Mechanisms of DNA damage-mediated neurotoxicity in neurodegenerative disease

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Abstract

Neurons are highly susceptible to DNA damage accumulation due to their large energy requirements, elevated transcriptional activity, and long lifespan. While newer research has shown that DNA breaks and mutations may facilitate neuron diversity during development and neuronal function throughout life, a wealth of evidence indicates deficient DNA damage repair underlies many neurological disorders, especially age-associated neurodegenerative diseases. Recently, efforts to clarify the molecular link between DNA damage and neurodegeneration have improved our understanding of how the genomic location of DNA damage and defunct repair proteins impact neuron health. Additionally, work establishing a role for senescence in the aging and diseased brain reveals DNA damage may play a central role in neuroinflammation associated with neurodegenerative disease.

Keywords DNA damage; DNA damage repair; inflammation; neurodegeneration; neuron

Subject Categories DNA Replication, Recombination & Repair; Neuroscience

Introduction

Post-mitotic neurons are the basic cellular unit of the nervous system. Their function controls primary aspects of human physiology, including movement, breathing, and heart rate, as well as higher order processes such as memory and attentional control. However, as a largely non-renewable resource, neurons must perform these essential tasks while also maintaining their cellular and genomic integrity over many decades of life. To survive the inexorable passage of time, neurons are equipped with accurate and efficient DNA damage response (DDR) pathways. Defective DDR pathways can result in toxic genomic rearrangements, transcriptional dysregulation, and the accumulation of unrepaired lesions (Hoeijmakers, 2009; Madabhushi et al., 2014; Chow & Herrup, 2015; McKinnon, 2017; Tubbs & Nussenzweig, 2017). These insults ultimately push cellular fate toward apoptosis, senescence, or uncontrolled cell division, all of which are hallmarks of age-associated disease (Hoeijmakers, 2009; Madabhushi et al., 2014; Chow & Herrup, 2015; McKinnon, 2017; Tubbs & Nussenzweig, 2017). We can appreciate the value of neuron viability in particular through the devastating effects of neurodegenerative diseases, which strip individuals of their memories, motor control, and autonomy. As of 2017, neurologic diseases are the third leading cause of death in the United States and the fifth leading cause of death worldwide (GBD, 2017 US Neurological Disorders Collaborators, 2021; GBD CompareIHME Viz Hub).

A well-established link exists between DNA damage and neurodegenerative diseases. In many cases, DNA damage seems to be one of the earliest indicators of neuropathology, suggesting it may be an initiating lesion of toxicity (Chow & Herrup, 2015; Simpson et al., 2015, 2016; Shanbhag et al., 2019). Recently, numerous findings have helped clarify the mechanisms by which DNA damage may mediate neuronal dysfunction. Broadly, these are lessons learned through both DNA damage repair disorders and models of age-associated neurodegenerative diseases. Additionally, through the advancement of sequencing techniques to map DNA lesions, rearrangements, and mutations, we are just beginning to appreciate the significance of a lesion’s genomic location in relation to its effect on neuronal activity and, ultimately, degeneration (Lodato et al., 2015, 2018; Wei et al., 2016; McConnell et al., 2017; Reid et al., 2021; Rodin et al., 2021; Wu et al., 2021). Finally, while both neuroinflammation and DNA damage are considered hallmarks and mediators of neurodegeneration, the mechanistic relationship between the two has yet to be fully realized. To this end, concepts from senescence cell biology are helping us inform how one might feed into the other (Bussian et al., 2018; Musi et al., 2018; Chow et al., 2019; Zhang et al., 2019; Gillispie et al., 2021). Here, we will cover the recent advances made in each of these subgenres of disease research and how they enhance our understanding of neuronal function and degeneration.

The DNA damage response (DDR)

Our genome continually incurs damage via exogenous agents and endogenous metabolic byproducts. In response to the constant onslaught of genomic lesions, mammalian cells have developed a myriad of DNA damage response (DDR) pathways, each specializing in the detection and correction of a different type of lesion. Although each pathway recruits different proteins and repair enzymes, the
basic DDR format remains the same. Lesions are first detected, then they are processed and/or excised by a nuclease. Lastly, a polymerase synthesizes new DNA to replace the missing nucleotides, and a ligase seals the resulting nick together. The following section briefly summarizes DNA lesions and their corresponding DDR pathway.

**Single-Strand Breaks (SSBs)**

While many different types of genomic injuries can occur, the majority ultimately manifest in the form of single-strand breaks (SSBs). This is largely by virtue of reactive oxygen species (ROS), which attack DNA to form oxidized bases and abasic sites (Lindahl & Barnes, 2000; Madabbushi et al., 2014; Tubbs & Nussenzweig, 2017). ROS-mediated DNA damage is greatest in the nervous system (Nakamura & Swenberg, 1999), likely because neurons exhibit substantial mitochondrial respiration, consuming approximately 20% of the body’s available oxygen (Attwell & Laughlin, 2001). ROS can generate SSBs directly through attacking the DNA backbone or indirectly through the generation of other DNA modifications whose repair requires transient break formation. One of the most abundant ROS-mediated DNA modifications is 8-oxo-7,8-dihydroguanine (8oxoG), a non-bulky lesion whose presence can dysregulate gene transcription and whose erroneous repair results in mutagenesis, a major contributor to aging and disease.

Non-bulky base modifications such as 8oxoG are resolved through base excision repair (BER), wherein a base-specific glycosylase detects and removes the damaged base, and the backbone is removed by apurinic/apyrimidinic endonuclease 1 (APE1) to generate an intermediate SSB. From here, the SSB can be resolved either through short-patch SSB repair (sp-SSBR) or long-patch SSBR (lp-SSBR). In short patch SSBR, polymerase β (POLβ) fills in the missing nucleotide and ligase III (LIG3) seals the nick. The alternative lp-SSBR replaces larger stretches of DNA (2-13 nucleotides), utilizing flap endonuclease 1 (FEN1), proliferating cell nuclear antigen (PCNA), and polymerase δ/ε (POL δ/ε) to open and replace the broken DNA strand. Ligase I (LIGI) then seals the nick.

In contrast to smaller base lesions, helix-distorting bulky lesions (which are most commonly caused by UV exposure) are detected by their steric distortion rather than their specific chemical structure. For example, bulky lesions are detected during transcription when their presence stalls RNA polymerase II, which with the help of proteins CSA and CSB (also known as ERCC6 and ERCC8, respectively) initiates transcription-coupled nucleotide excision repair (TC-NER). In non-transcribed or inactive regions of the genome, bulky lesions are detected by the XPC-RAD23B-CEN2 complex, which initiates global genomic NER (GG-NER). Beyond the mechanism of their initial detection, TC-NER and GG-NER share the same pathway. The transcription factor complex TFIHH is recruited to the lesion and opens up the DNA, further recruiting other NER repair factors to form a pre-incision complex. The damaged nucleotide is removed by ERCC1-XPF and XPG, generating an SSB. New DNA is synthesized by POLβ/δ/ε and then sealed by LIG1 or LIG3.

