Lecture 17. Gene Regulation

Michael Schatz

October 27, 2021

Advanced Biomedical Research

Updated schedule!

16.	Mo 10/25	Functional Analysis 5: Single Cell Genomics	* Ginkgo: interactive analysis and assessment of single-cell copy-number variations (Garvin et al, 2015, Nature Methods) * The dynamics and regulators of cell fate decisions are revealed by pseudotemporal ordering of single cells (Thapnell et al, Nature Biotech, 2014) * Eleven grand challenges in single-cell data science (Lähnemann et al, Genome Biology, 2020)	Assignment 5 & Preliminary Project Report
17.	We 10/27	Gene Regulation		
18.	Mo 11/1	Midterm Review		
19.	We 11/3	Midterm Exam		Take home exam
20.	Mo 11/8	Human Evolution	* An integrated map of genetic variation from 1,092 human genomes (1000 Genomes Consortium, 2012, Nature) * Analysis of protein-coding genetic variation in 60,706 humans (Lek et al, 2016, Nature) * A Draft Sequence of the Neandertal Genome (Green et al. 2010, Science) * Excavating Neandertal and Denisovan DNA from the genomes of Melanesian individuals (Vernot et al. 2016, Science)	Project Interim Report
21.	We 11/10	Human Genetic Diseases	* Genome-Wide Association Studies (Bush & Moore, 2012, PLOS Comp Bio) * The contribution of de novo coding mutations to autism spectrum disorder (Iossifov et al, 2014, Nature)	
22.	Mo 11/15	Cancer Genomics	* The Hallmarks of Cancer (Hanahan & Weinberg, 2000, Cell) * Evolution of Cancer Genomes (Yates & Campbell, 2012, Nature Reviews Genetics) * Comprehensive molecular portraits of human breast tumours (TCGA, 2012, Nature)	Project Presentations Scheduling
23.	We 11/17	Microbiome and Metagenomics	* Kraken: ultrafast metagenomic sequence classification using exact alignments (Wood and Salzberg, 2014, Genome Biology) * Chapter 12: Human Microbiome Analysis (Morgan and Huttenhower)	Project Report Assignment
	Mo 11/22	Thanksgiving Break		
	We 11/24	Thanksgiving Break		
24.	Mo 11/29	Project Presentations		
25.	We 12/1	Project Presentations		
26.	Mo 12/6	Project Presentations	Last Day of class	
	Mo 12/20	Final Project Report Due!		

https://github.com/schatzlab/biomedicalresearch2021

Assignment 5: RNAseq
Due Nov 1 @ 11:59pm

Assignment 5: RNA-seq

Assignment Date: Monday, Oct. 25, 2021
Due Date: Monday, Nov. 1, 2021 @ 11:59pm

Assignment Overview

In this assignment, you will explore a couple of aspects of RNA-seq (with a small introduction to clustering). For this assignment, you will have to generate some visualizations - we recommend R or Python, but use a language you are comfortable with!

Make sure to show your work/code in your writeup!

As a reminder, post any questions about this assignment to Piazza.

Question 1. Time Series (20 pts)

This file contains normalized expression values for 100 genes over 10 time points. Most genes have a stable background expression level, but some special genes show increased expression over the time course and some show decreased expression.

- Question 1a. Cluster the genes using an algorithm of your choice. Which genes show increasing expression and which genes show decreasing expression, and how did you determine this? What is the background expression level (numerical value) and how did you determine this? [Hint: K-means and hierarchical clustering are common clustering algorithms you could try.]
- Question 1b. Calculate the first two principal components of the expression matrix. Show the plot and color the points based on their cluster from part (a). Does the PC1 axis, PC2 axis, neither, or both correspond to the clustering?
- Question 1c. Create a heatmap of the expression matrix. Order the genes by cluster, but keep the time points in numerical order.
- Question 1d. Visualize the expression data using t-SNE.
- Question 1e. Using the same data, visualize the expression data using UMAP.
- Question 1f. In a few sentences, compare and contrast the (1) heatmap, (2) PCA, (3) t-SNE, and (4) UMAP results. Be sure to comment on understandability, relative positioning of clusters, runtime, and any other significant factors that you see.

https://github.com/schatzlab/biomedicalresearch2021

Class Project!
Proposal Due Nov 1

Project Proposal

Assignment Date: Monday Oct 25, 2021
Due Date: Monday, November 1 2021 @ 11:59pm

Review the Project Ideas page

Work solo or form a team for your class project of no more than 3 people.

The proposal should have the following components:

- Name of your team
- List of team members and email addresses
- Short title for your proposal
- 1 paragraph description of what you hope to do and how you will do it
- References to 2 to 3 relevant papers
- References/URLs to datasets that you will be studying (Note you can also use simulated data)
- Please add a note if you need me to sponsor you for a MARCC account (high RAM, GPUs, many cores, etc) or AnVIL account (WDL-based, T2T)

Submit the proposal as a 1 to 2 page PDF on GradeScope (each team member should submit the same PDF). After submitting your proposal, we can schedule a time to discuss your proposal, especially to ensure you have access to the data that you need. The sooner that you submit your proposal, the sooner we can schedule the meeting. No late days can be used for the project.

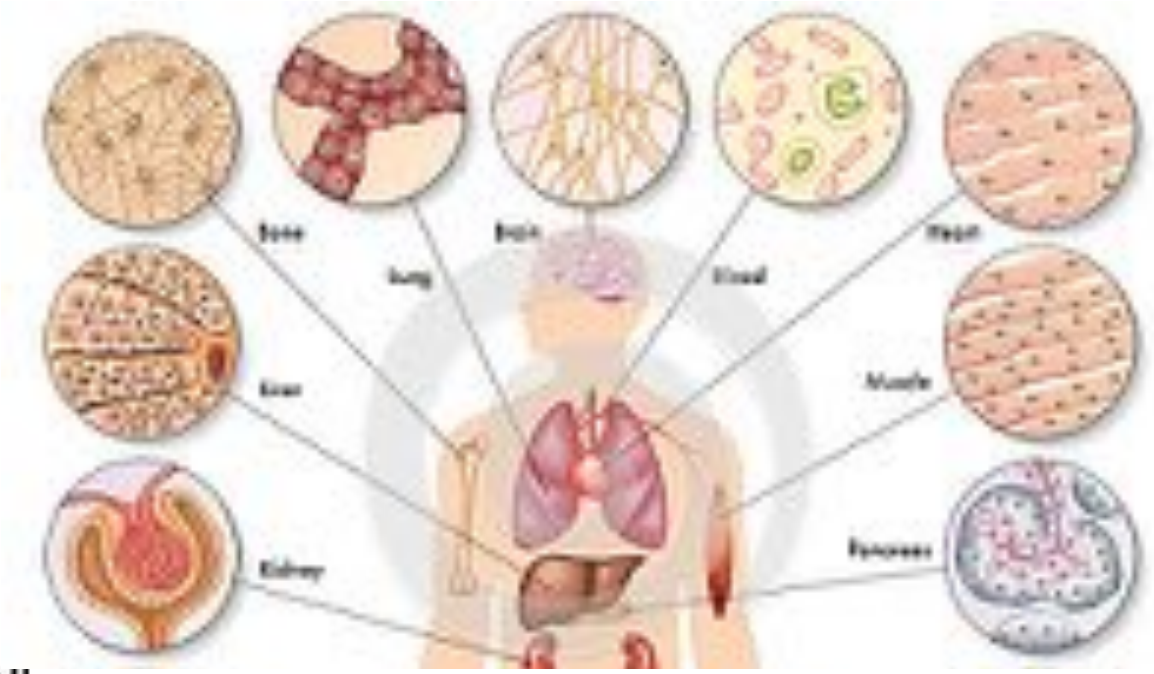
Later, you will present your project in class during the last week of class. You will also submit a written report (5-7 pages) of your project, formatting as a Bioinformatics article (Intro, Methods, Results, Discussion, References). Word and LaTeX templates are available at https://academic.oup.com/bioinformatics/pages/submission_online

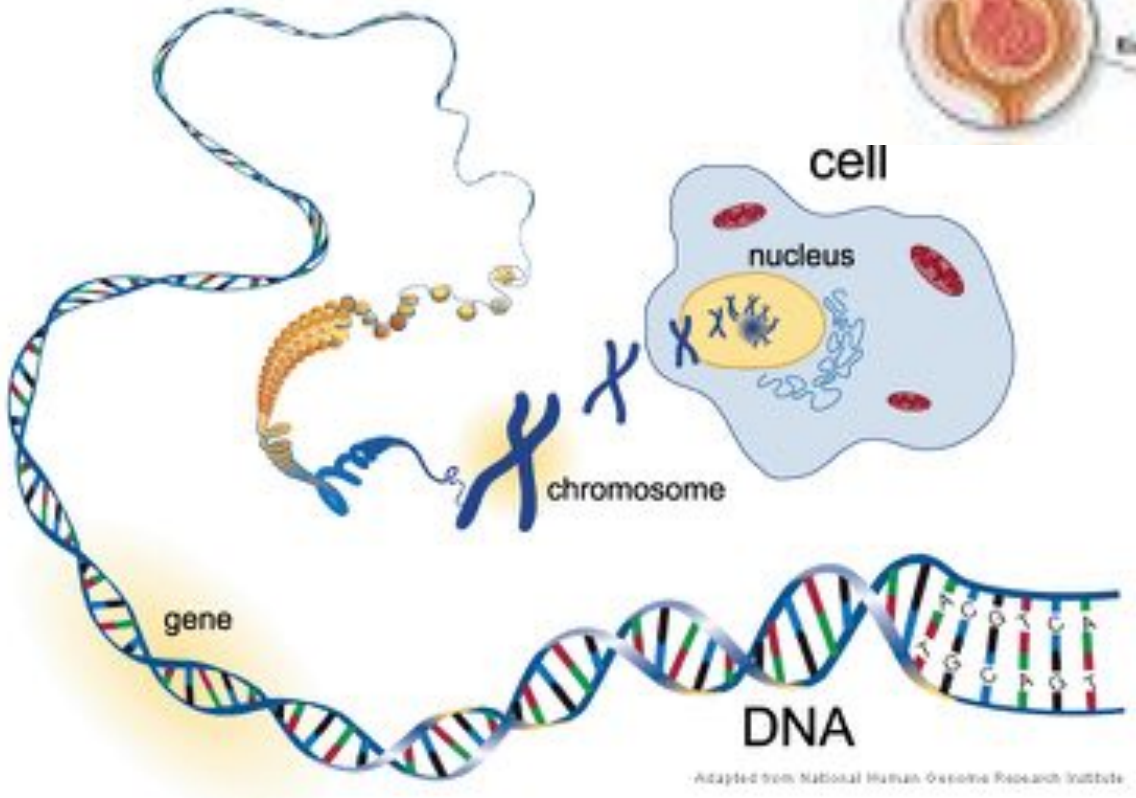
Please use Piazza to coordinate proposal plans!

https://github.com/schatzlab/biomedicalresearch2021/blob/main/project/proposal.md

Why Genes?

Each cell of your body contains an exact copy of your 3 billion base pair genome.





