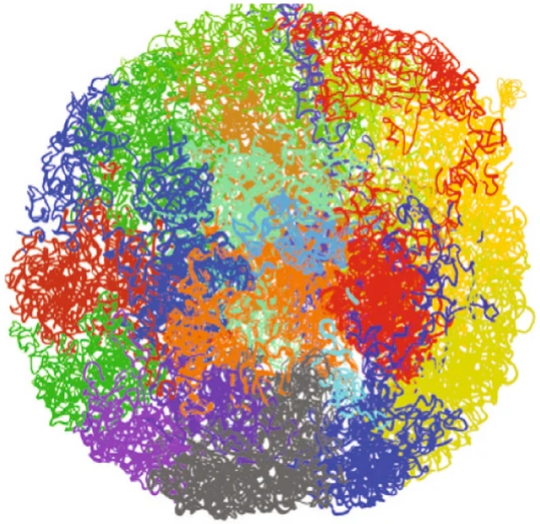
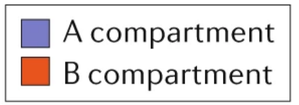
The Lamina and Gene Regulation

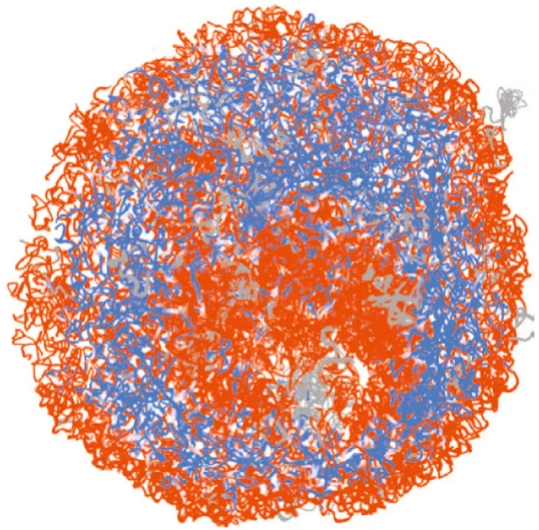
Al-Sady Lab Workshops

Can Goksal

10.09.2024





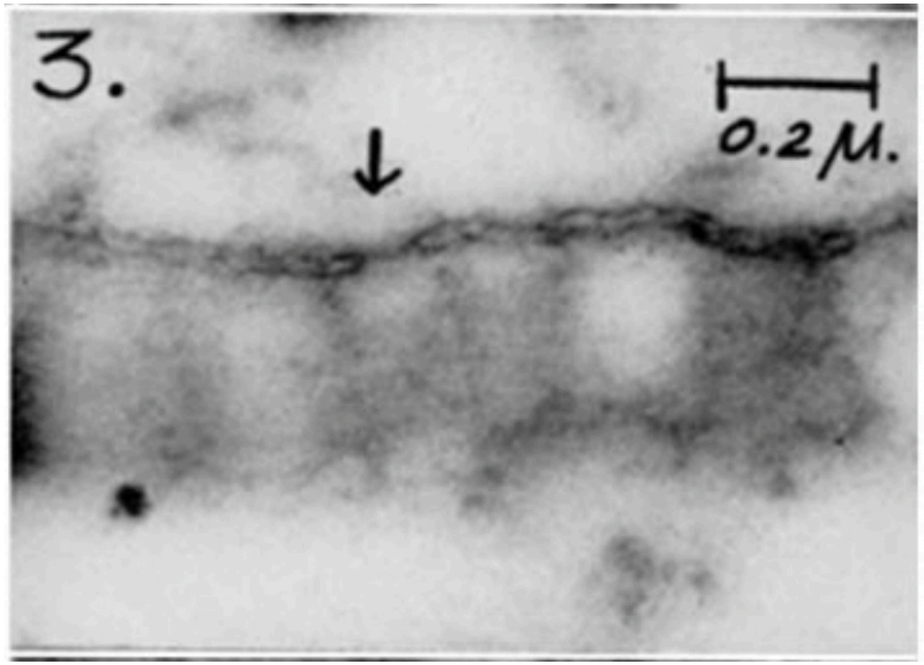


Content Covered

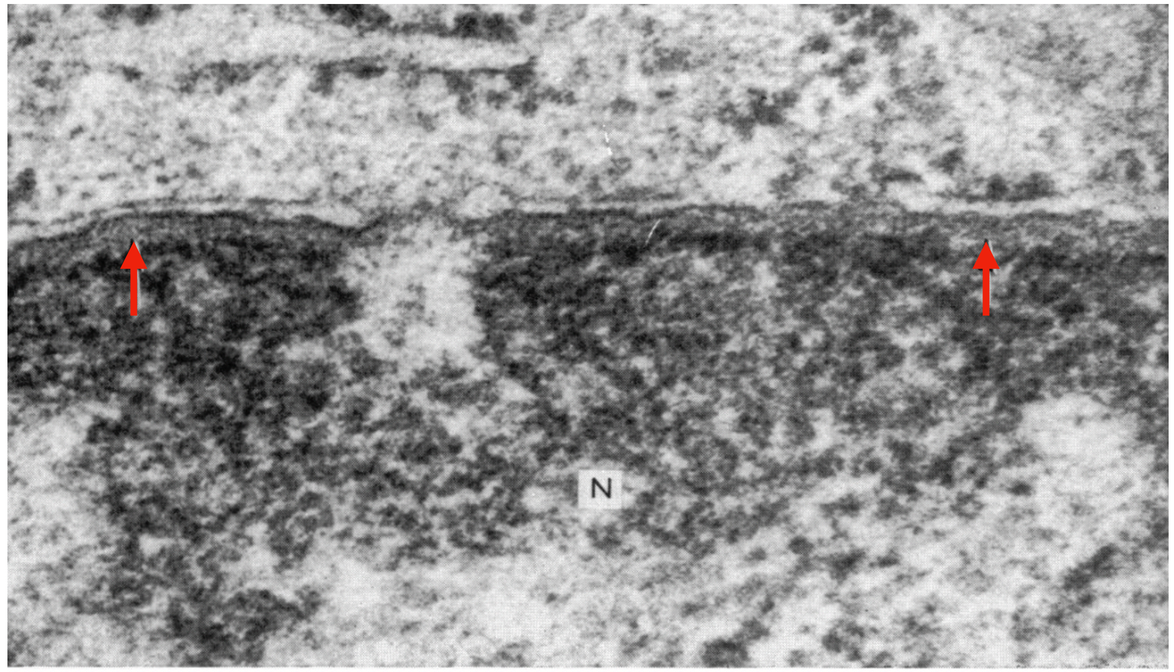
- *What is the nuclear lamina + background*
- *Why should I care about the lamina?*
- *Lamina and Gene Organization + Regulation*
- *Methods to Study Lamina Interactions*

History of the Lamina

- First nuclei observed in 1800s
- Nuclear envelope first described in 1913 by George Kite
- With development of electron microscopy, presence of fibrous nuclear lamina discovered later in mammalian cells in 1970s



1956 - EM image of Amoeba nuclei



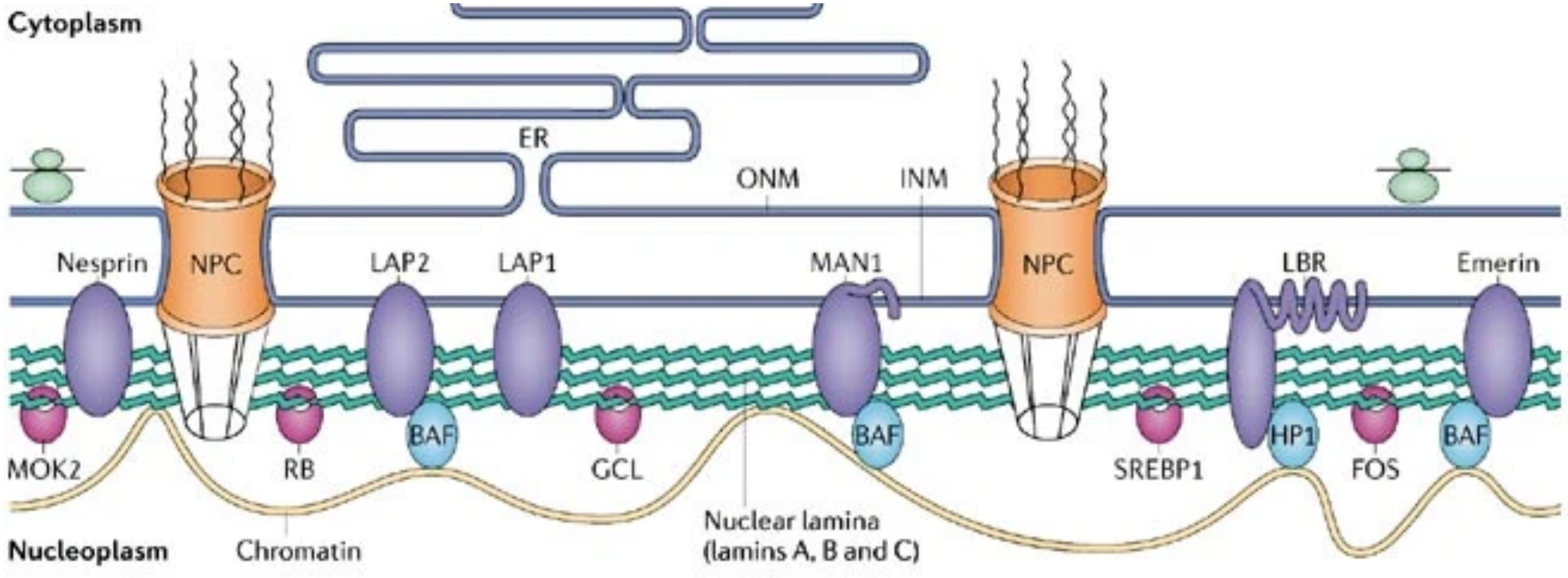
1972 - EM image of rabbit chondrocyte (cartilage cells) nuclei

Pappas, 1956

Ghadially et al., 1972

So what is the nuclear lamina?

- A meshwork of protein polymers beneath the inner nuclear membrane
- Provides mechanical and structural support for nucleus
- Consists of 4 core lamins: **Lamin A, Lamin C, Lamin B1, Lamin B2**



Cappell & Collines, 2006

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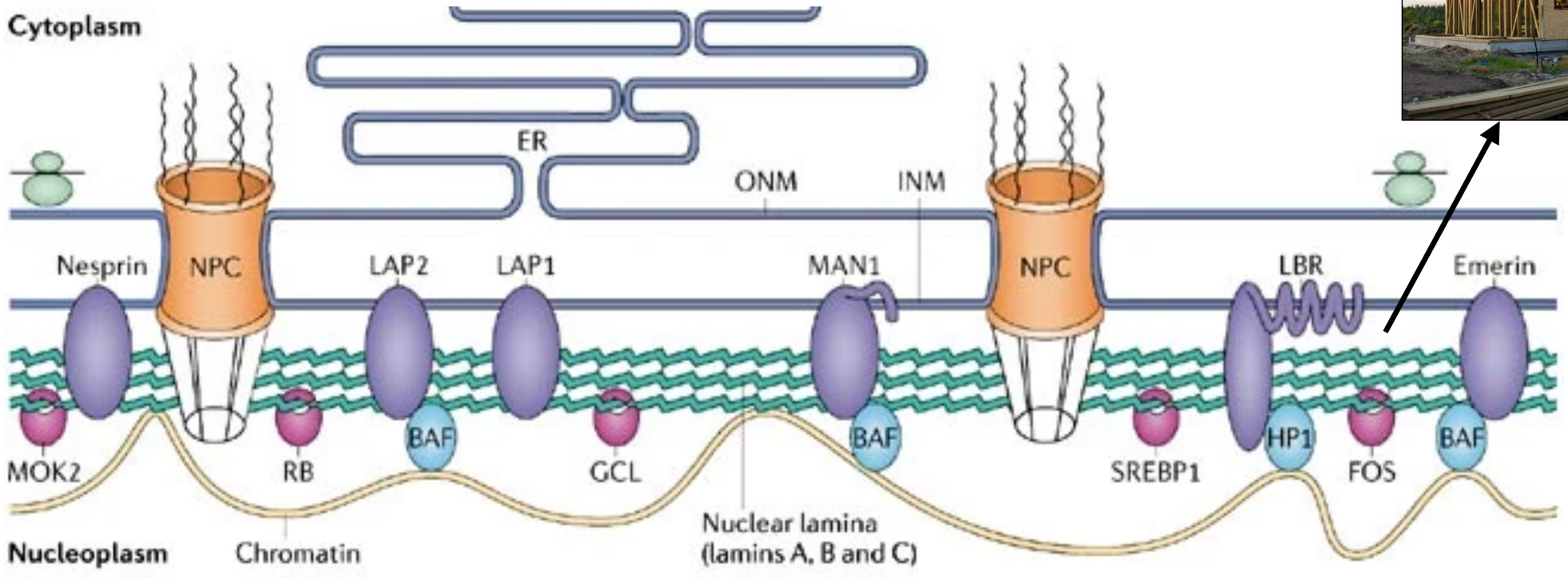

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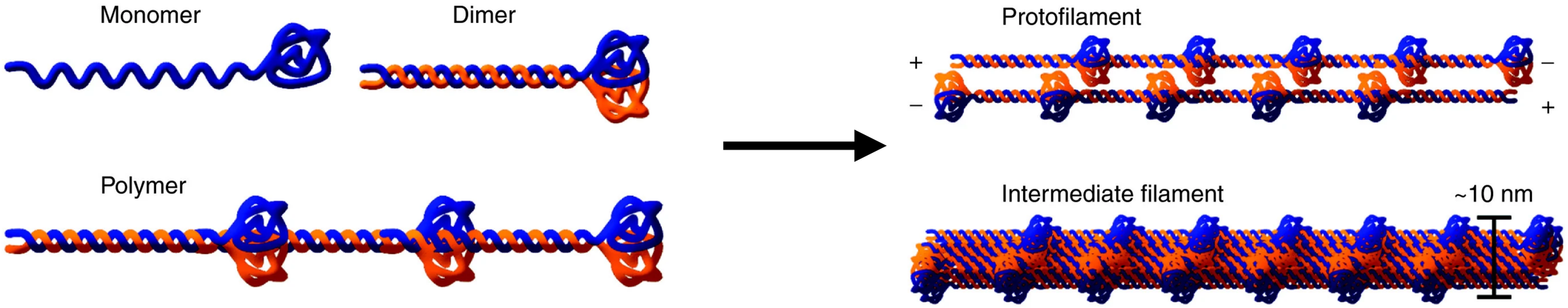
  - ***These lamins polymerize to form intermediate filaments***



