Send Orders for Reprints to reprints@benthamscience.ae

Current Genomics, 2017, *18*, 385-407

REVIEW ARTICLE

Aging as an Epigenetic Phenomenon

Vasily V. Ashapkin*, Lyudmila I. Kutueva and Boris F. Vanyushin

Belozersky Institute of Physico-Chemical Biology, Lomonosov Moscow State University, Moscow, Russia

ARTICLE HISTORY

Received: December 29, 2015
Revised: January 17, 2016
Accepted: February 09, 2016

DOI:
10.2174/1389202918666170412112130

Abstract: ***Introduction*:** Hypermethylation of genes associated with promoter CpG islands, and hypomethylation of CpG poor genes, repeat sequences, transposable elements and intergenic genome sections occur during aging in mammals. Methylation levels of certain CpG sites display strict correlation to age and could be used as "epigenetic clock" to predict biological age. Multi-substrate deacetylases SIRT1 and SIRT6 affect aging *via* locus-specific modulations of chromatin structure and activity of multiple regulatory proteins involved in aging. Random errors in DNA methylation and other epigenetic marks during aging increase the transcriptional noise, and thus lead to enhanced phenotypic variation between cells of the same tissue. Such variation could cause progressive organ dysfunction observed in aged individuals. Multiple experimental data show that induction of NF-κB regulated gene sets occurs in various tissues of aged mammals. Upregulation of multiple miRNAs occurs at mid age leading to downregulation of enzymes and regulatory proteins involved in basic cellular functions, such as DNA repair, oxidative phosphorylation, intermediate metabolism, and others.

***Conclusion*:** Strong evidence shows that all epigenetic systems contribute to the lifespan control in various organisms. Similar to other cell systems, epigenome is prone to gradual degradation due to the genome damage, stressful agents, and other aging factors. But unlike mutations and other kinds of the genome damage, age-related epigenetic changes could be fully or partially reversed to a "young" state.

Keywords: Epigenetics, Aging, DNA methylation, Histone modification, miRNA, Epigenetic age, Epigenetic reprogramming.

1. INTRODUCTION

Why we age has been a question of heated debate for a very long time [1]. Now it is widely understood that aging has multiple causes, such as accumulation of point mutations and other kinds of genetic damage both in nuclear and mitochondrial DNA, oxidative damage to macromolecules and cell structures, inflammatory damage to tissues, derangements of immunity and homeostasis, muscle weakness, osteoporosis and osteoarthritis, greatly increased frequency of cancer, cardiovascular, neurodegenerative and other diseases, and, last but not the least, epigenetic abnormalities, including changes in DNA methylation, both global and locus-specific ones. Nine hallmarks of aging that represent common denominators of aging in different organisms were proposed in a recent seminal review [2]. These hallmarks are genomic instability, telomere attrition, epigenetic alterations, loss of proteostasis, deregulated nutrient sensing, mitochondrial dysfunction, cellular senescence, stem cell exhaustion, and altered intercellular communication. One must take into account that these causes of aging are not independent. Epigenetic systems control gene activity and thus, either directly or indirectly, affect all other hallmarks. Their active components are encoded in genome and, thus, are themselves under epigenetic control. There are numerous links, both feedback and feed-forward ones, between different elements of epigenome. As an example, transcripts of genes encoding proteins of DNA methylation and histone modification systems could be targeted and controlled by miRNAs, whereas the expression of genes encoding miRNAs is controlled by cytosine methylation and histone modification. DNA methylation and histone modification systems are known to be coupled at both gene expression and target recognition levels. For example, 5mC (5-methylcytosine) and H3K4me3 are mutually exclusive epigenetic marks at gene promoters, where they have opposite effects on the promoter activity. An ability of epigenetic systems to affect all other drivers of aging puts them in a key position to affect the aging *per se*. On the other hand, the epigenetic systems are themselves subjected to aging-related deterioration, like any others in the living organisms. Whether alterations in DNA methylation and other epigenetic marks play a causal role in aging or are just manifestations of aging has yet to be elucidated.

2. DNA METHYLATION

2.1. Changes of DNA Methylation Levels During Aging

The first experimental data concerning age dependent changes in DNA methylation were obtained in our lab nearly half a century ago. DNA methylation levels in different organs of humpback salmon and rat have been found to gradually decrease with age [3, 4]. In a pioneering work at about the same time, Petro Volpe and Tamilla Eremenko have shown that regulatory regions of DNA are preferentially

*Address correspondence to this author at the Belozersky Institute of Physico-Chemical Biology, Lomonosov Moscow State University, Moscow 119991, Russia; Fax: + 7 (495) 939-3181;
E-mails: ashapkin@genebee.msu.ru; basilashapkin@gmail.com

1389-2029/17 $58.00+.00

methylated compared with protein-encoding ones [5]. As a matter of fact this work was the first experimental evidence that eukaryotic genes consist of intermittent "coding" and "non-coding" regions (named "exons" and "introns", respectively, few years later) [6]. The age-dependent loss of methylation mainly affects the heavily methylated repeat fraction of the mammalian genome [7], possibly leading to a general genome destabilization, since this genome fraction contains transposable elements. Repression of such elements is widely believed to be one of the main defensive functions of DNA methylation.

DNA methylation levels in different mice organs showed a correlation with the chronological age [8]. The rates of 5mC loss upon aging in DNA of two mouse species were inversely correlated with their maximal lifespans. A progressive decrease of DNA methylation levels was observed in cultured mouse, hamster and human fibroblasts [9]. Again, the rates of this hypomethylation were inversely correlated with cell lifespans (maximal number of cell population doublings before senescence). In dividing cells, DNA cytosine methylation occurs mostly parallel to DNA synthesis and continues for at least 30 min after its completion [10]. Methylation appears to semi-conservatively follow DNA replication [11]. The progressive 5mC loss in dividing cells might be mainly caused by errors in the maintenance DNA methylation that has fidelity ~95% per cell generation [12, 13]. Such loss of 5mC, essentially stochastic as it seems to be, could affect aging at both cellular and organismal levels by increasing the transcriptional noise and eventually leading to aberrant transcription of various genes. Methylation errors may not immediately lead to changes in gene expression. However, even such age-related drift of DNA methylation without effects on gene expression may enhance the likelihood of further methylation errors to result in aberrant gene expression or genomic instability. No changes in DNA methylation levels were observed in dividing immortal cell lines [9]. It seems inconceivable that fidelity of the maintenance DNA methylation is higher in these immortal cell lines compared with their normal counterparts. Similar losses of 5mC upon aging occur in mice tissues widely different in proliferative activity (liver, brain, and small intestine mucosa) [8]. Thus, the age related loss of 5mC could not be explained by maintenance methylation errors alone. Moreover, it was found that selective hypermethylation of some genes occurs upon aging, along with the global DNA hypomethylation [14]. Tumor suppressor genes, known to be hypermethylated in tumor cells, are often among the aging-hypermethylated ones. Promoter CpG islands (CGIs) can be variously methylated and contribute differently to the net transcription level; stable repression of transcription is usually correlated with CGI hypermethylation [6, 15]. Promoter CGIs of *LOX*, *CDKN2A* (also known as *p16*, *INK4a*, or $p16^{INK4a}$), *RUNX3*, and *TIG1* genes were found to be essentially unmethylated in normal stomach epithelial cells before the 50-yr patient age but progressively methylated between 50-yr and 80-yr ages [16]. This progressive methylation could explain the increase in tumor occurrences in aged people. Since the age dependent increase of DNA methylation level is non-linear in this case, it evidently must be caused by a specific process of some kind, not just by the accumulation of stochastic errors.

2.2. Changes of Genome Methylation Patterns Upon Aging

Genome-wide studies of DNA methylation have come into general practice with the advent of the Illumina Infinium HumanMethylation BeadChip hybridization assay platforms. An analysis of DNA methylation in human solid tissues showed an evident dependence of the methylation patterns on tissue source of DNA and on age [17]. A rather distinct correlation between age and methylation was observed in various tissues – increased methylation levels for CG sites inside CGIs and decreased methylation levels for those beyond CGIs. Genes known to be involved in aging mainly displayed increased methylation with age. Loci in genes involved in epigenetic regulation (*LAMB1*, *DNMT1*, *DNMT3B*, *HDAC1*, *HDAC7*) or telomere maintenance (*TERT*, *ERCC1*, *RAD50*) also tended to be increasingly methylated with aging, except for those in *DNMT3B* gene that were not located in CGIs and showed hypomethylation with age.

Similar results were obtained in a study of methylation of the unique gene associated CGIs in the mouse small intestine cells at 3 *versus* 35 month ages [18]. Out of about 3600 genes studied, 21% displayed hypermethylation with age and 13% displayed hypomethylation. Among genes devoid of the promoter CGIs, 7% displayed hypermethylation and 11% displayed hypomethylation. Genes known to be hypermethylated in colon cancer (*Cdh13*, *Dok5*, *Esr1*, *Igf2*, *Myod1*, *Nkx2-5*, *Cdkn2a*, *Pgr*, and *Tmeff2*) showed linear age-related hypermethylation in normal small intestine. Globally the hypermethylated gene group was enriched for genes involved in development and differentiation, whereas the hypomethylated gene group was not enriched for any specific functional category. Both tissue-specific and common age-dependent methylation loci were found. These age-dependent gene methylation patterns are partially conserved between humans and mice, and the conserved genes are mainly among the hypermethylated ones. A study of several representative members from both the groups showed that the age-dependent hypermethylation is usually accompanied by down-regulation of transcription, whereas the age-dependent hypomethylation is correlated with up-regulation of transcription.

A genome-wide study of DNA methylation in peripheral blood leukocytes from 93 healthy women of 49 to 75 year ages (including 31 twin pairs) found about 350 CpG loci differently methylated with age, ~200 of them being more methylated (hyper-aDMRs) and ~150 less methylated (hypo-aDMRs) [19]. More than 95% of these aging-associated differentially methylated regions (aDMRs) were located within 500 bp of the TSS (transcriptional start site). An essential share (>60%) of the hyper-aDMRs found were also present in purified fractions of CD14+ monocytes and CD4+ T-cells. The hypo-aDMRs set was significantly reproduced in T-cells, but not in monocytes. Since CD14+ monocytes are relatively short-lifespan (weeks) cells of the myeloid lineage, whereas CD4+ T-cells are long-lifespan (months–years) cells of the lymphoid lineage, it could be suggested that most hyper-aDMRs represent epigenetic perturbations inherent to the aging *per se*, whereas the hypo-aDMRs may reflect modifications associated both with aging *per se* and age-dependent changes in relative proportions of the blood cell subtypes.

The conservative hyper-aDMRs group has been found to be enriched for genes with tissue-specific functions, including neural cell-related processes. Most of these genes are moderately active or totally inactive in the peripheral blood cells. At least in T-cells, the hyper-aDMR promoters are poor in histone modification marks associated with transcriptional activity (H3K4me3, H2AZ) and RNA polymerase II, but are enriched for histone marks associated with transcriptional repression (H3K9me3, H3K27me3). These promoters significantly overlap those located in chromatin bivalent domains in embryonic stem cells, as well as those hypermethylated in various human cancers. Aberrant DNA methylation at chromatin bivalent domain promoters in cell culture was shown to cause a decreased capacity to differentiate and an increased ability to proliferate [20]. Similarly, in cancer cells such methylation leads to permanent silencing of genes required for differentiation, thereby stimulating cell proliferation. Hence, aging must also decrease the cell differentiation potential and increase the stem cell self-renewal. Possibly, this is one of the mechanisms that enhance cancer frequency at advanced ages.

In a study of DNA methylation in frontal cortex, temporal cortex, pons, and cerebellum of variously aged (1 to 102 years) donors, 589 CpG methylated loci showed age-dependence in one, 167 in two, 86 in three, and only 10 in all four brain divisions [21]. Of all age-related CpG sites found, ~82% were located within CGIs, ~11% not within CGIs, and ~7% in regions that could not be unequivocally defined as CGIs or non-CGIs. All 10 CpG sites that showed significant correlation with age in four brain regions were located within CGIs and their methylation levels were increased with age. Four of these sites were earlier identified as differentially methylated with age in other tissues [17, 19]. A positive correlation between the methylation level and age was observed also for majority (>95%) of remaining CpG sites. Of age-related CpG sites located within CGIs, 98% were hypermethylated. Compared with other tissues, brain contains mainly sites hypermethylated with age, most of them located in promoter-associated CGIs of genes encoding TFs (transcription factors). This part of the age-dependent DNA methylation could be involved in epigenetic program that affects genome expression in aging cells, whereas the age-dependent loss of total DNA methylation seems to represent mainly stochastic errors. This view gained support from a methylation study in MZ (monozygotic) twin pairs during the early life (from newborns to 18-months-olds) [22]. Intergenic regions and CpG poor promoters were found most likely to undergo changes in methylation during the early life, whereas methylation of CGIs and CpG rich promoters was most stable. Since environmental and life-style differences between MZ twins could be excluded at these ages, their discordance in DNA methylation should be a result of stochastic methylation errors.

Similar results were obtained in a study of the age-dependent DNA methylation in various organs (brain, blood, kidney, and skeletal muscle) [23]. CpG sites located within CGIs were found to predominate among those hypermethylated with age, whereas hypomethylation with age was more characteristic of CpG sites located beyond CGIs, and their methylation levels were more variable between tissues. Hence, age-related variations in methylation common for different tissues are mainly observed in CGIs and are usually represented by increase of the methylation level with age. On the other hand, tissue-specific variations in methylation are more characteristic of CpG sites located beyond CGIs and are often represented by decrease of the methylation level with age. Gene loci, functionally connected with regulation of transcription and control of morphogenesis, predominate among those hypermethylated with age in various tissues. These common age-related loci are frequently associated with chromatin bivalent domains containing both repressive (H3K27me3) and activating (H3K4me1, H3K4me3, H3K9Ac) epigenetic marks. The loci displaying hypomethylation with age are preferentially associated with active weak promoters and enhancers that are most enriched for H3K4me1 marks.

Discordance in DNA methylation profiles between MZ twins can be used to discriminate between age-related changes of DNA methylation that represent a cumulative result of stochastic errors and those that are a part of the hypothetical epigenetic program of aging [24]. Methylation levels of total lymphocyte DNA were found to be practically identical between MZ twins in 65% pairs and significantly different in 35% pairs. Identical DNA methylation levels were usually observed in young pairs, whereas aged pairs had most different ones. Thus, the DNA methylation difference between MZ twins gradually increases with age. An analysis of differentially methylated genome loci in most epigenetically discordant twin pairs showed that 43% of them are located in Alu family repeat sequences, 9% are in repeat sequences of other families (LINE, MER, MIR), 34% are in unidentified transcribed sequences, and 13% are in known unique genes. Generally, nearly identical methylation patterns were characteristic of young twin pairs that lived together for the most part of their life and had similar life styles, whereas most discordant methylation patterns were characteristic of older twin pairs that lived separately and had different life styles. Thus, large phenotypic discordance in MZ twin pairs may be caused by accumulated epigenetic differences. These differences could be due to both the effects of external and internal factors (smoking, physical activity, dietary preferences, *etc*.) and stochastic methylation errors (epigenetic drift) accompanying aging. Methylation errors probably occur much more often compared with mutations, since the fidelity of DNA methylation is by far lower compared with DNA replication and repair [6].

2.3. DNA Methylation Age Predictors

The aging rates are non-equal in different persons. Women are known to have a longer average lifespan compared with men. Nearly all super-centenarians that have reached the 110-yr age are women. Aging could be accelerated by unhealthy life habits, such as smoking, or slowed down by good ones, such as physical training. Molecular markers of aging that could be used to estimate biological, rather than passport, age are needed to evaluate more precisely degree of the age-dependent deterioration in physiological welfare. The age-related variance of DNA methylation seems to be a good contender for this role. The main obstacle in searches for the suitable epigenetic markers is a "needle in a haystack" problem. The age-related methylation loci in epigenomes of humans and other mammals are

masked by a plethora of epigenetic variations caused by other factors or stochastic methylation errors. In saliva samples of MZ twin pairs between 21 and 55 years of age, 88 CpG sites located inside or near 80 genes were found that have methylation levels significantly correlated with age [25]. Of these sites, 69 were positively correlated and 19 negatively correlated with age. Most (83%) age-correlated sites were within promoter CGIs. Three genes that showed most clear correlation with age and had the widest distribution of the methylation values, were tested in additional populations of both genders across a larger age range (18–70 years). All three genes tested, *Edaradd*, *NPTX2*, and *Tom1L1*, showed a clear correlation with age in men, but only *Edaradd* and *Tom1L1* showed such correlation in women. Methylation levels of *Edaradd* and *Tom1L1* were linearly decreasing with age, whereas the methylation level of *NPTX2* was increasing. Based on the methylation levels of just two CpG sites in *Edaradd* and *NPTX2* genes, age of the test subjects could be predicted with a 5-6-yr accuracy, whereas addition of one more site in *ELN* gene reduced the average error to ~3.5 years. It is worth a special note that the methylation level of *NPTX2* was shown to correlate with age in blood cells also [19]. Interestingly, no epigenetic drift was detected in the promoter CpG sites studied. This finding corroborates the view that stochastic methylation errors are mainly accumulated in repeat sequences and intergenic regions, whereas the gene and promoter methylation is under a more robust control.

Comparative studies of age-related methylation patterns in various tissues showed these patterns to be highly tissue-specific. Nevertheless, there are some loci that have methylation levels significantly correlated with age in various tissues. Obviously, these common methylated loci are of highest relevance for the mechanisms of aging *per se*, and their methylation status could be used as an epigenetic signature to estimate the biological age. Based on DNA methylation datasets from several independent studies that have used the same HumanMethylation27 BeadChip platform across different tissues, a non-cell-type-dependent epigenetic aging signature was elaborated [26]. Only 19 CpG sites showed correlation between the methylation level and age by the most stringent criteria ($p<10^{-13}$), all of them being hypermethylated upon aging. More than 450 age-correlated sites were found by a less stringent criteria ($p<10^{-5}$), most of them also being hypermethylated with age and only 25 hypomethylated. This is in accord with the view noted above that hypermethylation at specific sites is the predominant trend upon aging whereas hypomethylation seems to be less stringently regulated. CpG sites that showed a correlation with age across all tissues analyzed and had largest differences in DNA methylation levels between young and elderly donors were chosen to constitute the epigenetic aging signature. Most accurate age predictions were obtained when a set of four hypermethylated loci has been used, namely *TRIM58*, *KCNQ1DN*, *NPTX2*, and *GRIA2*. To further enhance the prediction accuracy a hypomethylated locus *BIRC4BP* was added to the set. When all five loci were used, the average prediction accuracy across all datasets was ±12.7 years, whereas the use of only three most reliable of them, *NPTX2*, *GRIA2*, and *KCNQ1DN*, enhanced the accuracy to ±11.4 years. For some individual datasets the accuracy was even better than ±6 years. It should be noted that in the work described the age prediction was applicable to various tissues and was gender-independent, whereas in the previous study described above [25] the prediction was based only on saliva samples. When blood samples were investigated, the set of CpG loci with a high predictive capability could be narrowed down to just three, *ITGA2B*, *ASPA*, and *PDE4C*, and accuracy of the age prediction was ±4.5 years [27].

