

The mechanism of ageing: primary role of transposable elements in genome disintegration

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Abstract Understanding the molecular basis of ageing remains a fundamental problem in biology. In multicellular organisms, while the soma undergoes a progressive deterioration over the lifespan, the germ line is essentially immortal as it interconnects the subsequent generations. Genomic instability in somatic cells increases with age, and accumulating evidence indicates that the disintegration of somatic genomes is accompanied by the mobilisation of transposable elements (TEs) that, when mobilised, can be mutagenic by disrupting coding or regulatory sequences. In contrast, TEs are effectively silenced in the germ line by the Piwi-piRNA system. Here, we propose that TE repression transmits the persistent proliferation capacity and the non-ageing phenotype (e.g., preservation of genomic integrity) of the germ line. The Piwi-piRNA pathway also operates in tumorous cells and in somatic cells of certain organisms, including hydras, which likewise exhibit immortality. However, in somatic cells lacking the Piwi-piRNA pathway, gradual chromatin decondensation increasingly allows the mobilisation of TEs as the organism ages. This can explain why the mortality rate rises exponentially throughout the adult life in most animal species, including humans.

Keywords Lifespan determination · Age-related diseases · Retroelements · Repetitive sequences · Chromatin relaxation · Non-coding small RNAs · Methylation · Cancer

Introduction

Understanding what actually causes ageing remains admittedly a fundamental and fascinating problem in biology [1]. Experimental data accumulated in the last three decades have led to the identification of various environmental and genetic factors, as well as chemical substances that influence lifespan in divergent eukaryotic species [1, 2]. Organisms normally age faster and hence live shorter under stress conditions that can lead to the generation of DNA mutations and, often as a consequence of mutations, damaged cytoplasmic constituents (including injured proteins, lipids, carbohydrates and organelles). Such types of damage can interfere with cellular functioning; thereby, they should be eliminated by effective repair and self-cleaning mechanisms to maintain cellular homeostasis. These mechanisms include DNA repair pathways, molecular chaperons, as well as the proteasome-ubiquitin system and lysosome-mediated autophagy, the main forms of cellular self-degradation [3]. This has led to the attractive model that the gradual, lifelong accumulation of unrepaired cellular damage drives the ageing process and determines the incidence of age-related fatal diseases [4, 5].

However, some observations contradict certain aspects of this damage-driven model of ageing. First, mutations should appear exponentially during the lifespan if they are to explain mortality patterns apparent at advanced ages (mortality rates often rise exponentially

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throughout adult life) [6]. Environmental and metabolic stress factors causing mutations, however, appear to be nearly constant during lifespan in any species examined. Thus, the causal role of mutations in the ageing process remains largely unclear [6]. Second, in the nematode *Caenorhabditis elegans* lowering mitochondrial respiration during adulthood only does not affect lifespan, even though it lowers the amount of cellular damage that are caused by reactive oxygen species generated during respiration [7]. Third, mice defective for *Pms2*, a DNA mismatch repair gene, display a 100-fold elevation in mutation frequency and a dramatically increased cancer rate, while not exhibiting an accelerated ageing phenotype [8]. One can argue that the early death stemming from the increased prevalence of cancer in *Pms2* mutant animals may be masking the signs of accelerated ageing [9]. However, *C. elegans* strains deficient in *msh-2*, another mismatch repair gene, have a lifespan identical to that of the wild type [10], and the soma of this organism is known to be devoid of cancer. It is also possible that many ageing phenotypes could be caused by genome rearrangements and not by point mutations [9]. Nevertheless, these examples show that the rate of ageing cannot be lowered by merely increasing the effectiveness of cellular repair mechanisms or diminishing the severity of factors responsible for damage. It may, therefore, be inferred that ageing is primarily caused by factors that are beyond the capabilities of cellular repair mechanisms.

Another attractive model of ageing is formulated by the “telomere shortening theory” [11]. The activity of the telomerase enzyme complex responsible for maintaining the structure of the chromosome ends (telomeres) at each round of cell division likewise affects lifespan in a number of model organisms [11, 12]. Still, the ageing process of postmitotic cells (like neurons) contradicts the theory. Furthermore, the somatic cells of adult *C. elegans* do not divide, meaning that the shortening of telomeric regions is not an issue even in the case of a complete absence of telomerase activity [13]. Regardless, the adult nematode ages and dies in about 2 weeks. Thus, the effect of telomere length on ageing appears to be rather complex.

