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DNA Strand Breaks, Neurodegeneration and Aging in the Brain

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Abstract

Defective responses to DNA single- or double-strand breaks can result in neurological disease, underscoring the critical importance of DNA repair for neural homeostasis. Human DNA repair-deficient syndromes are generally congenital, in which brain pathology reflects the consequences of developmentally incurred DNA damage. Although, it is unclear to what degree DNA strand-break repair defects in mature neural cells contribute to disease pathology. However, DNA single-strand breaks are a relatively common lesion which if not repaired can impact cells via interference with transcription. Thus, this lesion, and probably to a lesser extent DNA double strand breaks, may be particularly relevant to aging in the neural cell population. In this review we will examine the consequences of defective DNA strand break repair towards homeostasis in the brain. Further, we also consider the utility of mouse models as reagents to understand the connection between DNA strand breaks and aging in the brain.

Keywords

DNA damage; DNA repair; nervous system; aging; ATM; AOA1; SCAN1

INTRODUCTION

The mammalian nervous system is formed through continual cycles of proliferation, differentiation and maturation to generate the large number of cell types required for function (Jacobsen 1991). Although the human nervous system forms in a matter of months, neural tissues must be functional for decades of life, and the mature neurons bear the brunt of handling a lifetime of potential threats to the integrity of their DNA. Due to the substantial oxygen requirement for maintenance of CNS tissue, neurons must cope with oxidative and metabolic stress that can result in DNA strand breaks (Lombard et al. 2005; Barzilai 2007; Chen et al. 2007). Accordingly, neurons require efficient DNA strand-break surveillance and repair mechanisms to deal with these types of lesions. Human neurological syndromes resulting from defects in DNA repair highlight the importance of multiple repair pathways for maintaining homeostasis in the brain (Rolig and McKinnon 2000; McKinnon and Caldecott 2007; Subba Rao 2007). Hence, individuals who incur genetic mutations that inactivate these repair pathways show accelerated neuronal death, which can manifest as neurodegenerative disease. As most of these inherited syndromes are congenital, less is known about the effects of DNA repair deficiency during aging. Nonetheless, there are many

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studies reporting a link between aging and a decline in DNA repair activity (Intano et al. 2003; Lu et al. 2004; Vijg and Calder 2004; Imam et al. 2006; Gorbunova et al. 2007; Rutten et al. 2007; Wilson and Bohr 2007). However, the causal relationship between decreased DNA repair activity, increased mutations and an effect upon aging still has to be thoroughly evaluated. Clearly, understanding the role of DNA repair and aging in the brain will require suitable model systems and careful *in vivo* assessment of how the spatiotemporal changes in DNA repair capacity affects neural homeostasis.

This review will emphasize the requirement for DNA strand-break repair in the context of neural development. We will consider neurodegenerative diseases that are directly attributable to failure in strand-break responses and the importance of these pathways in the nervous system. We will also discuss the utility of mouse models of DNA repair deficiency as an important tool for understanding the impact of genomic instability in the brain, and how more refined genetic manipulation of the mouse will help us better understand the links between DNA repair deficiency and age-related disease of the CNS.

Neural development

Formation of mature neural tissue requires the expansion and differentiation of precursor cells into a variety of neural cell types that migrate, organize and stratify into distinct CNS structures. Figure 1 illustrates the CNS with expanded views of two representative tissues, the retina and cerebellum, illustrating the laminar structure of these tissues. In many DNA repair syndromes the cerebellum is often a target, and as the cerebellum is responsible for motor coordination, ataxia is associated with these syndromes (Frappart and McKinnon 2006; Lee and McKinnon 2007). Although the cerebellum only comprises 10% of the total brain mass, it contains approximately 50% of the neurons in the brain. The cerebellum is primarily composed of three general neuronal populations, the granule cells, the Purkinje cells and interneurons, with each type found in distinct cell layers; the inner granule layer, the Purkinje cell layer and interneurons which are found throughout the molecular layer and the inner granule layer (Fig. 1). During development, an external germinal layer is present and as cerebellar development progresses, granule neuron precursors generate granule cells that migrate inwards and populate the IGL as the external granule layer gradually diminishes (Goldowitz and Hamre 1998; Wang and Zoghbi 2001). The cell populations of the cerebellum are notable because granule cells are the most numerous neuronal cell type in the brain, while Purkinje cells are amongst the largest neuronal cell type in the brain. The outer molecular layer consists of interneurons (stellate and basket cells) together with Purkinje cell dendrites and parallel fibers arising from granule cells, making the molecular layer a synapse-rich area (Jacobsen 1991; Goldowitz and Hamre 1998). As the cerebellum serves primarily to control sensory-motor function, individuals with cerebellar neurodegenerative disorders, such as spinocerebellar atrophy and ataxia-telangiectasia, present with ataxia (impaired motor coordination) and eye-movement defects and speech disturbance (dysarthria) (Frappart and McKinnon 2006; Limperopoulos and du Plessis 2006).