Dittmer & Mistelli, 2011
Tenga & Medalia, 2020

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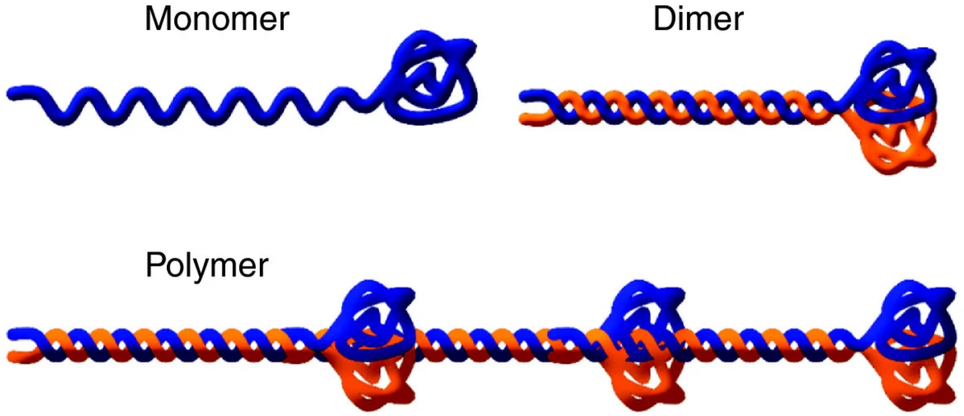
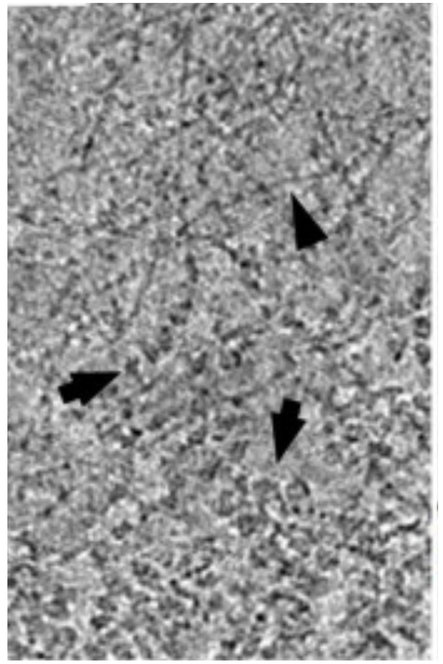


In vitro Lamin A polymers (cryo-ET)

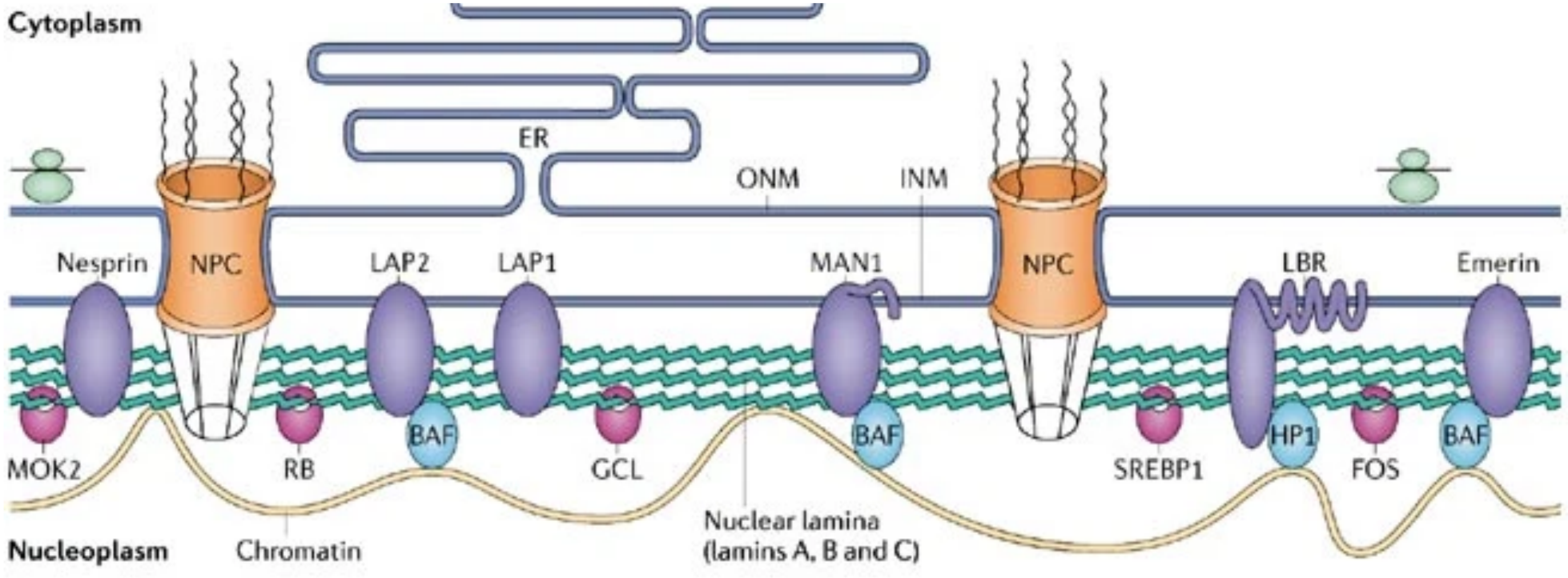


Native lamin filaments (cryo-ET)

Dittmer & Mistelli, 2011
Tenga & Medalia, 2020

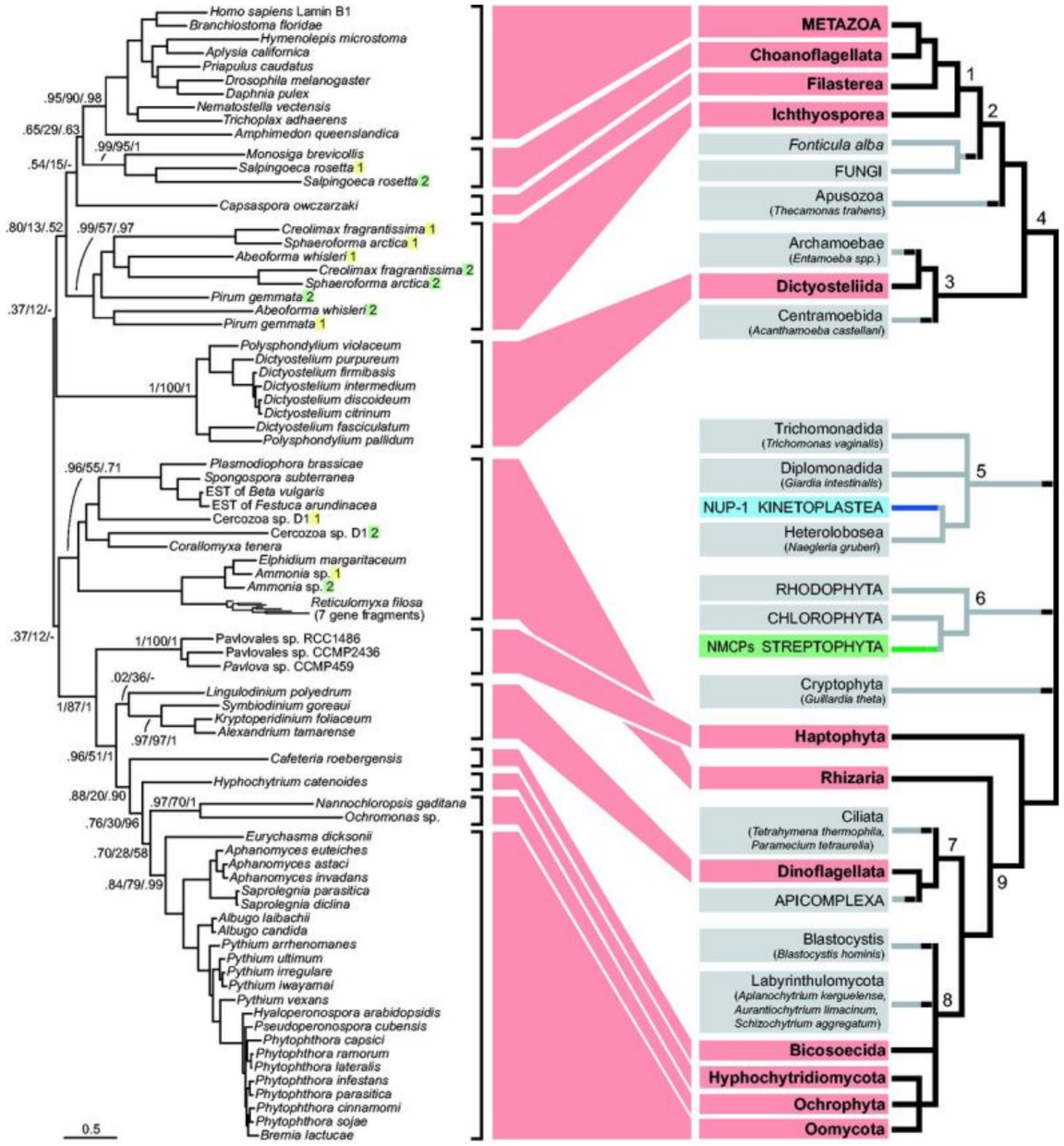
So what is the nuclear lamina?

- But the lamina isn't insular and has many neighbors— these include:
  - Inner nuclear membrane (INM) proteins → LEM family proteins, Lamin B receptor (LBR)
  - Nucleopore Complexes (NPCs)



Cappell & Collines, 2006

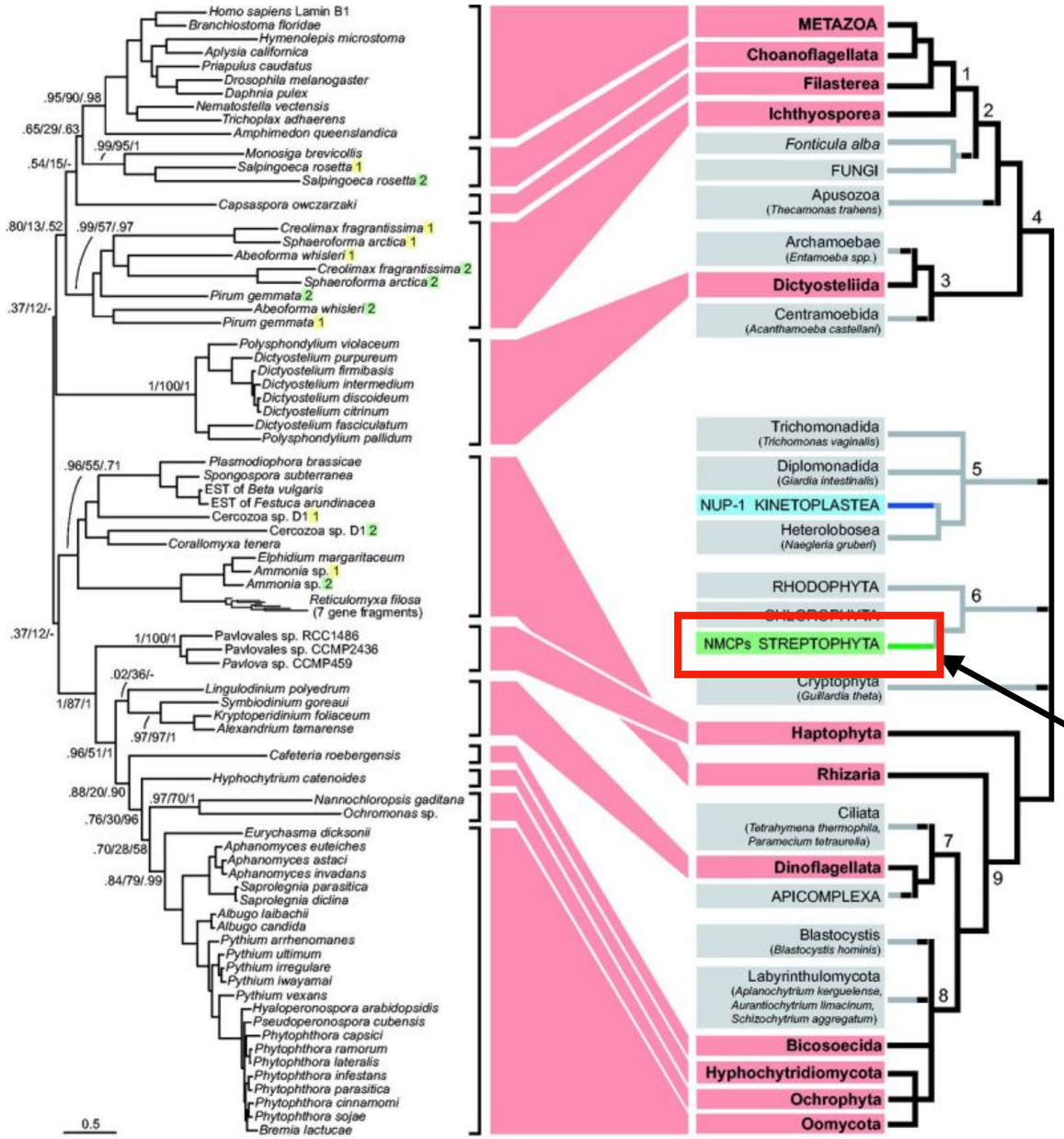
Evolution of the Lamina



- All intermediate filaments arose from a lamin ancestor
- Lamins exist across several eukaryotic lineages
  - Past understanding (until the 2010s) was that lamins arose in metazoans — With more genome assemblies more lamin homologs have been identified in non-metazoan eukaryotes

Koreny & Field, 2016

Evolution of the Lamina



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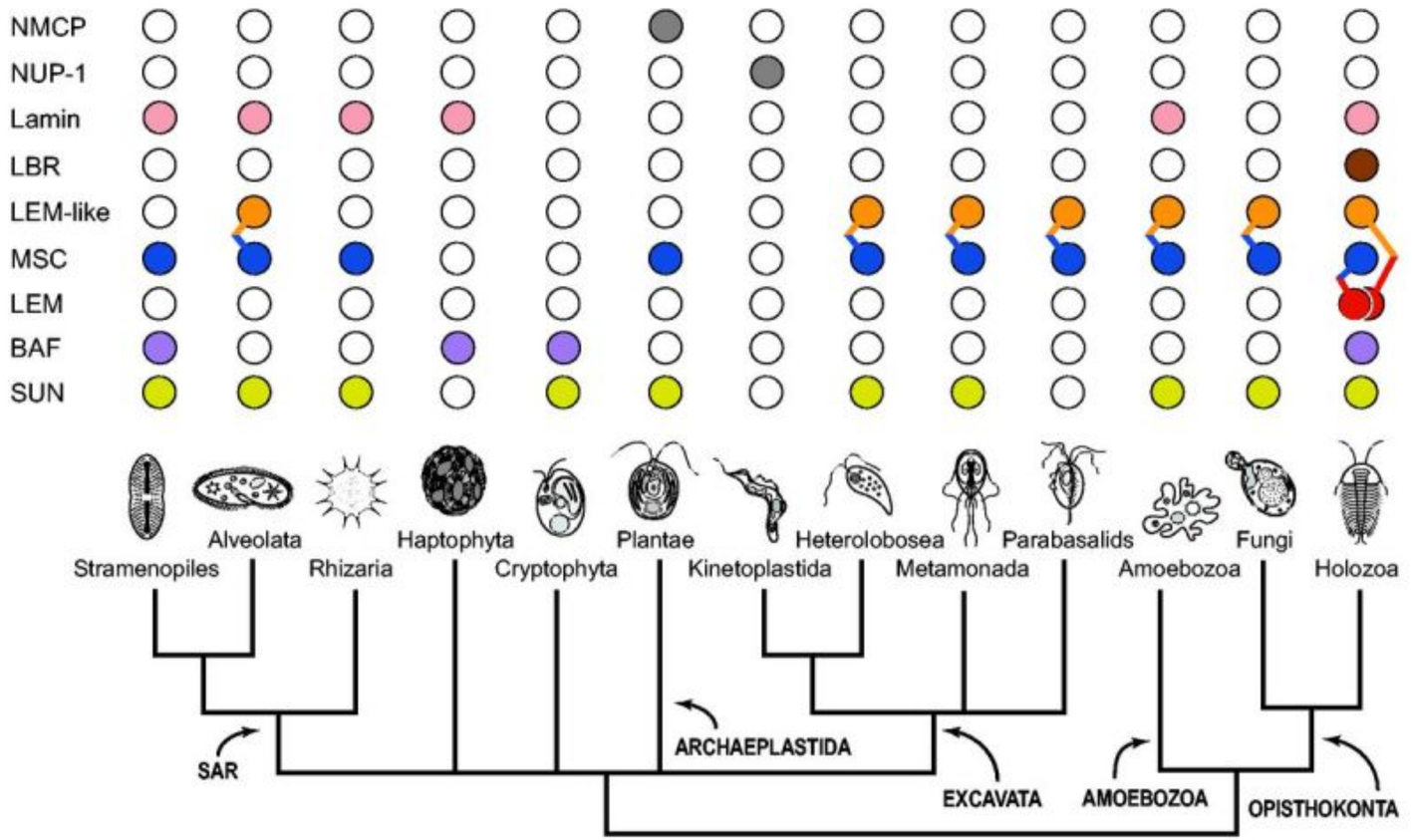
Plants lack lamins but have similar lamin-like NMCPs

