Epigenetics, Evolution and Development

Behavioral, Metabolic and Immune Implications

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University of Athens

Evolution *vs.* Development =,

The Evo-Devo Field

Phylogeny *vs.* Ontogeny

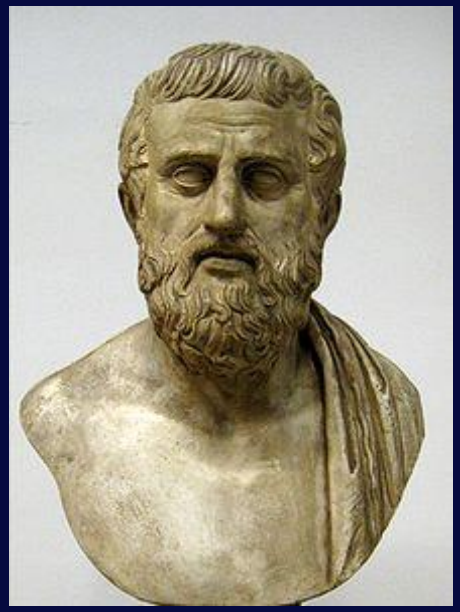
Genetics *vs.* Epigenetics ,

Genetics *vs.* Epigenetics

- **Complex Systems**
- **Evolutionary and Developmental Stressors**
- **Stress and Evo-Devo**

“Πολλά τα δεινά κ’ουδέν ανθρώπου δεινότερον πέλλει….”

‘There are many wonderful things and nothing is more wonderful than the human ...”



Σοφοκλής

Sophocles

496-406 BCE

HUMAN COMPLEXITY: POST(EPI)GENOMIC ERA

Human genome:

About 3 billion bases

About 20 thousand protein-coding genes

About 18 thousand ncRNA-coding genes

About 100-140 thousand transcripts (mRNA, ncRNA)

About 200-260 thousand proteins

Single nucleotide polymorphisms (snp's or snv's), microsatellites or copy number variants :

About >25 million snp's (snv's), 1.5 million indels

About 20 million microsatellites

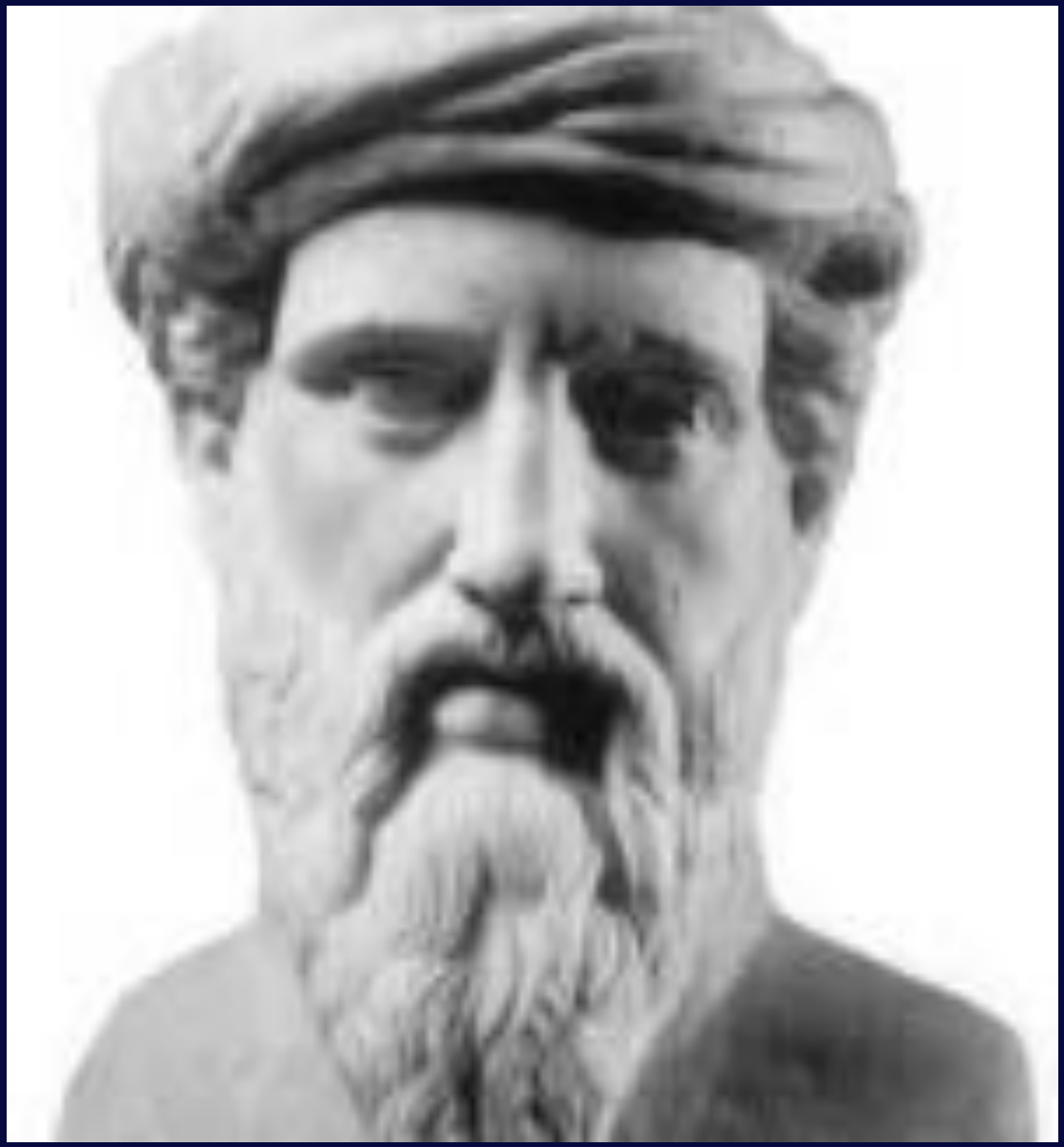
>5000 cnv's (many million bases)

Over 10 k disease-related mutations

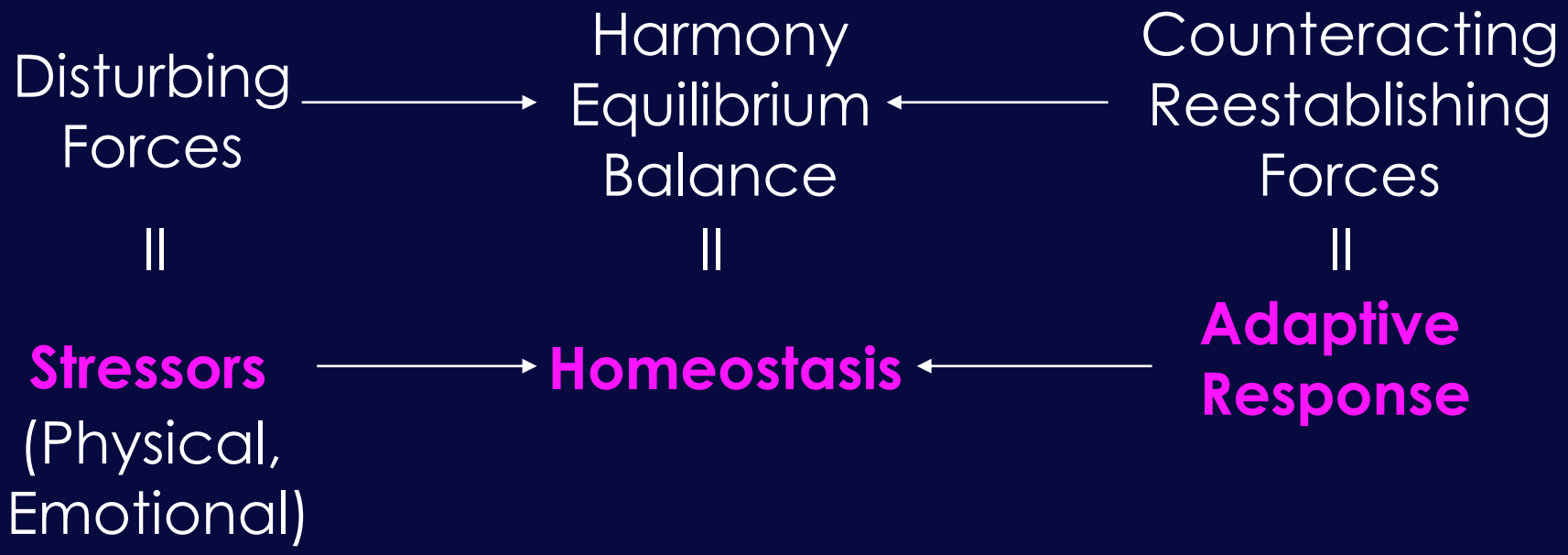
60% of promoters have CpG islands

HUMAN COMPLEXITY: SOME HUMAN BRAIN NUMBERS

- ~ 100 billion neurons ($100x10^{12)}$ x >10.000 synapses per neuron = $>10^{18}$ synapses)
- ~ 100.000 km of fibers
- ~ 1 trillion or more glial cells
- ~ 1.25 terabytes
- ~ 15 Watt lamp (2% of BW uses 20% energy)



Pythagoras 6th century BCE



Pythagoras= *Harmony*
Alcmaeon=*Iso-nomia*
Walter Cannon= Homeostasis

Stress is the State of Threatened (or Perceived Threatened) Homeostasis

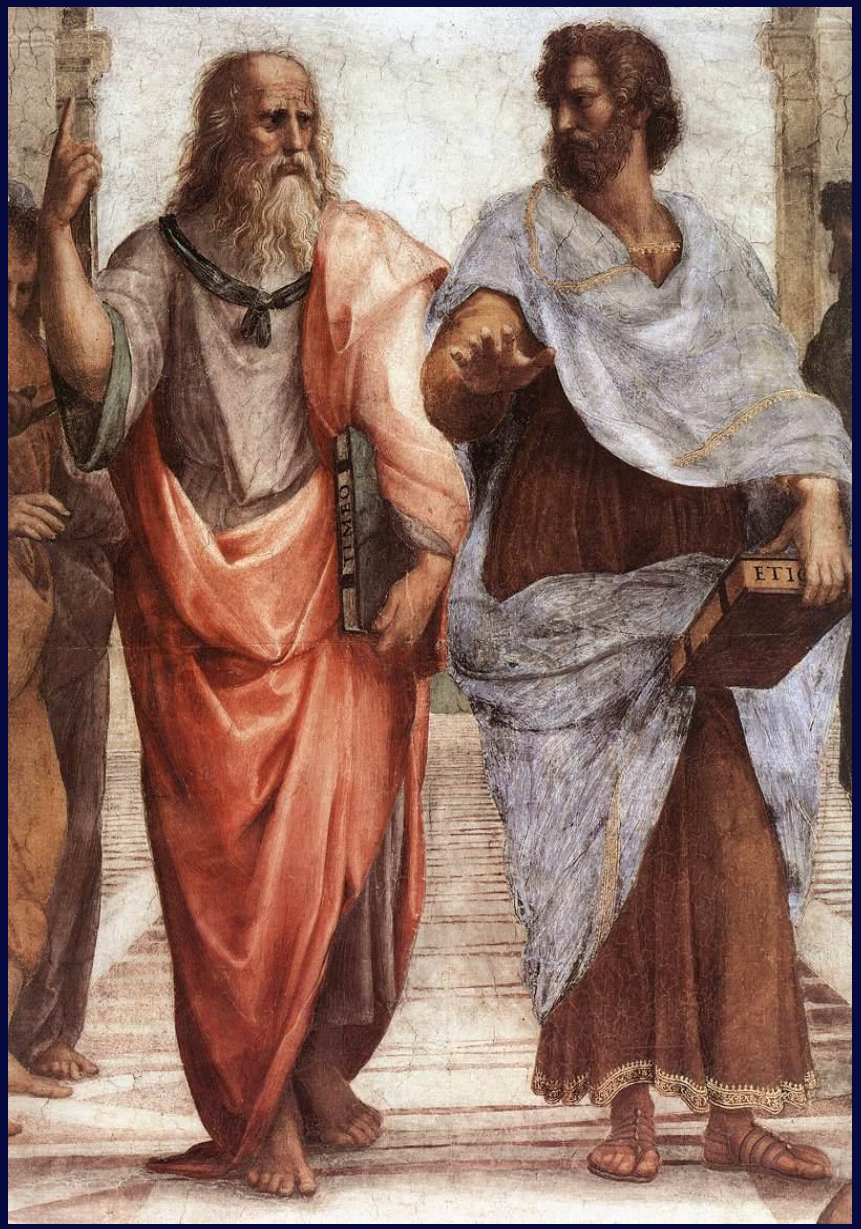
Genetics *vs.* Epigenetics

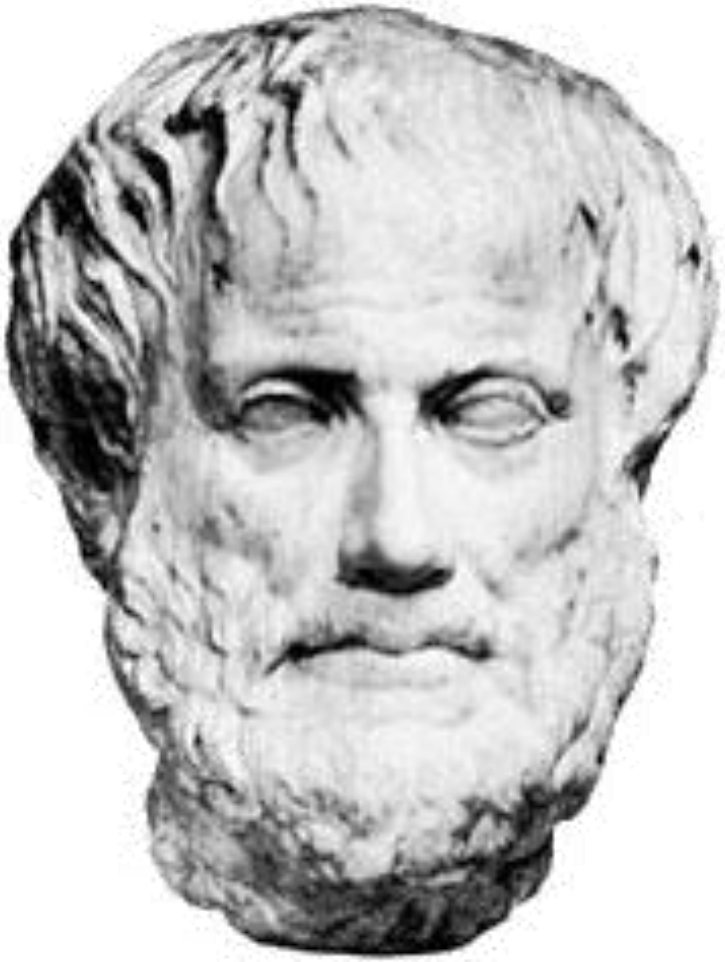
- Complex Systems
- Evolutionary and Developmental Stressors
- Stress and Evo-Devo

Evolutionary and Developmental Stressors

- Starvation
- Dehydration/hemorrhage (gastroenteritis, trauma)
- Injurious agents (infections, toxic substances)
- Adversaries (anticipation, minimization of exposure)
- Tissue injury

Raphael, 16th Century CE





Aristotle by Lysippos 4th Century BCE

The Human Brain

- Plato *(Meno)*

  The innate preformation theory

  (Genetic view)
- Aristotle

  The blank state theory

  (Epigenetic view)

Preformation (Plato)

(unfolding of preformed tissues)

Epigenesis (Aristotle)

(Embryology, Development)

Jean-Baptiste Lamarck (1744–1829)

Charles Robert Darwin (12 February 1809 – 19 April 1882)

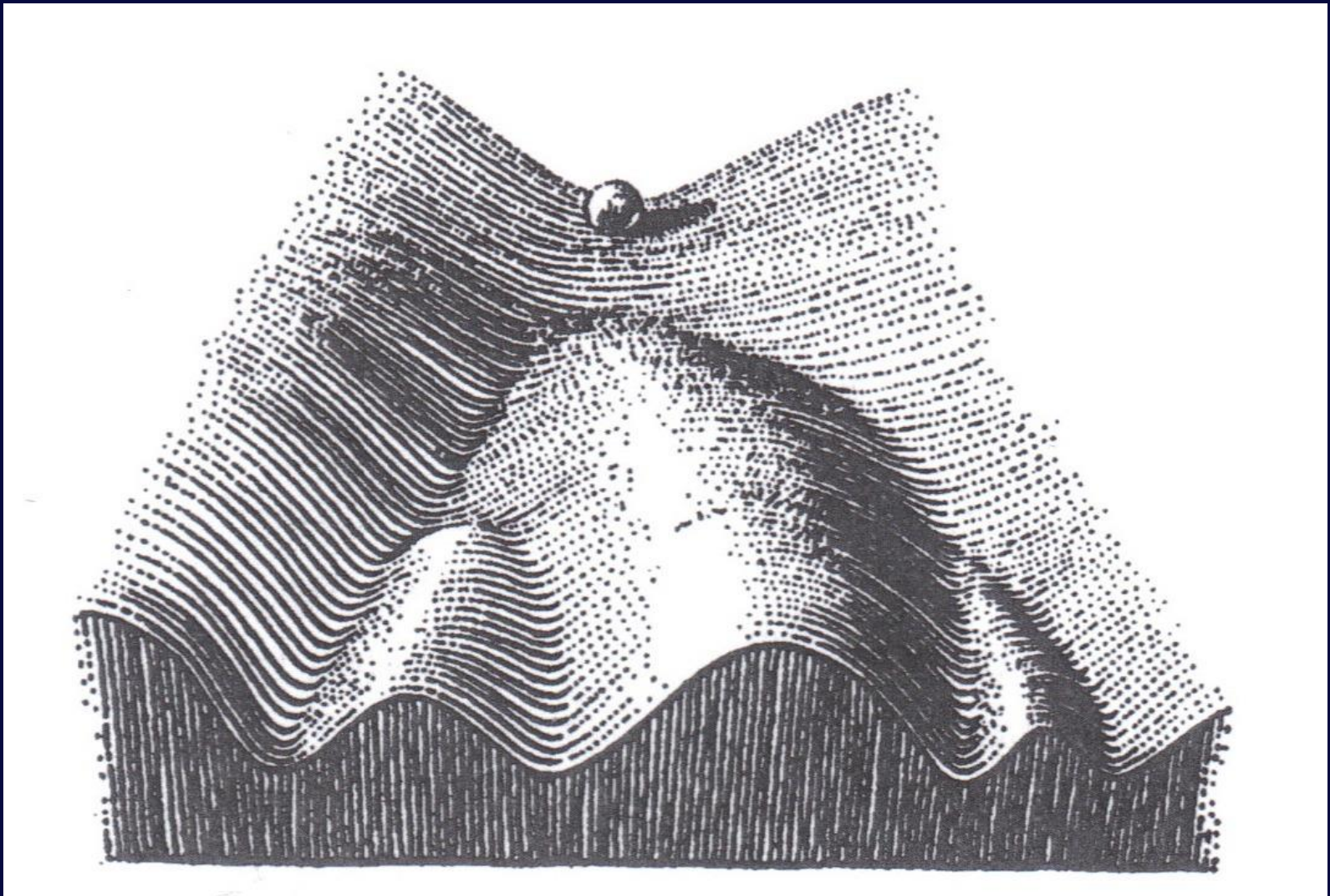
Modern definitions

"Epigenetics are the causal interactions between genes and their products which bring the phenotype into being" 1942

Conrad H Waddington 1900-1975

The Strategy of Genes, MacMillan 1957

“The Epigenetic Landscape”

