Pharmacogenomics

Module 2

Pharmacoepigenomics

PHARMACOGENOMICS

Programme of Module 2 Pharmacoepigenomics

-Introduction to pharmacoepigenomics: general aspects

-Interactions between genome and environment in the modulation of the pharmacological response

-Interactions between genome and environment in the modulation of side effects

-Pharmacoepigenomics deals with:

the influence that epigenetic alterations (DNA methylation, histone modifications, chromatin remodeling, non-coding RNAdysregulation) exert on drug efficacy and safety,

-the influence of the effects that drugs may have on the epigenetic machinery.

-Genes involved in pharmacogenomics are also affected by epigenetic modifications conditioning the therapeutic outcome.

-Drugs may modify epigenetic functions under normal and pathological conditions.

-Epigenetic drugs with potential effects in CNS disorders

-How epigenetic phenomena affect drug response in CNS diseases: addiction, chronic pain, neurodegenerative diseases, neuropsychiatric diseases.

-Epigenetic drugs: DNA methyltransferase inhibitors, histone deacetylase inhibitors, histone acetyltransferase modulators, histone methyltransferase inhibitors, histone demethylase inhibitors, non-coding RNAs (miRNAs)

-Ubiquitin proteasome system and interacions with epigenetic modifications.

-Animal models of human diseases

Epigenetics
And
Epigenetic drugs

What means epigenetic ????

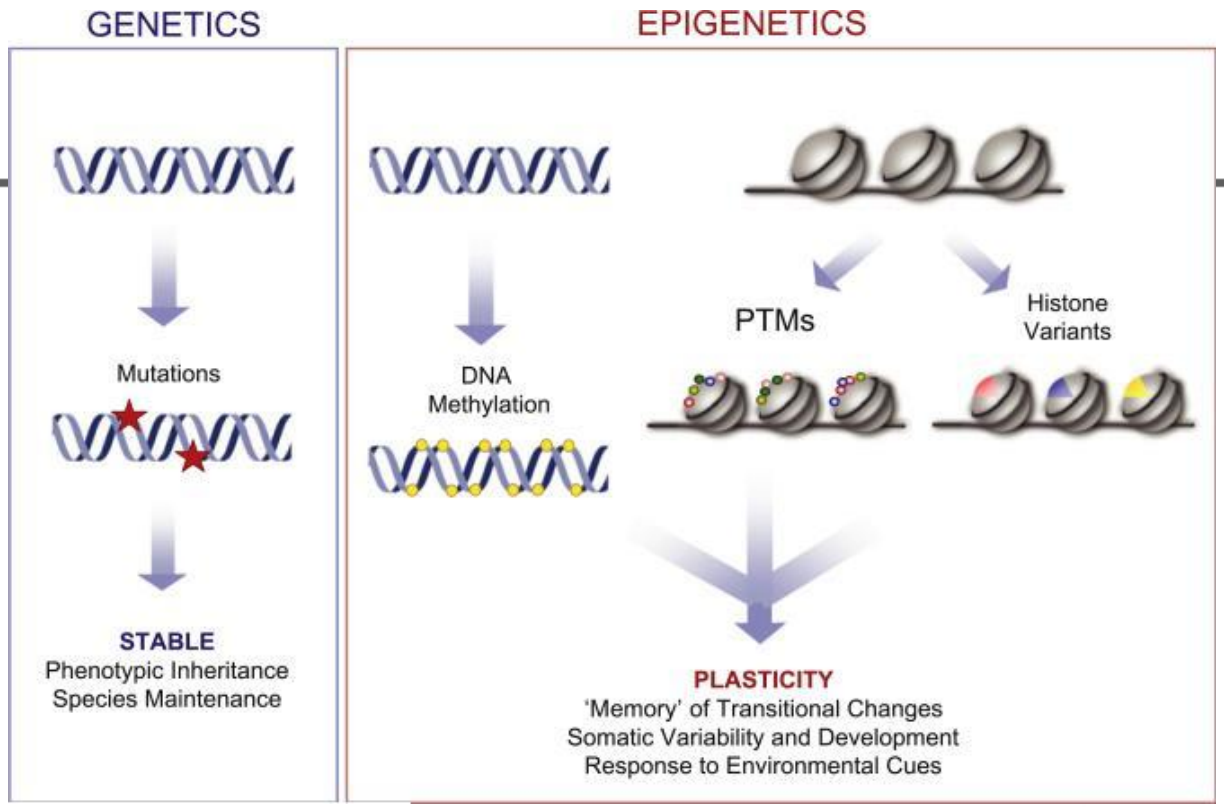
This term refers to changes altering the phenotype without altering the genotype of a person.

Epigenetics is a **branch of genetic** that is involved in the study of different kinds of alterations able to **affect gene expression**, **without altering the DNA** coding sequence.

Epigenetic signals and modifications are anyway inheritable even if it does not alter nucleotidic sequence, whereas it alters its activity.

They are inheritable phenomena and the phenotype is determined **not only** by the inheritable genotype **but also** by the overlapping of a mark, an influence of the functional behavior.

Genetics versus Epigenetic Control



Regulation of biological processes can be achieved via genetic and epigenetic programs. Variation in genetic information is obtained by mutagenesis of the DNA sequence that irreversibily change the encoded message. Epigenetic control operates either on DNA, via DNA methylation, or on chromatin.

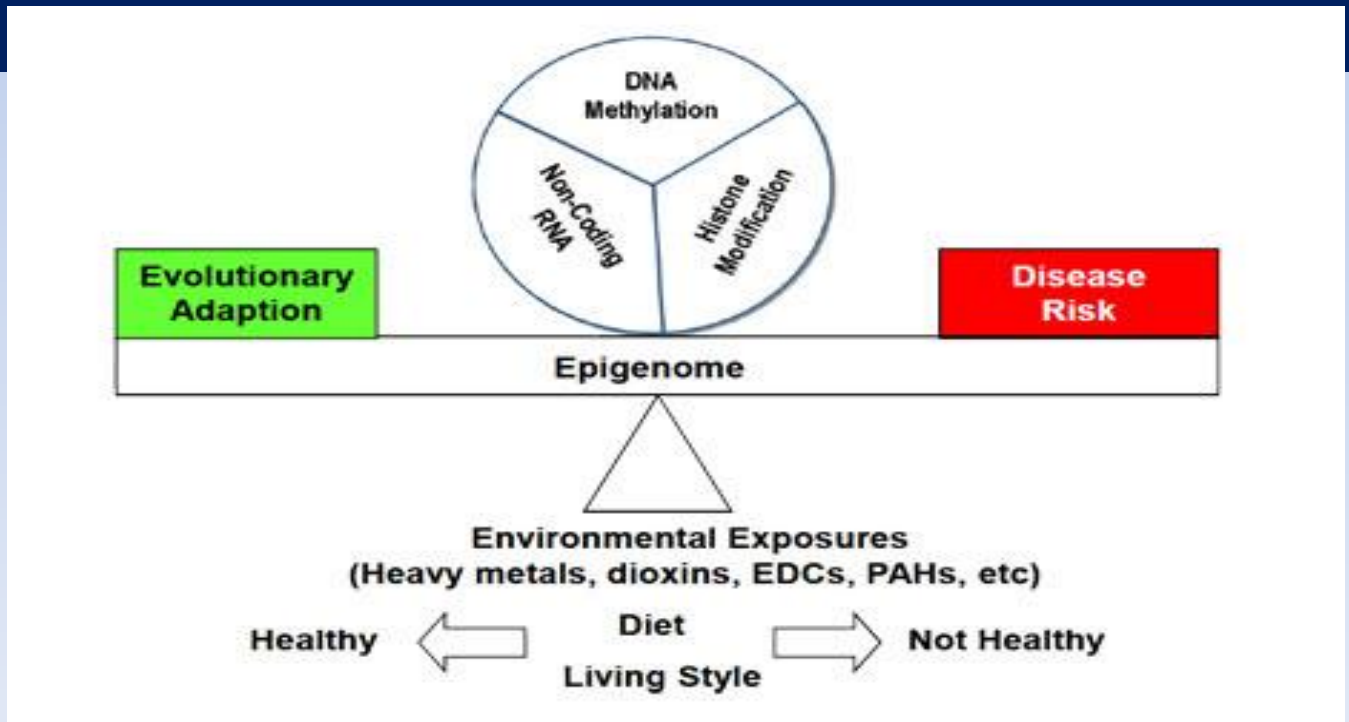
Borrelli et al., 2008, Neuron.

These changes are also called EPIMUTATIONS, influencing next generations of cells and individuals, without DNA mutations

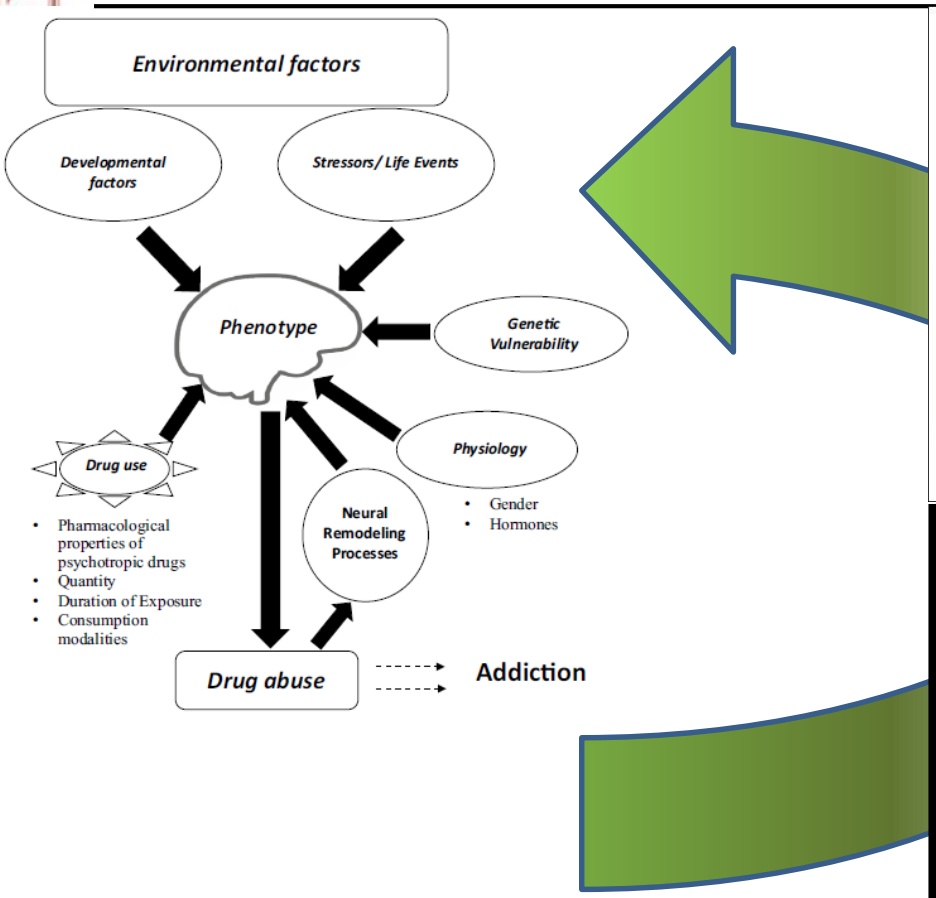
They are defined as

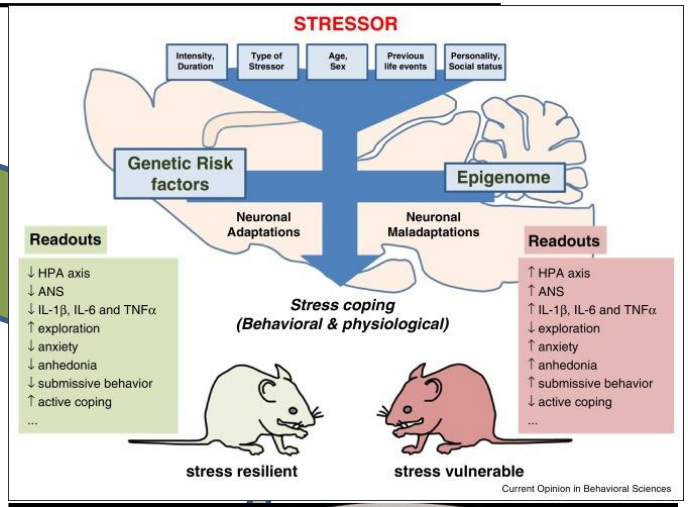
NONGENOMIC factors able to induce a different gene expression of the individuals

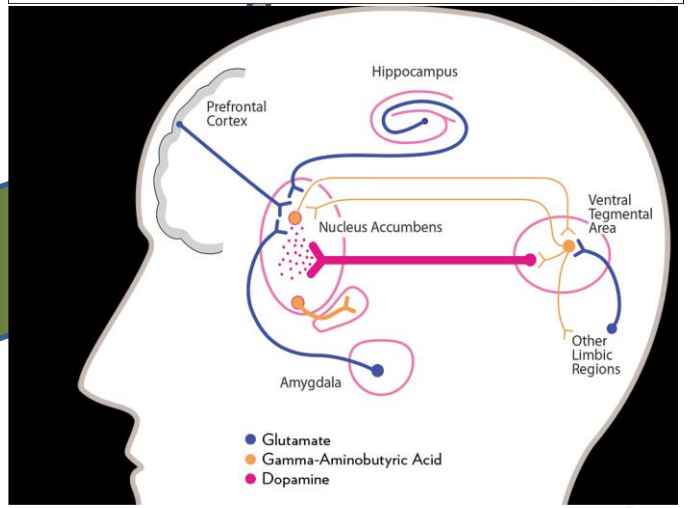
Gene-environment interactions are responsible of development trajectories alterations, leading to evolution adaptations toward some vulnerability or resilience for diseases

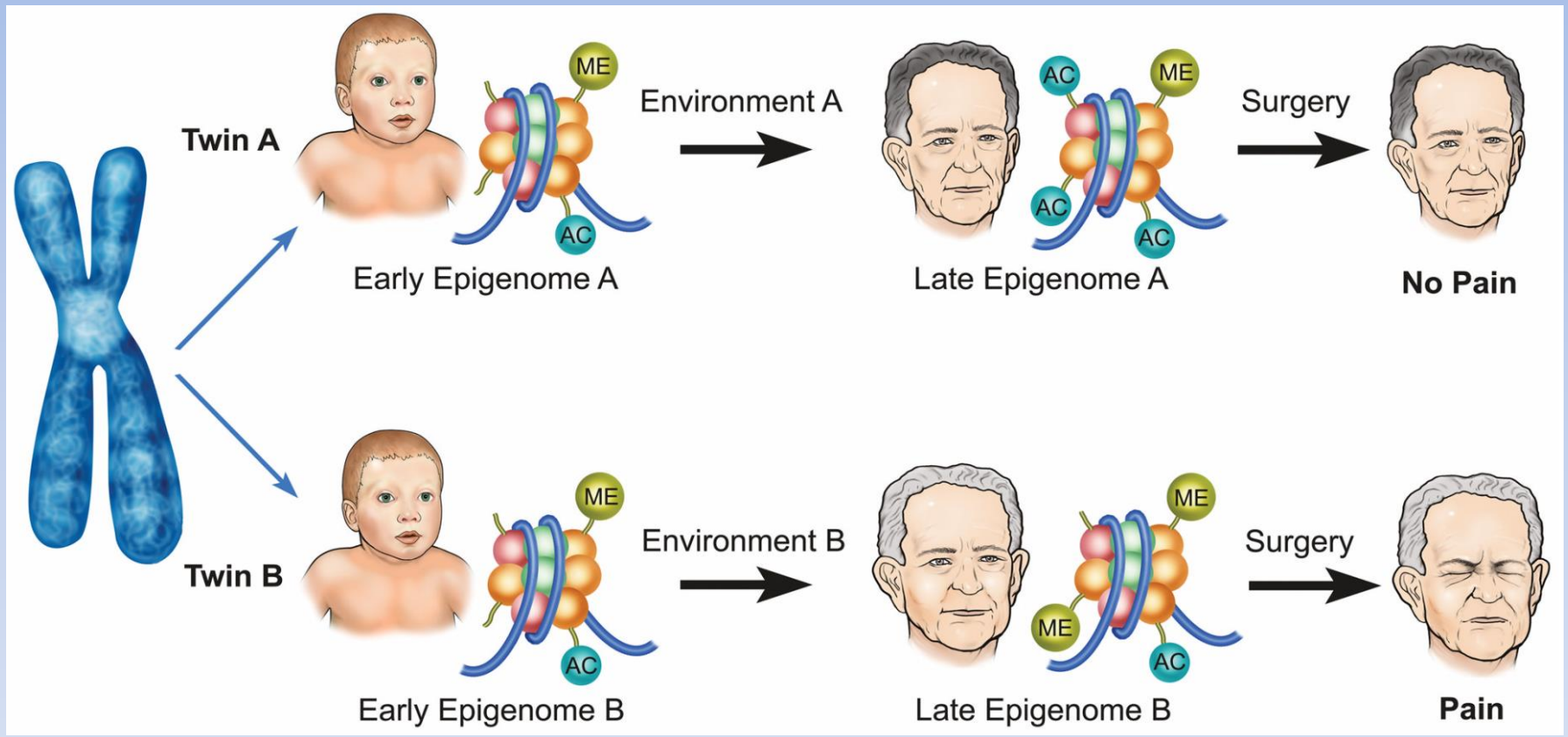


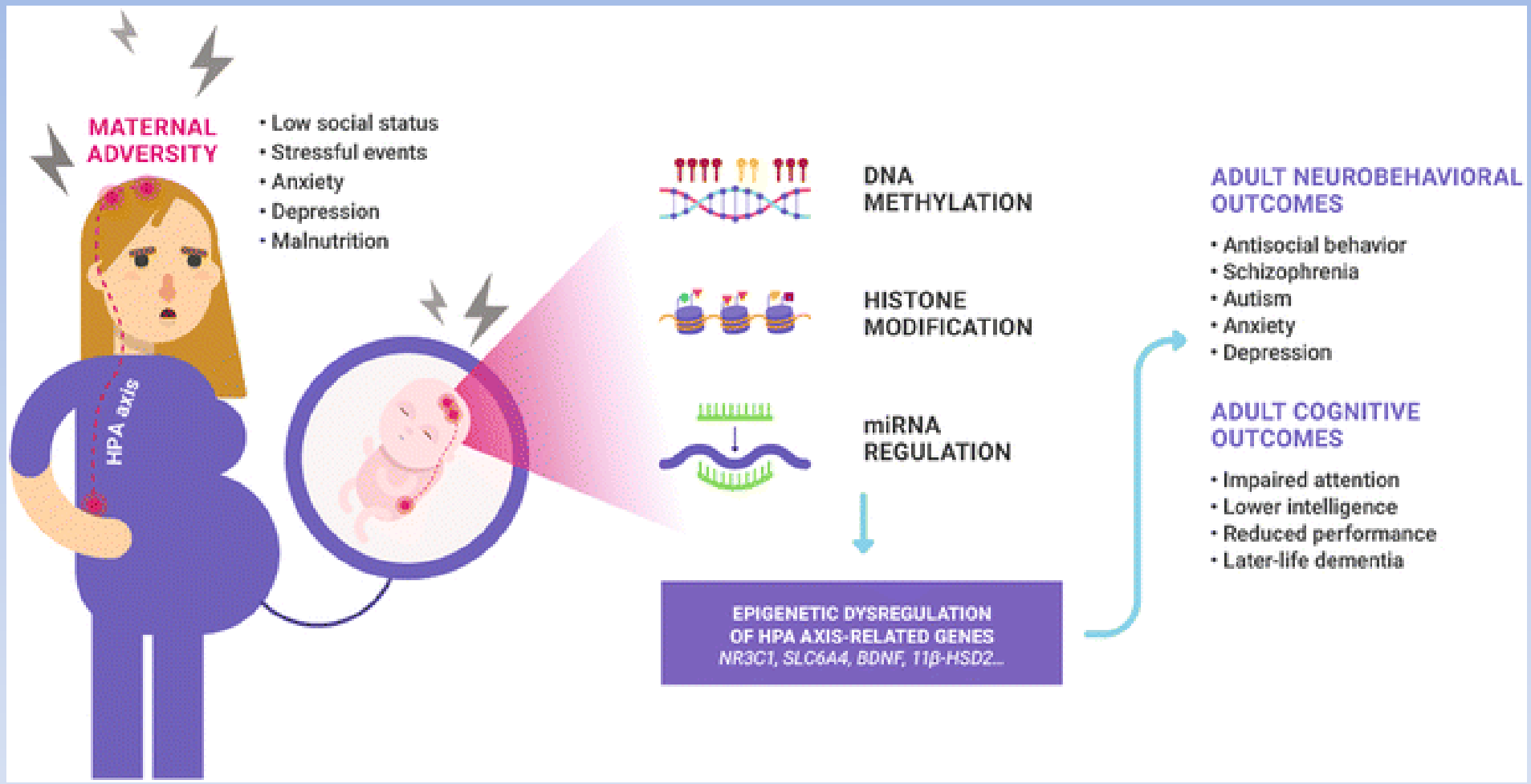
Environmental factor and Drug addiction

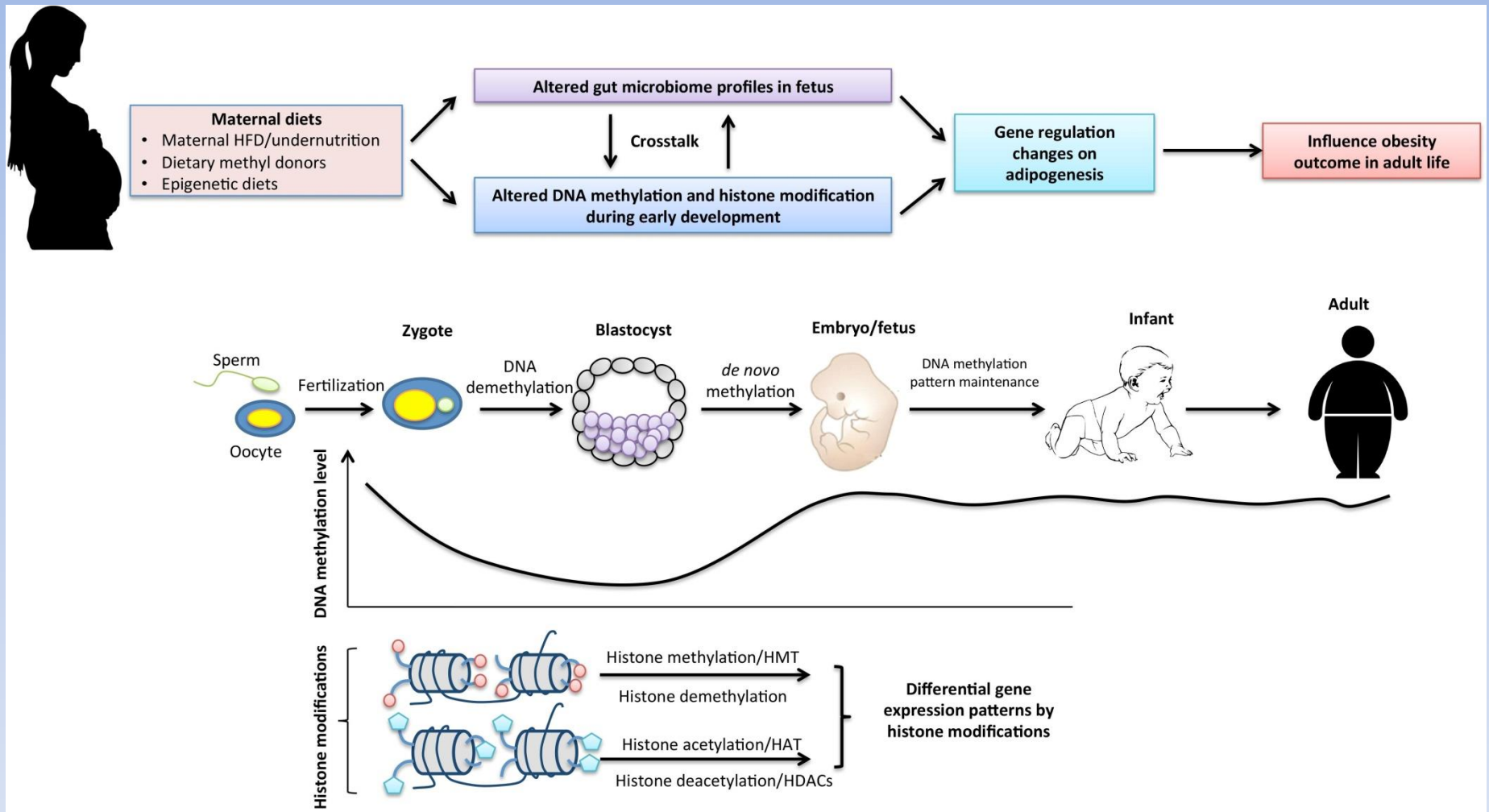




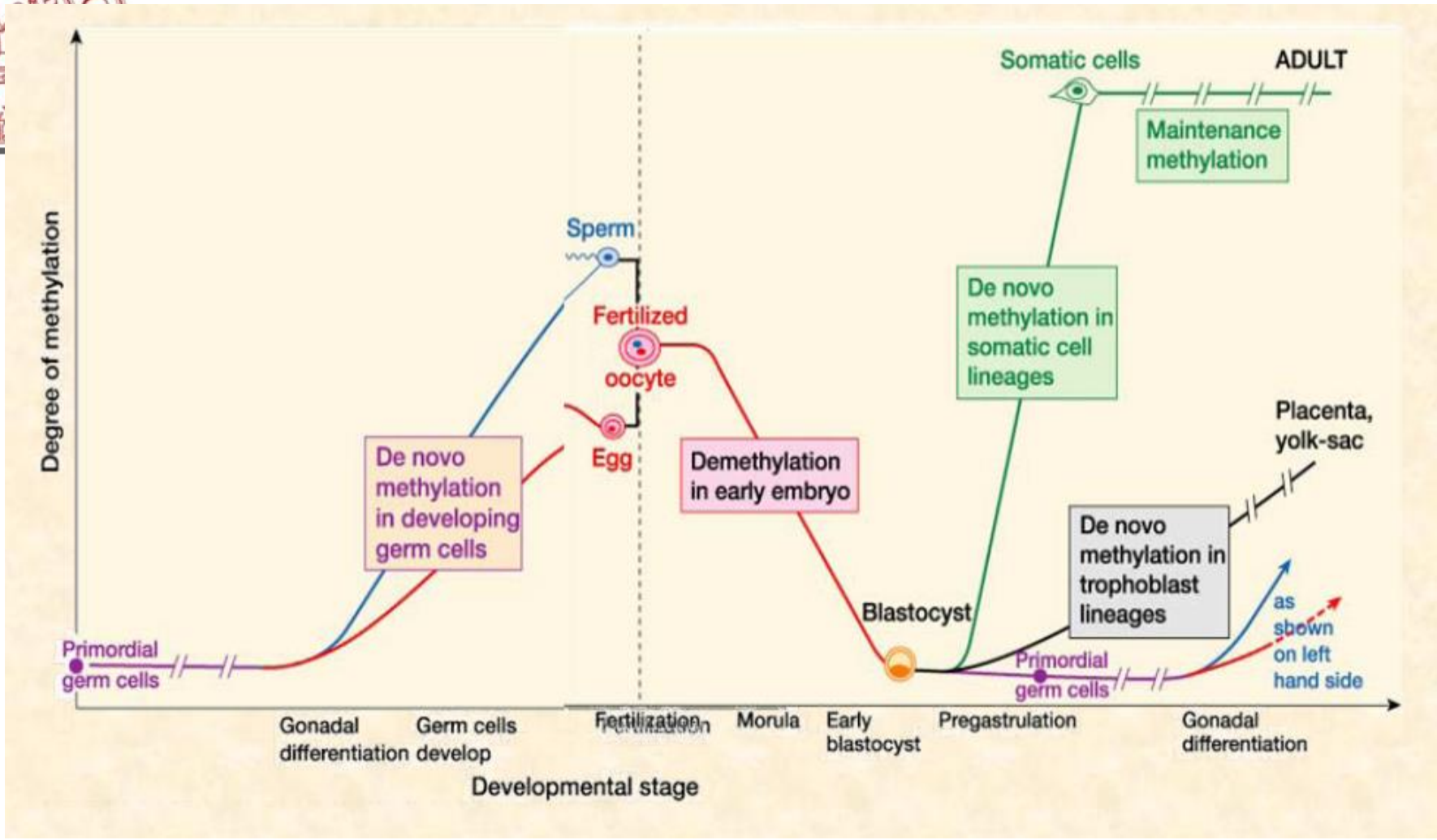



Twin A
ME
AC
Early Epigenome A
Environment A
AC
ME
AC
Late Epigenome A
Surgery
No Pain
Twin B
ME
AC
Early Epigenome B
Environment B
ME
ME
AC
Late Epigenome B
Surgery
Pain

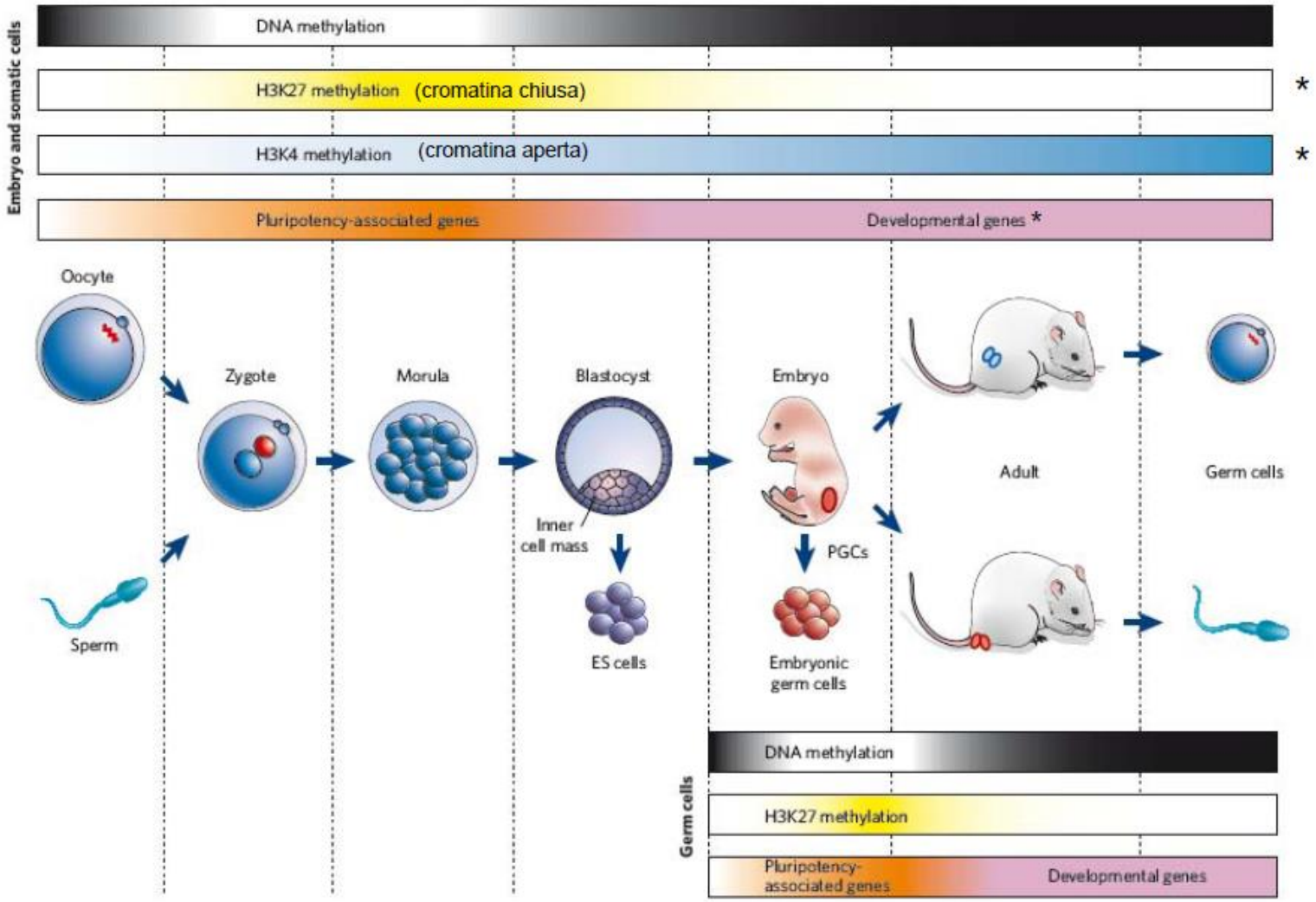

MATERNAL ADVERSITY
• Low social status
• Stressful events
• Anxiety
• Depression
• Malnutrition
HPA axis
DNA METHYLATION
HISTONE MODIFICATION
miRNA REGULATION
EPIGENETIC DYSREGULATION OF HPA AXIS-RELATED GENES
NR3C1, SLC6A4, BDNF, 11β-HSD2...
ADULT NEUROBEHAVIORAL OUTCOMES
• Antisocial behavior
• Schizophrenia
• Autism
• Anxiety
• Depression
ADULT COGNITIVE OUTCOMES
• Impaired attention
• Lower intelligence
• Reduced performance
• Later-life dementia


Maternal diets
• Maternal HFD/undernutrition
• Dietary methyl donors
• Epigenetic diets
Altered gut microbiome profiles in fetus
Crosstalk
Altered DNA methylation and histone modification during early development
Gene regulation changes on adipogenesis
Influence obesity outcome in adult life
Sperm
Oocyte
Fertilization
Zygote
DNA demethylation
Blastocyst
de novo methylation
Embryo/fetus
DNA methylation pattern maintenance
Infant
Adult
DNA methylation level
Histone modifications
Histone methylation/HMT
Histone demethylation
Histone acetylation/HAT
Histone deacetylation/HDACs
Differential gene expression patterns by histone modifications

Epigenetic regulation during development



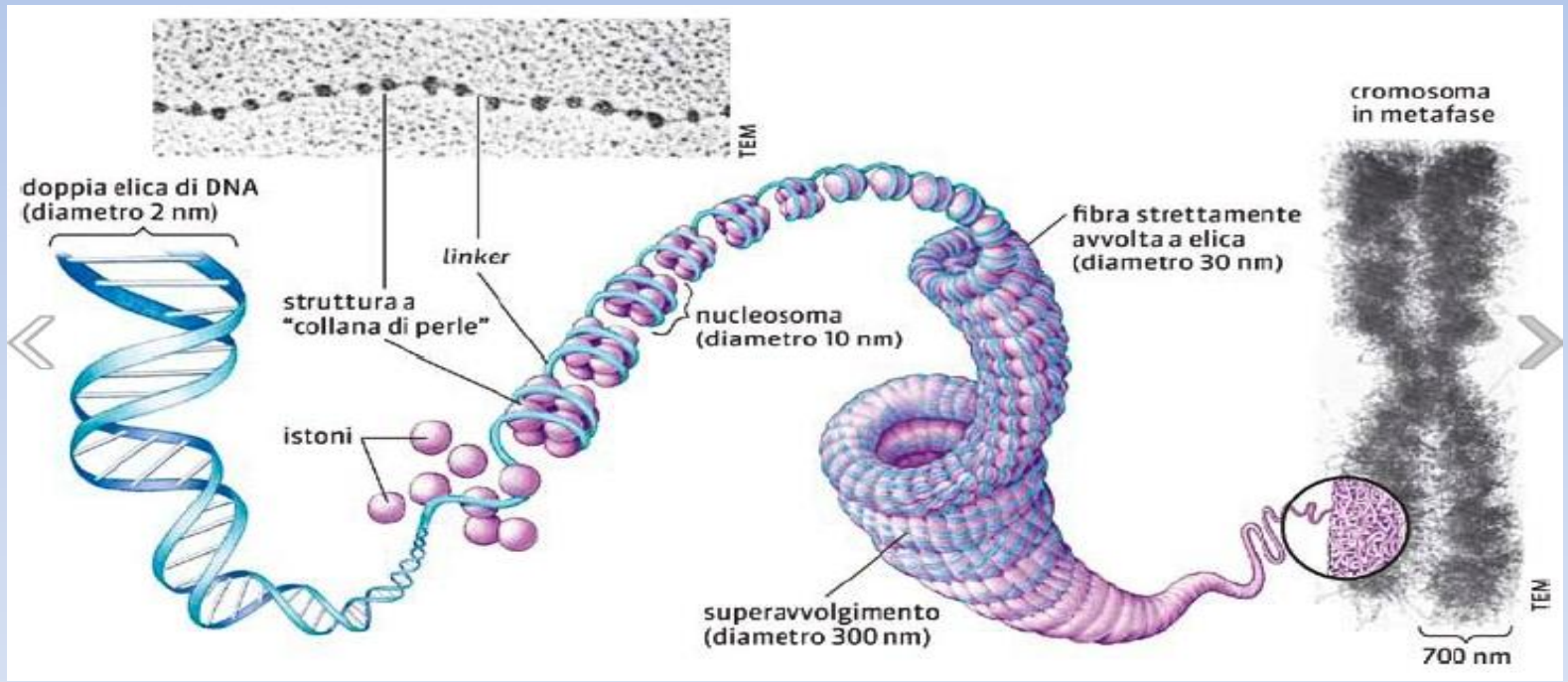
Epigenetic regulation during development

