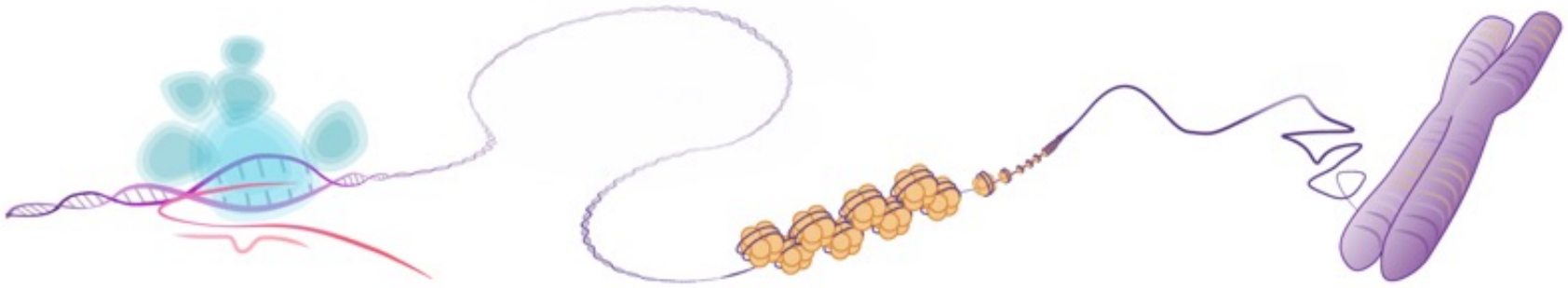
# Session 5: Epigenetics and Epitranscriptomics

Chris Tuggle



https://www.activemotif.com/epigenetics-101

Epi- “above or upon” so epigenetics is study of heritable *phenotypic* variation that does not involve changes to the DNA sequence.

Reversible molecular changes to the chromosome or RNA molecule that affect biological function- often due to environment changes.

Study of epigenetics: how the genome functions across cells, tissues, developmental stage, and environments.

**Mechanism for how our cells use our genomic information to respond to stimuli, interact, adapt and grow ....**

**your guide in 30 minutes(!)**

# Epigenetic effects on phenotype

Queen bee worker bee

Figure 21.4
*Genetics: A Conceptual Approach*, Fifth Edition
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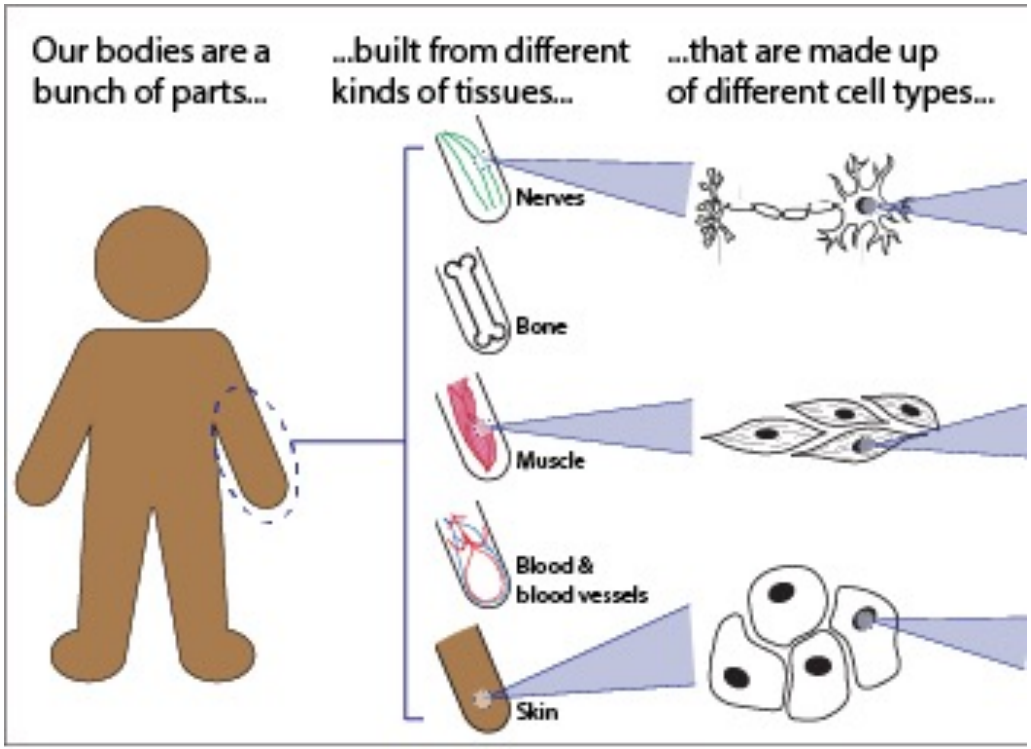
These two from the same colony have the same genes!
A different food causes this huge phenotypic difference- how?

# Selective Gene Expression defines function

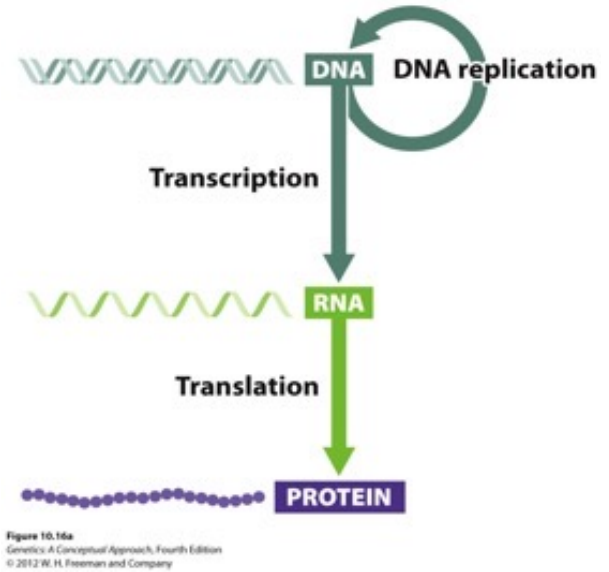



Each cell type/tissue is expressing different sets of genes from the same genome

A useful definition of a cell type or tissue:
the *pattern of genes* active in cells in that tissue!

# How are genes selectively expressed?

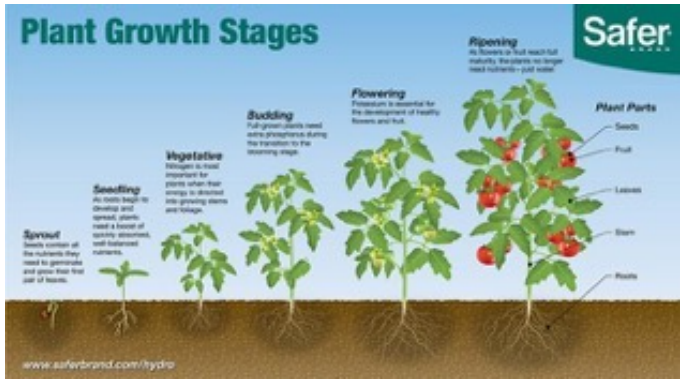
We have covered the “Central Dogma” and a bit about how genes *in general* are expressed:

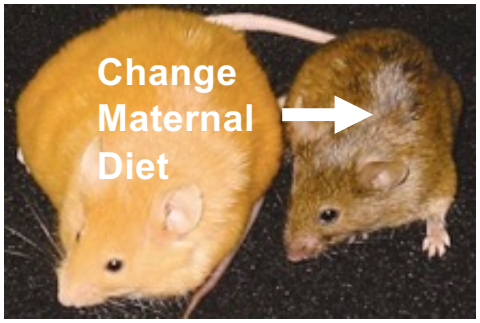



But how does selective gene expression work;
why some genes are on and some off?
How can we measure it?

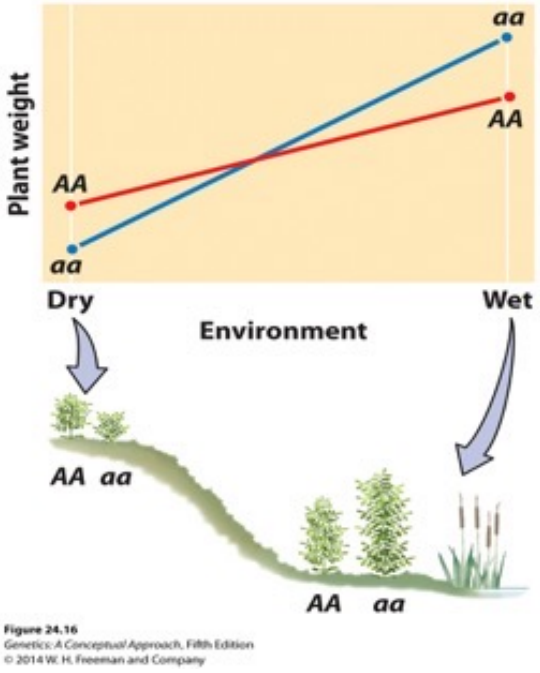
- to define cell types/tissues?
- across time, treatments, environments, genetic variants?
- and the interaction of all of these?

G2P... We want to predict phenotype under different conditions? Same G, different P???



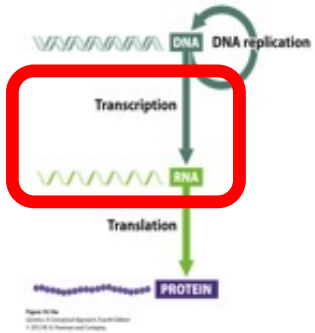




People have different responses to caffeine depending on genetic variants; image from waferboard at Creative Commons.



# Selective Gene Expression is controlled at Multiple Levels

- **Focus today:**




- **Where and when a gene is transcribed into RNA is the major level controlling gene expression patterns**

- **The compactness of the chromatin (DNA+protein) is important to allow transcription (or prevent it)**

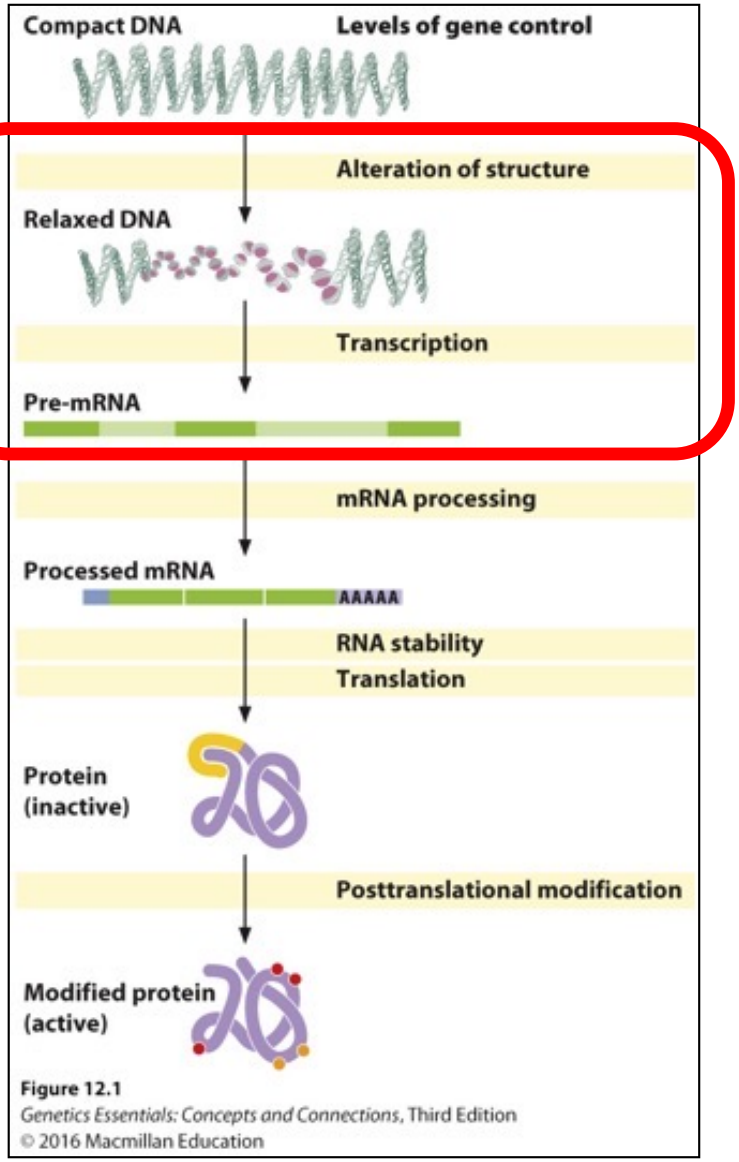
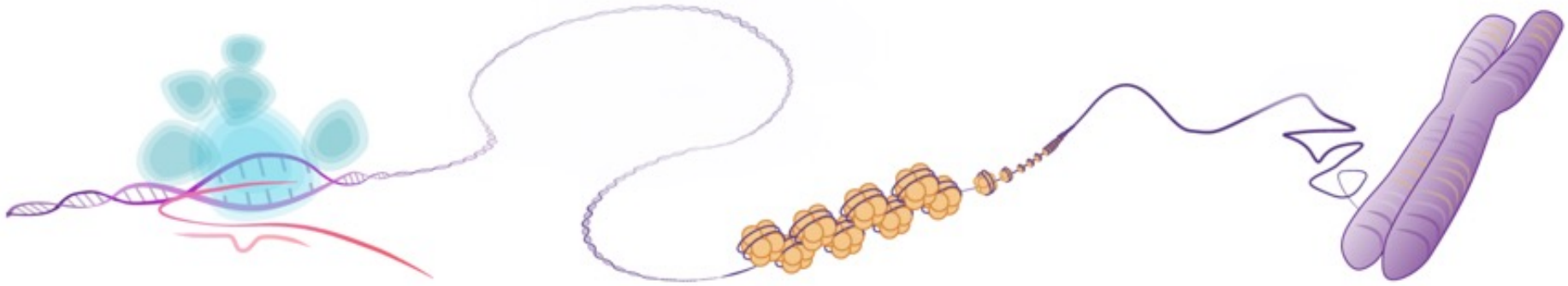



