Michael J Meaney PhD, FRSC, CQ, CM

Sackler Program for Epigenetics & Psychobiology
Ludmer Centre for Neuroinformatics & Mental Health
Douglas Mental Health Institute
McGill University
and
Singapore Institute for Clinical Sciences

The Integration of 'Big Data' Science and Informatics into the Study of Child Development

Sackler Program For Epigenetics and Psychobioogy

Patricia Silveira MD/PhD
Assistant Professor
Dept. Psychiatry
McGill University

Kieran O'Donnell PhD
Assistant Professor
Dept. Psychiatry
McGill University
CIFAR, Global Scholar

Tie-Yuan Zhang PhD
Assistant Professor
Dept. Psychiatry
McGill University

McGill

How are epigenetic measures used:

1. EWAS: discover ‘critical’ marks that associate with specific exposures
2. Define biological mechanisms that explain enduring environmental effects
3. Biomarkers for health research
4. Inform on the process of biological embedding of ”experience”

Developmental Origins of Adult Disease

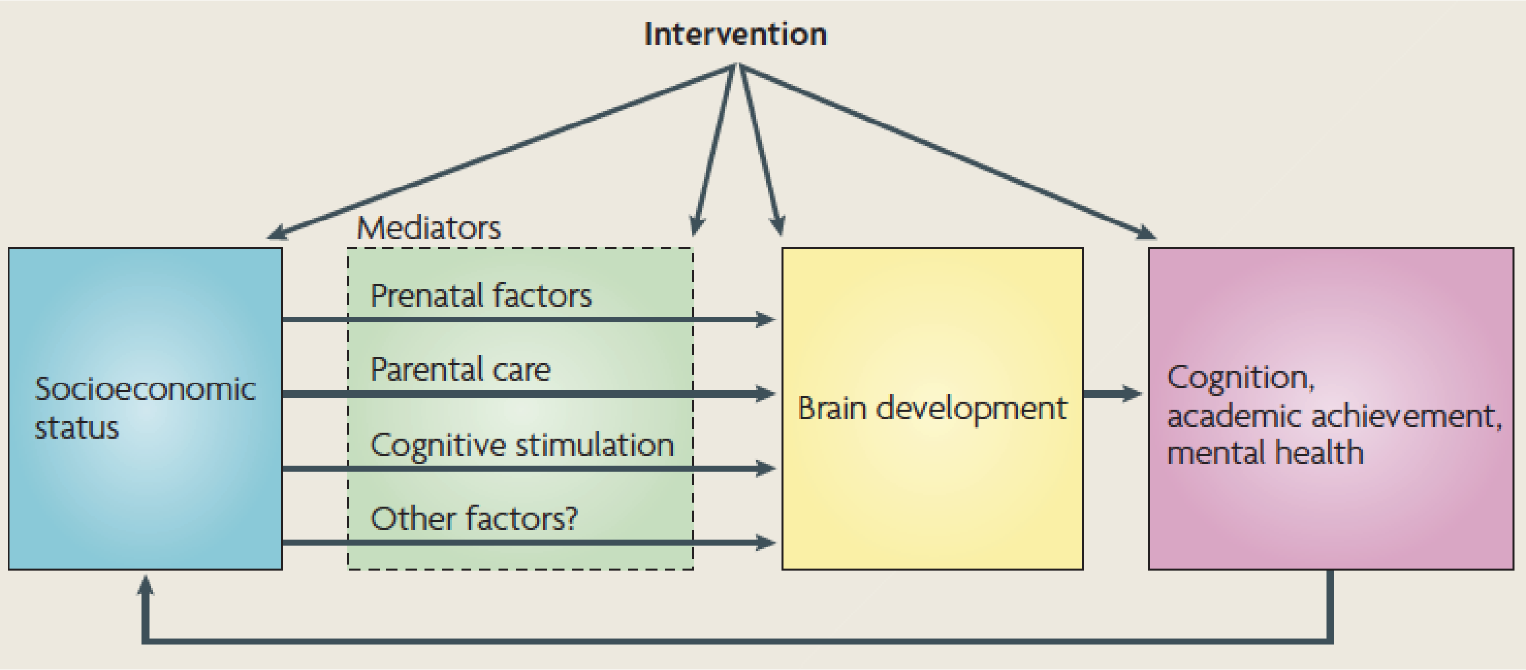
Early experience

- Abuse
- Family strife
- Emotional neglect
- Harsh discipline

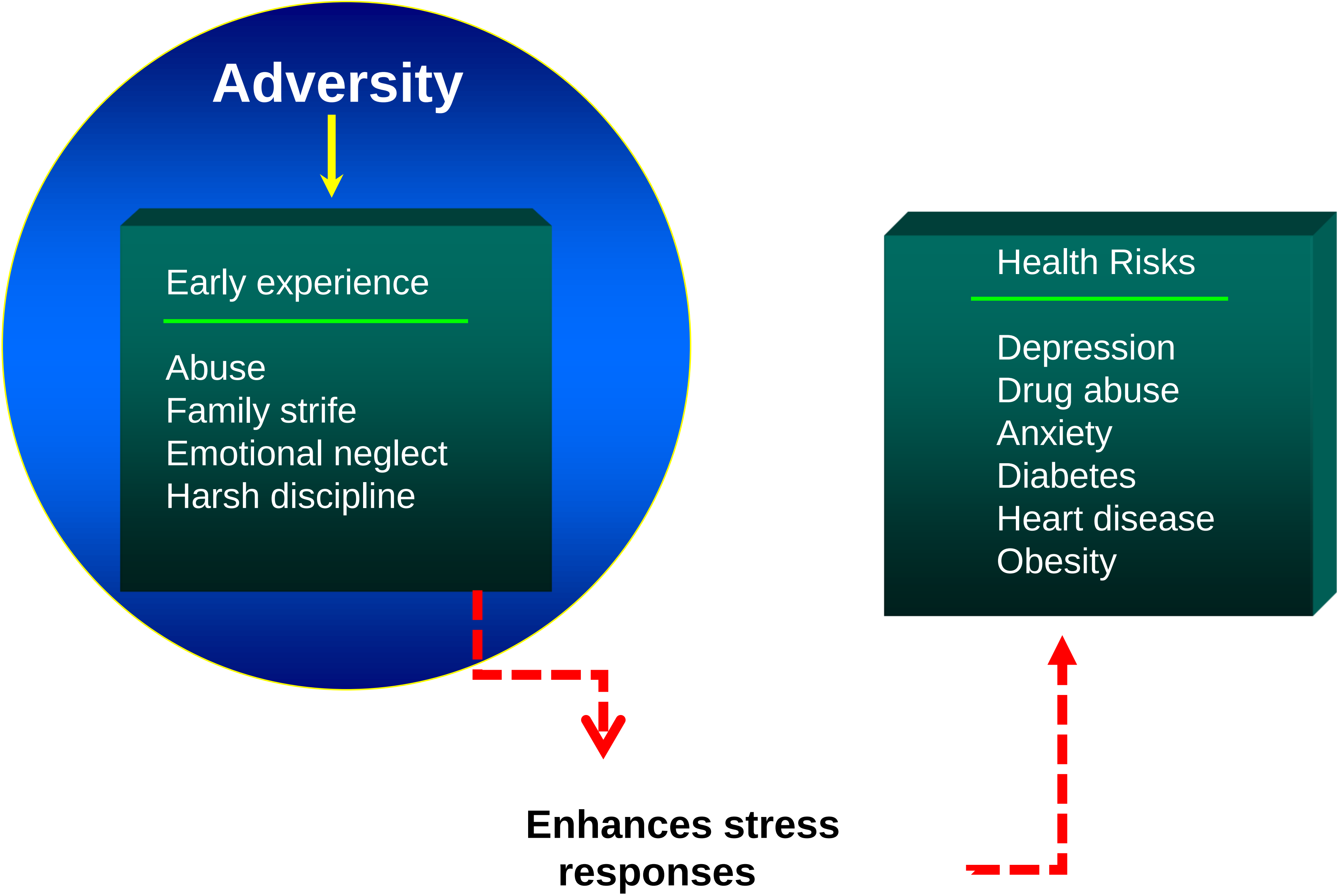
→

Health Risks

- Depression
- Drug abuse
- Anxiety
- Diabetes
- Heart disease
- Obesity



Hackman et al. Nature Reviews Neuroscience 2010



Adversity

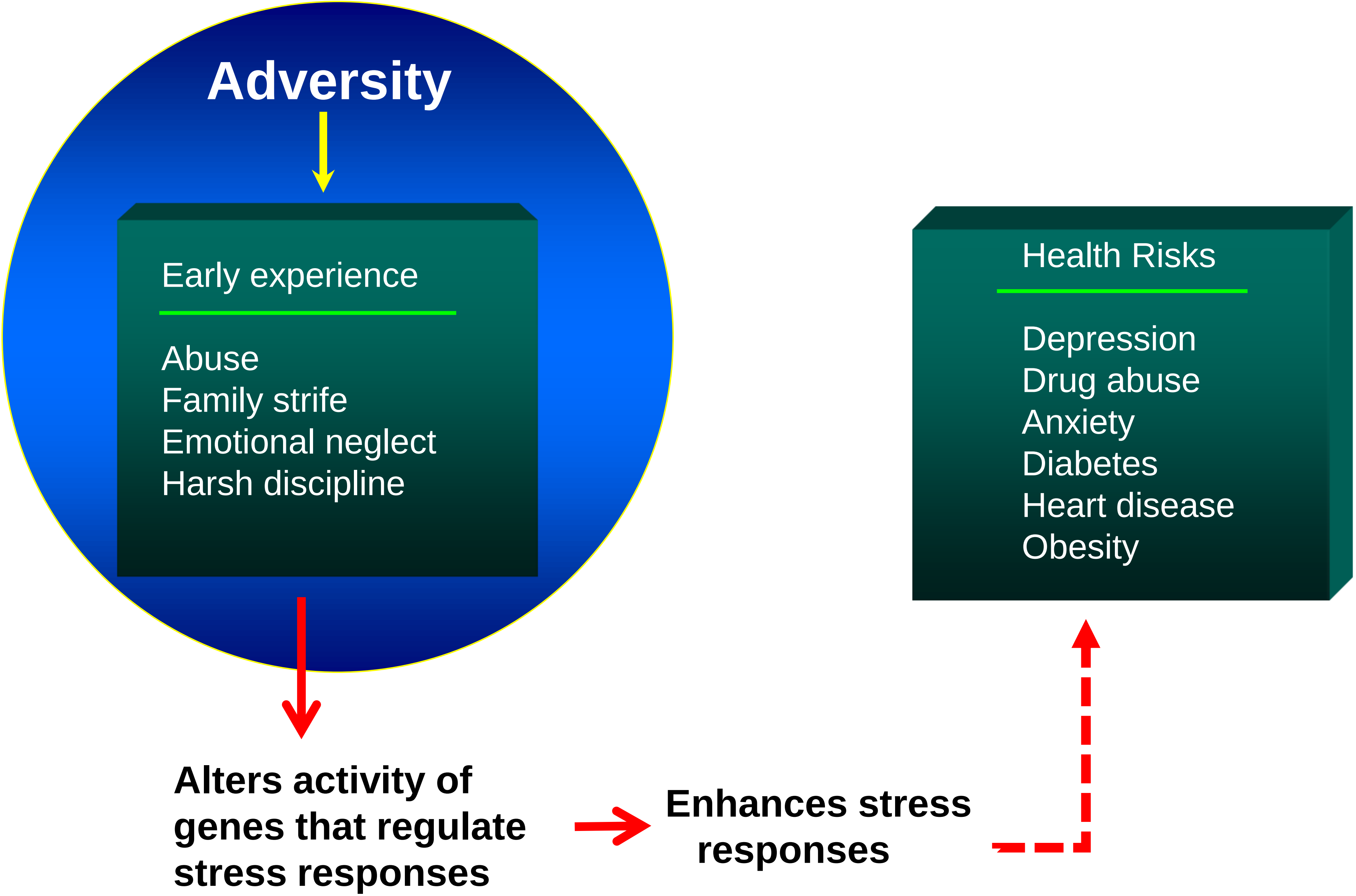
Early experience

Abuse
Family strife
Emotional neglect
Harsh discipline

Enhances stress responses

Health Risks

Depression
Drug abuse
Anxiety
Diabetes
Heart disease
Obesity



Adversity

Early experience

Abuse
Family strife
Emotional neglect
Harsh discipline

Health Risks

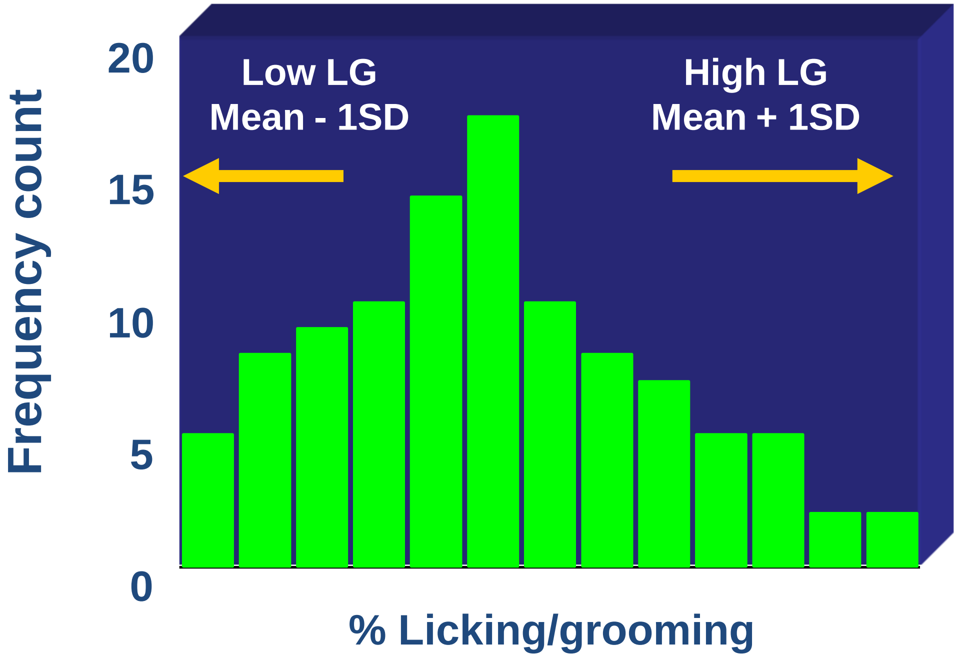
Depression
Drug abuse
Anxiety
Diabetes
Heart disease
Obesity

Alters activity of genes that regulate stress responses

Enhances stress responses

Maternal Care in Rat

Maternal Care in Rat



Broad range of parental effects

Stress responses

Neural development

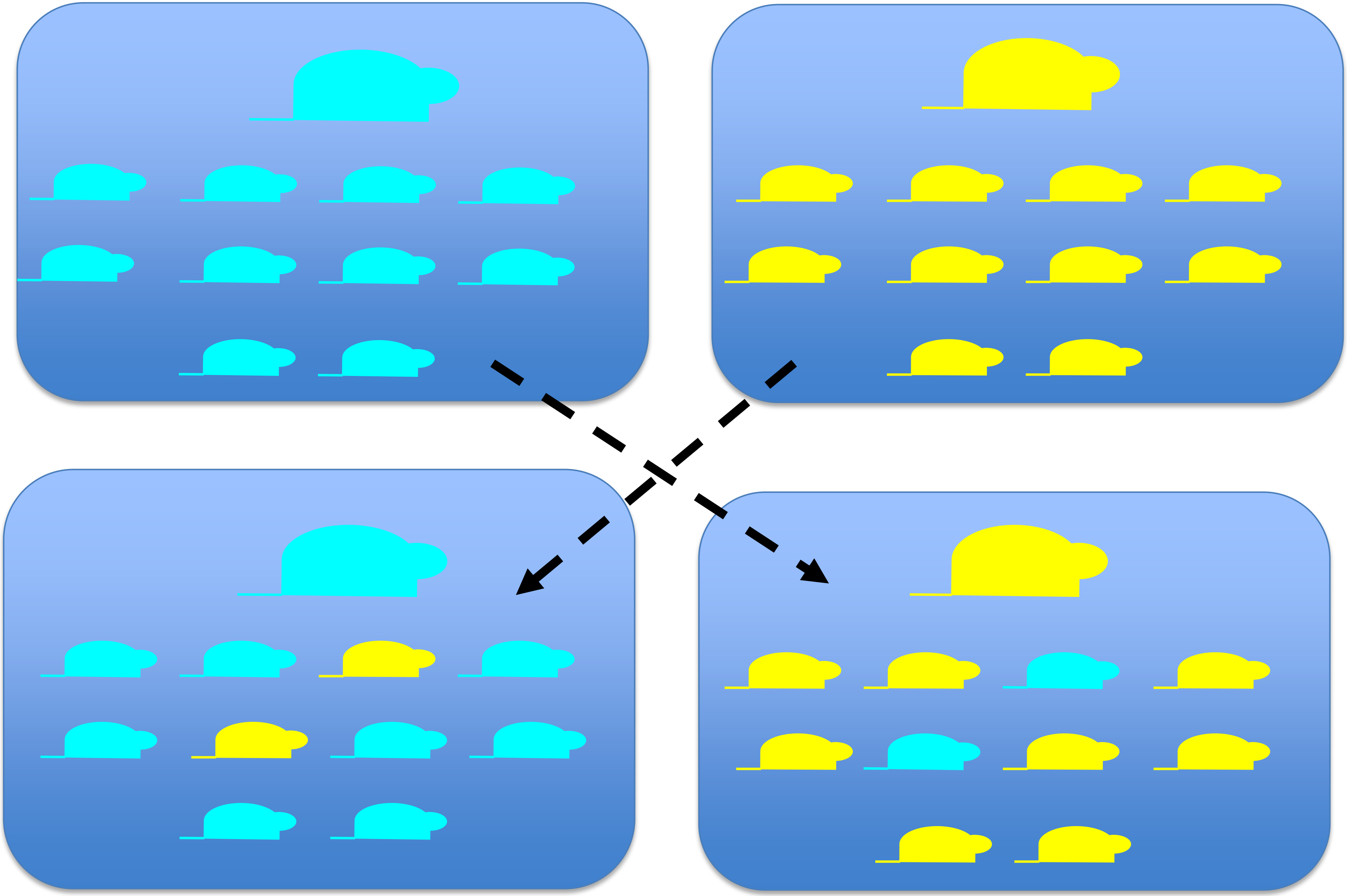
Learning & memory

Metabolism

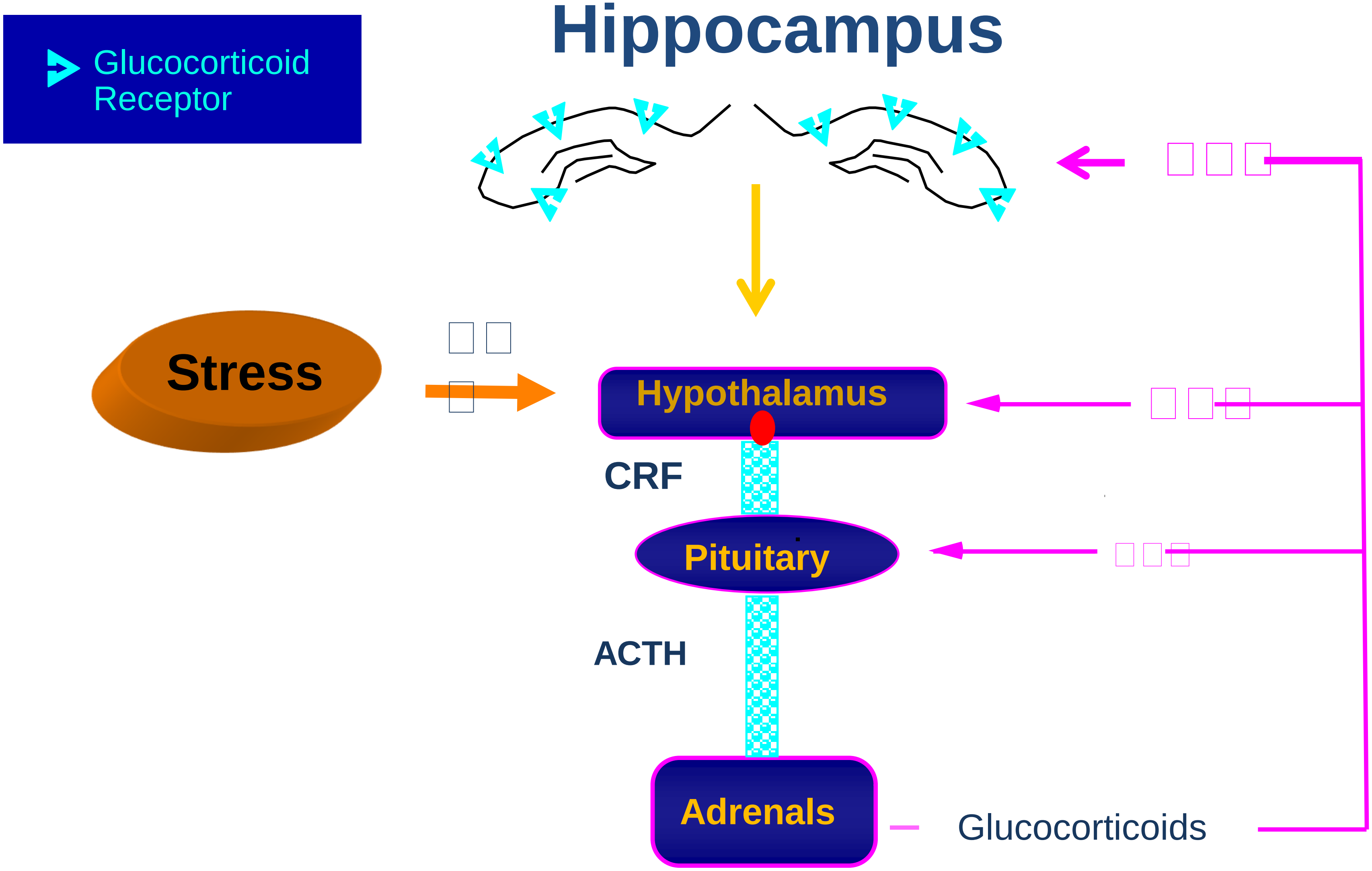
Reproduction (females)

