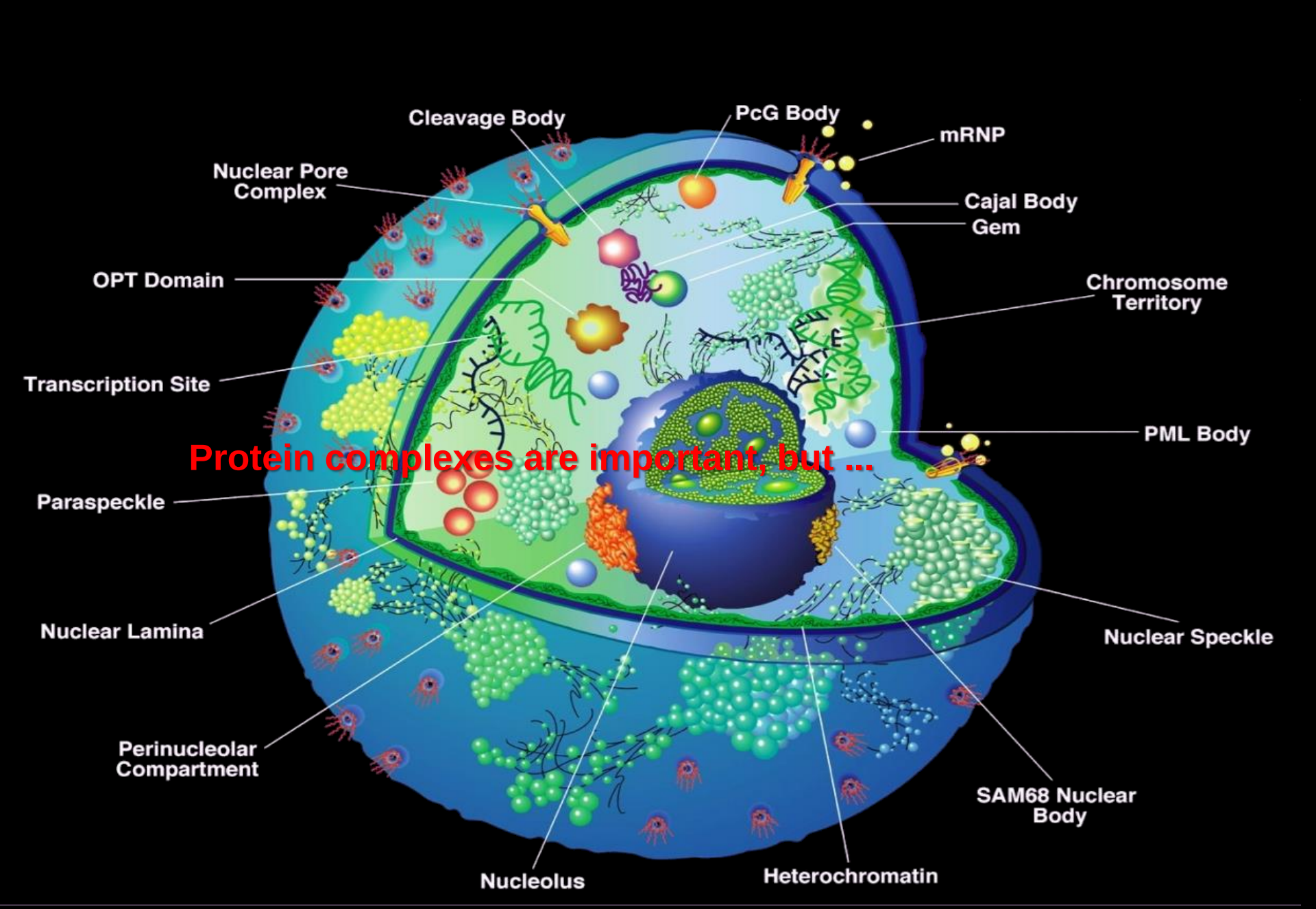
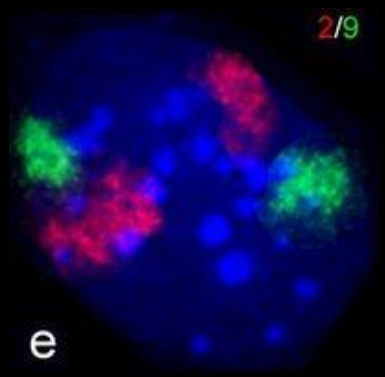
Nuclear phosphoinosites contribute to chromatin structure and regulation of gene expression

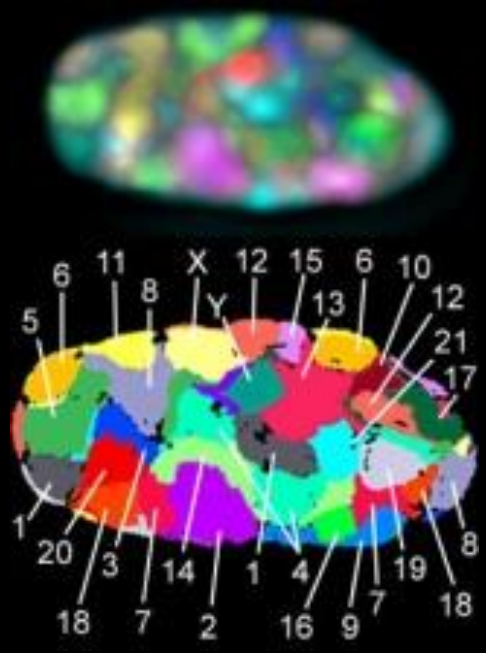
Pavel Hozák

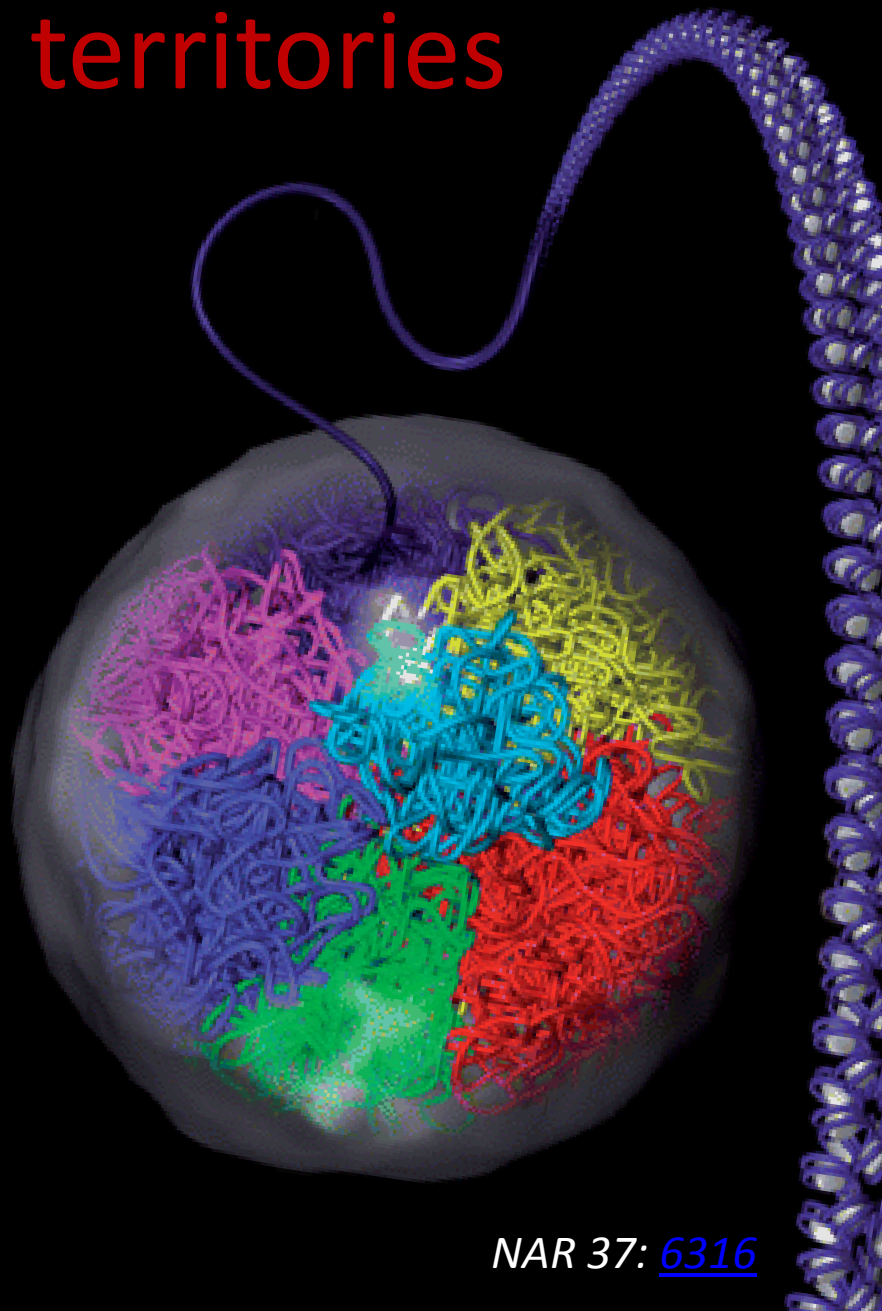
Department of Biology of the Cell Nucleus & Microscopy Centre, Institute of Molecular Genetics AS CR, Prague



