Genomic integrity and the ageing brain

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Abstract | DNA damage is correlated with and may drive the ageing process. Neurons in the brain are postmitotic and are excluded from many forms of DNA repair; therefore, neurons are vulnerable to various neurodegenerative diseases. The challenges facing the field are to understand how and when neuronal DNA damage accumulates, how this loss of genomic integrity might serve as a 'time keeper' of nerve cell ageing and why this process manifests itself as different diseases in different individuals.

Senescence
A state of cell cycle arrest that can arise in proliferating cells after a finite number of cell divisions. Senescence can also occur prematurely in dividing cells as a result of stress or a detrimental environment.

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All creatures age and yet the biology underlying this deceptively simple concept is not completely understood. We can describe ageing at the molecular, cellular and organismal levels, but defining the root causes of the process has proven difficult. One cellular feature that is consistently implicated in the ageing process is the accumulation of unrepaired DNA damage and the accompanying loss of genomic integrity. This process makes a great deal of intuitive sense. Accumulated damage to any cellular constituent might contribute to the process of ageing[1], but macromolecules such as lipids, proteins and carbohydrates are present in multiple copies in every cell and thus can easily be repaired or replaced. By contrast, each cell receives only a single genome. Repair is always possible, but once a gene is beyond repair it cannot be replaced; thus, DNA damage can only accumulate with age. The consequences of this vulnerability are clear. The cell will increasingly make errors in the manufacture of both its RNA and protein products. Compounding these problems still further, the loss of 'fitness' produced by this age-associated DNA damage — although genetic in nature — cannot be selected against by evolution because traits that arise after an organism has completed reproduction are not subject to selection, and thus will be neither selectively retained nor eliminated in its descendants[1].

Faced with the importance of maintaining DNA integrity, it is not surprising that all cells contain an elaborate array of DNA damage response proteins[2–4]. Each cell is equipped with overlapping networks of independent DNA repair mechanisms. During neuronal development, the efficient repair of DNA damage is crucial for maintaining genomic integrity in developing progenitors. Repair is mediated by four major pathways: nucleotide excision repair (NER), base excision repair (BER), homologous recombination (HR) and non-homologous end joining (NHEJ). Most single-stranded lesions are repaired by NER, whereas small alterations in bases are targeted by BER. Both NER and BER enable an error-free DNA repair by excising the injury and filling the resulting gap by DNA synthesis using the intact complementary strand as a template. NER offers a more versatile pathway to repairing lesions such as photoproducts formed by ultraviolet irradiation, cyclobutane pyrimidine dimers and bulky chemical adducts[5]. For lesions located within actively transcribed regions, stalling of the transcription fork signals the downstream recruitment of NER proteins and initiates transcription-coupled NER[6]. For damage located in non-transcribed regions, distortion of the DNA helix is detected by alternative complexes — UV-damaged DNA-binding protein and the xeroderma pigmentosum group C-containing complexes — to subsequently initiate the global genome NER[5]. For DNA double-stranded breaks, repair is mediated primarily by HR and NHEJ. HR-mediated repair is dependent on an intact second copy of the sequence on the sister chromatid; therefore, it is the predominant mode of double-stranded break repair used during S and G2 phases of the cell cycle. By contrast, NHEJ simply seals the breach in the helix and is more error-prone than HR because there is no consulting the normal sequence on the other chromosome; small deletions are unavoidable. NHEJ operates mainly in G1 phase but sometimes in S phase[7]. Once neurons mature and become postmitotic, HR is no longer an option for double-stranded break repair, and thus neurons rely almost exclusively on NHEJ (for details of multiple DNA repair pathways in the nervous system, readers may refer to an excellent recent review by McKinnon[8]). If DNA damage exceeds a certain threshold, the cell can also engage fail-safe mechanisms that trigger senescence or cell death[9,10]. These overlapping damage control systems are particularly important in differentiated somatic

Synapsis
The pairing of replicated homologous chromosomes during prophase I of meiosis.

Crossing over
The reciprocal exchange of genetic material between non-sister chromatids during synapsis of meiosis I.

cells such as neurons, which are morphologically and functionally specialized and are permanently postmitotic. Neurons cannot rely on cell division to replace a lost or disabled neighbour nor are they able to enhance DNA repair through the use of HR, which occurs most commonly during the DNA replication process. In a long-lived species such as *Homo sapiens*, a typical CNS neuron must survive for 80 years or more without the ability to utilize this more accurate DNA repair process. It is small wonder, therefore, that late-onset degenerative diseases such as Alzheimer disease have been suggested to be partially caused by inadequate DNA repair[11–16].

Adequate DNA repair maintains neuronal health

Although there is an intuitive appeal to the concept that the failure of DNA repair is a driving force in the process of ageing, it is critical to distinguish this model from one in which the accumulation of DNA damage is merely a by-product of the ageing process — a consequence but not a cause. First, we must subtract the background by estimating the extent to which the developmental process establishes a baseline burden of DNA lesions that cannot be repaired.

Planned DNA damage and chromosomal imbalance during development. Despite the hazard to the genome, several normal developmental processes transiently introduce complete double-stranded breaks in the DNA helix. During meiosis, for example, the process of synapsis and crossing over involves highly precise SPO11-catalysed DNA double-stranded break and repair (for details, see REF. 17). Similarly, T and B cell V(D)J recombination — a site-specific recombination event — relies on double-stranded breaks followed by an inaccurate repair process to generate diversity in the antigen recognition machinery[18,19]. Neurobiologists have been particularly intrigued by this ingenious and economical strategy of creating diversity given the enormous range of phenotypes among the cells of the brain[20]. Several key proteins of the V(D)J system are indeed expressed in the adult CNS[18]. RAG1 transcripts are found in the hippocampal formation and other limbic regions that are important for spatial learning and memory. Moreover, RAG1-deficient mice exhibit impaired social recognition memory, thus emphasizing the functional importance of these transcripts[19]. DNA ligase-dependent recombination events are also found in hippocampal extracts and are implicated in the consolidation of memory[21]. However, despite a search aimed specifically at detecting site-directed V(D)J-like effects in the brain, such activity is yet to be found[22]. In addition to planned damage events that occur during normal development, such as V(D)J recombination and meiosis, there are also unplanned lesions, such as replication-associated DNA damage[23] at gene loci or telomere regions[24], or chromosomal segregation defects that occur in rapidly proliferating progenitor cells (FIG. 1). Unrepaired, these larger genomic errors can give rise to aneuploidy in the neuronal precursors[25] and contribute to significant stress to the genome. This genomic stress can be seen in the repeated findings that mutations in DNA repair enzymes, such as DNA ligase 4, are lethal; abnormalities throughout the fetus are observed and the fetus dies mid-gestation.

