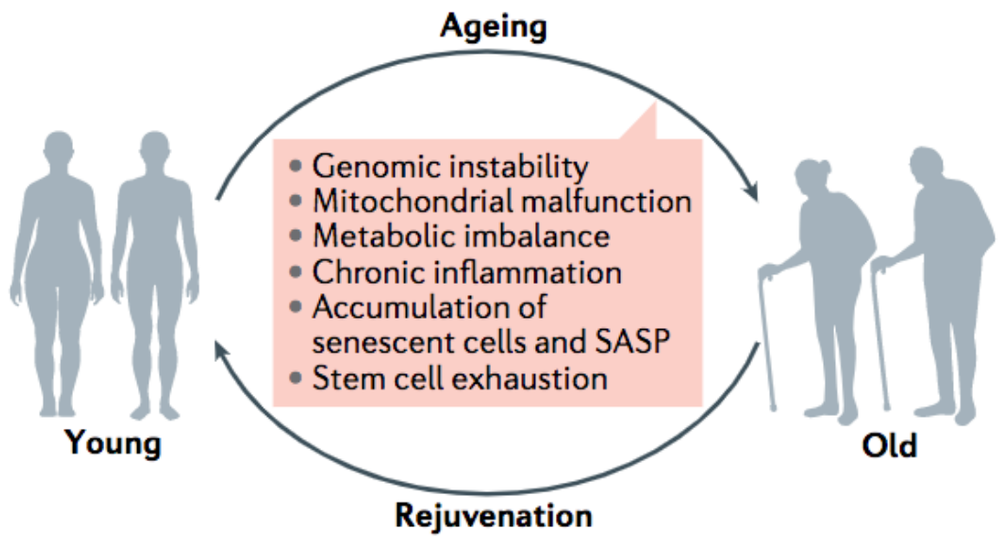


Epigenetics and Aging

Olivia Garry

MacMillan Group Meeting

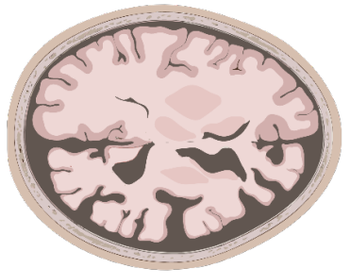
May 13th, 2020

Aging

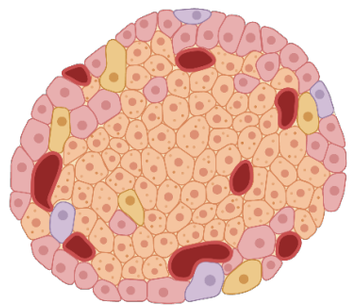
Aging = the time-dependent functional decline that affects most living organisms

Aging is the biggest risk factor for the majority of chronic diseases that increase morbidity or mortality

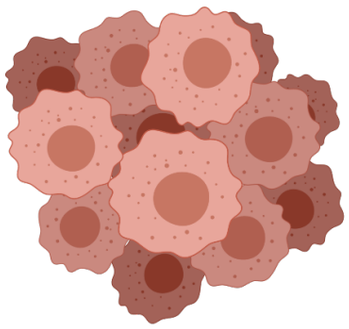
Interventions that extend lifespan may delay or prevent many chronic diseases



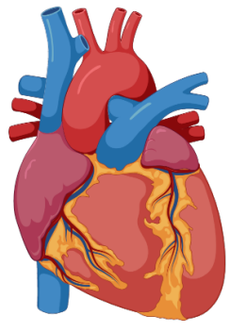
Alzheimer’s



Diabetes



Cancer



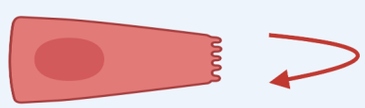
Heart failure

Kennedy, B. K.; Berger, S. L.; Brunet, A.; Campisi, J.; Sierra, F. et al. *Cell* **2014**, *159*, 709

Hallmarks of Aging

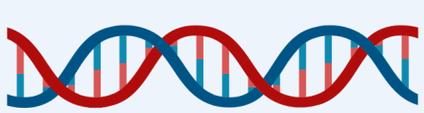
There are **nine hallmarks** that contribute to the aging process and determine the aging phenotype

Deregulated nutrient sensing

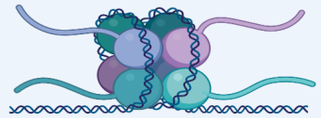


Stem cell exhaustion

Altered intercellular comm.



Genomic instability



Epigenetic alteration

Cellular senescence

Mitochondrial dysfunction

Telomere attrition

Loss of proteostatis

López-Otín, C.; Blasco, M. A.; Partridge, L.; Serrano, M.; Kroemer, G. *Cell* **2013**, *153*, 1194

Outline

Overview of Epigenetics

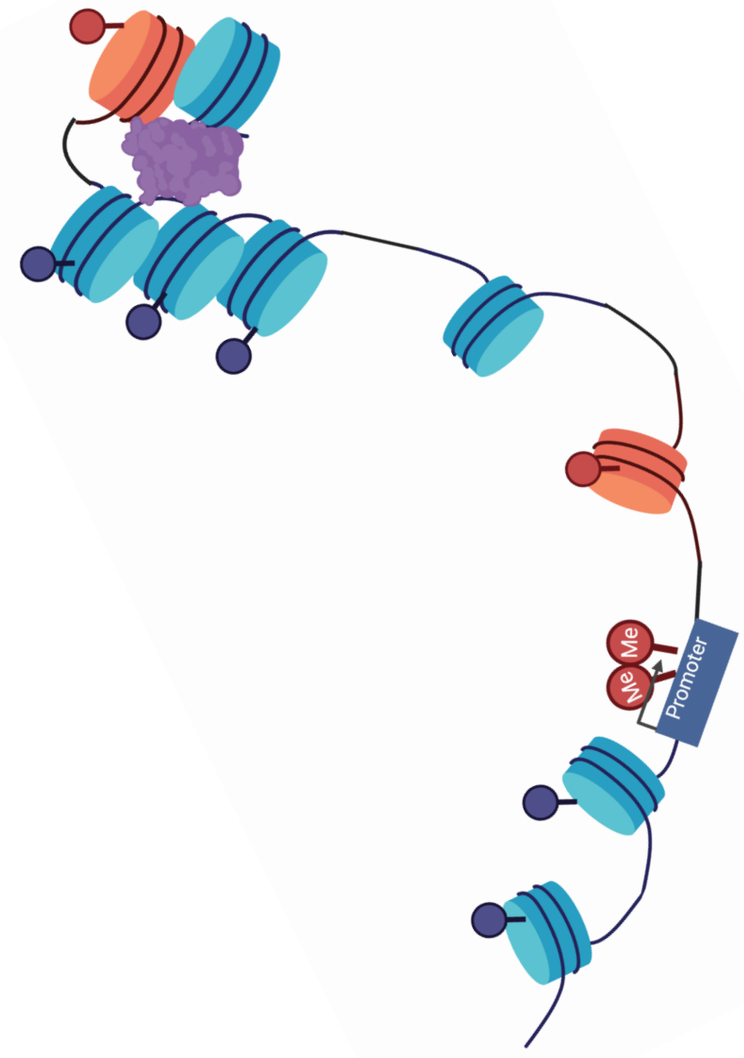
- Chromatin structure
- Epigenetic changes

The Aging Epigenome

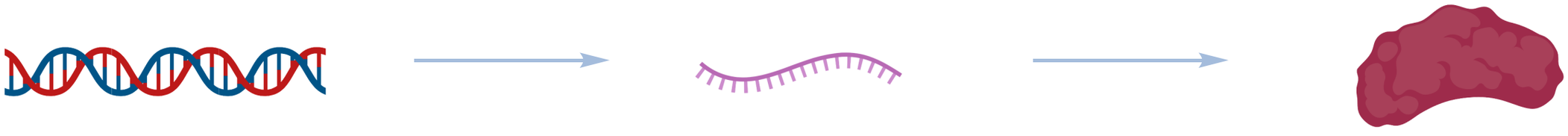
- Nucleosomes
- Histone PTMs
- DNA Methylation
- Heterochromatin
- Relocalization of Chromatin Modifiers

Reprogramming the Epigenome

- Calorie Restriction
- Small Molecules
- Cellular Reprogramming



The Central Dogma



What determines which genes are expressed?

Allis, D. C. et al. Epigenetics 2nd ed., *CSHL Press*, **2015**

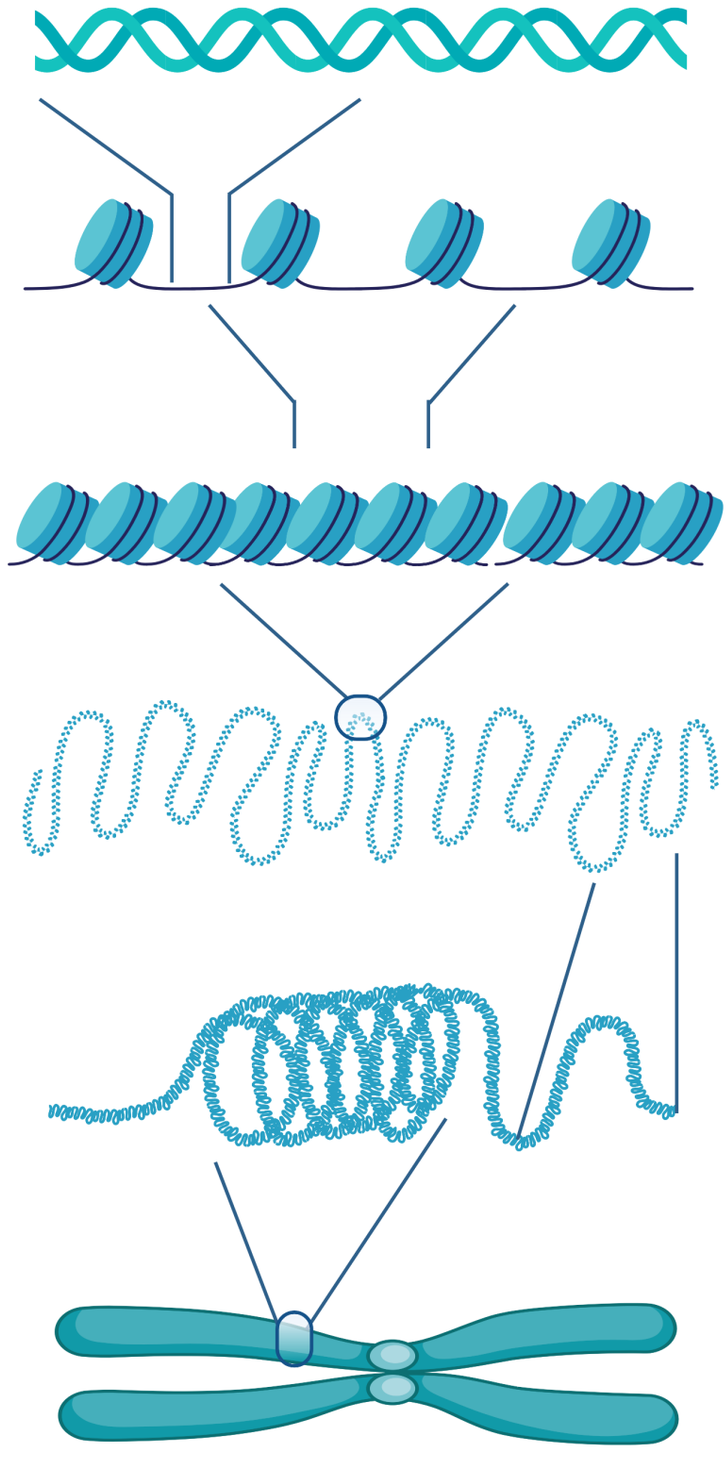
Chromatin

region of DNA double helix

“beads on a string” chromatin

condensed chromatin

entire mitotic chromosome



Chromatin consists of DNA bound to proteins including histones

histone

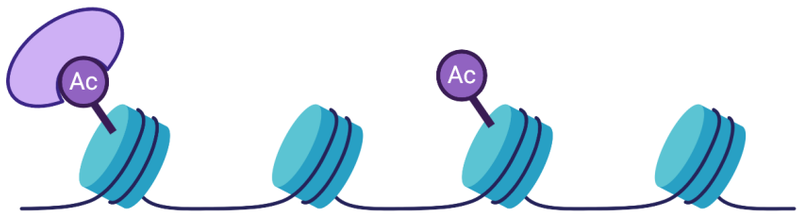
nucleosome

Chromosomes are composed of a very long DNA molecule and proteins that carries part of the hereditary information of an organism

Alberts, B.; Johnson, A.; Lewis J. et al. Molecular Biology of the Cell 4th ed., *Garland Science*; **2002**

Euchromatin vs. Heterochromatin

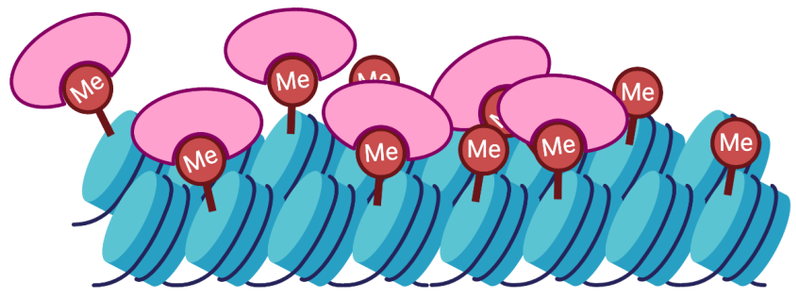
Chromatin is one of two subtypes → **euchromatin** or **heterochromatin**



euchromatin

loose structure → genes likely transcribed

"genes on"



heterochromatin

compacted structure → genes likely not transcribed

"genes off"

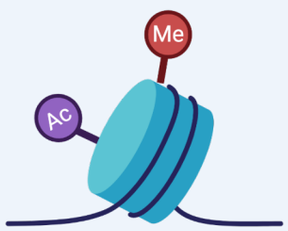
Wallrath, L. L. *Curr. Opin. Genet. Dev.* **1998**, *5*, 147

Grewal, S. I. S.; Moazed, D. *Science*, **2003**, *301*, 798

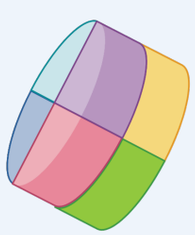
Epigenetics

Epigenetics = transmittable changes in gene “on-off” states through modulation of chromatin which is not brought about by changes in the DNA sequence

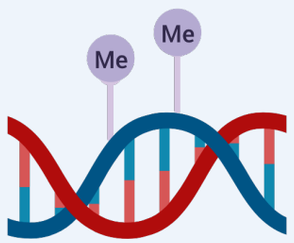
Epigenetic change - *chromatin modification*



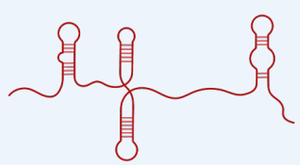
Histone modifications



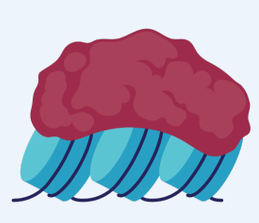
Histone variation



DNA methylation



Non coding RNAs

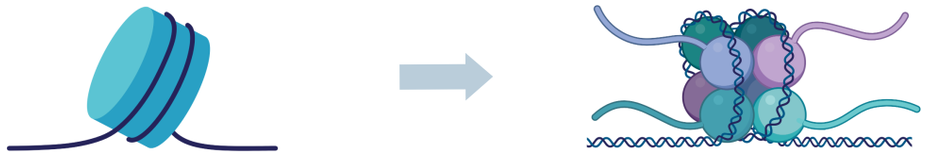


Chromatin remodeling

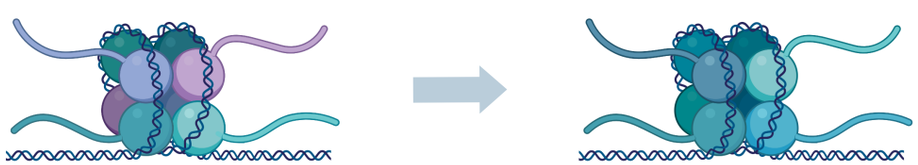
Allis, D. C. et al. Epigenetics 2nd ed., *CSHL Press*, **2015**

Nucleosome Remodeling

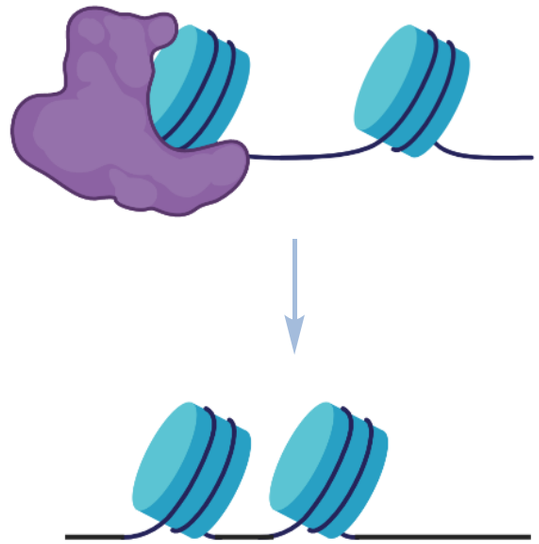
Nucleosomes consist of eight histone proteins (two each of H2A, H2B, H3 and H4)



Histone variants are proteins that substitute for the core histones (denoted as H3.#)



Nucleosome remodeling complexes use ATP hydrolysis to change chromatin and nucleosome composition non-covalently



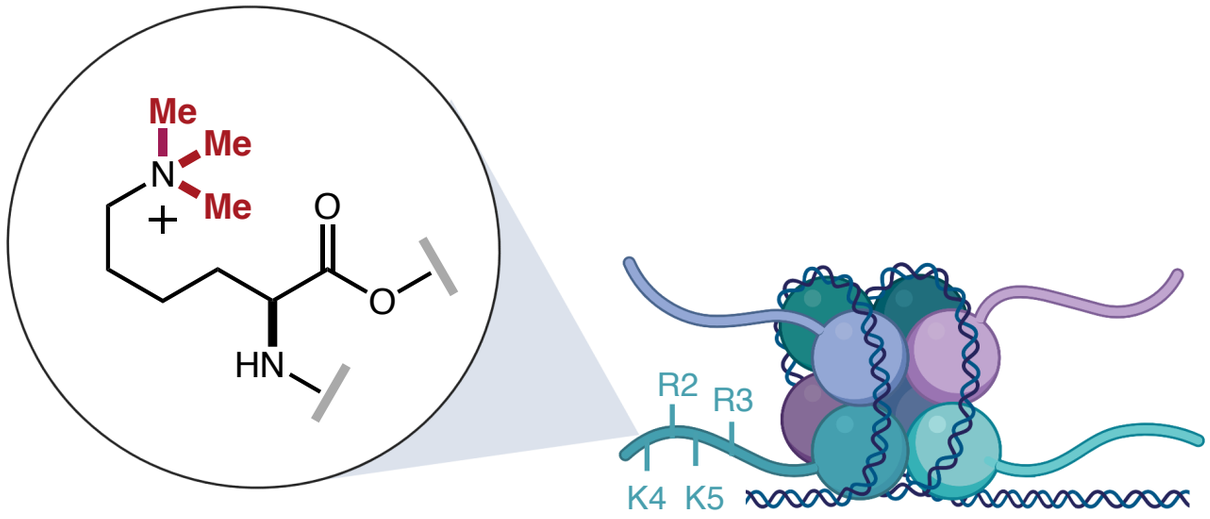
These complexes help chromatin accessibility for transcription factors and regulators

Allis, D. C. et al. Epigenetics 2nd ed., *CSHL Press*, **2015**

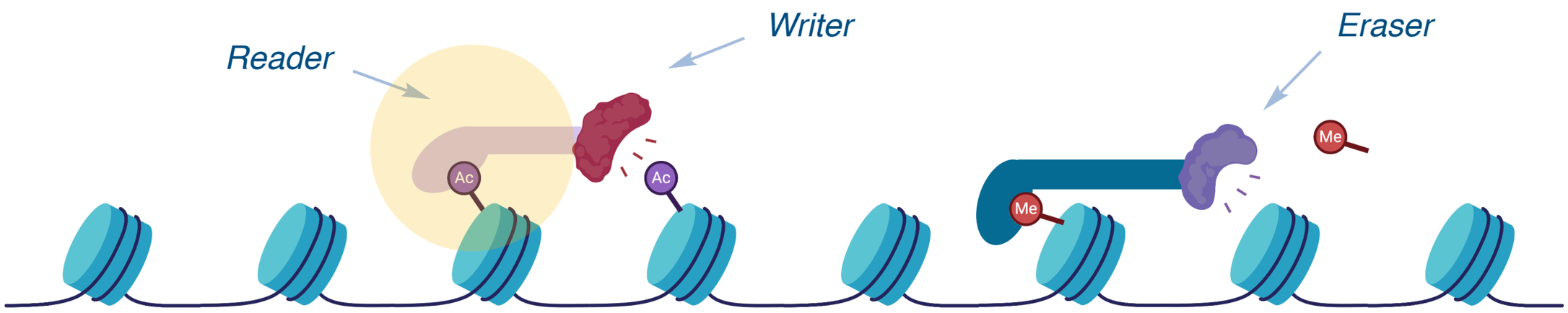
Histone Post-Translational Marks

Histone post-translation mark:
covalent modification of a histone amino acid

e.g. H3K4me3 = trimethylation of lysine 4 on histone 3



Proteins which have a specific affinity for a tail modification are called **readers**. Covalent modifications are effected by histone-modifying enzymes called **writers**, and removed by **erasers**

