Epigenetics

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Ntziachristos Lab

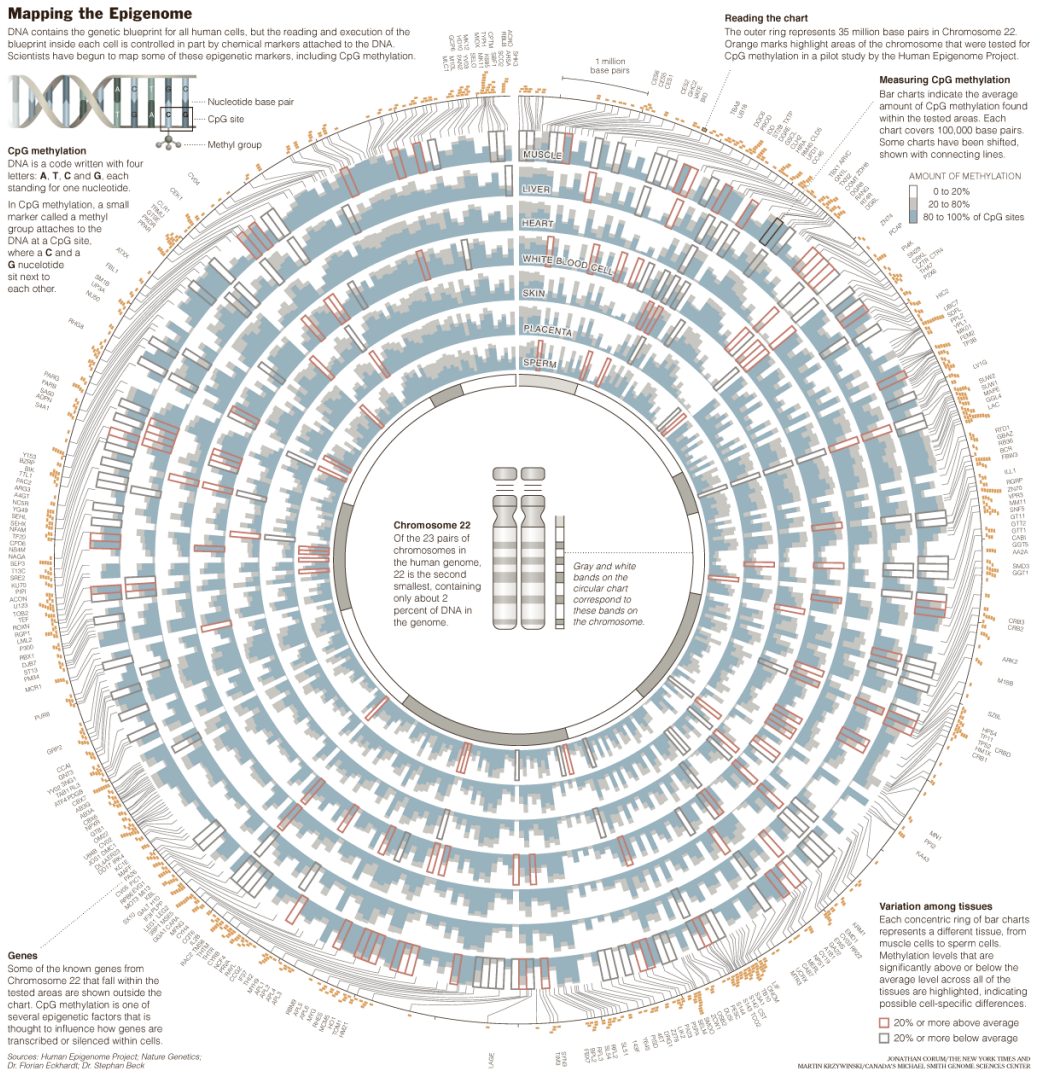
Overview

- Epigenetics introduction
- Techniques to study epigenetic marks
- Epigenetics in health and disease
- Epigenetic mechanisms of drug resistance and relapse

Epigenetics

De facto definition:

- *Transmission of information from a cell or organism to its descendants without the information being encoded in the nucleotide sequence.*
- A gene product expressed in a cell maintains activity of that gene. This activity is inherited in descendants of that cell. (non-coding RNAs)
- **Chemical modifications of chromatin/DNA copied with the DNA.**
- **Transgenerational Epigenetic Inheritance (TEI)** is the transmittance of epigenetic information from one generation to the next that affects the traits of offspring.



The New York Times, Nov 11, 2008

Transgenerational Epigenetic Inheritance: Myths and Mechanisms

Edith Heard[1,2,*] and Robert A. Martienssen[3,4,*]

Epigenetics in action

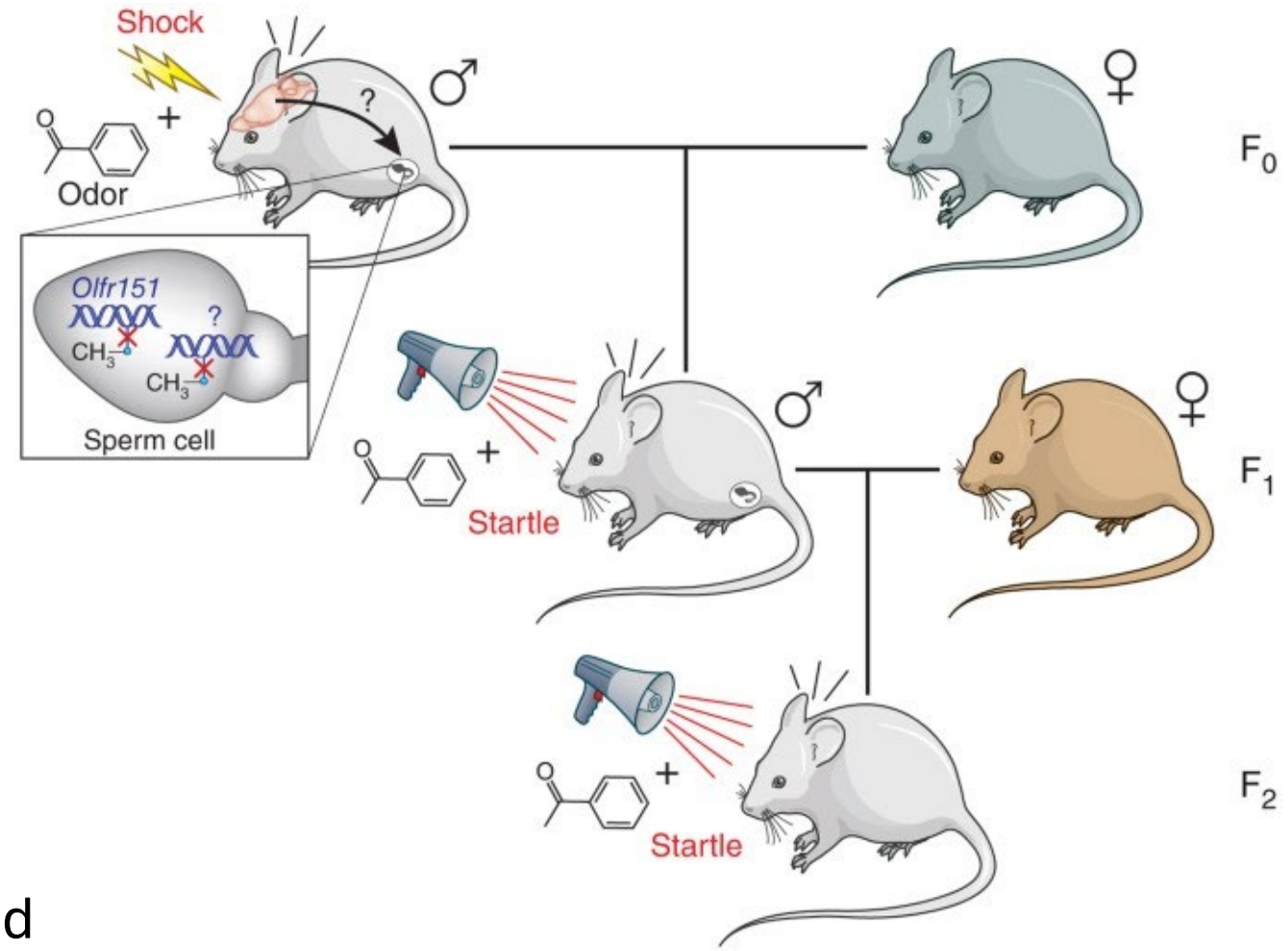
Article | Published: 01 December 2013

Parental olfactory experience influences behavior and neural structure in subsequent generations

Brian G Dias & Kerry J Ressler

Nature Neuroscience **17**, 89–96 (2014) | Cite this article

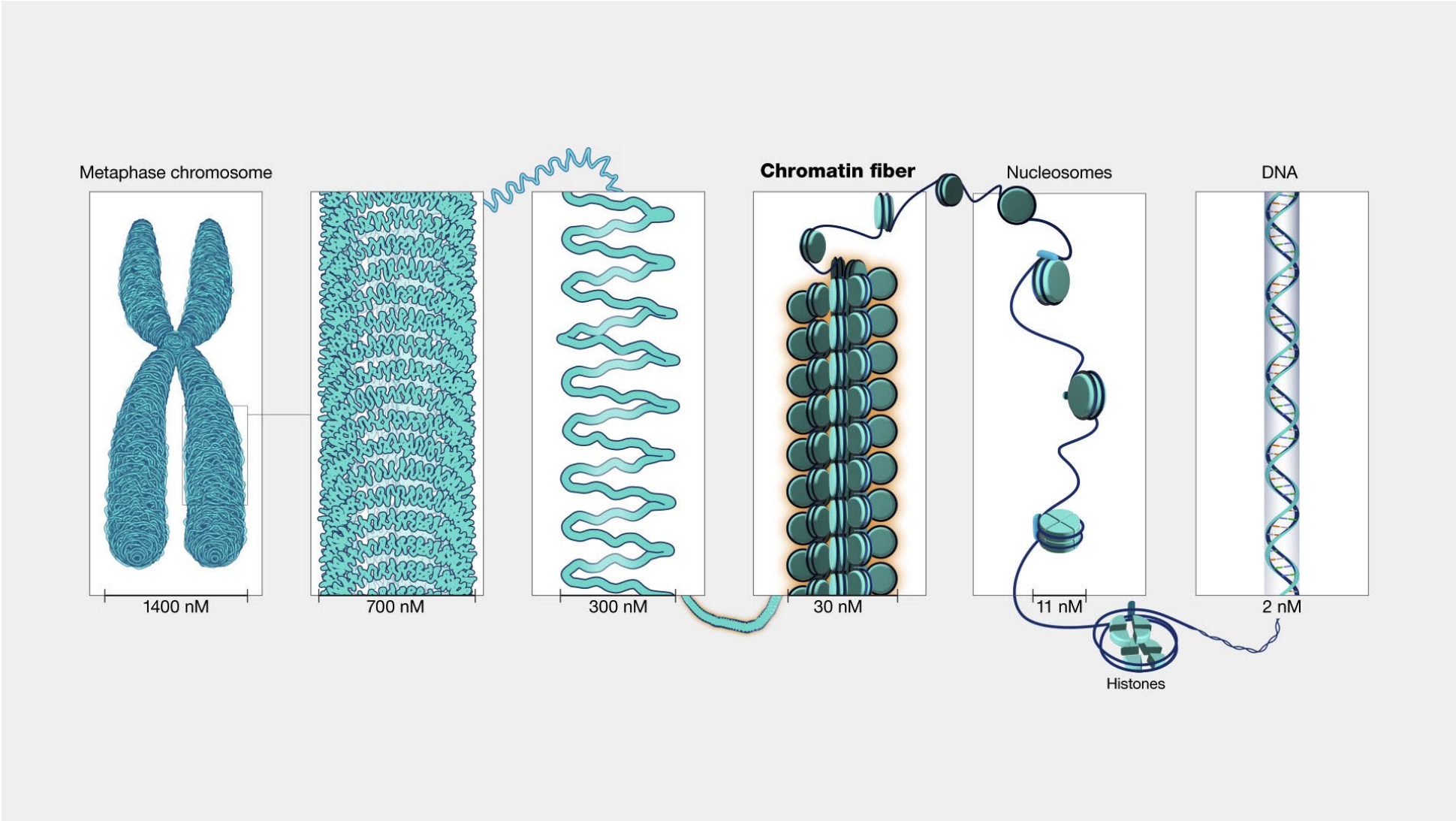
Acetophenon or Propanol



Lamarck revisited

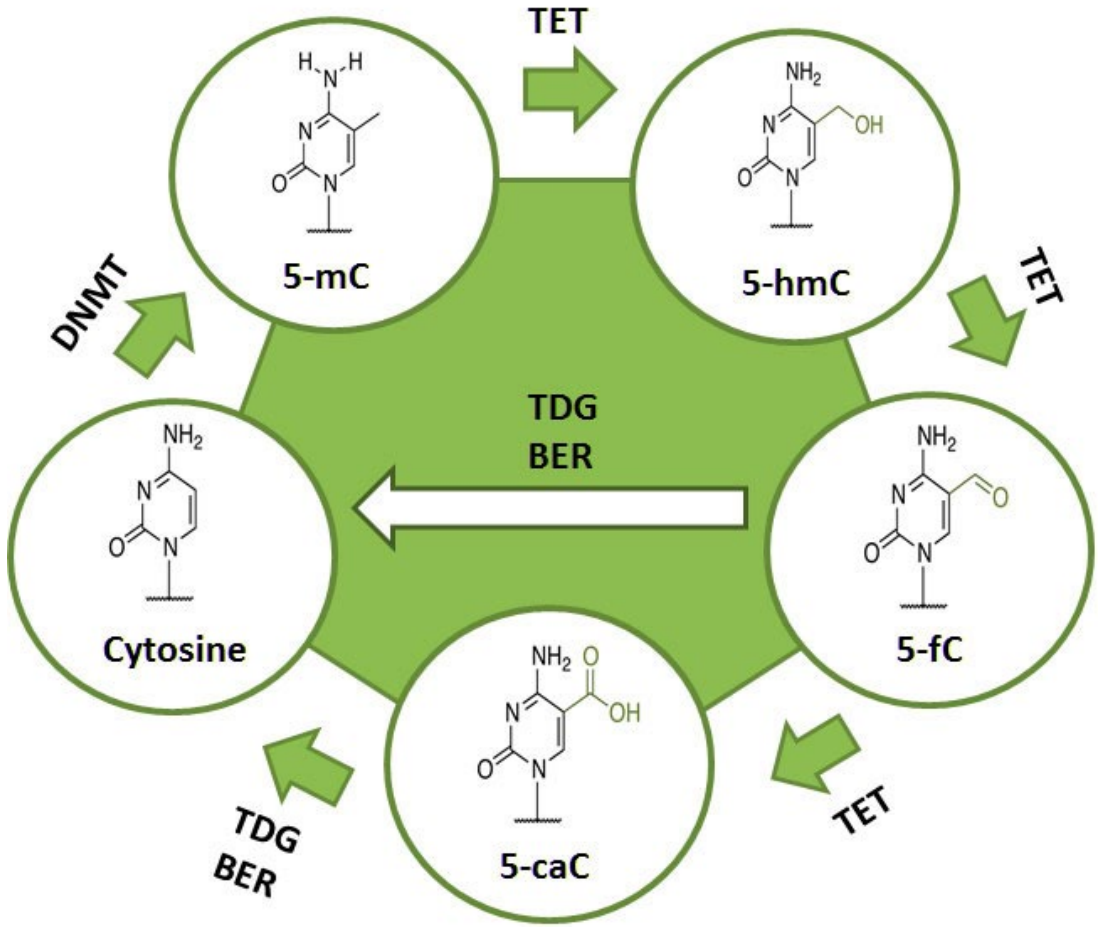
Szyf, Nature Neuroscience, 2014

Chromatin

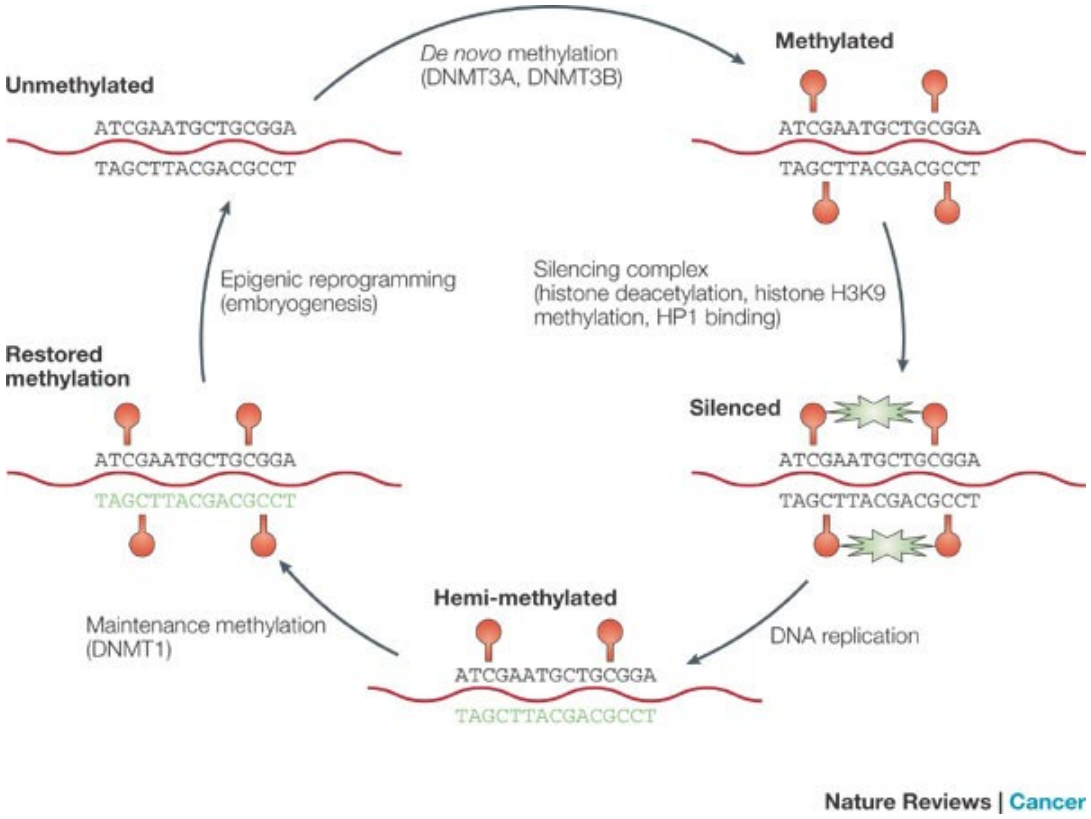


Paul Liu, NIH resources

DNA methylation is reversible



Modes of DNA methylation

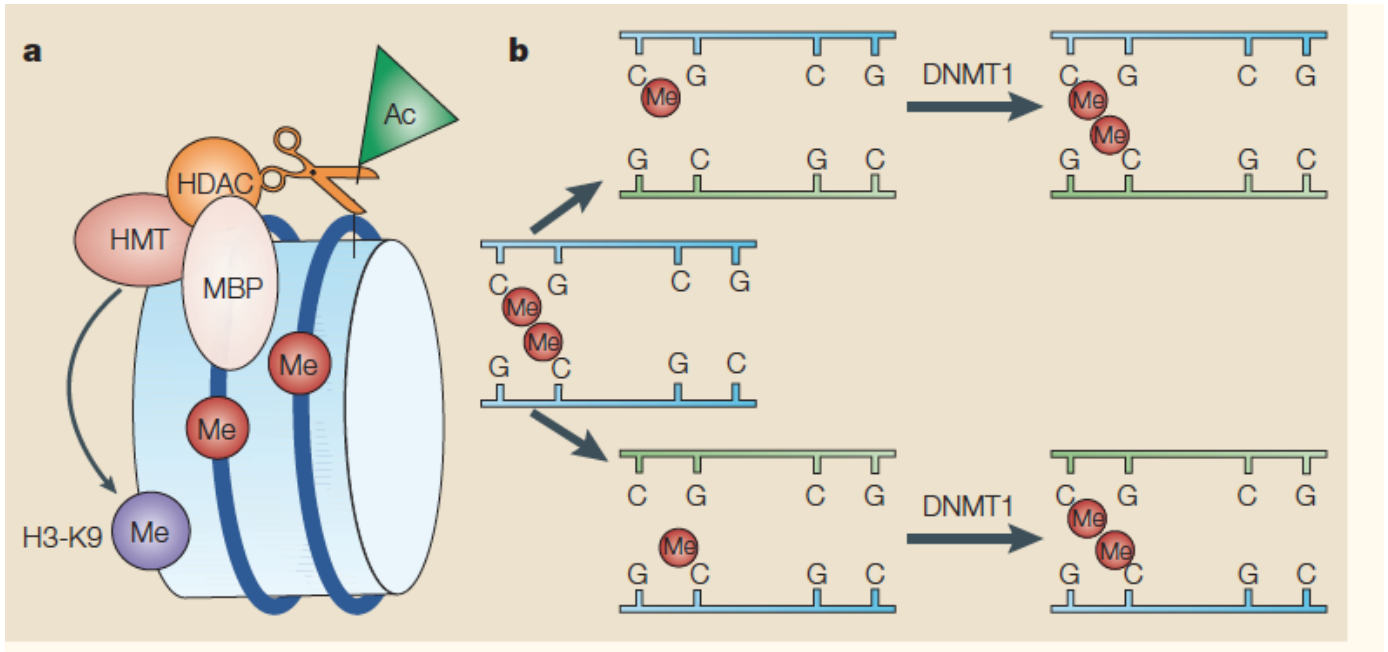


Dennis R Grayson and Alessandro Guidotti

DNA methylation can be added to new sites,
called de novo methylation
-via DNMT3A/B

