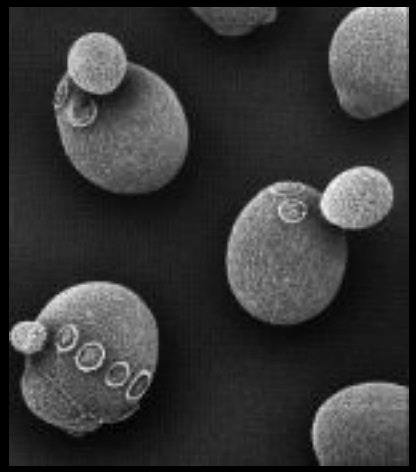
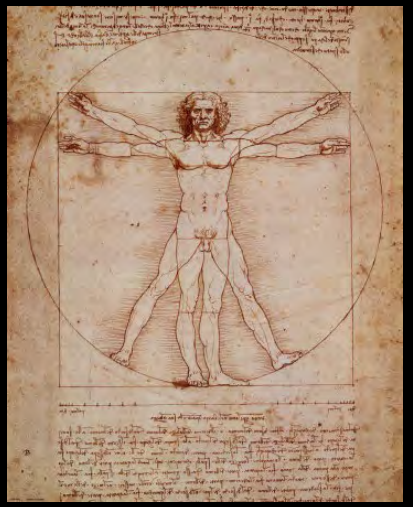
# *Exploring model of chromosome in vivo : ribosomal DNA and nucleolus*

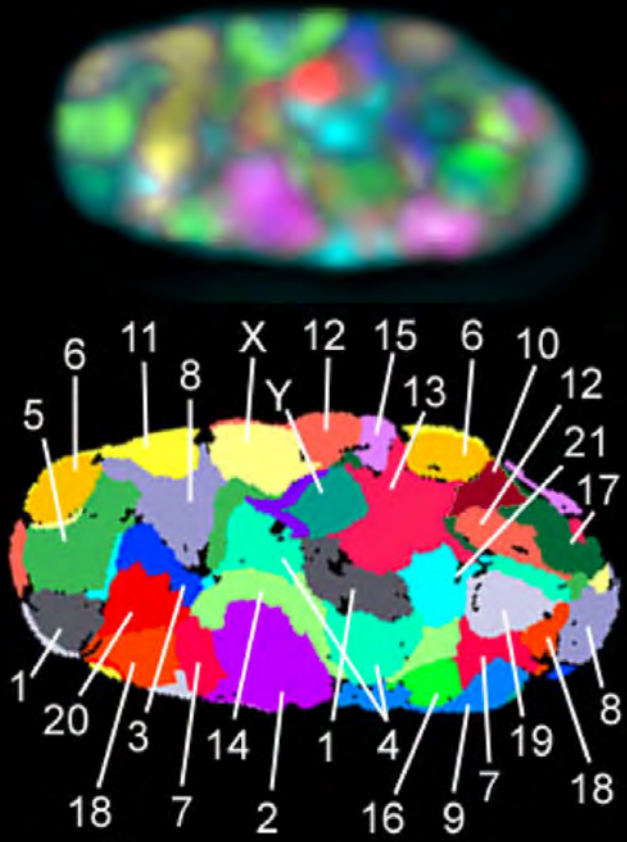
*Olivier Gadal,*

*MCD - CBI, Toulouse*





# Why studying nuclear organisation and intranuclear position of chromosomes ?

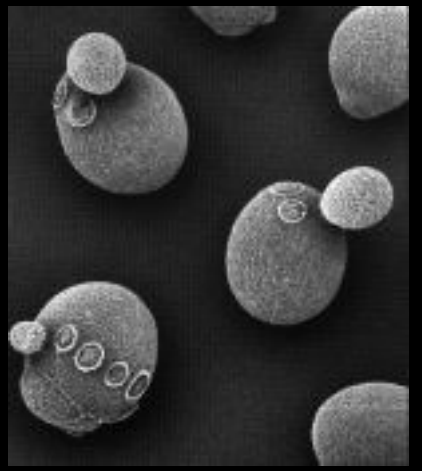



Bolzer et al., (2005)
PLoS Biol

Genetic material is not randomly organized

"Connected" to gene expression and genome stability

Underlying principle ?



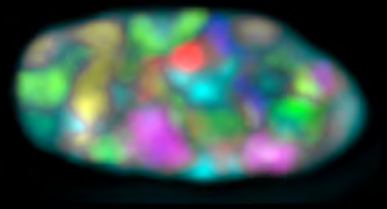
Yeast model

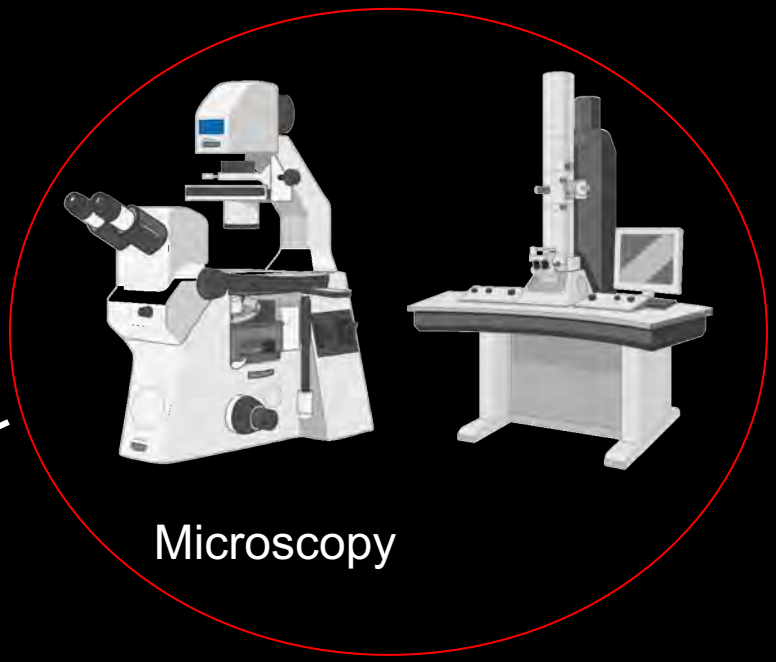
# What are the tools to explore genome organization ?

Poster(s) - Cohesin studies

Christophe Chapard
Henri Mboumba

Chromosome conformation capture (3C)



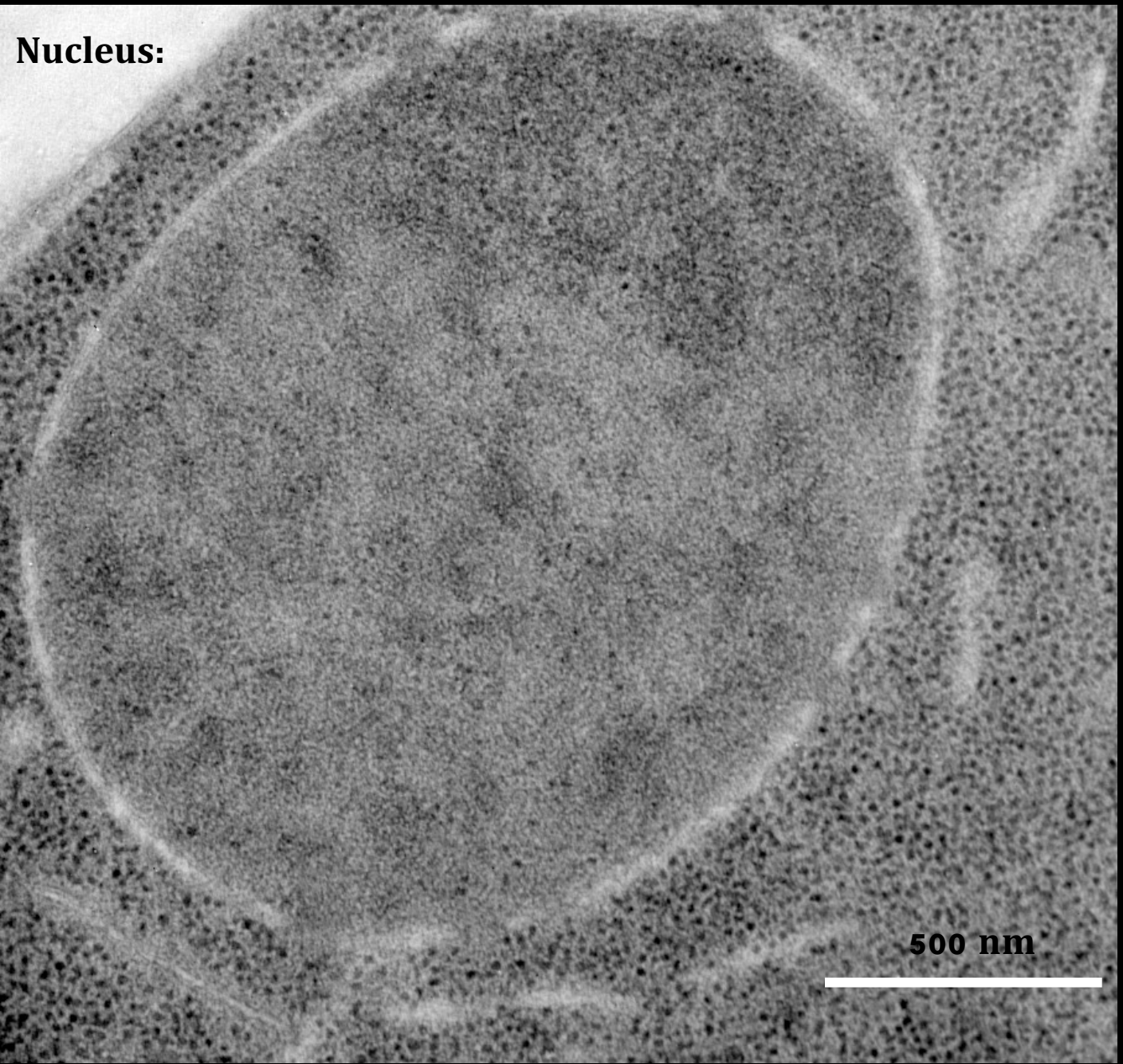


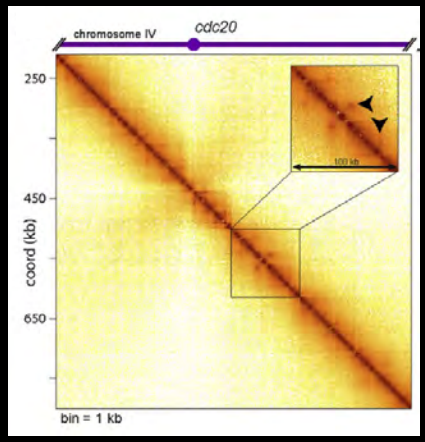
Microscopy

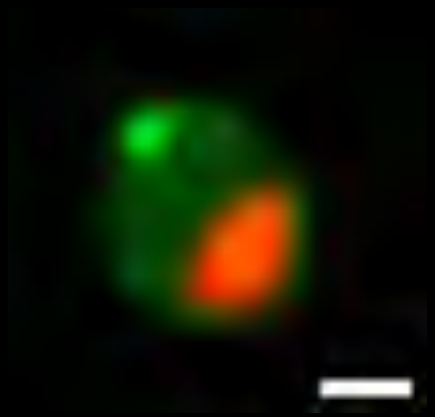
Modeling !

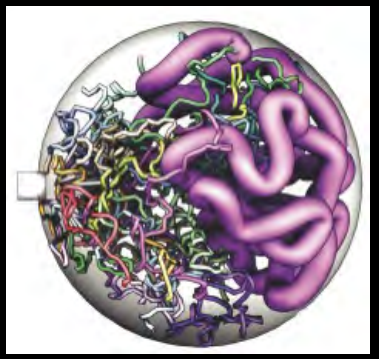
# A genetic model to explore genome organization ?

**Nucleus:**



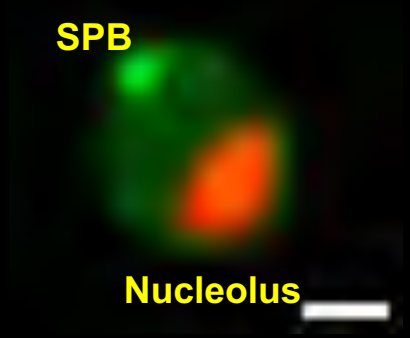


# Nuclear architecture in budding yeast

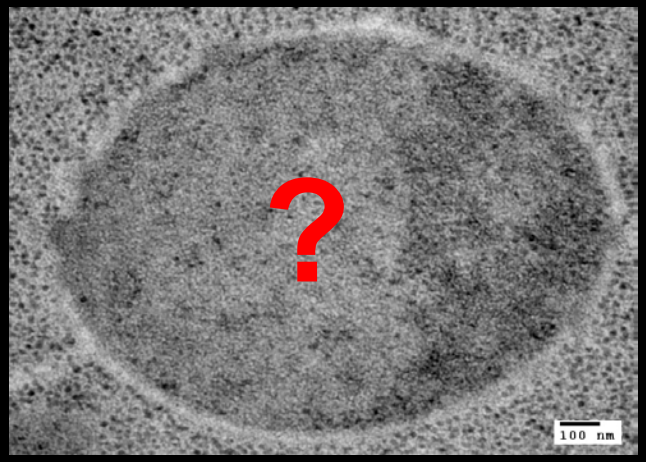
*S. cerevisiae* ~ 13 Mb, 16 chromosomes / 4µm$^3$

***Cryofixation/cryosubstitution – I. Leger-Silvestre***

*Diametrally opposed structure*

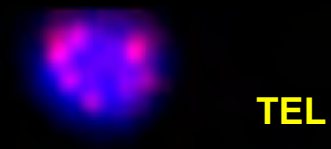


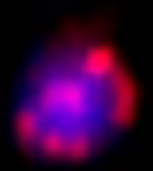

Yang et al., Chromosoma, 1989
Picture - A.B Berger




Léger-Silvestre *et al.*, Chromosoma, 1999

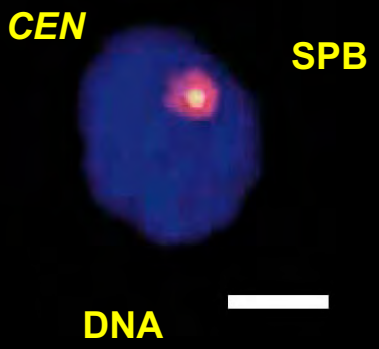
*Telomeres clustering*



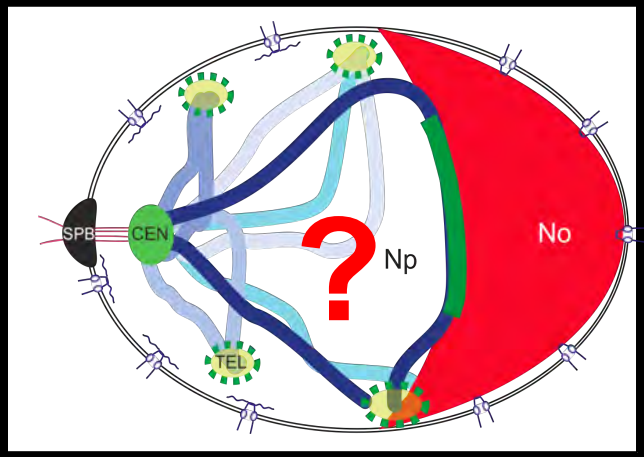




FISH Gotta et al., 1996
Picture - V. Galy

*Centromeres clustering*



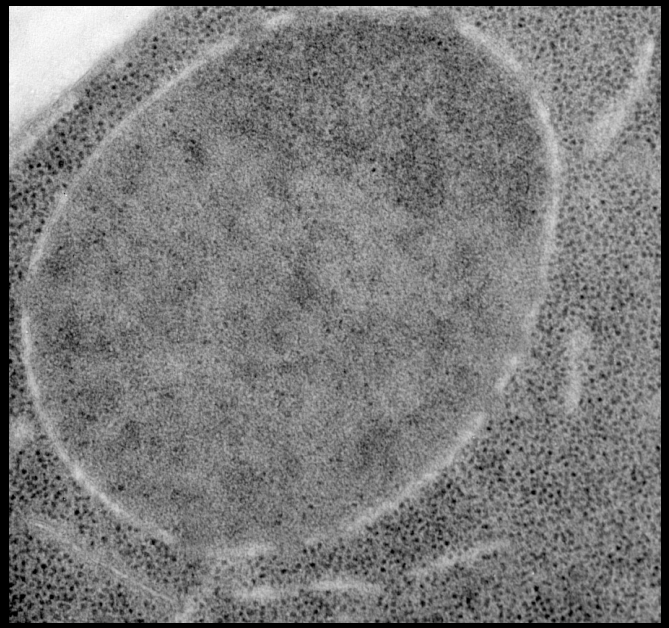

**FISH** Jin *et al.*, J. Cell Sci, 2000



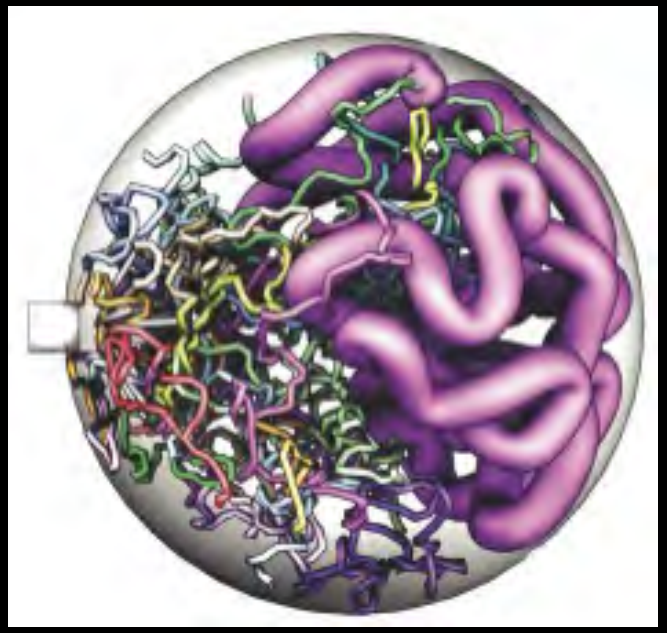

**Rabl conformation**

# Budding yeast chromosomes

*4 µm$^3$ – 16 chromosomes*



*In silico model of chromatin*



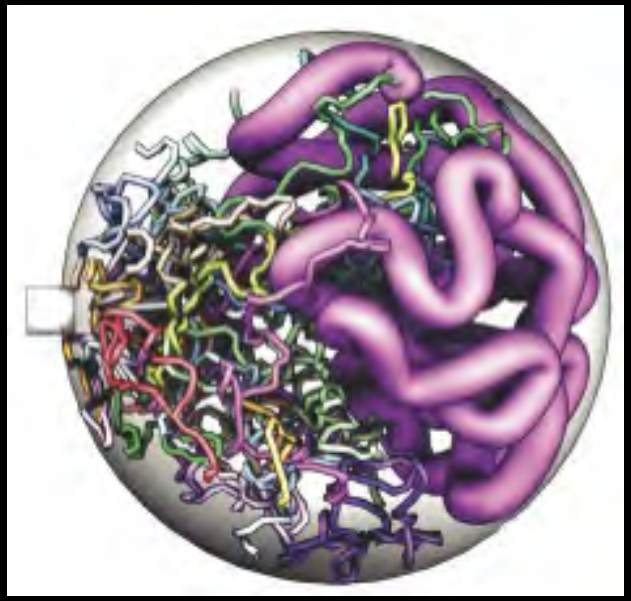
Coarse-grained model

*Wong et al., 2012*

Modeles proposed from « ensemble statistic »

# From budding yeast chromosomes to chromatin ?

*In silico model of chromatin*



*Wong et al., 2012*

Explore model prediction

Gene position ?

*In vivo* chromosome motion ?

Super-resolution microscopy ?

