3d molecular designs
Transforming Science Learning

Epigenetics

… tweaking your genetic destiny

Tim Herman

What is Epigenetics?

Epigenetics is the study of how your behaviors and environment can cause ***changes that affect the way your genes work***. Unlike genetic changes, epigenetic changes are reversible and do not change your DNA sequence, but they can change how your body reads a DNA sequence.

NO MORE EXCUSES

Don't blame it on genes

Workshop Outline

Epigenetics…chemical modifications of your genome

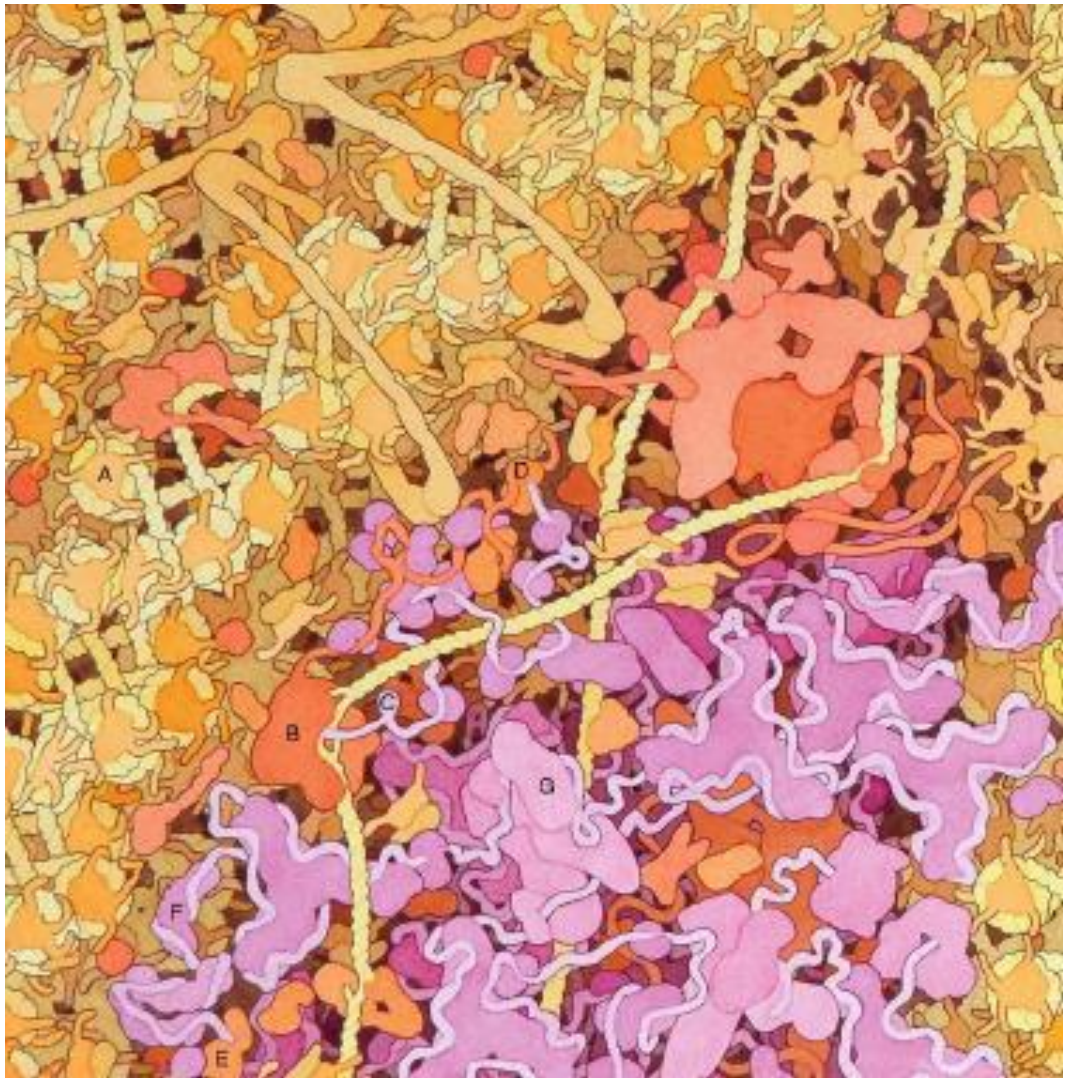
Writers / Erasers / Readers

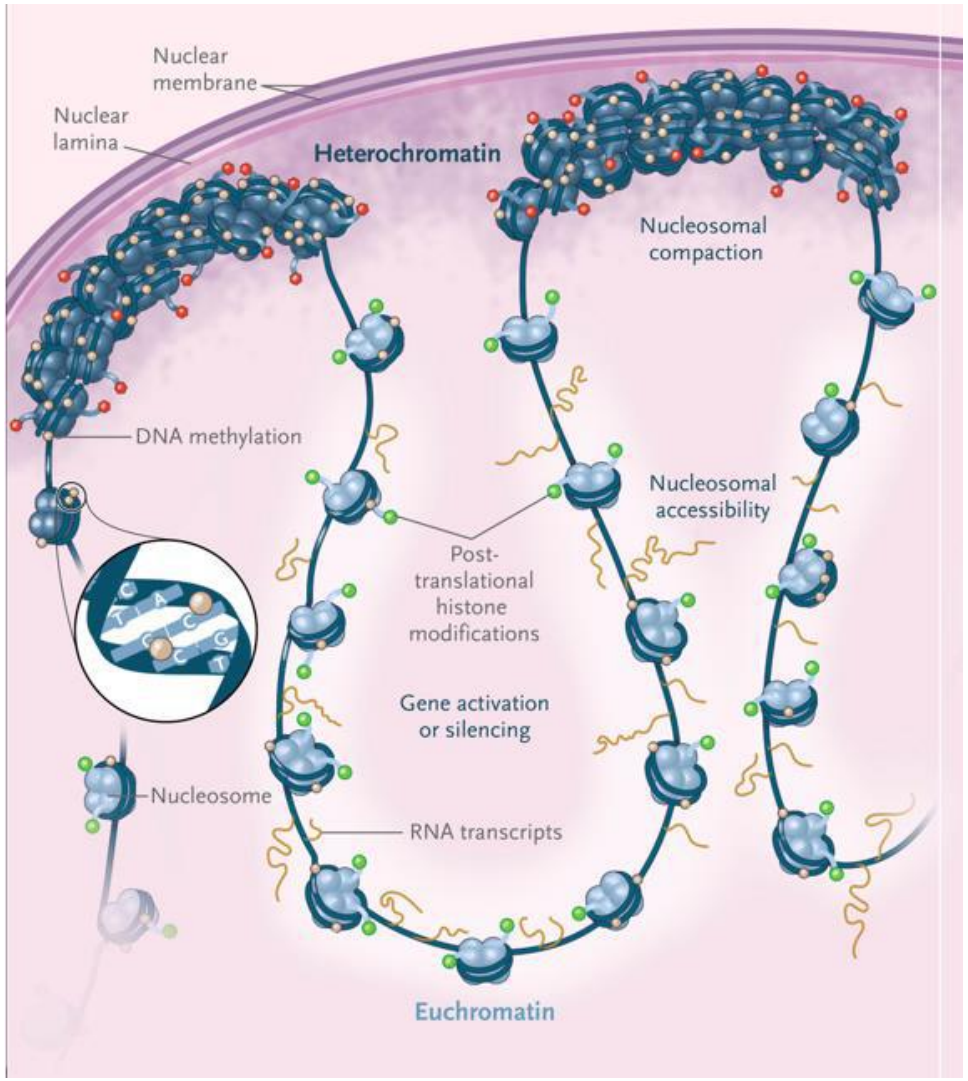
1. Histone modifications

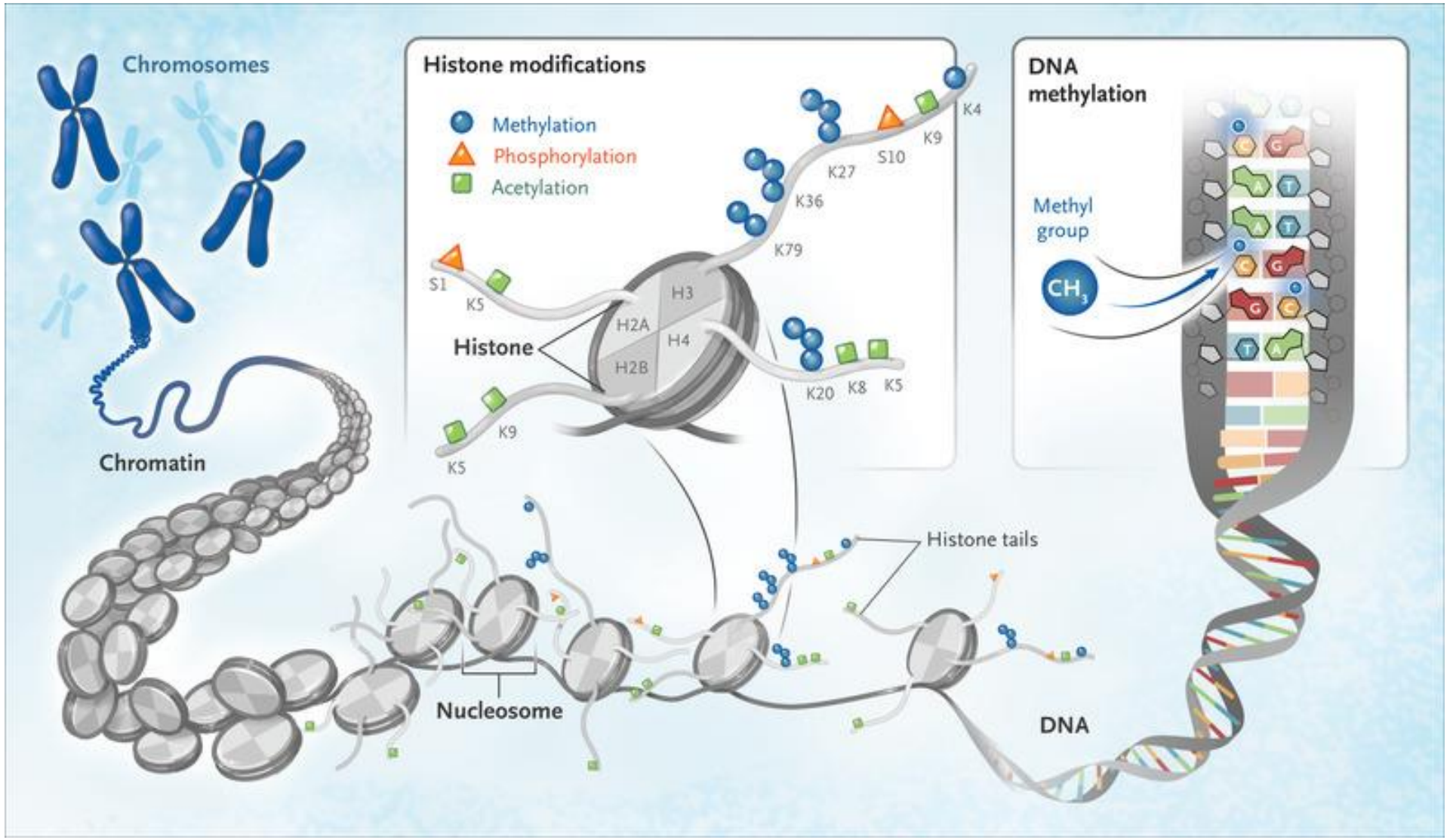
2. DNA modifications

… methylation of CpG

… a 3-D structure/function story


A
B
C
D
E
F
G


Nuclear membrane
Nuclear lamina
Heterochromatin
