中国科学院北京基因组研究所（国家生物信息中心）
BEIJING INSTITUTE OF GENOMICS CHINESE ACADEMY OF SCIENCES / CHINA NATIONAL CENTER FOR BIOINFORMATION

基因组学 - Genomics

第10章 表观基因组

章张 博士

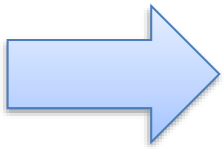
中国科学院大学
University of Chinese Academy of Sciences

国家基因组科学数据中心
National Genomics Data Center

内容提纲

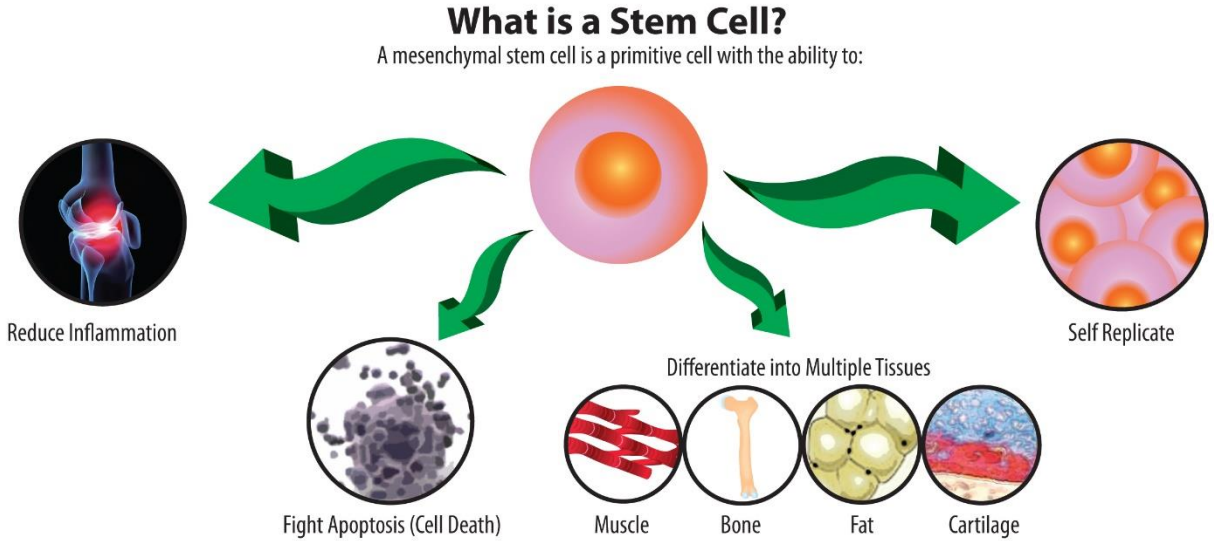
- 表观基因组学（Epigenomics）
- DNA修饰
- RNA修饰
- 蛋白质修饰
- 三维基因组
- 单细胞表观基因组学

一套基因组
One Genome



多套表观基因组
Many Epigenomes

人类有上亿个细胞，每个细胞的基因组都一样但却发挥不同的“角色”，每个细胞在特定状态或时间内开启并且利用不同的基因。



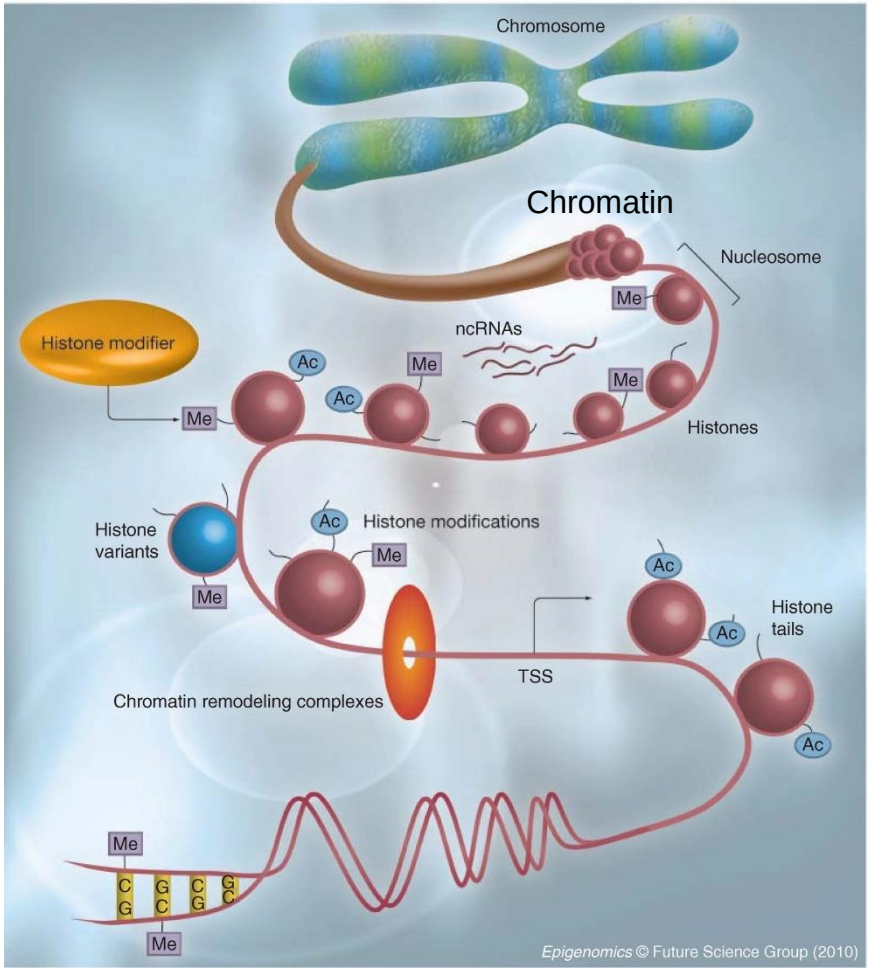
同卵双生的两人基因组完全相同，在同样的环境中长大后，在性格、健康等方面会有较大的差异。

表观基因组学（Epigenomics）

- 定义：在核苷酸序列不发生改变的情况下，研究基因组上的化学修饰和空间结构变化如何影响基因功能和表达调控的一门学科。
- 研究内容：
  - 化学修饰：DNA、RNA、蛋白质
  - 空间结构：核小体（nucleosome）、染色质（chromatin）、基因组
- 表观特点：DNA序列不变、可逆、可遗传、动态变化

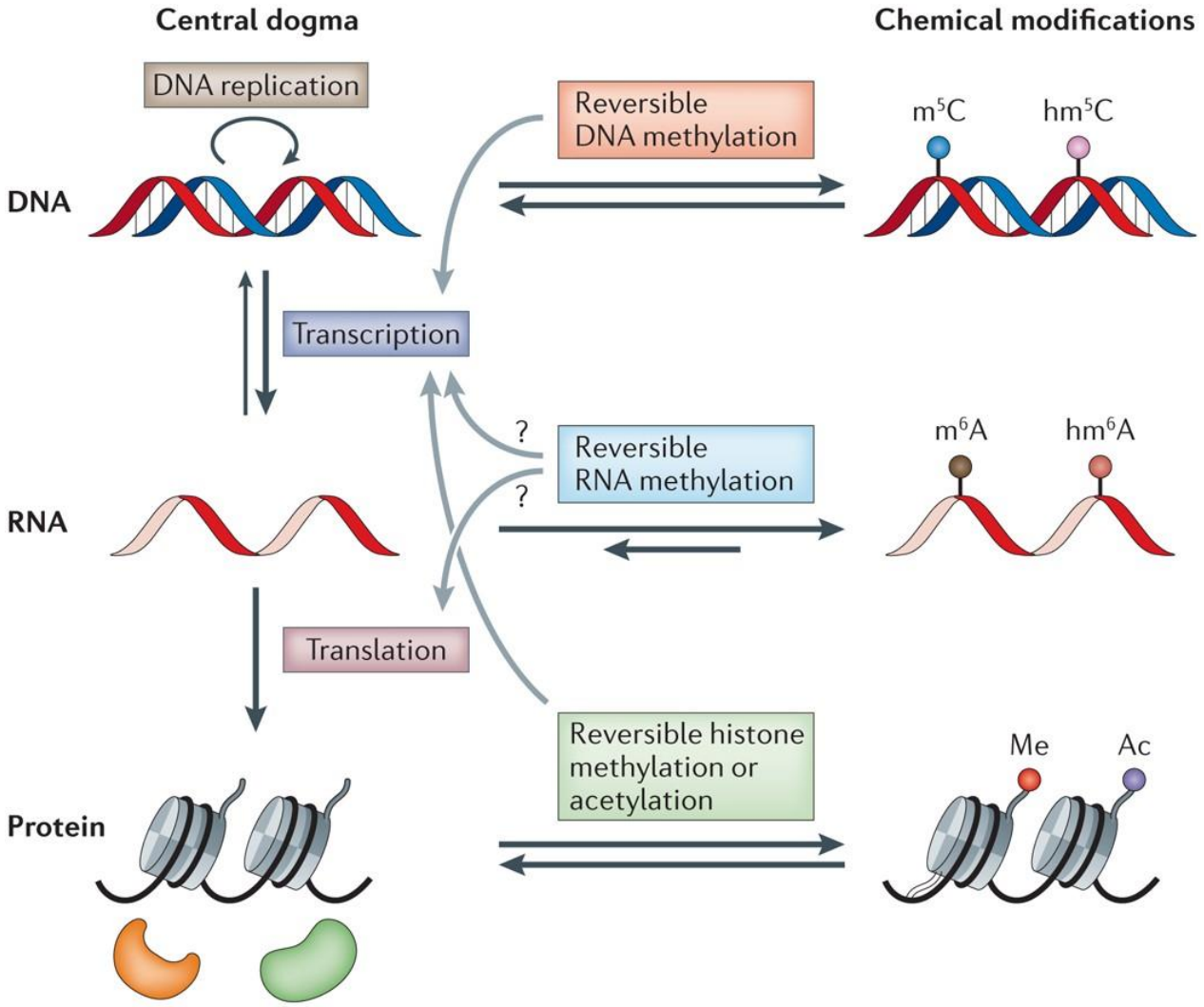
Epigenomics is to study chemical modifications to DNA/RNA/protein and physical changes to the structure of ncRNA, nucleosome, chromatin, and genome that regulate the expression of genes.

The systematic study of the global gene expression changes due to epigenetics processes and not due to DNA base sequence changes. https://www.ncbi.nlm.nih.gov/mesh/?term=epigenomics



Epigenetic control of inducible gene expression in the immune system. *Epigenomics* 2010. https://doi.org/10.2217/epi.10.55

表观化学修饰



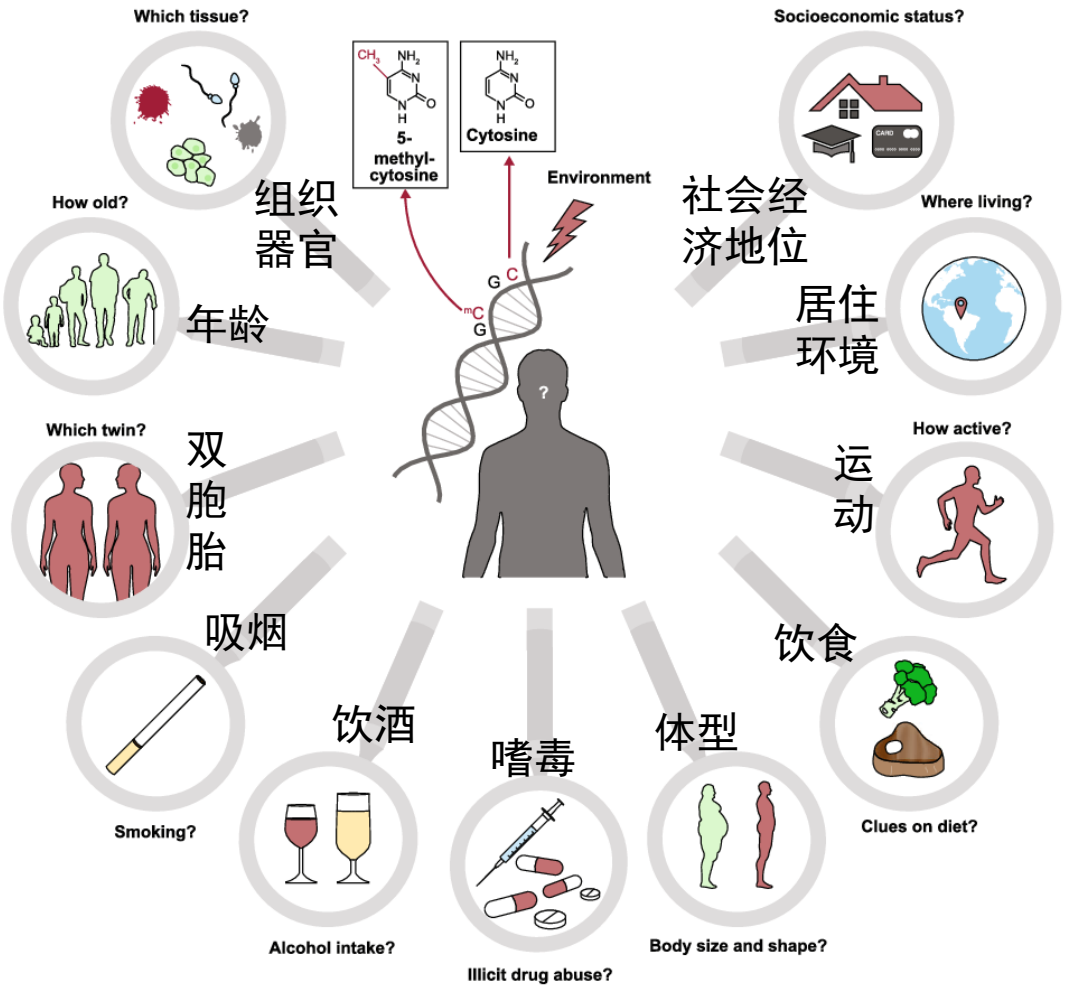
Gene expression regulation mediated through reversible m^{6}A RNA methylation. *Nature Reviews Genetics* 2014

引起表观基因组变化的因素

Lifestyle and environmental factors (such as smoking, diet and infectious disease) can expose a person to pressures that prompt chemical responses. These responses, in turn, often lead to changes in the epigenome, some of which can be damaging.

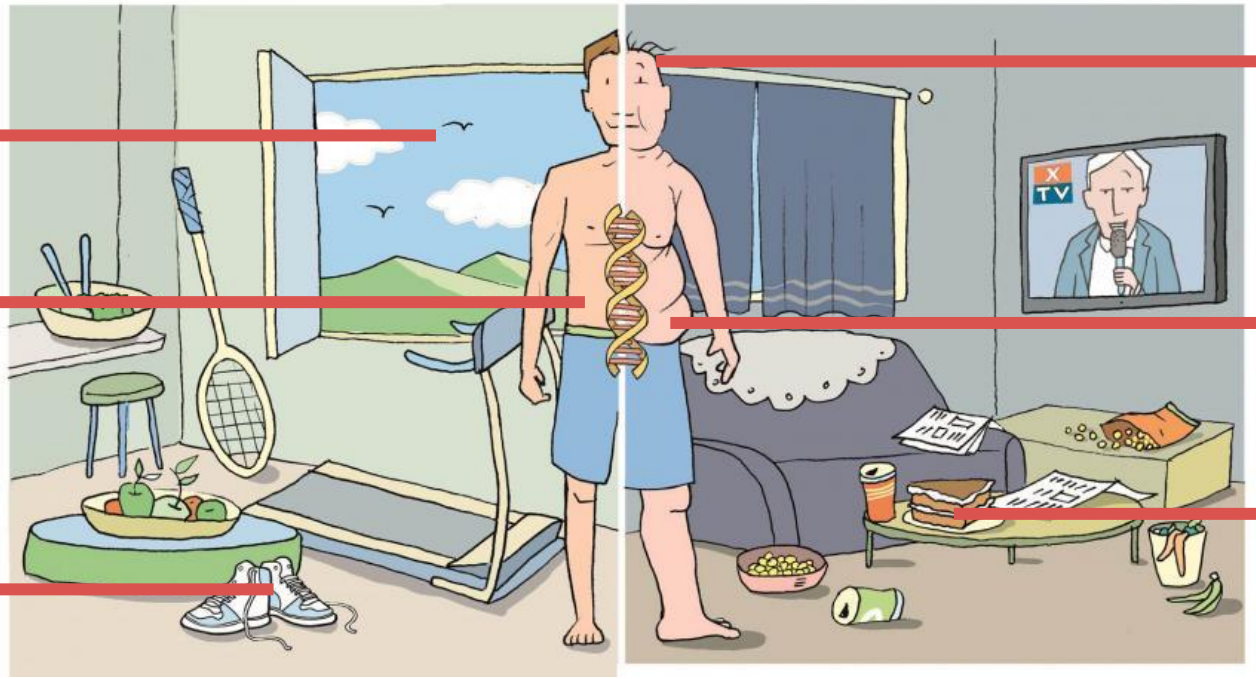
However, **the ability of the epigenome to adjust to the pressures of life appears to be required for normal human health**. Some human diseases are caused by malfunctions in the proteins that "read" and "write" epigenomic marks.

https://www.genome.gov/about-genomics/fact-sheets/Epigenomics-Fact-Sheet



From forensic epigenetics to forensic epigenomics: broadening DNA investigative intelligence. Genome Biology 2017. DOI:10.1186/s13059-017-1373-1

表观修饰与环境、表型、行为



表观修饰是表型、环境和行为的纽带

癌症表观基因组(Cancer Epigenome)

- **Cancers are caused by changes in the epigenome as well as the genome**. Changes in the epigenome can switch on or off genes involved in cell growth or the immune response. These changes can lead to uncontrolled growth, a hallmark of cancer, or to a failure of the immune system to destroy tumors.
- In a type of brain tumor called **glioblastoma**, doctors have had some success in treating patients with the drug **temozolomide**, which **kills cancer cells by adding methyl groups to DNA**. In some cases, methylation has a welcome secondary effect: it blocks a gene that counteracts temozolomide. **Glioblastoma patients whose tumors have such methylated genes are far more likely to respond to temozolomide than those with unmethylated genes (*MGMT*)**.
- Studies in the Cancer Genome Atlas (TCGA) are **comparing the genomes and epigenomes of normal cells with those of cancer cells**. Understanding all the changes that turn a normal cell into a cancer cell will speed efforts to develop new and better ways of diagnosing, treating and preventing cancer.

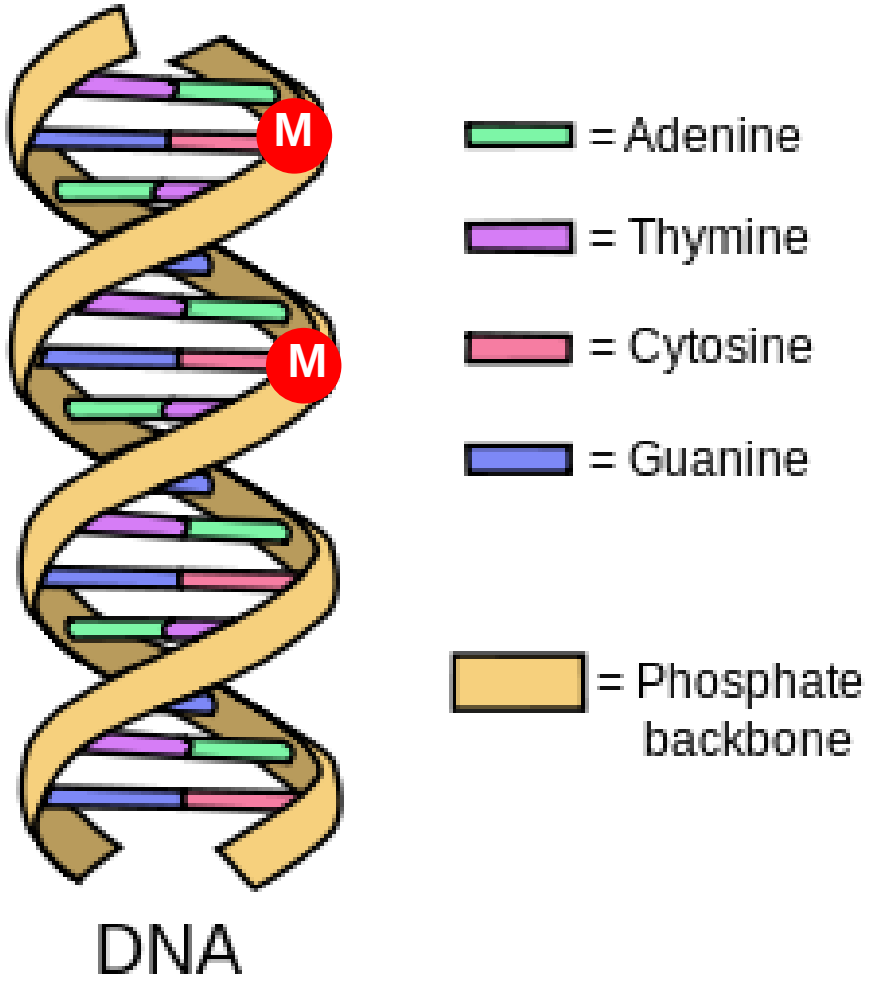
DNA修饰

DNA修饰–甲基化：给碱基戴帽子

甲基作为一个化学基团（-CH_3），能够结合在DNA上某些特定部位。

- 甲基和DNA结合过程叫**甲基化**
- 甲基从DNA上脱落的过程就叫做**去甲基化**

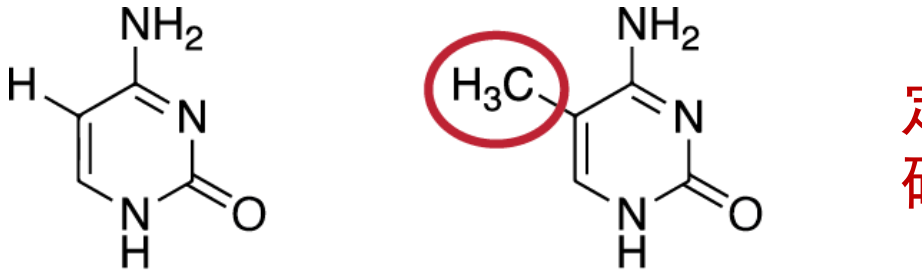
DNA甲基化能引起染色质结构、DNA构象、DNA稳定性及DNA与蛋白质相互作用方式的改变，从而控制基因表达。



DNA甲基化（Methylation）

DNA甲基化是指DNA碱基上特定位置的碳被添加甲基的过程。其主要形式有：N5-mC（胞嘧啶）、N6-mA（腺嘌呤）、N7-mG（鸟嘌呤）

- Cytosine methylation (5mC)



Cytosine　　methylated Cytosine

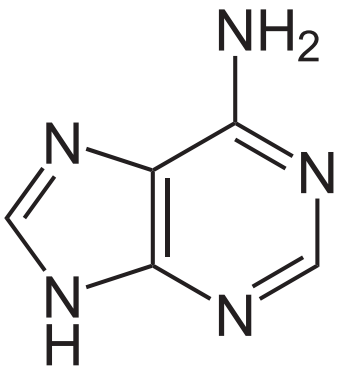
定量成熟，研究较多

CG甲基化：在DNA甲基转移酶（DNA methyltransferase）的作用下，在基因组CpG二核苷酸的胞嘧啶5'碳位共价键结合一个甲基基团（CH_3）

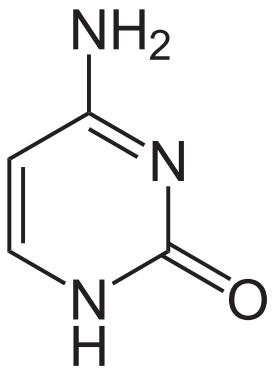
DNA甲基化一般不做特殊说明是指胞嘧啶形成5-甲基胞嘧啶的过程

DNA Methylation

Unmodified base

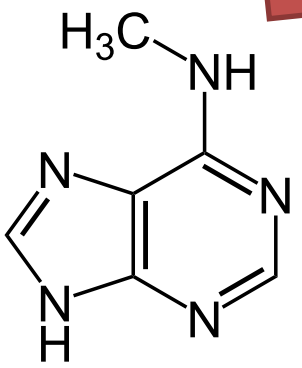


Adenine, A

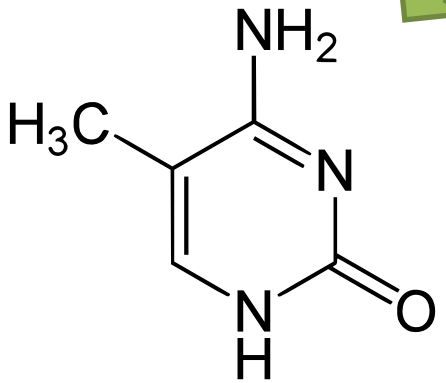


Cytosine, C

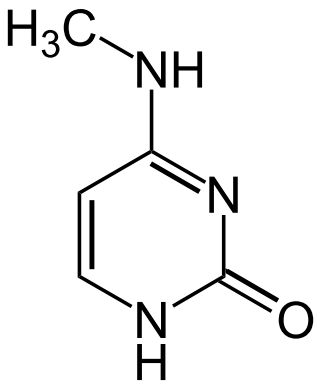
Modified base



***N*^{6}-Methyladenine, 6mA**



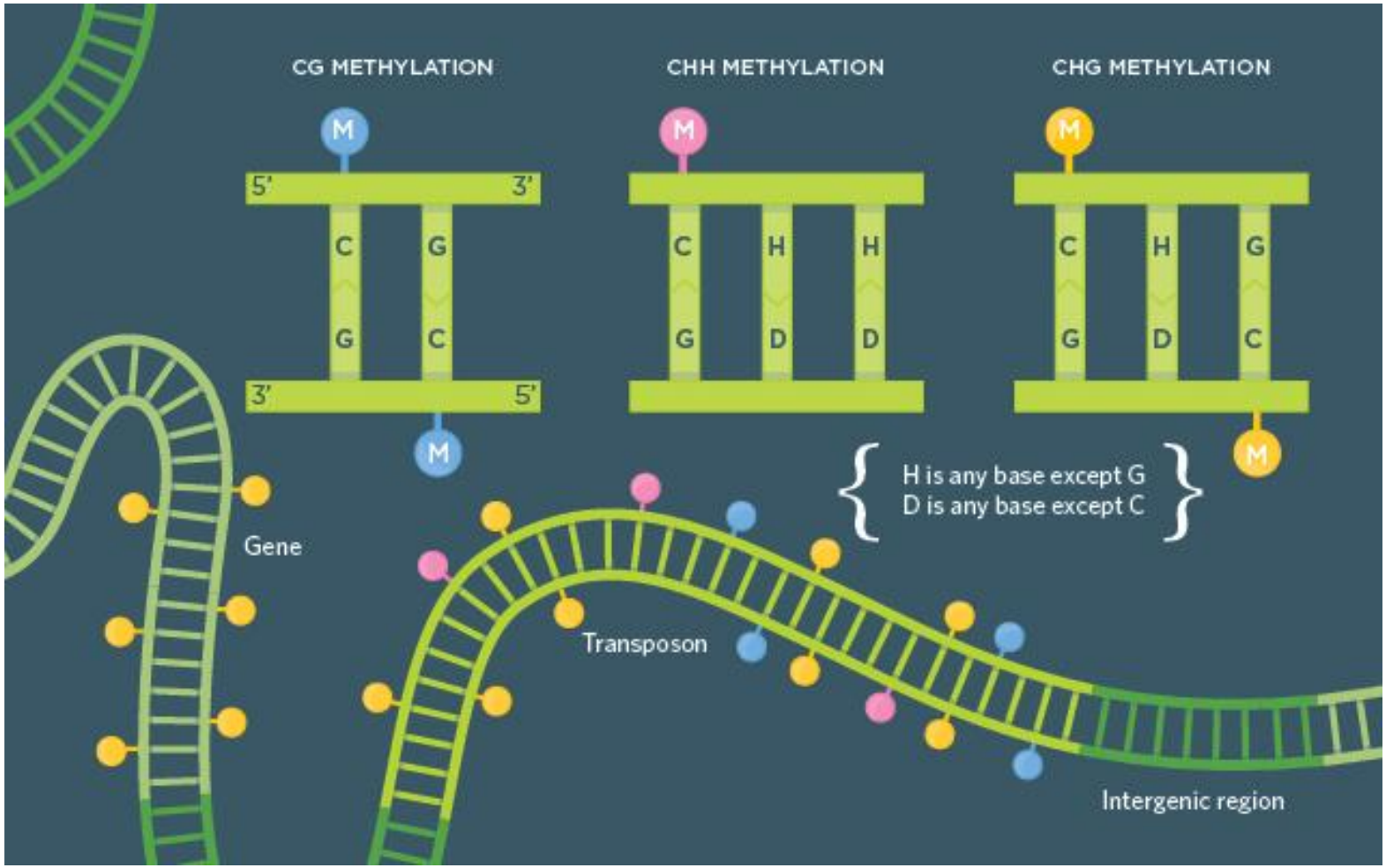
5-Methylcytosine, 5mC



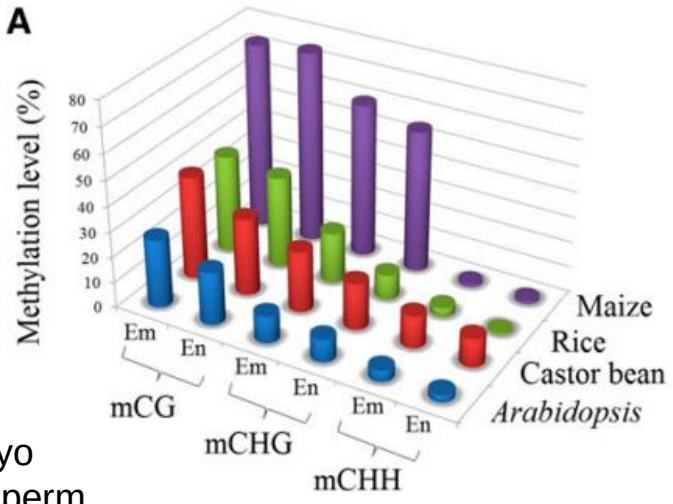
***N*^{4}-Methylcytosine, 4mC**

非CG甲基化

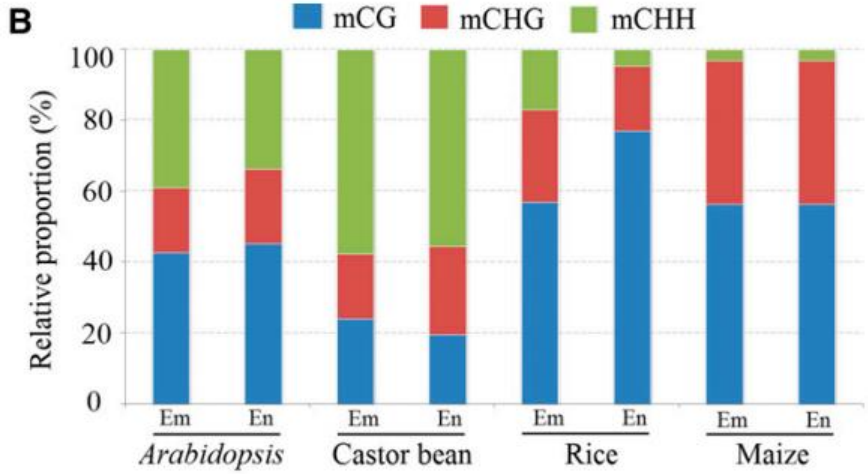
CHG、CHH （H=A、T、C）



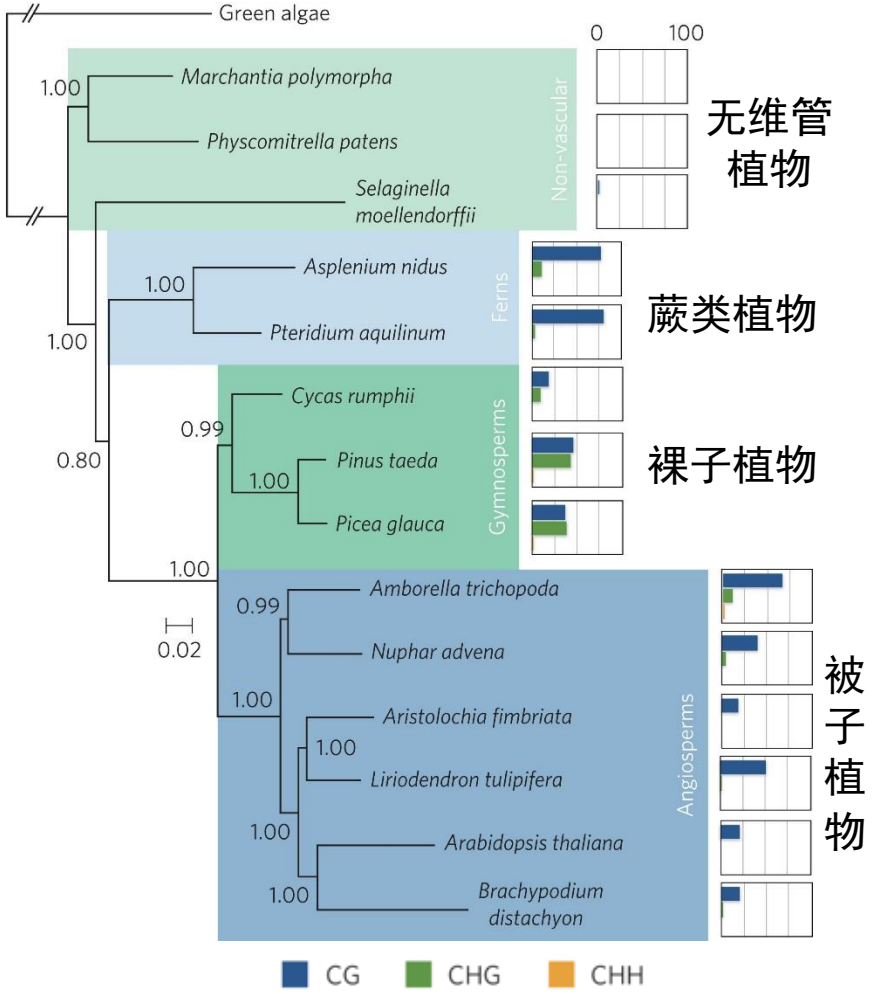
DNA methylation across plants



Em: embryo
En: endosperm



Genomic DNA Methylation Analyses Reveal the Distinct Profiles in Castor Bean Seeds with Persistent Endosperms. *Plant Physiol.* 2016



Evolutionary patterns of genic DNA methylation vary across land. *Nature Plants* 2016

“第五碱基”

Next-Generation DNA Sequencing/Review *Genome Research* 2009

Finding the fifth base: Genome-wide sequencing of cytosine methylation

Ryan Lister[1,2] and Joseph R. Ecker[1,2,3]

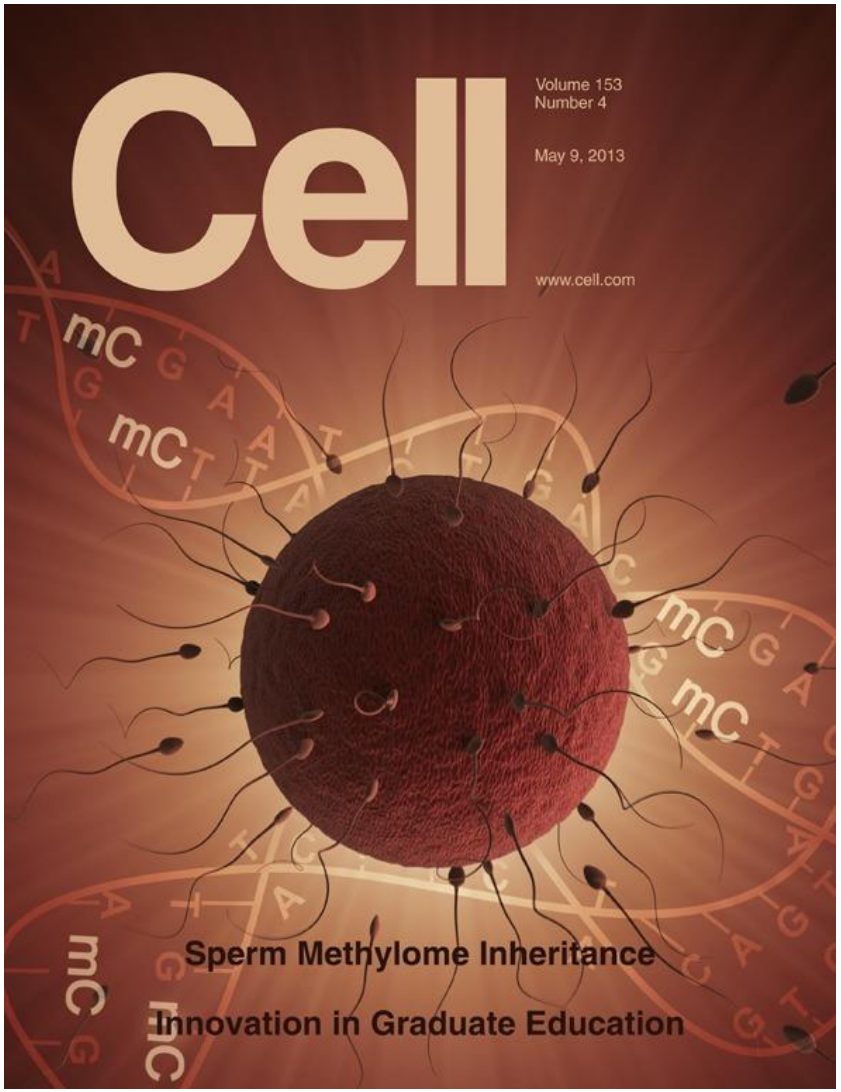
[1]*Genomic Analysis Laboratory, The Salk Institute for Biological Studies, La Jolla, California 92037, USA;* [2]*Plant Biology Laboratory, The Salk Institute for Biological Studies, La Jolla, California 92037, USA*