Figure 12.1
*Genetics Essentials: Concepts and Connections*, Third Edition

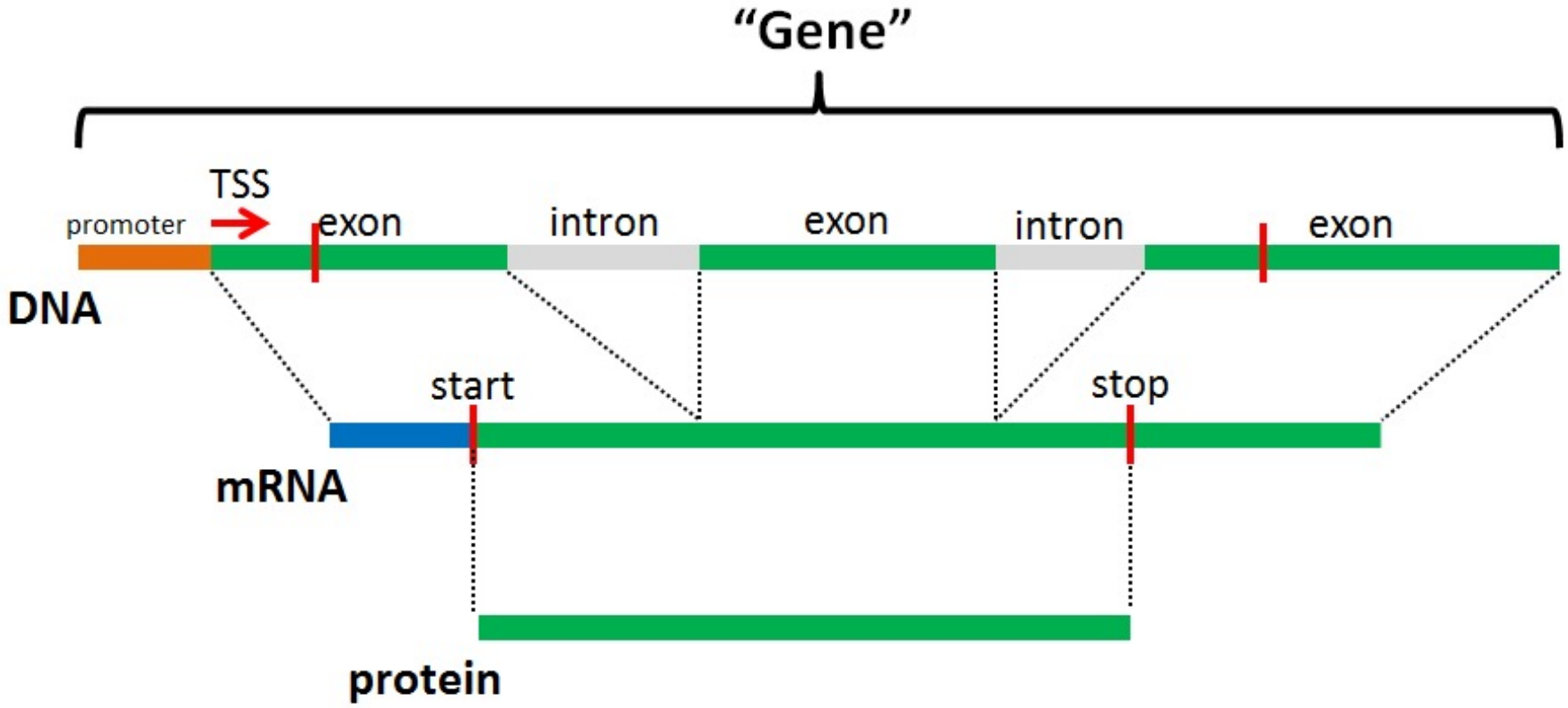
# Epigenetic Effects on Chromatin Influence Gene Expression

- Epigenetics works through molecular changes in chromatin structure, which alter gene expression

- Molecular mechanisms that alter chromatin structure
  - Changes in patterns of DNA methylation
  - Chemical modification of histone proteins
  - RNA molecules that affect chromatin structure and gene expression



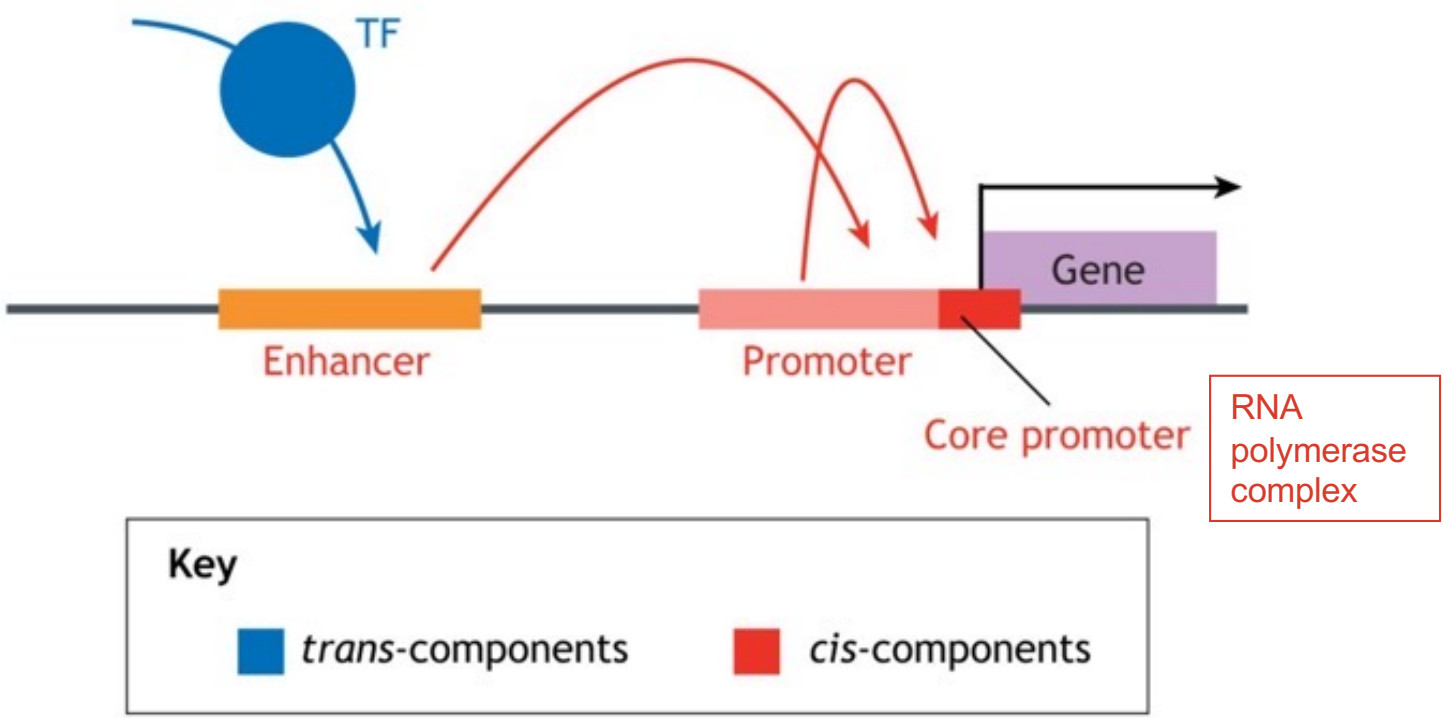
# Elements of a Gene as often drawn




(What is missing?)

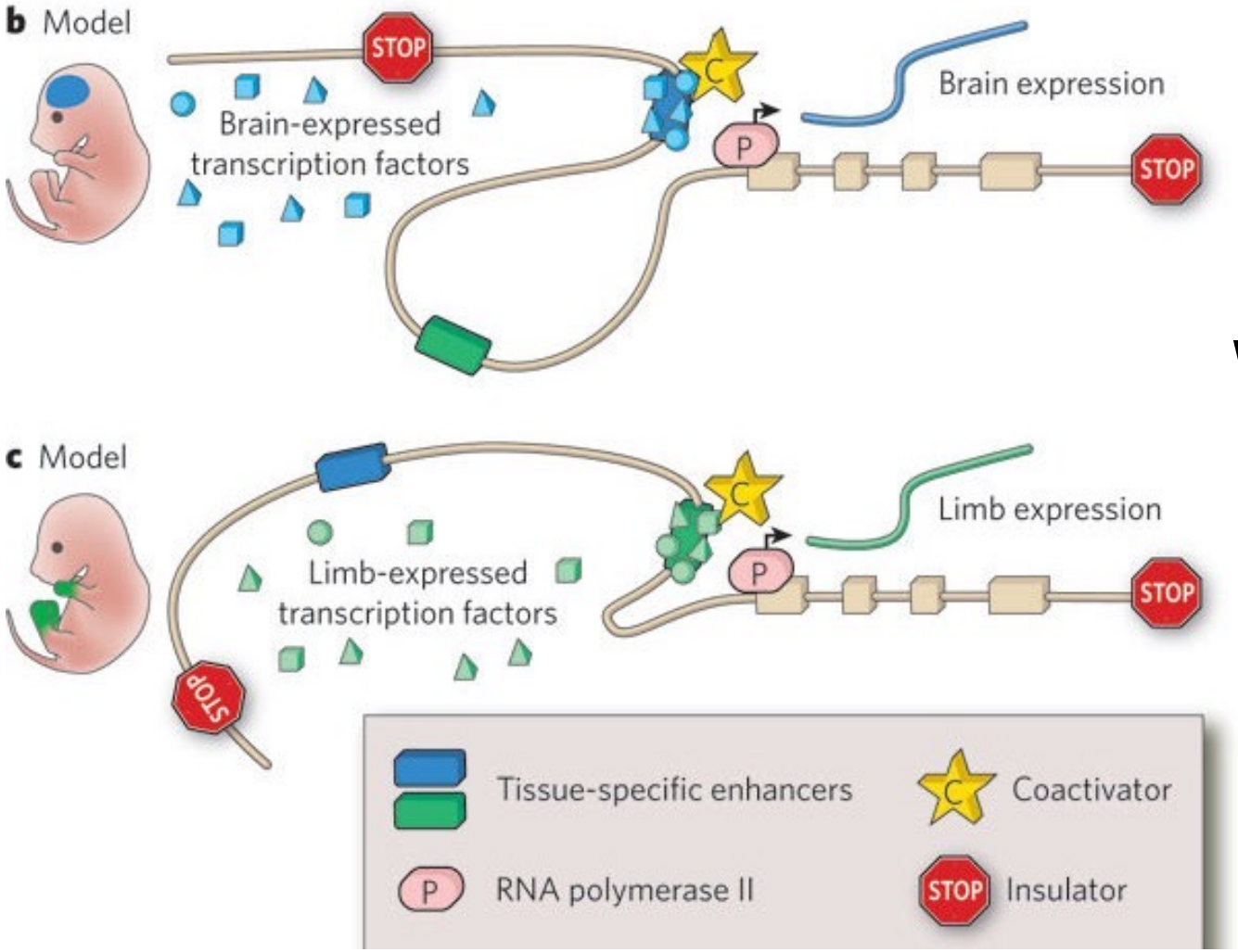
https://cpt.tamu.edu/bich464/slides/C1.html

# Gene Transcriptional Regulatory Elements




https://dnalc.cshl.edu/resources/animations/

# Selective gene expression models- a more complicated view of gene regulation




**What is missing from this view?**

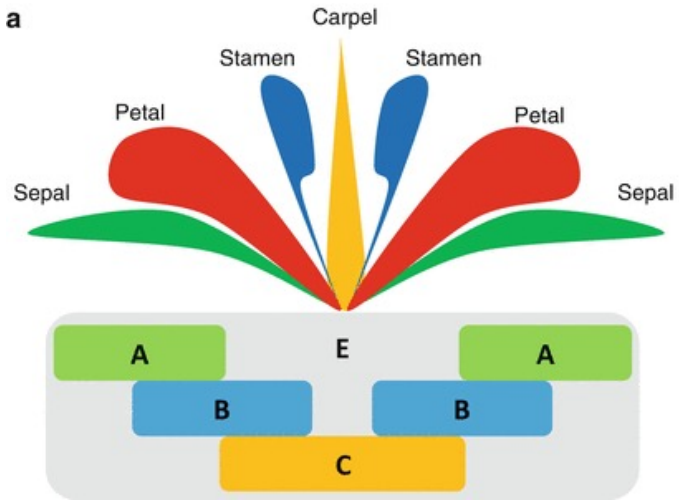
**Hint→**

**Logic problem for selective gene expression model: how do we explain tissue/cell-type specific regulation with transcription factors that themselves have to be specifically expressed?**

