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Long-term proteasome dysfunction in the mouse brain by expression of aberrant ubiquitin

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Abstract

Many neurodegenerative diseases are characterized by deposits of ubiquitinated and aberrant proteins, suggesting a failure of the ubiquitin–proteasome system (UPS). The aberrant ubiquitin UBB^{+1} is one of the ubiquitinated proteins accumulating in tauopathies such as Alzheimer's disease (AD) and polyglutamine diseases such as Huntington's disease. We have generated UBB^{+1} transgenic mouse lines with post-natal neuronal expression of UBB^{+1}, resulting in increased levels of ubiquitinated proteins in the cortex. Moreover, by proteomic analysis, we identified expression changes in proteins involved in energy metabolism or organization of the cytoskeleton. These changes show a striking resemblance to the proteomic profiles of both AD brain and several AD mouse models. Moreover, UBB^{+1} transgenic mice show a deficit in contextual memory in both water maze and fear conditioning paradigms. Although UBB^{+1} partially inhibits the UPS in the cortex, these mice do not have an overt neurological phenotype. These mouse models do not replicate the full spectrum of AD-related changes, yet provide a tool to understand how the UPS is involved in AD pathological changes and in memory formation.

Keywords: Ubiquitin–proteasome system; Neurodegenerative disease; Learning and memory; Alzheimer's disease

1. Introduction

A balance between protein synthesis and ubiquitin-mediated proteasomal degradation contributes to normal neuronal function (Fonseca et al., 2006; van Tijn et al., 2008). Ubiquitin is tagged to proteins via its C-terminal glycine residue, after which the target protein is degraded by the proteasome (reviewed in Pickart, 2001). Aberrations of the ubiquitin–proteasome system (UPS) have been implicated in the pathogenesis of neurodegenerative diseases (reviewed in Ciechanover and Brundin, 2003; de Vrij et al., 2004). Ubiquitinated proteins accumulate in neurodegenerative disease hallmarks (Mori et al., 1987) and an age- and disease-related decline of UPS activity has been reported (Bennett et al.,

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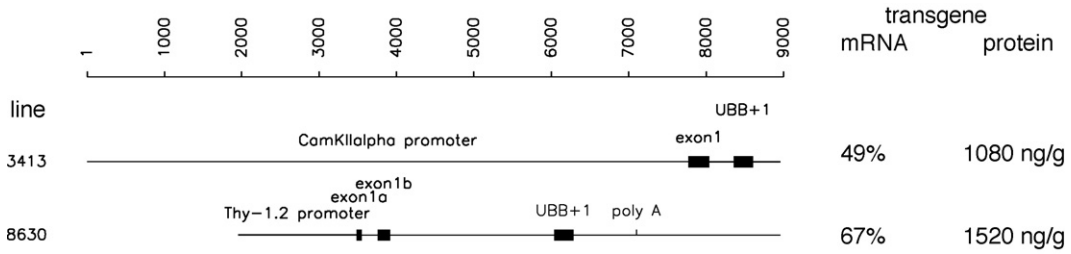

Fig. 1. Transgene expression on mRNA and protein level. Two different constructs used to generate transgenic mice. The first non-coding exon in both Thy-1.2 and CamkIIα promoter constructs is derived from Thy-1.2 or CamkIIα respectively. UBB^{+1} mRNA expression in the brain of mice ($n=4$ per genotype) was determined by Q-PCR. Transgene expression was measured with primers recognizing both the transgene and the endogenous UBB mRNA. Expression is relative to UBB levels of wild-type littermates (averages shown). UBB^{+1} protein expression in the brain of mice ($n=4$ per genotype) was measured with a radioimmunoassay, and is expressed at ng/g total protein content.

2007; Keck et al., 2003; Keller et al., 2000). Furthermore, it was reported that impairment of the UPS can be mediated by protein aggregation (Bence et al., 2001; Bennett et al., 2005), oxidative stress or oxidized proteins (Hyun et al., 2002; Okada et al., 1999) and amyloid-beta (Aβ) (Gregori et al., 1995; Kristiansen et al., 2007; Oh et al., 2005), leading to, e.g. accumulation of tau (Oddo et al., 2004; Song et al., 2003).

