

От организации хроматина к пониманию функционирования геномов эукариот

Алексей Константинович Шайтан

д.ф.-м.н., профессор, чл.-корр. РАН

кафедра биоинженерии

биологический факультет

МГУ имени М.В.Ломоносова

**Лекция 3.
Эпигенетика.**

Апрель 2023

Содержание лекции

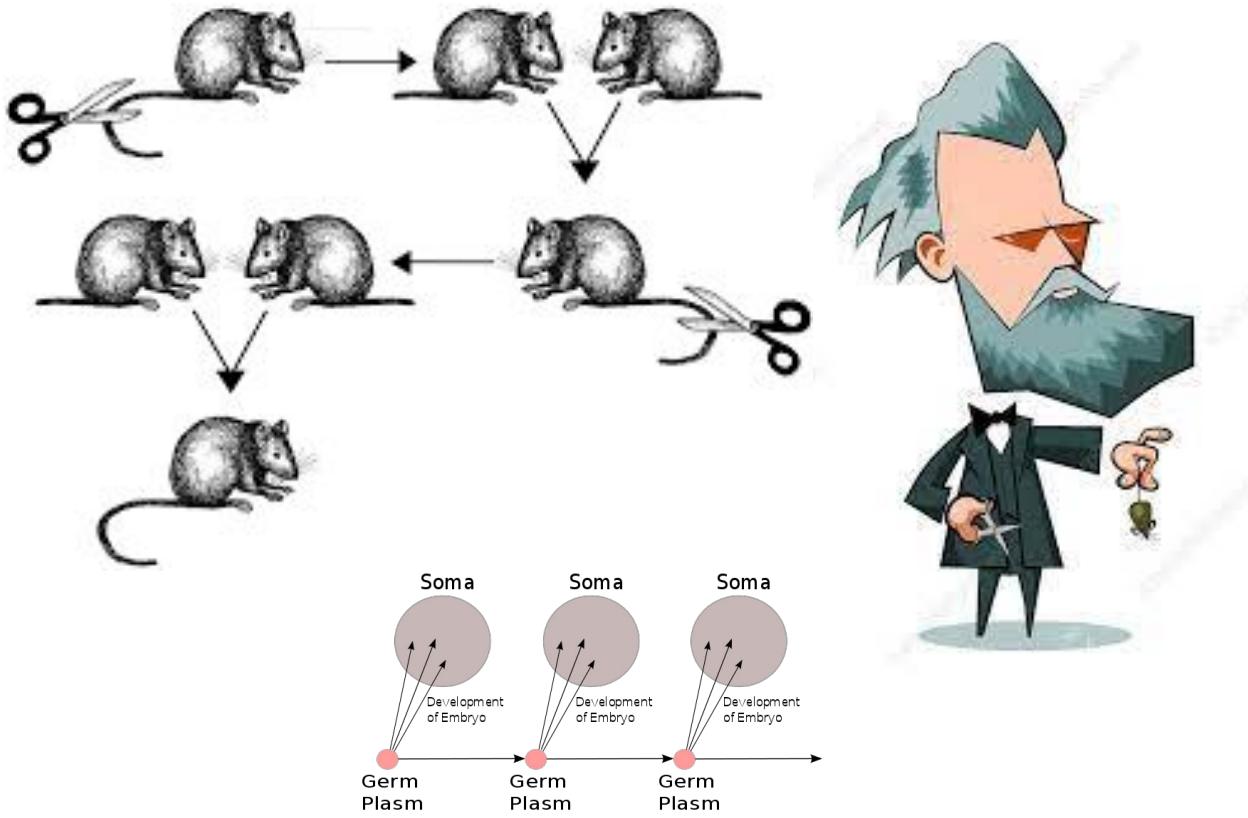
- История вопроса. К. Уаддингтон.
- ДНК метилирование. Эффекты. Ферменты. Промотеры и CpG островки. Мутации. Наследование. Импринтинг.
- ПТМ гистонов. Цвета хроматина.
- Ацетилирование, метилирование. Ингибиторы.
- Шапероны гистонов.
- Ремоделлеры. pBAF.
- Сопряжение меток и метилирования. CoREST?
- Сpreadинг гетерохроматина. Границы.
- Расположение нуклеосом. +1 нуклеосомы.

Эпигенетика

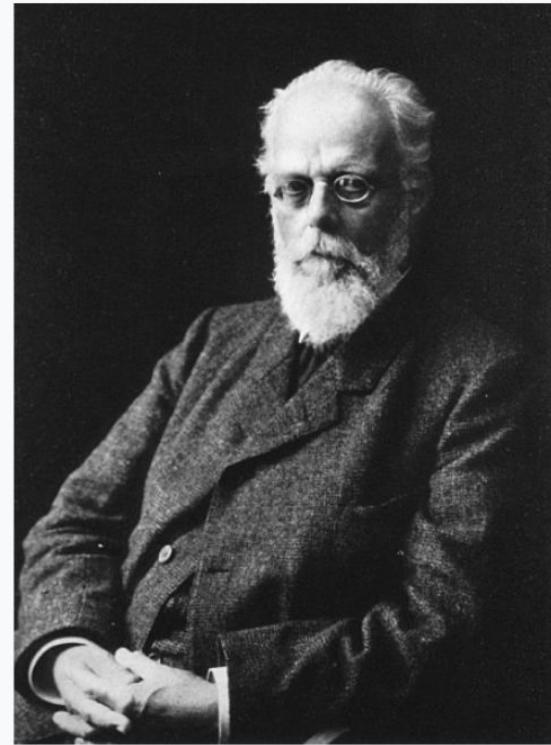
- Epigenetics (also sometimes called epigenomics) is a field of study focused on changes in DNA that do not involve alterations to the underlying sequence. The DNA letters and the proteins that interact with DNA can have chemical modifications that change the degrees to which genes are turned on and off. Certain epigenetic modifications may be passed on from parent cell to daughter cell during cell division or from one generation to the next. The collection of all epigenetic changes in a genome is called an epigenome. (NHGRI)

др.-греч. ἐπι— приставка, обозначающая пребывание на чём-либо
или помещение на что-либо

История вопроса



August Weismann



Born 17 January 1834
[Frankfurt am Main](#), Germany

Died 5 November 1914 (aged 80)
[Freiburg](#), Germany

Known for germ plasm theory

История вопроса

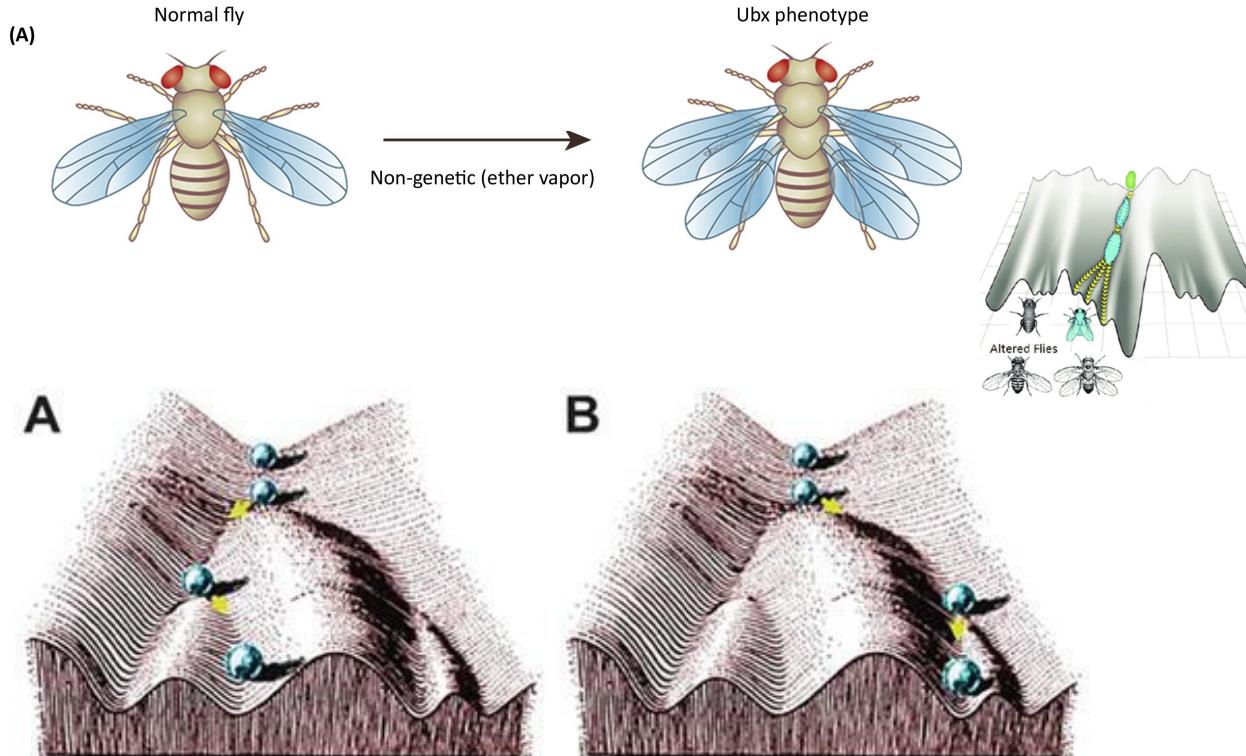
Conrad Hal Waddington

CBE FRS FRSE



Conrad Hal Waddington in 1934

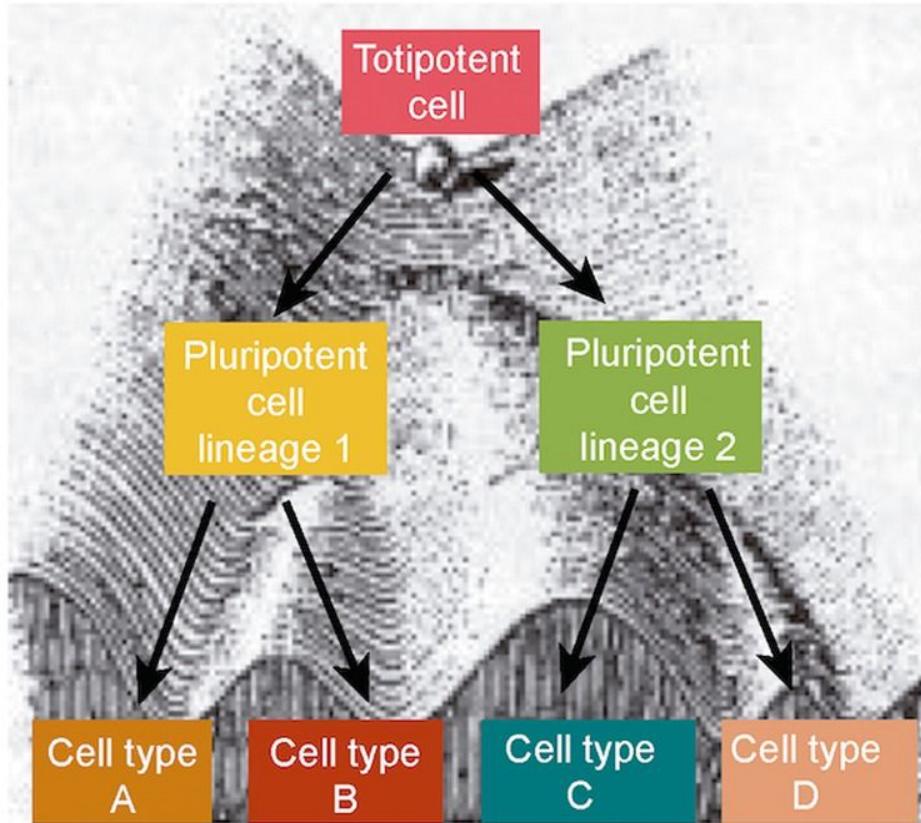
Born	8 November 1905 Evesham, Worcestershire, England
Died	26 September 1975 (aged 69) Edinburgh, Scotland



Понятие эпигенетического ландшафта развития, фенотипической пластичности, эпигенетического наследования, генетической ассимиляции

[10.1242/jeb.120071](https://doi.org/10.1242/jeb.120071)

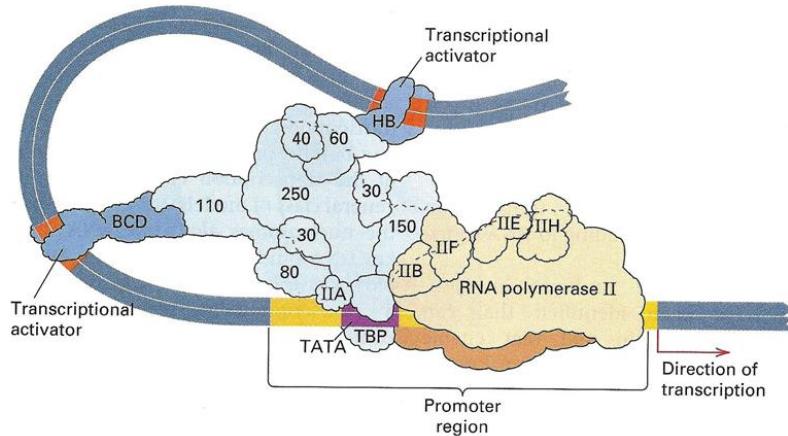
Multicellular eukaryote



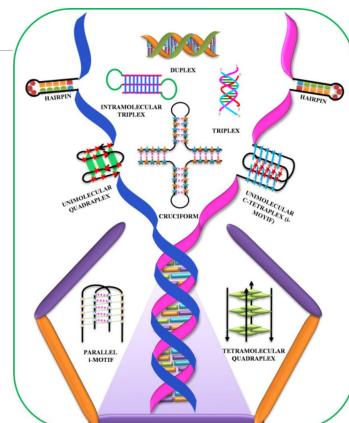
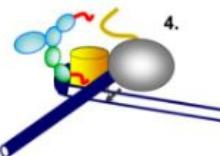
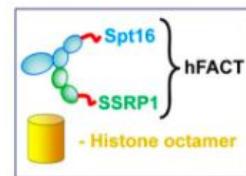
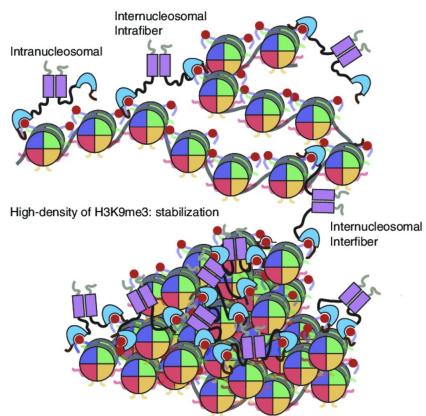
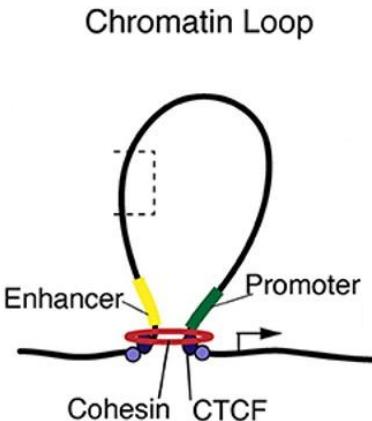
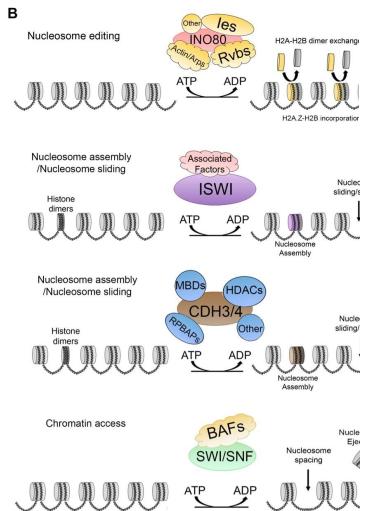
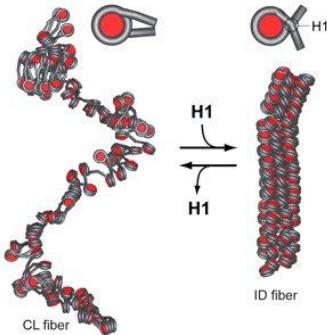
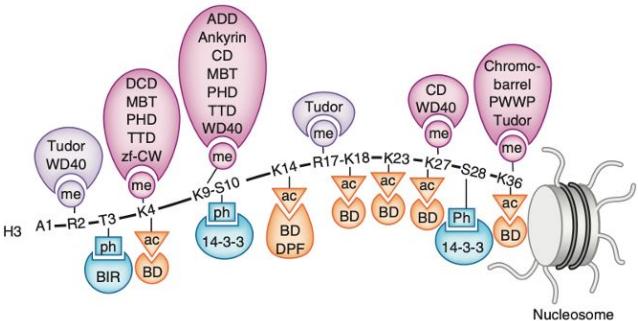
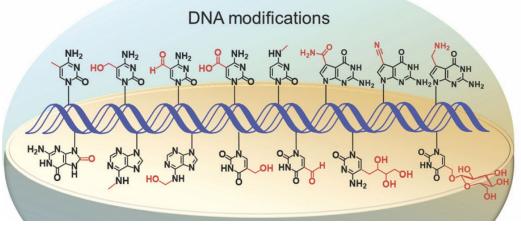
Молекулярные механизмы эпигенетики

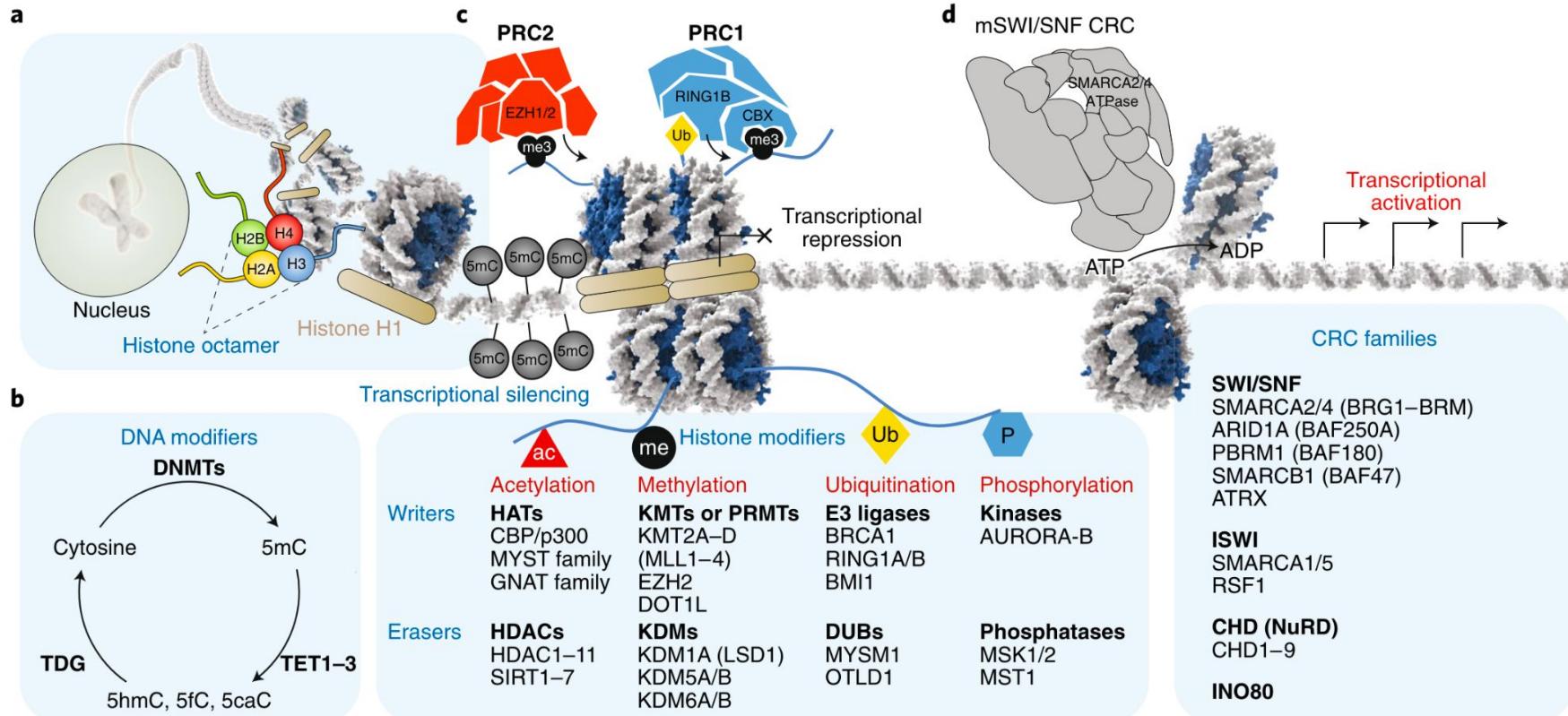
Что влияет на транскрипцию генов?

