nature aging

Perspective

https://doi.org/10.1038/s43587-023-00527-6

The Information Theory of Aging

Received: 28 April 2022

Accepted: 2 October 2023

Published online: 15 December 2023

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Information storage and retrieval is essential for all life. In biology, information is primarily stored in two distinct ways: the genome, comprising nucleic acids, acts as a foundational blueprint and the epigenome, consisting of chemical modifications to DNA and histone proteins, regulates gene expression patterns and endows cells with specific identities and functions. Unlike the stable, digital nature of genetic information, epigenetic information is stored in a digital–analog format, susceptible to alterations induced by diverse environmental signals and cellular damage. The Information Theory of Aging (ITOA) states that the aging process is driven by the progressive loss of youthful epigenetic information, the retrieval of which via epigenetic reprogramming can improve the function of damaged and aged tissues by catalyzing age reversal.

Over the past three decades, the field of aging research has made substantial strides, reaching a stage where we now possess a basic understanding of the underlying mechanisms that drive the aging process. Knowledge has extended to include techniques for quantifying aging, decelerating its progression and, in some cases, even reversing aspects of aging. At least twelve hallmarks of aging have been identified, including a loss of stem cells, reduced mitochondrial function, impaired protein and energy homeostasis, telomere shortening and increased cellular senescence[1,2]. But what causes these changes to happen in the first place? Is there an upstream process that drives them? Based on new findings linking yeast aging to mammals, we attempt to answer these questions and present a unifying hypothesis.

At its essence, life is information. There are two main ways biological information is stored. One is in the form of nucleic acids, with RNA or DNA encoding 'digital' information as strings of nucleotides. The other is 'digital–analog' information, encoded by the epigenome, a complex system of transcriptional networks, RNAs, DNA loops, DNA-binding proteins and chromatin modifications, which, together, control gene expression[3], cellular identity, DNA repair and responses to the cellular environment[4] (see Box 1 for definitions).

A major problem with analog-based information storage systems, whether electronic or biological, is that they are inherently susceptible to noise, which can obscure the original message. Biological analog information can easily be lost over time, as it is read, copied and disrupted by damage to the cell[5]. In 1948, a fundamentally important mathematical solution to preventing information loss was elucidated by communications engineer and mathematician Claude Shannon. In the communication of information, Shannon stated that a signal is sent by a sender to a receiver, during which noise can obscure the original signal. To preserve information during copying or transmission, Shannon introduced an 'observer' who has access to what we, today, would call a 'backup copy'. This observer sees both what is sent and what is received, notes any errors that occurred in transmission, and sends correction data to the receiver to restore the message to its original and true form, similar to how the internet and TCP/IP work to ensure all the original data survive transmission.

Based on results pointing to a role of epigenetic information loss in the aging of yeast and mammalian cells, and the observation that epigenetic information recovery exhibits potent rejuvenation, we apply Shannon's concepts to biology and formulate the ITOA (Fig. 1a), a theoretical framework to explain the underlying causes of numerous aging hallmarks[6–9]. In this Perspective, we explore the concept of the ITOA, which posits that the aging process is propelled by the progressive loss of cellular information, primarily in the form of epigenetic information, resulting in the erosion of cellular identity[10,11]. This information can be restored via partial epigenetic reprogramming, a system that may have evolved early in life's history to repair and rebuild damaged organs and tissues. The ITOA is attractive because, unlike the 'somatic mutation theory of aging'[12], it explains why different individuals display similar aging changes, even though they start out with individually unique genomes and accumulate mutations essentially randomly. One of the more interesting implications of the ITOA is the potential existence of a repository

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BOX 1

Definitions

Nucleosome: 147 bp of DNA wrapped around a histone octamer, comprising two H2A–H2B dimers and one H3–H4 tetramer.

Chromatin: A nucleosome–protein complex that packages 3-m-long DNA into a nucleus of 1–5 µm.

Euchromatin: An open and transcriptionally active chromatin state.

Heterochromatin: A condensed and transcriptionally silent chromatin state.

Biological digital information: A set of discrete sequential values, which in biology is encoded by sequences of nucleotides.

Biological digital–analog information: A continuous range of values, which in biology is primarily encoded by epigenetic modifications, protein–DNA interactions and the three-dimensional structure of chromatin, they are based on the digital genetic information but influenced by environmental signals and cellular damage.

Epimutation: Heritable alterations to the epigenome, including DNA and histone modifications and chromatin structural changes.

Exdifferentiation: Loss of cell identity caused by the introduction of epigenetic noise leading to epigenetic drift that disrupts gene expression. Also known as dysdifferentiation.

Partial reprogramming: The use of reprogramming factors to partially reverse the age of cells without them becoming stem cells or otherwise losing cellular identity.

TAD: A structural unit of chromatin characterized by high interaction frequency within the domain and lower frequency across different domains, 50 kb–2 Mb in size.

Rejuvenation: The process of restoring an aged cell or organism to a youthful state, which involves reversing the effects of aging, damage or deterioration, often leading to improved physical function.

Yamanaka factors: Four nuclear transcription factors, OCT4, SOX2, KLF4 and MYC (OSKM), that can turn a somatic cell into an iPSC and are canonical reprogramming factors.

DNA methylation clock: A set of DNA CpG methylation sites whose methylation status can be used to predict chronological age or mortality risk.

of youthful epigenetic information within each cell that enables gene expression to be restored such that cells regain their cellular identity. Based on recent discoveries demonstrating epigenetic age reversal in mammalian tissues and the resulting increases in tissue function and lifespan, we discuss directions for the development of epigenetic rejuvenation therapies to treat injuries, age-related diseases and aging itself.

Epigenetic information loss: a cause of aging in eukaryotes?

The somatic mutation theory of aging states that aging is due to the accumulation of mutations that change the amino acid sequence of proteins and gene expression patterns[12]. In support of this theory is the correlation between lifespan and mutation rates of mammals[13]. Moreover, DNA repair defects are seen in some syndromes that mimic premature aging, such as Werner syndrome and ataxia telangiectasia[14], and the artificial introduction of high-dose DNA breaks triggers premature aging in mice[15] (Fig. 1b). These studies, however, do not distinguish whether the underlying causes are due to changes in the genome or the epigenome, as both can be triggered by DNA damage[16]. Although mutations do occur and can affect the aging process, there is growing evidence that epigenetic changes might be primary. As examples, yeast cells accumulate less than one mutation per lifespan but still age[17]; humans with increased mutation burden and cancer risk sometimes do not exhibit any features of premature aging[18]; similarly, in mice, changes to the epigenome caused by low-level non-mutagenic DNA breakage (which ostensibly does not alter the genome or cause genotoxic stress)[19,20] accelerate aging-like changes, including increased DNA methylation age, age-related transcriptional changes, physiological changes and diseases, all reminiscent of aging[10]; and in numerous mammalian species, epigenetic drift during aging is remarkably similar across many loci, enabling the accurate development of universal epigenetic clocks[21,22]. Moreover, in mammals, a lower rate of epigenetic drift correlates with a greater maximum lifespan[23]. Other reasons for suspecting an epigenetic cause of aging include the observations that identical mice and human twins can age at different rates and that mammals cloned from old somatic cells can live healthy, normal lives[24,25]. More recently, the observation that old cells and tissues can be epigenetically reprogrammed to a more youthful state to achieve lifespan extension without apparently reversing mutations[26,27] argues that much of aging has a nongenetic origin.

Formulated by Sinclair and Oberdoerffer in 2009, an early form of the ITOA was called the 'relocalization of chromatin modifiers (RCM) hypothesis', in which chromatin factors move away from genes to DNA break sites in response to DNA damage signal and fail to return[6], resulting in the progressive loss of youthful gene expression patterns, especially at hotspots including developmental genes and transposons[6,10]. This study revealed that DNA damage is a driver of epigenetic information loss in mammals and proposed this as a cause of mammalian aging.