Apart from ROS, direct SSBs can also be generated by aborted topoisomerase I (TOP1) activity, which occurs when TOP1-initiated breaks meant to relax supercoiled DNA are not resolved. These persisting breaks are termed TOP1 DNA cleavage complexes (Top1cc) (El-Khamisy et al., 2005). Top1cc accumulation poses a significant threat to the nervous system. First, oxidative DNA damage has been shown to impede Top1cc resolution (Darouei et al., 2004), making neurons particularly sensitized to aborted TOP1 activity. Second, individuals with defunct Tyrosyl-DNA Phosphodiesterase 1 (TDP1), the SSB repair enzyme responsible for resolving Top1ccs, develop spinocerebellar ataxia with axonal neuropathy (SCAN1). This genetic disease is primarily defined by nervous system deficits such as ataxia, neuropathy, and cerebellar atrophy (Takashima et al., 2002; El-Khamisy et al., 2005).

**Double-Strand Breaks (DSBs)**

While SSBs are the more common form of DNA damage, double-strand breaks (DSBs) have the higher potential for toxicity. Indeed, it is popularly cited that just one DSB can induce cell cycle arrest and subsequent apoptosis (Huang et al., 1996). However, despite their toxicity, DSBs have also been shown to play important roles in cell physiology. For example, DSBs are required for T-cell receptor and antibody diversity, chromosomal recombination during meiosis, and in the case of neurons, assist in the expression of immediate early genes (Fig 1) (Suberbielle et al., 2013; Madabbushi et al., 2015; Alt & Schwer, 2018).

While replication is likely the primary cause of DSBs in cycling cells, postmitotic neurons presumably incur the majority of their DSBs through transcriptional activity. SSBs may form DSBs through their collision with the transcriptional machinery and replication forks, or through their close proximity to another SSB. DSBs are also directly generated by transcription (Cannan & Pederson, 2016), whereby topoisomerase II (TOP2) induces transient DSBs to relieve torsional stress and facilitate gene expression. These Top2 cleavage complexes (Top2ccs) are usually resolved immediately by Tyrosyl-DNA Phosphodiesterase 2 (TDP2). Similar to TDP1, mutations in TDP2 result in a rare neurological disease termed spinocerebellar ataxia autosomal recessive 23 (SCAR23), further underscoring the potential toxicity of topoisomerase-induced DNA damage in the nervous system (Zagnoli-Vieira et al., 2018; Gómez-Herreros et al., 2014; 2; Errichiello et al., 2020).

There are two methods of DSB repair: non-homologous end joining (NHEJ) and homologous recombination (HR). HR is considered an error-free method of DSB repair by which resected DNA strands utilize their sister chromatid as a template for DNA synthesis. First, the MRN (MRE11, RAD50, NBS1) complex binds to either side of the DSB to facilitate end resection by nucleases and helicases, including C-terminal binding protein-interacting protein (CtIP), EXO-nuclease 1 (EXO1), DNA replication helicase/nuclease 2 (DNA2), Werner syndrome helicase (WRN), and Bloom syndrome helicase (BLM). The resulting ssDNAs are coated by replication protein A (RPA) and RAD51, forming nucleoprotein filaments that invade the sister chromatid to look for sequence homologies. New DNA is then synthesized by a polymerase and ligated with LIG1 or LIG3.

Because HR requires sister chromatids, this pathway can only occur in cycling cells during or following S phase. In contrast, NHEJ operates in all phases of the cell cycle and thus is the only DSB repair pathway available to post-mitotic cells. In canonical NHEJ, DSBs are first bound on either end by Ku70/80 and DNA-dependent protein kinase (DNA-PK), then directly ligated back together with Ligase IV (LIG4), X-Ray Repair Cross Complementing 4 (XRCC4), and XRCC4-like factor (XLF). In an alternate form of NHEJ (alt-NHEJ), the broken strands are resected with the same nucleases and helicases used for HR (CtIP, EXO1, DNA2, BLM, WRN), resulting in single-strand overhangs at either side of the break site. These
overhangs then anneal at microhomologies, which are small stretches of complementary DNA, usually 5–20 bp long. Polymerase θ (POLθ) synthesizes new DNA which is then ligated by LIG3. Yet another alternative DSB repair pathway, termed single-strand annealing (SSA), searches for even larger homologies (> 25 bp). RAD52 mediates the annealing of resected DNA at these larger homologous sequences, and the resulting DNA flaps are excised by ERCC1-XPF. Both alt-NHEJ and SSA are inherently error-prone, as deletions of DNA and translocations must occur to facilitate strand annealing.

**SSB and DSB sensing**
Following break induction, chromatin is rapidly modified by DNA damage sensors to facilitate the recruitment of DNA repair proteins.

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**Figure 1. Sources of DNA damage in the brain.**
Transcriptional activities can result in topoisomerase cleavage complexes, which lead to the induction of SSBs or DSBs depending upon the topoisomerase in question. Additionally, metabolic activity by mitochondria generate ROS, which can scar DNA bases with oxidative modifications. Although less common in the adult brain, cell division is also a source of DNA damage. Proliferation increases the chance of replication fork and transcription complex collision, thereby inducing DSBs in the developing brain, this is a particular risk for NPCs, which harbor increased translocations in long genes (where these collisions are most likely to occur) important for neuronal function. Cognitively demanding tasks recruit specific neuronal ensembles whose plasticity is highly dependent upon immediate early gene transcription. Therefore, neurons generate topoisomerase II-mediated DSBs in response to learning and memory. Finally, the proteins responsible for various neurodegenerative diseases have also been found to play roles in DNA damage detection and repair. (Created with BioRender.com).
Poly(ADP-ribose) polymerase 1 (PARP1) and Ataxia telangiectasia mutated (ATM) are two major sensors of SSBs and DSBs. PARP1 senses both SSBs and DSBs, rapidly generating poly(ADP-ribose) (PAR) chain scaffolds (PARylation) on itself and other target proteins to recruit DNA repair proteins and relax chromatin at break sites. PARylation by PARP1 recruits X-Ray Repair Cross Complementing 1 (XRCC1) which is a crucial stabilizer for end-processing enzymes POL5 and LIG3. PARylation also facilitates MRN recruitment at DSBs. Similarly, following its activation by the MRN complex at DSB sites, the protein kinase ATM phosphorylates numerous downstream substrates such as histone variant H2A.X, checkpoint kinase 2 (CHK2), and p53 to initiate DSB repair, cell cycle arrest, and apoptosis, respectively. In particular, phosphorylation of H2A.X (γH2AX) by ATM is a critical post-translational modification, flanking DSB sites more than 500 kb upstream and downstream to form γH2AX foci, which function as docking sites for chromatin remodelers and DNA repair proteins.