High LG

Low LG



Hippocampus



Glucocorticoid Receptor

Stress

Hypothalamus

CRF

Pituitary

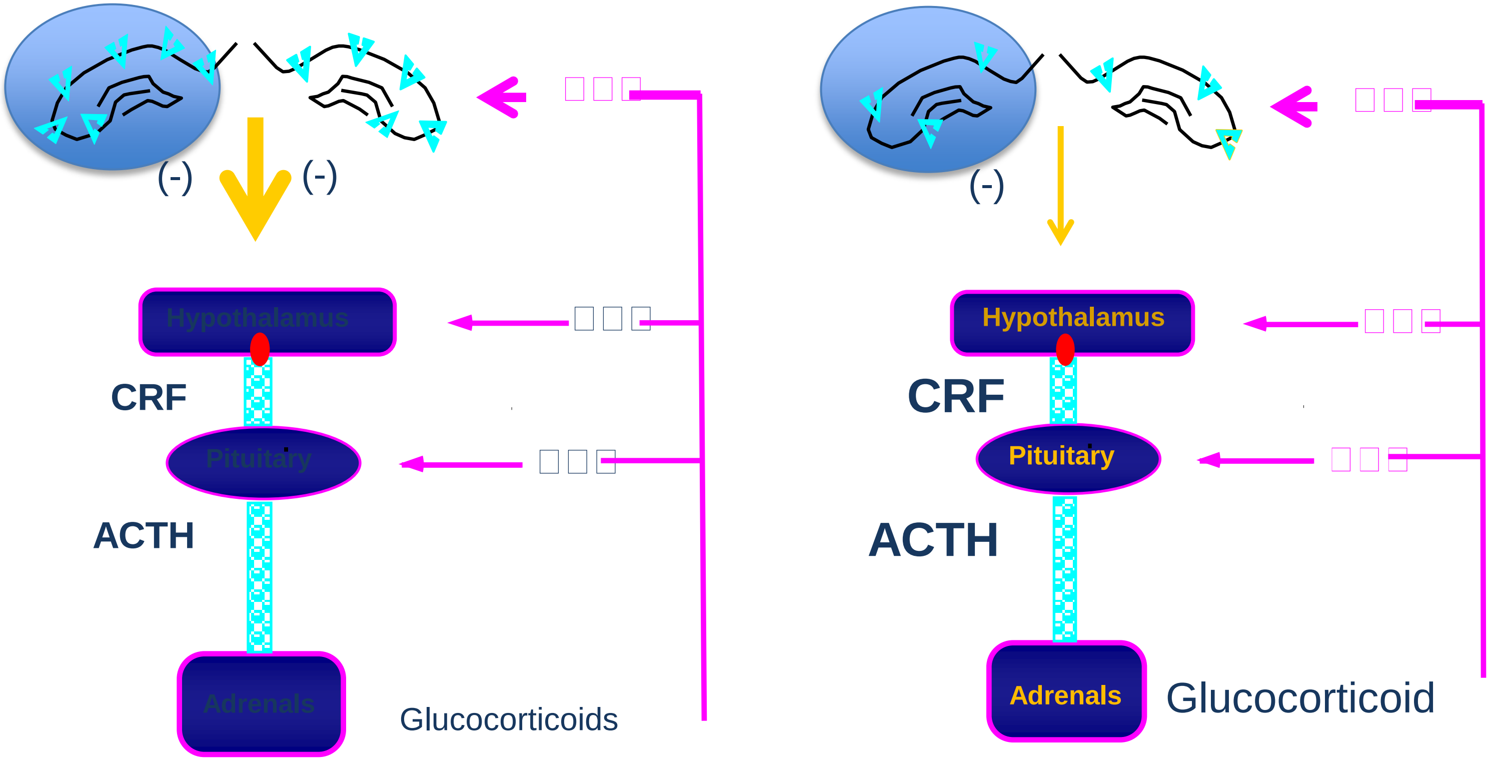
ACTH

Adrenals

Glucocorticoids

CRF: corticotropin releasing factor. ACTH: adrenocorticotropin

Individual differences in glucocorticoid receptor levels lead to altered pituitary-adrenal responses to stress



High LG

(-)

(-)

□□□

Hypothalamus

□□□

CRF

Pituitary

□□□

ACTH

Adrenals

Glucocorticoids

Low LG

(-)

□□□

Hypothalamus

□□□

CRF

Pituitary

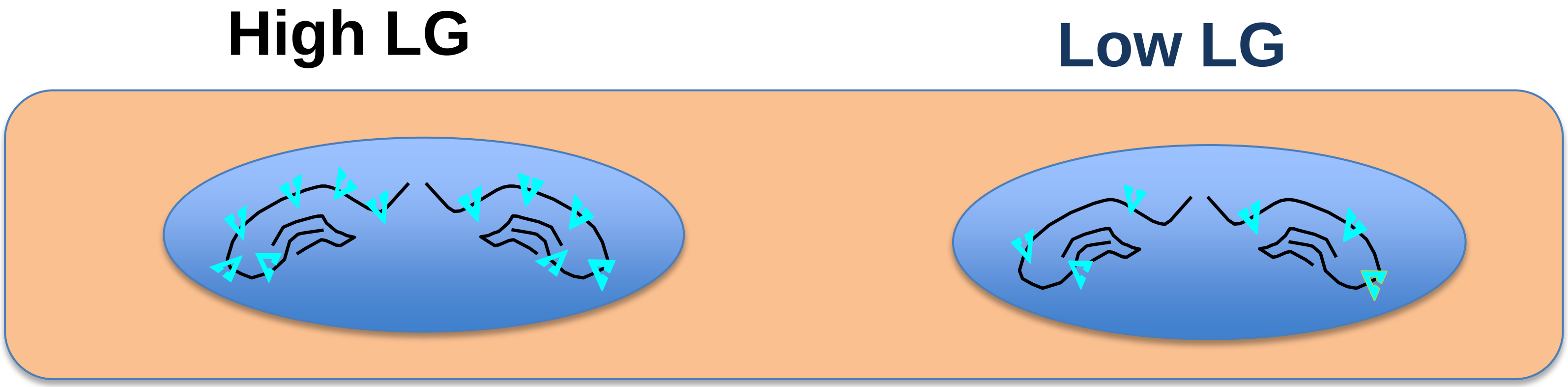
□□□

ACTH

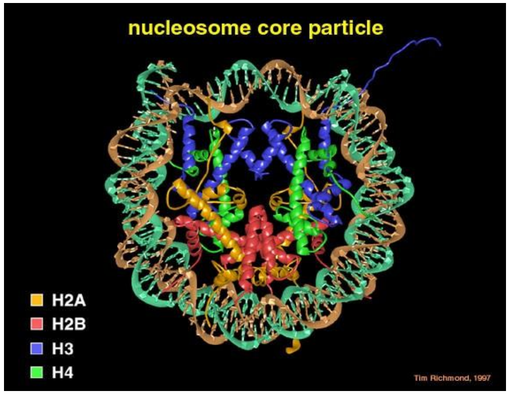
Adrenals

Glucocorticoid

How does maternal care over the first week of life stably alter the activity of the glucocorticoid receptor gene?



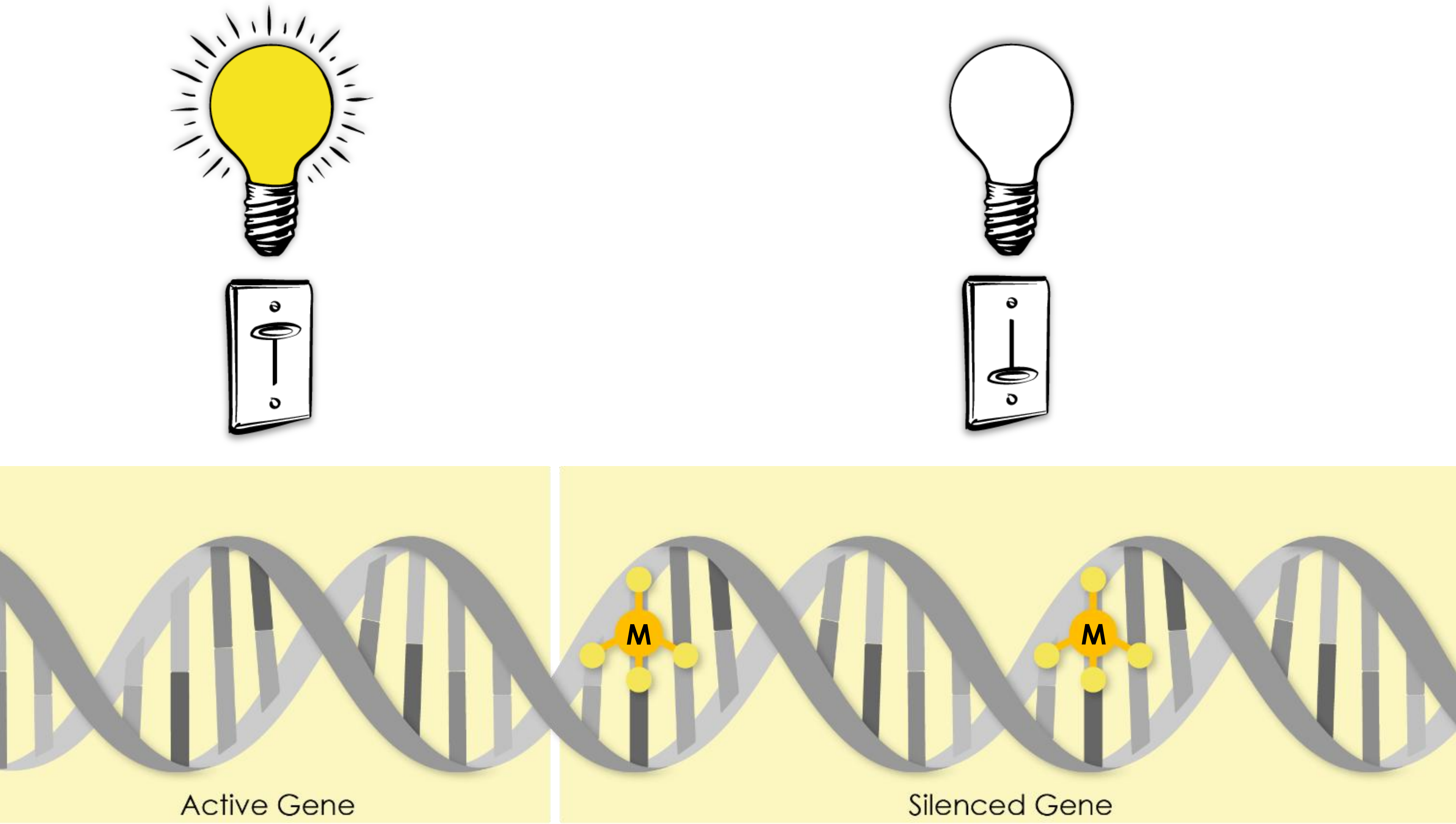
Parental signals as a source of phenotypic plasticity?



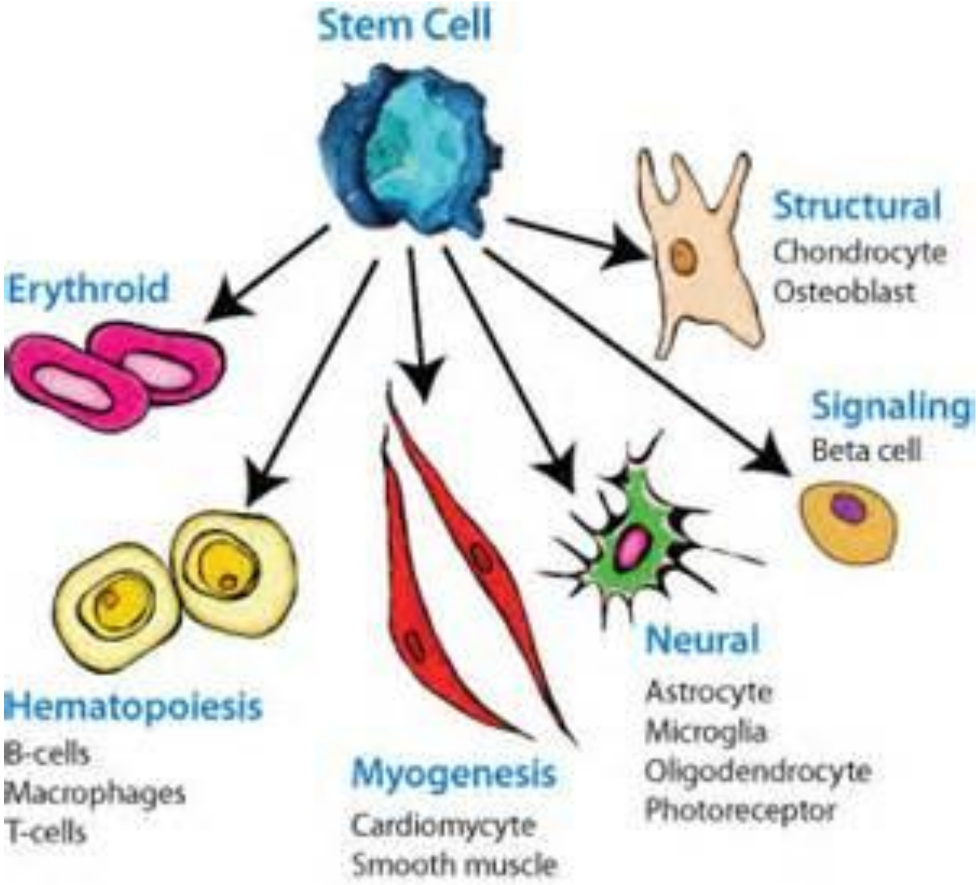
Parental care → Epigenetic marks → Gene expression → Phenotype

Environmental epigenetics hypothesis: Environmental events activate intracellular signals that remodel the epigenome, leading to sustained alterations in the structure and function of the genome, and stable effects on transcription.

DNA Methylation

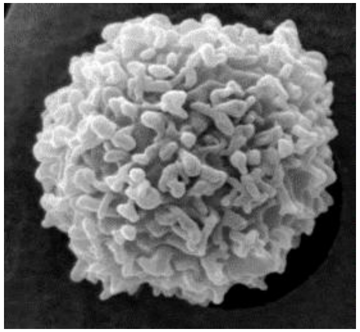


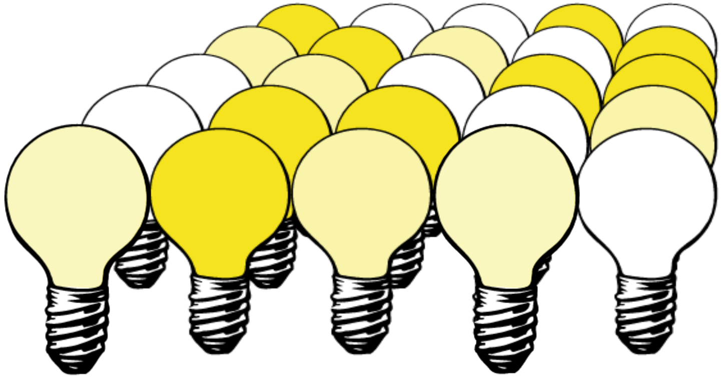
All tissues develop from a common DNA template