Chromosomal territories

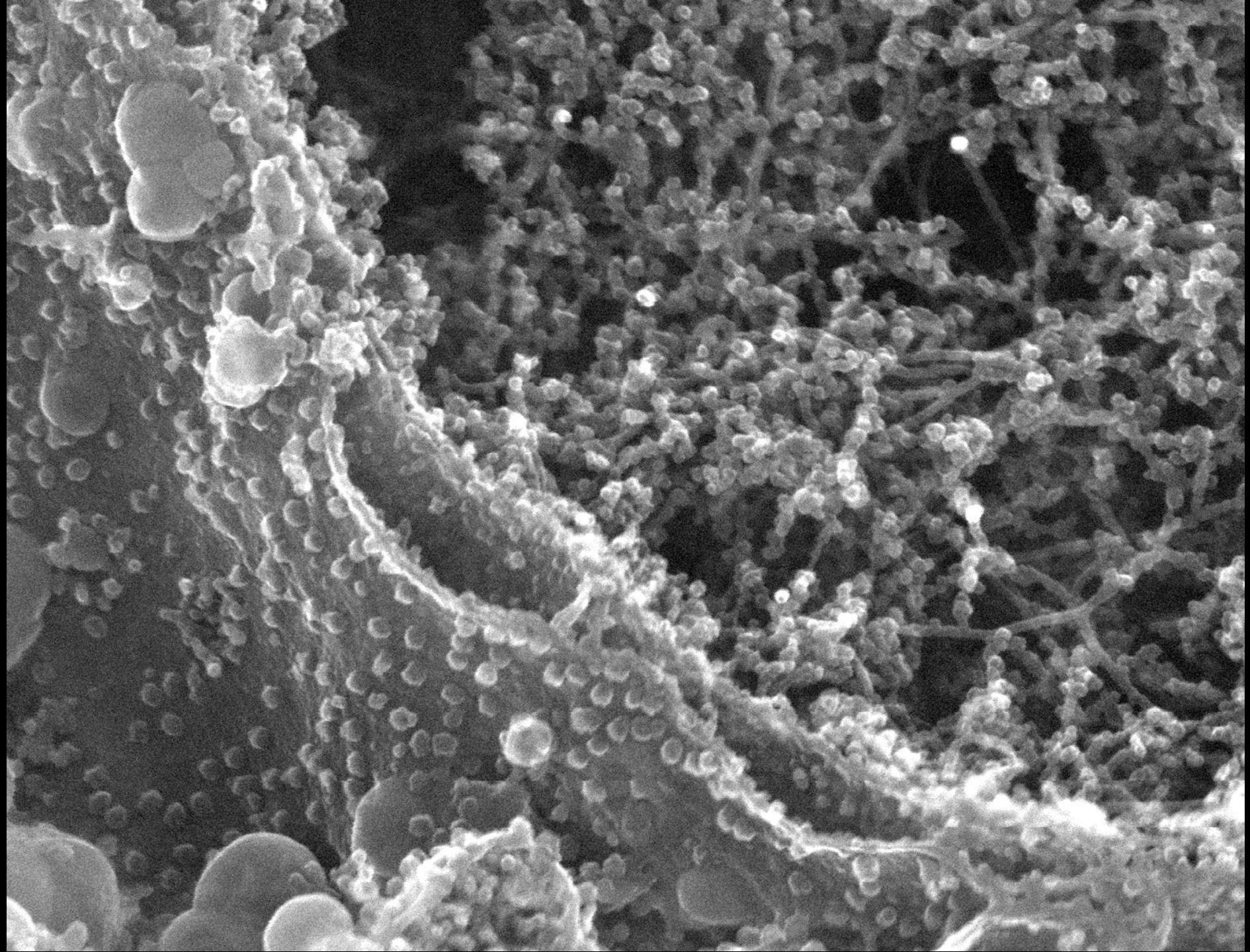






T. Cremer et al.

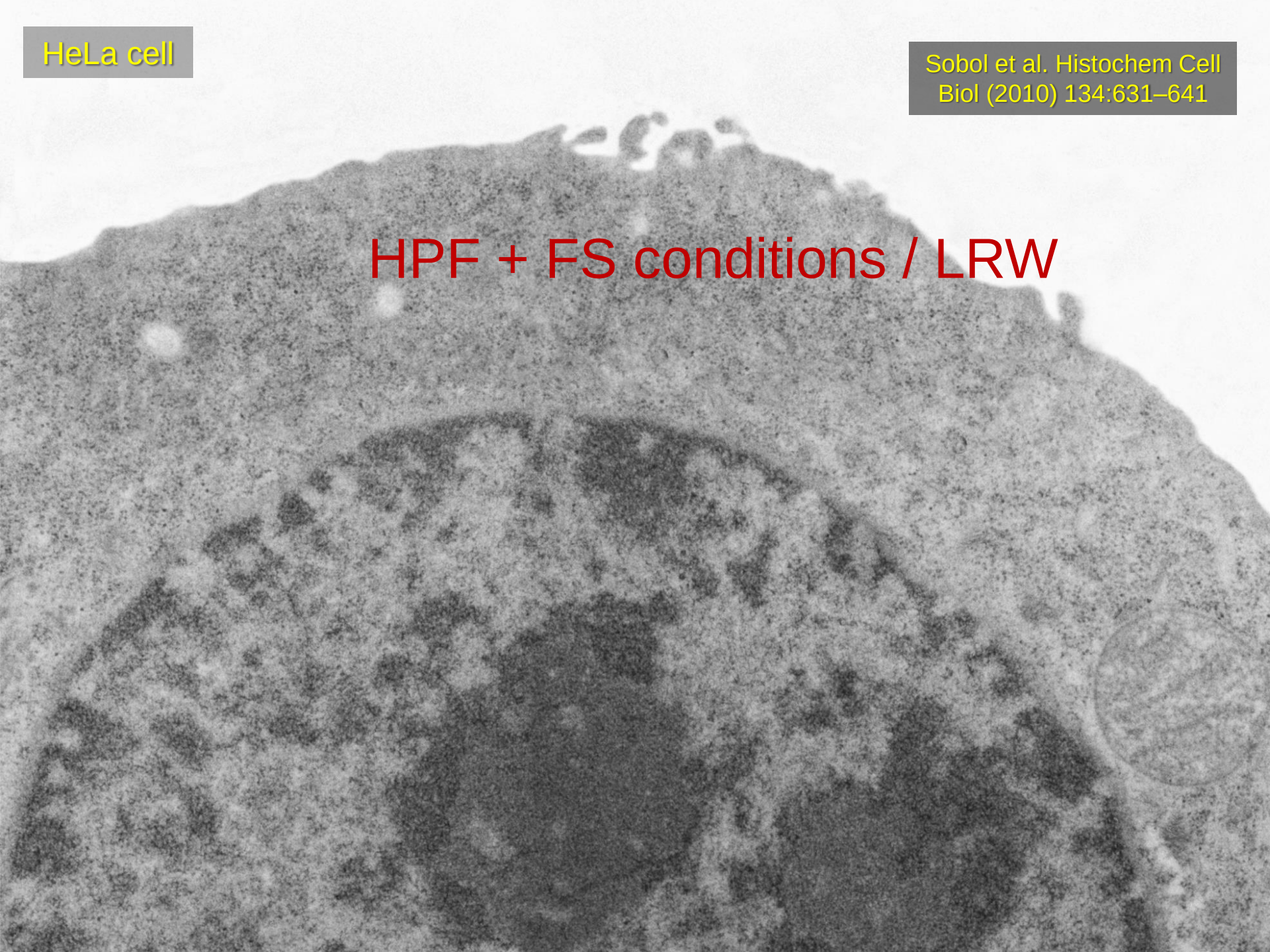
NAR 37: 6316



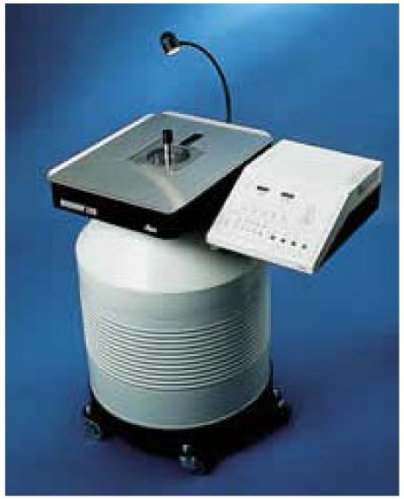

Leica
TRESTON

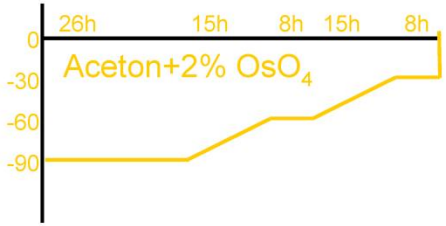
HeLa cell

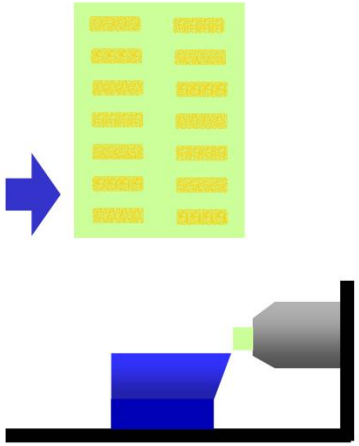
Sobol et al. Histochem Cell Biol (2010) 134:631–641

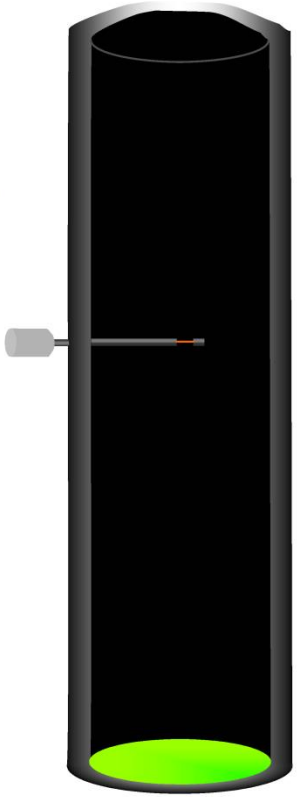


EM PACT and FS in AFS









HPF of specimen in tube holder

Leica EM PACT

FS

Leica EM AFS

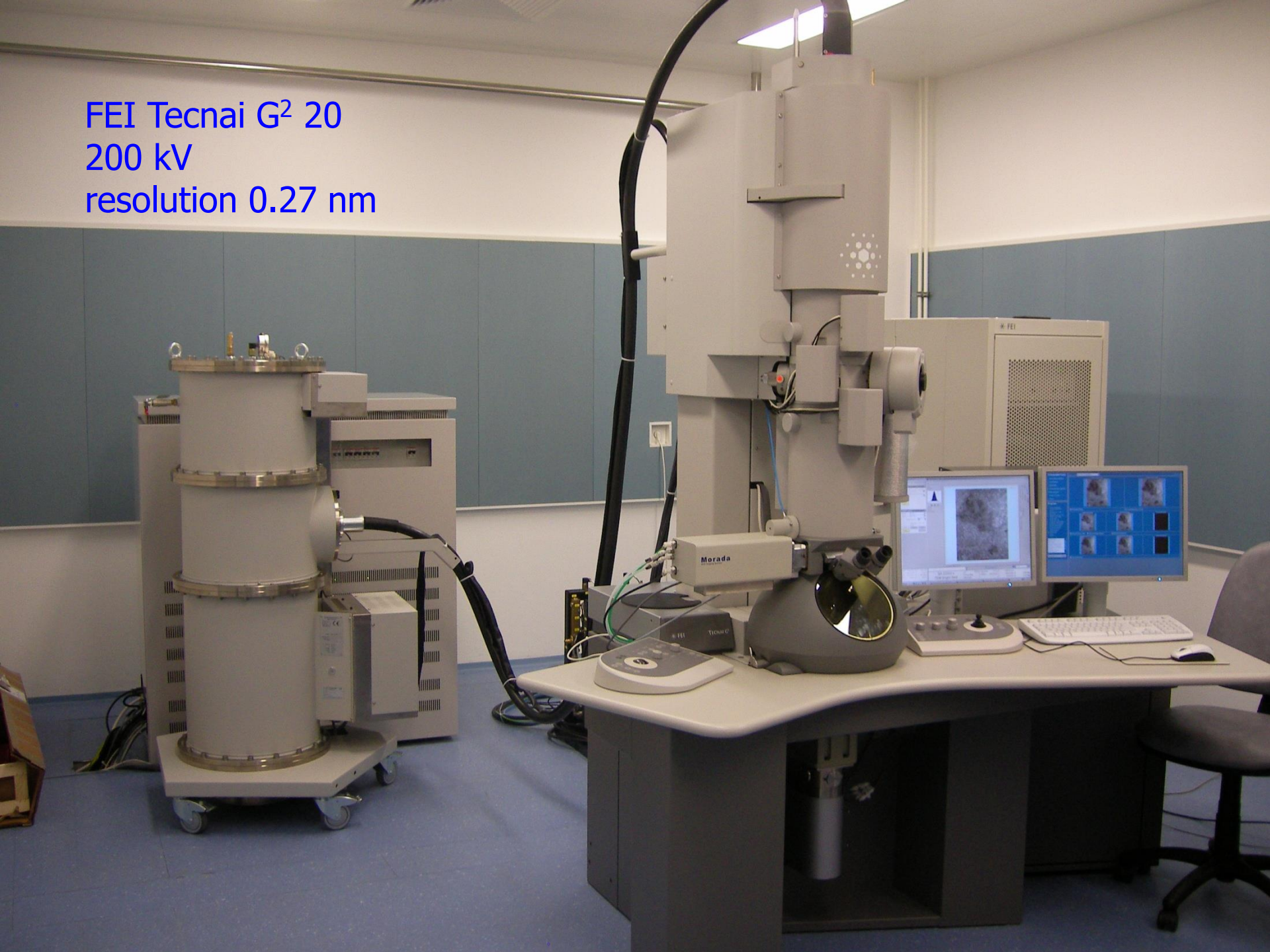
Embedding & polymerisation

EPON

Conventional sectioning

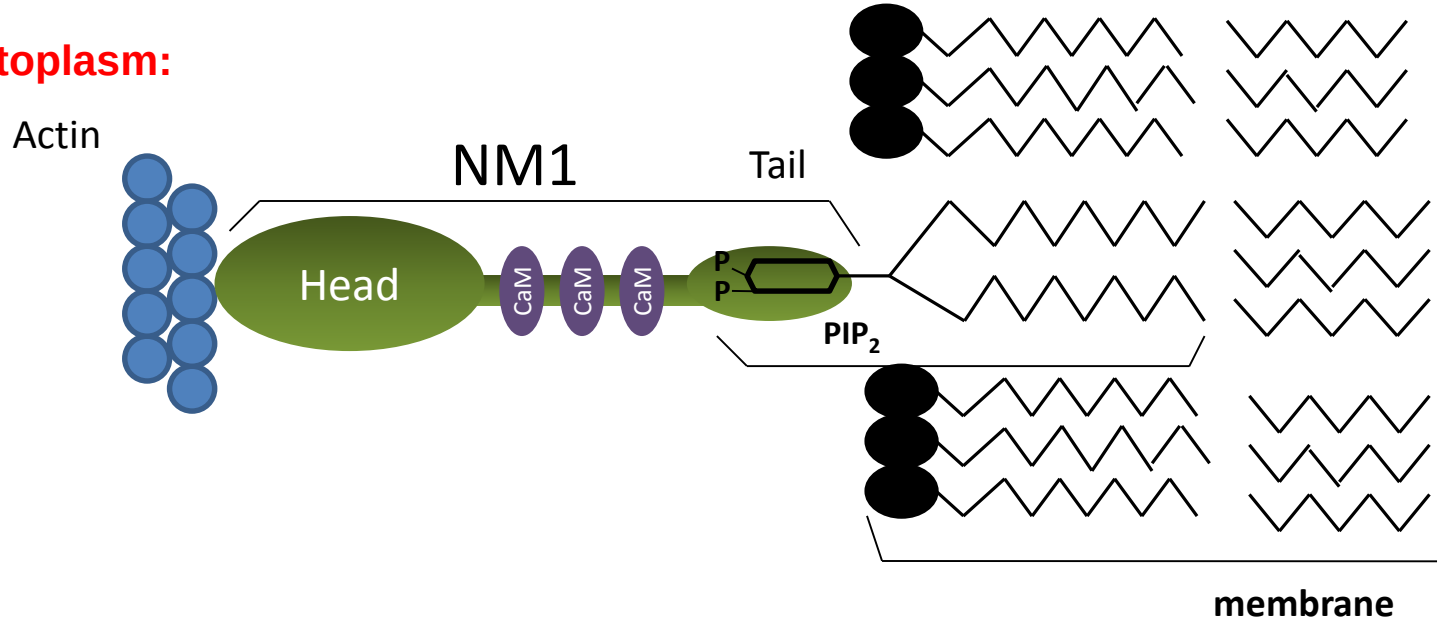
Leica UCT

TEM

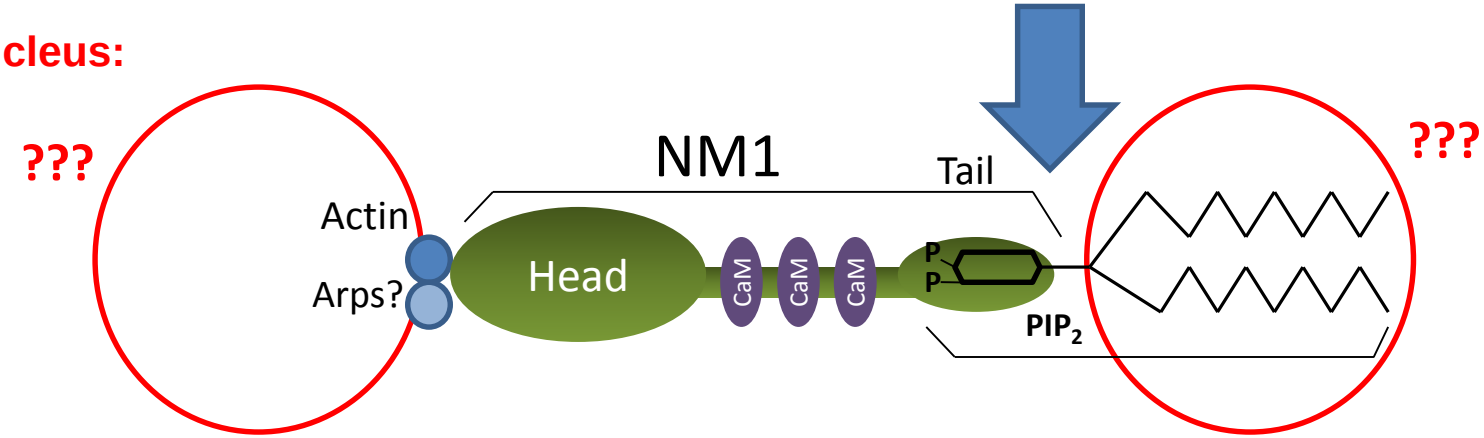

FEI Tecnai G^2 20
200 kV
resolution 0.27 nm
Morada

Binding partners of NM1-PIP_2?

