A primer in epigenetics

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Chair in Myocardial Genetics

Epigenetics

- Definition:

  Epigenetic phenomena are defined as heritable mechanisms that establish and maintain mitotically stable patterns of gene expression without modifying the base sequence of DNA.

Epigenetics

- The major epigenetic features of mammalian cells include DNA methylation, post-translational histone modifications and RNA-based mechanisms including those controlled by small non-coding RNAs (miRNAs).

Why study epigenetics?

- Despite the large amount of information gained in recent years defining the association between a vast number of genetic *loci* and chronic human diseases, the mechanisms through which the information enclosed in our genome becomes phenotypically expressed in physiologic and pathologic conditions is not yet fully understood.

Why study epigenetics?

- The impact of epigenetic mechanisms in cardiovascular pathophysiology is now emerging as a major player in the interface between genotype to phenotype variability.
- Examples:

  A genetic modification may have different effects depending on what “epigenetic” background exists. This may also help to explain incomplete penetrance of disease genes.

Dutch Hunger Winter Families Study

- enrolled individuals who were prenatally exposed to famine during the Dutch Hunger Winter, a severe wartime famine in which only 500 calories per day were consumed during the World War II. Preliminary results indicated that an increased CVD risk was associated with famine exposure in early gestation (Painter et al., 2006).

Dutch Hunger Winter Families Study

- A possible involvement of epigenetic dysregulation in the development of CVD has been studied by a Dutch group by evaluating the methylation of the region harbouring the insulin-like growth factor II (*IGF2*) in DNA extracted from whole blood (Heijmans et al., 2008). The *IGF2* gene is very important for human growth and development, and is regulated by maternal imprinting maintained through methylation of *IGF2* differentially methylated region (DMR) (Heijmans et al., 2008).

Dutch Hunger Winter Families Study

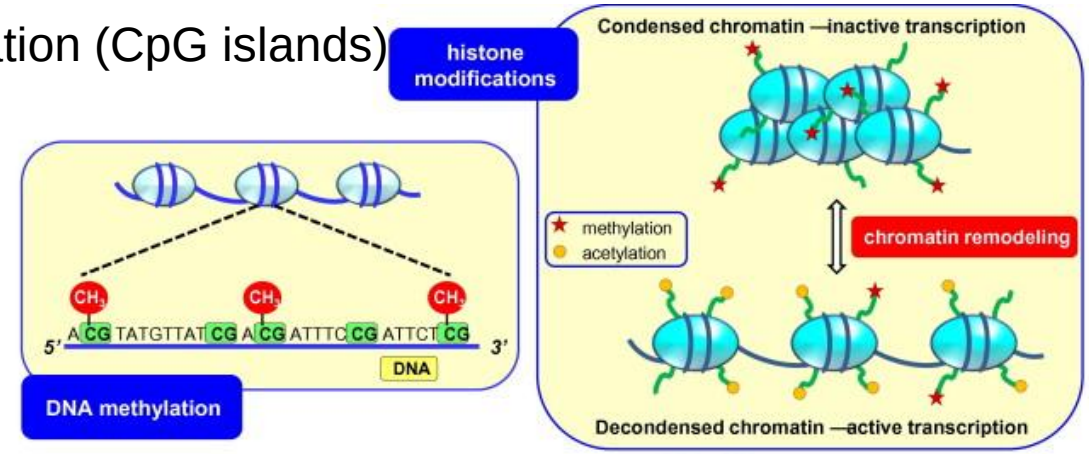
- The *IGF2* DMR was found hypomethylated in individuals exposed to famine, and this epigenetic mark persisted throughout life pointing out the importance of prenatal nutrition on chronic disease risk at later life through methylation of DNA (Heijmans et al., 2008).

Dutch Hunger Winter Families Study

- In individuals periconceptionally exposed to famine, compared to their unexposed same-sex siblings, a link between prenatal nutritional condition and DNA methylation (whole blood) was described by the same group, in genes involved in growth and metabolic disease: *IL10* (interleukin 10), *LEP* (leptin), *ABCA1* (ATP-binding cassette, sub-family A, member 1), *GNASAS* (GNAS antisense RNA 1, non-protein coding) and *MEG3* (maternally expressed 3, non-protein coding) were hypermethylated while locus *INSIGF* was hypomethylated (Tobi et al., 2009).

Principal epigenetic mechanisms of gene expression regulation

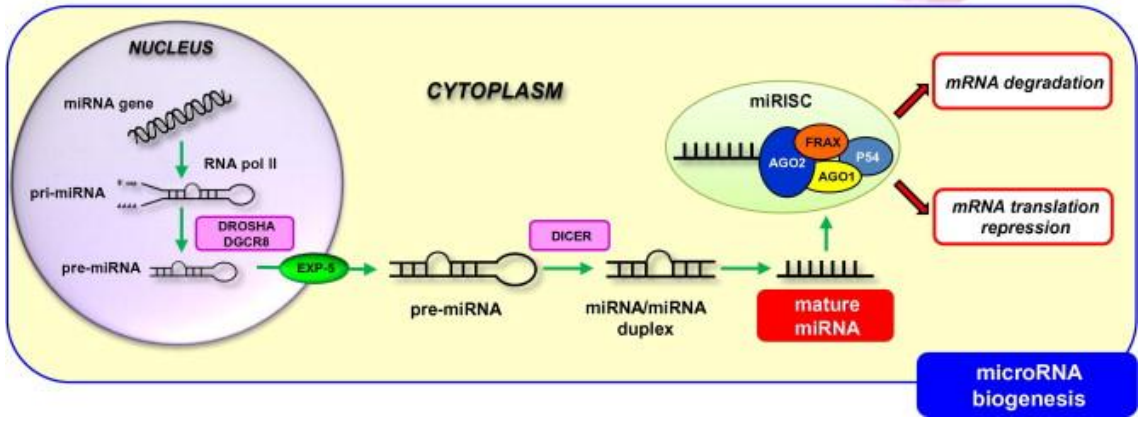
DNA Methylation (CpG islands)



Histone Methylation: transcriptional repression

Histone Acetylation: decondensed transcriptionally active

miRNA



DNA Methylation

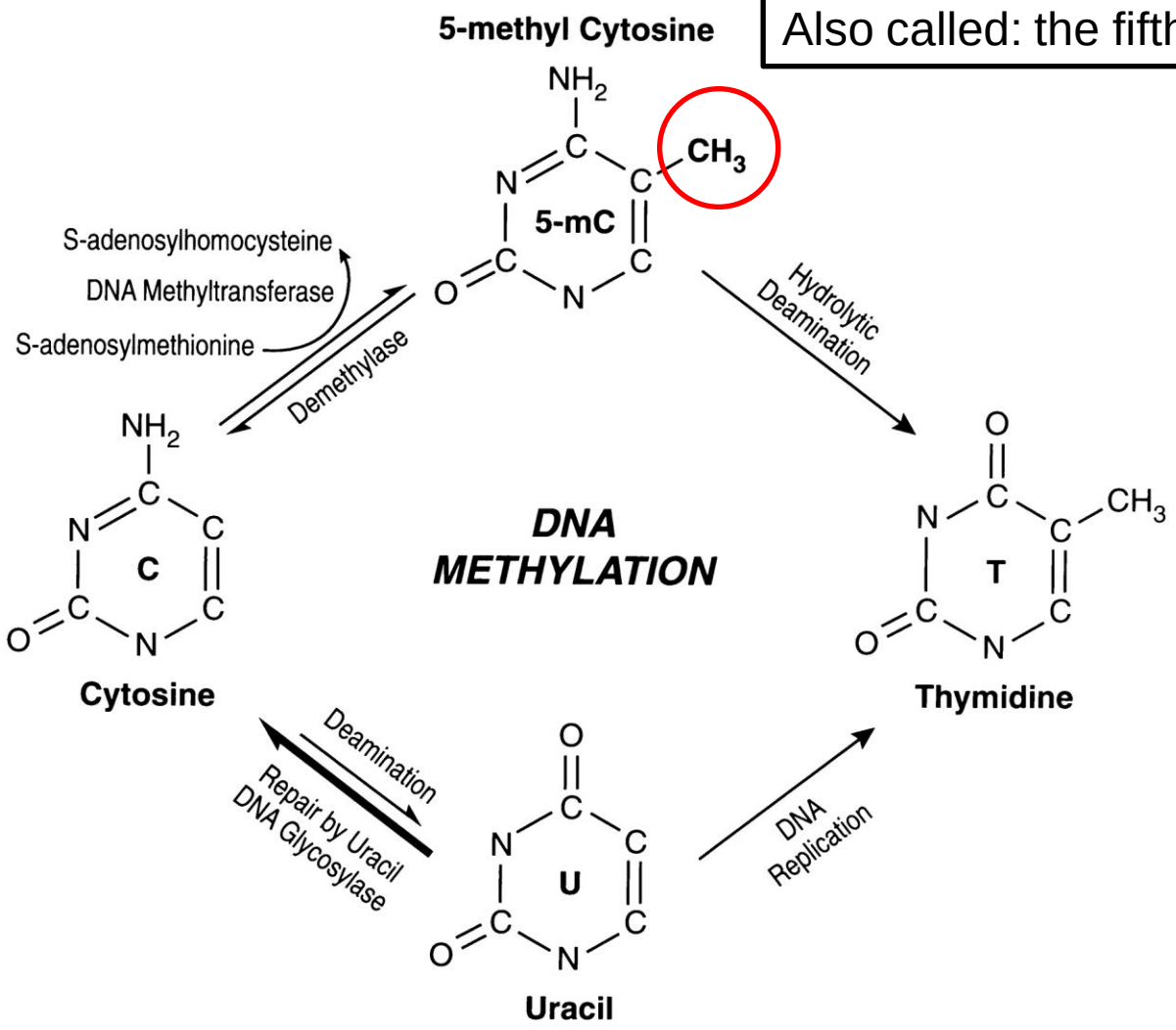
- In general (but not always) it is believed that methylation within the promoter decreases transcriptional activity
- Methylation in exons increases transcriptional activity
- (Methylation may also have effects on gene splicing.)

DNA Methylation

- DNA methylation is a long-term, fairly stable epigenetic modification that consists of the covalent binding of a methyl group to the 5′ carbon of cytosine mostly occurring at CpG dinucleotide sequences in the mammalian genome.