- Persistent loss of lamins in several lineages which might be due to high divergence

Koreny & Field, 2016

Evolution of the Lamina

Different components of the lamina have different evolutionary trajectories



Koreny & Field, 2016

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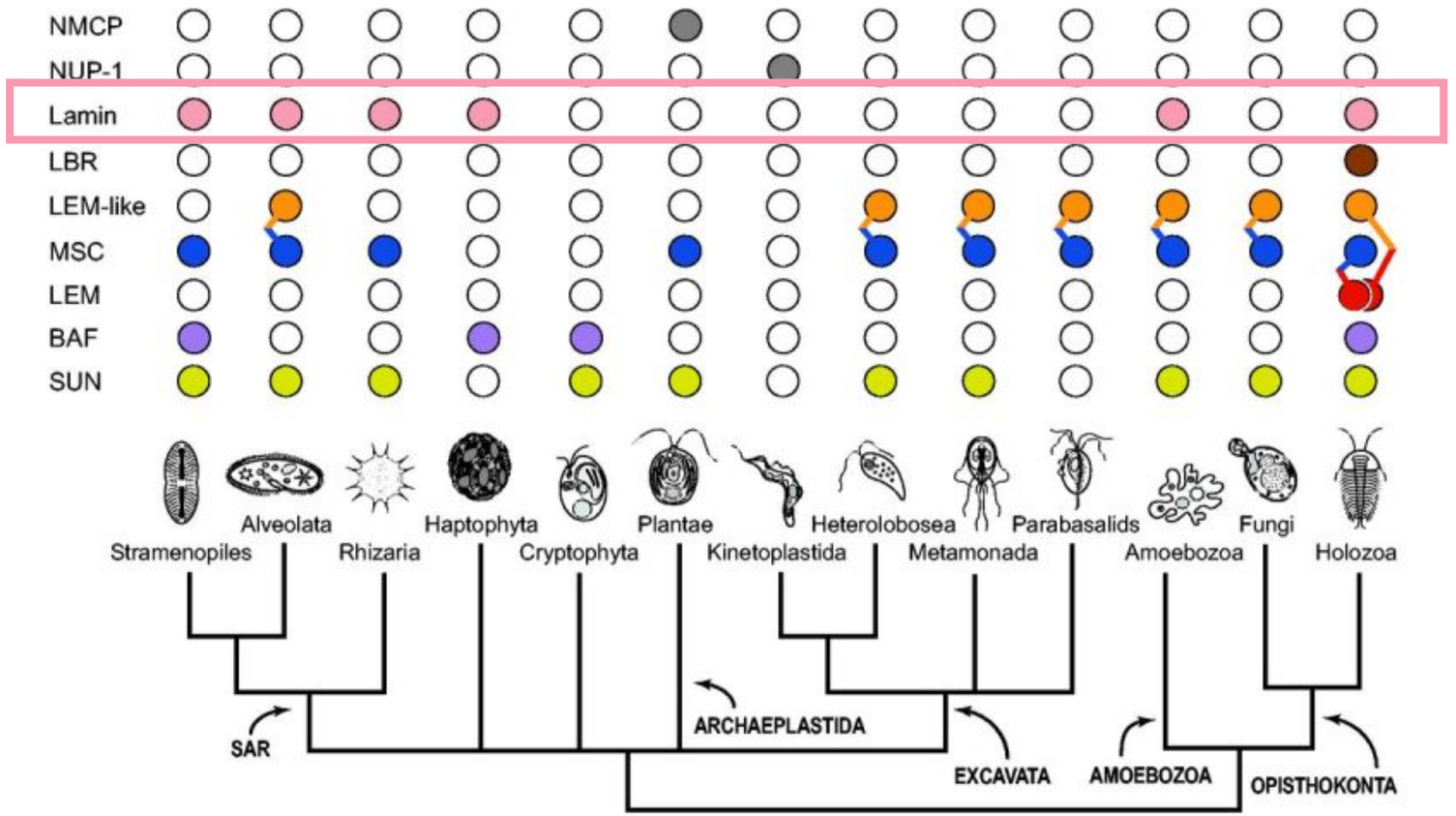

Lamins found across eukaryotes

Koreny & Field, 2016

Evolution of the Lamina

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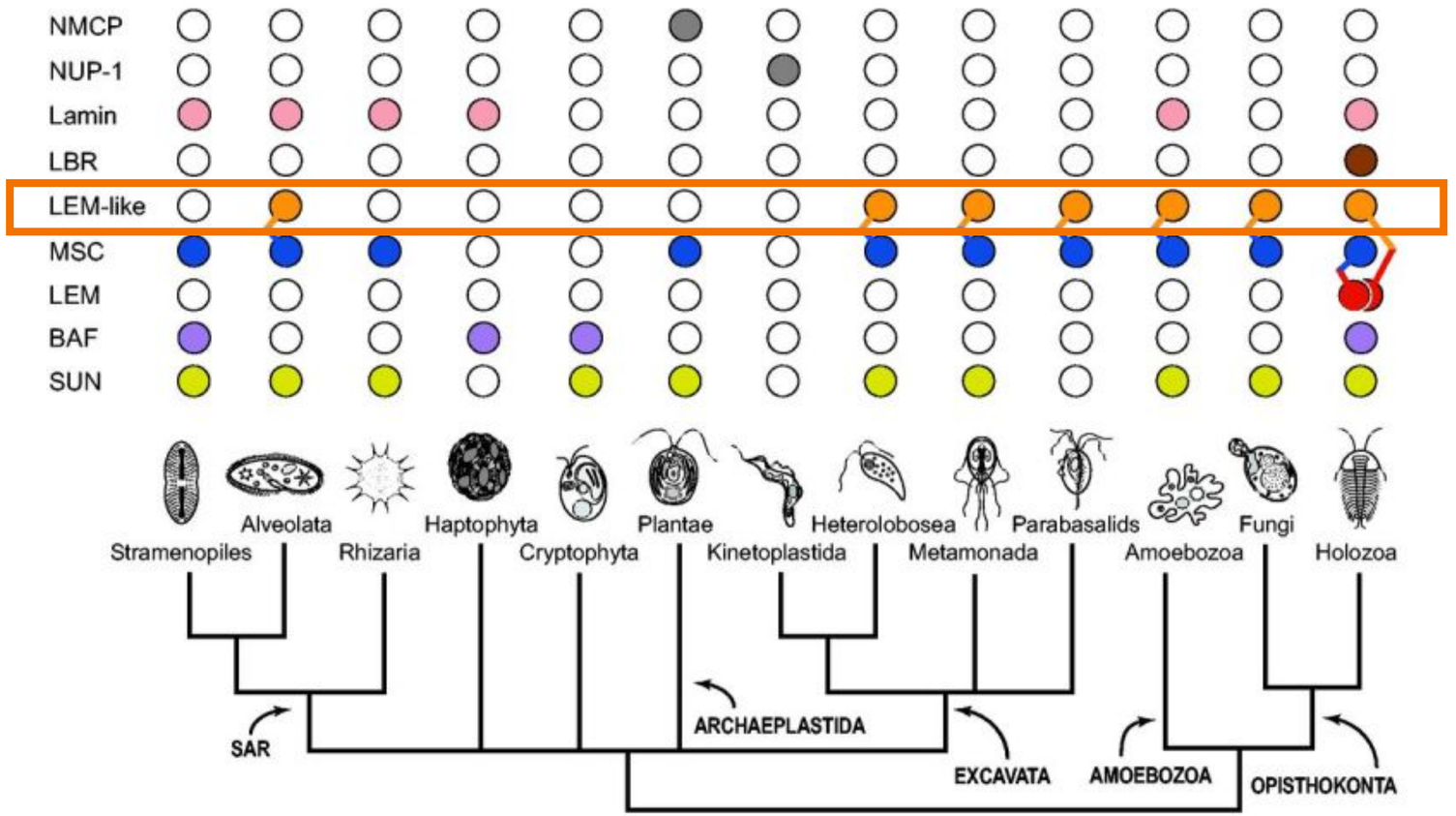

LEM family proteins are found in unicellular eukaryotes and metazoa

Koreny & Field, 2016

Evolution of the Lamina

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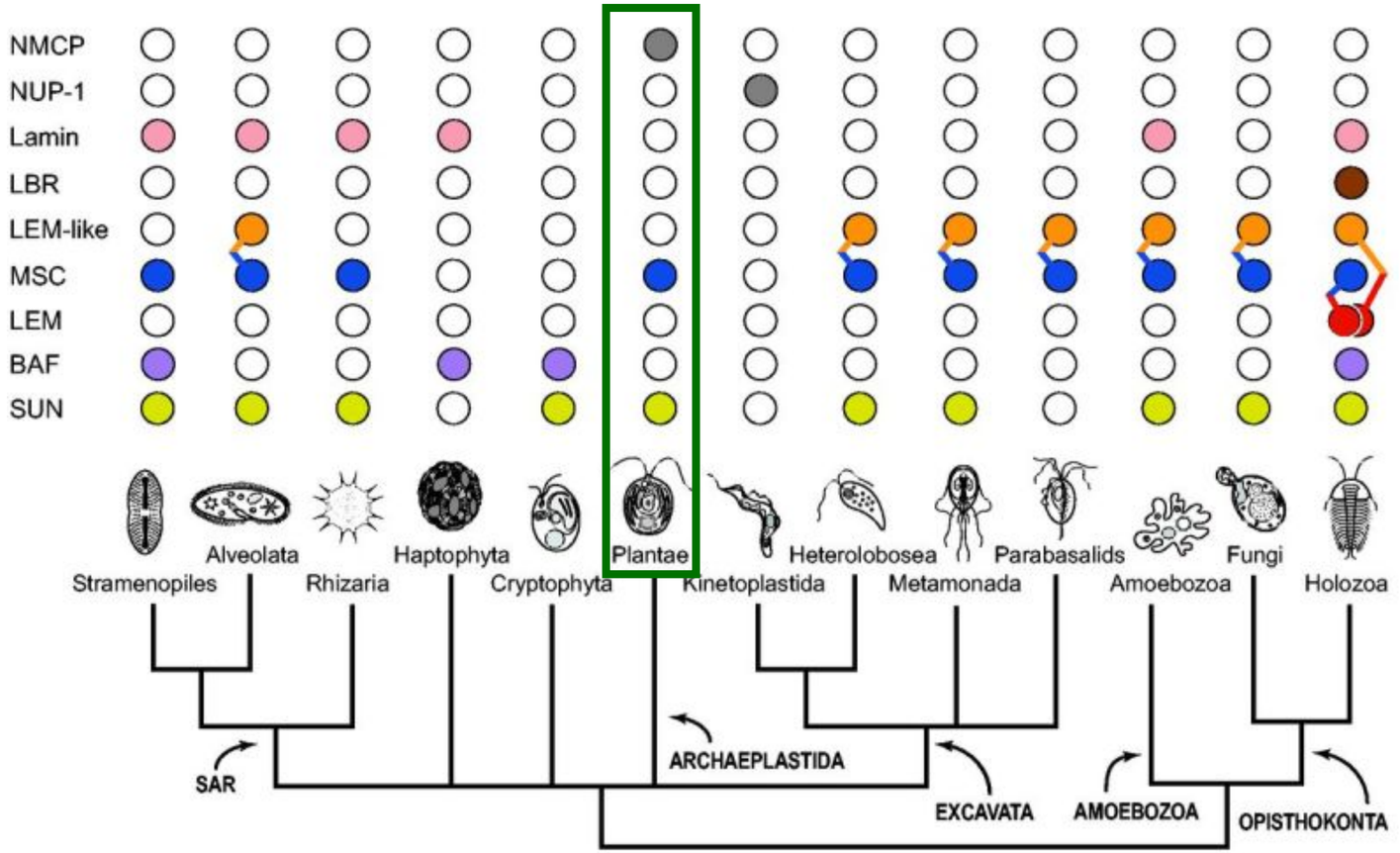
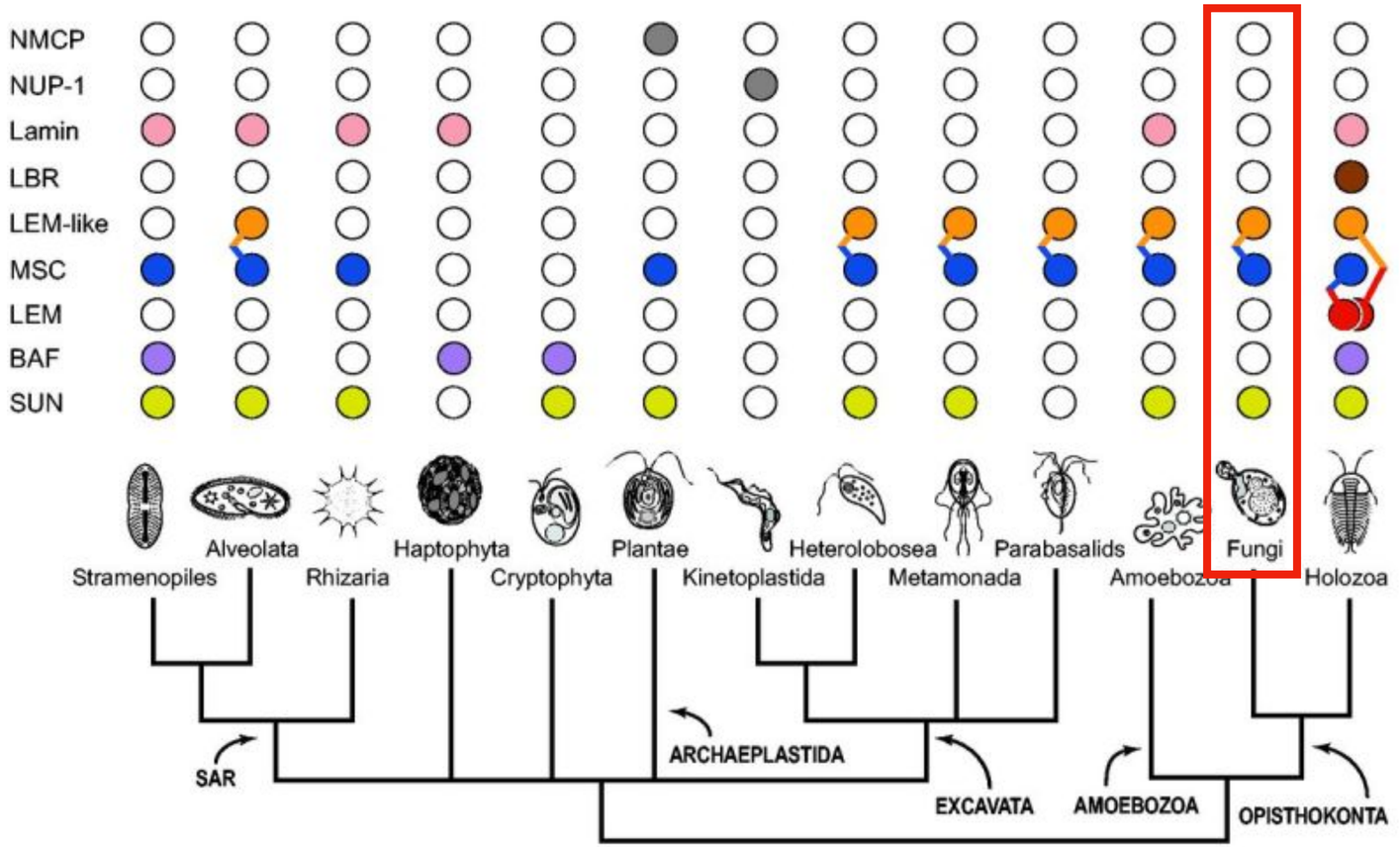