Unlike the soma, the germ line shows no sign of ageing. What is it that renders the germ line potentially immortal? The question is relevant as its cells also display metabolism and respond to diverse molecular and environmental stress factors. And how can certain organisms (such as certain hydras and the jellyfish *Turritopsis nutricula*) be immortal, when the above features define their somatic cells as well?

Age-related differences in genomic integrity between somatic and germ line cells

In most eukaryotic organisms, the genome of somatic cells progressively accumulates mutations, mostly genomic rearrangements, as the individual ages [14–16]; whereas the integrity of genetic material in germ line cells remains largely stable (the latter could result from either selection against mutations—damaged embryos just die at early stages—or a decreased mutation rate [14–16]). This suggests that uncovering the causes of this difference between the stability of the genetic information of these two cell types may help to understand the mechanisms that are actually responsible for ageing. This view is supported by a recent discovery of ectopic expression of germ line-specific genes in the soma of long-lived insulin/IGF-1 signalling-defective nematodes [16]. The longer lifespan can thus be characterised by soma-to-germ line transformation of cell fate.

The eukaryotic genome is organised into a highly ordered chromatin structure composed of DNA and histone proteins. Different regions of the genome exist in either the relatively loose euchromatin or tightly packaged heterochromatin state. The former represents transcriptionally active domains, while the latter represents transcriptionally silent chromosomal regions. Histone proteins can be covalently modified at specific amino acid side chains to modify gene expression. Hallmark features of heterochromatin formation include, for example, histone hypoacetylation, H3K9 (histone protein H3 lysine-9) hypermethylation, and recruitment of heterochromatin protein 1 (HP1) [17]. In the soma, heterochromatin domains established early in embryogenesis are progressively broken down during ageing [18, 19]. This age-associated gradual loss of heterochromatin contributes to the derepression of silenced genes at the affected loci. Although heterochromatinization at specific loci can also occur, ageing is associated with an overall net decrease in heterochromatin state in somatic cells [18, 19]. Indeed, overexpression of HP1 that promotes chromatin condensation extends lifespan in the fruit fly *Drosophila melanogaster* [20]. Genetic data indicate that longevity response triggered by caloric restriction is tightly coupled to the modification of heterochromatic domains in the somatic genome [21]. Furthermore, DNA methylation at the 5 position of cytosine (5-methylcytosine) also leads to epigenetic repression of gene expression, and markedly decreases as the organism ages (with an overall decrease in methylcytosine content, but with local increases in some cytosine guanine dinucleotide—CpG—islands) [22]. This phenomenon cannot be observed in immortal cell lines

[23, 24]. Hence, another common molecular feature associated with ageing is the global hypomethylation of genomic DNA [25].

Loss of heterochromatin structure and hypomethylation allow for the activation and relocation of transposable elements (TEs) that constitute a significant part of eukaryotic genomes (>45 % of the human genome consist of TEs [26–28]. For example, a progressive decline in DNA methylation during ageing preferentially takes place in TE-derived repetitive elements dispersed throughout the genome [29]. Mobilisation of TEs can lead to genomic instability: if a TE jumps into a functional (coding or regulatory) region of the genome, the insertion often results in loss of function, thereby, facilitating the death of the affected cell. The mass occurrence of transposition events can lead to various degenerative processes [30].

TEs, their mobilisation and silencing

TEs can be divided into two main groups, DNA transposons and retrotransposons [31]. DNA transposons are DNA stretches that encode an enzyme called transposase. In a so-called cut-and-paste transposition mechanism, the transposase binds to the inverted repeat sequences found at the ends of the transposon, and slices the DNA at these sites. The sliced DNA fragment is then integrated into a new genomic locus by the transposase.

Retrotransposons can be subdivided into two subgroups, retrotransposons with long terminal repeats (LTR retrotransposons) and without LTR (non-LTR retrotransposons). LTR retrotransposons are widely distributed in eukaryotes, and make up nearly 8 % of the human genome [28]. Non-LTR retrotransposons are the most abundant TEs in mammals, represented by the long and short interspersed nuclear elements (LINEs and SINEs). Retrotransposons can mobilise themselves through an element-derived RNA intermediate, which is converted into a complementary DNA (cDNA) by reverse transcription, followed by integration into a new genomic locus, thereby generating a second copy of the TE (copy-and-paste mechanism).