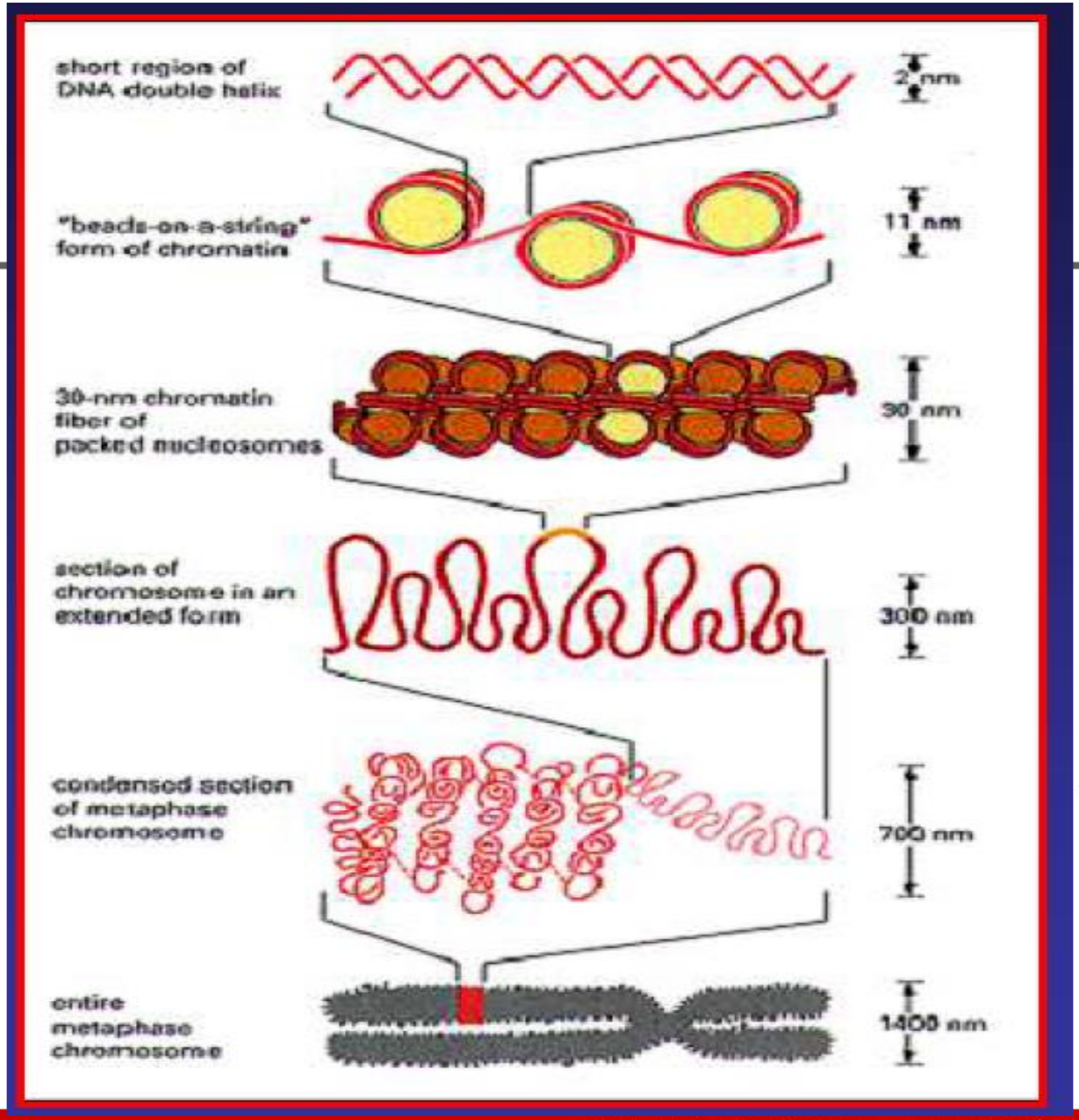


Genetics alone does not determine our health

ORGANIZATION and structure of CHROMATIN

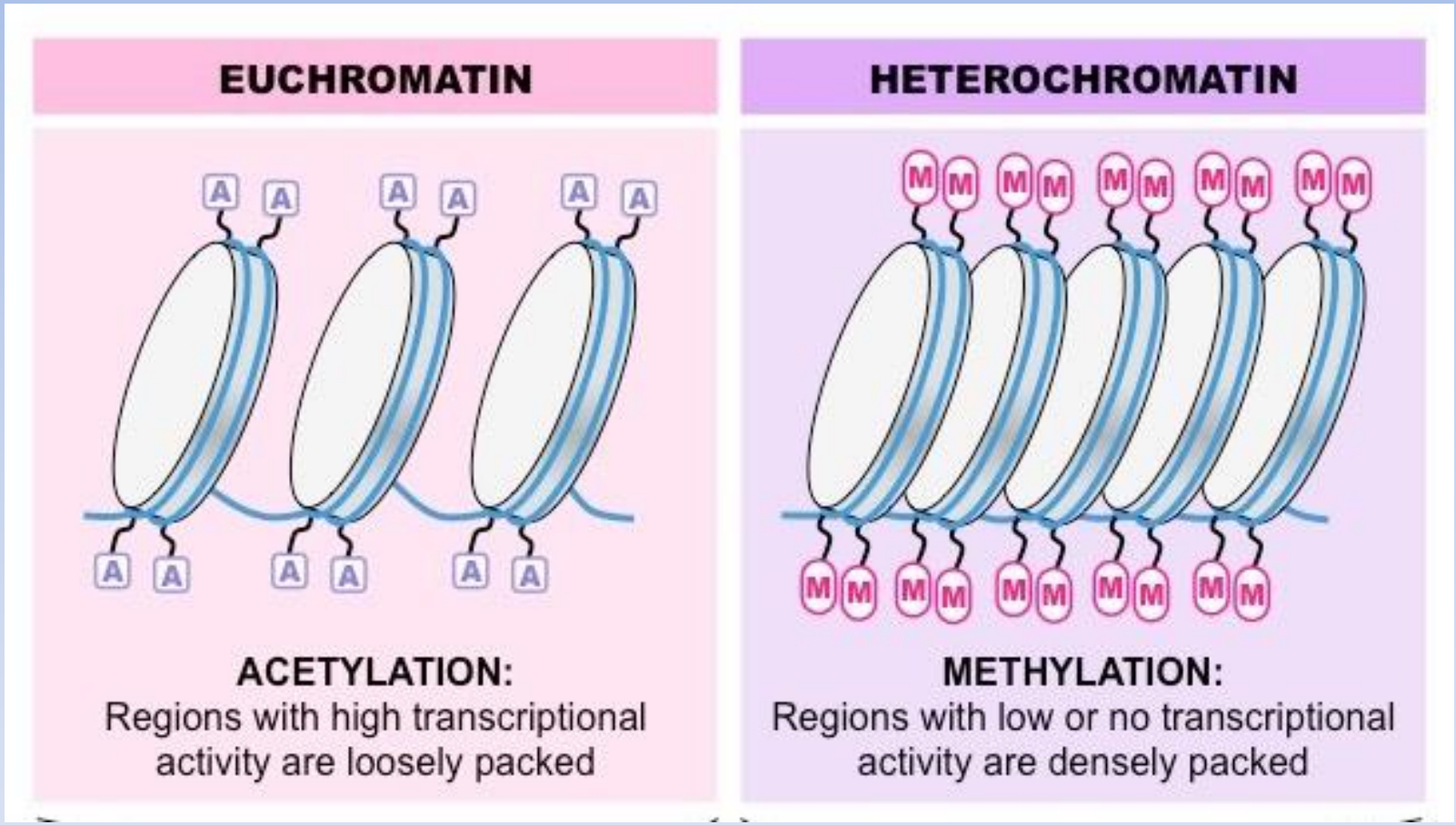
Chromatin is the structure of nucleic acids inside the cells.
In eucariotic cells chromatin is mainly consisting of:
<u>DNA wind up to proteins named Histone</u> (H) producing the NUCLEOSOMA

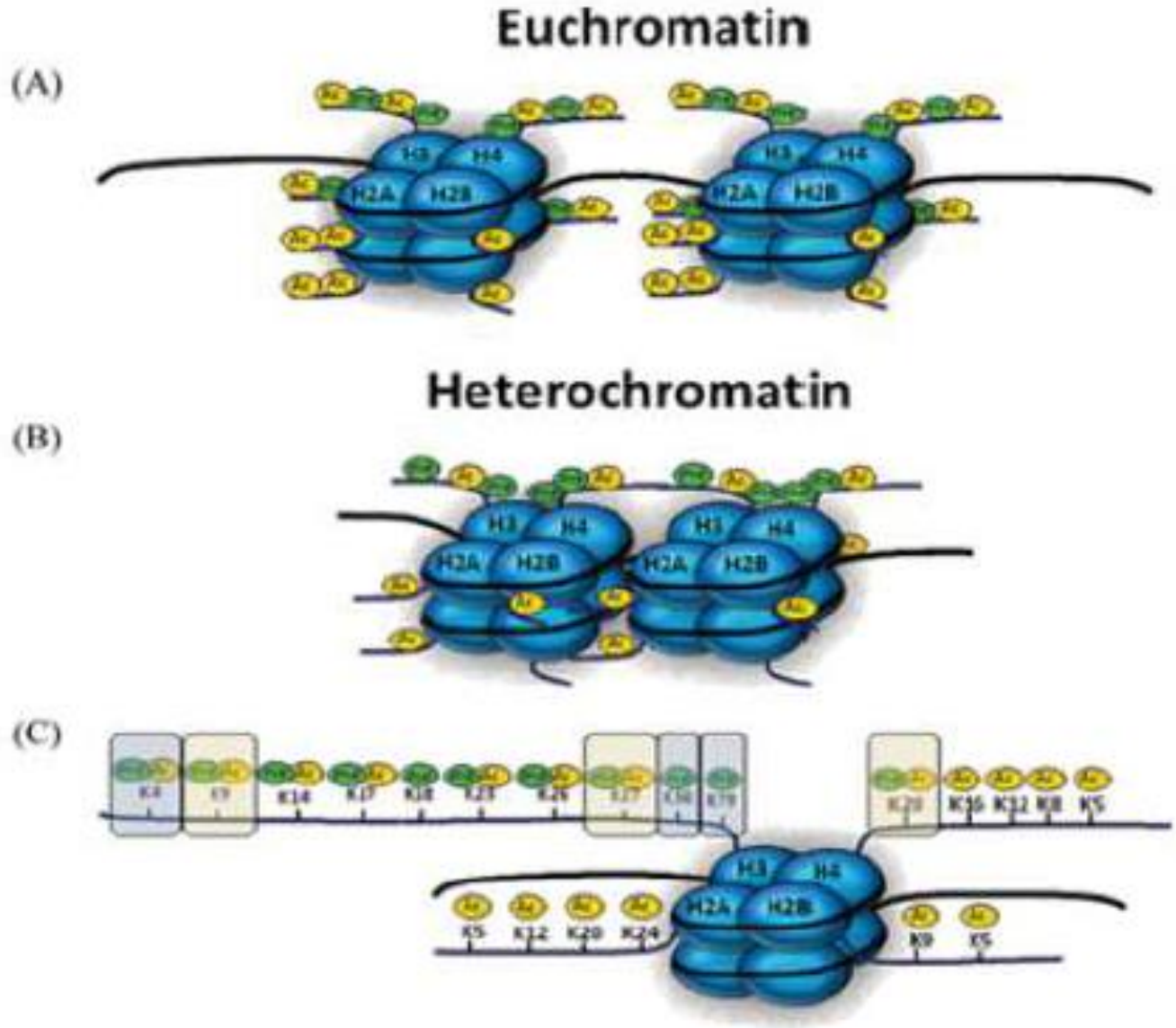


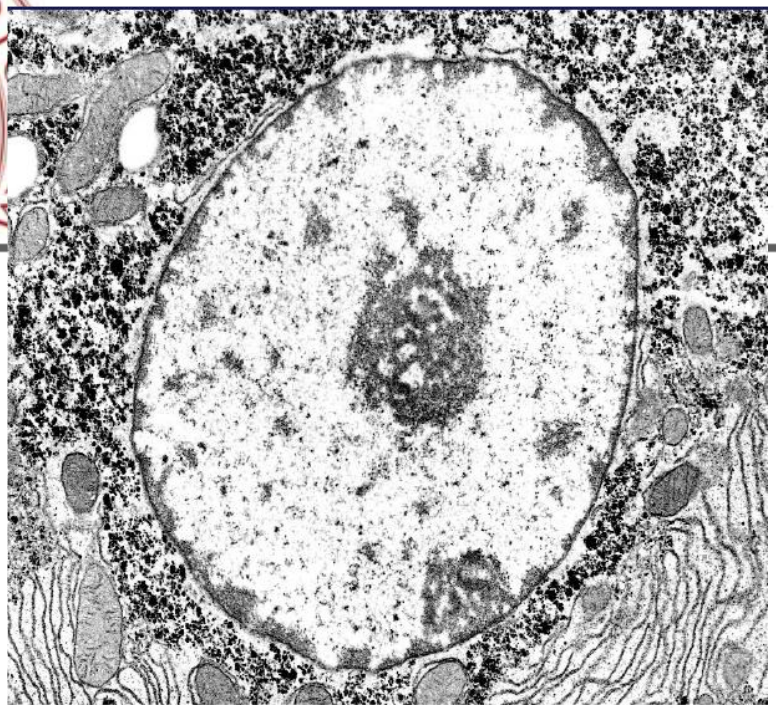


EUCHROMATIN vs HETEROCHROMATIN

ACTIVE **INACTIVE**

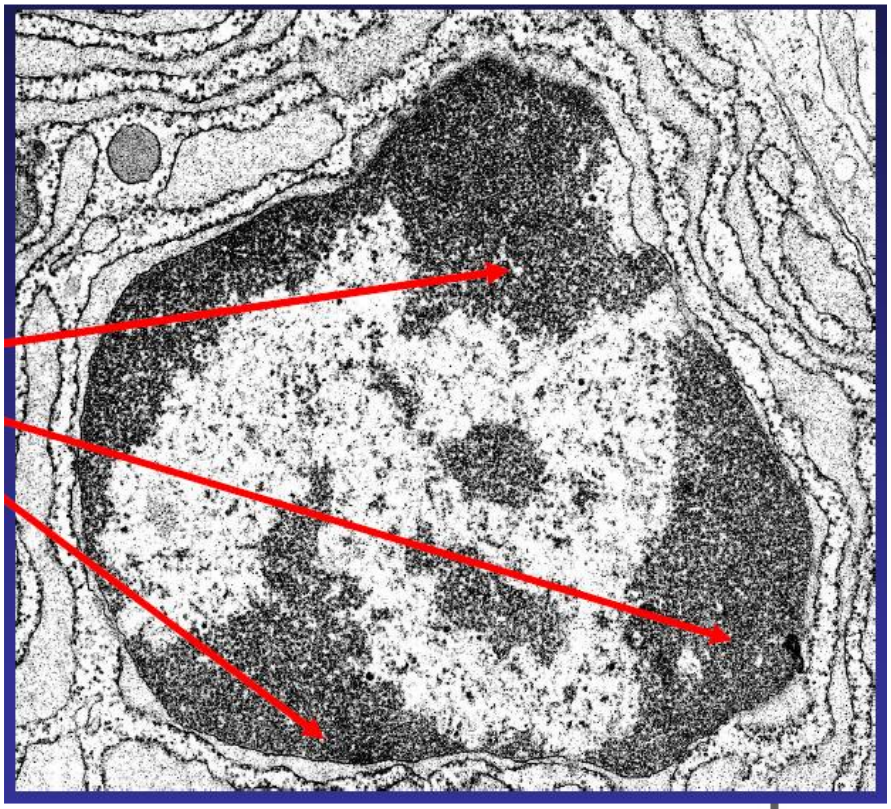






Relaxed chromatin

Condensed chromatin



3 Main epigenetics mechanisms:

1. **Histone modifications**
2. **DNA methylation**

They are **reversible** and **dynamic** also in adults.

3. non-coding RNA (ncRNA) = functional RNA encoding from DNA but not translated in protein → regulation of gene expression **at trascriptional and post-transcriptional level**.

ncRNA related to epigenetic include short-ncRNA (microRNAs (miRNAs), short interfering RNAs (siRNAs) and **long-ncRNA**.

1. Histone modifications are post-transcriptional

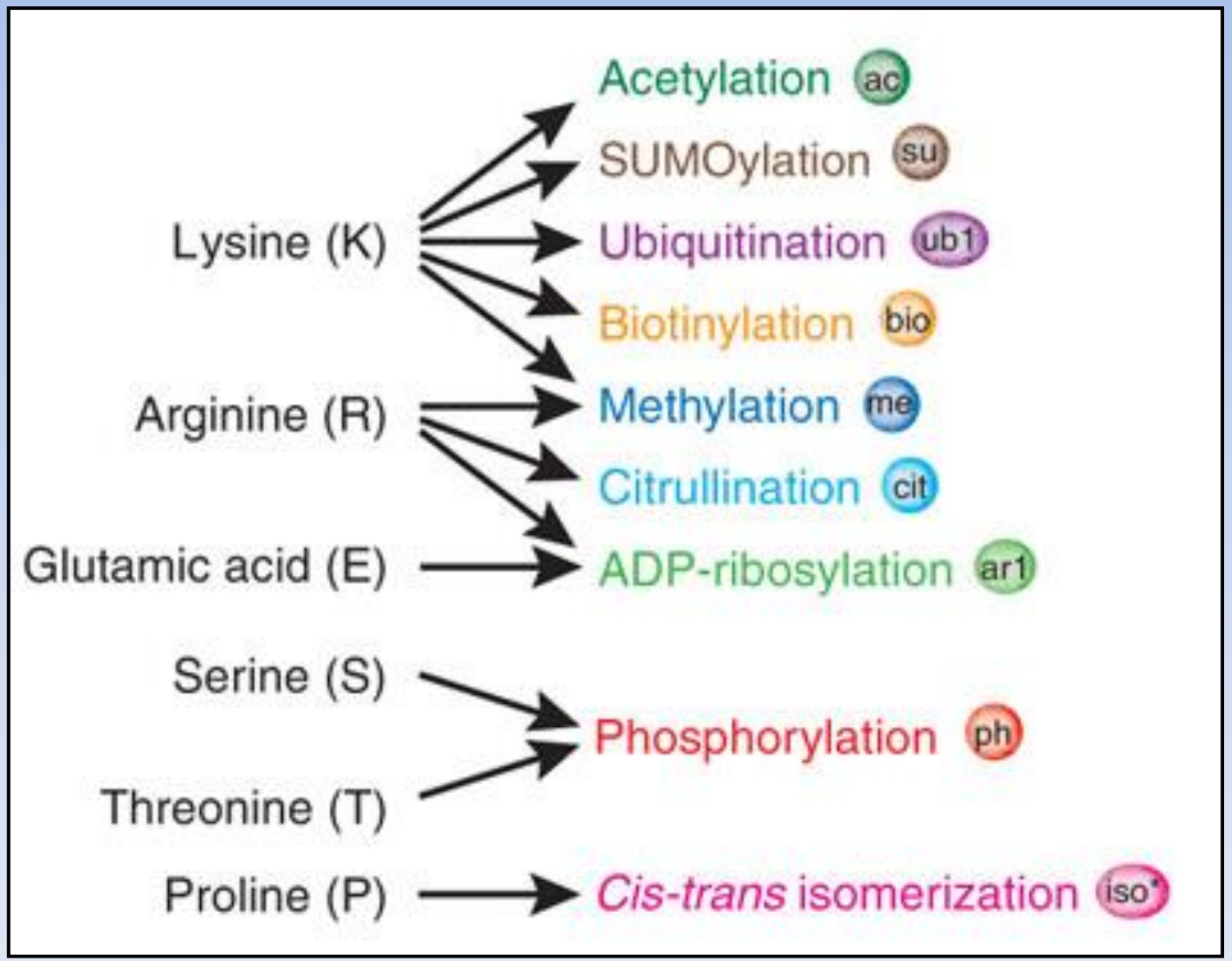
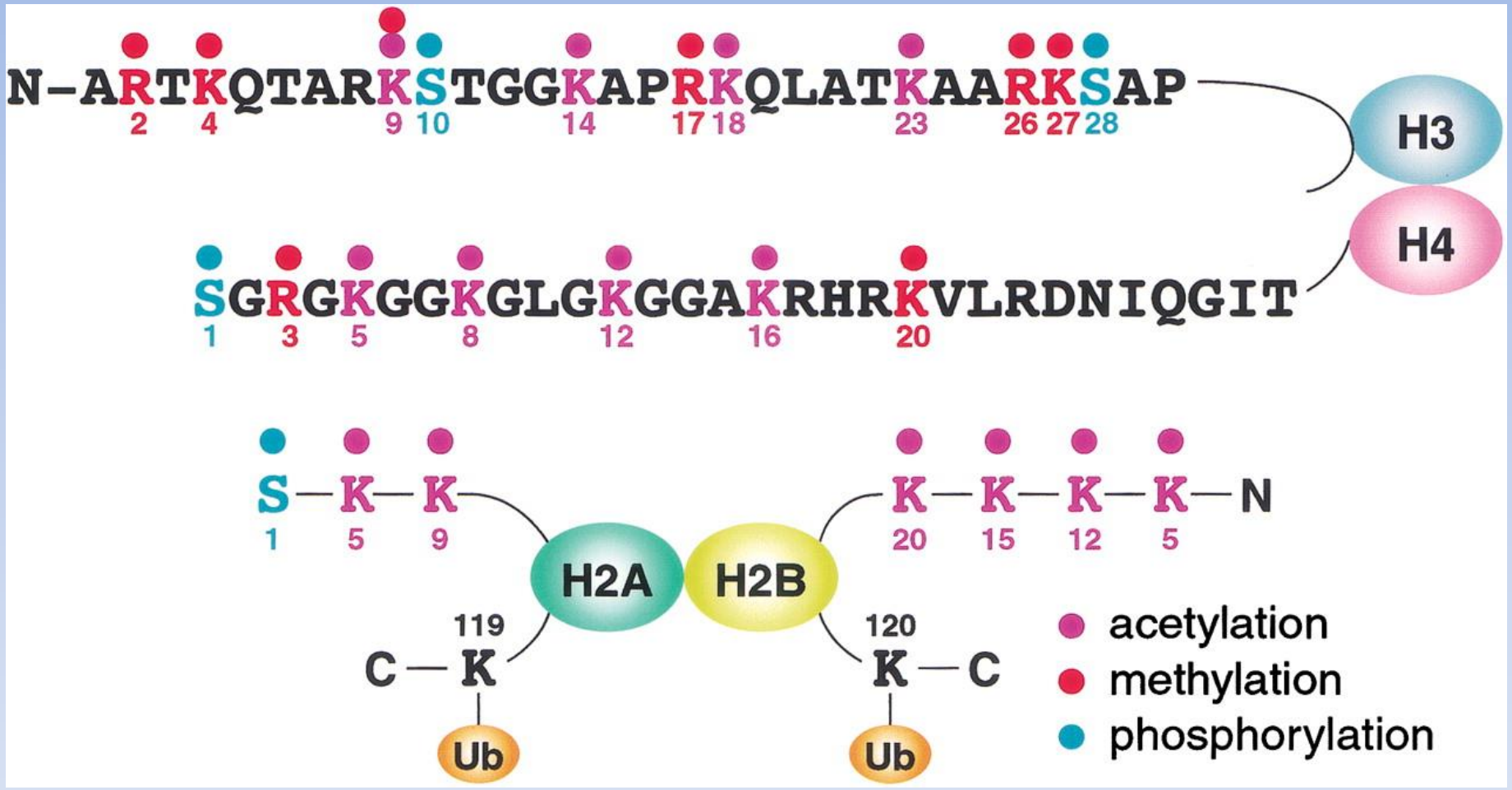


Table 1. Different Classes of Modifications Identified on Histones

Chromatin Modifications	Residues Modified	Functions Regulated
Acetylation	**K**-ac	Transcription, Repair, Replication, Condensation
Methylation (lysines)	**K**-me1 **K**-me2 **K**-me3	Transcription, Repair
Methylation (arginines)	**R**-me1 **R**-me2a **R**-me2s	Transcription
Phosphorylation	**S**-ph **T**-ph	Transcription, Repair, Condensation
Ubiquitylation	**K**-ub	Transcription, Repair
Sumoylation	**K**-su	Transcription
ADP ribosylation	**E**-ar	Transcription
Deimination	**R > Cit**	Transcription
Proline Isomerization	**P-cis > P-trans**	Transcription

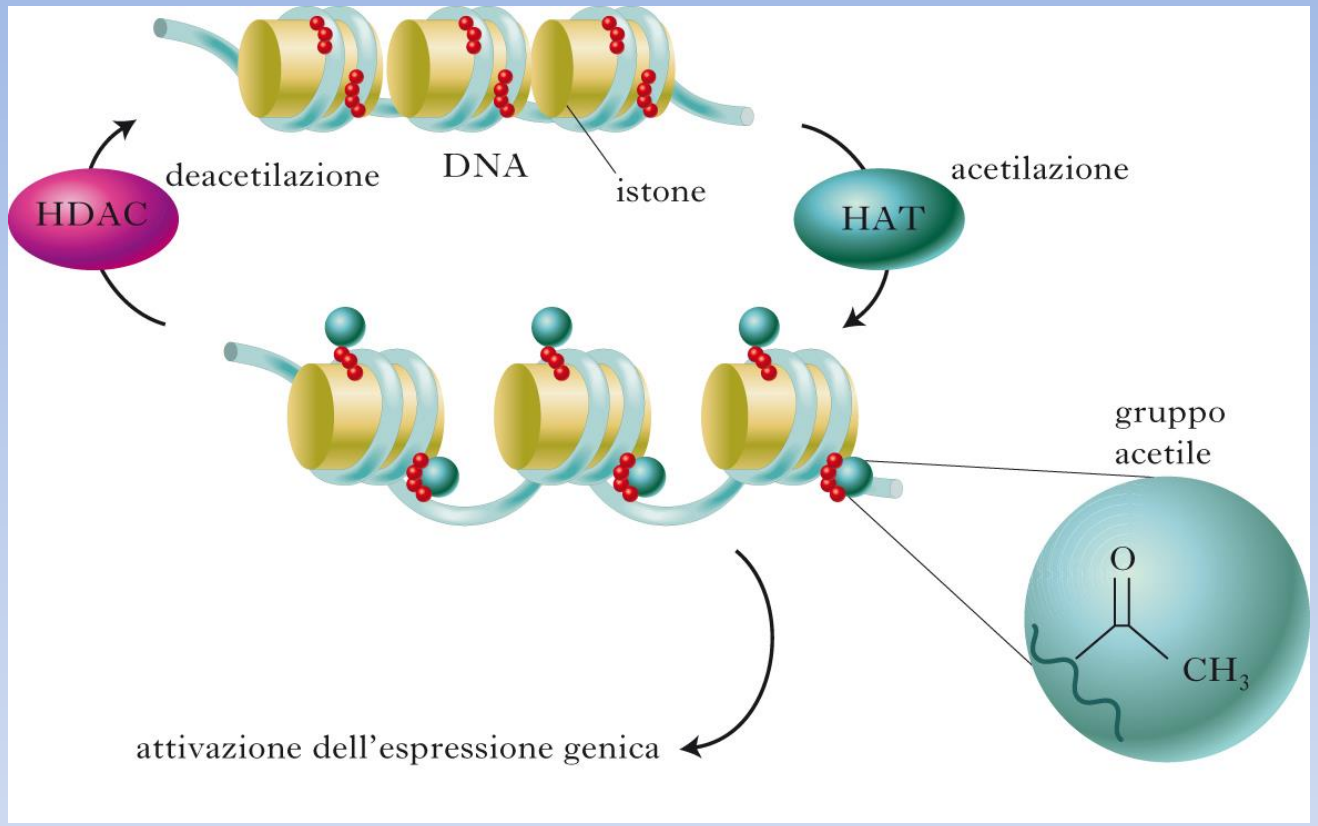
Overview of different classes of modification identified on histones. The functions that have been associated with each modification are shown. Each modification is discussed in detail in the text under the heading of the function it regulates.

Histone modifications



Lisine = K; Arginine = R; Serine = S

Acetylation and Deacetylation



HDACs
(many isoforms)

Deletion of acetyl groups is associated to chromatin condensation and gene transcription repression

HATs
(many isoforms)

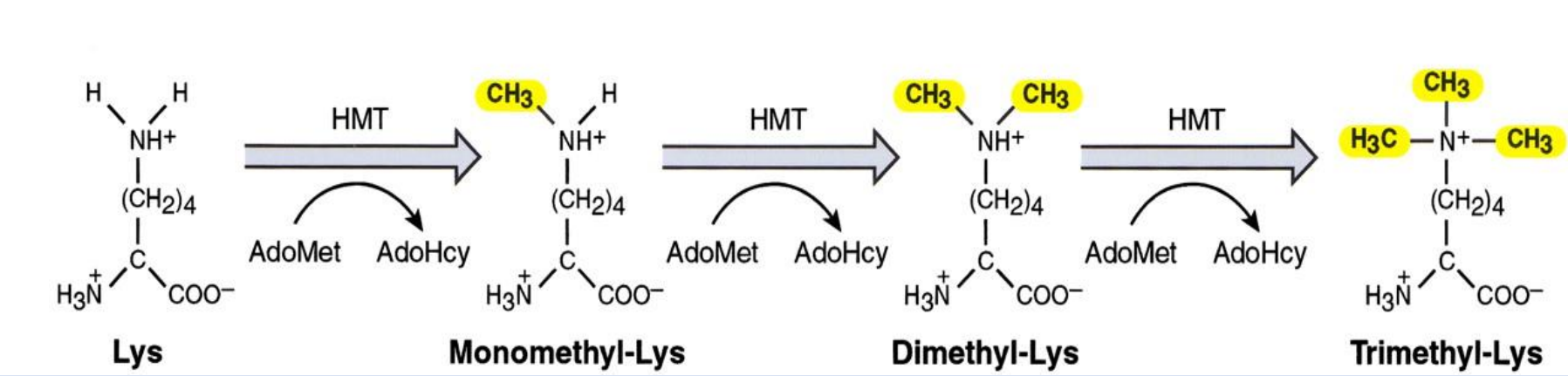
Acetylation is associated to chromatin relaxation and gene transcription enhancement

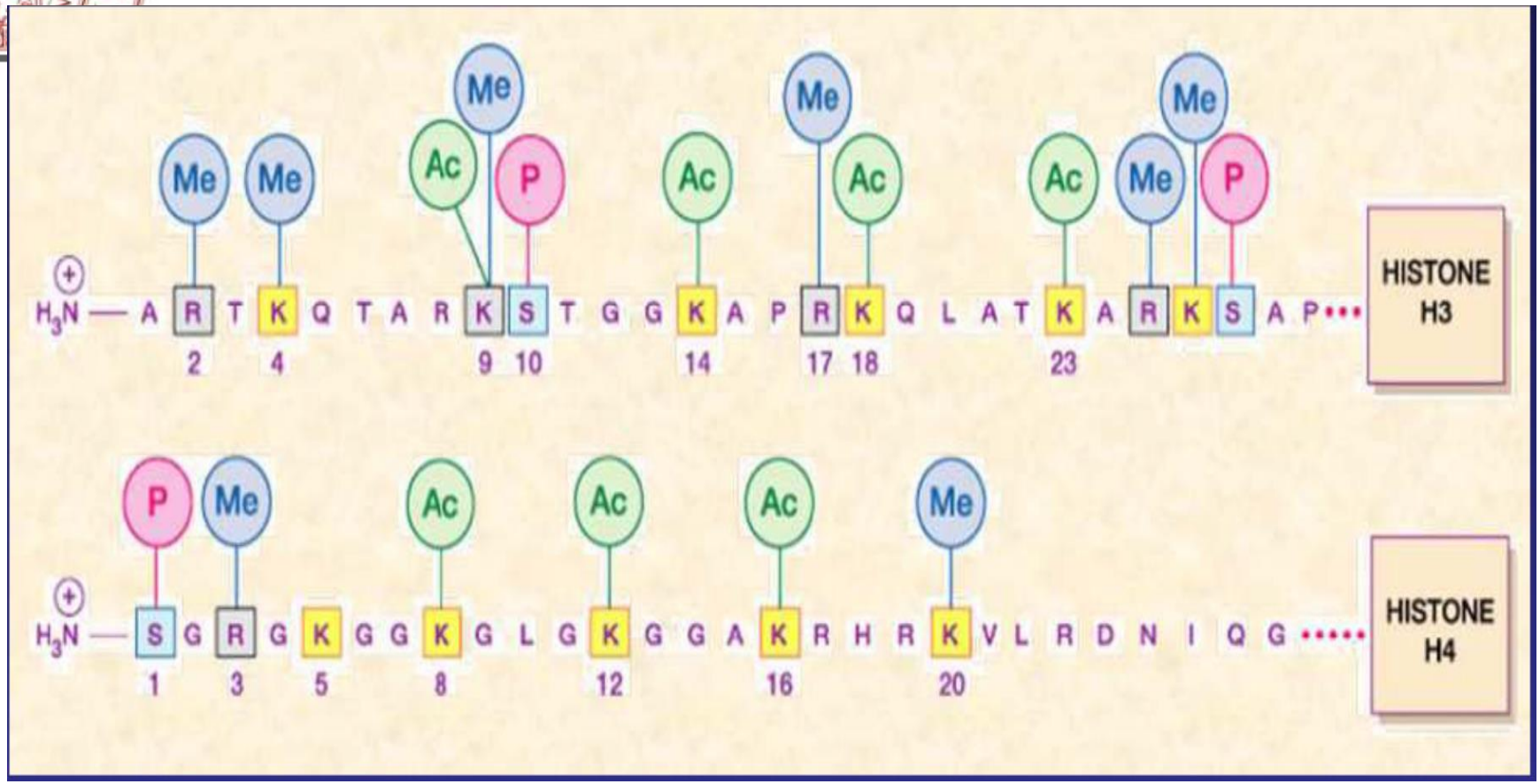
Histone methylation

histone-methyltransferase (HMTs)
substitute 1 H with 1 CH3 on residues of arginine (R) or lysine (K) →

