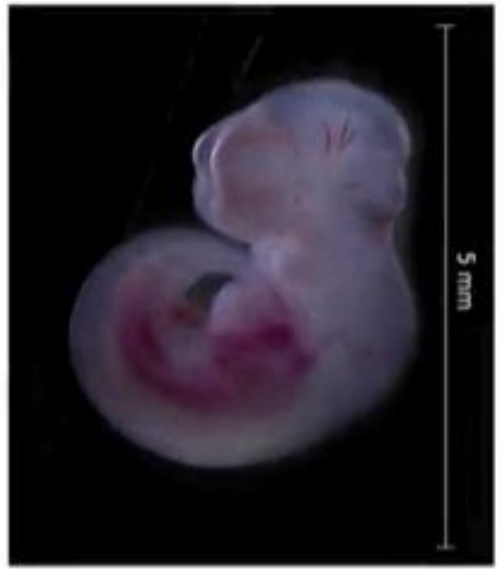
Эпигенетика

- Эпигенетические метки – митотически наследуемые изменения в экспрессии генов, не связаные с изменением нуклеотидной последовательности.

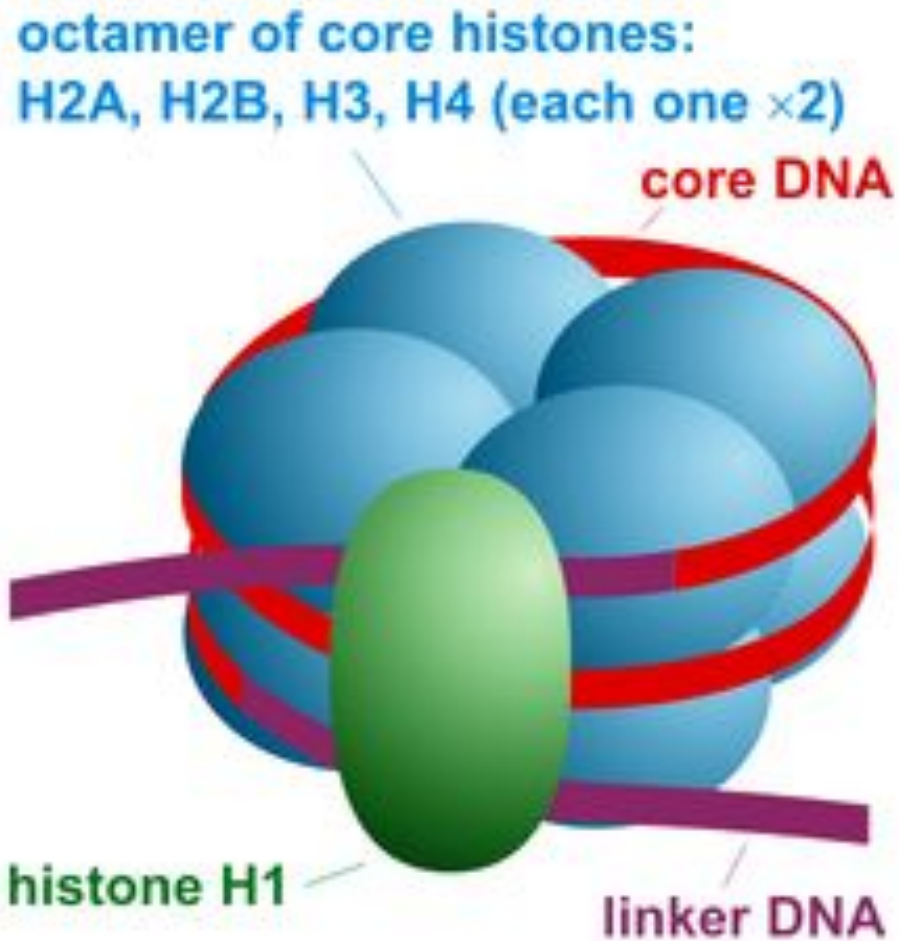
Single cell fertilised egg
1 cell type

Mouse embryo
Hundreds of cell types

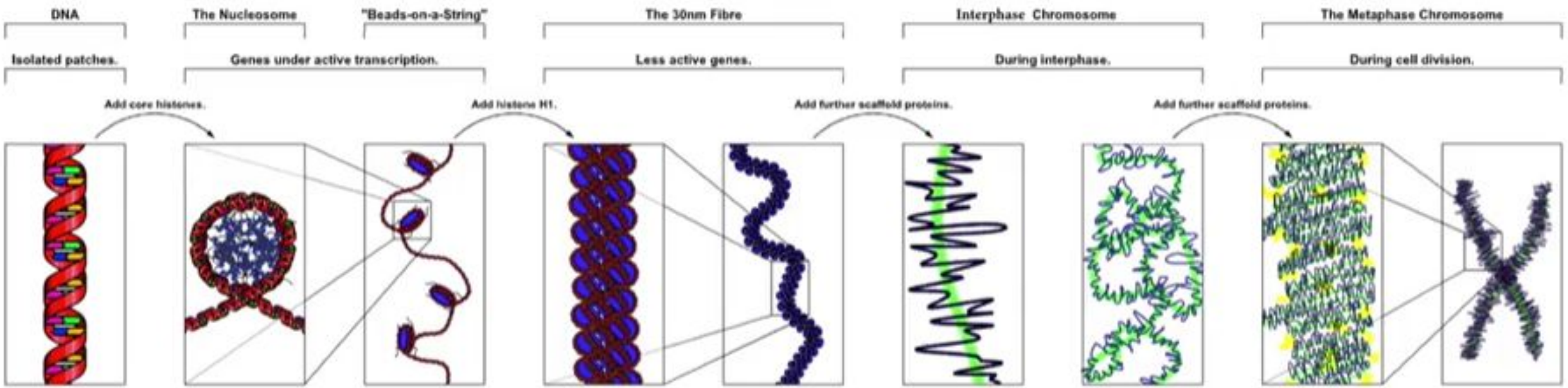


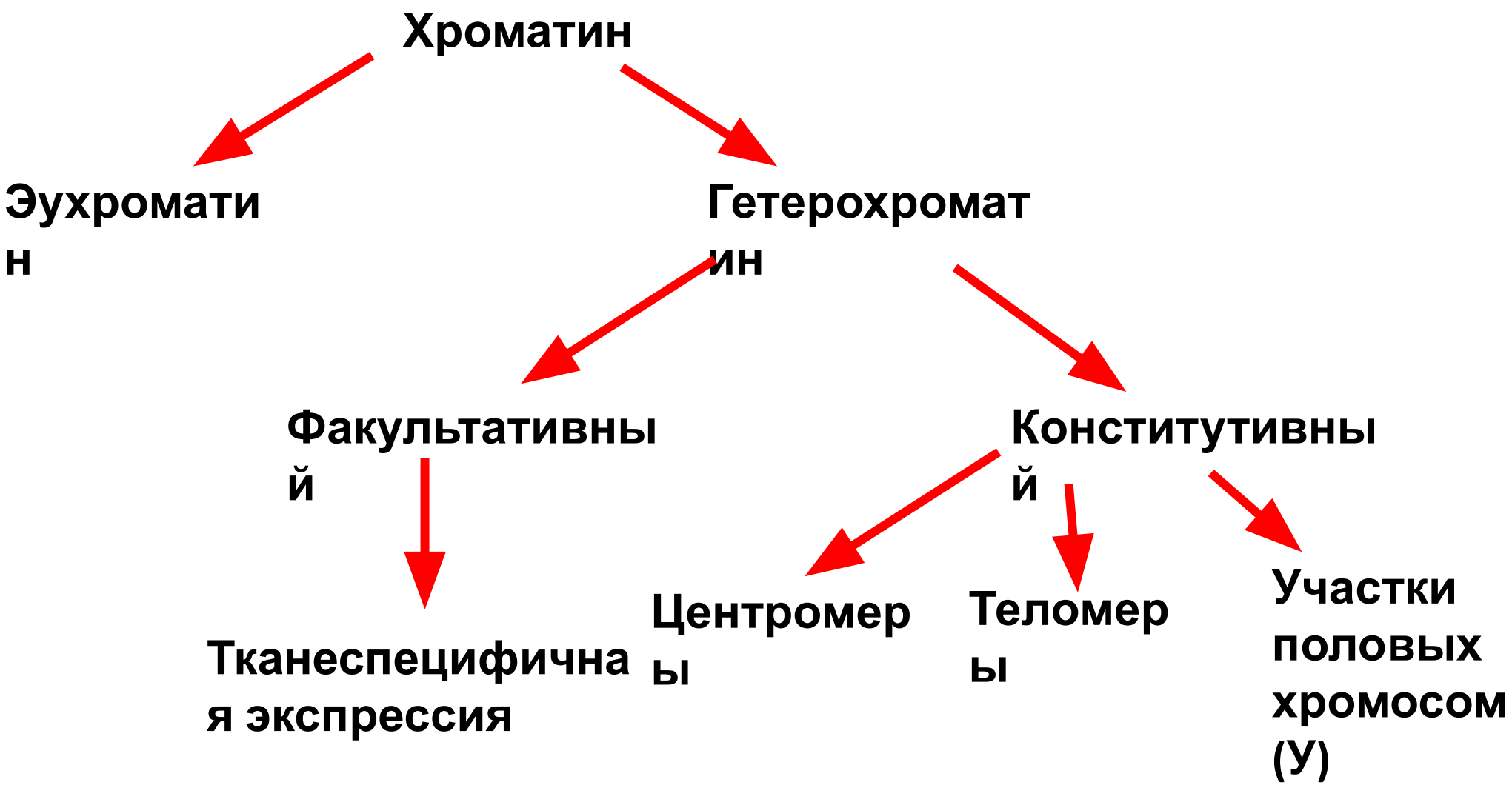
How does the same genetic information in every cell lead to so many different cell types, with each of their specialist functions?

Хроматин


octamer of core histones:
H2A, H2B, H3, H4 (each one ×2)
core DNA
histone H1
linker DNA

DNA packaging into chromatin: double helix to metaphase chromosome




Хроматин
Эухроматин
Гетерохроматин
Факультативный
Конститутивный
Тканеспецифичная экспрессия
Центромеры
Теломеры
Участки половых хромосом (У)

Specific epigenetic modifications

DNA methylation

Methylation of 5’ group of cytosines within CpG dinucleotides

Post-translational histone modifications

Methylation, ubiquitination, phosphorylation, sumoylation, acetylation of residues in the N-terminal tails of histones

Chromatin remodelling

ATP dependent chromatin remodelling complexes shift nucleosomes

Histone variants

Histones with varying stabilities or specialist domains that alter the function of the nucleosome

Noncoding RNAs

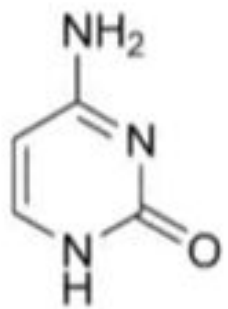
piRNAs and other siRNAs that can direct epigenetic machinery

Long noncoding RNAs – may direct epigenetic enzymes to sites in the genome

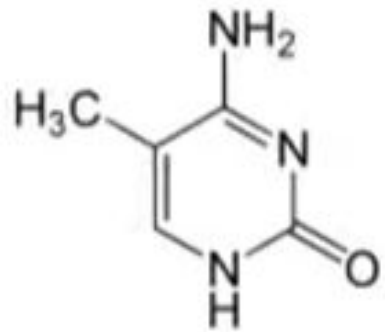
DNA methylation

Almost exclusively occurs at CpG dinucleotides in mammals

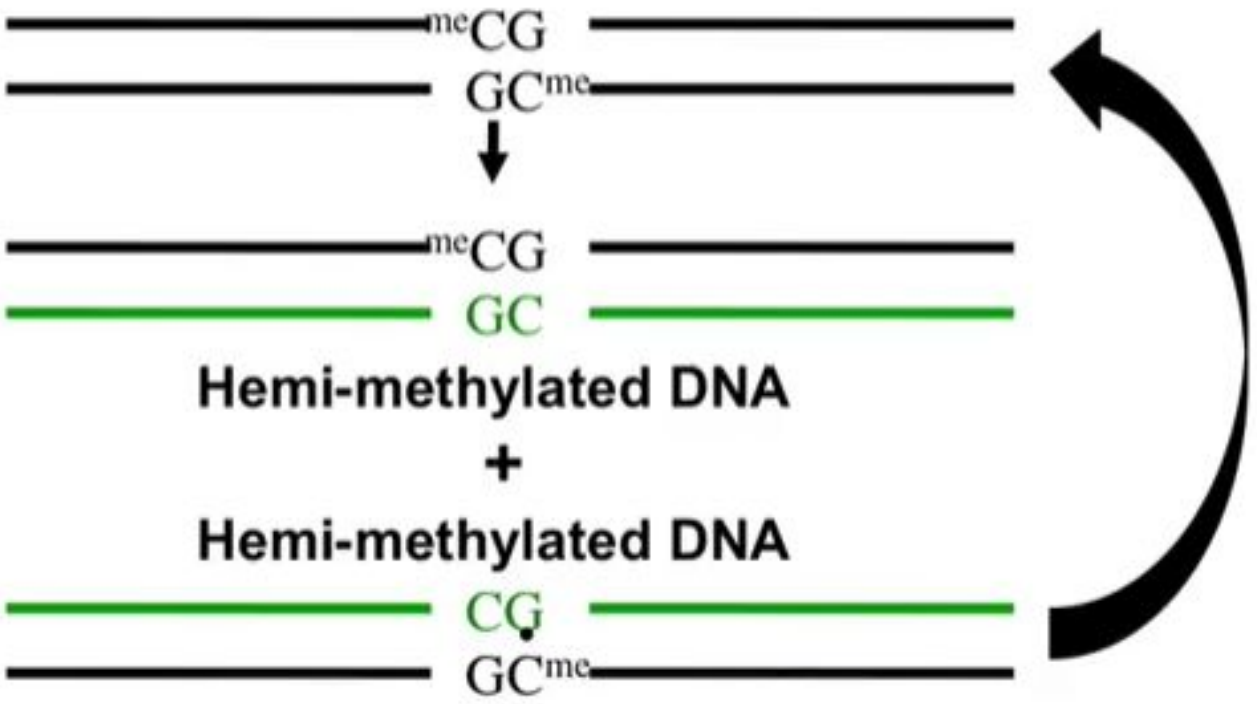
- Symmetrical, so able to be **maintained through cell division**



Cytosine



5- methyl cytosine



CpG острова часто находятся в промоторах.

DNMT 3 (De Novo Methyl Transferase)

How does DNA methylation lead to silencing?

- ^{me}CpG in a CpG island is associated with the formation of a repressive chromatin structure (**1º mechanism**)
  - ^{me}CpG can be bound by methylated CpG binding proteins e.g. MeCP1 & 2
  - MeCP proteins have a DNA binding domain and transcriptional repression domain
  - MeCP proteins can recruit other factors that condense the chromatin

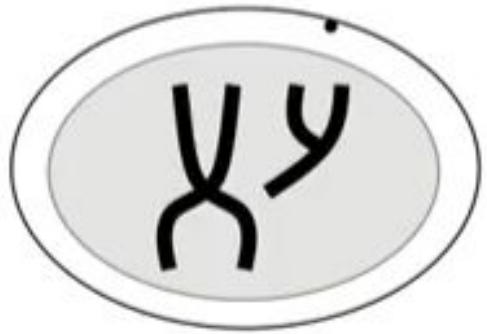
- ^{me}CpG can prohibit transcription factor binding, and alter gene expression (**2º mechanism**, probably for rare transcription factors, when CpG-poor promoters)

X inactivation – demonstrates mitotic heritability of DNA methylation

X inactivation is an epigenetic dosage compensation mechanism in mammals, so that males and females have the same dose of genes on the X chromosome

Random X inactivation occurs at gastrulation in the embryo, then this epigenetic state is mitotically inherited by all daughter cells

Female XX

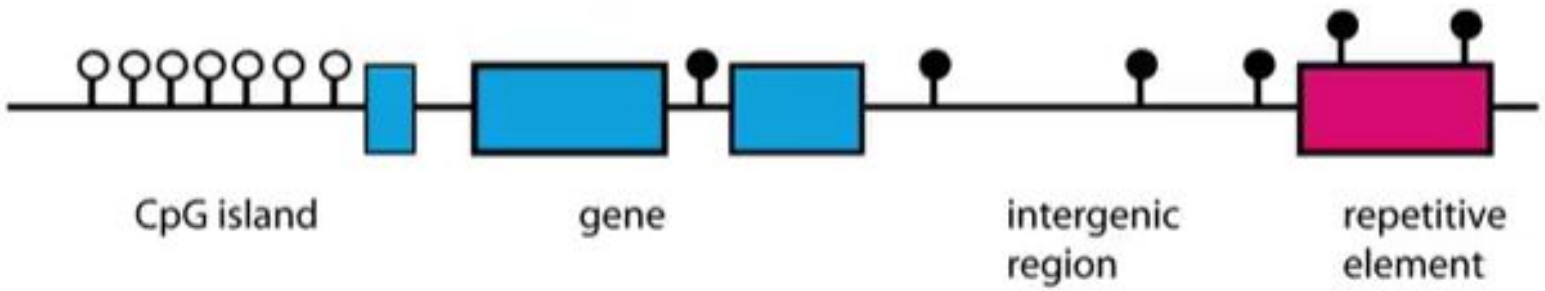


Male XY

Inactive X chromosome shows DNA methylation of CpG islands

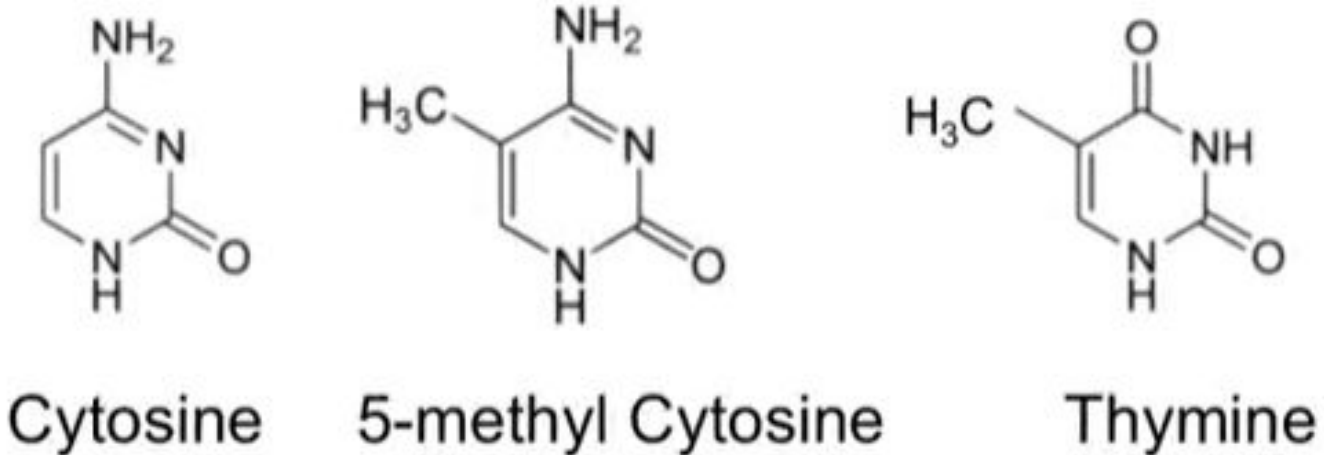
DNA methylation – where does it occur?