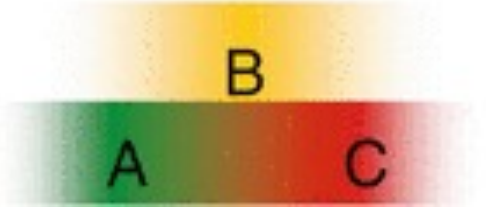
**Combinatorial model of gene regulation explains how we don't have "turtles all the way down" (solving the infinite regression model)...**

**Transcription factors combine together to create new selective expression patterns!**

**Example of combinatorial gene expression: the ABC(E) model of floral organ identity- three TF can specify >3 types**




**Beyond On/off: Variable inputs, many outputs**




https://link.springer.com/protocol/10.1007/978-1-4614-9408-9_4

# Selective Gene Expression is controlled at Multiple Levels

- **So the right TF may be expressed..**
- **Which parts of the genome are accessible to be bound and turned on by TF is also very important!**
- **How can we recognize:**

**accessible regions of the genome?**

**active versus inactive enhancers and promoters?**

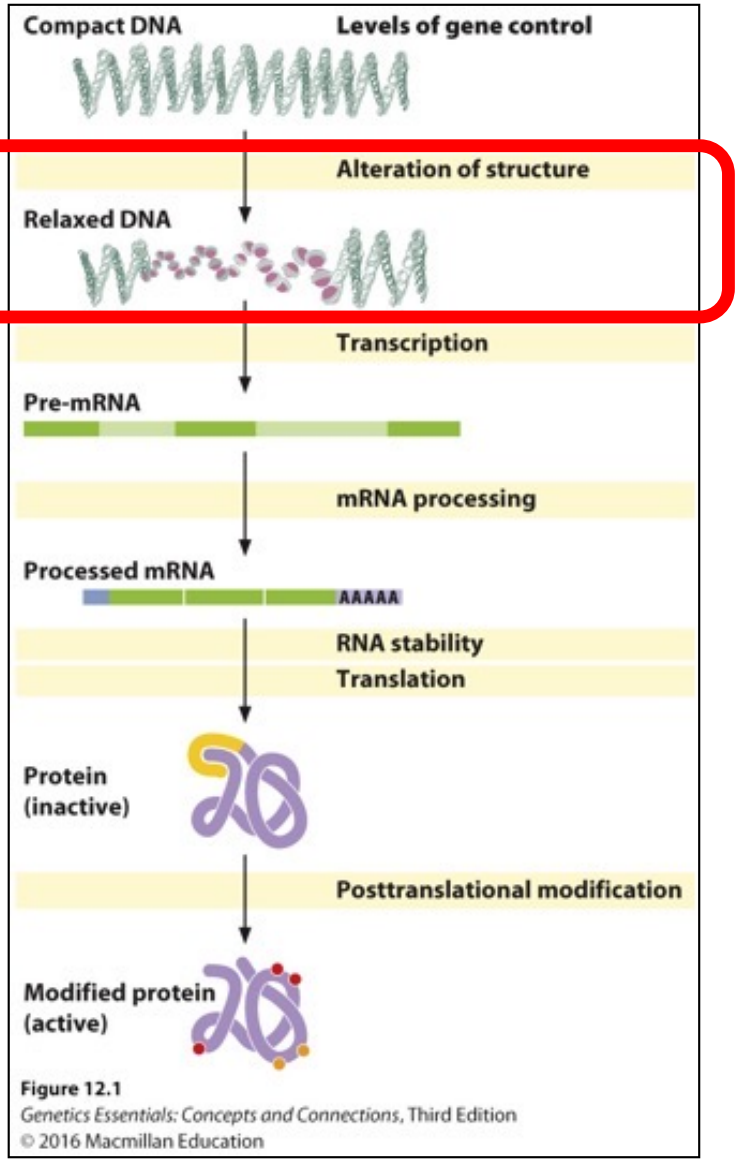



Figure 12.1
*Genetics Essentials: Concepts and Connections*, Third Edition

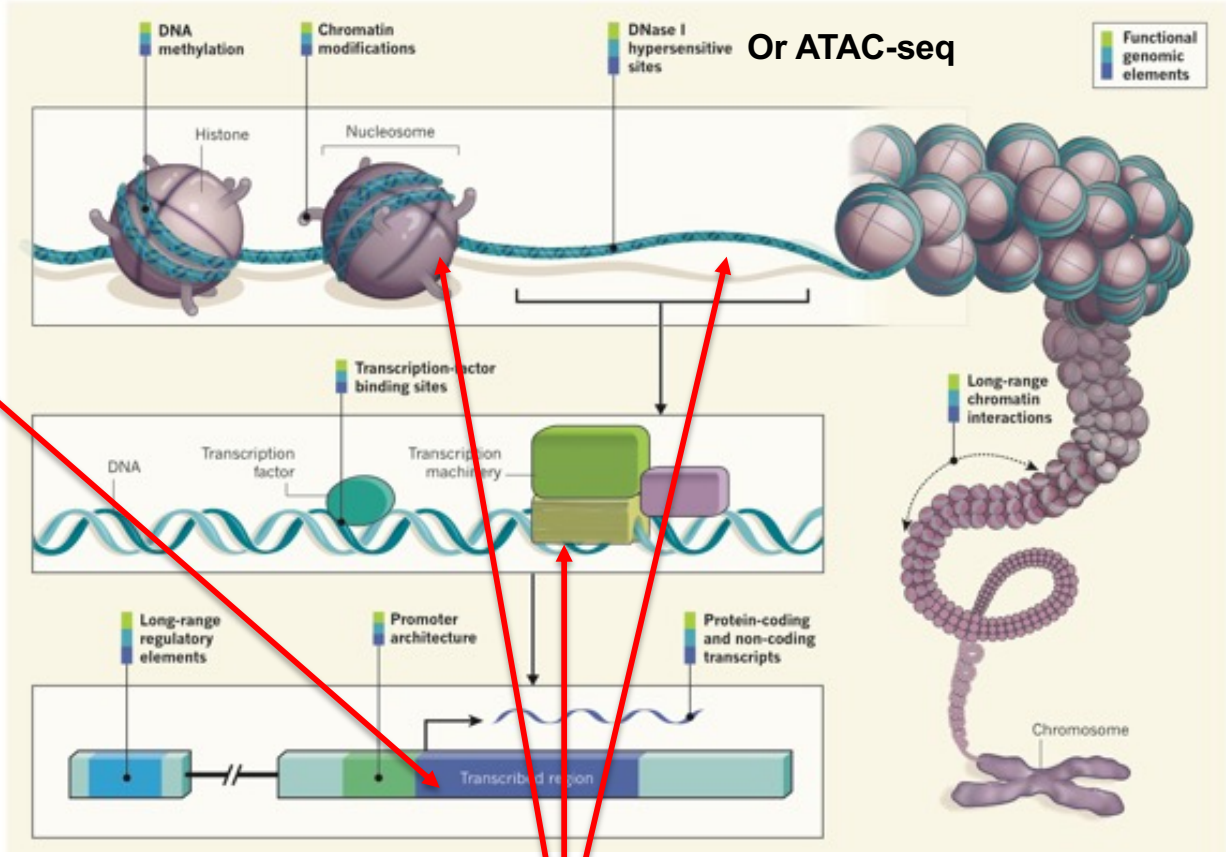
# The *human ENCODE project*

**$150 Million genome annotation: determining the function of all parts of genome across many tissues and cell types**

What part of genome is active (expressed)?

**RNA levels and location**

What part controls this expression? (specific tissue or cell type, response to infection, etc):




**Biochemical assays of Chromatin structure**

# Where does the RNA come from?

## *Steps in RNAseq*

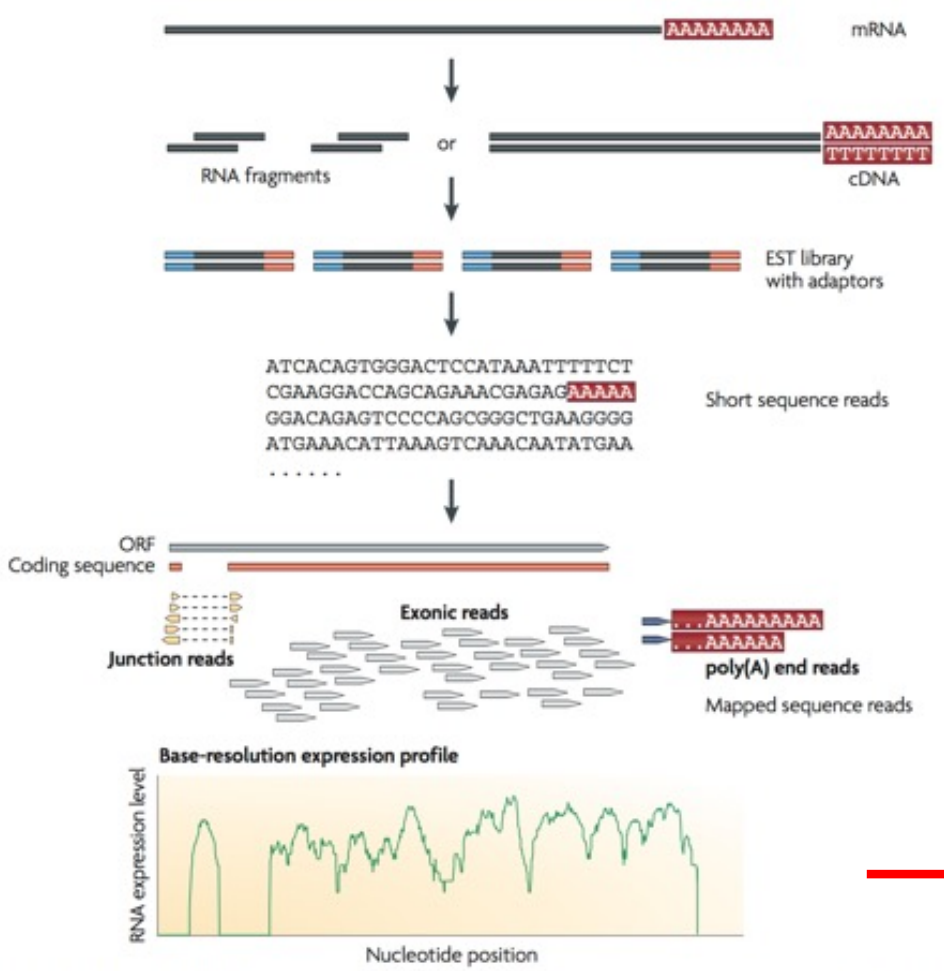
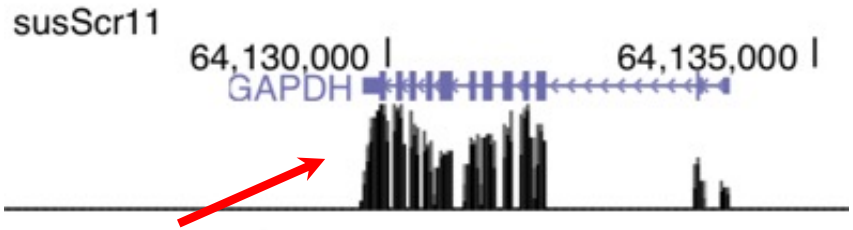



Figure 1 | **A typical RNA-Seq experiment.** Briefly, long RNAs are first converted into a library of cDNA fragments through either RNA fragmentation or DNA fragmentation (see main text). Sequencing adaptors (blue) are subsequently added to each cDNA fragment and a short sequence is obtained from each cDNA using high-throughput sequencing technology. The resulting sequence reads are aligned with the reference genome or transcriptome, and classified as three types: exonic reads, junction reads and poly(A) end-reads. These three types are used to generate a base-resolution expression profile for each gene, as illustrated at the bottom; a yeast ORF with one intron is shown.

