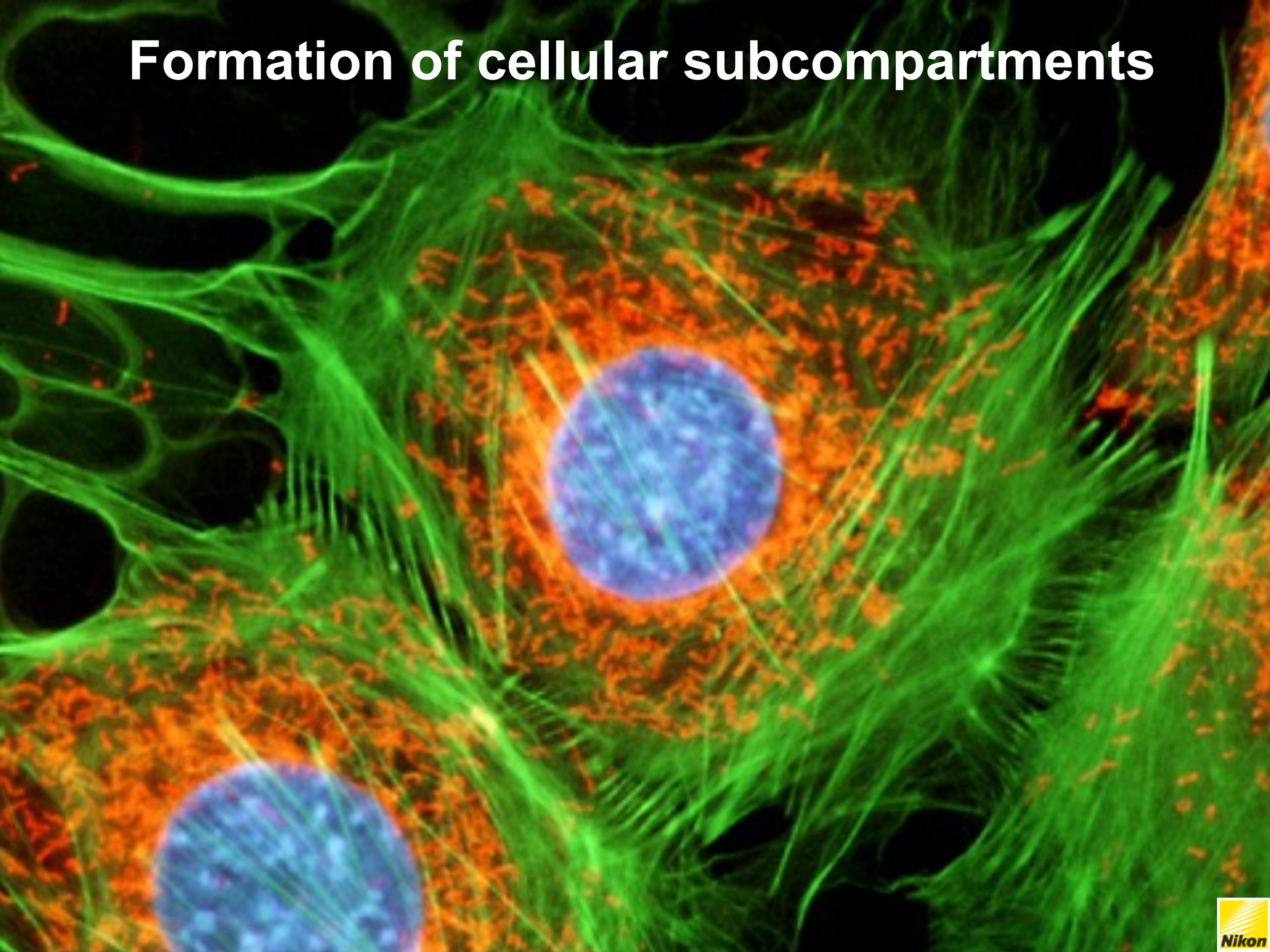
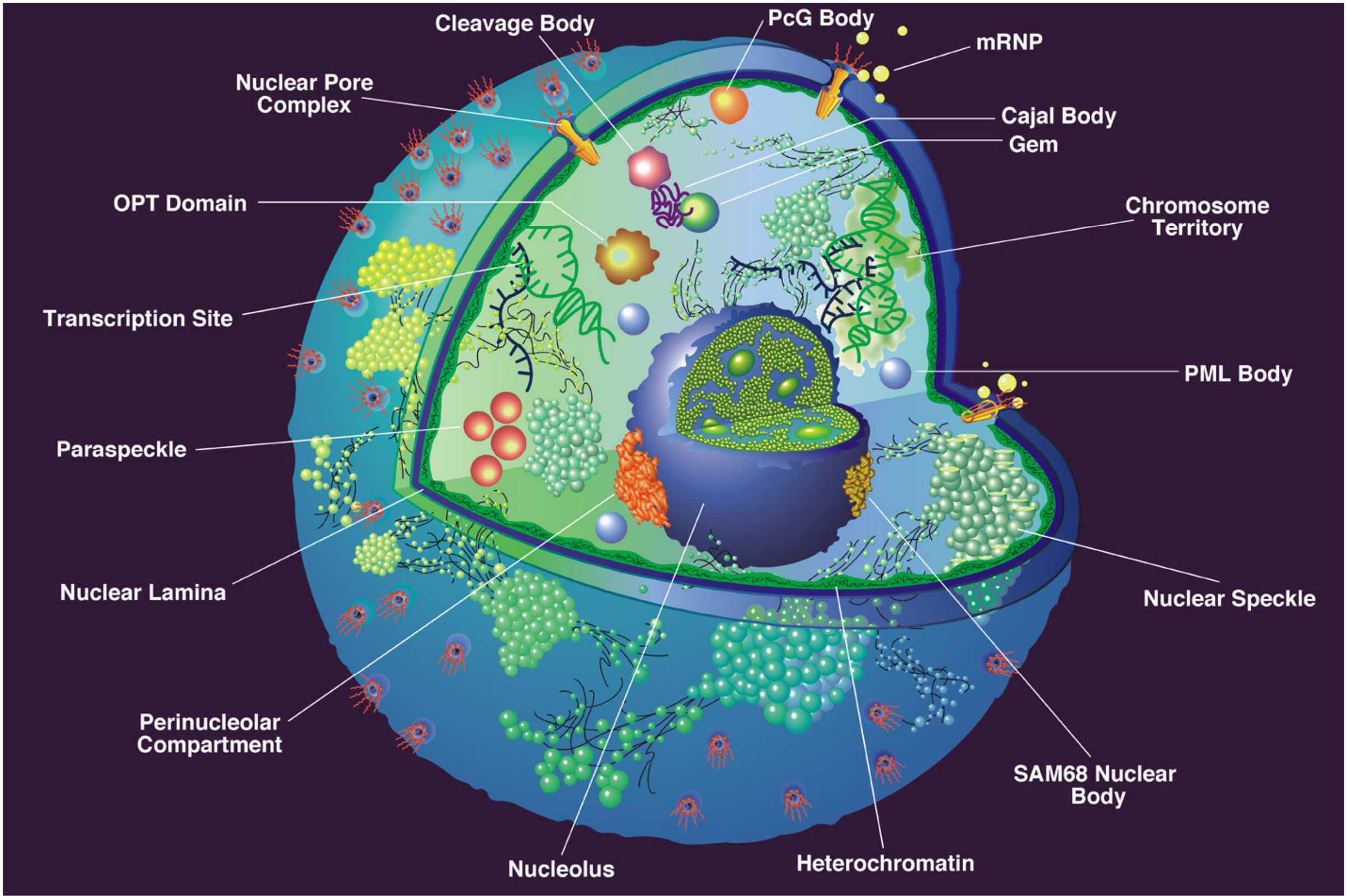
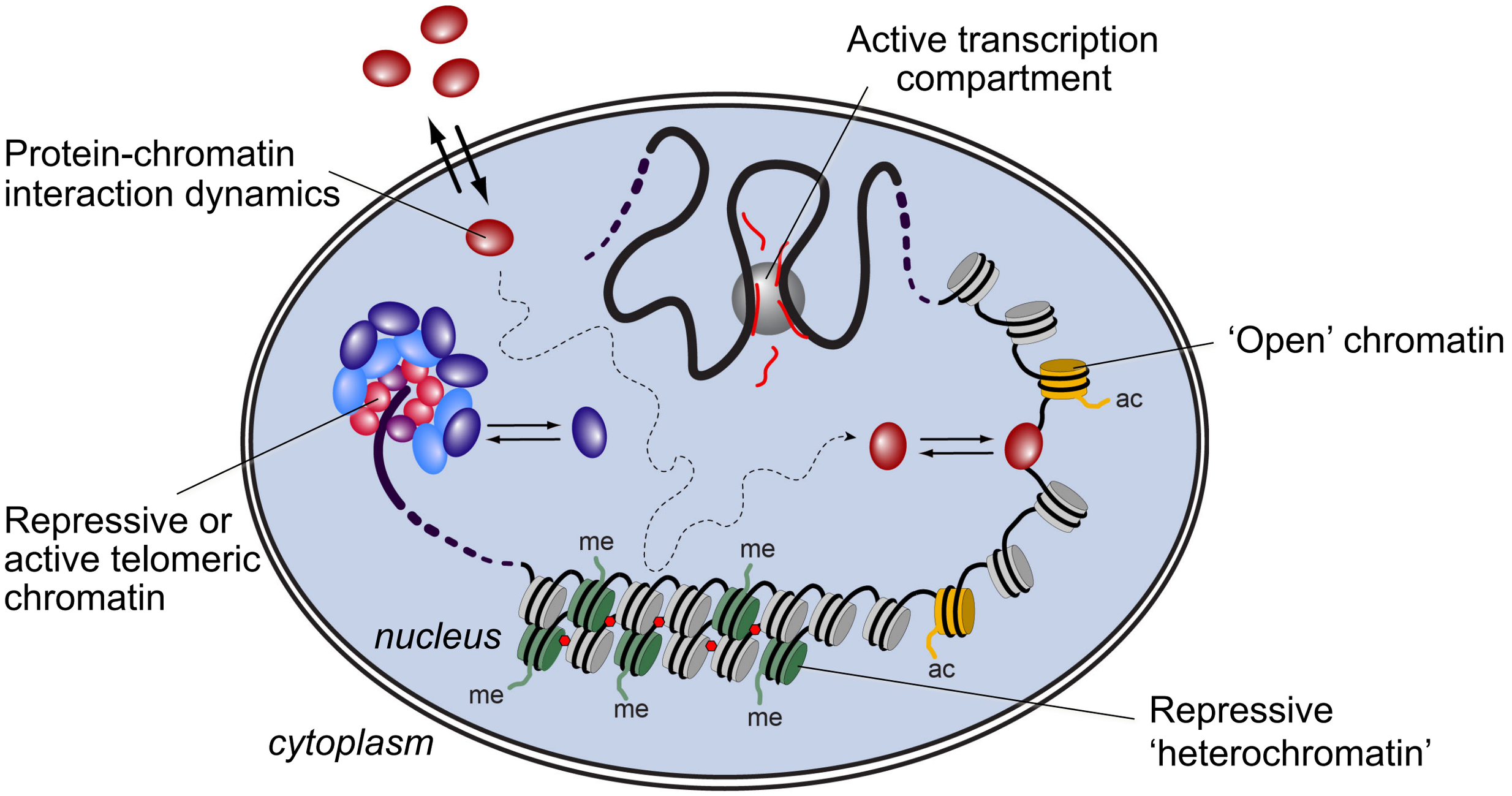
Formation of cellular subcompartments



The mammalian cell nucleus



Chromatin states and nuclear subcompartments



Gravity is not relevant for proteins in the cell…

… and everything should mix fast by diffusion

Self-assembly versus self-organization
(as defined by Tom Misteli)

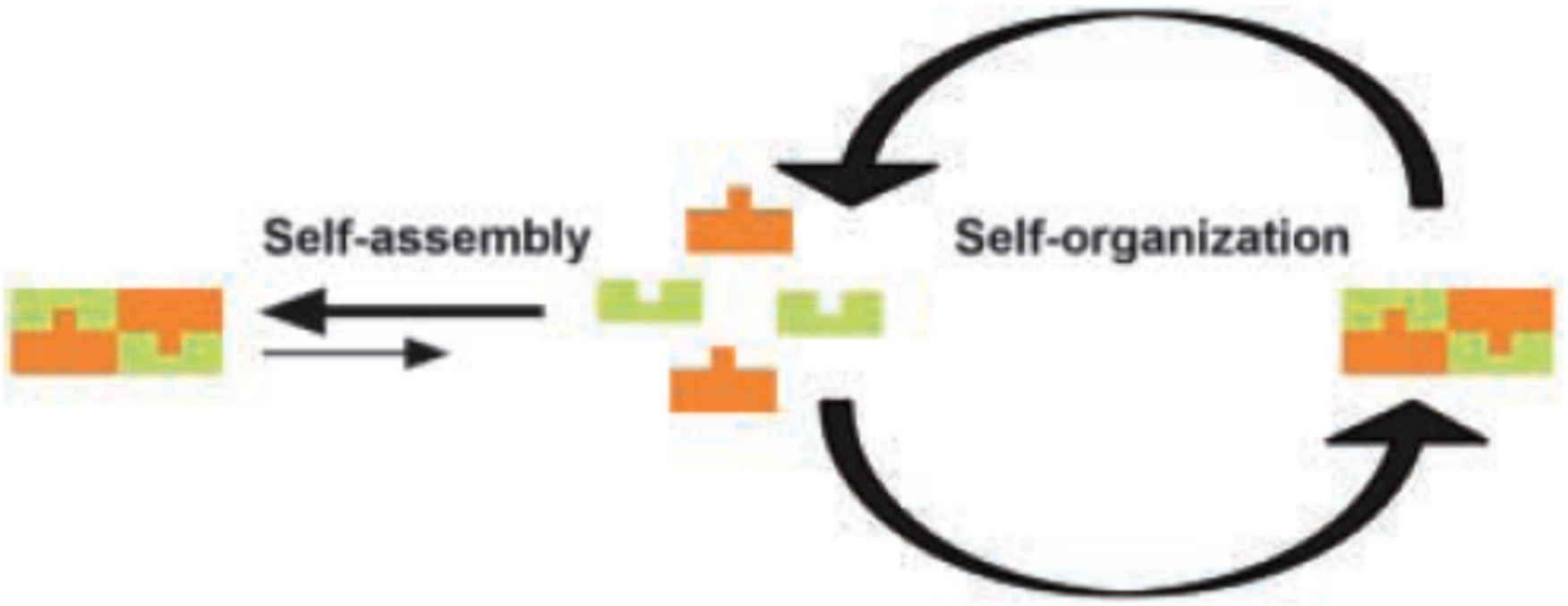
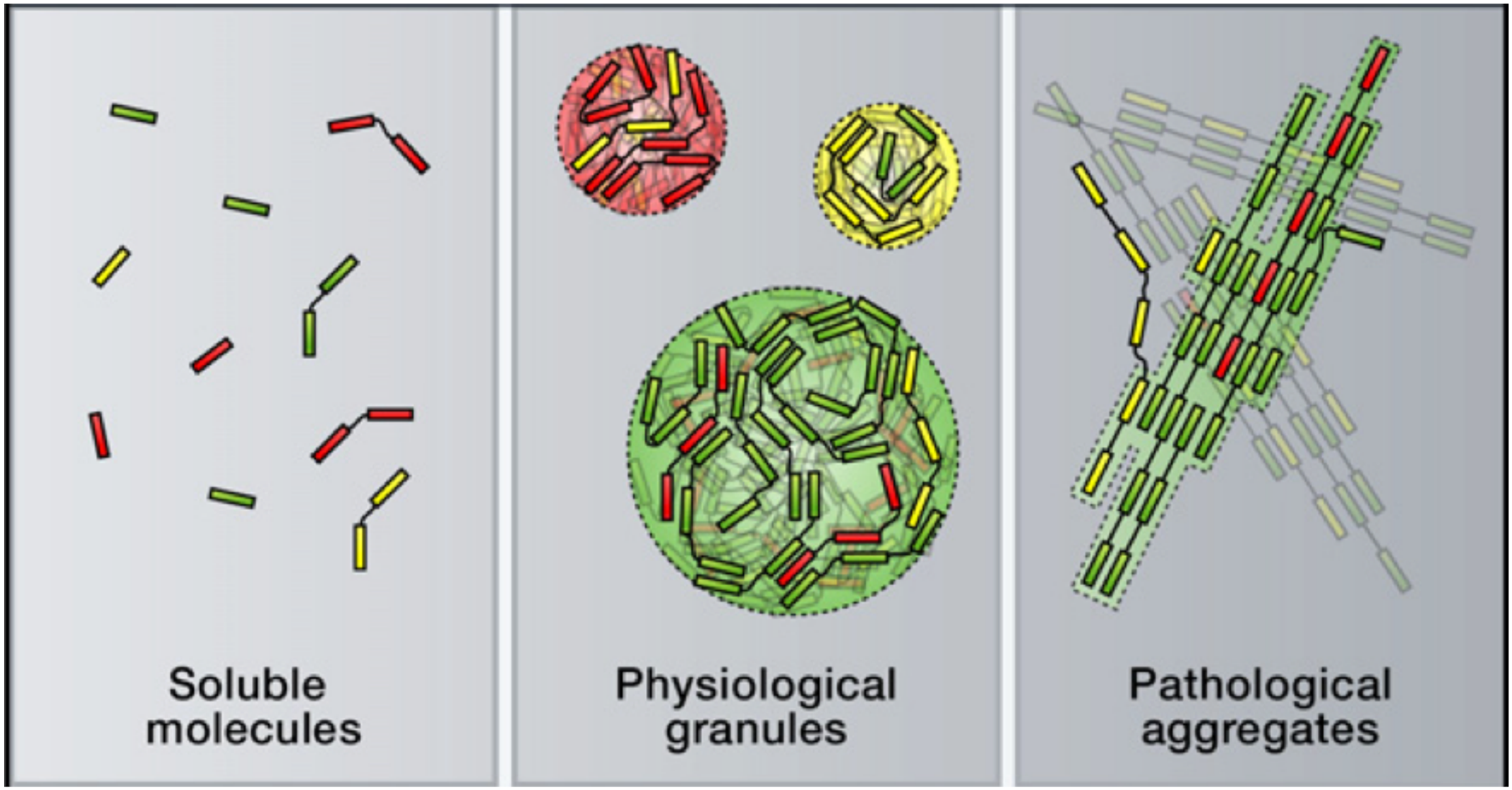