While the cerebellum is often affected in diseases associated with DNA strand break repair, progressive widespread neurodegeneration also occurs. In many cases these progressive changes are a later event than the effects upon the cerebellum. The likely scenario is that the cerebellum and perhaps granule cells in particular are very susceptible during postnatal neurogenesis to DNA damage. Furthermore, as defects in the cerebellum generally present as ataxia, an obvious movement disorder, this may cause milder cortical defects to be initially overlooked. As will be discussed later, mice with defective DNA strand break repair further reveal the cerebellum as a primary lesion in the nervous system.

Repair of DNA strand breaks

DNA strand breaks can occur as either single-strand breaks (SSBs) or double-strand breaks (DSBs) and biochemically distinct pathways repair these lesions. DNA DSBs are repaired by either non-homologous end-joining (NHEJ) or homologous recombination (HR), while SSBs are repaired by the DNA SSB repair (SSBR) pathway (Caldecott 2003; Lieber et al. 2003; West 2003; Wyman and Kanaar 2006; Helleday et al. 2007). In the nervous system DNA strand breaks can arise endogenously from normal cellular metabolism, during DNA replication or from exogenous agents such as ionizing radiation or chemicals in the environment. In differentiated neurons that do not divide, DNA DSBs clearly occur independently of replication and may result from the formation of adjacent SSBs via oxidative stress.

The choice between HR and NHEJ is related to the availability of a sister chromatid with which to use as an intact template for repair. Therefore, HR is restricted to replicating cells, and in the nervous system these are the progenitor populations that give rise to neurons and glia, and are found in the proliferative ventricular zones. Thus, HR promotes error-free DNA DSB repair and is the major DNA repair pathway utilized during early mammalian development, particularly in tissues that give rise to the nervous system (Orii et al. 2006). A large recombinase complex (composed of RAD50, RAD51, RAD54, XRCC2 and XRCC3) is recruited to the DNA break site in a BRCA2-dependent manner to mediate HR (West 2003; Wyman and Kanaar 2006; Helleday et al. 2007). The requirement for an undamaged sister-chromatid restricts usage of HR to the S and G2 phase of the cell cycle. In contrast, NHEJ utilizes end-processing enzymes to facilitate ligation of incompatible DNA ends. Major proteins involved in NHEJ include the Ku heterodimer (Ku70/80), DNA protein kinase (DNA-PK_{cs}) and the XRCC4/LigaseIV/XLF complex (Lees-Miller and Meek 2003; Lieber et al. 2003; Wyman and Kanaar 2006; van Gent and van der Burg 2007). The Ku complex binds to DNA ends and recruits the DNA-PK_{cs} protein kinase to facilitate efficient DNA ligation by XRCC4/LigIV/XLF. In differentiating and post-mitotic neurons, the repair of DSBs occurs via NHEJ (Orii et al. 2006).

Coincident with DNA repair is the activation of DNA damage-induced signaling pathways that serve to halt the cell cycle while DNA repair occurs, or alternatively to activate apoptosis where the damage is extensive (Shiloh 2001; Kastan and Bartek 2004; Ward and Chen 2004). Amongst the earliest signaling events resulting from DNA DSBs is the phosphorylation of histone H2AX (Sedelnikova et al. 2003). This event serves to retain DSB sensing factors at the DNA break sites, thereby promoting efficient DNA repair. Recruitment of the DNA damage sensing Mre11-Rad50-Nbs1 (MRN) complex to the DSBs is important for activating the apical signaling kinase ATM, which effects cell cycle arrest or apoptosis (Carson et al. 2003; Petrini and Stracker 2003; Uziel et al. 2003; Difilippantonio et al. 2005). In the developing nervous system the activation of apoptosis after genotoxic stress is often a preferred course as cell replacement can readily occur from widespread germinal zones throughout the embryonic nervous system and at some postnatal stages such as the maturing cerebellum (Lee and McKinnon 2007). However, defects in many components of the DNA DSB repair machinery can lead to neuropathology (see later).