## *Example data*

GAPDH gene



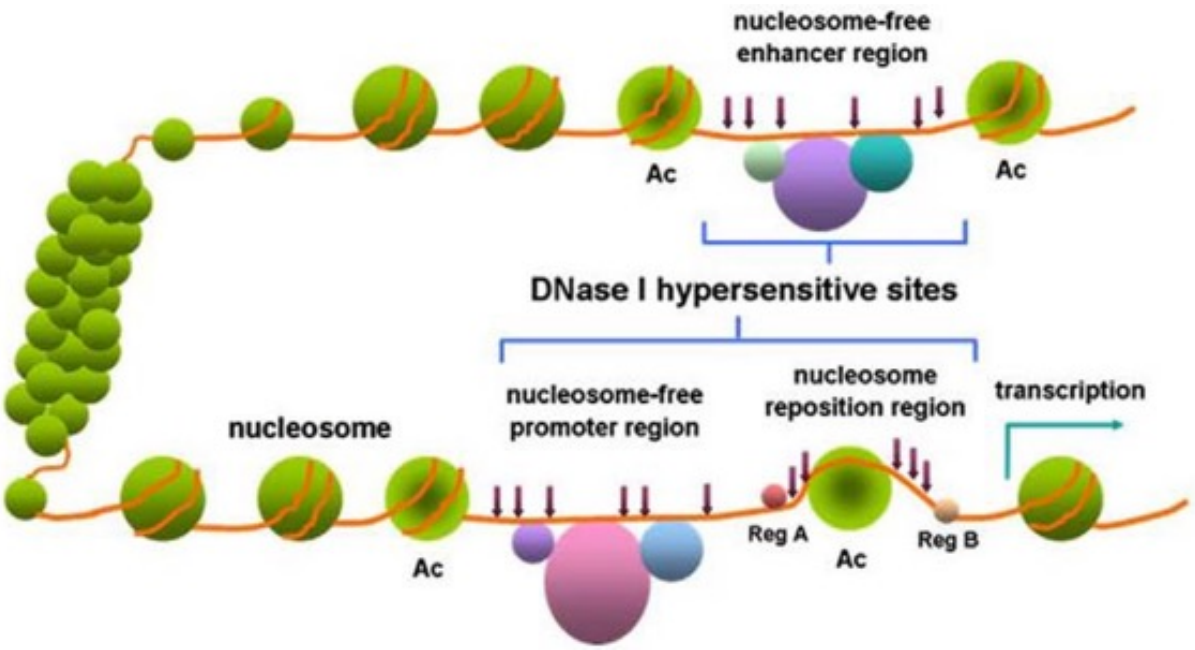

***RNAseq reads***

Kern, Zhou, et al., unpublished data

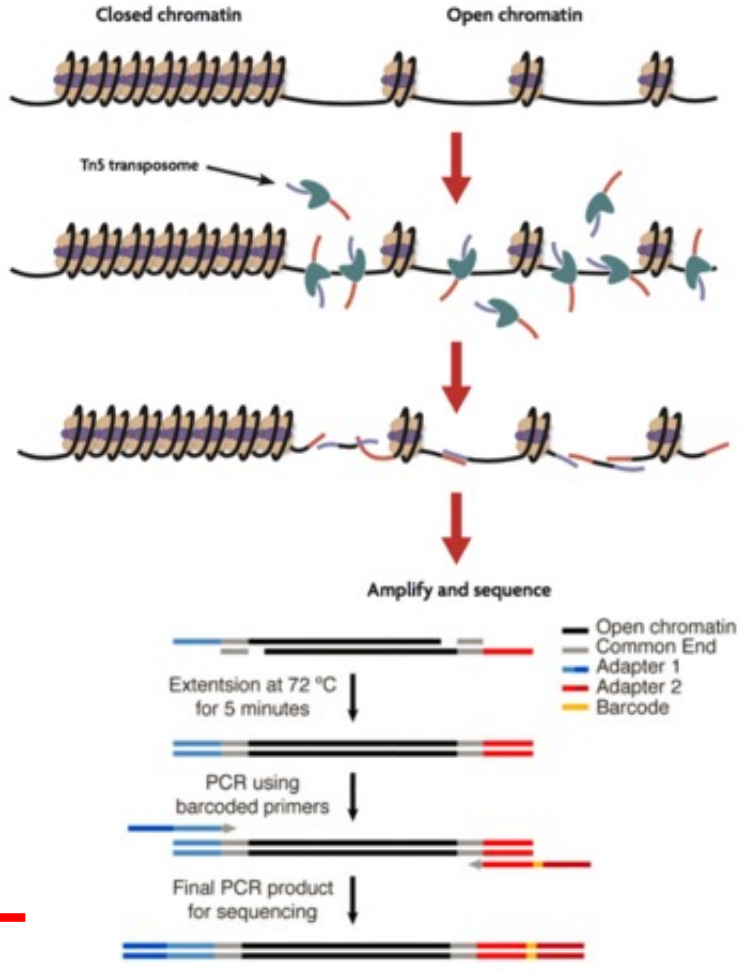
Alignment mapping data → exons + introns defined → gene structure models predicted → annotation of genome

# Measuring Chromatin Accessibility

**Regulatory Factors often require a nucleosome-free region to bind to specific DNA sequences**




**ATAC-seq (Assay for Transposase-Accessible Chromatin using sequencing)**



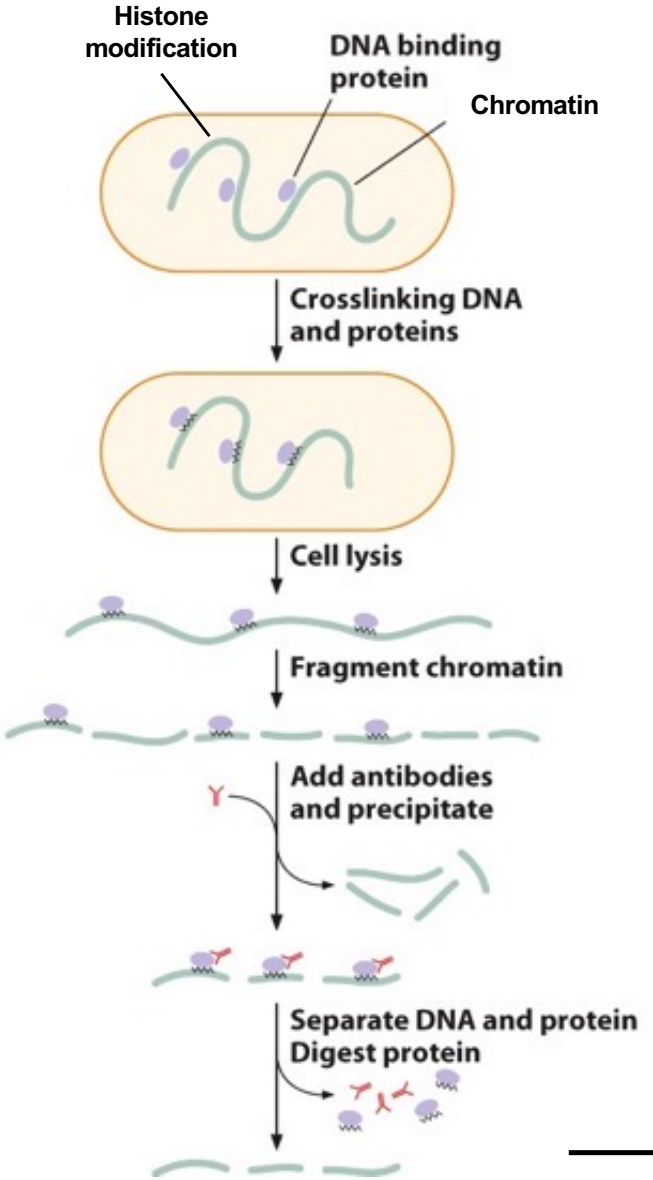

Map to genome → define peaks → Alignment mapping data identifies regions with accessibility in that sample/state

# Measuring chromatin modifications

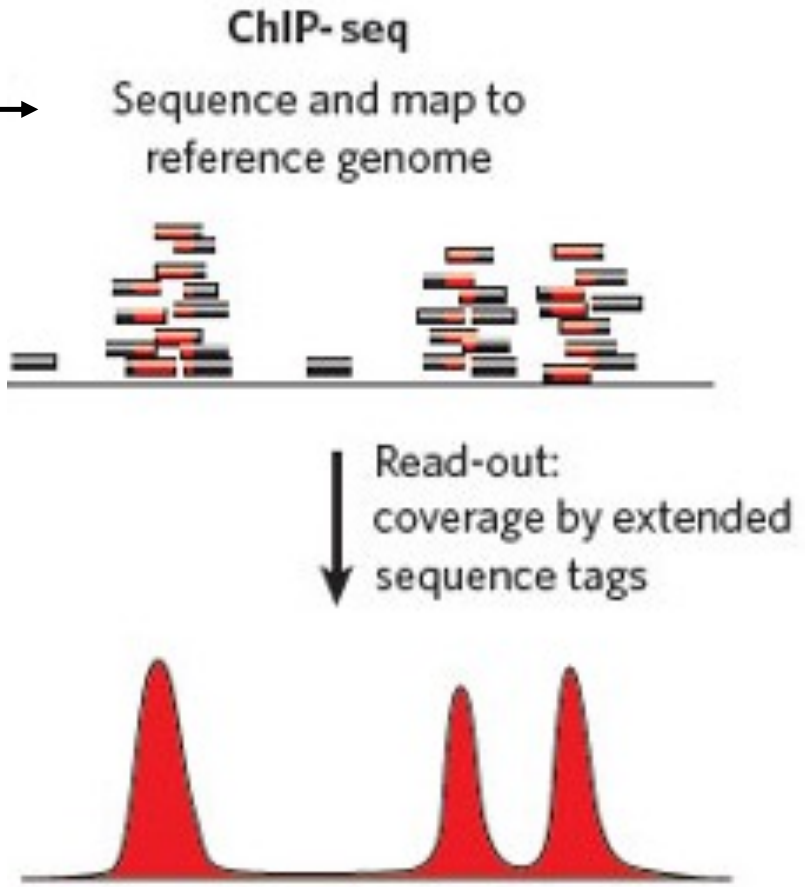
- **Histone modifications:** more than 100 different post-translational modifications of histone proteins

- **Modifications include the addition of:**
  - phosphates
  - methyl groups
  - acetyl groups
  - ubiquitin

# Chromatin Immunoprecipitation Principles

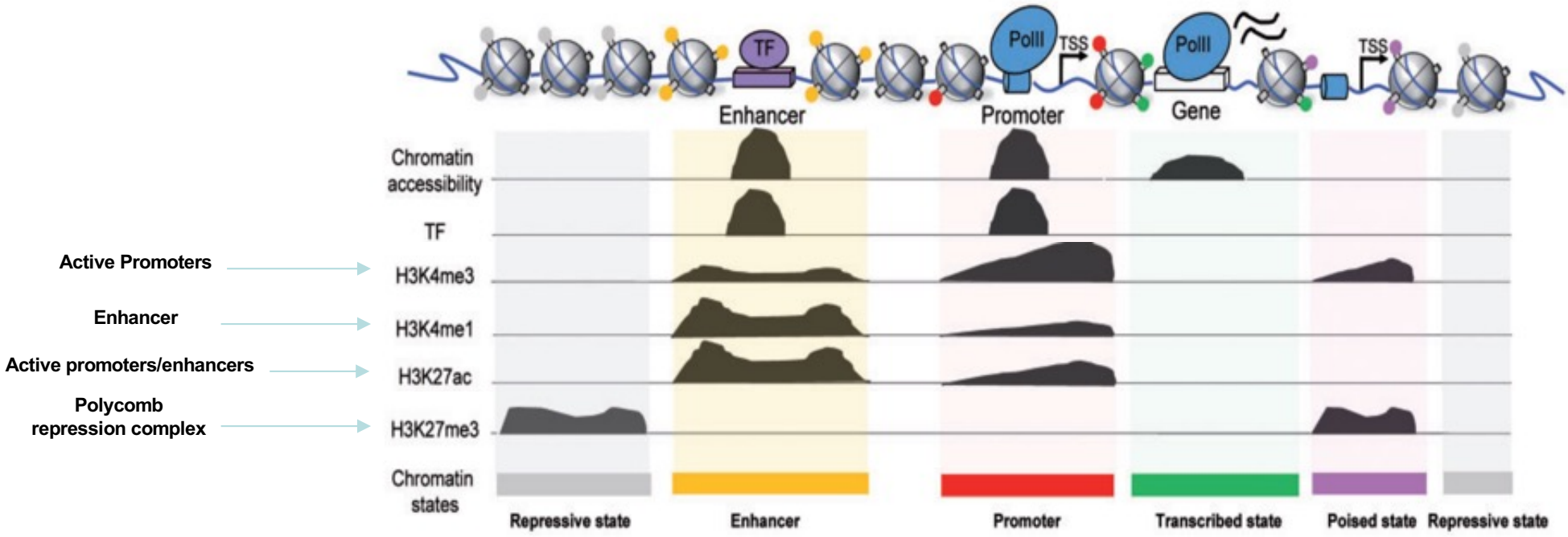



"Immuno" because an antibody is used to recognize either a DNA binding protein or a specific histone modification



