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Excelentísima Rectora Magnífica de la Universitat de València, ilustres autoridades académicas, estimados profesores y alumnos, queridos amigos y compañeros, señoras y señores;

My first words are words of gratitude. Gratitude to those here at the University of Valencia, that have considered appropriate to award me the highest honor that the University can confer. I am very deeply honored and humbled to be receiving this prestigious recognition. It is even more meaningful for me since the University of Valencia is my alma mater. It was here that I first began to truly delve into and expand my love of science and basic research. Precisely, this was under the guidance of professors Jose Cabo and Teresa Barber to whom I will always be indebted and grateful. I also want to thank you Teresa for your kind words and your heartfelt presentation. I appreciate it very much.

China, of the most ancient cultures, has a say that goes something like this: if you want to travel fast, travel alone, but if you want to travel far, travel together. My life journey is more of the second type, travel together. For this reason I would like to thank everyone who I have traveled with. Without them I will not be receiving this honor today. Friends that I made here at this university more than 35 years ago. Mentor and teachers here and throughout the world. Students, colleagues and scientists I have worked with. To all of you, many, many thanks for letting me traveling far with you and for allowing me to grow and together learn some of the secrets of nature.

I specially want to thank my wife, Concepcion Rodriguez Esteban, who unfortunately cannot be here today. I still remember myself around 40 years ago, here in Valencia, a kid struggling itself with identity and with which path to follow in life. Since the very first day when she helped me to do the first experiment at the Department of Biochemistry at the Facultad de Farmacia here in Valencia, until the last experiment we did together a few days ago, she has been my guide, my scientific colleague, my mentor, my teacher and my best life travel companion. I may not have been the obvious choice at the begining for her to choose me as her traveling partner, but it certainly worked out wonderfully. I want to thank Conchi publically for travelling that far together with me. She has been the true treasure of my life, and she is the heart and reason of this honor today.

I would like now to briefly summarize one aspect of the basic scientific research that is being carried out in my laboratory at the Salk Institute for Biological Studies in La Jolla, California. This is the topic of Ageing. Ageing is characterized by the functional decline of every organ in the body. A number of interventions have been proposed to rejuvenate cells and organs and to extend human lifespan. They include metabolic manipulations, heterochronic parabiosis, partial reprogramming, pharmaceutical administration, and the ablation of senescent cells. Epigenetic forms of gene regulation are central to the effectiveness of these interventions. Today, I would like to we discuss some current knowledge regarding the epigenetic mechanisms underlying lifespan-extending interventions, and outline questions to guide future research toward rejuvenating the epigenome and delaying ageing processes.

How long can a human being live? By analyzing global demographic data, a recent study suggested that there was a limit to human lifespan. However, human life expectancy has increased steadily over the past century in most countries. Improvements in healthcare and the environment are the major contributors to this increased life expectancy. Environmental factors affect the cellular epigenome to regulate gene expression and control cell fate. The progressive accumulation of age-associated epigenetic changes ultimately leads to the aberrant regulation of gene expression, metabolic instability, stem cell senescence and/or exhaustion, and disruptions in tissue homeostasis. Previous studies have shown how alterations in DNA methylation, post-translational modification (PTM) of histones, and chromatin remodeling influence lifespan. DNA methylation status can be used to predict chronological age, termed the epigenetic clock. Histones are subjected to various PTMs, such as methylation, acetylation, and lysine acylation. All of these modifications are critical to chromatin function, modulating the availability of DNA to transcriptional complexes. Nucleosome positioning also regulates chromatin accessibility (i.e., the open and closed state of chromatin), and is associated with cell-type specific gene expression programs. Non-coding RNAs (ncRNAs), including long noncoding RNAs (lncRNAs), microRNAs, and circular RNAs (circRNAs), provide an additional layer of epigenetic regulation. Recent studies have shown that aging results in substantial chromatin changes, which are typically accompanied by demethylation of active regulatory regions and progressive loss of constitutive heterochromatin. In fact, these epigenetic alterations are a hallmark of ageing. The notion that epigenetic factors regulate the ageing process is supported by numerous lines of

evidence, including epigenetic drift during ageing, intergenerational epigenetic effects on ageing, and most importantly, the modulation of ageing kinetics by environmental and epigenetic factors.

Ageing is reversible

Ageing is malleable and can be modulated by genetic, nutritional, or pharmacological interventions. Analysis of global methylation patterns in murine liver cells has demonstrated that the acquisition of epigenetic ageing signatures is slowed by lifespan-extending interventions, such as dietary restriction, exercise, and rapamycin supplementation. Stabilization of the fundamental chromatin structure is critical for slowing ageing processes. Normal ageing is accompanied by decreased levels of histones; conversely, restoring histone levels promotes lifespan extension. These data demonstrate that ageing can be delayed without modifying genomic sequences, suggesting that epigenetic forms of regulation are among the primary mechanisms affecting ageing. A better understanding of the proximal causes of age-related degeneration and disease, as well as the epigenetic targets of geroprotective interventions, is essential for developing more effective strategies to ameliorate age-associated disorders and extend lifespan. In this review, we will discuss in detail current knowledge concerning lifespan and healthspan-extending interventions, as well as how these interventions target chromatin and how high levels of diversity in chromatin organization influence cellular activity and organismal ageing. These topics will be discussed in the context of metabolic manipulation, partial reprogramming, heterochronic parabiosis, pharmaceutical administration, and the ablation of senescent cells. Furthermore, we will attempt to

dissect the epigenetic mechanisms underlying these rejuvenating processes, paying special attention to stem cell rejuvenation, the rescue of mitochondrial function, the resilience of activated retrotransposons, and the repression of age-related chronic inflammation.