In contrast to DNA DSBs, a more common strand break is a DNA SSB. Tens of thousands of DNA SSBs are estimated to occur daily within a cell due to oxidative stress, and it is this type of lesion that may well underpin much of the source of DNA damage in the brain (Caldecott 2003; Rass et al. 2007). Mammals are oxygen-consuming organisms, and reactive oxygen species (ROS) occur via normal cellular metabolic processes. ROS production represents the single greatest genotoxin to neurons as ROS-mediated attack of DNA results in direct DNA SSBs. Resolution of DNA SSBs utilizes a distinct DNA SSBR pathway (Caldecott 2003; McKinnon and Caldecott 2007). Detection of SSBs is mediated

by poly(ADP-ribose) polymerase (PARP) which acts to recruit the XRCC1 scaffolding protein accompanied with a variety of enzymes involved in DNA processing (TDP1, APTX, APE1, PKNP), gap-filling (DNA polymerase β) and ligation (ligase III) (McKinnon and Caldecott 2007; Wilson and Bohr 2007). Depending on the chemistry of the nucleophilic attack on the DNA backbone, DNA SSBs incur a multitude of 3' - and 5' - end-modifications, which must be processed by one or many end-processing enzymes prior to DNA, nick resealing (McKinnon and Caldecott 2007; Wilson 2007; Wilson and Mattson 2007). These processing enzymes are important in neural homeostasis as various neurodegenerative syndromes arise when germline mutations inactivate these enzymes. The inability to properly repair or process a SSB can lead to a variety of genotoxic consequences, including interference with DNA transcriptional machinery, formation of a DNA DSB upon encounter with the DNA replication machinery, or formation of either DNA SSB or DSB as a product of abortive Top1-DNA cleavage intermediates during DNA replication (Saxowsky and Doetsch 2006; el-Khamisy and Caldecott 2007; Katyal and McKinnon 2007). Notably, SSBs can be converted to DSBs in replicating cells via collision with replication forks, where HR is available as an adjunct to SSBR. However, such back-up mechanisms do not exist in post-mitotic neurons, and so the ability of these cells to repair SSBs will be very important for their survival (El-Khamisy et al. 2005; Katyal and McKinnon 2007).

Neurodegenerative disease and DNA strand break repair

Specific neuropathology is often present in syndromes involving DNA repair deficiency, highlighting the essential need for responding to genotoxic stress in the nervous system. As mentioned, the cerebellum is generally susceptible early in disease progression to the effects of DNA repair deficiency. This relative sensitivity may reflect the tremendous levels of neurogenesis that occur postnatally in this organ. Furthermore, the fact that neurons are post-mitotic, have critical higher-order functions and have demanding oxygen requirements makes them particularly sensitive to deficiency in resolving genotoxic stress. This section will consider the basis for the neurological defects in individuals with lesions associated with sensing or repairing DNA strand breaks. A representative list of syndromes associated with defective DNA strand break repair is presented in Table 1.

Ataxia-telangiectasia and related diseases

Ataxia telangiectasia (A-T) is amongst the best studied of the human neurological syndromes arising from DNA damage response defects. A-T results from mutation of ATM (ataxia telangiectasia, mutated) a large nuclear phosphoinositol-3-kinase-related serine/threonine protein kinase (Shiloh 2003). DNA DSBs activate ATM resulting in a multifaceted signaling response involving chromatin remodeling, DNA repair, cell cycle checkpoint activation and initiation of apoptotic pathways (Shiloh 2003; Lavin and Kozlov 2007; Matsuo et al. 2007). Individuals with A-T develop profound ataxia and are confined to a wheelchair before the first decade of life. Dysarthria, oculomotor apraxia, cerebellar atrophy and various extra-neurological features such as immunodeficiency, radiosensitivity and a predisposition to malignancy also feature in this disease (McKinnon 2004; Frappart and McKinnon 2006; McKinnon and Caldecott 2007). The DNA damage-response defects in A-T strongly affect the cerebellum as MRI and autopsy analysis reveal widespread loss of cerebellar Purkinje cell and granule neurons in A-T brains, that are subsequently accompanied by other cerebral and spinal defects (Frappart and McKinnon 2006).