Nucleosomal compaction
DNA methylation
Nucleosomal accessibility
Post-translational histone modifications
Gene activation or silencing
Nucleosome
RNA transcripts
Euchromatin

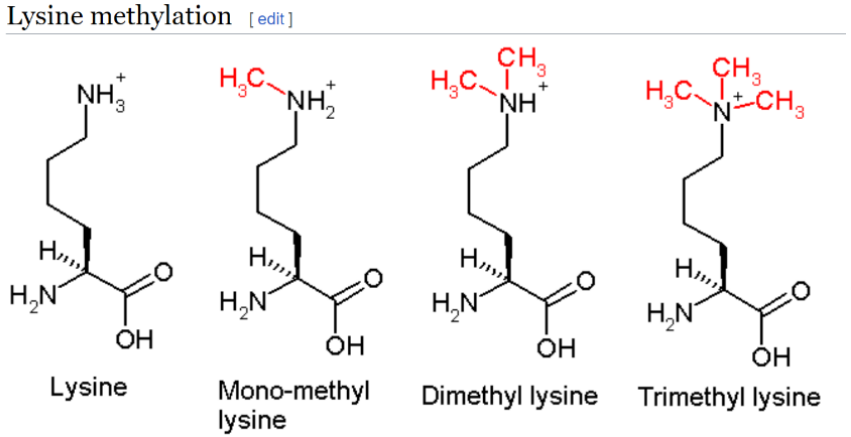

Chromosomes
Chromatin
Histone modifications
Methylation
Phosphorylation
Acetylation
K4
K9
S10
K27
K36
K79
S1
K5
H3
H2A
H4
H2B
Histone
K20
K8
K5
K9
K5
DNA methylation
Methyl group
CH3
Histone tails
Nucleosome
DNA

Lysine (K) #4 of histone H3 is tri-methylated….

H3K4me3 regulates RNA polymerase II promoter-proximal pause-release

- https://doi.org/10.1038/s41586-023-05780-

"Trimethylation of histone H3 lysine 4 (H3K4me3) is associated with transcriptional start sites and has been proposed to regulate transcription initiation[1,2]."

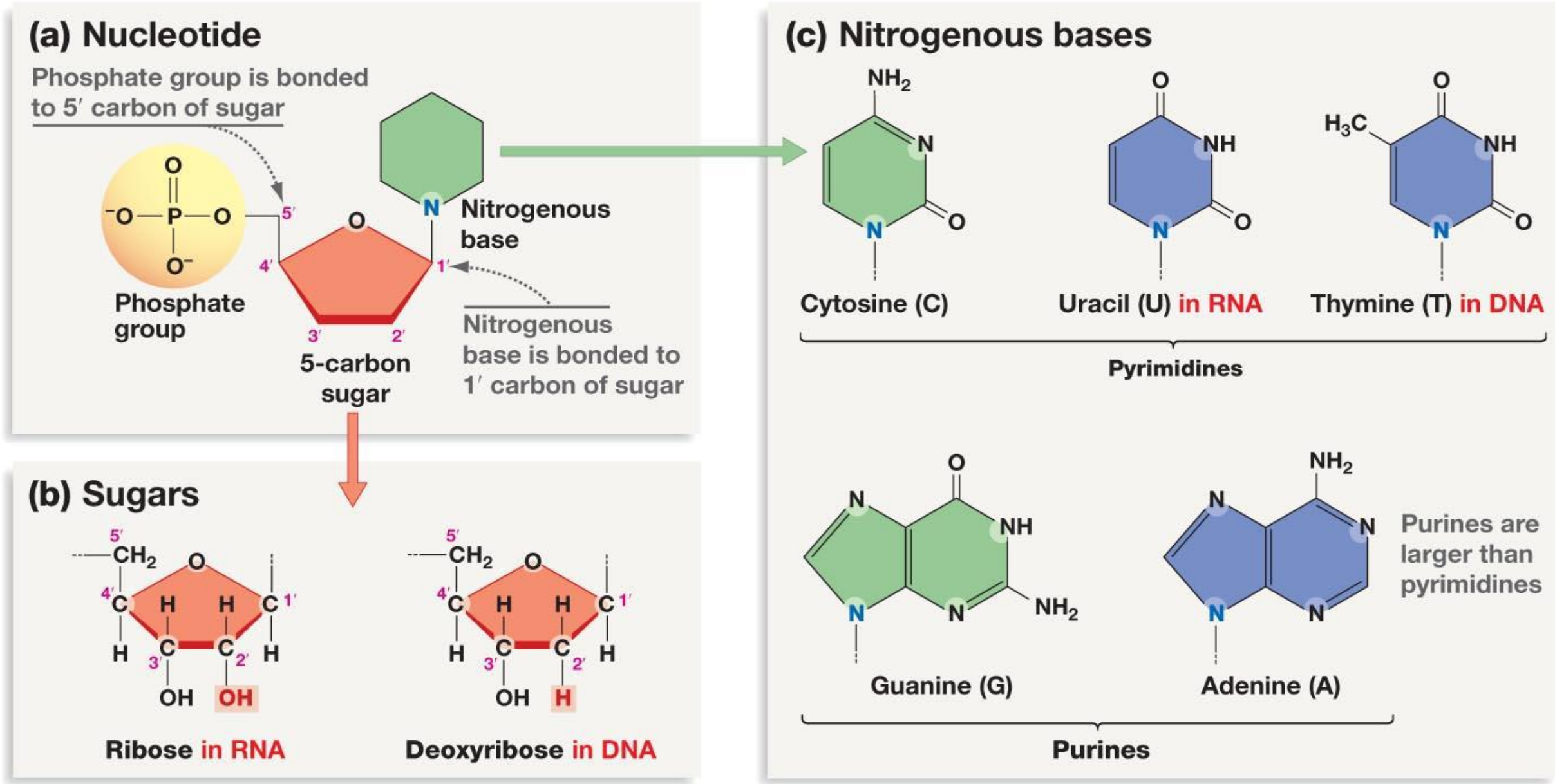


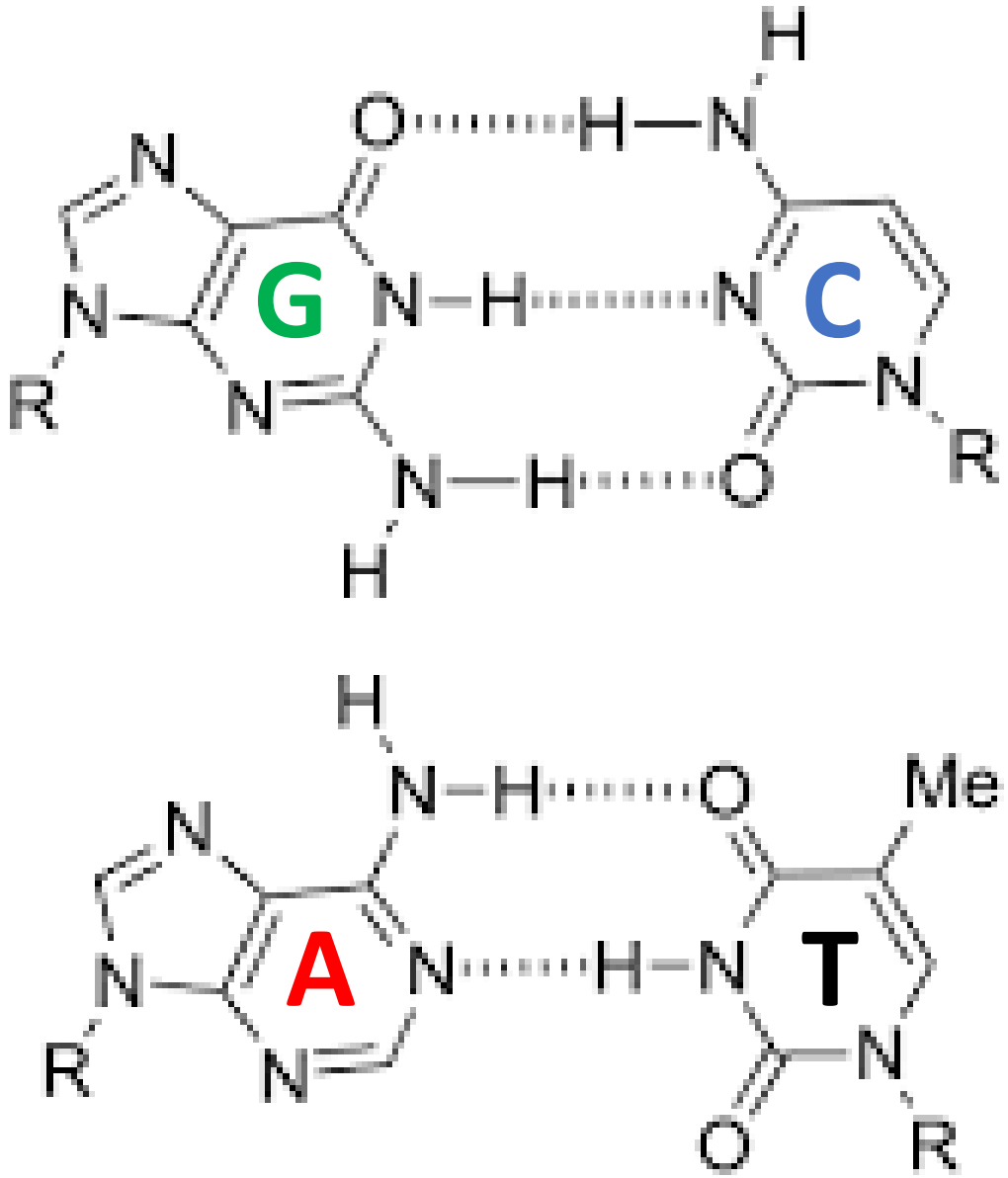
Modeling chromatin….