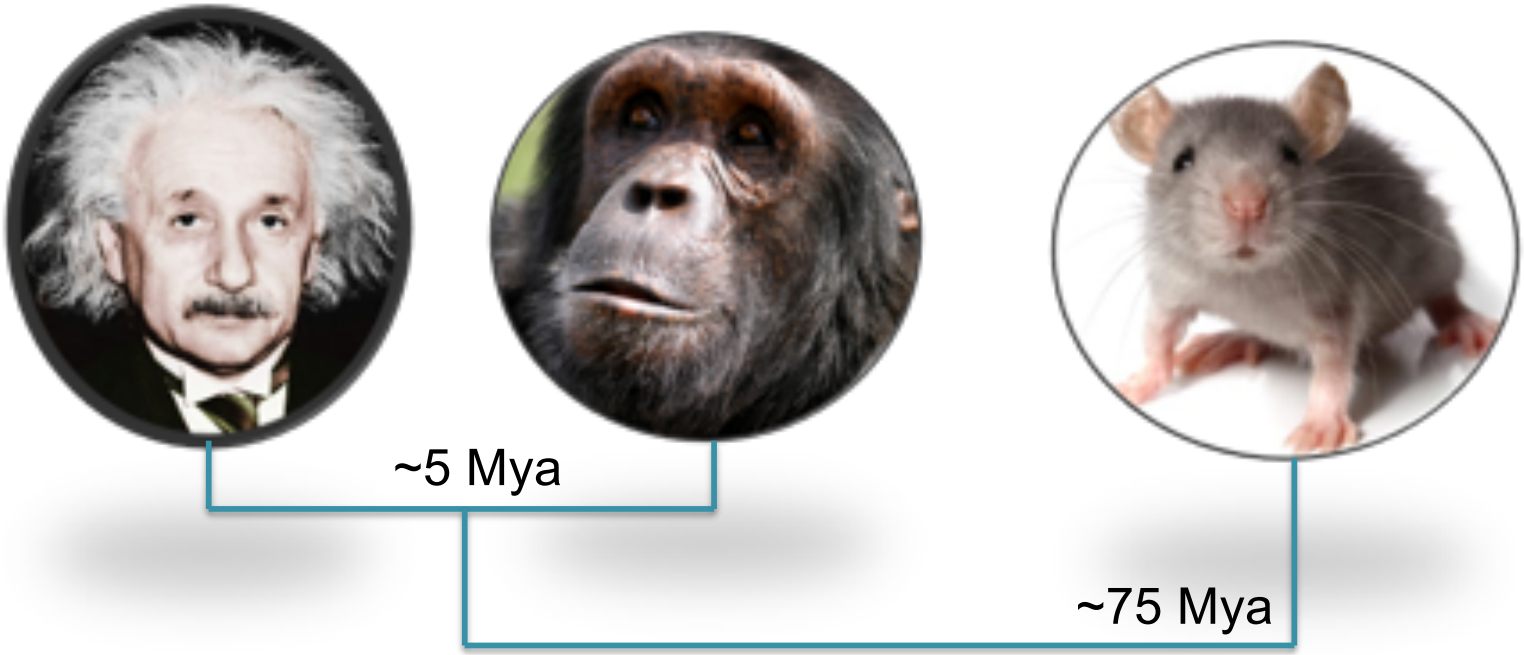
Your body has a few hundred (thousands?) major cell types, largely defined by the gene expression patterns

Human Evolution

- Humans and chimpanzees shared a common ancestor ~5-7 million years ago (Mya)
- Single-nucleotide substitutions occur at a mean rate of 1.23% but ~4% overall rate of mutation: comprising ~35 million single nucleotide differences and ~90 Mb of insertions and deletions
- Orthologous proteins in human and chimpanzee are extremely similar, with ~29% being identical and the typical orthologue differing by only two amino acids, one per lineage

Initial sequence of the chimpanzee genome and comparison with the human genome (2005) *Nature 437, 69-87 doi:10.1038/nature04072*

Human Evolution

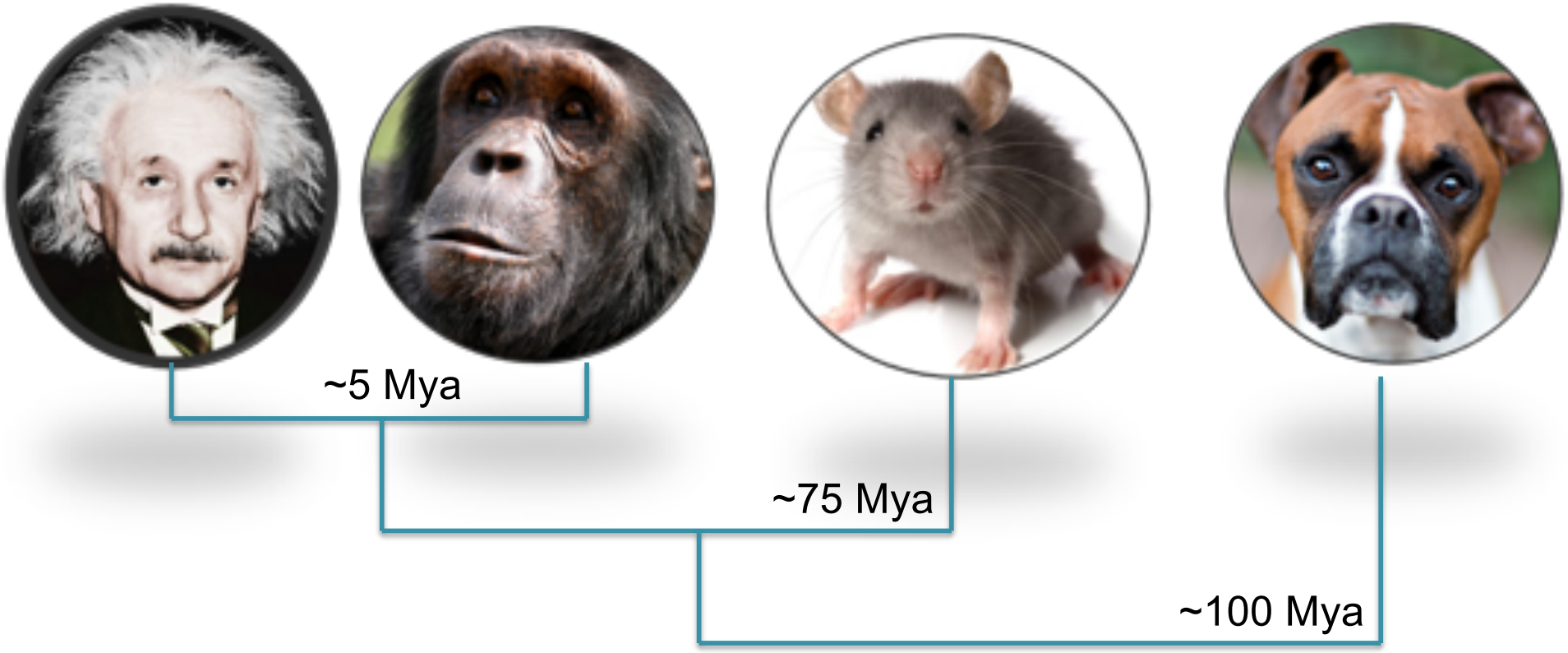


"In the roughly 75 million years since the divergence of the human and mouse lineages, the process of evolution has altered their genome sequences and caused them to diverge by ***nearly one substitution for every two nucleotides***"

"The mouse and human genomes each seem to contain about 30,000 protein-coding genes. These refined estimates have been derived from both new evidence-based analyses that produce larger and more complete sets of gene predictions, and new de novo gene predictions that do not rely on previous evidence of transcription or homology. The proportion of mouse genes with a single identifiable orthologue in the human genome seems to be approximately 80%. ***The proportion of mouse genes without any homologue currently detectable in the human genome (and vice versa) seems to be less than 1%.***"

Initial sequencing and comparative analysis of the mouse genome
Chinwalla et al (2002) *Nature.* 420, 520-562 doi:10.1038/nature01262

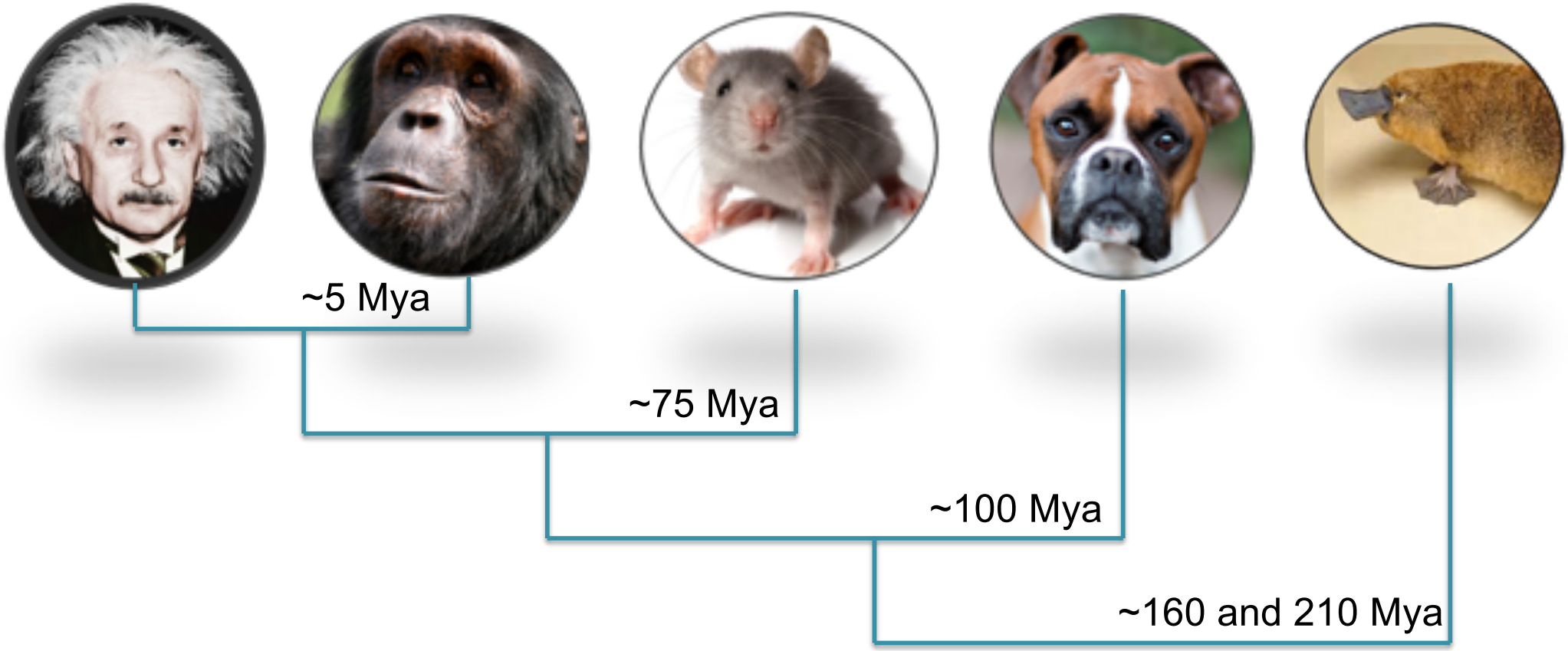
Human Evolution



"We generated gene predictions for the dog genome using an evidence-based method (see Supplementary Information). The resulting collection contains ***19,300 dog gene predictions, with nearly all being clear homologues of known human genes***. The dog gene count is substantially lower than the ~22,000-gene models in the current human gene catalogue (EnsEMBL build 26). For many predicted human genes, we find no convincing evidence of a corresponding dog gene. Much of the excess in the human gene count is attributable ***to spurious gene predictions in the human genome***"

Genome sequence, comparative analysis and haplotype structure of the domestic dog
Lindblad-Toh et al (2005) *Nature. 438, 803-819 doi:10.1038/nature04338*

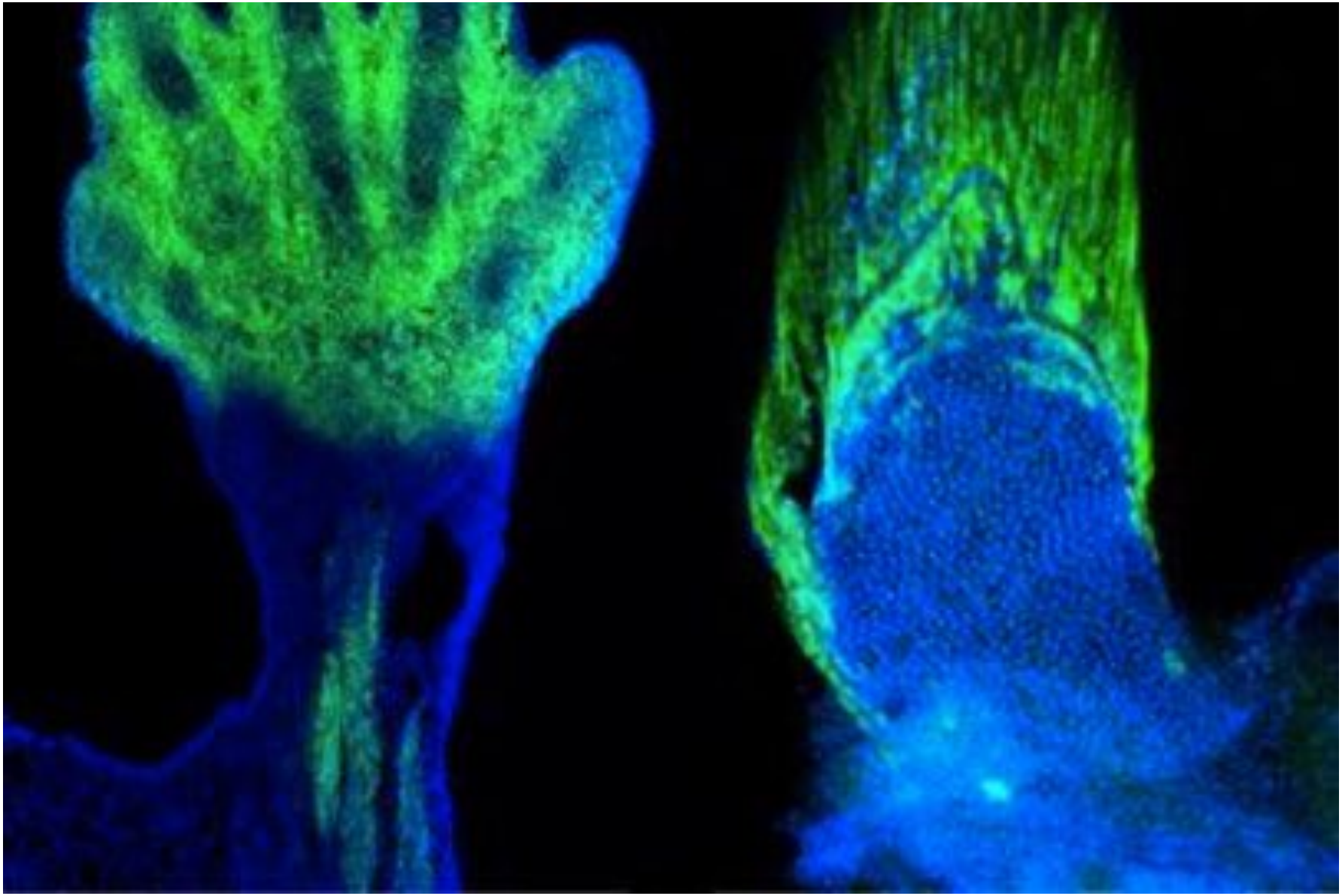
Human Evolution



As expected, the majority of platypus genes (82%; 15,312 out of 18,596) have orthologues in these five other amniotes (Supplementary Table 5). The remaining 'orphan' genes are expected to primarily reflect rapidly evolving genes, for which no other homologues are discernible, erroneous predictions, and true lineage-specific genes that have been lost in each of the other five species under consideration.

