Assembling the Genome:
Hierarchical Structure from the Basic Building Blocks

Plans for this morning

1.) Consider the fundamental building blocks for genome architecture

2.) Problems in assembly and how (we think) some of these are overcome

3.) How (we think) these are organized to produce a functioning transcriptional factory

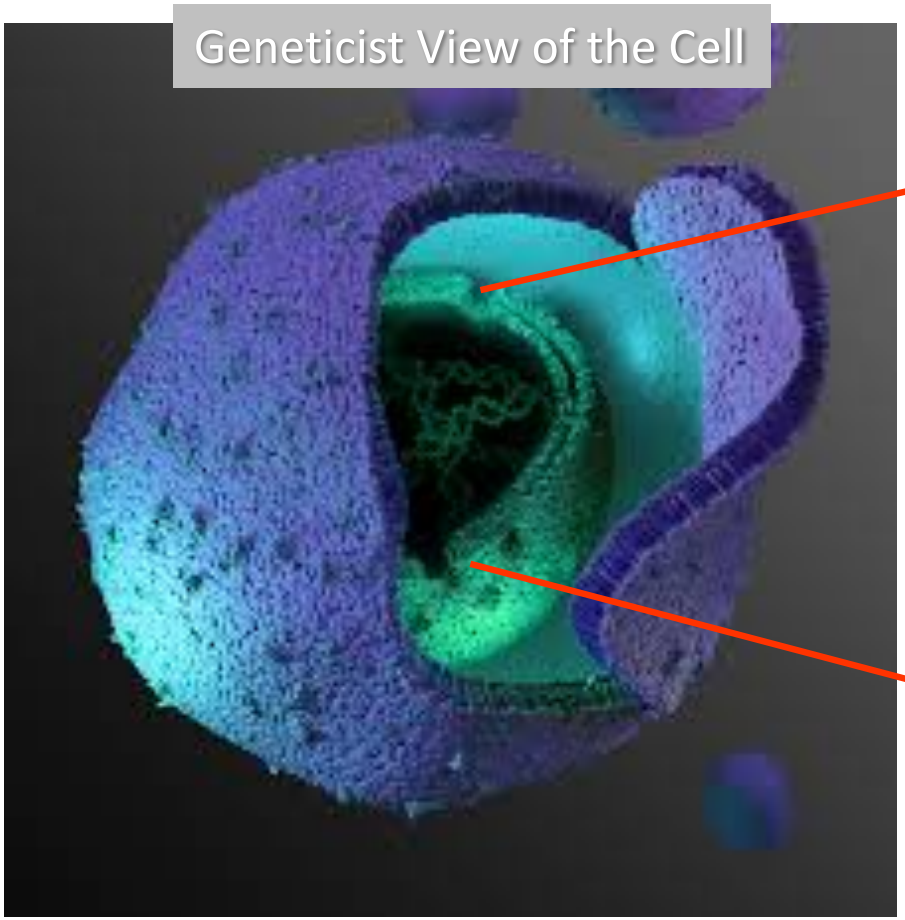
Group-based activity

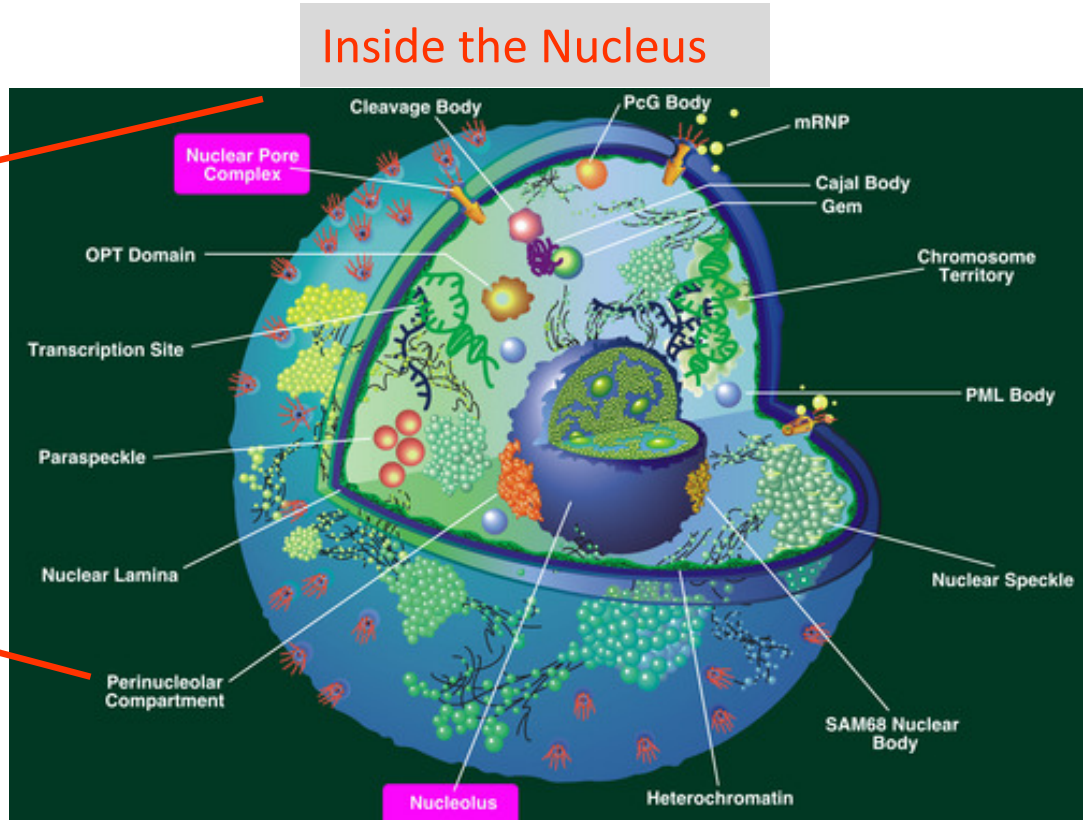
Write a grant

Central Library for Information

– the Blueprints for Carbon-Copy replicate

– the Program that converts Cell-Type and Fate





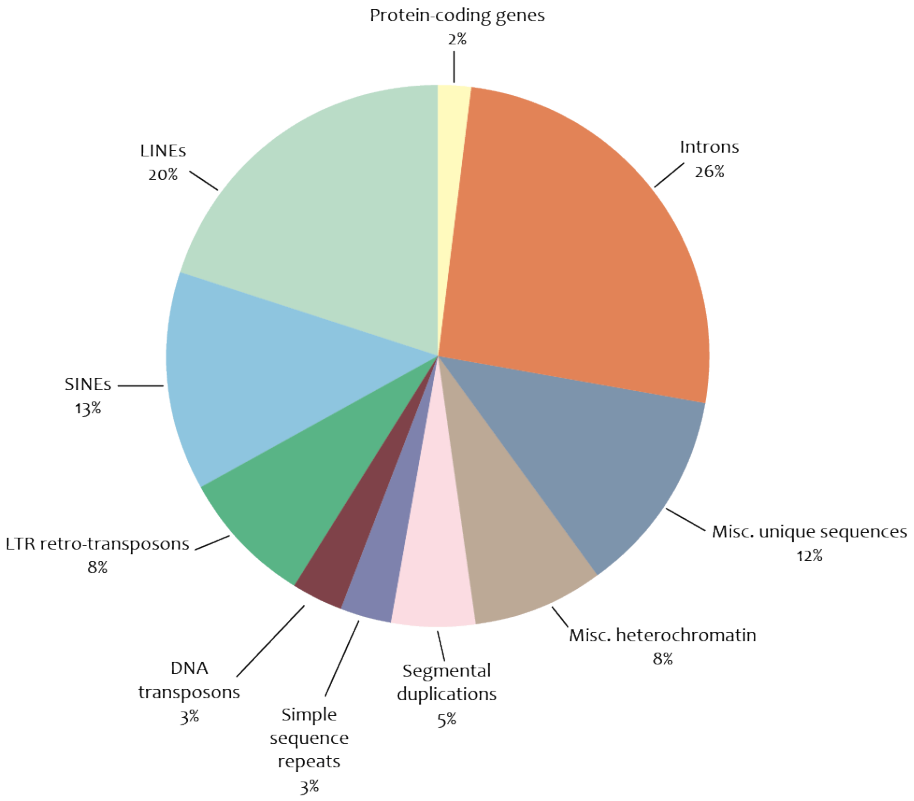
Largest organelle in the cell at ~6μm--focus of attention for most geneticists

Two Levels of Information Storage

Human genome

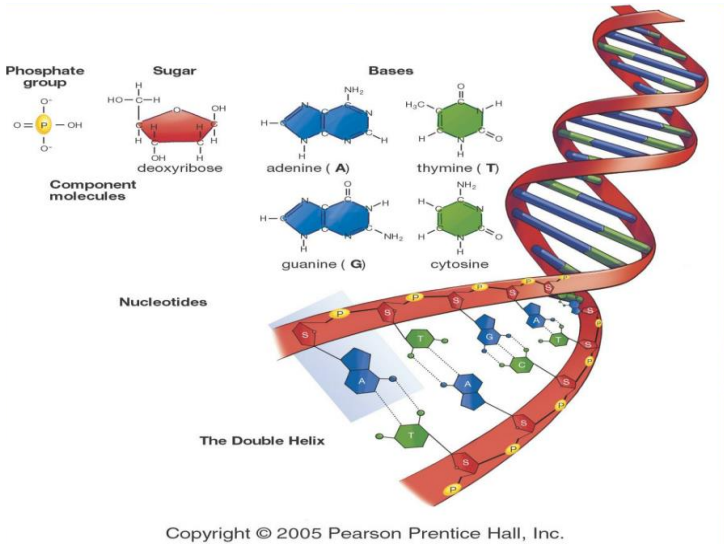
1. Coding Information (1.5% of the human genome)

2. Regulatory Information (estimates of 8% - 40%)



Fundamental components

DNA



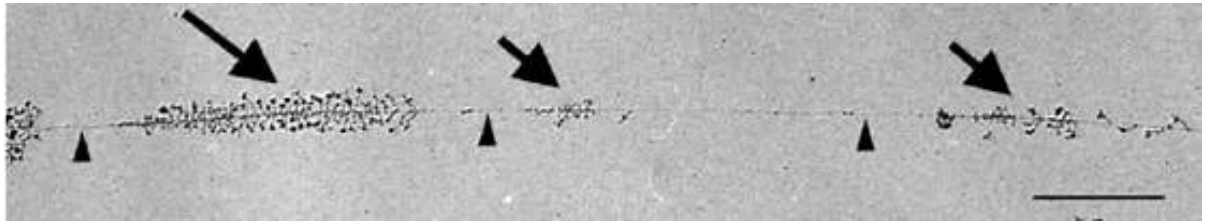
Protein

Histones-Major components

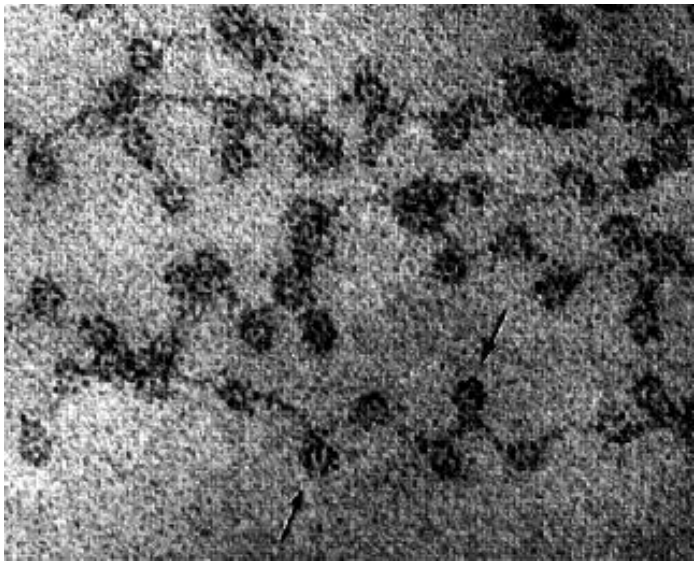
Non-Histones-minor components which include transcription factors and other structural components