Plants lack lamins but have functional non-homologous lamins

Koreny & Field, 2016

Evolution of the Lamina

Different components of the lamina have different evolutionary trajectories

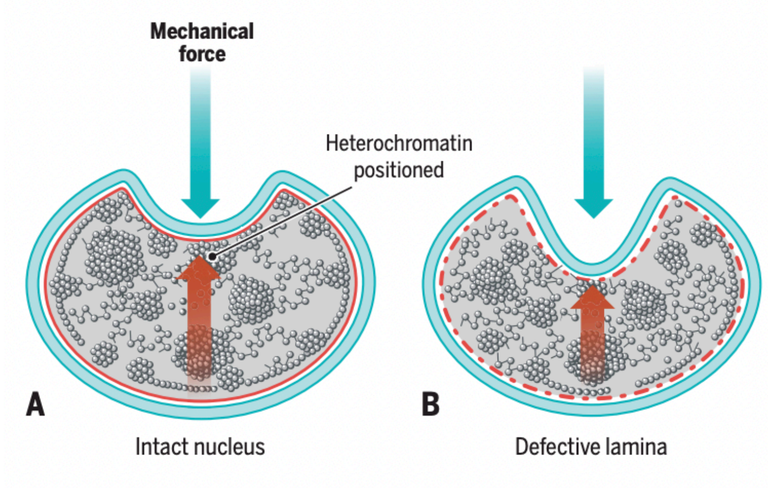


Lack of lamins compensated by nuclear envelope chromatin tethers? (LEM2, MAN1 in *S. pombe*)

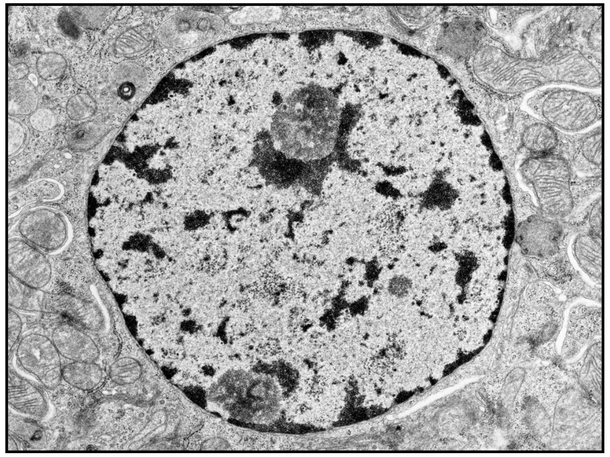
Koreny & Field, 2016

Why should I care about the lamina?

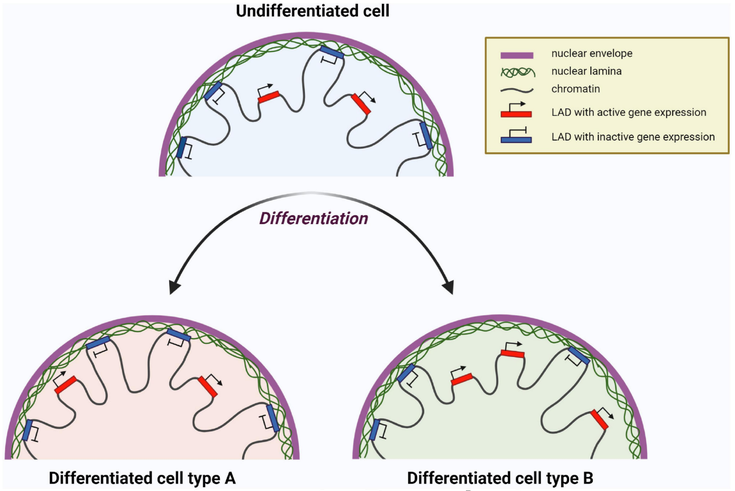
The lamina is a multifunctional component for proper development



Mechanical Support



Genome Organization



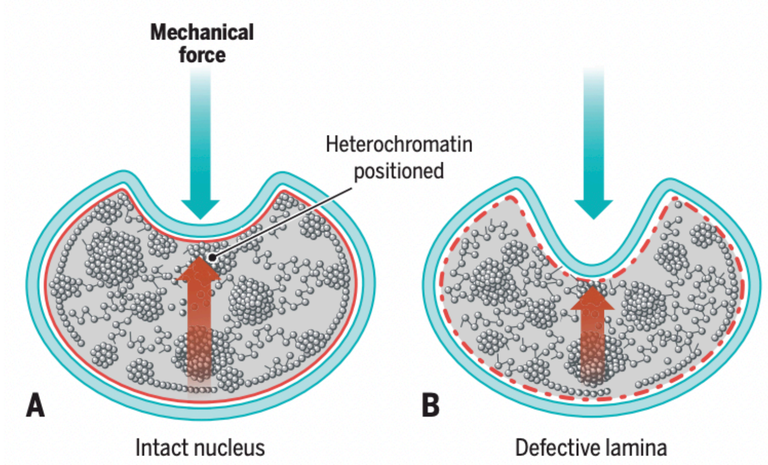
Epigenetic Regulation

Proper Developmental Plan

Bustin & Misteli, 2016

Why should I care about the lamina?

If you're only interested in chromatin biology these roles of the lamina might be more relevant 😬



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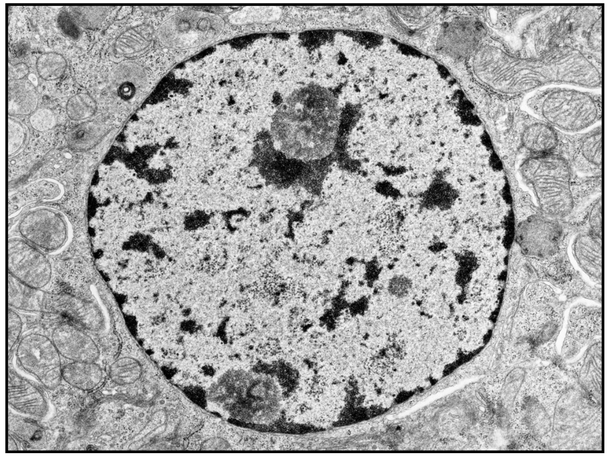

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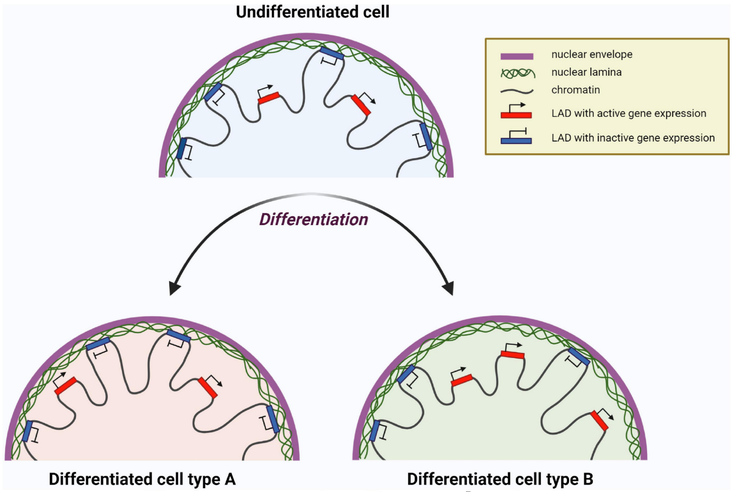


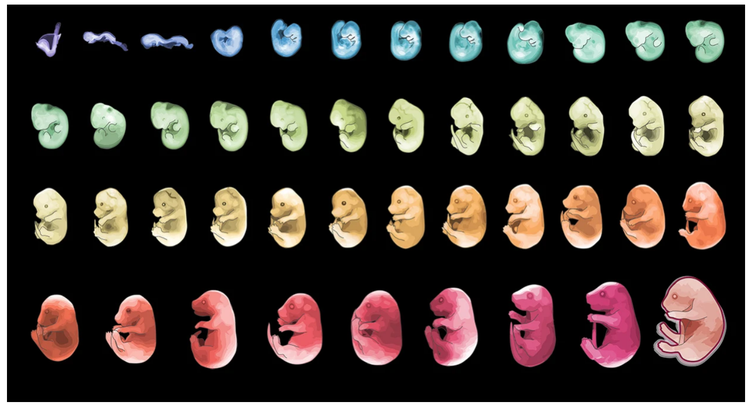
Epigenetic Regulation

Proper Developmental Plan

Bustin & Misteli, 2016

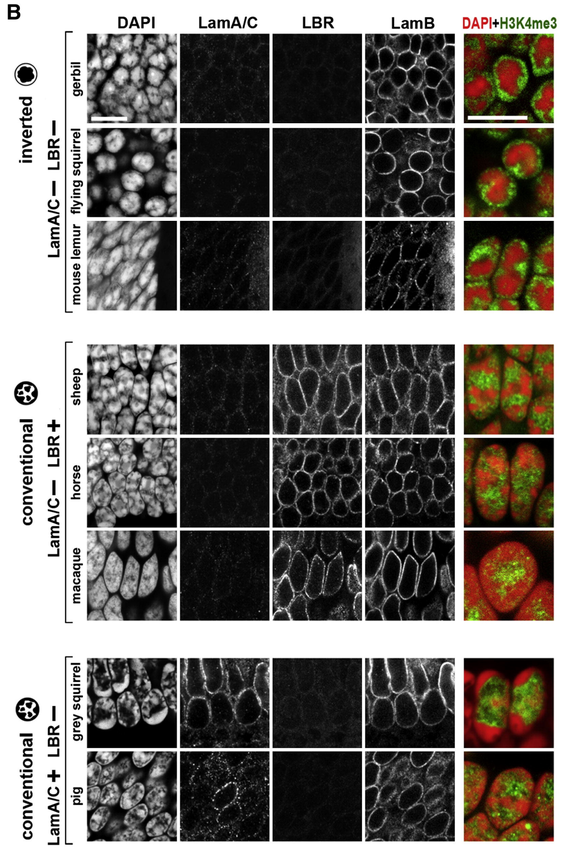
Lamina and Development

Lamin null mice are mostly non-viable



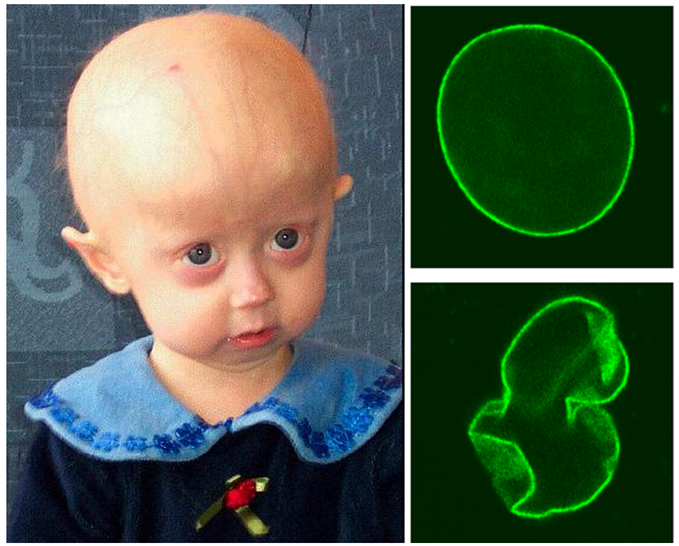
Lamins are needed for viability and development

LBR and Lamin A/C are differentially expressed



Lamins are differentially expressed in cell types/ differentiation

LmnA/C mutation leads to Progeria

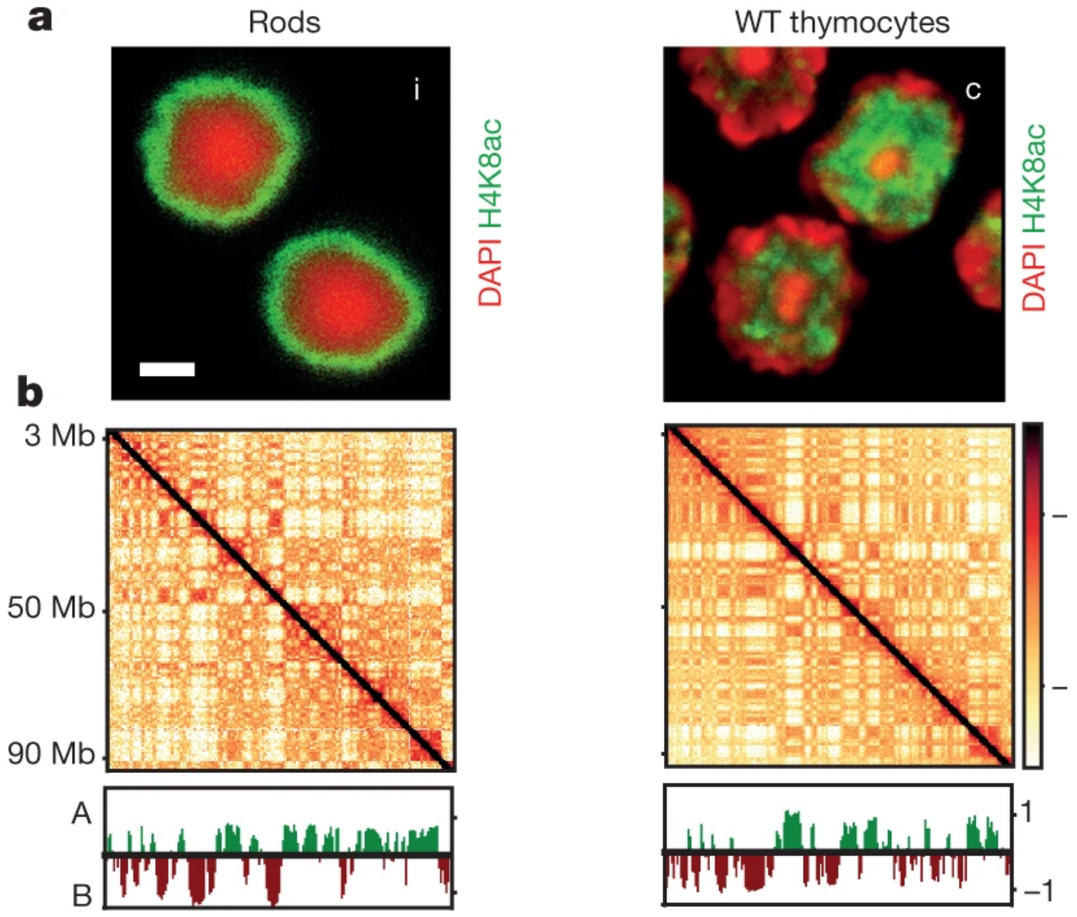


Loss of function mutations in lamins lead to disease

Solve et al, 2013

Lamina in Genome Organization

Inverted:
Rod cells have inverted organization → heterochromatin resides in the nuclear interior



