Journal Club

Editor's Note: These short reviews of recent *JNeurosci* articles, written exclusively by students or postdoctoral fellows, summarize the important findings of the paper and provide additional insight and commentary. If the authors of the highlighted article have written a response to the Journal Club, the response can be found by viewing the Journal Club at www.jneurosci.org. For more information on the format, review process, and purpose of Journal Club articles, please see http://jneurosci.org/content/preparing-manuscript#journalclub.

The Role of DNA Polymerase β in Neural Genome Stability

Zoltan Simandi
Sanford Burnham Prebys Medical Discovery Institute, Orlando, Florida 32827
Review of Onishi et al.

Development of the nervous system requires robust expansion of progenitor cells that will generate a diverse population of long-lived mature neurons. Precise transmission of the genomic information from parent cell to daughter cells and the faithful maintenance of this information in the terminally differentiated, postmitotic neurons is critically important to maintain normal cell functions throughout life. During early development, rapid proliferation of neural progenitors may result in alterations in the chemical structure of DNA due to DNA replication infidelity or unequal chromosome segregation (Barlow and Nussenzweig, 2014). DNA damage, such as a break in a strand of DNA, a missing base from the backbone of DNA, or a chemically changed base can also occur in postmitotic cells of the mature nervous system (Iyama and Wilson, 2013). These DNA alterations in nonproliferating cells are mainly induced by metabolism-related oxidative stress (Chow and Herrup, 2015; McKinnon, 2017); however, recent findings suggest that neuronal activity may also trigger DNA breaks (Madabhushi et al., 2015; Cholewa-Waclaw et al., 2016).

Mammalian cells have evolved at least six DNA damage–repair pathways to restore genome integrity after DNA damage. Among these, base excision repair is the major pathway responsible for coping with single-strand breaks (SSBs). SSBs can occur directly by disintegration of the oxidized sugar or indirectly during the base excision repair of alkylated or deaminated bases (Wilson and McNeill, 2007; Dianov and Hübscher, 2013). The importance of base excision repair is demonstrated by the fact that homozygous knockout of core base–excision–repair components leads to embryonic or postnatal lethality (Krokan and Bjørås, 2013). Furthermore, genetic variations resulting in reduction, but not complete loss, of base excision repair capacity, are associated with neurological disease risk, such as Alzheimer's disease and susceptibility to stroke-induced complications (Weissman et al., 2007; Sykora and Snow, 2008).

Base excision repair generally involves the following steps: (1) recognition and removal of damaged or inappropriate bases by DNA glycolysis (e.g., OGG1, MAG1, UNG); (2) incision at the resulting abasic site by AP endonucleases (e.g., APE1, APE2) to yield a 3′ hydroxyl adjacent; (3) removing of the remaining blocking groups from the DNA terminal ends by a lyase or phosphodiestarase (e.g., PNKP); (4) replacement of the missing nucleotides by DNA polymerases (Pols; e.g., Polβ or Polδ/ε); and (5) sealing of the nick by DNA ligase (Wilson and McNeill, 2007; Fig. 1). Base–excision–repair pathways are distinguished as "short patch" or "long patch" depending on whether a gap of a single nucleotide or a gap of 2–10 nt is generated and filled in the process (Fig. 1). Base excision repair typically proceeds via the short-patch pathway, which engages Polβ to replace the missing nucleotide. However, under special circumstances (e.g., 5′-terminal moiety is not a substrate for Polβ, ATP concentrations are low, or during the S phase of the cell cycle), base excision repair can proceed via the long-patch, strand-displacement synthesis process (Krokan and Bjørås, 2013). Long-patch base excision repair is most commonly performed with Polδ/ε (Fig. 1).