Mutation of two members of the MRN complex have also been linked to human disease. Patients with hypomorphic mutation of Mre11 develop a syndrome called ataxia telangiectasia like syndrome (A-TLD) and show neurological symptoms similar to A-T including ataxia, oculomotor apraxia and dysarthria (Petrini and Stracker 2003; Taylor et al. 2004). In contrast, hypomorphic mutation of NBS1, lead to Nijmegen breakage syndrome

(NBS) which is characterized by immunodeficiency and lymphoid malignancy and in the nervous system microcephaly rather than neurodegeneration is present (Weemaes et al. 1981; Demuth and Digweed 2007). MRE11 has DNA processing activity, while NBS1 contains specific protein interaction domains (BRCT and FHA) that mediate critical MRN interactions. Like A-T, cells from A-TLD and NBS patients show substantial radiosensitivity and increased genomic instability (Shiloh 1997; Kobayashi et al. 2004). Interestingly, symptoms pertaining to both A-TLD and NBS seem to make up the breadth of those found in A-T, confirming biochemical analyses that the MRN complex is important for ATM activity. Mre11 and Nbs1 mutations result in defective localization of MRN and ATM to DSBs, reduced ATM activation and defective ATM-dependent phosphorylation of downstream substrates (Carson et al. 2003; Petrini and Stracker 2003; Uziel et al. 2003; Kitagawa et al. 2004; Difilippantonio et al. 2005). However, most, if not all of the pronounced neurological phenotypes in A-T, A-TLD and NBS can be attributed to DSB processing defects during neural development. Further, it is also uncertain if the progressive decline of the nervous system in A-T solely reflects the consequence of a lack of this protein during development. As yet it's quite unclear as to what extent ATM, MRE11 and NBS1 are functionally important in the mature brain or as the brain ages.

DNA repair deficiency and microcephaly

In addition to neurodegeneration are DNA repair syndromes that lead to congenital brain development abnormalities resulting in microcephaly (when the brain size is 2 standard deviations below normal). Some individuals with Seckel syndrome, caused by hypomorphic ATR (ATM and Rad3-related) mutations, display severe growth retardation, microcephaly, mental retardation and "bird-like" facial features (Goodship et al. 2000; O'Driscoll et al. 2004; O'Driscoll and Jeggo 2006). Like ATM, ATR is a large PI3K-like protein kinase involved in the DNA damage response (Shiloh 2001; Paulsen and Cimprich 2007). Although ATM and ATR have similar downstream substrates, ATR is primarily involved in monitoring DNA replication stress during cell proliferation, such as stalled replication forks (Paulsen and Cimprich 2007). Therefore, the functional requirement for ATR in terminally differentiated or aging neural cells is unclear. It is conceivable that after proliferation has ceased ATR is no longer important, with perhaps ATM performing genomic surveillance functions.

Mutation of the centrosomal protein, pericentrin, has also been recently linked to the pathogenesis of Seckel syndrome (Griffith et al. 2007). These patients, like those with ATR-related Seckel syndrome, also show pronounced microcephaly. Mutant pericentrin results in its mislocalization from the centrosome resulting in replication fork defects. Disruption of this structural chromatin protein also results in defective DNA repair responses including those dependent on ATR (Griffith et al. 2007).

Patients with hypomorphic mutations in the essential NHEJ protein LIG4 show microcephaly, developmental and growth delay and immunological defects including lymphoid malignancy (O'Driscoll et al. 2004). The severity of Lig4 syndrome correlates with the extent of residual LIG4 activity (Girard et al. 2004). Inactivation of mouse *Lig4* results in early embryonic lethality associated with widespread neuronal apoptosis and defective lymphogenesis (Barnes et al. 1998; Gao et al. 1998). Additionally, mutation in another NHEJ factor cernunnos/XLF results in Human Immunodeficiency with Microcephaly (HIM), an autosomal recessive childhood disease characterized by microcephaly, developmental and growth delay, autoimmune defects and recurrent infection (Ahnesorg et al. 2006; Buck et al. 2006). XLF functions in NHEJ via association with the XRCC4/LIG4 complex (Andres et al. 2007; Li et al. 2008). Many other DNA repair deficient syndromes also show microcephaly including Cockayne's syndrome in which transcription coupled repair is defective and also NBS described above, indicating this brain

defect probably occurs as a result of cell loss during development from various types DNA damage.

While the above syndromes involve DNA repair or damage response factors, the syndromes are congenital and so the exact requirements for any of these factors during aging in the brain remain unclear. In fact one unresolved issue in hereditary DNA repair syndromes is determining the portion of the collective phenotype that is attributable to the DNA repair deficiency during aging. For example, as mentioned above, is the progressive neurodegeneration associated with A-T solely a result of developmentally incurred defects, or is there a component of aging involved; i.e. is the ATM pathway important in mature neural cells? Assessing the requirement for ATM and ATR in the mature nervous system will be important for understanding the relative roles ATM and ATR contribute to the DNA damage response and how this impacts neural homeostasis. Other DNA damage signaling factors such as TopBP1 (Kumagai et al. 2006) have also been implicated in modulating ATM and ATR function, and again it will be informative to determine how these factors modulate the DNA damage response in postmitotic cells.