# From budding yeast chromosomes to chromatin ?

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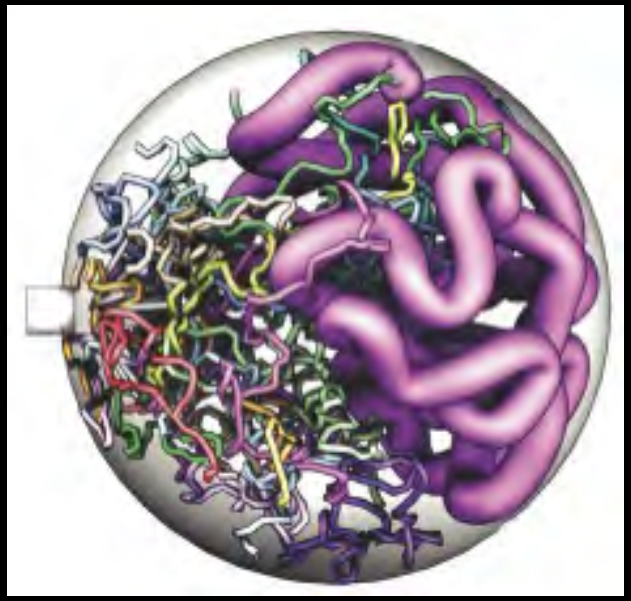

*Wong et al., 2012*

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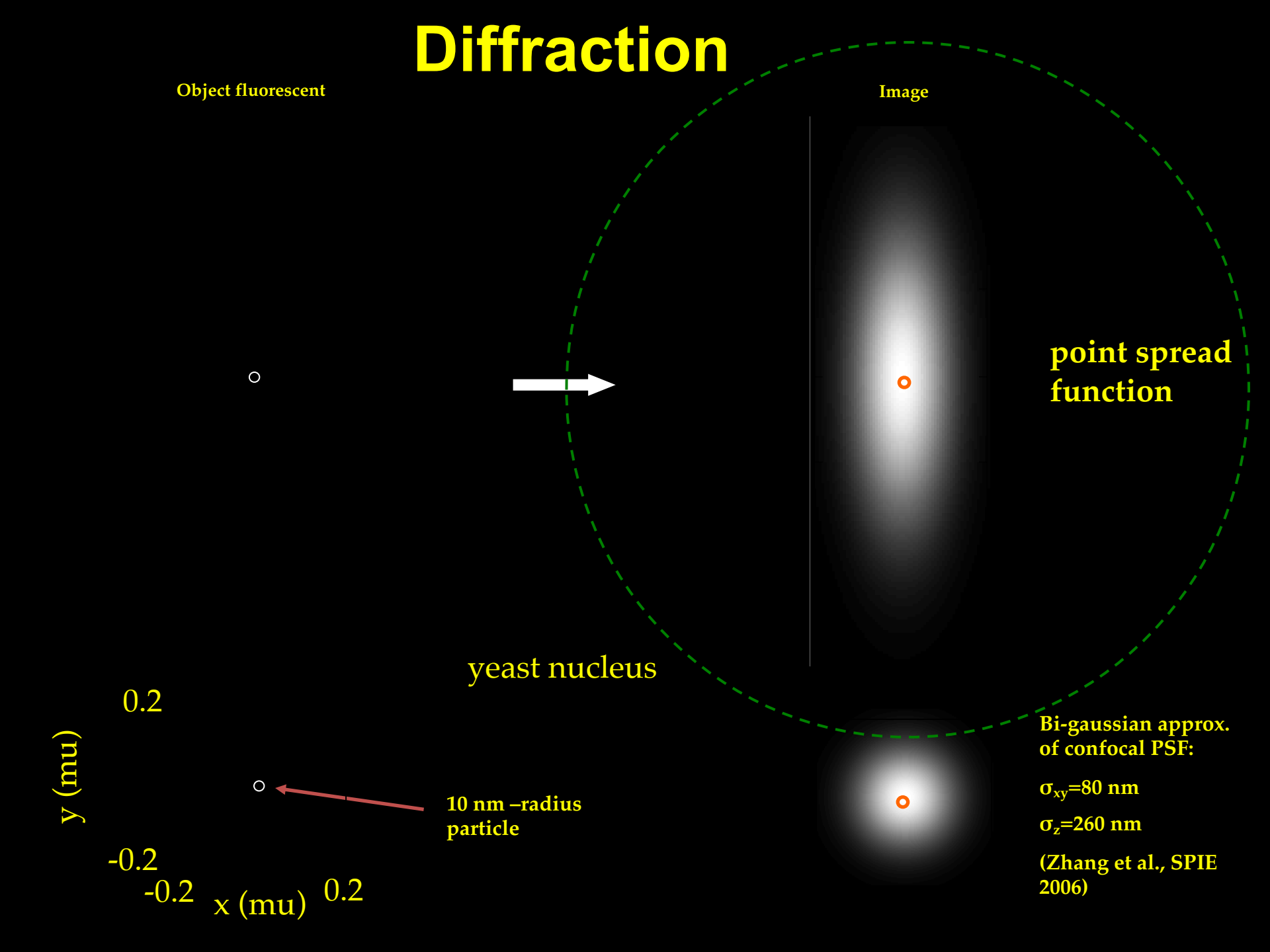
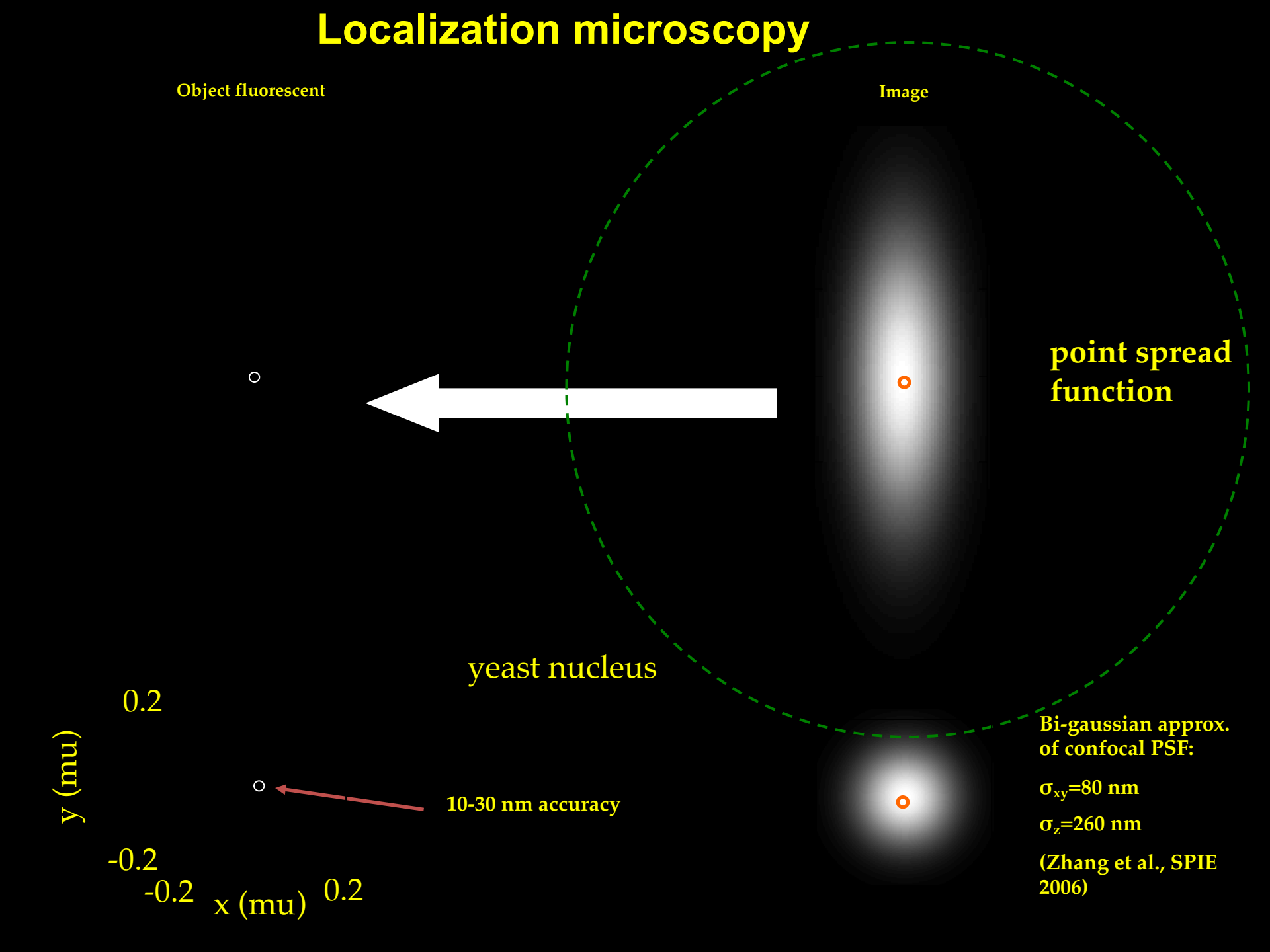
# Diffraction

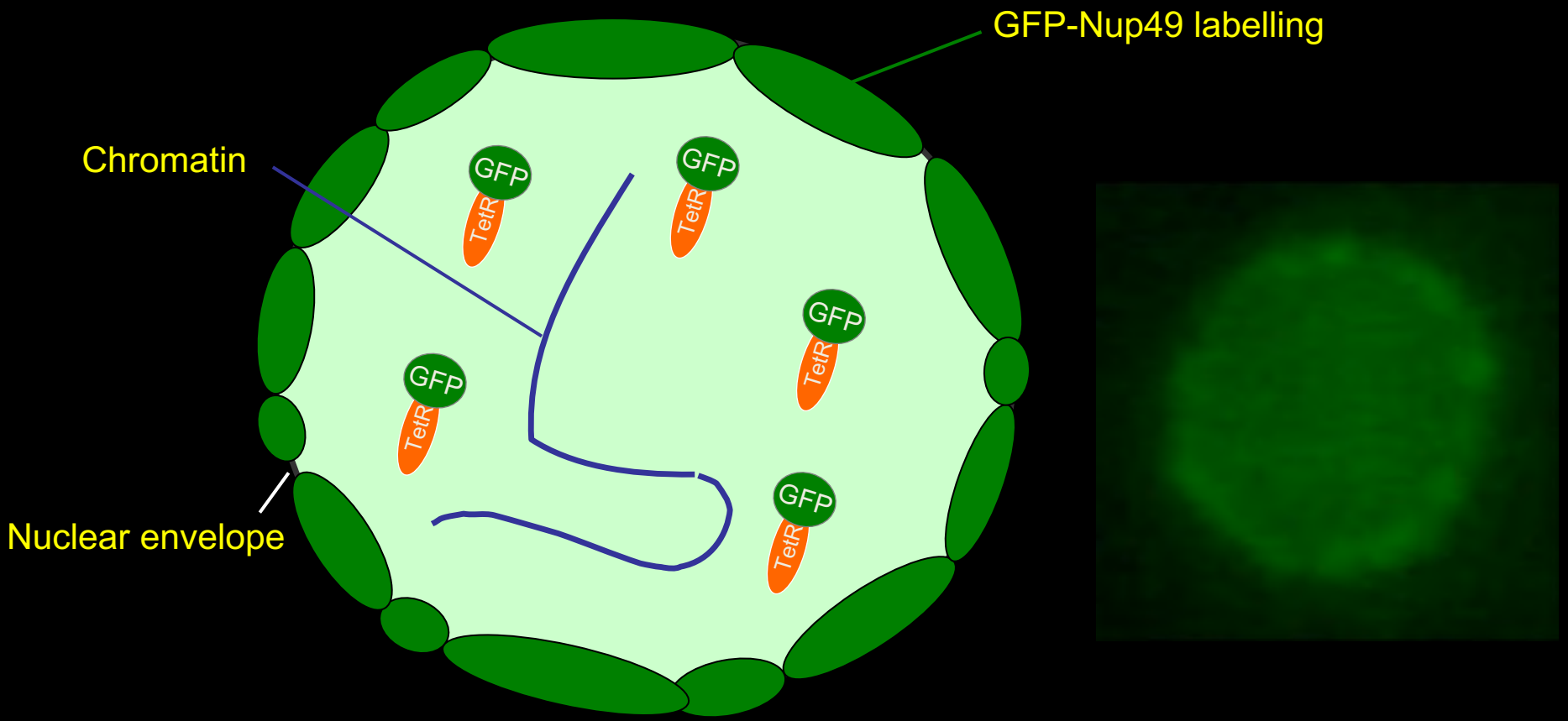


# Localization microscopy



# How to fluorescently label genes in the nuclear space ?

## Fluorescent repressor/operator system (FROS)




Visualization of a single chromosomal locus and nuclear envelope:

We Expressed the green fluorescent protein fused to tetR repressor (Michaelis *et al*., Cell, 1997)

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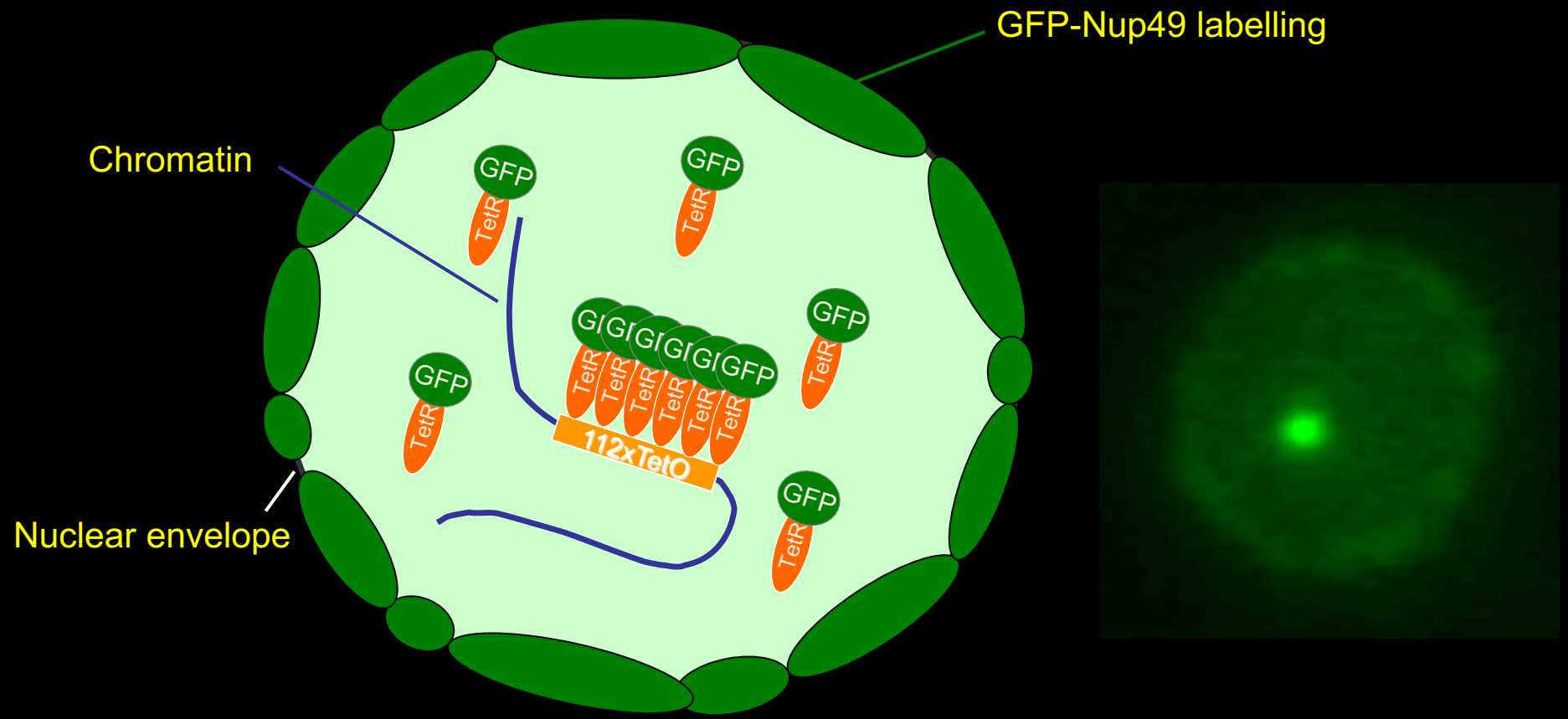


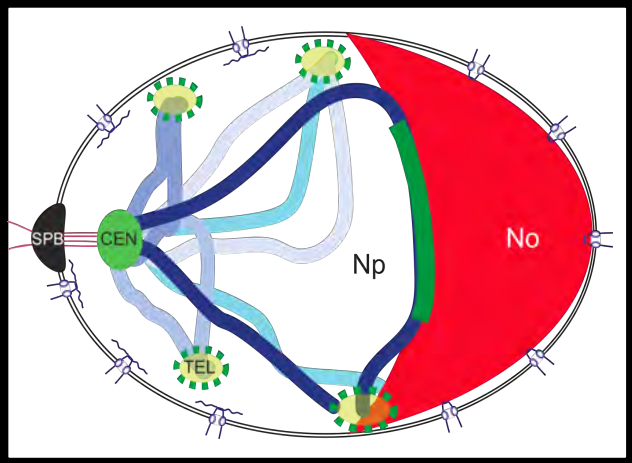

Visualization of a single chromosomal locus and nuclear envelope:

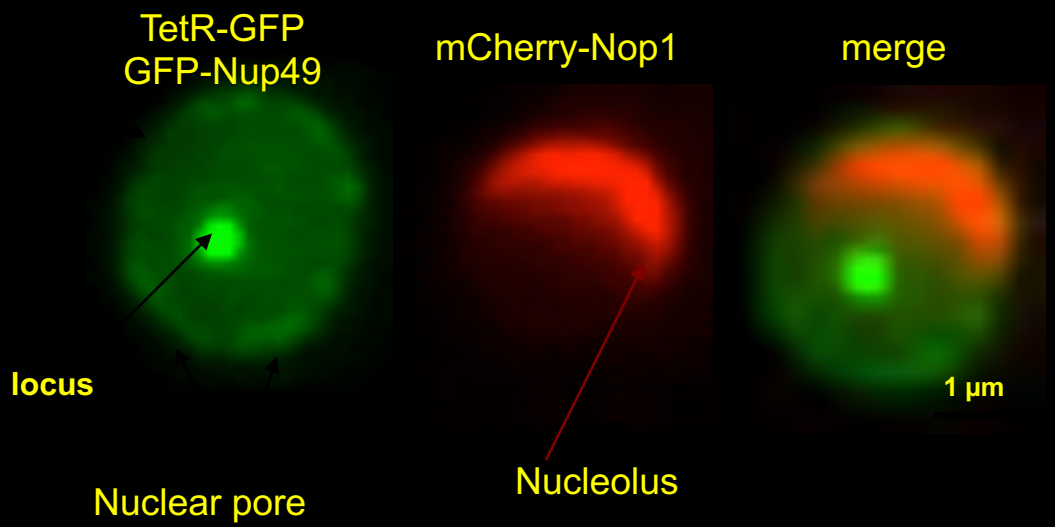
We expressed the green fluorescent protein fused to tetR repressor (Michaelis *et al.*, Cell, 1997)

We inserted of one array of 112 repeats of the bacterial tetO by homologous recombination

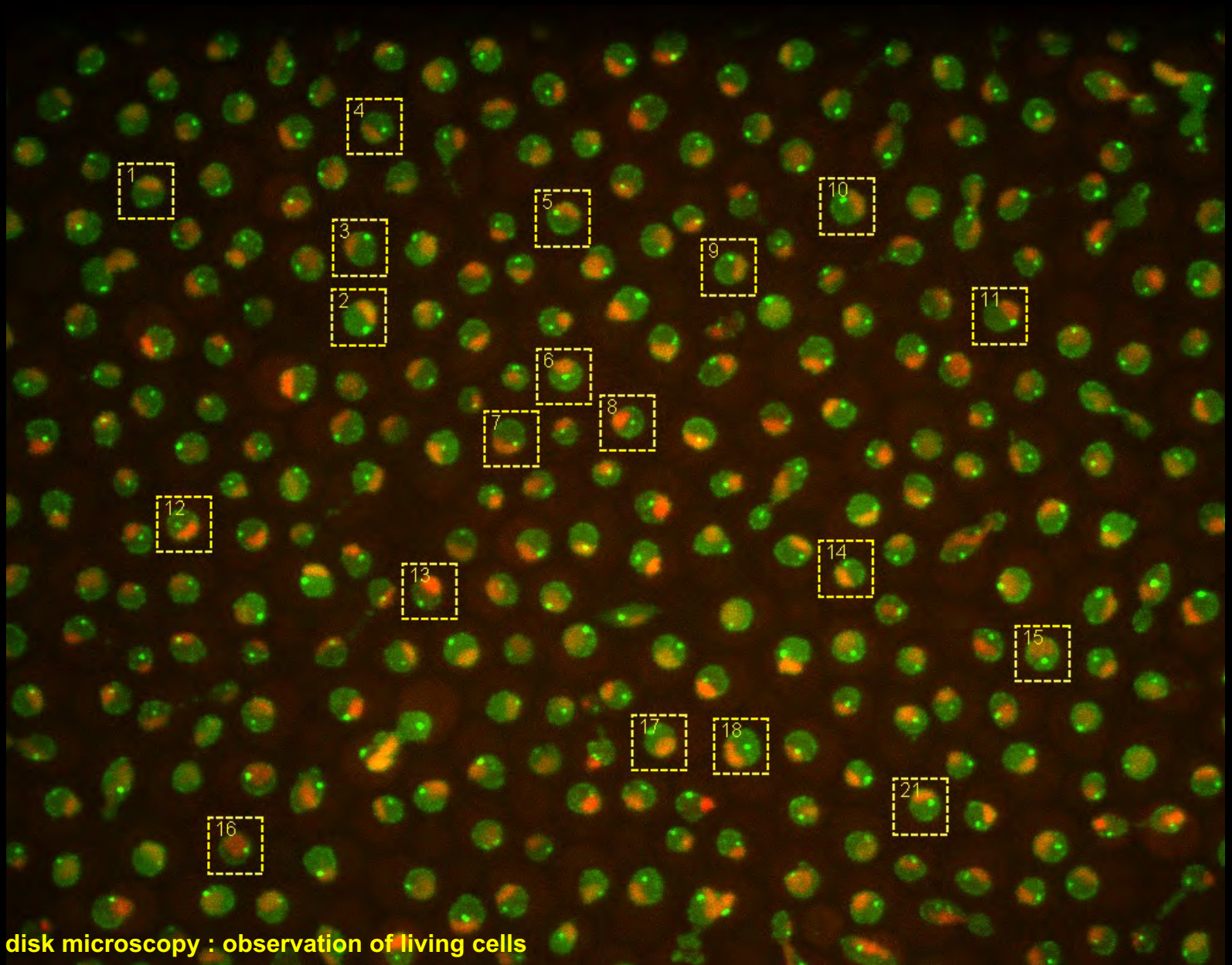
# Labeling Loci

- Fluorescent labelling:
  - Single locus (GFP-TetR)
  - NPC (GFP-Nup49)
  - Nucleolar protein (mCherry-Nop1p)