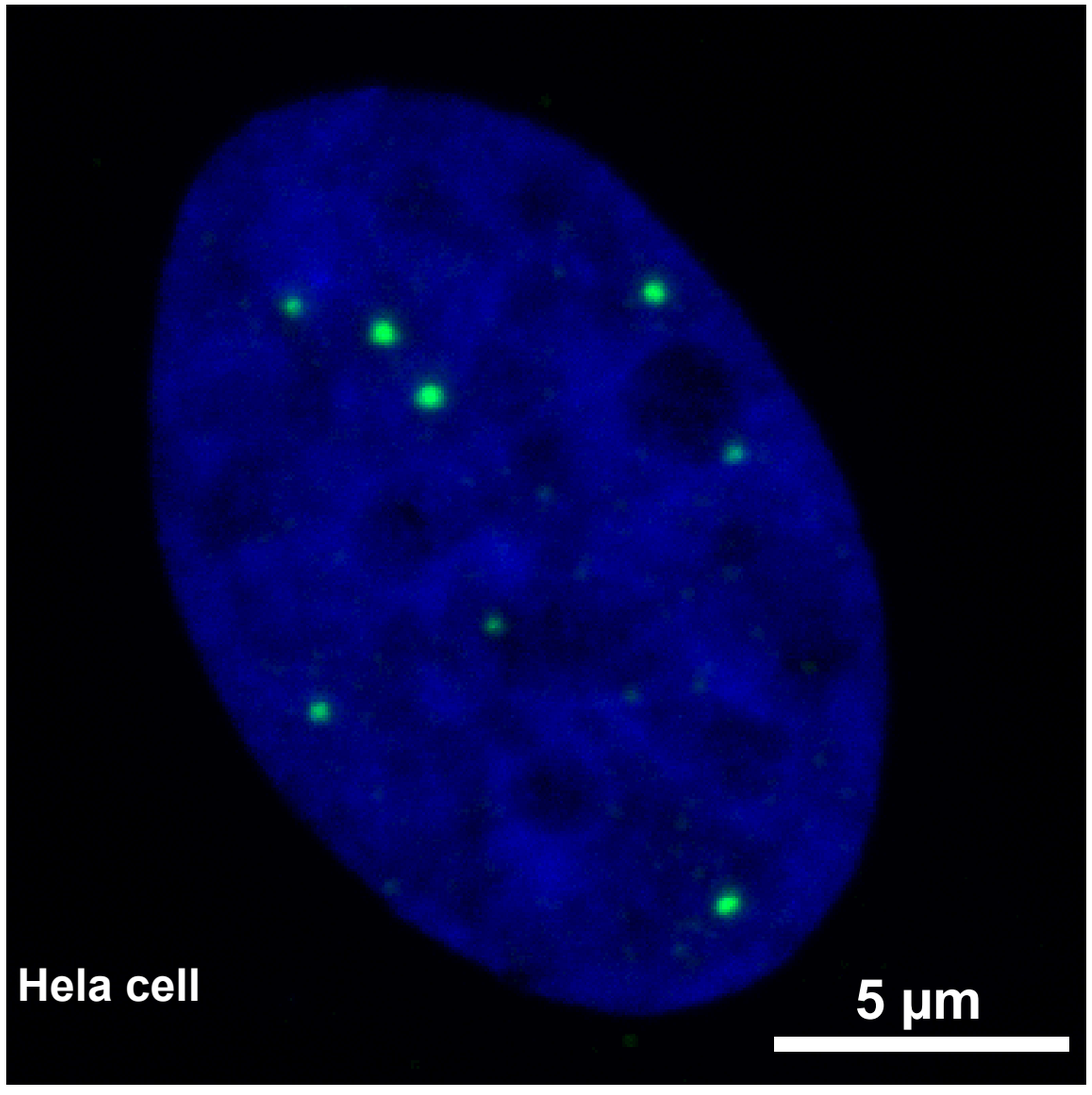
Figure 1. **Self-assembly versus self-organization.** In self-assembly, a set of components assembles into a stable, static structure that reaches a thermodynamic equilibrium. In self-organization, a set of components assembles into a steady-state, dynamic structure.

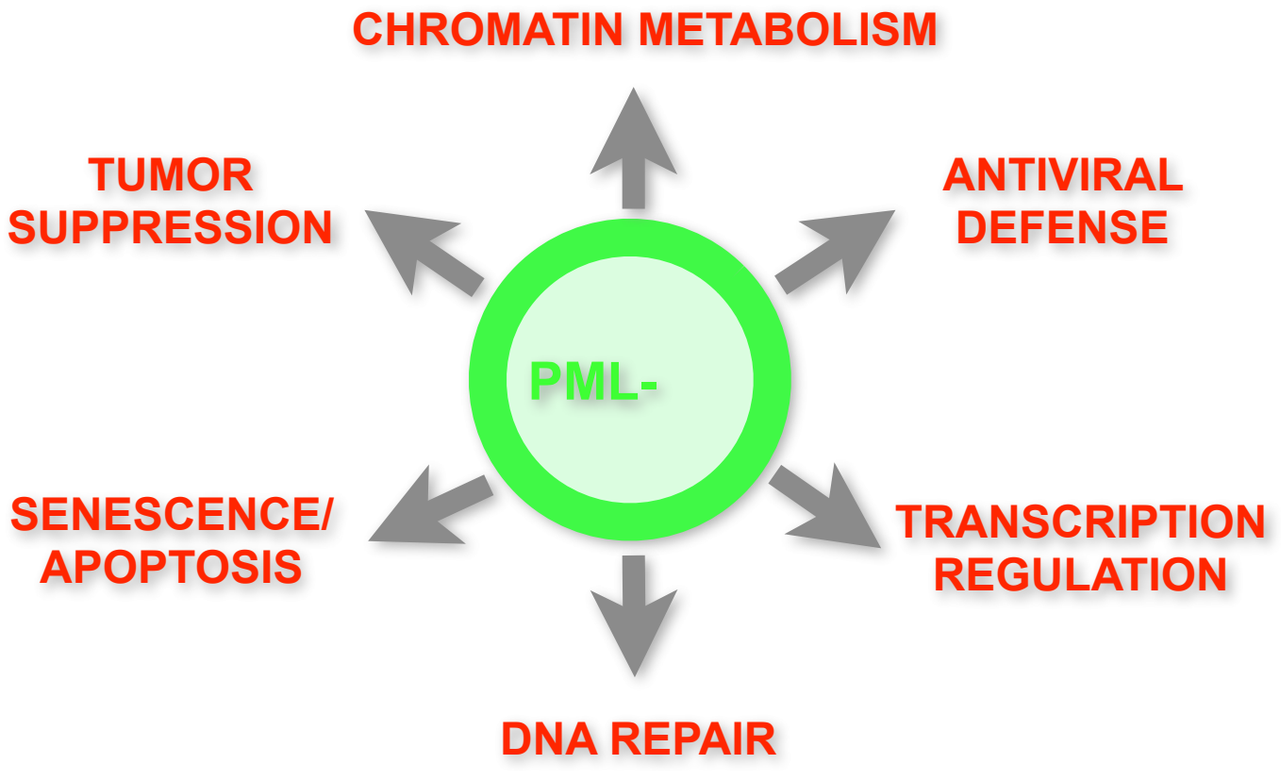
Subcompartment formation in the cell



Weber, 2012, Cell

The promyelocytic leukemia (PML) nuclear body



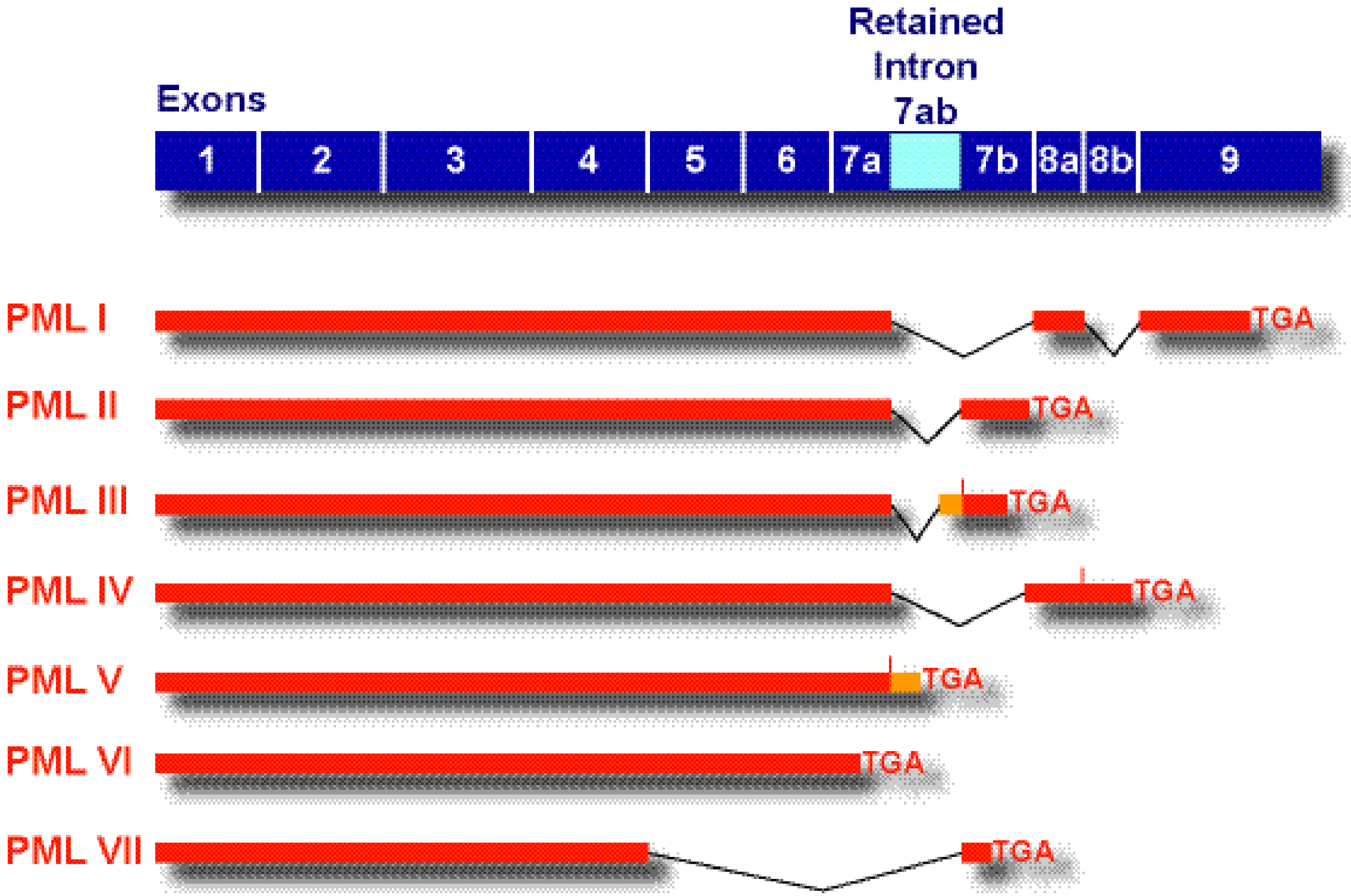


blue: DAPI; green: anti PML immunostaining

Görisch, Wachsmuth, Ittrich, Bacher, Rippe & Lichter (2004). Nuclear body movement is determined by chromatin accessibility and dynamics. *Proc Natl Acad Sci USA* **101**, 13221–13226 (2004).

