Chromatin Fiber Dynamics Workshop

Daniel Darling - November 2024

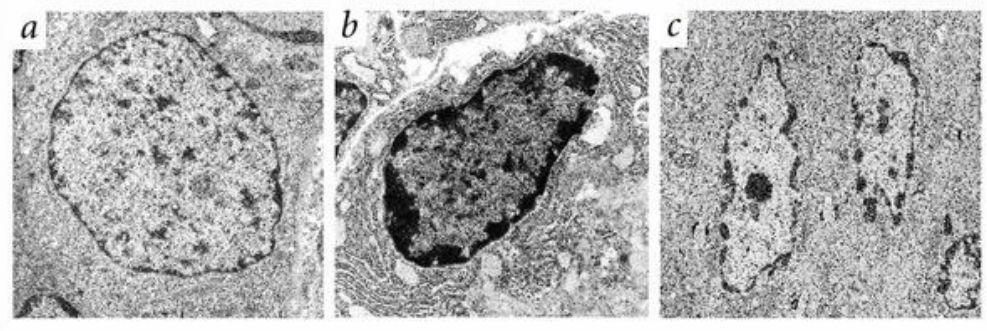
Al-Sady Lab

The challenge of DNA compaction

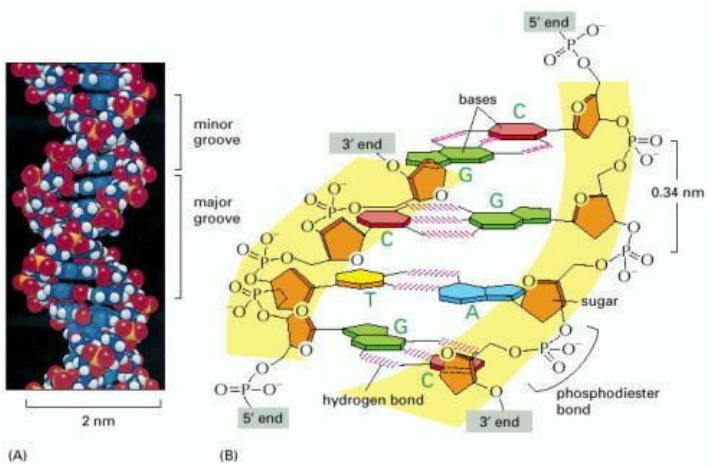
The Problem

- DNA per cell is ~2m long
- Nucleus diameter is 10uM
- DNA is negatively charged
- DNA needs to be very tightly regulated and maintained

The Solution Chromatin

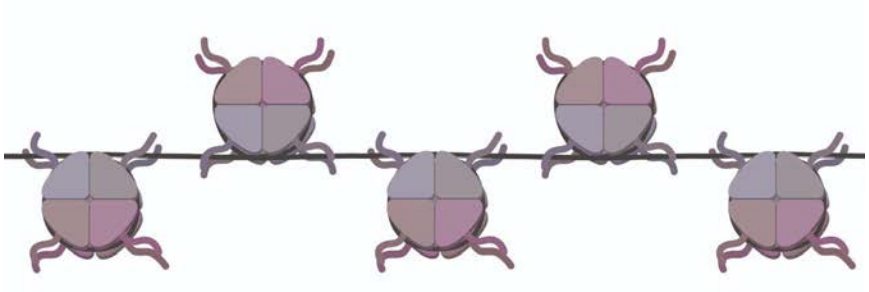


S. Pockwinse, *Nature Genetics* 2002



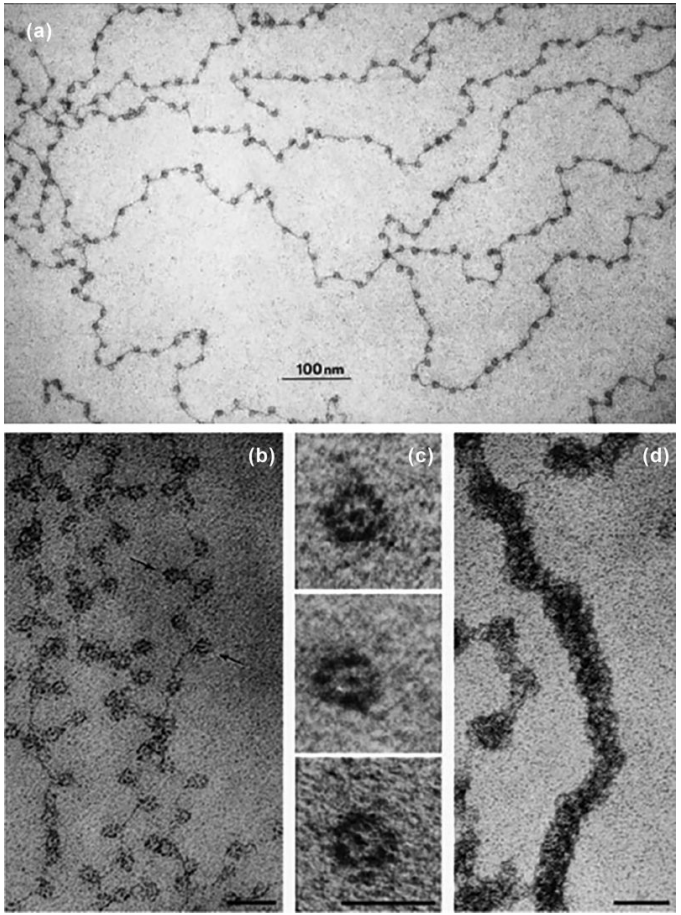
Bruce Alberts, Molecular Biology of the Cell. 4th Edition, 2002

Chromatin Components



- DNA
- Histones (H2A, H2B, H3, H4)

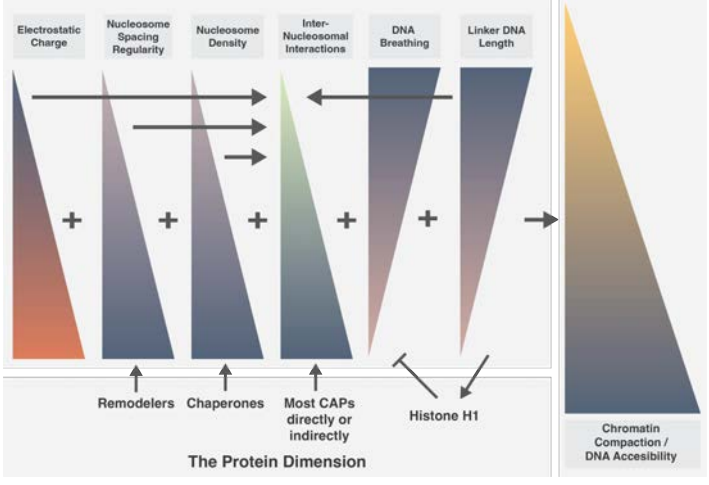
Basic subunit (Nucleosome) consists of ~147bp DNA wrapped around a central histone octamer core

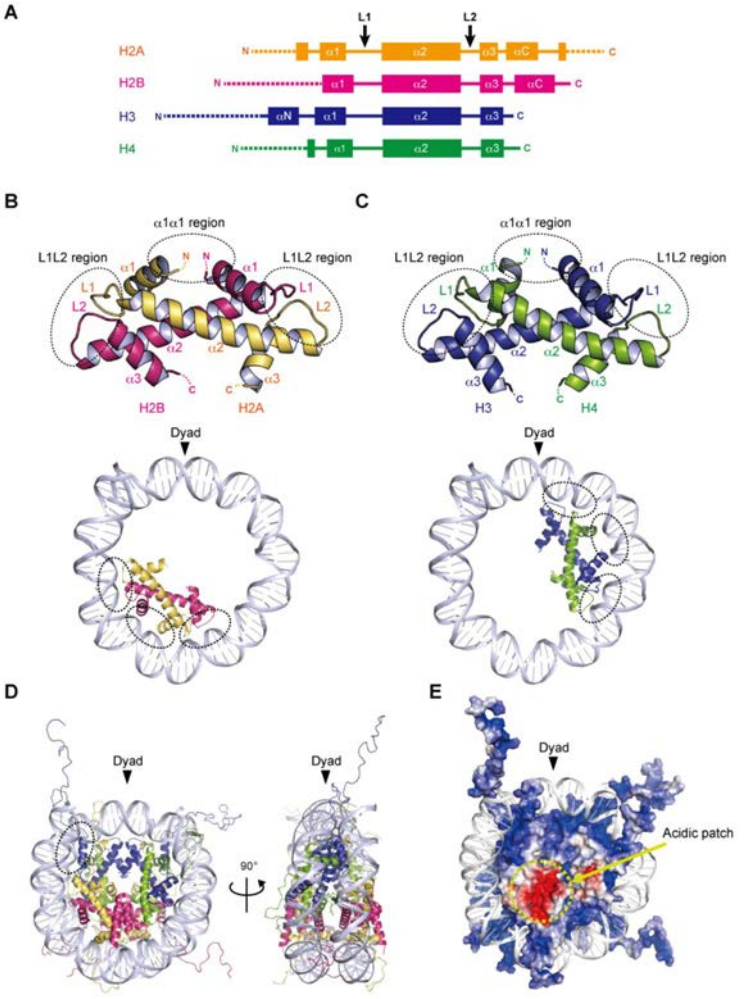


Ada and Don Olins *Nature* 1974

Nucleosome Highlights

- Positively charged residues interact with DNA
- Histone Tails
- Acidic Patch
- Histone H1
- Variable length of Linker DNA (~10-90bp)



