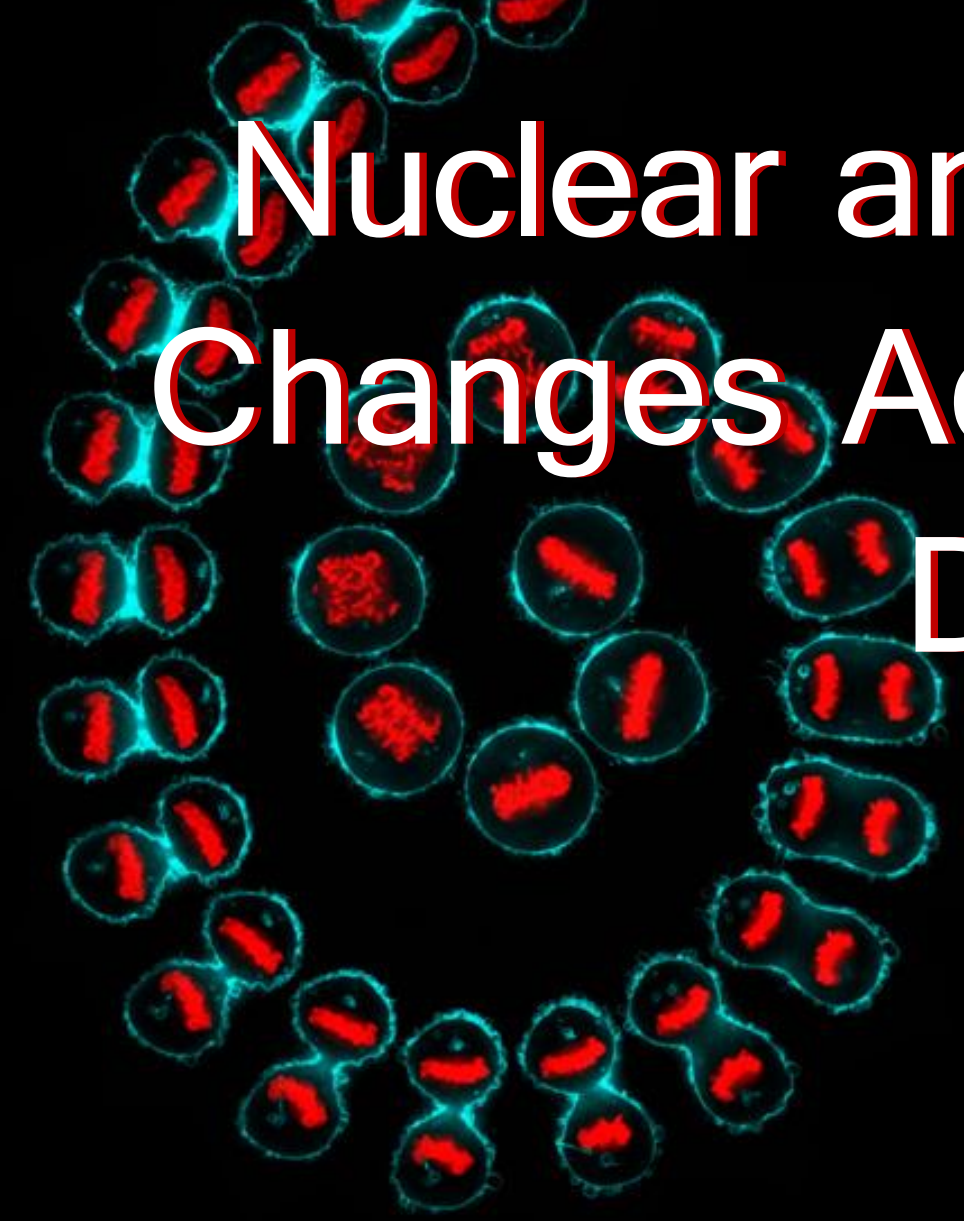


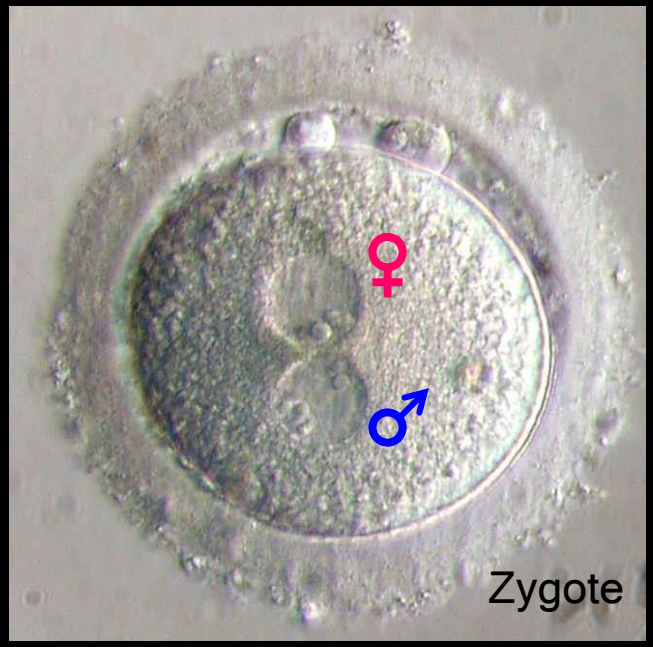
Nuclear and Chromatin Changes Accompanying Differentiation

Rui Pires Martins, PhD

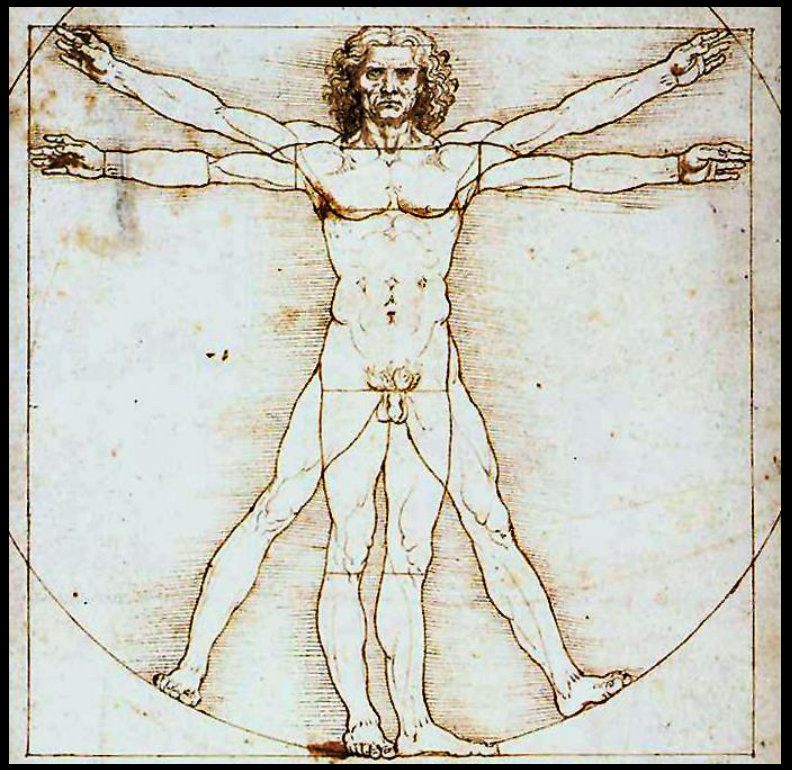
Queen Mary
University of London

Outline of today's talk...

- Brief introduction to:
  a. development/differentiation
  b. mechanotransduction
- The nucleus, from the inside-out.
- Chromatin archetecture
- Changes to chromatin and nuclear architecture that accompany differentiation in ES cells (including original work)

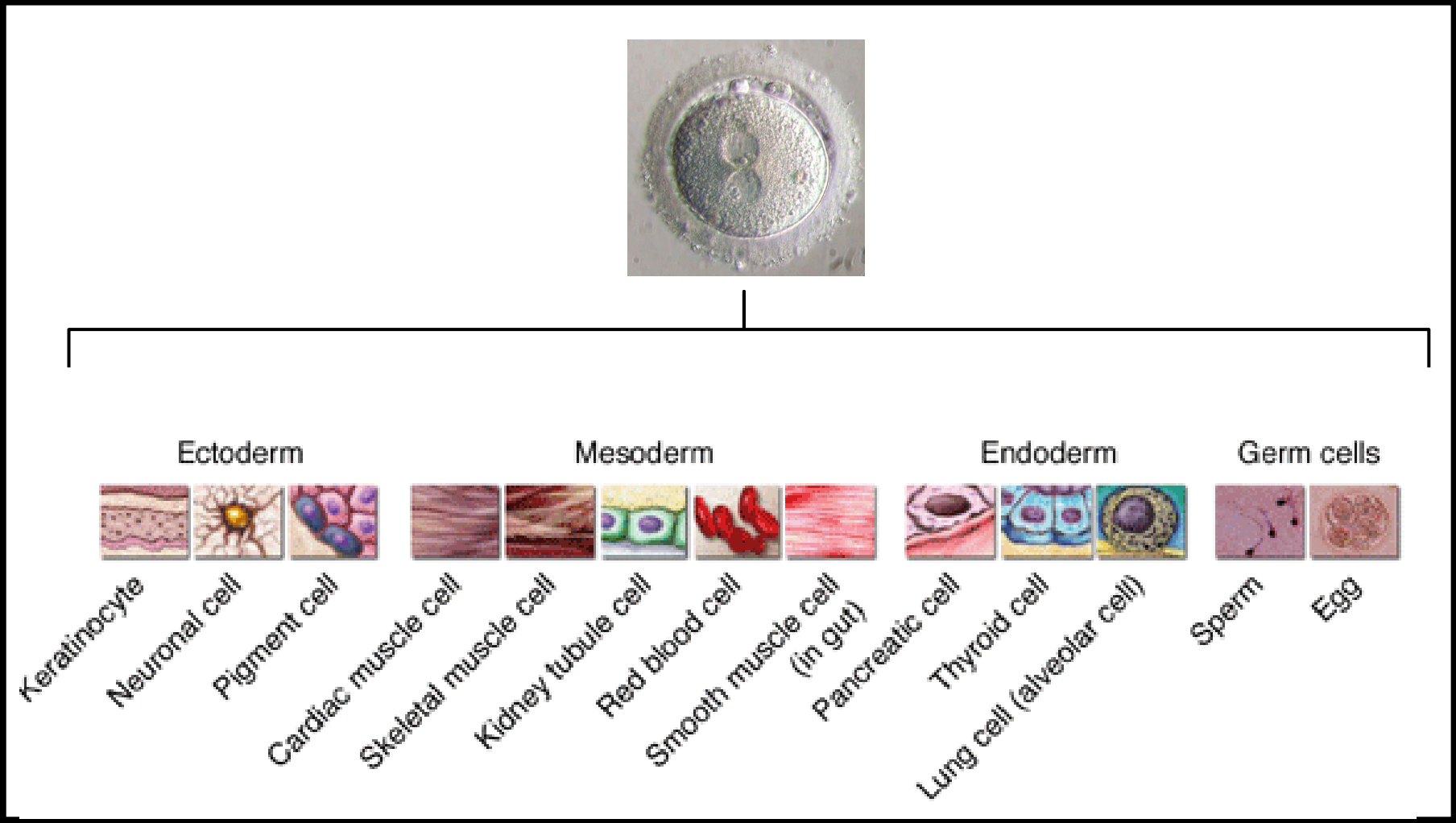


1 cell



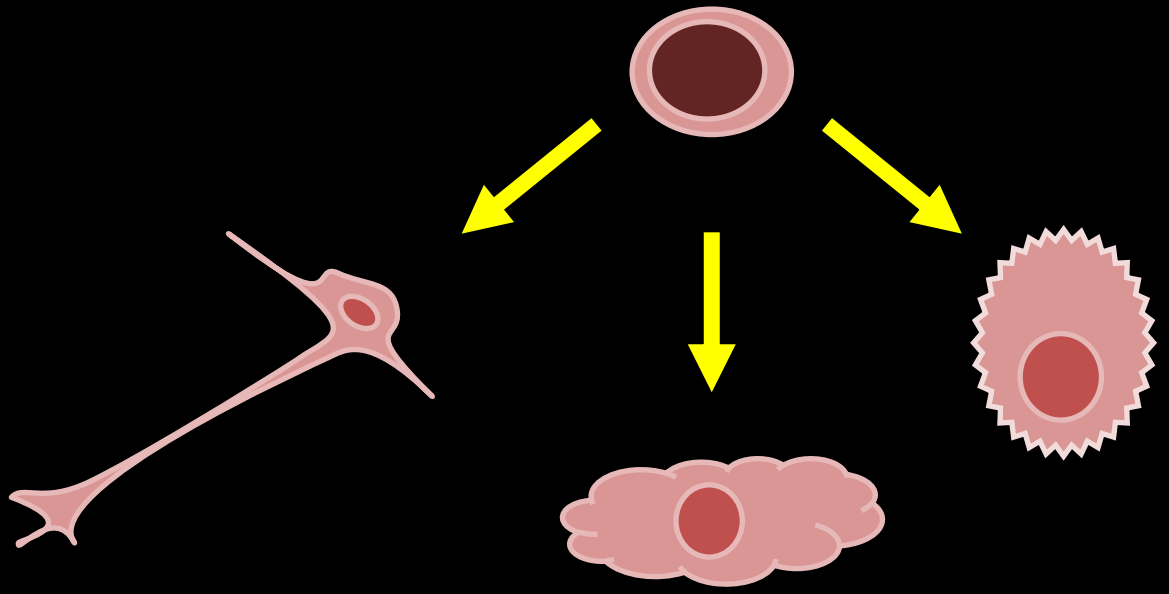
≈50-100 trillion (10^{14}) cells

There are potentially hundreds to thousands[1] of different cell *fates* or types in the Human Body

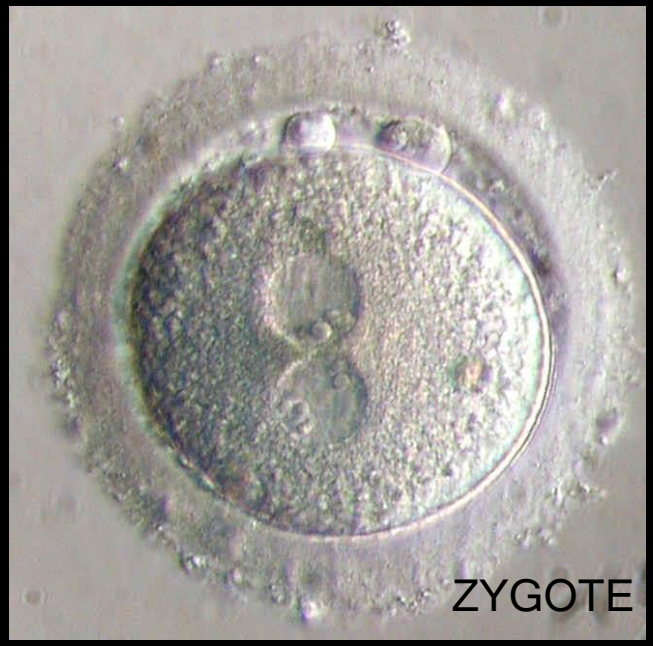


1. Cytokines & Cells Online Pathfinder Encyclopaedia. (http://www.copewithcytokines.de/cope.cgi?key=cell%20types)

Differentiation
is the progression from a naïve or unspecialised cellular state, to a more specialised state.

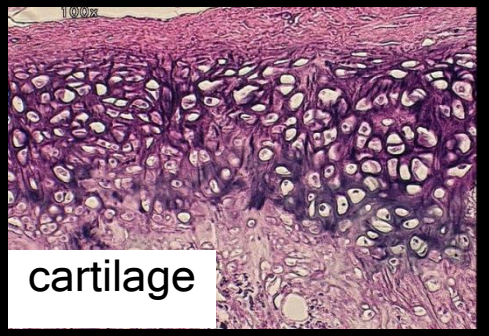


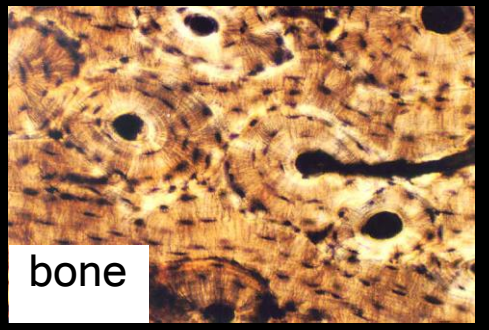
Potency
describes the developmental potential of a cell. Stem cells that can adopt multiple fates are said to be **pluripotent**.