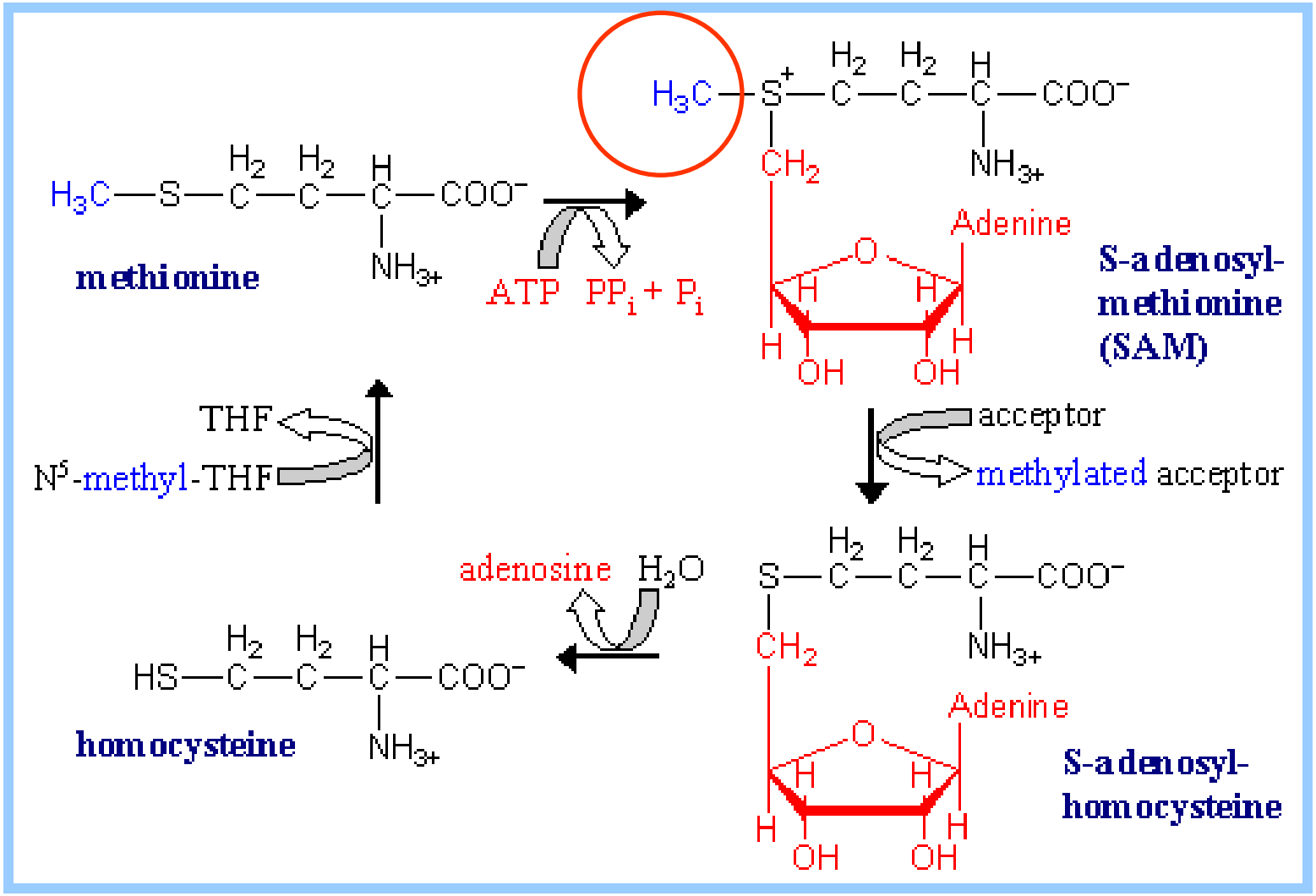
Schematic representation of the biochemical pathways for cytosine methylation, demethylation, and mutagenesis of cytosine and 5-mC.

Also called: the fifth nucleotide



Singal R , Ginder G D Blood 1999;93:4059-4070

S-Adenosylmethionine



DNA Methylation

- DNA methylation is achieved via de novo methyltransferases.
- DNA de-methylation enzymes exist, though they have not yet been unequivocally identified…

DNA Methylation

- The main function of DNA methylation is to modulate the expression of the genetic information by modifying the accessibility of DNA to the transcriptional machinery. In the cell, DNA methylation plays a crucial role in imprinting, X-chromosome inactivation and development.

DNA Methylation

- Among several pathologic conditions, the role of DNA methylation has been most extensively studied in cancer in terms of aberrant global or gene-specific DNA methylation

DNA Methylation

- Changes in both genome and site-specific DNA methylation have also been described in CVD although their specific function is still to be largely investigated. It is intriguing, in regards to epigenetic patterns, to take into account some fascinating similarities between carcinogenesis and atherosclerosis.

DNA Methylation

- Global DNA hypomethylation has been widely described in carcinogenesis. The atherosclerotic plaque can be compared to neoplastic tissue if considering the change of smooth muscle cells (SMCs) status from quiescence to that of monoclonal proliferation by the overexpression of growth factors genes similar to what occurs in tumor tissue. f disease

DNA Methylation

- Hiltunen and colleagues first reported that in advanced human, mouse and rabbit atherosclerotic lesions a global hypomethylation of DNA was present (Hiltunen et al., 2002).
- Whether global hypomethylation has a role in the development of atherogenesis, or is rather a consequence of proliferation of SMCs, remains to be determined.

DNA Methylation

- A major point pertains to the rather different cell types present in the atherosclerotic lesion, which may each have a unique DNA methylation pattern.
- In a mouse model, global DNA methylation was evaluated at early stages of atherosclerosis by comparing atherosclerosis-prone Apoe−/− mice with C57BL/6 control mice (Lund et al., 2004).

DNA Methylation

- Interestingly, in this rodent model, a modest but significant global hypomethylation status was observed in aortic samples, and this condition preceded any histological evidence of atherosclerosis, therefore leading to the hypothesis that not only in cancer disease but also in atherosclerosis, aberrations in DNA methylation may prelude to the clear manifestation of disease.

Cardiovascular diseases and epigenetics

- Atherosclerosis and coronary artery disease (CAD), which can result in myocardial infarction (MI)
- Arterial hypertension
- Heart failure
- Cerebrovascular stroke (ischemic or hemorrhagic)

How to analyze DNA methylation?

- To begin with: not trivial!
- Methylation sensitive restriction enzymes have been used in the past (of course, not precise).
- Some studies analyze certain loci which are thought to be representative for the whole genome.
- Bisulfite sequencing most accurate – but technically challenging.

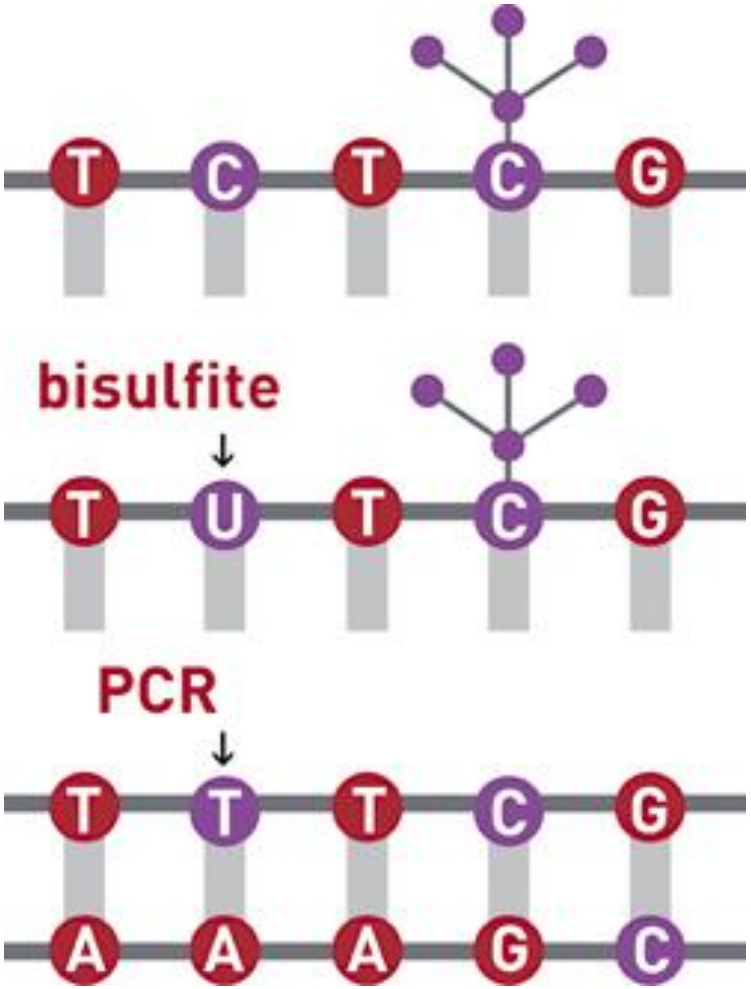
Bisulfite conversion

DNA methylation was the first discovered epigenetic mark and is the most widely studied topic in Epigenetics today. Many techniques have been developed to analyze DNA methylation, which can be divided into three groups:

- Chemical modification with bisulfite
- Affinity-based isolation of methylated DNA
- Treatment with methylation-sensitive restriction enzymes

Bisulfite modification of DNA is the most commonly used, "**gold standard**" method for DNA methylation studies. Since bisulfite treatment introduces specific changes in the DNA sequence depending on the methylation status of individual cytosine residues, single nucleotide resolution information can be obtained, the most prominent advantage of this method. Various analyses can be performed on the altered sequence to retrieve this information: bisulfite sequencing, methylation-specific PCR, high resolution melting curve analysis, microarray-based approaches, and next-generation sequencing.

Bisulfite conversion



Coagulation-related genes methylation and coronary artery disease

- Coagulation factor VII (*F7*) has a key role in the coagulation pathway, and elevated concentrations of plasma activated FVII have been associated with increased CAD risk. Different polymorphisms have been described as affecting plasma concentrations of activated coagulation factor VII (FVIIa).

Coagulation-related genes methylation and coronary artery disease

- The role of epigenetics by way of methylation at the *F7* promoter was evaluated in DNA extracted from peripheral blood mononuclear cells (PBMCs) of patients affected by angiografically documented CAD and CAD-free subjects. *F7* promoter hypomethylation correlated with higher plasma concentrations of FVIIa and higher risk for CAD.

Coagulation-related genes methylation and coronary artery disease

- All the subjects were also genotyped for a polymorphism known to influence FVIIa concentrations, the functional −323 *10bp del/ins* at the promoter site, demonstrating the genetic-epigenetic interaction at the *F7* promoter. In fact DNA methylation seemed to account for the altered plasma FVII concentrations only in patients not carrying the insertion in the promoter region (Friso et al., 2012).

Coagulation-related genes methylation and coronary artery disease

- This study also indicates that DNA promoter methylation status of PBMCs can be a maker that predicts the risk of CAD in those carrying a certain genotype (Friso et al., 2012).

DNA methylation

- To cut this point a little bit short here: Many examples of changes in methylation patterns in CVD could be provided here, but with almost no exception, they need to be repeated and confirmed.
- In other words: this is a very young field.