Or inherited through maintenance methylation
-via DNTM1

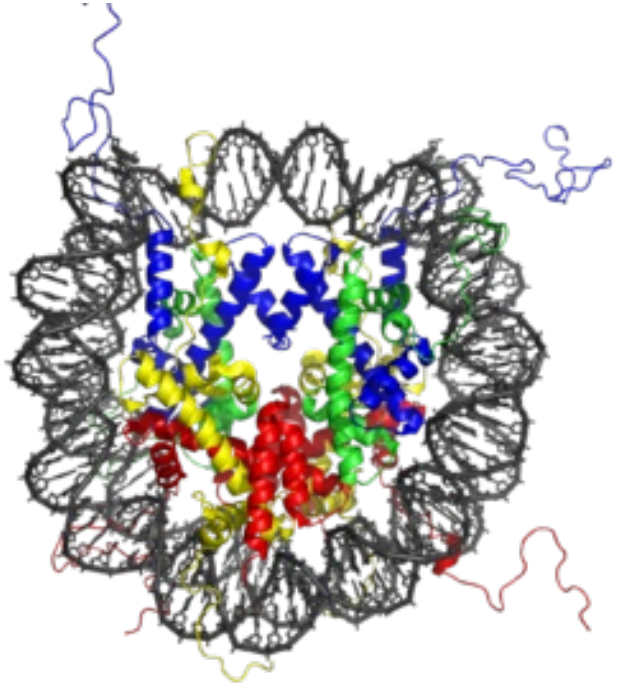
Repression of gene expression by methylation is mediated by crosstalk between DNA Methylation and Histone modifications



Chromatin

- Nucleosome is the basic unit of chromatin

Histone H3
Histone H4
Histone H2A
Histone H2B

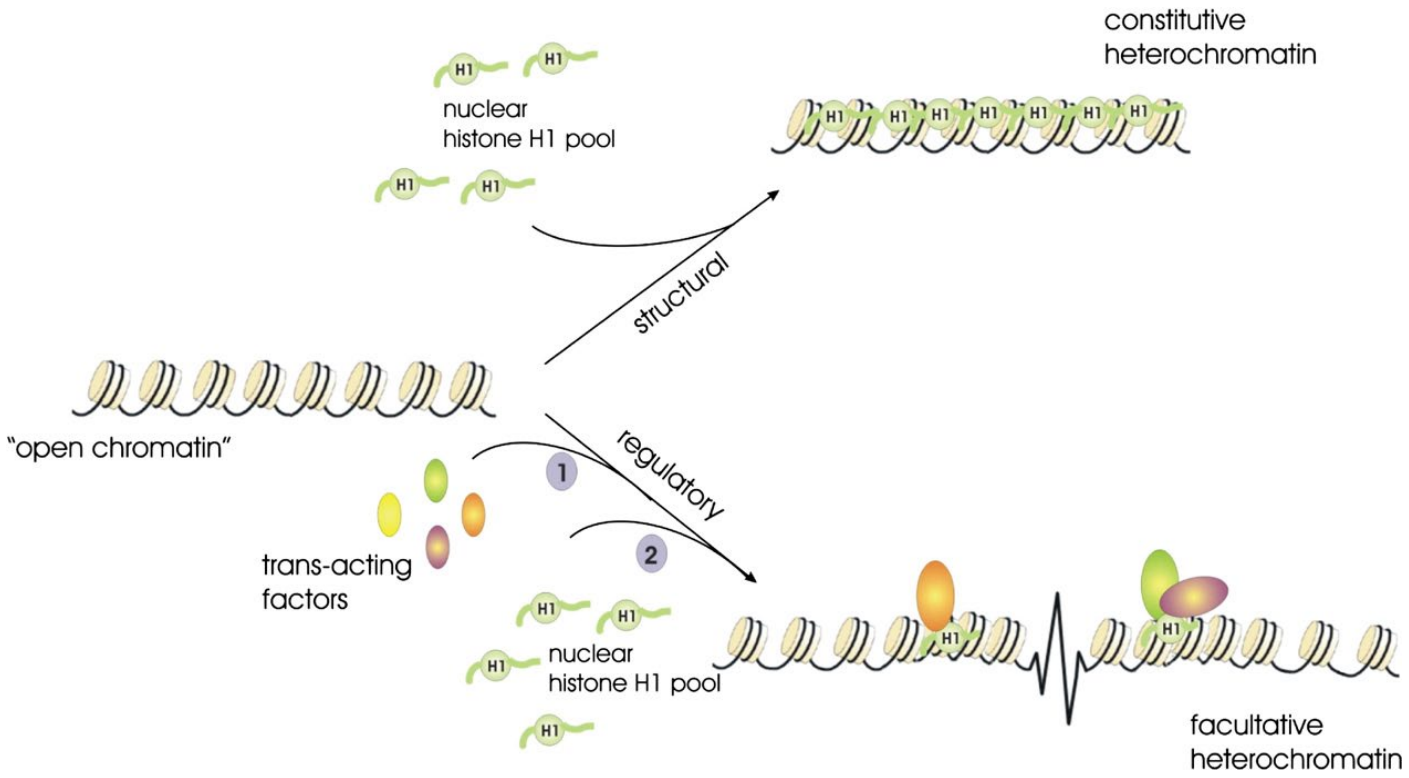


Assembly of the nucleosome regulates access of other proteins to the DNA

Wraps ~145 bps DNA
1.7x around the histone core octamer

Chromatin can exist in active and inactive states

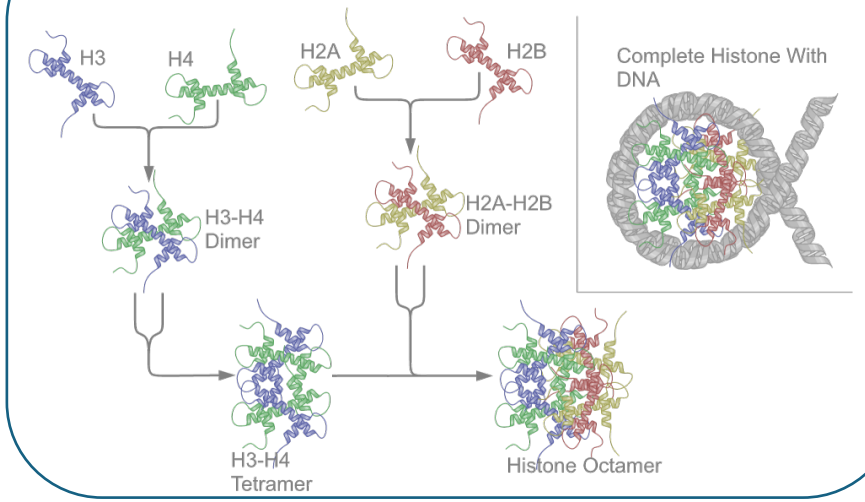
Euchromatin: Active, open
Heterochromatin: inactive/repressed, closed
-facultative: regulated
-constitutive: allways repressed



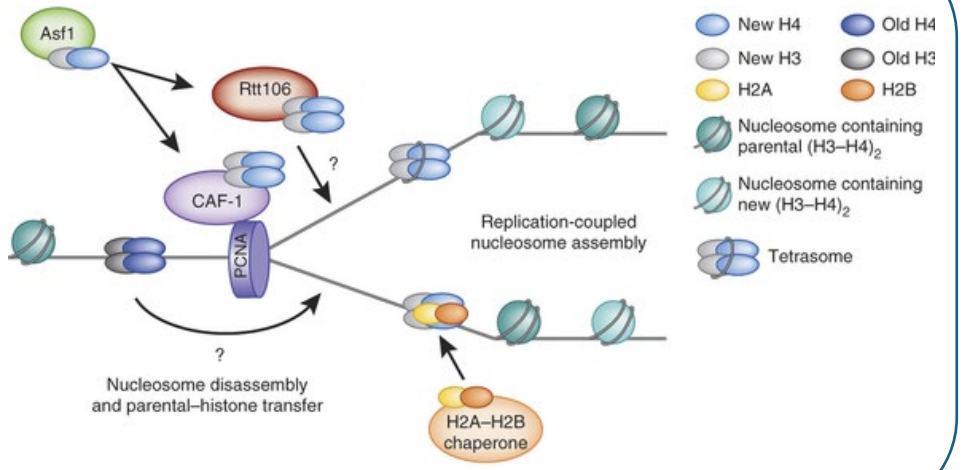
Nucleosomes must be assembled

-via histone chaperones

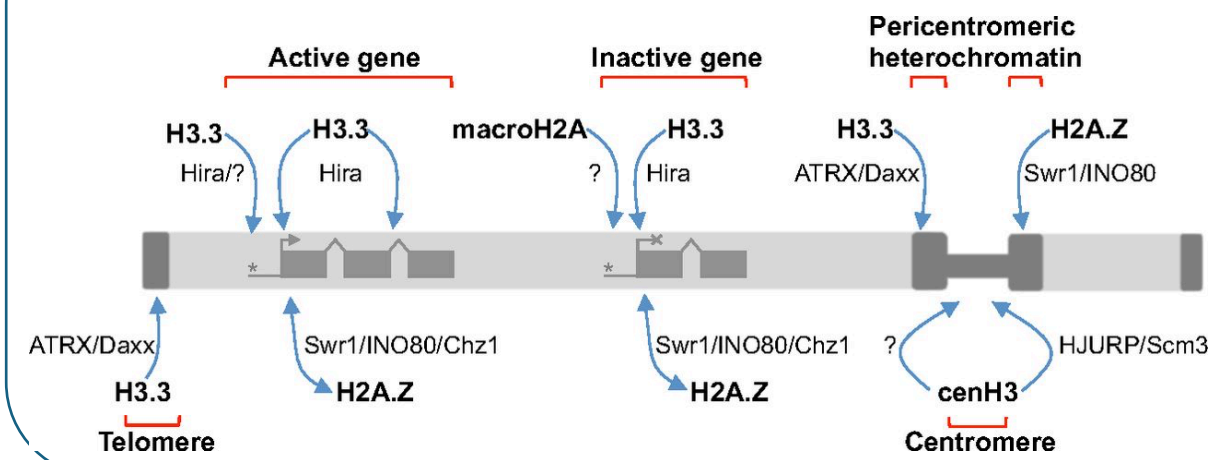
Histones are made from subunits



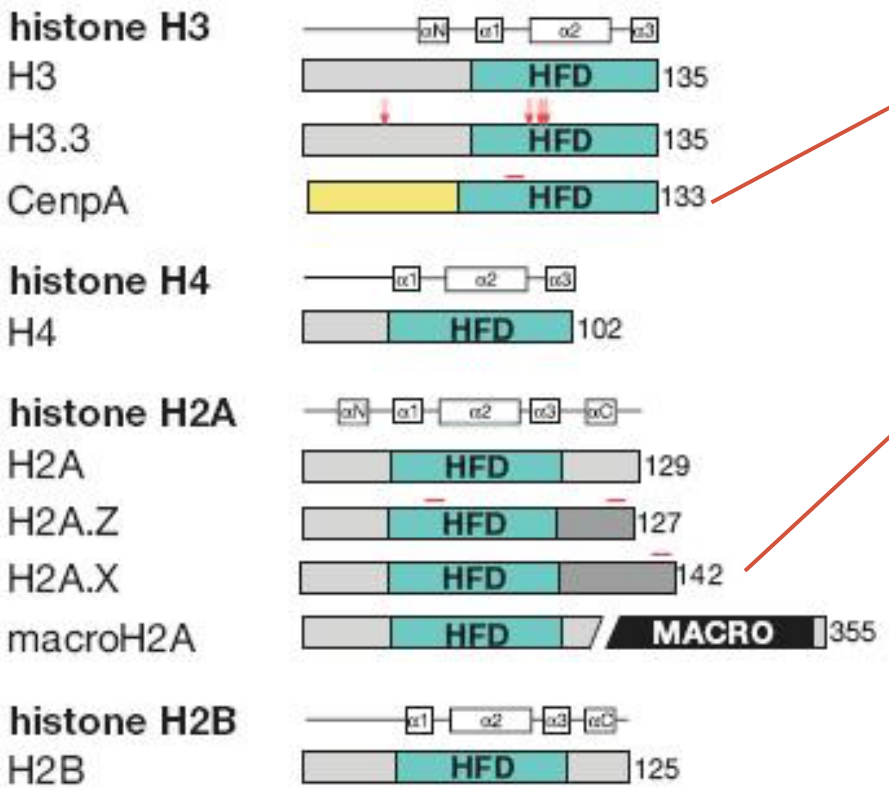
Subunits require chaperones

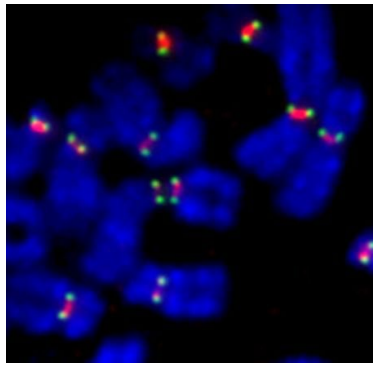


Histone Variants utilize distinct chaperones



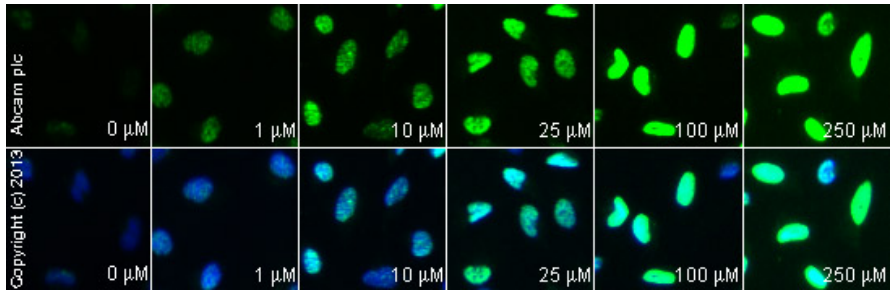
Differentiating chromatin function by histone variants

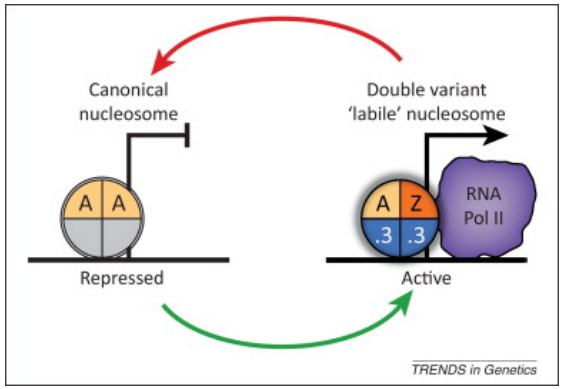




Centromeres

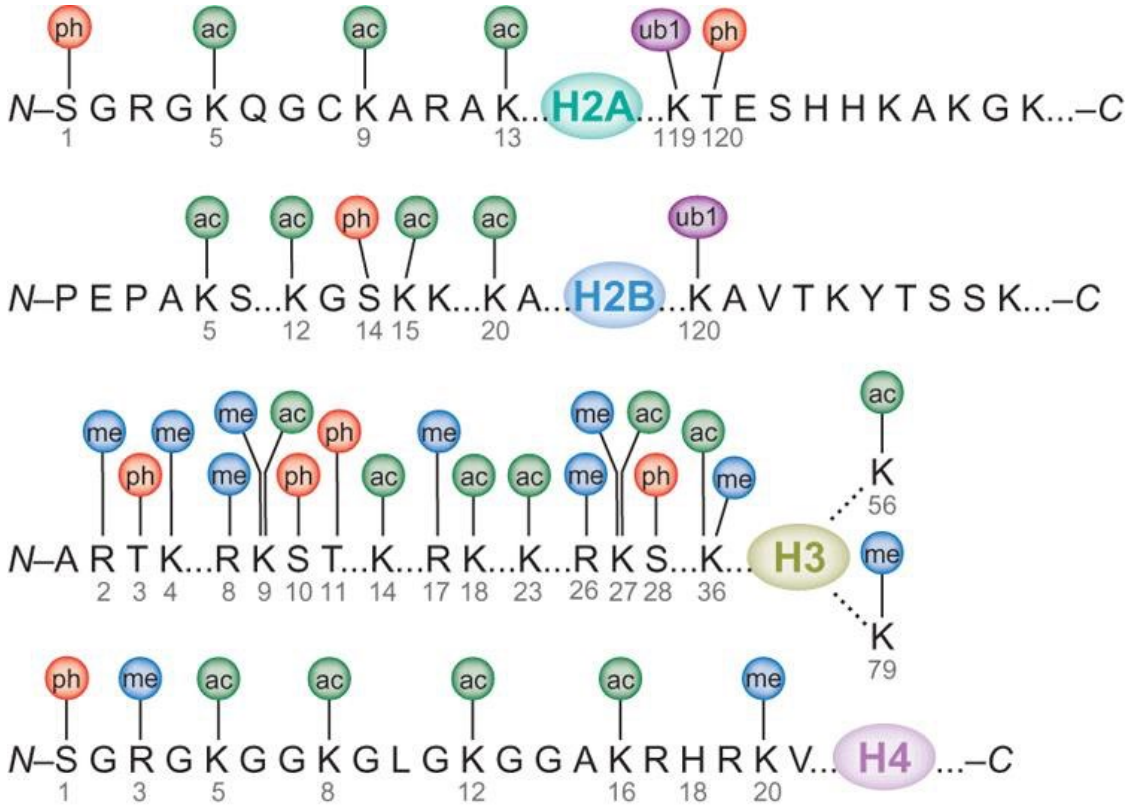
DNA damage



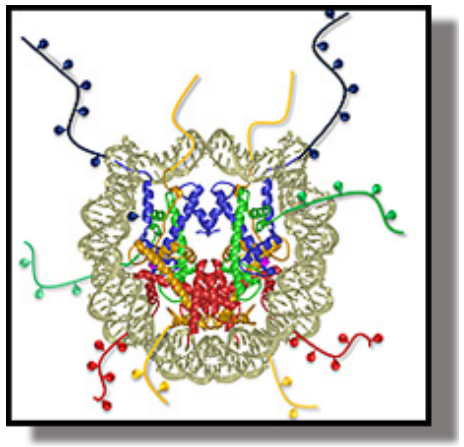


Promoters

Histone function can be changed by post translational modifications (PTMs)

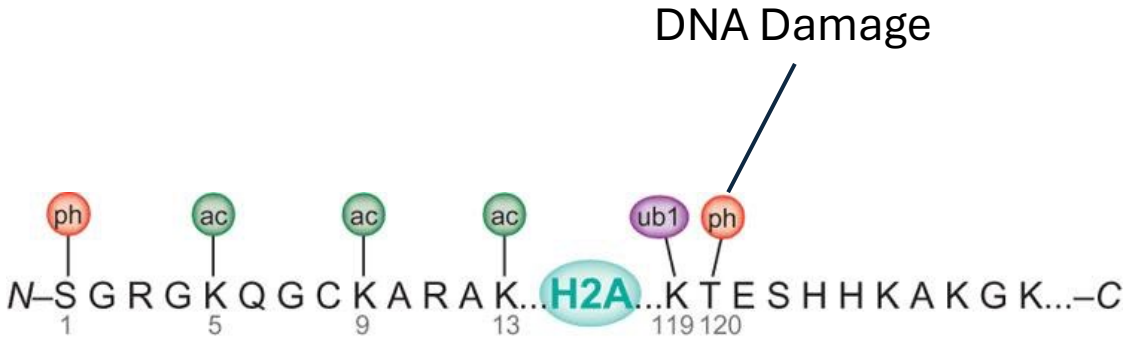


Histone proteins are modified by chemical additions to amino acid side chains



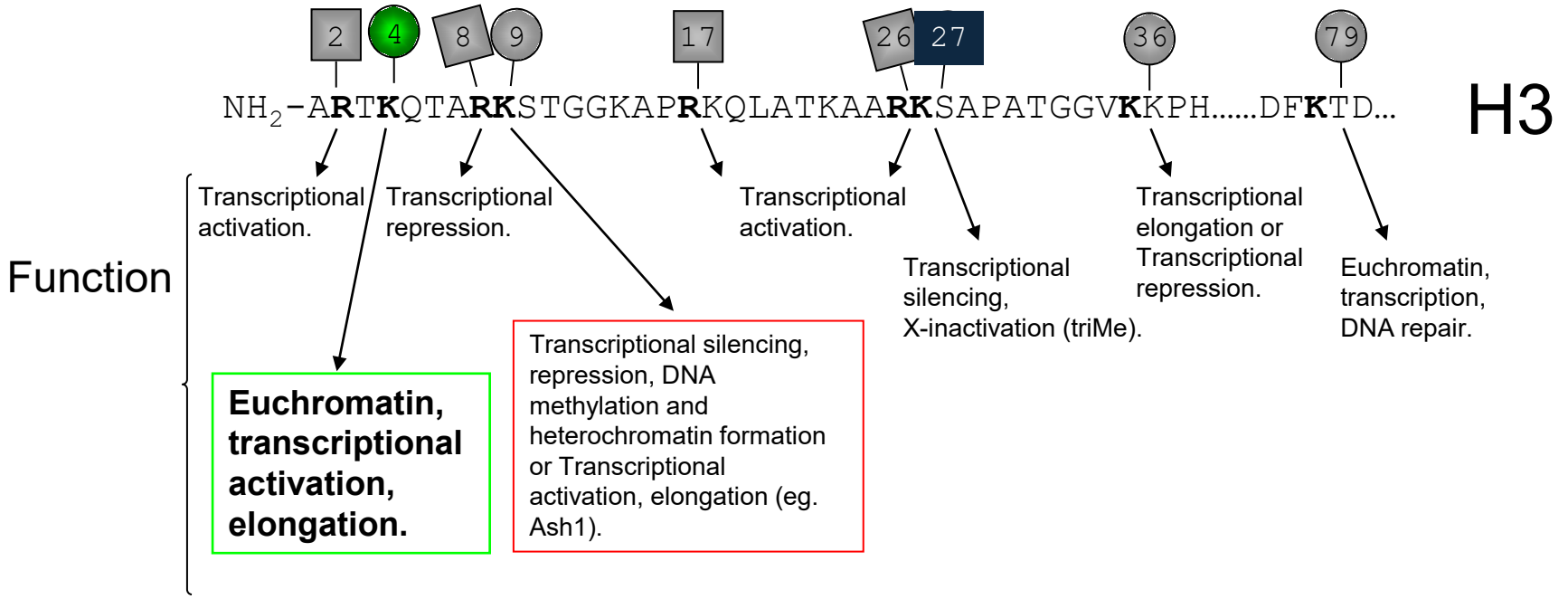
Acetylation
Methylation
Phosphorylation
Ubiquitylation
.....and several more