Structure of the PML protein

PML protein is present in 7 splicing variants

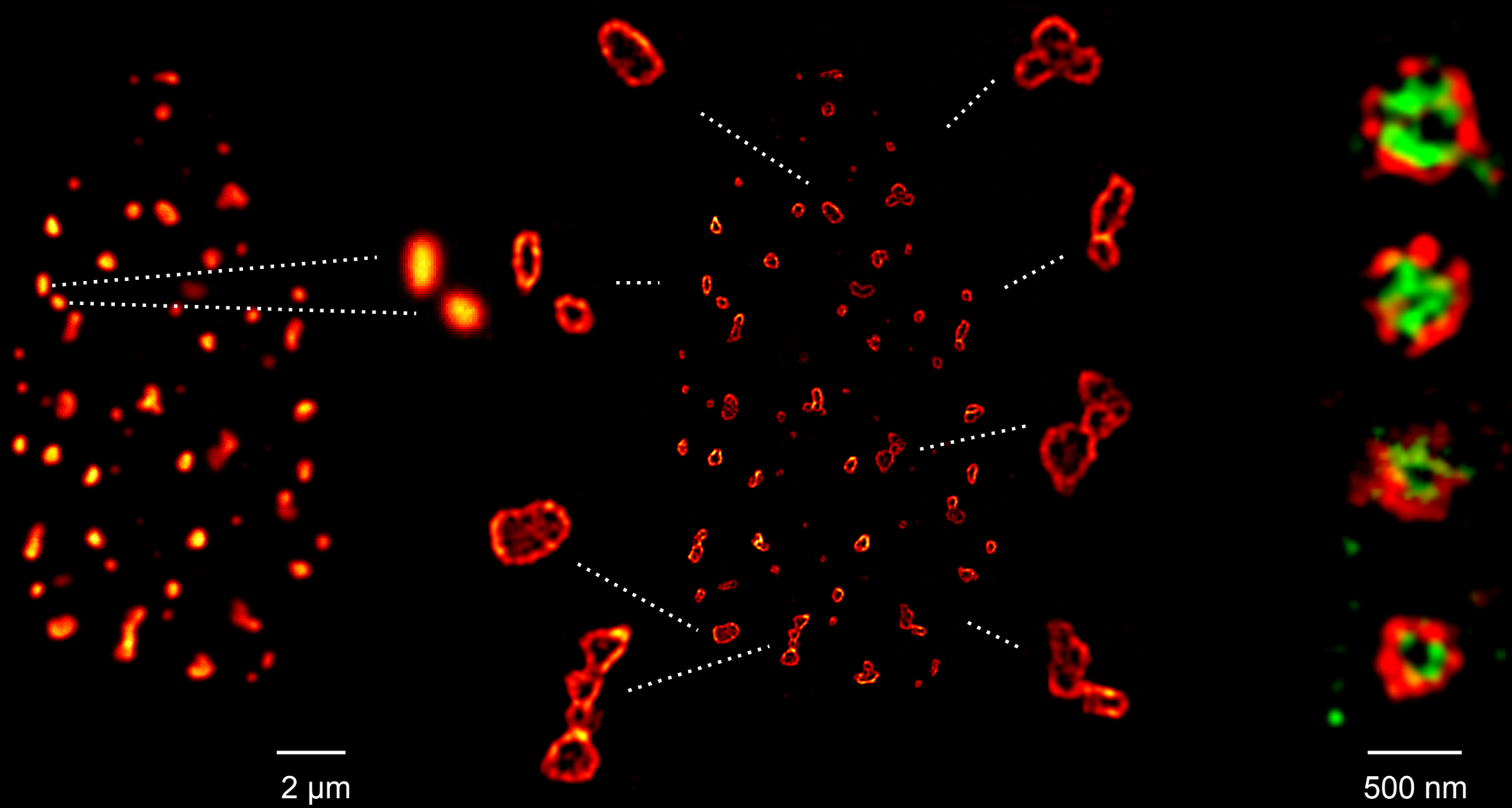


PML nuclear bodies and their complexes with telomeres

PML confocal microscope

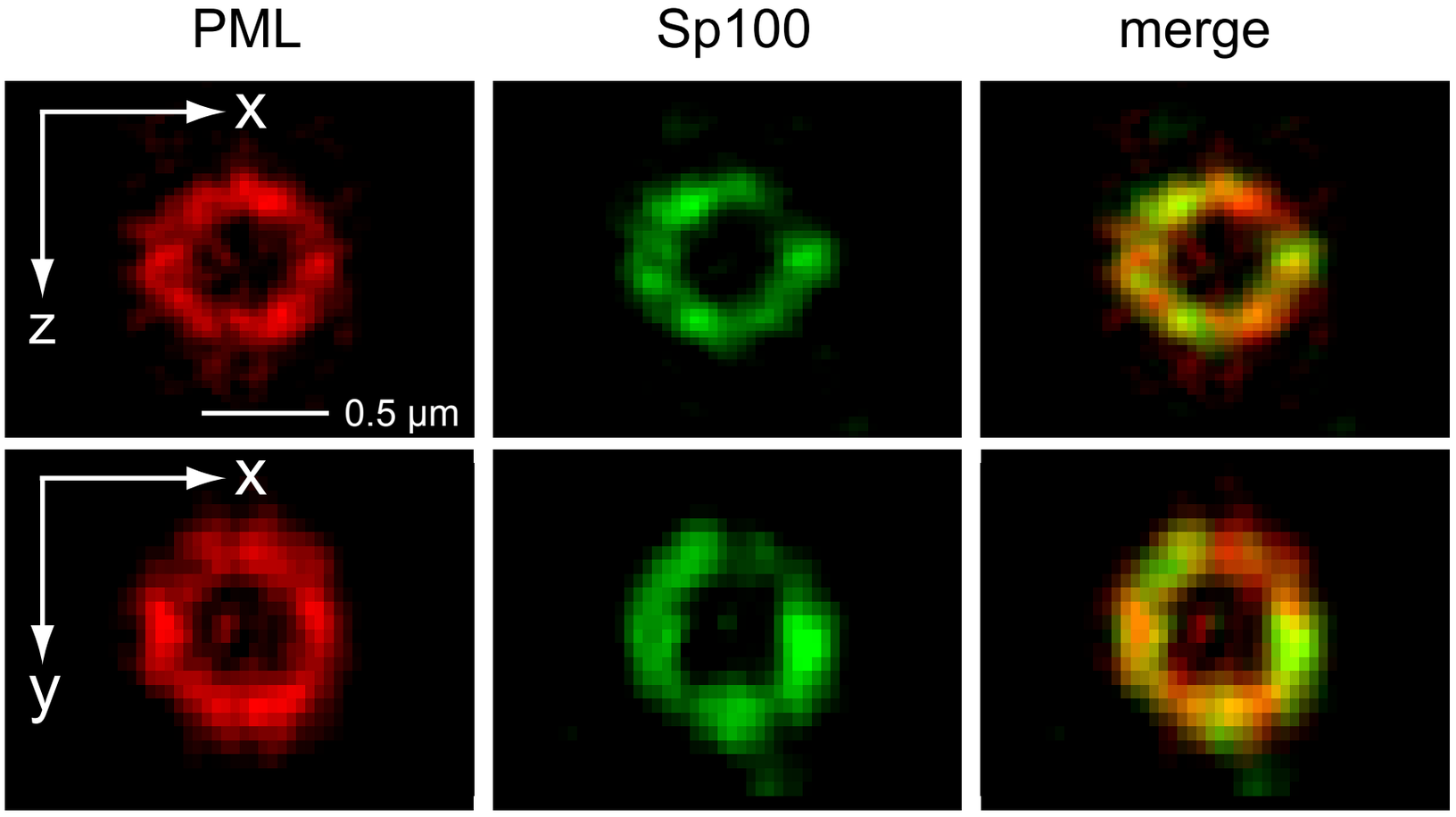
PML STED image

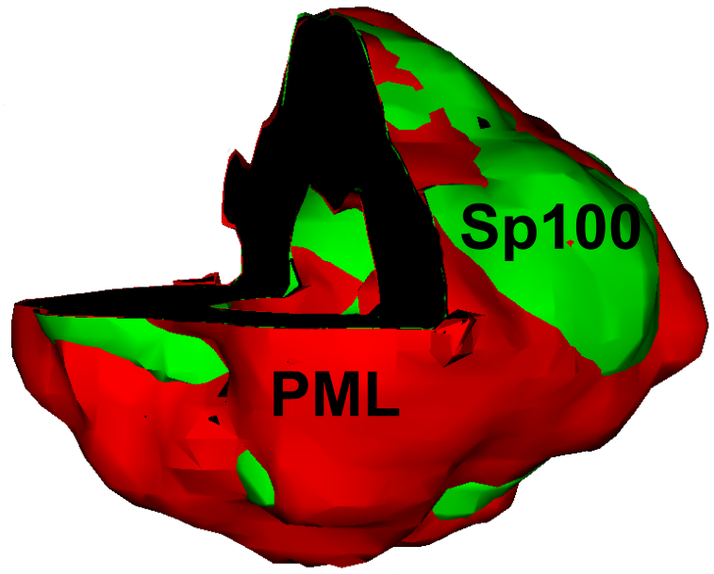
PML-telomere complexes



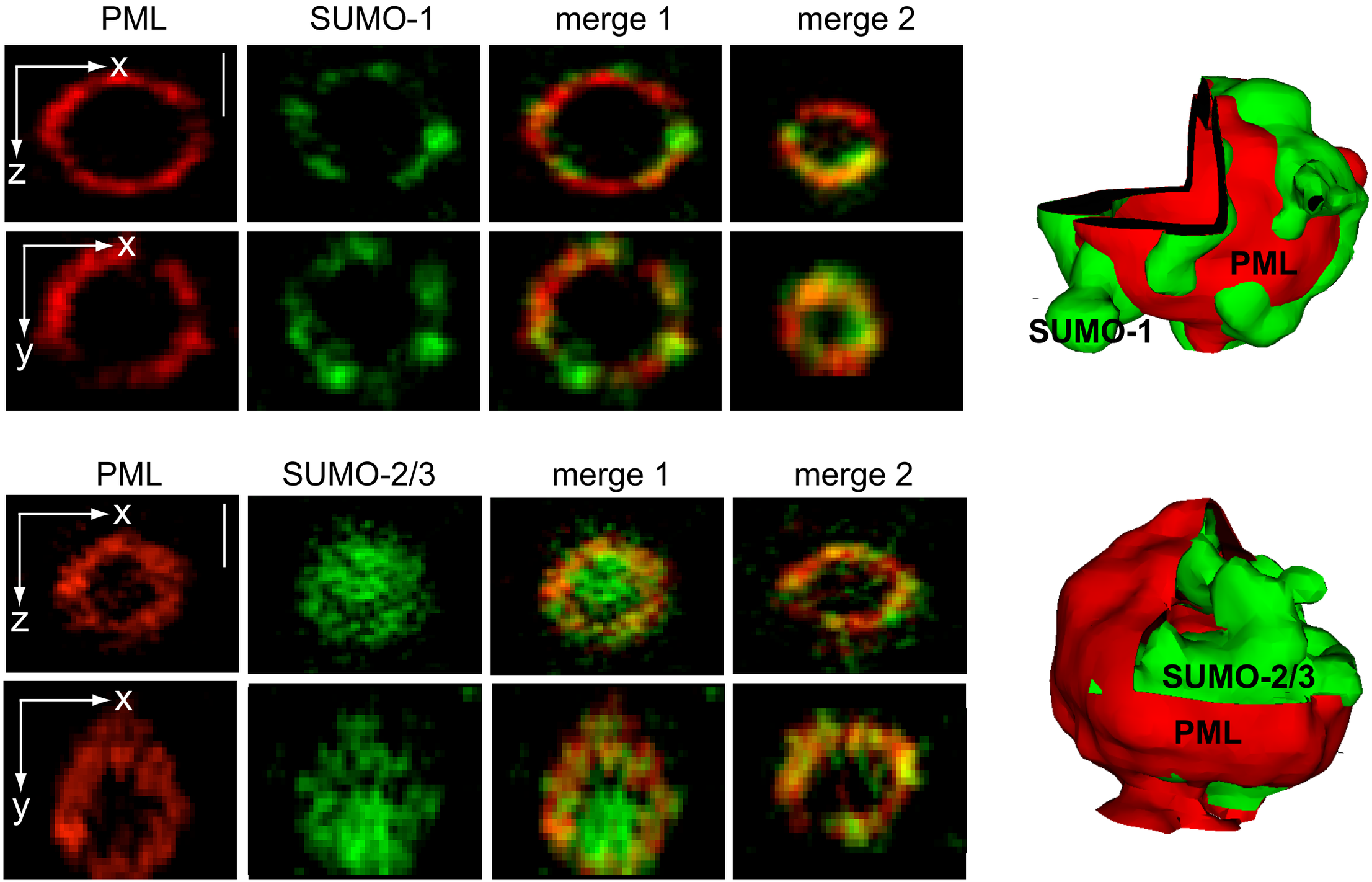
Matthias, Chung, Engelhardt, Hell & Rippe

PML and Sp100 proteins form distinct patches in the spherical shell of the PML nuclear body

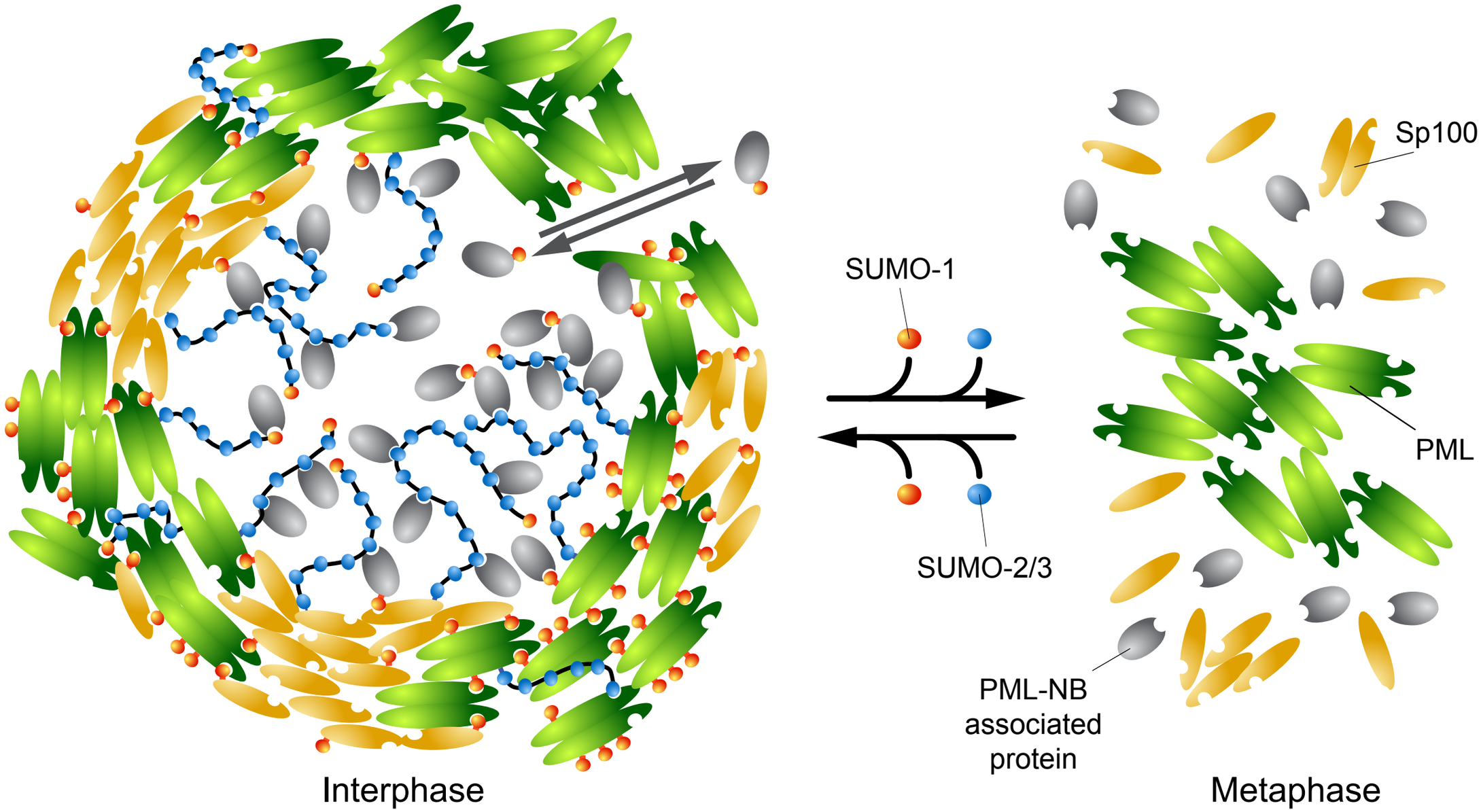




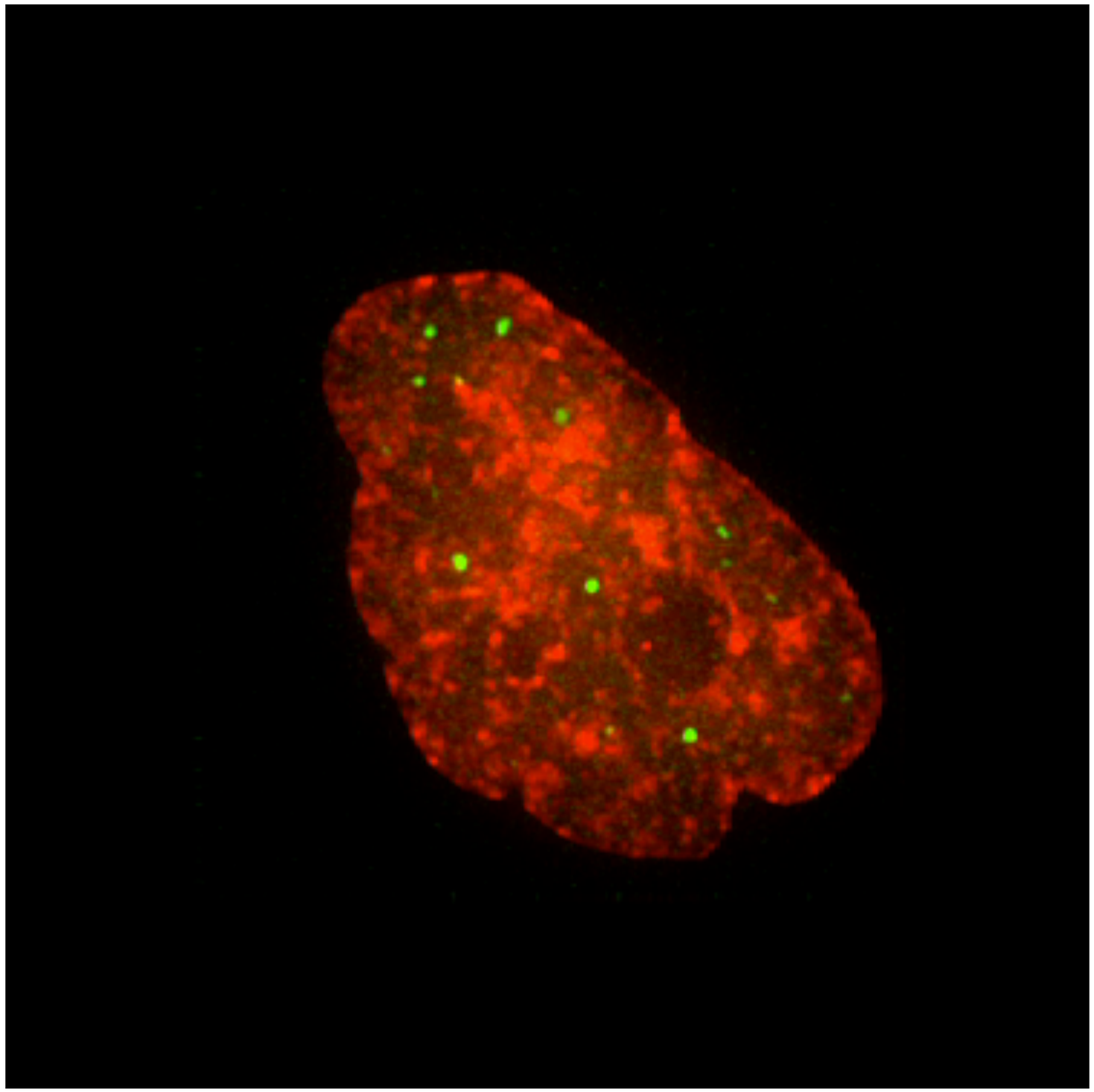
Localization of SUMO modification in PML-NBs



