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Metabolism, Longevity and Epigenetics

Claudia Cosentino¹ and Raul Mostoslavsky^{1,*}

¹The Massachusetts General Hospital Cancer Center-Harvard Medical School, 185 Cambridge St, Boston, MA USA

Abstract

Metabolic homeostasis, as well as interventions that influence nutrients' uptake, are well-established means to influence lifespan even in higher eukaryotes. Until recently, molecular mechanisms to explain such an effect remained scantily understood. Sirtuins represent a group of protein deacetylases that depend on the metabolic intermediate NAD⁺ as a co-factor for their function. For this reason they sense metabolic stress and in turn function at multiple levels to exert proper metabolic adaptation. Among other things, sirtuins could perform as histone deacetylases inducing epigenetic changes to modulate transcription and DNA repair. Recent studies indicate that beyond sirtuins, the activity of other chromatin modifiers, such as histone acetyl transferases (HATs), might also be tightly linked to the availability of their intermediate metabolite Acetyl-CoA. In this study, we will summarize current knowledge depicting emerging concepts indicating a close crosstalk between the epigenetic machineries able to sense metabolic stress, their adaptive metabolic responses and their potential role in longevity.

1. EPIGENETICS, SENESCENCE AND LIFESPAN

The term *epigenetics* derives from the Greek *epi-* (ἐπί- over, above, outer) –*genetics* (genetikos – γενετικός-, which come from genesis – γένεσις-, "origin"), and indicates changes in gene expression due to chromatin and histone modifications rather than changes in the DNA sequence. Even though a proper definition is yet to be agreed upon, it is safe to consider "epigenetic" those changes, both heritable (genomic imprinting) and acquired, that can be propagated through meiosis and mitosis [1]. These changes include DNA methylation as well as histone modifications, such as acetylation and methylation. Modifications of DNA and histone tails are specifically recognized by chromatin-remodeling complexes, such as members of the SWI/SNF family or the Polycomb group (PcG) proteins, and eventually transcription factors that would influence activation or silencing of chromatin regions.

a. Chromatin modifiers

Chromatin has been canonically divided into euchromatin and heterochromatin, which contain respectively expressed -"open"- and silenced -"closed"- regions. More recently it became clearer that chromatin actually exists in three states: active, poised and inactive [2]. Each state is determined by specific histone modifications, combined in a particular manner, usually referred to as the *histone code*. Typically, trimethylation of lysine 9 on histone H3 (H3K9me3) acts as a repressive mark and can be found on both regulatory elements and at gene bodies. Also H3K9 dimethylation (H3K9me2) can be present at gene bodies and enhancers, while promoters exhibit trimethylation of H3K27 (H3K27me3) to maintain a repressed status. Together with these histone methylation marks, DNA methylation is found at regulatory elements as well. The active state of chromatin is instead characterized by DNA hypomethylation, di- and trimethylation of H3K4 at the promoters, H3K36

*Correspondence should be addressed to: rmostoslavsky@mgh.harvard.edu.

trimethylation (H3K36me3) and H3K79 dimethylation (H3K79me2) at the gene bodies, and H3K4 mono- and dimethylation (H3K4me1 and me2) at the enhancers together with H3K27 acetylation (H3K27ac) [2]. In between these two states, there are regions in the chromatin that are “poised” to be activated, and this is of particular importance during development and tissue differentiation. Poised promoters present both activating and repressing marks, such as H3K4me3, H3K4me2 and H3K27me3 [3]. More recently, poised enhancers have also been identified. These enhancers, particularly important in early developmental stages, are characterized by the presence of H3K27me3 and H3K4me1 [4]. Recent work from Zhao and colleagues has shown that H3K5 acetylation is another important histone mark for regulating gene expression, marking those genes whose transcription is repressed just before mitosis, but that are needed to be immediately re-expressed once mitosis is complete [5].

A general concept appears to be emerging, in which it is becoming clear that acetylation and methylation compete for the same residue on histones. Therefore, gene expression of a particular cell in a particular moment results from the concerted action of multiple enzymes, including histone deacetylases, acetyltransferases, demethylases and methyltransferases.

i. Histone deacetylases—Histone deacetylases (HDACs) includes 18 family members divided into 4 classes (class I, IIA/IIB, III and IV). Classes I, II and IV comprise the 11 canonical HDACs (HDAC 1 to 11) which are sensitive to the inhibitor trichostatin A (TSA), while class III includes sirtuins, NAD⁺ dependent deacetylases that are not inhibited by TSA [6,7].

I. HDACs: HDACs were first identified as histone deacetylases. Their activity is mostly associated with condensed and therefore inactive chromatin. Nonetheless, in recent years many non-histone targets have been identified. In this context, not all of the HDACs are exclusively nuclear. For instance, HDAC6 and HDAC10 (class IIB) are cytoplasmic, while several other HDACs are both nuclear and cytoplasmic [6]. So far HDAC1/2/3 are the only ones found exclusively in the nucleus. Among the non-histone targets of HDACs there are several important transcription factors, such as p53, Stat3, Gata1 and Hif-1 α [6]. It is not surprising that many tumors show an aberrant expression of these enzymes and to date several HDAC inhibitors are being tested in clinical trials for cancer therapy ([8], see also last section).

HDACs in Metabolism: With regards to metabolism, multiple studies have link HDACs to glucose metabolism. HDAC1/4/5 inhibit expression of the GLUT4-glucose transporter [9,10], thereby lowering insulin-induced glucose uptake. HDAC4/5 are also involved in glucagon response, via recruitment of HDAC3 and concomitant FOXO deacetylation at promoters of gluconeogenic genes [11]. Importantly, inhibition of class II HDACs in mouse models of type II Diabetes have been shown to reduce glycemia [11]. Recent studies have also shown that HDAC1 promotes HNF4 expression and FOXO1 activity in the HepG2 liver cell line, in turn increasing expression of gluconeogenic genes [12]. In this context, it remains unclear how these usually “repressing” enzymes cause activation of these genes. In addition, recent studies in cancer cells have shown that HDAC4 deacetylates Hif-1 α , increasing its stability and therefore the expression of a subset of Hif-1 α target genes [13]. Considering that many glycolytic genes are among Hif-1 α targets, this could open the possibility that HDACs may participate in the metabolic changes observed in cancer cells, as described in detail below.

HDACs, senescence and aging: Recent studies showed that reduced expression of HDAC1/2 is associated with senescence of human multipotent stem cells (MSCs) [14], whereas HDAC1/5/6 are downregulated upon aging in hematopoietic stem cells (HSC) [15]. Cellular senescence has been postulated as a potential mechanisms underlying organismal

aging [16]. However, the role of HDAC1 in senescent differentiated cells seems quite opposite to what has been reported for MSCs and HSCs. In fact, in both human melanocytes and fibroblasts, HDAC1 activity increases upon senescence [17,18]. Further, overexpression of HDAC1 in human cervical adenocarcinoma cells (HeLa) induces senescence by deacetylating the transcription factor Sp1, which in turn interacts with p300. The p300/Sp1 complex is recruited to genes such as p16^{INK4}, leading to activation of the pRb tumor suppressor gene, in this way triggering senescence [19]. A recent study from Miller and colleagues, showed that also in human primary fibroblasts, senescence is accompanied by an increase in HDAC1/2 activity, with a concomitant decrease in H3K56 acetylation [20]. This histone mark appears to play a role not only in replicative senescence, but also in oncogene-induced senescence (OIS), since hypoacetylation of H3K56 is observed upon overexpression of oncogenic Ras in primary fibroblasts. In contrast, H4K16ac is increased during OIS, but decreased upon replicative senescence. This difference might be explained by the observation that H4K16ac is rapidly lost at DNA damage sites but then it increases during DNA repair, leading the authors to propose that during OIS, cells try actively to repair their DNA, while spontaneously-senescent cells fail to do so [20]. This would be in agreement with one of the current theories of aging, which proposes that during aging there is a decline in the ability of these senescent cells to repair DNA damage [21].