Neurotoxicity associated with DNA damage

The nervous system is particularly sensitive to loss-of-function mutations in DNA repair proteins. The explanation for this sensitivity may lie in the hallmarks of neuronal identity. That is, neurons perform transcriptionally and energetically demanding cellular functions and are post-mitotic and long-lived. The consequence of these features is elevated ROS byproducts, exclusion of error-free HR repair, age-associated decline in DNA repair enzyme efficiency, and an overall increased chance of somatic mutation. This is not to say that other cell types in the nervous system (i.e., astrocytes, oligodendrocytes, and microglia) are not susceptible to DNA damage. Indeed, DNA damage in glia plays demonstrable roles in neurodegeneration, as we will discuss later. However, compared to neurons, glial cells are replaceable, have lower energy requirements, and, in some cases, are able to re-enter the cell cycle, thus facilitating DNA repair. Combined, these features reduce the burden of DNA damage toxicity in glia. Therefore, in the following sections, we take a neuro-centric approach to interpreting the effects of DNA damage on the nervous system.

Functional roles for DNA damage in neuronal activity and development could lead to dysfunction later in life

Another reason why neurons are so susceptible to genomic toxicity stems from the fact that DNA breaks seem to serve a functional role in neuronal activity. Stimulating primary neurons with bicuculline or subjecting mice to fear learning results in the induction of DSBs at the promoters of immediate-early genes (Fig 1) (Madabhushi et al., 2015; Stott et al., 2021). Even simply introducing a mouse to a new environment is quickly followed by the induction of DSBs in neurons (Suberbielle et al., 2013, 2015). These activity-induced DSBs are hypothesized to facilitate the expression of immediate early genes through the rapid resolution of topological constraints at their transcription start sites. Previously, to identify the induction of DSBs at immediate-early gene promoters, researchers have utilized γH2AX chromatin immunoprecipitation (ChIP) sequencing (Madabhushi et al., 2015; Stott et al., 2021), which generates broad peaks associated with DSB detection. More recently developed technologies may help improve the resolution of activity-associated break induction in neurons (Rybin et al., 2021). For example, a DSB-mapping technique known as END-seq was recently used to identify strand breaks in human induced pluripotent stem cell (iPSC)-derived neurons, resulting in the finding that enhancers are hotspots for SSBs (Canéla et al., 2016; Wu et al., 2021). Newer break-mapping techniques include single nucleotide precision, Break Labeling In Situ and Sequencing (BLISS) for DSBs (Yan et al. 2017) and single-strand break mapping at nucleotide genome level (SSINGLE) for both SSBs and DSBs (Cao et al., 2019). However, the utility of these techniques has yet to be evaluated, as currently neither has been used to analyze the location of DNA breaks in neurons in physiologic or pathological conditions.

Crucially, while DNA breaks may serve a physiological function in learning and memory, their recurrence in neuron regulatory sequences makes these regions extremely vulnerable to mutation and translocation. One can imagine that over time, erroneous DSB repair could lead to mutations that result in transcriptomic dysfunction, which could further manifest at the cellular level as impaired synaptic signaling. In line with this hypothesis, DNA repair mapping reveals that postmitotic neurons do indeed accumulate breaks in regulatory elements associated with neuronal function (Fig 2) (Reid et al., 2021; Wu et al., 2021).

In addition to postmitotic activity, it is clear that somatic mutations induced by erroneous DNA damage repair or transposable elements are a common feature of neural development, giving rise to neuron diversity through genomic mosaicism (McConnell et al., 2013; Alt & Schwer, 2018; Lodato & Walsh, 2019). While most mutations are likely to be neutral, work has shown that some may underly neurodevelopmental and neurodegenerative disease. For example, work mapping translocations in iPSC-derived neural precursor cells (NPCs) under replicative stress reveal that DSB hotspots reside in long genes that are important for neuronal function and are risk factors for autism spectrum disorder and schizophrenia (Wei et al., 2016, 2018; Wang et al., 2020). This mapping was accomplished through a technique known as linear amplification-mediated high-throughput, genome-wide, translocation sequencing (LAM-HTGTS). Using this technique, endogenous DSBs are identified based on their translocation to a “bait” DSB located at a specific region in the genome. Furthermore, a study performing whole exome sequencing of hippocampal tissue from individuals with Alzheimer’s disease (AD) revealed that somatic single nucleotide variations (SNVs) increase with age and are enriched in genes that regulate tau phosphorylation (Park et al., 2019). Accumulation of SNVs at neurodegeneration risk genes could potentially increase risk of disease development.

DNA repair syndromes as proxies for aging and neurodegenerative disease

Some of the most long-standing evidence for the role of DNA damage in aging and neurodegeneration stems from inheritable DNA damage disorders, which frequently present with neurologic abnormalities. With the exception of AOA5, which seems to have manifested exclusively in adults so far (Hoch et al., 2017: 1; O’Connor et al., 2018: 1), the majority of DNA damage disorders typically present in early childhood. Why then would these disorders support the hypothesis that DNA damage plays critical roles in aging and neurodegeneration? First, as discussed in the previous section, we must acknowledge the pivotal role of DNA damage repair in
neurodevelopment, a period in which the rapid proliferation of NPCs results in profound transcriptional and replication-associated DNA damage. Deficient DNA damage repair in these vulnerable NPCs results in developmental abnormalities such as microcephaly, which is a feature of many DNA damage disorders. However, many DNA damage disorders are also defined by age-associated pathologies such as progressive brain atrophy and peripheral neuropathy. In these cases, it is likely that post-mitotic neurons are bearing the brunt of the DNA repair deficit. Thus, whether the resulting pathologies of a DNA repair deficit are developmental or age-associated likely depends on both the brain cell type composition at the time and the selective vulnerabilities of different neuronal subtypes to different repair deficits. Finally, the pathogenic load of a loss-of-function mutation accelerates the development of age-associated pathology, which may account for a DNA damage disorder presenting in childhood rather than later in life. In contrast, an individual devoid of DNA repair mutations must experience the progressive stress of aging (i.e. damage accumulation, oxidative stress, declining DNA repair enzyme efficiency) in order to recapitulate the pathogenic load of a DNA damage disorder. In the following

Figure 2. DNA lesions and mutations identified in neural genes and the techniques used to map them.