Model for the dynamic structure of a PML nuclear body



Movements of PML bodies (green) in the nucleus

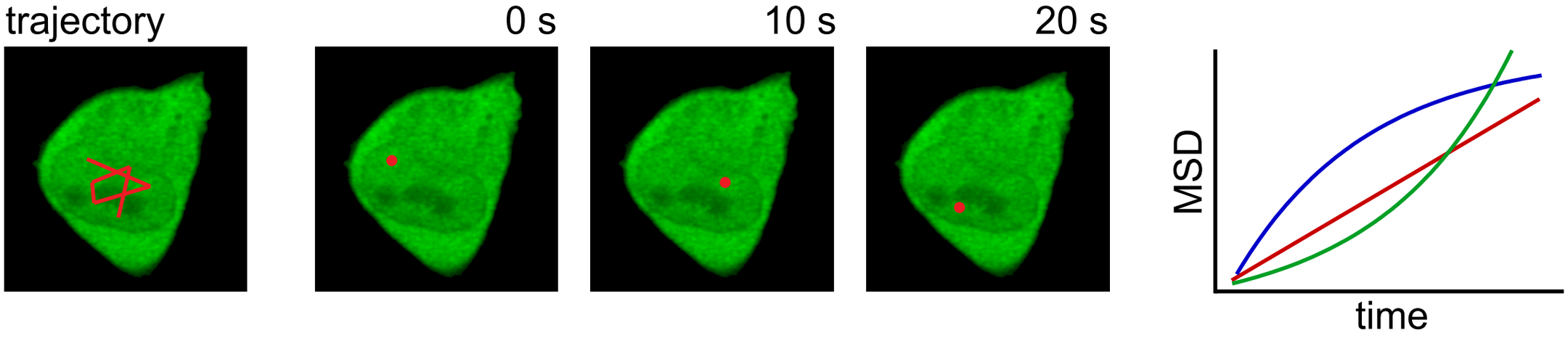


Single Particle tracking (SPT):
nuclear bodies, chromatin loci, proteins, RNA

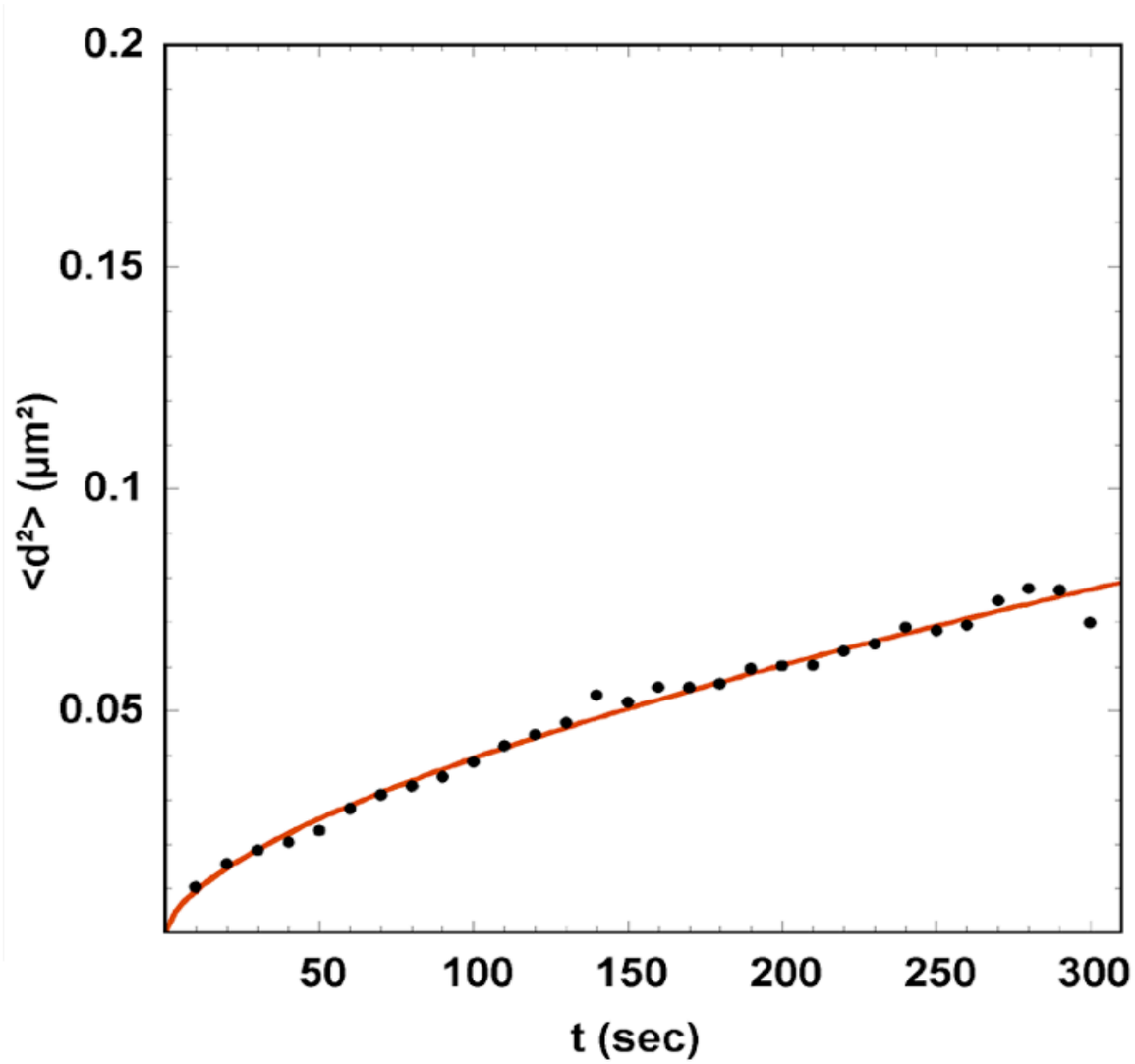
- Easiest approach to measure mobility:
  Directly watch single particles (over time)

- Prerequisites:
  Low concentration, bright & slow particles

SPT



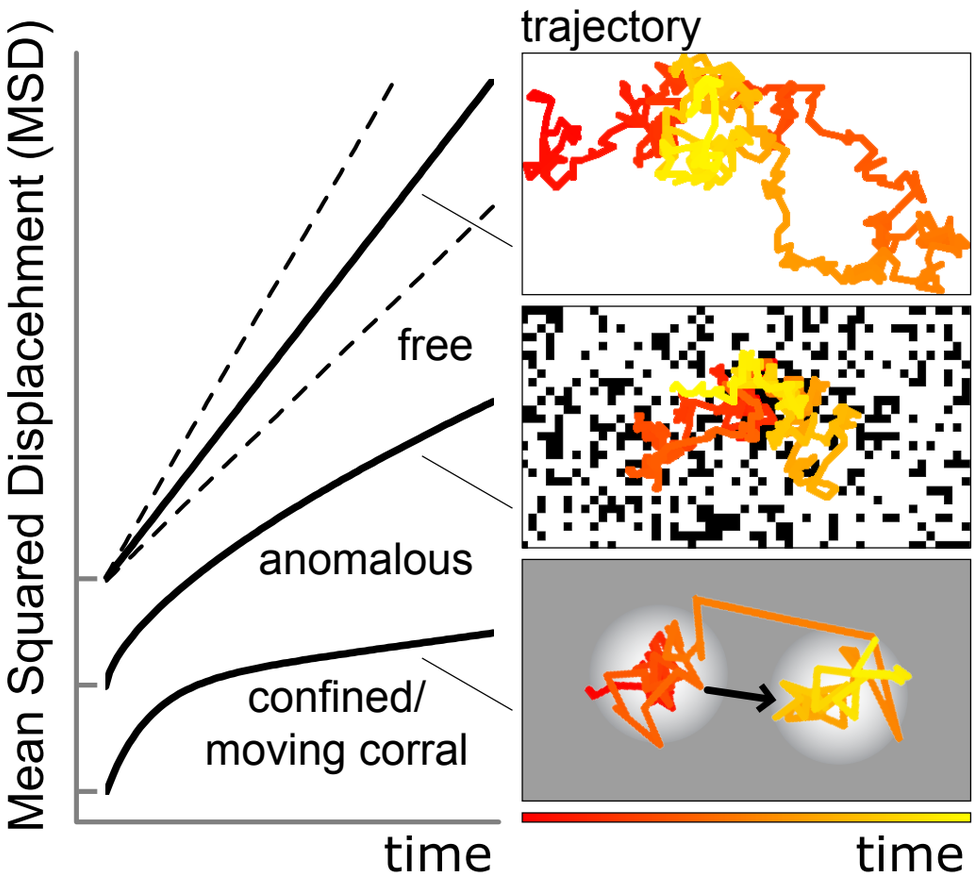
Due to obstructions and or binding the mean square displacement is no longer proportional to time

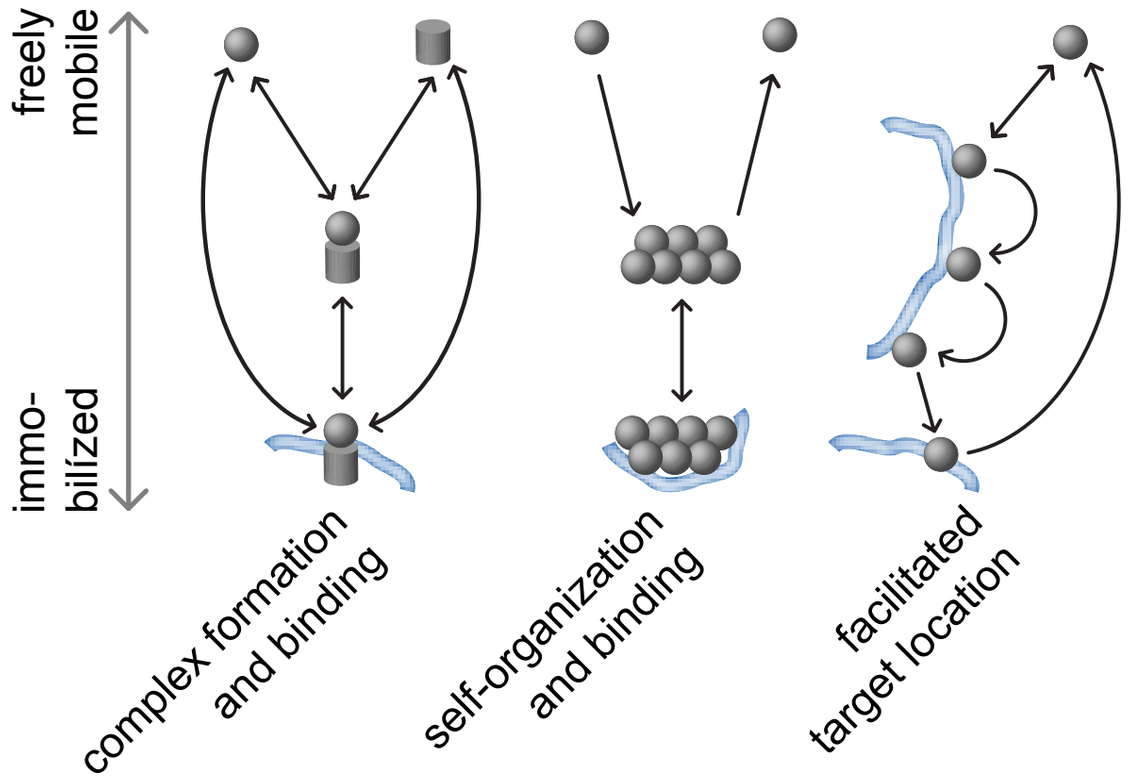


Movement of a PML body in the nucleus

Protein mobility and interactions in the cell

$$MSD = 6\,D\,t^{\alpha}$$





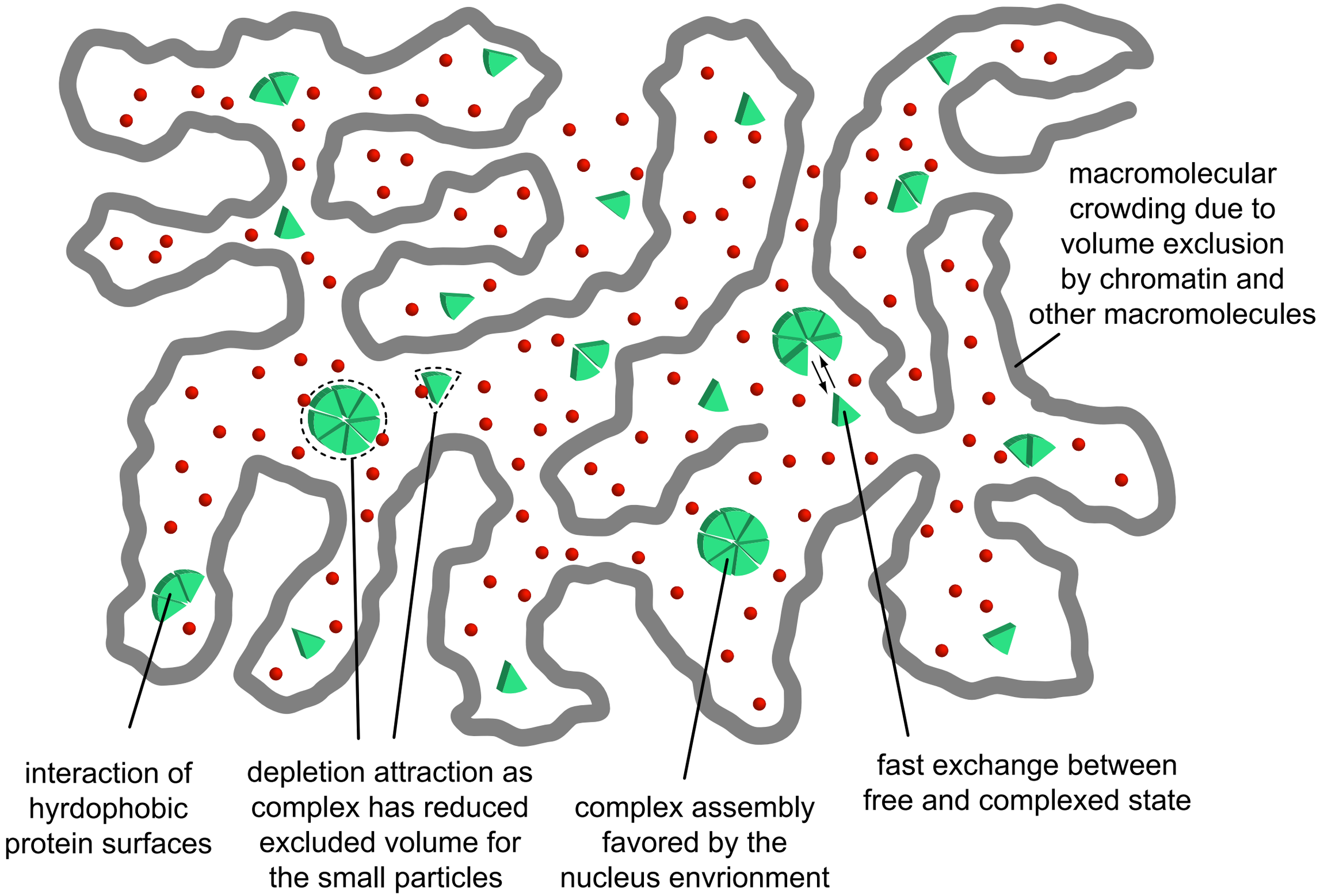
Dependence of diffusion coefficient D and molecular mass *M*

protein: $D \propto M^{-\frac{1}{3}}$ DNA: $D \propto M^{-\frac{1}{2}}$

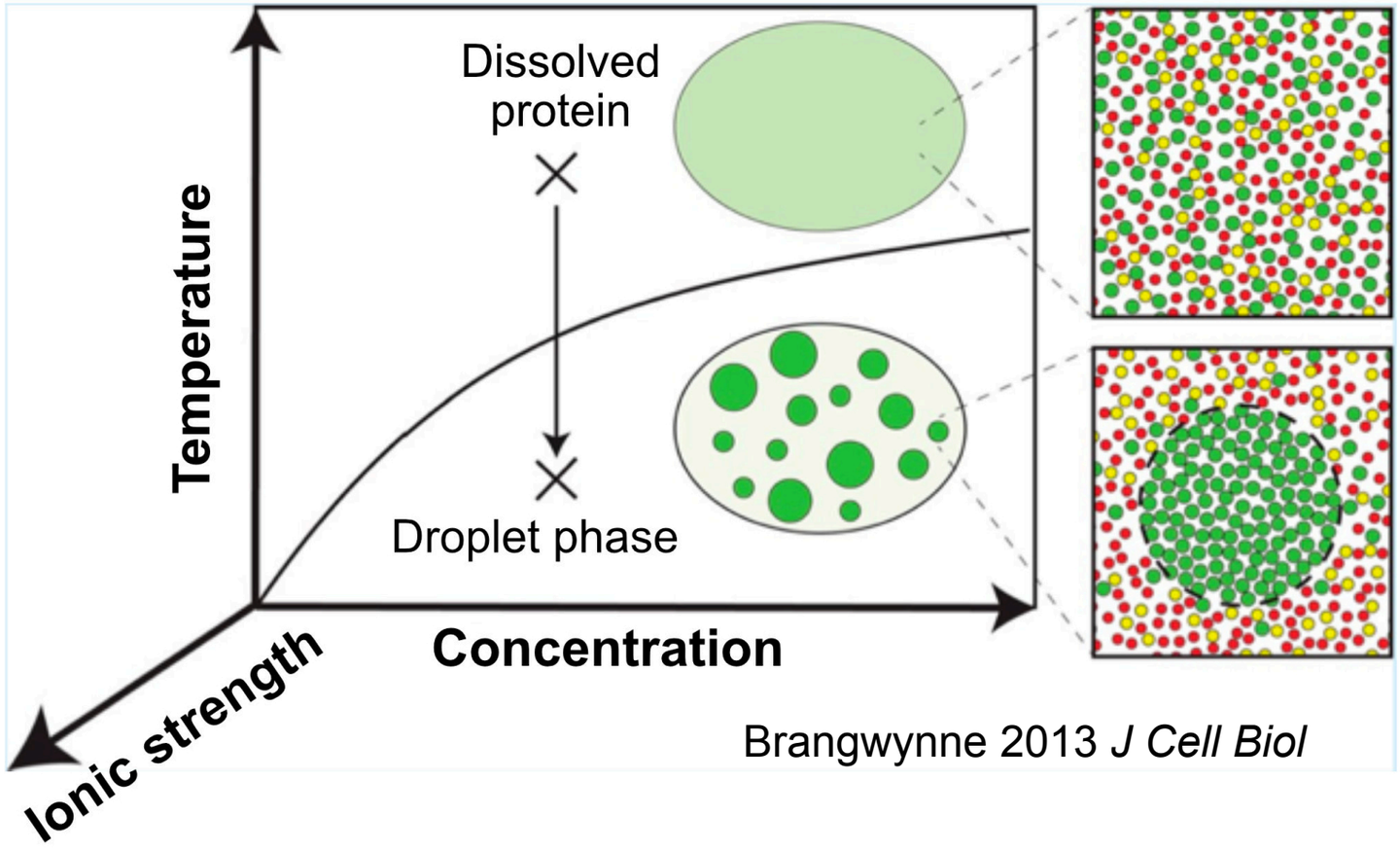
double mass *M* => 0.8 fold lower *D* double mass *M* => 0.7 fold lower *D*

Wachsmuth, M., Caudron-Herger, M. and Rippe, K. (2008). *Biochim. Biophys. Acta* **1783**, 2061-2079.