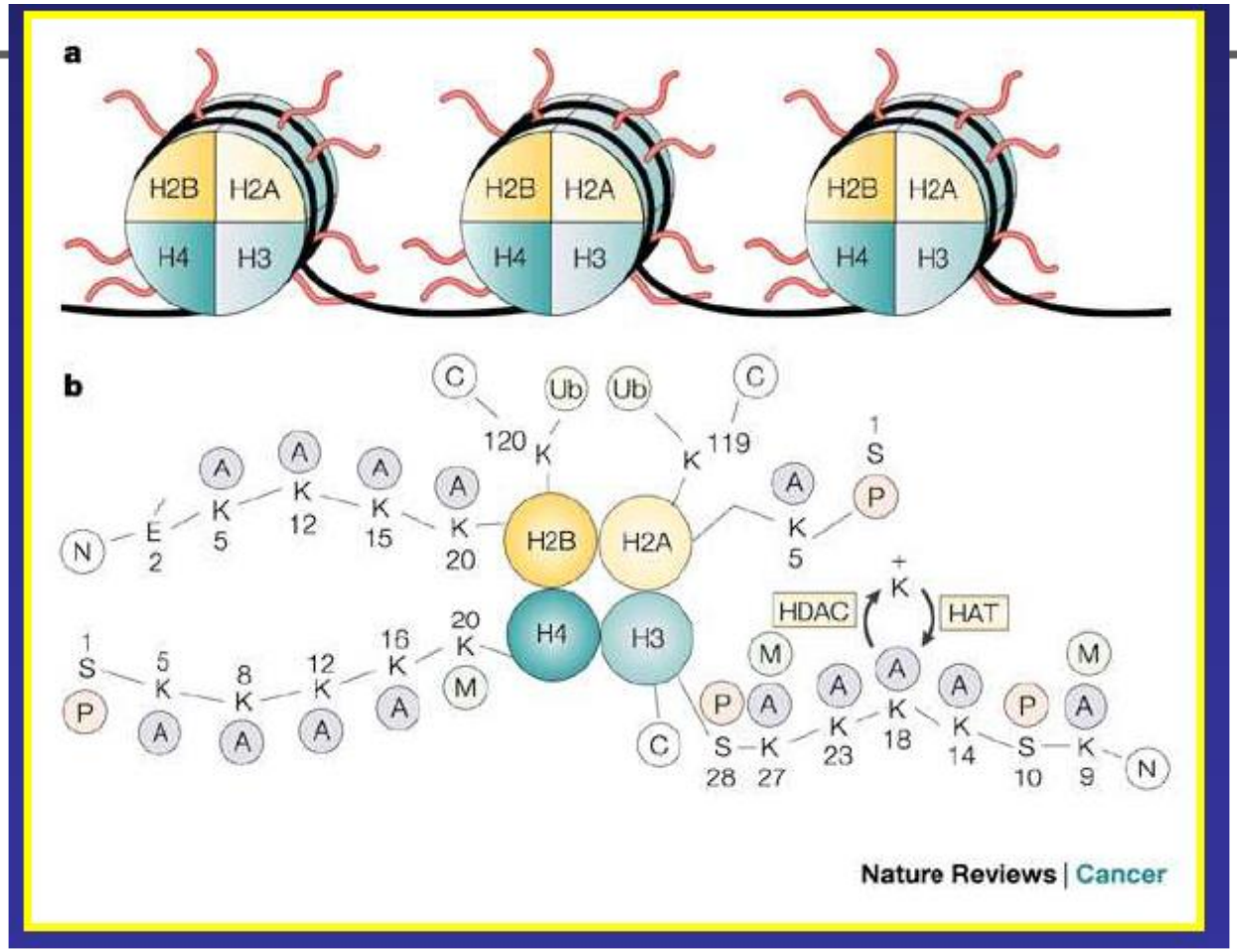
Usually, methylation of R activates gene transcription, of K inhibits; also number of –CH3 added is important

Lysin K can be MONO, DI o Tri methylated



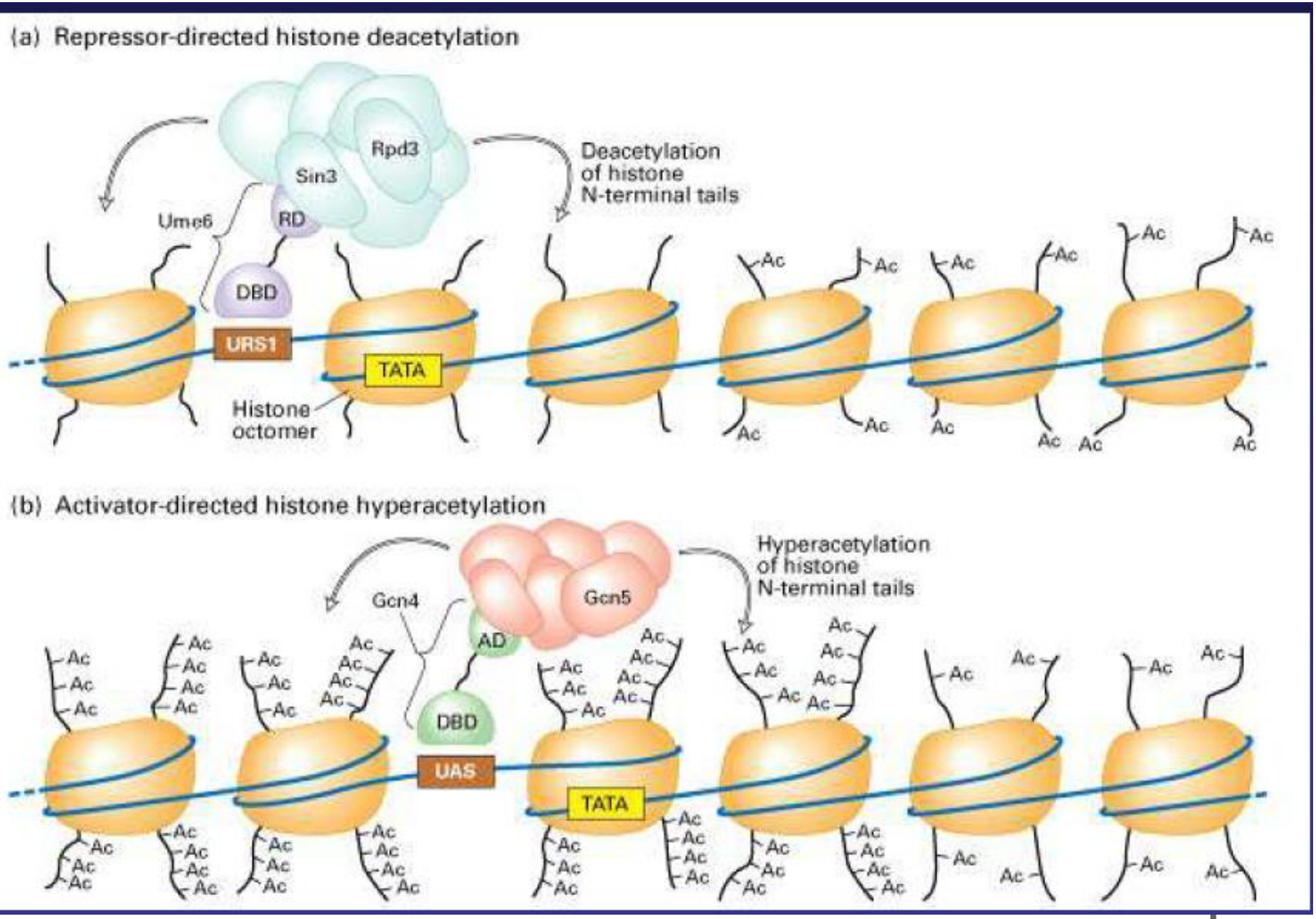


Examples



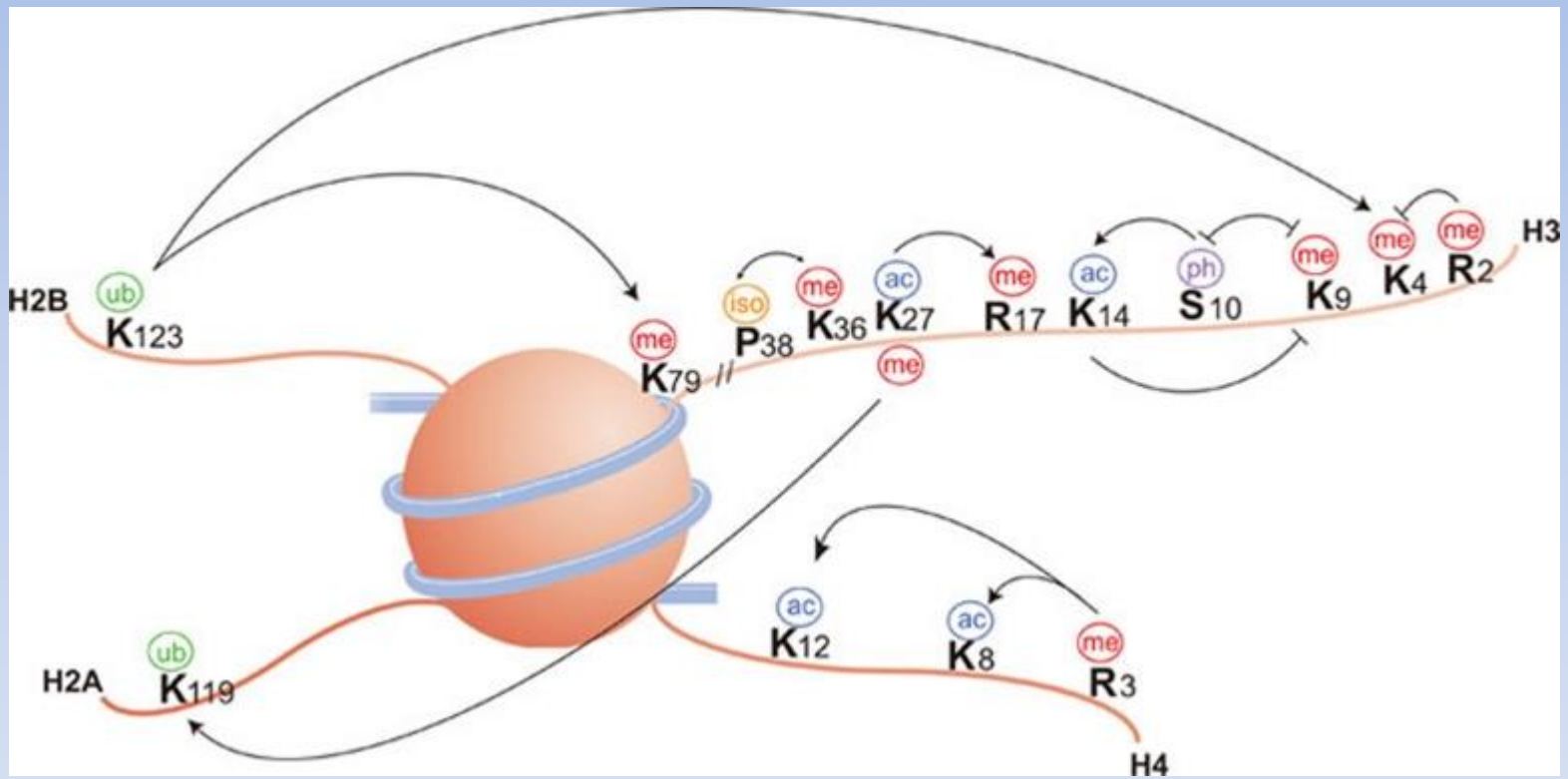
Tab.1 Epigenetic histone modifications

Histone PTMs		
Acetylated lysine (Kac)	H3 (9, 14, 18, 56), H4 (5, 8, 13, 16), H2A, H2B	Activation
Phosphorylated serine/ threonine (S/Tph)	H3 (3, 10, 28), H2A, H2B	Activation
Methylated arginine (Rme)	H3 (17, 23), H4 (3)	Activation
Methylated lysine (Kme)	H3 (4, 36, 79) H3 (9, 27), H4 (20)	Activation Repression
Ubiquitylated lysine (Kub)	H2B (123§/120¶) H2A (119¶)	Activation Repression
Sumoylated lysine (Ksu)	H2B (6/7), H2A (126)	Repression
Isomerized proline (Pisom)	H3 (30–38)	Activation/ repression



Cross-talk of histone modifications

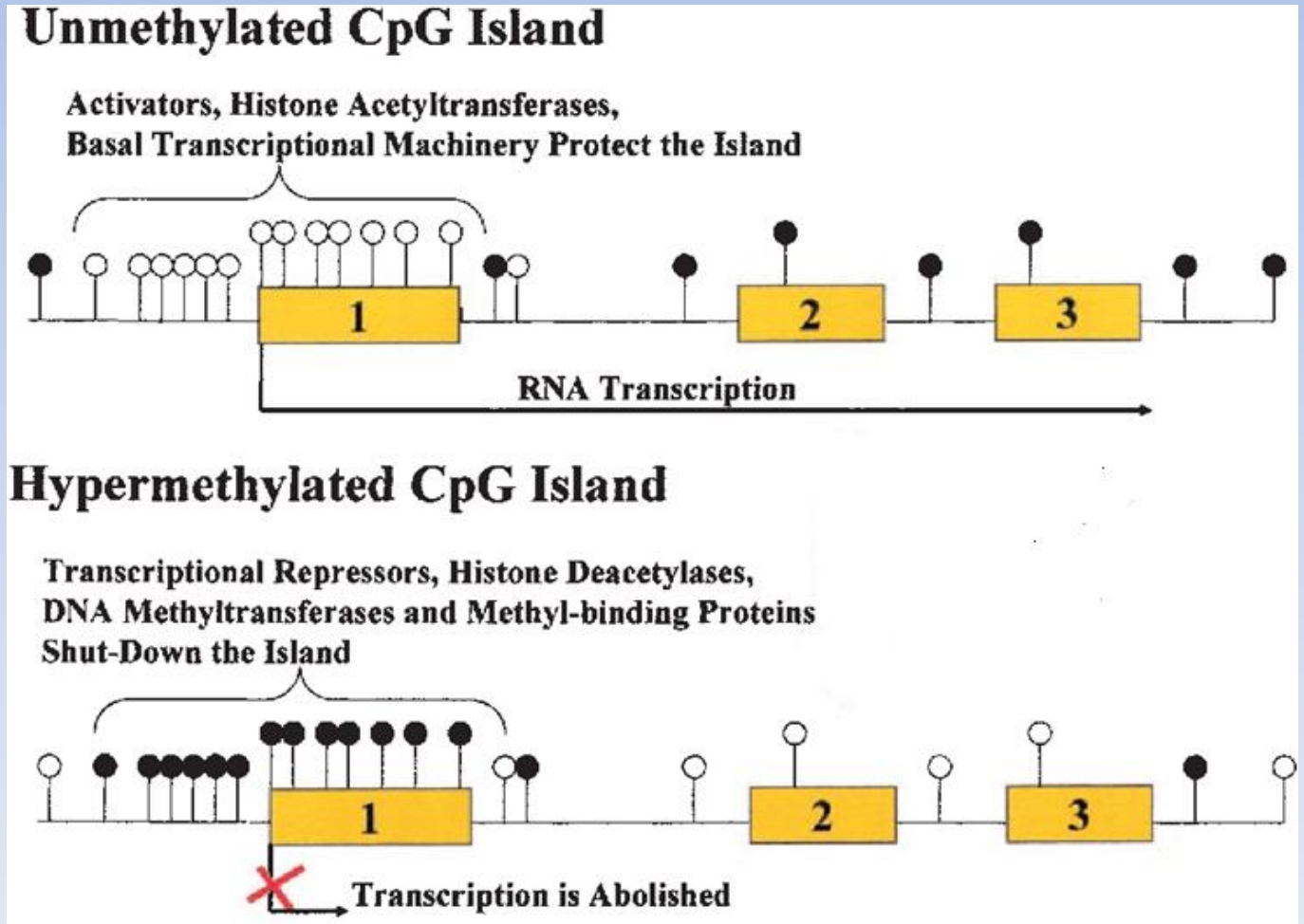
Also the histone modifications can affect other modifications.



Regulation of chromatin by histone modifications
Bannister and Kouzarides
(Cell Res 2011 Mar;21(3):381-95)

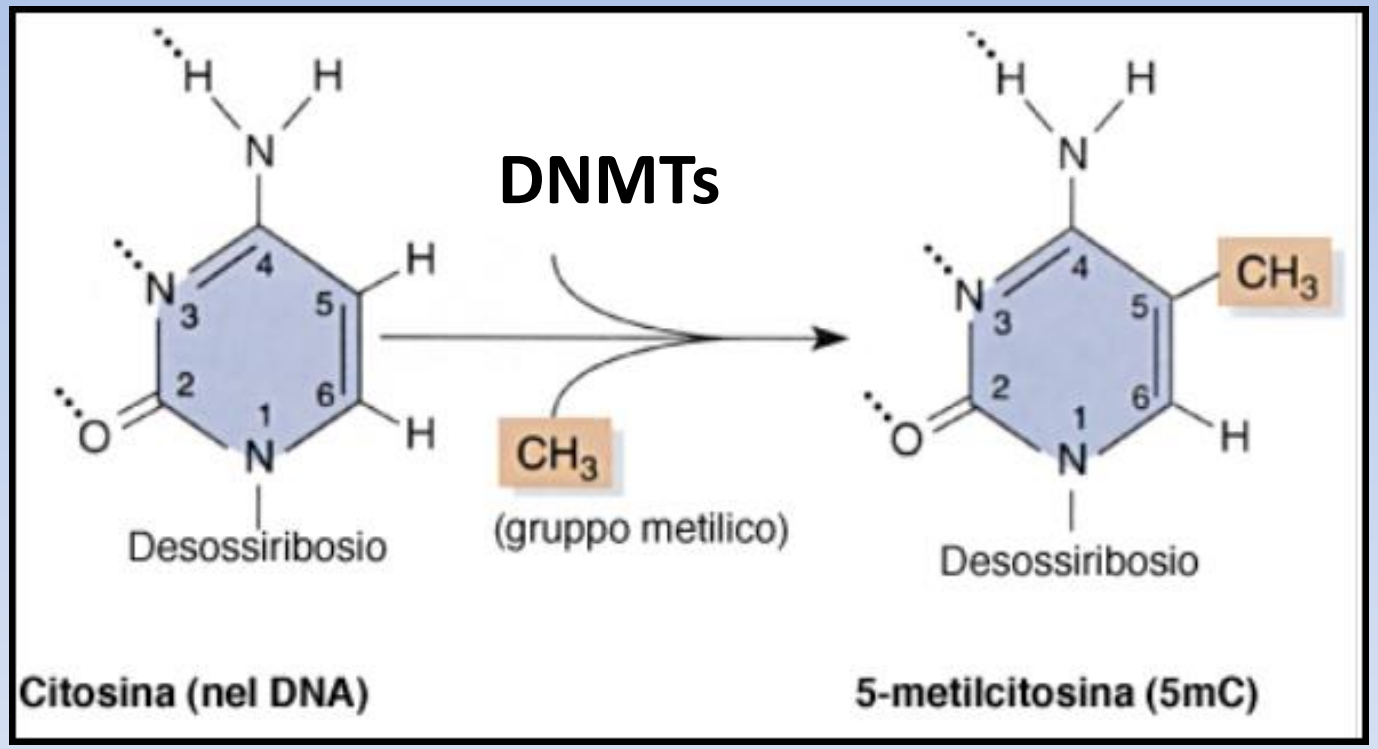
2. DNA methylation

Out of more than 3 billions of base pairs belonging to mammals genome,
About the 40% are CG and the 2-7% of them is methylated.
In eucariotics the methylation is on the cytosines (C) of the CpG islands.



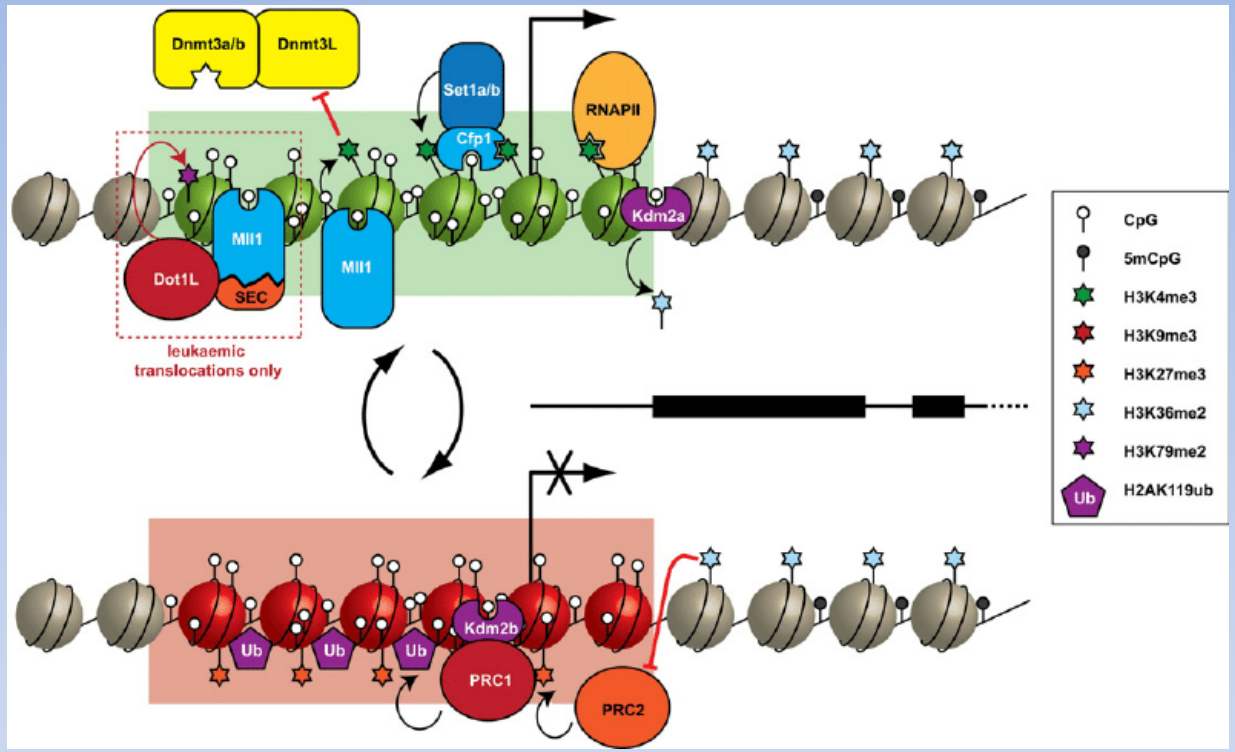
DNA methyltrasferase enzyme (DNMT) acts a methylation in position 5 of the Cytosine bases, producing 5-methyl cytosine.

There are different isoforms of these enzymes (DNMT1, DNMT3a, DNMT3b..)



CpG islands: genome DNA fragments carryng CpG sequences 10 fold more frequent, located overall close to CAP sites.

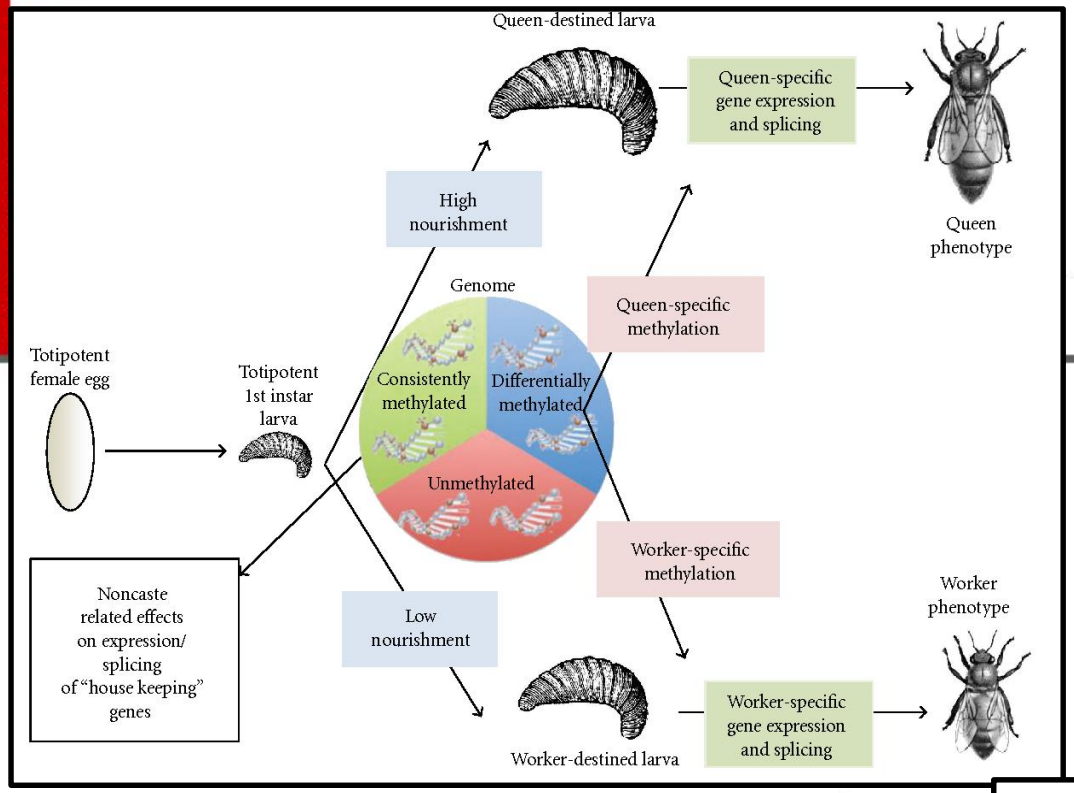
❑ DNA and histone lysine methylation systems are highly interrelated



❑ Also histone acetylation and DNA methylation are interregulated events

❑→ the first leads DNA access for demethylases enzymes, removing CH3 groups.

❑ → the second increases the affinity for histones deacetylases



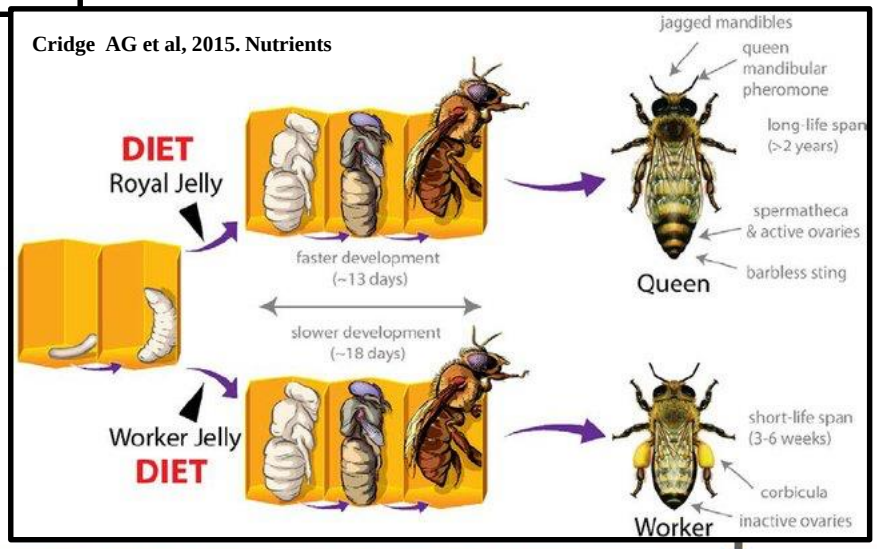
In honey bees the behaviorally and reproductively distinct queen and worker female castes derived from the same genome as a result of differential intake of **royal jelly** and are implemented in concert with DNA methylation.

Lyko F et al.., 2010, PloS Biol.

Royalactine

Since reproductive function is repressed in workers but not queens, it seems possible that DNA methylation results in repression of gene expression in workers. DNA methylation requires the enzyme DNMT3**. It was shown that silencing DNMTS expression in newly hatched honeybee larvae mimics the effect of royal jelly**, namely, the larvae destined to become workers develop into queens with fully developed ovaries.

Kucharski R et al., 2008, Science



EPIGENETIC FACTORS

Environmental Factors

- **Age**
- **Social stress**
- **Exposure to chemical substances**
- **Exposure to addictive drugs**
- **Neurodegenerative disorders**
- **Cancer**

Environmental factors

Social stress

Adults rats receiving high level of maternal care during the first months of life show less fear compared with lower level of maternal care and higher GR gene expression caused by lower DNA methylation on GR promoter.

Sexual abused children show high stress response and suicide risk increase.

The hippocampus of suicides sexually-abused in childhood show a strong decrease of GR mRNA expression associated to an hypermethylation

Epigenetics and suicide

Biological, developmental, social and environmental factors
interact each other and all together affect the complex phenomenon of suicide.

Brain studies suggest a role for epigenetic mechanisms affecting
gene expression and consequently affect behavior and predisposition to suicide.
Among them DNA methylation represents the most important
epigenetic modification in the neurobiology of suicide.

Decrease of GR gene expression levels is linked
to increase of site-directed DNA methylation in GR gene promoter.
This happens in the hippocampus of suicides, victims of adversity in childhood

(adversity history: severe physical and or sexual abuse, or severe neglect)

MCGowan et al., Hippocampus, 2009 GR showed for the 1° time:

- ▲ Methylation in NGFI-A binding site within GR promoter in hippocampus of suicides with history of abuse.
- ▼ GR expression in the same

Epigenetic and suicide

Suicide PFCx: show High levels of methylation of H3K27 on TrkB receptor gene promoter.

Alterations of 5-HT and GABA neurotransmission Are MARKERS of Major depression and suicide risk.

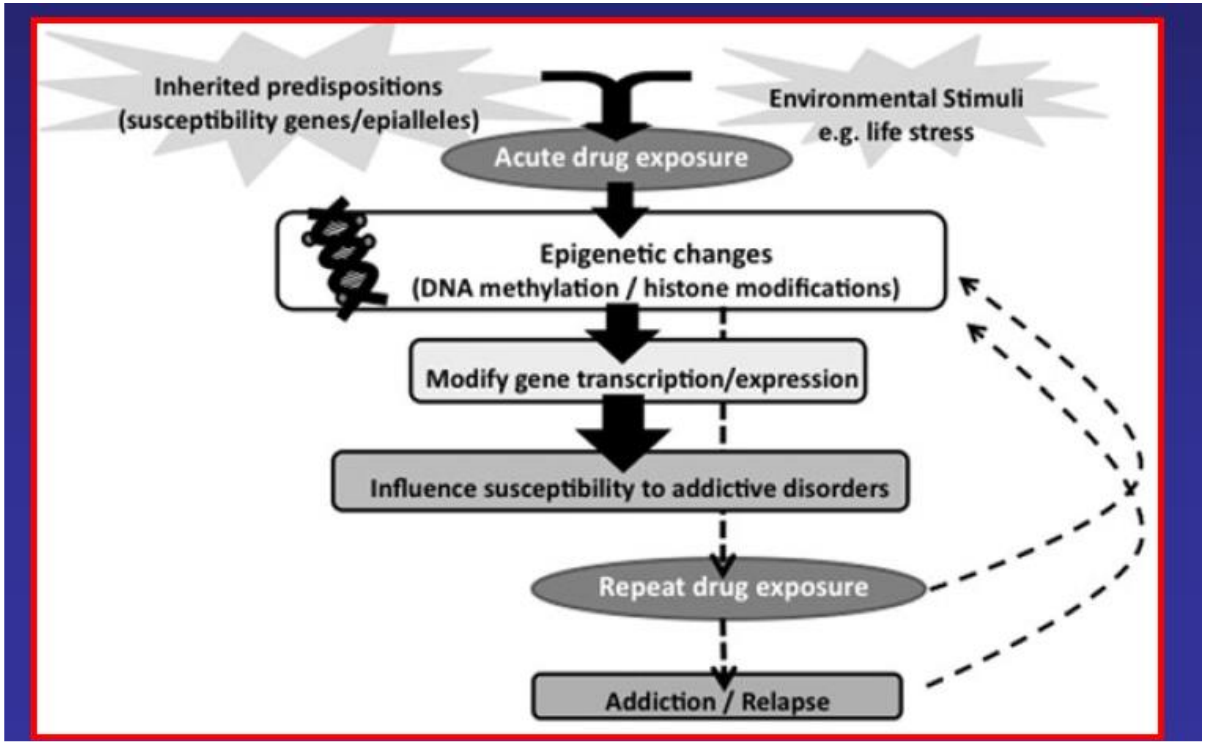
Alterations of DNMT (DNA methyl transferase) in FCX, limbic areas and brainstem of suicides

Poulter et al., 2008	Fronto-polar cortex (FPC) Hippocampus Amygdala Brain stem	$GABA_A$ α 1 DNMT1 DNMT3a DNMT3b	FPC: ↓ expression of DNMT1 mRNA in suicide completers ↑ expression of DNMT3b mRNA and protein levels in suicide completers ↑ increased methylation at two sites in the promoter region of GABAA receptor subunit α 1 in suicide completers Limbic system: ↓ expression of DNMT1 and DNMT3b mRNA levels in suicide completers Brain stem: ↓ expression of DNMT3b mRNA in suicide completers

Epigenetics and addictive drugs

Epigenetic alterations interact with genetic predisposition, environmental factors, exposure to addictive drugs.

All together induce long-lasting gene expression alterations influencing the typical behavior of addiction



Cocaine epigenetic effects

1 single cocaine administration ↑ in 30 min histone H4 acetylation (H4ac) e histone H3 phospho-acetylated (H3pac) levels in rat NAc

Self-administration of cocaine in the rat ↑ **H3ac and H4ac levels in NA** but only H3ac levels are related to motivation of the cocaine intake.

On the contrary, repeated exposure to cocaine ↓ **H3 dimethylation in Lysine in position 9 (H3K9me2), a repressive modification**.

Combining the two modifications (increase of acetylation and decrease of methylation on H3 determines the final effect: repeated exposure to cocaine induce a global increase of DNA transcription.

Therefore the chronic exposure to cocaine induce in the Nac neurons a global modification of chromatin that is favorable to transcription by means of increase of acetylation (H3ac, H4ac) and phosphoacetylation (H3pac) and a decrease of methylation (H3K9me2)

Epigenetic and disorders

Histone modifications

Many neurological disorders are related to a dysequilibrium of histones acetylation levels and to transcriptional dysfuctions.

→ Pharmacological treatment with **HDAC inhibitors** may correct these dysfunctions becoming useful therapeutic strategies for neurodegenerative diseases.

→ In fact nowadays we know that **HDAC inhibitors show neuroprotective, neurotrophic and antinfiammatory properties.**

→ **Environmental enrichment** **is also able to ameliorate learning and memory deficits in animals loosing hippocampal and cortical neurones**; a positive environment stimulates histone acetylation leading to an increase of BDNF levels causing an increase of the intact neuronal circuitry

Valproic acid is a drug used for epilepsy and bipolar disorders

Therefore it is considered as anticonvulsant and mood stabilizer.

Mechanisms of action:

- Increase of GAD enzyme activity, so more GABA synthesis
- Inhibition of GABA transporter (GAT-1)(antiepileptic)
- Decrease of Glutamate release by acting on Sodium and calcium channels.
- **HDACs Inhibition!!**

HDAC Inhibitors

Other possible disorders among the neurodegeneratives genetically based:

→ Huntington Chorea
→ Alzheimer disease
→amyotrophic lateral sclerosis SLA
→ spinal muscular atrophy (SMA)
→ Rett syndrome (a genetic type of autism)

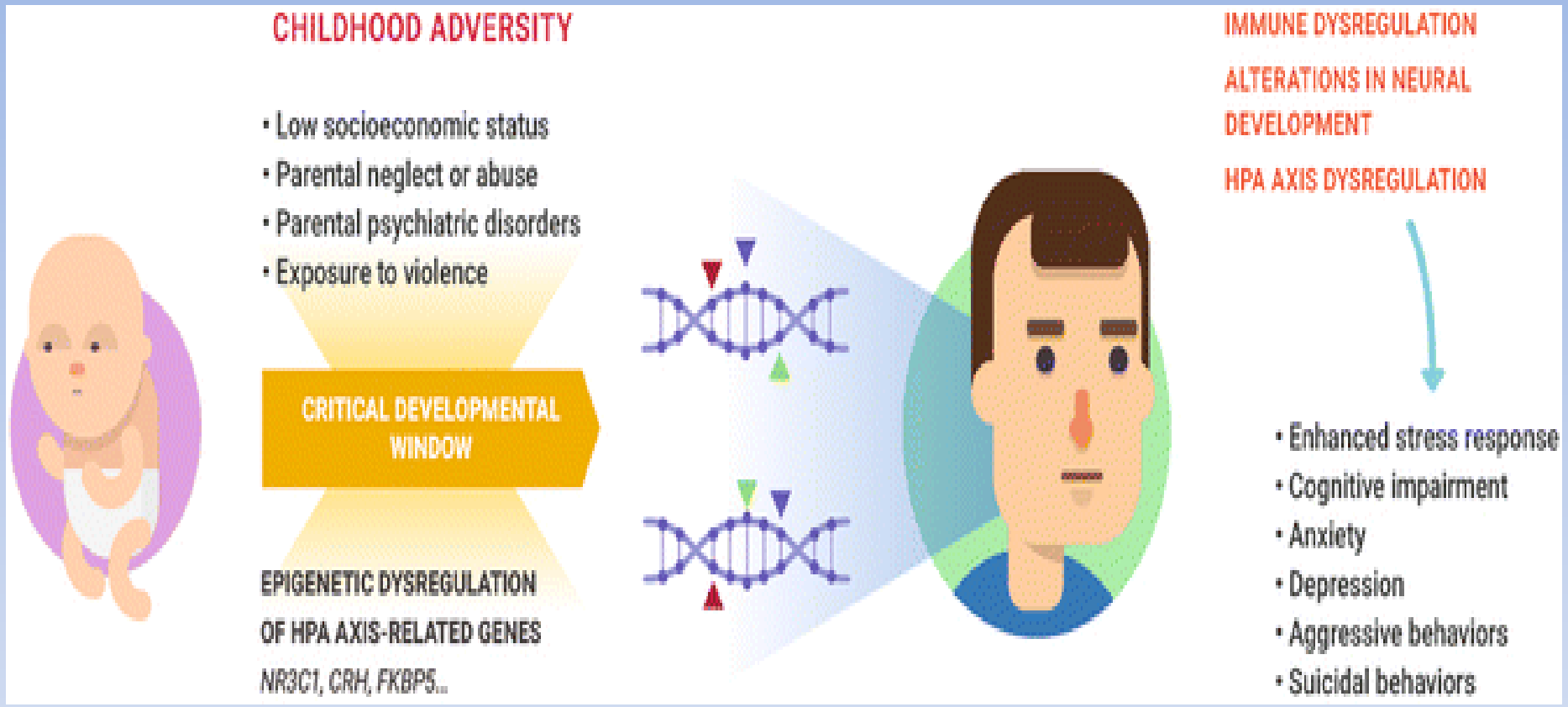
Epigenetic and diseases

→Emotional field patologies

Rat adolescents receiving **high levels** of **maternal/stimulation care**

Meet a GR (glucocorticoid receptor) gene demethylation, that causes an increase of histone acethylation in the hippocampus with a related increase of transcription rate for many genes.