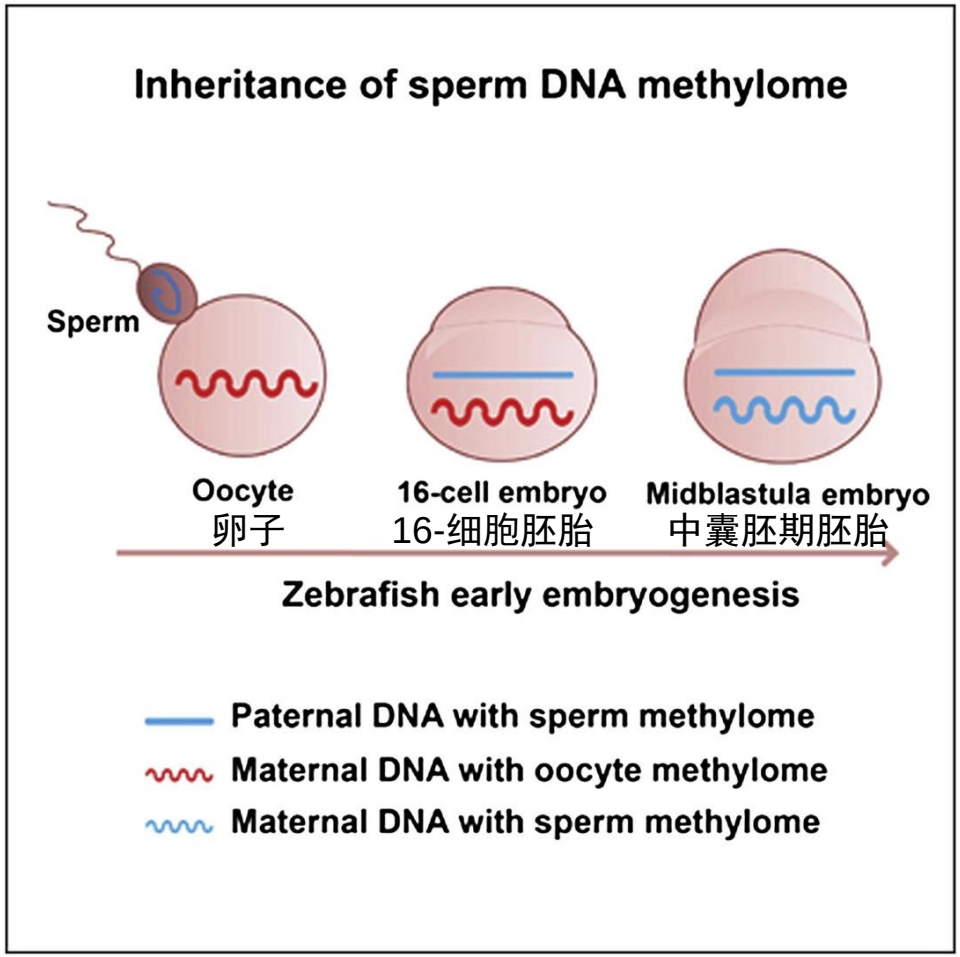
5甲基胞嘧啶（5mC）是表观遗传最重要的一种修饰，由于与基因的表达调控密切相关，又被誉为“第五碱基”

- 不影响复制过程中碱基配对
- 不影响转录过程中碱基配对

DNA修饰不能被看作新碱基

DNA甲基化遗传图谱

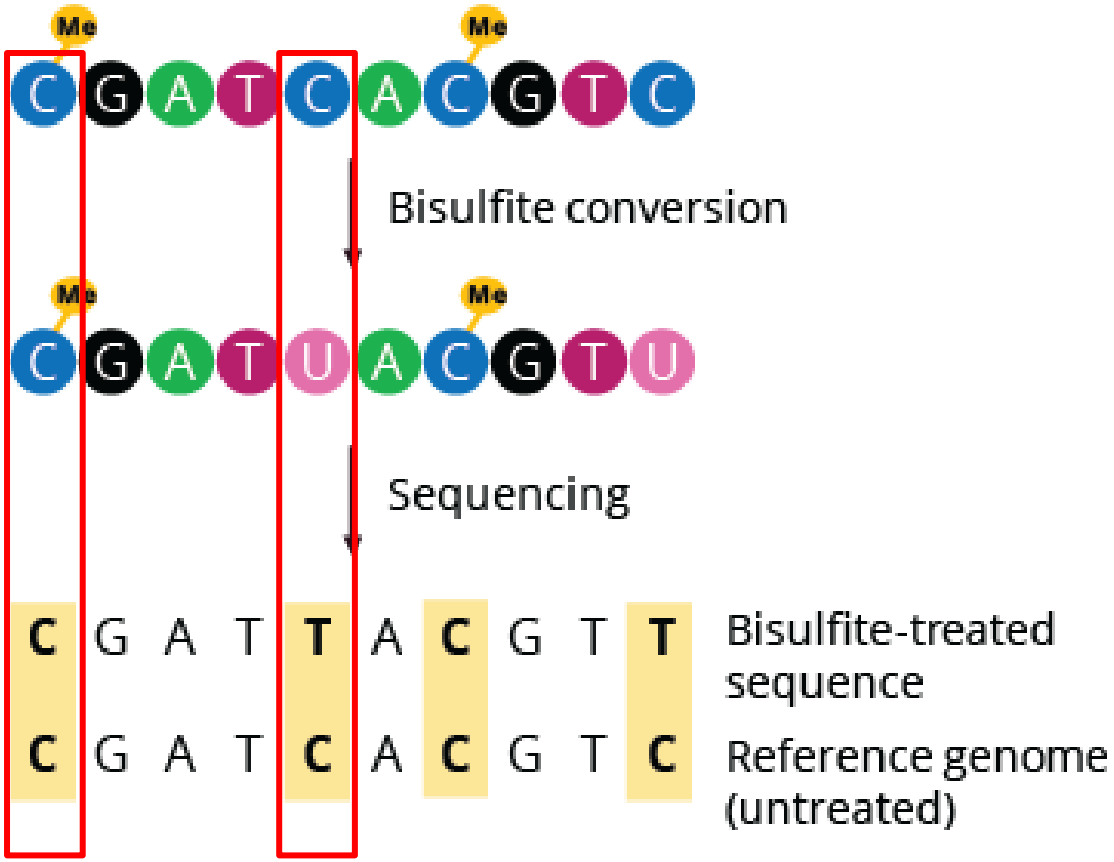




Sperm, but Not Oocyte, DNA Methylome Is Inherited by Zebrafish Early Embryos. *Cell* 2013

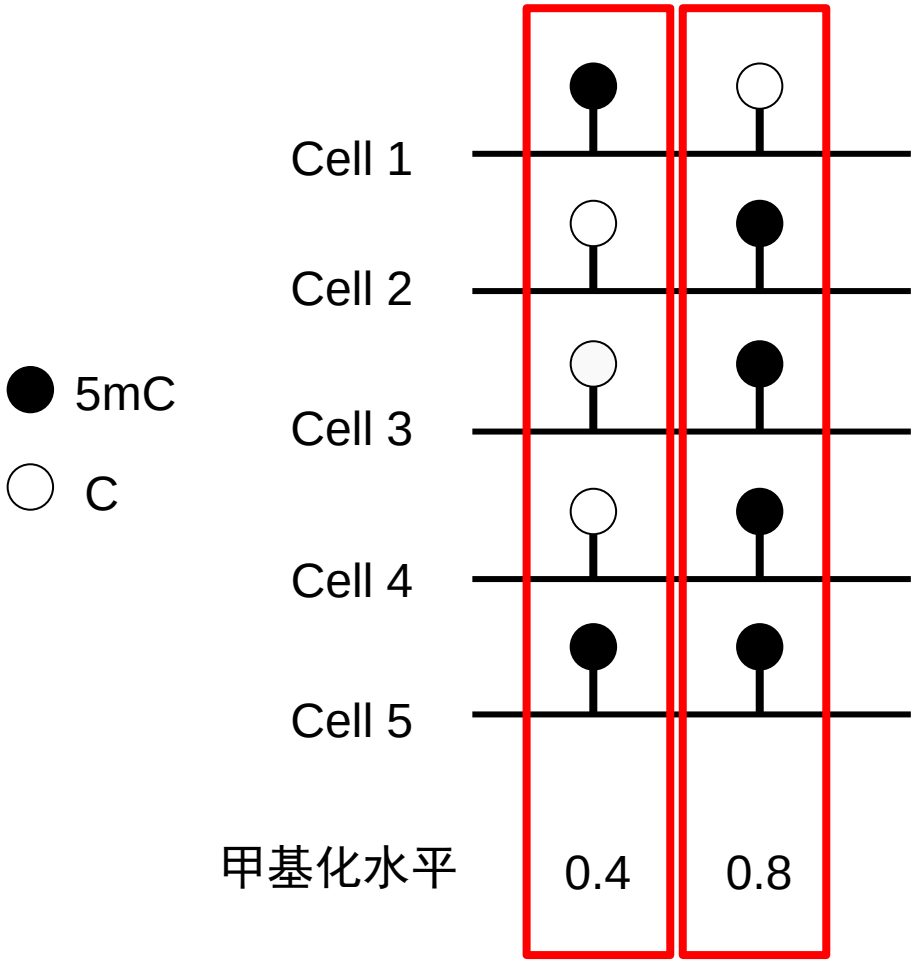
基因组所刘江团队

5mC测序



Bisulfite(重亚硫酸盐)处理能够将基因组中未发生甲基化的C碱基转换成U，进行PCR扩增后变成T，与原本具有甲基化修饰的C碱基区分开来，再结合高通量测序技术，可绘制单碱基分辨率的全基因组DNA甲基化图谱。

5mC甲基化水平的本质



单个位点甲基化水平本质上是不同甲基化状态细胞的比例。

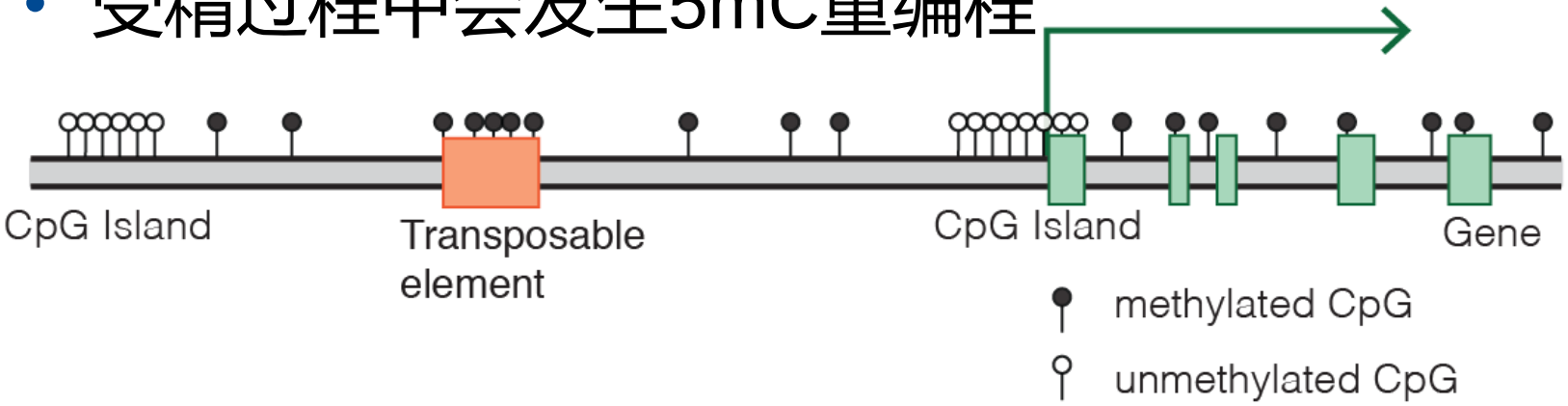
DNA甲基化测序

- 芯片适合大规模测序
- 全基因组甲基化测序适合探索性研究

	DNA甲基化芯片	全基因组甲基化测序
分析时间	两分钟	两天
测序成本	3000元	20000元/30X
捕获区域	~850,000个位点	全基因组
上样量	250 ng DNA	100 ng DNA
测序深度	相当于500X左右	通常不会超过100X

人类5mC甲基化的特点

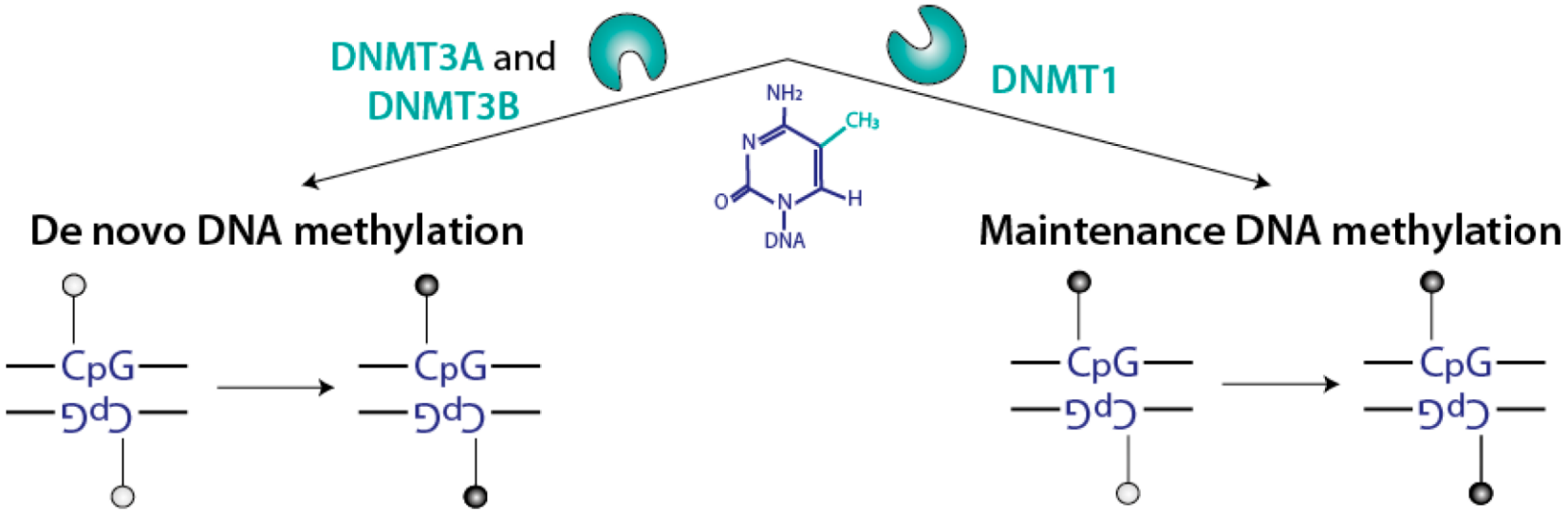
- 除了胚胎和脑组织，其它组织的5mC甲基化通常发生在CpG二核苷酸上
- 人类基因组中大约含有3千万个CpG二核苷酸
- CpG岛（一段CpG含量较高的区域）甲基化水平较低，非CpG岛甲基化水平较高
- 人类一生中5mC的含量动态变化
- 受精过程中会发生5mC重编程



DNA甲基化的建立与维持

DNA methyltransferase

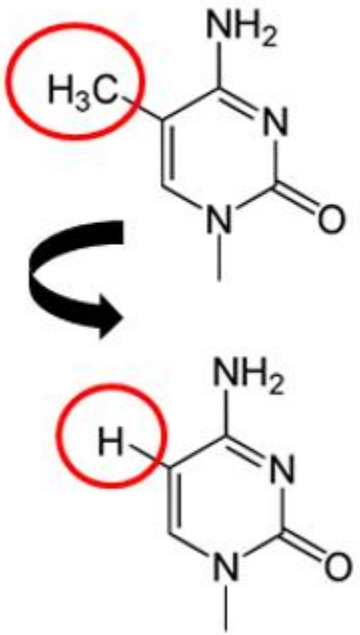
DNA甲基转移酶（DNMT）



DNMT3A和DNMT3B能够使胞嘧啶(C)发生从头甲基化

在复制过程中，DNMT1甲基转移酶会识别半甲基化的CpG，并催化形成5mC

DNA甲基化的去除



主动去甲基化：

- 通过酶(TET)进行去甲基化
- 通过DNA损伤修复去甲基化

被动去甲基化：

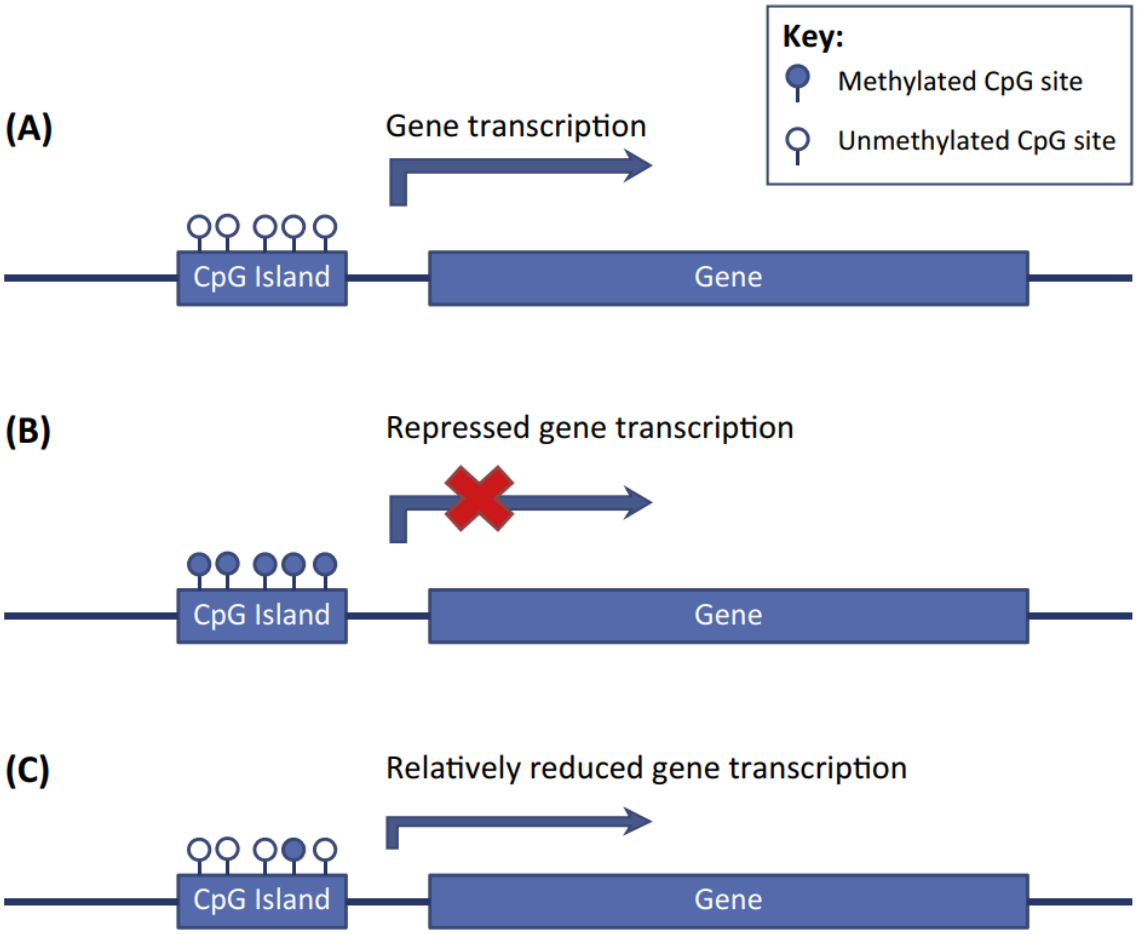
- 在复制过程中，DNMT1催化产生问题，导致DNA甲基化不能被维持

DNA甲基化功能：调控表达

启动子区域无DNA甲基化，基因通常表达

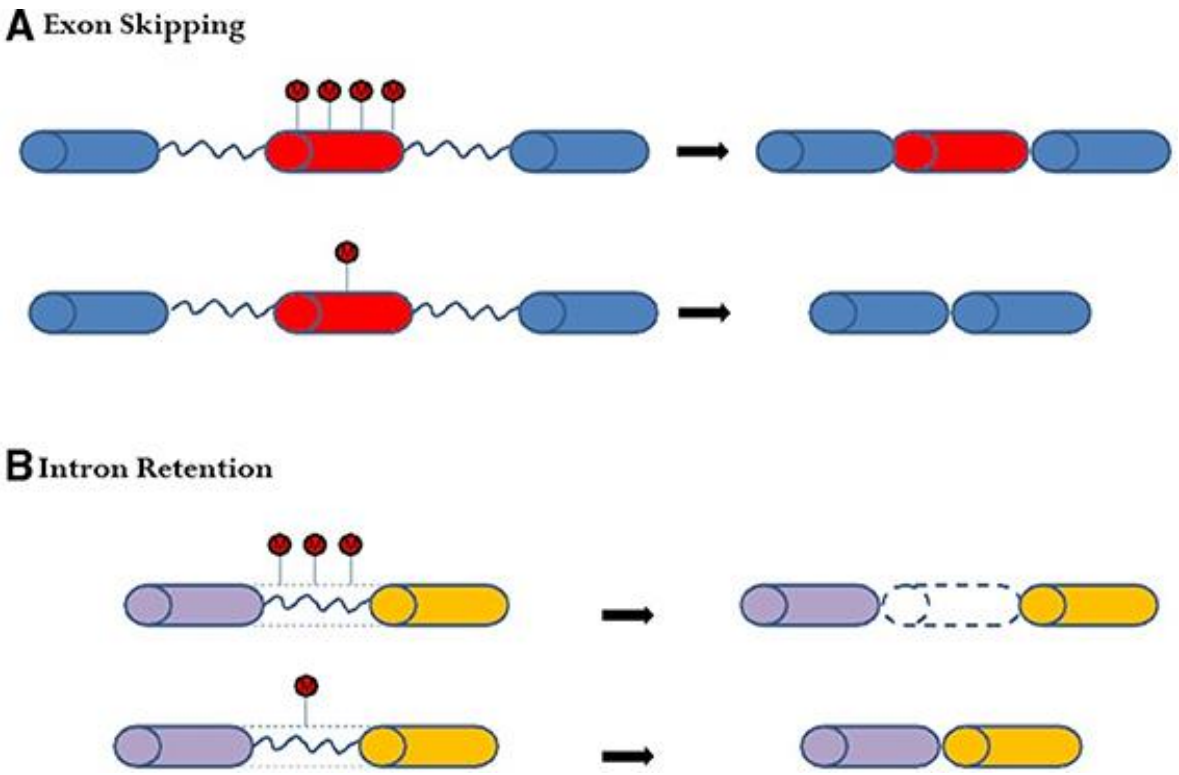
启动子出现 DNA 甲基化，基因通常沉默

启动子部分 DNA 甲基化，基因表达减弱



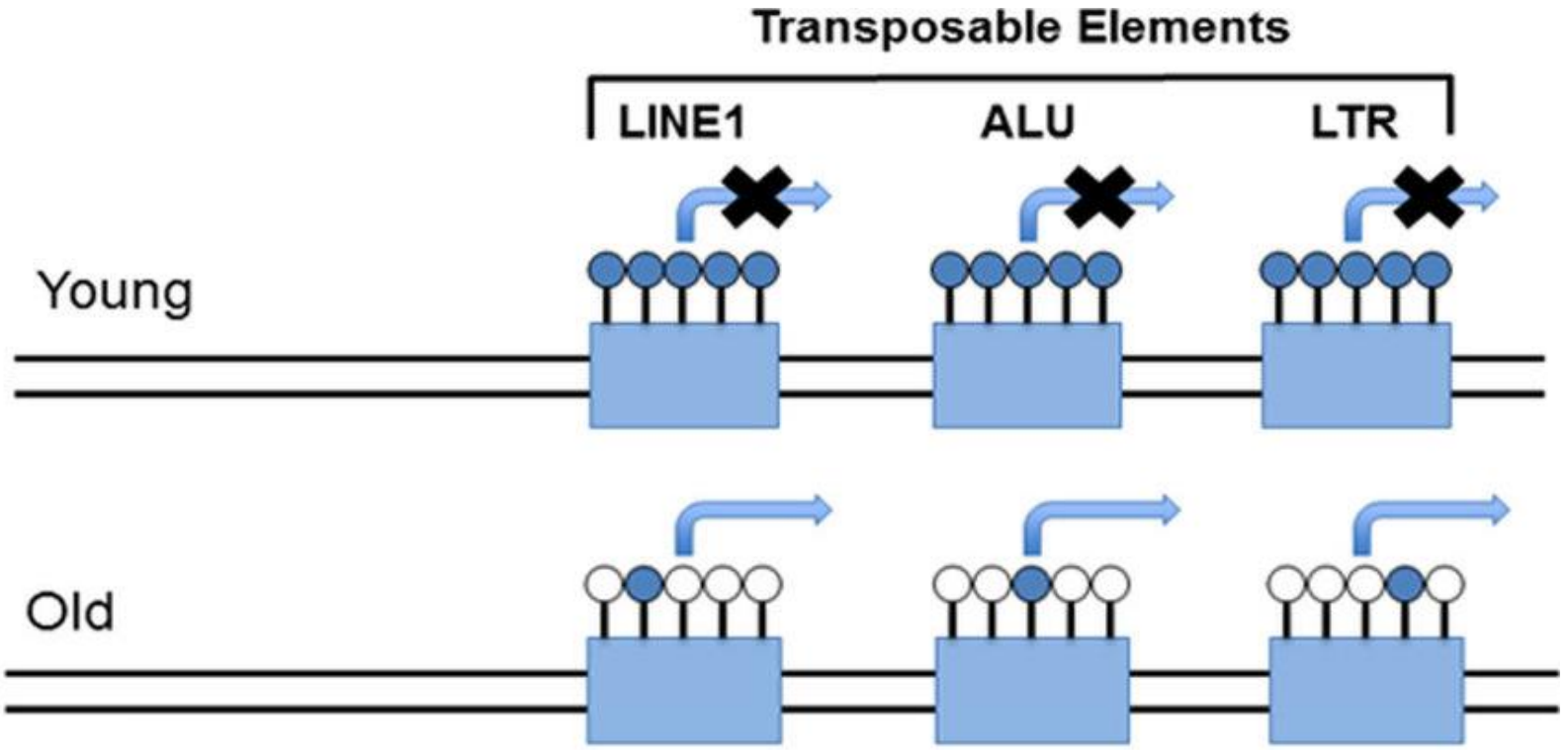
在大多数情况下启动子区域的甲基化与表达呈负相关

DNA甲基化功能：调控剪切



DNA甲基化调控剪切的机制还未明确，不同基因甲基化对剪切的影响也不同

DNA甲基化功能：抑制转座

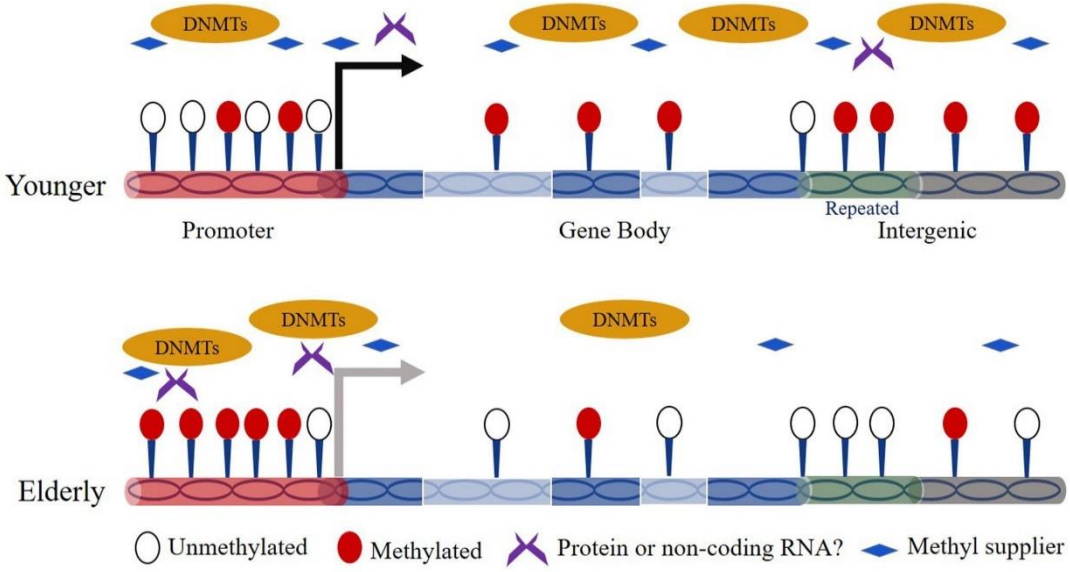


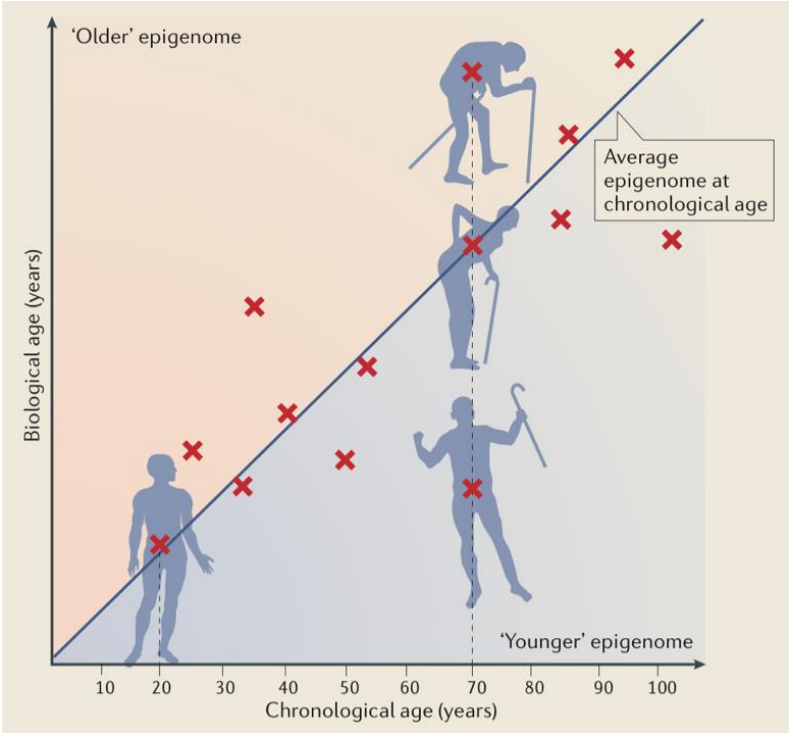
DNA甲基化能抑制新转座元件的转座，防止其对物种产生有害效应

DNA甲基化：衰老（Aging）

An epigenetic clock is a biochemical test based on DNA methylation levels that can be used to measure age (DNAmage).

- 利用DNA甲基化能够精准地预测实际年龄，总体误差小于三年
- 运动、良好生活习惯等因素能降低表观年龄
- 很多疾病患者，表观年龄大于实际年龄

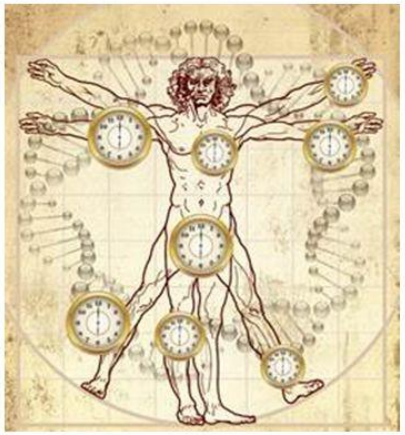




Clock Man

https://horvath.genetics.ucla.edu/html/dnamage/

DNA methylation age of human tissues and cell types.
Genome Biol. 2013 14(10):R115 PMID: 24138928

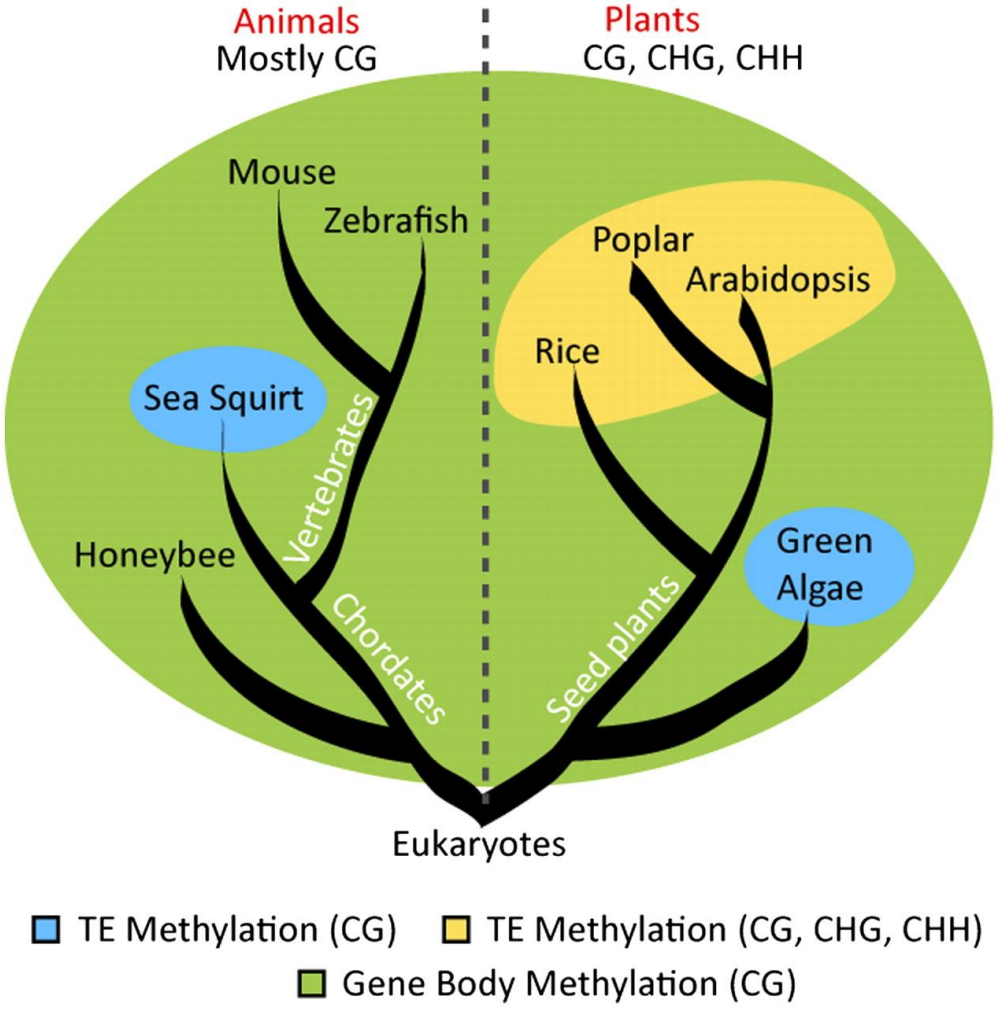


Citations: >4500 (as of May 2023)

The clock is defined as an age estimation method based on 353 epigenetic markers on the DNA. The 353 markers measure DNA methylation of CpG dinucleotides.