Post-transcriptional modification of DNA

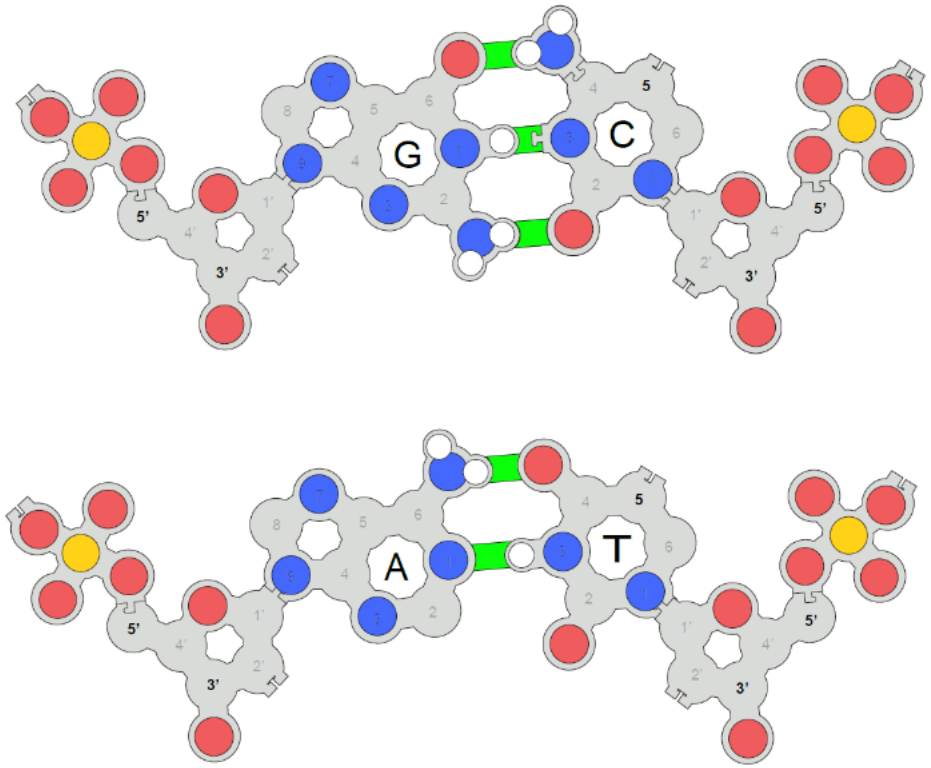
Methylation of CpG …. to $C^{Me}pG$

5’- C p G – 3’



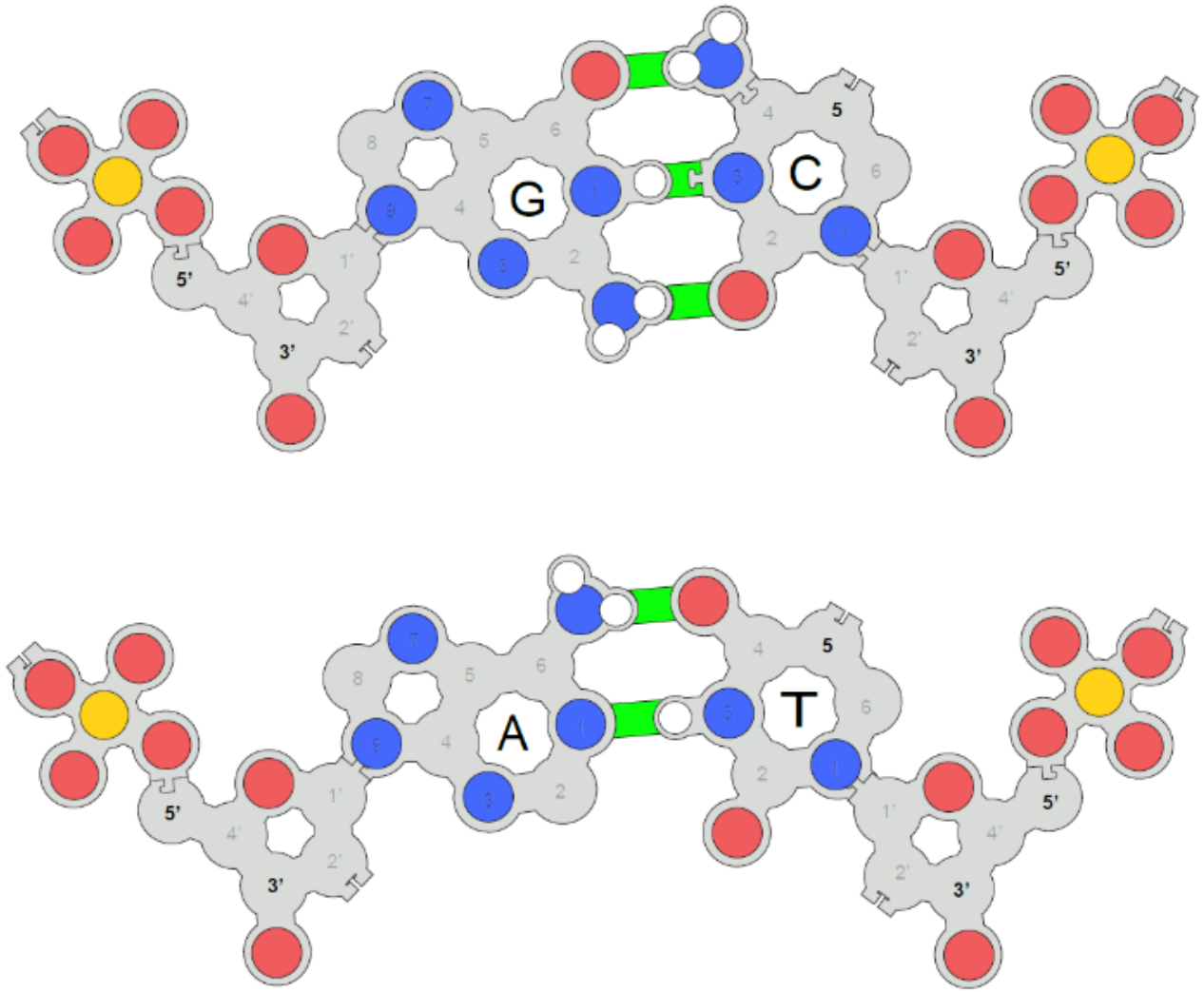

G
C
A
T
Me

3DMD Base Pair Models



What can you teach?

- *Why does RNA use U's… and DNA T's?*
- *What is pseudouridine?,… and why should I care?*
- *How does methylation of C regulate gene expression?*
- *Why are there surprisingly few CpG dinucleotides in our genes?*


G
C
A
T
5'
3'

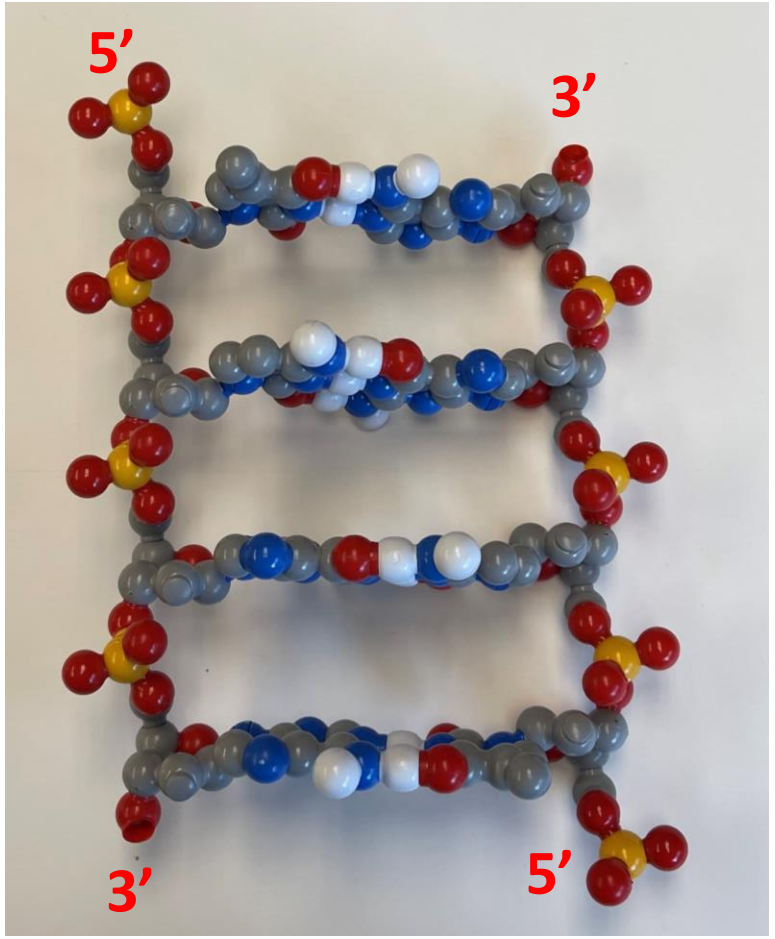
Looking for CpG's….