Together these compose the chromatin (functional) component of the genome

Early EM work formed initial view of chromatin



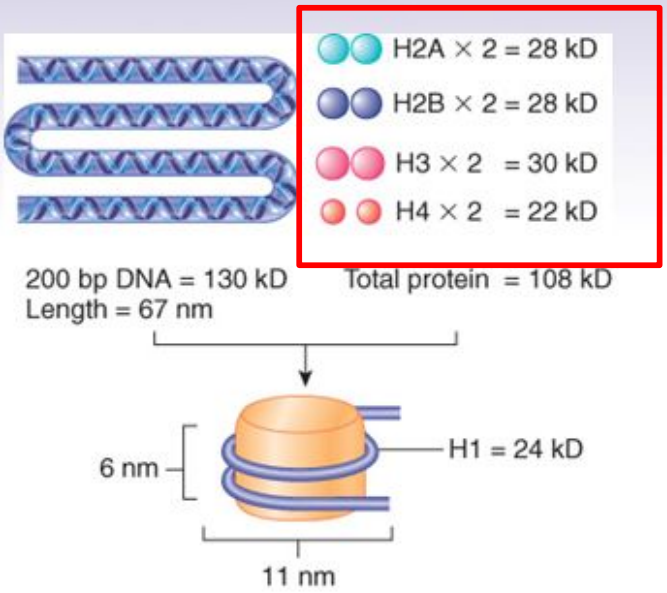
Miller spreads (1969)



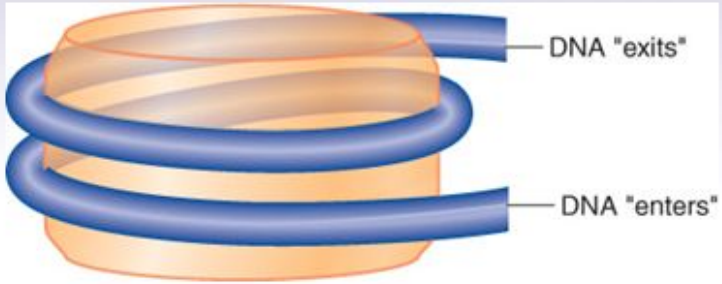
Beads on a string configuration
'Nu bodies'
Olins and Olins (1973)

Organization of components into Building Blocks

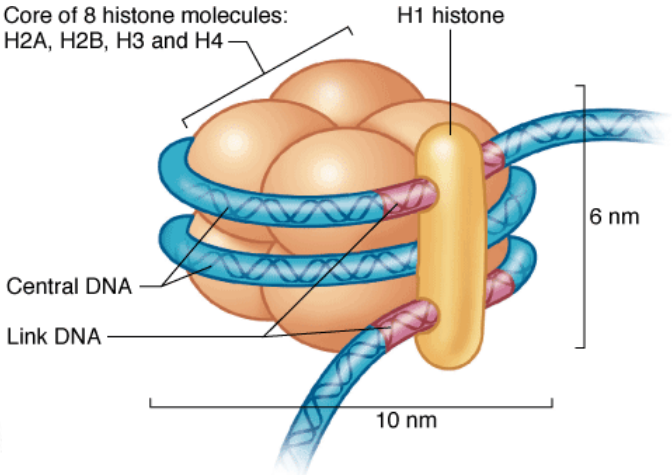
The Nucleosome Is the Subunit of All Chromatin



The nucleosome consists of approximately equal masses of DNA and histones (including H1). The predicted mass of the nucleosome is 262 kD.



The nucleosome is roughly cylindrical, with DNA organized into 1 3/4 turns around the surface.

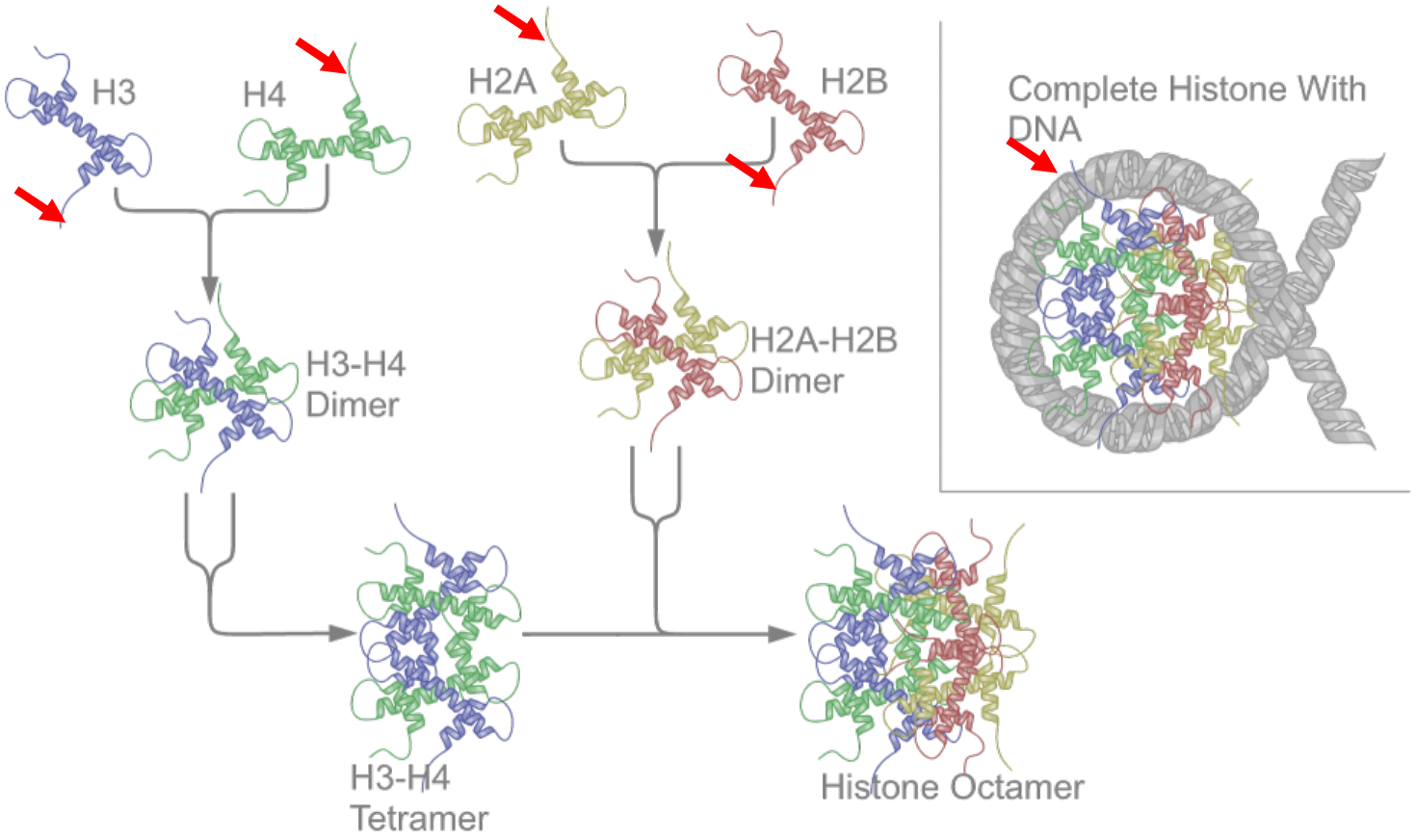


Source: Mescher AL: *Junqueira's Basic Histology: Text and Atlas, 12th Edition:* http://www.accessmedicine.com

Self-Assembly

Histone structure → Tetramer Formation → Nucleosome Productions

N & C-terminal tails



Roles—Compacting DNA

Compartmentalizing DNA (Histone code)

How much DNA in a cell?

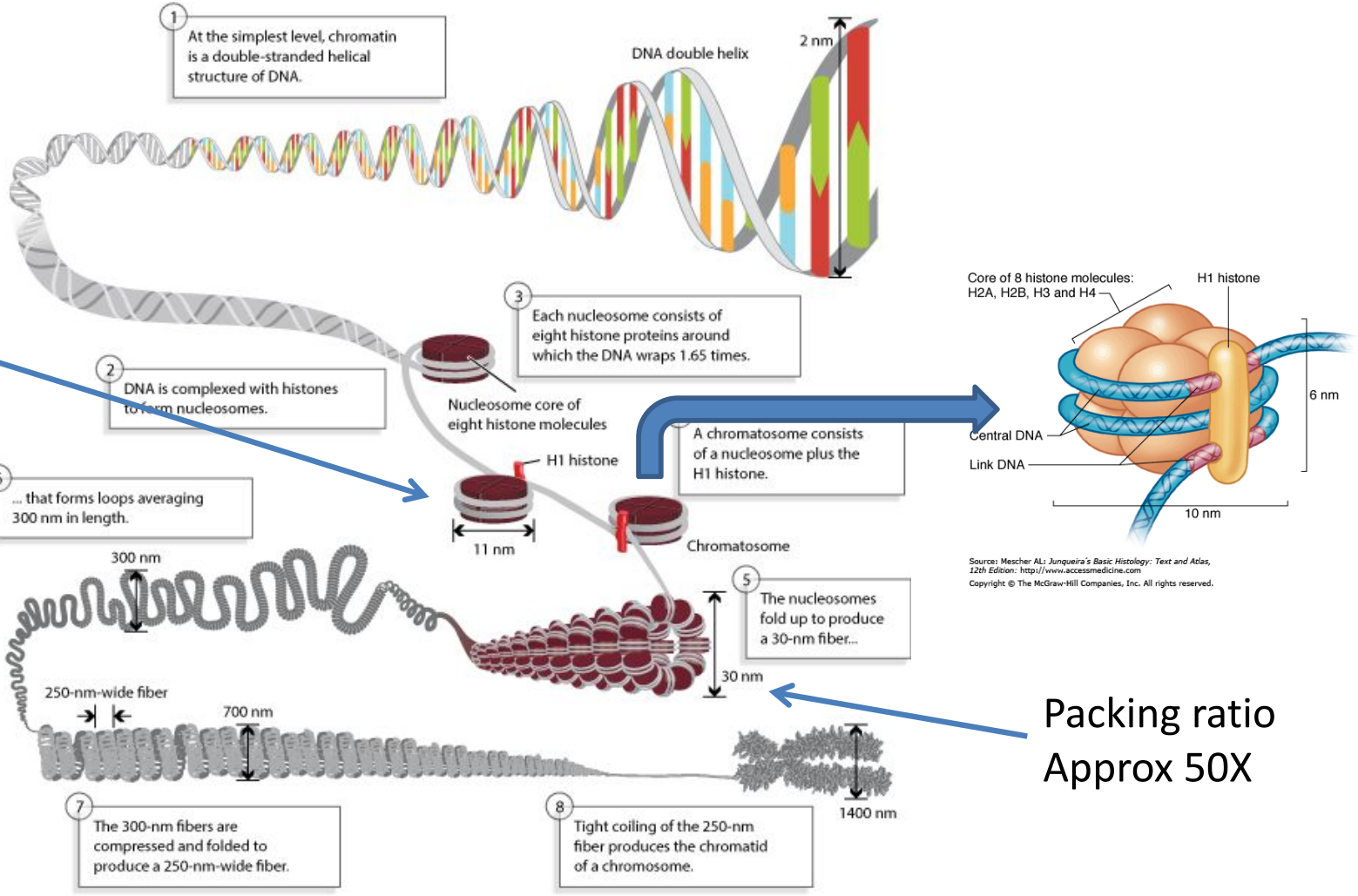
In Human	Female	6.06×10^9 bp
	Male	5.96×10^9
E coli		4.6×10^6
Yeast		12×10^6
S cerevisiae (1^{st} eucaryotic genome sequenced)		
Lungfish		130×10^9
(largest vert. genome)		
Amoeboid		670×10^9
(Largest known genome)		

~2 metres of DNA/cell
Initial sequence cost < $3 billion

Compaction of Two Meters of DNA (Human)

Proposed hierarchical structure of chromatin

Packing ratio
5-10X

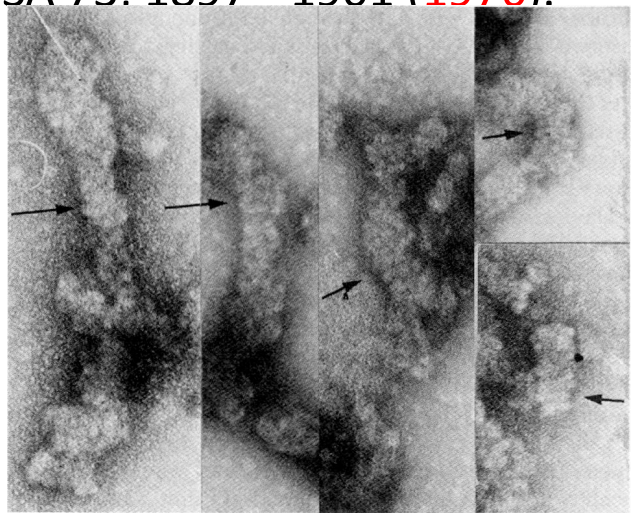


Packing ratio
Approx 50X

A bit of the History

Finch, J. T. & Klug, A. Solenoidal model for superstructure in chromatin. Proc. Natl Acad. Sci. USA 73. 1897–-1901 (1976).