Determining the specific requirements for the DNA damage response system during the life of neural cells is an area that warrants further attention. As discussed later, more refined animal models for DNA repair deficiency will be important for understanding the role of DNA DSB repair in mature neurons. In contrast however, DNA SSBs are likely to affect terminally differentiated neural cells as they occur at much greater frequency than DSBs and are likely to impact transcription (El-Khamisy et al. 2005; Saxowsky and Doetsch 2006; Katyal and McKinnon 2007; McKinnon and Caldecott 2007), perhaps pointing to a greater need for activity of this pathway in the aging neural population. Additionally, the potential interrelationships between DNA DSB and SSB (Clements et al. 2004) suggest a more complex scenario, and imply that cross-talk between pathways may also be important for neural homeostasis. In the following we consider the importance of DNA SSB in the nervous system.

DNA SSB repair deficiency in the nervous system

The importance of DNA SSB repair in maintaining neural homeostasis has recently been highlighted by the identification of defective repair enzymes as the underlying cause for several neurodegenerative syndromes. In contrast to DNA DSBs, phenotypes associated with SSB repair deficient syndromes are relatively specific to the nervous system. Two such diseases are spinocerebellar ataxia with axonal neuropathy (SCAN1) and ataxia with oculomotor apraxia (AOA1). These are inherited autosomal recessive syndromes with neurodegenerative phenotypes similar to A-T, including cerebellar atrophy and ataxia, dysarthria and oculomotor apraxia (only in AOA1) (Date et al. 2001; Moreira et al. 2001; Takashima et al. 2002). While SCAN1 is quite rare, AOA1 is among the most common autosomal recessive ataxic disorders in Japan and Portugal. AOA1 patients show loss of cerebellar Purkinje neurons, while MRI analysis of SCAN1 patients has shown reduced cerebellar size (Takashima et al. 2002; Sugawara et al. 2008). SCAN1 and AOA1 result from heritable mutations in the 3'-end processing enzyme tyrosyl DNA phosphodiesterase 1 (TDP1) and the 5'-end processing enzyme aprataxin (APTX), respectively (Date et al. 2001; Moreira et al. 2001; Takashima et al. 2002). TDP1 is involved in processing of a variety of damaged DNA ends, including 3'-phosphoglycolate, 3'-Top1 and other types of non-ligateable 3'-DNA ends generated after DNA oxidation, DNA replication or other genotoxic stress (El-Khamisy et al. 2005). APTX is a nucleotide hydrolase and studies using *APTX*^{-/-} cells have shown a failure to cleave a 5'-adenylate intermediate prior to sealing the nick, implicating a role for APTX in processing abortive ligation intermediates (Ahel et al. 2006; Rass et al. 2007). While the ability of HR to provide a backup repair pathway in proliferative tissues can circumvent SSB defects, the specific impact toward the nervous

system in defective SSBR syndromes probably reflects the high oxygen consumption of this tissue and the associated increased levels of oxidative damage (El-Khamisy et al. 2005; Katyal and McKinnon 2007; Rass et al. 2007).

Retinitis Pigmentosa (RP)

Retinitis pigmentosa (RP) is a heterogeneous neurodegenerative condition resulting in a gradual loss of photoreceptor neurons, leading to progressive constriction of the visual field and night blindness. Many RP gene groups and forms exist: including autosomal recessive, autosomal dominant and X-linked forms. However, mutation of RP2 accounts for the second most frequent cause of X-linked RP (Sharon et al. 2003). RP2 shares homology to nucleotide diphosphate kinases and *in vitro* oligo nuclease studies implicate RP2 as a novel 3'-5' exonuclease involved in SSBR. A recent study describes nuclear relocalization from the membrane of RP2 protein upon exposure to oxidative damage (Yoon et al. 2006). While a number of vision disorders are attributed to retinal degeneration including RP, age-related macular degeneration and cone-rod dystrophy, links to DNA repair deficiency have not been made in these diseases.