Other possible roles have been described for HDACs in aging and senescence, as shown by studies on regenerative potential of the liver in young and old mice. The liver is a very peculiar organ, with the ability to regenerate itself upon partial hepatectomy [18]. The regenerative potential of the liver decreases during aging, a decline that has been linked to increased levels of the c/EBP α /Brm complex in livers from old mice [18]. This complex includes HDAC1, which causes H3K9 deacetylation on E2F-target gene promoters, silencing these genes and inhibiting proliferation in liver from old mice [18]. These data could also explain previous observations by Wang and colleagues, who showed that liver-specific HDAC1 overexpression leads to hepatic steatosis, a hallmark of aging in liver, by modulating expression of lipid metabolic genes [18]. Despite this clear link between HDAC1 and senescence, surprisingly HDAC1 is actually overexpressed in human cancer [22,23]. One possibility could be that, in the context of tumors, upregulation of HDAC1 leads to the repression of tumor suppressor genes, such as cEBP α , in turn increasing cell proliferation [18]. Overall, the precise role for HDACs in senescence and cell proliferation appears to be cell type- and tissue context-dependent, an important point when considering HDACs modulators as therapeutic agents, as discussed further below.

II. Sirtuins: Sirtuins are class III histone deacetylases. There are 7 mammalian sirtuins (SIRT1–7), all homologs to the yeast protein Silent Information Regulatory 2 (Sir2) [7]. Sir2 is a NAD⁺ dependent deacetylase with a crucial role in regulation of both yeast metabolism and lifespan. It was actually first identified in a screening for silencing factors [24], and later shown to be required for extension of replicative lifespan in yeast upon calorie restriction [25]. Sir2 in *Saccharomyces cerevisiae* forms an active complex with Sir4 and binds acetylated H4K16 [26,27]. Following Sir2 mediated deacetylation of H4K16, Sir3 is recruited to this residue, causing compaction and silencing of the region [28]. The seven mammalian sirtuins share partial homology at the catalytic domain. Based on the phylogenetic classification of sirtuins made originally by Frye [29], all the Sir2 homologs fall into four classes (I–IV), with mammalian sirtuins divided as follows: SIRT1, SIRT2 and SIRT3 (Class I); SIRT4 (Class II); SIRT5 (Class III) and SIRT6 and SIRT7 (Class IV). The deacetylation reaction catalyzed by sirtuins is NAD⁺ dependent, and leads the formation of O-acetyl ADP ribose (AADPR), which can be used as a donor group in ADP-ribosylation reactions [30]. For this reason some of the sirtuins could also exhibit ribosyl transferase activity. Overall these proteins differ among themselves for localization and activity. SIRT1, SIRT2, SIRT3 and SIRT7 *in vivo* have only deacetylase activity, SIRT4 is mostly an ADP-

ribosyl transferase, while SIRT6 exhibit both activities [7]. Strikingly, a recent study demonstrated that SIRT5 appears to function as a desuccinylase and demalonylase *in vitro* and probably *in vivo* [31,32]. Such activities have never been described before in mammals, and therefore future studies addressing their physiological role will likely draw much attention. In terms of localization, SIRT1, SIRT6 and SIRT7 are mostly nuclear, SIRT2 is cytoplasmic and SIRT3, SIRT4 and SIRT5 are mainly mitochondrial [7]. The role of mammalian sirtuins has been mostly elucidated through generation of knockout mice [33]. Loss of SIRT1 leads in the majority of cases to perinatal lethality with associated retinal, bone and cardiac defects [33]. SIRT2 KO mice have been shown to develop tumors in several tissue upon aging, in a phenotype linked to genome instability associated to increased mitotic defects [34]. SIRT3 germline knockout show metabolic defects in several tissues (e.g. liver and muscle) associated, to mitochondrial protein hyperacetylation [33]. Interestingly, conditional knockout mice lacking SIRT3 in either muscle or liver show the molecular but not the metabolic defects observed in the germline knockout mice [35]. Also SIRT4 and 5 deficient mice are born normally but develop metabolic defects [33]. SIRT6 KO mice die within 4 weeks after birth due to a severe hypoglycemia [36]. SIRT7 deficient mice exhibit cardiac defects and reduced lifespan in a strain-specific manner [33], and recent studies indicate that SIRT7 deacetylates H3K18, enhancing proliferation in the context of tumor cells [37]. Overall, these observations strongly support a major role for sirtuins in modulating metabolism and potentially lifespan in mammalian organisms (Figure 1). Given the focus of this review on epigenetics, we will now discuss in detail some of the major functions for the two main sirtuins in the nucleus, SIRT1 and SIRT6.

Sirtuins in metabolism: SIRT1 is the closest mammalian homolog of ySir2. PGC1 α and FOXOs proteins have been extensively characterized as SIRT1 targets. Their deacetylation leads to increased mitochondrial respiration and lipid oxidation through regulation of genes such as ERR α (mitochondria regulatory gene), IDH3 α (TCA cycle), Cyt-c and COXVa (respiratory chain genes), MCAD, CPT-1 β and PDK4 (fatty acid utilization) and PGC1 α itself [38]. This activity of SIRT1 is particularly relevant when higher energy levels are needed (i.e. exercise) or during CR. It has been reported that SIRT1 protein levels increase upon CR in several tissues [39,40]. However, in other tissues, such as skeletal muscle, SIRT1 activity increases without noticeable changes in protein content [41–43]. Canto et al. suggested that hyperactivation of SIRT1 in skeletal muscle in low nutrient/exercise conditions depends on the crosstalk with another key metabolic enzyme, AMPK [41]. Both CR and exercise cause higher consumption of ATP in cells, which determines an increase in the AMP/ATP ratio. AMPK is a holoenzyme that can bind both ATP and AMP, the latter increasing its catalytic activity. Therefore, during CR or upon exercise, the higher AMP/ATP ratio activates AMPK, pushing the use of lipids as an energy source in order to satisfy the cellular energy demands. In addition, activation of AMPK causes increased expression of nicotinamide phosphoribosyltransferase (Nampt). Nampt is the rate-limiting enzyme in NAD biosynthesis and its increase leads to higher NAD⁺ production, which in turn activates SIRT1 (discussed by Posavec et al. in this same review series). In addition, SIRT1 deacetylation and activation of PGC1 α increases mitochondria biogenesis in muscle [43]. Overall, upon conditions of nutrient scarcity or higher energy demands, AMPK and SIRT1 start a positive loop aiming to sustain metabolic adaptations required to survive under those conditions. Another important protein in the response to metabolic stresses is mTOR, a master regulator of cell growth and metabolism. It is well established that AMPK activation leads to mTOR downregulation, in turn shutting down energy consuming processes, such as protein and glycogen synthesis, both stimulated by mTOR. Recent work suggests that SIRT1 could regulate mTOR as well, inhibiting its activity in a TSC2 dependent manner [44]. Interestingly, recent studies indicate that SIRT1 stimulates fatty acid oxidation in muscle and adipose tissue in response to activation of the cAMP-PKA pathway, rather than

to changes in NAD⁺ levels [45]. cAMP-PKA activates SIRT1 through phosphorylation of a highly conserved residue -serine 434-, and this response is also part of a mechanism underlying temperature control in mice. Indeed, cAMP-PKA dependent phosphorylation of SIRT1, and therefore its activity, is increased upon cold challenge, and SIRT1 transgenic mice have a better adaptation to cold [45].