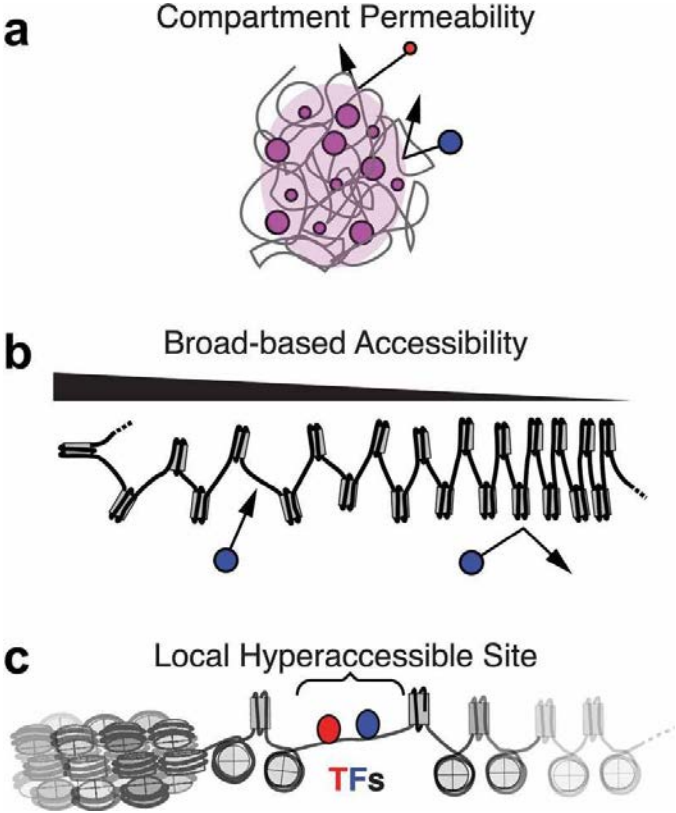


Sato, S.; Dacher, M.; Kurumizaka, H. *Epigenomes* 2022

Chromatin is a dynamic polymer that modulates DNA Accessibility

Variations in chromatin structural modulators yield variable dynamic states of chromatin with different DNA accessibilities and functions

- Nucleosomal DNA is inherently insulated
- There is a dynamic range of DNA accessibility within chromatin at different scales dependent on chromatin structural parameters

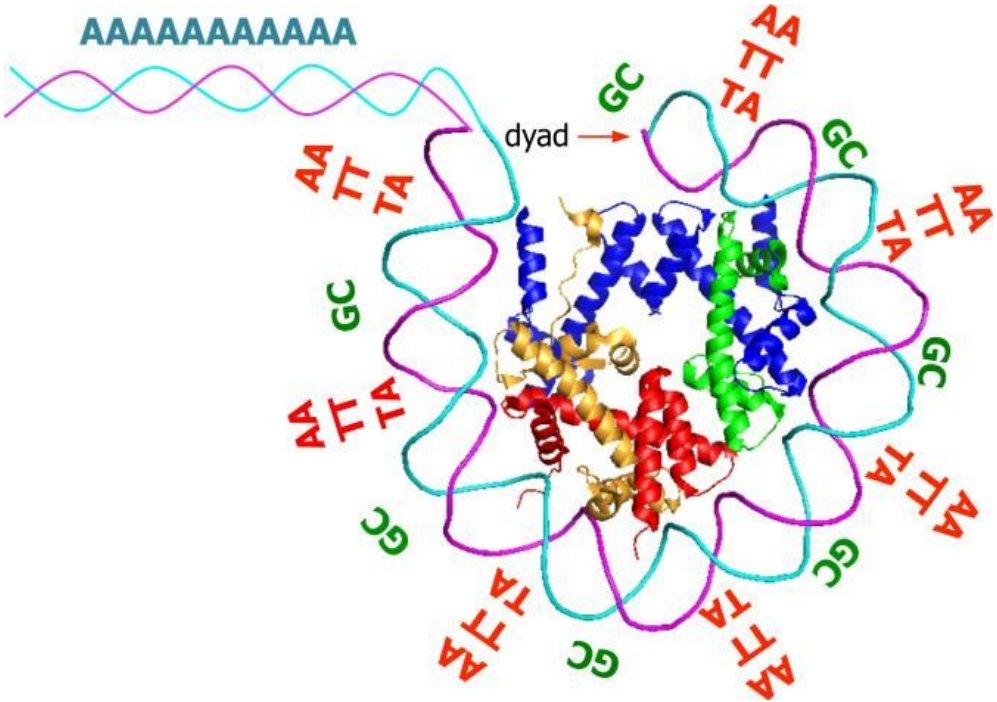


Mansisidor AR, Risca VI *Nucleus*. 2022

The effect of DNA sequence on Nucleosome Position

DNA sequence highly influences Nucleosome positioning

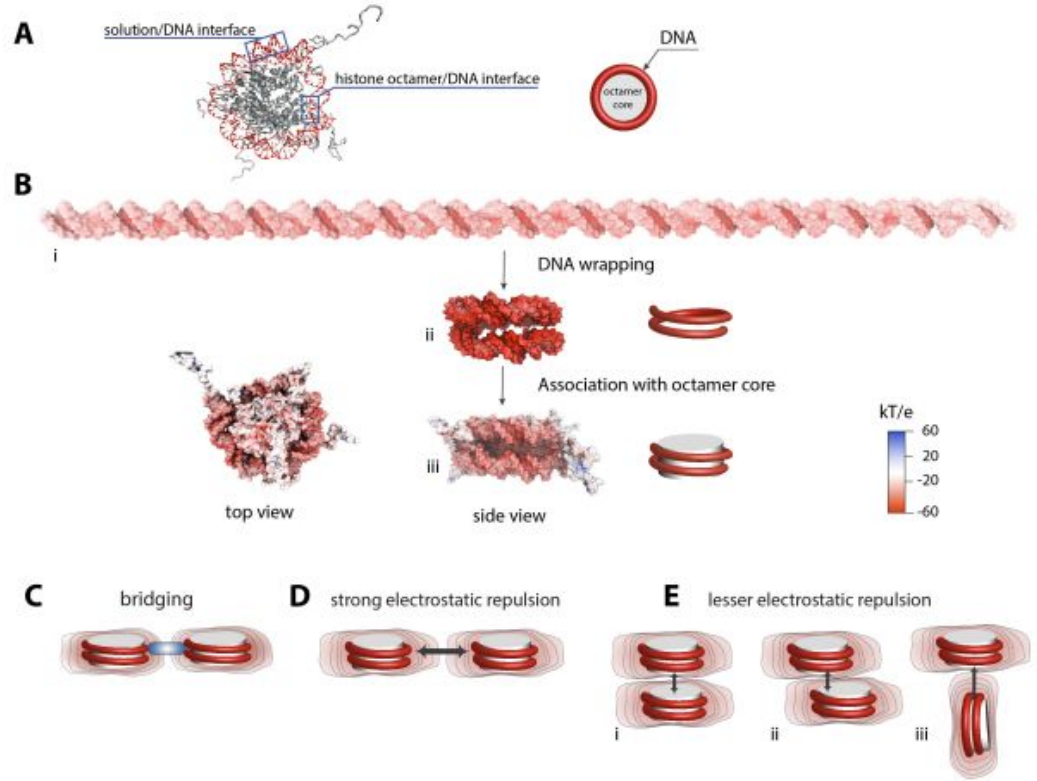
- Nucleosomes need to rotate the DNA ~600 degrees
- More bendy = more favorable
- 601 sequence
- Linker DNA length and DNA breathing are both highly affected by where the nucleosomes naturally position and how



Struhl K, Segal E. Mol Biol. 2013

Structure Modulators - Electrostatic State

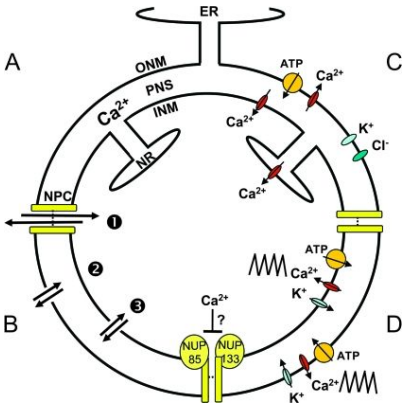
- The nucleosome has a net negative charge due to the strong negative charge of DNA
- The structure of the nucleosome creates a electrostatic field around the DNA limiting the potential orientations of nucleosome interactions