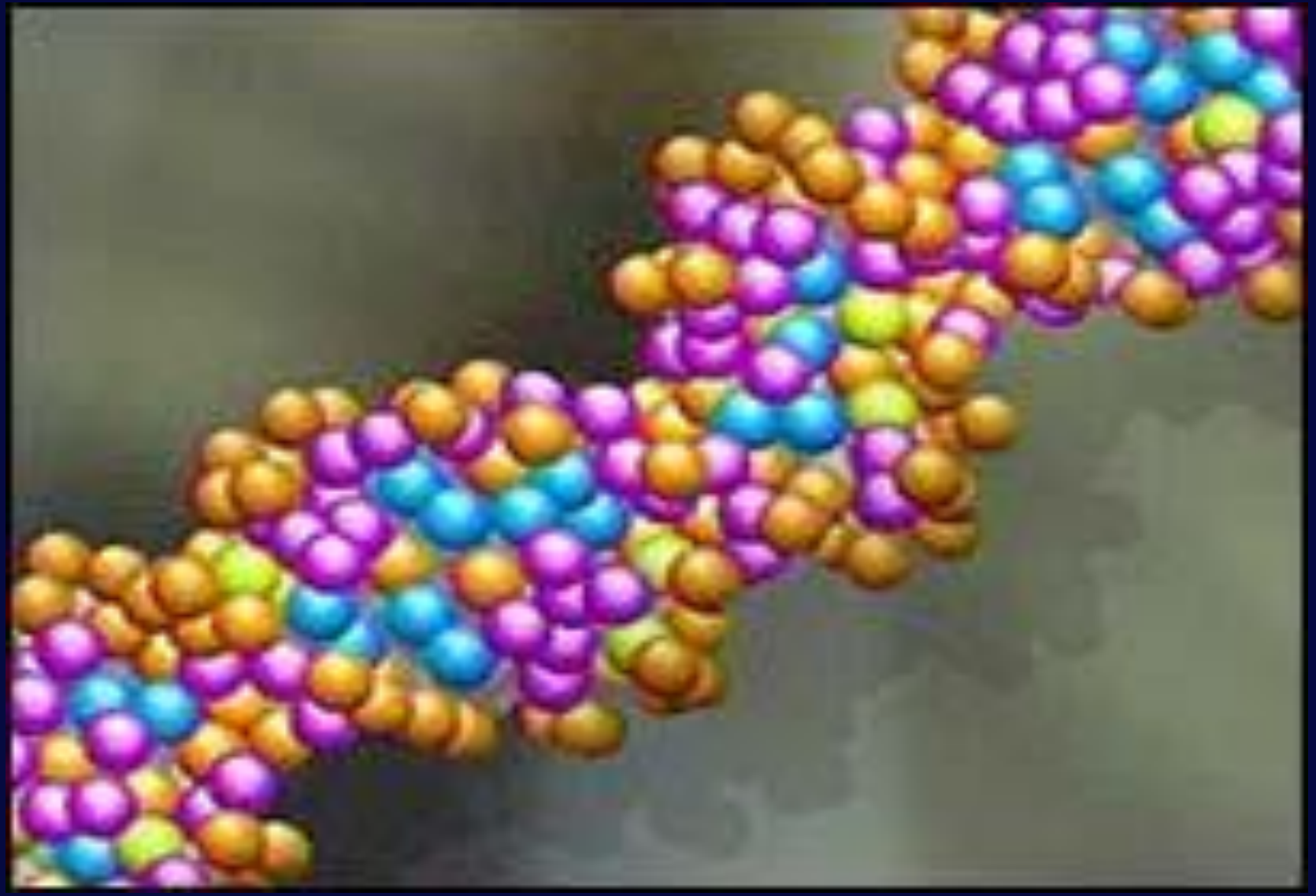


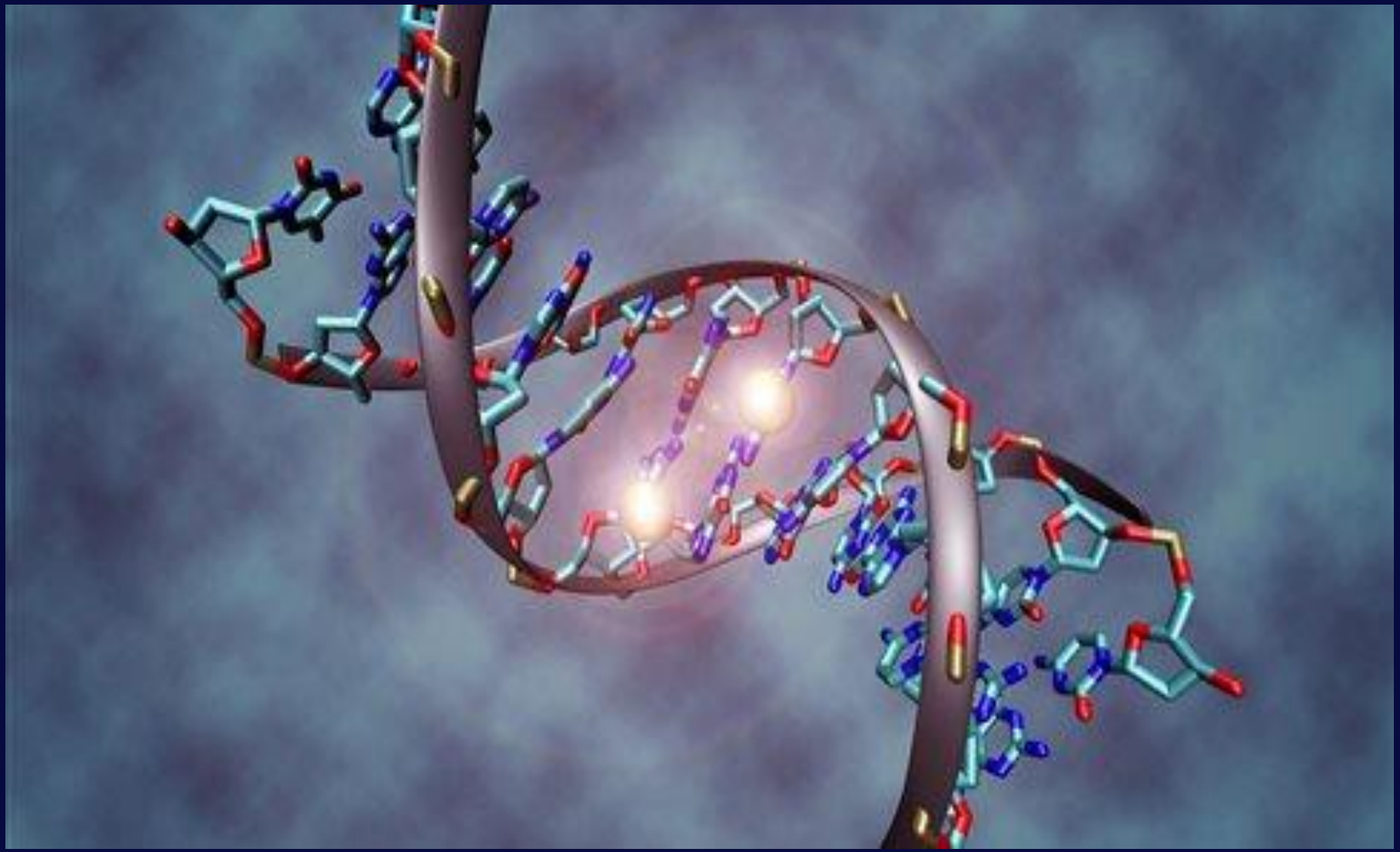
Conrad H Waddington 1900-1975

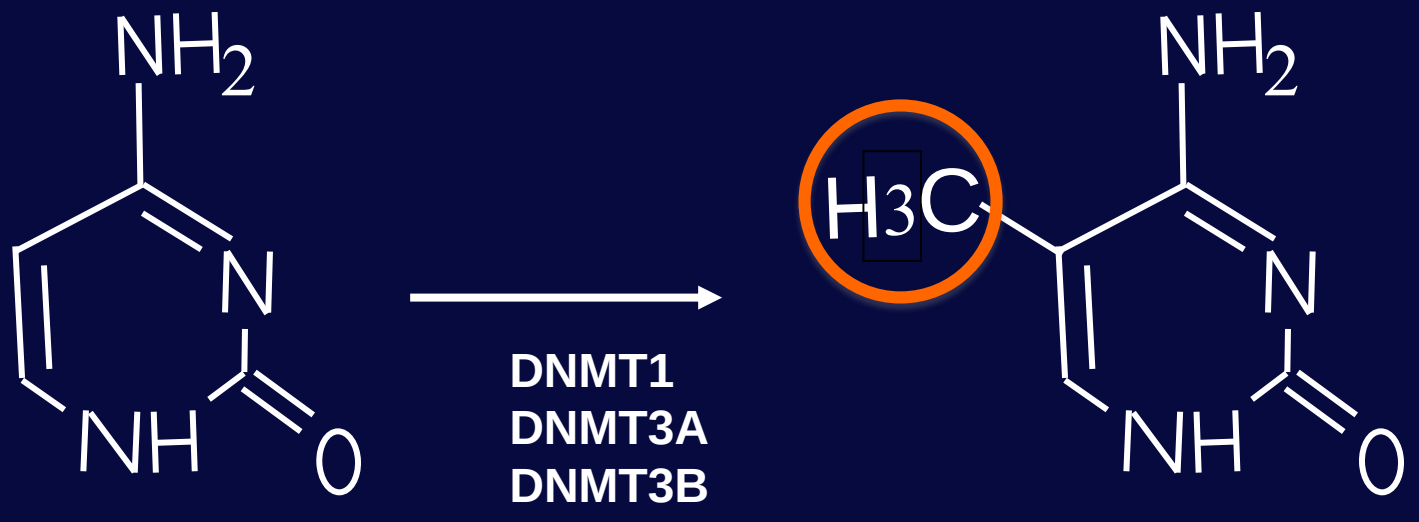
Modern definitions

"*Genetics proposes;*
Epigenetics disposes"

Medawar and Medawar 1983

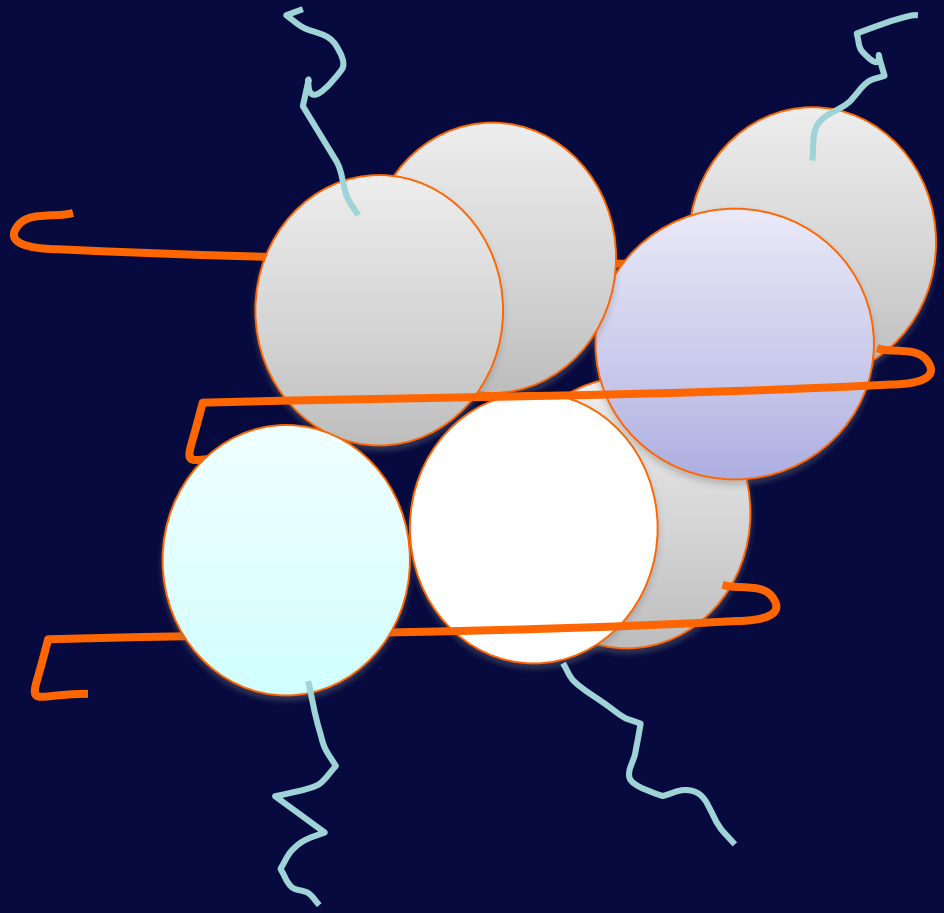


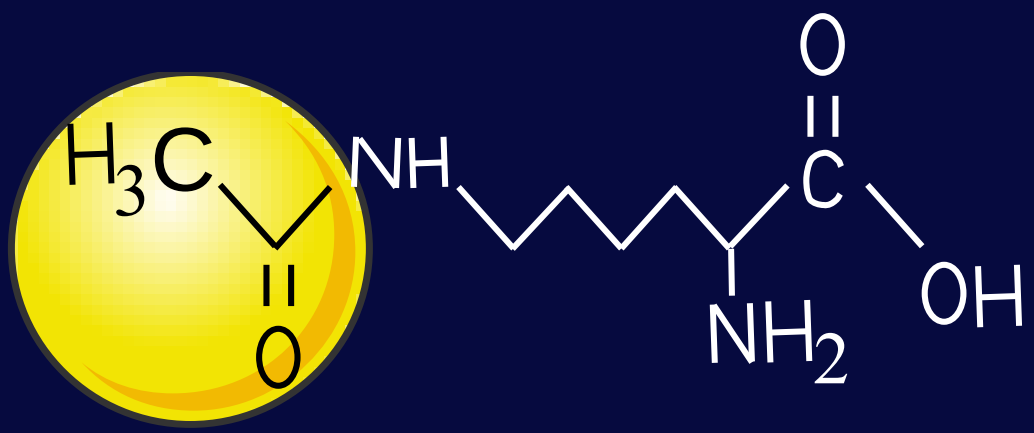




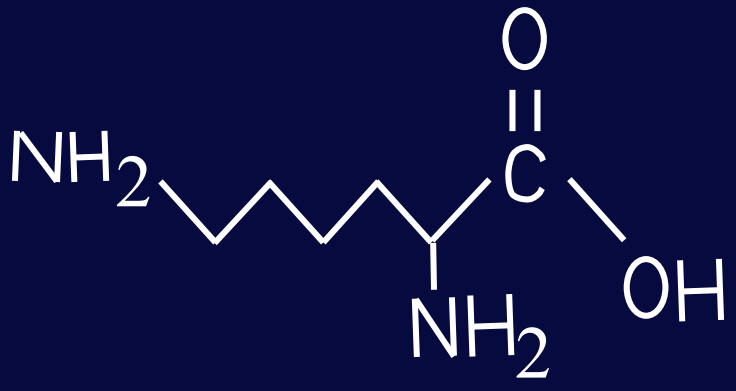
Cytosine

5-Methylcytosine





Acetyl-lysine



Lysine

Forms of Inheritance

- ***Genetic (blueprint)***
- ***Structural***
- ***Steady state***
- ***Epigenetic***
- ***Behavioral/Symbolic (memes)***

Components of Epigenetic Processes

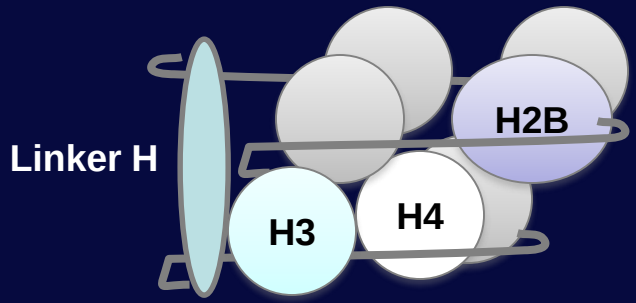
- ***Covalent bonds on DNA***
- ***Post-translational modification of proteins***
- ***DNA-binding proteins or complexes (Polycomb/Trithorax complexes (-/+ hox genes), Panoramix complex – other genes***
- ***miRNAs, other ncRNAs***

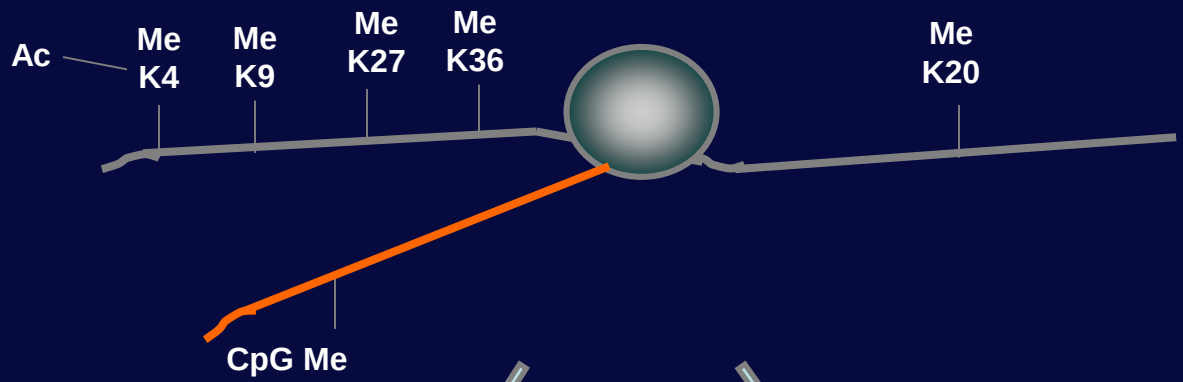
Epigenetic Mechanisms

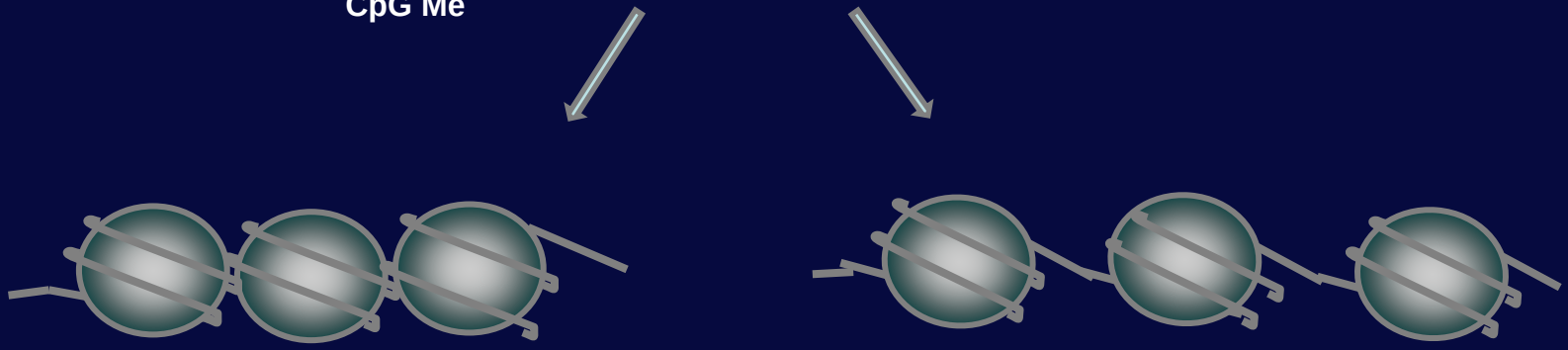
- ***DNA Methylation/demethylation, Acetylation/deacetylation***
- ***Covalent histone modifications***

(methylation, acetylation, phosphorylation, polyADP-ribosylation)

- ***Methyl-CpG domain-binding proteins***
- ***Chromatin compacting or unwinding complexes (polycomb, trithorax groups)***