Histone PTM Function



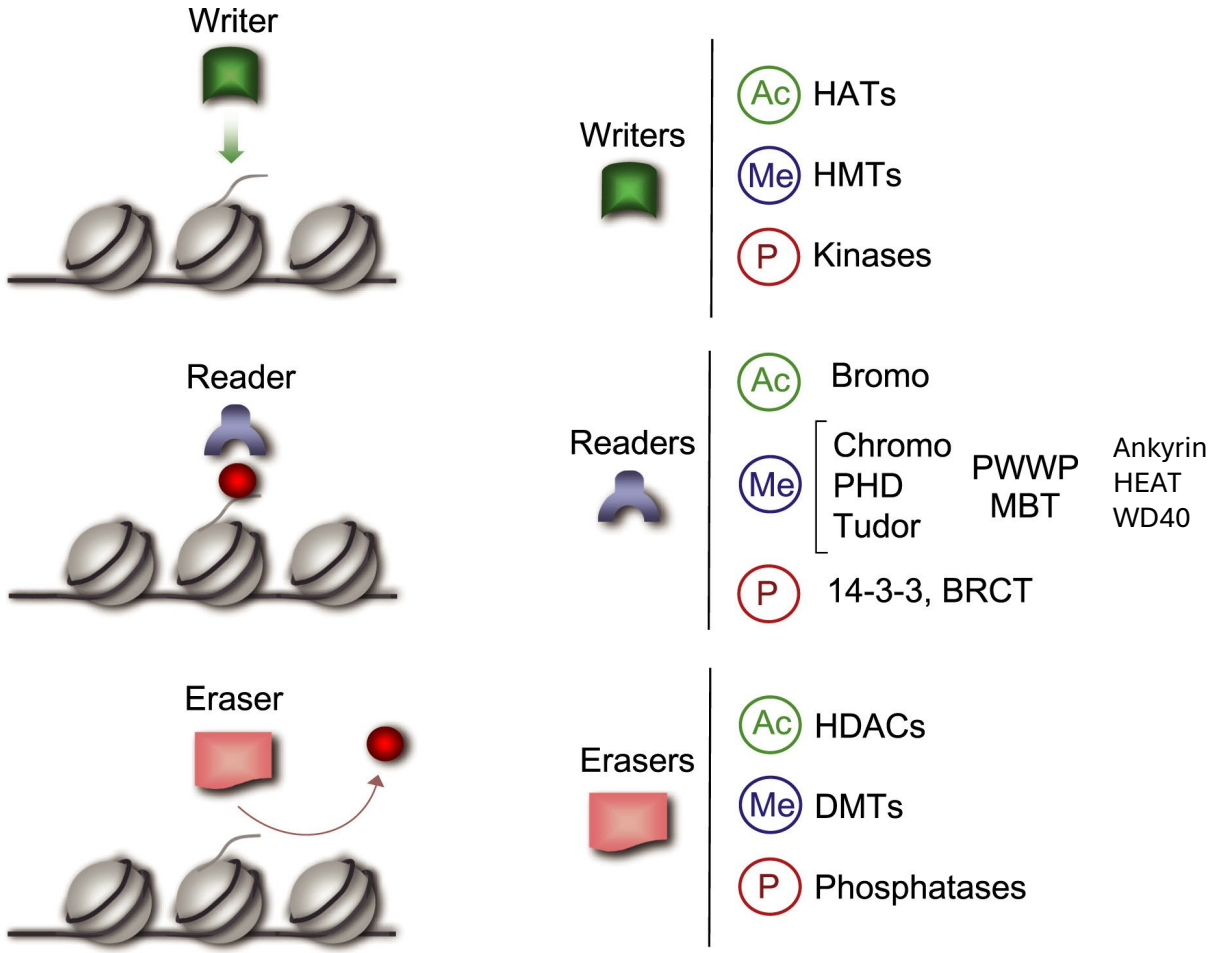
- Histone modifications are involved in almost all DNA associated events
  - Transcription/Activation/Repression
  - DNA Replication
  - Gene dosage/imprinting
  - DNA damage repair
  - Higher order chromatin organization

Histone PTM Function



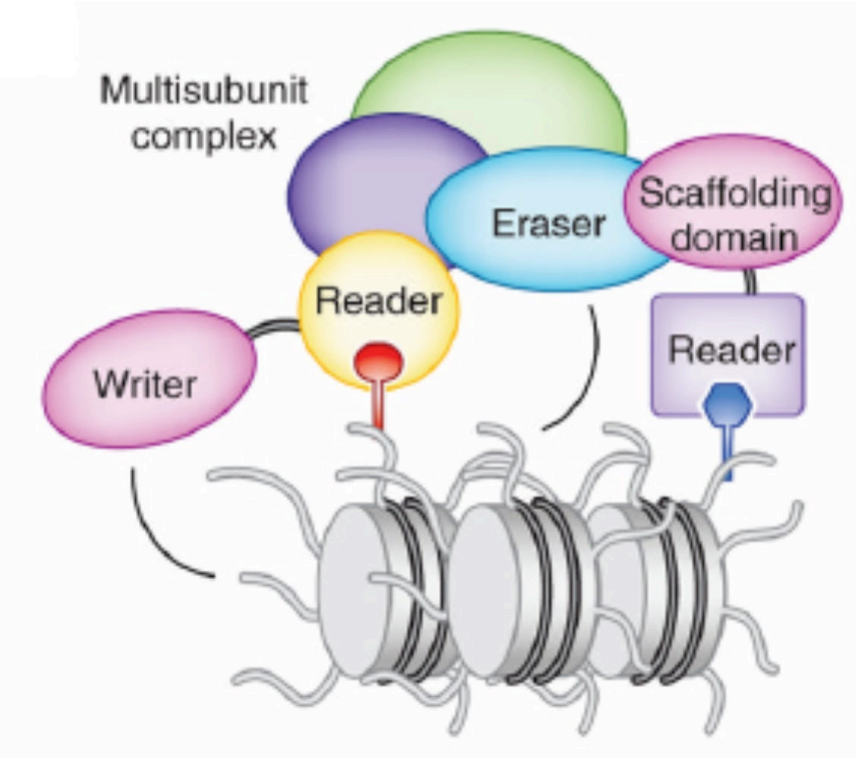
Patrick Grant

Writers, readers erasers...oh my.



Adapted from Borrelli et al *Neuron*, Volume 60, Issue 6, Pages 961-974E.

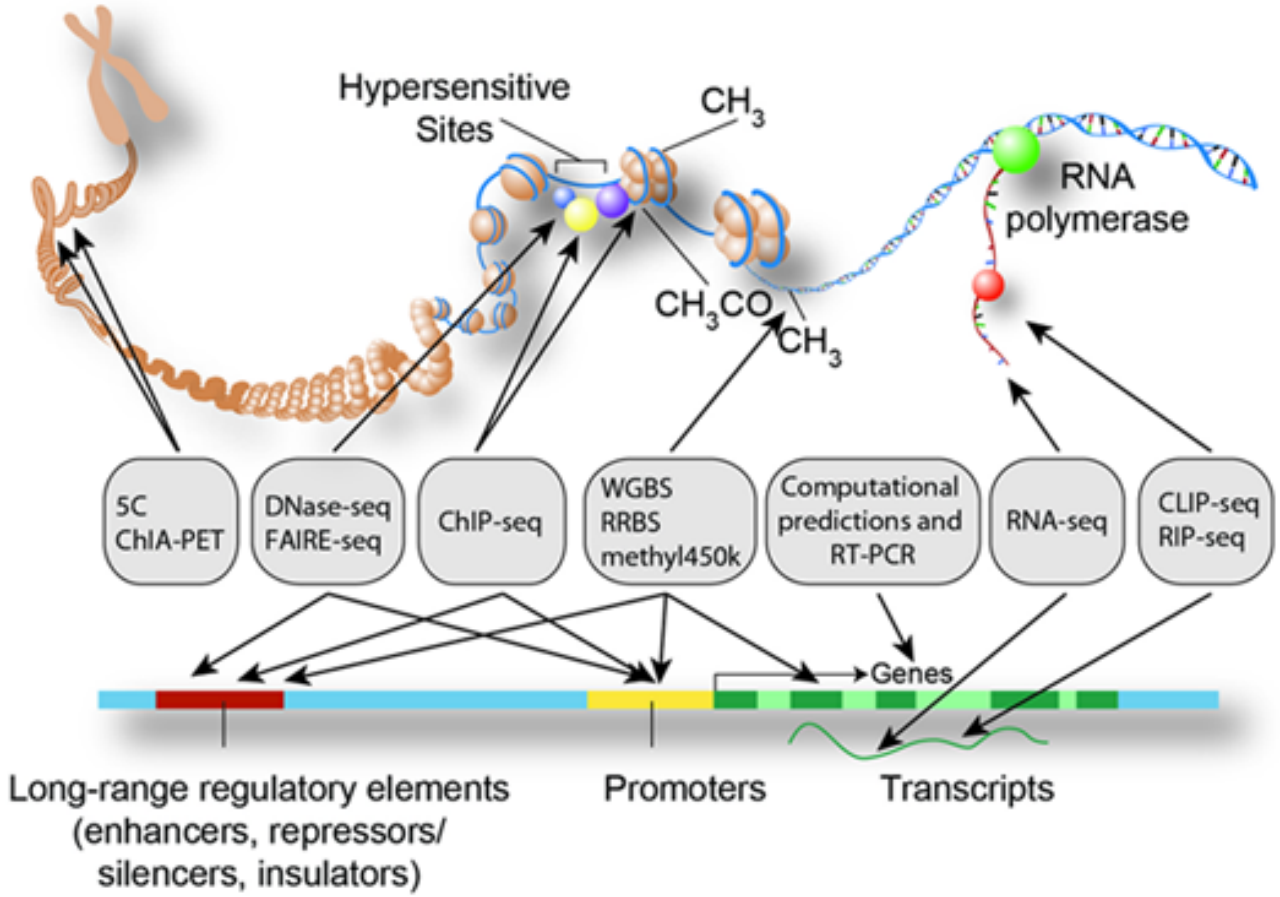
Writers, readers erasers...oh my.



Resources

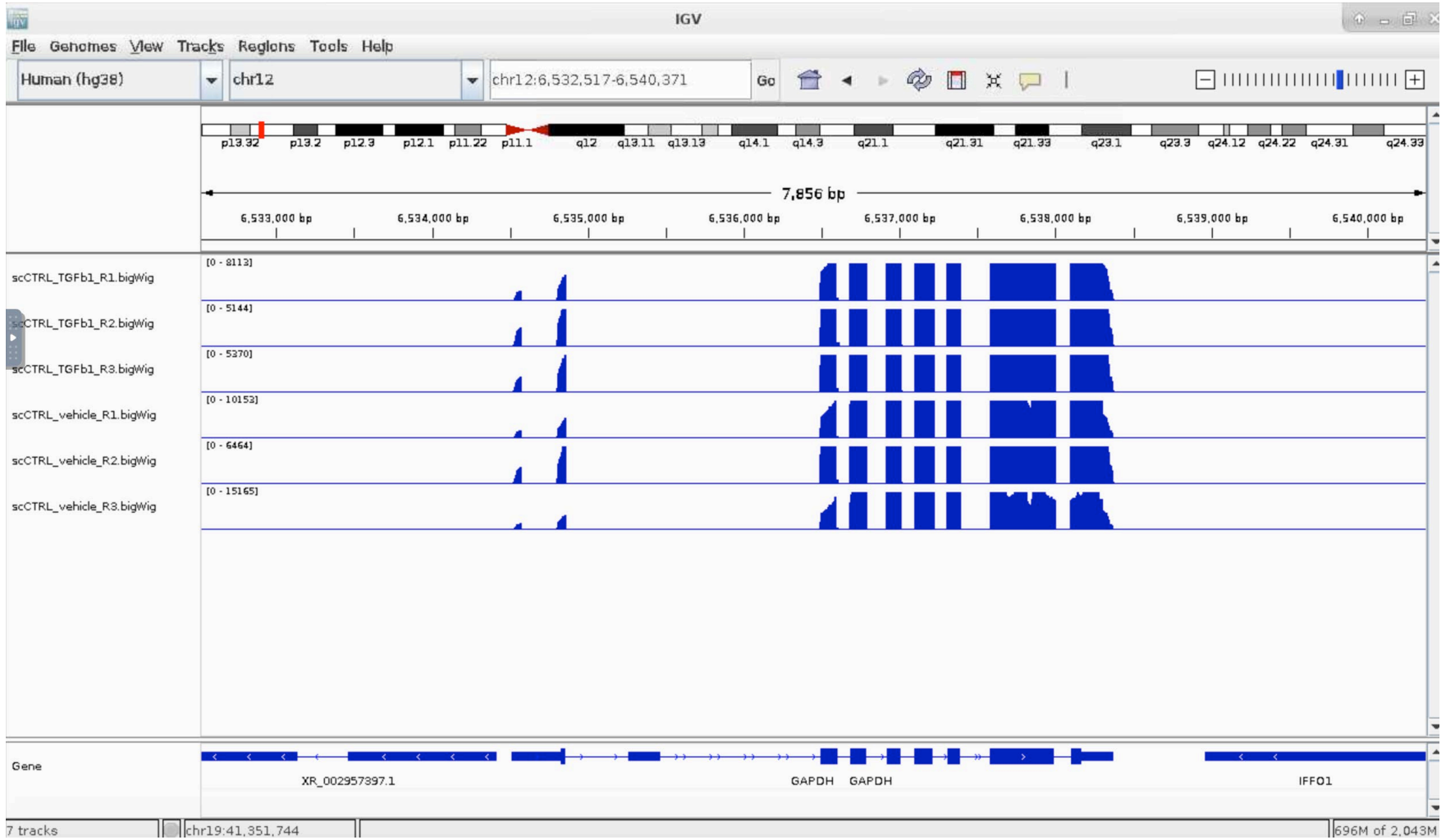
ENCODE consortium

Encyclopedia of DNA Elements

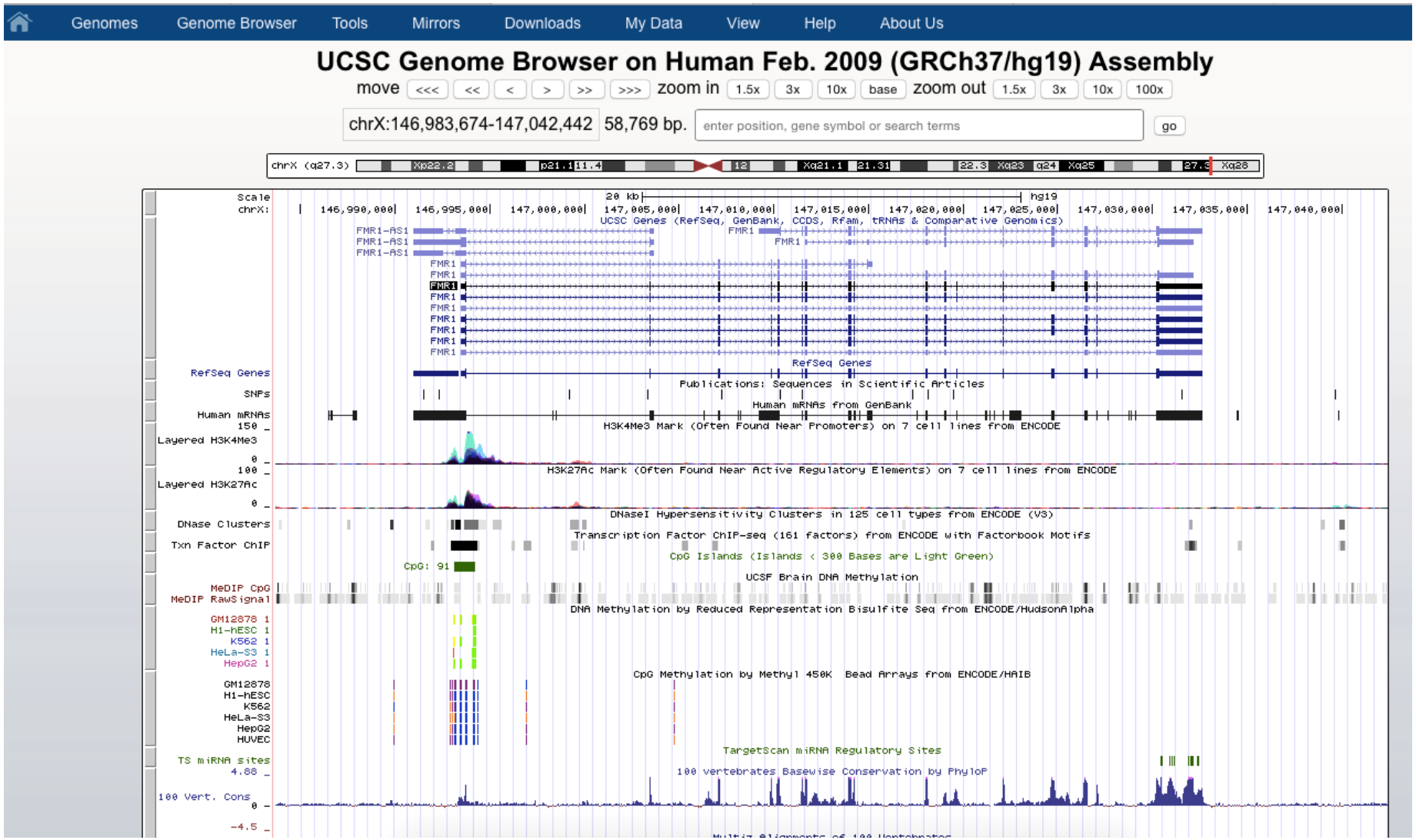


https://www.encodeproject.org

Integrated Genome Viewer (IGV)