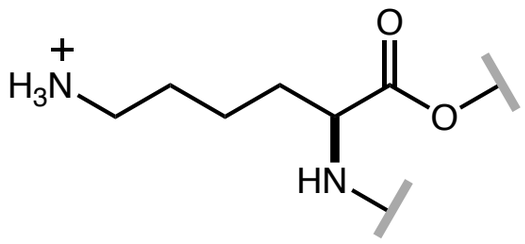


Allis, D. C. et al. Epigenetics 2nd ed., *CSHL Press*, **2015**

Example Modifications

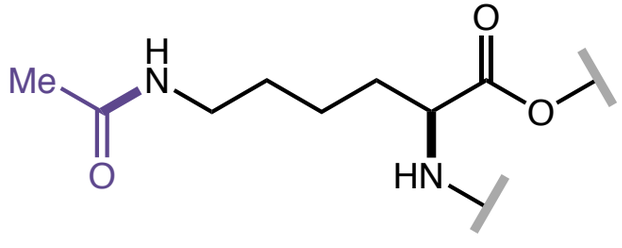
Covalent modifications alter chromatin structure or recruit histone modifiers leading to changes in gene expression

Lysine acetylation

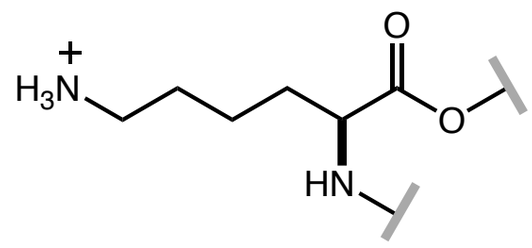


HAT
Histone acetyltransferase

HDAC
Histone deacetylase

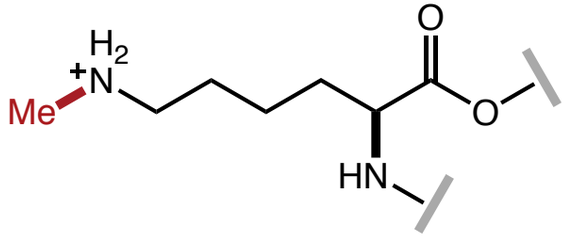


Lysine methylation



KMT
Histone lysine methyltransferase

KDM
Histone lysine demethylase

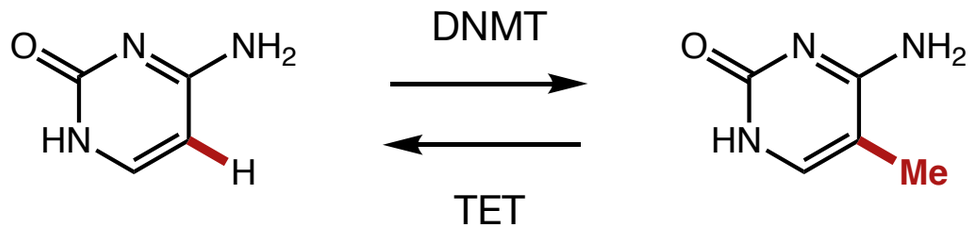


Allis, D. C. et al. Epigenetics 2nd ed., *CSHL Press*, **2015**

DNA Methylation

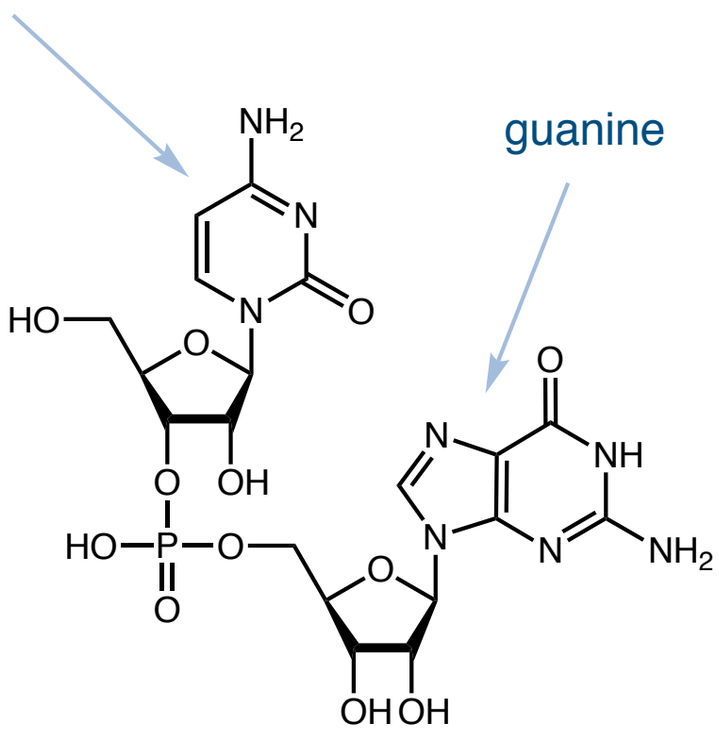
DNA methylation = addition of a methyl group to cytosine in the DNA template

Mainly occurs at CpG dinucleotides



cytosine

guanine



CpG dinucleotide = cytosine followed by guanine

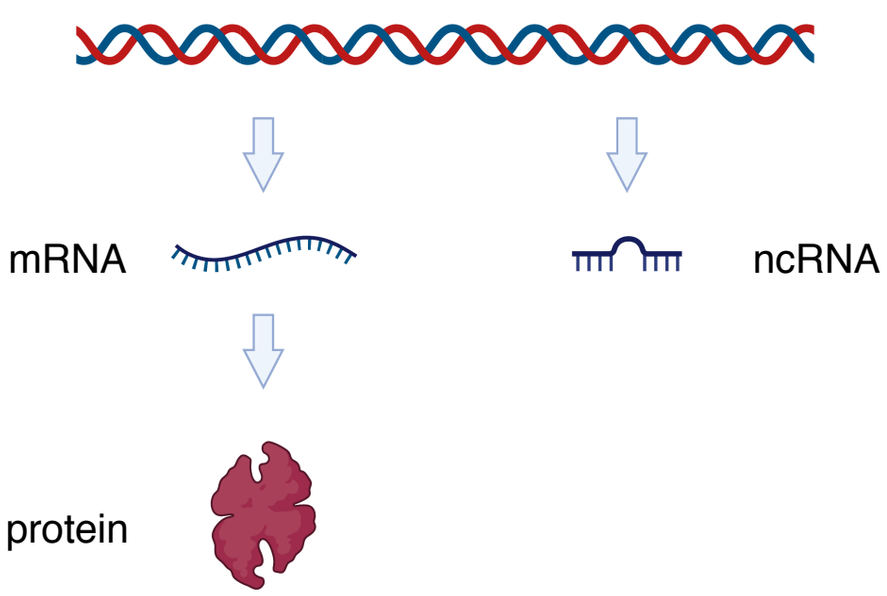
At *gene bodies*, DNA methylation tends to *promote expression* of the gene

Within *gene promoters* (at CpG islands), DNA methylation tends to *repress transcription*

Field, A. E.; Robertson, N. A.; Wang, T.; Havas, A.; Ideker, T.; Adams, P. D. *Mol. Cell.* **2018,** *71,* 882

Non-Coding RNA

ncRNA = Functional RNA molecule that is transcribed from DNA but not translated into proteins

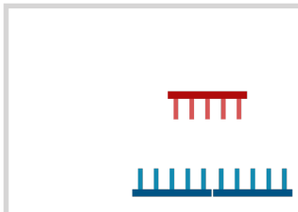


ncRNAs regulate gene expression at the transcriptional and post-translational levels

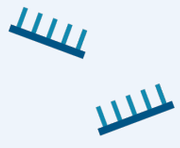
Epigenetic related ncRNAS include:

miRNA, siRNA, piRNA

These all carry out **RNA interference (RNAi)**



RNAi base pairs to target RNA

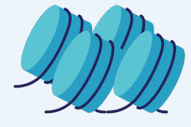


Cleavage of target RNA

or

Translational repression

or



Heterochromatin formation

Alberts, B.; Johnson, A.; Lewis J. et al. Molecular Biology of the Cell. 4th ed., *Garland Science*; **2002**

Outline

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Chromatin structure

Epigenetic changes

The Aging Epigenome

Nucleosomes

Histone PTMs

DNA Methylation

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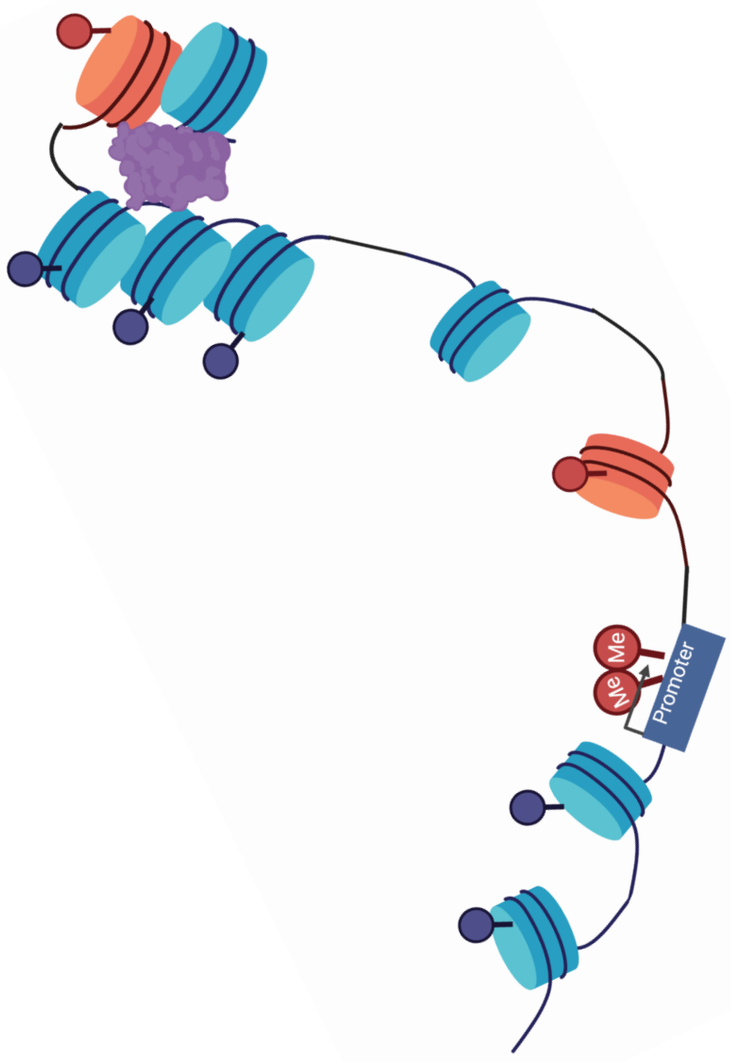
Relocalization of Chromatin Modifiers

Reprogramming the Epigenome

Calorie Restriction

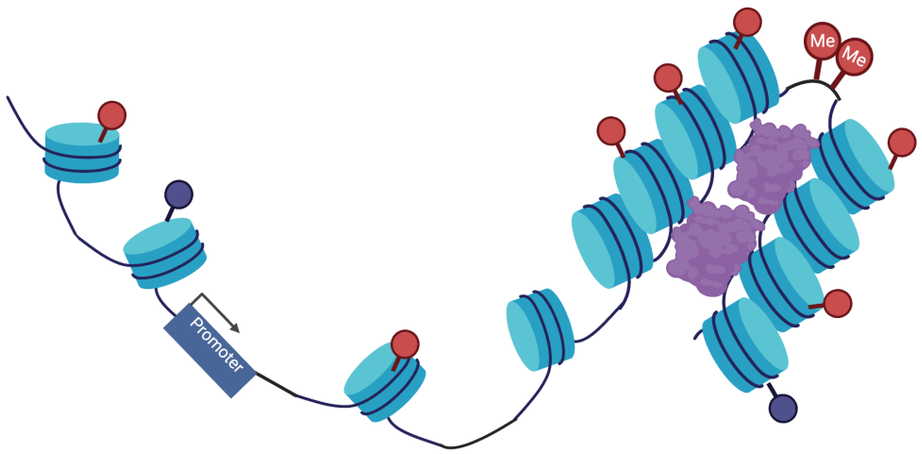
Small Molecules

Cellular Reprogramming

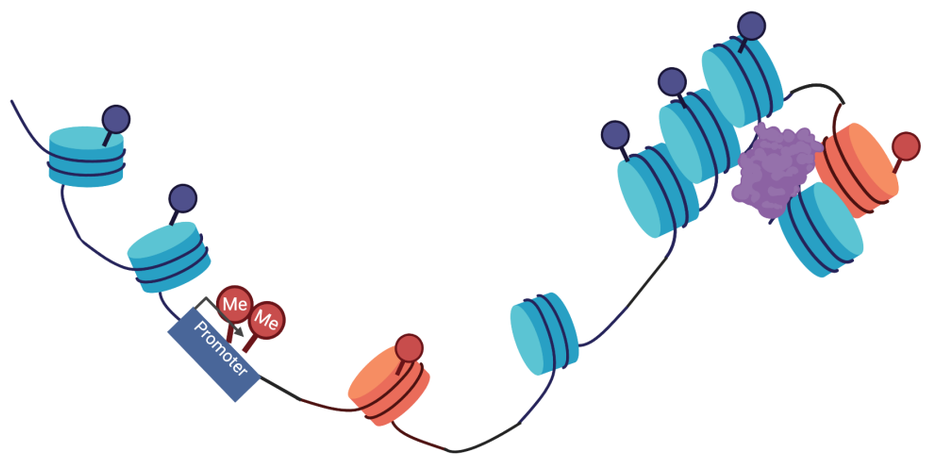


Epigenetics and Aging

Young epigenome



Old epigenome



With aging there are distinct changes to the epigenome

→

Increased genomic instability
Changes in gene expression
by e.g loss of silencing

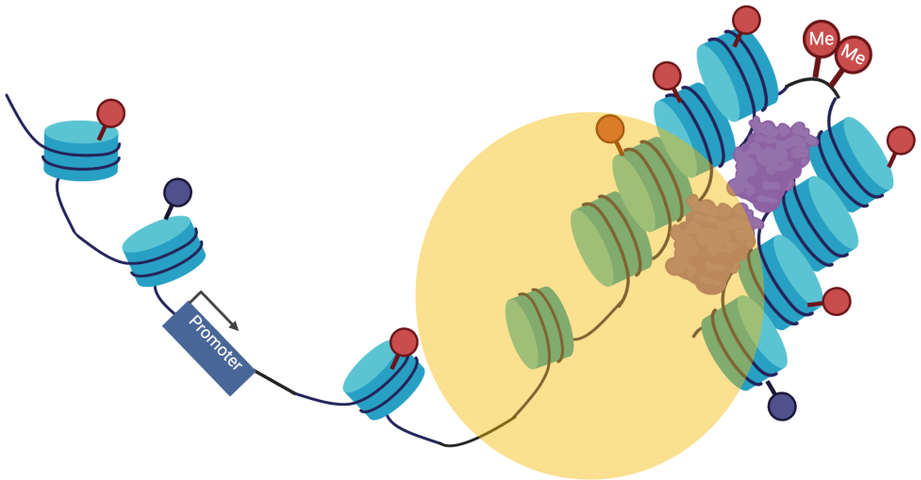
→

May contribute to hallmarks of aging

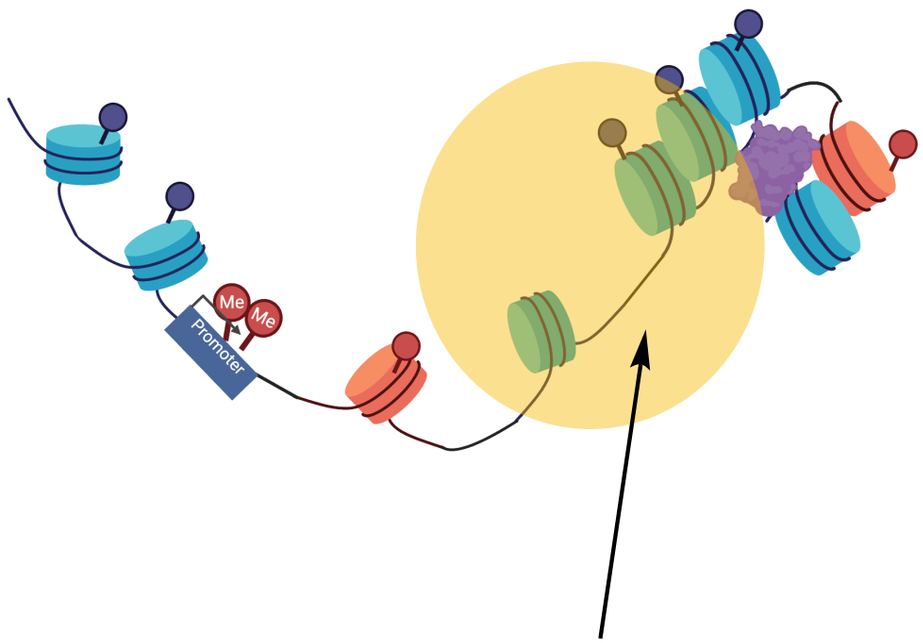
Kane, A. E.; Sinclair, D. A. *Crit. Rev. Biochem. Mol. Biol.* **2019**, *54*, 61

Epigenetics and Aging

Young epigenome

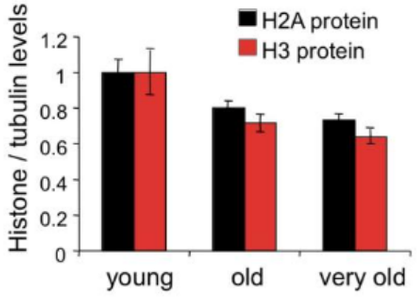


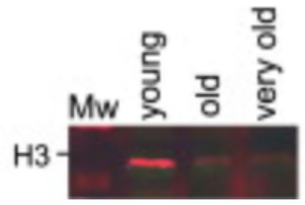
Old epigenome



Kane, A. E.; Sinclair, D. A. *Crit. Rev. Biochem. Mol. Biol.* **2019**, *54*, 61

Nucleosome Occupancy Loss in Yeast

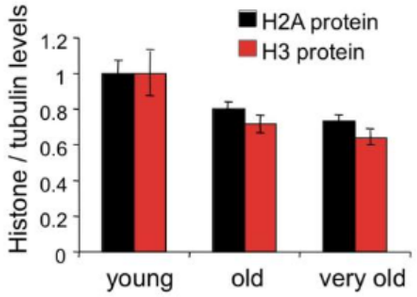


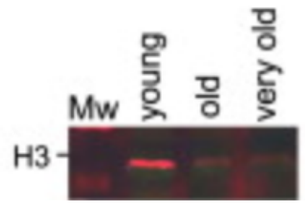


H3 and H2A protein decrease during aging

Feser, J.; Truong, D.; Das, C.; Carson, J. J.; Kieft, J.; Harkness, T.; Tyler, J. K. *Mol. Cell.* **2010**, *39*, 724