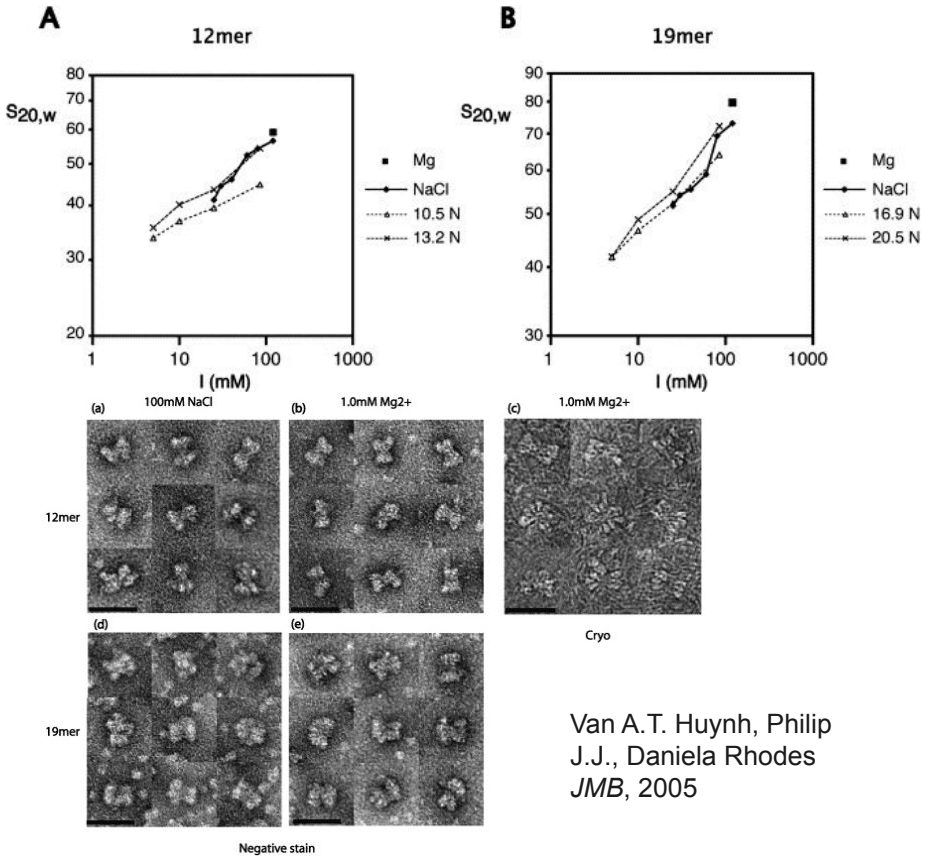
Gebala et al. *eLife* 2019

Structure Modulators - Cations Mg^{2+}, Ca^{2+}, Na^{+}, K^{+}

- The relative electrostatic negative forces of the nucleosome can be neutralized by cations
- Neutralization leads to greater chromatin compaction
- Cations concentrations are tightly regulated in the nucleus



Matzke AJ, Weiger TM, Matzke M. *Mol Plant.* 2010



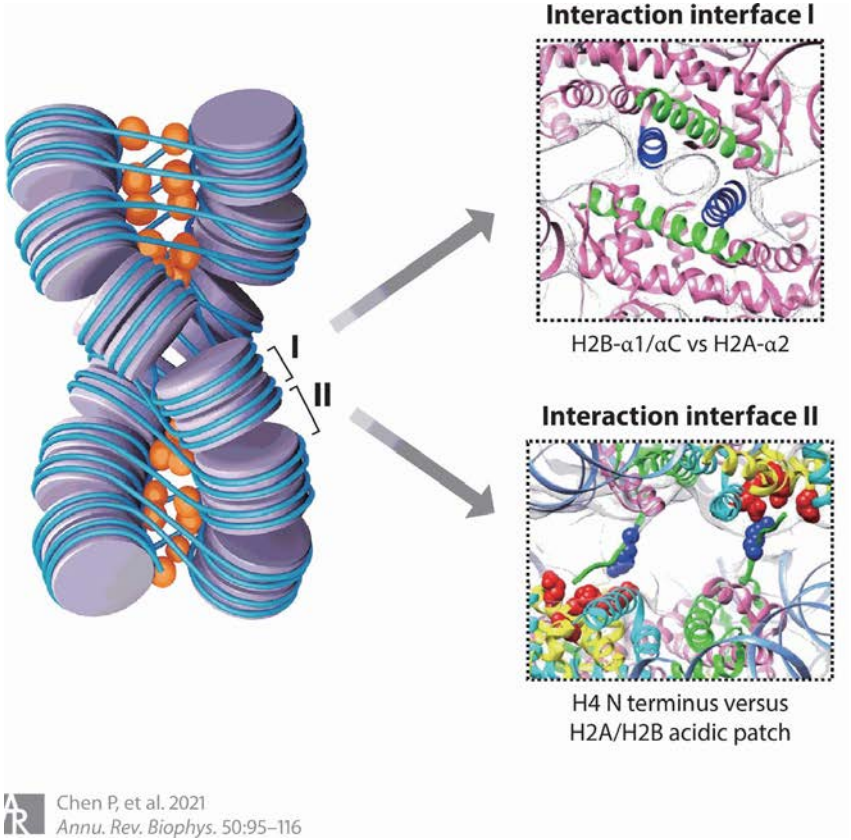
Van A.T. Huynh, Philip J.J., Daniela Rhodes *JMB*, 2005

Modes of Internucleosomal Interaction

Type 1 Internucleosomal Interactions
Faces of adjacent nucleosomes interact mediated by H2B-α1/αC of nucleosome 1 with the adjacent H2A-α2 of nucleosome 2

Type 2 Internucleosomal Interactions
Angled interaction between two adjacent nucleosomes mediated by the H4 tail of nucleosome 1 interacting with the H2A/H2B acidic patch of nucleosome 2

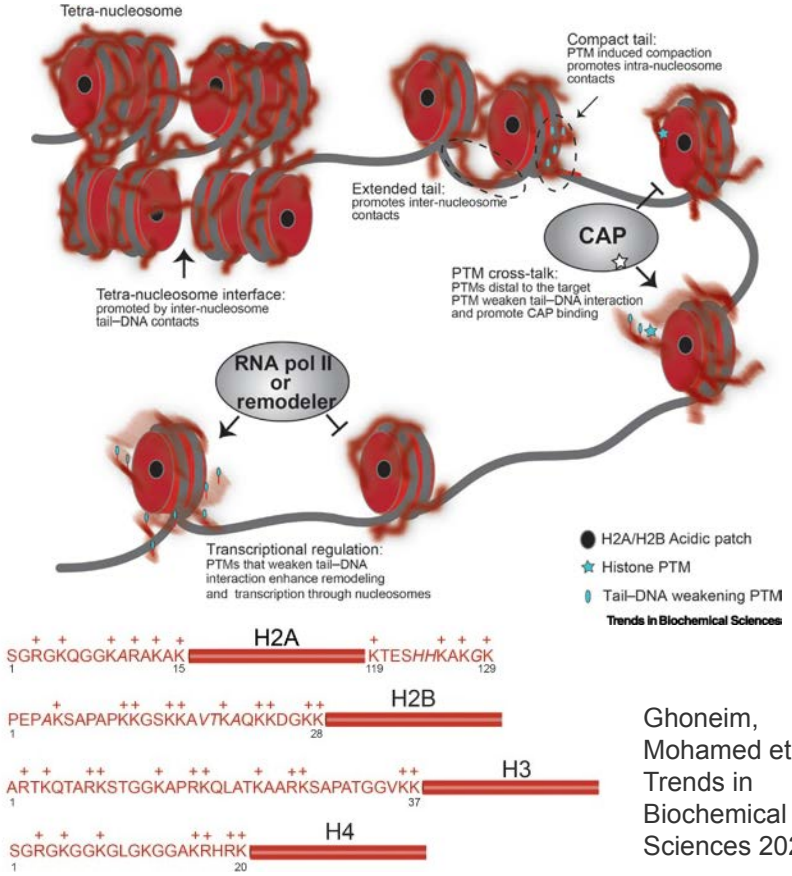
- These interactions can be transient and dynamic
- Chromatin associated proteins can modulate the stability of these interactions



Chen P, et al. 2021
Annu. Rev. Biophys. 50:95–116

Structure Modulators - Histone Tails

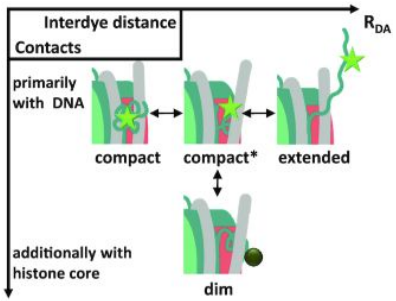
- Histone tails contain many positively charged residues and are intrinsically disordered (not resolved in structures)
- Histone tails adopt a range of conformations and exchange between these conformations (Fuzzy complex)
- Tails interact with nucleosomal DNA, linker DNA, chromatin associated proteins and nucleosomes in cis and trans
- PTMs can modulate the range of these conformations and interactions regulating chromatin structure



Ghoneim, Mohamed et al. Trends in Biochemical Sciences 2021

Structure Modulators - Individual Histone Tails

H3 - At entry/exit points of DNA, propensity to form helices, affects DNA breathing, proposed to have 4 main conformational states.