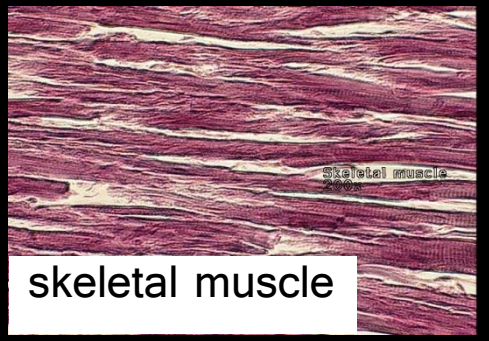


DIFFERENTIATION

REPROGRAMMING







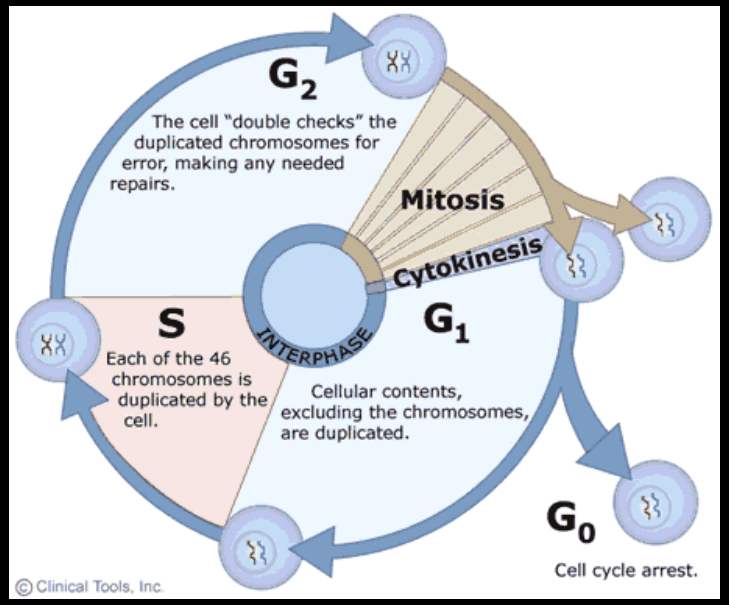
D E V E L O P M E N T A L P O T E N T I A L C O N T I N U U M

TOTIPOTENCY/
PLURIPOTENCY

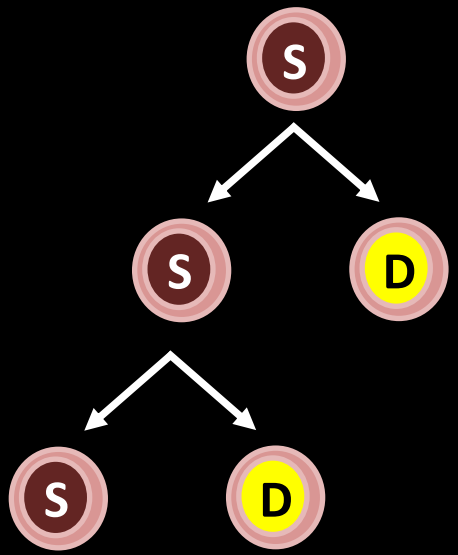
TERMINAL
DIFFERNTIATION

Self-renewal EXPAND

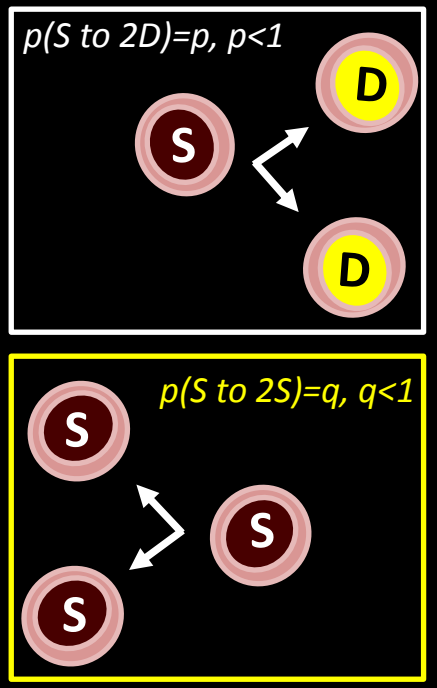
Embryos begin as a single cell and develop into multicellular organisms. Through mitotic cell division, cell numbers can expand exponentially, but self -renewal ensures that a stem cell population is maintained.



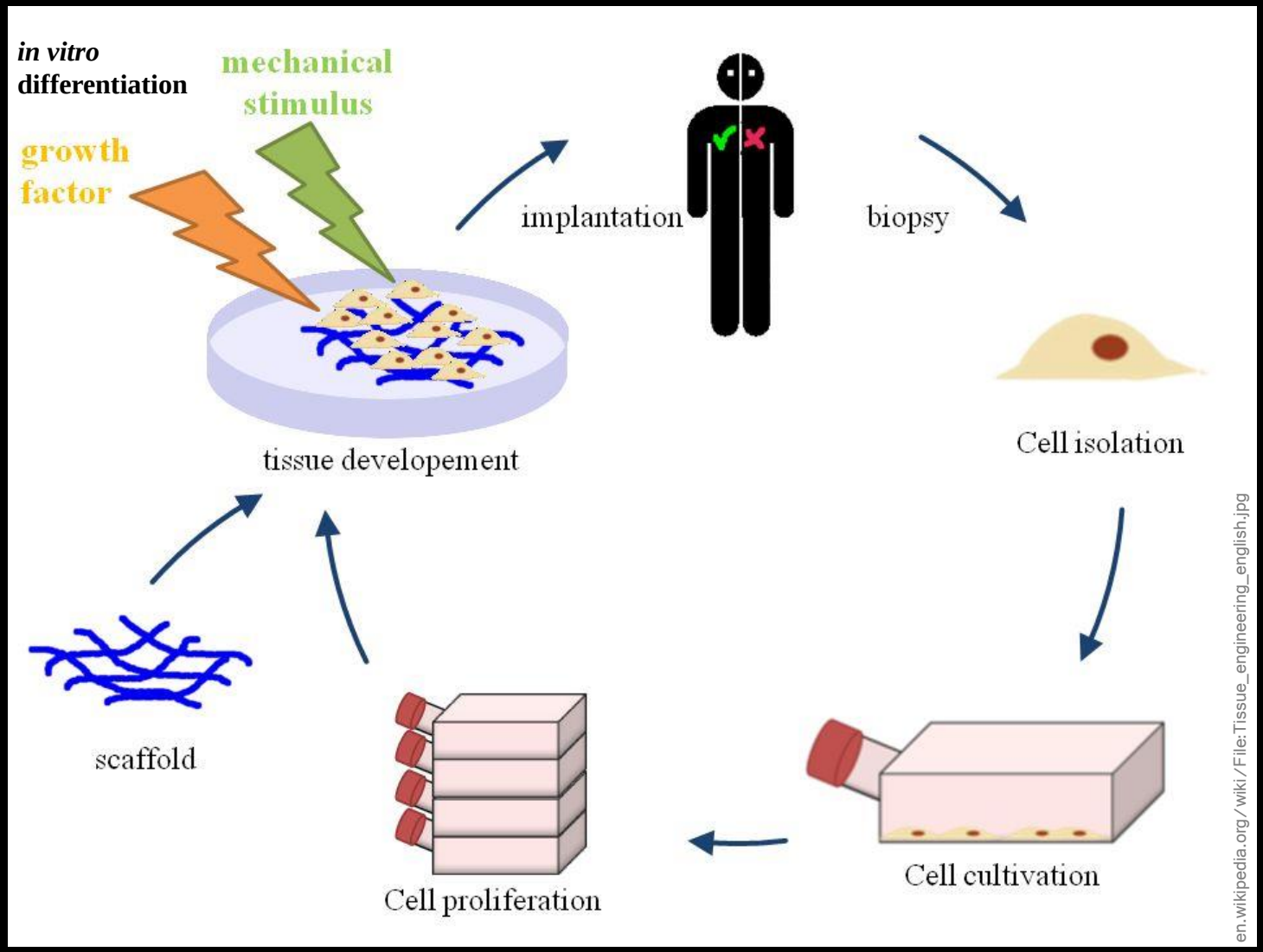
Asymmetric Division



Stochastic Mechanisms

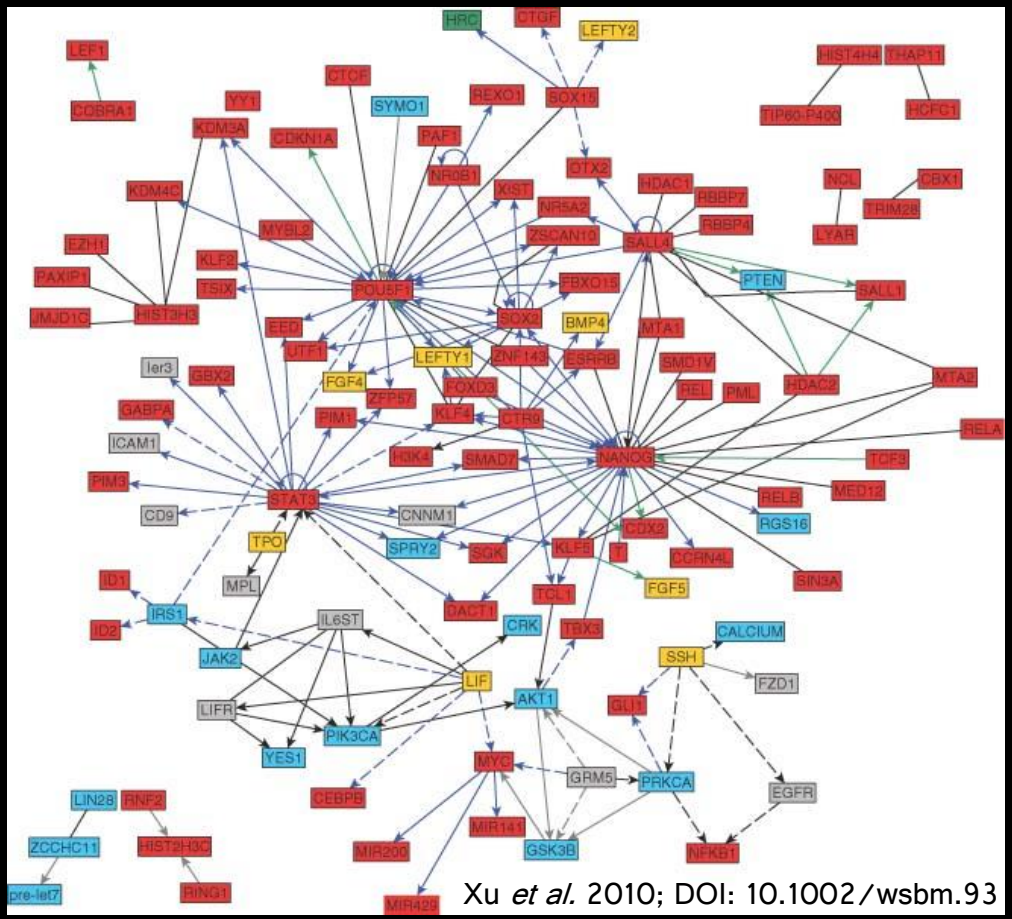


tissue engineering work-flow



Complex genetic/protein interaction pathways that govern the maintenance of pluripotency in stem cells are well characterised.

PLURIPOTENCY IN MESCs



Xu *et al.* 2010; DOI: 10.1002/wsbm.93

HOWEVER, how mechanical signals feed into these and other differentiative circuits are very much open questions.

Mechanotransduction

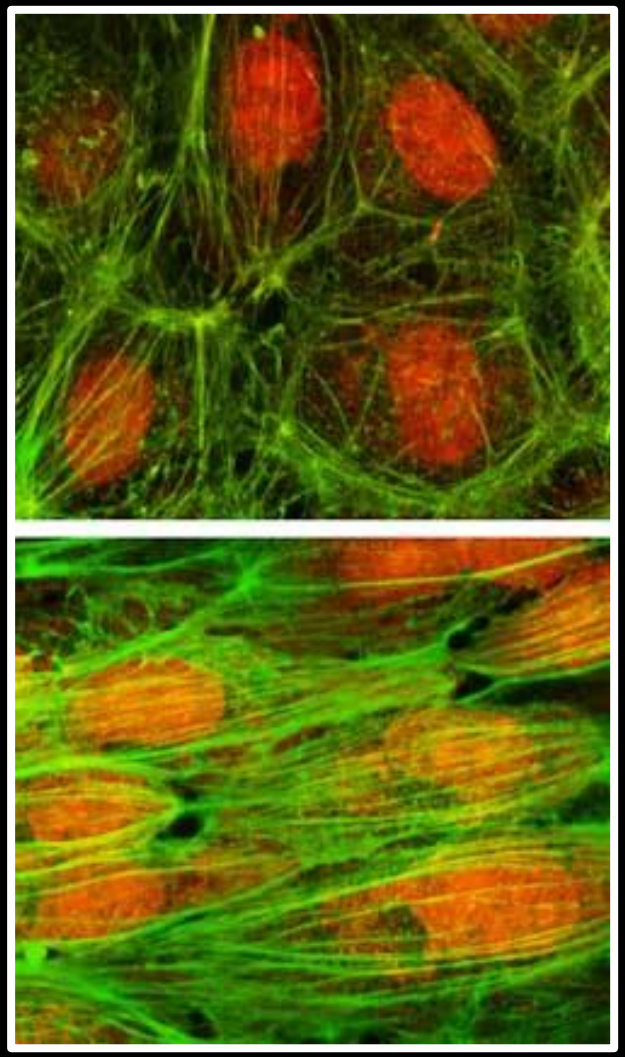
The process by which mechanical stimuli are converted to intracellular signals that control cellular physiology, and disease.

Sheer force on endothelial cells lining vessel walls

The force of blood flowing past an endothelial cell promotes changes in cell morphology: cells reorient their cytoskeletons in the direction of the blood flow, and cells send chemical signals to neighbouring endothelial cells that result in changes to local vessel stiffness.

Mechanotransduction may alter cellular physiology by stimulating an ion channel (e.g. Ca^{2+}) or activation of cellular signalling through cell surface receptors (e.g. integrins in the cell membrane)

Mechanical forces can also be transmitted along the various elements of the cytoskeleton on through to the nucleus.



Cytoskeleton (Actin), Nuclei (DNA)

Theodor and Marcella Boveri

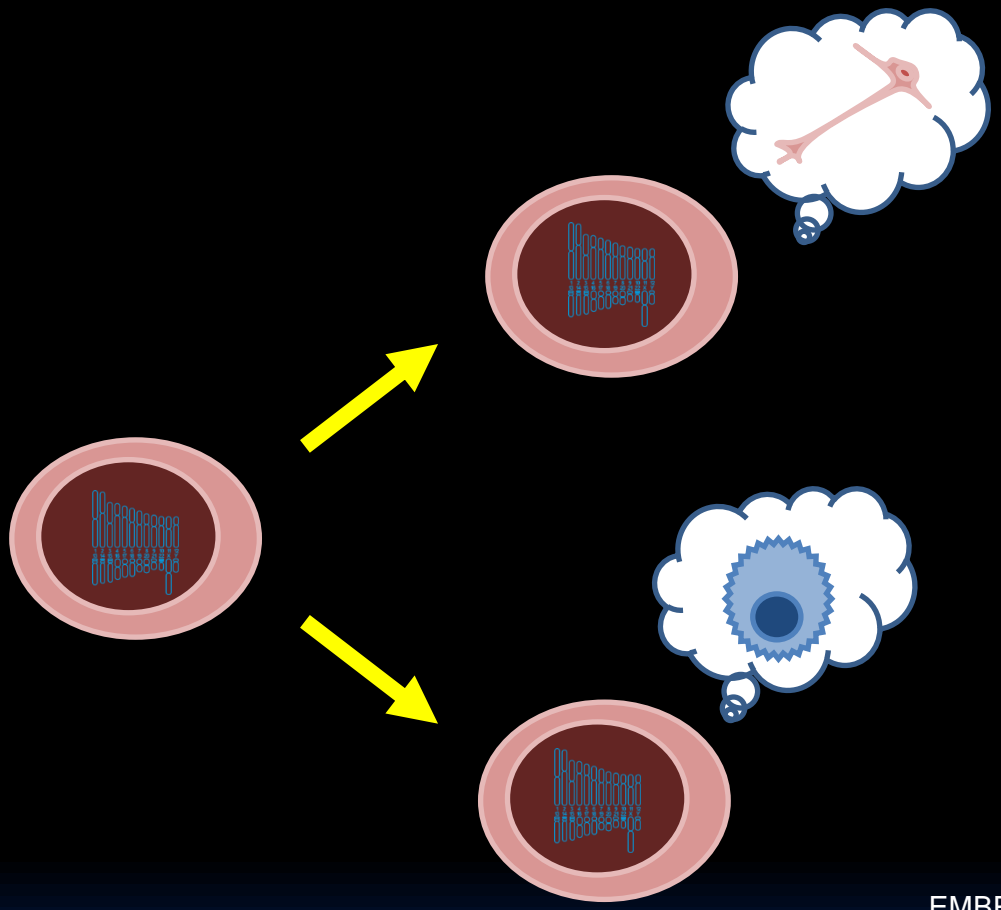
German and American cell and developmental biologists who worked in the late 1800s and early 1900s

The Boveris' seminal work in roundworms and sea urchins helped confirm the nucleus, and indeed chromosomes as the source of heredity.