Of the many types of DNA damage, one that is linked to aging more than all others is the DNA double-strand break (DSB). Because unrepaired DSBs are often lethal to the cell, the reaction to a DSB is swift and genome wide. It begins with a DNA damage signal that recruits the epigenetic regulators—including SIRT1, SIRT6 and HDAC1—to the DNA break site, where they facilitate the repair process by modifying chromatin and recruiting other DNA repair proteins such as RAD51 and NBS1 (ref. 6). Once DNA is repaired, these dual-function chromatin factors return to their original genomic locations to restore the previous pattern of gene expression. Over time, however, after cycles of damage, recruitment and return, not all the epigenetic regulators find their way back to their original loci, progressively altering the epigenome and changing gene expression. The ITOA posits that cellular responses to damage are a source of chromatin alterations and epigenetic dysregulation that make cells more susceptible to DNA damage, setting up a positive feedback loop that accelerates the gene expression changes that drive aging[10].

The initial idea that DSBs lead to the loss of epigenetic information stemmed from genetic studies on aging in budding yeast[28]. Research by Guarente and his team pinpointed 'silent information regulators' (*SIR1*–*SIR4*) as genes that control the mating type or gender of yeast cells. Not only that, but they also mend broken DNA and, when overexpressed, prolong the yeast's replicative lifespan. Of these, *SIR2* is the most conserved. It encodes an NAD^+-dependent histone deacetylase, which acts as a DSB repair factor, bolstering genome stability[29,30]. As cells get older, an abundance of DNA breaks, mostly at repetitive DNA loci such as the ribosomal DNA (rDNA), causes a protracted absence of Sir2 from silent mating-type loci[31]. This shift causes cells to express

https://doi.org/10.1038/s43587-023-00527-6

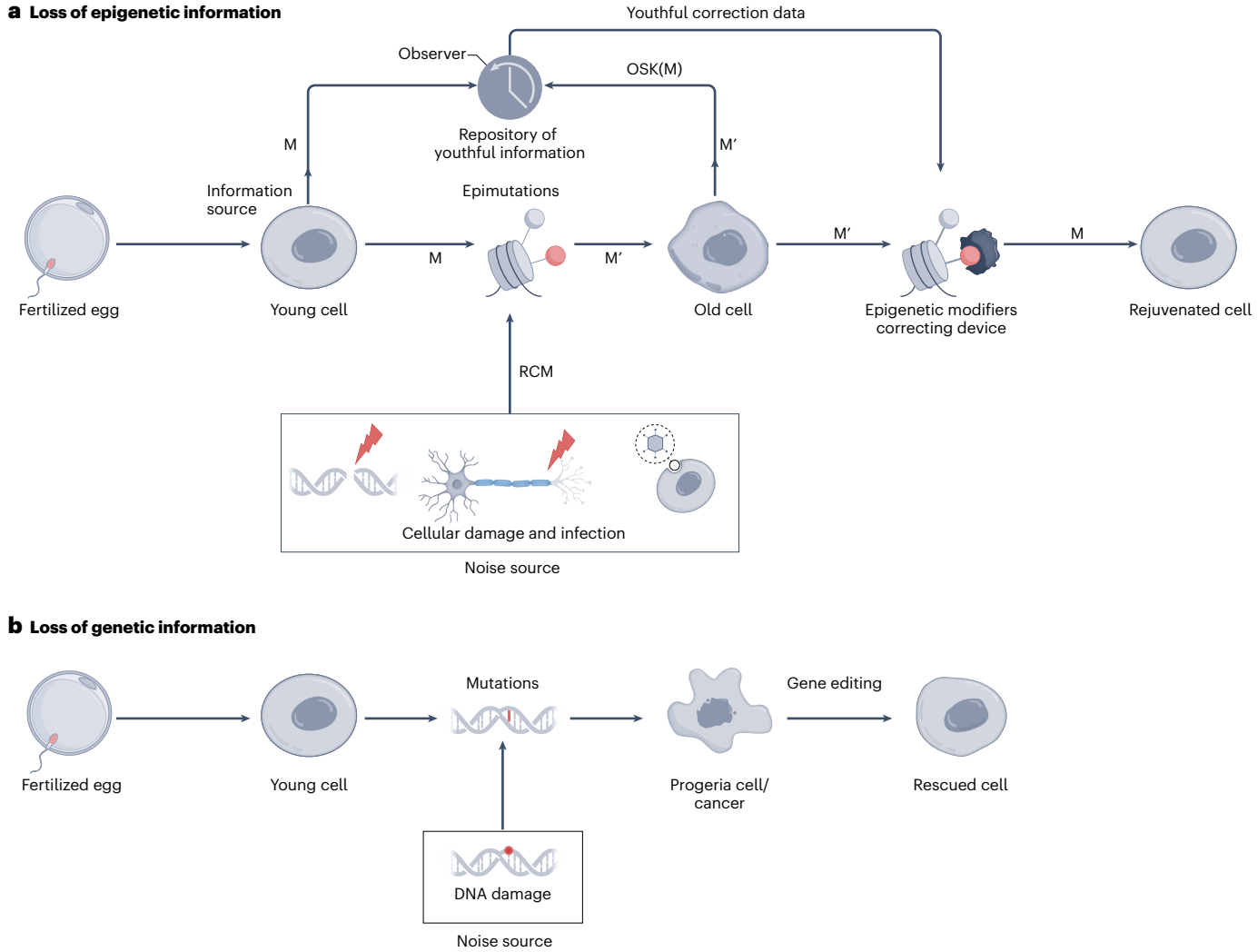


Fig. 1 | The ITOA. Biological information encoded in the genome and epigenome require different modes of transmission and preservation. A core concept of our theory is that the progressive loss of epigenetic information over time is a key driver of aging, and such information loss is reversible. **a**, Loss of epigenetic information during aging. Epigenetic information is laid down during embryonic development and is required for cells to maintain their identity and function. In contrast to mutations, which are caused by DNA damage, epimutations can arise from many types of cellular stress, including DNA DSB[10], viral infection[147] and physical or chemical damage[11,107]. These stresses induce the relocalization of chromatin modifying proteins (RCM)[6], alter histone and DNA modifications[10] and deregulate gene expression, particularly at developmental genes[6]. Fortunately, the loss of epigenetic information appears to be reversible by triggering an epigenetic reset system. Similar to Claude Shannon's 'observer', cells retain a 'backup copy' of youthful epigenetic information that can be accessed in adult tissues by expressing certain embryonic genes such as *Oct4*, *Sox2*, *Klf4* (OSK) and *Myc* (*Myc* is dispensable)[11]. These genes initiate a reset program that involves the activity of epigenetic modifiers to restore gene expression and function, essentially operating as Shannon's correcting device. The figure frame draws inspiration from Shannon's 1948 work, 'A Mathematical Theory of Communication'[5]. M, youthful message; M′, aged message. **b**, Loss of genetic information during aging. Genetic information is replicated during cell division, with minor errors arising from imperfect DNA polymerase activity and major errors stemming from DNA damage. The cell can restore original genetic information by duplicating the non-mutated copy of a locus, so long as the cell can identify which is the original (homologous recombination repair). Loss of genetic information could lead to progeria and cancer, and gene editing technologies have shown promise in repairing mutations and stopping disease progression[148,149].

both mating-type genes simultaneously, rendering them sterile, a hallmark of yeast aging[8,9]. This mechanism might have evolved to temporarily suspend a cell's mating capabilities while DNA damage is being addressed[6]. The rDNA also gives rise to extrachromosomal rDNA circles, which amplify and sequester the Sir2 enzyme as its abundance declines with age due to proteolysis[32]. Accordingly, an extra copy of *SIR2* (ref. 33), or the pulsed overexpression of *SIR2* (ref. 14), extends yeast lifespan by 30–82%.