In the cytoplasm:



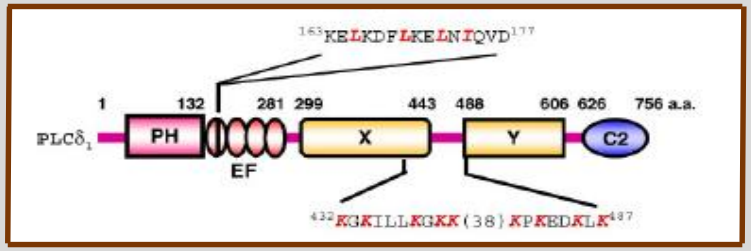
In the nucleus:



PIP2 imaging tools

1. PLCδ1PH

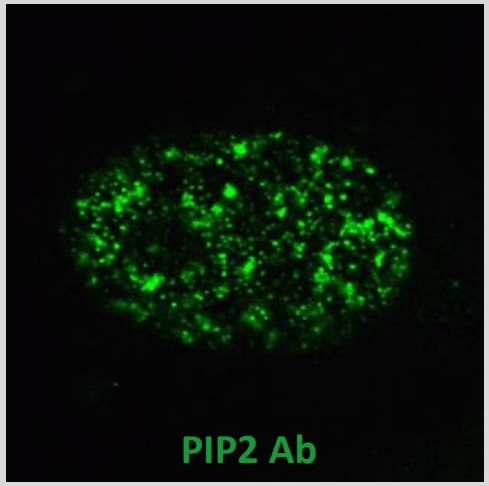
- ✓ Binds to PIP2 with a high affinity via PH domain
- ✓ R40A mutation in PH domain causes

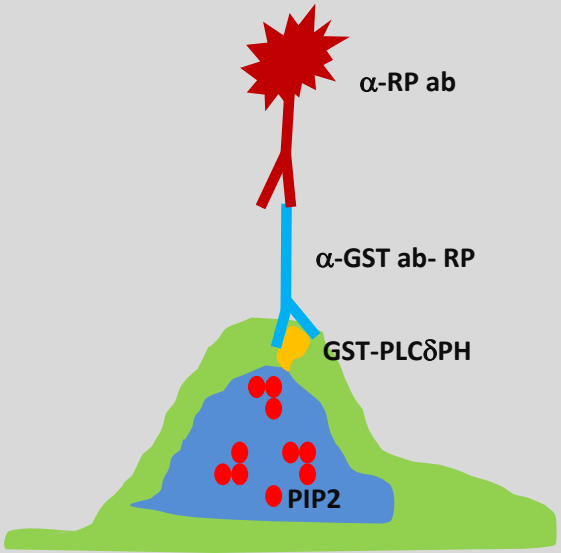


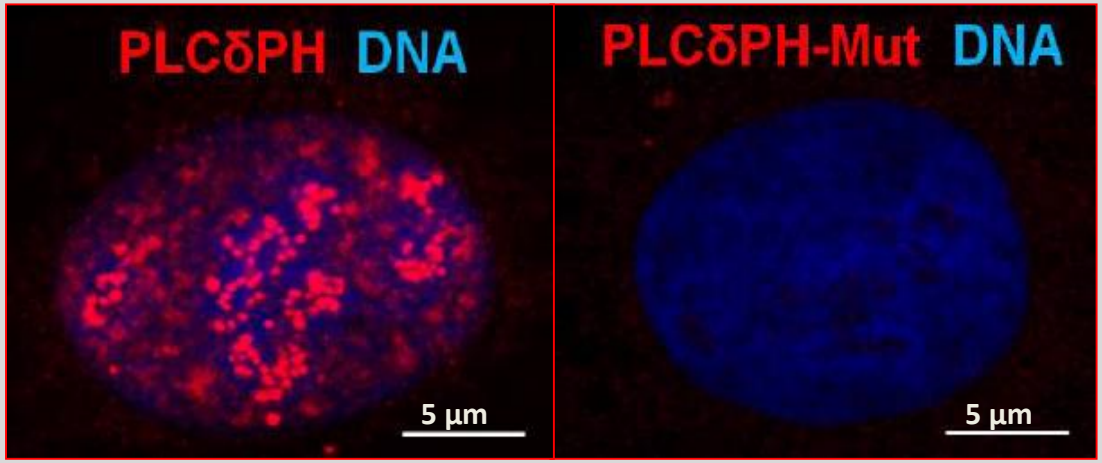
Yagisawa et al. 2006, Journal of Cellular Biochemistry

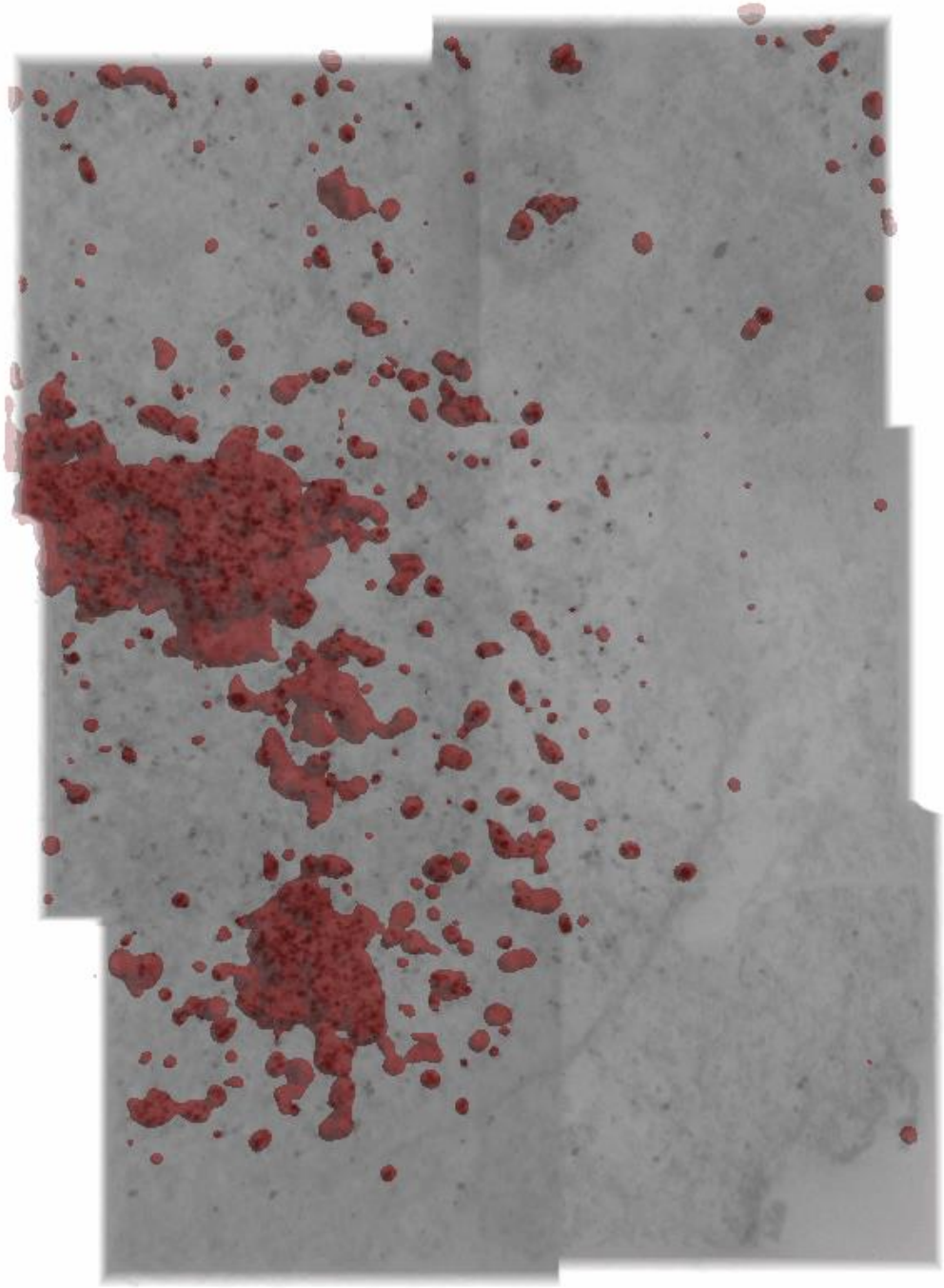
2. Anti-PIP2 monoclonal antibody Clone 2C11

Abcam
Echelon Biocsiences

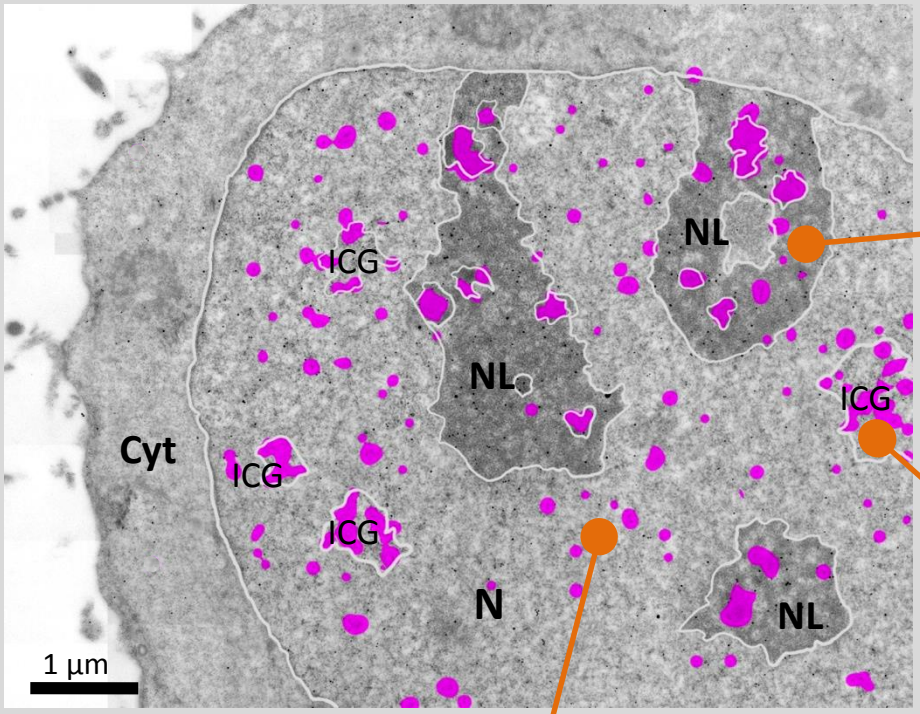


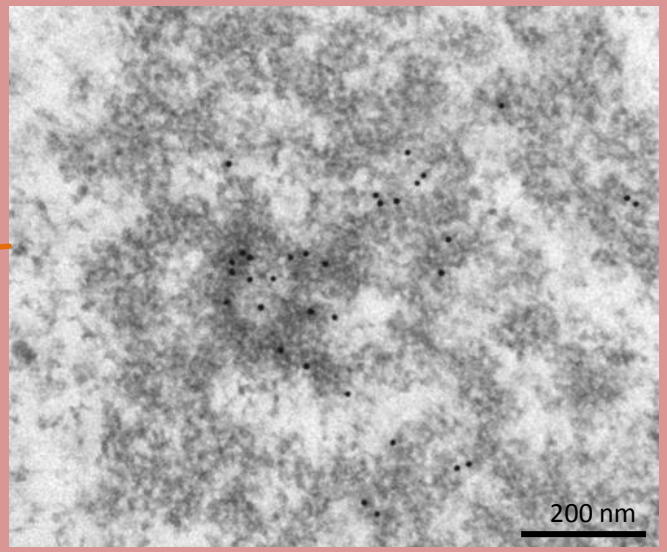






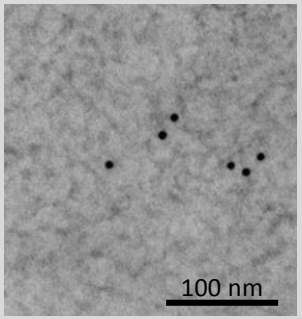
PIP2-containing structures in the cell nucleus