In a larger-scale investigation using the Illumina HumanMethylation450 BeadChip platform, methylation levels of 485577 CpG sites were analyzed in blood DNA samples from more than 650 volunteers of 19 to 101 years age [28]. A correlation with age was found for ~15% CpG sites. An age predictive model was built using a set of 71 methylation markers. Mean error of the age prediction by this model was ±3.9 years (96% correlation between the passport age and the predicted age). Of 71 markers chosen, most were associated with genes involved in aging-related processes (DNA damage, oxidative stress, tissue degradation) or diseases (Alzheimer's disease, cancer). The model was capable not only of predicting the age, but also of revealing the factors that affect the personal rate of aging. For example, the Body Mass Index (BMI) was found not to affect the aging rate. On the other hand, gender does affect the rate of aging: DNA methylome "ages" in men faster by ~4% than in women. The age prediction model worked with DNA samples from other organs (breast, lung, kidney, and skin) with the same accuracy as with blood samples, when a linear offset specific for each organ was used. When the epigenetic predictive models were constructed using the same algorithm but based on the age-related methylation data from other organs (breast, lung, and kidney), the main differences were in the sets of most informative CpG sites chosen. Only two CpG sites appeared to be common, namely those near *ELOVL2*, a gene involved in the skin cell aging. When the models constructed were used to estimate the age of tumors and respective normal cells, tumors appeared by ~40% more aged than normal cells of the same person. Not only methylation levels *per se* of the age-related CpG sites were changed with age, but also the variation limits of these methylation levels between different persons became larger for most sites. For any specific person the extent of deviation in these values from the population averages seems to be a fairly accurate measure of the individual aging rate.

A bioinformatics analysis of all publicly available datasets concerning age-related variations of DNA methylation in various tissues and cell lines (nearly 8000 samples, 51 tissue and cell types) was performed in attempt to build an ultimate age predictor [29]. The multi-tissue age predictor elaborated allows of estimating with high accuracy and reproducibility the epigenetic age (DNAm age) in various studies of aging. For example, it shows that the epigenetic age is reset when iPSCs (induced pluripotent stem cells) are produced from differentiated somatic cells. A total of 353 methylation sites were chosen, that allow of most reliable age prediction for various tissues and cells (96% correlation to the passport age, ±3.6 years accuracy). Blood cells that have very different lifespans, CD14+ monocytes (myeloid lineage) living several weeks at the most and CD4+ T cells (lymphoid lineage) living for months to years, have identical epigenetic ages in blood samples of healthy male subjects.

Hence, the epigenetic age reflects some internal methylome features related to the chronological age of the person, not just age-dependent peculiarities of respective blood cells. The mean epigenetic age is highly correlated with the chronological age in most tissues. The variation of the epigenetic age between different tissues of the same person is rather small. Two notable exceptions are breast tissue in women (epigenetically older compared with other tissues) and sperm in men (epigenetically younger compared with other tissues). Surprisingly, the system based on human data can be used to predict with fair accuracy the age of chimpanzee tissues, whereas its performance in gorillas is significantly worse. As could be expected, the epigenetic age of ESCs (embryonic stem cells) is close to zero. The same is true for iPSCs; therefore, these cells are much younger than somatic cells used for their production. When cells are maintained in culture, ESCs and iPSCs included, their epigenetic age increases with each passage. A functional analysis of 353 predictive CpG sites showed most of them to be associated with genes involved in the cell death-survival, growth-proliferation, organ-organism development, and cancer. Out of these 353 sites, 193 are positively correlated (hypermethylated) and 160 negatively correlated (hypomethylated) with age. Similar to findings of other studies, methylation levels of hypermethylated sites are less variable, whereas those of hypomethylated sites are more variable between different tissues. The epigenetic age is clearly not a reflection of mitotic age, since it correlates with the chronological age in tissues widely different in proliferative potential, including post-mitotic neurons. Neither is it related to the cell senescence, since its correlation to the chronological age is observed in immortal cell lines, such as ESCs. As the author of the model has proposed, the epigenetic age could be regarded as a quantitative measure of the cumulative work done by the epigenetic maintenance systems [29]. Thus, its value equals to zero in "newborn" ESCs. Epigenome of these cells, just formed, could be considered to be an epigenetic starting point. Early developmental stages involve massive cell division and differentiation events based on epigenetic changes. These events evidently demand high activity of the epigenetic maintenance systems. When early developmental stages end, quantity of epigenetic rearrangements stabilizes at a lower level, mainly limited to stem cell differentiation events in the somatic tissue renewal. These notions are in a good accord with the epigenetic clock being fastest in the first 18 years of life and slowing down to a constant rate thereafter. One of the non-trivial predictions from the model is that the epigenetic aging should be accelerated by factors that destabilize epigenome. The tumor tissue cells show evidence of significant aging acceleration. Enhanced activity of the epigenetic maintenance systems has a genome stabilizing effect; hence, there should be a decrease in the somatic mutation frequency in tumor cells. Indeed, the epigenetic age acceleration in various cancer samples is inversely correlated with the mutation frequency. The epigenetic age concept may be used in various developmental, aging and cancer studies. The *TP53* gene mutations were found to be associated with lower epigenetic aging in five different cancer types. It is in a good agreement with the p53 role as a trigger factor in the protective action of the epigenetic maintenance systems. In breast cancers, mutations of the estrogen receptor (*ER*) or progesterone receptor (*PR*) genes accelerate epigenetic aging, whereas amplification of *HER2/neu* gene does not affect the aging rate. Colorectal cancer samples with the *BRAF* gene mutations have higher aging accelerations than those with the *K-RAS* gene mutations. The mismatch repair gene *MLH1* promoter hypermethylation causes the most significant aging acceleration. The epigenetic age evidently could serve as a convenient marker in assessment of the rejuvenation treatments efficiency. Aging rates of different tissues of the same person can be used to identify those with the evidence of disease, uppermost cancer. An interesting example of application of the epigenetic age concept was estimation of the mortality risk [30]. It was found that acceleration of epigenetic aging to the five-year difference between the epigenetic and chronological age results in a 16% increase in mortality risk, irrespective of general health, life style and genetic factors.

The predictive accuracy can be enhanced when less universal models are used. For example, for blood cells it reached a value of 2.6 years when 17 marker CpG sites only were used [31]. In a follow-up study of the same persons 8 years later the predicted increases in methylation levels of hypermethylated sites and decreases in methylation levels of hypomethylated sites were observed.

3. HISTONE MODIFICATIONS

3.1. Sirtuins

Most abundant data concerning histone modification during aging are connected with the SIRT family deacetylases. Silent information regulator (Sir) proteins have been shown to affect lifespan in different species [32]. In yeast, an extra copy of *Sir2* extends the replicative lifespan by 50%, while deleting *Sir2* shortens lifespan. Sir2 exerts its silencing and lifespan-increasing effects *via* modulation of chromatin structure by catalyzing NAD^+-dependent deacetylation of histone H4K16ac [33]. Sir2 is required for the lifespan extension by CR (calorie restriction) in various model organisms. Mammals have seven Sir2 homologs, sirtuins (SIRT1–7) [32]. Unlike yeast Sir2, mammalian sirtuins are multitarget deacetylases that regulate a wide set of cellular processes. SIRT1, SIRT6, and SIRT7 are localized to the nucleus. SIRT1 has multiple substrates, known to be involved in aging (p53, NF-κB, FOXO family proteins, and others), and is widely believed to defend cells from DNA damage and oxidative stress. Many aging related functions altered by CR are regulated by SIRT1. SIRT1 affects differentiation of muscle cells, adipogenesis, fat storage and metabolism *via* PPARγ and PGC-α activity modulation. Thus, a connection could exist between SIRT1 activity and effects of diets that promote leanness and increase lifespan. Similar to yeast Sir2, SIRT1 has been shown to silence transcription *via* deacetylation of histones and some other proteins (H4K16ac, H3K9ac, H1K26ac, p300, and multiple TFs) [34]. Besides, SIRT1 activity decreases the level of a transcriptionally active chromatin mark H3K79me2 and increases levels of transcriptionally repressed chromatin marks H3K9me3 and H4K20me1 [35]. The major mammalian H3K9me3-specific histone methyltransferase SUV39H1 was shown to be a target of SIRT1 [36]. SIRT1 interacts directly with SUV39H1, recruits it to the respective chromatin loci, and stimulates its activity by deacetylating the K266 residue in its catalytic

SET domain. All these effects lead to an increase in H3K9me3 marks. SIRT1 deficiency results in the complete loss of the SUV39H1-dependent H3K9 methylation and prevents the heterochromatin protein 1 (HP1) localization to heterochromatic regions. Involvement of SIRT1 in DNA methylation at damaged CpG-rich promoters has also been demonstrated [37]. Recruitment of SIRT1, EZH2, DNMT1, and DNMT3B to the site of damage and promotion of H3K9me2, H3K9me3, and H3K27me3 modifications were observed at the E-cadherin promoter CGI upon an experimental induction of double strand breaks. In most cells the promoter activity was preserved following DNA repair, whereas in some cells a heritable silencing was observed. In these silenced cells, respective chromatin loci were enriched for DNMT1, DNMT3B, EZH2, and for the silent chromatin marks H3K9me2, H3K9me3, and H3K27me3, and the promoter CGI was DNA methylated. Interestingly, the enrichment for DNMT3B disappeared at later cell passages, whereas DNMT1 still remained enriched. Apparently, both DNMTs are needed for the initial promoter methylation, whereas DNMT1 alone suffices for its maintenance. Thus, normal DNA repair can occasionally cause aberrant silencing of CGI-containing promoters, events typical of aging and cancer. Treatment of mouse ESCs with non-cytotoxic levels of H_2O_2 greatly decreased the amount of SIRT1 bound to the pericentromeric major satellite repeats, coinciding with their increased transcription and increased H1K26ac content [38]. Thus, SIRT1 seems to be involved in the satellite repeats silencing, whereas oxidative damage counteracts its action. On the genome-wide scale, the SIRT1-bound promoter gene set was significantly enriched for few functional categories, such as chromatin assembly, transcriptional repression, ubiquitin-mediated protein breakdown, and the cell cycle regulation. Oxidative stress led to a massive redistribution of SIRT1, less than 10% of the original SIRT1-associated promoters remaining in the respective chromatin fraction in H_2O_2-treated cells. H_2O_2 increased expression of multiple genes that underwent SIRT1 loss. Interestingly, more than two-thirds of these genes are derepressed during normal aging in mice. Furthermore, SIRT1-target genes are significantly enriched for among the genes upregulated in aged animals. The DNA damage signaling through the ATM-dependent phosphorylation of H2AX is required for efficient SIRT1 recruitment to the damaged DNA loci. SIRT1, in its turn, recruits chromatin-modifying enzymes to the DNA damage sites to prepare them for incoming DNA repair factors. SIRT1 is recruited to double-strand breaks in response to genotoxic stress, resulting in a loss of silencing of SIRT1-target genes. Brain-specific overexpression of SIRT1 completely abolishes the age-dependent up-regulation of gene expression in mice brain. Thus, SIRT1 may provide a direct link between DNA damage and gene expression changes that occur during aging. Lower levels of DNA damage, decreased expression of the cell senescence marker gene $p16^{Ink4a}$, better overall health, and fewer tumors were observed at old ages in transgenic mice moderately overexpressing *Sirt1* compared with the wild-type controls [39].

SIRT6 is a chromatin-associated protein involved in the genomic integrity maintenance *via* the base excision repair (BER) pathway [40]. *Sirt6* deficient mice develop normally for the first two weeks but later undergo aging-like degenerative processes leading to death at around the postnatal day 24. SIRT6 was shown to function as a histone H3K9ac deacetylase at the telomeric chromatin in human cells [41]. The SIRT6 depletion leads to abnormal telomere structures that resemble defects observed in Werner syndrome (an accelerated aging disorder). Moreover, SIRT6 activity is required for stable association of RecQ (the helicase mutated in Werner syndrome) with the telomeric chromatin. SIRT6 was shown to physically interact with an NF-κB subunit, RELA [42]. Upon cell stimulation with TNF-α, a known activator of NF-κB, SIRT6 is recruited to the promoters of several NF-κB target genes leading to their repression. The SIRT6-repressed NF-κB target gene set overlaps with genes known to be upregulated in aged humans [43]. Thus, SIRT6 directly inhibits expression of NF-κB target genes, including those associated with aging. SIRT6 repression of transcription is mediated by its H3K9ac deacetylating activity that destabilizes the RELA association at the target chromatin loci leading to termination of the NF-κB signaling. Four gene sets that share specific binding motifs for NF-κB, CEBP, LFA1, and TEF in their promoters were found to be consistently induced in all tissues of $Sirt6^{-/-}$ mutant mice compared with the wild-type ones. These same gene sets were previously found to be associated with gene expression changes in mammalian aging [43]. Not all NF-κB target genes were hyperactive in $Sirt6^{-/-}$ mutant mice. The SIRT6-regulated subset of NF-κB target genes was selectively enriched for such functional categories as immune response, cell signaling, and metabolism. Furthermore, no SIRT6 association was detected at NF-κB target genes that were unaffected in $Sirt6^{-/-}$ mutant mice. Thus, SIRT6 appears to negatively regulate expression of a subset of NF-κB target genes by modifying chromatin at their promoters. The accelerated aging phenotype of *Sirt6-/-* mutant mice could be rescued by partial repression of NF-κB-dependent gene expression in heterozygous *RelA+/-* mice [42]. Hence this accelerated aging phenotype seems to be caused, at least partially, by overexpression of NF-κB-target genes. Since these NF-κB target genes are known to control many basic functions at both cellular and organismal levels, strict regulation of their activity seems to be very important. The NF-κB activity appears to be regulated by multiple mechanisms, and even disturbance of one of them, such as SIRT6 activity, can lead to the NF-κB-target genes overexpression and to accelerated aging. Several members of the NF-kB family of transcription factors were found to be overexpressed in tissues of $Sirt6^{-/-}$ mutant mice. These members may be involved in multiple links of feedback regulation. In transgenic male mice overexpressing *Sirt6* gene, a significant (~15%) increase in the average lifespan was observed, whereas the lifespan of transgenic female mice was not significantly changed [44]. Multiple genes were differently expressed between transgenic and control males, about 50% of them being also differentially expressed between wild-type males and females. Moreover, quite a number of genes that were either upregulated (*Lpin1*, *Lpin2*, *Gadd45g*, *Fkbp5*, *Dusp1*, and *Cebpd*) or downregulated (*Vnn1*, *Vnn3*, *Pctp*, *Vldlr*, *Car3*, and *G0s2*) in *Sirt6* transgenic males were similarly regulated in wild-type males fed a CR diet. Supposedly, *Sirt6* overexpression extended lifespan of male mice by reducing IGF1 signaling in adipose tissue. SIRT6 deficiency upregulated glucose uptake and activated a nutrient-stress response that included a switch in

glucose metabolism towards glycolysis, away from mitochondrial respiration [45]. SIRT6 has been suggested to be a Hif1a corepressor that acts by deacetylating H3K9ac at Hif1a target gene promoters, thus maintaining the glucose flux into the TCA cycle under normal nutrient conditions.

3.2. Histone Methylation

In aging brain cells of rhesus macaque, H3K4me2 mark has been found to positively correlate with gene expression [46]. As expected, H3K4me2 was concentrated at gene promoter sequences. Intriguingly, the fraction of H3K4me2 at these sequences gradually increased with age. Promoter-associated H3K4me2 peaks showed age-related increases both in intensity and breadth. Similar changes were found at enhancer regions. Thus, a global chromatin opening seems to occur in brain cells upon aging. Total number of genes that displayed increased H3K4me2 with age has been found to be in a great excess compared with those that showed decreased H3K4me2. That was very different from the age-related gene expression changes, about equal numbers of genes being upregulated or downregulated with age. Interestingly, sets of genes that displayed increases in expression or in H3K4me2 overlapped to a very significant extent, whereas no significant overlap was found between gene sets that showed decreased expression or H3K4me2. These data indicate that expression changes with aging are not always a direct result of changes in promoter H3K4me2 marks. This notion is particularly true for aging-downregulated genes. The gene set that showed age-related increase both in expression and in promoter H3K4me2 in the macaque brain significantly overlapped with the respective gene set in the human brain. However, age-related H3K4me2-downregulated macaque genes do not significantly overlap with H3K4me3-downregulated human genes. Thus, the promoter H3K4me2 up-regulation seems to be more relevant to aging than the promoter H3K4me2 down-regulation. Indeed, the H3K4me2-upregulated gene set is significantly enriched for aging-related functional categories, such as chromatin regulation, oxidative stress, DNA damage and repair, inflammation, and metabolism, whereas, the H3K4me2-downregulated gene set is not significantly enriched for any specific biological functions. The chromatin regulatory functions were significantly enriched among genes proximal to H3K4me2-upregulated enhancers. This last finding suggests that chromatin modifiers themselves are age-regulated by H3K4me2, indicating existence of an extensive cross-talk between different chromatin modification mechanisms. It was suggested that H3K4me2 marks at promoters and enhancers induce a progressive opening of the chromatin structure during development and aging [46]. Histone modifications are widely believed to play an important role in establishing and maintaining the epigenetic landscape. It would be interesting to study possible cross-talk between H3K4 methylation and other epigenetic modifications and their net effect on the chromatin structure. The finding that chromatin modifiers are themselves among the major targets of the age-related regulation of gene expression by H3K4 methylation suggests that more extensive changes to epigenome could occur upon aging.

4. GENE EXPRESSION

A comparative study of transcription profiles between middle-aged adult (6-day-old) and young adult (0-d-old) *C. elegans*, and between middle-aged adult (23-d-old) and young adult (3-d-old) *D. melanogaster*, showed that most aging-related changes in gene expression are species-specific [47]. Nevertheless, there is a conserved part in these expression profiles that includes several hundred *C. elegans–D. melanogaster* ortholog gene pairs. Aging in both *D. melanogaster* and *C. elegans* is accompanied by repression of genes functionally related to mitochondrial membranes, such as components of the respiratory chain, the ATP synthase complex, and the TCA cycle. Some genes encoding peptidases and proteins involved in DNA repair and catabolism are also similarly regulated in both species. These conserved changes in gene expression occur rather abruptly early in adulthood and are significantly correlated with responses to heat and oxidative stress in both organisms. Therefore these conserved changes in gene expression are not solely caused by the cumulative damage, but rather could present a strictly developmentally regulated part of the putative aging program.

Both downregulated and upregulated with aging gene sets were found in transcriptional profiles of the human frontal cortex in individuals ranging from 26 to 106 years of age [48]. Two groups of individuals, <42-yr-old and >73-yr-old, showed most homogeneous pattern of gene expression, and were negatively correlated with each other. The middle age group (45–71-yr-old) displayed a greater heterogeneity, with some individuals be more similar to the young group and others to the aged group. Thus, the rate of age-related changes in gene expression may be variable among adult humans. About 4% out of ~11,000 genes analyzed had significantly (≥1.5-fold) different expression levels between young and aged groups. Genes functionally related to learning and memory were mostly downregulated in aged individuals, whereas genes related to the stress response and repair functions, such as protein folding, antioxidant defense, metal ion homeostasis, and inflammation, were upregulated. Up-regulation of the genes encoding BER enzymes, 8-oxoguanine DNA glycosylase and uracil DNA glycosylase, corresponded with increased oxidative DNA damage in aged persons. Stably expressed and upregulated genes showed little promoter DNA damage (8-oxoguanine), whereas DNA damage in downregulated genes increased with aging. This increased DNA damage may be partially responsible for reduced expression of respective genes in the aged brain cells. Interestingly, essentially different sets of genes were downregulated or upregulated in aging cerebellum and caudate nucleus [49]. Even more surprising, the orthologs of both upregulated and downregulated human genes were found to be expressed in chimpanzee cortex at constant levels corresponding to those in young humans. Entirely different sets of genes changed expression in chimpanzee cortex during aging (1252 downregulated and 700 upregulated genes). No specific GO categories were enriched in the set of genes downregulated with age in chimpanzee cortex, although a number of significant enrichments were found in gene set upregulated with age, including mitochondrial localization, protein degradation, and several metabolic processes.