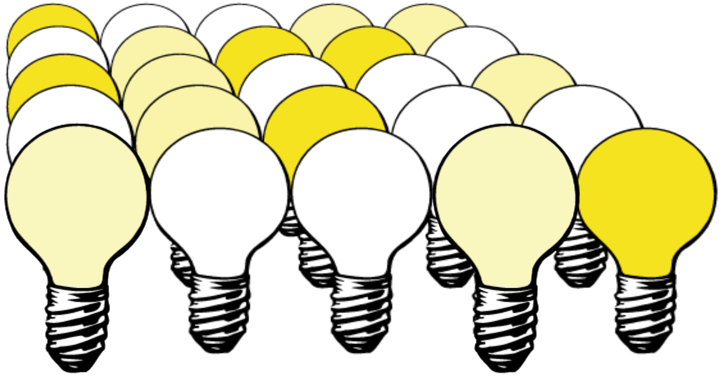


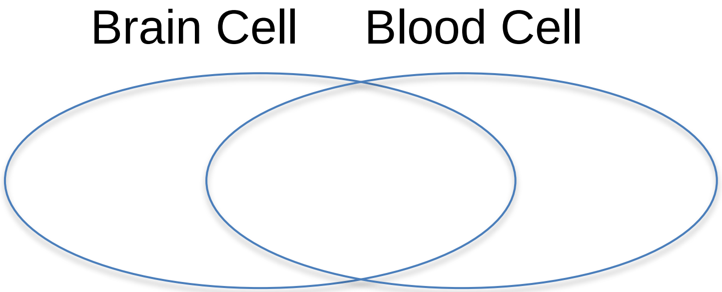
Brain Cell

Blood Cell

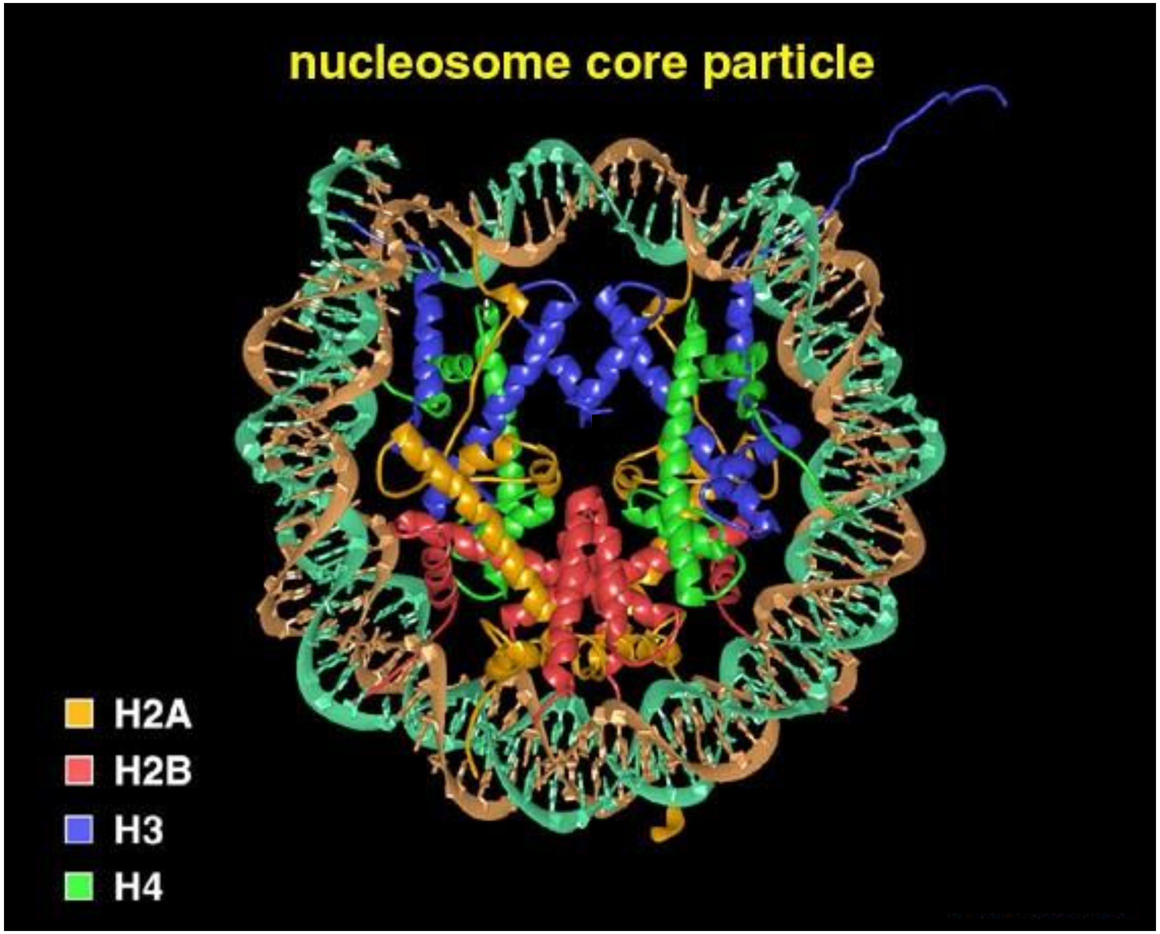








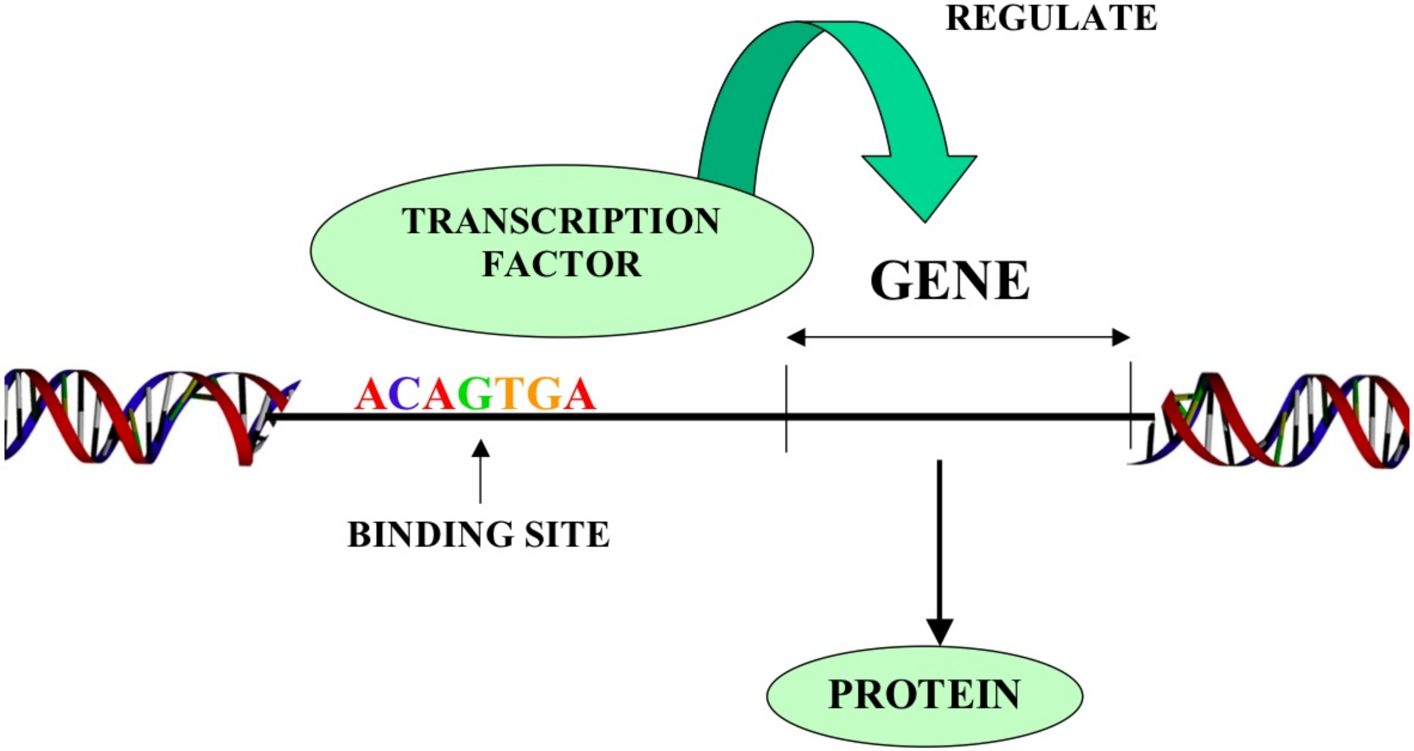
Expressed Genes



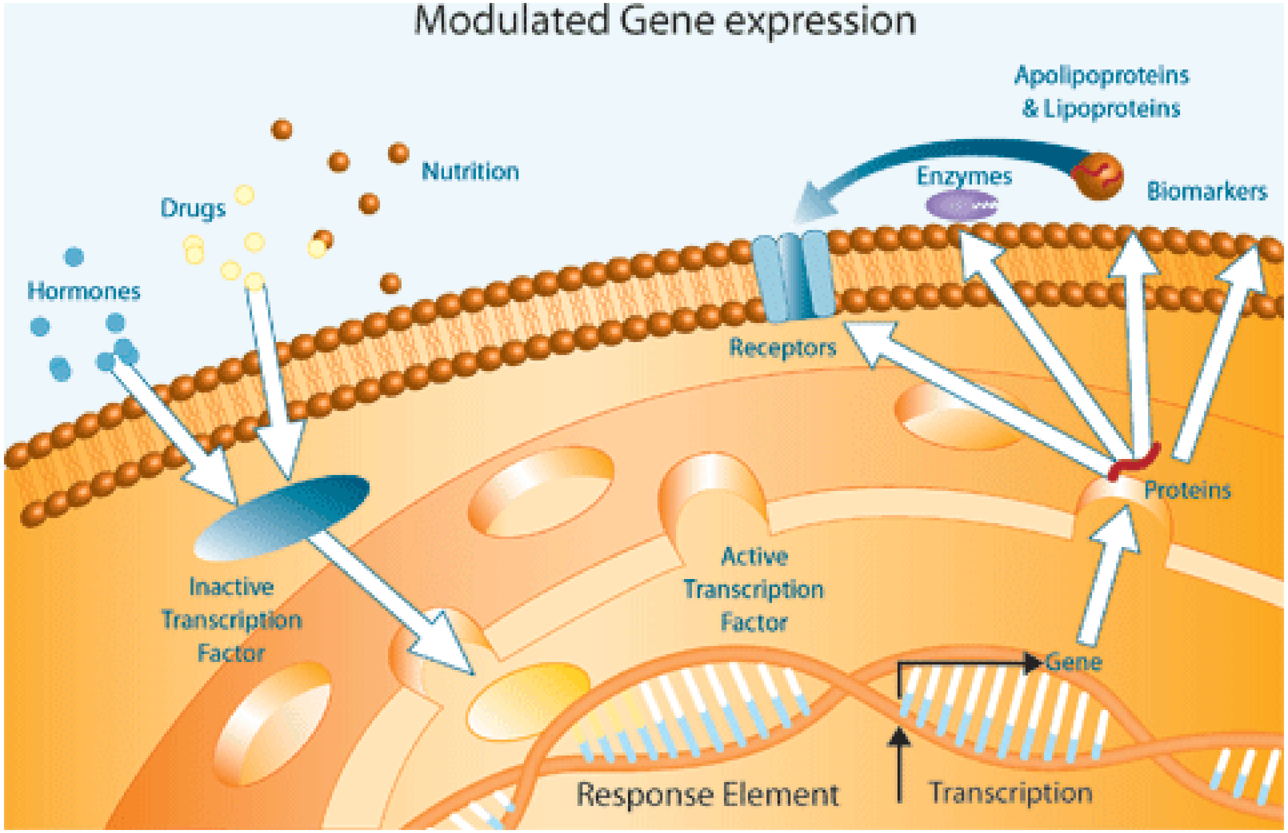
Prevents TF binding to DNA

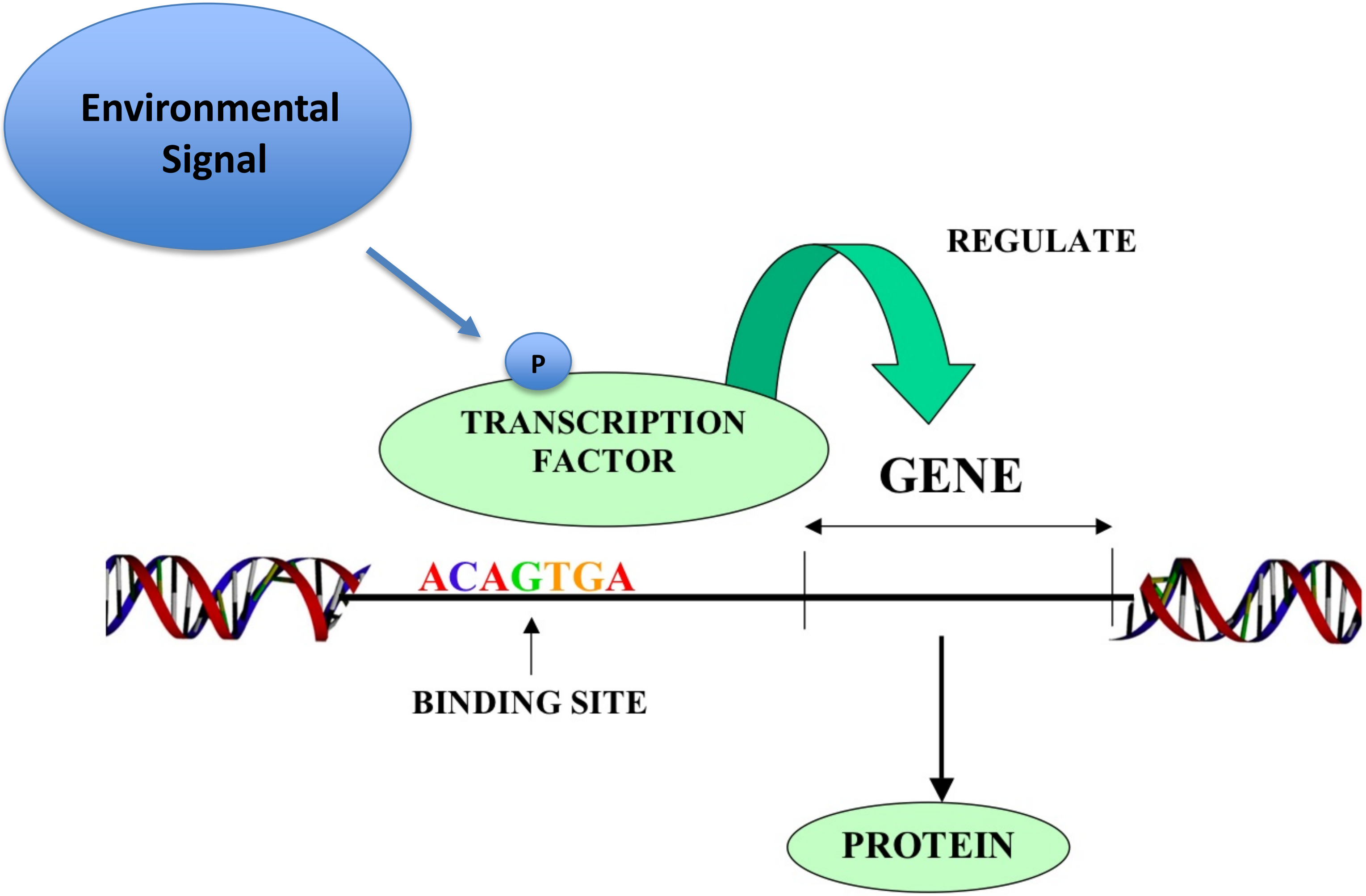
TF binding involves alteration of chromatin structure

Nucleosome core particle: ribbon traces for the 146-bp DNA phosphodiester backbones (brown and turquoise) and eight histone protein chains (Luger et al. Nature 1997).

REGULATE
TRANSCRIPTION FACTOR
GENE
ACAGTGA
BINDING SITE
PROTEIN

The activation of genes is a regulated process, influenced by “environmental” signals





Environmental Signal

P

TRANSCRIPTION FACTOR

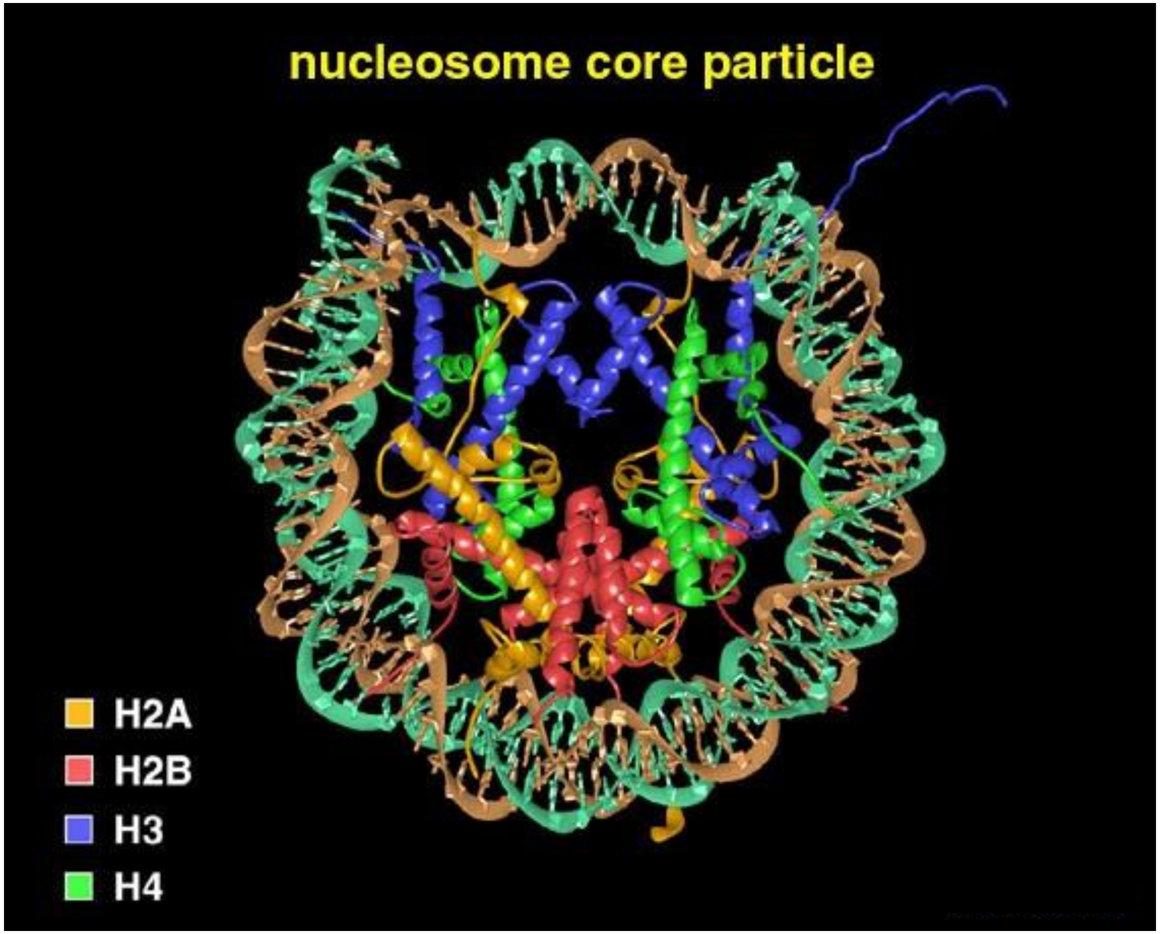
REGULATE

GENE

ACAGTGA

BINDING SITE

PROTEIN

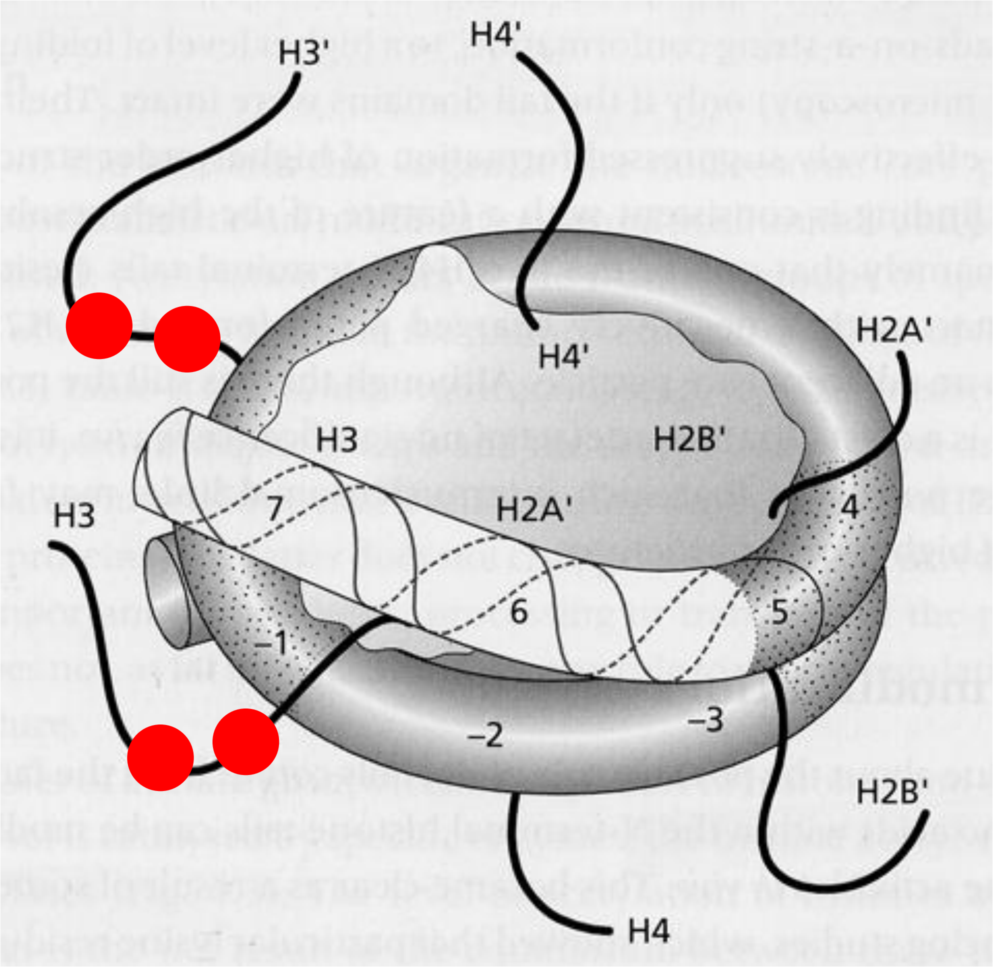


Prevents TF binding to DNA

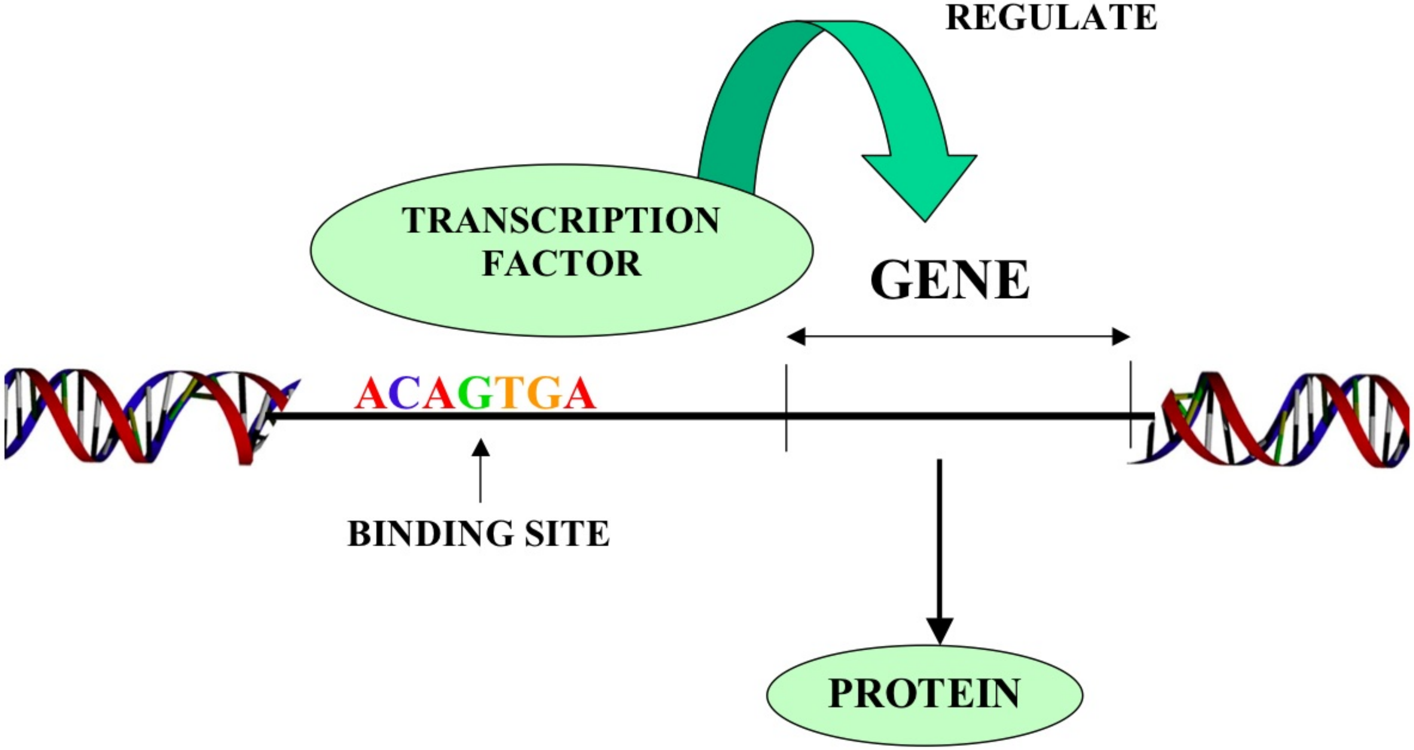
TF binding involves alteration of chromatin structure

Nucleosome core particle: ribbon traces for the 146-bp DNA phosphodiester backbones (brown and turquoise) and eight histone protein chains (Luger et al. Nature 1997).

● Acetyl group

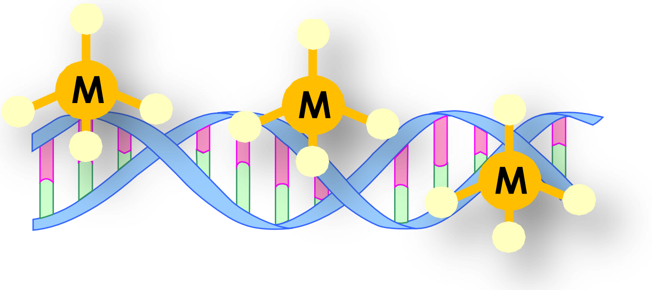


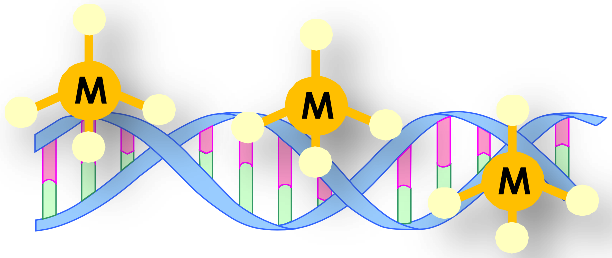
B. Turner. Chromatin structure and gene regulation. 2001.

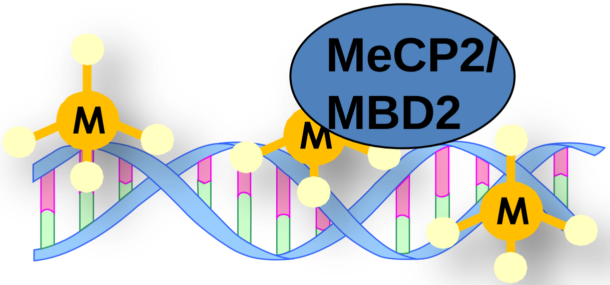

REGULATE
TRANSCRIPTION FACTOR
GENE
ACAGTGA
BINDING SITE
PROTEIN

DNA Methylation

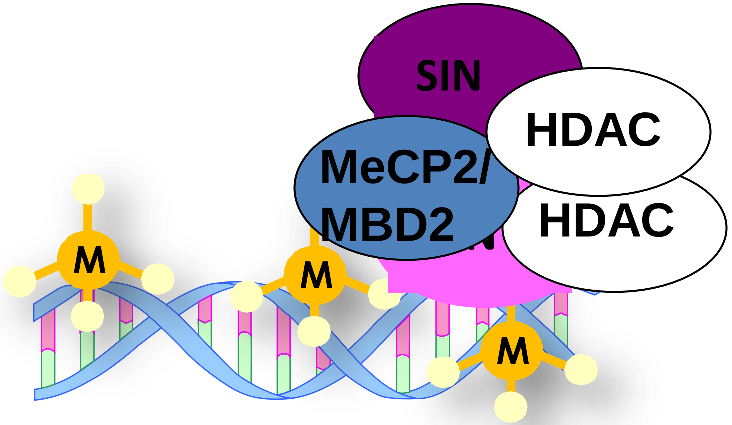
Epigenetic effects refer to modifications of the DNA that alter the activity of the gene, but not its function.