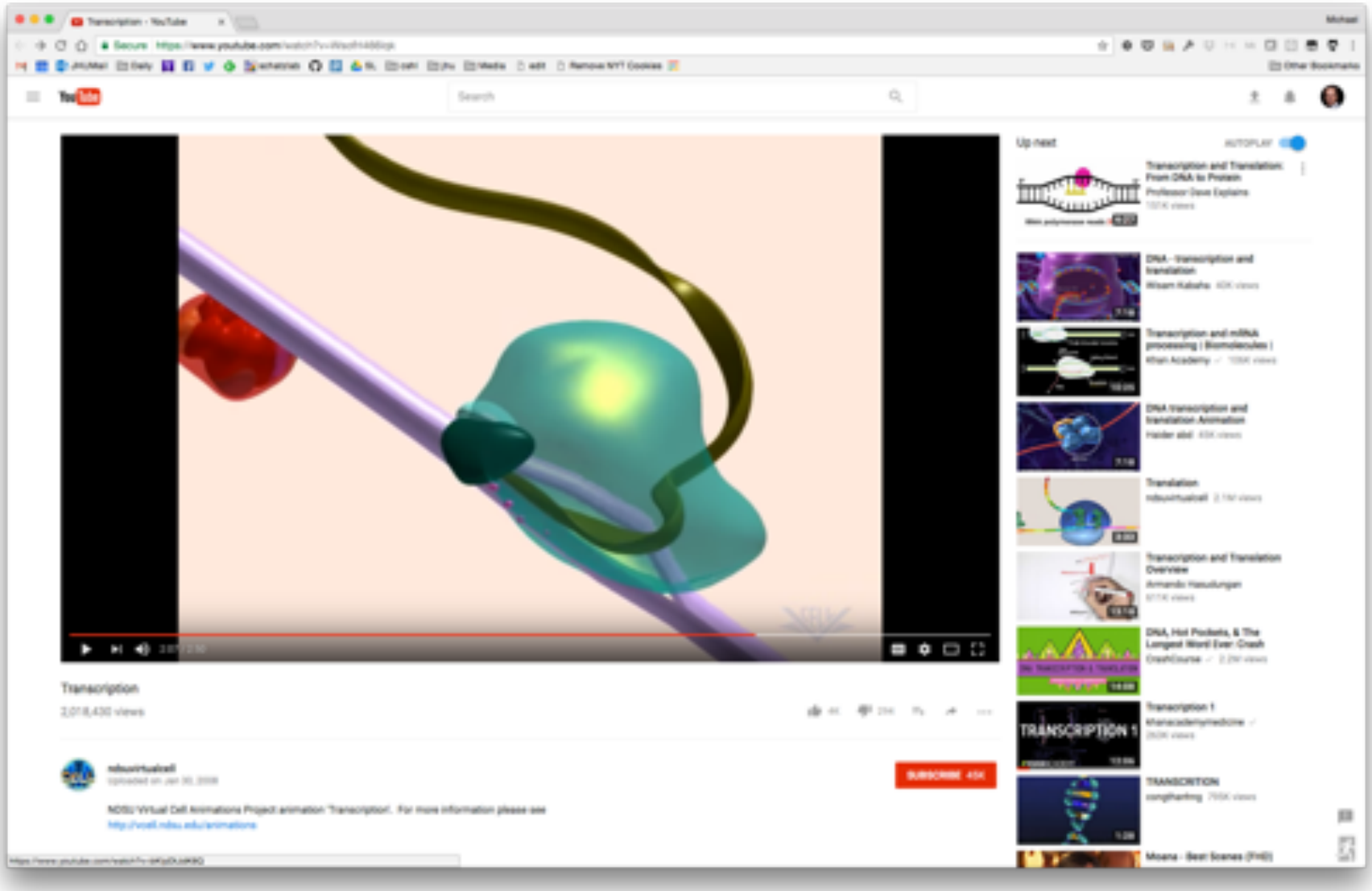
Genome analysis of the platypus reveals unique signatures of evolution
(2008) *Nature.* 453, 175-183 doi:10.1038/nature06936

Animal Evolution



Digits and fin rays share common developmental histories
Nakamura et al (2016) *Nature.* 537, 225–228. doi:10.1038/nature19322

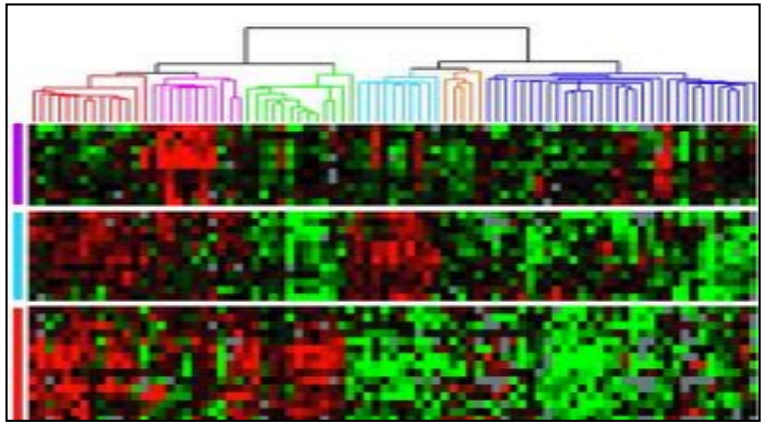
Transcription



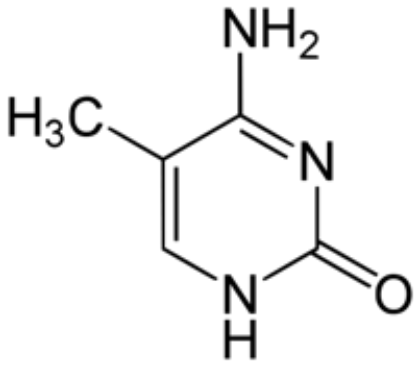
https://www.youtube.com/watch?v=WsofH466lqk

*-seq in 4 short vignettes

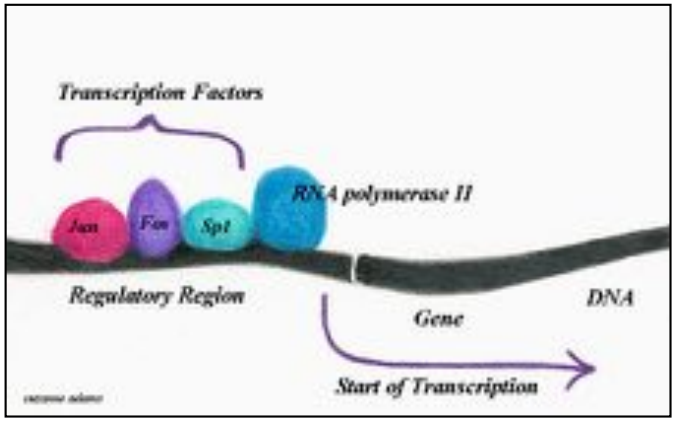
RNA-seq



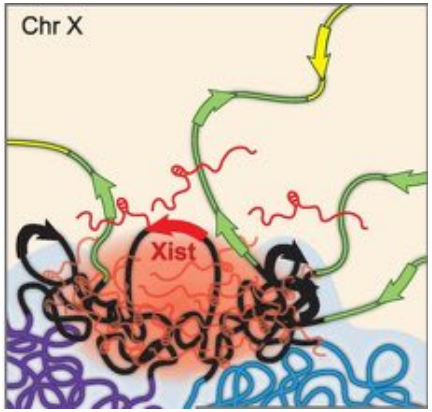
Methyl-seq



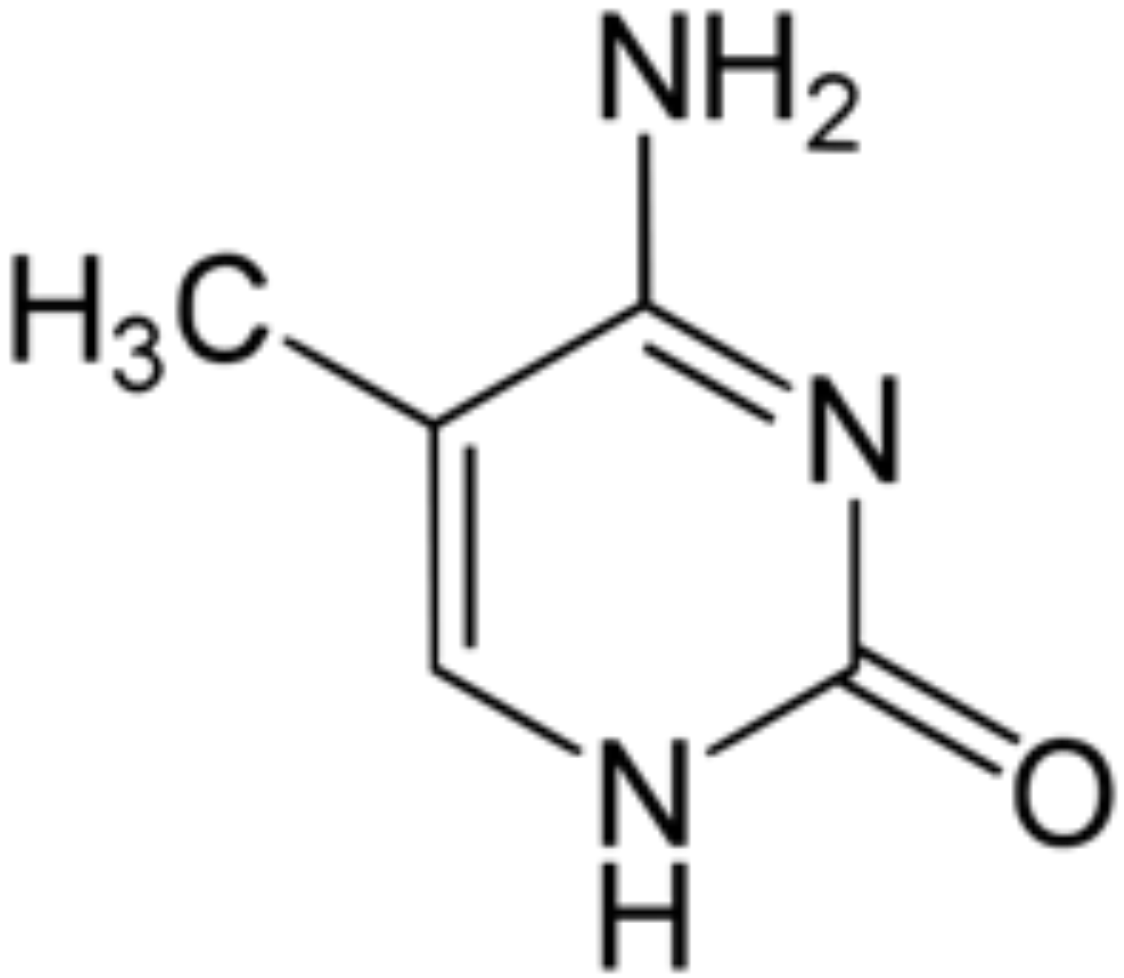
ChIP-seq



Hi-C

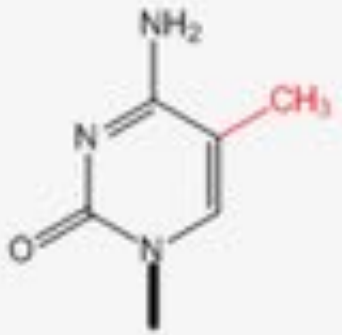


Methyl-seq

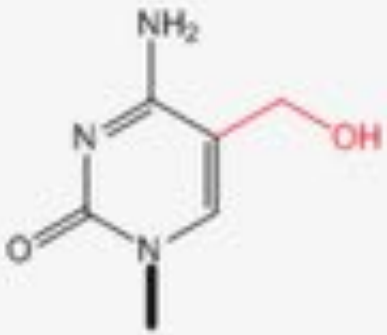


Finding the fifth base: Genome-wide sequencing of cytosine methylation
Lister and Ecker (2009) *Genome Research.* 19: 959-966