Isolated rat liver chromatin
Dialysed against 0.5mM $MgCl_2$.



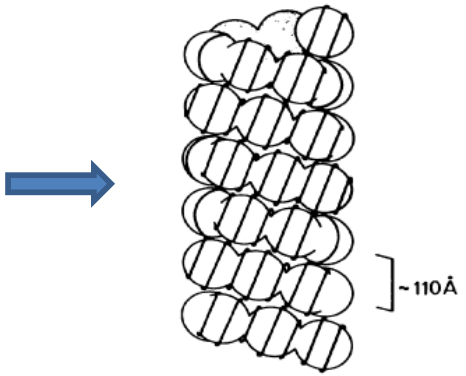
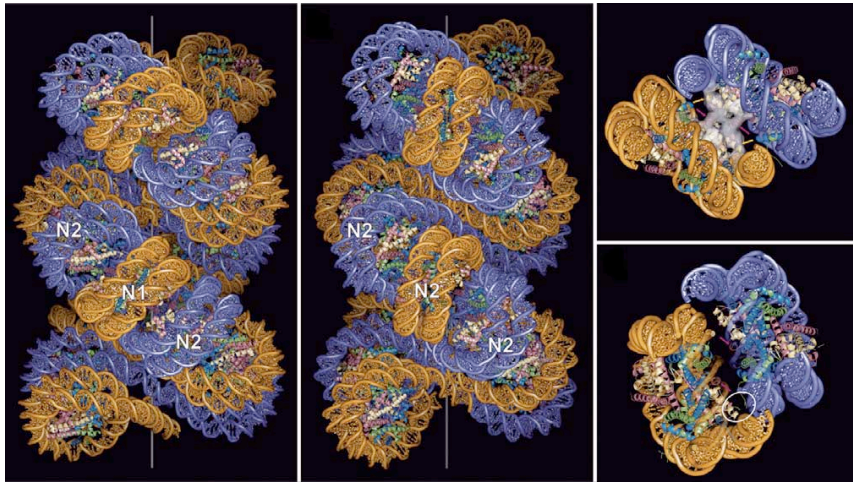


FIG. 7. Schematic diagram showing the folding of a nucleofilament into a solenoid. The thin line shown wound as a helix along the nucleofilament is intended to represent the folding of the DNA double helix on the outside of a protein core (5, 24, 8); it is highly schematic, since the path or fold is not known.

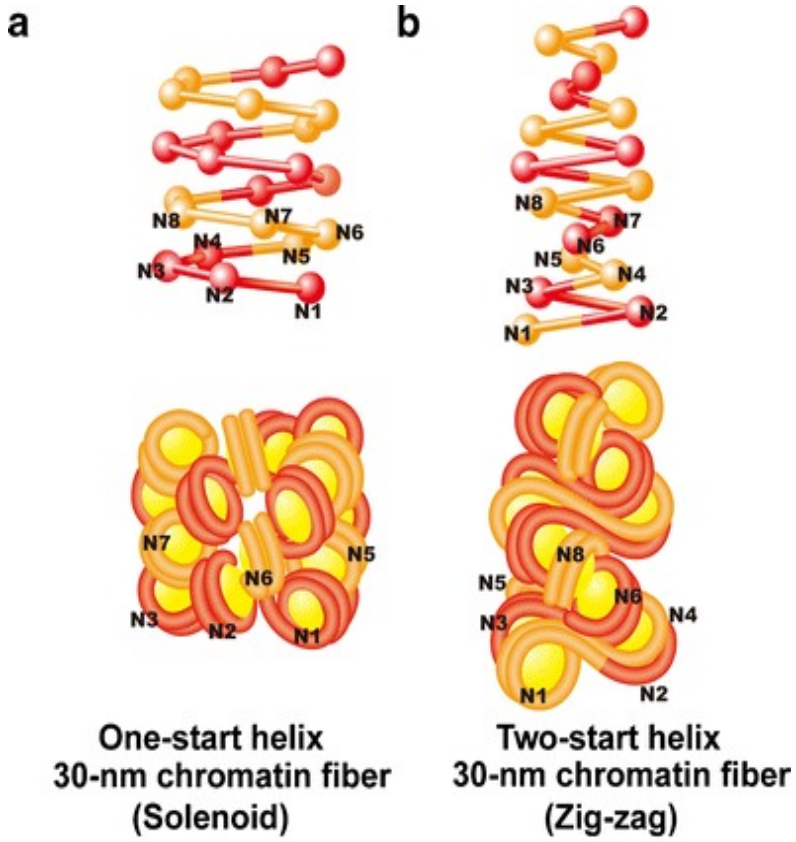
Schalch T, Duda S, Sargent DF, Richmond TJ (2005). X-ray structure of a tetranucleosome and its implications for the chromatin fibre. Nature. **436** (7047): 138–41



Determined by reconstitution of nucleosome (tetra nucleosome)

Two-start helix

Types of solenoidal structure



RESEARCH ARTICLE

Science (2017) **357,** eaag0025

CHROMATIN STRUCTURE

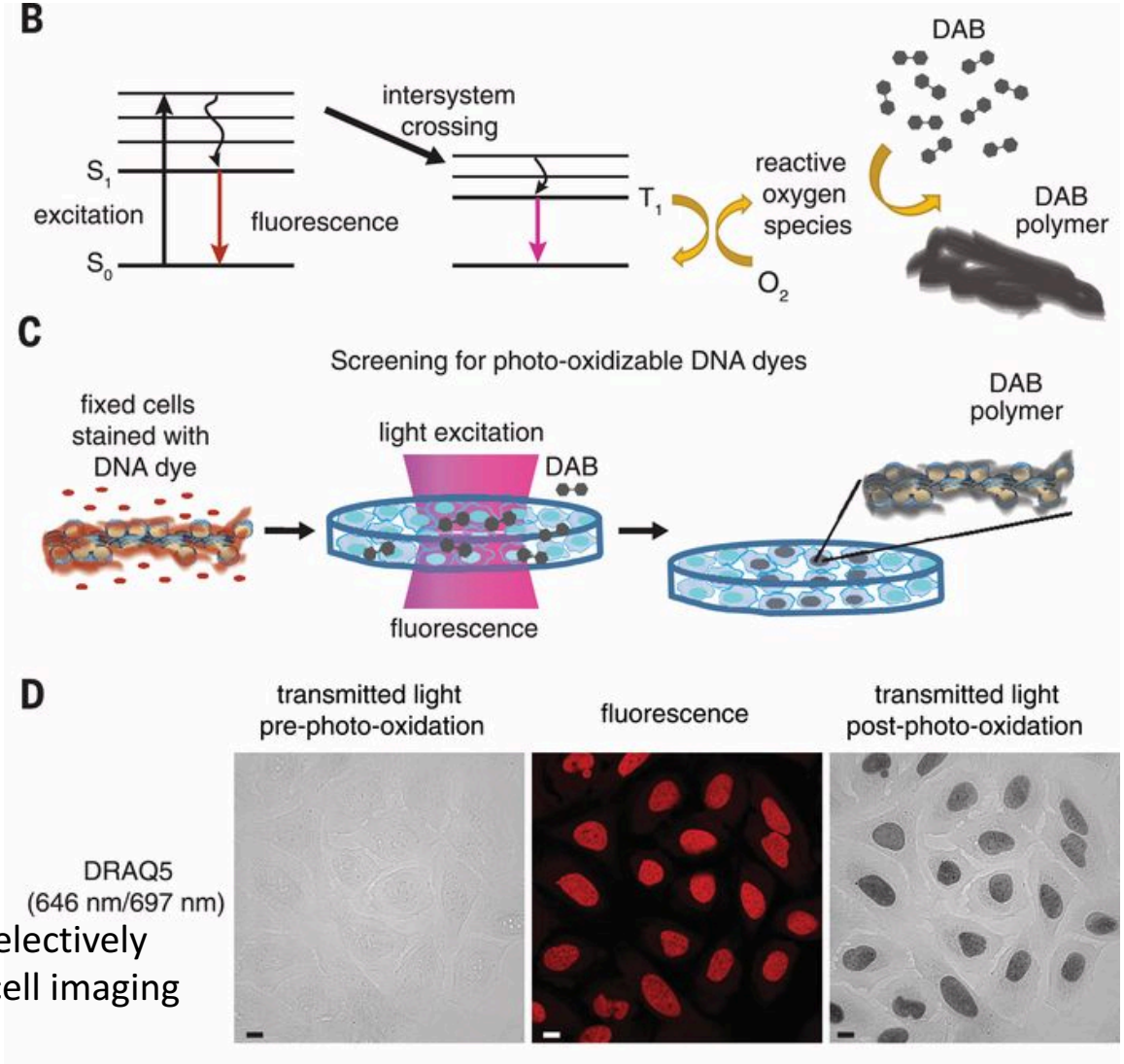
ChromEMT: Visualizing 3D chromatin structure and compaction in interphase and mitotic cells

Horng D. Ou,[1] Sébastien Phan,[2] Thomas J. Deerinck,[2] Andrea Thor,[2] Mark H. Ellisman,[2,3] Clodagh C. O'Shea[1]*

[1]Molecular and Cell Biology Laboratory, Salk Institute for Biological Studies, 10010 North Torrey Pines Road, La Jolla, CA 92037, USA. [2]National Center for Microscopy and Imaging Research, Center for Research in Biological Systems, University of California, San Diego, 9500 Gilman Drive, La Jolla, CA 92093, USA. [3]Department of Neurosciences, University of California, San Diego, 9500 Gilman Drive, La Jolla, CA 92093, USA.
*Corresponding author. Email: oshea@salk.edu

Fig. 1 A fluorescent DNA-binding dye that catalyzes local DAB polymerization on chromatin in the nucleus.

Traditionally, chromatin stained with electron dense, heavy metal stains such as OsO_4 which do not stain DNA selectively; prefer RNA, lipids and proteins



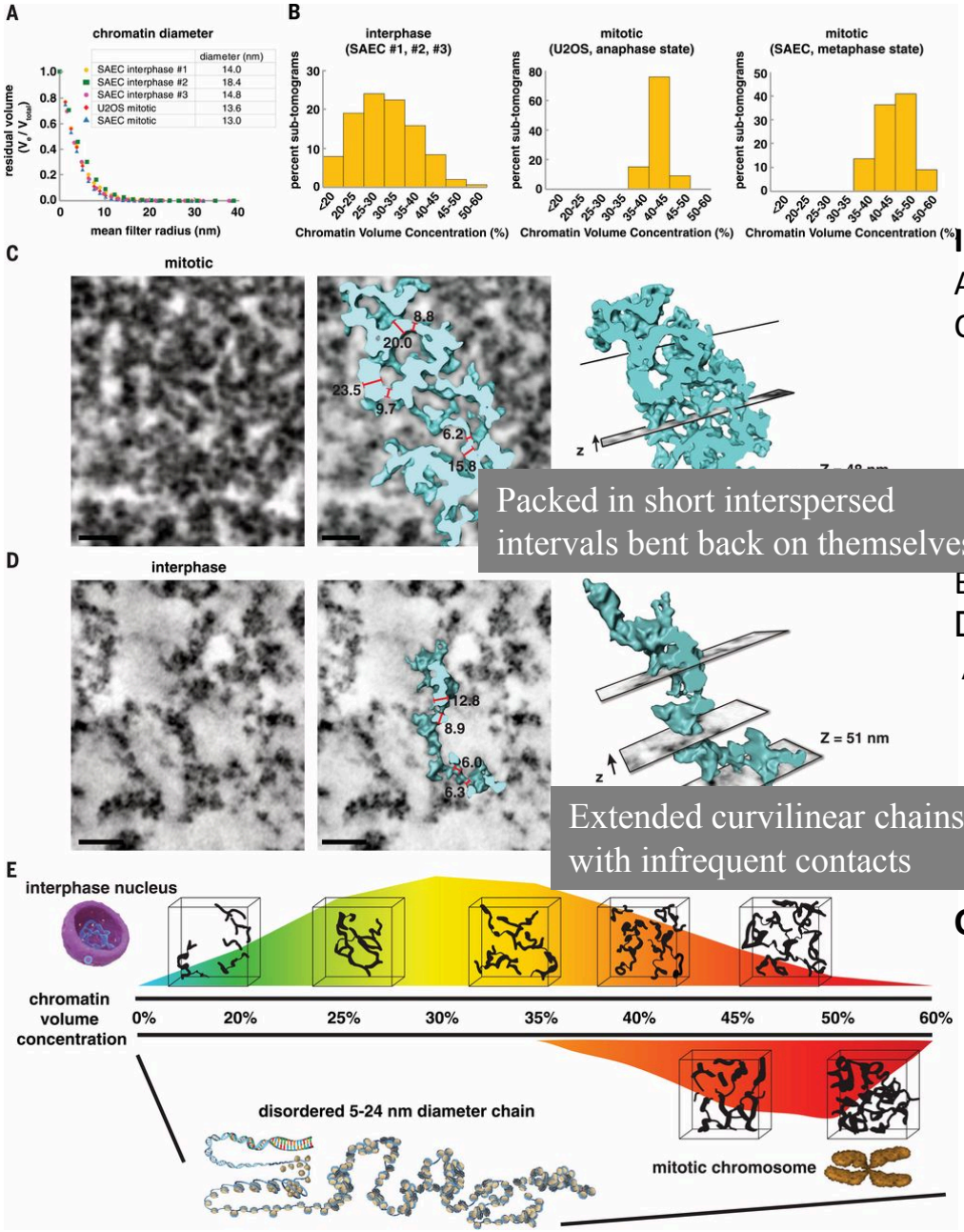
Membrane permeable dye that selectively binds dsDNA and is used for live cell imaging

Horng D. Ou et al. Science 2017;357:eaag0025

Two innovations - Staining technique and EM Tomography

Science AAAS

Disordered 5- to 24-nm-diameter chromatin chains are flexible and can be packed together at different concentration densities in interphase nuclei and mitotic chromosomes.