Effects of aging and CR on gene expression were studied in different tissues of young (4–6 months age), old (26–28 months age), and young CR (4–6 months age with 2.5–4.5 months of CR) mice [50]. Expression of 309, 1819, and 1085 genes was found to change significantly with aging in heart, liver, and hypothalamus, respectively. Only 9 genes changed expression with aging in all three tissues, some of them in opposite directions. CR significantly affected expression of 192, 839, and 100 genes, respectively, only 7 of them (3 upregulated, 4 downregulated) being common to all three tissues. Concerning the functional categories, up-regulation of antigen processing/presentation genes and down-regulation of stress response genes by CR were observed in all three tissues. Comparison between aging and CR showed that there are 389 genes, 18 biological processes, and 20 molecular functions in common. Aging affected gene expression more significantly and broadly than did CR in all tissues. Generally, partial overlaps of age-related gene expression profiles were observed between different tissues. Thus, both common and tissue specific changes in gene expression occur in response to aging and CR.

One of the consequences of the age-associated DNA damage accumulation in somatic cells could be stochastic deregulation of gene expression manifested as increased cell-to-cell variations in gene expression. A highly significant increase in such variations was observed in the ventricular heart tissue between young (6 months) and old (27 months) male mice for several nuclear genes (seven housekeeping genes, three heart-specific genes, and two protease-encoding genes), but not for three mitochondrial genes [51]. Spontaneous variation in transcription level (noise) seems to be an inherent feature of the basic mechanisms of transcription. It is especially evident for weakly expressed genes. Treatment of cultured fibroblasts with 0.1 mM H_2O_2 at early passages increased variation in gene expression levels between individual cells. The effect was absent at 6 h after treatment, but was significant at 48 h, when an increased number of senescent cells became obvious. At 9 days almost all cells reached senescence, and the enhanced variation in gene expression still remained highly significant. One may suggest that persistent forms of damage, including random changes in DNA methylation and chromatin modification, lead to a gradual increase in transcriptional noise, thus introducing progressively increased phenotypic variation among cells of the same tissue. Eventually, such progressively increased variation could lead to a complete failure of respective organs, which would explain many etiological features of aging, first of all the highly variable character of organ decline during chronological aging.

In a study of normal muscle samples from variously aged humans a molecular profile for aging consisting of 250 age-regulated genes was established [52]. A common signature for aging in diverse human tissues was defined by comparing these data with transcriptional profiles of aging in kidney and brain. This common aging signature involves genes of four genetic pathways (extracellular matrix, cell growth, complement activation, and cytosolic ribosome) that increase expression with age, and two genetic pathways (chloride transport and mitochondrial electron transport chain) that decrease expression with age. When the human transcriptional profile of aging was compared with those of mouse and fly, the electron transport chain pathway was found to be a common marker for aging across all three species. Great heterogeneity of the age-related changes of gene expression was found to exist between various murine tissues [53]. Some tissues displayed large transcriptional changes in aged mice, suggesting a strong contribution to organismal decline. Others displayed few changes in expression with age, showing a strong level of homeostasis even in aged animals. Based on these gene expression patterns, all tissues could be divided into three aging classes. Different tissues of the same individual were found to age in a coordinated fashion, such that certain individuals exhibit rapid aging, while others exhibit slow aging in all tissues. In the aging gene expression signatures in mice, humans, flies, and worms, the electron transport chain genes showed similar regulation in all four species, indicating that these genes may serve as exceptionally good markers of aging.

As has been noted above, chronological aging is accompanied by changed expression of large numbers of genes, the changed gene sets being to a great extent tissue- and species-specific. These age-dependent gene sets were compared for the presence of common upstream motifs for TF binding across diverse tissues samples (skin fibroblasts, kidney cortex, kidney medulla, abdominal muscle, skeletal muscle, and brain) obtained from variously aged (16 to 106 years) humans [43]. Hypothetically, motifs representing general regulators of aging should be those acting in multiple tissues. When such common motifs were compared between humans and mice, the strongest evidence for involvement in age-dependent gene regulation was obtained for those recognized by TFs of the NF-κB family. Coordinated induction of gene sets defined by the NF- κB recognition sites alone or their combinations with other TF binding sites tended to occur in humans after 40-yr age and was even more evident after 70-yr age. Moreover, putative NF-κB target genes were among the most upregulated ones in HGPS (Hutchinson-Gilford progeria syndrome) cells. Multiple NF-κB target genes were strongly upregulated in tissues and hematopoietic stem cells of aged mice. A high fat diet in mice increased expression of NF-κB target genes in liver, while concomitant treatment with resveratrol (known to reverse effects of high fat diet and to extend lifespan) repressed expression of these genes. It was suggested that NF-κB could be a master regulator of gene expression programs in mammalian aging [43].

A meta-analysis of age-related gene expression profiles from mouse, rat and human datasets revealed common signatures of aging, including 56 genes that showed increased expression in aged individuals (*APOD* being the most significant), and 17 genes whose expression decreased with age [54]. Concerning the functional categories, age-dependent gene expression most notably involved increased expression of inflammation, immune response, and lysosome genes, and decreased expression of genes associated with energy metabolism and collagen genes. Changes of the expression levels were also observed for genes related to apoptosis, cell cycle and cellular senescence. Supposedly, these molecular signatures of aging reflect not only a mix of degenerative processes but also adaptive responses of healthy cells to the aging-associated derangements.

In the human peripheral blood leukocytes, only 2% of genes were variably expressed at different ages [55]. Genes involved in messenger RNA splicing, polyadenylation, and other post-transcriptional events were among the most changed during aging. Thus, deregulation of mRNA processing may be one of the mechanisms of human cellular aging. As has been predicted, largest variations in expression levels with age were observed for genes involved in inflammatory responses or immune function [54]. Genes representing well known aging pathways, such as insulin and TOR signaling, oxidative stress, DNA repair, telomere maintenance, and some others, also were among the top 100 age-dependent ones. Only seven of the GO pathways were robustly associated with aging, of which four are involved in mRNA processing and three other are related to chromatin structure and to mRNA translation. Since disruptions to the proteins involved in mRNA processing appear to occur without transcriptome-wide changes in gene expression levels, it may be suggested that this disruption leads to changes in the relative abundance of alternatively spliced transcripts or increases the frequency of aberrant splicing events. Indeed, distortions of the isoform expression patterns with increasing age were observed for seven out of ten alternatively spliced transcripts studied. It may well be that stochastic DNA demethylation upon aging leads to increased frequency of aberrant splicing events, since the non-coding parts of genes (introns) were shown to be preferentially methylated compared with the coding parts (exons), thus providing a means to demarcate the sequences to be spliced out [5, 6].

Whole transcriptome sequencing allows comparing the transcription profiles of non-coding RNA and splice variants, besides protein-coding mRNAs. Comparison of the cerebral cortex transcriptomes between 6-, 12- and 28-month-old rats revealed differential expression of genes related to MHC II presentation and serotonin biosynthesis, as well as a wide group of non-coding genes [56]. Across the three age-groups, differential expression was found for 136 transcripts, 37 of which did not map to known exons. Fourteen of these transcripts were identified as novel lncRNAs (long non-coding RNAs). Evidence of isoform switching was also found. Therefore, in addition to changes in the expression of protein-coding genes, changes in transcript splicing, isoform usage and non-coding RNAs occur with age.

5. SMALL RNAs

Out of 114 *C. elegans* miRNAs cataloged in miRBase version 5.0, 34 have been found to change their expression levels during the post-young-adult aging from D4 (day 4) to D15 [57]. The relative abundance of several miRNAs changed very substantially across the adult lifespan. In particular, miR-231 was expressed at a high level and displayed striking variation over adulthood (increased from D4 to D11, decreased at D13, and greatly increased at D15). The level of miR-34 increased significantly between D6 and D8. Five age-regulated miRNAs, including let-7, decreased between D6 and D8. These miRNAs were suggested to influence aging and lifespan. The level of miRNA lin-4, known to affect lifespan [58], gradually declined from D6 to D11, and remained essentially constant thereafter. This observation fits with its effects on lifespan, corroborating the view that age-regulated miRNAs can regulate rate of aging and longevity. The level of let-7 was highest early in adulthood, rapidly decreased between D6 and D11 to minimal values and remained constant thereafter. The nuclear hormone receptor DAF-12 transcript has been shown to be a direct target of let-7. Since *daf-12* null mutations are known to extend lifespan in *C. elegans*, diminishing levels of let-7 with age could influence lifespan similar to lin-4. Highly similar expression patterns of miR-1 were observed in somatic muscle in *Drosophila*, zebrafish, chicken, mouse, and human. In mammals, miR-1 family members were implicated in cardiomyocyte and skeletal muscle development; in *Drosophila*, miR-1 has been shown to regulate cardiac cell differentiation in embryo. In *C. elegans*, concentration of miR-1 was found to exhibit a rapid decline during the adult life, correlating with the progressive decline of body-wall muscle during aging. Out of 204 *C. elegans* genes known to impact lifespan, 42 encode mRNAs that are potential targets of age-regulated miRNAs; 10 of them could be targeted by multiple age-regulated miRNAs. These genes encode proteins involved in gene expression, signal transduction, energy production, metabolism, cellular structure, and stress responses. Thus, age-dependent expression of multiple genes involved in aging could be regulated by age-dependent miRNAs.

A total of ~50 mature miRNAs (~30% out of 174 known miRNAs of *C. elegans*, annotated in miRBase release 14) were found to change in abundance between D0 (young adult) and D8 (old adult) ages, including 23 miRNAs upregulated and 16 miRNAs downregulated more than two-fold [59]. When miRNA maturation was inhibited in adult worms by an RNAi against *alg-1*, encoding an Argonaute protein, a significant decrease of lifespan was observed. Besides, miRNAs with increasing expression during aging, such as miR34, miR239a, and miR87, were disrupted in their expression, while those with decreasing expression, such as let-7, were not affected. Thus, age-regulated miRNAs, at least some of them, could play a significant role in aging and lifespan regulation. Compared with control animals, which were kept at 23°C during all life stages, animals shifted to lower temperature (15°C) at D0 showed a significantly longer lifespan, while those shifted to higher temperature (27°C) showed a shorter lifespan. Those miRNAs that increased expression during aging showed a significant delay in expression changes at 15°C, while at 27°C their expression changes were accelerated. As an example, miR-34 and miR-239a, both of which exhibited ~10−15-fold increase in expression from D0 to D8 in control worms, reached a similar fold increase at D15 in the long-lived (15°C) animals and at D3 in the short-lived animals (27°C). Thus, these miRNAs seem to be tightly regulated during aging, and their prolonged expression at lower levels might contribute to delay of aging at 15°C. In contrast, miRNAs with decreased expression during aging did not show a delay when shifted to prolongevity conditions at 15°C. Expression levels of these miRNAs, including let-7 and lin-4, decreased soon after animals were shifted to the lower temperature. Their down-regulation could be possibly programmed at earlier stages of development or controlled independently from lifespan. Interestingly, many miRNAs upregulated with age, at later stages of development displayed a wider range of tissues-specific expression compared with earlier stages, suggesting an age-dependent loss of proper regulation. Such ectopic

activation of miRNA expression might trigger unfavorable age-related decline, since alterations of miRNA expression levels may cause reciprocal changes in the expression of their multiple target genes. A total of 354 genes were predicted to be targets of at least one age-associated miRNA. Out of these genes, 58 are known to affect lifespan. Most proteins encoded in these genes are involved in aging-related pathways, such as phagosome and lysosome function, protein processing, splicing, oxidative phosphorylation, methionine metabolism, and others.

A temperature-sensitive allele of the miRNA biogenesis gene *pash-1* in *C. elegans* allows of rapid and reversible inactivation of miRNA synthesis [60]. The mean lifespan of *pash-1ts* adults shifted to the restrictive temperature was shorter by about 30% compared with similarly shifted wild-type worms. This defect was rescued by a wild-type *pash-1* transgene, confirming that it was caused by the *pash-1ts* lesion. Hence, miRNAs synthesized post-developmentally must have functions that affect lifespan. Decline in locomotion is a well-known marker of aging in *C. elegans*. It was noted that locomotion declined more rapidly in *pash-1ts* adult worms shifted to the restrictive temperature. Thus, miRNA synthesis deficiency appears to accelerate the aging. Very few (<20) genes were misregulated in *pash-1ts* mutants at the permissive temperature, whereas hundreds of genes became misregulated upon shift to the restrictive temperature. Genes that are known to be upregulated during normal aging in wild-type animals were rapidly upregulated in *pash-1ts* animals at the restrictive temperature. Conversely, genes normally downregulated in old animals were rapidly repressed. Genes that were upregulated, then downregulated, over the first 15 days of normal aging, were upregulated, then downregulated over the course of 24 hrs in *pash-1ts* animals at the restrictive temperature. These data corroborate the view that the reduced lifespan of animals deficient in miRNAs synthesis results from accelerated aging. Surprisingly, inactivation of the DAF-2 insulin/IGF-1 receptor extended lifespan even in animals deficient in miRNAs synthesis. This lifespan extension was *daf-16* dependent, indicating that FOXO can promote longevity *via* mechanisms that do not require synthesis of miRNAs. FOXO mediated effects of the IIS (insulin/IGF-1 signaling) pathway activity on lifespan are known to involve regulation of a large set of target genes. The data described suggest that this program of transcriptional regulation is sufficient to extend lifespan even in the absence of post-transcriptional regulation by miRNAs. Removal of miRNA expression reduced lifespan in a *daf-16* null mutant, in which IIS cannot regulate lifespan. Thus, miRNAs and IIS seem to regulate the aging *via* independent mechanisms.

In *Drosophila*, a hypomorphic mutation in loquacious (*loqs*), a key gene in fly miRNA processing, was found to lead to a significantly shortened lifespan and a late-onset brain morphological deterioration [61]. Out of 29 miRNAs expressed in the adult brain, most maintained a steady level or decreased with age, while one, namely miR-34, increased. miR-34 is a markedly conserved miRNA with identical seed sequence in orthologues among *Drosophila*, *C elegans*, mouse, and human. Flies null mutant for *mir-34* gene showed none obvious defects at early stages of development, but displayed a catastrophic decline in viability just after reaching mid-age (D30). Young (D3) mutants had normal locomotion and stress resistance, but showed dramatic climbing deficits and high stress-sensitivity at D20. Wild-type flies showed sporadic vacuoles in brain cells at advanced ages. *mir-34* mutants were born with normal brain morphology, but showed dramatic vacuolization with age, indicative of loss of brain integrity. A 9 kb genomic DNA fragment containing *mir-34* gene partially rescued the age-associated expression of miR-34 in mutant flies and lessened the mutation effects. Thus, loss of miR-34 seems to accelerate the brain aging. Transcription profiling of the fly brain at various ages showed that the majority of transcripts that increase with age display a faster increase in *mir-34* mutants compared with controls. These results confirm that *mir-34* mutation accelerates the brain aging. One of the miR-34 targets is *E74EF* gene encoding a protein of steroid hormone signaling pathways. Northern blot analysis indicated that *E74EF* is transcribed at a high level in adult flies, whereas the level of the encoded protein is very low. In flies lacking miR-34, the E74EF protein level is dramatically increased. In the wild type, E74EF protein is highly expressed in young flies, but undergoes a dramatic decrease within a 24 hrs time window, temporal pattern appearing to be opposite to that of miR-34. In *mir-34* mutant flies down-regulation of the E74EF protein during this critical period is lost. Thus, adult-onset expression of miR-34 seems to function to attenuate E74EF expression in young adults, and to maintain that repression through adulthood. When a *E74EF* hypomorphic mutation has been introduced into *mir-34* mutant flies, age-associated defects of *mir-34* mutation, including the shortened lifespan and the brain vacuolization, were mitigated. And vice-versa, when the adult activity of E74EF was upregulated with an *E74EF* transgene that lacked miR-34 binding sites, animals showed late-onset brain degeneration and a significantly shortened lifespan. Thus, deregulated expression of E74EF negatively impacts normal aging. One function of miR-34 is to silence E74EF in the adults to prevent its adult stage-specific deleterious effects on the brain integrity and viability. The findings described suggest that miR-34 is a key miRNA that couples maintenance of the brain long-term integrity with healthy aging. E74EF appears to have opposite effects on animal fitness at different life stages, being essential during pre-adult development, but harmful to the adults during aging. This biological property is known as antagonistic pleiotropy. miRNAs normally expressed in the adults may serve to down regulate the genes whose expression is needed at earlier developmental stages but promote age-associated decline. It has been noted that miR-34 is elevated with age in *C. elegans*, and mammalian miR-34 orthologues are highly expressed in the adult brain, increase with age, and are misregulated in degenerative diseases. Several studies have highlighted miR-34a as an important mediator of aging and cardiac and endothelial cell dysfunctions [62] and references therein. miR-34a expression increases in different organs of aged mice. Increasing levels of miR-34a are responsible for the endothelial cell senescence due to direct down-regulation of its target, SIRT1. Expression levels of miR-34a in aortas of old (21 month) mice are nearly twofold higher compared with those of young (2.5 month) mice [62]. Concomitant decrease of SIRT1 levels is observed both in endothelial and Vascular Smooth Muscle Cells (VSMCs) in aged aortas. The replica-

tive senescence of cultured VSMCs is also accompanied by a significant increase of miR-34a level and a concomitant decrease of SIRT1. Ectopic overexpression of miR-34a inhibits VSMCs proliferation and promotes senescence, and also significantly increases mRNA levels of pro-inflammatory cytokines. Thus, aging-associated up-regulation of miR-34a in VSMCs could lead to down-regulation of SIRT1 and induction of the senescence-associated secretory factors, thereby provoking cell senescence and inflammation that characterize arterial dysfunctions, such as vascular calcification and atherosclerosis.

