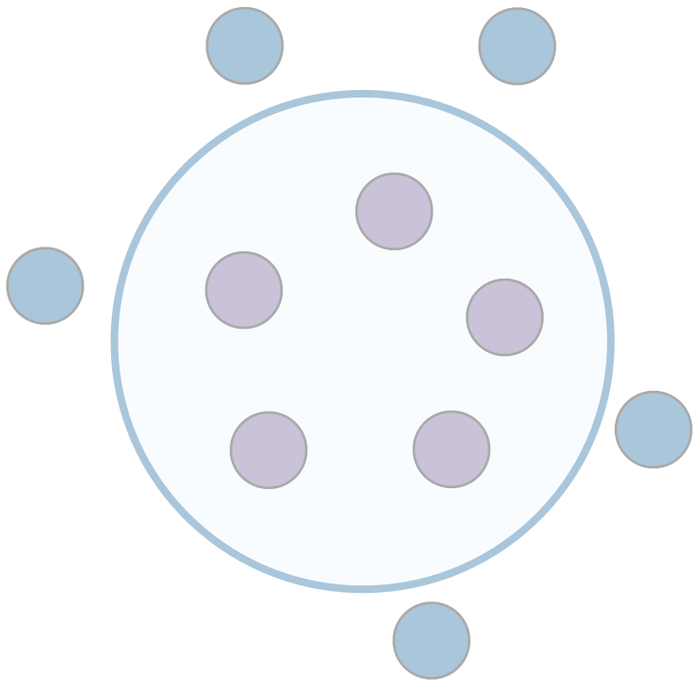
Larson, A. G. et al. *Nature* **2017**, *547*, 236.

Liquid–Liquid Phase Separation in Biology

the physiological functions of membraneless organelles

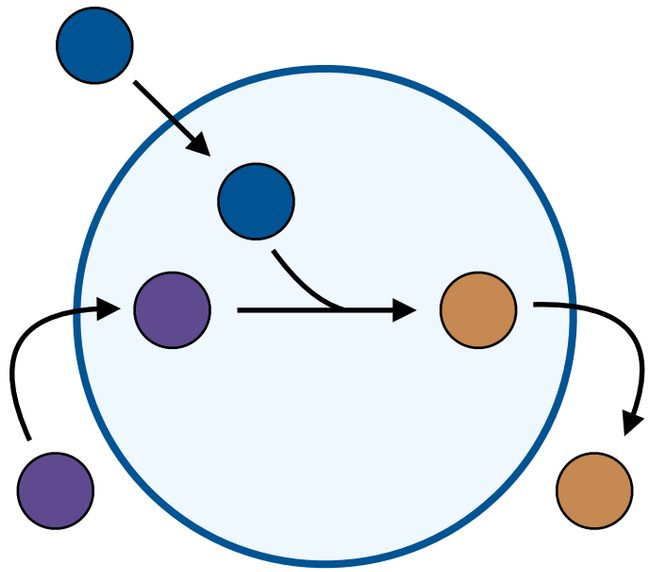
Functions of Intracellular Phase Separation

1. Organization



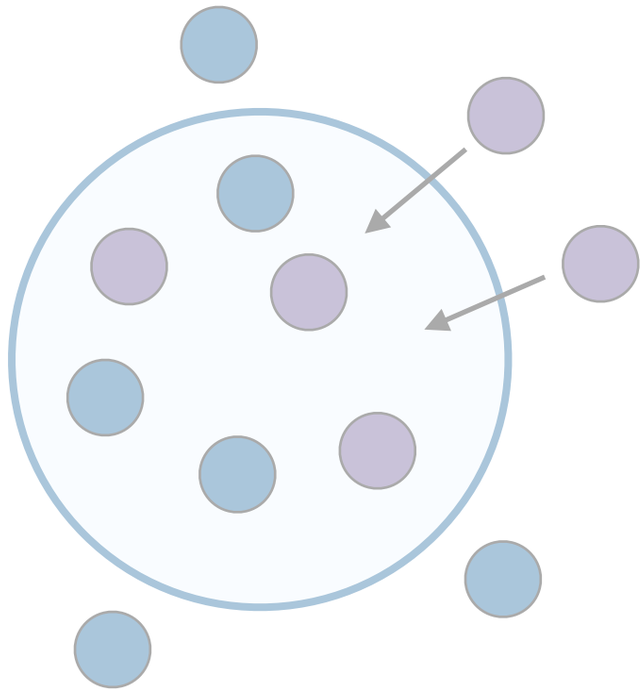
compartmentalize molecules

2. Reaction Crucible



promote reactions

3. Sequestration



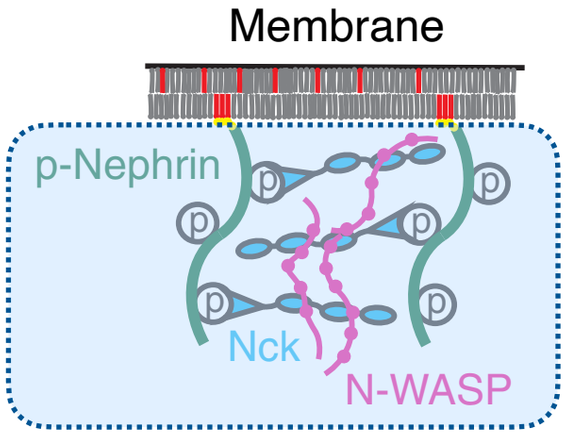
prevent reactions

Shin, Y.; Brangwynne, C. P. *Science* **2017**, *357*, eaaf4382.

Liquid–Liquid Phase Separation in Biology

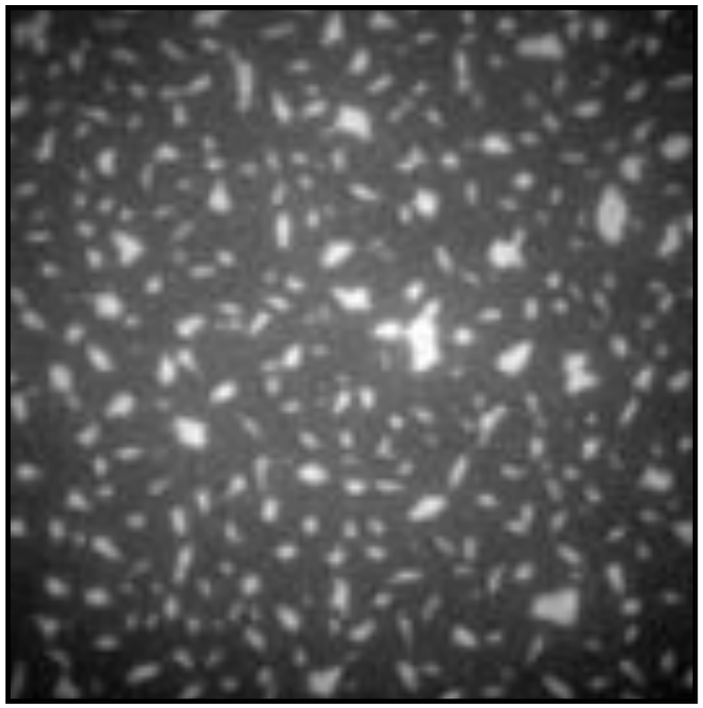
the physiological functions of membraneless organelles

2. Reaction Crucible



phase separation

p-Nephrin binds Nck, which binds N-WASP



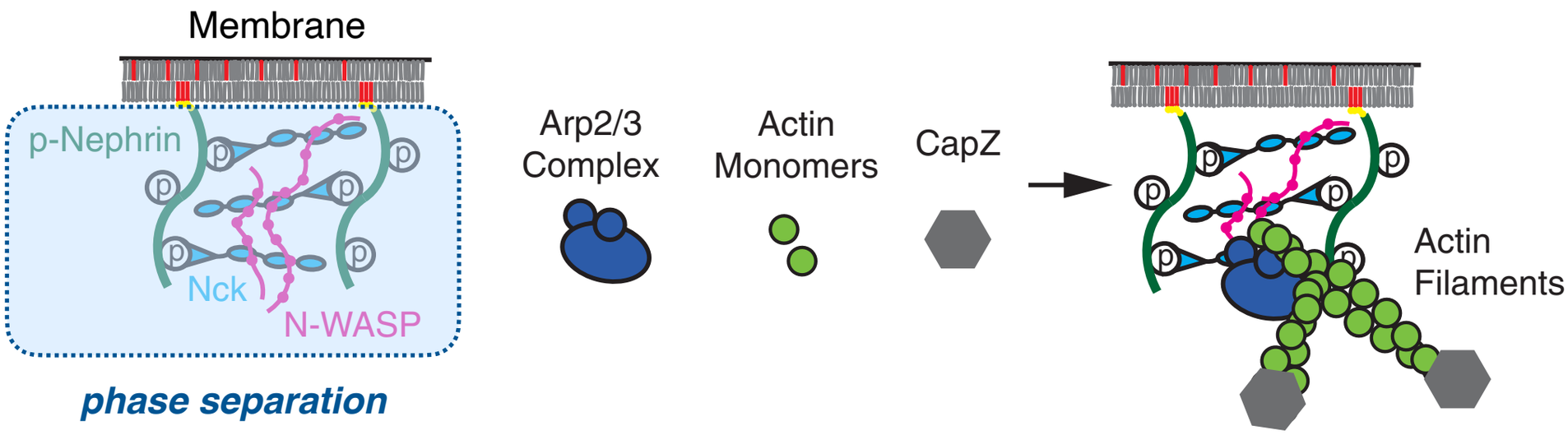
Nck and N-WASP undergo liquid-liquid phase separation

Case, L. B. et al. *Science* **2019**, *363*, 1093.