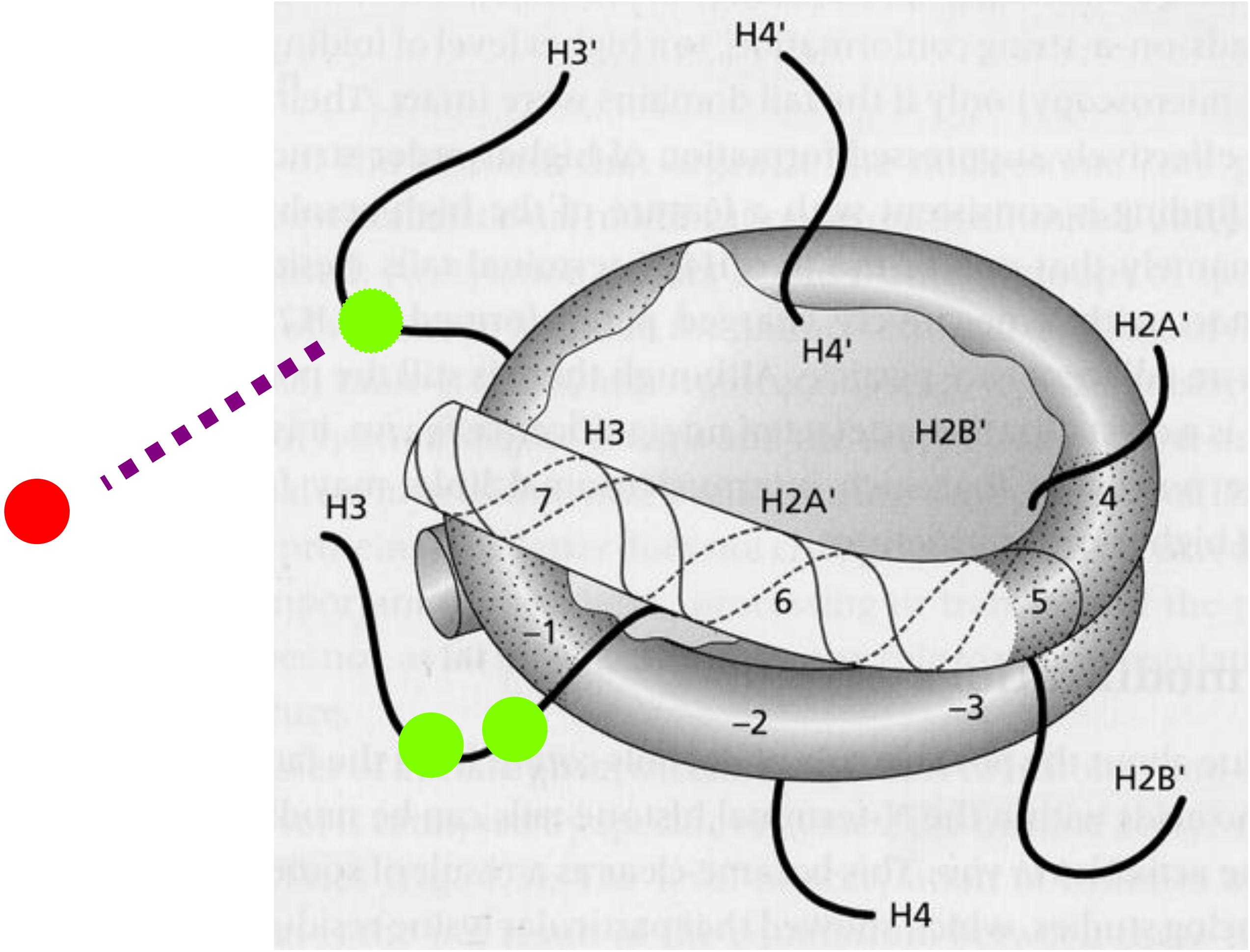


Methylated DNA
binding protein

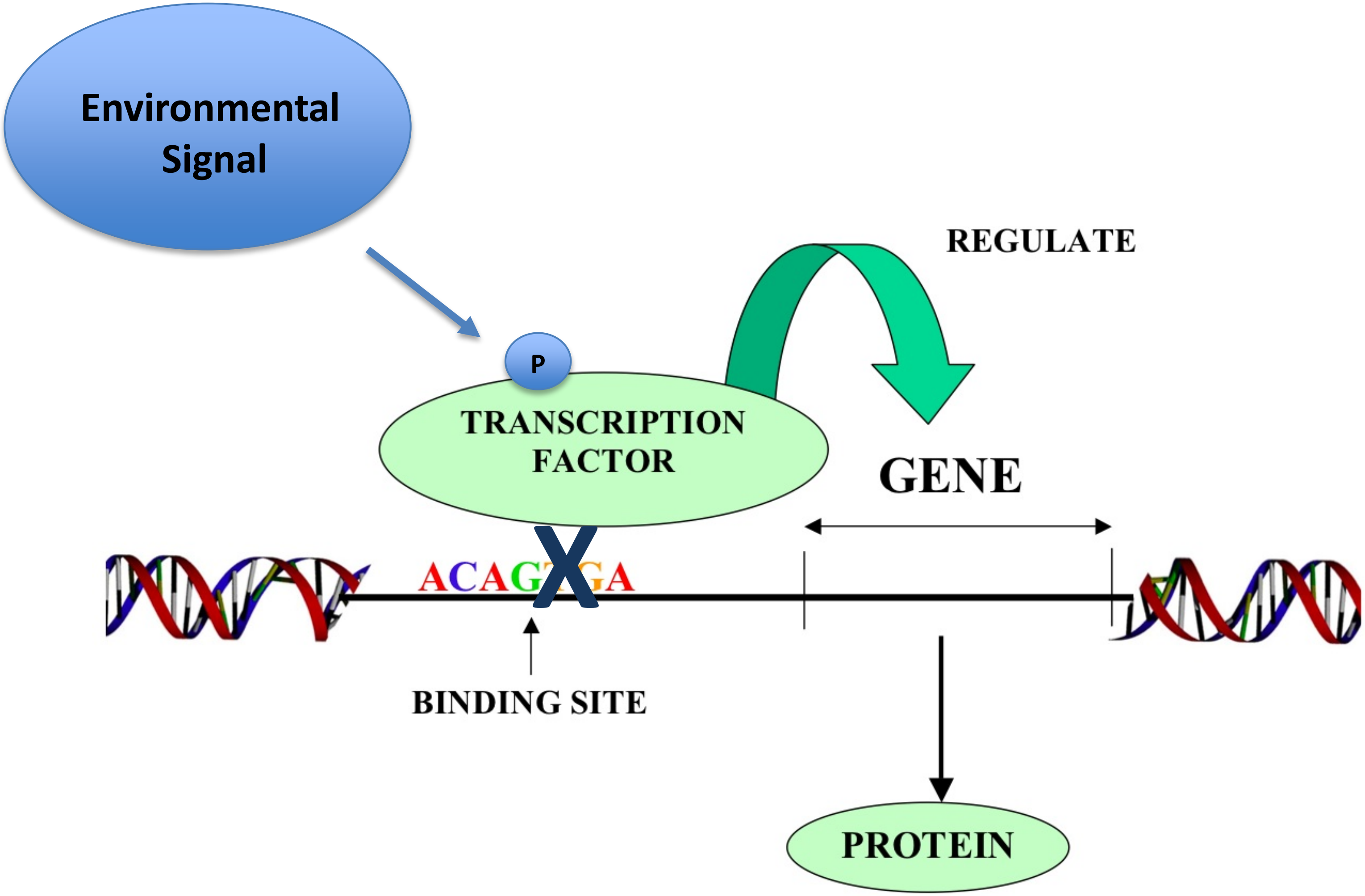


HDAC: Histone
deacetylase

🟢 HDAC



B. Turner. Chromatin structure and gene regulation. 2001.



Environmental Signal

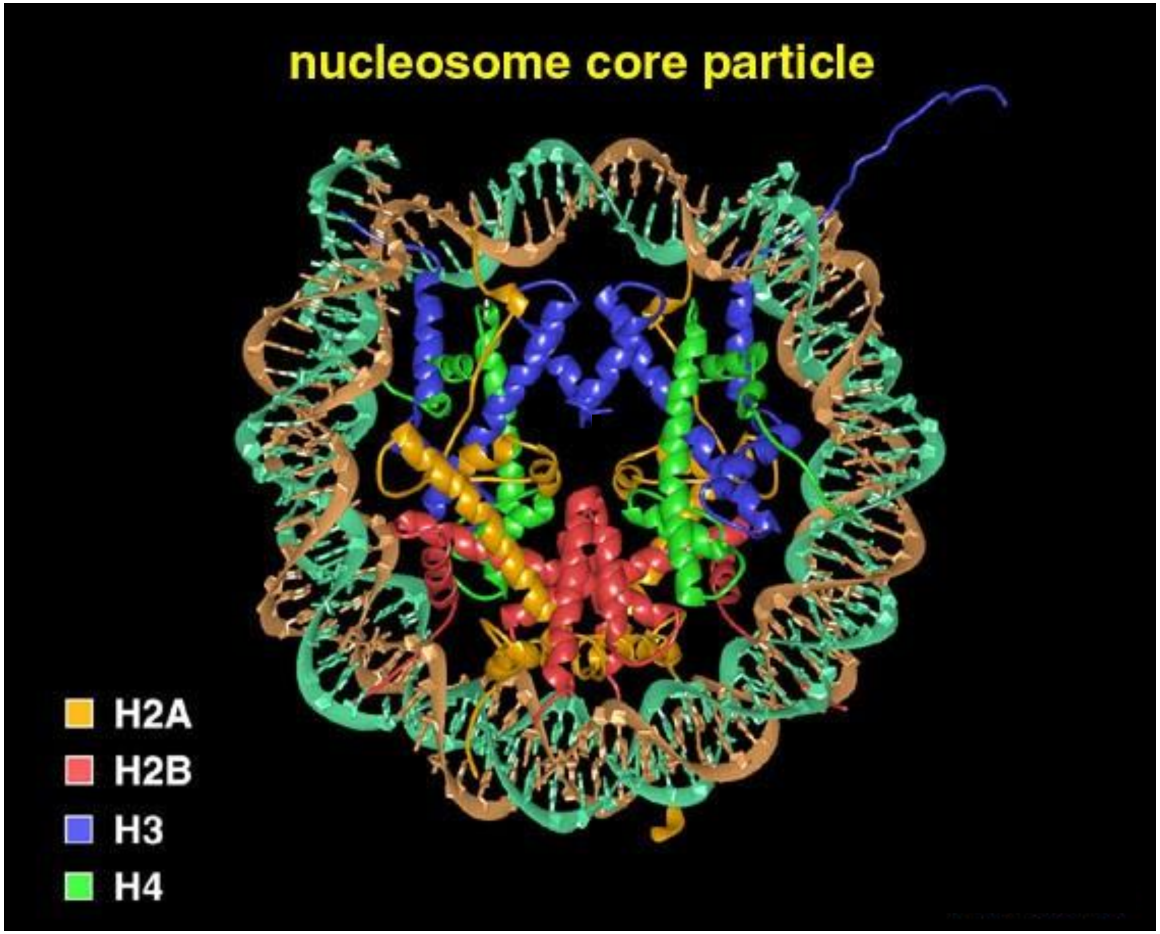
TRANSCRIPTION FACTOR

REGULATE

GENE

BINDING SITE

PROTEIN

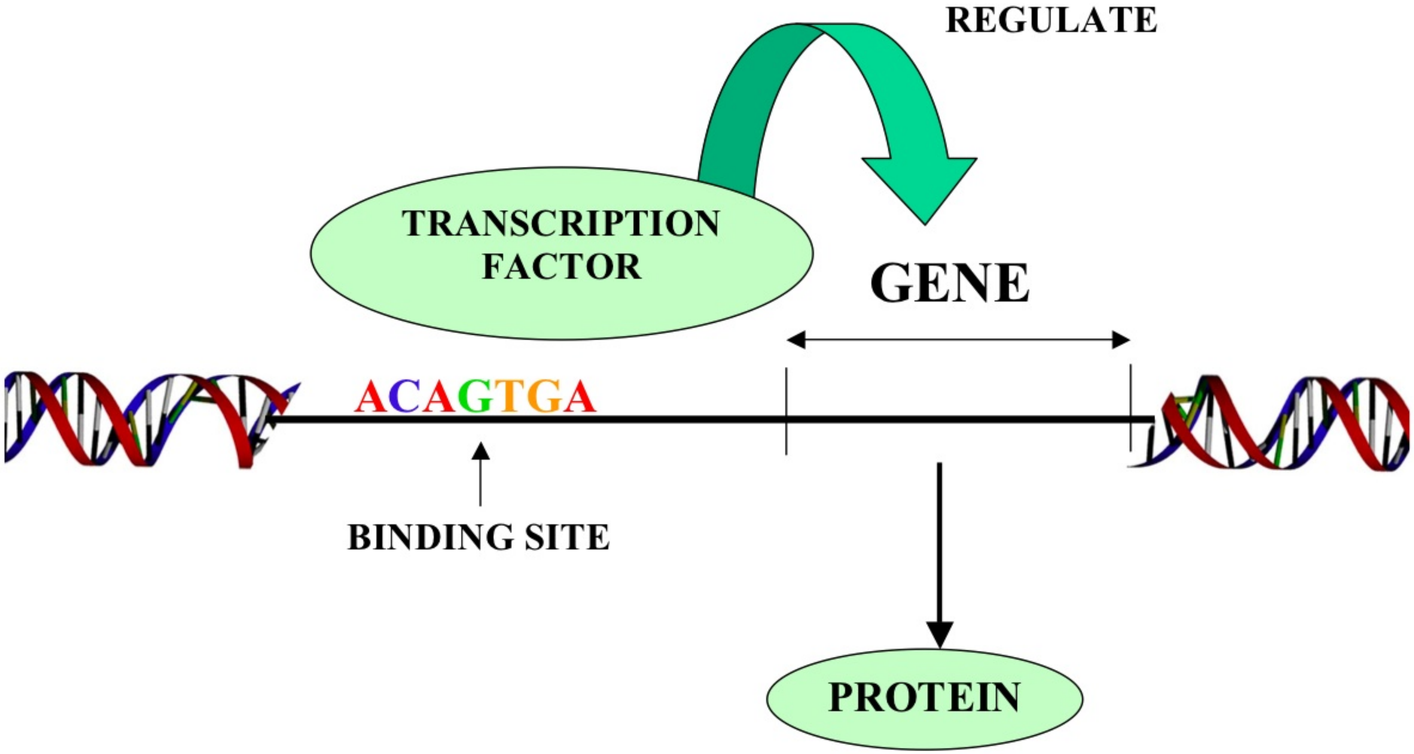


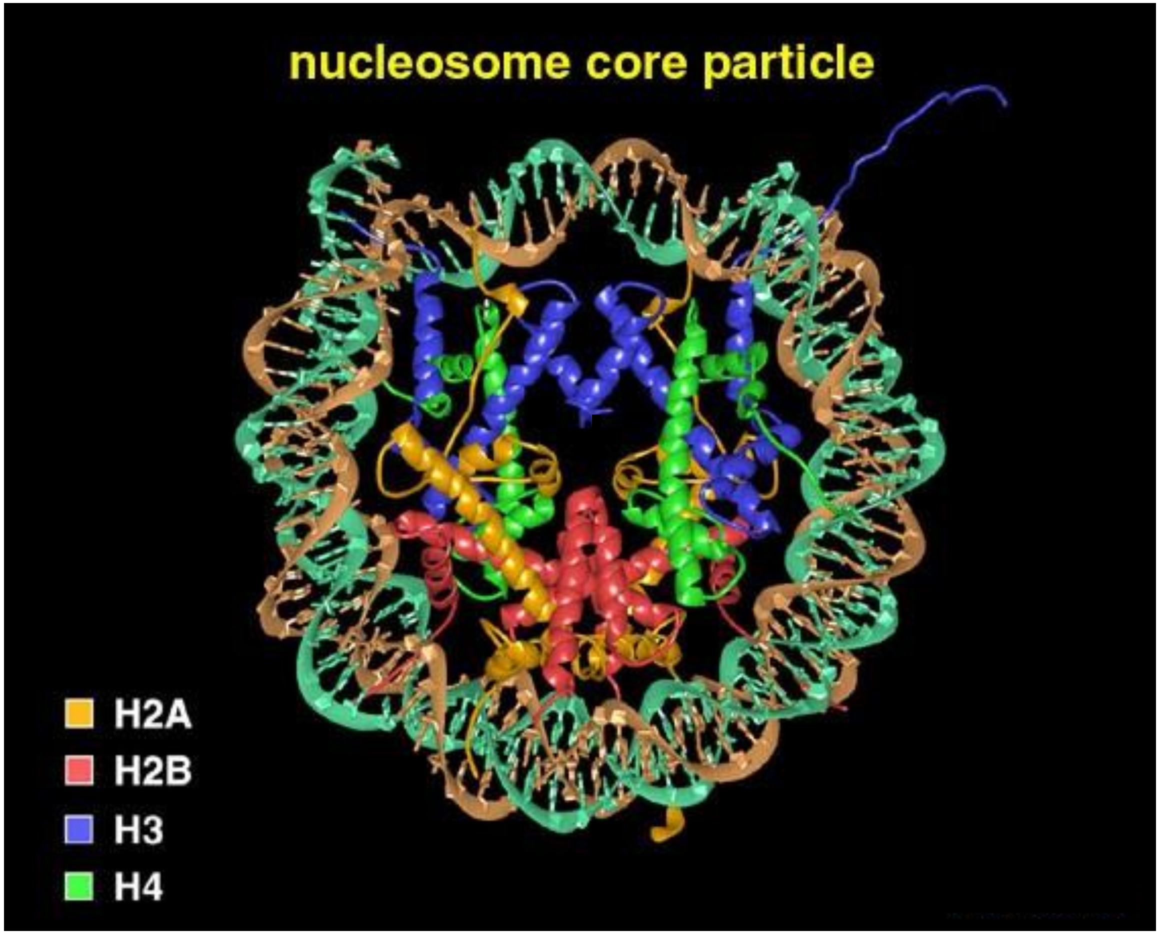
Prevents TF binding to DNA

TF binding involves alteration of chromatin structure

Nucleosome core particle: ribbon traces for the 146-bp DNA phosphodiester backbones (brown and turquoise) and eight histone protein chains (Luger et al. Nature 1997).

Epigenetic marks regulate the ability of these transcription factors to regulate gene activity



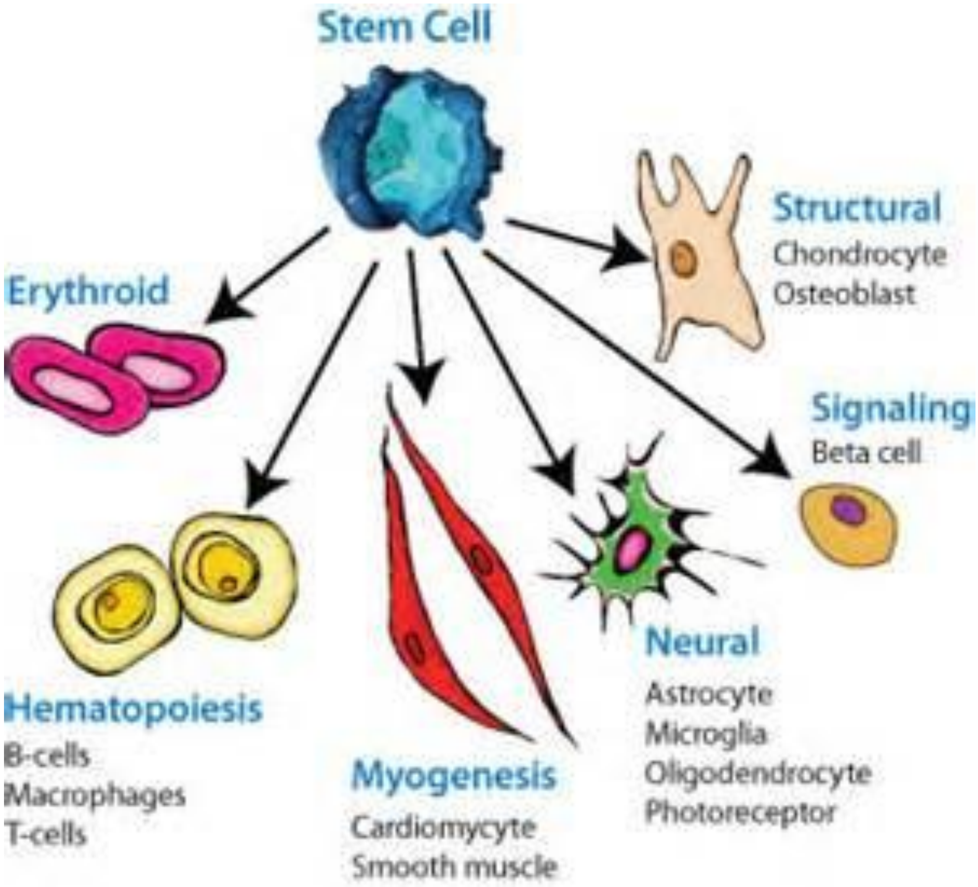


Prevents TF binding to DNA

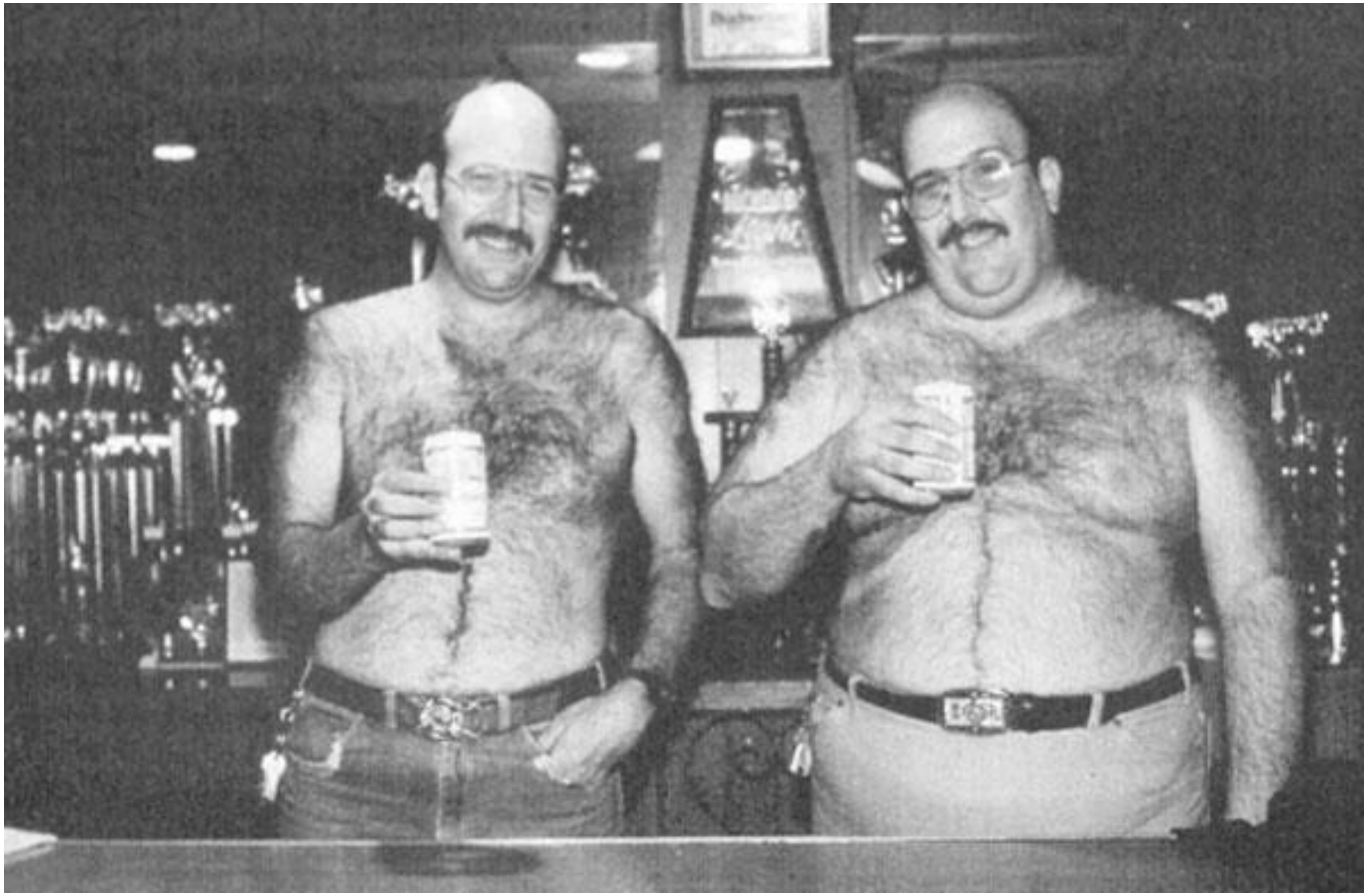
TF binding involves alteration of chromatin structure

Nucleosome core particle: ribbon traces for the 146-bp DNA phosphodiester backbones (brown and turquoise) and eight histone protein chains (Luger et al. Nature 1997).

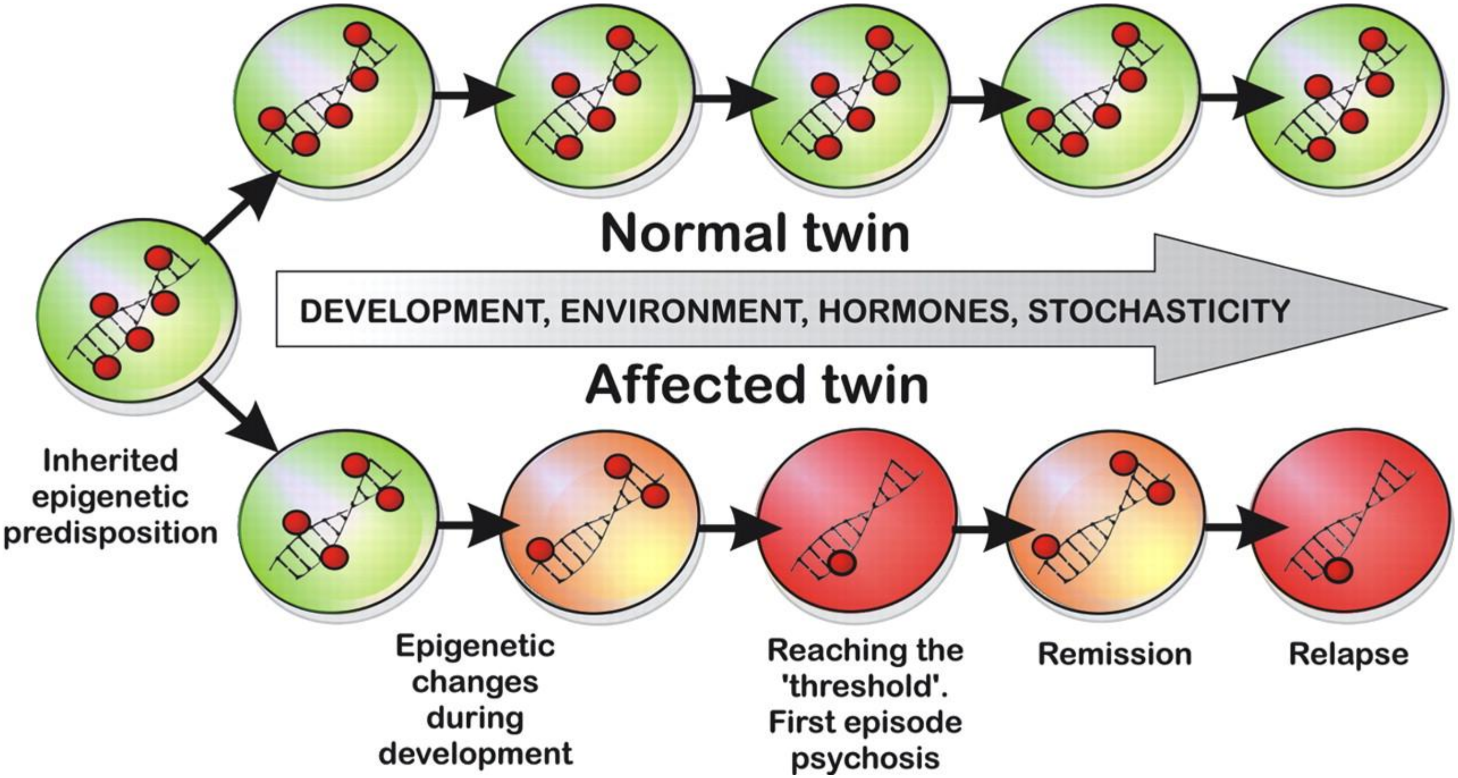
All tissues develop from a common DNA template

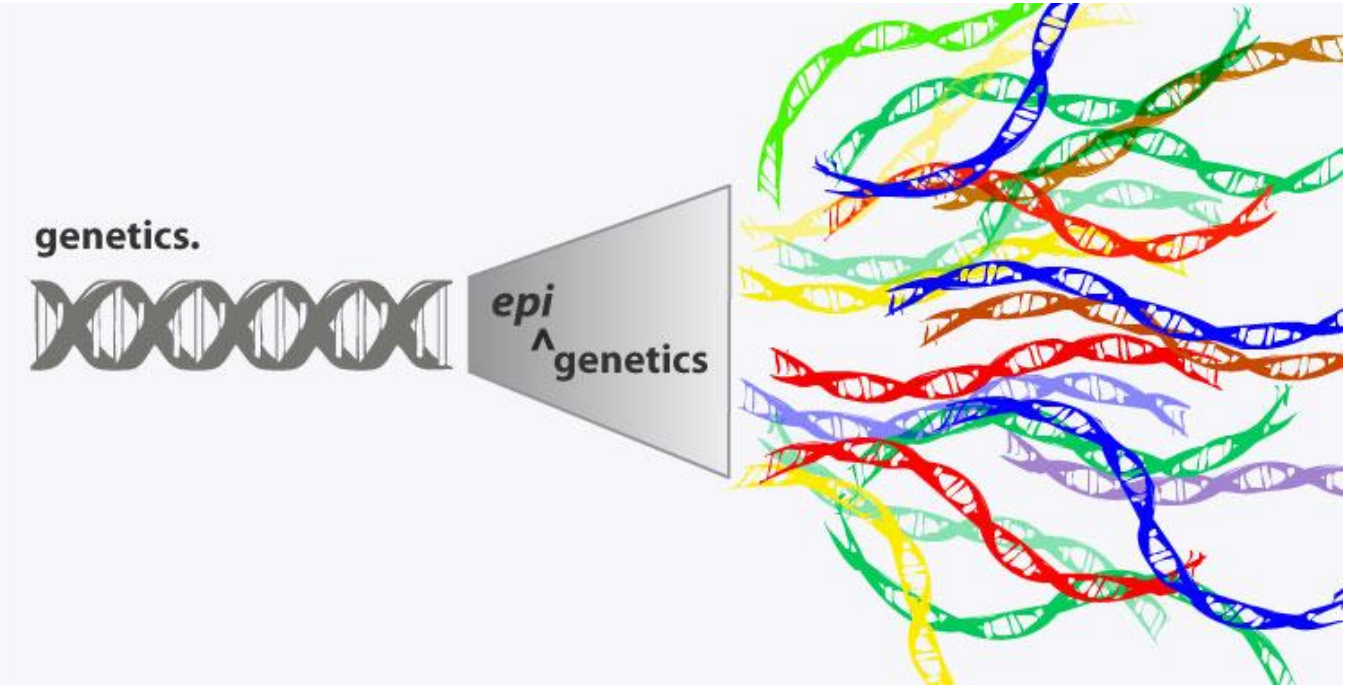


Multiple phenotypes from a common genotype



Every cell in your body has the same nuclear genes, but…?