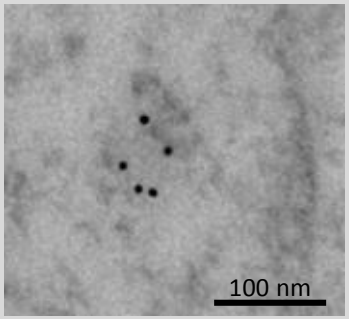


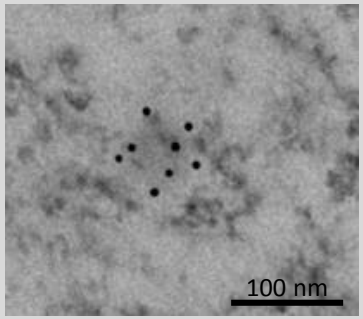


nucleolus

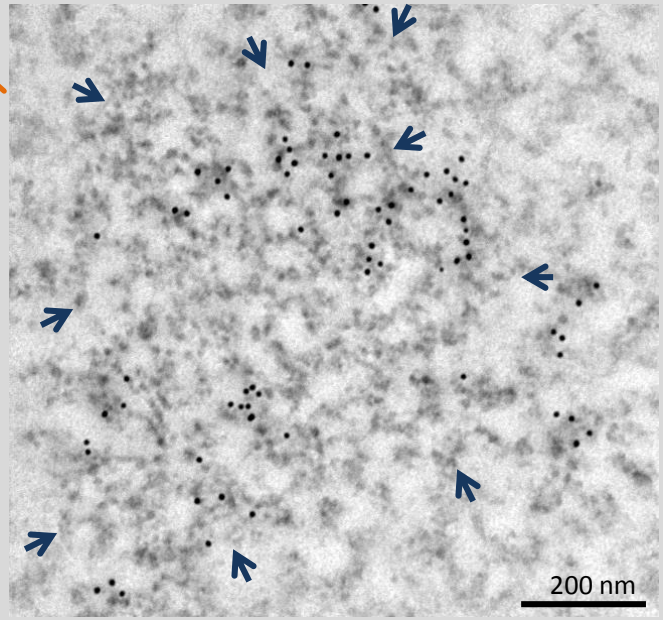
Nucleoplasmic foci





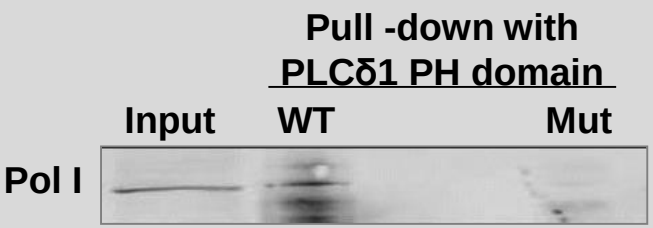


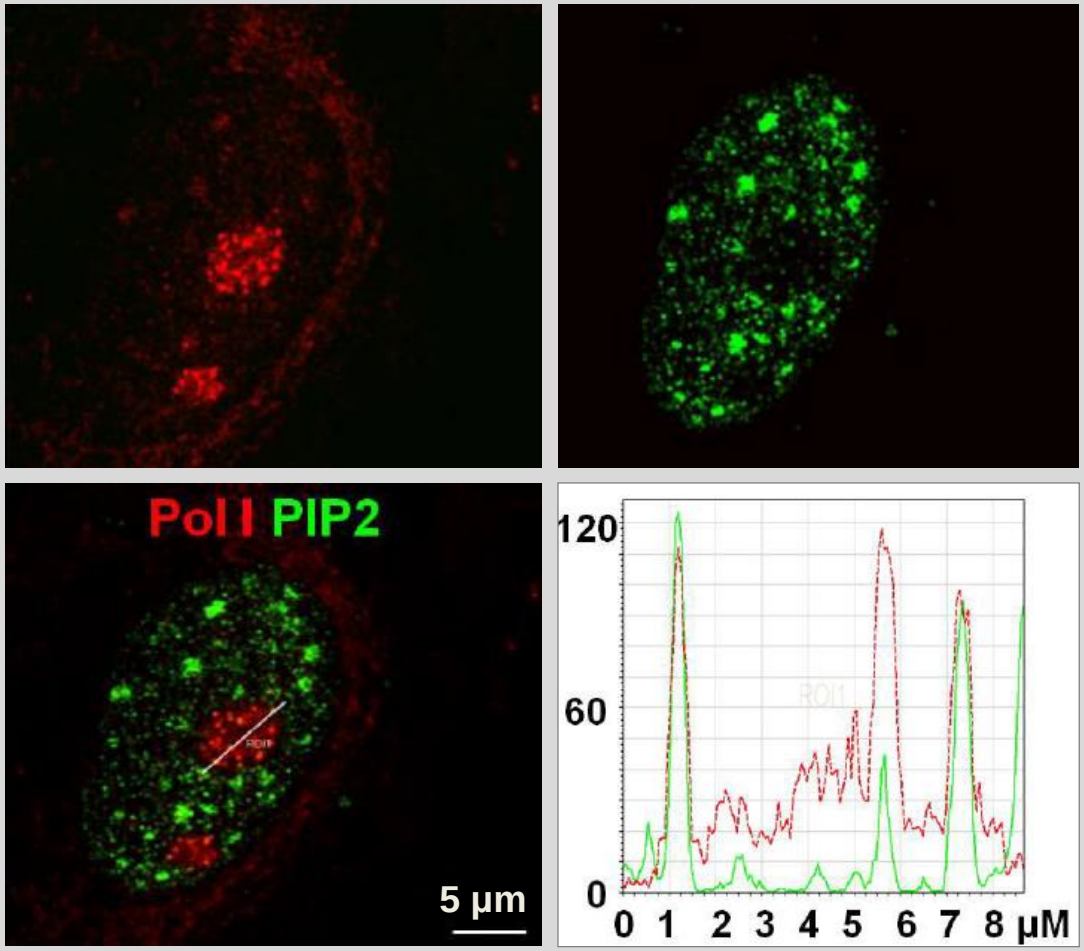
PIP2 “islets”



interchromatin granules

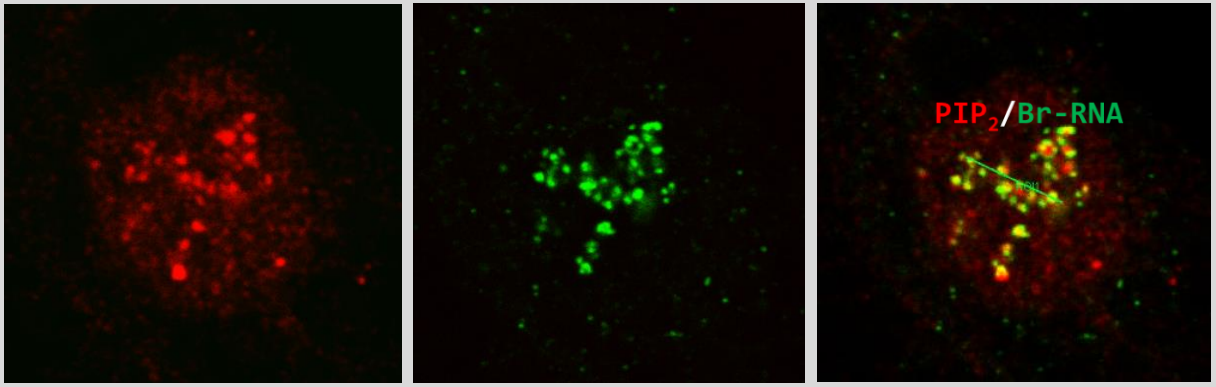
PIP2 participates in Pol I complex

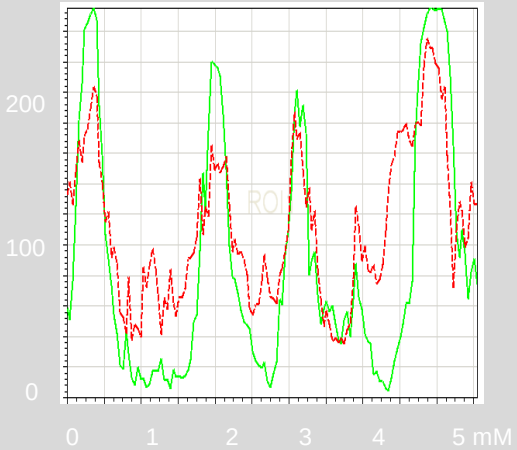


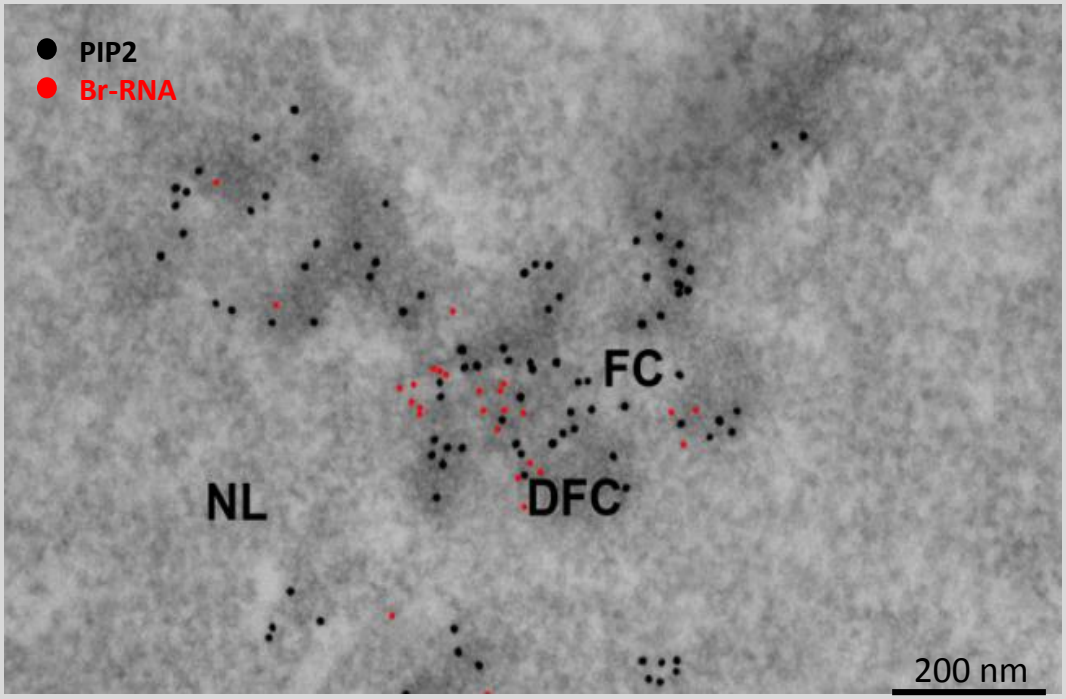


Yildirim et al., 2013, J Cell Sci

PIP2 colocalizes with nascent rRNA transcripts

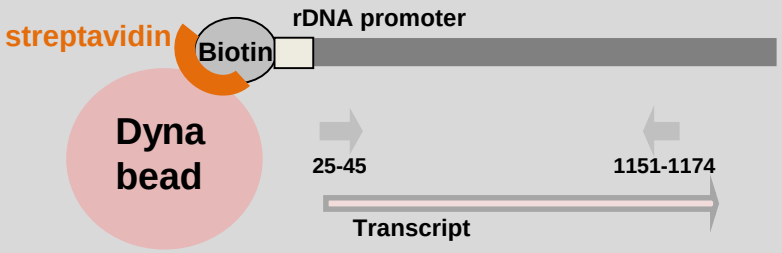


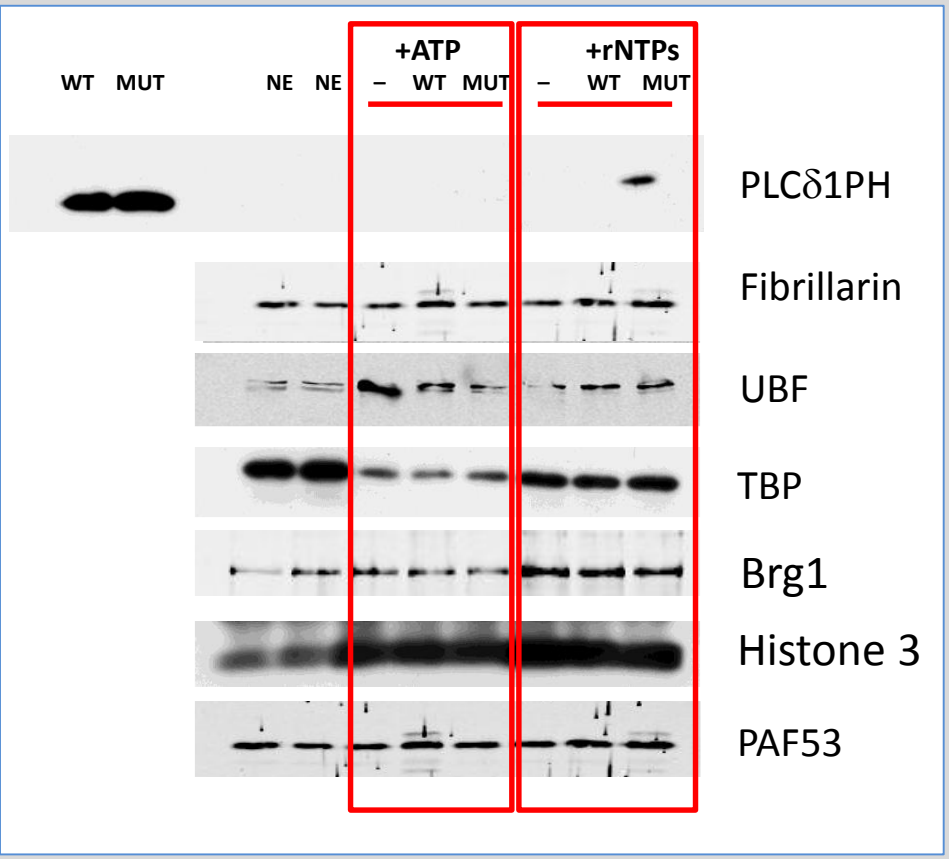




Yildirim et al., 2013, J Cell Sci

PIP2 in Pol I transcription



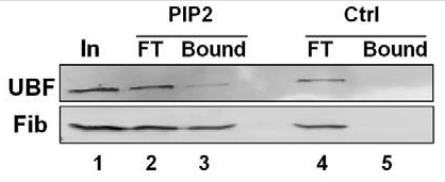


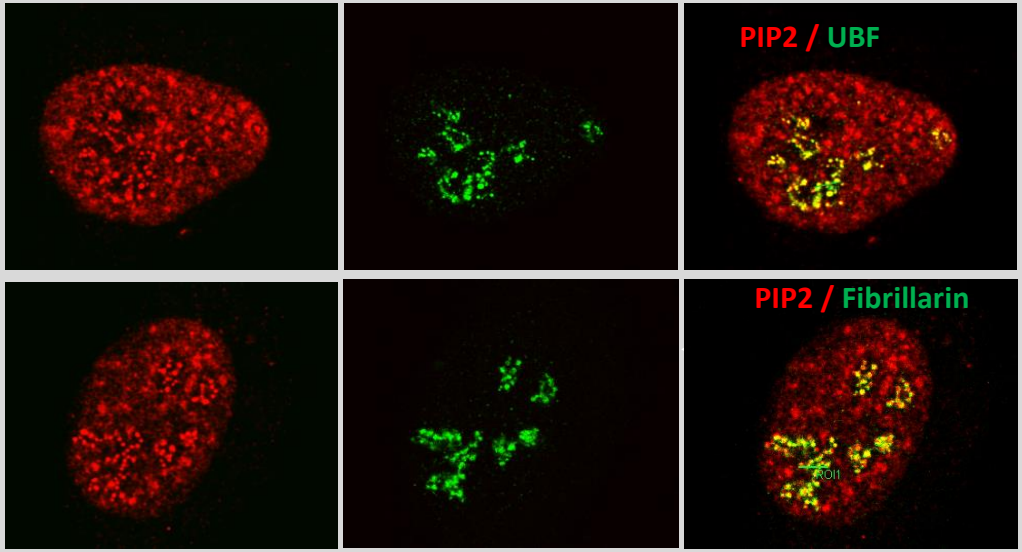
RNA Pol I	Threshold Ct Cycle Mean	Inhibition Fold
NE control	27.0±0.3	-
NE control+PIP2	26.6±0.6	~1
NEΔPIP2	30.3±0.3	~16
NEΔPIP2+PIP2	26.5±0.3	~1