While all cell types incur DNA damage and mutations, neurons in particular are susceptible due to activity-induced transcription. Immediate-early genes and other neuronal genes that enable synaptic function are highly transcribed. Accordingly, they accumulate DNA lesions and mutations in their gene body (Wei et al., 2016) and regulatory regions (Lodato et al., 2015; Reid et al., 2021; Wu et al., 2021). The induction of DSBs in the promoters of immediate-early genes facilitates gene expression (Madabhushi et al., 2015). Over time, these insults may impair neural function (Lu et al., 2004; Lodato et al., 2018; Pao et al., 2020). (Created with BioRender.com).
subsections, we highlight two recently developed models of DNA damage disorders (one driven by mutant XRCC1, and the other by mutant ATM, and APTX) that have clarified mechanisms of DNA damage-mediated neuronal dysfunction.

**Neurological diseases caused by SSBR mutations**

To date, loss-of-function mutations in SSBR proteins have manifested exclusively as neurodegenerative syndromes. Ataxia with Oculomotor Apraxia types 1 and 4 (AOA1, AOA4) are caused by mutations in DNA end-processing enzymes Apraxatin (APTX) and Polynucleotide Kinase 3'-Phosphatase (PNKP) respectively. As their names denote, both are progressive neurodegenerative diseases typified by cerebellar atrophy, ataxia, and oculomotor apraxia. SCAN1, caused by defective TDP1, is a similar neurodegenerative syndrome additionally characterized by peripheral neuropathy (Takashima et al., 2002; El-Khamisy et al., 2005). More recently, mutations have been identified in XRCC1 (Hoch et al., 2017; O’Connor et al., 2018: 1), the protein that complexes with and stabilizes all of these end-processing enzymes. Defective XRCC1 manifests in individuals as Ataxia with Oculomotor apraxia Type 5 (AOA5), another slow-progressing neurodegenerative disease.

If SSBs are left unpaired in cycling cells, they can form DSBs upon collision with DNA replication complexes (Ryan et al., 1991). Cycling cells with defective SSBR can access error-free HR during cell division to repair the resulting DSBs. However, post-mitotic neurons are not equipped with this alternate method of repair. This may explain why diseases of SSBR are exclusive to the nervous system; neurons are not able to mitigate SSB accumulation without the presence of a functioning SSBR. To this point, recent investigations into how mutant XRCC1 confers neuropathy have helped clarify the mechanisms by which defective SSBR could be neurotoxic. First, the study of patient fibroblasts from an individual with AOA5 revealed that in the absence of XRCC1, PARP1 becomes hyperactive, producing excessive amounts of poly(ADP-ribose) (Hoch et al., 2017). Unchecked PARP1 activity can induce cell death by progressive NAD+/ATP depletion and Parthanatos, a cell death signaling pathway triggered by excessive poly(ADP-ribose) (David et al., 2009).

PARP1 hyperactivity was further demonstrated through conditional deletion of XRCC1 in the mouse brain, which resulted in progressive cerebellar degeneration, ataxia, seizure-like activity, and dysregulated presynaptic calcium signaling in the hippocampus (Hoch et al., 2017; Komulainen et al., 2021). The mechanisms by which PARP1 hyperactivity could mediate neurotoxicity and dysregulated presynaptic calcium signaling have been explored in an expanded pair of recent publications. First, in the absence of XRCC1, PARP1 was found “trapped” at break intermediates produced during BER, thus impeding access of repair factors POLβ and LIG3 (Demin et al., 2021). This indicates XRCC1 is a crucial regulator of PARP1 activity. Second, PARP1 hyperactivity in XRCC1-deficient cells was shown to suppress transcription through the recruitment of ubiquitin protease USP3, leading to excessive deubiquitination of histone substrates (Adamowicz et al., 2021).

Suppressed transcription may account for the dysregulated calcium signaling observed in neurons from XRCC1<sup>Nes-Cre</sup> mice, specifically through the suppression of genes that regulate calcium homeostasis. To this point, a separate publication revealed that iPSC-derived neurons accumulate SSBs at enhancers regulating neuronal activity (Wu et al., 2021). These SSB hotspots were identified through genome-wide mapping of DNA damage repair, dubbed SAR-seq (Synthesis After Repair), whereby EdU incorporation into break sites serves as a molecular landmark for break repair, and END-seq (Wu et al., 2021). SSB accumulation at enhancers regulating neuronal activity provides a tempting mechanistic explanation for the neurodegenerative hallmarks of SSBR syndromes.

In contrast to mutations in the SSBR pathways, mutations that dysregulate the NER pathway are additionally characterized by symptoms occurring outside of the nervous system. For example, the hallmark feature of Xeroderma Pigmentosum, caused by XP gene mutations, is skin peeling and crusting due to the skin cells’ inability to repair bulky DNA modifications caused by UV exposure. Only about 20–30% of XP individuals develop progressive neurodegeneration (Nouspikel, 2008). Furthermore, individuals with Cockayne Syndrome (CS), who are diagnosed based on delayed development, light sensitivity, and progeria, or Trichothiodystrophy (TTD), whose hallmark feature is brittle hair, can present with neurodevelopmental defects such as microcephaly, dysmyelination, and intellectual disability (Diderich et al., 2011). Notably, while postmitotic neurons are able to repair bulky DNA modifications in both the template and non-template strand of transcribed genes, global NER is naturally attenuated in non-transcribed regions of the genome (Nouspikel & Hanawalt, 2000; Nouspikel, 2008). Combined with the added stressor of NER mutations, this may account for the neurodegenerative phenotypes observed in XP, CS, and TTD.

**Neurological diseases caused by DSBR mutations**

One of the most well-known DDR syndromes is Ataxia telangiectasia (AT), which is caused by mutations in ATM kinase. Individuals with AT exhibit profound immune deficiency and increased cancer susceptibility as well as progressive cerebellar atrophy, which results in ataxia by early childhood (McKinnon, 2012). Mutations in the MRN complex also produce syndromes with similar clinical phenotypes. Specifically, defective MRE11 causes AT-like disorder, which results in cerebellar atrophy, and defective NBS1 causes Nijmegen Breakage Syndrome, which results in microcephaly (Madabhushi et al., 2014; McKinnon, 2017).