An analysis of miRNA expression profiles between old and young murine liver samples revealed four miRNAs upregulated with aging, miR-669c (9.9-fold upregulation in 33-month old mice compared with 4-month old ones), miR-709 (7.6-fold), miR-214 (3.4-fold), and miR-93 (4.3-fold) [63]. Only miR-669c and miR-709 showed a gradual increase with age, whereas miR-93 and miR-214 showed biphasic expression at 10- and 18–33-month ages. Several miRNAs were found to decrease at 33 months compared with 10 months (miR-375, let-7i, and let-7g). Out of proteins significantly downregulated at 33 months, at least 24% were predicted to be the targets of one upregulated miRNA. A broad range of biological categories were found to involve genes targeted by age-upregulated miRNAs and to decline in old liver. Several downregulated glutathione S-transferases were predicted to be targeted by miR-93, miR-214, and miR-669c. Obviously, these miRNAs may be involved in aging mechanisms related to the oxidative stress. Since glutathione S-transferases are known to play major detoxification roles, their decreased activity in the liver of aged animals must expose them to higher toxin levels and oxidative stress. A decline in the oxidative protection may be correlated with the miR-93 targeted gene *Mgst1*, and the failure of the mitochondrial respiratory chain may be correlated with the miR-709 targeted gene *Uqcrc1*. Decreased respiratory chain activity is believed to result in enhanced ROS production observed in the mitochondria of old liver. *Igf1* was predicted to be targeted by miR-93; it may have a role in the IIS pathway similar to *lin-4* in *C. elegans*. Decreased IIS pathway activity in the liver has been observed at CR, and may promote its prolongevity effect. The gene for miR-669c is located within an intron of the gene encoding Sfmbt2, a Polycomb group protein, known to functionally interact with TF Yinyang1 (YY1), possibly forming a silencing complex [64]. The gene for miR-709 was found to be located within an intron of the gene encoding TF Rfx1, a known activator of virus gene expression and suppressor of cellular genes, such as *c-myc* and *PCNA*. miR-709 targets a protein encoded by the gene *BORIS* (Brother of the Regulator of Imprinted Sites), known to play an important role in epigenetic reprogramming during the male germ cell differentiation [65]. BORIS is a testis-specific paralogue of CTCF, a ubiquitous zinc finger protein involved in transcriptional silencing or activating in a context-dependent fashion, and organizing epigenetically controlled chromatin insulators that regulate imprinted genes [66]. CTCF and BORIS recognize the same or an overlapping spectrum of DNA sequences, but functional consequences of their binding are different. Their genes have been shown to be expressed in mutually exclusive modes during the male germ cell development. BORIS-positive spermatocytes that re-establish the paternal DNA methylation patterns and display genome-wide DNA demethylation were found to be negative for CTCF. At later stages of spermatogenesis, BORIS is silenced while CTCF is re-activated. It is known that in normal germline cells genome-wide erasure of DNA methylation coincides with *BORIS* up-regulation, whereas remethylation of DNA is associated with *BORIS* silencing [67]. *BORIS* is also expressed in multiple cancers and its conditional expression in normal fibroblasts activates cancer genes selectively. In human embryos significant *BORIS* transcription was detected in oocytes, followed by down-regulation at the early cleavage stages [68]. As a matter of fact, *BORIS* seems to be expressed in various somatic tissues, but the levels of its transcripts are lower by several orders of magnitude compared with those in testis and oocyte [69]. Of mouse somatic tissues studied, maximal levels of BORIS mRNA were detected in skin [70]. DNA-bound BORIS molecules were shown to recruit SET1A and BAT3 complexes to respective chromatin loci [71]. SET1A is a part of the H3K4 methyltransferase complex SET1A/ASH2. This may explain mechanisms of the BORIS mediated DNA demethylation, since DNA methylation and H3K4 methylation are widely known to be mutually exclusive epigenetic marks. BAT3, another BORIS binding partner, has been shown to recruit H3K79 methyltransferase to the DNA damage-induced chromatin foci of 53BP1 [72]. Context-specific H3K79 methylation by DOT1L is known to regulate a number of cellular activities, including gene transcription, embryonic development, embryonic stem cell division, and cardiac function. Thus, locus-specific effects of BORIS could be mediated by histone methylation. Knockout mice homozygous for the *BORIS* null allele have a defect in spermatogenesis that results in small testes and increased cell death [73]. The defect is evident as early as postnatal day 21 and is manifested by delayed production of haploid cells. Up-regulation of miR-709, targeting BORIS mRNA, may be the cause of age-dependent decline in spermatogenesis. Of a special interest is the finding that expression of *hTERT* gene in immortalized tumor cells could be due to BORIS binding [74].

Summing up, a mid-age up-regulation of miRNA expression leads to the down-regulation of proteins involved in the control of intermediate metabolism, apoptosis, DNA repair, oxidative defense, mitochondrial oxidative phosphorylation, and other cellular functions. Stress factors can induce upregulated miRNA expression [64]. In particular, permanent growth arrest, seen either in replicative senescence *via* serial passaging, or in premature senescence induced by peroxide treatment, is associated with significant up-regulation of miRNAs. This stress-induced activation of miRNAs may promote tissue aging. Certain miRNAs, including miR-210 and miR-373, decrease the expression of key DNA repair proteins [75]. Interestingly, most of >800 human miRNAs were found to be downregulated with age in peripheral blood mononuclear cells [76]. In 16 pairs of ~30-yr-old *vs* ~64-yr-old individuals, 16.5% of the miRNAs declined in abundance more than twofold, while only 2.5% increased. It has been shown that miR-24, significantly downregulated with age, can target *E2F2*, $p16^{INK4a}$, *MKK4,* and *H2AX* transcripts. Several miRNAs downregulated with age are known to be associated with cancers (miR-103, -107, -128, -155, and -

221). These findings underscore the importance of miRNA expression in age-related diseases. PI3K (phosphatidylinositol-3-kinase) is a known integrator of multiple signaling pathways that promote tumorigenesis. Down-regulation of miR-221 with age leads to increased PI3K mRNA and protein. Thus, miR-221 may be a modulator of pathways important in aging and tumorigenesis. Higher levels of c-Kit protein, known to be overexpressed and/or activated in various leukemias and other cancers, were observed in older individuals compared with younger ones. This could be due to age-dependent down-regulation of miR-221 that targets c-Kit mRNA.

In cerebellum and cortex of humans and non-human primates, miRNAs either upregulated or downregulated with aging were detected, their sets being both species- and tissue-dependent [77]. The only exception was miR-144; its expression was found to be upregulated in the aging cerebellum and cortex of all non-human primates studied, but not in the human cortex. Interesting, miR-144 level was found to be higher in the cortex of Alzheimer's patients compared with their healthy littermates. Multiple upregulated miRNAs were predicted to target a relatively conserved group of mRNAs related to transcription regulation and neuronal function. The mRNAs of ataxin 1 (ATXN1), a protein involved in pathogenesis of spinocerebellar ataxia (SCA), gamma-aminobutyric acid receptor A1 (GABARA1), and nuclear factor I/B (NFIB) were among the putative targets. Expression of miRNAs was markedly increased in the cortex and cerebellum of SCA patients compared with healthy individuals. ATXN1 mRNA was predicted to be targeted by miR-101, -130a, -19a, and -302. Both miR-144 and miR-101 reduced the ATXN1 protein expression. The sensitivity of a particular neuronal population to ATXN1 and other mutant proteins involved in neurodegeneration might depend on the effectiveness of their miRNA-mediated regulation. Thus, selective miRNA expression in the aging brain may be a protective mechanism, and its dysfunction may contribute to neurodegenerative disorders.

Numerous proteins exhibit age-dependent variations in mouse brain [78]. Among 190 proteins identified at a high confidence level, there were 13 subunits of the mitochondrial electron transport chain complexes or ATPase, of which 10 were downregulated. The sharpest decreases in protein expression levels were seen between 10 and 24 months. Interestingly, higher expression levels of oxidative phosphorylation proteins were observed in extremely old 33-month mice compared with 24-month mice. Three rate-limiting enzymes of glucose metabolism (hexokinase-1, pyruvate dehydrogenase, and citrate synthase) were increased at 18 and 24 months compared with 10 months. Mitochondrial oxidative defense proteins, peroxiredoxin-1, -2 and -6, and superoxide dismutase were increased at 33 months. Out of 367 miRNAs studied, 70 showed significant variations. Among them, 31 miRNAs exhibited consistent up-regulation and 17 were consistently downregulated in all aged groups compared with 10-month-old mice. Inconsistent changes across the age groups were observed for 22 miRNAs, including 3 miRNAs that exhibited increased expression at 18 months and 18 miRNAs that demonstrated increased expression at 24 months. Surprisingly, only 6 of the last ones were also upregulated at 33 months. Out of 70 age-variable miRNAs, 27 were predicted to target 10 mitochondrial subunits that were downregulated at 24 and/or 33 months. Among them, 16 miRNAs were consistently upregulated across all aged groups, whereas 4 were consistently downregulated. Out of the remaining 7 miRNAs, 2 increased at 18 months, 7 increased at 24 months, and 4 increased at 33 months. These data show that multiple miRNAs are upregulated during normal aging, while brains of the extremely old mice have less upregulated miRNAs than those of 24-month old mice. Expression levels of miR-22, -101a, -720, and -721 were inversely correlated with those of their predicted targets, Uqcrc2, Cox7a1, Atp5b, and Cox5b. One may suggest that there are two main groups of miRNAs involved in aging. The first group consists of crucial miRNAs that carry out tissue-specific regulation of genes, such as oxidative phosphorylation in brain or detoxification in liver, that leads to tissue-specific declines during aging. Another group consists of miRNAs that regulate common aging processes. Thus, upregulated miRNAs miR-30d, -34a, -468, -669b, and -709 were found both in aging liver and brain, whereas miR-22, -101a, -720 and -721 were found specifically in aging brain, and miR-669c, -712, -214, and -93 were specific to aging liver. Interestingly, the changes in expression of miRNAs and their target proteins observed in old mice (24 months) were inconsistent with those in extremely old ones (33 months). Since only a very small (~4%) proportion of mice population survived to 33 months, it may well be that some stochastic epigenetic events occurred in these mice, leading to reduced levels of miRNA deregulation and to an extended lifespan.

6. IS LIFESPAN EPIGENETICALLY CONTROLLED

Heterochronic genes *lin-4* and *lin-14* control the cell fate choices during *C. elegans* development. The *lin-4* gene encodes a miRNA that targets lin-14 mRNA, encoding a DNA associated nuclear protein, LIN-14. Mutations in *lin-4* and *lin-14* were found to affect aging [58]. A *lin-4* null-mutation led to a shorter lifespan compared with the wild type, whereas a *lin-14* null mutation had an opposite effect that was reproduced by RNAi against lin-14 mRNA. Thus, LIN-14 normally acts to shorten lifespan. A *lin-14* gain-of-function mutant that lacks the lin-4 target site and overexpresses LIN-14, closely phenocopied the short-lifespan phenotype of the *lin-4* mutant. An RNAi against lin-14 mRNA suppressed the short-lifespan effect of *lin-4* mutation. These data show that effects of *lin-4* on lifespan are mediated through LIN-14 repression. The *lin-4* mutant has been found to accumulate intestinal lysosomal deposits of lipofuscin, an established marker of aging, more rapidly than the wild type. This premature lipofuscin accumulation was suppressed by *lin-14* mutation. Thus, a miRNA that has a regulatory function in development can also regulate aging and lifespan.

Mature forms of 120 annotated miRNAs and 10 new miRNAs were detected in small RNA fractions from aged tissues of wild-type and *daf-2* mutant *C. elegans* by deep sequencing [79]. New miRNA, miR-4929, -4930, and -4933, had seed sequences homologous to those of higher eukaryote miRNAs; miR-4933 appeared to be a new member of the miR-34 miRNA family. As was described in the previous section, the miR-34 family miRNAs are among the most upregulated with aging. Several novel miRNAs were ex-

pressed in aged wild-type worms at higher levels compared with young wild-type and old *daf-2* worms. Interestingly, miR-4929, -4930, and -4934 were expressed only in *daf-2* mutant worms. These miRNAs could normally be suppressed by the IIS pathway activity. In wild-type animals, 8.6% of miRNAs increased and 28.4% decreased in expression more than twofold from D0 to D10. A nearly identical pattern was observed in *daf-2* animals, 9.8% of miRNAs increased and 25.6% miRNAs decreased with aging. miR-246 was the most upregulated miRNA in wild-type animals (6-fold increase). A strong increase in expression during aging was also observed for miR-71, miR-34, miR-253, miR-238, and miR-239a/b. Conversely, let-7 showed maximal (7.4-fold) decrease with aging. Expression of miR-41, -70, and -252 also decreased with aging. Most miRNAs showed similar changes in expression in wild-type and *daf-2* worms. The miRNAs most upregulated in *daf-2* mutants compared with wild-type animals, miR-237, -62, and -252, were among the most downregulated with aging. And vice-versa, miR-239b that showed decreased expression in *daf-2*, was among the most upregulated with aging. These *daf-2*-dependent miRNAs reveal an intersection between the IIS pathway and miRNA mediated gene regulation that affects lifespan. Four miRNAs that exhibited largest changes with aging, namely miR-71, -238, -239, and -246, also influenced lifespan. Deletions of *mir-71*, *mir-238*, and *mir-246* genes decreased lifespan, whereas deletion of *mir-239* gene increased it. Since levels of miR-71, -238, and -246 increased with aging, these miRNAs appear to promote longevity. Indeed, overexpression of miR-71 and miR-246 significantly increased lifespan, whereas overexpression of miR-239 decreased it. Overexpression of miR-238 was without effect. Thus, direct roles for miR-71 and miR-246 in pathways promoting longevity, and for miR-239 in those antagonizing longevity seem quite probable. Both miR-71 and miR-239 have been found to act *via* the IIS pathway; miR-71 has been also demonstrated to interact with the DNA damage response pathway. Thus, miR-71 may serve as a possible link between the IIS and the DNA checkpoint pathways. No discernible phenotypic effects were observed for deletion mutants of *mir-71*, *mir-239*, and *mir-246*, besides their effects on longevity. Thus, their encoded miRNAs may function as specific regulators of genetic pathways that affect lifespan.

High-level expression of miR-71 throughout the mid-adulthood (D3 to D7) has been found to promote longevity; animals with higher and/or longer-lasting miR-71 expression showed longer lifespans [80]. Collectively, these two parameters predicted 47% of the individual lifespan variation. This correlation was completely lost in *daf-16* mutant worms. Changes in miR-246 expression between D3 and D7 predicted 20% of the total lifespan variation. The mean lifespan of animals that have a high level of miR-239 expression was by about 10% shorter compared with those having a low level of miR-239 expression. The correlation of miRNA expression levels at early adult life stages with later longevity suggests that lifespan is determined to a very considerable extent by epigenetic mechanism of ‘‘robustness’’ [80]. In the case of miR-71 and miR-239, the mechanism of the individual lifespan determination may be *via* the well-known IIS pathway. Since deletion of *mir-71* has been shown to increase expression of IIS pathway components [79], whereas *daf-16* null mutation increases levels of miR-71 expression [80], one could suggest that DAF-16 and miR-71 activities are balanced *via* mutual regulation. Thus, individual features of miR-71 expression may directly influence IIS activity, and hence, the individual lifespan.

It has been shown that *mir-71* gene mutant worms have significantly increased sensitivity to heat shock and oxidative stress [81]. A wild-type *mir-71* transgene rescued both the short-lifespan and the increased-heat-stress-sensitivity phenotypes of these mutants. Obviously, miR-71 activity is required for both normal lifespan and normal stress-sensitivity. Moreover, extra copies of *mir-71* gene extended lifespan by about 20%. Thus, miR-71 seems to actively delay aging. The germline cell ablation was shown to extend lifespan in wild-type animals, but has been without effect in *mir-71* mutants. On the other hand, extra copies of *mir-71* modestly extended lifespan in germline-intact animals, and more significantly - in germline-deficient *glp-1* mutants. A partial loss-of-function mutation of the *daf-2* extended the lifespan in the wild-type and *mir-71* mutant backgrounds to similar extents (more than twofold). An analysis of cell-specific miR-71 deficient mosaics showed that its activity in the nervous system is necessary and sufficient to promote germline-mediated longevity. Since the miR-71 mediated lifespan extension was fully suppressed in *daf-16* null mutants but was unaffected in *daf-12* null mutants, miR-71 probably acts upstream of the transcription factor FOXO (product of *daf-16*) but independent of the steroid hormone receptor DAF-12. Interestingly, neuron-specific *daf-16* expression did not affect lifespan in *daf-16 glp-1* double mutants overexpressing miR-71. By contrast, intestinal expression of *daf-16* fully rescued the miR-71 mediated lifespan extension. Collectively, these data suggest that miR-71 acts in the nervous system to promote the lifespan extension by facilitating the activation of *daf-16* expression in intestine. Thus, regulation of localization and transcriptional activity of FOXO seems to be the major mechanism of promoting longevity by miR-71. These findings underscore the importance of signaling between different tissues in aging of the whole organism.

Genes of histone methyltransferases, SET domain containing proteins, and orthologues of some histone methylation regulator proteins have been found to affect lifespan in *C. elegans* [82]. Knockdown of *set-2*, *set-4*, *set-9*, *set-15*, and *ash-2* genes extended lifespan, effect of the *ash-2* being most significant. The encoded protein ASH-2 is a member of the evolutionary conservative H3K4 trimethylation (H3K4 me3) complex. In worms, *ash-2* knockdown decreased global levels of H3K4me3 marks. WDR-5 is a protein that interacts with ASH-2 and is important for H3K4 methylation. The *wdr-5* knockdown also decreased H3K4me3 levels and significantly (by about 30%) extended lifespan in *C. elegans*. Thus, ASH-2 and WDR-5 are involved in H3K4 methylation and normally limit lifespan. ASH-2 and WDR-5 are known to assist H3K4 methylation by several H3K4me3 methyltransferases of the SET1/MLL family. Out of four SET1/MLL orthologues (SET-1, -2, -12, and -16) in *C. elegans*, only SET-2 has been found to affect lifespan. The *set-2* knockdown worms had reduced H3K4me3 levels. These levels were unaffected in *set-9* and *set-15* knockdowns, though both have been shown to change lifespan. The bacte-

rially expressed SET-2 methyltransferase methylated histone H3 at lysine 4 *in vitro* to generate H3K4me2, whereas ASH-2 converted H3K4me2 to H3K4me3. Analysis of the lifespan-extending effects of combined mutations showed, that ASH-2, WDR-5 and SET-2 act as a complex in the same pathway to limit lifespan. RBR-2 is an H3K4me3 demethylase homologous to the human KDM5 H3K4me3 demethylases of the JARID family. The *rbr-2* mutant worms had increased H3K4me3 levels, and a significantly (by 15–25%) decreased lifespan, indicating that RBR-2 activity is necessary for normal longevity. The *ash-2* knockdown led to changes in expression of 847 genes at mid-adult ages. This set of ASH-2-controlled genes was most enriched for genes known to affect lifespan and to change expression during aging. These results show that members of the H3K4me3 methyltransferase complex ASH-2 and of the H3K4me3 demethylase complex RBR-2 regulate aging by controlling the expression of a specific subset of genes.