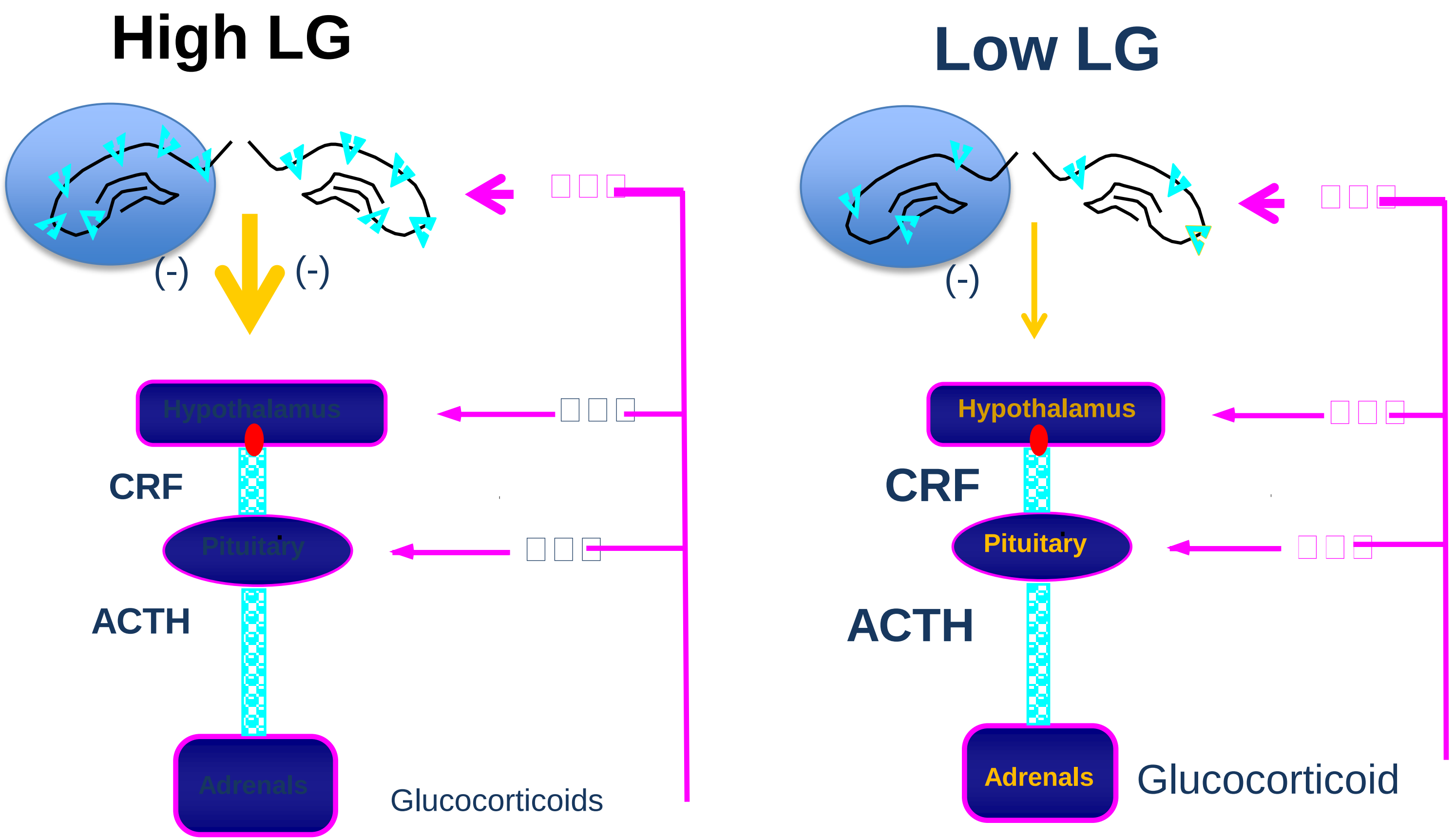

Normal twin
DEVELOPMENT, ENVIRONMENT, HORMONES, STOCHASTICITY
Affected twin
Inherited epigenetic predisposition
Epigenetic changes during development
Reaching the 'threshold'. First episode psychosis
Remission
Relapse


genetics.
epi
^genetics

Epigenetic hypothesis

Could environmental conditions produce epigenetic variations, that then might explain the enduring influence of clinically-relevant environmental conditions.

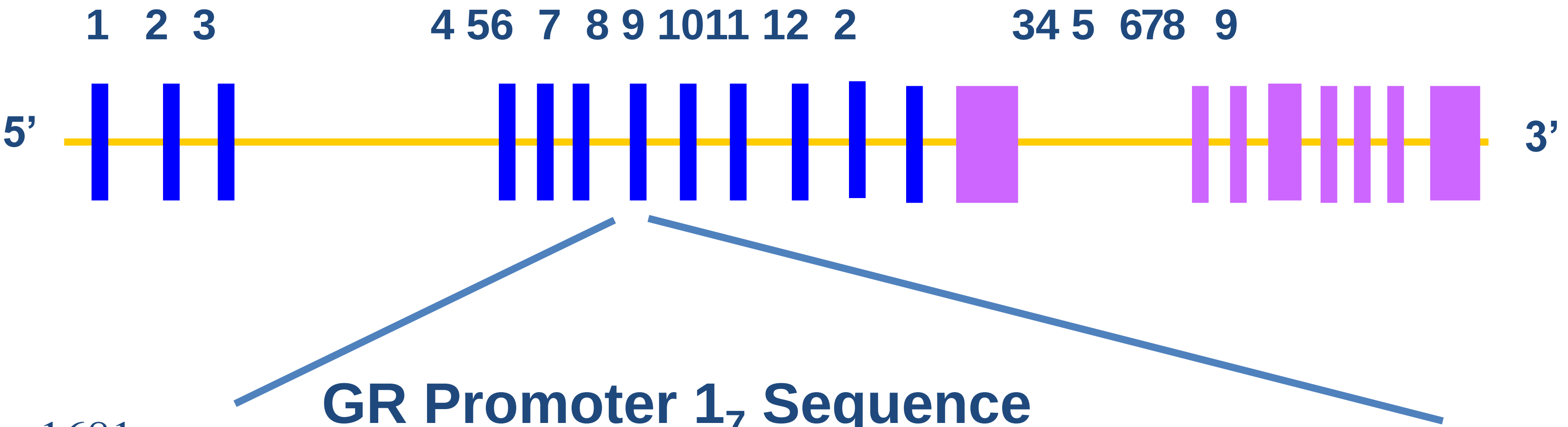
Individual differences in glucocorticoid receptor levels lead to altered pituitary-adrenal responses to stress



High LG

Low LG

DNA sites that regulate glucocorticoid receptor gene



GR Promoter 1_7 Sequence

1681 ccc

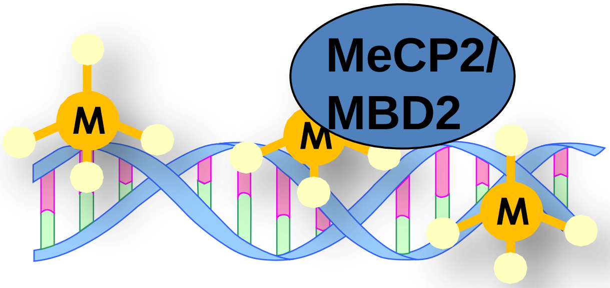
1741 ctctgctagt gtgacacact t^{1}**cg**^{2}**cg**caact c^{3}**cg**cagttgg ^{4}**cg**gg^{5}**cg**^{6}**cg**ga ccaccccctg^{7}**c**

1801 **g**gctctgc^{8}**cg** gctggctgtc accct^{9}**cg**ggg gctctggctg c^{10}**cg**accca^{11}**cg** ggg^{12}**cg**ggct

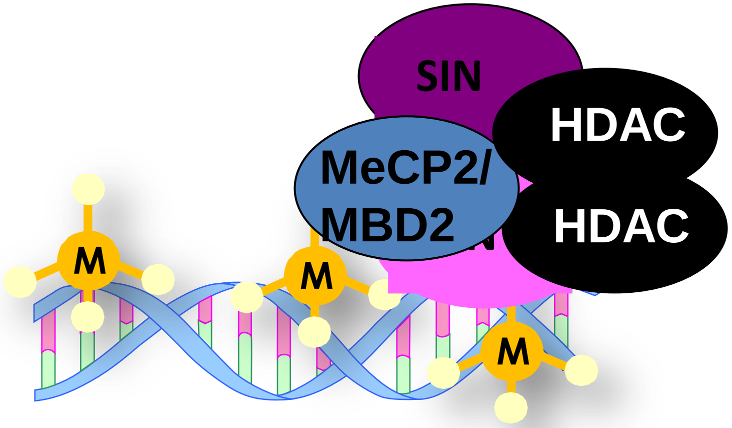
1861 c^{13}**cg**ag^{14}**cg**gtt ccaagcct^{15}**cg** gagtggg^{16}**cg** gggg^{17}**cg**ggag ggagcctggg agaa

NGFI-A

DNA Methylation can inhibit gene expression by blocking transcription factors binding

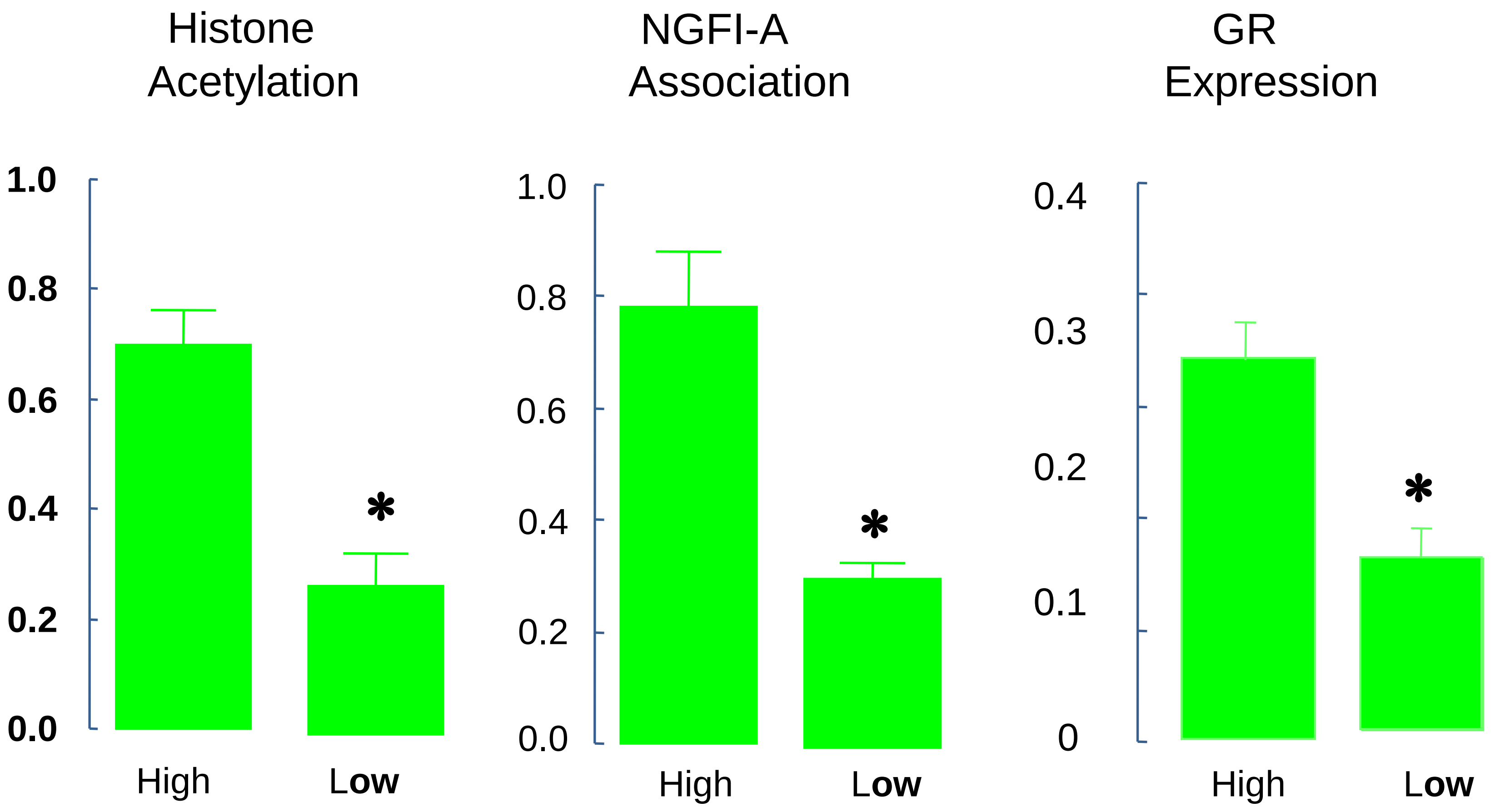


Methylated DNA binding protein



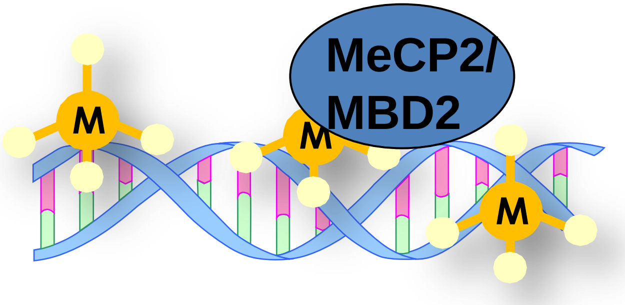
HDAC: Histone deacetylase

Increased methylation of the exon 1_7 GR promoter associates with decreased H3K9ac, reduced NGFI-A binding and GR expression



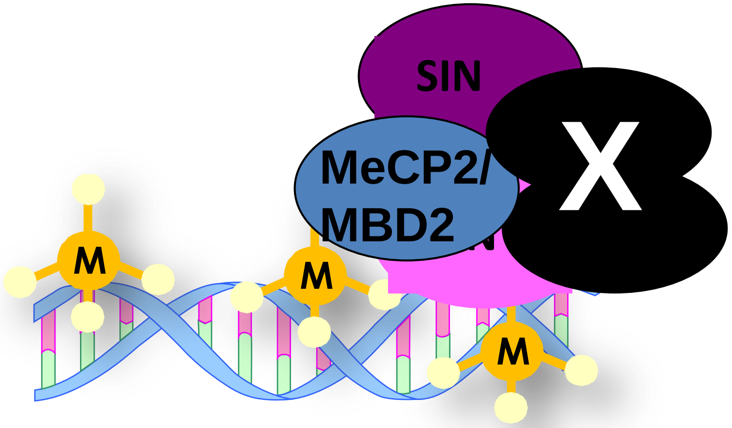
Difference in CpG methylation of the exon 1_7 promoter is completely reversed with cross-fostering

DNA Methylation can inhibit gene expression by blocking transcription factors binding



Methylated DNA
binding protein

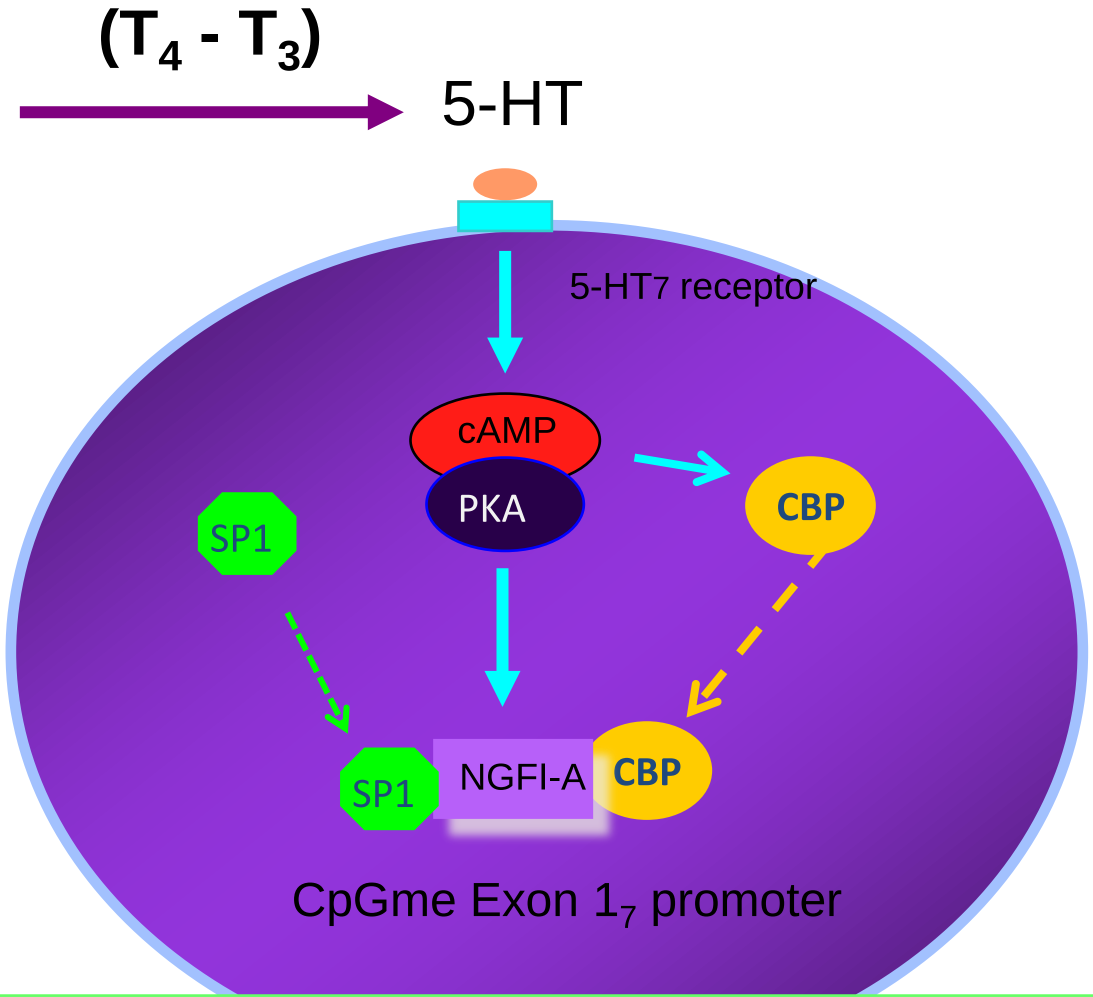
Chronic infusion
of TSA – HDAC
inhibitor



HDAC: Histone
deacetylase

Summary of *in vivo* and *in vitro* studies

Tactile stimulation
(maternal LG)



$(T_4 - T_3)$

5-HT

5-HT7 receptor

cAMP

PKA

CBP

SP1

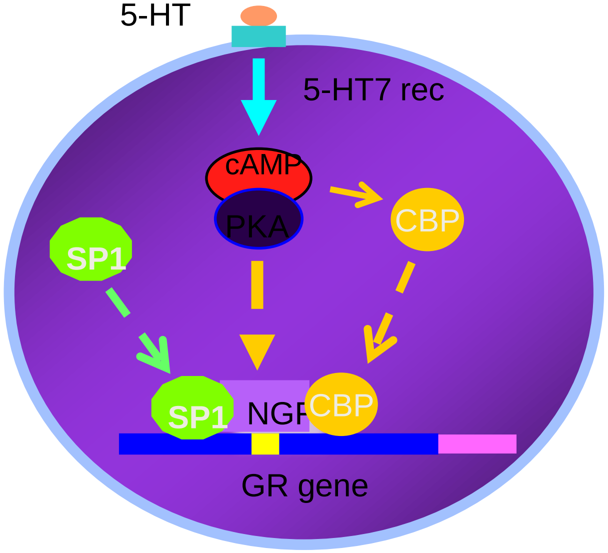
SP1

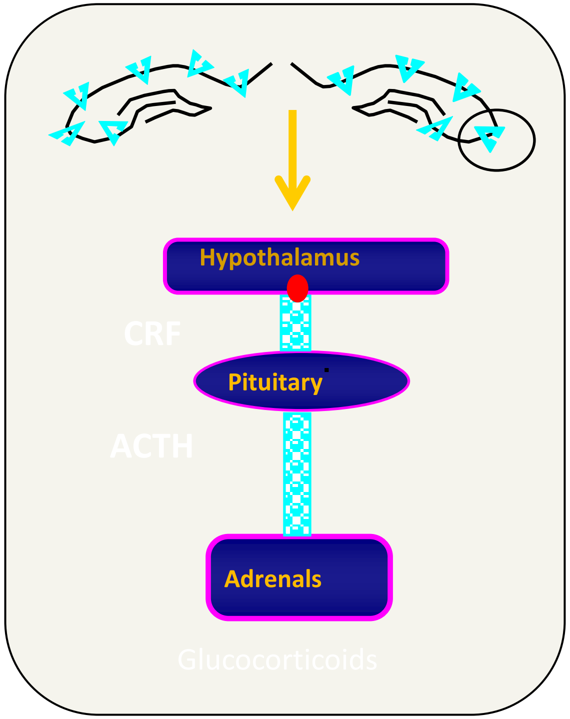
NGFI-A

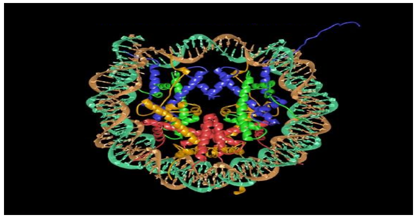
CBP

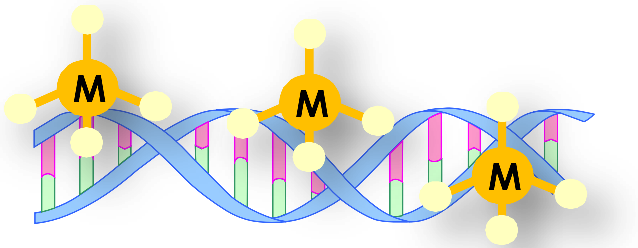
CpGme Exon 1_7 promoter

Maternal effect on DNA methylation status of the GR gene promoter in hippocampus

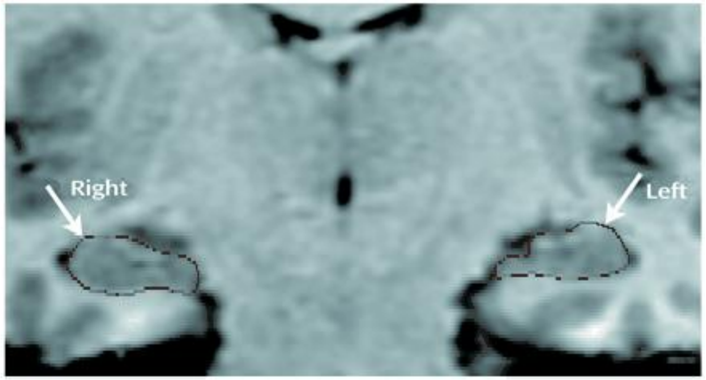

5-HT
5-HT7 rec
cAMP
PKA
CBP
SP1
SP1
NGF
CBP
GR gene


Hypothalamus
CRF
Pituitary
ACTH
Adrenals
Glucocorticoids



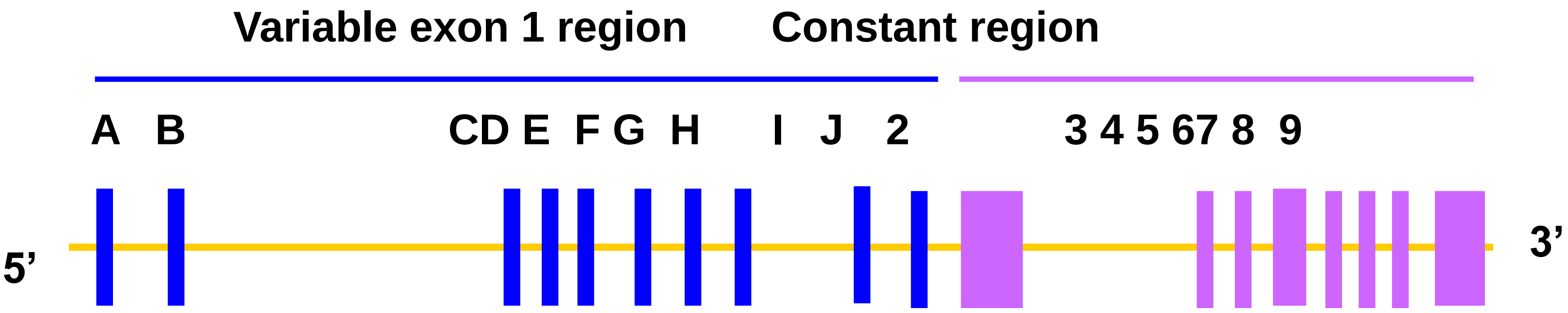

M
M
M

Do comparable processes occur in humans?