While murine knockout of ATM recapitulates the immune deficits observed in AT patients, these mutant mice seem to exhibit only mild ataxia and cerebellar atrophy (Barlow et al., 1996; Klijis et al., 1997). This has been a major limiting factor toward teasing out the molecular mechanisms of AT-related neurodegeneration. Nevertheless, studies utilizing ATM knockout mice have still revealed important roles for ATM in neuron health. First, abnormalities in lysosomal storage have been observed in Purkinje cells of ATM knockout mice, suggesting a pre-degenerative increase in cellular stress (Barlow et al., 2000). Additionally, enriched Top1ccs are also observed in the cerebellum, similar to what is observed in TDP1 knockout mice, suggesting ATM also plays a role in topoisomerase-1 mediated break sensing and repair (Katyal et al., 2007, 2014).

Finally, glial ATM seems to play crucial roles in neuronal health as well. Specifically, cerebellar cultures grown from ATM knockout mice exhibit disrupted network synchrony, which is rescued by culture with wild-type astrocytes (Kanner et al., 2018).

Notably, the development of a new mouse model of AT harboring both ATM nonsense mutation and APTX knockout was found to better recapitulate the neurologic deficits observed in AT (Perez et al., 2021). While progressive cerebellar atrophy and ataxia were
not observed in mice with individual mutations in either ATM or APTX, the combination of both mutations lowered the threshold for neuronal genomic instability, resulting in neurological deficits (Perez et al, 2021). This indicates that at least for murine models of AT, the ATM knockout alone is fairly well tolerated, and additional genomic stress is needed to bring about toxicity in neurons (Tal et al, 2018). A follow-up study utilizing this new mouse model explores an intriguing explanation for why certain neuronal populations seem selectively vulnerable to neurodegeneration observed in AT (and other DNA damage syndromes for that matter) (Kwak et al, 2021). While both cerebellar neuron subtypes (Purkinje and granule cells) experience DNA break repair deficiencies in the ATM/APTX double mutant mouse, ATAC-seq (assay for transposase-accessible chromatin using sequencing) revealed that Purkinje cells harbor uniquely open regions of chromatin that were perturbed by aberrant RNA splicing and subsequent R-loop formation (a three-stranded structure formed by an RNA:DNA template hybrid and the non-template DNA strand) (Kwak et al, 2021). The consequent disruption in Purkinje cell gene expression ultimately results in cerebellar atrophy and ataxia.

**DNA damage in neurodegenerative disease**

In addition to observations made from genetic disorders, work characterizing the molecular pathophysiology of age-associated neurodegenerative diseases further implicates DNA damage in brain aging and disease. In the following subsections, we highlight recent publications that explore the effects of DNA damage in the context of age-associated neurodegenerative disease, as well as recently developed DNA lesion mapping methodologies that may help us better define regions of the neuronal genome that are vulnerable to lesion accumulation and repair (Rybin et al, 2021).

**Oxidative DNA damage**

Increased oxidative DNA damage is observed in brain tissue from aged individuals (Mecocci et al, 1993) and patients with AD (Nunomura et al, 2001; Lovell & Markesbery, 2007; Weissman et al, 2007), Parkinson’s disease (PD) (Alam et al, 1997), Huntington’s disease (HD) (Brown et al, 1997), and amyotrophic lateral sclerosis (ALS) (Ferrante et al, 1997; Bogdanov et al, 2000). This is also accompanied by an age-associated decline in BER efficiency (Weissman et al, 2007; Xu et al, 2008; Sykora et al, 2015). Recently, a mechanism for the age and disease-associated increase in 8oxoG has been proposed. Specifically, the histone deacetylase HDAC1 has been shown to be critical for the repair of age-associated 8oxoG accumulation by increasing the activity of the DNA glycosylase OGG1 (Pao et al, 2020). In fact, the pharmacological activation of HDAC1 was shown to mitigate oxidative lesion accumulation in both aged mice and SFXAD mice (mice expressing human APP and PSEN1 with five familial AD mutations), and improve cognition in SFXAD mice (Pao et al, 2020). Importantly, the location of such oxidized bases has proven to be an important mediator of their neurotoxicity. Specifically, the aging brain contains increased 8oxoG at the guanine-rich promoters of genes regulating synaptic function, resulting in their transcriptional suppression (Fig 2) (Lu et al, 2004). 8oxoG is capable of repressing gene transcription through many mechanisms, including blocking transcription factor binding (Ghosh & Mitchell, 1999; Moore et al, 2016), recruiting chromatin remodelers that result in the methylation of gene promoters (Wang et al, 2018b; Xia et al, 2017, 4), and erroneous repair through BER, which could result in single nucleotide variations (SNVs). Accordingly, single-cell whole genome sequencing has revealed that somatic SNVs located near neuronal genes increase with age in human neurons, presumably due to errors in BER, NER, and transcription-associated repair (Fig 2) (Lodato et al, 2015, 2018). It is possible that at the population level, this accumulated genomic diversity in postmitotic neurons could result in neuronal dysfunction.

Aberrant DNA repair may also directly mediate the severity of neurodegenerative disorders like HD, whose toxicity is derived from the CAG trinucleotide expansion of the Huntingtin gene. Specifically, age-dependent CAG expansion occurs in the brains of individuals with HD, gradually increasing the toxicity of the Huntington protein in the striatum. Notably, CAG expansion has been found to be dependent upon DNA repair proteins such as OGG1, MLH1, and MSH2, whose detection of oxidized or mismatched bases in these repetitive elements could potentially elicit erroneous repair, leading to CAG expansion (Kovtun et al, 2007; Bettencourt et al, 2016; Pinto et al, 2013; Manley et al, 1999; Lee et al, 2017, 1). Gene-wide association studies have further identified SNPs in DNA maintenance genes that influence the age of onset in this disease (Lee et al, 2015; Moss et al, 2017). Therefore, erroneous DNA repair mechanisms also directly influence HD severity.

To further understand how DNA lesions accumulate in the brain, multiple sequencing techniques are now available that map these DNA lesions genome-wide. Previously, studies have approximated the location of 8oxoG lesions through OGG1 ChIP-seq (Hao et al, 2018), which is a somewhat indirect method as it captures lesion detection rather than the lesion itself. Newer antibody or biotin conjugate methods have also been developed to directly target 8oxoG (Ding et al, 2017; Amente et al, 2019); however, these techniques only permit a window of resolution of around 150 nucleotides. To this end, single-nucleotide resolution DNA lesion sequencing techniques are now being developed that could better define oxidative hotspots in the neuron genome. One such sequencing technique, Click-Code-seq, utilizes BER excision enzymes and click chemistry to specifically tag 8oxoG (Wu et al, 2018). Another sequencing technique, Nick-seq, is capable of detecting a variety of DNA modifications based on the enzyme or chemistry used to first convert the DNA modification to a strand break (Cao et al, 2020). It will be interesting to see if these new technologies can be applied to models of neurodegeneration as well as the postmortem human brain.