SIRT1 exerts profound effects in liver metabolism as well. Under condition of nutrient stress, SIRT1 deacetylation of PGC1 α and FOXO1 causes increased mitochondrial biogenesis and gluconeogenesis, in part through up-regulation of the gluconeogenic genes phosphoenolpyruvate carboxykinase (PEPCK) and glucose-6-phosphatase (G6Pase) [46]. In addition, SIRT1 increases fatty acid oxidation and inhibits lipogenesis and glycolysis in liver by modulating expression of another sirtuin, SIRT6 [46]. SIRT1 also deacetylates and inhibits the transcription factor HIF-1 α , thereby downregulating the expression of glycolytic genes [46]. Notably, the role for SIRT1 in gluconeogenesis has recently been challenged, with a study supporting a negative regulation of gluconeogenesis by SIRT1, secondary to up-regulation of Rictor, a component of the mTORC2 complex [47]. Further confirmation for a role of SIRT1 in regulating lipid metabolism comes from the analysis of liver-specific SIRT1 KO mice. These animals develop increased insulin resistance, decrease fatty acid oxidation and hepatic steatosis, secondary to PGC1 α inhibition [46], suggesting SIRT1 as a potential target against obesity and obesity-associated diseases. In support of this theory, treatment of mice with Resveratrol (RSV), a SIRT1 activator, improved their mitochondrial function, reducing the occurrence of diet-induced obesity and its associated insulin resistance [38]. More recently, a clinical study on 11 obese men with no other medical condition showed that 30 days of RSV treatment was sufficient to improve glucose homeostasis, mitochondrial function and systolic pressure in these patients [48]. These results support previous studies showing that SIRT1 overexpression prevents diabetes in mice [46]. Even though the clinical study with RSV was performed in a small cohort of human patients, it suggests that sirtuin activators could provide potential therapeutic benefit in the context of diabetes and obesity.

Another nuclear sirtuin with a very strong effect on metabolism is SIRT6. SIRT6 null mice die within 28 days from birth because of a severe hypoglycemia [36]. SIRT6 deficiency leads to a dramatic increase in glucose uptake both in culture cells as well as in brown adipose tissue and skeletal muscle in mice, explaining the hypoglycemic phenotype in these animals [49]. At the molecular level, SIRT6 functions as an histone H3K9 deacetylase to repress expression of multiple glycolytic genes, acting as a co-repressor of the transcription factor Hif1 α [49]. Among the genes affected, SIRT6 deficiency causes upregulation of the glucose transporter Glut-1 -responsible for the increase in glucose uptake-, phosphofructokinase 1 (PFK1), lactate dehydrogenase A and B (LDHA and B) and pyruvate dehydrogenase kinase (PDK1 and 4). From a biological standpoint, SIRT6 deficiency triggers a shift towards aerobic glycolysis, with increased production of lactate, and reduced mitochondrial respiration. The metabolic changes observed in Sirt6-deficient cells are reminiscent of the metabolic shift observed in cancer cells, which in fact rely on lactate glycolysis rather than mitochondrial respiration for their ATP production even in the presence of oxygen, the so-called *Warburg effect*. In agreement with this considerations, recent studies demonstrated that SIRT6 is indeed a powerful tumor suppressor, acting as a co-repressor of both Hif1 α and Myc to modulate glycolysis and protein synthesis [50]. SIRT6 has also been specifically deleted in liver. These animals developed fatty liver with increased triglyceride synthesis, secondary to higher expression of genes involved in lipid metabolism, including fatty acid translocase (FAT). As shown previously for glycolytic genes, SIRT6 appears to control H3K9 acetylation at the promoters of these genes. These results are in line with the observation that SIRT6 transgenic mice exhibit lower levels of cholesterol and triglycerides, compared to control animals [51]. Overall, the above results

indicate that SIRT6 plays a major role in controlling cellular metabolism, adapting glucose and lipid metabolism to promote survival under conditions of nutrient stress and counteracting the metabolic switch observed in cancer cells. Altogether, these observations support a critical role for SIRT6 in the context of normal as well as transformed cells. Future studies should provide further mechanistic details on its function, in order to translate this information into therapeutic and/or diagnostic tools, as we will further discuss below.

Sirtuins in aging: The role of the yeast Sir2 protein in lifespan may depend on its ability to compact chromatin, a function of particular importance at subtelomeric regions. Work from Dang and colleagues showed that H4K16 acetylation plays a key role in regulating lifespan of yeast cells [52]. They reported an age-associated increase in H4K16 acetylation, in particular at subtelomeric regions, which is accompanied with reduction of Sir2 protein levels. More importantly, Sir2 deletion in yeast and H4K16 mutation are epistatic in reducing cellular lifespan. It is important to consider that telomere dysfunction is associated with aging and genome instability both in yeast and higher eukaryotes. Telomeres are repetitive sequences at the end of chromosomes that are actively organized into structures that avoid recognition of such ends by DNA damage signaling pathways. Failure to maintain such structures leads to activation of DNA repair pathways and consequently p53-mediated senescence [53]. Together with changes in H4K16 acetylation, Dang and colleagues observed an age-dependent reduction of H3K56 acetylation at the same subtelomeric regions. Loss of this histone mark has been previously associated with genome instability [54], supporting the hypothesis that changes in histone acetylation at subtelomeric region might contribute to telomere-mediated aging.

The role that Sir2 homologs play in lifespan modulation in other species is as yet controversial. In *Caenorhabditis elegans*, overexpression of Sir2.1 was reported to induce a 15–50% increase in lifespan [55], however recent studies have challenged this notion, indicating that most of the effect was linked to a mutation in another locus. Indeed, when such a mutation was eliminated, the effect of Sir2.1 was either completely lost or minimal [56,57]. Other studies in *C. elegans* show that calorie restriction (CR) extends lifespan in worms; this effect depends on Sir2.1 activity since Sir2.1 mutations abolished the CR-mediated lifespan extension [56,57]. Overall, future studies will be important in deciphering the precise role for sirtuins in modulating lifespan in worms, and whether such discrepancies were due to differences in the assays or the strains utilized.

A similar controversy arises when we consider the effect of dSir2 on lifespan in *Drosophila melanogaster*. Rosenberg and Parkhurst showed that dSir2 is a histone deacetylase playing an important role in heterochromatic silencing, interacting with Deadpan (Dpn), a member of the bHLH euchromatic repressors that control sex determination and segmentation in *Drosophila*. For these reasons, loss of dSir2 led to sex specificity lethality, and the heterozygous flies showed both segmentation and sex ratio defects [58]. Further, dSir2 also participates in maintenance of heterochromatin silencing through its interaction with the PcG-complex histone methyltransferase E(Z) [7,59]. However, other groups were unable to demonstrate these effects [60]. It also remains controversial whether dSir2 influences lifespan in flies. One study has shown that overexpression of dSir2 in several fly strains extends lifespan [61], however other groups failed to observe such a phenotype [57,64].