DNA methylation patterns in eukaryotes

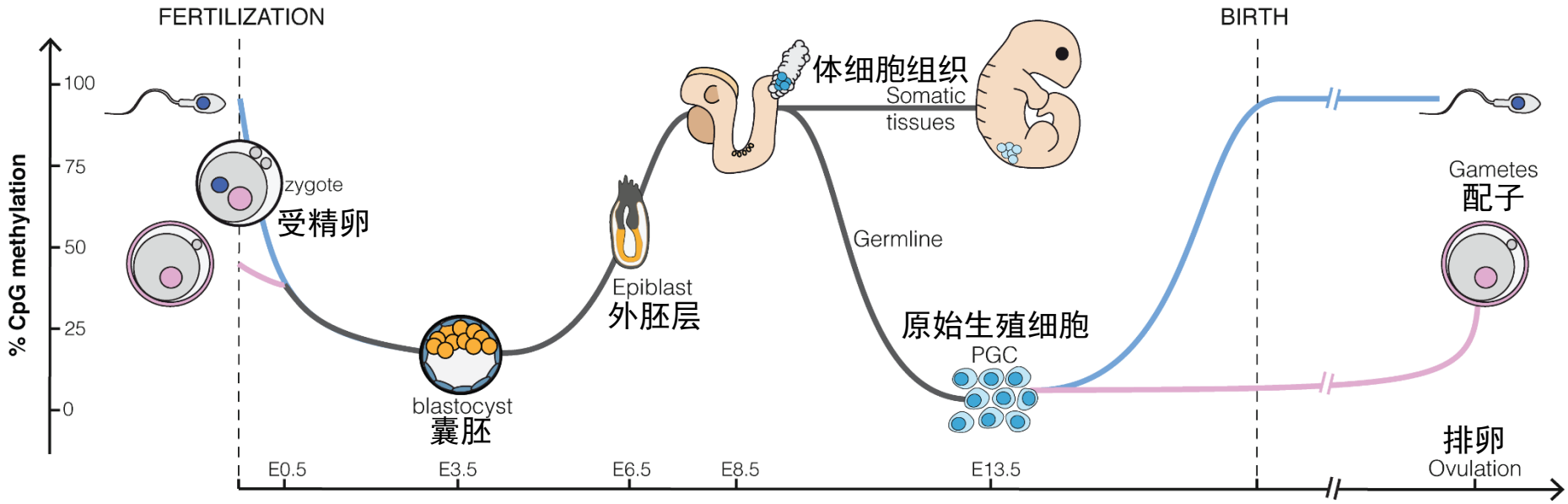
		CG	CHG	CHH
Metazoa	*Mus musculus* (mouse)	74.2	0.30	0.29
	Homo sapiens (human)	82.7	ND	ND
	Tetraodon nigroviridis (puffer fish)	65.5	0.25	0.34
	Danio rerio (zebrafish)	80.3	1.22	0.91
	Ciona intestinalis (sea squirt)	31.1	0.17	0.12
	Apis mellifera (honey bee)	0.51	0.11	0.16
	Drosophila melanogaster (fruit fly)	0.12	0.11	0.11
	Bombyx mori (silk moth)	0.71	0.08	0.09
Fungi	*Uncinocarpus reesii*	0.67	ND	ND
	Coprinopsis cinerea	12.2	ND	ND
	Phycomyces blakesleeanus	4.9	ND	ND
Eukaryota	*Chlorella* sp. NC64A	80.5	2.2	0.25
	Chlamydomonas reinhardtii	5.4	2.6	2.5
Viridiplantae	*Beta vulgaris* (beet)	92.6	81.2	18.9
	Selaginella moellendorffii	12.5	9.0	0.92
	Vitis vinifera (grape)	46.0	20.4	1.2
	Zea mays (maize)	86.0	74.0	5.4
	Sorghum bicolor (sorghum)	52.5	27.1	2.1
	Setaria viridis	44.5	23.3	1.6
	Oryza sativa (rice)	58.4	31.0	5.5
	Solanum tuberosum (potato)	70.9	42.2	15.8
	Solanum lycopersicum (tomato)	84.1	54.8	8.4
	Phaseolus vulgaris (green bean)	49.8	33.4	4.0
	Glycine max (soybean)	63.2	38.4	4.1
	Eutrema salsugineum	38.2	9.3	6.1
	Brassica oleracea (wild cabbage)	52.6	22.0	5.1
	Arabidopsis lyrata	41.0	21.0	6.5
	Arabidopsis thaliana	30.5	10.0	3.9
	Manihot esculenta (cassava)	51.5	30.4	1.9
	Populus trichocarpa (California poplar)	44.0	26.8	5.0
	Cucumis sativus (cucumber)	45.9	16.5	4.1
	Gossypium raimondii	72.0	57.8	13.2
	Theobroma cacao (cacao tree)	44.1	15.2	1.5
	Fragaria vesca (woodland strawberry)	48.4	20.6	2.3
	Prunus persica (peach)	50.2	19.6	3.7
	Malus x domestica Borkh. (apple)	63.5	44.1	4.6
	Cannabis sativa (cannabis)	75.6	65.0	8.7
	Physcomitrella patens	29.5	29.7	23.2
	Citrus × clementina (clementine)	45.8	25.1	8.3



Dynamic DNA Methylation in Plant Growth and Development. *Int J Mol Sci*. 2018

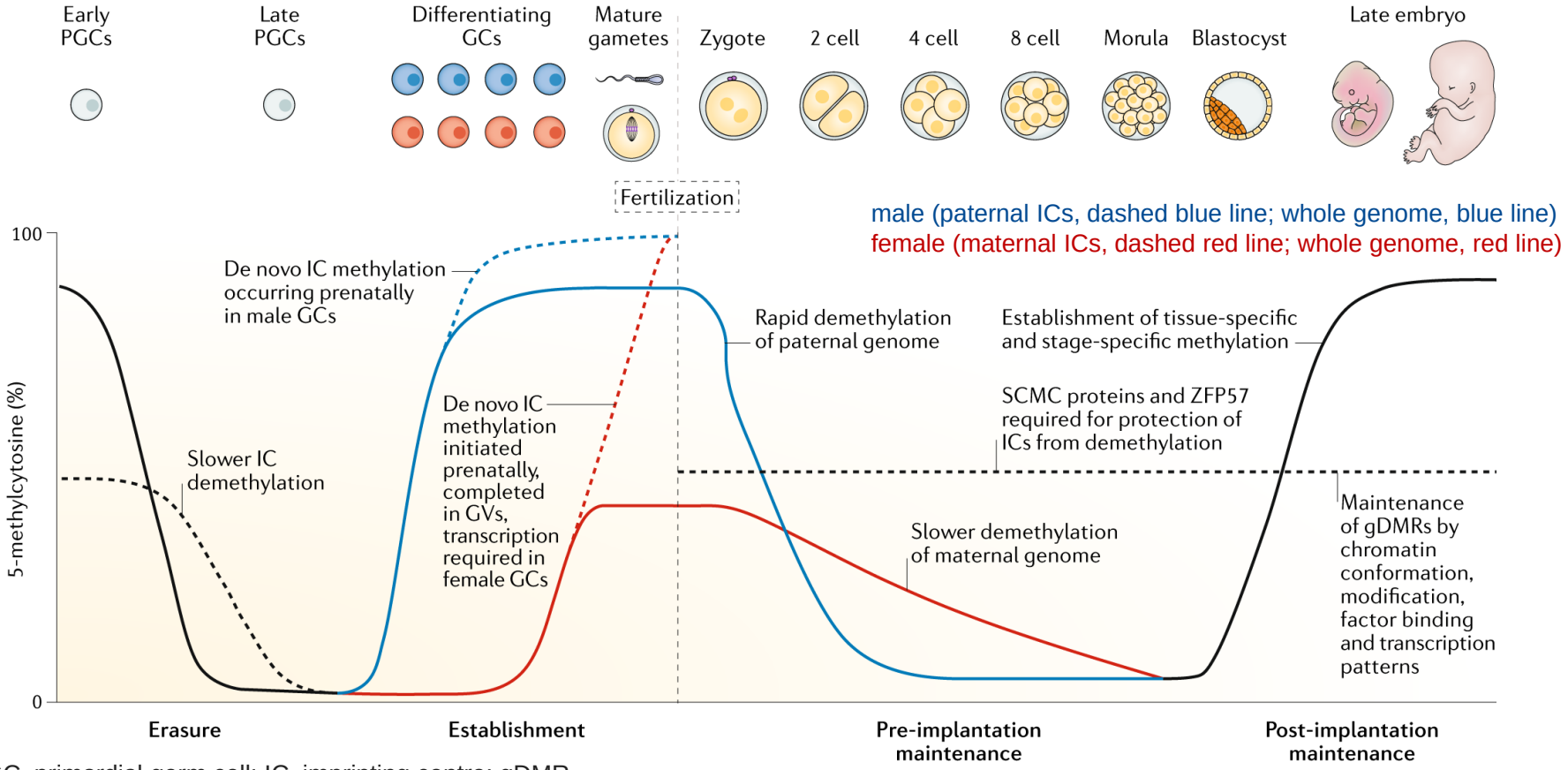
Conservation and divergence in eukaryotic DNA methylation. *PNAS* 2010

DNA methylation reprogramming during mouse development



E3.5-E6, etc., refer to days after fertilization
PGC: primordial germ cells

DNA methylation reprogramming during human development



PGC, primordial germ cell; IC, imprinting centre; gDMR, germline differentially methylated region; GV, germinal vesicle; SCMC, subcortical maternal complex

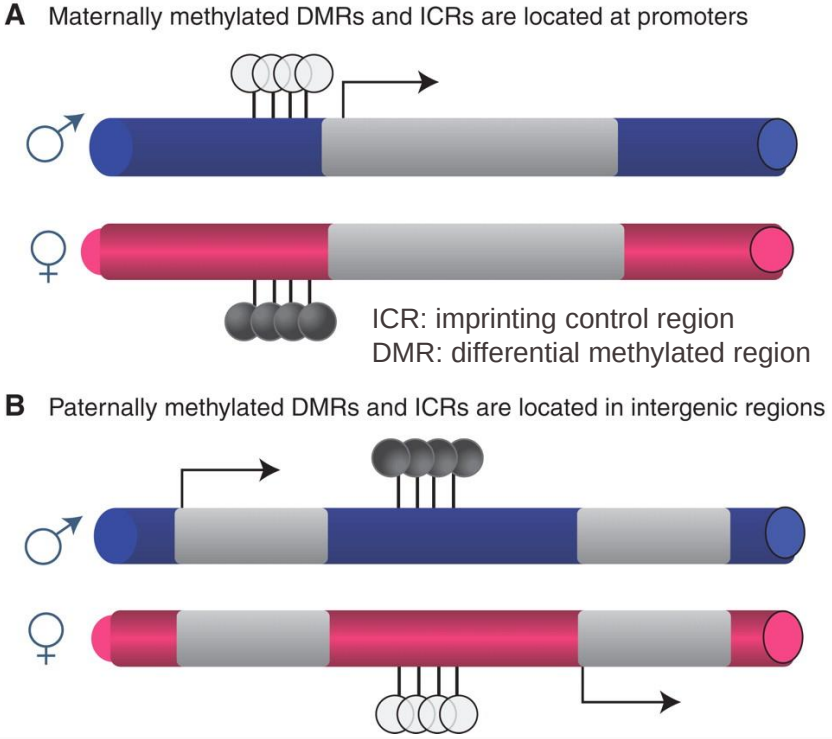
Genomic imprinting disorders: lessons on how genome, epigenome and environment interact. *Nature Reviews Genetics* 2019

DNA甲基化：基因组印记

基因组印记：基因表达具有亲本特异性的表观遗传现象

- **Genomic imprinting:** is an epigenetic phenomenon that causes genes to be expressed in a parent-of-origin-specific manner. It has been found in fungi, plants and animals.
- **Imprinted genes**: A small portion of genes are imprinted, meaning that gene expression occurs from only one allele. In contrast, for the vast majority of genes, expression occurs from both alleles simultaneously.
- **Independent of the classical Mendelian inheritance:** it represents a form of epigenetic regulation of gene expression in which one allele of a gene is preferentially expressed according to the parent-of-origin of the allele.

- The variable expression of a gene depending on whether it is of paternal or maternal origin, is a function of the DNA methylation pattern.
- Imprinted regions are observed to be more methylated and less transcriptionally active.



Genomic imprinting in biology & disease

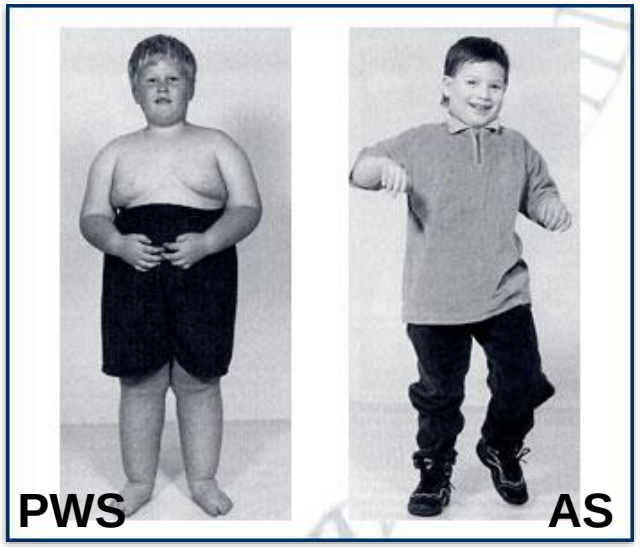
- Genomic imprinting results in monoallelic expression through gain or loss of DNA methylation.
- **Developmental physiology**: Imprinted genes have major effects on development, growth and survival, as well as on adult phenotypes.
- **Brain & behavior**: Many imprinted genes are expressed in the brain and affect diverse aspects of behaviour from birth onwards, from infant feeding to sleep and adult social behaviour.
- **Human diseases**: Abnormal expression of imprinted genes is a contributory factor in human diseases, such as intrauterine growth restriction, obesity, psychiatric disorders and cancer.
  - **Prader-Willi syndrome**, associated with poor muscle tone and constant hunger, **leading to obesity**.
  - **Angelman syndrome**, which **leads to intellectual disability, as well as motion difficulties**.

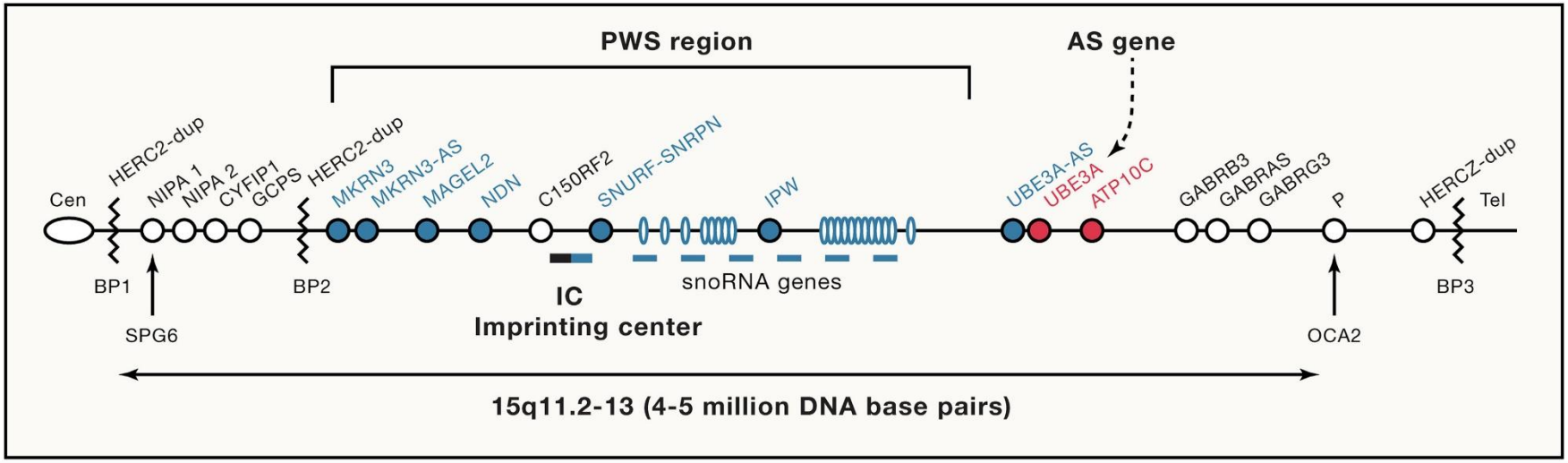
The role of genomic imprinting in biology and disease: an expanding view. *Nature Reviews Genetics* 2014

Imprinted genetic disorders

Both Prader-Willi syndrome (PWS) and Angelman syndrome (AS) are associated with **loss of the chromosomal region 15q11-13**, which contains the **paternally and maternally expressed genes**.

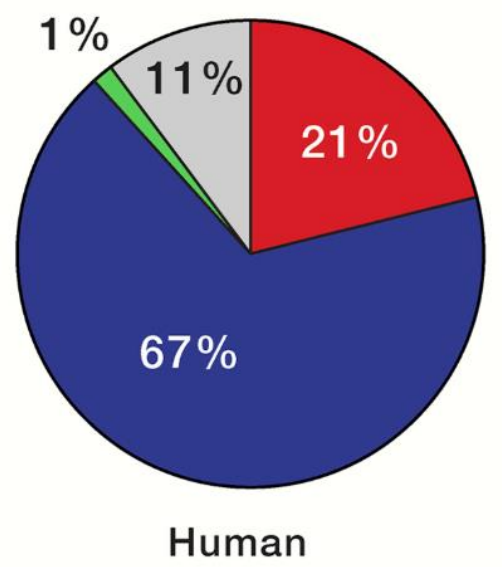
- Paternal inheritance: PWS
- Maternal inheritance: AS



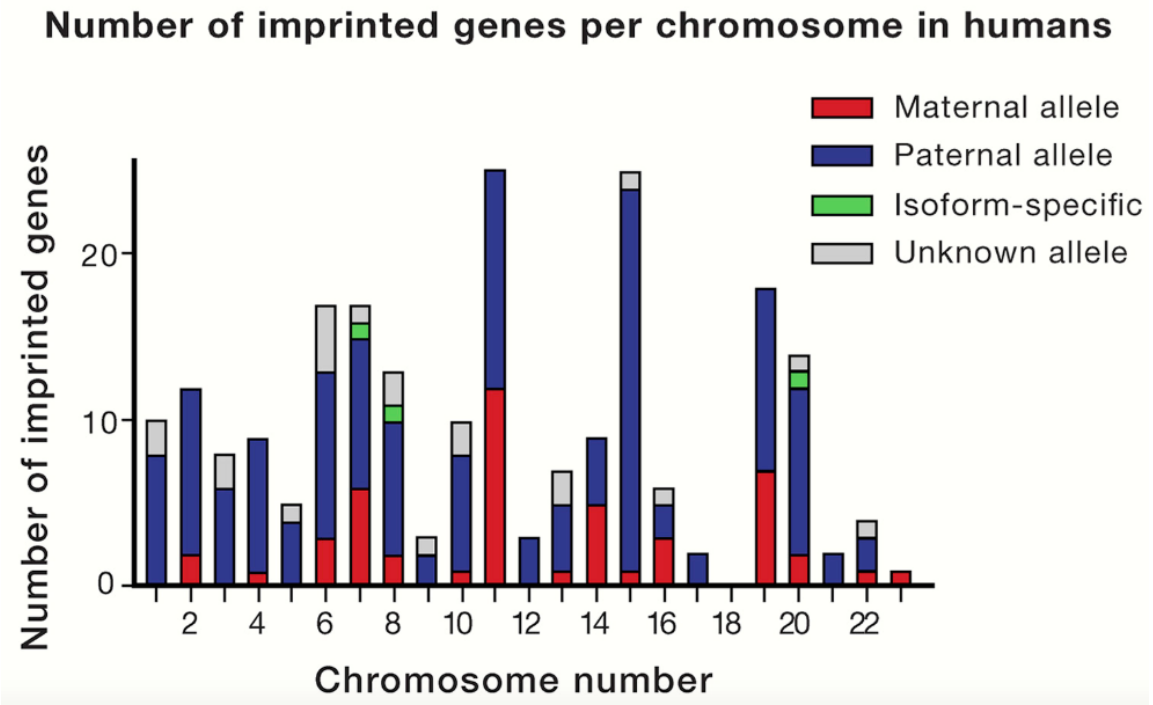


Imprinted genes in human

- Genomic imprinting plays a role in the nervous system, and some imprinted genes show imprinted expression in the brain.
- Imprinted genes reside mostly, but not exclusively, in clusters throughout the mammalian genome.
- Imprinted genes associate with circadian machinery.



Genomic Imprinting and Physiological Processes in Mammals. *Cell* 2019

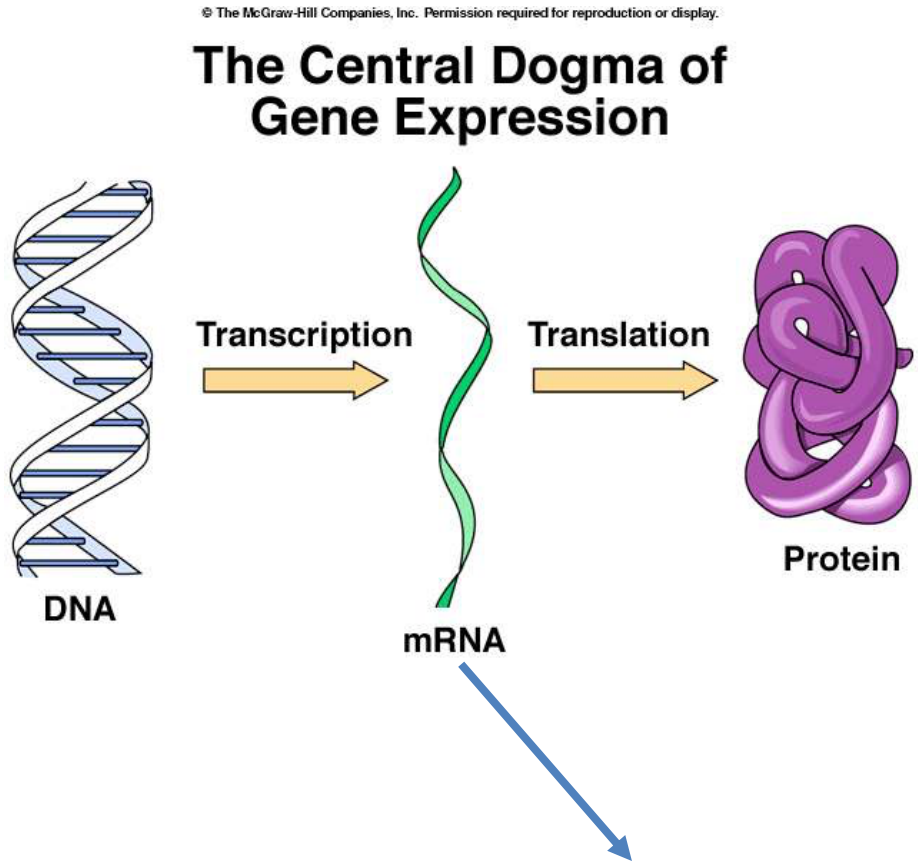


RNA修饰

开拓表观转录组学的新大陆

Epitranscriptomics: RNA revisited

By Charlotte Schubert | May. 17, 2019 , 2:00 PM



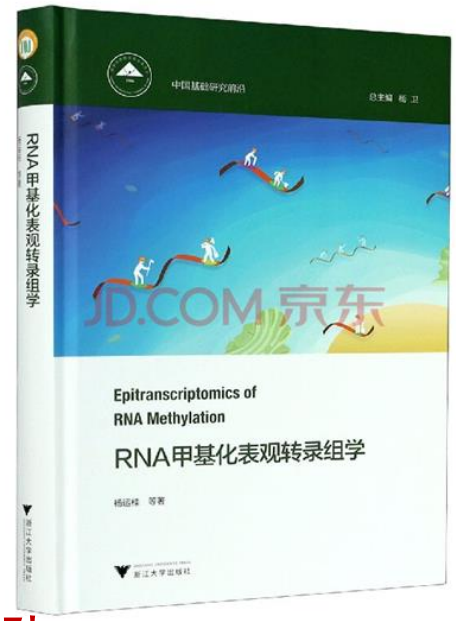
1492年哥伦布发现美洲新大陆

Chuan He-何川
University of Chicago

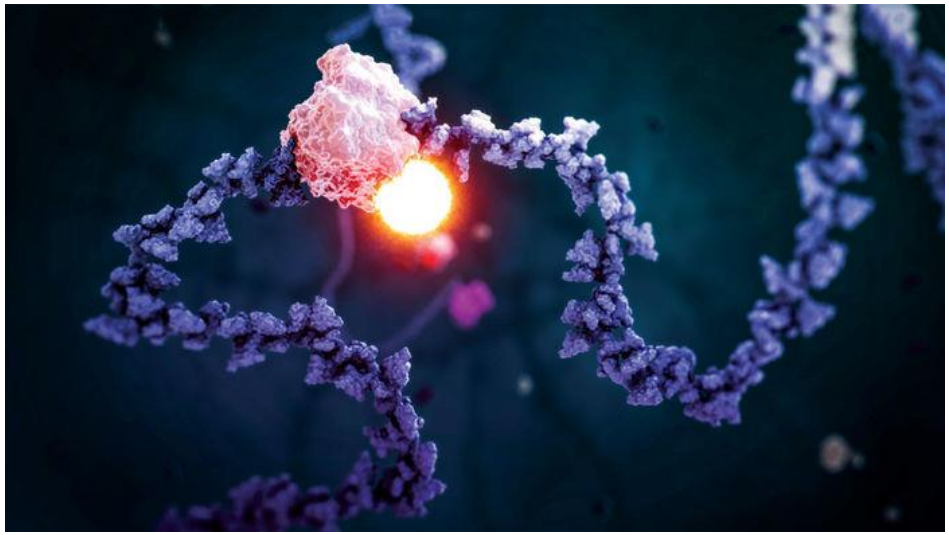
表观转录组学

表观转录组学 (Epitranscriptomics)

- 研究转录本上表观修饰机制和功能的新领域
- RNA上170多种化学修饰，个体发育与疾病
- 早在1970s就被发现，但功能研究相对滞后



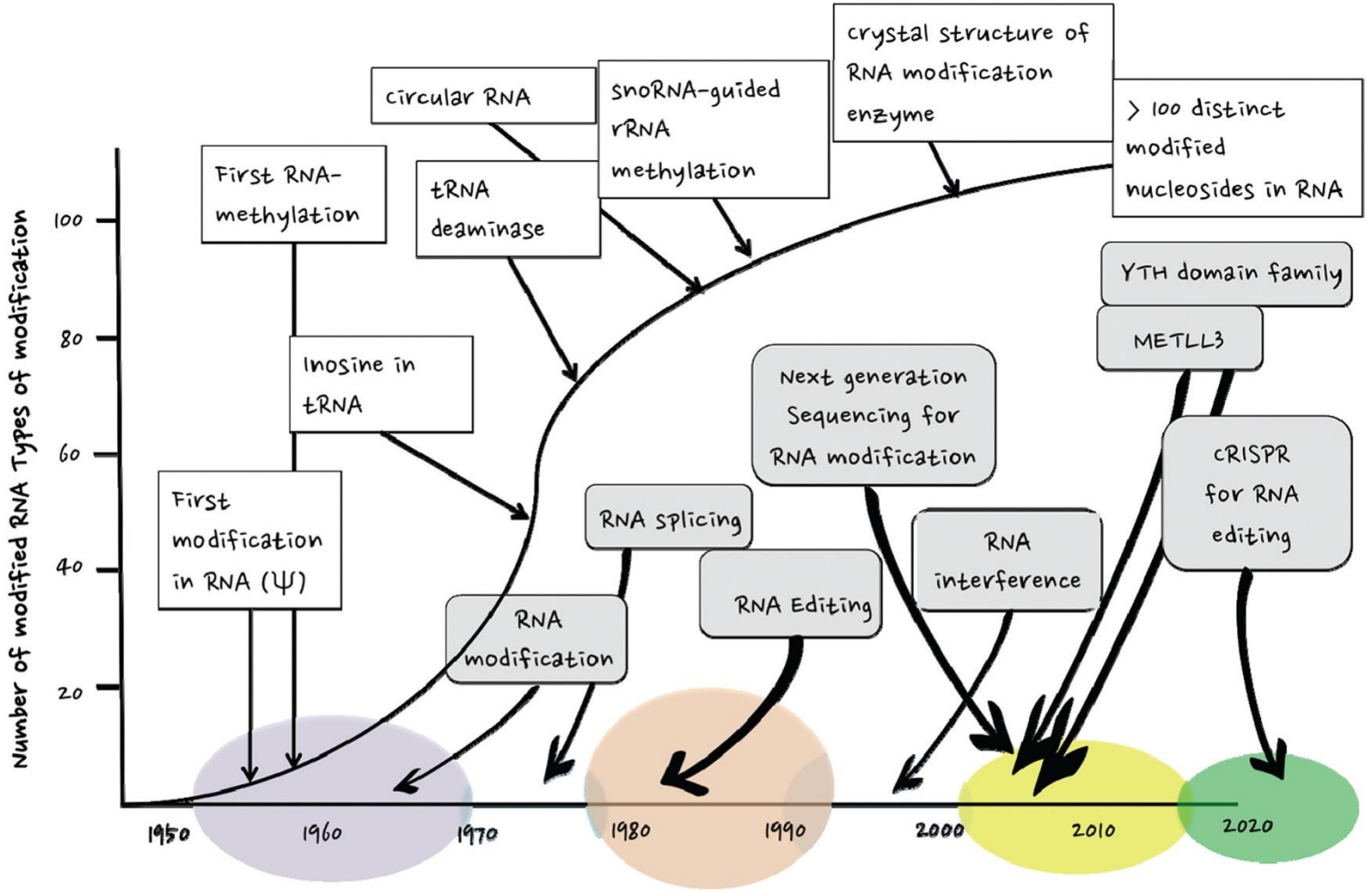
基因组所
杨运桂团队



一个核酶(粉色)正在将一个修饰基团(金色)
转移到mRNA (蓝色)上

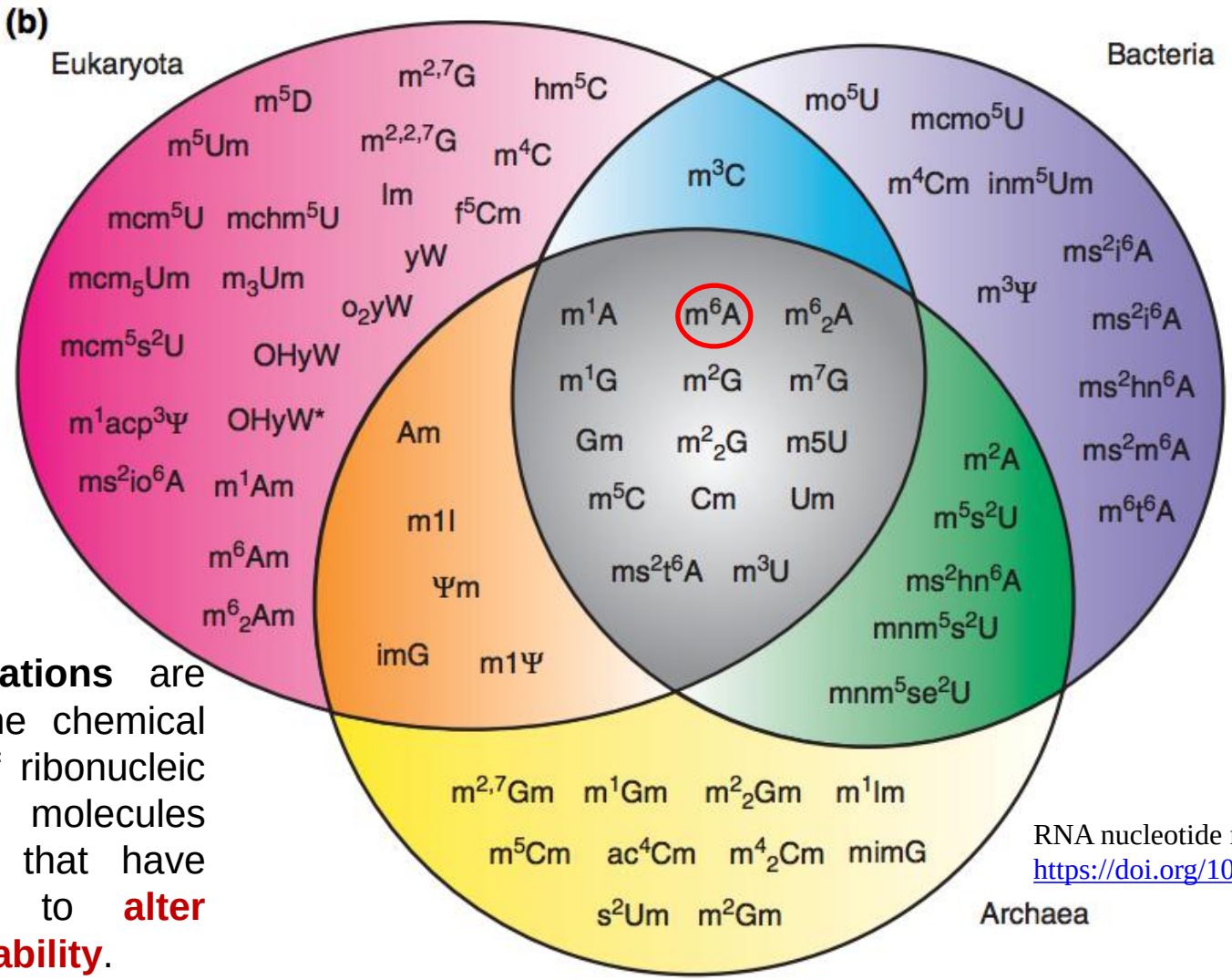
RNA modifications modulate gene expression during development. *Science* 2018

Timeline of RNA modification discoveries



Role of RNA modifications in brain and behavior
https://doi.org/10.1111/gbb.12444

Diversity of RNA modifications

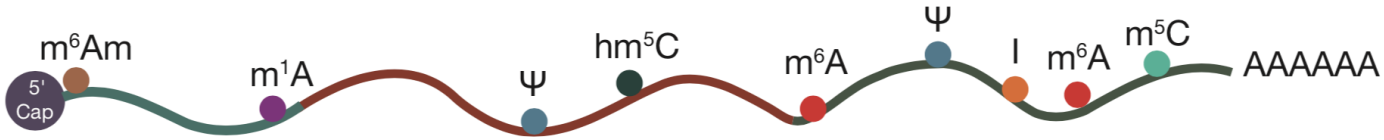


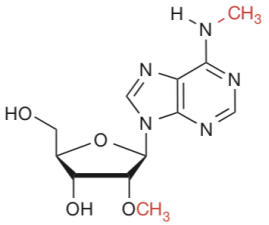
RNA modifications are changes to the chemical composition of ribonucleic acid (RNA) molecules post-synthesis that have the potential to **alter function or stability**.