T

C

G

A

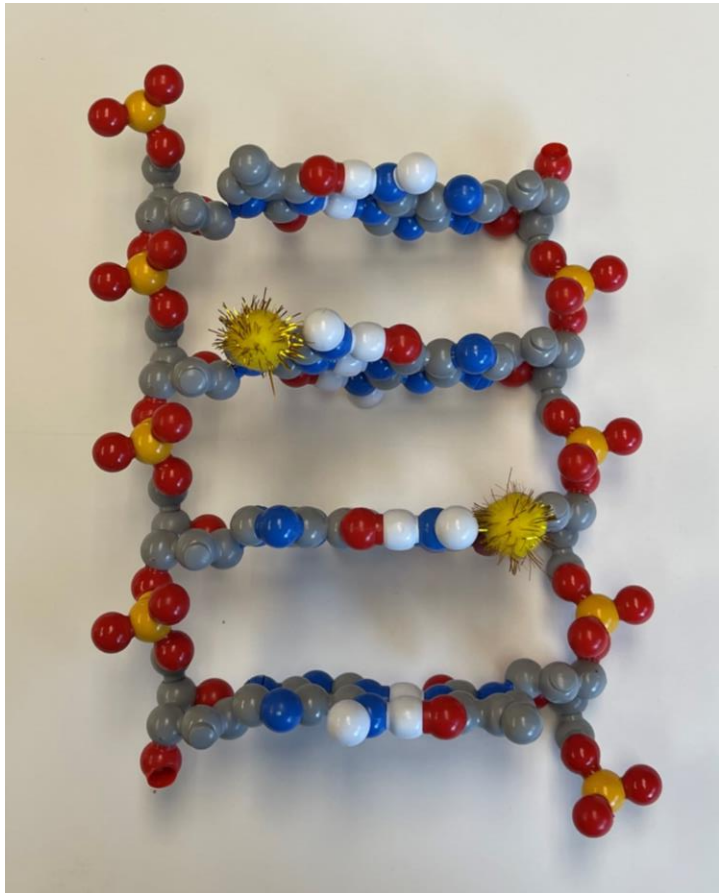


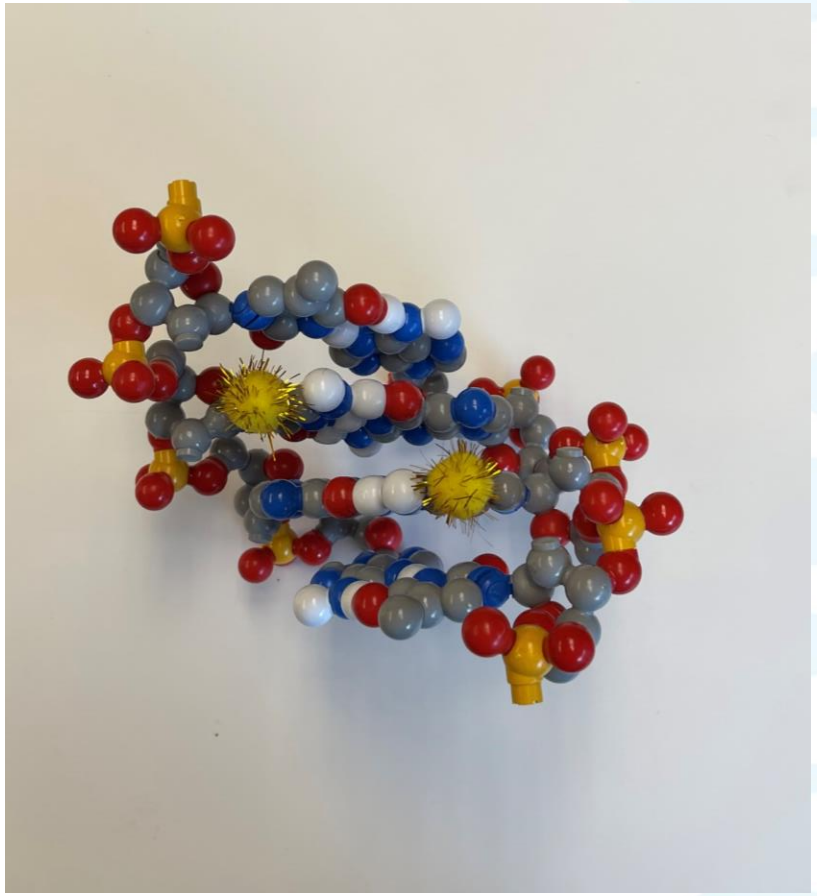
A

G

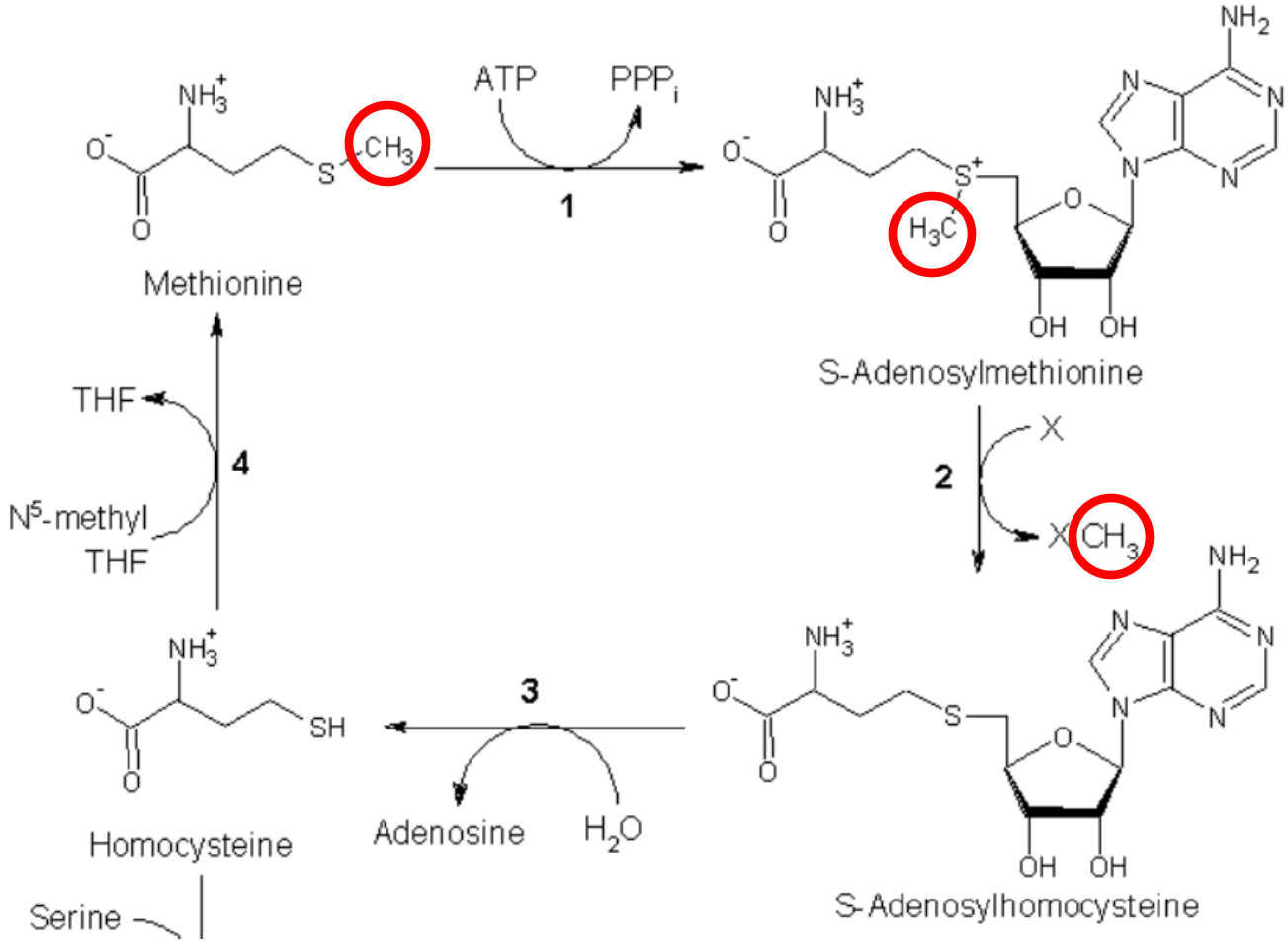
C

T

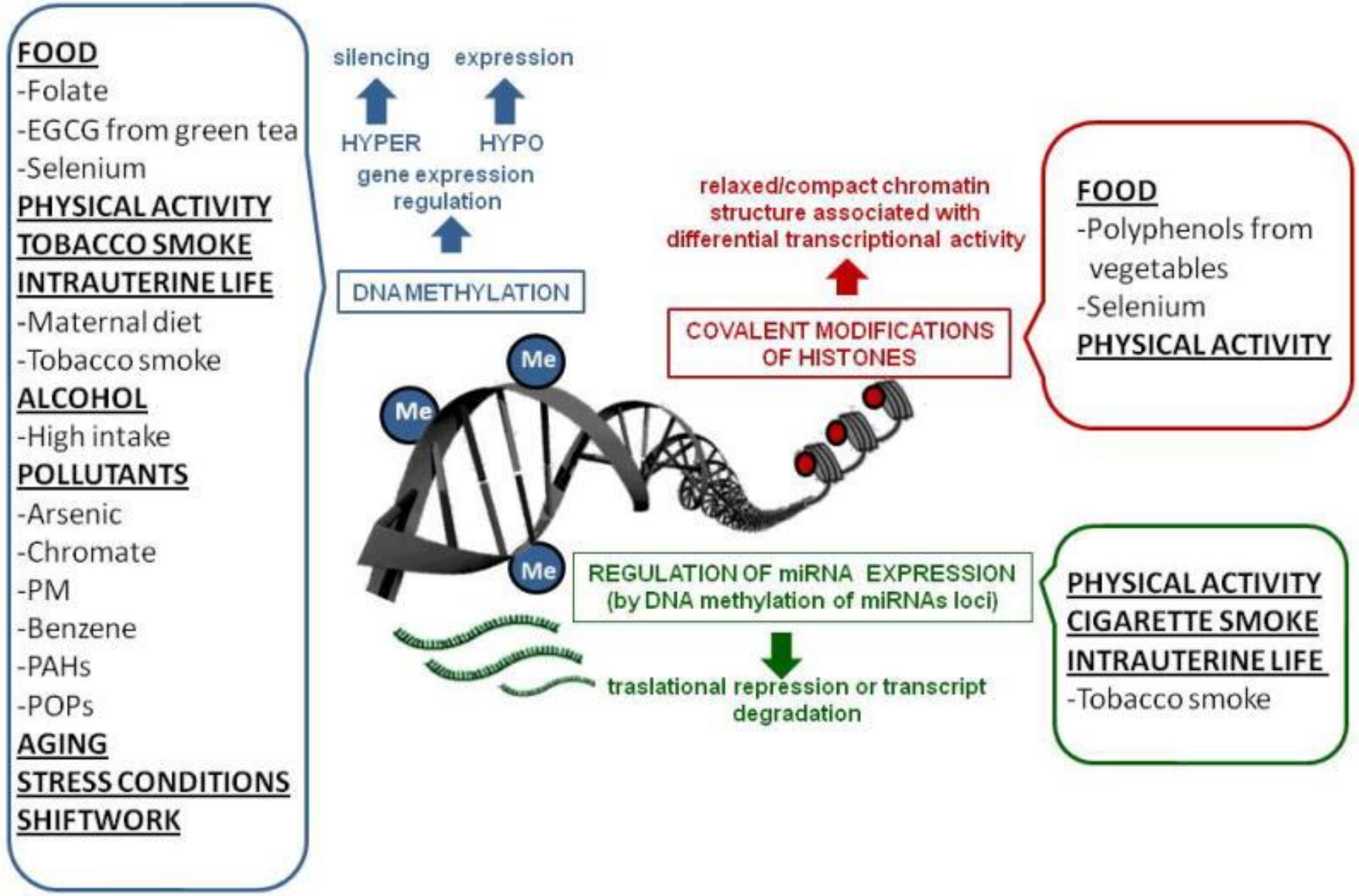




Methionine…. *is a methyl-group donor*



After all these years,… a kick in the pants.


FOOD
-Folate
-EGCG from green tea
-Selenium
PHYSICAL ACTIVITY
TOBACCO SMOKE
INTRAUTERINE LIFE
-Maternal diet
-Tobacco smoke
ALCOHOL
-High intake
POLLUTANTS
-Arsenic
-Chromate
-PM
-Benzene
-PAHs
-POPs
AGING
STRESS CONDITIONS
SHIFTWORK
silencing
expression
HYPER
HYPO
gene expression regulation
DNA METHYLATION
relaxed/compact chromatin structure associated with differential transcriptional activity
COVALENT MODIFICATIONS OF HISTONES
FOOD
-Polyphenols from vegetables
-Selenium
PHYSICAL ACTIVITY
Me
Me
Me
REGULATION OF miRNA EXPRESSION (by DNA methylation of miRNAs loci)
traslational repression or transcript degradation
PHYSICAL ACTIVITY
CIGARETTE SMOKE
INTRAUTERINE LIFE
-Tobacco smoke

Fragile X Syndrome …. *a genetic/epigenetic disorder??*

X linked triplet repeat (CGG) expansion disorder

Fragile X patients do not express FMR1 and have cognitive/autism spectrum disabilities

All human genomes have CGG repeats in FMR1 5’ untranslated region

Cytosines tend to become methylated at high repeat number (epigenetic modification!

Methylation of cytosines inhibits transcription of FMR1

CAUTION…*rabbit hole ahead!*

Histone / DNA cross-talk?

YES…

What's CRISPR got to do with it??

Epigenome editing: targeted manipulation of epigenetic modifications in plants.

Shin H, Choi WL, Lim JY, Huh JH (March 2022).
Genes & Genomics. **44** (3): 307–315.

Review

CRISPR/Cas-Based Epigenome Editing: Advances, Applications, and Clinical Utility

Jacob H. Goell [1] and Isaac B. Hilton [1,2,3,*,@]

The epigenome dynamically regulates gene expression and guides cellular differentiation throughout the lifespan of eukaryotic organisms. Recent advances in clustered regularly interspaced palindromic repeats (CRISPR)/Cas-based epigenome editing technologies have enabled researchers to site-specifically program epigenetic modifications to endogenous DNA and histones and to manipulate the architecture of native chromatin. As a result, epigenome editing has helped to uncover the causal relationships