Liquid–Liquid Phase Separation in Biology

the physiological functions of membraneless organelles

2. Reaction Crucible



p-Nephrin binds Nck, which binds N-WASP

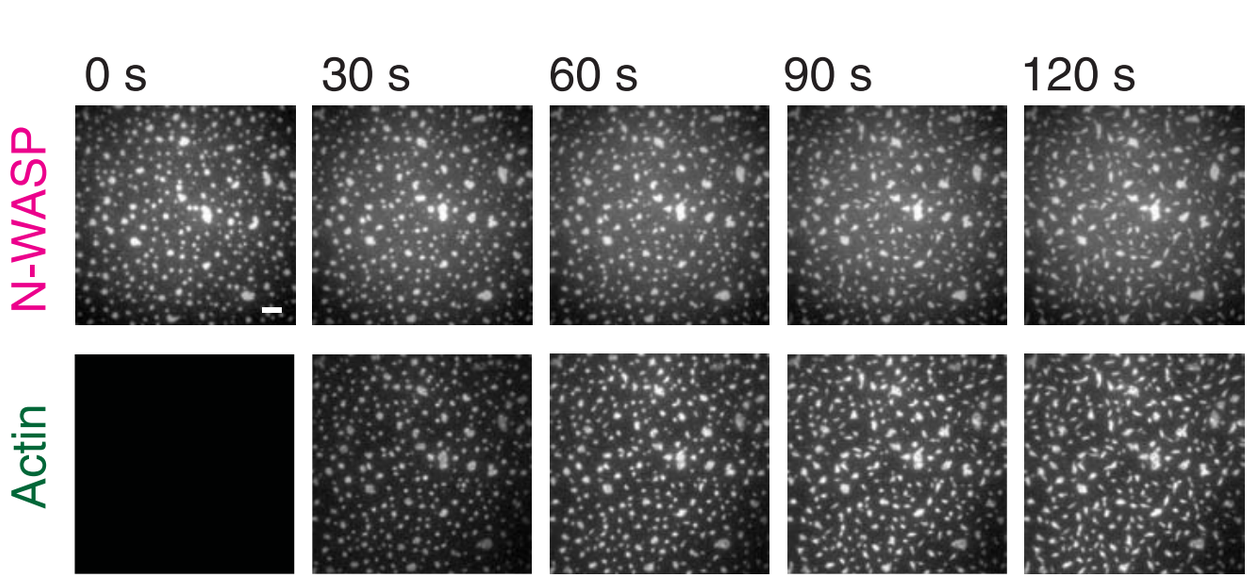
N-WASP stimulates actin polymerization by the Arp2/3 Complex

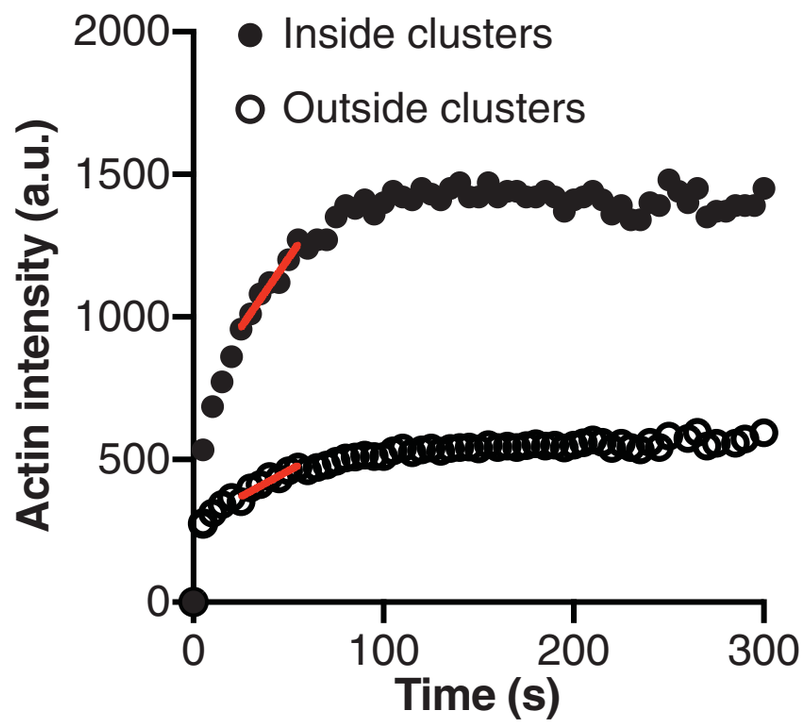
Case, L. B. et al. *Science* **2019**, *363*, 1093.

Liquid–Liquid Phase Separation in Biology

the physiological functions of membraneless organelles

2. Reaction Crucible





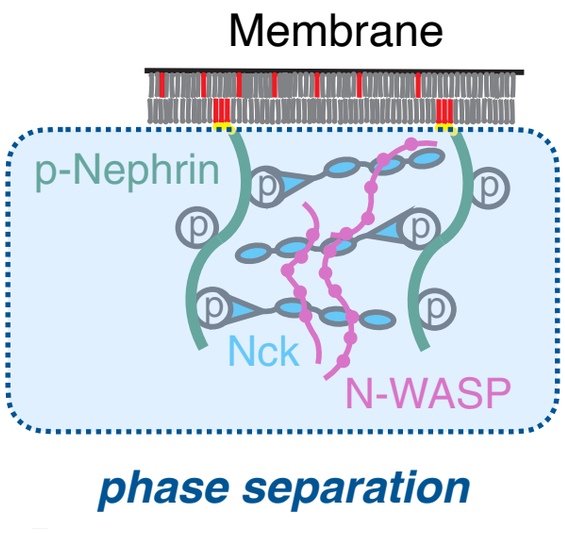
Activity of N-WASP to assemble actin is increased inside clusters

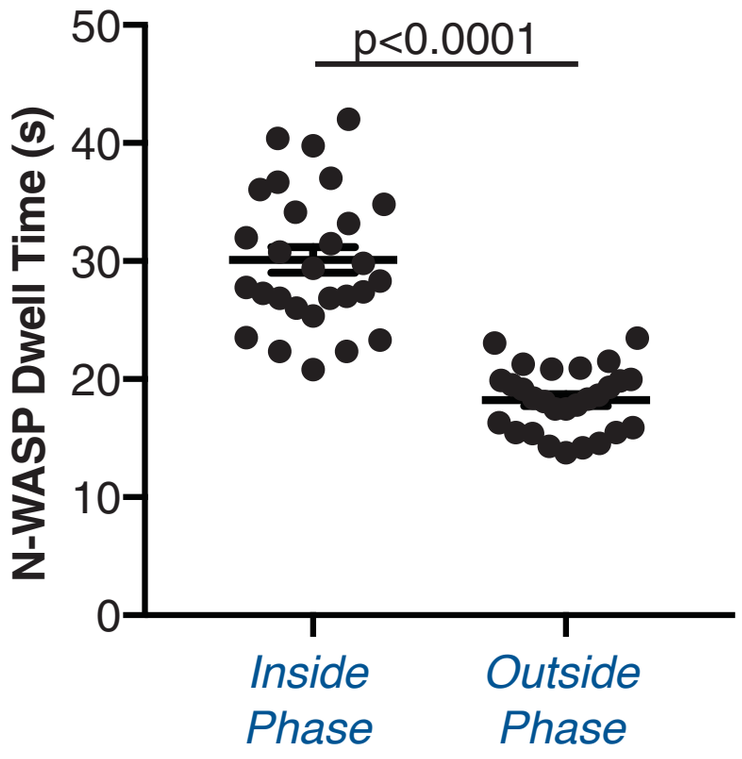
Case, L. B. et al. *Science* **2019**, *363*, 1093.

Liquid–Liquid Phase Separation in Biology

the physiological functions of membraneless organelles

2. Reaction Crucible





Liquid–liquid phase separation increases the residence time of N-WASP at the membrane, increasing actin assembly

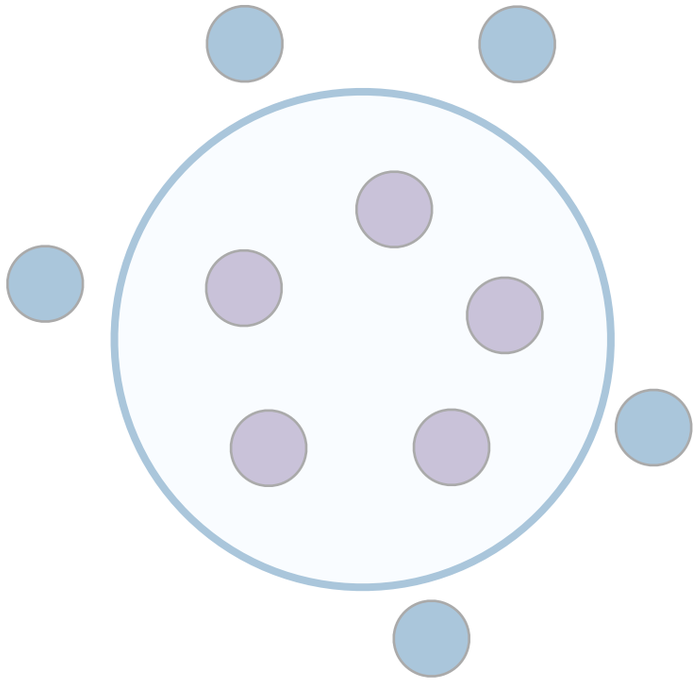
Case, L. B. et al. *Science* **2019**, *363*, 1093.

Liquid–Liquid Phase Separation in Biology

the physiological functions of membraneless organelles

Functions of Intracellular Phase Separation

1. Organization



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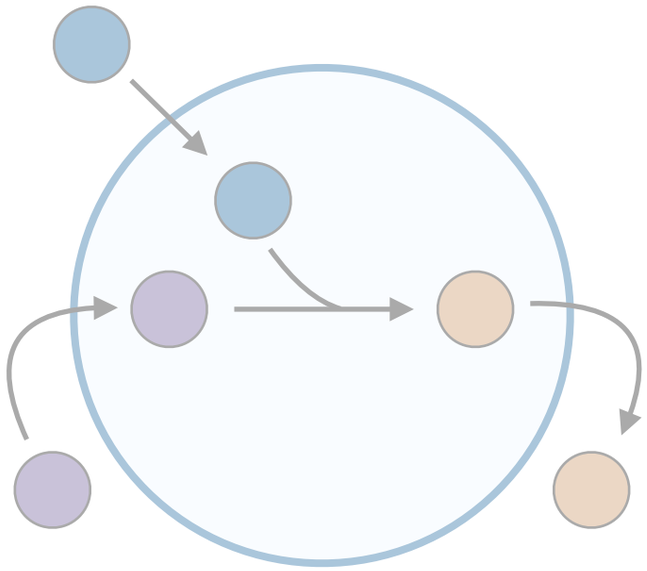
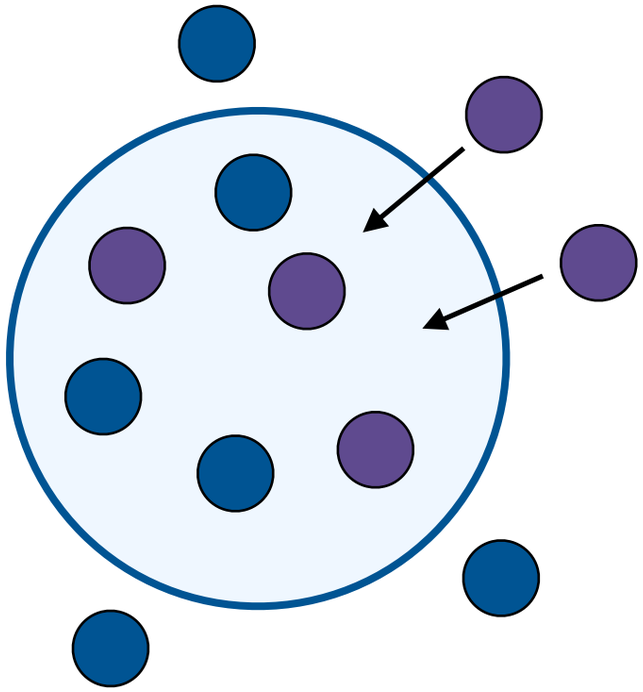