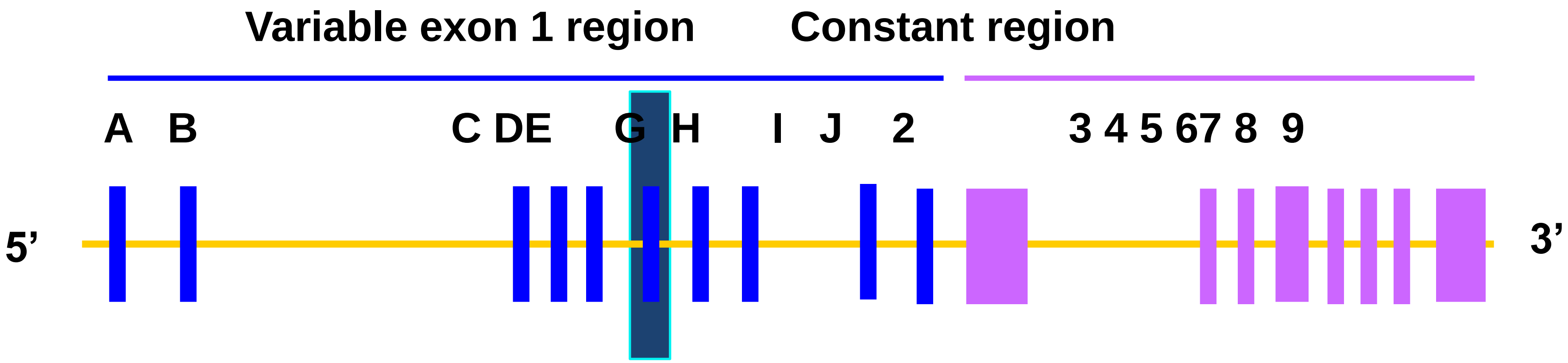
- Post-mortem studies of hippocampus.
- Samples from suicide victims/controls.
- QSBB (Gustavo Turecki) – forensic phenotyping.
- Human exon 1F promoter (Turner & Muller, 2005)

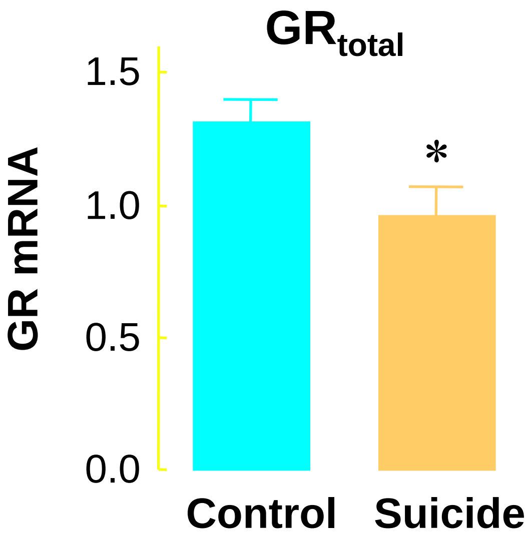
Human glucocorticoid receptor gene

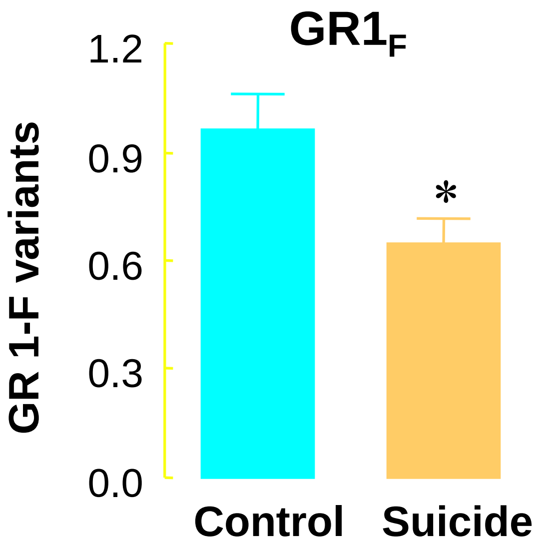


Exon 1_F is the human ortholog of the rat exon 1_7 GR promoter (70% homology) and contains an NGFI-A consensus sequence.

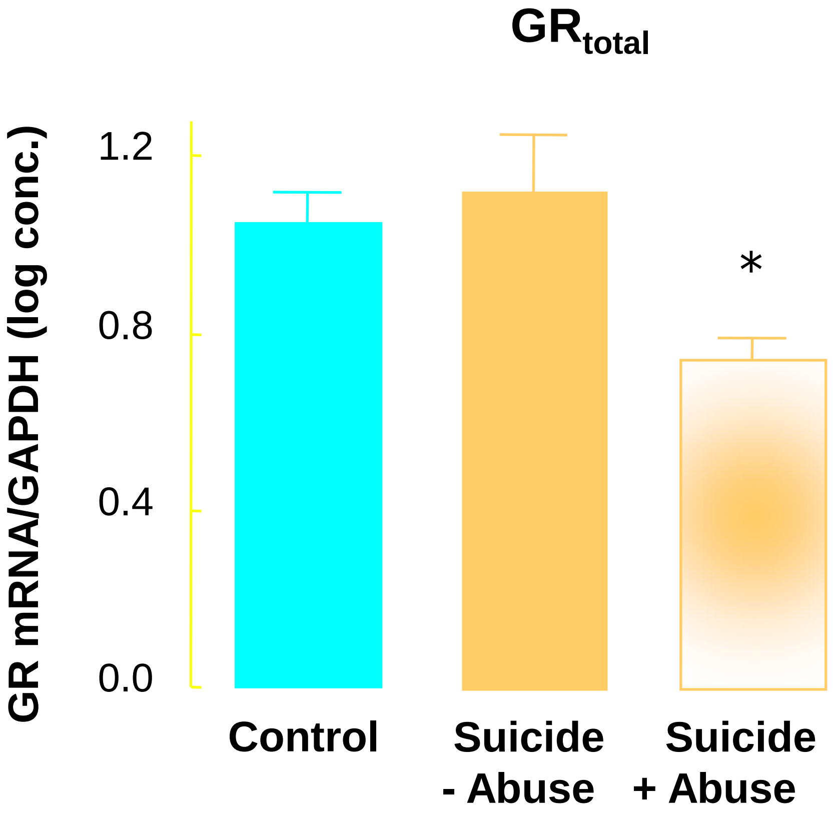
Human glucocorticoid receptor gene

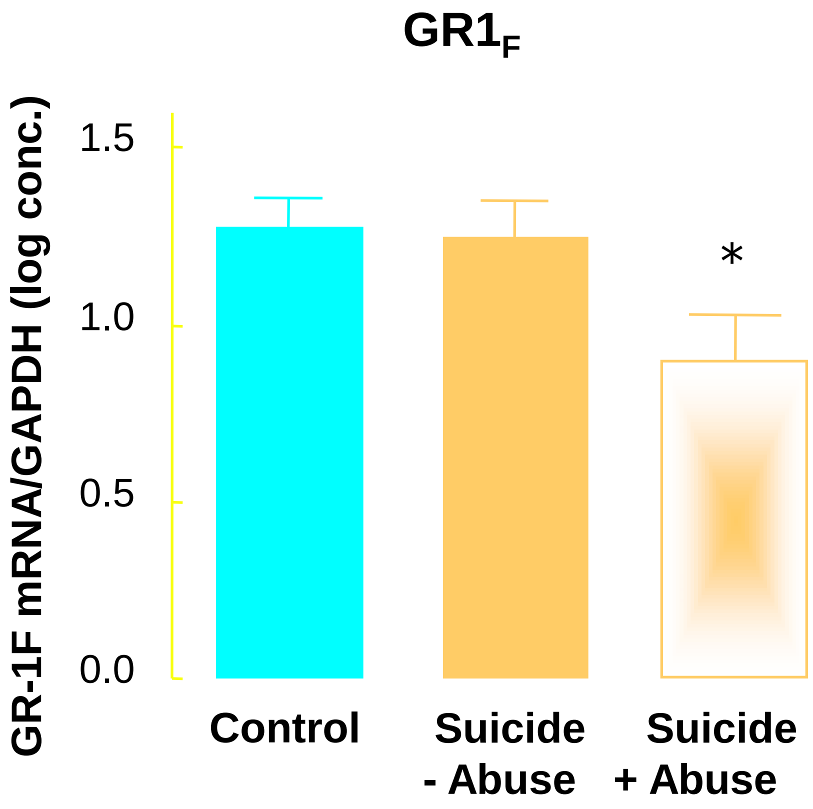


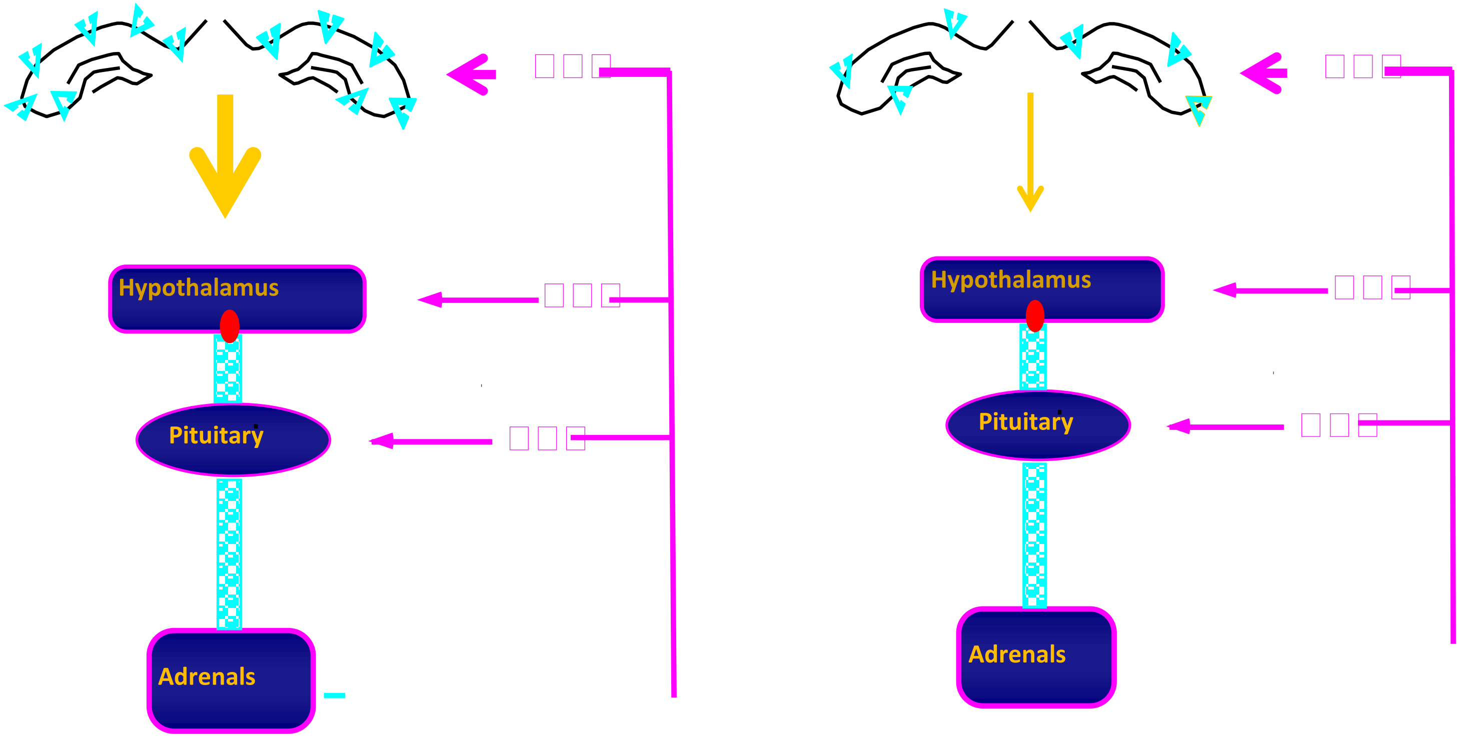

$\mathrm{GR}_{\mathrm{total}}$


$\mathrm{GR1}_{\mathrm{F}}$

Suicide vs abuse - GR expression

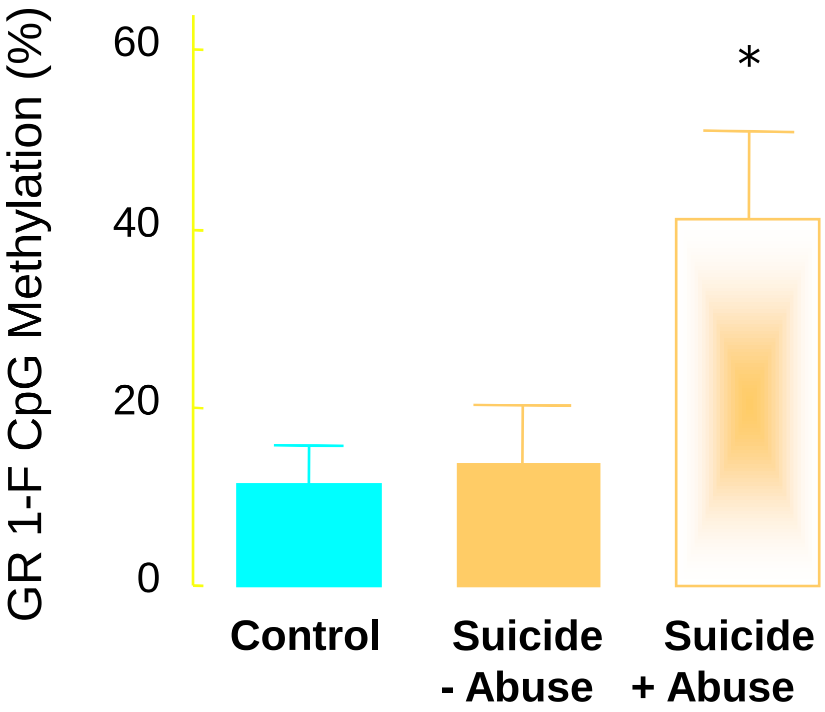




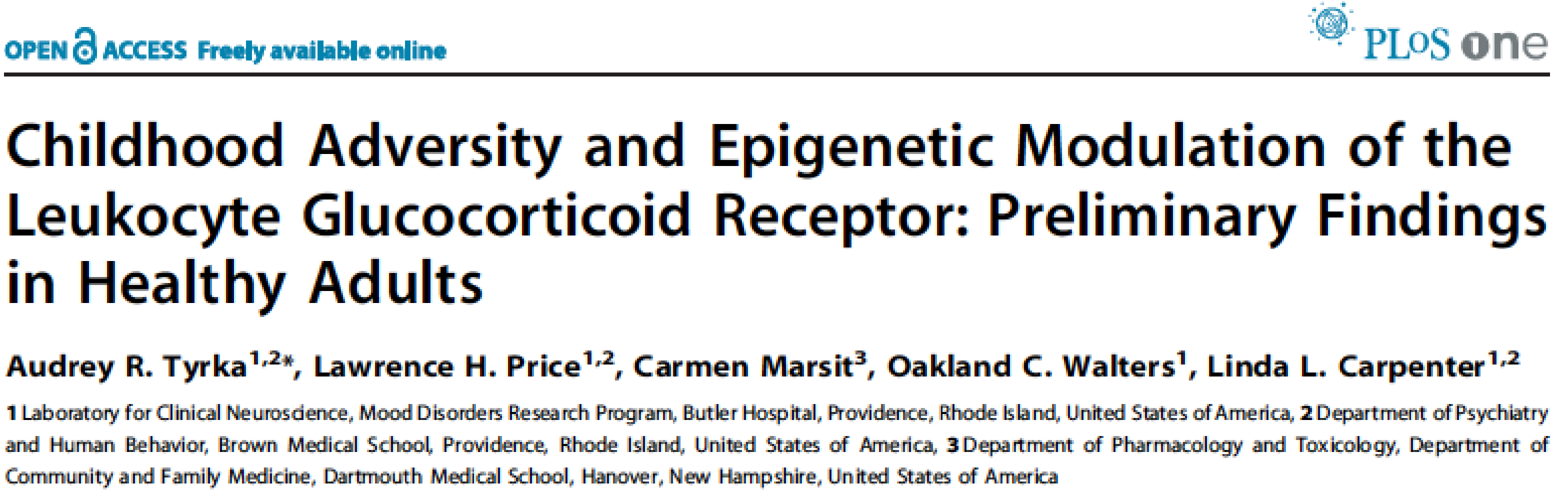


Childhood maltreatment associates with increased central CRF levels and greater HPA and autonomic responses to stress (DeBellis et al 1994; Heim et al 2000; Lee et al 2005)

Suicide vs abuse - CpG methylation



Childhood adversity and *NR3C1* promoter metnylation in DNA from periperhal samples



OPEN ACCESS Freely available online

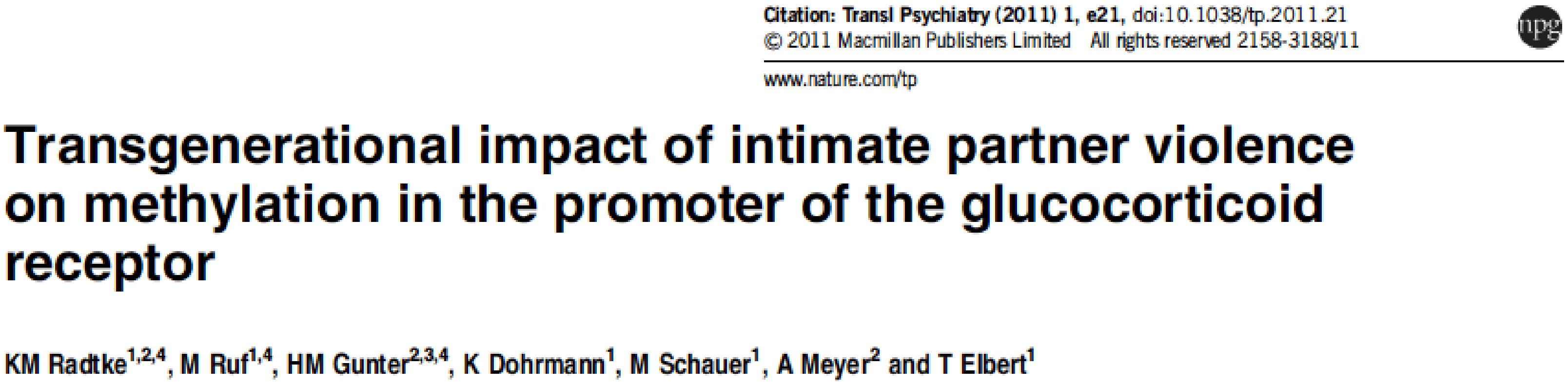
PLoS one

Childhood Adversity and Epigenetic Modulation of the Leukocyte Glucocorticoid Receptor: Preliminary Findings in Healthy Adults

Audrey R. Tyrka[1,2]*, Lawrence H. Price[1,2], Carmen Marsit[3], Oakland C. Walters[1], Linda L. Carpenter[1,2]

1 Laboratory for Clinical Neuroscience, Mood Disorders Research Program, Butler Hospital, Providence, Rhode Island, United States of America, **2** Department of Psychiatry and Human Behavior, Brown Medical School, Providence, Rhode Island, United States of America, **3** Department of Pharmacology and Toxicology, Department of Community and Family Medicine, Dartmouth Medical School, Hanover, New Hampshire, United States of America

---



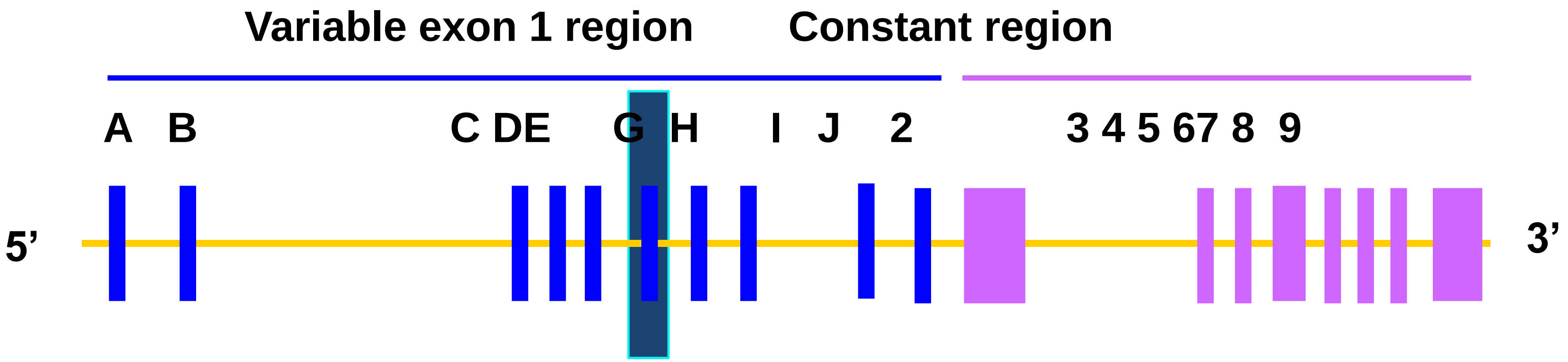
Citation: Transl Psychiatry (2011) 1, e21, doi:10.1038/tp.2011.21

www.nature.com/tp

Transgenerational impact of intimate partner violence on methylation in the promoter of the glucocorticoid receptor