Utilizing mouse models to understand DNA damage in the nervous system

Human DNA repair syndromes reveal the requirement for DNA repair during neural development, although these are less revealing about repair requirements in the mature nervous system. Detailed analysis of these processes will depend upon suitable experimental systems that are amenable to experimental manipulation. Perhaps the most significant model system that will directly expand our understanding of the biology of DNA repair in the brain is the mouse. Important information has been obtained using germline disruption of DNA repair factors, and more recently tissue specific gene ablation has helped refine our understanding to a much greater degree (Friedberg and Meira 2006). However, there are also limitations to the ability of the mouse to recapitulate human neurodegenerative disease phenotypes. For example, while *Atm*^{-/-} mice have been important for understanding many aspects of A-T, the substantial neuropathology present in humans is absent in *Atm*-null animals (Frappart and McKinnon 2006). The lack of discernable neuropathology suggests some fundamental differences between the response of the nervous system in mice and humans. It is not certain why the difference is pronounced in the nervous system, when in other physiological compartments there is equivalency after ATM loss between mouse and man. Similarly, while murine inactivation of either Mre11 or Nbs1 results in early embryonic lethality (Xiao and Weaver 1997; Zhu et al. 2001; Dumon-Jones et al. 2003), hypomorphic murine mutations that mimic the human gene mutation in these syndromes reflect the cellular and extra-neurological characteristics of the human disease, but not the neurological symptoms (Kang et al. 2002; Williams et al. 2002; Theunissen et al. 2003; Difilippantonio et al. 2005). Two potential reasons are that the mouse lifespan is insufficient to manifest age-accumulated stochastic lesions, or that the mouse nervous system is more resistant to DNA damage. Nonetheless, important lessons have been learnt from the mouse regarding ATM signaling in the nervous system. *Atm*^{-/-} mice have established that ATM is required for the induction of neuronal apoptosis selectively in immature, postmitotic neural cells after DNA damage (Herzog et al. 1998), suggesting that progressive neurodegeneration in A-T results from faulty neurons remaining integrated in neural tissue.

Additionally, *Tdp1*^{-/-} mice have been created in an effort to understand the role of TDP1 in preventing SCAN1. Cerebellar neurons from these mice have deficient DNA SSBR activity after damage induced by oxidative stress, ionizing radiation and Top1 inhibitors (Hirano et al. 2007; Katyal et al. 2007). Notably, in contrast to mouse models of DNA DSB response deficiency, *Tdp1*^{-/-} mice exhibit late-onset cerebellar atrophy, presumably due to accumulation of DNA lesions as result of neuronal repair deficiency (Katyal et al. 2007).

These mice are also extremely sensitive to Top1 inhibitors, particularly in hematopoietic and intestinal tissue.

These knockout mouse models highlight the potential differences in outcome of repair enzyme loss between mouse and human, underscoring relevant concerns in considering the mouse as a suitable model for studies of aging in the brain (Zahn et al. 2007). While this may be an issue for generating faithful disease models, the mouse is nonetheless the best currently available system for garnering a comparative understanding of the biological role of DNA repair pathways in the developing and mature brain. In particular, valuable data has been generated from mouse models in which inactivation of DNA repair factors show profound tissue-specific effects, particularly within the nervous system (Gao et al. 1998; Frappart et al. 2005; Orii et al. 2006; Frappart et al. 2007).

Refined mouse models to study DNA repair in the nervous system

The increasing availability of reagents for tissue specific gene inactivation has provided a more refined approach with which to selectively manipulate gene inactivation (Orban et al. 1992; Jonkers and Berns 2002). Tissue specific deletion of DNA repair genes in the nervous system has already provided valuable insights into the role of these factors during neural development (Garcia and Mills 2002; Gaveriaux-Ruff and Kieffer 2007). A standard approach to gene deletion in the nervous system via the Cre/LoxP system utilizes the Nestin promoter to drive the cre recombinase. Nestin is expressed at E10.5 a point at which neural development is commencing. As a result, gene deletion via nestin-driven Cre expression will result in gene deletion throughout CNS tissue (Bates et al. 1999). For example, while inactivation of Brca2 or Nbs1 leads to early embryonic lethality, selective ablation of these genes in the nervous system using Nestin-cre results in a viable animal, providing important insight into the respective roles of these genes in this tissue (Frappart et al. 2005; Frappart et al. 2007). A wide array of neural-specific Cre lines have been developed which exhibit Cre recombinase activation at specific stages of development of tissues (A comprehensive database is accessible at: <http://nagy.mshri.on.ca/cre/index.php> or www.jax.org). Depending on the spatiotemporal expression pattern of any given gene promoter, Cre recombinase activity can be pan-neuronal, CNS tissue- or cell-type specific. In addition, inducible tissue-specific Cre lines have been developed to allow increased temporal control of Cre expression (Kuhn et al. 1995; Feil et al. 1996; St-Onge et al. 1996; Garcia and Mills 2002).