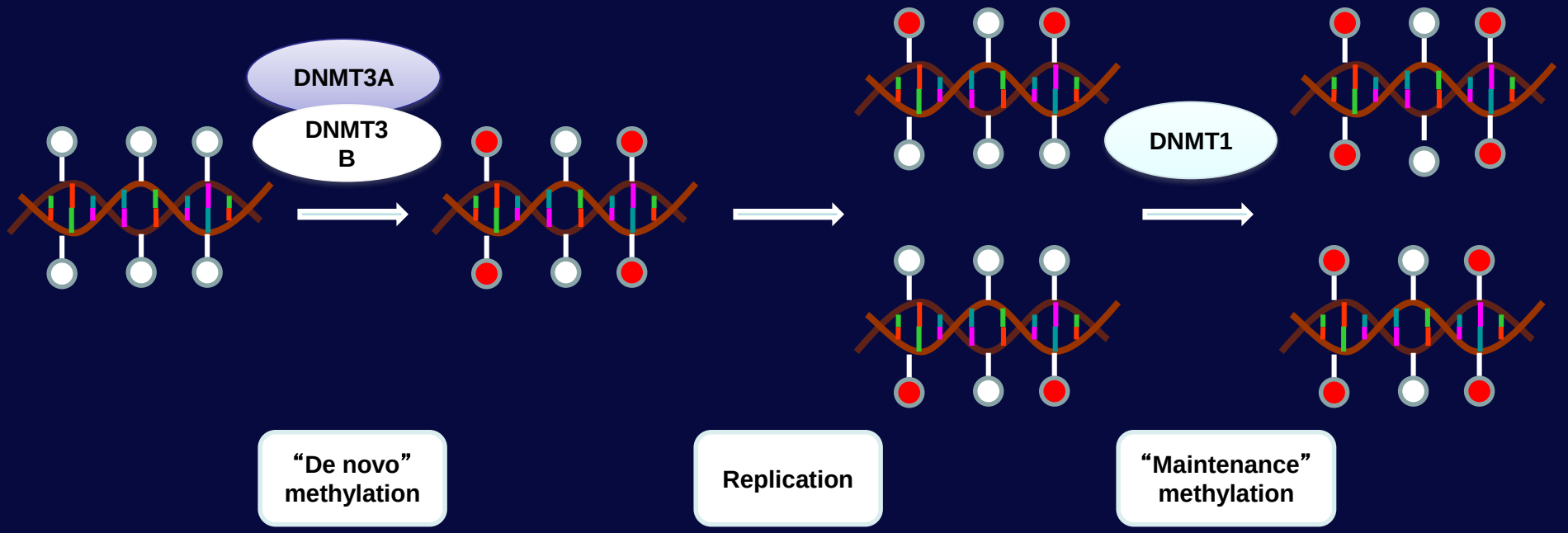


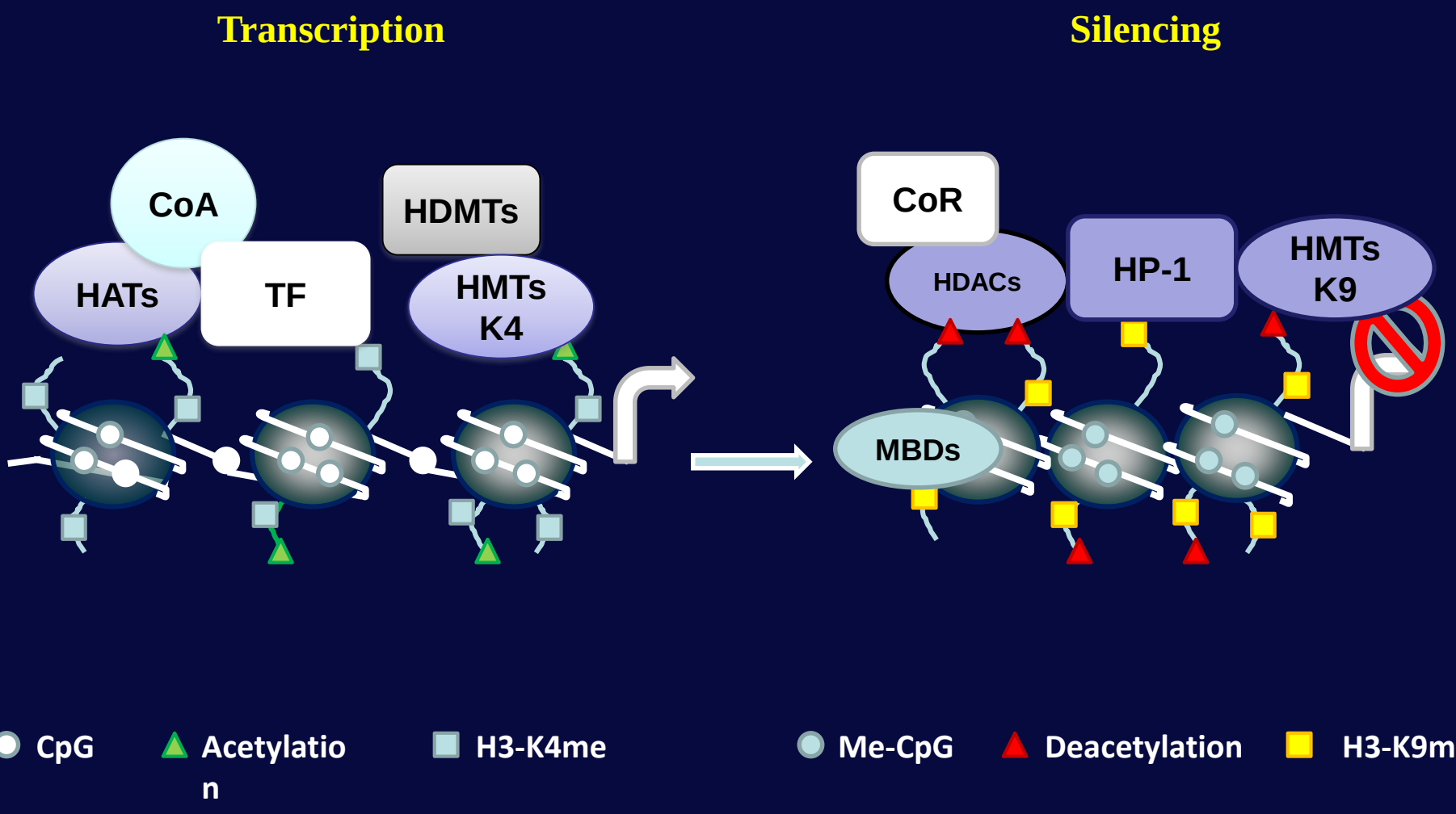




Gene Silencing

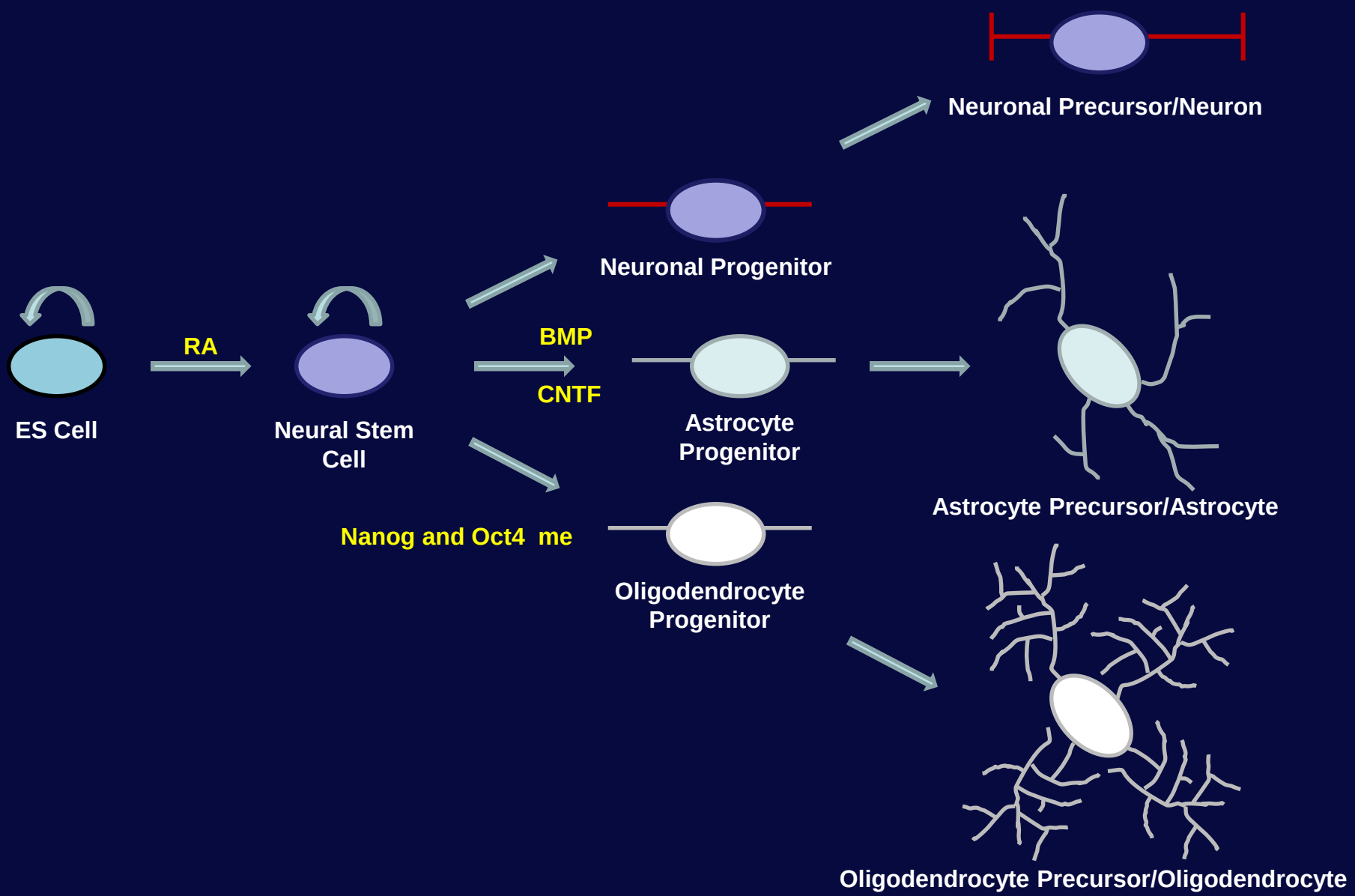
Gene Activation


DNMT3A
DNMT3
B
DNMT1
“De novo”
methylation
Replication
“Maintenance”
methylation


Transcription
Silencing
CoA
HATs
TF
HDMTs
HMTs
K4
CoR
HDACs
HP-1
HMTs
K9
MBDs
CpG
Acetylation
H3-K4me
Me-CpG
Deacetylation
H3-K9me

Epigenetic Functions

- *Cell differentiation*
- *Genomic imprinting*
- *X-chromosome inactivation*
- *Retrotransposon repression*
- *Puberty*
- *Sexual orientation*
- *Right/left handedness*
- *Labor and delivery*
- *Immune cell differentiation*


Neuronal Precursor/Neuron
Neuronal Progenitor
RA
ES Cell
Neural Stem Cell
BMP
CNTF
Astrocyte Progenitor
Astrocyte Precursor/Astrocyte
Nanog and Oct4 me
Oligodendrocyte Progenitor
Oligodendrocyte Precursor/Oligodendrocyte

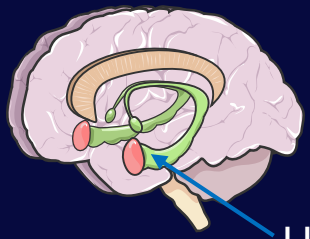
Genetic Imprinting

Source of chromosome 11	Both copies from mother: Mouse smaller than normal	One copy from each parent: Normal mouse	Both copies from father: Mouse larger than normal

Lots of licking and grooming of baby rat

Increased production of serotonin, the “happiness” neurotransmitter in the brain

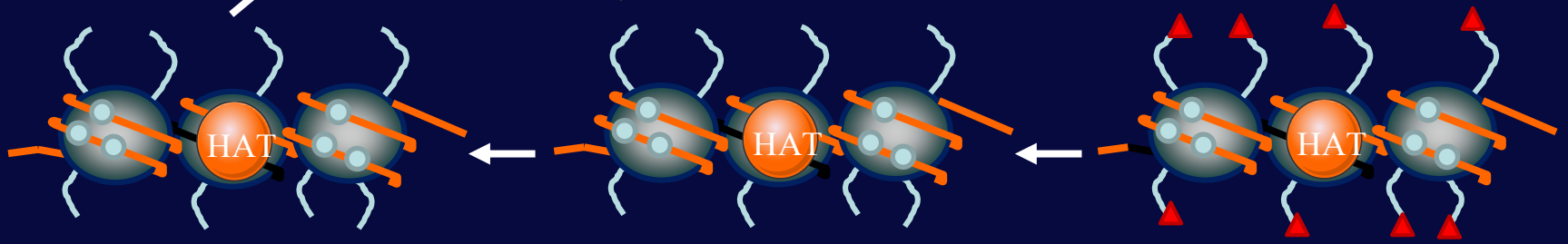
Serotonin signals to the hippocampus to increase production of an enzyme that acetylates histones (HAT)



Hippocampus

HAT

‘Chilled out rat’

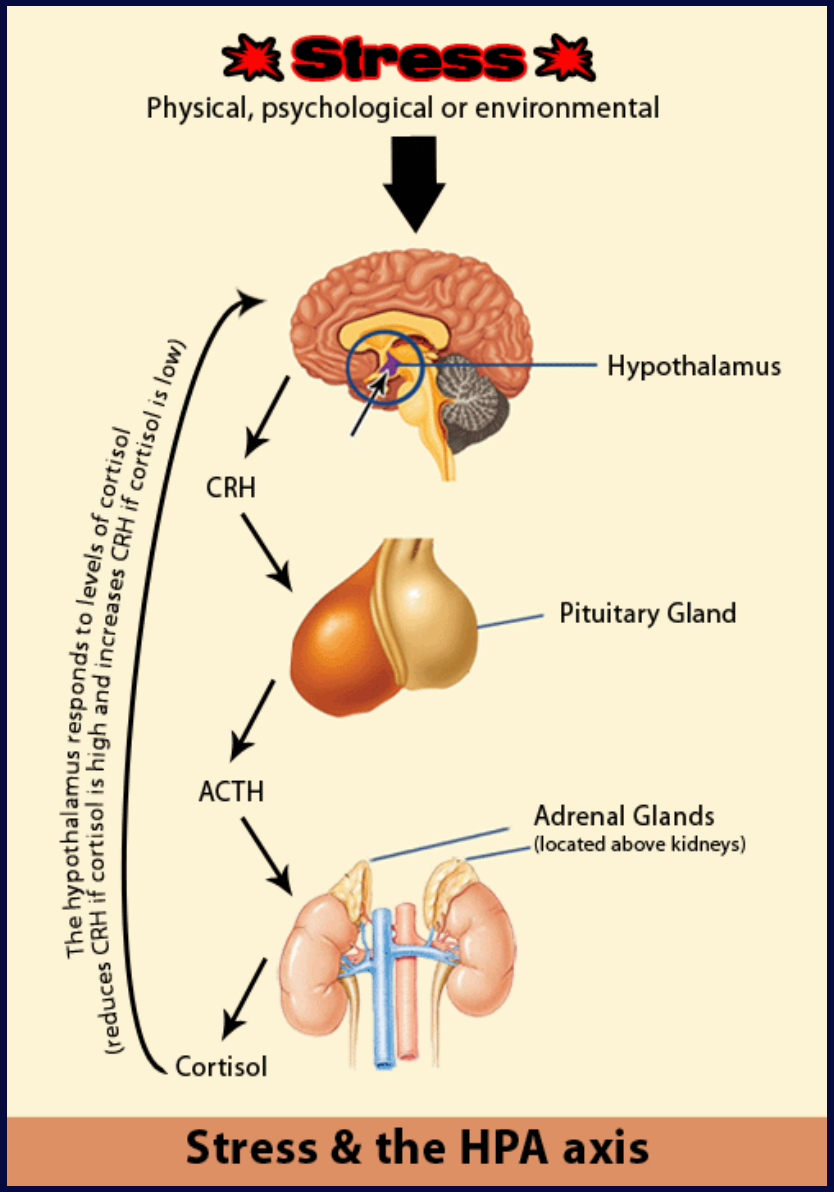


HAT binds to glucocorticoid receptor gene and adds acetyl groups to histone proteins

Histone acetylation creates a more relaxed chromatin environment. DNA methylation is removed

Decreased DNA methylation leads to higher expression of glucocorticoid receptor

‘Stressing’ the epigenome: glucocorticoids



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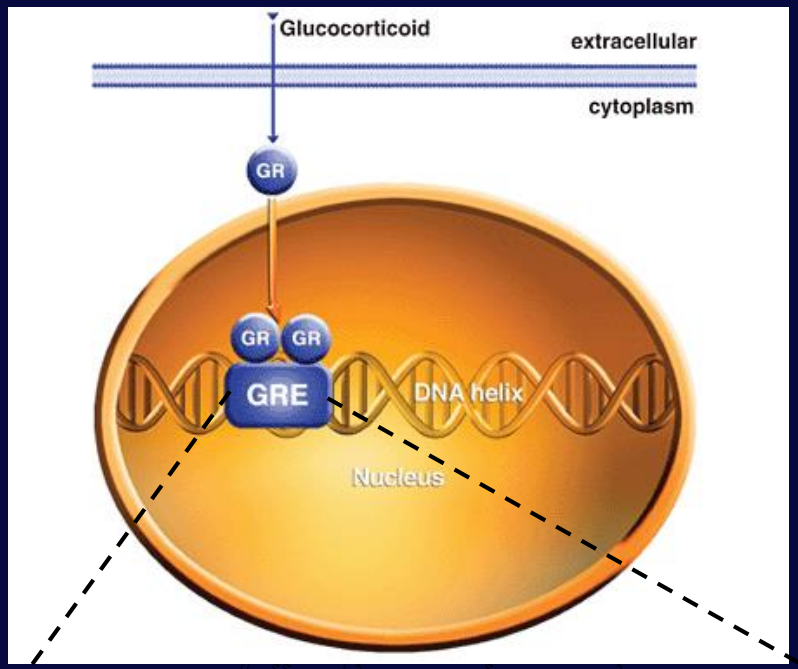

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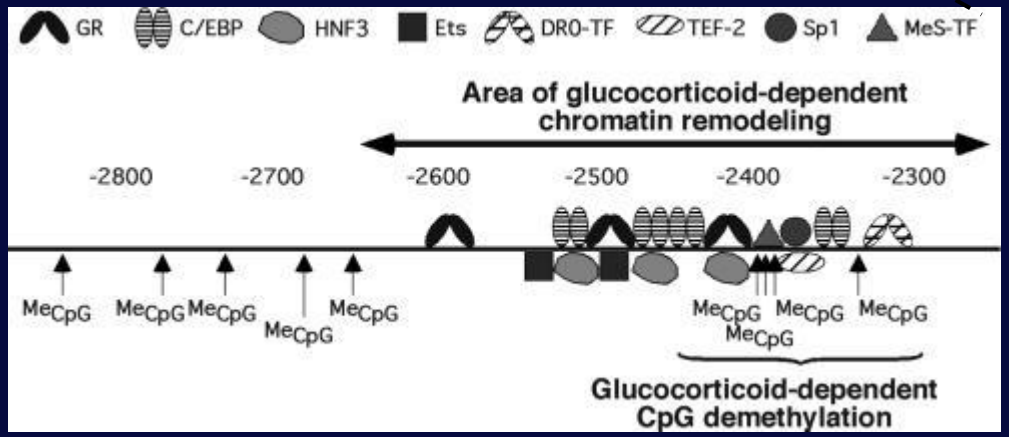
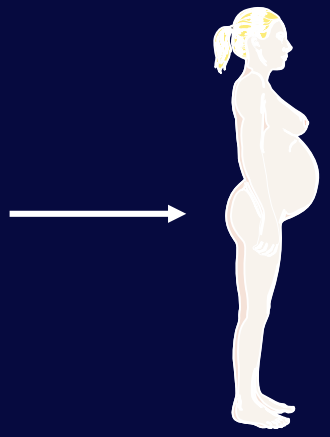


Τηομασσιν ετ αλ. 2001

Malnourished in FIRST trimester

Mary

Baby normal birthweight/MS



Helen

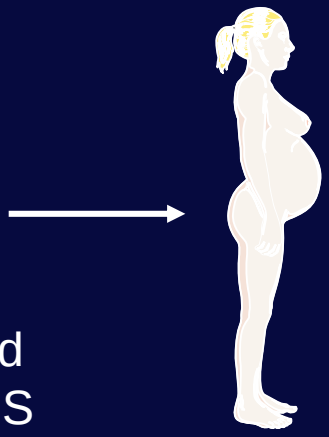
Baby likely to be heavier than average/ MS