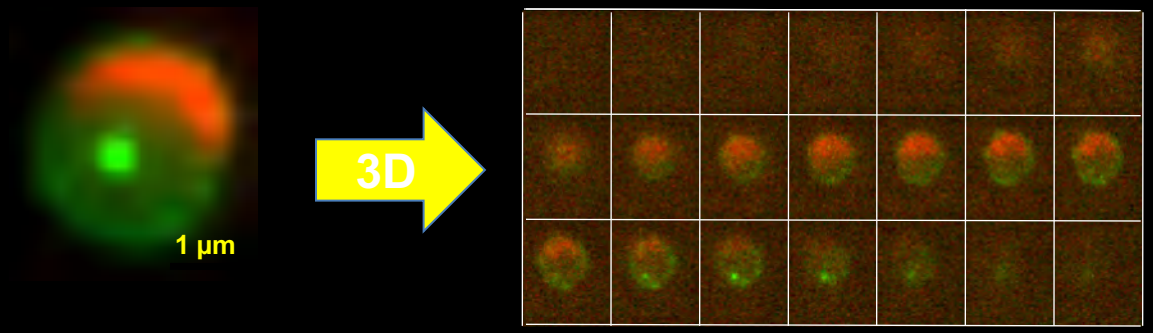
# 3D *in vivo* imaging




Spinning disk microscopy : observation of living cells

# Localization microscopy

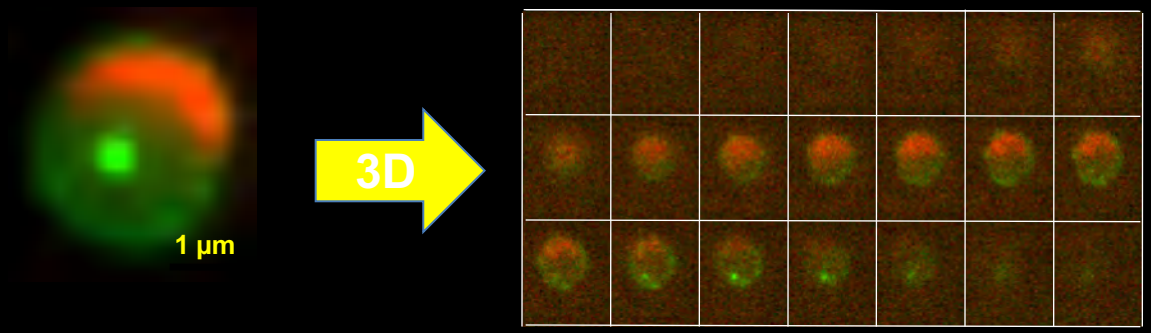
**Spinning disk microscopy : observation of living cells**



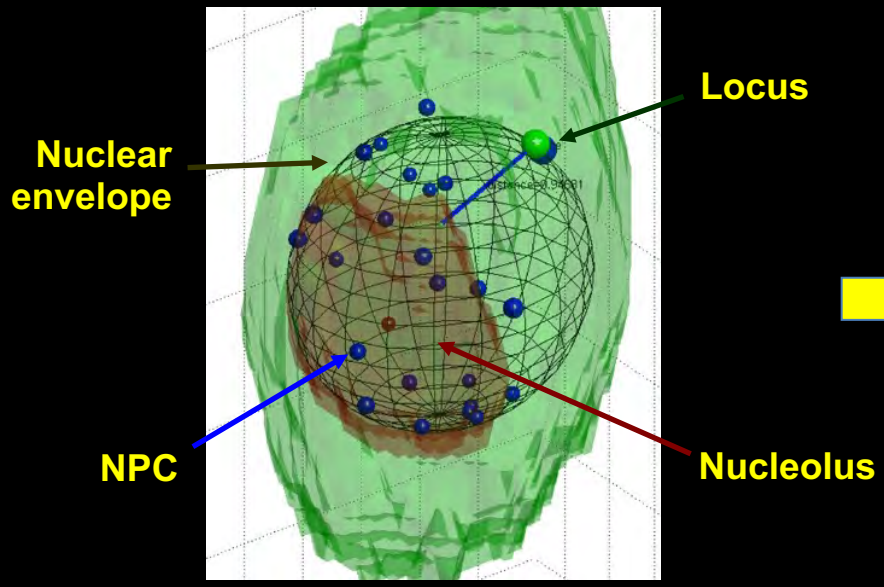

Berger et al., Nature Methods, 2008

# Statistical analysis of gene position

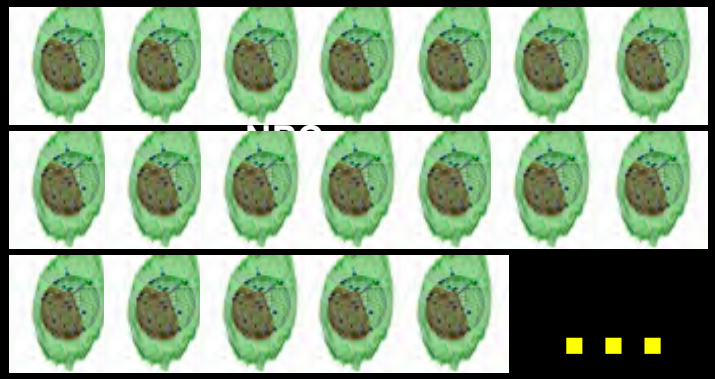
## Spinning disk microscopy : observation of living cells




## 3D position extracted from fluorescent images




• >1000 nuclei analyzed

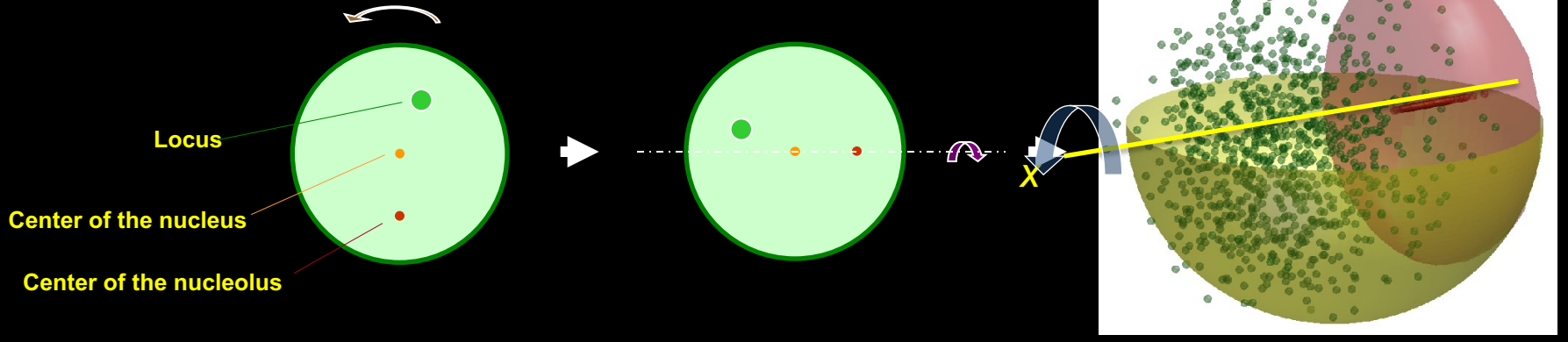


Berger et al., Nature Methods, 2008

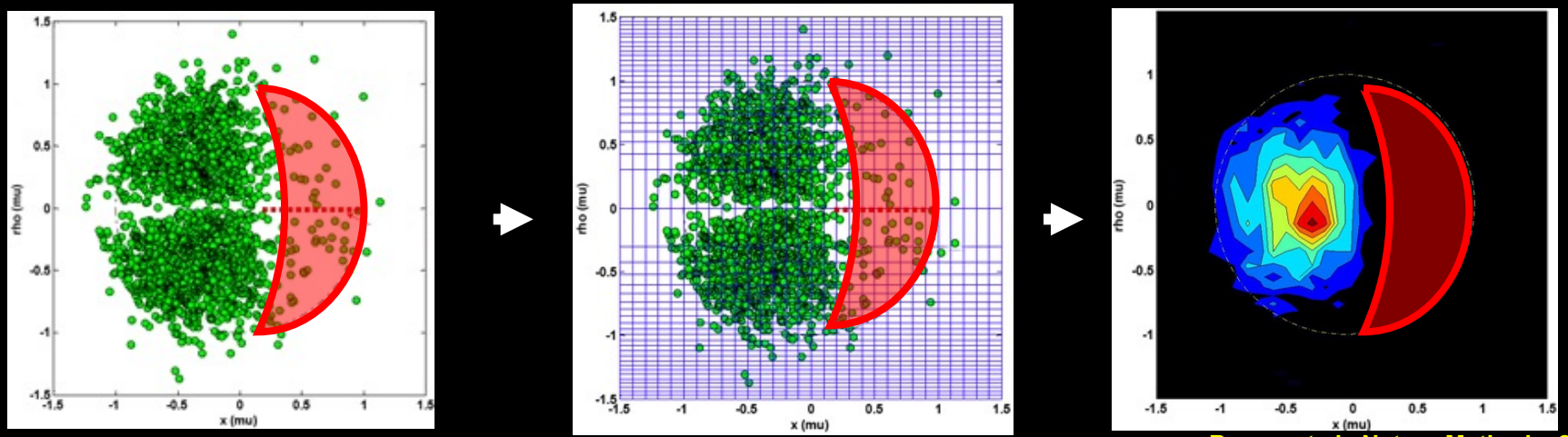
# Statistical analysis of gene position

- From individual position to statistical distribution

- Alignment of individual nucleus

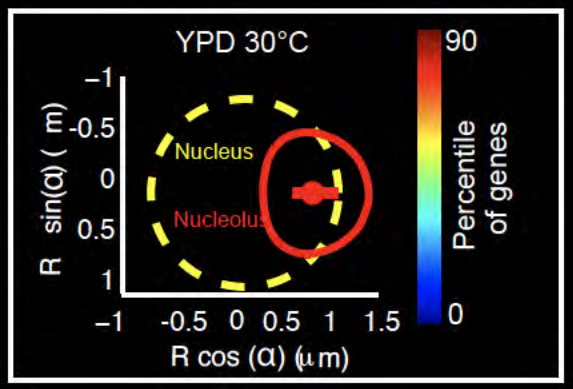


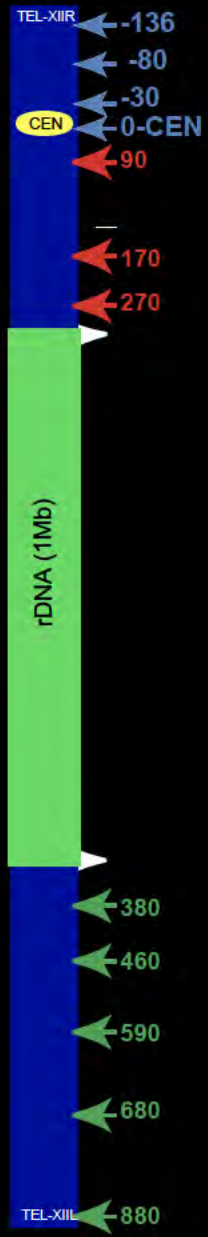

- Distribution probability

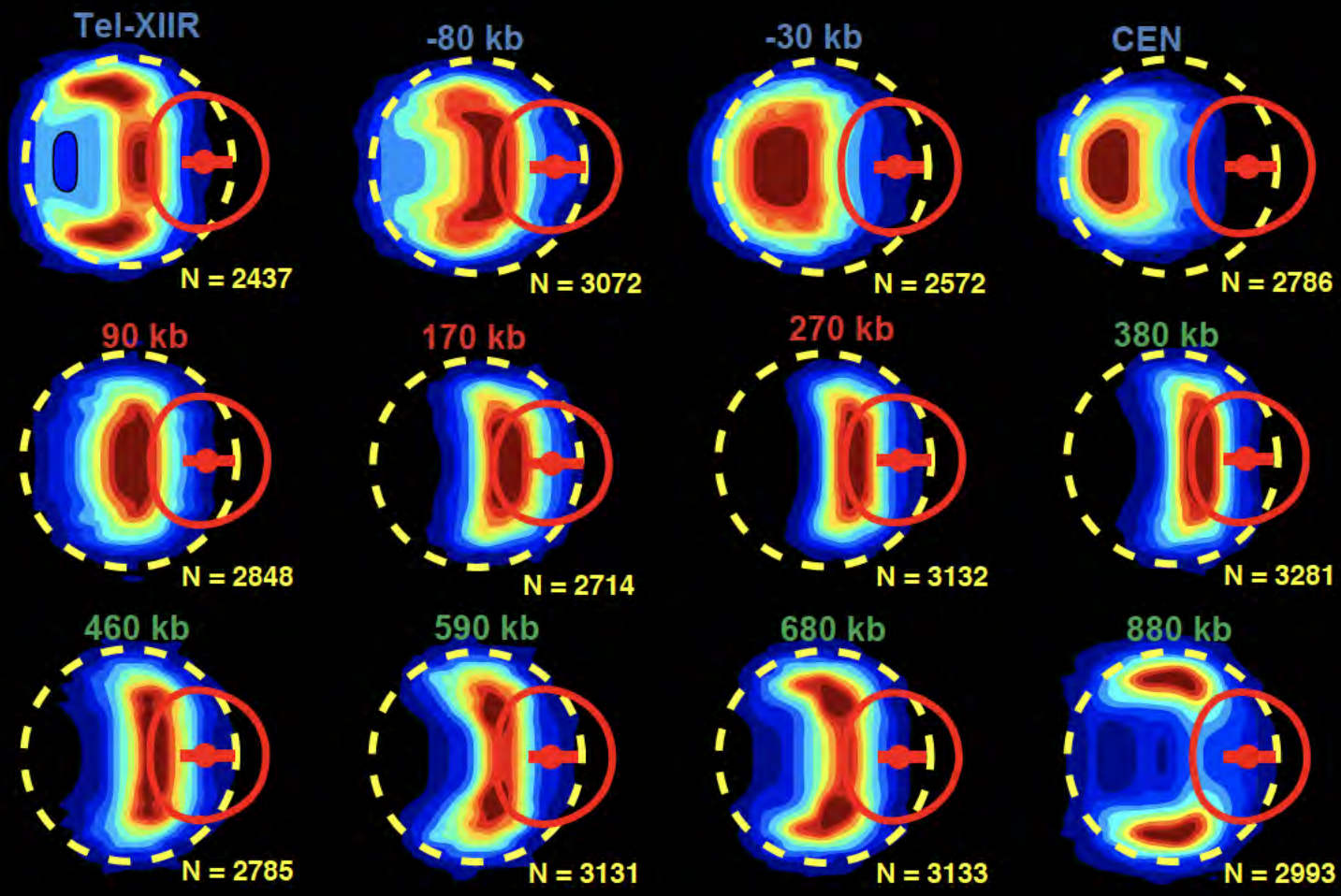



Berger et al., Nature Methods, 2008

# Chromosome XII: genemap



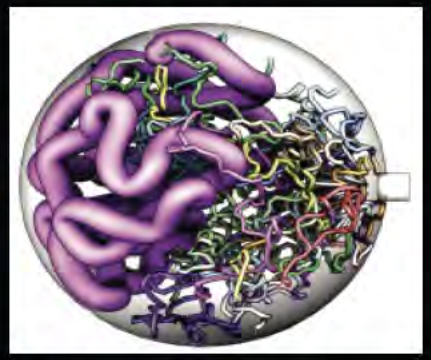

Albert et al., JCB, 2013

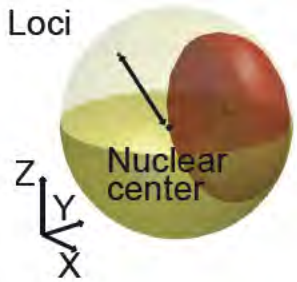
TEL-XIIL

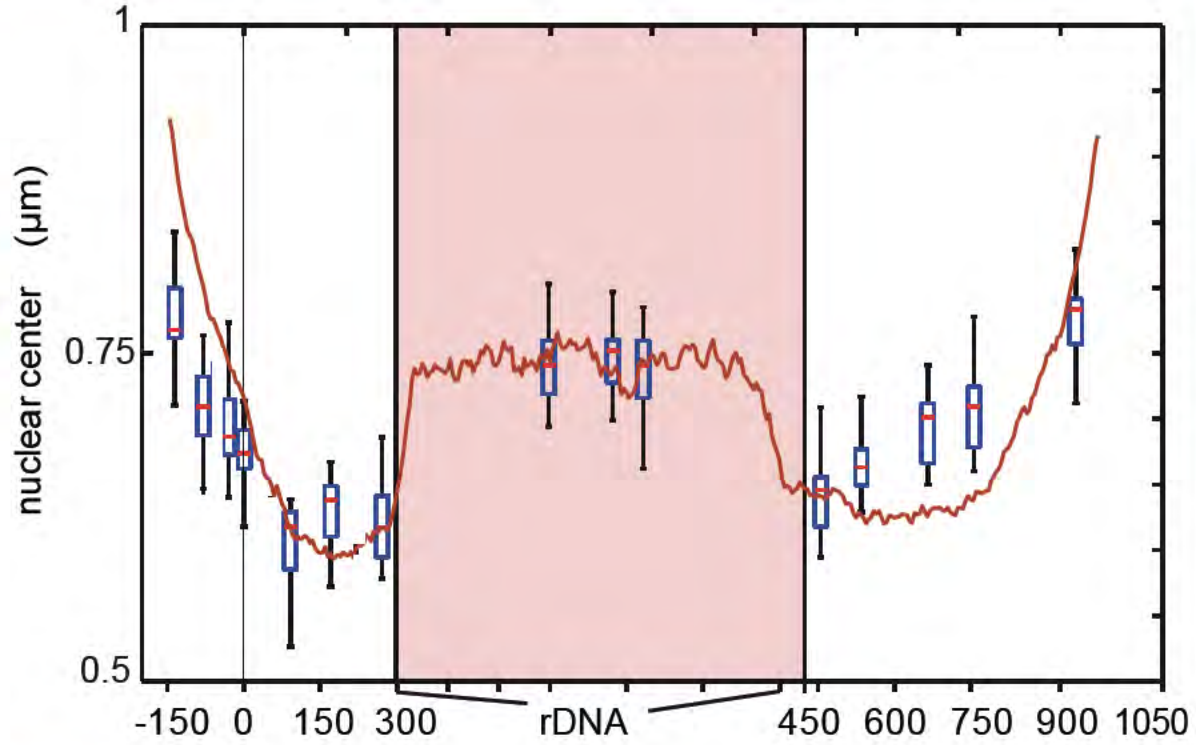
CEN

rDNA

TEL-XIIR






Red : Wong et al., 2012

Albert et al., JCB, 2013

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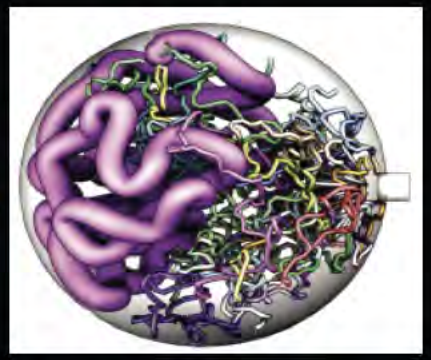
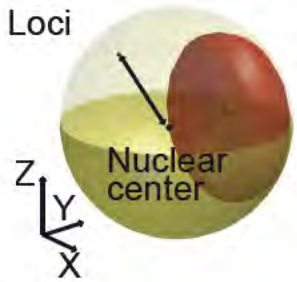
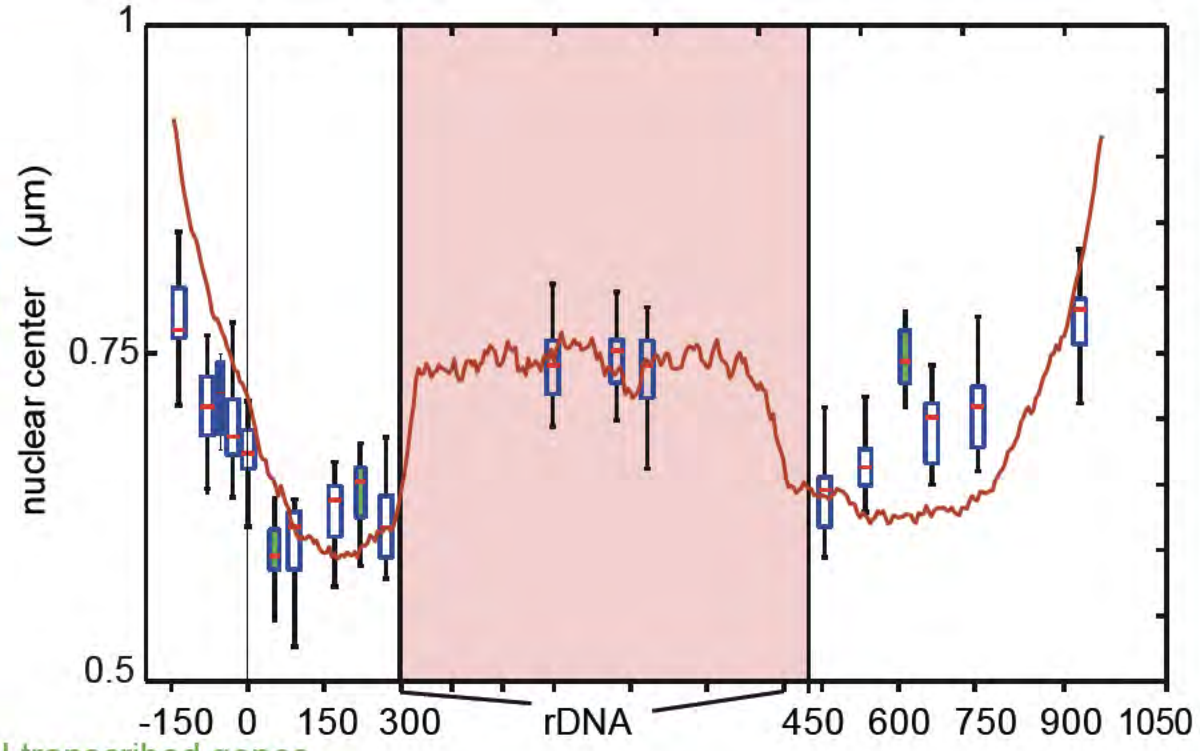