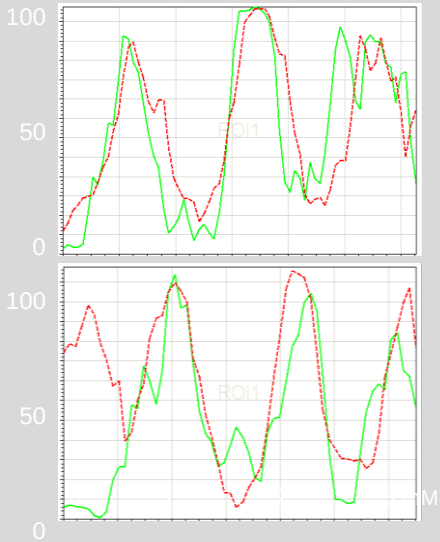
Yildirim et al., 2013, J Cell Sci

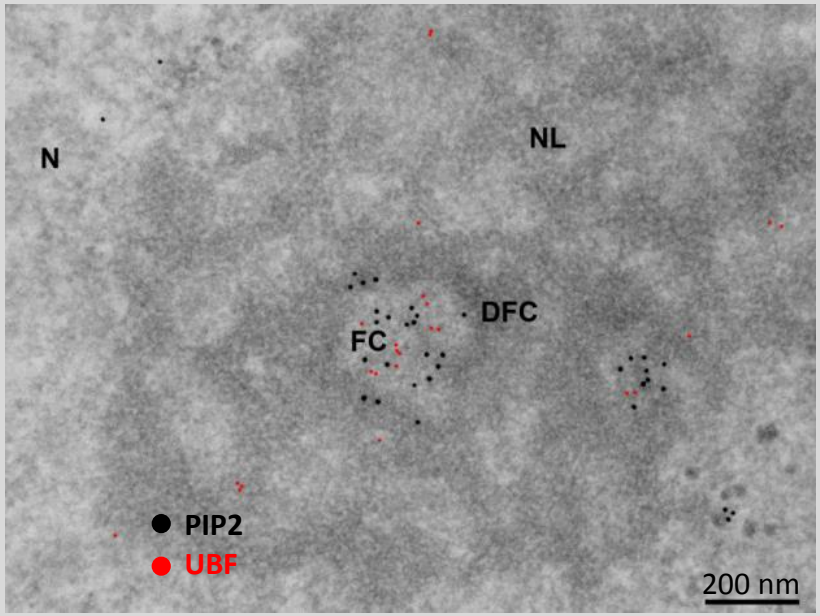
PIP2 makes complex with UBF and fibrillarin

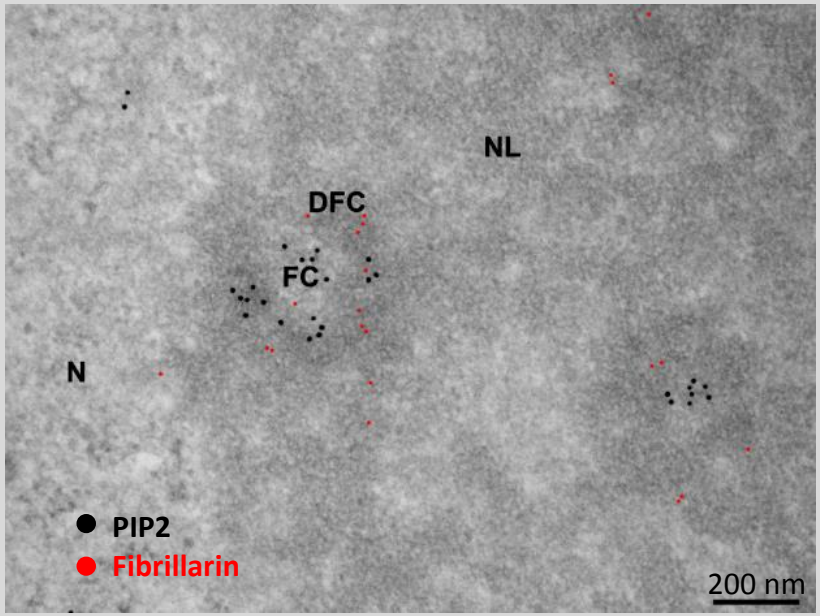
Pull-down via PIP2







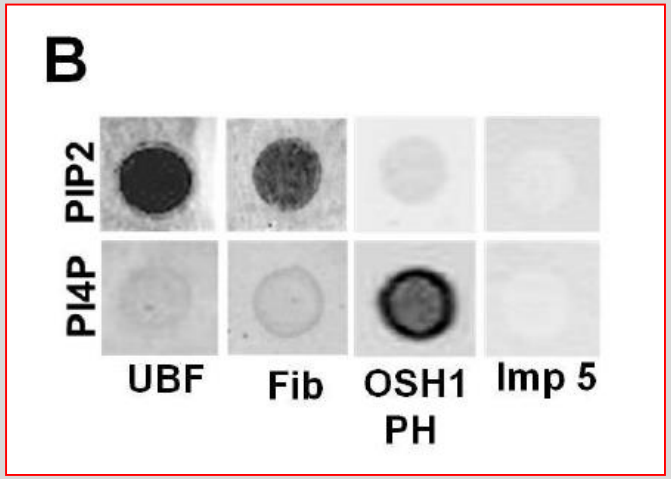




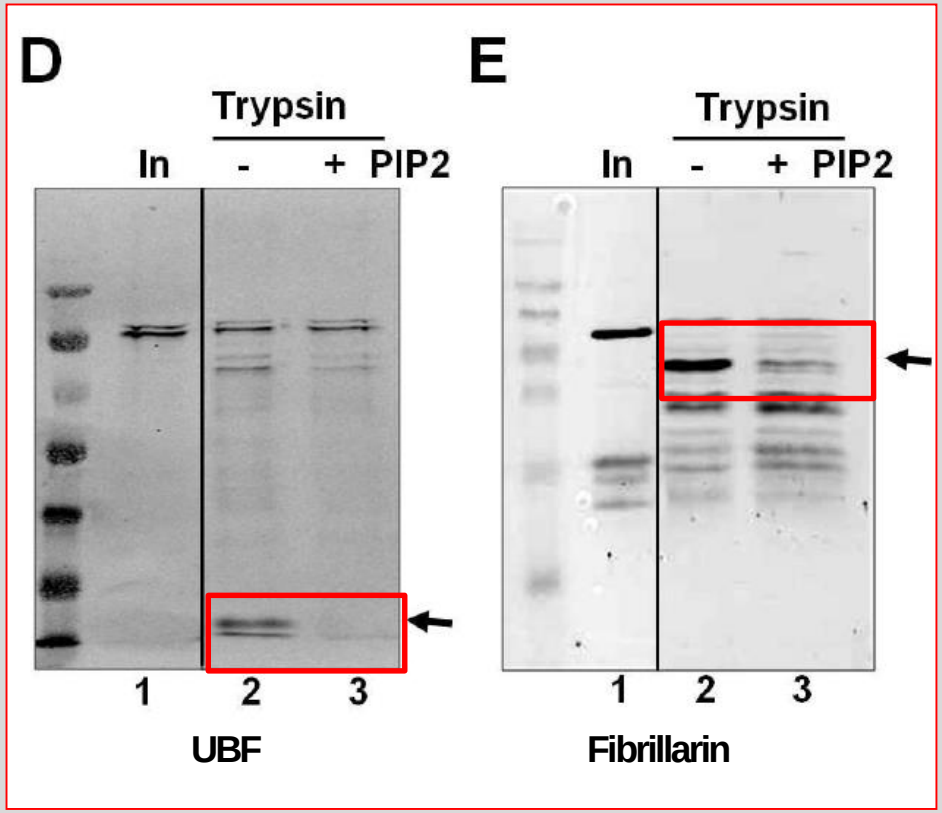
Yildirim et al., 2013, J Cell Sci

Direct binding of PIP2 leads to conformational changes in UBF and fibrillarin

Direct binding assay on membrane

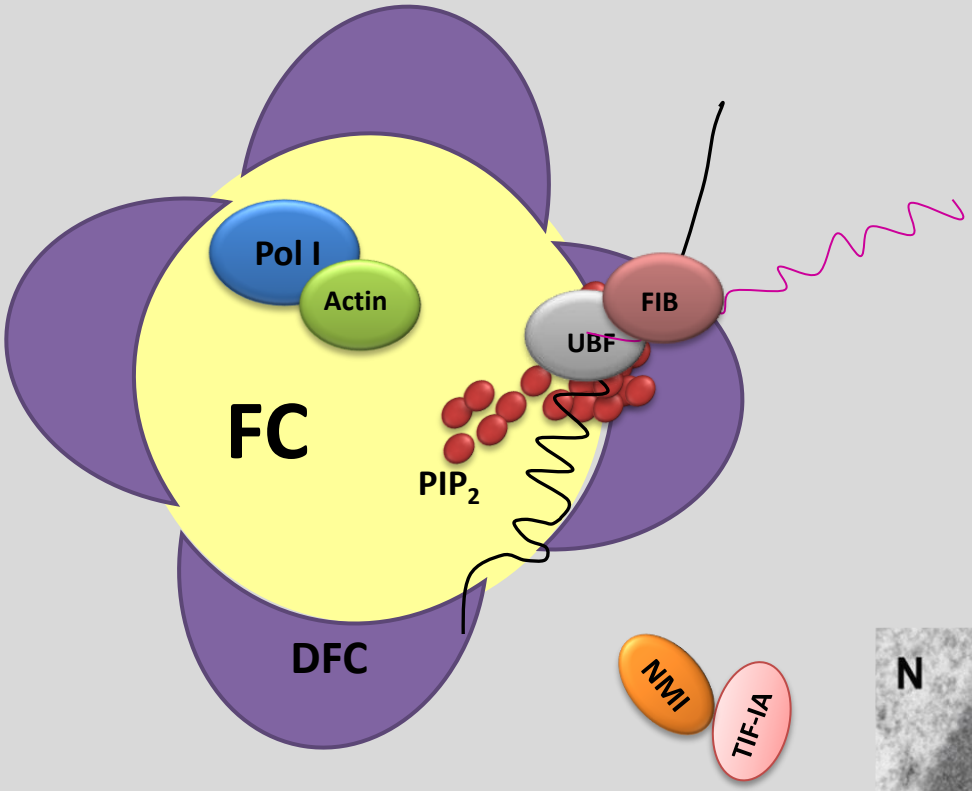


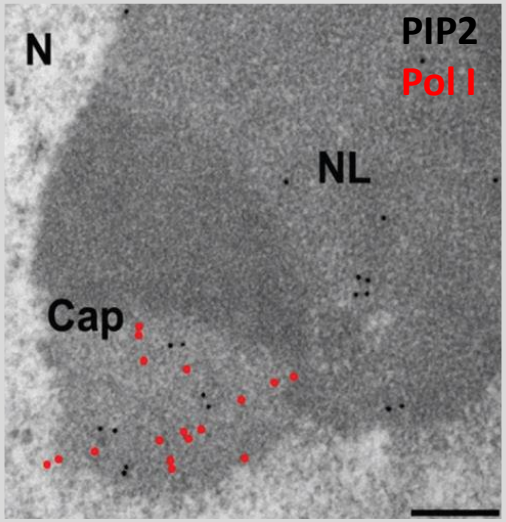
Limited protease digestion assay



Yildirim et al., 2013, J Cell Sci

PIP2-containing lipid islets may serve as anchoring points for NM1, UBF, and fibrillarin in RNA pol I transcription

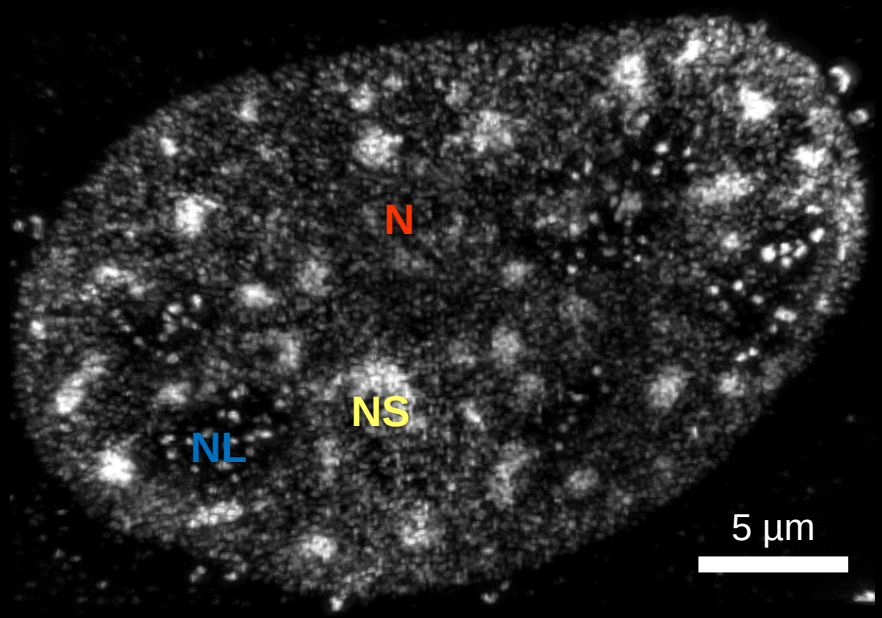


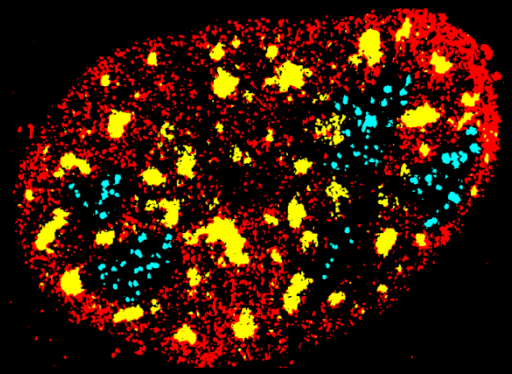


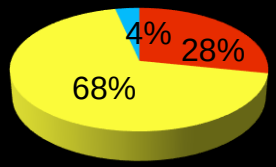
Sobol et al., 2013, Nucleus

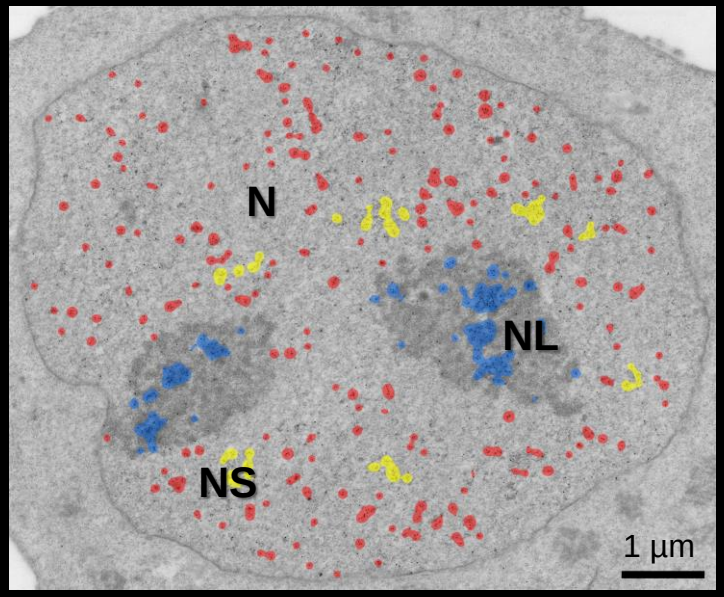
- PIP2 binding modifies conformation of proteins involved in rRNA synthesis and early processing
- PIP2 possibly participates in formation of nucleolar structure