RNA nucleotide methylation. 2011
https://doi.org/10.1002/wrna.79

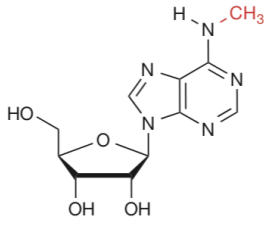
mRNA上有多种化学修饰

- 主要有7种化学修饰，它们具有不同的分布偏好性。

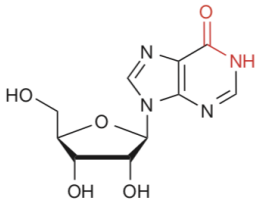




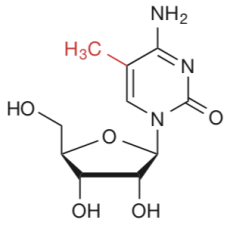
*N*6,2'-O-dimethyladenosine (m6Am)



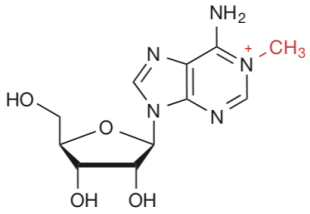
*N*6-methyladenosine (m6A)



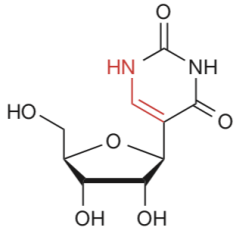
Inosine (I)



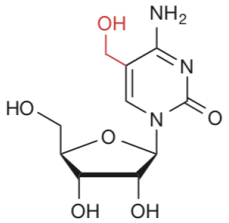
5-methylcytidine (m5C)



*N*1-methyladenosine (m1A)



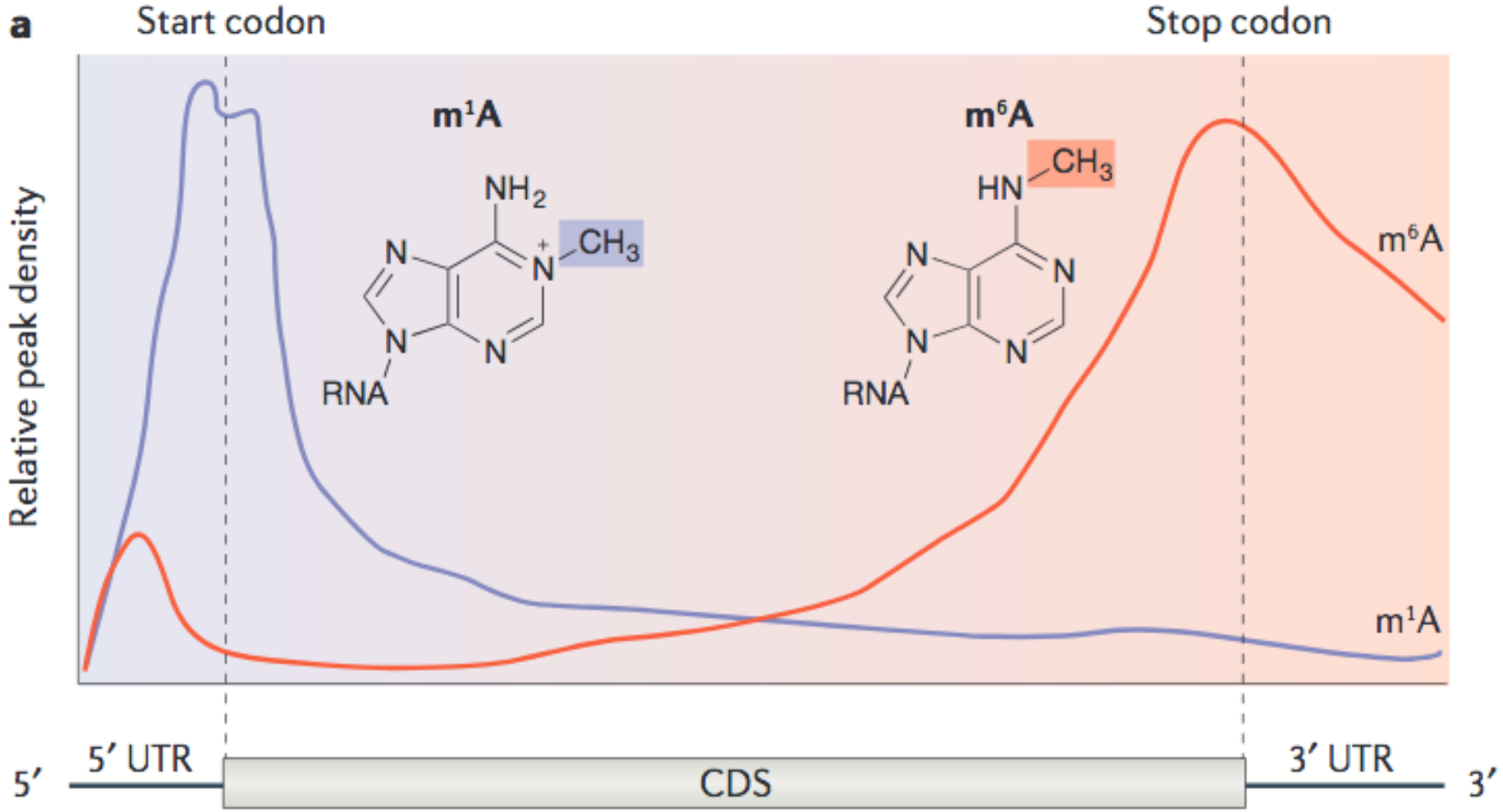
Pseudouridine (Ψ)



5-hydroxylmethylcytidine (hm5C)

Epitranscriptome sequencing technologies: decoding RNA modifications. *Nature Methods* 2017

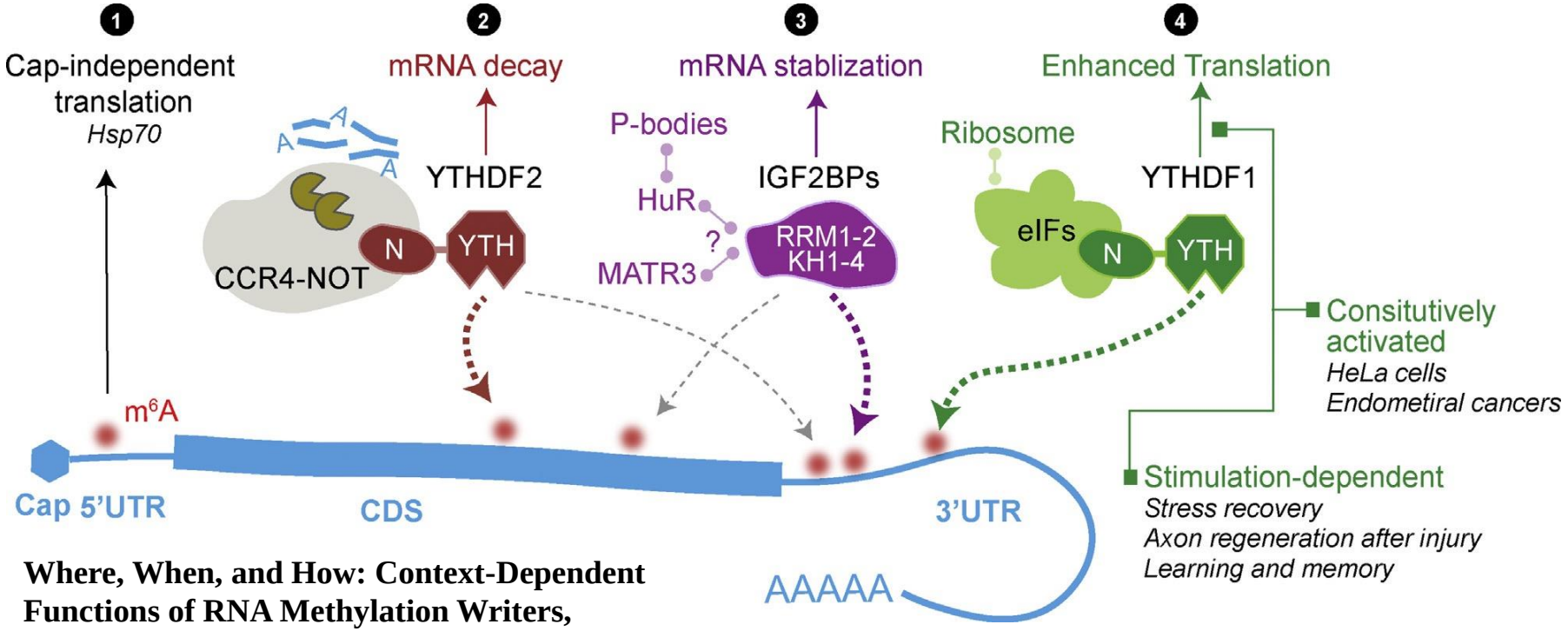
mRNA modifications: m^{1}A and m^{6}A



Post-transcriptional gene regulation by mRNA modifications. *Nature Reviews Molecular Cell Biology* 2016

mRNA修饰参与基因表达调控

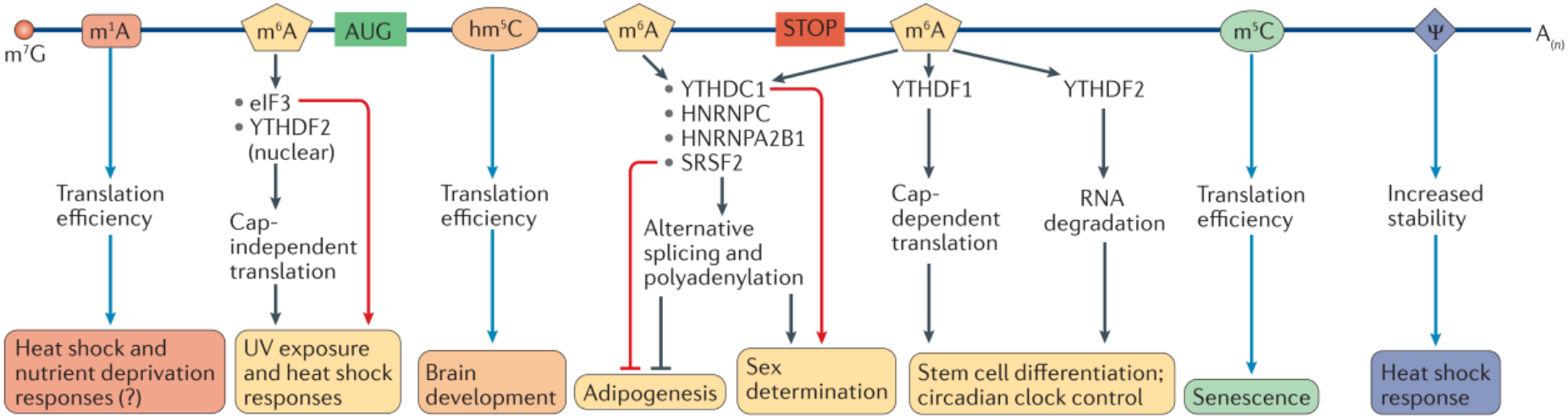
- 以m6A为例，其在识别蛋白的作用下会影响mRNA的稳定性、降解速率、翻译效率等。

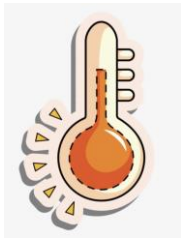
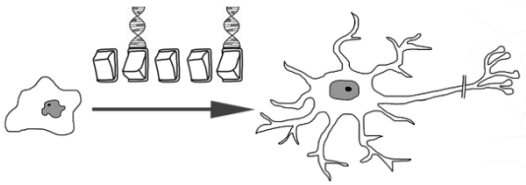
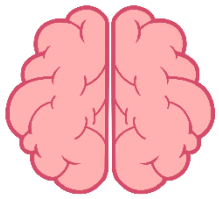


Where, When, and How: Context-Dependent Functions of RNA Methylation Writers, Readers, and Erasers. *Molecular Cell* 2019

mRNA修饰能够影响重要生物学进程

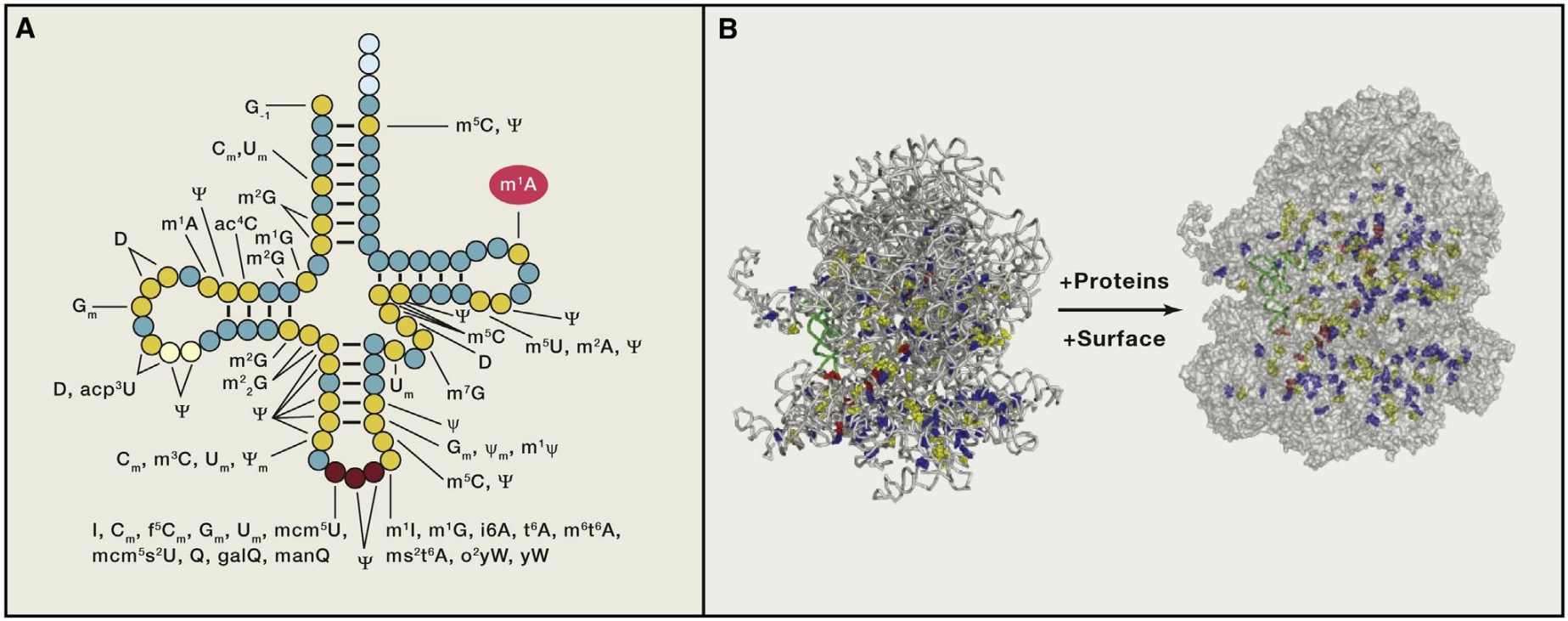
- 功能很重要，但角色尚未可知
- 热应激反应、脑发育、脂肪形成、生物钟、衰老等



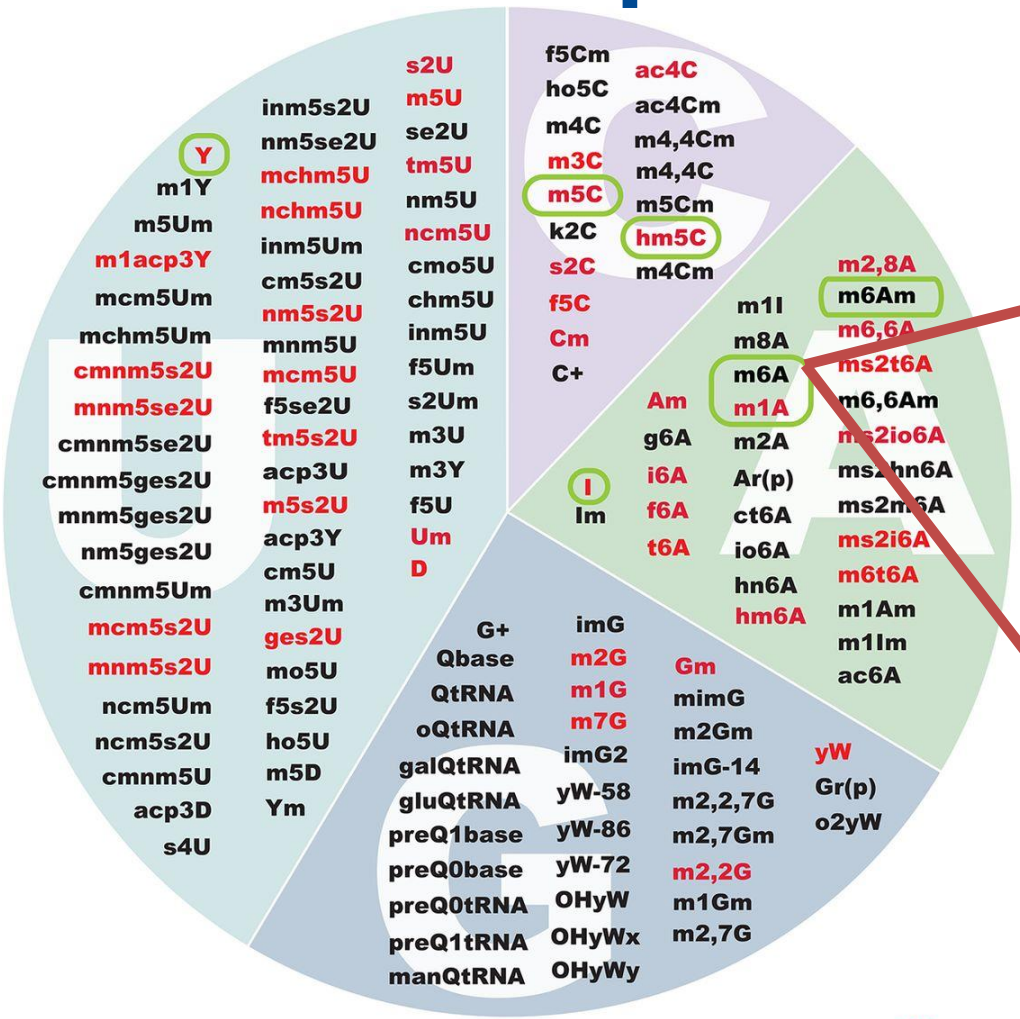





ncRNA修饰也有重要的生物学功能

- tRNA、rRNA、cirRNA、miRNA、lncRNA……



The landscape of RNA modifications



The RNA modification landscape in human disease. *RNA* 2017

Disease related

Detection technique available

Trends in Cell Biology

Volume 29, Issue 6, June 2019, Pages 487-499

CellPress

Review

Regulation of Gene Expression by N^6-methyladenosine in Cancer

Jun Liu [1, 2, 4], Bryan T. Harada [1, 2, 4], Chuan He [1, 2, 3]

[1] Department of Chemistry and Institute for Biophysical Dynamics, The University of Chicago, Chicago, IL 60637, USA

[2] Howard Hughes Medical Institute, Chicago, IL 60637, USA

[3] Department of Biochemistry and Molecular Biology, The University of Chicago, Chicago, IL 60637, USA

Review Article | Published: 16 March 2019

Importance of m N^6-methyladenosine (m^6A) RNA modification in cancer

Gulten Tuncel & Rasime Kalkan

Medical Oncology **36**, Article number: 36 (2019) | Cite this article

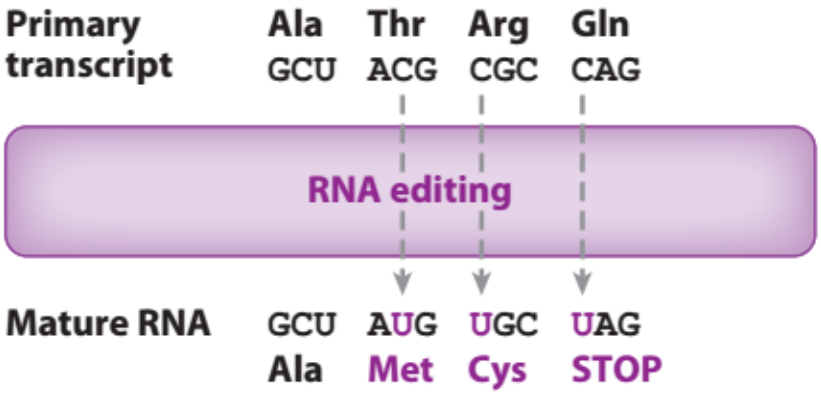
1055 Accesses | **5** Citations | Metrics

New discoveries, fast-evolving

日新月异

RNA编辑（RNA Editing）

RNA编辑是一种转录后加工过程，通过碱基替换、核苷酸插入和缺失修改RNA序列



RNA编辑在不同物种广泛存在，发生tRNA、rRNA、mRNA、miRNA等。

Class	Species	Genome Size (Mb)	RNA Editing Sites
病毒	丙型肝炎病毒	0.001677 Mb	2
细菌	大肠杆菌	5 Mb	16
真菌	河谷镰刀菌	37 Mb	26,000
	粉色面包霉菌	41 Mb	41,000
动物	线虫	100 Mb	65,543
	果蝇	138 Mb	57,065
	人类	3,000 Mb	~ 4,500,000
植物	拟南芥	0.37 Mb (MT) 0.15 Mb (Pltd)	490
	水稻	0.49 Mb (MT) 0.13 Mb (Pltd)	512

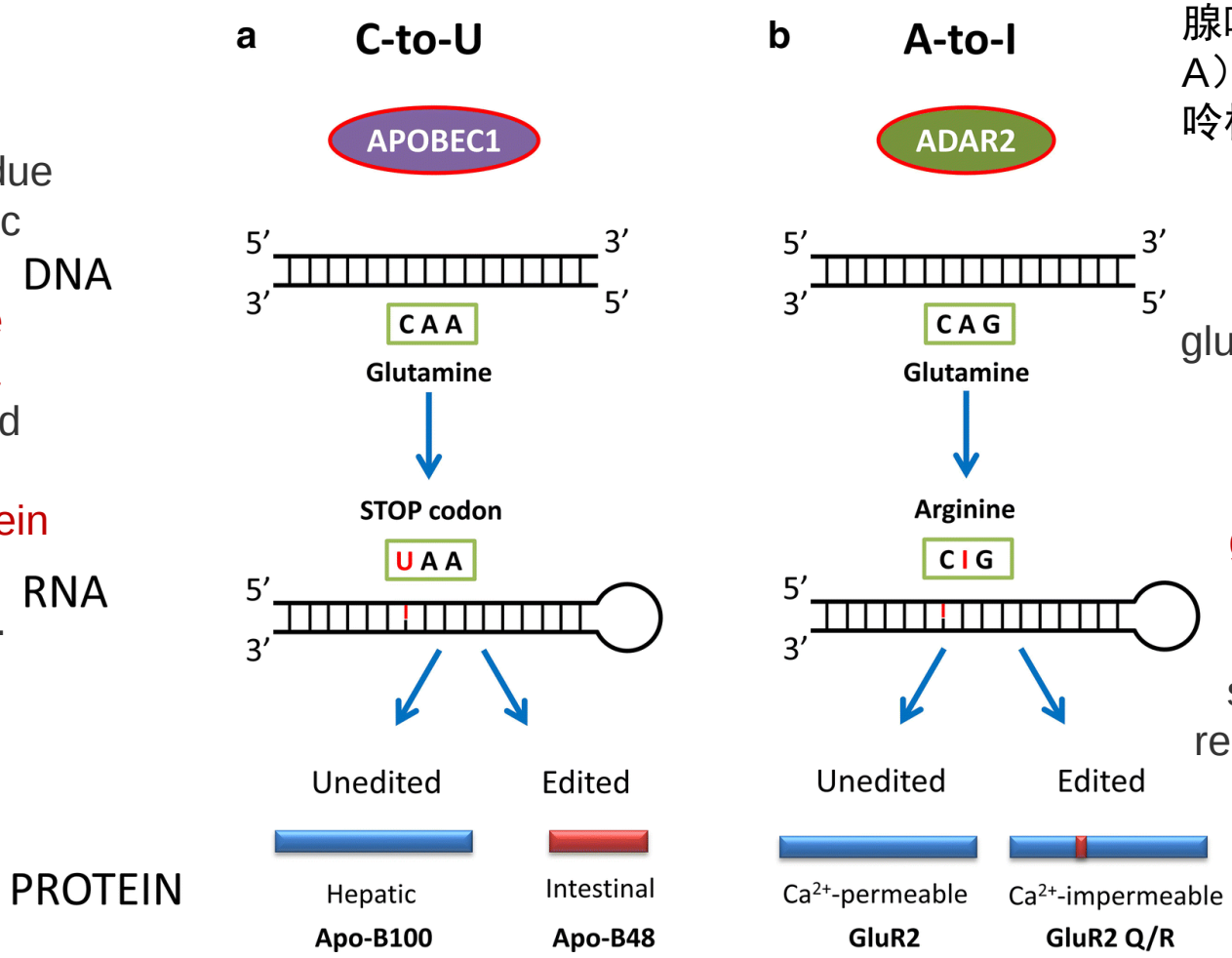
Bar-Yaacov et al., Genome Research, 2017; Liu et al., Genome Research, 2016; Picardi et al., Nucleic Acids Research, 2017; Giege & Brennicke, PNAS, 1999

https://ngdc.cncb.ac.cn/edk https://ngdc.cncb.ac.cn/ped

RNA Editing: C-to-U and A-to-I

腺嘌呤（Adenine, A）脱氨成次黄嘌呤核苷（Inosine, I）

The C-to-U editing at residue 2153 of hepatic Apo-B100 transforms the glutamate to a stop codon and produces a truncated protein Apo-B48 in intestinal cells.

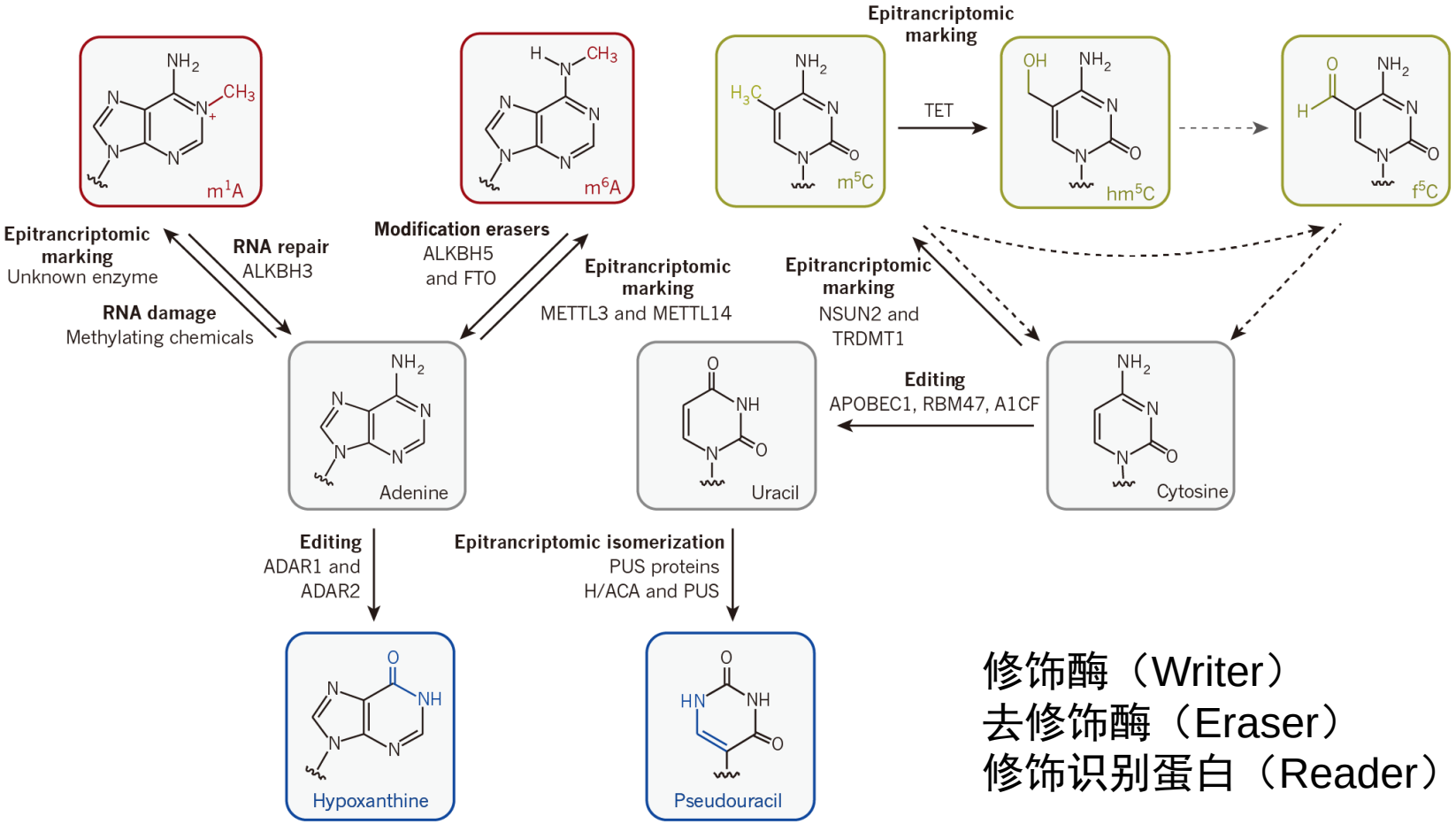


Editing of the glutamate receptor 2 (GluR2) transforms the C**A**G codon for glutamine (Q) to C**I**G for arginine (R) as (C**G**G), since ribosomes read inosine (I) as guanosine (G).

RNA editing in the forefront of epitranscriptomics and human health. *Journal of Translational Medicine* 2019

动态可逆的表观转录修饰途径

不同的修饰酶调控RNA修饰动态可逆



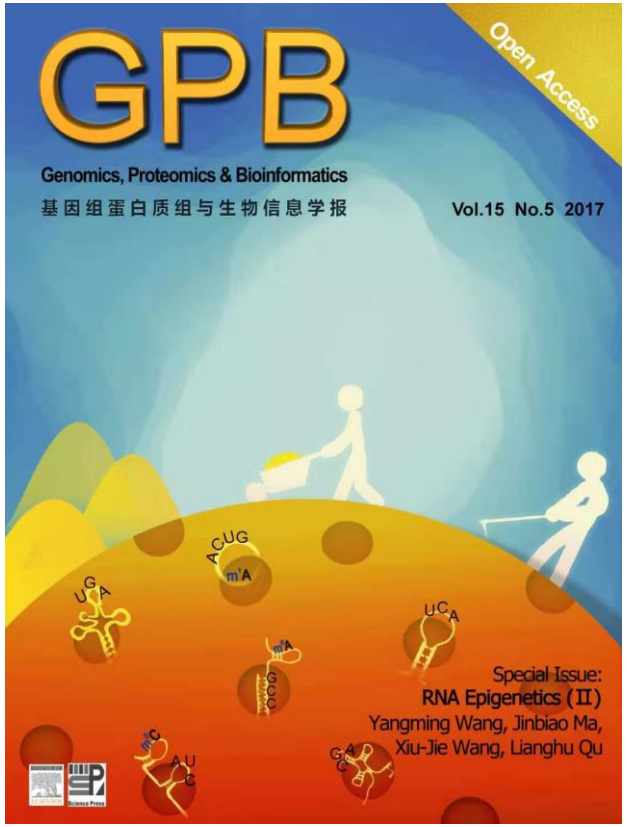
修饰酶（Writer）
去修饰酶（Eraser）
修饰识别蛋白（Reader）

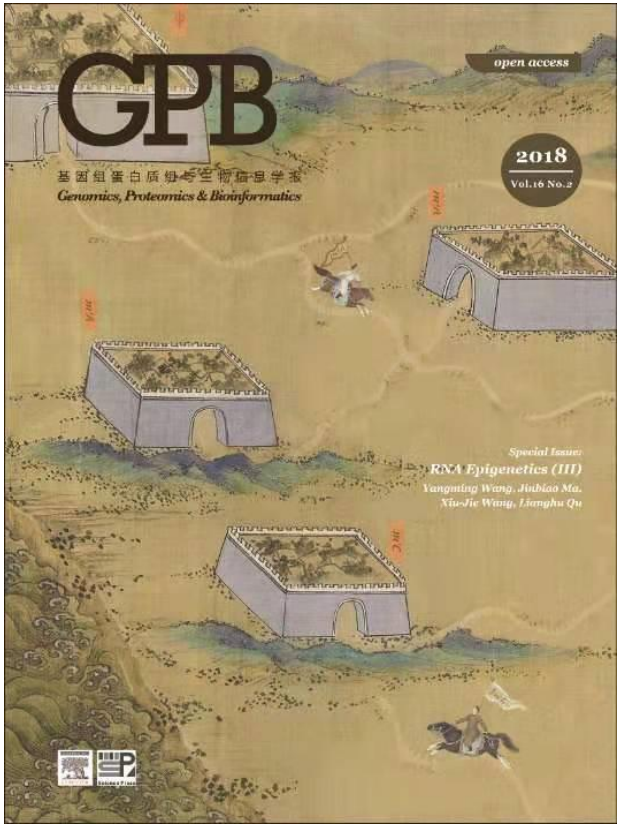
Nature Methods年度技术
——Epitranscriptome analysis

- 每年年底，Nature Methods都会对过去一年中推动生物学发展的技术方法做出回顾与总结，由此评选出当年最受瞩目、影响力最大的技术。
- 2016年，表观转录组学分析(Epitrans-criptome analysis)荣膺《自然 方法》年度生命科学技术。

GPB的RNA Epigenetics特刊

- RNA表观淘金热、RNA表观的丝路山水图





蛋白质修饰

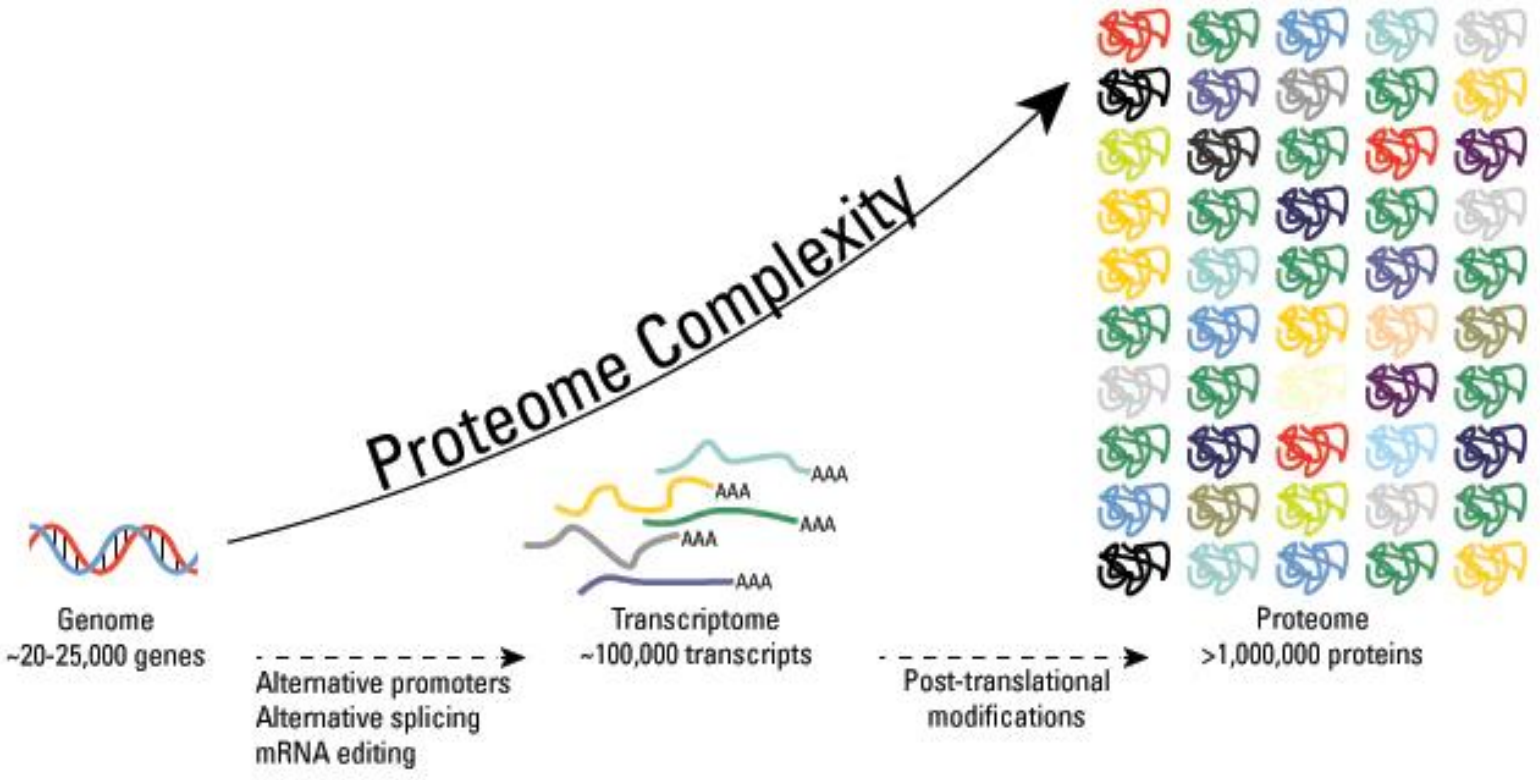
Thanks to 薛宇@HUST

翻译后修饰

翻译后修饰（Post-Translational Modification, PTM）：是共价化学反应过程，由蛋白酶水解或将修饰基团添加到一个氨基酸上。

- PTM作用：改变氨基酸的化学性质或结构， 扩阔蛋白质的功能，是细胞信号传导中的重要组成部分。
- 修饰阶段：蛋白质修饰可以发生在翻译过程中或翻译后，但大部分发生在翻译和折叠之后，因此统称为PTM。
- PTM类型：到目前为止，已发现350多种PTMs。它们通过改变大多数真核生物蛋白质的活性、定位、降解、以及与其他蛋白质的相互作用从而调节蛋白功能。
- PTM蛋白：目前已报道63,490个蛋白存在修饰现象，涉及1077种蛋白酶，被35,588篇文献报道。