Table 1
Gene-specific DNA methylation in cardiovascular disease.

Gene	DNA methylation	Gene expression	Biological process	Study design	Method	Reference
ALOX15	15-lipoxygenase promoter hypomethylation in atherosclerotic plaque	Upregulated	Lipid oxidation	Human arterial samples: 23 fatty streak, 29 advanced atherosclerotic lesions, 3 controls	Bisulfite sequencing	Hiltunen et al. (2002a,b)
ESR1	Estrogen receptor α promoter hypermethylation in coronary atherosclerotic plaque	Not measured	Cell proliferation control	Human coronary atherosclerotic plaques and normal proximal aorta	Southern blot analysis	Post et al. (1999)
ESR2	Estrogen receptor β promoter hypermethylation in coronary atherosclerotic tissues	Downregulated	Vascular function	Human vascular tissues: plaque and non-plaque regions	MS-PCR COBRA	Kim et al. (2007)
F7	Factor VII promoter hypomethylation in CAD patients *−323 10 bp ins/ins*	Increased protein levels	Blood coagulation	Genomic DNA from blood: 168 CAD, 88 CAD-free subjects	MS-PCR Bisulfite sequencing	Friso et al. (2012)
HSD11B2	11 beta-hydroxysteroid dehydrogenase 2 promoter hypermethylation associated with hypertension	Not measured	Blood pressure control	Genomic DNA from blood: 25 essential hypertensive patients, 32 patients undergoing glucocorticoid treatment	MS-PCR	Friso et al. (2008)
IGF2	Insulin-like growth factor II promoter hypomethylation in famine-exposed individuals	Not measured	Growth, development	Genomic DNA from blood: 60 cases, 60 controls	Mass spectrometry	Heijmans et al. (2008)
INS GNAS-AS1	Insulin and GNAS antisense RNA 1 hypermethylation associated to higher risk of MI in women	Not measured	Glucose metabolism, fetal growth	Genomic DNA from blood: 122 cases, 126 controls	Mass spectrometry	Talens et al. (2011)
MCT3	Monocarboxylate transporter promoter hypermethylation in cultured SMCs isolated from human coronary arteries and aortas	Downregulated	Mediates metabolic processes in vascular cells	Genomic DNA from SMCs isolated from 12 coronary arteries and 23 aortas, 10 controls	Bisulfite sequencing	Zhu et al. (2005)
Nr1h3	Liver X-receptor α promoter hypermethylation in protein-restricted pups	Downregulated	Cholesterol and fatty acid metabolism	Pregnant C57BL/6J mice exposed to low-protein diet; DNA extracted from fetal livers: 5 cases, 5 controls	DMH	van Straten et al. (2010)
PECAM1	Platelet/endothelial cell adhesion molecule 1 promoter hypermethylation in human heart failure	Downregulated	Angiogenesis	Human left ventricular tissues: 8 end-stage heart failure, 6 controls	MeDIP-Chip	Movassagh et al. (2010)
ARHGAP24	Hypermethylation within the gene body of Rho GTPase activating protein 24 in human heart failure	Upregulated				
AMOTL2	Hypomethylation within the gene body of angiomotin like 2 in human heart failure	Downregulated				
Ppara Nr3c1	Peroxisome proliferator-activated receptor α (*Ppara*) and Glucocorticoid receptor (*Nr3c1*) promoter hypomethylation in protein-restricted pups	Upregulated	*Ppara*: lipid and carbohydrate homeostasis *Nr3c1*: blood pressure	Pregnant Wistar rats fed with low-protein diet: 5 cases, 5 controls; genomic DNA extracted from offspring livers: 10 cases, 10 controls	Methylation-sensitive PCR	Lillycrop et al. (2005)
sAce	Somatic angiotensin-converting enzyme promoter hypermethylation associated with transcriptional repression	Downregulated	Blood pressure control	Human epithelial cell lines and Wistar Kyoto rats	Bisulfite sequencing	Riviere et al. (2011)
Slc12a2	Na^+–K^+–$2Cl^-$ cotransporter (NKCC1) promoter hypomethylation in spontaneously hypertensive rat	Upregulated	Membrane transport, blood pressure control	Aortae and hearts: spontaneously hypertensive rats, male Wistar Kyoto rats (control)	COBRA Bisulfite sequencing	Lee et al. (2010)

MS-PCR, methylation specific polymerase chain reaction; COBRA, COmbinated Bisulfite Restriction Analysis; CAD, coronary artery disease; MI, myocardial infarction; DMH, differential methylation hybridization; SMCs, smooth muscle cells; MeDIP, methylated DNA immunoprecipitation.

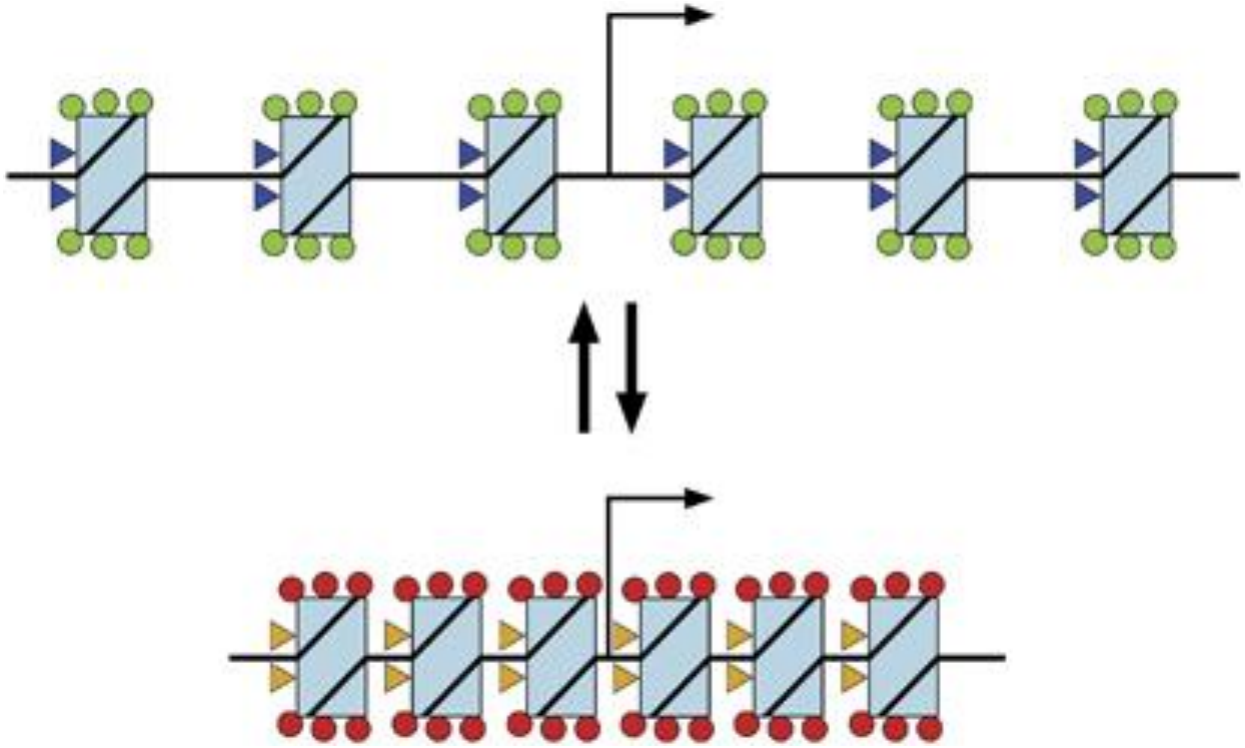
Udali et al., 2012 in press

Overview of miRNAs in acute myocardial infarction, coronary artery disease, and heart failure.

miRNA	Regulation	Study design	Species/No. samples	Source	Method	Reference
Acute myocardial infarction						
miR-21	Downregulated in infarcted area Upregulated in border areas	*In vitro, in vivo* observational	Rat/10	Myocytes	qRT-PCR	Dong et al. (2009)
miR-208	Upregulated (after isoproterenol s.c.)	Observational	Rat/8	Plasma	qRT-PCR	Ji et al. (2009)
miR-1, miR-133a, miR-208a miR-499	Upregulated	Case-control, observational	Human/33 MI, 16 CAD non MI, 17 other CVDs, 30 controls Rat/18	Plasma	qRT-PCR	Wang et al. (2010)
miR-208a miR-499 miR-223	Upregulated 1500–1600 fold Upregulated 90-100 fold Downregulated	Case-control	Human/32 STEMI, 36 controls	Plasma	qRT-PCR	Corsten et al. (2010)
miR-1, miR-133a/b miR-208	Downregulated Upregulated	Case-control	Human/50 MI, 8 controls, 9 fetuses	Heart tissue	qRT-PCR	Bostjancic et al. (2010)
miR-1	Upregulated	Case-control, observational	Human/93 MI (STEMI-NSTEMI), 66 controls	Plasma	qRT-PCR	Ai et al. (2010)
miR-1	Upregulated 200-fold Upregulated 100-fold 6 h after MI	Case control, observational, *in vitro*	Human/31 STEMI, 20 controls Rat/12	Serum, rat myocytes	qRT-PCR	Cheng et al. (2010)
miR-1	Downregulated 2 days post-MI, upregulated 5 days post-MI, elevated up to 90 days post-MI	Case-control, observational,	Human/12 post-MI, 12 controls	Plasma	qRT-PCR	Zile et al. (2011)
miR-21	Downregulated 2 days post-MI, upregulated 5 days post-MI	Prospective				
miR-208a, miR-133a	Upregulated 5 days post-MI, elevated up to 90 days post-MI					
miR-1, miR-133a/b, miR-499-5p miR-122, miR-375	Upregulated 15-140 fold Downregulated	Case-control, observational	Human/33 STEMI, 17 controls Mouse/5	Plasma	qRT-PCR	D'Alessandra et al. (2010)
miR-499	Upregulated 3–6 fold	Case-control, observational	Human/14 ACS (9 MI, 5 UA), 15 HF, 10 controls	Plasma	qRT-PCR	Adachi et al. (2010)
miR-1 miR-133a miR-208b miR-499-5p	Upregulated 300-fold Upregulated 70-fold Upregulated 3000-fold Upregulated 250-fold	Case-control, observational	Human/25 STEMI, 11 controls Pig/6	Plasma	qRT-PCR	Gidlof et al. (2011)
miR-1, miR-133a/b, miR-208b	Upregulated in STEMI-NSTEMI vs UA	Prospective	Human/444 ACS (196 STEMI, 131 NSTEMI, 117 UA)	Plasma	qRT-PCR	Widera et al. (2011)
miR-208b miR-499	Upregulated 5×10^5-fold Upregulated 3×10^5-fold	Case-control, prosepective	Human/510 MI (397 STEMI, 113 NSTEMI), 87 controls	Plasma	qRT-PCR	Devaux et al. (2012)
miR-1, miR-21, miR-133a, miR-423-5p	Upregulated 3- to 10-fold	Case-control	Human/92 NSTEMI, 81 acute HF, 99 controls	Plasma	qRT_PCR	Olivieri et al. (2012)
miR-663b, miR-1291 miR-30c, miR-145	Downregulated Upregulated	Case-control	Human/20 STEMI, 20 controls	PBMCs	Array	Meder et al. (2011)
miR-328 miR-328	Upregulated 10.9-fold (plasma) Upregulated 16.1-fold (whole blood)	Case-control, observational	Human/51 MI, 28 controls	Plasma, whole blood	qRT-PCR	Wang et al., 2011
miR-133	Upregulated 4.4-fold (plasma/whole blood)					