Nikolas

Malnourished in THIRD trimester

Vicky

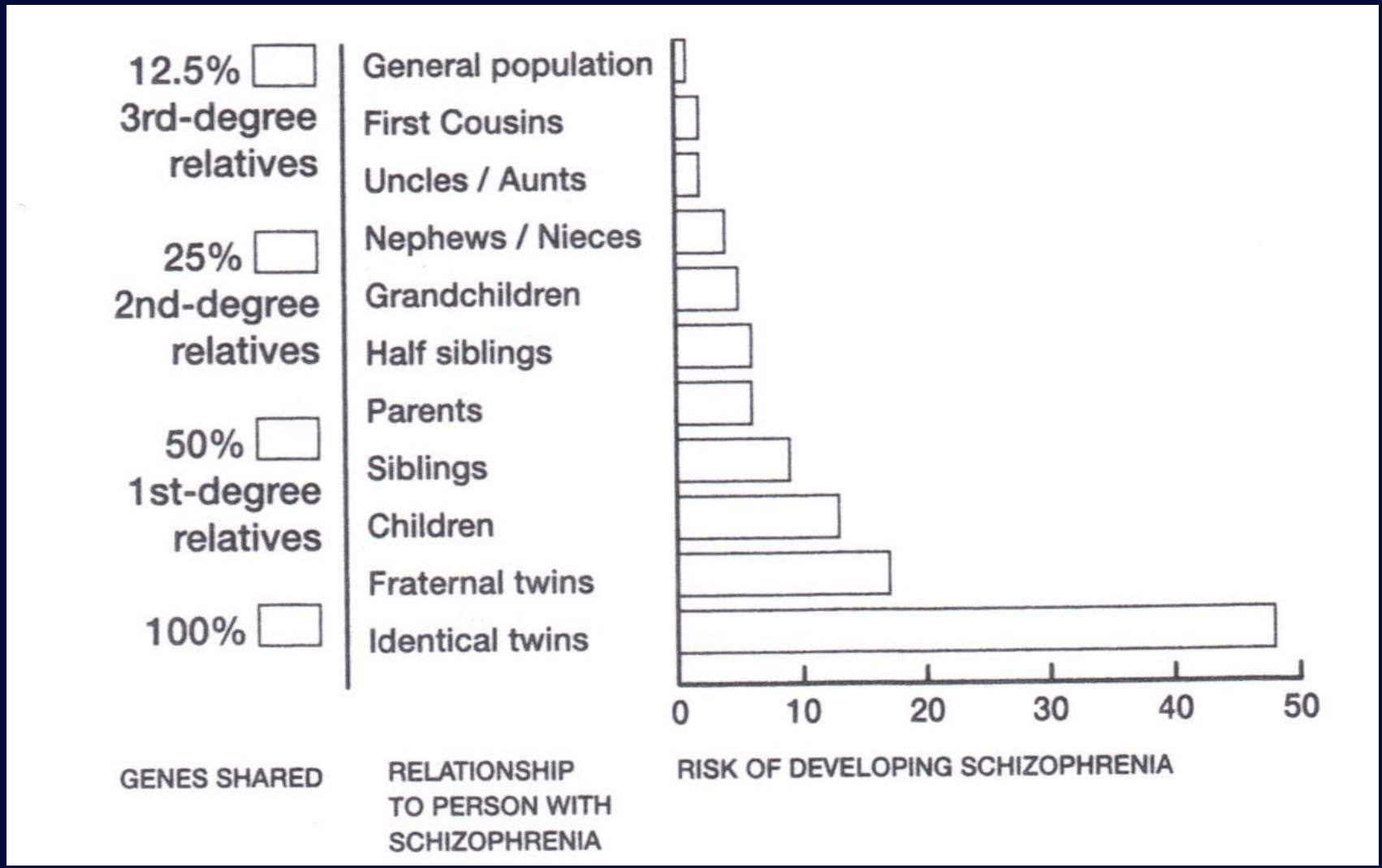
Baby reduced birthweight/MS



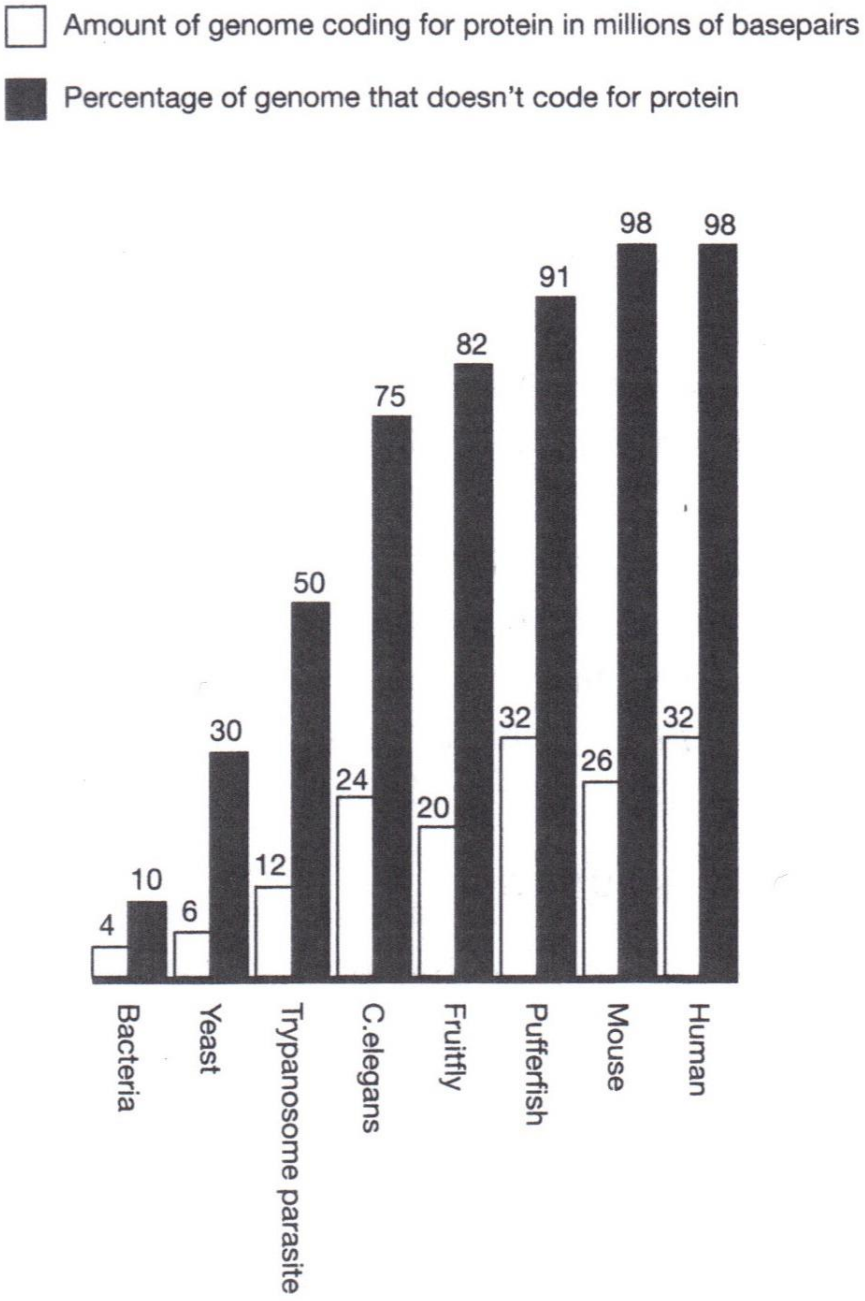
Kelly

Baby normal birthweight/ no MS

George

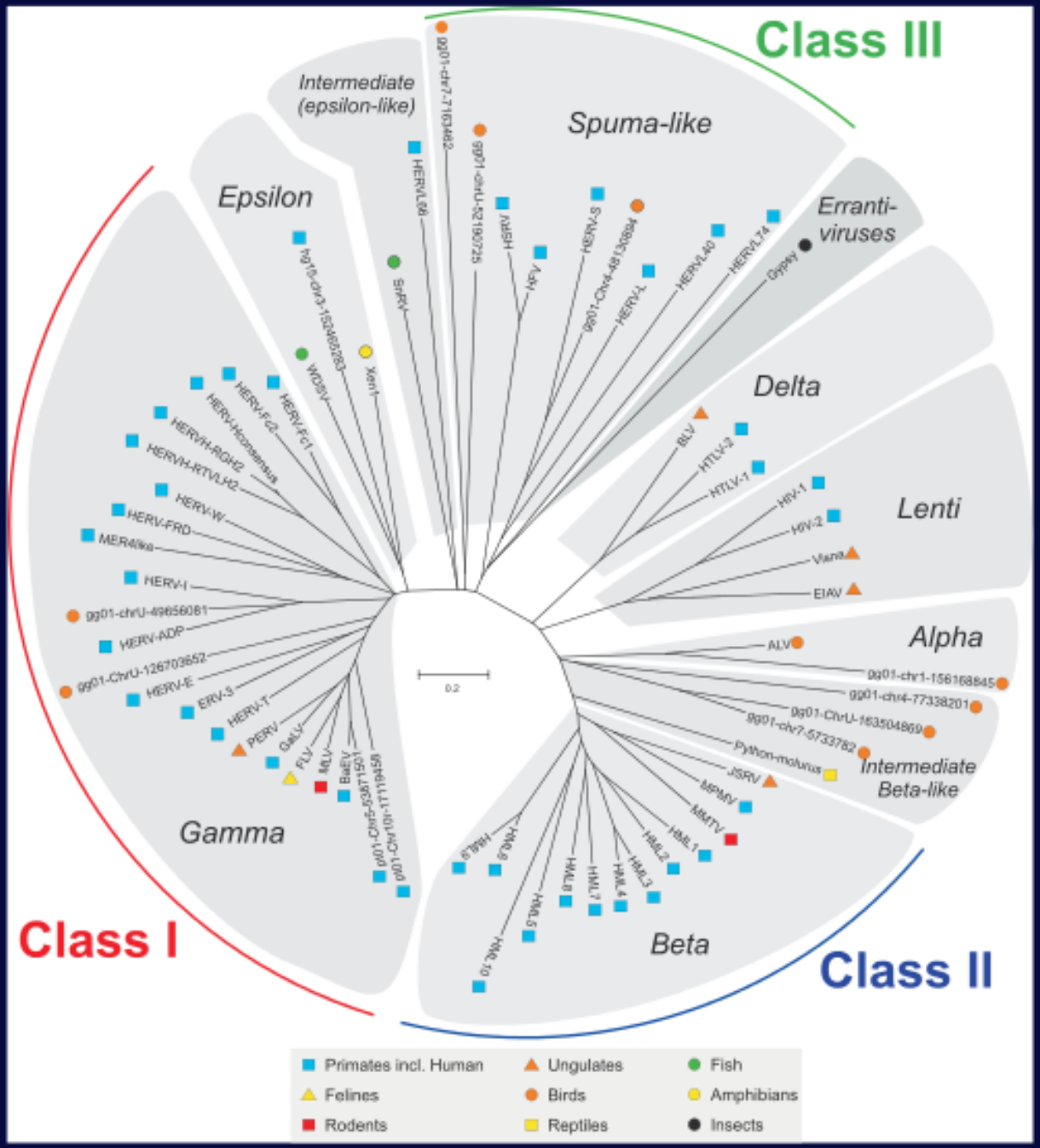


US Surgeon General's Report


Amount of genome coding for protein in millions of basepairs
Percentage of genome that doesn't code for protein
4
10
6
30
12
50
24
75
20
82
32
91
26
98
32
98
Bacteria
Yeast
Trypanosome parasite
C.elegans
Fruitfly
Pufferfish
Mouse
Human

Epigenetics of Retrotransposons (piRNAs)

- *40-60% of genome of retroviral origin*
- *10% of genome Alu repeats*
- *10,000 HERV-K retrotransposons*
- *5,000 SVA retrotransposons*



:ative unrooted Pol neighbor joining (NJ) dendrogram

Genetics *vs.* Epigenetics

- Complex Systems –Stress Concepts
- Evolutionary and Developmental Stressors
- Stress and Evo-Devo

Genotype + Environment = Phenotype/Disease Phenotype

- *Epigenetic control mechanisms evolve*
- *There is a Lamarckian dimension in evolution*
- *Imprints and methylation marks are erased and reestablished de novo stochastically in each generation*

Epigenetic Regulation of Pediatric "Endocrine"-related Genes

GRalpha, hippocampus, NGF-IP (stress, sexual abuse)

GRalpha, liver (obesity)

PPARalpha, liver (obesity)

Pdx1, islets of Langerhans (diabetes mellitus type 2)

ERalpha, hypothalamus (female behavior)

AR brain (male behavior), skin (hirsutism)

FTO (obesity) -> IRX3 homeobox transcription factor

Nanog, Oct 4 (neural stem cell differentiation)

DLK1-MEG3 (obesity, diabetes type 1)

Lxralpha (obesity, carbohydrate intolerance)

Kisspeptin –puberty

CRH- labor and delivery

FKBP5-Depression Rx

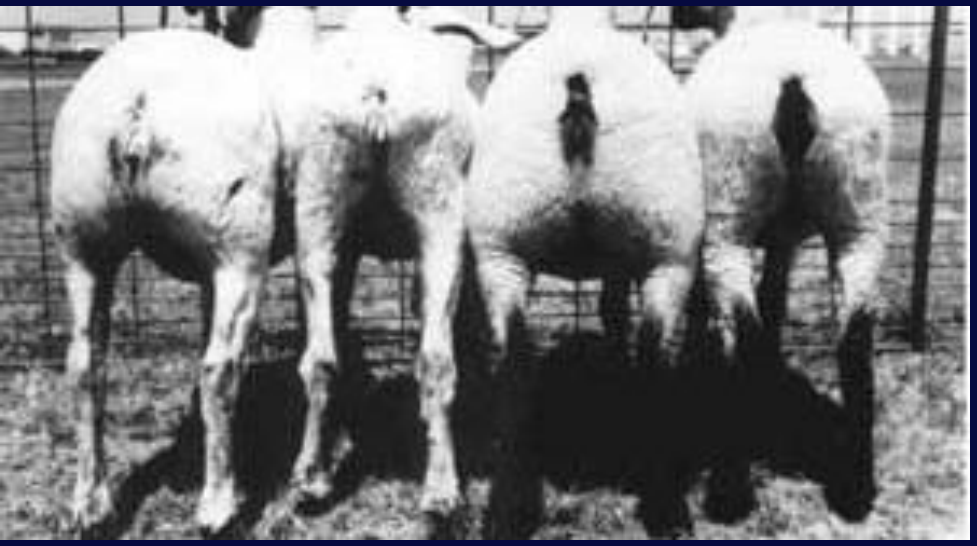
Human endogenous retroviral element K10 (HERV-K10) is altered in *in vitro* handled blastocysts

Decreases methylation of the imprinted DLK1-MEG3 gene region on chromosome 14q32.2.