Similar to yeast, mammalian Sir2 homologs, SIRT1, SIRT6 and SIRT7, move to sites of DNA damage to assist with DSB repair, causing the ectopic transcription of hundreds of genes, satellite repeat RNA and transposons that can increase inflammation[6,29,31] (Fig. 2). Because the epigenomic landscape is not completely reset each time chromatin modifiers leave their post, epigenetic noise is introduced, leading to a loss of cellular identity and cellular senescence[10,34,35]. As in yeast, the RCM process is thought to have evolved to coordinate gene expression with DNA repair[6].

The RCM concept is by no means limited to sirtuins. In recent years, other proteins have been implicated in this age-related loss of epigenomic information, including PARP-1, HDAC1, Wnt, the REST complex, the Polycomb repressive complex 2 (PRC2) and DNA methyltransferase (DNMT) 1 (refs. 36–39). Abundant evidence supports this idea, including observations that: (i) DSBs accelerate the DNA methylation clock[10]; (ii) increased expression of DNA repair genes is

https://doi.org/10.1038/s43587-023-00527-6

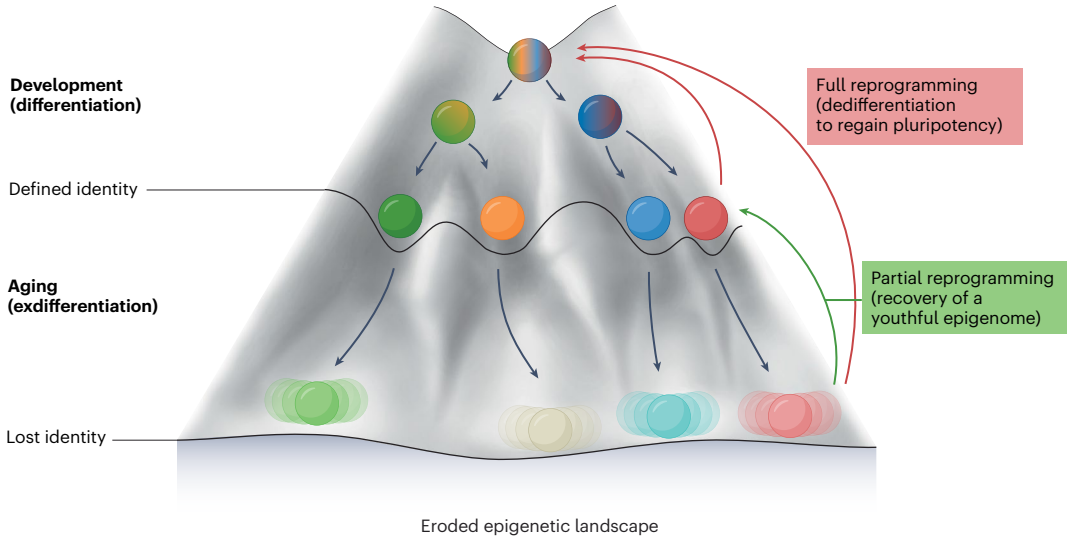


Fig. 2 | The epigenetic landscape of development, aging and rejuvenation. In the original Waddington landscape metaphor[150], valleys represent cell-type specificity, starting with a pluripotent cell at the highest point and ending at the lowest point when a differentiated state is reached. During development, a complex set of epigenetic changes, including DNA methylation and posttranslational histone modifications, dictates patterns of gene expression, providing cells with a defined cellular identity[151]. By extending this landscape forward to include post-developmental events, we can represent changes to cell-type specificity that occur during aging. Distinct from another theory suggesting aging is an intrinsic feature of the developmental program[103], the ITOA posits that DNA damage and various cellular insults lead to temporary alterations in the epigenetic landscape that induce a specific pattern of gene expression aimed at enhancing cell survival. These changes, however, are not fully reset after insults, leading to the landscape becoming eroded over time and cellular identities drifting away from their original state of differentiation[10,34], a process called exdifferentiation or dysdifferentiation[34,152]. For reasons that are unclear, developmental genes are particularly susceptible to deregulation. The resulting accumulation of epimutations reduces the function and resilience of cells and tissues[10], the rate of which negatively correlates with maximum lifespan in mammals[23]. Partial epigenetic reprogramming restores the epigenome to a younger state without erasing cell identity, perhaps due to robustness of certain epigenetic marks such as the methylation of cell-type-specific enhancers[144], thereby restoring lost functions, reversing age-related diseases and extending maximum lifespan[26].

correlated with an increased lifespan among species[40]; (iii) DSB repair efficiency correlates with longevity across different rodent species; (iv) SIRT6 is more active in the naked mole rat[41], a rodent species known for its highly stable epigenomic landscape and exceptional longevity[42]; and (v) the overexpression of *dSir2*/*Sirt1* and *Sirt6* extends lifespan in flies and mice[43–45].

The initial yeast studies led to the ITOA, which states that disturbances in the epigenome, termed 'epigenomic noise,' have a critical role in aging, affecting not just yeast but also multicellular organisms[7]. The theory posits that aging may stem from an evolutionary mechanism designed to balance both genetic and epigenetic reactions to cellular damage known as the 'survival circuit'[7]. Over time, this can disrupt gene expression networks and result in the loss of epigenetic information. The theory encompasses the idea that there are hotspots for gene dysregulation caused by DNA breaks and other threats to survival, especially developmental genes[7,10].

The ITOA is also consistent with antagonistic pleiotropy, an evolutionary aging theory proposed by George Williams[46], which states that an adaptive, beneficial process that enhances fitness and reproduction in young organisms is detrimental later in life. RCM is clearly an adaptive mechanism given the recruitment of the sirtuins to the DNA damage site requires DNA damage checkpoint signaling including γ-H2AX and Mec1 or ATM[9,47]. The recruitment of chromatin factors to DNA breaks may have evolved to keep young cells alive during adversity but, over time, disrupts the epigenome and drives aging.

Plasticity of the epigenome and aging

The ITOA, based on Claude Shannon's work, has a surprising corollary. If information loss is the cause of aging, is there a backup copy that can be used to reset the cell? In the animal kingdom, numerous examples provide evidence that aging is not only epigenetically driven but also reversible. Fertilization and the early stages of embryogenesis reset the biological age of the germ line for subsequent generations without correcting somatic mutations[48]. Cloning also shows that age can be reset: in 1958, John Gurdon and colleagues cloned adult frogs by transferring the nucleus of an adult frog cell into an enucleated egg[49], and these cloned frogs went on to live a normal lifespan. Gurdon's work was extended to larger animals, with Dolly the sheep being perhaps the best-known example[50]. Since then, dozens of cloned mammals have been generated and found to live a normal, healthy lifespan[24,25].

In 2006, Shinya Yamanaka and his research team identified four nuclear transcription factors, OCT4, SOX2, KLF4 and MYC (OSKM), capable of reprogramming somatic cells into induced pluripotent stem cells (iPSCs). These iPSCs are notable, not only because they can be coaxed into numerous cell types, but also because they have an epigenetic age of zero and display rejuvenated characteristics[51,52] (Fig. 2). Expression of the four Yamanaka factors plus two others, Nanog and LIN28, reprograms senescent and centenarian fibroblasts into iPSCs with the signature of young cells, characteristics that are retained even after they have been converted back to fibroblasts[53]. Similarly, the reprogramming of aged stem cells to a pluripotent state and back to somatic cells leads to functional rejuvenation[54], but not if done by direct lineage conversion[55]. These experiments collectively provided evidence that the epigenetic age of a cell has plasticity and can be reset, independent of mutations, and catalyzed research efforts to rejuvenate cells by epigenetic reprogramming without them losing cellular identity (Table 1).