Эффективность инициации транскрипции, эффективность элонгации



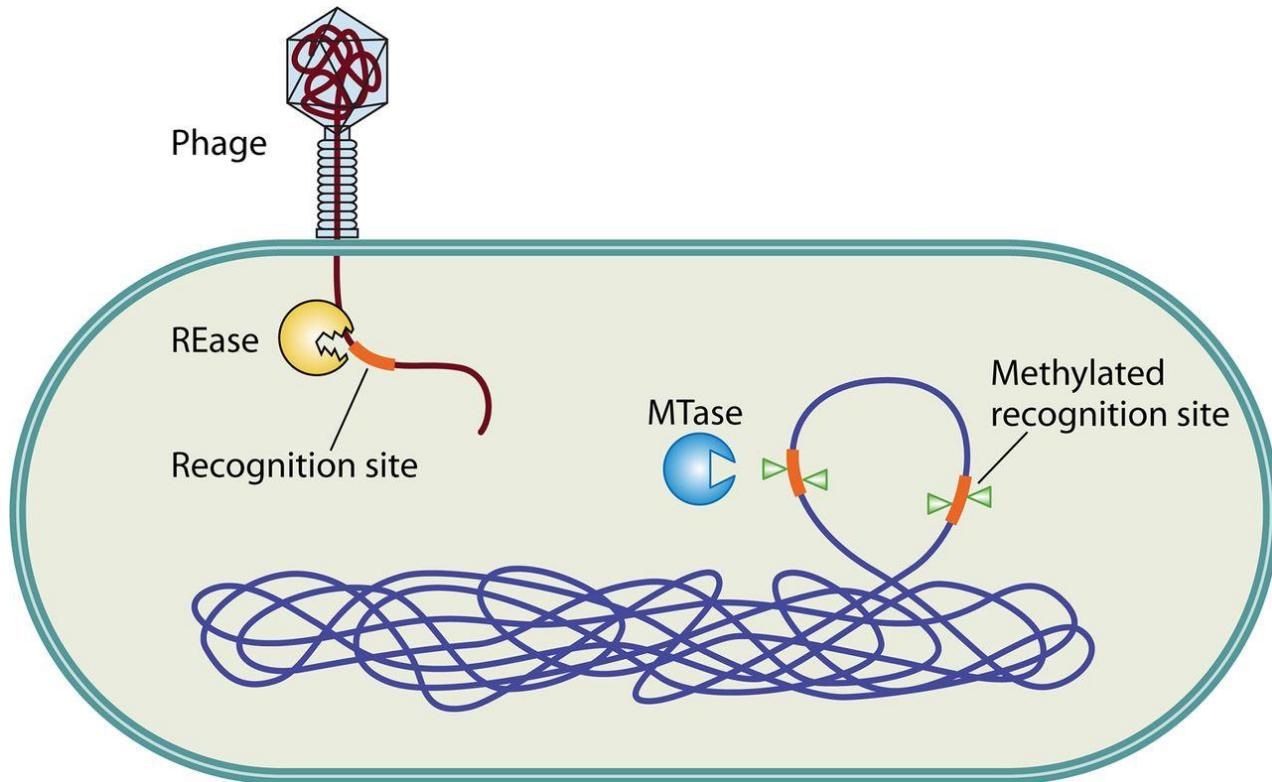
- 1) Доступностью ДНК для связывания (расположение нуклеосом, компактность хроматина)
- 2) Легкостью транскрипции через нуклеосомы
- 3) Легкостью формирования петель
- 4) Модификациями ДНК помогающими или затрудняющими связывания факторов
- 5) Модификациями гистонов помогающими или затрудняющими связывания факторов





.1 | Chromatin regulatory processes in mammalian cells. **a**, DNA is wrapped around a histone octamer containing two copies each of histones H2A,

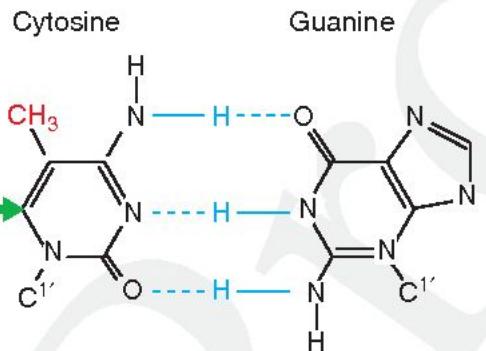
Системы рестрикции-модификации в бактериях



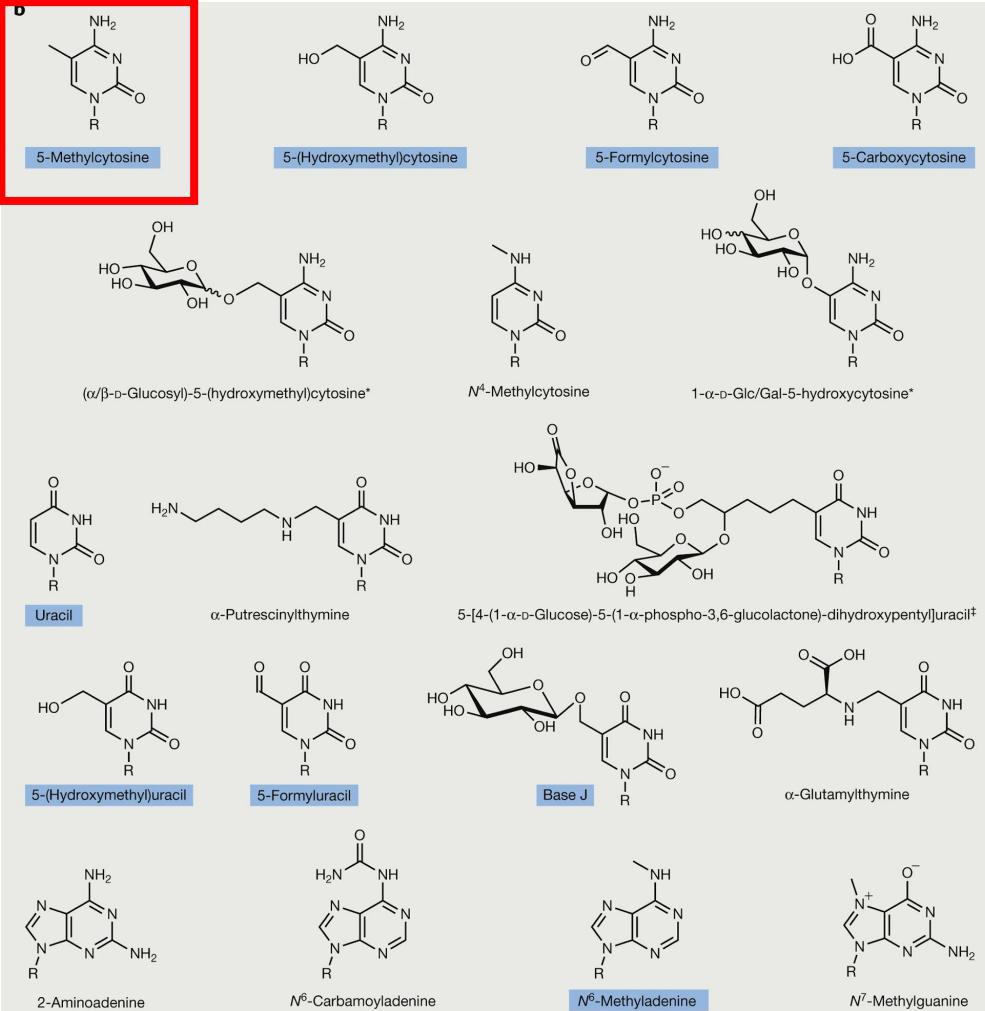
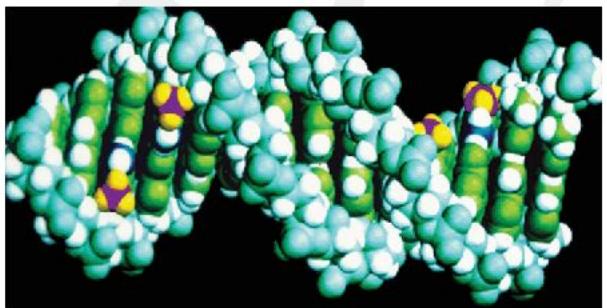
1960-ые годы

Метилирование ДНК

A



B

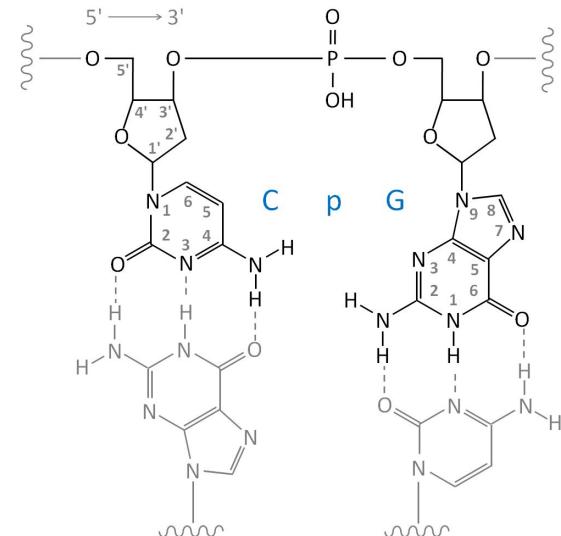
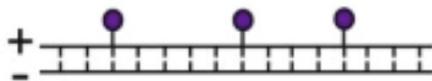
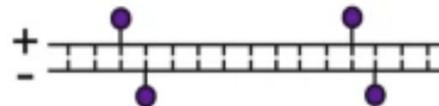
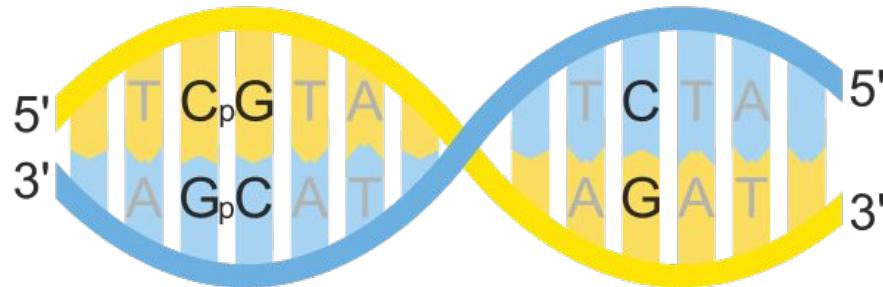


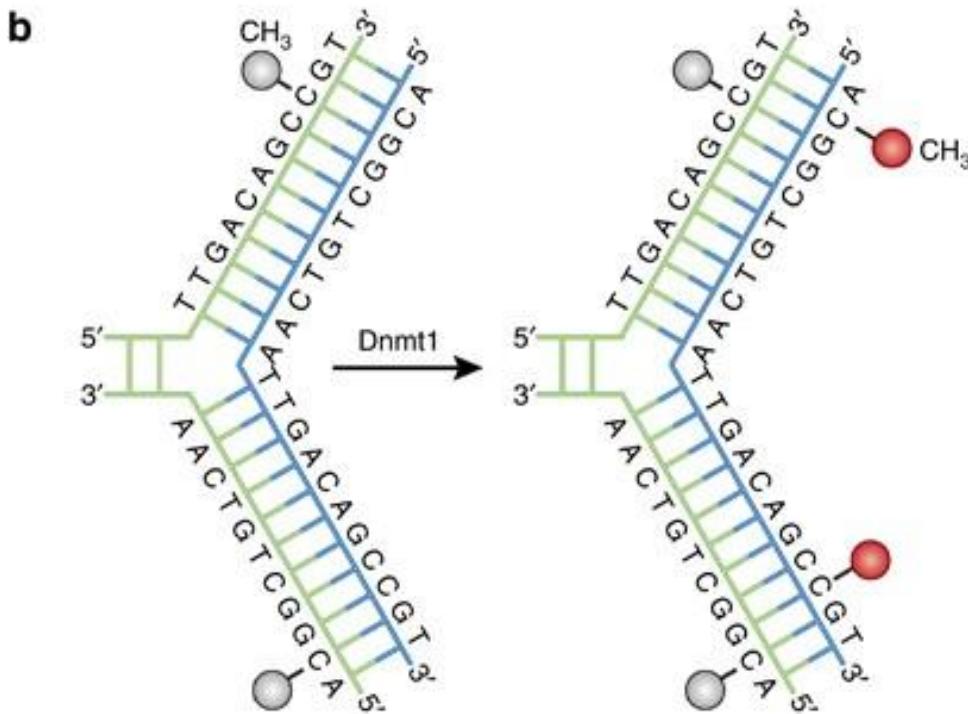
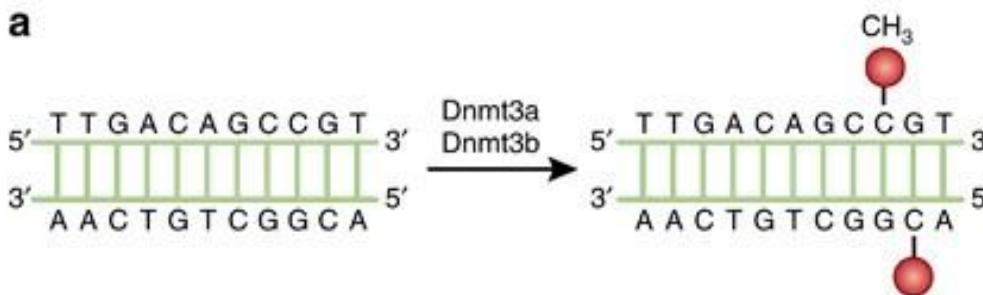
Метилирование ДНК у млекопитающих -- история

- 1970-ые: Метилирование распределено неравномерно по геному, разное в разных тканях, мешает транскрипции генов
- 1980-ые: метилирование идет в контексте CpG, они распределены неравномерно, существуют островки CpG, открыты ферменты – метилтрансферазы. Метилирование промоторов подавляет транскрипцию.
- 1990-ые: открыты белки узнающие метилированную ДНК, открыты де novo метил трансферазы
- 2000-ые: Большинство промоторов не регулируется посредством метилирования – для понимания регуляции генов необходимо рассматривать метилирование во взаимодействии с модификациями гистонов. Транспозоны активно регулируются метилированием ДНК.

CpG

- Метилирование у млекопитающих идет в контексте CpG





ДНК метилтрансферазы

m6A - those that generate N6-methyladenine EC 2.1.1.72 ↗

m4C - those that generate N4-methylcytosine EC 2.1.1.113 ↗

m5C - those that generate C5-methylcytosine EC 2.1.1.37 ↗

мС5 ДНК метилтрансферазы человека:

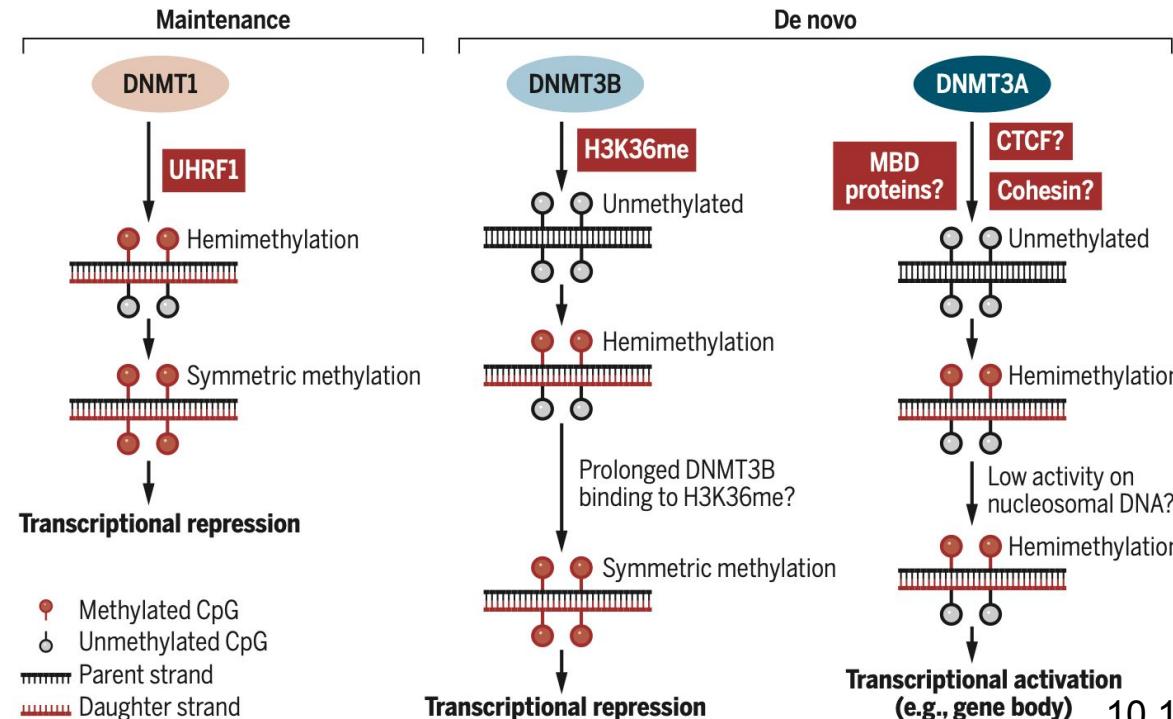
1. DNMT1
2. DNMT3a1, DNMT3a2
3. DNMT3b
4. DNMT3c (в половых клетках?)
5. DNMT3L (вспомогательная функция к DNMT3a)

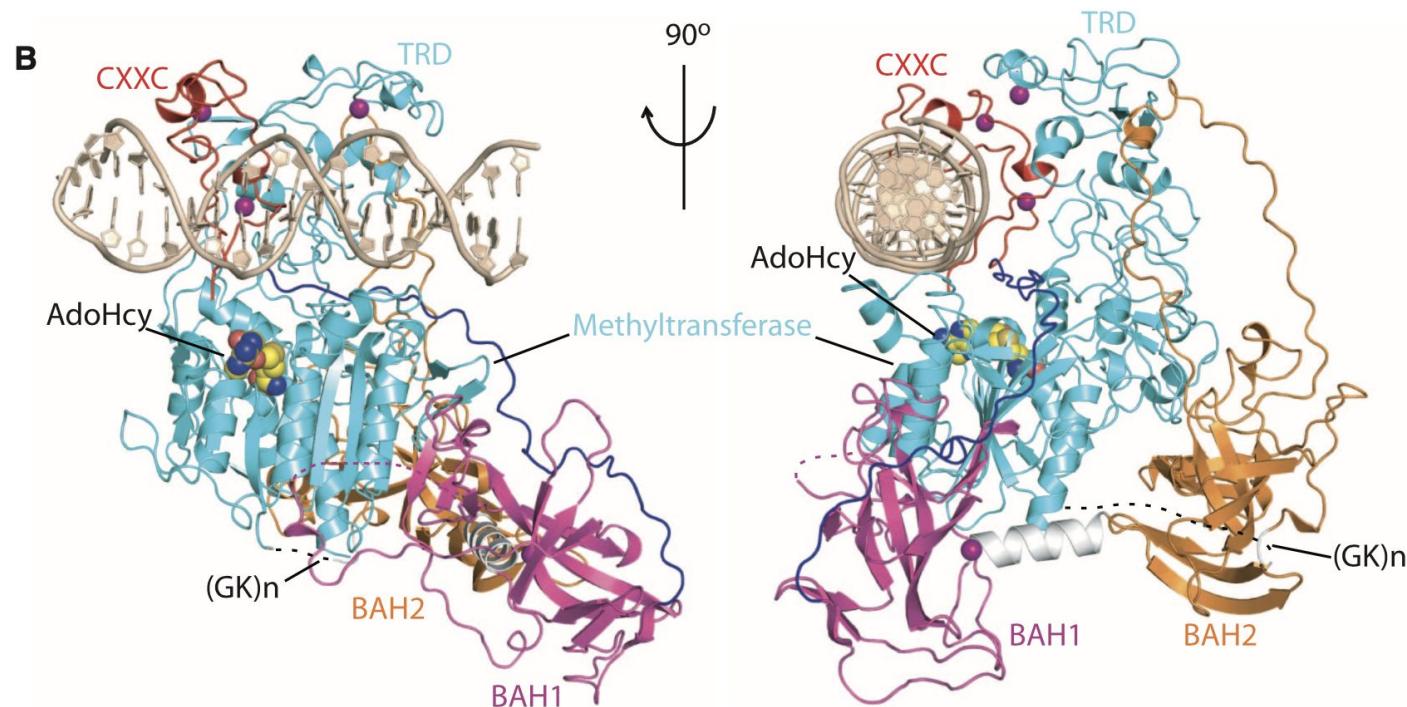
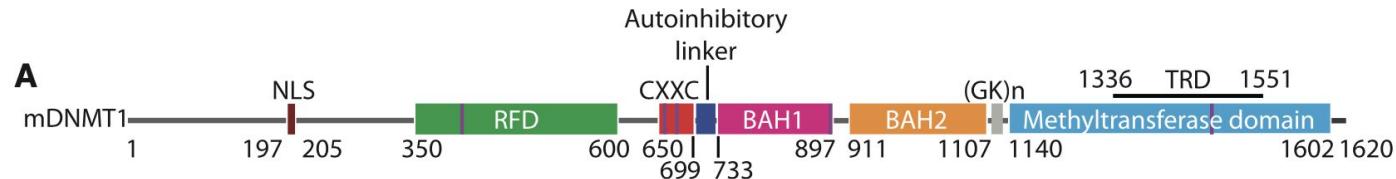
ДНК метилтрансферазы

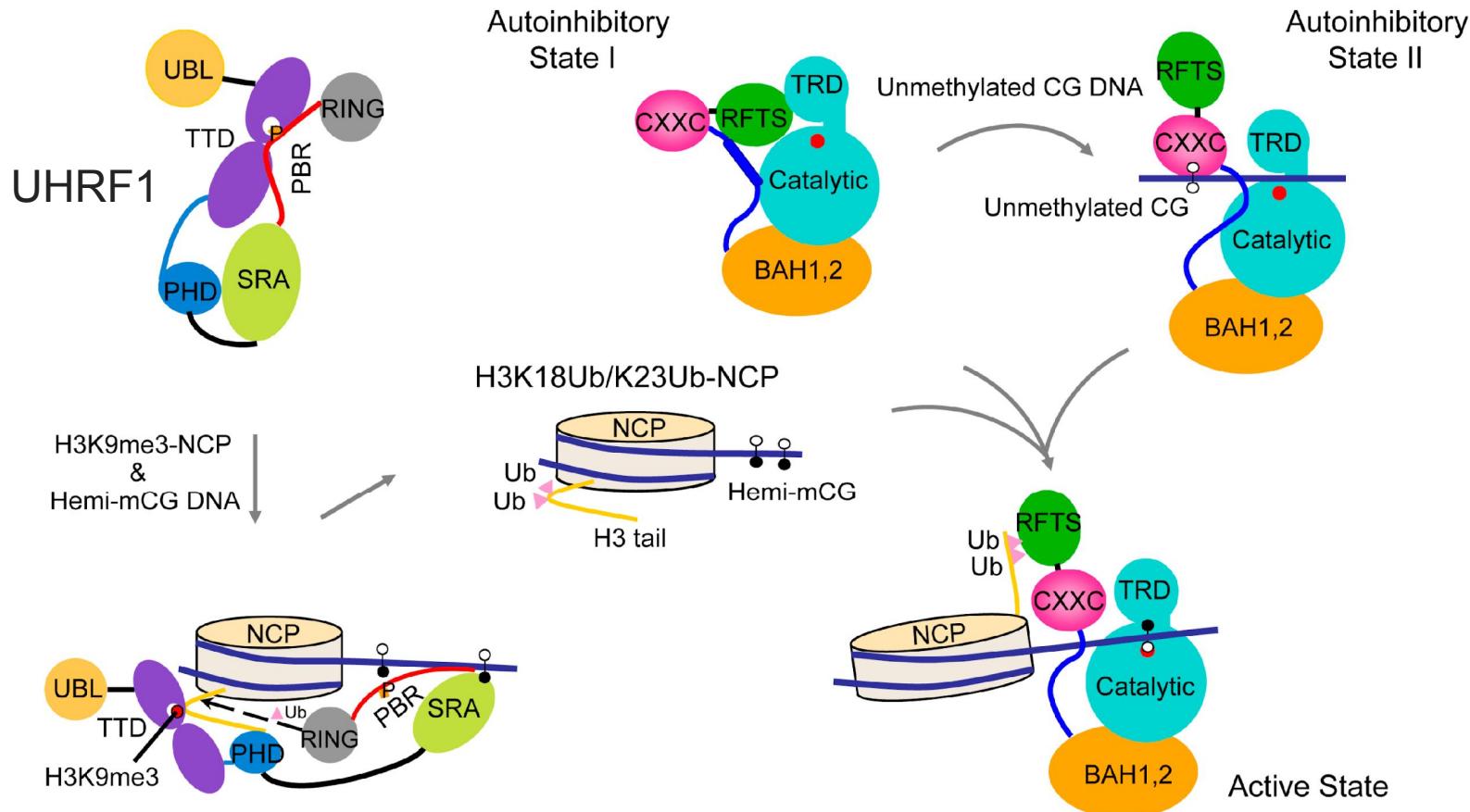
The fate of hemimethylated DNA

After DNA replication, hemimethylated CpGs are converted to symmetrical methylation by DNMT1.