- CpG islands – usually unmethylated
- Intergenic regions – usually methylated
- Repetitive elements – usually methylated



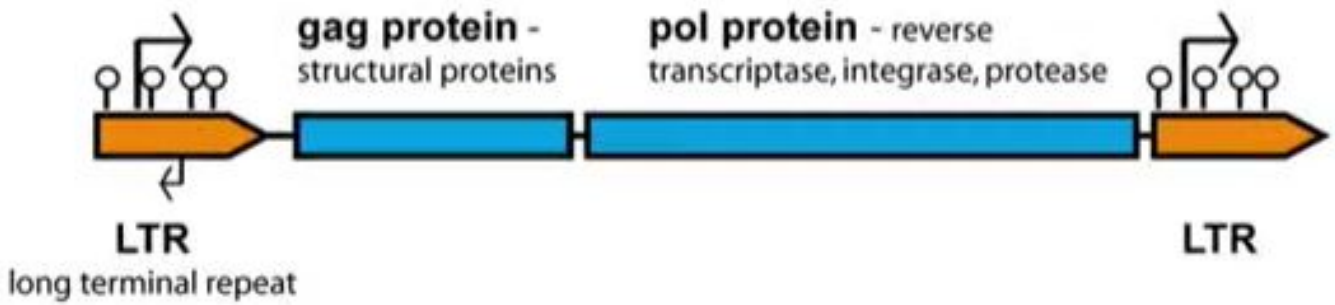
Метилирование CpG мутагенно

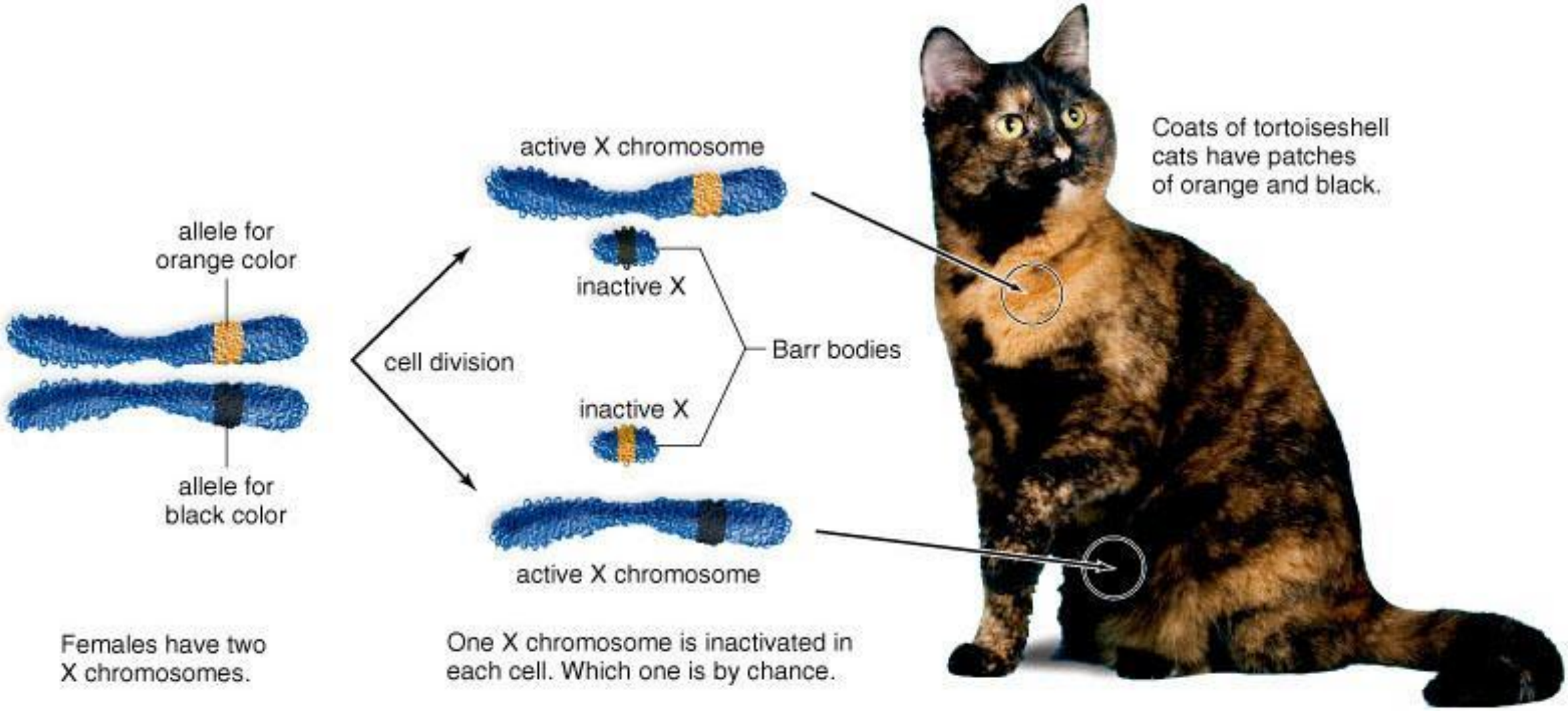
- В геноме CG пар меньше, чем можно было ожидать вероятностно, т.к. metC переходит в T.



What is the function of DNA methylation at repetitive elements?

- **Maintain genomic integrity**
  - silencing of repeats to prevent transposition
  - mutation of the repeats (meC to T) to prevent transposition
  - silencing of repeats, so avoid transcriptional interference from strong promoters
  - methylation of repeats may prevent illegitimate recombination

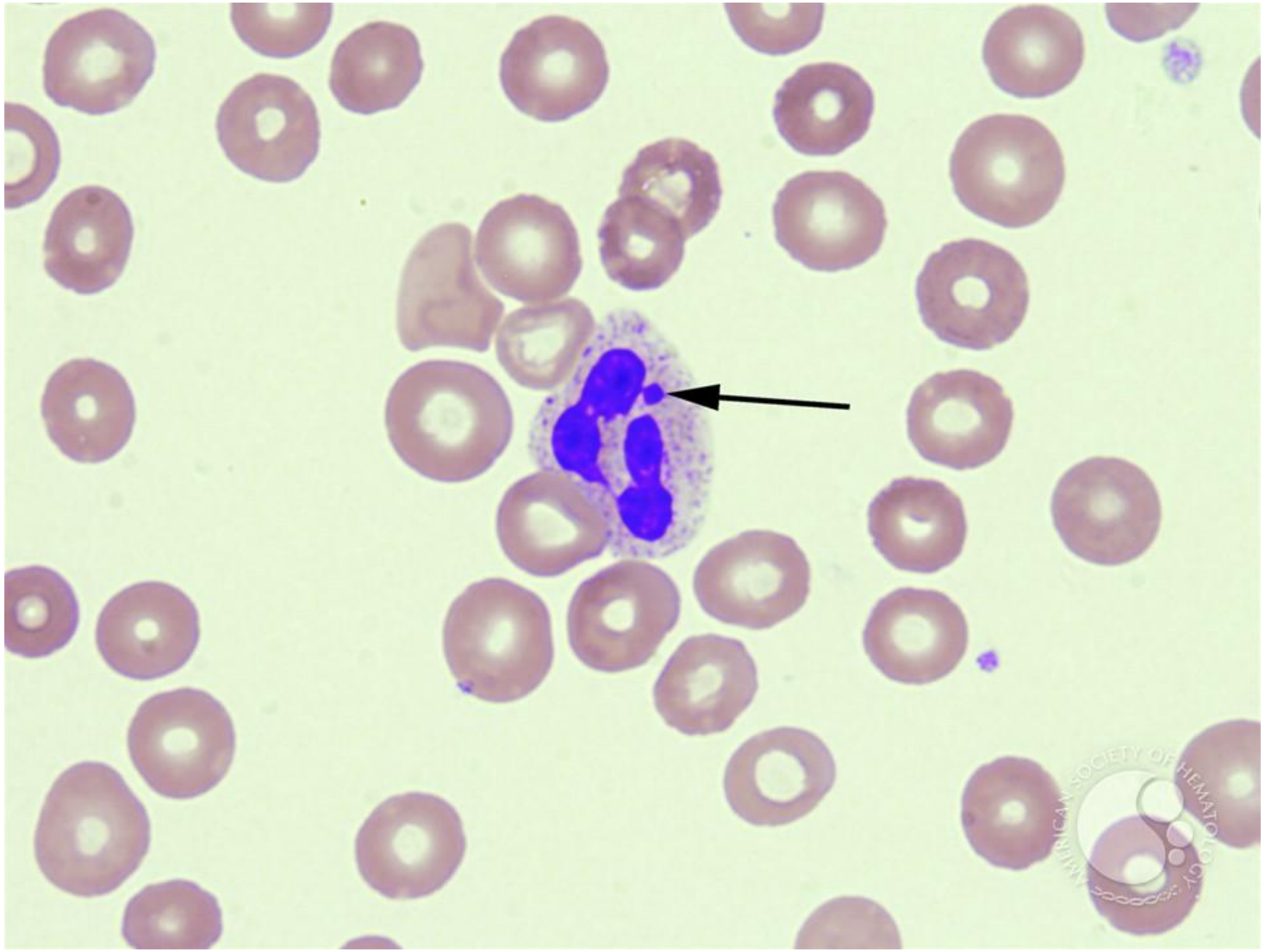




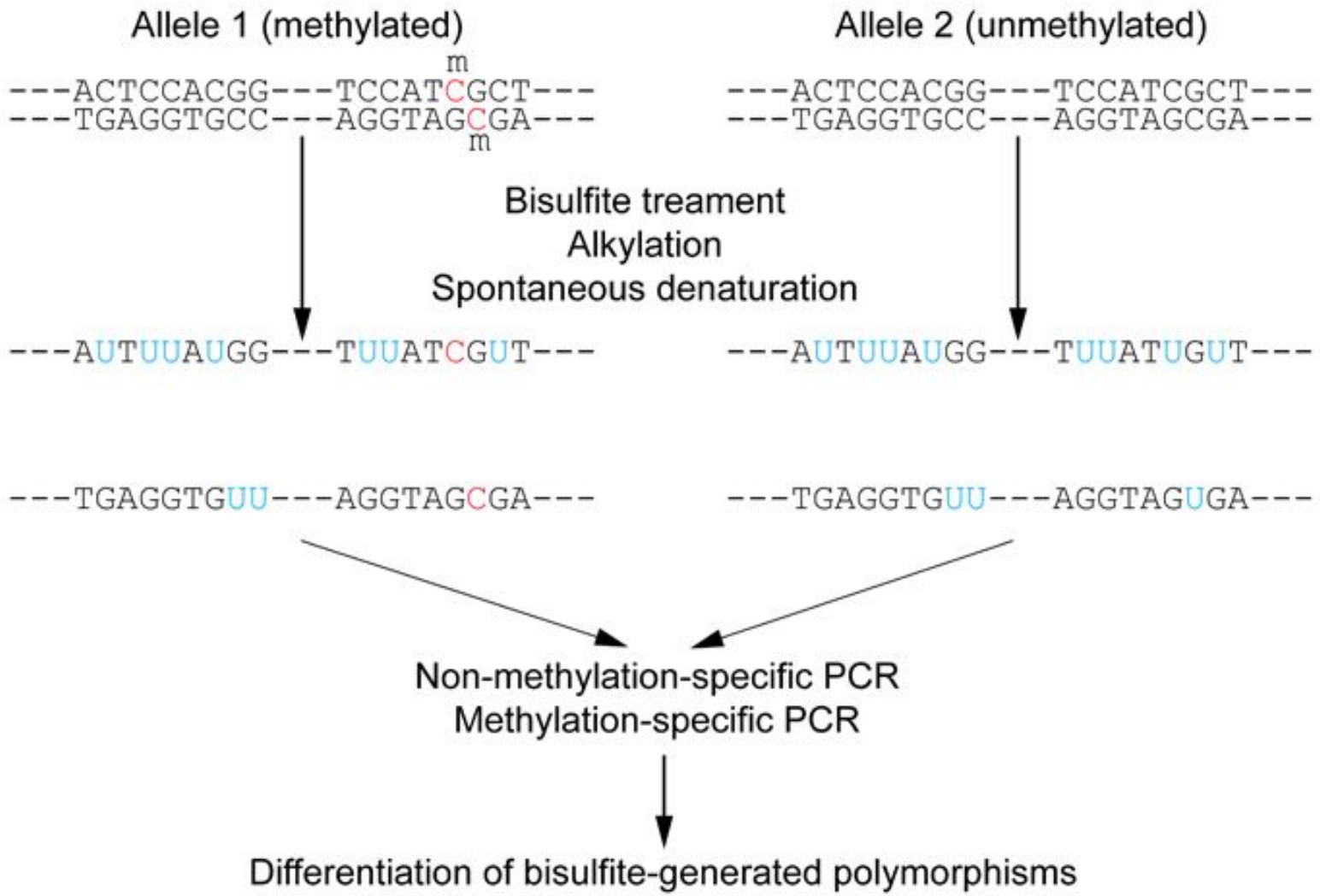
Females have two X chromosomes.

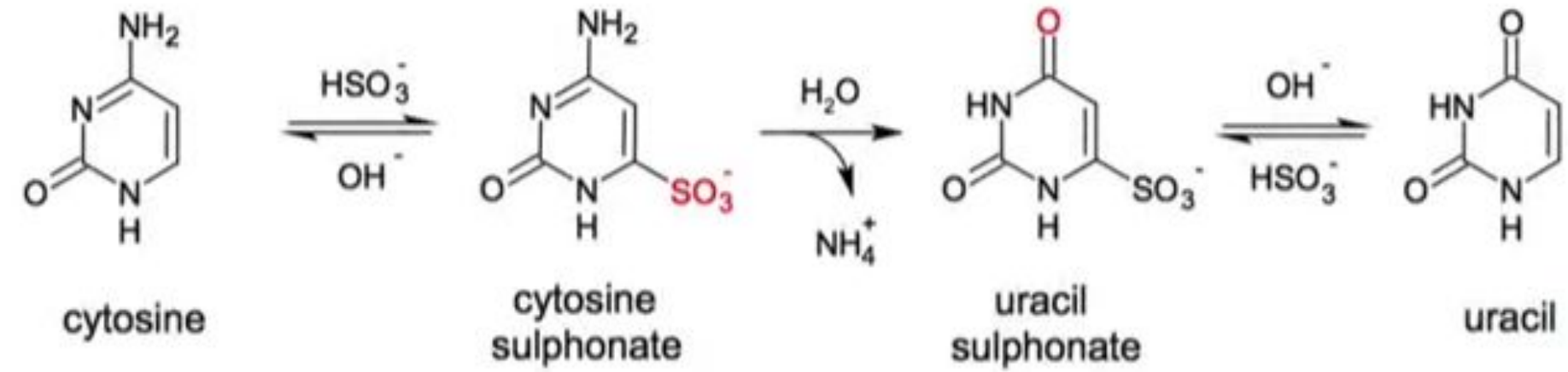
One X chromosome is inactivated in each cell. Which one is by chance.