In both mitotic and interphase-

Ave diameter ~14nm

Chromatin is in disordered primary polymer chains ranges from 5-24nm

Interphase—packed at higher densities

Suggest that scaffolding factors constrain architecture

Basic structure

Disordered 5- to 24-nm-diameter chromatin chains are

Advantage—

Flexible and can be packed together at different concentration densities in interphase nuclei and mitotic chromosome

May explain speed at which chromosome condense

Questions-

Heterochromatin assoc with high CVC

Is [3D chromatin] a universal principle that determines functional activity & accessibility of genomic DNA

Science

AAAS

Why is this important?

Chromatin is the functional component of the genome

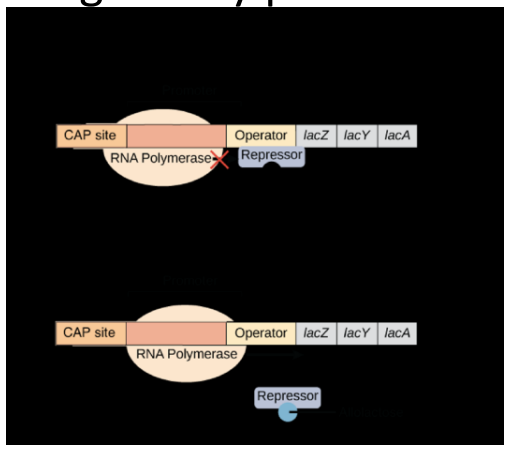
Chromatin structure is important for gene activity

This notion of chromatin structure provides different problems to confront

Why regulate genes?

Unicellular organisms-

Regulatory promoters



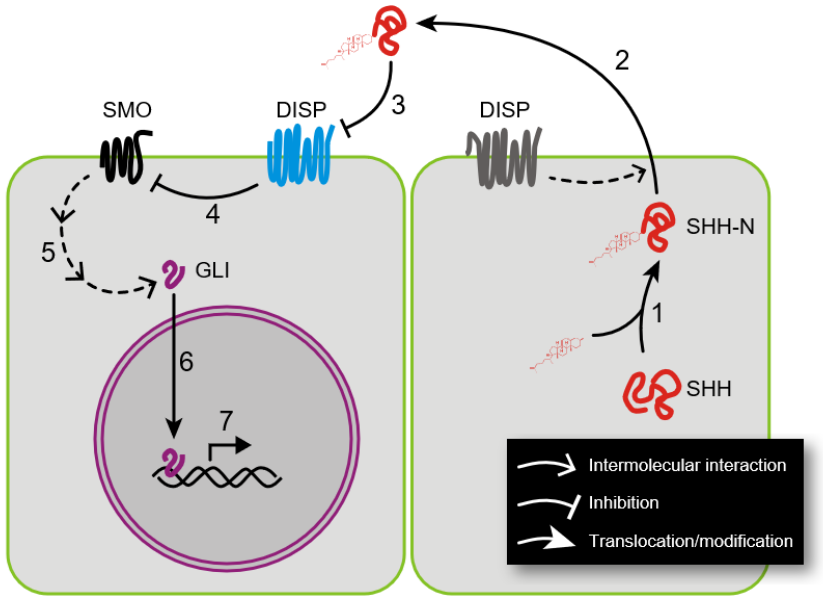
Puts the genome in touch with its environment

A great evolutionary inventions that enabled multicellularity?

Distal *cis*-regulators

General Role of Cis-regulators

1. Links the genome to the world outside the cell.



2. Enables the gene to respond to multiple complex signals

Properties of an enhancer

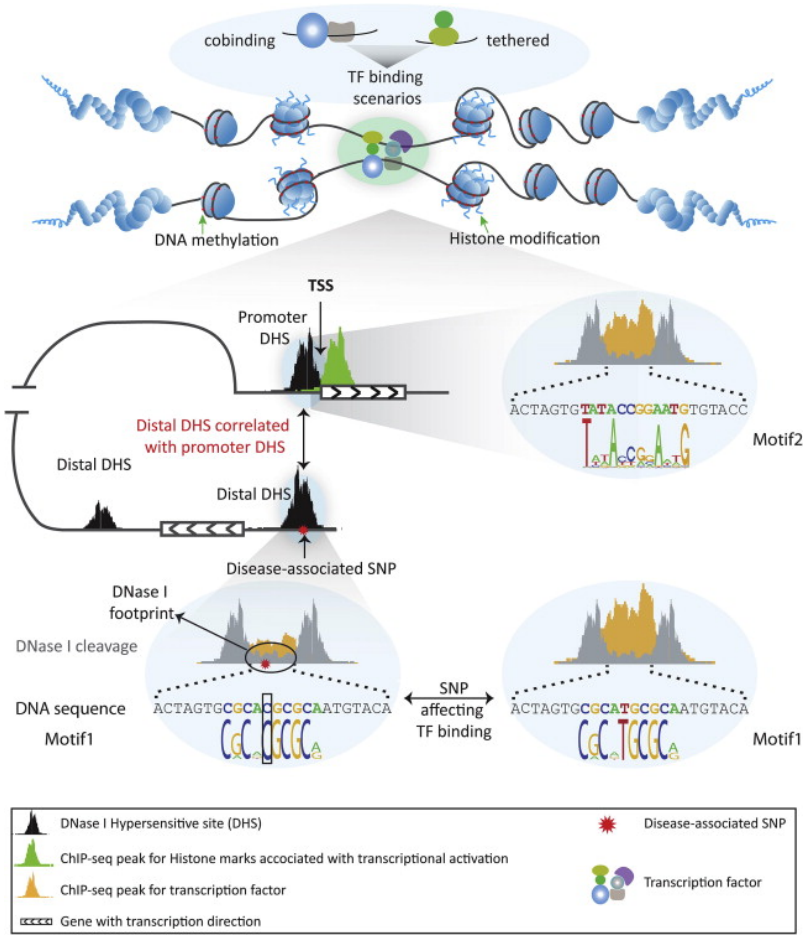
Cis-acting element that increases (the likelihood) of transcription of a gene.
Its targets are gene promoters
Operates from a distance
Are found upstream, downstream or inside a gene

Exploiting properties of cis-regulatory elements

ENCODE- Methodologies used to study enhancers

National Human Genome Research Institute

Multi-dimensional regulation of gene expression



1.) clustering of TF sites--200TFs used by ChIP-Seq
2.) DNAse hypersensitivity – over 150 cell lines and tissues
3.) Histone modifications – H3K4me1, H3K4me3, H3K27ac; in addition p300.

>400,000 cis-regulatory elements

Hongzhu Qu, Xiangdong Fang (2013) **A Brief Review on the Human Encyclopedia of DNA Elements (ENCODE) Project** Genomics, Proteomics & Bioinformatics, Volume 11, Issue 3, 2013, 135–141

Calo E, Wysocka J. (2013) **Modification of Enhancer Chromatin: What , How, and Why**? Molecular Cell 49, 825.

Next-generations sequencing
or
High-throughput sequencing

Whole genome analysis dependent on NG-sequencing

Illumina (Solexa) sequencing
Roche 454 sequencing
Ion torrent: Proton / PGM sequencing
SOLiD sequencing

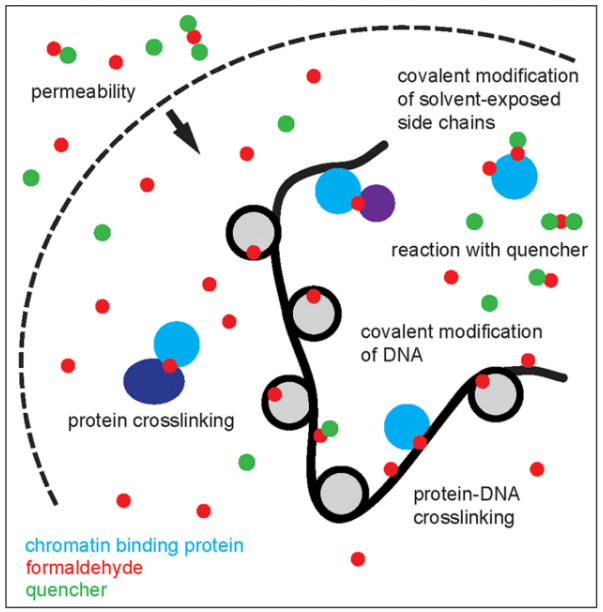
(MinION sequencer – Handheld)

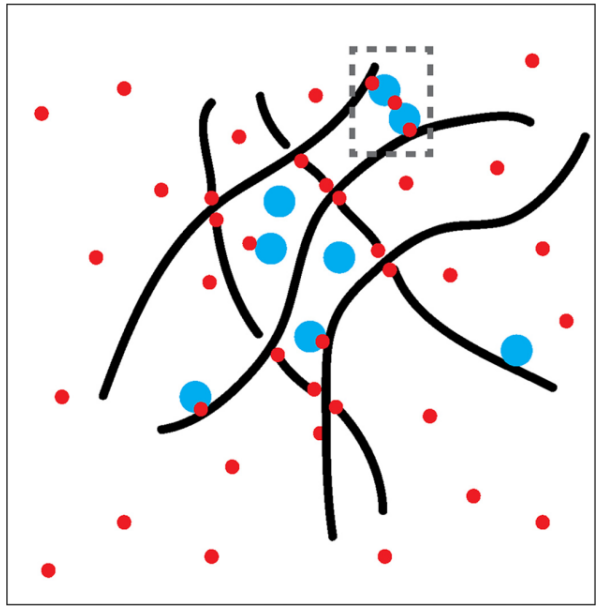
Capacity—
Whole genome sequencing (30X coverage) overnight

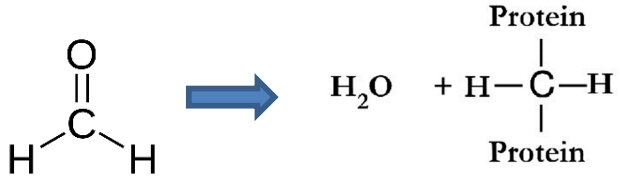
HiSeqX system (£700 genome)
COMING soon – NovaSeq 5000 & 6000 (£100 genome)

Many techniques examining chromatin interactions use crosslinking with formaldehyde

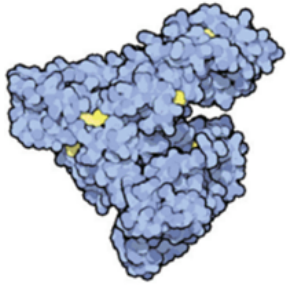
Hoffman et al. 2015 Formaldehyde Crosslinking: A Tool for the Study of Chromatin Complexes THE JOURNAL OF BIOLOGICAL CHEMISTRY 290, 26404 – 26411,