between epigenetic marks and gene expression. As epigenome editing tools have continued to develop, researchers have applied them in new ways to explore the function of the epigenome in human health and disease. In this review, we discuss the recent technical improvements in CRISPR/Cas-based epigenome editing that have advanced clinical research and examine how these technologies could be improved for greater future utility.

Highlights

Epigenome editing enables researchers to activate and repress endogenous gene expression and can provide graded control over gene regulation.

Recruitment of epigenome editing effector domains using CRISPR/Cas systems allows site-specific control over modifications to DNA, histones, and chromatin architecture.

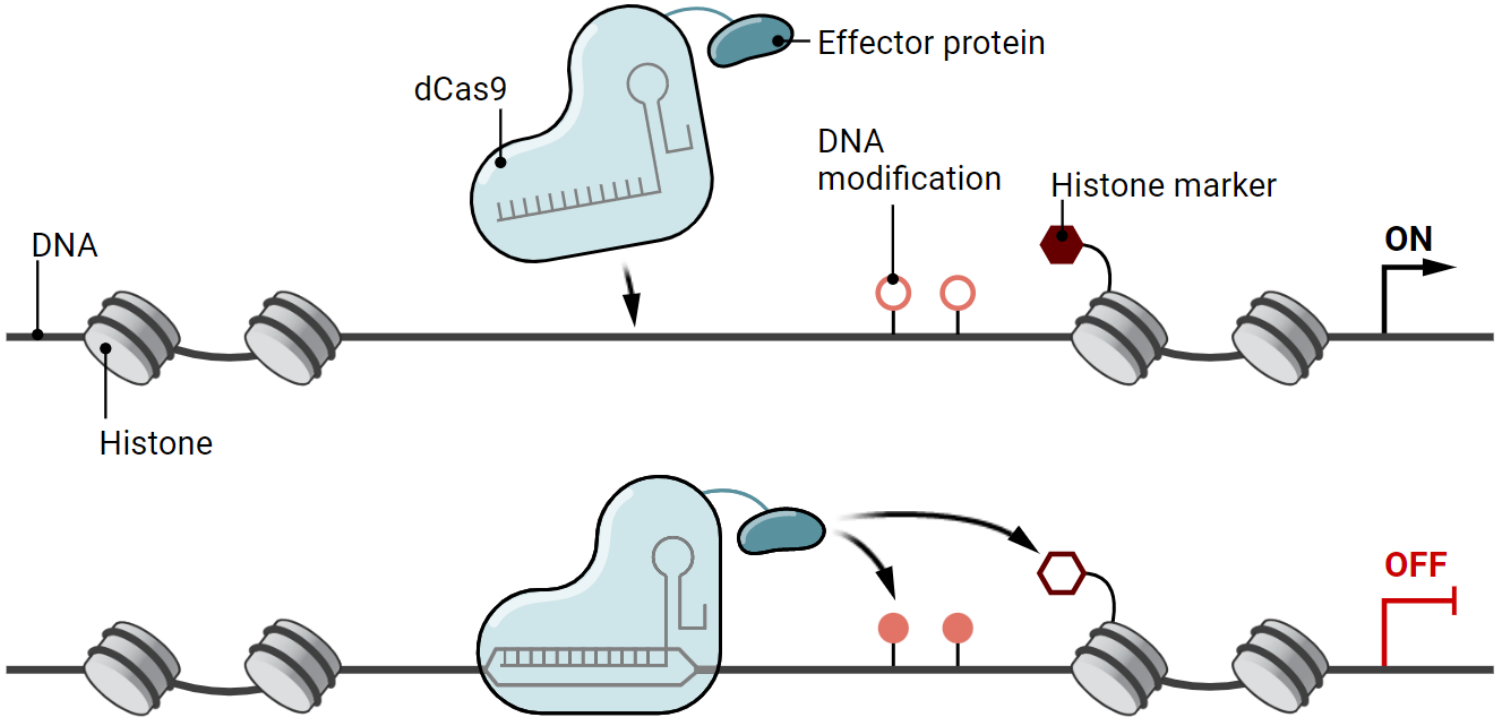
The combined use of patient-derived induced pluripotent stem cells and epigenome editing permits precision disease

Trends in Biotechnology, July 2021, Vol. 39, No. 7
https://doi.org/10.1016/j.tibtech.2020.10.012

Regulation of Gene Expression and the Elucidative Role of CRISPR-Based Epigenetic Modifiers and CRISPR-Induced Chromosome Conformational Changes

Abstract

In complex multicellular systems, gene expression is regulated at multiple stages through interconnected complex molecular pathways and regulatory networks. Transcription is the first step in gene expression and is subject to multiple layers of regulation in which epigenetic mechanisms such as DNA methylation, histone tail modifications, and chromosomal conformation play an essential role. In recent years, CRISPR-Cas9 systems have been employed to unearth this complexity and provide new insights on the contribution of chromatin dysregulation in the development of genetic diseases, as well as new tools to prevent or reverse this dysregulation. In this review, we outline the recent development of a variety of CRISPR-based epigenetic editors for targeted DNA methylation/demethylation, histone modification, and three-dimensional DNA conformational change, highlighting their relative performance and impact on gene regulation. Finally, we provide insights on the future developments aimed to accelerate our understanding of the causal relationship between epigenetic marks, genome organization, and gene regulation.