Since TEs serve as potent mutagenic factors causing genomic instability, organisms have evolved diverse molecular mechanisms to protect their genomes against TE activity [32]. Defects in these mechanisms can cause sterility and accelerated ageing. One of the main mechanisms blocking the movement of TEs is RNA-induced posttranscriptional gene silencing, during which small RNA molecules—small interfering RNAs (siRNAs) and Piwi (P-element induced wimpy testis in *Drosophila*) protein-interacting RNAs (piRNAs)—mediate the degradation of TE transcripts (Fig. 1). In the siRNA pathway, a

TE transcript-derived double-stranded RNA (dsRNA) is cleaved into 20–22 nucleotide-long siRNA fragments by the enzyme Dicer, an endoribonuclease of the RNase III family. One strand of the siRNA is loaded onto the AGO2-RISC (Argonaute family protein 2—RNA-induced silencing complex) complex to mediate the degradation of TE transcripts with complementary sequence (Fig. 1a). The siRNA pathway operates in both soma and germ line. In the piRNA pathway, transcripts of piRNA-encoding genes clustered in the genome are processed by an endonuclease (Zucchini in *Drosophila*) into primary piRNA fragments (Fig. 1b). The primary piRNA is then loaded into the Aub (Aubergine) or Piwi protein to mediate the degradation of the TE transcript with a complementary sequence. Fragments of the degraded TE mRNA are loaded onto the AGO3 complex to mediate the generation of the secondary piRNA from a piRNA cluster transcript in a “ping-pong” amplification cycle [33]. Although the piRNA pathway operates predominantly in the germ line, only some specific somatic cells (e.g., stem cells and tumorous cells) have the capacity to express *piwi* genes and Piwi-interacting RNAs, suggesting somatic functions of the pathway in tissue regeneration and possibly cancer [34].

The piRNA pathways can also repress TE activity at the transcriptional level through RNA-mediated chromatin modification collectively called RNA-mediated epigenetic silencing. piRNAs not only promote degradation of TE mRNA, but also they can direct the recruitment of chromatin modifying factors to specific loci [35, 36].

Changes in chromatin structure and TE activity during ageing

Epigenetic changes influencing TE activity can be uniformly observed during ageing. For example, the murine intracisternal A-particle (IAP) retrotransposon has been found to become active upon ageing, and this change is associated with a progressive demethylation of the IAP promoter during the lifespan [37]. DNA methylation in humans also decreases as the individual ages [29]. A significant decline in average Alu (the most common SINE in primates) methylation over the lifespan was detected; age is thus negatively associated with methylation levels of Alu sequences [38]. Furthermore, whole-genome bisulfite sequencing in humans revealed that the centenarian DNA has significantly lower methylation content, as compared with the newborn DNA [39]. Together, these observations may reveal a general epigenetic process that could account for a series of events associated with ageing. Indeed, genome-wide methylation profiles were recently used to quantify individual ageing rates in humans [22].