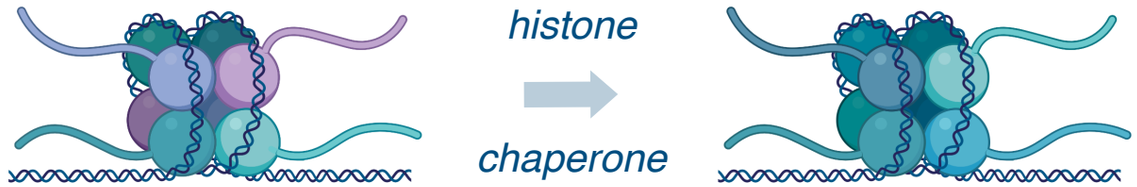
Nucleosome Occupancy Loss in Yeast

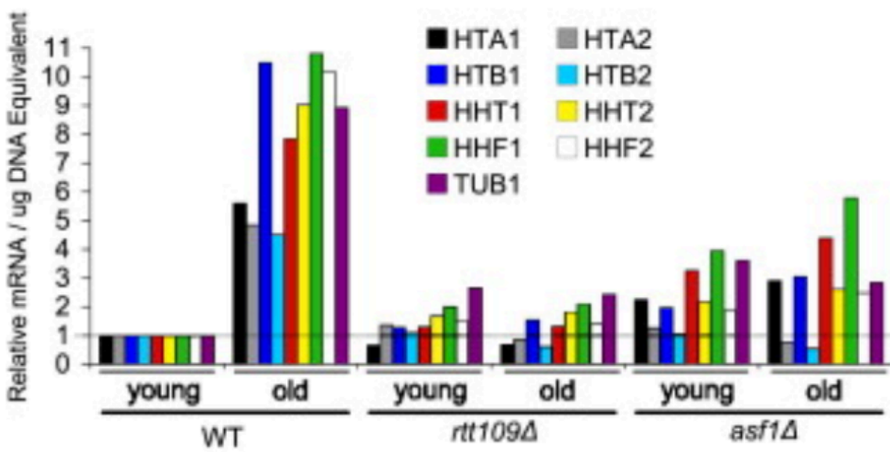




H3 and H2A protein decrease during aging

Mutant yeast bred which lacked ASF1, a gene encoding for a histone chaperone (these were shorter lived)



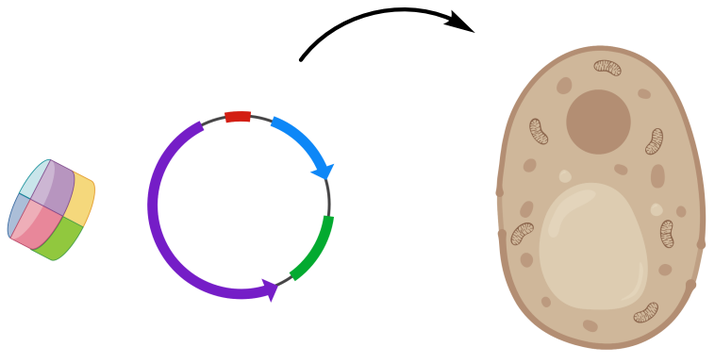


A significant increase in histone transcripts was found in the aging population of WT yeast but not mutants

Feser, J.; Truong, D.; Das, C.; Carson, J. J.; Kieft, J.; Harkness, T.; Tyler, J. K. *Mol. Cell.* **2010**, *39*, 724

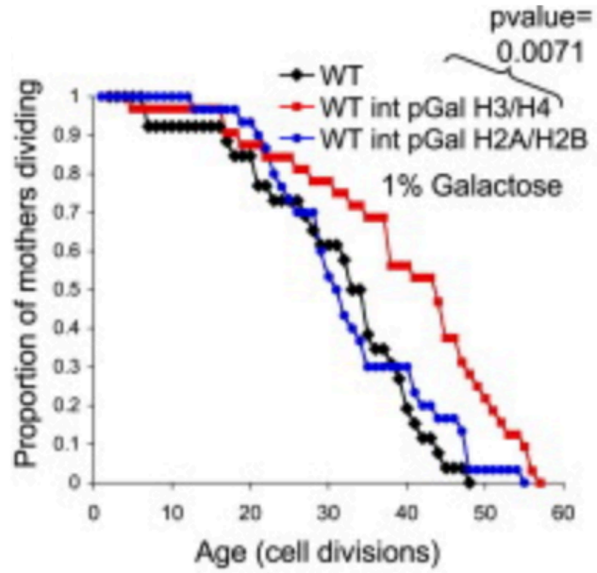
Nucleosome Occupancy Loss in Yeast

Hypothesized that the the reduced histone expression in the short-lived mutants is a cause of their shortened life span



A plasmid encoding all four core histones extended the median life span of asf1 mutants by 65%

To determine which core histones impart anti-aging properties, added extra copies of genes encoding H3/H4 or H2A/H2B

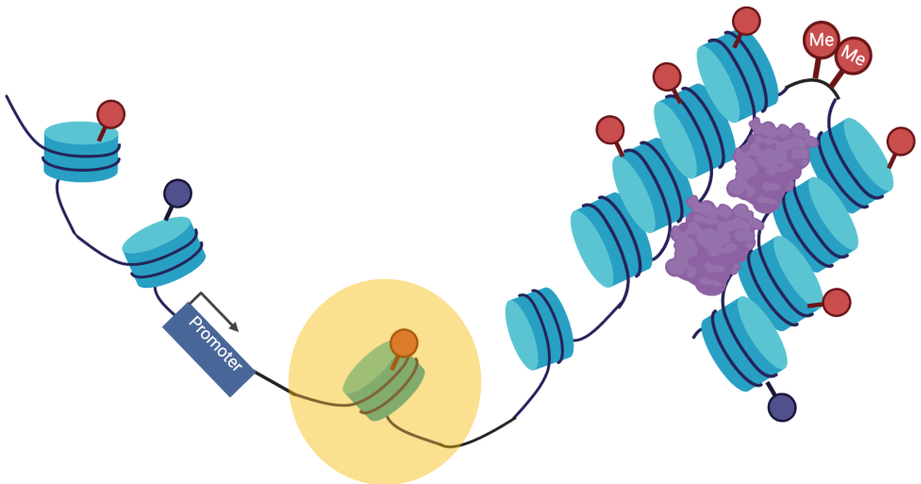


Overexpression of genes encoding H3/H4 extended WT life span by 30%

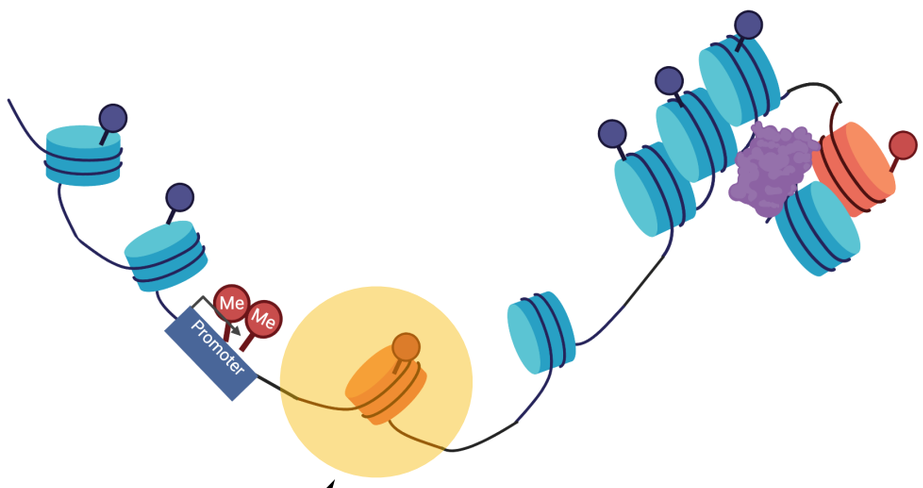
Feser, J.; Truong, D.; Das, C.; Carson, J. J.; Kieft, J.; Harkness, T.; Tyler, J. K. *Mol. Cell.* **2010**, *39*, 724

Epigenetics and Aging

Young epigenome



Old epigenome



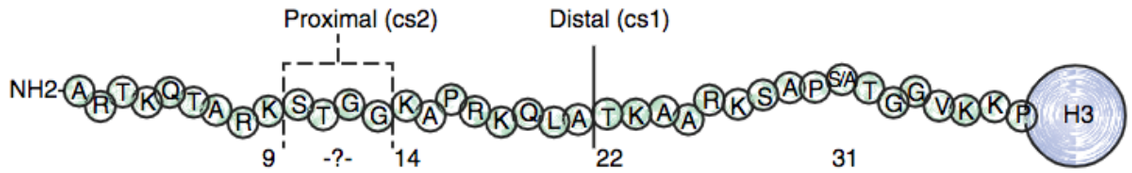
Increase in histone variants

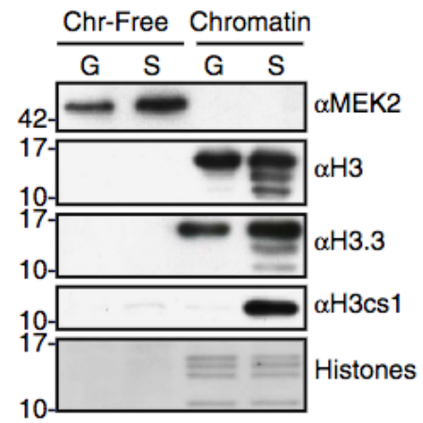
Kane, A. E.; Sinclair, D. A. *Crit. Rev. Biochem. Mol. Biol.* **2019**, *54*, 61

Histone Variants e.g H3.3

Histone variants H3.3 and its cleaved products (H3.3cs#) are found in an increased amount during senescence

Senescence = cells stop dividing without undergoing cell death - a hallmark of aging

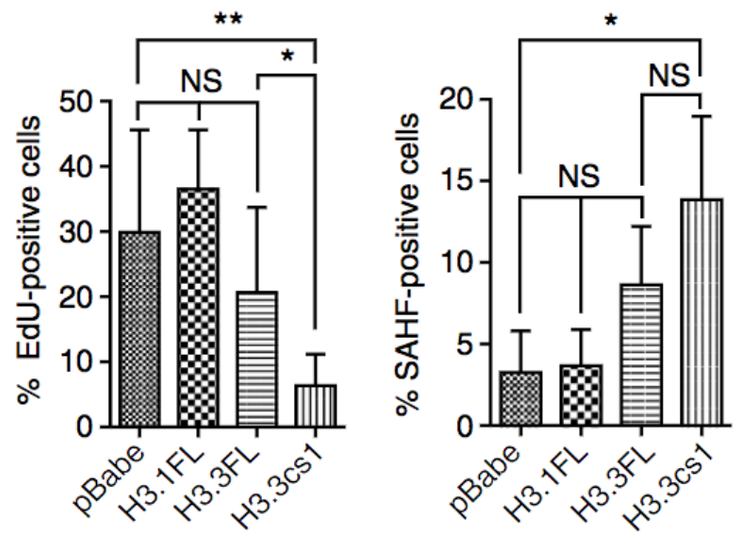




G = growing

S = senescent

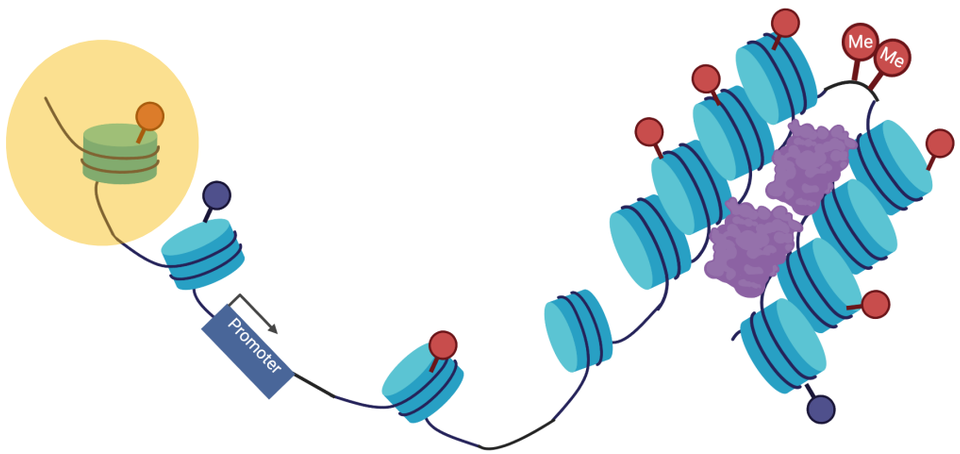
Overexpression of these variants induced senescence, indicating that these histone changes may drive aging



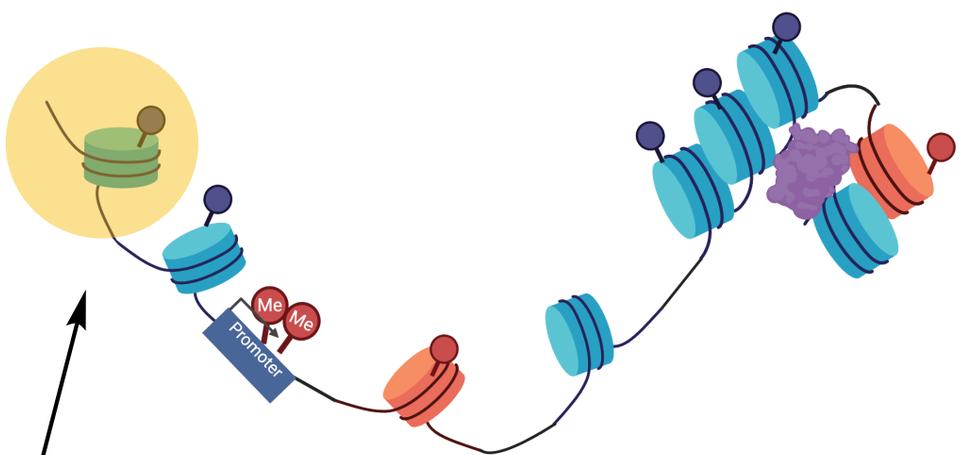
Duarte, L. F.; Young, A. R. J.; Wang, Z.; Wu, H.-A.; Panda, T.; Kou, Y.; Kapoor, A.; Hasson, D.; Mills, N. R.; Bernstein, E. *Nat. Comm.* **2014**, *5*, 5210

Epigenetics and Aging

Young epigenome



Old epigenome



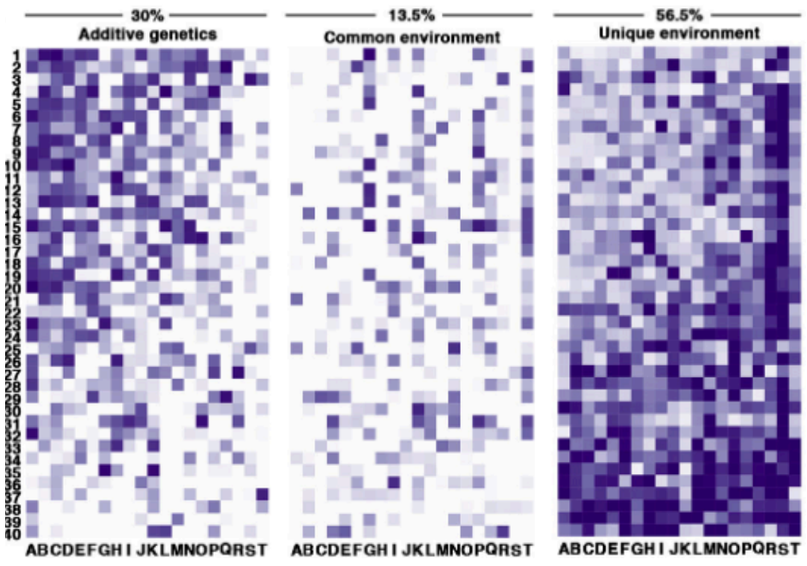
Changes in histone post-translational modifications

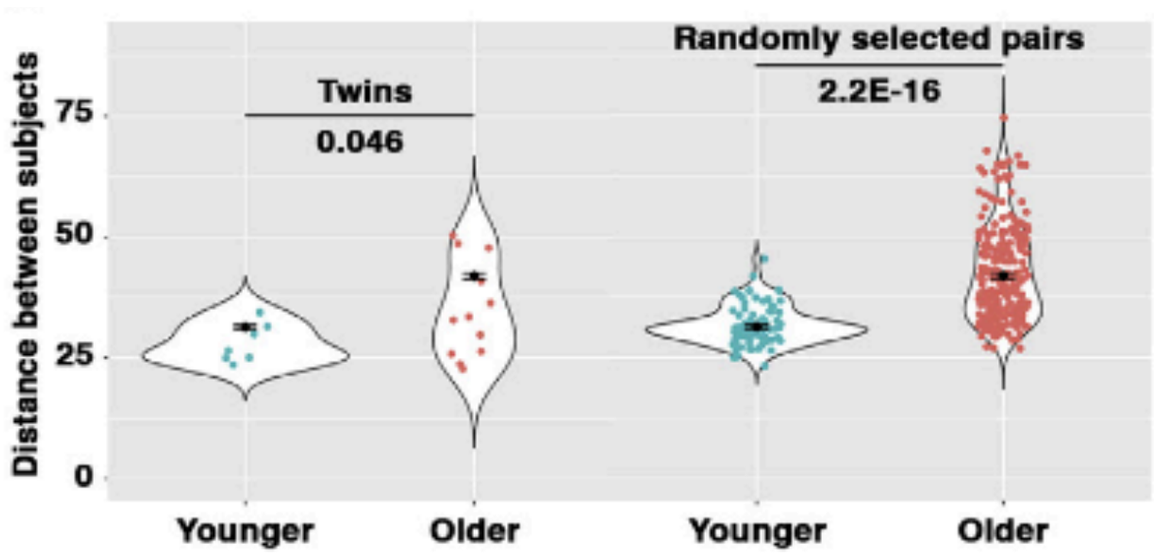
Kane, A. E.; Sinclair, D. A. *Crit. Rev. Biochem. Mol. Biol.* **2019**, *54*, 61

Histone Post-Translational Marks and Aging

Global increase in active histone marks and decrease in repressive histone marks

Specific pattern of histone modifications differs between organisms, tissues of same individuals, and cells of same tissue





In human peripheral blood mononuclear cells there is **increased heterogeneity in histone modifications** between both cells and individuals with age

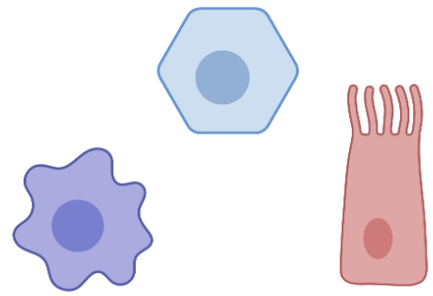
Benayoun B.; Pollina, E.A.; Brunet, A. *Nat. Rev. Mol. Cell Biol.* **2015**, *16*, 593

Cheung, P.; Vallania, F.; Warsinske, H. C.; Donato, M.; Schaffert, S.; Chang, S. E.; Dvorak, M.; Dekker, C. L.; Kuo, A. J. et al. *Cell* **2018**, *173,* 1385

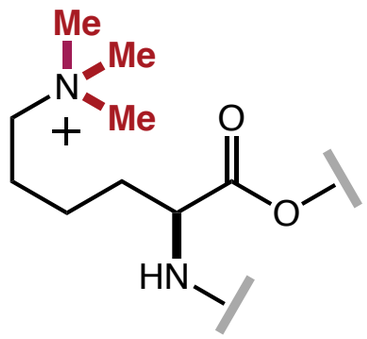
Example: H3K27me3

H3K27me3 is a repressive mark with various roles

important in ES cells establishing and maintaining cell identity



H3K27me3 is altered in a variety of cell types and species during aging

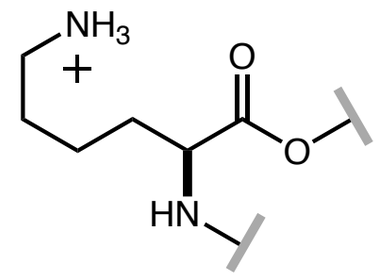


Increased levels of H3K27me3 with aging

Kilifish brain

Mouse muscle stem cells

Mouse brain tissue



Decreased levels of H3K27me3 with aging

C.elegans

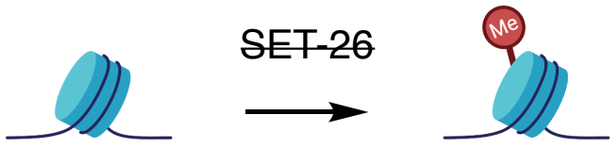
Fibroblasts from patients with age-acceleration disorders

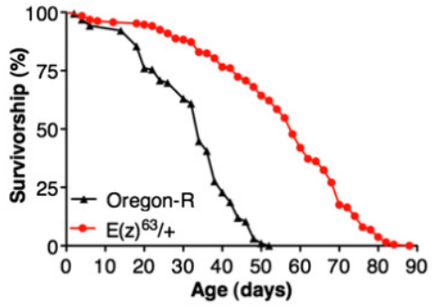
Kane, A. E.; Sinclair, D. A. *Crit. Rev. Biochem. Mol. Biol.* **2019**, *54*, 61

Example: H3K27me3

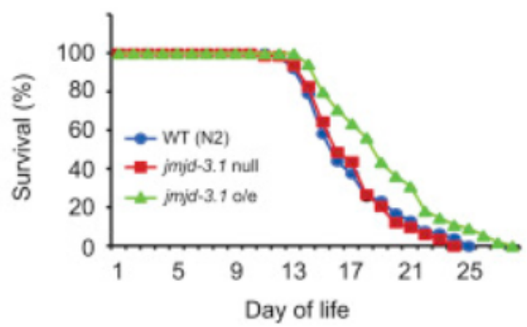
H3K27me3 methylation is controlled by demethylases UTX-1, KDM6B/JMJD-3, PHF8/JMJD-1.2 and the methyltransferases Polycomb and SET-26