De novo symmetric methylation by DNMT3B is possibly mediated by H3K36me binding. DNMT3A maintains hemimethylated DNA at specific loci, potentially marked by CTCF-cohesin and MBD proteins.



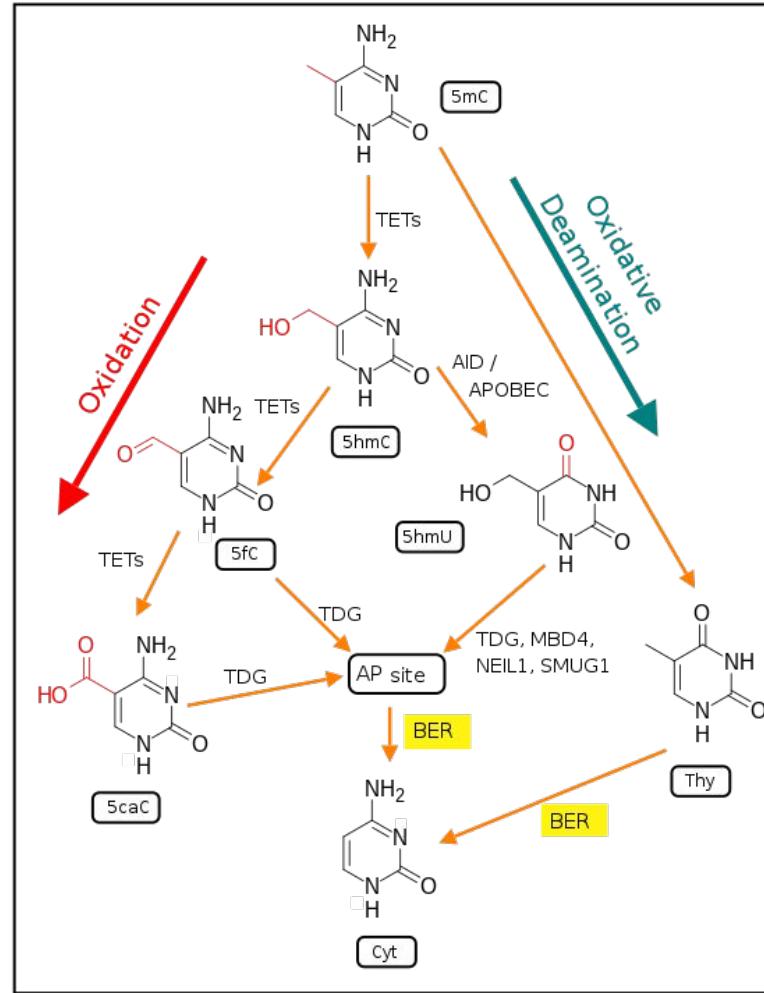


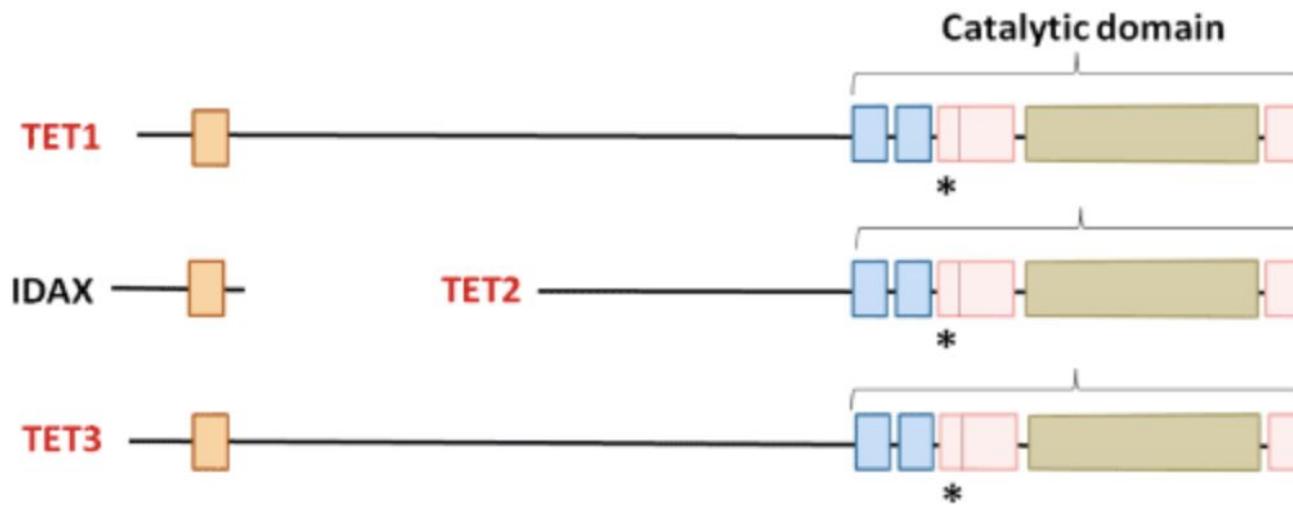


Деметилирование

Ten-eleven translocation (Tet) proteins catalyze 5-methylcytosine (5 mC) conversion to 5-hydroxymethylcytosine (5 hmC)

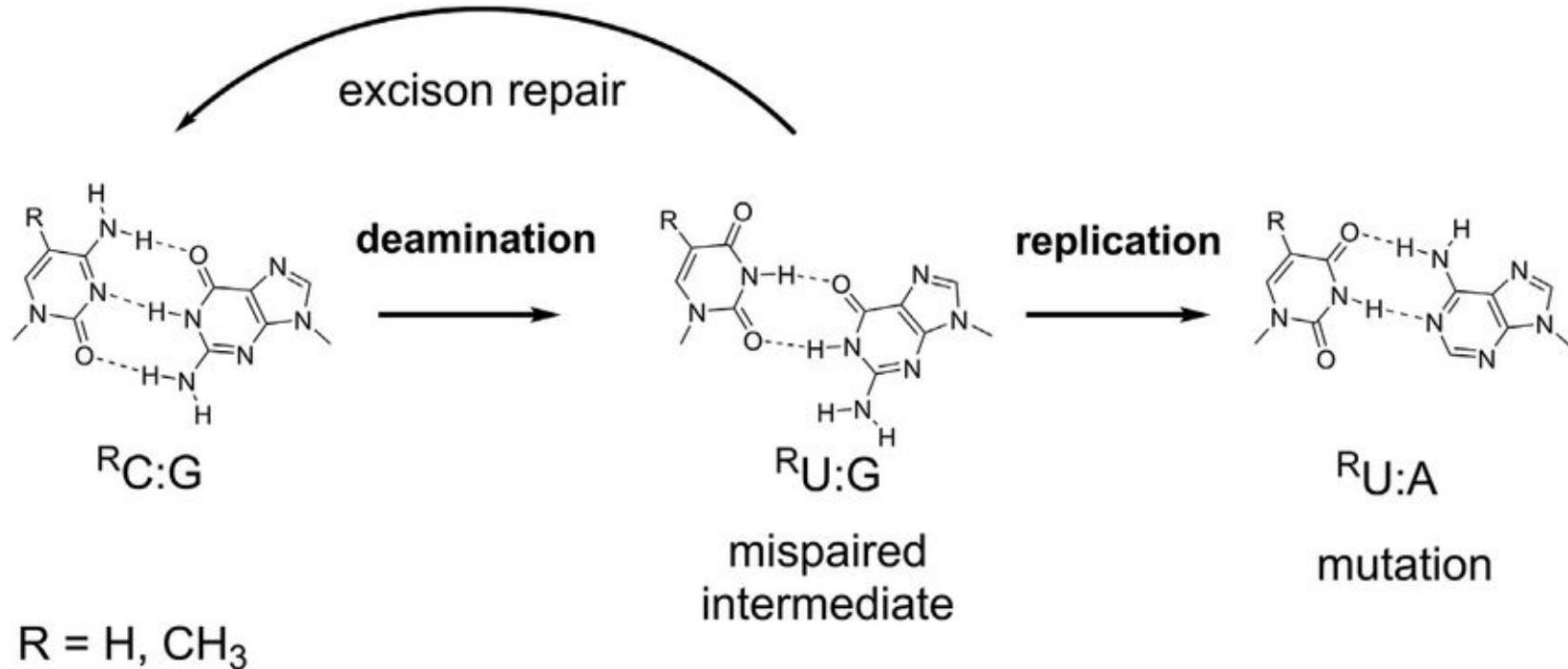
Thymine-DNA glycosylase
(TDG)





CXXC (binding to CpG Island)	Cys-rich (Methyl-C dioxygenase activity)	DSBH (Methyl-C dioxygenase activity)	Spacer (unkown function)	* Fe(II) Interacting
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CpG и мутации (C>T)



Распределение CpG

- У человека GC-состав ~40%. Теоретическая вероятность CpG $0.2 \times 0.2 = 0.04$
- Реальная встречаемость CpG 1%.
- У млекопитающих, 70% - 80% CpG сайтов метилировано
- У человека около 70% промоторов, расположенных около сайта начала транскрипции содержат CpG островки (island).

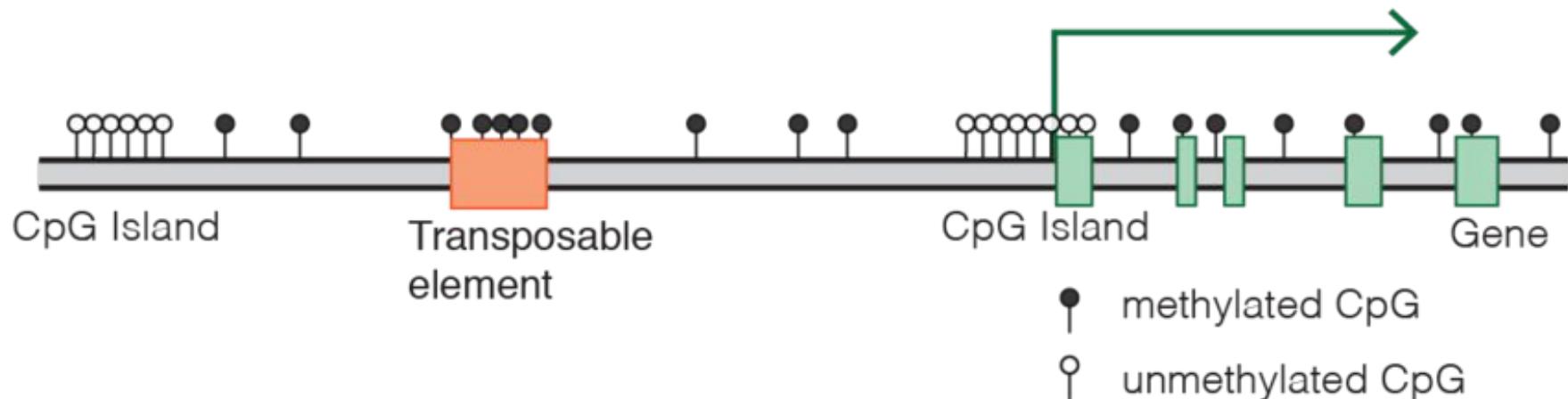
CpG-островки

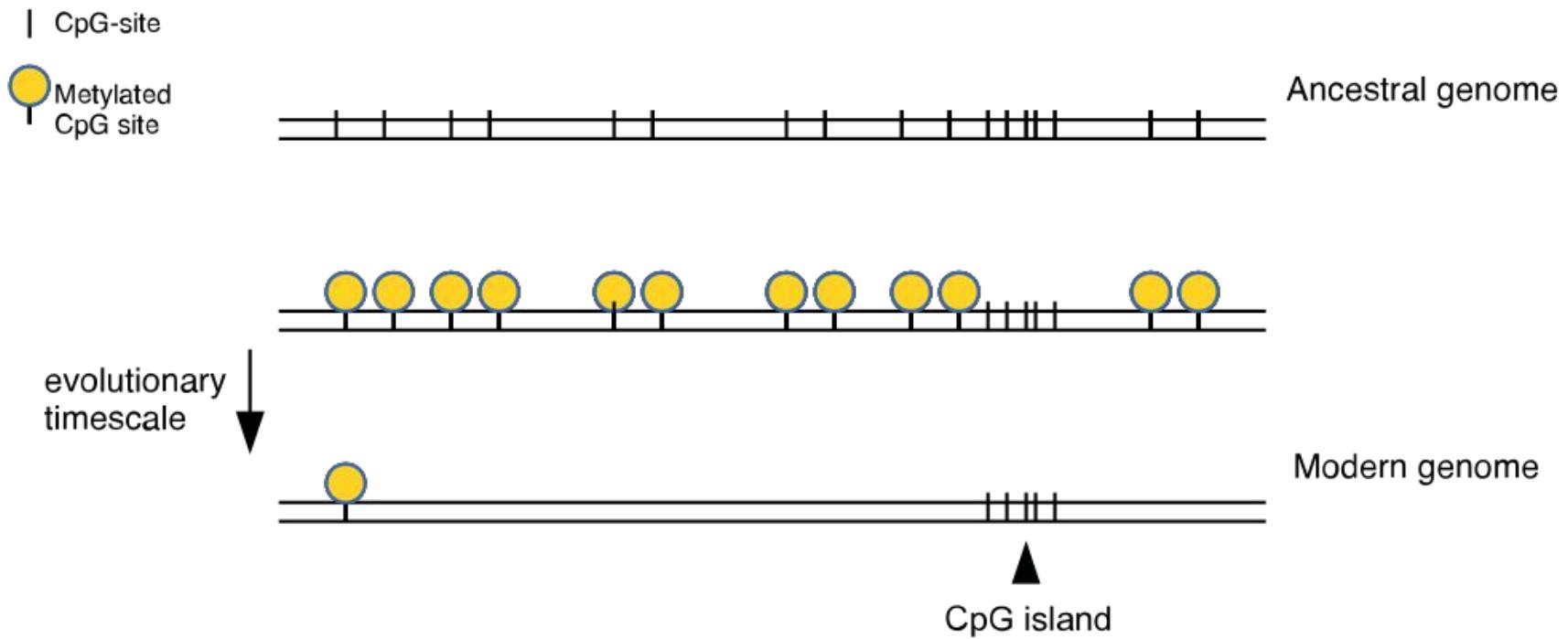
- Регион длиной около 200 п.н.
- GC состав более 50%
- Наблюдаемое количество CpG по отношению к ожидаемому при случайном перемешивании последовательности более 60%
- Часто располагаются вблизи начала гена в районе промоторов.

CpG sites

GpC sites

Typical mammalian DNA methylation landscape

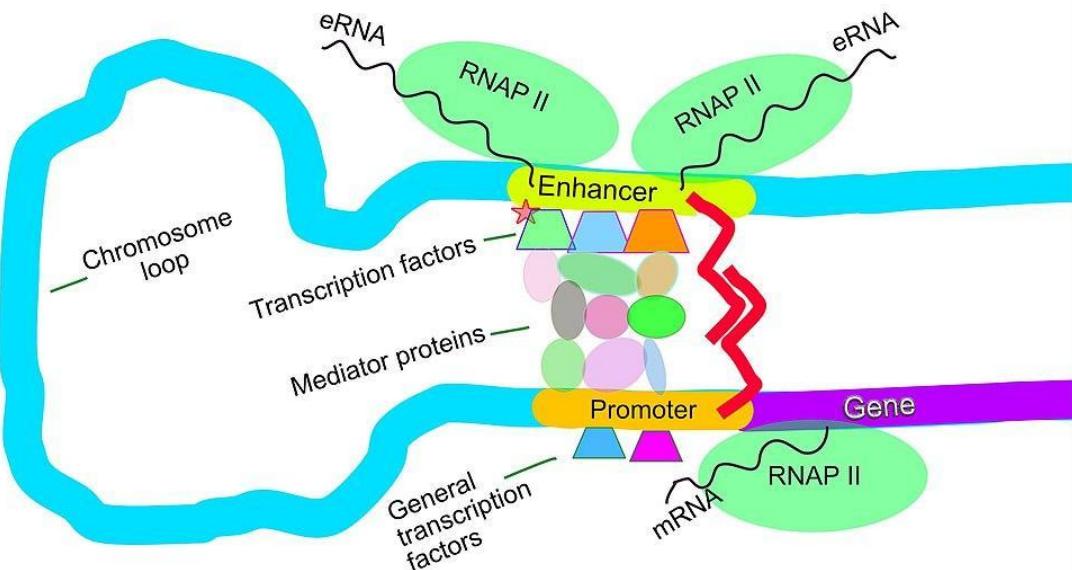




Функциональная роль метилирования

- Метилирование CpG-островков вблизи промоторов коррелирует с репрессией транскрипции.
- Однако, оно не обязательно для репрессии.
- В теле активно транскрибуемых генов наблюдается высокий уровень метилирования ДНК.

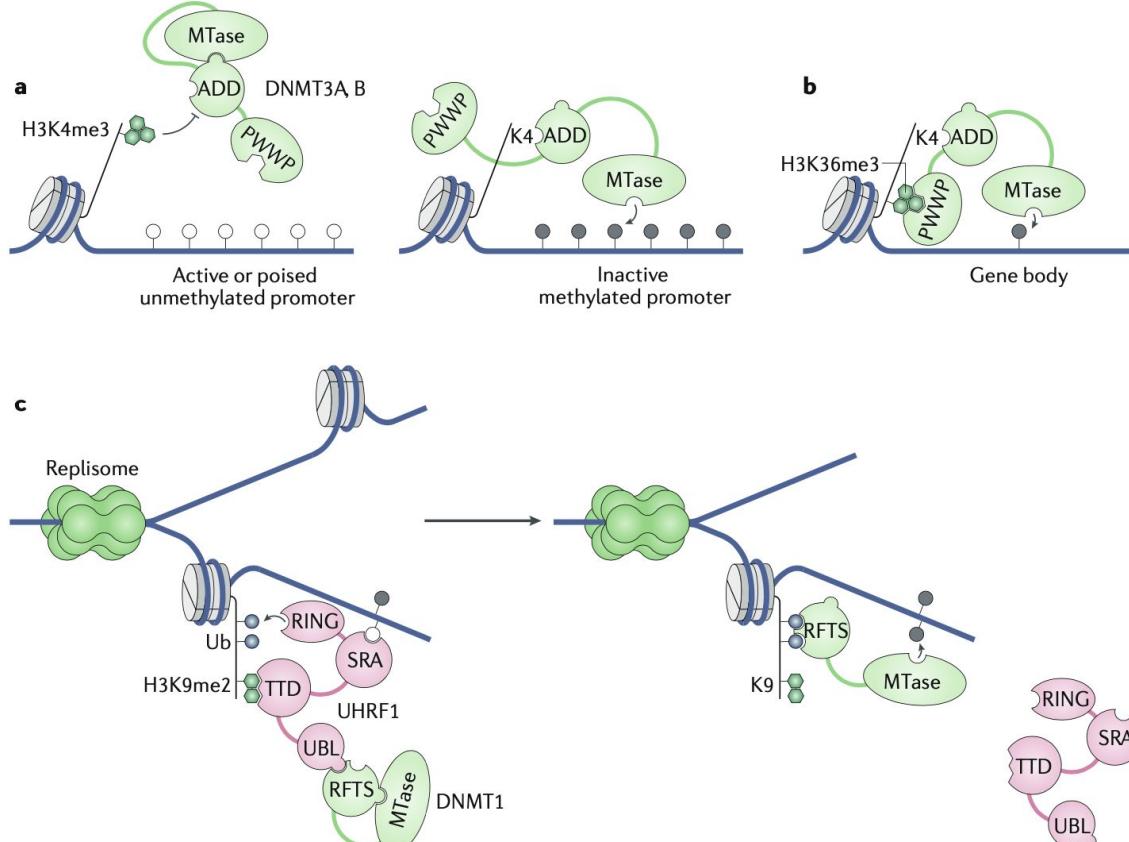
Каким образом метилирование управляет экспрессией генов?