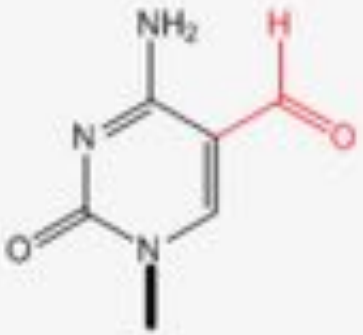
Epigenetic Modifications to DNA



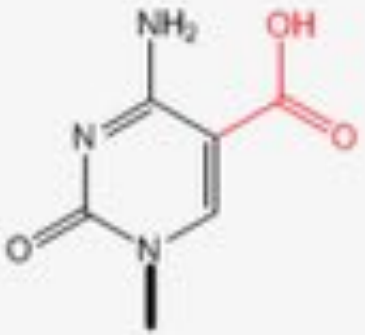
5-mC



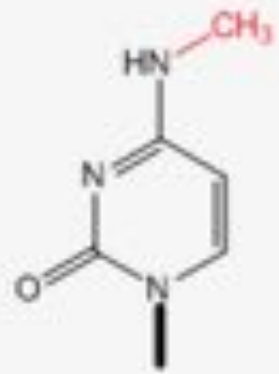
5-hmC



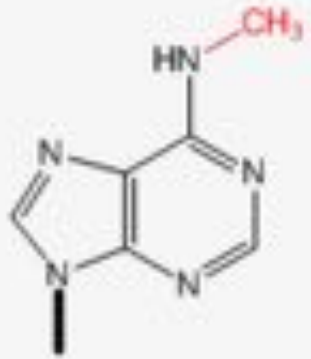
5-fC



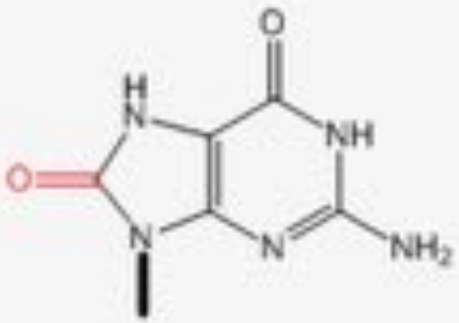
5-caC



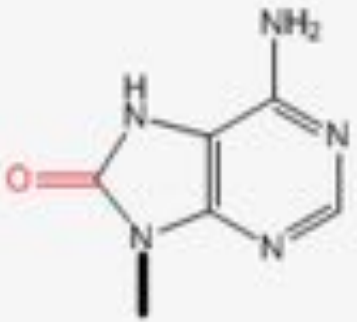
4-mC



6-mA

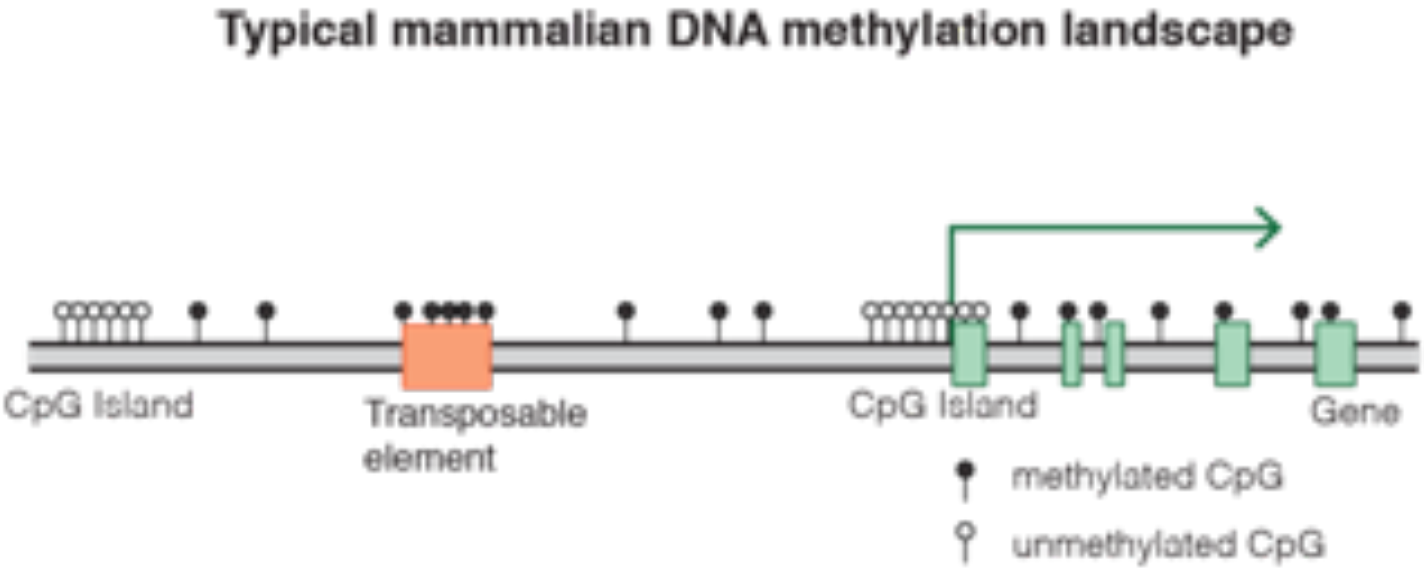


8-oxoG



8-oxoA

Methylation of CpG Islands



CpG islands are (usually) defined as regions with

1) a length greater than 200bp,
2) a G+C content greater than 50%,
3) a ratio of observed to expected CpG greater than 0.6

Methylation in promoter regions correlates negatively with gene expression.

- CpG-dense promoters of actively transcribed genes are never methylated
- In mouse and human, around 60-70% of genes have a CpG island in their promoter region and most of these CpG islands remain unmethylated independently of the transcriptional activity of the gene
- Methylation of DNA itself may physically impede the binding of transcriptional proteins to the gene
- Methylated DNA may be bound by proteins known as methyl-CpG-binding domain proteins (MBDs) that can modify histones, thereby forming compact, inactive chromatin, termed heterochromatin.

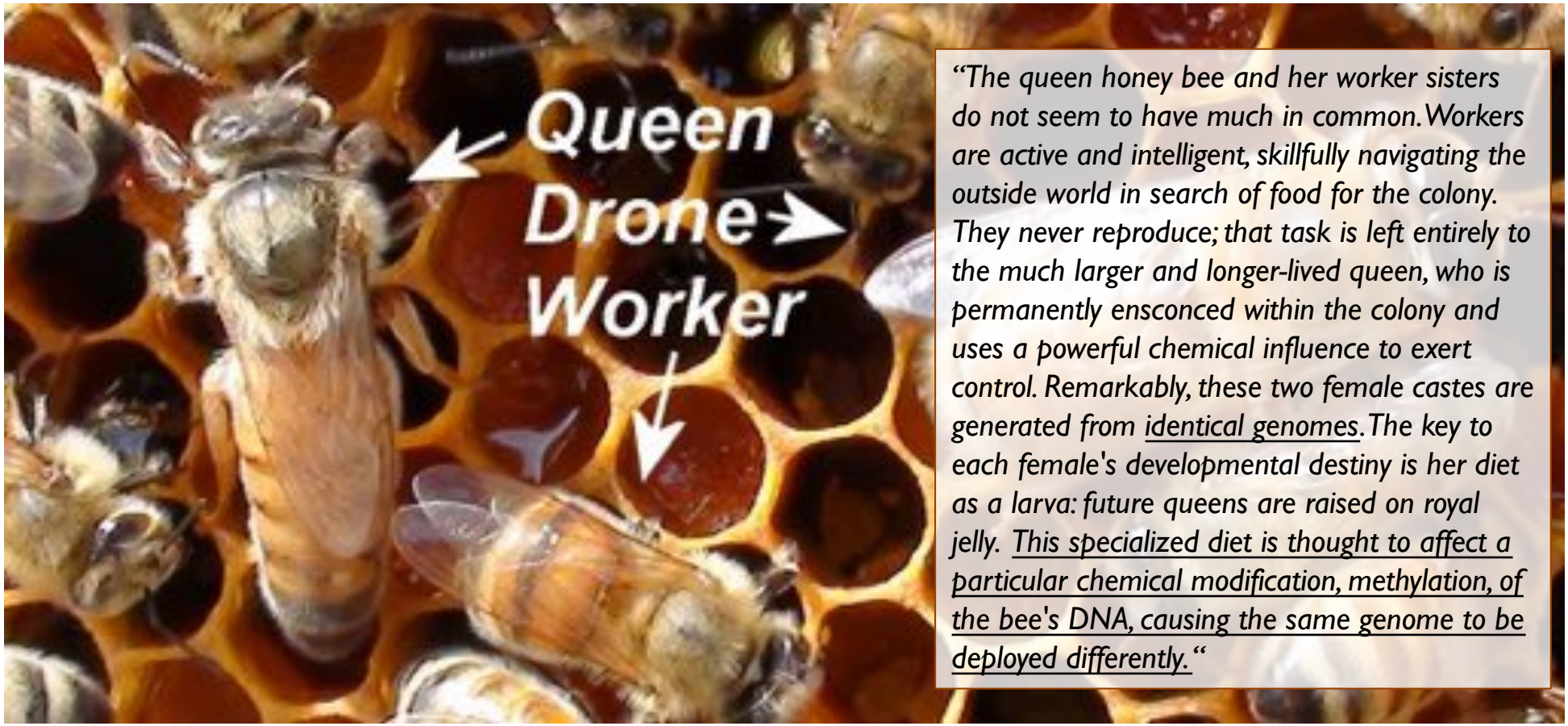
OPEN ACCESS Freely available online

PLoS BIOLOGY

The Honey Bee Epigenomes: Differential Methylation of Brain DNA in Queens and Workers

Frank Lyko[1☯], Sylvain Foret[2☯], Robert Kucharski[3], Stephan Wolf[4], Cassandra Falckenhayn[1], Ryszard Maleszka[3*]

1 Division of Epigenetics, DKFZ-ZMBH Alliance, German Cancer Research Center, Heidelberg, Germany, 2 ARC Centre of Excellence for Coral Reef Studies, James Cook University, Townsville, Australia, 3 Research School of Biology, the Australian National University, Canberra, Australia, 4 Genomics and Proteomics Core Facility, German Cancer Research Center, Heidelberg, Germany



"The queen honey bee and her worker sisters do not seem to have much in common. Workers are active and intelligent, skillfully navigating the outside world in search of food for the colony. They never reproduce; that task is left entirely to the much larger and longer-lived queen, who is permanently ensconced within the colony and uses a powerful chemical influence to exert control. Remarkably, these two female castes are generated from identical genomes. The key to each female's developmental destiny is her diet as a larva: future queens are raised on royal jelly. This specialized diet is thought to affect a particular chemical modification, methylation, of the bee's DNA, causing the same genome to be deployed differently."