In mammals, the precise role for sirtuins in lifespan regulation is yet to be fully defined. Although a direct effect for Sirt1 in lifespan extension is yet to be described, Sirt1 regulates multiple age-related pathways that suggest a beneficial healthspan effect for this sirtuin. For instance, Sirt1 deficiency in liver has been associated with an increased production of reactive oxygen species (ROS) [47]. ROS accumulation leads to oxidation of macromolecules in cells, including DNA, structural protein and lipids. Notably, oxidative

damage and impaired redox control are among the factors that has been linked to aging in several organisms, suggesting that SIRT1 might play a role in modulating lifespan through regulation of ROS. In support of this hypothesis, SIRT1 deficiency is associated with increased replicative senescence in human primary fibroblasts [62], and SIRT1 protein levels decreases in culture cells upon senescence, at least in part due to a negative feedback loop between PPAR γ and SIRT1 [63]. A separate study evaluated how SIRT1 positioning changes on chromatin during aging. Oberdoerffer and colleagues observed that during aging, SIRT1 redistributes on chromatin, moving from its gene-targets towards site of DNA breaks, in this way affecting not only gene expression but also genome stability. Interestingly, the changes SIRT1 undergoes during aging are overlapping with those occurring during DNA damage, in particular those observed upon oxidative damage [64]. The role of SIRT1 in DNA damage is also supported by other studies showing that SIRT1 is involved in DSBs repair through deacetylation of NBS1, in nucleotide excision repair (NER) through deacetylation of XPA and XPC and in telomere maintenance by modulation of histone modifications [65]. *In vivo* data also demonstrated that SIRT1 overexpression in the context of p53 deficiency in mice improved post-irradiation survival and protected against tumorigenesis, supporting a role for SIRT1 in maintenance of genome stability upon oxidative damage [64]. These results suggest a role for SIRT1 as a tumor suppressor acting on multiple levels, although such a role has recently been disputed, as discussed further below.

Another important protein that plays critical roles in oxidative stress responses and aging is the mitochondrial protein p66Shc. Inactivation of p66Shc is associated with increased resistance to oxidative stress, protection from aging-associated vascular disease, and increased lifespan [66]. Recent work demonstrated that SIRT1-overexpressing transgenic animals are protected from age related endothelial dysfunctions [66]. Moreover, when diabetes was induced in both control and transgenic mice with streptozotocin, the transgenic mice exhibited less oxidative stress. Both these effects depend on SIRT1 mediated regulation of p66Shc. SIRT1 binds in fact to the promoter of the p66Shc gene, deacetylating histone H3 and downregulating expression of this gene [66]. In the context of vascular diseases, it has also been shown that SIRT1 appears to stimulate angiogenesis by deacetylating Foxo1 and Notch1 [67]. In contrast to these results, SIRT1 overexpression in another study was shown to increase triglyceride and cholesterol synthesis in the liver, sustaining proatherogenic changes in lipid metabolism [68]. Overall, SIRT1 might influence angiogenesis and atherosclerosis acting on numerous substrates, with the precise outcome likely depending on which of the phenotypes may dominate over the others.

It is important to consider though, that SIRT1 could also affect age-related phenotypes through modulation of other transcription factors. For instance, SIRT1 deacetylates and inhibits p53 –the first target described for this sirtuin- reducing genotoxic stress and DNA damage induced apoptosis [69]. SIRT1 also binds, deacetylates and inhibits p65/RelA, a component of the NF- κ B complex [69], in this way protecting against NF- κ B-induced apoptosis and senescence. Considering that the same stimuli that activate SIRT1 (genotoxic and oxidative stress) activate NF- κ B as well, it is possible that under stress conditions SIRT1 tries to counteract NF- κ B action. Consistently with the proposed stimulatory effect of CR on SIRT1 and its putative beneficial consequences on lifespan [25,40,73], CR has also been shown to inhibit NF- κ B [70]. However, it remains to be determined whether such an effect on NF- κ B is SIRT1-dependent. Recent work from the de Cabo's laboratory has shown that in *rhesus monkeys* CR does not increase lifespan, although it delays the onset of several age-related diseases, suggesting the possibility that CR may extend healthier aging without affect lifespan *per se* [71]. Whether SIRT1 plays a role in these phenotypes remains to be determined.

Remarkably, the first mammalian sirtuin to be linked to lifespan is SIRT6. In a recent study, Kanfi and colleagues demonstrated that male transgenic mice overexpressing SIRT6 live longer, however the mechanisms behind these observations remain unclear [72]. Original data suggested a role for SIRT6 in base excision repair (BER), yet how SIRT6 modulates BER at the molecular level remains unclear [36]. More recently, SIRT6 has also been involved in non-homologous end joining (NHEJ) and homologous recombination (HR) pathways [73,74]. These two pathways play critical roles in repairing double strand breaks (DSBs) [75]. Kaidi and colleagues demonstrated that SIRT6 is recruited to sites of damage to deacetylate the end-processing factor CtIP, an event necessary for recruitment of downstream effectors. Consistently with this observation, HR and resistance to DSBs generating agents are severely impaired in cells lacking SIRT6 [73]. More recently, it has been shown that SIRT6 overexpression improves not only HR but also NHEJ efficiency, through a mechanism that involves SIRT6-specific mono ADP-ribosylation of PARP1 [74]. Finally, SIRT6 has been implied in maintenance of telomere structures in human cells, by keeping H3K9 in a deacetylated state in telomere regions. Cells lacking SIRT6 show abnormal telomere structures, resembling the phenotypes observed in other progeroid diseases, such as Werner (WRN) syndrome [76]. Even though the precise function for SIRT6 in DNA repair remains to be fully established, it is clear that this chromatin factor plays an important role in multiple DNA repair pathways. Whether the progeroid phenotypes in the SIRT6 deficient mice and the extension of lifespan in the transgenic SIRT6 animals are due to a protective role for SIRT6 against genomic instability, depend on its function in glucose metabolism, or a combination of both, remains to be established as well. Interestingly, SIRT6 can also mediate H3K9 deacetylation at NF- κ B target gene promoters, repressing transcription of genes that has been previously linked to aging [69]. Future work will be required in order to decipher how SIRT6 coordinates in a concerted fashion the metabolic adaptations and DNA repair activities required in order for cells to survive in the face of nutrient and oxidative stress.

ii. Acetyltransferases—Cellular deacetylases are counteracted by the activity of acetyltransferases (HATs). In mammalian cells, several HATs have been identified, including CBP/p300, GCN5/pCAF and the MYST acetyltransferases such as TIP60. Deletion of GCN5, p300 and CBP in mice are embryonic lethal, indicating a critical role for these enzymes during development. A recent article showed that deletion of GCN5/pCAF in mammalian cells dramatically reduced H3K9ac, while CBP/p300 deletion affected mainly H3K18 and H3K27 acetylation [77]. On the other hand, *in vitro* studies propose H3K9, H3K27 and H3K36 as targets for p300 [78]; acetylation at these residues induces opening of chromatin, and as such these modifications have been associated with increased accessibility of RNA polymerase and concomitant increased in gene expression.