1. Мешает связыванию белков с ДНК
2. Привлекает регуляторные белковые комплексы
3. Стабилизирует нуклеосомы (?)

Примеры молекулярных механизмов (репрессия)

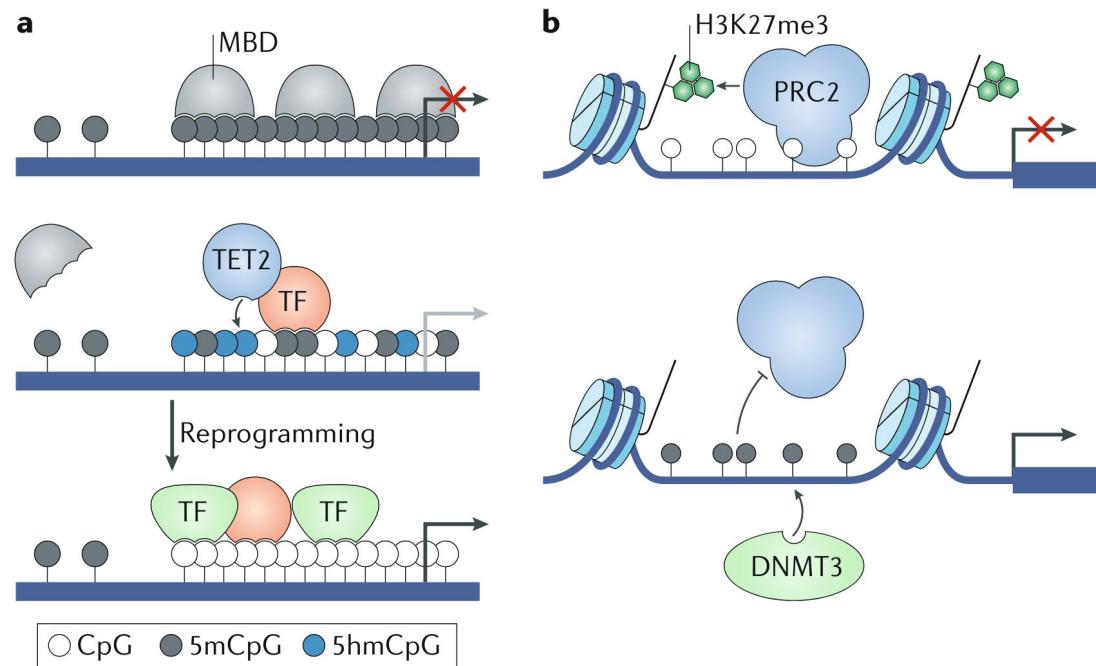
30



Примеры молекулярных механизмов (активация)

31

cell pluripotency factors KLF4 and OCT4, the homeobox proteins HOXB13, NKX neural patterning factors



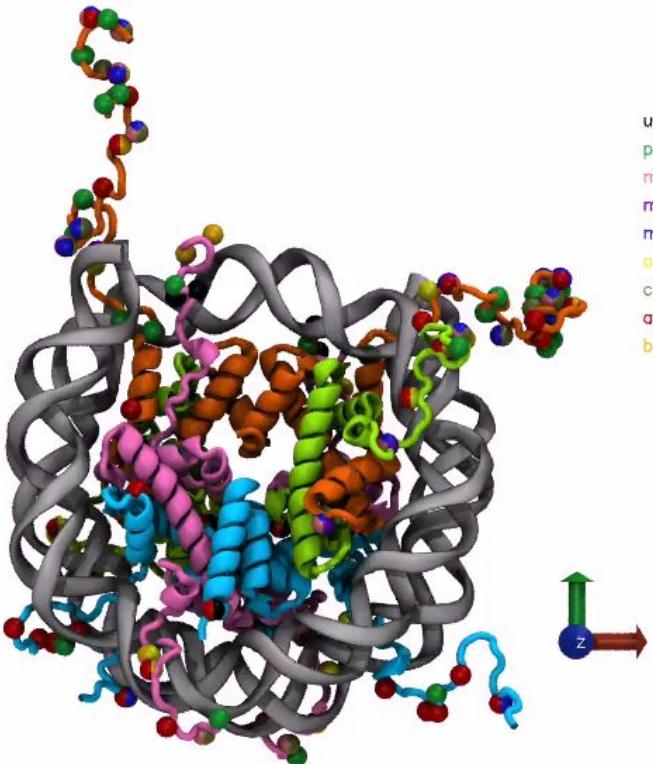
DNA methylation-mediated transcription activation

10.1038/s41580-019-0159-6

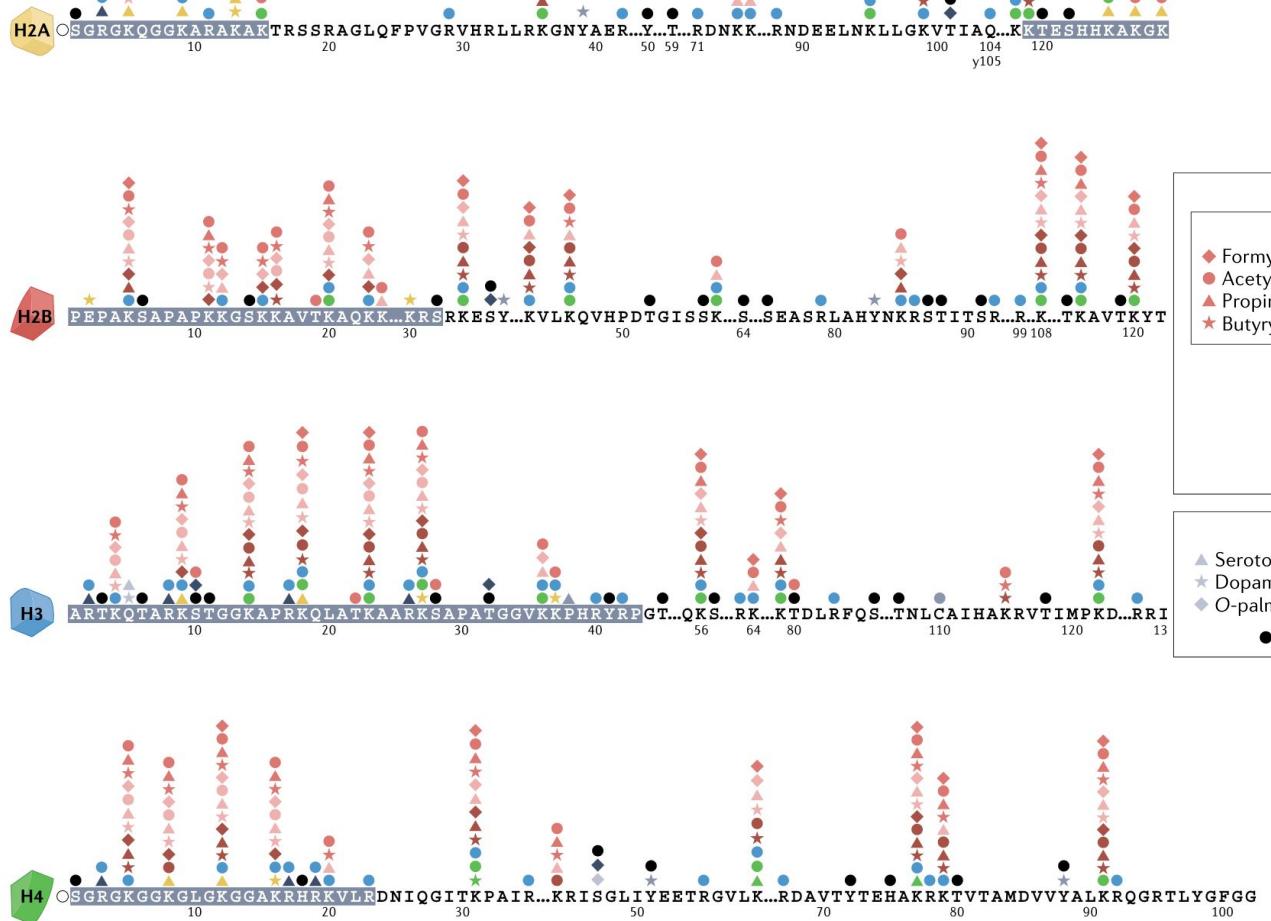
Пост-трансляционные модификации гистонов

Histone post-translational modifications

Nucleosome structure (1KX5)



H2A	H2B	H3	H4
H2AS1ph	H2BK5ac	H3R2me1	H4S1ph
H2AR3me2	H2BK5me1	H3R2me2	H4R3me1
H2AR3ci	H2BK12ac	H3R2ci	H4R3ci
H2AK5ac	H2BS14ph	H3T3ph	H4R3ci
H2AK9ac	H2BK15ac	H3K4ac	H4K5ac
H2AK9bio	H2BK16ac	H3K4me1	H4K8ac
H2AK13bio	H2BK20ac	H3K4me2	H4K8bio
H2AK13or	H2BK30or	H3K4me3	H4K12ac
H2AK119ub	H2BK46ac	H3S6ph	H4K12bio
H2AT120ph	H2BK120ac	H3T6ph	H4K16ac
H2AK121ub	H2BK120ub	H3R8ci	H4K16or
H2AK125bio	H2AK127bio	H3K9ac	H4K20me1
H2AK129bio	H2AS137ph	H3K9me1	H4K20me2
H2AS139ph	H2AS139ph	H3K9me2	H4K20me3
H2AY142ph	H2AY142ph	H3K9me3	H4K91ac
		H3K9bio	H4K91ub
		H3S10ph	
		H3T11ph	
		H3K14ac	
		H3R17me1	
		H3R17me2	
		H3R17ci	
		H3K18ac	
		H3K18bio	
		H3K23ac	
		H3R26me1	
		H3R26ci	
		H3K27ac	
		H3K27me1	
		H3K27me2	
		H3K27me3	
		H3K27ar	
		H3S28ph	
		H3S31ph	
		H3K36ac	
		H3K36me3	
		H3K36me1	
		H3K36me2	
		H3K37ar	
		H3Y41ph	
		H3T45ph	
		H3K56ac	
		H3K79me1	
		H3K79me2	

a

Lysine PTMs (and other aa as indicated)

Acylations		
Formylation (K)	Crotonylation (K)	Lactylation (K)
Acetylation (K, S, T)	Benzoylation (K)	Malonylation (K)
Propinylaion (K)	2-Hydroxyisobutyrylation (K)	Succinylation (K)
Butyrylation (K)	Hydroxybutyrylation (K)	Glutarylation (K)

Ubiquitin-like

Ubiquitylation (K)
Sumoylation (K)
Ufmylation (K)

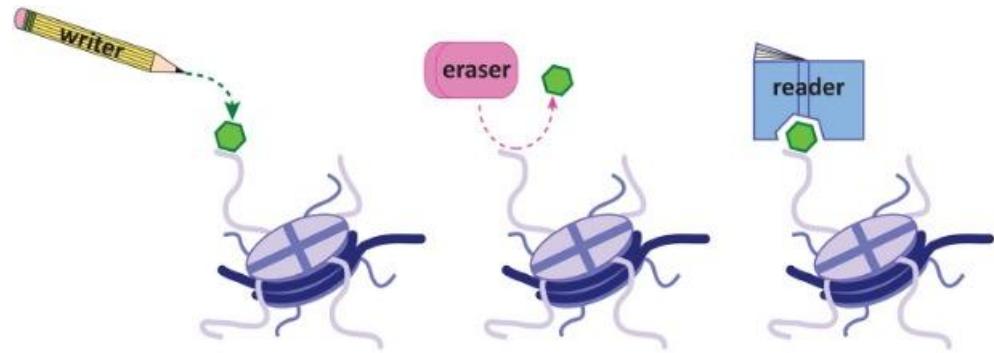
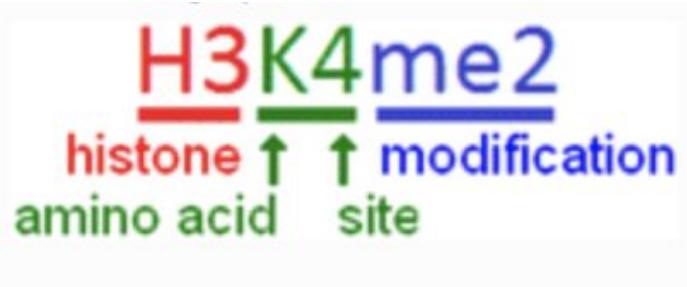
Others

Methylation (K, R)
Biotinylation (K)
ADP ribosylation (K, E)

Non-lysine PTMs

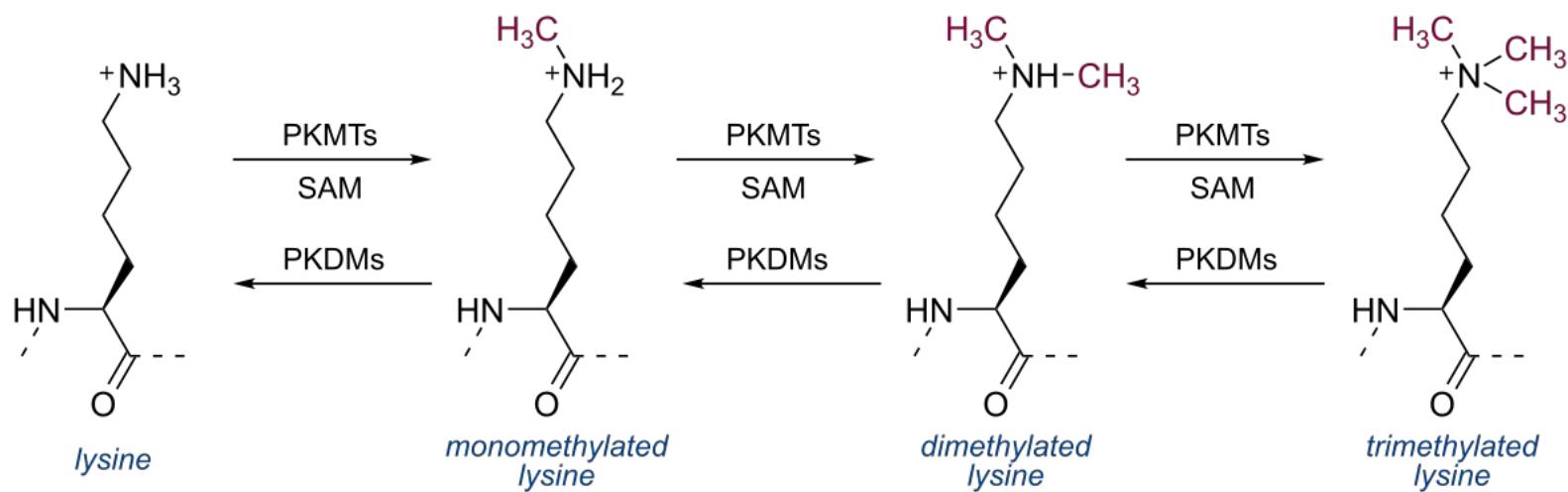
Serotonylation (Q)	S-palmitoylation (C)	O-GlcNAcylation (S, T)
Dopaminylation (Q)	Isomerization (P)	Deimination (R)
O-palmitoylation (S)	Hydroxylation (Y)	
● Phosphorylation (S, T, Y, H)	○ N-terminal acetylation (S)	

Brno nomenclature



Писатели, стиратели, читатели

Метилирование лизинов



Protein lysine methyltransferases (PKMTs)
Заряд +1 не меняется

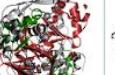
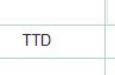
Домены: писатели, стиратели, читатели.
SET domain, chromodomain???

Histone Methylation Domains

Histone Methyltransferase (HMT)
nonSET
PRMT
SET1
SET2
RIZ
EZ
SUV39
SUV4-20
SMYD
HMT_other

Histone Demethylase (HDM)
LSD1_KDM1
JARID
JHDM1
JHDM2
JHDM3_JMJD2
PHF2_PHF8
UTX_UTY
JmjC_only

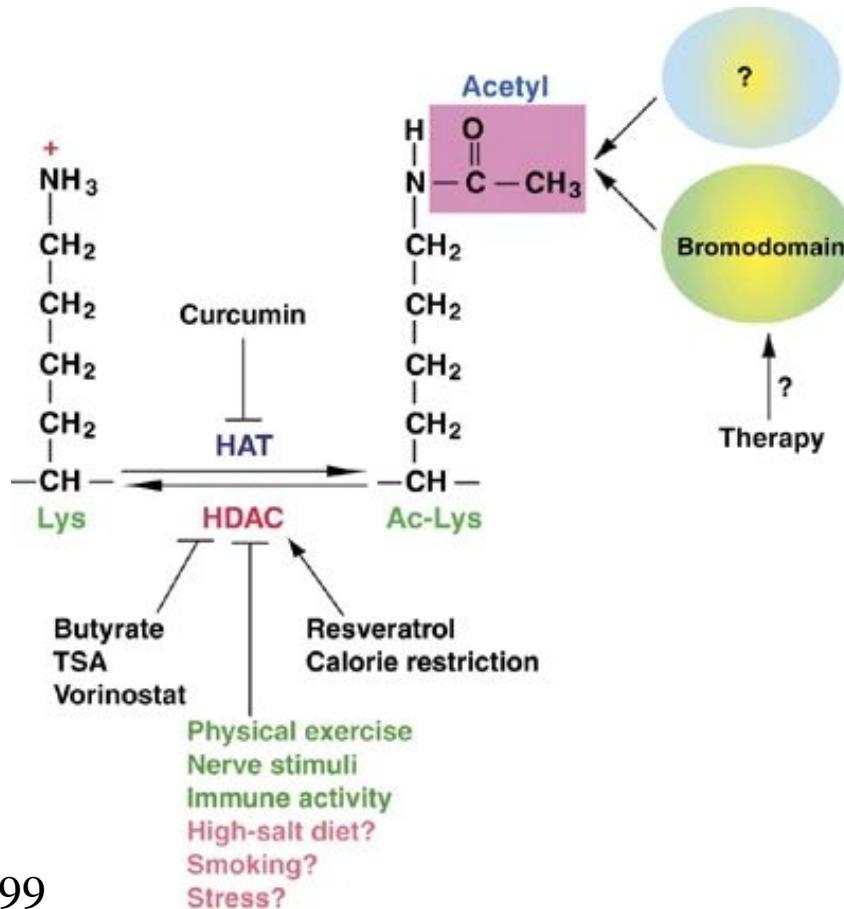
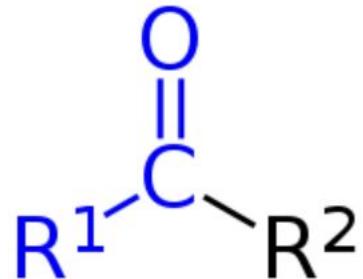
Histone Methylation Reader (Me_Reader)
ADD
Ankyrin
BAH
Chromodomain
PWWP
TTD
Tudor
WD40
ZF-CW
PHD
Chromo-Barrel
DCD
MBT
Me_Reader_other