When they are adults they show higher levels of GR and a less stress related phenotype, since this gene is responsible of the regulation of the stress response circuit (activated by the axis HPA)


CHILDHOOD ADVERSITY
• Low socioeconomic status
• Parental neglect or abuse
• Parental psychiatric disorders
• Exposure to violence
CRITICAL DEVELOPMENTAL WINDOW
EPIGENETIC DYSREGULATION
OF HPA AXIS-RELATED GENES
NR3C1, CRH, FKBP5...
IMMUNE DYSREGULATION
ALTERATIONS IN NEURAL DEVELOPMENT
HPA AXIS DYSREGULATION
• Enhanced stress response
• Cognitive impairment
• Anxiety
• Depression
• Aggressive behaviors
• Suicidal behaviors

Also BDNF function is crucial for synaptic neuronal plasticity , and it is regulated by epigenetic mechanisms.

→ **In inactive neurones, BDNF promoter is methylated and it is linked to MECP2 factor** (*Methyl Cytosine phosphate guanine binding Protein 2*) and to **a transcription repressor complex**.

→Environmental stimuli cause neuronal depolarization and cause BDNF gene demethylation, inducing unbinding from MECP2-dependent transcription repressor complex.

→ Mutations causing the loss of **MECP2 gene function** are responsible in humans for serious deficits of motor coordination and for socialization and cognitive abilities (**Rett syndrome**).

DNA methylation and cancer

Anormal DNA methylation is associated to a non programmed gene inhibition (silencers)

Many of them are related to many human cancers:

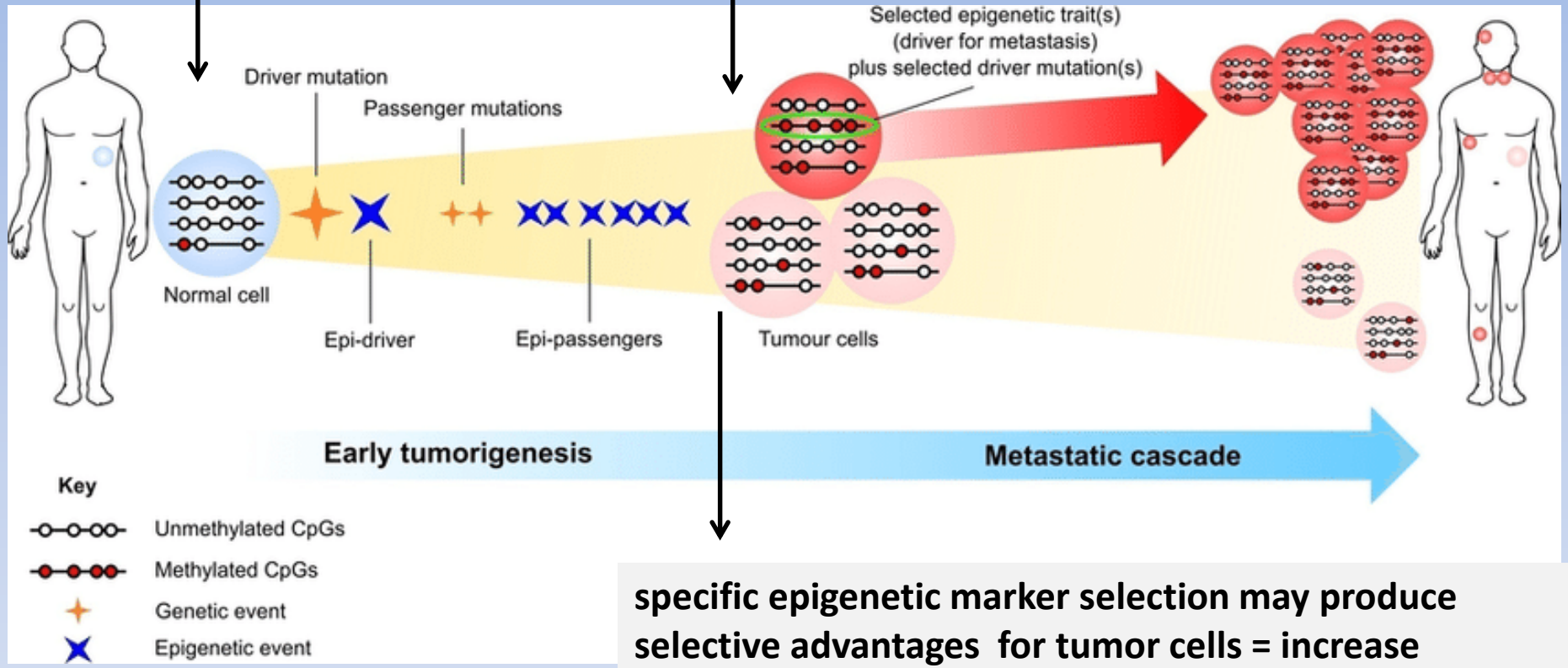
Genes may appear hyper or hypo methylated vs normal tissues

→ DNA hypermethylation is repressing transcription usually in a oncorepressor gene promoter **= this causes an increase of cell proliferation**

→ DNA hypomethylation enhances transcription of an oncogene **= this causes an increase of cell proliferation**

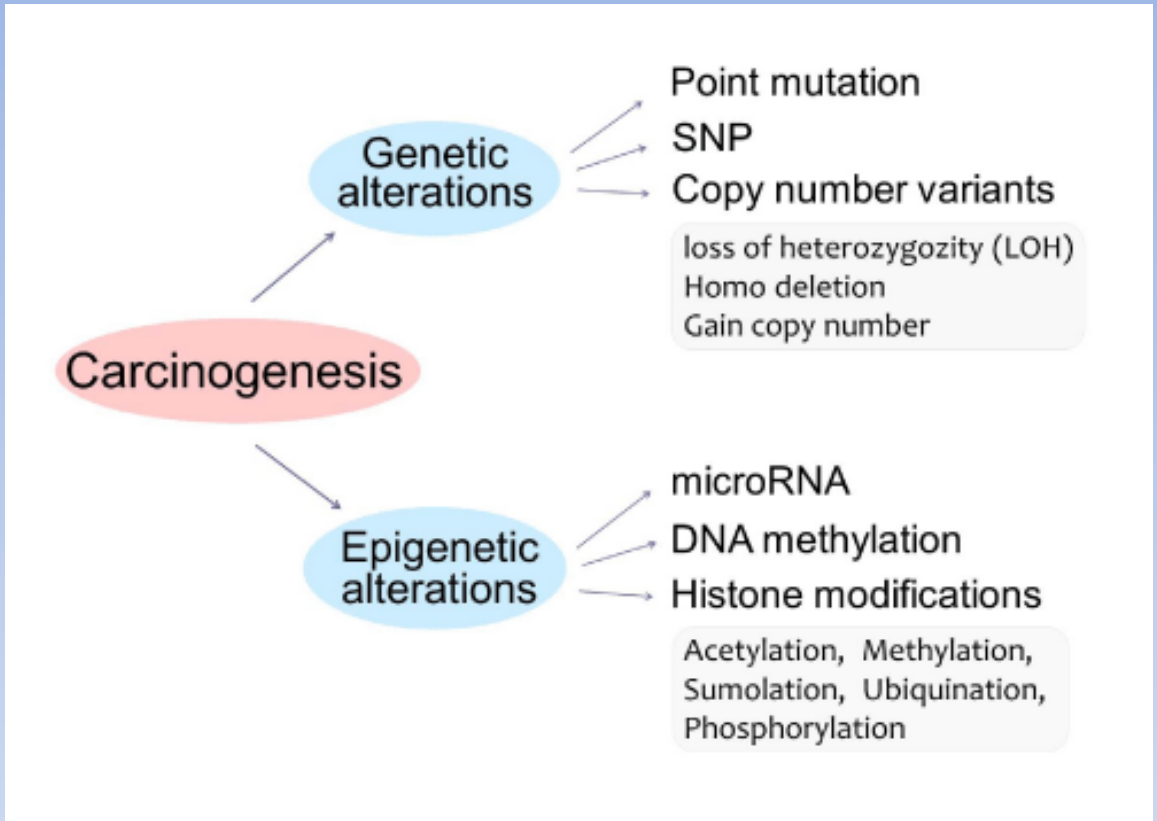
Early epigenetic changes

Late epigenetic changes



specific epigenetic marker selection may produce selective advantages for tumor cells = increase metastatic cascade.

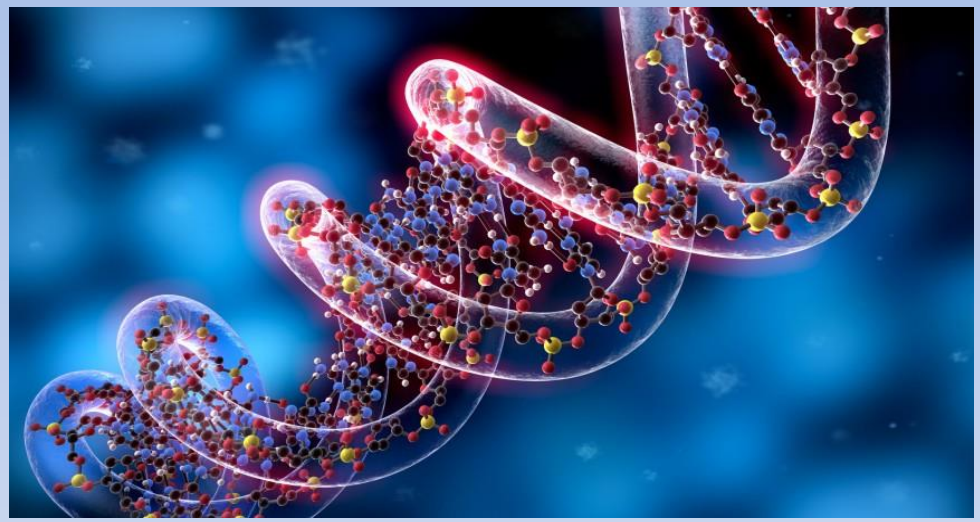
To look for these epi-drivers might be important for the understanding of metastatic risk in a tumor.



VALPROIC acid is studied also for blood liquid tumors.
miRNAs = utilized as diagnostic and prognostic markers.
Dual molecules acting both as HDACs and DNA methylation inhibitors

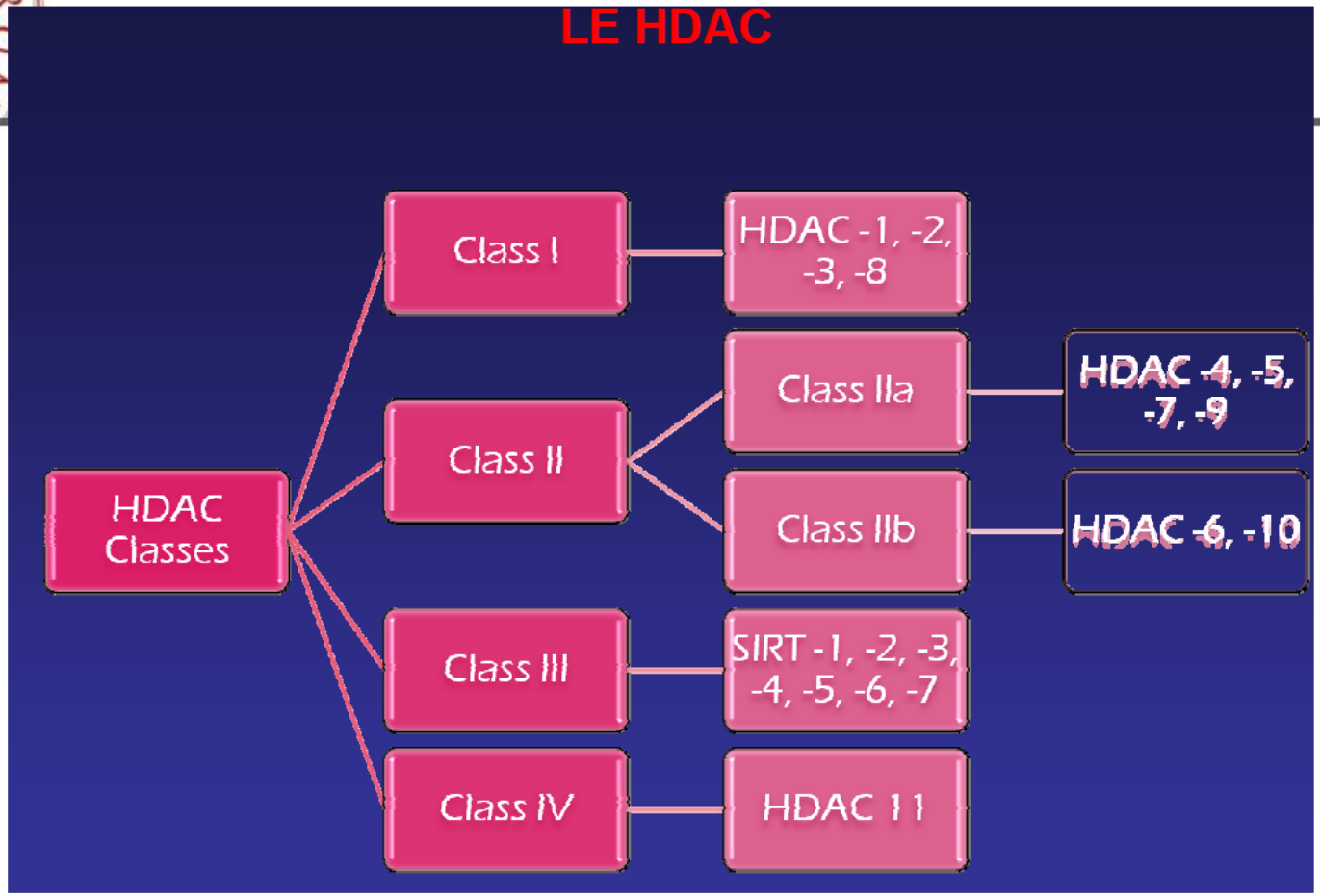
Drug discovery for new dual molecules acting both as **HDACs and DNA methylation inhibitors** have shown a potent antineoplastic effect for melanoma**.**

More advantages,
more specificity,
More activity
at epigenetic level, usually potentiated in cancer



→ Strategy to understand better new **targets**

LE HDAC



Classe I: HDAC 1, 2, 3 e 8

Class I		
HDAC 1	RPD3 deacetylase	Nucleus
HDAC 2	RPD3 deacetylase	Nucleus
HDAC 3	RPD3 deacetylase	Nucleus, cytoplasm and in association with the membrane
HDAC 8	RPD3 deacetylase	Nucleus

LE HDAC

Classe III

Dalla **SIRT 1 alla SIRT 7**

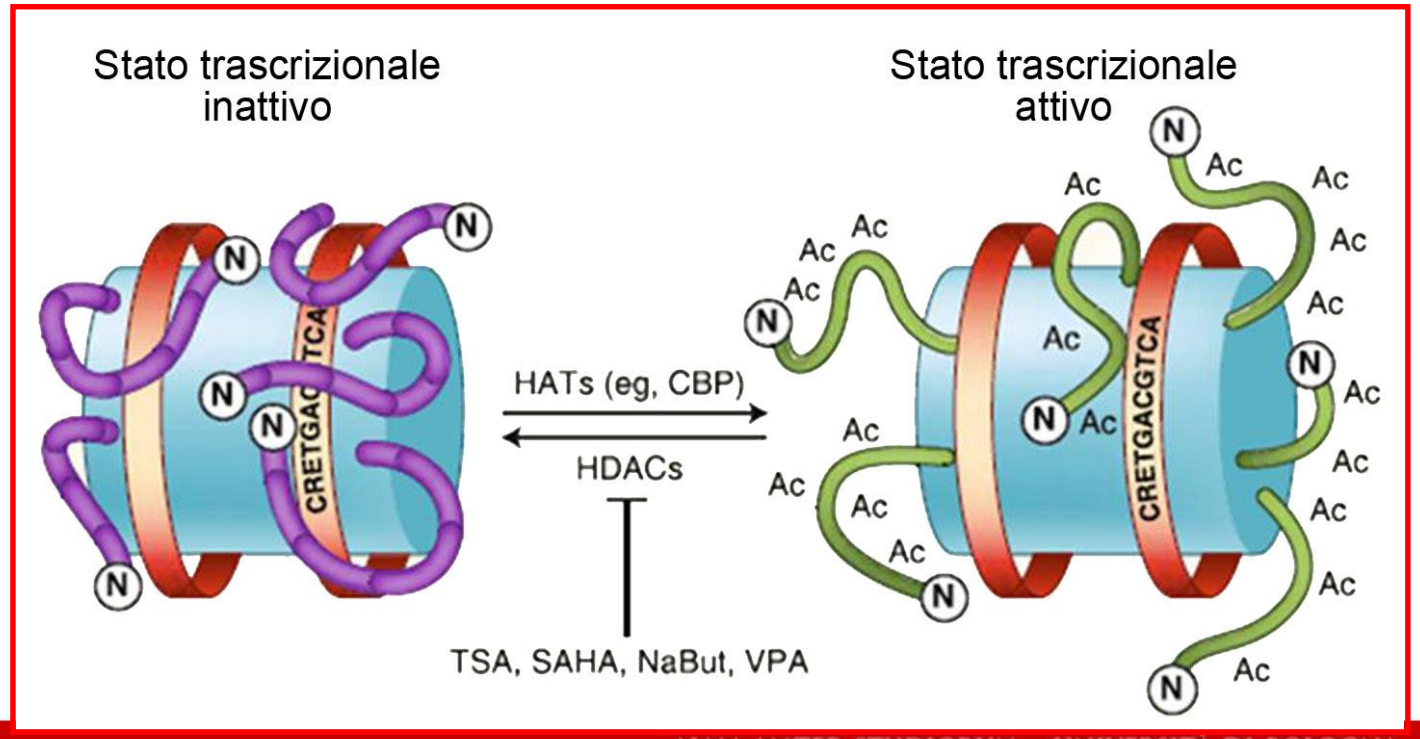
Questa classe ha un solo meccanismo catalitico che richiede il cofattore NAD+ per l'attività.

Class III		
SIRT 1	Silent information regulator 2 family	Nucleus
SIRT 2	Silent information regulator 2 family	Cytoplasm
SIRT 3	Silent information regulator 2 family	Nucleus and mitochondria
SIRT 4	Silent information regulator 2 family	Mitochondria
SIRT 5	Silent information regulator 2 family	Mitochondria
SIRT 6	Silent information regulator 2 family	Nucleus
SIRT 7	Silent information regulator 2 family	Nucleus

SIRT1 PIN1 APOE1 APP PSEN

Histone modifications

Competitive inhibitors of HDACs (tricostatin A [TSA], hydrossamic acid suberoilanilide [SAHA], sodium butirrate [NaBut] and valproic acid [VPA]) interact directly and prevent deacetylation of Lysines by HDACs, therefore inducing a hyperacetylation and active transcription.



Review

Multiple roles of HDAC inhibition in neurodegenerative conditions

De-Maw Chuang, Yan Leng, Zoya Marinova, Hyeon-Ju Kim and Chi-Tso Chiu

Molecular Neurobiology Section, National Institute of Mental Health, National Institutes of Health, 10 Center Drive MSC 1363, Bethesda, MD 20892-1363, USA

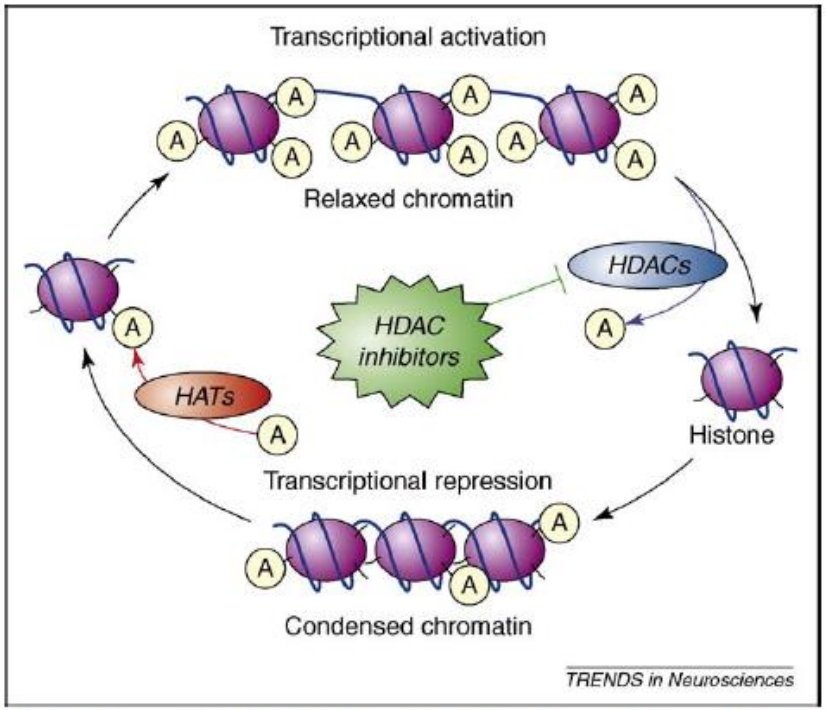
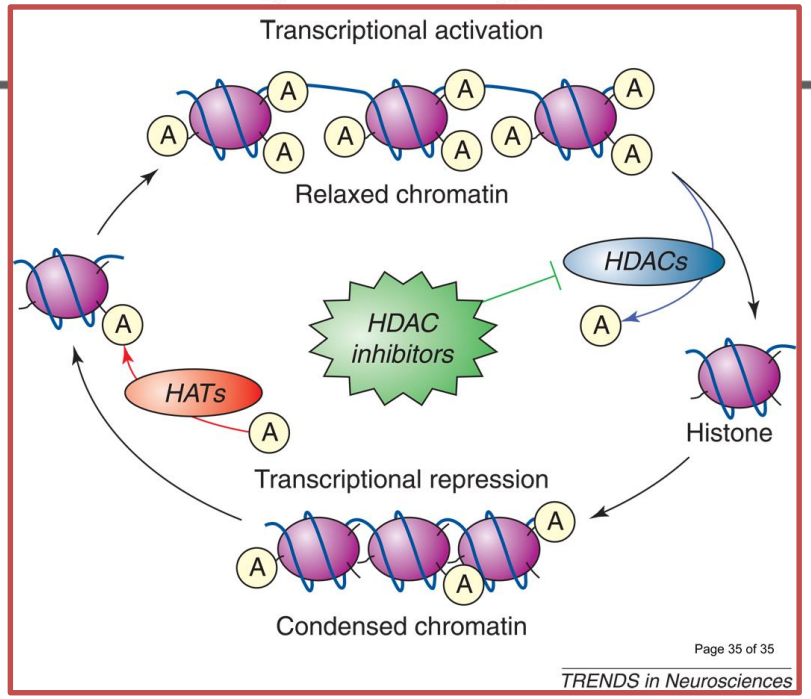


Figure 1. Effects of HDAC inhibitors on chromatin remodeling. Levels of histone acetylation at Lys residues on histone-tails are determined by interplays of acetylation and deacetylation catalyzed by histone acetyltransferases (HATs) and histone deacetylases (HDACs), respectively. Inhibition of HDACs by HDAC inhibitors results in a net increase in histone acetylation levels and a more open, relaxed chromatin conformation that favors transcriptional activation. By contrast, chromatin with a compact conformation is transcriptionally inactive. Ⓐ, acetylated Lys residues of histone-tail proteins.

Trends Neurosci. 2009 Nov;32(11):591-601. doi: 10.1016/j.tins.2009.06.002. Epub 2009 Sep 21.

Multiple roles of HDAC inhibition in neurodegenerative conditions.

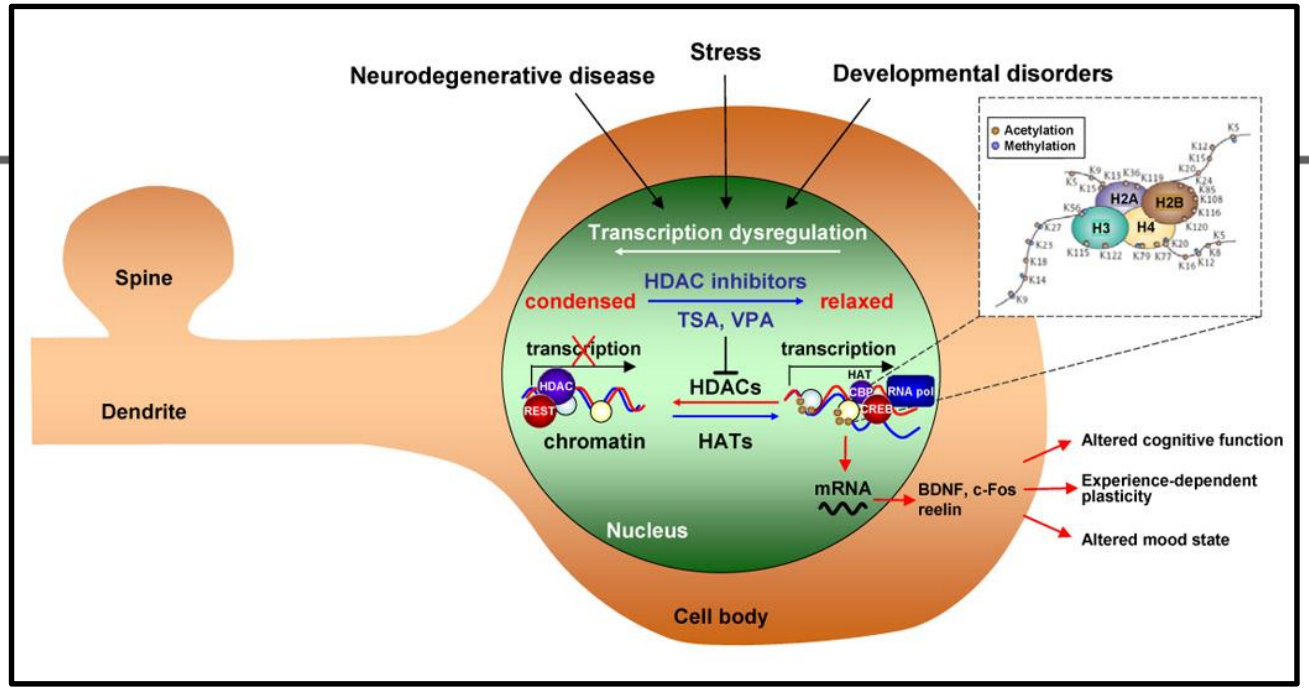


A large number of neurodegenerative conditions *in vivo* and *in vitro* involve functional imbalance in HATs and HDACs, resulting in histone hypoacetylation and transcriptional dysfunction.

Treatment with Class I, II and, more recently, III HDAC inhibitors restores these deficiencies.

Curr Opin Pharmacol. 2008 Feb;8(1):57-64. doi: 10.1016/j.coph.2007.12.002.

Epigenetic targets of HDAC inhibition in neurodegenerative and psychiatric disorders.



Scheme showing that neurological and psychiatric disorders involve epigenetic modifications of key neuronal genes and intervention by HDAC inhibitors

Neurodegenerative diseases (Huntington's disease, Parkinson's disease and ischemia), psychiatric disorders (depression, stress and anxiety) and neurodevelopmental disorders can involve **aberrant acetylation and methylation of histones and/or DNA methylation**. These epigenetic modifications can be influenced by experience and determine the transcriptional state of regulatory genes critical to synaptic plasticity, cognition and mood. **Histone deacetylase inhibitors amelioration of plasticity and cognitive deficits.**

J Neurochem. 2015 Aug;134(4):642-51. doi: 10.1111/jnc.13171. Epub 2015 Jun 4.

Hippocampal chromatin-modifying enzymes are pivotal for scopolamine-induced synaptic plasticity gene expression changes and memory impairment.

Scopolamine administration drastically up-regulated DNA methyltransferases (DNMT1) and HDAC2 expression. HDAC inhibitor sodium butyrate and DNMT inhibitor Aza-20deoxycytidine recovered scopolamine-impaired hippocampal-dependent memory consolidation with concomitant increase in the expression of synaptic plasticity genes Brain-derived neurotrophic factor (BDNF) and Arc and level of histone H3K9 and H3K14 acetylation and decrease in DNA methylation level.

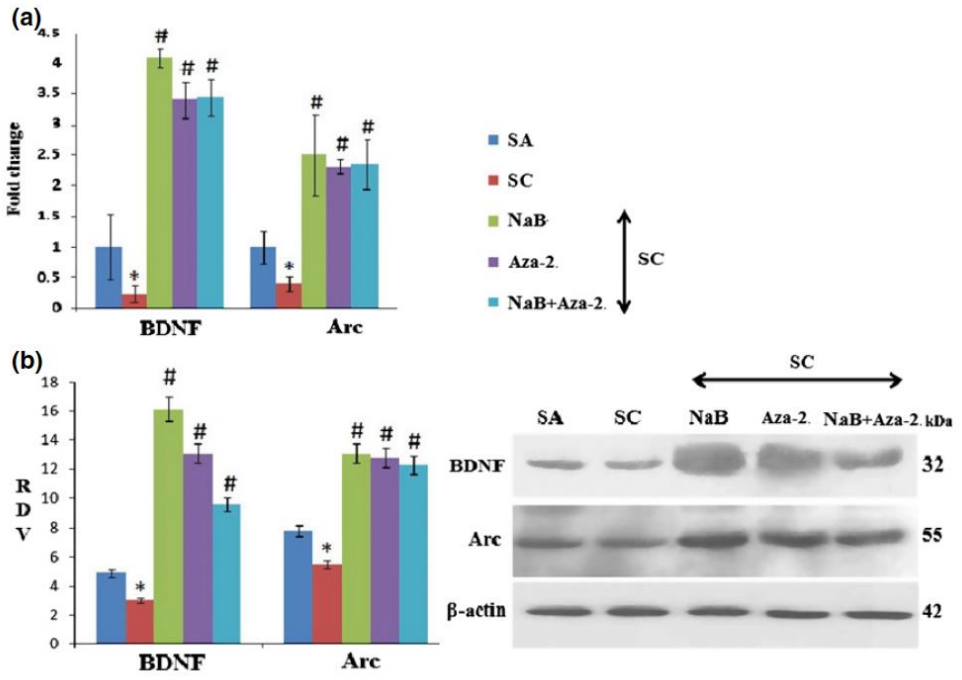


Fig. 2 (a) Quantitative real-time PCR of BDNF and Arc expression in scopolamine (SC)-treated mice as compared to saline control (SA) and Sodium butyrate (NaB), Aza-2′deoxycytidine (Aza-2) and NaB+Aza-2-treated mice. (b) Western blot analysis of BDNF and Arc proteinexpression. Histogram represents relative density value (RDV) (IDV of BDNF/Arc/IDV of β-Actin) from three independent experiments. Data are represented as Mean ± SEM and '*' and '#' denote significant differences ($p < 0.05$) as compared to SA and SC, respectively (one-way ANOVA followed by Tukey's test).

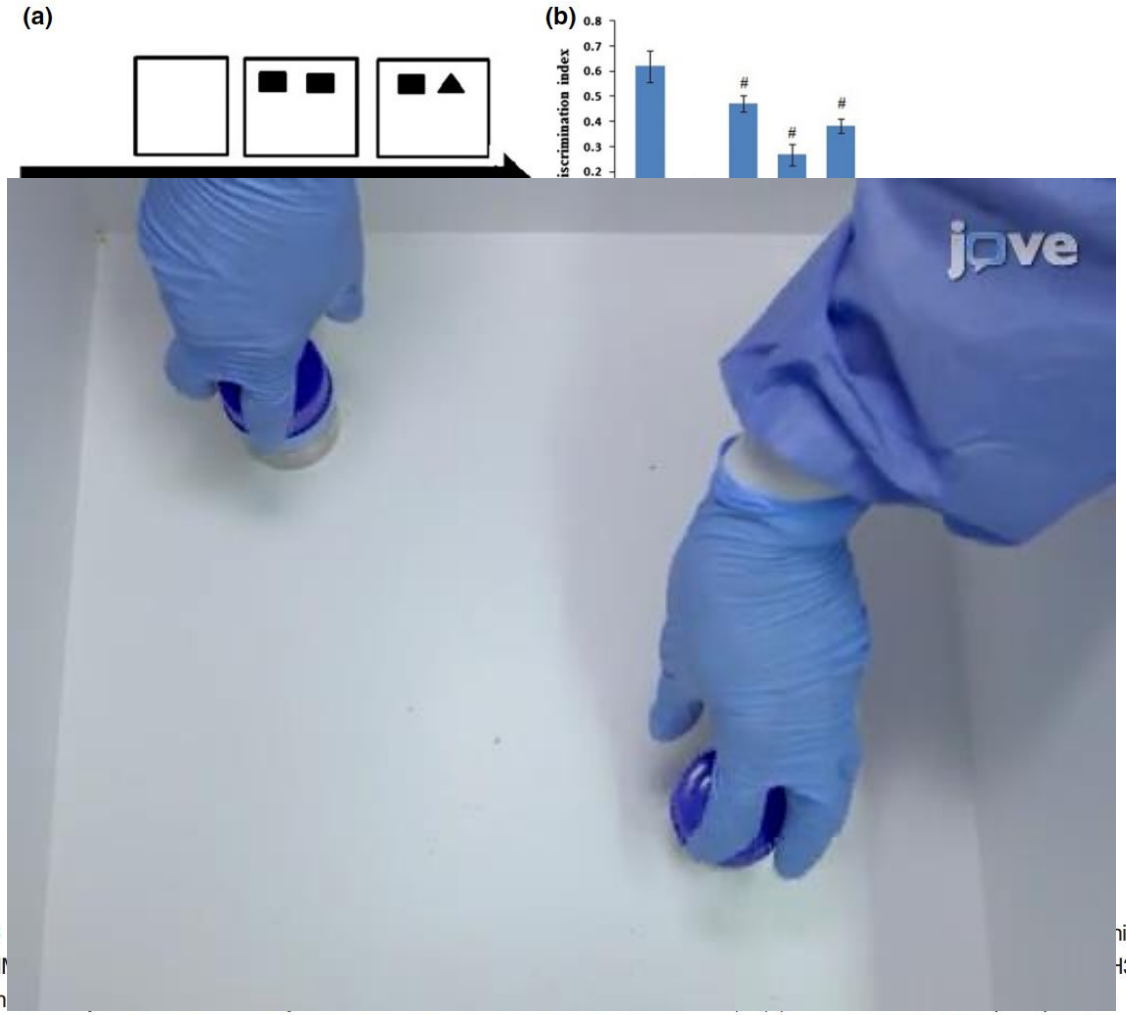
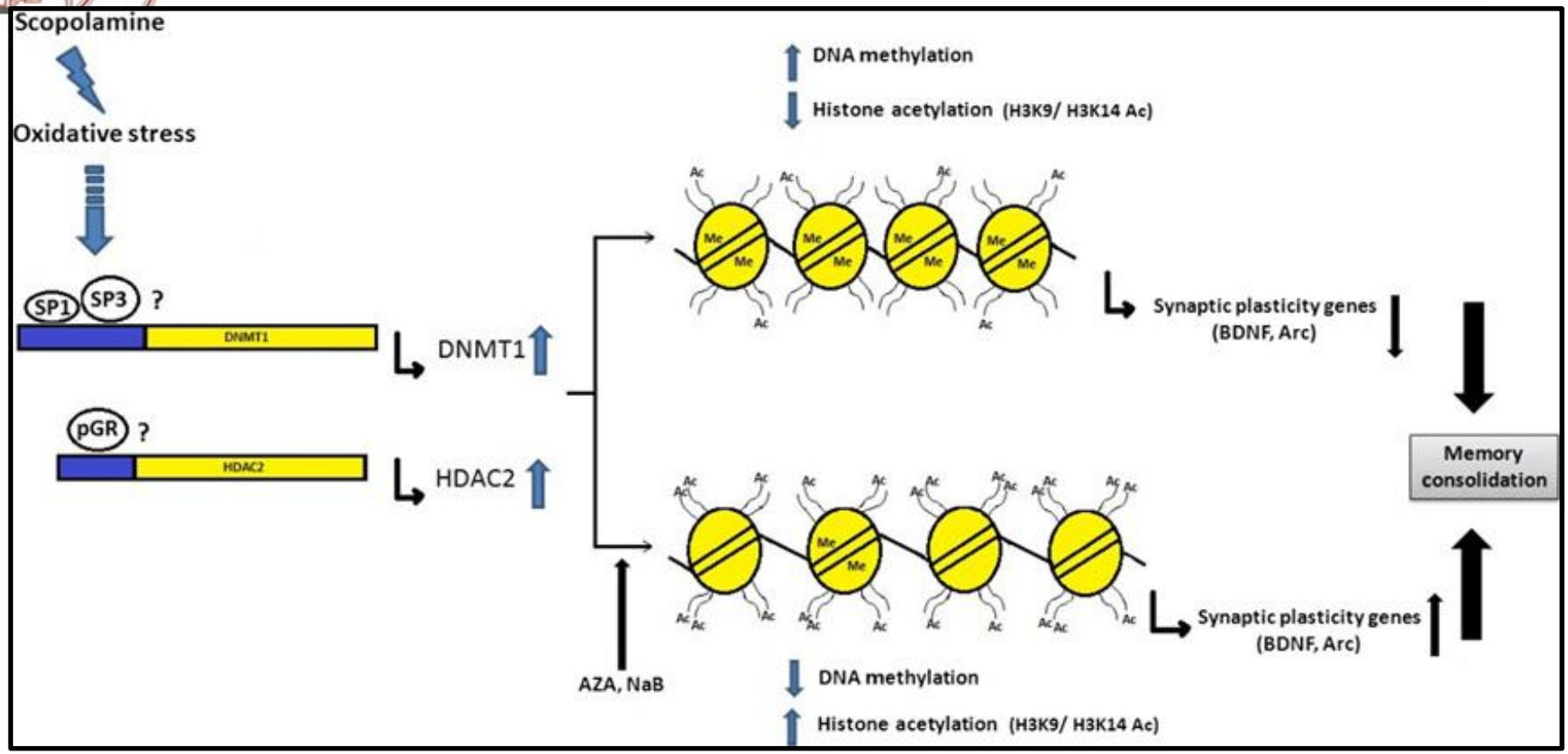


Fig. 3 Effect of HDAC … istogram methyltransferases (DN… H3K9 Ac/ novel object recognition … analysis methylation level in scopolamine (SC)-treated mice. (a) Experimental design: mice were treated with respective drug for 7 days. On 5th and 6th day, mice were habituated in the open box. On 7th day, two similar objects were placed in the box and mice were allowed to interact. On 8th day, one object was replaced by novel one and mice were allowed to interact with both the objects (b) Discrimination index (DI). (c) showing histone acetylation level at the promoter of BDNF and Arc. (e) Methylated DNA immunoprecipitation (MeDIP) analysis showing DNA methylation level at the promoter of BDNF and Arc. Data are represented Mean ± SEM. '*' and '#' denote significant differences ($p < 0.05$) as compared to SA and SC, respectively (one-way ANOVA followed by Tukey's test).