Dimitriadou et al., Stress 2013

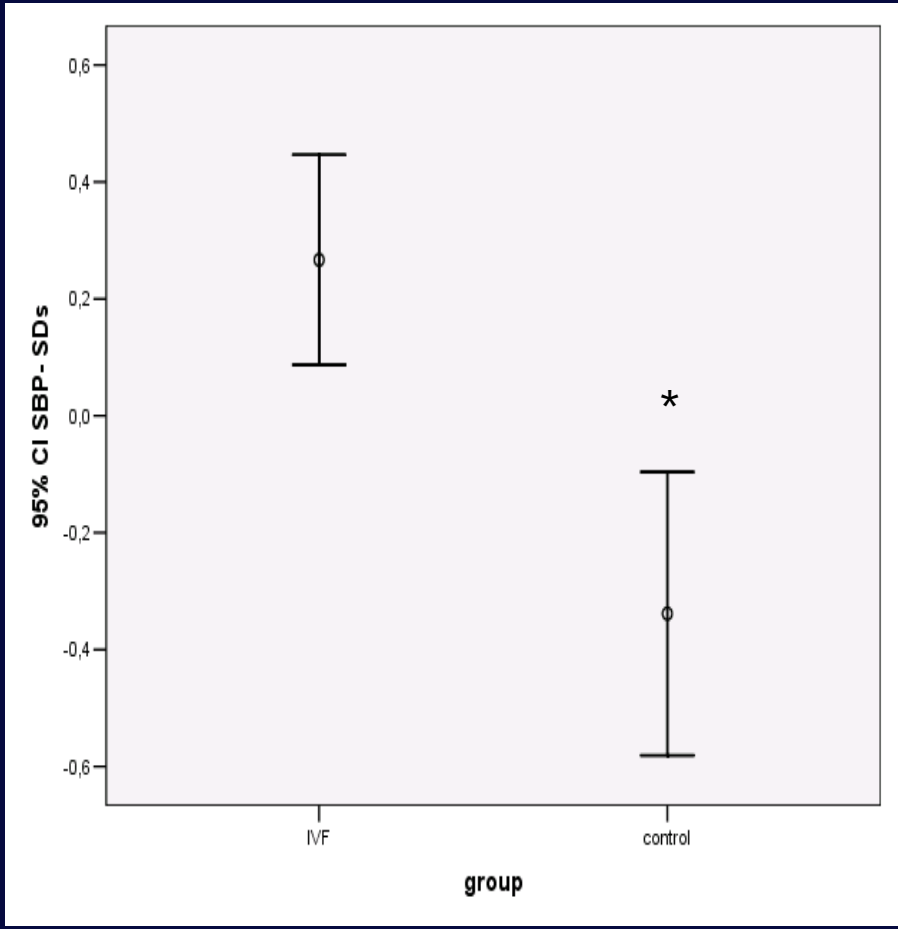
Venus Callipyge

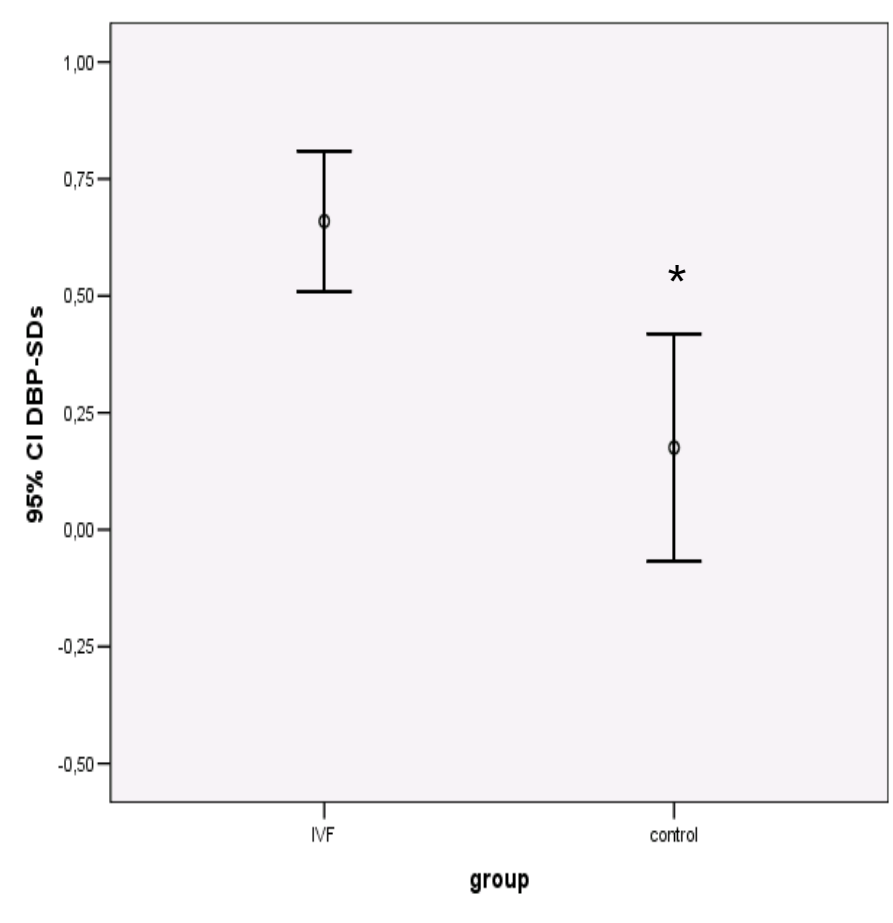
Callipyge sheep



Callipyge

Systolic and Diastolic Blood Pressure-SDS



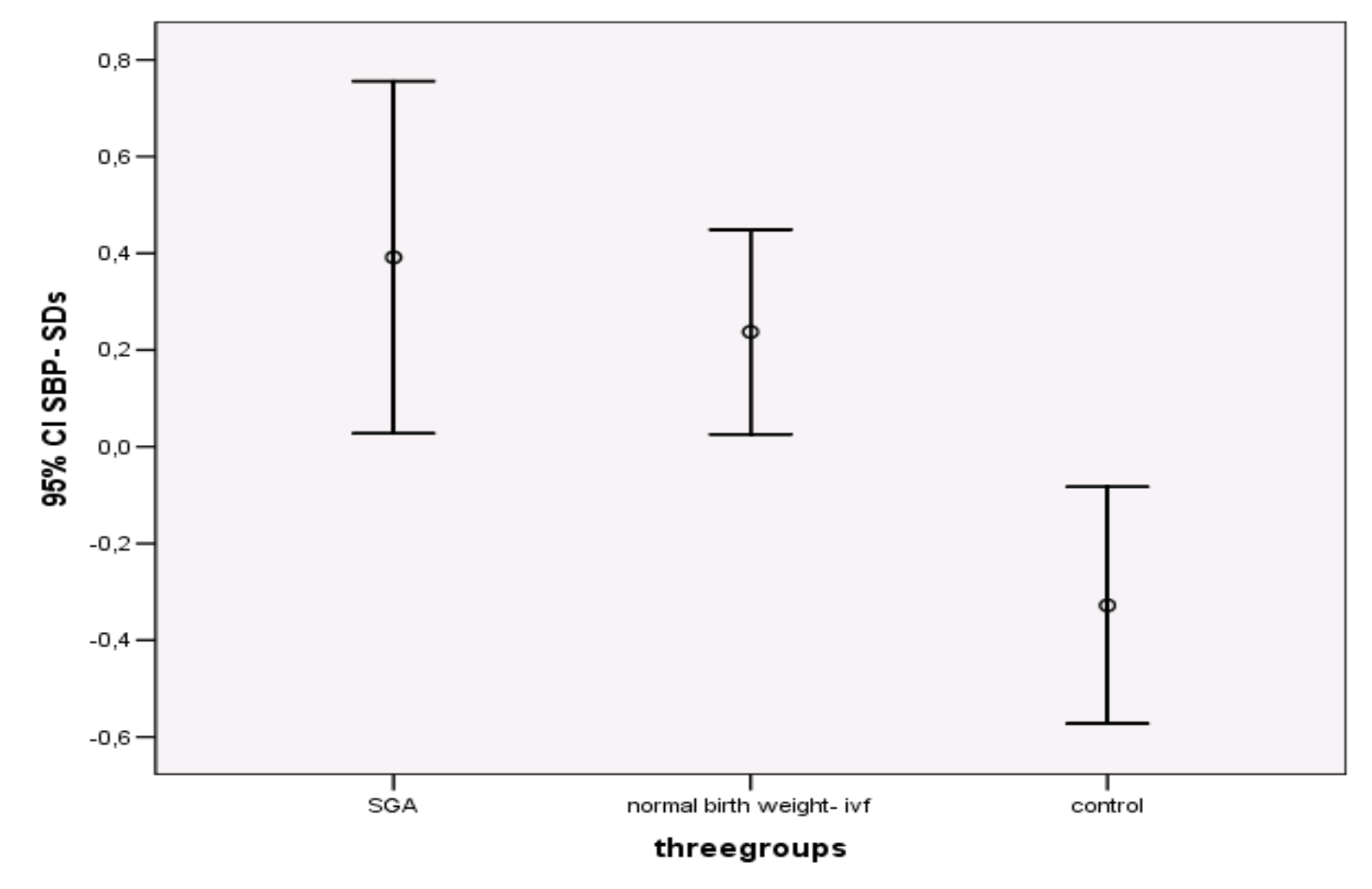


p=<0.001

p=<0.001

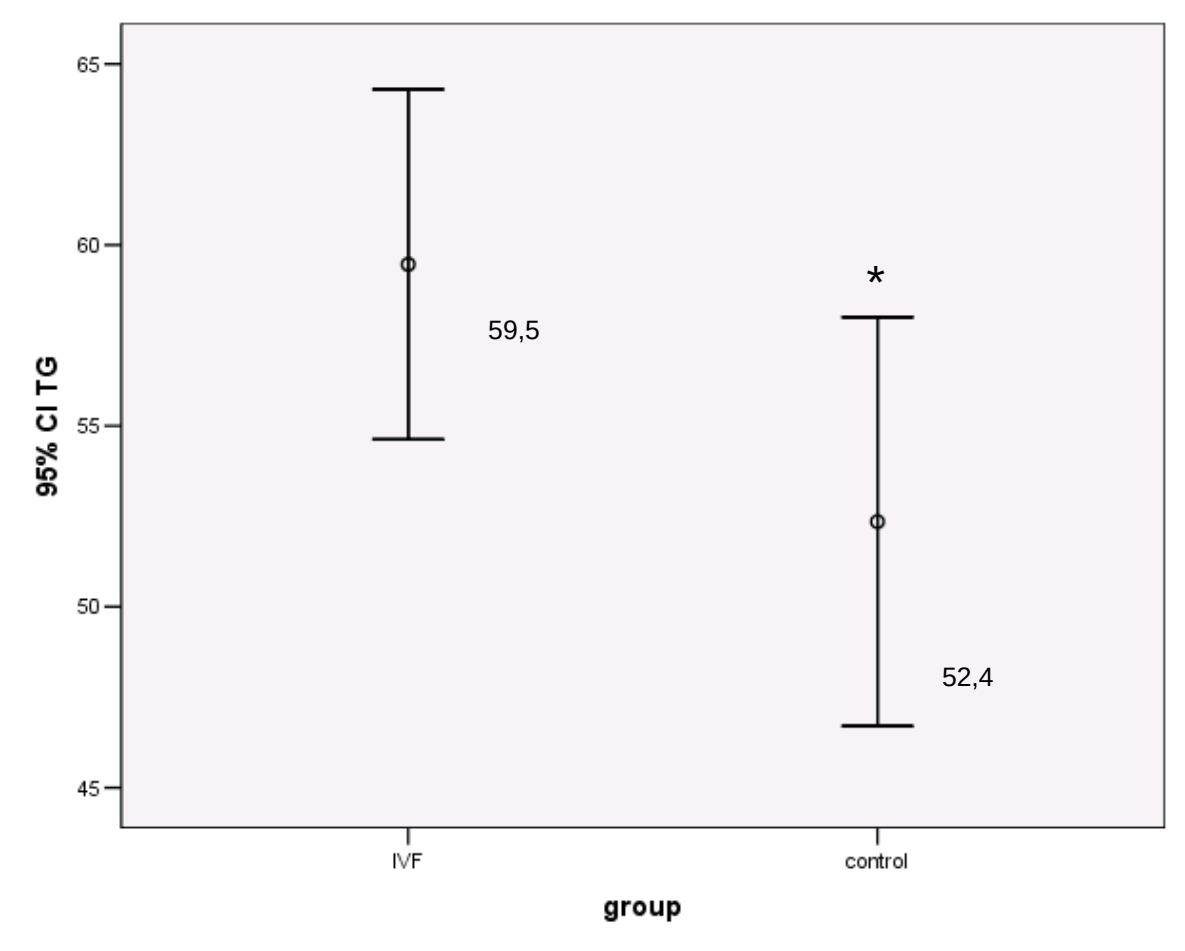
Sakka *et al*. Fertility Sterility 2010

Results
Comparison between SGA-IVF, AGA-IVF and controls



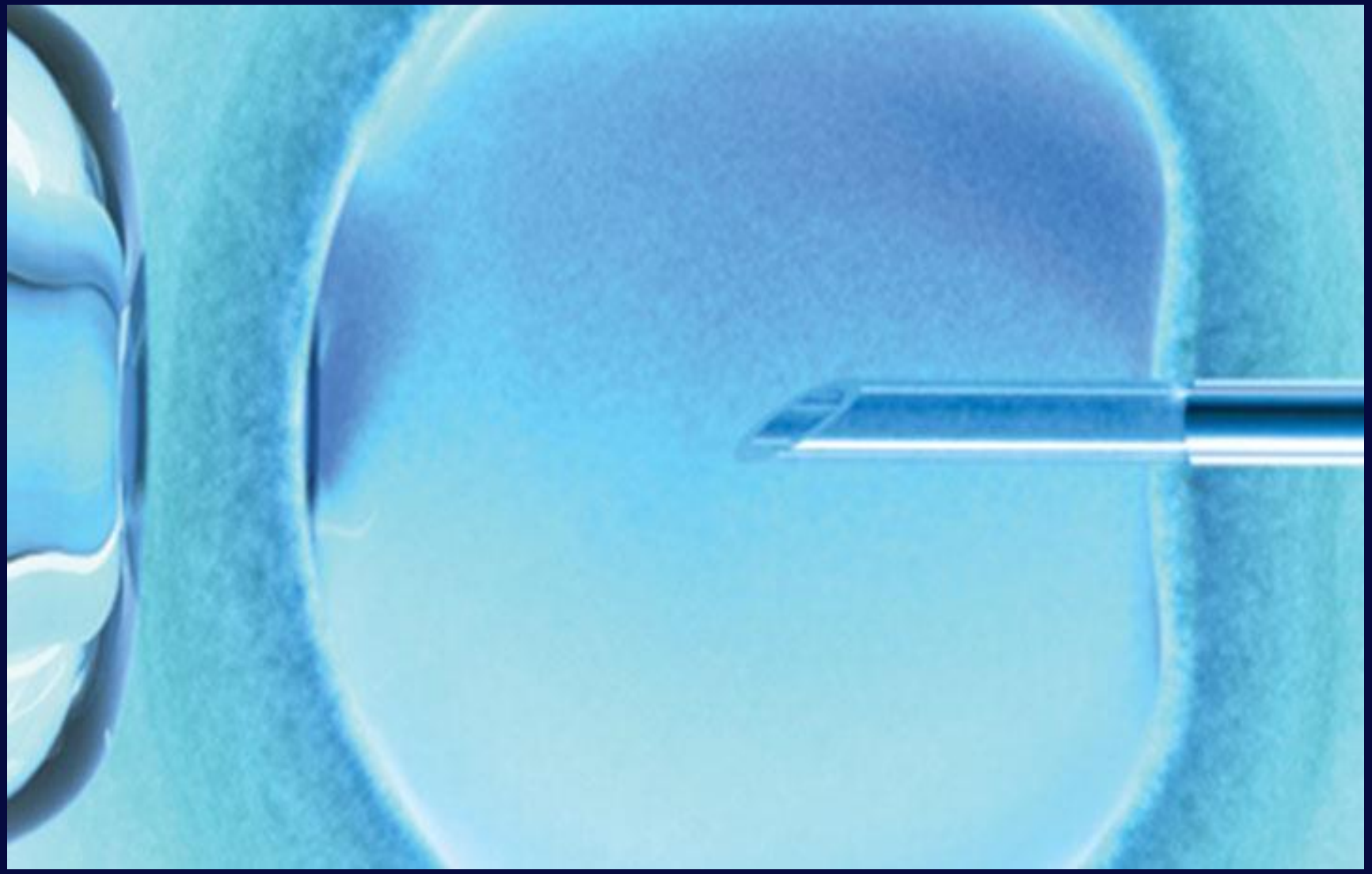
Sakka *et al*. Fertility Sterility 2010

Triglycerides



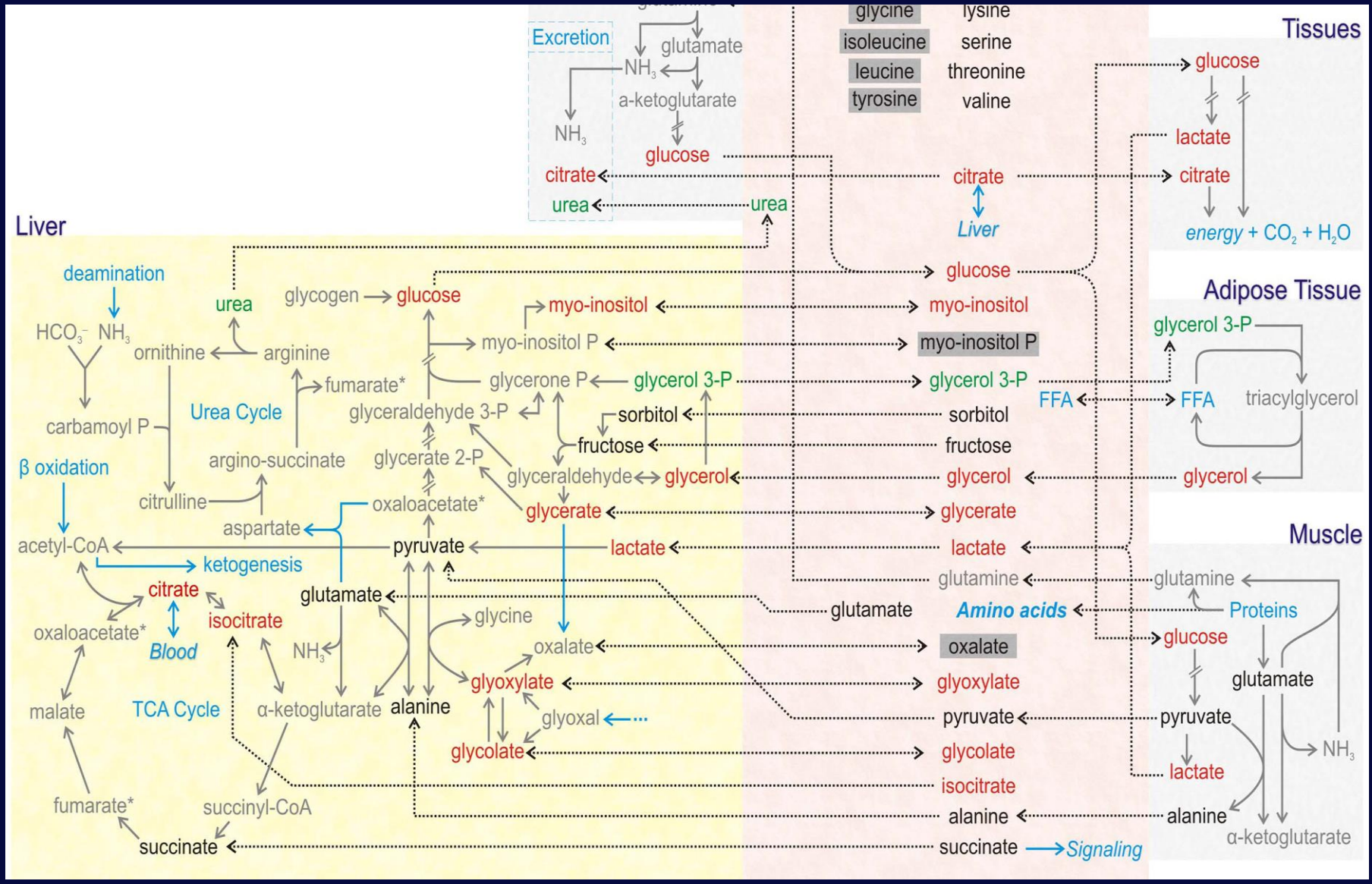
Sakka *et al*. Fertility Sterility 2010

p= 0,031

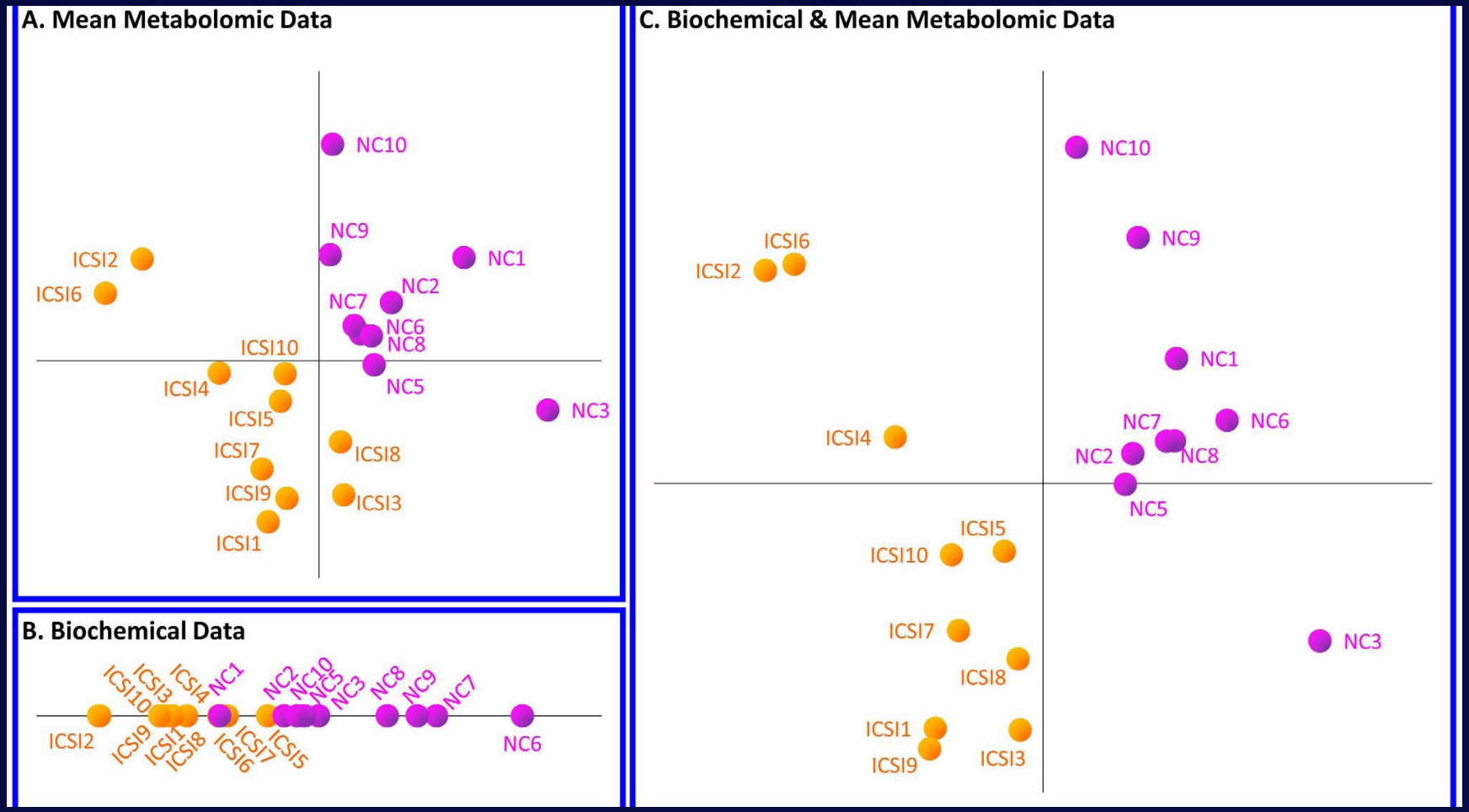


Parameters	Control N=42	ICSI N=42	Difference
Glucose (mg/dL)	83.7± 9.3	81±7.7	0.36
Insulin (mU/L)	6.9± 6.7	5.5± 2.5	0.9
HOMA index	1.5± 1.8	1.1±0.5	0.9
Total Chol (mg/dL)	172.7±24.5	167.7±25.3	0.35
Triglycerides (mg/dL)	54.2±22.6	45.4± 16.5	0.07
HDL-C (mg/dL)	60.8±12	63.9±8.9	0.17
LDL-C (mg/dL)	100.9±22	94.6± 21.2	0.18
ApoA1 (mg/dL)	156.1±19.8	153±21.1	0.5
ApoB (mg/dL)	74.2± 14.9	75.7±14.7	0.65
Lp(a) (mg/dL)	15.4± 20.3	11.8± 14.8	0.16
IGF-1 (ng/mL)	190.5±92.5	193.2± 115.3	0.58
YKL-40 (ng/mL)	27.08±15.5	15.45± 8.9	0.0002
hs-IL6 (pg/mL)	1.6± 1.5	2.3±4	0.38
hs-CRP (mg/L)	0.78± 0.87	0.44±0.3	0.022

Gkourogianni *et al*. PLoS One 2015

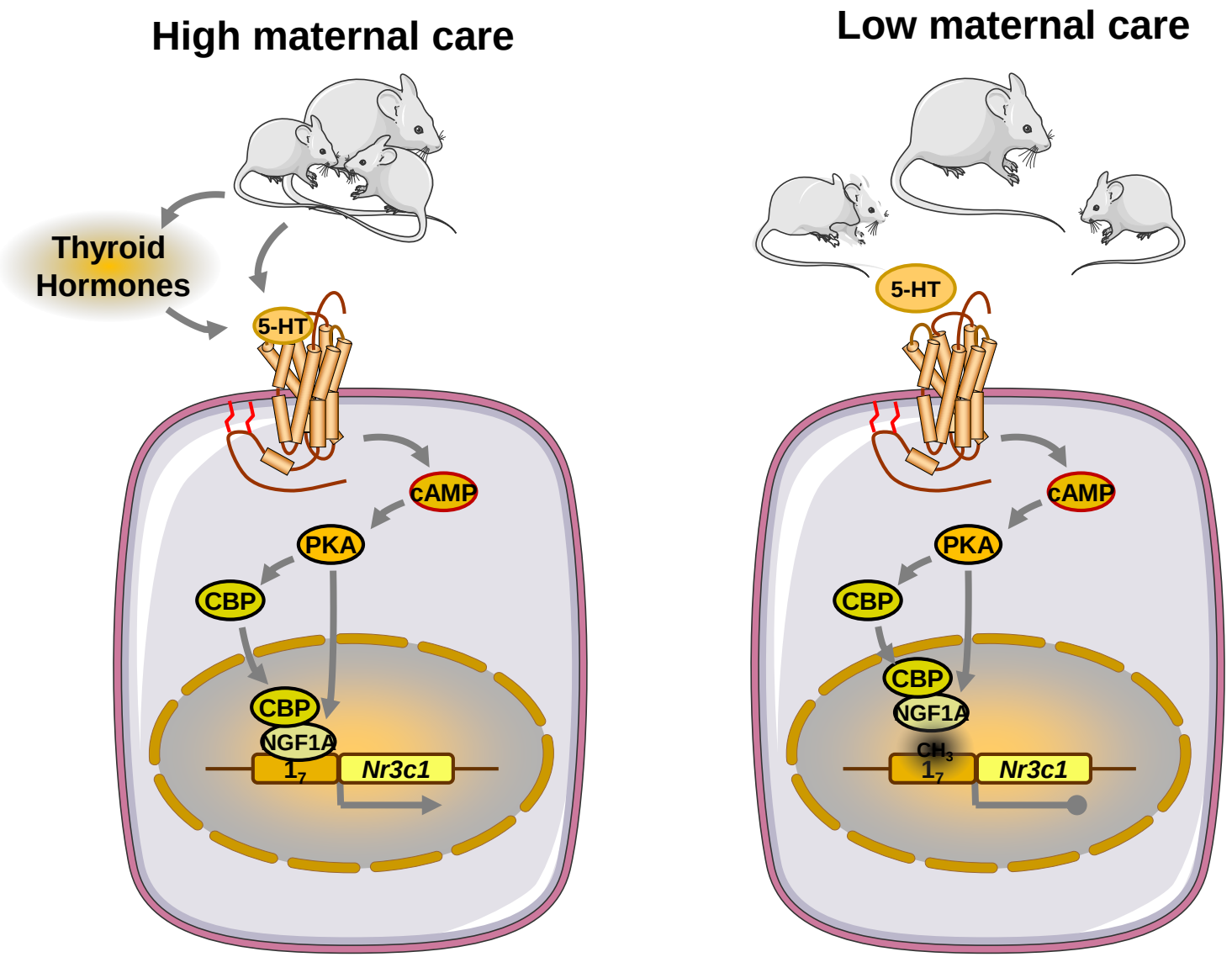


Gkourogianni et al. PLoS One 2015



Gkourogianni et al. PLoS One 2015

EPIGENETICS AND SEPARATION



Champagne FA. *Behavioral Neuroscience* 2013; 127(5): 628–636

EPIGENETICS AND CHILDHOOD ABUSE ⊃)

Epigenetic regulation of the glucocorticoid receptor in human brain associates with childhood abuse

Patrick O McGowan[1,2], Aya Sasaki[1,2], Ana C D'Alessio[3], Sergiy Dymov[3], Benoit Labonté[1,4], Moshe Szyf[2,3], Gustavo Turecki[1,4] & Michael J Meaney[1,2,5]

McGoan PO et al. *Nature Neuroscience* 2009; 12(3): 342-348

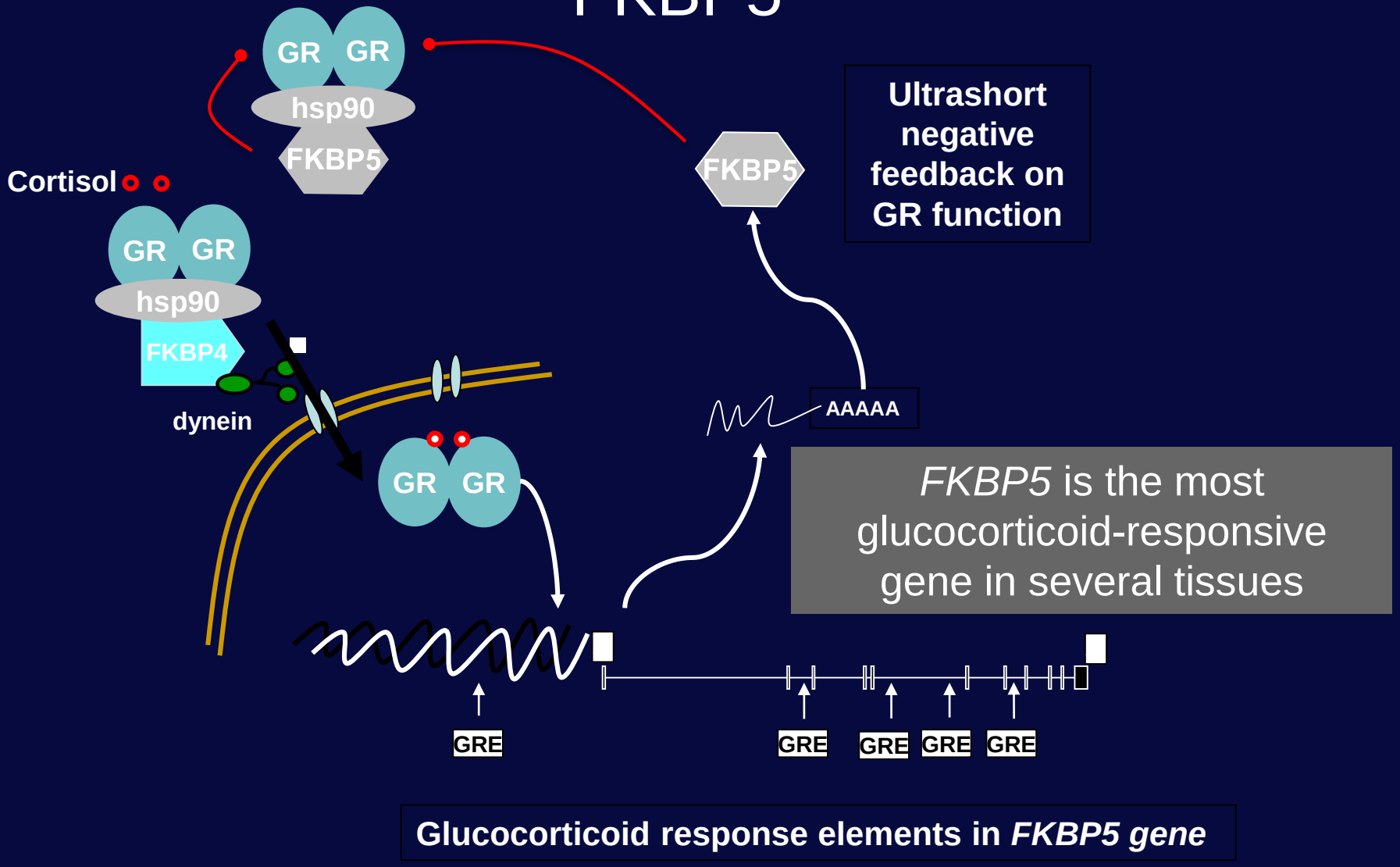
EPIGENETICS AND CHILDHOOD ABUSE

Increased methylation of glucocorticoid receptor gene (*NR3C1*) in adults with a history of childhood maltreatment: a link with the severity and type of trauma

N Perroud[1], A Paoloni-Giacobino[2], P Prada[3], E Olié[4,5,6], A Salzmann[1], R Nicastro[3], S Guillaume[4,5,6], D Mouthon[2], C Stouder[2], K Dieben[3], P Huguelet[3], P Courtet[4,5,6] and A Malafosse[1,2]

Perroud N et al. *Transl Psychiatry* 2011; (1): e59.