promote reactions

3. Sequestration



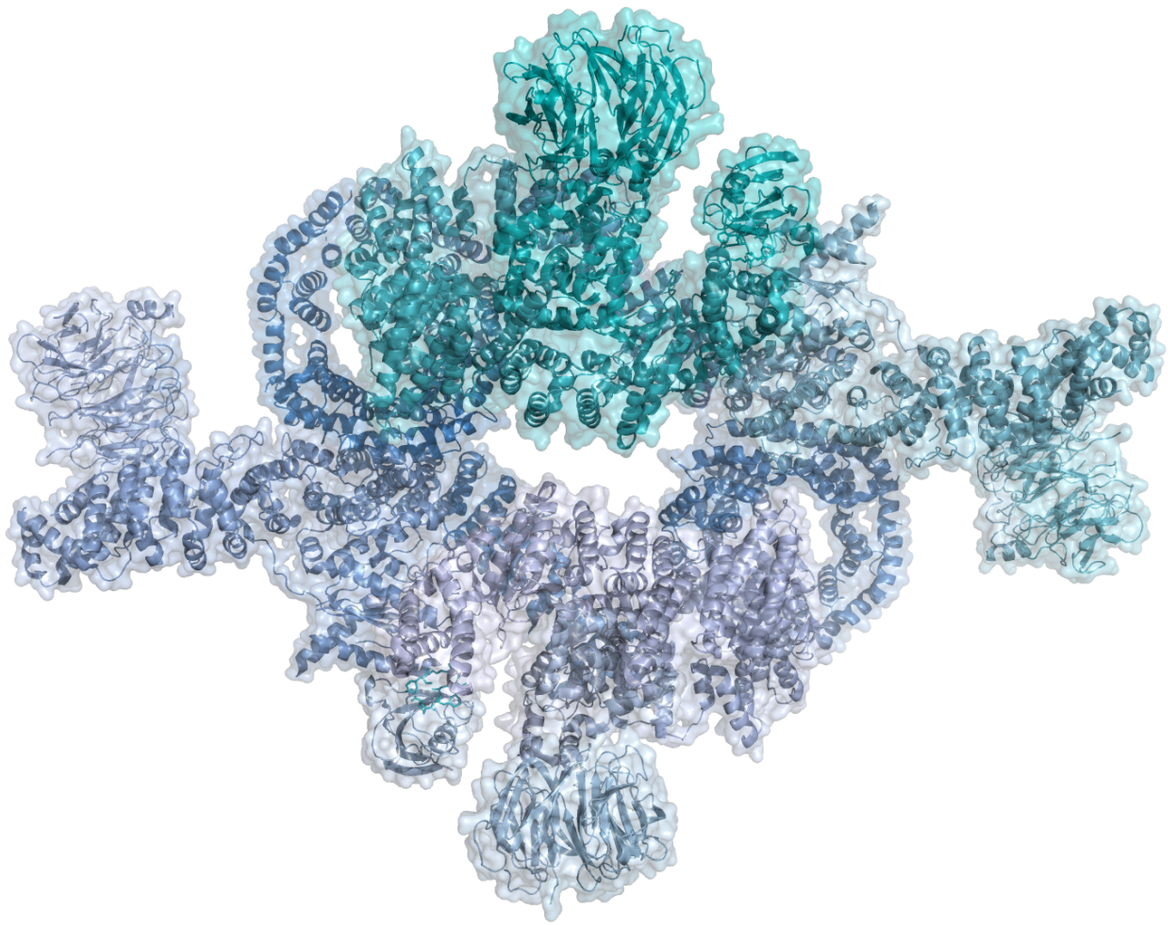
prevent reactions

Shin, Y.; Brangwynne, C. P. *Science* **2017**, *357*, eaaf4382.

Liquid–Liquid Phase Separation in Biology

the physiological functions of membraneless organelles

3. Sequestration



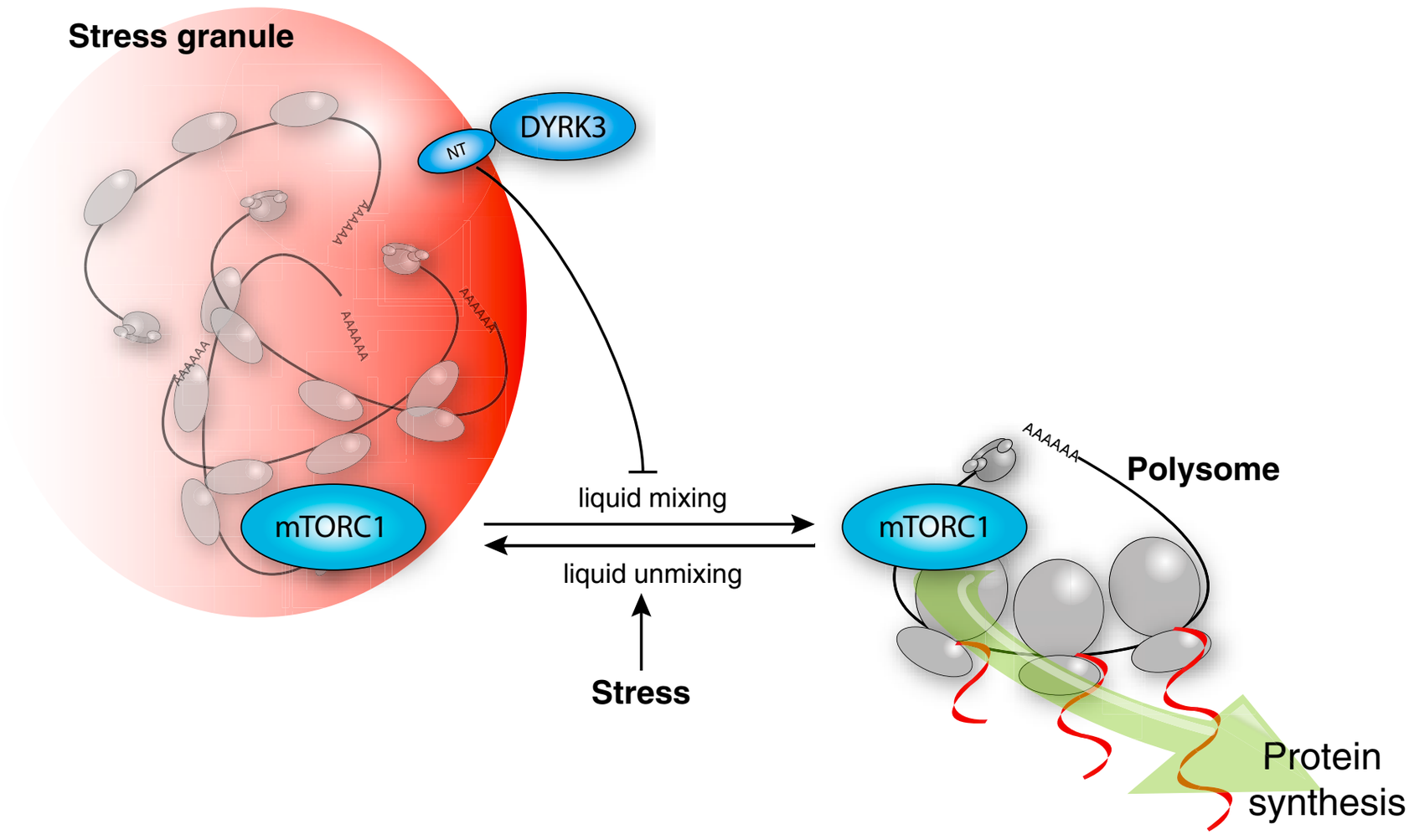
mammalian target of rapamycin complex 1 (mTORC1) controls translation of proteins

Wippich, F. et al. *Cell* **2013**, *152*, 791.

Liquid–Liquid Phase Separation in Biology

the physiological functions of membraneless organelles

3. Sequestration



stress granules sequester mTORC1, a key modulator of cell signaling

Wippich, F. et al. *Cell* **2013**, *152*, 791.

Liquid–Liquid Phase Separation in Biology

the physiological functions of membraneless organelles

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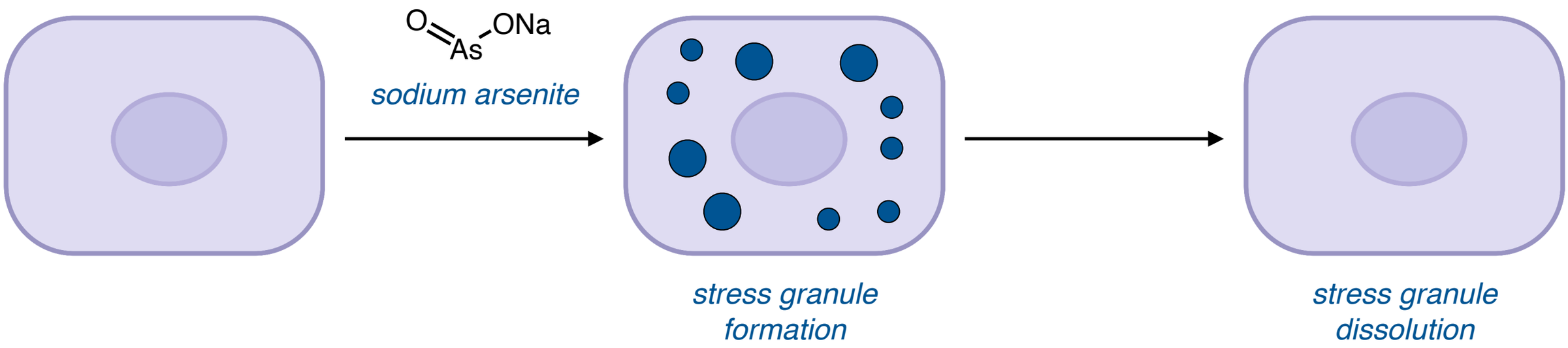


stress granules protect RNA when the cell is under stress

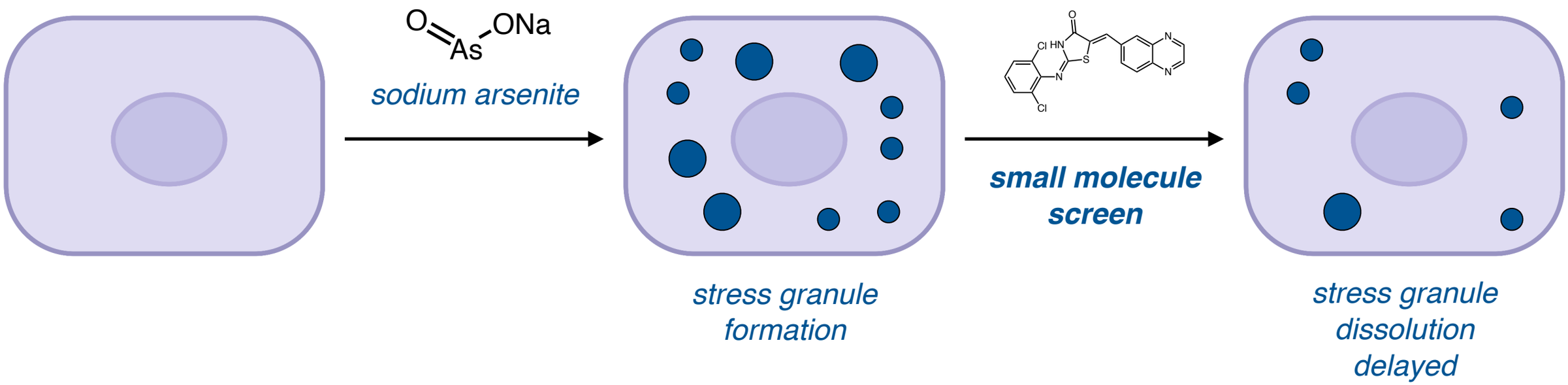
Wippich, F. et al. *Cell* **2013**, *152*, 791.