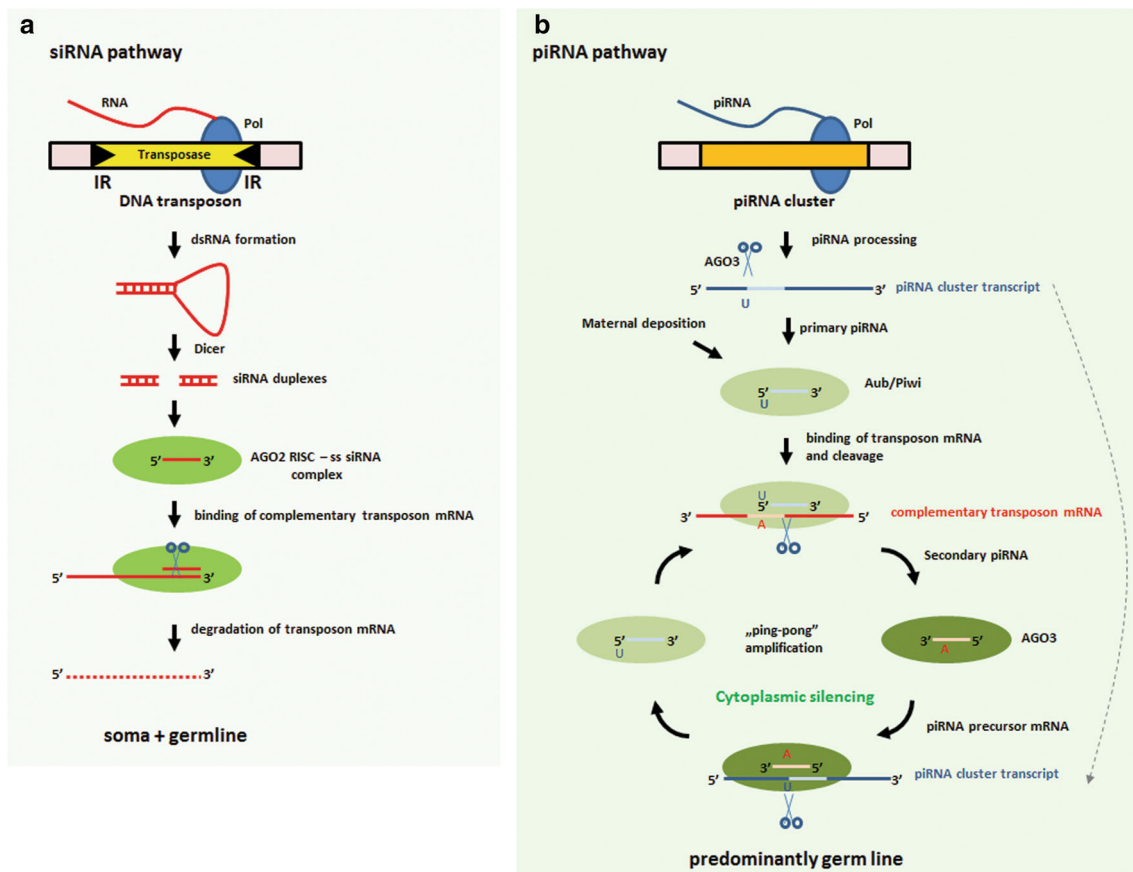


Fig. 1 RNA-induced posttranscriptional silencing of transposons in *Drosophila*. A transposon can be targeted by destroying the mRNAs, which it encodes, (posttranscriptional silencing) or by altering its chromatin structure (transcriptional silencing). Destroying of transposon transcripts can be achieved by the siRNA and piRNA pathways. **a** The siRNA pathway is triggered by double-stranded RNAs, which, in case of transposons, are derived from pairing of the inverted repeats (IR; *black triangles*) of a DNA transposon transcript, bidirectional transcription of a TE, or read-through transcription of two adjacent transposons present in inverted orientation. Transposon transcript-derived dsRNA then is cleaved into 20–22 nt-long small interfering RNAs (siRNAs) by the Dicer family of enzymes. Then, a single-stranded (ss) siRNA is generated that is incorporated into an Argonaute (AGO2) containing RNA-induced silencing complex (RISC), and directs the complex to destruct transposon mRNAs that are complementary to the siRNA sequence. **b** The piRNA pathway begins with a primary piRNA transcript generated from a piRNA

cluster that contains sequences of transposon origin. The primary piRNA is processed into 26–31 nt-long mature piRNAs (*light blue*) with uracil (U) at the 5' end. The piRNA fragment binds an Argonaute family protein, Aubergine/Piwi (Aub/Piwi), and then is directed to a transposon transcript that is complementary to its sequence. Endonucleolytic cleavage of the transposon mRNA [at 10 nt upstream of an adenine (A) whose position corresponds to the U site of the mature piRNA] produces a secondary sense transposon piRNA, which associates with the Argonaute 3 (AGO3) protein. This complex is targeted to another piRNA cluster transcript that is endonucleotically cleaved at a U that corresponds to the A site of the sense transposon piRNA (*the dashed, grey arrow*), thereby generating a secondary piRNA fragment. The latter binds again an Aub/piwi protein and is directed to a transposon transcript. This so-called “ping-pong” amplification cycle of piRNAs destructs eventually the transcripts of those transposons that have been inserted into the piRNA cluster