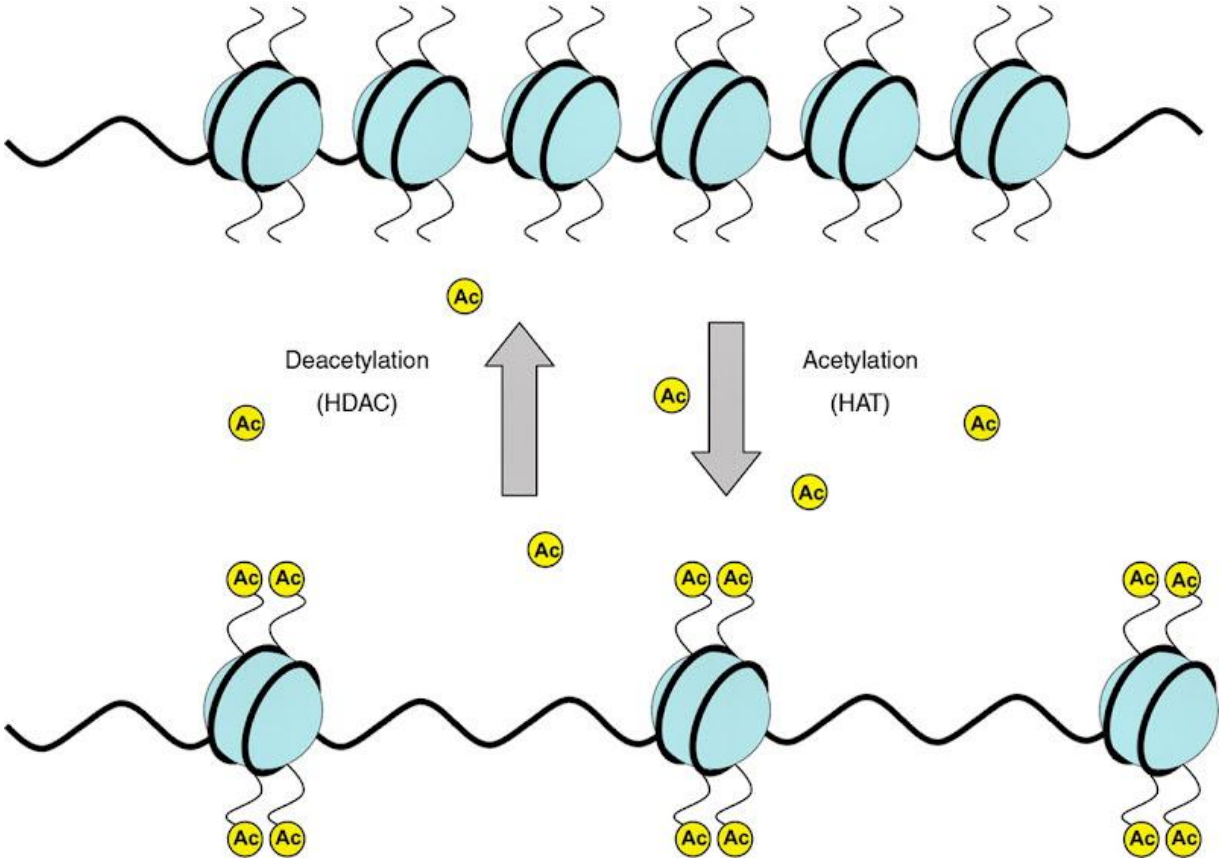
Udali et al., 2012 in press

Histones



Open and closed chromatin configurations are influenced by post-translational histone modifications. In the upper panel, DNA is wrapped around histones that possess activating modifications (green circles and blue triangles). In the lower panel, DNA is wrapped around histones with repressing modifications (red circles and orange triangles). The bent arrow indicates a transcription start site; this is more accessible to RNA polymerase in the open chromatin configuration.

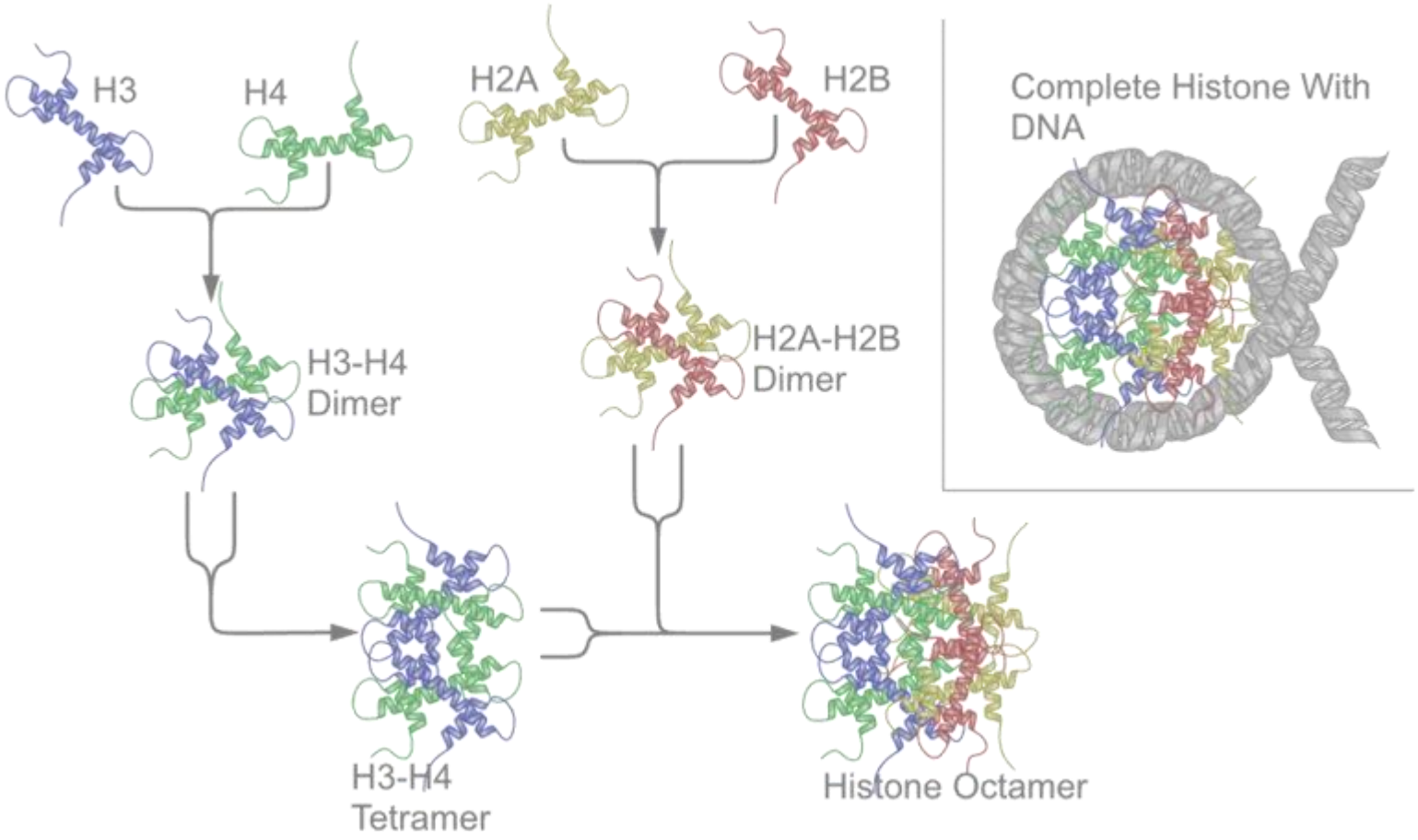
Histones


Ac
Deacetylation
(HDAC)
Acetylation
(HAT)
Ac
Ac
Ac
Ac
Ac
Ac
Ac
Ac
Ac
Ac
Ac
Ac
Ac
Ac

Histones

- highly alkaline proteins found in eukaryotic cell nuclei that package and order the DNA into structural units called nucleosomes. They are the chief protein components of chromatin, acting as spools around which DNA winds, and play a role in gene regulation. Without histones, the unwound DNA in chromosomes would be very long (a length to width ratio of more than 10 million to one in human DNA).
- For example, each human cell has about 1.8 meters of DNA, but wound on the histones it has about 90 micrometers (0.09 mm) of chromatin, which, when duplicated and condensed during mitosis, result in about 120 micrometers of chromosomes.