Liquid–Liquid Phase Separation in Biology

the physiological functions of membraneless organelles

3. Sequestration

screen for inhibitors that delay stress granule (SG) dissolution



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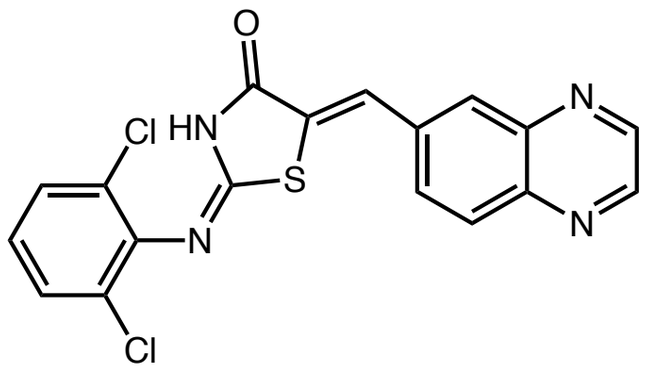
Liquid–Liquid Phase Separation in Biology

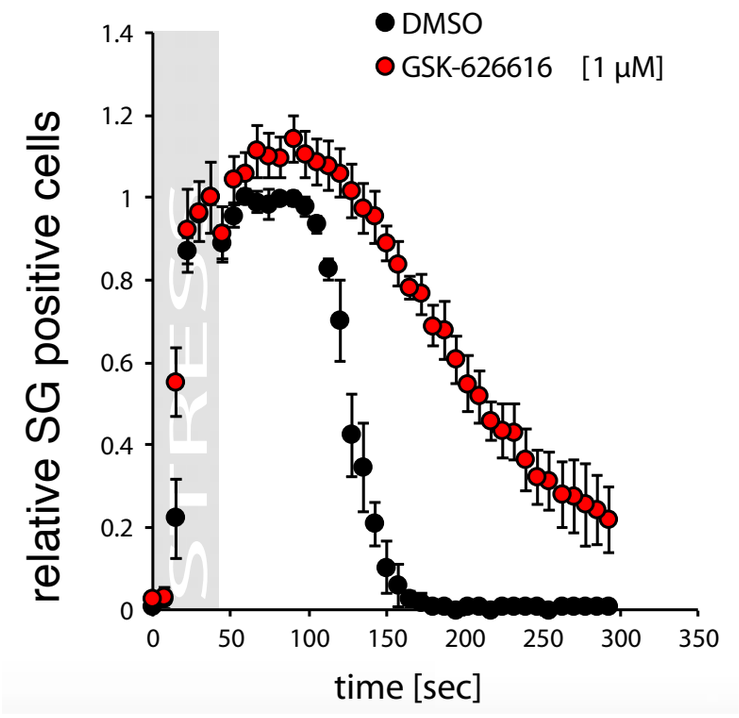
the physiological functions of membraneless organelles

3. Sequestration



GSK-626616

0.7 nM for the kinase DYRK3



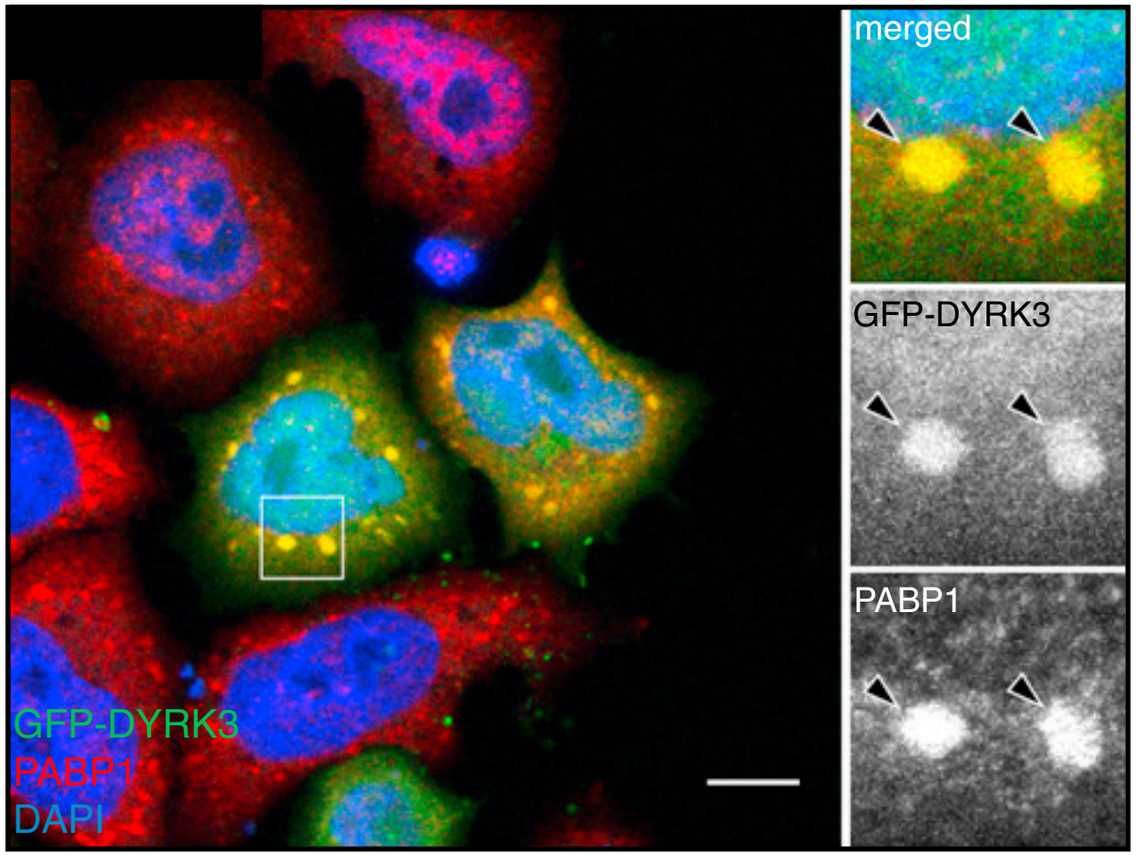
GSK-626616 delays stress granule dissolution

Wippich, F. et al. *Cell* **2013**, *152*, 791.

Liquid–Liquid Phase Separation in Biology

the physiological functions of membraneless organelles

3. Sequestration



PABP1 is recruited to stress granules upon stress

DYRK3 kinase partitions in stress granules

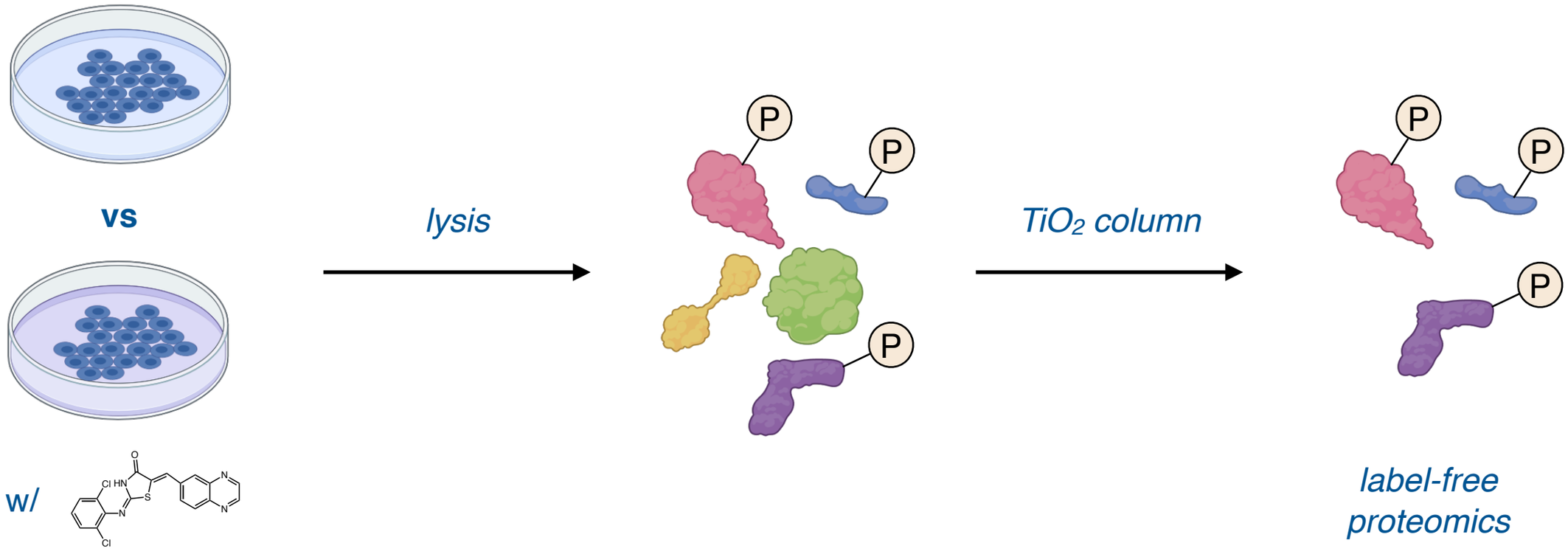
Wippich, F. et al. *Cell* **2013**, *152*, 791.

Liquid–Liquid Phase Separation in Biology

the physiological functions of membraneless organelles

3. Sequestration

How does GSK-626616 modulate phosphorylation in cells?



seven of the fifteen affected proteins reported to associate with RNA or RNA granules

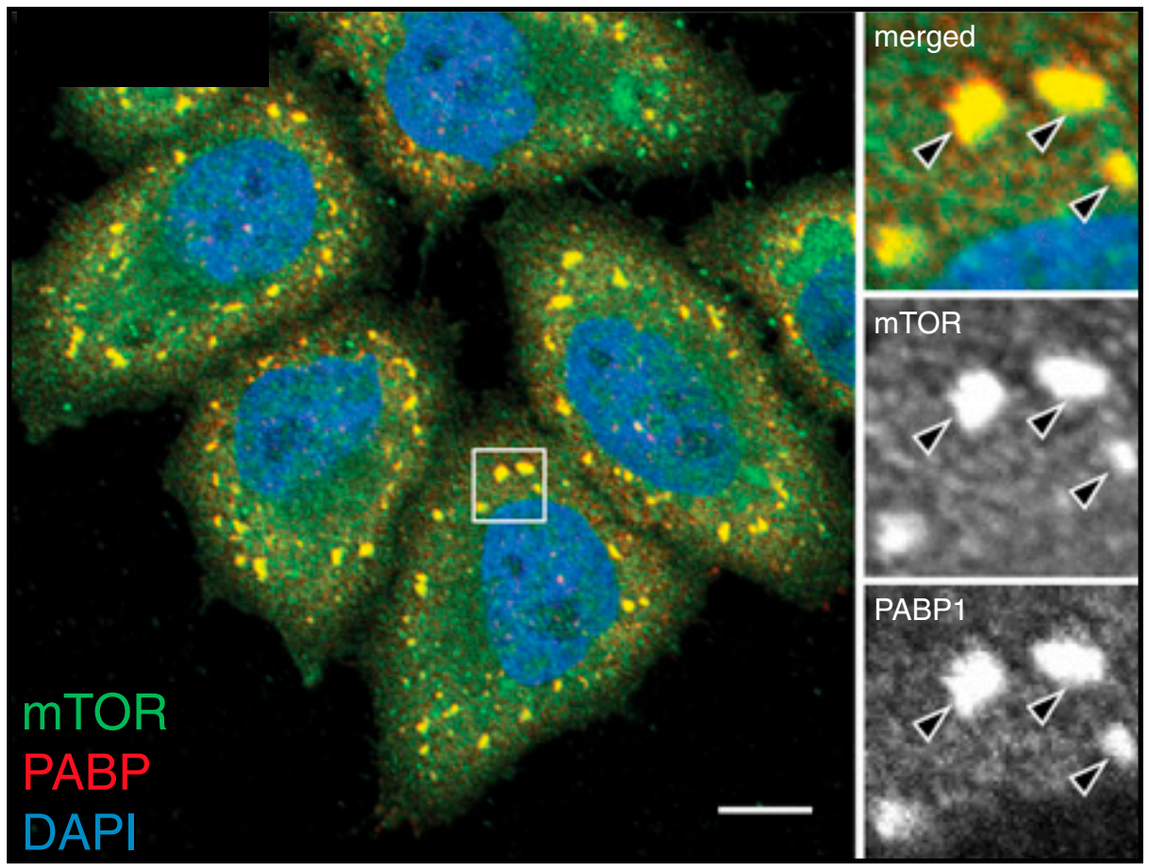
five of the fifteen proteins are proteins downstream of mTORC1 signaling

Wippich, F. et al. *Cell* **2013**, *152*, 791.