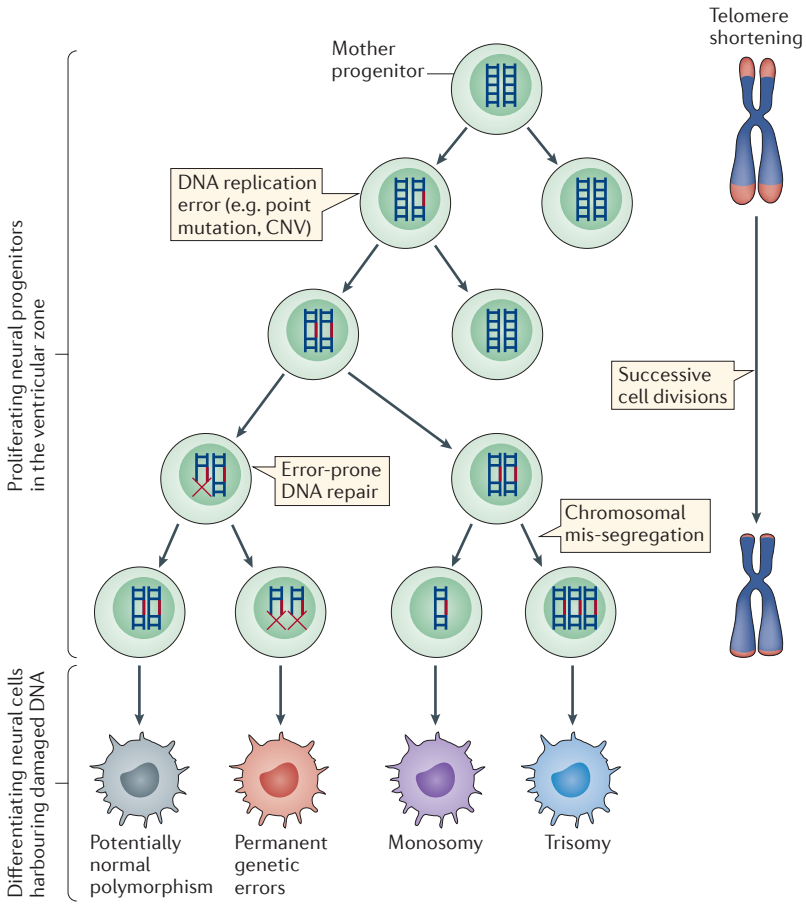

Figure 1 | **DNA breakage is a part of normal development.** This schematic presents the possible events during which DNA damage might occur in neurogenesis; the expansion of a typical neuronal lineage in the ventricular zone is shown. During the proliferation of neural progenitors, lesions (red portion of the DNA backbone) can be introduced during DNA replication and these can be propagated. Scanning by the repair machinery may detect other lesions, but if error-prone DNA repair processes are used, permanent mistakes (red X) are inserted into the genome and are retained. In addition to these microlesions, mis-segregation of chromosomes can also occur, leading to aneuploidy that cannot be corrected. One final form of DNA damage that might be expected during development would be progressive telomere shortening with increasing cell divisions, potentially leading to epigenetic modifications, both at telomeric and subtelomeric chromatin regions. CNV, copy number variation.

The state of the neuronal genome in adults

As paradoxical as it might sound, the integrity of the genome in the adult neuron is not certain and has become an object of intense scrutiny[26]. The absence of direct evidence for a specific V(D)J process rules out only one specific type of DNA arrangement. Other break and repair strategies are certainly possible and may be used by different cell types under different conditions. Although it is unclear what these strategies might be, there are features of the adult neuronal genome that suggest that alternative break and repair strategies are both present and regularly used. Multiple lines of evidence point to variations in chromosome

V(D)J recombination
Also known as somatic recombination, this process occurs in B and T lymphocytes that are generated during early development via somatic assembly of component gene segments. V(D)J recombination enables diversity in the antigen recognition machinery.

Aneuploidy
The presence of an abnormal number of chromosomes in a cell.

Microaneuploidies
Genomic alterations that result in unbalanced copy numbers of subchromosomal regions.

Copy number variation
Refers to when the number of copies of a particular gene varies from one individual to the next.

Chromosomal mosaicism
Refers to when an individual has two or more cell populations with a different chromosomal makeup.

DNA supercoiling
Refers to the over- or under-winding of a DNA strand.

number throughout the neurons of the brain. This variation begins in development when aneuploid neuroblasts are estimated to account for as much as 33% of the total population that eventually gives rise to the cerebral cortex[27]. Although the majority of these aneuploid progenitors may eventually be eliminated by a process of developmental cell death, postmitotic neurons with abnormal numbers of chromosomes can be identified in the normal mature brain[27–35]; their numbers may differ with respect to the species and brain region analysed, the technique used and the chromosome queried[32,36–38]. In the mouse brain, locus-specific fluorescence *in situ* hybridization (FISH) data suggest that ~1–6% of the cells show gain or loss of sex chromosomes[27,32]. In humans, FISH, immunocytochemistry and cell sorting assays have led to similar estimates of ~4% aneuploidy with respect to chromosome 21 (REF. 25), although a more recent study suggested a 13% rate of aneuploidy in chromosome 21 in a normal adult brain[39]. Higher estimates (6–19%) have also been reported based on an analysis of chromosome 17 in the entorhinal cortex[32]. Although some of the increased incidence of aneuploidy in the ageing brain is no doubt in non-neuronal cells[40], these numbers still demonstrate that the adult brain contains and tolerates a small but significant percentage of neurons with chromosomal aneuploidy, and a larger number of cells with microaneuploidies. If the search for aneuploidy is reduced to a subchromosomal scale, thus including copy number variation, the estimates of imbalance reach as high as 13–41%[26]. This percentage is the background produced by errors in DNA replication in the neural precursors during the developmental process itself. We would predict that most such lesions would be propagated to the entire lineage and lead to a wide range of chromosomal mosaicism effects (FIG. 1).

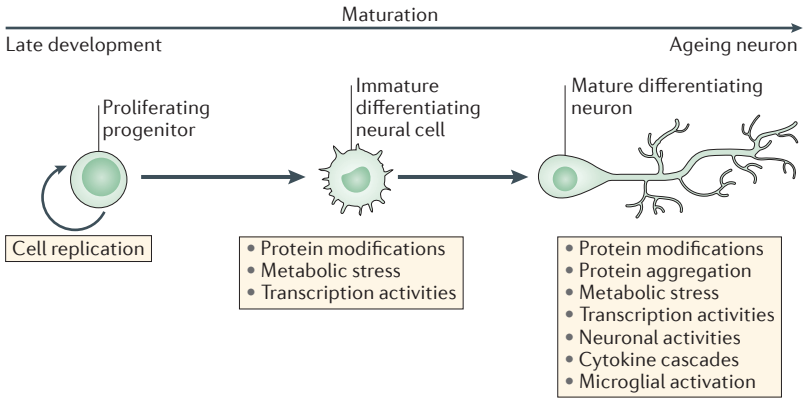

Figure 2 | **Causes of DNA damage in the developing, mature and ageing nervous system.** During early development, active replication of proliferating progenitors is the main cause of DNA lesions by the mechanisms outlined in FIG. 1. However, even after neurogenesis is completed, other sources of DNA damage can take their toll on the genome. As immature neural cells begin to express different genes specific to their adult fate, it is likely that stresses introduced by active metabolism and different protein modifications contribute additional DNA damage. Finally, during ageing, a mature differentiated neuron is continuously subjected to the genomic stress of different protein modifications, oxidation, metabolic stress, transcriptional and neuronal activities. These stresses are often brought on by extrinsic influences such as inflammatory cytokines and microglial activation.