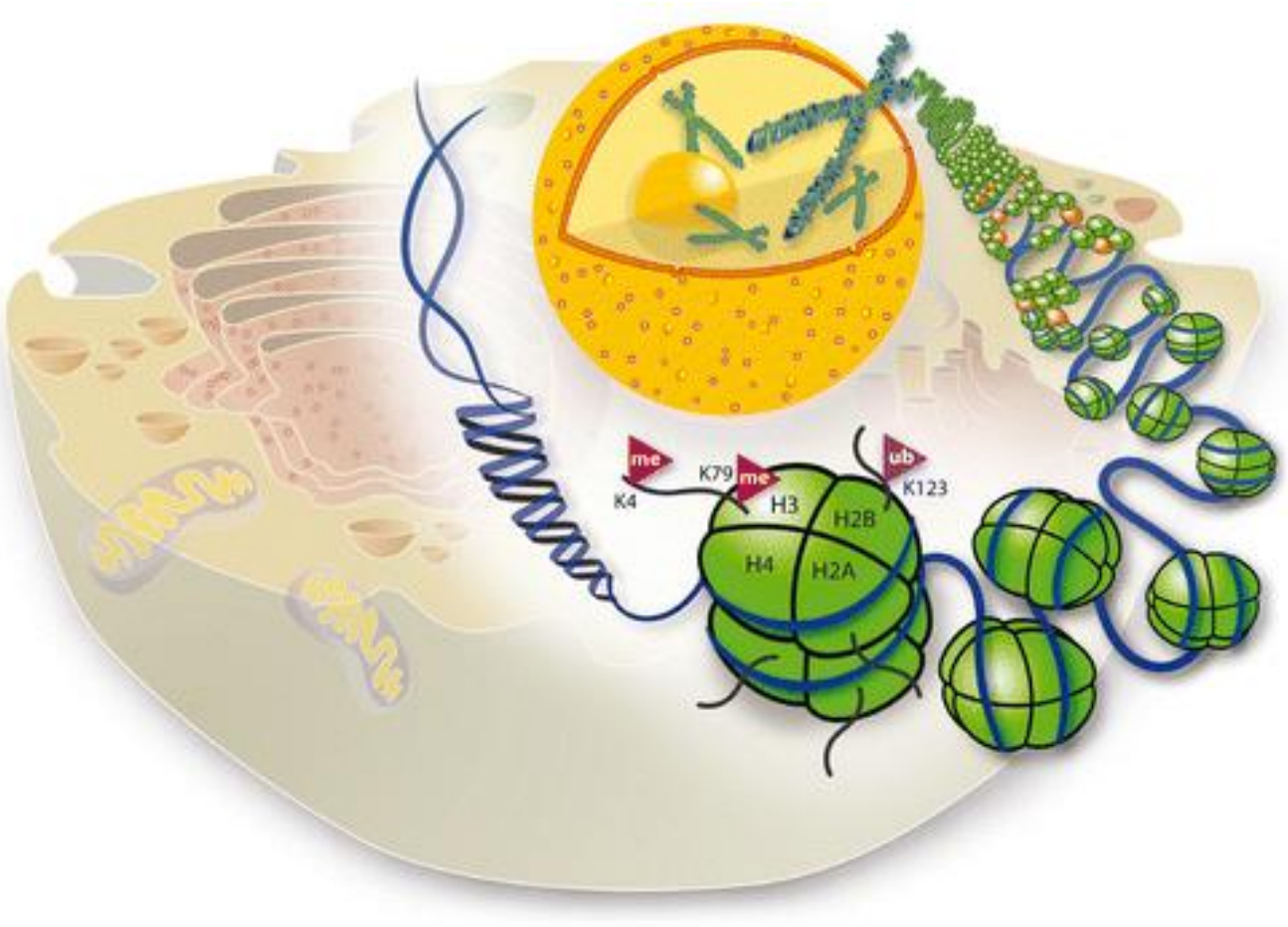
Histones


H3
H4
H3-H4
Dimer
H2A
H2B
H2A-H2B
Dimer
H3-H4
Tetramer
Histone Octamer
Complete Histone With
DNA

Histones

- Five major families of histones exist: H1/H5, H2A, H2B, H3, and H4. Histones H2A, H2B, H3 and H4 are known as the core histones, while histones H1 and H5 are known as the linker histones.

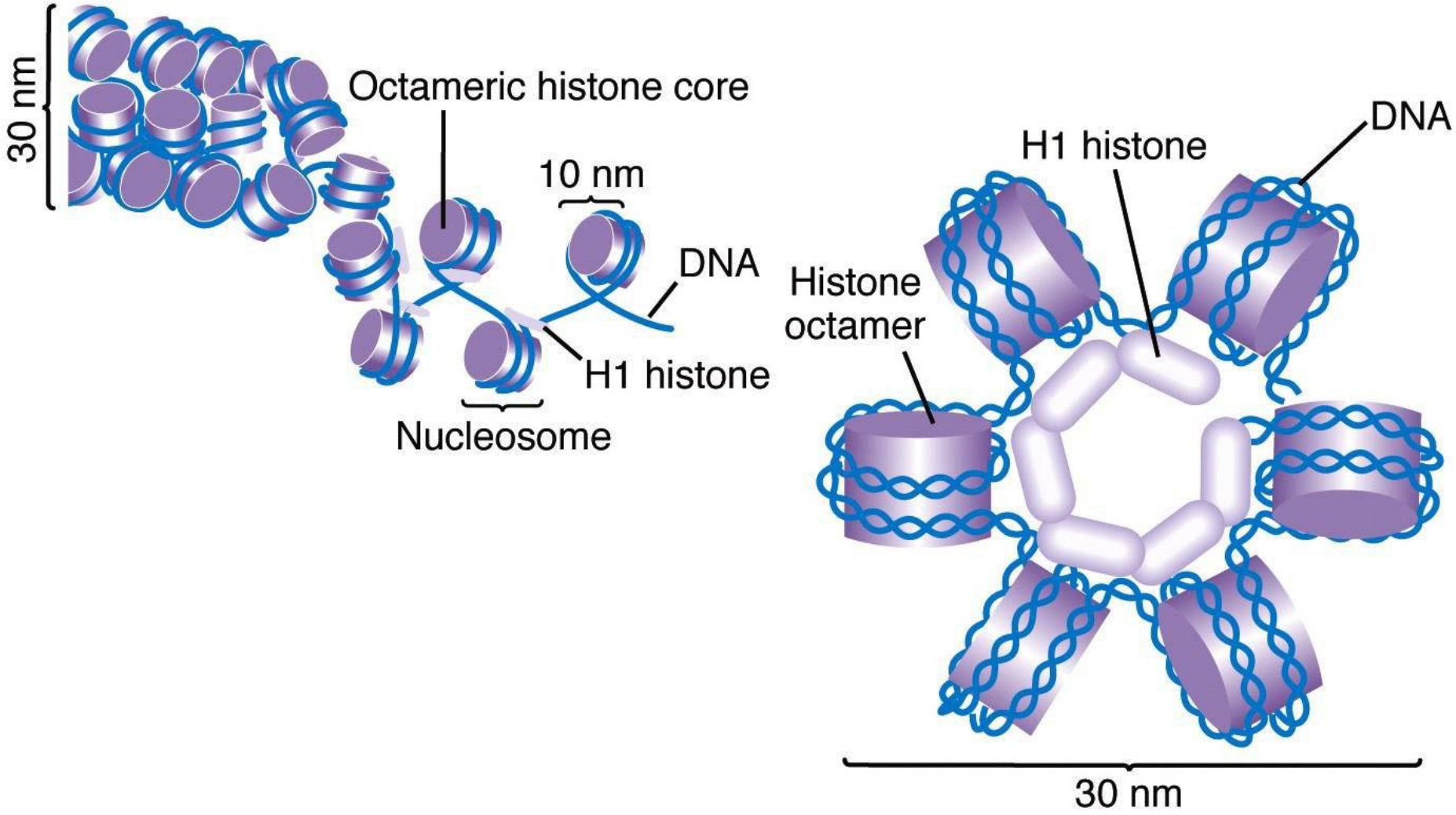
Chromatin, histone modifications, and gene expression



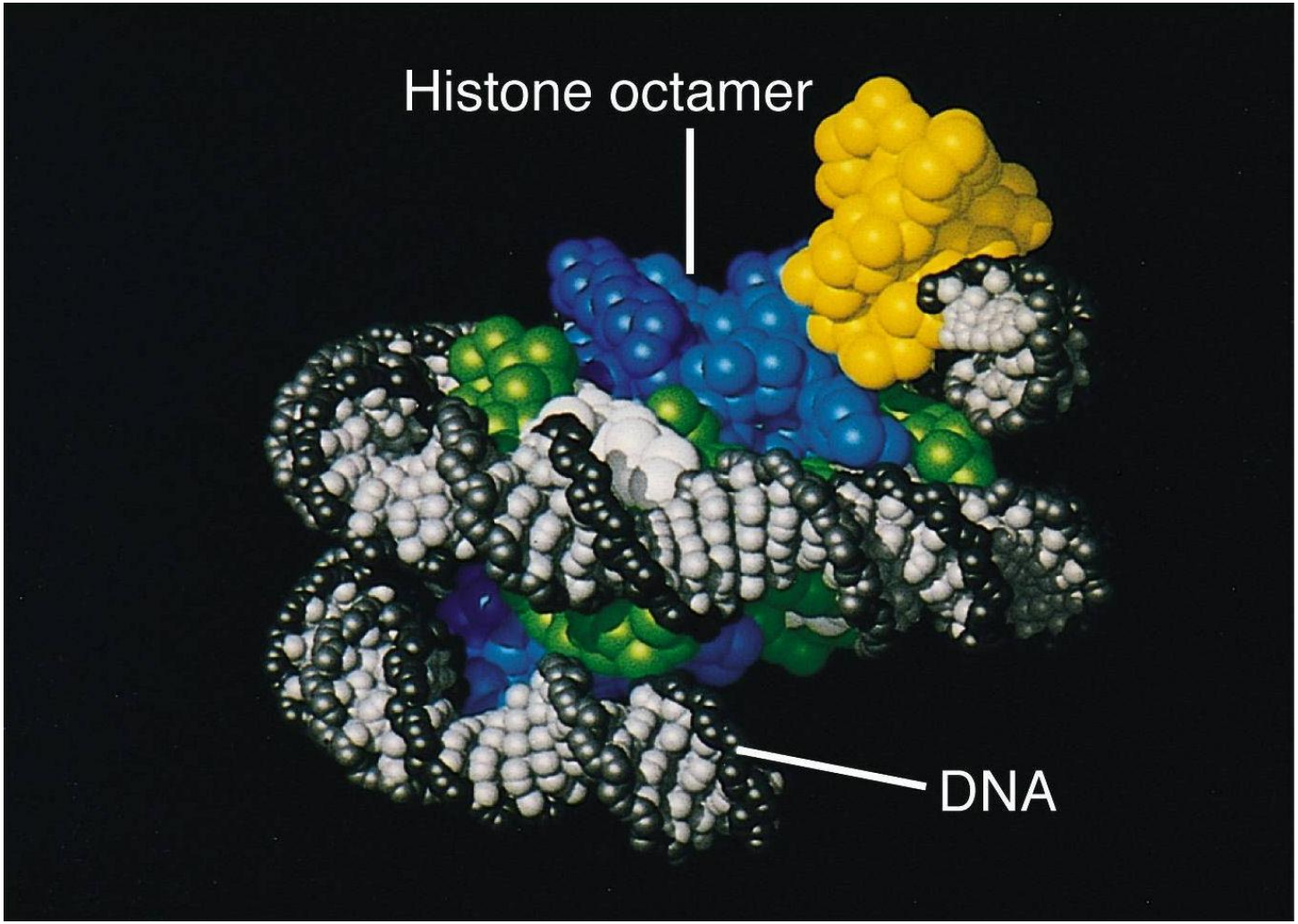
Shilatifard A. 2012.
Annu. Rev. Biochem. 81:65–95

In this figure, sites of posttranslational modifications on histone tails for histone H3 lysine 4 (H3K4) methylation and histone H2B monoubiquitination by the COMPASS family of H3K4 methylases and the Rad6/Bre1 complex, respectively, are shown. Abbreviations: me, methylation; ub, ubiquitin.