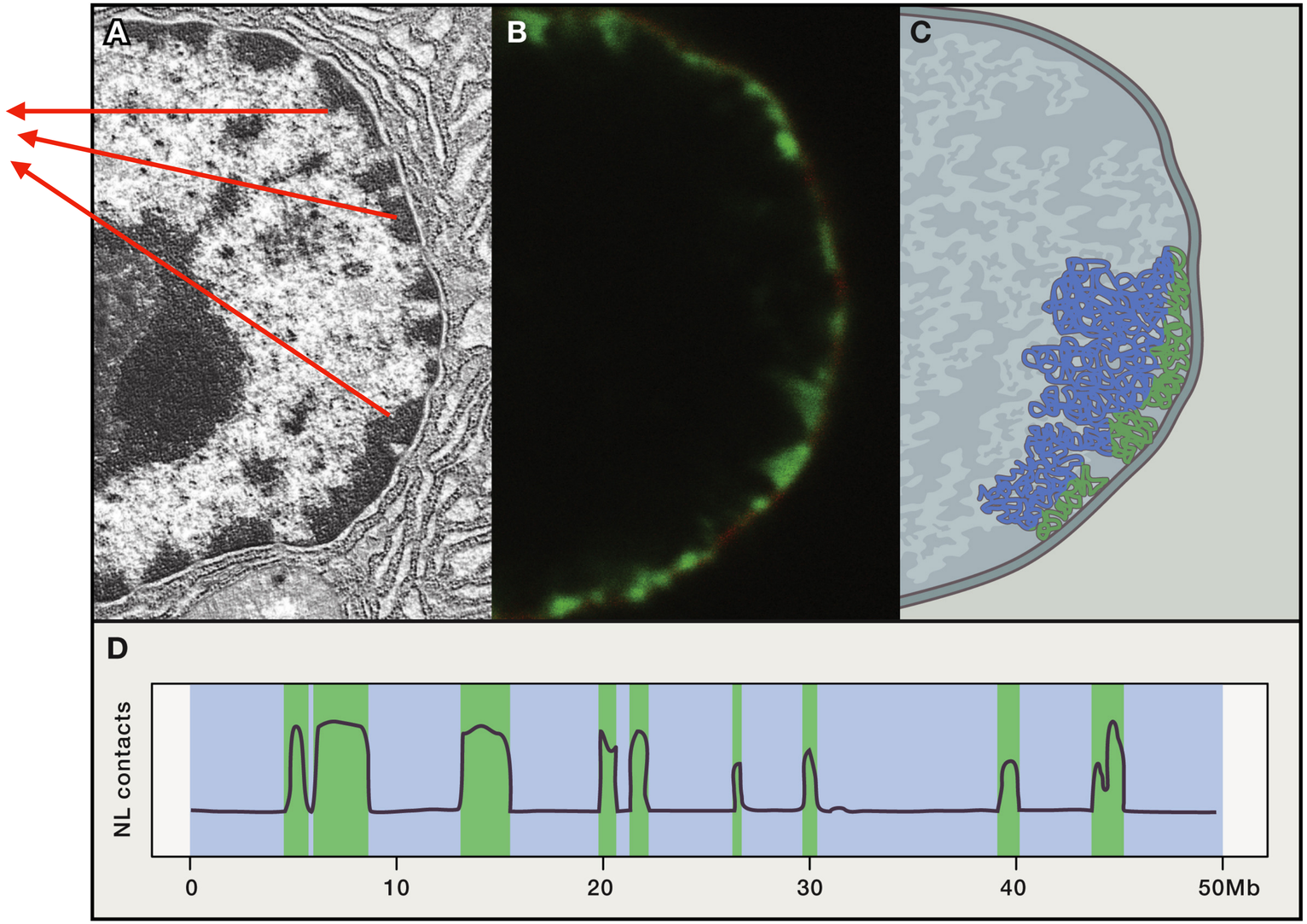
Conventional:
Most other cells have conventional nuclei → heterochromatin is peripheral and sparsely in the interior

However intra-genome contacts and TADs are mostly the same!

Lamina in Genome Organization - LADs

- Lamin associated domains (LADs) —genomic regions that contacts the lamina. These were identified via DamID (see Methods section)
  - Identified in human, mouse, worms, and flies

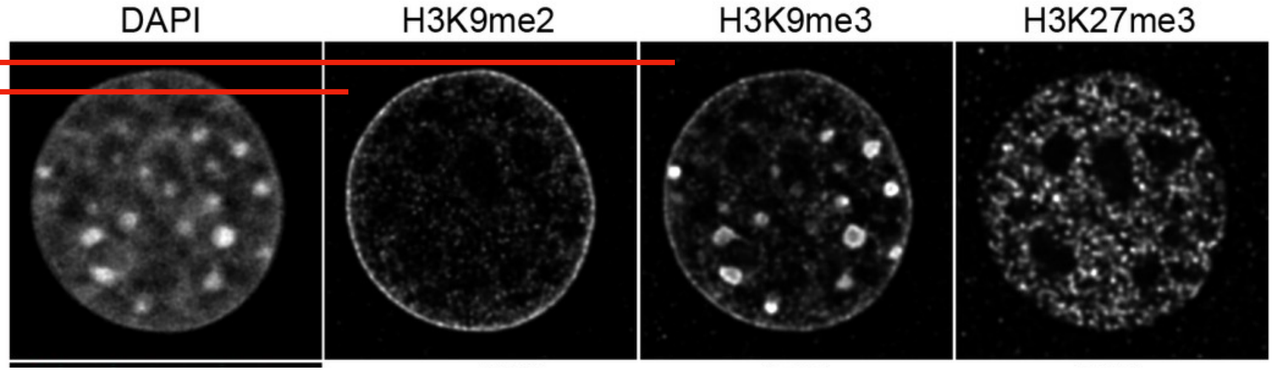
- How LADs are positioned to the periphery is a question under **active investigation**



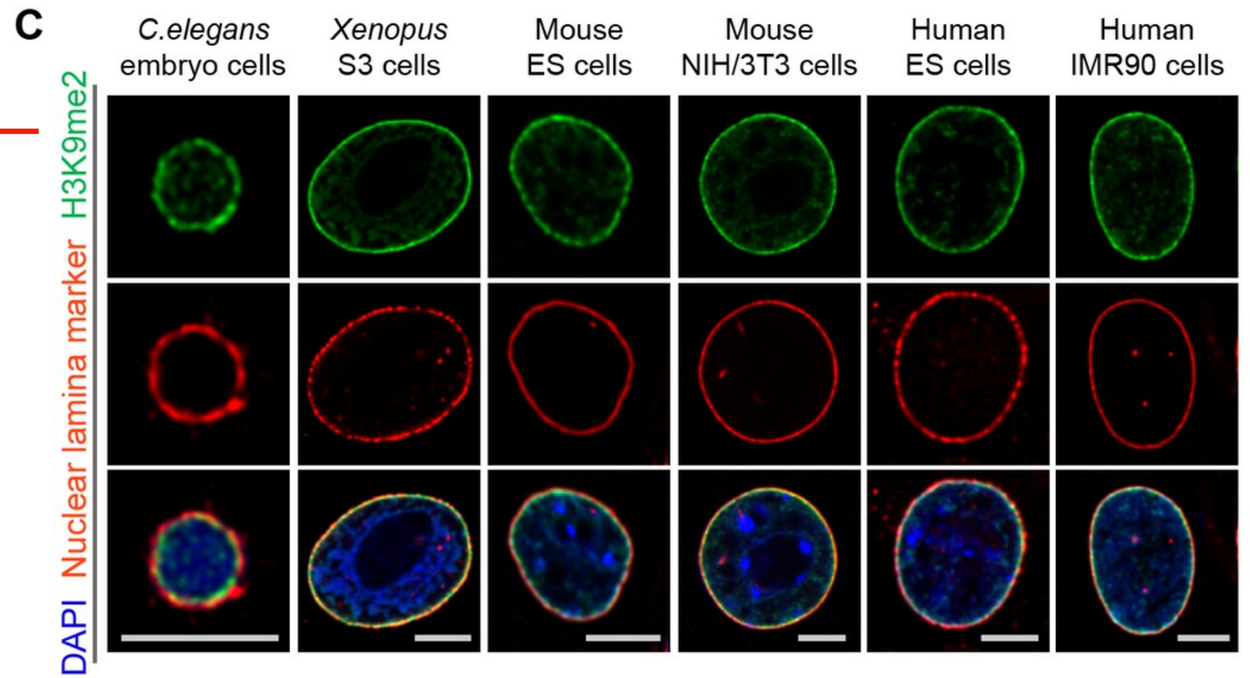
Van Steensel & Belmont, 2017

Lamina in Genome Organization - PTMs

- LADs are enriched for key histone PTMs H3K9me2 and H3K9me3



- Particularly, H3K9me2 is one major PTM that is uniquely enriched at the nuclear periphery
  - Evolutionarily conserved



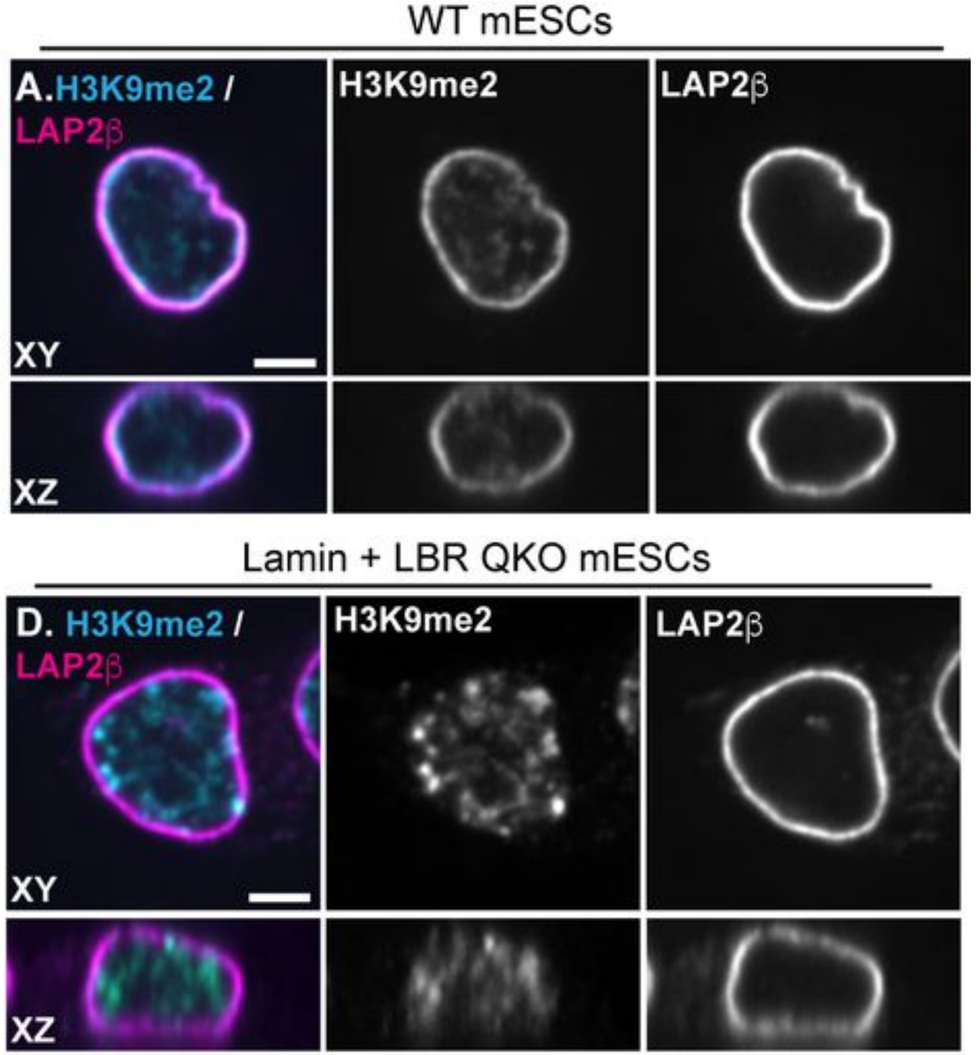
- ***What are the functional roles of spatially positioned heterochromatic marks?***

Poleshko et al, 2019

Lamina in Genome Organization - PTMs

- H3K9me2 is uniquely enriched at the nuclear periphery
- H3K9me2 marked domains are redundantly tethered to the periphery by the lamina + LBR
- Perturbing chromatin-lamina interactions leads to inverted nuclei-like organization

- ***How LADs and H3K9me2 marked domains are tethered to the periphery is not clear … yet!***

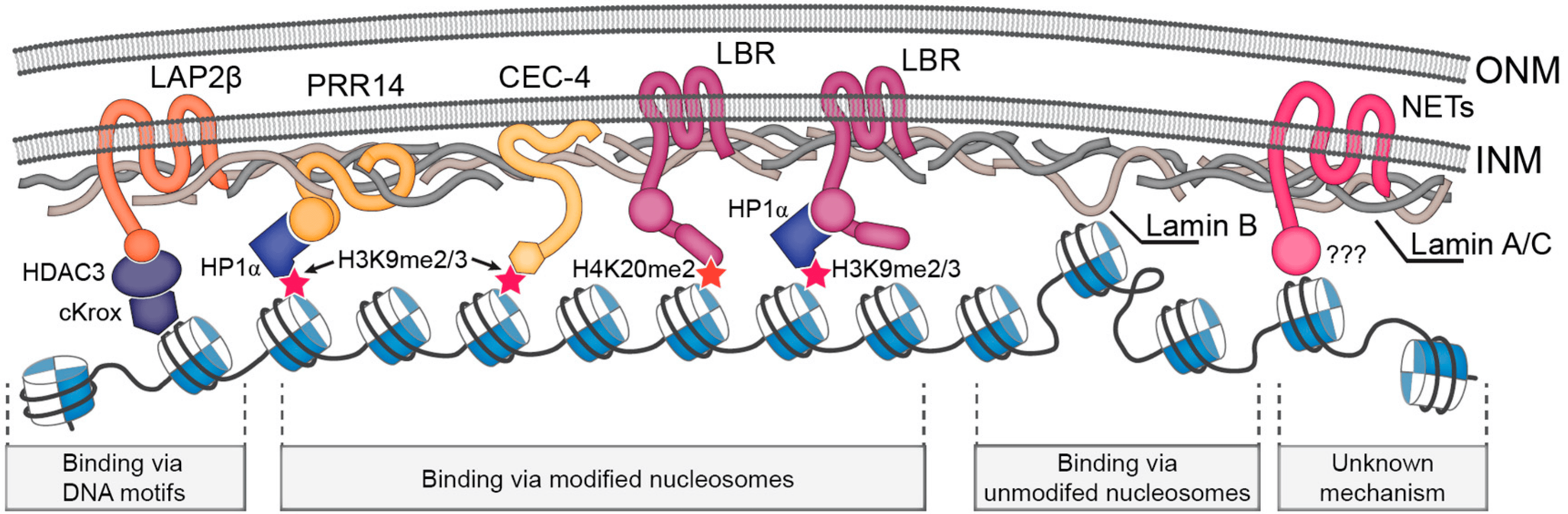


Marin et al, 2024

The nuclear periphery is a transcriptionally repressive environment—how is this achieved?