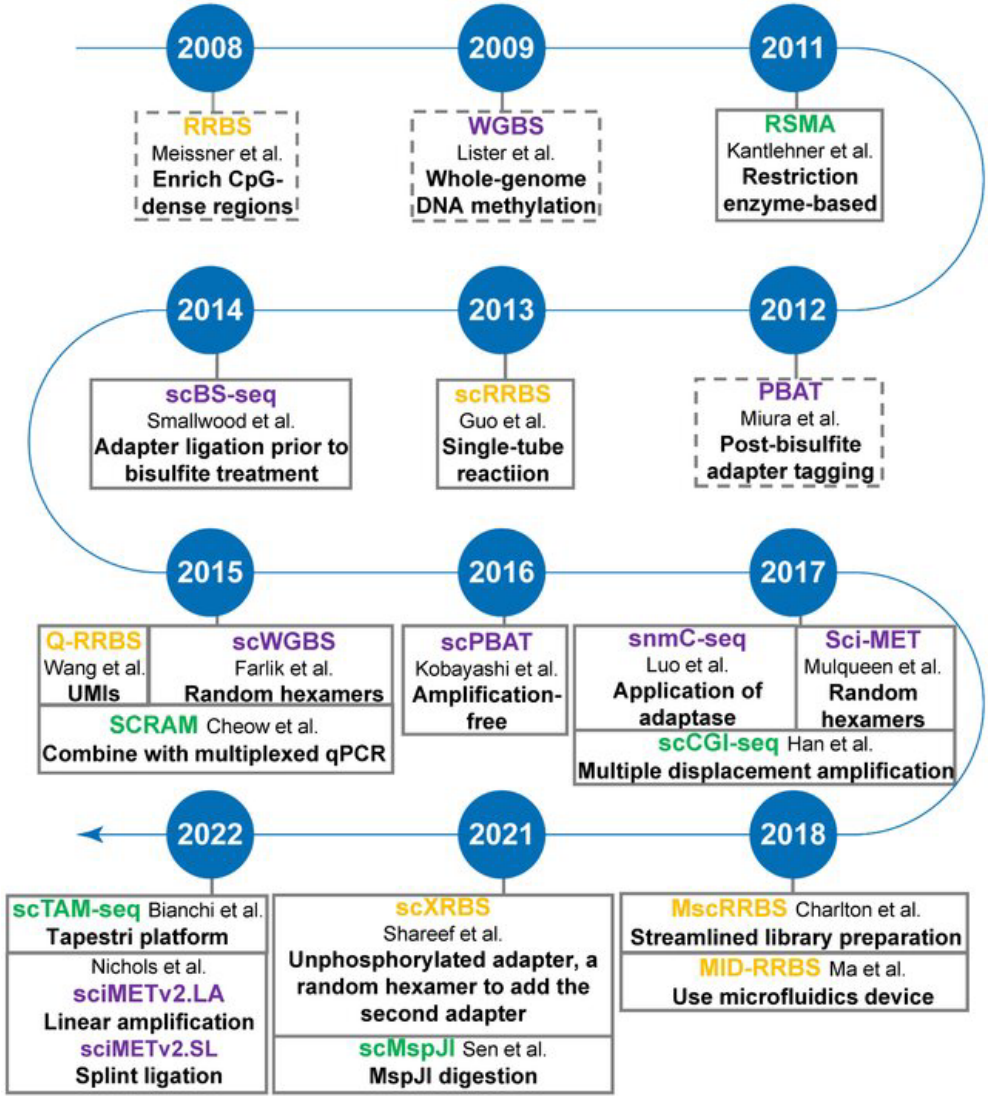
The UCSC Genome Browser



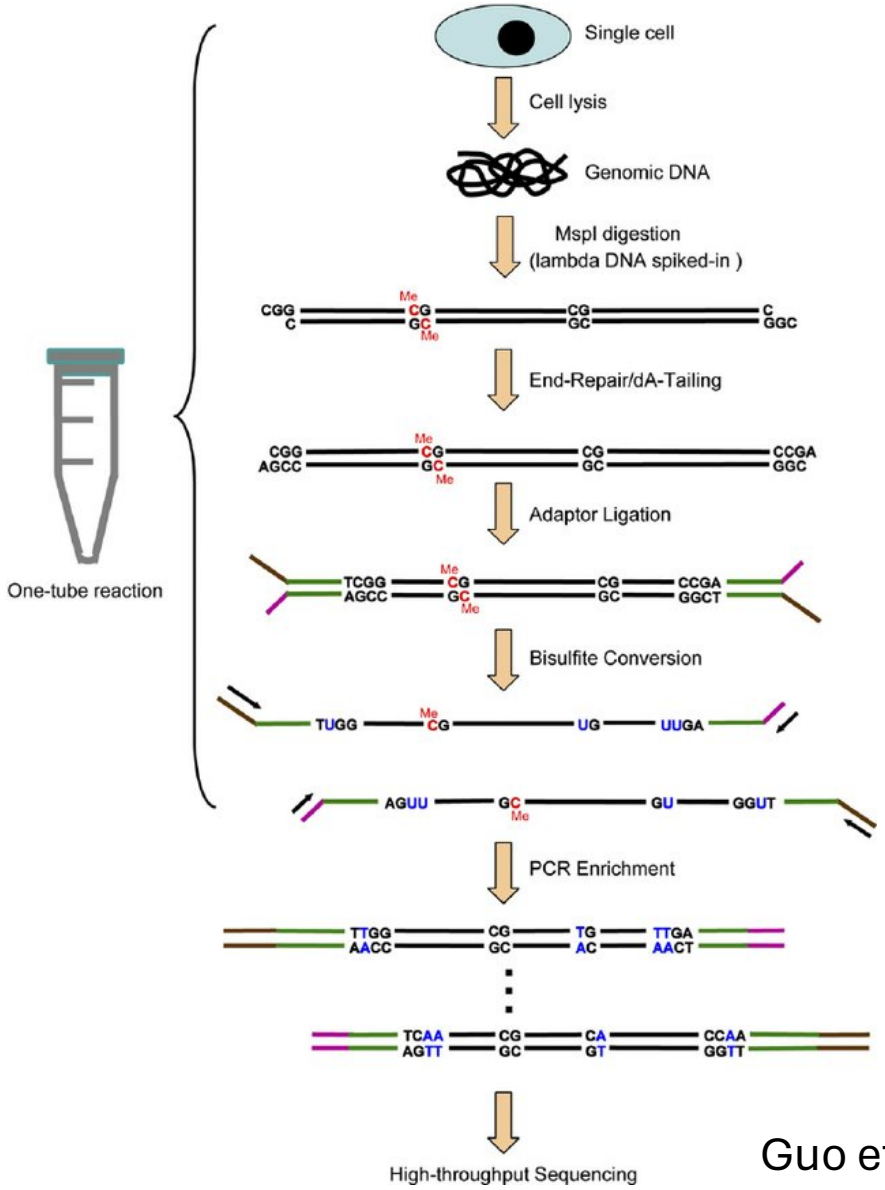
Technologies

DNA methylation

Bisulfite conversion techniques



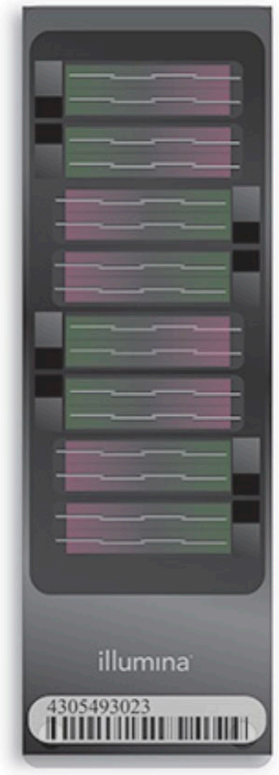
Lit et al., Theranostics 2023



Guo et al., Genome Research 2013

DNA methylation

Methylation array- EPIC2.0



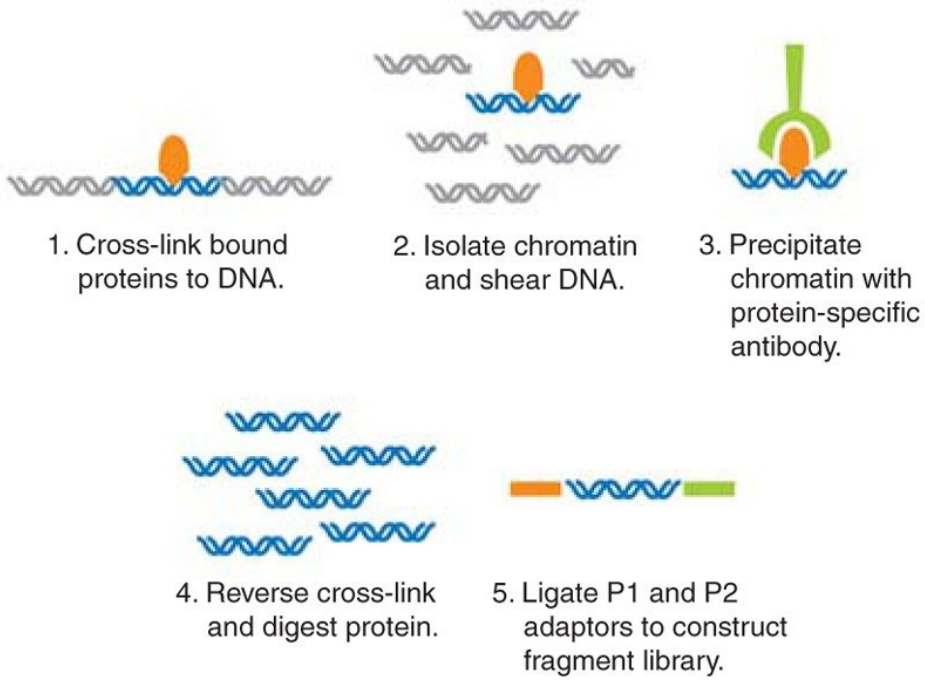
The Infinium MethylationEPIC v2.0 BeadChip Kit is a genome-wide methylation screening tool that targets over 935,000 CpG sites in the most biologically significant regions of the human methylome, while maximizing backwards compatibility with it's predecessor,

Guided by expert evaluations of EPIC v1.0, poor-performing probes have been removed and replaced with cutting-edge content to enable greater discovery power for epigenetics studies. The additional 186,000 CpGs on EPIC v2.0 target enhancers and super-enhancers, additional CTCF-binding sites, CNV detection regions, CpG islands insufficiently covered on EPIC v1.0, and common cancer driver mutations. The updated v2.0 beadchip also profiles open regions of chromatin identified by ATAC-Seq and ChIP-seq experiments. A more extensive description of the content covered by MethylationEPIC v2.0 is described in the product datasheet.