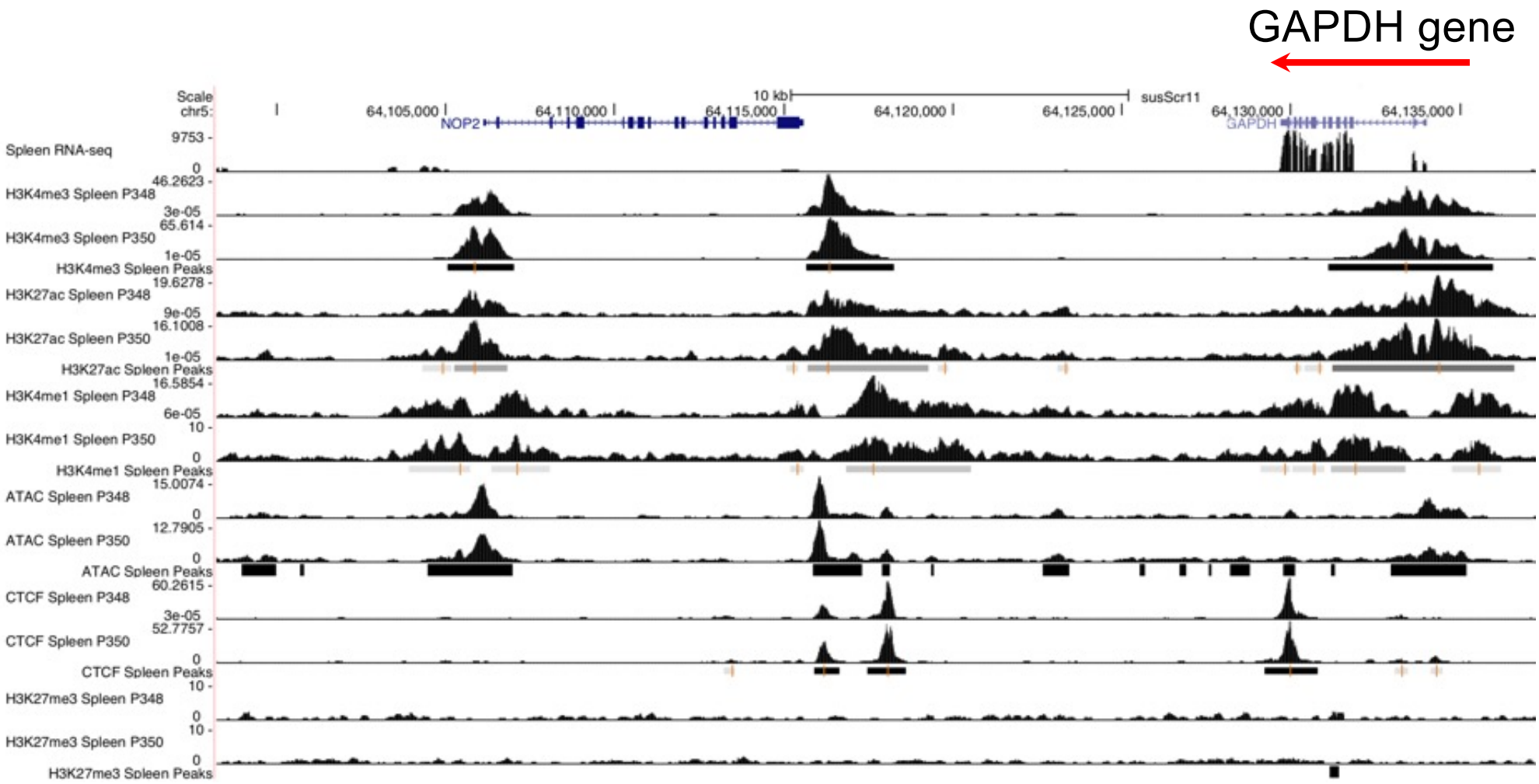
# Putting it all together to predict genome function

## The ”histone code” can predict function of genome regions- chromatin accessibility reinforces/validates these predictions




Jiang, S and Mortazavi, A. 2015 . Briefings in Functional Genomics, 17(2), 2018, 104–115

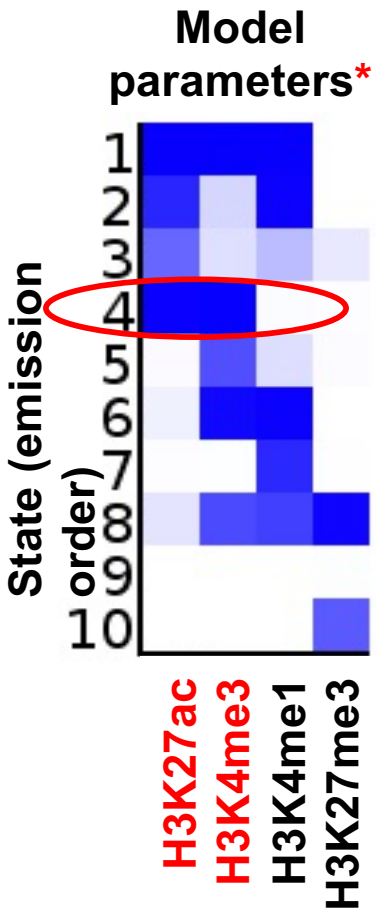
# *Example data for UCD pilot FAANG*



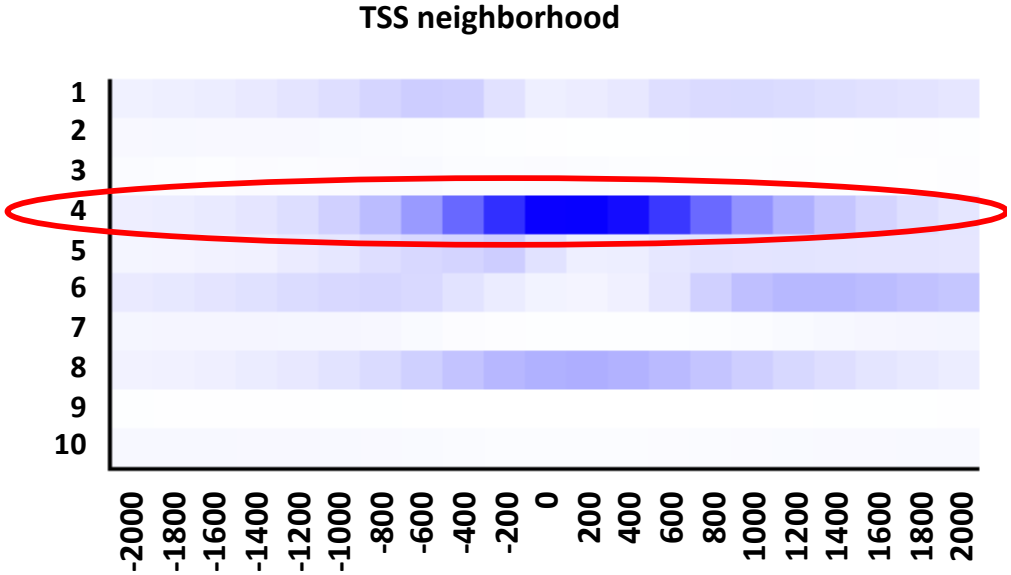

Kern, Zhou, et al., unpublished data

# Assigning function* to genomic regions: chromatin states prediction from ChromHMM

*** Algorithmic model uses data from all four histone marks to predict the regulatory function of a region**

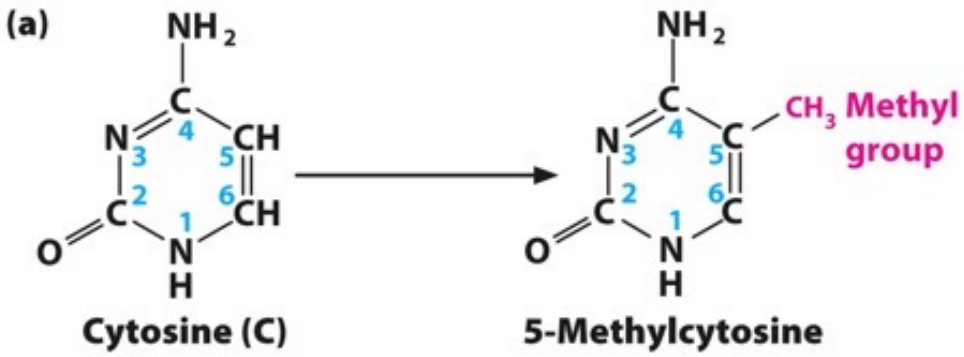


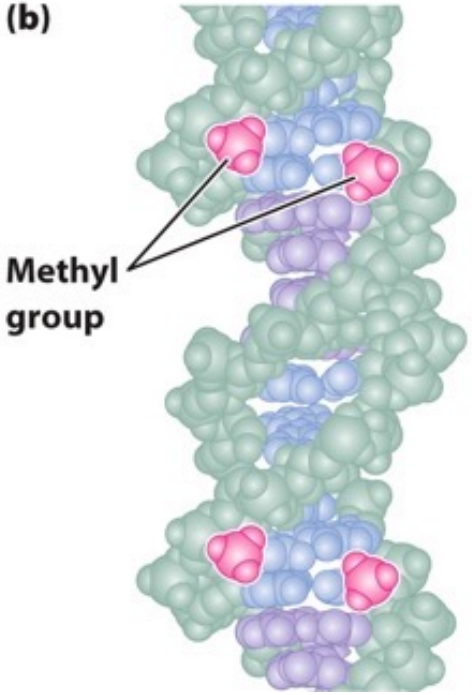

1. Flanking active promotor
2. Strong enhancer
3. Weak/bivalent enhancers
4. Active promotor
5. Weak promotor
6. Weak promotor
7. Weak/poised enhancer
8. Bivalent/poised promotor
9. Heterochromatin; low signal
10. Repressed polycomb



# The DNA itself is also Modified!

- **DNA methylation:** addition of methyl groups to nucleotide bases
- **Most common:** methylation of cytosine to produce 5-methylcytosine at CpG sequences



# DNA Methylation Assay

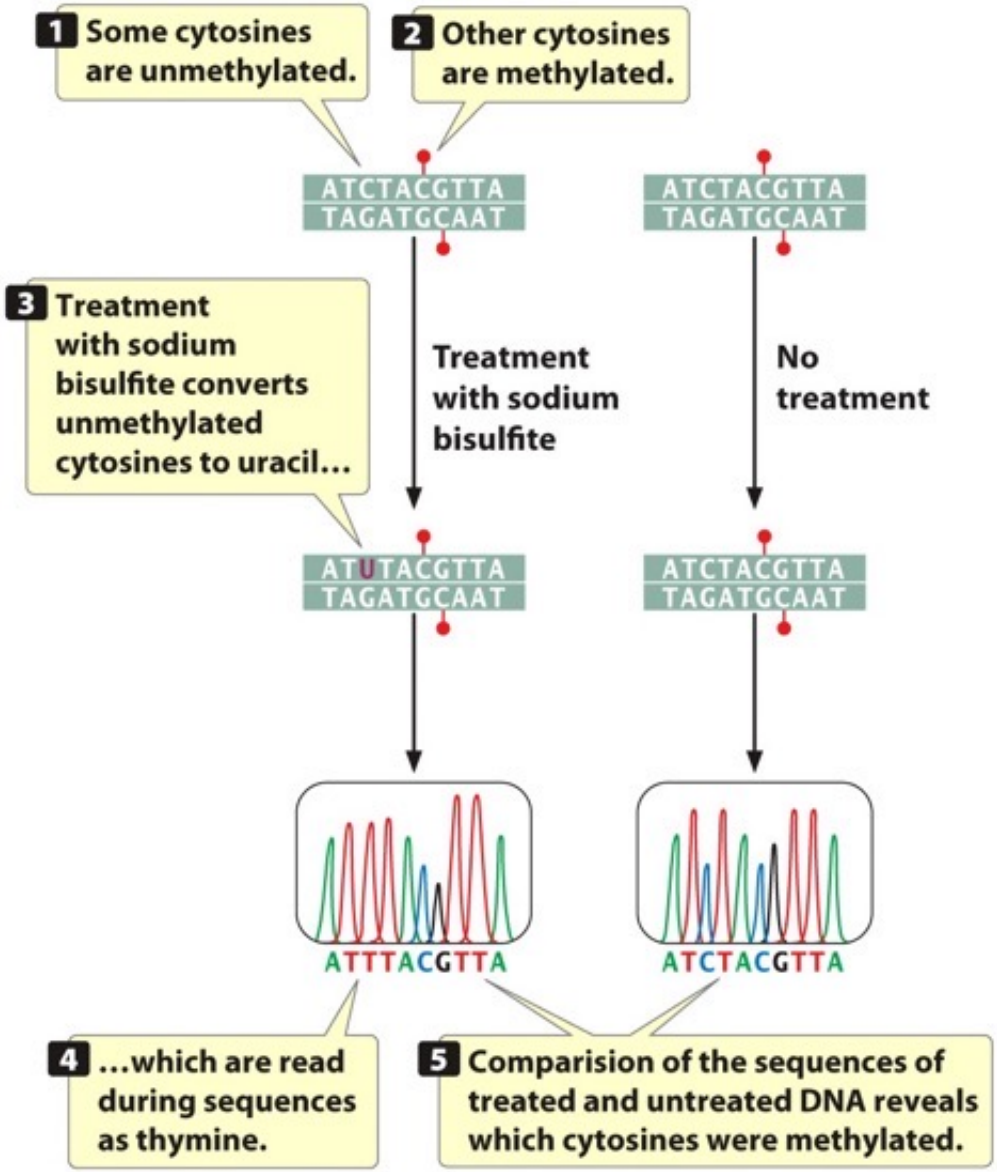
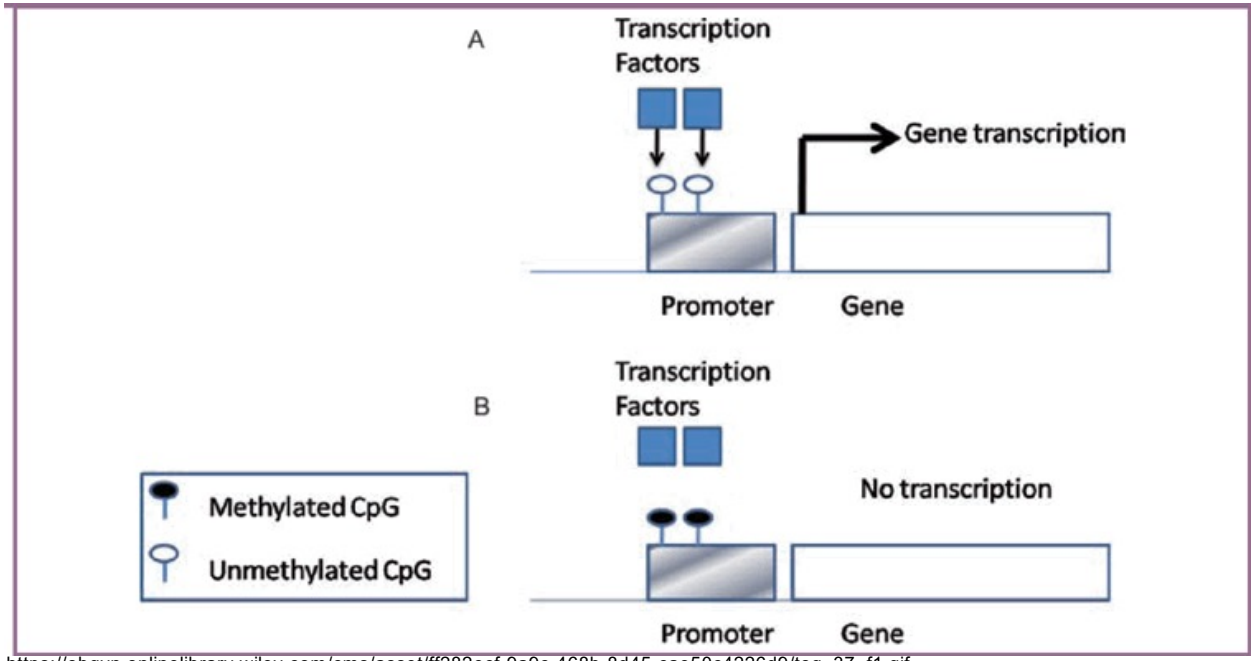