There are several examples establishing a relationship between TE activity and ageing in different eukaryotic organisms ranging from fungi to humans. In yeast, retrotransposition of Ty1 (an LTR-retrotransposon) is associated with genomic instability during chronological ageing; Ty1 mobility occurs at elevated rates in old populations [40]. Transposition in mitochondrial DNA also causes senescence in *Neurospora* [41]. Thus, a correlation between retrotransposition and genome instability exists during fungal ageing. Furthermore, transcriptional profiling

of ageing in *C. elegans* revealed that only less than 1 % of the genome shows significant changes in transcript levels as the organism ages [42]. Among these genes, expression of certain transposases markedly increases in older worms. In addition, somatic excision of Tc1, a DNA transposon in *C. elegans*, increases by more than 14-fold during the organism's lifespan [43]. In an inbred *D. melanogaster* line, a significant age dependence of the transposition rate of the retrotransposon *copia* was documented [44]. Consistent with these findings, reverse transcriptase inhibitors caused

retardation of ageing in this species [45]. In addition, it was recently found that several TEs were highly active in the *Drosophila* brain during normal ageing, suggesting that TE activation with age contributes to neuronal decline [46]. In humans, somatic expression of LINE1 elements has also been widely detected, and associated with the induction of DNA damage [47]. In the adult human brain, somatic retrotransposition reshapes the genetic circuitry in an individual pattern, underlying various pathological processes at advanced ages [48]. Senescence-associated somatic expression of LINE1 elements has also been widely detected [49]. Moreover, repression of LINE1 by SIRT6 into transcriptionally inactive heterochromatin fails to occur during the course of ageing [50]. Together, these data show that genetic damage generated by TE mobilisation in somatic cells may be a characteristic feature of ageing. Thus, TEs may have an important, suicidal role in age-related genomic instability [51–53].

Somatic activity of the Piwi-piRNA pathway and its potential role in extreme longevity

The Piwi-piRNA pathway effectively silences TE activity in the germ line, contributing considerably to genome integrity and immortality of this tissue. Here, we hypothesise that somatic expression of the pathway is responsible for the absence of the ageing phenotype in certain organisms, particularly lower eukaryotes. A Cnidarian, the freshwater hydra *Hydra vulgaris*, exhibits an almost unlimited regeneration capacity and immortality [54]. Its adult body largely consists of populations of stem cells with an unlimited proliferating and self-renewal capacity. These types of hydrozoan somatic cells are characterised by the abundant expression of Piwi proteins and Piwi-interacting RNAs [55, 56], suggesting low levels of TE activity in their genome. Another Cnidarian, the jellyfish *T. nutricula*, is also regarded as an immortal organism, as the sexually mature adult is able to transform itself back into a post-larval polyp stage, and repeat this cycle indefinitely [57]. Somatic cells of the closely related hydrozoan *Podocoryne carnea*, which can also alternate between the mature medusa and post-larval polyp stages, accumulate Piwi-like proteins (Cniwi) in somatic cells during all developmental stages [58]. This organism is likewise capable of continuously renewing its tissues. Considering that these Cnidarians are subjected to the same external DNA damaging factors as all other eukaryotic organisms, and that their DNA repair systems are not known to exhibit extraordinary effectiveness, we suggest that the activity of the Piwi-piRNA system is the cause of the non-ageing phenotype of their soma. In addition, some planarians (flatworms) display an extraordinary ability to regenerate

lost body parts [59]. This phenomenon is used in asexual reproduction: the animal detaches its tail ends and each half regrows the lost parts by regeneration. During the process, adult stem cells divide and differentiate, and their activities are functionally associated with Piwi-like proteins [60]. As they have an apparently limitless regenerative capacity, planarians are considered to be effectively immortal. The amphibian *Mexican axolotl* also represents an animal species that lives unusually long (up to 25 years). In this organism, abundant activation of germ line-specific *Piwi* genes in the soma, especially in the wound epidermis, is required for injury-induced effective regeneration of limbs [61]. Thus, somatic activity of Piwi proteins and piRNAs is likely to contribute to increased longevity and regeneration capacity in these organisms.

TE activity and the mechanism of ageing

It has been widely accepted that ageing is associated with the progressive, lifelong accumulation of unrepaired cellular damage [1, 2]. Cells respond to molecular stress by activating various defence mechanisms and eventually by dying. Massive levels of cell loss then lead to tissue deterioration and, eventually, to organismal death [62].