Reduction of DNA integrity in ageing neurons. The DNA of brain cells is constantly subjected to various types of damage. Much of this damage is random and is attributable to stressors such as radiation or reactive oxygen species; however, other processes also contribute (FIG. 2). For example, the high transcription rates of a neuron would put its genome in jeopardy because of the generation of DNA damage through topoisomerase I cleavage complexes[41]. These complexes are topoisomerase I-linked DNA single-stranded breaks formed endogenously during active transcription when DNA supercoiling is removed by topoisomerase I; normally, the efficient turnover of topoisomerase I is facilitated by ataxia telangiectasia mutated (ATM) kinase-independent activities[41]. Another example of DNA damage in neurons is the recent suggestion that simple changes in neuronal activity can also produce lesions in the genome. Suberbielle *et al.*[42] demonstrated that double-stranded breaks can result from little more than the enhanced brain activity seen during the exploration of an enriched environment in 4–7 month-old wild-type mice. This seemingly innocuous behaviour caused widespread but transient increases in neuronal double-stranded breaks followed by efficient repair. The extent of the DNA damage was directly related to level of activity, and, underscoring the importance of the repair process, the authors also observed a similar level of damage in a mouse model of Alzheimer disease, but the repair process was slower[42].

Another example of non-random DNA damage involves the telomeres. The Hayflick effect — an upper limit on the number of possible cell divisions — is often attributed to the progressive shortening of telomeres that occurs with each cell division. The telomeres of neurons, however, should remain stable because these cells no longer divide[43]. However, perturbed telomere function is linked to human neurological disorders[44–46] perhaps because of genomic, but non-telomeric, functions of telomerase and other telomere-associated proteins in regulating cell survival[47]. One such example is the change in gene expression caused by spreading telomere heterochromatin[48]. In budding yeast, telomeres are maintained by the constitutive expression of telomerase, but the long distance looping of telomeres was found to repress genes up to 20 kb from the end[49,50]. In humans, the expression of several genes located at three different subtelomeric ends (1p, 6p and 12p) are affected by this process, including interferon-stimulated ISG15, desmoplakin and complement C1s subcomponent[48]. It is also possible that telomere shorting could occur by other means, thus influencing the rate of ageing. DNA damage response proteins such as ATM[51], the Werner helicase and Nijmegen breakage syndrome protein 1 (NBS1; also known as nibrin)[52] are associated with healthy telomeres where they collaborate with telomerase to repair DNA or remodel the chromatin structure in response to stress[53–55]. Telomere dysfunction is also reported in leukocytes from patients with age-related neurodegenerative disorders, including Alzheimer disease[56,57]; although this observation is at most an indirect effect, the linkage is intriguing. Viewed together, it appears that the maintenance of telomere integrity, including a functional telomerase enzyme is indispensable for normal

neuronal health. Therefore, despite the lack of cell division in adult neurons, the slow loss of telomeric integrity may account for some features of brain ageing.

Finally, selective repair represents an additional source of non-random DNA damage. Because neurons are unable to divide during the lifetime of the organism, neurons have no option but to repair double-stranded break through NHEJ rather than the more accurate HR pathway. Faced with this problem, it appears that post-mitotic adult neurons have adopted a selective approach: repair at the level of the whole genome is reduced while genes that remain transcriptionally active are repaired more effectively[58]. This approach has profound implications for the process of ageing. The transcriptional pattern of any two neurons is unlikely to be identical; therefore, the poorly repaired genomic regions probably vary from cell to cell. Furthermore, if active neurons break and repair their DNA more frequently than their 'quiet' neighbours[42], broad differences in accumulated damage will become increasingly apparent with time. If this damage is not random but is instead related to each cell's individual pattern of transcriptional activity (see above), then, as the brain ages, different neurons in different regions would acquire different patterns of unmended 'scars' in their genome. Potentially, therefore, the genome of each brain cell is unique and results both from its genetic inheritance, its developmental history and the epigenetic scars that result from natural variations in its electrical and biochemical activity. When exposed to a specific disease chemistry, one could imagine that these scars creates a feed-forward degenerative cycle whereby genetic damage produces a vulnerability that increases activity in certain disease situations (for example, neuroinflammation). This activity then further damages the genome in specific locations that further increases the initial vulnerability and so on. This feed-forward process is an attractive model to explain the different molecular phenotypes that are found in different neurodegenerative diseases.

Compromised DNA repair leads to age-related disease. When the balance between DNA damage and repair tips too far towards unrepaired damage, evidence suggests that age-related cognitive decline and progressive loss of normal neuronal physiology are the inevitable result[59]. The relationship between insufficient DNA repair and neurodegeneration was first suggested after the discovery of premature neuronal death and neurological symptoms in patients with xeroderma pigmentosum[60]. This connection was further supported when the mutations responsible for other neurodegenerative disorders were also discovered to involve DNA damage-response proteins (for an excellent review, see the work by McKinnon[61]). The idea that this relationship has relevance for late-onset neurodegenerative disease can be found in the work Sykora *et al.*[62] who reported that triple transgenic (3xTg; homozygous for the presenilin 1 (*Psen1*) mutation and for the co-injected APP_{Swe} and tau_{P301L} transgenes) mice with haploinsufficiency for DNA polymerase-β (*Polb*$^{+/-}$) have compromised BER, elevated DNA damage and enhanced neuronal death. Furthermore, transcriptome profiles of humans with Alzheimer diseases are more similar to 3xTg-*Polb*$^{+/-}$ mice than to either *Polb*$^{+/-}$ or 3xTg mice[62]. The role of failed repair as a driver of the process of ageing is further emphasized by the neurological consequences of deficiency of excision repair cross-complementation group 1 (*Ercc1*), which is an essential component of multiple DNA repair pathways, including BER. Homozygous *Ercc1*-null mutants die during gestation; hypomorphs survive but accumulate multiple types of DNA lesions at an accelerated rate[63]. That *Ercc1* hypomorphs display a neurodegenerative phenotype — progressive loss of synaptic plasticity and cognitive decline — is a significant finding[64]. Thus, multiple lines of evidence point to the conclusion that defective DNA repair both accelerates the ageing process and leads to neurodegenerative disease.

In the context of the suggestion that different neurodegenerative disorders have their roots in a genetic and epigenetic signature caused by scars in different areas of the genome, it is notable that relatively little is known about changes in the capacity for DNA repair in ageing adult neurons. If the non-random nature of the genomic damage is compounded by a unique signature of repair deficiencies, the emergence of regional and cell-type specificity in different diseases is almost a direct prediction. This hypothesis is difficult to test. In mice, it has been known for many years that loss of essential components of the NHEJ pathway, such as KU70 (also known as XRCC6) and KU80 (also known as XRCC5)[65], XRCC4 (REF. 66) and ligase 4 (REF. 67), results in massive apoptosis of early postmitotic cells immediately following their exit from the ventricular zone, whereas mitotic progenitor cells in the ventricular zone itself are unaffected. Thus, genetic loss of the capacity for NHEJ, the only way a neuron can repair double-stranded break lesions, is lethal to a cell as soon as it starts the process of maturation. Furthermore, recent data have suggested that the requirement for a robust NHEJ capacity is lifelong. It is increasingly evident that there is an age-related reduction in DNA damage response proteins such as ATM[68], MRE11 (REF. 69) and DNA-PKcs (the catalytic subunit of the DNA-dependent kinase)[70,71], and that this decreased activity may be linked to late-onset neurodegenerative diseases such as Alzheimer disease[12,42].