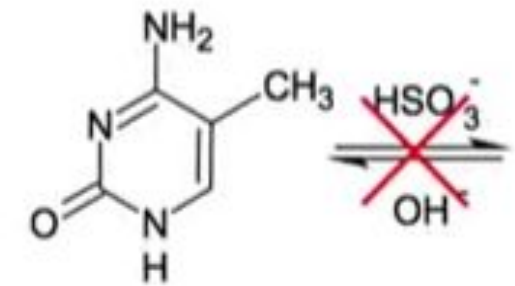
Тельце Барра



Бисульфитное секвенирование – определение метилированных CpG

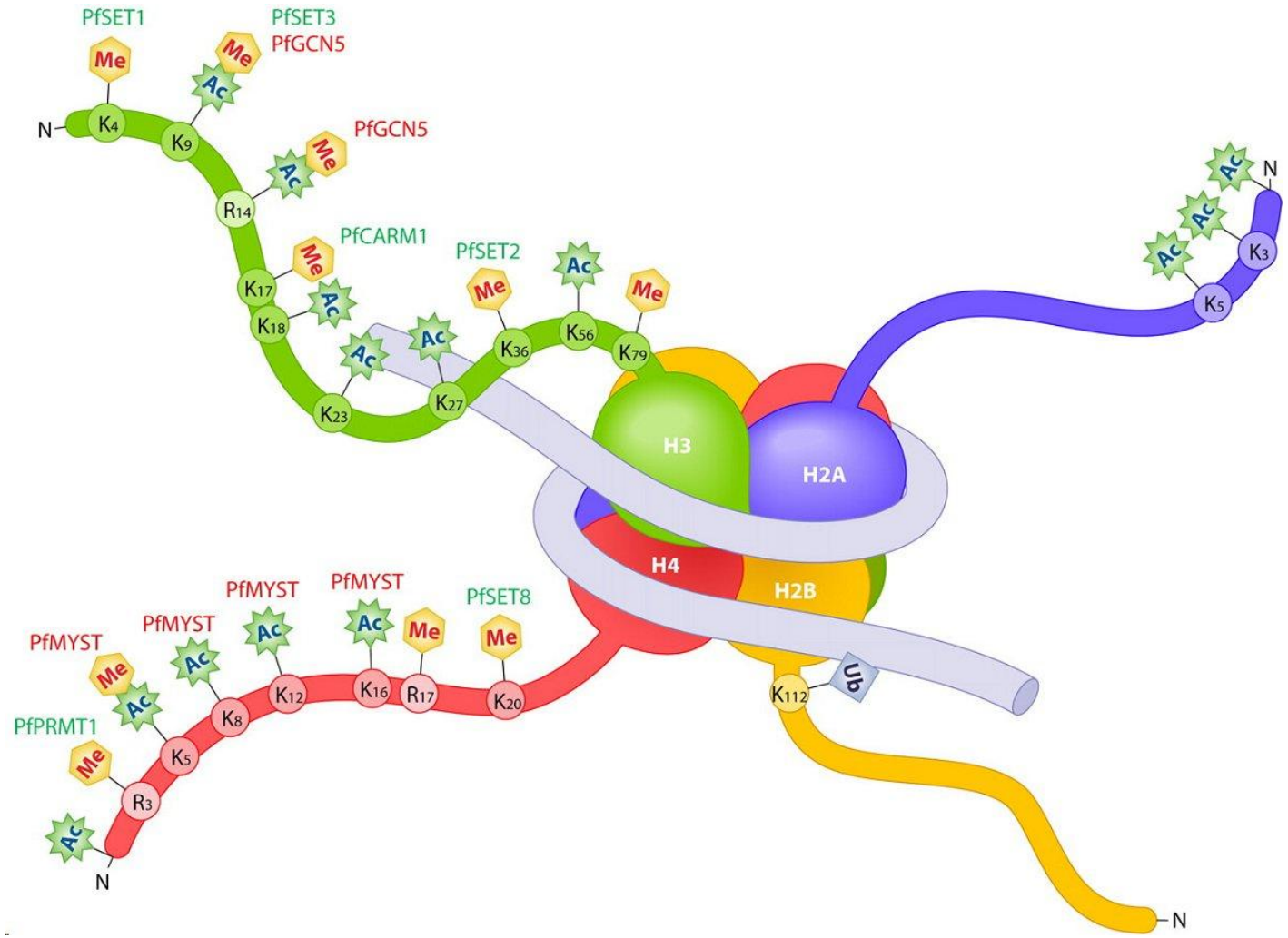






5-methylcytosine

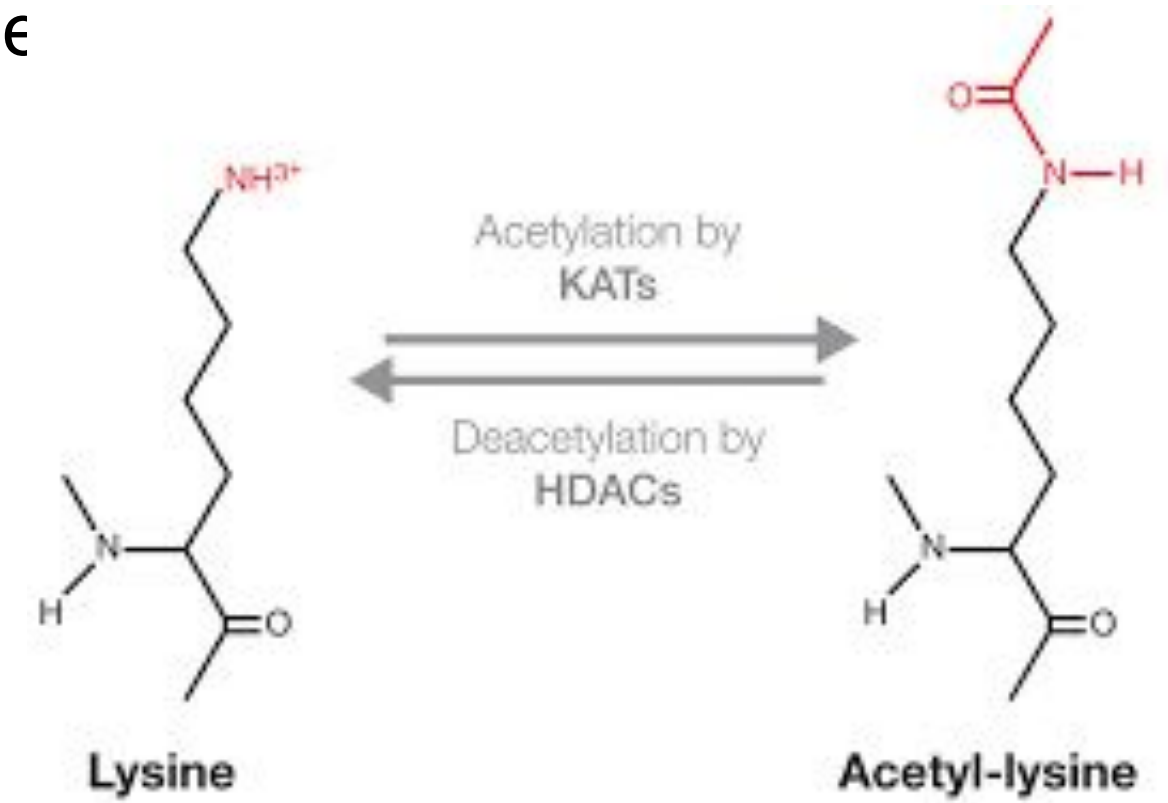
Хвосты гистонов



Histone modification	Which residues?	Functions
Methylation (me, mono, di or tri me)	Lysines (K) and Arginines (R)	Transcription, Repair (K)
Acetylation (ac)	K	Transcription, Repair, Replication, Condensation
Ubiquitination (ub)	K	Transcription
Sumoylation (su)	K	Transcription
ADP-ribosylation	Glutamate (E)	Transcription
Phosphorylation (Ph)	Serine (S) and Threonine (T)	Transcription, Repair, Condensation
Citrullination (Cit)	R converts to Cit	Transcription

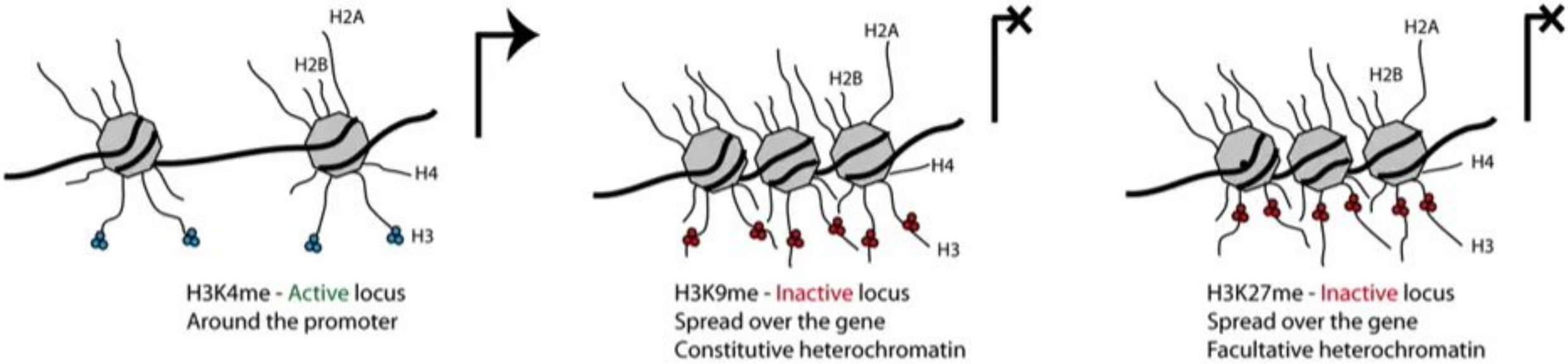
Ацетилирование гистонов

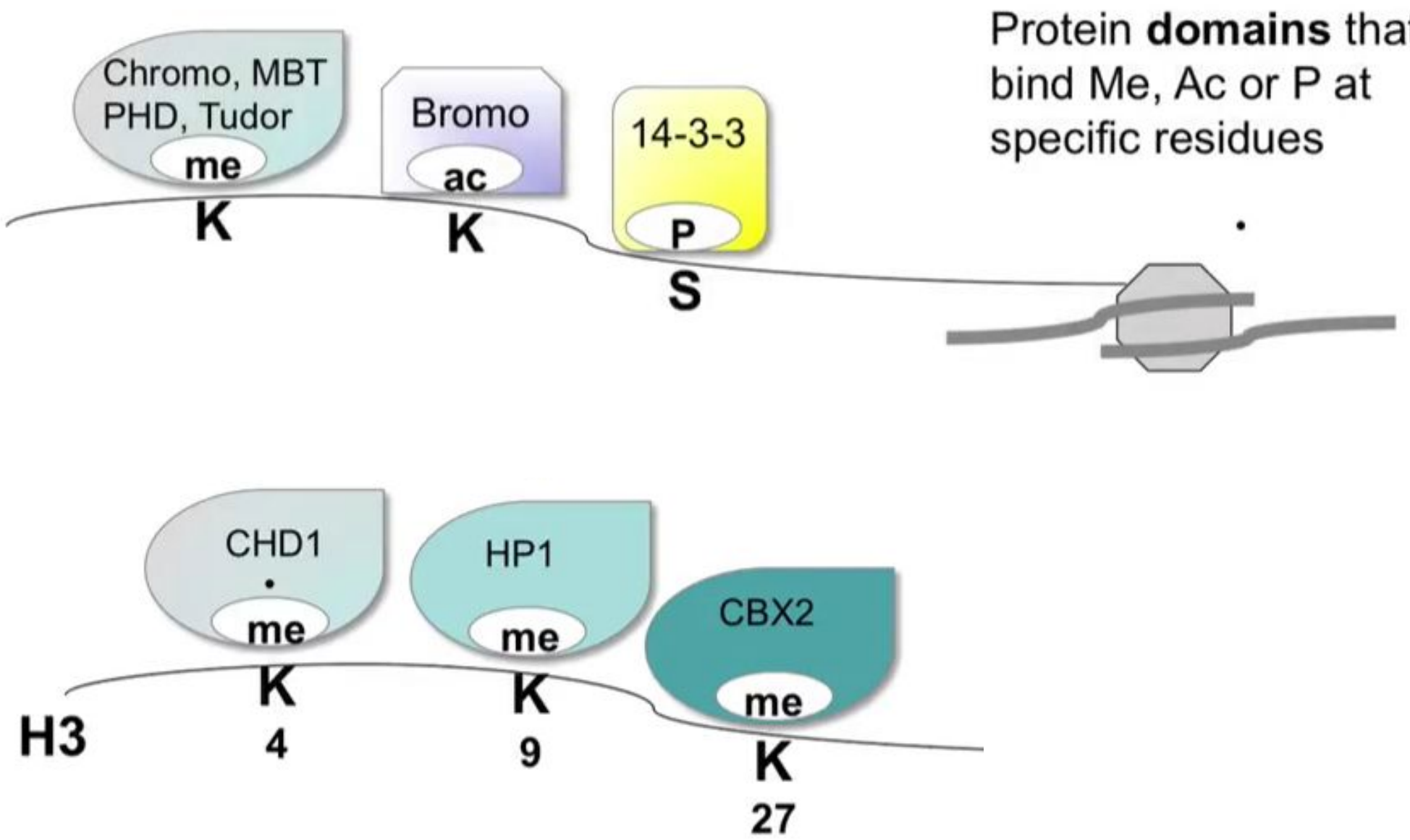
- Эухроматинизация
- Рекрутирование комплексов ремоделлинга через бромодомены
- НЕ эпигенє митотически



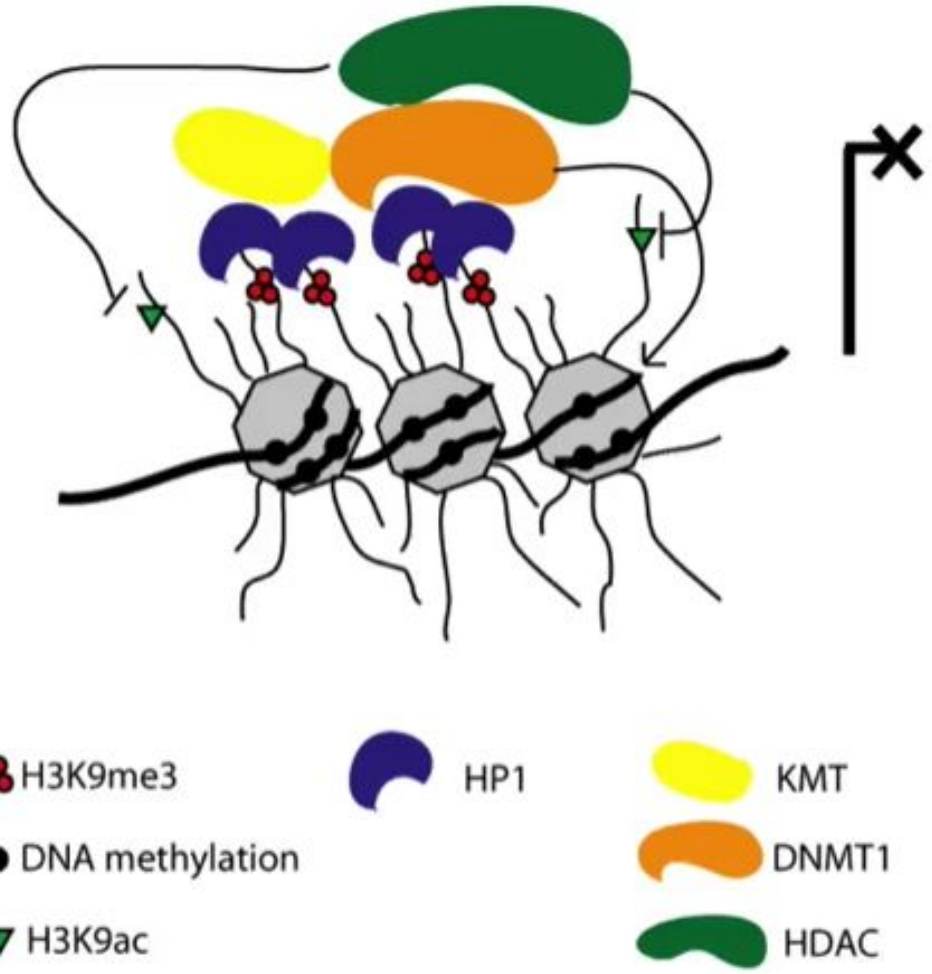
Метилирование гистонов

- Может как активировать так и ингибировать транскрипцию
- Не меняет заряд




Protein **domains** that bind Me, Ac or P at specific residues
Chromo, MBT
PHD, Tudor
me
K
Bromo
ac
K
14-3-3
P
S
CHD1
me
K
4
HP1
me
K
9
CBX2
me
K
27
H3

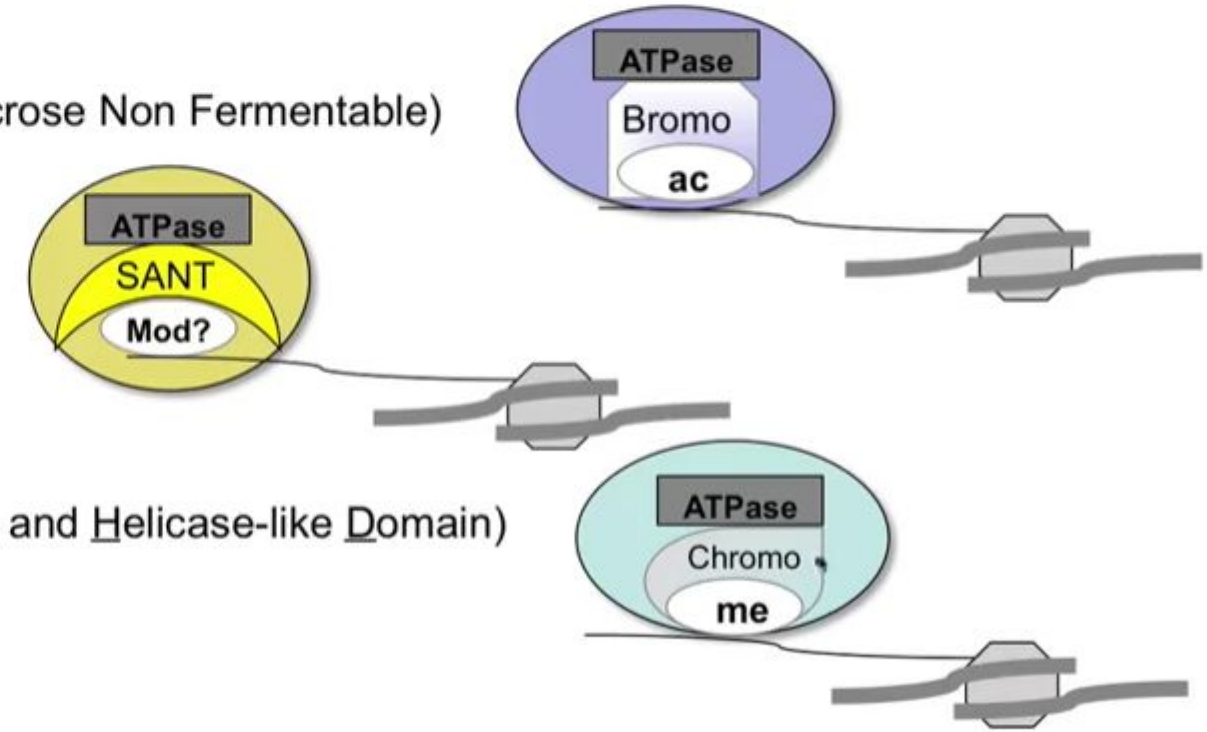
- H3K9me рекрутирует DNMT, HDAC (Histone DeAcetylase), HP1 (рекрутирует HMT, Histone Methyl Transferase, для распространения метки).



Комплексы ремоделлинга – АТФ-зависимое передвижение нуклеосом

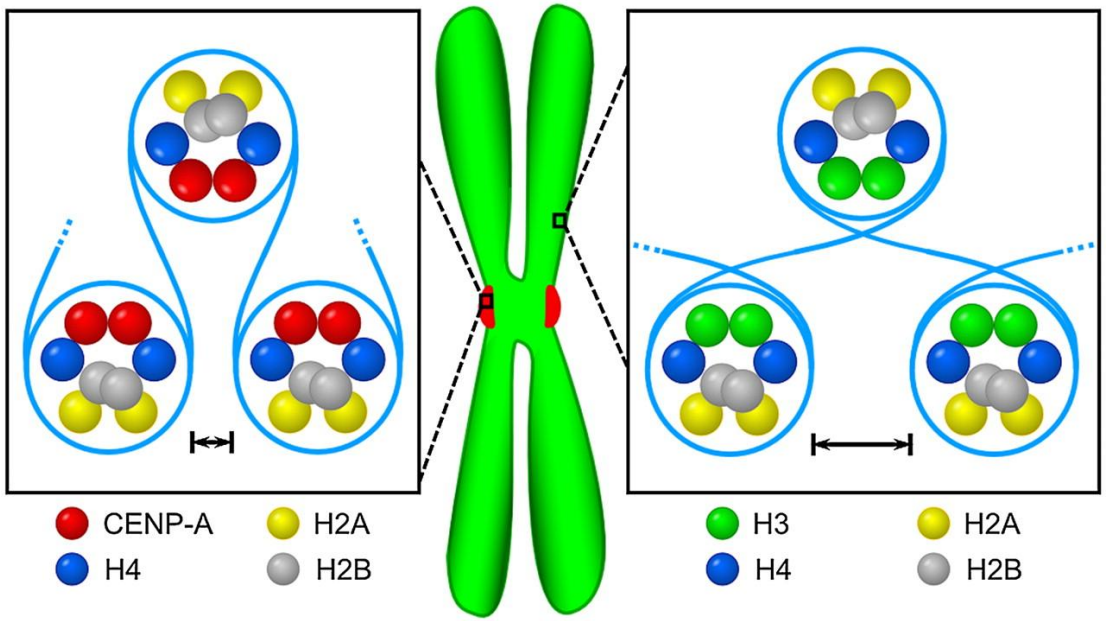
Three main types of multi-protein complex, each with specific binding domains and ATPases

- **SWI-SNF** (SWItch/Sucrose Non Fermentable)
- **ISWI** (Imitation SWI)
- **CHD** (Chromo domain and Helicase-like Domain)