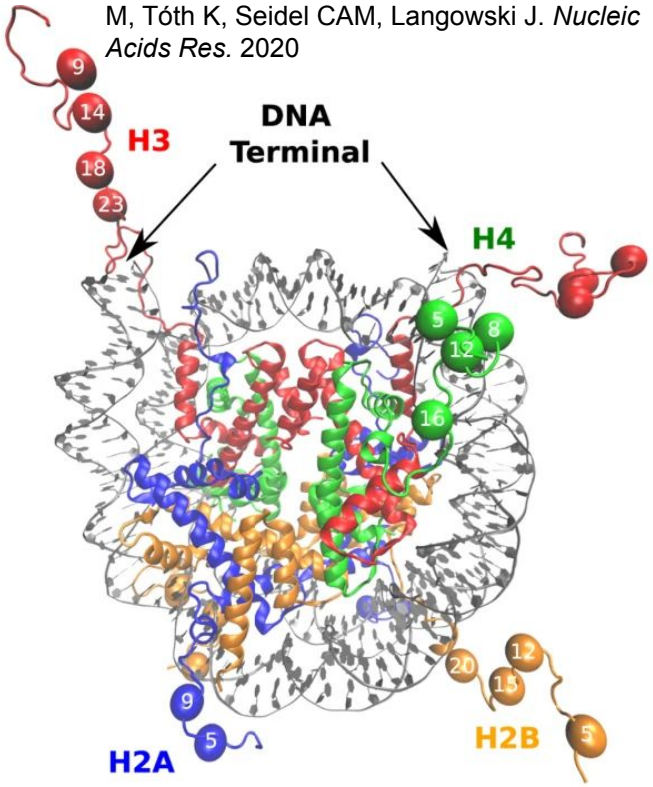


H4 - Extend through the face of the nucleosome. Contains two distinct dynamical regions. Interacts with nucleosomal DNA and the acidic patch in cis and trans.

H2A - Has both N and C terminal tails, N tail interacts primarily with nucleosomal DNA, C terminal tail interacts with nucleosomal and linker DNA.

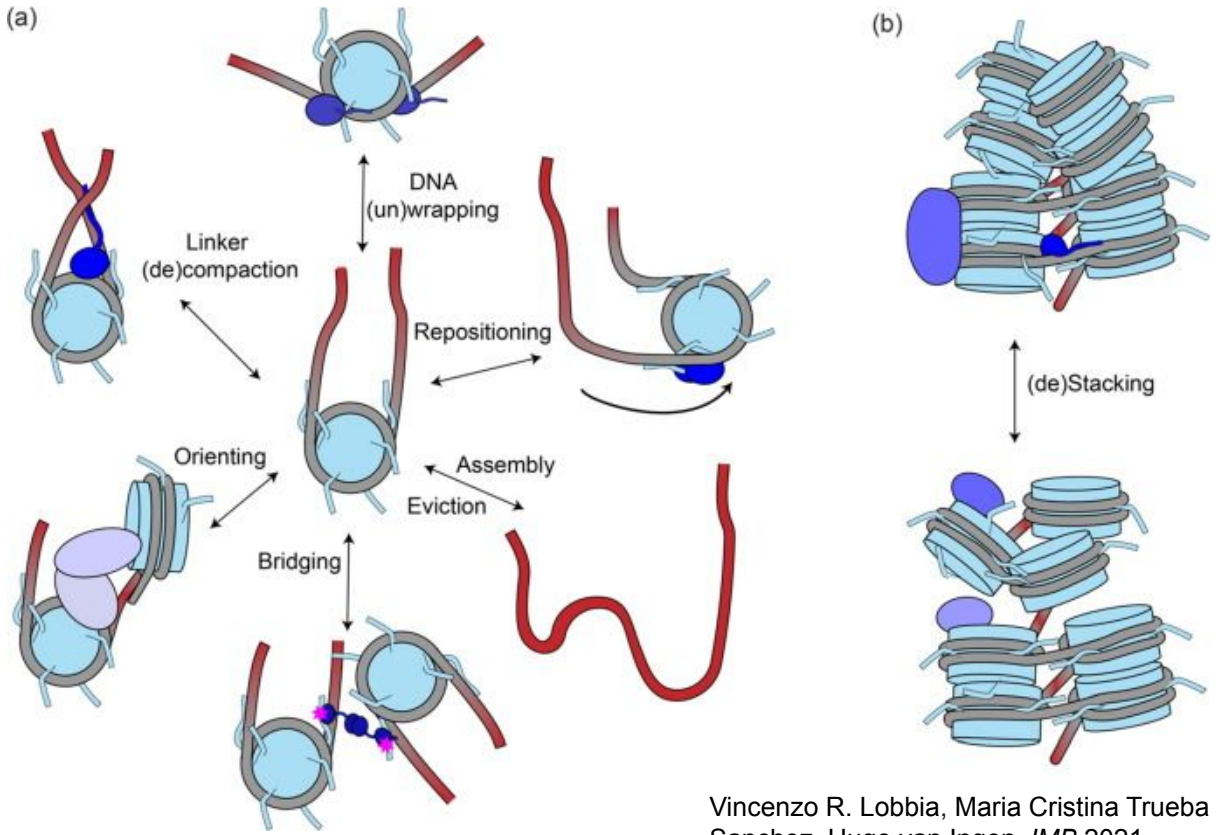
H2B - Protrude from the DNA gyre on the opposite side of H3. Contains two distinct dynamical regions

Lehmann K, Felekyan S, Kühnemuth R, Dimura M, Tóth K, Seidel CAM, Langowski J. *Nucleic Acids Res.* 2020



Chang, L., Takada, S. *Sci Rep* 2016

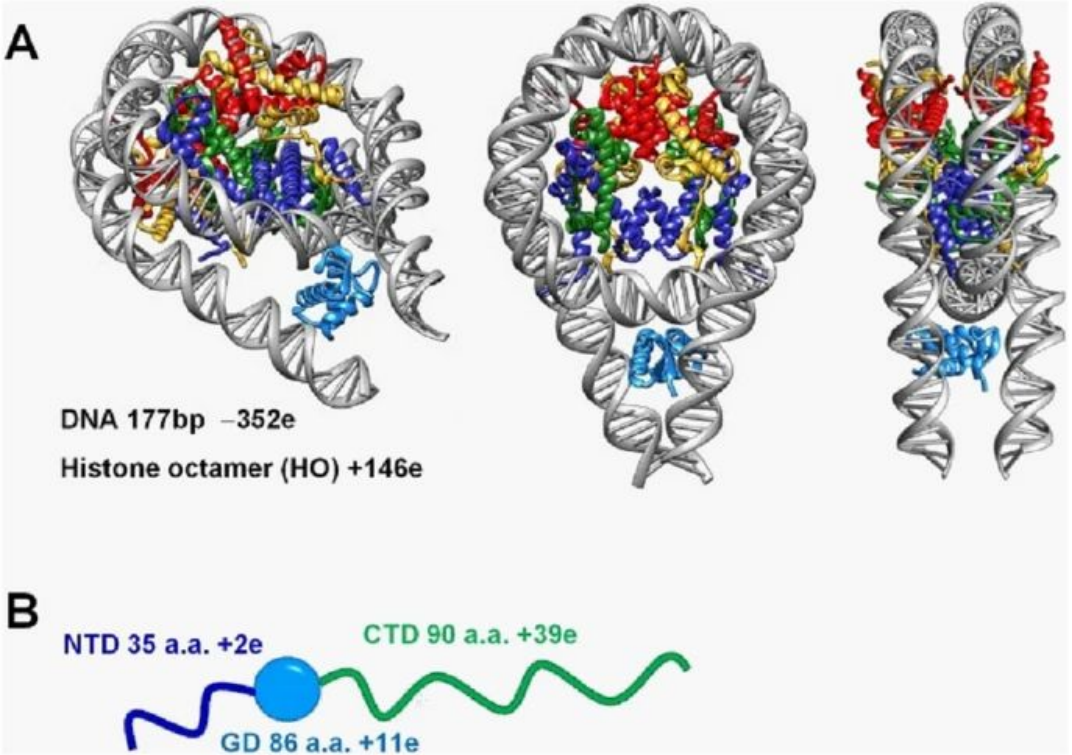
Structural Modulators - Protein interactors