PTM是增加蛋白质多样性的关键机制



人的基因组中存在20,000-25,000个基因，但据估算蛋白质的类型超过1百万。通过可变剪接与RNA编辑可大大增加转录本的类型，而丰富多样的翻译后修饰则使蛋白质的类型发生指数级增长。

翻译后修饰在不同物种中的分布

	Substrates (protein)	Substrates (proteoforms)	Sites	Enzymes	Enzyme-substrate pairs	Enzyme-substrate-site	PTM-dependent PPI	PMIDs	Variants
Homo sapiens (Human)	18596	8649	444147	520	9467	18176	1064	25261	75853
Mus musculus (Mouse)	15234	725	147394	225	1127	1967	270	5337	0
Rattus norvegicus (Rat)	7170	99	39708	100	529	953	26	2495	0
Saccharomyces cerevisiae	3949	0	34533	65	671	1495	26	1122	0
Drosophila melanogaster (Fruit fly)	852	0	3483	6	5	9	1	235	0
Caenorhabditis elegans	802	0	1994	9	11	17	0	137	0
Arabidopsis thaliana (Mouse-ear cress)	8653	343	32730	30	113	294	34	412	0
Zea mays (Maize)	100	0	137	0	0	0	0	16	0
Oryza sativa subsp. japonica (Rice)	1808	14	4376	0	0	0	0	21	0

https://research.bioinformatics.udel.edu/iptmnet/

iPTMnet v5.1
Updated on November 19, 2019

翻译后修饰类型

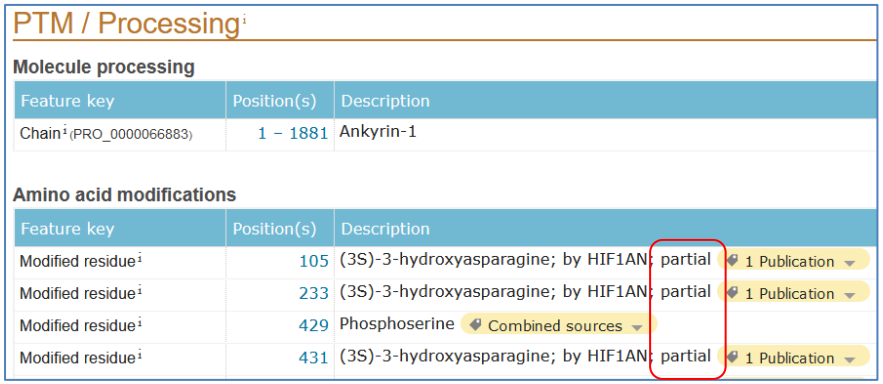
	Substrates (protein)	Substrates (proteoforms)	Sites	Enzymes	Enzyme-substrate pairs	Enzyme-substrate-site	PTM-dependent PPI	PMIDs	Variants
Phosphorylation 磷酸化	57204	10799	507157	1052	11967	23042	737	31576	50881
Acetylation 乙酰化	17416	1023	52702	19	135	258	0	1210	4249
Ubiquitination 泛素化	21479	917	132816	1	2	4	0	274	14601
Methylation 甲基化	7570	255	19521	4	4	7	0	381	6216
N-Glycosylation N-糖基化	2496	30	6022	0	0	0	0	1591	401
O-Glycosylation	1423	17	4927	1	1	2	0	304	670
C-Glycosylation	20	1	197	0	0	0	0	17	20
S-Glycosylation	5	0	5	0	0	0	0	6	0
Sumoylation 类泛素化	2712	118	8357	0	2	2	0	19	1251
Myristoylation 肉豆蔻酰化	284	20	296	0	0	0	0	202	16
S-Nitrosylation S-亚硝化	2921	0	5803	0	0	0	0	150	397

https://research.bioinformatics.udel.edu/iptmnet/

iPTMnet v5.1
Updated on November 19, 2019

翻译后修饰类型

- 常见的修饰包括：磷酸化、甲基化、乙酰化、酰胺化
- 根据蛋白质分子是否全部被修饰、同一个氨基酸的修饰种类、修饰的稳定性，制定了以下分类和描述。
  - 部分修饰（ partial ）：细胞中某一蛋白的部分分子被修饰
  - 可变修饰（alternate）：同一个氨基酸位点含有多种修饰类型
  - 瞬时修饰（transient）：不稳定的修饰状态



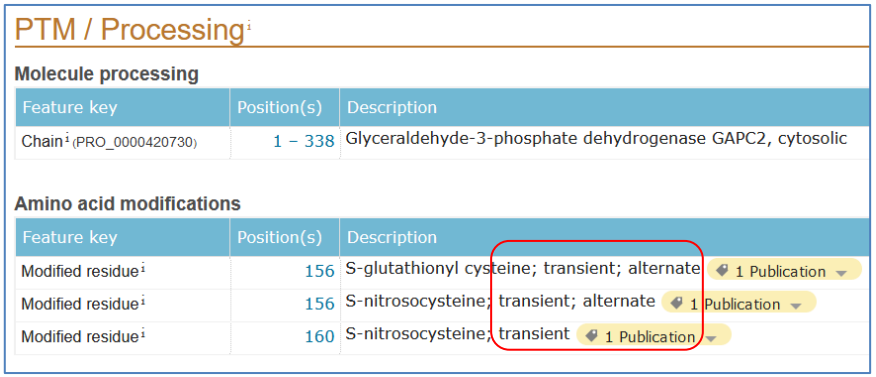
PTM / Processing

Molecule processing

Feature key	Position(s)	Description
Chain (PRO_0000066883)	1 – 1881	Ankyrin-1

Amino acid modifications

Feature key	Position(s)	Description
Modified residue	105	(3S)-3-hydroxyasparagine; by HIF1AN; partial — 1 Publication
Modified residue	233	(3S)-3-hydroxyasparagine; by HIF1AN; partial — 1 Publication
Modified residue	429	Phosphoserine — Combined sources
Modified residue	431	(3S)-3-hydroxyasparagine; by HIF1AN; partial — 1 Publication



PTM / Processing

Molecule processing

Feature key	Position(s)	Description
Chain (PRO_0000420730)	1 – 338	Glyceraldehyde-3-phosphate dehydrogenase GAPC2, cytosolic

Amino acid modifications

Feature key	Position(s)	Description
Modified residue	156	S-glutathionyl cysteine; transient; alternate — 1 Publication
Modified residue	156	S-nitrosocysteine; transient; alternate — 1 Publication
Modified residue	160	S-nitrosocysteine; transient — 1 Publication

翻译后修饰的审编与可视化

磷酸化修饰（Phosphorylation）

- 磷酸化修饰：是指γ-磷酸基团转移到氨基酸上的过程，它是真核细胞和原核细胞信号转导的关键机制。
- 磷酸化的氨基酸残基包括：
  - 丝氨酸、苏氨酸、酪氨酸的羟基
  - 精氨酸、组氨酸、赖氨酸的氮
  - 天冬氨酸的羧基
  - 半胱氨酸的巯基
- 磷酸化可发生：在三界物种，细胞质和细胞核内：
  - 真核生物：丝氨酸、苏氨酸和酪氨酸的磷酸化最常见，尤其是丝氨酸
  - 原核生物：组氨酸和天冬氨酸的磷酸化
- 功能：调控蛋白活性，影响多种重要生命过程，如细胞周期。
  - 细胞周期蛋白依赖性蛋白激酶CDK(cyclin-dependent kinases) 对细胞周期蛋白的丝氨酸/苏氨酸磷酸化，驱动细胞周期。
  - CDK活性也受到磷酸化和去磷酸化的影响。

磷酸化修饰

- 研究最广泛的翻译后修饰
- 可逆性：
  - Protein kinase: 磷酸化- Writer
  - Phosphotase: 去磷酸化- Eraser
  - Phospho-binding domain: 识别特定磷酸化位点- Reader

Edmond H. Fischer

Edwin G. Krebs

The Nobel Prize in Physiology or Medicine 1992

“for their discoveries concerning reversible protein phosphorylation as a biological regulatory mechanism”

甲基化修饰

- 通过多种方式将S-腺苷蛋氨酸中的甲基转移到细胞质或细胞核蛋白上。
- 在羧基上发生的甲基化反应是可逆的，可以调节目标蛋白的活性，而在N末端和侧链上的氮原子上发生的甲基化通常是不可逆的。
  - 羧基甲基化
  - 氮甲基化
  - 组氨酸甲基化
  - 赖氨酸甲基化
  - 精氨酸甲基化
  - 其他罕见的侧链甲基化

乙酰化修饰

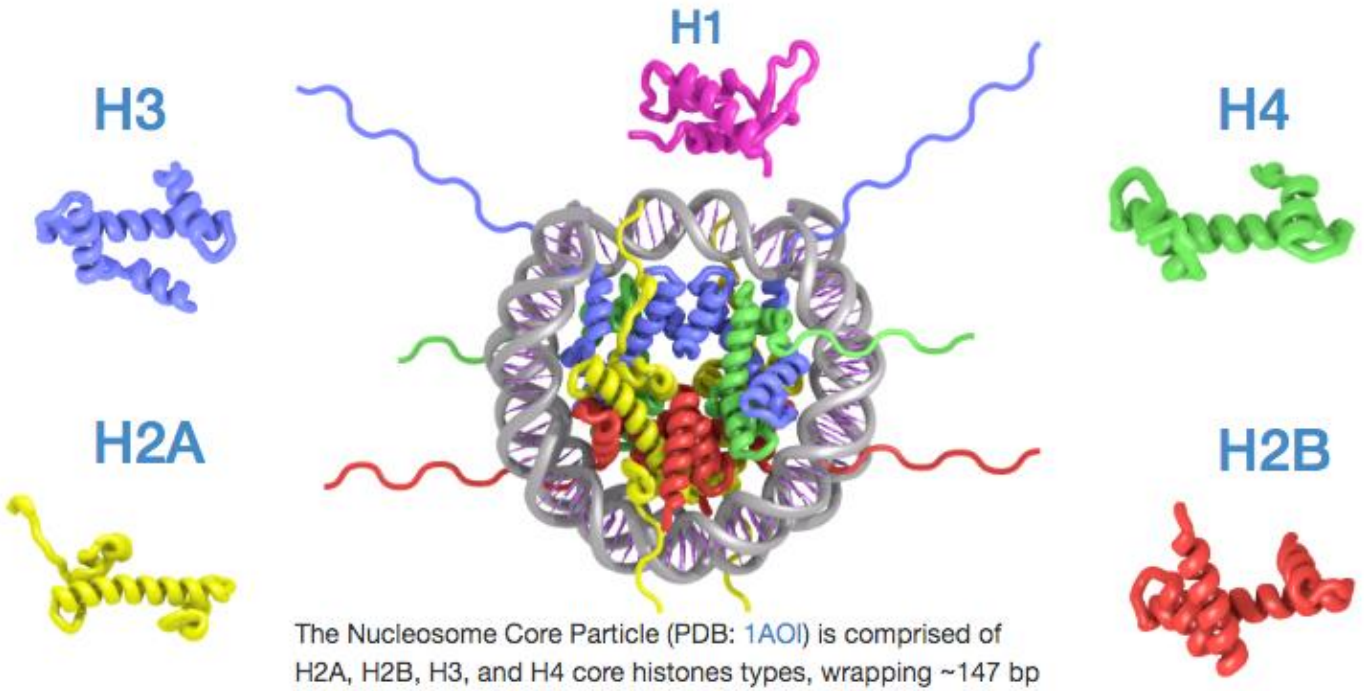
- N-末端乙酰化
  - N-末端乙酰化是将乙酰辅酶A中的乙酰基加入到蛋白质第一个残基的α-氨基中的过程，通常发生在起始蛋氨酸剪切之后。
  - N-末端乙酰化发生在细胞质中，是真核生物中最常见的翻译后修饰之一，在原核生物中很少见。
  - 最常见的乙酰化残基是甘氨酸、丙氨酸、丝氨酸或苏氨酸。
- 内部乙酰化
  - 内乙酰化是将乙酰辅酶A中的N-α-乙酰基添加到赖氨酸残基侧链上的过程。
  - 在真核生物中，内部乙酰化通常发生在细胞核中，主要影响组蛋白。这种修饰也存在于原核生物中。

蛋白质修饰—组蛋白修饰

Thanks to 薛宇@HUST

组蛋白（Histone）

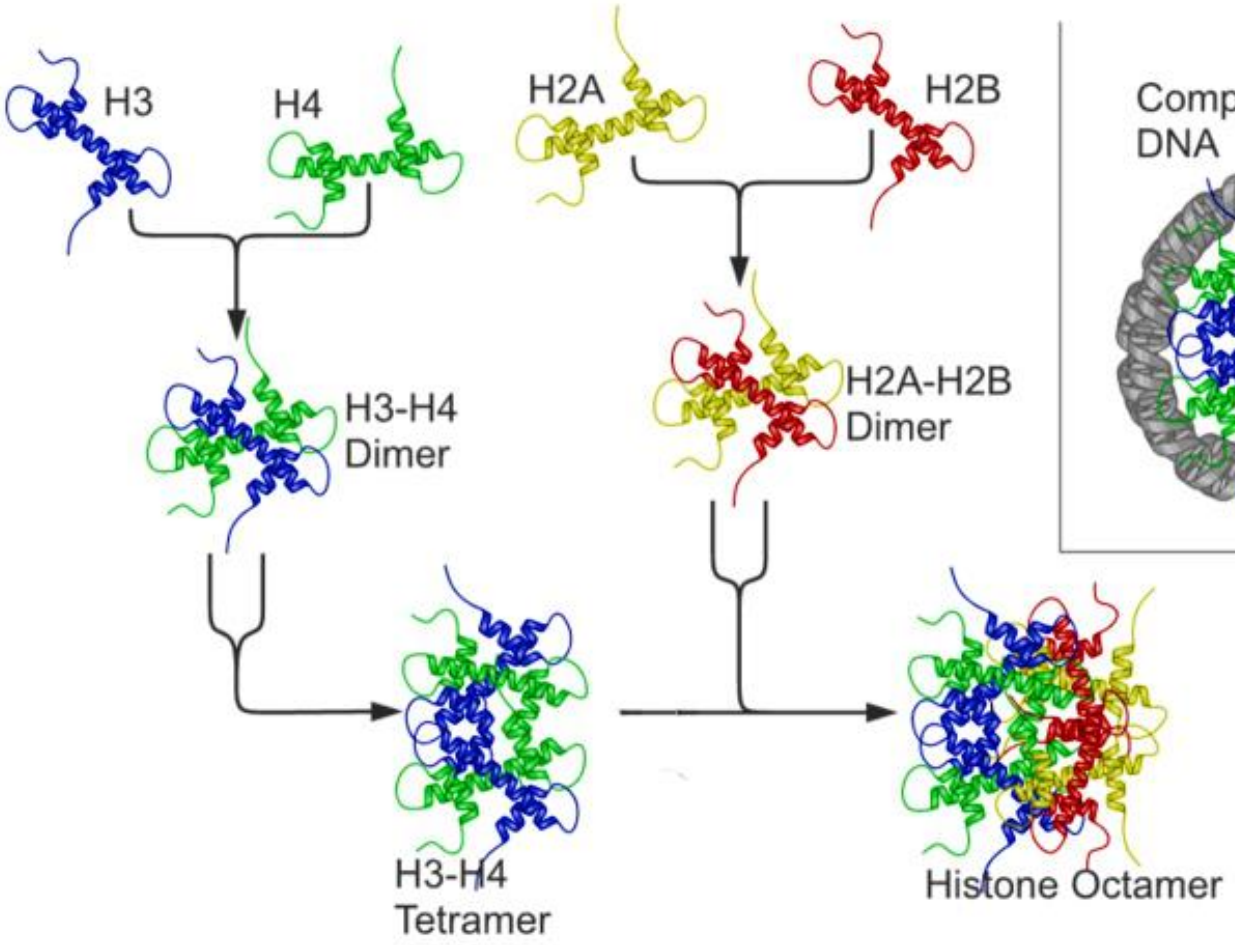
- 组蛋白：是包装DNA的一类蛋白质，能够改变DNA的结构；真核生物细胞染色质与原核细胞中的碱性蛋白质，和DNA共同组成核小体结构。
- 存在五个主要的组蛋白家族： H1/H5、H2A、H2B、H3和H4。
  - H2A、H2B、H3和H4被称为核心组织蛋白（Core Histone）
  - H1/H5被称为连接组织蛋白（Linker Histone）

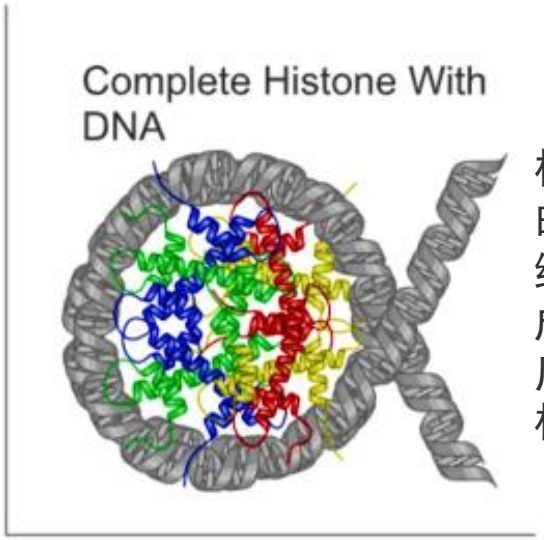


The Nucleosome Core Particle (PDB: 1AOI) is comprised of H2A, H2B, H3, and H4 core histones types, wrapping ~147 bp of DNA. H2A-H2B form a dimer and H3-H4 form a tetramer.

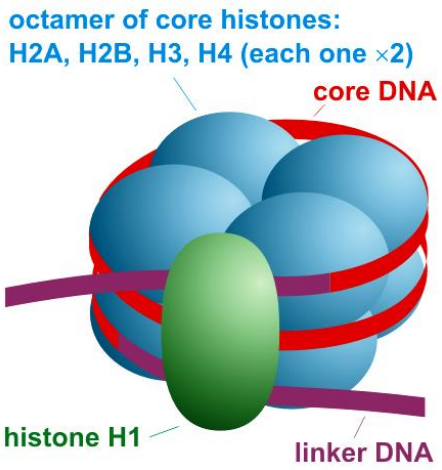
https://www.ncbi.nlm.nih.gov/research/HistoneDB2.0/

组蛋白八聚体和DNA构成核小体





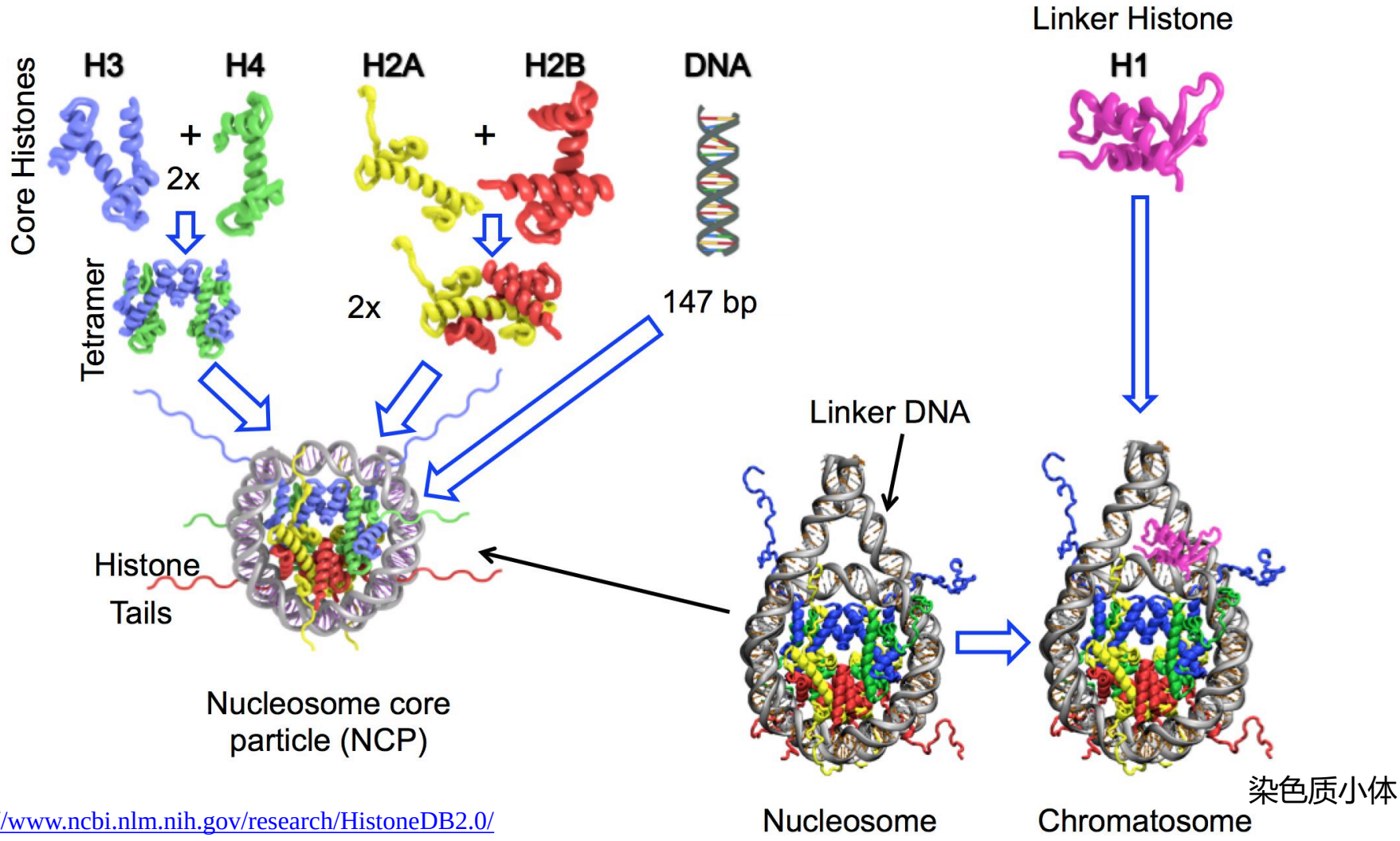
核小体是由DNA和组蛋白形成的染色质基本结构单位。



每个核小体由~147bp的DNA缠绕组蛋白八聚体1.65圈形成

https://en.wikipedia.org/wiki/Histone

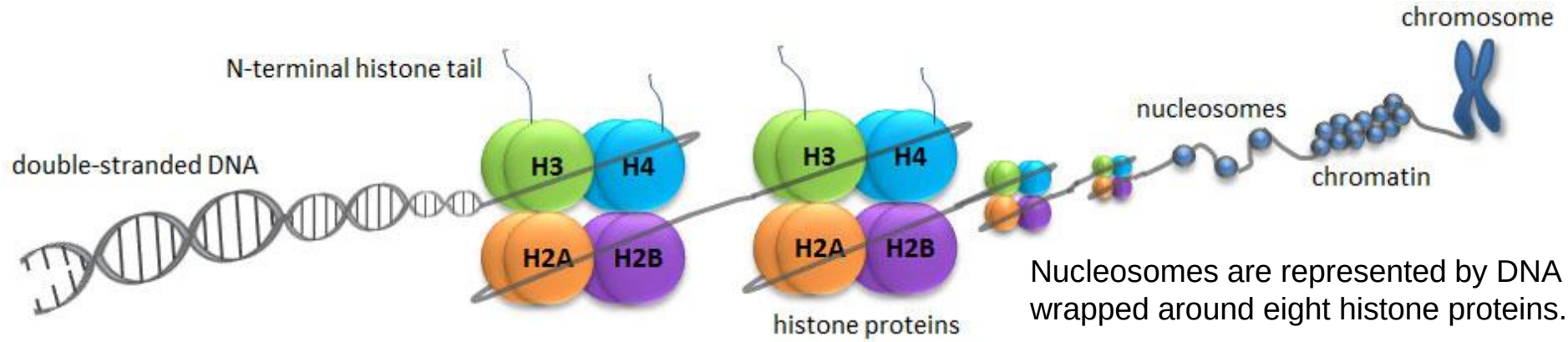
Nucleosome structure



https://www.ncbi.nlm.nih.gov/research/HistoneDB2.0/

组蛋白修饰

组蛋白修饰（Histone Modifications）是组蛋白翻译后发生的共价修饰，包括甲基化、磷酸化、乙酰化、泛素化、类泛素化等。



Nucleosomes are represented by DNA wrapped around eight histone proteins.

- 组蛋白与DNA是染色质的稳定成分
- 非组蛋白与RNA的含量则随细胞生理状态不同而变化（染色体中组蛋白以外的蛋白质成分称非组蛋白，绝大部分非组蛋白呈酸性）

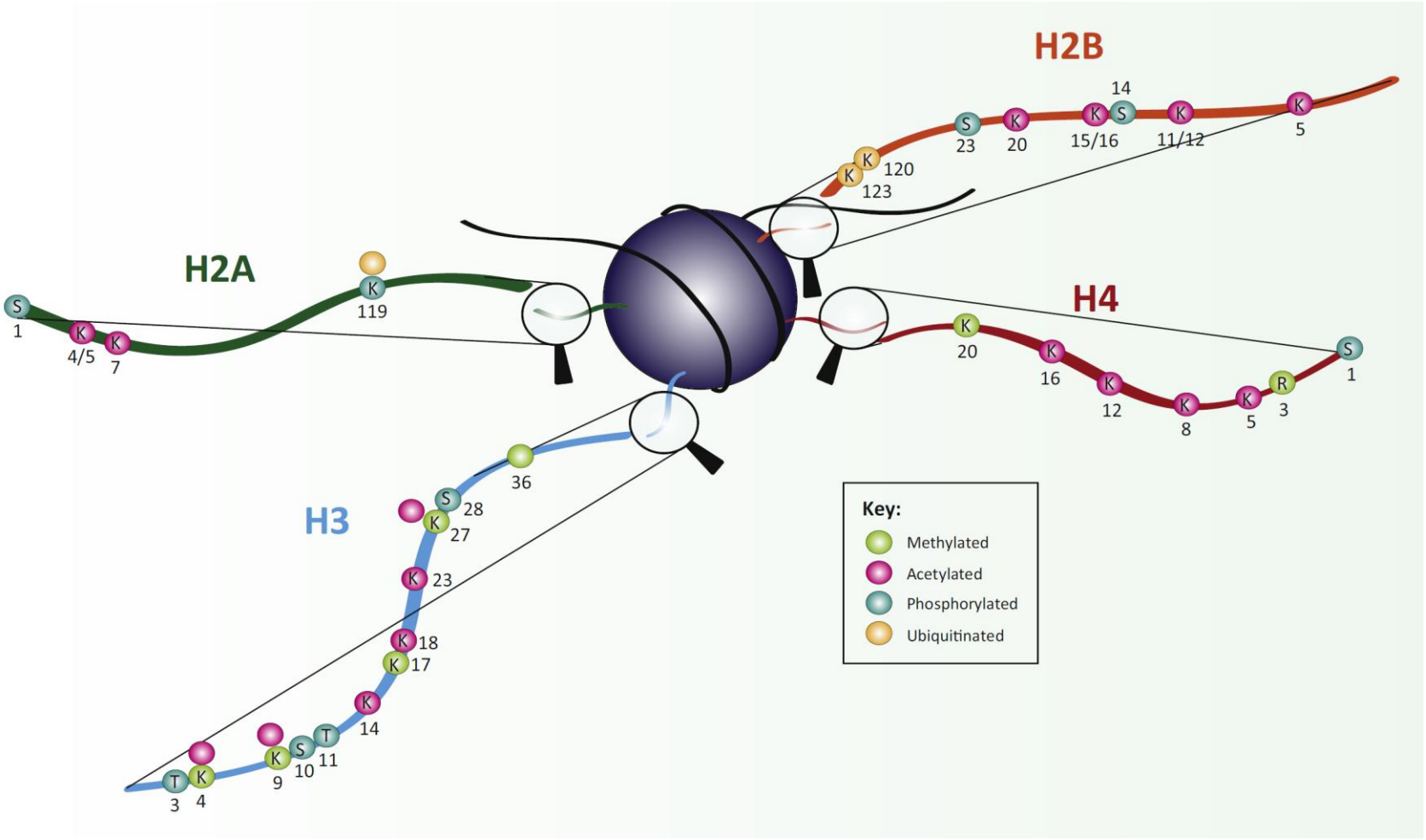
- N-末端氨基酸残基可发生多种共价修饰
- 组蛋白修饰作用：
  - 改变与DNA及其他核蛋白的相互作用
  - 改变染色质结构和活性
  - 调控基因表达

Histone Modifications & Roles

Histone	Modification	Role
H2A	H2AS1P	Mitosis; chromatin assembly
	H2AK4/5ac	Transcriptional activation
	H2AK7ac	Transcriptional activation
	H2AK119P	Spermatogenesis
	H2AK119uq	Transcriptional repression
H2B	H2BS14P	Apoptosis
	H2BS33P	Transcriptional activation
	H2BK5ac	Transcriptional activation
	H2BK11/12ac	Transcriptional activation
	H2BK15/16ac	Transcriptional activation
	H2BK20ac	Transcriptional activation
	H2BK120uq	Spermatogenesis/meiosis
	H2BK123uq	Transcriptional activation
H3	H3K4me2	Permissive euchromatin
	H3K4me3	Transcriptional elongation; active euchromatin
	H3K9me3	Transcriptional repression; imprinting; DNA methylation
	H3R17me	Transcriptional activation
	H3K27me3	Transcriptional silencing; X-inactivation; bivalent genes/gene poising
	H3K36me3	Transcriptional elongation
	H3K4ac	Transcriptional activation
	H3K9ac	Histone deposition; transcriptional activation
	H3K14ac	Transcriptional activation; DNA repair
	H1K18ac	Transcriptional activation; DNA repair; DNA replication
	H3K23ac	Transcriptional activation; DNA repair
	H3K27ac	Transcriptional activation
	H3T3P	Mitosis
	H3S10P	Mitosis; meiosis; transcriptional activation
	H3T11/S28P	Mitosis
H4	H4R3me	Transcriptional activation
	H4K20me1	Transcriptional silencing
	H4K20me3	Heterochromatin
	H4K5ac	Histone deposition; transcriptional activation; DNA repair
	H4K8ac	Transcriptional activation; DNA repair; transcriptional elongation
	H4K12ac	Histone deposition; telomeric silencing; transcriptional activation; DNA repair
	H4K16ac	Transcriptional activation; DNA repair
	H4S1P	Mitosis

Lateral Thinking: How Histone Modifications Regulate Gene Expression.
Trends in Genetics 2015
https://doi.org/10.1016/j.tig.2015.10.007

Modifications of the Histone Tails



Lateral Thinking: How Histone Modifications Regulate Gene Expression. *Trends in Genetics* 2015
https://doi.org/10.1016/j.tig.2015.10.007

组蛋白甲基化

- 组蛋白甲基化：组蛋白甲基转移酶将一至三个甲基基团从S-腺苷-L-蛋氨酸转移到组蛋白的赖氨酸或精氨酸残基上的过程。
- 基因表达调控：在细胞核内，组蛋白甲基化将影响DNA的转录活性，激活或抑制基因的表达，例如H3-K9与H3-K27 通常抑制基因表达。
- 甲基转移酶
  - H3-K4: SET1、SET7/9、Ash1、ALL-1、MLL、ALR、Trx、SMYD3
  - H3-K9: ESET、G9a、SUV39-h1、SUV39-h2、SETDB1、Dim-5、Eu-HMTase
  - H3-K27: G9a与polycomb group enzymes 如 EZH2

组蛋白去甲基化

- 组蛋白甲基化是一个动态过程，可通过组蛋白去甲基化酶去除修饰组蛋白中的甲基。
- 已发现两个主要的去甲基酶家族：
  - 赖氨酸特异性脱甲基酶1（LSD1）
  - 包含Jumonji结构域（JmjC结构域）的组蛋白去甲基酶（JMJD2、JMJD3/UTX和JARIDs）
- 去甲基酶具有特异性：
  - LSD1（BHC110，KDM1）：H3-K4me、H3-K4me2
  - JARID（1A-1D）：H3-K4me3
  - JMJD3和UTX（KDM6A）：H3-K27me2、H3-K27me3
  - JMJD1：H3-K9me， H3-K9me2
  - JMJD2：H3-K9me3

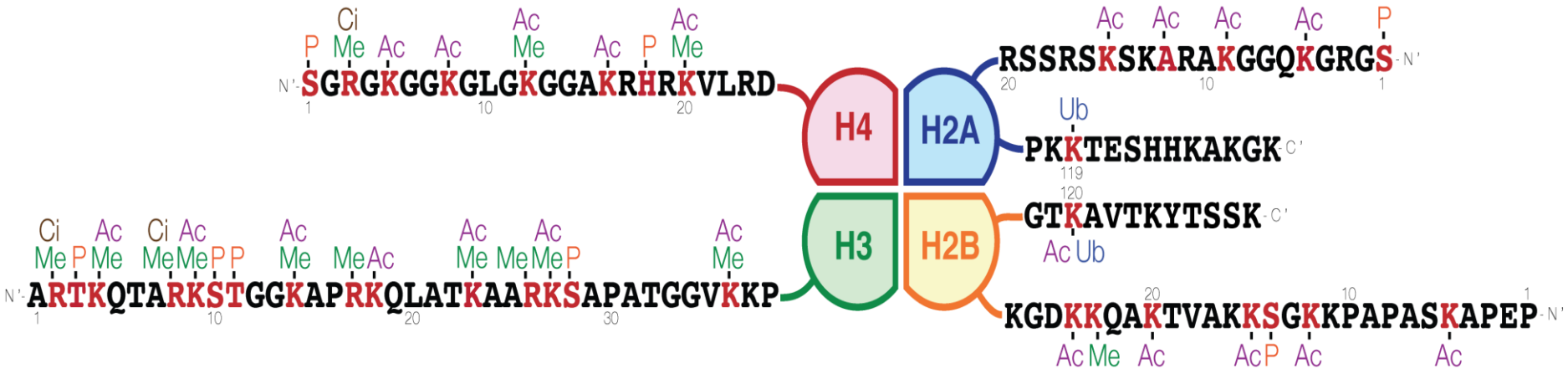
组蛋白乙酰化

- 组蛋白乙酰化：通过酶催化使乙酰辅酶A的乙酰基（COCH3）转移至组蛋白上的过程。
- 功能：与许多细胞过程的调控密切相关，包括染色质动力学、基因转录、基因沉默、细胞周期、凋亡、分化、DNA复制、DNA修复、入核、神经元抑制。
- 乙酰转移酶：已鉴定出20余种乙酰转移酶，分为5组，GNAT1、 MYST、TAFII250、P300/CBP、ACTR等核受体辅活化子。

组蛋白去乙酰化

- 由组蛋白去乙酰化酶催化组蛋白赖氨酸残基水解去除乙酰基
- 已鉴定出至少4 种去乙酰化酶
  - Class I： HDACs 1，2，3，8
  - Class II： HDACs 4，5，6，7，9，10
  - Class III： sirtuins，需要NAD+ 辅助因子，包含 SIRTs 1-7
  - Class IV： HDAC11

组蛋白修饰及其命名规则



Me甲基化　Ac乙酰化　Ci瓜氨酸化
Ub泛素化　P磷酸化

命名规则：

[组蛋白名称][氨基酸单字符简称][氨基酸位置][修饰种类]

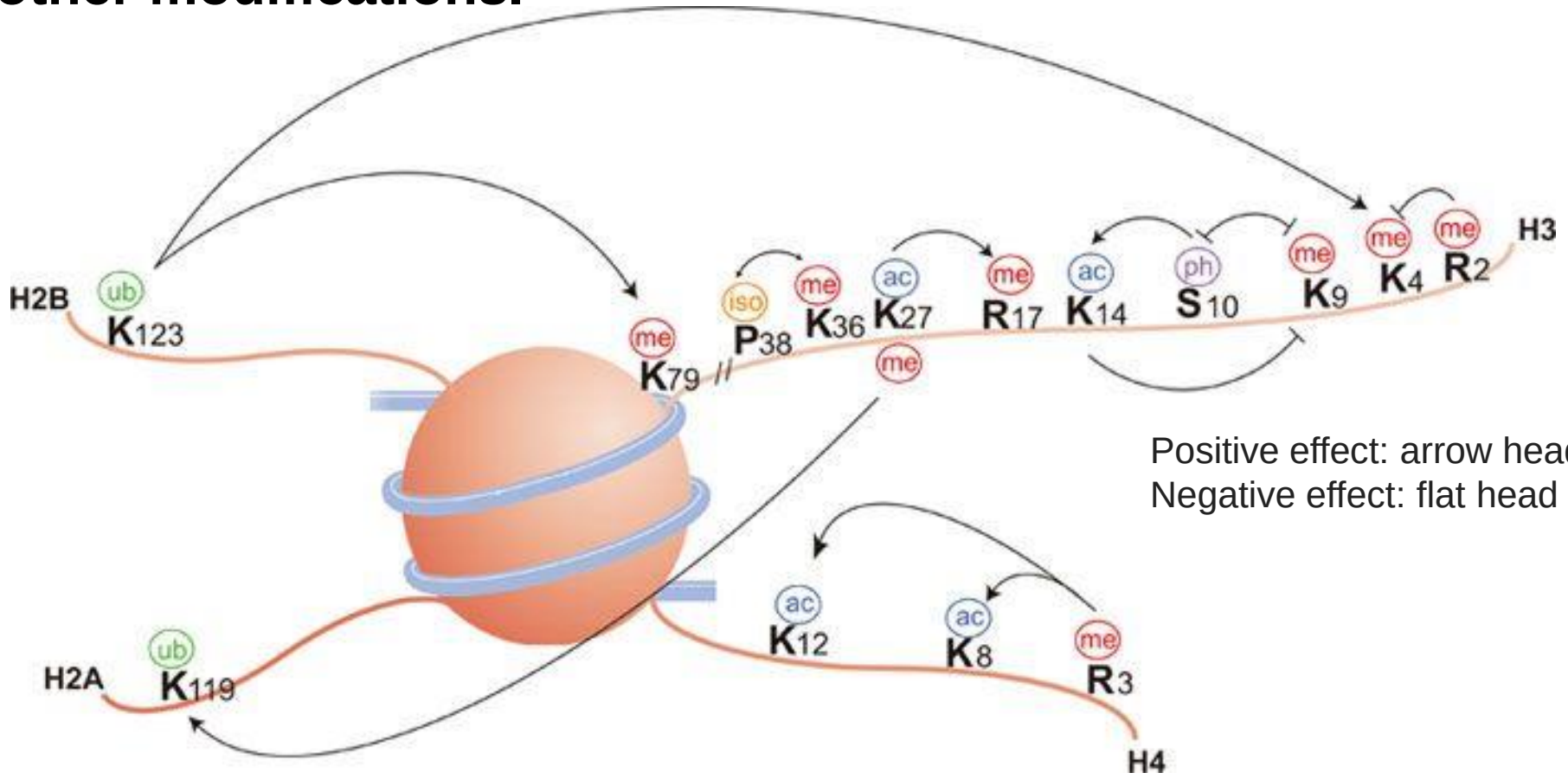
H3　　K　　4　　Me

H3K4Me=组蛋白H3从N端开始起计第4个赖氨酸的甲基化

A huge catalogue of histone modifications have been described, but a functional understanding of most is still lacking.

