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# Mitochondrial base excision repair in mouse synaptosomes during normal aging and in a model of Alzheimer's disease

Ricardo Gredilla[a,1], Lior Weissman[b], Jenq-Lin Yang[b,c], Vilhelm A. Bohr[b], Tinna Stevnsner[a,*]

[a] Danish Center for Molecular Gerontology and Danish Aging Research Center, Department of Molecular Biology, University of Aarhus, Aarhus, Denmark

[b] Laboratory of Molecular Gerontology, National Institute on Aging, NIH, Baltimore, MD, USA

[c] Laboratory of Neurosciences, National Institute on Aging, NIH, Baltimore, MD, USA

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**Abstract**

Brain aging is associated with synaptic decline and synaptic function is highly dependent on mitochondria. Increased levels of oxidative DNA base damage and accumulation of mitochondrial DNA (mtDNA) mutations or deletions lead to mitochondrial dysfunction, playing an important role in the aging process and the pathogenesis of several neurodegenerative diseases. Here we have investigated the repair of oxidative base damage, in synaptosomes of mouse brain during normal aging and in an AD model. During normal aging, a reduction in the base excision repair (BER) capacity was observed in the synaptosomal fraction, which was associated with a decrease in the level of BER proteins. However, we did not observe changes between the synaptosomal BER activities of presymptomatic and symptomatic AD mice harboring mutated amyolid precursor protein (APP), Tau, and presinilin-1 (PS1) (3xTgAD). Our findings suggest that the age-related reduction in BER capacity in the synaptosomal fraction might contribute to mitochondrial and synaptic dysfunction during aging. The development of AD-like pathology in the 3xTgAD mouse model was, however, not associated with deficiencies of the BER mechanisms in the synaptosomal fraction when the whole brain was analyzed.


*Keywords:* DNA repair; Mitochondria; Synaptic dysfunction; Neurodegeneration

## 1. Introduction

Brain aging is characterized by cognitive and behavioral decline. Decrease in spine densities (Dickstein et al., 2007), alterations in signaling pathways (Dröge and Schipper, 2007; Foster, 2007) and neurotransmitter systems (Mora and Segovia, 2007) occur with normal aging, favoring general neuronal dysfunction. These changes are described to occur mainly in cerebral cortex and hippocampus (Mora and Segovia, 2007), but also in cerebellum (Huang et al., 2006; Jernigan et al., 2001). Moreover, these changes are exacerbated in specific brain regions when neurodegenerative disorders such as Alzheimer's disease (AD) develop during aging (Dickstein et al., 2007; Mattson, 2004).

Mitochondrial DNA (mtDNA) mutations and mitochondrial dysfunction are thought to play an important role in the aging process (Barja, 2004; Cantuti-Castelvetri et al., 2005; Kujoth et al., 2007); and increased levels of oxidative modifications and mutations in mtDNA occur in the brain during normal aging (Beal, 2005; Melov, 2004; Vermulst et al., 2007) and in AD (Gabbita et al., 1998; Morocz et al., 2002).

Base excision repair (BER) is the primary DNA repair pathway for small DNA modifications caused by alkylation, deamination, or oxidation in nuclei and mitochondria and it has been described to play a major role in the development

* Corresponding author at: Danish Center for Molecular Gerontology and Danish Aging Research Center, Department of Molecular Biology, University of Aarhus, C.F. Moellers Allé 3, bldg. 1130, 8000 Aarhus C, Denmark. Tel.: +45 8942 2657; fax: +45 8942 2650.

*E-mail address:* tvs@mb.au.dk (T. Stevnsner).

[1] Current address: Complutense University, Faculty of Medicine, Department of Physiology, Ciudad Universitaria s/n, 28040 Madrid, Spain.

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and maintenance of the central nervous system (CNS) (Weissman et al., 2007a). The BER pathway includes 4 distinct steps (Bohr, 2002). First, DNA glycosylases are responsible for recognition and removal of the modified bases, rendering an abasic site, which is then processed by apurinic/apyrimidinic (AP) endonuclease (APE). Repair can then proceed through 1 of 2 subpathways: short- or long-patch BER, both taking place in the nucleus and in the mitochondrion (Akbari et al., 2008; Bohr, 2002; Liu et al., 2008). The short-patch BER involves the incorporation of a single nucleotide into the gap by DNA polymerase followed by strand ligation by DNA ligase, while long-patch BER involves incorporation of several nucleotides, typically 2 to 7, followed by cleavage of the resulting 5′ flap and ligation. In mitochondria, polymerase gamma (pol γ) is the only polymerase present, being involved both in replication and repair events. Although mitochondria possess an independent BER machinery, the mitochondrial BER components are encoded by nuclear genes (Bohr, 2002).