Effector protein
dCas9
DNA
modification
Histone marker
DNA
ON
Histone
OFF

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    A Virus Collection,.. including CoV-2
    Antibodies/vaccines /and your immune system
    mRNA vaccines and multi-valent, ultra-potent vaccines…from synthetic biology.
  - Stories, stories and more stories….
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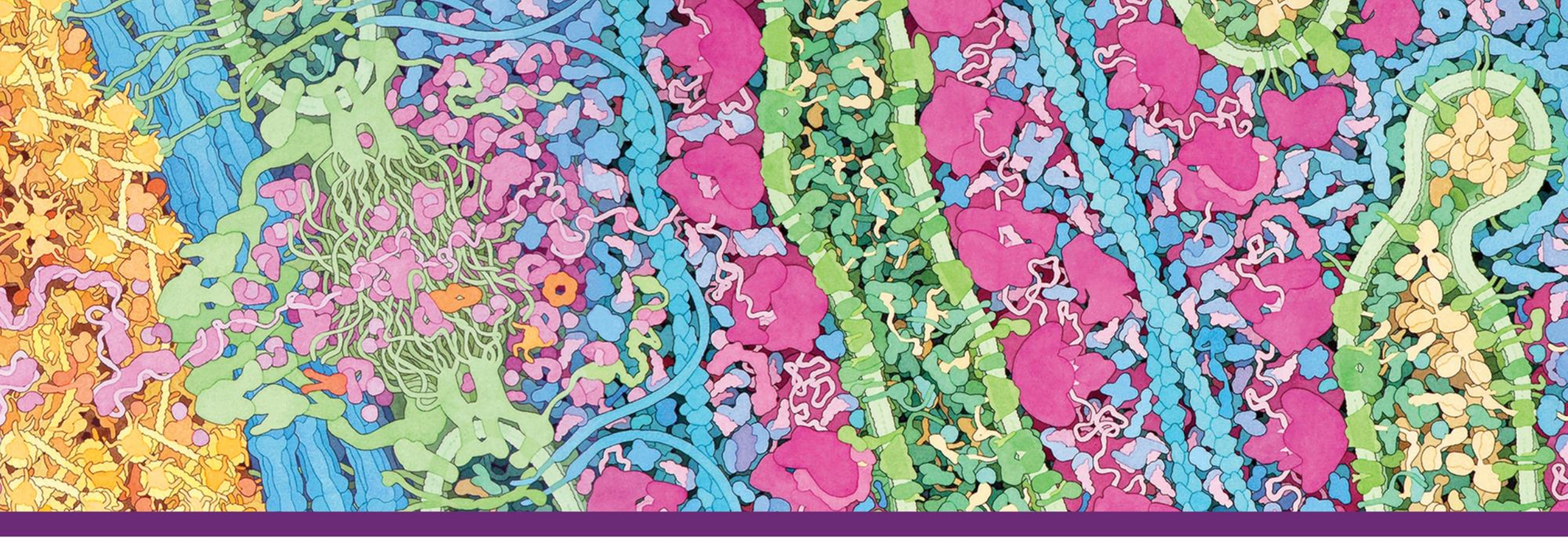

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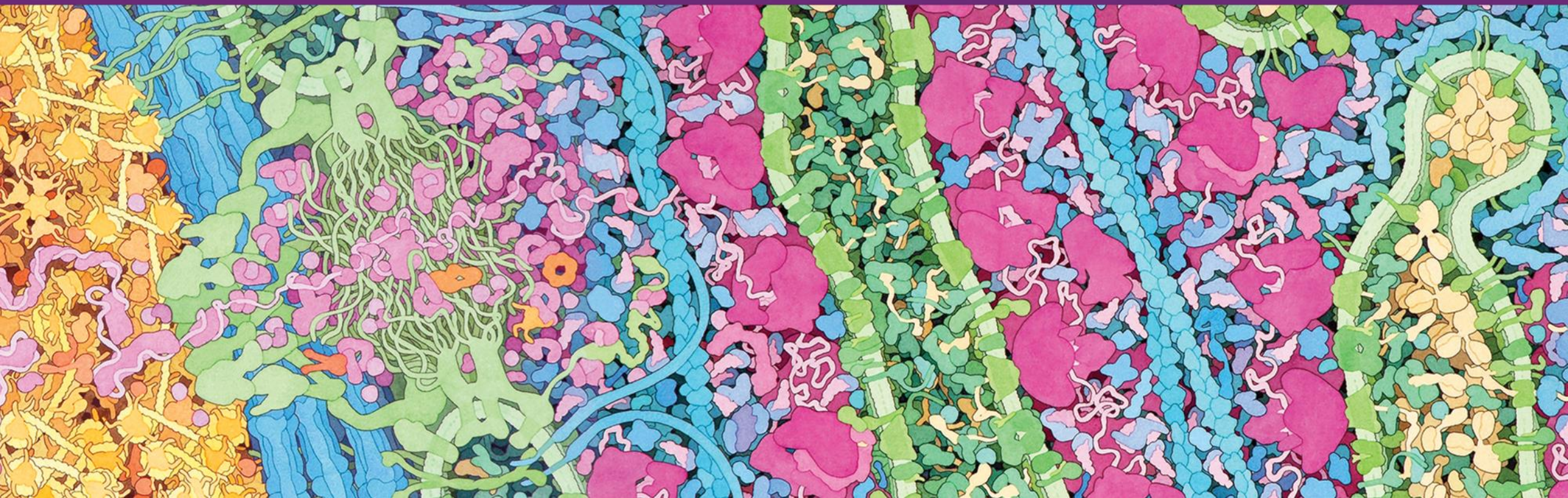

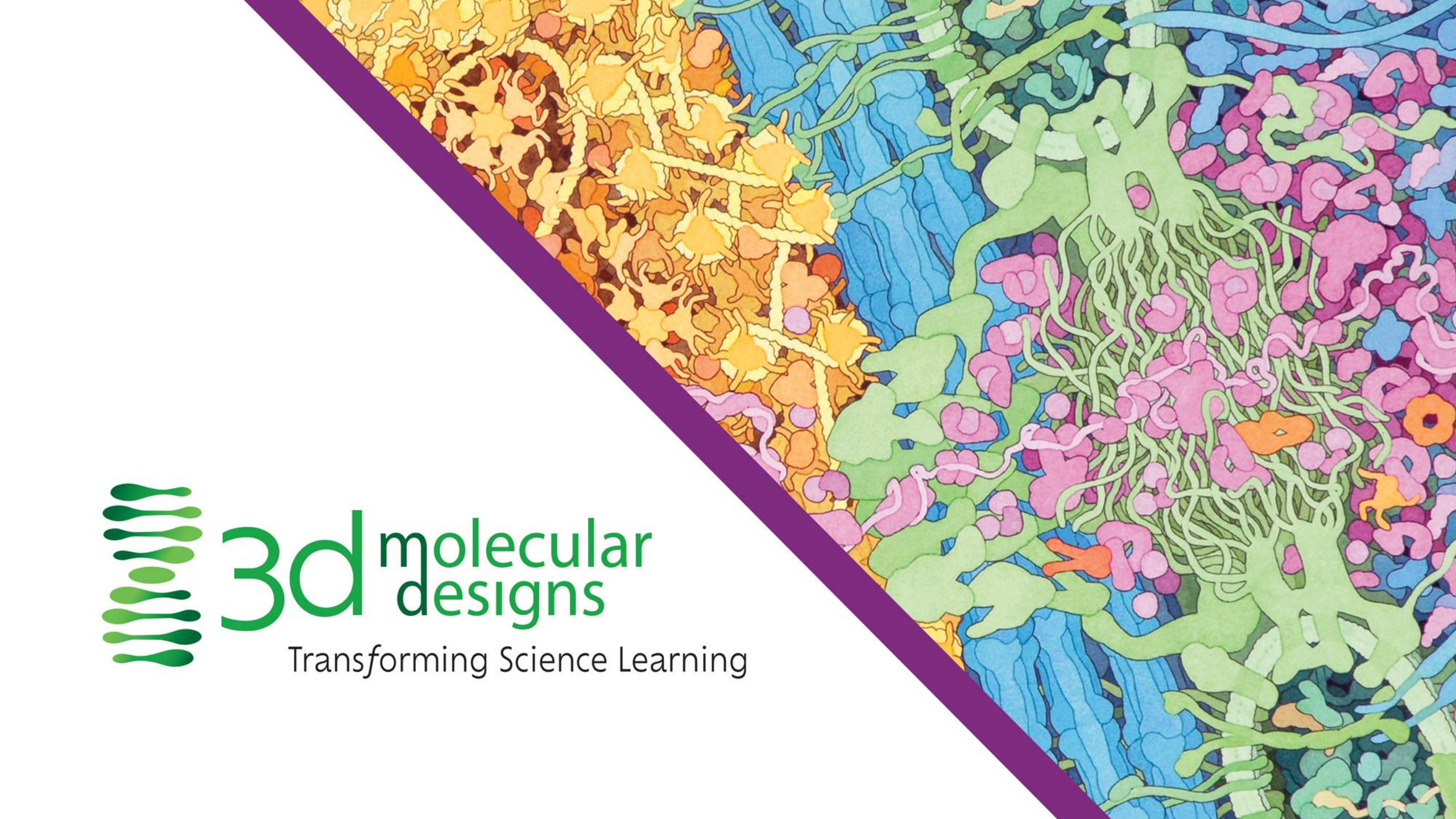

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WHY…did T replace U in DNA?

…if we had not switched…

spontaneous oxidative deamination of C

AGUCGAAGUCAACCGAGGCUGCGCUAC
UCAGCUUCAGUUGGCUCCGACGCGAUG

AGUCGAAGU$^{U}_{x}$AACCGAGGCUGCGCUAC
UCAGCUUCA$_{G}$UUGGCUCCGACGCGAUG

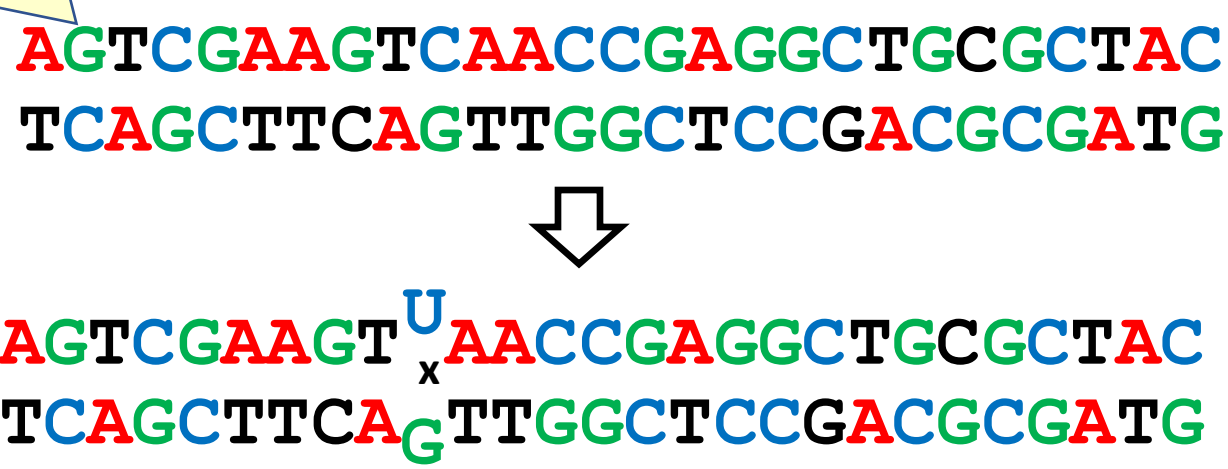
A DNA repair system can remove “U” from DNA.
But,…how can we distinguish between a
“deaminated C” and a normal U?