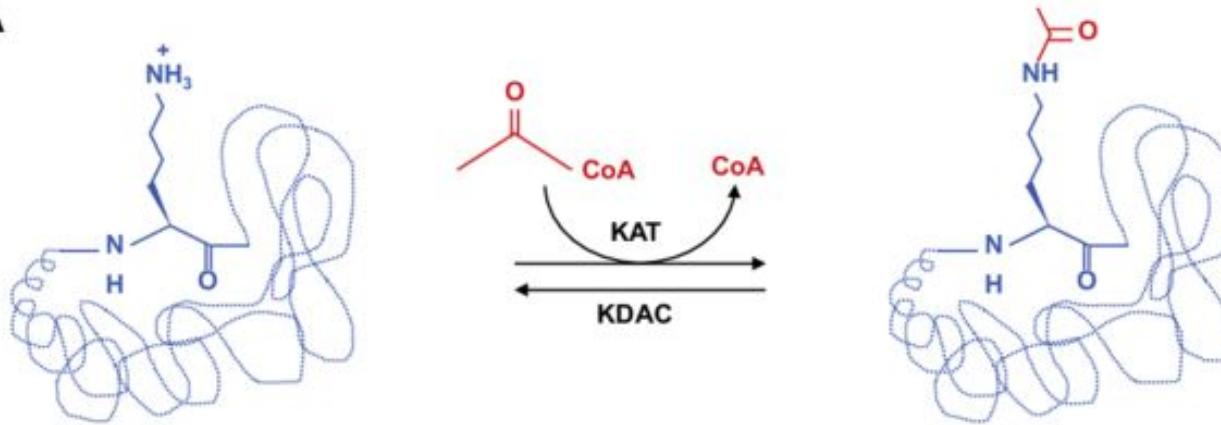
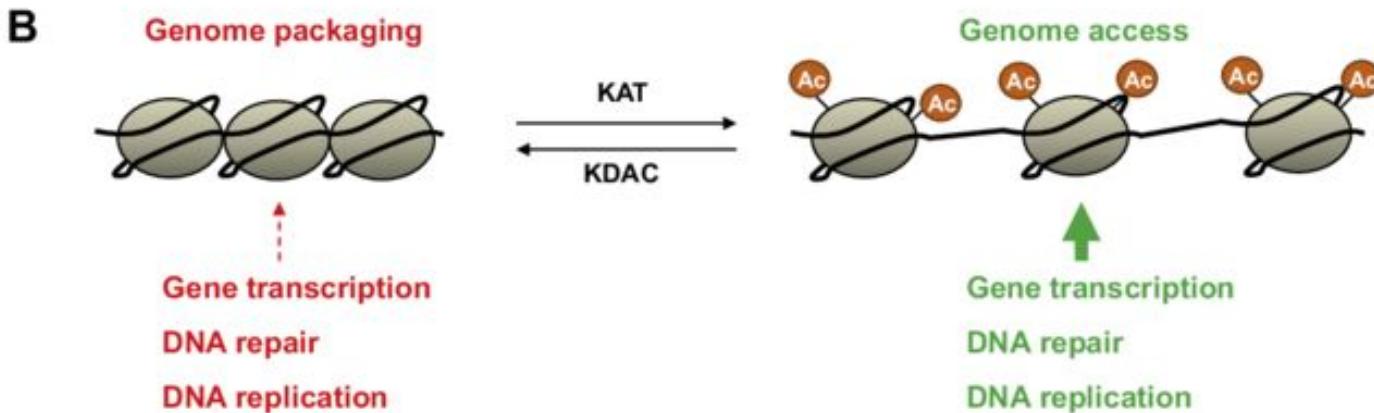
HMT				N/A	
SET1					nonSET
SUV39					
HDM					
e_Reader	N/A	N/A			
	ADD	Ankyrin	BAH	Chromodomain	
PWWP		N/A			
TTD			Tudor	WD40	
PHD					
Chromo-Barrel					
DCD					
MBT					

H3K4me3, H3K9me3, H3K27me3, H3K36me3

H3K4me1 + H3K27me3	repressed enhancers in stem cells
H3K4me1	enhancer
H3K4me3	active promoter regions
H3K4me1 + H3K27ac	active enhancer
H3K27Ac	Enhancers, promoters
H3K36me3	Gene body - [H3K36me2 (which is distributed broadly in intergenic regions and weakly transcribed regions, for moderate levels of DNA methylation); H3K36me3, which essentially marks the actively transcribed regions, for high levels of methylation.]
H3K9me3	конститутивный гетерохроматин
H3K27me3	факультативный гетерохроматин

Ацилирование (ацетилирование)



A**B**

Histone Acetylation Domains

Histone Acetyltransferase (HAT)

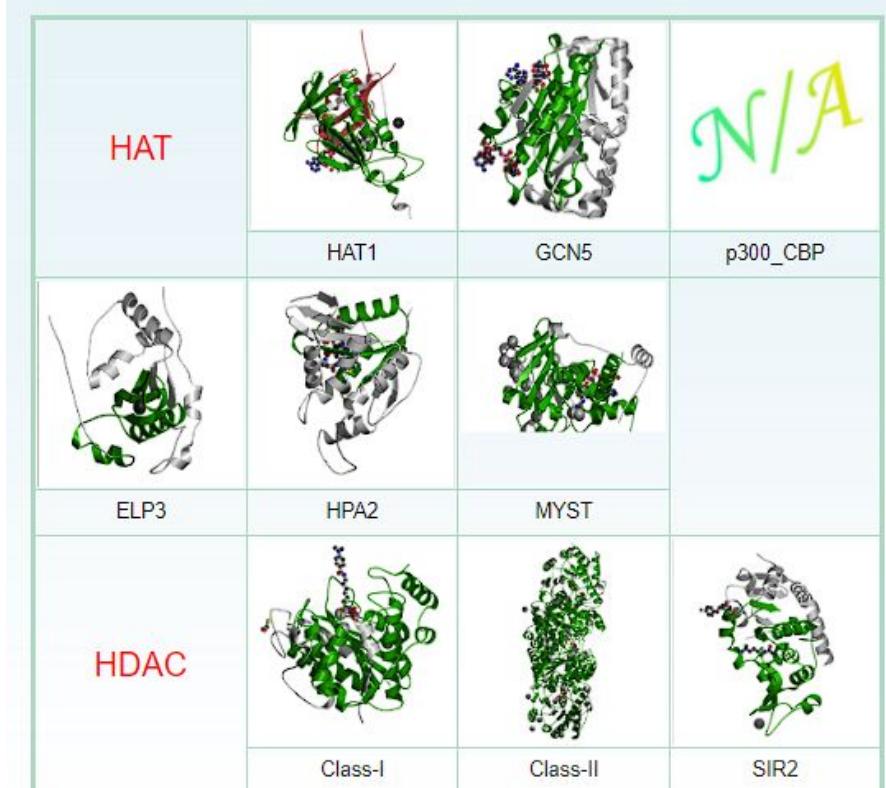
- p300_CBP
- MYST
- HAT_other
- GCN5
- HAT1
- HPA2
- ELP3
- GNAT_other

Histone Deacetylase (HDAC)

- Class-I
- Class-II
- SIR2
- Class-IV
- HD2

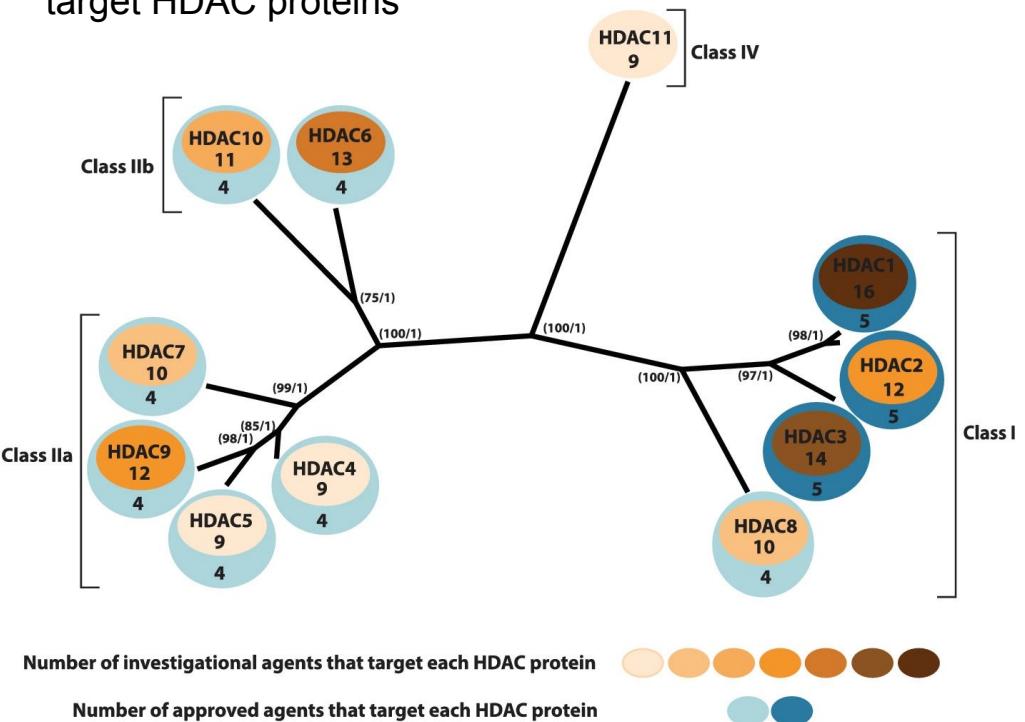
Histone Acetylation Reader (Ac_Reader)

- Bromodomain
- Tandem-PHD

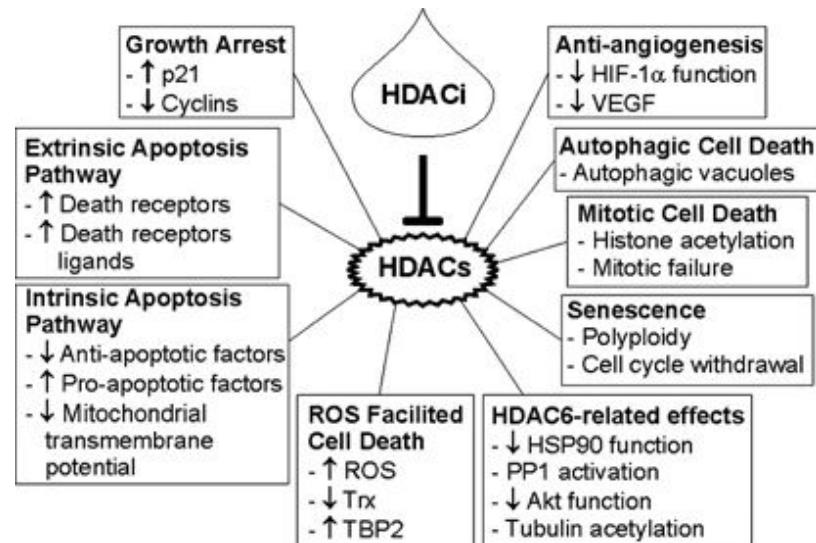


HDAC/HAC inhibitors

Number of investigational and approved agents that target HDAC proteins



Multiple HDACi-activated antitumor pathways



Number of investigational agents that target each HDAC protein



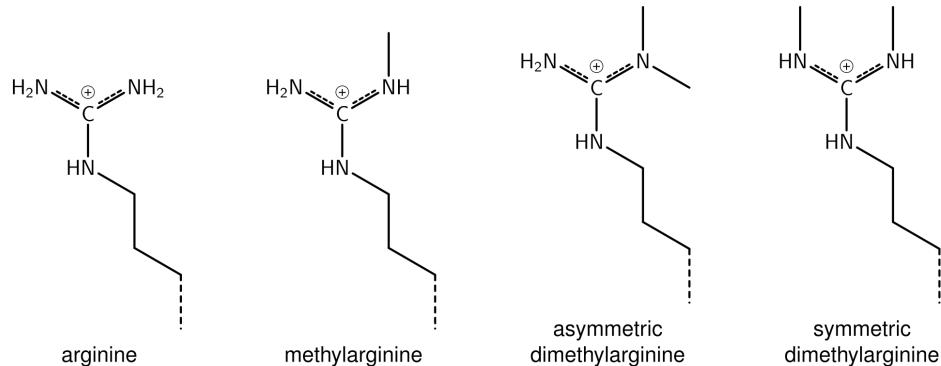
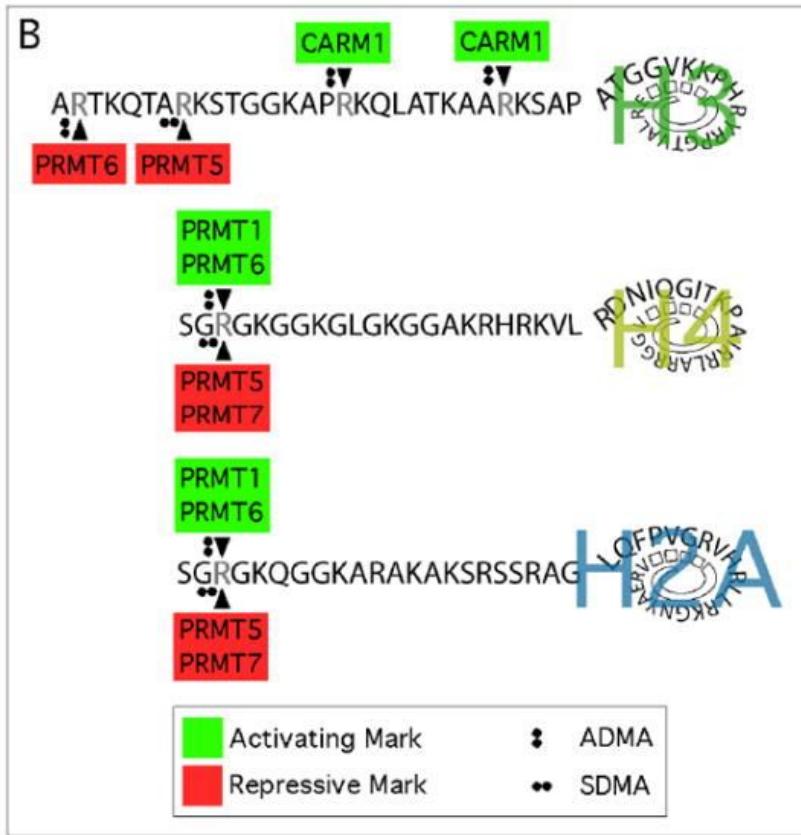
Number of approved agents that target each HDAC protein



HDACi

Drug name	Enzyme specificity	Indications investigated	Highest CT phase	Drug name	Chemical class	Enzyme specificity	Indications investigated	Highest CT phase
Abexinostat	HDAC 1, 2, 3, 6, 10	DLBCL, MCL, AML, ALL, FL, RCC, MDS, sarcoma, skin cancers, NSCLC	Phase 3	Tacedinaline (CI-994)	Benzamide	HDAC 1	Solid and haematological cancers	Phase 3 (discontinued)
Fimepinostat (CUDC-907)	HDAC 1, 2, 3, 10	Lymphomas, brain tumours	Phase 1/2	Entinostat	Benzamide	Class 1 HDACs	PC, BC, BIC, AML, CRC, LL, RCC, melanoma, NSCLC, gynaecological cancers, CNS tumours, MDS, pancreatic cancer, NE tumours	Phase 2
Quisinostat (INJ26481585)	HDAC 1, 6, 9	Ovarian cancer, CTCL, NSCLC	Phase 2	Domatinostat	Benzamide	Class 1 HDACs	CTCL	Phase 1
Ricolinostat (ACY-1215)	HDAC 6	MM, DNP, lymphomas, BC, gynaecological cancers, CLL	Phase 2	RG2833	Benzamide	HDAC 3	Friedreich's ataxia	Phase 1
Trichostatin A	HDAC 7, 8	Haematological cancers	Phase 1	Givinostat	Benzamide	Pan-HDAC	MDS, PV, JIA	Phase 2
Nanatinostat (VRx-3996)	HDAC 9	EBv-associated malignancies	Phase 1/2	KA2507	Cyclic peptide	HDAC 6	Melanoma	Phase 1
CG200745	HDAC 9, 11	MDS, pancreatic cancer	Phase 1/2	Mocetinostat	Benzamide	Pan-HDAC	UC, NSCLC, HL, DLBCL, FL, leiomyosarcoma, melanoma	Phase 2
Pracinostat	Pan-HDAC	MDS, AML, MF, PC, sarcoma	Phase 3	OBP-801	Cyclic peptide	Pan-HDAC	LC, lymphoma, RC, glaucoma	Phase 1a
Resminostat	Pan-HDAC	CTCL, HCC, HL, CRC, pancreatic cancer, NSCLC	Phase 2	AR-42	Benzamide	Pan-HDAC	RCC, sarcoma, meningioma, VS, AML	Phase 1
CUDC-101	Pan-HDAC	Advanced solid tumours	Phase 1 (discontinued)				
MPT0E028	Pan-HDAC	Advanced solid tumours	Phase 1					

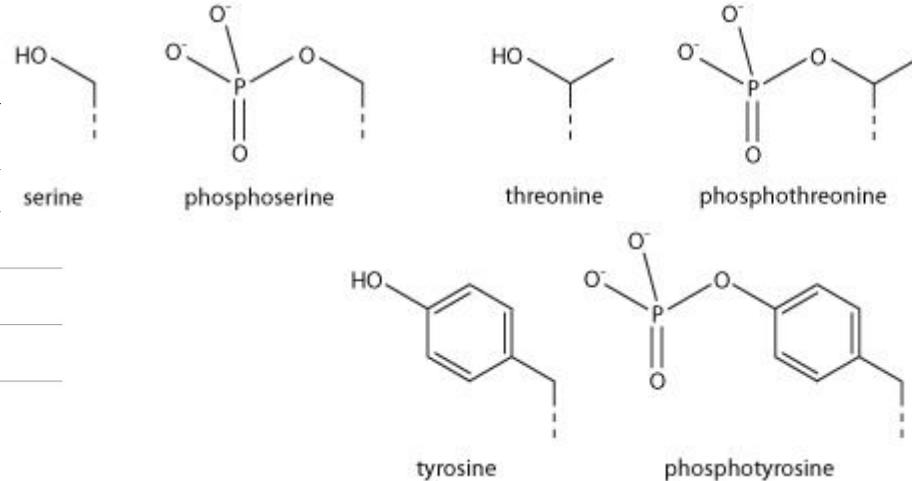
Метилирование аргининов



(A) Arginine residues in the tails of histones can be **monomethylarginines (MMA)**, **asymmetric dimethylarginines (ADMA)**, and **symmetric dimethylarginines (SDMA)**. The MMA form of arginine is generally regarded as an intermediate on its way to the dimethylated state and is not depicted here. (B) The known sites of histone H3, H4, and H2A arginine dimethylation are shown. Red denotes transcriptional repressor activity and green denotes transcriptional activator activity.

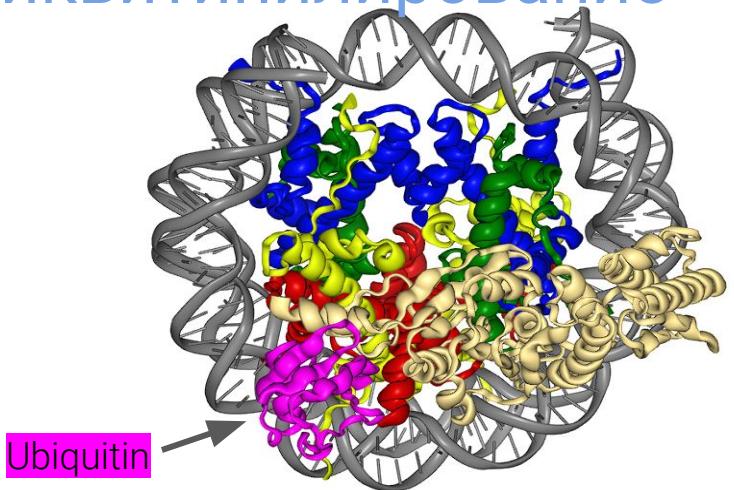
Фосфорилирование

Histone	Phosphorylated residue	Kinases	Role
H2A	S1	RSK2 Bub1, NHK-1 (Dm) Mec1, Tel1 (Sc) / ATM, ATR, DNA-PK (Hs) Y142 (H2AX)	Mitosis EGF signaling DNA repair/Mitosis/Meiosis DNA repair Apoptosis DNA repair
	S16		
	S122*(Sc)/T120(Hs)		
	S129* (Sc)/S139(Hs,H2AX)		
	Y142 (H2AX)		
H2B	S10 (Sc)/S14 (Hs)	Ste20 (Sc)/Mst1 (Hs) (Ipl1?)	Apoptosis Meiosis
	S32	RSK2	EGF signaling
	S36	AMPK	Transcription
	S1	CKII Sps1	DNA repair, Transcription Meiosis, Transcription
H4	S47	PAK2	(H3.3-H4) Deposition
	S/T	CDK2	Mitosis Transcription
H1			

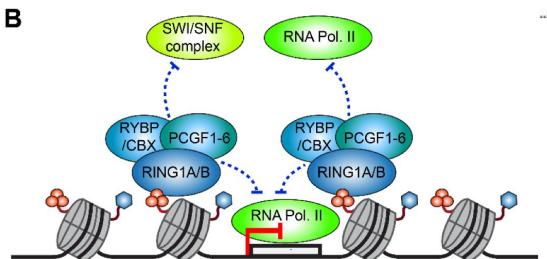
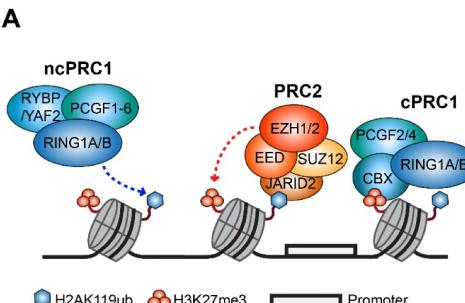


H3	T3	Haspin	Mitosis
	T6	PKC β	Transcription
	S10	Ipl1 (Sc) / AuroraB (Hs), RSK2, MSK1, ERK1, p38, Fyn, Chk1, PRK1	Transcription, Chromatin condensation, UVB response
	T11	Mek1 (Sc) / Dlk (Hs, ?) PRK1, PKM2 Chk1	Meiosis (Sc), Mitosis (Hs) Transcription DNA damage response
	S28	AuroraB, ERK1/2, p38 MLTK- α , JNK1/2, MSK1	Meiosis Mitosis, Transcription
	Y41	JAK2	Transcription
	T45	PK-C δ	Apoptosis