Illumina

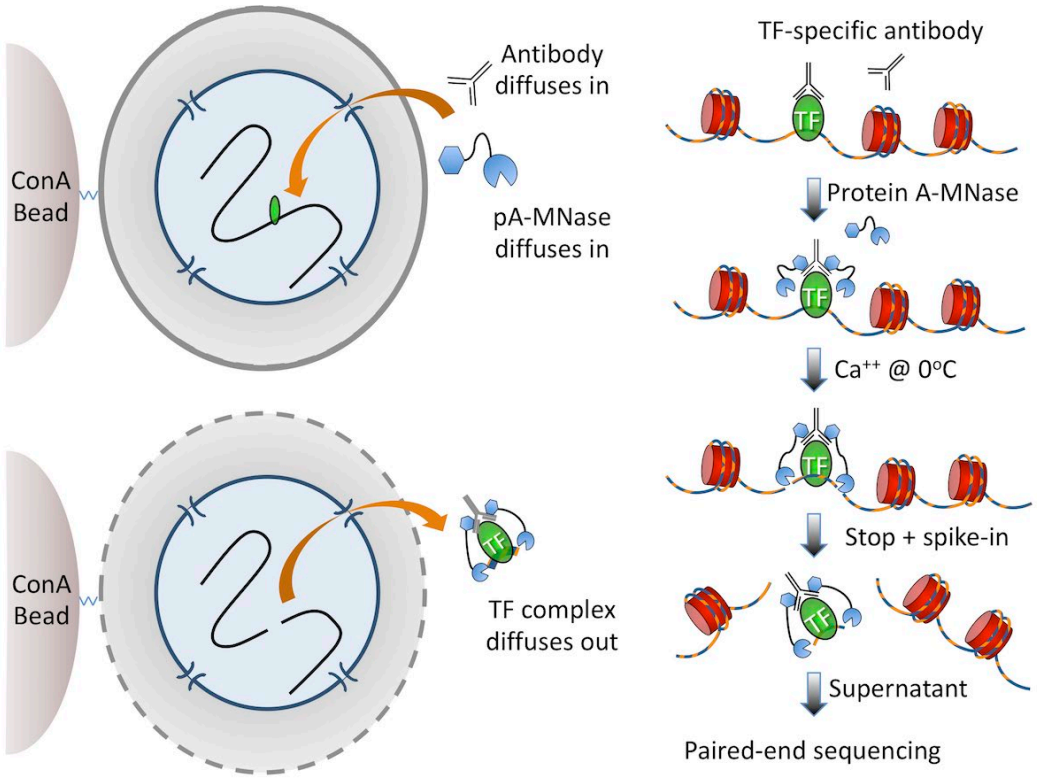
Histone modifications

ChIP-Seq



Shahn, Nature Protocols, 2009

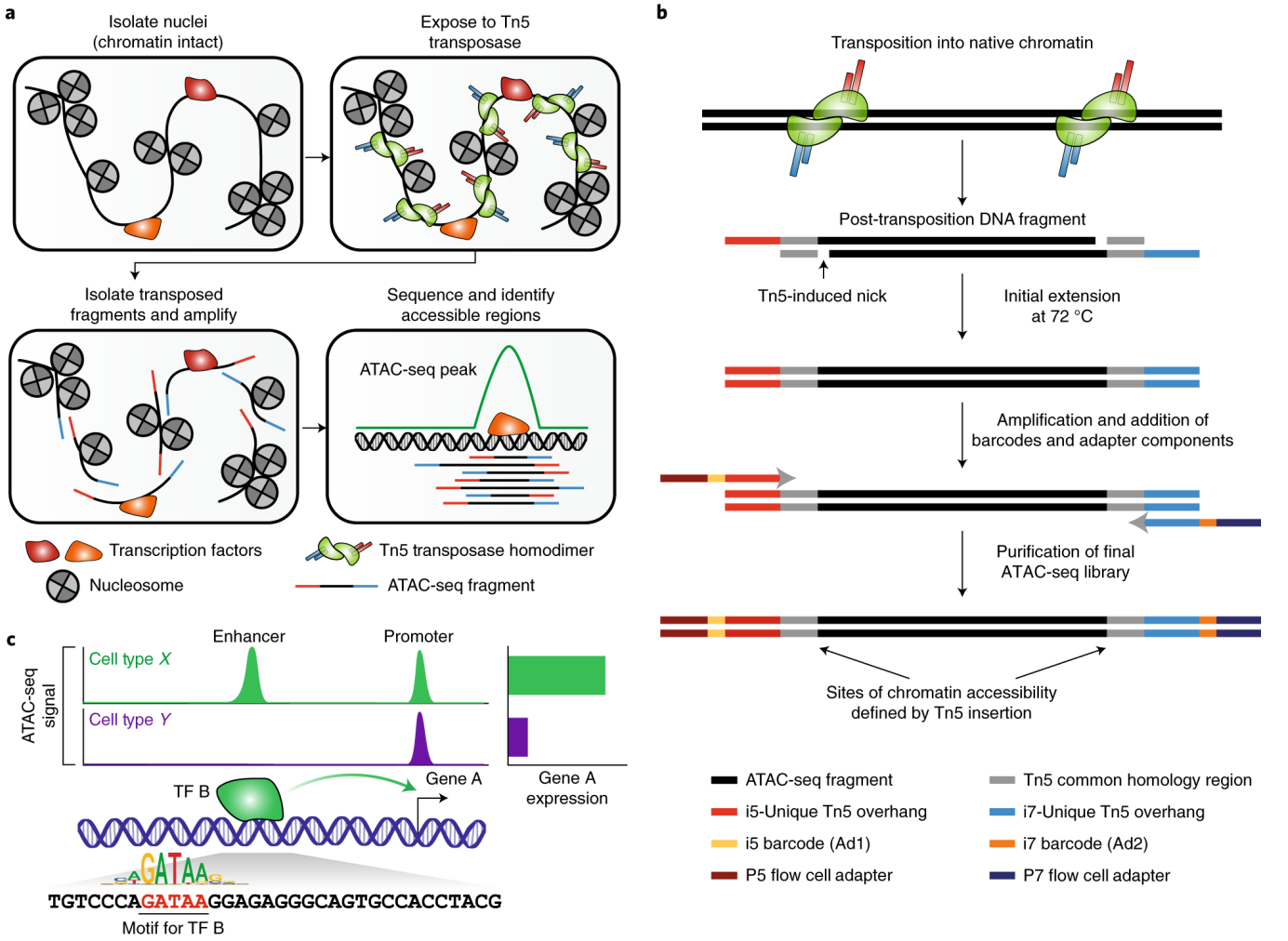
Cut and Run



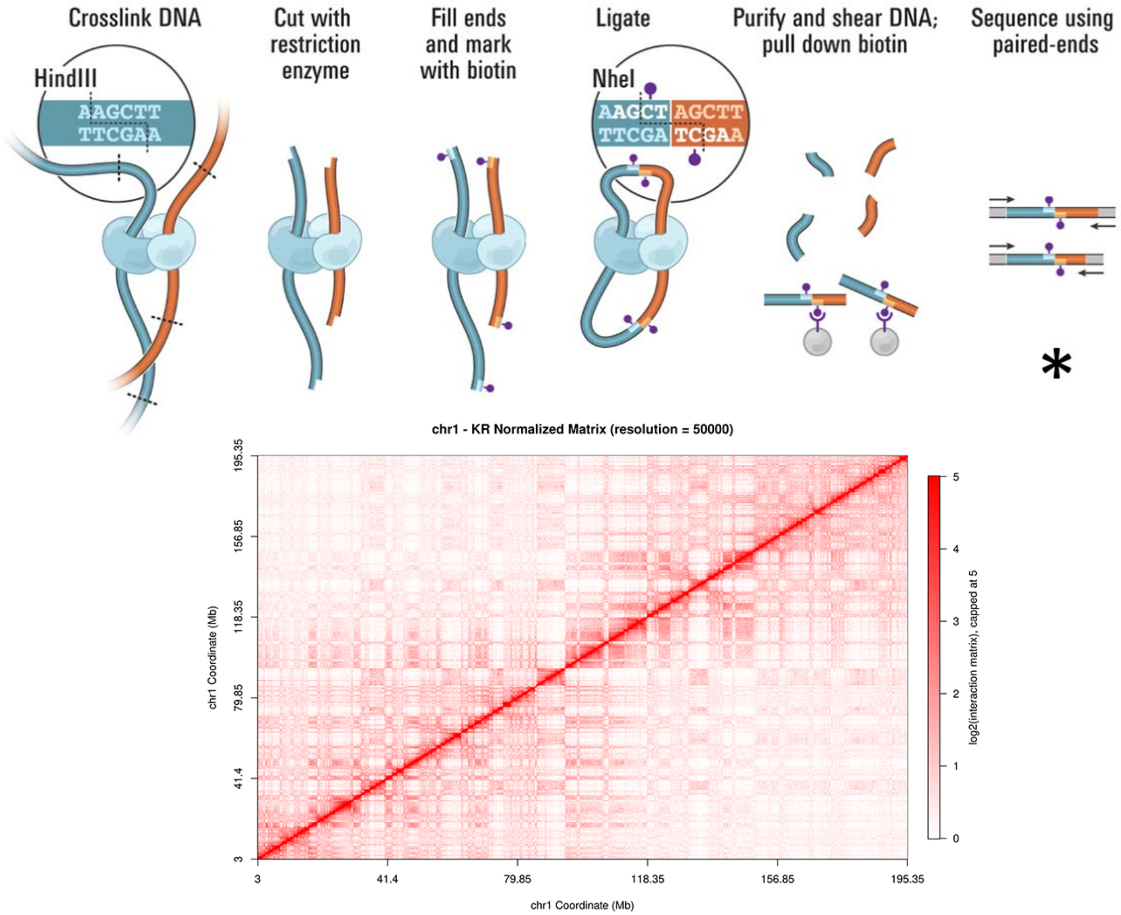
Skene, eLIFE, 2017

Chromatin organization

ATAC-Seq

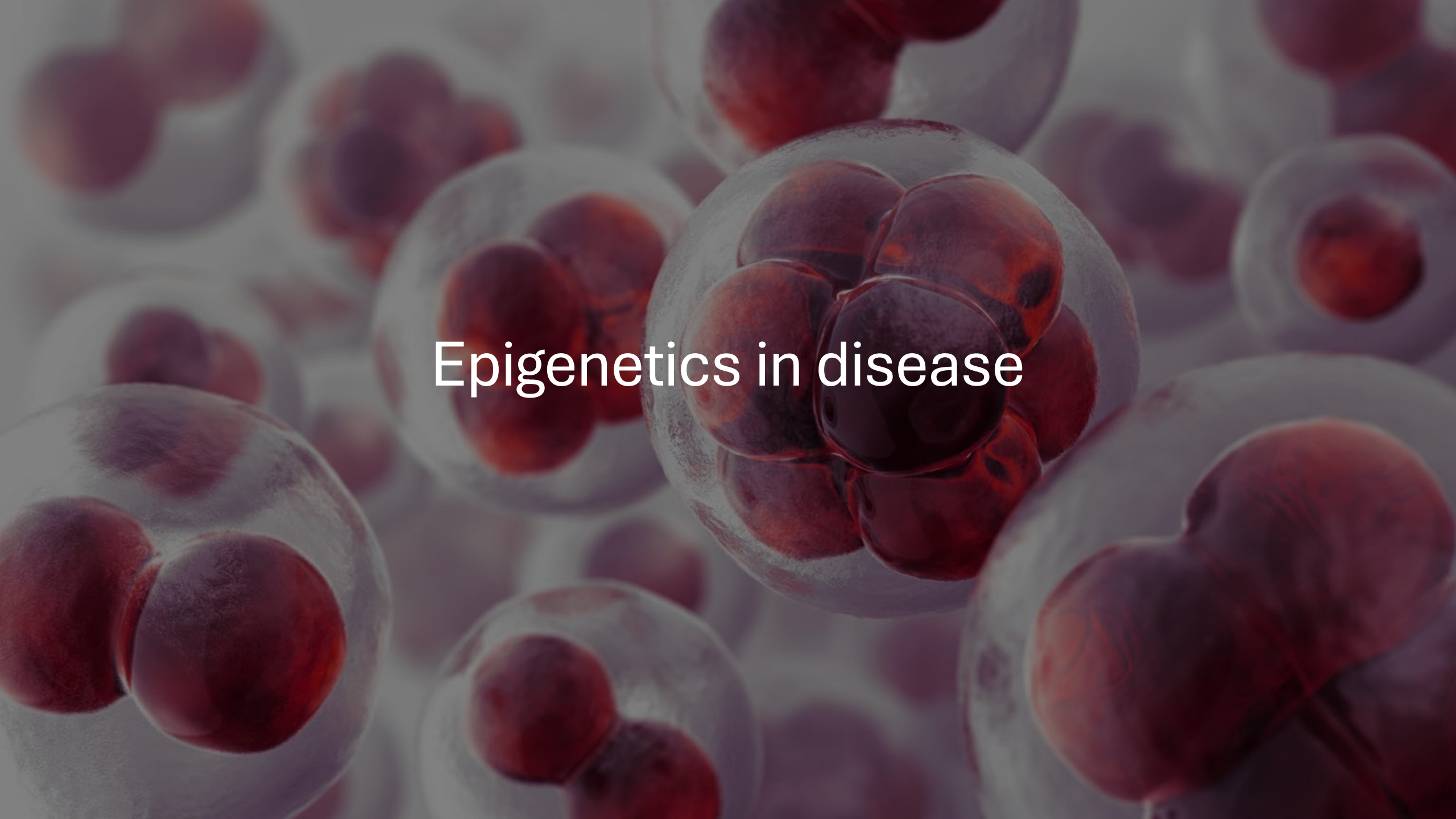


HiC-Seq



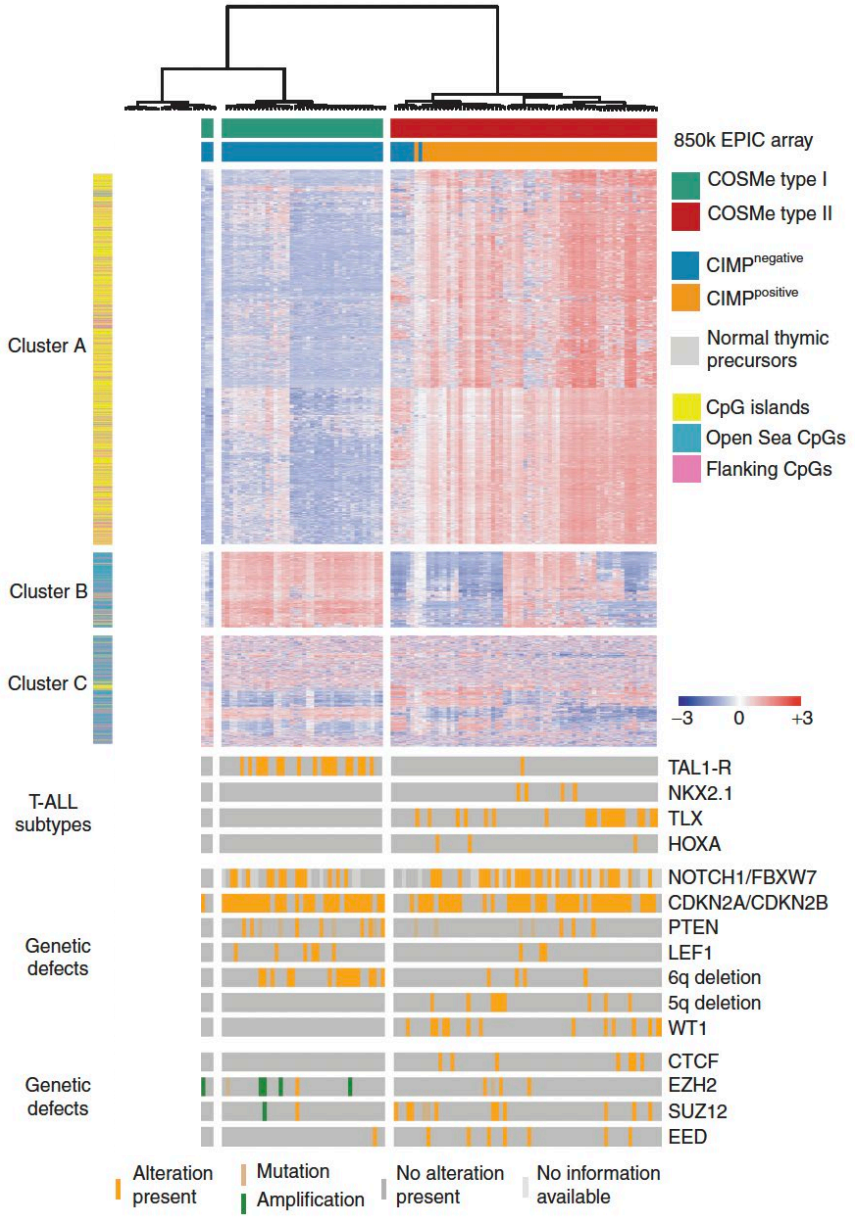
Grandi et al., Nature Protocols, 2022

Arima Genomics



Epigenetics in disease

A CpG Island and Open Sea DNA Methylation Signature in Human T-ALL

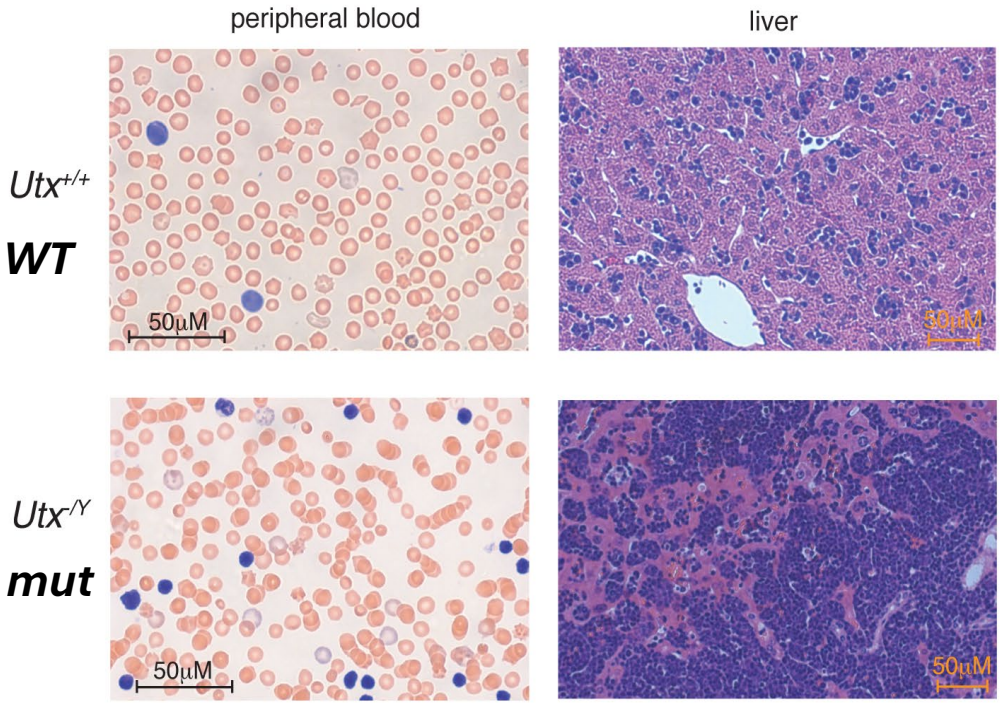


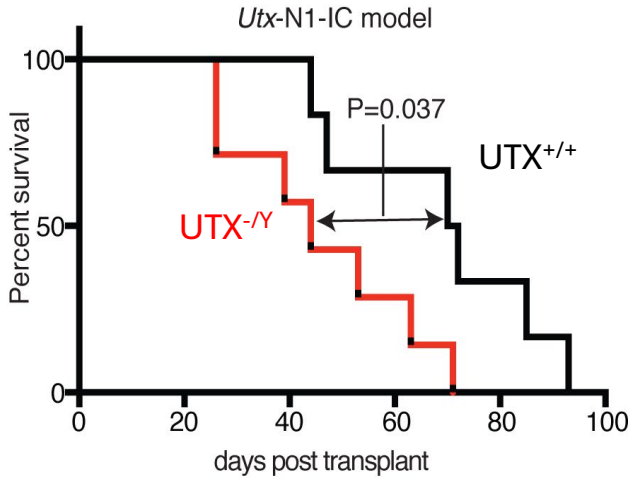
CIMP = CpG island methylator phenotype

- DNA methylation profiling using the 850k EPIC array platform, using unsupervised clustering of the 5,000 most variably methylated CpGs
- **COSMe** (CpG island and Open Sea Methylation) type I and II = methylation-based categories
- COSMe-II T-ALLs showed enrichment for genetic aberrations associated with double-negative or early-cortical T-ALLs: 5q deletions and loss-of-function of WT1, CTCF, and PRC2 members EZH2, SUZ12, or EED
- Cluster B CpG sites displayed hypermethylation in almost all COSMe-I T-ALLs, but in only a subset of COSMe-II leukemias

Histone demethylation in leukemia

UTX acts as a tumor suppressor in T cell leukemia

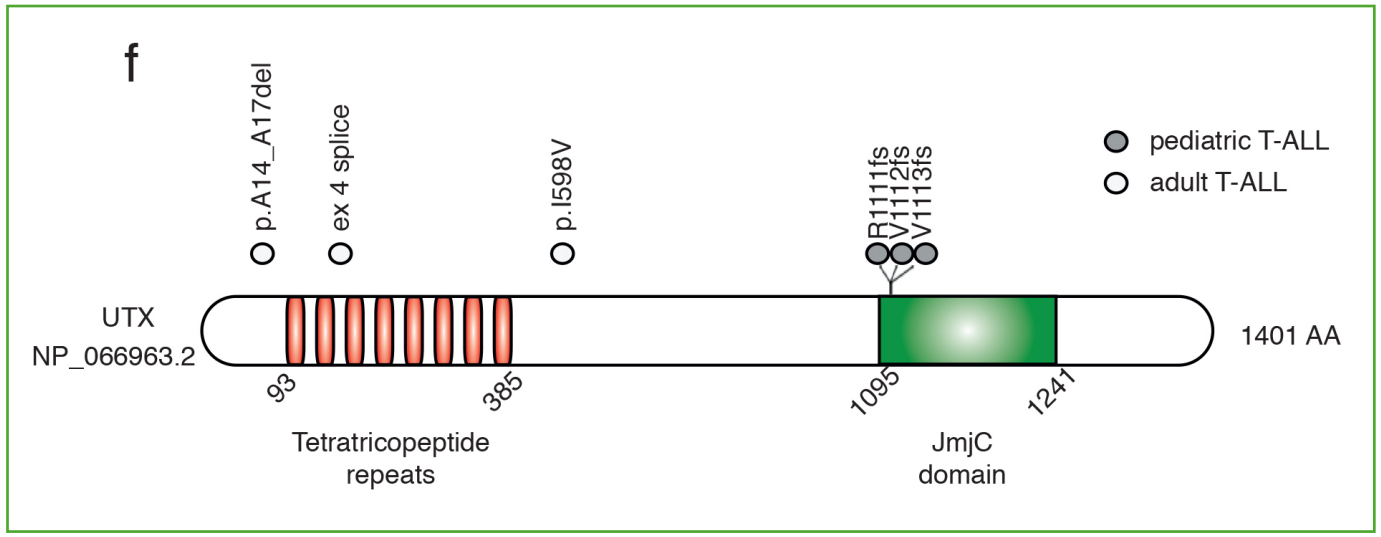




Ntziachristos et al., Nature, 2014

Histone demethylation in leukemia

UTX is a novel X-linked tumor suppressor targeted by deletions and mutations in both pediatric and adult T-ALL



Ntziachristos et al., Nature, 2014

Epigenetic regulators and cancer

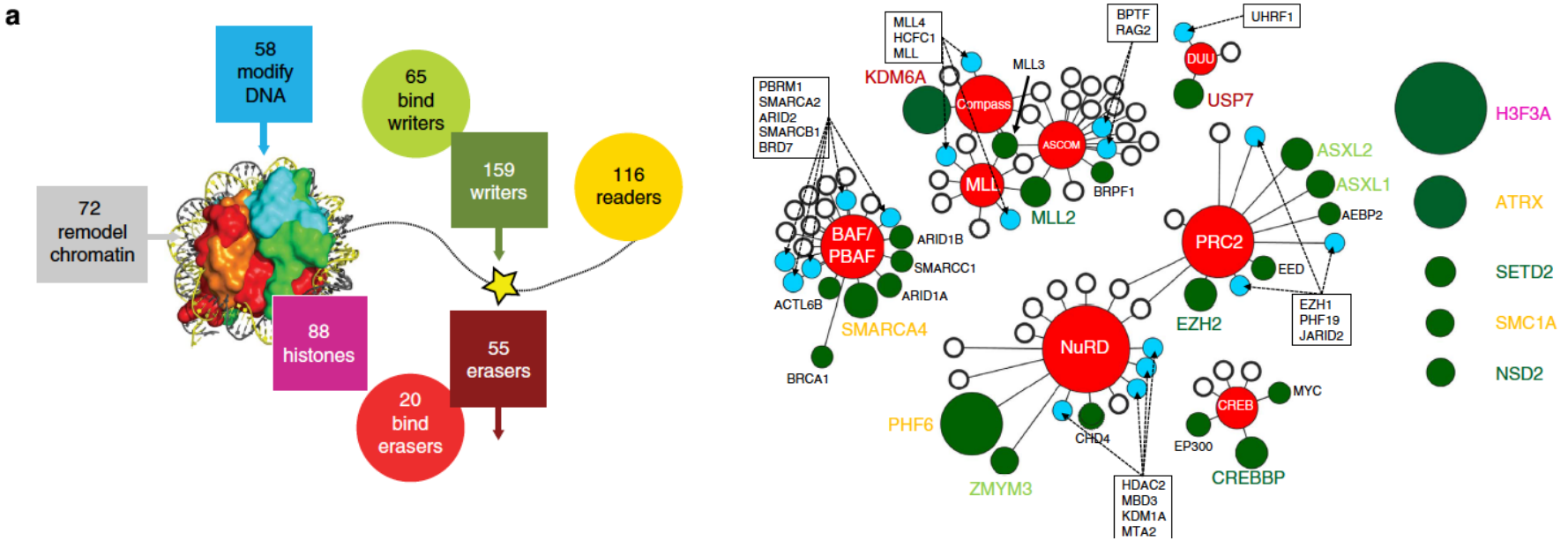
ARTICLE

Received 24 Sep 2013 | Accepted 12 Mar 2014 | Published 8 Apr 2014

DOI: 10.1038/ncomms4630

The landscape of somatic mutations in epigenetic regulators across 1,000 paediatric cancer genomes

Robert Huether[1,*], Li Dong[2,*], Xiang Chen[1], Gang Wu[1], Matthew Parker[1], Lei Wei[1], Jing Ma[2], Michael N. Edmonson[1], Erin K. Hedlund[1], Michael C. Rusch[1], Sheila A. Shurtleff[2], Heather L. Mulder[3], Kristy Boggs[3], Bhavin Vadordaria[3], Jinjun Cheng[2], Donald Yergeau[3], Guangchun Song[2], Jared Becksfort[1], Gordon Lemmon[1], Catherine Weber[2], Zhongling Cai[2], Jinjun Dang[2], Michael Walsh[4], Amanda L. Gedman[2], Zachary Faber[2], John Easton[3], Tanja Gruber[2,4], Richard W. Kriwacki[5], Janet F. Partridge[6], Li Ding[7,8,9], Richard K. Wilson[7,8,9], Elaine R. Mardis[7,8,9], Charles G. Mullighan[2], Richard J. Gilbertson[10], Suzanne J. Baker[10], Gerard Zambetti[6], David W. Ellison[2], Jinghui Zhang[1] & James R. Downing[2]



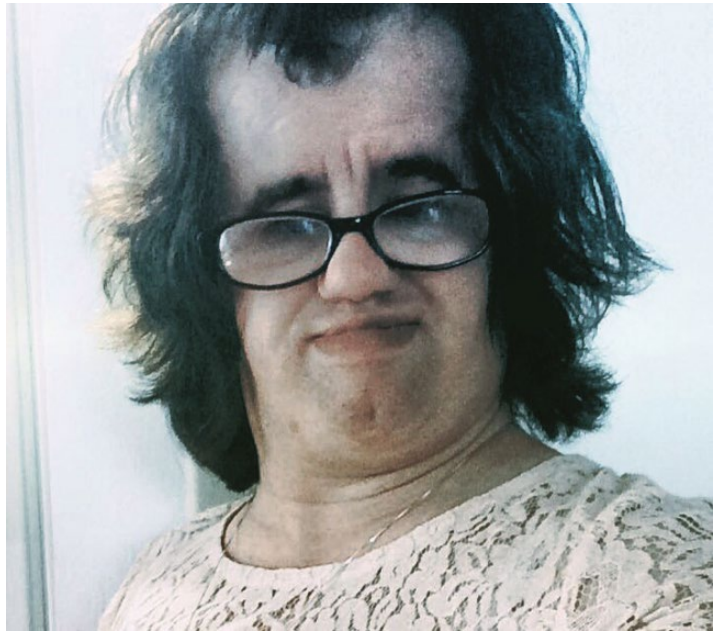
Case study: Cofin-Siris Syndrome (SWI/SNF)

Mutations in chromatin "remodelers" can lead to developmental syndromes

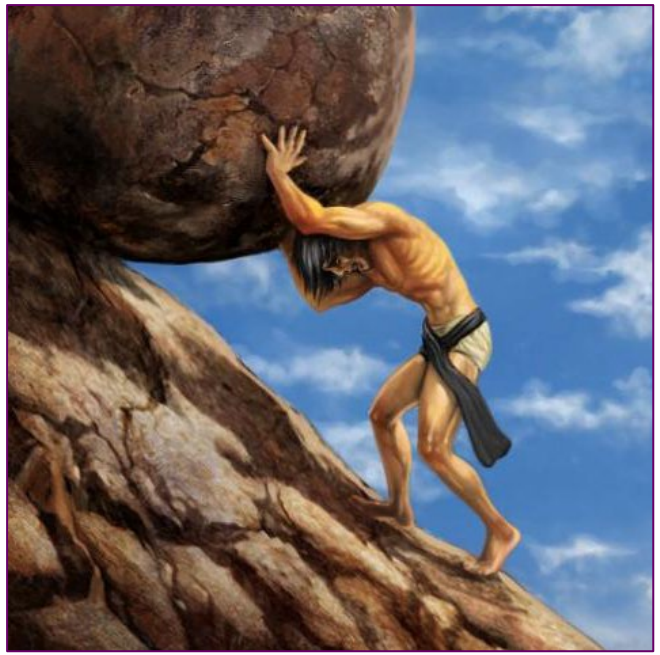
*Although there are many variable signs and symptoms, hallmarks of this condition include developmental disability, abnormalities of the fifth (pinky) fingers or toes

*Severe intellectual disability or delayed development of speech and motor skills such as sitting and walking

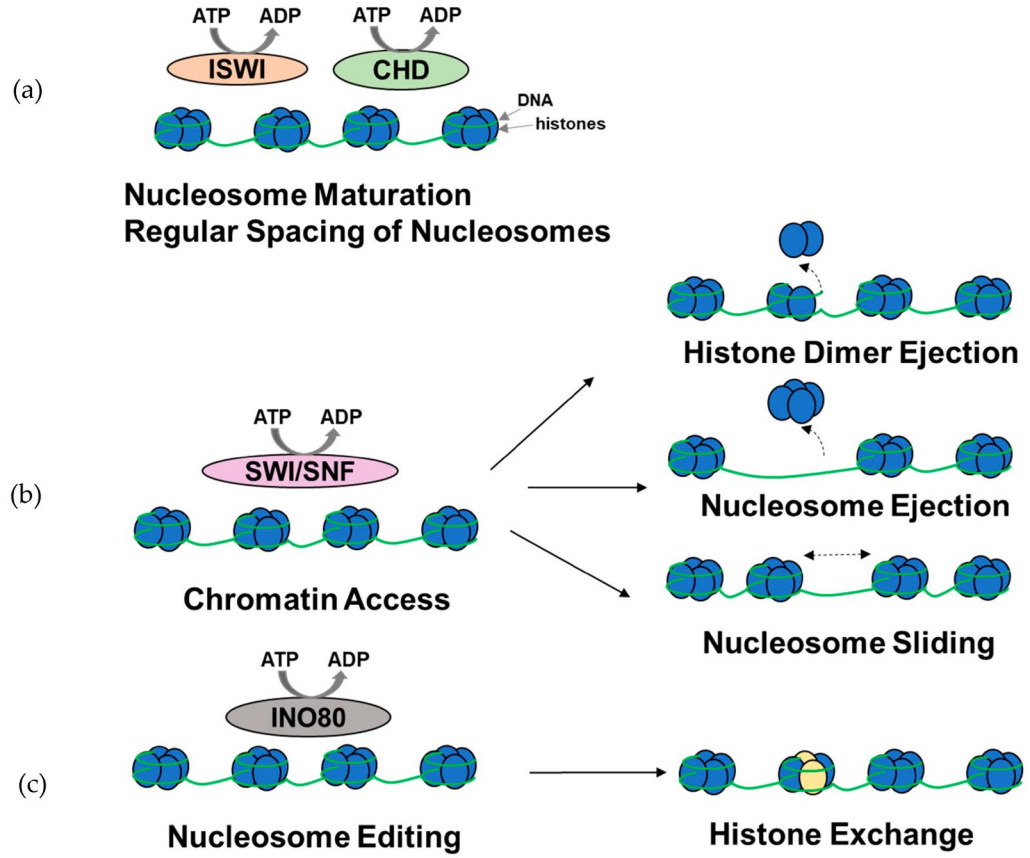
*Wide nose with a flat nasal bridge, a wide mouth with thick lips, and thick eyebrows and eyelashes



Many different proteins can be mutated in complexes-- The example of Chromatin Remodeling Complexes