Cell cycle re-entry — going beyond G1 as a sign of insufficient repair. In cycling cells, cell cycle control and DNA damage repair are intricately linked; proteins that take part in cell cycle regulation also alter the expression of components of DNA repair[72,73]. The regulation goes in both directions. Not only are some of the same proteins used in both processes, but in response to DNA damage, activation of cell cycle checkpoints prevents a cell from replicating misinformation and thus propagating the mutations to the daughter cells[7]. For example, in response to a double-stranded break, ATM activates cell cycle checkpoints by phosphorylating a cascade of mediator molecules, including checkpoint kinases (for example, CHK2)[74–76], γH2AX and the checkpoint mediator protein tumour suppressor p53-binding protein (TP53BP1), as well as the double-stranded break

Hypomorphs
Mutations in genes that have a similar but weaker effect than the corresponding wild-type gene.

Box 1 | **Cell cycle re-entry and DNA repair in postmitotic neurons**

In postmitotic neurons, reactivation of the cell cycle machinery may be an essential part of the non-homologous end joining (NHEJ) response to DNA damage. Subtoxic concentrations of certain stressors, such as hydrogen peroxide, produce double-stranded breaks in postmitotic cortical neurons, and G1 phase proteins are activated in response. The linkage of this process to DNA repair can be seen in cells in which the cyclin-dependent kinases CDK4 and CDK6 (essential components of G0 to G1 transition) are simultaneously knocked down. Such knock down results in an increase in DNA damage, suggesting that DNA repair is less reliable when cell cycle processes are inhibited[177].

Similarly, a cyclin C-directed, phosphorylated RB-dependent G0 exit is proposed to activate NHEJ repair in postmitotic neurons[178]. Forcing G1 entry while simultaneously blocking the G1 to S transition triggers a full NHEJ response, even in the absence of DNA damage. These data strongly argue that shifting from the quiescent G0 phase of the cell cycle to the active G1 phase may be part of the means by which a postmitotic neuron initiates the DNA repair response. When DNA damage is too severe, however, apoptosis of postmitotic neurons is observed, accompanied by DNA replication and CDK2 and cyclin E expression. This response hints at a relationship between S phase progression and neuronal death, as blocking CDK2 activity not only prevents S phase progression but also blocks neuronal apoptosis. Significantly, CDK2 inhibition has no effect on the efficacy of DNA repair[178,179]. One model that would tie these various observations together is if cell cycle re-entry from G0 to G1 was used by non-mitotic neurons to facilitate DNA repair. If the process is not stopped, however, S phase is initiated, leading to neuronal death. The increased presence of cell cycle markers in neurons of Alzheimer disease brains is a predicted consequence of such a scheme and further highlights the role of DNA damage in the aetiology of age-related diseases.

recognition factor NBS1 (REFS 77,78). During this process, double-stranded break repair and signalling molecules form discrete nuclear foci that stimulate repair and amplify checkpoint responses[79,80].

In neurons, the interrelationship between cell cycle re-entry and DNA repair is particularly complex (BOX 1). Although cell cycle re-entry seems to be part of DNA repair in postmitotic neurons, there is a growing body of evidence suggesting that individual neurons in populations that are at high risk for neurodegeneration show evidence of having re-entered a cell cycle process. Cell cycle-related proteins are unexpectedly expressed in neurons of patients with Alzheimer disease[81–83] and in neurons of many other neurodegenerative diseases[84–91]. This phenomenon involves true DNA replication and not just DNA repair; Yang *et al.*[92] used FISH to show that 4% of the hippocampal pyramidal neurons from patients with Alzheimer disease are hyperploid (three to four fluorescent spots for each unique genomic probe) instead of the expected diploid. In their study, the background aneuploidy in the non-Alzheimer disease cases was very low. This result has now been repeated by multiple research groups. Mosch *et al.*[32] showed that a population of cyclin B1-positive tetraploid neurons constitutes approximately 2% of all neurons in Alzheimer disease brains. Furthermore, this phenomenon of ectopic DNA replication is not unique to Alzheimer disease[88,93].

Thus, over and above the developmental background, the progression of several different neurodegenerative diseases is associated with evidence of enhanced cell cycle-related DNA replication leading to increased aneuploidy. However, although the existence of increased aneuploidy in diseases such as Alzheimer disease is well established, the consequences for brain function and cognition during ageing are less clear. Aneuploidy may potentially contribute to functional diversity in domains such as learning and behaviour, but it can also lead to functional decline and predisposition to disease. To this end, aneuploidy has been implicated as one of the causes of Alzheimer disease, in particular changes in chromosome 21 (REFS 27,39,94). Chromosomal aneuploidy could logically originate from at least two sources: a mitotic non-disjunction during development or the consequence of an incomplete cell cycle event triggered by a disease process in the adult. As discussed above, there is a background of chromosomal aneuploidy in the brain, but that would be expected to be more evenly distributed between affected and unaffected individuals. Therefore, it is likely that most disease-related aneuploidy results from lost cell cycle control in at-risk postmitotic neurons. Although the evidence is strong that re-initiation of cell cycle activity is an integral part of the disease process, we must also ask what the consequences of this aneuploidy for adult brain function might be. This question is a particularly acute one to answer because, during the course of various neurodegenerative diseases[30,86,88,95], the hyperploid neurons do not die as rapidly as they do during development[23,82]. In adult-onset disorders such as Alzheimer disease, for example, it is estimated that the neurons that have replicated all or most of the DNA persist for many months[96,97].

Changes in non-genomic factors

In a typical cell, genome integrity includes not just DNA and the four possible bases, but also differential patterns of base modification (for example, cytosine methylation) as well as changes in the DNA-bound histones with or without chemical modifications of their own (for example, acetylation or methylation). Other factors that affect genomic integrity include intracellular levels of sirtuin, the levels of oxidative stress and, it is increasingly recognized, a network of small non-coding RNAs. As with the double helix itself, alterations of these factors can contribute to the speed and fidelity of DNA repair and also alter the ageing phenotype.