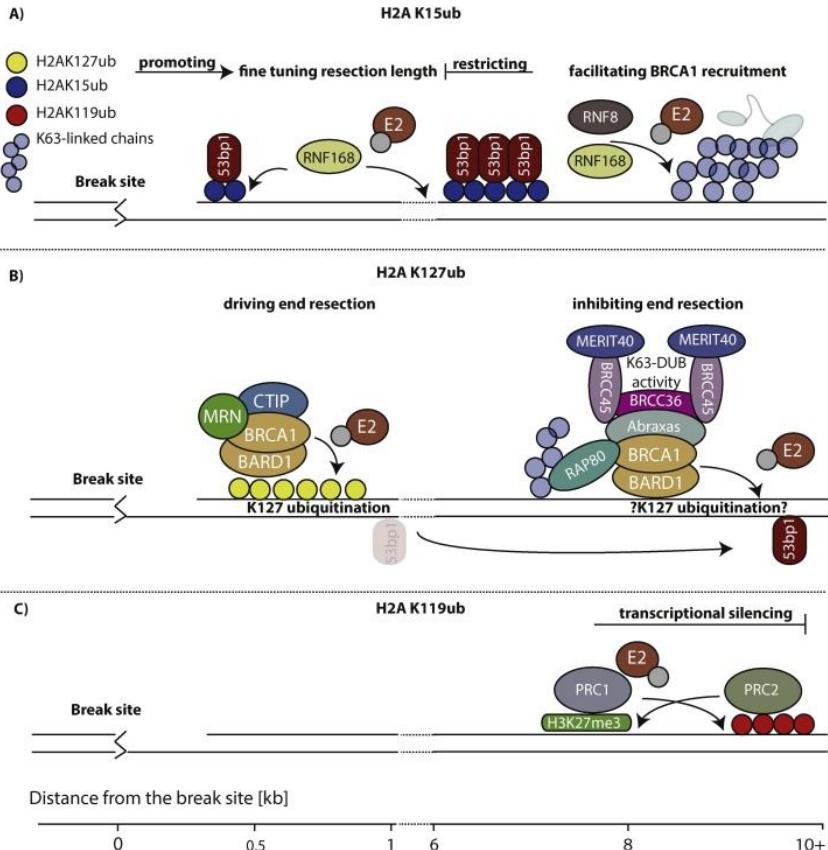
ЮБИКВИТИНИЛИРОВАНИЕ

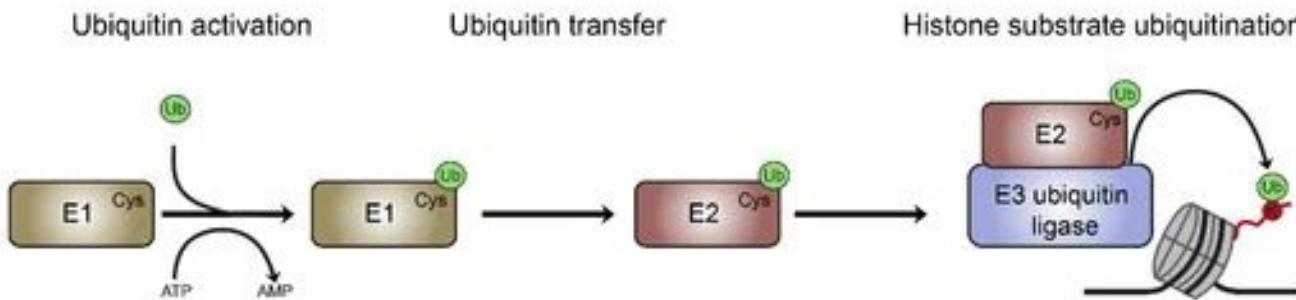
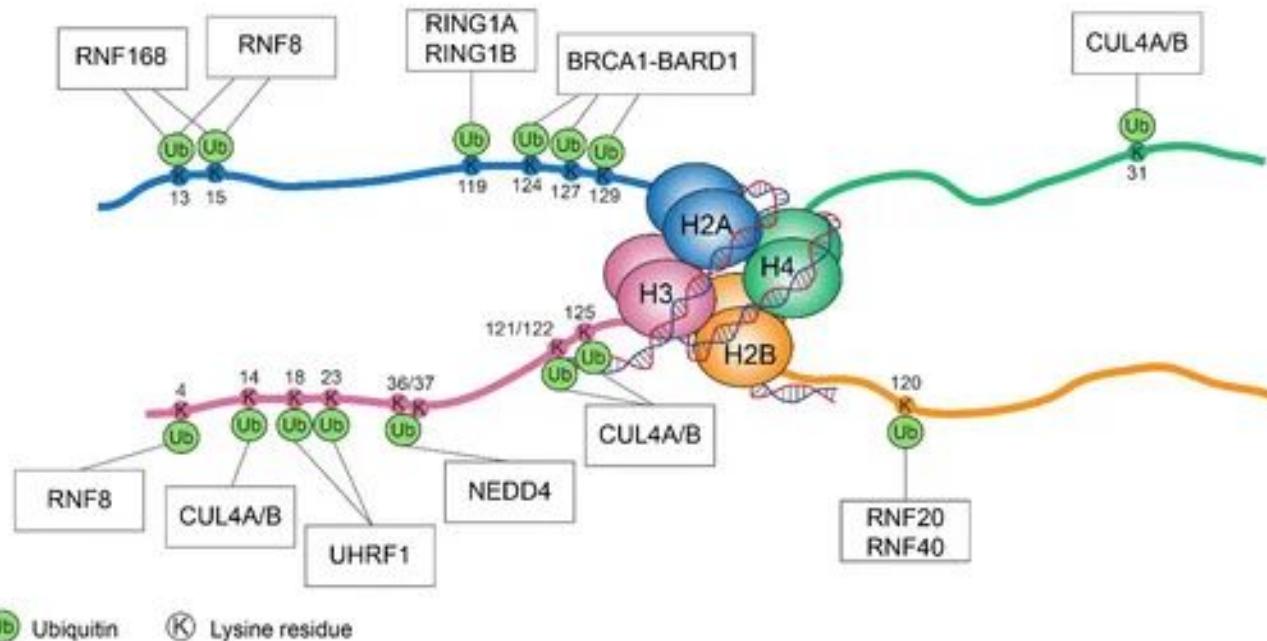


Transcriptional regulation by PRC1-mediated H2AK119ub



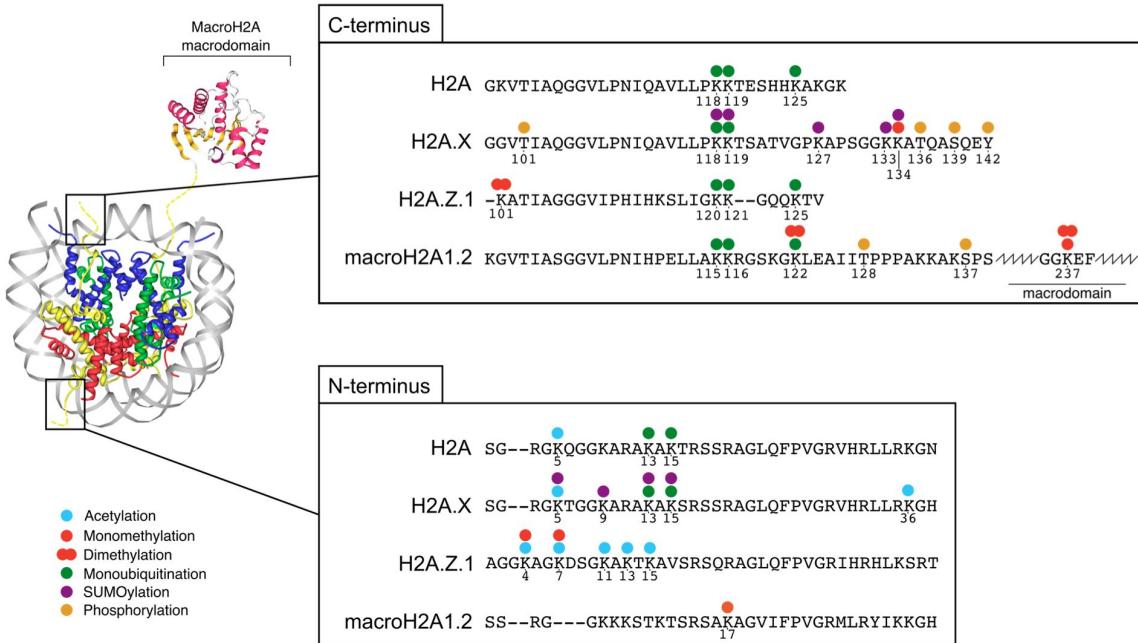
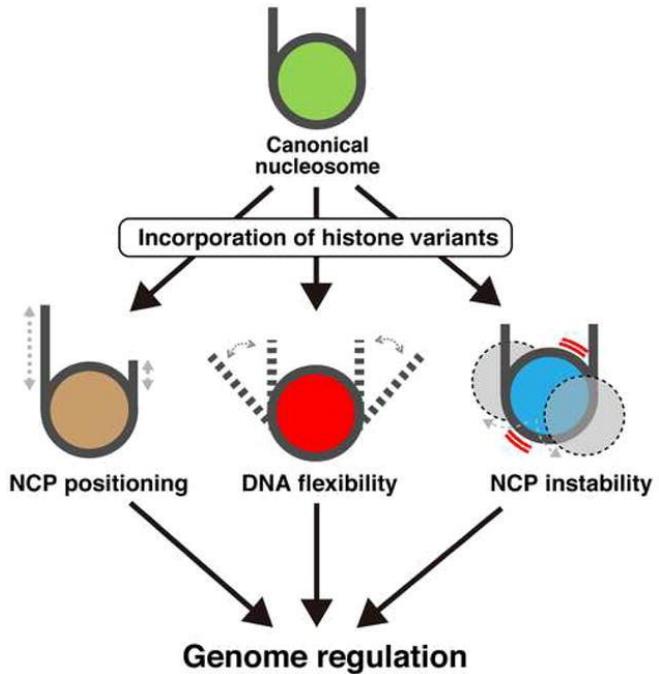
Model of site-specific ubiquitination in the DNA damage response

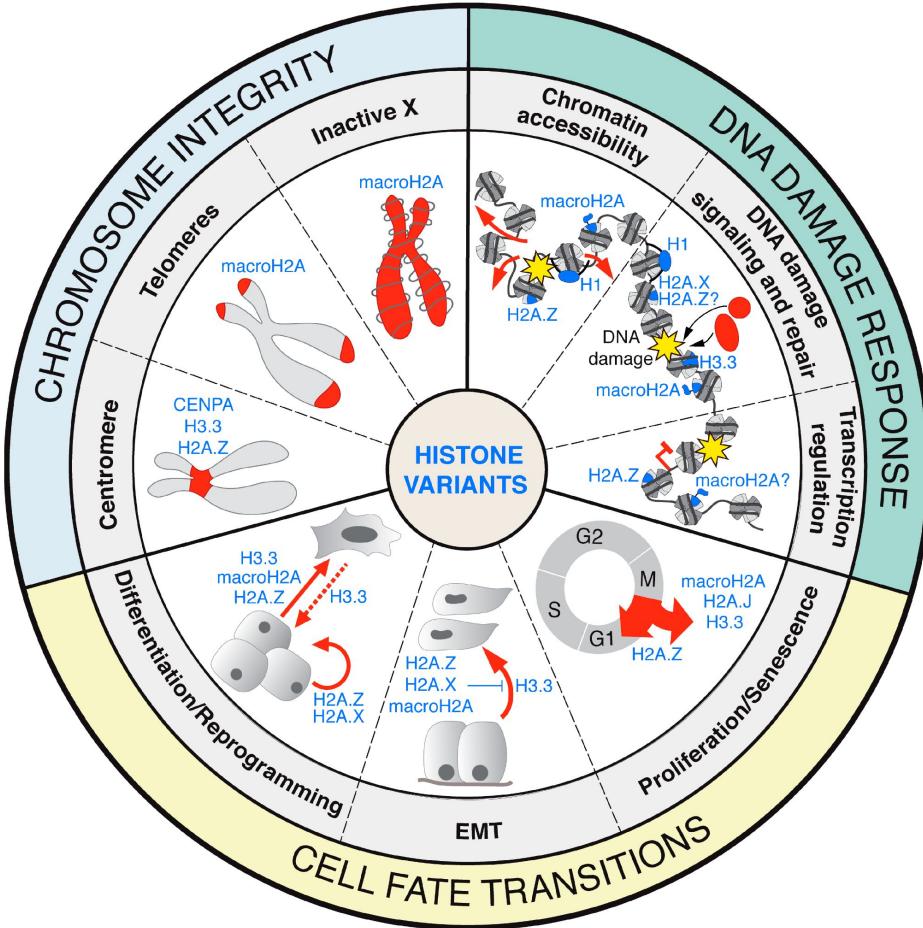


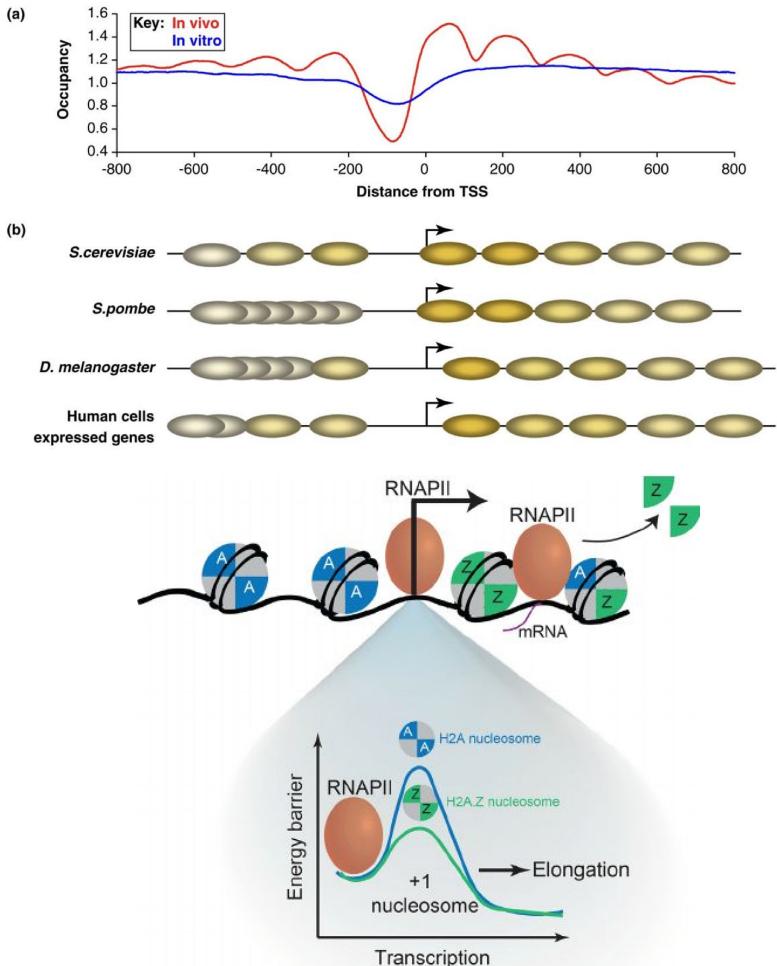
A**B**

Сводная таблица по меткам

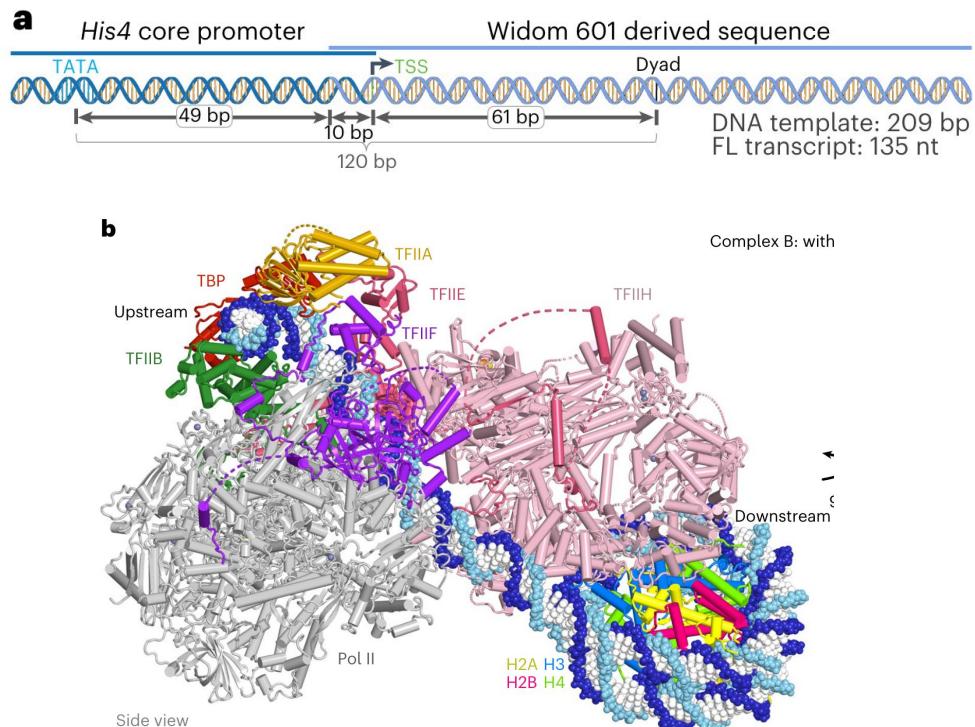
Гистоновые варианты и регуляция транскрипции







Структура преинициационного комплекса с +1 нуклеосомой



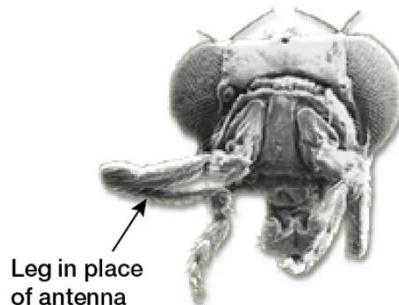
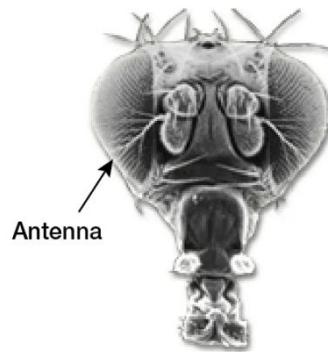
Ремоделирование хроматина (Chromatin remodeling)

Ремоделирование хроматина - это динамическая модификация архитектуры хроматина, обеспечивающая доступ белков к конденсированной геномной ДНК и тем самым контролирующая экспрессию генов. Такое ремоделирование в основном осуществляется путем

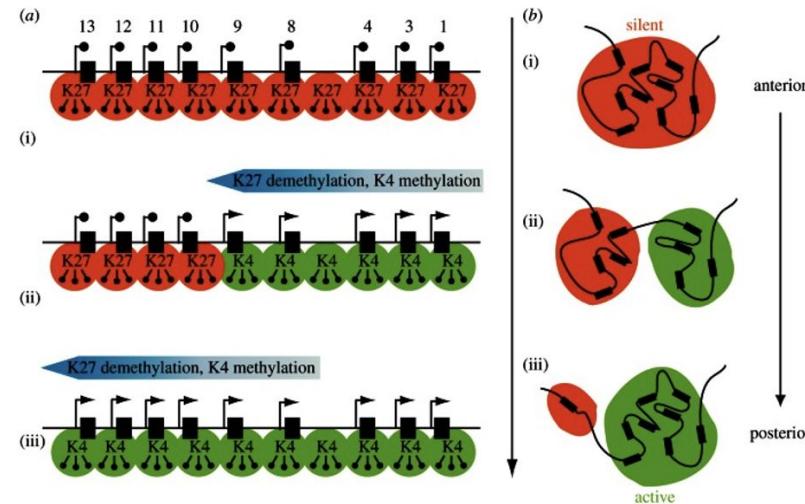
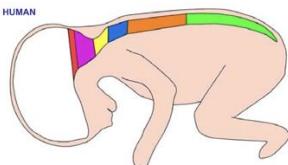
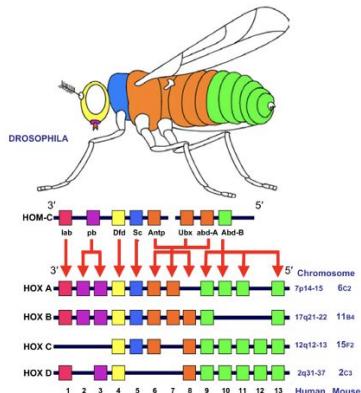
- 1) ковалентных модификаций гистонов специфическими ферментами, например гистонацетилтрансферазами (HATs), деацетилазами, метилтрансферазами и киназами, и
- 2) АТФ- зависимыми комплексами ремоделирования хроматина, которые либо перемещают, выбрасывают, либо реструктурируют нуклеосомы.