The major mammalian DNA polymerase in the brain is Polβ, which is primarily responsible for replacing single nucleotides during base excision repair. Polβ is required for the normal development of cortex (Sugo et al., 2000). Mice deficient in Polβ throughout the body show extensive apoptosis of the immature postmitotic cells in the intermediate zone and cortical plate (CP) of the developing cortex, whereas the progenitor cells in the ventricular zone (VZ) are less affected (Sugo, et al., 2000). This suggests that cell death resulting from Polβ deletion mainly occurs in immature neurons (Sugo et al., 2000). The role of Polβ has been also studied in mature neurons in the context of aging. Oxidative damage-dependent induction of Polβ gradually declines with aging, which is consistent with a reduced ability of aging neurons to repair DNA damage (Bosshard et al., 2012). These results indicate an important role for Polβ in postmitotic neurons as well as immature neurons and progenitors. A detailed

Received Aug. 22, 2017; revised Oct. 2, 2017; accepted Oct. 9, 2017.
The author declares no competing financial interests.
Correspondence should be addressed to Zoltan Simandi, 6400 Sanger Road, Orlando, FL 32827. E-mail: zsimandi@sbpdiscovery.org.
DOI:10.1523/JNEUROSCI.2380-17.2017
 0270-6474/17/3711069-03$15.00/0

characterization of how Polβ contributes to prevent DNA damage accumulation in differentiating neurons remains to be determined, however.

A recent article (Onishi et al., 2017) provides important insight regarding the role of Polβ-dependent DNA repair in the developing cortex. To investigate the function of Polβ in neural progenitors versus terminally differentiated postmitotic cells, Onishi et al. (2017) established mouse lines in which Polβ was deleted primarily in precursor cells (Emx1-Cre/Polβ$^{fl/fl}$ mice), selectively in postmitotic neurons (Nex-Cre/Polβ$^{fl/fl}$ mice), or throughout the body (Sycp1-Cre/Polβ$^{fl/fl}$ mice). Whole-body Polβ-knock-out mice died shortly after birth, while precursor (Emx1-Cre/Polβ$^{fl/fl}$) and postmitotic (Nex-Cre/Polβ$^{fl/fl}$) Polβ knock-out mice were obtained at the expected Mendelian ratios, indicating that conditional knockout did not affect survival. Next, the authors examined the number of apoptotic cells at various stages. In precursor Polβ knock-out mice, cleaved caspase-3-positive apoptotic cells were observed predominantly in more mature neurons but not in neural progenitors in the ventricular zone. Strikingly, only a few apoptotic cells were detected in any layer of the developing cortex in postmitotic Polβ knock-out mice, suggesting that cell death is caused by the loss of Polβ in the progenitors (Onishi et al., 2017).

Next, Onishi et al. (2017) investigated how apoptosis is triggered in Polβ-deficient cells. Normally, SSBs are produced enzymatically during the incision step of the base excision repair, and these SSBs are repaired by the action of Polβ and DNA ligase (Fig. 1). In line with this, Polβ deficiency is associated with accumulation of SSBs (Wilson and McNeill, 2007). Unrepaired SSBs can convert into double-stranded breaks (DSBs) in replicating cells due to the collapse of DNA replication fork (Kuzminov, 2001). DSBs are one of the most dangerous DNA lesions, as even a single DSB can directly inactivate key genes, leading to serious chromosomal aberrations and apoptosis (Kaina, 2003). To determine whether the frequency of DSB formation was increased in Polβ-depleted mice, Onishi et al. (2017) investigated the number of γH2AX foci, a biomarker of DSBs (Kuo and Yang, 2008). γH2AX signal was detected in the CP and VZ of precursor Polβ knock-out mice but not in postmitotic Polβ knock-out mice, leading to a key conclusion that primarily the neural progenitors are vulnerable to Polβ deficiency and that Polβ deficiency