Types of epigenetic information loss during aging

The epigenome, functioning as a digital–analog system, inherently possesses a relatively high degree of instability, exacerbated by environmental influences, such as the passage of time, nutrient availability and adverse conditions. The ITOA is fundamentally grounded in the notion of progressive loss of epigenetic information over time. Like the introduction of genetic noise in the form of mutations, there are multiple ways epigenetic noise can be introduced as epimutations that

Table 1 | Examples of epigenetic information loss being recovered by epigenetic reprogramming

Epigenetic factor	Epigenetic function	Change during aging	Intervention	Intervention outcome	Context	Experiment type
Histone level	Nucleosome assembly	Decrease	OSK	Increase	Fibroblasts isolated from old mice[11]	In vitro
Lamin B1	Lamina-associated domain formation	Decrease	OSK	Increase	Fibroblasts isolated from old mice[11]	In vitro
HP1γ	Heterochromatin maintenance	Decrease	OSKMLN	Increase	Fibroblasts and endothelial cells from aged humans[116]	In vitro
H3K9me3	Heterochromatin maintenance, transcriptional silencing	Decrease	OSK	Increase	Kidney of epigenetically aged mouse[10]	In vivo
			OSKM	Increase	Progeroid fibroblasts, kidney and spleen (mouse), high-passage fibroblasts (mouse, human)[105]	In vitro and in vivo
			OSKMLN	Increase	Fibroblasts and endothelial cells from aged humans[116]	In vitro
H4K20me3	Heterochromatin maintenance, transcriptional silencing	Increase	OSKM	Decrease	Progeroid fibroblasts, kidney and spleen (mouse)[105]	In vitro and in vivo
DNA methylation clock (and transcriptome)	Biological age	Increase	OSK	Decrease	RGCs of old mice[11], or of young mice after injury	In vivo
			OSKM	Decrease	Pancreas and liver[112], skin and kidney[111]	In vivo
			OSKMLN	Decrease	Fibroblasts and endothelial cells from aged humans[116]	In vitro

OSKMLN denotes OCT4, SOX2, KLF4, MYC, LIN28 and Nanog.

alter transcription factor binding, chromatin structure, RNA–DNA hybrids, histone modifications and DNA methylation.

Transcription factor dysregulation

Transcription factors that bind to specific DNA sequences (and their associated proteins and RNAs) establish cell identity during embryogenesis, locking in cell-type-specific transcriptional profiles during the early life of the organism. Over time, some of the most universal changes seen across species are modifications, proteolytic degradation and dysregulation of transcription factors. For example, the HOXA locus, comprising 13 transcription factors that control body polarity during development, is dysregulated during aging in mammals due to changes in histone acetylation, histone methylation and a loss of long-range enhancer–promoter interactions[10]. Changes to transcription factor binding efficiency are also seen during aging, as is the case for JUN and FOXO[56,57]. Recent large-scale transcriptome profiling in aged mouse tissues has revealed dysregulation of transcription factor regulatory networks in a tissue-specific manner[58].

Noncoding RNAs

Noncoding RNAs (ncRNAs) establish gene expression patterns during development. Long ncRNAs can activate or repress gene transcription by interacting with the enhancers or recruiting chromatin modifiers to their target sites to remodel the chromatin state[59]. Another major type of ncRNAs, known as microRNAs, predominantly suppresses gene expression by blocking protein translation or degrading mRNA targets[60]. Several aging-associated pathways, including DNA damage responses, IGF-1 signaling, sirtuin gene regulation, mTOR and mitochondrial signaling are controlled, in part, by ncRNAs[61], suggesting a causal role of ncRNAs in regulating the aging process. Indeed, certain microRNAs have been found to affect lifespan in *Caenorhabditis elegans*, *Drosophila* and mammals[61]. For example, overexpressing miR-17 (ref. 62) or miR-455-3p[63] extends lifespan in mice. ncRNAs can also form RNA–DNA hybrids, known as R-loops. They are generally seen as deleterious structures that promote mutations[64], but emerging data indicate they also regulate gene expression. In fission yeast, R-loops seem to be triggered by an age-dependent derepression of Sir2-mediated silencing[65]. In flies, R-loops are required for the maintenance of gene expression, neuronal function and vision during aging[66]. The role of R-loops in mammalian aging, however, is poorly understood.

Alterations to chromatin structure

Chromatin is organized into functional compartments within the nucleus to control gene expression patterns across different cell types, known as heterochromatin and euchromatin. These compartments are maintained, in part, by phase separation and the nuclear lamina[67]. In 1997, two papers, one by Villeponteau and one by Imai and Kitano, proposed that a loss of heterochromatin may underlie aging[68,69], a theory that has gained traction in recent years. In model organisms, including yeast[35], *C. elegans*[70], *Drosophila*[71,72] and mice[73], there is a global loss of heterochromatin during aging. Two of the most important regulators of heterochromatin are heterochromatin protein 1 (HP1) and trimethylated histone H3 Lys9 (H3K9me3), both of which decline during aging in multiple species[74], leading to inappropriate relaxing of chromatin and the ectopic expression of genes that confer other cell types. In mammals, an age-dependent loss of heterochromatin also relieves silencing at repetitive elements such as retrotransposons[73,75,76] and endogenous retroviral elements[77], triggering an inflammatory response. The loss of cell identity during aging may be due, in part, to the reduced expression of nuclear lamin B1 (ref. 78) and the accumulation of truncated lamin A[79], as well as compromised lamina-associated domains that are essential for stabilizing chromatin[80]. Other possible causes include DNA damage-induced movement of the chromatin-associated proteins SIRT1, SIRT6 and Polycomb repressive complexes[39] away from developmental genes and other RCM hotspots[10].

Changes to the epigenome during aging are not just at the gene level. Long-range enhancer–promoter interactions, facilitated by chromatin looping and insulated by topologically associating domains (TADs), change with age[81], as the distinction between silent and active compartments is progressively lost[82]. Why TADs and chromatin compartmentalization patterns change over time is unclear, but candidate

sources include DNA breaks, changes in acetylated histone H3 Lys27 (H3K27ac) patterns[10], and reduced levels of TAD boundary anchor proteins CTCF and cohesin[83,84]. Notably, chromatin changes during aging are not limited to heterochromatin, but can also occur in euchromatin, decreasing global chromatin accessibility and smoothening the epigenetic landscape[85].

Histone modifications and abundance

Histone-modifying enzymes decorate histone proteins with over 100 types of chemical groups[86]. The two most abundant modifications are histone methylation (me) and acetylation (ac). H3K9me3, H3K27me3 and H4K20me2 are associated with silent genes, and H3K4me3 and H4K16ac are associated with active genes. In yeast[87], worms[70], mouse quiescent stem cells[88] and replicatively senescent human cells[89], increased transcription across the genome occurs as a result of a decline in the abundance of histone proteins during aging. In parallel, the heterochromatin mark H3K9me3 and its corresponding methyltransferase, SUV39H1, also decline in abundance over time[74,79,90]. Levels of H3K27me3, H3K4me3, H3K36me3, H4K20me3, H3K56Ac and H4K16ac also undergo changes, the directions of which are tissue dependent[91,92]. Consistent with histone alterations driving the aging process, increasing the expression of histones extends yeast replicative lifespan[93], and changing the abundance of specific histone marks by manipulating the levels of histone 'writers' and 'erasers' extends lifespan in both yeast and worms[91,92,94].

DNA modifications

As a stable epigenetic mark, DNA methylation has a vital role in establishing epigenetic landscapes and defining cell identity during and after development. Recent studies have revealed that DNA methylation patterns change during aging in predictable ways. The most common DNA modification in mammals is 5-methylcytosine (5mC) at CpG dinucleotides. In mice and humans, there is a global decline in DNA 5mC[95] and an increase in DNA 5mC at a subset of CpGs, including Polycomb-group protein targets and bivalent promoters[96]. These bidirectional changes in methylation and demethylation during aging serve as the basis for using DNA methylation profiles as a biomarker of aging.

The use of DNA methylation to predict age was first achieved in specific cell and tissue types, including human saliva[97] and blood samples[98]. DNA methylation patterns were then identified as a universal biomarker of aging across different tissues within an individual, often referred to as 'Horvath clocks'[21]. Although clocks were originally based on chronological age, they can also serve as markers of biological age that predict health and future lifespan[99]. DNA methylation clocks have been developed for dozens of species including mice[100], dogs[101], naked mole rats[102], rats, bats, sheep and humans[22]. The fact that the same clocks can be used on diverse mammalian species[22] and that age reversal via epigenetic reprogramming requires active DNA methylation in mouse and human cells[11] indicates that epigenetic information loss at the level of DNA methylation may not simply be a marker of aging but a contributor to the aging process.