**SSBs and DSBs**

A plethora of studies document increased levels of both SSBs and DSBs in AD, HD, PD, and ALS (Mullaart et al, 1990; Adamec et al, 1999; McKinnon, 2013, 2017; Madabhushi et al, 2014; Alt & Schwer, 2018; Shanbhag et al, 2019; Thadathil et al, 2021), and their accumulation seem to correlate with important milestones in disease progression. Specifically, increased DDR foci are observed in neurons from individuals with mild cognitive impairment (MCI) and AD compared to aged counterparts (Shanbhag et al, 2019). Similarly, DSB marker proteins correlate with cognitive impairment in individuals with low levels of amyloid and tau pathology (Simpson et al, 2015). More recently, increased ROS and DSBs have also been documented in neurons derived directly from sporadic AD patient fibroblasts, indicating that age-dependent features of genomic
instability are recapitulated in human in vitro models of disease (Mertens et al., 2021). These findings have led to the hypothesis that DNA strand breaks may be a contributing factor to disease progression (Chow et al., 2019).

As previously discussed, one mechanism of DNA break-mediated neurodegeneration may stem from PARP1 hyperactivity. Increased PARP1 activation and elevated poly(ADP-ribose) has been observed in numerous neurodegenerative disorders and their associated animal models (Thapa et al., 2021; Mao & Zhang 2022). For example, oxidative damage-induced poly(ADP-ribose) mediates α-synuclein aggregation and consequent neurodegeneration in PD (Kam et al., 2018), facilitates the formation of cytoplasmic TDP-43 foci in ALS (McGurk et al., 2018a, 2018b), and can mediate neuroinflammatory activity in AD (Kauppinen et al., 2011). In combination with studies utilizing XRCC1-deficient neurons, these findings have motivated the development of PARP inhibitors as a potential therapeutic for a variety of nervous system disorders (Thapa et al., 2021).

In addition to PARP1 activity, many genes that encode the building blocks of neurodegenerative proteinopathies have been found to play a role in DDR as well. For example, the genes whose mutations can cause ALS or frontotemporal dementia (fused in sarcoma (FUS) and transactive response DNA binding protein 43 (TDP-43)) are involved in SSB and DSB detection and repair. FUS is an RNA/DNA binding protein that has been found to facilitate DSB repair through its colocalization with HDAC1 (Wy et al., 2013, 1). Furthermore, phosphorylation of FUS occurs in response to DSB detection by proteins ATM and DNA-PK (Gardiner et al., 2008; Deng et al., 2014), and FUS activates and recruits the XRCC1-LIG3 complex to sites of oxidative DNA damage in a PARP1-dependent manner (Naumann et al., 2018; Wang et al., 2018a). Recently, TDP-43 was also found to be directly involved in NHEJ through its recruitment and stabilization of the XRCC4-LIG4 complex at DSBs (Guerrero et al., 2019; Mitra et al., 2019). The Huntingdon protein may also play a role in DNA damage detection, potentially serving as a scaffold for ATM at sites of oxidized DNA (Mauri et al., 2017), and ATXN3 and PNKP at sites of transcription-coupled DNA break repair (Gao et al., 2019).

Finally, α-synuclein, whose cytoplasmic aggregation is a hallmark of PD, is able to bind double-strand DNA and facilitate NHEJ in homeostasis (Scherer et al., 2019). However, toxic aggregation of α-synuclein in the nucleus or cytosol elicits the accumulation of DSBs (Vasquez et al., 2017; Milanese et al., 2018). Tau may also play a role in DSB repair as well, as its deletion in the mouse brain leads to the accumulation of DSB foci in neurons (Violet et al., 2014; Mansuroglu et al., 2016).

While these findings have firmly established DNA breaks as a mechanism of neurodegenerative disease, the mechanisms by which break location may contribute to disease progression are not well described. To this end, genome-wide profiling of DNA breaks in post-mitotic neurons have revealed that their locations are likely major mediators of their toxicity. As described earlier, the use of nucleoside analog incorporation to map DNA repair has led to the observation that postmitotic neurons harbor SSBS and other forms of breaks in the regulatory regions of genes that modulate synaptic plasticity and neuronal function (Reid et al., 2021; Wu et al., 2021). It follows that while these breaks may be functionally relevant, over time, their erroneous repair could lead to disruptive translocations, mutations, and genomic instability.

Neuroinflammation associated with DNA damage

In addition to DNA damage, neuroinflammation has emerged as a core feature and mechanism of neurodegenerative disease. Indeed, many of the risk genes associated with AD mediate their effects through microglia, the brain-resident macrophages (Glass et al., 2010; Heneka et al., 2015; Nott et al., 2019). More recently, researchers have identified senescent-like brain cells in murine models of AD and tauopathy, and their removal via senolytic drugs seems to mitigate pathology and improve cognition (Bussian et al., 2018; Musi et al., 2018; Zhang et al., 2019; Ogrodnik et al., 2021). Thus, senescence is now considered as a potential driver of neuroinflammation and neurodegeneration. Importantly, while not well-studied in the context of the degenerating brain, the mechanistic link between DNA damage and senescence has already been defined in great detail (d’Adda di Fagagna, 2008; Miyamoto, 2011; Li & Chen, 2018). Below, we discuss recent mechanistic insights between DNA damage and neuroinflammation, and how DNA damage may influence neurodegeneration through the activation of senescent-like signaling from different brain cell types.