Vincenzo R. Lobbia, Maria Cristina Trueba Sanchez, Hugo van Ingen *JMB* 2021

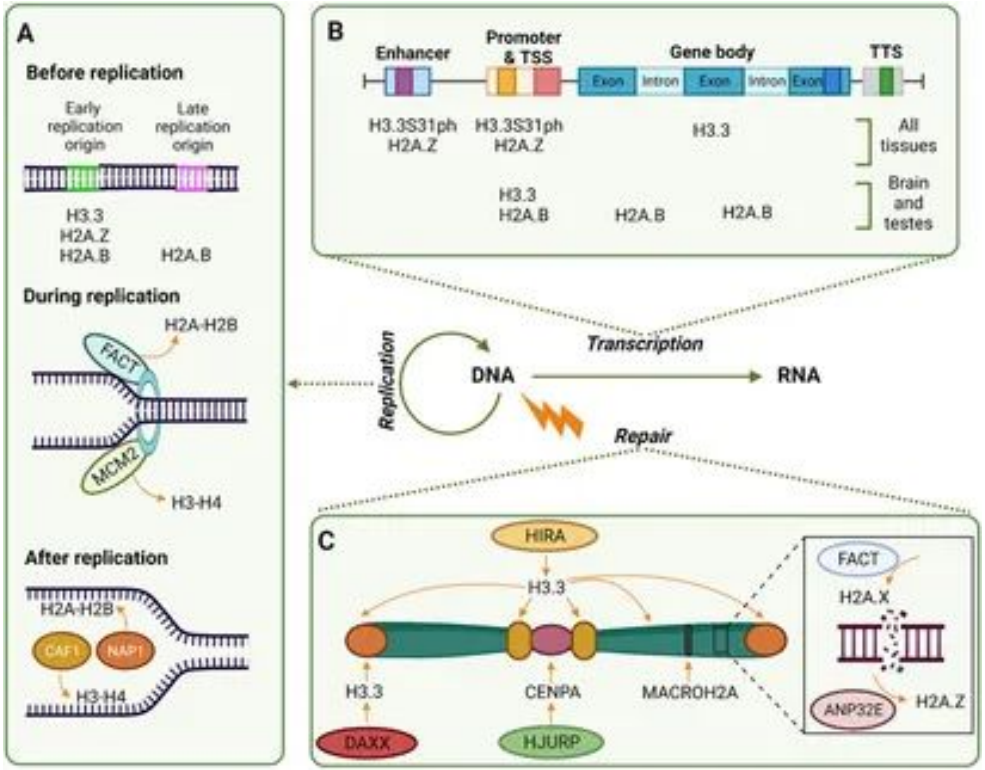
Structure Modulators - Histone H1

- H1 binds to the nucleosome at the dyad interacting with linker DNA forming the chromatosome
- H1 binding leads to linker compaction (less accessible)
- There are 11 different subtypes in humans
- They have many PTMs like core histones
- Interact with other chromatin associated proteins



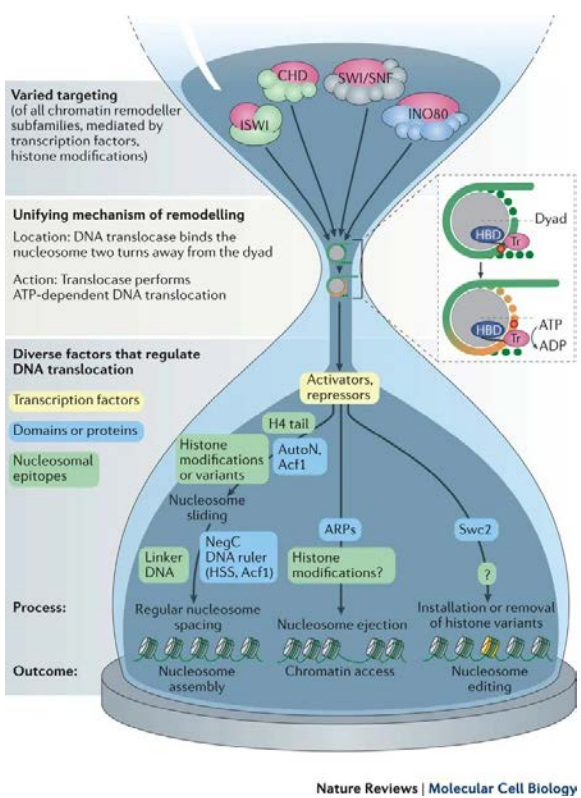
Wang, S., Vogirala, V.K., Soman, A. *et al. Sci Rep* 2021

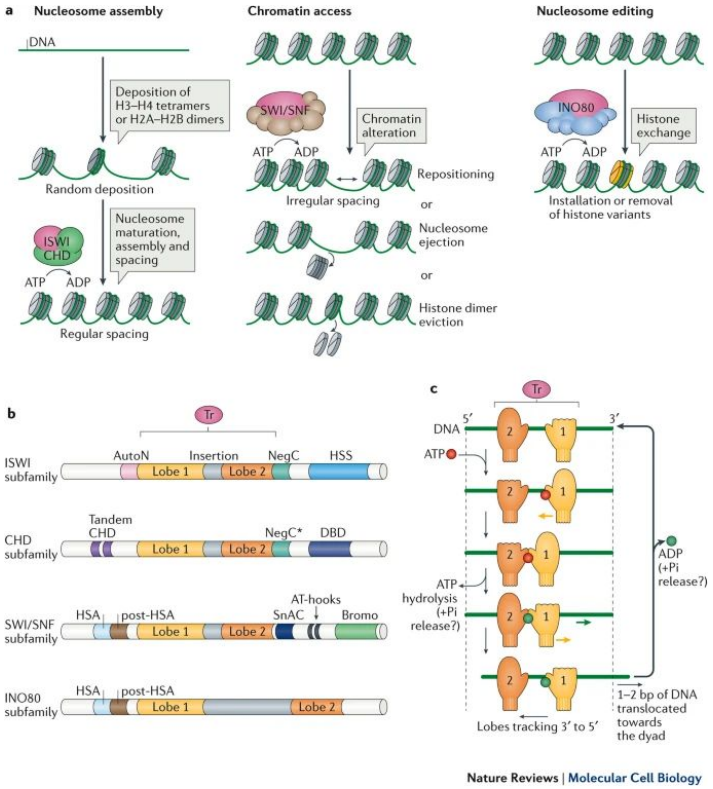
Structural Modulators - Histone Chaperones



Torres-Arciga K, Flores-León M, Ruiz-Pérez S, Trujillo-Pineda M, González-Barrios R and Herrera LA *Front. Genet.* 2022

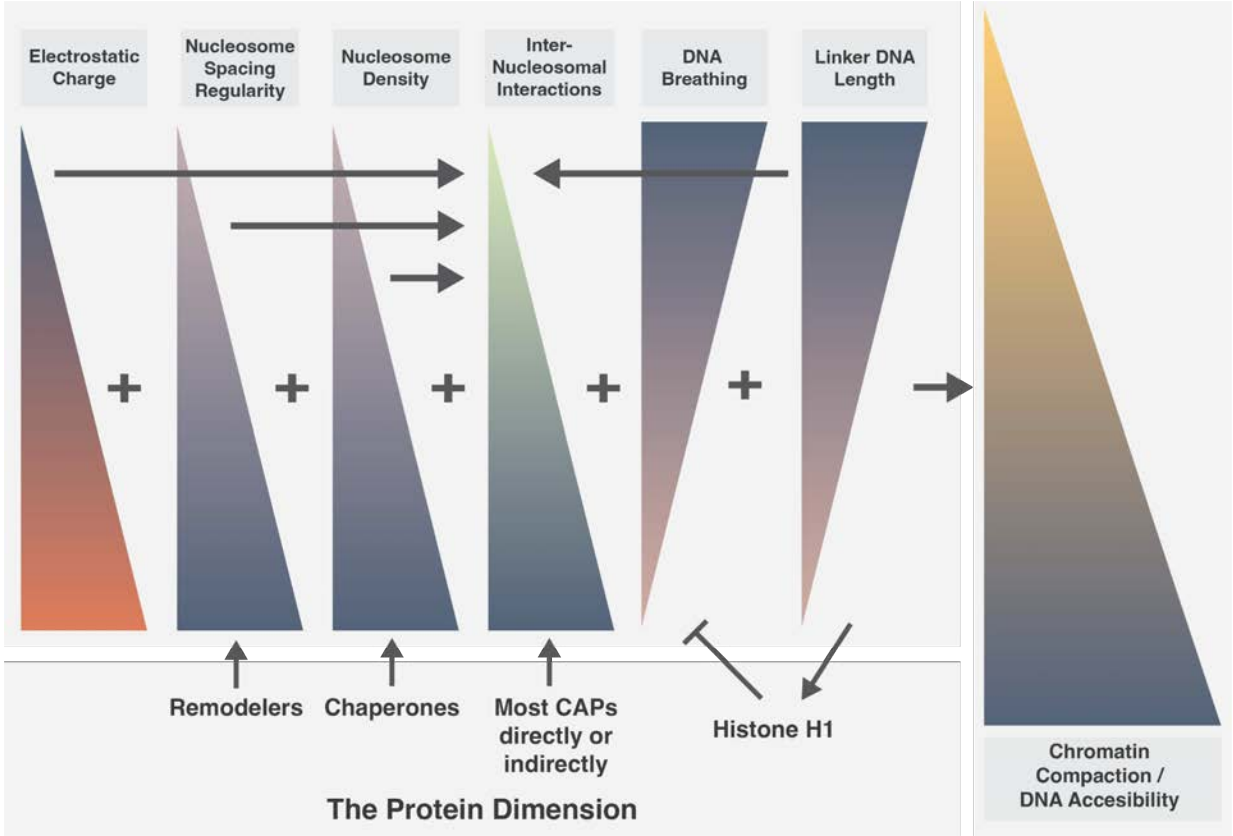
Structural Modulators - ATP dependant remodelers