Lamina in Gene Expression - Readers

Several chromatin readers are associated with the periphery



Shevelyov & Ulyanov, 2019

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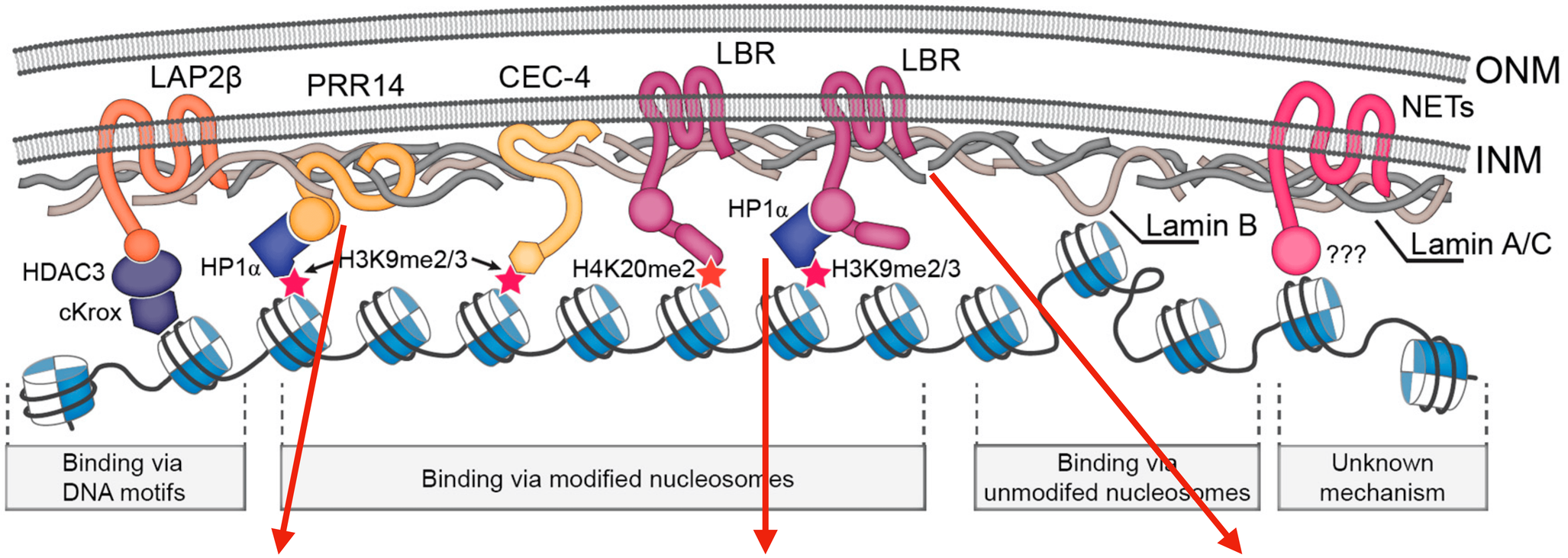


Proline Rich 14 (PRR14)

- *H3K9me3 binding*
- *Lamina binding*
- *Interaction with HP1*

Heterochromatin Protein 1 (HP1)

- *H3K9me2/3 binding*
- *Transcriptional repression*
- *Compaction*
- *Oligomerization*

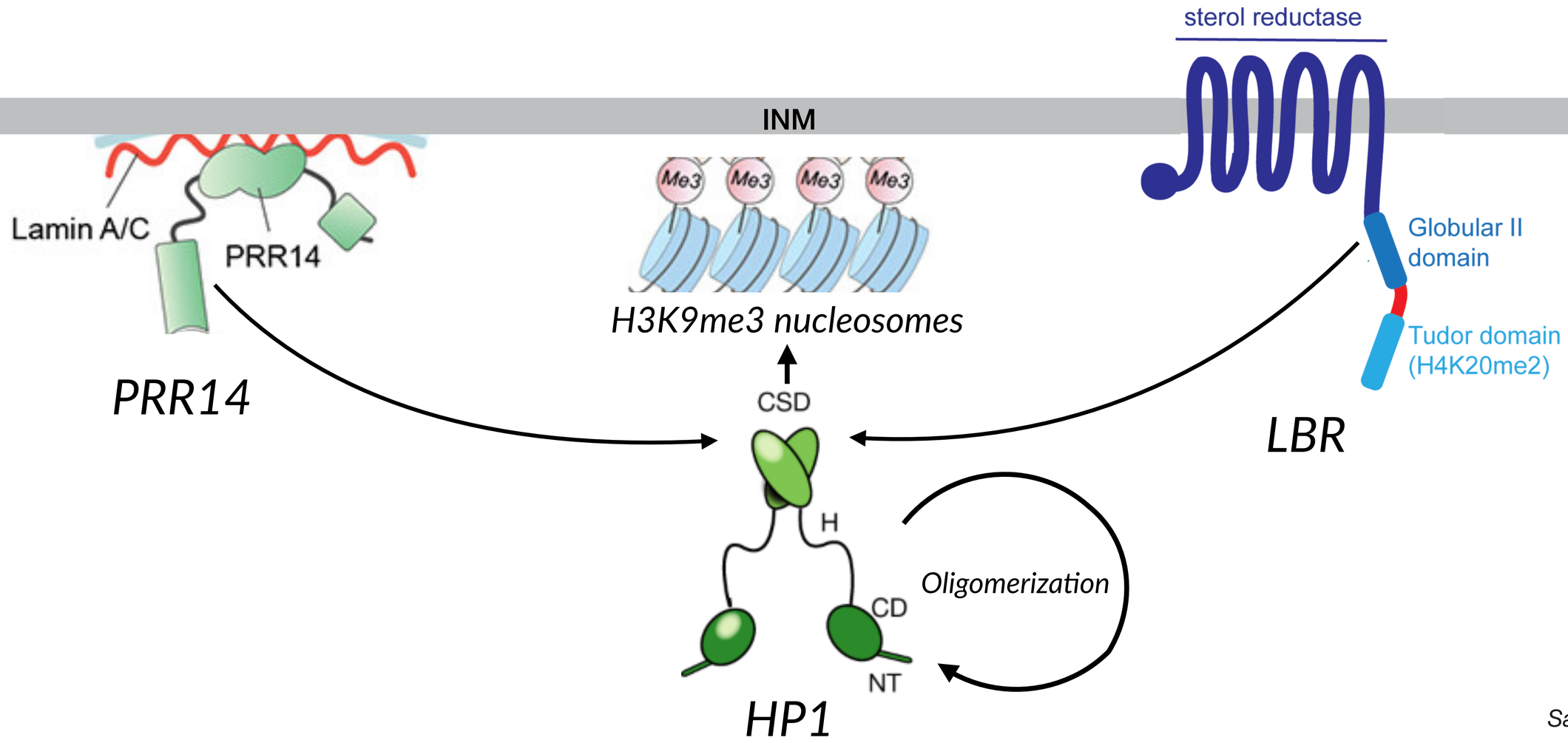
Lamin B receptor (LBR)

- *H4K20me2 binding*
- *Transcriptional repression*
- *Compaction*
- *Oligomerization*
- *Membrane-bound domain*
- *Interaction with HP1*

Shevelyov & Ulyanov, 2019

Lamina in Gene Expression - Readers

HP1 is redundantly recruited to the nuclear periphery

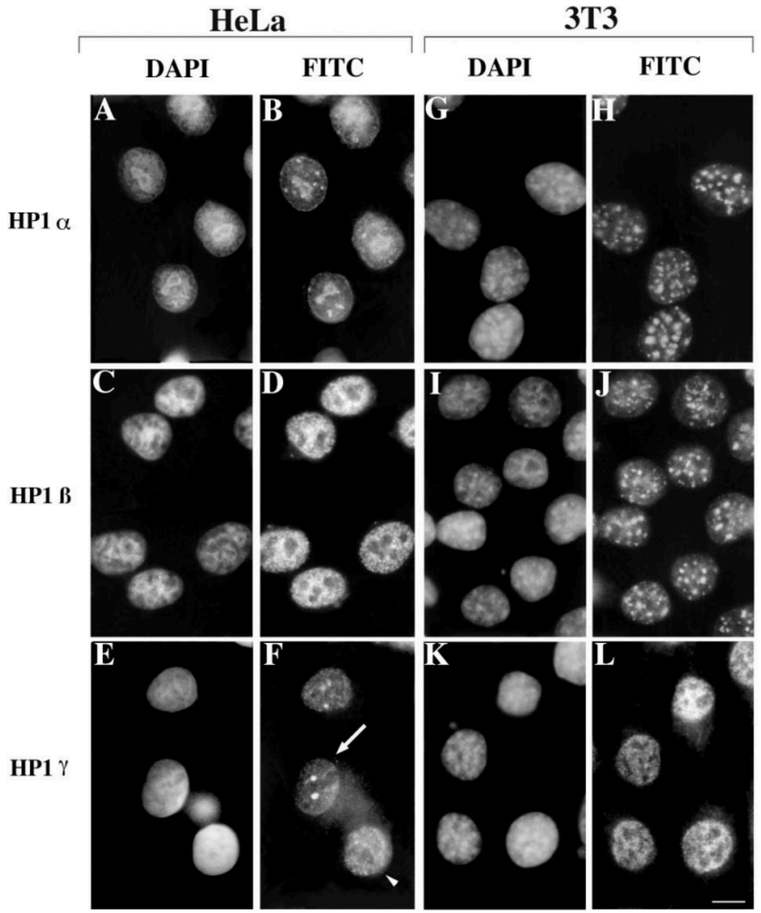


Sanulli et al, 2019

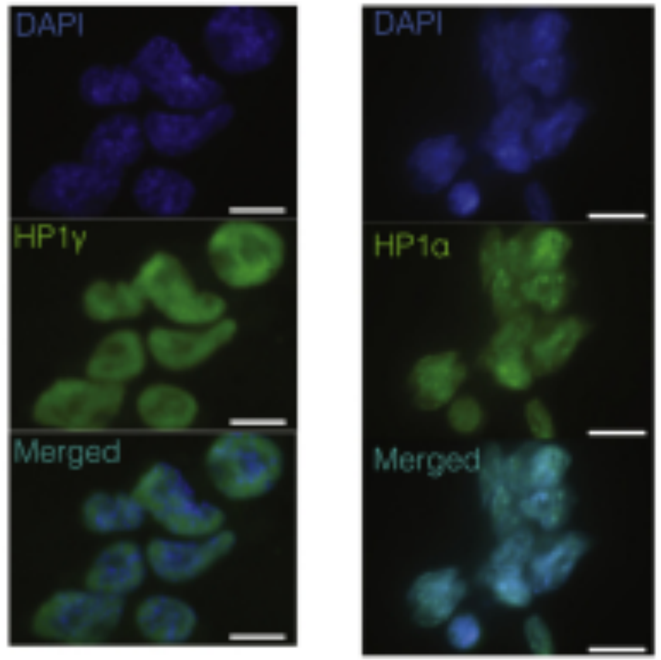
Kiseleva et al, 2023

Lamina in Gene Expression - Readers

HP1 paralogs are not necessarily enriched at the nuclear periphery



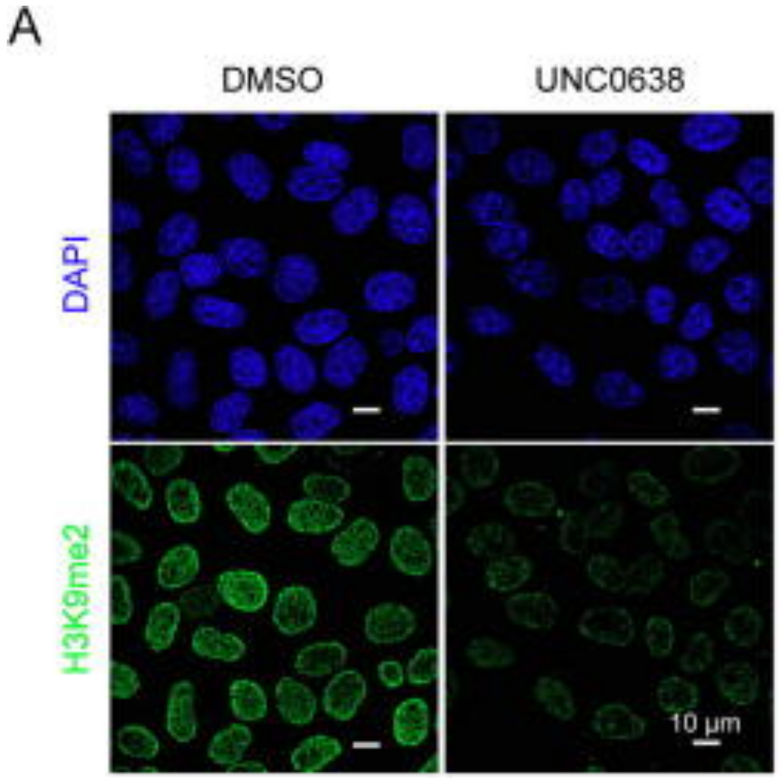
Mouse ESCs



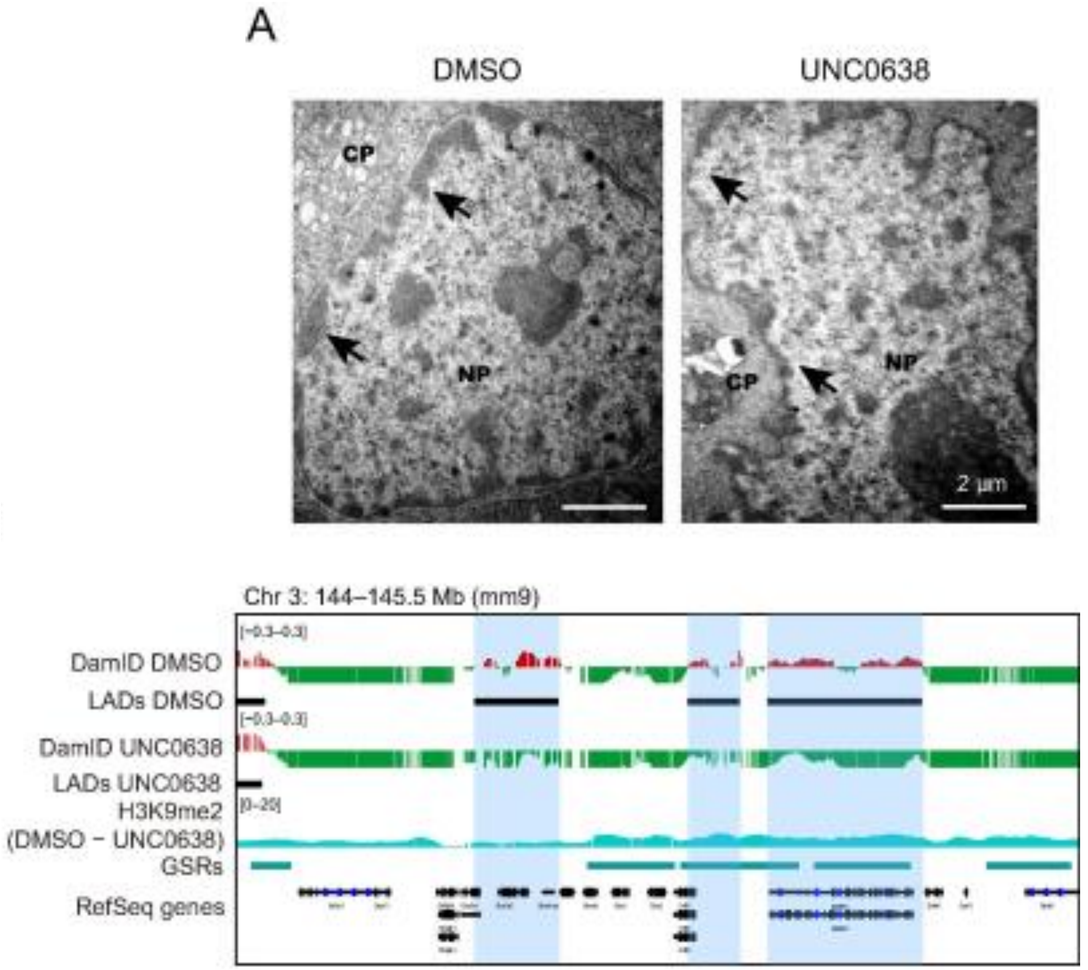
Minc et al, 1999
Zaidan et al, 2018

Lamina in Gene Expression - Writers

Histone methyltransferases play a role in lamina-chromatin contacts



G9a/GLP inhibition reduces global H3K9me2



G9a/GLP inhibition reduces chromatin lamina contacts

Yan et al, 2020

Lamina in Gene Expression - Writers

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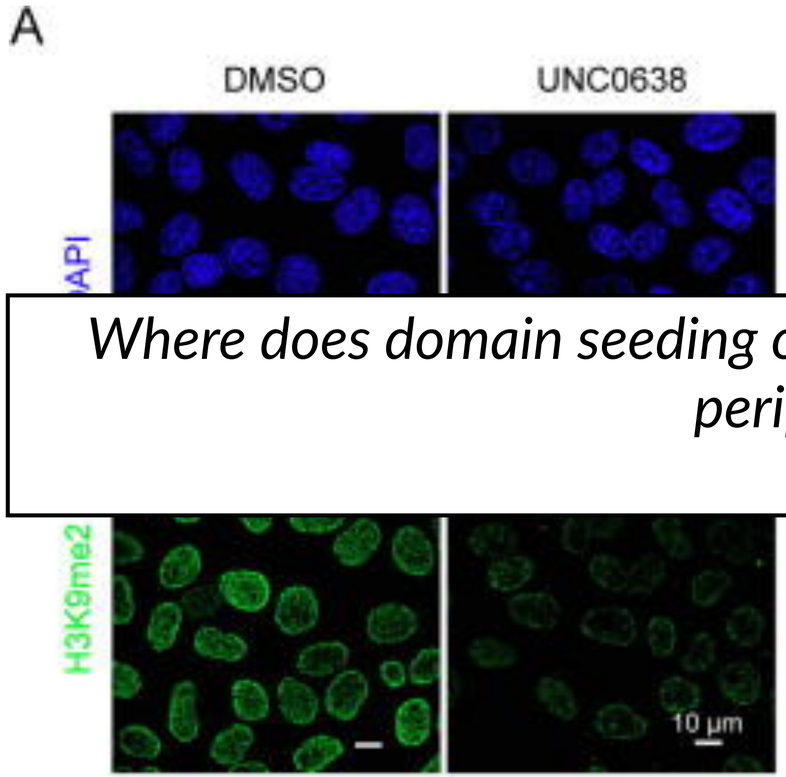