green : RNA Pol III transcribed genes

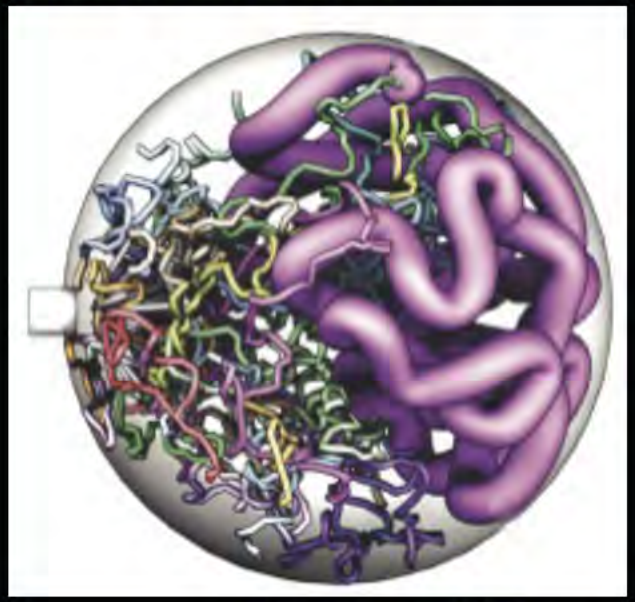
Red : Wong et al., 2012

Belagal et al.J. Cell; Science, 2016

Albert et al., JCB, 2013

# From budding yeast chromosomes to chromatin ?

*In silico model of chromatin*



*Wong et al., 2012*

Explore model prediction

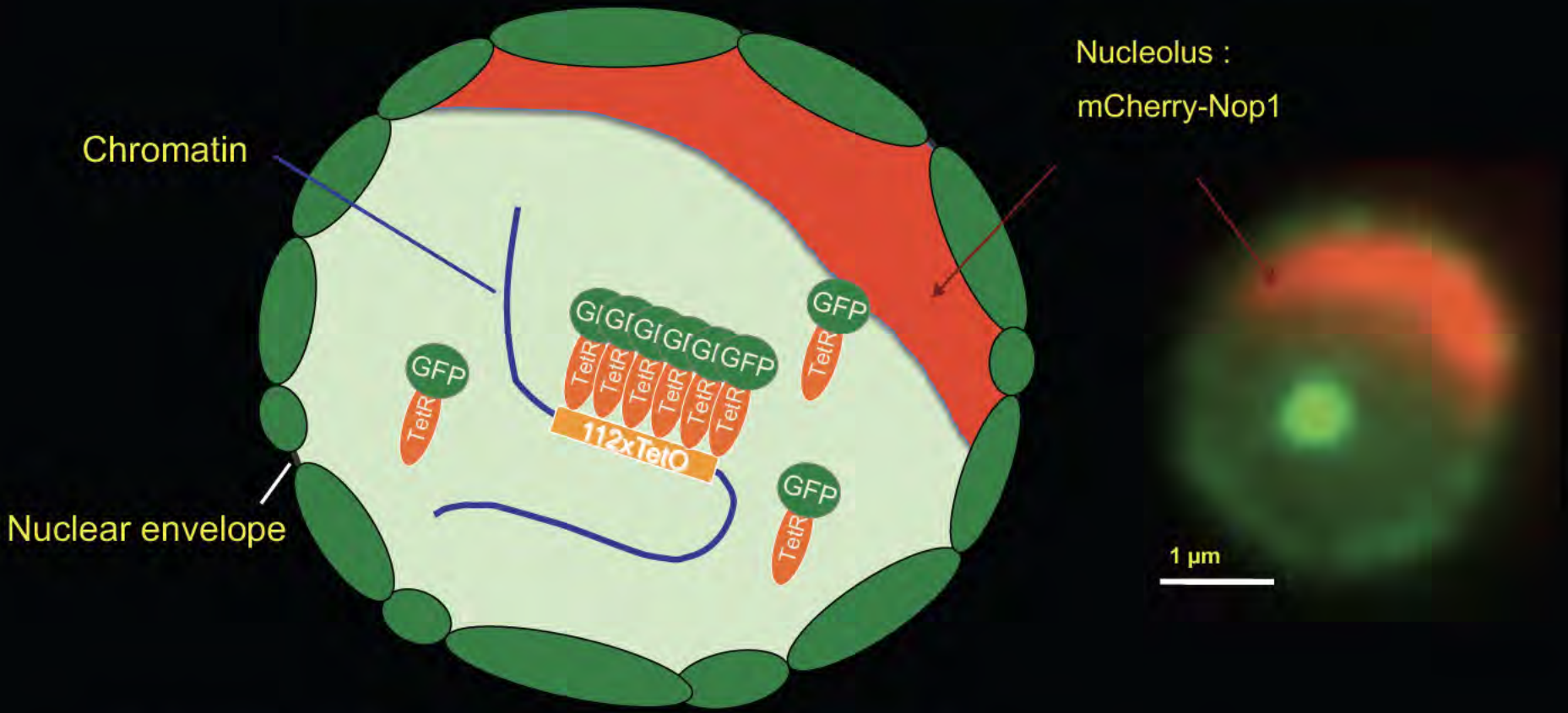
Gene position ?

*In vivo* chromosome motion ?

Super-resolution microscopy ?

## from theory to practice :

## Fluorescent repressor/operator system (FROS) + nucleolus



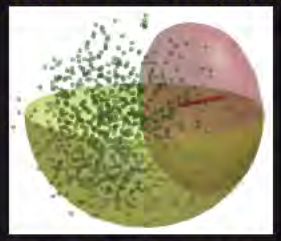

Visualization of a single chromosomal locus, nuclear envelope and nucleolus:

We expressed the green fluorescent protein fused to tetR repressor (Michaelis *et al.*, Cell, 1997)

We inserted of one array of 112 repeats of the bacterial tetO by homologous recombination

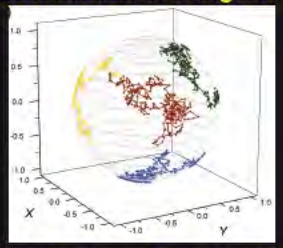
# 15 loci tagged along chromosome XII


Loci position in cell population



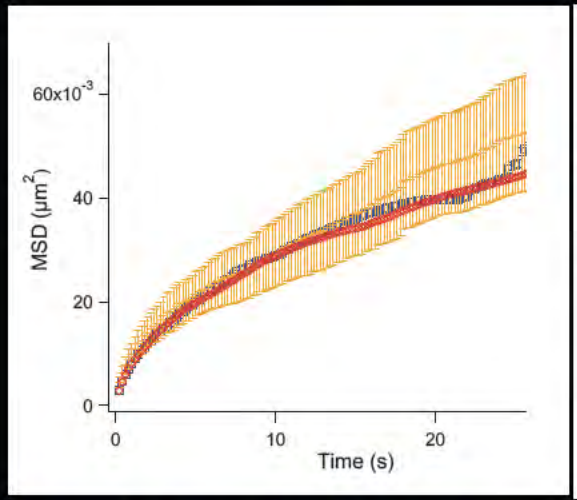
Albert et al., J. Cell. Biol., 2013

Loci motion in living cell

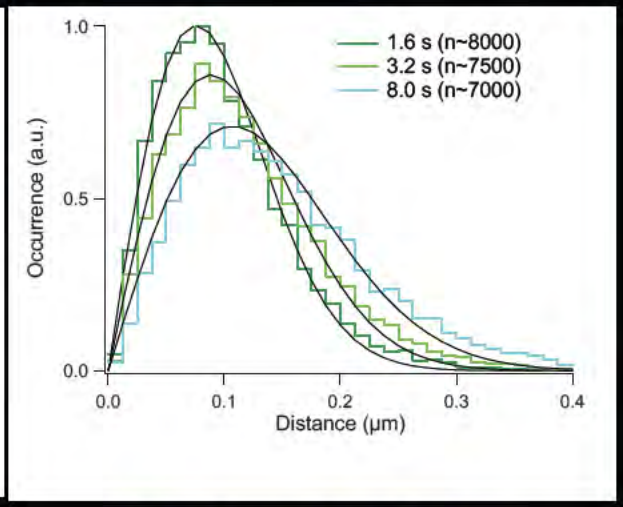


Hajjoul et al., Genome Research, 2013

## Measure chromatin fluctuation !



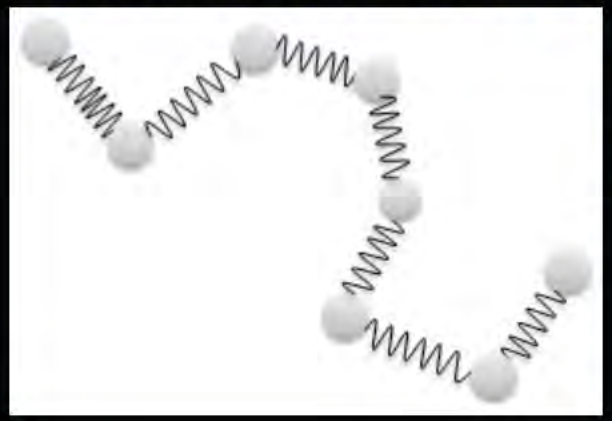

Mean square displacement (MSD)




Step distribution function (SDF)

# Models to describe chomatin motion ?
# Theoretical MSD

## Standart model : Rouse polymer

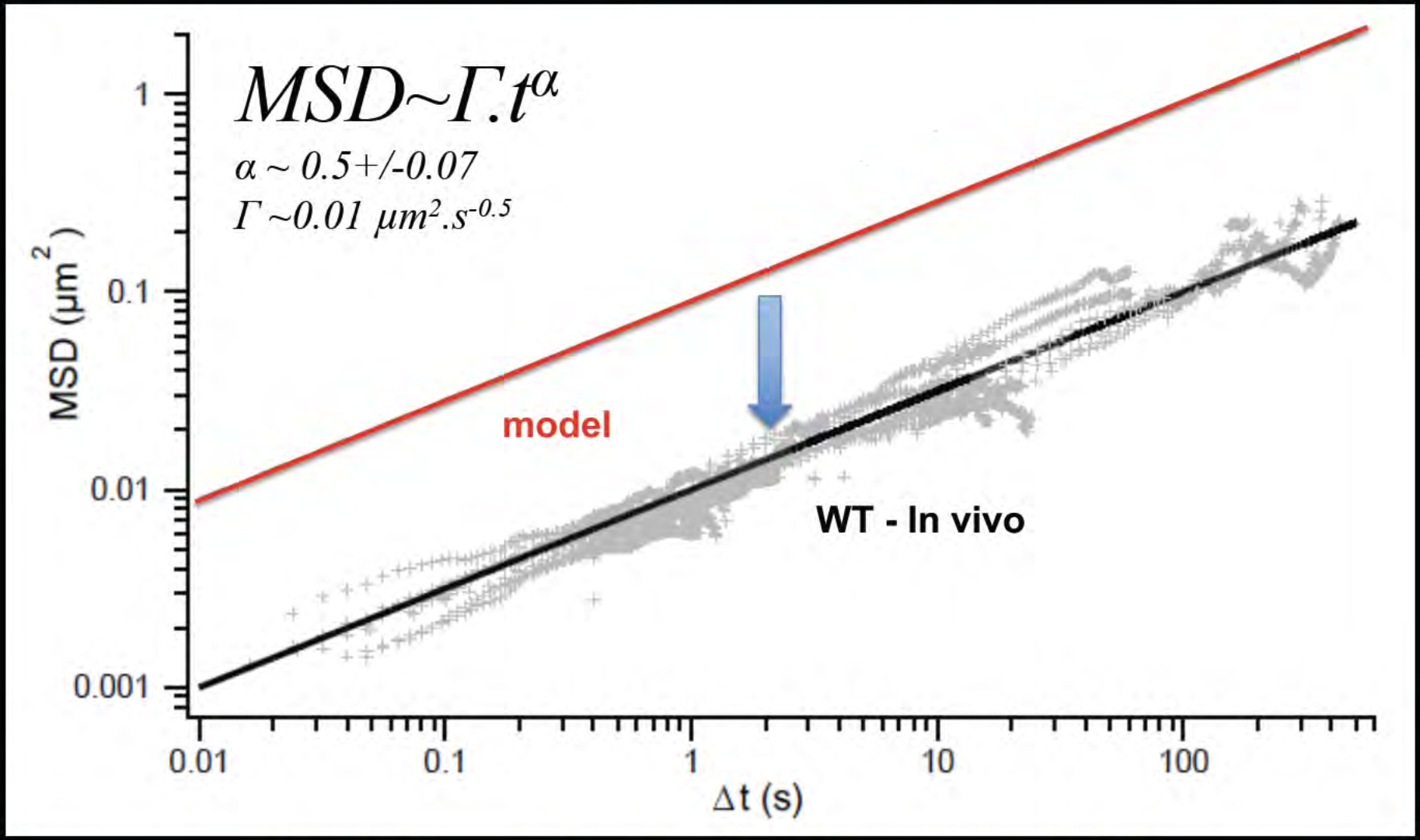


Stiffness of the spring – Persistence length

Persistence length (Lp) defines the stiffness of a polymer.
2 x Lp = Kuhn length (Lk)

Lk = labelled gene

# Comparing chromatin fluctuation In vivo and in vitro




**What is missing in the Rouse model ?**

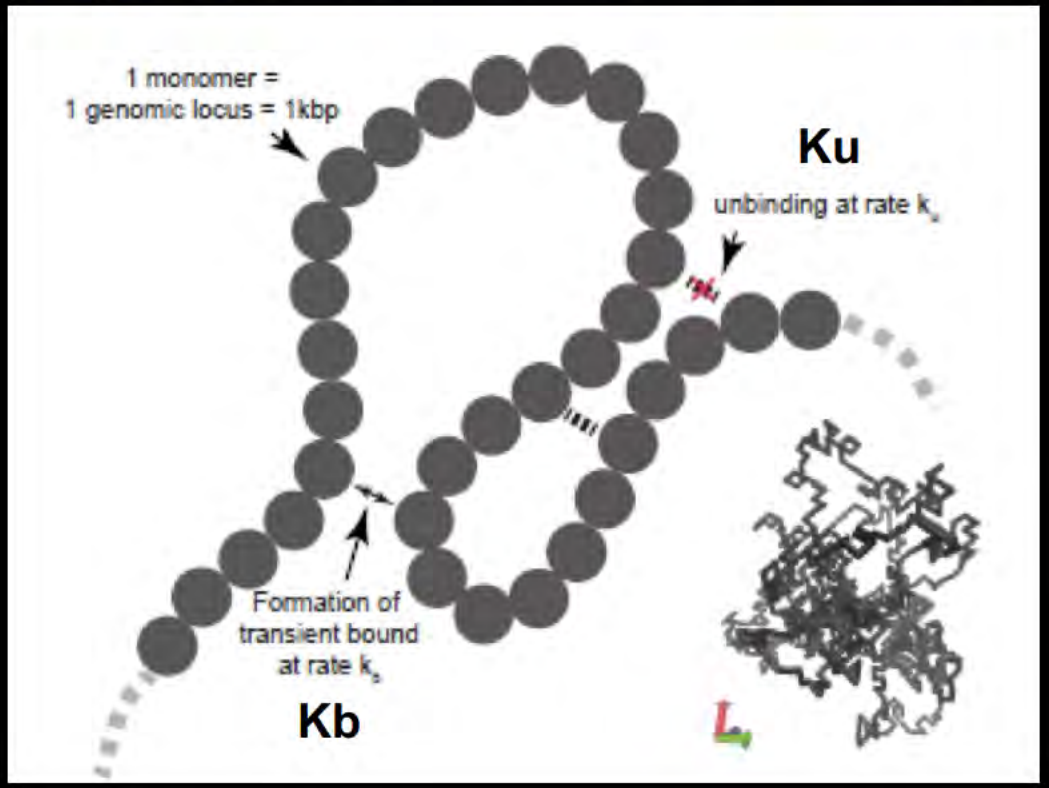
# Transient contact along fiber may explain chromatin fluctuation In vivo and in vitro

**Daniel Jost**

Physical biology of chromatin

at LBMC, CNRS, ENS Lyon, University of Lyon

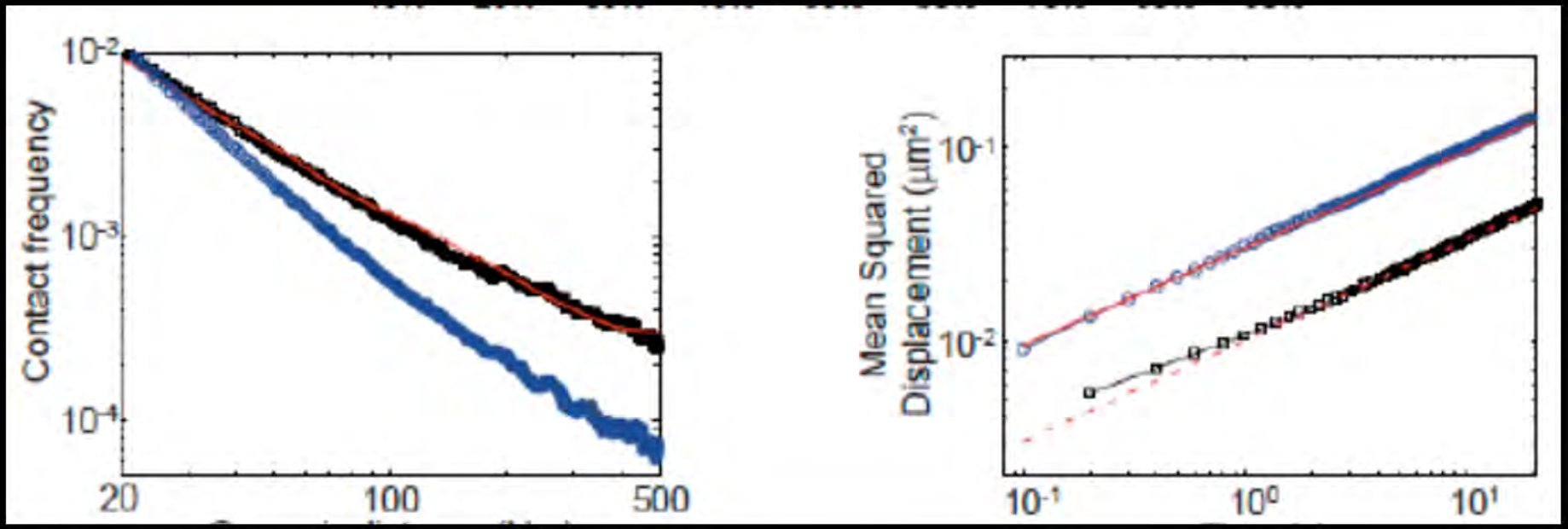
$K_b / k_u$ ratio determined from 3C dataset