Epigenetic reprogramming to reverse age-related information loss

According to the ITOA, cellular reprogramming is a normal biological process that allows tissues to regenerate after injury, inflammation or aging. We have previously compared epigenetic rejuvenation by partial reprogramming to the polishing of scratched compact discs to access the digital information or the reinstallation of software to revive an old computer[7], a concept that has been adapted and expanded[103].

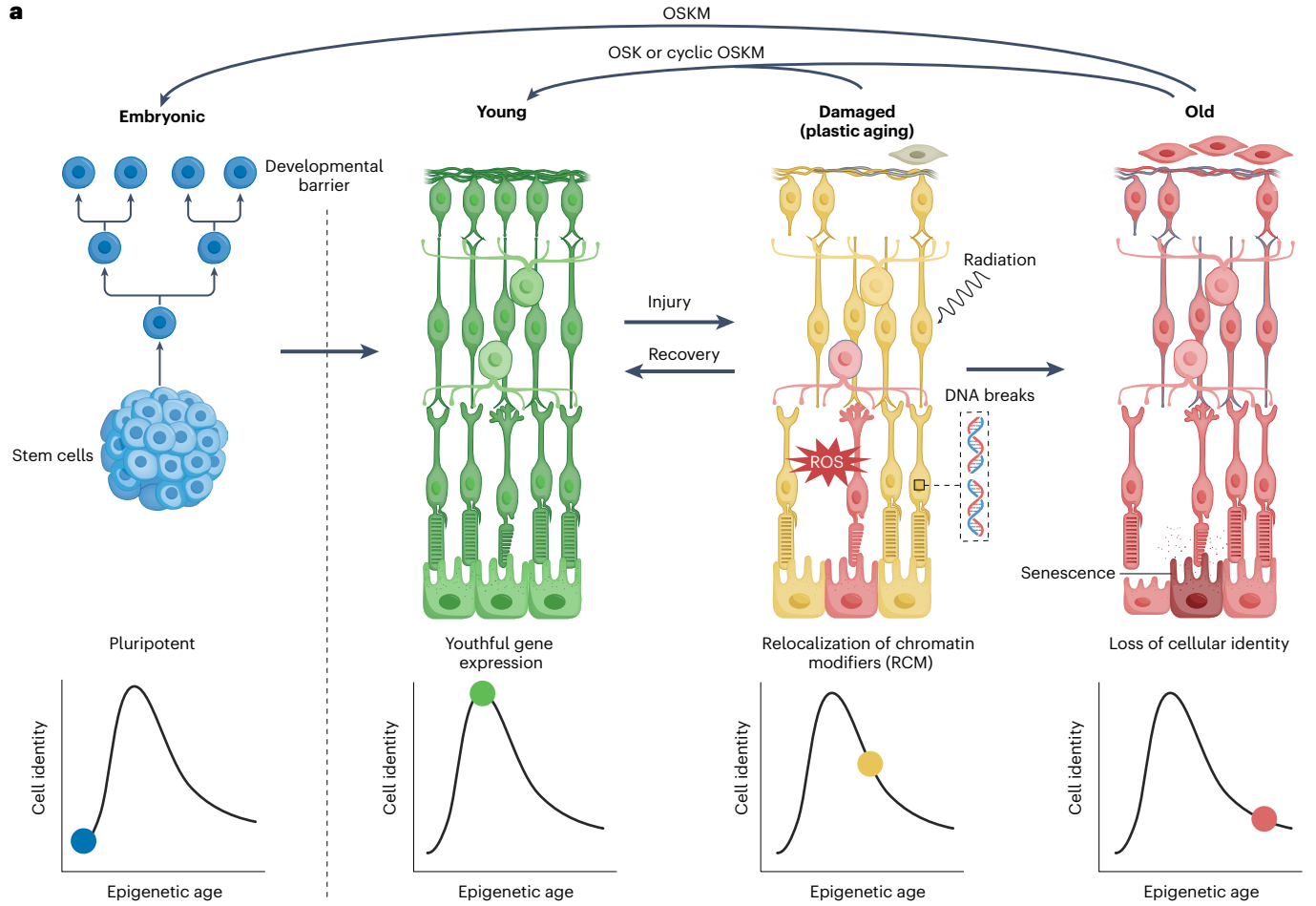
Although the Yamanaka reprogramming factors were first discovered in 2006, it was not obvious that they could be used to reverse aging in a safe manner. OSKM reprogramming of adult somatic cells into iPSCs allows for an aged epigenome to be reset to age zero[11,21], but this involves the complete resetting of the epigenome and the loss of cellular identity, leading to runaway cell growth and cancer. When reprogramming was first attempted in mice, the loss of cell identity resulted in teratomas and rapid death[104]. But by transiently expressing Yamanaka factors for a few days, or by turning on only a subset of them, typically OSK, it is possible to partially reset the epigenome and imbue tissues with youthful capacities without cell identity being lost (Table 1).

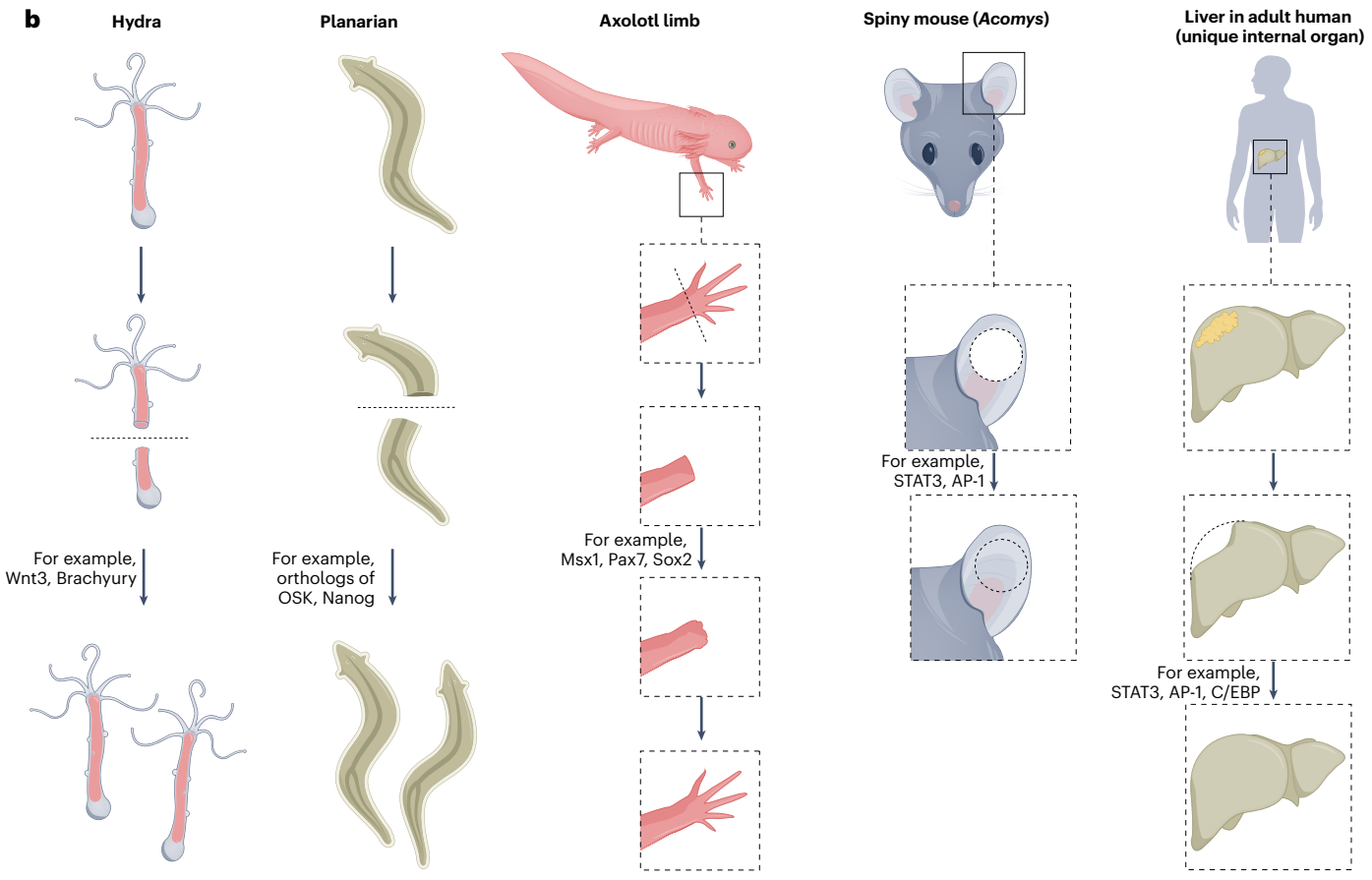
The first successful experiment to show rejuvenation by in vivo reprogramming was carried out by the Belmonte group in a strain of mouse carrying a loss-of-function mutation in the *Lmna* gene that modeled Hutchison–Gilford syndrome, a progeria[105]. When the genetically integrated OSKM cassette was induced for over a week, the mice either died, ostensibly owing to hepatic and intestinal failure[106], or, with a longer exposure, developed teratomas[104]. However, when OSKM was induced cyclically for only 2 days in a week, symptoms of the disease were alleviated in multiple organs and the mice lived 40% longer[105]. A later study showed that even when OSKM is only induced for two and half weeks early in life, the progeroid mice still live longer, albeit only 15%[27].