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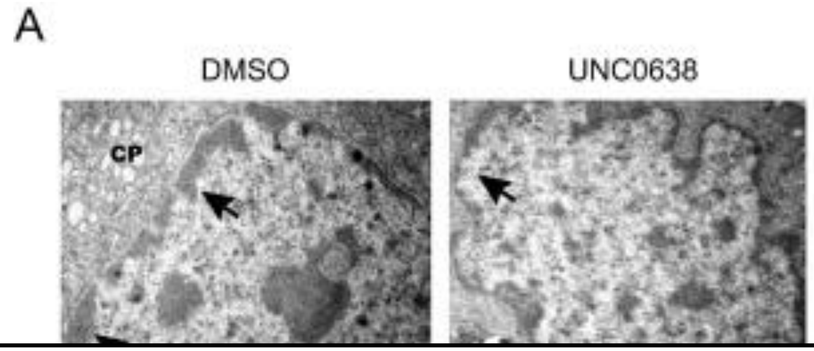
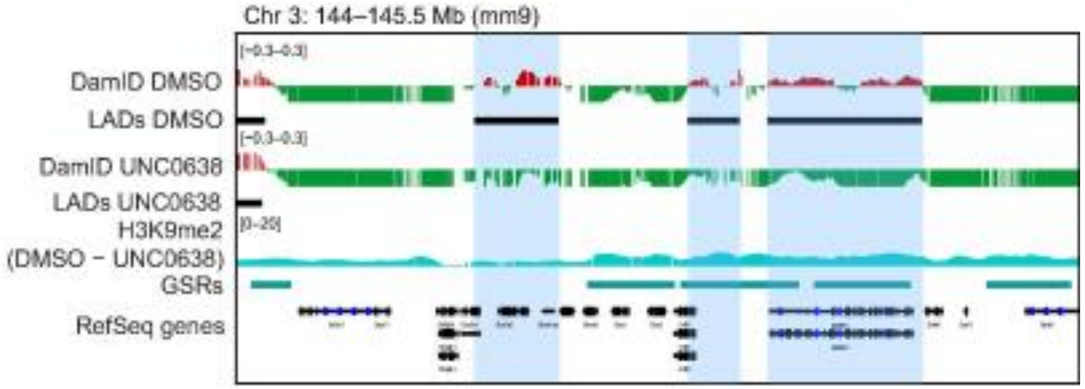


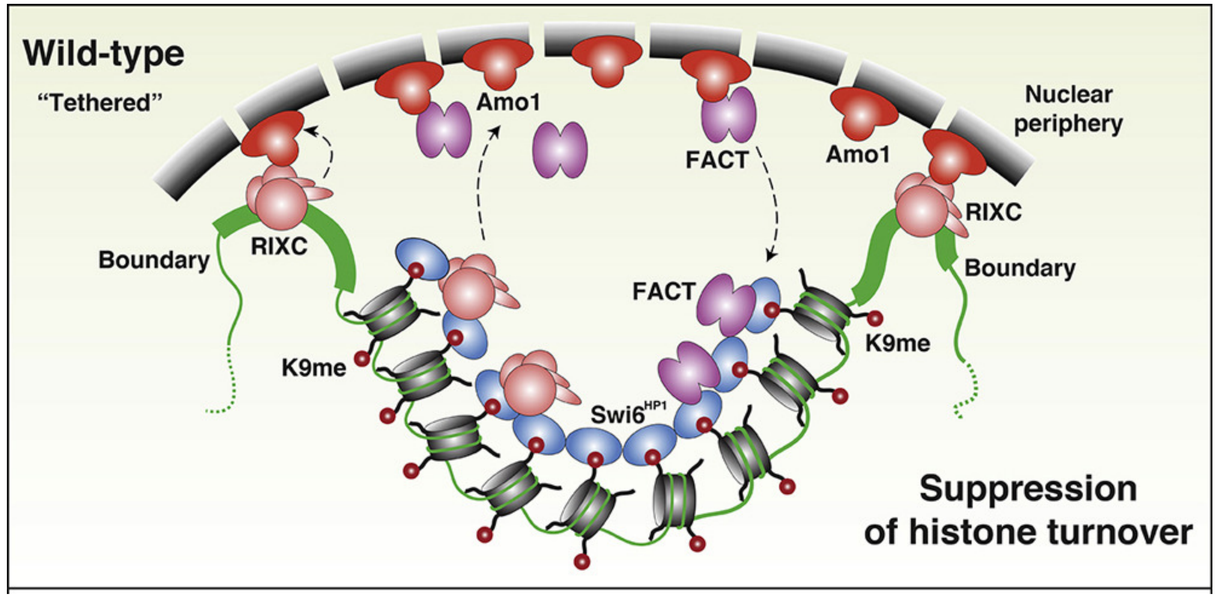
Where does domain seeding occur? Does G9a/GLP convert H3K9me2 → me3 at the periphery through differentiation?

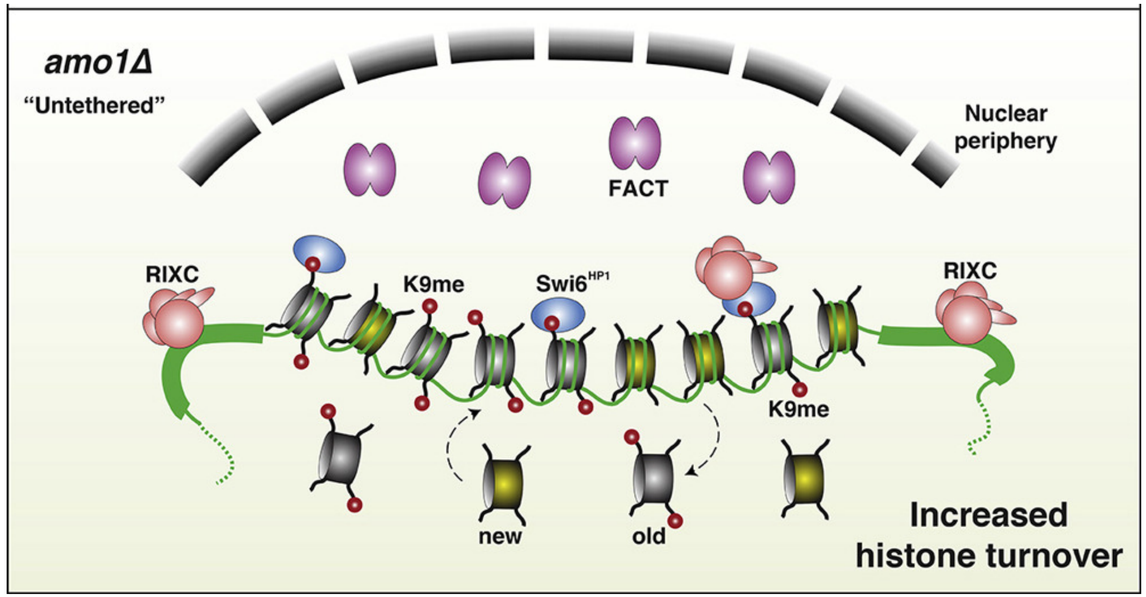


G9a/GLP inhibition reduces chromatin lamina contacts

Yan et al, 2020

Lamina in Gene Expression - Turnover?





In S. pombe, nuclear rim protein *Amo1* promotes Swi6-FACT association to inhibit histone turnover and maintain repression

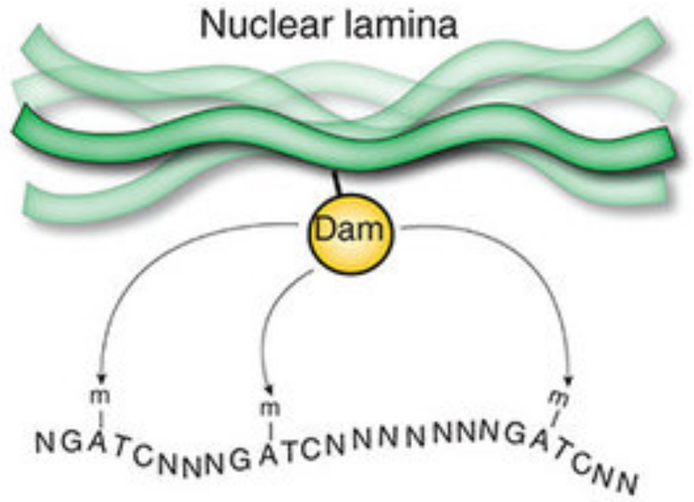
Does the metazoan nuclear periphery confer repression through a similar mechanism?

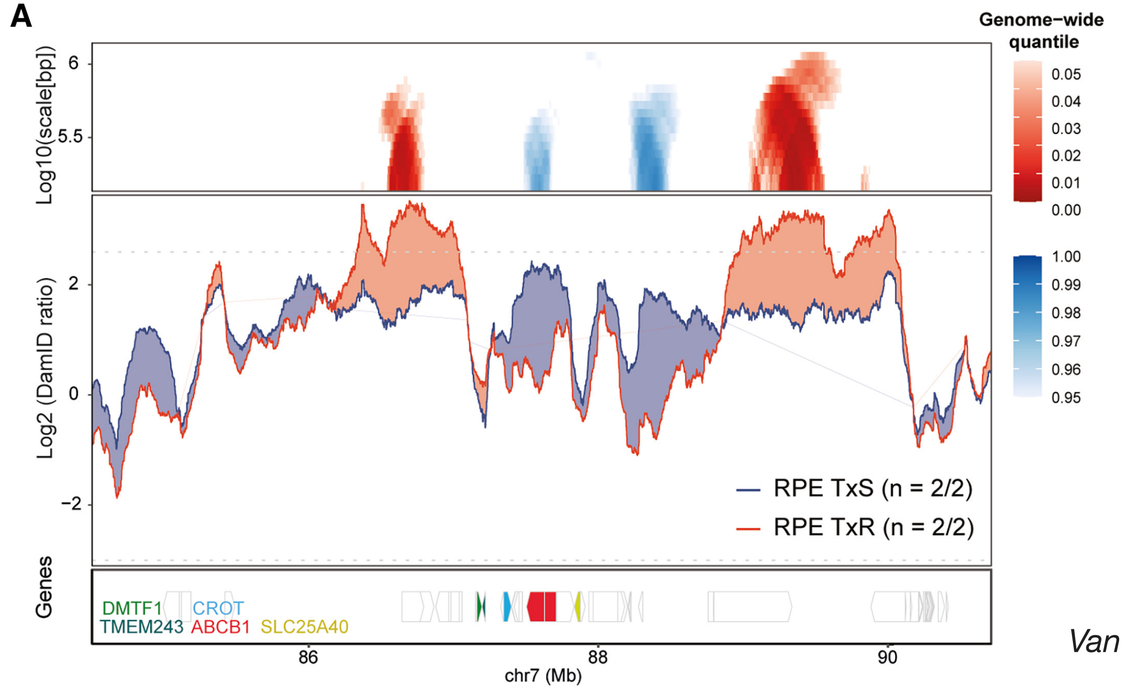
Holla et al, 2020

Methods to Study the Lamina

DNA Adenine Metyhltransferase - DamID

- DNA Adenine Methyltransferase (Dam) fused to a component of the lamina (eg. Lamin B1)
  - Now more commonly Dam fused to pA which binds antibody fold
- Useful for identifying base LAD state as well as change in chromatin-lamina contacts





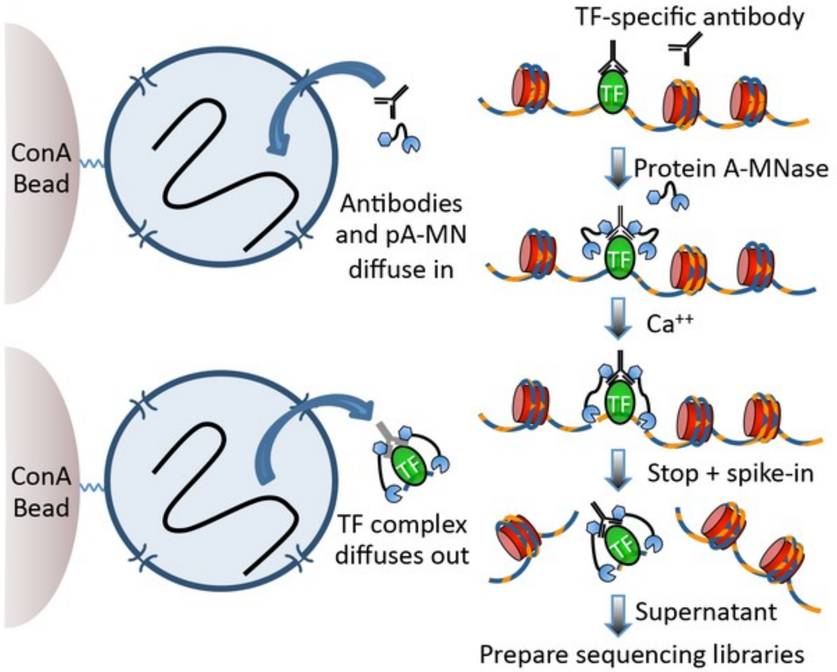
Van Steensel & Henikoff, 2000

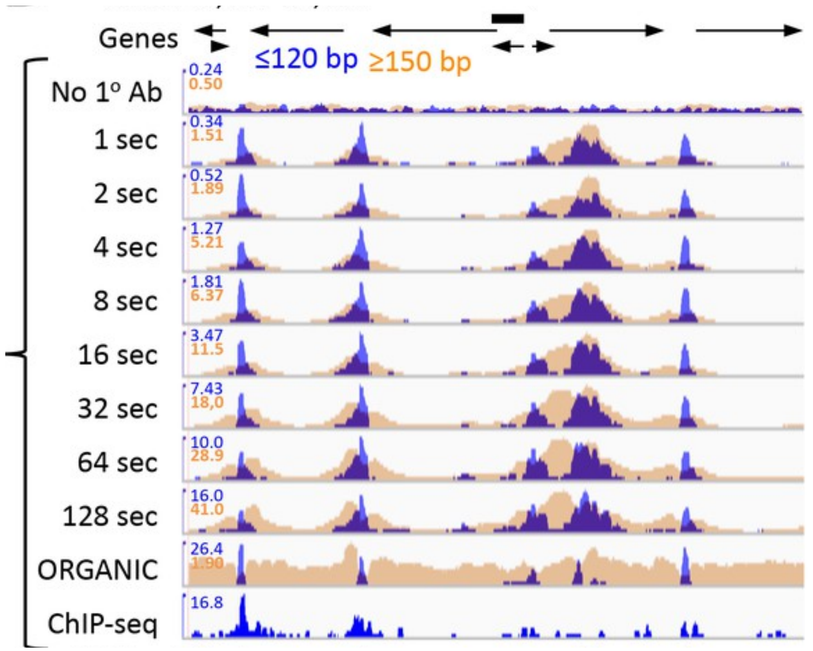
Manjon et al, 2023

Methods to Study the Lamina

Cut & Run

- Permeabilized cells are incubated with antibody against lamina target
- pA-MNase is added which diffuses into cells, binds to antibody fold and cuts DNA on both sides of antibody binding site, freeing DNA
- Cut DNA diffuses out of cell and are purified for library and sequencing prep
- Useful for identifying LADs as well as change in chromatin-lamina contacts