Evidence that increased demethylation is beneficial





Knockout of genes for methyltransferases Polycomb and SET-26 increases lifespan in Drosophila



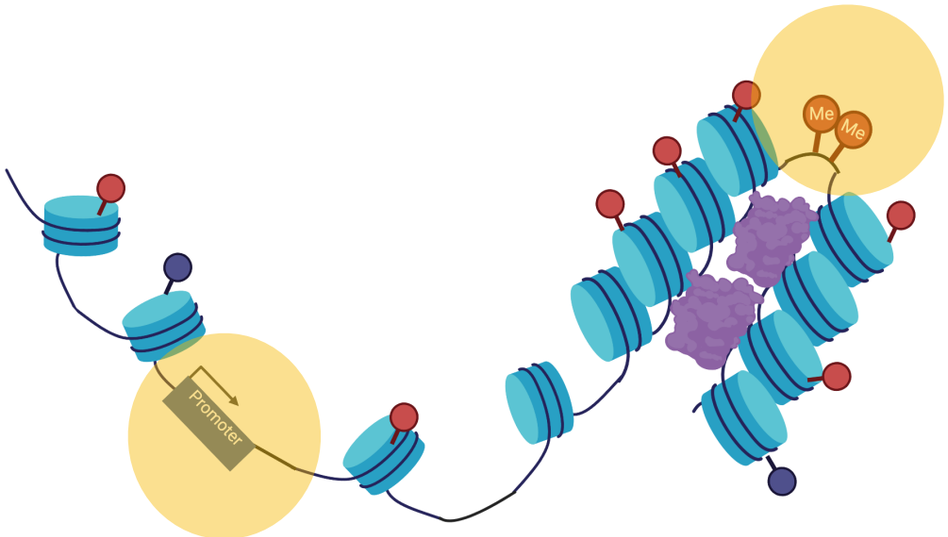
Overexpression of demethylases KDM6B/ JMJD-3 or PHF8/JMJD-1.2 increases lifespan in C.elegans

Siebold, A. P.; Banerjee, R.; Tie, F.; Kiss, D. L.; Moskowitz, J.; Harte, P. J. *PNAS* **2010**, *107*, 169

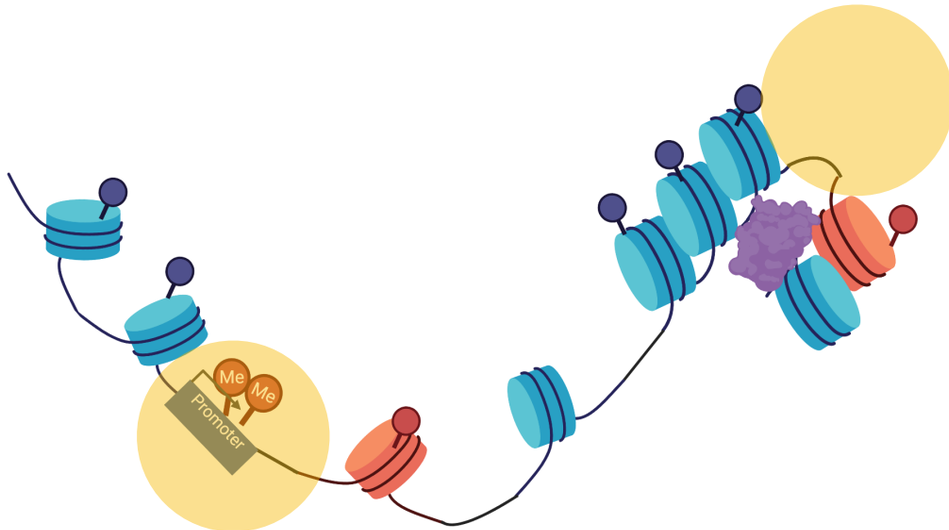
Labbadia, J.; Morimoto, R. I. *Mol. Cell.* **2015**, *59*, 639

Epigenetics and Aging

Young epigenome



Old epigenome

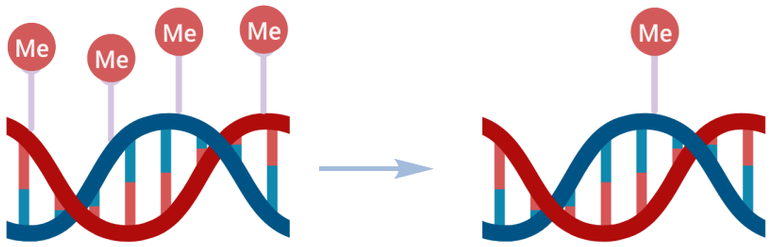


Location-specific DNA methylation changes

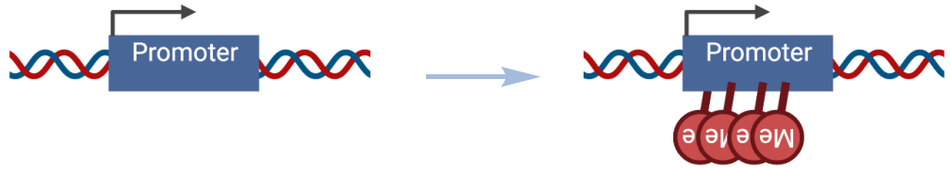
Kane, A. E.; Sinclair, D. A. *Crit. Rev. Biochem. Mol. Biol.* **2019**, *54*, 61

DNA Methylation Changes with Age

Two general changes in DNA methylation with age which lead to repression



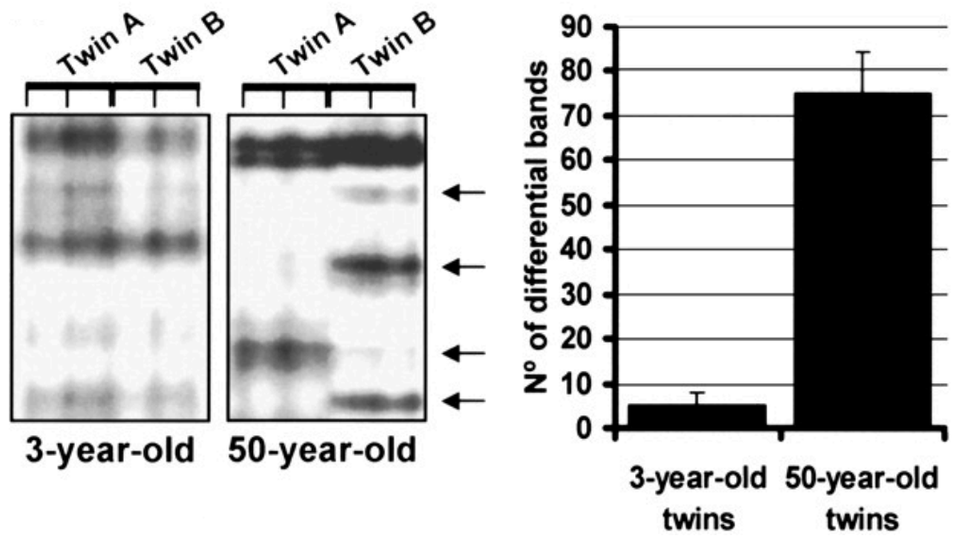
Global DNA hypomethylation



Specific areas of DNA hypermethylation

Identical twins show large differences in the overall content and distribution in DNA methylation with age

Epigenetic drift = epigenetic modifications occurring as consequence of age

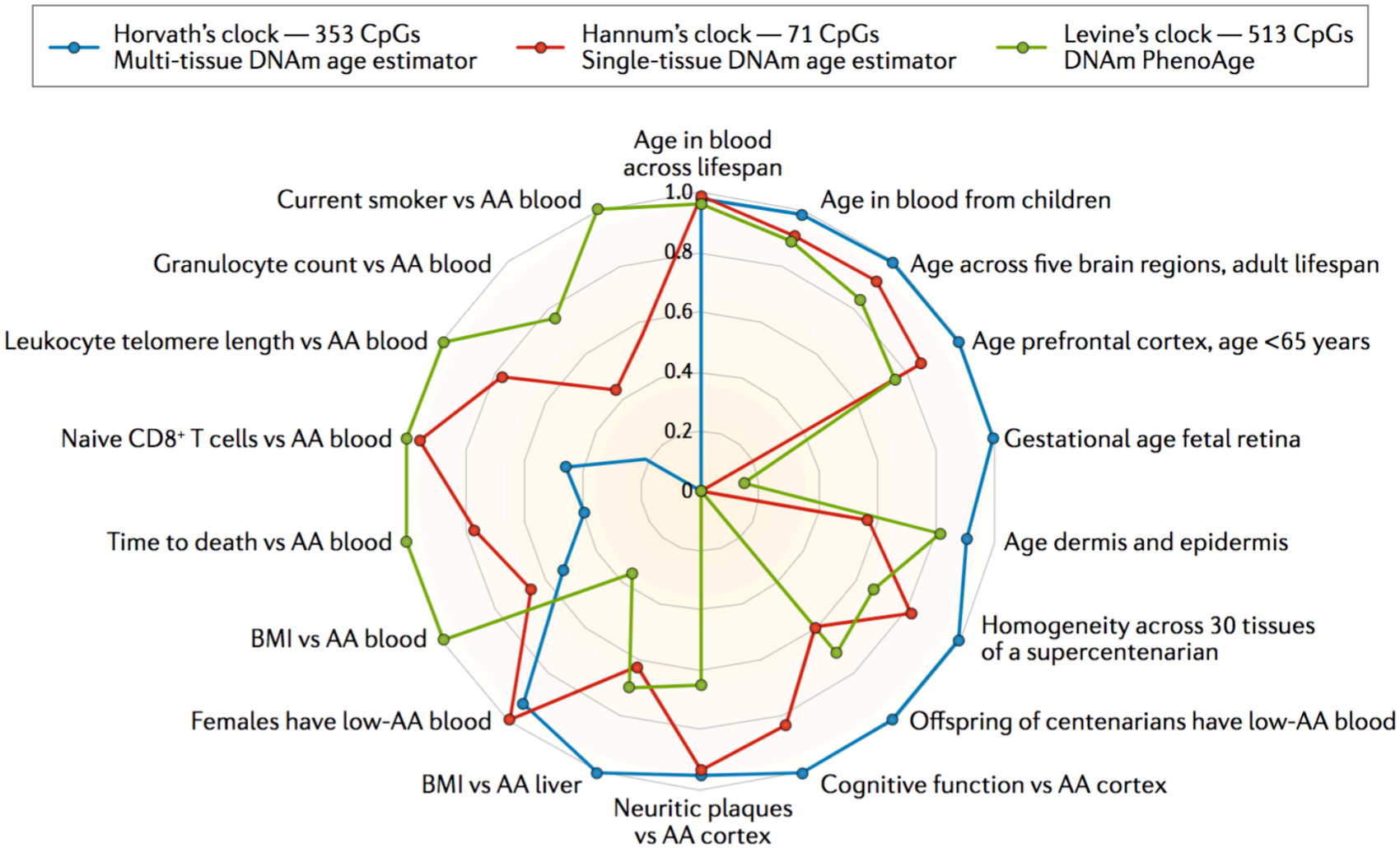


Kane, A. E.; Sinclair, D. A. *Crit. Rev. Biochem. Mol. Biol.* **2019**, *54*, 61

Fraga, M. F.; Ballestar, E.; Paz, M. F.; Ropero, S.; Setien, F.; Ballestar, M. L.; Plass, C.; Esteller, M. et al. *PNAS* **2005**, *102*, 10604

DNA Methylation and Epigenetic Clocks

Epigenetic age estimators = mathematical algorithms that use values assigned to the methylation state of specific CpGs in the genome to estimate the age of a person or biological sample



Horvath, S.; Raj, K. *Nat. Rev. Genet.* **2018**, *19*, 371

DNA Methylation and Age Estimation

Positive epigenetic age acceleration, where an epigenetic age is older than expected (on the basis of their chronological age), i*s correlated with many age-related conditions*

Alzheimer disease	Prefrontal cortex	Horvath's clock
Amyloid load and neuropathology	Prefrontal cortex	Horvath's clock
Blood pressure (systolic)	Blood	Hannum's clock
Body mass index	Liver	Horvath's clock
Cancer	Blood	All clocks
Cardiovascular disease	Blood	DNAm PhenoAge
Coronary heart disease	Blood	DNAm PhenoAge
Cellular senescence (oncogene-induced)	Various	Horvath's clock
Centenarian (offspring status)	Blood	Horvath's clock
Cholesterol, HDL (not LDL)	Blood	Hannum's clock and DNAm PhenoAge
Cognitive performance	Blood and brain	Horvath's clock and DNAm PhenoAge
C-reactive protein	Blood	All
Diet (carotenoids)	Blood	Hannum's clock and DNAm PhenoAge
Dementia	Blood	DNAm PhenoAge
Down syndrome	Blood and brain	Horvath's clock
Education	Blood	Hannum's clock and DNAm PhenoAge
Exercise (recreational)	Blood	Hannum's clock and DNAm PhenoAge
Frailty	Blood	Horvath's clock and DNAm PhenoAge
Gender	Blood and brain	All
Gestational week	Blood and brain	Horvath's clock
Glucose	Blood	All
Huntington disease	Blood and brain	Horvath's clock
Income	Blood	Hannum's clock and DNAm PhenoAge
Insulin levels	Blood	All
Menopause	Blood and saliva	Horvath's clock
Mortality (all-cause)	Blood	All
Obesity	Liver and blood	All clocks
Osteoarthritis	Cartilage	Horvath's clock
Parkinson disease	Blood	All
Pubertal development	Blood	Horvath's clock
Sleep	Blood	Hannum's clock
Smoking	Blood	DNAm PhenoAge
TERT expression	Blood and fibroblasts	Horvath's clock
Triglycerides	Blood	All
Walking speed	Blood	DNAm PhenoAge
Werner syndrome	Blood	Hannum's clock and Horvath's clock

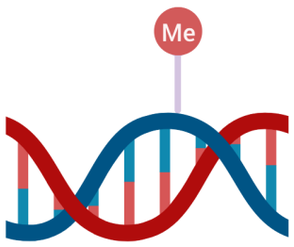
DNA methylation-based biomarkers are the most promising molecular estimators of biological age

Horvath, S.; Raj, K. *Nat. Rev. Genet.* **2018**, *19*, 371

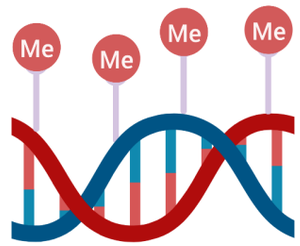
Is DNA Methylation a Contributor or a Consequence of Aging?

More research in mammals required to determine whether DNA methylation changes are a consequence of aging, or a contributor

DNA methylation probably affects age-related changes in transcription factors and histone-modifying enzymes and vice versa



DNA hypomethylation more common in regions with H3K9Ac, H3K27Ac, H3K3me1, H3K4me2, H3K4me3

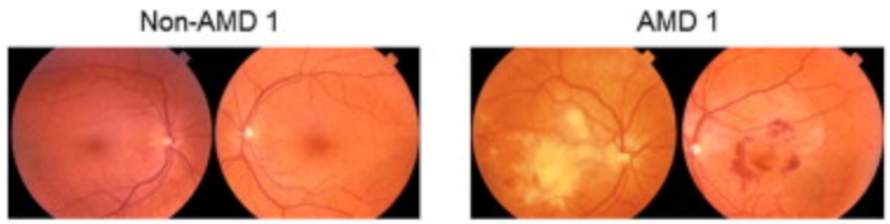


DNA hypermethylation more common in regions with H3K4me3 and H3K27me3

McClay, J. L.; Aberg, K. A.; Clark, S. L.; Nerella, S.; Kumar, G.; Xie, L. Y.; Hudson, A. D.; Harada, A. et al. *Hum. Mol. Genet.* **2014**, *23*, 1175
Raddatz, G.; Hagemann, S.; Aran, D.; Söhle, J.; Kulkarni, P. P.; Kaderali, L.; Hellman, A.; Winnefeld, M.; Lyko, F. *Epigenetics Chromatin* **2013**, *6*, 36

DNA Methylation and Macular Degeneration

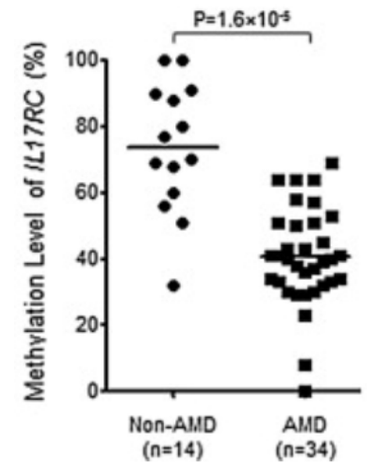
Reduced DNA methylation at certain sites can lead to pathological effects e.g macular degeneration



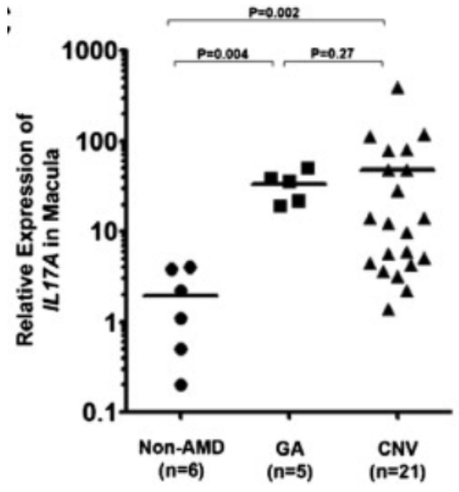
While majority of identical twins get end-stage AMD together, some do not

231 genes with different methylation patterns on their promoters

Fewer methylated CpG sites in the promoter regions of IL17RC in AMD patients



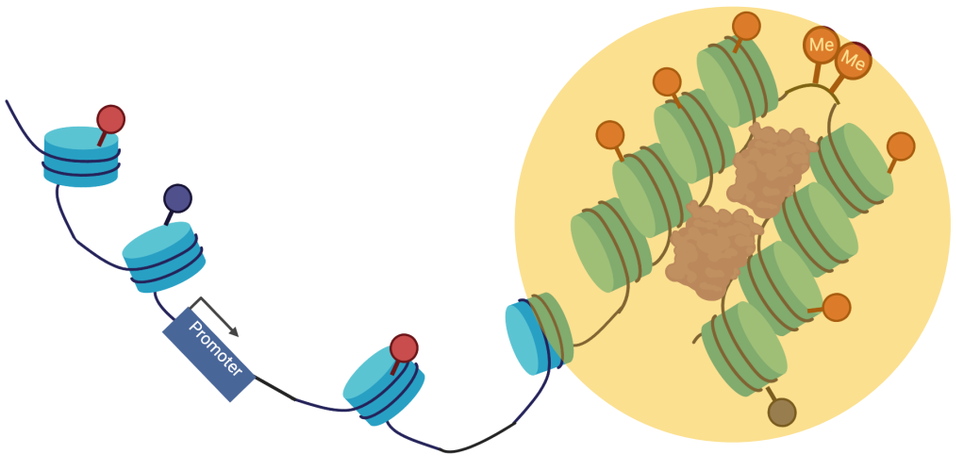
Hypomethylation of this promoter led to elevated IL-17RC expression in AMD patients



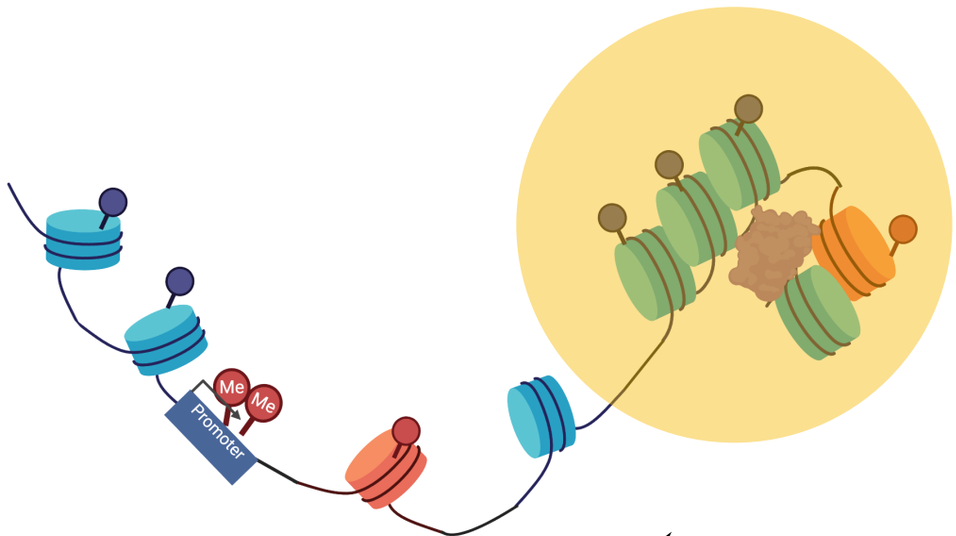
Wei, L.; Liu, B.; Tuo, J.; Shen, D.; Chen, P.; Li, Z.; Liu, X.; Ni, J.; Dagur, P.; Sen, H. N.; Jawad, S.; Ling, D.; Nussenblatt, R. B. et al. *Cell Reports* **2012**, *2*, 1151