Histone modification cross-talk

Histone modifications can positively or negatively affect other modifications.

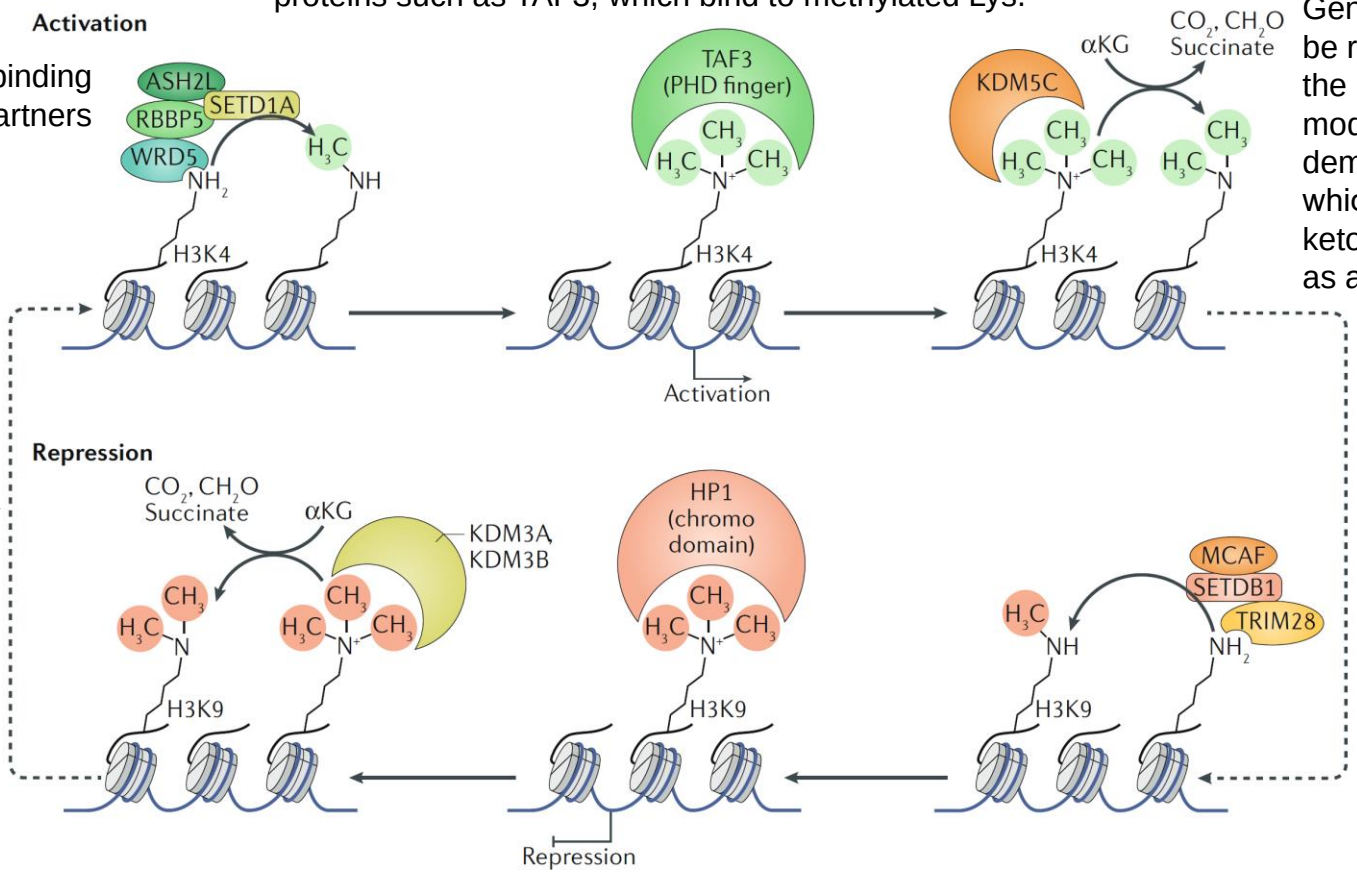


Regulation of chromatin by histone modifications. *Cell Research* 2011. https://doi.org/10.1038/cr.2011.22

Gene expression regulation by histone modification

SETD1A methyltransferase complex

H3K4me3 is recognized by PHD finger domains in proteins such as TAF3, which bind to methylated Lys.



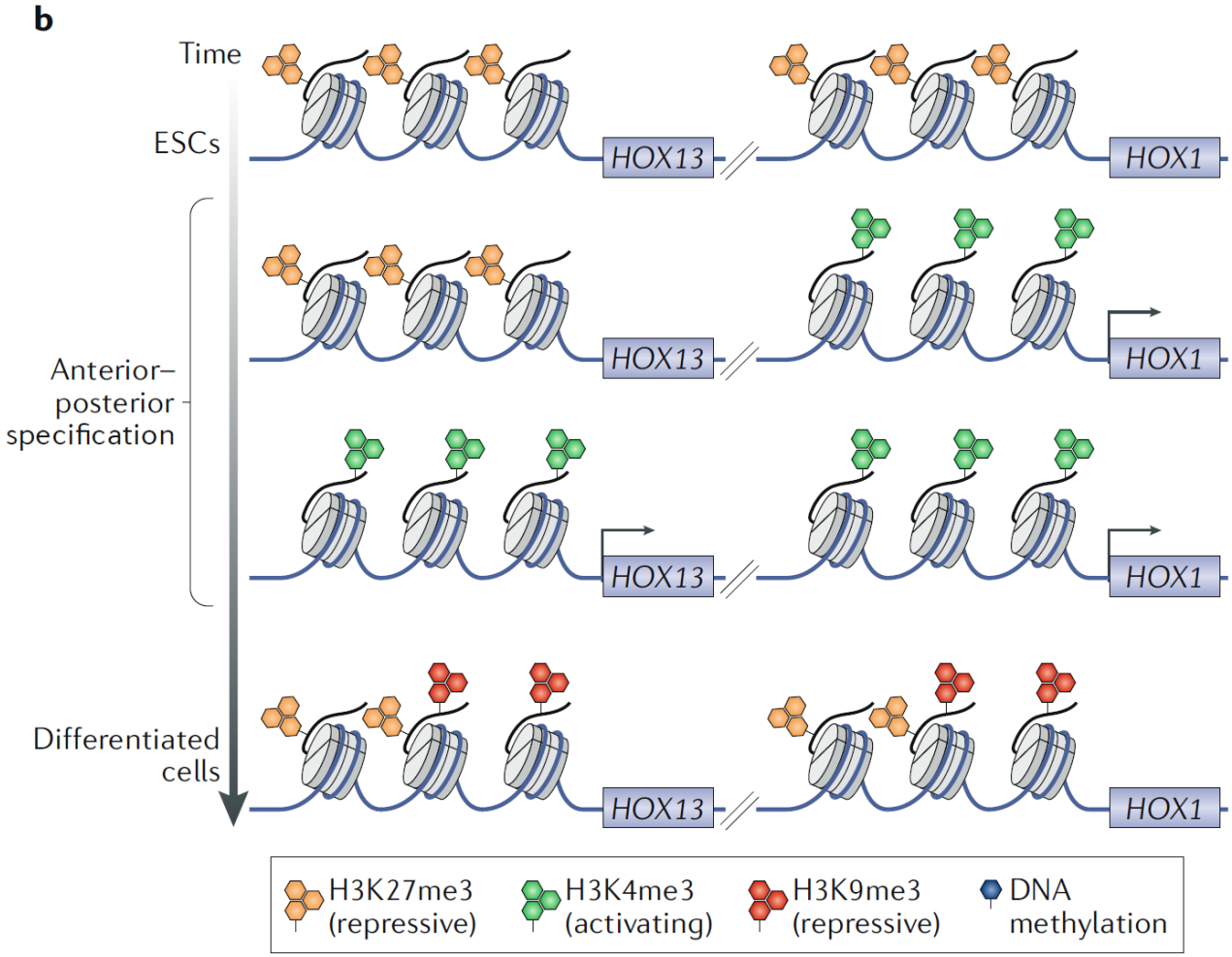
Gene activation can be reversed through the removal of this modification by the demethylase KDM5C, which utilizes α-ketoglutarate (αKG) as a cofactor.

H3K9me3 is recognized by the chromodomain in HP1 proteins and can be removed by the KDM3A and/or KDM3B demethylase in the presence of αKG as a cofactor, to allow for gene activation.

Gene-repressive states can be established by the deposition of H3K9me3 by the SETDB1 histone methyltransferase complex (together with a regulator, MCAF and a reader protein, TRIM28).

Roles and regulation of histone methylation in animal development. *Nat Rev Mol Cell Biol* 2019

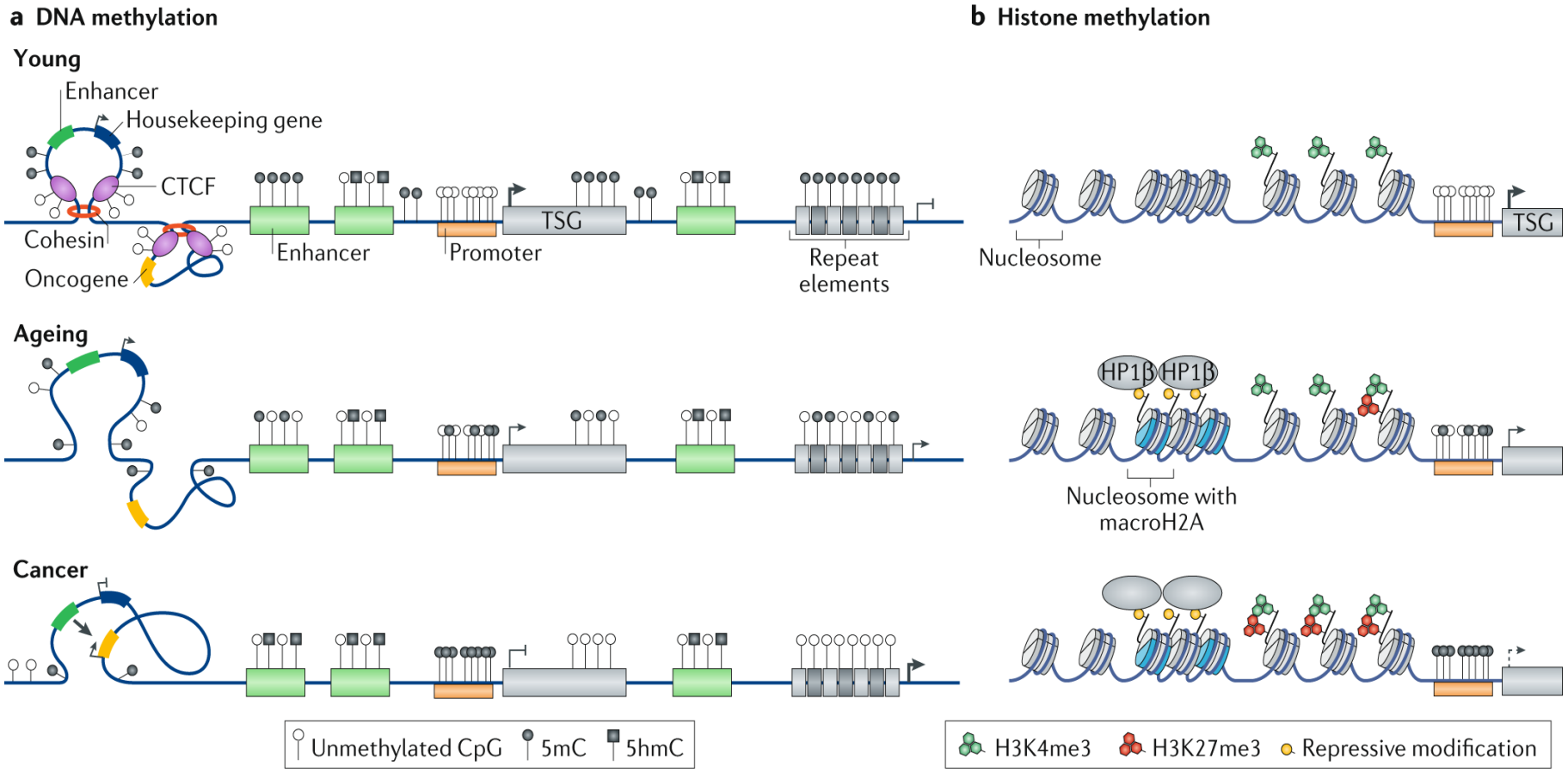
Developmental processes regulated by histone methylation



Regulation of HOX genes by histone methylation. In embryonic stem cells (ESCs), histone tri-methylation at H3K27 represses all HOX genes. At later stages of differentiation, early HOX genes are activated along the anterior–posterior axis by removal of H3K27me3 and addition of H3K4me3. In differentiated cells, HOX genes are again repressed by tri-methylation at H3K9 and H3K27.

Roles and regulation of histone methylation in animal development. *Nat Rev Mol Cell Biol* 2019
https://doi.org/10.1038/s41580-019-0151-1

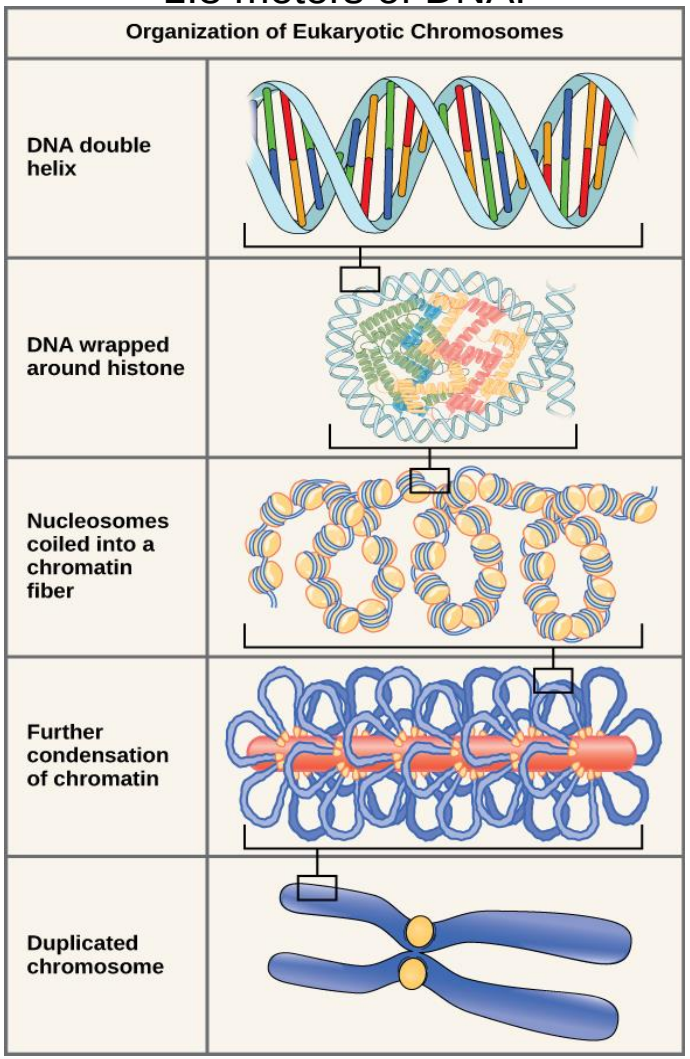
DNA and histone modifications



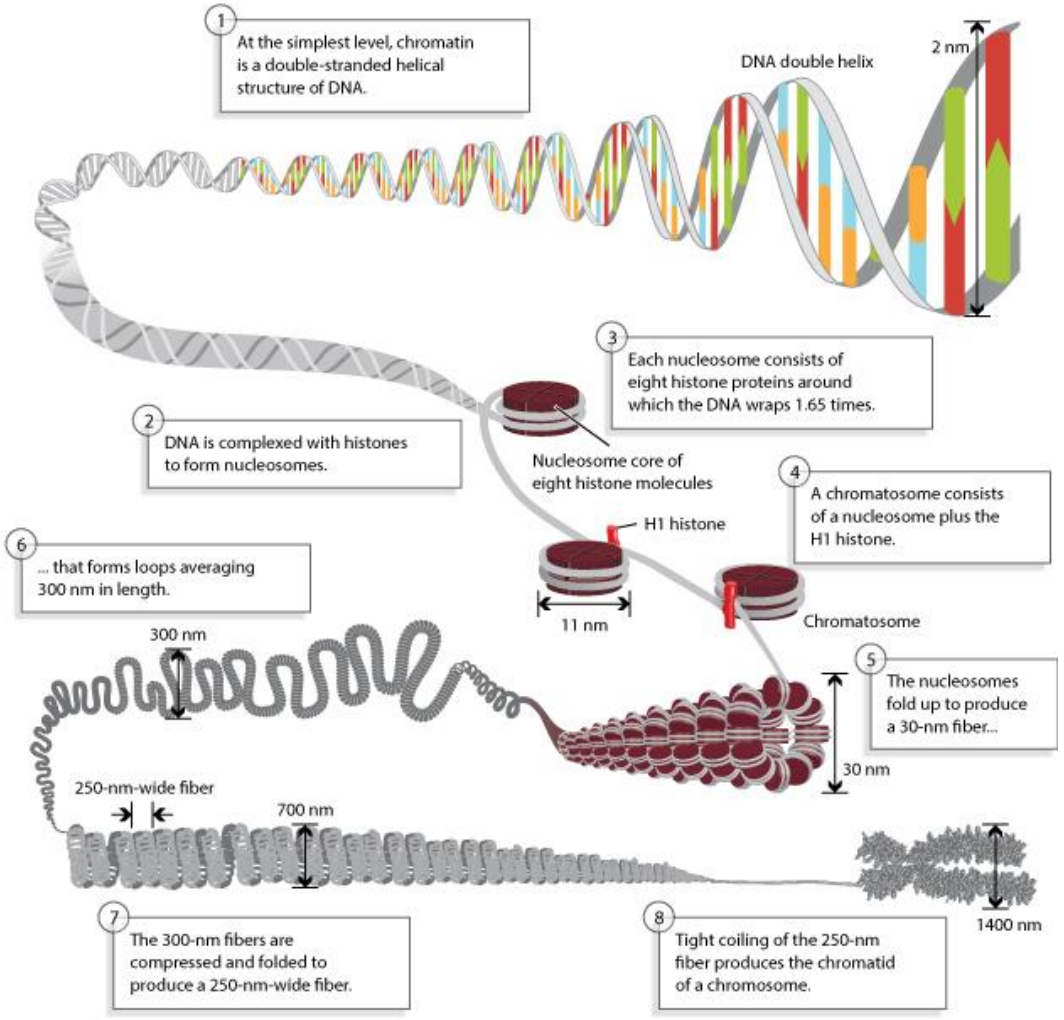
The roles of DNA, RNA and histone methylation in ageing and cancer. *Nature Reviews Molecular Cell Biology* 2019

DNA packaging: histone, nucleosome and chromatin

Each human diploid cell has about 1.8 meters of DNA.



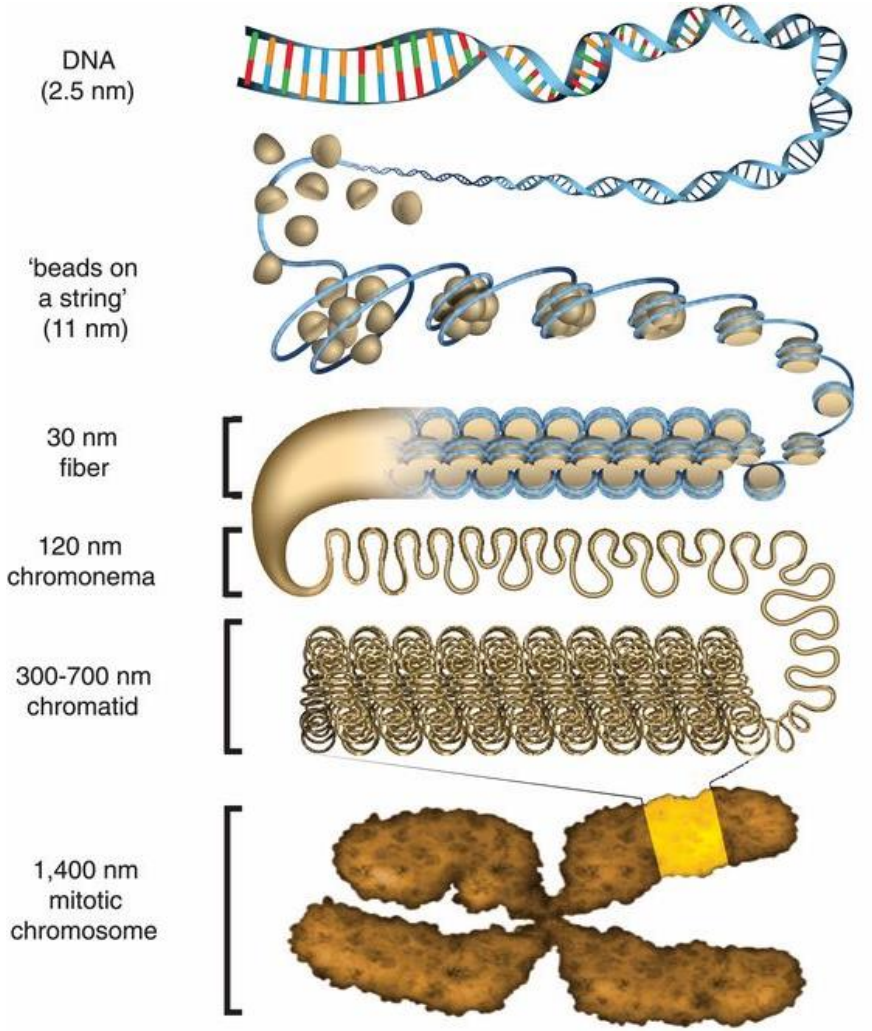
How is DNA packaged so tightly into chromosomes and squeezed into a tiny nucleus?



三维基因组学
Three-Dimensional Genomics
3D Genomics

基因组三维空间结构

- 染色体由DNA与组蛋白共同组成
- 染色体从一级结构（绳珠模型）到四级超螺旋折叠结构
- DNA分子一共被压缩了8400倍左右，形成了复杂的三维空间结构
- 正是这些折叠和压缩，使得基因在细胞中的分布复杂而又有序



ChromEMT: Visualizing 3D chromatin structure and compaction in interphase and mitotic cells. *Science* 2017

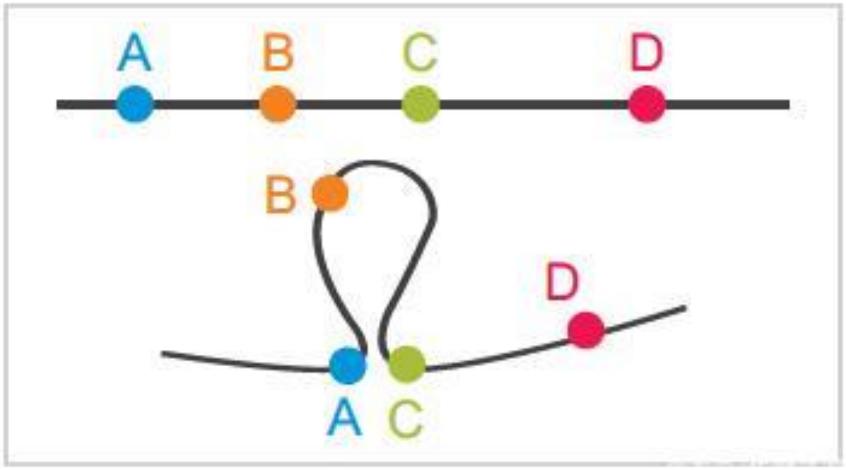
三维基因组学（3D Genomics）

- 基因组三维空间结构与功能的研究简称**三维基因组学**

  在考虑基因组序列、基因结构及其调控元件的同时，对基因组序列在细胞核内的三维空间结构，及其对基因转录、复制、修复和调控等生物过程中功能的研究。

- 为什么要研究三维基因组？

  科学家们发现，调控元件在空间结构上并不是在染色体上呈线性地一字依次排开，这些离散的调控元件并不能有效地解释很多基因的调控结果和机制。由此，猜测其与基因组的三维空间结构相关。



三维基因组基本实验技术

- 荧光显微实验方法：荧光原位杂交技术Fluorescence in situ hybridization (FISH)，2D-FISH、3D-FISH
- 染色体构象捕获：通过消化和重连空间上接近的染色体片段来确定不同位点之间的空间交互
  - Chromosome Conformation Capture (3C)
  - Chromosome Conformation Capture on Chip (4C)
  - Chromosome Conformation Capture Carbon Copy (5C)
  - High-throughput Chromosome Conformation Capture (Hi-C)
  - Chromatin Interaction Analysis by Paired-End Tag sequencing (ChIA-PET)：加入了免疫共沉淀技术，鉴定特定蛋白介导的染色质交互和空间结构

Job Dekker
UMASS

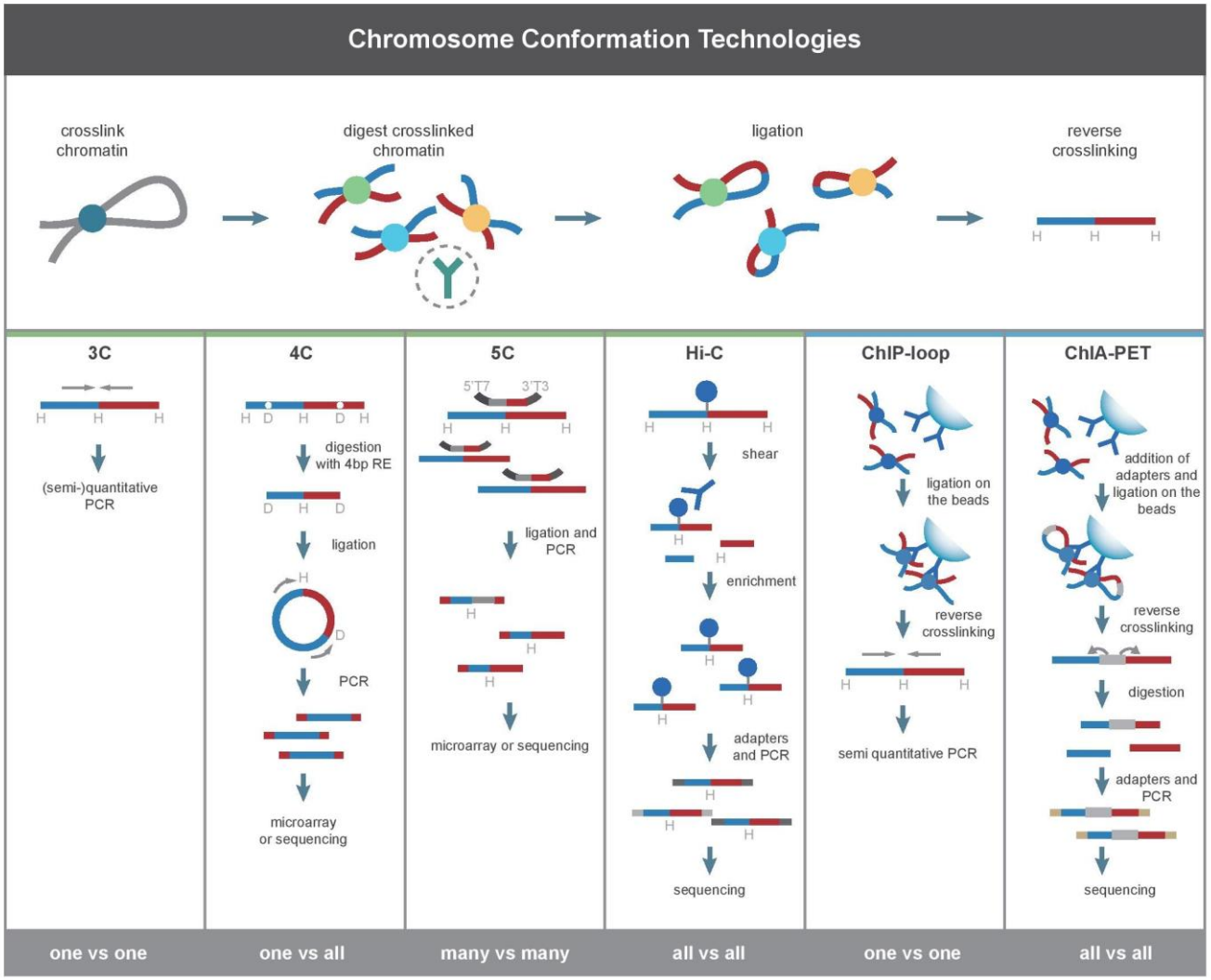
Dekker J, Rippe K, Dekker M, Kleckner N. **Capturing Chromosome Conformation**. *Science* 2002

染色体构象捕获

	互作方式	覆盖范围	检测方法	技术限制	研究应用
3C	单点对单点	通常小于1Mb	位点特异性PCR	通量低	确定已知启动子和增强子之间的相互作用
4C	单点对多点	全基因组范围	高通量测序	仅限于一个视角	与已知LCR（locus control regions)互作的全部基因和基因组元件
5C	多点对多点	通常小于1Mb	高通量测序	覆盖有限	确定染色体特定区域内的完整高级结构
Hi-C	全部互作	全基因组范围	高通量测序	—	所有染色体内和染色体外的相互左右
ChIP	特定蛋白介导的单点对单点	通常小于1Mb	位点特异性PCR	依赖于染色质交联的蛋白因子，忽略了其他的互作	依靠已知启动子和增强子之间的相互左右，确定特殊转录因子的功能
ChIA-PET	特定蛋白介导的全部互作	全基因组范围	高通量测序		构建已知转录因子介导的染色质互作网络

3C技术

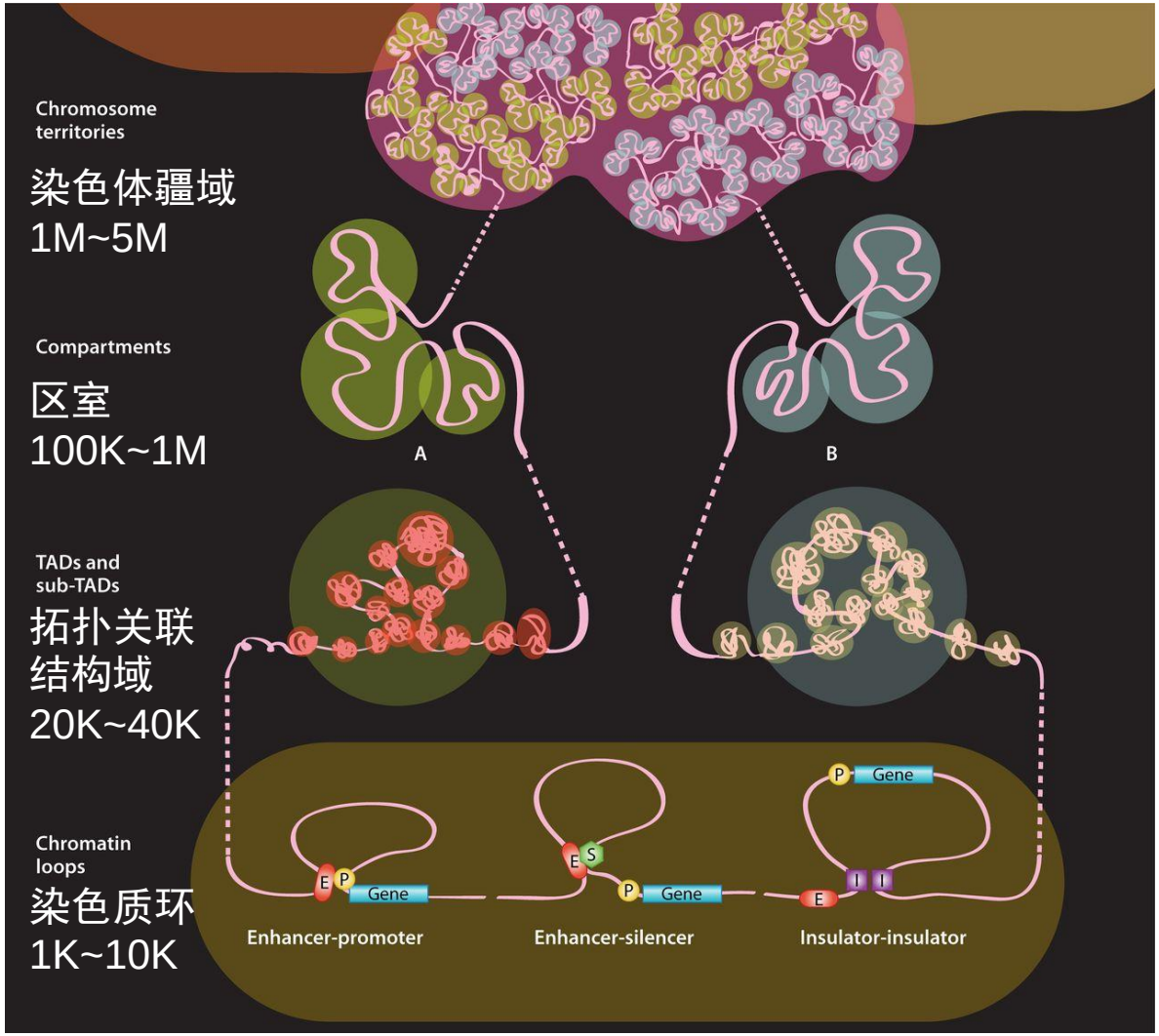
染色体构象捕获技术是通过一种定量手段（PCR产物的有和无、产量的高和低）对DNA之间是否存在相互作用这一定性问题进行研究，主要经过甲醛交联、限制性酶切、稀释和连接、解交联、DNA纯化与PCR鉴定。通过一对分别与选定的2段DNA配对的引物进行PCR扩增，通过PCR产物的有无、产量的高低等，就可以对是否存在相互作用进行判断。