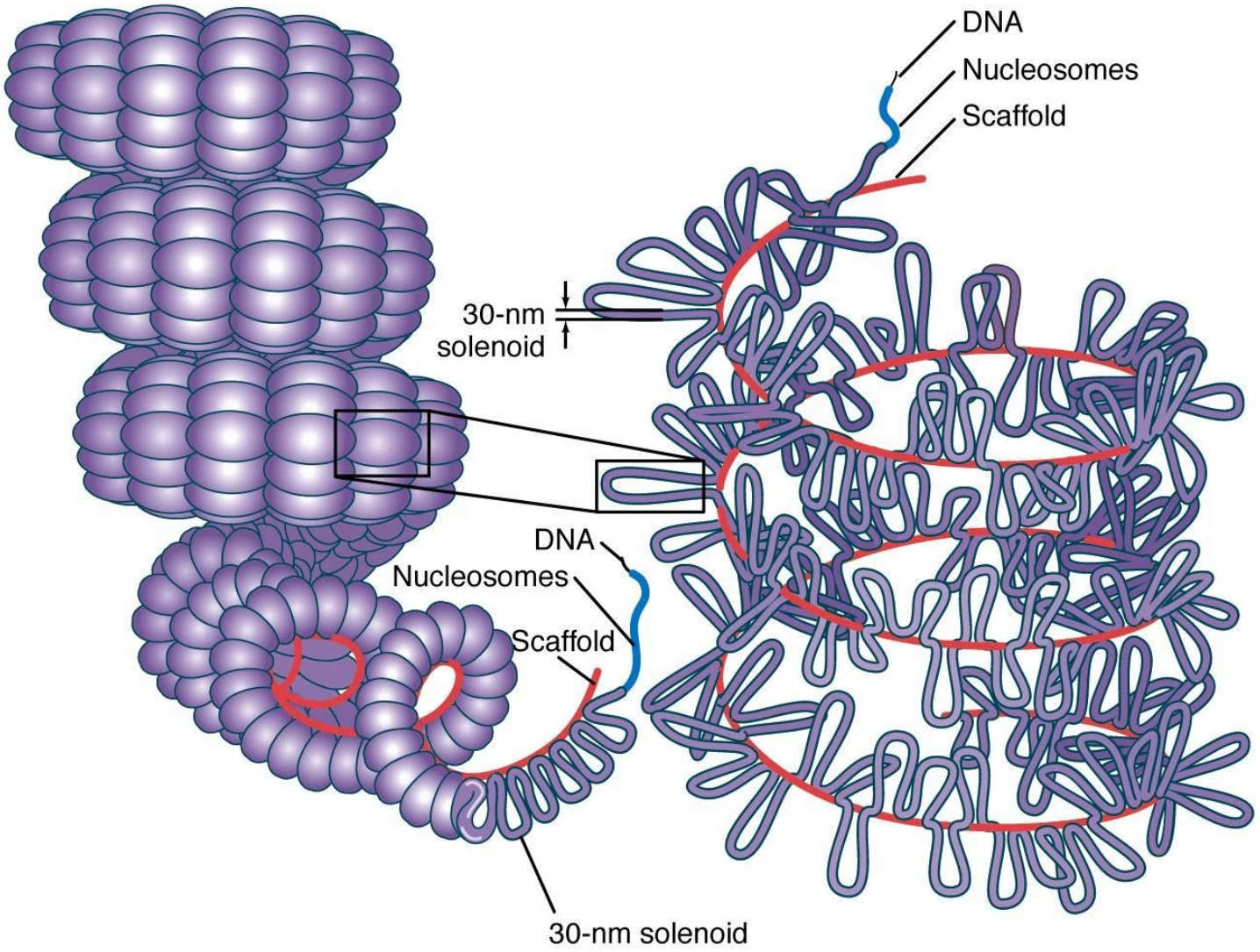
Histones


30 nm
Octameric histone core
10 nm
DNA
H1 histone
Nucleosome
DNA
H1 histone
Histone
octamer
30 nm