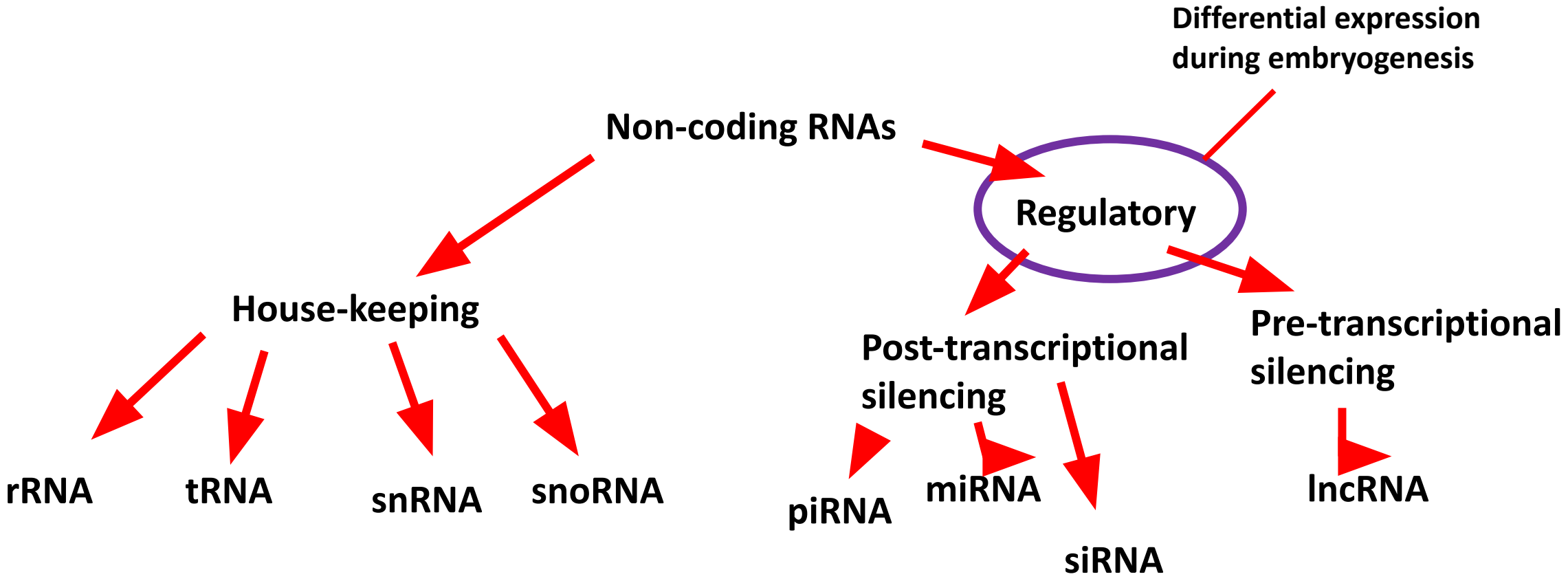


Варианты гистонов

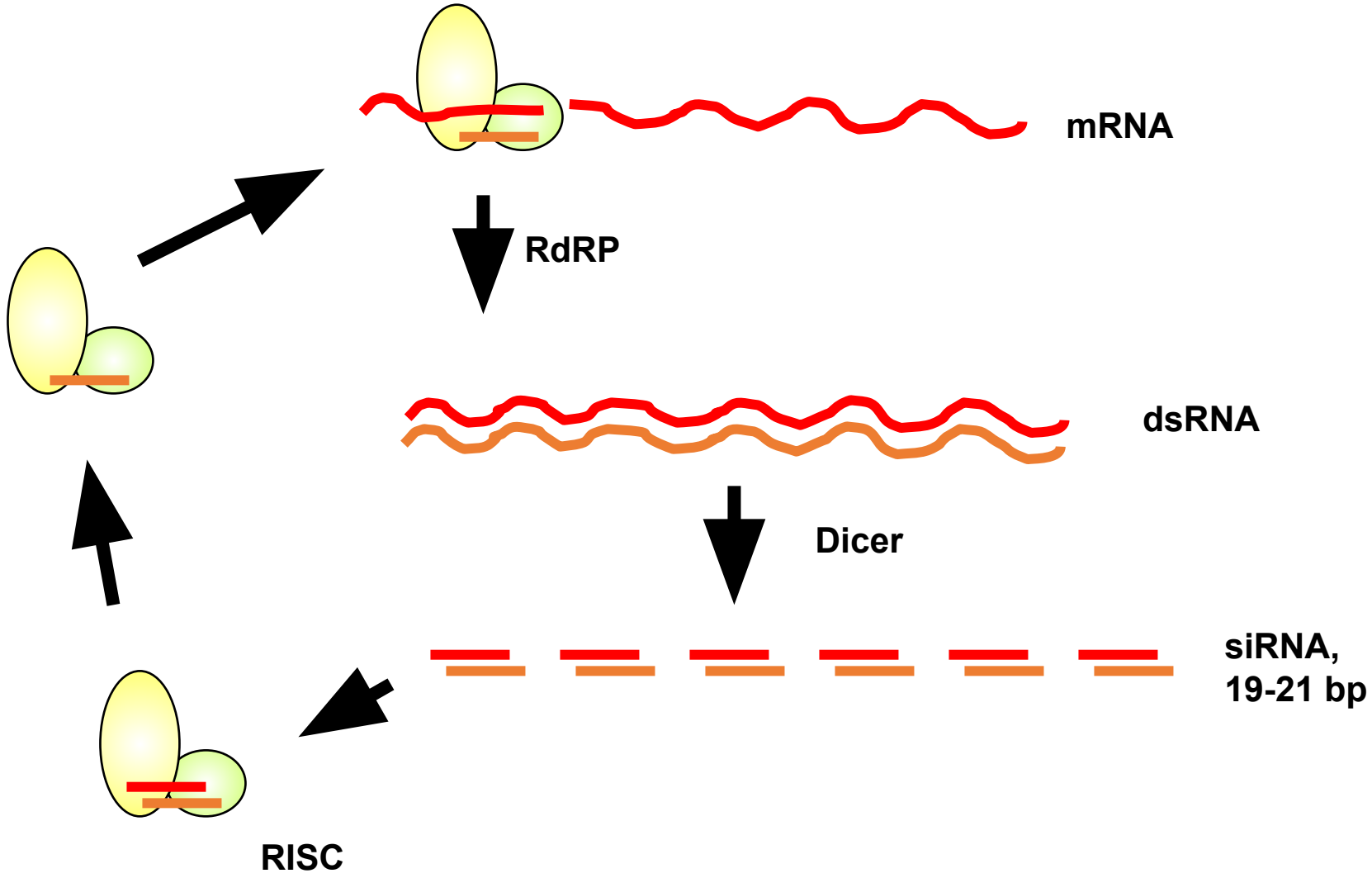
- Гистоны H2A, H3 и H1 бывают в виде нескольких вариантов.
- CENP-A (centromere specific histone)
- H2A.X – репарация (доп. сайт фосф., после репарации отщепляется фосфатазой)
- macroH2A – неактивная X хромосома



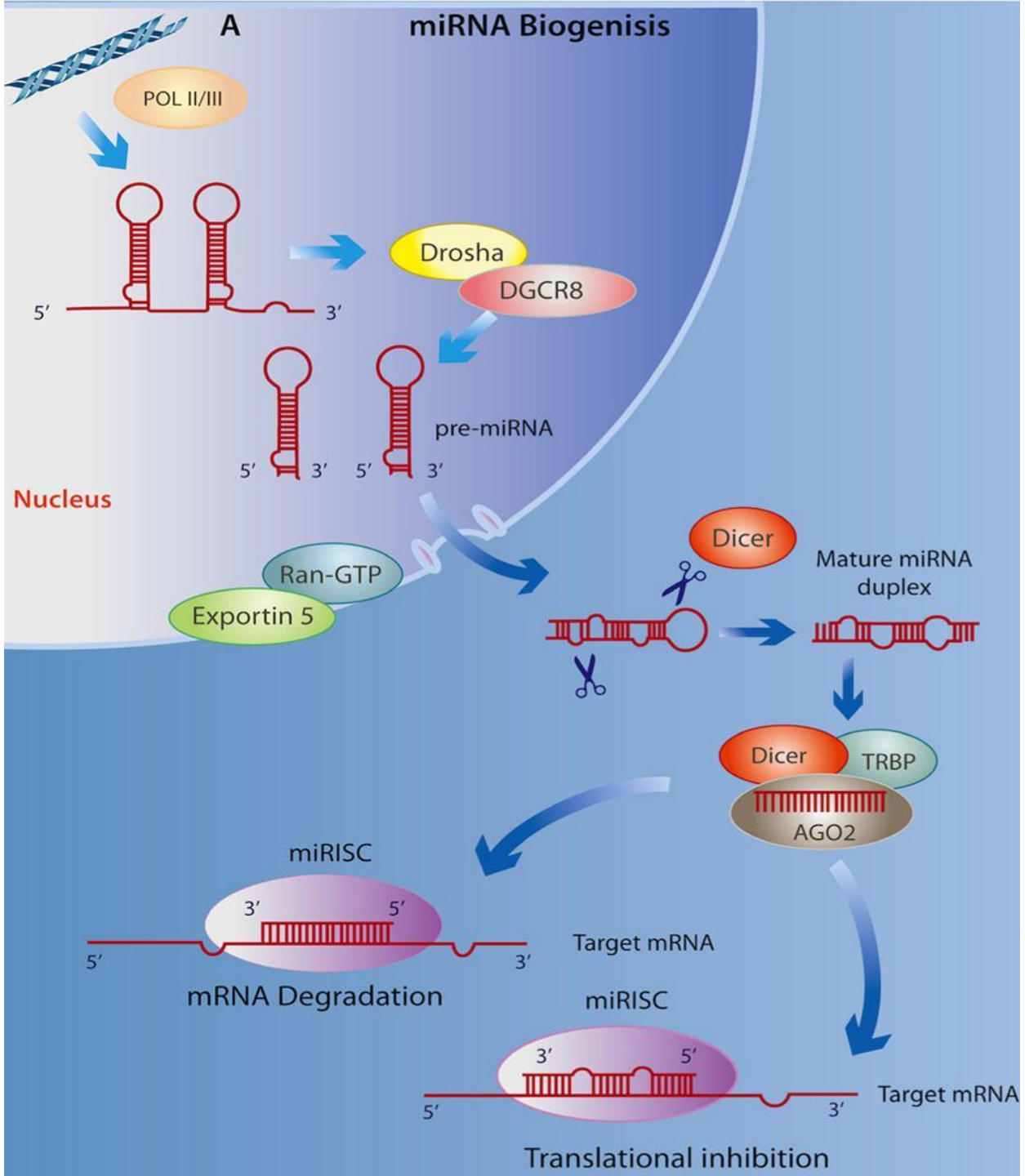
Некодирующие РНК

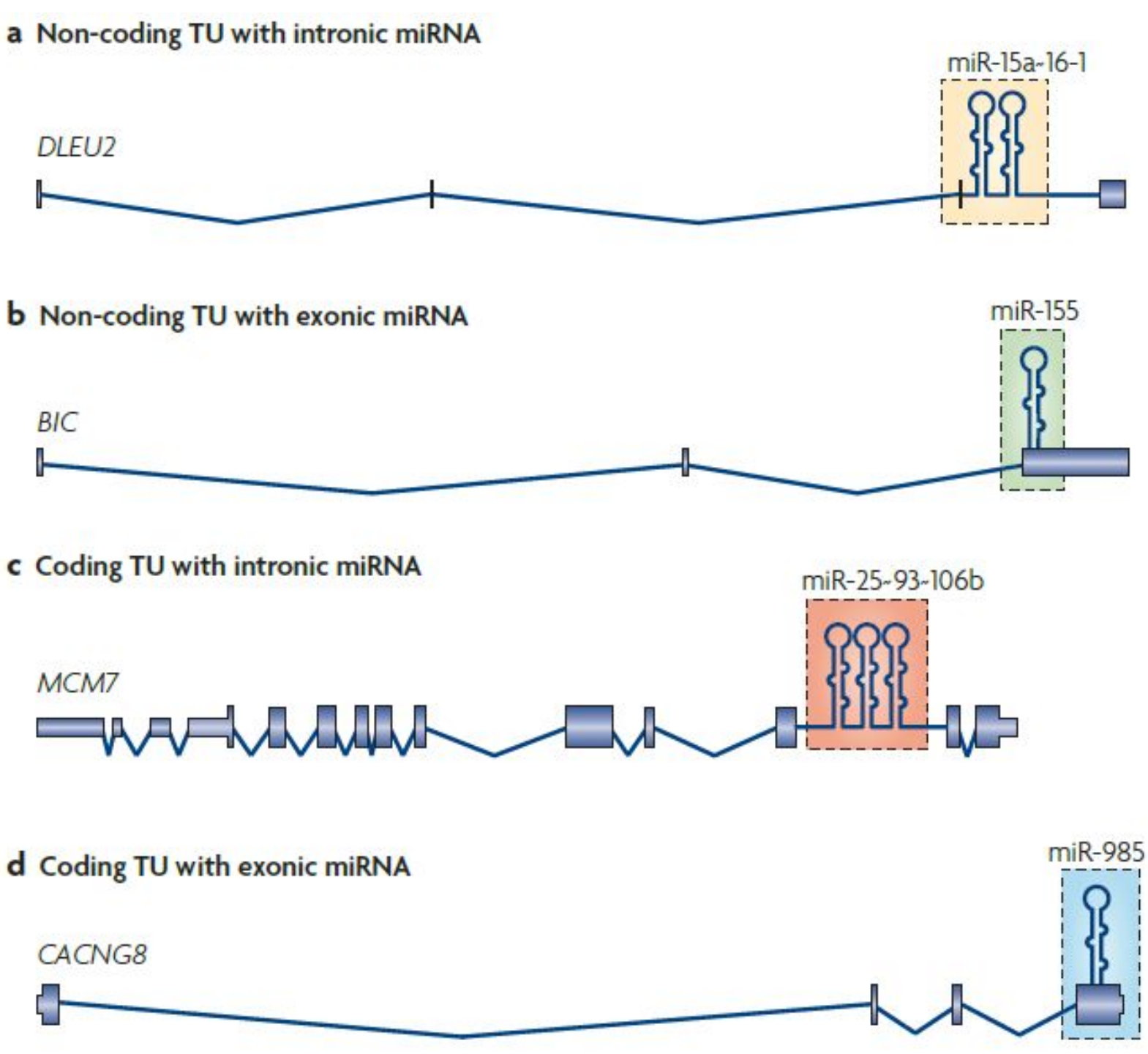


РНК-интерференция - siRNA



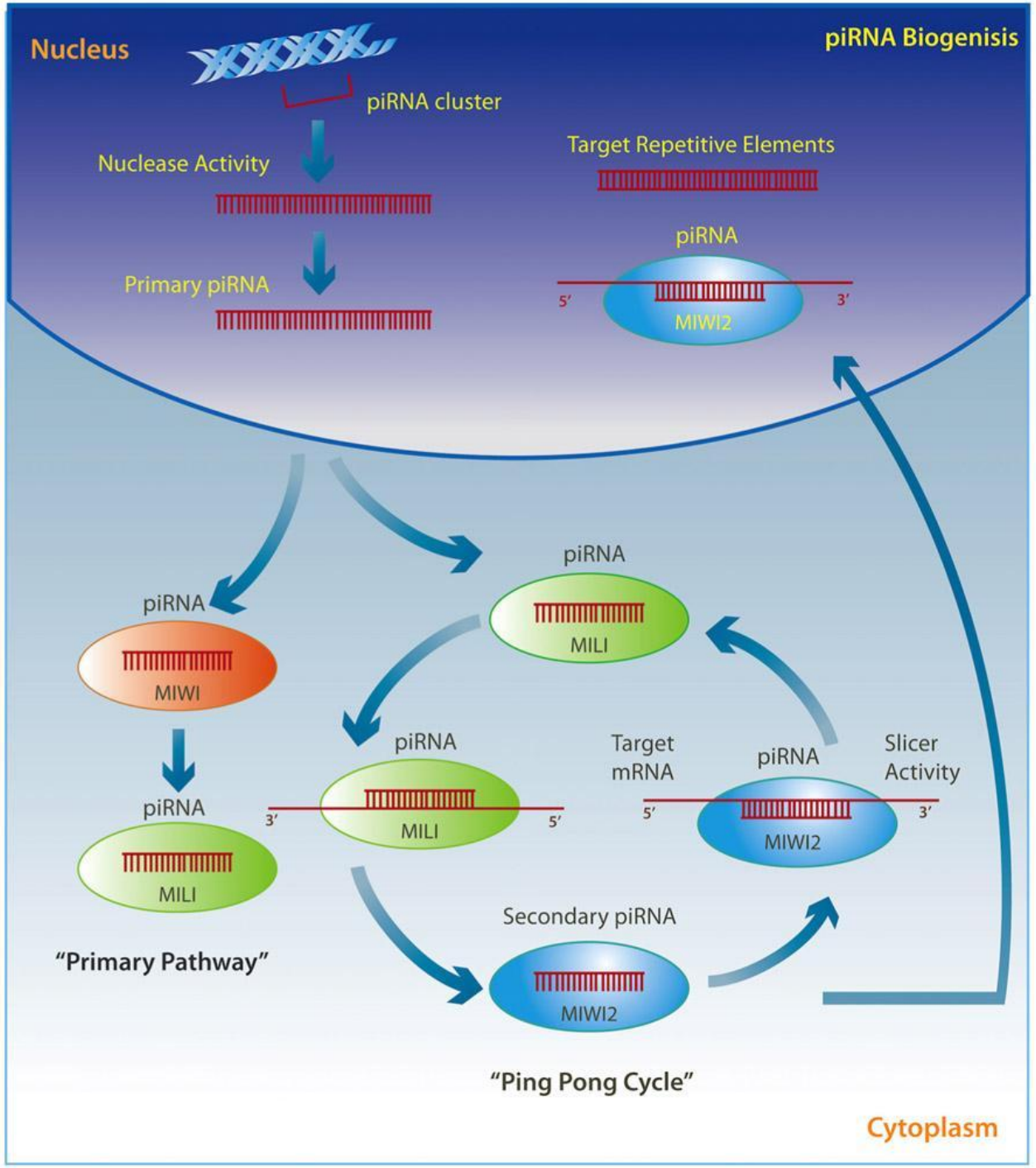
microRNA

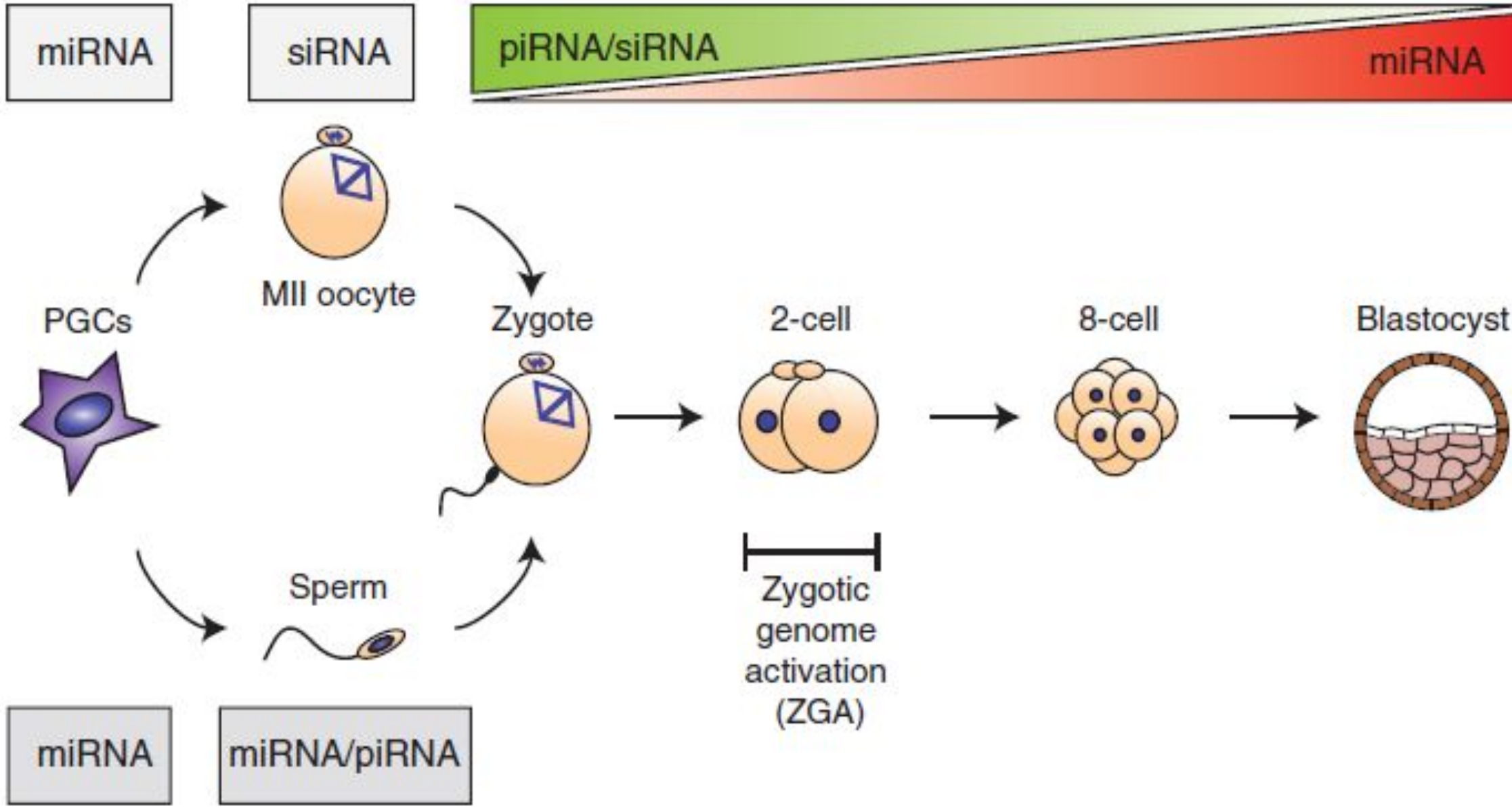

A
miRNA Biogenisis
POL II/III
5′
3′
Drosha
DGCR8
pre-miRNA
Nucleus
Ran-GTP
Exportin 5
Dicer
Mature miRNA
duplex
Dicer
TRBP
AGO2
miRISC
Target mRNA
mRNA Degradation
miRISC
Target mRNA
Translational inhibition


a Non-coding TU with intronic miRNA
miR-15a~16-1
DLEU2
b Non-coding TU with exonic miRNA
miR-155
BIC
c Coding TU with intronic miRNA
miR-25~93~106b
MCM7
d Coding TU with exonic miRNA
miR-985
CACNG8