Epigenetic factors

Before arguing that epigenetic mechanisms should be targeted to combat aging-associated disorders, we provide two more lines of evidence supporting epigenetic regulation as a critical hallmark of aging. First, environmental changes result in elevated epigenomic noise, which leads to substantial heterogeneity in gene expression at the cellular level, and divergent phenotypes at the organismal level during the ageing process. Recent studies have shown that transcriptional and epigenetic heterogeneity is higher in aged identical twins compared with young pairs of twins. Elder identical twins show substantial heterogeneity in levels of global or locus-specific DNA methylation, histone acetylation, and tri-methylation of histone 3 at lysine 27 (H3K27me3), which is mediated by polycomb-repressive complex 2 (PRC2), leading to higher transcriptional noise during aging. With advancements in single-cell sequencing technologies, increased cell-to-cell variation and gene-expression noise is has been observed in ageing heart, muscle, pancreas, and dermal cells. Similarly, single-cell analyses of memory CD4+ T cells from young and aged mice uncovered that variations in the immune system are largely driven by non-heritable factors. Moreover, single-cell chromatin modification profiling revealed elevated levels of cellular epigenetic heterogeneity in PRC2 complex-mediated modifications (e.g., H3K-27me2/3, and ubiquitylation of histone 2A at lys119 (H2AK119ub))

across a broad array of immune cell types during ageing. Thus, environmental effects can provoke "epigenetic drift" at both cellular and organismal levels, resulting in altered gene expression, disrupted cellular function, and phenotypic discordance during aging.

Second, environmental perturbations can cause epigenetic changes that are heritable, influencing lifespan and the development of aging-related diseases in both the parents and offspring. In Drosophila melanogaster, males fed high levels of sugar express genes embedded in heterochromatin, leading to intergenerational reprogramming of metabolic networks and obesity in offsprings. In mice, the offspring of an aged father live shorter lives and develop aging phenotypes earlier than offspring of a young father. Similarly, maternal exposure to suboptimal nutrition leads to progressive epigenetic silencing of hepatocyte nuclear factor 4α (Hnf4α) in pancreatic islets, eventually resulting in diabetes in offsprings. High-fat maternal diet induces hypermethylation of the PGC1 promoter, causing age-dependent metabolic dysfunction in offspring that can be prevented by exercise. Interestingly, the transgenerational inheritance of chromatin marks increases lifespan for multiple generations, providing another strong piece of evidence for the role of epigenetic regulation in the complicated and disordered aging process. Together, as further discussed in the next sections, the epigenetic regulatory apparatus represents important targets for counteracting aging, and it is becoming clear that any lifespan-extending intervention must rejuvenate the aged epigenome.

Metabolic manipulation

An abundance of key nutrients, such as glucose, fatty acids, and amino acids, directly influence organismal longevity. Previous studies have shown that impairing glycolysis can extend lifespan, and supplementation of D-glucosamine, an antagonist of glucose that can impair glucose metabolism, extends lifespan in C. elegans and mice. In addition, amino acid and lipid metabolism strongly correlates with age and can be taken as an indicator of healthspan, as revealed by LC-MS metabolomics analysis of plasma from healthy young and older individuals. Whole-body overexpression of genes involved in fatty acid oxidation extends lifespan in D. melanogaster. Notably, dietary supplementation of mono-unsaturated fatty acids (MUFAs) is sufficient to extend lifespan. Moreover, a deficiency in the H3K-4me3 methyltransferase complex specifically promotes the accumulation of MUFAs and extends lifespan in C. elegans.

Dietary restriction, including daily or intermittent calorie restriction (CR), is a simple, non-invasive metabolic manipulation that can be utilized to improve both healthspan and lifespan. Two pilot clinical trials in healthy humans showed that dietary restriction decreases systemic biomarkers of ageing and lowers multiple risk factors for cancer and cardiovascular diseases, supporting the notion that dietary restriction promotes healthspan in humans. Critically, CR has profound effects on adult stem cells. CR preserves the function of neural stem cells, mesenchymal stem cells (MSCs), intestine stem cells (ISCs), endothelial progenitor cells, and hematopoietic stem cells (HSCs) in the bone marrow. In old mice, metabolic interventions lower levels of insulin-like growth factor 1 (IGF-1), limits Pro-

tein kinase A (PKA) activity, elevates levels of the neuron-specific transcription factor NeuroD1, promotes neurogenesis in the hippocampus, and improves cognitive performance. Additionally, short-term fasting promotes ISCs and progenitor cell function in young and aged mice by inducing a robust peroxisome proliferator-activated receptor PPAR-mediated fatty acid oxidation program. Short-term CR also enhances skeletal muscle stem cell function. Although studies concerning the mechanisms by which CR provides benefits have focused on evolutionarily conserved pathways that regulate energy metabolism and growth (e.g., insulin/IGF-I signaling and the target of rapamycin (TOR) pathway), increasing data support the notion that nutritional and metabolic factors affect the epigenetic program and play critical roles in regulating stem cell function and ageing process.

Both chromatin and metabolic states influence lifespan. However, how they interact with each other to regulate lifespan has only recently been explored. Effects of CR on healthspan and lifespan, at least in part, result from preventing ageing-associated global changes in DNA methylation, histone modification, and chromatin remodeling. First, CR is generally protective against age-related changes in DNA methylation. For macaques and mice subjected to CR, their "methylation age" is younger than their chronological age. The DNA methylation age of old rhesus macaques exposed to CR for more than 10 years is 7 years younger than their chronologic age. For aged mice subjected to metabolic interventions, DNA methylation patterns within their hepatocytes shift to include protein coding regions of genes involved in lipid metabolism, suppressing the expression of these genes and lowering systemic levels of triglyceride.