CpG island methylation. DNA methylation, in addition to having a well-defined role in altering gene transcription, has a potential role in DNA damage repair. The DNA methylation reaction is catalysed by DNA methyl transferase 1 (DNMT1), and mouse cells lacking DNMT1 are genetically unstable[98–100]. In human cells, global loss of DNA methylation results in genome instability[101]. This effect is most likely a direct effect as DNA methylation is part of the damage response. DNA double-stranded breaks trigger the recruitment of DNMT1, DNMT3, nuclear protein 95 (NP95; also known as UHRF1) and growth arrest and DNA-damage-inducible, alpha (GADD45A) to the site of the lesion, where they help modulate the methylation pattern. As DNA repair is less efficient when DNA methylation is abnormal, it is noteworthy that over the genome as a whole, DNA methylation decreases with age (reviewed in REF. 102). The suggestion is that this loss of DNA methylation and the resulting reduction in DNA repair contributes both to the ageing process itself and to

neurological diseases of ageing. Demethylation also is a factor in establishing the epigenome; 5-hydroxymethylcytosine (5hmC) is produced by the actions of the ten-eleven translocation (TET) family of proteins and serves as an intermediate in the pathway to demethylation[103]. In Alzheimer disease, both 5mC and 5hmC have a negative correlation with amyloid plaque load in the hippocampus[104]; however, these are early days in the exploration of this phenomenon and uncertainties remain.

Histone modification. Changes in chromatin proteins occur throughout brain development, continue through the process of ageing and contribute in specific ways to DNA repair and hence to ageing and the pathogenesis of neurodegenerative disease[105]. Histone phosphorylation, at the right location and time, facilitates DNA repair. Phosphorylation of histone H2AX by ATM and DNA-PKcs at serine 139 is one of the hallmarks of the DNA damage response, producing the well-known DNA damage signal, γH2AX[106]. γH2AX appears in nuclear foci during double-stranded break repair, and it facilitates the recruitment of other repair factors such as NBS1, breast cancer type 1 susceptibility protein (BRCA1) and checkpoint proteins such as mediator of DNA damage checkpoint protein 1 (MDC1) and TP53BP1 to the site of damage[80]. Despite evidence showing that the absence of H2AX only mildly affects the process of double-stranded break repair, the formation of foci facilitated by γH2AX is important for the activation of cell cycle checkpoints in response to mild DNA damage[80]. Other examples of the modification of histones during the DNA damage response include the differential phosphorylation of serine 10 and threonine 11 of histone H3 (REFS 107,108). Upon UV irradiation, these two residues are dephosphorylated early in the damage response then rephosphorylated shortly after the damage has been repaired[107,108]. Methylation of histones at different lysine residues is also implicated as a part of DNA repair and neurodegeneration. In the context of DNA repair, both H3K79 and H4H20 methylations are involved in recruiting TP53BP1 and CRB2 to nuclear foci after double-stranded break induction[109–113]. Finally, histones are generally hyperacetylated after UV irradiation, and repair of DNA is more efficient with hyperacetylated nucleosomes[114,115]. A recent study showed that during NHEJ, histone deacetylase 1 (HDAC1) is recruited and activated by sirtuin 1 (SIRT1) as part of the process of double-stranded break repair[68]. However, mutations in *HDAC1* mimic a constitutively acetylated state and render neurons more susceptible to DNA damage[68].

It is likely that these three different types of histone modification function together during the process of DNA repair. For example, the DNA damage response protein ATM targets enzymes responsible for both histone acetylation[116] and methylation[117], thus enhancing the former and inhibiting the latter. The net result is that ATM tends to open the chromatin, which would be expected to improve access for the DNA repair proteins. In the case of EZH2, this effect has been shown directly[117]; for HDAC4, the effect appears to be indirect[116]. In addition to their role in the DNA damage response, changes in histone phosphorylation, acetylation and methylation increasingly appear in lists of molecular mechanisms underlying neurodegenerative disease. Phosphorylated histone proteins, normally located in association with the DNA double helix can on occasion be found ectopically localized in cytoplasm. This mis-positioning is correlated with unscheduled cell cycle activity in hippocampal neurons of Alzheimer disease brains, possibly driving them towards neuronal dysfunction, mitotic catastrophe and death[118]. Global levels of histone acetylation are reportedly lower in the temporal lobe of individuals with Alzheimer disease compared with age-matched controls[119]; although for all of the current interest, surprisingly little research has been conducted in this area. In mouse models of Alzheimer disease, HDAC2 malfunctions are part of the loss of regulation of genes that are crucial for learning and memory[120,121], including immediate early genes as well as other genes that are crucial for synaptic plasticity[120]. Application of HDAC inhibitors further highlights the importance of maintaining proper histone acetylation in disease pathogenesis. Thus, treatment with valproic acid reduces the plaque burden of PSAPP (APP_{V717F}) transgenic mice[122]. In addition, injection of sodium butyrate or trichostatin A induces dendritic sprouting, increases the number of synapses, and restores learning and long-term memory in CK-p25 mice[123]. Consequently, HDAC inhibitors have been suggested as promising therapeutics for Alzheimer disease[124].

Sirtuins and DNA repair. The sirtuins are class III HDACs that regulate various cellular functions[125]. Sirtuins have long been associated with the process of ageing[126] as mutations in the genes encoding these proteins can extend lifespan considerably. In contrast to class I and II HDACs that target the histone proteins themselves, sirtuins can bind to multiple factors and target many different protein substrates. Among the seven sirtuin homologues found in humans, SIRT1 is the most widely studied member and is reduced in Alzheimer disease[127]. During the process of DNA repair, SIRT1 is recruited to sites of DNA damage along with other histone-modifying enzymes to trigger epigenetic changes near the break, which results in chromatin remodelling[128]. In addition, SIRT1 deacetylates a number of proteins and thus facilitates the initiation of the DNA repair response. For instance, SIRT1 deacetylates NBS1 and modulates γH2AX, BRCA1, RAD1 and NBS1 foci formation[129–131]. SIRT1 also stimulates ATM autophosphorylation activity and stabilizes ATM at the break site[68]. Furthermore, while in association with ATM, SIRT1 recruits and activates HDAC1 to facilitate NHEJ[68]. SIRT1 may also mediate double-stranded break repair independent of the ATM pathway through a mechanism involving the Werner helicase[132] or the deacetylation of KU70 during NHEJ[133]. For single-stranded break repair, SIRT1 deacetylates xeroderma pigmentosum group A (XPA) proteins. This process is important because cells deficient for XPA show significantly higher sensitivity to UV light, which is partly due to the reduced activity of the NAD^+–SIRT1–PGC1α (peroxisome proliferator-activated

receptor-γ coactivator 1α) axis[134]. From the perspective of neurodegenerative disease, the interaction between SIRT1 and XPA is potentially at the core of an important nuclear–mitochondrial crosstalk circuit.

In addition to SIRT1, SIRT6 is also involved in DNA repair, in particular the BER pathway. Enhanced chromosomal breaks, as well as ultra-sensitivity to genotoxins caused by knock down of *SIRT6* can be rescued by the introduction of a fragment of polymerase-β, a DNA polymerase that takes part in 'short patch' BER[135]. Mechanistically, poly(ADP-ribose) polymerase 1 (PARP1) is also suggested to be involved in DNA repair, as it is activated by SIRT6 in response to double-stranded breaks[136]. SIRT6 also facilitates chromatin opening at the site of DNA damage by recruiting sucrose nonfermenting protein 2 homologue (SNF2H; also known as SMARCA5)[137], and it facilitates the recruitment of downstream factors, such as TP53BP1, BRCA1 and replication protein A (RPA)[137]. In Alzheimer disease, levels of different members of the sirtuin family are dysregulated[138,139] and it has been suggested that modulation of sirtuin levels or activity, through pharmacological innervation or calorie restriction, may offer new approaches to the prevention or treatment of neurodegenerative disorders. Collectively, these data suggest that the sirtuins, long associated with alterations in the rate of ageing, are also important in maintaining genomic integrity via their effects on the DNA repair process. Whether these two activities are merely correlated or perhaps constitute an unrecognized driver of the ageing process itself remains to be determined.