染色质三维空间结构

随着测序分辨率的增加，研究者发现基因组的三维空间结构从大到小依次分为：

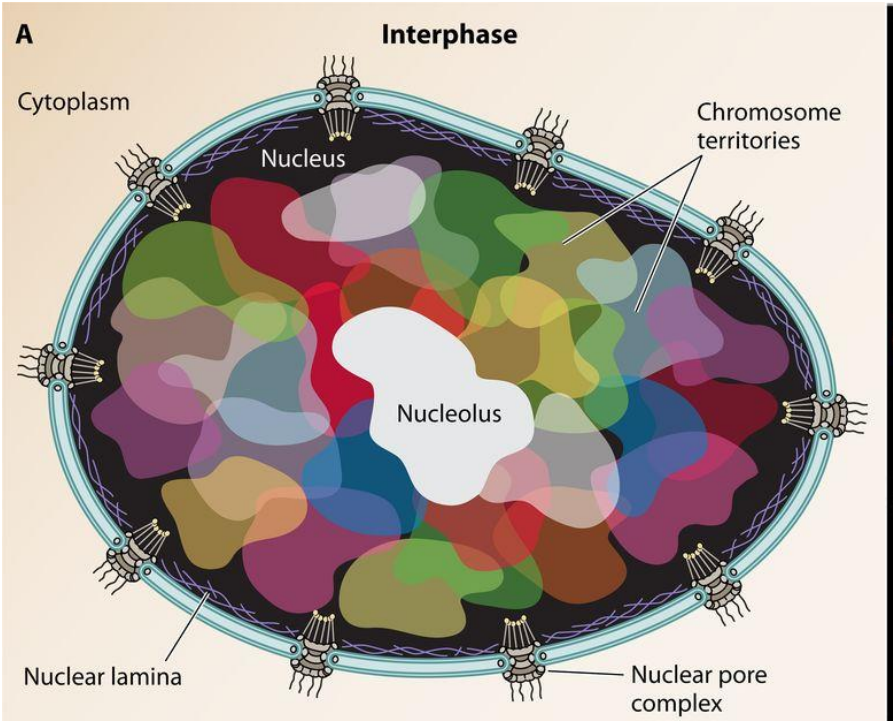
- **染色体疆域**
- **区室**
- **拓扑关联结构域**
- **染色质环**



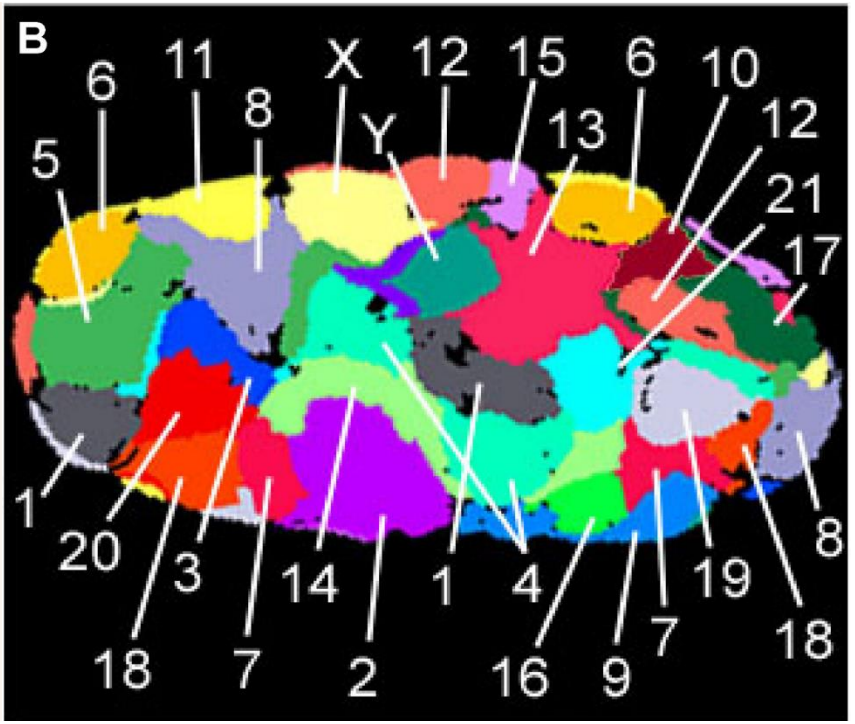
An Overview of Genome Organization and How We Got There: from FISH to Hi-C. *Microbiol Mol Biol Rev* 2015

染色体疆域

染色体疆域（Chromosome Territories）：指每条染色体都占据着一个独特的区域，同一染色体上的交互频率高于不同染色体之间的交互频率。交互频率随着基因座之间的线性距离增加而呈指数级下降。



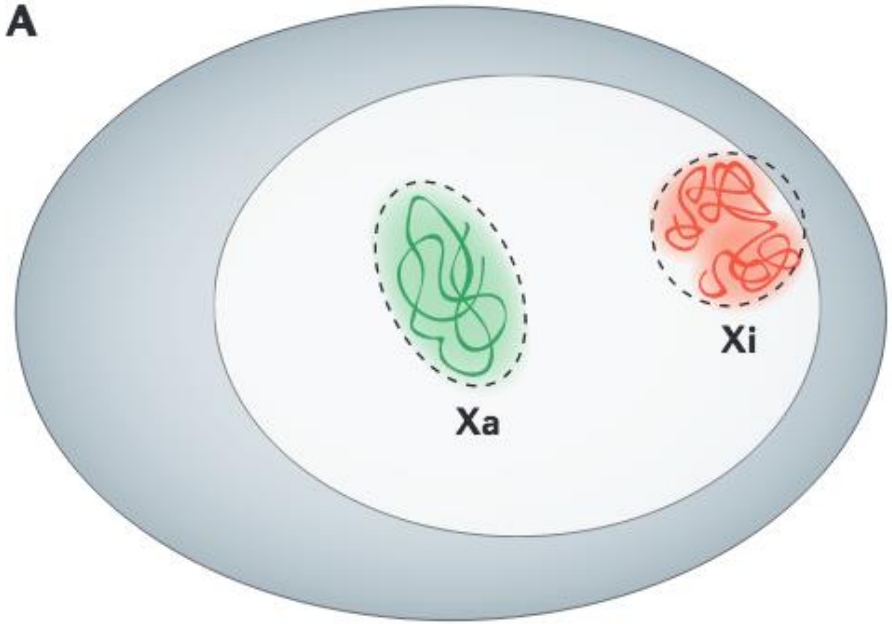
Development 2016, 143 (6) :910



PLoS Biology 2005, 3(5): e157

Chromosome Territories

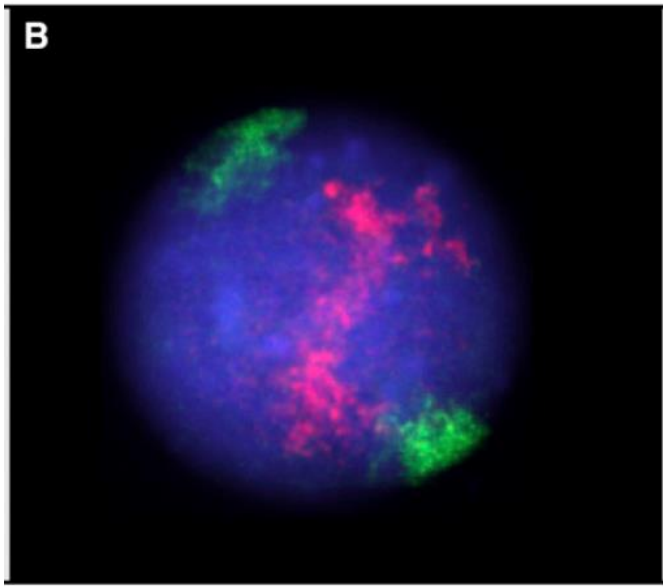
Distinct 3D X-chromosome structures: more condensed Xi



Active and inactive X chromosomes (**Xa, Xi**)

The X chromosome in space. *Nature Reviews Genetics* 2017

Gene-rich chromosomes in the center: allowing more contacts

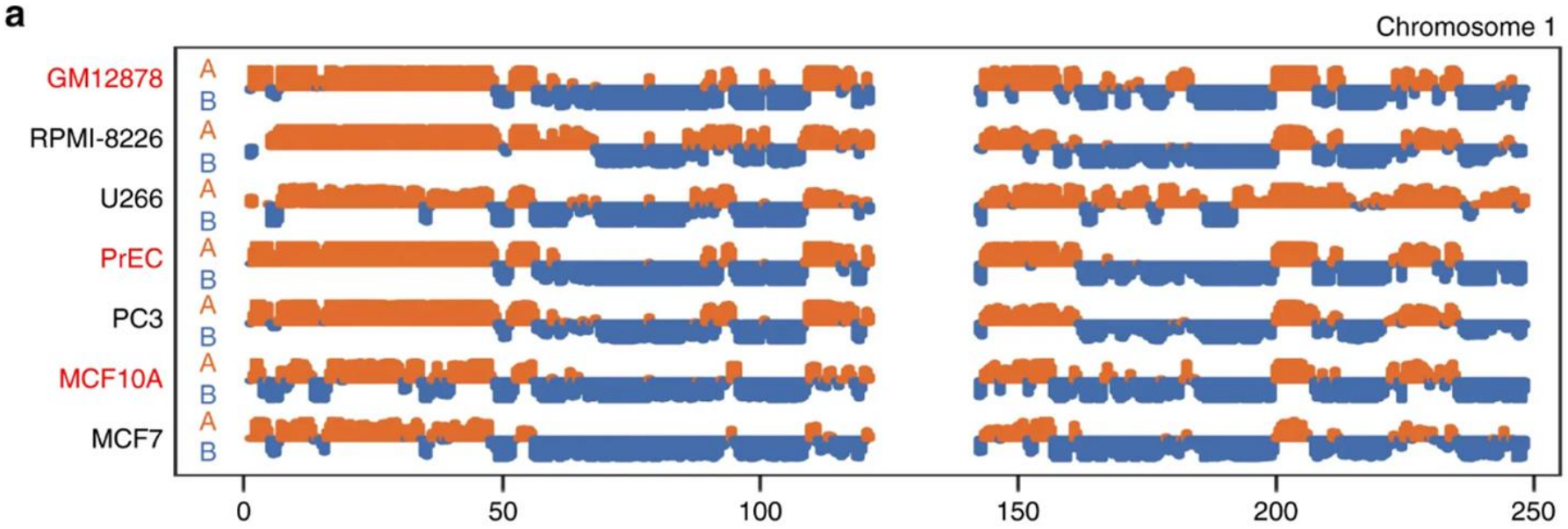


Gene-rich human chromosome 19 (red)
Gene-poor human chromosome 18 (green)

Genome Architecture: Domain Organization of Interphase Chromosomes. *Cell* 2013

区室

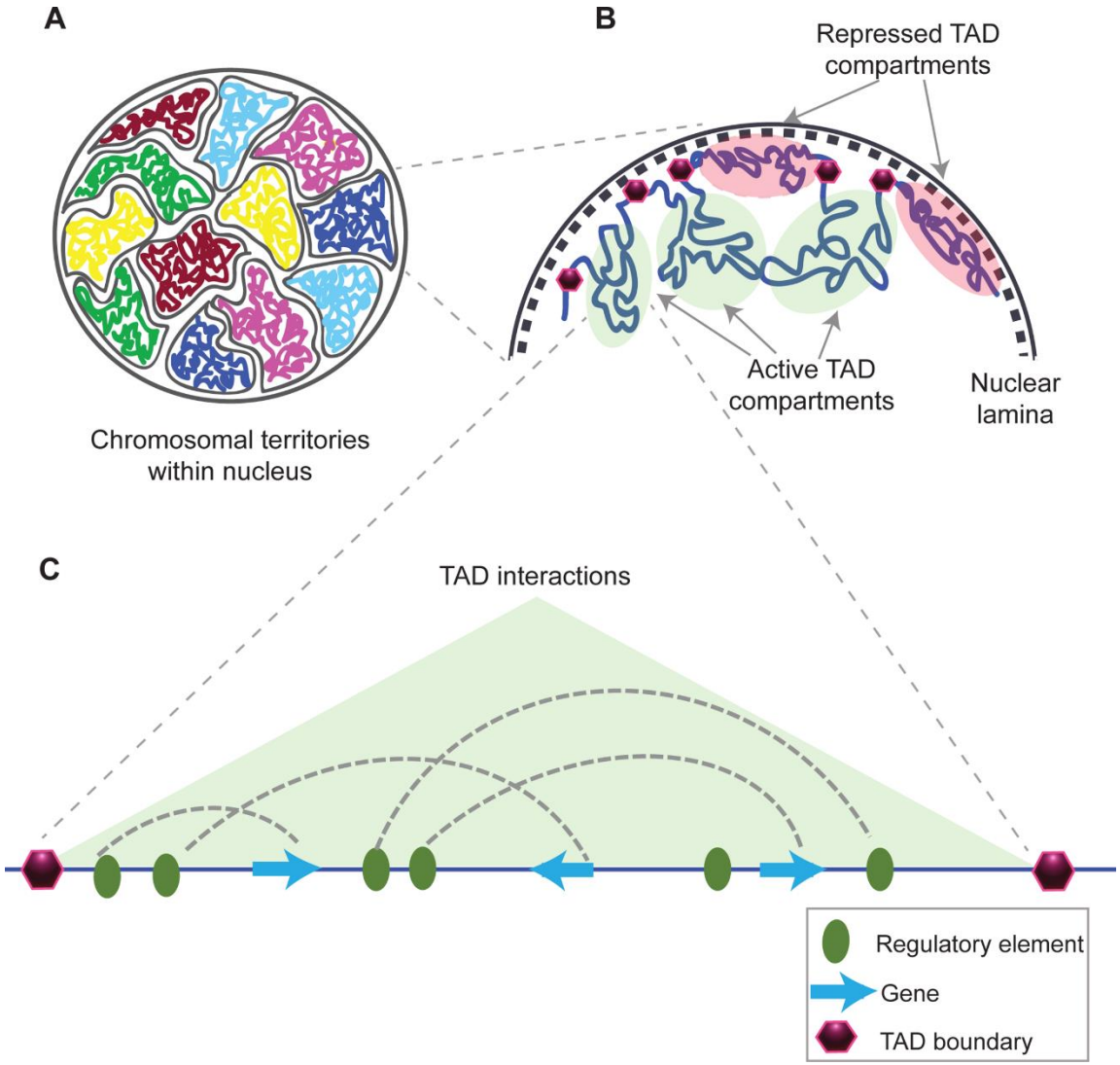
区室（Compartments A/B）：根据互作图谱，能够将基因组近似分为A（常染色质-转录活跃区域），B（异染色质-转录非活性区）。A/B区室在染色体上相间分布，并且在不同细胞状态下可以互相转变。



3D genome of multiple myeloma reveals spatial genome disorganization associated with copy number variations. *Nature Communications* 2017

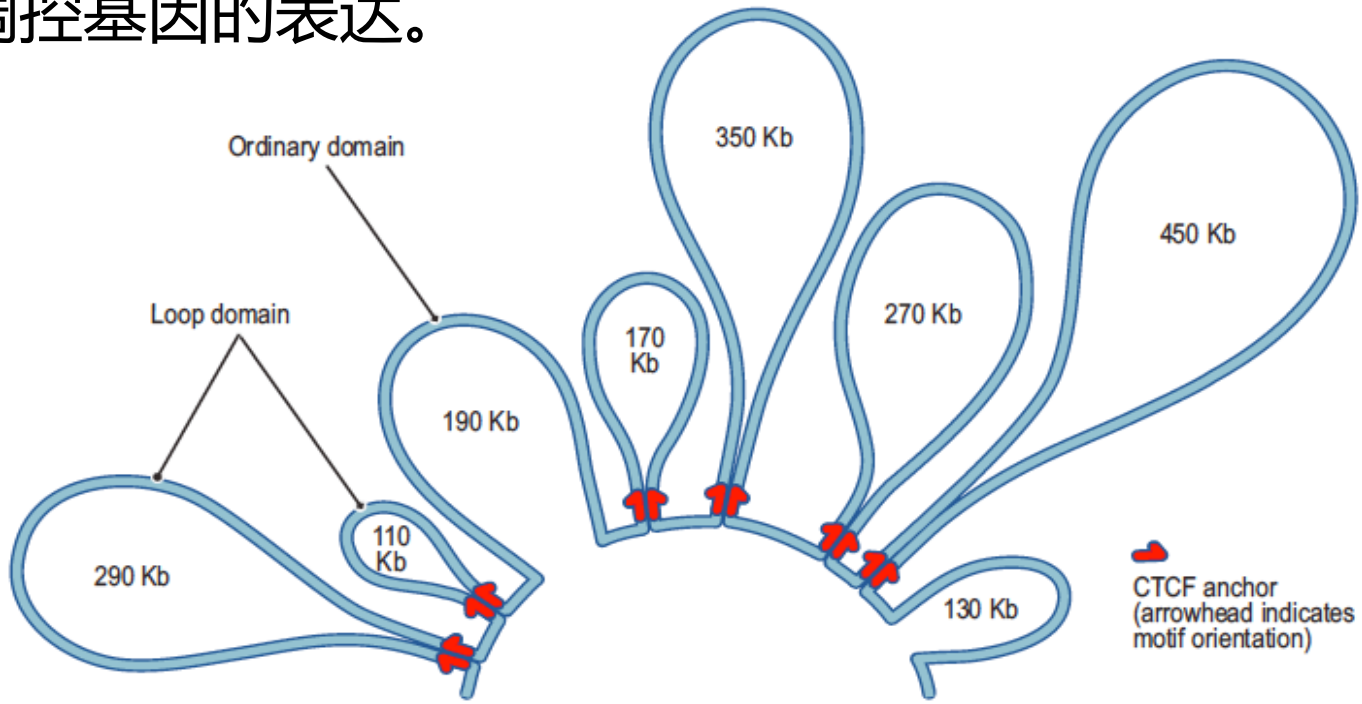
拓扑关联结构域

拓扑关联结构域（Topologically Associating Domains, TAD）：是区室下的亚结构，长度为300Kb-1Mb，具有TAD内部互作频率高，TAD间互作频率低的特点。其边界富集CTCF、持家基因、tRNAs、SINE 反转录转座子等DNA元件。



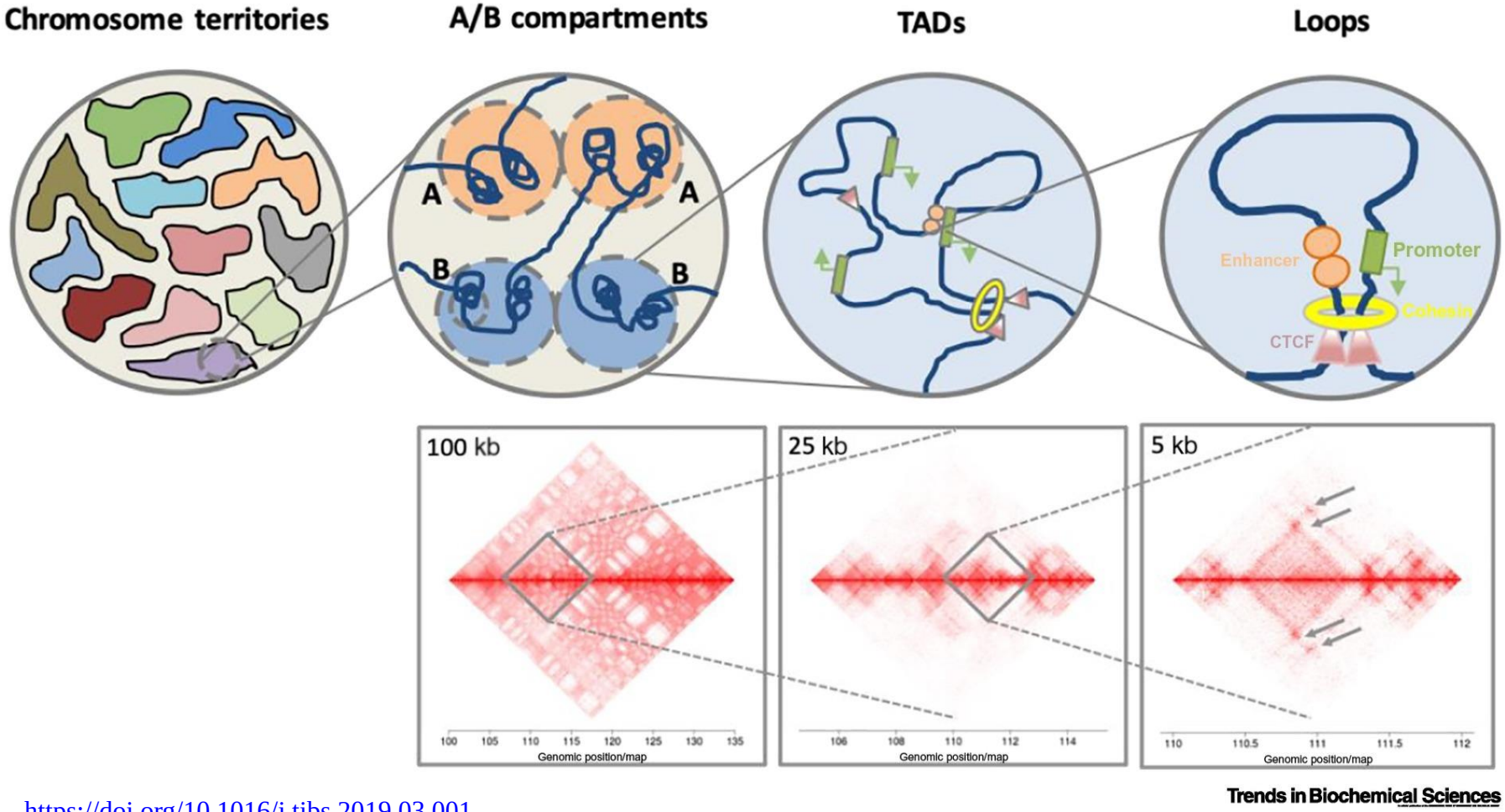
染色质环

染色质环（Chromatin loop）：也可称为交互峰（interaction peaks），由染色体上相距较远的两个片段构成，其在线性空间中虽相距较远，但在三维空间结构中却具有显著的近距交互作用。调控元件（如增强子）便可以通过这种结构远距离调控基因的表达。



CTCF is a transcription factor encoded by the CTCF gene and involved in regulation of chromatin architecture.

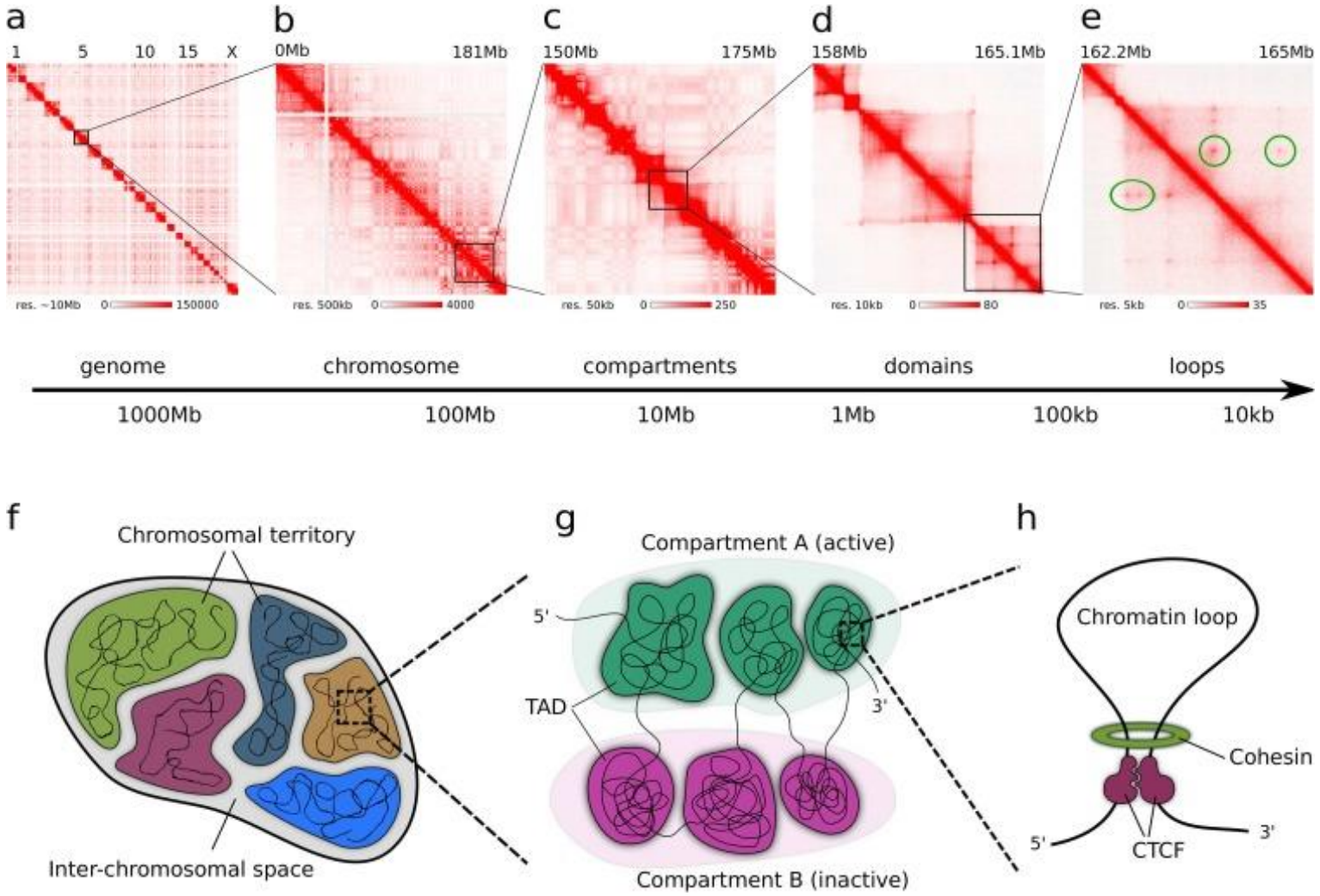
Chromosome structure at multiple scales



https://doi.org/10.1016/j.tibs.2019.03.001

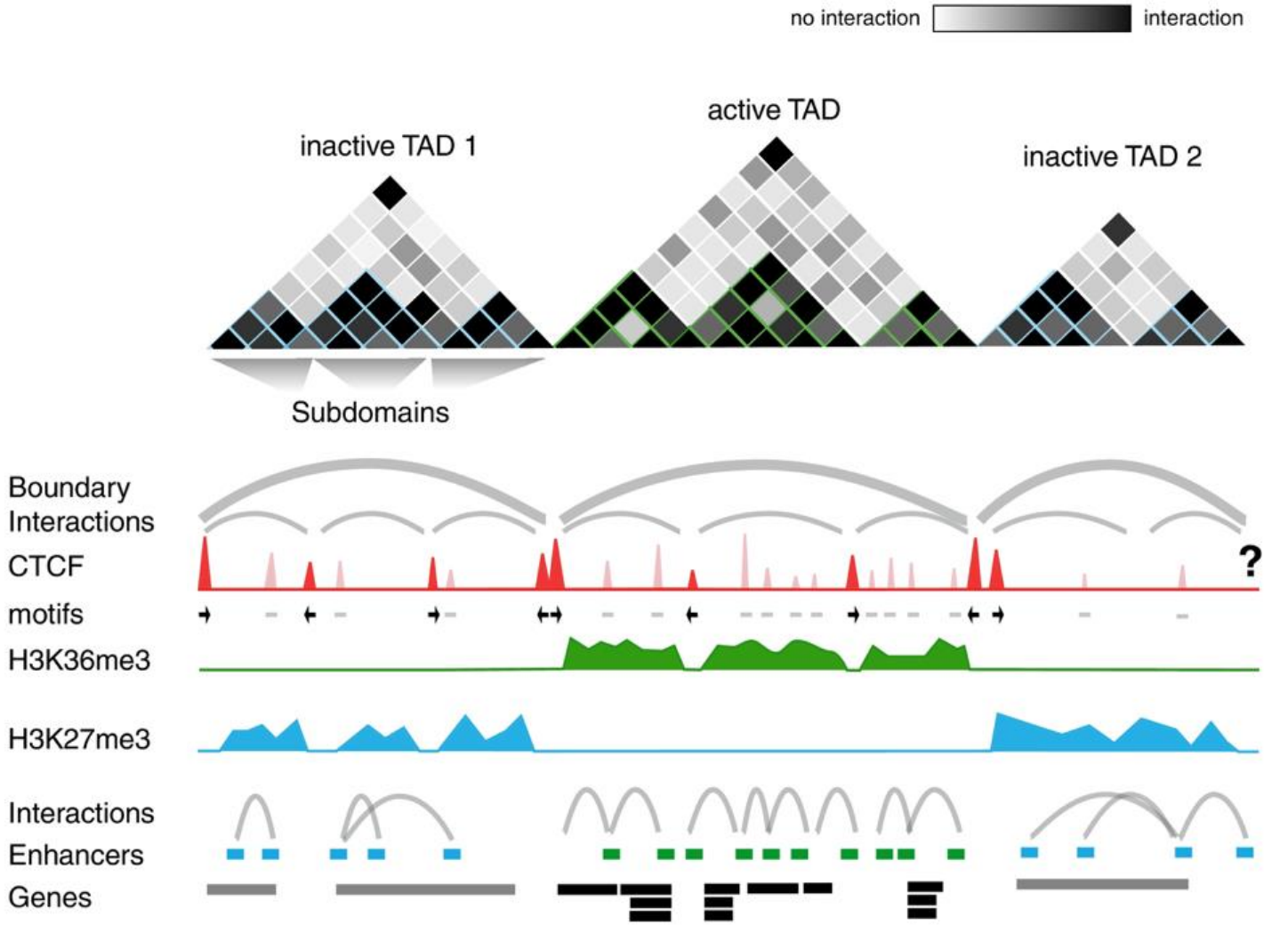
Trends in Biochemical Sciences

Hierarchical genome organization



Three-dimensional organization and dynamics of the genome. *Cell Biol Toxicol.* 2018

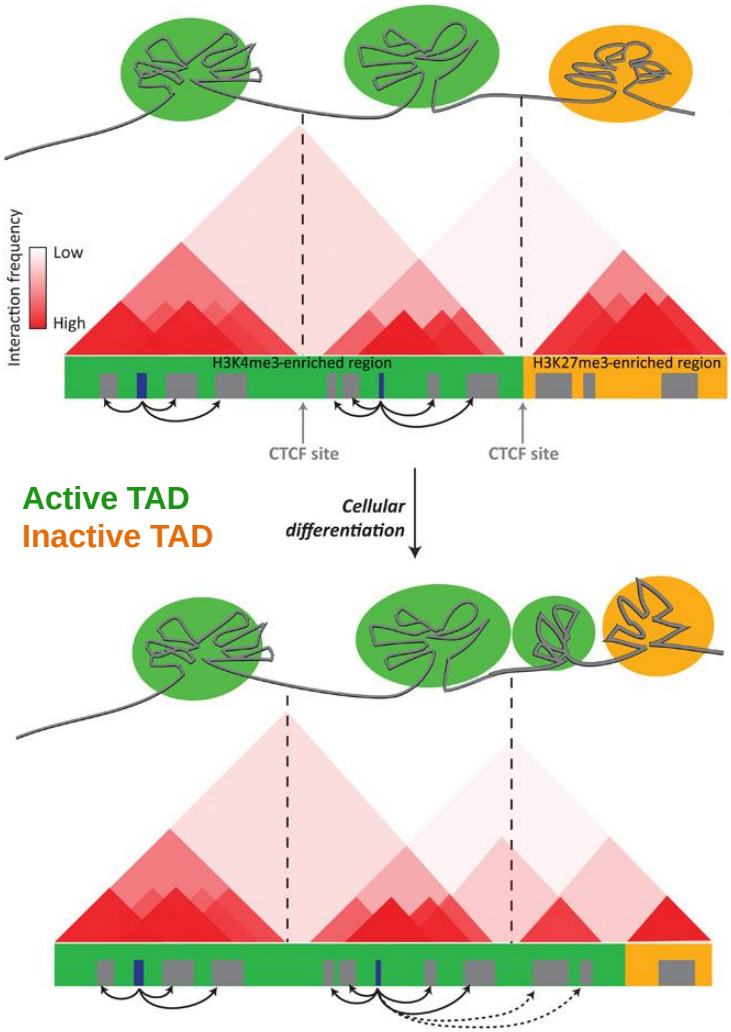
TADs with histone modifications



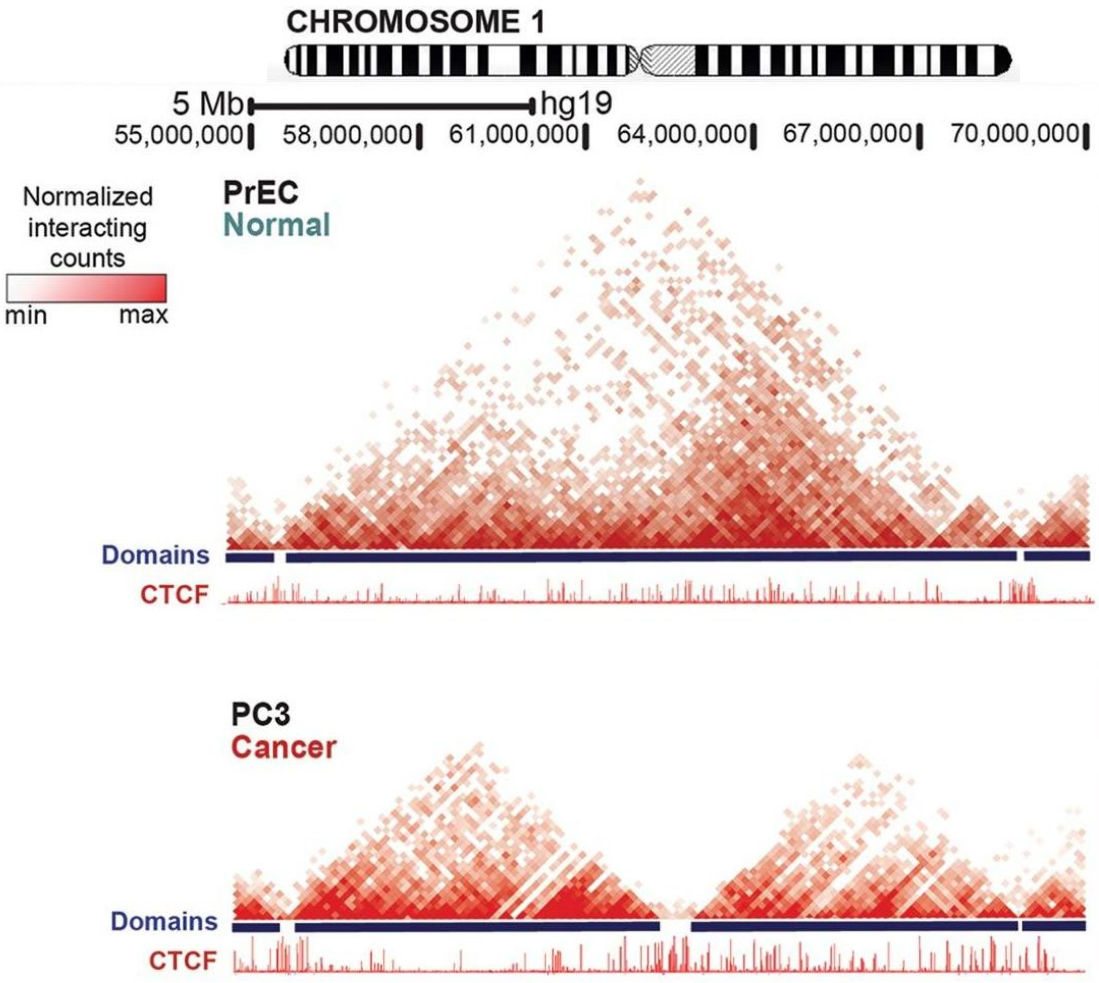
https://doi.org/10.1016/j.gde.2015.11.009