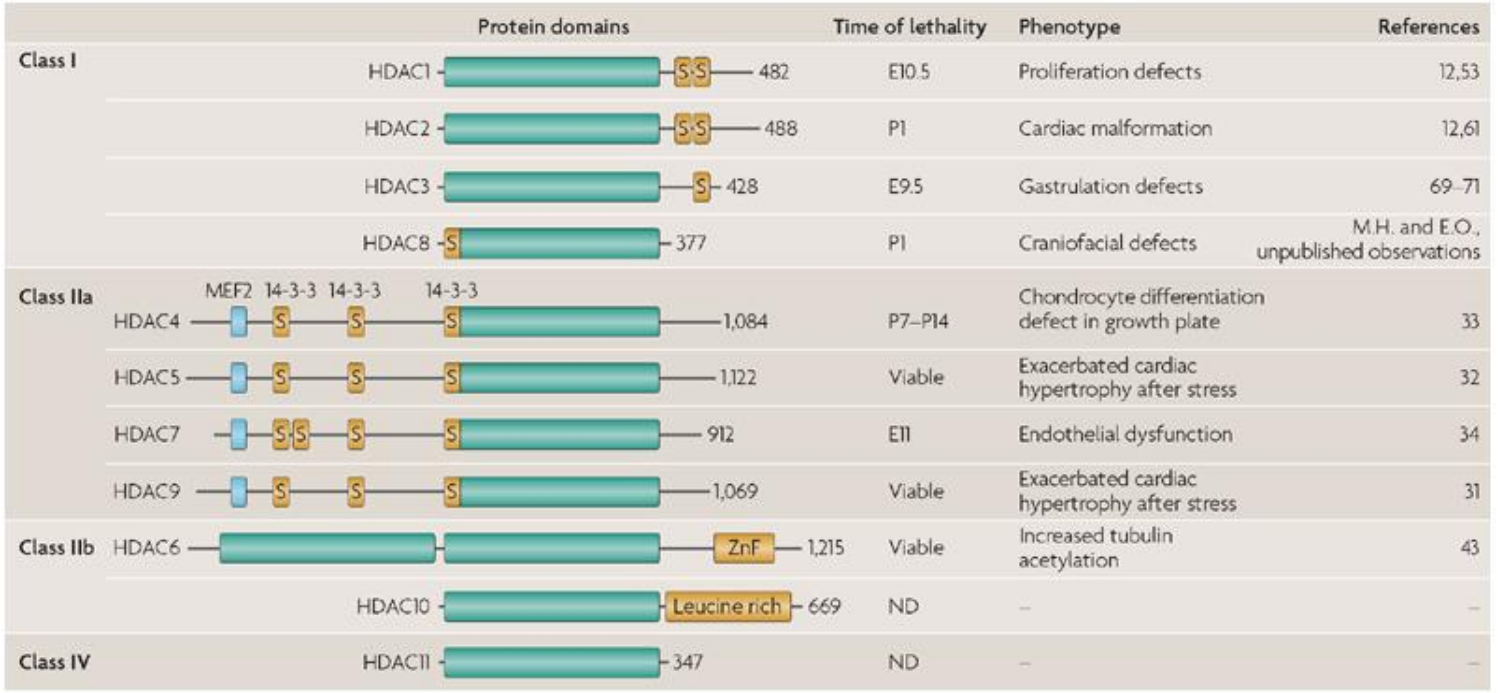
Histones



Histones

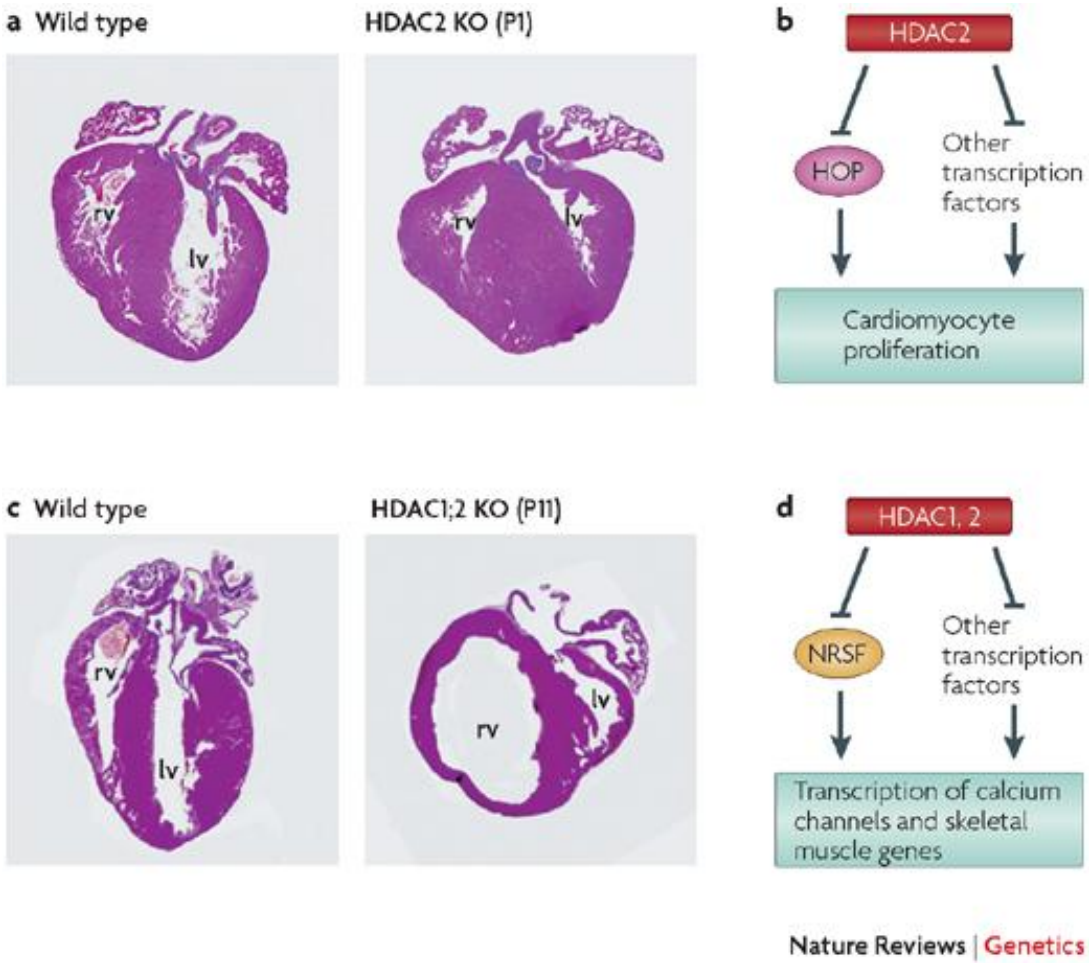

DNA
Nucleosomes
Scaffold
30-nm
solenoid
DNA
Nucleosomes
Scaffold
30-nm solenoid

Histones in the heart



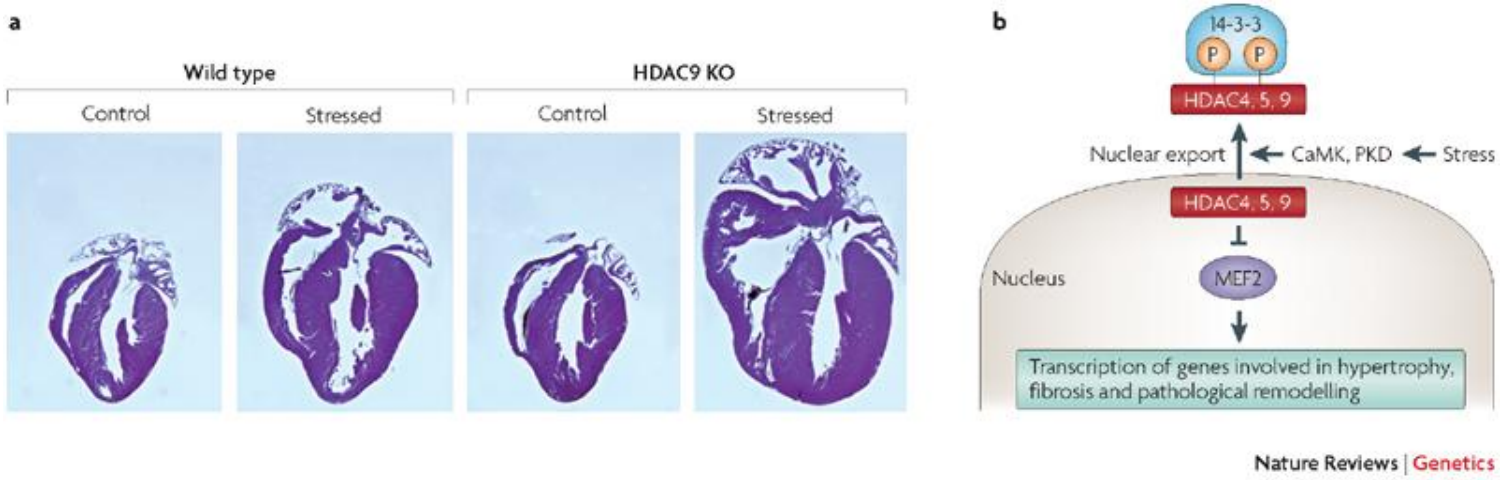
Green rectangles indicate the conserved HDAC domain; numbers following the HDAC domain indicate the number of amino acids. Myocyte enhancer factor 2 (MEF2)-binding sites are marked by a blue square, and binding sites for the 14-3-3 chaperone protein are also shown. E, embryonic day; ND, not determined; P, days postnatal; S, serine phosphorylation sites; ZnF, zinc finger.

Histones and the heart



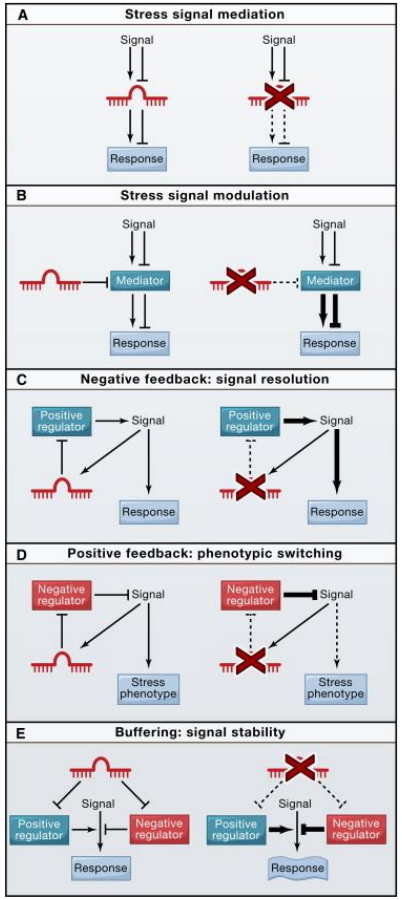
a | Histological sections of hearts from wild type and HDAC2 knockout (KO) mice at postnatal day 1 (P1). Note the excessive number of cardiomyocytes in the mutant heart, which fill the chambers of the left ventricle (lv) and right ventricle (rv). b | Schematic of the role of HDAC2 in the repression of cardiomyocyte proliferation through inhibition of homeodomain-only protein (HOP). c | Histological sections of hearts from wild-type mice and mice with a cardiac deletion of HDAC1 and 2 at P11. Note the dilatation of the right ventricle in the mutant, which is indicative of heart failure. d | Schematic of the redundant roles of HDAC1 and 2 in regulation of calcium channel and skeletal muscle genes in cardiomyocytes via repression of neuron-restrictive silencer factor (NRSF) and other transcription factors.

Histones and the heart



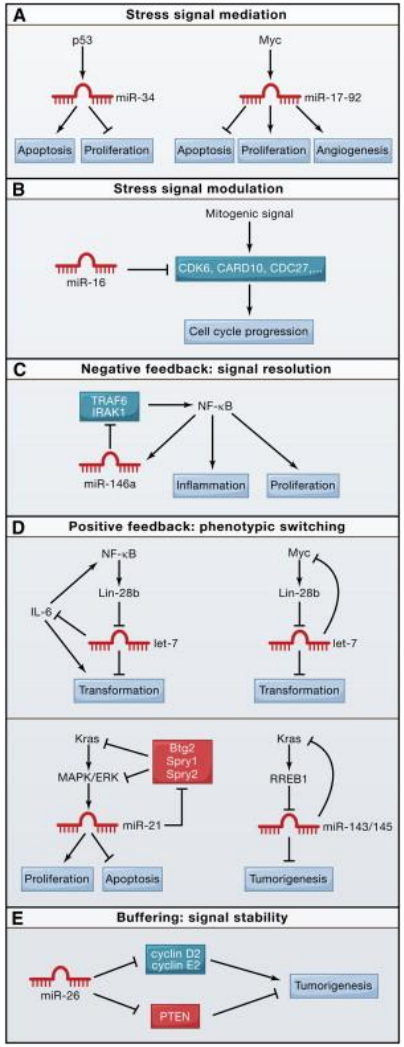
a | Histological sections of hearts from wild-type and HDAC9 knockout (KO) adult mice. Mice were subjected to cardiac stress by expression of a cardiac-specific transgene encoding activated calcineurin, which drives pathological hypertrophy. Note that HDAC9 knockout mice have normal hearts in the absence of stress, but display cardiomegaly in response to stress, owing to loss of the growth-inhibitory function of HDAC9. b | Schematic of the repressive influence of class IIa HDACs on myocyte enhancer factor 2 (MEF2) and pathological cardiac remodelling. Stress-inducible kinases, such as calcium/calmodulin-dependent protein kinase (CaMK) and protein kinase D (PKD), induce the phosphorylation of class IIa HDACs, which creates docking sites for the 14-3-3 chaperone protein, resulting in nuclear export with consequent activation of MEF2 and its downstream target genes, which are involved in cardiac remodelling.

miRNAs



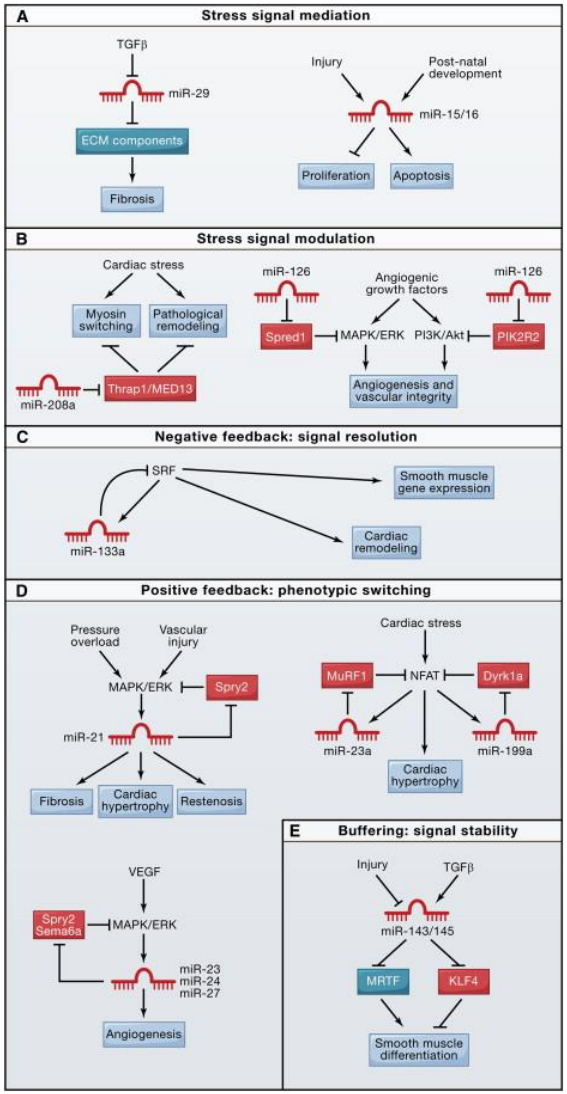
Potential Mechanisms through which miRNAs Regulate Stress Signaling Pathways and the Consequent Effects of miRNA Deletion on Pathway ActivityA miRNA can perform a stress signal mediation function (A) in which it acts as a critical intermediate in a signaling pathway or it may act as a stress signal modulator (B) in which it titrates a signaling intermediate. A miRNA may participate in a negative (C) or positive (D) feedback loop that serves to dampen or amplify a signal, respectively. Lastly, a miRNA may target both positive and negative regulators of a pathway (E), thereby buffering pathway activity from stochastic fluctuation. For each mode of regulation, the consequences of deletion of the miRNA on pathway activity are schematized on the right.