Acetyltransferases in metabolism: Recent studies demonstrated a critical role for GCN5 in metabolic homeostasis. GCN5 interacts with and acetylates PGC1 α [79], counteracting SIRT1 in modulating glucose homeostasis. GCN5 overexpression in hepatocytes reduces the expression of the gluconeogenic genes PEPCK and G6Pase, triggering the same response observed in SIRT1 knockout hepatocytes [80]. These results suggest that GCN5 can induce metabolic changes depending on nutrient availability. This idea is further supported by recent studies in yeast. When exposed to low-glucose condition, budding yeast undergo a cycle of growth/stand-by that can be divided in three phases: oxidative (OX), reductive building (RB) and reductive charging (RC) [81]. During the OX phase, cells are actively synthesizing proteins involved in growth -for instance ribosomal genes- and they exhibit high levels of Acetyl-CoA and enhanced mitochondrial respiration. During the RB phase, yeast cells slow down their mitochondrial respiration, replicate their DNA and divide. Lastly, at the RC phase, mostly survival and stress genes are expressed. In other terms, these

cells undergo a phase of growth (OX), cell division (RB) and quiescence (RC). Each of these phases is characterized by a specific gene expression profile. Interestingly, the GCN5/SAGA complex is essential to trigger the expression of the growth related genes in the OX phase [81]. Analysis of histone acetylation throughout their cell-cycle revealed a peak of histone acetylation during the OX phase, which persisted into the RB phase. Acetylation of these histone residues occurred in growth-related genes, which were the ones where the GCN5/SAGA complex peaked [81]. These observations demonstrated a strong link between mitochondrial metabolism and cell growth mediated by epigenetic mechanisms.

Acetyltransferases in aging: As it has been shown for histone deacetylases, also the expression of HATs changes upon senescence. In fact, levels of p300 decrease in human melanocytes as the number of cell passages increase [82]. In this context, downregulation of p300 leads to stable repression of the cyclin E promoter, with consequent induction of senescence. Interestingly, it has also been shown that p33ING levels are increased in high passage cells, leading to the binding of p300 to p53 [83]. p300 acetylates and activates p53, thereby inducing replicative senescence. Similarly, CBP also activates p53 by acetylation, a step required for oncogene-induced senescence [84]. All together, these observations reinforce the concept that a fine balance between these chromatin enzymes is essential for controlling cellular homeostasis and growth.

Histone acetyltransferases have also been involved in DNA repair and genome stability maintenance. Das and colleagues observed increased CBP/p300-dependent H3K56 acetylation at site of DNA breaks, although Miller et al. reported an opposite observation [20]. The dynamics of H3K56 acetylation upon damage remains still to be fully elucidated. An intriguing hypothesis is that changes in levels and distribution of H3K56ac are linked to reposition of nucleosomes upon damage, in order to prevent further breakage to allow repair. For example, GCN5 mediated H3K56ac has been reported to be an essential step for nucleosomes assembly during DNA replication, and efficient nucleosomes re-assembly is required for maintaining genome stability [85]. GCN5 maintains genome stability also by stabilizing TRF1, a component of the shelterin complex, thereby contributing to telomere protection [86]. As we will discuss further below, maintenance of telomere structure is a crucial feature to prevent genome instability and senescence. Work from yeast support the idea that GCN5 may link metabolism, DNA repair and lifespan through modulation of the retrograde response [87]. Whether similar mechanisms take place in higher eukaryotes remain to be determined.

Although a direct role for the MYST family of acetyltransferases in aging remains to be established, these enzymes have also been involved in control of DNA repair and replication processes, and defects in these enzymes have been associated with cancer [88]. For instance, Tip60 controls the expression of several oncogenes and tumor suppressors and is a crucial player in the repair of DSBs. Tip60 in fact acetylates and activates both p53 and ATM, enhancing the DNA-damage signaling cascade [89]. Recent studies indicate that Tip60 also mediates the recruitment of ribonucleotide reductase to sites of damage, inducing a local increase in dNTPs pool, required for efficient repair [90].

iii. Methyltransferases—Another important epigenetic modification, which can actually occur both on DNA and proteins, is methylation. As acetylation, DNA and histone methylation play critical roles in multiple biological processes, including DNA replication, DNA repair, gene transcription and chromatin accessibility. This modification is regulated by a fine-balance between the activities of methyl-transferases and histone demethylases (and the newly emerging group of hydroxylases/carboxylases). Emerging evidence supports a role for these enzymes in metabolism, senescence and aging, as described elsewhere in this review series (see Chiacchiera et al.).

b. Epigenetic Cross-talk and regulation of senescence

As discussed before, gene expression or silencing is influenced by multiple epigenetic modifications, including histone marks and DNA methylation. Although we treated them separately in the previous section, it is clear that chromatin-remodeling enzymes act in a concerted manner to activate or repress gene transcription. Indeed, these enzymes form part of large multiprotein complexes, such as NuRD and NoRC [91,92]. These complexes are usually recruited to chromatin through recognition of particular histone and DNA methyl-marks already in place; once bound, the different complexes might either maintain the *status quo* or determine further changes in those epigenetic marks, thereby activating or repressing gene transcription. In order to better understand the dynamics and the interactions between these different modifications, we will consider a model gene locus that plays a key role in cellular senescence: the INK4-ARF locus (Figure 2).

The INK4-ARF locus encodes three proteins: p16^{INK4a}, p15^{INK4b} and p19^{ARF}. p16^{INK4a} and p15^{INK4b} are cyclin-dependent kinase inhibitors of CDK6, essentially blocking cell cycle progression by preventing phosphorylation of pRB; p19^{ARF} sustains cell cycle arrest and apoptosis mainly by stabilizing p53 [93]. The expression of these three proteins increases during senescence -both physiological and oncogene induced senescence- triggering cell cycle arrest as a protective mechanism against stress [94]. Considering the importance of this locus it is not surprising that it is tightly regulated during cellular lifespan, representing one of the most common silenced loci in cancers [95].

In normal-dividing cells, the INK4-ARF locus is usually repressed due to binding of the Polycomb repressive complex 2 (PRC2) at the locus. PRC2 contains the lysine methyltransferase EZH2, which maintains high levels of H3K27me3. H3K27me3 in turn is recognized by the Polycomb repressive complex 1 (PRC1), which binds to this modified residue with high specificity. PRC1 contains the RING protein RING1b and BMI1, both ubiquitin ligases that monoubiquitinate H2A on lysine 119. Together with PRC1 and PRC2, DNMTs are also recruited to the locus to methylate DNA. Overall, H3K27me3, H2AUb and DNA methylation provide a stable, heritable lock, maintaining the INK4-ARF locus in a repressed state for as many generations as necessary. Further studies have shown that the histone deacetylases HDAC3/4 and the histone demethylase JHDM1b also participate in silencing this locus by guaranteeing, respectively, deacetylation of H3 and H4 and demethylation of H3K36 [96,97]. While repression of this locus is faithfully maintained through many cellular divisions, multiple signals (oxidative stress, oncogenes, replicative stress) can cause de-repression of the INK4-ARF locus, with concomitant cellular senescence. One of the first steps in this process is the displacement of the Polycomb protein complexes, a process mediated in part by downregulation of ANRIL, a non-coding RNA important for the recruitment of the PRC components to the INK4-ARF locus. At the same time, the SWI/SNF chromatin-remodeling complex is recruited, which helps to evict the PRC from the locus [93]. Removal of the PRC complex in turn induces CpG demethylation and acetylation of H4K16, an active chromatin mark. In addition, the H3K27me3 demethylase JMJD3 is activated, removing this key-PRC recognition mark, further enhancing activation of the locus (Figure 2) [93].