RNAi inhibition of expression of the *utx-1* gene, encoding an H3K27me3 demethylase, in *C. elegans* extended the mean lifespan by about 30% [83]. No apparent developmental anomalies were observed, indicating the lifespan extension to be mainly caused by delayed aging. On the contrary, RNAi against another H3K27me3 demethylase gene transcript, F18E9.5, the closest homologue of the human JMJD3 demethylase, shortened lifespan, which may be explained by its known requirement in gonadal development. Thus, H3K27me3 may be a regulatory signal in aging, opposite effects of different H3K27me3 demethylases being a consequence of their different target genes. Compared with control worms, *utx-1* RNAi worms were more resistant to heat (35ºC), DNA damage (UV), and oxidative (paraquat) stresses. By an analysis of *utx-1* RNAi effects in mutant backgrounds, the lifespan extension was shown to be mediated by activity of the IIS pathway, *daf-2* being the exact target of UTX-1. The *daf-2* expression level increases dramatically during aging, lagging behind the increase of the *utx-1* expression. Apparently, transcription of *daf-2* might be regulated *via* H3K27me3 demethylation. UTX-1 regulation of IIS pathway genes and of lifespan may well be relevant to mammals, since both UTX and IIS are highly conserved. Indeed, when the endogenous *Utx* expression in murine 3T3-L1 cells was downregulated by a shRNA, a remarkable decrease of the *Igf1r* expression was detected. Similar decreases were also observed for IIS downstream genes. As could be expected, H3K27me3 levels along the *Igf1r* locus increased several-fold. Thus, utx-1 regulation of the IIS pathway is conserved between worms and mice. A significant decrease of the *IGF1R*-associated H3K27me3 levels was found in macaque muscle samples during aging. Interestingly, a global decrease of H3K27me3 at promoter regions was also observed. It has been restored by *utx-1* RNAi, proving the global loss of H3K27me3 to result from increased *utx-1* expression. Therefore, UTX-1 may not specifically target IIS genes, but instead, IIS genes may serve as sensors for global changes in epigenome that regulate cell growth and stress resistance accordingly [83].

In *Drosophila*, E(Z) is the catalytic subunit of Polycomb Repressive Complex 2 (PRC2) and its H3K27 trimethylation activity is essential for the establishment and maintenance of Polycomb silencing. Flies heterozygous for loss-of-function mutant alleles of *E(z)* exhibited a substantially increased lifespan compared with wild-type controls [84]. Mutations of *escl*, encoding another component of PRC2, had similar effects on longevity. TRX and other Trithorax group (TrxG) proteins antagonize PRC2-induced silencing. TRX is an H3K4me3 methyltransferase. It is also known to interact with CBP, a H3K27 acetyltransferase that directly blocks H3K27 trimethylation by E(Z), because H3K27me3 and H3K27ac are mutually exclusive marks. A *trx* null mutation partially (by about 75%) suppressed the long-lifespan phenotype of *E(z)* mutants. Obviously, increased lifespans of heterozygous PRC2 mutants are caused by reduced H3K27me3 and defects in PRC2-induced silencing. The *E(z)* and *escl* heterozygotes displayed substantially greater resistance to acute oxidative stress (paraquat) and to starvation compared with controls. Both phenotypes were suppressed by *trx* mutation, showing them also to be caused by impaired PRC2 silencing. These data point to an important role of histone H3 methylation in the lifespan regulation.

There are few direct indications of existing links between DNA methylation and lifespan. In contrast to mammals, *Drosophila* has a single cytosine DNA methyltransferase, Dnmt2. Overexpressing the *Dnmt2* gene in several transgenic lines leads to a 16-58% increase in lifespan compared with control flies [85]. Conversely, in a *Dnmt2* mutant line, lifespan decreases by ~27%. The expression levels of several genes encoding small heat shock proteins (a mitochondrial protein Hsp22, and cytosolic proteins Hsp23 and Hsp26) were increased about threefold in the *Dnmt2* overexpressing lines, and decreased two- to threefold in the mutant line. These proteins are chaperones affecting the aging. Overexpression of *Hsp22*, *Hsp23*, and *Hsp26* has been shown to increase lifespan. Overexpression of *Hsp22* and *Hsp26* also increased the resistance to oxidative and heat stress. Thus, modulation of *Hsp22*, *Hsp23*, and *Hsp26* expression may be mediating, at least partially, the effects of Dnmt2 on lifespan. Whether these findings are applicable to mammals and to what extent is presently unclear. The exact biological role of Dnmt2 in mammals is still unknown. A popular view is that Dnmt2 functions not as a DNA methyltransferase, but rather as a RNA methyltransferase methylating 38th cytosine residue in anticodon loop of the asparaginic acid tRNA [86].

CR is one of the most thoroughly studied ways to increase lifespan in mammals and other organisms [87]. Besides increasing lifespan, it decreases the frequencies of quite a few of the aging-associated diseases, such as various cancers, cardiovascular and neurodegenerative diseases, atherosclerosis, diabetes. The culturing on a glucose-free medium enhanced the replicative lifespan of normal fibroblasts, whereas that of immortalized fibroblasts was greatly diminished [88]. Glucose restriction increased expression of *hTERT* gene encoding a key component of telomerase and decreased expression of $p16^{INK4a}$ gene in normal fibroblasts. The opposite effects were found in immortalized fibroblasts. The change of the *hTERT* gene expression level in normal fibroblast was correlated with an increase of active histone marks (acetyl-H3, acetyl-H4, and H3K4me2) and a decrease of repressive marks (H3K9me3 and HDAC1) at its promoter. Essentially opposite results were obtained for the $p16^{INK4a}$ gene. The DNA methylation pattern of the *hTERT* promoter CGI was not significantly changed by glucose restriction. In

the $p16^{INK4a}$ gene promoter, two CpG sites located inside the E2F-1 transcription factor binding sequence showed changed methylation on glucose-free medium in normal fibroblasts, but not in immortalized cells. A DNA methylation inhibitor, AzaC, was found to stimulate activity of the $p16^{INK4a}$ gene promoter and to suppress activity of the *hTERT* gene promoter in normal fibroblasts. These effects were stronger on glucose-free medium compared with the control one. The effects of AzaC on the promoter activity of both genes were insignificant in immortalized cells. Thus, immortalization could be suggested to somehow disturb the epigenetic control of gene activity at glucose restriction and lead to a rapid cell death. The $p16^{INK4a}$ gene promoter methylation, that suppresses E2F-1 binding, causes its repression in normal fibroblasts, thus ensuring an escape from early senescence and apoptosis. Apparently, the normal cell response to CR includes activity of epigenetic systems that modulate gene expression to optimize cell growth and survival.

Human longevity is influenced by multiple genetic and environmental factors. Approximately 20–30% of the overall variation in human longevity is genetically determined, though it becomes evident only at advanced ages [89]. The searches for gene loci affecting longevity were essentially unsuccessful. In a genome-wide screen of the expression profiles of 863 miRNAs in the whole blood samples from 15 long-lived individuals (mean age 96.4 years) and 55 younger individuals (mean age 45.9 years), 80 differentially regulated miRNAs were found, 16 of them upregulated and 64 downregulated with age [90]. A total of 4957 mRNAs could be potentially targeted by these miRNAs, 1233 by upregulated miRNAs and 4229 by downregulated ones. There are 505 mRNAs that could be potentially targeted both by upregulated and downregulated miRNAs. No significantly enriched cellular pathways were found for the set of targets of upregulated miRNAs, whereas for the downregulated miRNAs, four significantly enriched target pathways were detected, including the p53 signaling cascade, cancer pathways, the citrate acid cycle, and the hedgehog signaling pathway. A total of 14 diseases were correlated with 64 downregulated miRNAs, most of them cancers. In contrast, no enrichment for specific diseases was found for 16 upregulated miRNAs. Obviously, changes in the expression levels of miRNAs during aging may serve to suppress age-related pathologies, first of all cancers. Some age-dependent miRNAs were shown to affect senescence, a mechanism that prevents cancer. miRNAs shown to decrease in senescent cells, such as miR-17, -20a, -106a, and -21, were also downregulated in long-lived individuals. Interestingly, miR-21 has been shown to be differentially regulated in about 40 different diseases; very likely its deregulation plays key roles in all of these diseases by a common mechanism.

Possible significance of DNA methylation as a factor controlling the human longevity was studied in leukocytes of female centenarians (>100-yr ages), their daughters of about 70-yr age selected from pairs, where the father was also long-lived (died at the age of >77 years), females of about 70-yr age, whose parents were both non-long-lived (mothers died at ≤72-yr age, fathers at ≤67-yr age), and a control group of young (17-34 years) women [91]. Clinical histories showed centenarians' daughters to have a much better health status (age-related diseases, permanent usage of prescribed drugs, *etc*.) compared with daughters of non-long-lived parents. Evidently, the probability to become long-lived is inheritable to a very significant degree. Global DNA methylation levels were significantly decreased in all three aged groups compared with the control group of young women, but to different extents. Maximum hypomethylation was observed in daughters of non-long-lived parents, minimal in centenarians' daughters, intermediate in centenarians themselves. About 700 CpG sites (located in ~600 genes) were detected that were hypermethylated in all three aged groups, to similar extents in daughters of centenarians and non-long-lived parents, and to a greater extent in centenarians themselves. Multiple genes among these hypermethylated ones were functionally related to organ development, cell differentiation and transcription regulation. A total of 330 CpG sites (located in 326 genes) were found that were hypomethylated in aged subjects, to similar extents in both daughters' groups and to a greater extent in centenarians' group. Large numbers of genes involved in defense responses, acute inflammation and signal transduction were found among these hypomethylated loci. A detailed comparison of the methylation patterns revealed ~150 CpG sites (located in 124 genes), that were significantly hypermethylated in centenarians' daughters compared with daughters of non-long-lived parents. Genes functionally related to nucleotide metabolism, nucleic acids synthesis, and cellular signaling were enriched in this group. On the other hand, 67 CpG sites (located in 65 genes) were found that showed significant hypomethylation in centenarians' daughters compared with daughters of non-long-lived parents. The genes related to downstream processes of the signal transmission were predominant in this group. Most strongly pronounced differences in methylation levels were found for twelve CpG sites (ten hypermethylated and two hypomethylated in centenarians' daughters) located in nine genes. Six of the hypermethylated genes (*SLC38A4*, *SLC22A18*, *MGC3207*, *ECRG4*, *ATP13A4*, and *AGPAT2*) are involved in metabolic processes, one hypermethylated, *DUSP22*, is a tumor-suppressor gene, still another one hypermethylated gene, *ZNF169*, encodes a zinc finger DNA binding protein with unknown function; the function of the only hypomethylated gene, *FLJ32569*, is also unknown. As a whole the genome methylation in centenarians' daughters is obviously more stable compared with daughters of non-long-lived parents. Thus, epigenome stability and a more robust epigenetic control for nucleotide metabolism, nucleic acids synthesis and signal transduction may contribute to increase of lifespan and healthy aging in centenarians.

7. ACCELERATED AGING SYNDROMES

One of the most extensively studied diseases of accelerated aging is Hutchinson-Gilford progeria syndrome (HGPS) [92]. Characteristic features of this syndrome are multiple pathologies arising within the first few years of life that are collectively often referred to as an aging-like phenotype. Skeleton, body fat, skin, hair, and the cardiovascular system are organs most affected in HGPS. These disturbances eventually lead to death at the age of 13 on average, usually from progressive atherosclerosis of the coronary and cerebrovascular arteries. In most cases HGPS is caused by mutations of *LMNA* gene encoding nuclear fibrillar proteins lamins A and C. Lamins are important constituents of the nuclear lamina, a

proteinaceous network adjacent to the inner surface of the nuclear envelope and participating in the nuclear structure maintenance, chromatin organization, gene transcription and other genetic processes. About 20 different mutations of *LMNA* gene have been reported to cause HGPS, but most (~90%) of the cases are caused by a *de novo* 1824 C → T mutation in germline cells [93, 94]. The 1824 C → T mutation leads to active usage of a cryptic splice site in the exon 11 of *LMNA* gene, resulting in production of progerin, a truncated by 50 amino acid residues partially processed form of lamin A. The cryptic splice site appears to be also used in wild-type cells, but at a much lower frequency. There are characteristic disturbances in the nuclear structure characteristic of HGPS cells [95]. Similar disturbances are present in cells of normal elderly persons, though their anomalous features are never as severe as those in HGPS cells. In nuclei of the late-passage HGPS cells, an almost complete loss of the peripheral heterochromatin and a partial loss of the internal heterochromatin occur. When a bacterially expressed mutant form of lamin A, progerin, is injected into control fibroblasts, about 30–35 min later multiple nuclei display an anomalous morphology characteristic of the late-passage HGPS cells. Thus, the mutant protein directly and quickly affects nuclear morphology. Injection of the normal lamin A into anomalous cells does not rescue the anomalous phenotype induced by previous progerin injection. Thus, progerin is a dominant factor relative to normal lamin A. Severity of HGPS symptoms were noted to positively correlate with the progerin concentration relative to normal lamin A. Progerin was not detected in control fibroblast cultures at early passages, whereas it appeared and accumulated at later ones [96]. In skin biopsies of variously aged (22-97 years) persons of both sexes progerin mRNA was detected by RT-PCR. A screen of 40 biopsies using monoclonal antibodies to progerin showed its small quantities to be present in samples obtained from elderly donors, whereas it was not detected in samples from young ones. Obviously, production of progerin does occur in normal fibroblasts but at a rather small level. In most cases studied, progerin-positive cells in normal subjects are those that have reached terminal differentiation and/or senescence.

In primary fibroblast cultures obtained from HGPS patients, age-matched unaffected controls, and unaffected parents of HGPS patients, all three splice forms of the *LMNA* transcript were detected [97]. Lamin C is the most highly expressed mRNA in all samples (1500-2000 copies per 1 μg of total RNA), its concentrations being about the same (~1500) in HGPS and control fibroblasts and somewhat higher (~2000) in the HGPS parent's fibroblasts. Levels of lamin A mRNA are practically equal in all three groups (~500). Level of progerin mRNA is similar to that of lamin A mRNA in HGPS patients and ~200-fold lower (~2.5) in control and HGPS parent's fibroblasts. Apparently progerin, even in relatively small quantities, is very active in inducing anomalous phenotype features in aging cells. Similar to HGPS cells, progerin accumulation in normal cells leads to heterochromatin delocation from nuclear envelope, global chromatin decondensation and its restructuring. All these changes promote fixation of terminally differentiated or senescent states. Progerin accumulation in HGPS cells leads to premature terminal differentiation and/or senescence. This, in its turn, leads to premature depletion of mitotically active cells and to clinical features of the accelerated aging. Thus, studies of HGPS cells could be useful to understand mechanisms of the normal aging and to elaborate methods to cope with it. Generally, progressive accumulation of progerin with the normal aging could be one of the causes of cell aging and genome destabilization observed in aged cells. Progerin leads to changes in levels and sub-nuclear localization of chromatin remodeling factors, TFs, DNA repair enzymes and many other proteins. It affects nuclear morphology, chromatin structure and gene expression. These changes disturb cell proliferation, leading to apoptosis and organ dysfunction. Cardiovascular disorders similar to those occurring in normally aged atherosclerotic patients are among the phenotypic manifestations of progerin-induced disturbances [98]. Large quantities of progerin are accumulating in arterial walls of HGPS patients. Progerin was also detected in coronary arteria of non-HGPS individuals, where its quantity increased with age. One may suggest that progerin accumulation in vascular cells leads to increased cell death and inflammation response to oxidative stress and other insulting factors. In HGPS patients most pronounced defects are observed in VSMCs. There are characteristic changes in these cells including calcification, lipid accumulation, fibrosis, and VSMC attrition in arteries and arterioles. All these defects are very reminiscent of those occurring in common age-associated vascular pathologies.

Damage to the telomeric chromosome sequences in fibroblasts of healthy humans activates progerin production and cell aging [99]; and vice versa, progerin expression in diploid fibroblasts leads to telomeres aggregation, damage of their DNA, and chromosome aberrations [100]. In fibroblasts obtained from advanced age donors (81-96 years), morphologic anomalies, similar to those in HGPS cells, are regularly observed, including decreases in immunoreactive H3K9me3 and HP1 [101]. The frequency of cells with H3K9me3 and HP1 decreased in aged individuals is similar to that in HGPS patients, though the extent of H3K9me3 and HP1 depletion in the latter ones is much larger. The histone H2AX phosphorylation foci serve as markers of the unrepaired DNA damage. Similar to HGPS cells, their quantities in fibroblasts from aged donors are significantly larger compared with fibroblasts from young donors. Thus, normal aging and HGPS have a number of nuclear defects in common. When the cryptic splice site use in aged donor cells was inhibited with a modified complementary oligonucleotide, HP1 and H3K9me3 were increased to the levels corresponding to young donor cells at the same passages. Decrease in the expression of aging markers, *p21*, *IGFBP3*, and *GADD45B*, and increase in proliferative activity to the young donor levels were also observed. Hence, the accelerated aging in HGPS patients could be a consequence of excessive activity of some progerin-dependent mechanism involved in the normal aging.

Mechanisms of the progerin action are not quite clear. Probably these mechanisms involve defects in functional chromosome organization, such as changes in heterochromatin quantity and its sub-nuclear distribution, telomeres anomalies, and global changes in gene expression. Expression levels of 361 genes changed at least twofold (193 increased, 168 decreased) in several HGPS fibroblast lines

compared with control fibroblasts of the similar age [102]. Genes encoding TFs were predominant among these differentially expressed genes. The most highly affected gene, *MEOX2/GAX* (29-fold upregulated in HGPS), encodes a homeobox factor that functions as a negative regulator of proliferation in several mesodermal tissues. Genes encoding two regulators of histone acetylation, *ING1* and *TWIST2*, a heterochromatin silencer, *SALL1*, and some other chromatin related proteins, are among the negatively affected ones. In terms of tissue and organ function, the largest category shows clear vascular and atherosclerosis involvement. The second most prevalent category is involved in skeletal, limb and cartilage tissue functions.

Progerin expression *via* an inducible transgene in a human skin fibroblast line, immortalized with telomerase reverse transcriptase (hTERT), led to significant progerin accumulation at 5 days, reaching a plateau at 10 days [103]. Disturbances of the nuclear structure, loss of LAP2 protein, and increased DNA damage, features typical of HGPS, were evident. A microarray analysis of gene expression revealed changes in 194 genes at 5 days after induction of progerin expression and in 1013 genes at 10 days. One notable early event was activation of the Notch cell-signaling pathway, a major regulator of the stem cell differentiation. When a progerin transgene was introduced into immortalized human mesenchymal stem cells (hMSCs), multiple cells aberrantly expressed cell differentiation markers, indicating that spontaneous differentiation has occurred. For example, ~10% of cells were expressing collagen IV, a basement membrane marker of epithelial and endothelial cells, or nestin, a neuroprogenitor marker. An endothelial marker MCAM, angiogenic factors endothelin (ET-1) and vascular endothelial growth factor receptor VEGFR1, and an osteogenic marker osteopontin were significantly upregulated. This mechanism could explain multiple phenotypic features of HGPS. Quite possibly, small quantities of progerin accumulated during the normal aging distort the Notch cell-signaling pathway activity, thus, disrupting regulation of MSCs differentiation and tissue homeostasis.