Epigenetics and Aging

Young epigenome



Old epigenome



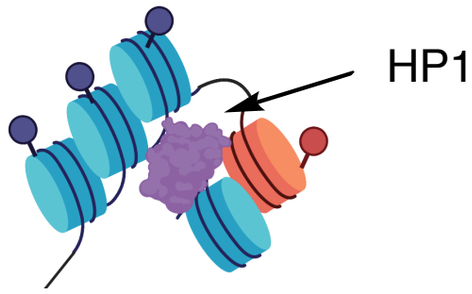
Heterochromatin changes

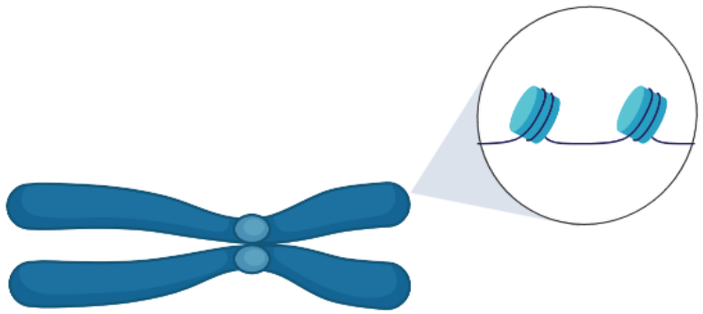
Kane, A. E.; Sinclair, D. A. *Crit. Rev. Biochem. Mol. Biol.* **2019**, *54*, 61

Heterochromatin Changes with Age

Heterochromatin characterized by presence of:

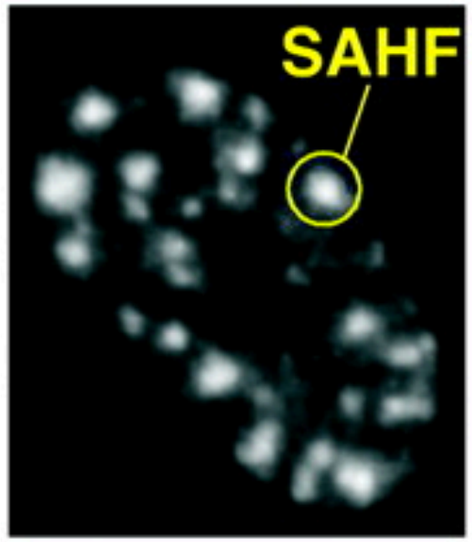
1. HP1 2. Decreased histone acetylation 3. Increased H3K9me3





Global reduction in HP1 and H3K9me3 levels, lamin A changes

Regions that are normally heterochromatic (e.g telomeres) become more euchromatic

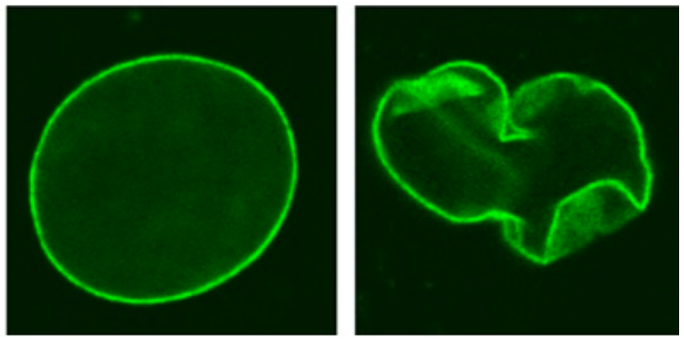


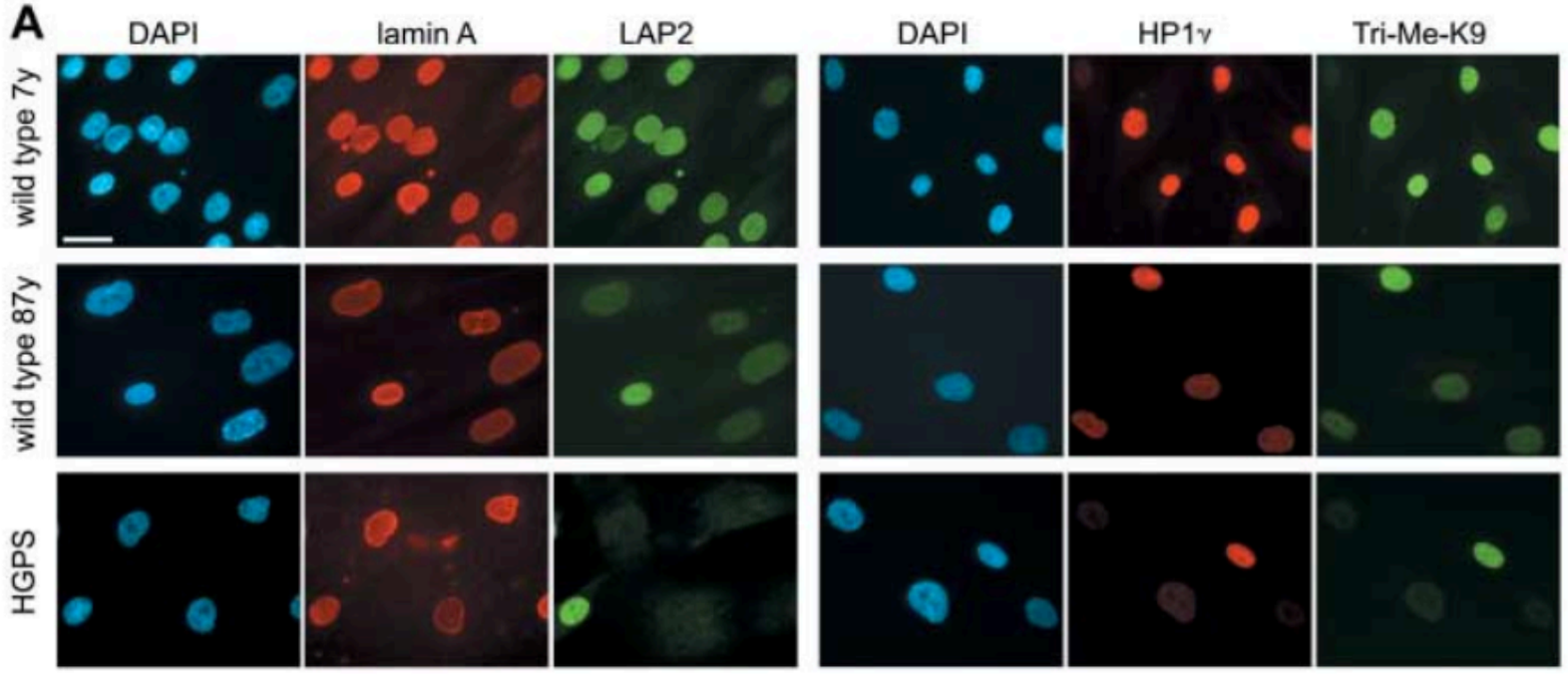
Domains of heterochromatin form called senescent-associated heterochromatin foci (SAHFs)

Kane, A. E.; Sinclair, D. A. *Crit. Rev. Biochem. Mol. Biol.* **2019**, *54*, 61

Heterochromatin Changes and HGPS

HGPS is caused by mutations in the nuclear structural protein lamin A, that results in a reduction in heterochromatin, HP1 and H3K9Me3 marks





These changes are also seen in normal aging

It is unknown which change drives the aging

Scaffidi, P.; Misteli, T. *Science*, **2006**, *312*, 1059

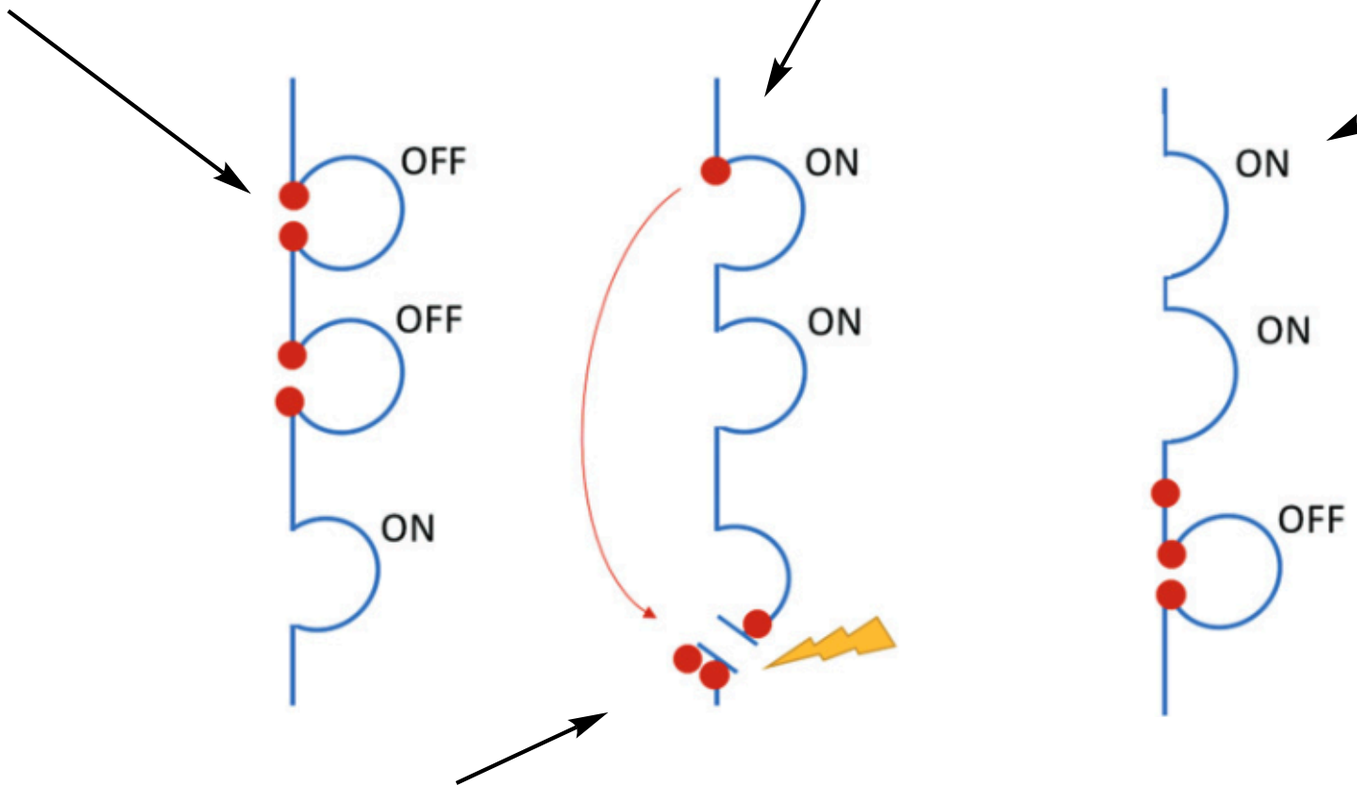
Re-localization of Chromatin Modifiers (RCM)

The delocalization of chromatin modifying factors (RCM) concept is a hypothesis for aging

Chromatin modifying factors (e.g SIRT 1, Sir2) are located at particular loci where they modulate transcription

Normally, these factors quickly return to their original sites

Over time, the factors that do not return to their original sites leads to alterations in gene expression



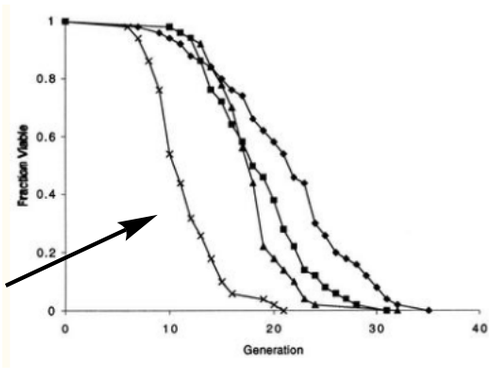
When DNA is damaged, these factors are recruited to assist in repair

Causes loss of cellular identity, cellular dysfunction and aging

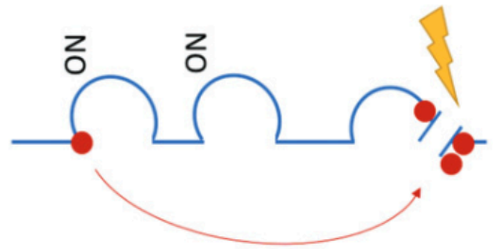
Kane, A. E.; Sinclair, D. A. *Crit. Rev. Biochem. Mol. Biol.* **2019**, *54*, 61

RCM and Yeast

First evidence for RCM in yeast

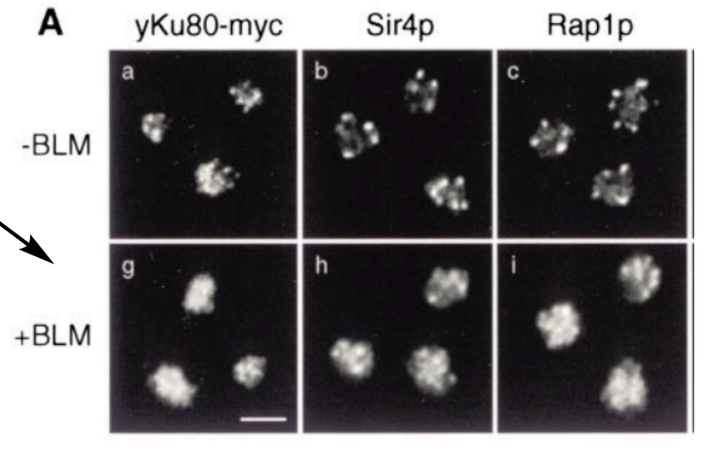


Addition of null alleles for the histone deacetylase Sir2 dramatically decreased lifespan



Sir2 was identified as a DNA repair protein that relocalizes from silent loci to sites of DNA repair

Diffuse pattern upon DNA damage



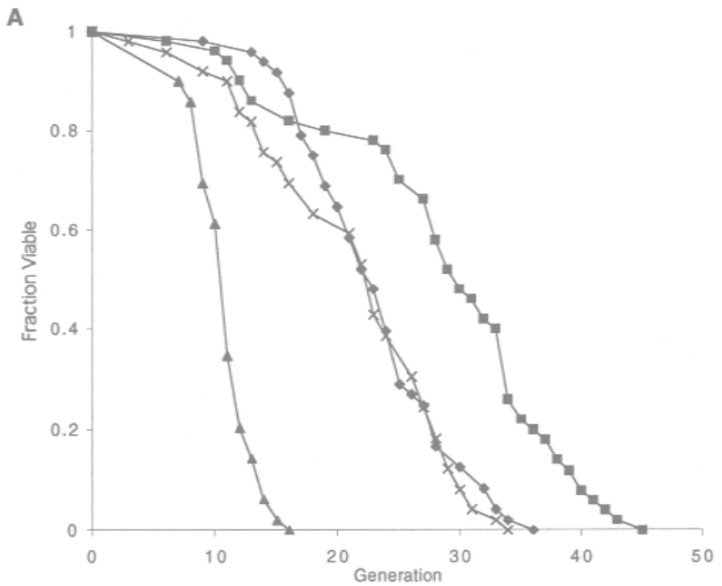
Martin, S. G.; Laroche, T.; Suka, N.; Grunstein, M.; Gasser, S. M. *Cell,* **1999**, *97*, 621
Kaeberlein, M.; McVey, M.; Guarente, L. *Genes Dev.* **1999**, *13,* 2570

RCM and Yeast

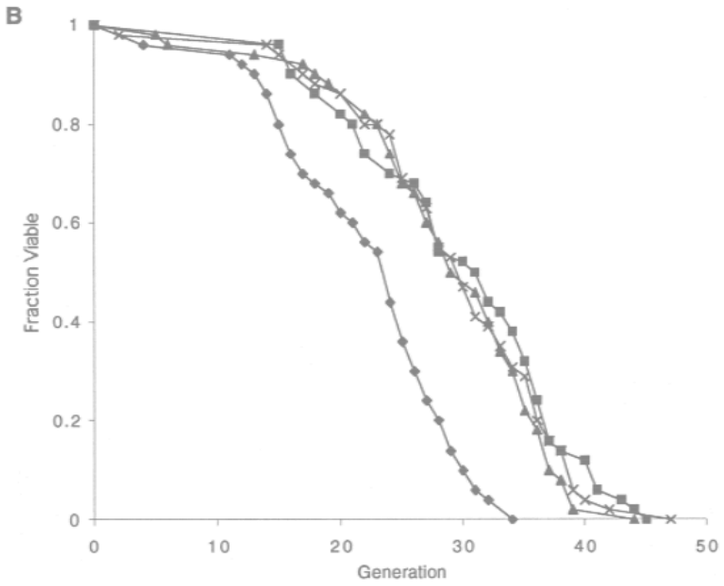
The absence of Sir2 at silent mating-type loci caused nuclear changes similar to aging yeast

Genotype	LOH rate per 10,000 cell divisions (95% CI)			
	Young		Old	
	MET15	*SAM2*	*MET15*	*SAM2*
Wild type	7 (5–10)	1 (0.5–2.0)	300 (100–500)	200 (50–400)
fob1Δ/fob1Δ	7 (4–10)	1 (0.4–3.0)	150 (90–230)	80 (30–200)
sir2Δ/sir2Δ	160 (120–200)	1 (0.4–3.0)	200* (50–300)	†

*The *sir2Δ/sir2Δ* rate of *MET15* LOH in old cells was calculated by half-sector frequency. †No *sir2Δ/sir2Δ* mother cell produced more than a single daughter colony with a *SAM2* LOH event.