Notably, the SIRT1 deacetylase also binds to this locus, however it appears to play a dual role: it represses p16^{INK4a} expression while activating p19^{ARF} [98,99]. How SIRT1 causes such phenotypes remains unclear. Original data on SIRT1 deletion show that KO MEFs compared to WT cells are more resistant to replicative but not oncogene-induced senescence [99]. This is supported by the observation that telomerase immortalized human fibroblasts and mouse hematopoietic stem cells also exhibit increased growth capacity upon SIRT1 silencing, through a mechanism involving the nutrient-stress kinase AMPK [100]. Even though the above results would suggest that reduced levels of SIRT1 would be beneficial to

avoid cellular senescence, other results are inconsistent with such an effect. In fact SIRT1 transgenic mice, compared to controls, exhibit lower degree of age-associated phenotypes, such as osteoporosis, impaired glucose tolerance and accumulation of DNA damage. These phenotypes are at least in part ascribable to lower levels of p16^{INK4a} [101], suggesting that SIRT1 repression of p16 might dominate over the other functions. Consistent with these results, over-expression of SIRT1 rescued PML-mediated premature senescence in mouse cells, through deacetylation and inactivation of p53 [102]. In addition, SIRT1 may also inhibit replicative senescence by deacetylating H1, reducing the formation of heterochromatin [103] and through activation of the Erk-S6K MAP kinase-signaling pathway [98]. Such discrepancies might reflect different experimental conditions; regardless, they raise a word of caution with regards to modeling beneficial effects of putative sirtuin activators.

The accumulation of senescent cells in tissues and organs is now considered an integral part in the mechanisms contributing to organismal aging, as we already discussed. Most importantly, it appears to also play a role in several degenerative diseases associated with aging [104]. Strikingly, a recent study reported a mark delay in the onset of age-related phenotypes, such as lordokyphosis and cataracts, in a progeroid murine model where p16^{INK4a}-expressing cells were selectively killed [105]. Further, clearance of senescent cells caused a beneficial effect even in mice that already presented age-related dysfunctions. This study strongly supports the causal link between senescence and age-related diseases, and further suggests that modulation of p16^{INK4a} could be sufficient to revert some of these degenerative phenotypes.

c. Chromatin topology and aging

Beyond epigenetic modifications, chromatin could also be classified as euchromatin and heterochromatin, historically considered as the open/active and the close/repressed configurations, respectively. From a structural point of view, “euchromatin” is formed by nucleosomes assembled in the familiar “beads-on-a-string” configuration. Each nucleosome encompasses 147bp of DNA wrapped around an octamer of histones, in turn formed by two H3/H4 dimers and two H2A/H2B dimers. In between nucleosomes, linker DNA adds up to 80bp. The addition of other proteins, including the linker histone H1, enables the primary conformation to coil into 30nm fibers or higher-order, compacted conformation, as seen in heterochromatin [106]. In order for cells to either duplicate or transcribe its DNA, histones need to be evicted from the DNA to allow the passage of the replication- and transcription-machineries and then promptly reassembled on DNA. This requires a very fast and effective “histone exchange” process. Even though the precise mechanisms governing this process remain to be elucidated, several lines of evidence suggest that such histone dynamics may play a role during aging as well, as discussed below.

i. Histone expression and aging—Recent studies showed that during aging, as well as during chronic exposure to damaging agents, histone levels in yeast are reduced [107–109]. In addition, deletion of ASF1, a histone chaperone involved in histone exchange, reduces replicative lifespan in yeast cells. A similar effect was observed in cells lacking H3K56 acetylation, a chromatin mark in newly synthesized histones. This mark is recognized by ASF1, allowing this chaperone to transfer H3-H4 dimers to the CAF1 chaperone, which in turn incorporate them into nucleosomes [107]. Cells lacking ASF1 or with impaired acetylation of H3K56, exhibited lower levels of histones compared to control cells, suggesting that defects in total histones levels is sufficient to drive aging. Consistent with this idea, overexpression of histones in yeast cells was sufficient to dramatically extend lifespan in yeast [107], clearly indicating that at least in this organism, histone levels play a crucial role during aging.

Similarly to what happens in yeast, human primary fibroblasts exhibit reduced histone levels following replicative senescence [108]. This process is also accompanied by significant changes in histone modifications. Indeed, H3K56ac, H3K9me2, H3K9me3 and H4K20me3 levels decrease, whereas H3K9me1 and H4K20me2 increase in cells undergoing senescence [108]. Due to these changes, overall nucleosome number decreases [110], causing major gene expression changes and increased susceptibility to DNA damage, explaining at least in part why senescent cells exhibit higher levels of pH2AX and increased genomic instability. Moreover, DNA damage itself can also affect histone expression, rendering a vicious cycle with cellular senescence as endpoint, as we will discuss below [108]. Recent studies have shown that during aging, learning decline was associated with specific decrease in H4K12 acetylation. Remarkably, the phenotype was rescued upon intrahippocampal injection of the SAGA acetyltransferase [111]. These compelling results suggest the tantalizing possibility that correcting epigenetic changes may suffice to restore function in the aging brain. Overall, these studies demonstrate that maintenance of histone content in a cell is an actively regulated process that plays critical role in modulating proper gene expression and maintaining genome integrity, a process that seems to go awry with age.

ii. Senescence associated heterochromatic foci—In 2003, Narita and colleagues observed that human-senescent fibroblasts accumulate distinct heterochromatic structures, termed senescence associated heterochromatic foci (SAHF). SAHFs exhibit highly methylated DNA, binding of the heterochromatin protein HP1, hypoacetylated histones and high level of H3K9me3 and H4K20me3 [93,112]. Most importantly, these foci present a very peculiar histone variant: histone macroH2A; deletion of macro H2A in senescent cells induces disappearance of SAHFs. Work from Zhang *et al.* showed that the histone chaperone ASF1 and HIRA are required to form SAHF and that these structures are not a simply consequence of senescence. Notably, cells expressing mutant forms of HIRA and/or ASF1 still undergo senescence, despite the fact that SAHF are not formed [112]. It was thought that SAHFs formation contributes to inducing and/or maintaining senescence, activating pRB-mediated silencing of E2F genes [113,112]. However, recent work indicates that SAHFs are not observed in all cell types upon senescence. Further, their appearance depends on the type of insult triggering senescence [114]. For example, MRC5 fibroblasts would develop SAHF both in oncogene- and DNA damage-induced senescence, while BJ fibroblasts would exhibit SAHF only following oncogene insult. All together, these studies indicate that SAHF is an integral characteristic of at least some forms of senescence, however their precise role in this process, and whether they play a role during normal aging remains to be determined.