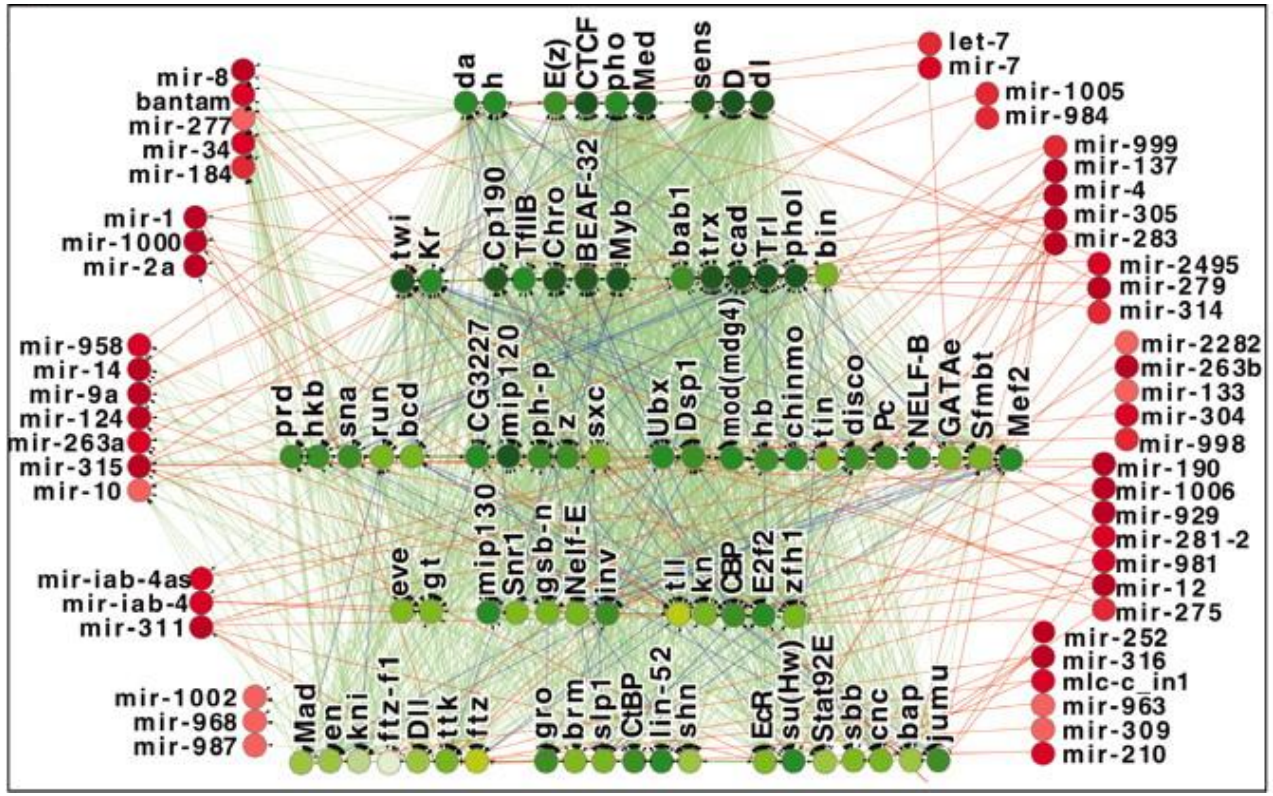
Piwi RNA

piRNA стимулируют метилирование повторов




miRNA
siRNA
piRNA/siRNA
miRNA
MII oocyte
PGCs
Zygote
2-cell
8-cell
Blastocyst
Sperm
Zygotic genome activation (ZGA)
miRNA
miRNA/piRNA

miRNAs are integrated in the regulatory networks

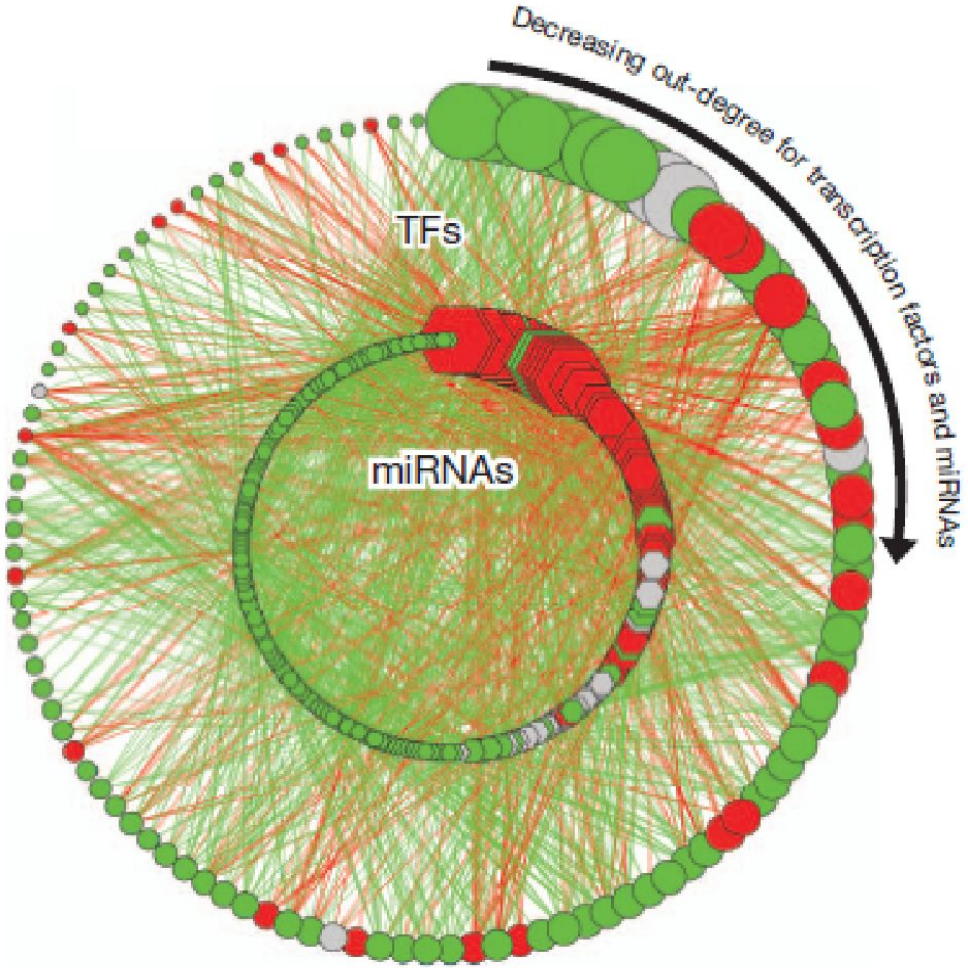


Regulatory network in *Drosopila* cells

Transcription factor gene

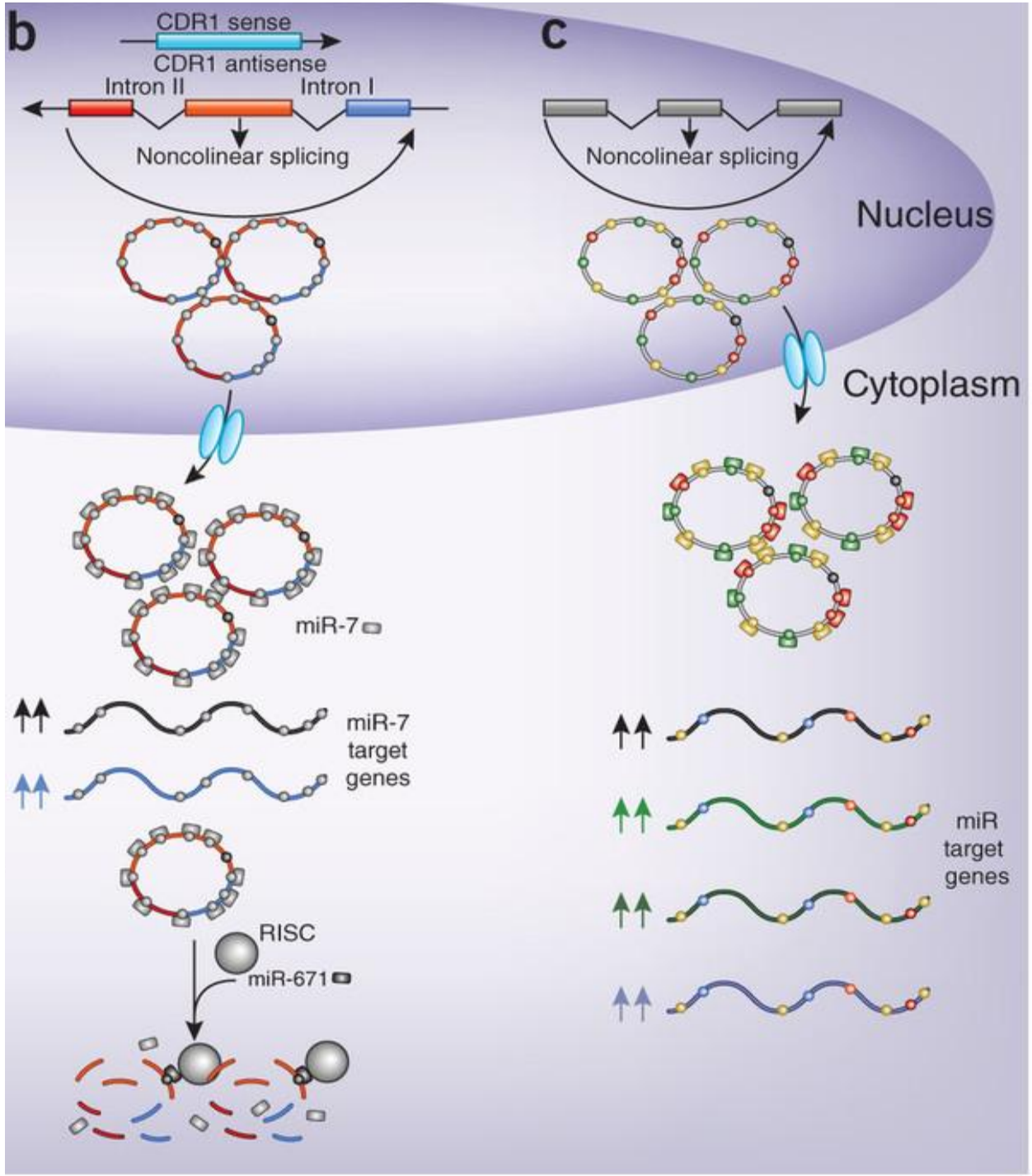
miRNA gene

Target gene



Regulatory network in human cells

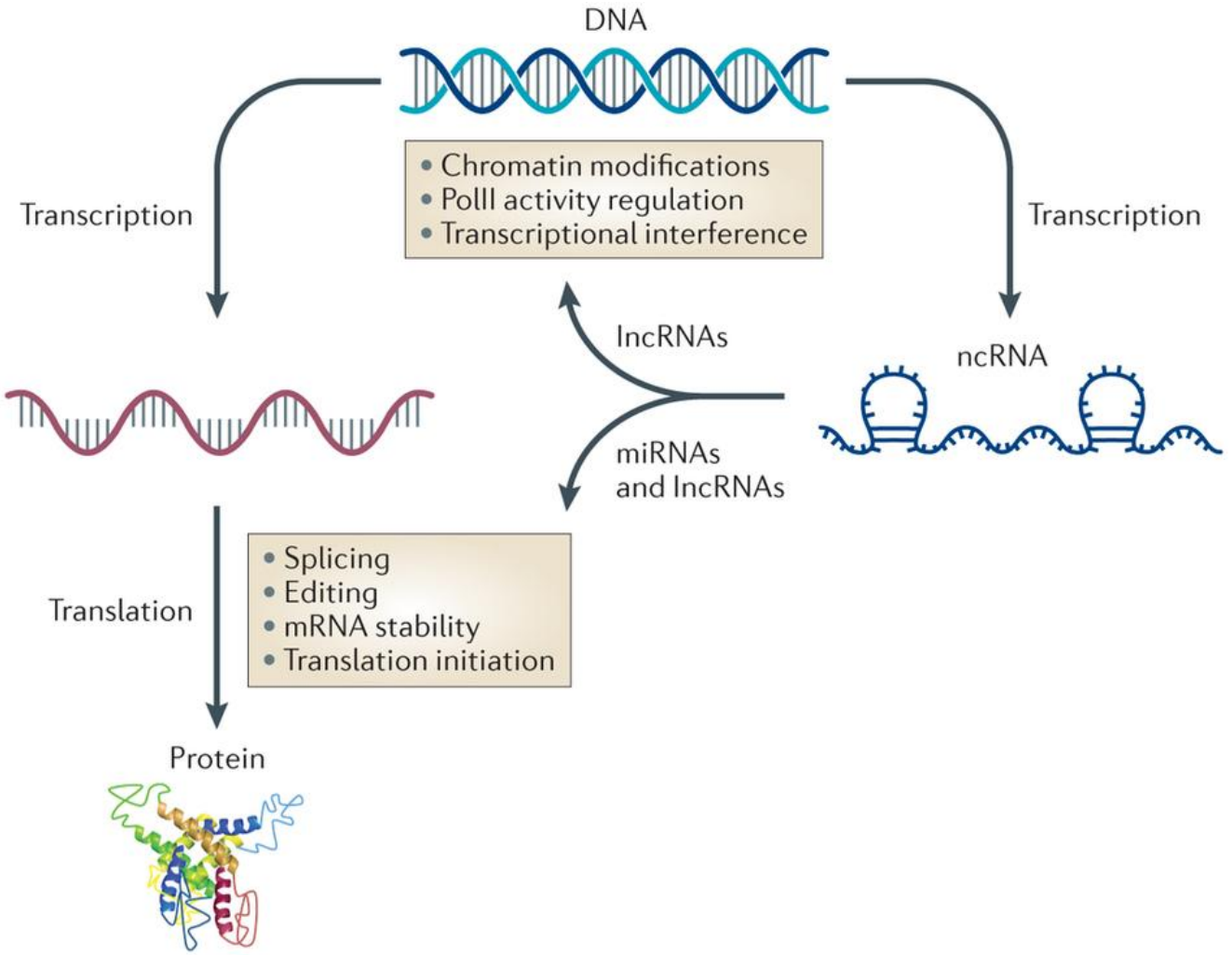
circRNA


b
CDR1 sense
CDR1 antisense
Intron II
Intron I
Noncolinear splicing
c
Noncolinear splicing
Nucleus
Cytoplasm
miR-7
miR-7
target
genes
RISC
miR-671
miR
target
genes


circRNA
miRNA

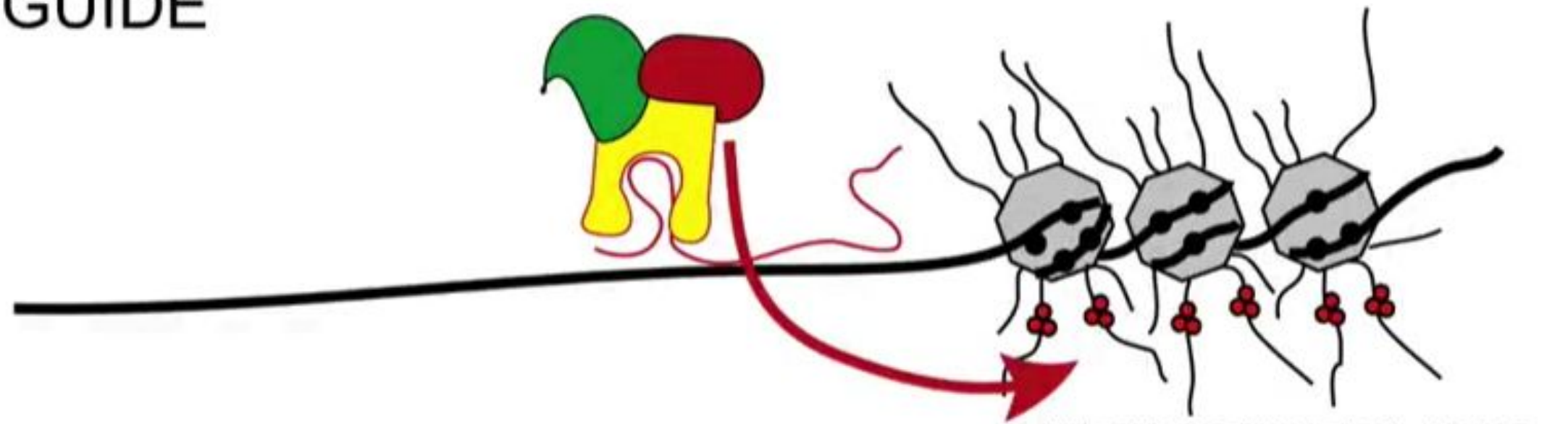
lncRNA – длинные некодирующие

- Прошли сплайсинг, кэпирование и полиаденилирование
- >200 b
- Обеспечивают специфичность комплексов ремоделинга
- Как in cis так и in trans
- Могут обеспечивать транскрипционную интерференцию
- Могут работать как Guide или Scaffold