The prevailing wisdom governing development and differentiation was that only germ cells received a complete set of chromosomes as these needed to reproduce the whole organism. Differentiated cells of other lineages only received the genetic information pertinent to their function.

The Boveris' Chromosome Theory demonstrated that there was equal (genomic) contribution between parents (at fertilisation) and that all, not just a subset of the chromosomes were transmitted from germ cells to somatic cells during differentiation.

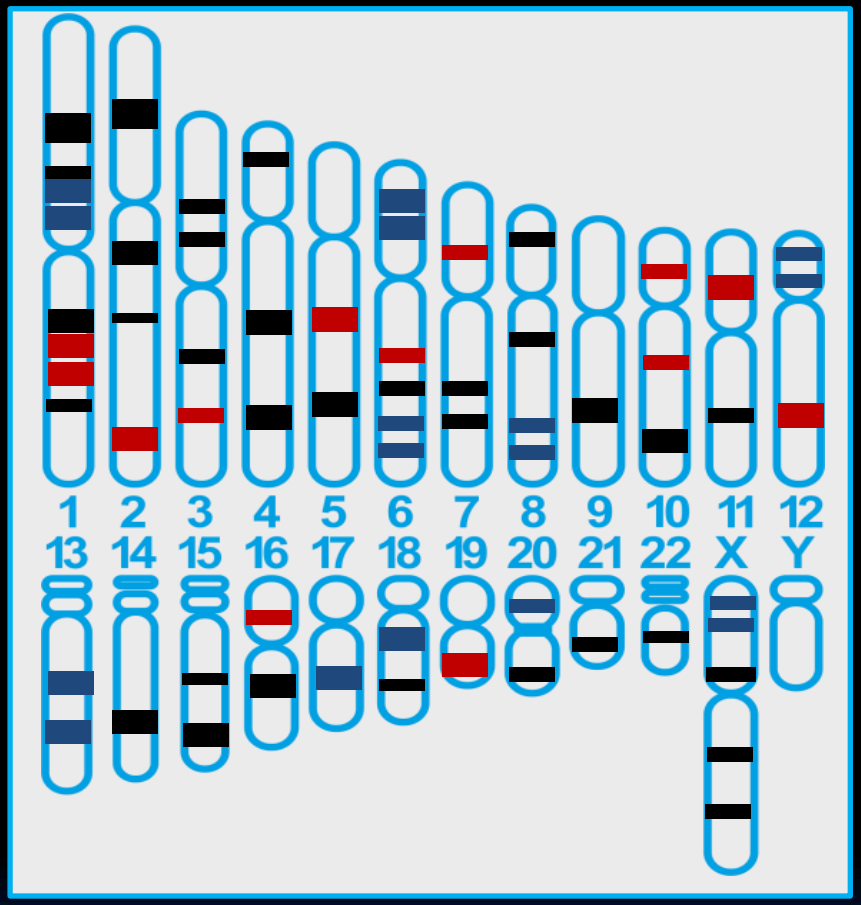
Fundamantally, differentiation operates at a genetic level. Genomically, cells in the body are clonal. What *differentiates* one cell type from another is the how each type utilises that genome.



Humans have 23 pairs of chromosomes, one inherited from each parent.

These are composed of 22 *autosomes*, and two sex chromosomes, X and Y.

These chromosomes code for approximately 21+K genes. That are arranged enigmatically through the genome. This is only 1% of the total information coded within.



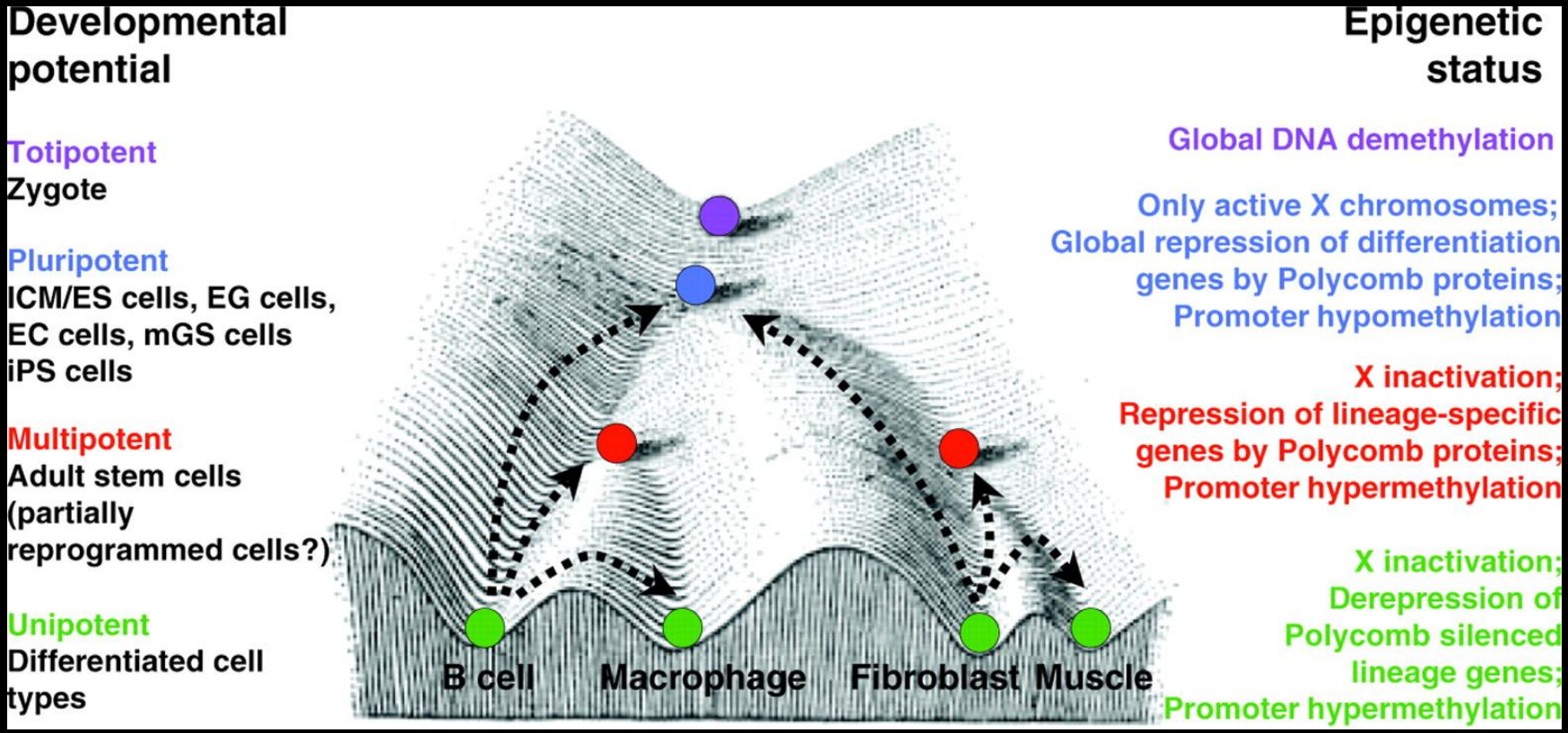
Housekeeping Genes: govern basic cell function, structure and metabolism. These generally function in ALL cell types.

Lineage Specific or Determinant Genes: genes that impart *fate* ie. Turn on a genetic programme or contribute form or function to a cell lineage

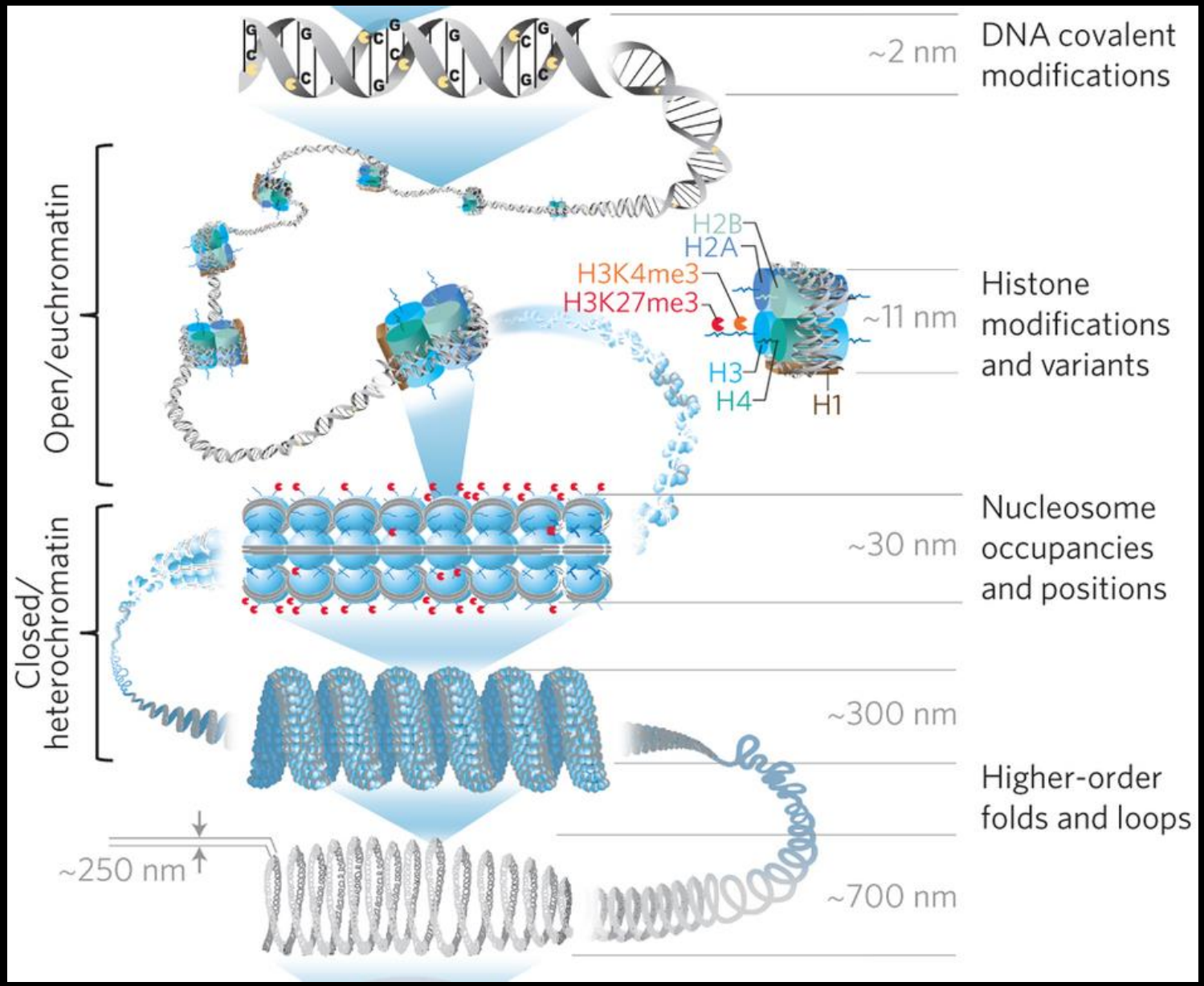
Waddington's Canalisation Concept that cell fate is progressively fixed/restricted by epigenetics

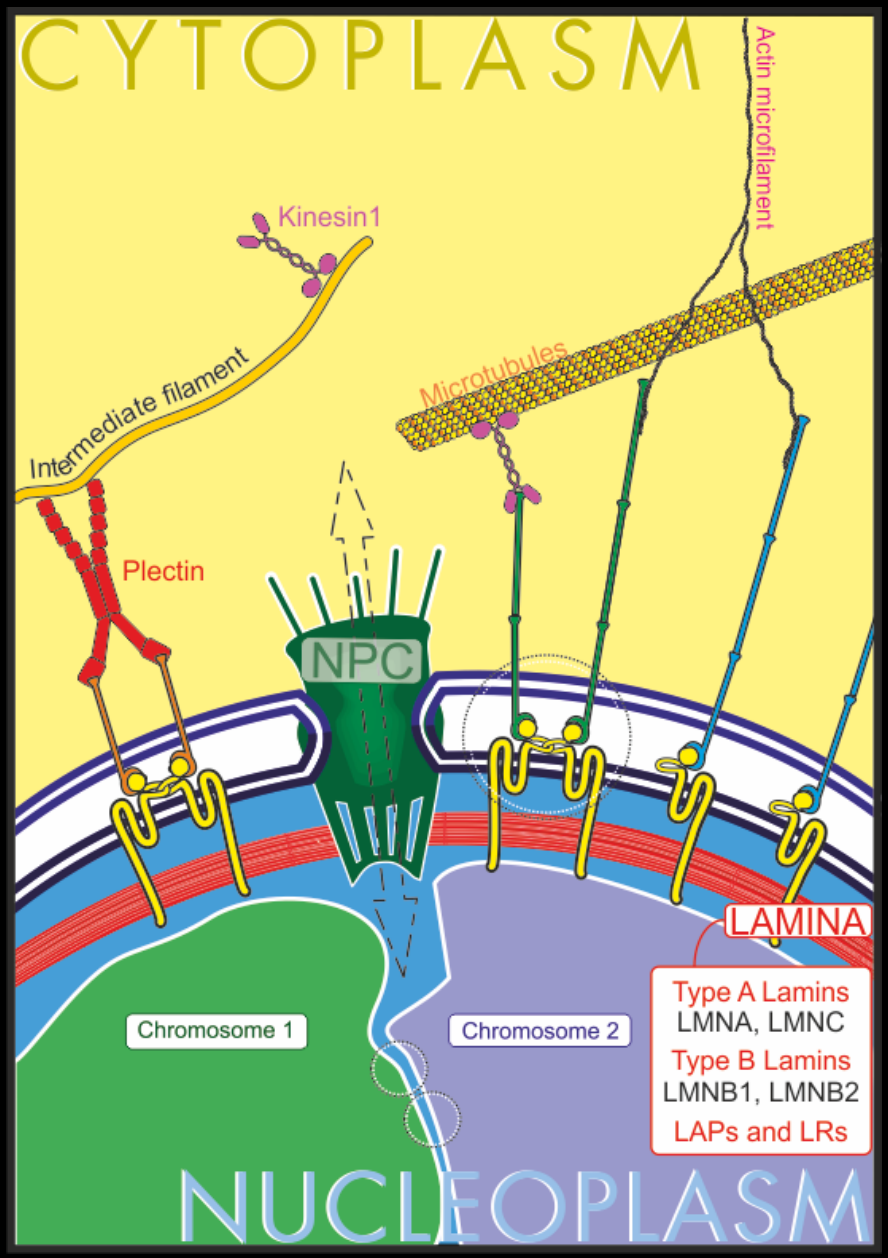
TOTIPOTENCY / PLURIPOTENCY

TERMINAL DIFFERNTIATION



Hochedlinger and Plath 2009; DOI:10.1242/dev.020867


~2 nm
DNA covalent modifications
Open/euchromatin
H2B
H2A
H3K4me3
H3K27me3
~11 nm
Histone modifications and variants
H3
H4
H1
Closed/ heterochromatin
~30 nm
Nucleosome occupancies and positions
~300 nm
Higher-order folds and loops
~250 nm
~700 nm



The Nucleus

Is the largest compartment of the cell, it houses the genome (chromatin/chromosomes) and is the site of all genomic transcription.