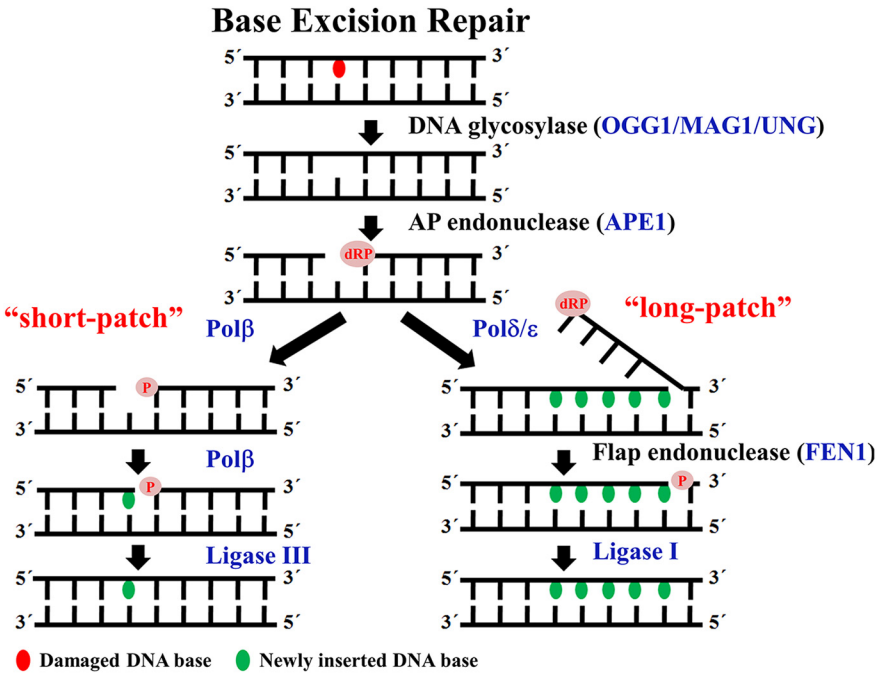

Figure 1. Overview of the base excision repair. Damaged or inappropriate bases are recognized and removed by DNA glycolysases, forming an AP site. These sites are cleaved by AP endonucleases, resulting in single-stranded breaks (SSBs). SSBs are processed by either "short-patch" (single nucleotide) or "long-patch" (2–10 new nucleotides) repair. Polβ is the main polymerase that catalyzes "short-patch" base excision repair.

can indeed increase the frequency of DSBs. The comparable number of γH2AX foci between CP and VZ cells in precursor Polβ knock-out mice indicated that the majority of unrepaired DSBs in progenitors remain unrepaired in postmitotic cells. In an elegant *in vitro* experiment, comparing the transformation of SSBs to DSBs in control versus Polβ-deficient cortical progenitor cells exposed to an alkylating agent, Onishi et al. (2017) were able to prove the protective role of Polβ against replication-associated DSB formation.

To investigate the distribution and impact of Polβ deficiency-induced DSB formation and apoptosis, Onishi et al. (2017) performed a systematic immunohistochemistry analysis on cortical cytoarchitecture using anti-γH2AX and layer-specific antibodies. γH2AX foci-positive cells were more abundant in deep layers in precursor Polβ-deficient mice compared with control animals. Moreover, the thickness of deep layers and the number of cells within these layers was significantly reduced, likely due to the ongoing extensive apoptosis. Since DSBs were frequently observed in surviving cells, Onishi et al. (2017) next investigated whether the accumulation of DSBs can affect the differentiation potential of cortical cells in deep layers. In neurons that survived in precursor Polβ knock-out mice, axon length was significantly shorter than normal. Gene expression analysis revealed dysregulation of axon growth-related genes in these cells, suggesting that even the surviving cortical cells of precursor Polβ knock-out mice are dysfunctional and unable to properly differentiate (Onishi et al., 2017).

Cells accumulating DSBs are typically undergoing a p53-dependent blockage of proliferation, and, depending on the severity of damage, p53 either relays a wide range of prosurvival signals or promote apoptosis (Chipuk and Green, 2006). Onishi et al. (2017) found that depletion of p53 could suppress neural apoptosis in Polβ-deficient mice, supporting the conclusion that DSB-dependent activation of p53 pro-apoptotic signals are the primary cause of cell death in the Polβ knock-out mice.

Onishi et al. (2017) clarify an important question regarding the functions of base excision repair and Polβ in proliferating versus postmitotic cells. According to the results, unrepaired SSBs increase the prevalence of DSBs in Polβ-deficient precursor cells, which remain unrepaired in postmitotic cells, leading to developmental problems and p53-mediated cell death. The study raises some interesting questions. First, it remained unaddressed by Onishi et al. (2017) whether postmitotic Polβ knock-out mice develop any neurological problems with age. Further experiments with Nex-Cre/Polβ$^{fl/fl}$ mice will help to clarify the extent to which ge-

nome integrity in postmitotic cells relies on base excision repair and Polβ. Second, it is noteworthy that while previous studies indicate that Polβ is the predominant DNA polymerase in neurons isolated from developing, adult, and aging brain (Chow and Herrup, 2015), significant levels of polymerases α and δ/ε have also been detected. Whether Polα and Polδ/ε show any compensatory mechanisms in the animal models established by Onishi et al. (2017) remains to be determined.