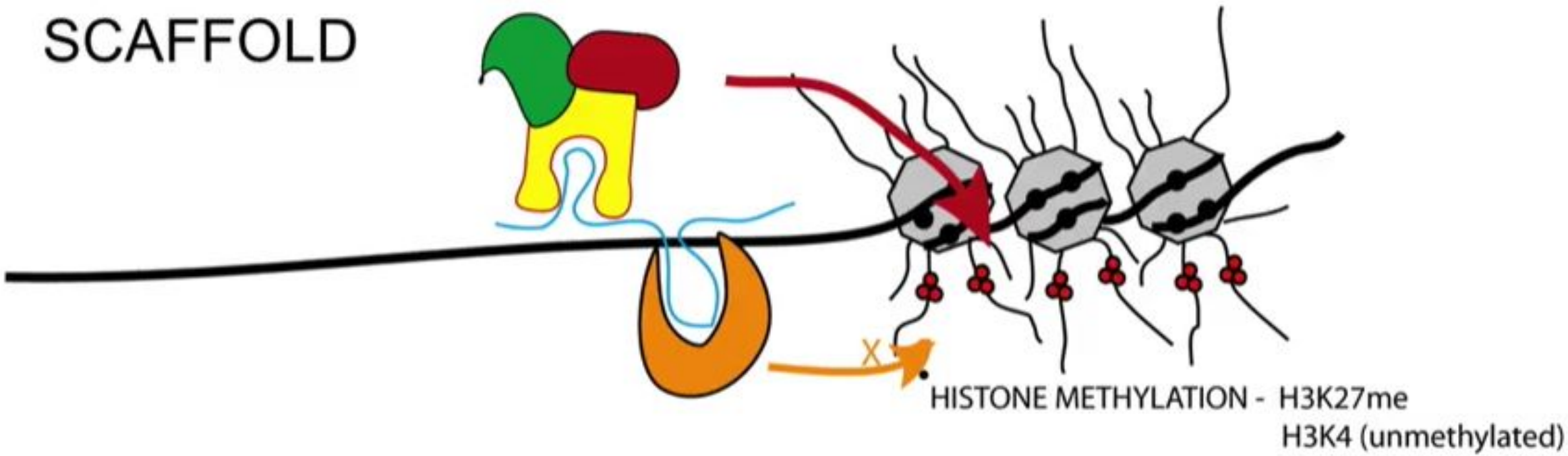
Nature Reviews | Drug Discovery

GUIDE



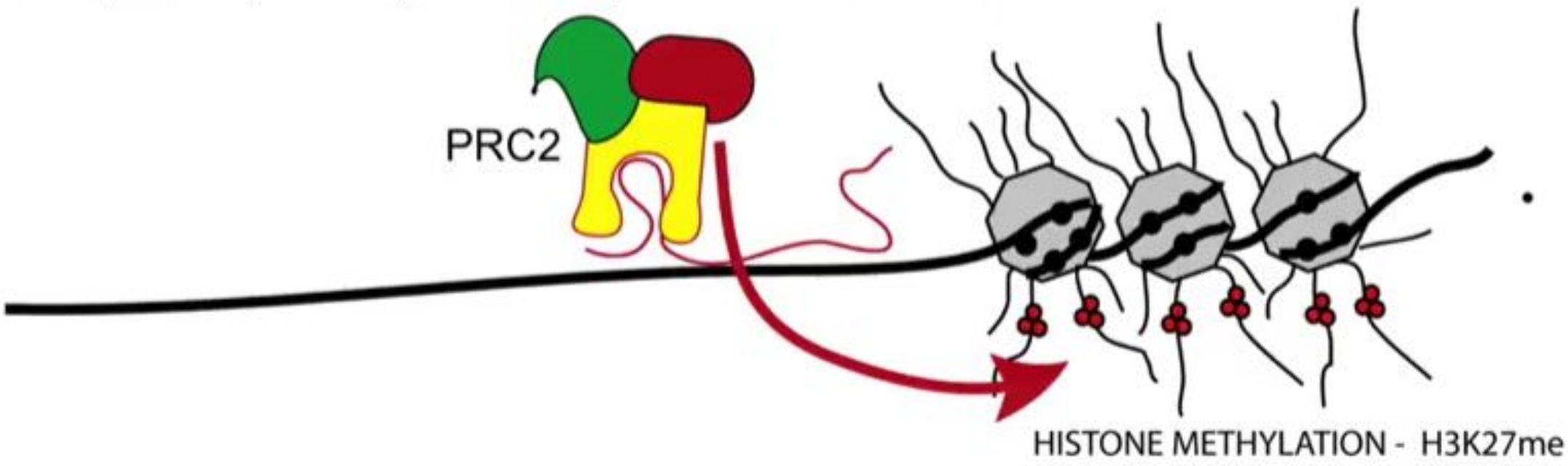
HISTONE METHYLATION - H3K27me

- Специфичность по последовательности необязательна.



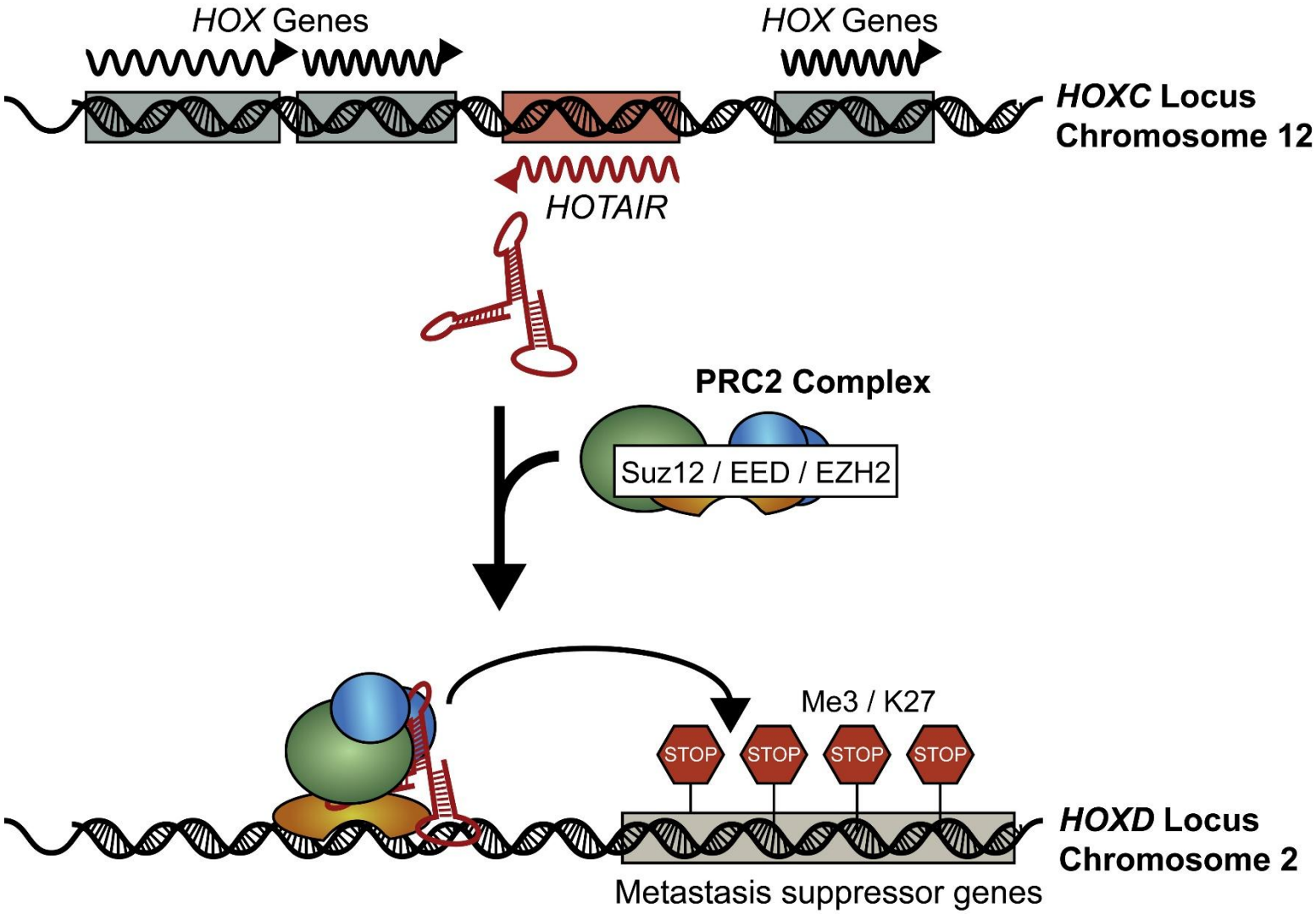
XIST – X-Inactivation Specific Transcript

- Экспрессируется только с 1 X-хромосомы – работает in cis
- Рекрутирует Polycomb Repressive Complex 2 => guide

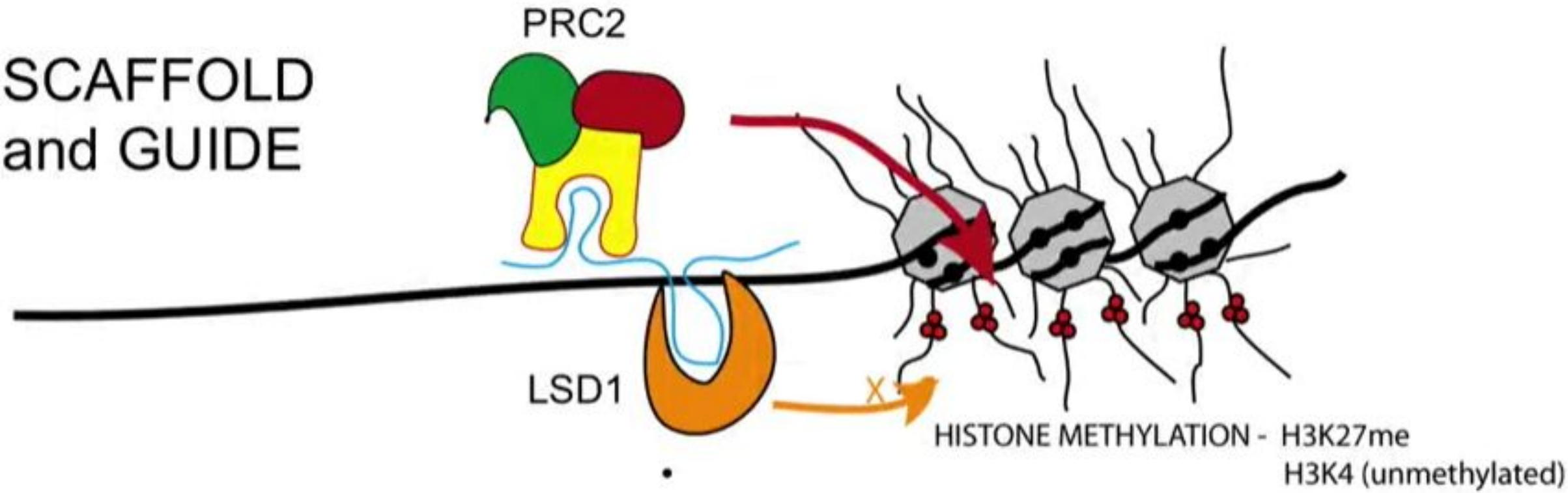


HOTAIR – HOX Transcript Antisense RNA.

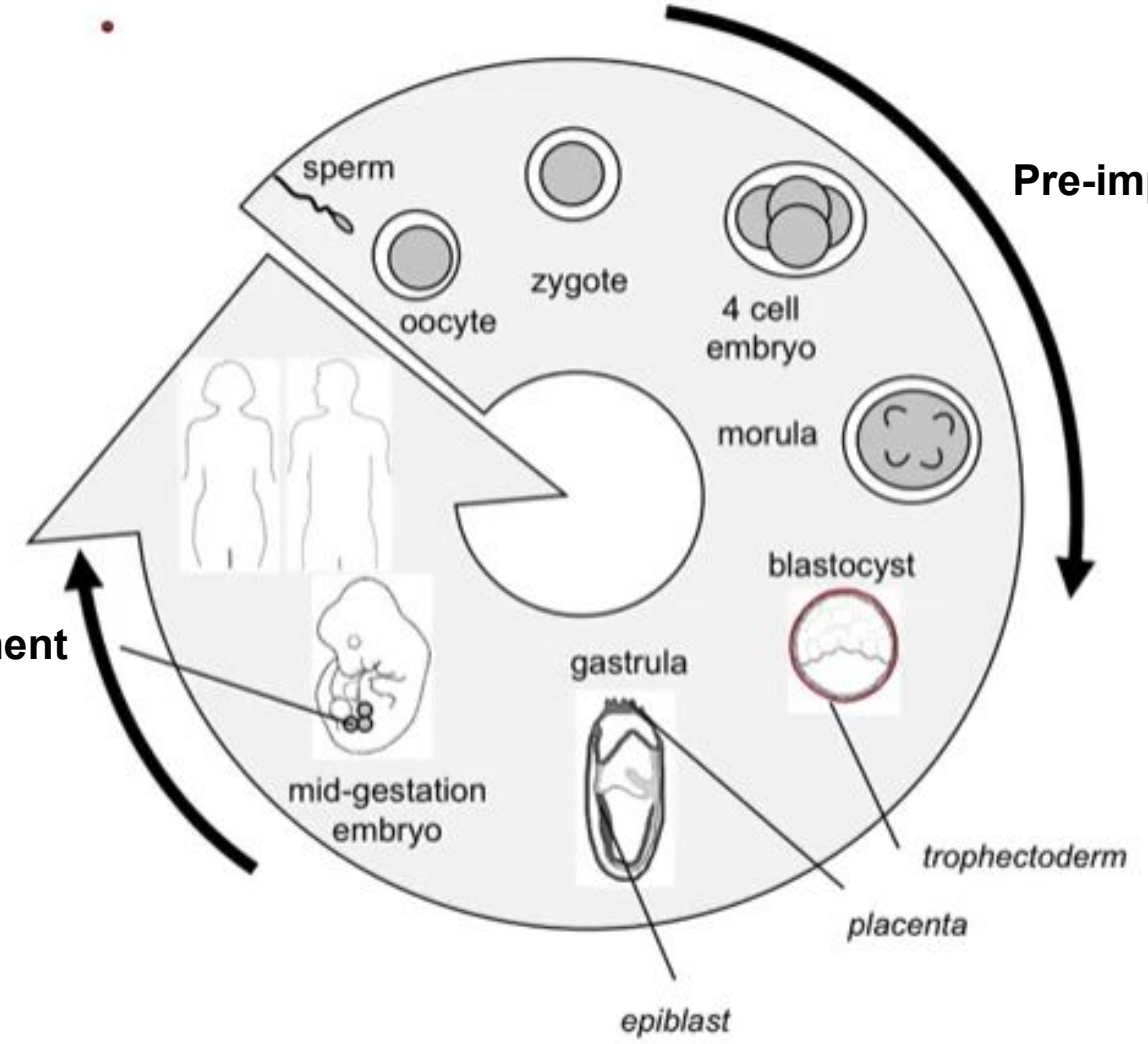
- In trans (HoxC -> HoxD)
- И Guide и Scaffold