It is surrounded by the nuclear envelope, composed of:

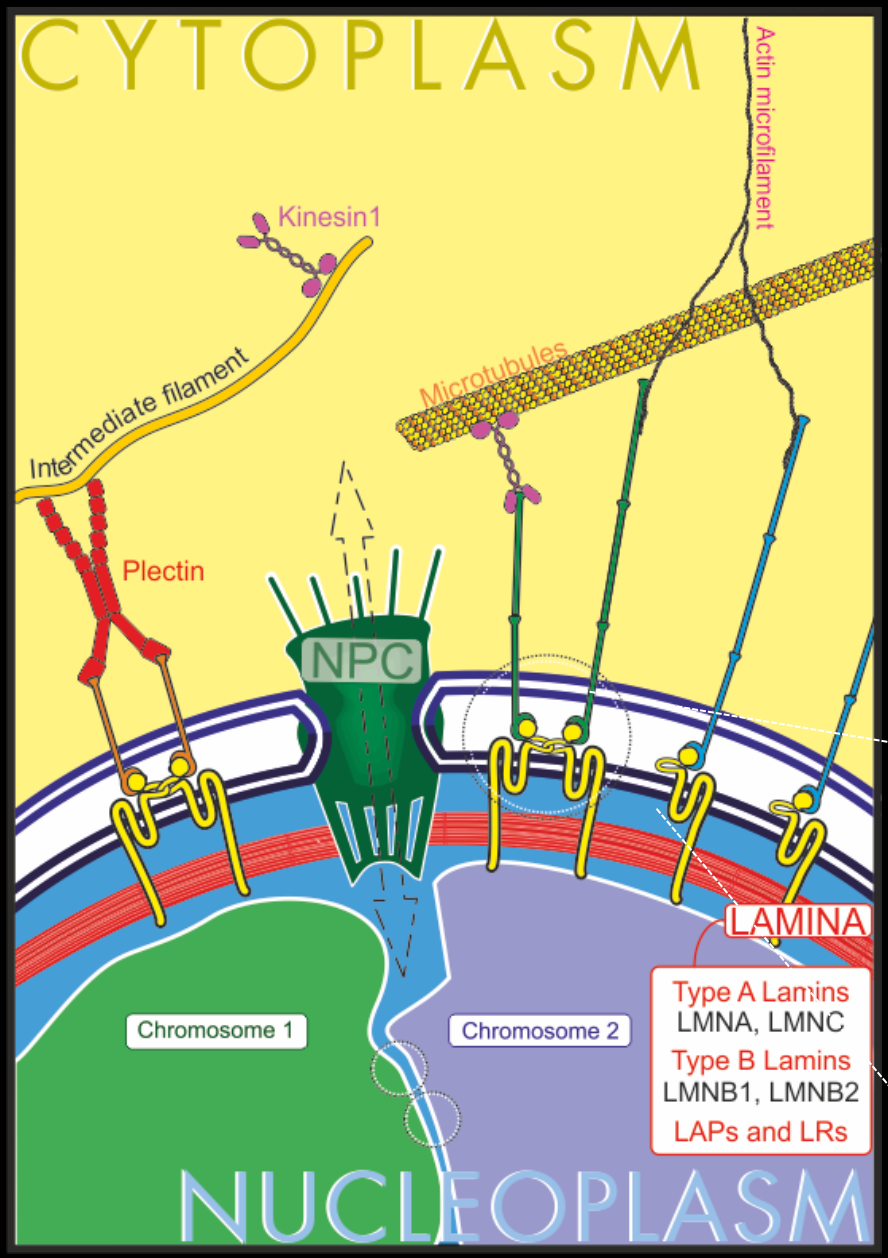
- The nuclear membrane, semi-permeable double lipid bi-layer
- large nuclear pore complexes control trafficking in and out of the nucleus
- the nuclear lamina, the major support structure of the nucleus, composed of intermediate filaments

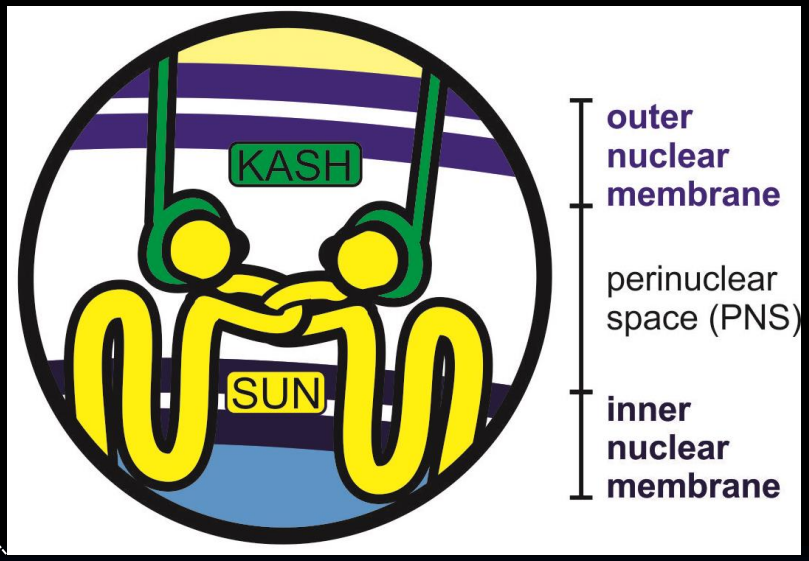
The LINC complex traverses the nuclear envelope and connects the cytoskeleton to the nuclear lamina.

- It involved in cell polarisation and nuclear positioning
- Force transmission through the cell is dependent on an intact LINC complex

The LINC complex

- SUN-domain proteins on the inner nuclear membrane that connect the lamina
- KASH-domain proteins (Nesprins) on the outer membrane that bind to elements of the cytoskeleton





Cytoskeleton

The major support structure in the cell with three principal components

- **Actin** rapidly polymerises into **microfilaments** that are cross-linked to form **filaments** that are mobilised in the cell to apply forces to neighbouring cells or its environment, or to induce cell migration/movement

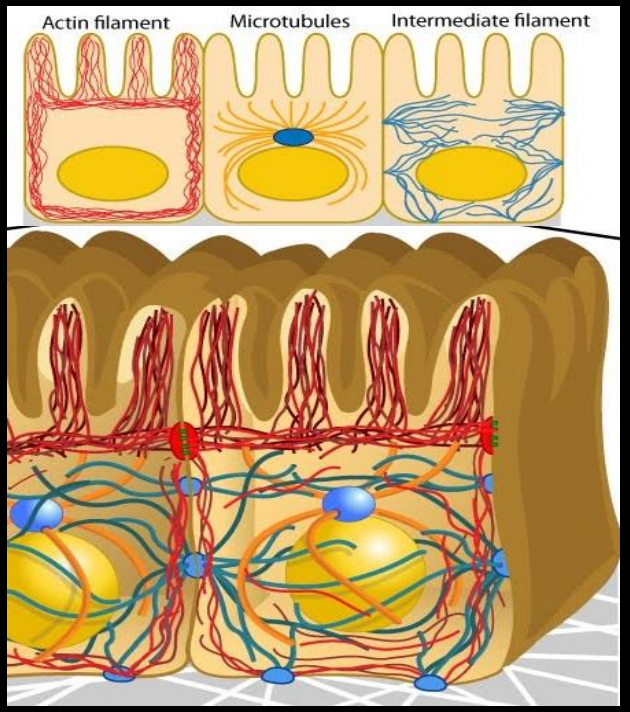
  http://www.youtube.com/watch?v=y6Ro_gxl_HE

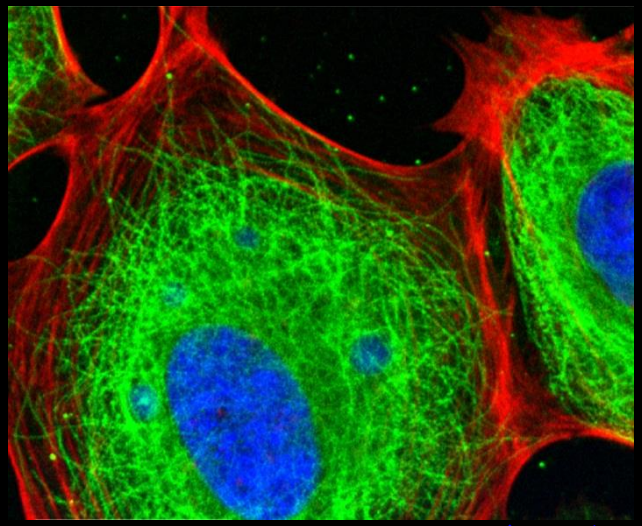
- **Microtubules** are polymers of **α and β-tubulin** that are involved in organelle localisation, cell division, motility (cilia, flagella) and mechanosensation (?)

  http://www.youtube.com/watch?v=2-L-Ts6fsks

- **Intermediate filaments** form fibrous structures that are flexible but resistant to fracture or extension and connect to neighbouring cells through desmosomes.

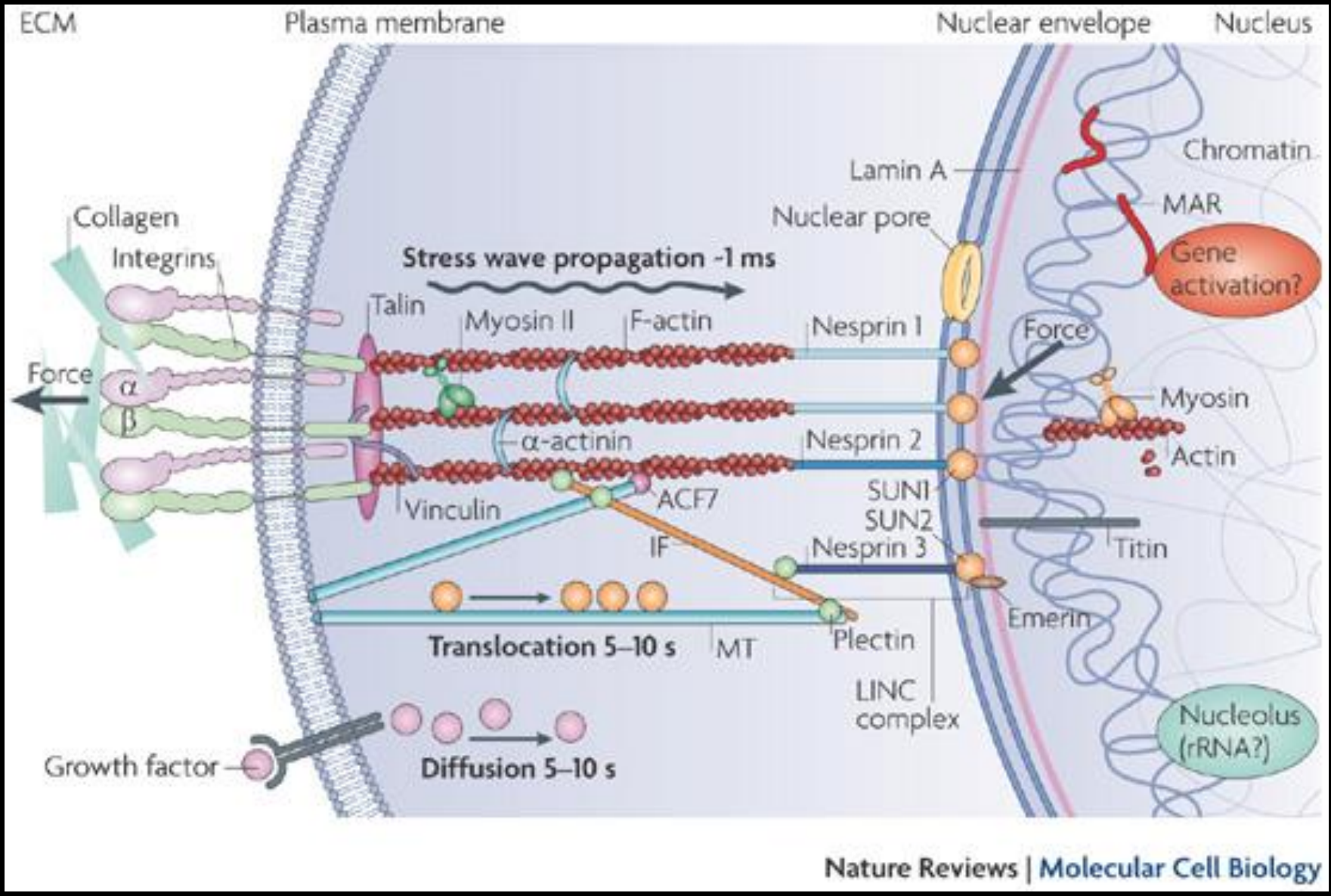
  http://www.youtube.com/watch?v=FoDniO676Dw





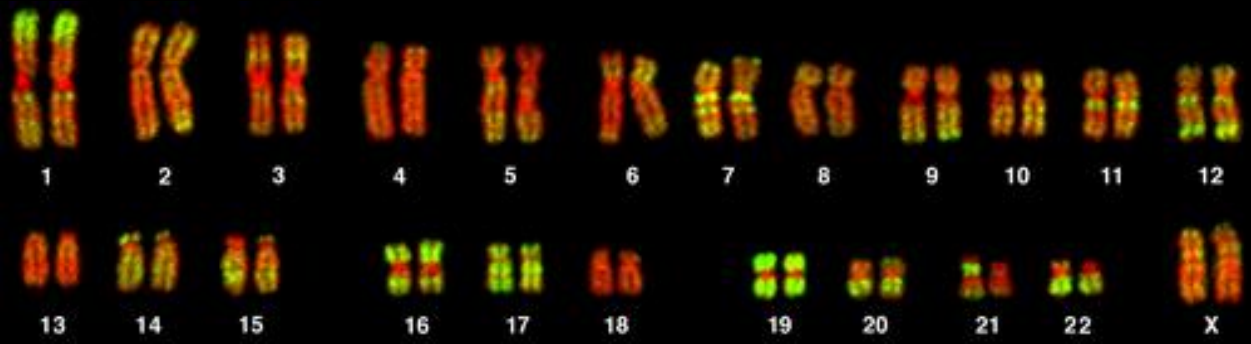
Actin Tubulin Nuclei (DNA)

Mechanotransduction on the nucleus

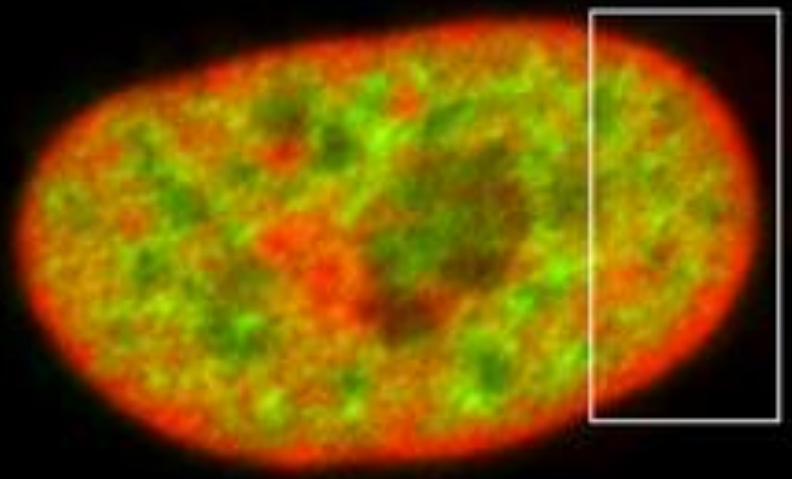


Wang et al. 2009 doi:10.1038/nrm2594

Chromosomes decondense after cell division



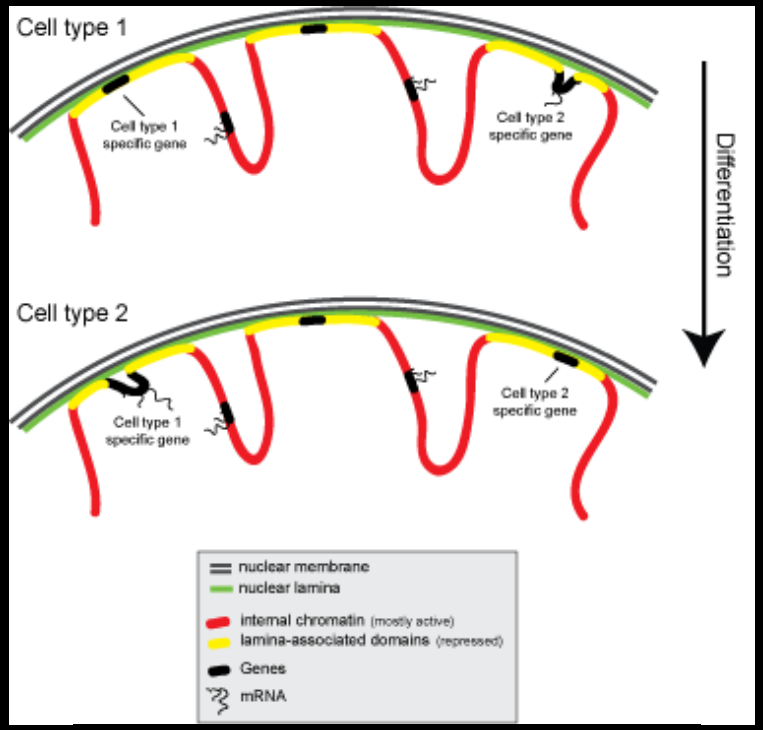
metaphase chromosomes from a female lymphocyte (Karyotype: 46, XX)