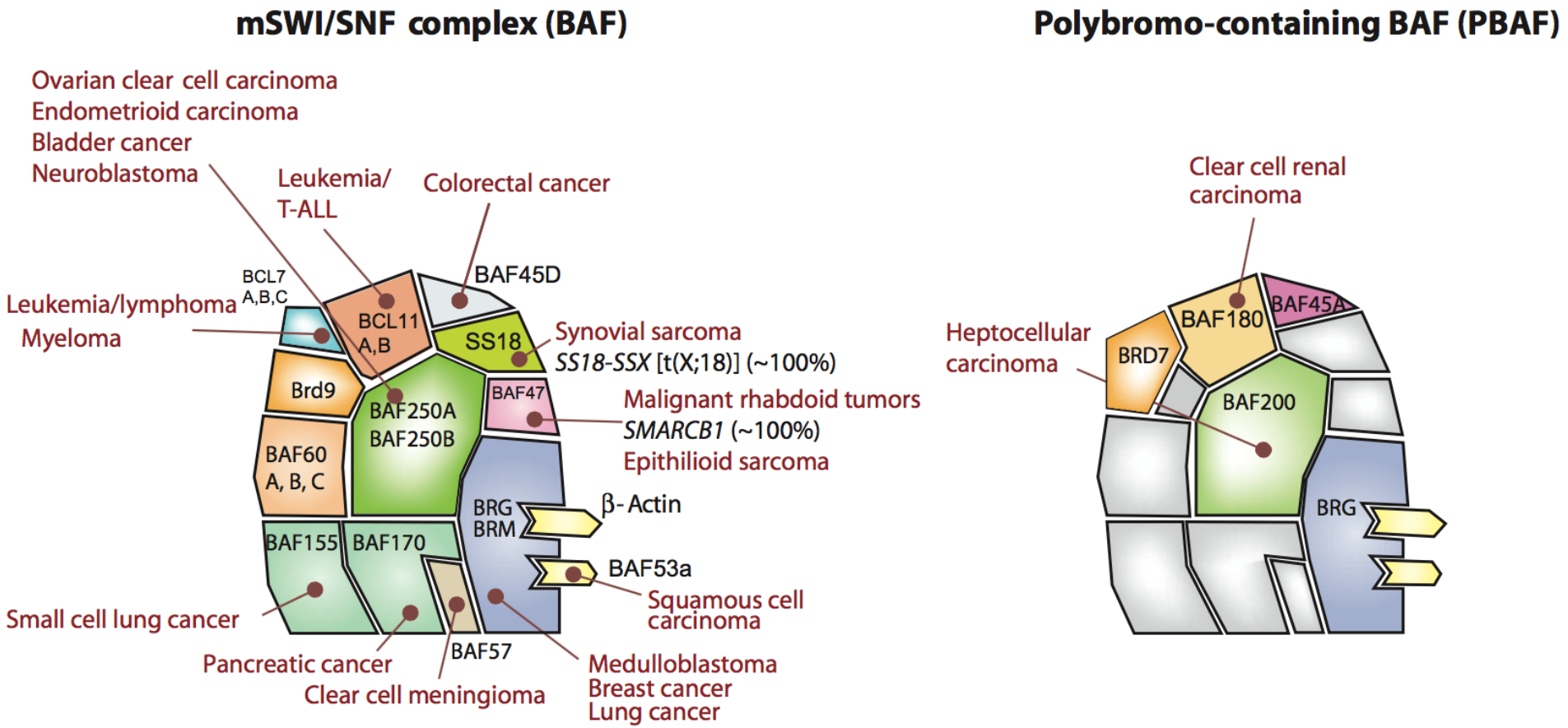


*moving chromatin around to free up genes areas

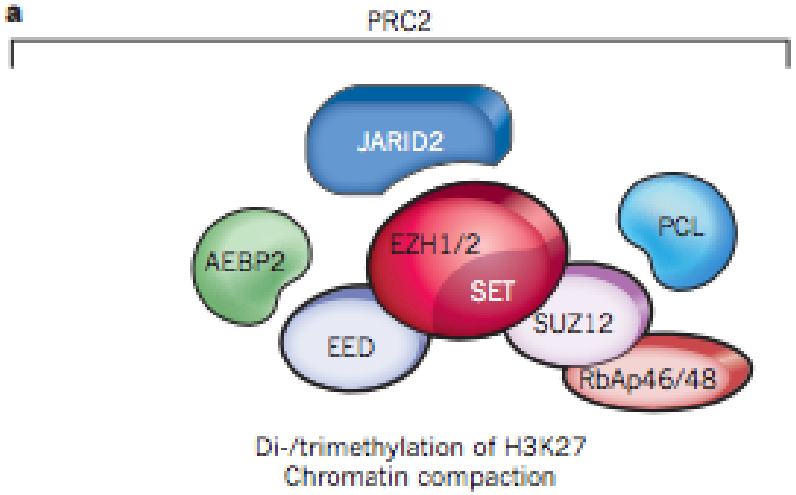


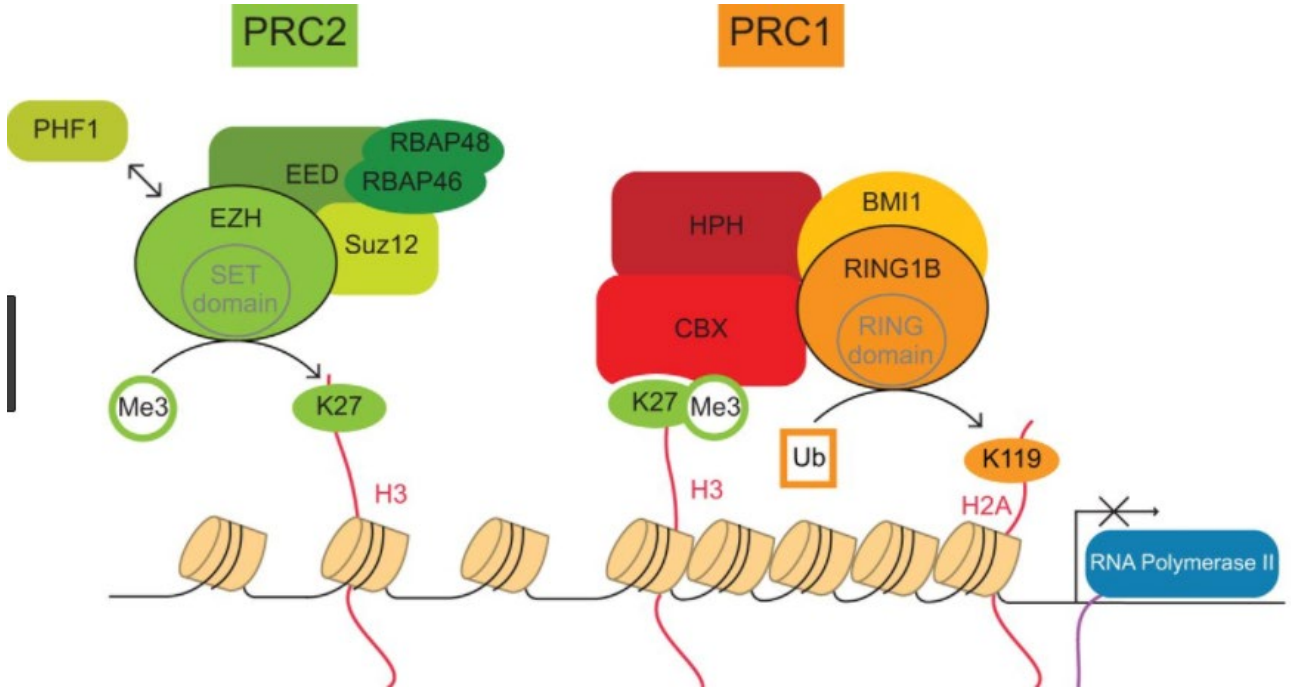
Hassan and Ahuja, 2019

Many different proteins can be mutated in complexes-- The example of Chromatin Remodeling Complexes



EZH2 is a member of the polycomb repressive complex





Mutations and role of the polycomb complex in blood cancers

LETTERS

nature genetics

Inactivating mutations of the histone methyltransferase gene *EZH2* in myeloid disorders

Thomas Ernst[1–3,11], Andrew J Chase[1,2,11], Joannah Score[1,2], Claire E Hidalgo-Curtis[1,2], Catherine Bryant[1,2], Amy V Jones[1,2], Katherine Waghorn[1,2], Katerina Zoi[4], Fiona M Ross[1,2], Andreas Reiter[5], Andreas Hochhaus[3], Hans G Drexler[6], Andrew Duncombe[7], Francisco Cervantes[8], David Oscier[9], Jacqueline Boultwood[10], Francis H Grand[1,2] & Nicholas C P Cross[1,2]

LETTERS

nature medicine

Genetic inactivation of the polycomb repressive complex 2 in T cell acute lymphoblastic leukemia

Panagiotis Ntziachristos[1,2,20], Aristotelis Tsirigos[3,20], Pieter Van Vlierberghe[4–6,20], Jelena Nedjic[1,2], Thomas Trimarchi[1,2], Maria Sol Flaherty[4], Dolors Ferres-Marco[7], Vanina da Ros[7], Zuojian Tang[8,9], Jasmin Siegle[1,2], Patrik Asp[2], Michael Hadler[4], Isaura Rigo[4], Kim De Keersmaecker[10,11], Jay Patel[12], Tien Huynh[3], Filippo Utro[3], Sandrine Poglio[13–16], Jeremy B Samon[4–6], Elisabeth Paietta[17], Janis Racevskis[17], Jacob M Rowe[18], Raul Rabadan[19], Ross L Levine[12], Stuart Brown[8,9], Francoise Pflumio[13–16], Maria Dominguez[7], Adolfo Ferrando[4–6,20] & Iannis Aifantis[1,2,20]

Polycomb repressive complex 2 is required for MLL-AF9 leukemia

Tobias Neff[a,b], Amit U. Sinha[a,b], Michael J. Kluk[b,c], Nan Zhu[a,b], Mohamed H. Khattab[a,b], Lauren Stein[a,b], Huafeng Xie[a,b], Stuart H. Orkin[a,b,d,e,1], and Scott A. Armstrong[a,b,e,1]

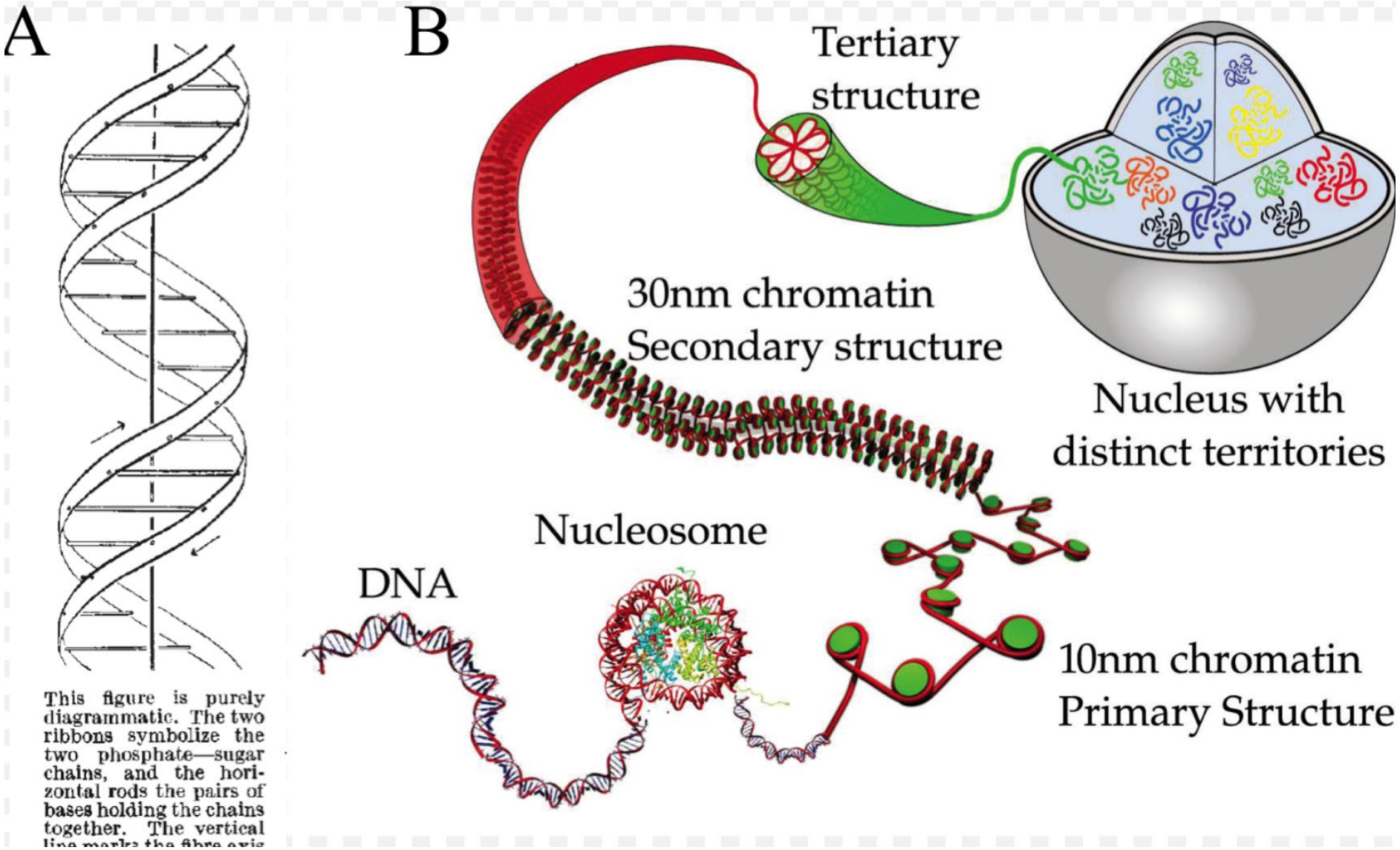
LYMPHOID NEOPLASIA

Somatic mutations at EZH2 Y641 act dominantly through a mechanism of selectively altered PRC2 catalytic activity, to increase H3K27 trimethylation

Damian B. Yap,[1,2] Justin Chu,[2] Tobias Berg,[3] Matthieu Schapira,[4] S.-W. Grace Cheng,[5] Annie Moradian,[5] Ryan D. Morin,[5] Andrew J. Mungall,[5] Barbara Meissner,[6] Merrill Boyle,[6] Victor E. Marquez,[7] Marco A. Marra,[5] Randy D. Gascoyne,[1,6] R. Keith Humphries,[3,8] Cheryl H. Arrowsmith,[4,9] Gregg B. Morin,[5,10] and Samuel A. J. R. Aparicio[1,2]

Case study: Chromatin Folding (3D chromatin structure), more than meets the eye

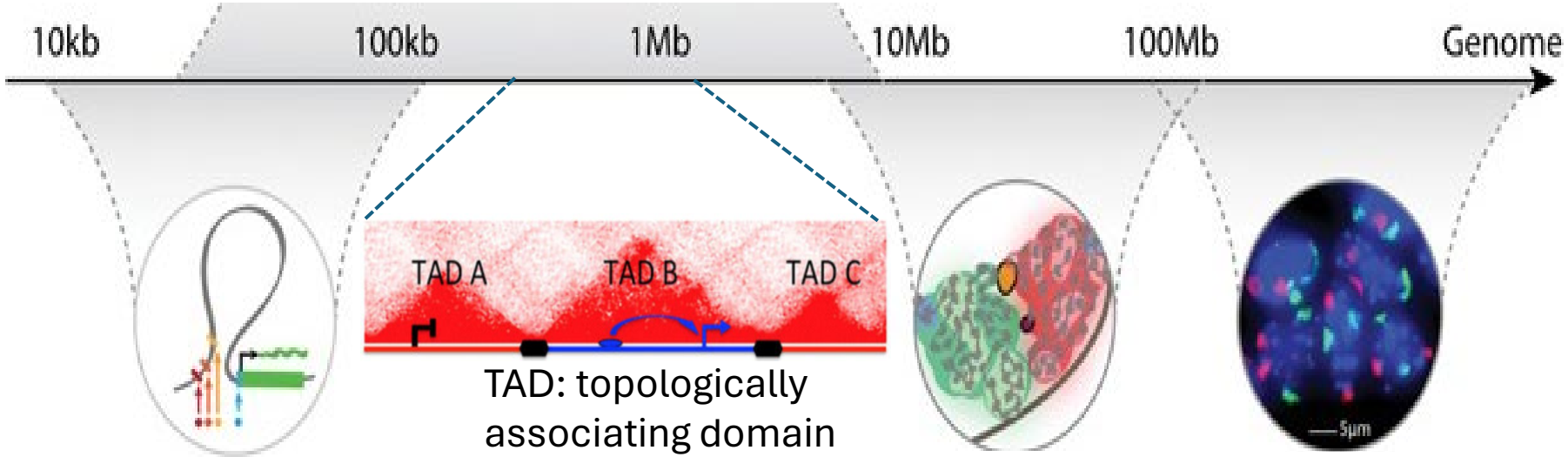
DNA folding creates unique opportunities and vulnerabilities



DNA folding creates unique opportunities and vulnerabilities

Scales of genome architecture

Resolution scale



Local DNA contacts
eg promoter enhancer

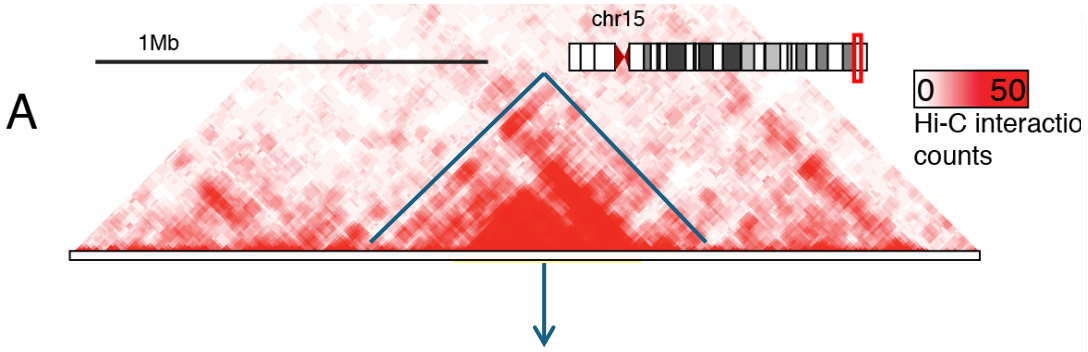
TAD: topologically associating domain

Folding in chromosome territories
eg domains of active and inactive chromatin

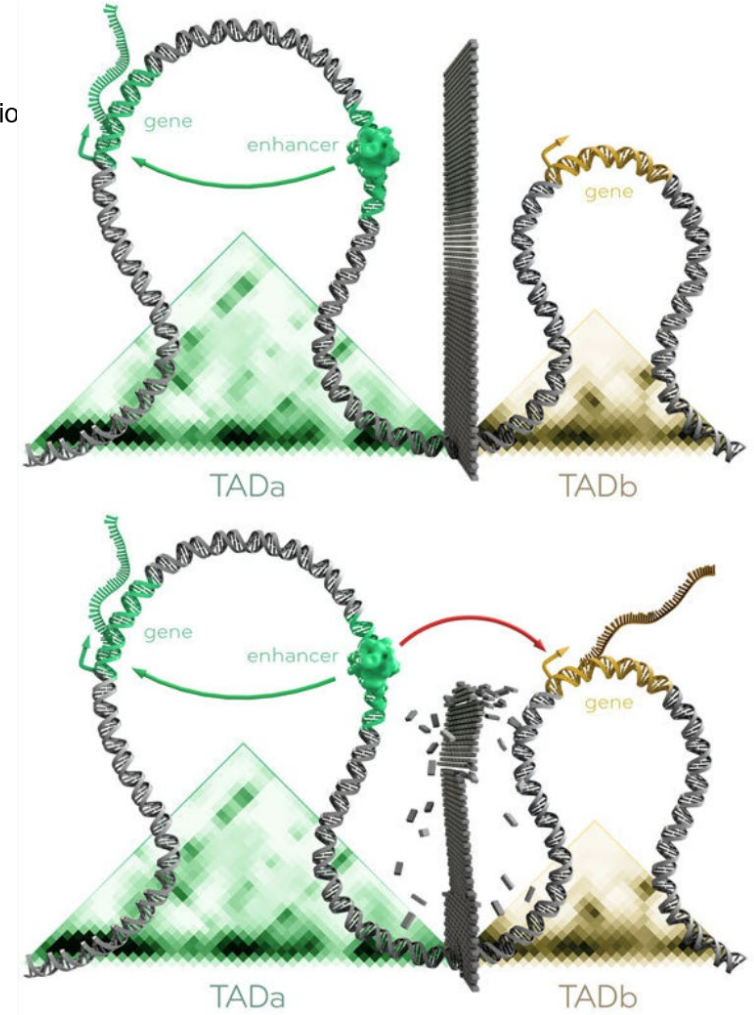
Chromosome positioning
eg spatial rearrangement

Dekker and Heard, Bioassays, 2013

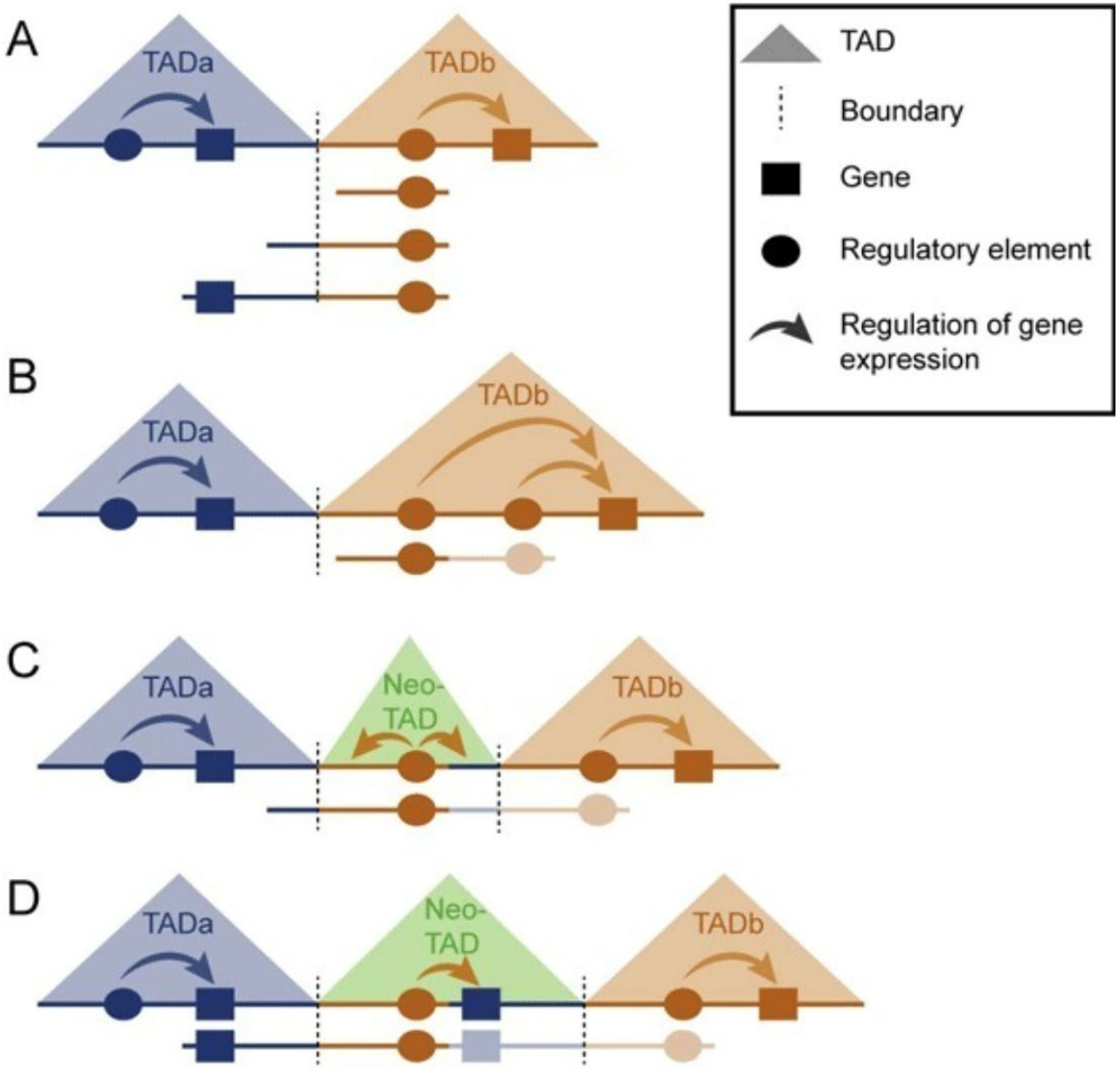
DNA folding creates unique opportunities and vulnerabilities