# General conclusion – internal friction is the key to understand !

**From 1 to 2 parameter :**

- **Persistence length fixed !**
- **On/off rate ajusted from Hi-C and MSD**

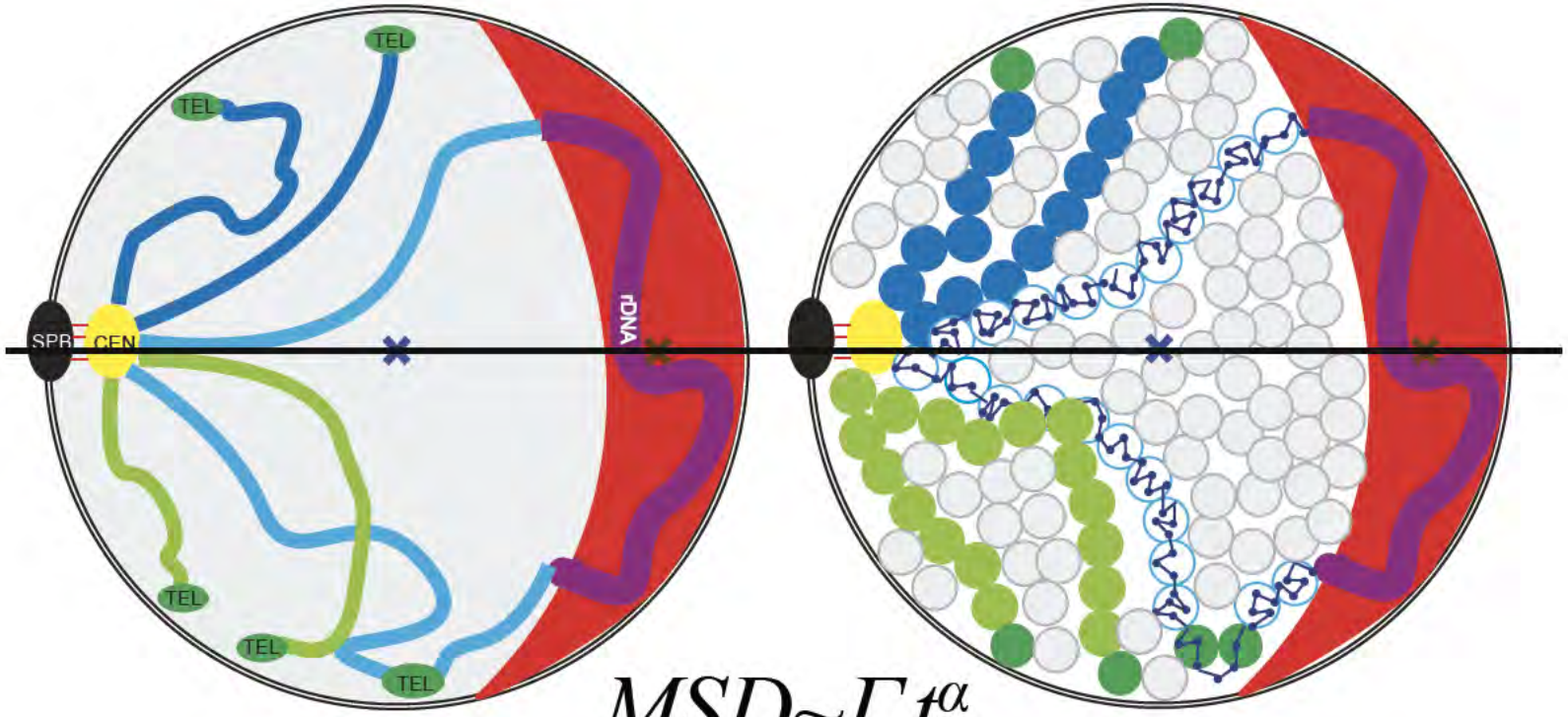



**Rouse TIC:**

Rouse model with transient intramolecular contacts on a timescale of seconds.

## Rouse models to describe chomatin motion:

## From global to local – good prediction of chromatin properties




**Nucleoplasmic :**
Rouse model with Transient Internal Contacts

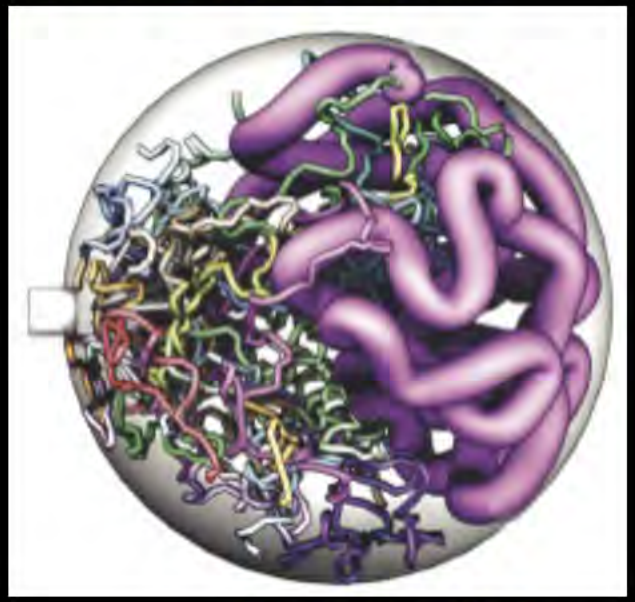
$\alpha \sim 0.5 +/- 0.07$ *Contact ~ seconds*
$\Gamma \sim 0.01\ \mu m^2.s^{-0.5}$ *attractive energy of ~0.5 kBT*

**Nucleolar chromatin ?**

$\alpha \sim 0.25$ $<5s$
$\alpha \sim 0.7$ $>5s$

# From budding yeast chromosomes to chromatin ?

*In silico model of chromatin*



*Wong et al., 2012*

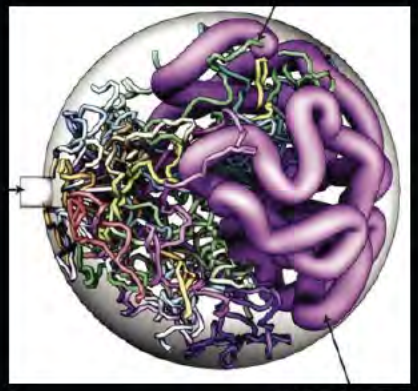
Explore model prediction

Gene position ?

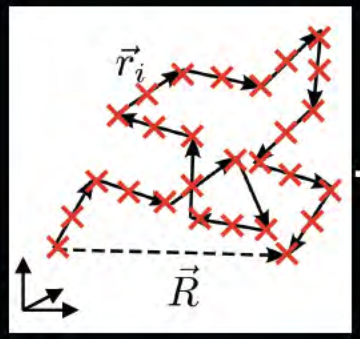
*In vivo* chromosome motion ?

Super-resolution microscopy ?

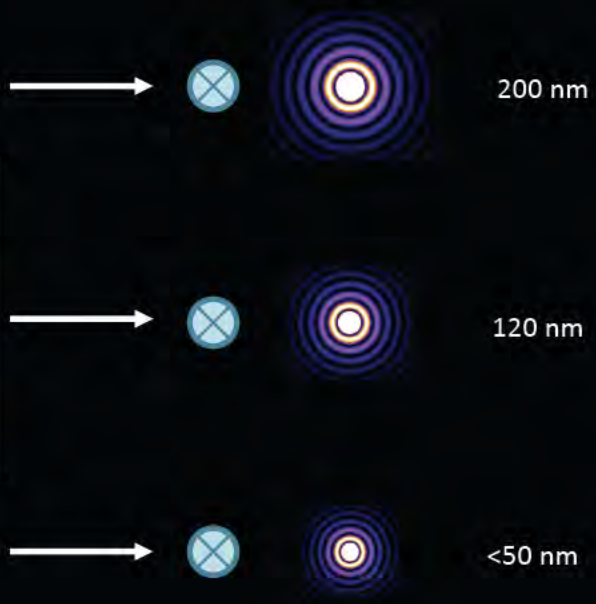
# Virtual microscopy : from model to microscopic image

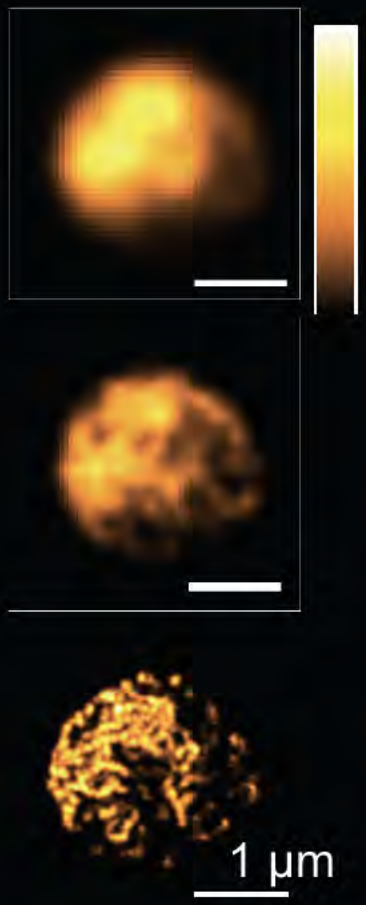


Wong et al, 2012

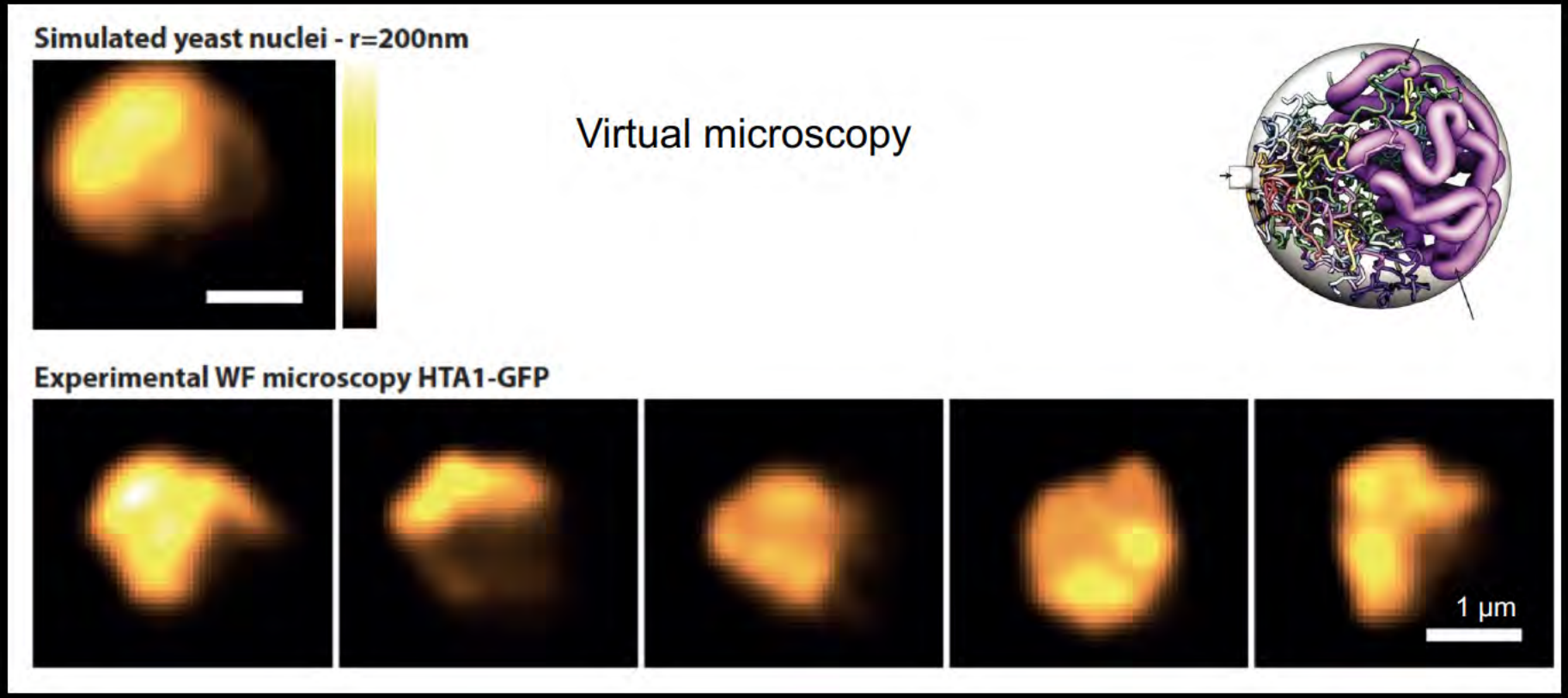



PSF
3D



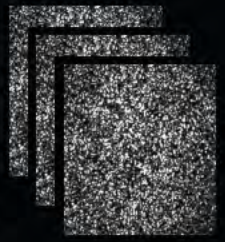



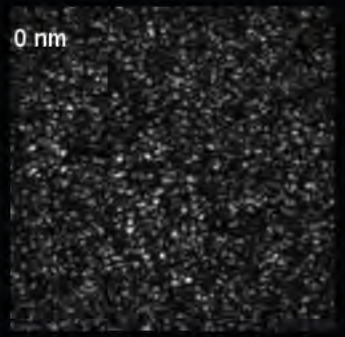
# Chromatin visualized at 200 nm resolution
# Little informations !



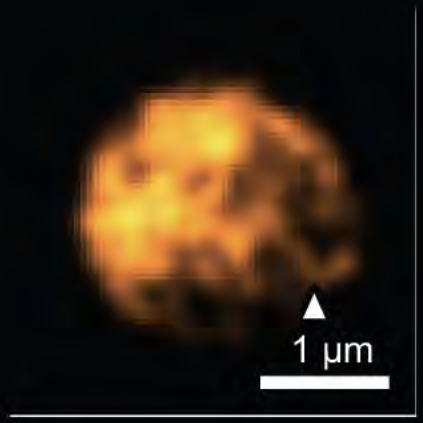
# Chromatin visualized at 150 nm resolution

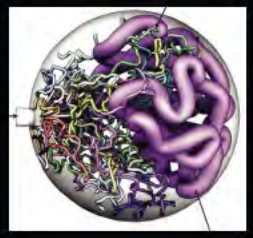
Random Illumination Microscopy



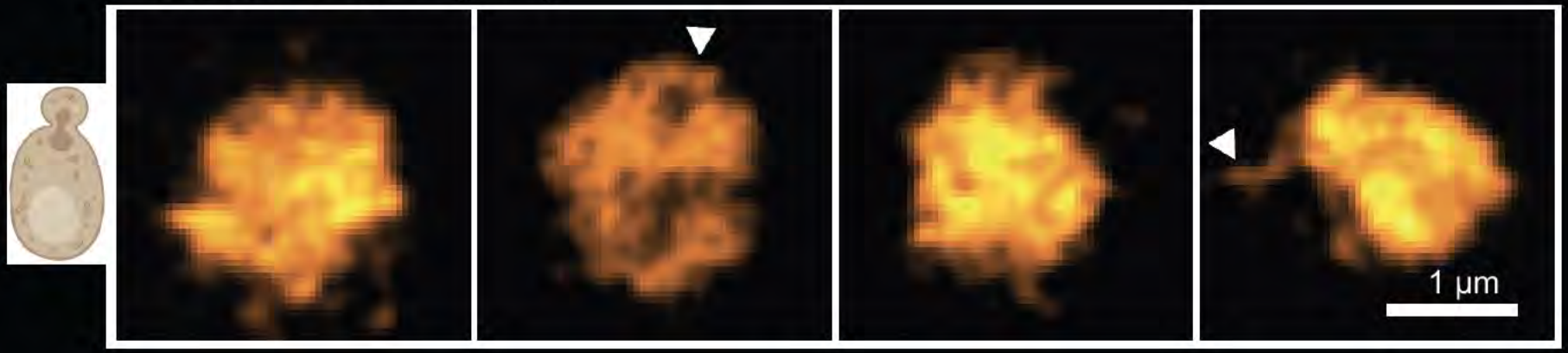



Virtual microscopy



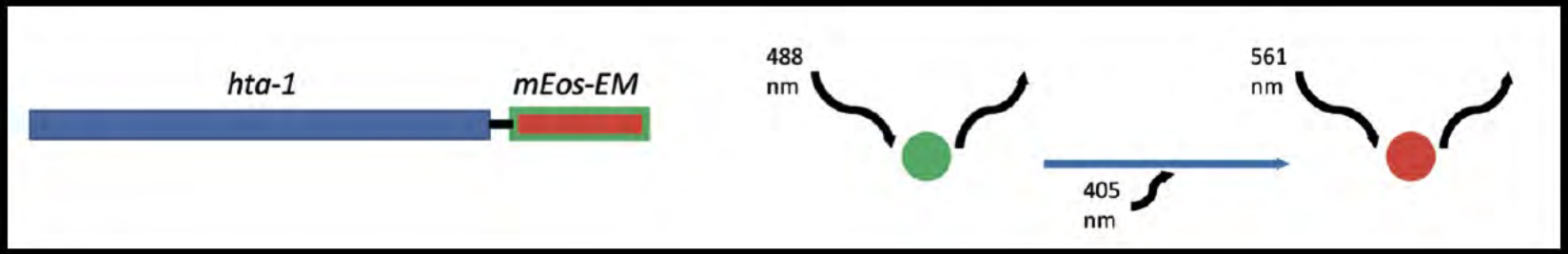



Yeast chromatin (RIM)

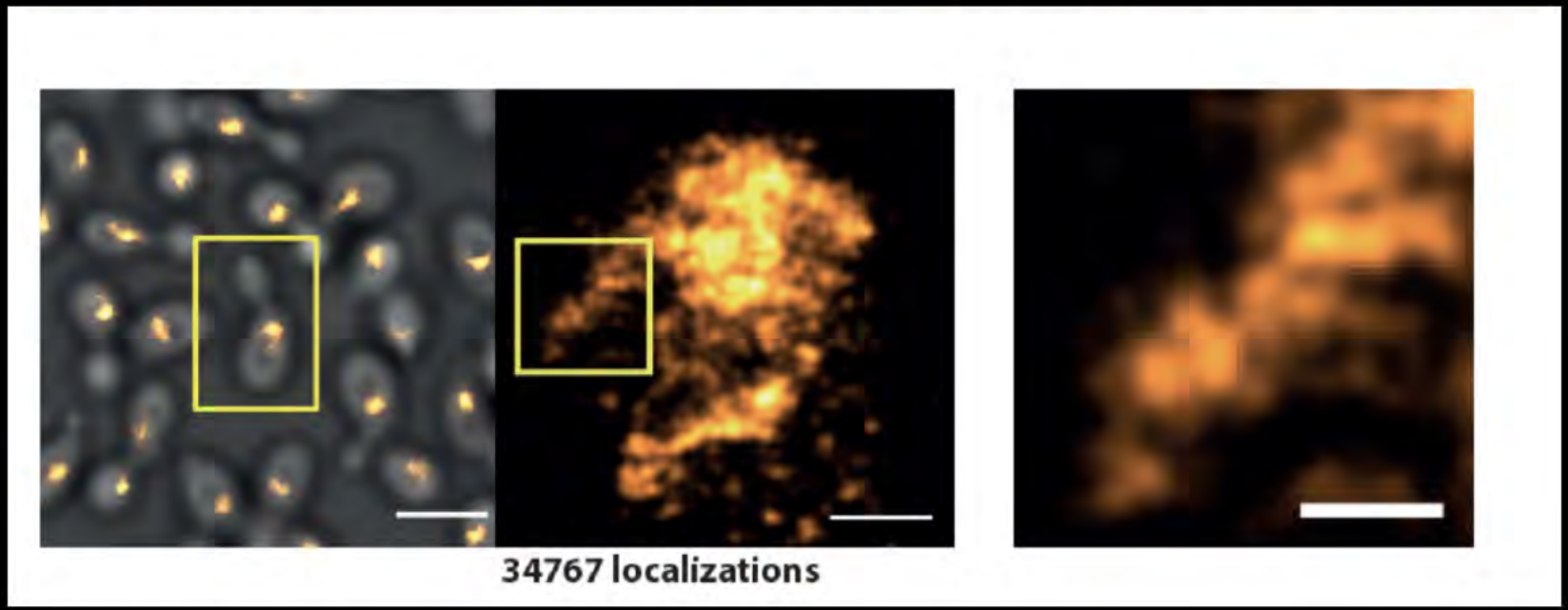



Visualisation of chromosomal arms ?