In the early-passage fibroblasts cultures obtained from a HGPS female patient, 57% of cells contained an inactivated X chromosome (Xi) associated with H3K27me3, an epigenetic mark of facultative heterochromatin, whereas at passage 21 the share of such cells was reduced to 36% [104]. In the control fibroblast cultures, these values were 94 and 88%, respectively. In the 9-13-passage HGPS cells, 58% of the H3K27me3-positive Xi's consisted of loose arrays of discrete H3K27me3 foci, probably representing an intermediate state during the loss of this epigenetic mark. In general, the H3K27me3 loss precedes the appearance of clear-cut anomalies in the cell nuclear morphology. As it has been determined by fluorescent *in situ* hybridization (FISH), the H3K27me3-positive Xi's also contained XIST RNA. When H3K27me3 was lost, the FISH fluorescence has become more dispersed. A significant reduction in the H3K27me3-methylase EZH2 and its encoding mRNA was observed in the 14-passage HGPS cells compared with the control ones. The EZH2 mRNA level was decreased tenfold in the 25-passage HGPS cells relative to the control cells. No differences in the XIST RNA levels were found between the HGPS and control cells. When progerin was transiently expressed in human embryonic kidney cells (HEK293) a reduction of H3K27me3 associated with Xi and nuclear lamina was observed, whereas the association between Xi and XIST RNA was intact. Thus, expression of progerin is accompanied by a loss of H3K27me3 from the facultative heterochromatin, reduction of EZH2 methylase levels and subsequent global structural anomalies. In the control cell cultures at all passages and in the HGPS cells at early passages, H3K9me3 marks of the constitutive heterochromatin were detected mainly as small foci interspersed with few larger foci both in the nucleoplasm and in the lamina regions. At later passages, quantity of H3K9me3 marks in the HGPS cells was significantly diminished, especially in the lamina region. A similar decrease was observed upon transient progerin expression in normal cells. In control cells the H3K9me3 fluorescence foci mostly matched those of the heterochromatin protein HP1α. In the middle-passage HGPS cells mutual association of these marks was lost and their quantities decreased. These changes preceded the appearance of morphological anomalies. A reduction in transcripts of *SUV39H1* and *SUV39H2* genes, encoding H3K9me3-methylases, occurred in these cell cultures at later passages. The quantity of another mark of the pericentromeric heterochromatin, H4K20me3, was found to be upregulated in the later-passage HGPS cells and in normal cells transiently expressing exogenous progerin. Effects described could lead to the changed epigenetic program observed in progeroid cells.

A still another mechanism of progerin action could be disturbance of the sirtuin SIRT6 activity [105]. Lamin A has been shown to physically interact with SIRT6. This interaction enhances the histone H3 deacetylation at lysine residues 9 and 56 by SIRT6. Progerin exhibits a stronger binding to SIRT6, compared with lamin A, but does not stimulate its deacetylating activity. A recruitment of SIRT6 to chromatin occurs when DNA is damaged. This recruitment was found to be dependent on the lamin A presence, whereas progerin interfered with it. The SIRT6 participation in repair of dsDNA breaks also appeared to be lamin A-dependent and dominantly inhibited by progerin. One of the essential activities of SIRT6 involved in DNA repair is PARP1 mono-ADP-ribosylation. Again, lamin A stimulated this activity, whereas progerin inhibited it. Practically all SIRT6 activities noted above are disrupted in HGPS fibroblasts. Perhaps, exactly these effects of progerin explain DNA repair defects in HGPS.

Changes in genome loci methylation may play a significant role in phenotypes of accelerated and normal aging. In patients with different progerias (Werner syndrome, WS, often caused by mutations in *WRN* gene encoding the helicase RecQ, and HGPS) multiple CpG sites were found to be differentially methylated compared with control cells [106]. Some of these sites are common to both progeria syndromes, but there are also specific ones. Some patients have all features characteristic of HGPS or WS, but do not possess known mutations in *LMNA* or *WRN* genes. The differences between these patients and control individuals in genome loci methylation seem to be smaller compared with those between mutant patients and control subjects. More than 1300 differentially methylated CpGs were found to be associated with *WRN* gene mutations. Methylation of 144 CpGs regularly changes both during normal aging and in WS. Sites

in promoter regions of the NFκB-signaling pathway genes (*CASP8*, *IL1RL1*, and *LGALS1*), as well as of the extracellular matrix protein genes (*ADAMTS4*, *LGALS1*, *PODNL1*, and *ZP3*) are among these dynamically methylated CpGs. All these genes could be regarded as being potentially involved in aging mechanisms. Out of 18,480 differentially methylated CpGs that were identified in samples of WS caused by *LMNA* gene mutations, 485 were also differentially methylated during the normal aging. Some of these last CpGs are associated with the NF-κB-signaling pathway genes (*PRKCG*, *NLRP12*). Evidently, this pathway plays an essential role in aging. In three patients with a non-mutant version of HGPS (a father and two daughters) 78 common differentially methylated CpGs were found. Four of them are located in promoter of gene encoding a long non-coding RNA (lncRNA), *LOC149837*, in close proximity to TSS. Evidently, these sites can belong to a differentially methylated region (DMR), controlling transcription of the gene. Unfortunately, the function of this lncRNA is unknown. The respective locus (chr20:5461000–5500000) also encodes a group of PIWI-associated RNAs (piRNAs). Since *LOC149837* epimutations were detected in all progeria samples analyzed (both mutant and non-mutant ones), it could be suggested that anomalous hypomethylation of this locus induces synthesis of the respective non-coding RNAs, leading to a progeroid phenotype. Promoters differentially methylated in non-mutant progeria samples were found to be enriched for sequence motifs recognized by the transcription factor MYB. The epigenetic changes observed may play an important role in the accelerated aging phenotypes, partially due to changed activity of the NF-κB-signaling pathway genes and of those regulated by the transcription factor MYB.

Interestingly, many organs, such as liver, kidney, lung, brain, gastrointestinal tract, and bone marrow, are not affected in HGPS. Some of the normal aging phenotypic features, for example, disturbances of intellectual activity, are also absent. In a mouse model of HGPS, producing large quantities of progerin, a typical accelerated aging phenotype is observed, but the nervous system remains quite normal [107, 108]. Lamin A is expressed in brain cells at a very low level due to the brain-specific miRNA miR-9 that recognizes a site in the 3′-UTR sequence of the pre-lamin A mRNA and causes its quick degradation. Lamin A is also not expressed in iPSCs produced from fibroblasts of HGPS patients and control subjects [109]. Expression of lamin A, as well as of progerin in the case of HGPS cells, was observed in a variety of cells derived from iPSCs, including keratinocytes, melanocytes, retinal pigment epithelial cells, and MSCs. Progerin expression was accompanied by characteristic features of HGPS cells, early loss of proliferation, replicative senescence, and the nuclear structure anomalies. No expression of lamin A was observed in neurons derived from iPSCs, whereas the expression of miR-9 was readily detectable. When pre-miR-9 has been injected into MSCs, lamin A expression was inhibited threefold. An injection of pre-miR-9 into HGPS MSCs inhibited expression of both lamin A and progerin and significantly reduced anomalies of nuclei morphology. Evidently, brain-specifically expressed miR-9 protects nervous cells from damaging effects of progerin. When a human progerin transgene was expressed in the mouse hippocampal neurons its age-dependent accumulation and characteristic anomalies in nuclear morphology were observed [110]. However, unlike the progerin-expressing fibroblasts, neurons did not display any visible changes in the chromatin ultrastructure, including heterochromatin association to the internal surface of the nuclear envelope. Gene expression was also essentially unchanged (only 5 genes out of the 16,572 investigated showed at least twofold changes in expression levels). Memory in behavioral test and postnatal neurogenesis in the hippocampal dentate gyrus were also unaffected. Thus, neurons are resistant to progerin action, even when it is expressed at a high level.

8. IS IT POSSIBLE TO SLOW DOWN, ARREST OR REVERSE AGING?

A natural example of the full reversal of aging is fertilization. In humans and other mammals fusion of an oocyte and a sperm cell results in a zero-age zygote. There are no reasons to believe the germinal cells to be specifically protected from chronological aging. Thus, species would age with each generation, suffering the burden of aging of all antecedent generations in their cells, if there is no such aging reset. A similar age reset takes place when nucleus of a somatic cell is transferred to cytoplasm of an enucleated oocyte (SCNT), resulting in development of a new individual. The oocyte cytoplasm seems to possess a capability to "erase" all the aging features accumulated in the somatic cell nucleus. These examples prove the age-related features of the cell nuclei, whatever is their nature, to be principally reversible. Epigenome of any cell (germinal cells included) is a complex mosaic of epigenetic marks, where age-related marks are intermixed with a plethora of others (invariant, developmentally changed, arisen as responses to external factors or as results of spontaneous errors, *etc*.). In this situation a most easy way to reset the age-related epigenetic changes is to fully erase all existing epigenetic information and then rebuild it from scratch in a form corresponding to the zero age. Such erasure takes place during the first hours following fertilization [111-113]. The picture described does not exclude accumulation of some irreversible changes during chronological aging, provided that these changes are not the specific causes of the aging *per se*. Evidently gradual accumulation of mutations increases the genetic variability of humans as a biological species, and most of these mutations are not directly related to aging.

A still another example of the biological clock experimental resetting is production of the induced pluripotent stem cells [114]. Recent observations showed that iPSCs generated from senescent cells or centenarian donor cells rejuvenate telomeres, gene expression profiles, oxidative stress, and mitochondrial metabolism to levels characteristic of ESCs [115, 116]. In addition, as was noted earlier, iPSCs are fully rejuvenated epigenetically.

In all cases described above resetting of the aging clock was coupled to cell de-differentiation. Is it possible to rejuvenate a cell without disturbing its differentiation status? In an experimental procedure, known as heterochronic parabiosis, a shared circulatory system between young and old mice is established, thus exposing old mouse to factors present in young serum. Heterochronic parabiosis was found to restore

the activity of the Notch signaling pathway, and the proliferation and regenerative capacity of aged skeletal muscle satellite cells [117]. Furthermore, heterochronic parabiosis increases proliferation of the aged hepatocytes and restores their cEBP-α levels to values seen in young animals. Thus, the age-related decline of the progenitor cell activity could be modulated by systemic factors that change with age. The number of newly born neurons, proliferating cells and neural progenitors in the dentate gyrus of hippocampus decreases in the young heterochronic parabiont mice and increases in the old ones [118]. Systemic environment seems to influence to a significant degree the biological age of cells. Most likely, its effects are caused by changes in the activity of main signaling pathways (Notch, Wnt, and TGFβ) due to changed concentrations of various cytokines and chemokines. Systemic factors can also affect the aging-associated decline of the neural stem cells (NSCs) activity that leads to impaired cognitive function in elderly. In a mouse heterochronic parabiosis model, remodeling of the cerebral vasculature has been observed to occur in the aged parabiont mouse, resulting in significantly higher blood flow, enhanced NSCs proliferation, and improved olfactory function [119]. GDF11, a circulating TGF-β family member that has been reported to reverse cardiac hypertrophy in aged mice [120], also stimulated vascular remodeling and increased neurogenesis in aged mice [119].

As was noted above, NF-κB is the most promising candidate to play the master regulator role in gene expression programs of mammalian aging [43]. Effects of the temporal NF-κB activity inhibition were tested in chronologically aged transgenic mice with *NF-κB* gene conditionally repressed in epidermal skin cells. Comparison of global expression profiles between young and old skin samples revealed more than four hundred genes that changed (mostly increased) activity in the old skin. About 50% of these age-dependent genes were targets of NF-κB, thus confirming the proposed role of NF-κB in aging. After a 2-wk long *NF-κB* repression in the old skin, activity of about 54% out of the age-dependent genes returned to "young" levels. By global expression profiles *NF-κB*-quenched aged skin samples were closer to the young skin than to control aged skin samples. Thus, NF-κB activity appears to be required to maintain a substantial part of the aging-associated gene expression in skin cells. Besides gene expression, *NF-κB* blockade increased epidermal thickness of the old skin to a degree intermediate between the young and the old skin, increased cell proliferation, and significantly decreased expression of *SA-β-gal* and $p16^{INK4A}$. Skin constitution and general condition improved without any visible disturbances of the normal skin structure and differentiation. Cell proliferation was observed predominantly in the basal layer of epidermis, and the normal stratification was intact. Thus, continuous activity of NF-κB is required to maintain the gene expression profile of the chronologically aged skin. These data suggest that many features of mammalian aging may not be due to passive accumulation of stochastic cellular damages and errors but rather are actively enforced by special gene expression programs and, thus, can be substantially reversed by selective gene expression interventions. The NF-κB action in skin aging appears to be cell-autonomous since reversion of the aging features was possible in limited patches of epidermis in otherwise old animals. As a TF that is responsive to oxidative stress, DNA damage, growth signals, and immune activation, on the one hand, and acting on a great number of various target genes, on the other hand, NF-κB seems to be in an ideal position to transduce diverse extracellular signals to adaptive changes in gene expression and tissue homeostasis. The contribution of specific NF-κB target genes to aging remains unknown. The biological effect of NF-κB may be mediated by combined effects of a large number of NF-κB responsive genes, individual target genes having at best very modest influence on aging.

HSCs decrease function with age, showing as reduced self-renewal, hematopoiesis, and lymphopoiesis [121]. In mammals, the target of rapamycin (mTOR) pathway integrates multiple signals from nutrients, growth factors, and oxygen to regulate the cell growth, proliferation, and survival [122, 123]. Conditional *Tsc1* gene deletions in HSCs of young adult mice drive them from quiescence into rapid cycling, increased mitochondrial biogenesis and elevated levels of reactive oxygen species [124]. Importantly, these deletions dramatically reduce both hematopoiesis and self-renewal of HSCs and lead to constitutive activation of mTOR. In murine HSCs, mTOR activity increases with age [125]. Treatment of old mice with an mTOR inhibitor, rapamycin, significantly increases their lifespan. Moreover, the treatment causes a significant increase in the proliferative activity of HSCs and a decrease in the expression of $p16^{Ink4a}$ and $p19^{Arf}$, known markers of the cell aging. Old mice have an impaired B cell generation due to decreased numbers of pre-B cells. The rapamycin treatment enhances generation of B cells due to fourfold increase in the number of pre-B cells.

Thus, rejuvenation of differentiated or committed cells can be achieved without disturbing their differentiation programs. These results suggest the possibility of targeted therapies to reverse individual features of aging and to alleviate age-related pathologies in the elderly.

CONCLUSION

Multiple lines of evidence described above show that aging is accompanied and probably caused to a significant extent by epigenetic changes. Epigenetic perturbations are seen in progeroid syndromes and provoke progeroid phenotype in model organisms. The roles of DNA methylation and other epigenetic systems in triggering the differentiated states of cells are widely accepted. Traditionally, epigenome of a terminally differentiated cell has been looked at as a stably maintained entity. Unexpectedly, multiple studies in the last few years showed that in fact epigenome is a dynamically regulated system involved in aging, or at least affected by it, and responsive to various external and internal factors.

Small non-coding RNAs, in particular miRNAs, have been implicated in various aspects of animal development. Targeting of single genes by multiple miRNAs and of multiple genes by single miRNAs makes this epigenetic system very versatile and efficient. Ironically, these very features could cause serious derangement of gene expression programs when an improper activity of miRNA systems occurs, such as changed miRNA levels observed during aging.

Studies of miRNA, mRNA and protein expression in animal tissues over their entire lifespans showed that many changes in miRNA and gene expression that occur during aging initiate well before the adulthood and appear to represent reversals or extensions of the developmental programs. Similarly to those of gene expression, many changes in epigenome proceed continuously from the early embryo across the entire lifespan. Epigenetically aging should be regarded to start at earliest stages of the embryo development, when global reprogramming of epigenome just has occurred. Figuratively speaking, the epigenetic clock starts ticking from the very beginning of our prenatal development.

Studies of the aging details using predictive epigenetic models could have many practical implications, from health assessment to forensic analysis. Similar to the analysis of the gender influence on aging rates described above (section **2.3**), effects of various environmental and life style factors, such as smoking, alcohol consumption, dietary preferences and many others, could be assessed. As the predictive accuracy of the models improves, it seems quite probable that the biological age, measured by epigenetic markers, might become more useful in the clinical practice than the passport age. And of course the epigenetic age could become indispensable in evaluating efficiency of new rejuvenation procedures.

Possibility of the aging clock to be reset suggests that epigenetic signals are not the mere correlates of the aging process, but rather a substantial part of the aging mechanism. Links between epigenome and aging can be mutual; epigenome is changed by age factors, but it also affects aging. Epigenome can be viewed as a general sensor of cellular dysfunction, responsive to any changes in genome and the internal milieu, including those related to aging. On the other hand, epigenome determines changes in gene expression patterns that underlie the aging phenotype. Similar to other cell systems, epigenome is prone to gradual degradation due to the genome damage, stressful agents and other aging factors. But unlike mutations and other kinds of the genome damage, age-related epigenetic changes could be fully or partially reversed to a "young" state. To say it in few words, knowing epigenome and being able to manipulate it hold promise for curing age-related pathologies and extending the healthy lifespan.

CONFLICT OF INTEREST

The authors declare no conflict of interest, financial or otherwise.

ACKNOWLEDGEMENTS

This work was supported by grant No 14-50-00029 from Russian Science Foundation (RSF).

REFERENCES

[1] Holliday, R. Aging is no longer an unsolved problem in biology. *Ann. N.Y. Acad. Sci.*, **2006**, *1067*, 1-9.

[2] López-Otín, C.; Blasco, M.A.; Partridge, L.; Serrano, M.; Kroemer, G. The hallmarks of aging. *Cell*, **2013**, *153*, 1194-1217.

[3] Berdyshev, G.D.; Korotaev, G.K.; Boyarskikh, G.V.; Vanyushin, B. F. Nucleotide composition of DNA and RNA from somatic tissues of humpback salmon and its changes during spawning. *Biokhimiya*, **1967**, *32*, 988-993.

[4] Vanyushin, B.F.; Nemirovsky, L.E.; Klimenko, V.V.; Vasiliev, V. K.; Belozersky, A.N. The 5-methylcytosine in DNA of rats: Tissue and age specificity and the changes induced by hydrocortisone and other agents. *Gerontologia*, **1973**, *19*, 138-152.

[5] Volpe, P.; Eremenko, T. Preferential methylation of regulatory gene sequences in HeLa cells. *Febs Lett.*, **1974**, *44*, 121-126.

[6] Volpe, P. The unexpected existence of coding and non-coding fragments along the eukaryotic gene. *Adv. Biol. Chem.*, **2015**, *5*, 98-125.

[7] Romanov, G.A.; Vanyushin, B.F. Methylation of reiterated sequences in mammalian DNAs: Effects of the tissue type, age, malignancy and hormonal induction. *Biochim. Biophys. Acta,* **1981**, *653*, 204-218.

[8] Wilson, V.L.; Smith, R.A.; Mag, S.; Cutler, R.G. Genomic 5-methyldeoxycytidine decreases with age. *J. Biol. Chem.*, **1987**, *262*, 9948-9951.

[9] Wilson, V.L.; Jones, P.A. DNA methylation decreases in aging but not in immortal cells. *Science*, **1983**, *220*, 1055-1057.

[10] Geraci, D.; Eremenko, T.; Cocchiara, R.; Granieri, A.; Scarano, E.; Volpe, P. Correlation between synthesis and methylation of DNA in HeLa cells. *Biochem. Biophys. Res. Commun.*, **1974**, *57*, 353-361.

[11] Volpe, P.; Eremenko, T. Cell and virus macromolecular modifications during the cell life. In: *Macromolecules in the Functioning Cell*; Bayev, A.A.; Ed.; Nauka Publishers: Moscow, **1982**; Book 1, pp. 179-195.

[12] Wigler, M.; Levy, D.; Perucho, M. The somatic replication of DNA methylation. *Cell*, **1981**, *24*, 33-40.