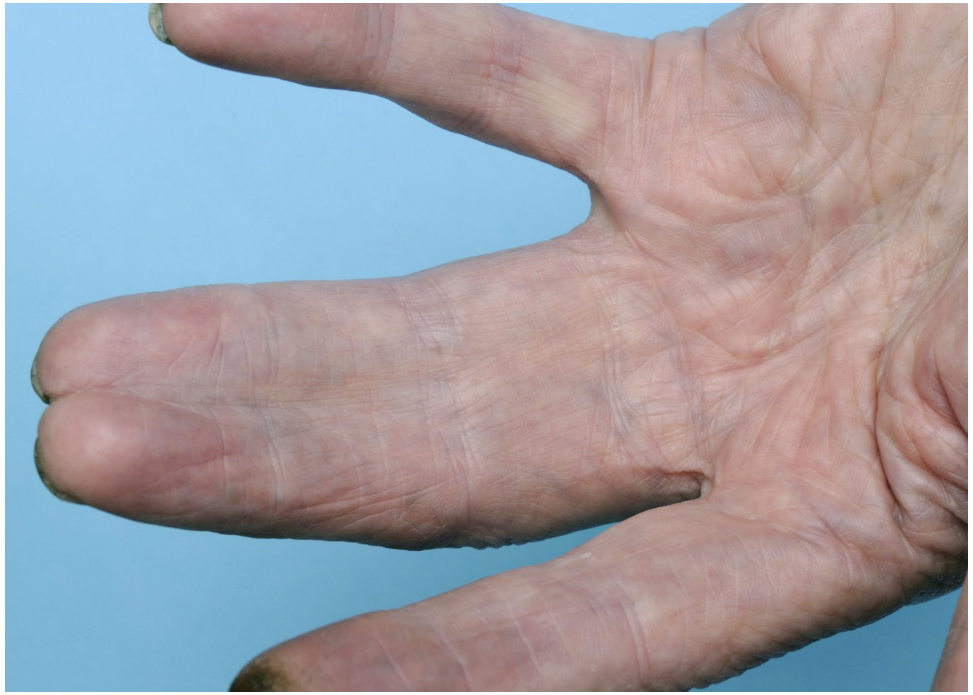
Topologically associated domains (TADs)

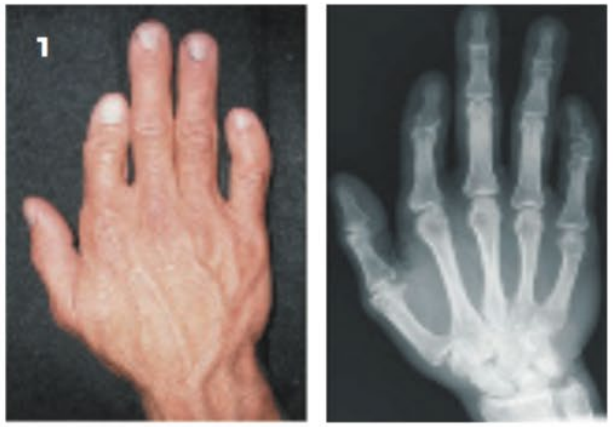


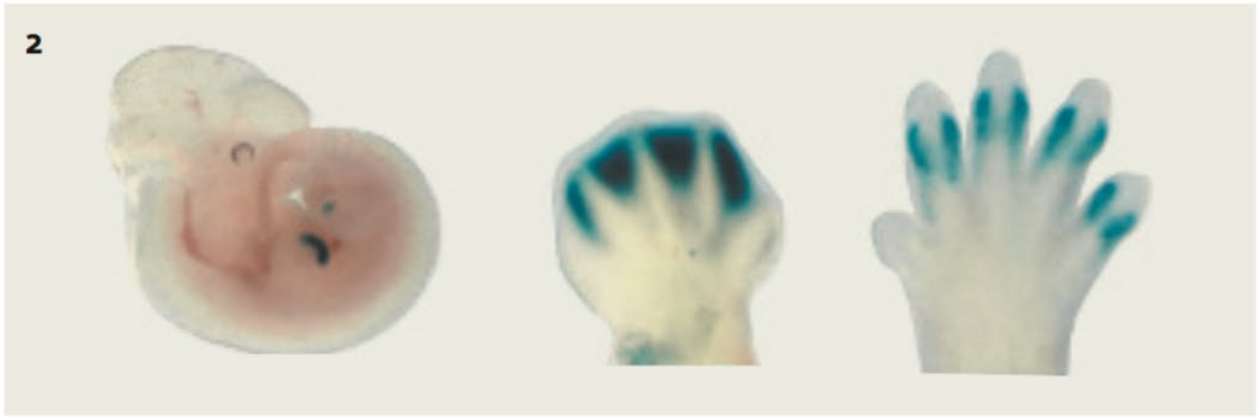
DNA folding creates unique opportunities and vulnerabilities



DNA folding creates unique opportunities and vulnerabilities



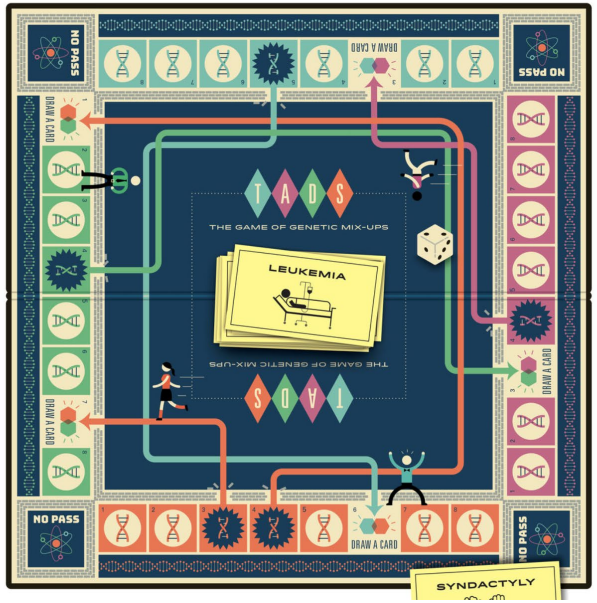




DNA folding creates unique opportunities and vulnerabilities

A Family's Shared Defect Sheds Light on the Human Genome

Basics
By NATALIE ANGIER JAN. 9, 2017



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MATTER
Telling Jewels From Junk in DNA
JAN. 21, 2016

Stefan Mundlos of the Max Planck Institute for Molecular Genetics in Germany studies the origin and development of limb malformations, some of which are caused by a novel class of genetic defects.

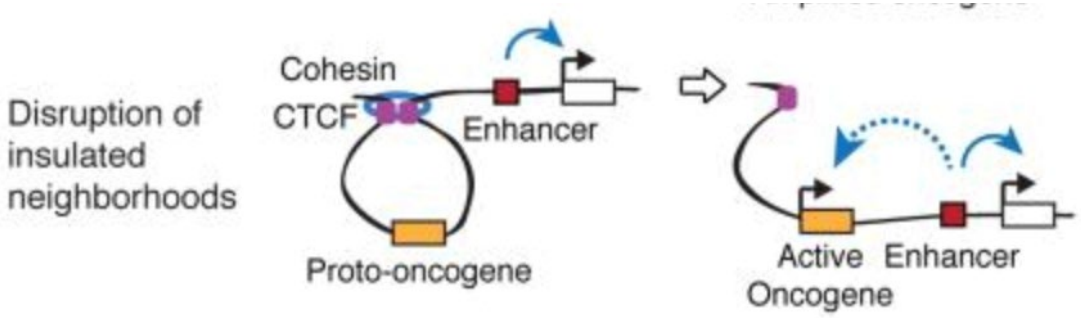
Norbert Michalke/Max Planck Institute for Molecular Genetics, Berlin

Stefan Mundlos, TADs

DNA folding creates unique opportunities and vulnerabilities

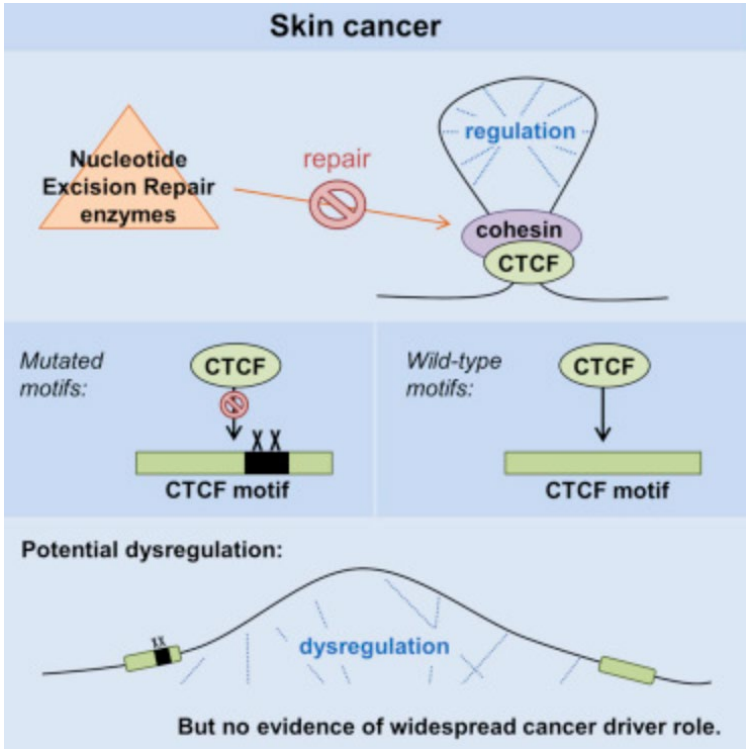
Even small alterations in DNA sequence can lead to extensive expression changes

Leukemia



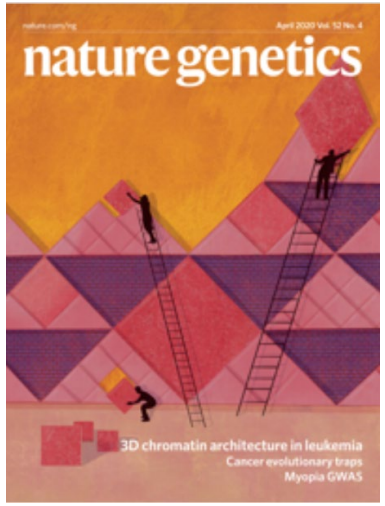
Hnisz et al., Science, 2016

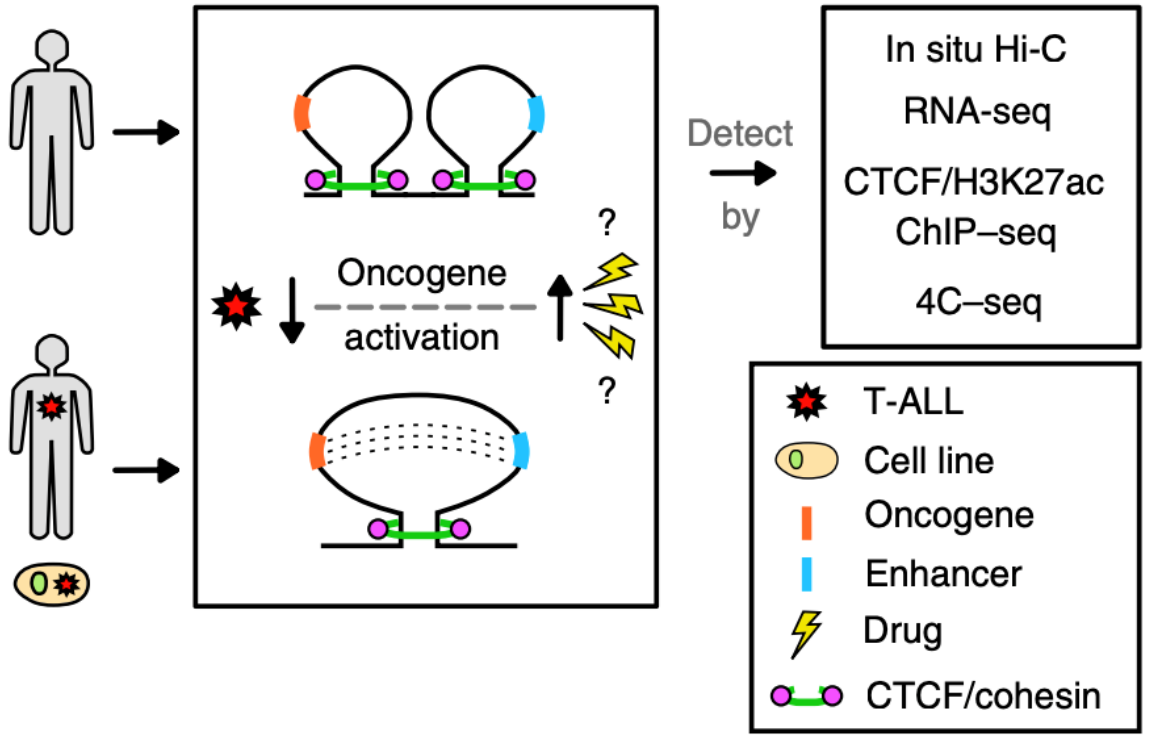
Melanoma



Poulos et al., Cell Reports, 2016

Oncogenic fusion of three-dimensional chromatin neighborhoods in T cell acute lymphoblastic leukemia





Andreas Kloetgen, Palaniraja Thandapani*, Panagiotis Ntziachristos*, et al., Nature Genetics, 2020*

Cancer-type specific CTCF binding

Fang et al. Genome Biology (2020) 21:247
https://doi.org/10.1186/s13059-020-02152-7

Genome Biology

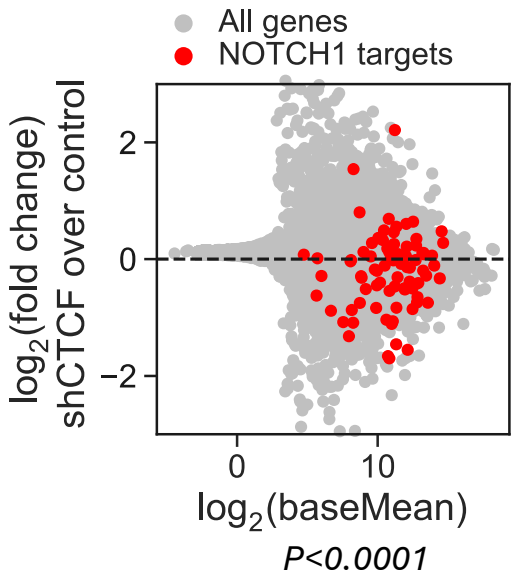
RESEARCH Open Access

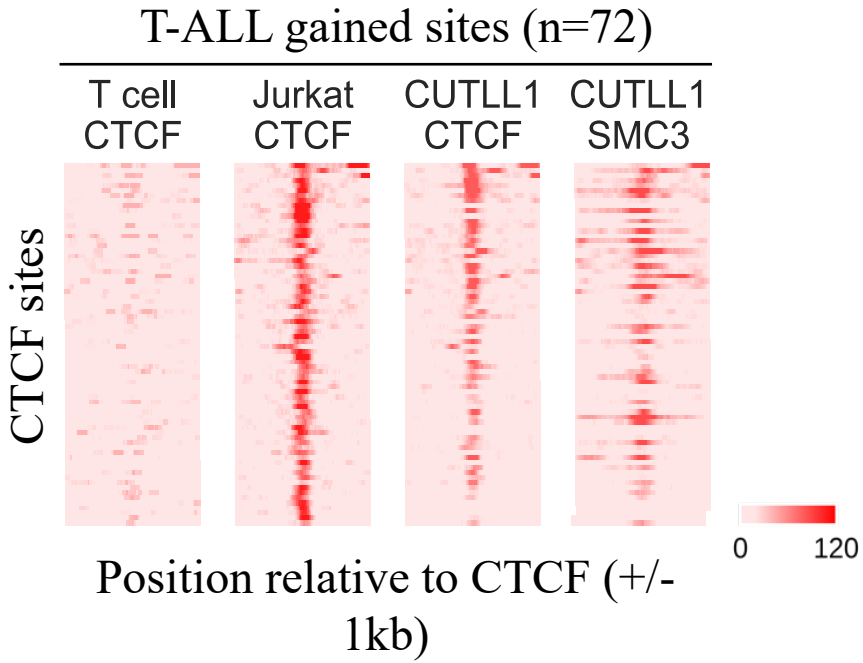
Cancer-specific CTCF binding facilitates oncogenic transcriptional dysregulation

Celestia Fang[1,2†], Zhenjia Wang[3†], Cuijuan Han[1,2], Stephanie L. Safgren[4], Kathryn A. Helmin[5], Emmalee R. Adelman[6,7], Valentina Serafin[8], Giuseppe Basso[8,9], Kyle P. Eagen[1,2], Alexandre Gaspar-Maia[4], Maria E. Figueroa[6,7], Benjamin D. Singer[1,2,5], Aakrosh Ratan[3,10,11], Panagiotis Ntziachristos[1,2,12*†] and Chongzhi Zang[3,10,11*†]

CTCF levels and genomic binding control oncogenic expression

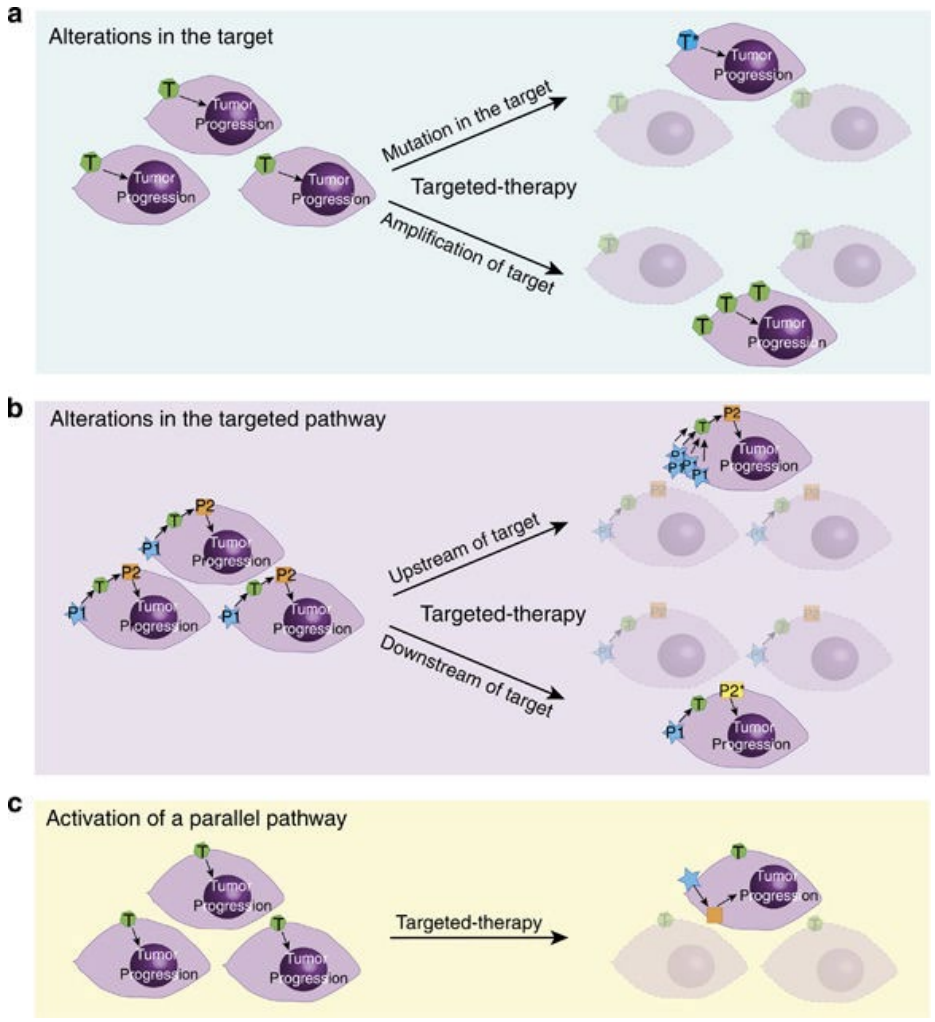
RNA-seq upon silencing of CTCF





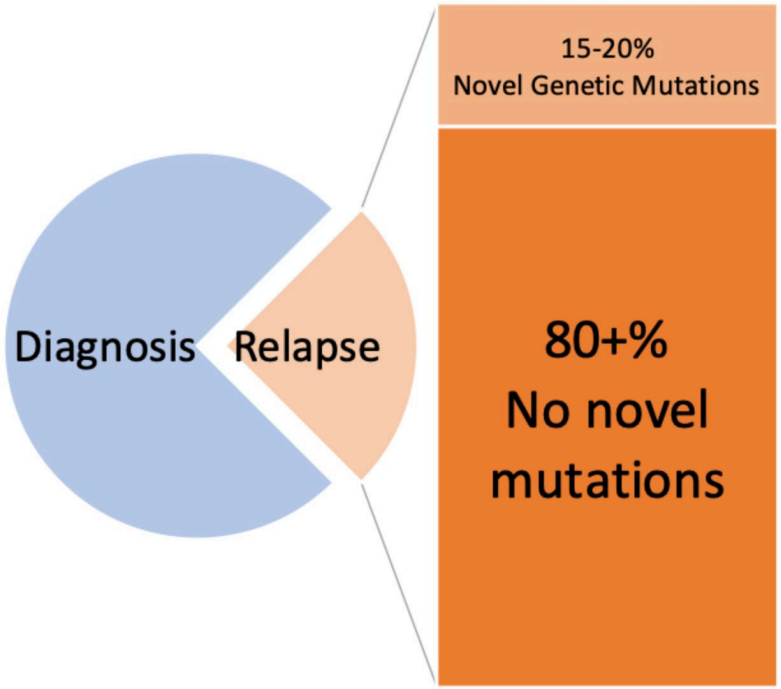
Relapse and therapy resistance

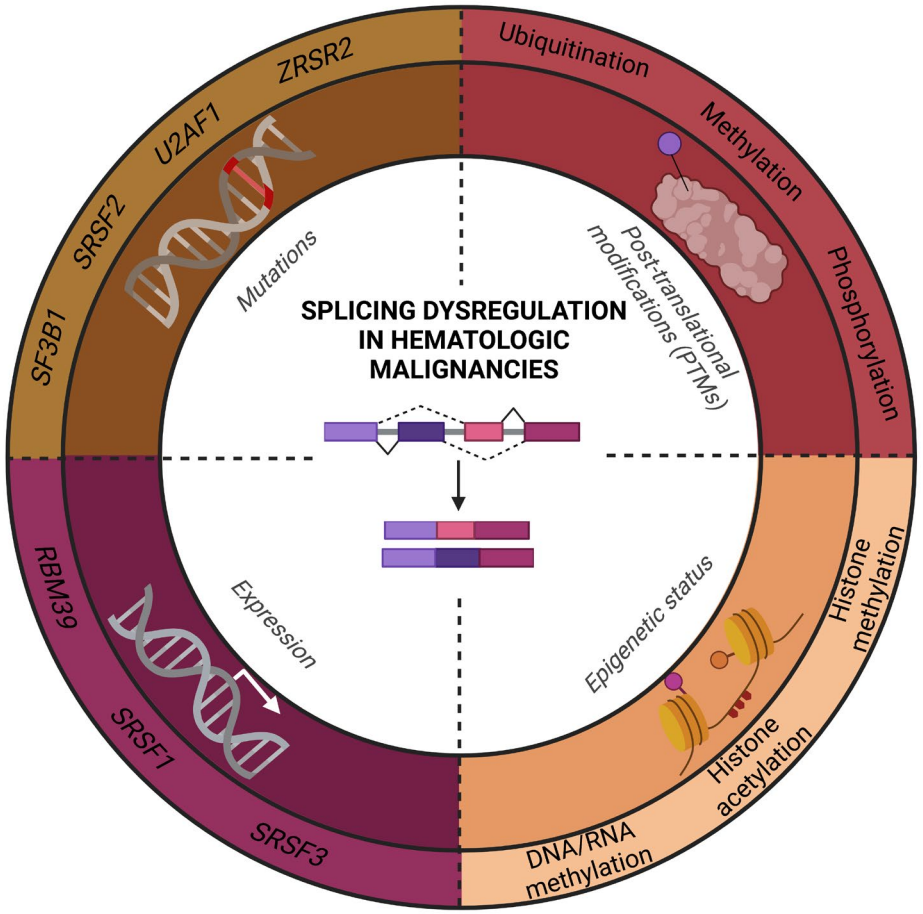
Cell-intrinsic mechanisms of resistance:



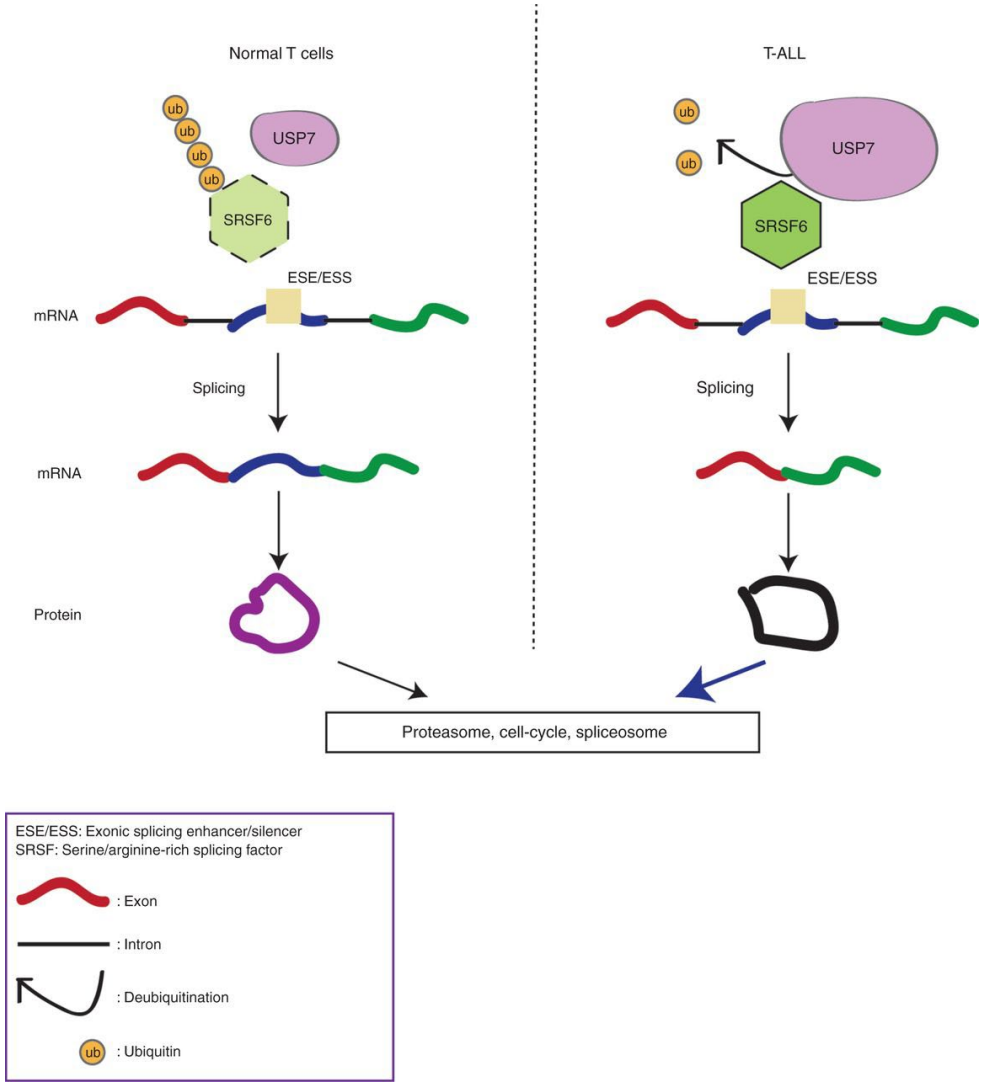
Ramos and Bentires-Alj, *Oncogene* 2014

T-ALL therapy resistance

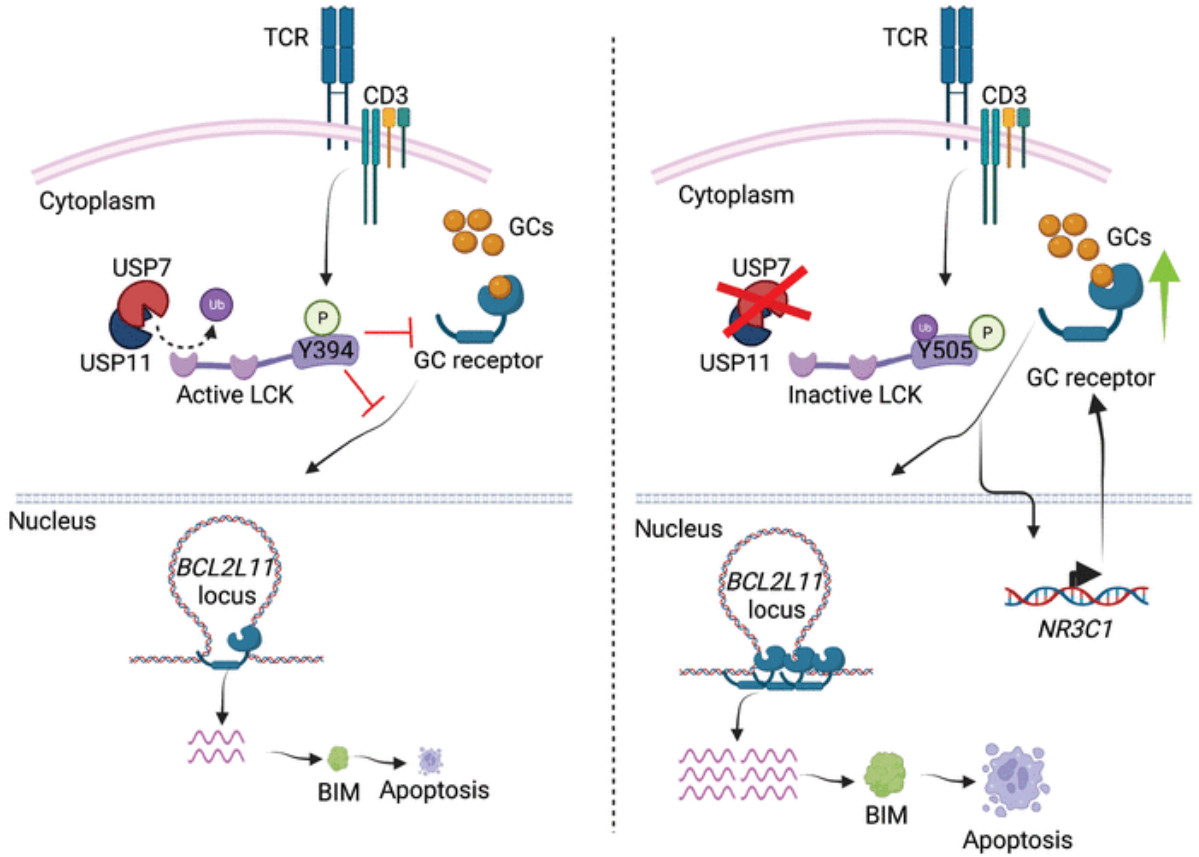




T-ALL therapy resistance

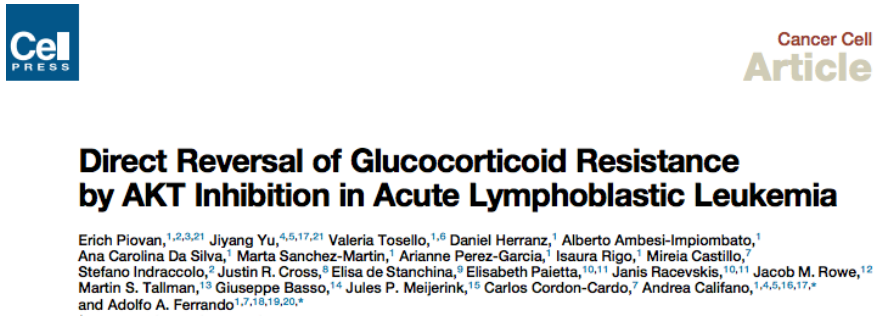


Cancer Discov. 2020;10(9):1388-1409. doi:10.1158/2159-8290.CD-19-1436



Science Advances 2022; DOI: (10.1126/sciadv.abq8437)

Off-target MAPK pathway activation inhibits glucocorticoid function and creates resistance



Cancer Cell
Article

Direct Reversal of Glucocorticoid Resistance by AKT Inhibition in Acute Lymphoblastic Leukemia

Erich Piovan,[1,2,3,21] Jiyang Yu,[4,5,17,21] Valeria Tosello,[1,6] Daniel Herranz,[1] Alberto Ambesi-Impiombato,[1] Ana Carolina Da Silva,[1] Marta Sanchez-Martin,[1] Arianne Perez-Garcia,[1] Isaura Rigo,[1] Mireia Castillo,[7] Stefano Indraccolo,[2] Justin R. Cross,[8] Elisa de Stanchina,[9] Elisabeth Paietta,[10,11] Janis Racevskis,[10,11] Jacob M. Rowe,[12] Martin S. Tallman,[13] Giuseppe Basso,[14] Jules P. Meijerink,[15] Carlos Cordon-Cardo,[7] Andrea Califano,[1,4,5,16,17,*] and Adolfo A. Ferrando[1,7,18,19,20,*]

Regular Article

blood

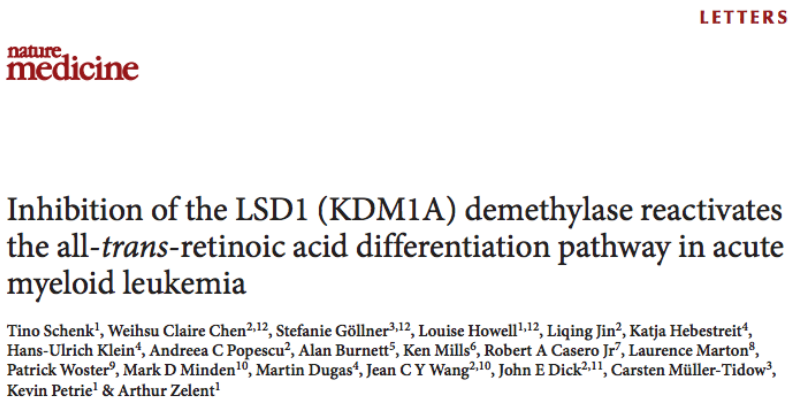
LYMPHOID NEOPLASIA

Glucocorticoid resistance is reverted by LCK inhibition in pediatric T-cell acute lymphoblastic leukemia

Valentina Serafin,[1] Giorgia Capuzzo,[1] Gloria Milani,[1] Sonia Anna Minuzzo,[2] Marica Pinazza,[3] Roberta Bortolozzi,[1] Silvia Bresolin,[1] Elena Porcù,[1] Chiara Frasson,[1-4] Stefano Indraccolo,[3] Giuseppe Basso,[1,*] and Benedetta Accordi[1,*]

[1]Department of Woman's and Child's Health and [2]Immunology and Oncology Section, Department of Surgery, Oncology, and Gastroenterology, University of Padova, Padova, Italy; [3]Immunology and Molecular Oncology Unit, Istituto Oncologico Veneto IRCCS, Padova, Italy; and [4]Istituto di Ricerca Pediatrica Città della Speranza, Padova, Italy

Resistance to targeted therapy: the role of epigenetics

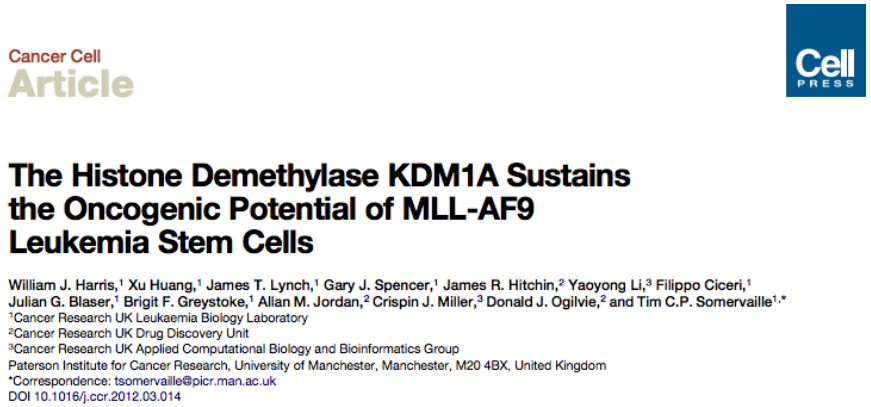


LETTERS

nature medicine

Inhibition of the LSD1 (KDM1A) demethylase reactivates the all-*trans*-retinoic acid differentiation pathway in acute myeloid leukemia

Tino Schenk[1], Weihsu Claire Chen[2,12], Stefanie Göllner[3,12], Louise Howell[1,12], Liqing Jin[2], Katja Hebestreit[4], Hans-Ulrich Klein[4], Andreea C Popescu[2], Alan Burnett[5], Ken Mills[6], Robert A Casero Jr[7], Laurence Marton[8], Patrick Woster[9], Mark D Minden[10], Martin Dugas[4], Jean C Y Wang[2,10], John E Dick[2,11], Carsten Müller-Tidow[3], Kevin Petrie[1] & Arthur Zelent[1]



Cancer Cell
Article

Cell PRESS

The Histone Demethylase KDM1A Sustains the Oncogenic Potential of MLL-AF9 Leukemia Stem Cells

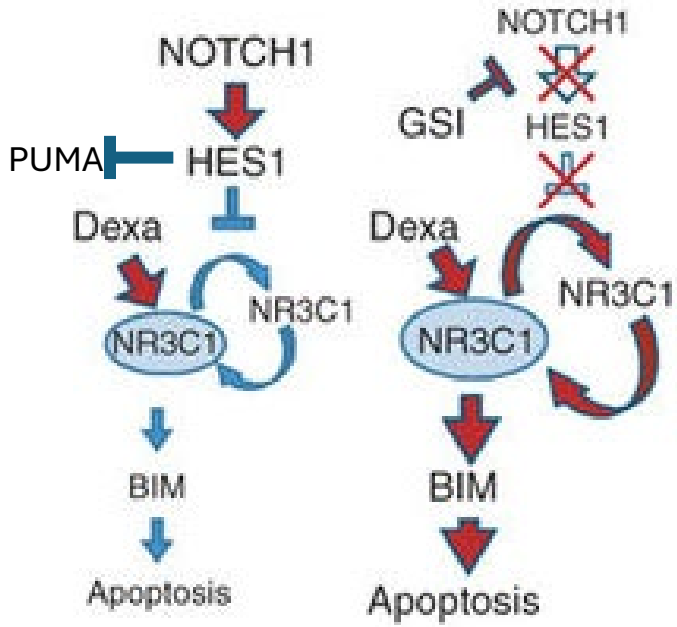
William J. Harris,[1] Xu Huang,[1] James T. Lynch,[1] Gary J. Spencer,[1] James R. Hitchin,[2] Yaoyong Li,[3] Filippo Ciceri,[1] Julian G. Blaser,[1] Brigit F. Greystoke,[1] Allan M. Jordan,[2] Crispin J. Miller,[3] Donald J. Ogilvie,[2] and Tim C.P. Somervaille[1,*]
[1]Cancer Research UK Leukaemia Biology Laboratory
[2]Cancer Research UK Drug Discovery Unit
[3]Cancer Research UK Applied Computational Biology and Bioinformatics Group
Paterson Institute for Cancer Research, University of Manchester, Manchester, M20 4BX, United Kingdom
*Correspondence: tsomervaille@picr.man.ac.uk
DOI 10.1016/j.ccr.2012.03.014

Off-target activation of the NOTCH pathway generates resistance to glucocorticoids

nature medicine

γ-secretase inhibitors reverse glucocorticoid resistance in T cell acute lymphoblastic leukemia

Pedro J Real[1,2], Valeria Tosello[1,11], Teresa Palomero[1,3,11], Mireia Castillo[3], Eva Hernando[4], Elisa de Stanchina[5], Maria Luisa Sulis[1,6], Kelly Barnes[1], Catherine Sawai[7], Irene Homminga[8], Jules Meijerink[8], Iannis Aifantis[7], Giuseppe Basso[9], Carlos Cordon-Cardo[3], Walden Ai[10] & Adolfo Ferrando[1,3,6]



On-target upstream mutations create resistance to nucleotide analogues

LETTERS

nature medicine

Activating mutations in the *NT5C2* nucleotidase gene drive chemotherapy resistance in relapsed ALL

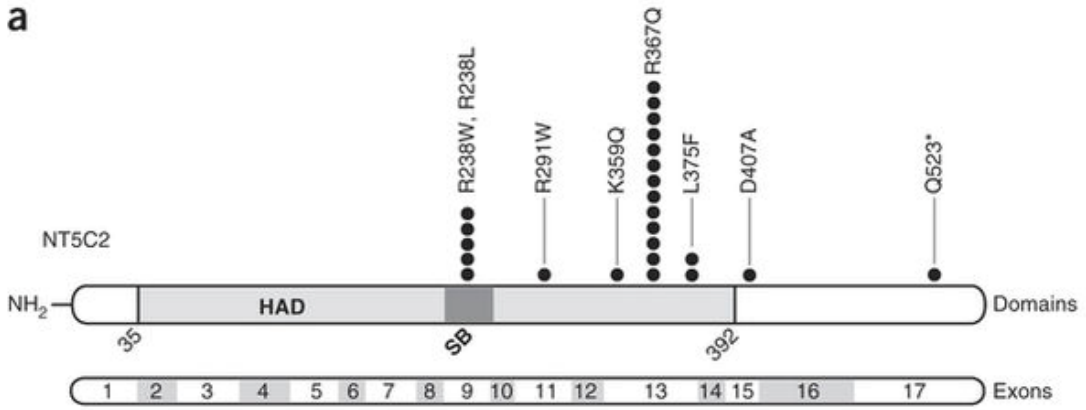
Gannie Tzoneva[1,12], Arianne Perez-Garcia[1,12], Zachary Carpenter[2,12], Hossein Khiabanian[2], Valeria Tosello[3], Maddalena Allegretta[1], Elisabeth Paietta[4], Janis Racevskis[4], Jacob M Rowe[5,6], Martin S Tallman[7], Maddalena Paganin[8], Giuseppe Basso[8], Jana Hof[9], Renate Kirschner-Schwabe[9], Teresa Palomero[1,10,12], Raul Rabadan[2,12] & Adolfo Ferrando[1,10–12]

LETTERS

nature genetics

Relapse-specific mutations in *NT5C2* in childhood acute lymphoblastic leukemia

Julia A Meyer[1,2], Jinhua Wang[1,3], Laura E Hogan[1,4,13], Jun J Yang[5], Smita Dandekar[1,4], Jay P Patel[6], Zuojian Tang[3], Paul Zumbo[7,8], Sheng Li[7,8], Jiri Zavadil[1,3], Ross L Levine[6,9], Timothy Cardozo[10], Stephen P Hunger[11,12], Elizabeth A Raetz[1,4], William E Evans[5], Debra J Morrison[1,4], Christopher E Mason[7,8] & William L Carroll[1,2,4]



Antimetabolite-related resistance due to on-target mutations

nature medicine

ARTICLES

Negative feedback–defective PRPS1 mutants drive thiopurine resistance in relapsed childhood ALL

Benshang Li[1–3,12], Hui Li[1,3,4,12], Yun Bai[2,12], Renate Kirschner-Schwabe[5,6], Jun J Yang[7], Yao Chen[1,3], Gang Lu[2], Gannie Tzoneva[8], Xiaotu Ma[7], Tongmin Wu[1,3,4], Wenjing Li[9], Haisong Lu[10], Lixia Ding[1,3], Huanhuan Liang[1], Xiaohang Huang[1], Minjun Yang[2], Lei Jin[2], Hui Kang[2], Shuting Chen[2], Alicia Du[10], Shuhong Shen[1,3], Jianping Ding[9], Hongzhuan Chen[4,11], Jing Chen[1], Arend von Stackelberg[5], Longjun Gu[1], Jinghui Zhang[7], Adolfo Ferrando[8], Jingyan Tang[1], Shengyue Wang[2,11] & Bin-Bing S Zhou[1,3,4,11]

Summary

-Histones, DNA and RNA can be modified

-Those modifications are important for gene expression and can be disturbed in cancer and developmental disorders

-Epigenetic enzymes can add, erase and read modifications

-A lot of syndromes and diseases implicate genetic alterations affecting genes of epigenetic regulators

-Nevertheless, there are some diseases and disease states caused exclusively by epigenetic alterations without any obvious mutations

-Epigenetic enzymes act in complexes and mutations affecting different members of the complex can lead to similar, different and/or context-specific phenotypes

-Epigenetic alterations in disease can lead to drug resistance against systemic and targeted therapies

Thank you!
Questions?

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