Wild type yeast



Mutant missing a gene that leads to aging-related DNA damage

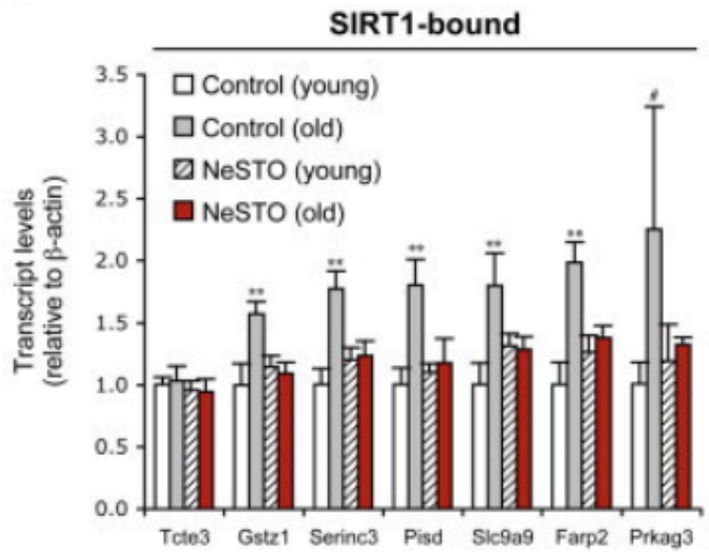
The addition of extra copy of Sir2 gene prevented the age-related relocalization and increased lifespan by 30%

McMurray, M. A.; Gottschling, D. E. *Science* **2003**, *301*, 1908
Kaeberlein, M.; McVey, M.; Guarente, L. *Genes Dev.* **1999**, *13,* 2570

RCM in Mammals

In mammals, the sirtuins (SIRT 1 and SIRT 6) also relocalize from silent loci to sites of damage to facilitate DNA repair

Leads to transcription changes in 100s of genes, including changes characteristic of aging



Overexpression of SIRT1 or SIRT6 in aging mice can prevent these changes in gene expression

However, while RCM is established as a cause of aging in yeast, it is not in mammals and may or may not be reversible

Oberdoerffer, P.; Michan, S.; McVay, M.; Mostoslavsky, R.; Vann, J.; Park, S.-K.; Hartlerode, A.; Stegmuller, J.; Sinclair, D. A. et al *Cell* **2008**, *135,* 907

Outline

Overview of Epigenetics

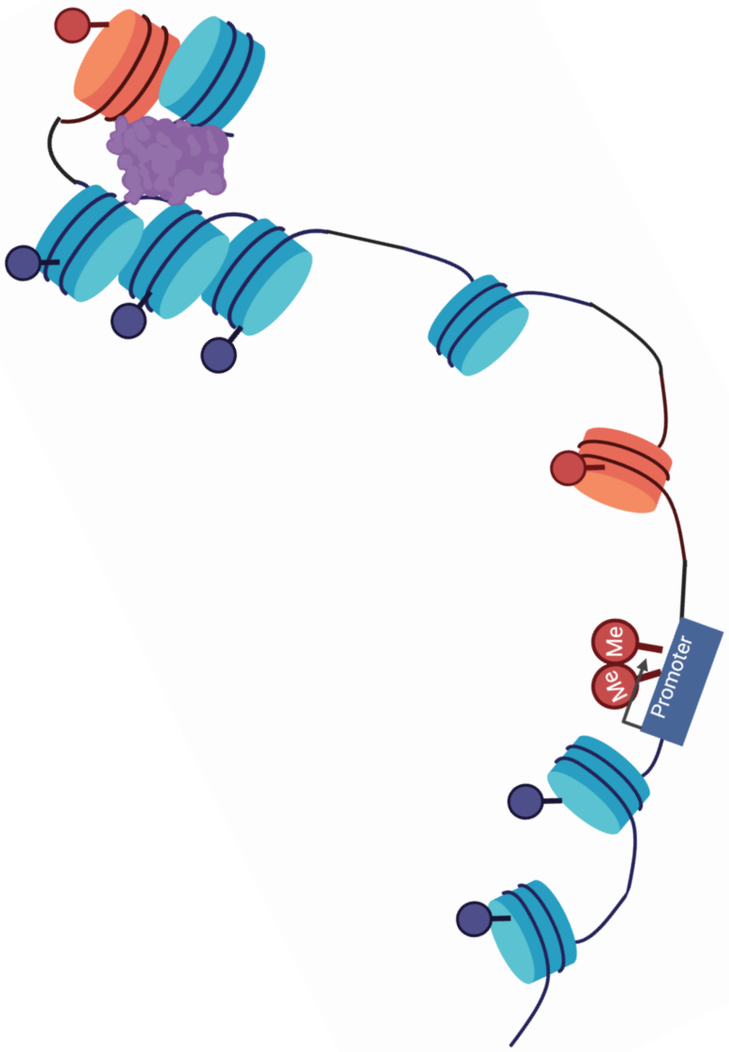
- Chromatin structure
- Epigenetic changes

The Aging Epigenome

- Nucleosomes
- Histone PTMs
- DNA Methylation
- Heterochromatin
- Relocalization of Chromatin Modifiers

Reprogramming the Epigenome

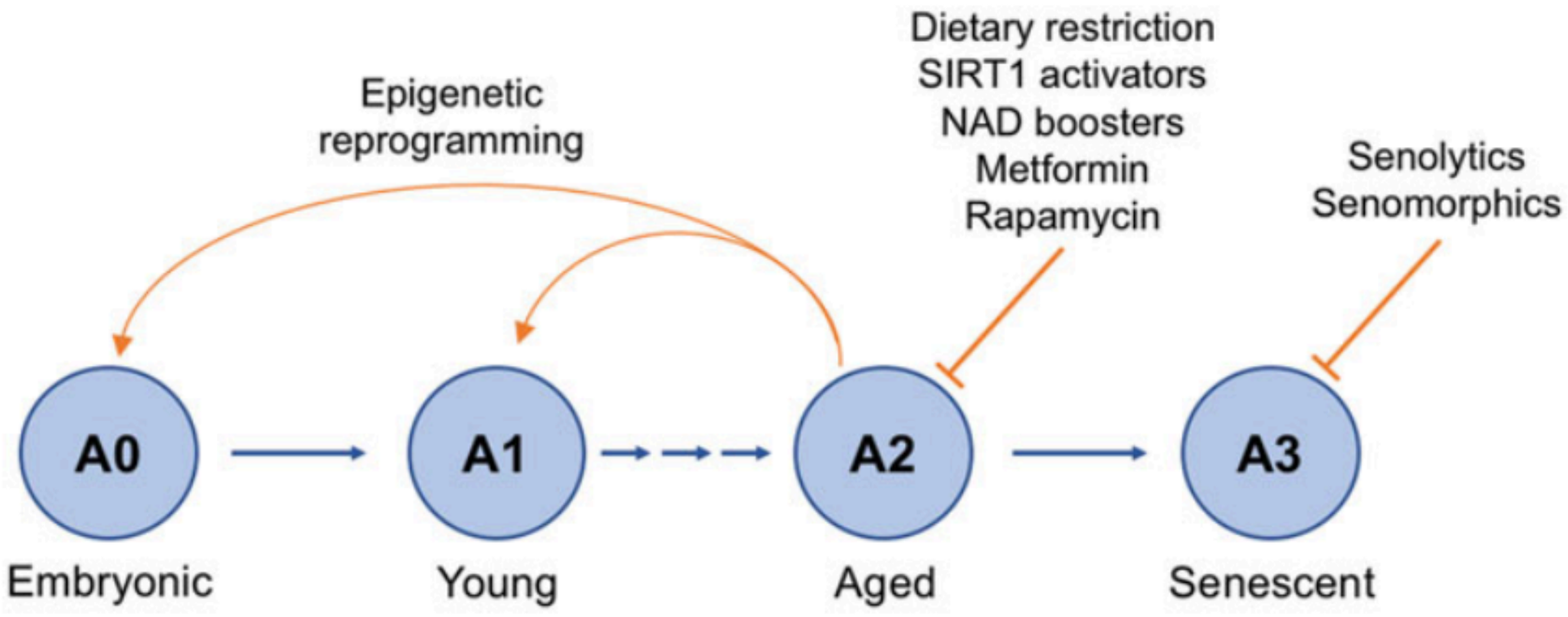
- Calorie Restriction
- Small Molecules
- Cellular Reprogramming



Slowing and Reversing Epigenetic Change

There are **four main strategies** for slowing and reversing epigenetic change:

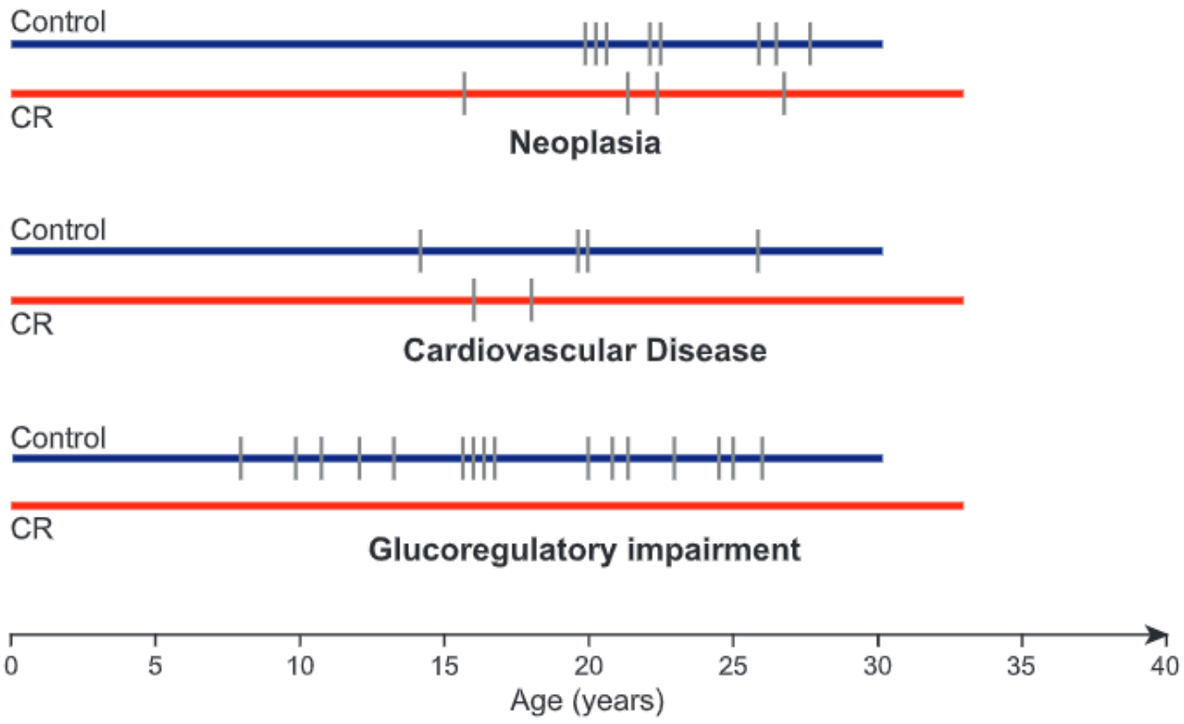
Calorie restriction, small molecules, overexpression of epigenetic regulators and cellular reprogramming

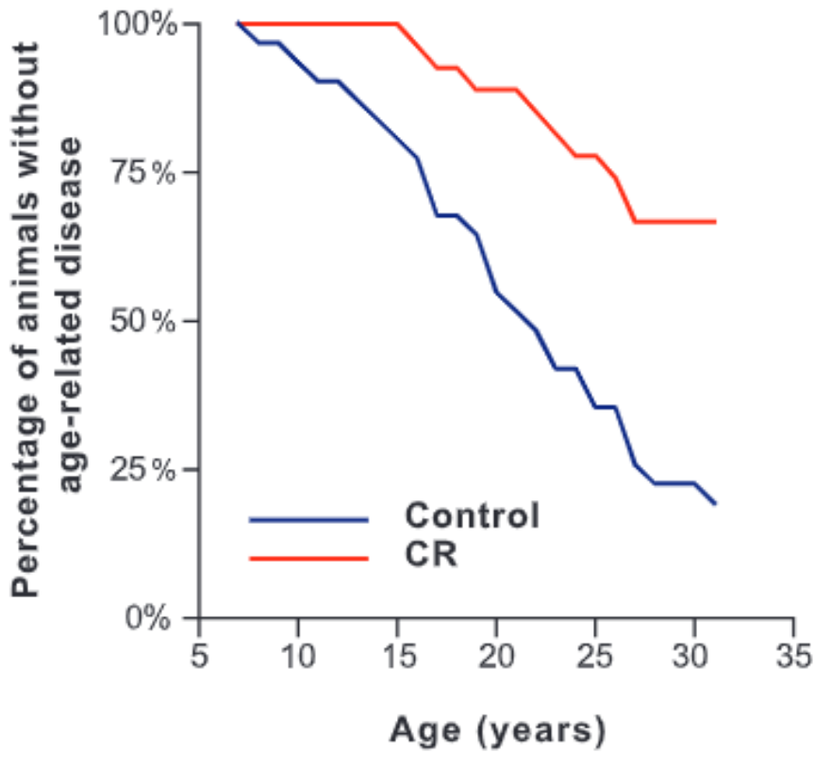


Oberdoerffer, P.; Sinclair D. A. *Nat. Rev. Mol. Cell Biol.* **2007** *8,* 692

Calorie Restriction

Calorie restriction, a reduction in food intake without malnutrition, is the strongest intervention for increasing lifespan across a wide range of species



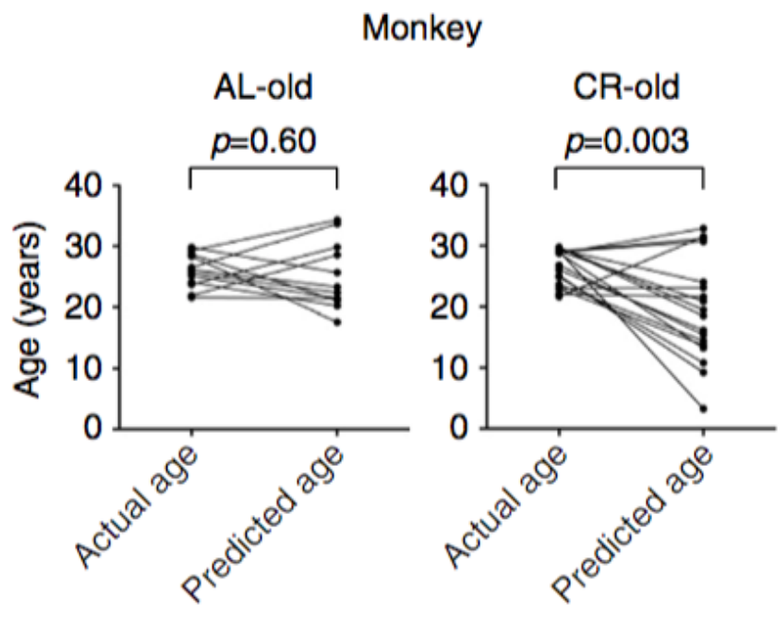


First study in primates with 30% calorie restriction showed a remarkable **difference in the onset and prevalence of age-related conditions**

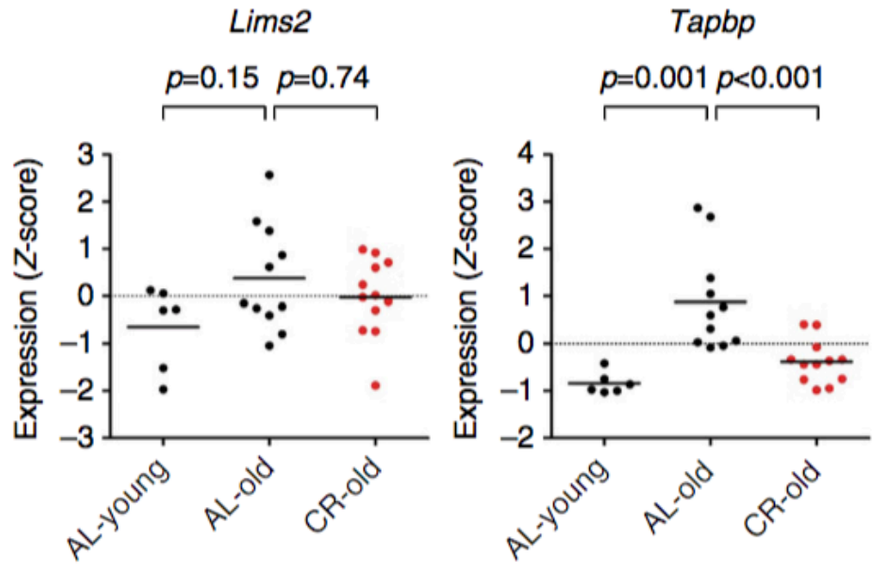
Colman, R. J.; Anderson, R. M.; Johnson, S. C.; Kastman, E. K.; Kosmatka, K. J.; Beasley, T. M.; Allison, D. B.; Weindruch, R. et al *Science* **2009**, *325,* 201.

Calorie Restriction and DNA Methylation

Rhehus macaques exposed to 30% calorie restriction for 15–20 years showed ***less epigenetic methylation drift***

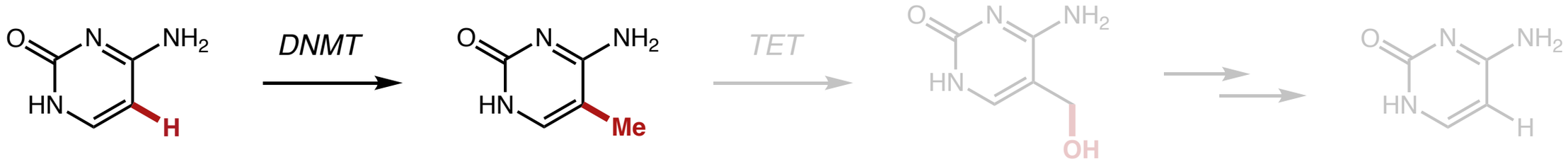


Effects of caloric restriction on DNA methylation was correlated with ***gene expression changes*** *in mouse liver*

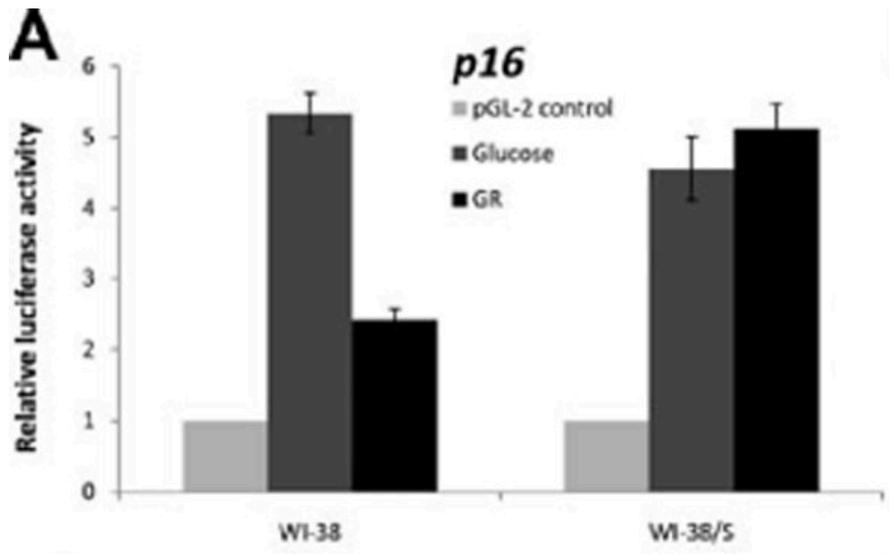


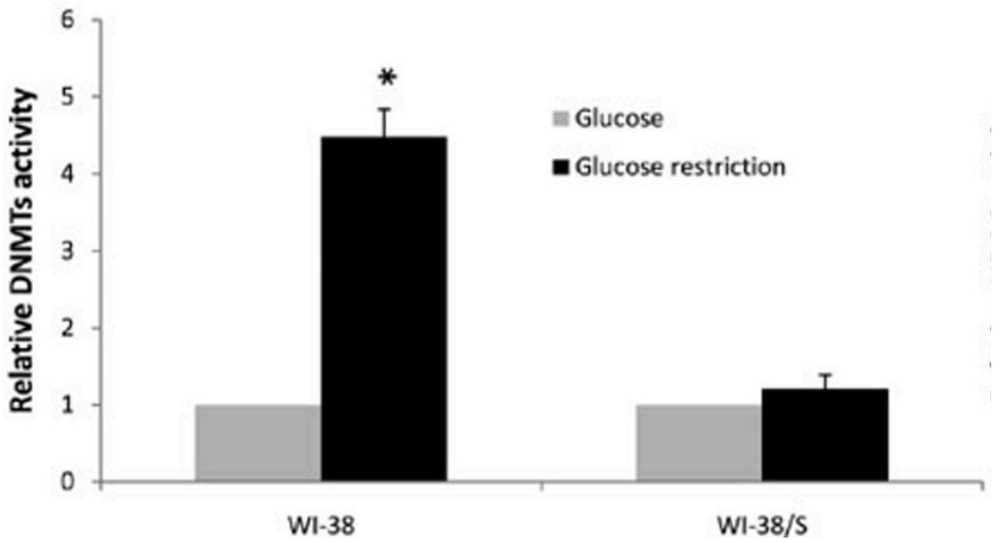
Maegawa, S.; Lu, Y.; Tahara, T.; Lee, J. T.; Madzo, J.; Liang, S.; Jelinek, J.; Colman, R. J.; Issa, J.-P. J. *Nat. Comm.* **2017**, *8*, 1

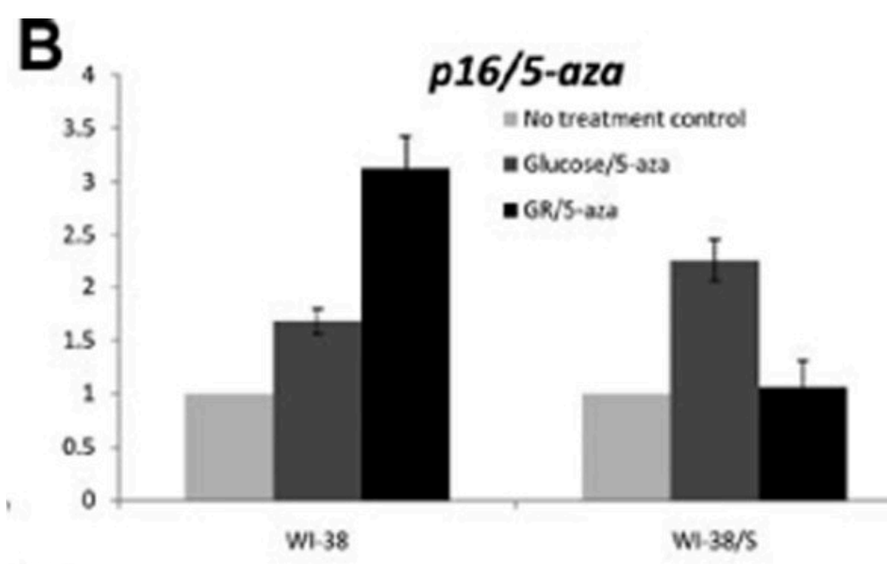
How does Calorie Restriction effect DNA Methylation?