Clapier, C., Iwasa, J., Cairns, B. *et al. Nat Rev Mol Cell Biol* 2017

Review - What Affects Chromatin Dynamics



Unraveling Chromatin Structure - 30nm Fiber

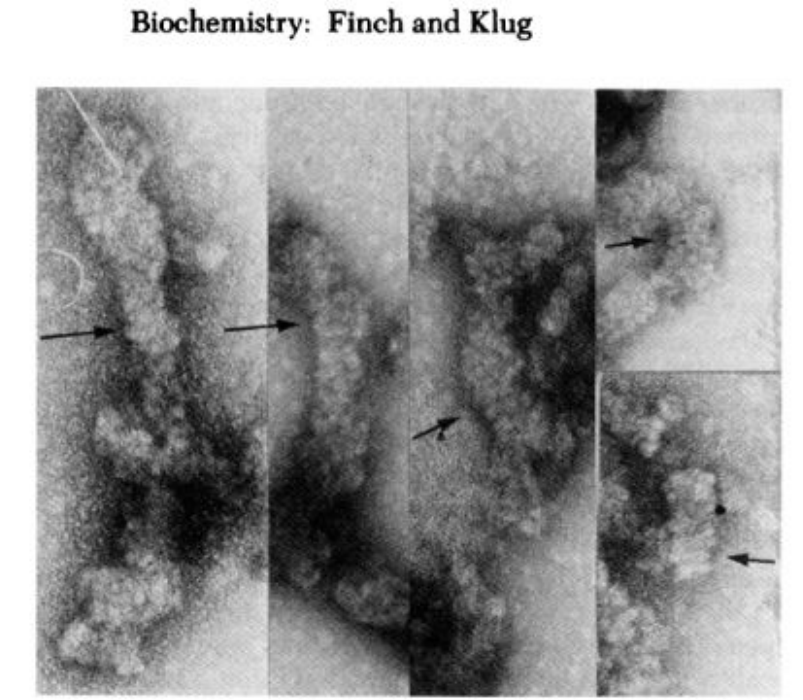
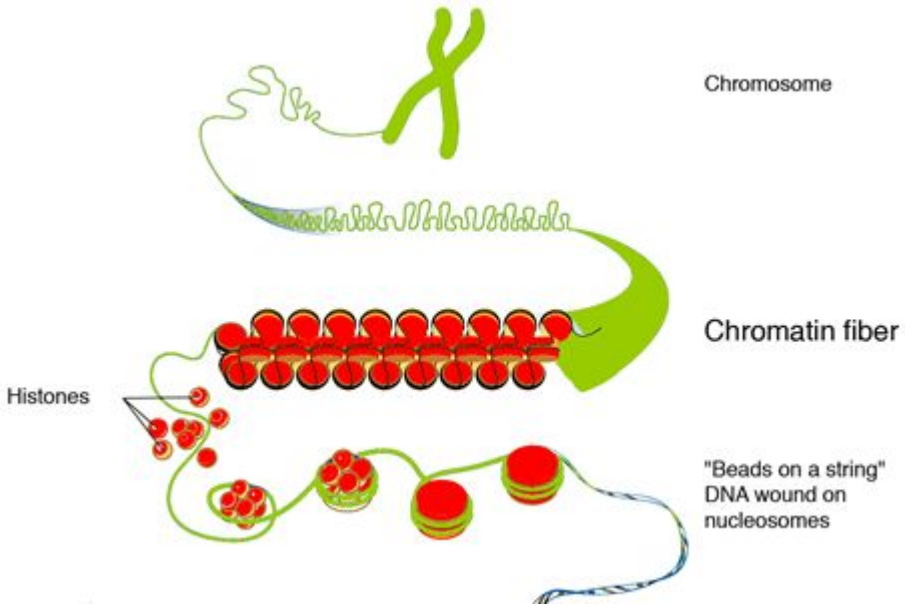


FIG. 3. Chromatin in 0.5 mM Mg^{++}, negatively stained. Arrows indicate transverse striations across elongated particles. ×140,000.

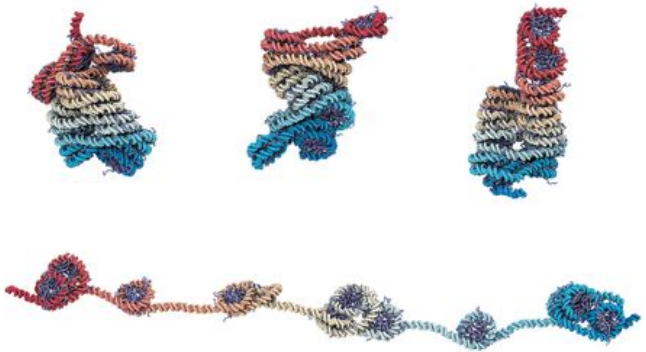
Finch JT, Klug A. *Proc Natl Acad Sci USA.* 1976



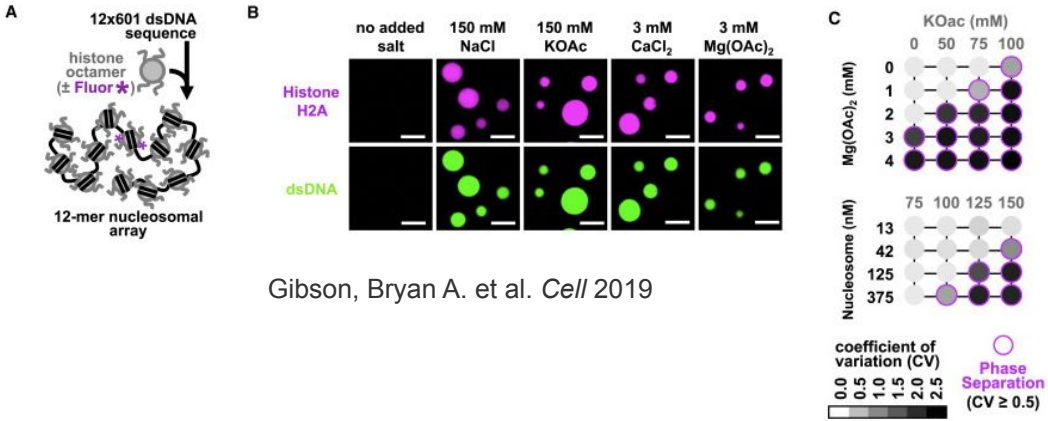
Genome.gov

Unraveling Chromatin Structure - Liquid-Like Nucleosome Clutches

- In reality the data suggests that under physiological conditions chromatin is a lot more dynamic and the properties of chromatin are “liquid-like”
- Nucleosomes are often grouped in “clutches” that are highly variable in structure
- Structure/accessibility is dependant on the combination of chromatin modifiers described earlier