Review

Multiple roles of HDAC inhibition in neurodegenerative conditions

De-Maw Chuang, Yan Leng, Zoya Marinova, Hyeon-Ju Kim and Chi-Tso Chiu

Molecular Neurobiology Section, National Institute of Mental Health, National Institutes of Health, 10 Center Drive MSC 1363, Bethesda, MD 20892-1363, USA

- Histone hypoacethylation and transcriptional disfunctions are involved in many neurodegenerative disorders and conditions *in vivo* e *in vitro.*

- iHDAC normalize these deficiencies and protect against neurodegeneration.

- many genes are involved in neuroprotection and neurotrophic mechanisms and they are regulated by inhibition of HDAC

- iHDAC suppress neuroinflammation by inhibiting microglial activation

Review

Multiple roles of HDAC inhibition in neurodegenerative conditions

De-Maw Chuang, Yan Leng, Zoya Marinova, Hyeon-Ju Kim and Chi-Tso Chiu

Molecular Neurobiology Section, National Institute of Mental Health, National Institutes of Health, 10 Center Drive MSC 1363, Bethesda, MD 20892-1363, USA

Ictus or stroke

In a model of stroke induced by occlusion of middle cerebral artery (MCA): ↓ Hac on lysine in the mouse or rat ischemic brain.

These alterations decrease by means of iHDAC therapy causing a reduction of stroke volume.

Review

Multiple roles of HDAC inhibition in neurodegenerative conditions

De-Maw Chuang, Yan Leng, Zoya Marinova, Hyeon-Ju Kim and Chi-Tso Chiu

Molecular Neurobiology Section, National Institute of Mental Health, National Institutes of Health, 10 Center Drive MSC 1363, Bethesda, MD 20892-1363, USA

Amyotrophic lateral sclerosis

4-phenylbutirrate administered at beginning of symptoms in a mouse transgenic model of SLA increase survivor and admeliorate symptoms.

Alzheimer Disease

In a mouse transgenic model of AD the administration of 4-phenylbutirrate :

- restore deficits of spatial memory by means of normalization of hyperphosphorylation of Tau protein in the hippocampus

- improve the dramatic loss of H4 acethylation in the cortex.

Review

Multiple roles of HDAC inhibition in neurodegenerative conditions

De-Maw Chuang, Yan Leng, Zoya Marinova, Hyeon-Ju Kim and Chi-Tso Chiu

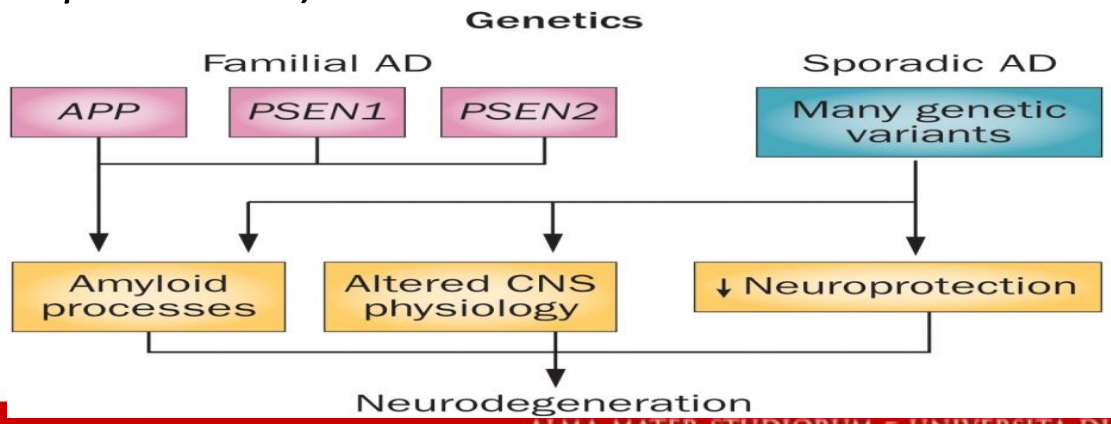
Molecular Neurobiology Section, National Institute of Mental Health, National Institutes of Health, 10 Center Drive MSC 1363, Bethesda, MD 20892-1363, USA

Disease model	Histone hypo-acetylation/ transcriptional dysfunction	Microtubule dysfunction	HDAC inhibitors examined	Beneficial effects after treatment
Stroke	Yes	Yes	Valproic acid, vorinostat, sodium butyrate, TSA, 4-phenylbutyrate	Restored histone hypoacetylation and transcriptional dysfunction; enhanced neurogenesis; decreased infarct volume, neuroinflammation and neurological deficits
Huntington's disease (HD)	Yes	Yes	Vorinostat, sodium butyrate, 4-phenylbutyrate, TSA, HDACi 4b, nicotinamide	Restored histone hypoacetylation and transcriptional dysfunction; normalized striatal atrophy and degeneration; increased BDNF vesicular transport; improved motor performance and survival
Amyotrophic lateral sclerosis (ALS)	Yes	?	4-Phenylbutyrate, valproic acid, 4-phenylbutyrate+antioxidant, valproic acid+lithium, 4-phenylbutyrate+riluzole	Restored histone hypoacetylation and CBP loss; suppressed motor neuronal death; improved motor function and survival
Spinal muscular atrophy (SMA)	Yes	?	Sodium butyrate, 4-phenylbutyrate, valproic acid, vorinostat, TSA, romidepsin (FK-228)	Increased SMN_2 expression; induced Bcl-2, Bcl-X_L and BDNF; suppressed spinal motor neuronal degeneration and muscle atrophy; prolonged life span
Parkinson's disease (PD)	Yes	Yes	Valproic acid, sodium butyrate, TSA, vorinostat, AGK2	Increased GDNF and BDNF expression; reduced neuroinflammation and dopaminergic neuronal death; increased acetylation of α-tubulin
Alzheimer's disease (AD)	Yes	Yes	Valproic acid, sodium butyrate, 4-phenylbutyrate, nicotinamide, vorinostat	Restored histone hypoacetylation; increased synaptic plasticity; decreased Aβ production and *Tau* hyperphosphorylation; reinstated learning and memory; reversed spatial memory deficits

AD biomarkers

- ↓ BDNF in progressive decline of cognition in AD

- Sirt1 as IHDAC involved in epigenetic mechanisms → decreases β-A e Tau
- ↓ Pin1, peptidil-prolil cis/trans isomerasis *(modulate plaques formation)*
- Psen1 as component of complex γ-secretasis *(production of β-A and Tau phosphorilation)*



Contents lists available at ScienceDirect

Experimental and Toxicologic Pathology

journal homepage: www.elsevier.de/etp

Treatment with the neurotoxic Aβ (25–35) peptide modulates the expression of neuroprotective factors Pin1, Sirtuin 1, and brain-derived neurotrophic factor in SH-SY5Y human neuroblastoma cells

Francesca Lattanzio, Lucia Carboni*, Donatella Carretta, Sanzio Candeletti, Patrizia Romualdi

Department of Pharmacy and Biotechnology, Alma Mater Studiorum University of Bologna, via Irnerio 48, 40126 Bologna, Italy

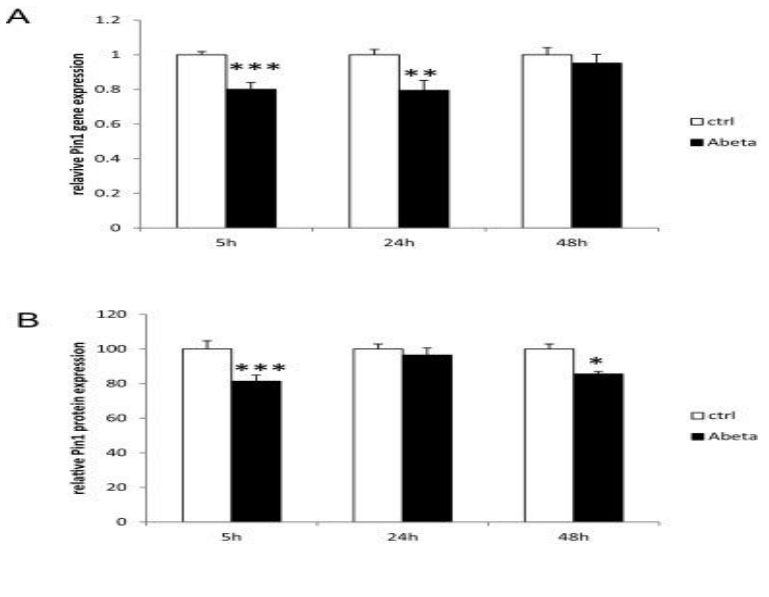


Fig. 1. (A) Pin1 mRNA expression after 25 μM Aβ (25–35) treatment for 5, 24, or 48 h. Gene expression was measured by real-time PCR. Data represent 2−DDCt values normalized to GAPDH levels. Data are expressed as mean ± SEM of controls of three independent experiments.

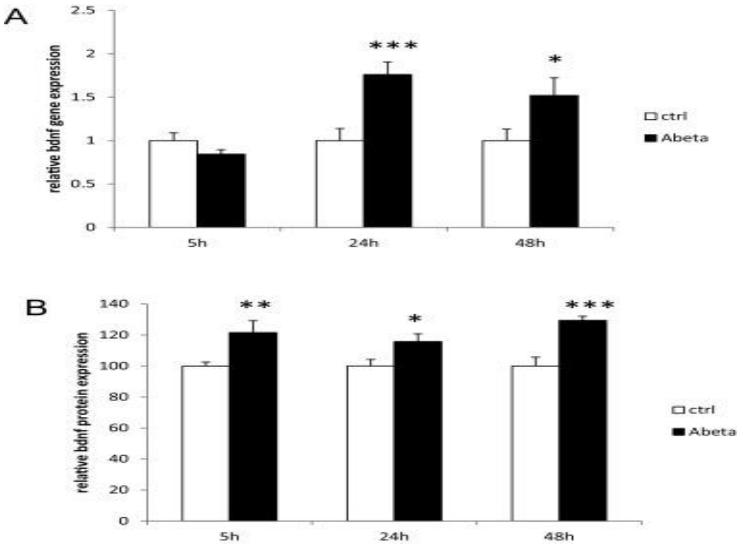
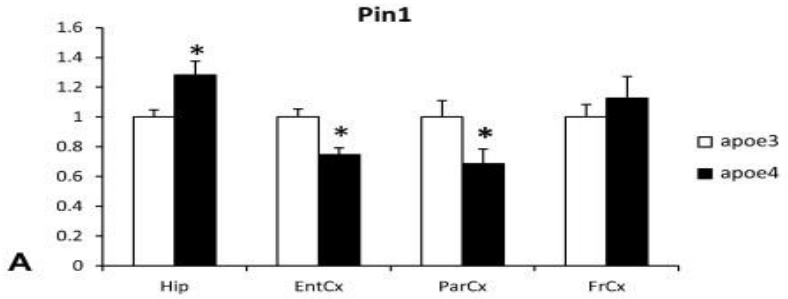


Fig. 3. (A) Bdnf mRNA expression after 25 μM Aβ (25–35) treatment for 5, 24, or 48 h. Gene expression was measured by real-time PCR. Data represent 2−DDCt values normalized to GAPDH levels. Data are expressed as mean ± SEM of controls of three independent expe...

Neuroscience
Volume 256, 3 January 2014, Pages 360–369

Human apolipoprotein E4 modulates the expression of Pin1, Sirtuin 1, and Presenilin 1 in brain regions of targeted replacement apoE mice

F. Lattanzio[a], L. Carboni[a], D. Carretta[a], R. Rimondini[b], S. Candeletti[a], P. Romualdi[a]



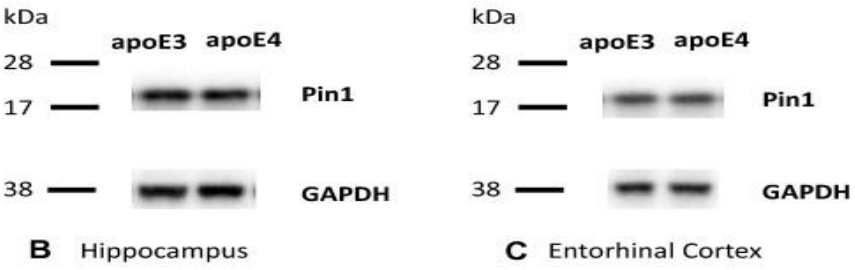
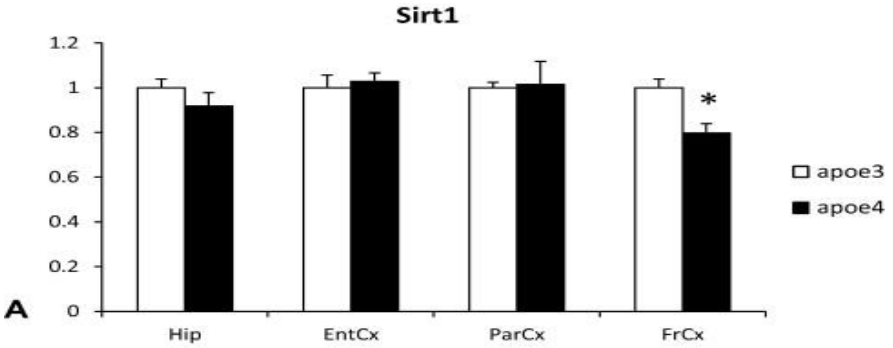


Fig. 1. (A) Pin1 relative gene expression (mRNA) levels were determined by real-time PCR in the hippocampus, entorhinal cortex, parietal cortex, and frontal cortex of apoE3 and apoE4 transgenic mice.



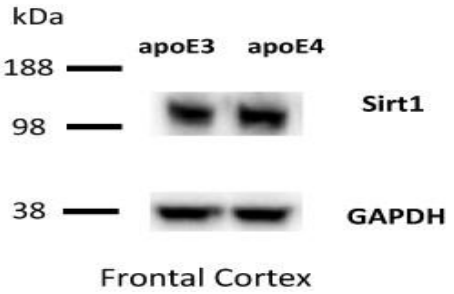


Fig. 2. (A) Sirt1 relative gene expression (mRNA) levels were determined by real-time PCR in the hippocampus, entorhinal cortex, parietal cortex, and frontal cortex of apoE3 and apoE4 transgenic mice.

ApoE: Fattore a maggior rischio genetico di AD sporadico

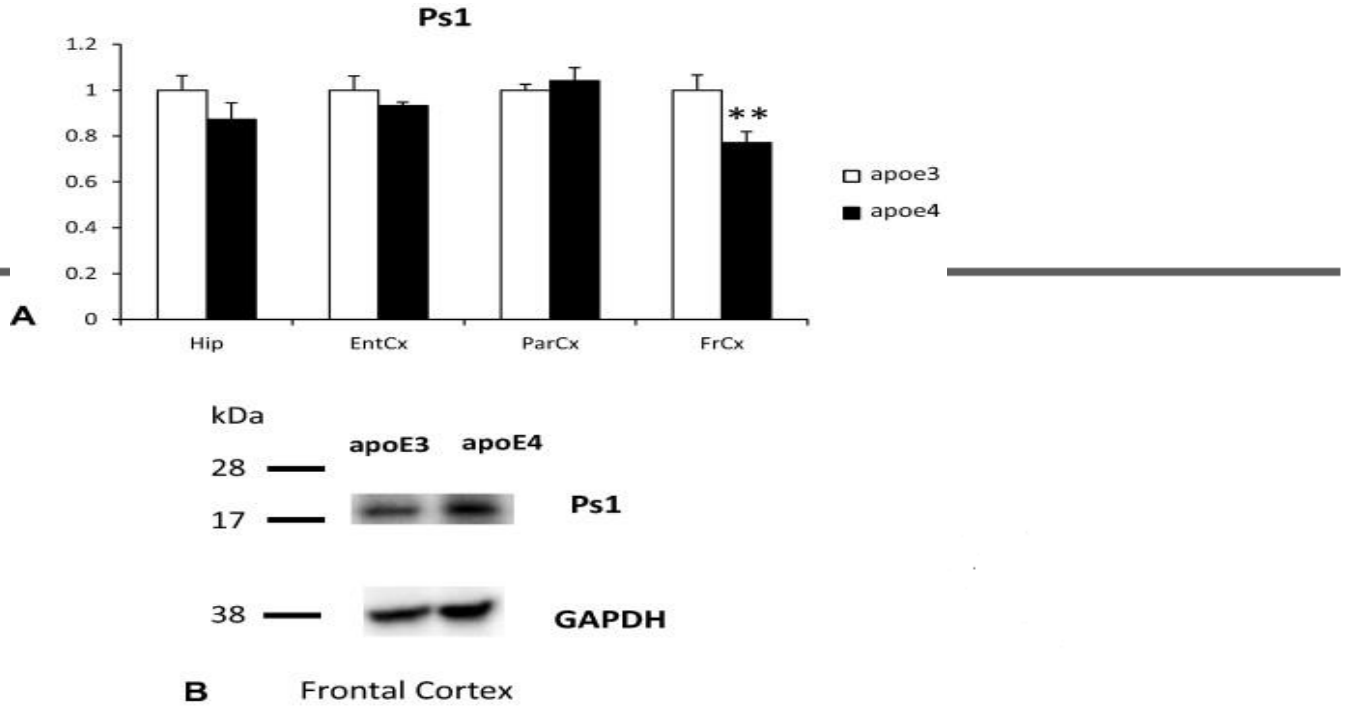
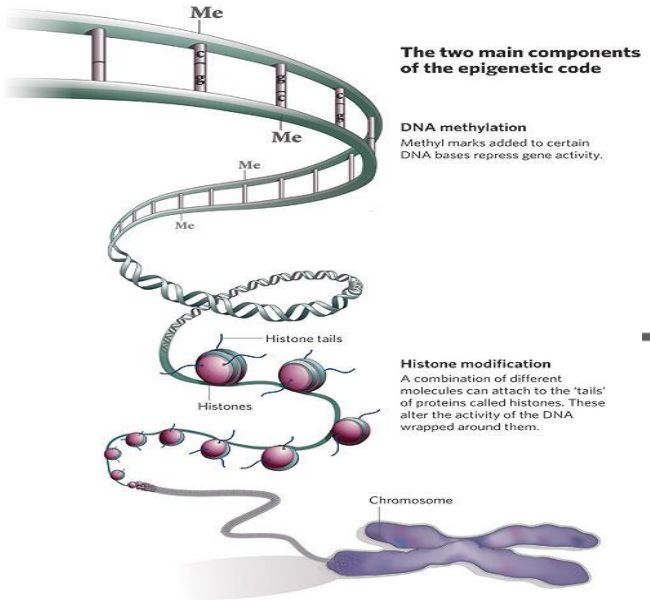


Fig. 3. (A) PS1 relative gene expression (mRNA) levels were determined by real-time PCR in the hippocampus, entorhinal cortex, parietal cortex, and frontal cortex of apoE3 and apoE4 transgenic mice.

in vitro and in vivo data demonstrate the gene expression modulation for the investigated proteins and this has something to do in the molecular mechanisms underlying Alzheimer D.



Neurodegener Dis. 2012;10(1-4):207-11. doi: 10.1159/000333799. Epub 2012 Jan 17.

Pin1 contribution to Alzheimer's disease: transcriptional and epigenetic mechanisms in patients with late-onset Alzheimer's disease.

Arosio B[1], Bulbarelli A, Bastias Candia S, Lonati E, Mastronardi L, Romualdi P, Candeletti S, Gussago C, Galimberti D, Scarpini E, Dell'Osso B, Altamura C, MacCarrone M, Bergamaschini L, D'Addario C, Mari D

Author information

2.1. Study subjects

Patients with a clinical diagnosis of AD and elderly controls were enrolled from the longitudinal "Conselice study" [24] as described elsewhere [25]. Cognitive performance was measured according to the Mini-Mental State Examination (MMSE). Clinical diagnosis of AD followed the criteria of the Diagnostic and Statistical Manual of Mental Disorders and the National Institute of Neurological and Communicative Disorders and Stroke and the Alzheimer's Disease and Related Disorders Association (now called the Alzheimer's Association). Cases and controls were selected in order to reduce sample variability as much as possible. Therefore, the demographic characteristics in both groups were very similar (Table 1) and only male subjects were included. Genomic DNA from circulating leukocytes was purified according to standard phenol chloroform protocols.

Table 1.
Demographic characteristics of subjects (ave ± stdev).

	Number	Age	BMI	MMSE	Cholesterol (mg/dL)	HDL (mg/dL)	Triglycerides (mg/dL)	ApoE4 carriers
CTR	19	74 ± 7	27 ± 3	28 ± 1	230 ± 45	55 ± 16	111 ± 54	6 (31.6%)
AD	20	76 ± 7	27 ± 4	17 ± 7	230 ± 48	55 ± 12	125 ± 84	7 (35.0%)

BMI: body mass index; MMSE: Mini-Mental State Examination scores; HDL: high density lipoproteins; CTRL: controls. Statistical analysis showed no significant difference between groups except in MMSE scores ($p < 0.001$).

Neuroscience Letters 605 (2015) 44–48

Contents lists available at ScienceDirect

Neuroscience Letters

journal homepage: www.elsevier.com/locate/neulet

Research paper

Peripheral leukocyte expression of the potential biomarker proteins Bdnf, Sirt1, and Psen1 is not regulated by promoter methylation in Alzheimer's disease patients

Lucia Carboni[a,*,1], Francesca Lattanzio[a,1], Sanzio Candeletti[a], Elisa Porcellini[b], Elena Raschi[b], Federico Licastro[b], Patrizia Romualdi[a]

[a] Department of Pharmacy and Biotechnology (FaBIT), Alma Mater Studiorum University of Bologna, Bologna, Italy
[b] Department of Experimental, Diagnostic and Specialty Medicine (DIMES), Alma Mater Studiorum University of Bologna, Bologna, Italy

HIGHLIGHTS

- Promoter methylation was investigated in leukocytes of Alzheimer's disease cases.
- Bdnf promoter methylation levels did not differ between patients and controls.
- Sirtuin1 promoter methylation levels were low and did not differ between groups.
- Psen1 methylation showed large variability and no significant difference in cases.
- No correlation between methylation levels and cognitive deterioration was detected.

The identification of Alzheimer's disease (AD) biomarkers is crucial to support drug discovery. Within putative biomarkers, peripheral Bdnf levels correlate with cognitive decline and AD, although conflicting findings are reported. Sirtuin 1 (Sirt1) serum levels are lower in AD patients and Presenilin 1 (Psen1) is expressed by blood cells. DNA methylation is altered in AD patients, suggesting that epigenetic mechanisms play a role in AD pathophysiology. The objective of this study was to investigate promoter methylation levels of potential biomarkers in AD cases and controls. Peripheral blood DNA methylation levels were analysed by methylation-specific primer real-time PCR.

Bdnf promoter methylation levels did not differ between AD patients and controls. Similarly, Sirt1 promoter revealed minimal levels of methylation which did not display significant differences between groups. No significant difference was revealed between AD patients and controls also in Psen1 methylation, showing a large variability of values among subjects. Although peripheral Bdnf expression is associated with differential promoter methylation in psychiatric and neurological disorders, our results suggest that different mechanisms take place in AD. The finding that the control of Sirt1 protein levels in blood is not exerted through the repression of mRNA expression by promoter hypermethylation is in agreement with previous data. In contrast, other studies reported that Psen1 methylation may be increased or decreased in AD patients, suggesting that additional studies are required. In conclusion, this study shows that peripheral levels of the potential AD biomarker proteins Bdnf, Sirt1, and Psen1 are not regulated by different promoter methylation.

RESULTS

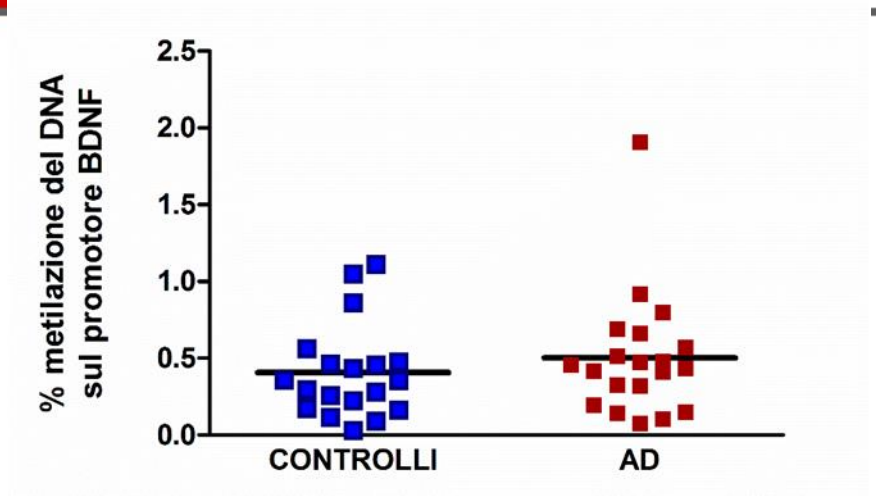


Fig. 1. Percentage of BDNF promoter methylation levels in controls and AD patients.

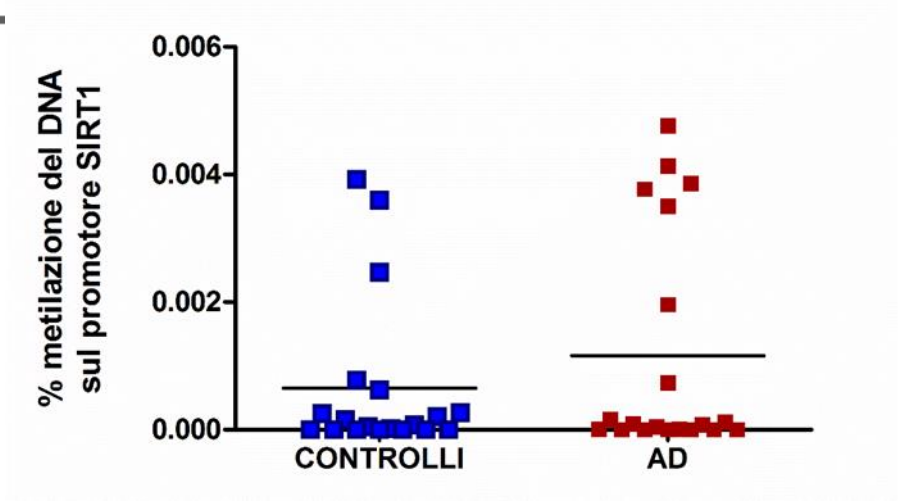


Fig. 2. Percentage of Sirt1 promoter methylation levels in controls and AD patients.

Research paper

Peripheral leukocyte expression of the potential biomarker proteins Bdnf, Sirt1, and Psen1 is not regulated by promoter methylation in Alzheimer's disease patients

Lucia Carboni[a,*,1], Francesca Lattanzio[a,1], Sanzio Candeletti[a], Elisa Porcellini[b], Elena Raschi[b], Federico Licastro[b], Patrizia Romualdi[a]

[a] Department of Pharmacy and Biotechnology (FaBiT), Alma Mater Studiorum University of Bologna, Bologna, Italy
[b] Department of Experimental, Diagnostic and Specialty Medicine (DIMES), Alma Mater Studiorum University of Bologna, Bologna, Italy

Research paper

Peripheral leukocyte expression of the potential biomarker proteins Bdnf, Sirt1, and Psen1 is not regulated by promoter methylation in Alzheimer's disease patients

Lucia Carboni[a,*,1], Francesca Lattanzio[a,1], Sanzio Candeletti[a], Elisa Porcellini[b], Elena Raschi[b], Federico Licastro[b], Patrizia Romualdi[a]

[a] Department of Pharmacy and Biotechnology (FaBiT), Alma Mater Studiorum University of Bologna, Bologna, Italy
[b] Department of Experimental, Diagnostic and Specialty Medicine (DIMES), Alma Mater Studiorum University of Bologna, Bologna, Italy

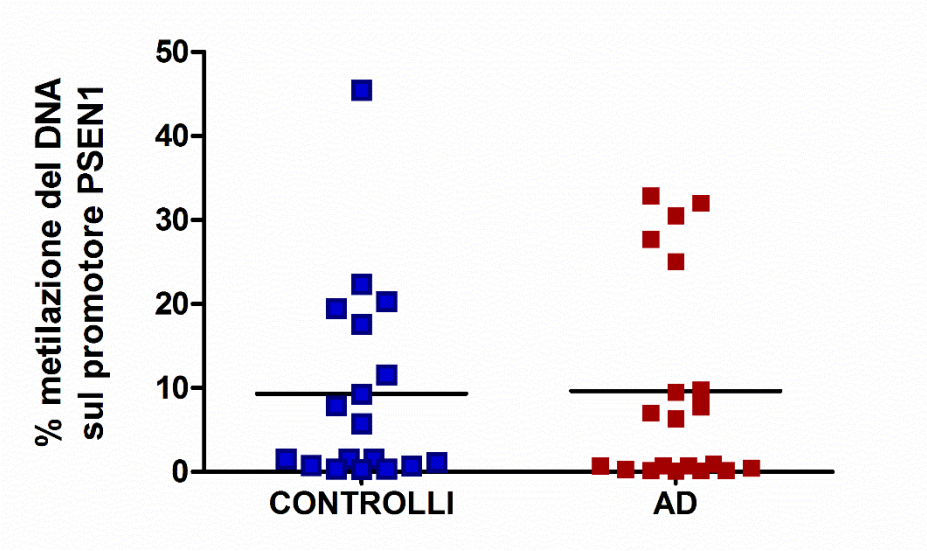


Fig. 3. Percentage of Psen1 promoter methylation levels in controls and AD patients.

Journal of the Neurological Sciences 372 (2017) 211–216

Contents lists available at ScienceDirect

Journal of the Neurological Sciences

journal homepage: www.elsevier.com/locate/jns

Clinical Short Communication

Transcriptional and epigenetic phenomena in peripheral blood cells of monozygotic twins discordant for alzheimer's disease, a case report

Claudio D'Addario [a,b,*], Sussy Bastias Candia [c], Beatrice Arosio [d,e], Martina Di Bartolomeo [a], Carlo Abbate [e], Alessandra Casè [d], Sanzio Candeletti [f], Patrizia Romualdi [f], Sarah Damanti [e,g], Mauro Maccarrone [b,h,1], Luigi Bergamaschini [d,i,1], Daniela Mari [d,e,1]

[a] Faculty of Bioscience and Technology for Food, Agriculture and Environment, University of Teramo, Teramo, Italy
[b] European Center for Brain Research (CERC)/Santa Lucia Foundation, Rome, Italy
[c] Departamento de Biología, Facultad de Ciencias, Universidad de Tarapacá, Arica, Chile
[d] Geriatric Unit, Department of Medical Sciences and Community Health, University of Milan, Italy
[e] Fondazione Ca'Granda, IRCCS Ospedale Maggiore Policlinico, Milan, Italy
[f] Department of Pharmacy and Biotechnology, University of Bologna, Italy
[g] Nutritional Sciences, University of Milan, Milan, Italy
[h] Department of Medicine, Campus Bio-Medico University of Rome, Rome, Italy
[i] A.S.P Pio Albergo Trivulzio, Milan, Italy

Target genes in Alzheimer's disease (AD) have been identified. In monozygotic twins discordant for AD we analysed the expression of selected genes, and their possible regulation by epigenetic mechanisms in peripheral blood mononuclear cells, possibly useful to discover biomarkers. Amyloid precursor protein, sirtuin 1 and peptidyl prolyl isomerase 1 gene expressions were highly up-regulated in the AD twin *versus* the healthy one. Consistently with sirtuin 1 role in controlling acetylation status, we observed a substantial reduction of the acetylation on histone 3 lysine 9, associated with gene transcription in the AD twin. Noteworthy in the AD twin we also observed an increased gene expression in two histone deacetylases (HDACs) isoforms: HDAC2 and HDAC9. A general DNA hypomethylation of all gene promoters studied was also observed in both twins. Our results unravel transcriptional and epigenetic differences potentially helpful to better understand environmental factors and phenotypic differences in monozygotic twins.

Contents lists available at ScienceDirect

Journal of the Neurological Sciences

journal homepage: www.elsevier.com/locate/jns

Clinical Short Communication

Transcriptional and epigenetic phenomena in peripheral blood cells of monozygotic twins discordant for alzheimer's disease, a case report

Claudio D'Addario [a,b,*], Sussy Bastias Candia [c], Beatrice Arosio [d,e], Martina Di Bartolomeo [a], Carlo Abbate [e], Alessandra Casè [d], Sanzio Candeletti [f], Patrizia Romualdi [f], Sarah Damanti [e,g], Mauro Maccarrone [b,h,1], Luigi Bergamaschini [d,i,1], Daniela Mari [d,e,1]

Epigenetic mechanism in leucocites of discordant twins

sirt 1 and pin1 are up-regulated in AD twin

A significant decrease of acetylation on H3K9 in agreement with gene expression.