**Figure 21.13**
*Genetics: A Conceptual Approach*, Fifth Edition

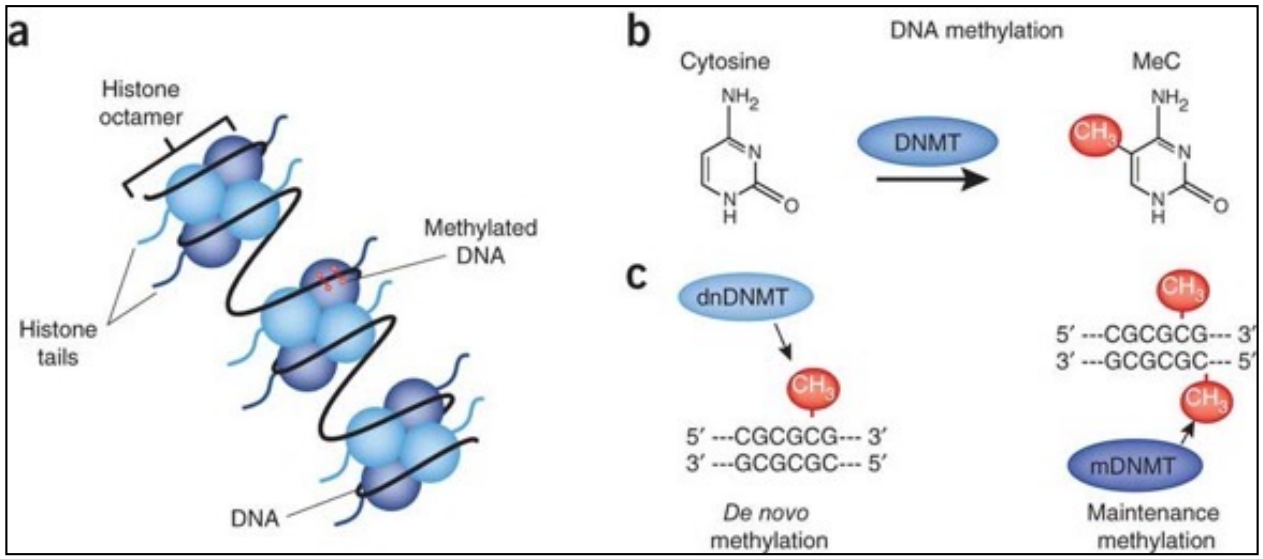
# Methylation of Genomic DNA: A regulated signal

**Methylation of Genomic DNA is Usually a Repressive Signal to nearby genes**




https://obgyn.onlinelibrary.wiley.com/cms/asset/ff282ecf-9a9c-468b-8d45-cae50c4226d9/tog_37_f1.gif

**Methylation of Genomic DNA is altered by DNA Methyl-transferase enzymes (DNMT)**

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# Epigenetic effects on phenotype

Returning to our question on the bees... A phenotype caused by epigenetics- a difference due to nutrition.
Future queens are fed "royal jelly" during development ☺- workers get regular food ☹

How does food create a queen?

**What is the epigenetic effect?**

Answer: Substances in the Royal jelly turn off a DNA methyl-transferase gene, so Royal jelly decreases methylation on DNA so more genes are expressed!

Figure 21.4
Genetics: A Conceptual Approach, Fifth Edition
© 2014 W. H. Freeman and Company

Genes that create a queen!

# Epigenetic effects on phenotype: imprinting

In mammals, the male and female genomes found in sperm and egg are not equivalent; you cannot create a viable embryo from 2 haploid genomes from eggs or from sperm; you need one of each! True even for inbred mice- where there is one genome.... Has to be epigenetic!

The Maternal Conflict hypothesis: these genomes are in a tug-of-war for maternal resources

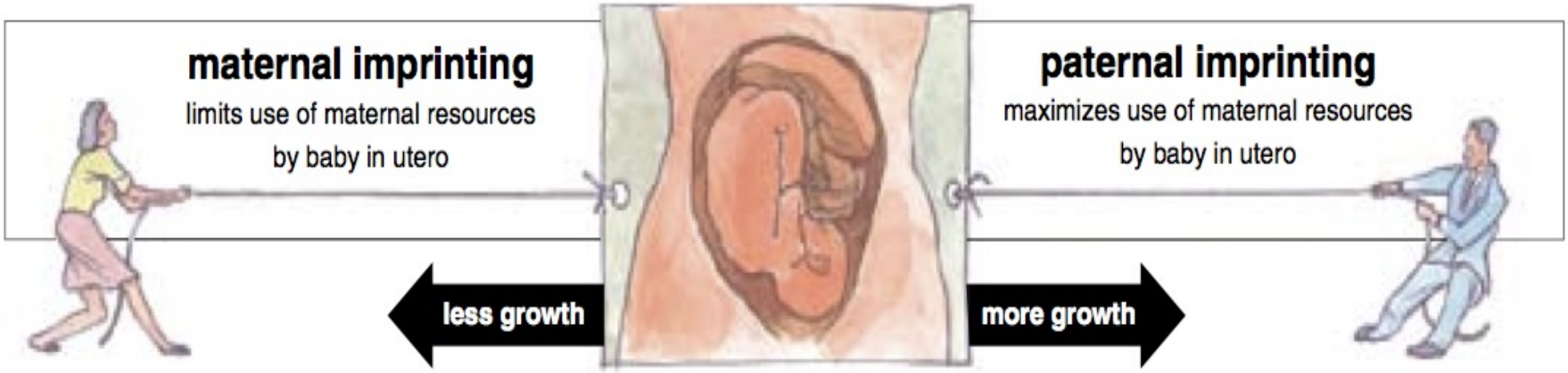
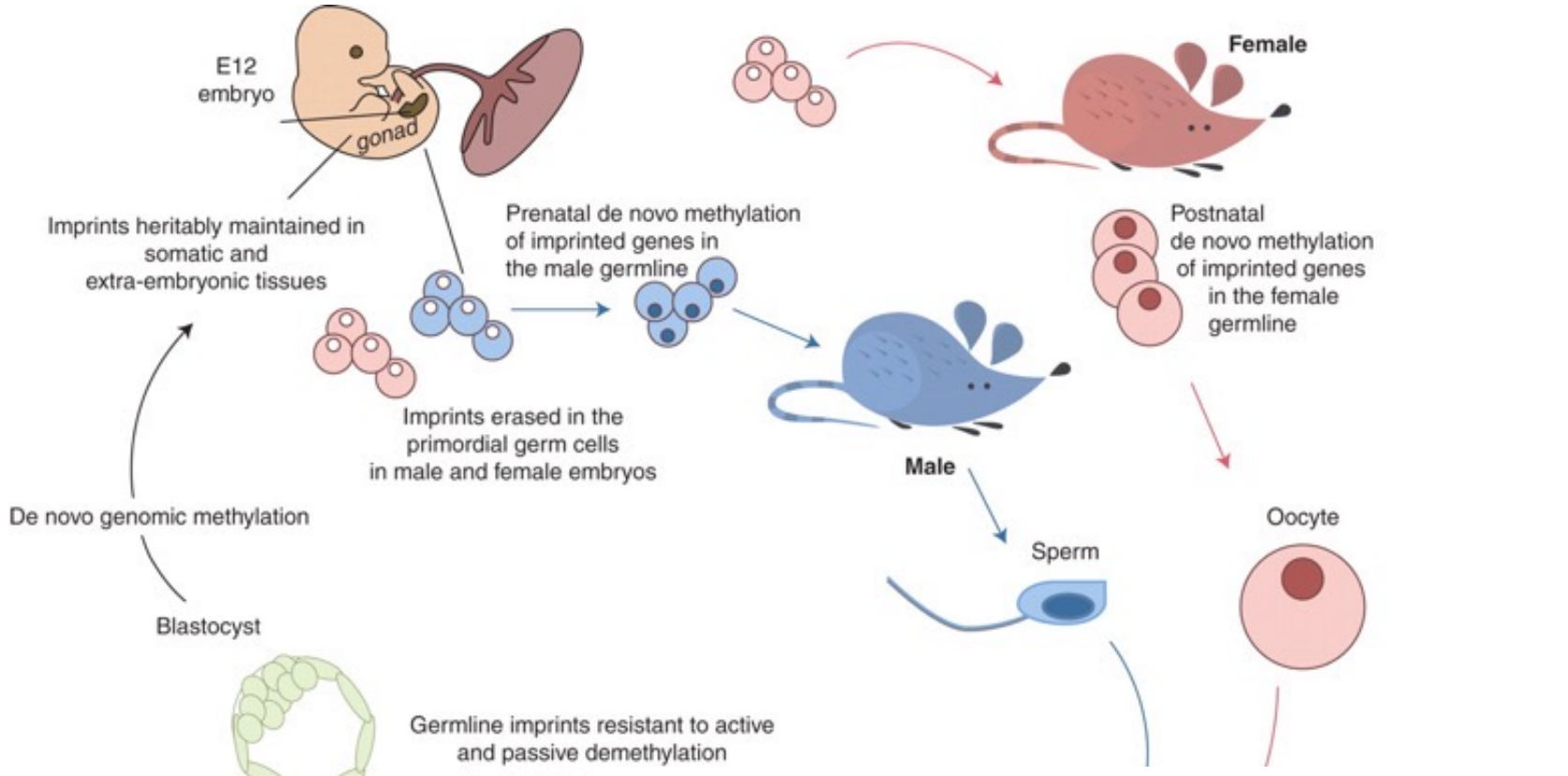



Figure 4. The conflict hypothesis suggests that imprinting arose because of a genomic tug-of-war between mothers and fathers over the use of maternal resources by the fetus. In mammals that bear live offspring, the male's evolutionary fitness is maximized if his offspring monopolizes the female's energy reserves during gestation. The female's best strategy demands that she not invest all of her resources in a single offspring. If the embryo were a car on the highway of growth and development, paternal imprinting would try to speed the car up; maternal imprinting would try to slow it down.

# Epigenetic effects on phenotype: imprinting

A major mechanism for this “imprinting” difference is epigenetic methylation of the genomic DNA during gametogenesis




REVIEW

## Genomic imprinting in plants—revisiting existing models

Rita A. Batista and Claudia Köhler

Department of Plant Biology, Uppsala BioCenter, Swedish University of Agricultural Sciences and Linnean Centre for Plant Biology, Uppsala SE-750 07, Sweden

GENES & DEVELOPMENT 34:24–36 (2020)

# Many epigenetic effects on phenotype

- Epigenetic changes induced by maternal behavior
- Epigenetic effects of early stress in humans
- Epigenetics in cognition
- Epigenetic effects of environmental chemicals
- Epigenetic effects in monozygotic twins
- Transgenerational epigenetic effects on metabolism

# Transgenerational epigenetic effects

Records of food shortages/excesses and health records over many years in Sweden…. Link between famine in parent and obesity in offspring…

The “thrifty phenotype” hypothesis- what is it?