Increased neuronal mtDNA instability is especially important when occurring in mitochondria located at the synaptic terminals, which are characterized by high-energy requirements. Mitochondria at that location provide energy for critical processes such as exocytosis/endocytosis events of synaptic vesicles or the preservation of ionic strength. Thus, these mitochondria play a central role in synaptic activity and therefore in the proper function of the central nervous system (Brown et al., 2006; Foster, 2007; Ly and Verstreken, 2006). Various investigations have reported important biochemical differences between synaptic mitochondria and those located in the neuronal soma (Borrás et al., 2003; Brown et al., 2006; Lai et al., 1977). They also behave differently when physiological conditions change (Martinez et al., 2009), suggesting that these mitochondrial subpopulations might also have different vulnerability to aging.

Various studies suggest that synaptic impairment is associated with mitochondrial dysfunction during aging and as an early event in AD (Fontán-Lozano et al., 2008; Mattson et al., 1998; Selkoe, 2002), but it is not known whether mtDNA repair mechanisms play a significant role in this process. The first reports describing the presence of a DNA repair protein, pol γ, in synaptic brain mitochondria were published in the seventies (Hübscher et al., 1977, 1979). However, those investigations only focused on the role of pol γ in mtDNA replication, because, at that time, it was believed that mitochondria lacked the enzymes necessary for DNA repair (Clayton et al., 1974). More recently, Cortina et al. (2005) have described the regulation of pol γ and 8-oxoguanine-DNA glycosylase levels in synaptic mitochondria in photoreceptors after retinal damage induced by light. Yet, the DNA repair mechanisms in the synaptic fraction of the brain have not previously been investigated.

In this study, we investigated BER activities of the synaptic fraction from whole mouse brains and whether they may play a significant role in the synaptic loss associated with aging and AD. We approached this study by purifying synaptosomes from normal aged mice and from an AD mouse model, 3xTgAD mice carrying $PS1_{M146V}$, $APP_{swe}$, and $Tau_{P301L}$ transgenes (Oddo et al., 2003a). Synaptosomes have been widely used in neurobiology as an approach to synaptic research and have been extensively characterized (Borrás et al., 2003; Brown et al., 2006; Hübscher et al., 1979; Lai et al., 1977; Mattson et al., 1998; Schrimpf et al., 2005). Our findings show that a significant age-related decline of BER capacity occur specifically at the synapses, partly due to a reduction in the level of BER proteins in that fraction. However, the development of synaptic impairment that is observed in 3xTgAD mice is not reflected in a decline in the BER capacity at the synapses, at least when the whole brain is investigated.

## 2. Methods

### 2.1. Animals

Male 129B6F1 mice were bred and fed at the University of Aarhus. Animals were sacrificed by decapitation at age 5 weeks (young), 5 months (adult), and 23 months (old) and whole brains were rapidly removed. Synaptosomal and free brain mitochondrial (FBM) fractions were purified and used to investigate the role of BER in synaptic loss during normal aging. On the other hand, the role of BER in synaptic impairment during AD was investigated by using male 3xTgAD mice carrying $PSI_{M146v}$, $APP_{Swe}$, and $Tau_{P301L}$ transgenes (Oddo et al., 2003a). Male C57BL/6 age-matched animals served as controls. The 3xTgAD mice were bred to homozygosity and backcrossed to C57BL/6 mice for 7 generations. In 3xTgAD animals, amyloid beta (Aβ) accumulation is first detectable in cortical brain regions, primarily frontal cortex and hippocampus at 6 months of age (Oddo et al., 2003b) along with impairment of synaptic transmission (Oddo et al., 2003a). Because the purpose of the investigation was to study the association between the appearance of Aβ pathology, synaptic failure, and BER, we compared 3-month-old presymptomatic mice to 12-month-old animals in which Aβ pathology is clearly evident. Animals were sacrificed by decapitation and brains were immediately removed for synaptosomal purification.

All mice were fed regular Purina animal chow (Purina Mills, Summit, MO) ad libitum and kept in a 12-hour light/dark cycle. All experiments were approved by the NIA IACUC and were performed in accordance with the guidelines for the use and care of laboratory animals (NIH Publications 85–23).

### 2.2. Purification of synaptosomal and free brain mitochondrial fractions

Synaptosomes were isolated as previously described elsewhere (Lai and Clark, 1979). This purification protocol of synaptosomes allows simultaneous isolation of nonsyn-