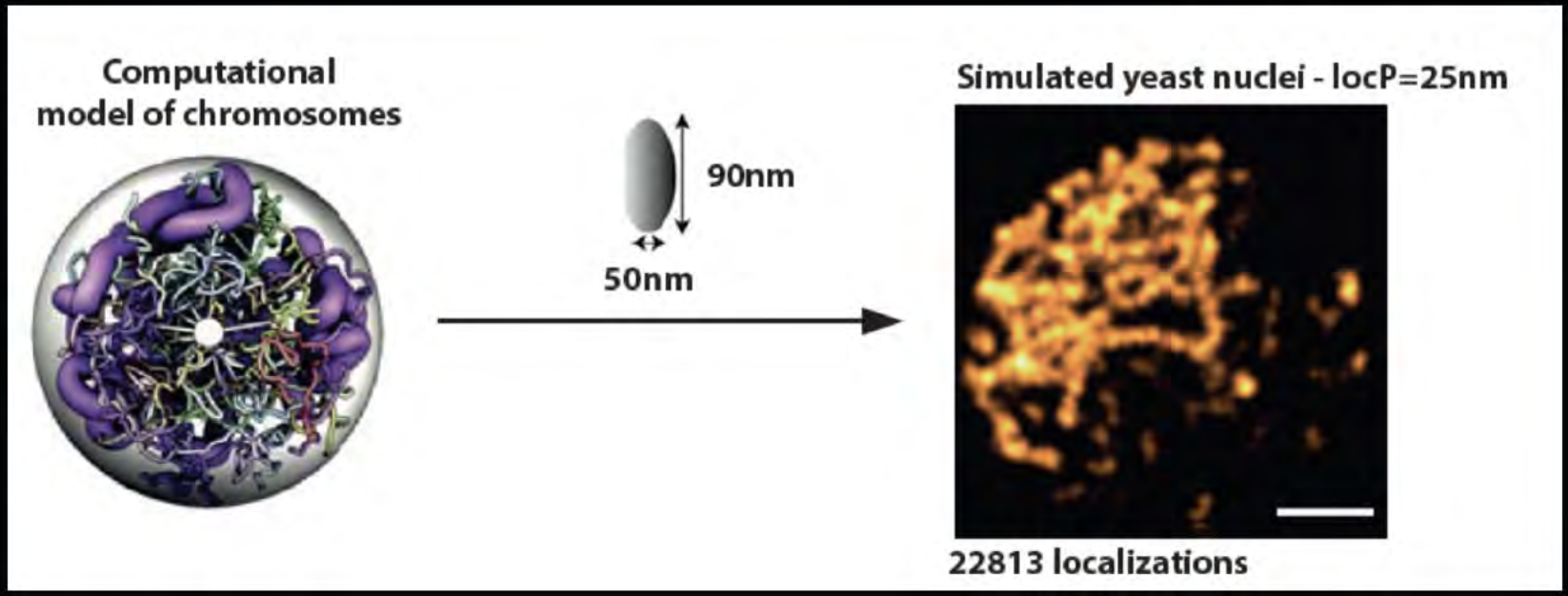
# Chromatin visualized at 50 nm resolution



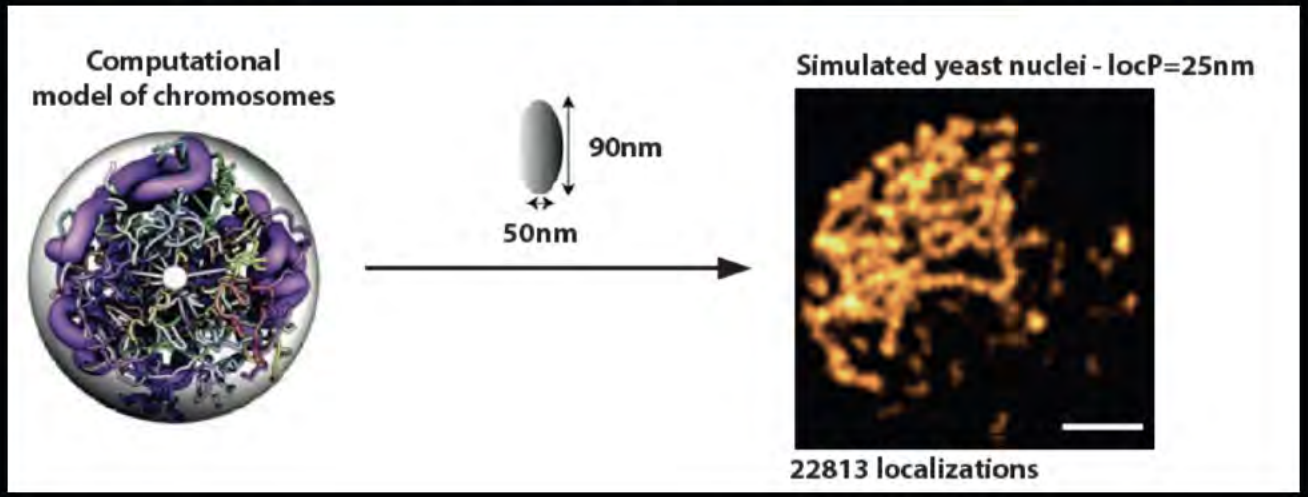

# Single molecule localisation microscopy (SMLM) - PALM



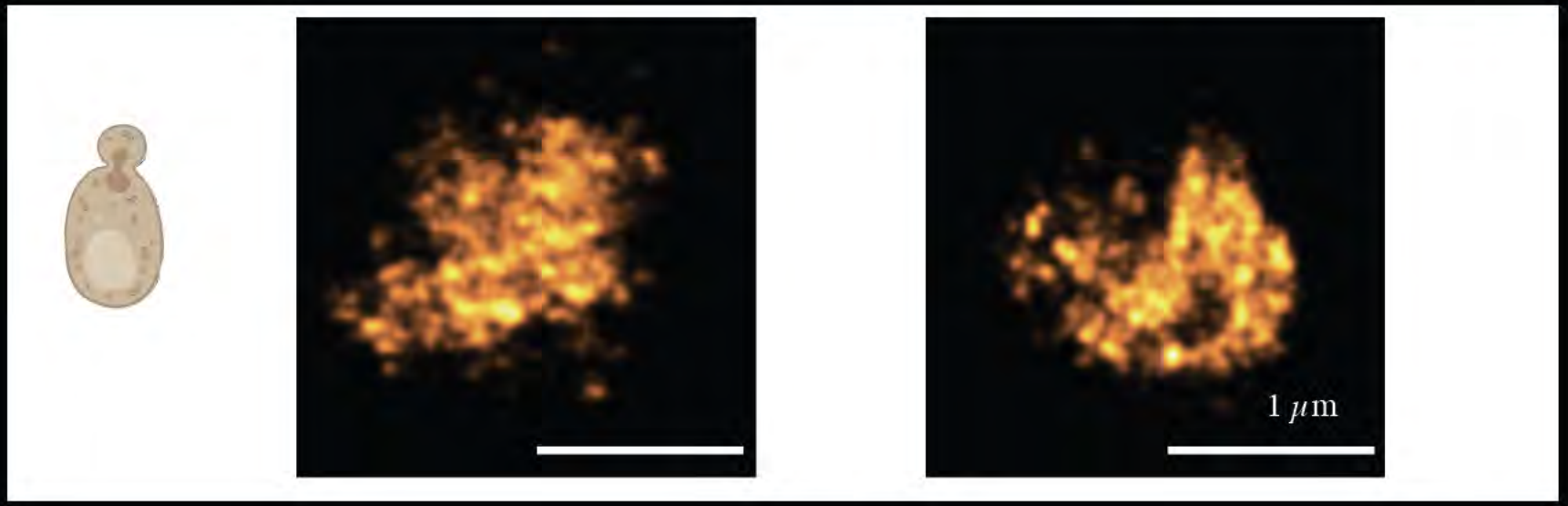
# Chromatin visualized at 50 nm resolution



# Chromatin visualized at 50 nm resolution

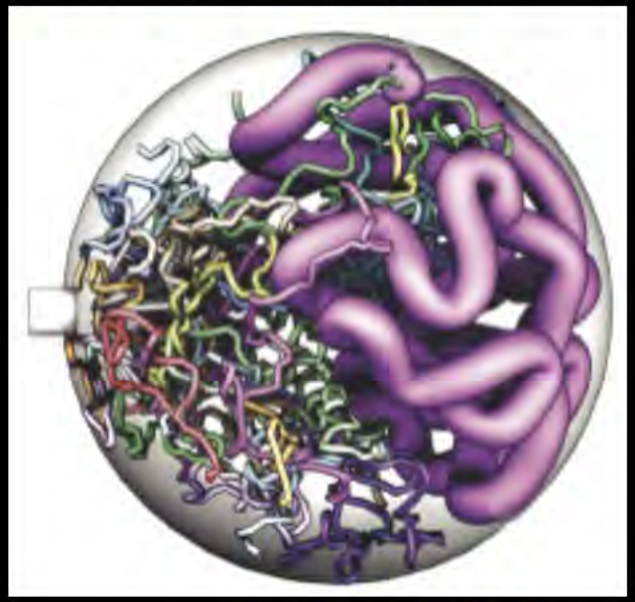



Clear discrepancy between virtual microscopy of coarse grain model and SMLM imaging



# Summary and working hypothesis

*In silico model of chromatin*



*Wong et al., 2012*

Explore model prediction

Super-resolution microscopy

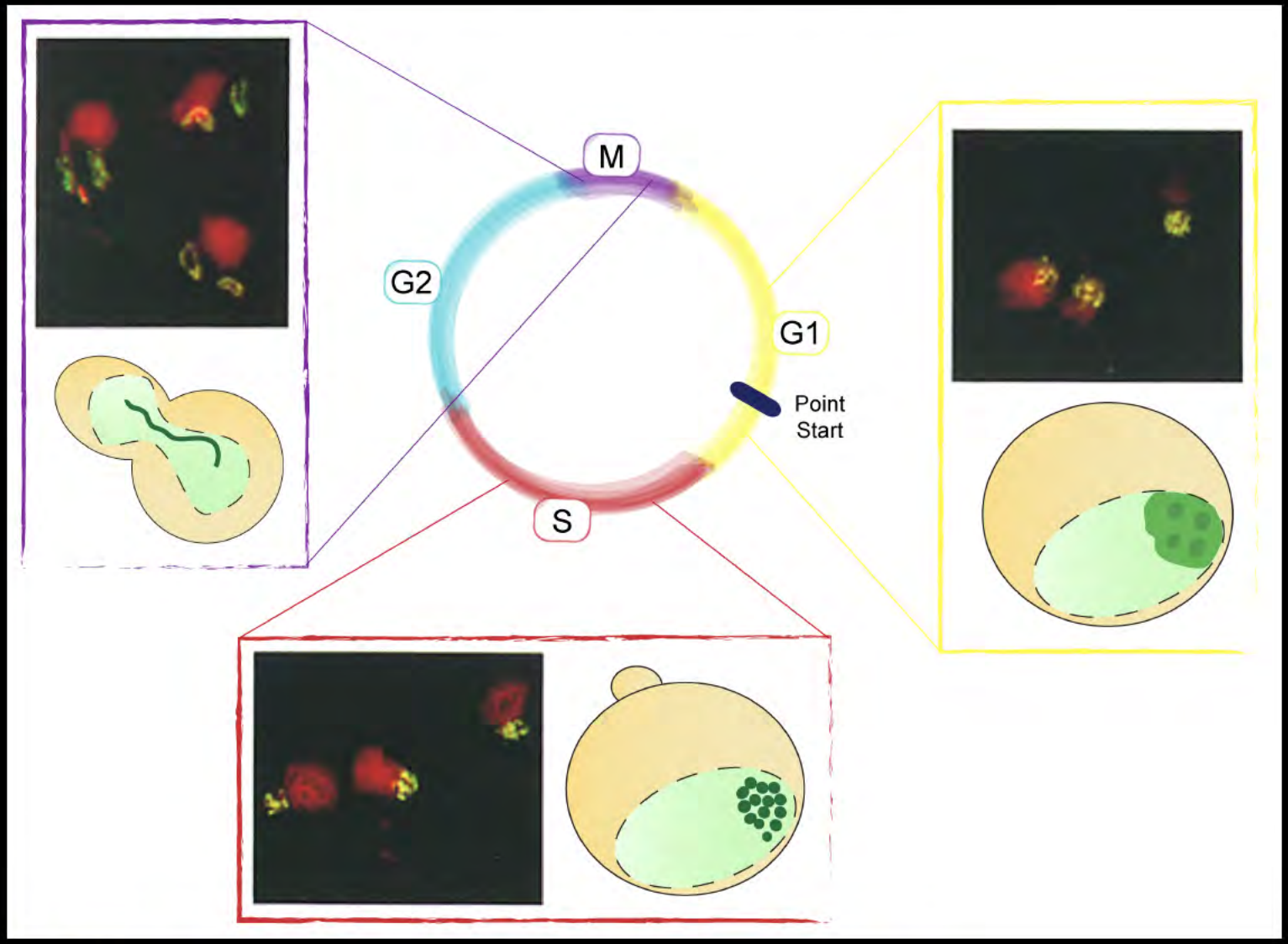
150 nm – 50 nm ?

Transcriptional activities ?

Loop formation ?

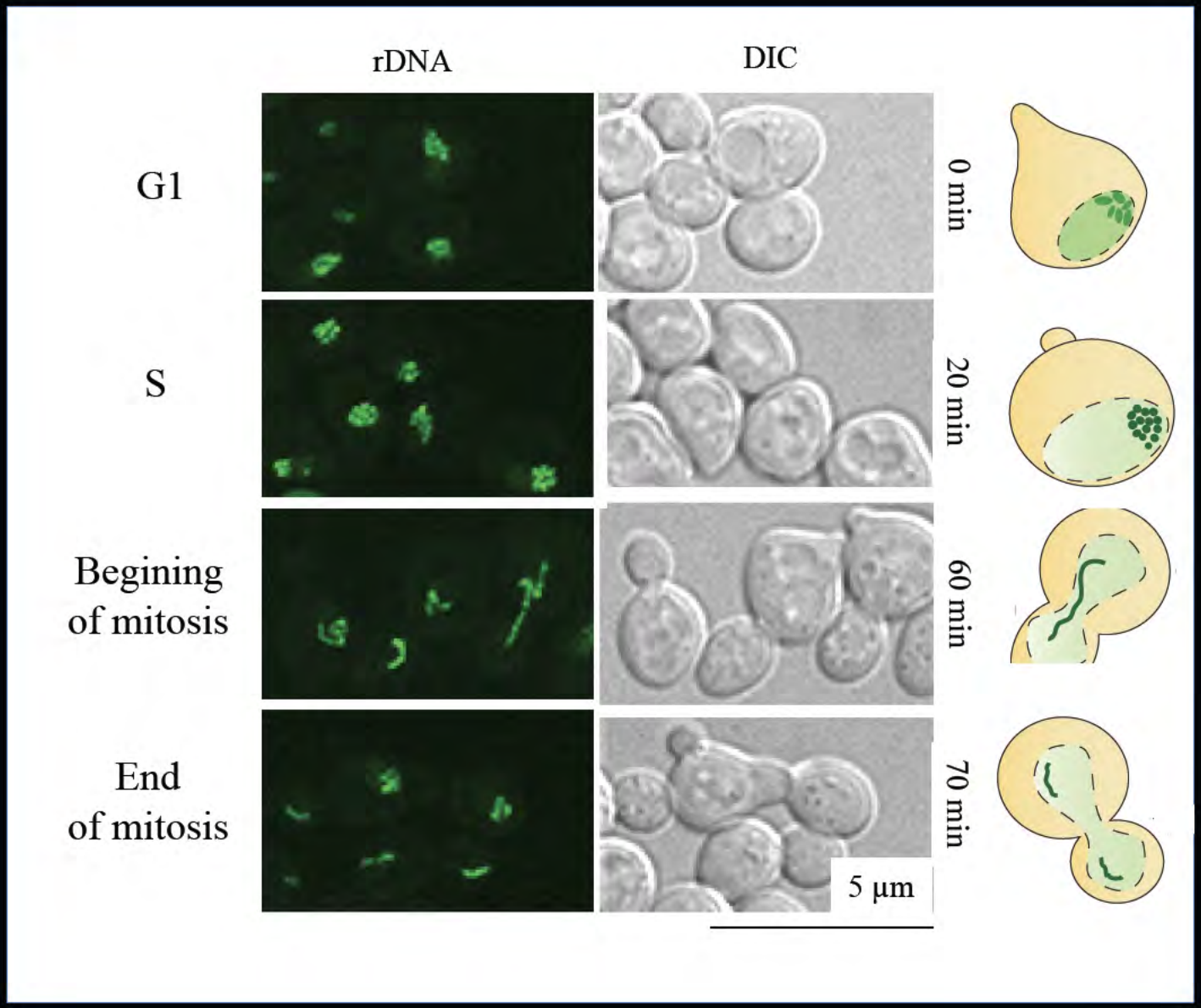
The nucleolar chromatin !

# What about nucleolar chromatin?




rDNA morphology is re-organized during cell cycle

# What about nucleolar chromatin?

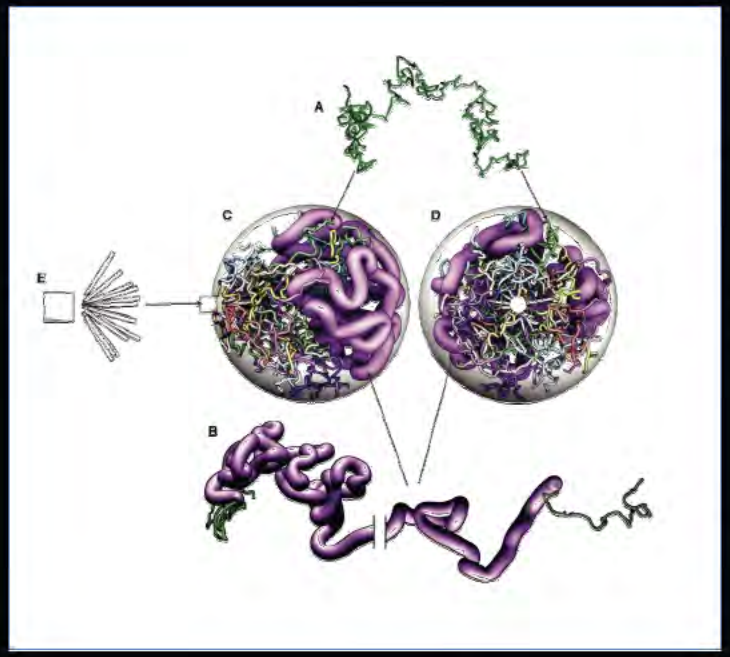


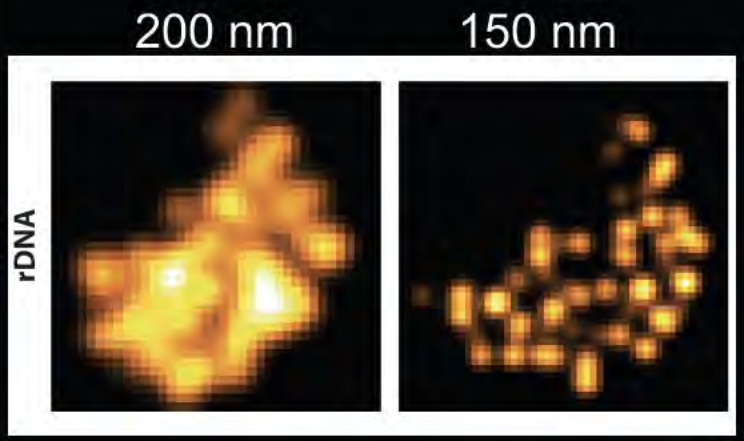
# Nucleolar Chromatin : a region to explore !

The highest transcription rate of the genome

1-2 Mb Chromatin organized by SMC complexes (Cohesin, Condensin…)

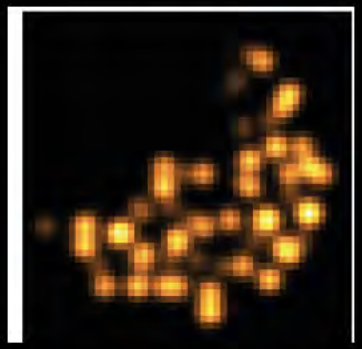
Amenable to microscopic observation !