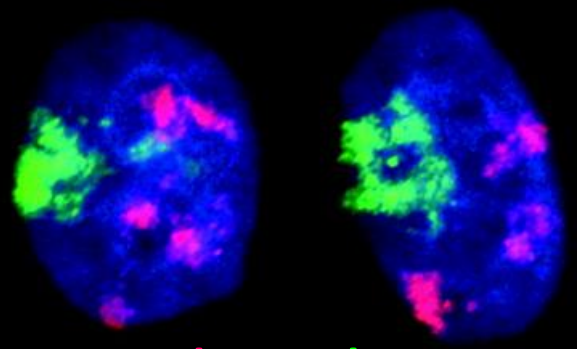


decondensed chromosomes
from a female lymphocyte

Bolzer et al. PLoS Biol. 3(5):e157. doi:10.1371/journal.pbio.0030157

- The organisation of the genome within the (interphase) nucleus is non-random
- Chromosomes occupy specific *Territories* within the nucleoplasm
- Radial 3D arrangement (ie. localisation towards the nuclear interior vs. periphery) has been associated with gene activity and can be clonally inherited.
- Proximity to the nuclear periphery is associated with developmental gene silencing.
- Changes in intranuclear 3D localisation of specific chromosome territories (and their adjacent "chromsome neighbourhood") have been correlated with different cell states
- Mechanism is still unclear, but both active (molecular motors) and passive movements involving a cell division have been suggested

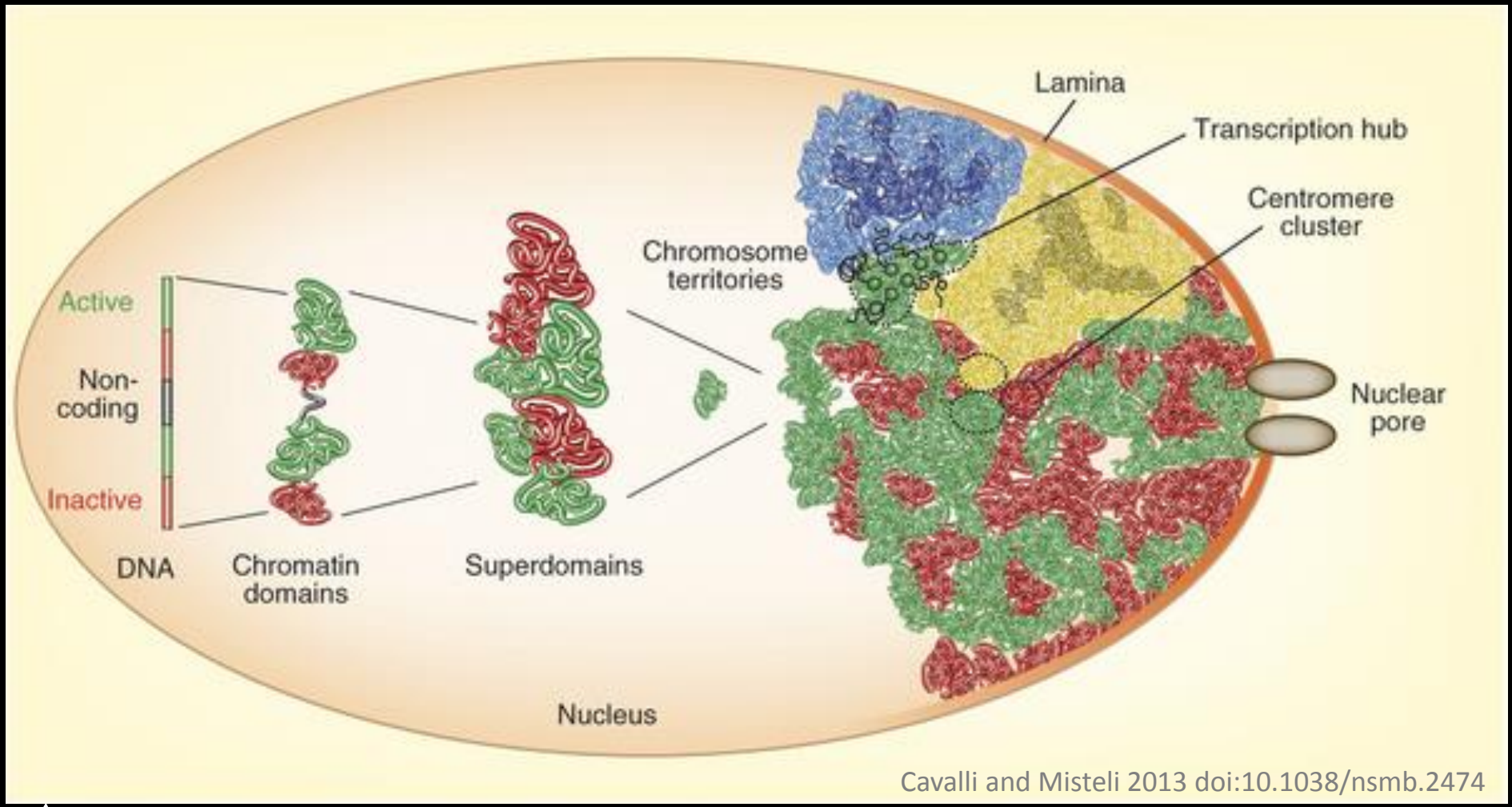




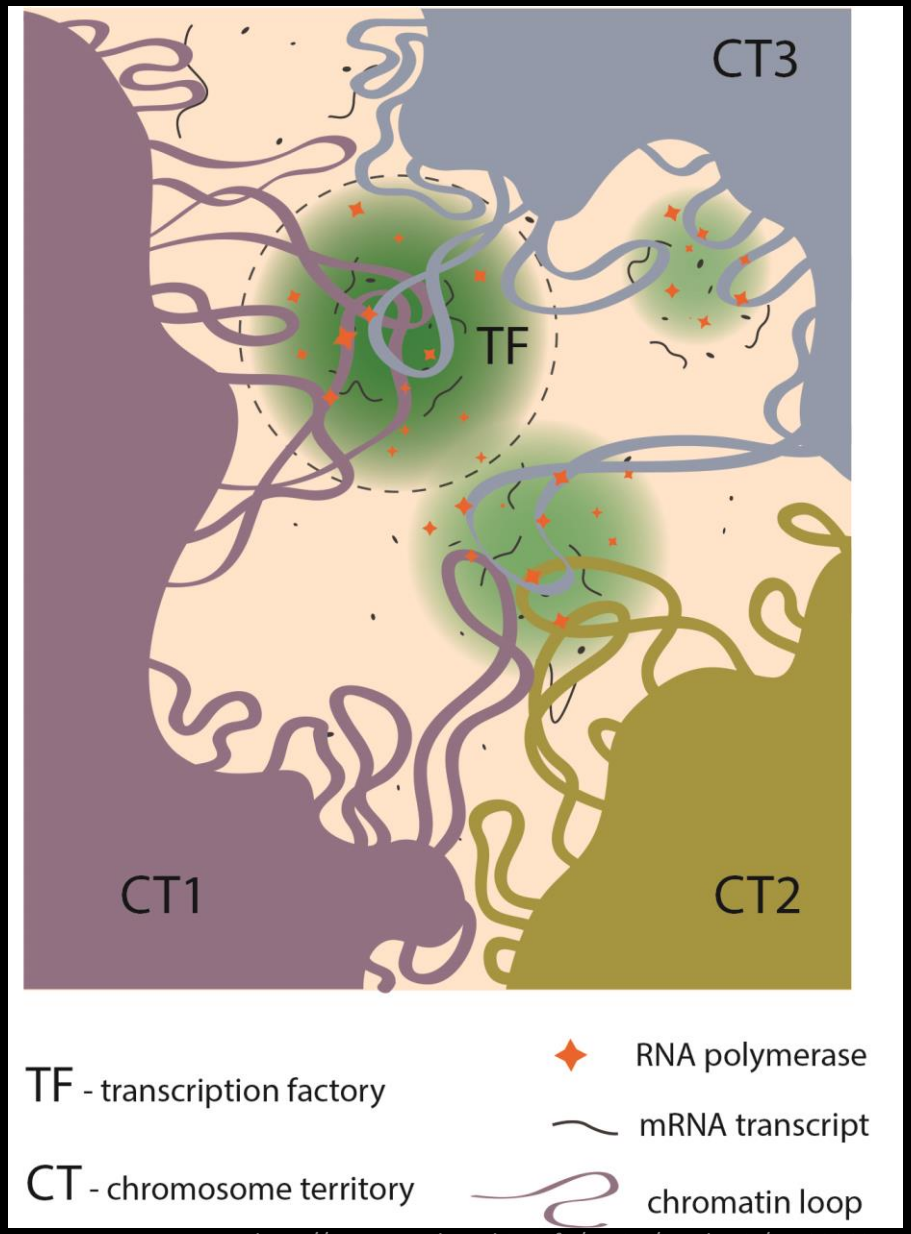
Chr10 Chr7

Walter et al 2003 doi: 10.1083/jcb.200211103

Chromatin architecture: Chromosome domains and neighbourhoods facilitate the silencing or transcription of co-regulated genes that are genomically distant…



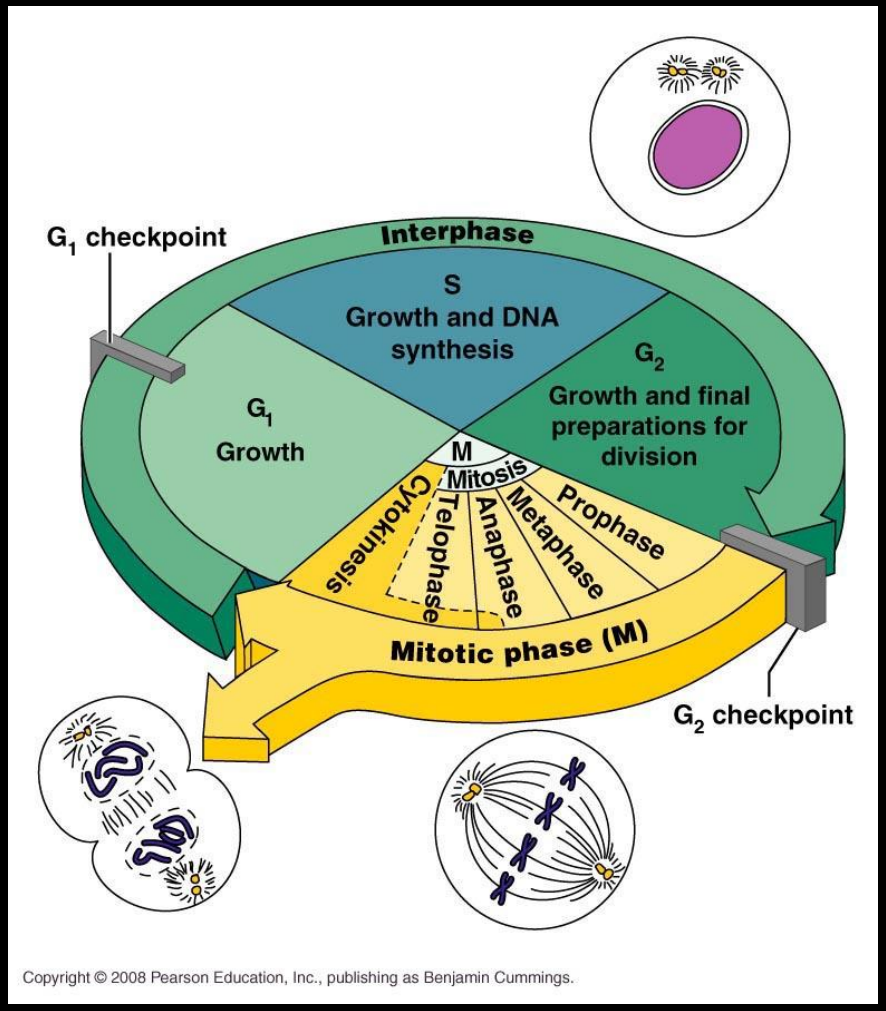
Cavalli and Misteli 2013 doi:10.1038/nsmb.2474



http://www.mechanobio.info/topics/synthesis/go-0006351

…placing them in a region (subnuclear domain) that is rich in the factors necessary to promote their intended regulation for that cell type/developmental state.

The Cell Cycle and Cell Division



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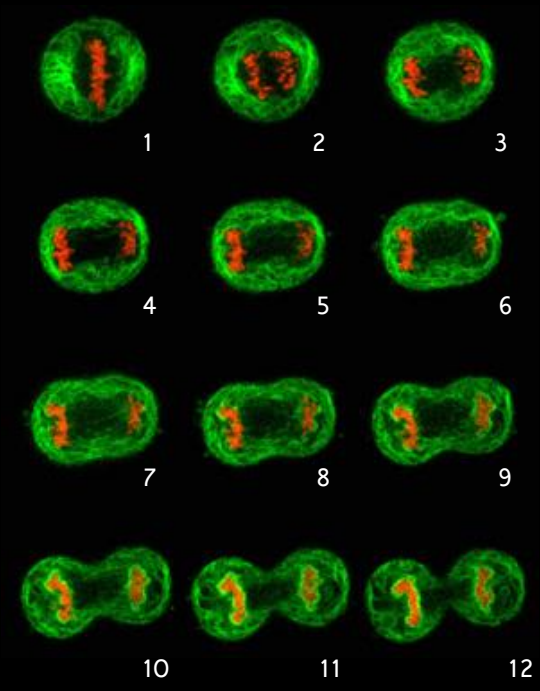
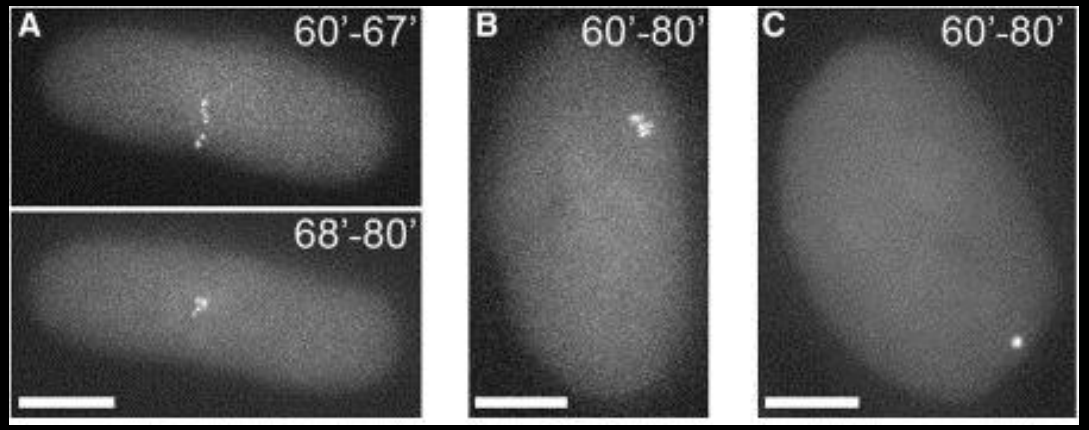
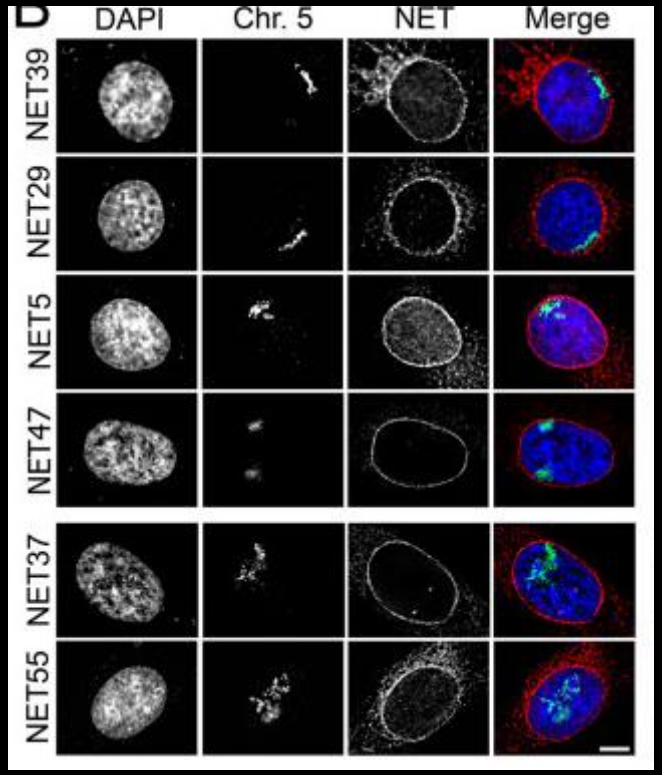


Spindle microtubules (Tubulin), Nuclei (DNA)

"Migration of an interphase chromosome site from the nuclear periphery to the interior 1–2 hr after targeting at transcriptional activator to this site. Spot redistribution is perturbed by specific actin or nuclear myosin I mutants."