A parallel effort by our laboratory to understand whether lost epigenetic information could be recovered to restore tissue function in old cells was based on an inducible adeno-associated virus (AAV) system developed to express only three of the Yamanaka factors, OSK, excluding the *Myc* oncogene[11]. Overexpression of OSK in human neurons protected them from cell death in a DNA demethylase-dependent manner, and when expressed in old mouse fibroblasts, they restored youthful gene expression patterns (Table 1). Importantly, overexpression of

Fig. 3 | The rejuvenation of old and damaged cells via epigenetic reprogramming. a, Epigenetic reprogramming reverses age- and injury-related cell identity loss. The ITOA states that stressors such as cellular injury, infection and DNA breaks cause chromatin modifiers to relocalize and expedite the loss of epigenetic information, leading to age-related tissue dysfunction[10,11]. Aging progresses from pluripotent cells to young, functional tissues, to damaged plastic states, old non-plastic states, and eventually senescence (top). At the molecular level, epigenetic changes during aging contribute to an increase in epigenetic age and a loss of cell identity and function (lower). Physical injury to retinal neurons is also known to increase DNA methylation age and a loss of cellular identity leading to a loss of function (orange circle, line graph)[11]. Similar effects occur with exposure to chemotherapy[11], elevated pressure[153] or maybe even loud noises[154]. Recoverable injuries such as surgery and severe coronavirus disease 2019, temporarily accelerate DNA methylation age, but over time, aging effects become locked in (green circle, line graph)[147]. **b**, Epigenetic rejuvenation may mediate natural tissue and organ regeneration. The ITOA posits that epigenetic rejuvenation is a normal biological process that allows tissues to recover from injury or degeneration. Hydra and planarians can regenerate body parts and have an extremely slow or nonexistent pace of aging[155,156], zebrafish can regrow fins, heart and kidney throughout their lives[157], and axolotls, a species of salamander, can replace complex body parts such as limbs at any age[158]. Among mammals, mice can regrow toe tips[159], and African spiny mice can regenerate a variety of tissues[160,161] and their cells are protected from cellular senescence[162,163]. Even in humans, a resected human liver can regenerate to its original shape and size[164]. It is likely that certain cells of these regenerative species naturally retain the ability to rejuvenate by expressing pluripotency factors or somatic cells can turn on factors capable of inducing epigenetic rejuvenation, allowing them to remain epigenetically young, similar to human embryonic stem cells and iPSCs[48], whereas non-regenerative species have lost this ability and require ectopically expressing the pluripotency factors to initiate this process of rejuvenation and regeneration. In planarians, Oct4 targets are necessary for stem cell 'neoblasts' to regenerate body parts[165] and homologs of Oct4, Sox2, Klf4 and Nanog are expressed throughout regenerating tissue[118]. The transcription factor MSX1, which is highly expressed in regenerating limb blastemas of axolotls, can partially restore youthful gene expression in mouse myogenic cells[110], and STAT3, a transcription factor rapidly induced during liver regeneration, promotes a youthful epigenetic state in human chondrocytes partially through repressing DNMT3B[166].

https://doi.org/10.1038/s43587-023-00527-6





OSK systemically in mice via AAV9 for up to 18 months did not increase tumor incidence or cause negative effects on overall health. When expressed in old postmitotic retinal ganglion cells (RGCs), transcription and DNA methylation signatures were restored to a more youthful state, independent of cell proliferation, allowing the RGCs to regenerate axons and improve visual function in old and glaucomatous mice, an effect that was not seen with overexpression of one or two Yamanaka factors or the *Tet1* DNA demethylase alone[11]. Continuous OSK expression in the RGCs of glaucomatous mice provided year-long improved visual function without any obvious detrimental effects[107]. To our knowledge, this is currently the only in vivo epigenetic rejuvenation method capable of resetting both the transcriptome and DNA methylome to promote a long-term functional recovery, while avoiding runaway cellular proliferation, toxicity or risk of cancer, even when the genes are expressed continuously. Since our original findings in 2020, the same AAV-OSK system has been used in other disease models and species. For example, AAV-OSK has extended the remaining lifespan of 2-year-old mice by 109%[26]; it has also reduced vision loss in a mouse model of multiple sclerosis[108] and improved vision in a nonhuman primate model of non-arteritic anterior ischemic optic neuropathy[109].

Impressively, OSK and OSKM have been shown to restore youthful transcription profiles and promote the regeneration of multiple cell types and tissues. In aged adipocytes and mesenchymal stem cells, for example, single or dual factors have little to no rejuvenation effect, but combining three of four Yamanaka factors, including OSK, can restore the transcriptome to a more youthful pattern[110]. Organs that have now been rejuvenated by OSK(M) reprogramming include kidney[10,111], liver[112,113], skin[111], heart[114], brain[115] and pancreas and muscle[10,105,116,117]. A reversal of age-related changes to histone modifications is also seen[105]. In addition, the intramuscular injection of DNA plasmid carrying OSKM increases the regeneration of damaged muscle and reduced fibrosis without causing dysplasia or tumorigenesis[117]. Similarly, the introduction of OSKM mRNA into aged human fibroblasts and endothelial cells, plus two other stem cell factors, *LIN28* and *NANOG*, recovers levels of HP1γ and H3K9me3 and reverses the DNA methylation clock[116] (Table 1).