Perspective & Conclusion

Neurodegenerative syndromes highlight the importance of responding to DNA strand breaks in the nervous system. However, the precise requirement for DNA repair factors during aging of the brain is not necessarily revealed by the phenotype of human DNA repair syndromes. While progeria is associated with some DNA repair syndromes (e.g. Werner's), it is not strongly associated with DNA strand break repair deficiency syndromes. DNA DSB syndromes appear to primarily affect developing tissues, and most if not all of the neurological phenotype can be attributed to defects associated with development. In comparison however, the DNA SSBR deficiency syndromes may reflect a greater link to the process of aging. In particular, the high frequency of this lesion and the ability of the damage to impact transcription point to this lesion as one that is likely to be important during aging, perhaps in a synergistic manner with other common age-related neurological diseases. The exact requirements for DNA DSB and SSBR in the aging brain is not clear, but as outlined above mouse models will be extremely important for generating a basic understanding of how various repair pathways participate in cellular homeostasis in the brain.

A general reduction in DNA repair activity has been associated with brain aging in humans and rodents raising the possibility that this is a normal aspect of aging (Intano et al. 2003; Lu et al. 2004; Imam et al. 2006; Gorbunova et al. 2007; Rao 2007; Rutten et al. 2007). However, a correlative relationship between repair activity and aging doesn't necessarily imply that an assayable decrease in enzyme activity would be associated with increased DNA lesions or accumulation of mutations. Interestingly, relative to the liver the brain was found to be resistant to accumulated DNA mutations (Dolle et al. 1997; Stuart et al. 2000).

Defective DNA repair is being increasingly linked with diseases with striking neuropathology that are associated with aging, such as Alzheimer's and Parkinson's disease (Nospikel and Hanawalt 2003; Bender et al. 2006; Hegde et al. 2006; Kraytsberg et al. 2006; Shackelford 2006; Weissman et al. 2007). Studies of DNA repair from brains of Alzheimer's individuals have shown reduced DNA DSB repair during NHEJ that was attributed to reduced DNA-PK α levels, while protein levels of the MRN complex have also been reported to be diminished (Jacobsen et al. 2004; Shackelford 2006). Reduced base excision repair capacity was also found in Alzheimer's disease brains compared to age-matched controls (Weissman et al. 2007). In Parkinson's disease (PD) patients, increased DNA damage was also found in the mitochondria in substantia nigra neurons (a neuronal population lost in PD), implying a relationship between PD and mitochondrial DNA damage (Bender et al. 2006; Kraytsberg et al. 2006; Reeve et al. 2008).

Other considerations for aging in the nervous system includes the requirement for DNA repair in neural stem cells, perhaps those that potentially contribute towards new neurogenesis in regions of the mature brain (Toni et al. 2007; Zhang et al. 2008). Certainly, recent data points to a strong requirement of DNA DSB repair in some stem cell populations (Nijnik et al. 2007; Rossi et al. 2007). However, the functional role for stem cells in the aging brain is unclear, as is the requirement for new neurogenesis as a component of normal brain function.

While DNA repair syndromes highlight the importance of maintaining genomic integrity in the brain, the role of DNA damage in normal aging processes is less clear. As mentioned previously, reports documenting a loss of repair capacity with age suggest that DNA damage may be involved in aging, although many other reports find no clear defects in DNA repair associated with normal aging. Perhaps, assessment of other DNA damage parameters may be more informative for understanding the contribution of DNA damage to aging? For example, telomeres feature prominently in eliciting a DNA damage response when disrupted, and telomere erosion is a consequence of cellular proliferation (Verdun and Karlseder 2007). Telomeres are structures present at the end of chromosomes, and serve a critical function to ensure that DNA ends of chromosomes are not detected as DNA breaks (DePinho and Wong 2003; Blasco 2005). Intriguingly, telomere shortening has been found in cells from individuals experiencing emotional stresses often associated with aging, suggesting a strong environmental link to maintenance of genome integrity (Epel et al. 2004; Sapolsky 2004; Epel et al. 2006). However, the need for telomere maintenance in non-replicating tissue such as the nervous system or the potential impact of loss of function of telomere protection factors has not yet been carefully assessed *in vivo*. Many new insights to address these questions will be forthcoming as new mouse models of DNA repair deficiency are generated. These insights will be important guides as we plan therapeutic strategies to potentially alleviate neuropathology associated with DNA repair deficiency and aging.