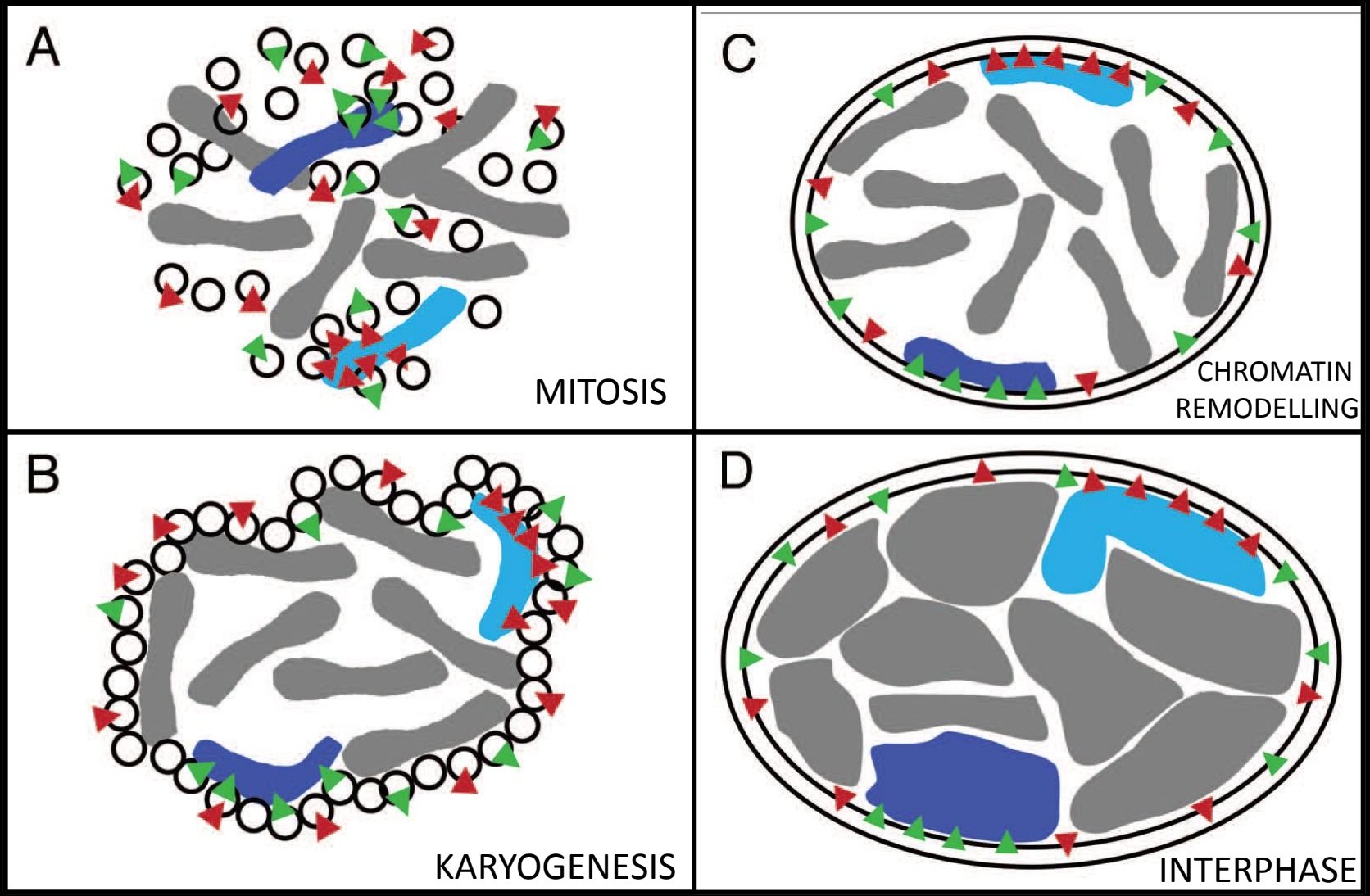
Chuang et al 2006 http://dx.doi.org/10.1016/j.cub.2006.03.059



Zuleger et al 2013 doi:10.1186/gb-2013-14-2-r14

"The discovery of nuclear envelope transmembrane (NET) proteins that can modulate chromosome position and have restricted patterns of expression may enable dissection of the functional relevance of tissue-specific patterns of radial chromosome positioning."

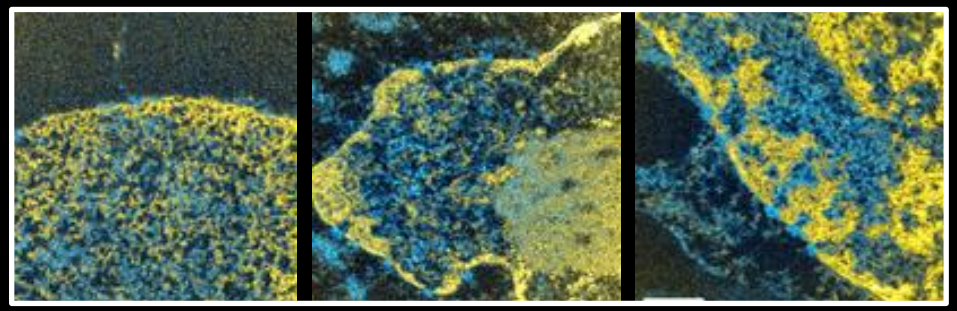
Speculative affinity mechanism for establishment of spatial chromosome organizational patterns.



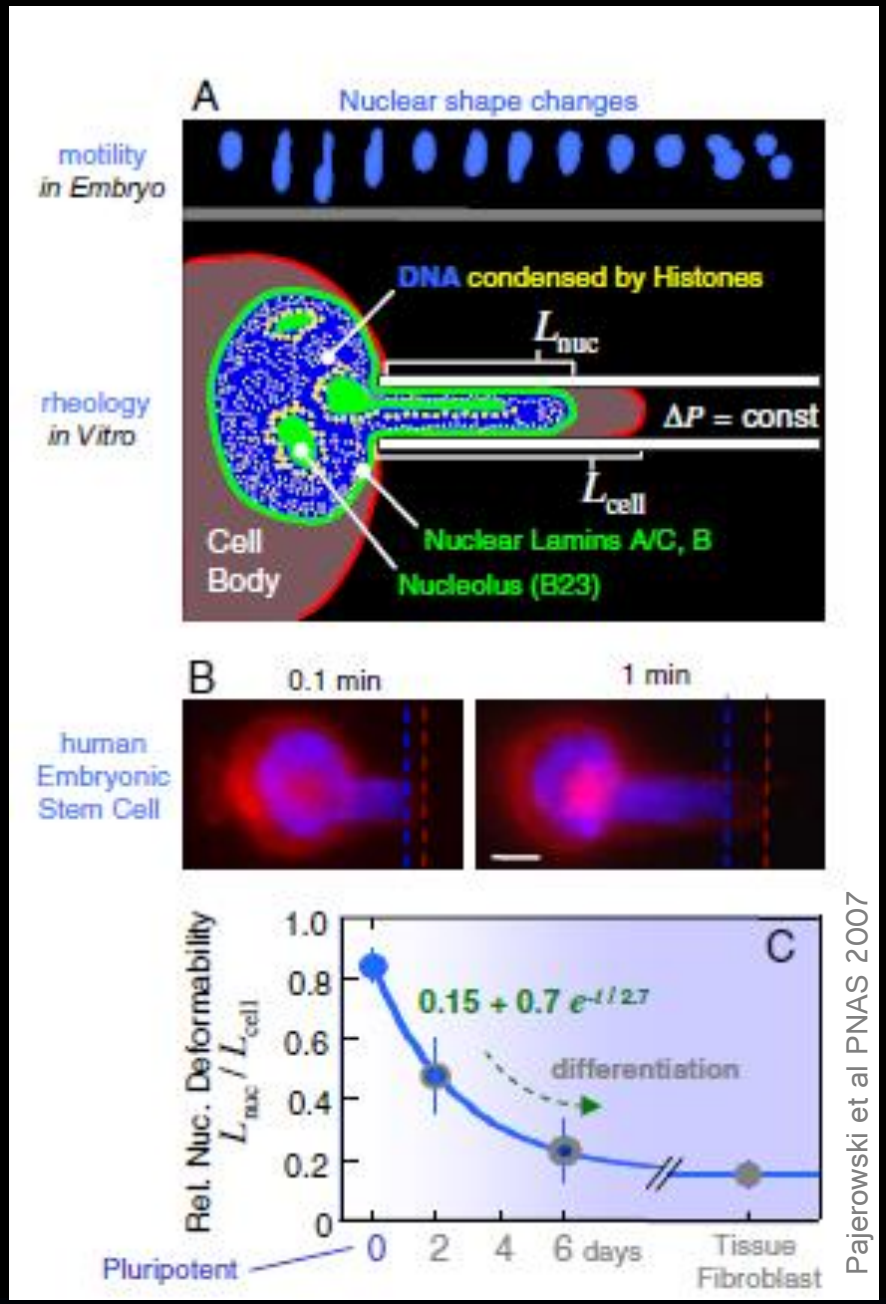
- As cells differentiate, nuclei also become more stiff (less deformable)

- As cells differentiate, the chromatin becomes more complex, and accumulations of highly complex DNA begin to form around the nuclear periphery

ES cells (undifferentiated) | TS cells (placental) | XEN cells (endoderm)

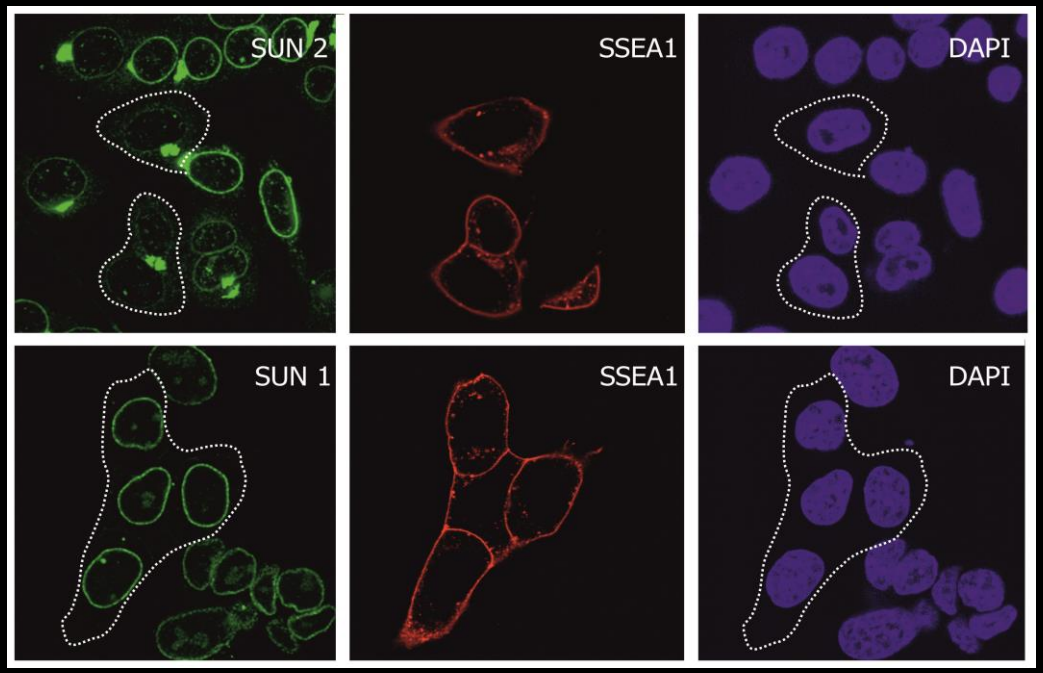


Ahmed et al . (2010 PLoS ONE 5(5): e10531

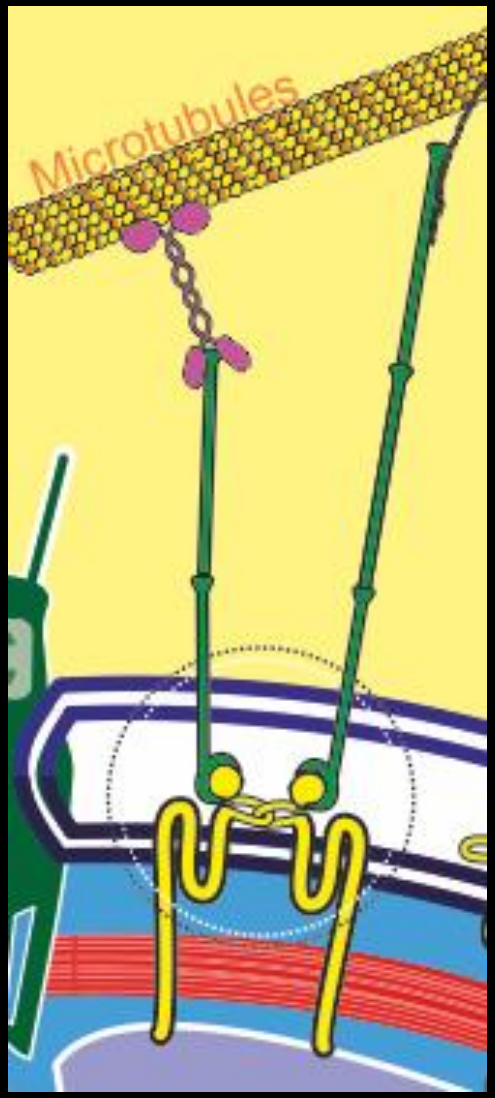


LINC complex & Differentiation

- SUN2 is part of the LINC, connecting the lamina (the major nuclear support structure) and the nuclear membrane and the cytoskelton through the Nesprin proteins.
- Implicated in nuclear positioning and DNA anchorage
- SUN2 is only expressed after ES cells differentiate



(pluripotent ES cells)



Summary

- Chromosomes occupy specific *Territories* within the nucleoplasm and 3D arrangements of neighbouring chromatin can be inherited between from mother cell to daughters.

- At differentiation the composition of the lamina and nuclear envelopes change as A-type Lamins, type-II Suns are up-regulated.