Loss of Karma transposon methylation underlies the mantled somaclonal variant of oil palm
Ong-Abdullah, et al (2015) *Nature. doi:10.1038/nature15365*

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Somaclonal variation arises in plants and animals when differentiated somatic cells are induced into a pluripotent state, but the resulting clones differ from each other and from their parents. In agriculture, somaclonal variation has hindered the micropropagation of elite hybrids and genetically modified crops, but the mechanism responsible remains unknown. The oil palm fruit 'mantled' abnormality is a somaclonal variant arising from tissue culture that drastically reduces yield, and has largely halted efforts to clone elite hybrids for oil production. Widely regarded as an epigenetic phenomenon, 'mantling' has defied explanation, but here we identify the MANTLED locus using epigenome-wide association studies of the African oil palm Elaeis guineensis. DNA hypomethylation of a LINE retrotransposon related to rice Karma, in the intron of the homeotic gene DEFICIENS, is common to all mantled clones and is associated with alternative splicing and premature termination. **Dense methylation near the Karma splice site (termed the Good Karma epiallele) predicts normal fruit set, whereas hypomethylation (the Bad Karma epiallele) predicts homeotic transformation, parthenocarpy and marked loss of yield.** *Loss of Karma methylation and of small RNA in tissue culture contributes to the origin of mantled, while restoration in spontaneous revertants accounts for non-Mendelian inheritance. The ability to predict and cull mantling at the plantlet stage will facilitate the introduction of higher performing clones and optimize environmentally sensitive land resources.*

Loss of Karma transposon methylation underlies the mantled somaclonal variant of oil palm
Ong-Abdullah, et al (2015) *Nature. doi:10.1038/nature15365*

Hypomethylation distinguishes genes of some human cancers from their normal counterparts

Andrew P. Feinberg & Bert Vogelstein

Cell Structure and Function Laboratory, The Oncology Center, Johns Hopkins University School of Medicine, Baltimore, Maryland 21205, USA

It has been suggested that cancer represents an alteration in DNA, heritable by progeny cells, that leads to abnormally regulated expression of normal cellular genes; DNA alterations such as mutations[1,2], rearrangements[3–5] and changes in methylation[6–8] have been proposed to have such a role. Because of increasing evidence that DNA methylation is important in gene expression (for review see refs 7, 9–11), several investigators have studied DNA methylation in animal tumours, transformed cells and leukaemia cells in culture[8,12–30]. The results of these studies have varied; depending on the techniques and systems used, an increase[12–19], decrease[20–24], or no change[25–29] in the degree of methylation has been reported. To our knowledge, however, primary human tumour tissues have not been used in such studies. We have now examined DNA methylation in human cancer with three considerations in mind: (1) the methylation pattern of specific genes, rather than total levels of methylation, was determined; (2) human cancers and adjacent analogous normal tissues, unconditioned by culture media, were analysed; and (3) the cancers were taken from patients who had received neither radiation nor chemotherapy. In four of five patients studied, representing two histological types of cancer, substantial hypomethylation was found in genes of cancer cells compared with their normal counterparts. This hypomethylation was progressive in a metastasis from one of the patients.

and (3) *Hpa*II and *Hha*I cleavage sites should be present in the regions of the genes.

The first cancer studied was a grade D (ref. 43), moderately well differentiated adenocarcinoma of the colon from a 67-yr-old male. Tissue was obtained from the cancer itself and also from colonic mucosa stripped from the colon at a site just outside the histologically proven tumour margin. Figure 1 shows the pattern of methylation of the studied genes. Before digestion with restriction enzymes, all DNA samples used in the study had a size >25,000 base pairs (bp). After *Hpa*II cleavage, hybridization with a probe made from a cDNA clone of human growth hormone (HGH) showed that significantly more of the DNA was digested to low-molecular weight fragments in DNA from the cancer (labelled C in Fig. 1) than in DNA from the normal colonic mucosa (labelled N). In the hybridization conditions used, the HGH probe detected the human growth hormone genes as well as the related chorionic somatotropin

Table 1 Quantitation of methylation of specific genes in human cancers and adjacent analogous normal tissues

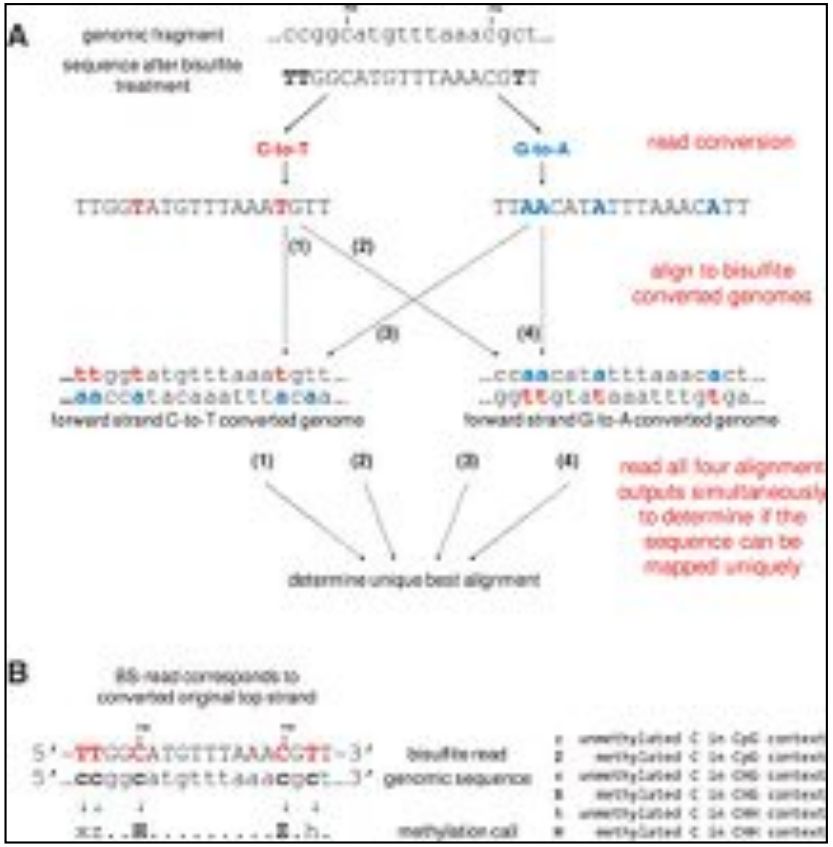
Patient	Carcinoma	Probe	Enzyme	% Hypomethylated fragments		
				N	C	M
1	Colon	HGH	*Hpa*II	<10	35	—
			*Hha*I	<10	39	—
		γ-Globin	*Hpa*II	<10	52	—
			*Hha*I	<10	39	—
		α-Globin	*Hpa*II	<10	<10	—
			*Hha*I	<10	<10	—
2	Colon	HGH	*Hpa*II	<10	76	—
			*Hha*I	<10	85	—
		γ-Globin	*Hpa*II	<10	58	—
			*Hha*I	<10	23	—
		α-Globin	*Hpa*II	<10	<10	—
			*Hha*I	<10	<10	—
3	Colon	HGH	*Hpa*II	<10	41	—
			*Hha*I	<10	38	—
		γ-Globin	*Hpa*II	<10	50	—

Nature 301, 89 - 92 (06 January 1983); doi:10.1038/301089a0

Bisulfite Conversion

Treating DNA with sodium bisulfite will convert unmethylated C to T

- 5-MethylC will be protected and not change, so can look for differences when mapping
- Requires great care when analyzing reads, since the complementary strand will also be converted (G to A)
- Typically analyzed by mapping to a "reduced alphabet" where we assume all Cs are converted to Ts once on the forward strand and once on the reverse



Bismark: a flexible aligner and methylation caller for Bisulfite-Seq applications
Krueger and Andrews (2010) *Bioinformatics*. 27 (11): 1571-1572.

Bisulfite Conversion

Tr
w

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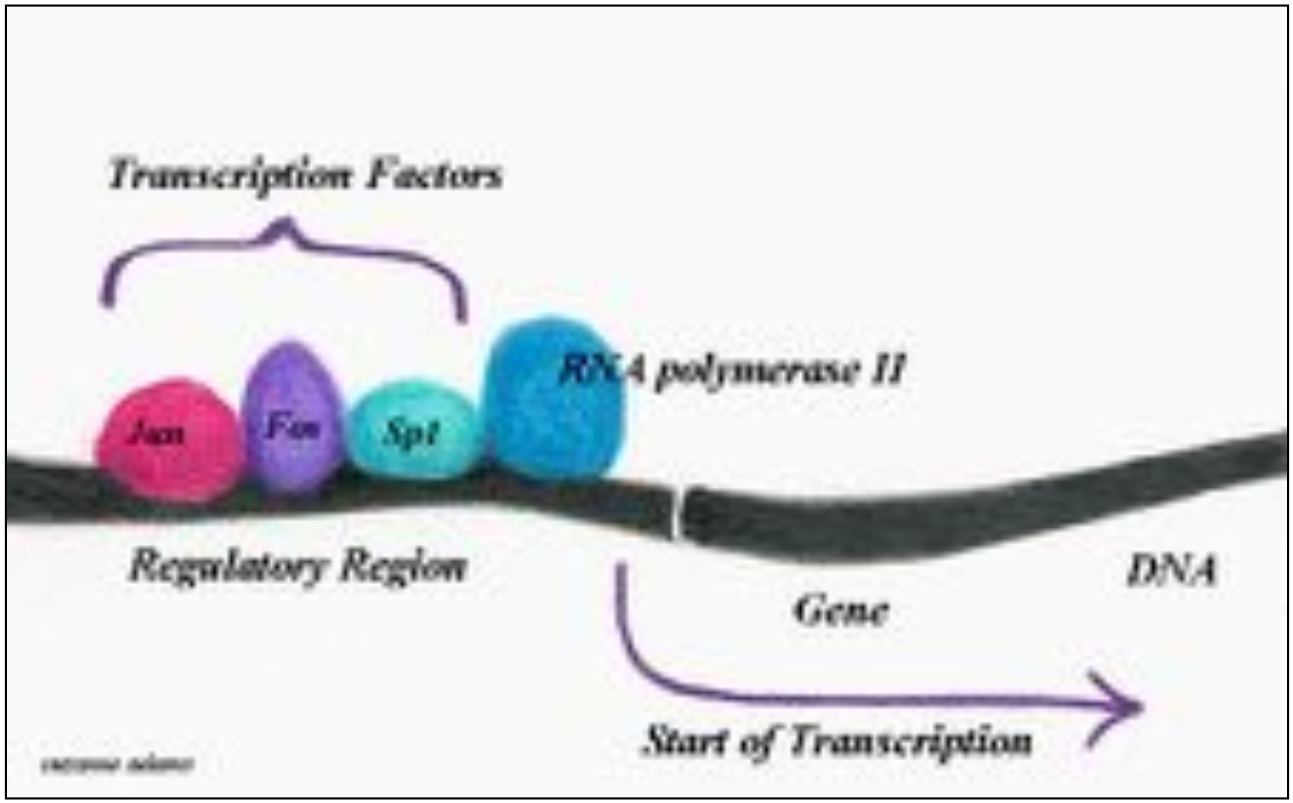
Bismark: a flexible aligner and methylation caller for Bisulfite-Seq applications
Krueger and Andrews (2010) *Bioinformatics*. 27 (11): 1571-1572.

ChIP-seq

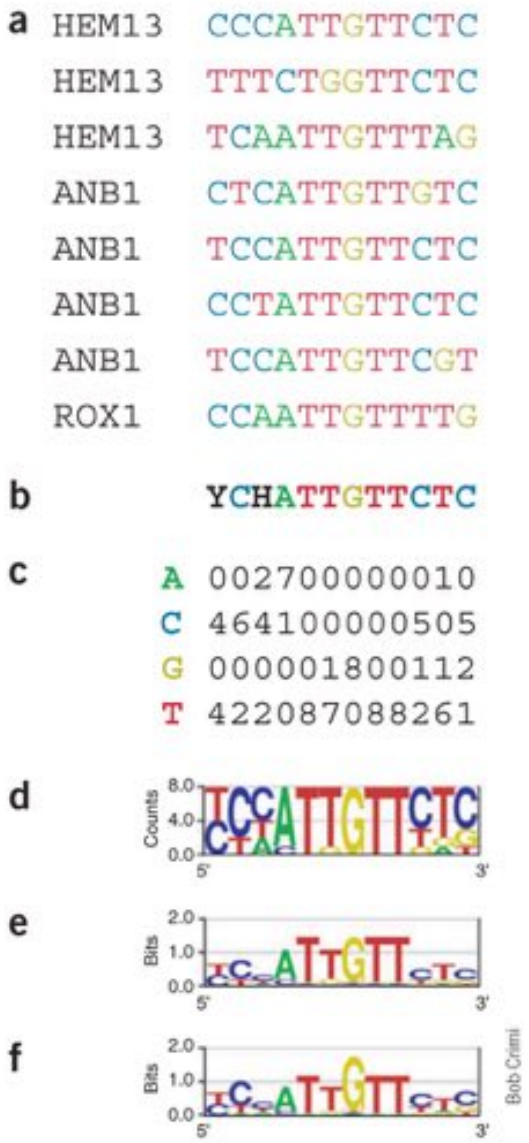


Genome-wide mapping of in vivo protein-DNA interactions.
Johnson *et al* (2007) *Science.* 316(5830):1497-502