The germ line, cancer cells and certain Cnidaria are considered to be effectively immortal. They are also subjected to ionising radiation, harmful factors generated by their metabolism, or high temperature and oxidative stress—yet they do not show any signs of ageing. These immortal systems all share the activity of the Piwi-piRNA system whose main function is to silence TEs. Thus, we suggest that ageing is primarily caused by transposition-associated genomic instability. Indeed, suppression of Alu by RNA interference in aged adult stem cells can reverse the senescent phenotype and reinstat the cells' self-renewing properties [63]. Other factors causing molecular damage do not influence the rate of ageing to a great extent, as the damage is either repaired, or the damaged cell is eliminated from the tissue through cell loss (Fig. 2).

Mutations induced by physical and chemical mutagens occur independently from TEs (biological mutagens) in both germ line and somatic cells, but the majority of these are repaired by effective molecular mechanisms, which include DNA repair pathways, molecular chaperones, autophagy and the ubiquitin–proteasome system (Fig. 2a–c). These cell repair mechanisms are likely to be equally effective in the soma and germ line, but they do operate with some imperfection. This imperfection, however, is inconsequential, as the damaged cells are eliminated at the population level (microevolution) [6]. The primary difference is that in the germ line, the TEs are largely silenced by the Piwi-piRNA pathway; while in the soma, TEs become