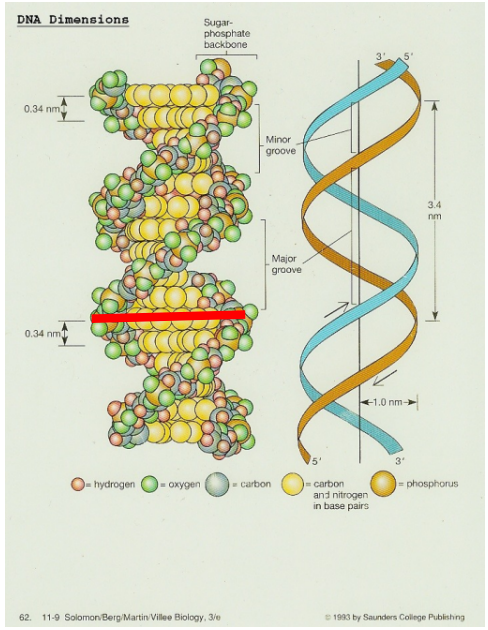
The small size of formaldehyde dictates its linkage of groups that are approximately 2 Å apart, making it well suited for capture of Interactions between macromolecules that are in close proximity



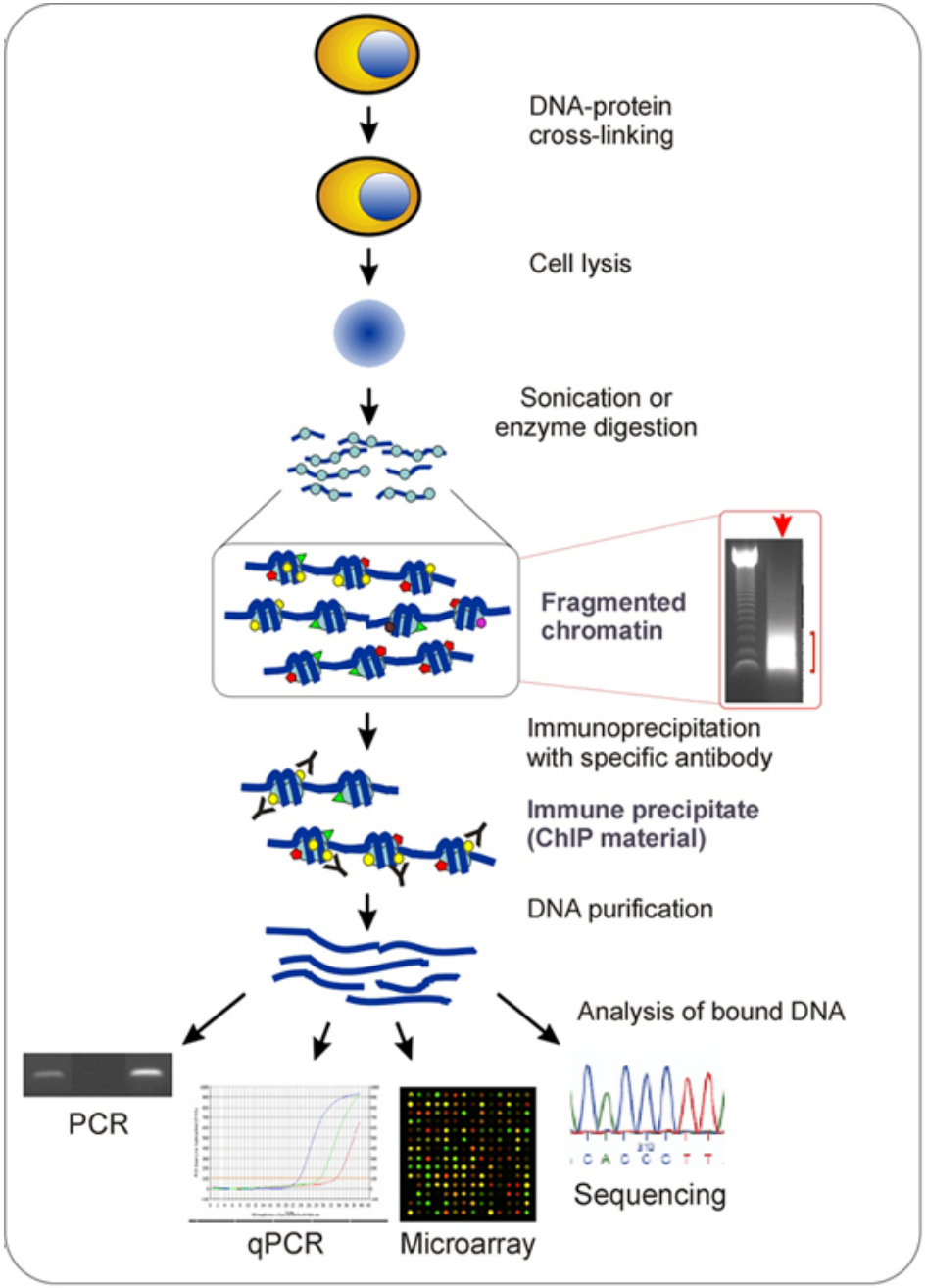
serum albumin (1e7i)

70Å

25Å width



Chromatin Immunoprecipitation

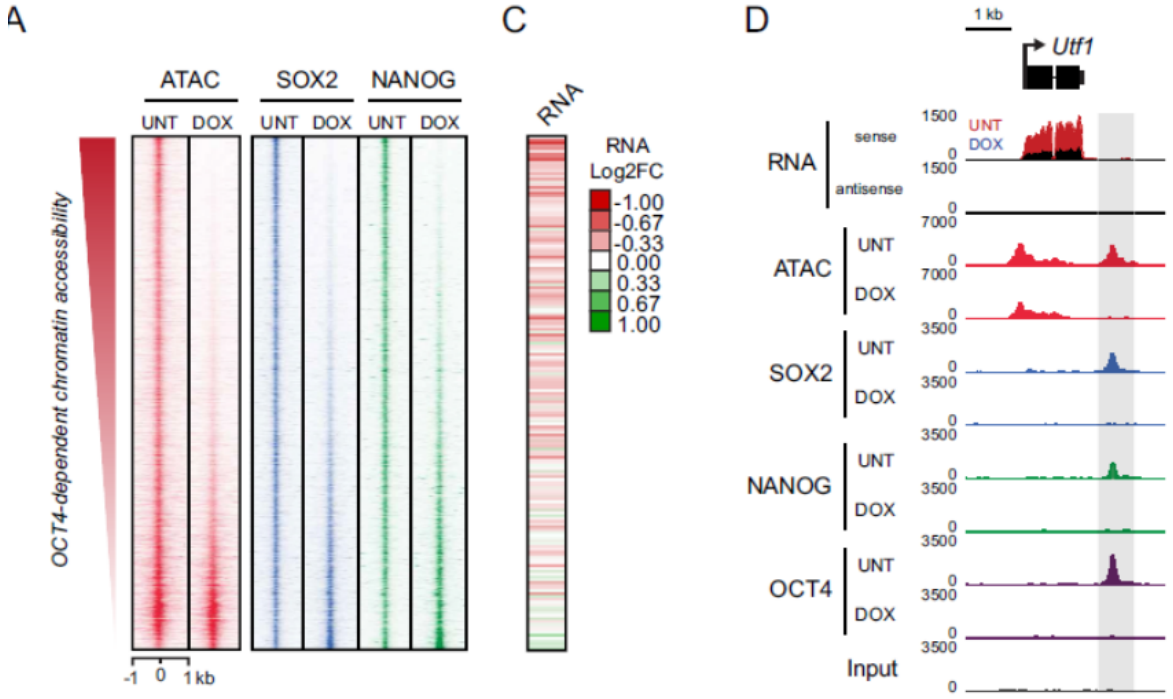


eLIFE elifesciences.org

RESEARCH ARTICLE

The pioneer factor OCT4 requires the chromatin remodeller BRG1 to support gene regulatory element function in mouse embryonic stem cells

Hamish W King, Robert J Klose* eLife 2017; 6:e22631

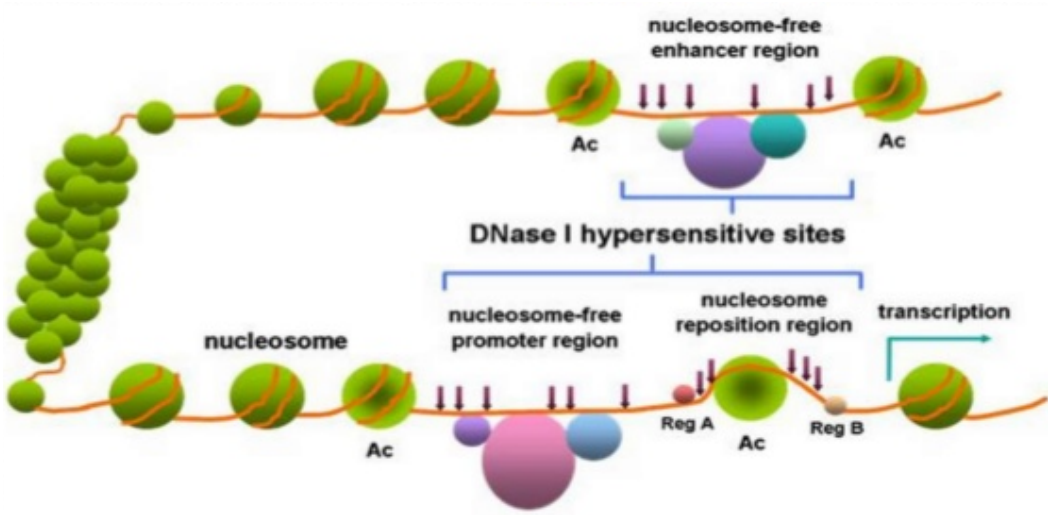


DNAseI Sensitivity is an Assay for Distal Regulators

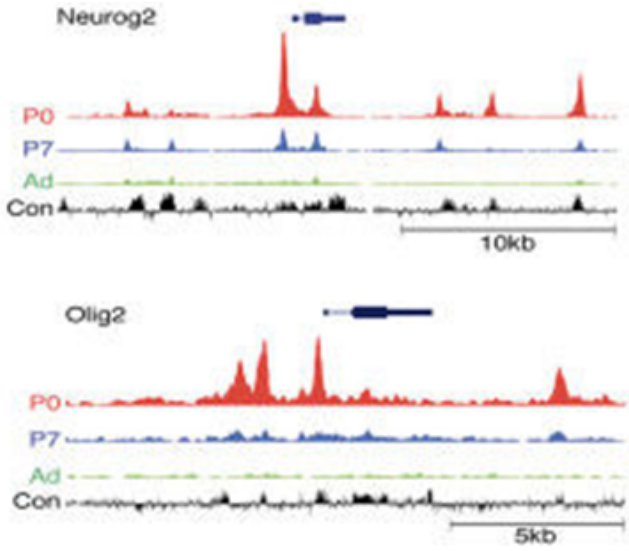
Nucleases or ATAC techniques

Hypersensitive sites are short regions of chromatin which are are detected by their sensitivity to DnaseI or other nucleases