miRNAs

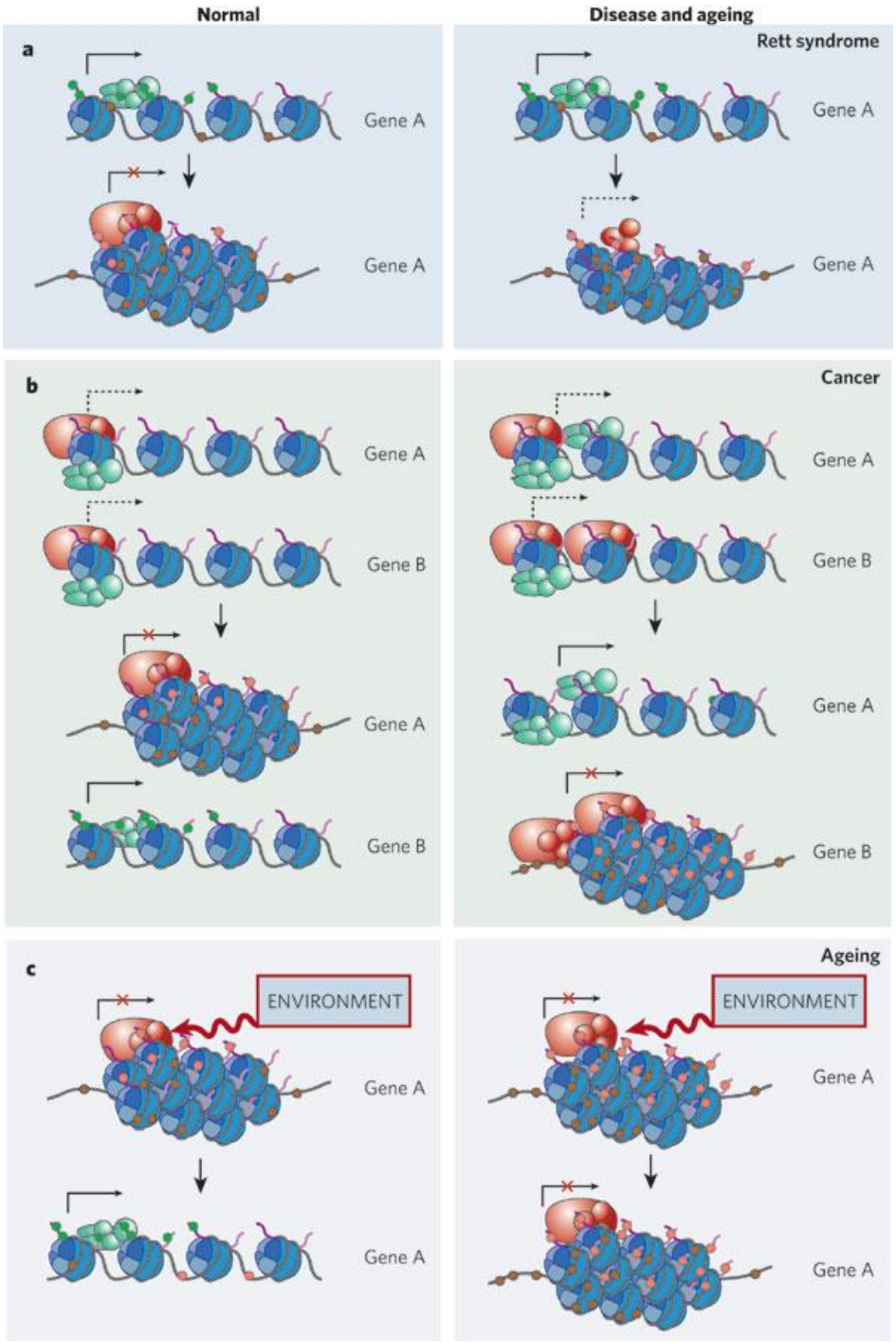


miRNAs that Promote or Inhibit Tumorigenesis Provide Diverse Functions within Oncogenic and Tumor Suppressor Signaling Pathways(A) miR-34 family members and the miR-17-92 cluster function as signal mediators for the p53 and Myc pathways, respectively.(B) miR-16 negatively regulates multiple components of mitogenic pathways and thereby provides an inhibitory signal modulation function.(C) miR-146a is activated by NF-κB signaling and negatively feeds back on the pathway by repressing upstream activators of NF-κB. In this capacity, miR-146a restrains excessive NF-κB activity, which can lead to tumorigenesis.(D) Let-7, miR-21, and the miR-143/145 cluster participate in positive feedback loops that function to stably enforce cellular transformation programs upon activation of oncogenes such as NF-κB, *KRAS*, and *MYC*.(E) miR-26 can repress both pro-tumorigenic targets (cyclins D2 and E2) and antitumorigenic targets (PTEN). These opposing activities endow miR-26 with context-dependent positive or negative effects on tumorigenesis.

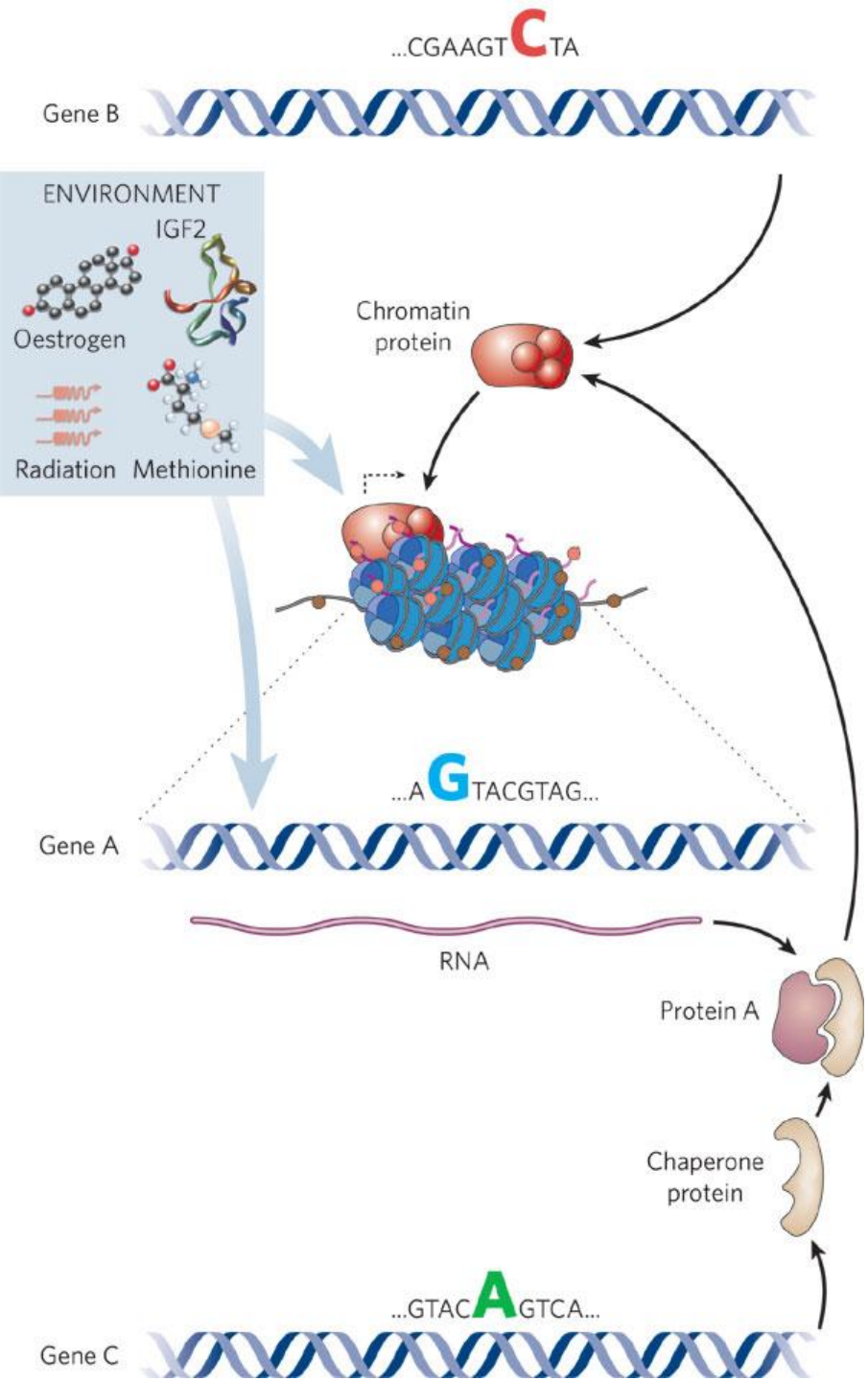
miRNAs



Examples of miRNAs that Function in Cardiac Stress Signaling Pathways(A) miR-29 and miR-15 family members act as mediators of stress signaling pathways that regulate fibrosis and cardiomyocyte proliferation and survival, respectively.(B) miR-208a and miR-126 titrate regulators of cardiac remodeling and angiogenesis and thereby function as stress signal modulators.(C) miR-133a directly targets its activator SRF and in this manner restrains excessive SRF activity in adult cardiomyocytes, which can lead to heart failure.(D) miR-21, miR-199a, and the miR-23a/27a/24-2 cluster participate in positive feedback loops, which serve to stably activate signaling pathways that lead to pathologic cardiac remodeling and angiogenesis.(E) The miR-143/145 cluster targets both positive and negative regulators of smooth muscle differentiation. Through this buffering activity, these miRNAs maintain the characteristic phenotypic plasticity of this cell type, allowing smooth muscle cells to proliferate in response to injury.



A common feature of epigenetic lesions in human disease is that they affect a cell's ability to change its phenotype. **a**, In monogenic disorders such as Rett syndrome, a defect in the normal epigenetic apparatus itself impedes normal development. DNA methylation (brown circles on the DNA) proceeds normally but is not recognized owing to the absence of the MeCP2-methylation-interacting protein (large red oval). This leads to failure to completely silence genes appropriately during development (dashed arrow). **b**, Cancer involves many epigenetic lesions that could affect a pluripotent programme in tissue-specific stem cells, possibly leading to an incorrect distribution of differentiated cell lineages (indicated by the bivalent euchromatin and heterochromatin proteins shown in the upper left panel) and normal tissue-specific silencing of gene A and activation of gene B after differentiation (lower left panel). Examples of epigenetic lesions found in cancer include changes in chromatin proteins in stem cells caused by increased expression of *MLL1* in leukaemia (upper right panel, green complex above gene A representing *HOX* genes), leading to aberrant *HOX* expression in differentiated cancer lineages (lower right panel). Another epigenetic lesion found in cancer is increased expression of *EZH2* (upper right panel, red complex above gene B, representing diverse tumour suppressor genes), leading to aberrant silencing of these genes in differentiated cancer lineages (lower right panel). **c**, Ageing involves a loss of the normal plasticity of response to internal and external environmental signals. The epigenome could have an important role in ageing if the aged epigenome is less responsive to such signals. For example, a gene (at this point hypothetically) showing increased H3K9-methylation (upper right panel, red circles on nucleosomes) or DNA methylation (brown circles on DNA), might be relatively refractory to environmentally induced activation (lower right panel) than if the gene had not undergone age-dependent epigenetic change (left panels).



According to the common disease genetic and epigenetic (CDGE) hypothesis, the epigenome may modulate the effect of genetic variation (example shown is the large nucleotide in gene A, which could be C or G), either by affecting the gene's expression through the action of chromatin proteins or DNA methylation, or by modulating protein folding of the gene product of the variant locus or chromatin protein. The epigenome may, in turn, be affected by sequence variation in the genes encoding chromatin or chaperone proteins (genes B and C, respectively). Environmental factors (such as toxins, growth factors, dietary methyl donors and hormones) can affect the genome and the epigenome.