The question of why OSK(M) expression seemingly works universally to improve regeneration in multiple species and in different tissues with distinct gene expression patterns is an intriguing one. An aspect of the ITOA is that epigenetic rejuvenation is a natural, inherent biological process that exists to allow tissues to recover and regenerate after injury (Fig. 3a). Consistent with this, OCT4, SOX2, KLF4 and Nanog are involved in planarian body regeneration[118], regulate pro-longevity genes among 26 species[40] and are enriched in the blood mononuclear cells of centenarians[119]. The ability of a species to regenerate and rejuvenate probably depends on how advantageous it has been for the species' survival. Species with high rates of predation may benefit more than those that are less likely to be fatally injured (Fig. 3b). The ultimate size and shape of the rejuvenated tissue is probably dependent on an interplay between the OSK program, chemical gradients and bioelectrical signaling between cells[120]. Evidence that injury accelerates aging came unexpectedly from our studies of the mouse eye, where nerve crush altered DNA methylation patterns in a way that mirrored accelerated aging[11]. OSK induction counteracted this effect, providing a molecular explanation for how epigenetic reprogramming robustly improves tissue function in both aging and injury. Importantly, the DNA demethylases TET1 and TET2 were required for OSK to both regenerate neurons after injury and restore vision in aged mice, indicating that rewriting the DNA methylome is necessary for the epigenetic information recovery from both damaged and old states. Thus, preventing DNA hypermethylation during injury through inhibition of DNMTs may alleviate tissue damage and improve repair. For example, DNMT3a inhibition reactivates the regeneration potential of RGCs[121], and protects against noise-induced hearing loss[122]. Uncovering the mechanisms by which natural regeneration occurs, while testing the factors involved, may suggest novel rejuvenation interventions and lead to breakthroughs in medicines to safely treat injuries, diseases and aging itself (Fig. 3b).

The next frontier: secretory factor and chemical rejuvenation

Traditionally, epigenetic reprogramming factors, including OCT4, SOX2 and KLF4, are delivered to specific tissues via viral vectors. However, widespread rejuvenation across the entire body is limited by viral tropism. For example, most AAVs deliver their DNA cargo into the liver and much less into muscle, brain and testes. Thus, secretory factors and chemicals possess an inherent advantage because they can reach multiple tissues via the bloodstream and far more evenly.

The ability of parabiosis or young blood plasma transfusion to reduce DNA methylation age[123,124] suggests that there may be secretory factors or exosomes that can induce epigenetic rejuvenation. Some blood factors have been reported to slow or reverse specific aspects of aging in tissues, such as GDF15[125], eNAMPT[126], Klotho[127] and clusterin[128]. Although it remains unknown whether these factors function at least partly via an epigenetic mechanism, one recent study reported that SOX2 and MYC or OCT4 can be replaced by secreted and membrane-bound antibodies[129], serving as an example of how extracellular proteins might be used to rejuvenate tissues.

Using small molecules for reprogramming is also a promising strategy because of their ease of delivery, low cost and cell permeability. Chemical cocktails containing components that target epigenetic modulators, such as the HDAC inhibitor valproic acid, the LSD1 inhibitor tranylcypromine and the GSK-3β inhibitor CHIR-99021, can initiate a step-wise process converting mouse and human fibroblasts into iPSCs[130,131]. Although some toxicity exists in adult cells, it appears that short exposures to these chemical cocktails can partially restore age-related epigenetic changes without losing cell identity or causing the runaway cell growth seen with iPSCs[132,133] and can extend the lifespan of *C. elegans*[134]. Boosters that increase iPSC efficiency, including sodium butyrate and α-ketoglutarate, show an additive rejuvenation effect and reverse transcriptional age, while maintaining cell identity[132]. Interestingly, α-ketoglutarate is a TET co-substrate that extends the lifespan of worms and mice[135,136] and reverses the blood DNA methylation clock in humans[137], echoing the involvement of TETs during OSK-mediated rejuvenation. Other potential candidates for rejuvenating chemicals include trichostatin A, suberoylanilide hydroxamic acid[138], vitamin C (a histone demethylase KDM6B activator) and DNMT inhibitors 5-azazcytidine and RG108 (ref. 122).

The mechanisms of epigenetic rejuvenation

The ITOA posits that there is a backup copy of youthful information stored in every cell, akin to Shannon's 'observer'. This store of original epigenetic information may be accessed in aged or damaged adult cells to recover lost epigenetic information, promote resilience and healing and restore youthful functions (Fig. 1). Results from our laboratory and others indicate that this backup information may indeed exist. One of the most remarkable facts about partial epigenetic reprogramming is it is possible to safely reset gene expression patterns to years earlier[11,112,116], targeting not only the correct loci but also the direction and fold-change. In the case of old RGCs,for example, the induction of OSK restores 90% of the aging-altered genes back to youthful levels[11]. Two key questions remain to be answered: how this backup information is being accessed, and by what mechanism it is recorded and stored.

Clues to how it is accessed have come from epigenetic reprogramming studies. A process that is critical for both iPSC formation and somatic cell cloning is DNA demethylation[139,140], carried out by the DNA demethylases TET1–TET3 and the DNA glycosylase TDG[141]. Increasing evidence supports an important role for DNA demethylation in the rejuvenation process as well. For example, we find that OSK-mediated rejuvenation of postmitotic RGCs requires TET1, TET2 and TDG[11]. Similarly, restoring TET2 in the adult hippocampal neurogenic

https://doi.org/10.1038/s43587-023-00527-6

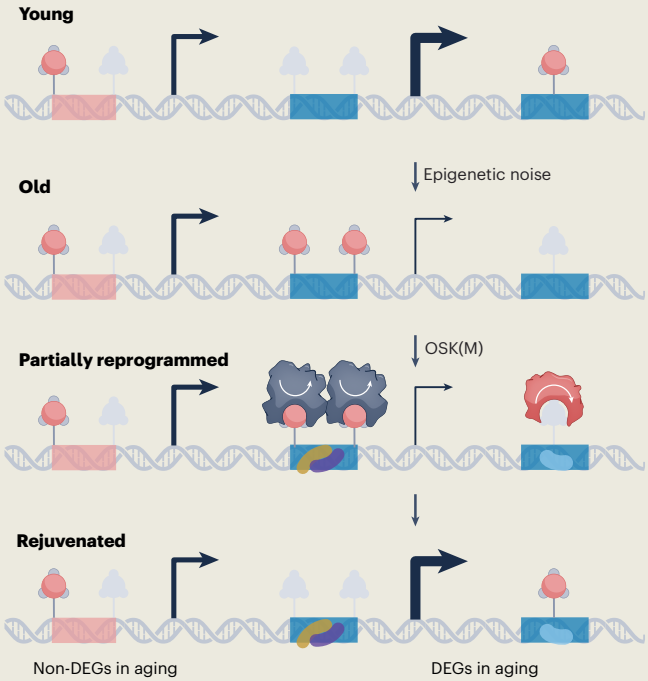
BOX 2

A hypothetical working model of the repository of youthful epigenetic information

Inspired by Shannon's 'information theory of communication' from the 1940s, we hypothesize there is a biological 'observer' storing youthful epigenetic information even in old cells[5]. One type of observer is passive, and the other is active (as illustrated). The passive observers mark DNA early and stay inactive during reprogramming, whereas the active observers mark DNA regions altered during aging and interact with master regulators and epigenetic modifiers during reprogramming.

Passive observers act as barriers during rejuvenation, marking specific regions as inaccessible to the reprogramming machinery. Possible forms of passive information storage include DNA modifications and DNA segments rich in CpG dinucleotides. Genes with CpG islands in their promoters typically have widespread expression and show consistent expression levels throughout aging (non-differentially expressed genes; non-DEGs)[167]. By contrast, developmental genes at shores of CpG islands[145] or lacking CpG islands[167] are more susceptible to disrupted heterochromatin formation during aging, leading to global dysregulation in aged cells and tissues (differentially expressed genes (DEGs) in aging; arrow width represents transcription frequency). QSER1, which interacts with TET1 and prevents de novo methylation at bivalent promoters, could also act as a passive observer safeguarding transcriptional and developmental networks[168]. We imagine that enhancers linked to cellular identity genes contain passive observers and stay hypomethylated, allowing old cells to regain their identity efficiently[144].