Self-organization in the nucleus

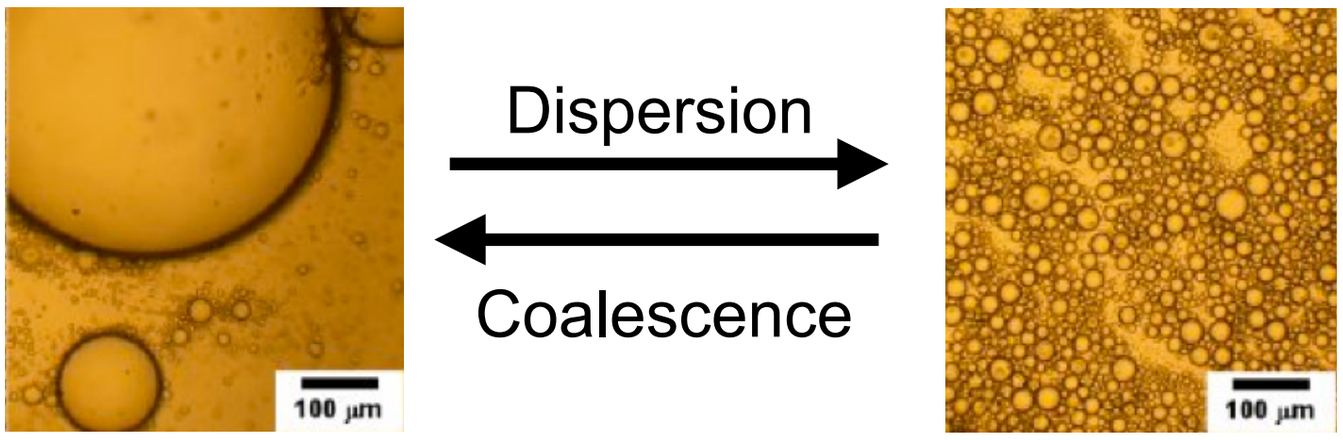


Liquid-liquid phase separation (LLPS) in the cell



Brangwynne 2013 *J Cell Biol*

The “oil droplets in water” model



LLPS for chromatin

Nucleolus
Brangwynne 2011 *PNAS*
Feric 2016 *Cell*

(Peri)centromeres/ heterochromatin
Larson 2017 *Nature*
Strom 2017 *Nature*
Cerase 2019 *Nat Struct Mol Biol*
Wang 2019 *Mol Cell*
Trivedi 2019 *Nat Cell Biol*
Sanulli 2019, *Nature*

Telomeres
Shin 2018 *Cell*
Min 2019 *Genes Dev*

“Transcriptional condensates”
Hnisz 2017 *Cell*
Sabari 2018 *Science*
Boija 2018 *Cell*
Boehning 2018 *Nat Struct Mol Biol*
Cho 2018 *Science*
Lu 2018 *Nature*
Chong 2018 *Science*
Shrinivas 2019 *Mol Cell*

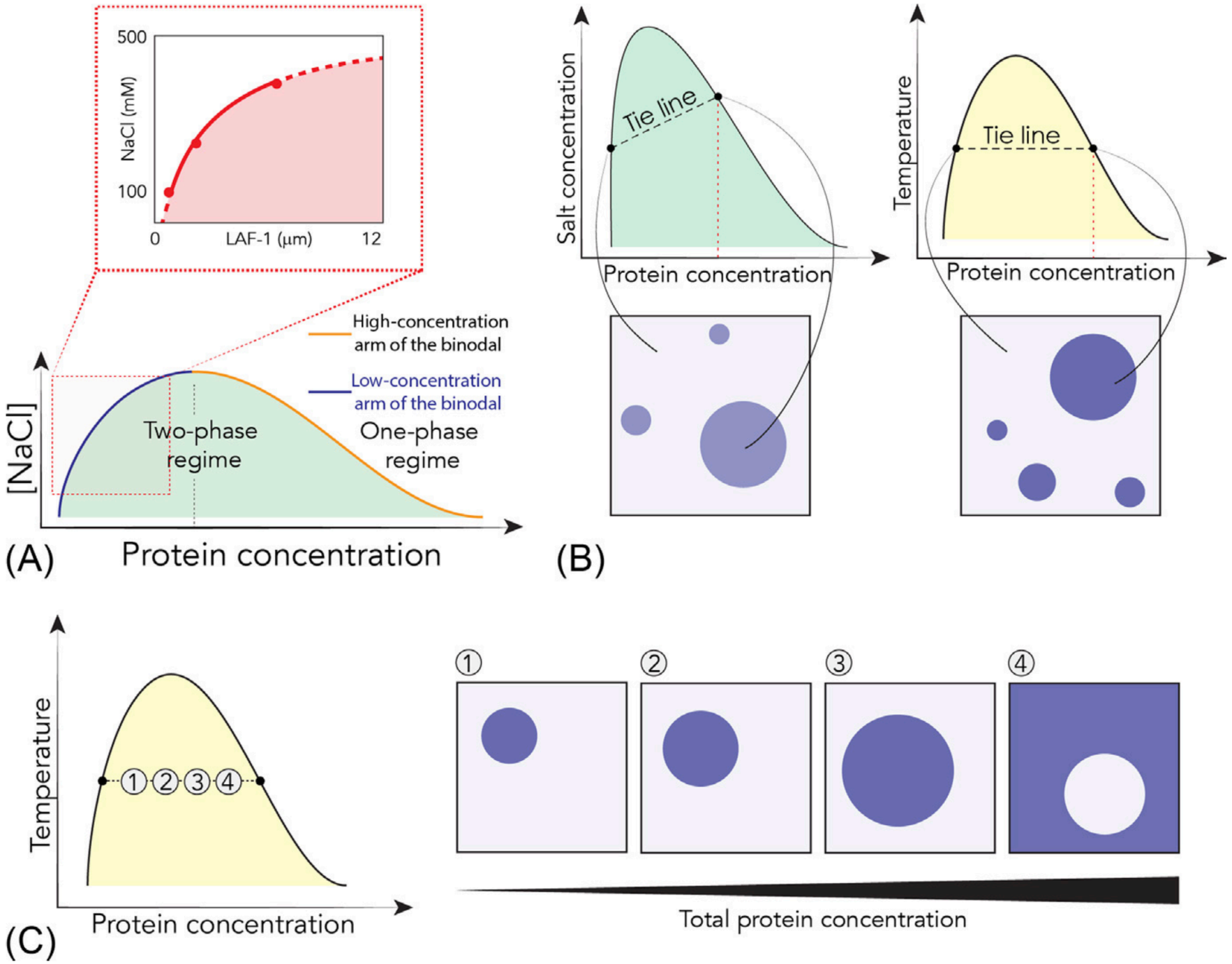
Chromatin in general
Gibson 2019 *Cell*

Does chromatin look like a lava lamp?

Dolgin 2018 *Nature*

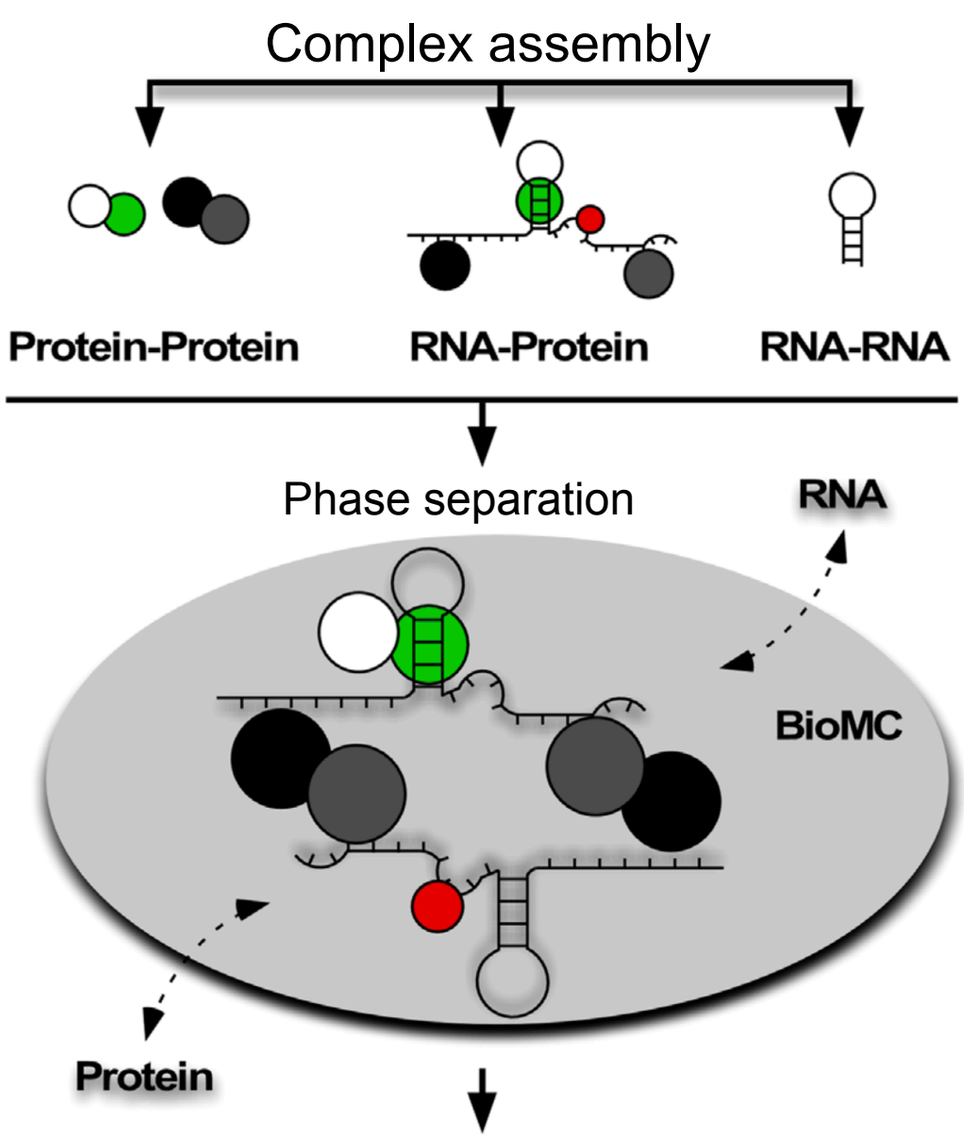
and what would this mean in terms of function?

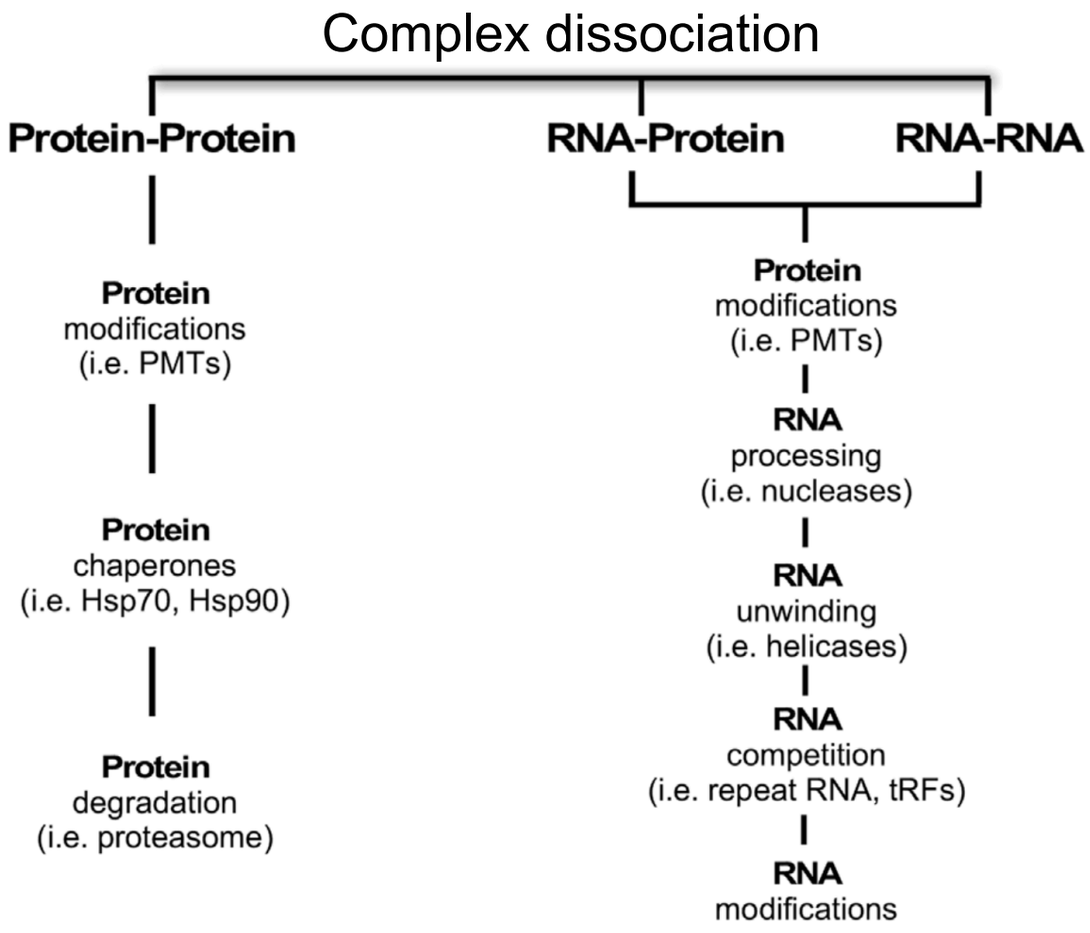
Phase diagram representation



Holehouse 2019 *Intrinsically Disordered Proteins*

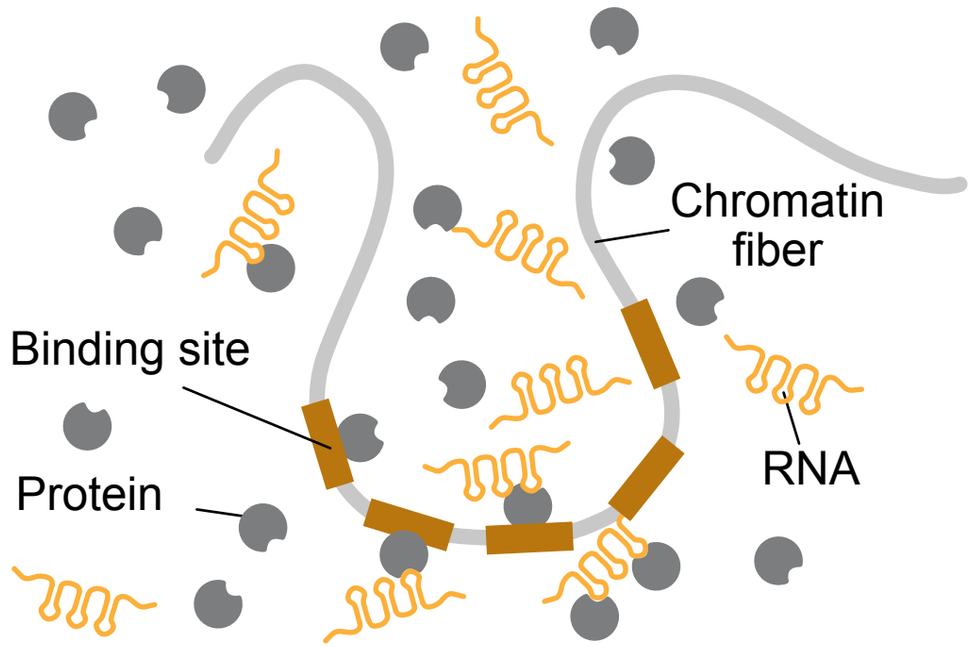
RNA can act as a glue to drive phase separation