SCAFFOLD
and GUIDE

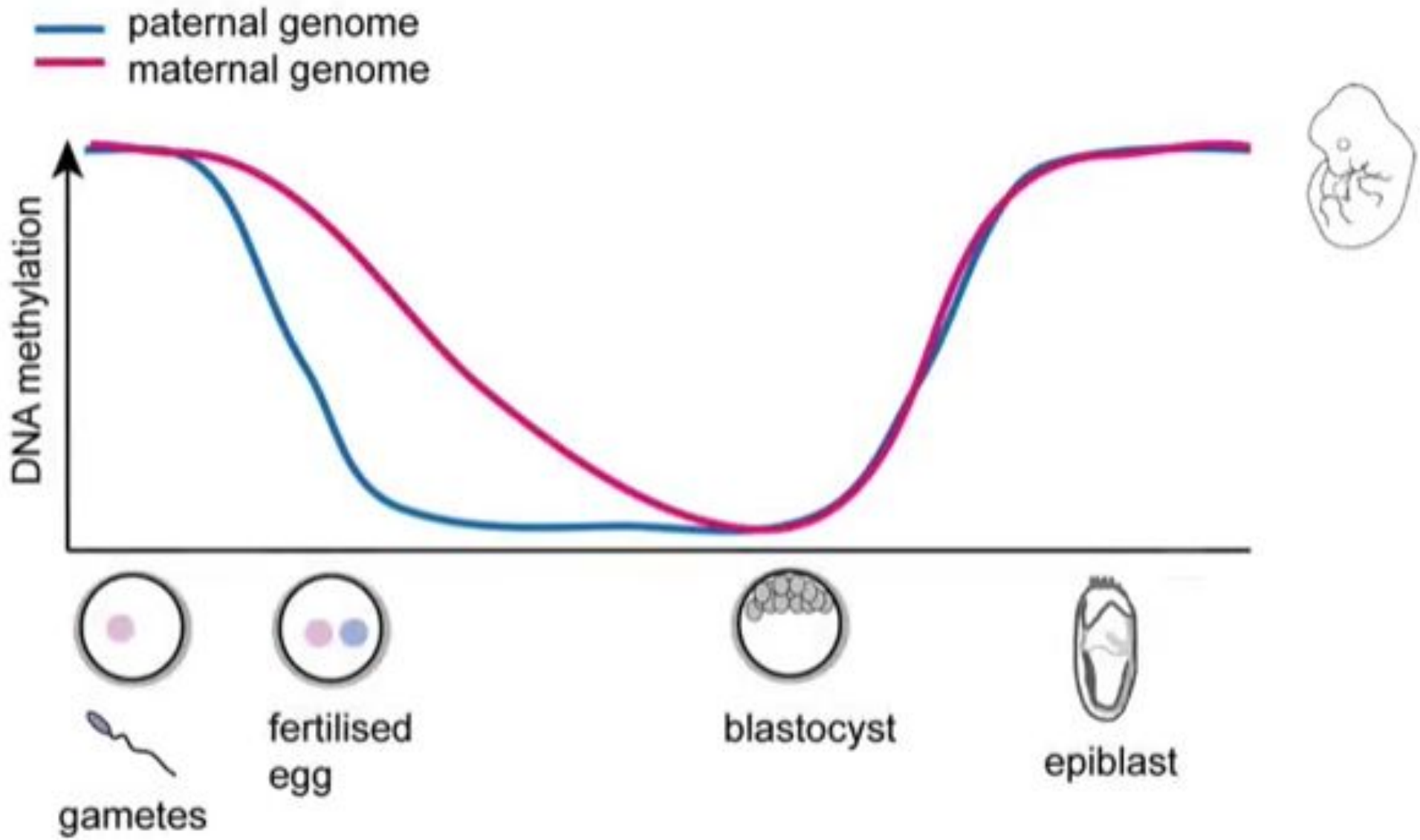


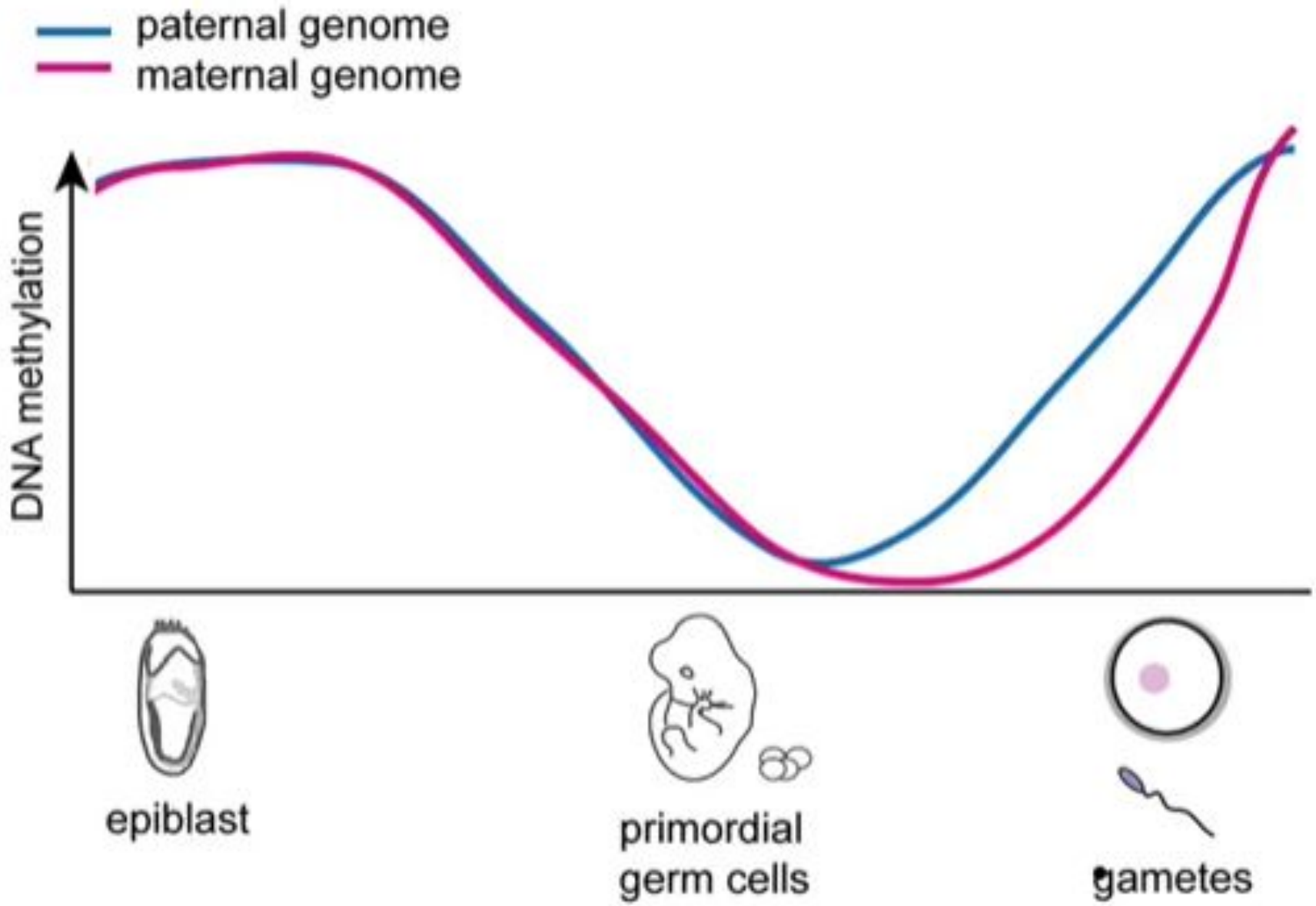
Эпигенетическое репрограммирование – восстановление тотипотентности



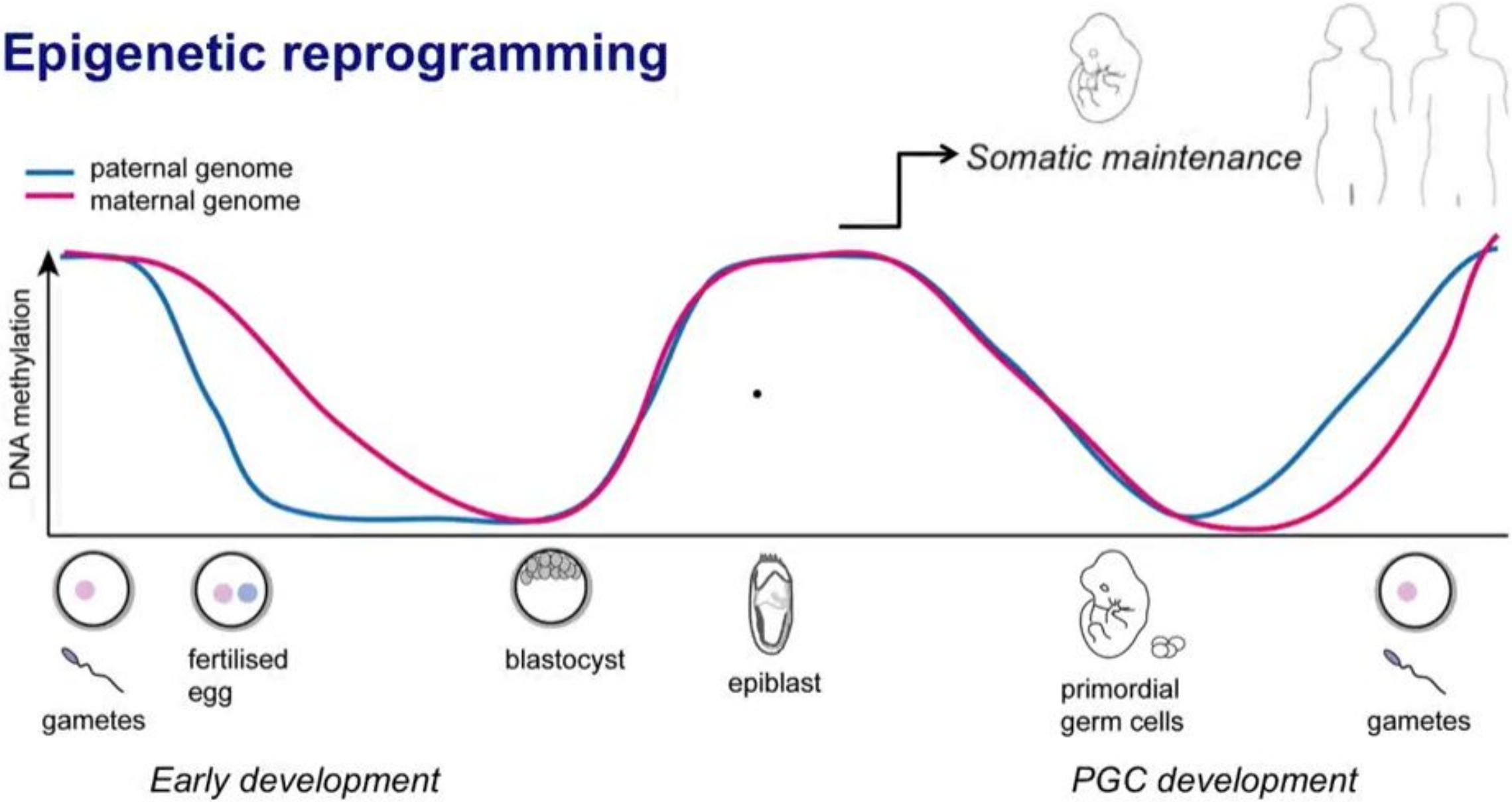
Pre-implantation development

Primordial germ cell development

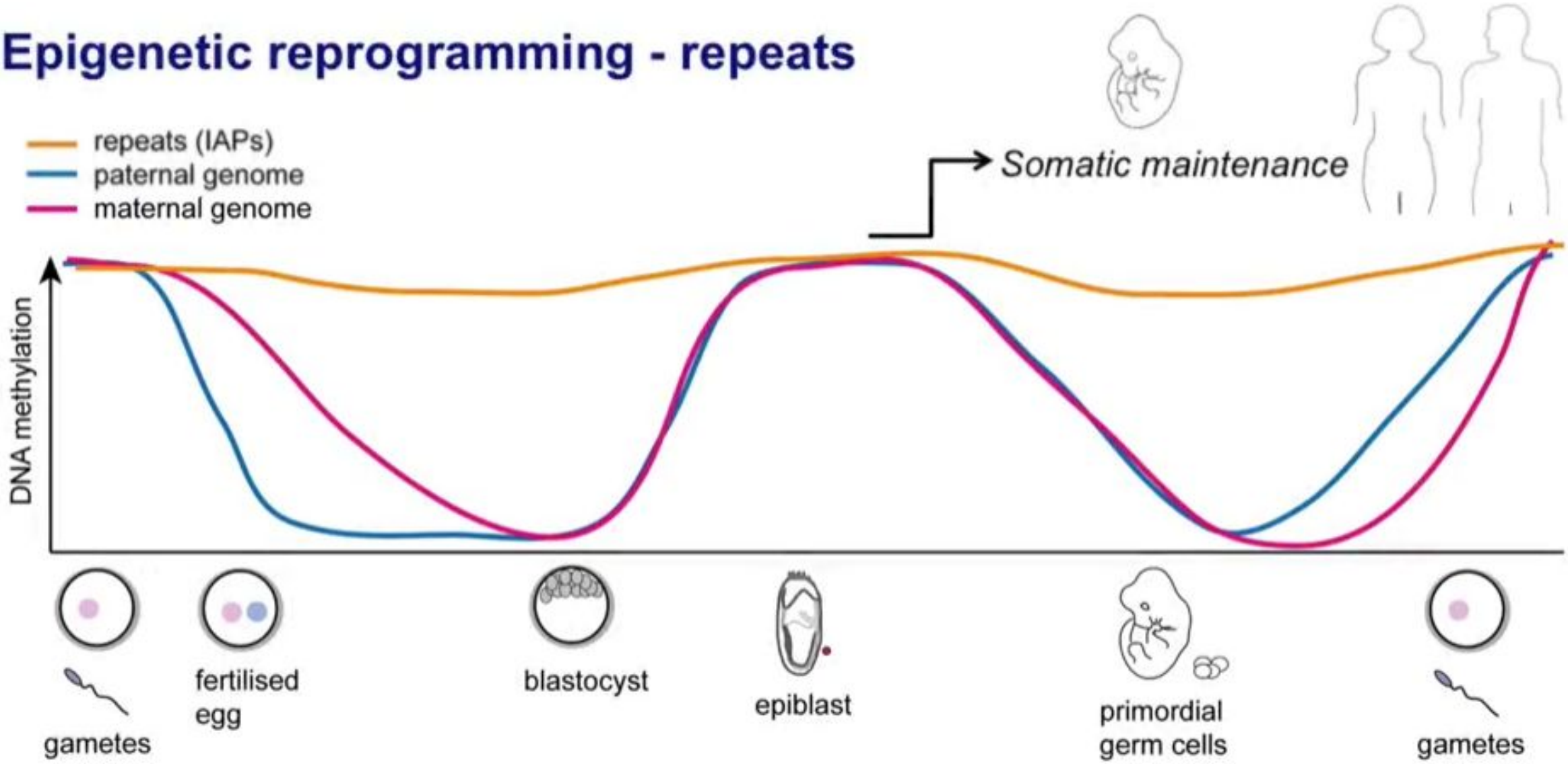

paternal genome
maternal genome
DNA methylation
gametes
fertilised egg
blastocyst
epiblast


paternal genome
maternal genome
DNA methylation
epiblast
primordial
germ cells
gametes

Epigenetic reprogramming

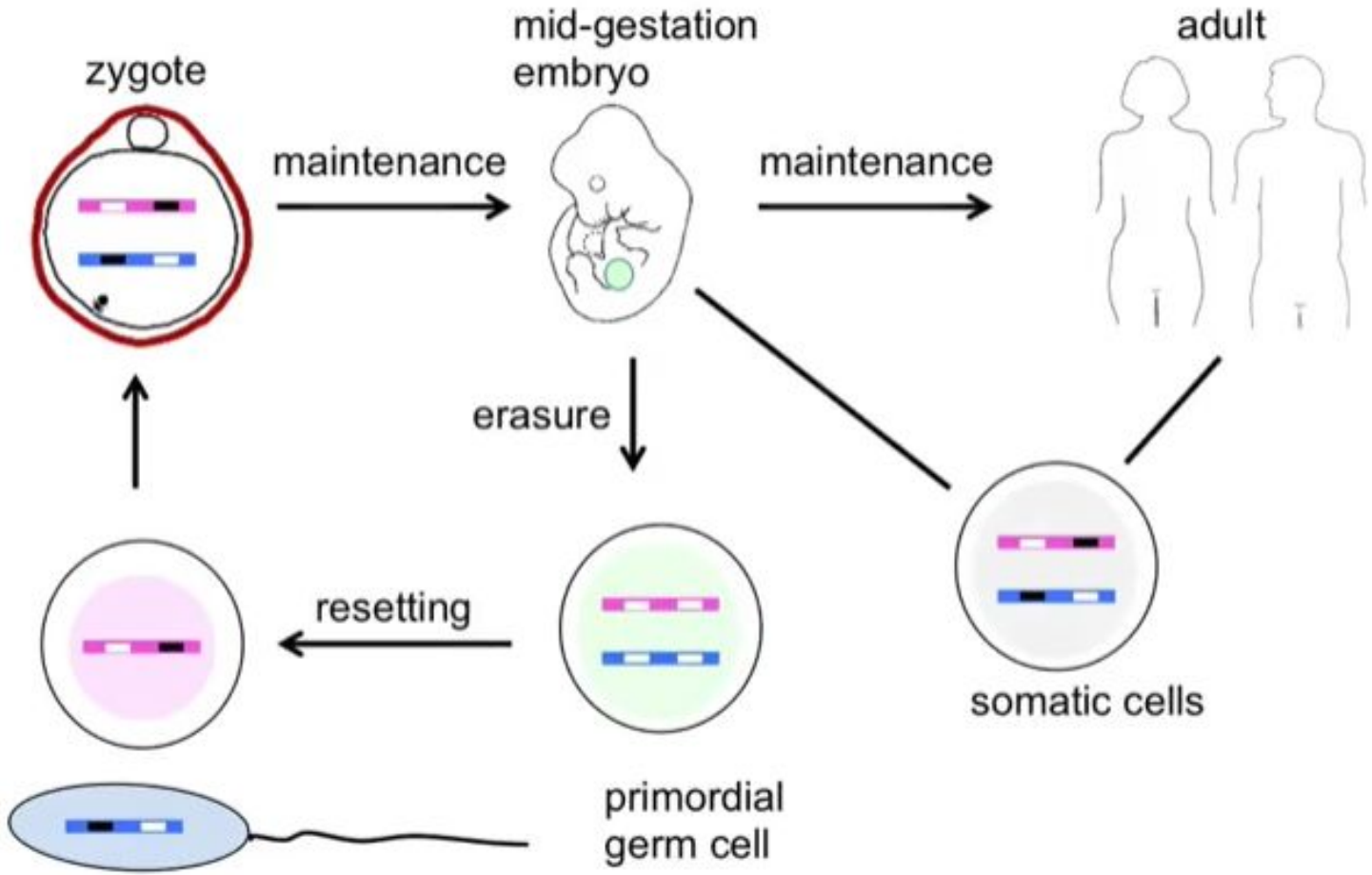


Epigenetic reprogramming - repeats

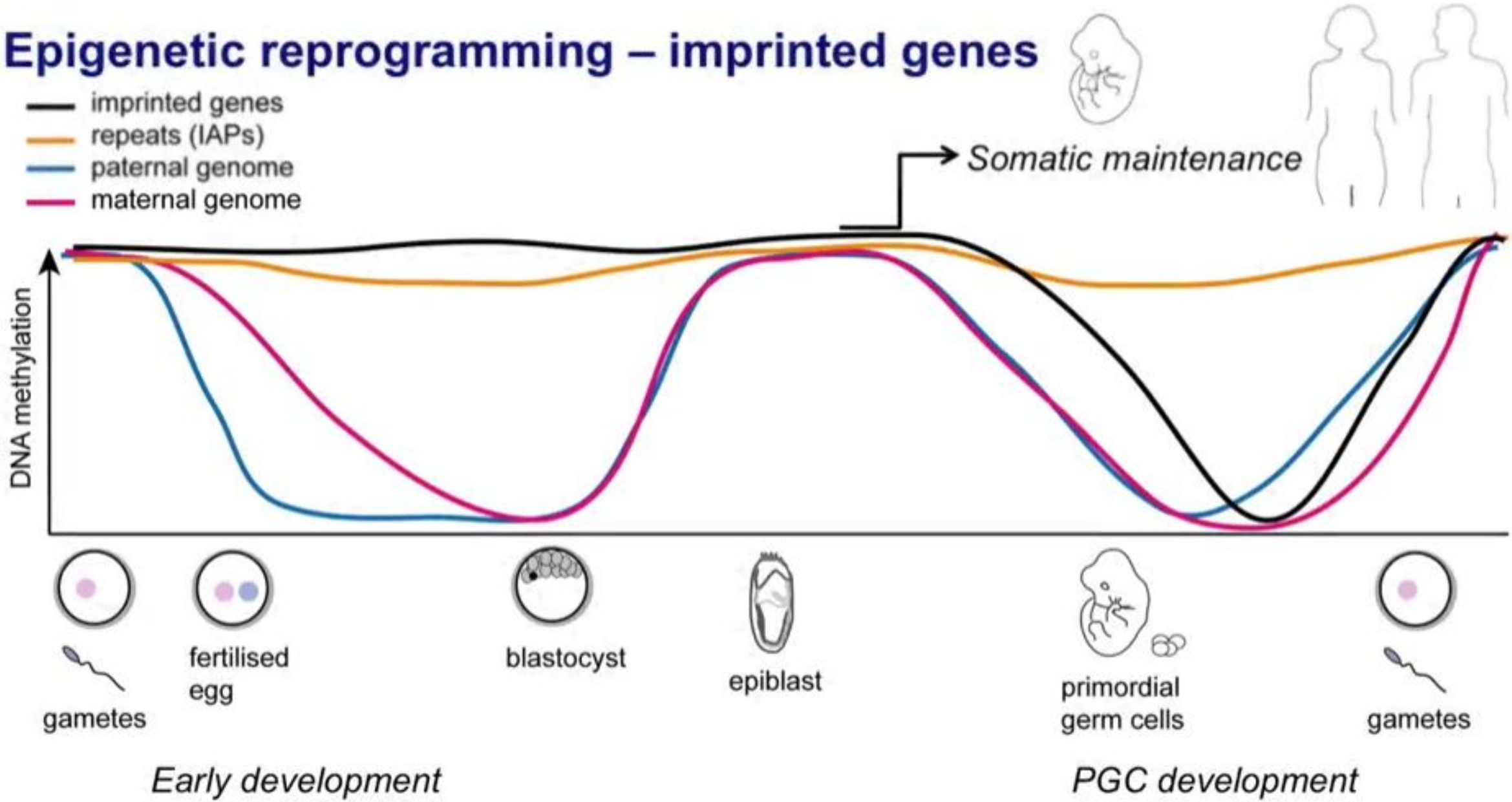


Геномный импринтинг

- Экспрессия гена только с одной из хромосом в паре (parent-of-origin specific gene expression).