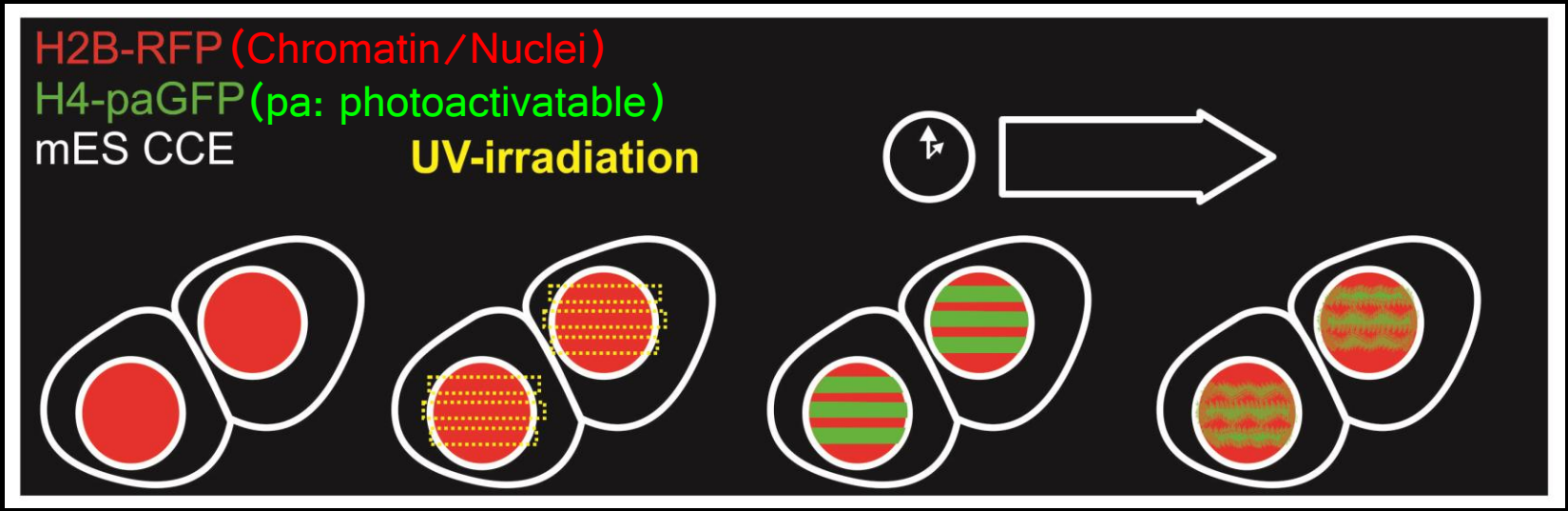
- Nuclei of embryonic stem cells stiffen when they differentiate. This is in part explained by changes to the lamina and envelope (Laminopathic cell data)

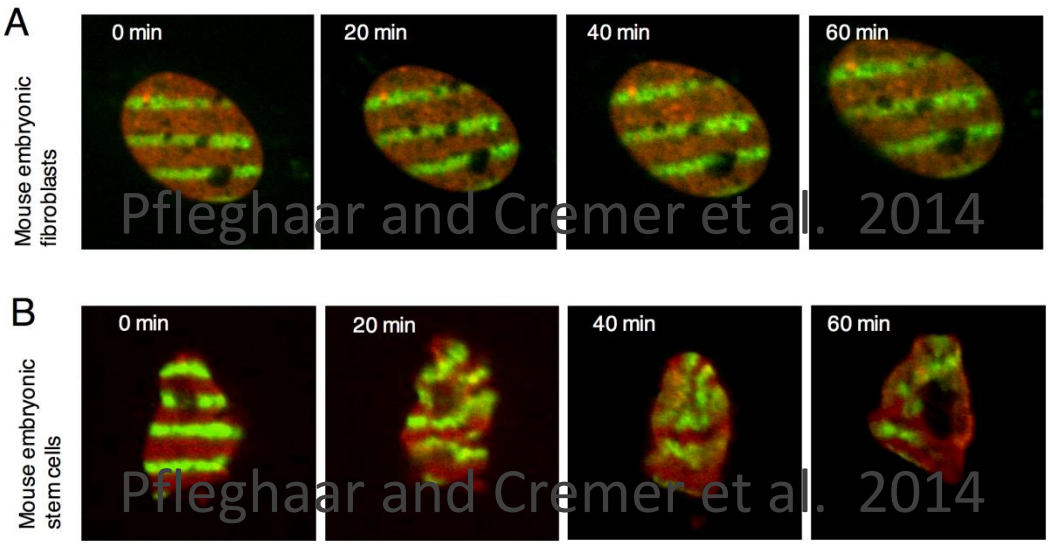
What about the chromatin, does it contribute structure to the nucleus?

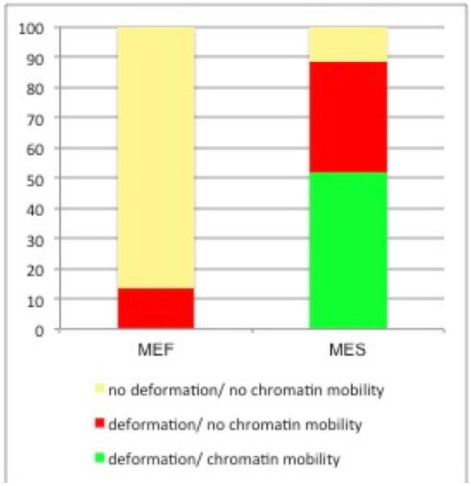
(we'll revisit this in a moment...)

Q: How does nuclear deformability relate to chromatin organisation.

ES cells have more fluid chromatin (chromosomes)







But what is an ES cell?
And what does it mean, (epi)genomically to be a stem cell?
Is there more than one state to pluripotency?

Cell

The Transcriptional and Epigenomic Foundations of Ground State Pluripotency

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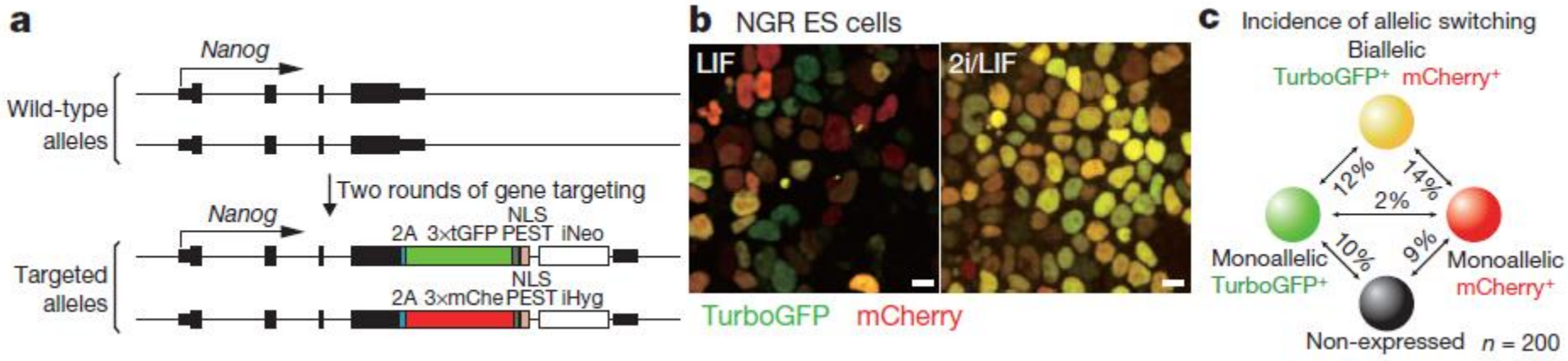
SUMMARY

Mouse embryonic stem (ES) cells grown in serum exhibit greater heterogeneity in morphology and expression of pluripotency factors than ES cells cultured in defined medium with inhibitors of two kinases (Mek and GSK3), a condition known as "2i" postulated to establish a naive ground state. We show that the transcriptome and epigenome profiles of serum- and 2i-grown ES cells are distinct. 2i-treated cells exhibit lower expression of lineage-affiliated genes, reduced prevalence at promoters of the repressive histone modification H3K27me3, and fewer bivalent domains, which are thought to mark genes poised for either up- or downregulation. Nonetheless, serum- and 2i-grown ES cells have similar differentiation potential. Precocious transcription of developmental genes in 2i is restrained by RNA polymerase II promoter-proximal pausing. These findings suggest that transcriptional potentiation and a permissive chromatin context characterize the ground state and that exit from it may not require a metastable intermediate or multilineage priming.

Ground-State Pluripotency is mediated by bi-allelic *nanog* expression



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Chromatin Decondensation and Nuclear Softening Accompany Nanog Downregulation in Embryonic Stem Cells

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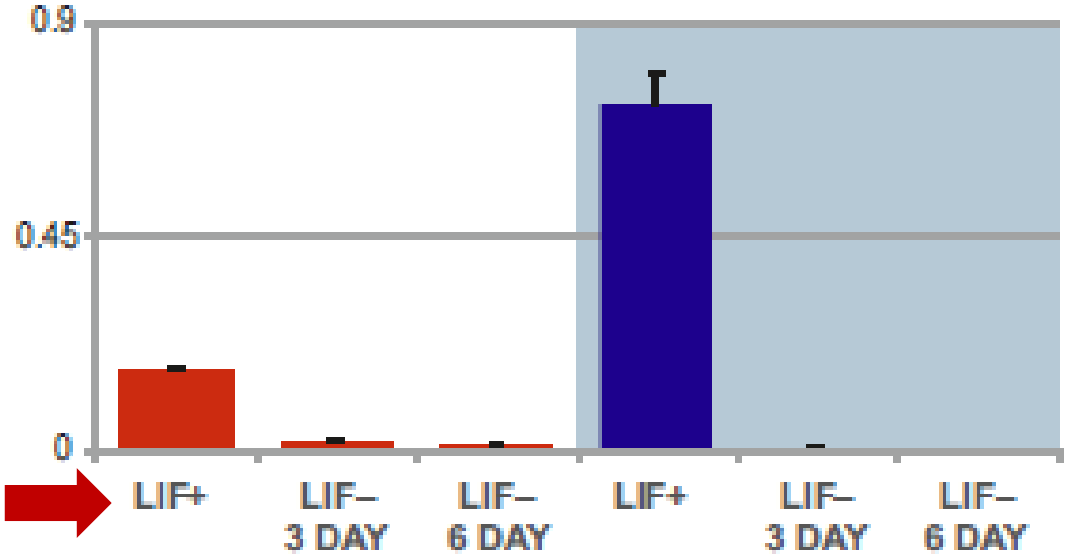
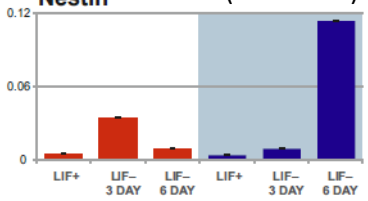
STD ESC CULTURE

2i: EMB GROUND STATE

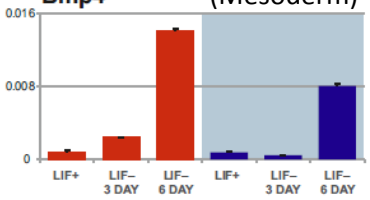
Nanog (Main Pluripotency Regulator)



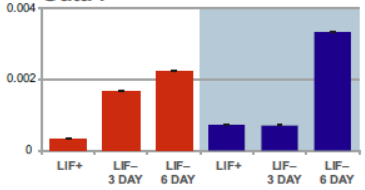
Nestin (Ectoderm)



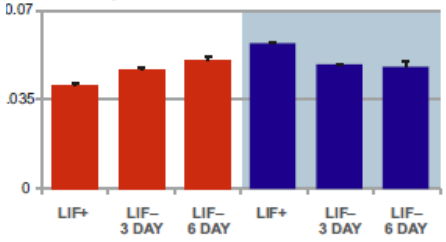
Bmp4 (Mesoderm)



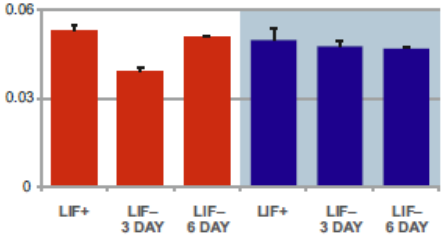
Gata4 (Endoderm)



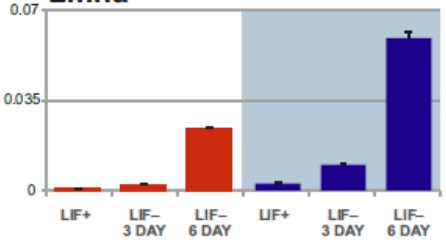
Lmnb1



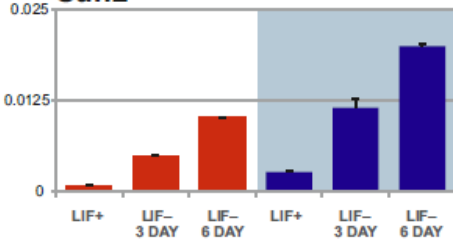
Sun1



Lmna



Sun2



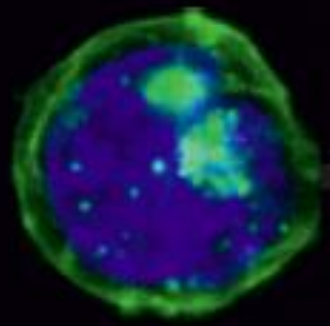
- Pluripotency in ES cells has two phases that are regulated by the level of Nanog expression.

- Monoallelic (and stochastic)-Nanog cells are primed for differentiation, quite heterogeneous, but still capable of self-renewal. These cells have soft nuclei and highly motile chromatin, despite an increased amount of negative epigenetic marks.

- Bi-allelic Nanog cells are in the pluripotent ground state. In essence, the state defined as not being anything else (yet). Here they essentially self-renew, and have quite stiff nuclei.

- It has been suggested that the stiffness is contributed through specific repression of lineage-specification programmes.

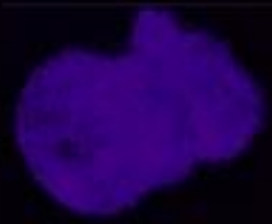
1. Cell selection



mESCs

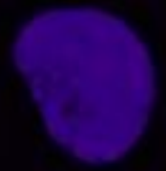
Hoecsht

αSSEA1



MEFs

Hoecsht

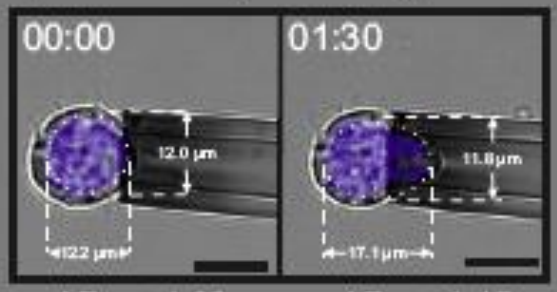


NSCs

Hoecsht

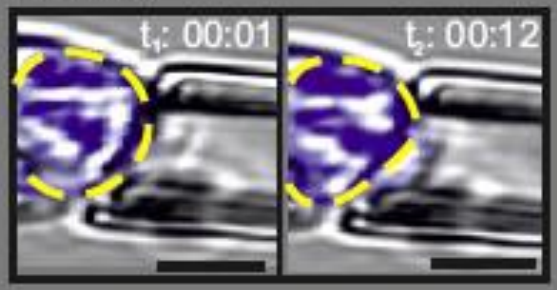
2. Aspiration and imaging

1) Aspect Ratio (AR = x/y)