Transcription Factors

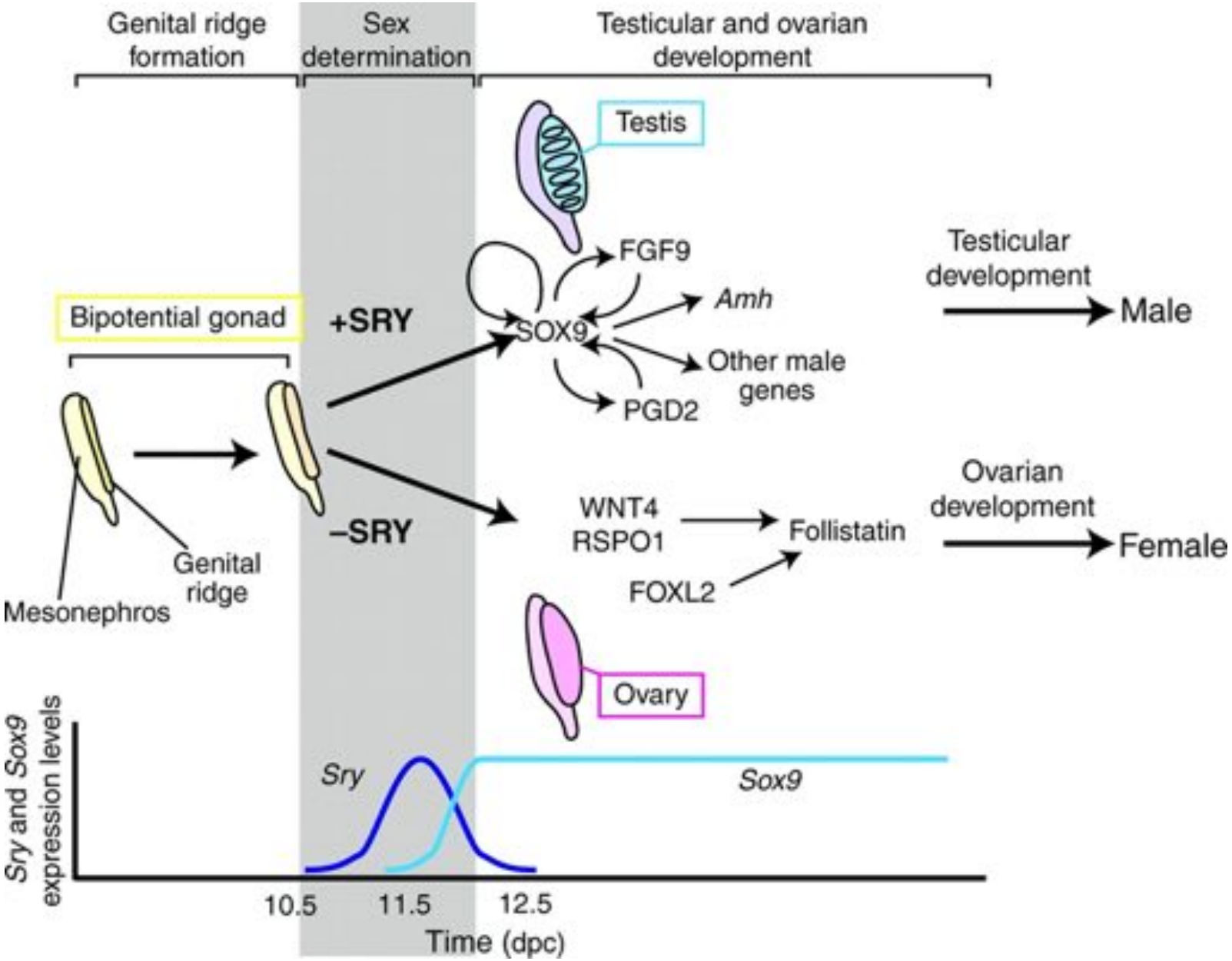
A transcription factor (or sequence-specific DNA-binding factor) is a protein that controls the rate of transcription of genetic information from DNA to messenger RNA, by binding to a specific DNA sequence.

- Transcription factors work alone or with other proteins in a complex, by promoting (as an activator), or blocking (as a repressor) the recruitment of RNA polymerase to specific genes.
- A defining feature of transcription factors is that they contain at least one DNA-binding domain (DBD)
- Figure (a) Eight known genomic binding sites in three S. cerevisiae genes. (b) Degenerate consensus sequence. (c,d) Frequencies of nucleotides at each position. (e) Sequence logo (f) Energy normalized logo using relative entropy to adjust for low GC content in S. cerevisiae.

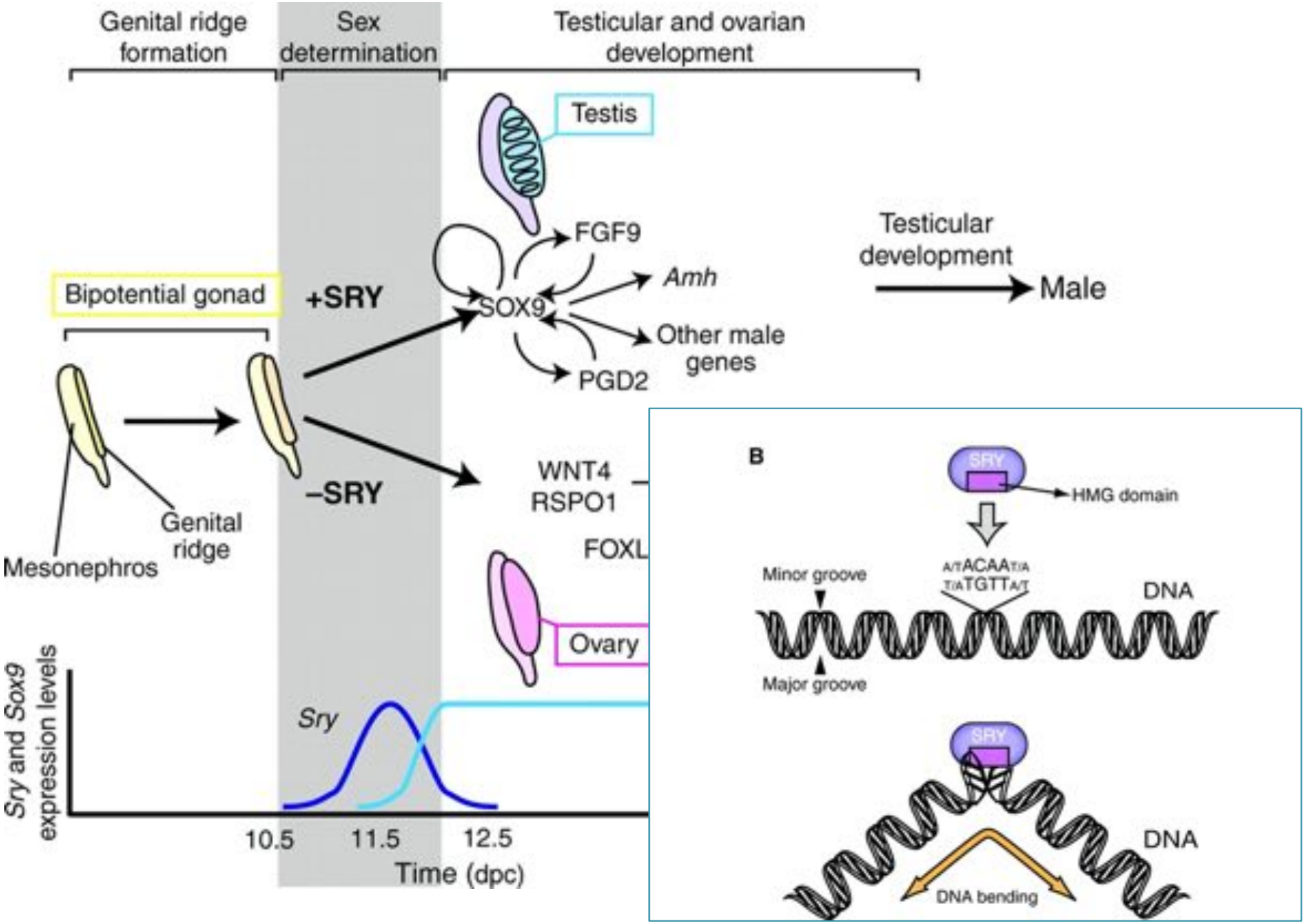


What are DNA sequence motifs?

D'haeseleer (2006) Nature Biotechnology 24, 423 – 425 doi:10.1038/nbt0406-423



SRY: The master switch in mammalian sex determination
Kashimada and Koopman (2010) Development 137: 3921-3930; doi: 10.1242/dev.048983



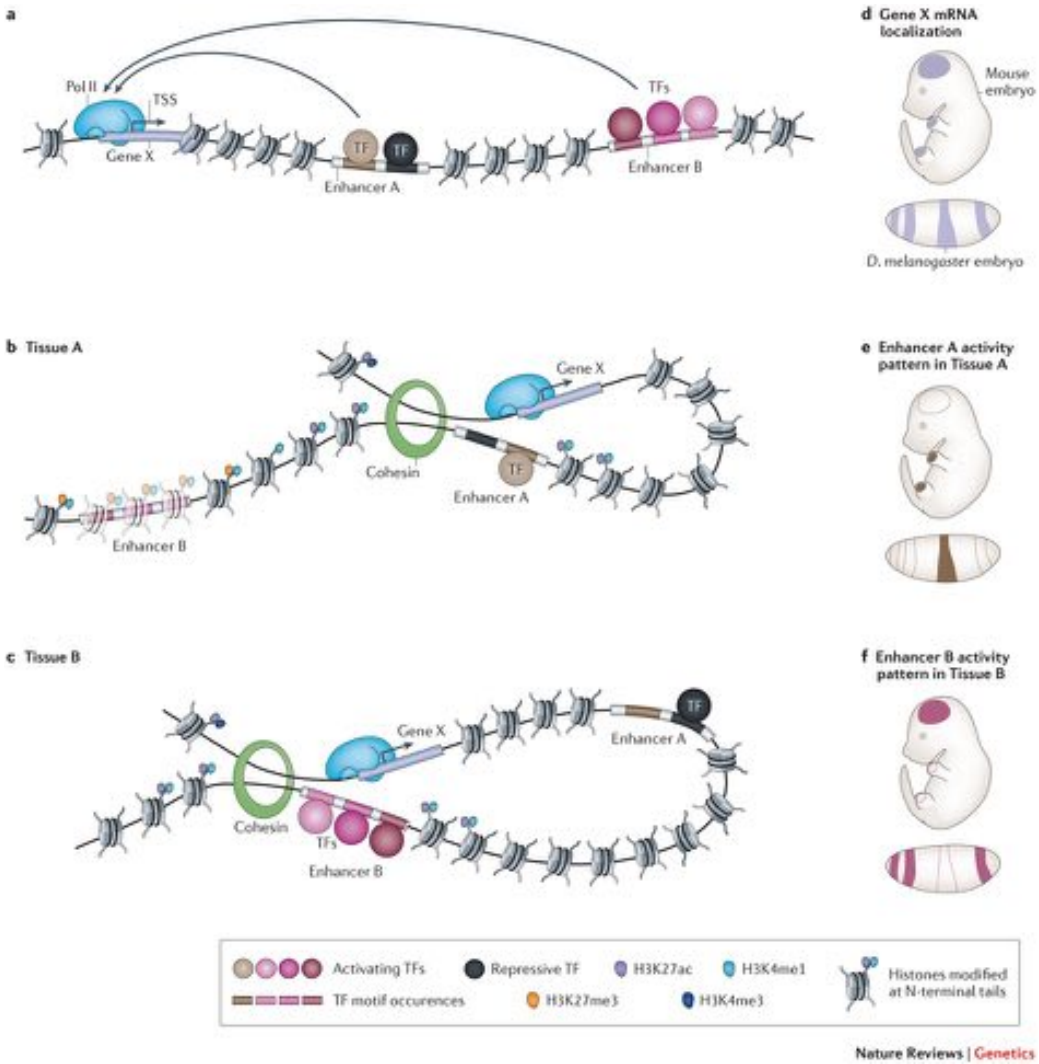
SRY: The master switch in mammalian sex determination

Kashimada and Koopman (2010) Development 137: 3921-3930; doi: 10.1242/dev.048983

Enhancers

Enhancers are genomic regions that contain binding sites for transcription factors (TFs) and that can upregulate (enhance) the transcription of a target gene.