A significant increase of HDAC2 and HDAC9 gene expression.

Biomarkers predictive of AD.

Drug and Alcohol Dependence 155 (2015) 68–75

Contents lists available at ScienceDirect

Drug and Alcohol Dependence

journal homepage: www.elsevier.com/locate/drugalcdep

Effects of acute ethanol exposure on class I HDACs family enzymes in wild-type and BDNF$^{+/-}$ mice

F.F. Caputi[a], M. Palmisano[a], C. D'Addario[b,c], S. Candeletti[a], P. Romualdi[a,*]

[a] Department of Pharmacy and Biotechnology, Alma Mater Studiorum—University of Bologna, Irnerio 48, 40126 Bologna, Italy
[b] Faculty of Bioscience and Technology for Food, Agriculture and Environment, University of Teramo, Teramo, Italy
[c] Karolinska Institutet, Department of Clinical Neuroscience, Center for Molecular Medicine, Stockholm, Sweden

ARTICLE INFO

ABSTRACT

Background: Alterations of brain-derived neurotrophic factor (BDNF) have been associated with the development of addiction to different drugs of abuse, including ethanol (EtOH). EtOH exposure activates the BDNF-signaling cascade in dorsal striatum, which in turn affects further EtOH intake. Different alcohol exposures have been widely demonstrated to modulate chromatin remodeling, affecting histone acetylation/deacetylation balance. Recently, class I histone deacetylases (HDACs) inhibition has been reported to modulate BDNF mRNA expression and to attenuate morphological and behavioral phenomena related to EtOH exposure. However, the role played by different HDAC isoforms in EtOH-induced plasticity is still unclear.

Methods: We investigated the effects induced by acute EtOH exposure on the protein levels of class I HDAC 1–3 isoforms of wild-type (WT) and BDNF heterozygous mice (BDNF$^{+/-}$), in nuclear and cytoplasmic extracts of specific brain regions associated with EtOH addiction.

Results: Nuclear HDAC 1–3 levels were markedly reduced after acute EtOH treatment in the caudate putamen (CPu) of WT mice only. Furthermore, CPu basal levels of nuclear HDAC isoforms were significantly lower in BDNF$^{+/-}$ mice compared to WT. With the exception of nuclear HDAC 3, no significant changes were observed after acute EtOH treatment in the prefrontal cortex (PFCx) of BDNF$^{+/-}$ and WT mice. In this area, the nuclear HDAC basal levels were significantly different between the two experimental groups.

Conclusions: These results provide details about EtOH effects on class I HDAC isoforms and strongly support a correlation between BDNF and class I HDACs, suggesting a possible influence of BNDF on these enzymes.

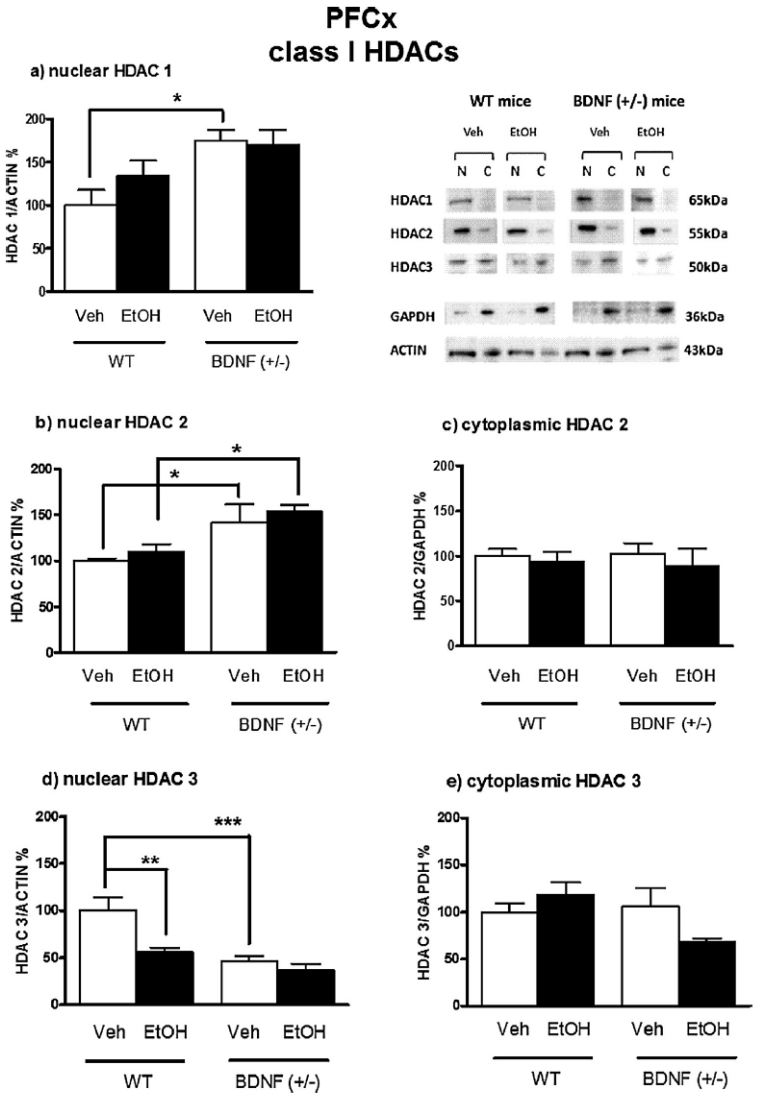
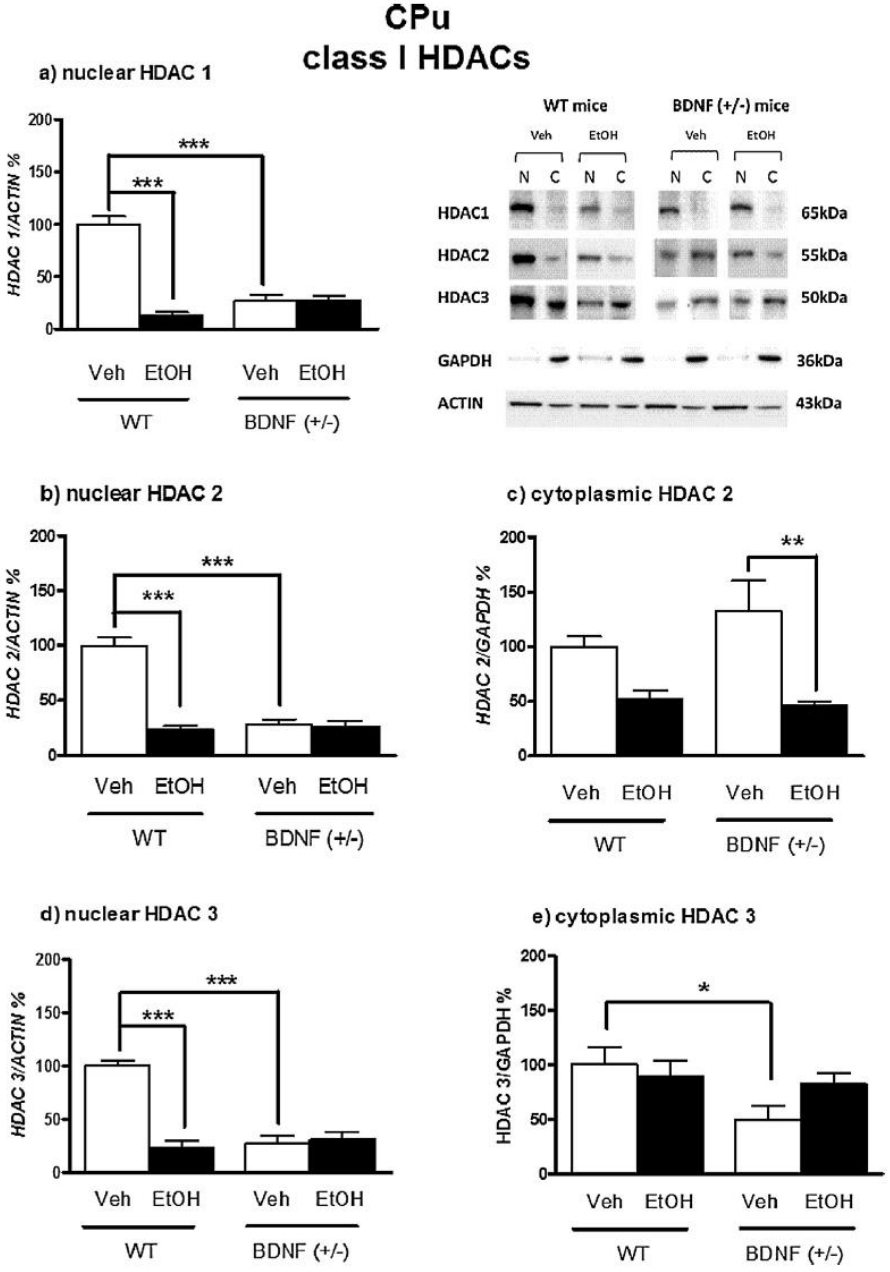


Fig. 3. Western blot of PFCx lysates after acute EtOH i.p. (2 g/kg) or vehicle (Veh) in nuclear (N) and cytoplasmic (C) fraction of WT and BDNF$^{+/-}$ mice. The HDAC 1, HDAC 2 and HDAC 3 protein levels were assessed using selected antibodies (see Materials and Methods section for details) compared to Actin and GAPDH, respectively used to assess nuclear and cytoplasmic fraction. Data are presented as mean ± SEM ($n = 6$ mice per group) and by analyzed by two-way ANOVA (*** $p < 0.001$; ** $p < 0.01$; * $p < 0.05$). In the upper right panel, representative immunoblots of HDAC 1/2/3 were reported.



Neuropsychopharmacology (2012) 37, 1647–1655

www.neuropsychopharmacology.org

Selective DNA Methylation of BDNF Promoter in Bipolar Disorder: Differences Among Patients with BDI and BDII

Claudio D'Addario*[,1,8], Bernardo Dell'Osso*[,2,8], Maria Carlotta Palazzo[2], Beatrice Benatti[2], Licia Lietti[2], Elisabetta Cattaneo[2], Daniela Galimberti[3], Chiara Fenoglio[3], Francesca Cortini[3], Elio Scarpini[3], Beatrice Arosio[4], Andrea Di Francesco[1], Manuela Di Benedetto[5], Patrizia Romualdi[5], Sanzio Candeletti[5], Daniela Mari[4], Luigi Bergamaschini[6], Nereo Bresolin[3], Mauro Maccarrone[1,7,9] and A Carlo Altamura[2,9]

[1]Department of Biomedical Sciences, University of Teramo, Teramo, Italy; [2]Department of Clinical Psychiatry, Università degli Studi di Milano, Fondazione IRRCS Cà Granda, Ospedale Maggiore Policlinico, Department of Mental Health, Department of Psychiatry, Milano, Italy; [3]Department of Neurological Sciences, Centro Dino Ferrari, Università degli Studi di Milano, Fondazione IRRCS Cà Granda, Ospedale Maggiore Policlinico, Department of Neurology, Milano, Italy; [4]Geriatric Unit, Fondazione IRCCS Cà Granda Osp Maggiore Polidinico, University of Milan, Milano, Italy; [5]Department of Pharmacology, University of Bologna, Bologna, Italy; [6]University of Milan, A.S.P. Pio Albergo Trivulzio, Milano, Italy; [7]European Center for Brain Research (CERC)/Santa Lucia Foundation, Rome, Italy

Table 1 Demographical and Clinical Characteristics of Patients Enrolled for DNA Methylation Studies

Sample	Total (n)	M	F	Mean age ± SD	Current phase
BD I	49	27	22	49.34 ± 12.65	A = 9
					B = 16
					C = 21
					D = 3
BD II	45	11	34	53.58 ± 12.04	A = 22
					B = 17
					C = 4
					D = 1
Controls	52	21	31	68.99 ± 1.89	

Keys: A, euthymia; B, depression; C, mania/hypomania; D, mixed state.

Table 2 List of Drugs in the Study Divided for Class, Dose, and % of Patients Under Treatment

DRUGS	CLASS	DOSE (mg)	% PATIENTS
Quetiapine	Mood stabilizer	25–1000	53
Valproate	Mood stabilizer	300–2000	51
Lithium	Mood stabilizer	300–1500	24
Aripiprazole	Mood stabilizer	5–25	17
Olanzapine	Mood stabilizer	5–40	17
Gabapentin	Mood stabilizer	300–1200	7
Pregabalin	Mood stabilizer	25–150	6
Venlafaxine	SNRI	75–300	15
Citalopram	SSRI	10–30	10
Duloxetine	SNRI	30–120	10
Clomipramine	TCA	50–225	6
Sertraline	SSRI	50–100	3
Escitalopram	SSRI	10–20	3

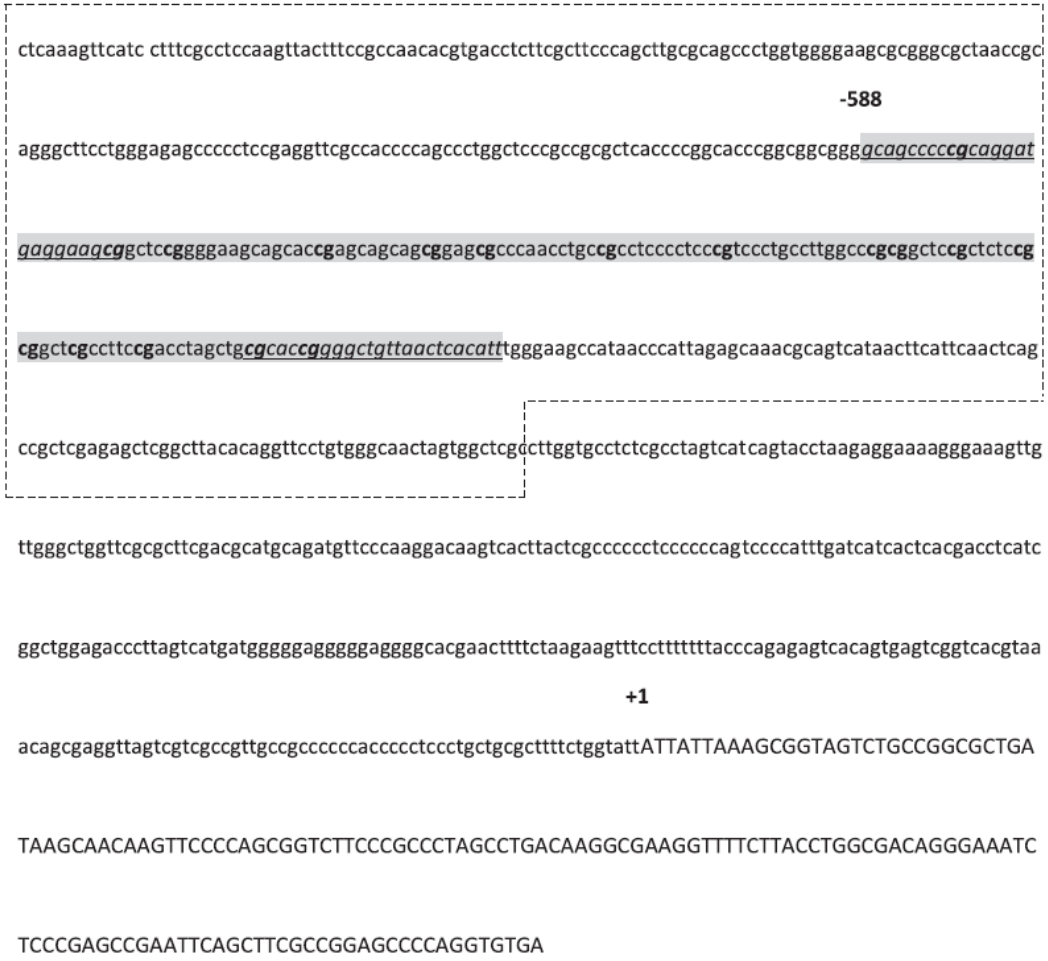


Figure 1 Sequence of the human brain-derived neurotrophic factor (BDNF) exon I promoter (chr11: 27 743 605–27 744 379). Amplicon is highlighted with the 17 cytosine guanine dinucleotide (CpG) sites present (also bolded) and primer sequences (methylated) underlined. The transcriptional start site (+1) is indicated and the predicted CpG island is shown (criteria used: island size >100, % GC >50.0, obs/exp >0.60).

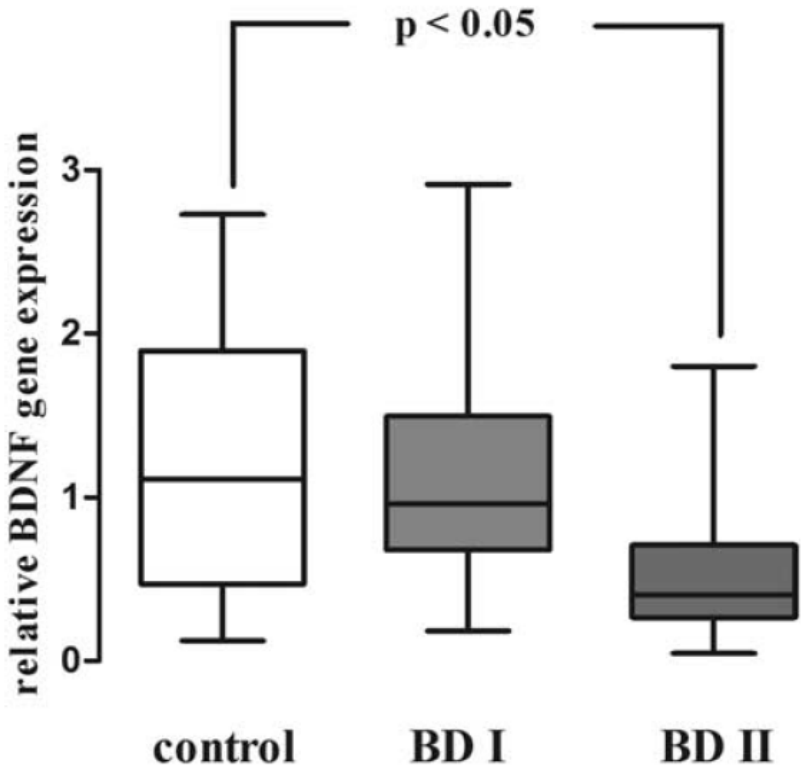


Figure 2 Levels of brain-derived neurotrophic factor (BDNF) mRNA in peripheral blood mononuclear cells from patients diagnosed with bipolar disorders type I (BD I; $n = 16$) and BD type 2 (BD II; $n = 16$). Box plots with whiskers from minimum to maximum represent 2^{-DDCt} values calculated by the Delta-Delta Ct (DDCt) method. Means of mRNA levels are expressed relative to control subjects ($n = 14$).

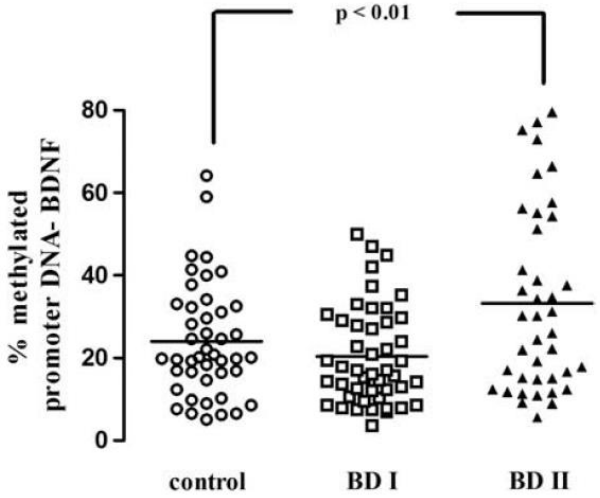


Figure 3 Amount of methylated DNA in the promoter region of brain-derived neurotrophic factor (BDNF) in controls, patients diagnosed with bipolar disorders type I (BD I; $n=16$) and BD type 2 (BD II; $n=16$). Scatter dot plots with mean values are shown.

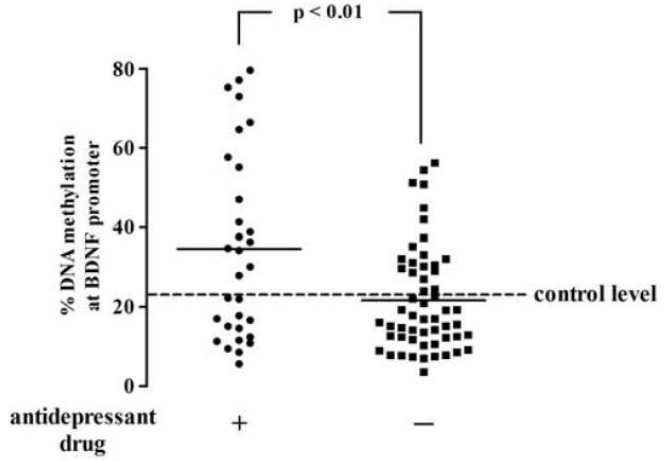


Figure 5 Amount of methylated DNA in the promoter region of brain-derived neurotrophic factor (BDNF) in peripheral blood mononuclear cells from patients diagnosed with bipolar disorders type I (BD I) + BDs type 2 (BD II) in therapy with (+) or without antidepressant drug (–). Scatter dot plots with mean values are shown.

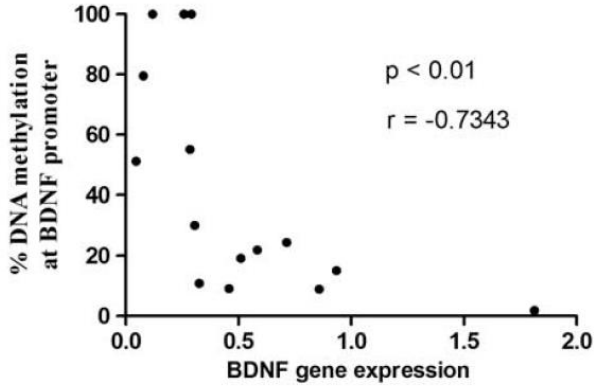


Figure 4 Correlation between brain-derived neurotrophic factor (BDNF) gene expression and percentage change in DNA methylation at BDNF promoter in bipolar disorders type 2 (BD II) subjects. Data are compared by Spearman's rank correlation coefficient ($P<0.01$, $r=-0.7343$).

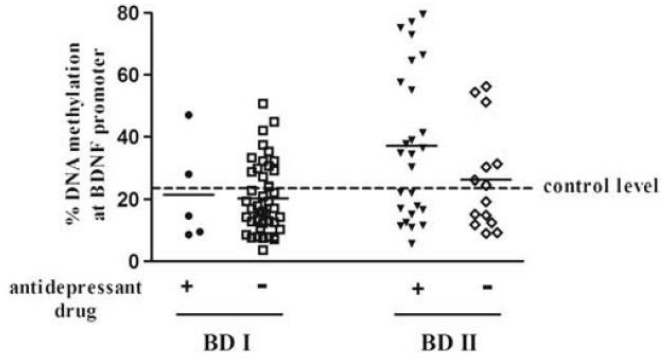


Figure 6 Amount of methylated DNA in the promoter region of brain-derived neurotrophic factor (BDNF) in peripheral blood mononuclear cells from patients diagnosed with bipolar disorders type I (BD I) or BD type 2 (BD II) in therapy with (+) or without antidepressant drug (–). Scatter dot plots with mean values are shown.

Neuron
Review

Chronic Pain: Emerging Evidence for the Involvement of Epigenetics

Franziska Denk[1,*] and Stephen B. McMahon[1]
[1]King's College London, Wolfson Centre for Age-Related Diseases, Guy's Campus, London SE1 1UL, UK
*Correspondence: franziska.denk@kcl.ac.uk
DOI 10.1016/j.neuron.2012.01.012

PAIN® 154 (2013) 15–23

PAIN®

www.elsevier.com/locate/pain

Research papers

Chronic opioid use is associated with increased DNA methylation correlating with increased clinical pain

Alexandra Doehring [a], Bruno Georg Oertel [a,b], Reinhard Sittl [c], Jörn Lötsch [a,*]

[a] Institute of Clinical Pharmacology, Goethe-University, Theodor Stern Kai 7, 60590 Frankfurt am Main, Germany
[b] Fraunhofer Project Group Translational Medicine and Pharmacology (IME-TMP), Theodor Stern Kai 7, D-60590 Frankfurt am Main, Germany

Environmentally caused changes in chromosomes that do not alter the DNA sequence but cause phenotypic changes by altering gene transcription are summarized as epigenetics. A major epigenetic mechanism is methylation or demethylation at CpG-rich DNA islands. DNA methylation triggered by drugs has largely unexplored therapeutic consequences. Here we report increased methylation at a CpG rich island in the *OPRM1* gene coding for μ-opioid receptors and at a global methylation site (LINE-1) in leukocytes of methadone-substituted former opiate addicts compared with matched healthy controls. Higher DNA methylation associated with chronic opioid exposure was reproduced in an independent cohort of opioid-treated as compared to non-opioid-treated pain patients. This suggests that opioids may stimulate DNA methylation. The *OPRM1* methylation had no immediate effect on μ-opioid receptor transcription and was not associated with opioid dosing requirements. However, the global DNA methylation at LINE-1 was significantly correlated with increased chronic pain. This suggests inhibitory effects on the transcription of still unspecified nocifensive gene products. It further implies that opioids may be causally associated with increased genome-wide DNA methylation, although currently there is no direct evidence of this. This has phenotypic consequences for pain and may provide a new, epigenetics-associated mechanism of opioid-induced hyperalgesia. The results indicate a potential influence of opioid analgesics on the patients' epigenome. They emphasize the need for reliable and cost-effective screening tools and may imply that high-throughput screening for lead compounds in artificial expression systems may not provide the best tools for identifying new pain medications.

EPIGENETICS and cancer

European Journal of Pharmacology 625 (2009) 131–142

Contents lists available at ScienceDirect

European Journal of Pharmacology

journal homepage: www.elsevier.com/locate/ejphar

Review

Epigenetics and cancer treatment

Lasse Sommer Kristensen, Helene Myrtue Nielsen, Lise Lotte Hansen *

Institute of Human Genetics, The Bartholin Building, University of Aarhus, 8000 Aarhus C, Denmark

DNA hypoMETHYLATION

Cancer cells have **20–60% less CpG methylated sites compared to normal cells**; this represents the first epigenetic alterations shown in cancer cells.

- Hypomethylation grade increases across tumor evolution, from a benigne lesion to an invasive tumor

- cancerogenesis role: loss of imprinting, cromosomes instability generation, activation of **oncogenes normally methylated.**

- meccanisms not well clarified

DNA Hypermethylation

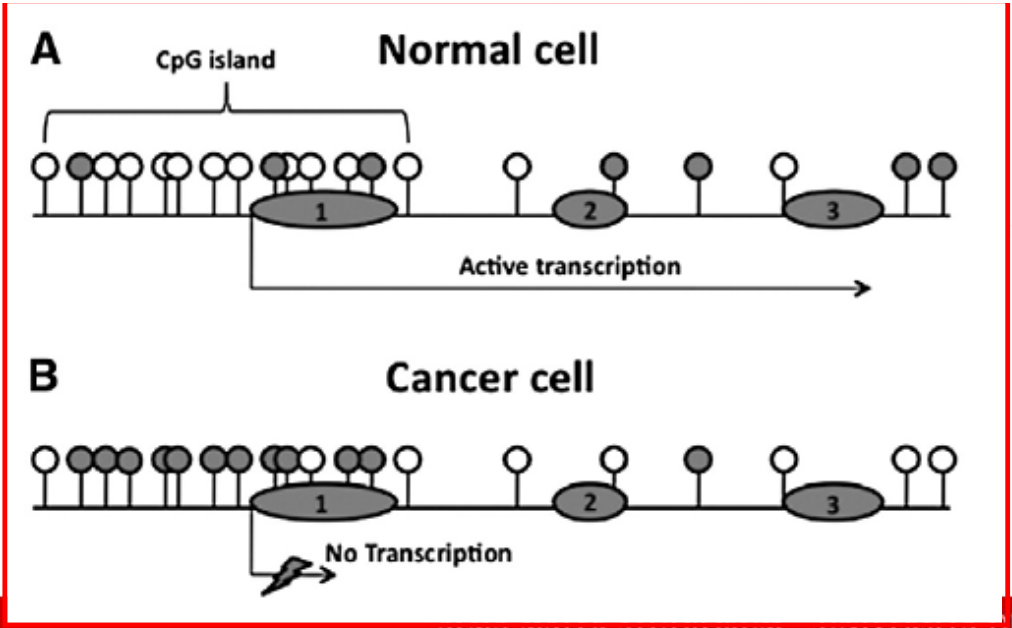
Hypermethylation of CpG islands and the silencing of ***tumor suppressor genes*** **are causal factors both in early and in late stages of** cancerogenesis.

Hypermethylation of *tumor suppressor gene* promoters is observed in the majority of tumors.

EPIGENETICs and cancer

A) CpG sites in CpG islands are usually non methylated in normal cells, whereas CpG sites out of islands are usually methylated. The CpG islands non methylated are usually actively transcribed.

B) Transcritional silencing of *tumor suppressor genes* in cancer cells is often associated to hyp**ermethylation of their CpG islands in the promotor**. Then CpG sites out of islands often are hypomethylated in cancer cells.



EPIGENETICs and cancer

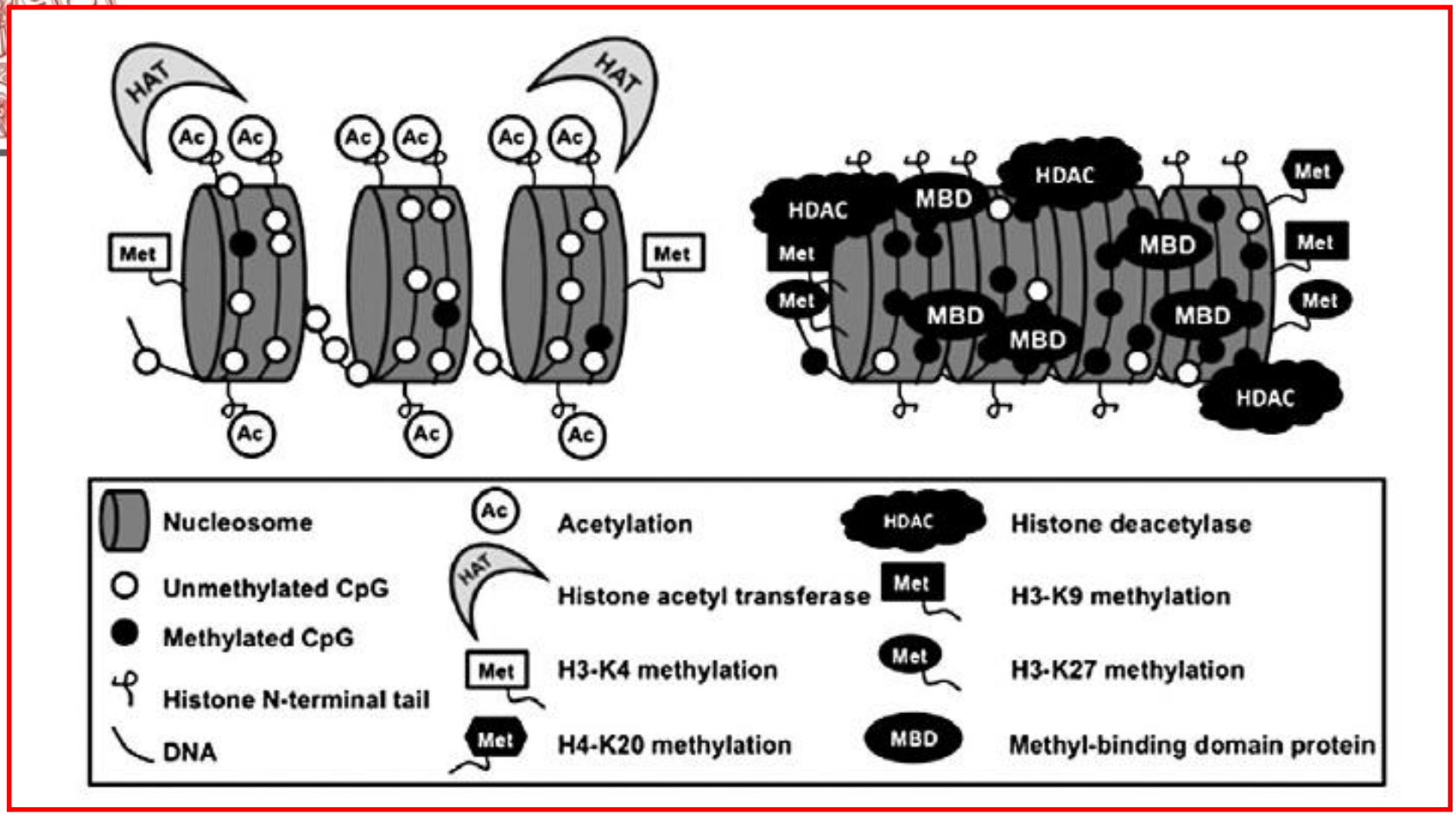
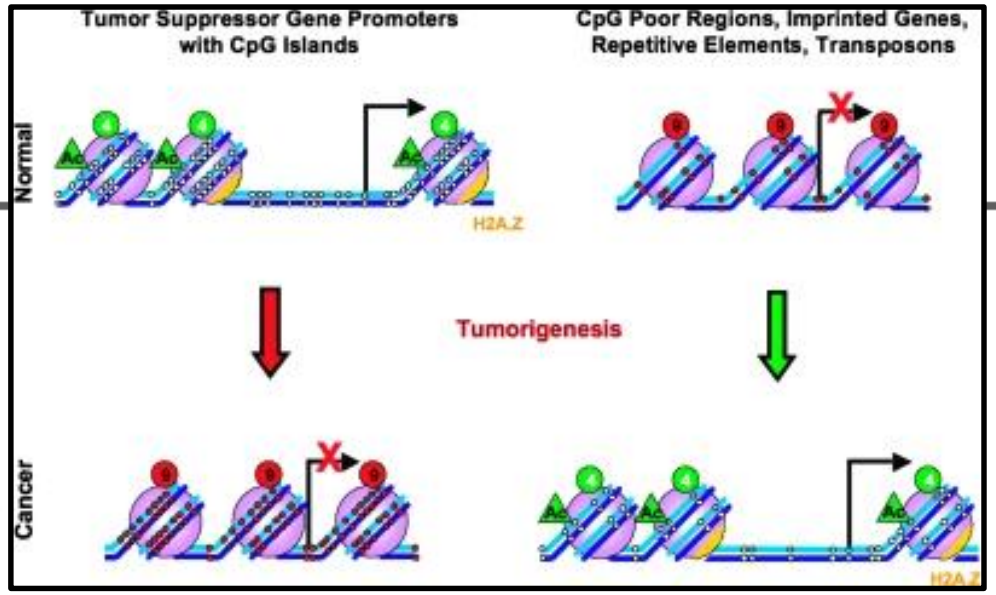


Fig. 2. Common epigenetic changes in cancer. To the left, the promoter region of an actively transcribed gene in a normal cell is shown. The acetylation of histone N-terminal tails by histone acetyl transferases favors an open chromatin structure making the promoter accessible for transcription factors. To the right, the promoter region of an epigenetically silenced gene in a cancer cell is shown. The CpG island is methylated and methyl-binding domain proteins (MBDs) recruit histone deacetylases, which remove acetyl groups from the histone N-terminal tails. This and other histone modifications favor a closed chromatin structure, which is inaccessible for the transcription machinery.