Практические примеры

- Мутации в НОХ-генах



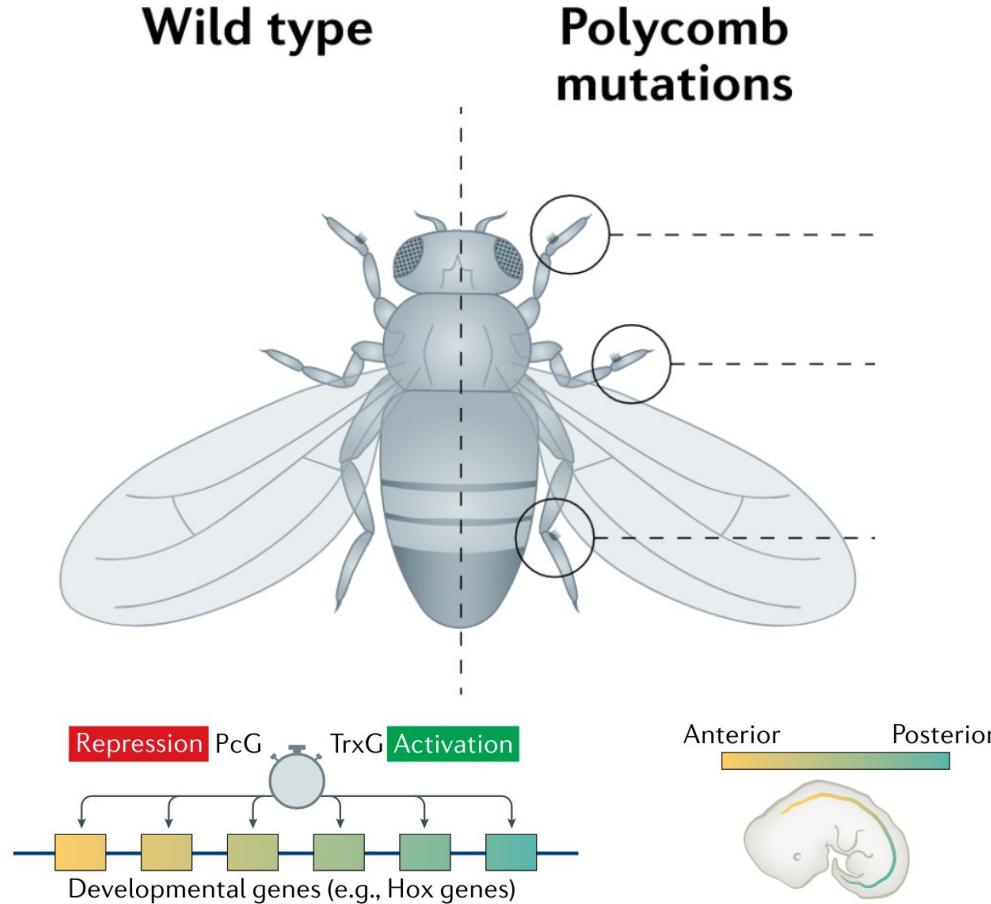
- Контроль развития организма вдоль оси голова-хвост



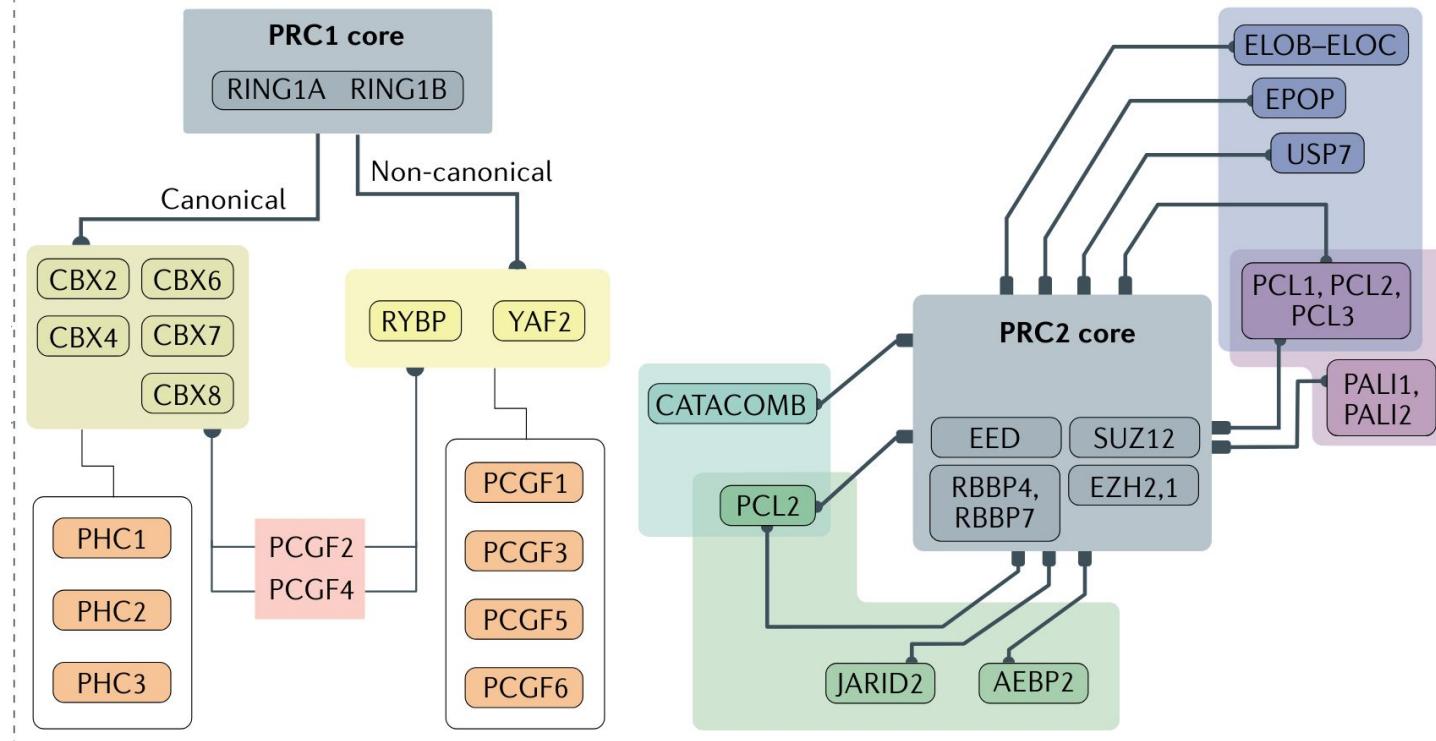
«Колinearность» – гены, расположенные вдоль ДНК, последовательно активируются в сегментах тела от головы к хвосту

Ноx-гены

Polycomb repressor group proteins



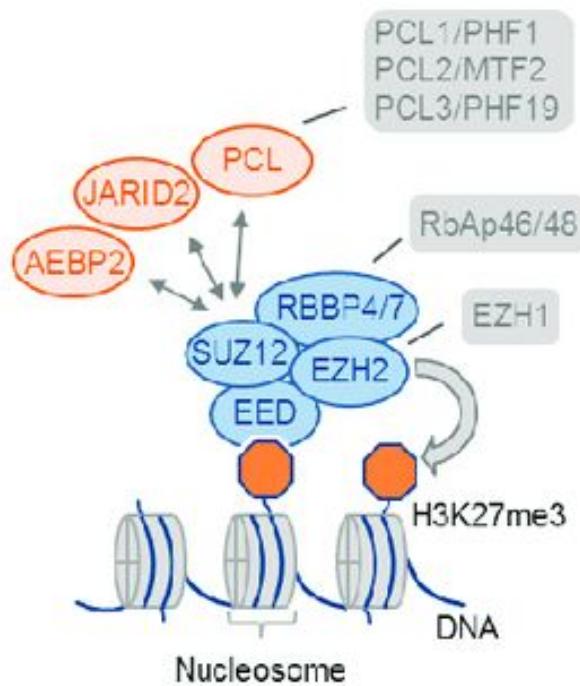
Polycomb repressor group proteins



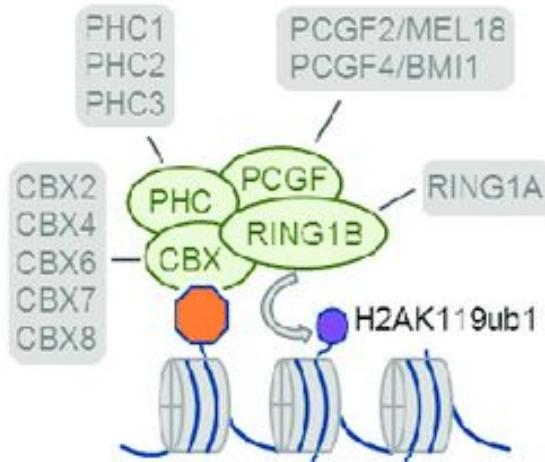
involved in stable and heritable transcriptional silencing

A

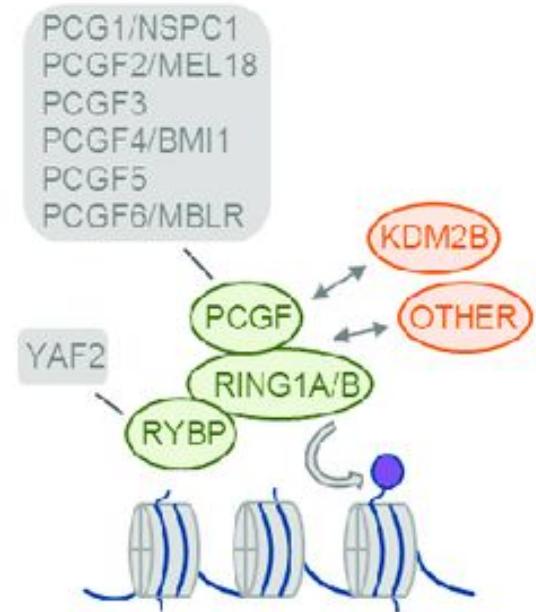
PRC2

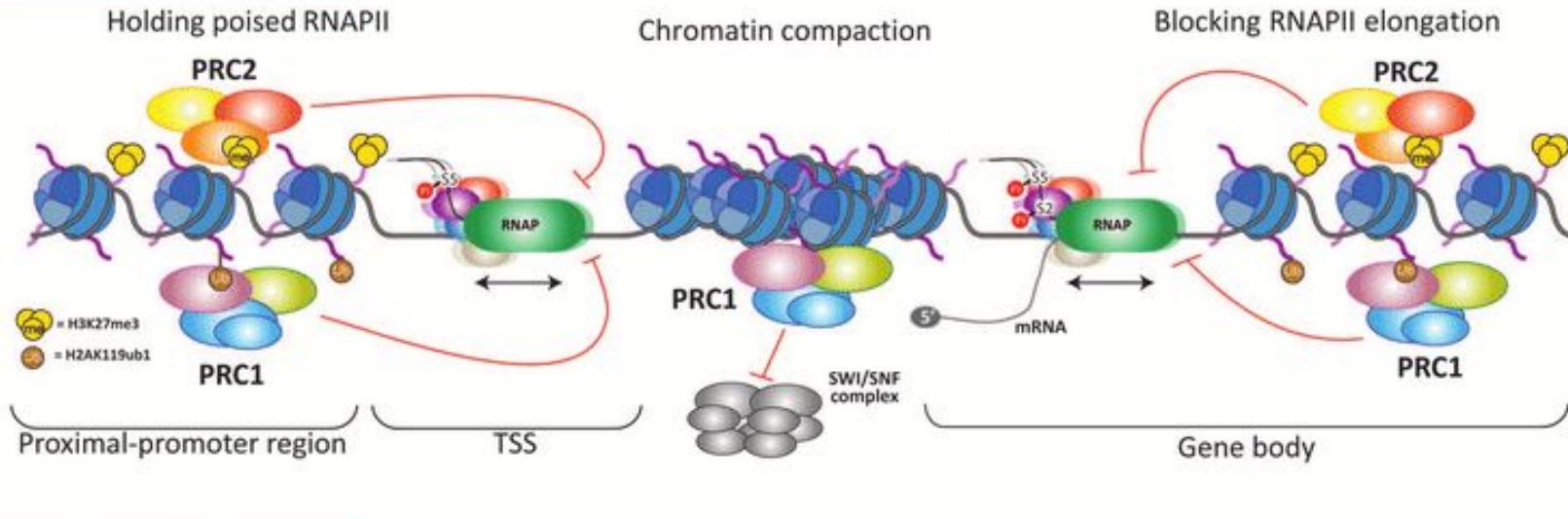
**B**

Canonical PRC1

**C**

Non-canonical PRC1

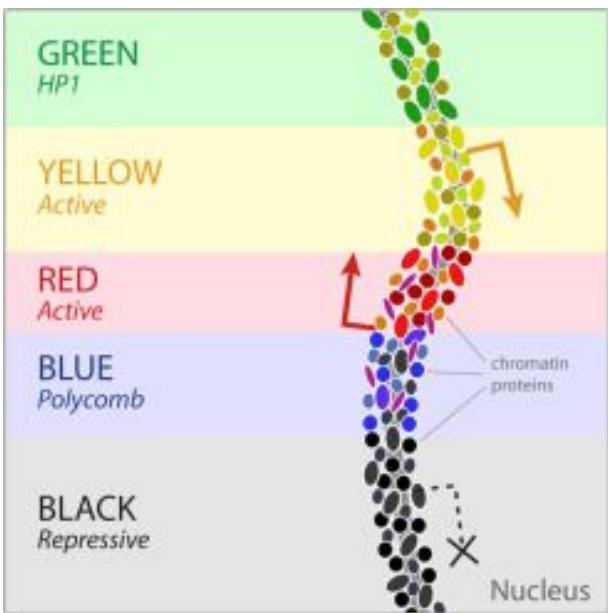


A**PcG-mediated transcriptional repression****B**

Цвета хроматина

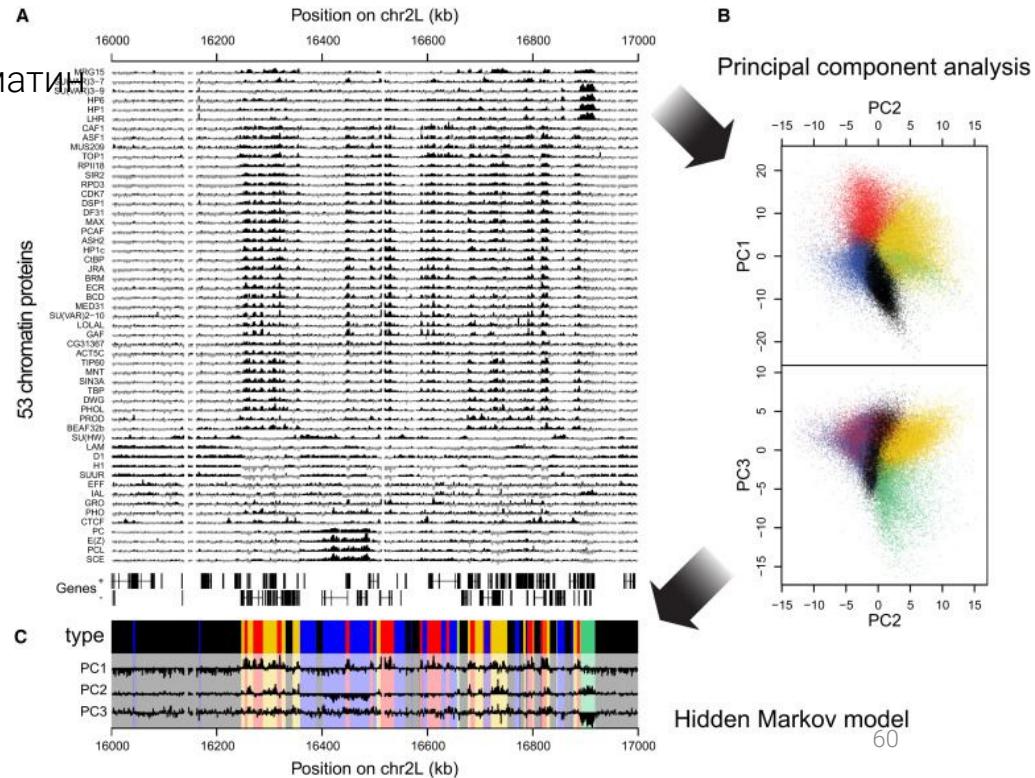
53 профиля белков на хроматине после Dam-метилирования (идентификация ДНК-аденин-метилирования) объединены в цвета хроматина:

- красный, желтый - активный хроматин,
- зеленый, синий, черный - неактивный хроматин



Systematic Protein Location Mapping Reveals Five Principal Chromatin Types in *Drosophila* Cells

Guillaume J. Filion,^{1,5} Joke G. van Bemmel,^{1,5} Ulrich Braunschweig,^{1,5} Wendy Talhout,¹ Jop Kind,¹ Lucas D. Ward,^{3,4,6} Wim Brugman,² Inès J. de Castro,^{1,7} Ron M. Kerkhoven,² Harmen J. Bussemaker,^{3,4} and Bas van Steensel^{1,*}



BLUE and GREEN Chromatin Correspond to Known Heterochromatin Types

GREEN chromatin corresponds to classic heterochromatin that is marked by SU(VAR)3-9, HP1, and the HP1-interacting proteins LHR and HP6.

BLUE chromatin corresponds to PcG chromatin, as shown by the extensive binding by the PcG proteins PC, E(Z), PCL, and SCE.

BLACK Chromatin Is the Prevalent Type of Repressive Chromatin

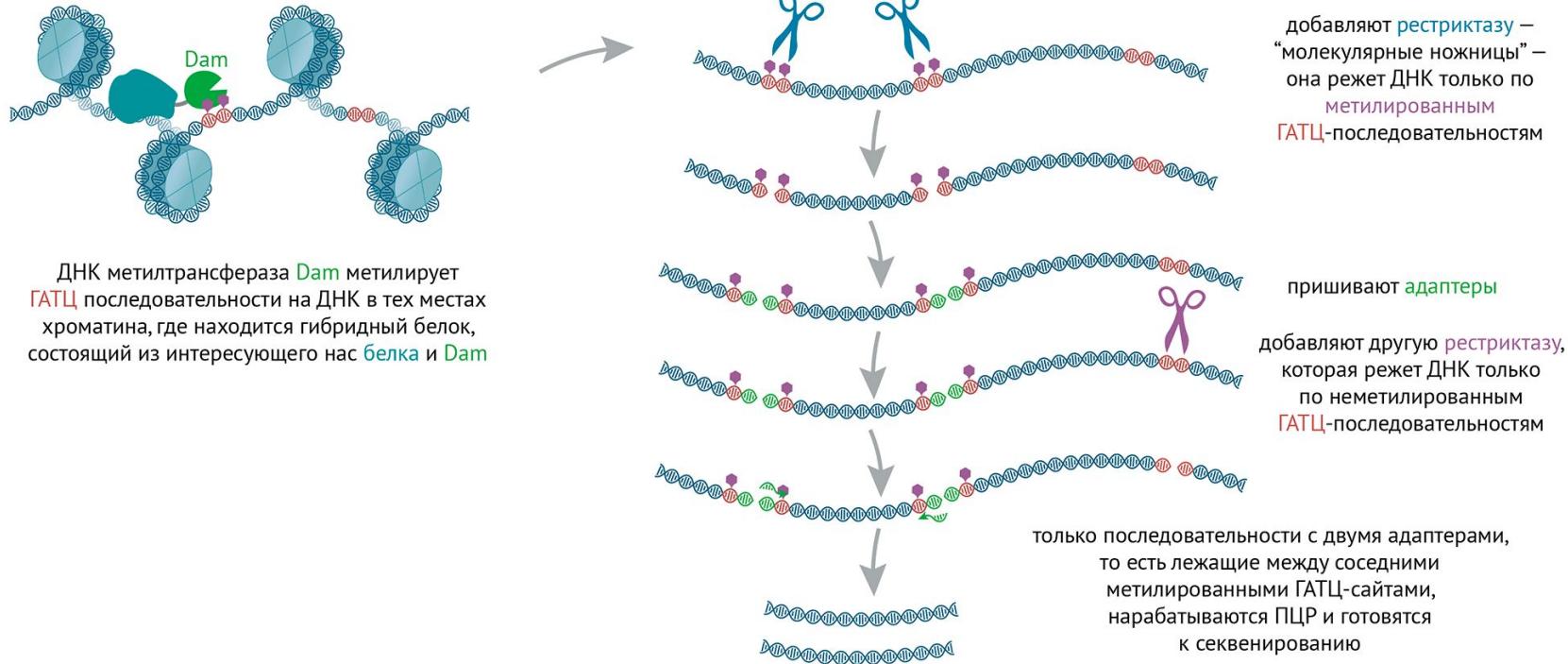
BLACK chromatin is overall relatively gene poor

BLACK chromatin is almost universally marked by four of the 53 mapped proteins: histone H1, D1, IAL, and SUUR, whereas SU(HW), LAM, and EFF are also frequently present

YELLOW and RED Chromatin Are Two Distinct Types of Euchromatin

Genes with universal cellular functions such as “ribosome,” “DNA repair,” and “nucleic acid metabolic process” are almost exclusively found in YELLOW chromatin (Figure 6B), whereas genes in RED chromatin are linked to more specific processes such as “receptor binding,” “defense response,” “transcription factor activity,” and “signal transduction”

Схема Dam-метилирования



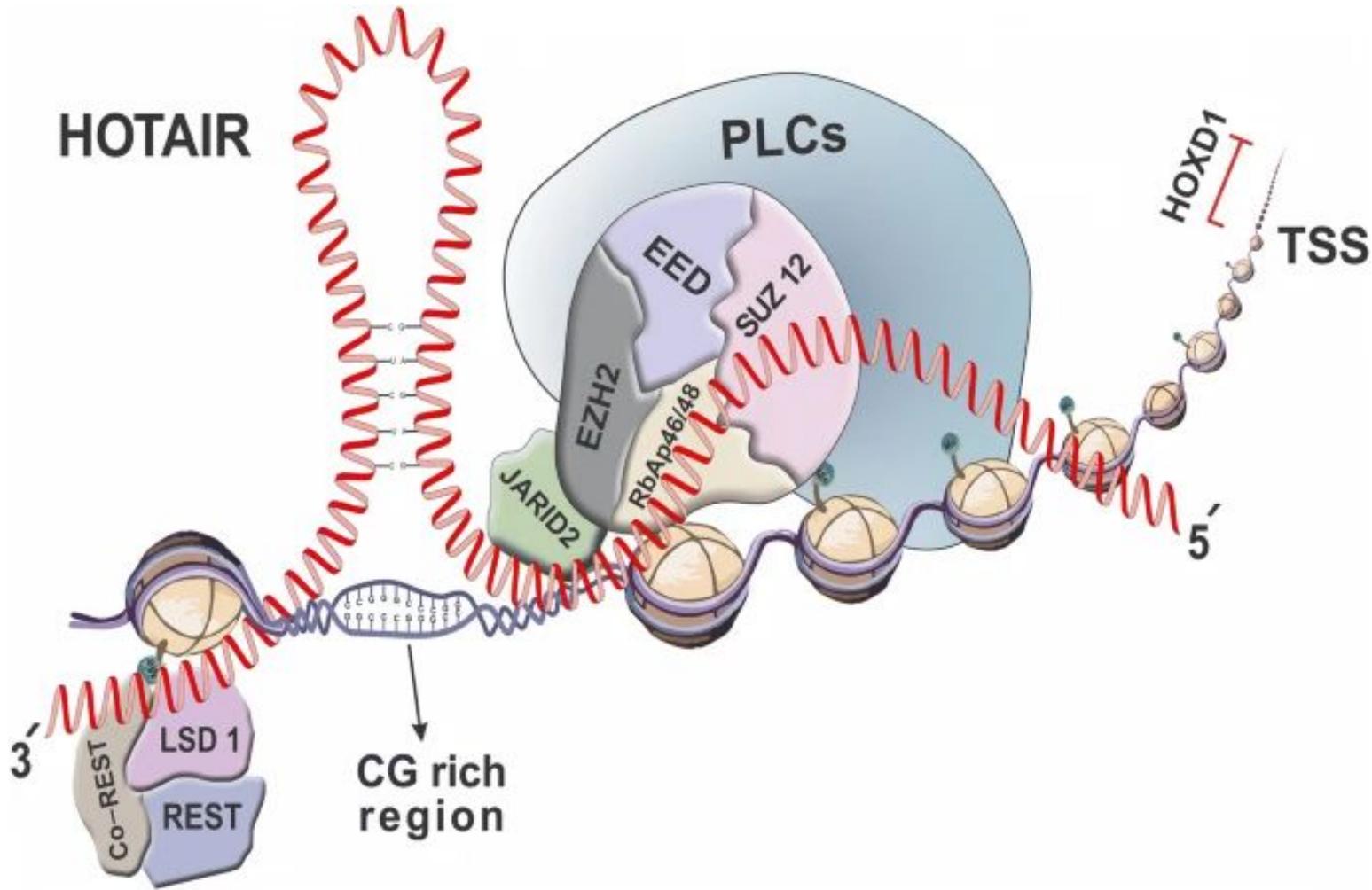
Роль длинных некодирующих РНК

Длинные некодирующие РНК (днкРНК, lncRNAs) — некодирующие РНК, которые как правило имеют длину более 200 нуклеотидов, и расположены в ядре или в цитоплазме.