Liquid–Liquid Phase Separation in Biology

the physiological functions of membraneless organelles

3. Sequestration



PABP1 is recruited to stress granules upon stress

mTORC1 is recruited to stress granules during stress

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Liquid–Liquid Phase Separation in Biology

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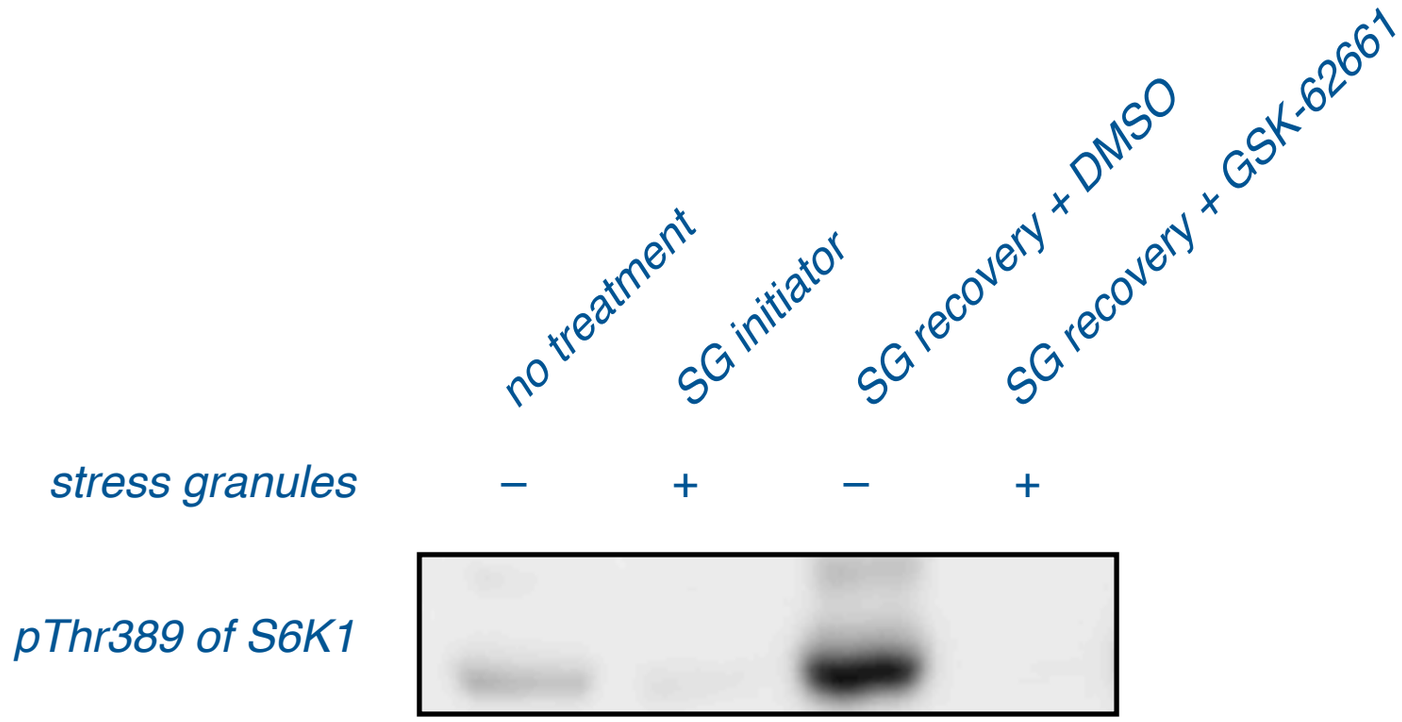

pThr389 of S6K1 is a direct readout for mTORC1 activity

Wippich, F. et al. *Cell* **2013**, *152*, 791.

Liquid–Liquid Phase Separation in Biology

the physiological functions of membraneless organelles

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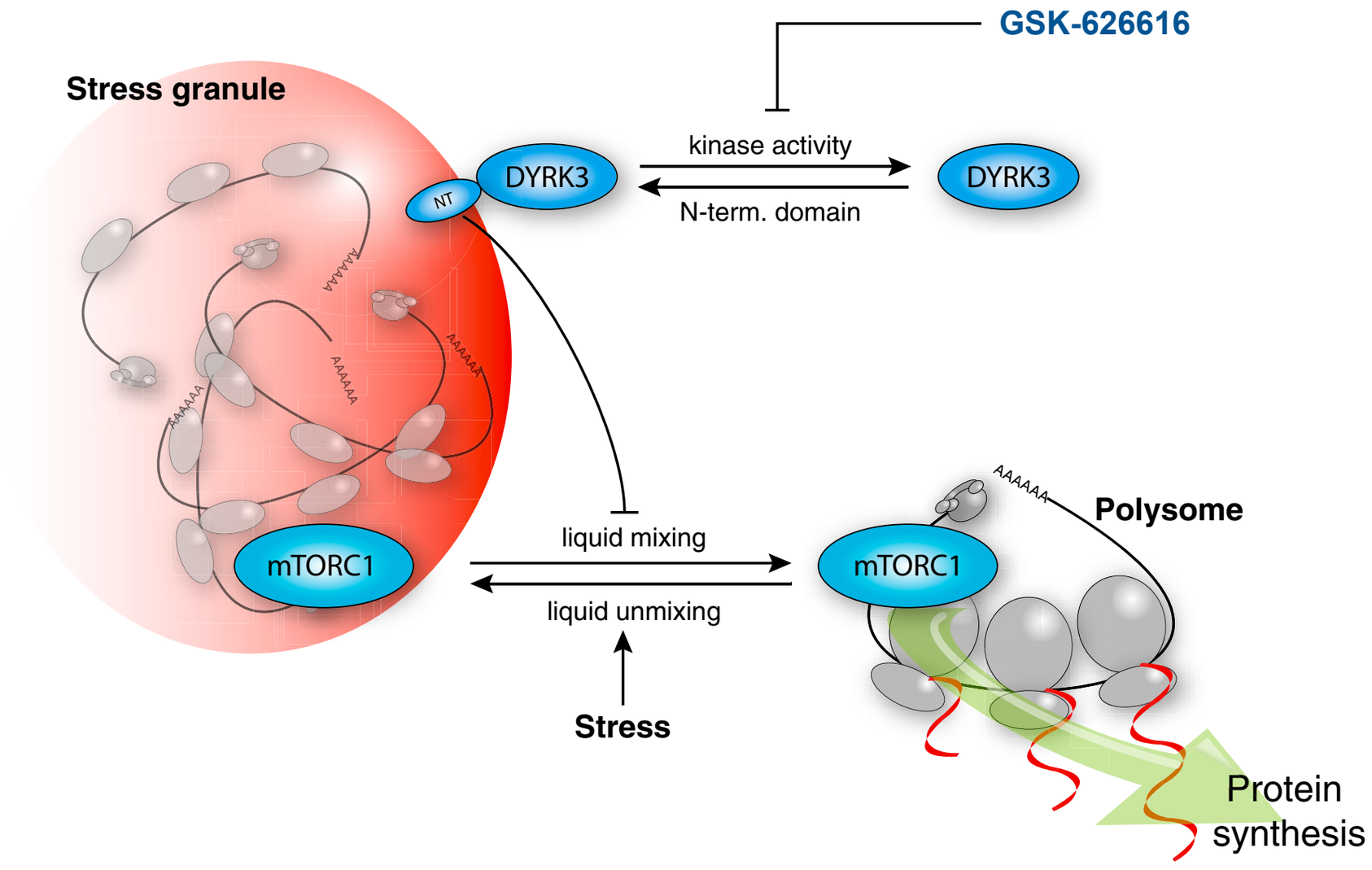

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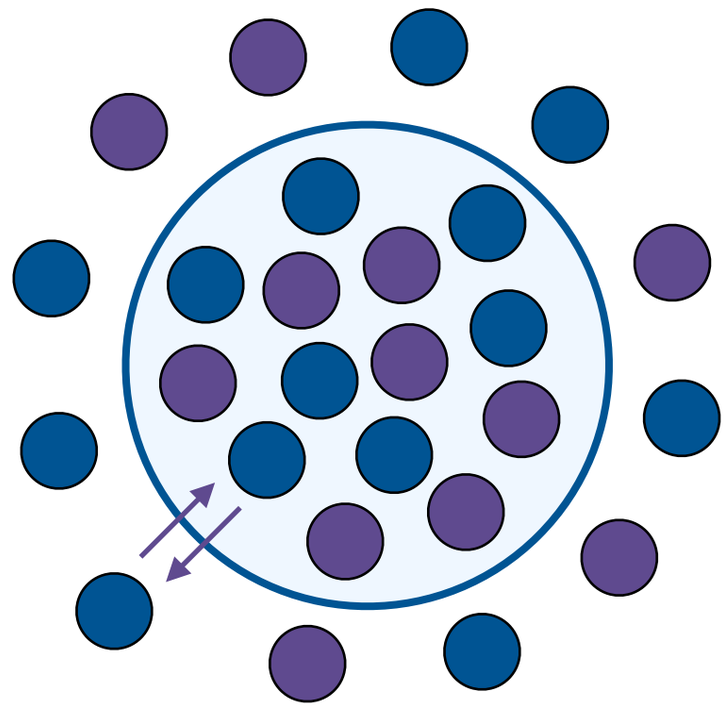
Liquid–Liquid Phase Separation in Biology