DNA methylation changes in cancer

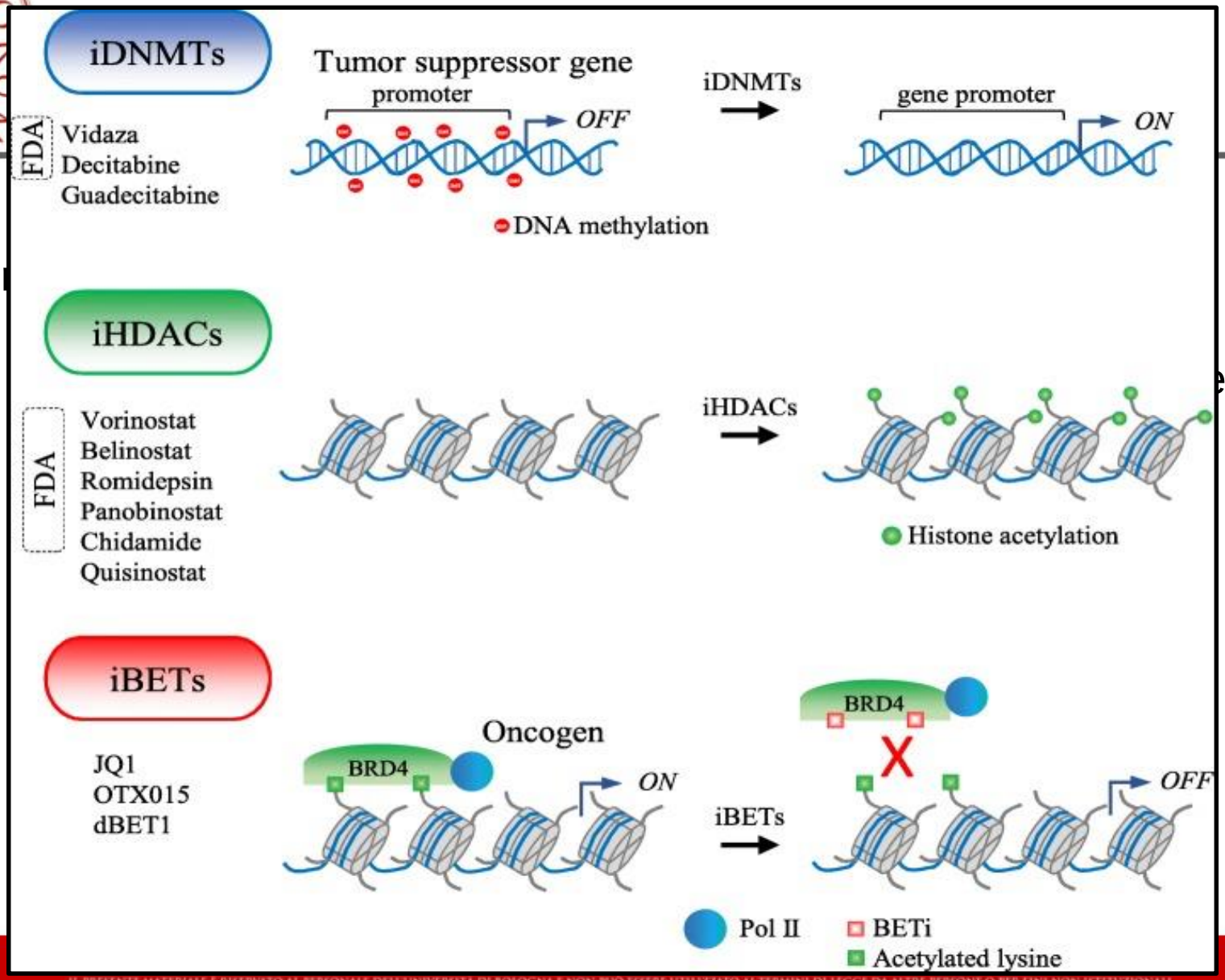


Epigenetics in cancer

Shikhar Sharma,[1,2] Theresa K. Kelly,[1] and Peter A. Jones[1,*]

In normal cells, CpG island promoters are generally unmethylated and when active, as in the case of tumor suppressor genes, are accompanied by active histone marks such as acetylation and H3K4 methylation (green circles, 4) allowing for a transcriptionally active open chromatin structure.
However, repetitive regions, transposons, CpG poor intergenic regions and imprinted gene promoters are heavily methylated and accompanied by repressive histone marks such as H3K9 methylation (red circles, 9) that together form a silent chromatin state. **During tumorigenesis, tumor suppressor gene promoters with CpG islands become methylated, resulting in the formation of silent chromatin structur**e and aberrant silencing (indicated by the red arrow). In contrast, the repetitive sequences, transposons and imprinted gene promoters become hypomethylated resulting in their aberrant activation (indicated by the green arrow).

EPIGENETICS and cancer



EPIGENETICS and CANCER

- Drugs able to “reverse” epigenetic alterations may be useful
- inhibiting DNMTs to oppose the DNA hypermethylation
- inibiting HDACs to oppose the histone hypoacetylation

epigenetics: therapy

In cancer cells epigenetic alterations leading silencing of “tumor suppressor genes” produce new drugs able to revert aberrant patterns of DNA methylation and histone acetylation by inhibiting **DNMTs and HDACs**.

European Journal of Pharmacology 625 (2009) 131–142

Contents lists available at ScienceDirect

European Journal of Pharmacology

journal homepage: www.elsevier.com/locate/ejphar

Review

Epigenetics and cancer treatment

Lasse Sommer Kristensen, Helene Myrtue Nielsen, Lise Lotte Hansen *

Institute of Human Genetics, The Bartholin Building, University of Aarhus, 8000 Aarhus C, Denmark

epigenetic: therapy

DNA methyltransferase inhibitors

Name	Chemical nature	Clinical status
Azacitidine	Nucleoside analogue	Approved myelodysplastic syndrome Phases I, II, III
Decitabine	Nucleoside analogue	Approved myelodysplastic syndrome Phases I, II, III
Zebularine	Nucleoside analogue	Not yet in clinical trial
5-fluoro-2′-deoxycytidine	Nucleoside analogue	Phase I
(−)-epigallocatechin-3-gallate	Non-nucleoside analogue	Phases I, II
Hydralazine	Non-nucleoside analogue	Phases I, II, III
RG108	Non-nucleoside analogue	Phase

epigenetic: therapy

Among the iHDAC, FDA has approved **vorinostat**, for the treatment of cutaneous linphoma T-cell.

HDAC inhibitors discussed within this review.

Name	Chemical nature	Clinical status
Sodium phenylbutyrate	Short-chain fatty acid	Phases I, II
Sodium butyrate	Short-chain fatty acid	In clinical trial
Valproic acid	Short-chain fatty acid	Phases I, II
OSU-HDAC42	Short-chain fatty acid	Not yet in clinical trial
Trichostatin A	Hydroxamic acid	Not in clinical trial
Vorinostat	Hydroxamic acid	Approved (CTCL) Phases I, II, III
Panobinostat	Hydroxamic acid	Phases I, II, III
Belinostat	Hydroxamic acid	Phases I, II
Romidepsin	Cyclic peptide	Phases I, II
Entinostat	Benzamide	Phases I, II
MGCD-0103	Benzamide	Phases I, II

miRNA

1993 first miRNA isolated in C.elegans Lin4

1998 second miRNA in C.elegans Lin-7

In 15 years more than 2000 miRNA identified

smallRNA:

smallRNA

siRNA

miRNA

smRNA

tncRNA

miRNA:

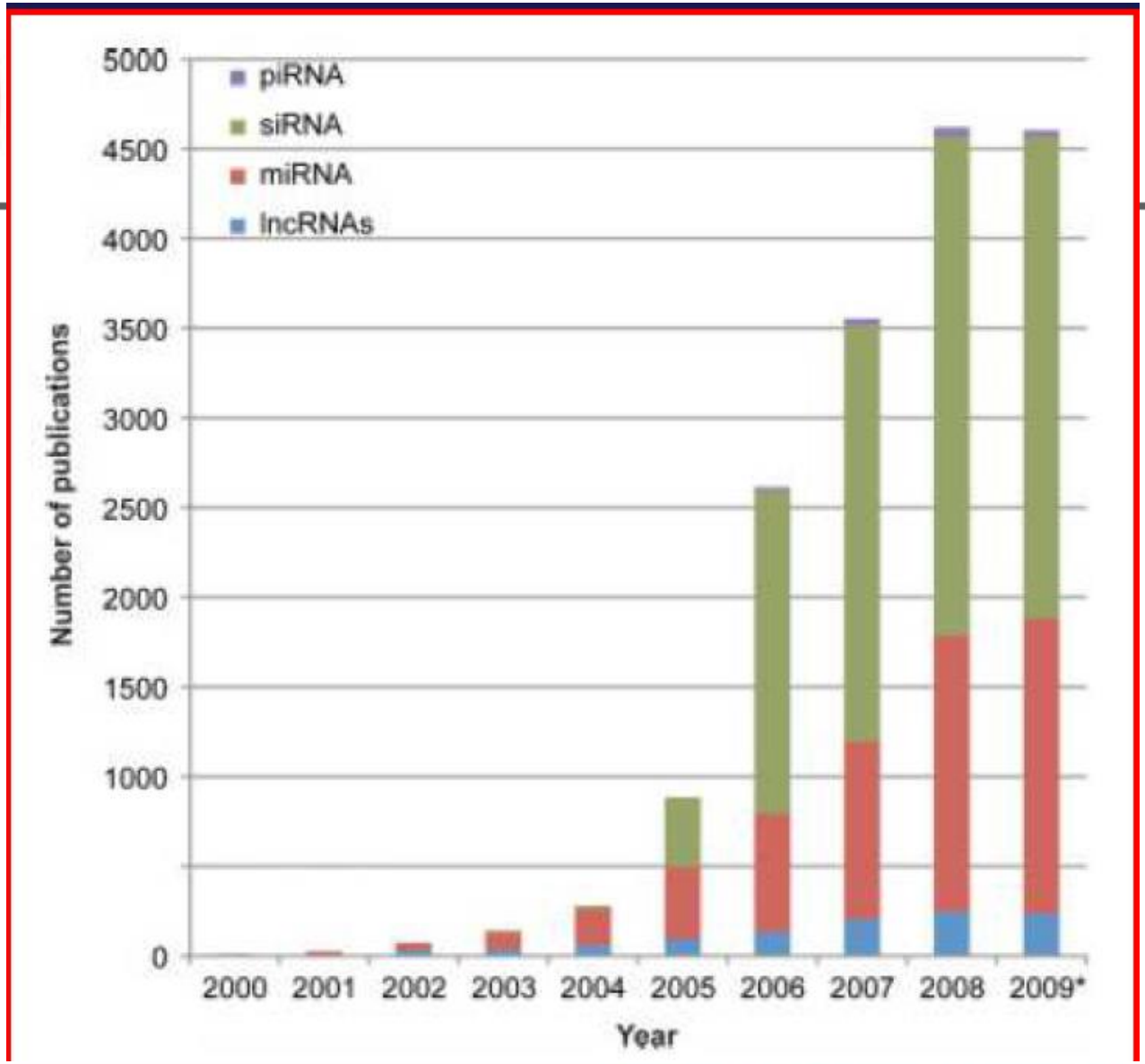
miRNA (MicroRNA)

- small molecule of single strend RNA
- 19-25 nucleotides lenght
- non coding but regulating of translation without degradation
- non perfectly complementary to their mRNA target.
- genes codifying for miRNA are 1% out of the total.

RNA non coding (ncRNA)

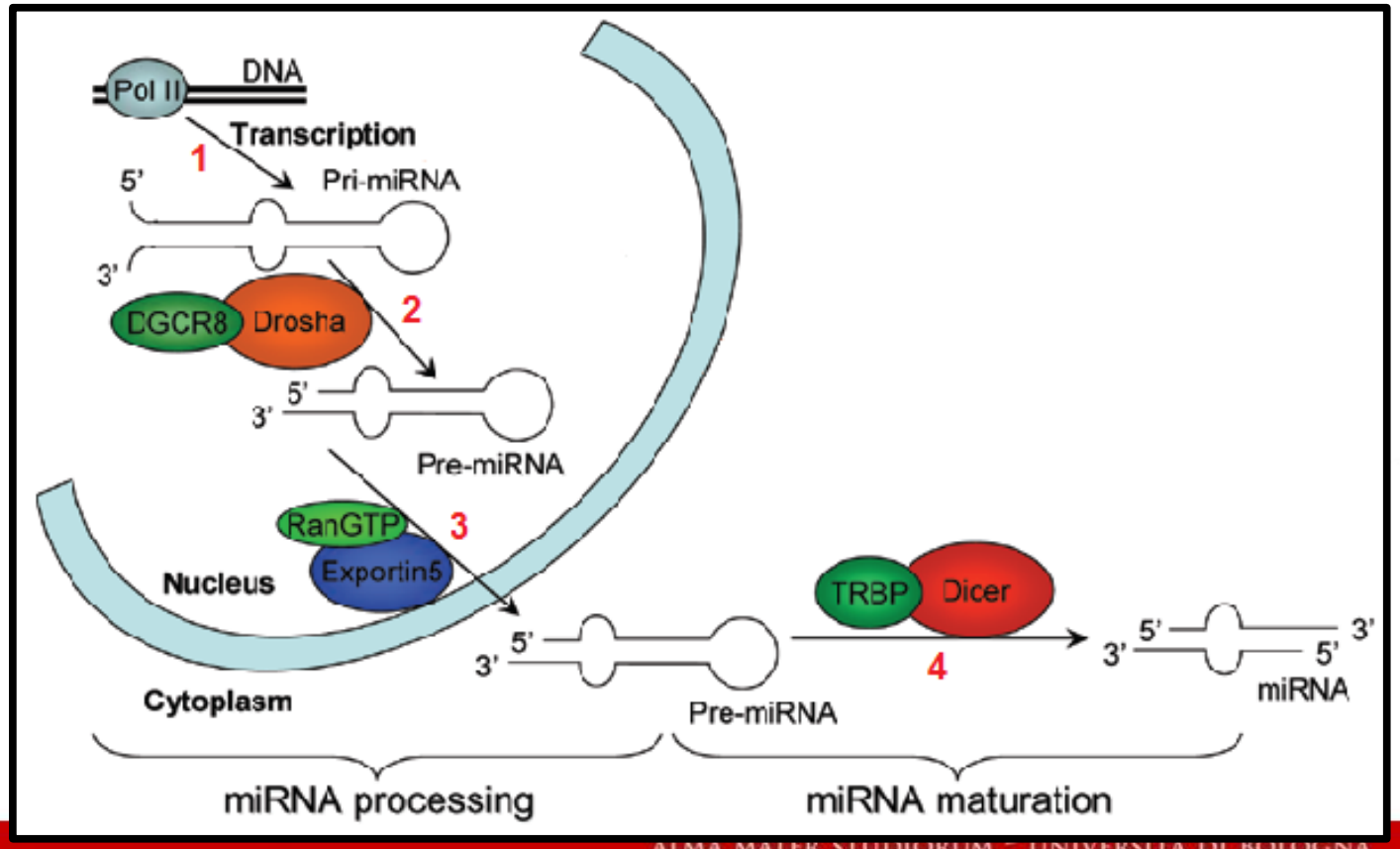
tRNA (RNA transfer) and rRNA (ribosomial RNA). They are important for protein synthesis.
Also….

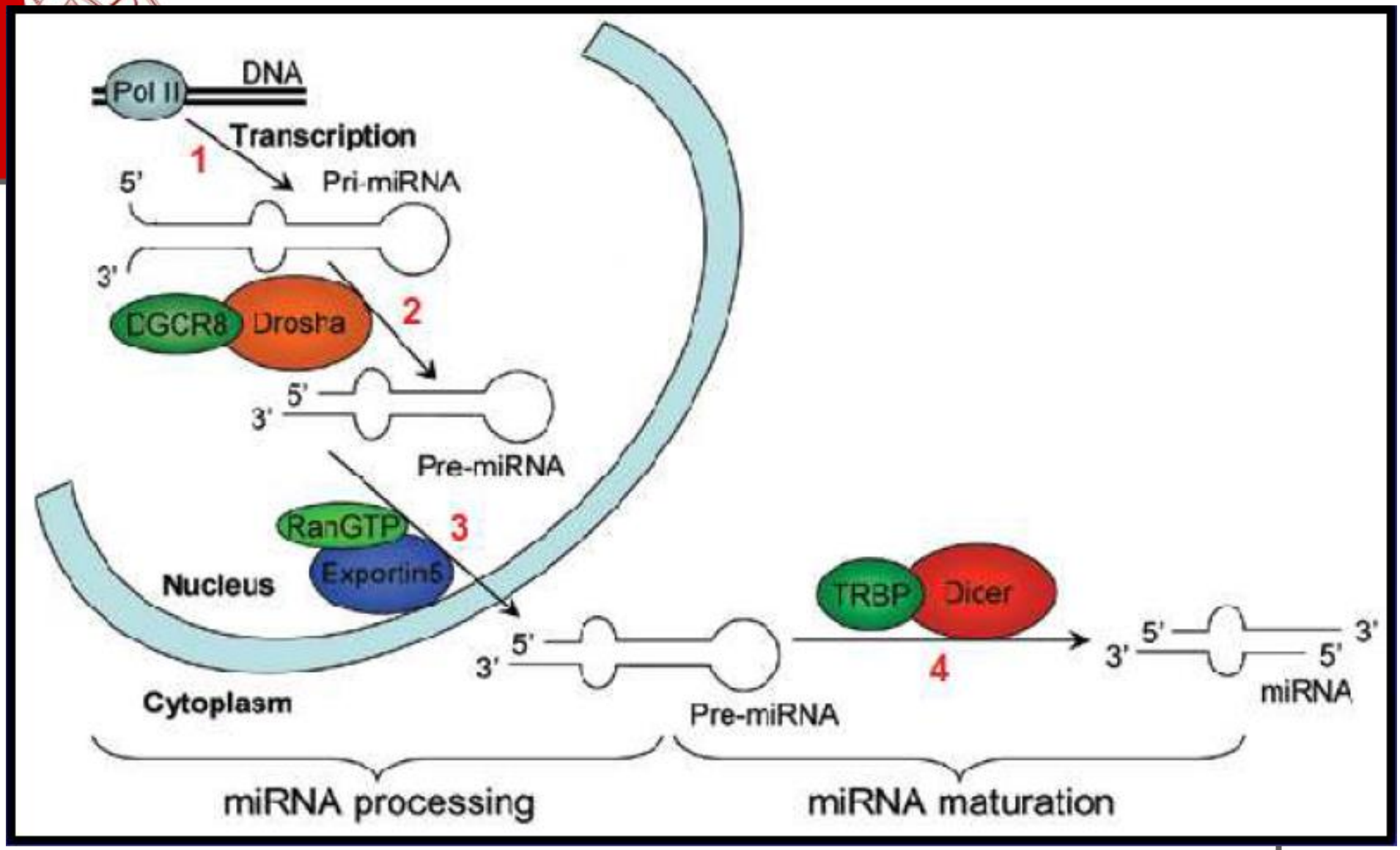
NcRNA class	Characteristics
Established ncRNA classes	
Long (regulatory) non-coding RNAs (lncRNAs)	The broadest class, lncRNAs, encompass all non-protein-coding RNA species >~200 nt, including mRNA-like ncRNAs. Their functions include epigenetic regulation, acting as sequence-specific tethers for protein complexes and specifying subcellular compartments or localization
Small interfering RNAs (siRNAs)	Small RNAs ~21–22 nt long, produced by Dicer cleavage of complementary dsRNA duplexes. siRNAs form complexes with Argonaute proteins and are involved in gene regulation, transposon control and viral defence
microRNAs (miRNAs)	Small RNAs ~22 nt long, produced by Dicer cleavage of imperfect RNA hairpins encoded in long primary transcripts or short introns. They associate with Argonaute proteins and are primarily involved in post-transcriptional gene regulation



miRNA: how are born

1. transcription from RNA pol II to form pre-miRNA
2. clivage from Drosha to form pre-miRNA
3. translocation out of nucleus by esportin 5
4. clivage from Dicer to miRNA mature





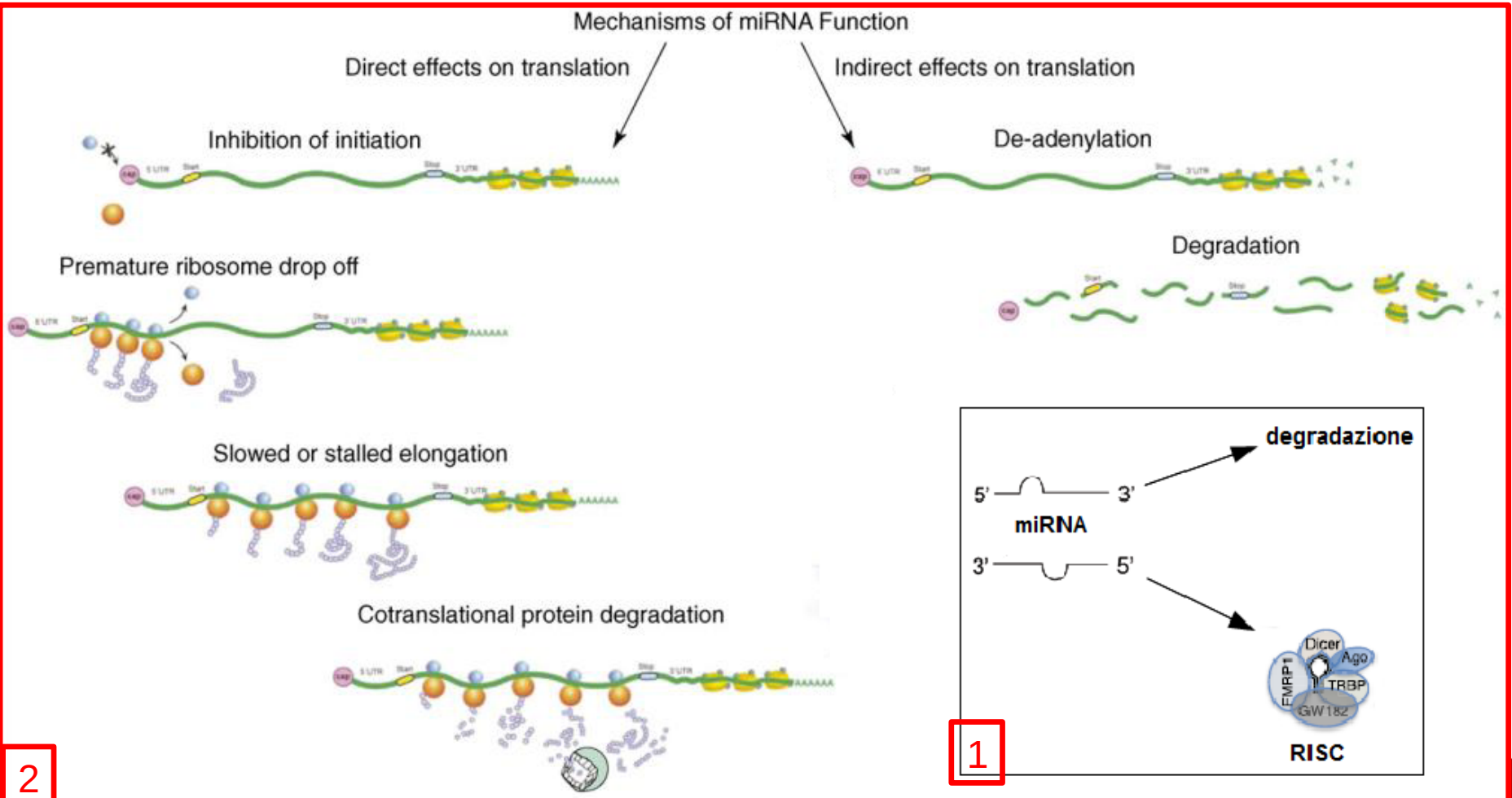
miRNA: how do they work

After formation of silencing complex induced by RNA (RISC):

a. inhibition of mRNA-ribosome complex formation

b. protein synthesis alterations

c. degradation of the poliA tail of mRNA



The functions of microRNAs in pluripotency and reprogramming

Trevor R. Leonardo, Heather L. Schultheisz, Jeanne F. Loring and Louise C. Laurent

Pluripotent stem cells (PSCs) express a distinctive set of microRNAs (miRNAs). Many of these miRNAs have similar targeting sequences and are predicted to regulate downstream targets cooperatively. These enriched miRNAs are involved in the regulation of the unique PSC cell cycle, and there is increasing evidence that they also influence other important characteristics of PSCs, including their morphology, epigenetic profile and resistance to apoptosis. Detailed studies of miRNAs and their targets in PSCs should help to parse the regulatory networks that underlie developmental processes and cellular reprogramming.

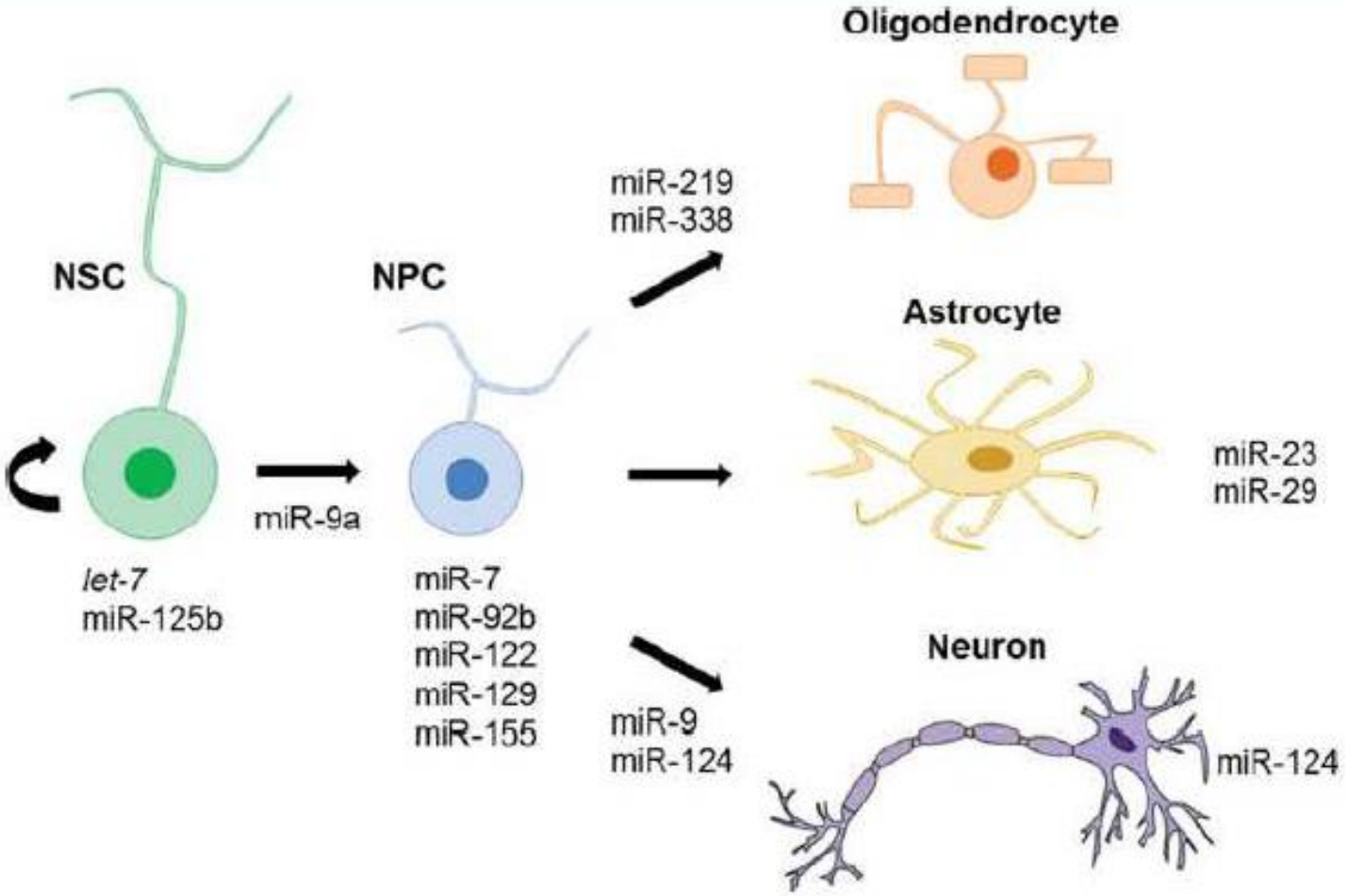
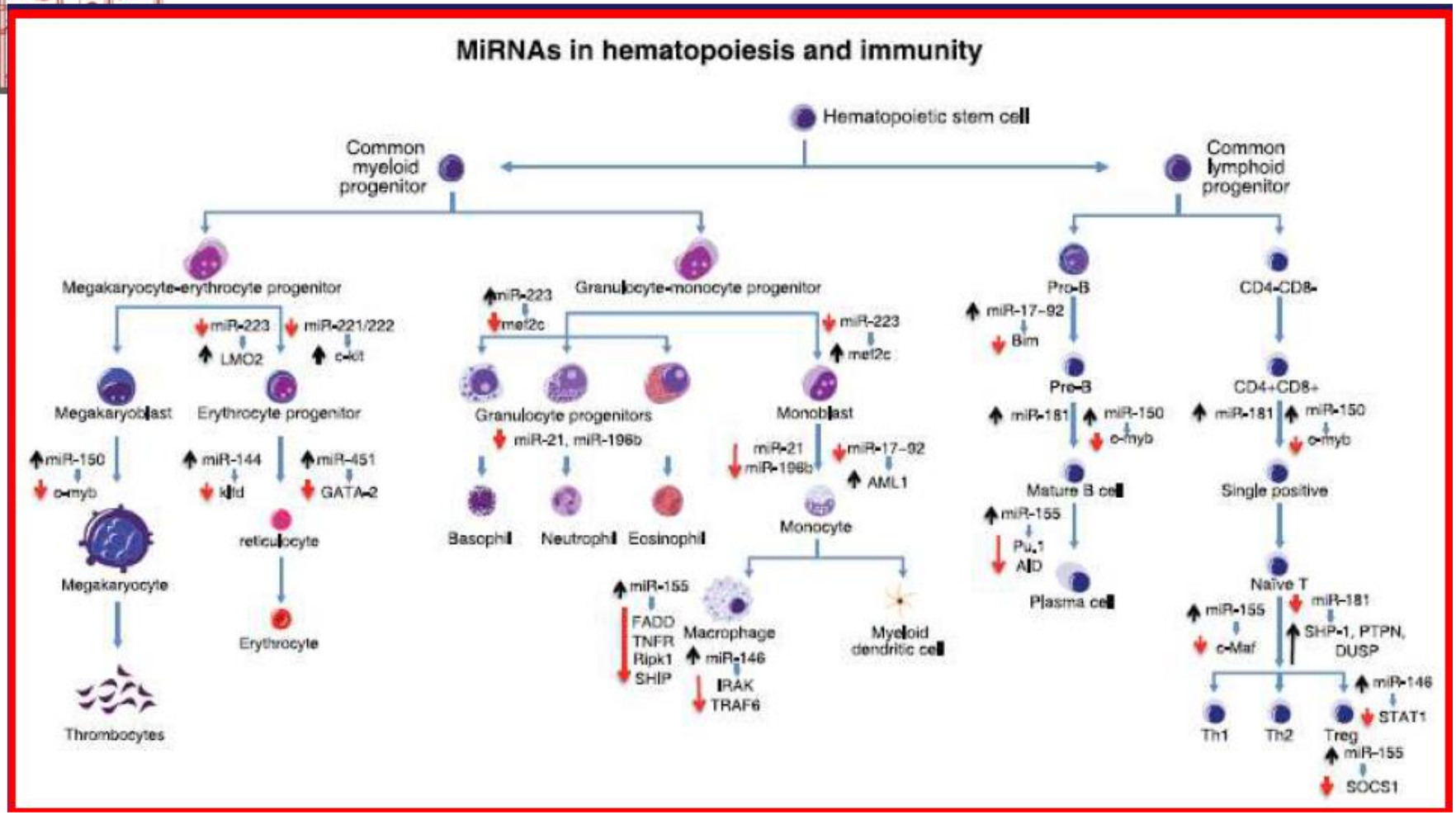


Fig. 3. miRNAs involved in CNS development. Different miRNAs participate during the differentiation process of neuronal and glial phenotypes in a variety of organisms. Cell type-specific miRNA expression (miRNAs below/beside each cell type) is necessary to maintain exquisite control of gene expression within specific spatiotemporal windows. NSC, neural stem cell; NPC, neural progenitor cell. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]



miRNA: functions

- embrional development
- haematopoiesis
- apoptosi
- neoplastic genesis and progression
- cardiac elettrophysiology
- cardiac myopathies
- Endocrine mechanisms

miRNA: functions

- Embrional development (miR51 family),
- electrical cardiac functions (miR-1, miR-2)
- synapsis formation and maturation (miR-134, miR-138)
- haematopoiesis

miRNA FUNZIONI

Abnormal miRNA expression profiles have been identified in several pathologies, suggesting their role in cellular events underlying start and progression of the illness.

- cancer
- autoimmune diseases
- SNC diseases

Table 1. MiRNAs involved in addiction

Drug	miRNAs involved	Mechanisms affected	References
Cocaine	miR-212	Decreases activity of CREB and TORC1, and controls expression of gene encoding BDNF via interaction with MeCP2	[4,15]
	miR-181a	Upregulated by cocaine; affects expression of genes encoding BDNF, DAT, CREB, Homer1 and Drd3	[10,11]
	let-7d	Downregulated by cocaine; targets semaphorins, BDNF, neuropilins and mGluR5	[10,11]
	miR-124	Downregulated by cocaine; targets Drd3, DAT and FosB	[10,11]
	miR-324-5p	Induced by Arg2 in Drd2 neurons; regulates cdk5r1, FosB and Mef2d	[5,9]
	miR-369-3p	Induced by Arg2 in Drd2 neurons; regulates cdk5r1, FosB and Mef2d	[5,9]
Nicotine	miR-140	Induced by nicotine; regulates dynamin-1, regulates synaptic endocytosis	[12,16]
	miR-504	Upregulates gene encoding Drd1	[12,16]
Opiates	miR-23b	Induces mOR	[19]
	miR-190	Induced by mOR by upregulation of talin2	[13,19]
	miR-15b, miR-181b	Induced by morphine	[20,21]
		Induced by morphine	
	miR-133b	Decreased by morphine; this increases expression of gene encoding Pitx3 and induces TH and DAT	[6]
Antidepressants, alcohol and CYP3A4	miR-133b	Specifically expressed in dopaminergic neurons; downregulates expression of gene encoding Pitx3; Pitx3 induces production of TH and DAT and regulates maturation of midbrain dopaminergic neurons	[6,21]
	miR-16	Induced by SSRI in serotonergic neurons; reduces expression of gene encoding SERT	[23]
	miR-9	Induced by alcohol; downregulates BK channels	[4]
	miR-212	Induced by alcohol; decreases ZO-1, affects cell permeability	[22]
	miR-27b	Downregulates CYP3A4	[17]
	miR-298	Downregulates CYP3A4	[17,24]

Arg2, arginase, type II; cdk5r1, cyclin-dependent kinase 5, regulatory subunit 1; CREB, cAMP-responsive element binding protein; BDNF, brain-derived neurotrophic factor; CYP3A4, cytochrome P_{450} 3A4; DAT, dopamine transporter; Drd1, dopamine receptor D1R; Drd2, dopamine receptor D2R; Drd3, dopamine receptor D3R; FosB, FBJ murine osteosarcoma viral oncogene homolog B; MeCP2, methyl CpG binding protein 2; Mef2d, myocyte enhancer factor 2D; mGluR5, metabotropic glutamate receptor 5; SSRI, selective serotonin reuptake inhibitor; TH, tyrosine hydroxylase; TORC1, transducer of regulated CREB activity 1; ZO-1, tight junction protein.