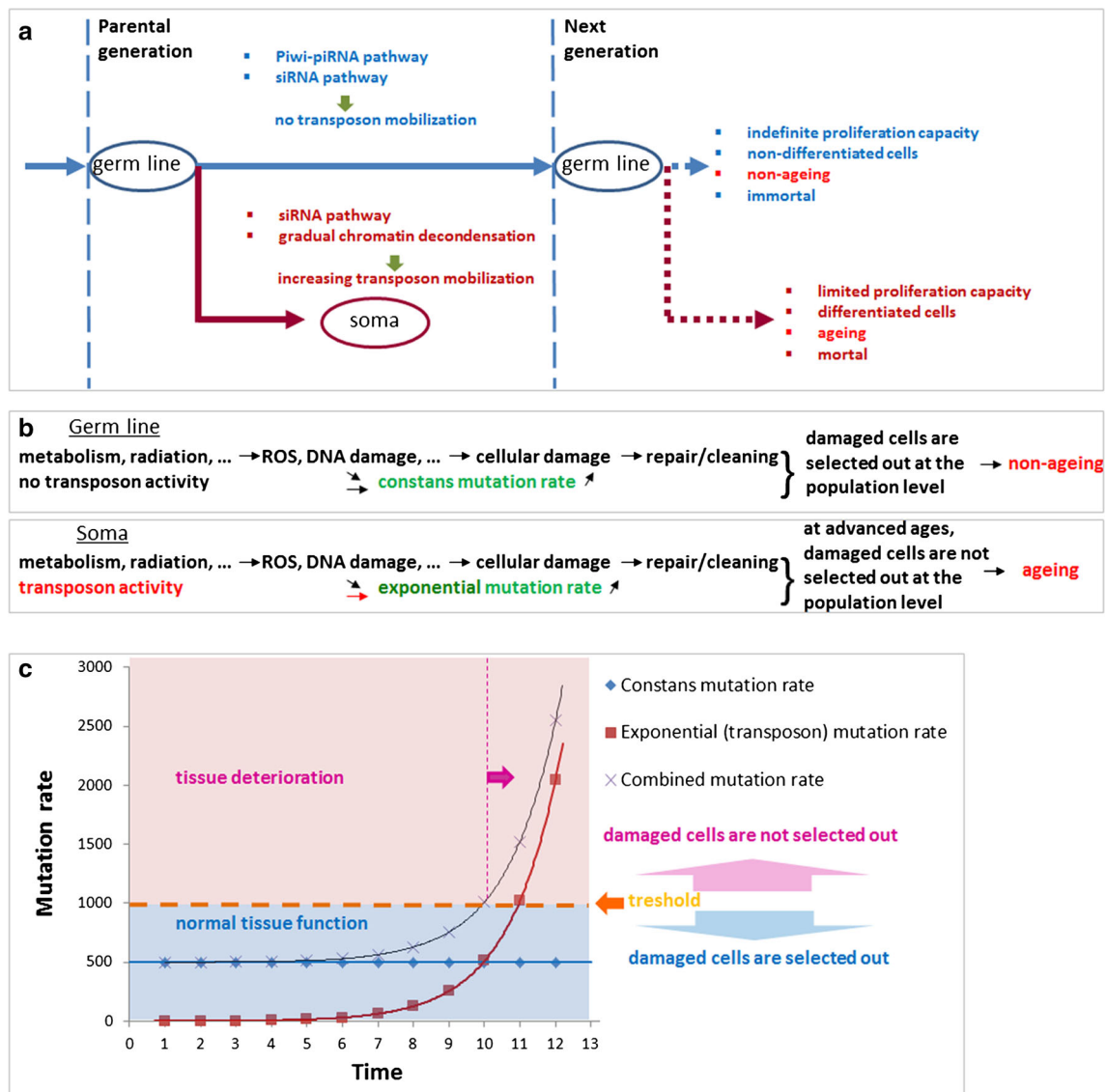


Fig. 2 Transposon activity primarily causes ageing in the soma. **a** In the germ line, the Piwi-piRNA system effectively silences transposons. Due to the lack of transposon mutagenesis, the germ line genome remains constantly stable. As a consequence, the germ line is essentially immortal. The Piwi-piRNA system does not operate in the soma (except from tumour and some stem cells). Gradual DNA demethylation and decrease in global heterochromatin state (chromatin decondensation) over the lifespan stimulate transposon mobilisation, leading to genome instability. As a result, the soma undergoes an age-related deterioration. **b** Both soma and germ line encounter various environmental and molecular stress factors (physical and chemical mutagens, ROS, high temperatures, etc.) that lead to the generation of cellular damage. Cellular damage can be removed by the repair (e.g., DNA repair pathways) and self-cleaning (e.g., autophagy) mechanisms. If damage is not eliminated, the affected cells are effectively selected out from the tissue (population) via microevolution until a given threshold. The remaining undamaged

cells maintain tissue function. Over the threshold, due to the progressive accumulation of transposon insertions, abundant cell loss compromises tissue function. In the germ line, transposons are silenced, so the mutation rate is constant in time. **c** Unrepaired mutations caused by chemical or physical agents arise in both soma and germ line at a nearly constant rate during the lifespan of a given organism. In the example, this translates to 500 unrepaired, damaged cells in a single unit of time (purple line). The “fitness” of the cell containing the unrepaired mutation diminishes, allowing undamaged cells to overgrow it (microevolution). The population’s selection capability (yellow dashed line) allows for 1000 damaged cells to be removed in a unit of time, meaning that 0–1000 damaged cells may be selected out (blue area) in that timeframe. However, mutations generated by replicative transposition occur with an exponential rate as the organism ages (green line). As a result, the number of unrepaired damaged cells increases at a rate surpassing the population’s selection rate, so the damaged cells cannot all be selected

progressively active during the lifespan. Replicative transposition is self-duplicating, enabling the number of retrotransposon-induced mutations to grow exponentially

in the somatic genome over time, and explaining why the mortality rate rises exponentially throughout adult life in most animal species (Fig. 2c). Since TEs constitute a

significant portion of eukaryotic genomes, the mutagenic effect of their activity may be considerable [46, 50, 63], thereby providing a plausible explanation for the difference in mutation rates between somatic and germ line genomes. Thus, TE activity may be the main factor in ageing. In the germ line, the mutation rate is constant due to the silenced TEs, in contrast to the exponential rate of TE-derived mutagenesis in the soma. As a result of the exponential mutation rate, damaged somatic cells cannot be eliminated after a while, since the rate of mutation surpasses the rate of elimination, a process that manifests in ageing (Fig. 2b, c). Note that factors modulating the accumulation of cellular damage generally affect transposition as well. For instance, caloric restriction leads to attenuation of metabolism (including respiration) and, through reduced transcription and cell proliferation rate, lowered transposition activity [64]. The constant mutation rate of the germ line does not exceed the elimination rate, allowing all damaged cells to be eliminated from the population through cell loss or early embryo death (Fig. 2c) (in *C. elegans*, for example, nearly half of the germ cells undergo apoptosis [65]). This is why mutant or damaged cells cannot accumulate in the germ line, allowing it to attain the quality of immortality.

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