outline

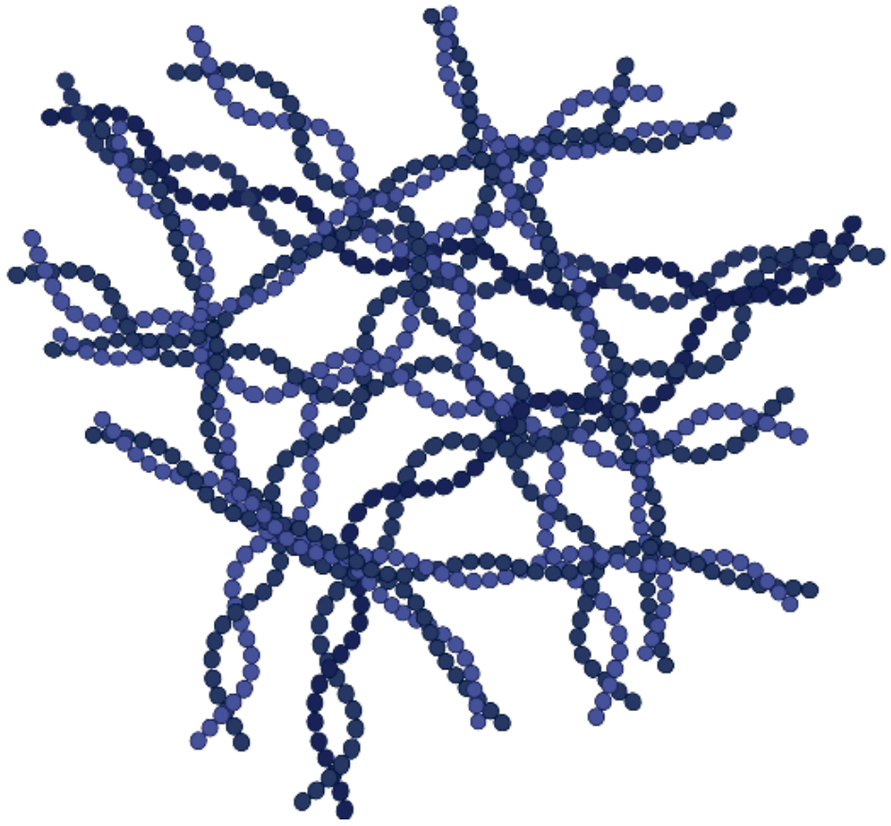
- Introduction to liquid–liquid phase separation (LLPS)
- The first observation of phase separation in cells
- Physiological functions of phase separation
  - Organization
  - Promote reactions
  - Sequestration
- Aberrant phase separation can cause disease
  - Amyotrophic lateral sclerosis (ALS)
- Conclusion and outlook



Liquid–Liquid Phase Separation in Biology

aberrant phase separation can cause disease

Neurodegenerative Diseases



Tau fibrils

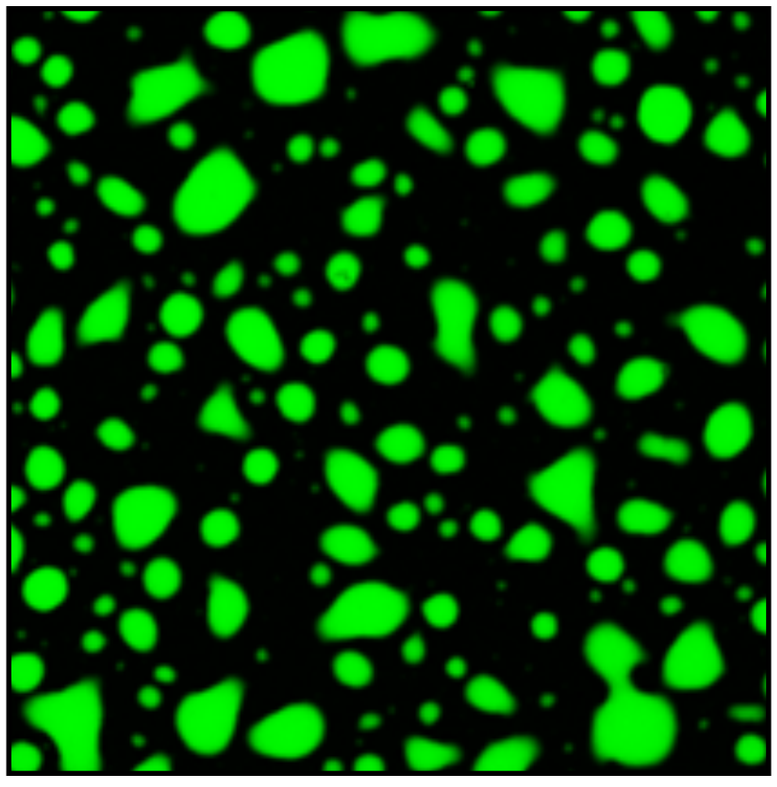
many neurodegenerative diseases exhibit pathological protein aggregates

Kanaan, N. M. et al. *Nat. Commun.* **2020**, *11*, 2809.

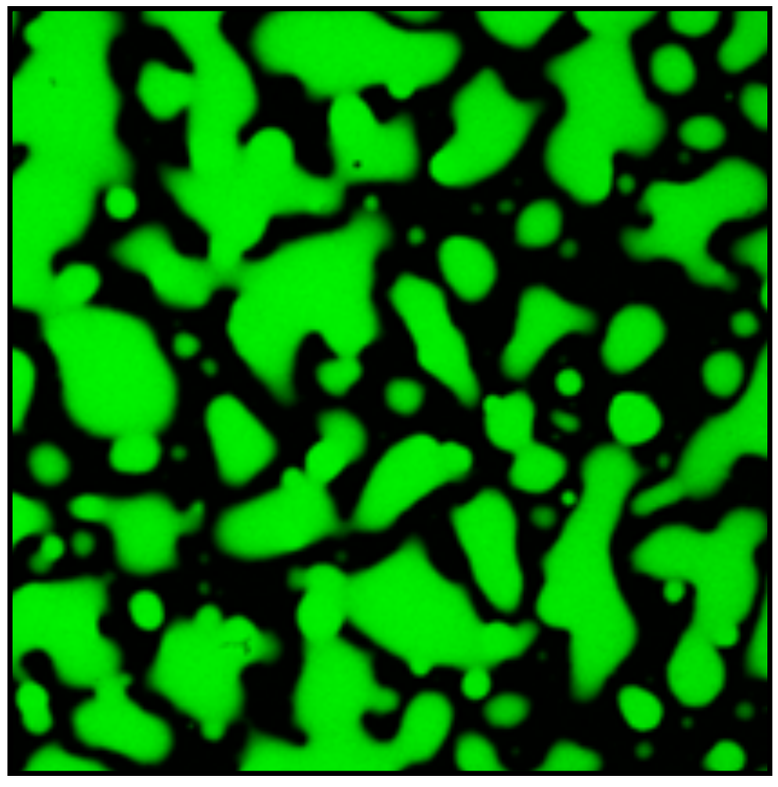
Liquid–Liquid Phase Separation in Biology

aberrant phase separation can cause disease

Neurodegenerative Diseases



tau undergoes phase separation in vitro



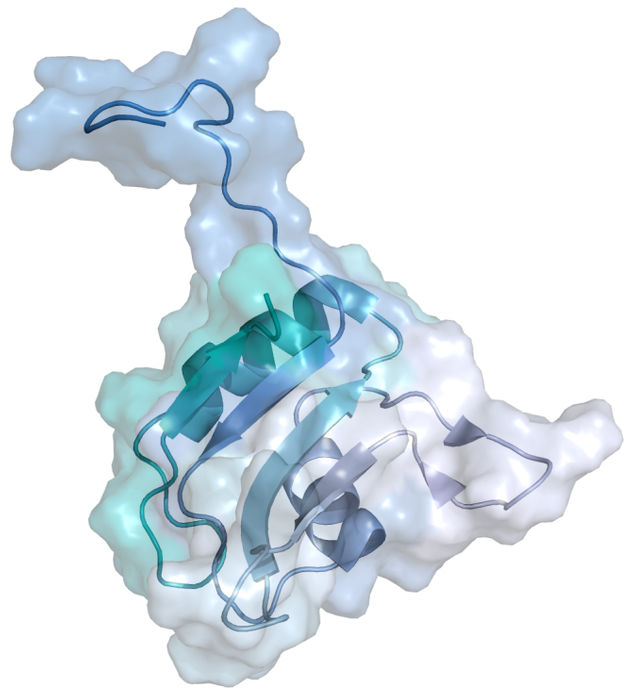
disease-related mutations lead to tau oligomers

Kanaan, N. M. et al. *Nat. Commun.* **2020**, *11*, 2809.

Liquid–Liquid Phase Separation in Biology

LLPS in amyotrophic lateral sclerosis

Neurodegenerative Diseases



Fused in Sarcoma Protein (FUS)

FUS is involved in transcription, DNA repair, and RNA biogenesis

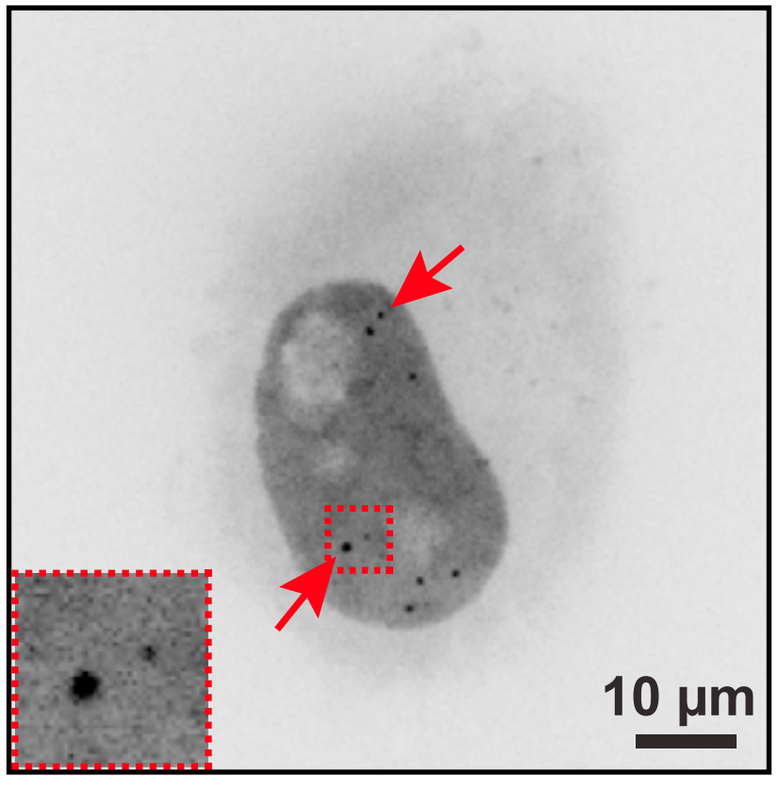
Mutations in the protein FUS are associated with ALS

Patel, A. et al. *Cell* **2015**, *162*, 1066.