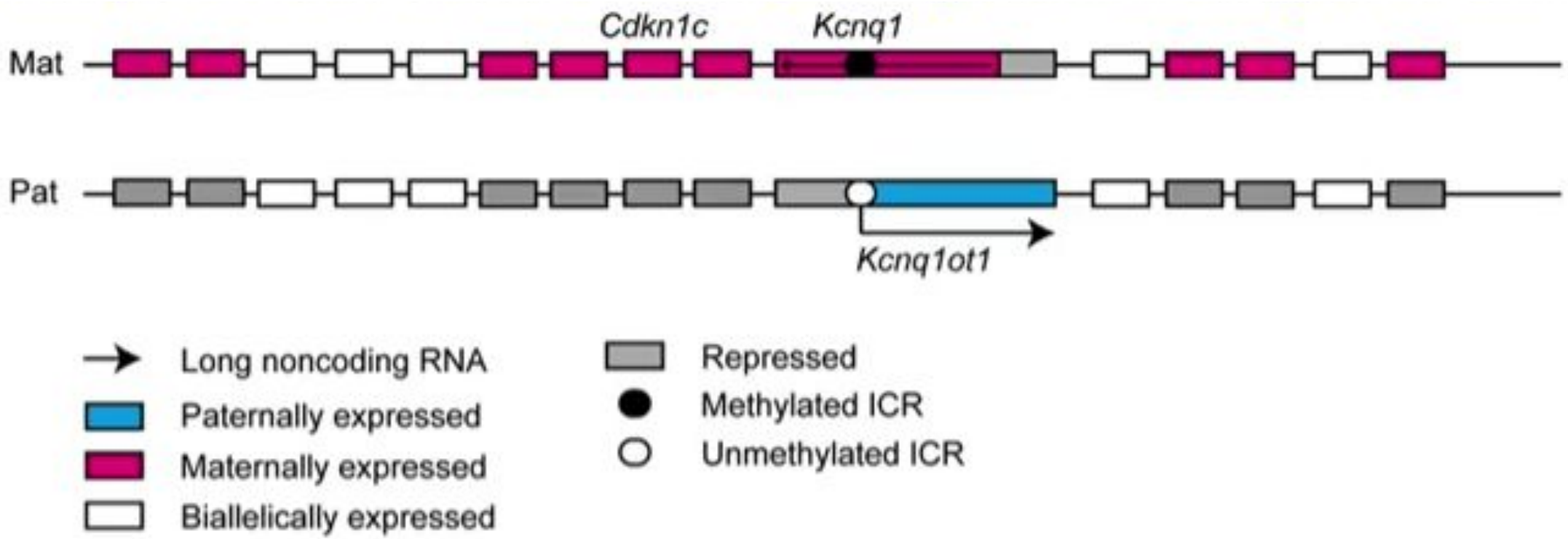


Epigenetic reprogramming – imprinted genes

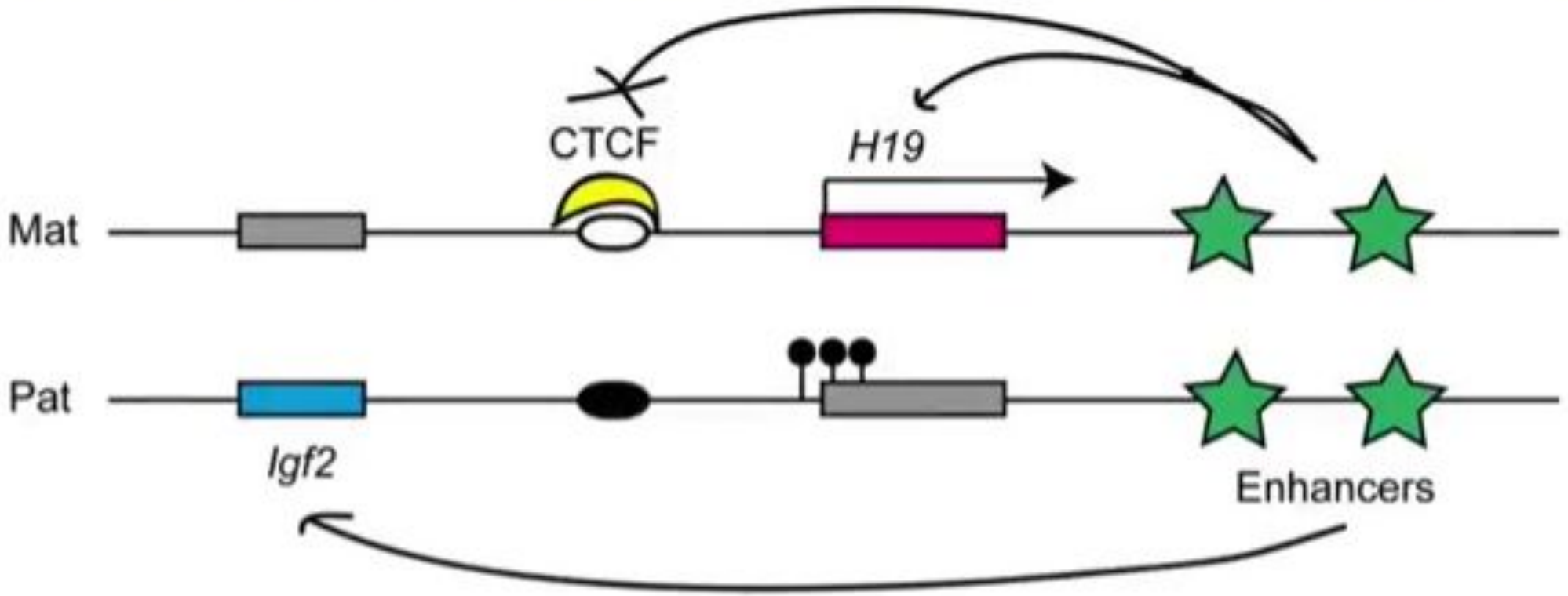


ICR – Imprint Control Region в промоторе

Kcnq1 – lncRNA, сайленсинг in cis



Блокирование энхансеров



- CTCF is an insulator protein, insulates *Igf2* from downstream enhancers
- DNA methylation at ICR blocks binding of CTCF binding
- Without CTCF, DNA methylation spreads to *H19* promoter to silence and enhancers can access *Igf2* to activate

Эволюция импринтинга

- Разделение на социальные группы – родство не 1, а 1/2

Hamilton's rule

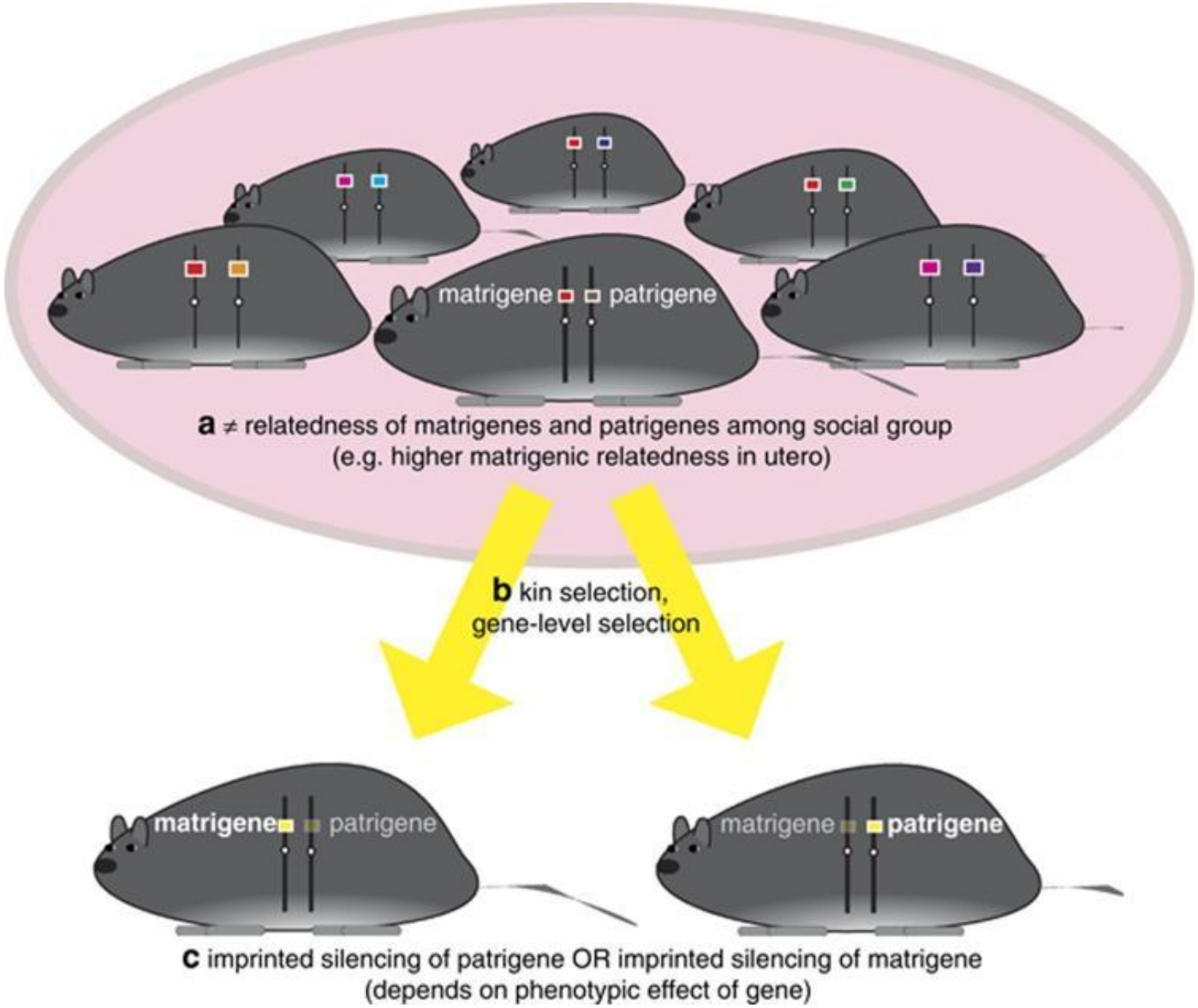
Altruism is favored by natural selection when:

$$r\,B > C$$

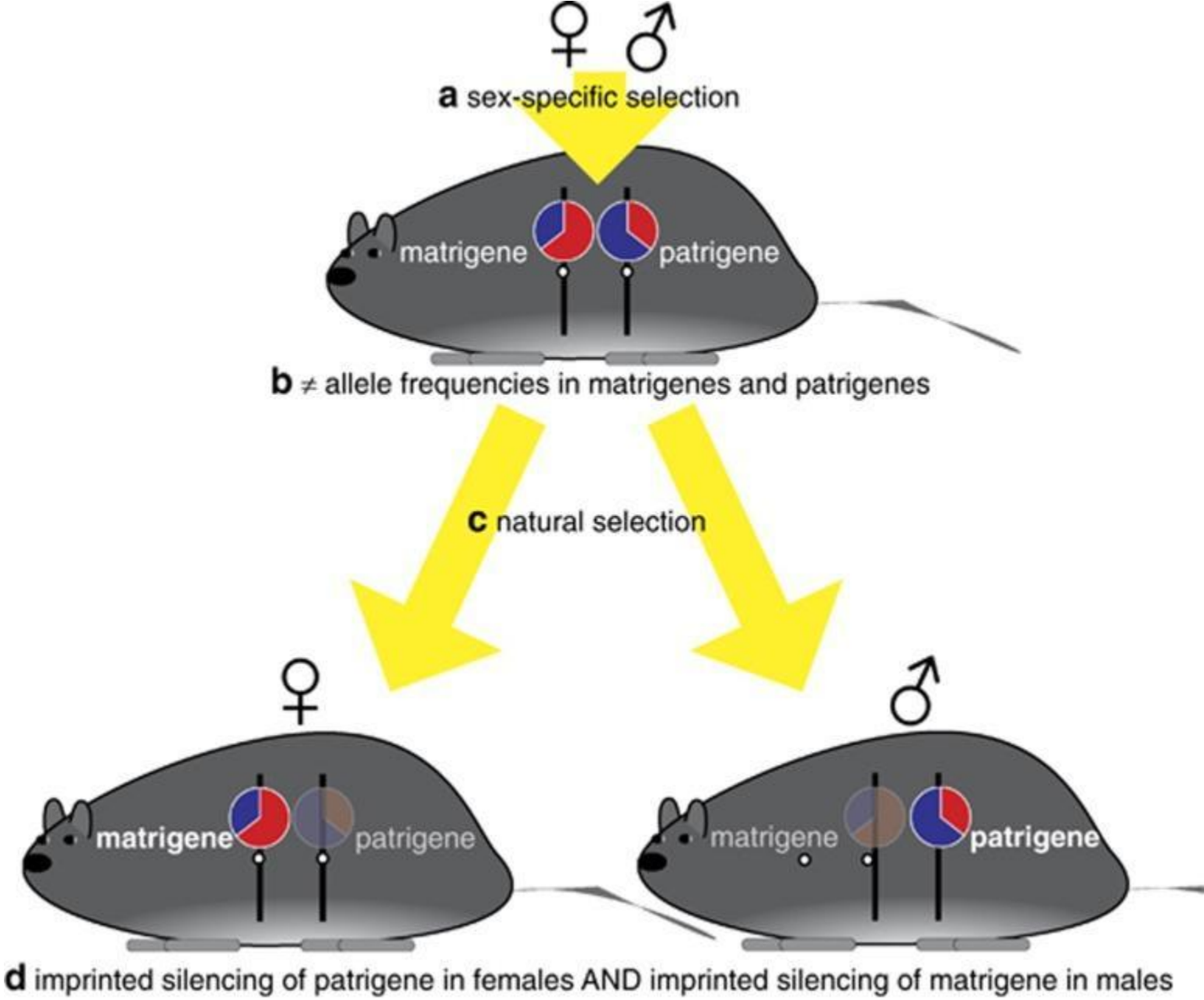
Relatedness (proportion of shared genes)

Benefit to the recipient (how many more offspring are produced)

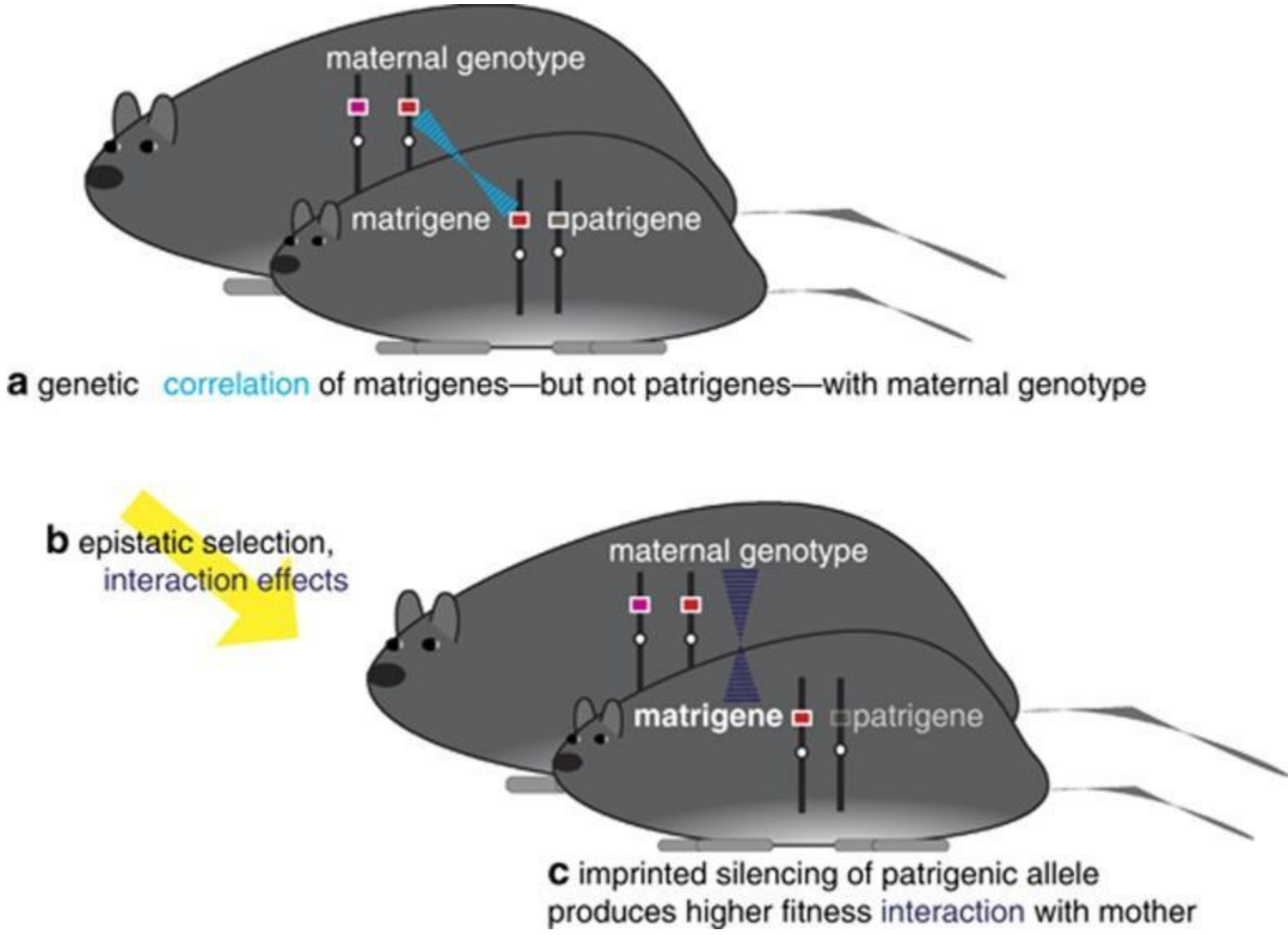
Cost to the altruist



(**a**, **b**) The sexual antagonism theory of genomic imprinting starts with sexually antagonistic selection, which produces different allele frequencies, shown as pie charts, for genes of maternal and paternal origin. (**c**, **d**) Natural selection favors individuals that are able to express the fitter of the two alleles at a locus, which for males will be the patrigenic allele and for females will be the matrigenic allele.



(**a**) The maternal–offspring coadaptation theory of genomic imprinting relies on the correlation of genes in the mother and genes of maternal origin in the offspring (shown in light blue). (**b**) Fitness of offspring is determined by the interaction (shown in dark purple) between the phenotypes of mothers and offspring. (**c**) Imprinted silencing of the patrigenic allele can be favored for either of two reasons, depending on the genetic architecture of the interacting phenotypes. First, when a single gene governs the interaction and phenotypic matching between mothers and their offspring produces high fitness, then silencing of the patrigenic allele is beneficial to offspring because it raises the probability of producing a match. Second, if different loci are involved in the phenotypic interaction, past correlational selection will have produced a covariance between them, generating haplotypes with combinations of alleles that interact well together. (N.B. This multi-locus interaction is not depicted in the figure.) The offspring is more likely to inherit from its mother an allele that interacts well with the alleles in the mother's genotype. This also favors the imprinted silencing of the patrigenic allele because it raises the probability that the offspring expresses an allele that makes for a good interaction with the maternal phenotype.



Спасибо за внимание!