In summary, Onishi et al. (2017) demonstrated that Polβ-dependent base excision repair in neural progenitors is required for cellular viability and ability to properly function as mature neurons. Considering the results of the work from Onishi et al. (2017), along with other studies that are connecting disrupted Polβ to aging and neurodegeneration, there is an urgent need to better understand the molecular mechanisms regulating the level and activity of Polβ and contributing to its age-dependent decline.

References

Barlow JH, Nussenzweig A (2014) Replication initiation and genome instability: a crossroads for DNA and RNA synthesis. Cell Mol Life Sci 71:4545–4559. CrossRef Medline

Bosshard M, Markkanen E, van Loon B (2012) Base excision repair in physiology and pathology of the central nervous system. Int J Mol Sci 13:16172–16222. CrossRef Medline

Chipuk JE, Green DR (2006) Dissecting p53-dependent apoptosis. Cell Death Differ 13:994–1002. CrossRef Medline

Cholewa-Waclaw J, Bird A, von Schimmelmann M, Schaefer A, Yu H, Song H, Madabhushi R, Tsai LH (2016) The role of epigenetic mechanisms in the regulation of gene expression in the nervous system. J Neurosci 36:11427–11434. CrossRef Medline

Chow HM, Herrup K (2015) Genomic integrity and the ageing brain. Nat Rev Neurosci 16:672–684. CrossRef Medline

Dianov GL, Hübscher U (2013) Mammalian base excision repair: the forgotten archangel. Nucleic Acids Res 41:3483–3490. CrossRef Medline

Iyama T, Wilson DM 3rd (2013) DNA repair mechanisms in dividing and non-dividing cells. DNA Repair (Amst) 12:620–636. CrossRef Medline

Kaina B (2003) DNA damage-triggered apoptosis: critical role of DNA repair, double-strand breaks, cell proliferation and signaling. Biochem Pharmacol 66:1547–1554. CrossRef Medline

Krokan HE, Bjørås M (2013) Base excision repair. Cold Spring Harb Perspect Biol 5:a012583. CrossRef Medline

Kuo LJ, Yang LX (2008) Gamma-H2AX-a novel biomarker for DNA double-strand breaks. In Vivo 22:305–309. Medline

Kuzminov A (2001) Single-strand interruptions in replicating chromosomes cause double-strand breaks. Proc Natl Acad Sci U S A 98:8241–8246. CrossRef Medline

Madabhushi R, Gao F, Pfenning AR, Pan L, Yamakawa S, Seo J, Rueda R, Phan TX, Yamakawa H, Pao PC, Stott RT, Gjoneska E, Nott A, Cho S, Kellis M, Tsai LH (2015) Activity-induced DNA breaks govern the expression of neuronal early-response genes. Cell 161:1592–1605. CrossRef Medline

McKinnon PJ (2017) Genome integrity and disease prevention in the nervous system. Genes Dev 31:1180–1194. CrossRef Medline

Onishi K, Uyeda A, Shida M, Hirayama T, Yagi T, Yamamoto N, Sugo N (2017) Genome stability by DNA polymerase β in neural progenitors contributes to neuronal differentiation in cortical development. J Neurosci 37:8444–8458. CrossRef Medline

Sugo N, Aratani Y, Nagashima Y, Kubota Y, Koyama H (2000) Neonatal lethality with abnormal neurogenesis in mice deficient in DNA polymerase beta. EMBO J 19:1397–1404. CrossRef Medline

Sykora P, Snow ET (2008) Modulation of DNA polymerase beta-dependent base excision repair in cultured human cells after low dose exposure to arsenite. Toxicol Appl Pharmacol 228:385–394. CrossRef Medline

Weissman L, Jo DG, Sørensen MM, de Souza-Pinto NC, Markesbery WR, Mattson MP, Bohr VA (2007) Defective DNA base excision repair in brain from individuals with Alzheimer's disease and amnestic mild cognitive impairment. Nucleic Acids Res 35:5545–5555. CrossRef Medline

Wilson DM 3rd, McNeill DR (2007) Base excision repair and the central nervous system. Neuroscience 145:1187–1200. CrossRef Medline