Liquid–Liquid Phase Separation in Biology

LLPS in amyotrophic lateral sclerosis

Neurodegenerative Diseases



FUS forms granules in cells

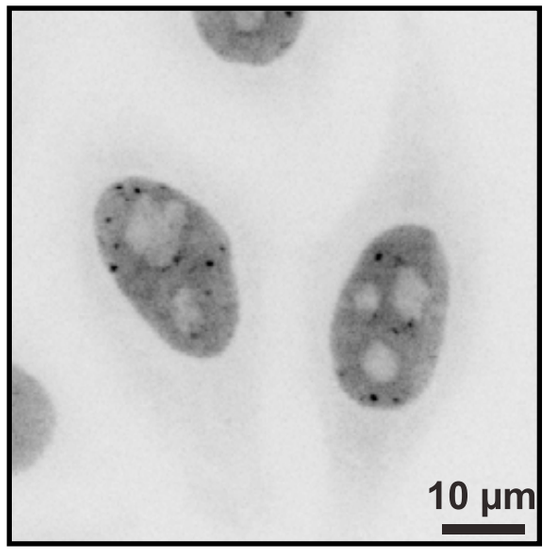
Patel, A. et al. *Cell* **2015**, *162*, 1066.

Liquid–Liquid Phase Separation in Biology

LLPS in amyotrophic lateral sclerosis

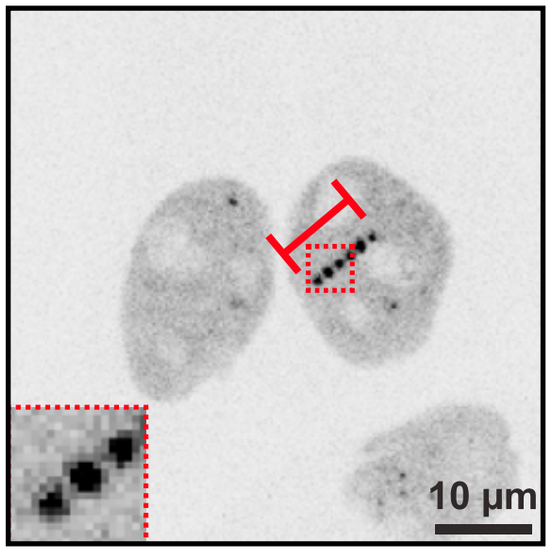
FUS Forms Several Dynamic Compartments in Cells

control



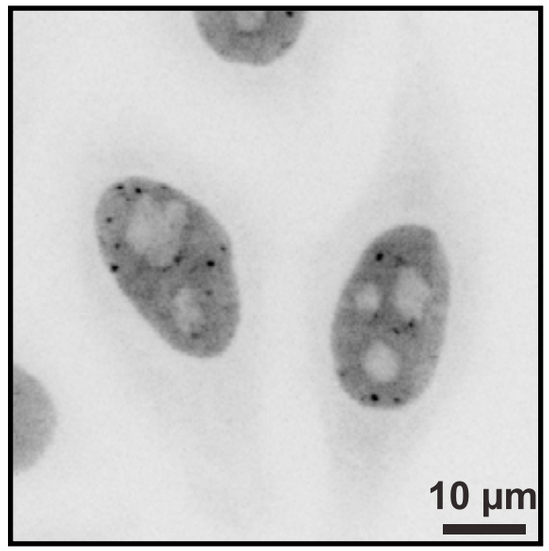
droplets localize to nucleus

DNA damage



droplets accumulate at DNA lesions

heat stress



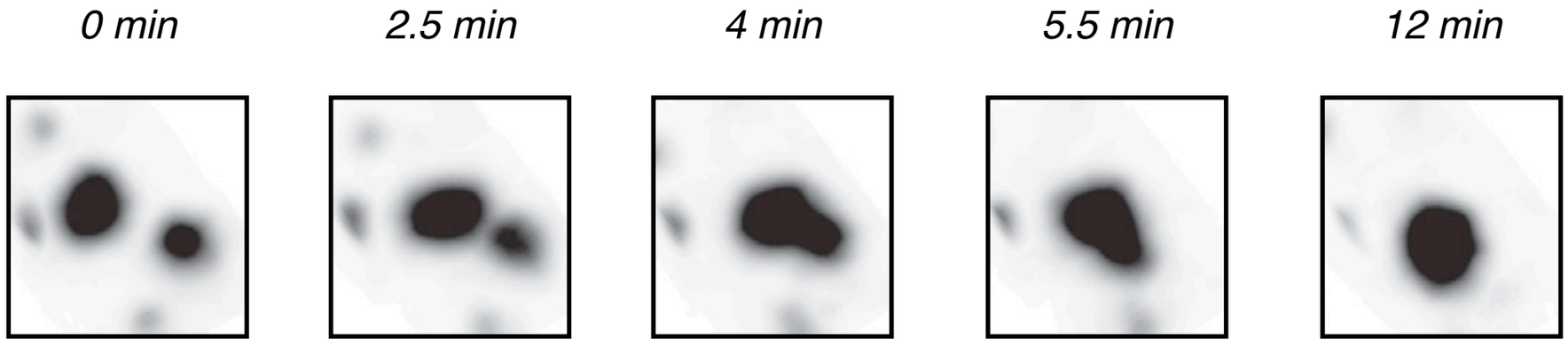
droplets accumulate in cytoplasm

Patel, A. et al. *Cell* **2015**, *162*, 1066.

Liquid–Liquid Phase Separation in Biology

LLPS in amyotrophic lateral sclerosis

FUS Compartments have Liquid-like Properties in Cells



FUS granules undergo fusion events in cells

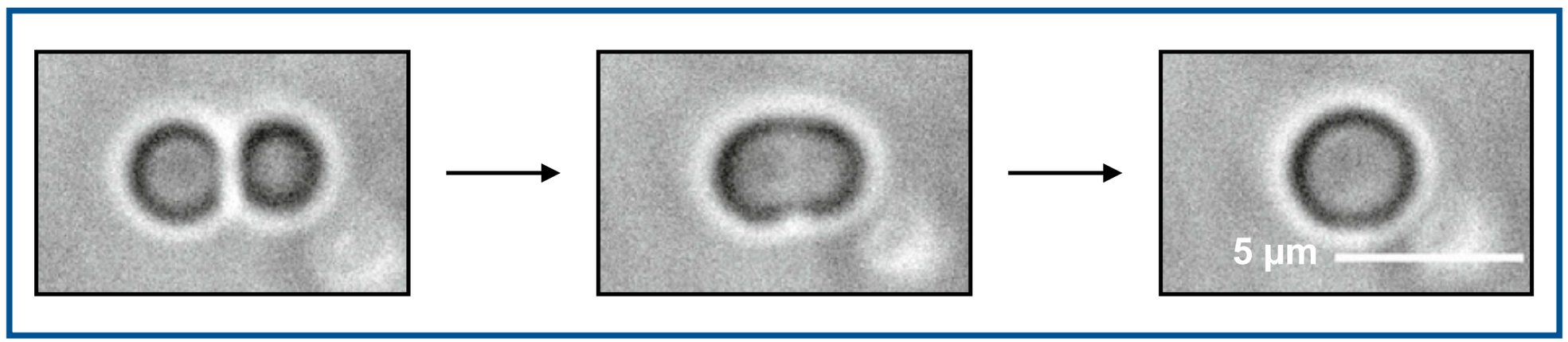
Patel, A. et al. *Cell* **2015**, *162*, 1066.

Liquid–Liquid Phase Separation in Biology

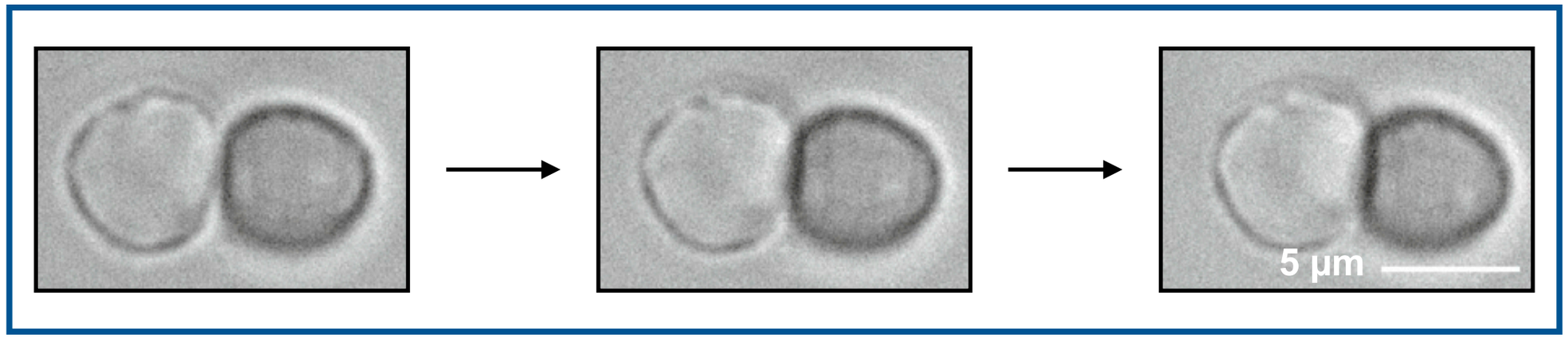
LLPS in amyotrophic lateral sclerosis

in vitro fusion of droplets via optical trap experiments

fusion



no fusion



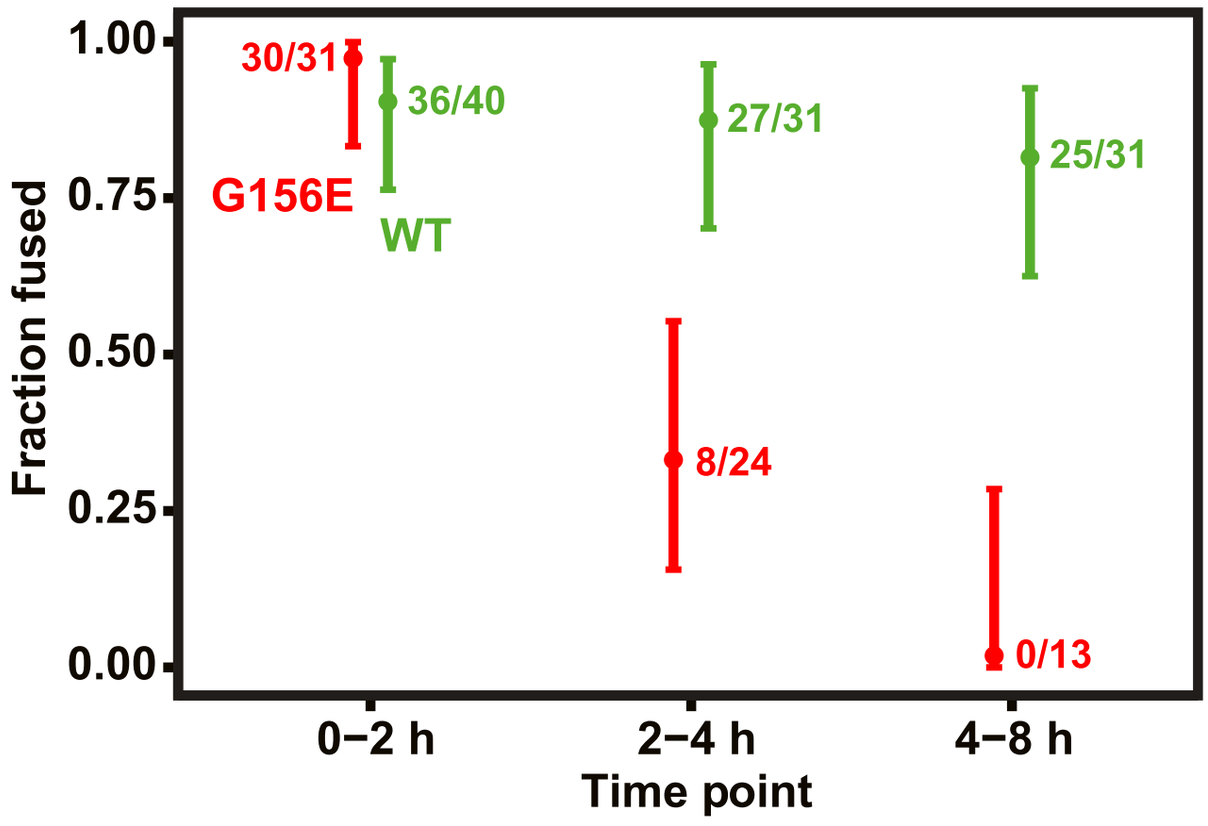
droplets lose ability to fuse over time

Patel, A. et al. *Cell* **2015**, *162*, 1066.