ANSWER: We can’t.

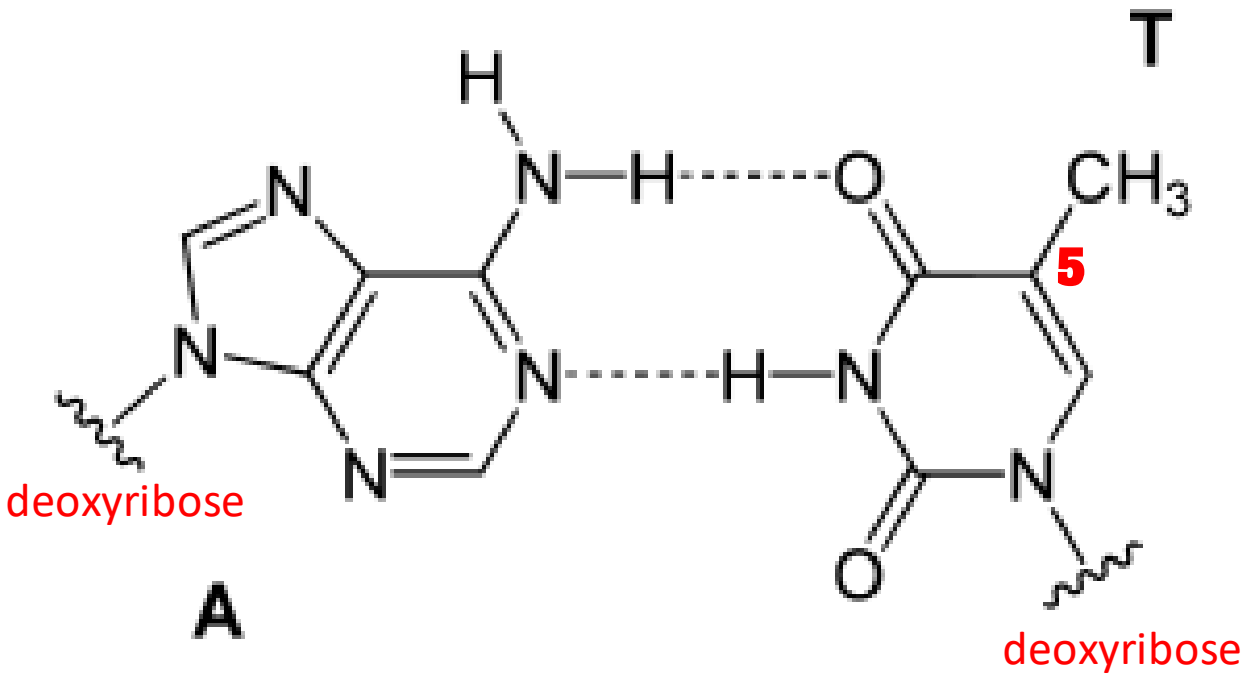
WHY…did T replace U in DNA?

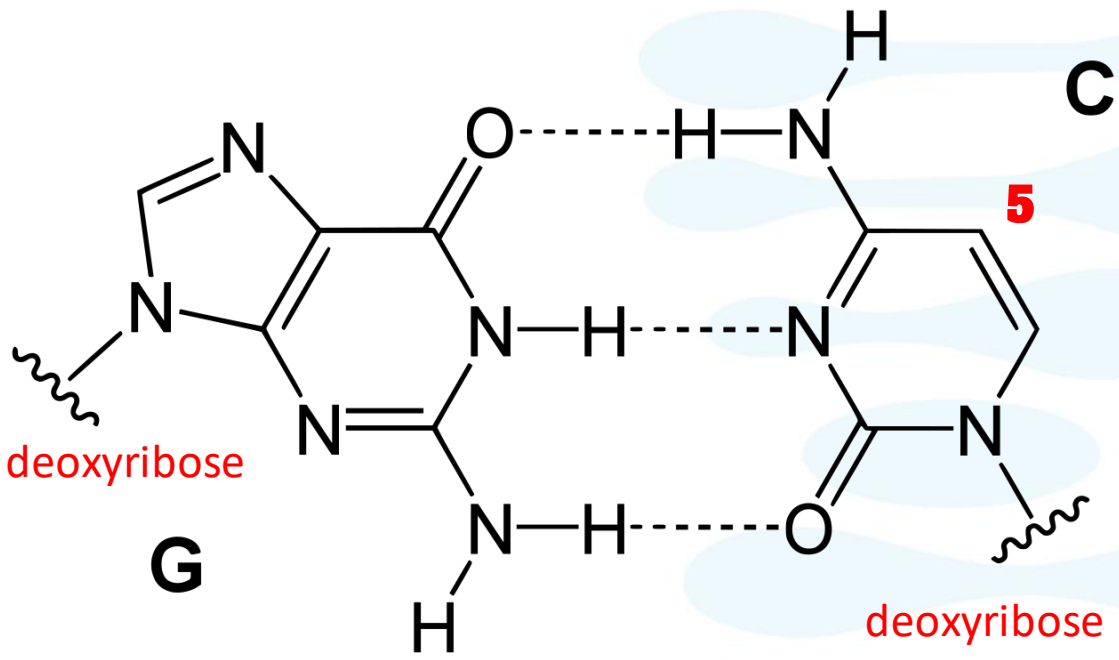
…but after we switched…

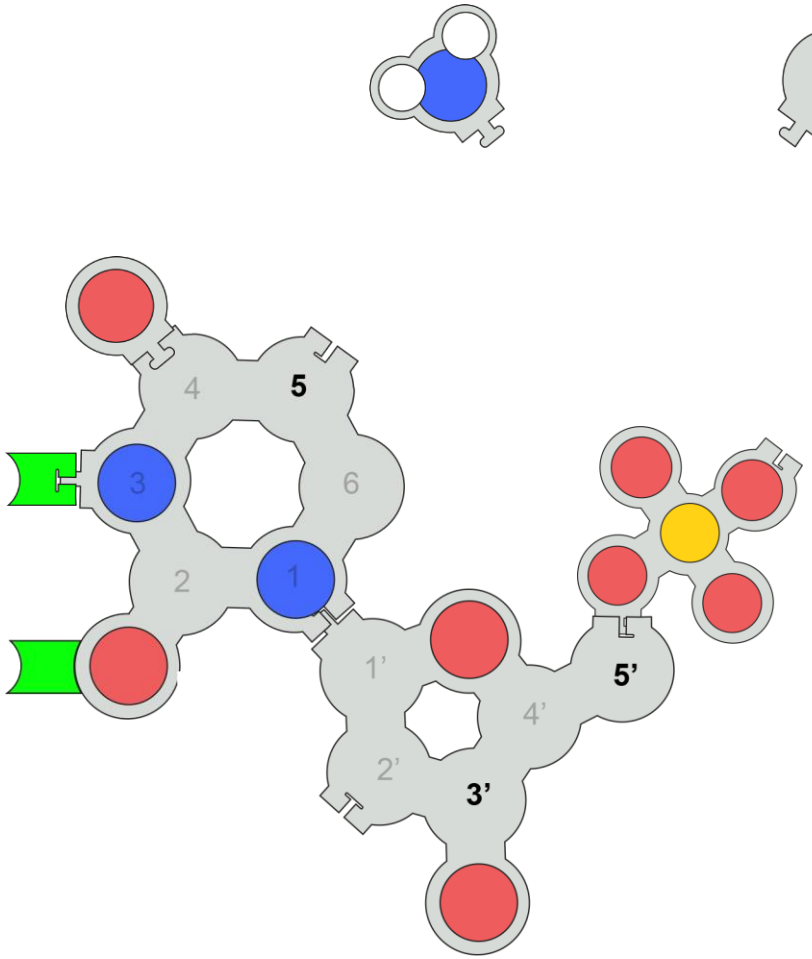
spontaneous oxidative deamination of C



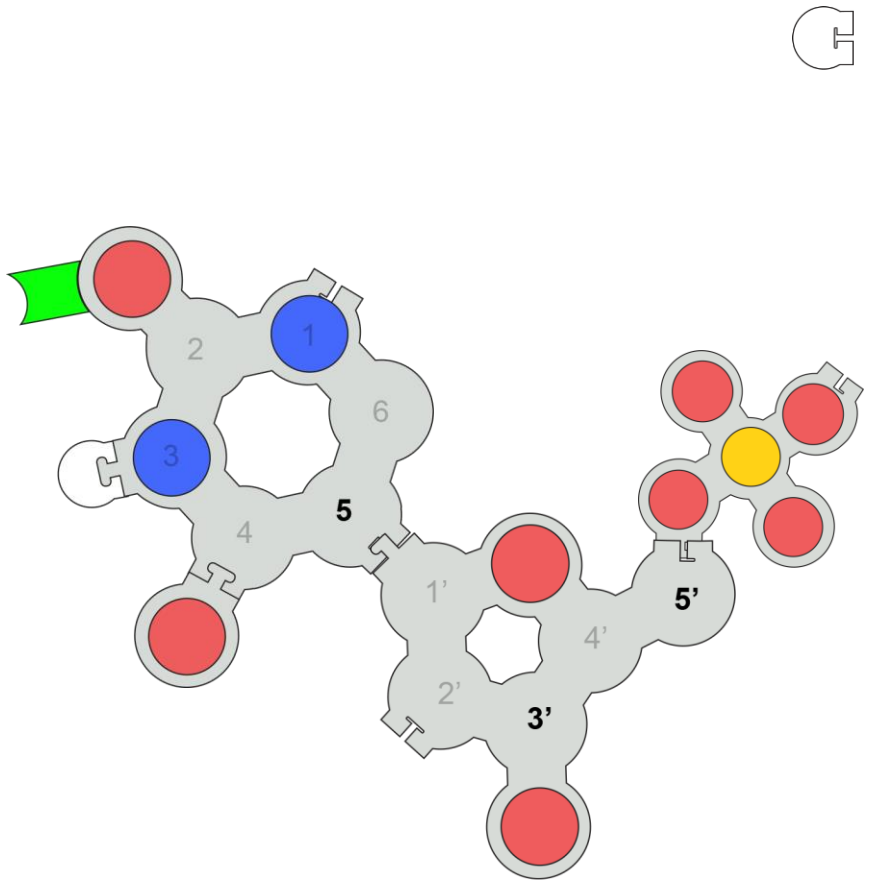
Uracil glycosidase constantly scans the DNA….looking for U's.
Any U … is a bad U…and should be repaired.