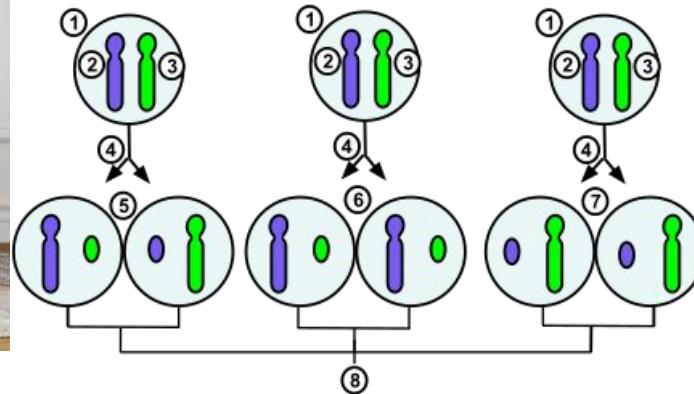
LncBook 2.0

Integrating human long non-coding RNAs with multi-omics annotations

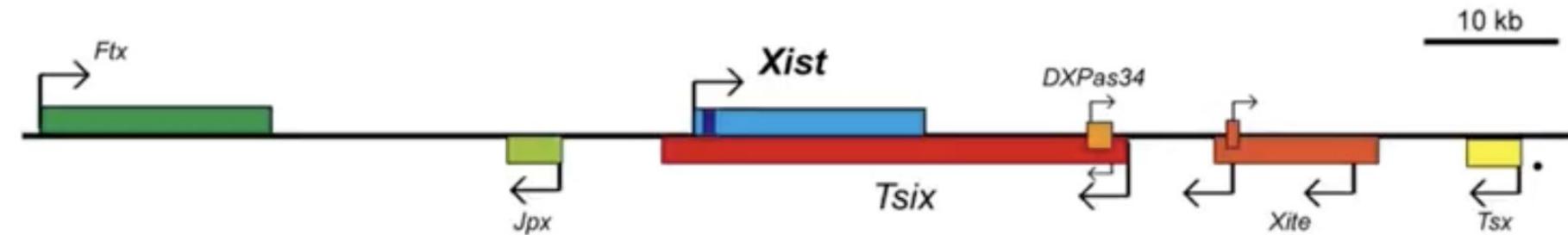
LncBook accommodates a high-quality collection of 95,243 human lncRNA genes and 323,950 lncRNA transcripts, and incorporates their abundant annotations at different omics levels, thereby enabling users to decipher functional signatures of lncRNAs in human diseases and different biological contexts.



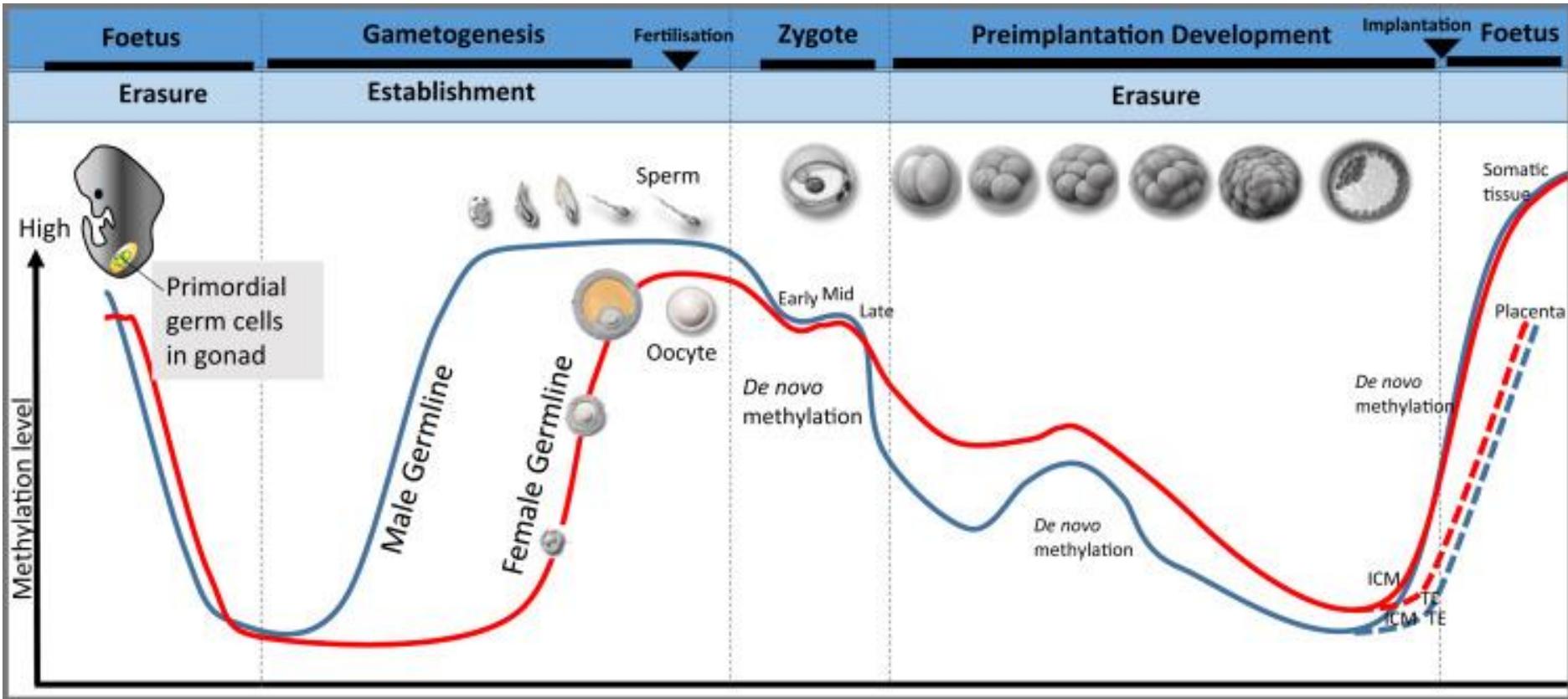
Xist (X-inactive specific transcript)



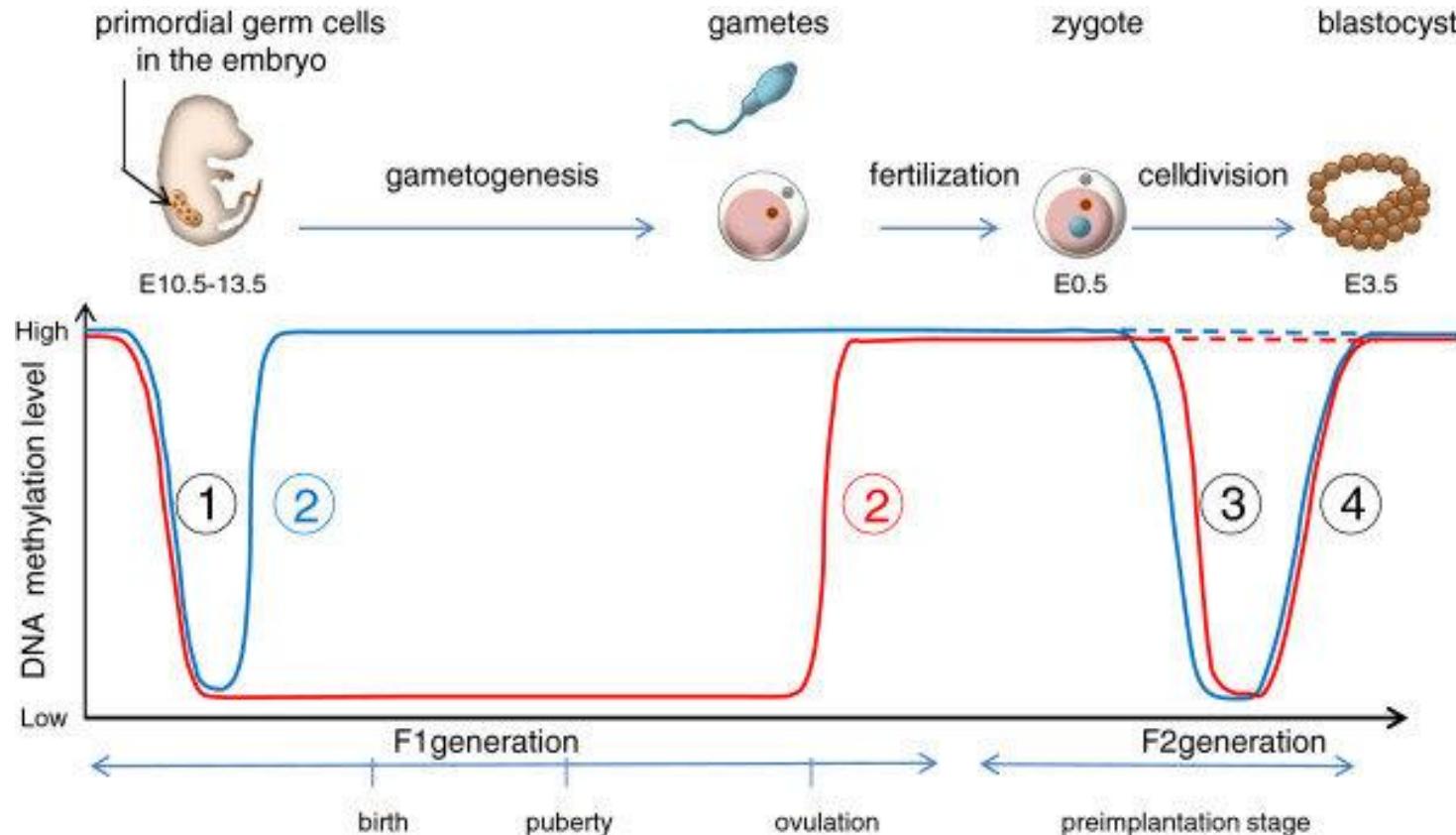
X inactivation centre (XIC)



Epigenetic genome wide reprogramming

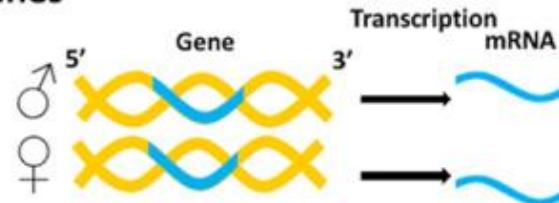


Epigenetic genome wide reprogramming

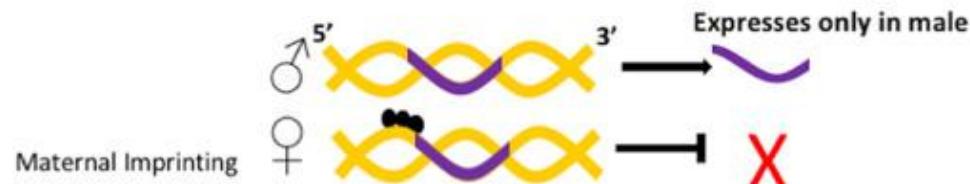


Импринтинг

Non-imprinted genes



Imprinted genes



----- OR -----



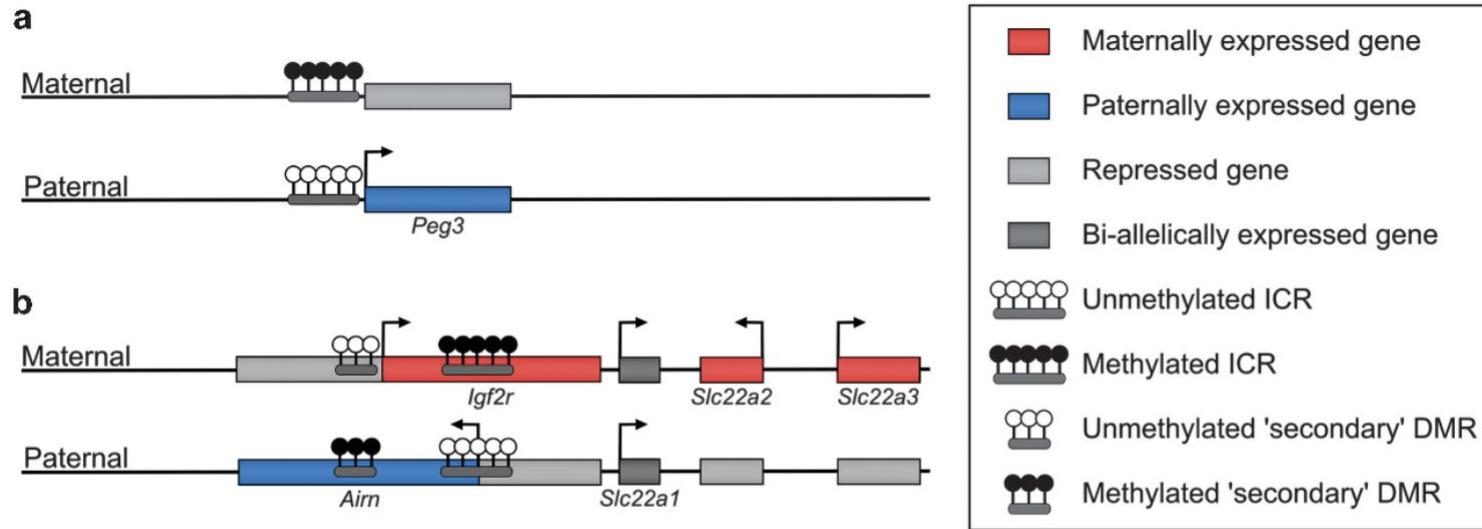
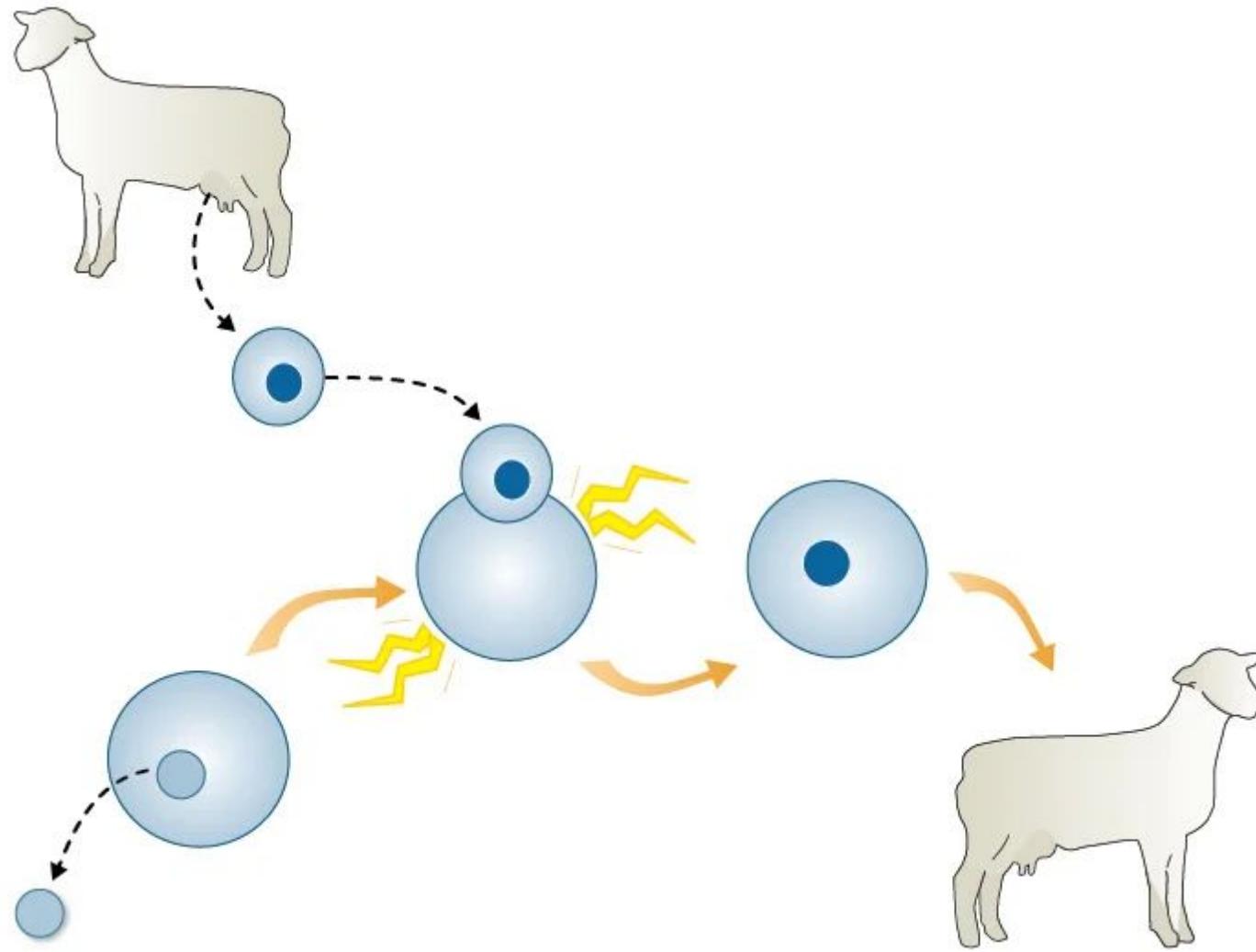
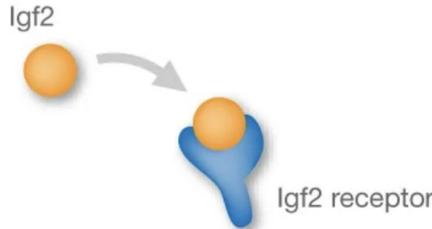


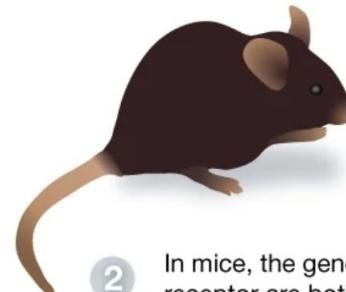
Figure 1 Examples of directly and indirectly regulated imprinted regions. Schematic representation of the (a) *Peg3* imprinted gene on chromosome 7 and (b) the *Igf2r* imprinted cluster on chromosome 17. The expression status of the genes on the maternal and paternal alleles is illustrated; active promoters are represented by horizontal arrows. (a) The differentially methylated ICR established during germ cell development is located at the promoter of the *Peg3* gene and directly regulates the monoallelic transcription of this gene. (b) The maternally methylated ICR indirectly regulates the monoallelic expression of the adjacent genes at this locus, partially mediated by the monoallelic methylation acquired at the nearby secondary DMR at the *Igf2r* promoter.



AN EXAMPLE OF IMPRINTING



- 1 In mammals, the growth factor Igf2 interacts with the Igf2 receptor.



Genes from mom:

Igf2 receptor - ON

Igf2 - OFF

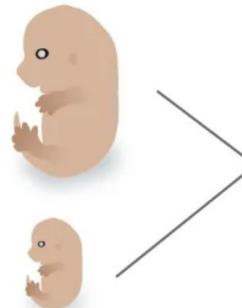
Genes from dad:

Igf2 receptor - OFF

Igf2 - ON

- 2 In mice, the genes for Igf2 and the Igf2 receptor are both imprinted.

Deleting the mother's Igf2 receptor gene produces overly large offspring.



Deleting the mother's Igf2 receptor gene AND the father's Igf2 gene produces normally sized offspring.

Deleting the father's Igf2 gene produces dwarf offspring.

- 3 The imprints on the Igf2 and Igf2 receptor genes normally cancel each other out. Changing the imprint on one copy of the gene has a dramatic effect on the size of the offspring. This result supports the genetic conflict hypothesis

Спасибо за внимание!