KM Radtke[1,2,4], M Ruf[1,4], HM Gunter[2,3,4], K Dohrmann[1], M Schauer[1], A Meyer[2] and T Elbert[1]

Human glucocorticoid receptor gene



Variable exon 1 region **Constant region**

A B C DE G H I J 2 3 4 5 67 8 9

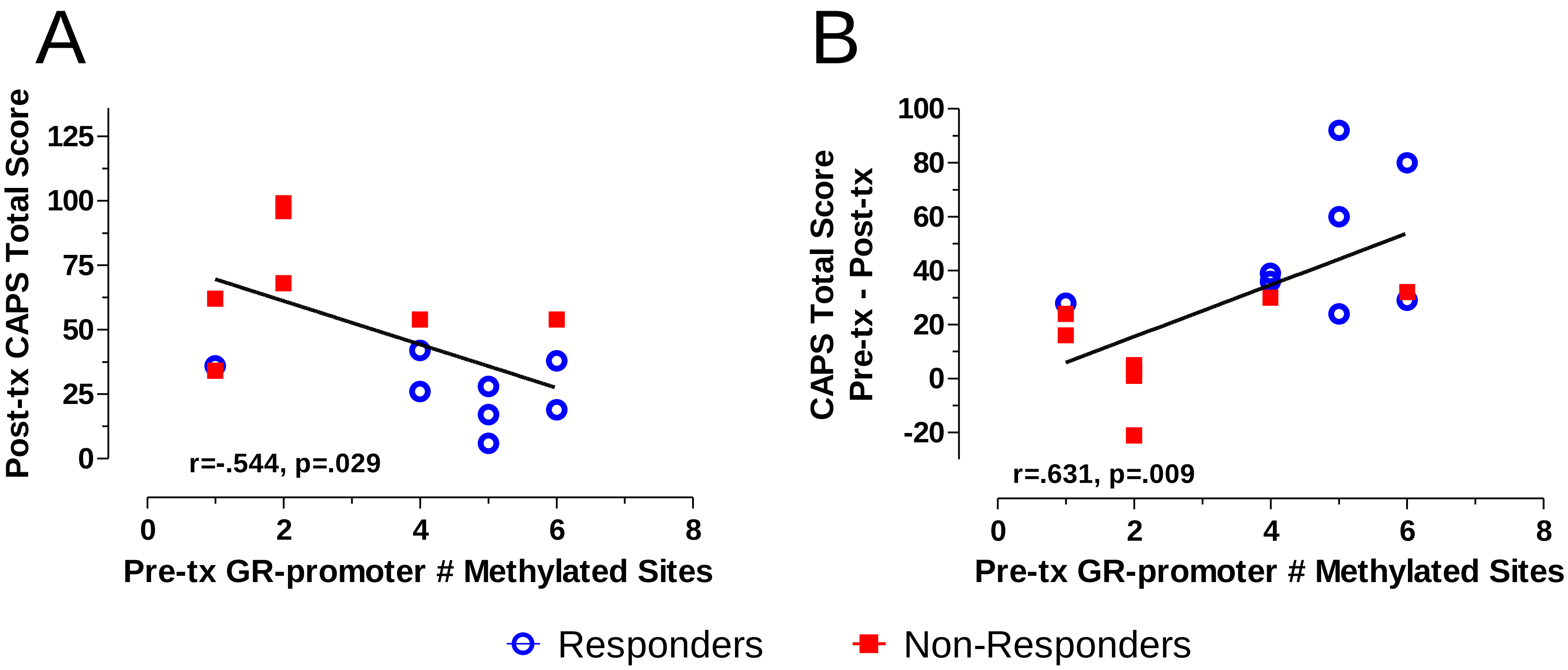
5’ **3’**

102 US combat veterans (Iraq, Afghanistan); 51 w/ PTSD, 51 w/o PTSD*

- Differ significantly in exon 1_F GR promoter methylation (PMBCs) and correlated with combat events (p= .004)
- Exon 1_F GR methylation correlated significantly w/…..
  - Glucocorticoid negative feedback (p=.007)
  - PTSD symptom severity (p=.03)
  - Avoidance symptoms (p=.007)
  - Depression symptoms (p=.02)
  - Peritraumatic dissociation (p=.006)

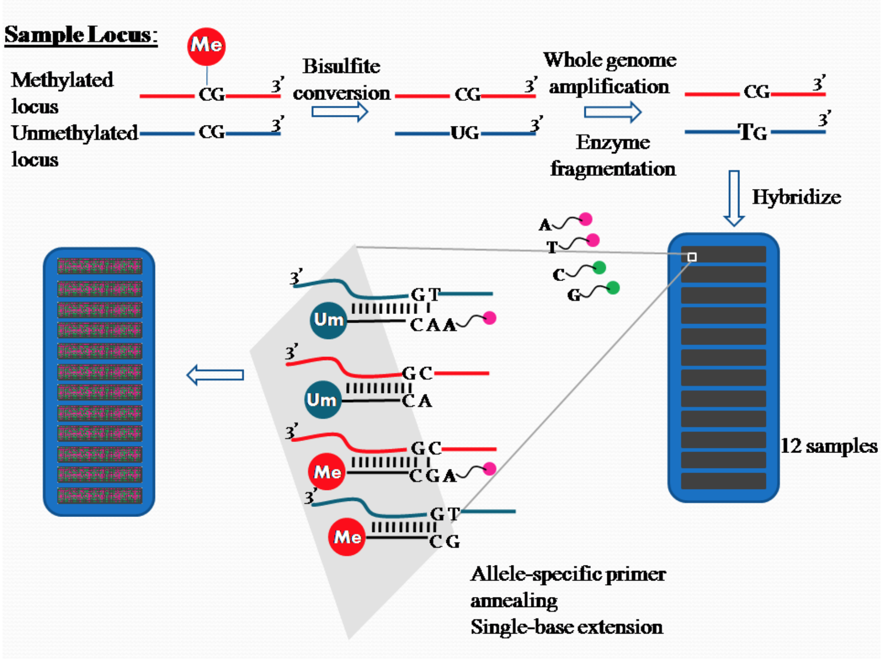
*Controlled for cell type and medication

Pre-Tx Exon 1_F GR Promoter Methylation and PTSD Symptoms at Post-Tx

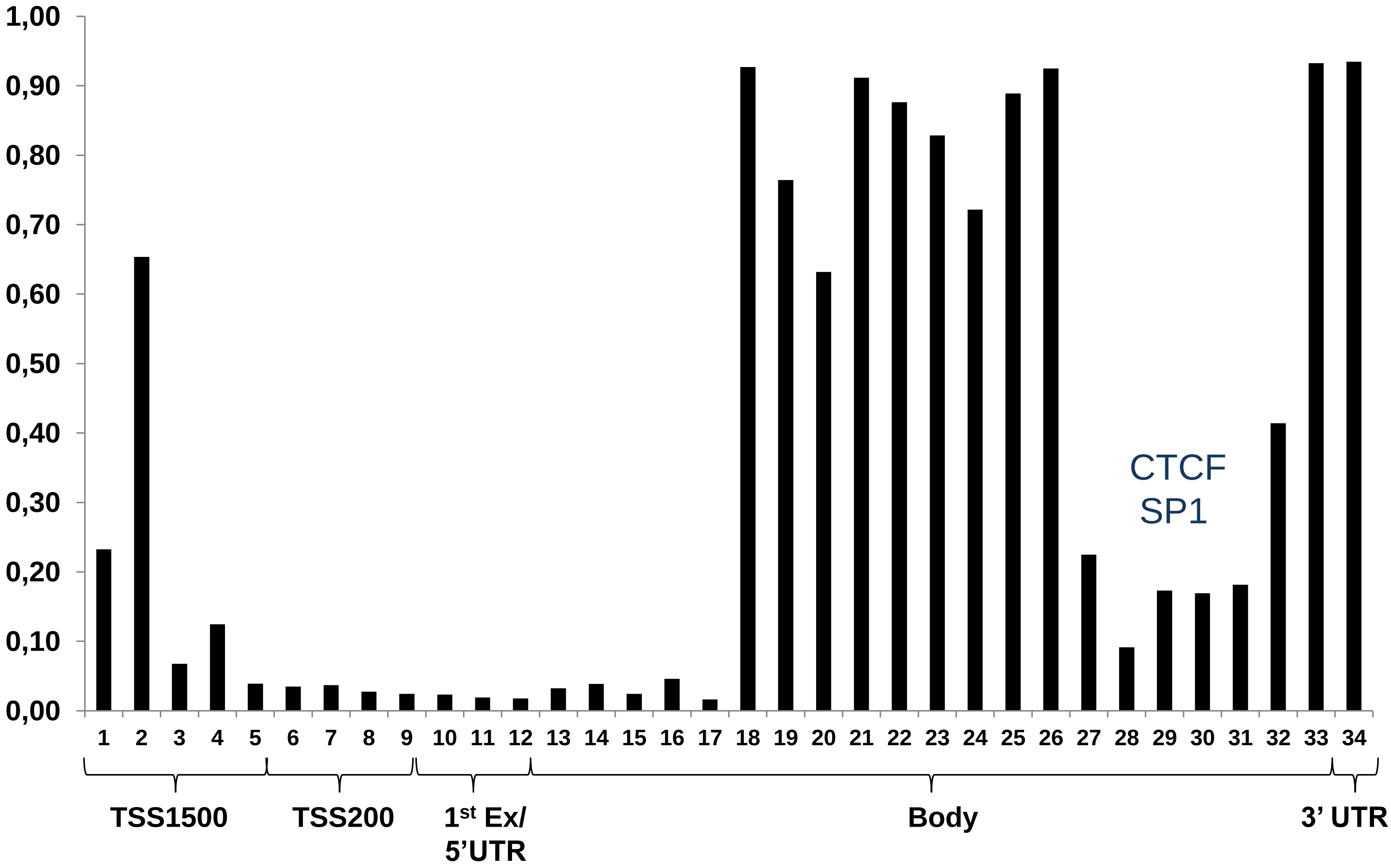


Does the human epigenome reflect variations in the quality of the early social environment?

~488,000 CpG's
~3,000 CpN's



Mitogen-activated protein kinase kinase kinase (MAP3K6)



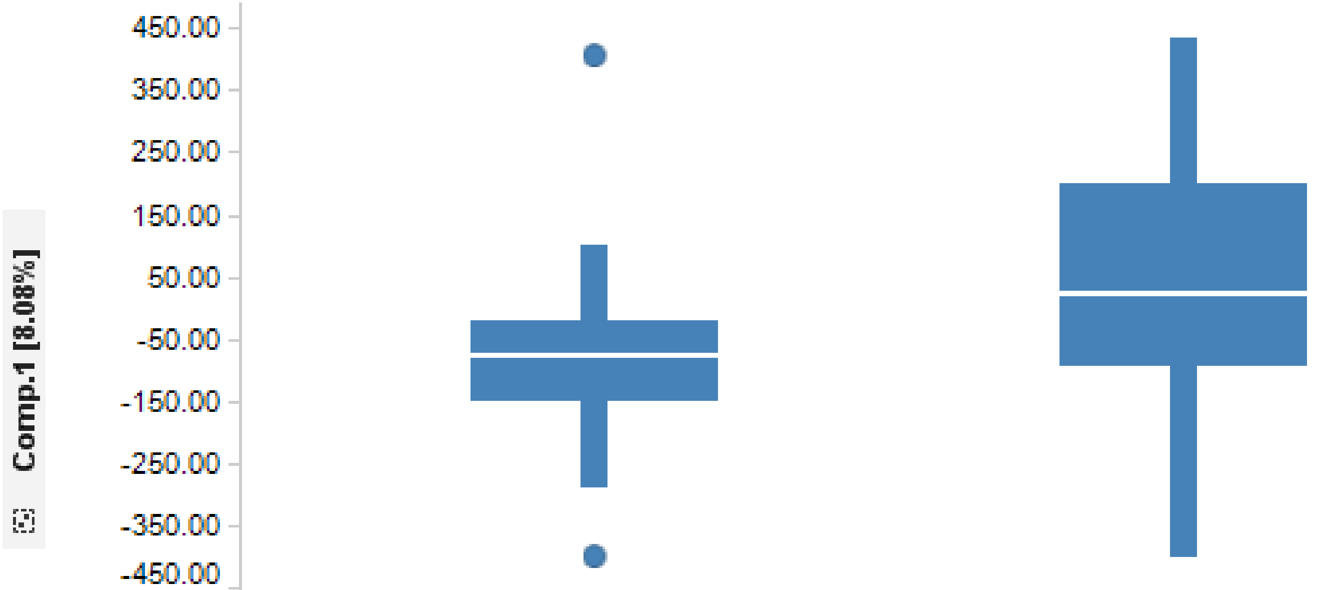
Nurse-Family Partnership
Helping First-Time Parents Succeed®

- ~200 Unique Samples
- Infinium 450K Array
- Blood Samples
- 27 years old
- Variables:
  - methy_grp (0= control/no treatment ;1=intervention)
  - TREATMENT
  - CHILDGENDER
  - Child Abuse at age 4 and age 15

Olds DL, et al JAMA 1997 278:637-643.
Olds DL, et al JAMA 1998 280:1238-1244.
Kitzman H, et al JAMA 2000 283:1983-1990.

Child abuse is physical, sexual or emotional mistreatment or neglect

NFP Intervention and Genome-Wide DNA Methylation



No Treatment
(88)

Treatment
(98)

1461 Differential Variable CpGs (FDR<0.05)

Relative contribution of genetic and environmental Conditions to DNA methylome profiles

Singapore birth cohort (n= 237)

Umbilical chord gDNA

Genotyping: Illumina HumanOmniExpress BeadChip

Multiple environmental measures

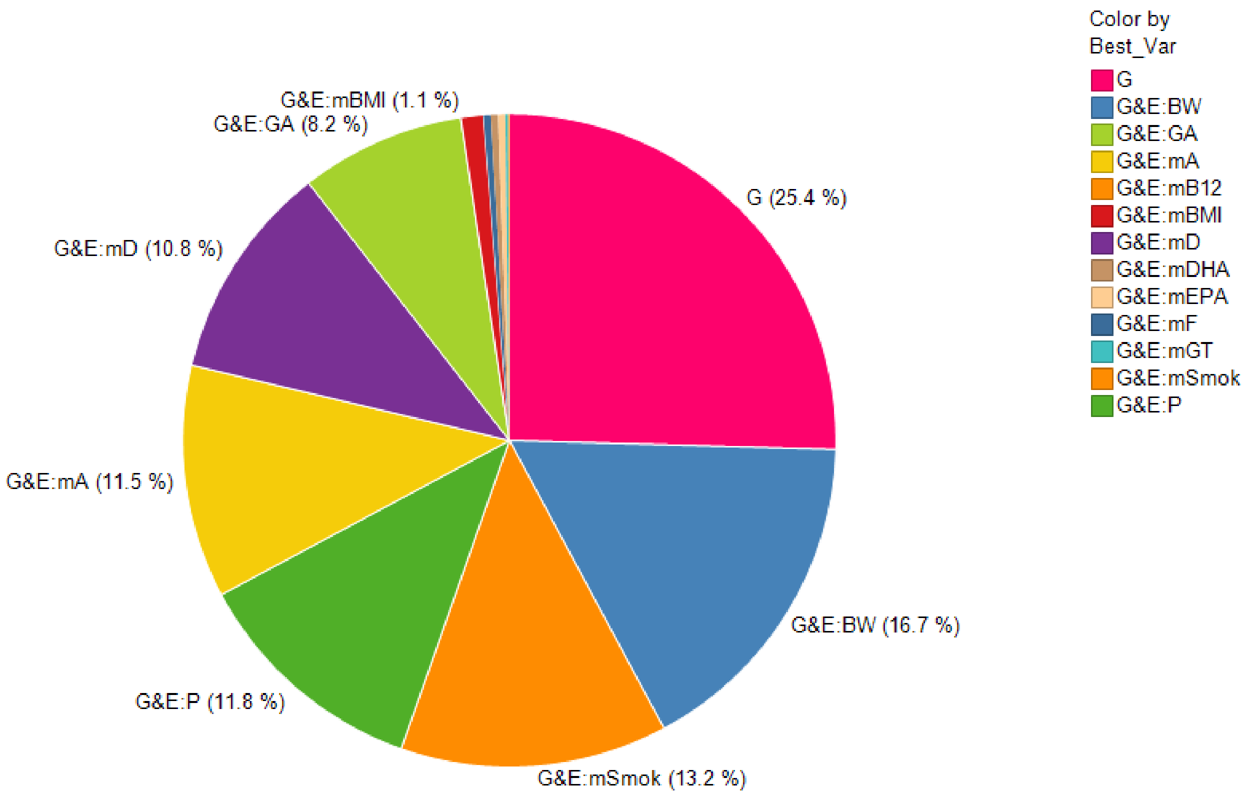
Challenge: Alterations across the entire genome

- Can we identify a global influence of environmental conditions during fetal development across the genome?

- Are these effects moderated by genotype?

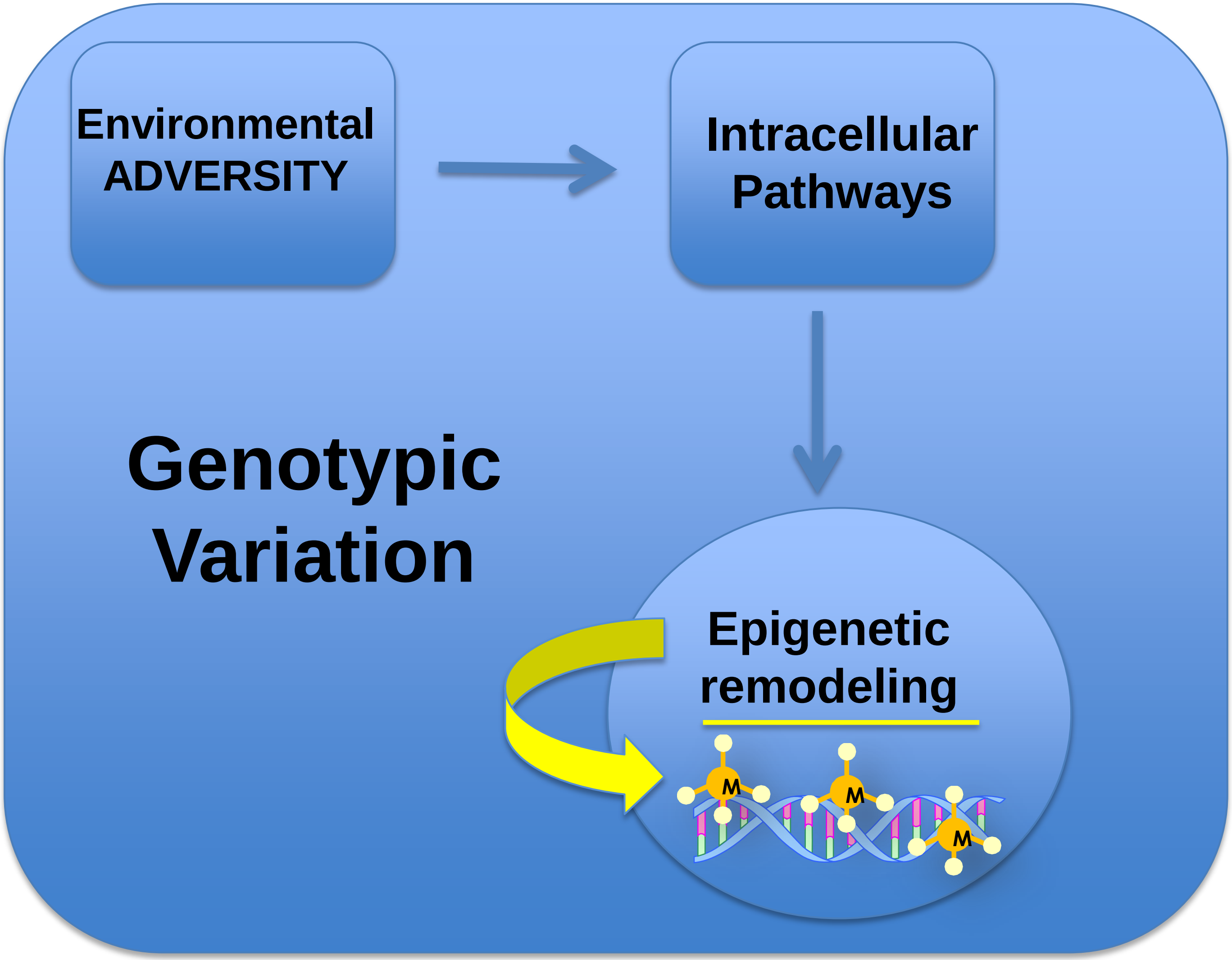
- Do such effects associate with functional outcomes?

Proportions of the 1423 VMRs best explained by G vs GxE regression models



Note: Multi-ethnic composition of the sample enhances the “genotype” alone contribution; among ethnic Chinese, estimate is ~16%.

Epigenetic Modifications as “Biomarker” of the Impact of Adversity at the Level of the Individual



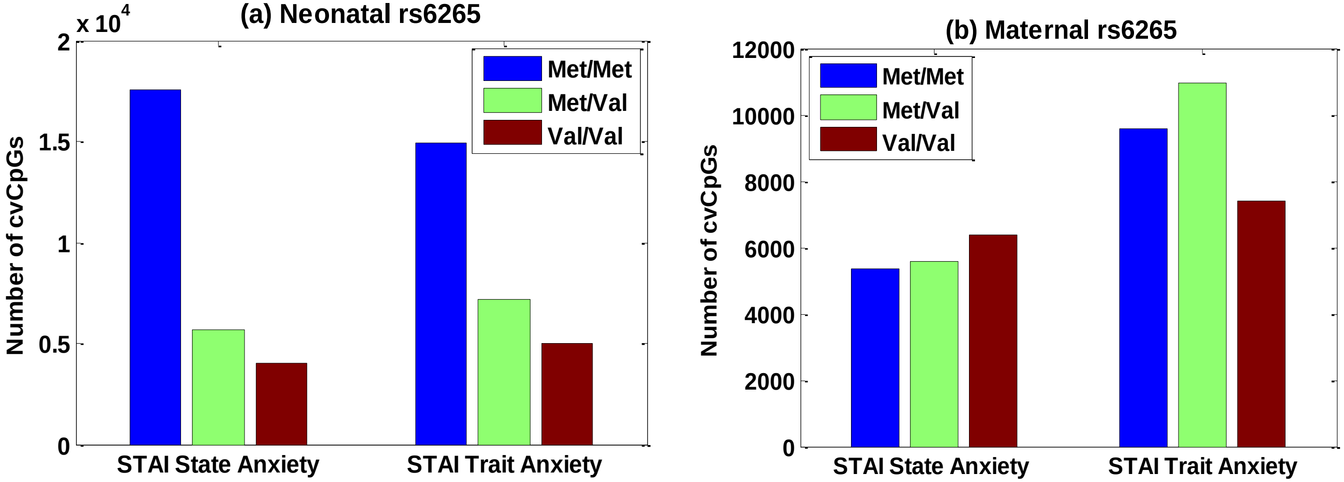
Environmental ADVERSITY

Intracellular Pathways

Genotypic Variation

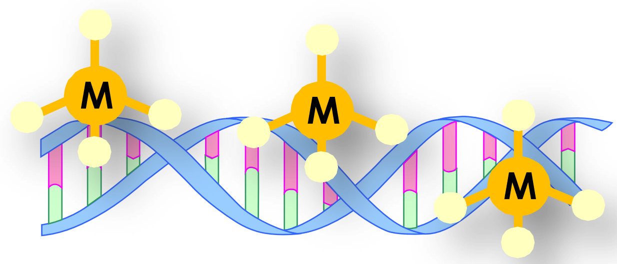
Epigenetic remodeling

Prenatal Maternal Anxiety x infant BDNF genotype



More than 10% of vCpGs significantly co-varied with anxiety scores in Met/Met group, suggesting that antenatal maternal anxiety was a significant source of variation in DNA methylation across the genome among Met/Met carriers.