CR transcriptionally regulates Tet methylcytosine dioxygenase 2 (Tet2) and Tet methylcytosine dioxygenase 3 (Tet3), which catalyze the oxidation of 5-methylcytosine (5mC) to 5-hydroxymethylcytosine (5hmC) and subsequent demethylation of DNA. At the level of histone modification, dietary restriction inhibits histone deacetylase (HDAC) activity and increases the acetylation of histone 3 lysine 9 (H3K9ac), a marker of active transcription, in aged mouse. Inhibition of HDAC and H3K9ac accumulation together upregulate levels of Forkhead box O3 (FOXO3A) and its targets involved in antioxidant responses. CR also reduces the level of nicotinamide adenine dinucleotide (NAD)+-dependent deacetylase sirtuin-1 (SIRT1) enrichment at nutrition-responsive genes, accompanied by accumulation of H3K9ac. This change enhances the expression of transcription factor hairy and enhancer of split-1(HES1) and promotes adult neurogenesis. In addition, the effect of CR on stress response pathways is partially mimicked by deletion of ISW2, a component of the ATP-dependent chromatin remodeling complex in C. elegans and human fibroblasts. Dietary restriction also regulates longevity via RNA splicing, suppressing mTORC1 and regulating Splicing factor 1 (SFA-1), which modulates pre-mRNA splicing homeostasis and longevity in C. elegans. Taken together, dietary restriction shapes the DNA methylation and histone modification landscapes, affects RNA splicing, and delays age-related epigenetic drift.

Furthermore, ageing and nutrient sensing are naturally linked to circadian rhythms, which drive physiological and cellular adaptations to day and night cycles. The oscillating diurnal transcriptome is rewired in epidermal and muscle stem cells of aged mice, switching from genes involved in homeostasis to those responsible for

stress adaption. Interestingly, this age-associated reprogramming of the oscillatory transcriptome is prevented by long-term CR in a SIRT1-dependent manner. Mice lacking SIRT1 in the brain recapitulate these age-dependent circadian changes, whereas mice overexpressing SIRT1 in the brain are protected from disturbed circadian rhythms during aging. Thus, the lifespan-extension effect of CR may be, in part, attributed to reprogramming of the circadian clock.

Cellular reprogramming

Reprogramming to pluripotency, through ectopic expression of defined transcription factors, promotes epigenetic reprogramming and resets senescent cells back to an embryonic-like state. Recently, human induced pluripotent stem cells (iPSCs) were successfully generated from fibroblast collected from centenarians, supercentenarians, and individuals with progeroid syndromes, including Hutchinson-Gilford progeria syndrome (HGPS), Nestor-Guillermo Progeria syndrome (NGPS), Werner syndrome (WS), Xeroderma pigmentosum (XP), Fanconi anemia (FA), and Ataxia-telangiectasia (AT), albeit with reduced levels of efficiency compared to young fibroblasts. Senescence imposes a barrier to successful reprogramming. Inhibition of senescence-promoting factors, including TP53, p16INK4a, p21WAF1/Cip1, mTOR, nuclear factor-κB (NF-Kb), and DOT1L (DOT1-like histone H3-K79 methyltransferase), improves the efficiency of iPSC generation from senescent cells. Silencing certain ncRNAs, such as lncRNA Zeb2-NAT and microRNA-195 in aged cells also facilitates reprogramming. Remarkably, DOT1L inhibitors extend the lifespan of progeroid mice, suggesting that epigenetic barriers to reprogramming are potential targets for rejuvenation-based therapies.

Somatic cell reprogramming resets molecular and cellular characteristics of aged cell, including telomere size, gene expression profiles, oxidative stress, and mitochondrial metabolism, thereby erasing age-associated features. This cellular rejuvenation suggests that aging may be a reversible process. The transcriptomic profile of fibroblasts re-differentiated from iPSCs derived from centenarians are similar to fibroblasts derived from human embryonic stem cells (hESCs), indicating a successful 'rejuvenation' of the transcriptome. While HGPS and WS PSCs are able to differentiate into MSCs without an apparent decline in differentiation efficiency, these MSCs exhibit accelerated senescence and re-establish an aged epigenome after extensive passaging in vitro. However, age-associated DNA methylation patterns (known as the "epigenetic clock") are not erased by transdifferenation. Neurons transdifferentiated from fibroblasts of old mice retain biomarkers of ageing. Nonetheless, how reprogramming by pluripotency factors rejuvenates the transcriptional and epigenetic program of the aged cell is poorly studied. One challenge is that the elimination of senescent features is accompanied by loss of cellular identity during reprogramming, making it difficult to discriminate the fundamental epigenetic changes needed to rejuvenate aged cells, from the ones controlling the switch in identity.

In a thought-inspiring study, Ocampo et al. showed that the cyclic expression of reprogramming factors extends the lifespan of a premature ageing murine model, and is beneficial to the health of physiologically aged wild-type mice. Moreover, partial reprogramming restored the number of both satellite cells in skeletal muscle and hair follicle stem cells in skin. Additionally, partial reprogramming via Adeno-associated virus (AAV) vectors expressing Yamanaka factors

dramatically improved axon regeneration after damage. Epigenetic changes may be at the core of these ageing-associated reversions. The reprogramming factors induce a complete remodeling of the chromatin landscape, including restoring levels of H3K9me3 and Histone H4 trimethyl Lys20 (H4K20me3). Another study showed that partial reprogramming led to a steady reduction in epigenetic age before the loss of somatic identity. In time-course experiments, the epigenetic changes preceded the reversion of DNA damage and senescence markers, and inhibition of H3K9me3 methyltransferases abrogated the rejuvenation effect. Hence, pluripotency factors hold the potential to epigenetically reprogram the cells to a younger state at the cellular, tissue and organismal level.