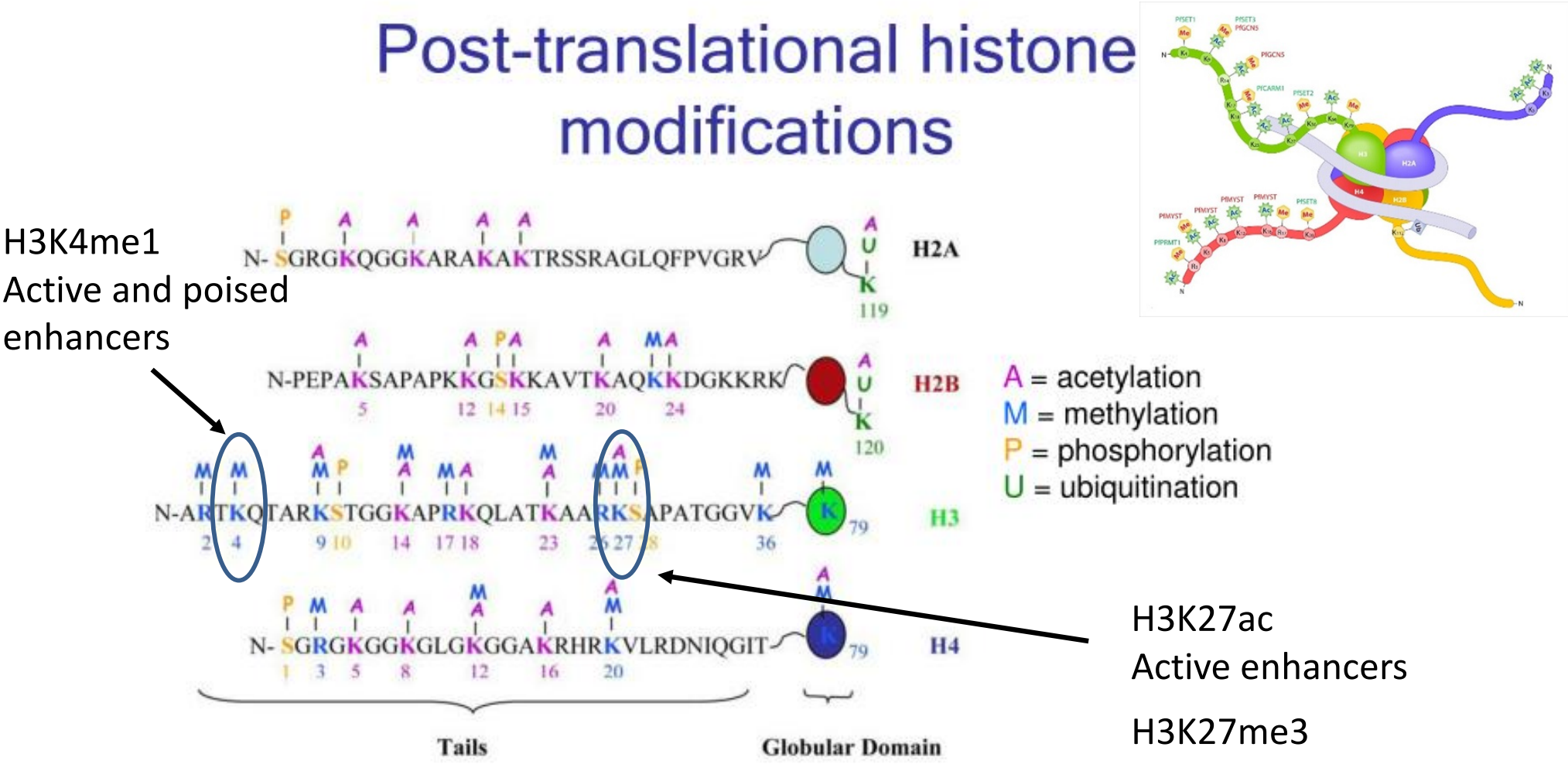
Target is less compacted nucleosomal structure

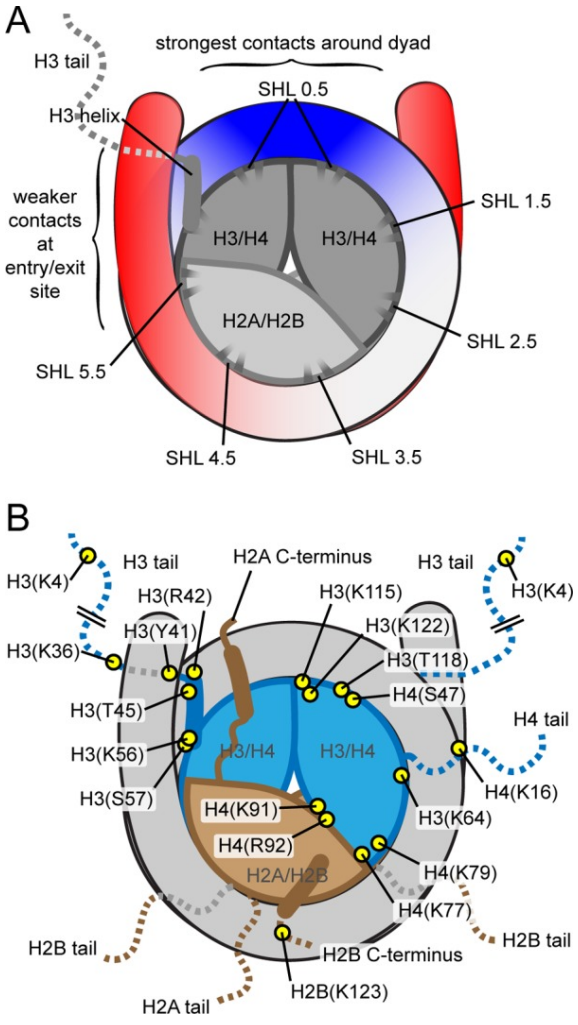


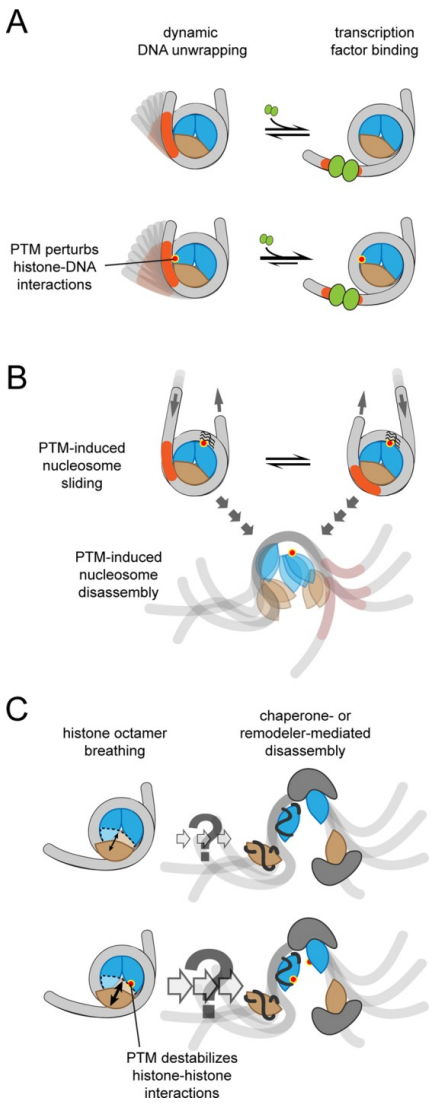
DNAseI profiles



Histone modifications



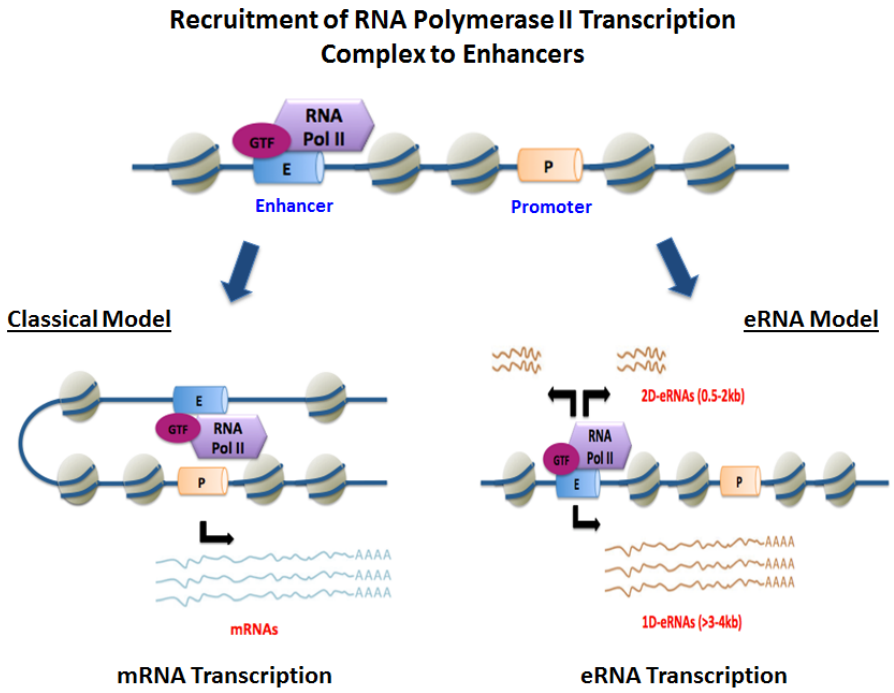




Gregory D. Bowman*† and Michael G. Poirier. (2015) Post-Translational Modifications of Histones That Influence Nucleosome Dynamics Chem Rev. Mar 25; 115(6): 2274–2295.

Properties of an enhancer

Enhancer RNAs

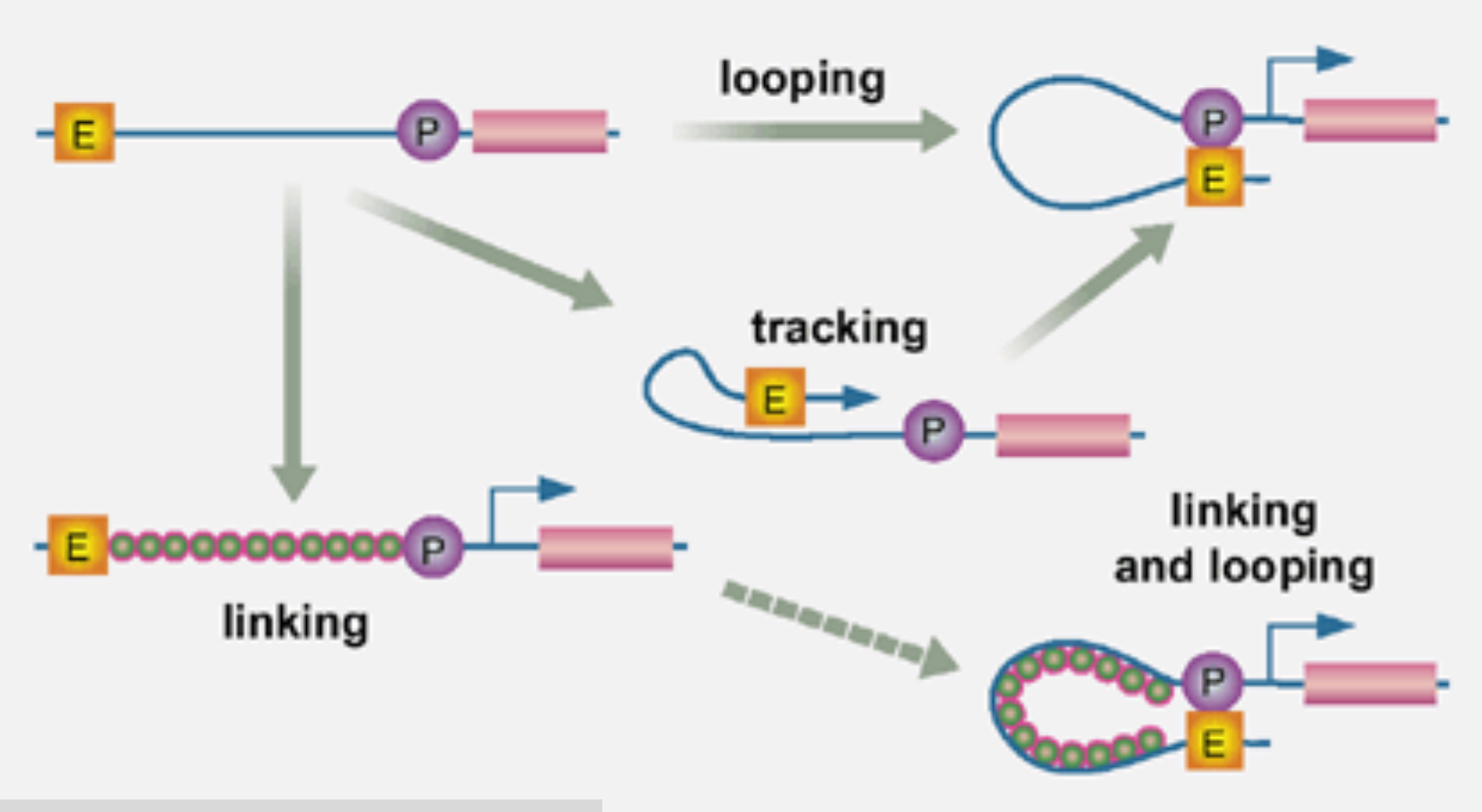


Prior to promoter activation

- Enhancers recruit RNA PolII and TFs to form PIC
- Transcribes 1D or 2D - produces short unstable transcripts

Not all enhancers have been associated with eRNAs (only 25% of 12,000 neuronal enhancers)