Glucose restriction **increases DNMT 1 activity**, leading to hypermethylation and subsequent **repression of age-related genes** such as p16

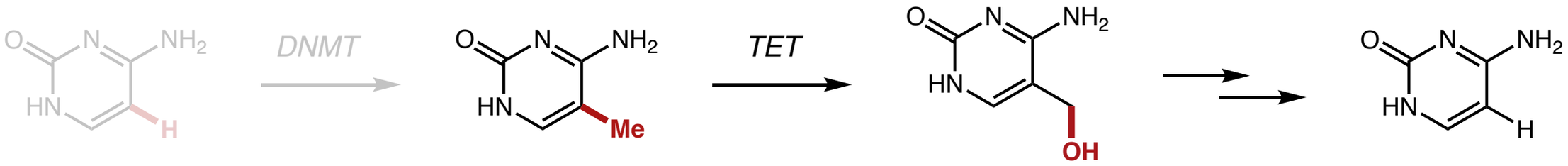




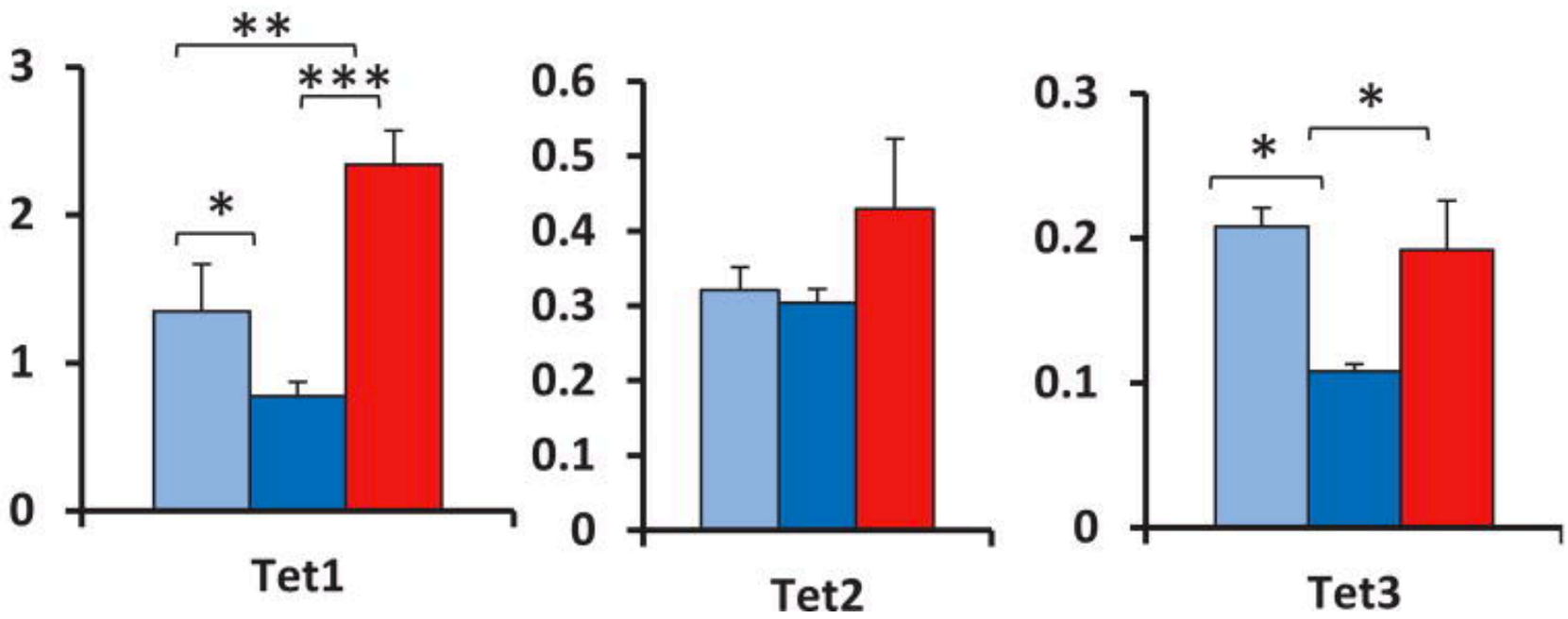


Li, Y.; Liu, L.; Tollefsbol, T. O. *The FASEB Journal* **2010**, *24*, 1442

How does Calorie Restriction effect DNA Methylation?



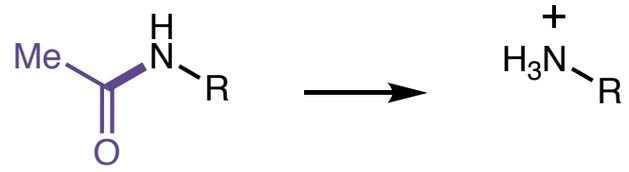
Calorie restriction causes **increase in gene expression of methylcytosine deoxygenate TET1 and TET3** in mouse colon mucosa

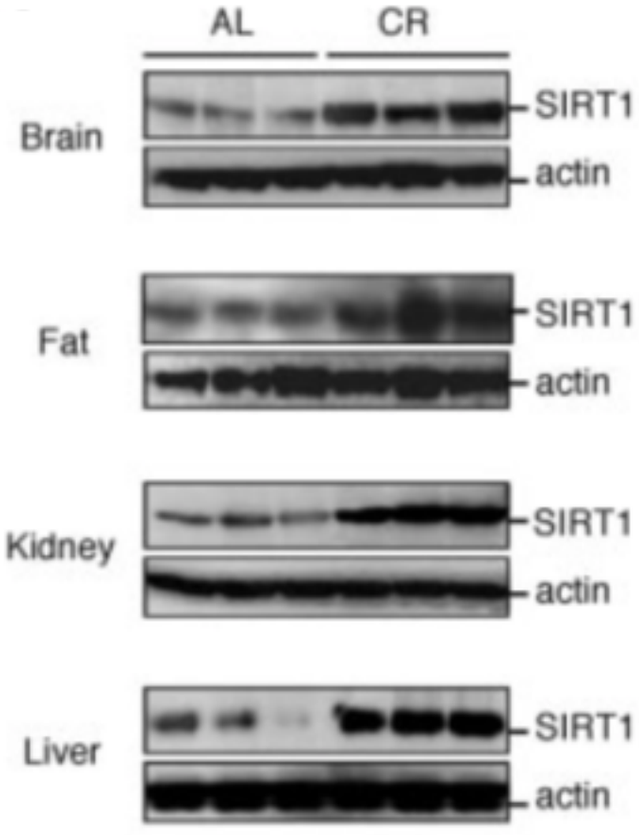


Unnikrishnan, A.; Hadad, N.; Masser, D. R.; Jackson, J.; Freeman, W. M.; Richardson, A. *Ann N Y Acad. Sci.* **2018**, *1418*, 69

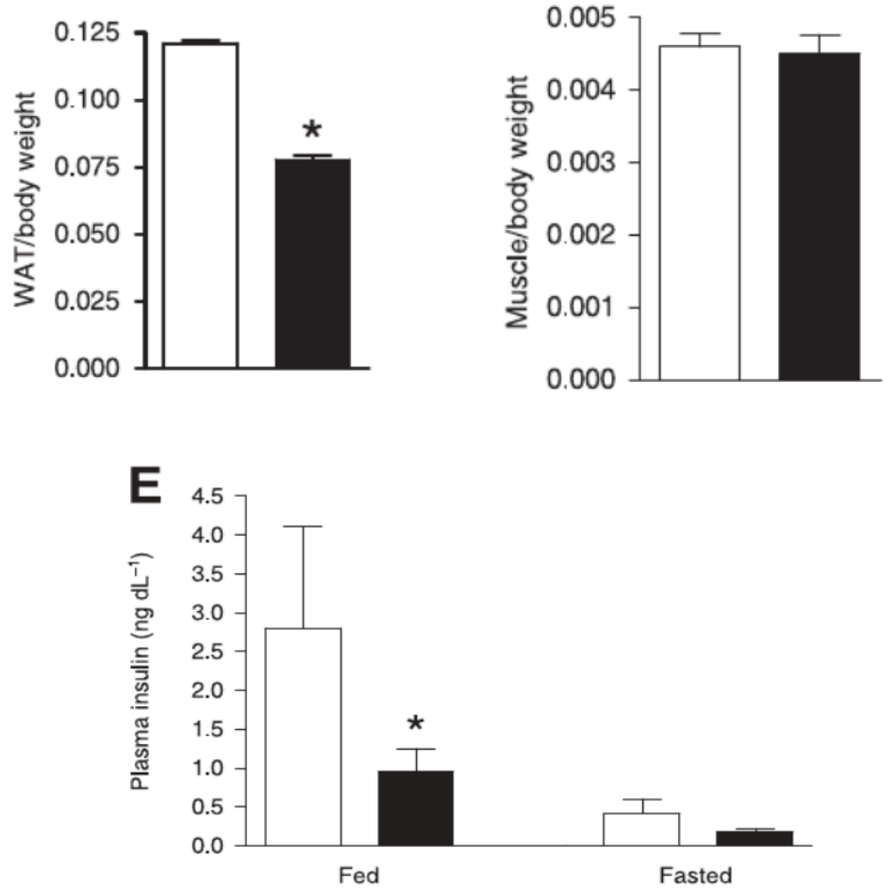
Calorie Restriction and Sirtuins

Many of the sirtuin family (SIRT1-SIRT7) which are deacetylases have shown to be regulators of aging and calorie restriction





Calorie restriction induces SIRT1 expression in mammalian cells



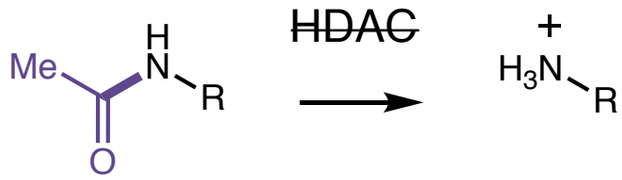
Overexpression of SIRT1 mimics calorie restriction phenotypes, delaying aging in mice

Bordone, L.; Cohen, D.; Robinson, A.; Motta, M. C.; van Veen, E.; Czopik, A.; Steele, A. D.; Crowe, H.; Marmor, S. et al *Aging Cell* **2007**, 6, 759

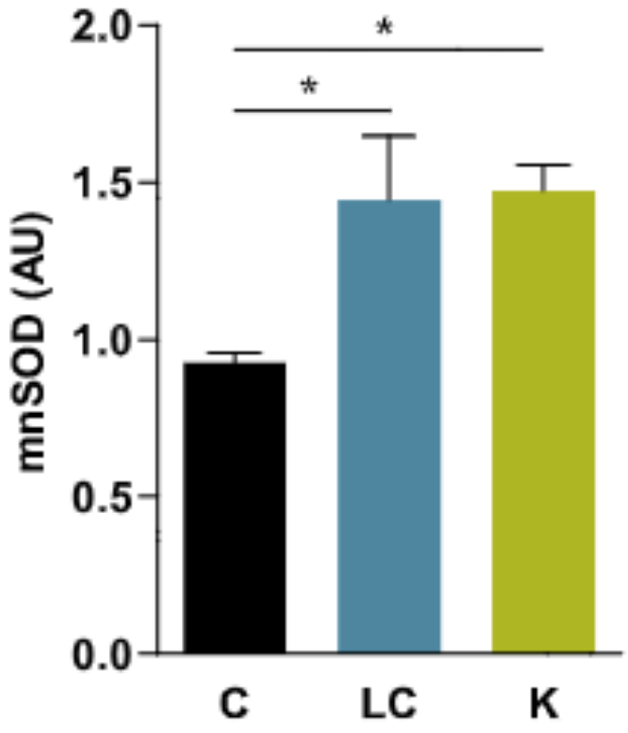
Cohen, H. Y.; Miller, C.; Bitterman, K. J.; Wall, N. R.; Hekking, B.; Kessler, B.; Sinclair, D. A. et al *Science* **2004**, *305*, 390

Calorie Restriction and other HDACs

Calorie restriction inhibits HDAC activity, and increases H3Lys9 acetylation, a marker of active transcription



Upregulates transcription factor FOXO3 and its targets, which activate antioxidant responses



Prevents accumulation of cellular damage during aging

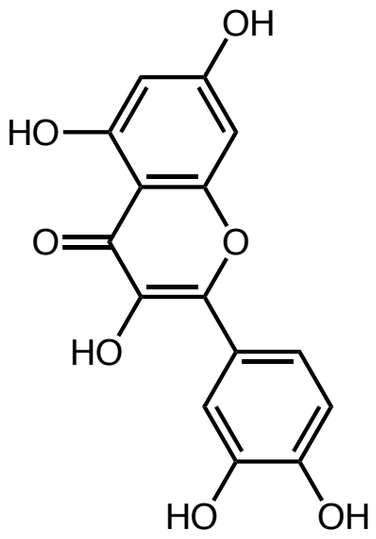
mnSOD = protein of target gene
Of FOXO3

Roberts, M. N.; Wallace, M. A.; Tomilov, A. A.; Zhou, Z.; Marcotte, G. R.; Tran, D.; Perez, G.; Lopez-Dominguez, J. A. et al *Cell Metab.* **2017**, *26*, 539

Cao, T.; Zhou, X.; Zheng, X.; Cui, Y.; Tsien, J. Z.; Li, C.; Wang, H. *Front. Aging Neurosci.* **2018**,

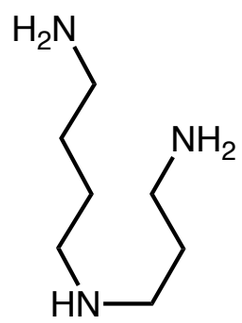
Chemicals Against Aging

Small molecule approaches that mimic the effects of calorie restriction:



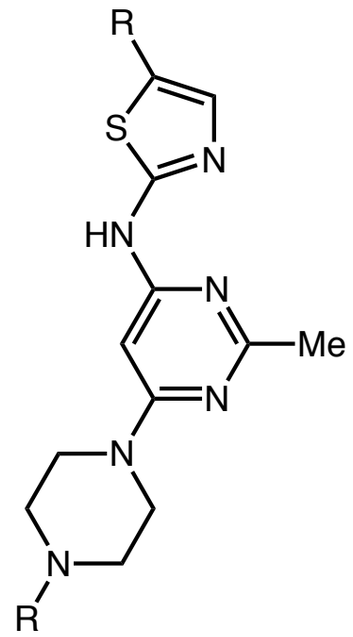
Sirtuin-activating compounds (STACs)

Activate SIRT1



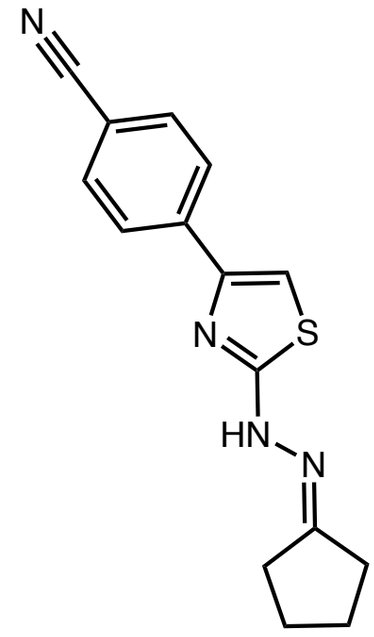
Histone deacetylase (HDAC) Inhibitors

Inhibit HDACs



Senolytics

Ablate senescent cells

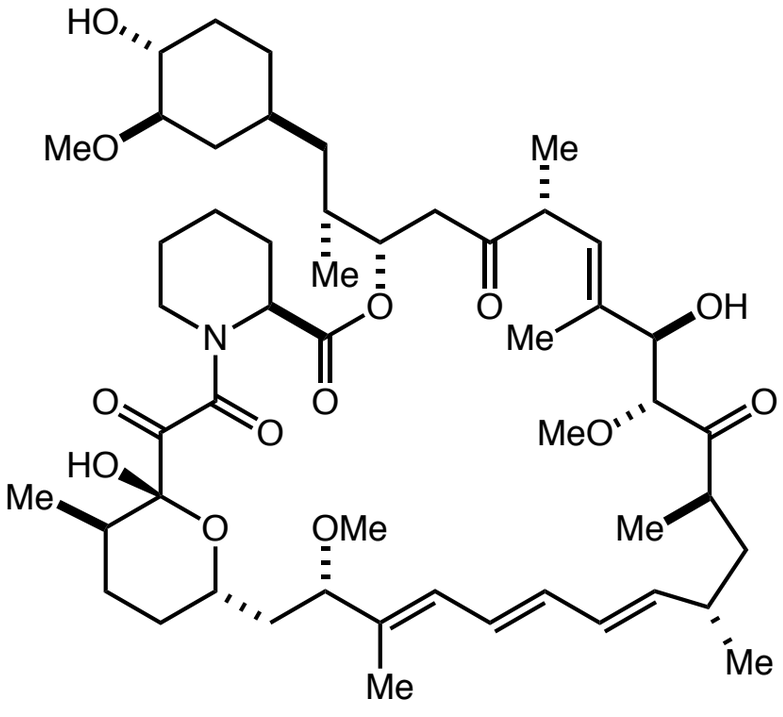


Other

Compound dependent

Zhang, W.; Qu, J.; Liu, G.; Belmonte, J. C. I. *Nat. Rev. Mol. Cell Biol.* **2020**, *21*, 137

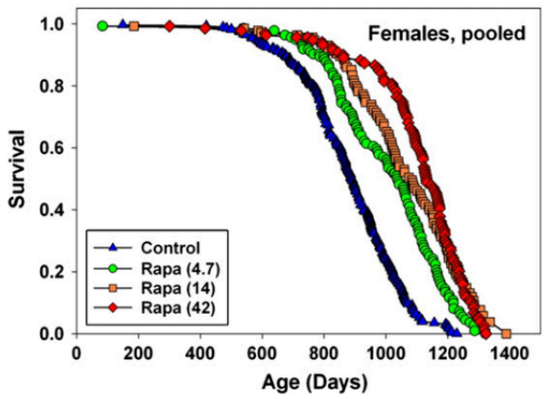
Example: Rapamycin



An mTOR inhibitor which is used in treatment of multiple conditions

Rapamycin = general anti-aging drug?

Rapamycin **extends lifespan by 10-30%** in mice



Rapamycin **suppresses cell senescence** and **delays or treats most age-related diseases**

e.g cancer, neurodegeneration

PERSPECTIVE | ALZHEIMER'S DISEASE

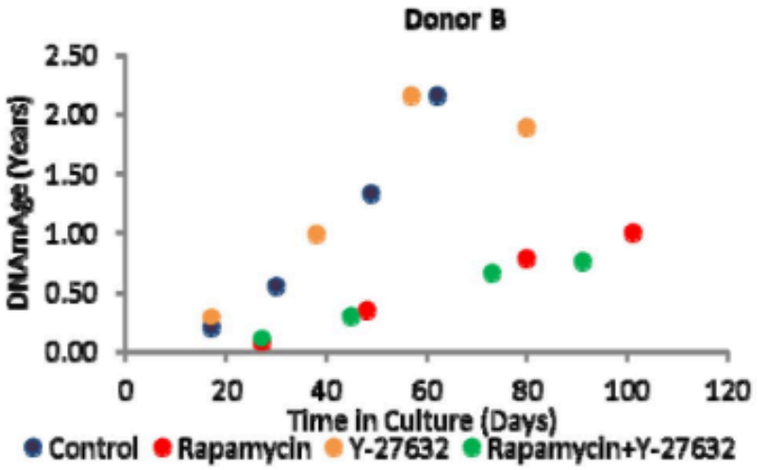
Rapamycin and Alzheimer's disease: Time for a clinical trial?