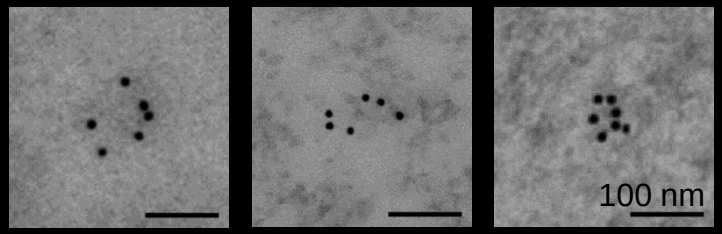
The nuclear pool of PIP2 is distributed among the nuclear speckles, nucleoli and islets









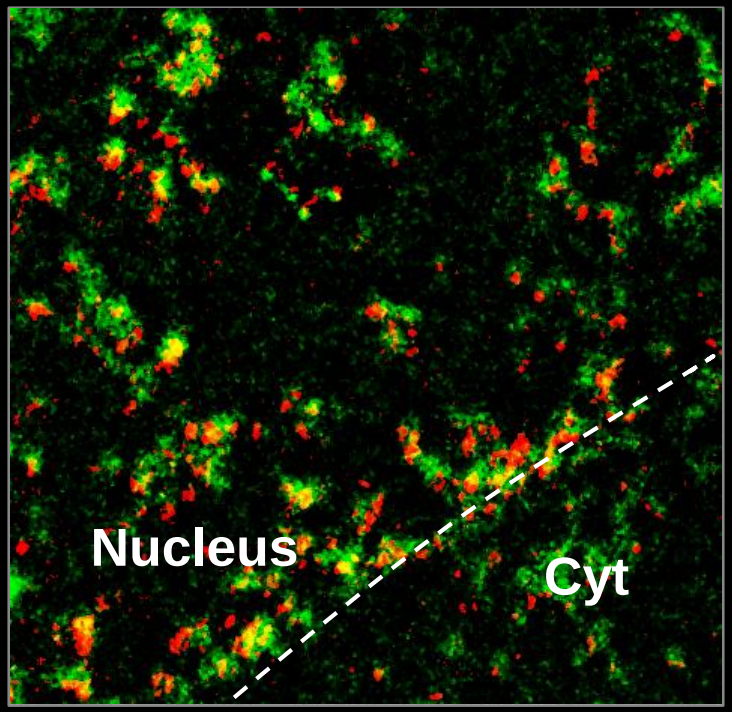


super-resolution SIM: PIP2 - labeling

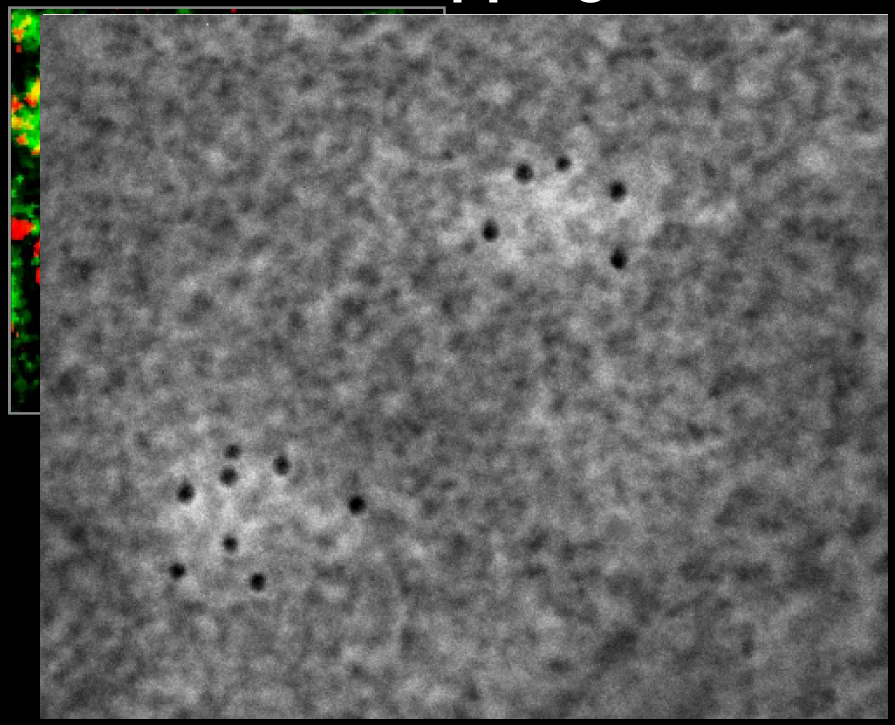
immunogold electron microscopy: PIP2 - labeling

PIP2 islets are lipidic structures surrounded by nucleic acids and proteins

P, N mapping



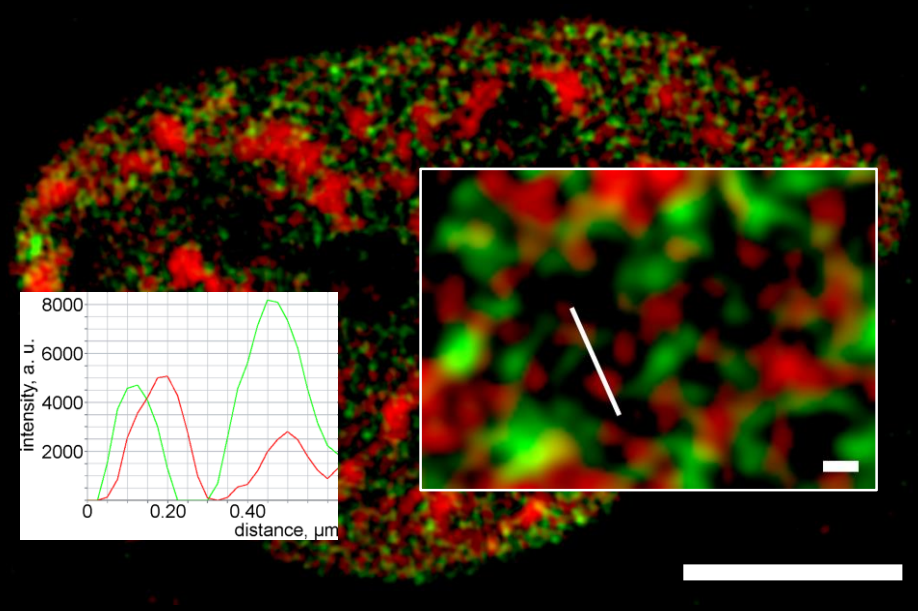
C mapping



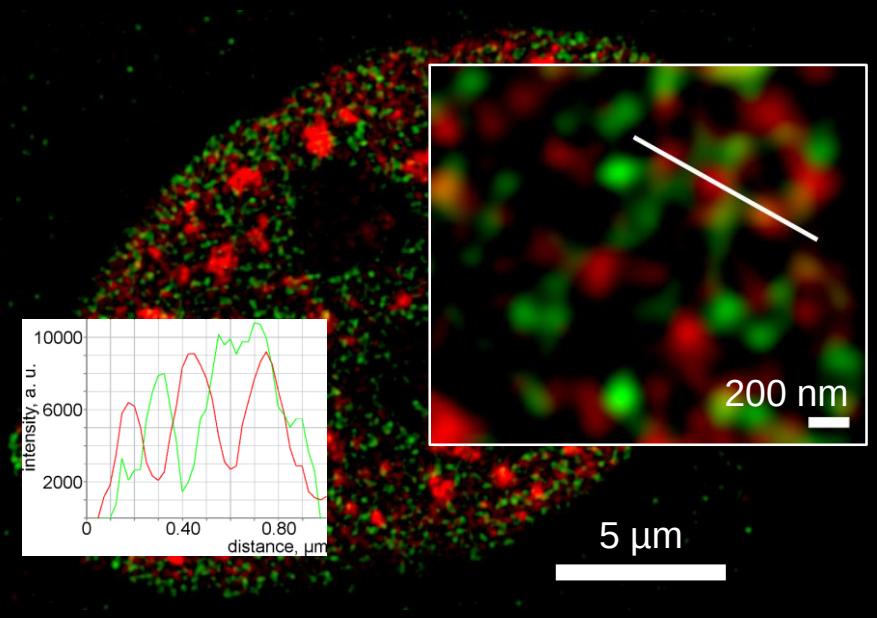
FEI TECNAI G2 20 LaB6 EM - EELS elemental mapping

PIP2 is located close to transcriptionally active genes marked by H3K4me2 and heterochromatin regions marked by H3K9me2