[13] Stein, R.; Gruenbaum, Y.; Pollack, Y.; Razin, A.; Cedar, H. Clonal inheritance of the pattern of DNA methylation in mouse cells. *Proc. Natl. Acad. Sci. U.S.A.*, **1982**, *79*, 61-65.

[14] Boyd-Kirkup, J.D.; Green, C.D.; Wu. G.; Wang, D.; Han, J.- D.J. Epigenomics and the regulation of aging. *Epigenomics*, **2013**, *5*, 205-227.

[15] Cacciamani, T.; Virgili, S.; Centurelli, M.; Bertoli, E.; Eremenko, T.; Volpe, P: Specific methylation of the CpG-rich domains in the promoter of the human tissue transglutaminase gene. *Gene*, **2002**, *297*, 103-112.

[16] So, K.; Tamura, G.; Honda, T.; Homma, N.; Waki, T.; Togawa, N.; Nishizuka, S.; Motoyama, T. Multiple tumor suppressor genes are increasingly methylated with age in non-neoplastic gastric epithelia. *Cancer Sci.*, **2006**, *97*, 1155-1158.

[17] Christensen, B.C.; Houseman, E.A.; Marsit, C.J.; Zheng, S.; Wrensch, M.R.; Wiemels, J.L.; Nelson, H.H.; Karagas, M.R.; Padbury, J. F.; Bueno, R.; Sugarbaker, D. J.; Yeh, R. -F.; Wiencke, J.K.; Kelsey, K.T. Aging and environmental exposures alter tissue-specific DNA methylation dependent upon CpG island context. *PLoS Genet.*, **2009**, *5*, e1000602.

[18] Maegawa, S.; Hinka, G.; Kim, H.S.; Shen, L.; Zhang, L.; Zhang, J.; Zhang, N.; Liang, S.; Donehower, L.A.; Issa, J.-P. J. Widespread and tissue specific age-related DNA methylation changes in mice. *Genome Res.*, **2010**, *20*, 332-340.

[19] Rakyan, V.K.; Down, T.A.; Maslau, S.; Andrew, T.; Yang, T. -P.; Beyan, H.; Whittaker, P.; McCann, O.T.; Finer, S.; Valdes, A.M.; Leslie, R.D.; Deloukas, P.; Spector, T.D. Human aging-associated DNA hypermethylation occurs preferentially at bivalent chromatin domains. *Genome Res.*, **2010**, *20*, 434-439.

[20] Meissner, A.; Mikkelsenm, T.S.; Gu, H.; Wernig, M.; Hanna, J.; Sivachenko, A.; Zhang, X.; Bernstein, B.E.; Nusbaum, C.; Jaffe, D. B.; Gnirke, A.; Jaenisch, R.; Lander, E.S. Genome-scale DNA methylation maps of pluripotent and differentiated cells, *Nature*, **2008**, *454*, 766-770.

[21] Hernandez, D.G.; Nalls, M.A.; Gibbs, J.; Arepalli, S.; van der Brug, M.; Chong, S.; Moore, M.; Longo, D.L.; Cookson, M.R.; Traynor, B.J.; Singleton, A.B. Distinct DNA methylation changes highly correlated with chronological age in the human brain. *Hum. Mol. Genet.*, **2011**, *20*, 1164-1172.

[22] Martino, D.; Loke, Y.J.; Gordon, L.; Ollikainen, M.; Cruickshank, M.N.; Saffery, R.; Craig, J.M. Longitudinal, genome-scale analysis of DNA methylation in twins from birth to 18 months of age reveals rapid epigenetic change in early life and pair-specific effects of discordance. *Genome Biol.*, **2013**, *14*, R42.

[23] Day, K.; Waite, L.L.; Thalacker-Mercer, A.; West, A.; Bamman, M.M.; Brooks, J.D.; Myers, R.M.; Absher, D. Differential DNA methylation with age displays both common and dynamic features across human tissues that are influenced by CpG landscape. *Ge-*

nome Biol., **2013**, *14*, R102.

[24] Fraga, M.F.; Ballestar, E.; Paz, M.F.; Ropero, S.; Setien, F.; Ballestar, M.L.; Heine-Suner, D.; Cigudosa, J.C.; Urioste, M.; Benitez, J.; Boix-Chornet, M.; Sanchez-Aguilera, A.; Ling, C.; Carlsson, E.; Poulsen, P.; Vaag, A.; Stephan, Z.; Spector, T.D.; Wu, Y.-Z.; Plass, C.; Esteller, M. Epigenetic differences arise during the lifetime of monozygotic twins. *Proc. Natl. Acad. Sci. U.S.A.*, **2005**, *102*, 10604-10609.

[25] Bocklandt, S.; Lin, W.; Seh, M.E.; Sanchez, F.J.; Sinsheimer, J.S.; Horvath, S.; Vilain, E. Epigenetic predictor of age. *PLoS One*, **2011**, *6*, e14821.

[26] Koch, C.M.; Wagner, W. Epigenetic aging signature to determine age in different tissues. *Aging*, **2011**, *3*, 1018-1027.

[27] Weidner, C.I.; Lin, Q.; Koch, C.M.; Eisele, L.; Beier, F.; Ziegler, P.; Bauerschlag, D.O.; Jöckel, K.-H.; Erbel, R.; Mühleisen, T.W.; Zenke, M.; Brümmendorf, T.H.; Wagner, W. Aging of blood can be tracked by DNA methylation changes at just three CpG sites. *Genome Biol.*, **2014**, *15*, R24.

[28] Hannum, G.; Guinney, J.; Zhao, L.; Zhang, L.; Hughes, G.; Sadda, S.; Klotzle, B.; Bibikova, M.; Fan, J.-B.; Gao, Y.; Deconde, R.; Chen, M.; Rajapakse, I.; Friend, S.; Ideker, T.; Zhang, K. Genome-wide methylation profiles reveal quantitative views of human aging rates. *Mol. Cell*, **2013**, *49*, 359-367.

[29] Horvath, S. DNA methylation age of human tissues and cell types. *Genome Biol.*, **2013**, *14*, R115.

[30] Marioni, R.E.; Shah, S.; McRae, A.F.; Chen, B.H.; Colicino, E.; Harris, S.E.; Gibson, J.; Henders, A.K.; Redmond, P.; Cox, S.R.; Pattie, A.; Corley, J.; Murphy, L.; Martin, N.G.; Montgomery, G. W.; Feinberg, A.P.; Fallin, M.D.; Multhaup, M.L.; Jaffe, A.E.; Joehanes, R.; Schwartz, J.; Just, A.C.; Lunetta, K.L.; Murabito, J.M.; Starr, J.M.; Horvath, S.; Baccarelli, A.A.; Levy, D.; Visscher, P.M.; Wray, N.R.; Deary, I.J. DNA methylation age of blood predicts all-cause mortality in later life. *Genome Biol.*, **2015**, *16*, 25.

[31] Florath, I.; Butterbach, K.; Müller, H.; Bewerunge-Hudler, M.; Brenner, H. Cross-sectional and longitudinal changes in DNA methylation with age: An epigenome-wide analysis revealing over 60 novel age-associated CpG sites. *Hum. Mol. Genet.*, **2014**, *23*, 1186-1201.

[32] Haigis, M.C.; Guarente, L.P. Mammalian sirtuins - emerging roles in physiology, aging, and calorie restriction. *Genes Dev.*, **2006**, *20*, 2913-2921.

[33] Dang, W.; Steffen, K.K.; Perry, R.; Dorsey, J.A.; Johnson, F.B.; Shilatifard, A.; Kaeberlein, M.; Kennedy, B. K.; Berger, S. L. Histone H4 lysine 16 acetylation regulates cellular lifespan. *Nature*, **2009**, *459*, 802-808.

[34] Vaquero, A.; Reinberg, D. Calorie restriction and the exercise of chromatin. *Genes Dev.*, **2009**, *23*, 1849-1869.

[35] Vaquero, A.; Scher, M.; Lee, D.; Erdjument-Bromage, H.; Tempst, P.; Reinberg, D. Human SirT1 interacts with histone H1 and promotes formation of facultative heterochromatin. *Mol. Cell*, **2004**, *16*, 93-105.

[36] Vaquero, A.; Scher, M.; Erdjument-Bromage, H.; Tempst, P.; Serrano, L.; Reinberg, D. SIRT1 regulates the histone methyltransferase SUV39H1 during heterochromatin formation. *Nature*, **2007**, *450*, 440-444.

[37] O´Hagan, H.M.; Mohammad, H.P.; Baylin, S.B. Double strand breaks can initiate gene silencing and SIRT1-dependent onset of DNA methylation in an exogenous promoter CpG island. *PLoS Genet.*, **2008**, *4*(8), e1000155.

[38] Oberdoerffer, PH.; Michan, SH.; McVay, M.; Mostoslavsky, R.; Vann, J.; Park, S.-K.; Hartlerode, A.; Stegmuller, J.; Hafner, A.; Loerch, P.; Wright, S.M.; Mills, K.D.; Bonni, A.; Yankner, B.A.; Scully, R.; Prolla, T.A.; Alt, F.W.; Sinclair, D.A. SIRT1 redistribution on chromatin promotes genomic stability but alters gene expression during aging. *Cell*, **2008**, *135*, 907-918.

[39] Herranz, D.; Muñoz-Martin, M.; Cañamero, M.; Mulero, F.; Martinez-Pastor, B.; Fernandez-Capetillo, O.; Serrano, M. Sirt1 improves healthy ageing and protects from metabolic syndrome-associated cancer. *Nat. Commun.*, **2010**, *1*, 3.

[40] Mostoslavsky, R.; Chua, K.F.; Lombard, D.B.; Pang, W.W.; Fischer, M.R.; Gellon, L.; Liu, P.; Mostoslavsky, G.; Franco, S.; Murphy, M.M.; Mills, K.D.; Patel, P.; Hsu, J.T.; Hong, A.L.; Ford, E.; Cheng, H.L.; Kennedy, C.; Nunez, N.; Bronson, R.; Frendewey, D.; Auerbach, W.; Valenzuela, D.; Karow, M.; Hottiger, M.O.; Hursting, S.; Barrett, J.C.; Guarente, L.; Mulligan, R.; Demple, B.; Yancopoulos, G.D.; Alt, F.W. Genomic instability and aging-like phenotype in the absence of mammalian SIRT6. *Cell*, **2006**, *124*, 315-329.

[41] Michishita, E.; McCord, R.A.; Berber, E.; Kioi, M.; Padilla-Nash, H.; Damian, M.; Cheung, P.; Kusumoto, R.; Kawahara, T.L.; Barrett, J.C.; Chang, H.Y.; Bohr, V.A.; Ried, T.; Gozani, O.; Chua, K. F. SIRT6 is a histone H3 lysine 9 deacetylase that modulates telomeric chromatin. *Nature*, **2008**, *452*, 492-496.

[42] Kawahara, T.L.; Michishita, E.; Adler, A.S.; Damian, M.; Berber, E.; Lin, M.; McCord, R.A.; Ongaigui, K.C.; Boxer, L.D.; Chang, H.Y.; Chua, K.F. SIRT6 links histone H3 lysine 9 deacetylation to NF-κB-dependent gene expression and organismal life span. *Cell*, **2009**, *136*, 62-74.

[43] Adler, A.S.; Sinha, S.; Kawahara, T.L.A.; Zhang, J.Y.; Segal, E.; Chang, H.Y. Motif module map reveals enforcement of aging by continual NF-κB activity. *Genes Dev.*, **2007**, *21*, 3244-3257.

[44] Kanfi, Y.; Naiman, Sh.; Amir, G.; Peshti, V.; Zinman, G.; Nahum, L.; Bar-Joseph, Z.; Cohen, H.Y. The sirtuin SIRT6 regulates lifespan in male mice. *Nature*, **2012**, *483*, 218-221.

[45] Zhong, L.; D´Urso, A.; Toiber, D.; Sebastian, C.; Henry, R.E.; Vadysirisack, D.D.; Guimaraes, A.; Marinelli, B.; Wikstrom, J.D.; Nir, T.; Clish, C.B.; Vaitheesvaran, B.; Iliopoulos, O.; Kurland, I.; Dor, Y.; Weissleder, R.; Shirihai, O.S.; Ellisen, L.W.; Espinosa, J. M.; Mostoslavsky, R. The histone deacetylase Sirt6 regulates glucose homeostasis *via* Hif1a. *Cell*, **2010**, *140*, 280-293.

[46] Han, Y.; Han, D.; Yan, Zh.; Boyd-Kirkup, J.D.; Green, Ch.D.; Khaitovich, Ph.; Han, J.-D.J. Stress-associated H3K4 methylation accumulates during postnatal development and aging of rhesus macaque brain. *Aging Cell*, **2012**, *11*, 1055-1064.

[47] McCarroll, S.A.; Murphy, C.T.; Zou, S.; Pletcher, S.D.; Chin, Ch.-Sh.; Jan, Y.N.; Kenyon, C.; Bargmann, C.I.; Li, H. Comparing genomic expression patterns across species identifies shared transcriptional profile in aging. *Nat. Genet.*, **2004**, *36*, 197-204.

[48] Lu, T.; Pan, Y.; Kao, SH.-Y.; Li, CH.; Kohane, I.; Chan, J.; Yankner, B.A. Gene regulation and DNA damage in the ageing human brain. *Nature*, **2004**, *429*, 883-891.

[49] Fraser, H.B.; Khaitovich, Ph.; Plotkin, J.B.; Pääbo, S.; Eisen, M. B. Aging and gene expression in the primate brain. *PLoS Biol.*, **2005**, *3*(9), e274.

[50] Fu, CH.; Hickey, M.; Morrison, M.; McCarter, R.; Han, E.-S. Tissue specific and non-specific changes in gene expression by aging and by early stage CR. *Mech. Ageing Dev.*, **2006**, *127*, 905-916.

[51] Bahar, R.; Hartmann, C.H.; Rodriguez, K.A.; Denny, A.D.; Busuttil, R.A.; Dollé, M.E.T.; Calder, R.B.; Chisholm, G.B.; Pollock, B.H.; Klein, CH. A.; Vijg, J. Increased cell-to-cell variation in gene expression in ageing mouse heart. *Nature*, **2006**, *441*, 1011-1014.

[52] Zahn, J.M.; Sonu, R.; Vogel, H.; Crane, E.; Mazan-Mamczarz, K.; Rabkin, R.; Davis, R.W.; Becker, K.G.; Owen, A.B.; Kim, S.K. Transcriptional profiling of aging in human muscle reveals a common aging signature. *PLoS Genet.*, **2006**, *2*(7), e115.

[53] Zahn, J.M.; Poosala, S.; Owen, A.B.; Ingram, D.K.; Lustig, A.; Carter, A.; Weeraratna, A.T.; Taub, D.D.; Gorospe, M.; Mazan-Mamczarz, K.; Lakatta, E.G.; Boheler, K.R.; Xu, X.; Mattson, M.P.; Falco, G.; Ko, M.S.; Schlessinger, D.; Firman, J.; Kummerfeld, S.K.; Wood, W.H. 3rd; Zonderman, A.B.; Kim, S.K.; Becker, K.G. AGEMAP: A gene expression database for aging in mice. *PLoS Genet.*, **2007**, *3*(11), e201.

[54] de Magalhães, J.P.; Curado, J.; Church, G.M. Meta-analysis of age-related gene expression profiles identifies common signatures of aging. *Bioinformatics*, **2009**, *25*, 875-881.

[55] Harries, L.W.; Hernandez, D.; Henley, W.; Wood, A.R.; Holly, A.C.; Bradley-Smith, R.M.; Yaghootkar, H.; Dutta, A.; Murray, A.; Frayling, T.M.; Guralnik, J.M.; Bandinelli, S.; Singleton, A.; Ferrucci, L.; Melzer, D. Human aging is characterized by focused changes in gene expression and deregulation of alternative splicing. *Aging Cell*, **2011**, *10*, 868-878.

[56] Wood, S.H.; Craig, TH.; Li, Y.; Merry, B.; de Magalhães, J.P. Whole transcriptome sequencing of the aging rat brain reveals dynamic RNA changes in the dark matter of the genome. *Age*, **2013**, *35*, 763-776.

[57] Ibáñez-Ventoso, C.; Yang, M.; Guo, S.; Robins, H.; Padgett, R.W.; Driscoll, M. Modulated microRNA expression during adult lifespan in *Caenorhabditis elegans*. *Aging Cell*, **2006**, *5*, 235-246.

[58] Boehm, M.; Slack, F. A developmental timing microRNA and its target regulate life span in *C. elegans*. *Science*, **2005**, *310*, 1954-1957.

[59] Kato, M.; Chen, X.; Inukai, S.; Zhao, H.; Slack, F.J. Age-

associated changes in expression of small, noncoding RNAs, including microRNAs, in *C. elegans*. *RNA*, **2011**, *17*, 1804-1820.

[60] Lehrbach, N.J.; Castro, C.; Murfitt, K.J.; Abreu-Goodger, C.; Griffin, J.L.; Miska, E.A. Post-developmental microRNA expression is required for normal physiology, and regulates aging in parallel to insulin/IGF-1 signaling in *C. elegans*. *RNA*, **2012**, *18*, 2220-2235.

[61] Liu, N.; Landreh, M.; Cao, K.; Abe, M.; Hendriks, G.J.; Kennerdell, J.R.; Zhu, Y.; Wang, L.S.; Bonini, N.M. The microRNA miR-34 modulates aging and neurodegeneration in *Drosophila*. *Nature*, **2012**, *482*, 519-523.

[62] Badi, I.; Burba, I.; Ruggeri, C.; Zeni, F.; Bertolotti, M.; Scopece, A.; Pompilio, G.; Raucci, A. MicroRNA-34a induces vascular smooth muscle cells senescence by SIRT1 downregulation and promotes the expression of age-associated pro-inflammatory secretory factors. *J. Gerontol. A. Biol. Sci. Med. Sci.*, **2015**, *70*, 1304-1311.

[63] Maes, O.C.; An, J.; Sarojini, H.; Wang, E. Murine microRNAs implicated in liver functions and aging process. *Mech. Ageing Dev.*, **2008**, *129*, 534-541.

[64] Liang, R.; Bates, D.J.; Wang, E. Epigenetic control of microRNA expression and aging. *Curr. Genomics*, **2009**, *10*, 184-193.

[65] Tamminga, J.; Kathiria, P.; Koturbash, I.; Kovalchuk, O. DNA damage-induced upregulation of miR-709 in the germline downregulates BORIS to counteract aberrant DNA hypomethylation. *Cell Cycle*, **2008**, *7*, 3731-3736.

[66] Klenova, E.M.; Morse, H.C. III, Ohlssonc, R.; Lobanenkov, V.V. The novel BORIS + CTCF gene family is uniquely involved in the epigenetics of normal biology and cancer. *Semin. Cancer Biol.*, **2002**, *12*, 399-414.

[67] Vatolin, S.; Abdullaev, Z.; Pack, S.D.; Flanagan, P.T.; Custer, M.; Loukinov, D.I.; Pugacheva, E.; Hong, J.A.; Morse, H. 3rd.; Schrump, D.S.; Risinger, J.I.; Barrett, J.C.; Lobanenkov, V.V. Conditional expression of the CTCF-paralogous transcriptional factor BORIS in normal cells results in demethylation and derepression of MAGE-A1 and reactivation of other cancer-testis genes. *Cancer Res.*, **2005**, *65*, 7751-7762.