Liquid–Liquid Phase Separation in Biology

LLPS in amyotrophic lateral sclerosis

G156E is a patient derived mutation in FUS

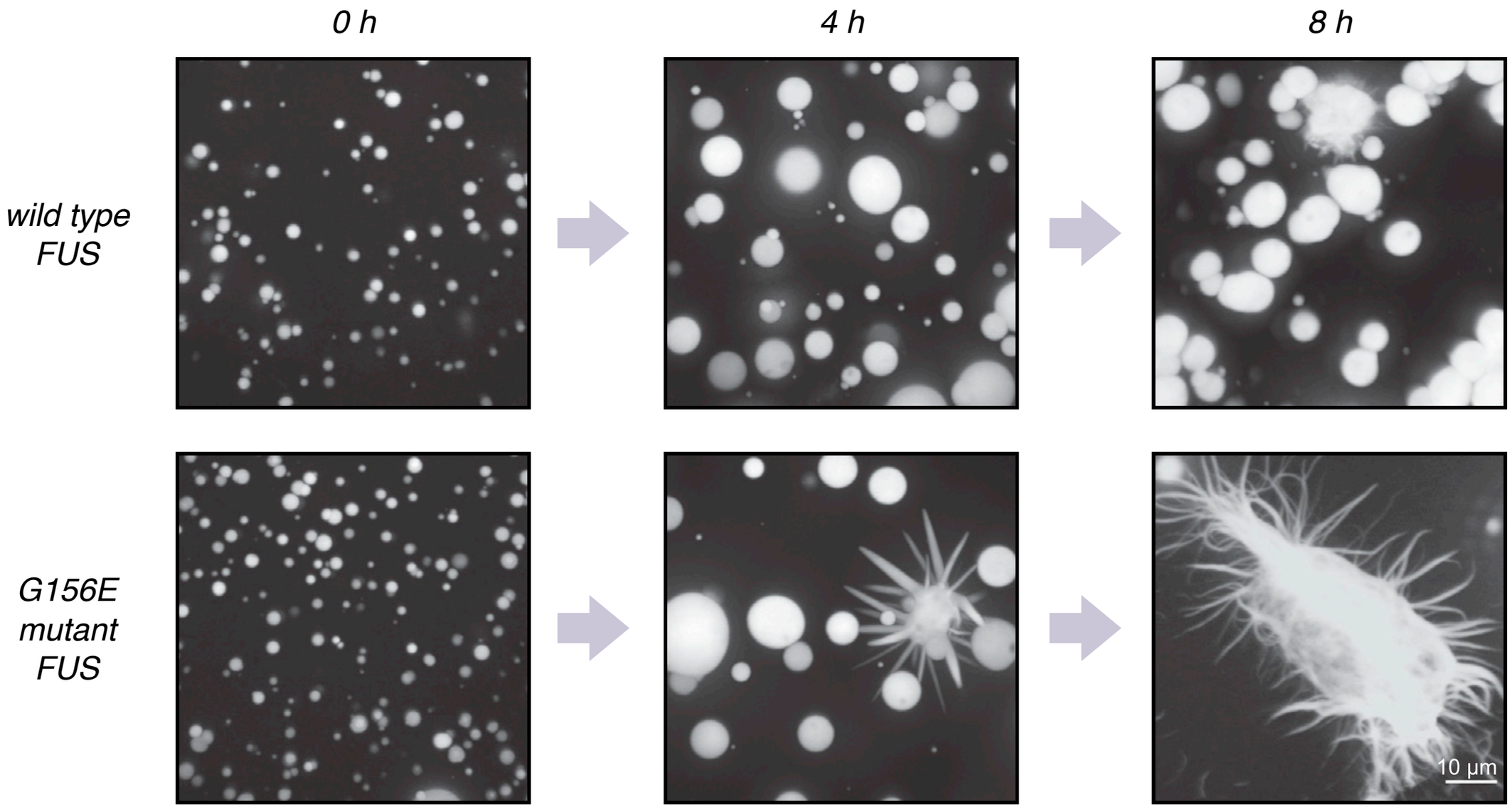


Mutated FUS droplets lose the ability to fuse more quickly than wild type droplets

Patel, A. et al. *Cell* **2015**, *162*, 1066.

Liquid–Liquid Phase Separation in Biology

LLPS in amyotrophic lateral sclerosis

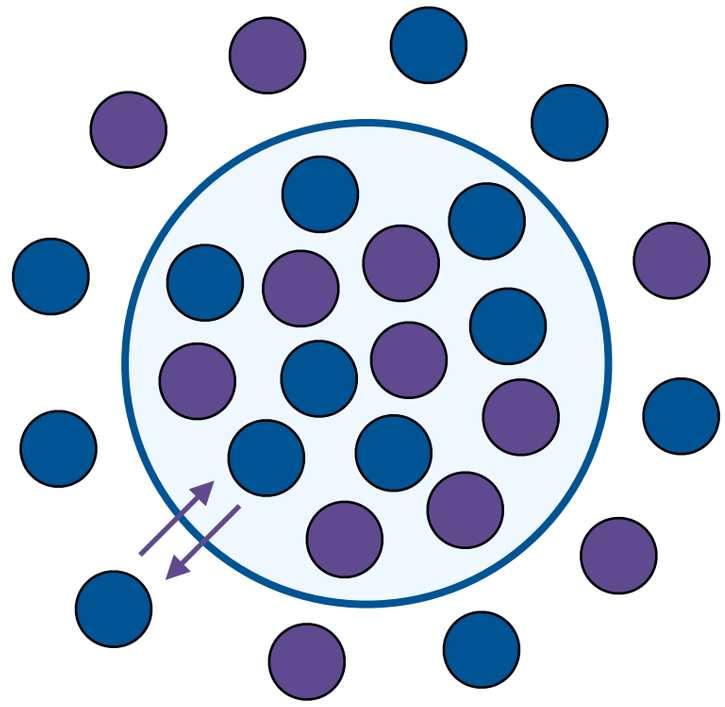


FUS granules convert to aggregated, fibrous state more quickly in mutant

Patel, A. et al. *Cell* **2015**, *162*, 1066.

Liquid–Liquid Phase Separation in Biology

outlook



More tools to control phase separation in cells (e.g. optogenetics)

Uncovering regulatory mechanisms that control LLPS

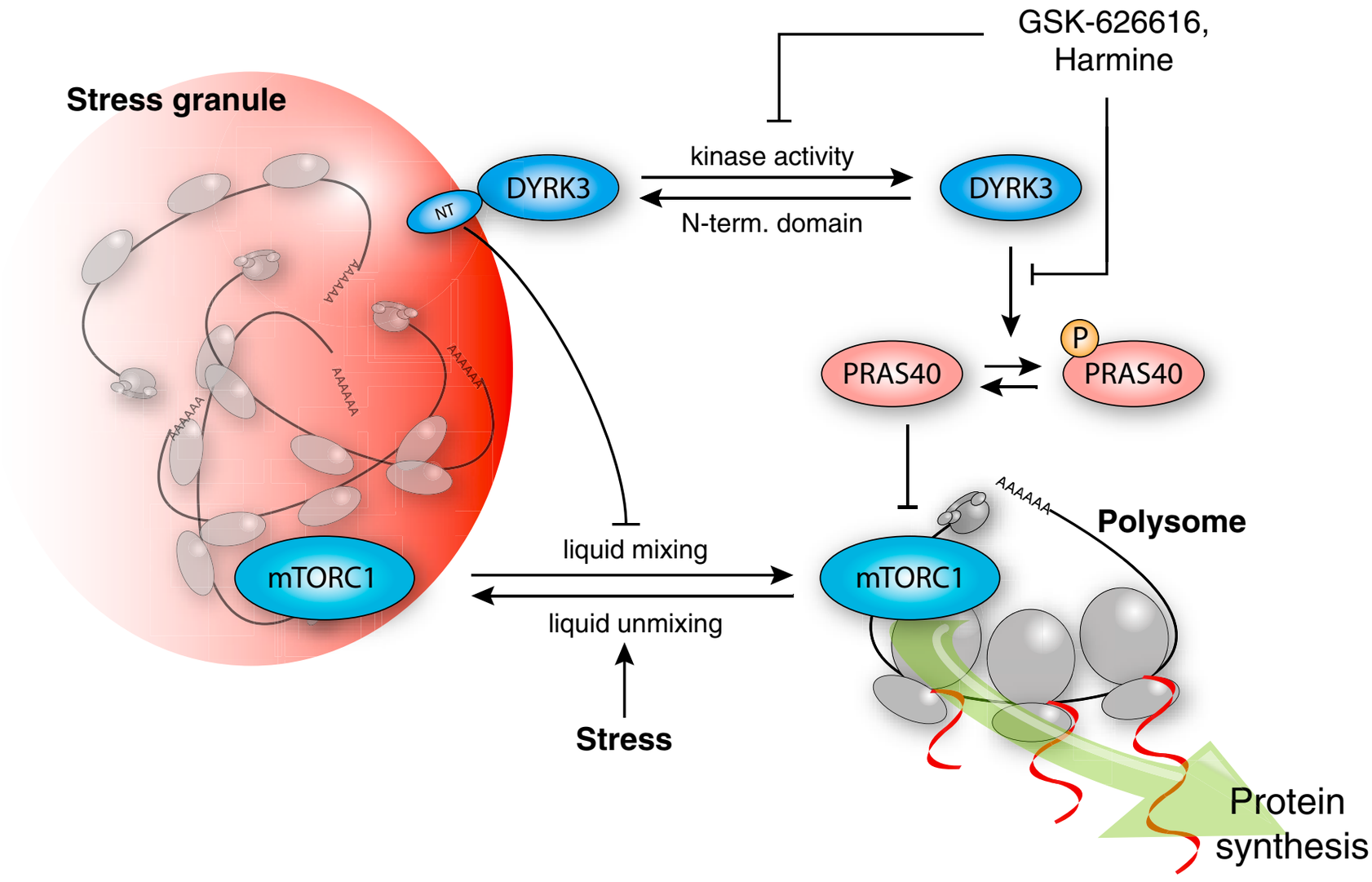
More studies on the physiological functions of LLPS are necessary

Alberti, S.; Gladfelter, A.; Mittag, T. *Cell* **2019**, *176*, 419.

Liquid–Liquid Phase Separation in Biology

the physiological functions of membraneless organelles

3. Sequestration



full mechanism for mTORC1 modulation