Active observers mark the youthful state of genes early in life. They may also mark genes that have changed their expression over time. Potential modalities of information storage include DNA–RNA hybrids such as R-loops, DNA modifications, protein–DNA interactions and histone modifications. H3K27me3, a product of the PRC2 complex, probably serves as part of an active observer system, being enriched at bivalent promoters of developmental genes[10,145] and methylation clock sites[11,21,169] that become dysregulated over time. H3K27me3 may facilitate rejuvenation by recruiting PRC2 and TETs to specific loci[11]. Supporting this, the CpG sites altered during aging and reset by OSK in RGCs possess an enrichment of PRC2 binding sites and H3K27me3 (ref. 11). In addition, PRC2 binding regions account for most age-dependent DNA methylation gain, making them age predictors[170]. We envision that master regulators OCT4, SOX2 and potentially PRC2 and TOP2A, bind to regions marked by active observers such as H3K27me3, H3K9me or DNA:RNA hybrids[66], recruiting epigenetic modifiers like TETs, TDG, DNMTs and KDM6B to reset DNA methylation and histone modifications.



	Lead candidates
Passive observer	CpG island, QSER1, RNAs
Active observer	H3K27me3, H3K9me3
Master regulator	OCT4, SOX2, PRC2 complex, TOP2a
Modifiers	TETs, TDG, DNMTs, KDM6B
Markers	5mC, histone modifications, RNAs

niche can counteract an age-related decline in neurogenesis and restore cognition in mice[142]. TDG also contributes to cellular identity reestablishment through its function at neuronal lineage-specific enhancers[143]. Additionally, DNA methylation by DNMTs can also have a role in rejuvenation. In the aged pancreas, for example, partial reprogramming re-methylates a similar number of CpGs as those that are demethylated[112], and in human fibroblasts, the promoter region of an embryonic development gene (*IRX5*) gets demethylated during aging and re-methylated by partial reprogramming[144].

The DNA methylation–demethylation machinery is believed to require master regulators to guide them to specific sites on the genome[11]. It seems likely that pioneer transcription factors OSK(M) activate other master regulators during the rejuvenation process (Box 2), including the embryonic regulator PRC2, which associates with RNAs and DNA sequences that are known to be differentially methylated during rejuvenation[11]. Another candidate is TOP2A, a crucial regulator of the epigenome that is highly induced by Yamanaka factors and necessary for TET1 upregulation and in vivo reprogramming of liver[113].

In our view, the most important question in the field is when and where youthful epigenetic information is recorded and stored, allowing in some cases for a reset decades later. Although the precise physical nature of the biological information back-up, or the 'observer', remains elusive, we hypothesize that the information storage mechanism may require passive observers that protect essential genes and the enhancer regions of cell identity genes, alongside active observers that record youthful epigenetic status and mark regions experiencing epigenetic alterations during aging (Box 2). In one model, only active observers engage with the rejuvenation machinery, composed of master regulators and epigenetic modifiers, to reset the epigenetic landscape and

transcription machinery. Potential forms of youthful information storage include DNA–RNA hybrids such as R-loops, DNA modifications, protein–DNA interactions and histone modifications. Although this initial model of the repository of youthful epigenetic information will be subject to refinement with emerging data, it offers a foundation for elucidating rejuvenation's biological mechanisms.

Conclusions and future directions

According to the ITOA, the progressive deterioration of organismal function culminating in mortality, the process we call 'aging', is primarily attributed to the gradual loss of information established during development.

There are a number of predictions the ITOA makes, the testing of which will help to support or refute the theory. An increasing number of studies indicate that dysregulation of developmental pathways and loss of cell identity are common occurrences in mammalian aging. In aging human brain tissue, for example, there is a general upregulation and alteration of CpG methylation near developmental genes[22,145]. As our mouse model with inducible epigenetic changes showed, developmental genes are hotspots for epigenetic changes during aging, including those caused by DSBs[10]. We suggested this might occur because developmental genes are activated as part of the RCM response when cells are damaged, as a way to temporarily increase cell repair and survival. This on-and-off cycling makes them more susceptible to epigenetic changes over time. A recent interpretation of these findings that aging is a programmed extension of development[103], a proposition that is in alignment with a recent analysis showing that developmental genes are hotspots for DNA methylation changes[22]. If so, then enhancing DNA DSB repair would be unlikely to affect the rate of epigenetic aging or lifespan. Yet, long-lived species have more efficient DSB repair[41] and overexpression of *Sirt6*, a DSB repair factor, makes mice live longer, arguing that DSB repair is a part of the normal aging process and aging is not simply an extension of development[43]. The ITOA predicts that reducing other types of cellular damage that alter the epigenome will also lead to lifespan extension.

Targeting the epigenome alone has demonstrated an impressive capacity to reverse various aging hallmarks, including genomic instability and epigenetic alterations[11,105], mitochondrial and lysosome dysfunction[116], inflammation[116] and deregulated nutrient sensing[112]. If the ITOA proves correct, in vivo epigenetic reprogramming might also be capable of reversing recently nominated aging hallmarks, such as dysbiosis and impaired macroautophagy[2].

The ITOA also predicts there is a structure or molecule within cells that retains a memory of an earlier state of the epigenome. Finding this backup copy, which we are calling the biological observer, will lend considerable support for the theory and greatly speed up development of ways to control biological age. Finding the putative observer could be achieved by genetic screening or by studying animals that can innately reverse aging signatures, such as flatworms and jellyfish. Unraveling the nature of the observer would not only address a longstanding question in biology, but also contribute to the development of more accurate and efficacious approaches for rejuvenating epigenomes and restoring youthful functions of tissues.

Reversing aging in a single organ can provide benefits for tissue-specific diseases but may not result in substantial increases in lifespan. It will be crucial to establish efficient delivery methods to introduce the necessary genetic material for in vivo cell reprogramming or identify chemical compounds or cocktails capable of achieving similar outcomes without causing cell dysfunction, death or cancer.

It is also important that research in the field develops more precise, reproducible and well-accepted methods for assessing aging and calculating biological age. Presently, there are several aging clocks available. None, however, offer a comprehensive assessment of the entire individual and many necessitate taking blood or biopsies, posing limitations to their widespread application in animal research and clinical trials. Better clocks will help determine the optimal timing for implementing reprogramming interventions and evaluating their effectiveness in patients. Although clock readouts are informative, ultimately rejuvenation should only be declared when the function of a cell, tissue or individual is restored.

Substantial strides in our ability to control aging have been achieved in recent years, and the discovery of alternative approaches to rejuvenate tissues will undoubtedly accelerate the use of in vivo reprogramming outside the laboratory and in human clinical trials[146]. Strategies such as functional genomic screening and comprehensive analysis of established rejuvenation methods like parabiosis, chemical reprogramming and tissue and limb regeneration, offer promising avenues for identifying novel reprogramming techniques. Combined with the ability to screen trillions of compounds and combinations in silico using artificial intelligence, these advances hold great promise for advancing our understanding of why and how we age, and the application of rejuvenation therapies to treat injuries, age-related diseases and ultimately aging itself.

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Acknowledgements

We thank E. Smith for figure illustrations and R. Rogers-Hammond and M. LaPlante for manuscript advice. Y.R.L. was supported by postdoctoral fellowships from Glenn/AFAR (by Michael Shen, MIT'13) and the Life Sciences Research Foundation (by Lei-Luo Life Science Fund). X.T. was supported by a NIH/NIA award K99AG068303. This work was also funded by NIH/NIA R01AG019719, Harvard Medical School Epigenetics Grants, gifts from M. Chambers, R. Rozenkrantz, T. Robbins, P. Diamandis, S. Aoki, D. and S. Hoff, and the Glenn Foundation for Medical Research to D.A.S.

Author contributions

Y.R.L., X.T. and D.A.S. wrote this Perspective.

Competing interests

Y.R.L., D.A.S. and X.T. are inventors on patent applications licensed to Life Biosciences, a company developing epigenetic reprogramming-based therapies, in which Y.R.L. and D.A.S. have equity. Complete details of all relationships for profit and not-for-profit for D.A.S. can be found in the Supplementary Information.

Additional information

Supplementary information The online version contains supplementary material available at https://doi.org/10.1038/s43587-023-00527-6.

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Peer review information *Nature Aging* thanks Vera Gorbunova and the other, anonymous, reviewer(s) for their contribution to the peer review of this work.