How Distal Regulatory Elements Activate Target Genes



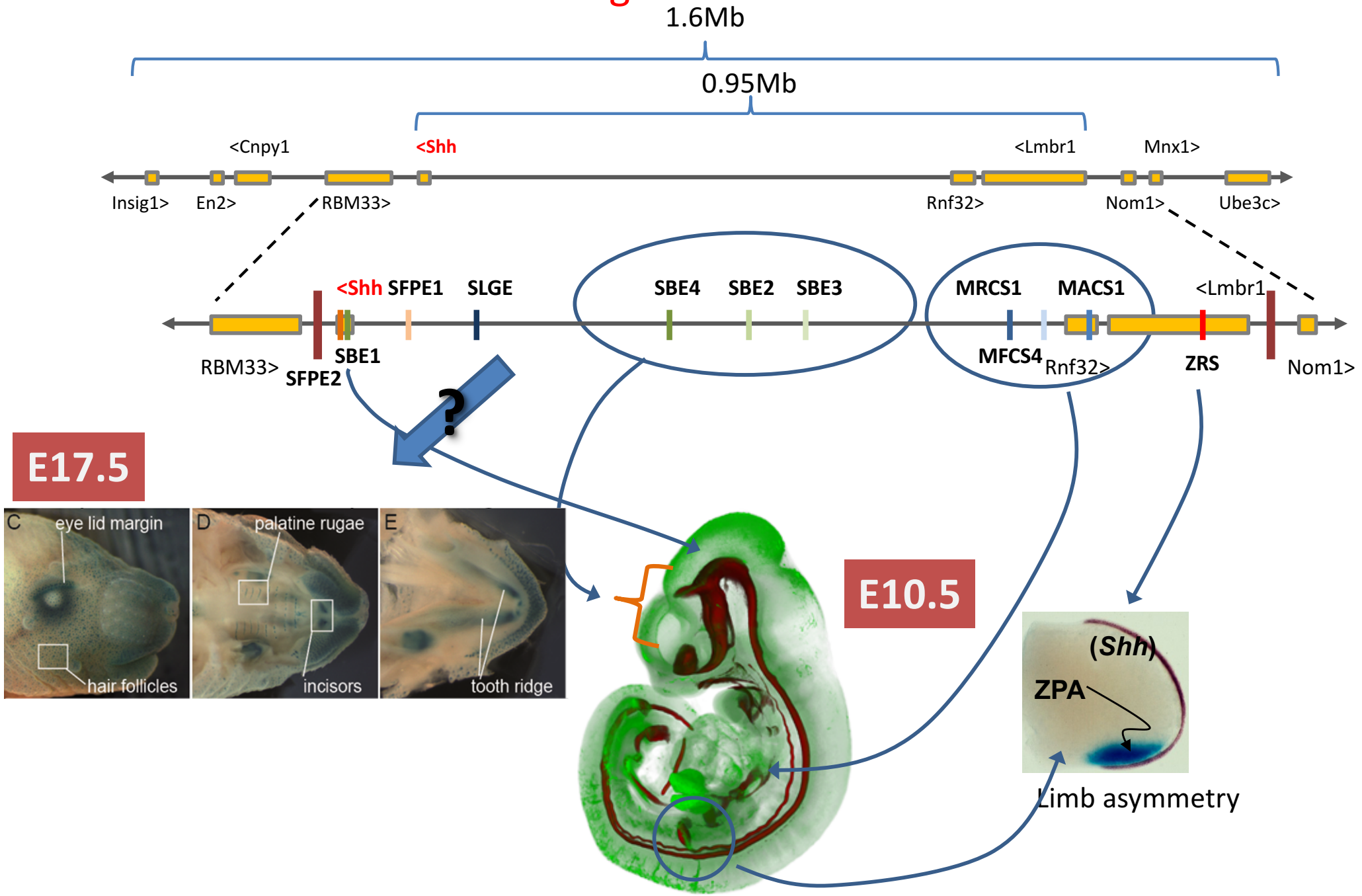
Modified from- *Nature Genetics* **32**, 555–556 (2002)

Does Chromatin Organisation Play a Role in Gene Regulation?

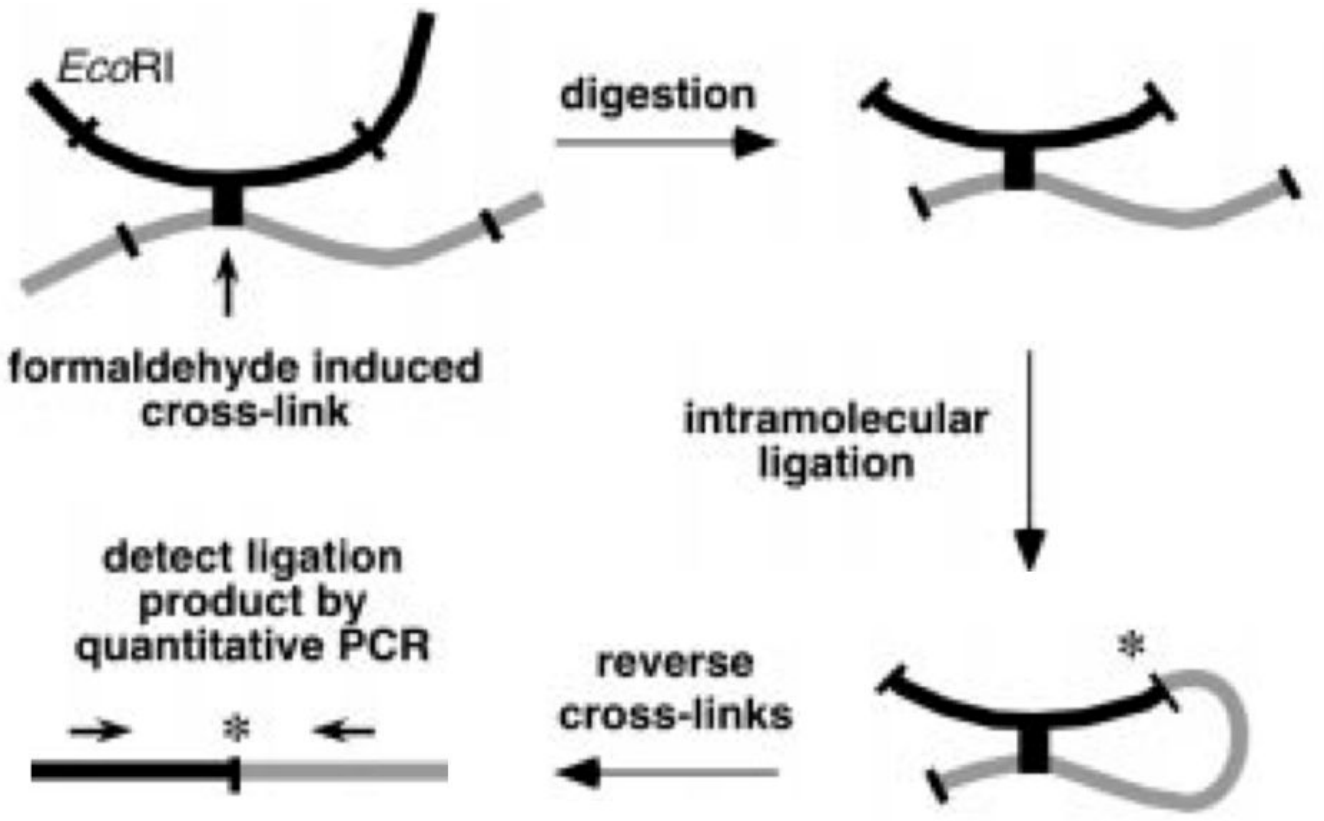
How can we assay Hierarchical Structure across the genome?

Long-Range Locus Composition

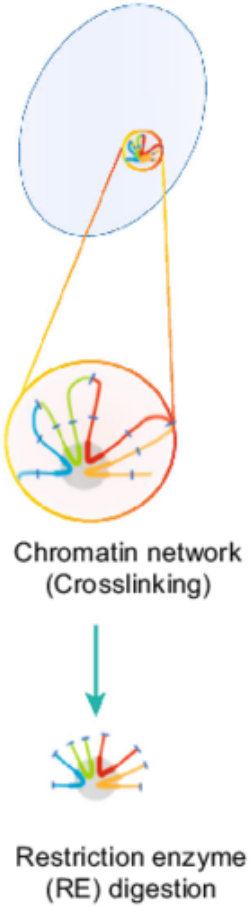
Design-A-Locus



Chromatin Conformation Capture (3C)



Different 3C Approaches



3C	Intramolecular DNA ligation	Reverse crosslink	PCR amplification	
4C	Intramolecular DNA ligation	Reverse crosslink and second RE digestion	Circulation and inverse PCR	Microarray
5C	Intramolecular DNA ligation	Reverse crosslink	Oligo annealing and ligation	PCR amplification and deep sequencing or microarray
Hi-C	Biotin fill in and intramolecular DNA ligation	Reverse crosslink	Terminal biotin removal sonication and size selection	Pull down biotin, adapter ligation and deep sequencing

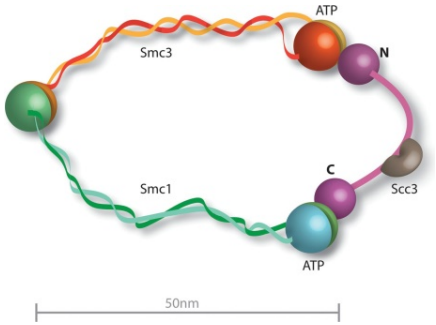
Specific interaction

All interactions from one viewpoint

All interaction over a limited domain

All interactions

Organization of Chromatin into Topologically Associated Domains TADs



Stabilized by CTCF and cohesin rings

Loop-extrusion model

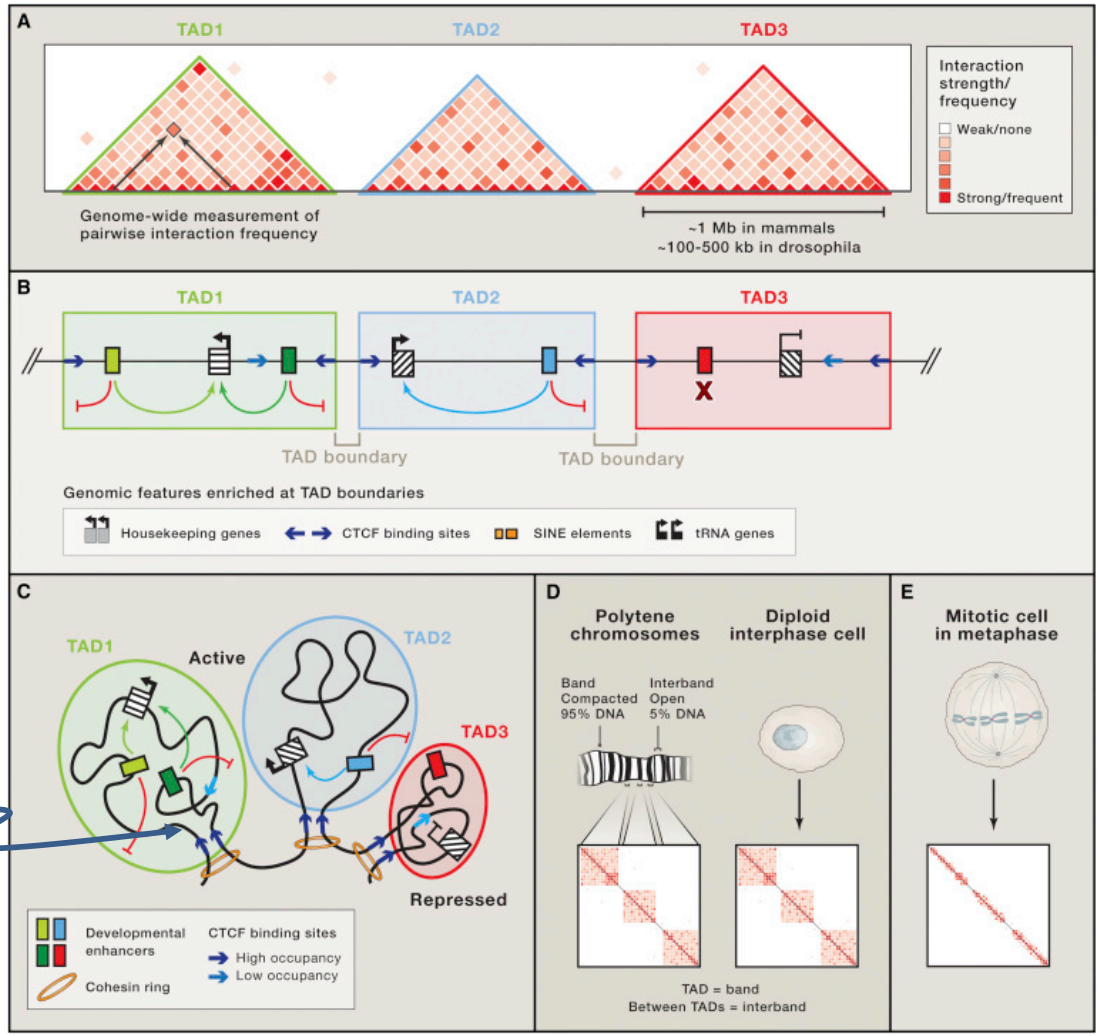


Figure 3. Organization of Chromatin into Topologically Associated Domains(A) Hi-C or 5C heatmaps visualize three-dimensional interactions or compartmentalization of chromosomes into TADs, visible as triangular blocks of increased interaction frequencies.(B) TA...