[68] Monk, M.; Hitchins, M.; Hawes, S. Differential expression of the embryo/cancer gene *ECSA*(*DPPA2*), the cancer/testis gene *BORIS* and the pluripotency structural gene *OCT4*, in human preimplantation development. *Mol. Hum. Reprod.*, **2008**, *14*, 347-355.

[69] Jones, T.A.; Ogunkolade, B.W.; Szary, J.; Aarum, J.; Mumin, M.A.; Patel, SH.; Pieri, CH. A.; Sheer, D. Widespread expression of BORIS/CTCFL in normal and cancer cells. *PLoS One*, **2011**, *6*(7), e22399.

[70] Rosa-Garrido, M.; Ceballos, L.; Alonso-Lecue, P.; Abraira, C.; Delgado, M.D.; Gandarillas, A. A cell cycle role for the epigenetic factor CTCF-L/BORIS. *PLoS One*, **2012**, *7*(6), e39371.

[71] Nguyen, P.; Bar-Sela, G.; Sun, L.; Bisht, K.S.; Cui, H.; Kohn, E.; Feinberg, A.P.; Gius, D. BAT3 and SET1A form a complex with CTCFL/BORIS to modulate H3K4 histone dimethylation and gene expression. *Mol. Cell. Biol.*, **2008**, *28*, 6720-6729.

[72] Wakeman, T.P.; Wang, Q.; Feng, J.; Wang, X. -F. Bat3 facilitates H3K79 dimethylation by DOT1L and promotes DNA damage-induced 53BP1 foci at G1/G2 cell-cycle phases. *EMBO J.*, **2012**, *31*, 2169-2181.

[73] Suzuki, T.; Kosaka-Suzuki, N.; Pack, S.; Shin, D.M.; Yoon, J.; Abdullaev, Z.; Pugacheva, E.; Morse, H.C. 3rd; Loukinov, D.; Lobanenkov, V. Expression of a testis-specific form of *Gal3st1* (*CST*), a gene essential for spermatogenesis, is regulated by the *CTCF* paralogous gene *BORIS*. *Mol. Cell. Biol.*, **2010**, *30*, 2473-2484.

[74] Renaud, S.; Loukinov, D.; Alberti, L.; Vostrov, A.; Kwon, Y.W.; Bosman, F.T.; Lobanenkov, V.; Benhattar, J. BORIS/CTCFL-mediated transcriptional regulation of the *hTERT* telomerase gene in testicular and ovarian tumor cells. *Nucleic Acids Res.*, **2011**, *39*, 862-873.

[75] Crosby, M.E.; Kulshreshtha, R.; Ivan, M.; Glazer, P.M. MicroRNA regulation of DNA repair gene expression in hypoxic stress. *Cancer Res.*, **2009**, *69*, 1221-1229.

[76] Noren Hooten, N.; Abdelmohsen, K.; Gorospe, M.; Ejiogu, N.; Zonderman, A.B; Evans, M.K. microRNA expression patterns reveal differential expression of target genes with age. *PLoS One*, **2010**, *5*(5): e10724.

[77] Persengiev, S.; Kondova, I.; Otting, N.; Koeppen, A.H.; Bontrop, R.E. Genome-wide analysis of miRNA expression reveals a potential role for miR-144 in brain aging and spinocerebellar ataxia pathogenesis. *Neurobiol. Aging*, **2011**, *32*, 2316.e17-e27.

[78] Li, N.; Bates, D.J.; An, J.; Terry, D.A.; Wang, E. Up-regulation of key microRNAs, and inverse down-regulation of their predicted oxidative phosphorylation target genes, during aging in mouse brain. *Neurobiol. Aging*, **2011**, *32*, 944-955.

[79] de Lencastre, A.; Pincus, Z.; Zhou, K.; Kato, M.; Lee, S.S.; Slack, F.J. MicroRNAs both promote and antagonize longevity in *C. elegans*. *Curr. Biol.*, **2010**, *20*, 2159-2168.

[80] Pincus, Z.; Smith-Vikos, T.; Slack, F.J. MicroRNA predictors of longevity in *Caenorhabditis elegans*. *PLoS Genet.*, **2011**, *7*(9), e1002306.

[81] Boulias, K.; Horvitz, H.R. The microRNA acts in neurons to promote germline-mediated longevity through regulation of DAF-16/FOXO. *Cell Metab.*, **2012**, *15*, 439-450.

[82] Greer, E.L.; Maures, T.J.; Hauswirth, A.G.; Green, E.M.; Leeman, D.S.; Maro, G.S.; Han, S.; Banko, M.R.; Gozani, O.; Brunet, A. Members of the H3K4 trimethylation complex regulate lifespan in a germline-dependent manner in *C. elegans*. *Nature*, **2010**, *466*, 383-387.

[83] Jin, C.; Li, J.; Green, C.D.; Yu, X.; Tang, X.; Han, D.; Xian, B.; Wang, D.; Huang, X.; Cao, X.; Yan, Z.; Hou, L.; Liu, J.; Shukeir, N.; Khaitovich, P.; Chen, C.D.; Zhang, H.; Jenuwein, T.; Han, J.D. Histone demethylase UTX-1 regulates *C. elegans* life span by targeting the insulin/IGF-1 signaling pathway. *Cell Metab.*, **2011**, *14*, 161-172.

[84] Siebold, A.P.; Banerjee, R.; Tie, F.; Kiss, D.L.; Moskowitz, J.; Harte, P.J. Polycomb Repressive Complex 2 and Trithorax modulate *Drosophila* longevity and stress resistance. *Proc. Natl. Acad. Sci. U.S.A.*, **2010**, *107*, 169-174.

[85] Lin, M.-J.; Tang, L.-Y.; Reddy, M.N.; Shen, C.-K.J. DNA methyltransferase gene *dDnmt2* and longevity of *Drosophila*. *J. Biol. Chem.*, **2005**, *280*, 861-864.

[86] Goll, M.G.; Kirpekar, F.; Maggert, K.A.; Yoder, J.A.; Hsieh, C.-L.; Zhang, X.; Golic, K.G.; Jacobsen, S.E.; Bestor, T.H. Methylation of $tRNA^{Asp}$ by the DNA methyltransferase homolog Dnmt2. *Science*, **2006**, *311*, 395-398.

[87] Sinclair, D.A. Toward a unified theory of caloric restriction and longevity regulation. *Mech. Ageing Dev.*, **2005**, *126*, 987-1002.

[88] Li, Y.; Liu, L.; Tollefsbol, T.O. Glucose restriction can extend normal cell lifespan and impair precancerous cell growth through epigenetic control of hTERT and p16 expression. *FASEB J.*, **2010**, *24*, 1442-1453.

[89] Hjelmborg, J. VB.; Iachine, I.; Skytthe, A.; Vaupel, J.W.; McGue, M.; Koskenvuo, M.; Kaprio, J.; Pedersen, N.L.; Christensen, K. Genetic influence on human lifespan and longevity. *Hum. Genet.*, **2006**, *119*, 312-321.

[90] ElSharawy, A.; Keller, A.; Flachsbart, F.; Wendschlag, A.; Jacobs, G.; Kefer, N.; Brefort, T.; Leidinger, P.; Backes, C.; Meese, E.; Schreiber, S.; Rosenstiel, P.; Franke, A.; Nebel, A. Genome-wide miRNA signatures of human longevity. *Aging Cell*, **2012**, *11*, 607-616.

[91] Gentilini, D.; Mari, D.; Castaldi, D.; Remondini, D.; Ogliari, G.; Ostan, R.; Bucci, L.; Sirchia, S.M.; Tabano, S.; Cavagnini, F.; Monti, D.; Franceschi, C.; Di Blasio, A.M.; Vitale, G. Role of epigenetics in human aging and longevity: genome-wide DNA methylation profile in centenarians and centenarians' offspring. *Age*, **2013**, *35*, 1961-1973.

[92] Baek, J.-H.; McKenna, T.; Eriksson, M. Hutchinson-Gilford Progeria Syndrome. In: *Genetic Disorders*; Puiu, M.; Ed.; InTech: Rijeka, Croatia, **2013**, vol. 1, pp. 65-87.

[93] De Sandre-Giovannoli, A.; Bernard, R.; Cau, P.; Navarro, C.; Amiel, J.; Boccaccio, I.; Lyonnet, S.; Stewart, C.L.; Munnich, A.; Le Merrer, M.; Lévy, N. Lamin A truncation in Hutchinson-Gilford progeria. *Science*, **2003**, *300*, 2055.

[94] Eriksson, M.; Brown, W.T.; Gordon, L.B.; Glynn, M.W.; Singer, J.; Scott, L.; Erdos, M.R.; Robbins, C.M.; Moses, T.Y.; Berglund, P.; Dutra, A.; Pak, E.; Durkin, S.; Csoka, A.B.; Boehnke, M.; Glover, T.W.; Collins, F.S. Recurrent *de novo* point mutations in lamin A cause Hutchinson-Gilford progeria syndrome. *Nature*, **2003**, *423*, 293-298.

[95] Goldman, R.D.; Shumaker, D.K.; Erdos, M.R.; Eriksson, M.; Goldman, A.E.; Gordon, L.B.; Gruenbaum, Y.; Khuon, S.; Mendez, M.; Varga, R.; Collins, FS. Accumulation of mutant lamin A causes progressive changes in nuclear architecture in Hutchinson-Gilford progeria syndrome. *Proc. Natl. Acad. Sci. U.S.A.*, **2004**, *101*, 8963-8968.

[96] McClintock, D.; Ratner, D.; Lokuge, M.; Owens, D.M.; Gordon, L. B.; Collins, F.S.; Djabali, K. The mutant form of lamin A that causes Hutchinson-Gilford progeria is a biomarker of cellular aging in human skin. *PLoS One*, **2007**, *2*(12), e1269.

[97] Rodriguez, S.; Coppede, F.; Sagelius, H.; Eriksson, M. Increased expression of the Hutchinson-Gilford progeria syndrome truncated lamin A transcript during cell aging. *Eur. J. Hum. Genet.*, **2009**, *17*, 928-937.

[98] Olive, M.; Harten, I.; Mitchell, R.; Beers, J.K.; Djabali, K.; Cao, K.; Erdos, M.R.; Blair, C.; Funke, B.; Smoot, L.; Gerhard-Herman, M.; Machan, J.T.; Kutys, R.; Virmani, R.; Collins, F.S.; Wight, T. N.; Nabel, E.G.; Gordon, L.B. Cardiovascular pathology in Hutchinson-Gilford progeria: correlation with the vascular pathology of aging. *Arterioscler. Thromb. Vasc. Biol.*, **2010**, *30*, 2301-2309.

[99] Cao, K.; Blair, C.D.; Faddah, D.A.; Kieckhaefer, J.E.; Olive, M.; Erdos, M.R.; Nabel, E.G.; Collins, F.S. Progerin and telomere dysfunction collaborate to trigger cellular senescence in normal human fibroblasts. *J. Clin. Invest.*, **2011**, *121*, 2833-2844.

[100] Benson, E.K.; Lee, S.W.; Aaronson, S.A. Role of progerin-induced telomere dysfunction in HGPS premature cellular senescence. *J. Cell Sci.*, **2010**, *123*, 2605-2612.

[101] Scaffidi, P.; Misteli, T. Lamin A-dependent nuclear defects in human aging. *Science*, **2006**, *312*, 1059-1063.

[102] Csoka, A.B.; English, S.B.; Simkevich, C.P.; Ginzinger, D.G.; Butte, A.J.; Schatten, G.P.; Rothman, F.G.; Sedivy, J.M. Genome-scale expression profiling of Hutchinson-Gilford progeria syndrome reveals widespread transcriptional misregulation leading to mesodermal/mesenchymal defects and accelerated atherosclerosis. *Aging Cell*, **2004**, *3*, 235-243.

[103] Scaffidi, P.; Misteli, T. Lamin A-dependent misregulation of adult stem cells associated with accelerated ageing. *Nat. Cell Biol.*, **2008**, *10*, 452-459.

[104] Shumaker, D.K.; Dechat, T.; Kohlmaier, A.; Adam, S.A.; Bozovsky, M.R.; Erdos, M.R.; Eriksson, M.; Goldman, A.E.; Khuon, S.; Collins, F.S.; Jenuwein, T.; Goldman, R.D. Mutant nuclear lamin A leads to progressive alterations of epigenetic control in premature aging. *Proc. Natl. Acad. Sci. U.S.A.*, **2006**, *103*, 8703-8708.

[105] Ghosh, S.; Liu, B.; Wang, Y.; Hao, Q.; Zhou, Z. Lamin A is an endogenous SIRT6 activator and promotes SIRT6-mediated DNA repair. *Cell Rep.*, **2015**, *13*, 1396-1406.

[106] Heyn, H.; Moran, S.; Esteller, M. Aberrant DNA methylation profiles in the premature aging disorders Hutchinson-Gilford Progeria and Werner syndrome. *Epigenetics*, **2013**, *8*, 28-33.

[107] Yang, S.H.; Bergo, M.O.; Toth, J.I.; Qiao, X.; Hu, Y.; Sandoval, S.; Meta, M.; Bendale, P.; Gelb, M.H.; Young, S.G.; Fong, L.G. Blocking protein farnesyltransferase improves nuclear blebbing in mouse fibroblasts with a targeted Hutchinson-Gilford progeria syndrome mutation. *Proc. Natl. Acad. Sci. U.S.A.*, **2005**, *102*, 10291-10296.

[108] Jung, H.J.; Coffinier, C.; Choe, Y.; Beigneux, A.P.; Davies, B.S.; Yang, S.H.; Barnes, R.H. 2nd; Hong, J.; Sun, T.; Pleasure, S.J.; Young, S.G.; Fong, L.G. Regulation of prelamin A but not lamin C by *miR-9*, a brain-specific microRNA. *Proc. Natl. Acad. Sci. U.S.A.*, **2012**, *109*, 423-431.

[109] Nissan, X.; Blondel, S.; Navarro, C.; Maury, Y.; Denis, C.; Girard, M.; Martinat, C.; De Sandre-Giovannoli, A.; Levy, N.; Peschanski, M. Unique preservation of neural cells in Hutchinson-Gilford progeria syndrome is due to the expression of the neural-specific *miR-9* microRNA. *Cell Rep.*, **2012**, *2*, 1-9.

[110] Baek, J.-H.; Schmidt, E.; Viceconte, N.; Strandgren, CH.; Pernold, K.; Richard, T.J.C.; Van Leeuwen, F.W.; Dantuma, N.P.; Damberg, P.; Hultenby, K.; Ulfhake, B.; Mugnaini, E.; Rozell, B.; Eriksson M. Expression of progerin in aging mouse brains reveals structural nuclear abnormalities without detectible significant alterations in gene expression, hippocampal stem cells or behavior. *Hum. Mol. Genet.*, **2015**, *24*, 1305-1321.

[111] Surani, M.A.; Hayashi, K.; Hajkova, P. Genetic and epigenetic regulators of pluripotency. *Cell*, **2007**, *128*, 747-762.

[112] Albert, M.; Peters, A.H.F.M. Genetic and epigenetic control of early mouse development. *Curr. Opin. Genet. Dev.*, **2009**, *19*, 113-121.

[113] Zhou, L.; Dean, J. Reprogramming the genome to totipotency in mouse embryos. *Trends Cell Biol.*, **2015**, *25*, 82-91.

[114] Yamanaka, S.; Blau, H.M. Nuclear reprogramming to a pluripotent state by three approaches. *Nature*, **2010**, *465*, 704-712.

[115] Marión, R.M.; Blasco, M.A. Telomere rejuvenation during nuclear reprogramming. *Curr. Opin. Genet. Dev.*, **2010**, *20*, 190-196.

[116] Lapasset, L.; Milhavet, O.; Prieur, A.; Besnard, E.; Babled, A.; Aït-Hamou, N.; Leschik, J.; Pellestor, F.; Ramirez, J.-M.; De Vos, J.; Lehmann, S.; Lemaitre, J.-M. Rejuvenating senescent and centenarian human cells by reprogramming through the pluripotent state. *Genes Dev.*, **2011**, *25*, 2248-2253.

[117] Conboy, I.M.; Conboy, M.J.; Wagers, A.J.; Girma, E.R.; Weissman, I.L.; Rando, T.A. Rejuvenation of aged progenitor cells by exposure to a young systemic environment. *Nature*, **2005**, *433*, 760-764.

[118] Villeda, S.A.; Luo, J.; Mosher, K.I.; Zou, B.; Britschgi, M.; Bieri, G.; Stan, T.M.; Fainberg, N.; Ding, Z.; Eggel, A.; Lucin, K.M.; Czirr, E.; Park, J.-S.; Couillard-Despres, S.; Aigner, L.; Li, G.; Peskind, E.R.; Kaye, J.A.; Quinn, J.F.; Galasko, D.R.; Xie, X.S.; Rando, T.A.; Wyss-Coray, T. The ageing systemic milieu negatively regulates neurogenesis and cognitive function. *Nature*, **2011**, *477*, 90-94.

[119] Katsimpardi, L.; Litterman, N.K.; Schein, P.A.; Miller, CH.M.; Loffredo, F.S.; Wojtkiewicz, G.R.; Chen, J.W.; Lee, R.T.; Wagers, A.J.; Rubin, L.L. Vascular and neurogenic rejuvenation of the aging mouse brain by young systemic factors. *Science*, **2014**, *344*, 630-634.

[120] Loffredo, F.S.; Steinhauser, M.L.; Jay, S.M.; Gannon, J.; Pancoast, J.R.; Yalamanchi, P.; Sinha, M.; Dall'Osso, C.; Khong, D.; Shadrach, J.L.; Miller, Ch. M.; Singer, B.S.; Stewart, A.; Psychogios, N.; Gerszten, R.E.; Hartigan, A.J.; Kim, M.-J.; Serwold, Th.; Wagers, A.J.; Lee, R.T. Growth differentiation factor 11 is a circulating factor that reverses age-related cardiac hypertrophy. *Cell*, **2013**, *153*, 828-839.

[121] Rossi, D.J.; Bryder, D.; Zahn, J.M.; Ahlenius, H.; Sonu, R.; Wagers, A.J.; Weissman, I.L. Cell intrinsic alterations underlie hematopoietic stem cell aging. *Proc. Natl. Acad. Sci. U.S.A.*, **2005**, *102*, 9194-9199.

[122] Wullschleger, S.; Loewith, R.; Hall, M.N. TOR signaling in growth and metabolism. *Cell*, **2006**, *124*, 471-484.

[123] Gough, N. R. Focus issue: TOR signaling, a tale of two complexes. *Sci. Signal.*, **2012**, *5*(2170), eg4.

[124] Chen, CH.; Liu, Y.; Liu, R.; Ikenoue, T.; Guan, K.-L.; Liu, Y.; Zheng, P. TSC - mTOR maintains quiescence and function of hematopoietic stem cells by repressing mitochondrial biogenesis and reactive oxygen species. *J. Exp. Med.*, **2008**, *205*, 2397-2408.

[125] Chen, C.; Liu, Y.; Liu, Y.; Zheng, P. mTOR regulation and therapeutic rejuvenation of aging hematopoietic stem cells. *Sci. Signal.*, **2009**, *2*, ra75.