- Enhancers can be located at any distance from their target genes (up to ~1Mbp)
- In a given tissue, active enhancers (Enhancer A in part b or Enhancer B in part c) are bound by activating TFs and are brought into proximity of their respective target promoters by looping
- Active and inactive gene regulatory elements are marked by various biochemical features
- Complex patterns of gene expression result from the additive action of different enhancers with cell-type- or tissue-specific activities

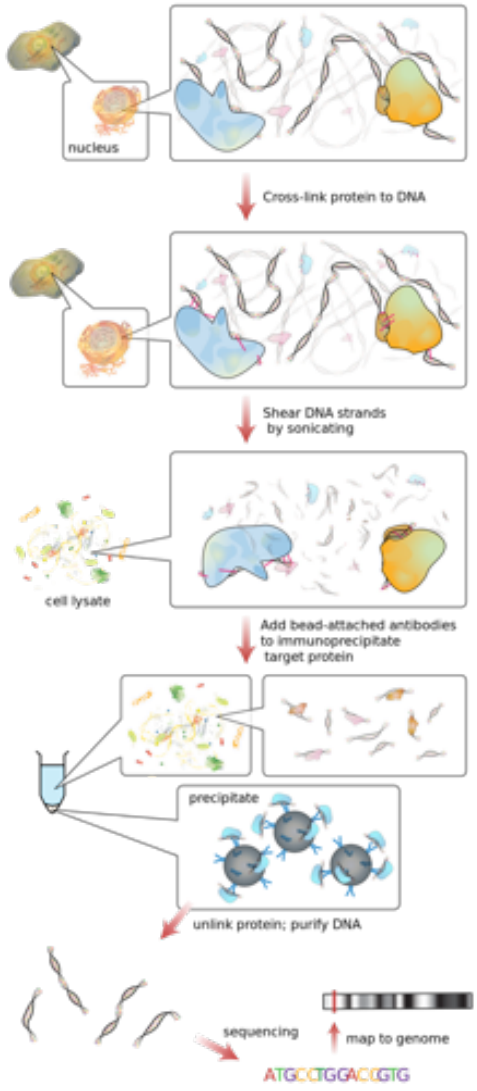


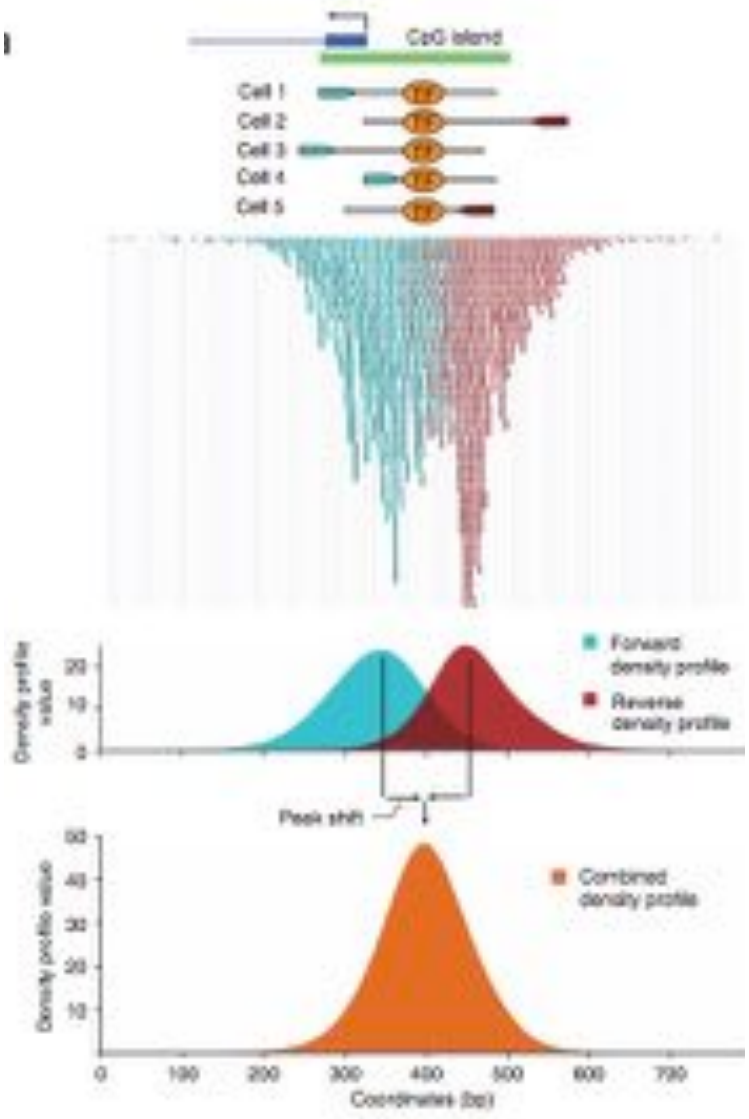
Transcriptional enhancers: from properties to genome-wide predictions
Shlyueva et al (2014) *Nature Reviews Genetics* 15, 272–286

ChIP-seq: TF Binding

Goals:

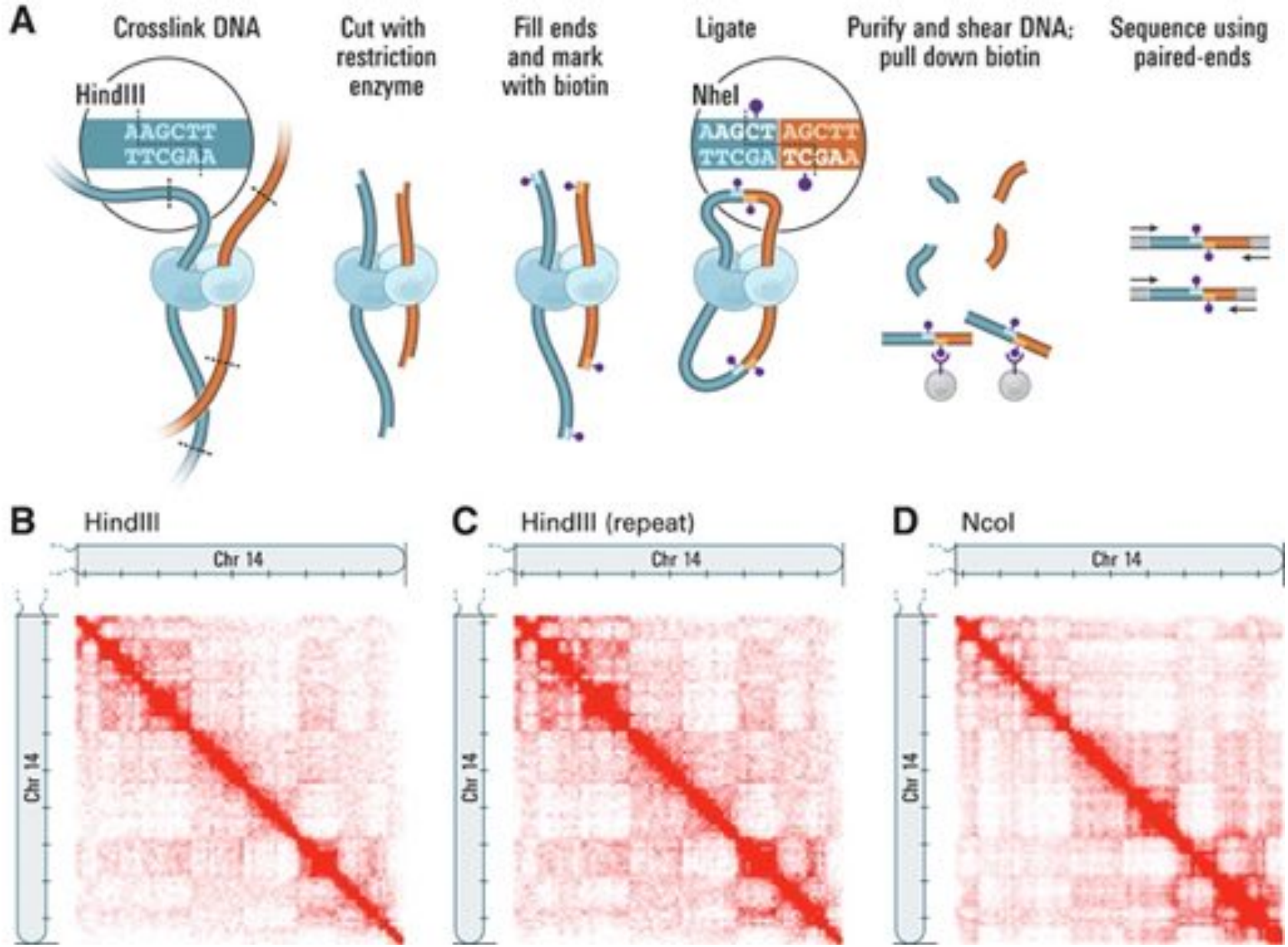
- Where are transcription factors and other proteins binding to the DNA?
- How strongly are they binding?
- Do the protein binding patterns change over developmental stages or when the cells are stressed?





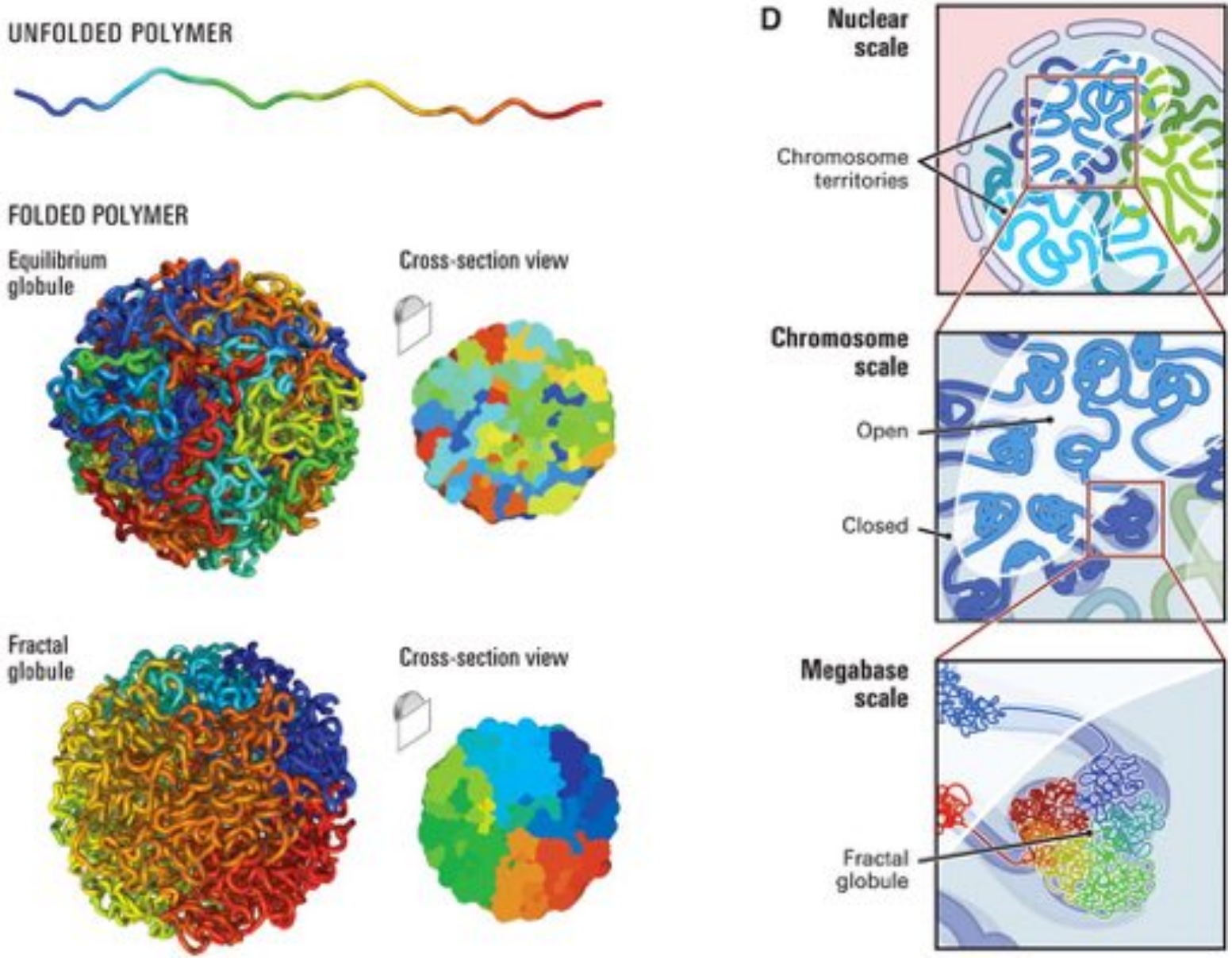
Genome-wide analysis of transcription factor binding sites based on ChIP-Seq data
Valouev et al (2008) *Nature Methods*. 5, 829 - 834

HI-C: Mapping the folding of DNA



Comprehensive Mapping of Long-Range Interactions Reveals Folding Principles of the Human Genome
Liberman-Aiden et al. (2009) *Science*. 326 (5950): 289-293

HI-C: Mapping the folding of DNA



Comprehensive Mapping of Long-Range Interactions Reveals Folding Principles of the Human Genome
Liberman-Aiden et al. (2009) *Science*. 326 (5950): 289-293