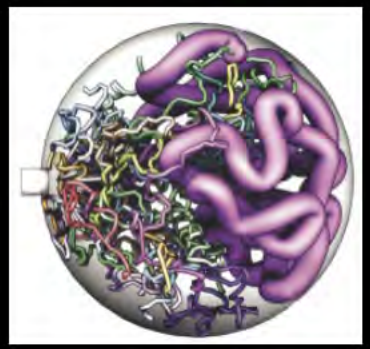


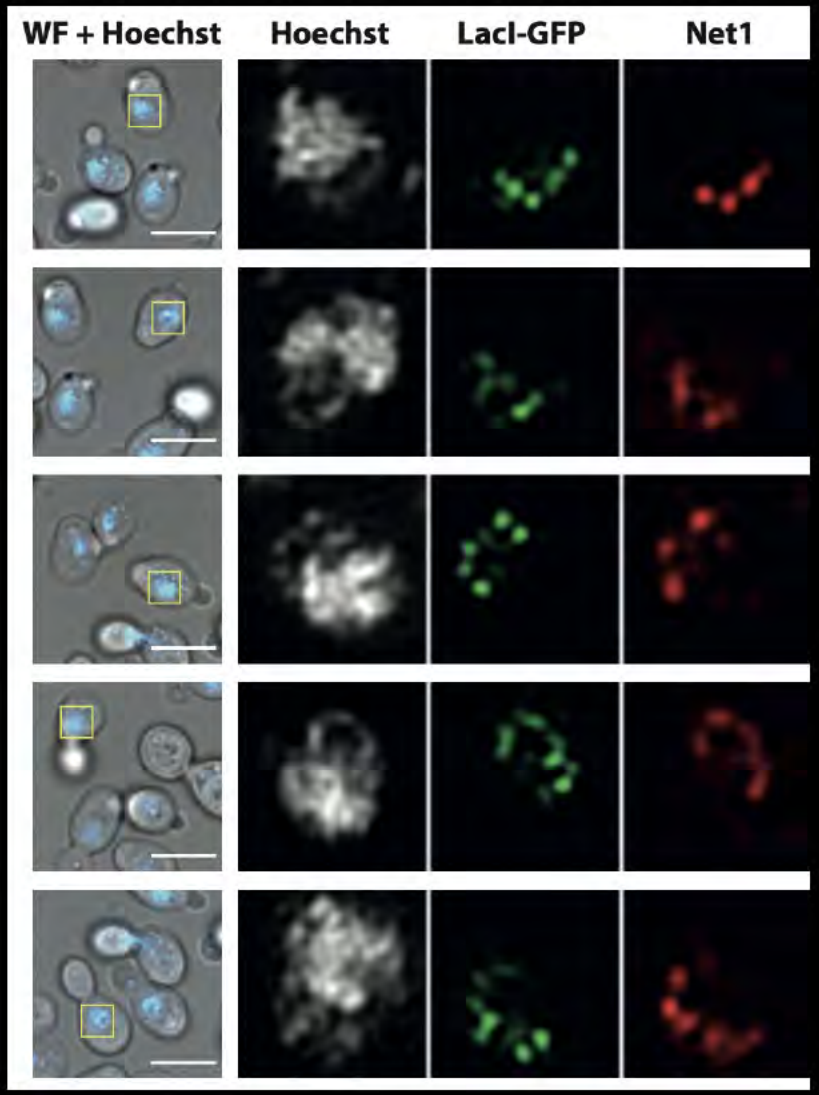



# Nucleolar Chromatin : a region to explore !

150 nm



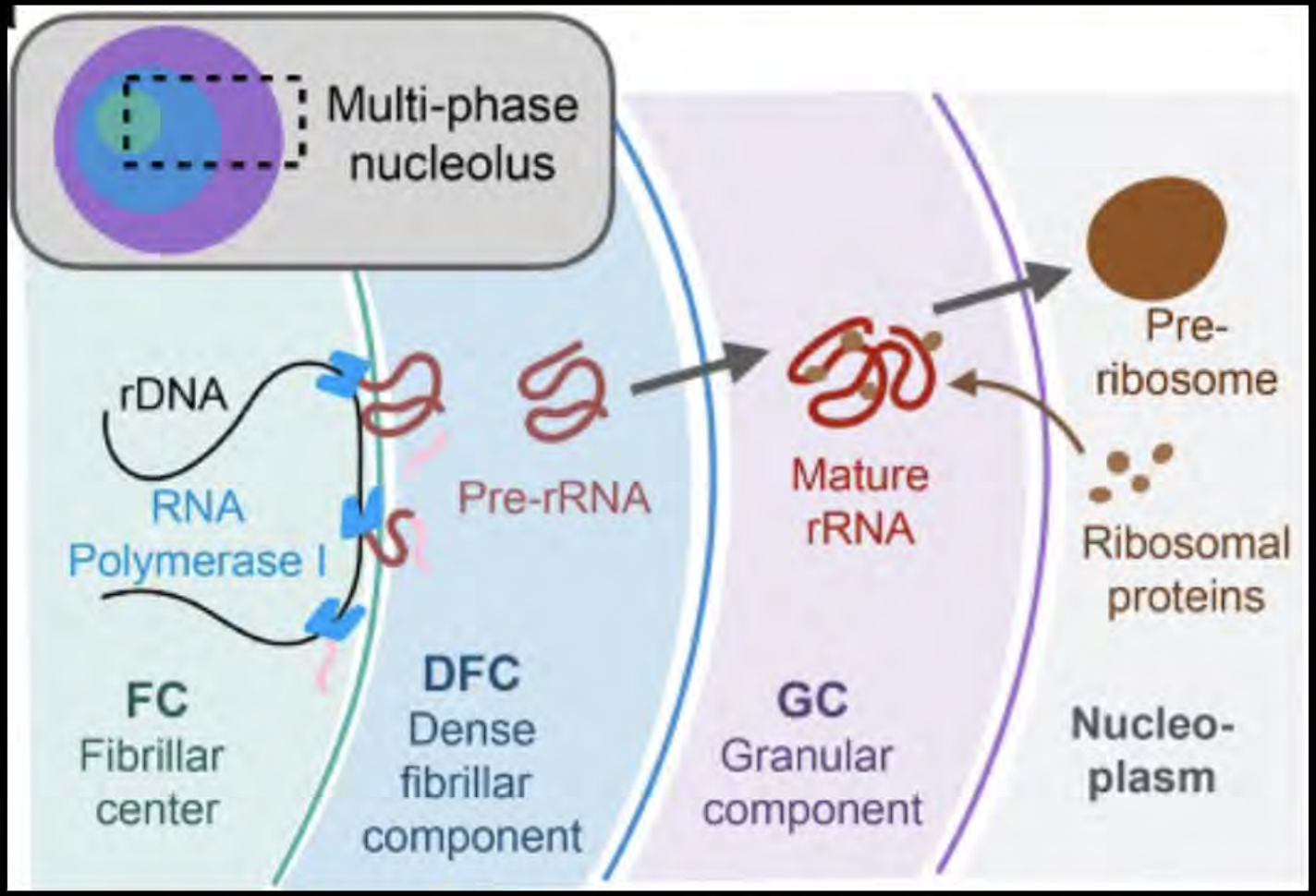





Large scale
Re-organization
during cell cycle

Sub-domain visible !

## Nucleolar chromatin is at the center of the nucleolar sub-compartment

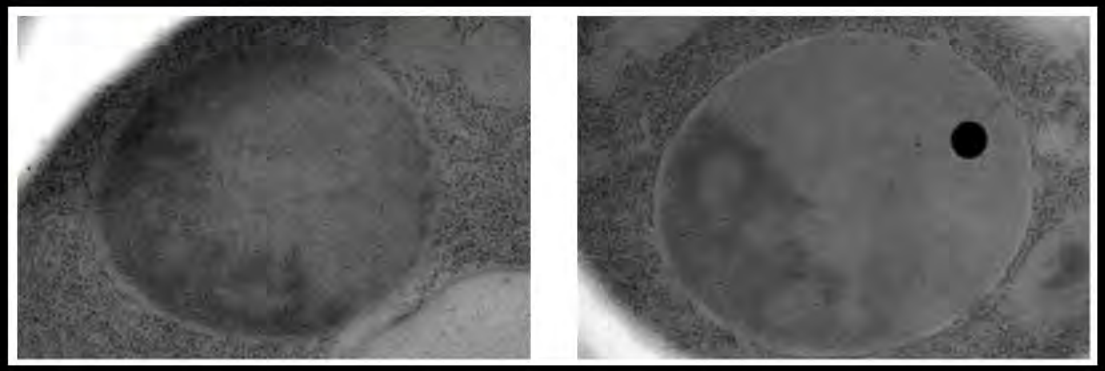



The three distinguishable phases observed by electron microscopy are the fibrillar center (FC), the dense fibrillar component (DFC) and the granular component (GC). The outward flux of transcribed ribosomal RNA (rRNA) is met by a countervailing inward flux of ribosomal proteins (rProteins) in the GC.

# Nucleolar Chromatin center of the condensate !

Correlative light and electron microscopy !

TEM observation

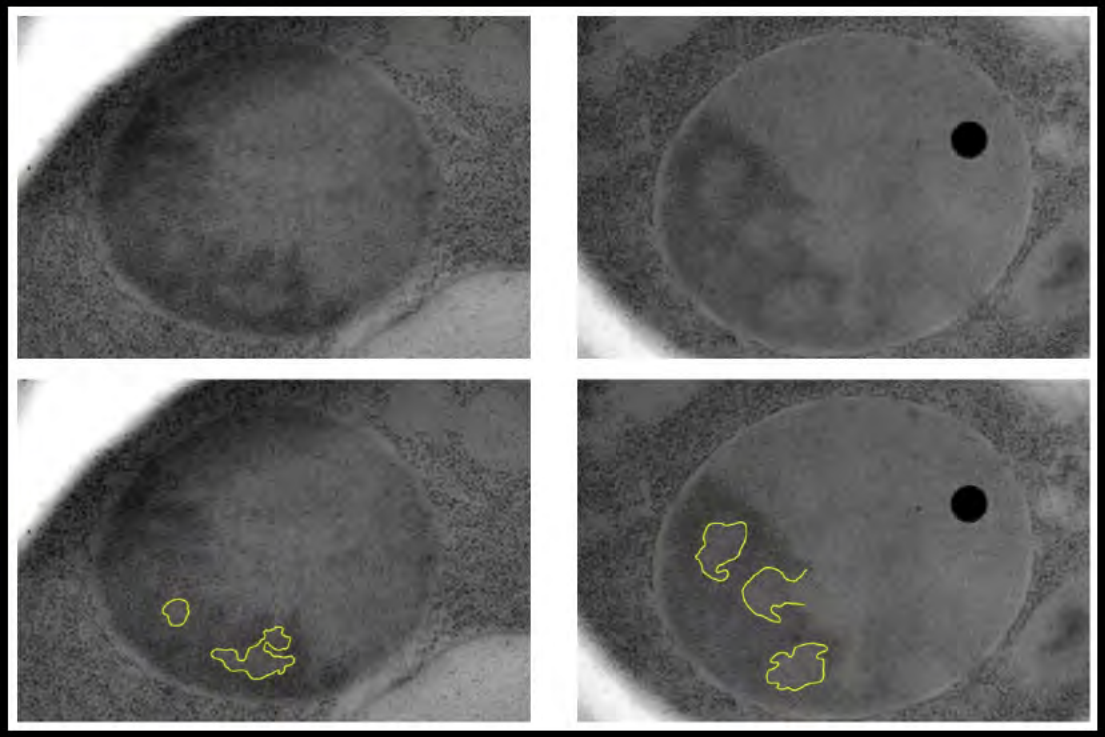


# Nucleolar Chromatin : a region to explore !

Correlative light and electron microscopy !

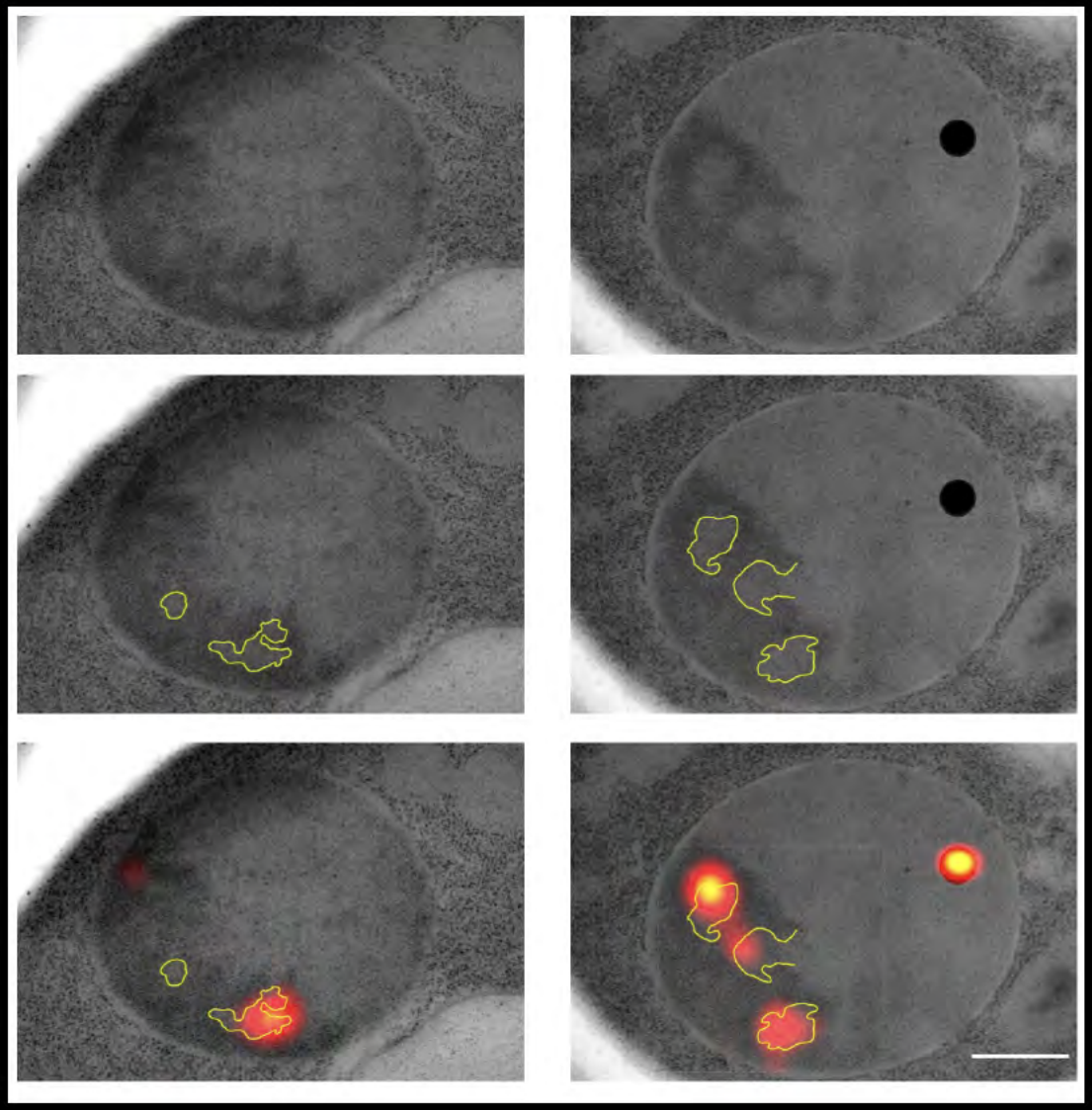
TEM observation

Identification of nucleoplasmic like region?



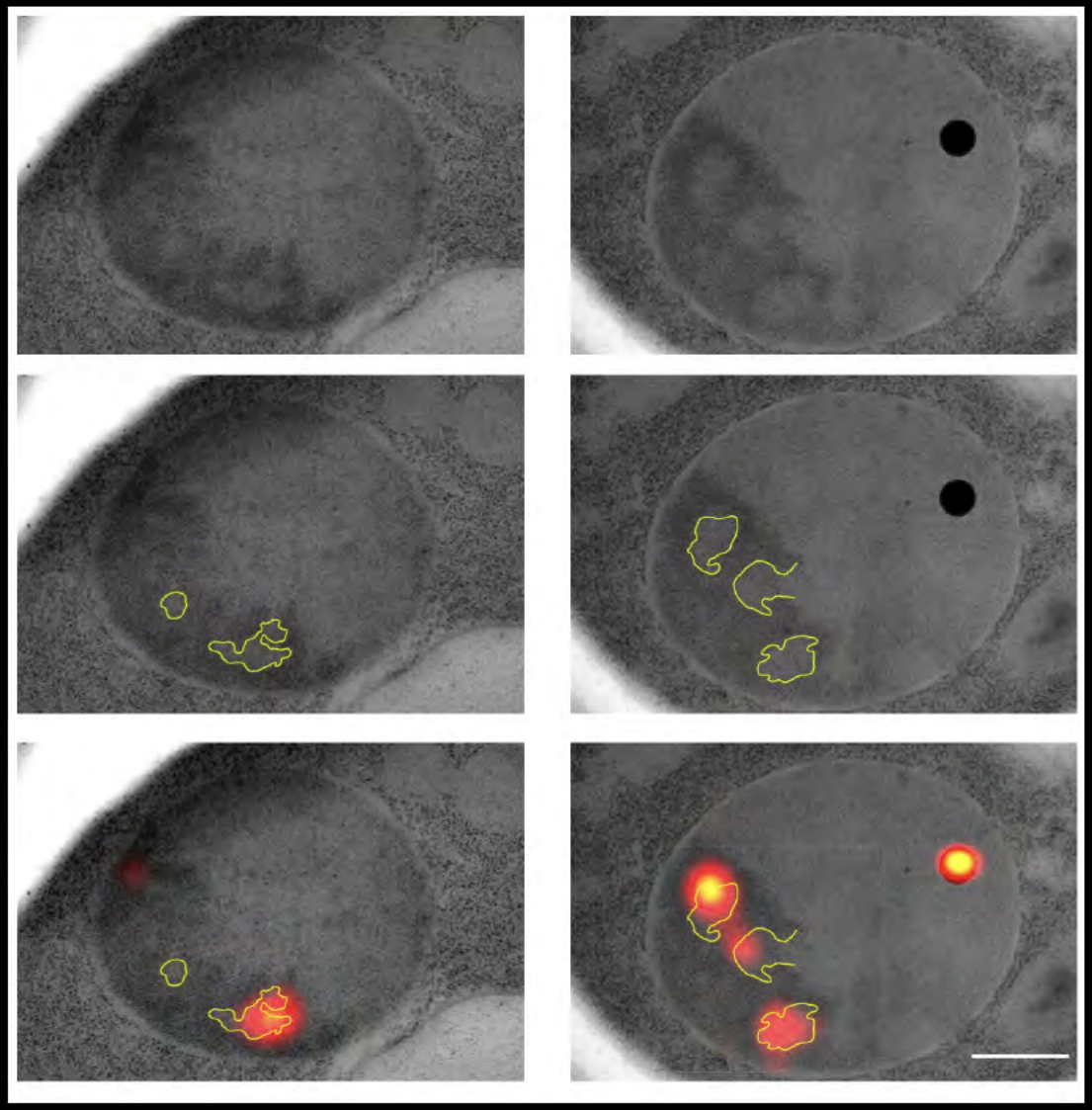
# Nucleolar Chromatin : a region to explore !

Correlative light and electron microscopy !



# Nucleolar Chromatin : a region to explore !

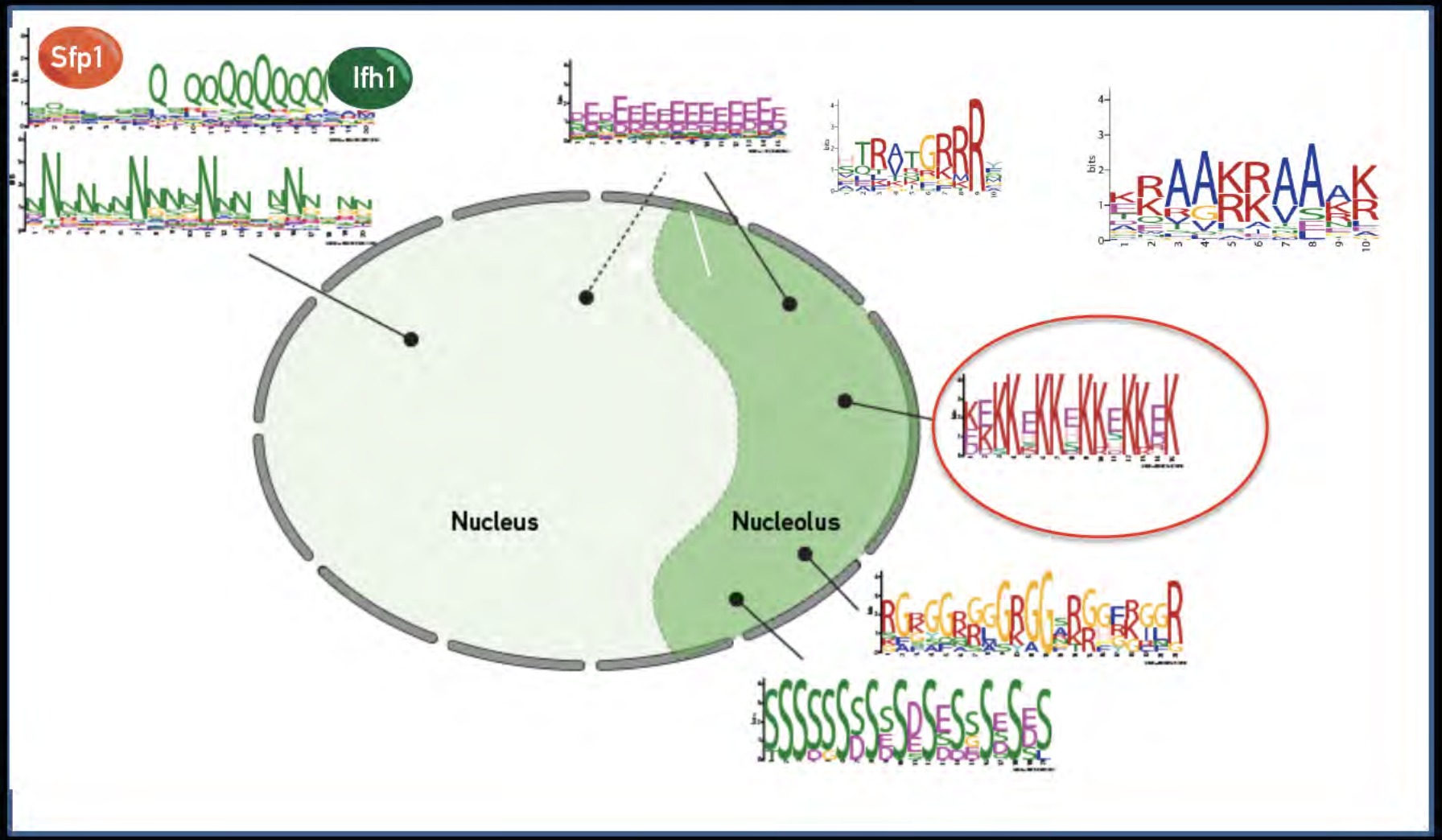
Correlative light and electron microscopy !