PIP2 H3K4me2

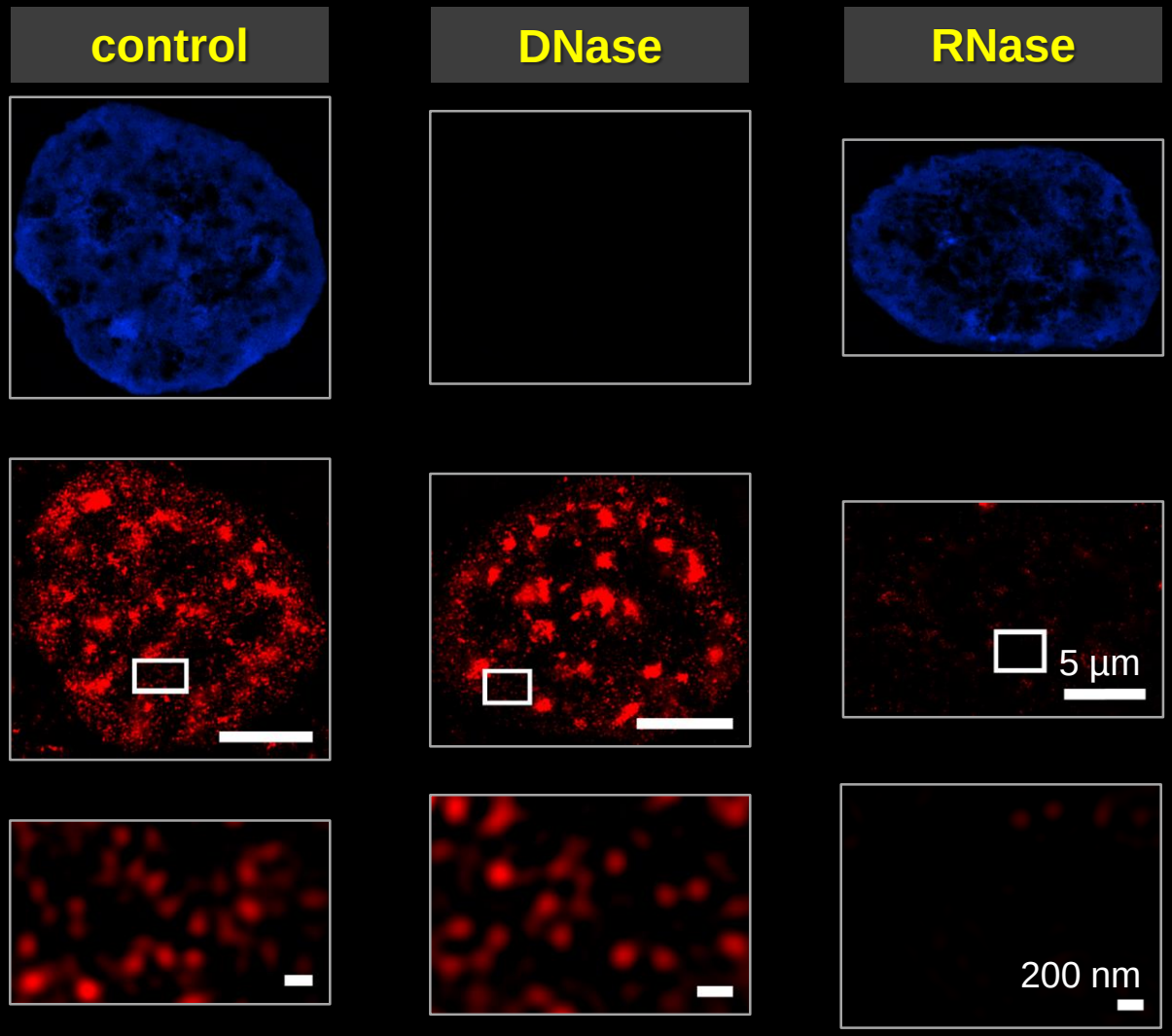


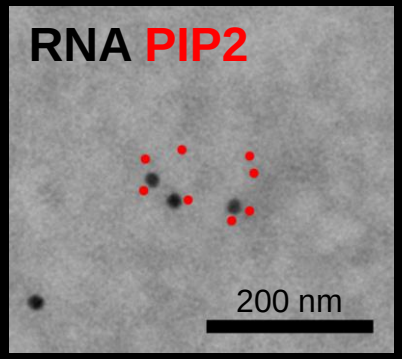
PIP2 H3K9me2

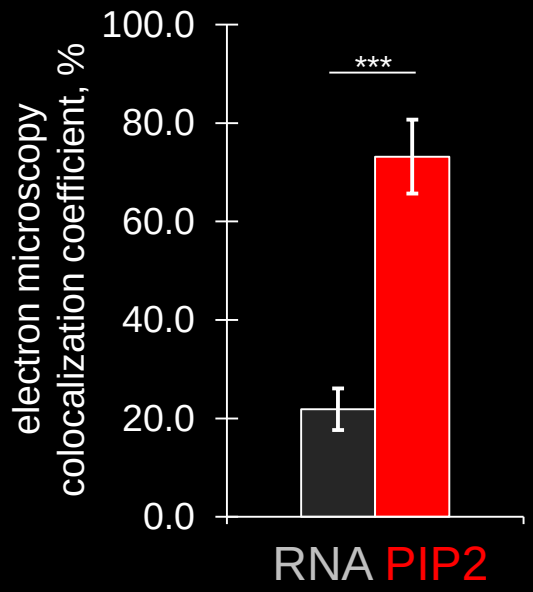


SIM

PIP2 islets are resistant to DNase treatment but are RNA-dependent





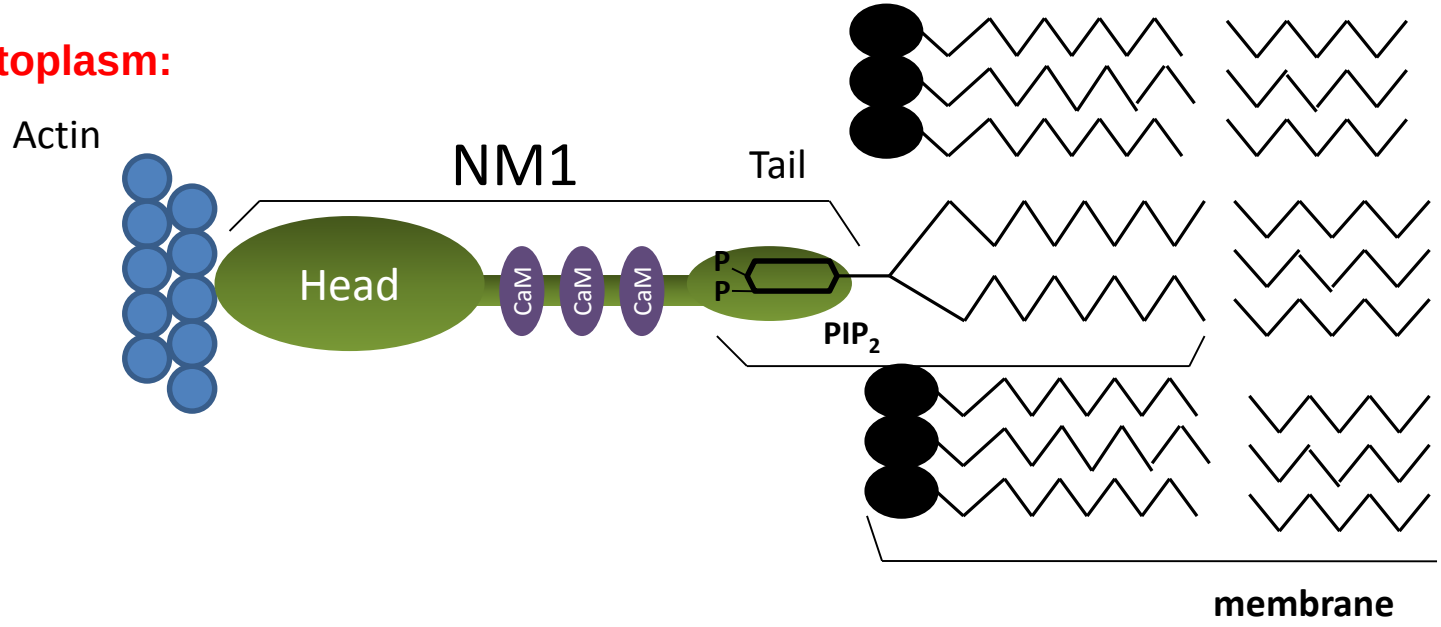


SIM: PIP2 DAPI

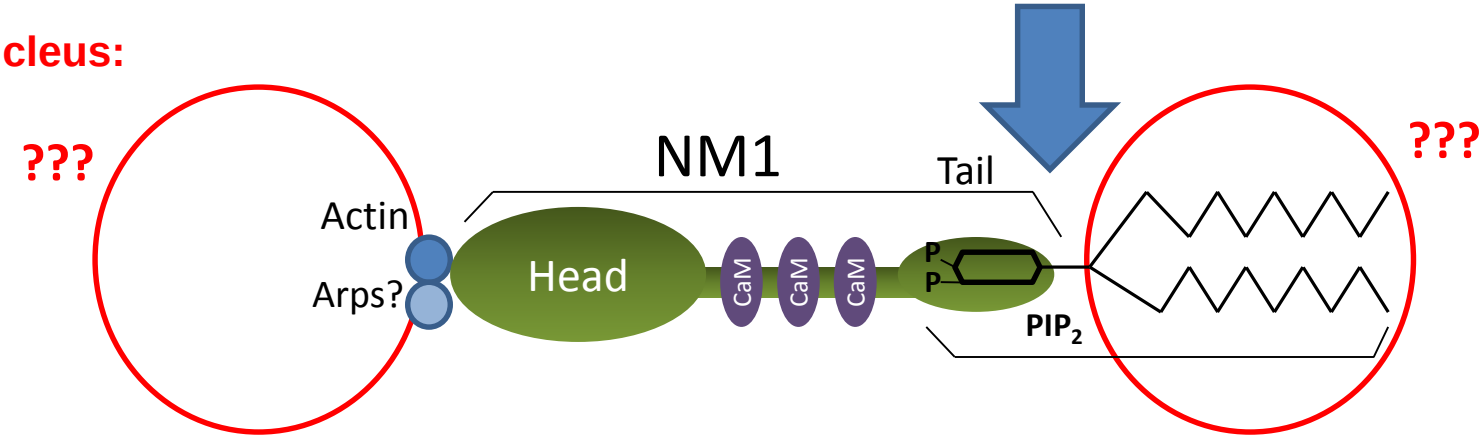
IEM

Binding partners of NM1-PIP_2?

In the cytoplasm:

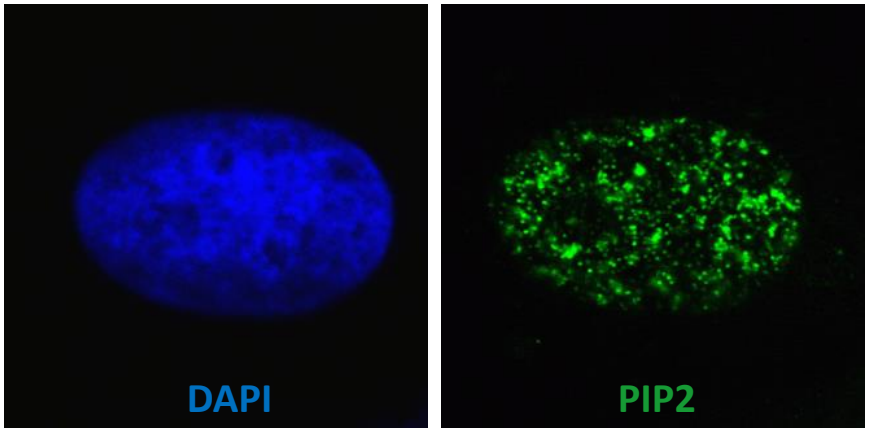


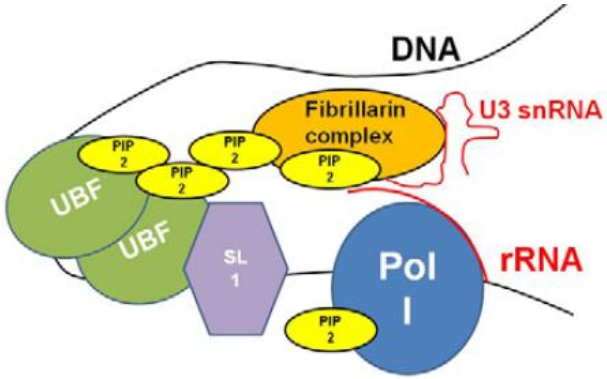
In the nucleus:



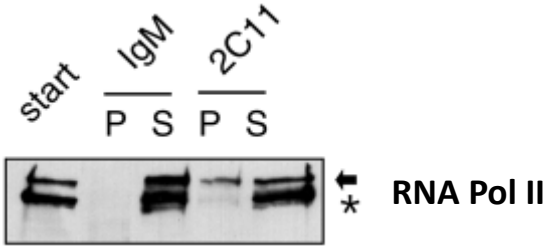
Phosphatidylinositol 4,5-bisphosphate (PIP2) (nucleus)

- localizes to the nucleus
- binds directly **Pol I**, UBF and fibrillarin
- binds hyperphosphorylated **Pol II**
- chromatin remodeling (BAF complex)
- splicing



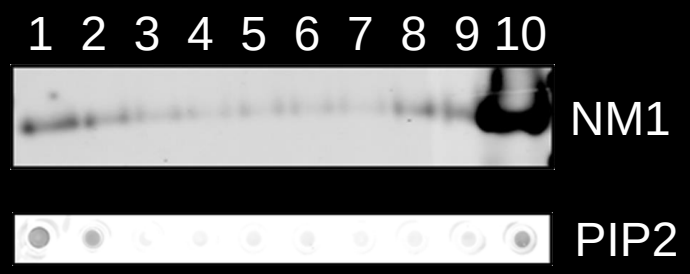


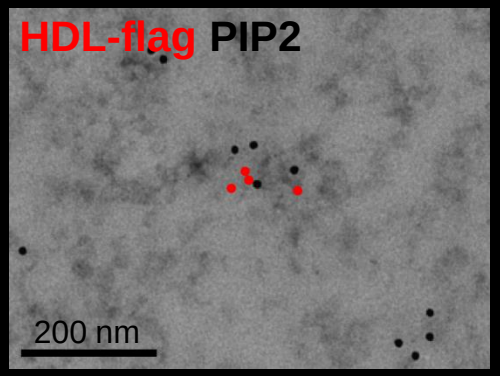
Yildirim et al., JCS, 2013

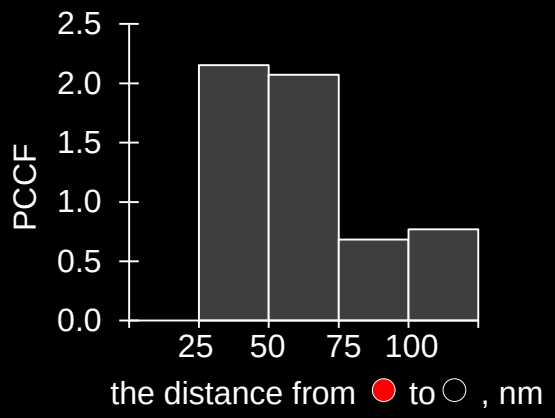


Osborne et al., JCS, 2001

PIP2 colocalizes with NM1 in nuclear lipid complexes



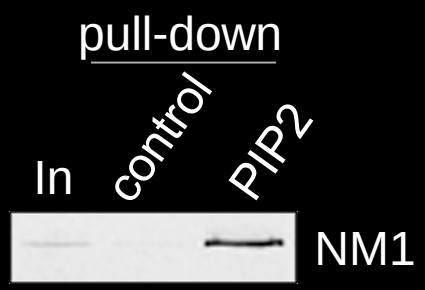




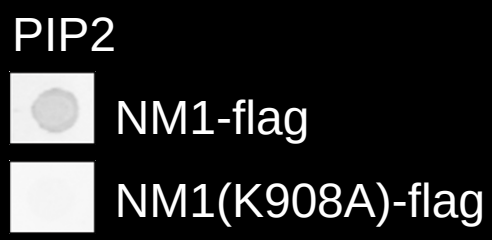
Nuclei extraction → flotation in sucrose density gradient

IEM

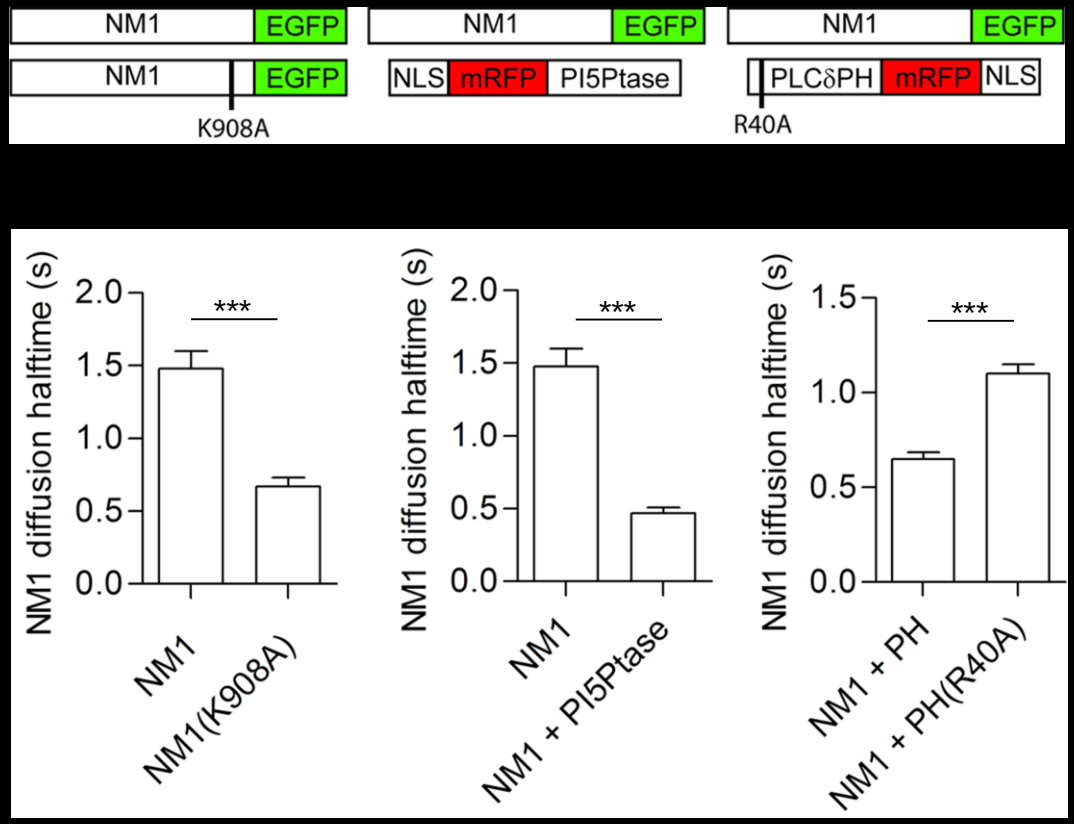
PIP2 anchors NM1 into larger complexes inside of the cell nucleus