Molecular modulators of the stress response: FKBP5



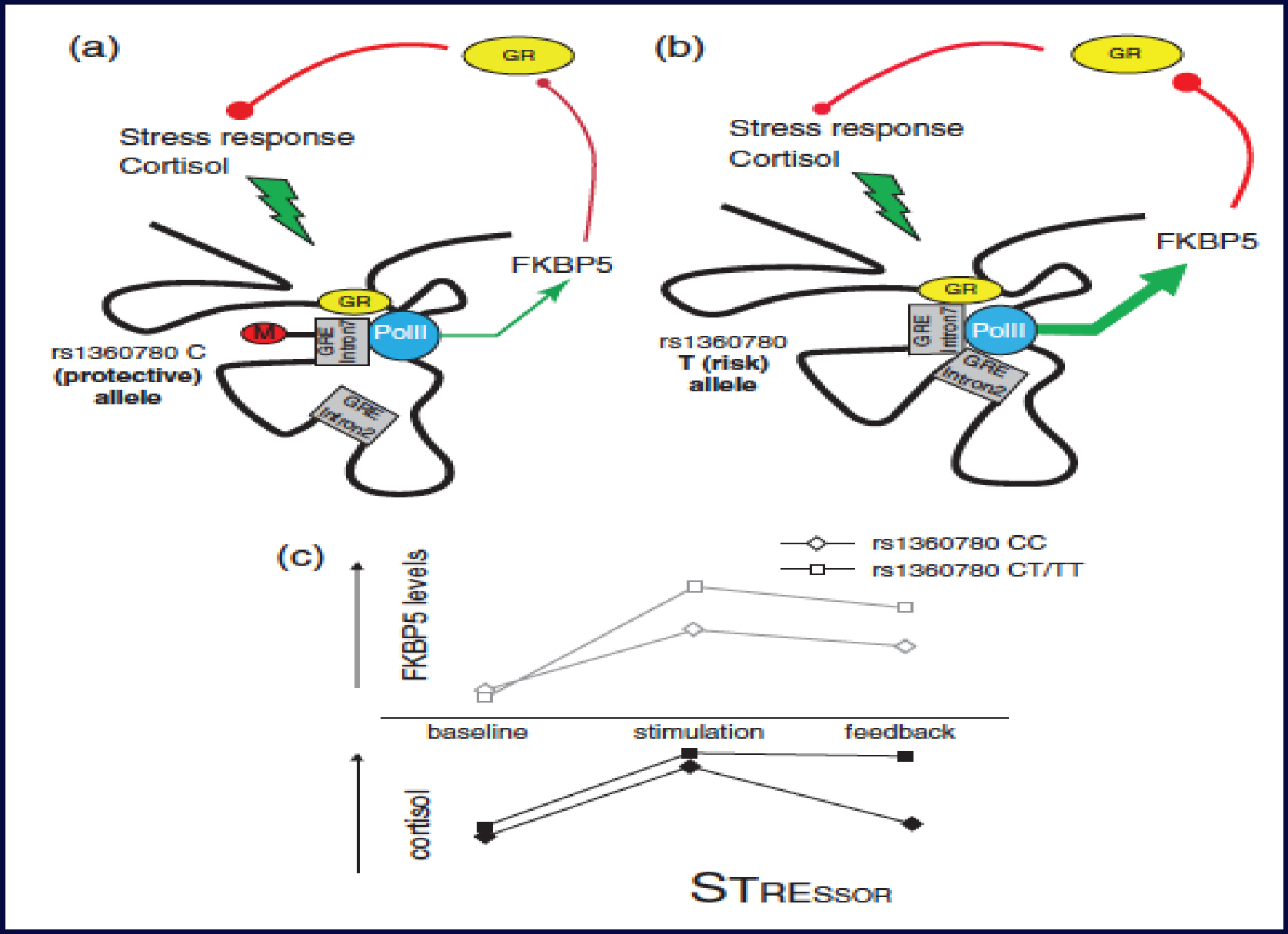
Ultrashort negative feedback on GR function

FKBP5 is the most glucocorticoid-responsive gene in several tissues

Glucocorticoid response elements in *FKBP5 gene*

Binder Nat. Genet. 2004, Klengel Nat Neurosc., 2013, Menke et al,. Genes, Brain and Behavior 2013, Scharf et al, PLOSone 2011, Zannas et al. 2015

FKBP5 disinhibition by gene-environment-epigenetic interactions



Zannas & Binder 2014

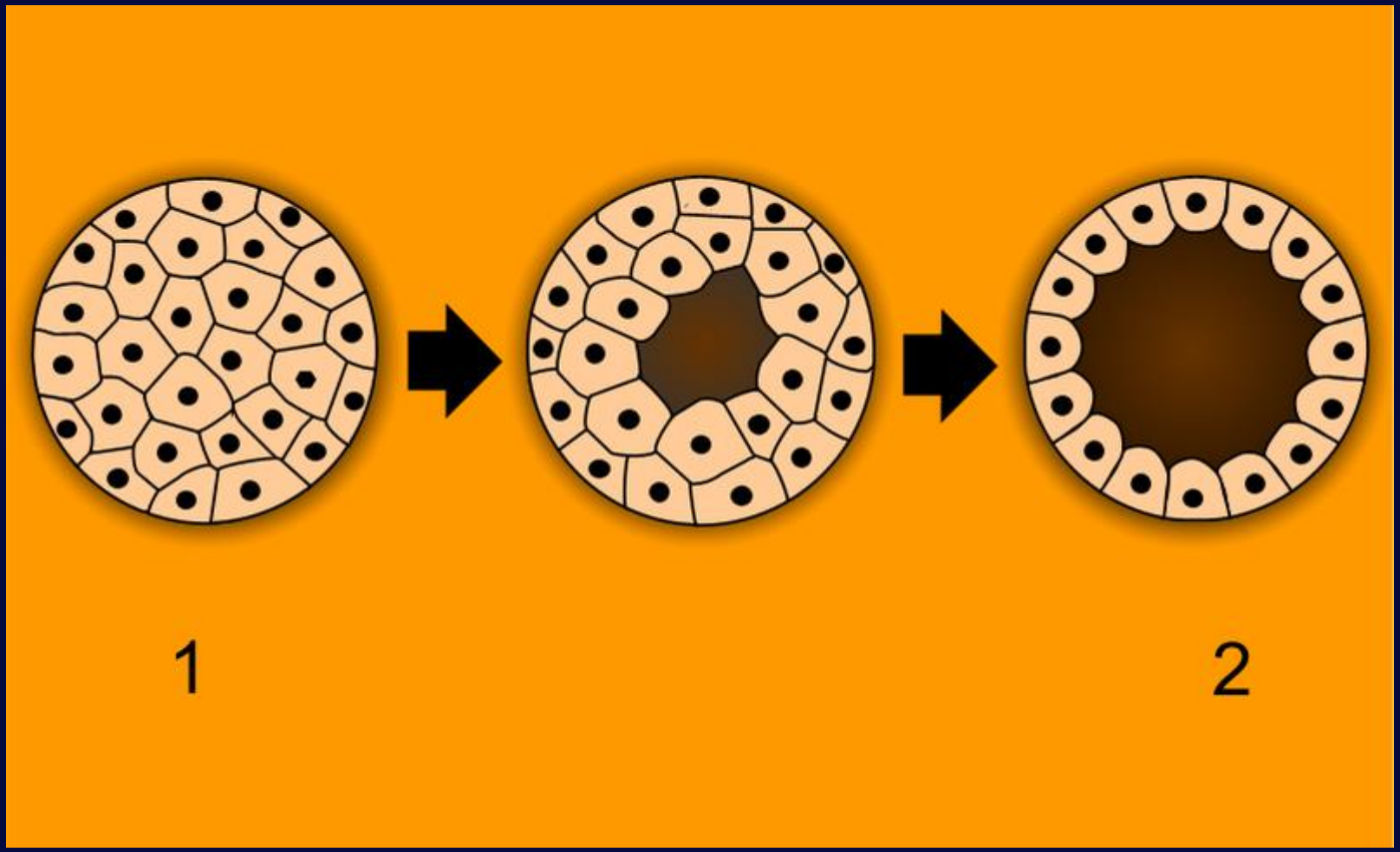
Epigenome

- *CpG islands in 60% of gene promoters*
- *25% of methylation in stem cells is in non-CpG context*
- *Global demethylation of the trophoblast*
- *Global demethylation in aging*
- *Global demethylation in cancer*

Epigenome

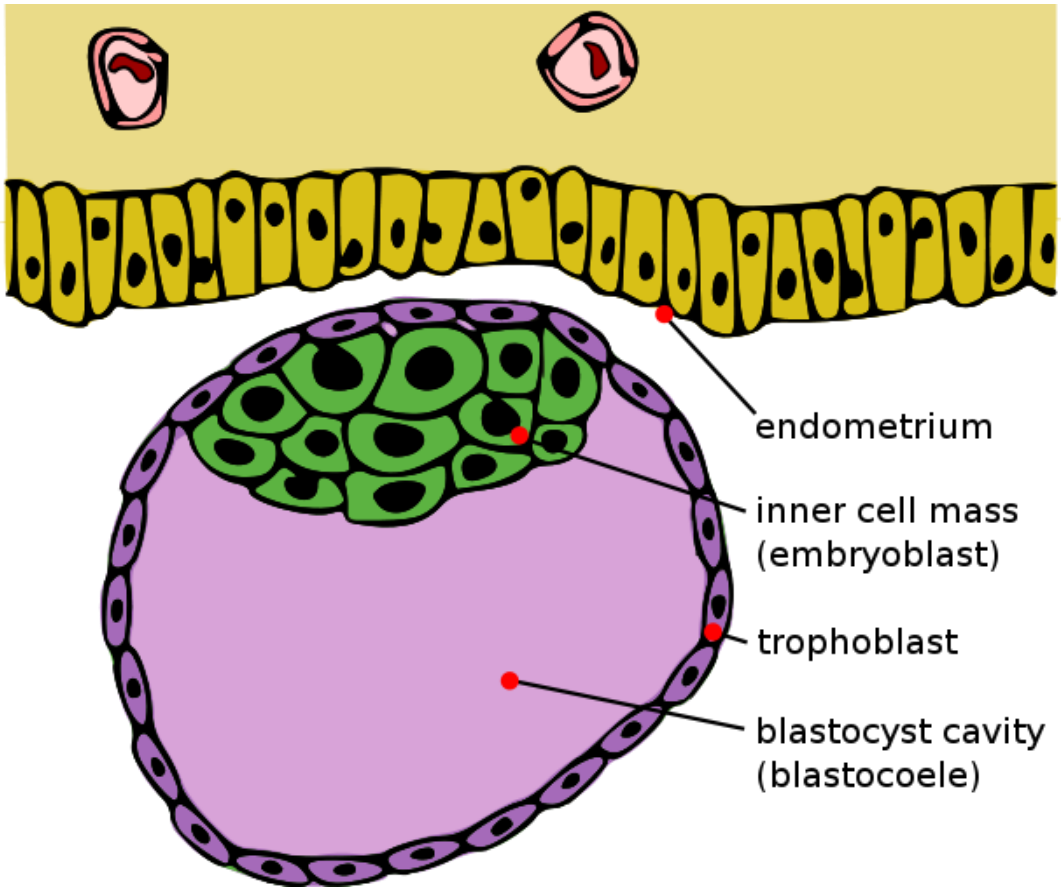
- ***Preimplantation: Methylome erasure***
  - ***Paternal first, maternal ensues***
- ***Remethylation: Morula-Blastocyst***
- ***Inner cell mass: hypermethylation***
- ***Trophoblast: hypomethylation***
- ***Gametogenesis: epigenetic reprogramming/ erasure-remethylation***

Blastulation: 1 - morula, 2 - blastula.

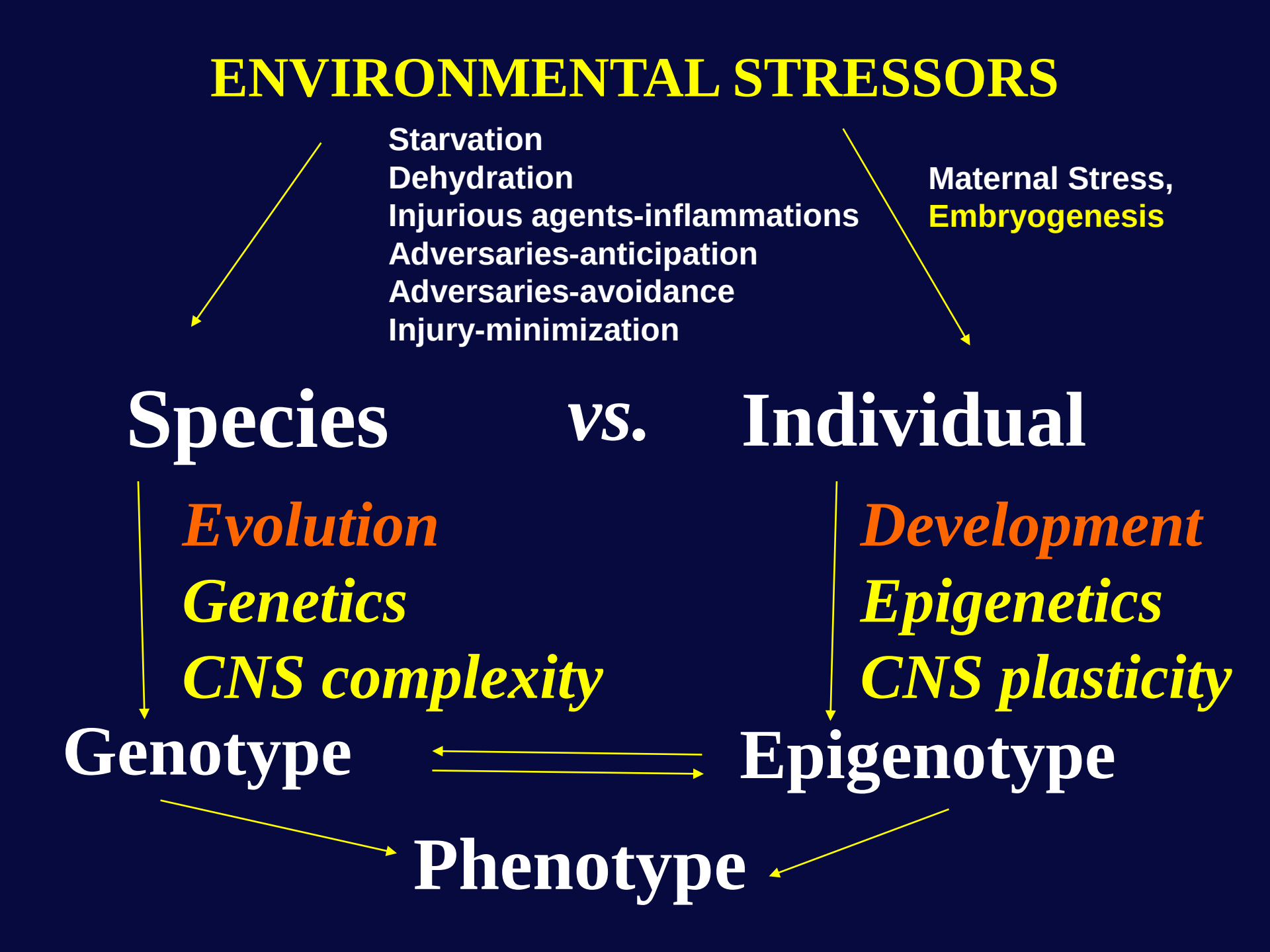


- Oocyte to 2 cells 32 genes
- Two to 4 cells 129 genes

Tohonen et al. Nat Comm 2015


endometrium
inner cell mass
(embryoblast)
trophoblast
blastocyst cavity
(blastocoele)

Blastocyst


ENVIRONMENTAL STRESSORS
Starvation
Dehydration
Injurious agents-inflammations
Adversaries-anticipation
Adversaries-avoidance
Injury-minimization
Maternal Stress,
Embryogenesis
Species
vs.
Individual
Evolution
Genetics
CNS complexity
Development
Epigenetics
CNS plasticity
Genotype
Epigenotype
Phenotype

The Economist

Gore anoints Dean
PAGES 12 AND 33

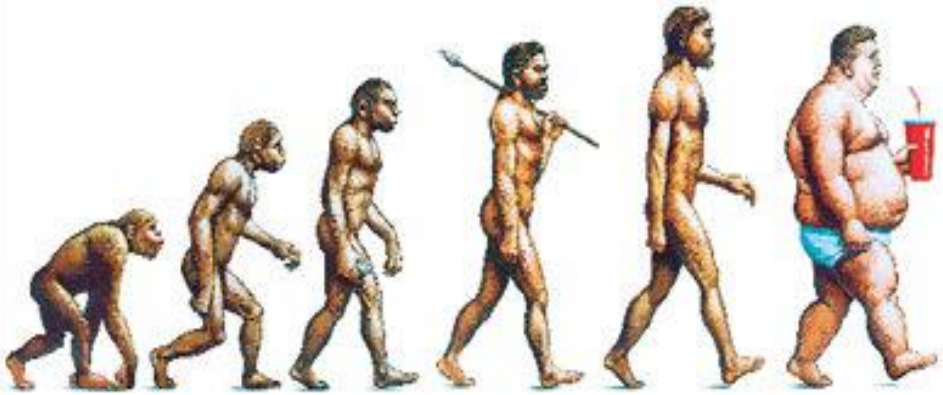
America's Taiwan test
PAGES 12 AND 29

The future of flight
PAGES 79-81

A SURVEY OF FOOD
AFTER PAGE 52

DECEMBER 13TH-19TH 2003 www.economist.com

The shape of things to come



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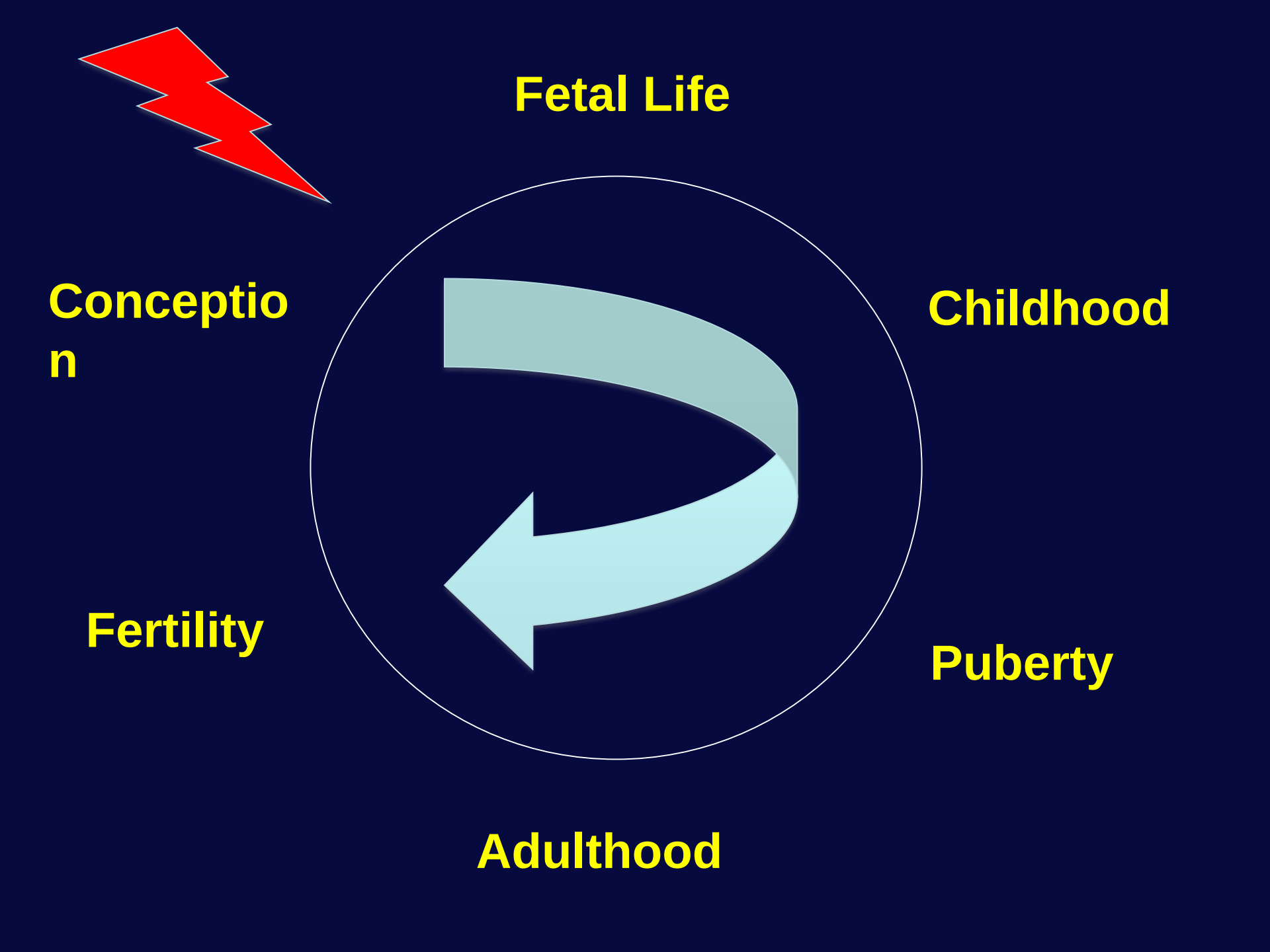
Selections of Gene and Epigenetic Networks Participating in Functions Important for Human Survival and Species Preservation

RESPONSE TO SURVIVAL THREAT	SELECTIVE ADVANTAGE	CONTEMPORARY DISEASE
Combat starvation	Energy conservation	Obesity/metabolic syndrome
Combat dehydration	Fluid and electrolyte conserve	Hypertension
Combat injurious agents	Potent immune reaction	Autoimmunity/Allergy
Anticipate adversaries	Arousal/fear	Anxiety/insomnia
Minimize exposure to danger	Withdrawal	Depression
Prevent tissue strain/damage	Retain tissue integrity	Pain and fatigue syndromes

Chrousos, Amer J Med 2004


Fetal Life
Childhood
Puberty
Adulthood
Fertility
Conceptio
n

ΕΠΙΚΤΗΤΟΥ
ΕΓΧΕΙΡΙΔΙΟΝ.

EPICTETI
ENCHIRIDION.

ΚΕΦ. α'.

Τῶν ὄντων τὰ μὲν ἐφ' ἡμῖν, τὰ δ' οὐκ ἐφ' ἡμῖν. ἐφ' ἡμῖν μὲν ὑπόληψις, ὁρμή, ὄρεξις, ἔκκλισις· καὶ ἐν ἡμῖν λόγῳ, ὅσα ἡμέτερα ἔργα. οὐκ ἐφ' ἡμῖν δὲ, τὸ σῶμα, ἡ κτῆσις, δόξαι, ἀρχαί. καὶ ἐν λόγῳ, ὅσα οὐχ ἡμέτερα ἔργα.

CAP. I.

Res quædam in potestate nostra sunt, quædam non sunt. In nostra potestate est opinio, appetitio, desiderium, aversatio; &, ut uno complectar verbo, quæcunque nostræ actiones. Nostri arbitrii non sunt corpus, pecunia, gloria, imperia: ad summam, ea quæ ipsi non agimus, omnia.