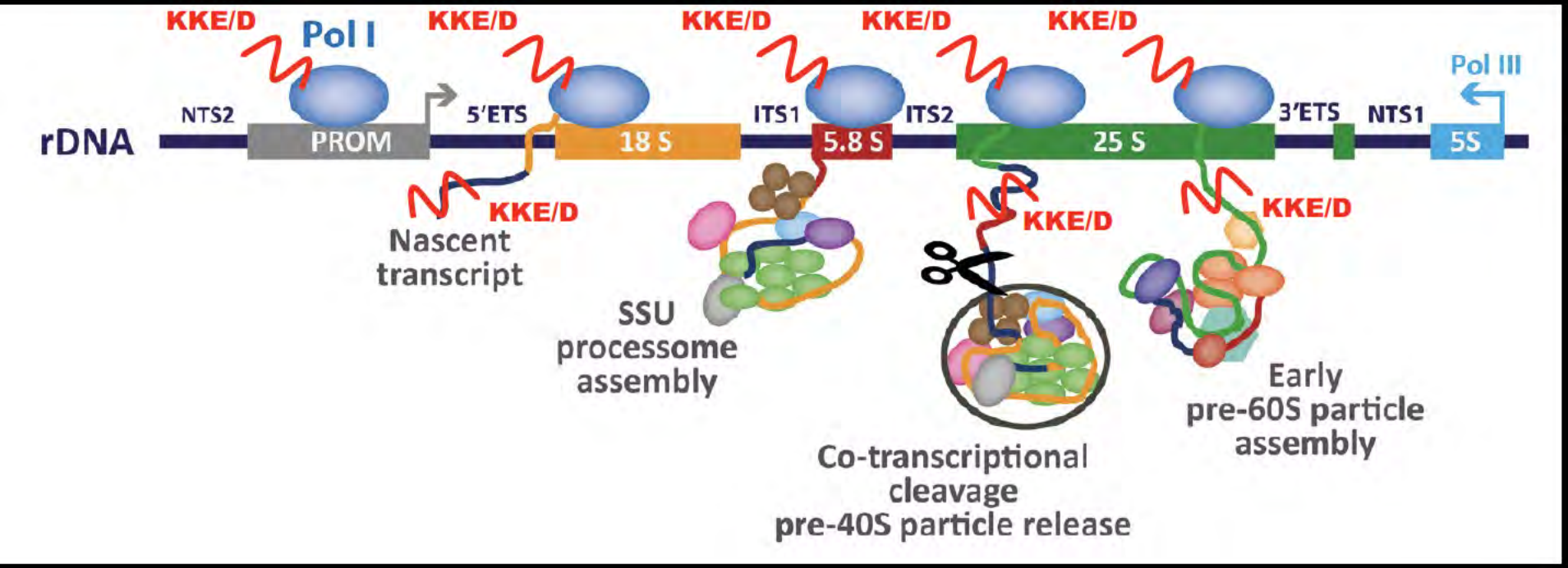
Colocalisation
With rDNA
Bound
Protein (GFP)

# Specific nucleolar IDR present ?

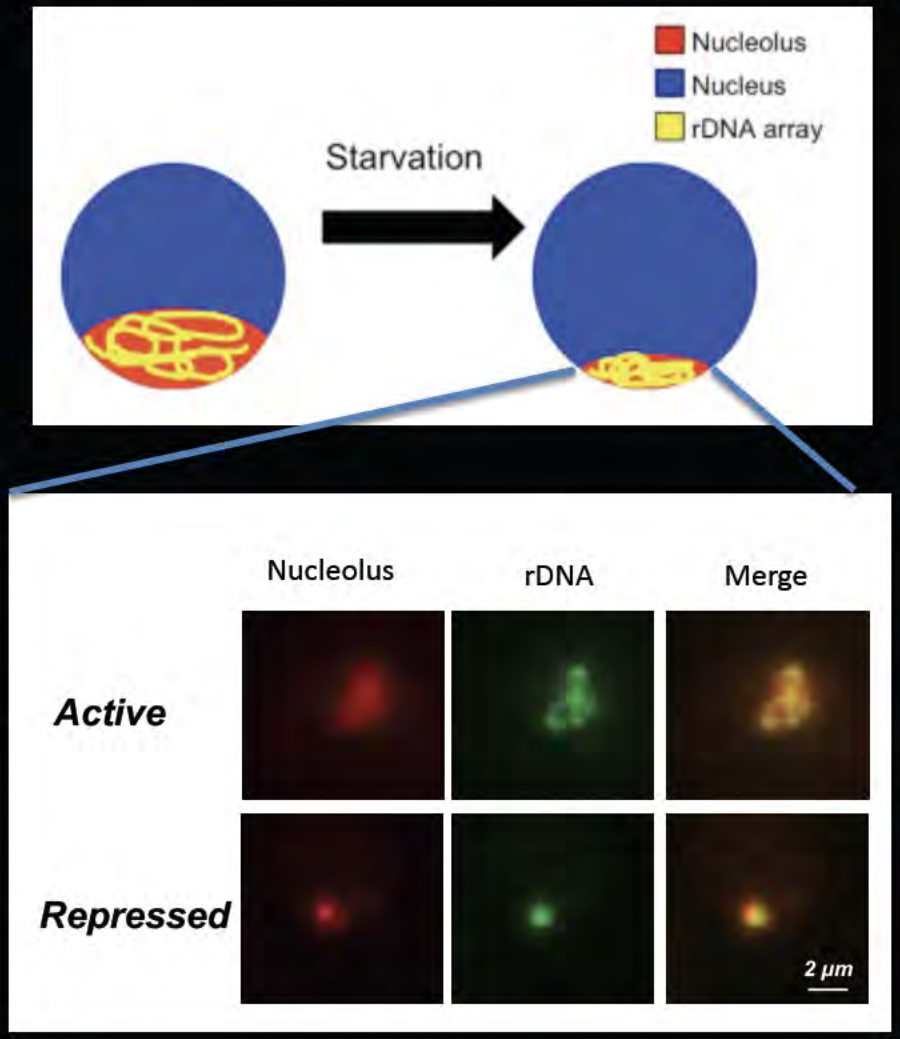
*Disorder motif enrichment in nucleus (n=1206, yeast GFP-data Base)*



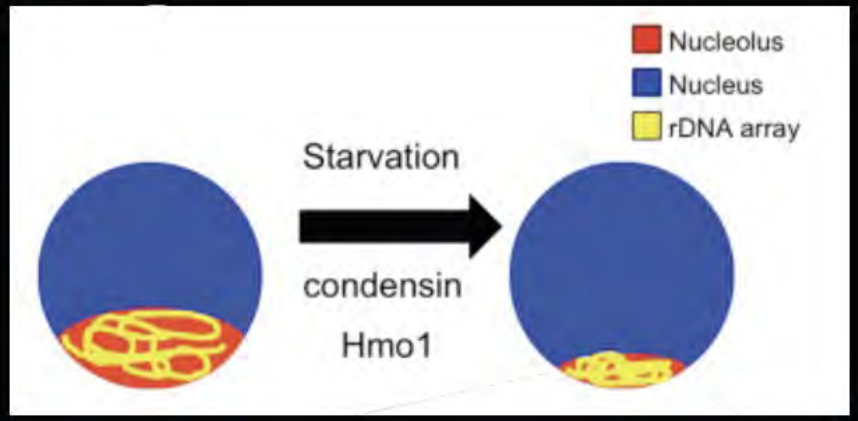
# KKE/D tail is enriched in factors involved in early steps of rDNA transcription

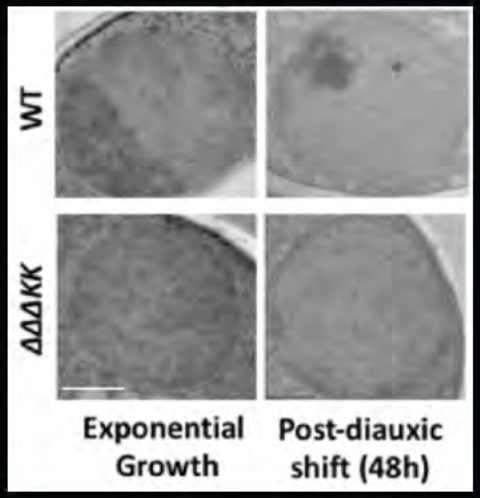


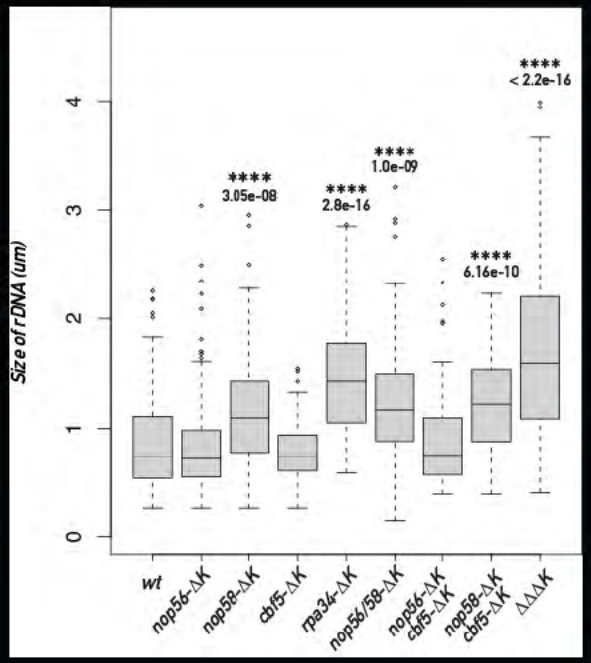
# *Nucleolar chromatin is compacted upon inactivation of ribosome production*

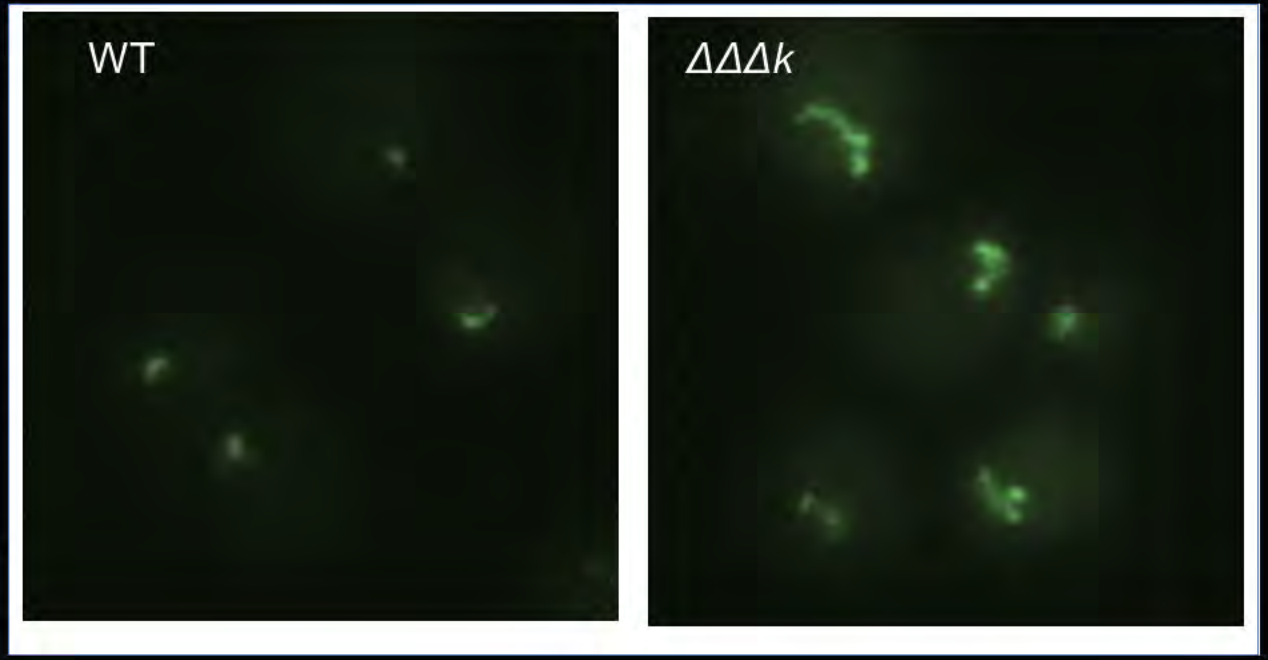


# In absence of KKE/D tail, nucleolar structure is massively altered

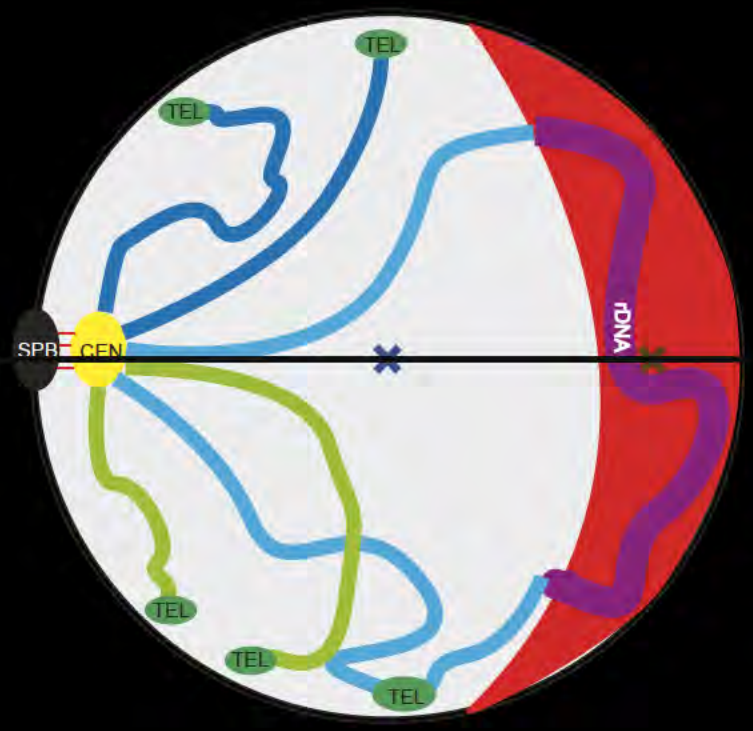


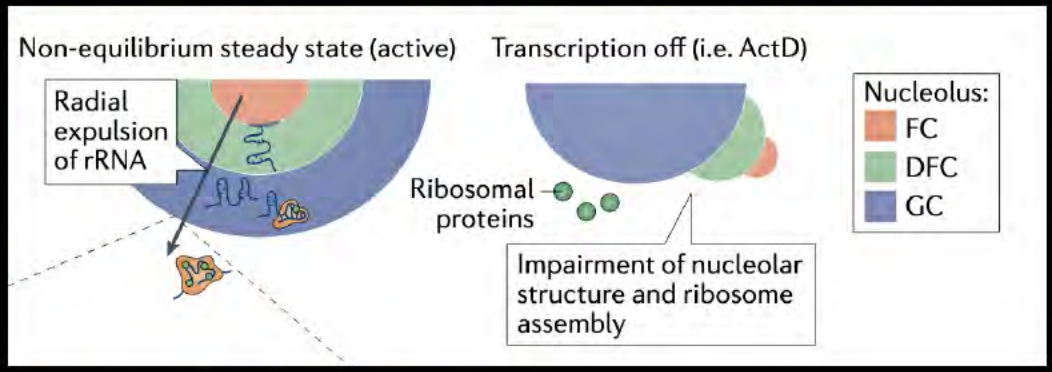



# How to manipulate nucleolar chromatin ?




Nuclear condensate genetically amenable !

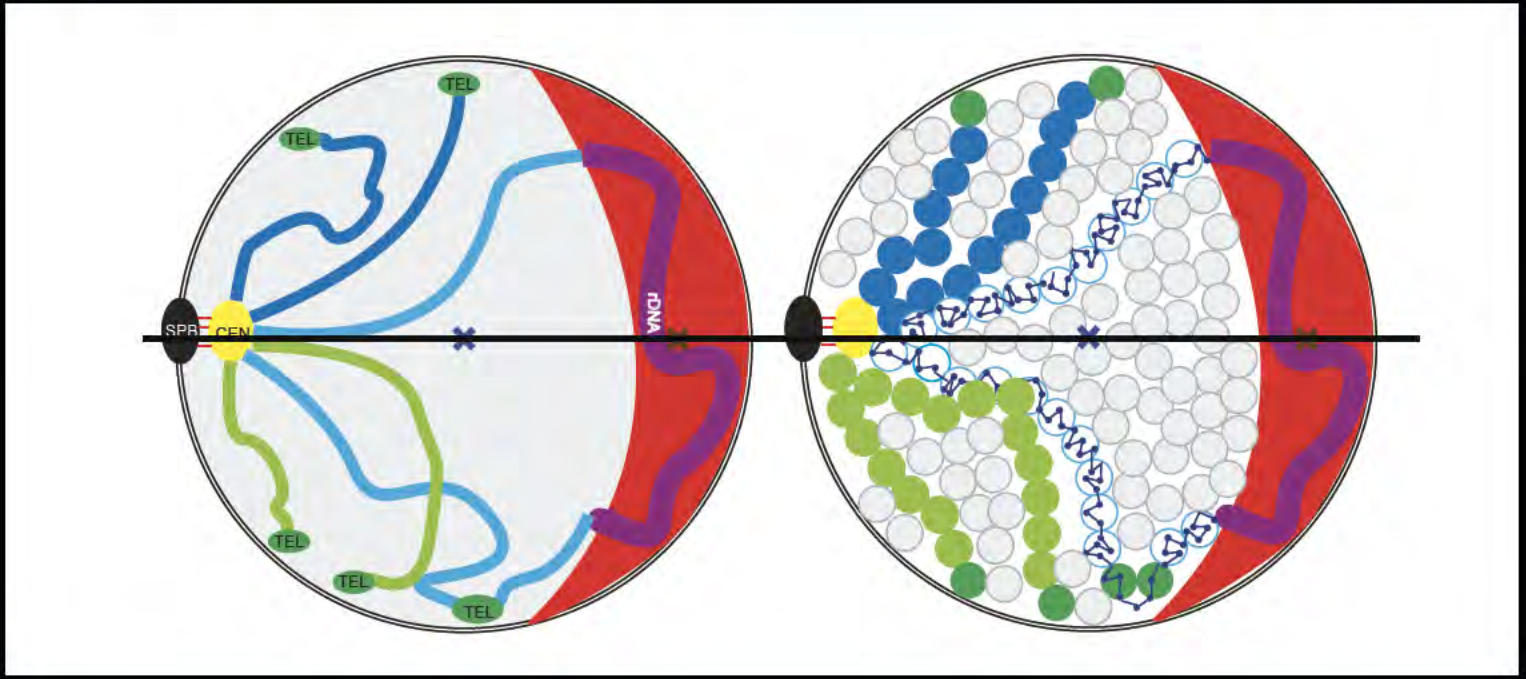



Sarah Danché - Poster

# Conclusion – Perspectives

**Nucleoplasmic :**
Rouse model with Transient Internal Contacts

**Nucleolar chromatin ?**




**The nucleolus – a mysterious condensate :**

- **Organisation of rDNA chromatin ?**
- **Contribution of specific IDR in rDNA organization?**

Acknowledgements

Organisation et dynamique nucléaire
MCD

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Lise Dauban – live rDNA

Claudie Carron – RIM, SMLM

Sarah Danché – IDR and nucleolus

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# Organisation et dynamique nucléaire