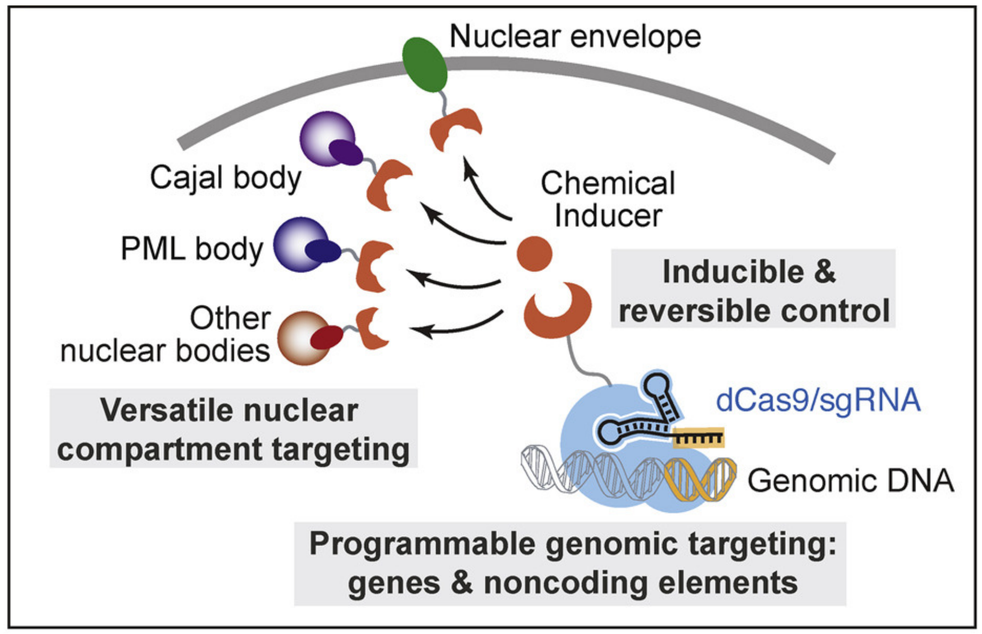


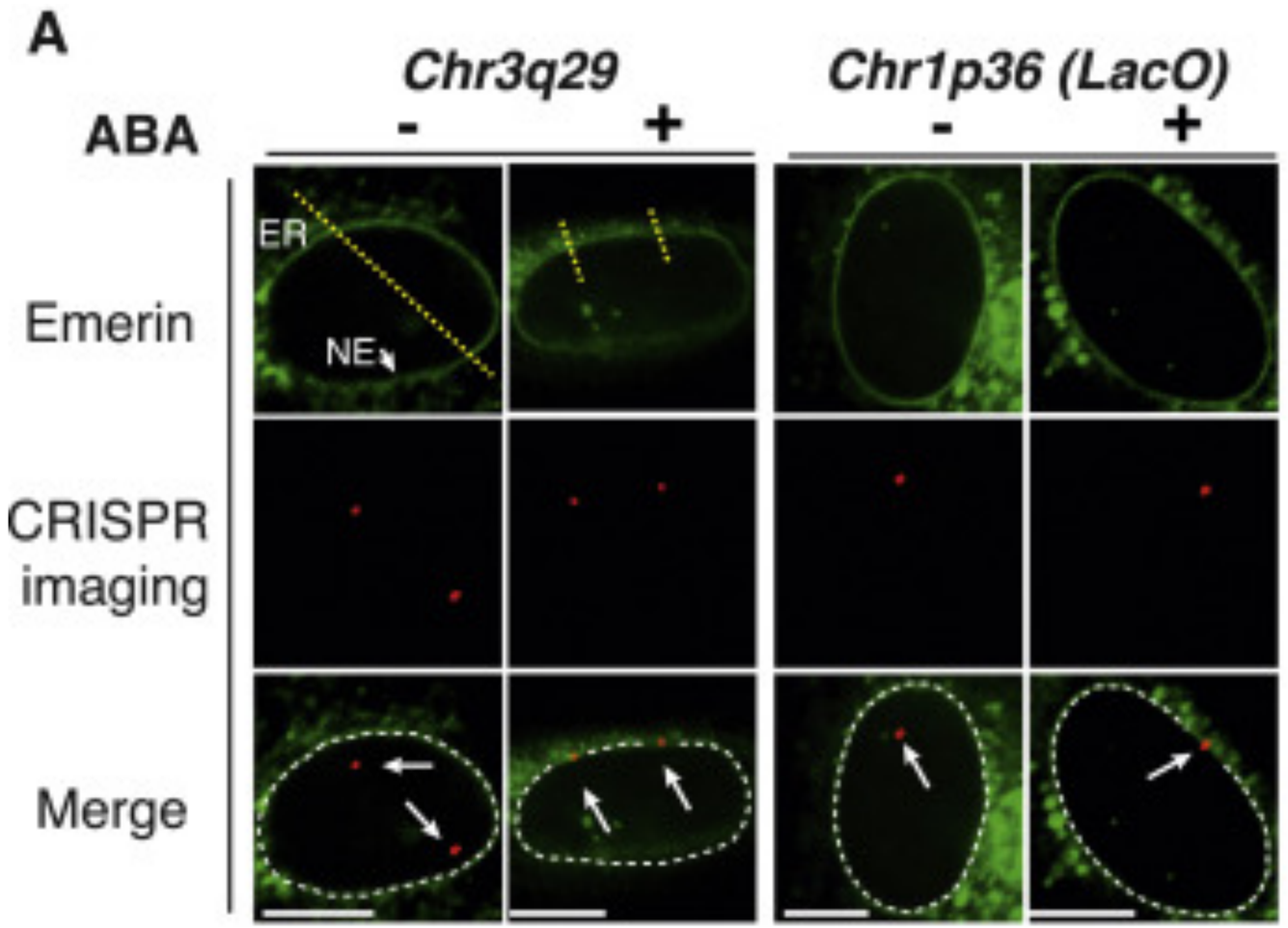
Skene et al, 2017

Methods to Study the Lamina

Synthetic lamina tethering via Cas9

- dCas9 inducibly tethered to the NE via ligand
- Useful for repositioning specific loci within nucleus and assaying repressive capability of periphery



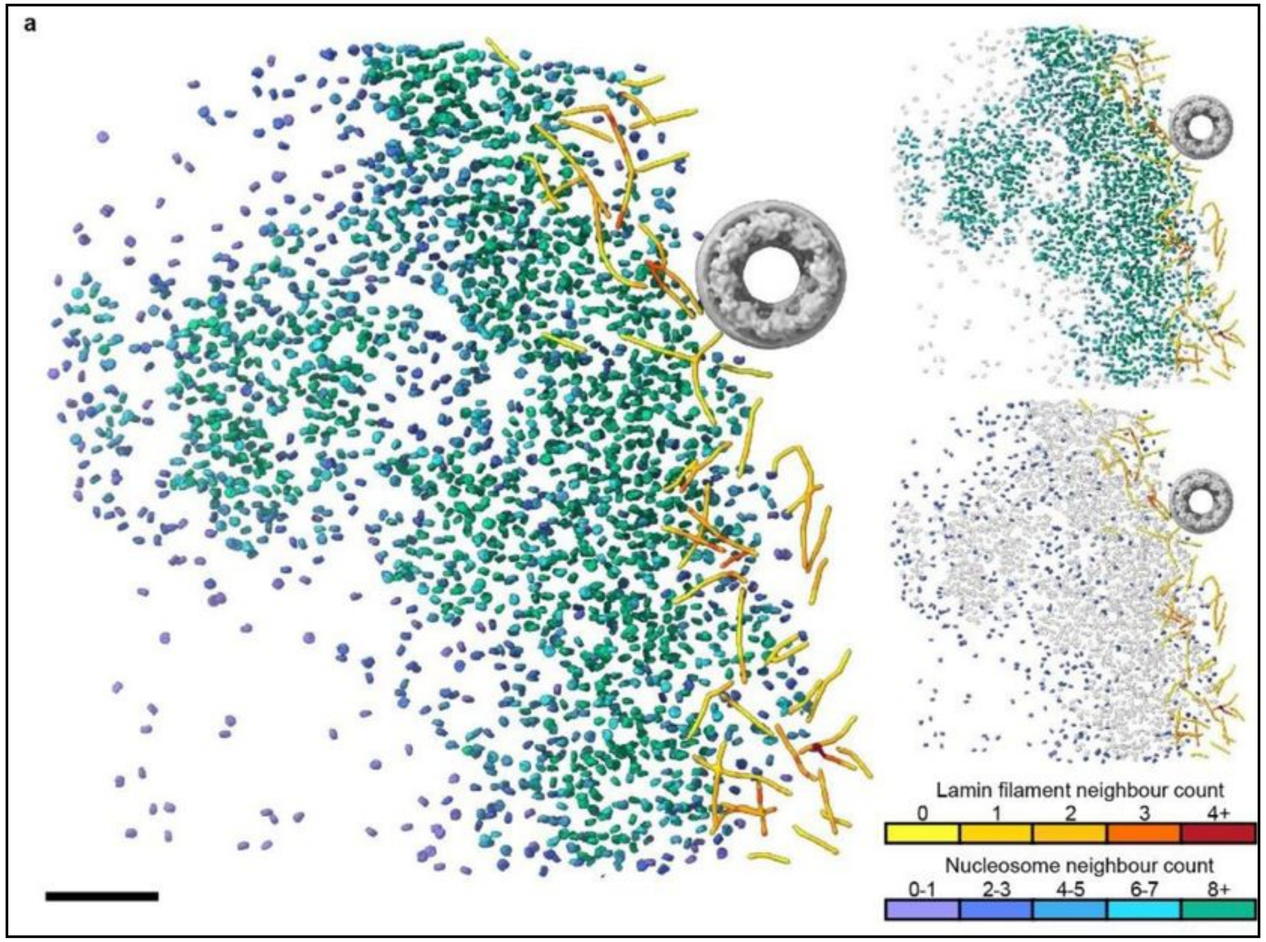


Wang et al, 2018

Methods to Study the Lamina

Cryo-ET

- Allows in situ imaging of vitrified cells, tissues, even organisms in native state
    - Needs cryo-FIB milling to produce thinner subsamples
- Useful to measure nucleosome or lamin density, lamina structure/shape



Wang et al, 2024

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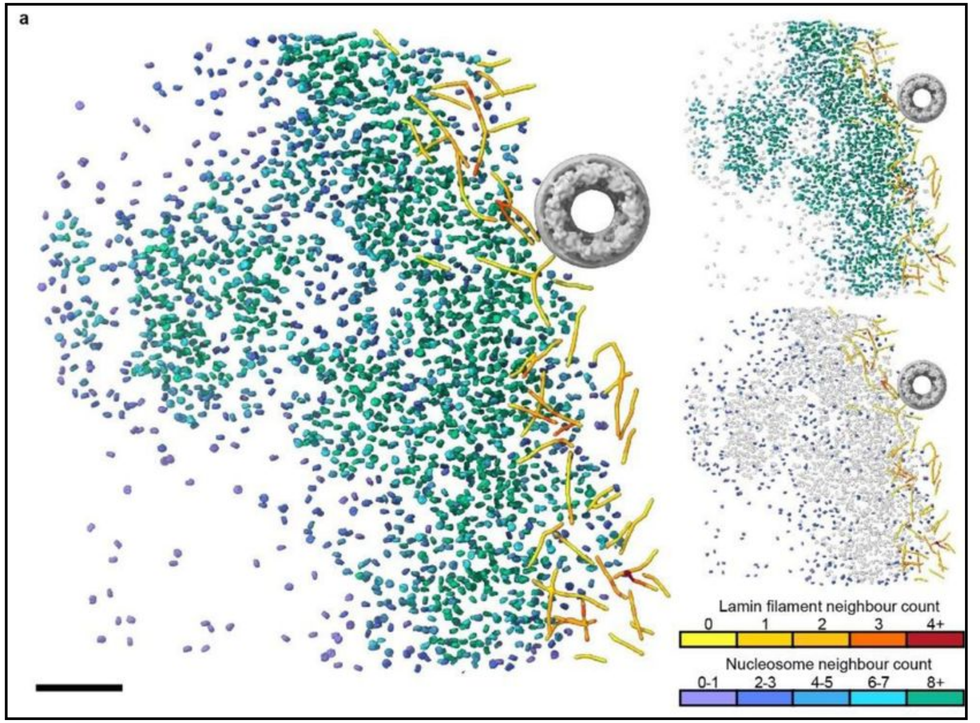

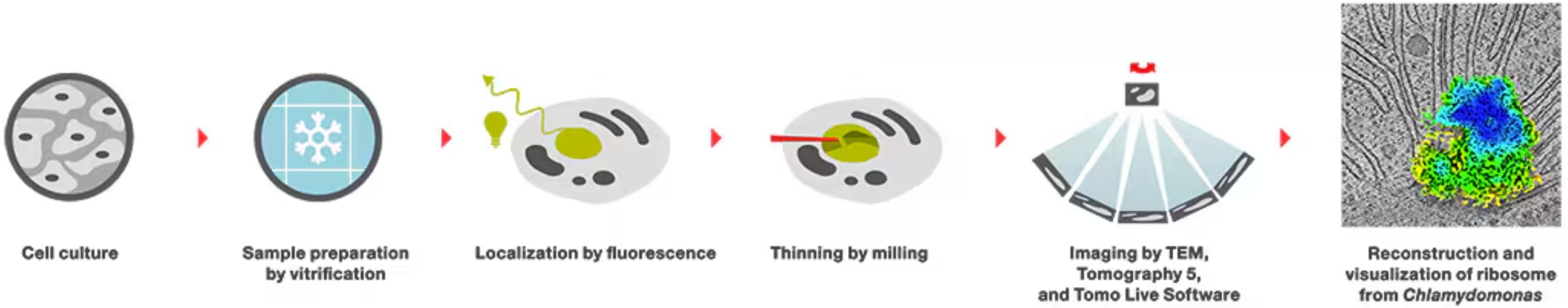

Wang et al, 2024

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