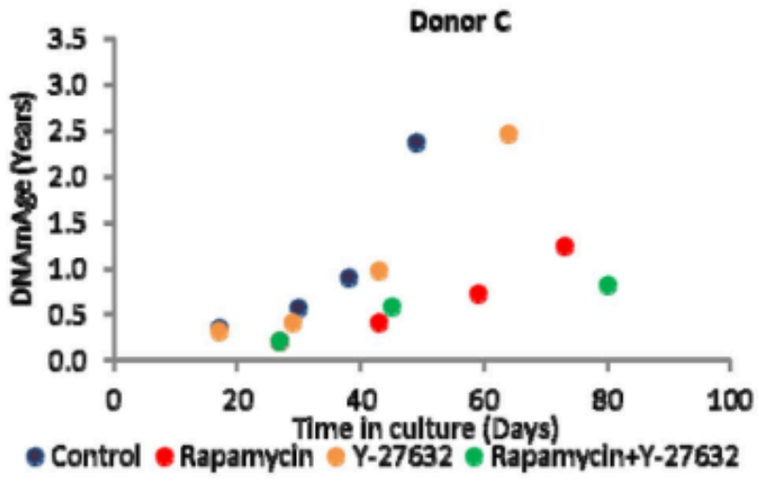
Blagosklonny, M. V. *Aging* **2019**, *11,* 8048

Miller, R. A.; Harrison, D. E.; Astle, C. M.; Fernandez, E.; Flurkey, K.; Han, M.; Javors, M. A.; Li, X. et al. *Aging Cell* **2014**, *13,* 468

Example: Rapamycin

Rapamycin causes changes to histone modifications, heterochromatin and gene silencing





Rapamycin shown to slow epigenetic aging in human keratinocytes independently of its effects on replicative senescence and proliferation

the life-extending property of rapamycin may include suppression of epigenetic aging

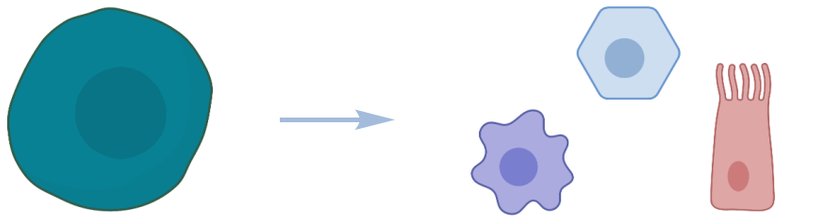
Kane, A. E.; Sinclair, D. A. *Crit. Rev. Biochem. Mol. Biol.* **2019**, *54*, 61

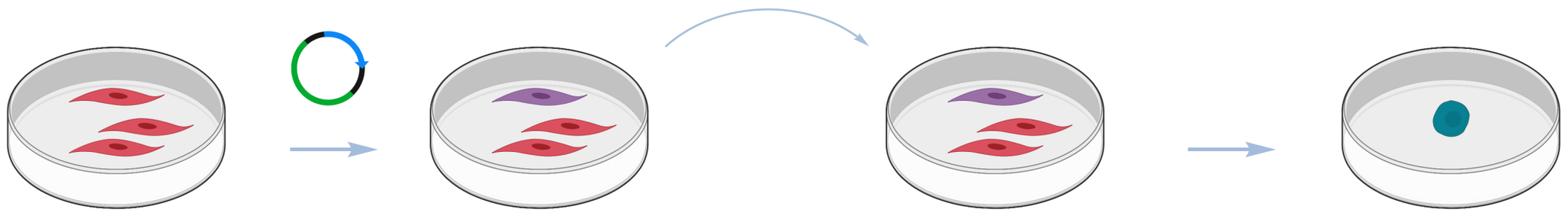
Horvath S.; Lu, A.T.; Cohen, H.; Raj, K. *Aging* **2019**, *11*, 3238

Cellular Reprogramming via iPSC overview

Human induced pluipotent stem cells are another strategy to reprogram the epigenome

Pluripotent stem cells = proliferative, unspecialized cells with the capacity to differentiate into many other different cell types in the body





Induced pluripotent stem cells (iPSCs) have been **generated through forced expression of four transcription factors** (Oct4, Sox2, cMyc, Klfc - OSKM) that are known to maintain pluripotency during embryonic development

http://www.addgene.org/collections/stemcell/

Cellular Reprogramming via iPSC overview

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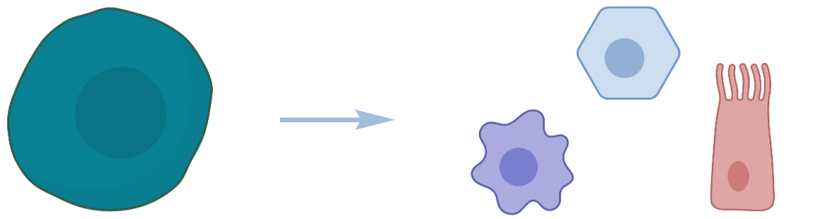
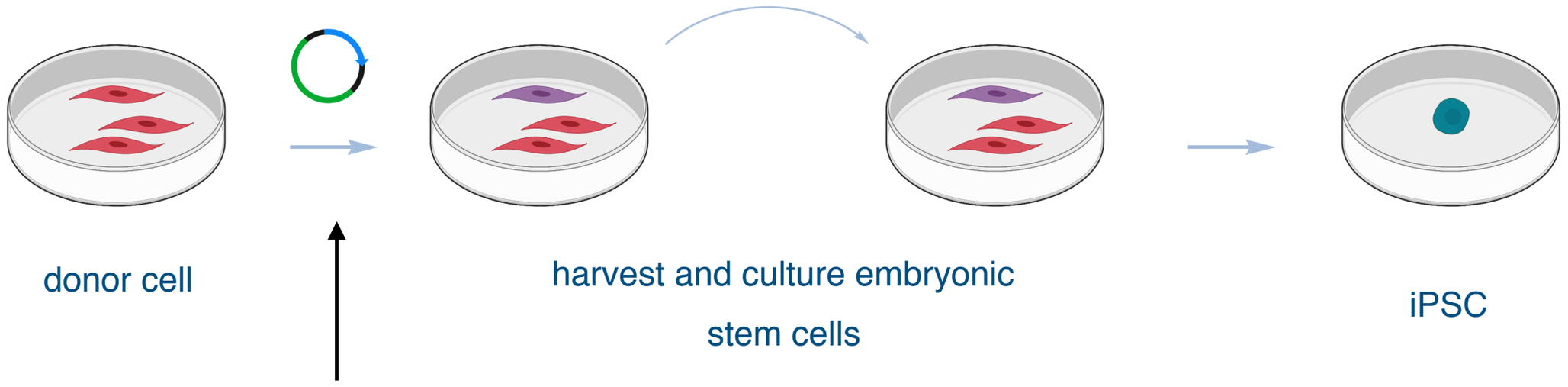
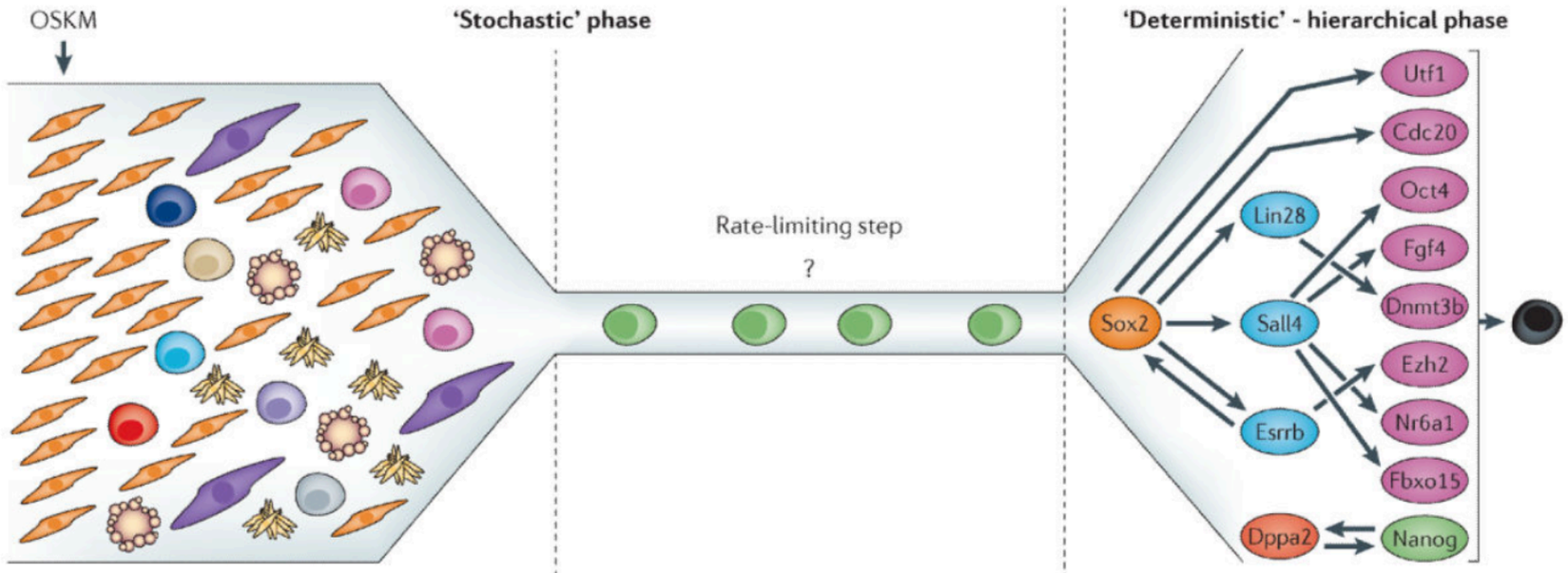




http://www.addgene.org/collections/stemcell/

Molecular Mechanisms of Reprogramming

Reprogramming believed to occur in two phases: **stochastic** and **deterministic**



Once activated, these pluripotent proteins maintain the induced pluripotent stem cell (iPSC)

After induction with OSKM, stochastic gene expression leads to multiple cell fates

In rare cases in reprogrammable cells, early pluripotency genes become activated leading to Sox2 activation

Sox2 leads to activations of the rest of core pluripotency circuitry

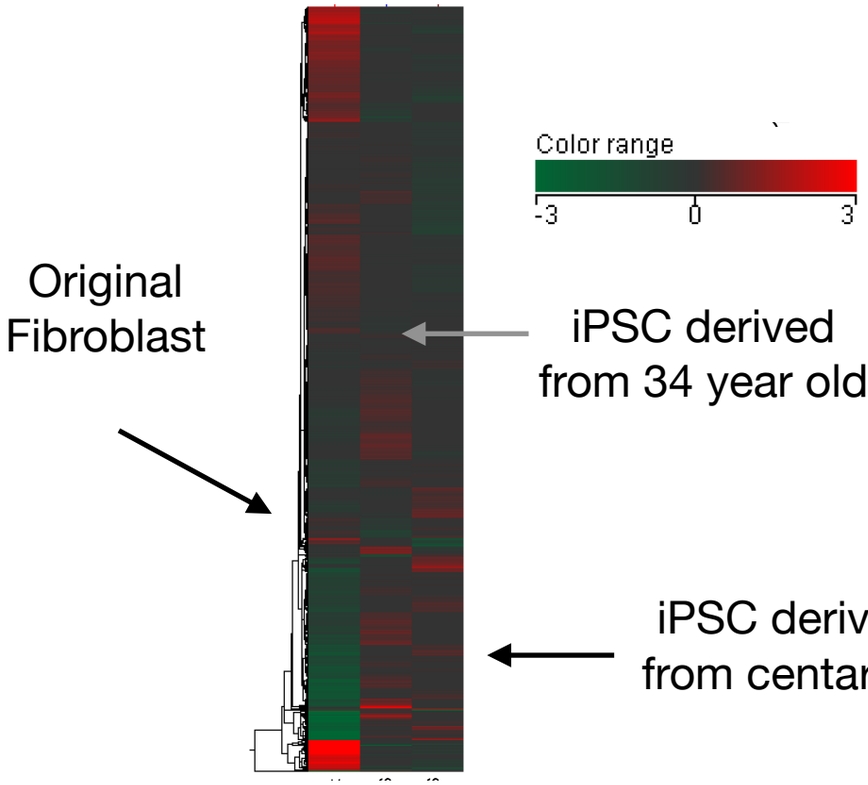
Major epigenetic remodeling in both phases	*Stochastic:* H3K4me3/H3K27me3	*Deterministic:* miRNA changes and DNA methylation

Buganim, Y.; Faddah, D. A.; Jaenisch, R. *Nat. Rev. Genet.* **2013**, *14* , 427

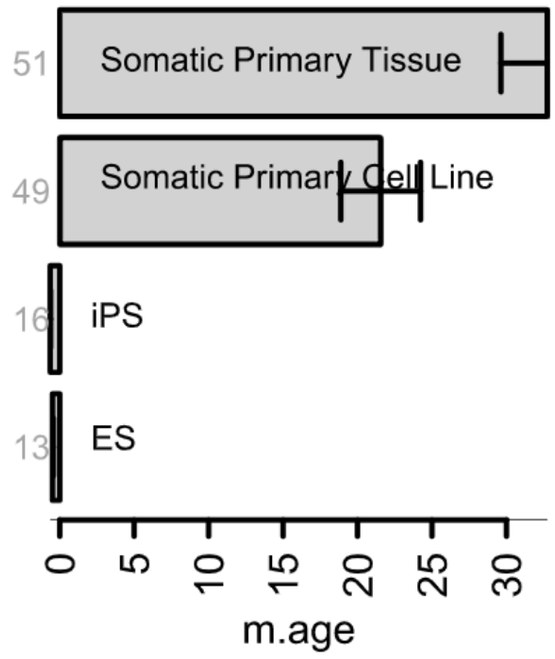
Polo, J. M.; Anderssen, E.; Walsh, R. M.; Schwarz, B. A.; Nefzger, C. M.; Lim, S. M.; Ramaswamy, S.; Hochedlinger, K. et al *Cell* **2012**, *151*, 1617

Cellular Reprogramming In Vitro

Epigenetic changes of aging can be slowed or reversed by reprogramming in human cells



Human senescent + centarian fibroblasts returned to iPSCs **restores gene expression profiles to those of young cells**



Horvath DNA methylation clock is reset in iPSCs to that of embryonic stem cells

Horvath S. *Genome Biol*. **2013** 14:R115

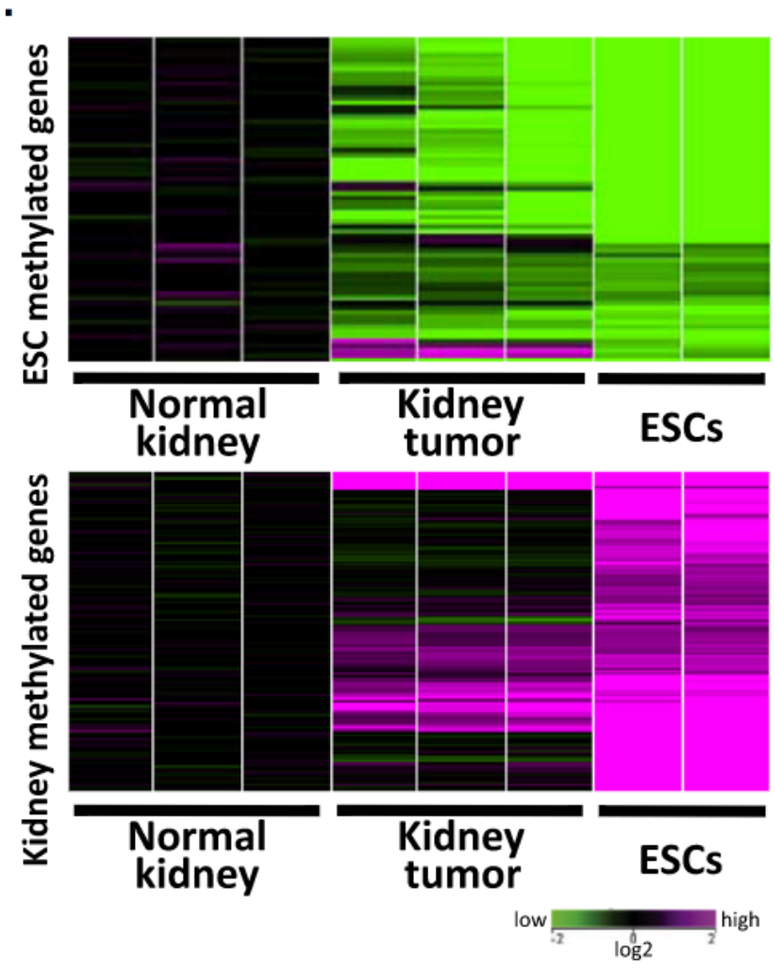
Yagi, T.; Kosakai, A.; Ito, D.; Okada, Y.; Akamatsu, W.; Nihei, Y.; Nabetani, A.; Ishikawa, F.; Arai,Y.; Hirose, N. et al. *PLoS One.* **2012** 7:1

Cellular Reprogramming In Vivo

Yamanaka factors can induce in vivo reprogramming but not without side effects

Teratoma formation is generally a big safety issue

Whole-body introduction of OSKM factors in mice led to Nano expression in many tissues, but results in tumor development (teratomas)



altered epigenetics relating to somatic cell reprogramming drove tumorigenesis

Ohnishi, K.; Semi, K.; Yamamoto, T.; Shimizu, M.; Tanaka, A.; Mitsunaga, K.; Okita, K.; Osafune, K.; Yamada, Y. et al *Cell* **2014**, *156*, 663

Conclusions and Outlook

Elucidated many patterns of change of epigenetics during aging, but better understanding of specific rather than global change needed

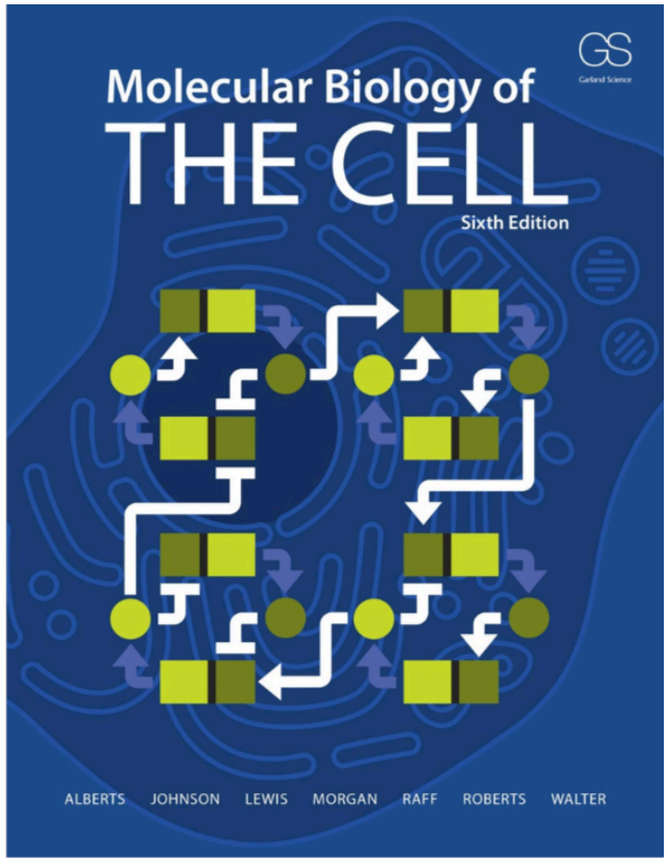
→ *More research needed on understanding how these epigenetic changes cause aging and what drives them*

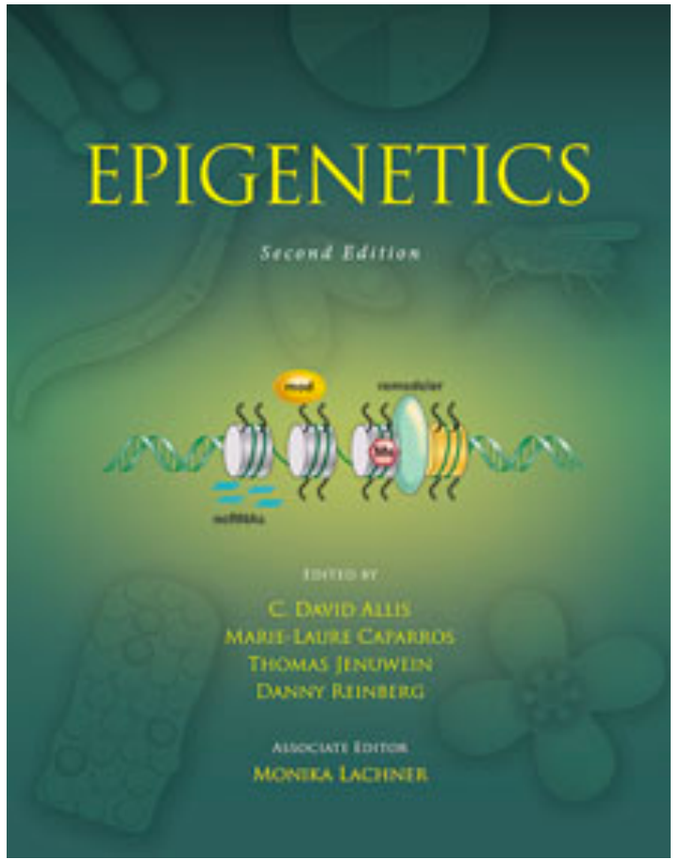
If epigenetic changes do contribute to aging, then we have exciting results that these changes can be prevented or reversed

→ *Further study on the epigenetic mechanisms underlying these intervention strategies will open new avenues for therapeutic strategies*

Kane, A. E.; Sinclair, D. A. *Crit. Rev. Biochem. Mol. Biol.* **2019**, *54*, 61

Questions?





Epigenetic changes during aging and their reprogramming potential:

Kane, A. E.; Sinclair, D. A. *Crit. Rev. Biochem. Mol. Biol.* **2019**, *54*, 61

The ageing epigenome and its rejuvenation:

Zhang, W.; Qu, J.; Liu, G.; Belmonte, J. C. I. *Nat. Rev. Mol. Cell Biol.* **2020** *21*, 137