Heterochronic parabiosis

In heterochronic parabiosis, the circulation system of young and aged animals are surgically attached, thereby facilitating the exchange of immune cells and secreted factors present in the blood. Heterochronic parabiosis can "rejuvenate" several signatures of ageing in old mice, including altered immune responses, and stem cell exhaustion. Independent laboratories confirmed that heterochronic parabiosis has rejuvenating effects in aged mice, ameliorating age-associated dysfunction in multiple tissues, including muscle, liver, brain, pancreas, heart, bone, and arteries. Although mechanisms underlying the youth-promoting effects of heterochronic parabiosis remain elusive, circulating factors such as FGF21 are believed to be partially responsible for the ageing-delaying effects. Young blood rejuvenates the regenerative performance of aged stem cells, and restores the molecular signatures of an old cell to a more youth-

ful state, suggesting that exposure to a youthful microenvironment via heterochronic parabiosis can reverse or mitigate the appearance of age-associated epigenetic alterations. In line with this notion, decreased levels of Tet2 in the aged hippocampus are reversed in a heterochronic parabiosis model of brain rejuvenation. Heterochronic parabiosis reverses an imbalance in the epigenetic status in the central nervous system of aged mice by reducing H3K27me3 levels. However, it will be interesting to determine whether young blood is capable of reprogramming the chromatin landscape of old cells to a youthful state while retaining its intrinsic cellular identity. Specifically, characterization of epigenetic profiles, including DNA methylation and histone modification of cells exposed to heterochronic parabiosis may help to determine the molecular mechanism of epigenetic reprogramming, help compare this system to other rejuvenation approaches, and facilitate the development health-span extending treatments.

Chemicals against ageing

Geroprotective compounds, such as metformin and rapamycin, hold great promise in treating ageing-related conditions and in delaying ageing. They have therefore received great attention from the pharmaceutical industry. Metformin modulates the activation of AMP-activated protein kinase (AMPK), which directly catalyzes numerous epigenetic enzymes, such as histone acetyltransferases (HATs), HDAC, and DNA methyltransferase (DNMT). Metformin restores AMPK-mediated phosphorylation and stabilizes TET2, thereby preventing changes in 5hmC levels. On the other hand, rapamycin treatment slows the accumulation of epigenetic ageing

signatures in liver cells of mice, similar to CR. Aspirin supplementation has also been shown to recapitulate features of CR. Mechanistically, the aspirin metabolite, salicylate, may competitively inhibit the histone acetyltransferase p300 and trigger cardioprotective mitophagy (as seen in mice and nematodes). Screening for chemicals capable of delaying cellular senescence in a stem cell model of WS identified ascorbate as a powerful small molecule for alleviating ageing defects. Ascorbate resets gene expression profiles, restores heterochromatin structure, and alleviates ageing defects, potentially in a reactive oxygen species (ROS)-scavenger independent but epigenetics-dependent manner. Ascorbate is a cofactor for Jumonji C domain-containing demethylases (JHDMs) in promoting histone demethylation, which facilitates erasure of epigenetic memory at the beginning of reprogramming. Additionally, ascorbate maintains unusually high levels of HsCs in humans and mice, helping to promote TET2 activity, maintain the HSC pool, and suppress leukaemogenesis in vivo.

Senolytics selectively eliminate senescent cells and represent a new class of drugs that could potentially slow the ageing process. Baker and colleagues found that eliminating p16INK4a-expressing cells increases lifespan in both prematurely aging models and wild type mice, regardless of the gender or strain of the mouse examined. Clearing senescent cells in obese mice partially prevents depletion of neural stem cells. This intervention ameliorates a range of age-dependent, disease phenotypes, including cancer onset, cataracts, kidney dysfunction, and abnormalities of heart and fat tissue. However, this method of eliminating p16INK4a-expressing cells has only been tested in mouse models and has limited clinical applications. Senolytics was developed to ablate senescent zombie cells and

reverse age-related phenotypes in a cell type-specific manner. For example, dasatinib eliminates senescent fat cell progenitors, whereas quercetin effectively eliminates senescent human endothelial cells and mouse bone marrow stem cells. A chemical screen showed that quercetin functions as a geroprotector by enhancing self-renewal and restoring heterochromatin architecture in aged MSCs. Although all these geroprotective compounds have been linked to a "younger" chromatin architecture, further studies are required to unravel how these longevity-promoting drugs target epigenetic networks to delay the aging process.

Interplay between epigenetic reprogramming and other factors during rejuvenation

Restoration of a youthful epigenome is vital for maintaining tissue function and extending the lifespan of aged animals. Epigenetic rejuvenation is indispensable in the aforementioned healthspan-extension strategies. Notably, a global view of these strategies has revealed that somatic stem cells may be more amenable to rejuvenation, and that all interventions discussed here help to maintain stem cell pools. Therefore, it will be intriguing to study how aged stem cells exposed to rejuvenating interventions restore youthful function, as well as the convergent epigenetic mechanisms that contribute to this process. Second, we will highlight that epigenetic rejuvenation functions by reversing other hallmarks of ageing, such as mitochondrial dysfunction, genomic instability caused by retrotransposon activation, and chronic inflammation to extend lifespan. In the following section, we dissect the interconnectedness between epigenetic regulation and these cytoplasmic and nuclear events, and elaborate the

contributions of epigenetic reprogramming to ageing rejuvenation by focusing on these key biological pathways.