iii. Role of telomeres structure in senescence—Telomeres are segment of repetitive DNA at the extremities of each chromosome. During S-phase, the DNA polymerase machinery can fully replicate the leading DNA strand, while the lagging-strand poses a serious challenge. In order to prevent, or at least slow down, telomere shortening, cells take advantage of telomerase, an enzyme capable of adding a repetitive sequence at the end of the chromosome using RNA as a template [115]. In 2003, Weinberg, Hahn and colleagues described that growing and pre-senescent fibroblasts exhibit telomerase activity, while senescent cells instead bare almost undetectable activity [116]. These results prompted these authors to propose that incomplete replication at the end of the lagging strand, in association with a very low telomerase activity, could cause telomeres shortening in human cells, activating a checkpoint that causes replicative senescence [117]. These studies have now been extended to clearly demonstrate a critical role for telomere structure in preventing senescence. In this context, telomeres are fully covered by the shelterin complex [53], which mainly avoid telomeres to be recognized as DNA breaks, preventing in this way the activation of the ATM signaling cascade. Indeed, mice deleted for any of the shelterin

complex proteins exhibit signs of tissue degeneration and neoplastic transformation, even in the presence of normal telomeres' length, suggesting that persistent activation of DNA damage-signaling pathways, rather than telomere length *per se*, is crucial in promoting senescence and transformation [118].

From an epigenetic point of view, telomeres are characterized by heterochromatic markers: CpG methylation, H3K9me3 and H4K20me3. Recent studies indicate that during aging, DNA hypomethylation and changes in levels of deacetylases and methyltransferases can affect these marks, therefore leading to telomere dysfunction [119,93]. Furthermore, telomere shortening has also been associated with downregulation of H3 and H4 histone levels and destabilization of the histone chaperone ASF1 [108]. Reduction in ASF1 levels causes chromatin assembly defects, which in turn will further reduce H3 and H4 levels, starting a vicious cycle that could affect telomere structure [108]. When telomere shortening passes certain threshold, it activates ATM signaling, leading to cell cycle arrest and cellular senescence. Interestingly, recent observations indicate that telomere dysfunction influences as well the metabolic and mitochondrial changes that usually accompany the organismal decline observed with aging. Indeed, ATM mediated-p53 activation in telomerase-deficient mice leads to repression of PGC1 α , with concomitant decrease in mitochondrial biogenesis and function, decreased gluconeogenesis, cardiomyopathy, and increased reactive oxygen species [120]. This is in contradiction with previous studies reporting a positive action of p53 on mitochondrial respiration [121]. It is possible that the effect of p53 on mitochondrial respiration depend on the specific context of the cell and interaction of the different signaling pathways.

Overall, it is clear that telomere biology plays a key role in cellular senescence and as such it represents an essential component of human aging. Remarkably, the progeroid phenotype observed in mTERT deficient animals was basically reverted by inducing the re-expression of mTERT *in vivo* [122]. This study provides evidence that aging may actually be a reversible process and could represent a milestone in the field of regenerative medicine.

2. MITOCHONDRIAL METABOLISM AFFECTS HISTONE ACETYLATION

Acetylation of histones relies on availability of Acetyl-CoA as metabolite donors, as well as on the presence of NAD⁺ as cofactor for deacetylases. Given that those factors play critical roles in cellular metabolism and their availability is subjected to nutrient availability, food intake and cellular energy status can directly impinge on epigenetic processes, as we will discuss in this section.

Acetate freely diffuses through cell membranes, therefore in order to maintain a discrete acetate pool in different cellular compartments, it is necessary to conjugate acetate to coenzyme-A to form acetyl-CoA. The conversion of acetate to acetyl-CoA is catalyzed by the acetyl-CoA synthetase (AceCS in mammalian cells, ACS in bacteria) enzyme, while the opposite reaction requires acetyl-CoA hydrolase. AceCS is present in two isoforms in mammalian cells: cytosolic AceCS1 and mitochondrial AceCS2. Both enzymes are regulated by acetylation and are sirtuins' targets. [123,124]. Acetyl-CoA in the mitochondria is mainly generated from pyruvate as part of glucose metabolism, however under fasting or hypoxic conditions citrate is generated from lipids or glutamine rather than glucose [125,126]. Once acetylCoA is formed, it will enter the TCA cycle to be converted into citrate. Citrate will provide either the first step in the TCA cycle to form ATP, or else it will be shuttled outside the mitochondria, where the ATP-citrate lyase (ACL) enzyme will convert it back to acetyl-CoA for lipid synthesis in the cytoplasm [127].

The acetyl-CoA pool is necessary not only to sustain many metabolic pathways in the cells, but also as acetate donor in the acetylation of proteins, therefore mutations in pathways

producing acetyl-CoA may affect protein acetylation. In this context, Wellen and colleagues showed in fact that silencing of ACL causes a dramatic reduction on H2B, H3 and H4 acetylation, which can be rescued by adding acetate to the culture media. Most importantly, depletion of ACL and the resulting hypoacetylation of histones impair both cell growth and differentiation [128], indicating that this cross-talk between mitochondria and chromatin plays important homeostatic functions. Cells lacking ACL exhibit also impaired lipogenesis and glucose-dependent growth, a phenotype that confers these cells with resistance against PI3K-induced tumorigenesis [127]. In support of these data, chemical inhibition of ACL can suppress *in vitro* and *in vivo* tumor growth [129]. ACL activity might itself be affected by the availability of citrate, therefore changes in mitochondria metabolism can lead to changes in cytosolic acetyl-CoA, in turn influencing histone acetylation.

These observations support the idea that cell metabolism modulates epigenetic states in cells [130]. Moreover, these changes may influence levels of metabolic genes, in turn affecting metabolism in another example of a feedback-cycle. This represents a very important loop that can be relevant, for example, in cancer and metabolic syndrome. Undoubtedly, a better understanding of the crosstalk between these mechanisms could open new therapeutic and diagnostic horizons, and its potential we are only starting to grasp.

3. IMPACT OF EPIGENETICS IN AGE-RELATED DISEASES

Aging is a complex process associated with a progressive decline of the organism and its ability to react to external stresses. Many factors contribute to aging and among these, increased oxidative stress, accumulation of DNA damage and changes in gene expression appear to have a particular impact [104]; as we have discussed so far, chromatin dynamics seems to play key roles in modulating those factors.