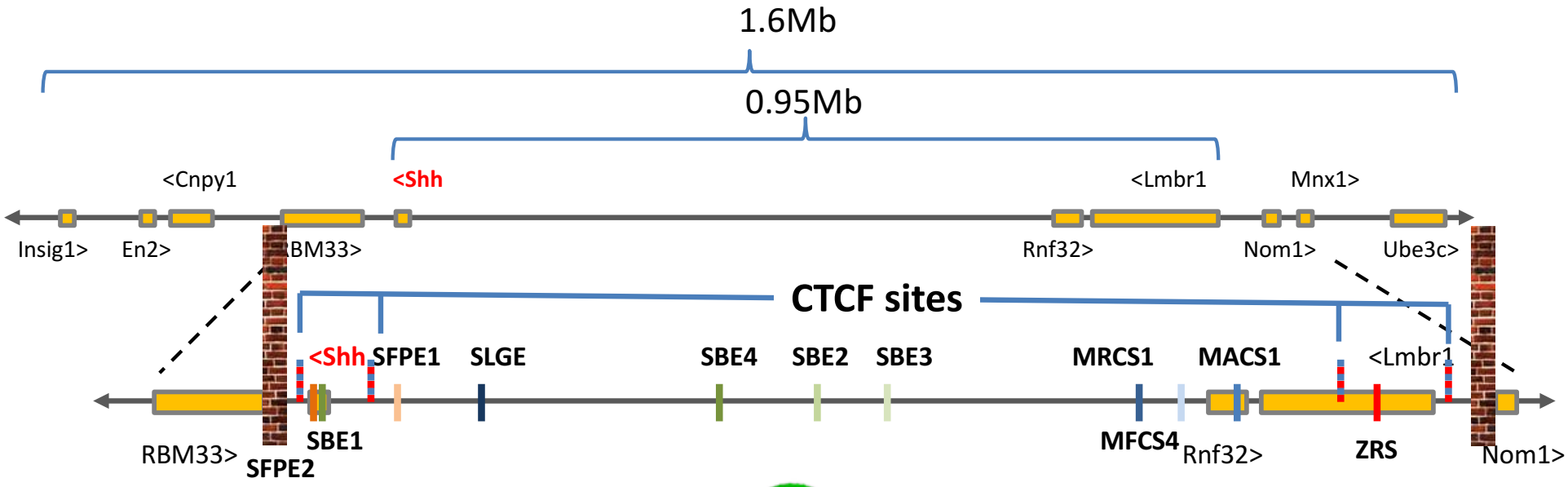
Hannah K. Long, Sara L. Prescott, Joanna Wysocka (2016) Ever-Changing Landscapes: Transcriptional Enhancers in Development and Evolution **Cell 167; 1170-1187.**

Outlining the Basics for a Research Grant

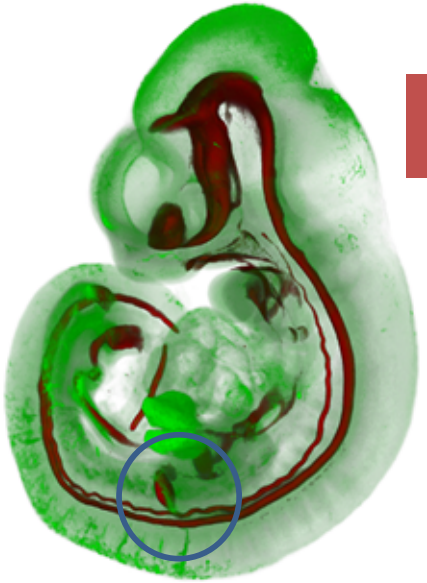
The Francis Crick Foundation has announced a new initiative to fund the very best of our young scientist.

Initiative entitled “Healthy living through Genetics”. Funding 5 year programs (worth up to £5 million) to investigate basic problems in understanding how our genomes work

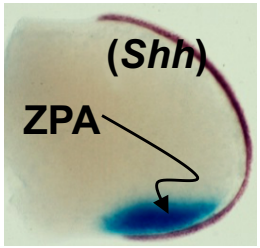
Model System



Shh and its Regulatory Domain



E10.5



Limb asymmetry

Outline of a Grant Proposal

Overarching (General) Scientific Question-

Hypotheses

Goals (Specific Aims)

Experimental Procedures

Overarching (General) Scientific Question-

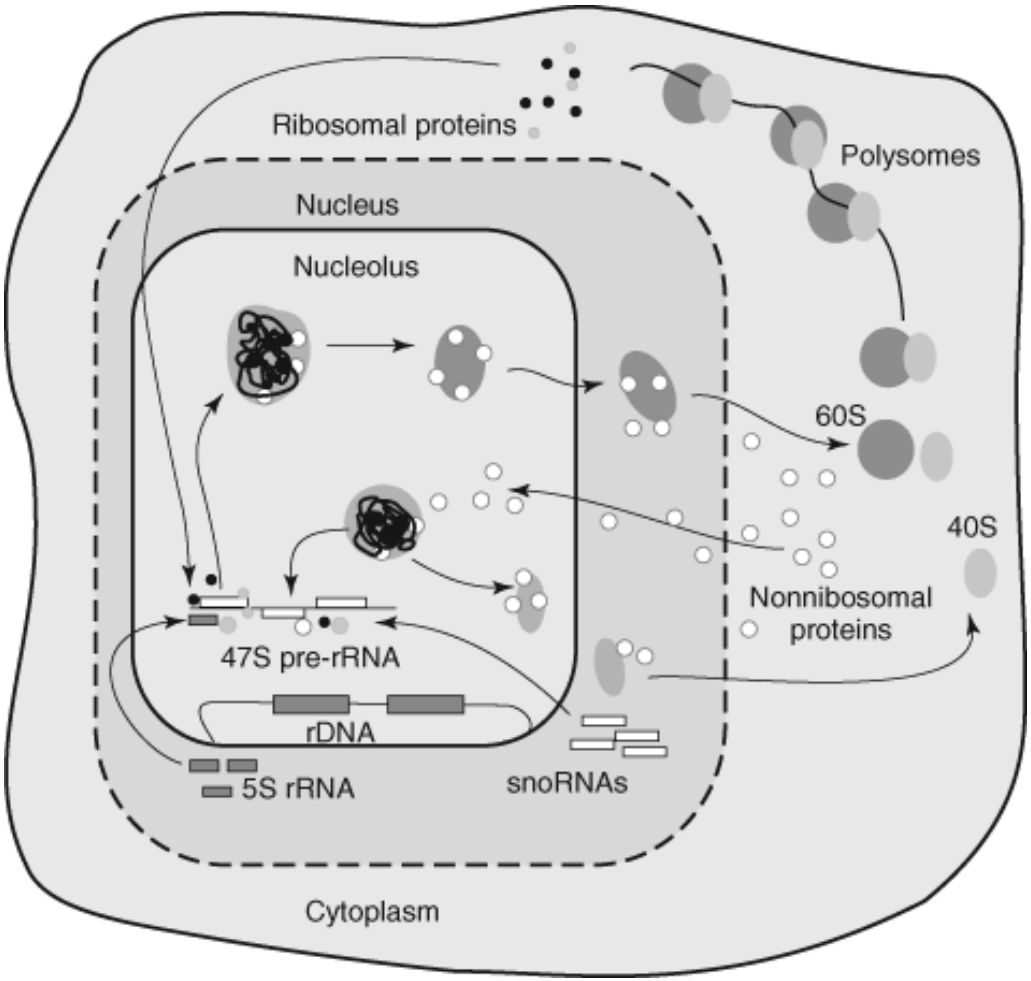
1.) How specific are the enhancers for Shh promoter?
2.) How do the enhancers activate the promoter?
3.) How are the enhancers activated
4.) How do your restrain regulatory activity within the locus
5.) What transcription factors are working at one or all of the enhancers
    Can these lead to identification of the key signaling pathways
6.) What are the boundary elements for this locus
    and are CTCF sites the insulators for the locus

Hypotheses

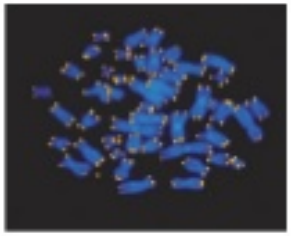
Goals (Specific Aims)

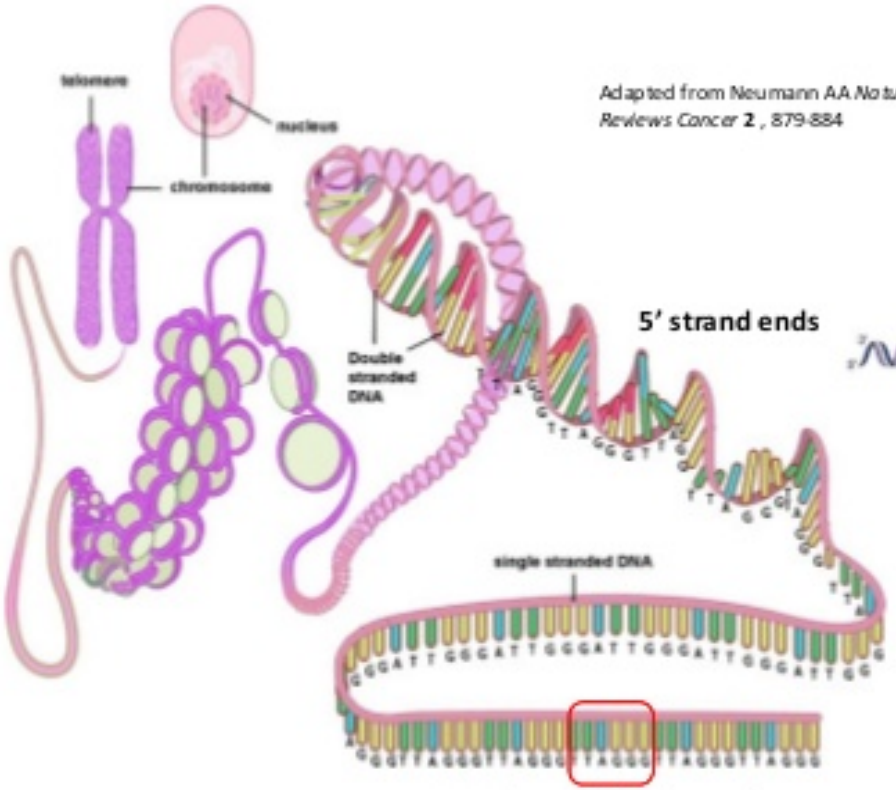
Experimental Procedures

Role of nucleolus



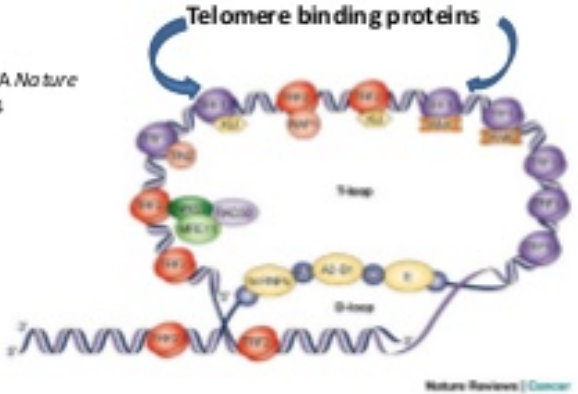
Telomere Basics: Structure

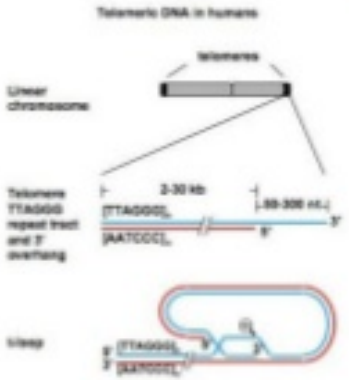




Adapted from Oeseburg Eur J Physiol (2010) 459: 259–268

Adapted from Neumann AA *Nature Reviews Cancer* **2**, 879-884





Telomere caps

What do Telomeres do?

- Serve as chromosome end-caps to protect the integrity of our genes.
- Keep chromosomes from degrading to prevent fusion and massive genomic instability.
- Allow cells to replicate (cells can not divide when telomeres get too short)

Bottom Line: Telomeres protect cells from DNA mutations, senescence and death.