Epigenetic regulation of mitochondria during rejuvenation

Dysfunctional mitochondria often accumulate in aged cells. Mitochondrial restoration is seen with CR, partial reprogramming, heterochronic parabiosis, and pharmaceutical administration. CR increases SIRT1 levels, which results in mitochondrial biogenesis in tissues such as muscle and white fat. Indeed, feeding mice the putative SIRT1 activator, resveratrol, upregulates mitochondrial numbers in muscle, improves physical activity, and lengthens the average life span in mice. Cellular reprogramming rejuvenates mitochondria similar to the state observed in ESCs. Heterochronic parabiosis reduces mitochondrial swelling and vacuolization in skeletal muscle of old mice.

Mitochondria-dependent mechanisms of lifespan extension include attenuation of oxidative phosphorylation (OXPHOS), changes in reactive oxygen species (ROS), Ca2+ signaling, and the mitochondrial unfolded protein response (UPRmt). Mechanically, mitochondria regulate longevity by supplying substrates for epigenetic modifications, thereby serving as a central platform for metabolism, epigenetic regulation, and ageing. Metabolic intermediates and by-products of the tricarboxylic acid (TCA) cycle serve as cofactors and substrates for various epigenetic enzymes. These include acetyl-CoA, which is used for acetylation, and methionine, which is used for methylation. In addition, the TCA cycle intermediate α-ketoglutarate (α-KG) induces DNA and histone demethylation by activating Jumonji C domain-containing demethylases and lysine demethylases. A

recent study showed that elevated levels of α-KG activate JMJD3 (a histone H3K27 demethylase) and PHF8 (a histone lysine demethylase specific for H3K9me1/2), resulting in the removal of repressive marks and the induction of UPRmt gene expression. This was sufficient to extend lifespan in C. elegans.

Another important metabolite that connects epigenetic regulation to mitochondria is NAD+, the cofactor for the sirtuin HDACs. High levels of NAD+ improve mitochondrial function, replenish stem cell pools, and enhance lifespan in mice. Nicotinamide riboside (NR) and nicotinamide mononucleotide (NMN) are NAD+ precursors. Plasma levels of NMN decrease with age, and administration of these NAD+ precursors to aged mice ameliorates senescence of muscle, neural, and melanocyte stem cells, delays reductions in mitochondrial and arterial function, mitigates ageing-associated physiological declines (e.g., type 2 diabetes and cognitive impairment), and increases lifespan. ROS generated by oxidative phosphorylation in mitochondria also influences epigenetic signaling, and ROS-mediated epigenetic changes may in turn alter the expression of genes regulating mitochondrial metabolism, and eventually regulate ageing and longevity. Mitochondrial ROS reduces level of the H3K36 demethylase Rph1p, resulting in increased levels of H3K36me3 at subtelomere regions, which promotes binding of the silencing protein Sir3p to repress subtelomeric transcription and longevity.

Therefore, mitochondria closely interact with the nuclear epigenome in rejuvenation and ageing. In one aspect, the environment affects the epigenome to modulate mitochondrial function in the opposite direction. Mitochondrial byproducts are essential mediators

of epigenetic enzymes and participate in promoting longevity. Understanding the bidirectional mito-nuclear communication underlying longevity-promoting strategies would potentially help develop mechanism-based interventions to promote longevity and healthy ageing.

Epigenetic regulation of retrotransposable elements during rejuvenation

Retrotransposable elements consist of non-long terminal repeat (LTR) retrotransposons and two sub-types of non-LTR retrotransposons, long interspersed elements (LINEs) and short interspersed elements (SINEs), which together comprise almost half the human genome. Retrotransposable elements are silenced by heterochromatin in young cells and organisms, but they are activated during cellular senescence and organismal ageing due to deficiencies in the regulation of higher-order chromatin structure. Lifespan-extending interventions, such as CR, counteract the increased expression of retrotransposons in aged mice. In liver and muscle cells of old mice, CR delays the loss of constitutive heterochromatin and suppresses the expression of repetitive elements, including LINE-1 and satellite elements, which are repetitive sequences localized to centromeric, pericentromeric and telomeric regions. CR represses interactions between miRNA and the chromatin remodeling factor Chd1, thus safeguarding against the activation of retrotransposons induced by ageing and poor diet.

In principle, suppression of retrotransposable elements is dependent on the integrity of heterochromatin. Guo et al. showed that chromatin relaxation activates transposable elements in Alzheimer's disease, promoting Tau neurofibrillary tangle pathology. Heterochromatin decay has also been reported during normal ageing and in progeroid syndromes. Cells from aged individuals or from patients with HGPS or WS exhibit progressive loss of heterochromatin, indicated by a progressive loss of the constitutive heterochromatin mark, H3K9me3, and epigenetic silencers such as HP1α, the nucleosome remodeler and deacetylase (NuRD) chromatin remodeling complex, and the PGC compelx. The loss of heterochromatin in regions of retrotransposable elements results in reactivation and transposition of retrotransposable elements. Notably, suppression of heterochromatin decay and retrotransposable elements reverses senescence, both in vitro and in vivo. Overexpression of the Histone-lysine N-methyltransferase, SUV39H1, or HP1 rescues heterochromatin decay and premature senescence in WS MSC. The argonaute family protein, Piwi, maintains heterochromatin, prevents retrotransposon activity, and protects the D. menanogaster intestinal system from age-associated dysregulation of stem cell function and loss of tissue homeostasis. Accordingly, the NAD+-dependent protein deacetylase, sirtuin-6 (SIRT6), mono-ADP ribosylates KAP1 and facilitates its interaction with HP1 in packaging of LINE-1 elements into repressive heterochromatin. Another known regulator of heterochromatin, pRb, antagonizes the activation of LINE-1 in senescent cells, and occupancy of pRb on the LINE-1 promoter decreases in senescent human cells and ageing mouse tissues. Repression LINE-1 expression by the homeoprotein transcription factor, engrailed, blocks