DNA integrity in mitochondria. Nuclear DNA is not the only DNA in the cell for which integrity must be maintained in the adult neuron. Although many copies are present in each cell, mitochondrial DNA (mtDNA) mutations increase during normal ageing and may play a part in both ageing and neurodegenerative disease. From 42 to 97 years of age, human cells experience a progressive increase in the levels of 8-hydroxy-2′-deoxyguanosine (8-OHdG), and the magnitude of the age-related damage is approximately tenfold greater in mtDNA than in nuclear DNA[140]. Oxidative damage is one of the most significant risk factors for neurodegeneration, and as early as 1994, Mecocci *et al.*[141,142] reported an age-dependent increase in the levels of 8-OHdG in nuclear DNA and mtDNA in specimens from the cerebral cortex of normal control subjects[141], and a higher level in Alzheimer disease. Other groups have reported similar results[15,143]. These oxidative lesions are leading rather than lagging indicators of Alzheimer disease progression because 8-OHdG appears elevated in early Alzheimer disease, and the levels reached are comparable to those observed during end-stage disease. Although the repair of mtDNA relies mainly on the BER pathway[144], SIRT1 and other members of the sirtuin family have important roles in mitigating mtDNA damage[145] through stimulating mitochondrial biogenesis[146], reducing superoxide generation from the respiratory chain[147] and enhancing the expression of antioxidant enzymes[148].

The role of microRNAs in DNA repair and neurodegeneration. It is increasingly being recognized that disease pathogenesis can be modulated through non-protein coding microRNA (miRNA) species. The total number of different mature miRNAs in humans is likely to exceed 1,000 (REF. 149). Although not translated, miRNAs bind to the 3′-UTR or, less commonly, to other regions of mRNAs[150], thus regulating their expression. The usual effect of miRNA binding is to silence, although occasionally the opposite effect is observed[151]. This family of regulatory RNAs is relevant to the current discussion because certain miRNAs undergo age-associated changes that affect brain function and are likely to have roles in neurodegenerative disease[152]. For example, miR-16 and miR-193B are linked to post-transcriptional control of the amyloid precursor protein (APP) expression[153,154]. In Alzheimer disease, overexpression of these two miRNAs reduces the efficiency of *APP* mRNA translation both *in vivo* and *in vitro*. A third entity, miR-124 regulates the *APP* mRNA splicing process and is downregulated during the progression of Alzheimer disease[155]. Binding sites for miR-107, among other miRNAs are found in the 3′-UTR region of the transcript of β-secretase 1 (*BACE1*). As this binding would be expected to reduce BACE1 production, it is a significant finding that reduced there is reduced expression of miR-107 even at the very early stage of Alzheimer disease[156]. A current summary of the different miRNAs that are altered in Alzheimer disease brains are listed in TABLE 1.

A linkage between miRNAs and the DNA damage response also exists, but it is an indirect one mediated by the downregulation of many of the DNA damage response genes. Examples of genes that are subject to inhibitory regulation by miRNAs[157] include *ATM*[158], *H2AX*[159], *RAD52* (REF. 160), *RAD23B*[160], *MSH2* (REF. 161) and *BRCA1* (REF. 162). We propose that with increasing research, this linkage will prove to be more direct and more coordinated. Thus, the same miRNAs that are dysregulated in Alzheimer disease brains also play direct parts in altering the expression of DNA repair genes. In ataxia telangiectasia[163], the expression of the ATM kinase is downregulated by miR-421. This connection between the ageing process, DNA damage and expression of miRNAs is highlighted in a cell's response to both the nature and intensity of DNA damage[164]. For example, members of the miR-34 family are identified as a direct transcriptional target of p53, a DNA damage responsive factor[165]. Ectopic expression of miR-34 genes causes G1 phase cell cycle arrest and the downregulation of genes involved in promoting cell cycle progression[165]. In addition, miR-34a is upregulated in mouse models of Alzheimer disease, and is proposed to inhibit *Bcl2* translation, resulting in higher levels of activated caspase 3 (REF. 166). Expression of other clusters of miRNAs, including miR-192, miR-194, miR-215 and miR-17-92, are also upregulated by p53 after DNA damage, further facilitating p53-induced cell cycle arrest[165,167,168]. Thus, it may be that the loss of DNA repair efficiency that occurs in late-onset neurodegenerative disease is partly due to changes in the expression of different miRNAs[169].

Table 1 | **Changes in expression of miRNA in Alzheimer disease and their targets involved in DNA repair**

Brain region	miRNA	Technique	Consequences for DNA damage or repair targets
Upregulation			
Hippocampal CA1	miR-9	DNA array and Northern blot analyses[180]	Downregulation of BRCA1 (REF. 181)
	miR-128	DNA array and Northern blot analyses[180]	Downregulation of SIRT1 (REF. 182)
Hippocampus, cerebellum, medial frontal gyrus	miR-26a	Microarray[183]	• Downregulation of ATM[184] • Downregulation of PTEN[185]
	miR-27a	Microarray[183]	Downregulation of ATM[186]
	miR-27b	Microarray[183]	Unknown
	miR-30c	Microarray[183]	Unknown
	miR-30e-5p	Microarray[183]	Unknown
	miR-34a	Microarray[183]	• Downregulation of TP53BP1 (REF. 187) • Downregulation of SIRT (REF. 188) • Downregulation of E2F1 and E2F3 (REF. 189)
	miR-92	Microarray[183]	Unknown
	miR-125	Microarray[183]	Downregulation of TP53 (REF. 190)
	miR-145	Microarray[183]	Downregulation of RAD18 (REF. 191)
	miR-200c	Microarray[183]	Unknown
	miR-381	Microarray[183]	• Downregulation of WEE1 (REF. 192) • Downregulation of TP53 (REF. 193)
	miR-422a	Microarray[183]	Unknown
	miR-423	Microarray[183]	Unknown
Temporal cortex	mir-26b	qRT-PCR[194]	Downregulation of ATM[195]
Downregulation			
Frontal cortex	miR-29a	qRT-PCR[196]	Upregulation of TP53 (REF. 197)
Hippocampus, cerebellum, medial frontal gyrus	miR-9	Microarray[183]	Downregulation of BRCA1 (REF. 181)
	miR-132	Microarray[183]	Downregulation of RB[198]
	miR-146b	Microarray[183]	Downregulation of BRCA1 (REF. 199)
	miR-212	Microarray[183]	Unknown
Anterior temporal cortex	miR-124	qRT-PCR[155]	Downregulation of KU70 (REF. 200)
Cerebral cortex	miR-107	Microarray, Northern blot analysis, *in situ* hybridization[156]	Downregulation of RAD51 (REF. 201)

ATM, ataxia telangiectasia mutated; BRCA1, breast cancer type 1 susceptibility protein; miRNA, microRNA; qRT, quantitative real time; SIRT1, sirtuin 1; TP53BP1, tumour suppressor p53-binding protein.