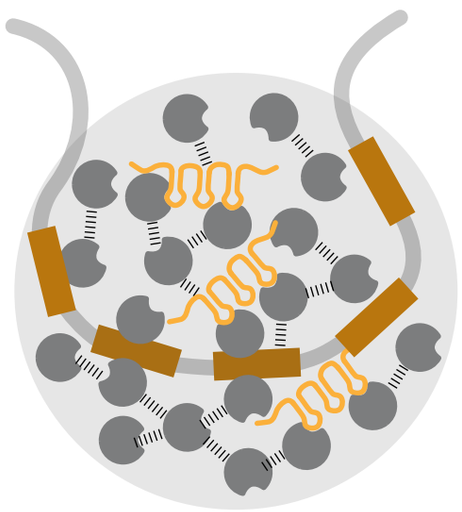
Drino 2018 *BioEssays*

Mechanisms for the formation of chromatin subcompartments

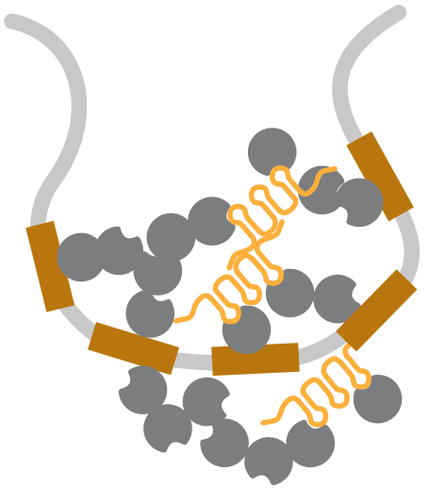


Specific chromatin binding

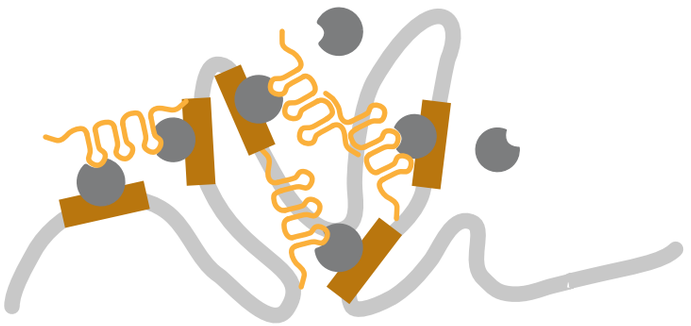
Phase separation (PS)



Liquid-liquid phase separation (LLPS)

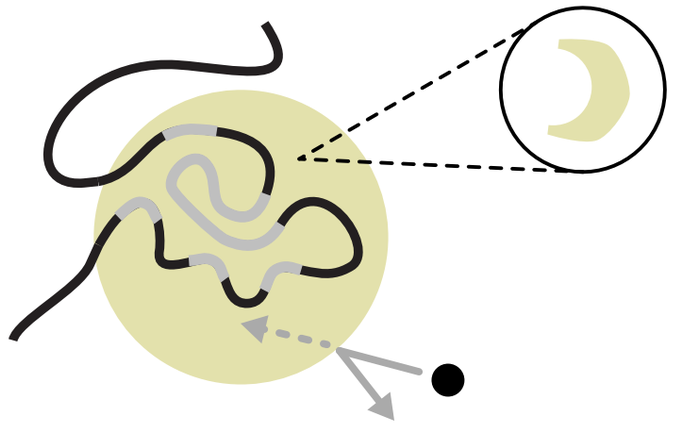


Liquid-gel phase separation (LGPS)

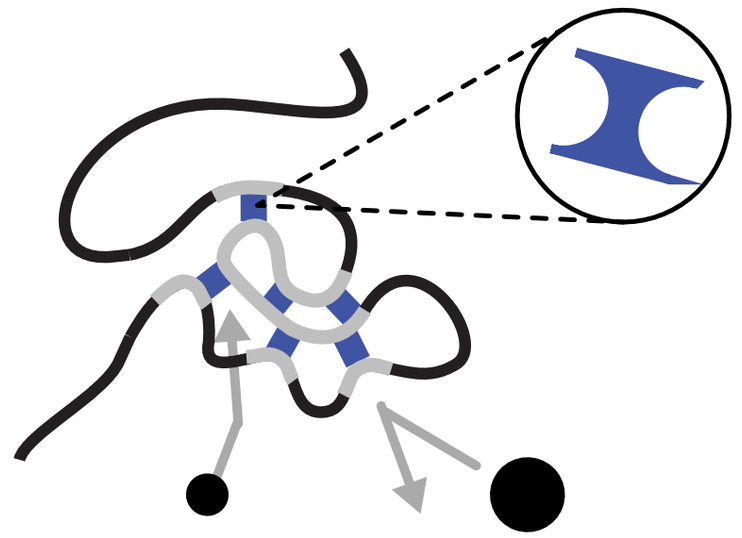


Polymer-polymer phase separation (PPPS)

Features of a liquid-liquid versus polymer-polymer phase separation mechanisms

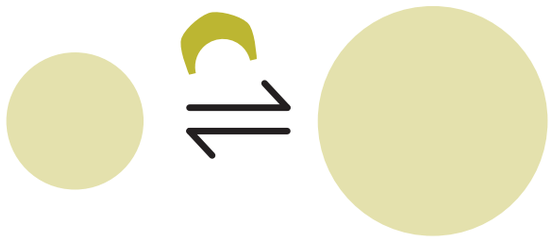


- Homogeneous “liquid” phase
- Fast internal mixing
- Exclusion (chemical properties)
- Different viscosity
- Coalescence/fusion

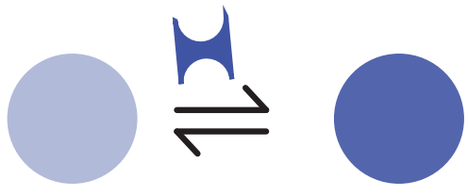


- Colocalization with chromatin
- Accessible to soluble factors
- Size dependent exclusion
- Soluble fraction like nucleoplasm
- Coalescence/fusion

Concentration dependence



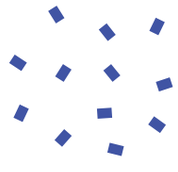
Concentration buffering



stable size

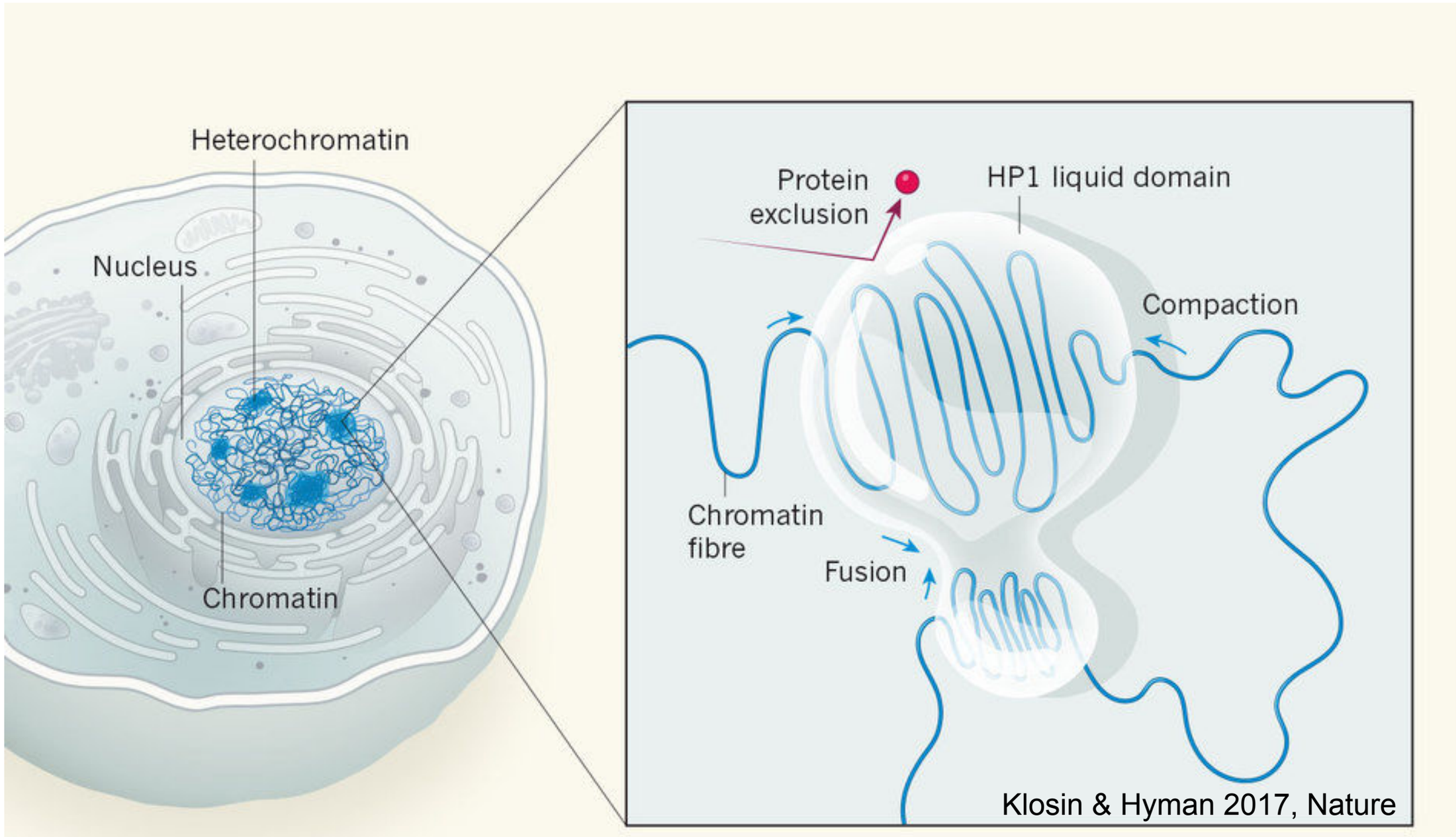
Stability without chromatin

Stable droplet



Dissociation

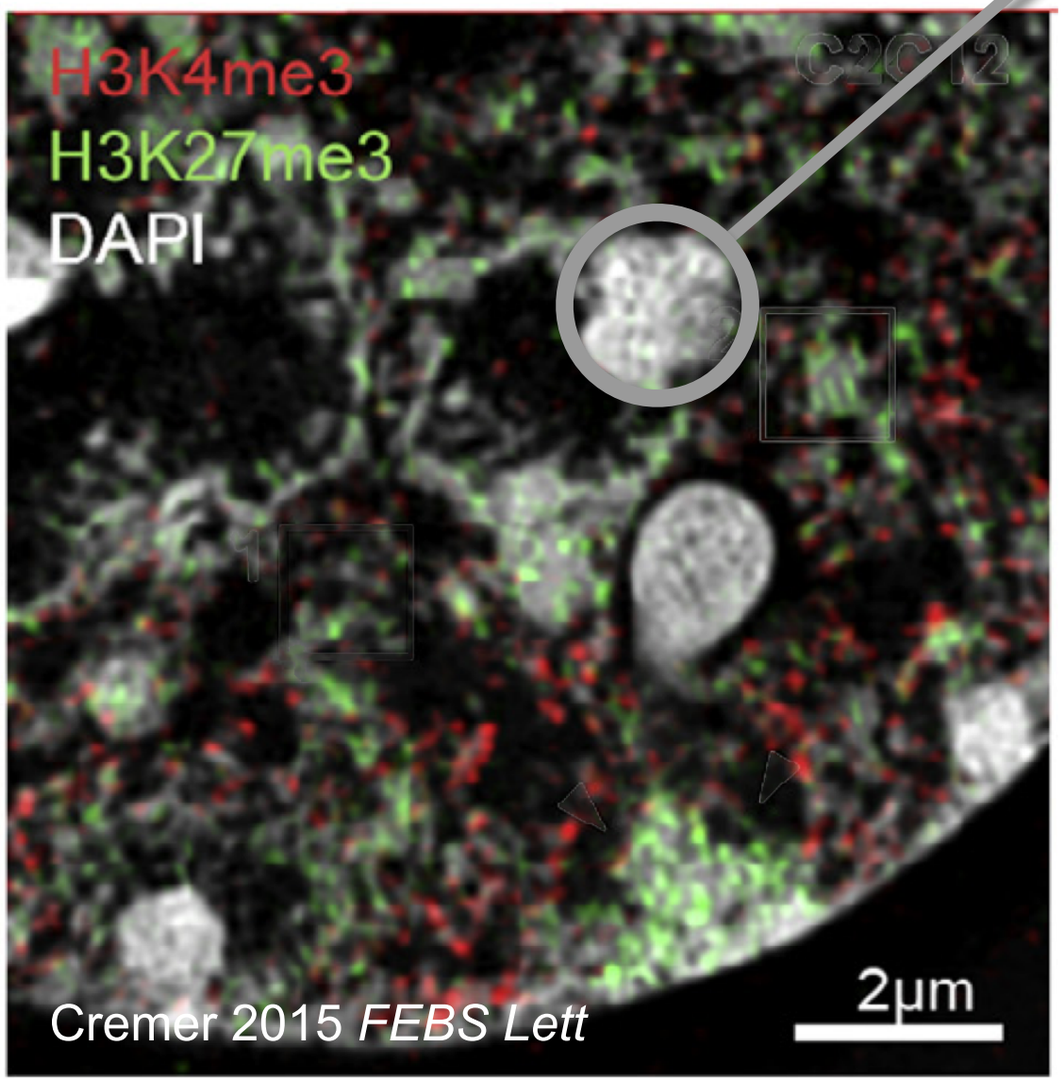
Formation of heterochromatin domains by a liquid-liquid like phase separation mechanism?