degeneration in adult dopaminergic neurons. Moreover, partial overexpression of histone proteins H3 and H4 reverse the transcriptional defects observed during ageing and reduce retrotransposition, indicating that the increased retrotransposition in old yeast is a consequence of histone loss during ageing. In addition, CRISPR/Cas9 screening has provided a genome-wide survey of genes involved in the control of LINE-1 retrotransposition, revealing that HUSH (a component of the human silencing hub complex) and MORC family CW-type zinc finger protein 2 (MORC2) promote deposition of H3K9me3 to silence the transcription of LINE-1 elements. A recent study showed that repressing the transposition of LINE-1 by the nucleoside reverse transcriptase inhibitor, lamivudine, counteracts cellular senescence. Hence, blocking the transcription of endogenous retrotransposable elements by dietary restriction and pharmaceutical administration can ameliorate age-associated phenotypes, supporting the notion that retrotransposons contribute to ageing or age-associated disorders, and hence are potential targets for therapeutic interventions. Collectively, these findings suggest a critical role for epigenetic remodeling in rejuvenation processes, perhaps by improving epigenomic stability (particularly heterochromatin) to prevent the activation and mobilization of retrotransposons. However, genome-wide quantitative analyses will add new insights into the frequency, structure, and location of retrotransposon transpositions during ageing, and further dissect their overall contribution to ageing and rejuvenation.

Epigenetic regulation of inflammation during rejuvenation

Age-associated accumulation of senescent cells results in activation of innate immune activity with increased levels of pro-inflammatory cytokines, such as IL6 (Interleukin 6), tumor necrosis factor alfa (TNF-α), IL1 Interleukin 1, and IFN- (Interferon), a phenomenon that has been termed inflammaging. CR and heterochronic parabiosis reduce levels of pro-inflammatory cytokines, and recent studies have shown that ablation of senescent cells via genetic approach or senolytics reduces inflammation across tissues. Small lymphoid nodules are observed in the splenic white pulp of progeria mice after partial reprogramming. Despite the fact that inflammation is attenuated by CR, heterochronic parabiosis, partial reprogramming, ablation of senescent cells, and life-extending drugs, the epigenetic mechanisms underlying inflammaging remain elusive.

Gene-specific and age-dependent epigenetic changes control the expression of inflammaging-related genes, such as NF-κB, C/EBPβ, GATA-binding factor 4 (GATA4), C-X-C motif chemokine 10 (CXCL10), TNF-α, Krüppel-like factor 14 (KLF14), and cyclic GMP–AMP synthase (cGAS)/STING. cGAS is silenced through epigenetic mechanisms, which can be reversed by inhibitors of DNA methylation. More importantly, there is evidence that heterochromatin decay and inflammaging are intertwined by cytosolic DNA or RNA sensing pathways. As discussed earlier, loss of heterochromatin leads to the transcription and translocation of endogenous retroviruses, resulting in increased levels of cytosolic DNA and RNA, which stimulates the cGAS/STING or MAVS pathways, respectively. Both pathways trigger a powerful innate immune response

and elicit the production of type I interferons (IFN-I), pro-inflammatory cytokines associated with late senescence that contribute to the development of the senescence-associated secretory phenotype (SASP). Therefore, it is not surprising that the cGAS/STING cytosolic DNA sensing pathway is upregulated in HGPS and physiological aged cells and organisms. Recently, two groups demonstrated that cytoplasmic LINE-1 cDNA drives IFN expression in senescent cells and promotes age-associated inflammation in aged mice. These effects are antagonized by inhibitors of the LINE-1 reverse transcriptase. However, much remains to be learned about the biochemical mechanisms and physiological functions of how heterochromatin influence the cGAS-cGAMP-STING pathway and inflammation response. In addition, decreased levels of DICER1 or the accumulation of Alu-RNA triggers the release of mtDNA into the cytosol, which engages cGAS and drives noncanonical-inflammasome activation in a mouse model of age-related macular degeneration. Inflammaging is unequivocally considered a major hallmark of organism ageing, and reducing inflammation is critical for life-extending interventions to be effective. To date, however, it is still unkown how to modulate inflammaging by epigenetic manipulation. Therefore, monitoring 3D architectural changes to chromatin in rejuvenated cells after life-extending interventions will help reveal ways to inhibit chronic inflammation and combat ageing.

Conclusions and perspectives

The fact that rejuvenation strategies enumerated in this review can facilitate "epigenetic reprogramming" provides strong evidence that the epigenetic program, which is reset during development, can

also be experimentally shifted to a younger state at later periods of life. Simultaneous analysis of epigenetic changes in stem cells from a bi-directional perspective (namely both ageing and rejuvenation) will help to identify the epigenetic determinants of human healthspan and lifespan. These analyses will eventually help answer key questions, including "what are the convergent and distinct epigenetic regulations triggered by different therapeutic interventions?", and "can we mimic the effects of different types of rejuvenation interventions by directly targeting the epigenetic machinery?" More importantly, a deeper understanding of the epigenetic mechanisms will provide critical links between the regulation of gene expression and multiple cell biological processes (e.g., the maintenance of mitochondrial homeostasis, the repression of retrotransposable elements, and the amelioration of inflammation) to promote a young state in cells and organisms. Dissecting the dynamic interplay between these biological pathways and epigenetic parameters in adult stem cells, tissues, and organisms that have been subjected to rejuvenation strategies will deepen and broaden our understanding of how to make our body younger with minimal side effects.