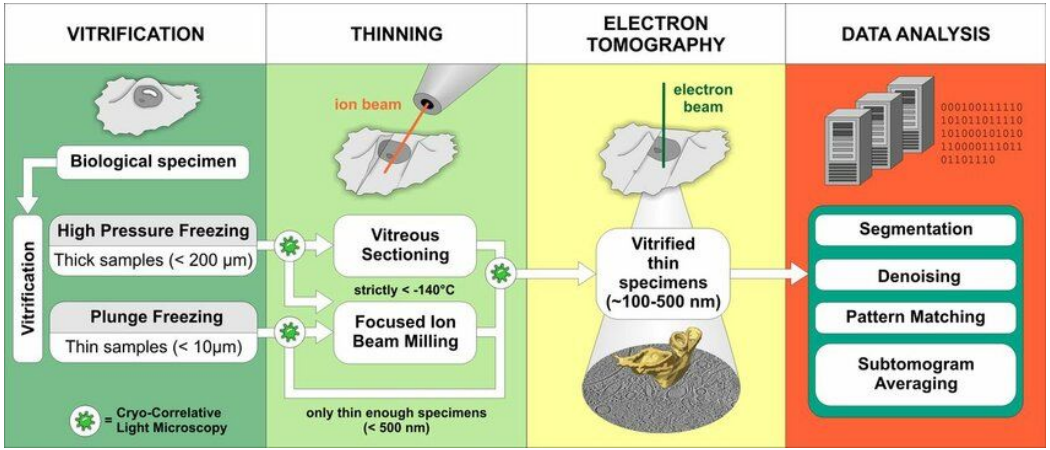


Shuming Liu, Xingcheng Lin, Bin Zhang, *Nucleic Acids Research* 2022



Gibson, Bryan A. et al. *Cell* 2019

Chromatin Structure Techniques - Cryo-FIB/CryoET

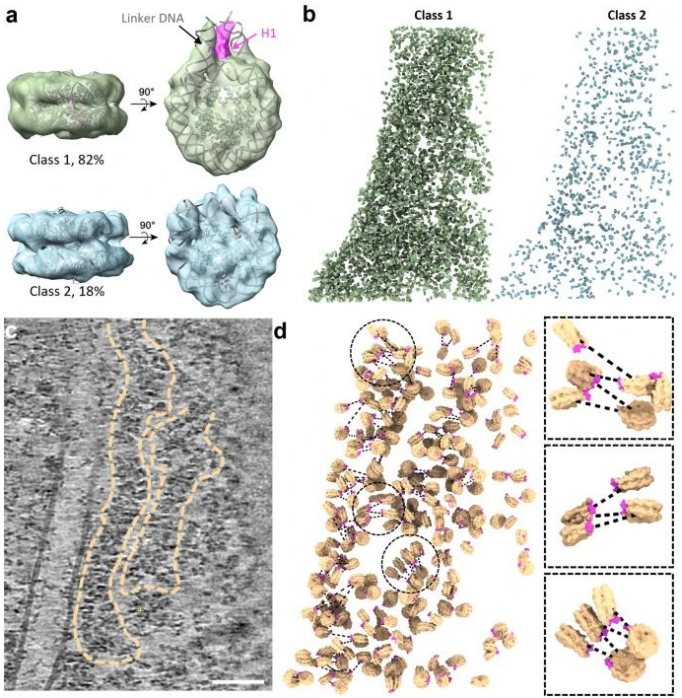


Vladan Lučić, Alexander Rigort, and Wolfgang Baumeister. *JCB* 2013

Article | Open access | Published: 10 October 2023

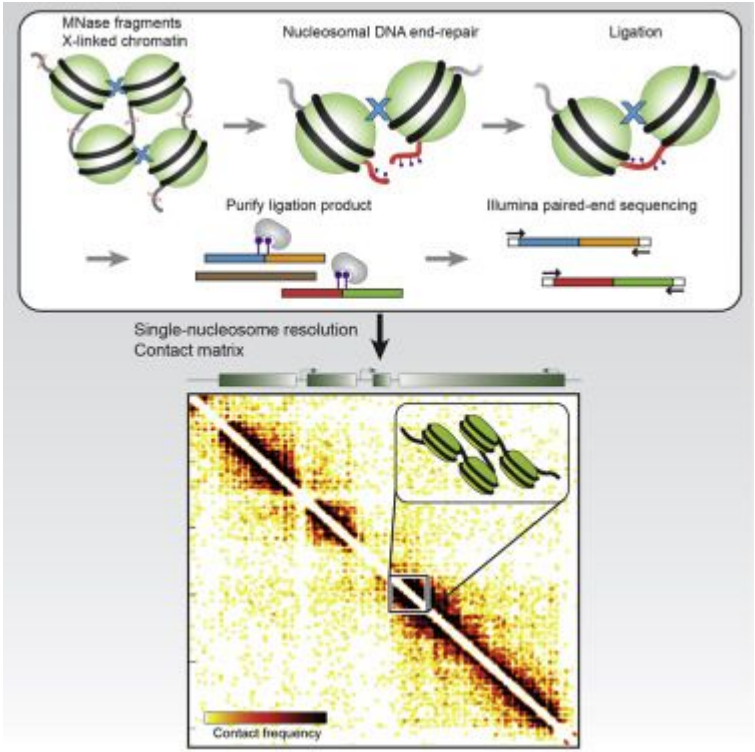
Structure of native chromatin fibres revealed by Cryo-ET in situ

Zhen Hou, Frank Nightingale, Yanan Zhu, Craig MacGregor-Chatwin & Peijun Zhang



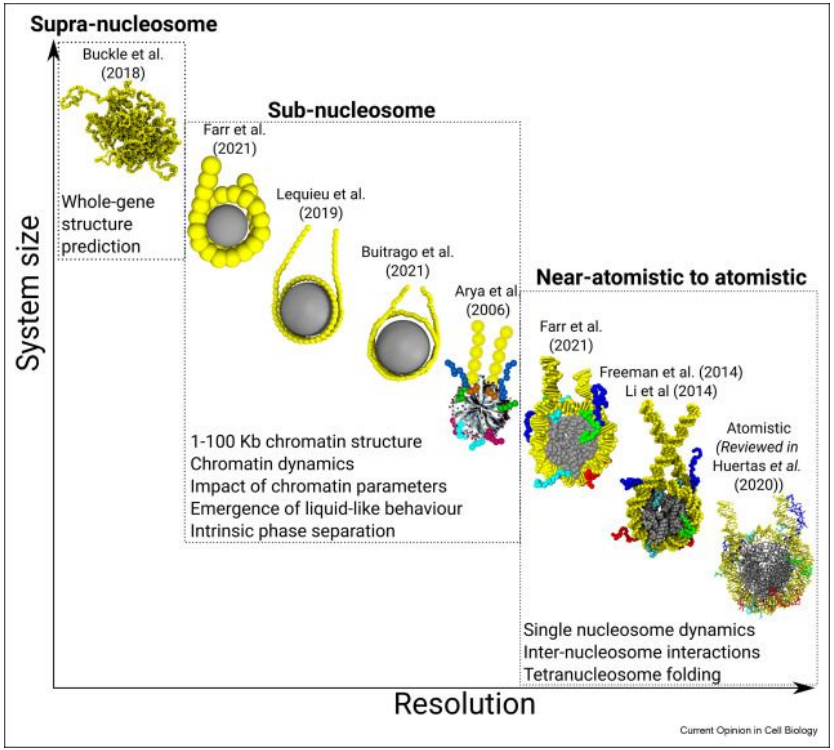
Unraveling Chromatin Structure - Micro-C

- Micro-C like its predecessor Hi-C gives information on DNA contacts
- Hi-C uses restriction enzymes that give ~4kb resolution with variable distances between cut sites.
- Micro-C gets around this limitation by instead using MNase-digestion to get down to nucleosomal resolution
- Has been combined with Capture-C to allow for greater depth at targeted genomic regions of interested (Micro-Capture-C)

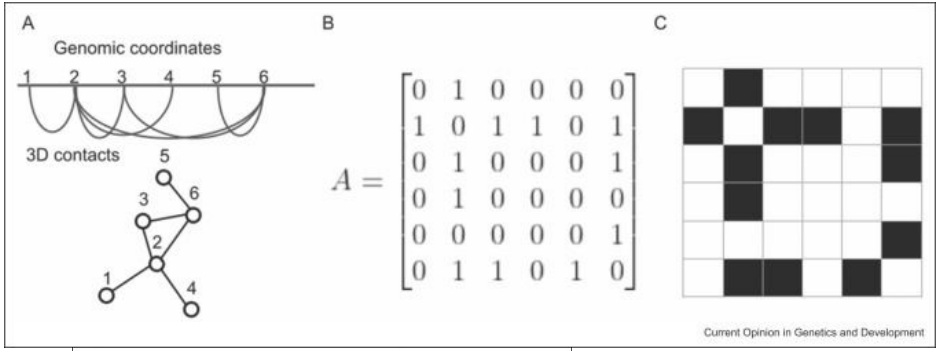


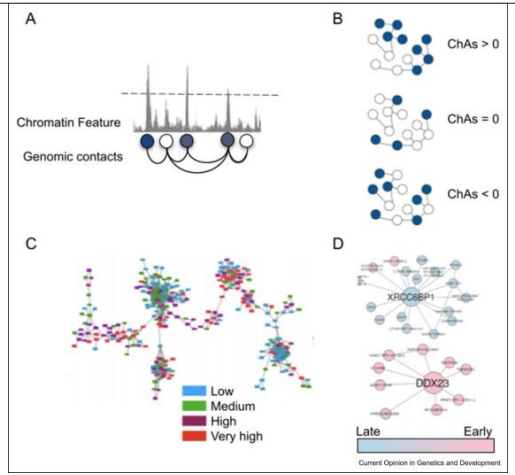
Hsieh, Tsung-Han S. et al. Cell 2015

Models of Chromatin Structure - Network Modeling



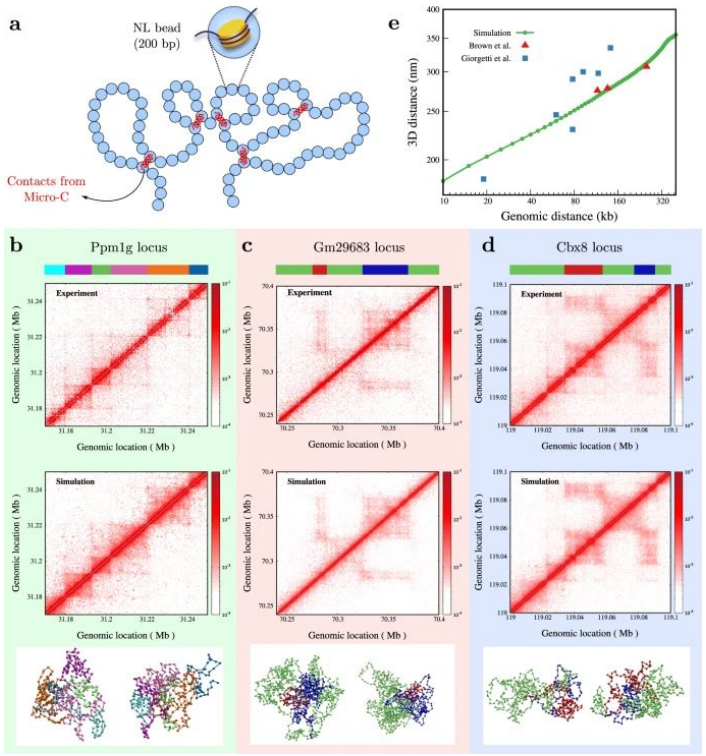
Jan Huertas, Esmae J. Woods, Rosana Collepardo-Guevara
Current Opinion in Cell Biology 2022