Current Opinion in Genetics & Development

TAD with cell differentiation and cancer

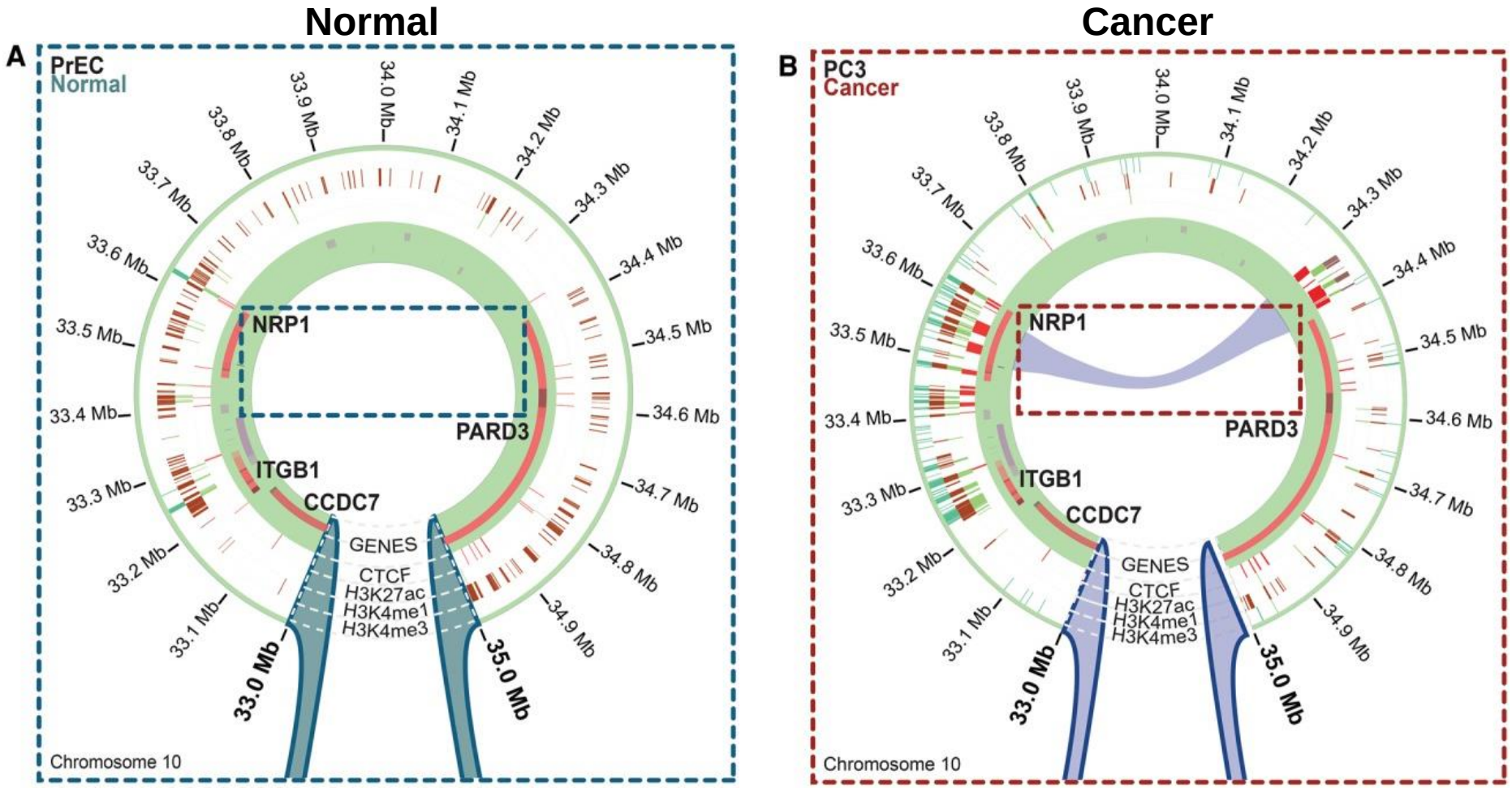


Regulation of gene transcription by Polycomb proteins. *Science Advances* 2015



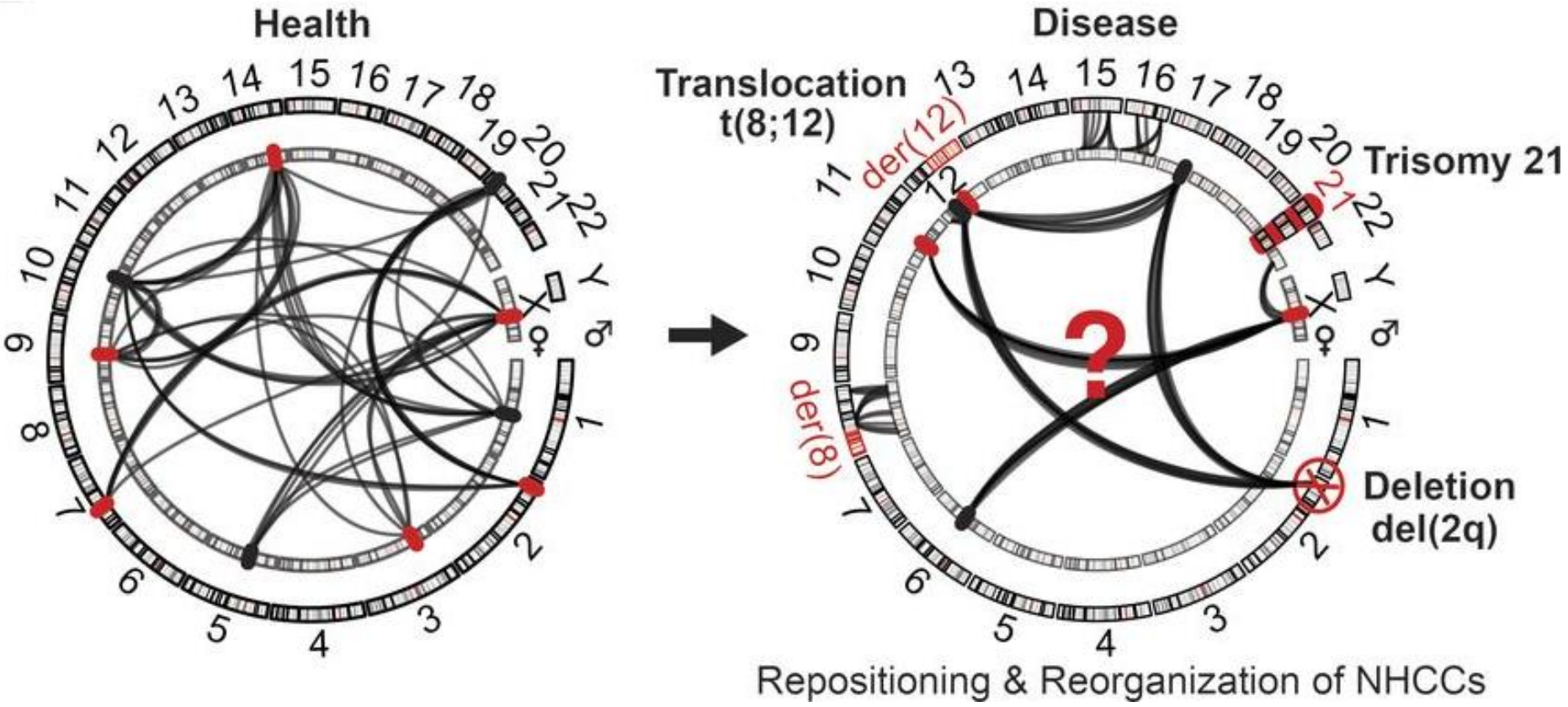
Three-dimensional disorganization of the cancer genome occurs coincident with long-range genetic and epigenetic alterations. *Genome Research* 2016

Differential chromatin interactions



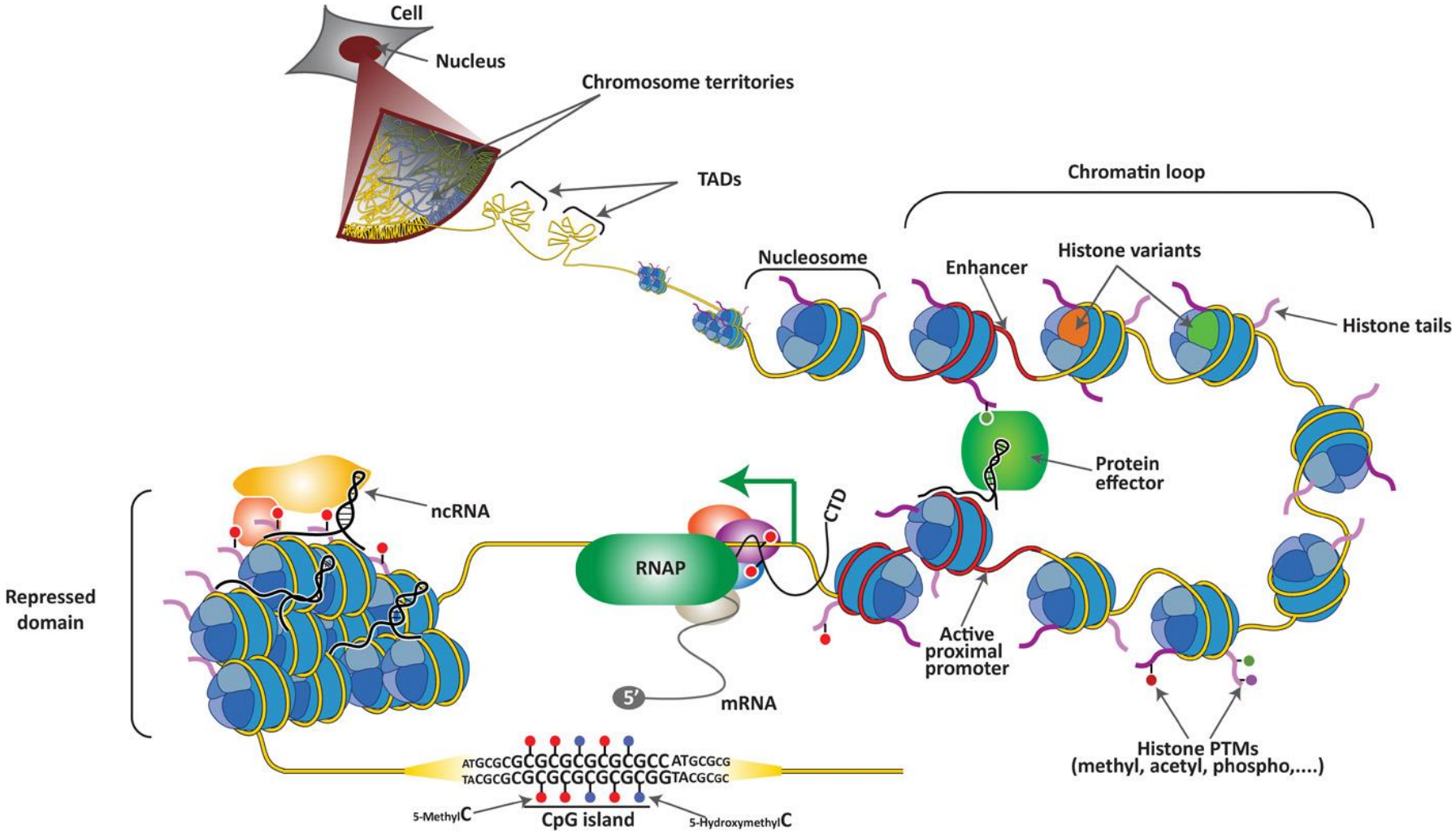
Three-dimensional disorganization of the cancer genome occurs coincident with long-range genetic and epigenetic alterations. *Genome Research* 2016

Dynamics of chromosome interactions in health and disease



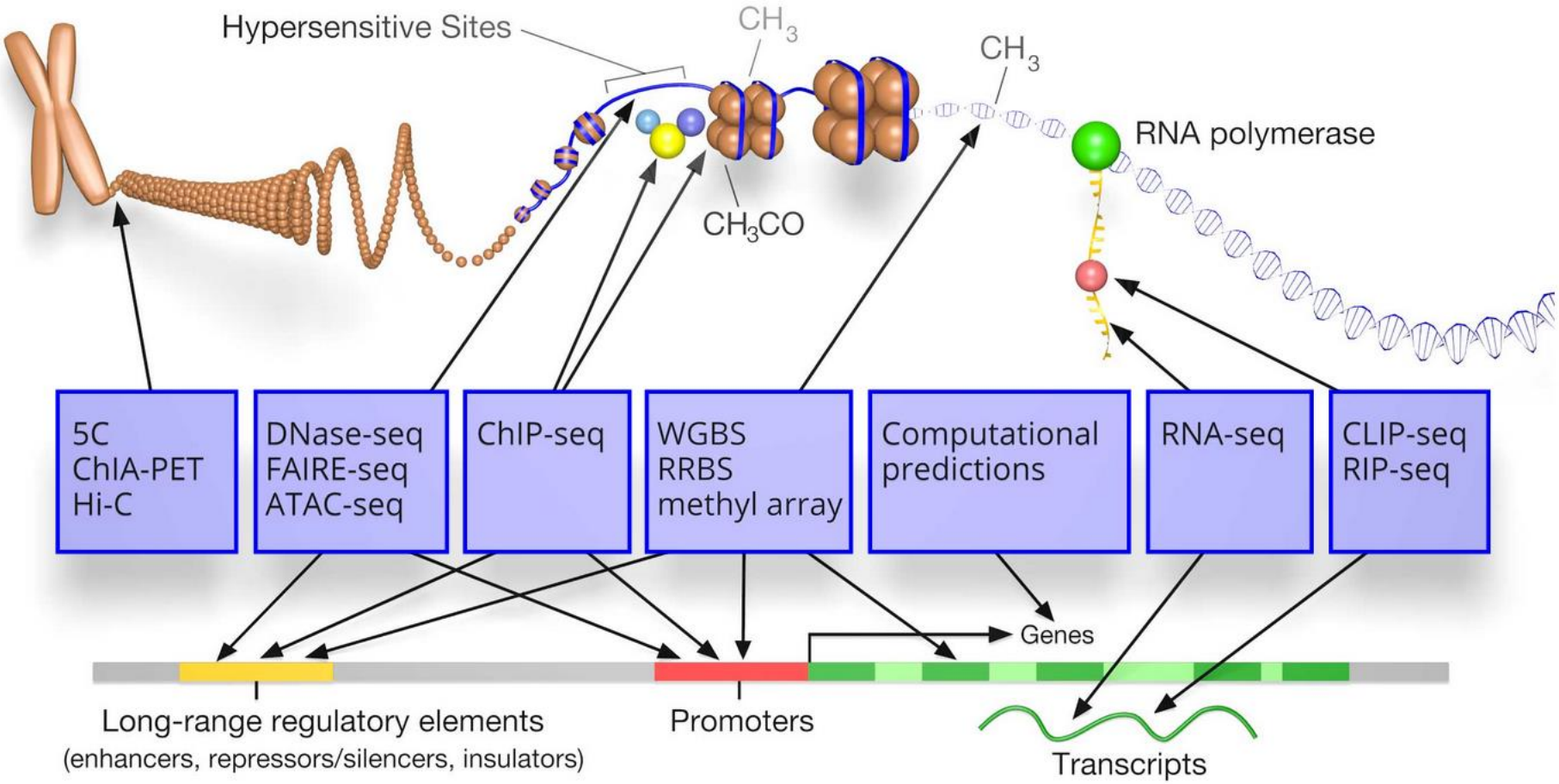
Interchromosomal interactions: A genomic love story of kissing chromosomes. *J Cell Biol* 2019

Chromosome conformation



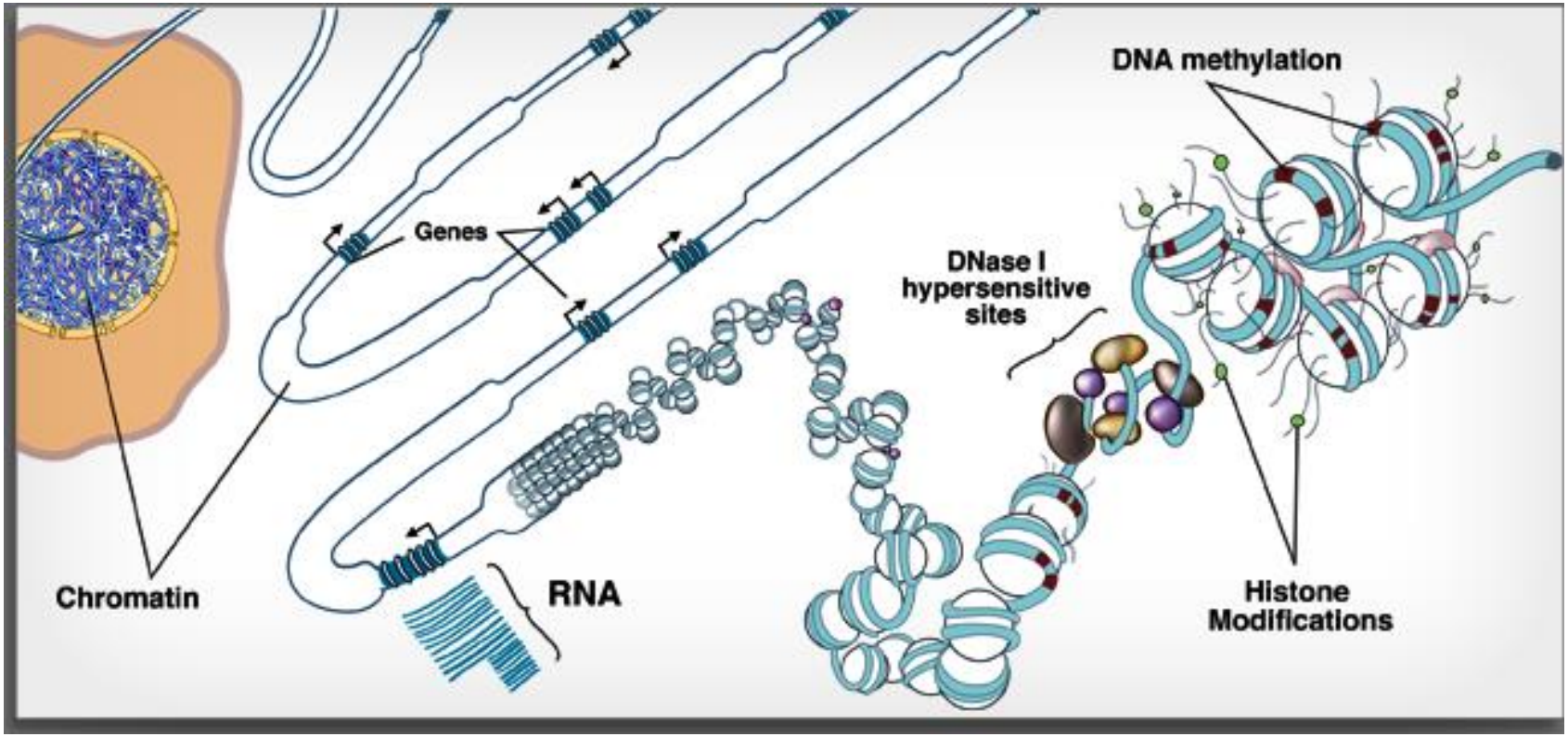
Regulation of gene transcription by Polycomb proteins. *Science Advances* 2015

ENCODE



https://www.encodeproject.org

Roadmap Epigenomics Project



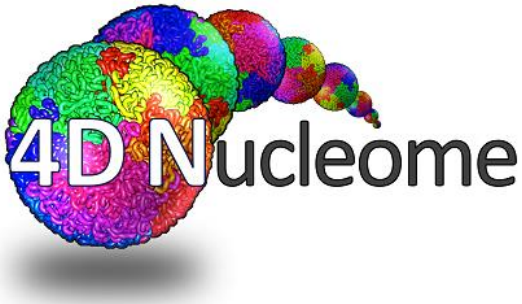
The NIH Roadmap Epigenomics Mapping Consortium was launched with the goal of producing a public resource of human epigenomic data to catalyze basic biology and disease-oriented research.

http://www.roadmapepigenomics.org

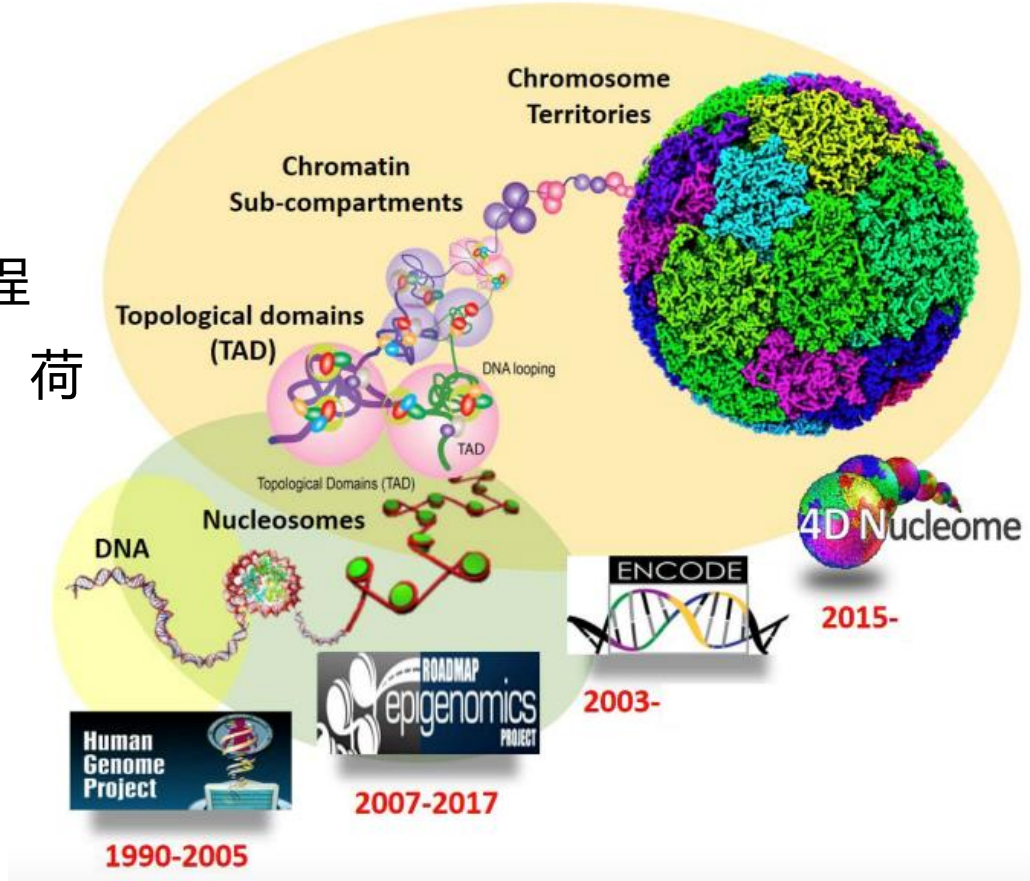
4D基因组学

基因表达和调控随着时间的变化而变化，研究时间动态变化下的基因组三维结构和功能，称之为4D基因组学

- 细胞周期过程
- 细胞发育和分化的不同阶段
- 正常细胞向疾病细胞转变过程
- 细胞受到外界刺激（如用药、荷尔蒙、温度变化、病毒入侵）



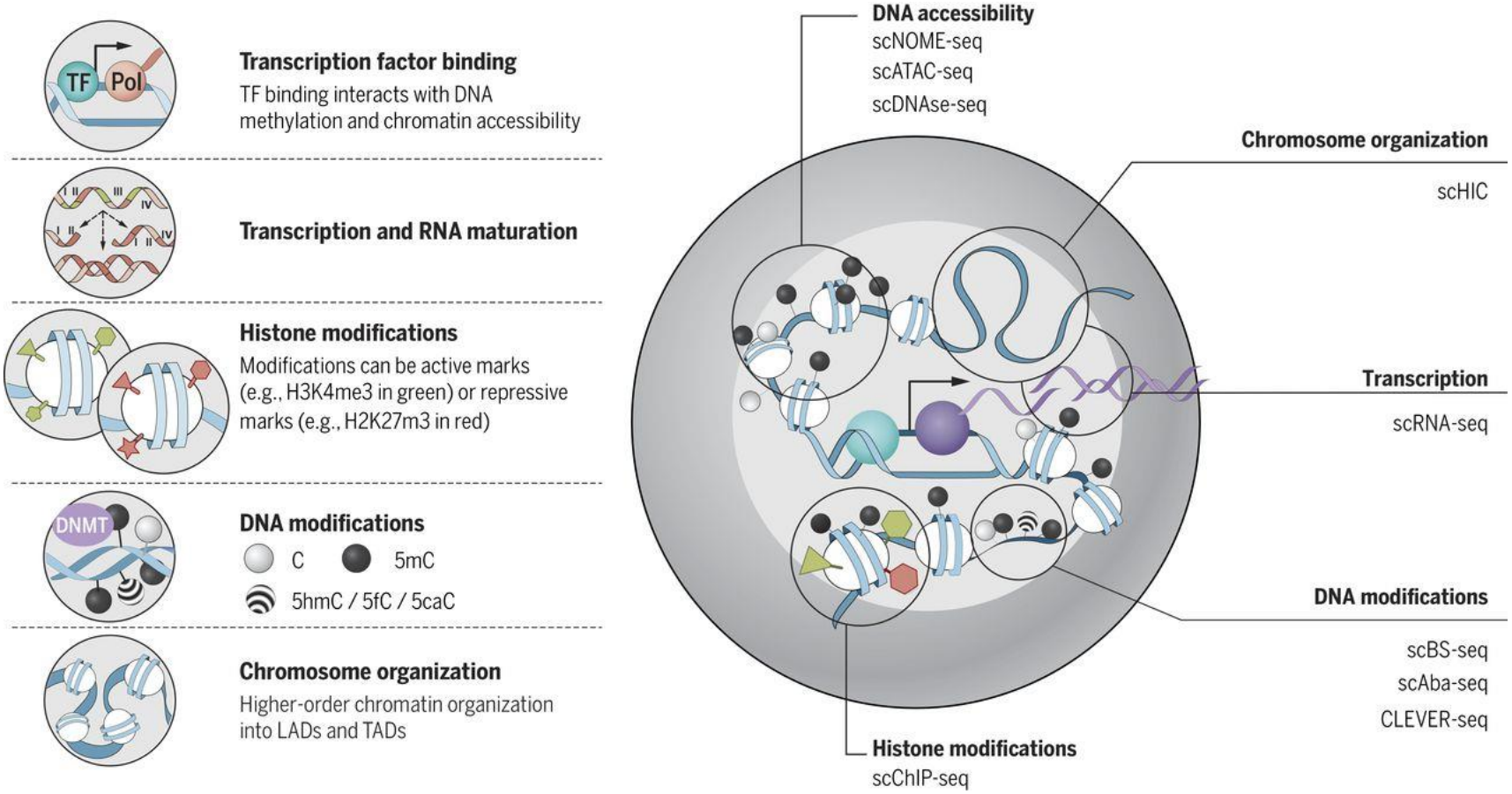
https://www.4dnucleome.org



单细胞表观基因组学

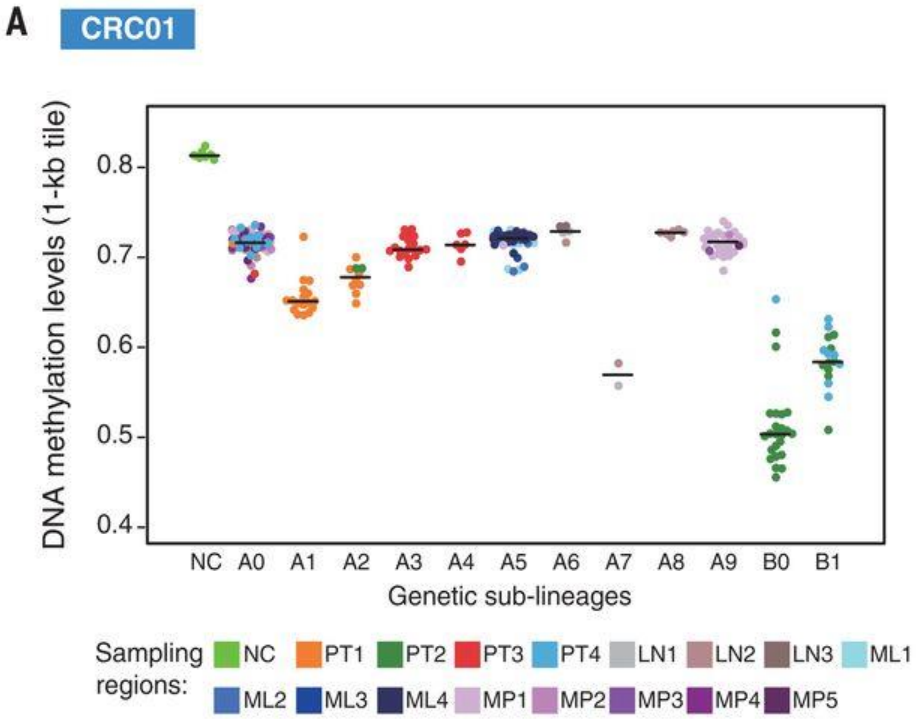
Single-cell epigenomics

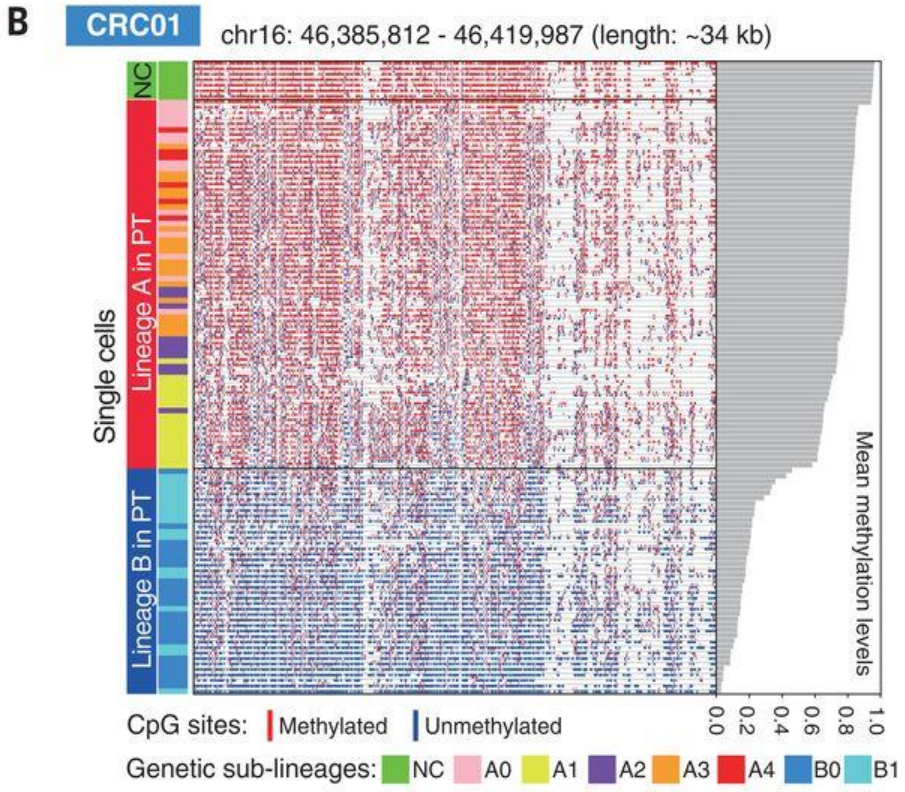
Single-cell epigenomics is the study of epigenomics in individual cells by single cell sequencing.



Single-cell epigenomics: Recording the past and predicting the future. *Science* 2017

Single-cell DNA Methylation

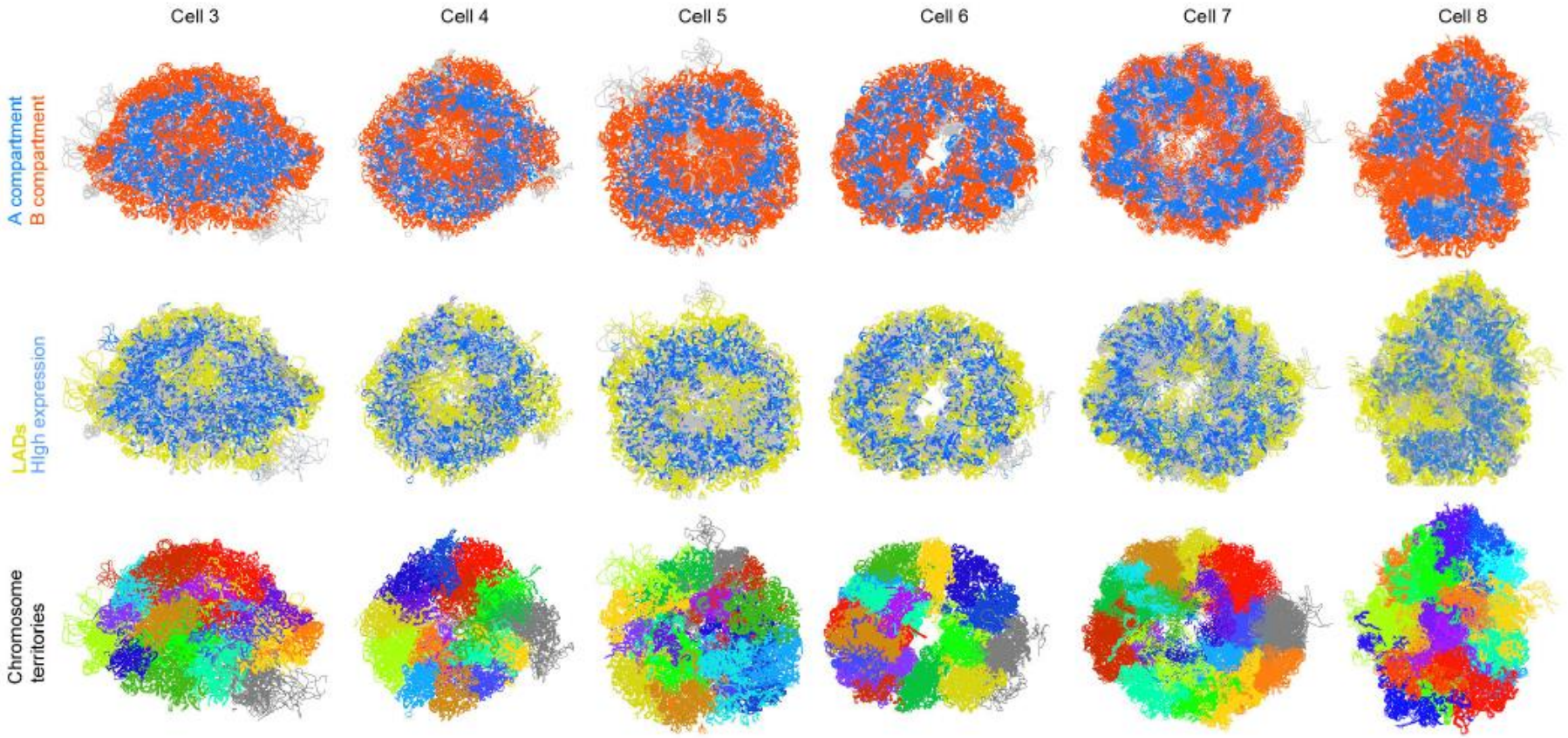




For patient CRC01, we obtained 534 single cells (after quality control) from adjacent normal colon (NC) tissue and 16 tumor regions, including the primary tumor (PT), the lymph node metastasis (LN), the liver metastasis (ML), and a posttreatment liver metastasis (MP) after chemotherapy.

Single-cell multiomics sequencing and analyses of human colorectal cancer. *Science* 2018

Single-cell Hi-C



3D structures of individual mammalian genomes studied by single-cell Hi-C. *Nature* 2017

Summary

- 表观基因组学的定义、研究内容以及特点
- DNA修饰：DNA甲基化、DNAmage、基因组印记
- RNA修饰：mRNA修饰、m^6A、RNA编辑
- 蛋白质修饰：
  - 翻译后修饰（Post-Translational Modification）
  - 组蛋白修饰（Histone Modification）
- 空间变化
  - 三维基因组
  - FISH & 染色体构象捕获（3C）
  - 染色质疆域、区室、TAD、染色质环
- 单细胞表观基因组学（Single-cell Epigenomics）

Further Reading

- **The roles of DNA, RNA and histone methylation in ageing and cancer**. *Nature Reviews Molecular Cell Biology* 2019
- **Genomic imprinting disorders: lessons on how genome, epigenome and environment interact**. *Nature Reviews Genetics* 2019
- **RNA modifications modulate gene expression during development**. *Science* 2018
- **How best to identify chromosomal interactions: a comparison of approaches.** *Nature Methods* 2017
- **Single-cell epigenomics: Recording the past and predicting the future**. *Science* 2017
- **Epigenetics and aging.** *Science Advances* 2016
- **An Overview of Genome Organization and How We Got There: from FISH to Hi-C**. *Microbiol Mol Biol Rev* 2015
- **The Transcriptome and DNA Methylome Landscapes of Human Primordial Germ Cells**. *Cell* 2015
- **A 3D Map of the Human Genome at Kilobase Resolution Reveals Principles of Chromatin Looping.** *Cell* 2014