To achieve this goal, we need cutting-edge stem cell isolation technologies, multi-omic analyses (e.g., transcriptomic and epigenomic), artificial intelligence and integrative approaches for analyzing these data to unraveling the complexities of ageing across cell types and tissues. Finally, CRISPR-based, genome-wide, gain- and loss-of-function screens can be used to identify functional genomic elements that are indispensable for cellular/organismal rejuvenation by life-extending interventions. In summary, scientists now have many new tools in hand to reveal the mechanisms of epigenetic regulation

of ageing and rejuvenation. Further studies focusing on the epigenetic mechanisms underlying these interventions will open new avenues to develop therapeutic strategies for delaying the onset of age-related diseases and for improving health and longevity.

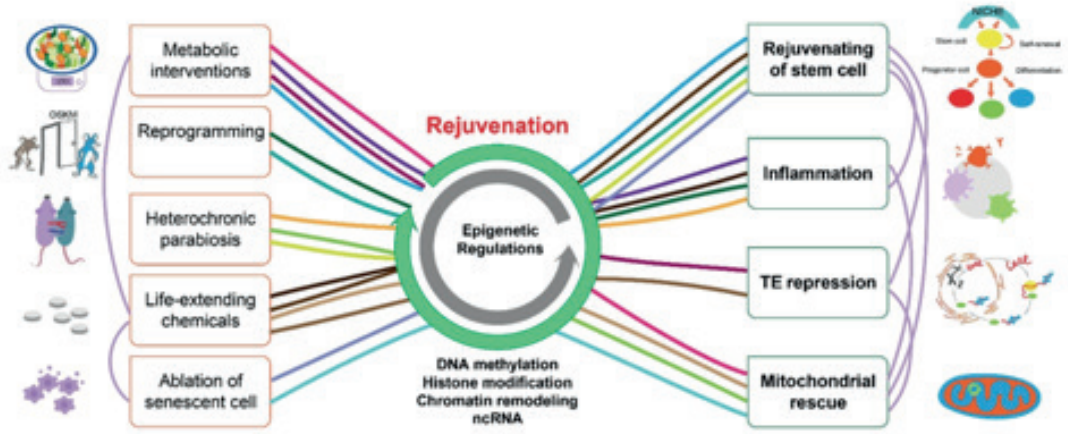

Figure 1. **Epigenetic regulation at the nexus of ageing rejuvenation.** Five life-extending strategies are discussed in this review, including metabolic manipulation, epigenetic reprogramming, heterochronic parabiosis, pharmaceutical administration, and ablation of senescent cells. The effects of these interventions convey on epigenetic reprogramming to gene expression and reprogram aged cells to a younger state. Stem cell rejuvenation is a common feature of all these rejuvenation interventions. In addition, epigenetic program interplays with important molecular pathways such as maintenance of mitochondrial homeostasis, suppression of retrotransposon elements, and amelioration of inflammation to counteract ageing.