T
H
N
H
O
CH3
5
N
N
N
H
N
N
N
O
deoxyribose
A
deoxyribose

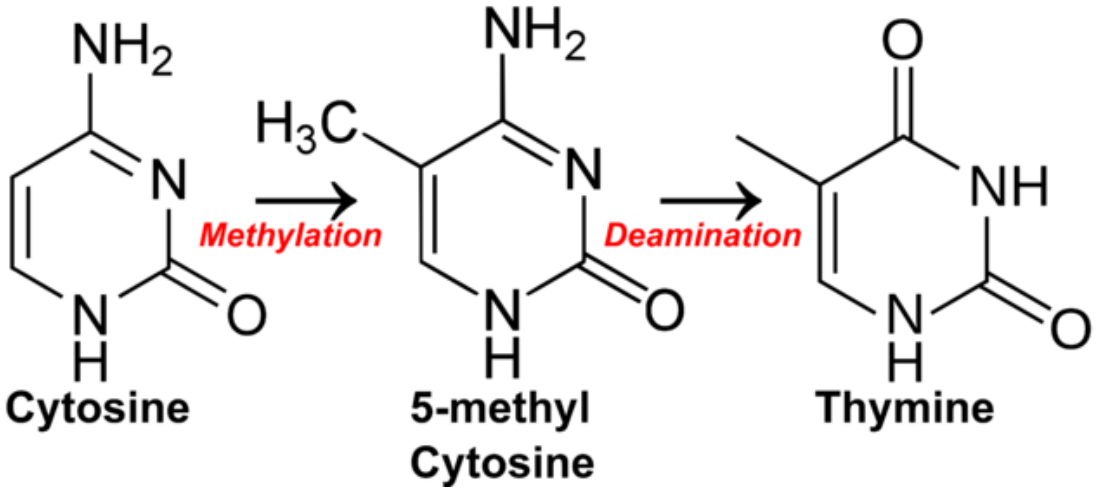

H
C
N
O
H
N
5
N
N
H
N
N
N
N
N
H
O
deoxyribose
G
H
deoxyribose

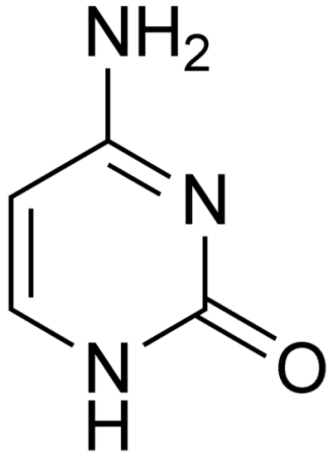


Uridine

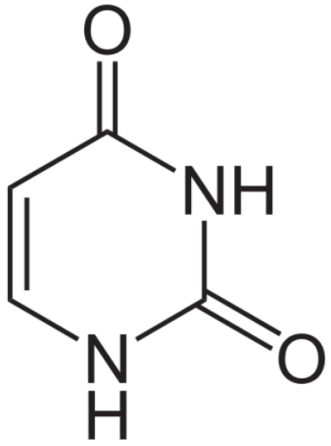


Pseudo-Uridine

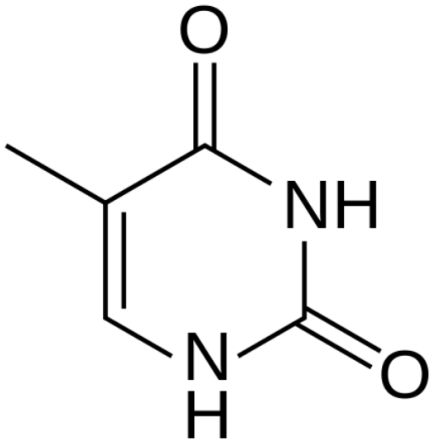

NH2
N
N
H
O
Cytosine
Methylation
H3C
NH2
N
N
H
O
5-methyl
Cytosine
Deamination
O
NH
N
H
O
Thymine



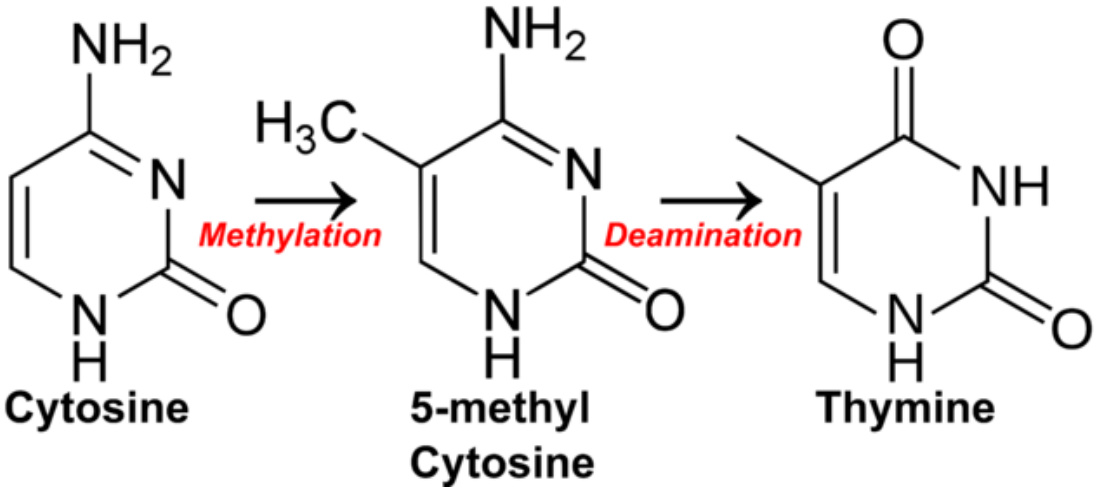
Cytosine

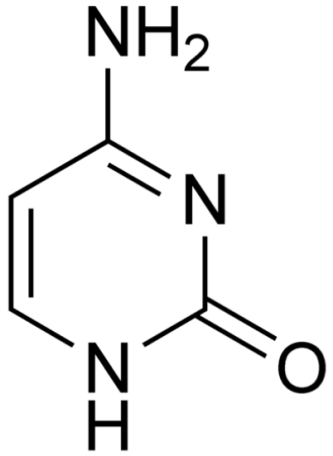


Uracil

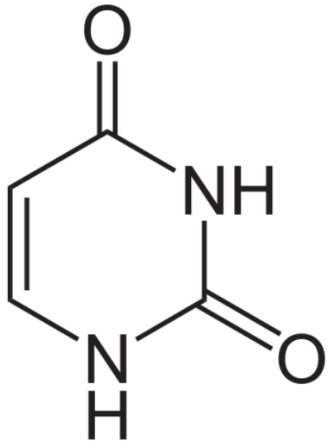


Thymine

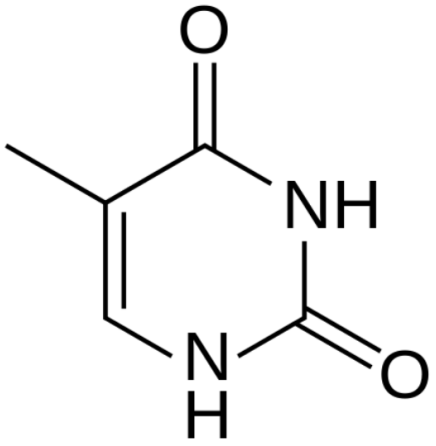




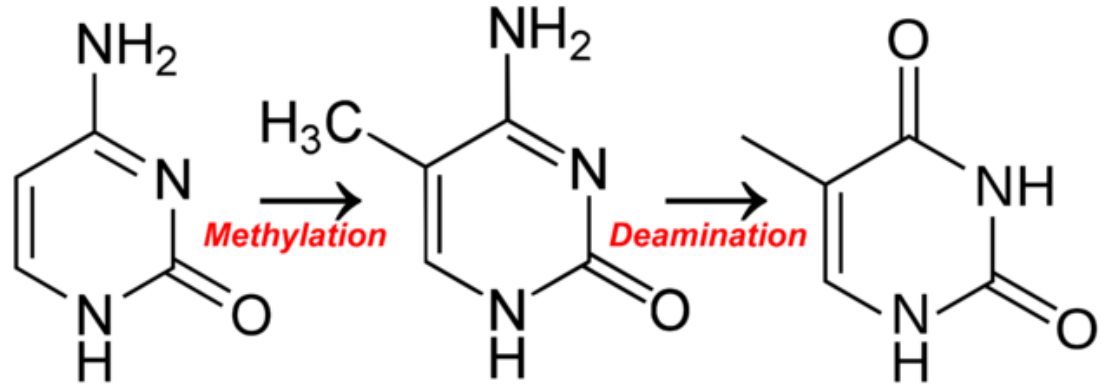
Cytosine



Uracil



Thymine



Cytosine **5-methyl Cytosine** **Thymine**