Perspectives and conclusion

Age is by far the most common risk factor for most adult-onset neurodegenerative diseases. Even the most aggressive familial forms of these diseases rarely strike before the age of 40 years. DNA damage accumulates with age and it is likely that this increasing loss of genomic integrity is one of the causative factors in the ageing process itself (FIG. 3). This notion raises the real possibility that there is a feed-forward relationship between DNA damage and the initiation and progression of neurological disease. Thus, misfolded protein aggregates not only seem to drive regional neuronal vulnerability but they also shape the patterns of DNA damage in different diseases. The neuropathological hallmarks of Alzheimer disease are the formation of amyloid plaques and neurofibrillary tangles. It may be significant, therefore, that the amyloid-β peptide can inhibit DNA-PK and thus hamper DNA repair through the NHEJ pathway[170]. In a similar vein, unphosphorylated tau, the major constituent of the neurofibrillary tangle, binds to the minor groove of the DNA double helix where its presence protects DNA from oxidative damage[171,172]. Phosphorylation of tau reduces its ability to prevent DNA thermal denaturation and reduces its protection of DNA from reactive oxygen species[173]. Finally, accumulation of α-synuclein in Parkinson disease is associated with increased mtDNA deletions and oxidative DNA damage[174].

As mutations in DNA repair genes are associated with premature ageing, it is logical that a time-dependent somatic loss of activity of such genes might underlie the ageing process itself. If true, the resulting loss of repair capacity would represent an additional age-related factor

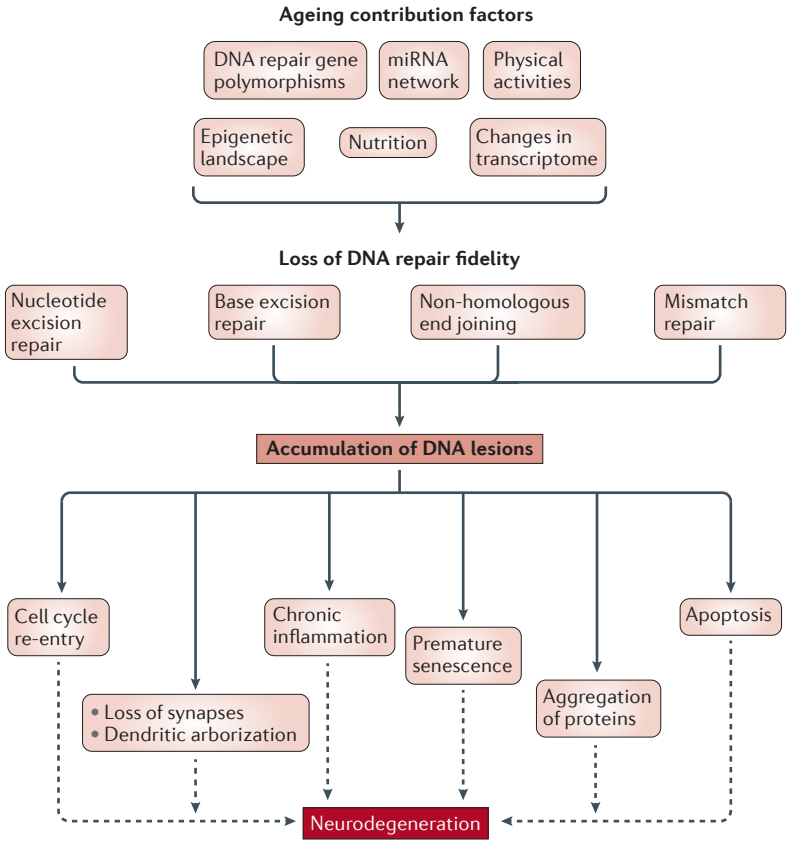

Figure 3 | **Neurodegeneration in ageing neurons resulting from a reduction of DNA integrity.** Ageing contributes to the loss of DNA integrity in numerous ways, including lifestyle factors such as nutrition, chemical exposure and physical activity. These systemic stresses add to the molecular stresses brought about by polymorphisms (hypomorphs) in DNA repair genes, changes in the epigenetic landscape, shifts in the microRNA (miRNA) network, as well as changes in the rate and pattern of transcription. All of these stresses influence the fidelity of DNA repair in neurons. As a result, impairment of various DNA repair pathways, including nucleotide excision repair, base excision repair, non-homologous end joining and mismatch repair, stimulates the accumulation of DNA lesions in the neuronal genome. As one possible response to accumulating DNA damage, neurons may re-enter the cell cycle or become prematurely senescence or go on to die. These changes can also create a feed-forward loop with the potential to catalyse additional harmful changes in the neuronal genome, further disturbing patterns of gene expression. These degenerative changes are then 'read out' to result in a loss of synapses or dendritic arborization, in the triggering of chronic inflammation or in the accumulation and aggregation of misfolded proteins. These downstream outcomes further combine in various ways to contribute to the degeneration of neurons.

in the pathogenesis of late-onset neurodegenerative disease. Changes in DNA methylation, histone modification and the networks of miRNA expression further expand this relationship. These epigenetic changes affect the process of DNA repair and vice versa. As these same processes are found in a range of neurodegenerative diseases, it seems likely that inadequate DNA repair links the ageing process with neurodegeneration. It is clear that cause and consequence need to be untangled in this complex web of interactions, but the case is strengthening that the lifelong accumulation of DNA damage in brain cells is a key factor that acts almost like an ageing clock and relentlessly increases the risk of a wide variety of late-onset neurological disorders.

However, even as evidence of this linkage accumulates, many important questions remain. One particularly vexing question is where are the regional specificities of diseases such as Alzheimer disease and Parkinson disease determined? DNA repair is a critical function of every cell in our bodies; thus, it is difficult to imagine how such a seemingly ubiquitous function can, in and of itself, be the source of the observed regional differences in different neurodegenerative diseases. A general 'snowball' model might serve as a starting point to understand the dynamics of the interaction between DNA damage and the ageing process. In this conceptualization, there is a slow accumulation of DNA damage with age, much as a snowball gradually grows in size as it rolls down a gentle slope. Once an age-related disease starts, however, the disease progress itself drives additional non-random DNA damage in the cells involved, creating a feed-forward effect (as if the snowball had veered off and started down a much steeper slope) (FIG. 4a). Other models are also compatible with the observed data. One is that the loss of DNA integrity is simply the ageing clock itself. All cells can read the time, but intrinsic vulnerabilities, such as polymorphisms in DNA repair genes, might predispose an individual towards different late-onset neurodegenerative disorders during the process of ageing. For example, in Parkinson disease, a mutant variant located at the poly-Q-track region of DNA polymerase subunit γ1 (*POLG1*) DNA repair gene represents a risk factor for the disease[175]; ageing might interact with such an intrinsic vulnerability to favour the development of Parkinson disease. In different individuals, haplotypes -3TT/4CC in *PARP1* or a Ser326Cys polymorphism at 8-oxoguanine DNA glycosylase (*OGG1*) might be carried. The first is significantly associated with an increased risk of developing Alzheimer disease[124] and the latter with an increased risk of developing amyotrophic lateral sclerosis[176]. When combined with the ageing process, these intrinsic vulnerabilities might trigger disease-specific cascades of neurodegenerative events. And because the ageing clock applies to all processes, environmental factors such as dietary changes and physical activity might ultimately lead to biochemical changes such that in some individuals the 'Alzheimer alarm' goes off on the clock, whereas in others the 'Parkinson alarm' goes off first. Another alternative is that the brain activity-induced DNA damage described by Suberbielle *et al.*[42] imposes a circuit-specific pattern to the DNA damage that drives the normal age-related process faster in certain areas than in others (FIG. 4b). As described above, this pattern could lead to a circuit-specific vulnerability towards degeneration. Note that these alternatives are not mutually exclusive; the final outcome is probably a mixture of all three models.