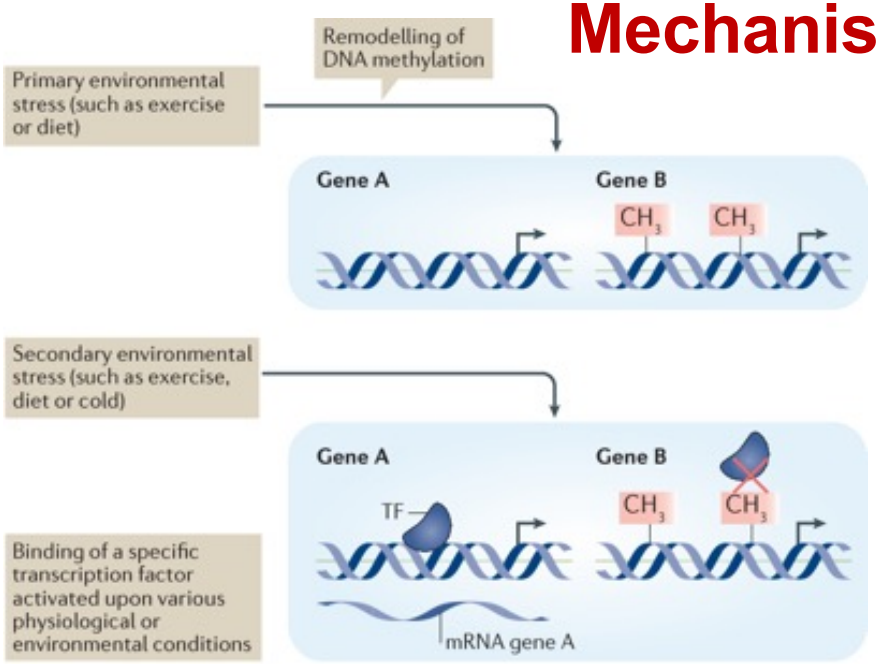
Food deprivation in utero → efficiently store excess calories

Also seen in Dutch famine in WWII:

Offspring of women who were pregnant during the “hunger winter” had increased metabolic and cardiovascular disease. Also, they found increased neonatal adiposity among the grandchildren of these women.

https://doi.org/10.1111/1471-0528.12136

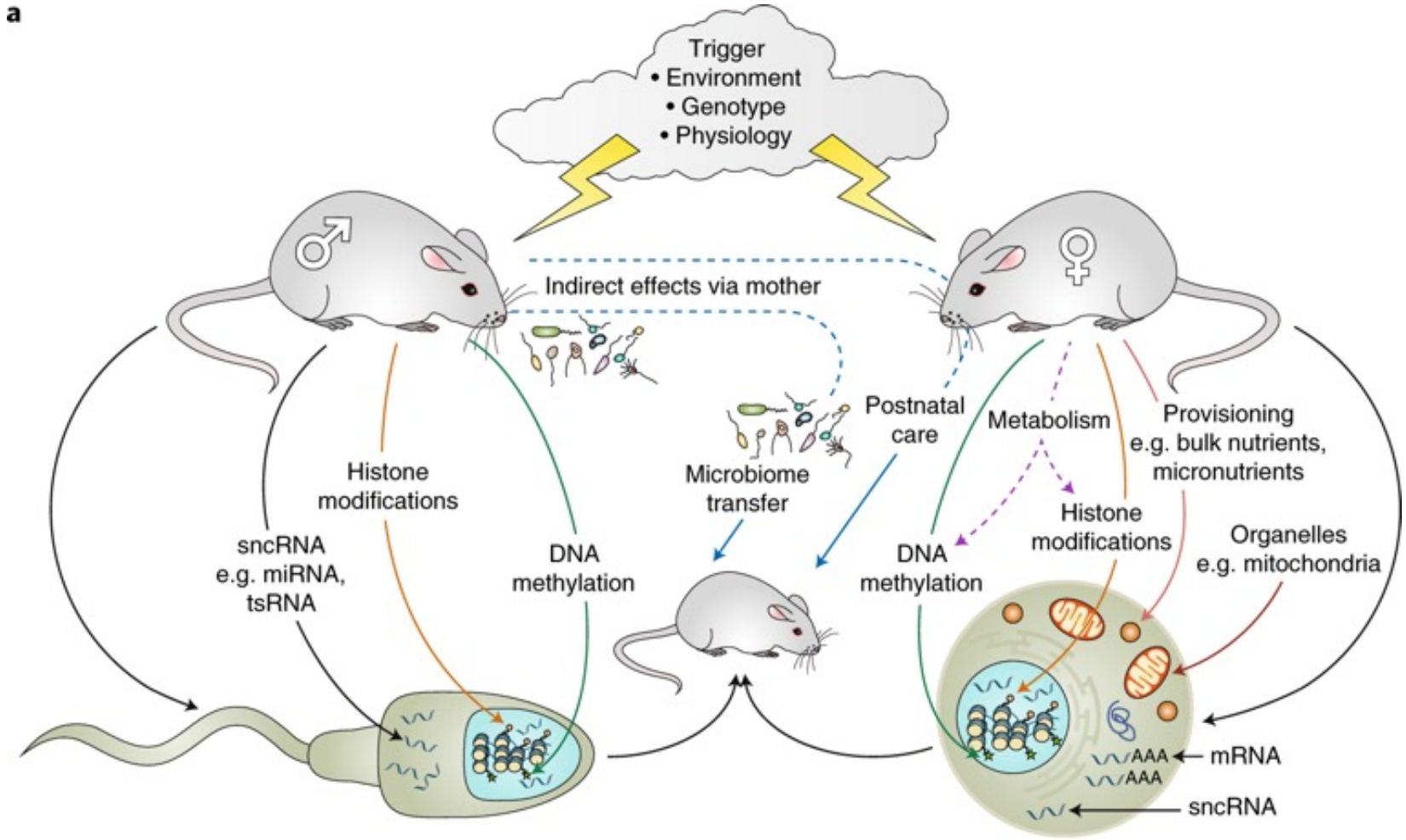
**Mechanism?**




Published in Nature Reviews Endocrinology 2016
The role of diet and exercise in the transgenerational epigenetic landscape of T2DM R. Barrès, J. Zierath

# Transgenerational epigenetic effects

## many possible mechanisms

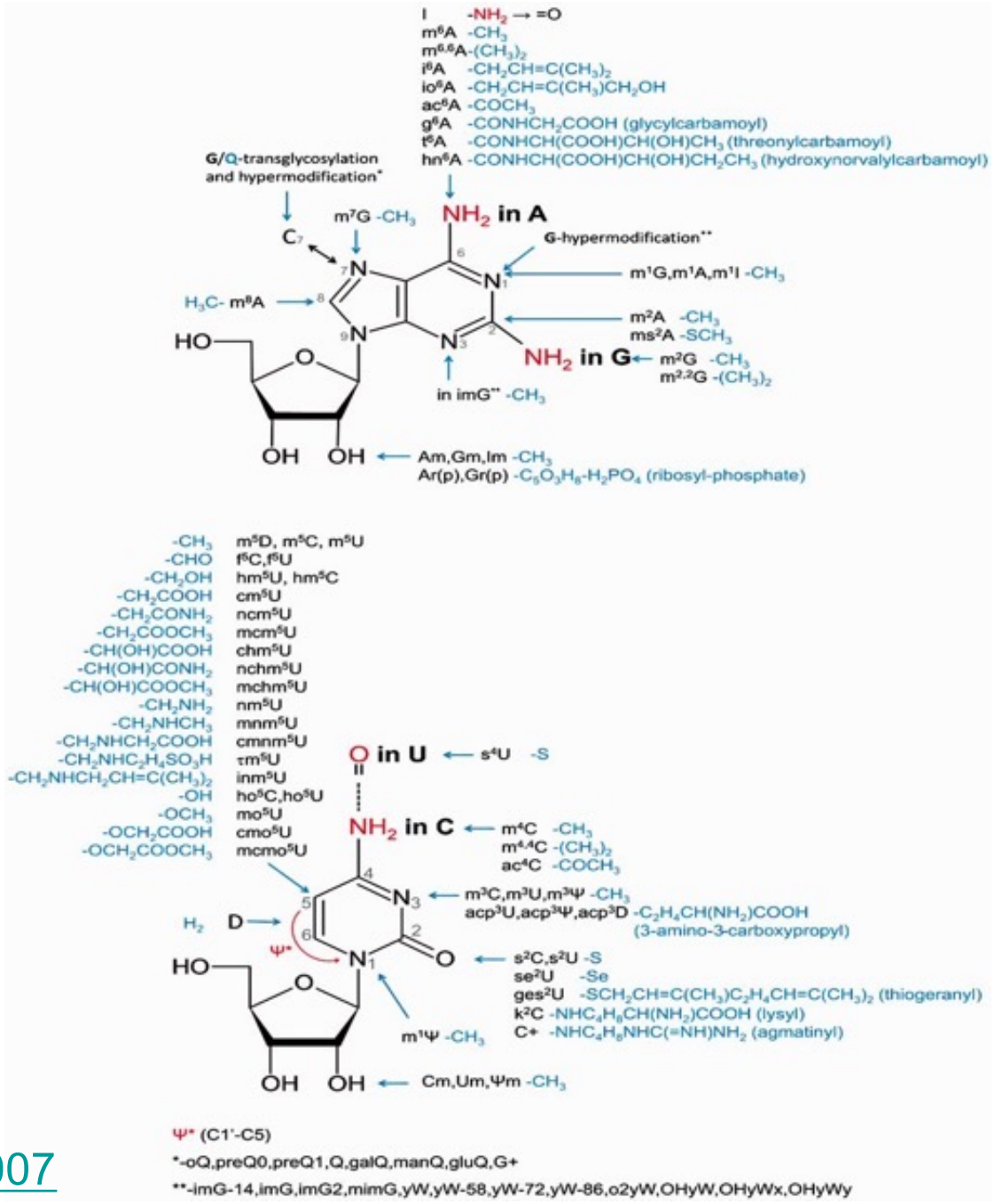



*Perez and Lehner, Nature Cell Biology*
**volume 21**, pages143–151(2019)