Gene Regulation in 3-dimensions

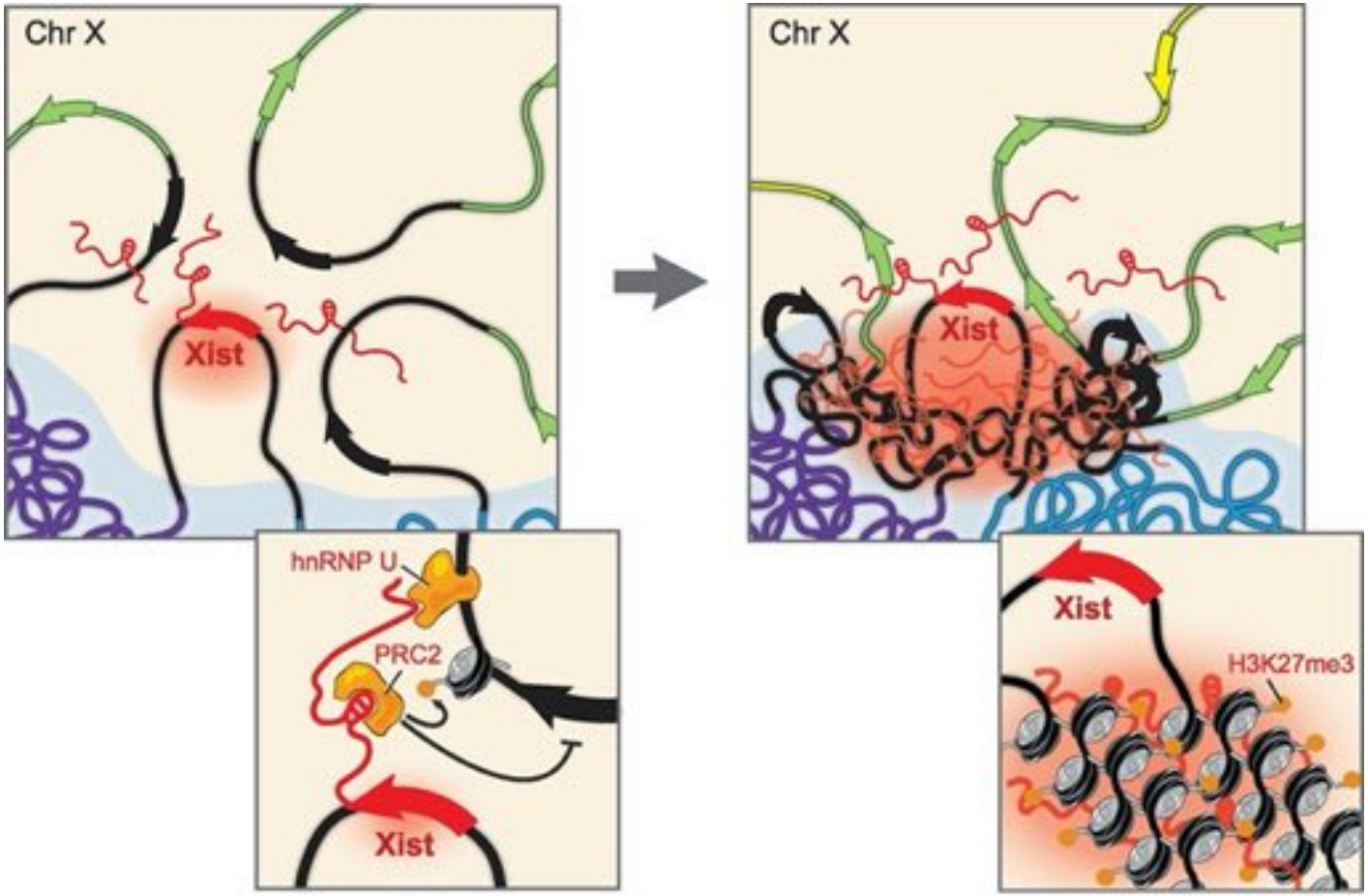
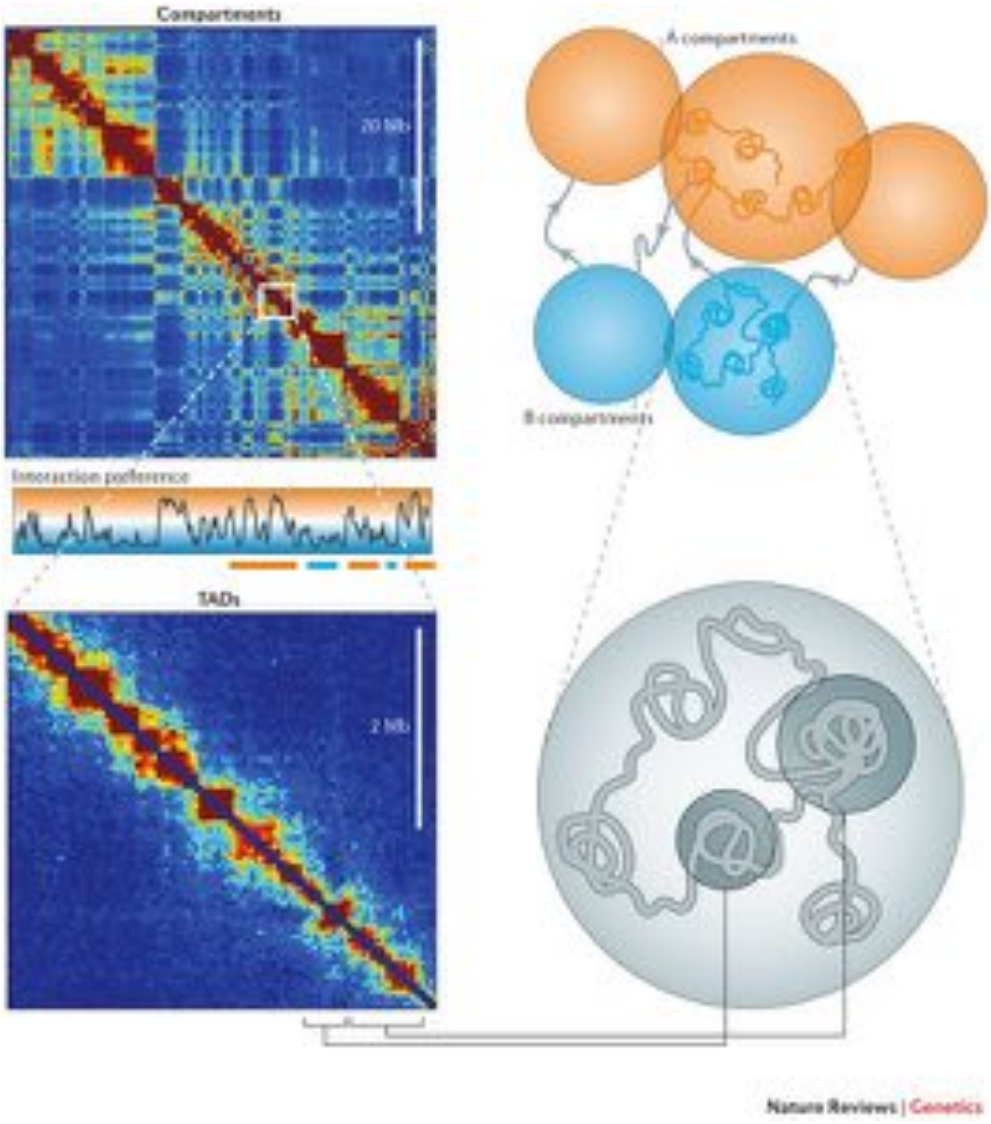


Fig 6. A model for how Xist exploits and alters three-dimensional genome architecture to spread across the X chromosome.

The Xist lncRNA Exploits Three-Dimensional Genome Architecture to Spread Across the X Chromosome
Engreitz et al. (2013) *Science*. 341 (6147)

Genome compartments & TADs



Mammalian genomes have a pattern of interactions that can be approximated by two compartments called A and B

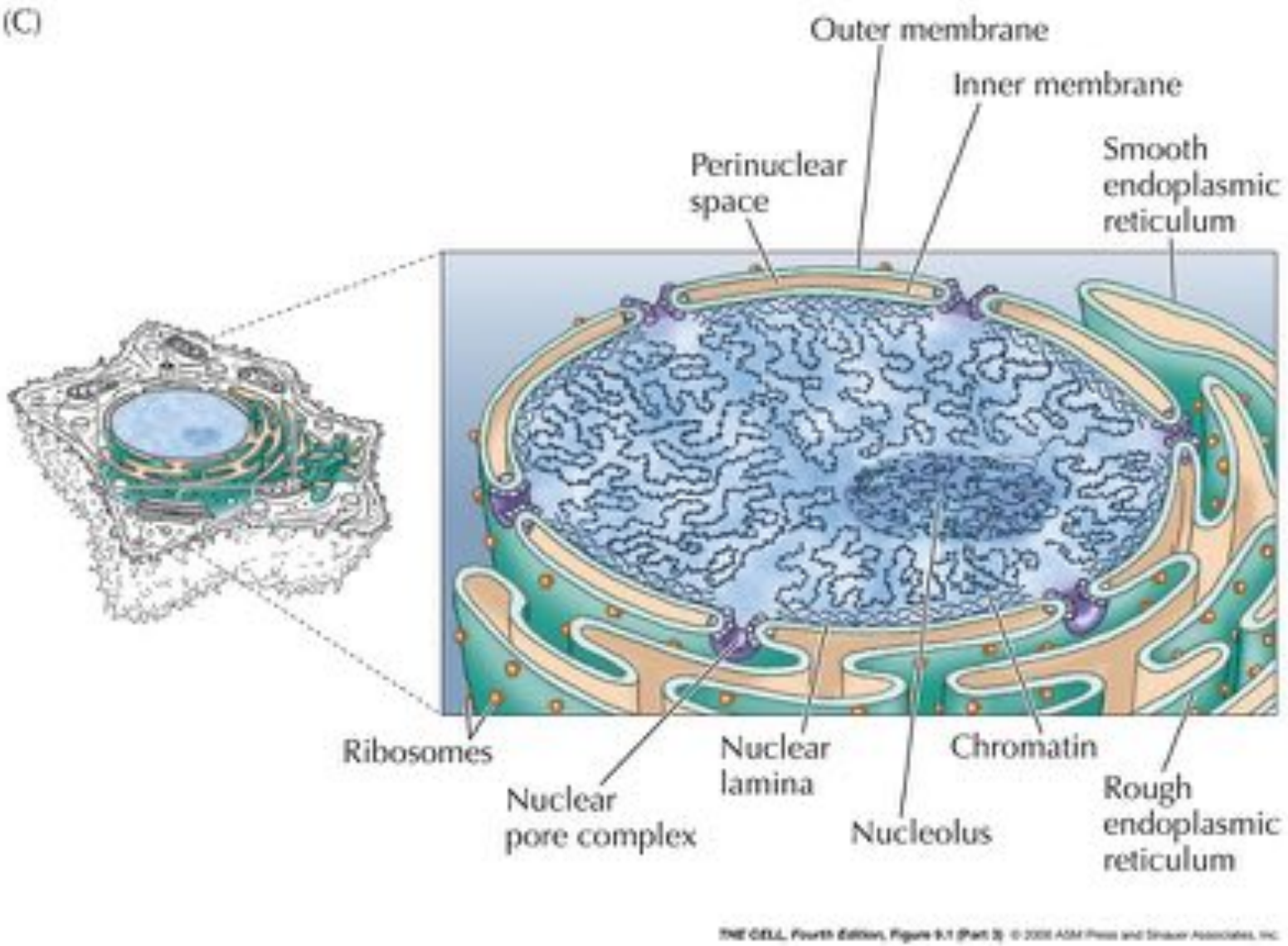
- alternate along chromosomes and have a characteristic size of ~5 Mb each.
- A compartments (orange) preferentially interact with other A compartments; B compartments (blue) associate with other B compartments.
- A compartments are largely euchromatic, transcriptionally active regions.

Topologically associating domains (TADs)

- TADs are smaller (~400–500 kb)
- Can be active or inactive, and adjacent TADs are not necessarily of opposite chromatin status.
- TADs are hard-wired features of chromosomes, and groups of adjacent TADs can organize in A and B compartments

Exploring the three-dimensional organization of genomes: interpreting chromatin interaction data
Dekker et al. (2013) *Nature Reviews Genetics 14, 390–403*

“Lamina-Associated Domains are the B compartment”



Chromosome Conformation Paints Reveal the Role of Lamina Association in Genome Organization and Regulation
Luperchio et al. (2017) bioRxiv. *doi: https://doi.org/10.1101/122226*