1 Tribuitur hoc Enchiridion Epicteto, quamvis ipse id non scripserit, sed Arrianus, qui & uberiorem in id commentarium edidit, quo disputationes Epicteti plenius prosequitur. Testatur id Simplicius in præfatione commentarii ad hunc libellum hisce verbis: Τὸ δὲ βιβλίον τοῦτο τὸ Ἐπικτήτου ἐγχειρίδιον ἐπιγράφεται, ᾧ τάττουσι συνιστᾶσιν οἱ Λόγιοι, τὰ κεφαλαιώτατα τῶν διατριβῶν τοῦ Φιλοσόφου, καὶ κινητικώτατα τῆς ψυχῆς ἐπιλεξάμενον. Hoc est: *Idem Arrianus & hunc libellum, quod Enchiridion inscribitur composuit, delectu ex Epicteti disputationibus philosophiæ locis maxime idoneis ac necessariis, & animos vehementissime permoventibus. Senecanus.*

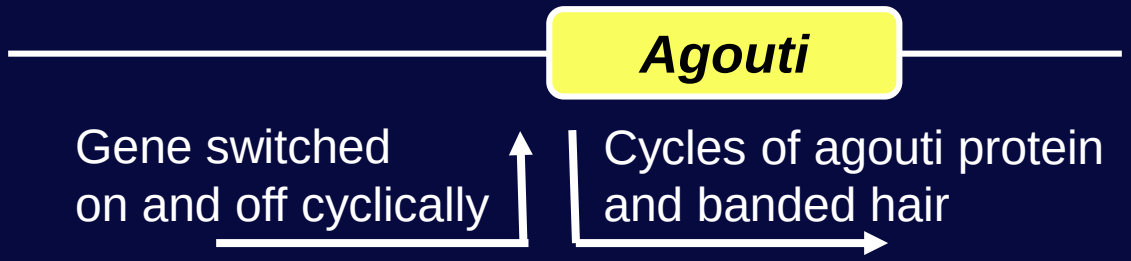
2 Non solum pugio Græcis hoc nomine vocatur, sed etiam quidquid ad manum est, & in usum promptum & obvium, ut instrumenta quælibet ac vasa ἐγχειρίδια, quæ parata & in manu semper gestanda. Hesychius, Ἐγχειρίδια, σκεύη ἃ ἔστιν ἐπιφόρια, ἤγουν δι-

A

εσ

[illegible]

'Be equanimous and remember not to believe easily'



Normal

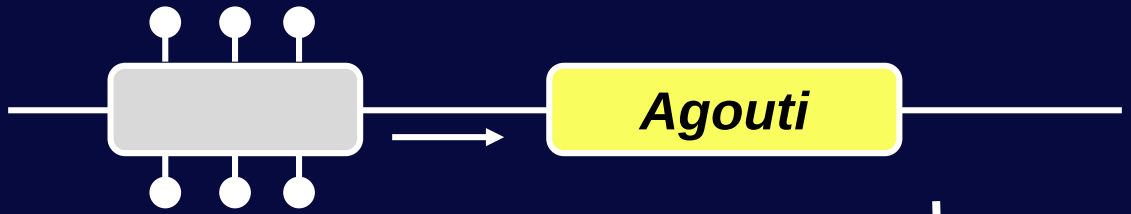
Gene switched on and off cyclically

Cycles of agouti protein and banded hair

A^{vy} unmethylated

Inserted retrotransposon expresses an abnormal RNA and agouti is switched on permanently

Yellow hair

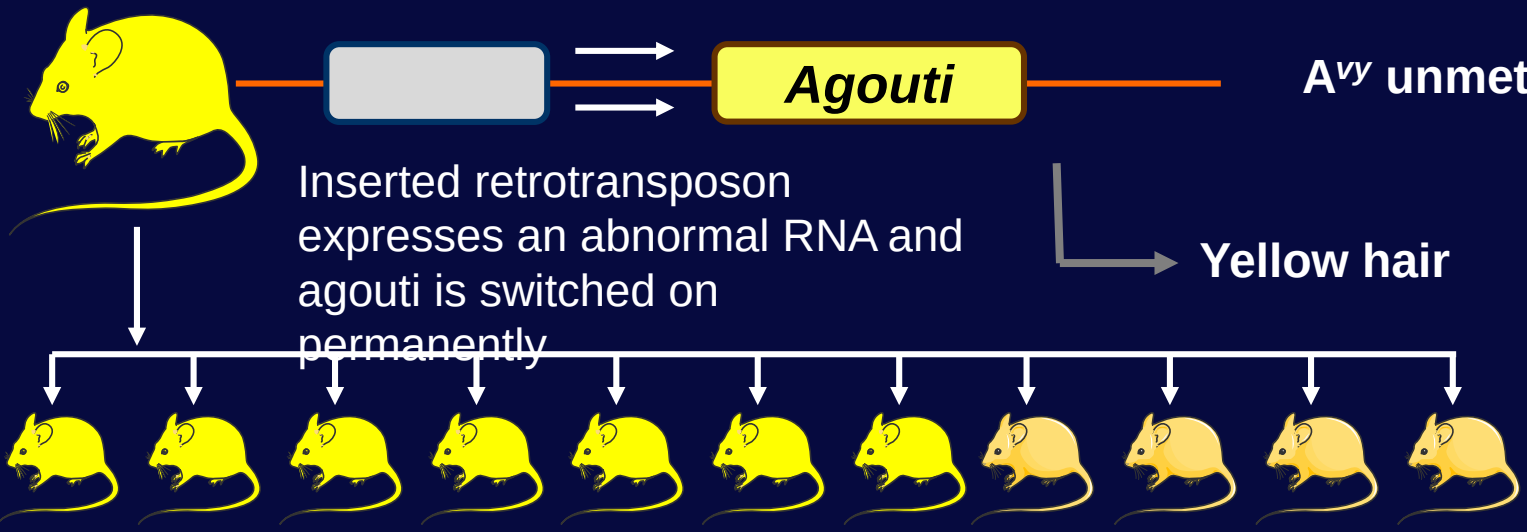


A^{vy} methylated

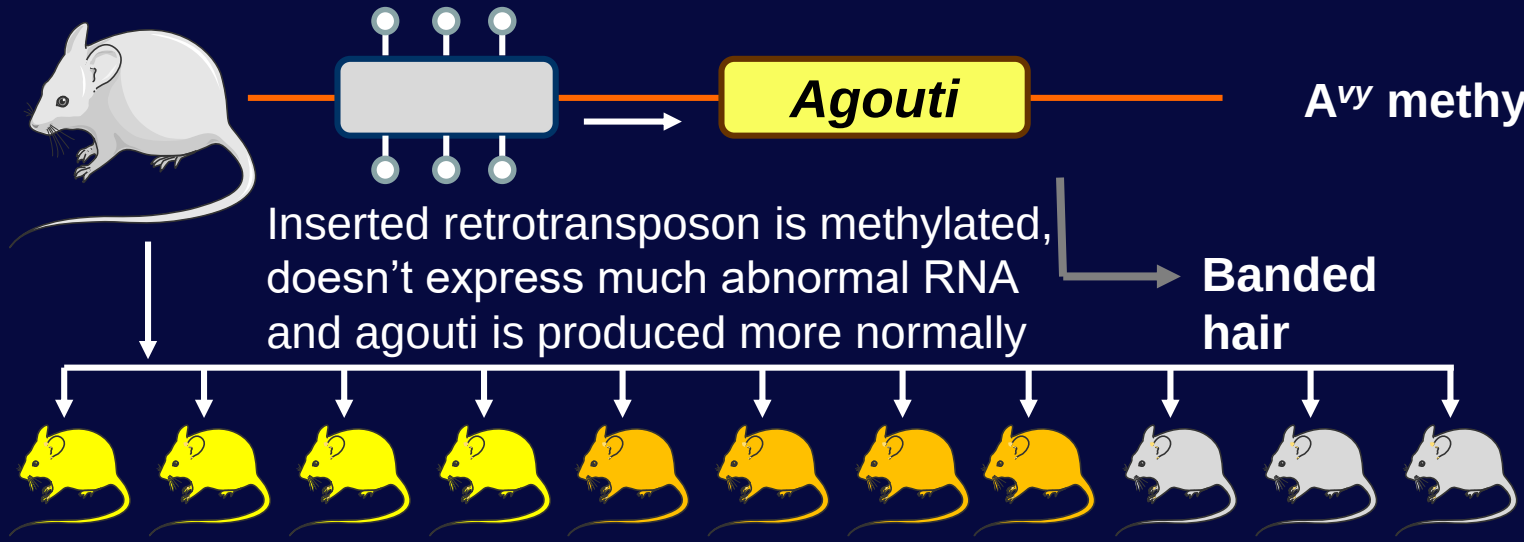
Inserted retrotransposon is methylated, doesn't express much abnormal RNA and agouti is produced more normally

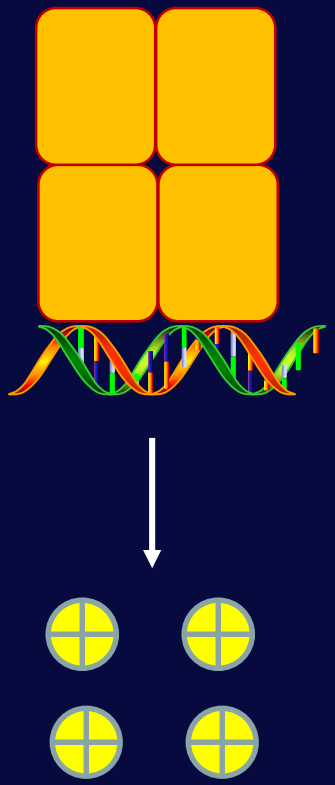
Banded hair

A yellow mother only gives birth to yellow or lightly colored pups

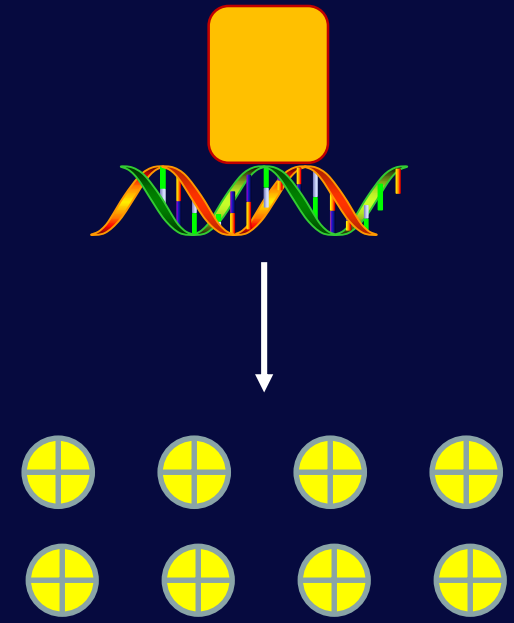


A dark mother gives birth to yellow, lightly colored and dark pups

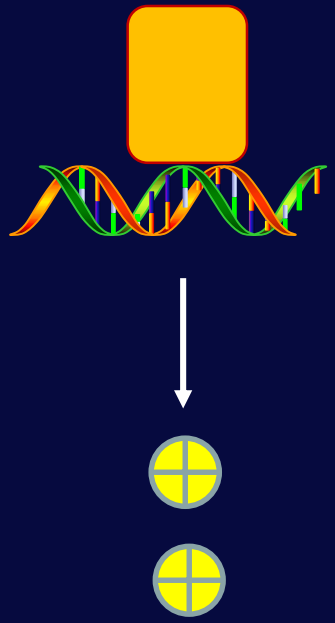




Hypothesis:
ncRNA represses expression of target gene

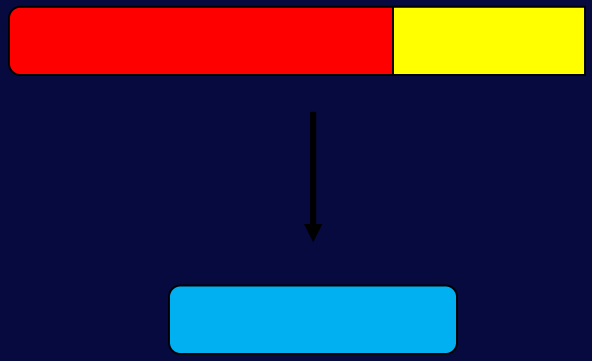


Prediction:
Decreasing levels of ncRNA leads to increased expression of target gene



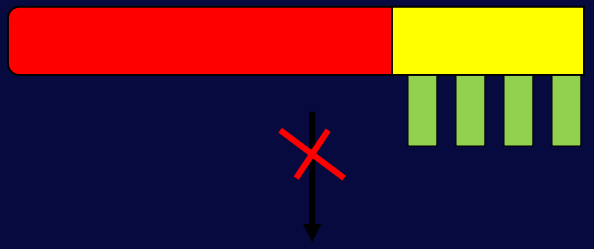
Actual outcome:
Decreasing levels of ncRNA leads to decreased expression of target gene

Embryo and first larval stage



LIN-14 mRNA is translated into protein

Second larval stage



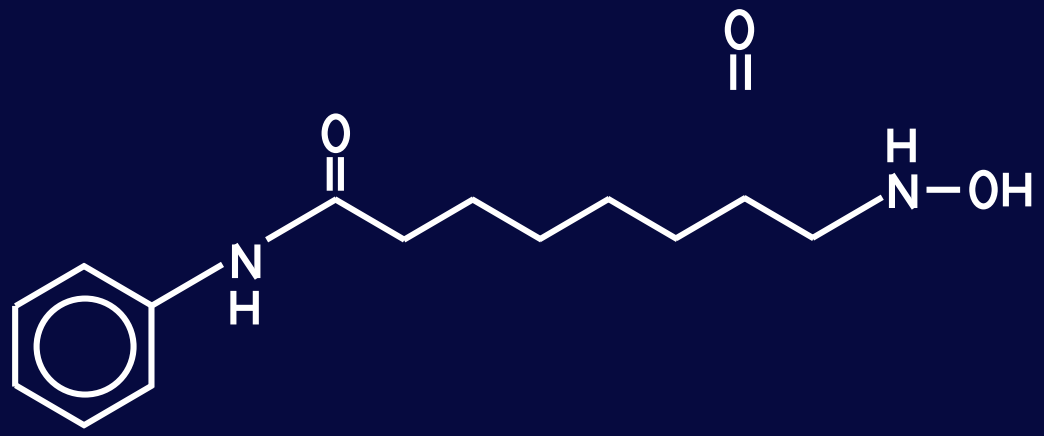
Binding of Lin-4 ncRNA to 3’ UTR of LIN-14 mRNA prevents translation of protein

Region of LIN-14 mRNA that gets translated into protein

Region of LIN-14 mRNA that DOES NOT get translated into protein
= 3’ untranslated region
= 3’ UTR

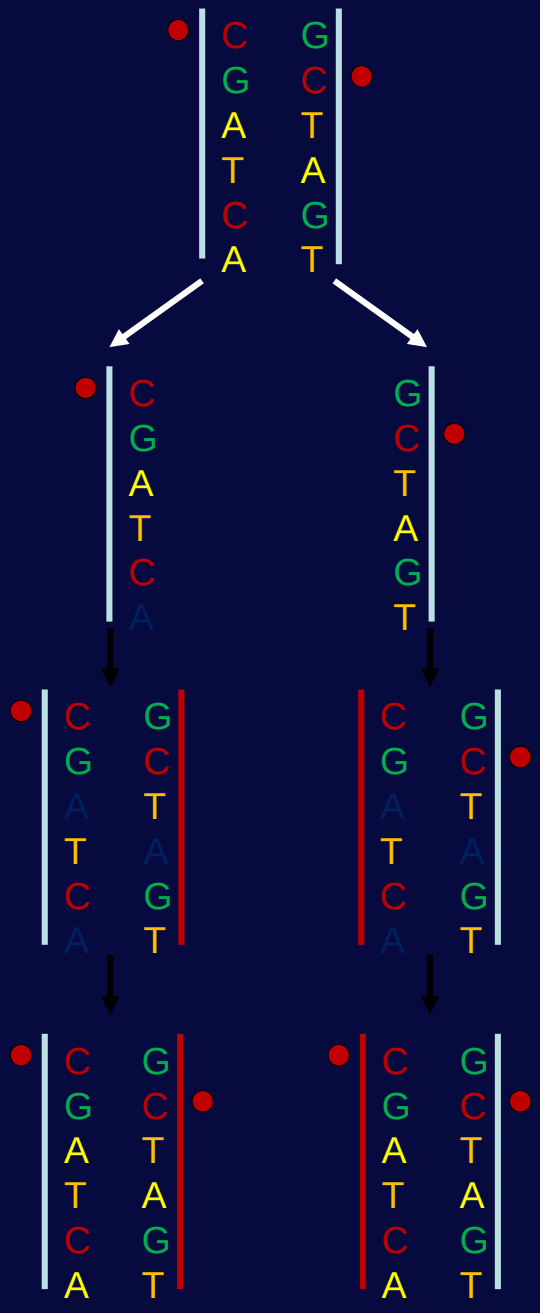
LIN-14 protein

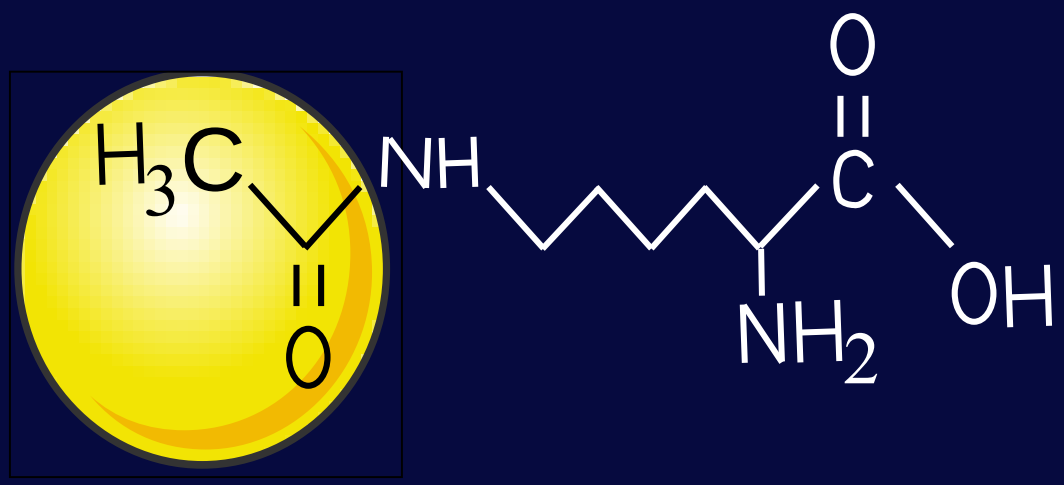
Lin-4 non-coding RNA



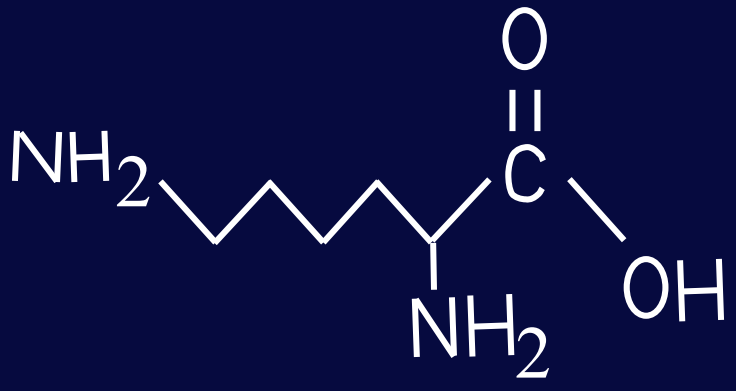
SAHA

10HDA

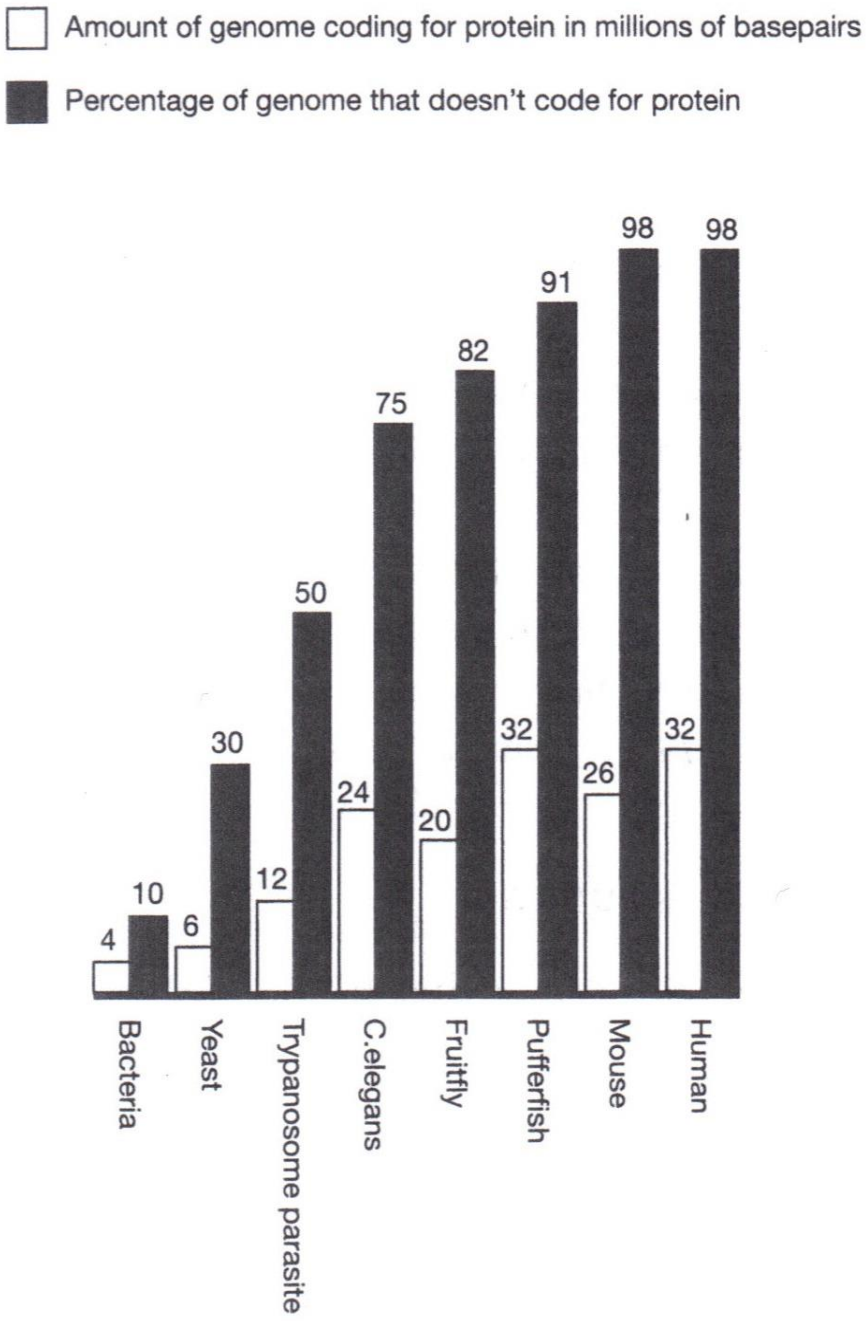

C G A T C A
G C T A G T
C G A T C A
G C T A G T
C G A T C A
G C T A G T
C G A T C A
G C T A G T
C G A T C A
G C T A G T
C G A T C A
G C T A G T