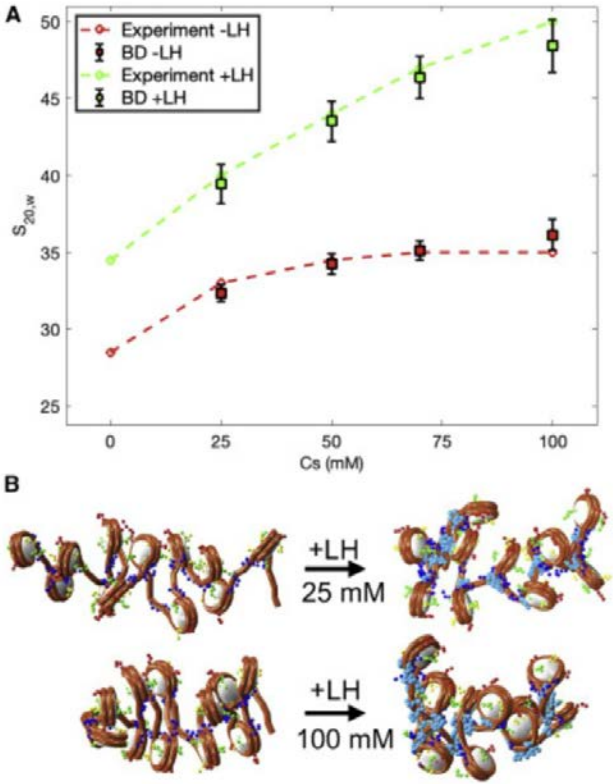


Vera Pancaldi Current Opinion in Genetics & Development 2023

Models of Chromatin - Examples at different scales



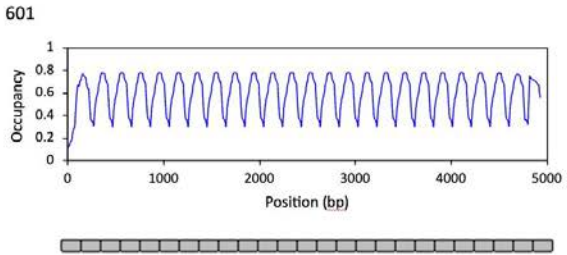
Kadam, S., Kumari, K., Manivannan, V. *Nat Commun* 2023

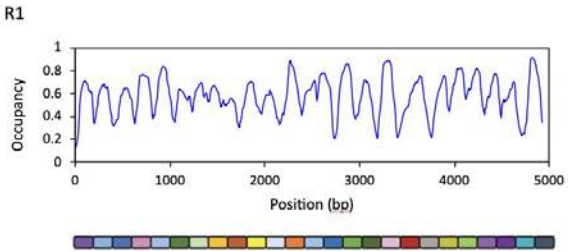


Zilong Li, Stephanie Portillo-Ledesma, Tamar Schlick. Biophysics Journal 2023

Models of Chromatin Structure - Gilbert Lab Arrays

Aim: Investigate how nucleosome positioning irregularity affects the structural and dynamic properties of chromatin

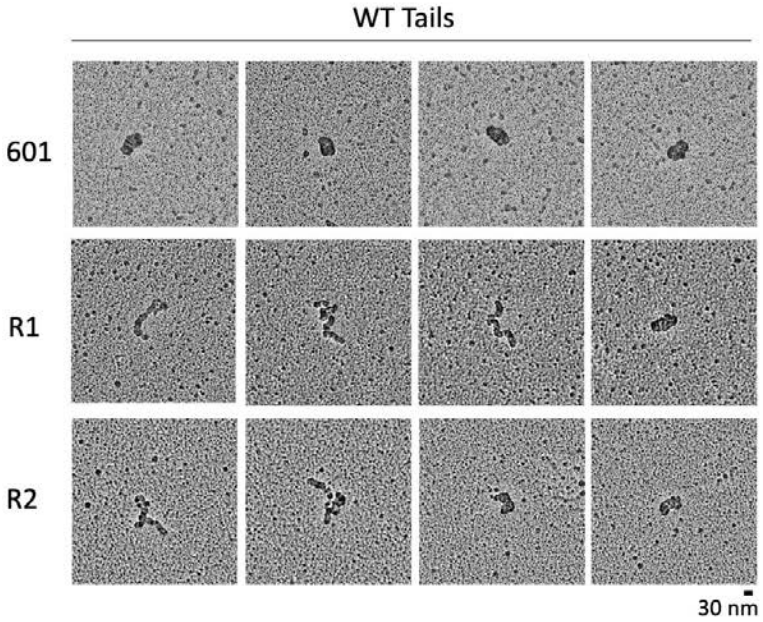


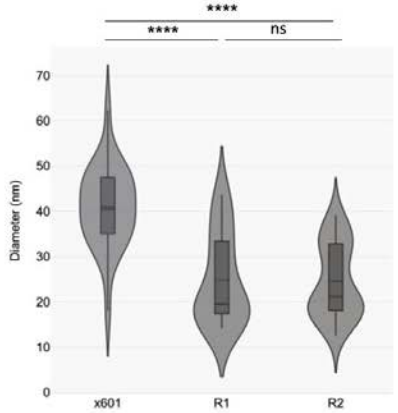


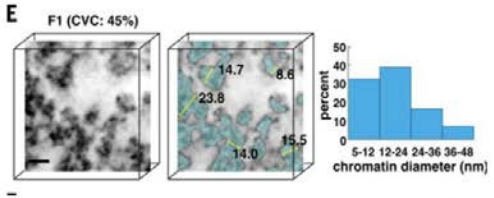
Predictions based on algorithm by van der Heijden et al., 2012

Models of Chromatin Structure - Gilbert Lab Arrays

Irregular nucleosome spacing destabilizes chromatin folding



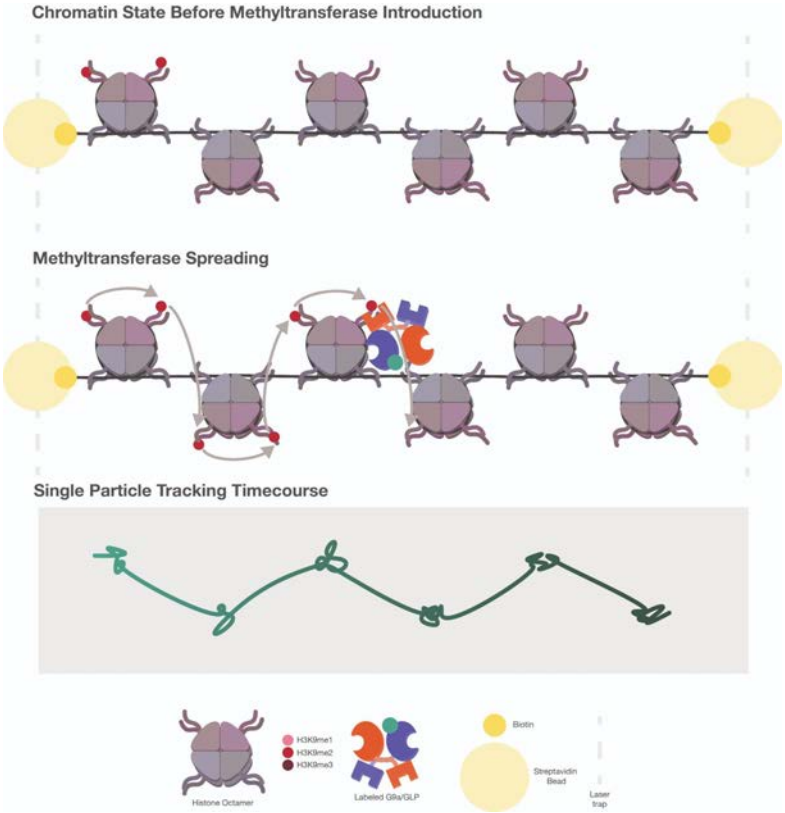


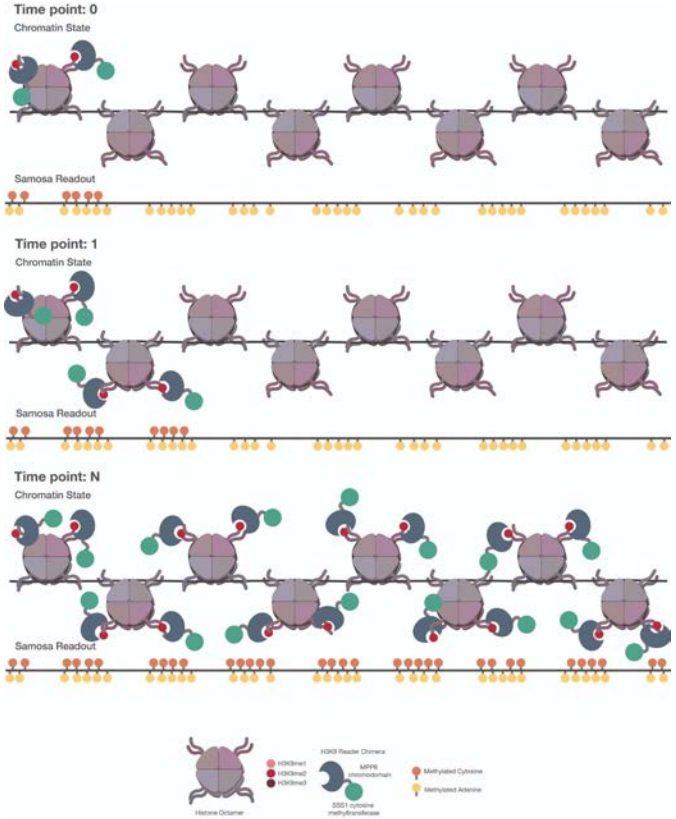


- 601 arrays fold to canonical ~30-40 nm fibre

(Ou et al., 2017, Science)

Models of Chromatin Structure - Current Outlook





Acknowledgements