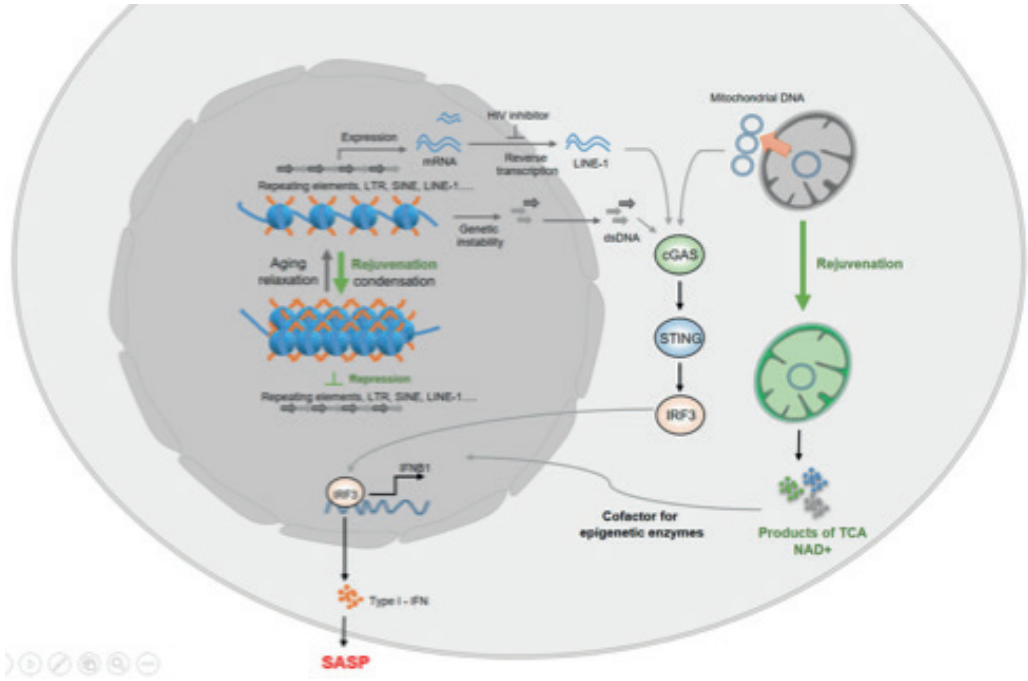

Figure 2. **The chain reaction of heterochromatin loss, retrotransposon activation, and inflammation are critical targets for rejuvenation.** In aged cells, heterochromatin loss leads to a decondensed state of chromatin, and derepression of the expression of retrotransposon elements in the human genome, including LTR, LINE-1, SINE-1, etc. Transcribed mRNA of retrotransposon elements is converted into cDNA by reverse transcriptase in the cytoplasm. Retrotransposon elements may jump into new sites of genome, therefore cause DNA damage and genomic instability, which increases dsDNA leakage. The excessive cDNAs are sensed by cGAS/sting pathway, which activates IRF3. The activated IRF3 translocate from cytoplasm to nucleus, and drives the gene expression of IFN family, a downstream mediator of the senescence-associated secretory phenotype (SASP), and eventually causes chronic inflammation and declined function in aged organisms. Rejuvenation strategies such as CR

can repress heterochromatin decay, and impedes the chain reaction from the epigenetic origin. In addition, administration of reverse transcriptase inhibitor prevents the activation of cGAS/sting DNA sensing pathway and SASP. Furthermore, CR maintains the health of mitochondria, and prevent the mtDNA divulge from damaged mitochondria. Renewed mitochondria release the proper amount of NAD+ and other products of TCA cycle, which functions as the co-factors for epigenetic enzymes that are important for rejuvenating epigenetic status of aged cells.

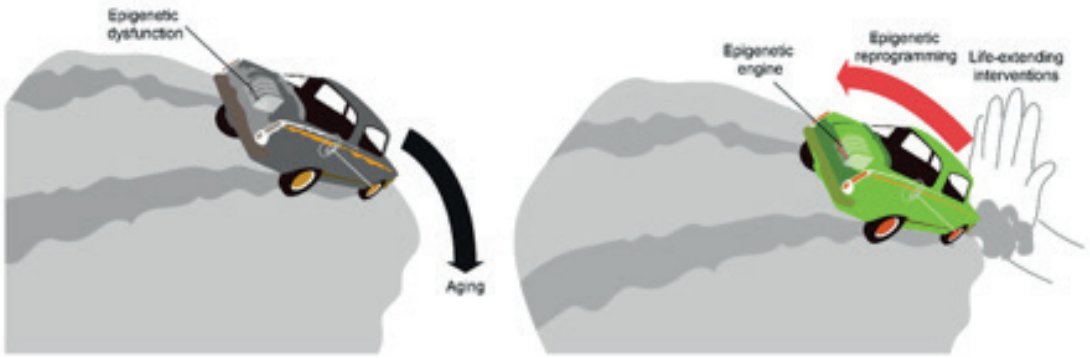

Figure 3. **A model depicting that epigenetic engine powered by various life-extending interventions.** Four wheels represents stem cell rejuvenation, repression of retrotransposon elements, amelioration of inflammation, and mitochondrial homeostasis, get power from the engine to overcome the inclination of age-related dysfunction.

Before finishing my intervention I would like to briefly reflect on the importance of fundamental basic science for humankind.

Basic science is risky and sometimes the value in what one is studying is not immediately apparent and some of the more basic science experiments I have indicated above are clear examples. Other example I would like to cite is a US federally funded project to study the mating habits and aging process of the screwworm. What is a screwworm you ask and why would anyone care what their mating habits are? I am certain most people cannot answer those questions and more relevant, do not care what the answer is. One might be concerned that the US government has frivolously thrown away hard-earned tax money from its citizens in supporting such a project. However, the screwworm turned out to be an agricultural pest and based on this research, its eradication was made possible by using sterile insects and the US cattle industry was saved approximately $20 billion.

Basic science research is not always straightforward and the results are uncertain. The lack of a defined end result may be seem daunting, as no one wants to waste years of their life, but there is also the glimmer and hope of being able to discover something that is truly life changing. I hope to be able to specially inspire the young people that I see in the room to not be discouraged if your research is not going exactly as you had envisioned. The route to discovery may have many twists and turns, but it is an individual's determination and perseverance that will see them to the end. After all, where would we be if Thomas Edison had given up on inventing the light bulb? When famously asked about how it felt to fail 1,000 times, he

replied “I didn’t fail 1,000 times. The light bulb was an invention with 1,000 steps.” In many ways this is what my professional career has been. I have been very lucky, as seemingly failures have eventually led to new concepts and discoveries.

The path to the next miracle cure or drug starts at the bench top and fingertips of the basic scientist. It is concerning that in today’s fast-paced, application driven society, our government officials, and our society in general, are too focused on the end therapies and treatments, that they forget about the wealth of knowledge that first needs to be uncovered before any therapy can be created and safely applied in a human. In fact, many times the knowledge gained from basic science has applications beyond human health and medicine, such as in agriculture, food industry, energy, or generation of household products, which make our everyday lives easier. Additionally, I am convinced that the basic science discoveries made in the last few years that allow for modifying our genome and epigenome will alter the very essence of humankind and will change human evolution at an accelerated pace never experienced before. Thus, I would like to take the opportunity that the University of Valencia (the temple of knowledge and the place where most fundamental basic research is carried out) gives me today, to ask our government authorities to reflect deeply on the importance that basic science and knowledge is having and will have for our lives, and prioritize and support basic fundamental science as one of the major endeavors that we humans can undertake.

I would like to finish my intervention by thanking all of you for your patience and attention. I consider todays' recognition, not a personal but rather a recognition to fundamental science in general, and to those scientists in particular that day after day dedicate their lives to increase our knowledge.

I want to reiterate my highest heartfelt gratitude, and proudness for the honor bestowed on me today from this loved and admired University of Valencia.

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