Assuming that specific patterns of DNA damage can determine the clinical signatures of different diseases, we would then predict that the patterns of damage to the genetic landscape are specific. By contrast, if DNA damage is just a clock, the extent of damage may be roughly equal in each cell. We would urge the development of comprehensive data sets of

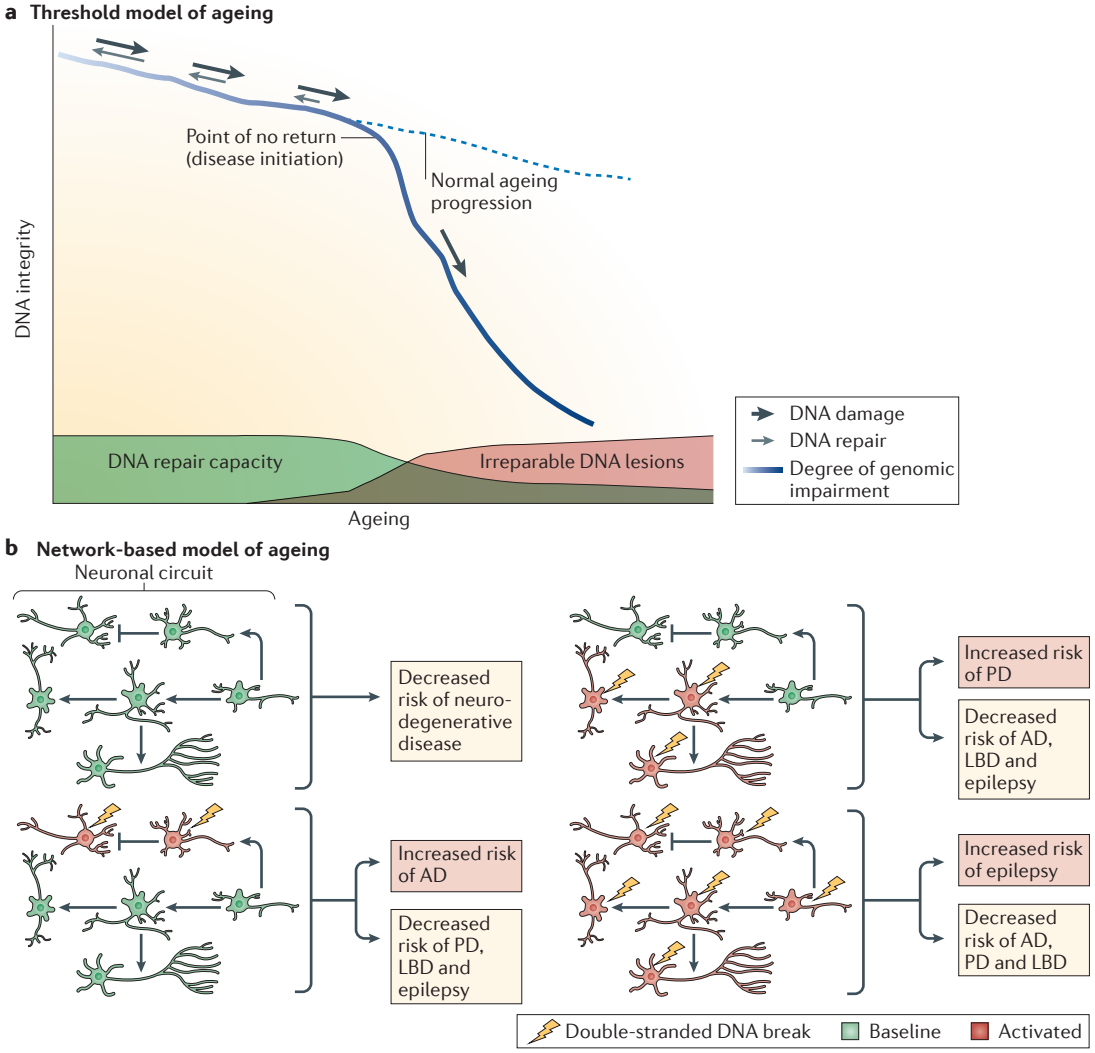

Figure 4 | **DNA damage and the onset of specific neurodegenerative diseases.** **a** | As we age, all of our neurons experience increasing amounts of irreparable DNA damage. The accumulating damage is induced by products of cell metabolism and other destructive activities (black arrows) coupled with a reduced capacity for DNA repair (grey arrows). Disease initiation then arises as a result of an additional insult, specific to the particular degenerative condition, which, coupled with the damage already present, precipitates the emergence of disease. Without that insult, a slow but benign descent into ageing would continue without serious clinical consequences (as indicated by the dashed line). Once the activity of DNA repair can no longer keep pace with the rate at which DNA damage is generated, damage accumulates at an increased pace and a point of no return is reached, eventually leading to neuronal death. **b** | An alternative, but not mutually, exclusive conceptualization involves a network-based model of DNA damage. If the relative activity levels of different circuits of neurons leads to the accumulation of specific unrepaired DNA lesions in the participating cells[42], the predicted consequence would be regional variability in the rates of DNA damage, leading to different rates of neuronal ageing and hence to specific selections of neurodegenerative events. For instance, during the development of Alzheimer disease (AD), aberrant activities of neurons in the hippocampal network might result in the lethal accumulation of DNA damage in certain cells. Within the same brain, Purkinje cells in the cerebellum, engaged in a different pattern of physiological activity, would show minimal accumulation of such damage and be spared. After many years, the loss of genomic integrity in the most affected hippocampal neurons would lead to a pattern of cell dysfunction and death that would be more pronounced than that in the cerebellum. A similar branching network model with different initiation points could be envisioned for other diseases, including Parkinson disease (PD), Lewy body disease (LBD) and epilepsy.

transcriptome, epigenome and miRNA profiles sampled with high anatomical specificity, using single cell technology where possible[26], to provide the most comprehensive view possible of which specific subset (or subsets) of genes predispose an individual to which specific diseases. The prize for teasing out the connections between DNA damage, the ageing process and the various neurodegenerative disorders will be the opening of new avenues of understanding as well as fertile areas for future drug development.

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Competing interests statement
The authors declare no competing interests.