Alzheimer Disease

miRNA-146 increase chronic inflammation

Proinflammatory cytochines induce NF-kB Activation

Activation of miRNA-146 expression
Causes an increase of the inhibition of CFH (H complement factor) that represses cerebral inflammatory processes

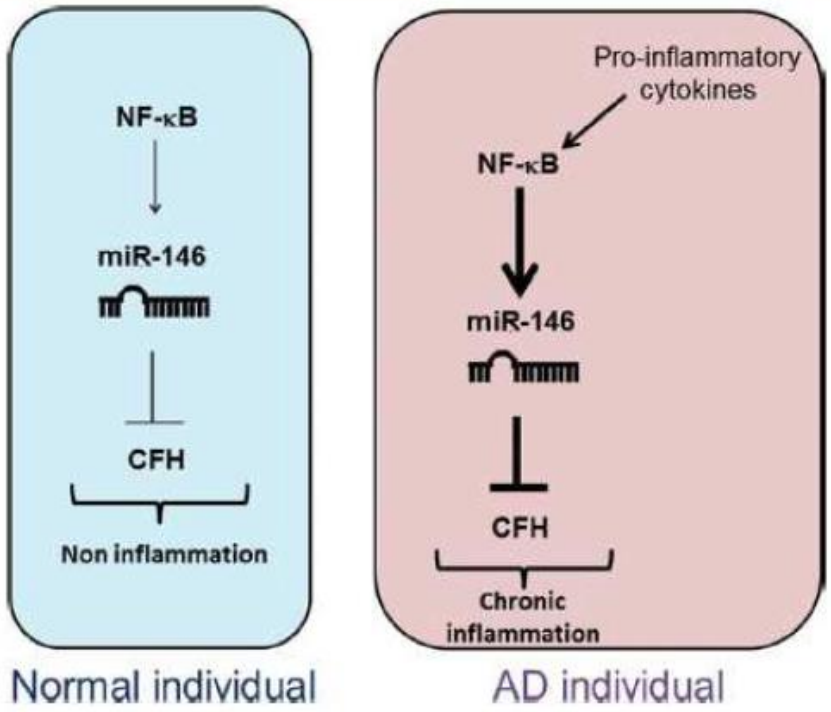


Fig. 4. miR-146 promotes chronic inflammation in AD. In normal individuals, the activity of NF-κB is normally regulated and thus the expression of miR-146 is moderated and maintains normal levels of complement factor (CFH). However, in AD, proinflammatory cytokines promote the activation of the NF-κB transcription factor, which in turn activates the miR-146, resulting in permanent inhibition of CFH and leading to chronic inflammation. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

miRNA and Cancer

↓ let-7	**increase of tumoral growth**
↓ miR-15a/16-1	**decrease of apoptosis**
↑ miR-424	**increase of angiogenesis**
↑ mirR-103/107	
↓ miR-200	**metastasis**
↑ miR-21	**gemcytabine resistance**

Table I. miRNAs implicated in select human malignancies

Condition	Overexpressed	Downregulated
Lung cancer	miR-155, miR-21, miR-17-92, miR-221/222	let-7, miR-1, miR-29, miR-126
Breast cancer	miR-155, miR-21, miR-182, miR-17-92, miR-200, miR-9	let-7, miR-143/145, miR-10b, miR-125b. miR-126. miR-9
Hepatocellular cancer	miR-21, miR-221/222	miR-1, miR-26a,
Colorectal cancer	miR-155, miR-21, miR-17-92	let-7, miR-34
Pancreatic cancer	mir-21, miR-155	let-7, miR-15a/16-1, miR-34
Prostate cancer	miR-21	let-7, miR-15a/16-l, miR-221
CLL	miR-155	miR-15a/16-1, miR-29b, miR-181b, miR-34
AML	miR-10a/b, miR-29, miR-155	miR-181, miR-204

miRNAs in lung cancer

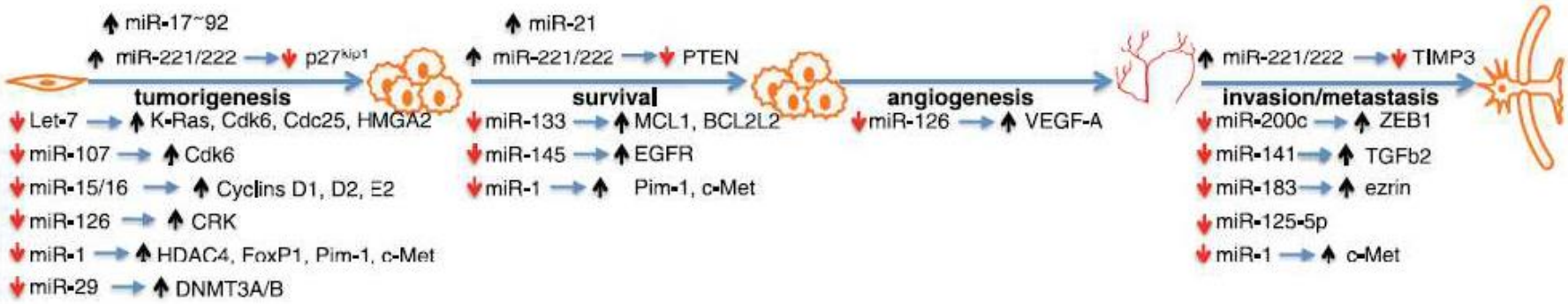


FIGURE 7 A diagram showing miRNAs and their targets in lung cancer. The diagram displays the different miRNAs and their targets that are involved in transformation, survival, angiogenesis, and invasion/metastasis of lung cancer. Upregulation or downregulation of a specific miRNA is represented by an upward (black) or a downward (red) arrow, respectively. The changes in the expression levels of a target gene inversely correlate with that of the targeting miRNA and are similarly represented by an up or down arrow. All listed targets have been validated. These include the following: cell division cycle 25 (Cdc25), cyclin-dependent kinase 6 (Cdk6), high mobility group AT-hook 2 (HMGA2), K-Ras, v-crk sarcoma virus CT10 oncogene homolog (CRK), histone deacetylase 4 (HDAC4), forkhead box P1 (FoxP1), proviral integration site 2 (Pim-1), hepatocyte growth factor receptor (c-Met), DNA methyltransferase 3A/B (DNMT3A/B), myeloid cell leukemia sequence 1 (MCL1), Bcl2-like 2 (BCL2L2), epidermal growth factor receptor (EGFR), vascular endothelial growth factor A (VEGF-A), zinc finger E-box binding homeobox 1 (ZEB1), and transforming growth factor beta 2 (TGFb2).

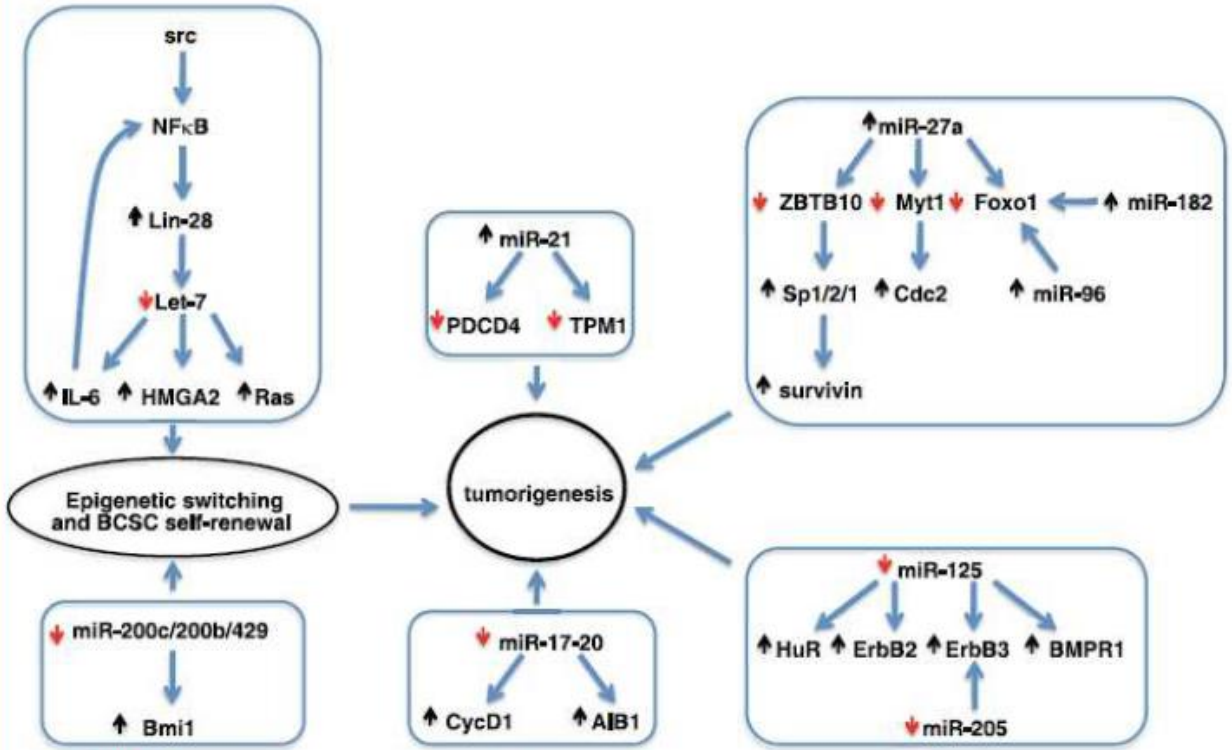
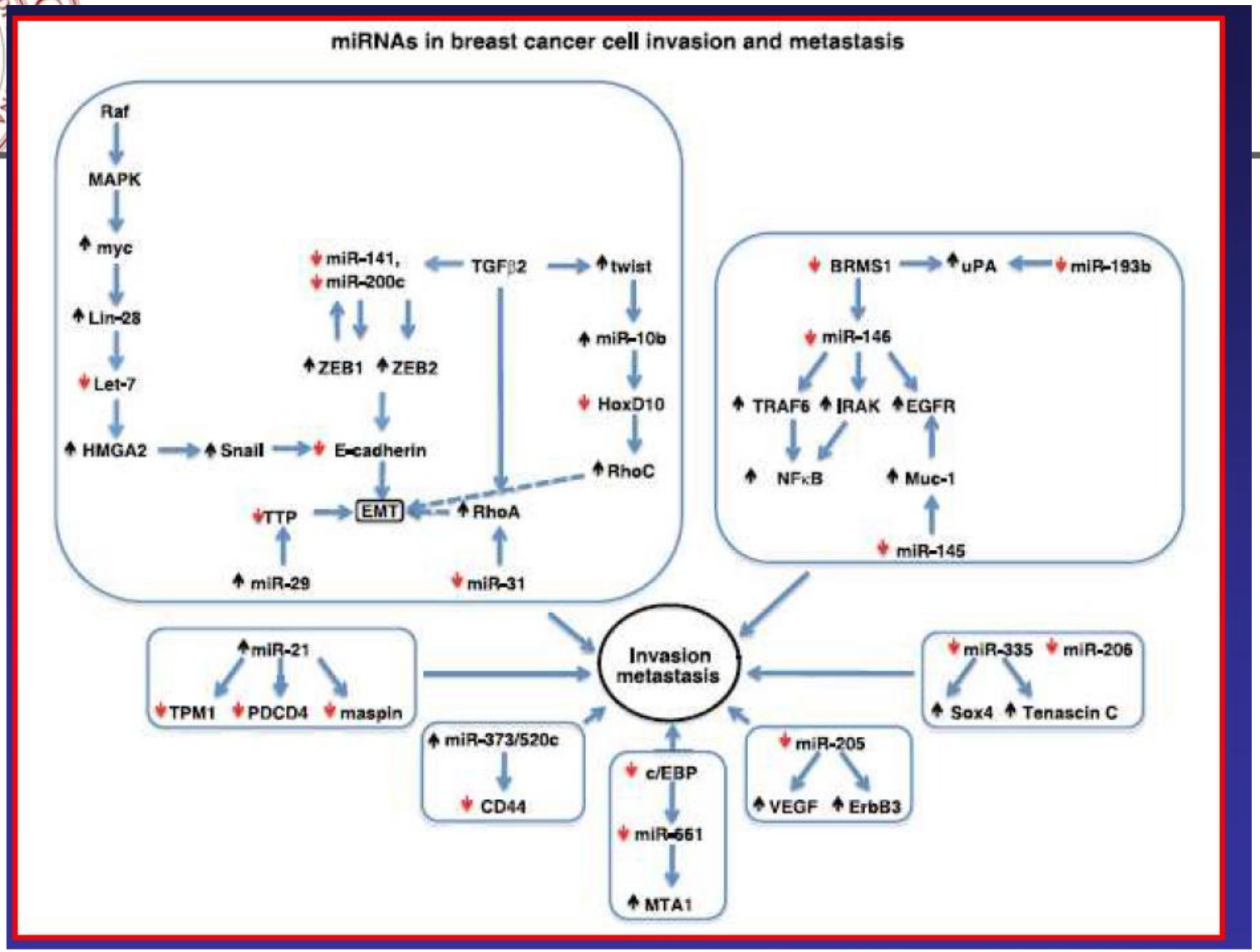


FIGURE 9 A diagram showing miRNAs and their targets in initiation and growth of breast cancer. The diagram displays the different miRNAs and their targets that are involved in breast cancer stem cells (BCSC) self-renewal and tumor growth. This also includes some of the identified upstream molecules that regulate their expression. Upregulation or downregulation of a specific miRNA is represented by an upward (black) or a downward (red) arrow, respectively. The changes in the expression levels of a target gene inversely correlate with that of the targeting miRNA and are similarly represented by an up or down arrow. All listed targets have been validated. The targets listed include the following: v-src sarcoma (Schmidt-Ruppin A-2) viral oncogene homolog (src), nuclear factor of kappa light polypeptide gene enhancer in B-cells 1 (NFκB), interleukin-6 (IL-6), high mobility group AT-hook 2 (HMGA2), Ras oncogene, signal transducer and activator of transcription 3 (STAT3), programmed cell death 4 (PDCD4), tropomyosin 1 (TPM1), zinc finger and BTB domain containing 10 (ZBTB10), myelin transcription factor 1 (Myt1), forkhead box O1 (Foxo1), Sp1 transcription factor, cell division cycle 2 protein (Cdc2), Hu antigen R (HuR), v-erb-b2 erythroblastic leukemia viral oncogene homolog 2 (ErbB2) and homolog 3 (ErbB3), bone morphogenetic protein receptor type 1B (BMPR1B), cyclin D1 (CycD1), and amplified in breast cancer 1 protein (AIB1).



ncRNA: therapeutical advanges

miRNA as diagnostical biomarkers

miRNA pattern of expression is able to identify neoplastic formation scarsely differentiated, very difficult to see with mRNA or Protein expression

The pattern of expression of more than 200 miRNAs in plasma, saliva, tissues can help to make early diagnosis of hidden tumors

ncRNA therapeutical advantages

miRNA as therapeutical target

RNA molecules can be used for therapeutical aims
by mimiking or regulating miRNA activity in tumors.

- Introduce underexpressed miRNA

- Inhibit the overexpression of miRNA
(by antagomirs, complementary or binding to miRNA or by Sponges containing several artificial binding sites for miRNAs)

miRNA: Diseases

cancer

miRNAs may have both oncogene or oncosupressor functions.

Surprisingly, one specific miRNA can behave as oncogene or oncosuppressor depending upon the type of alteration, type of cell or transcriptional or post transcriptional regulation of target genes.

miRNA: diseases

Cancer

↓ let-7 → ↑ tumoral growth,

↓ miR-15a/16-1 → ↓ apoptosis,

↑ miR-424 → ↑ angiogenesis,

↑ miR-103/107, ↓ miR-200 → metastasis,

↑ miR-21 → gemcitabine resistance

Table I. miRNAs implicated in select human malignancies

Condition	Overexpressed	Downregulated
Lung cancer	miR-155, miR-21, miR-17-92, miR-221/222	let-7, miR-1, miR-29, miR-126
Breast cancer	miR-155, miR-21, miR-182, miR-17-92, miR-200, miR-9	let-7, miR-143/145, miR-10b, miR-125b. miR-126. miR-9
Hepatocellular cancer	miR-21, miR-221/222	miR-1, miR-26a,
Colorectal cancer	miR-155, miR-21, miR-17-92	let-7, miR-34
Pancreatic cancer	mir-21, miR-155	let-7, miR-15a/16-1, miR-34
Prostate cancer	miR-21	let-7, miR-15a/16-l, miR-221
CLL	miR-155	miR-15a/16-1, miR-29b, miR-181b, miR-34
AML	miR-10a/b, miR-29, miR-155	miR-181, miR-204

Table 2 | **Therapeutic use of miRNAs and antagomirs *in vivo***

miRNA‡	Delivery method	Model used	Phenotypes
let-7	Intranasal delivery of viral particles	$Kras^{G12D/+}$ autochthonous NSCLC mouse	Suppression of lung tumour initiation when delivered at the same time as transgene activation
	Intratumoral injection of lipid-based mimetic	Subcutaneous H460 NSCLC xenografts	Interfered with further tumour growth
	Intranasal delivery of viral particles	$Kras^{G12D/+}$ autochthonous NSCLC mouse	Reduced burden of tumours that were allowed to preform 10 weeks before *let-7* therapy
	Systemic delivery of lipid-based mimetic	$Kras^{G12D/+}$ autochthonous NSCLC mouse	Reduced burden of tumours that were allowed to preform 10 weeks before *let-7* therapy
	Transfected into cells before transplantation	Subcutaneous human U251 or U87 glioblastoma cells	Reduced tumour formation
	Transduced into cells before transplantation	Chemotherapy-resistant breast tumour initiating cells	Reduced tumour formation and metastasis

miRNA: diseases

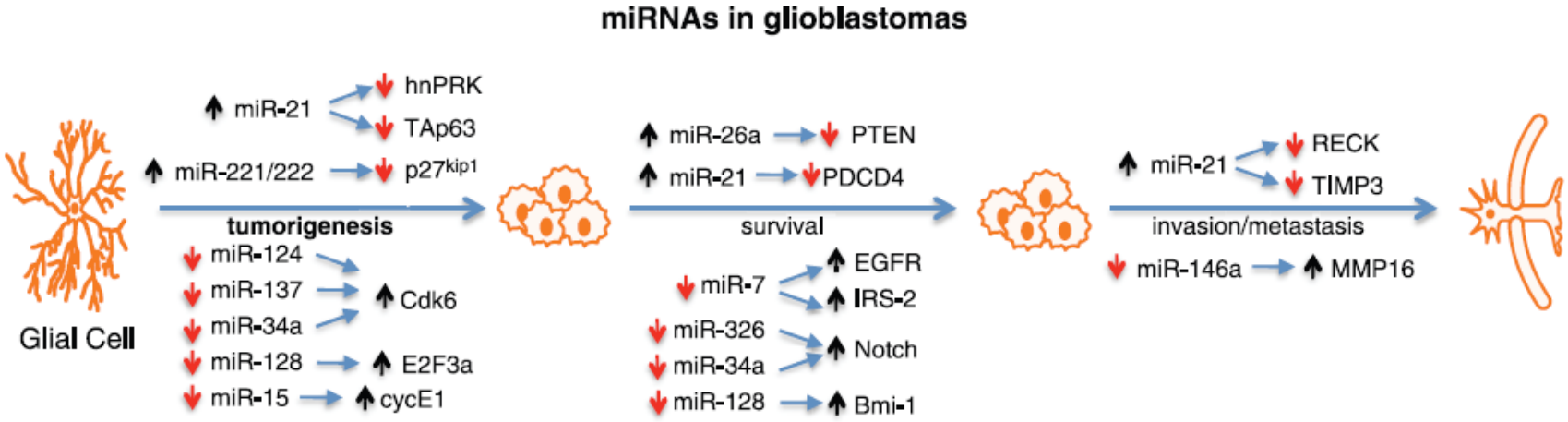


FIGURE 6 A diagram showing miRNAs and their targets in glioblastomas. The diagram displays the different miRNAs and their targets that are involved in transformation, survival, and invasion/metastasis of glioblastomas. Upregulation or downregulation of a specific miRNA is represented by an upward (black) or a downward (red) arrow, respectively. The changes in the expression levels of a target gene inversely correlate with that of the targeting miRNA and are similarly represented by an up or down arrow. All listed targets have been validated. These include the following: heterogeneous nuclear ribonucleoprotein K (hnRPK), tumor protein p63 (TAp63), cyclin-dependent kinase 6 (Cdk6), E2F transcription factor 3 (E2F3a), phosphatase and tensin homolog (PTEN), programmed cell death 4 (PDCD4), epidermal growth factor receptor (EGFR), insulin receptor substrate 2 (IRS-2), BMI1 polycomb ring finger oncogene (Bmi-1), reversion-inducing-cysteine-rich protein with kazal motifs (RECK), tissue inhibitor of metalloproteinase 3 (TIMP3), and matrix metallopeptidase 16 (MMP16).

miRNAs in lung cancer

FIGURE 7 A diagram showing miRNAs and their targets in lung cancer. The diagram displays the different miRNAs and their targets that are involved in transformation, survival, angiogenesis, and invasion/metastasis of lung cancer. Upregulation or downregulation of a specific miRNA is represented by an upward (black) or a downward (red) arrow, respectively. The changes in the expression levels of a target gene inversely correlate with that of the targeting miRNA and are similarly represented by an up or down arrow. All listed targets have been validated. These include the following: cell division cycle 25 (Cdc25), cyclin-dependent kinase 6 (Cdk6), high mobility group AT-hook 2 (HMGA2), K-Ras, v-crk sarcoma virus CT10 oncogene homolog (CRK), histone deacetylase 4 (HDAC4), forkhead box P1 (FoxP1), proviral integration site 2 (Pim-1), hepatocyte growth factor receptor (c-Met), DNA methyltransferase 3A/B (DNMT3A/B), myeloid cell leukemia sequence 1 (MCL1), Bcl2-like 2 (BCL2L2), epidermal growth factor receptor (EGFR), vascular endothelial growth factor A (VEGF-A), zinc finger E-box binding homeobox 1 (ZEB1), and transforming growth factor beta 2 (TGFb2).

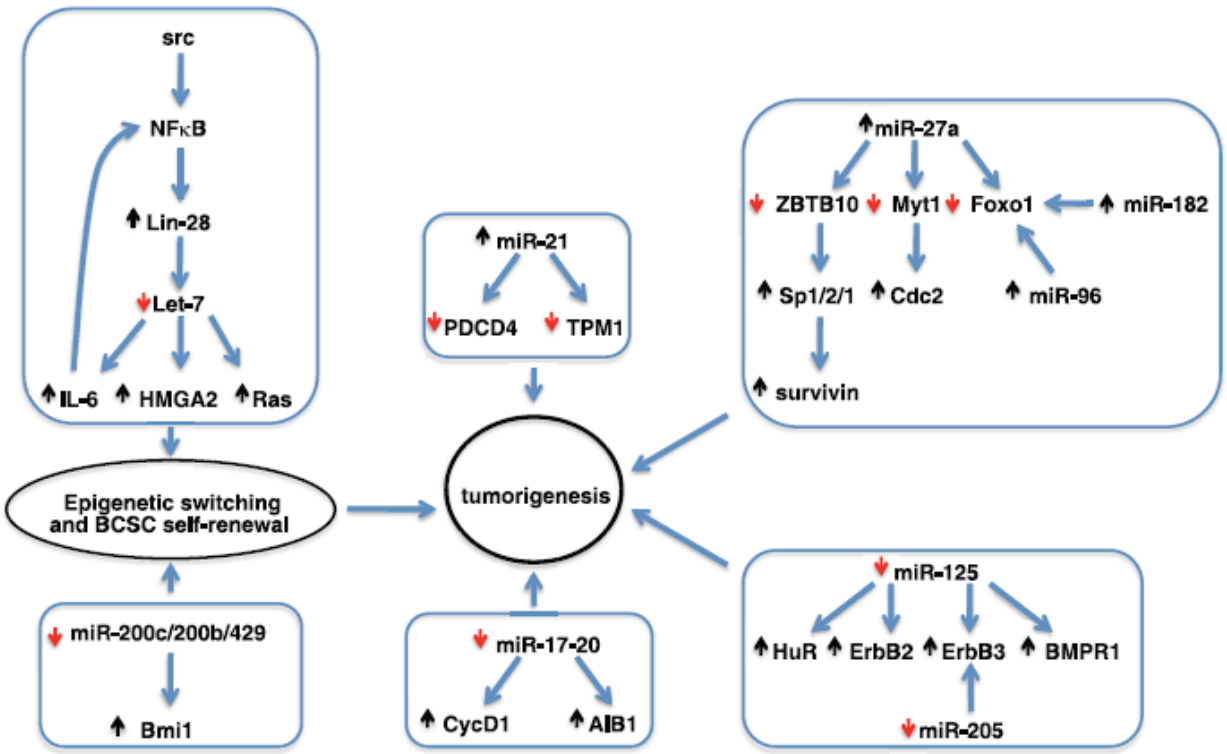
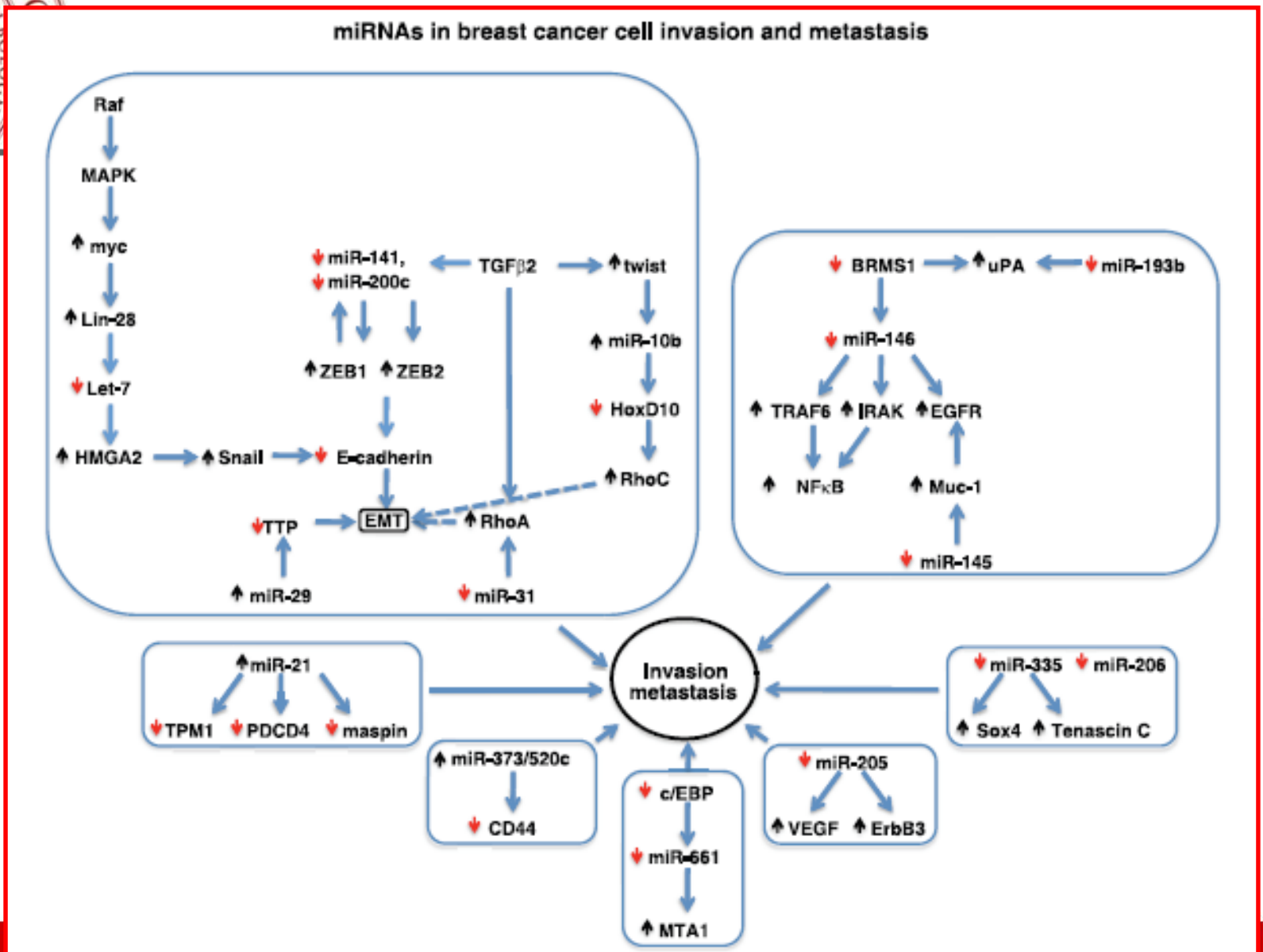


FIGURE 9 A diagram showing miRNAs and their targets in initiation and growth of breast cancer. The diagram displays the different miRNAs and their targets that are involved in breast cancer stem cells (BCSC) self-renewal and tumor growth. This also includes some of the identified upstream molecules that regulate their expression. Upregulation or downregulation of a specific miRNA is represented by an upward (black) or a downward (red) arrow, respectively. The changes in the expression levels of a target gene inversely correlate with that of the targeting miRNA and are similarly represented by an up or down arrow. All listed targets have been validated. The targets listed include the following: v-src sarcoma (Schmidt-Ruppin A-2) viral oncogene homolog (src), nuclear factor of kappa light polypeptide gene enhancer in B-cells 1 (NFκB), interleukin-6 (IL-6), high mobility group AT-hook 2 (HMGA2), Ras oncogene, signal transducer and activator of transcription 3 (STAT3), programmed cell death 4 (PDCD4), tropomyosin 1 (TPM1), zinc finger and BTB domain containing 10 (ZBTB10), myelin transcription factor 1 (Myt1), forkhead box O1 (Foxo1), Sp1 transcription factor, cell division cycle 2 protein (Cdc2), Hu antigen R (HuR), v-erb-b2 erythroblastic leukemia viral oncogene homolog 2 (ErbB2) and homolog 3 (ErbB3), bone morphogenetic protein receptor type 1B (BMPR1B), cyclin D1 (CycD1), and amplified in breast cancer 1 protein (AIB1).



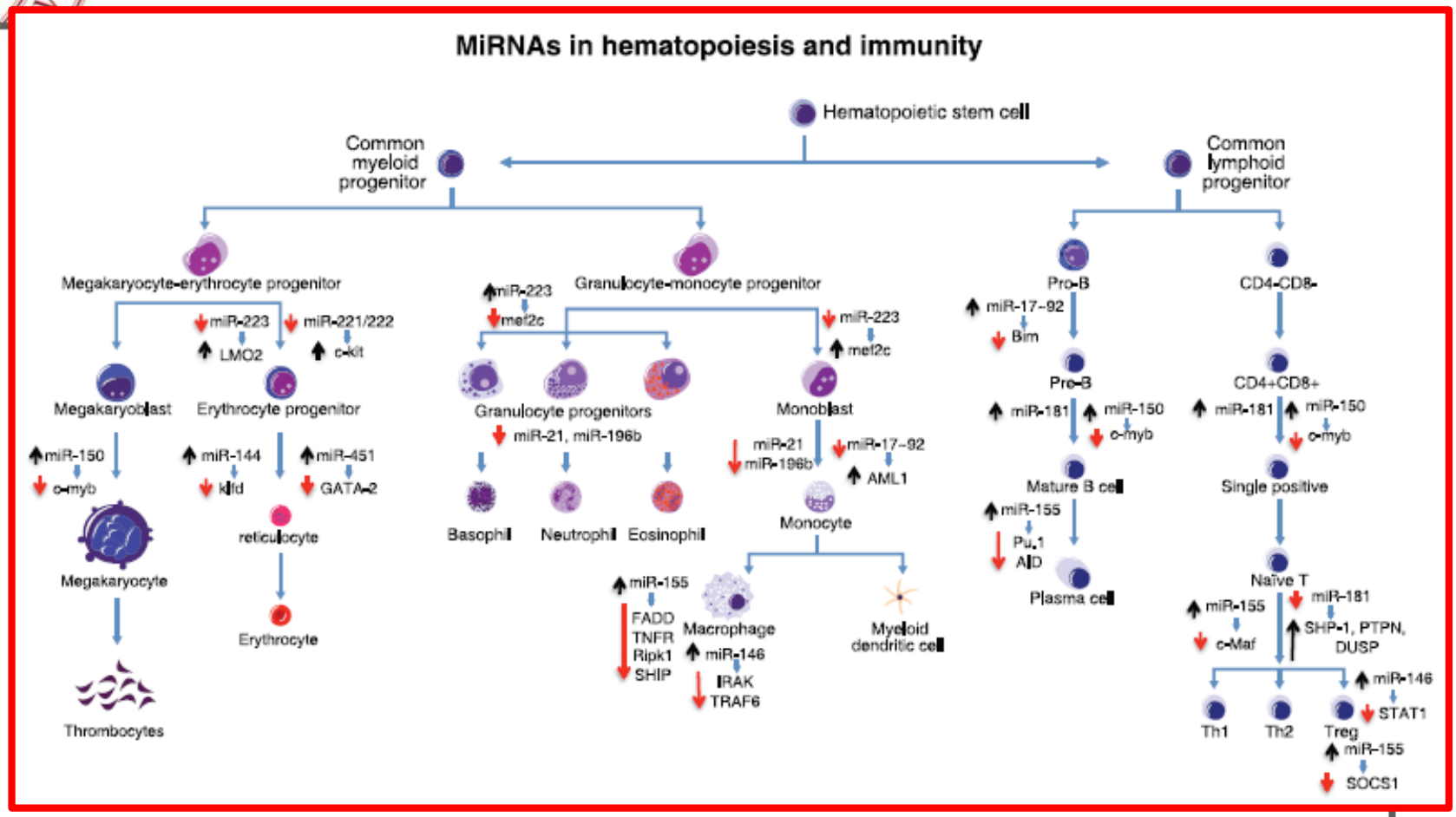
ncRNA: therapy

miRNA as diagnostic biomarker

Sometimes miRNA expression pattern is able to identify the origin of neoplastic formations scarcely differentiated (differently from mRNA encoding protein).

The expression pattern of more than 200 miRNA (in the plasma, salive, tissues) can help in the early diagnosis of hidden tumors.

miRNA: functions

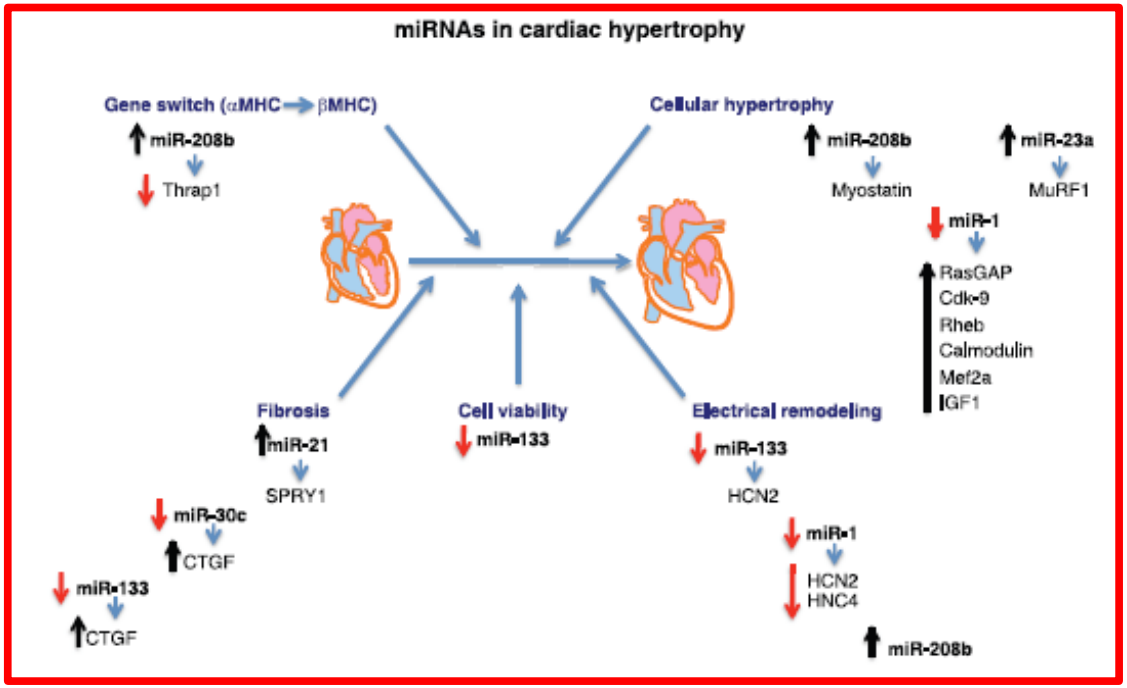


miRNA: diseases

. Alzheimer: ↓ miR-29b → ↑ BACE1 → ↑ βamiloid plaques

. Parkinson: absence of miR-133 → ↑ apoptosi DN, ↓ miR-7 → ↑ α-sinucleine

. Cardiac hypertrophy



. HIV: interaction Tat_{viral}-$Dicer_{human}$ → ↓ miR-N367→ ↑ virulence, ↓ miR-Tar 3p and miR-Tar 5p→ viral transcription active, ↓ miR-17-92 → ↑ viral replication.

miRNA: therapy option

Prevention:

. miRNA as biomarker

Terapy:

. By introducing miRNA under expressed

. By inhibiting overexpression of miRNA

Problems:

. Low stability *in vivo*

. targeting

. Cell entrance

. Well knowledge of miR action both utilized/supressed

ncRNA: therapy

miRNA come therapeutic targets

RNA molecule can be used therapeutically,
By mimiking or regulating the activity of miRNA in tumors

- ✓ by adding miRNA under expressed
- ✓ by inhibiting miRNA over expression

… it would be easier and more useful to repair the regulation software (i.e. ncRNAs) than tray to correct the hardware (i.e. protein-coding genes).