DNA and histone modifications participate not only in physiological aging, but they might also contribute to the occurrence of age-related diseases, such as diabetes, neurodegeneration, cancer and Progeria-like syndromes. Indeed, many trials with drugs regulating HDACs, sirtuins and DNMTs are ongoing for the potential treatment against these diseases; in the case of cancer, HDACi has already been approved for particular cases. In this section, we will discuss in detail the link between epigenetic changes and age-related degenerative diseases. Considering that diabetes and progeroid diseases have been extensively discussed above, we will focus our attention on neurodegeneration and cancer.

a. Neurodegeneration

As mentioned above, changes in H4K12 acetylation may play an important role in learning decline during aging [111]. Recent studies have also showed that HDAC1 protects neurons in a mouse model of neurodegeneration [131]. Neurotoxic stimuli, as in AD and ischemia/stroke, induces calpain-mediated cleavage of p35, in turn forming the active form p25. p25 forms a complex with cdk5 and among other things, it inhibits HDAC1. HDAC1 inhibition leads to accumulation of DNA double strand breaks and expression of cell cycle genes [131], leading these postmitotic neurons to undergo apoptosis [132]. These observations partially contradict previous studies in which CBP was shown to protect neurons from apoptosis in neurodegeneration-mouse models [133]. In addition, SIRT1 activation can also protect neurons from apoptosis in several models of neurodegeneration, including Alzheimer's disease (AD), amyotrophic lateral sclerosis (ALS) and primary neurons exposed to neurotoxic insults [134]. A different study suggested instead that SIRT1 inhibition in mice restored cognition in AD by stabilizing tau protein [135]. According to Liu and colleagues such discrepancy might be linked to cellular NAD⁺ status [136]. They proposed that during cerebral hypoxia/ischemia, levels of ATP and oxygen in neurons are reduced, determining a higher release of glutamate. Glutamate receptor overstimulation

determines both bioenergetic and oxidative stress, leading to DNA breaks and SIRT1 activation. In this context, SIRT1 activation, instead of protecting cells, further reduces the NAD⁺ pool, worsening the bio-energetic defects and triggering apoptotic programs. In such a background, SIRT1 inhibition might actually protect cells from apoptosis. In other words, it appears that SIRT1 could protect neurons from neurotoxic insults when cells are not pre-sensitized by severe bioenergetic impairments. For all these reasons, even if sirtuin and HDAC modulators represent putative therapeutic tools for neurodegenerative diseases, their use might need to be adapted to specific cases and bioenergetic contexts. Of note, deacetylase inhibitors affect not only neurons but also astrocytes and glia cells. For example, it has been shown that both HDAC3 and SIRT1 inhibit NF- κ B transcription with opposing outcomes: enhancement of the inflammatory response in response to HDAC3 and diminished release of inflammatory molecules -such as iNOS- in response to SIRT1 [137]. Again, such results raise a word of caution, indicating that the net effect of these modulators might be context- and cell-dependent.

It is important to notice that DNA-damage accumulation is a common feature in the pathogenesis of many neurodegenerative disorders [75]. Given that chromatin affects also the susceptibility of cells to DNA damage, it is likely that the epigenetic changes that occur with age, as described before, may contribute to the development of neurodegenerative diseases through its effects on genome stability.

b. Cancer

Human tumors rely on the acquisition of multiple “hallmarks”: sustained proliferative signals, the ability to escape growth suppression, apoptosis and immune response; activation of angiogenesis and invasion/metastasis; immortalization and a metabolic shift towards glycolysis and lactate production, a phenomenon known as the “Warburg Effect” [138]. Epigenetic changes can be associated to many, if not all, of these characteristics, as we have previously discussed.

SIRT1, 2, 3, 6 and 7 have all been shown to modulate tumorigenesis in mouse models. Haploinsufficiency of SIRT1 leads to genome instability and when associated to haploinsufficiency of p53 determines a higher rate of spontaneous tumors compared to controls [139]. Wang and colleagues also showed that SIRT1 is downregulated in human tumor samples. In contrast, other studies have shown that knockdown of SIRT1 in pancreatic tumor cells induced senescence and apoptosis, reducing invasiveness while enhancing chemosensitivity [140]. It is important to notice that the latter was an *in vitro* study, and in this system, persistent p53 activation (secondary to SIRT1 knockdown) might have a dominant effect. Considering that the *in vivo* evidence collected so far supports mostly an anti-tumor role for SIRT1 [141], it is not surprising that several SIRT1 activators are in clinical trials for cancer therapy [142,143]. As discussed above, also other sirtuins, mainly SIRT6, play a role in preventing tumorigenicity. In this context, overexpression of SIRT6—at least *in vitro*-induces apoptosis selectively in cancer cells [144]. Moreover, as indicated before, in recent studies SIRT6 was shown to work as a tumor suppressor, inhibiting the Warburg effect in a mouse model of colon cancer, and higher levels of SIRT6 strongly correlate with a better prognosis in human colorectal cancer patients [50], suggesting that SIRT6 could represent not only a therapeutic target, but also a prognostic tool.

Several cancers express high level of HDACs. Indeed, *in vitro* experiments showed that inhibition or silencing of HDACs induces cell cycle arrest and apoptosis in cancer cells [6]. *In vivo*, HDAC inhibitors (HDACi) exhibit selective cytotoxicity towards tumor cells. This effect might be explained by the different cell cycle dynamics and checkpoints between normal and tumor cells, but also by the recent finding that HDACi seem to increase the

expression of antigens recognized by cells of the immune system [145,146]. However, if administered systemically, HDACi instead reduce the inflammatory response and the innate immunity against the tumor, through mechanisms that remain unclear [145]. Further studies are required to optimize the use of HDACi. Yet, they are emerging as a valid therapeutic option against this disease, and for this reason there are many ongoing trials with HDAC inhibitors in cancer therapy, both alone and in combination with other drugs [142,6]. In this context, the first such a drug, the HDACi Vorinostat (SAHA), has already been approved for treatment of T-cell lymphoma [147], and it is conceivable that it will be the first from many to come.

4. CONCLUDING REMARKS

Chromatin dynamics appear to play key roles in virtually every cellular process. In this context, the activity of histone and DNA modifiers seems closely intertwined to cellular metabolism, and this is evident at least at three levels: 1) epigenetic modifications affect expression of metabolic genes; 2) multiple histone modifiers have, among their non-histone targets, metabolic proteins; 3) metabolic intermediates and cofactors influence the activity of histone and DNA modifiers. Changes in epigenetic marks can be dictated by metabolic changing conditions (i.e. nutrient availability and oxidative stress). In turn, defects in chromatin dynamics could impinge on cellular metabolic pathways, influencing processes beyond their obvious effects in the nucleus. Misregulation of histone and DNA modifications appear to play causal roles in aging and age-related diseases such as diabetes mellitus, neurodegeneration and cancer. As such, drugs that modulate chromatin enzymes provide promising therapeutic alternatives in such diseases. Considering that many of these enzymes act in a highly concerted manner, a better understanding of the precise mechanisms governing epigenetic dynamics may help to design better therapeutic strategies against those devastating diseases.

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Figure 1. Role of Sirtuins in organismal homeostasis and disease

Graphical summary depicting the inhibitory and activating roles of the different mammalian sirtuins in metabolism, cancer, neurodegeneration and aging.

Figure 2. Epigenetic modifications at the INK4-ARF locus

In replicating cells (A) ANRIL non-coding RNA mediates PRC2 recruitment to the gene locus. The PRC2 subunit EZH2 trimethylates H3K27. H3K27me₃ recruits PRC1, which in turn ubiquitinates H2AK119. Other factors recruited to the *INK4-ARF* locus are DNMTs (which methylate the DNA), HDAC3, HDAC4 (deacetylate histones) and JHDM1b (demethylates H3K4). These histone- and DNA-marks are necessary to maintain the locus in a repressed state. In senescent cells (B) ANRIL degradation and the recruitment of the SWI/SNF complex displaces the DNMTs, the HDACs and the PRC1 complex. JMJD3 is recruited at the site and demethylates H3K27, removing the signal for PRC1 recruitment and therefore H2AK119ub is lost. SIRT1 is recruited, but its action is not fully defined. The net

result is the activation of p16^{INK4a}, p15^{INK4b} and p19^{ARF} expression and induction of senescence, which at the organismal level will lead to age-related dysfunction. See main text for details.