DNA damage induces senescence

DNA damage is a potent activator of inflammatory signaling and senescence (Coppé et al., 2008; Rodier et al., 2009; Brzostek-Racine et al., 2011; Hårtlova et al., 2015). Initially defined by the permanent cessation of cell division and evasion of apoptosis, senescence is an age-associated cellular state that is thought to contribute to organismal aging, cancer, and more recently, neurodegeneration (Di Micco et al., 2021). Importantly, removal of senescent cells promotes healthy aging in mice, making senescence-associated inflammatory signaling an attractive target of therapeutic intervention for aging and age-related disease (Tilstra et al., 2012; Childs et al., 2015; Baker et al., 2016; Ogrodnik et al., 2021). Senescent cells can be identified by a variety of factors, one being the secretion of pro-inflammatory cytokines (termed senescence-associated secretory phenotype (SASP)) to modulate their microenvironment. Initially, induction of persistent DDR signaling in cell cultures including fibroblasts (Coppé et al., 2008; Rodier et al., 2009), bone marrow-derived macrophages (BMDMs) (Hårtlova et al., 2015), and primary monocytes (Brzostek-Racine et al., 2011) was found to elicit the SASP (Coppé et al., 2008). This secretory profile was found to be at least partially ATM-dependent, implying the importance of DSB repair specifically in immune activation (Rodier et al., 2009; Brzostek-Racine et al., 2011). Previous work with AT fibroblasts had shown that the canonical immune transcription factor NF-kB is activated in an ATM-dependent manner following treatment with a radiomimetic, ionizing radiation, or topoisomerase inhibitor (Lee et al., 1998; Piret et al., 1999; Li et al., 2001). This suggested NF-kB was critical for the expression of pro-inflammatory signaling molecules following DNA damage. Subsequent studies confirmed that following DSB induction, activated ATM couples with a number of protein intermediates leading to its cytoplasmic transport and downstream activation of NF-kB (Hinz et al., 2010; Wu et al., 2010; Miyamoto, 2011). In addition to inducing the expression of inflammatory signaling proteins, NF-kB also exerts anti-apoptotic activity through the expression of caspase inhibitors such as FLIP, XIAP, and c-XIAP. This directly counters pro-apoptotic activity mediated by DNA damage-induced p53 signaling. Thus, the interplay between NF-kB and p53...
strongly influence cellular fate following DNA damage accumulation (Karin & Lin, 2002).

Interestingly, a subsequent study revealed that DNA damage can also evoke type-I interferon signaling in AT cells due to the accumulation of cytosolic DNA fragments, suggesting activation of inflammatory signaling via DNA damage can occur independent of the DDR. Cytosolic DNA, the source of which can be DNA damage (Dou et al., 2017; Erdal et al., 2017; Glück et al., 2017; Yang et al., 2017), micronuclei rupture (Härtlova et al., 2015), or de-repression of retrotransposable elements (De Cecco et al., 2019). In addition to genomic DNA, mitochondrial DNA (mtDNA) is also a major source of innate immune elements (De Cecco et al., 2015), or de-repression of retrotransposable elements (De Cecco et al., 2019).

DNA damage can be sensed by the nucleic acid sensor cyclic GMP-AMP synthase (cGAS) which produces the second messenger cGAMP upon binding to free-floating DNA. The production of cGAMP in turn activates Stimulator of IFN Genes (STING). The activation of STING mediates a number of downstream signaling cascades, including the activation of both IRF3 and NF-κB, thus resulting in the expression of interferons and pro-inflammatory cytokines (Abe & Barber, 2014; Dunphy et al., 2018; Li & Chen, 2018). Therefore, DNA damage has direct molecular links to senescence-associated inflammatory signaling, both through DDR pathway activation and the mis-localization of DNA itself (Fig 3).

Additional mechanisms exist to elicit inflammatory gene transcription following the detection of cytosolic nucleic acids (Miller et al., 2021). This includes TLR9 (Toll-like receptor 9), which binds to double-stranded DNA engulfed in endosomes, particularly bacterial DNA or mtDNA. This recruits adaptor protein MYD88 (myeloid differentiation primary response 88) to activate NFκB and stimulate the expression of inflammatory genes. STING also facilitates the formation of the IKK complex, which phosphorylates IκB to activate NFκB. TLR9 pathway: Endosomal double-strand DNA is bound by TLR9, activating MyD88, which interacts with and activates IRAK1 and IRAK4. IRAK1 and 4 dissociate from MyD88 and activate TRAF6. TRAF6 ubiquitinates NEMO, a member of the IKK complex that results in NFκB translocation into the nucleus. Inflammasome pathway: NLPR3 detects cytosolic DNA, leading to the assembly of the NLPR3, ASC, pro-Caspase 1 inflammasome. pro-Caspase 1 autoproteolytically matures to functional Caspase 1, which cleaves pro-IL1b and pro-IL18 to generate functional IL1b and IL18.

**Figure 3. DNA damage initiates inflammatory signaling through DDR and cGAS-STING.** ATM–NF-κB pathway. The activation of ATM by DSBs leads to its coupling with NEMO in the nucleus. NEMO is phosphorylated by ATM and SUMOylated by PIASy in a PARP1-dependent manner, which is not shown here. These modifications lead to NEMO monoubiquitination, and the ATM–NEMO complex is transported to the cytoplasm. Here, NEMO partners with IKKβ and IKKα to form the active Inhibitor of KappaB Kinase (IKK) complex. The IKK phosphorylates IκB, allowing NFκB to be transported to the nucleus. The most common form of NFκB is the heterodimer p50–p65, shown here. The phosphorylation of IκB leads to its polyubiquitination and subsequent degradation. cGAS–STING pathway: DSBs result in the leakage of self-DNA into the cytosol, which is sensed by cGAS. cGAS generates second messenger cyclic GAMP. cGAMP binds to STING, which activates TANK-binding kinase 1 (TBK1), which in turn activates IFN Regulatory Factor 3 (IRF3). Homodimerized IRF3 transports to the nucleus and activates the expression of inflammatory genes. STING also facilitates the formation of the IKK complex, which phosphorylates IκB to activate NFκB. TLR9 pathway: Endosomal double-strand DNA is bound by TLR9, activating MyD88, which interacts with and activates IRAK1 and IRAK4. IRAK1 and 4 dissociate from MyD88 and activate TRAF6. TRAF6 ubiquitinates NEMO, a member of the IKK complex that results in NFκB translocation into the nucleus. Inflammasome pathway: NLPR3 detects cytosolic DNA, leading to the assembly of the NLPR3, ASC, pro-Caspase 1 inflammasome. pro-Caspase 1 autoproteolytically matures to functional Caspase 1, which cleaves pro-IL1b and pro-IL18 to generate functional IL1b and IL18.
expression of inflammatory cytokines. An additional mechanism of cytosolic DNA-mediated inflammation is through the NLRP3 inflammasome, which senses cell stressors (such as cytosolic DNA) to activate the cleavage of pro-IL1β and pro-IL18 via Caspase I to elicit their secretion. In particular, microglial NLRP3 has been shown to be a major driver of amyloid beta and tau toxicity in AD, suggesting its activation has important implications for disease progression (Halle et al., 2008; Venegas et al., 2017; Ising et al., 2019).