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Figure 1. The adult mammalian CNS

The mature nervous system contains a myriad of different cell types and tissues. DNA repair processes impact substantially during neural development leading to defective neurogenesis and development. However, less is known regarding the requirement for DNA repair processes in mature neural cell. Inset panels are hematoxylin and eosin stained retinal and cerebellar sections that show cell organization in these tissues. The retina is laminar in nature and cell-types are stratified into three distinct nuclear layers: outer, inner and ganglion. The outer nuclear layer contains the photoreceptor (rods and cones) neurons. The inner nuclear layer contains various signal processing cell types: bipolar, horizontal, amacrine, interplexiform and the Müller glia. The ganglion cells carry the visual signal via its axons through the optic nerve and project onto the brain. The cerebellum is stratified into three primary layers: inner granule cell layer, the Purkinje cell layer and the molecular layer. Excitatory sensory signals originating from the cerebellum are ultimately transmitted through granule-Purkinje synapses and out of the cerebellum through Purkinje neuron axons to affect normal control of movement.

Table 1

DNA repair Syndromes resulting from DNA strand break deficiency.

Disease/Syndrome	Gene	Neurological	Extra-neurological	Mouse mutant(s)	Phenotype	References
DNA DSB Sensing and Repair						
Ataxia-telangiectasia	<i>ATM</i>	ataxia, neurodegeneration telangiectasia, dysarthria,	immunological defects, malignancy, sterility	<i>Atm</i> ^{-/-}	growth retardation, infertility, immunological defects, radiosensitivity, malignancy	(Barlow et al. 1996) (Elson et al. 1996) (Xu and Baltimore 1996) (Herzog et al. 1998) (Spring et al. 2001) (Borghesani et al. 2000)
Seckel syndrome	<i>ATR</i> <i>PCNT</i>	microcephaly, mental retardation	growth defects, abnormal facial features	<i>ATR</i> ^{-/-} none reported	embryonic lethal (<E7.5)	(Brown and Baltimore 2003)
Nijmegen breakage syndrome	<i>NBS1</i>	microcephaly	immunological defects, lymphoid malignancy	<i>Nbs1</i> ^{-/-} <i>Nbs1</i> ^{B/B} <i>Nbs1</i> ^{ins-6/ΔTg-Mx-Cre} <i>Nbs1</i> ^{Δ/Δ} <i>6 neslin-Cre</i> <i>Nbs1</i> ^{C/C}	embryonic lethal (<E7.5) immunological defects, lymphoid malignancy defects in lymphogenesis, marrow, spleen, thymus ataxia, cerebellar and growth defects, microcephaly defective apoptotic response after IR	(Zhu et al. 2001) (Williams et al. 2002) (Demuth et al. 2004) (Frappart et al. 2005) (Stracker et al. 2007) (Diflippantonio et al. 2005)
Ataxia-telangiectasia-like disorder	<i>MRE11</i>	ataxia, neurodegeneration dysarthria, oculomotor apraxia	mild immunological defects	<i>Mre11</i> ^{-/-} <i>Mre11</i> ^{ATLD/ATLD}	embryonic lethal reduced embryonic viability	(Xiao and Weaver 1997) (Theunissen et al. 2003)
LIG4 syndrome	<i>LIG4</i>	microcephaly	immunodeficiency, lymphoma, developmental/growth delay	<i>LIG4</i> ^{-/-}	embryonic lethal (<E16.5); neuronal apoptosis and defective lymphogenesis	(Frank et al. 2000) (Barnes et al. 1998)
Fanconi anemia	<i>BRCA2</i>	medulloblastoma	bone marrow and congenital defects	<i>Brcal</i> ^{2loxP/loxP neslin-Cre}	defects in neurogenesis, tumor prone	(Frappart et al. 2007)
Human Immunodeficiency with microcephaly	<i>XLFI</i>	microcephaly	immunological defects, infection developmental/growth delay	<i>Xlf</i> ^{-/-} ES cells only none reported	genomic instability, chromosomal translocations	(Zha et al. 2007)
DNA SSB Repair						
Spinocerebellar ataxia with axonal neuropathy	<i>TDPI</i>	ataxia, neurodegeneration muscle weakness	hypercholesterolemia, hypalbuminemia	<i>Tdp1</i> ^{-/-}	mild cerebellar atrophy, hematopoietic/intestinal	(Katyal et al. 2007) (Hirano et al. 2007)

Disease/Syndrome	Gene	Neurological	Extra-neurological	Mouse mutant(s)	Phenotype	References
Ataxia with oculomotor apraxia 1	<i>APTX</i>	ataxia, neurodegeneration oculomotor apraxia	hypercholesterolemia, hypalbuminemia	<i>Aptx</i> ^{-/-}	sensitivity to topoisomerase-1 inhibitors (Topotecan) Defective resolution of 5 - end abortive ligation intermediates, murine analysis in progress	(Ahel et al. 2006)