We have reported on the occurrence of an aberrant ubiquitin B^{+1} (UBB^{+1}) that selectively accumulates in neurodegenerative diseases such as the tauopathies like Alzheimer's disease (AD) and the polyglutamine disease Huntington's disease (HD) (De Pril et al., 2004; Fischer et al., 2003; van Leeuwen et al., 1998). UBB^{+1} is translated from an aberrant mRNA that is present at low frequency both in the brains of control subjects as well as in patients with neurodegenerative diseases (Fischer et al., 2003; Gerez et al., 2005). UBB^{+1} has lost the ability to ubiquitinate proteins (de Vrij et al., 2001), but is ubiquitinated itself and is both a substrate (Lindsten et al., 2002), and an inhibitor of the UPS (Lam et al., 2000). High levels of prolonged UBB^{+1} expression with viral vectors eventually lead to apoptosis in neuroblastoma cell lines (De Pril et al., 2004; de Vrij et al., 2001). The dual substrate/inhibitor property of UBB^{+1} has provided us with a tool to chronically inhibit the activity of the UPS (Hol et al., 2005; van Tijn et al., 2007).

We have generated several lines of transgenic mice expressing UBB^{+1} in neurons and analyzed these mice for gross neuropathology and changes in life-span. We identified a reduction in proteasome activity in the brains of these mice. By two-dimensional (2D) gel electrophoresis followed by mass spectrometry we identified proteins that are misregulated or proteins that are post-translationally modified as a result of the transgene expression and the subsequent chronic proteasome inhibition. Furthermore, we analyzed the impact of chronic proteasome inhibition on learning and memory, which is one of the salient features of AD. To our knowledge, no studies on chronic impairment of the *in vivo* proteasome and consequent proteomic changes have been published so far. Hence, this study gives the first insights into the consequences of long-term proteasome inhibition in a transgenic mouse model harboring a mutation relevant to neurodegeneration, and reveals some of the pathways that are affected as a consequence.

2. Materials and methods

2.1. Generation of transgenic mice

Two different promoters were used to drive expression in transgenic mice: the murine Thy-1.2 promoter (Caroni, 1997) and the murine CamKIIα promoter (Mayford et al., 1996). The *UBB^{+1}* cDNA, encoded by the first ubiquitin sequence and the C-terminus in the +1 reading frame (van Leeuwen et al., 1998) was either cloned directly in the Thy-1.2 cassette with XhoI, or with a flanking 5′ intron (Choi et al., 1991) and 3′ polyadenylation site (bovine growth hormone) in the CamkIIα cassette by NotI (Fig. 1A). Before injection, inserts were excised from the plasmid, purified from gel by electroelution and ethanol precipitated. Constructs were injected into fertilized oocytes of FVB/N (line 8630) or C57Bl/6 (line 3413) mice. The lines were maintained on their respective genetic background by breeding hemizygous mice to wild-type mice. The founder of line 8630 was highly mosaic (1/104 F1 screened); F1 mice from line 3413 were generated by *in vitro* fertilization. From F2 onwards Mendelian ratios were observed in the offspring. Mice were kept in group housing on a 12/12 h light–dark cycle with food and water ad libitum in specific pathogen free conditions (Nicklas et al., 2002). Mice were genotyped on DNA isolated from ear-snips using the QIAamp DNA mini kit (Qiagen); primers are listed in Supplementary information. The copy-number of the transgene (3413: 13 copies, 8630: 2 copies) was determined by Southern blotting and analysis on a Storm 860 phosphorimager (Molecular Dynamics). All animal experiments were performed conforming to national animal welfare law and under guidance of the animal welfare committee of the Royal Netherlands Academy of Arts and Sciences.

2.2. RNA isolation and qPCR

Mice were euthanized by carbon dioxide asphyxiation, the brain was immediately dissected and hemispheres