Interestingly, work characterizing the neurodevelopmental defects observed in the neuroinflammatory disorder Aicardi-Goutières Syndrome (AGS), a type I interferonopathy, indicates that DNA damage can mediate cellular toxicity through multiple different mechanisms in the same disease. AGS is caused by mutations in genes that regulate nucleic acid metabolism. For example, deficits in RNASEH2, an enzyme responsible for removing ribonucleotides from DNA and resolving R-loops, comprise more than half of AGS diagnoses (Crow & Manel, 2015). Accordingly, RNASEH2 murine models of AGS reveal that cGAS-STING sensing of micronuclei is the cause for type I IFN signaling in this disease (Mackenzie et al., 2016, 2017; Pokatayev et al., 2016). However, recent work investigating the cause of AGS neurotoxicity revealed that it is the DDR-mediated activation of p53, not cGAS-STING, that causes neuron cell death, although increased micronuclei and R-loops were observed in astrocytes (Aditi et al., 2021). This indicates that different phenotypes of DNA damage response deficits are mediated by different pathways, which may have preferential function in different cell types.

**Senescence-associated inflammation in brain aging and neurodegeneration**

It has since been shown that removal of senescent cells in the brain mitigates neurofibrillary tangle (NFT) burden, amyloid burden, neuronal loss, and cognitive decline in mouse models of tauopathy (Bussian et al., 2018; Musi et al., 2018), AD (Zhang et al., 2019), PD (Chinta et al., 2018), and normal aging (Ogrodnik et al., 2021). Notably, each of these studies attribute different cell types as the primary sources of senescence. For example both microglia (Bussian et al., 2018), astrocytes (Bussian et al., 2018), and neurons (Musi et al., 2018) have been found to display senescent-like phenotypes in Tau P301S mice, and have been proposed as drivers of cognitive decline and neurodegeneration. Additionally, senescent astrocytes have been identified as modulators of PD pathology (Chinta et al., 2018). Senescent-like phenotypes have also been identified in oligodendrocyte precursor cells (OPCs) in APP/PS1 mice, and their removal has been shown to mitigate amyloid load and cognitive decline (Zhang et al., 2019). Finally, removal of senescent microglia from aged mice has been found to improve cognition (Ogrodnik et al., 2021). In total, these findings place senescence as an encouraging target for therapeutic intervention in neurodegenerative disease, but bring into question how senescence presents in different brain cell types, and how this might affect the progression of various diseases.

Studies have also made more direct links between DNA damage, neuroinflammation, and neurotoxicity. For example, ATM-deficient microglia have been shown to accumulate cytosolic DNA, thus activating the cGAS-STING pathway to elicit pro-inflammatory and neurotoxic signals (Quek et al., 2017; Song et al., 2019). Interestingly, sequencing the cytosolic DNA fragments from these microglia revealed that most were derived from the nuclear DNA as intergenic repetitive elements, suggesting that the source of cytosolic DNA may not be random (Song et al., 2021). Recent single-nucleus RNA sequencing of AT cerebella reveals that microglial activation likely precedes neuronal degeneration, further emphasizing the role of microglia in ATM-mediated neuropathology (Lai et al., 2021).

**The role of neuronal DNA damage in neuroinflammation**

Notably, the concept of a senescent-like phenotypes in neurons has been particularly controversial, presumably because senescence was initially defined in the context of cycling cells (Gillispie et al., 2021; Sah et al., 2021). Nevertheless, observations of senescent-like phenotypes in neurons have been reported for quite some time. As early as 2012, the term “senescence” was used to describe pathological features of neurons in the aged mouse brain, including increased DSBs, lipid peroxidation, and senescent-associated β-galactosidase staining (Jurk et al., 2012). Here, DSBs were considered the major driver of these senescence-associated phenotypes because knock-out of p21, a protein activated by p53 to initiate DSB-induced cell cycle arrest, was able to mitigate their enrichment. Interestingly, re-inspection of older transcriptional data also revealed similar enrichment in AD neurons. In a 2006 study, neurons with high levels of tau pathology or no tau pathology were isolated from AD brain tissue and transcriptionally characterized (Dunckley et al., 2006). This dataset was later re-analyzed to show that neurons with high levels of tau were enriched for signatures of DNA damage and senescence (Dunckley et al., 2006; Musi et al., 2018). Senescent-like phenotypes in neurons have also been noted in other models of aging and neurodegeneration (Chow et al., 2019; Moreno-Blas et al., 2019).

Because DNA damage accumulates in neurons early on in AD, and because it is also a primary driver of senescence, it is worth hypothesizing that DNA lesions mediate neurodegeneration at least in part through neuronal senescence, although senescence in other cell types clearly also plays important roles in neurodegeneration. Nevertheless, future research will have to dissect how different cell types react to senescent neurons, and how this may facilitate the development of neurodegenerative disease. Importantly, as DNA damage is a well-established source of inflammatory signaling, this uncovers a previously unstudied aspect of neuronal response to DNA damage. Compared to microglia and astrocytes, the capacity for inflammatory signaling in neurons has received little attention. Indeed, the protein machinery required to detect cytosolic DNA such as STING is reported to be low in neurons compared to glial cells (Mathur et al., 2017; Song et al., 2019). However, work examining the immune response to neurotropic viral infections reveals neuron-derived inflammatory signaling is a critical feature of the antiviral response (Klein et al., 2005; Chakraborty et al., 2010; Di Liberto et al., 2018). The detection of viral nucleic acids utilizes the same cGAS-STING pathway as that described for detection of self-DNA within the cytosol of senescent cells, suggesting that the mechanism of innate immune signaling in DSB-bearing neurons may be of some significance. Indeed, observations of neuron-derived immune signaling at early stages of neurodegenerative disease indicate that this may play a role in disease progression (Dutta et al., 2020; Weliko-vitch et al., 2020). Notably, neuronal cGAS-STING has been shown to be activated in models of HD and ALS, suggesting that toxic protein aggregates may stimulate DNA release (mitochondrial or
In need of answers

1. Are neuron subtypes differentially vulnerable to DNA damage? The wide range of activity and metabolic demands encompassed by neuronal subtypes may predispose certain populations to genomic toxicity. Single cell sequencing techniques are likely to play a crucial role in dissecting the differential responses to lesion accumulation.

2. What is the functional role of DNA damage-mediated senescence in neurons? It will be important to determine if neurons harboring DNA damage play meaningful roles in age-associated neuroinflammation and neurodegeneration.

3. How does lesion and mutation accumulation in neuronal genes result in neurotoxicity? Numerous studies reveal scars of DNA damage are most abundant around genes important for synaptic function. However, it is unclear how these mutations modify transcription. While this technology is still in its infancy, simultaneously profiling of the genome and transcriptome at single-cell resolution would clarify the functional readout of these mutations.

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