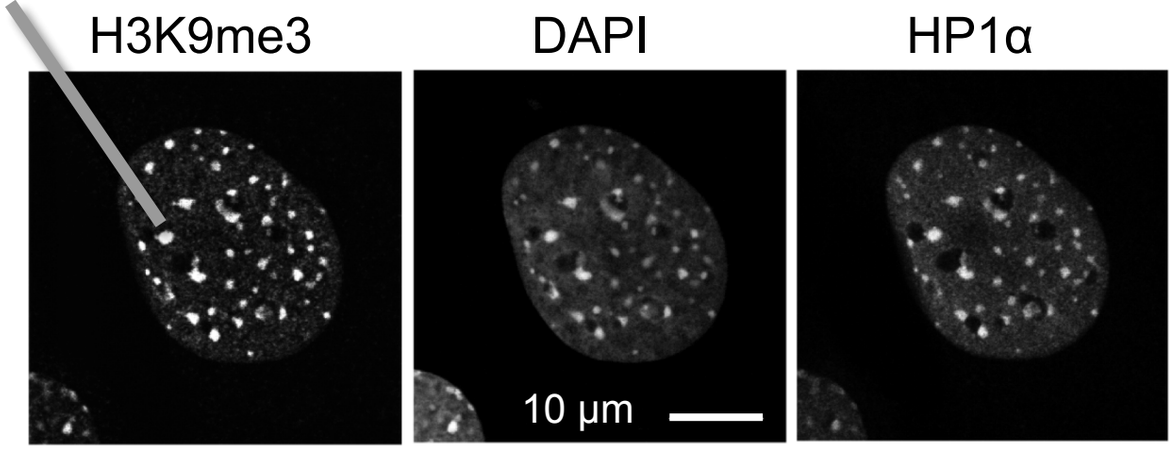


Larson, Narlikar 2017 *Nature* (Human HP1)
Strom, Karpen 2017 *Nature* (Drosophila HP1)

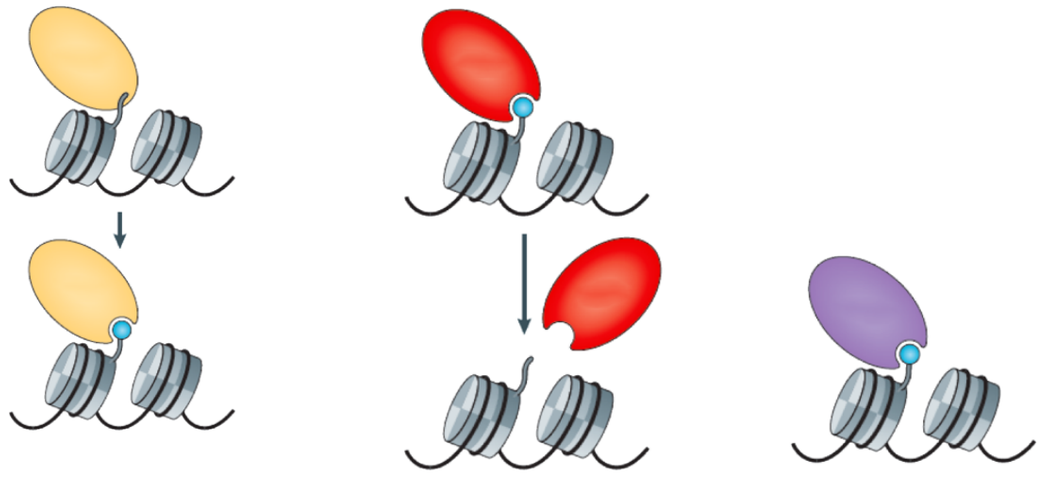
Mouse pericentric heterochromatin - a model system for a large silenced chromatin domain

Pericentric heterochromatin ("chromocenters")





H3K9me3 modification



Writer
SUV39H1/H2

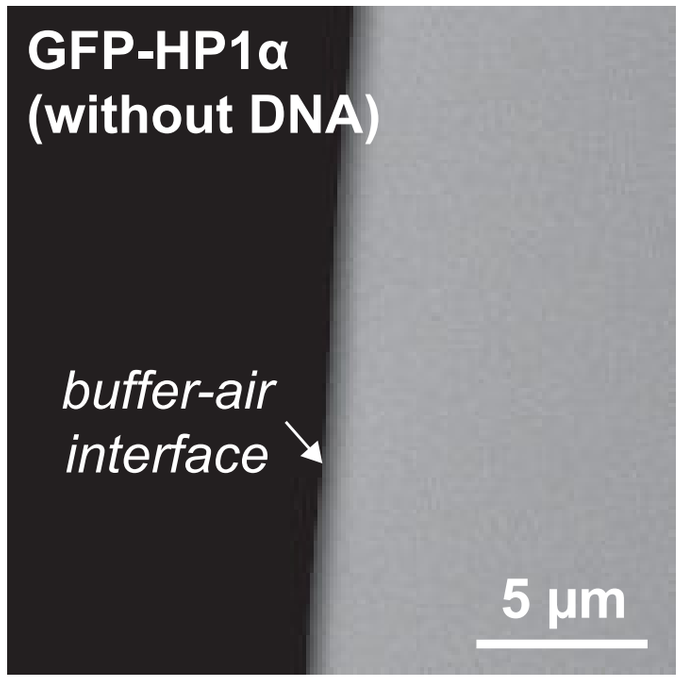
Eraser
JMJD2

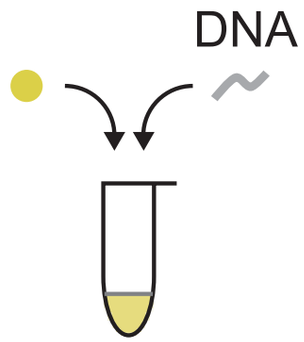
Readers
HP1α/β/γ
SUV39H
ATRX

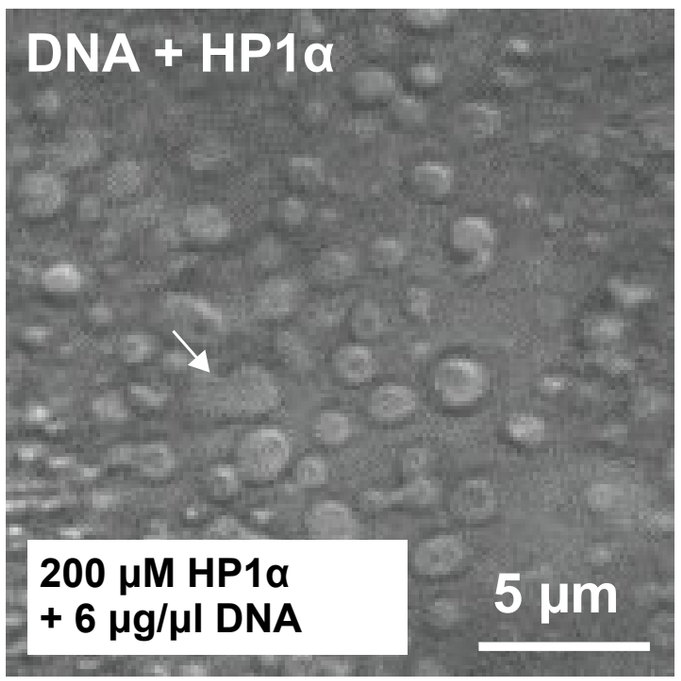
Mouse chromocenters
- Nucleosomes: 230 µM
- HP1α/β total: 10 µM dimer

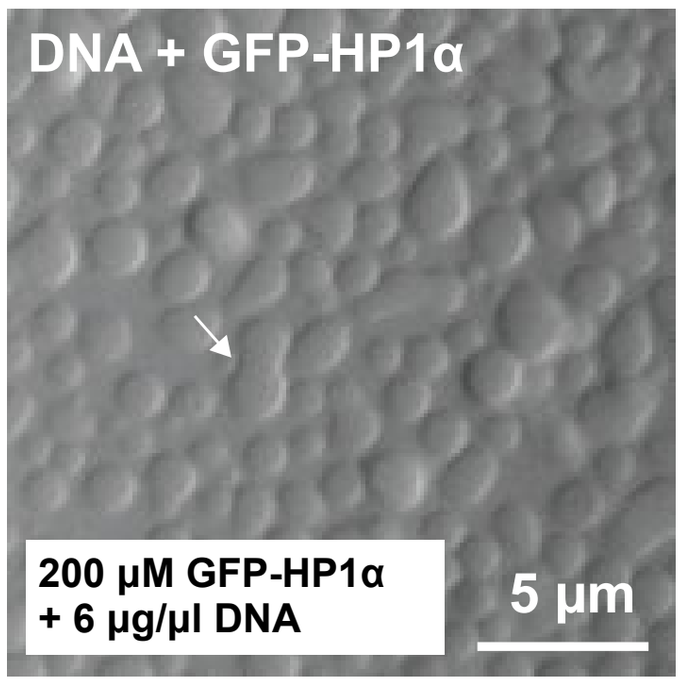
HP1α with DNA makes liquid droplets in vitro

(HP1β and HP1γ have a lower droplet formation propensity)

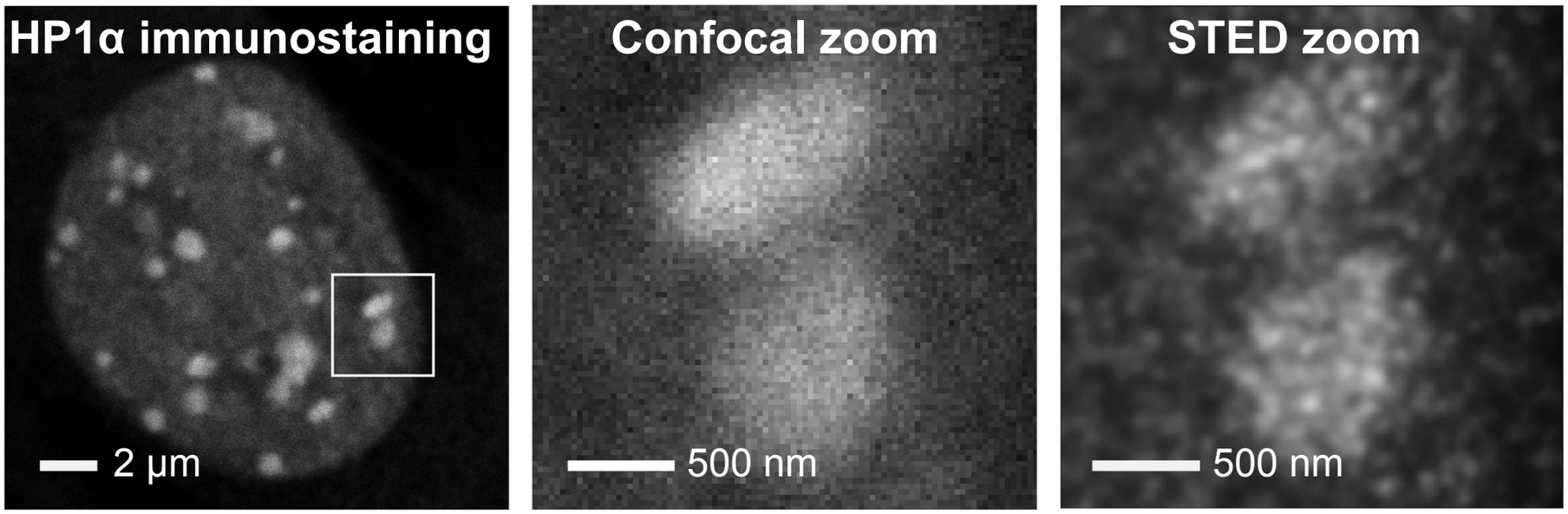




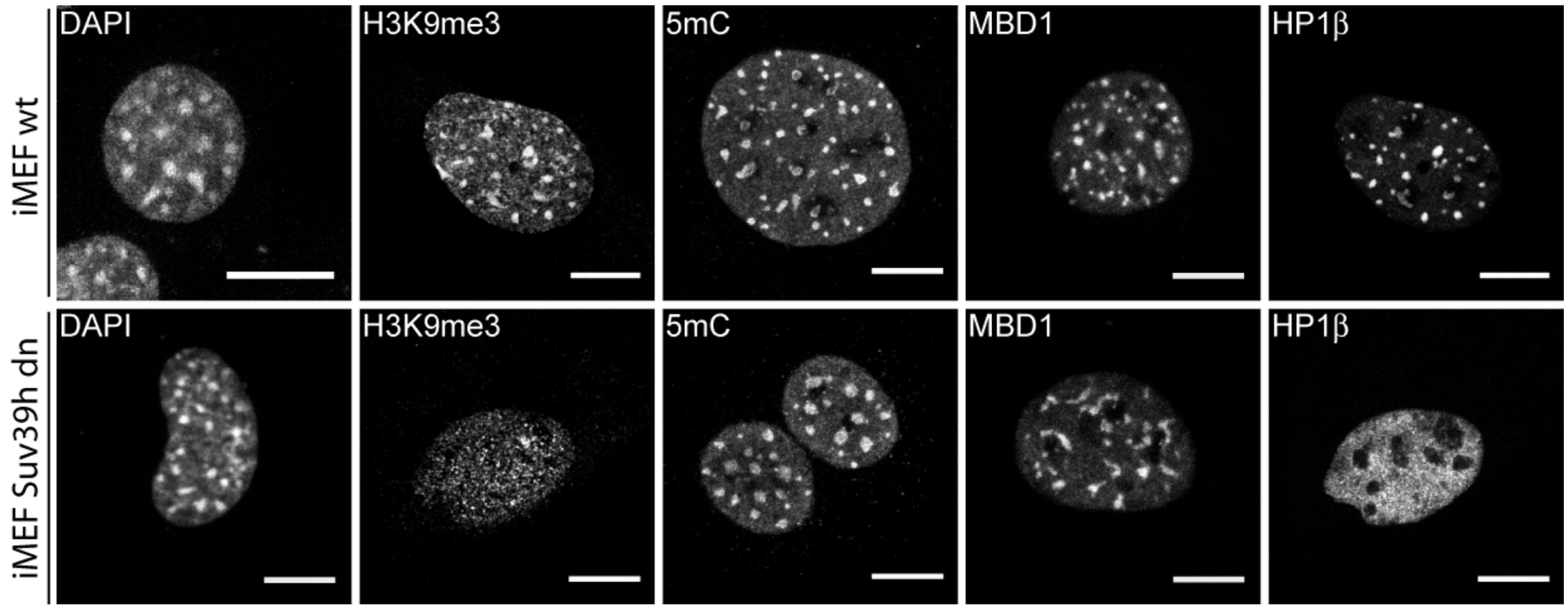




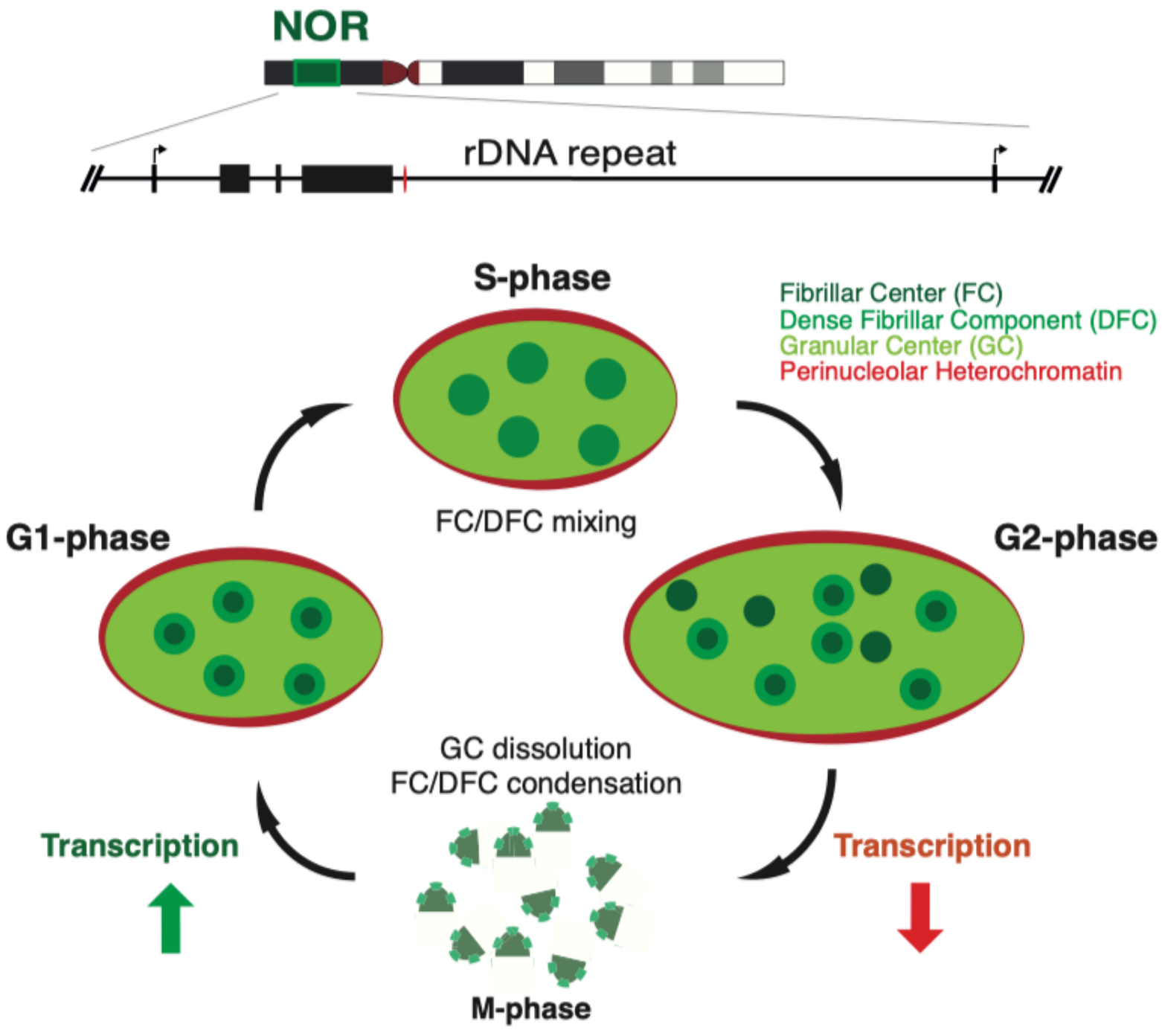
HP1 displays a granular structure and is not required for chromocenter condensation