Individual differences in epigenetic marks reflect gene x environment interactions



Conclusions

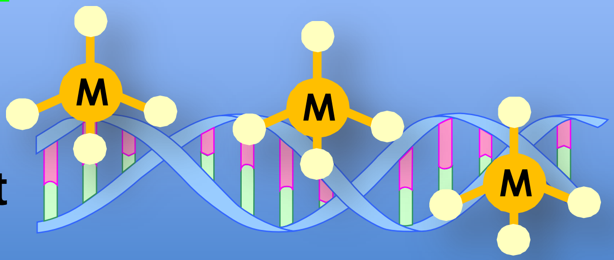
- The function of the genome is regulated by epigenetic signals that are influenced by environmental regulation.
- These epigenetic signals reflect the quality of the early environment, and guide the genomic function in the brain.
- These environmental influences on the epigenome appear to be highly moderated by genotype, thus reflecting G x E interaction effects.
- A G x E perspective implies that 'consequences' are not necessarily related directly to the 'severity' of the adversity.

Translational Opportunities

Epigenetic Modifications as “Biomarker” of the Treatment Outcomes in Mental Health

Early experience

Abuse
Family strife
Emotional neglect
Harsh discipline



Health Risks

Depression
Drug abuse
Anxiety
Diabetes
Heart disease
Obesity

Can we use the epigenetic marks to define the biology that underlies the impact of social adversity?

Association between prenatal SES and Variation in DNA Methylation

SES-sensitive VMRs at different p-value thresholds

p<.001 | p<.01 | p<.05

Look for SES-sensitive VMRs that are also associated with other MMH and birth outcome variables

Check for other variables associations at different p-value threshold

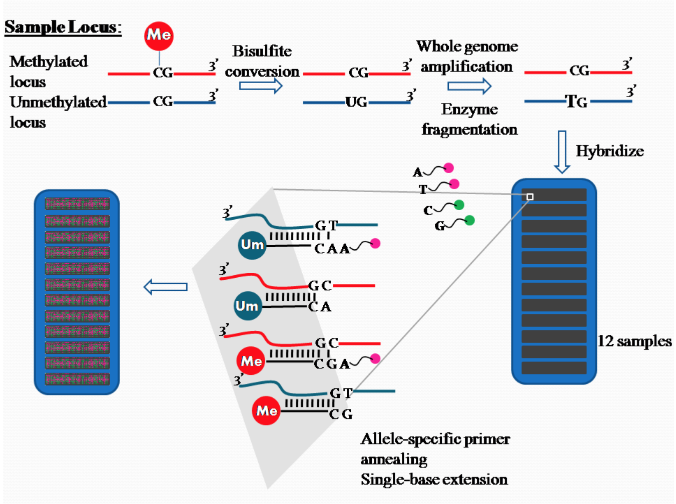
List VMRs that are both found sensitive to SES and other variable

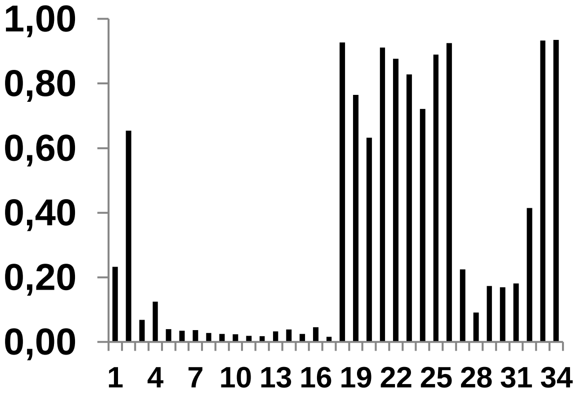
Place unique genes in Panther

Search for biological pathways (corrected for Bonferroni p-value <.05)

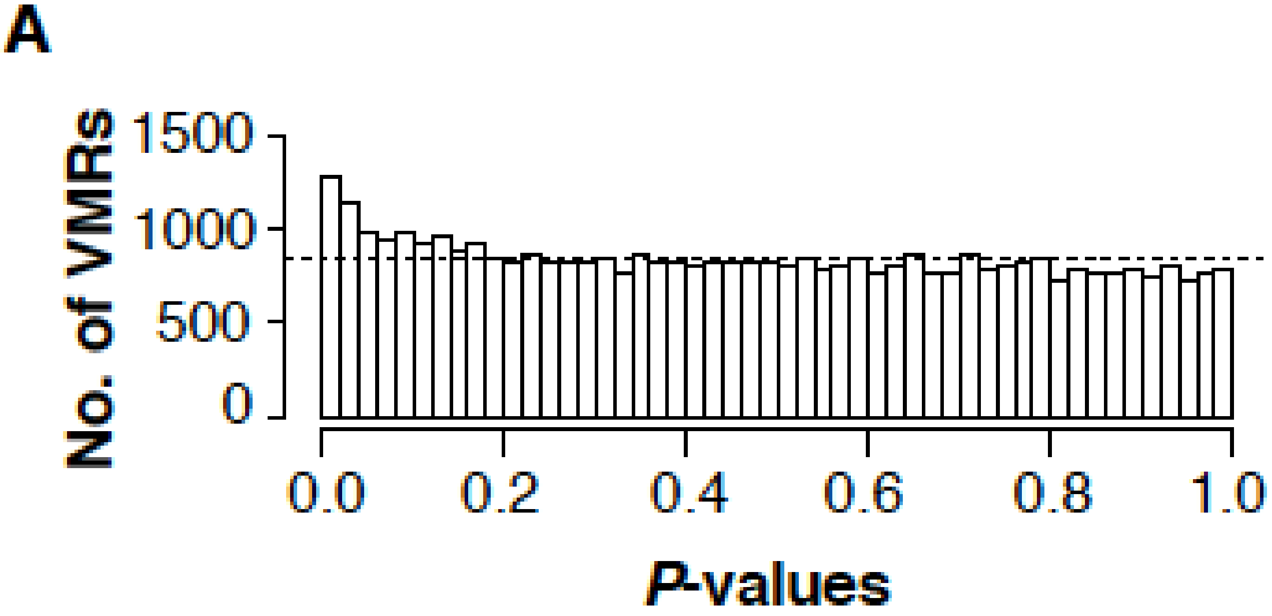
Place genes found in biological pathways in Genemania

Search for top biological function (pass FDR p-value <.05)

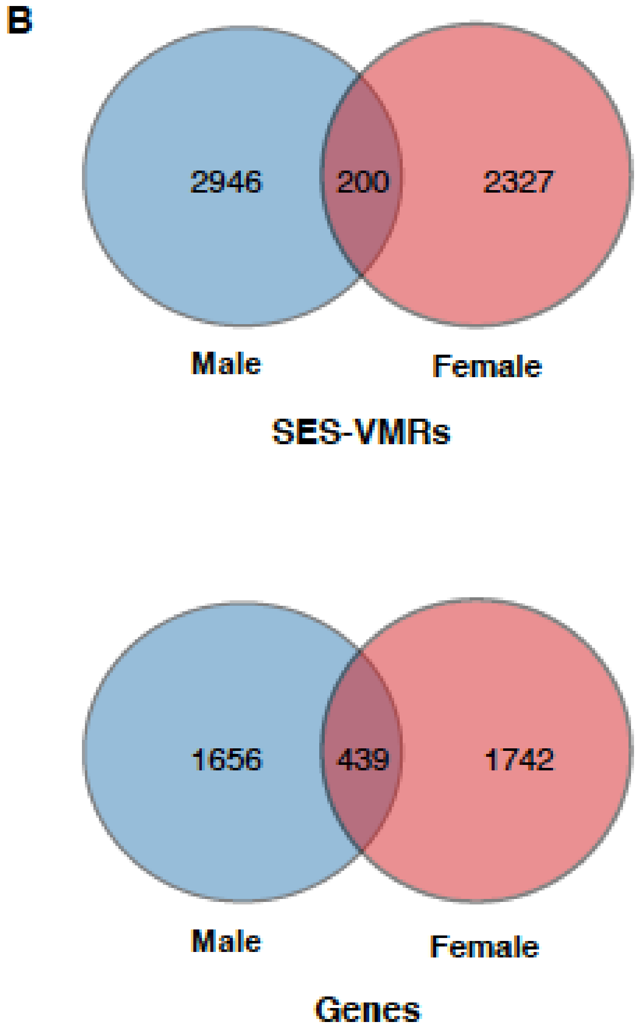




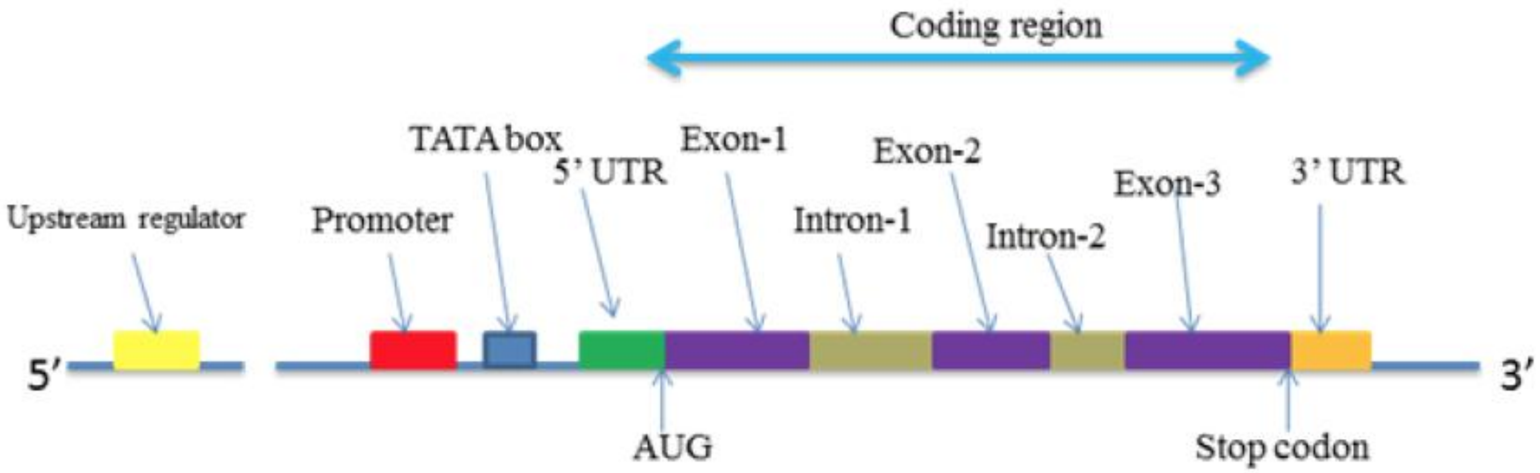
Prenatal SES associates with inter-individual variation in DNA methylation

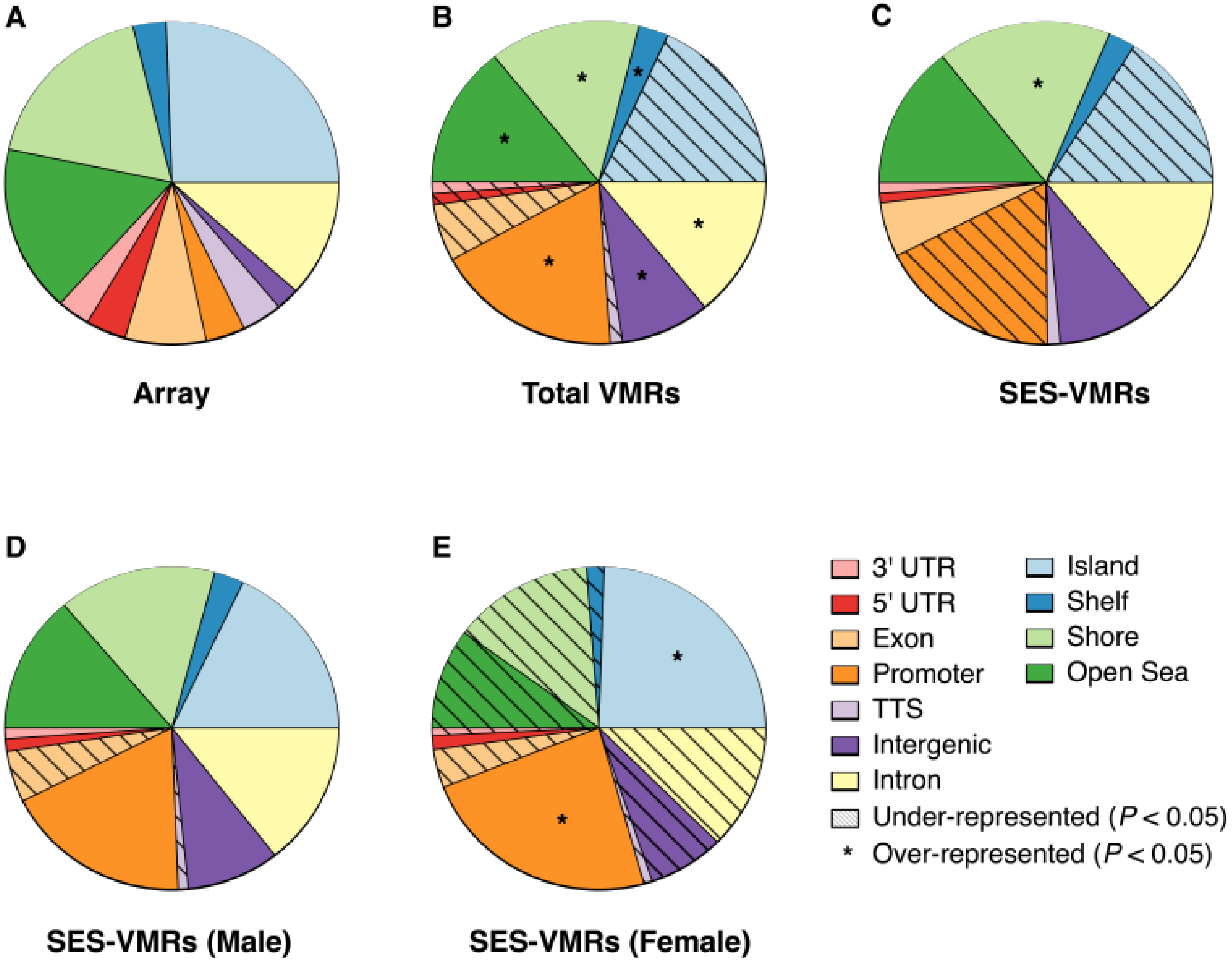


SES-related VMR's are highly sex dependent



Where do these prenatal SES-VMR’s appear across the genome?





Array

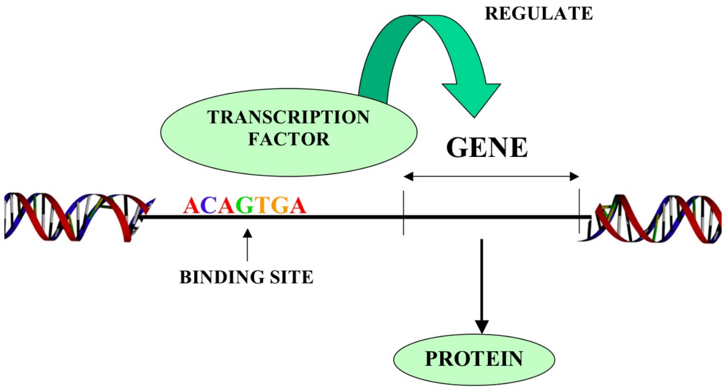
Total VMRs

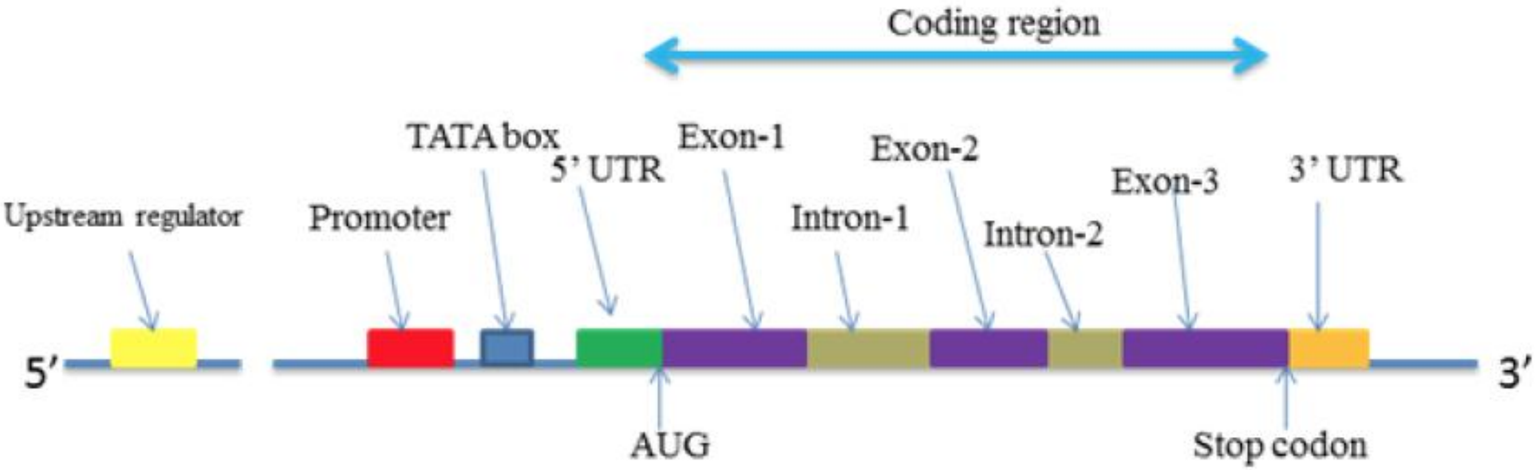
SES-VMRs

SES-VMRs (Male)

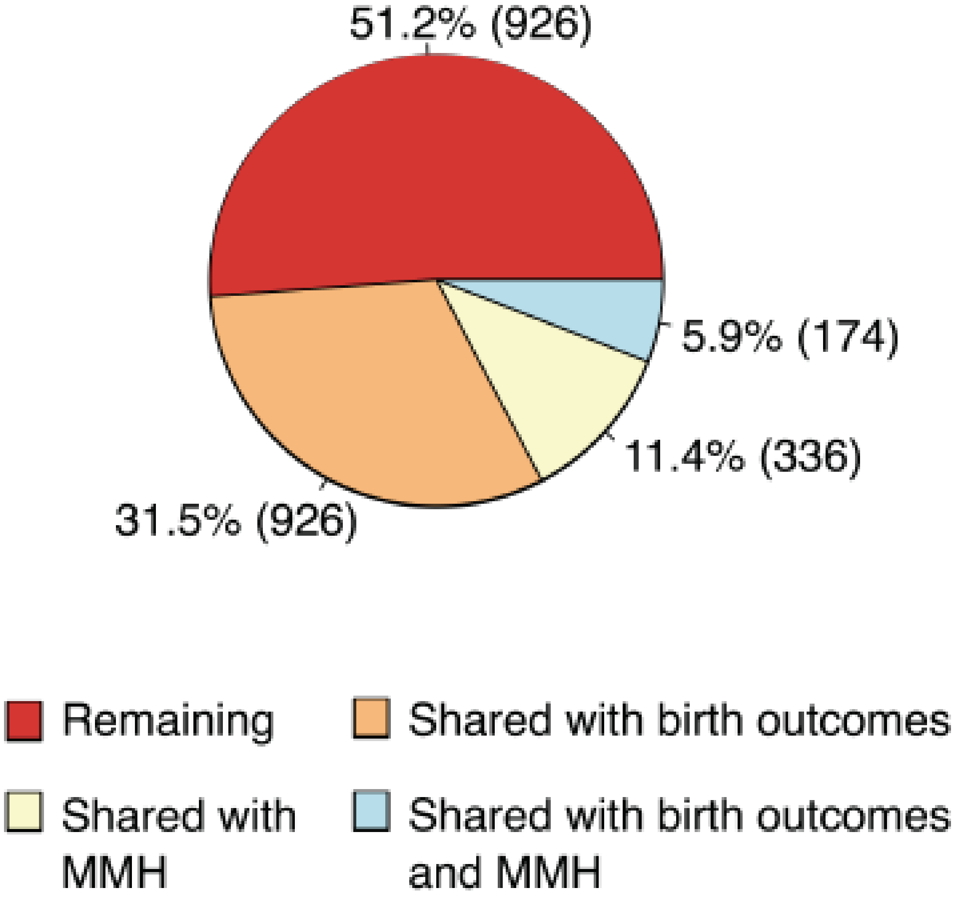
SES-VMRs (Female)

Transcription factors bind to regulatory elements to activate gene transcription and to initiate remodeling of epigenetic states

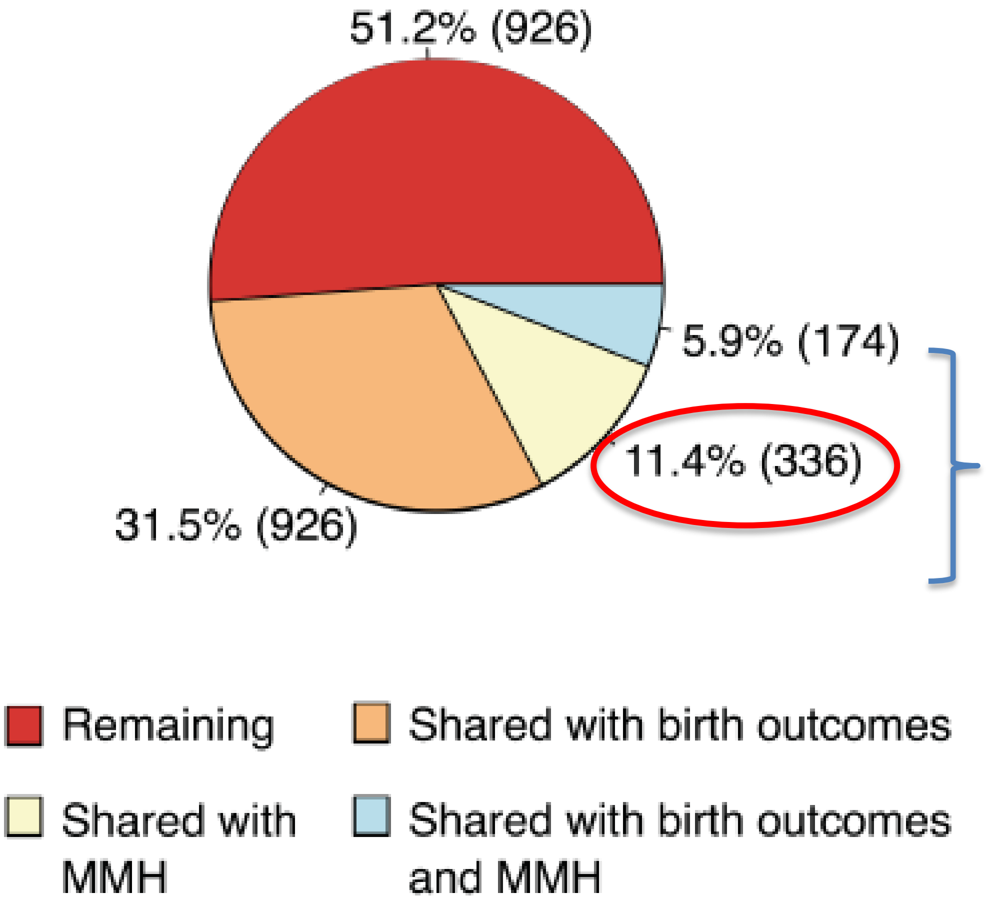




SES-related VMR’s (~50%) associate with birth outcomes and/or maternal mental health



SES-related VMR’s (~50%) associate with birth outcomes and/or maternal mental health



These VMR’s are enriched for GR sites

Challenges for Translational Medicine

- Maternal mental health is an issue of population health
- Demands collaborations across Obstetrics, Psychiatry, Pediatrics
- What are the determinants of antenatal maternal mood?
  - Are these determinants common to mood disorders?
  - What is the pre-conceptual state?
  - Are there effects of peri-conceptual mood?
  - Effects of gender?

Predictive models for identification of vulnerable children
will need to incorporate measures of child genotype –
Precision Intervention?