# RNA can be epigenetically modified!

**“Epitranscriptomics”**

**There are over 170 known modifications to RNAs that have been identified**




https://doi.org/10.1093/nar/gks1007

# RNA can be epigenetically modified!

## Effect of RNA Modifications on RNA function

a) Protein binding
b) Stability
c) RNA translation
d) RNA folding

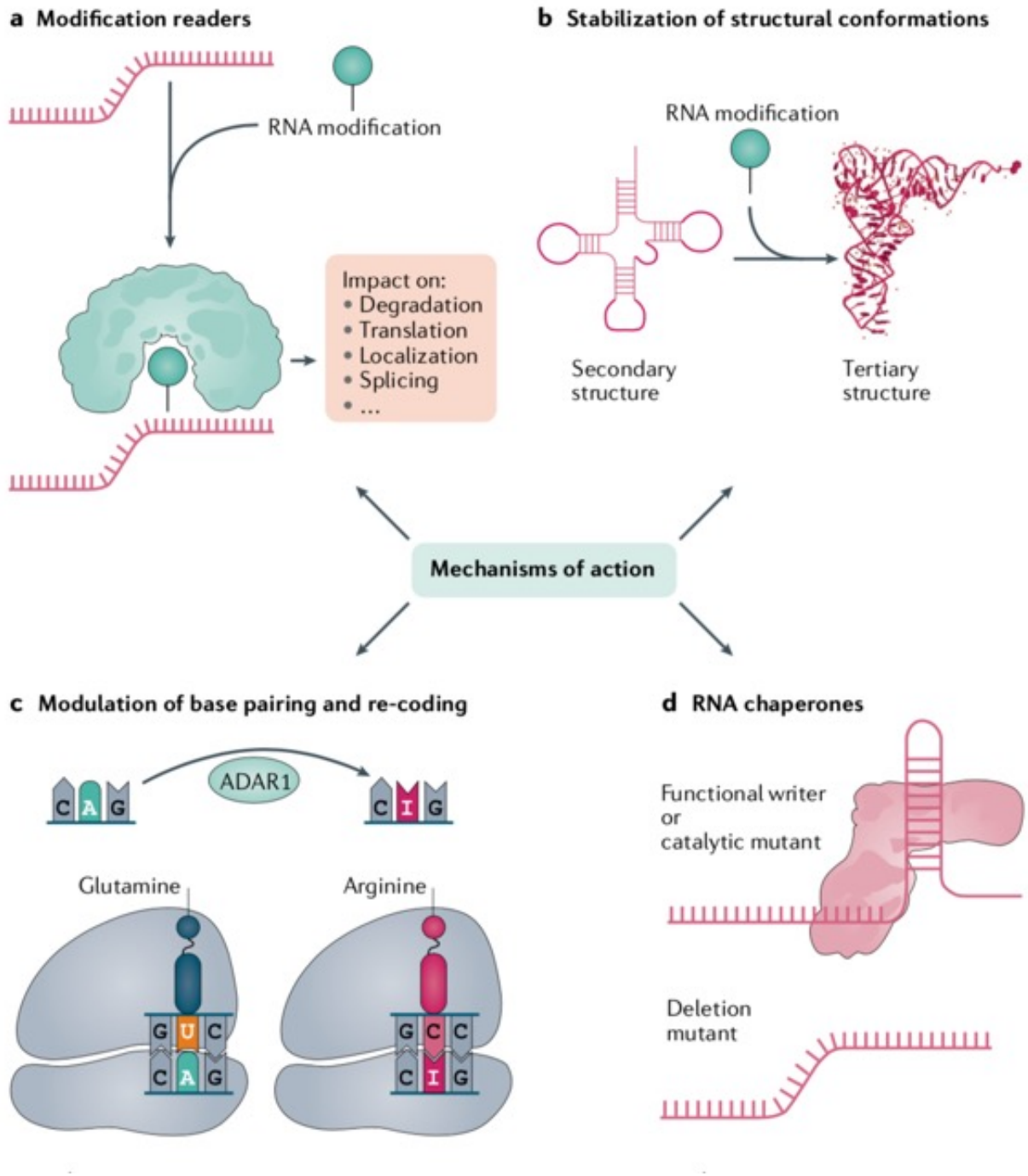



Fig. 4 | **Mechanisms through which RNA modifications can act. a** | A specialized protein ('reader') specifically binds the modification and triggers different molecular phenotypes downstream. **b** | The chemical properties of the modification help to stabilize a specific RNA structure. **c** | The modification leads to altered Watson–Crick interactions, which can lead, for example, to a difference in the decoding of the modified base by the translational machinery. This is illustrated for an adenosine base that can be edited into inosine, which will be decoded as a guanosine. **d** | The modification 'writer' serves as a chaperone for proper folding of the RNA. The modification itself is not strictly required for such chaperoning activity and, as a consequence, catalytic mutants lacking the modification will allow similar folding activity as the wild type but different than in a full deletion of the modification writer.

Nature Reviews Genetics 22: 119 (2021)

# Functional Genomics Projects in Animals and Plants

Andersson et al. Genome Biology (2015) 16:57
DOI 10.1186/s13059-015-0622-4

Genome Biology

OPEN LETTER Open Access

## Coordinated international action to accelerate genome-to-phenome with FAANG, the Functional Annotation of Animal Genomes project

The FAANG Consortium, Leif Andersson[1,2], Alan L Archibald[3], Cynthia D Bottema[4], Rudiger Brauning[5], Shane C Burgess[6], Dave W Burt[3], Eduardo Casas[7], Hans H Cheng[8], Laura Clarke[9], Christine Couldrey[10], Brian P Dalrymple[11], Christine G Elsik[12], Sylvain Foissac[13], Elisabetta Giuffra[14*], Martien A Groenen[15], Ben J Hayes[16,17,18], LuSheng S Huang[19], Hassan Khatib[20], James W Kijas[11], Heebal Kim[21], Joan K Lunney[22], Fiona M McCarthy[23], John C McEwan[24], Stephen Moore[25], Bindu Nanduri[26], Cedric Notredame[27], Yniv Palti[28], Graham S Plastow[29], James M Reecy[30], Gary A Rohrer[31], Elena Sarropoulou[32], Carl J Schmidt[33], Jeffrey Silverstein[34], Ross L Tellam[35], Michele Tix[illegible], Stephen N White[37,38], Shuh[illegible]

*Annual Review of Animal Biosciences*

## Functional Annotation of Animal Genomes (FAANG): Current Achievements and Roadmap

Elisabetta Giuffra,[1] Christopher K. Tuggle,[2] and the FAANG Consortium*

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ARTICLE

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## Functional annotations of three domestic animal genomes provide vital resources for comparative and agricultural research

Colin Kern[1], Ying Wang[1], Xiaoqin Xu[1], Zhangyuan Pan[1], Michelle Halstead[1], Ganrea Chanthavixay[1], Perot Saelao[1], Susan Waters[1], Ruidong Xiang[2,3], Amanda Chamberlain[3], Ian Korf[4], Mary E. Delany[1], Hans H. Cheng[5], Juan F. Medrano[1], Alison L. Van Eenennaam[1], Chris K. Tuggle[6], Catherine Ernst[7], Paul Flicek[8], Gerald Quon[9], Pablo Ross[1✉] & Huaijun Zhou[1✉]

## pENCODE: A Plant Encyclopedia of DNA Elements

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Amanda K. Lane,[1] Chad E. Niederhuth,[1] Lexiang Ji,[1,2] and Robert J. Schmitz[1,2]
[1]Department of Genetics, University of Georgia, Athens, Georgia 30602; email: schmitz@uga.edu
[2]Institute of Bioinformatics, University of Georgia, Athens, Georgia 30602

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REVIEW PAPER

## Epigenetics: possible applications in climate-smart crop breeding

Serena Varotto[1], Eleni Tani[2], Eleni Abraham[3], Tamar Krugman[4], Aliki Kapazoglou[5], Rainer Melzer[6], Aleksandra Radanović[7] and Dragana Miladinović[7,*]

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## Integrative analysis of reference epigenomes in 20 rice varieties

Lun Zhao, Liang Xie, Qing Zhang, Weizhi Ouyang, Li Deng, Pengpeng Guan, Meng Ma, Yue Li, Ying Zhang, Qin Xiao, Jingwen Zhang, Hongmeijuan Li, Shunyao Wang, Jiangwei Man, Zhilin Cao, Qinghua Zhang, Qifa Zhang, Guoliang Li ✉ & Xingwang Li ✉

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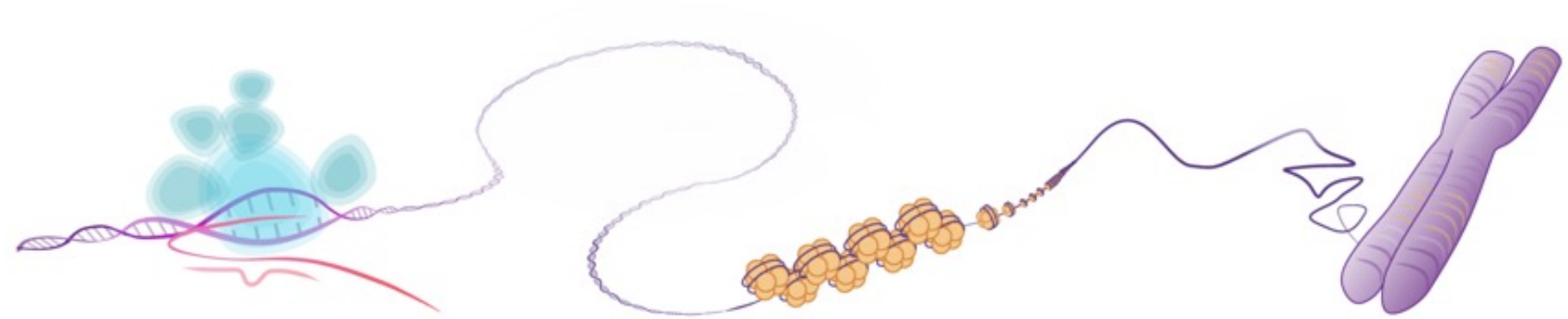
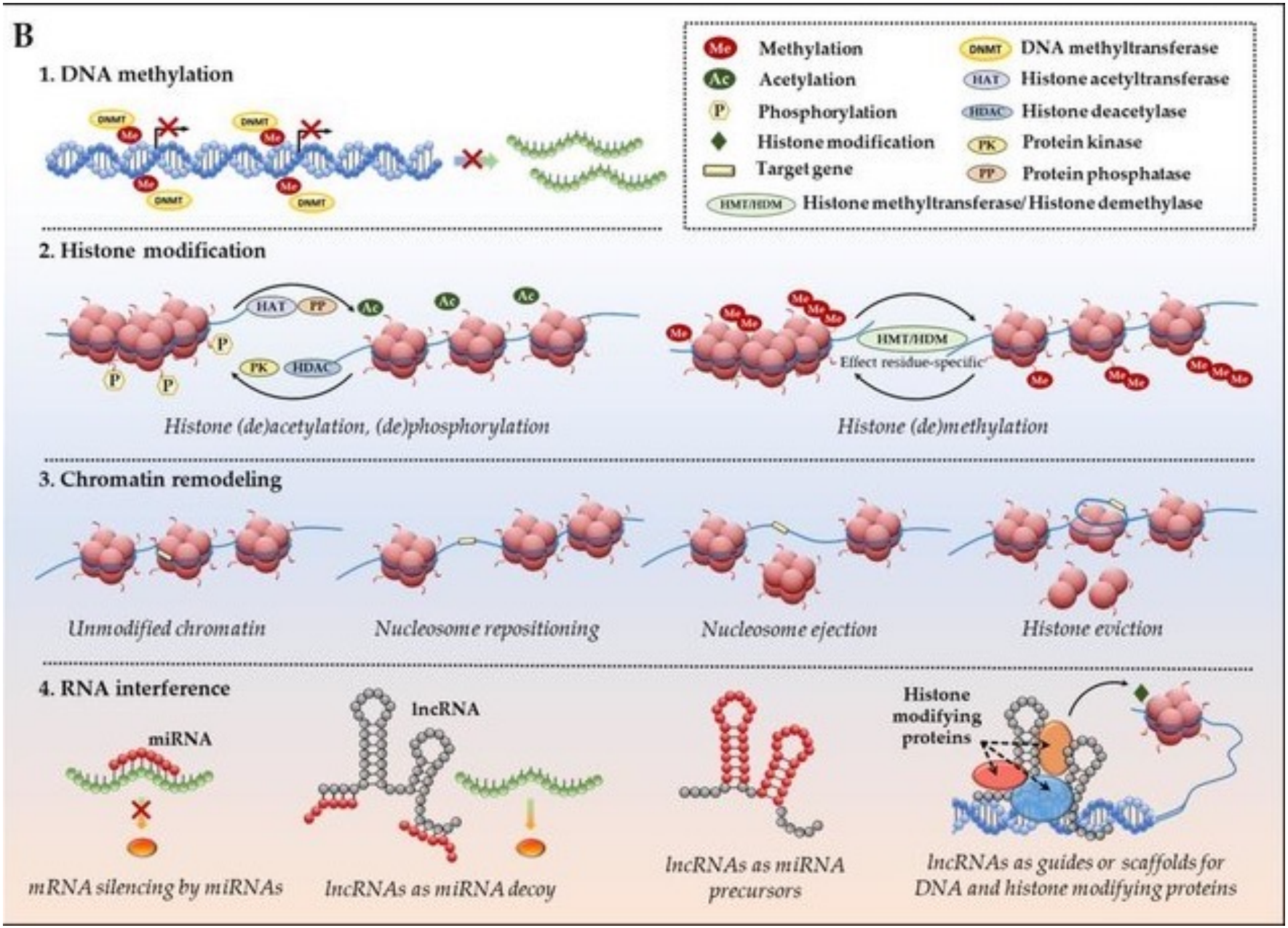
# Resources for epigenetics

- Nature reviews genetics series on epigenetics:
- https://www.nature.com/collections/jgywtpkhrv/
- “Techniques that interrogate DNA methylation, histone modifications or chromatin conformations are enabling unprecedented analyses of epigenomes, including at the single-cell level. The articles in this series discuss insights into chromatin biology and the components and mechanisms of diverse epigenetic processes in health and disease, from fundamental roles in gene regulation to broader biological phenomena such as reprogramming, imprinting and transgenerational inheritance.”



https://www.activemotif.com/epigenetics-101

# Many molecular modifications affect genome function




https://www.researchgate.net/publication/327326465/figure/fig2/AS:665497179791362@1535678300469/Epigenetic-regulation-of-gene-expression-A-DNA-packing-in-a-eukaryotic-cell-a-DNA_W640.jpg

# Epigenetics Concept Summary

- Epigenetic effects—inherited changes in gene expression not due to changes in the DNA base sequence—are frequently caused by DNA methylation and alterations in chromatin structure. Epigenetic changes are stable but can be affected by environmental factors.
- Many epigenetic phenotypes result from changes to chromatin structure. Epigenetic effects occur through DNA methylation, histone modifications, and RNA molecules.
- Early life experiences can produce epigenetic changes that have long-lasting effects on behavior. Environmental chemicals may produce epigenetic effects that are passed to later generations. Phenotypic differences between genetically identical monozygotic twins may result from epigenetic effects.
- The epigenome is the complete set of chromatin modifications possessed by an individual organism.

# Discussion Questions

1. If the genetic information is the same, why are a brain and a kidney (or a root and a flower petal) so different?

2. Describe two different ways genes are activated and two ways they are silenced.

3. What is the basis for genomic imprinting, and why does it occur?

4. How can your grandfather's diet affect your health?