$AR_{00:00}$=1.02 $AR_{00:00}$=1.45

2) calculate aspiration pressure (AP) required to initially deform the nucleus

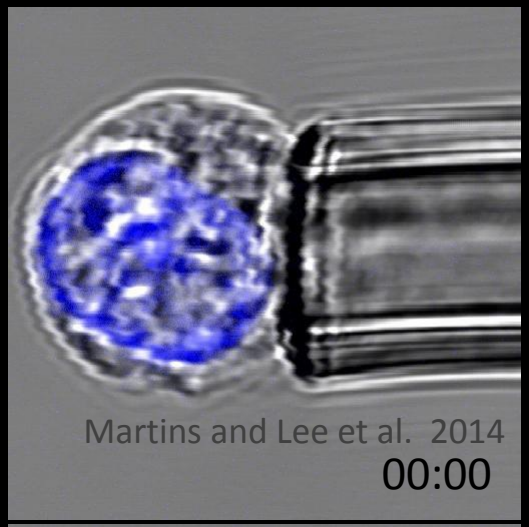


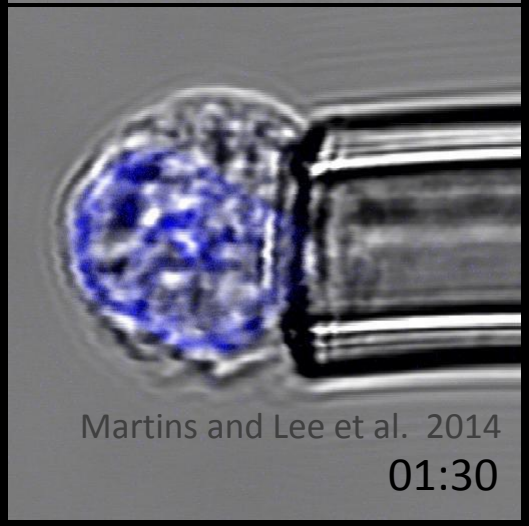
$AP = (t_2-t_1) \times 0.245$ kPa/s

= 27.5 kPa

2i-Pluripotent ES cell medium containing LIF

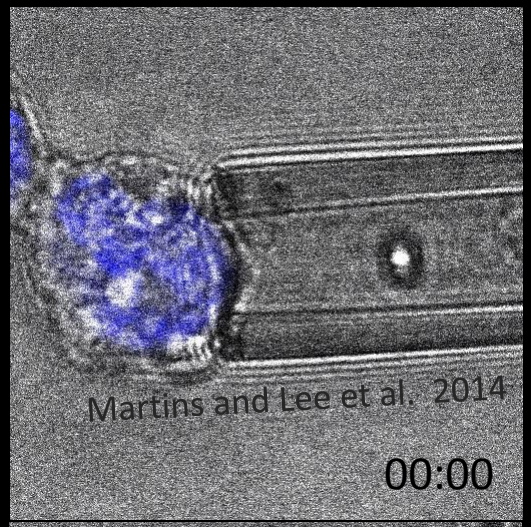
Bi-allelic Nanog

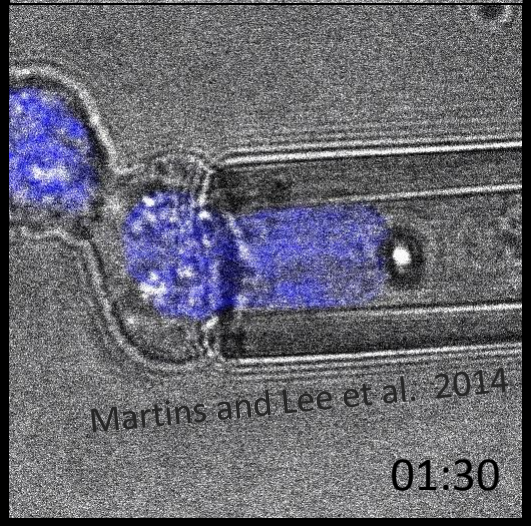




Standard ES cell medium containing serum and LIF

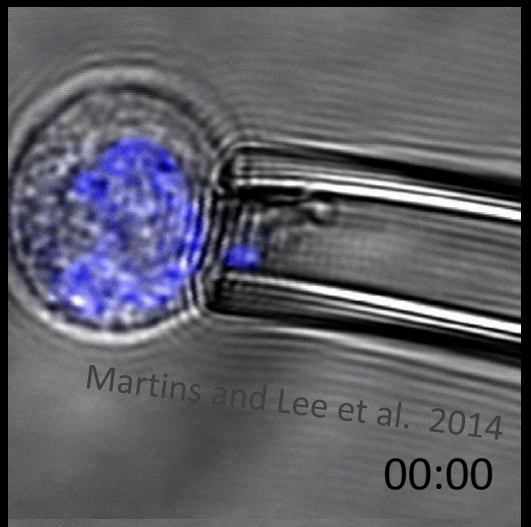
Monoallelic+stochastic

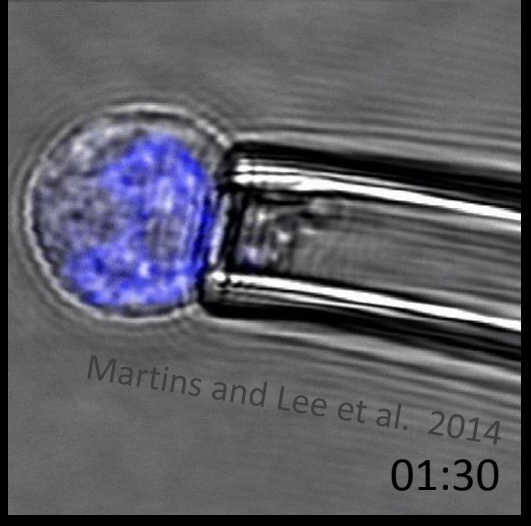


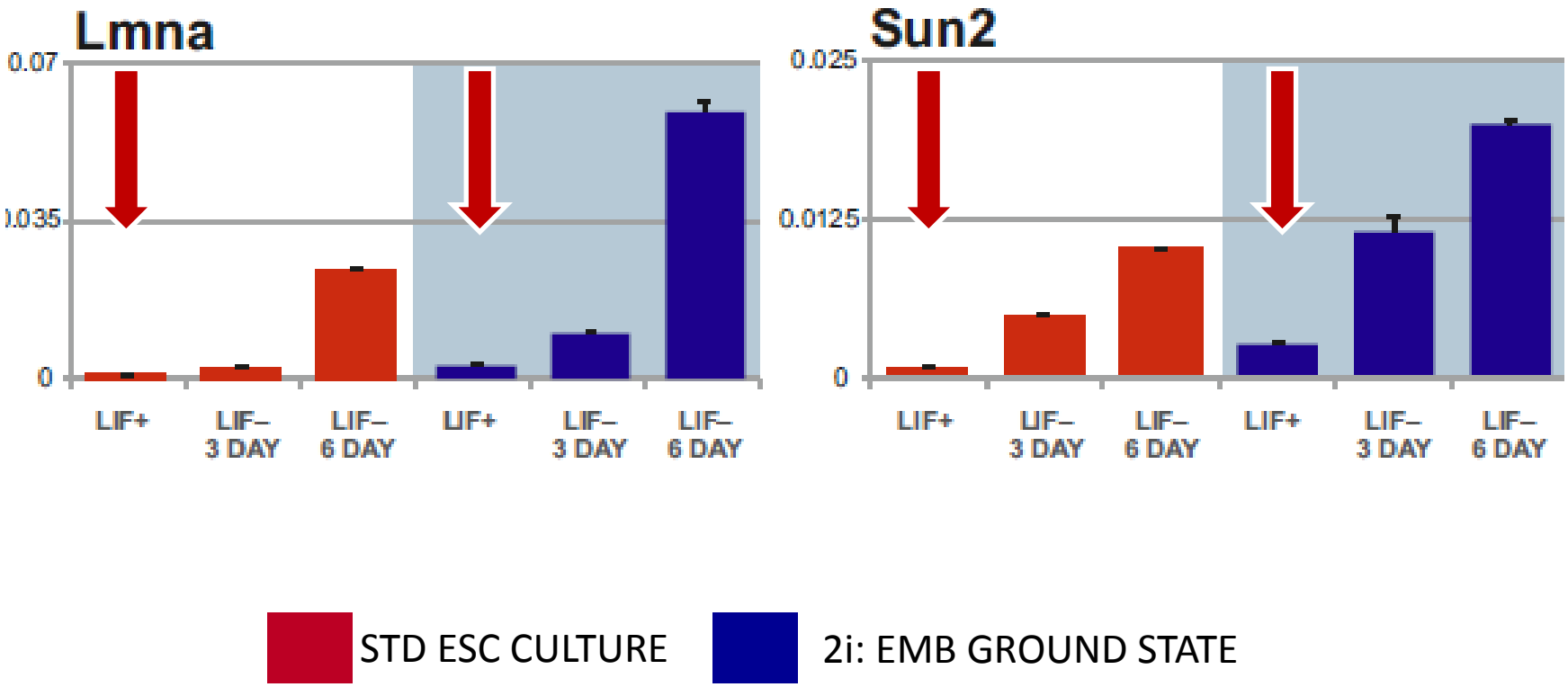


Differentiation conditions no LIF, 6 Days

Nanog downregulated



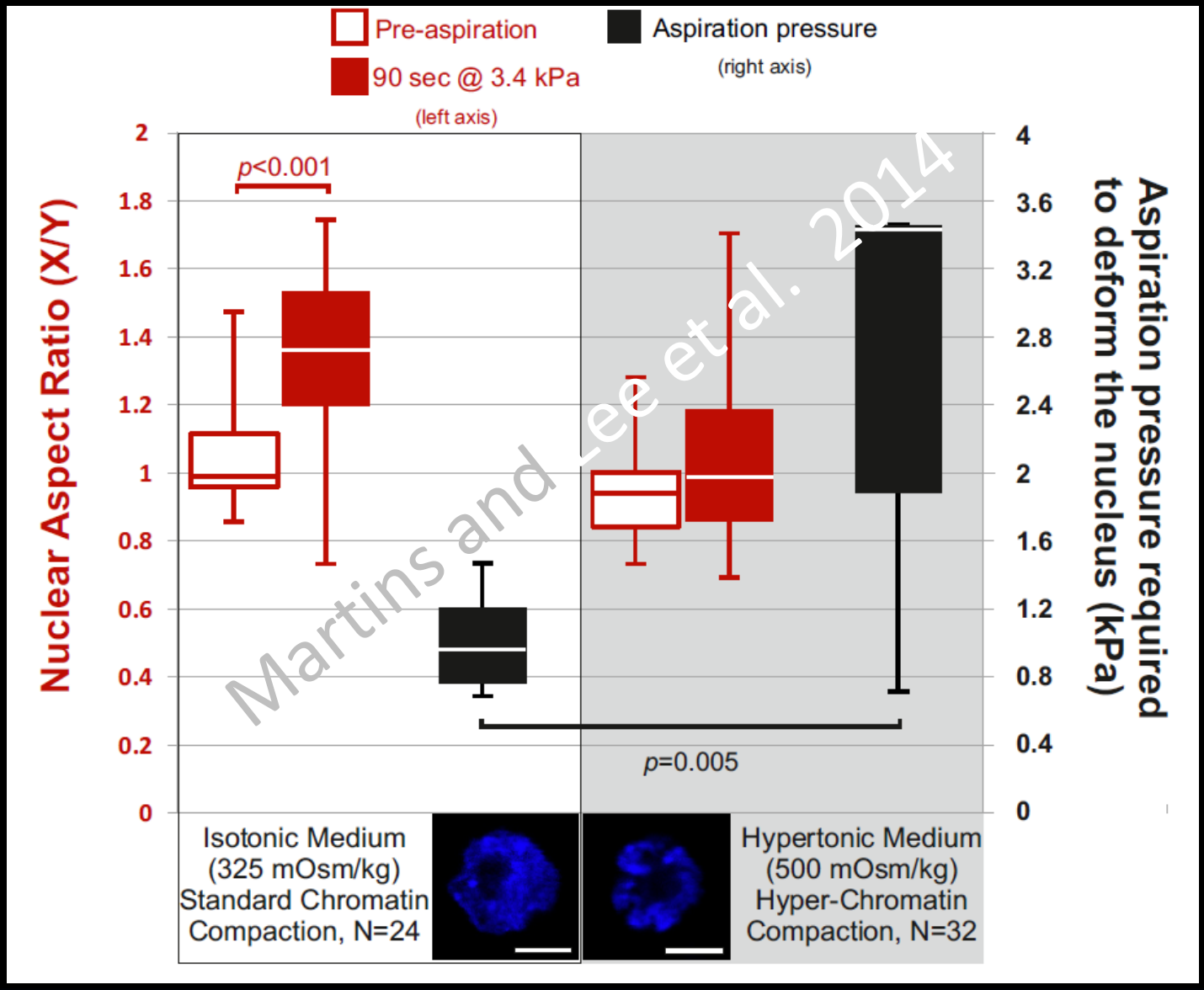




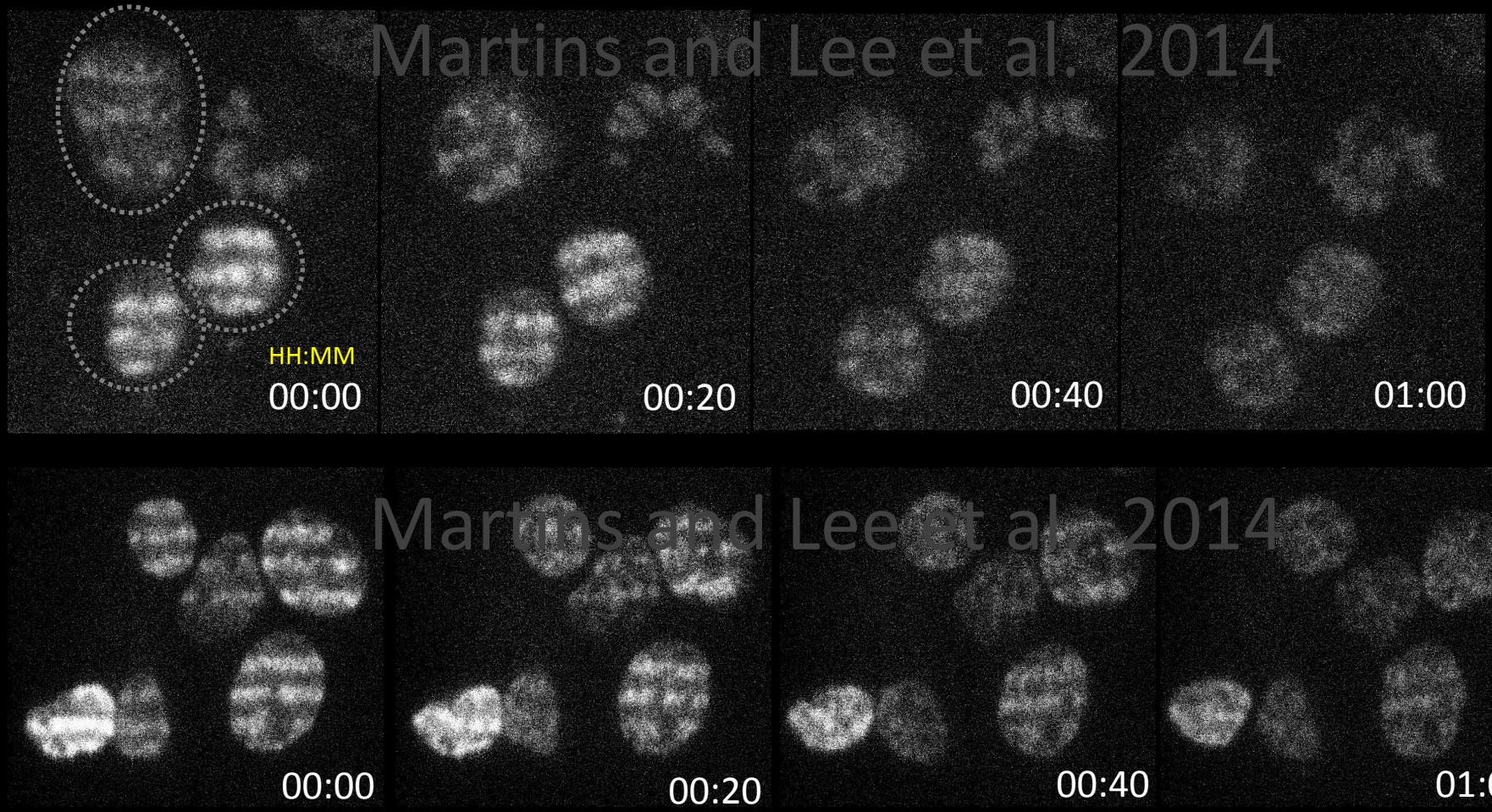
STD ESC CULTURE

2i: EMB GROUND STATE

Q: Can chromatin condensation alone explain some of the nuclear stiffness?


Pre-aspiration
90 sec @ 3.4 kPa
(left axis)
Aspiration pressure
(right axis)
Nuclear Aspect Ratio (X/Y)
Aspiration pressure required to deform the nucleus (kPa)
2
1.8
1.6
1.4
1.2
1
0.8
0.6
0.4
0.2
0
4
3.6
3.2
2.8
2.4
2
1.6
1.2
0.8
0.4
0
p<0.001
p=0.005
Martins and Lee et al. 2014
Isotonic Medium
(325 mOsm/kg)
Standard Chromatin
Compaction, N=24
Hypertonic Medium
(500 mOsm/kg)
Hyper-Chromatin
Compaction, N=32

Q: ES cells grown in 2i/LIF which promotes bi-allelic Nanog conditions are stiffer, but does that translate to non-motile chromatin, as is evident in differentiating/ed cells?



- Chromatin mobility is much lower in stiffer nuclei- no gross rearrangements visible during interphase.

- Changes in the nuclear envelope composition when ES cells differentiate (Lamin A and Sun 2) provides more putative anchoring points to stabilise chromosomal architecture

- Changes to the overall level of chromatin condensation; with highly condensed chromatin being able to significantly stiffen ES cell nuclei.

So why is it important to maintain a fluid chromatin architecture while in a state primed for differentiation?

Acknowledgements

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