Acetyl-Lysine

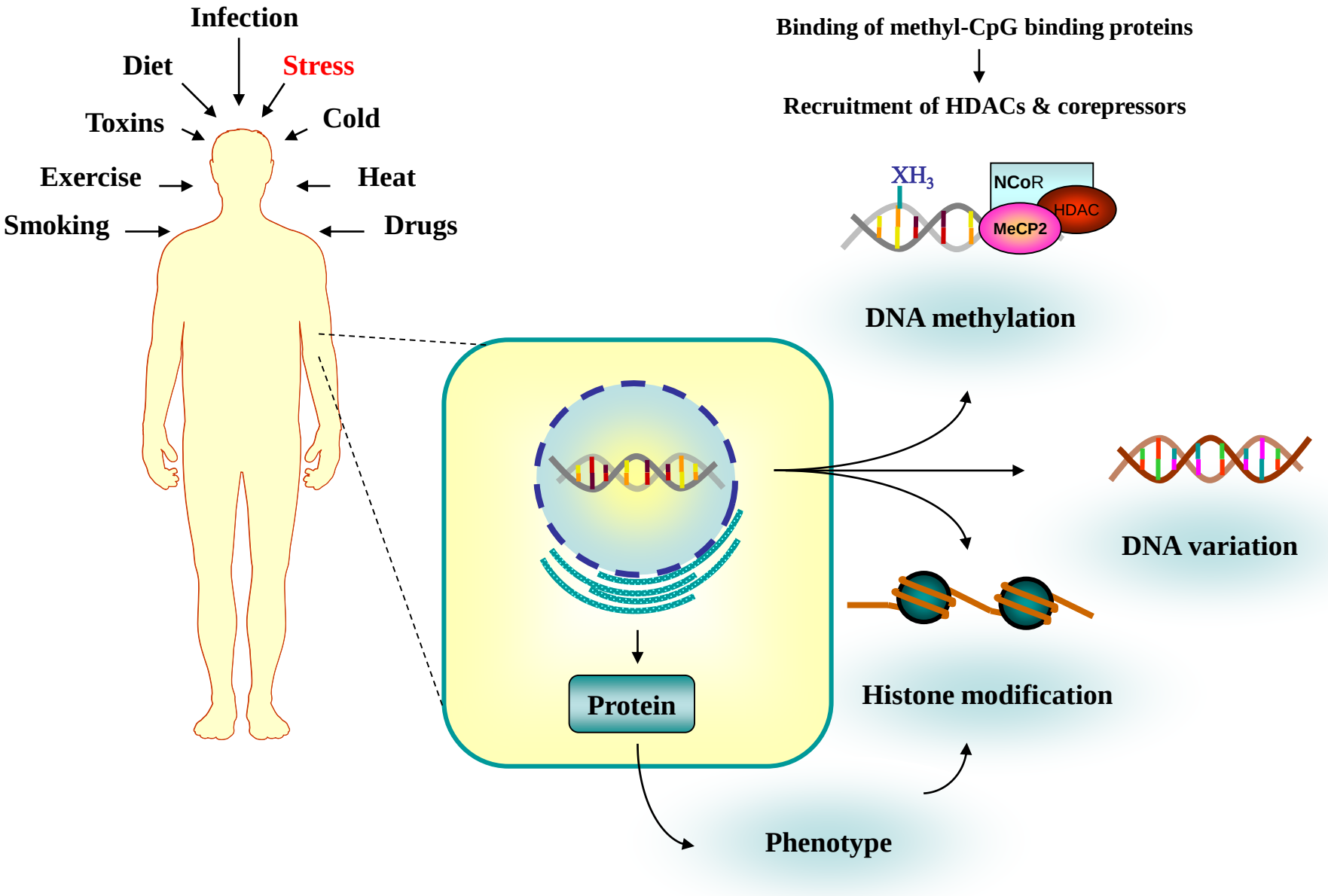


Lysine


Amount of genome coding for protein in millions of basepairs
Percentage of genome that doesn't code for protein
4
10
6
30
12
50
24
75
20
82
32
91
26
98
32
98
Bacteria
Yeast
Trypanosome parasite
C.elegans
Fruitfly
Pufferfish
Mouse
Human

ENVIRONMENTAL STRESSORS

GENETIC MACHINERY



Epigenetics at the Early Stages of Childhood

Evo Devo: Genetics *vs.* Epigenetics ,

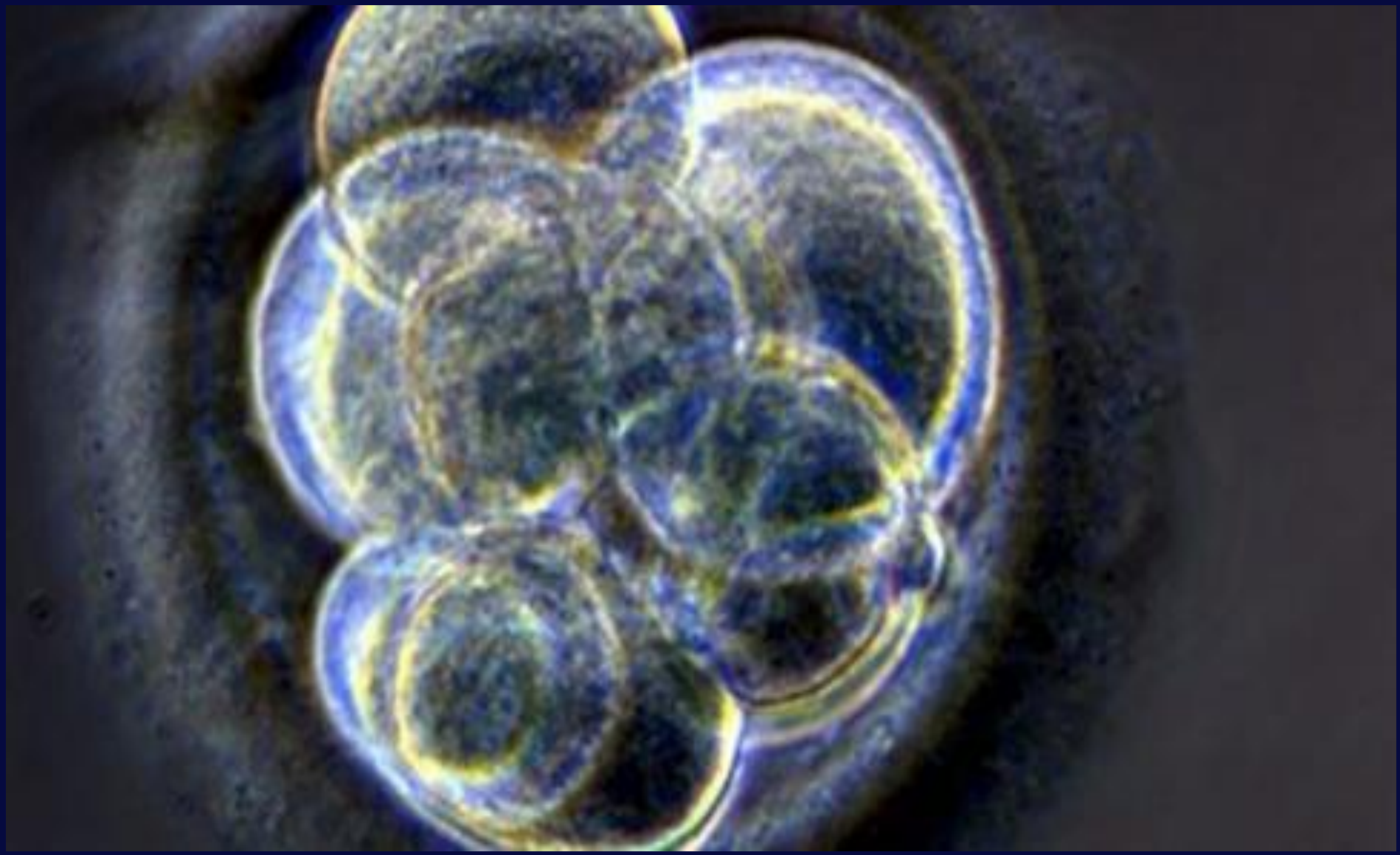
George P. Chrousos, MD,

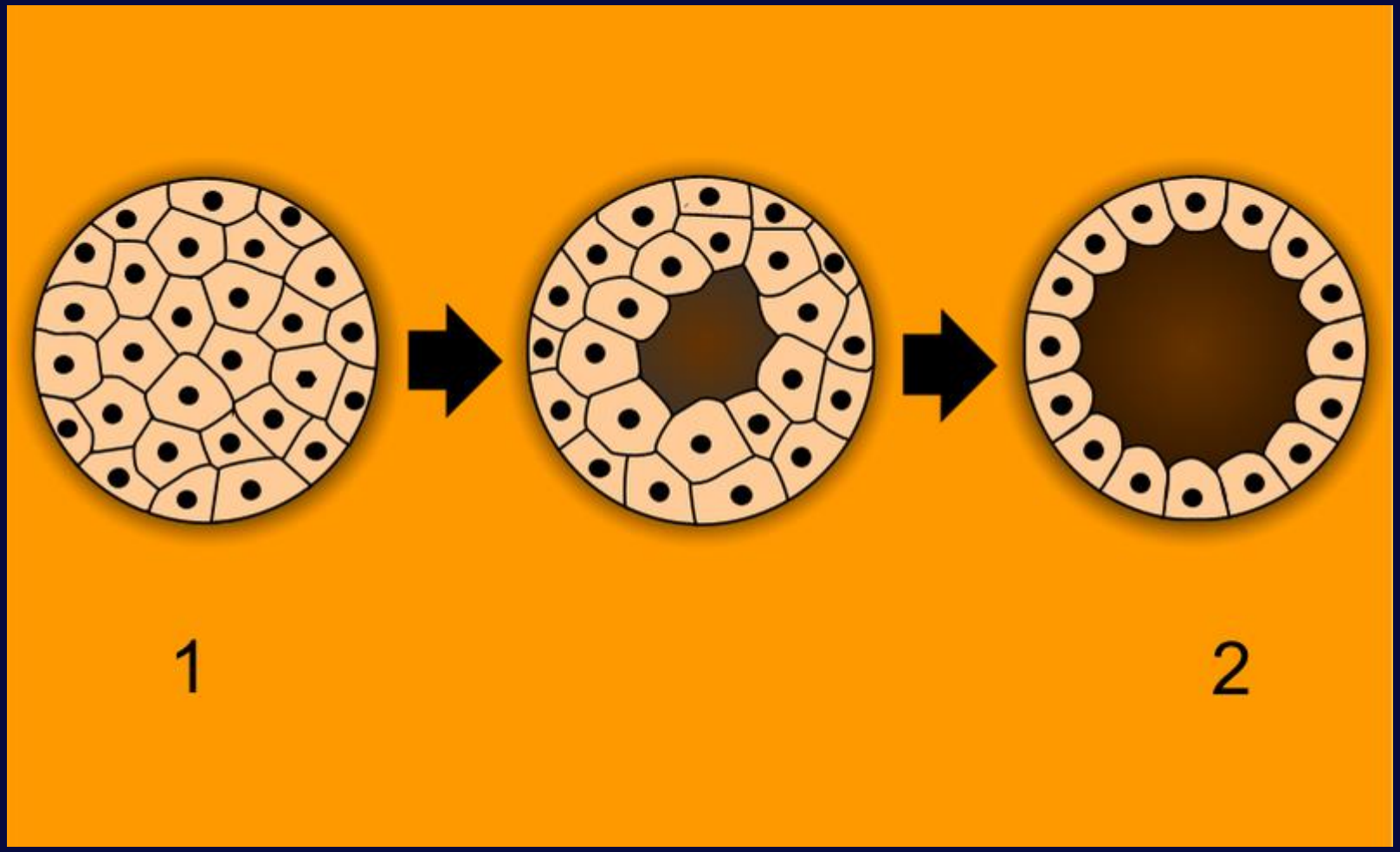
University of Athens,

Athens, Greece

(No Disclosures)

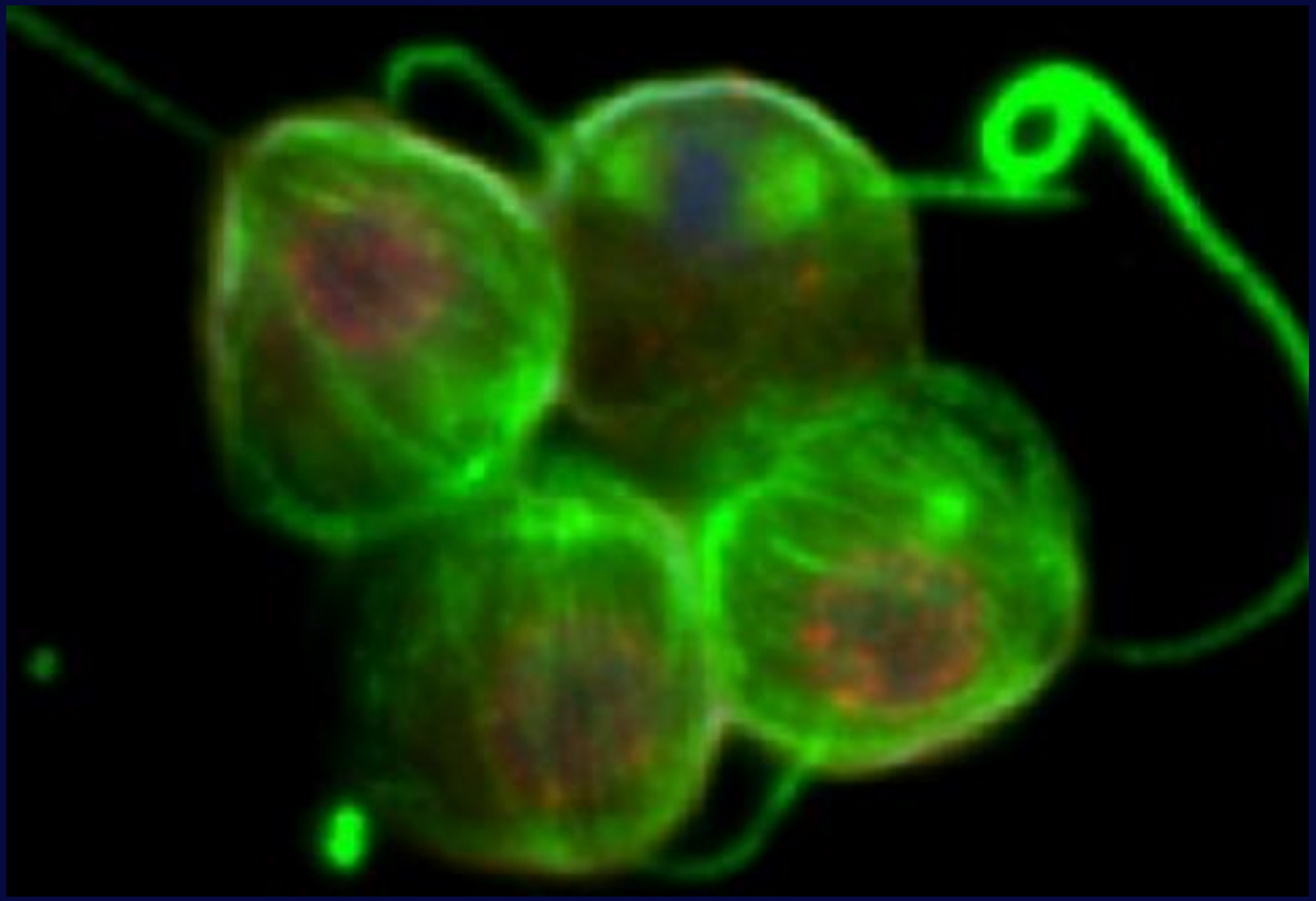
Early Embryon

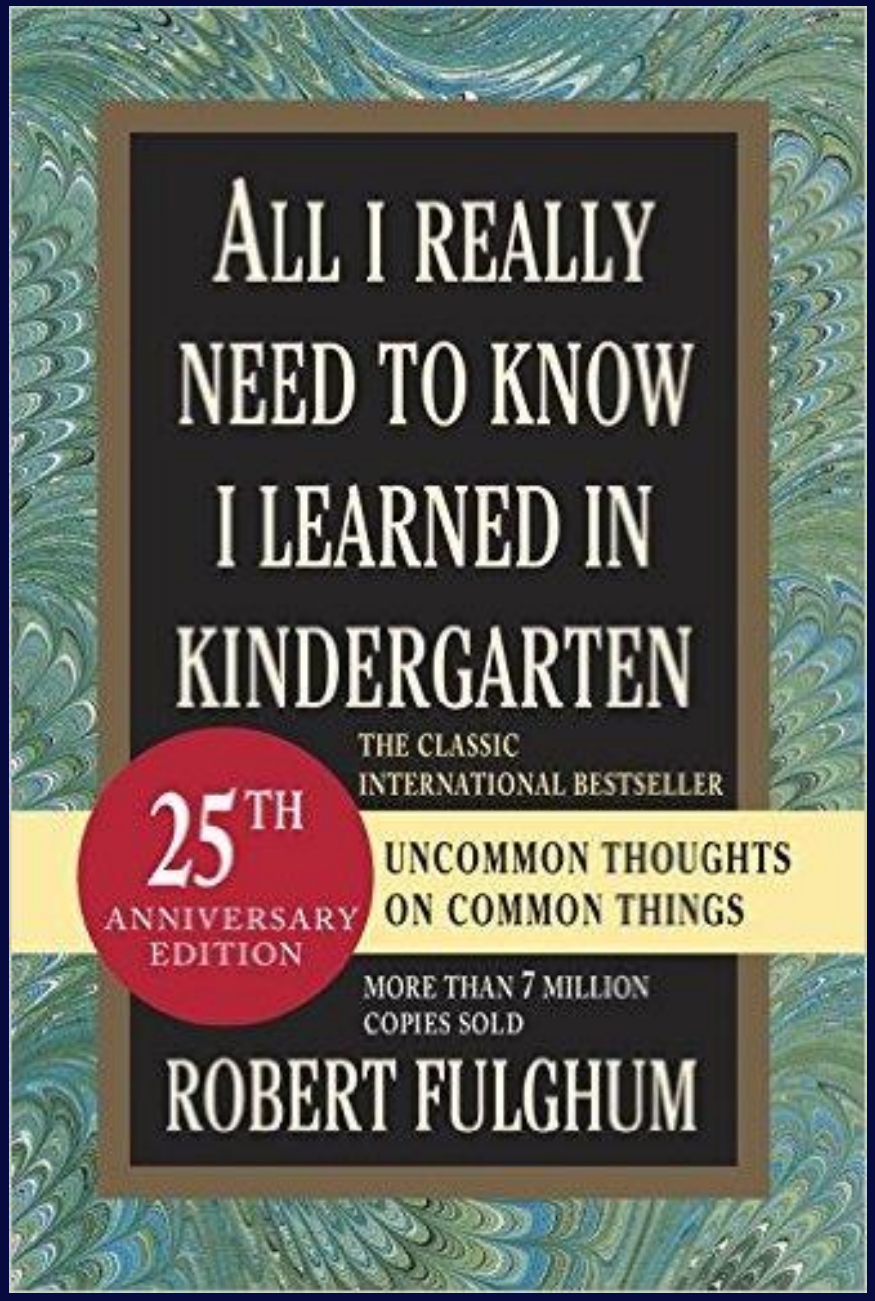
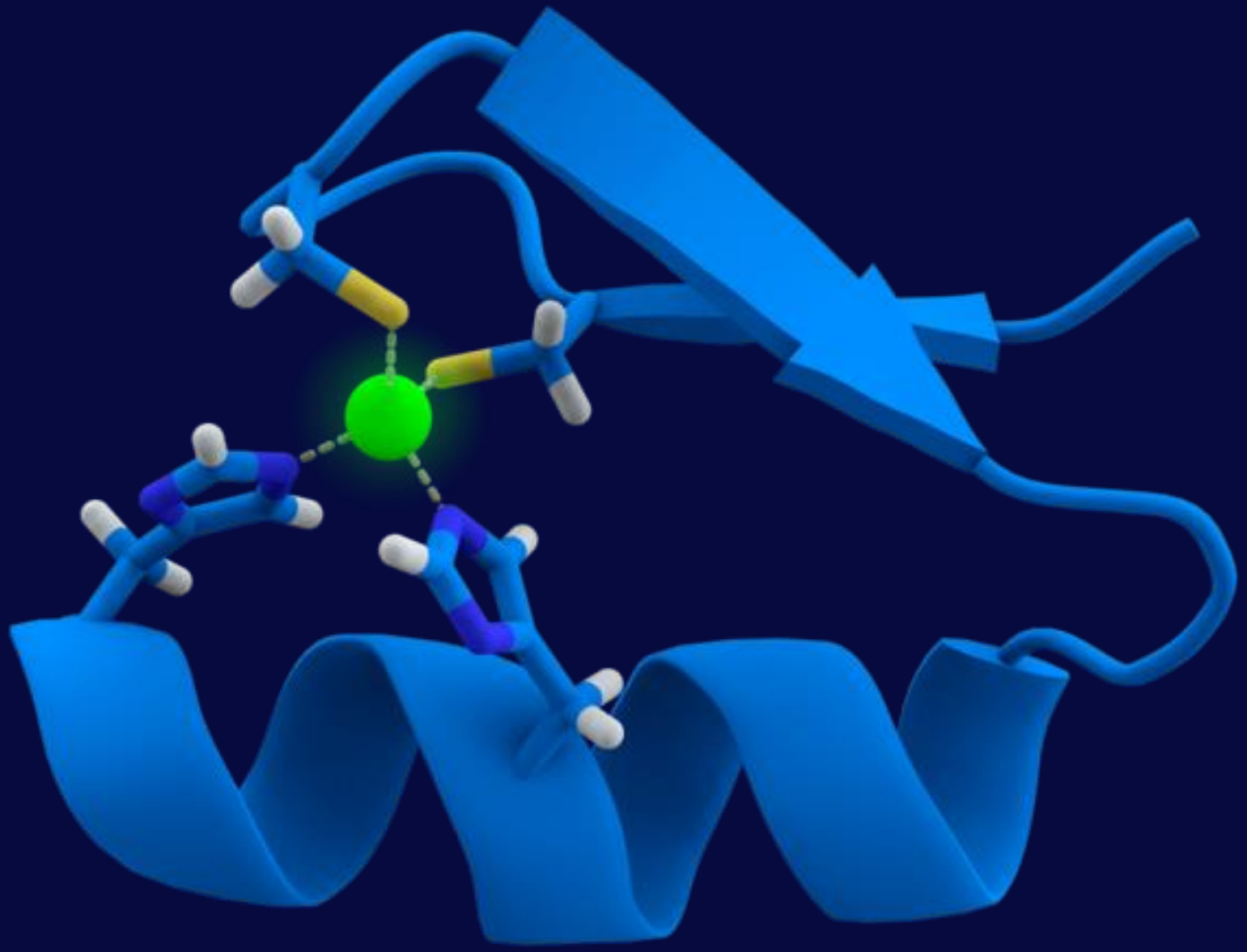




Blastulation: 1 - morula, 2 - blastula.

Choanoflagellatess




ALL I REALLY NEED TO KNOW I LEARNED IN KINDERGARTEN
THE CLASSIC INTERNATIONAL BESTSELLER
25TH ANNIVERSARY EDITION
UNCOMMON THOUGHTS ON COMMON THINGS
MORE THAN 7 MILLION COPIES SOLD
ROBERT FULGHUM

Tandem Zinc Finger Proteins