Suv39h1/h2 knock-out leads to H3K9me3 and HP1 loss but not to decondensation

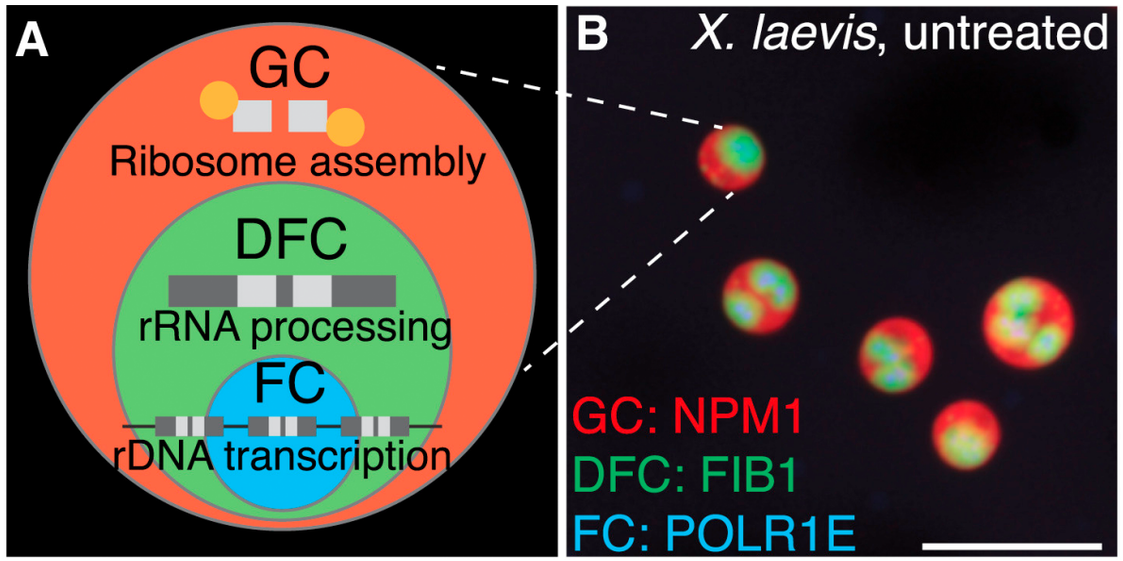


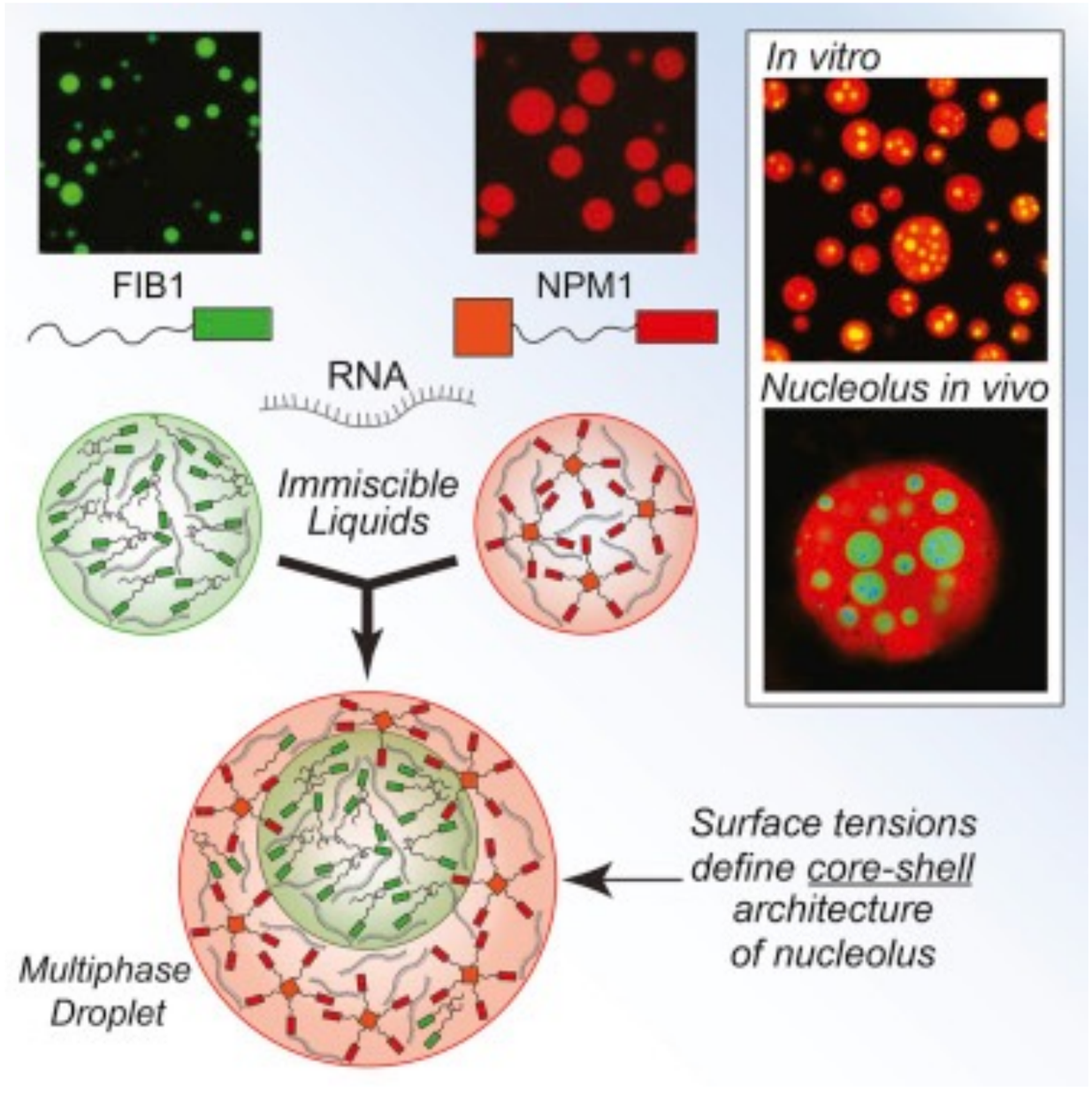
The nucleolus continuously (dis)assembles during the cell cycle



Nemeth & Grummt, 2018

Coexisting liquid phases underlie nucleolar subcompartments



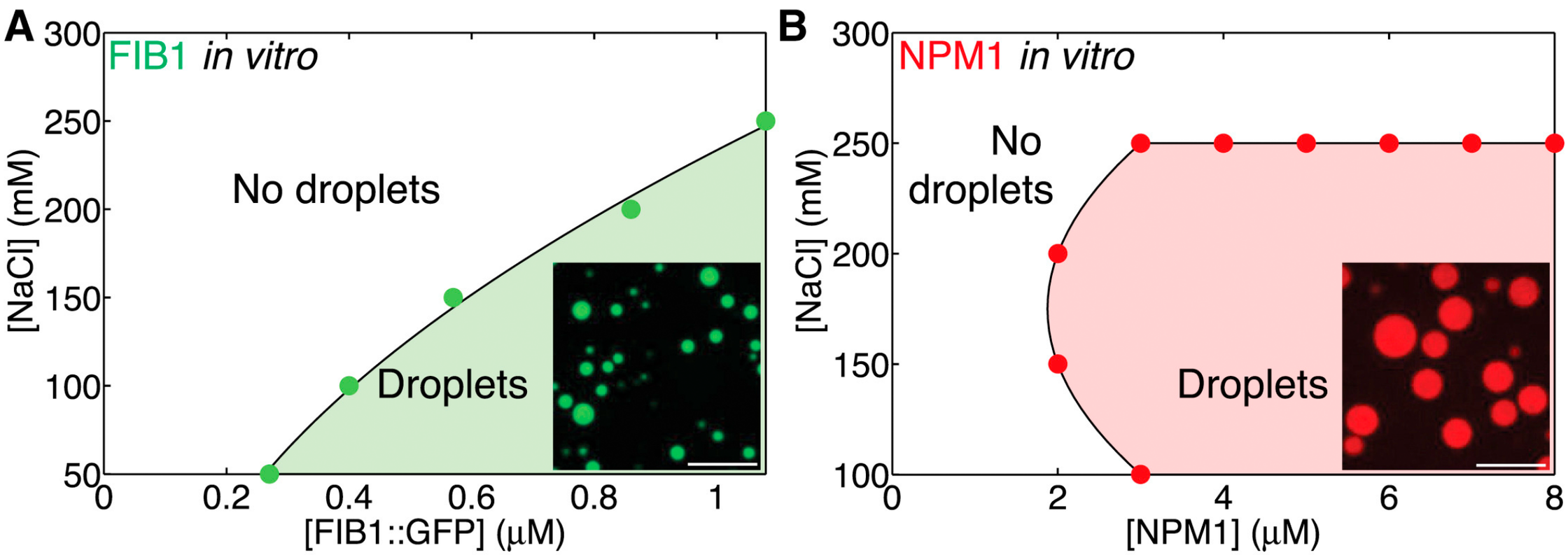


(A) Schematic diagram of ribosome biogenesis in nucleolus.

(B) Nucleoli in an untreated *X. laevis* nucleus. Scale bar, 20 μm. For all images, granular component (GC) is visualized with NPM1 (nucleophosmin, red), dense fibrillar component (DFC) with FIB1 (fibrillarin, green), and fibrillar center (FC) with POLR1E (blue).

Marina Feric et al., Cell 2016

Purified nucleolar proteins can phase separate into droplets with different biophysical properties

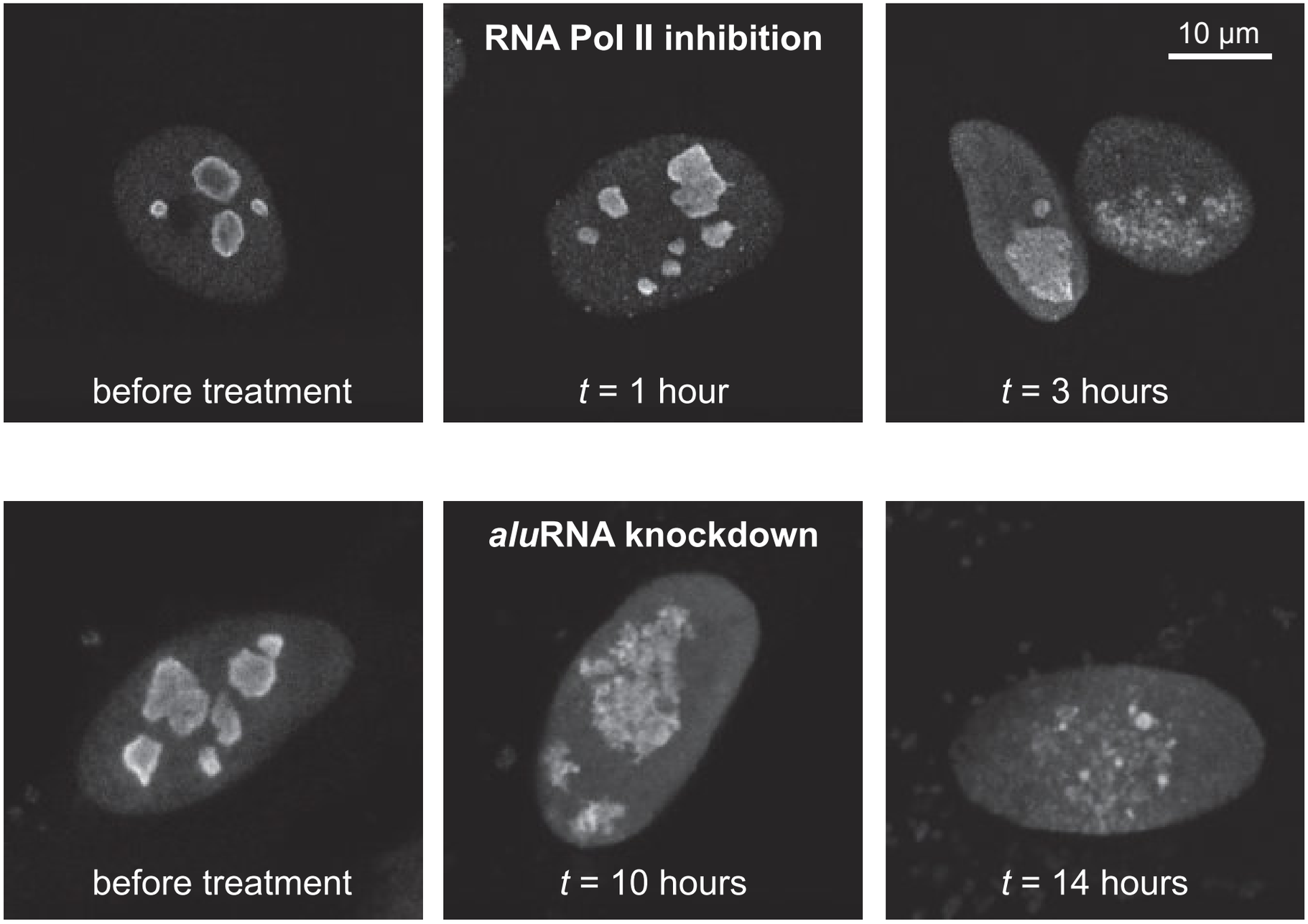


(A) Phase diagram of purified FIB1 in the presence of 5 μg/ml of rRNA. Inset: FIB1 droplets. Scale bar, 10 μm.

(B) Phase diagram of purified NPM1 in the presence of 100 μg/ml of rRNA. Inset: NPM1 droplets. Scale bar, 10 μm.

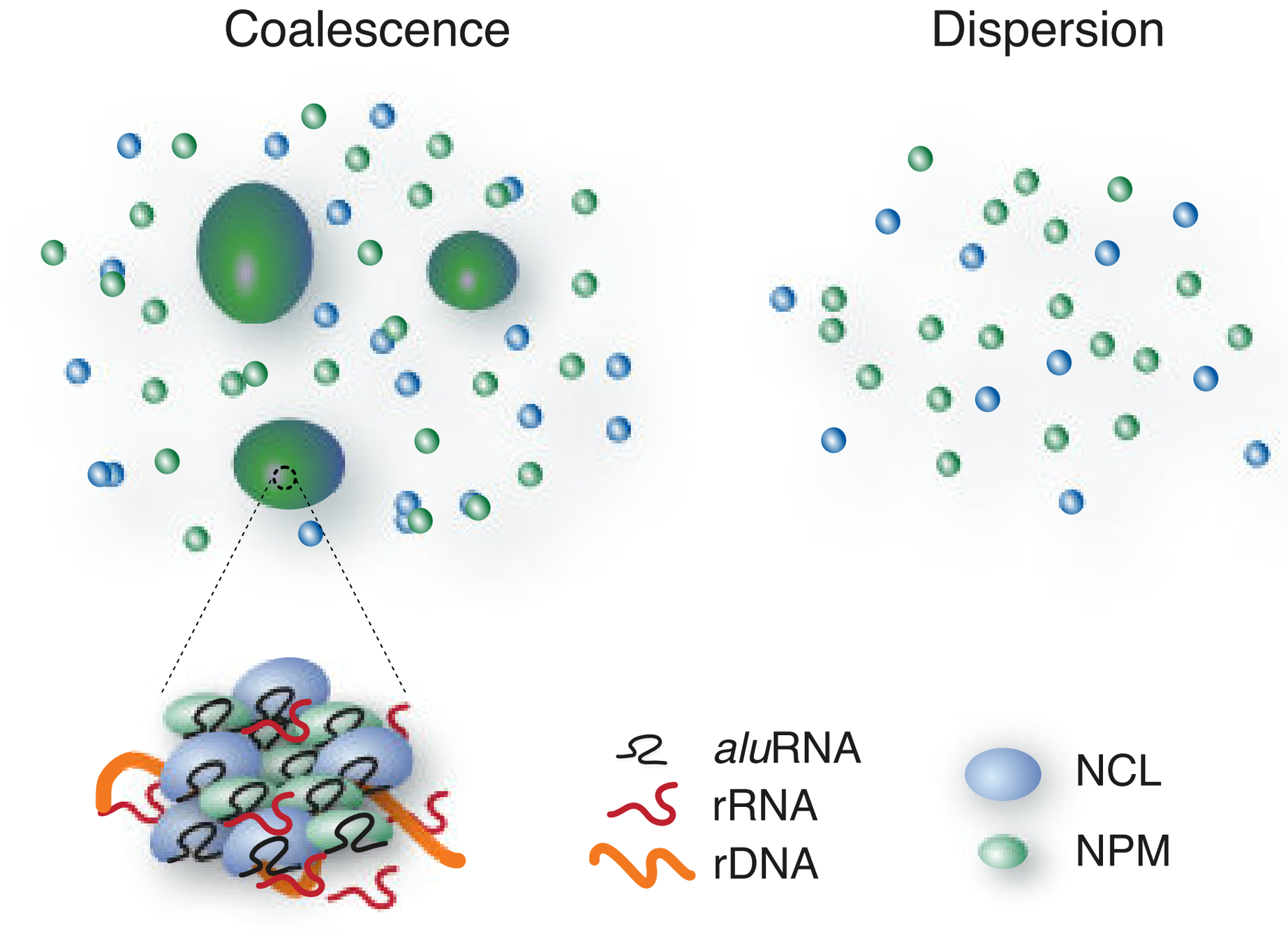
Marina Feric et al., Cell 2016

*alu*RNA-driven phase transition of the nucleolus



Caudron-Herger 2015, EMBO J
Caudron-Herger 2016, Nucleus

*alu*RNA-driven phase transition of the nucleolus



Caudron-Herger 2015, EMBO J
Caudron-Herger 2016, Nucleus