pull down

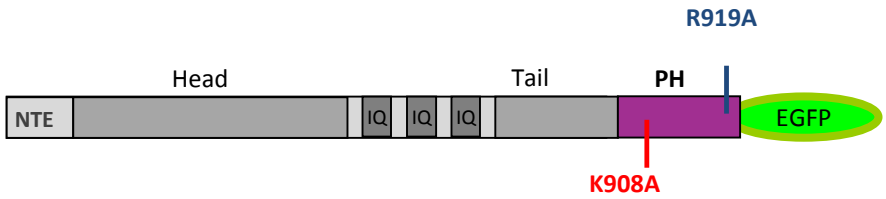


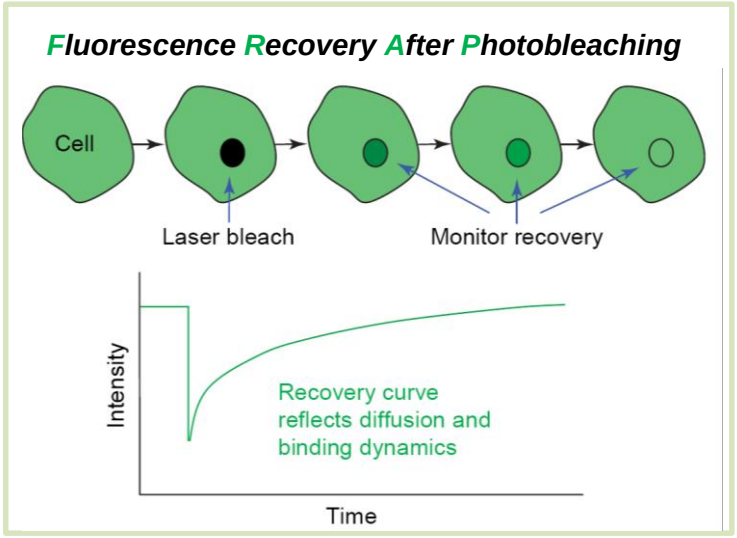
dot blot

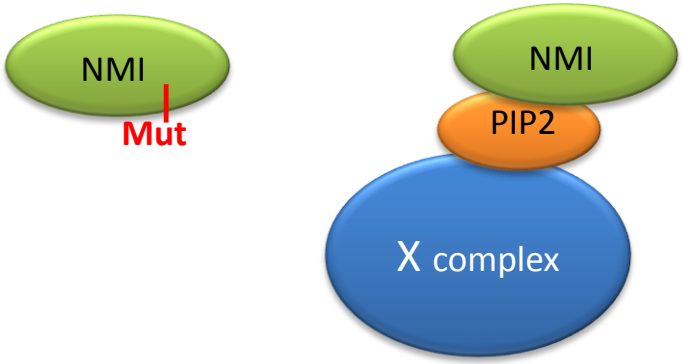


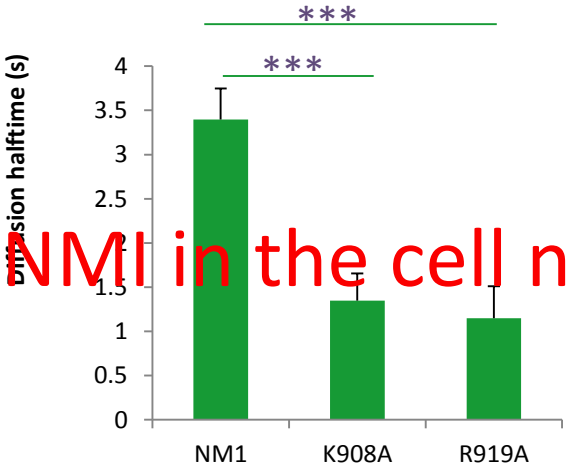
FRAP

NM1 mobility in the cell nucleus is restricted by PIP2 binding



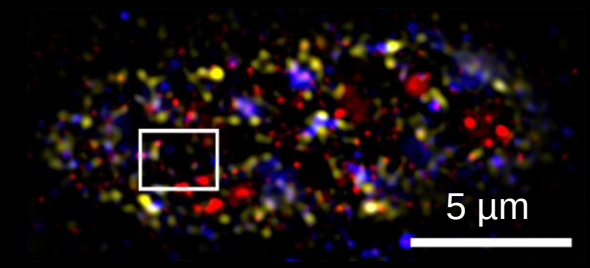


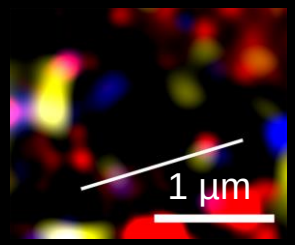


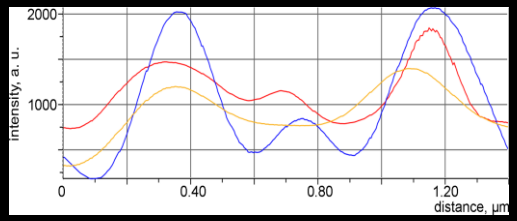


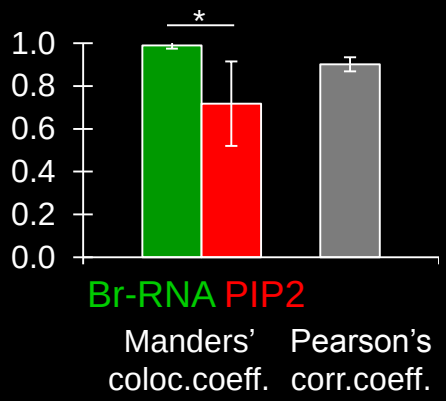
FCS, FRAP: PIP2 anchors NMI in the cell nucleus

Colocalization between PIP2 molecules and nascent RNA transcripts is significant at the distance of 25 - 75 nm

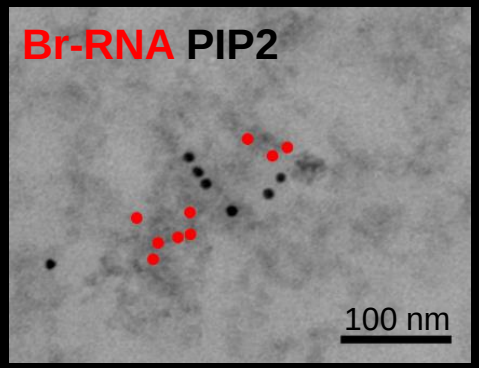


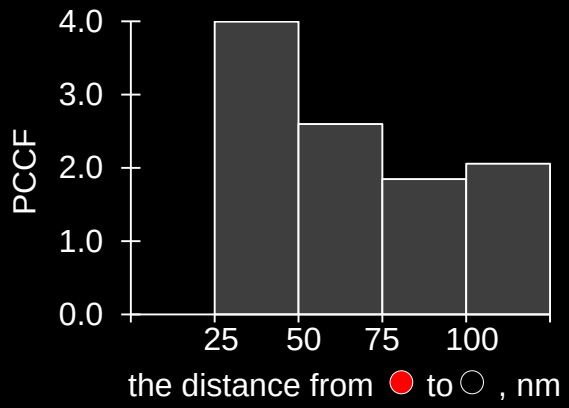


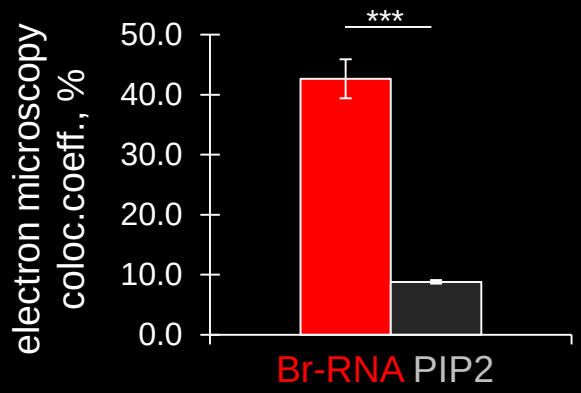




SIM: Br-RNA PIP2 RNA Pol II CTD



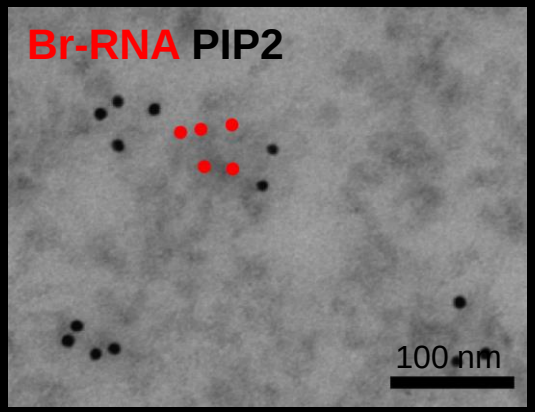




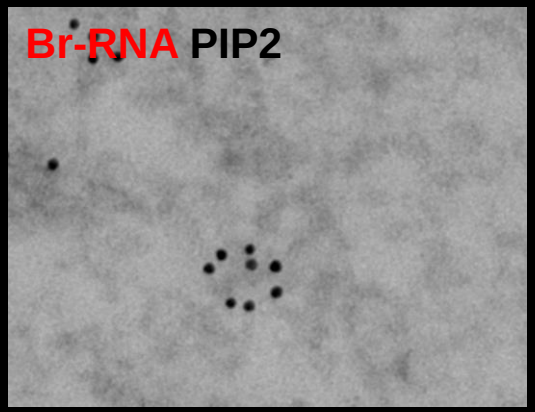
IEM: BrUTP incorporation

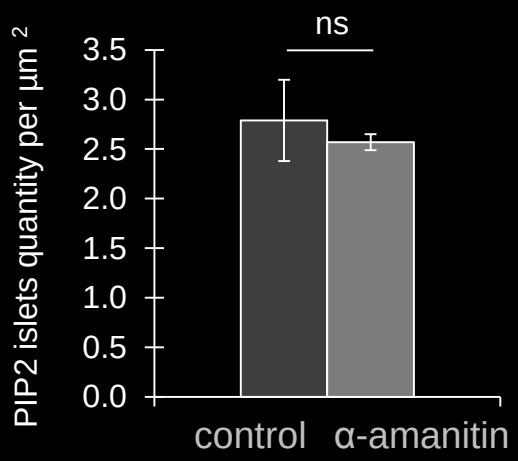
Inhibition of Pol II transcription does not affect the quantity and the pattern of PIP2 islets

control



α-amanitin

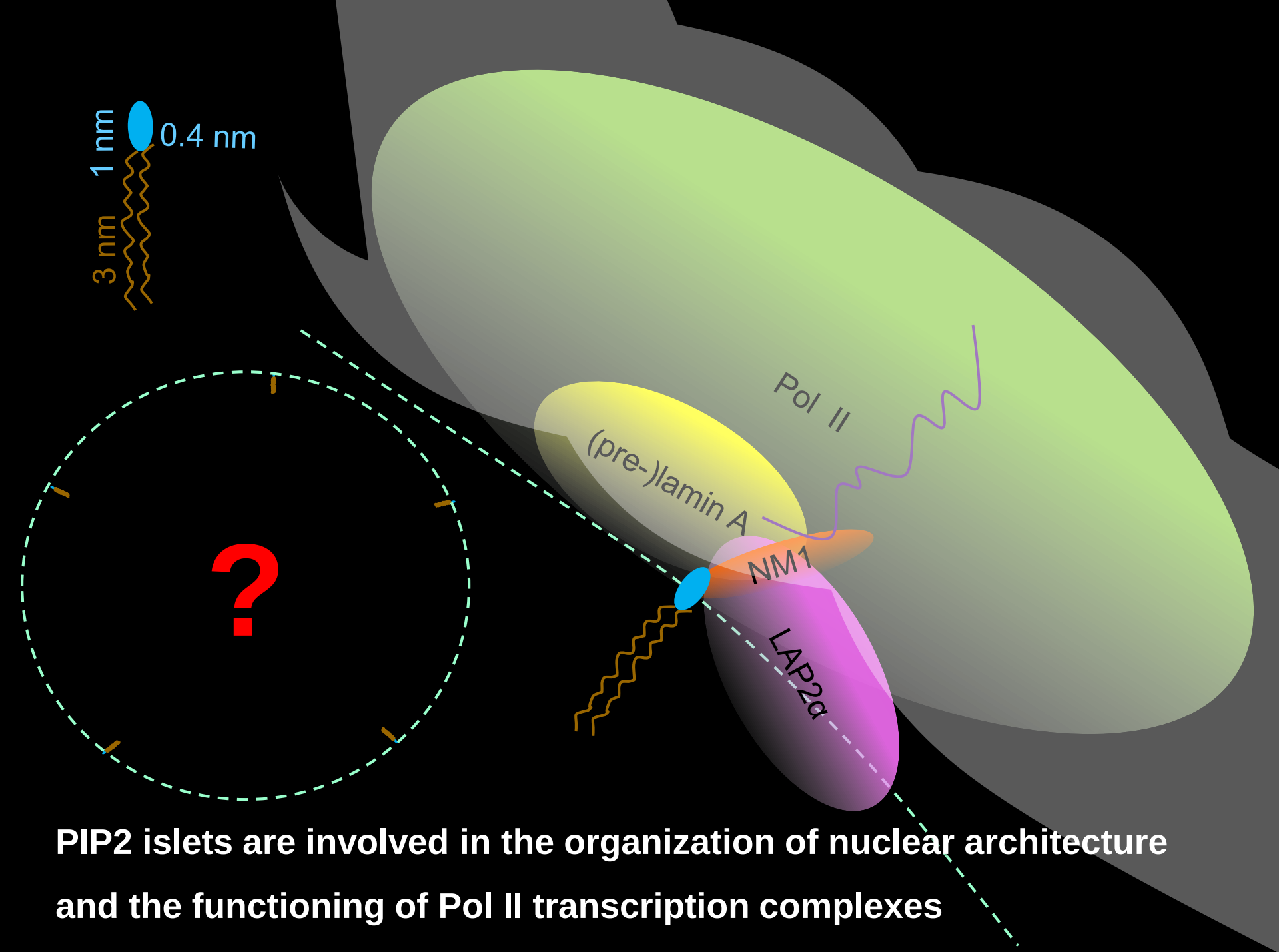




IEM: BrUTP incorporation

CONCLUSIONS

- PIP2 islets – novel significant structures of cell nuclei
- attached structural proteins involved in chromatin remodeling
- attached active/inactive chromatin, Pol II transcription complexes and nascent transcripts
- disruption of PIP2 islets reduces level of transcription by Pol II
- NM1/Myo1c and PIP2 co-fractionates with active CTD
- interaction between NM1/Myo1c and PIP2 is required for efficient Pol II transcription *in vivo*



PIP2 islets are involved in the organization of nuclear architecture and the functioning of Pol II transcription complexes

Acknowledgements

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