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DETERMINING NUCLEAR LOCALIZATION OF ALPHA-SYNUCLEIN IN MOUSE BRAINS

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Abstract—Alpha-synuclein (α-Syn) is a major component of Lewy bodies, abnormal protein aggregates that are present in neurons of patients with Parkinson's disease and other neurological disorders. Despite intensive investigation, the *in vivo* role of α-Syn in physiological and pathological processes is not fully understood. This study addresses a current debate on the nuclear localization of α-Syn protein in the brain. To assess the specificity of various α-Syn antibodies, we compared their staining patterns in wild-type mouse brains with that of the α-Syn knock-out mice. Among five different α-Syn antibodies tested here, two generated intensive nuclear staining throughout the normal mouse brain. However, nuclear staining by these two antibodies was also present in neurons of the α-Syn knock-out mice. This provides evidence that the nuclear signal is not specifically related to the presence of α-Syn, but it rather results from the cross-reactivity of the two antibodies to some unknown antigens in neuronal nuclei. In mouse brain neurons, endogenous α-Syn proteins are primarily localized to neuronal processes and nerve terminals but present only at low levels in the cell bodies. This is different from a generally uniform distribution of exogenously expressed α-Syn in both cytoplasm and nuclei of heterologous cells and suggests that the neuritic enrichment of α-Syn in neurons may be mediated by their specific interactions with certain structural or molecular components in the neuropil.

Key words: α-synuclein, mouse brain, nuclear localization, antibodies.

Alpha-synuclein (α-Syn) is a small protein implicated in the pathogenesis of a number of neurodegenerative disorders including Parkinson's disease (PD) (Recchia et al., 2004; Rampello et al., 2004; Wenning and Jellinger, 2005). Mutations in the α-Syn gene (A53T, A30P, and E46K) cause autosomal-dominant hereditary PD (Polymeropoulos et al., 1997; Kruger et al., 1998; Spira et al., 2001; Zarranz et al., 2004). Recent genome-wide association studies also revealed the involvement of α-Syn gene variants in sporadic PD (Venda et al., 2010). Moreover, α-Syn is a major protein component of Lewy bodies (LB), eosinophilic cytoplasmic inclusions developed in brain neurons of PD and other disorders (Spillantini et al., 1997; Giasson et al., 2000b; Ma et al., 2003; Lin et al., 2004; Cantuti-Castelvetri et al., 2005).

α-Syn was initially identified from the electric ray *Torpedo californica* as a neuronal-specific protein, which is localized to the presynaptic nerve terminal and nucleus (Maroteaux et al., 1988). Subsequent studies have shown that α-Syn proteins are abundantly expressed and widely distributed in mammalian central and peripheral nervous systems (Bayer et al., 1999; Mori et al., 2002). In neurons, α-Syn proteins are enriched at synapses and may play a regulatory role in presynaptic vesicle cycling and neurotransmitter release (Abeliovich et al., 2000; Chandra et al., 2004; Totterdell and Meredith, 2005; Lee et al., 2008; Watson et al., 2009). However, the nuclear localization of α-Syn remains controversial, as conflicting results have been obtained on the existence of endogenous α-Syn proteins in nuclei of mammalian brain neurons (Li et al., 2002; Yu et al., 2007; Zhang et al., 2008; Vivacqua et al., 2009, 2011; Zhong et al., 2010). Based on published reports, this discrepancy is possibly attributable to different α-Syn antibodies used in various studies, but the underlying cause is still unknown. On the other hand, experiments conducted in cultured primary neurons and transfected mammalian cells have consistently demonstrated the localization of α-Syn proteins in the nucleus where they may act to inhibit histone acetylation and promote neurotoxicity (McLean et al., 2000; Goers et al., 2003; Kontopoulos et al., 2006). In addition, nuclear localization of α-Syn has been observed in transgenic mice expressing a mutant form of α-Syn (A53T). Interestingly, these studies noted nuclear accumulation of phosphorylated α-Syn (Pser129) in specific brain regions of the transgenic mice, suggesting a potential role of nuclear α-Syn in neuropathology (Wakamatsu et al., 2007; Schell et al., 2009).

In this study, we examined the subcellular localization of endogenous α-Syn in mouse brains using a panel of antibodies. Our data suggested that nuclear staining by some of the α-Syn antibodies may result from their nonspecific cross-reactivity to other antigen(s) with epitope(s) similar to that of α-Syn. We also determined that endogenous α-Syn was minimally expressed in neuronal cell bodies, whereas it was enriched in neuropil throughout the mouse brain. Our findings should help resolve the ongoing debate on nuclear localization of α-Syn and contribute to our understanding of α-Syn's role in the brain.

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Abbreviations: cDNA, complementary DNA; DAPI, 4′,6-diamidino-2-phenylindole; LB, Lewy bodies; PBS, phosphate-buffered saline; PD, Parkinson's disease; PMT, photomultiplier; SN, substantia nigra; α-Syn, alpha-Synuclein.

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EXPERIMENTAL PROCEDURES

Antibodies and cDNAs

Polyclonal anti-α-synuclein antibodies were generated by immunizing rabbits with a glutathione S-transferase fusion protein containing the C-terminal region (residues 94-140) of human α-synuclein and purified using an affinity column containing the same fusion protein. In addition, the following antibodies were used in this study: monoclonal anti-α-synuclein antibody 3D5 (Yu et al., 2007), polyclonal anti-α-synuclein antibody C-20 (Santa Cruz Biotechnology, Inc., Santa Cruz, CA, USA), polyclonal anti-α-synuclein antibody AB5038P (Millipore, Billerica, MA, USA), polyclonal anti-α-synuclein antibody (Cell Signaling Technology, Inc., Danvers, MA, USA), monoclonal anti-α-synuclein (phosphorylated Ser129) antibody (Wako Chemicals, Inc., Richmond, VA, USA), monoclonal anti-α-synuclein (nitrated Tyr123/133) antibody (Novus Biologicals, Littleton, CO, USA), and monoclonal anti-tyrosine 3-hydroxylase antibody (Epitomics, Inc., Burlingame, CA, USA). The flag-tagged and EGFP-fused α-synuclein complementary DNA (cDNAs) were constructed by either cloning the human α-synuclein coding sequence into a mammalian expression vector pcDNA3, or fusing EGFP to the carboxyl terminus of α-synuclein coding region in the pEGFP-N1 vector.

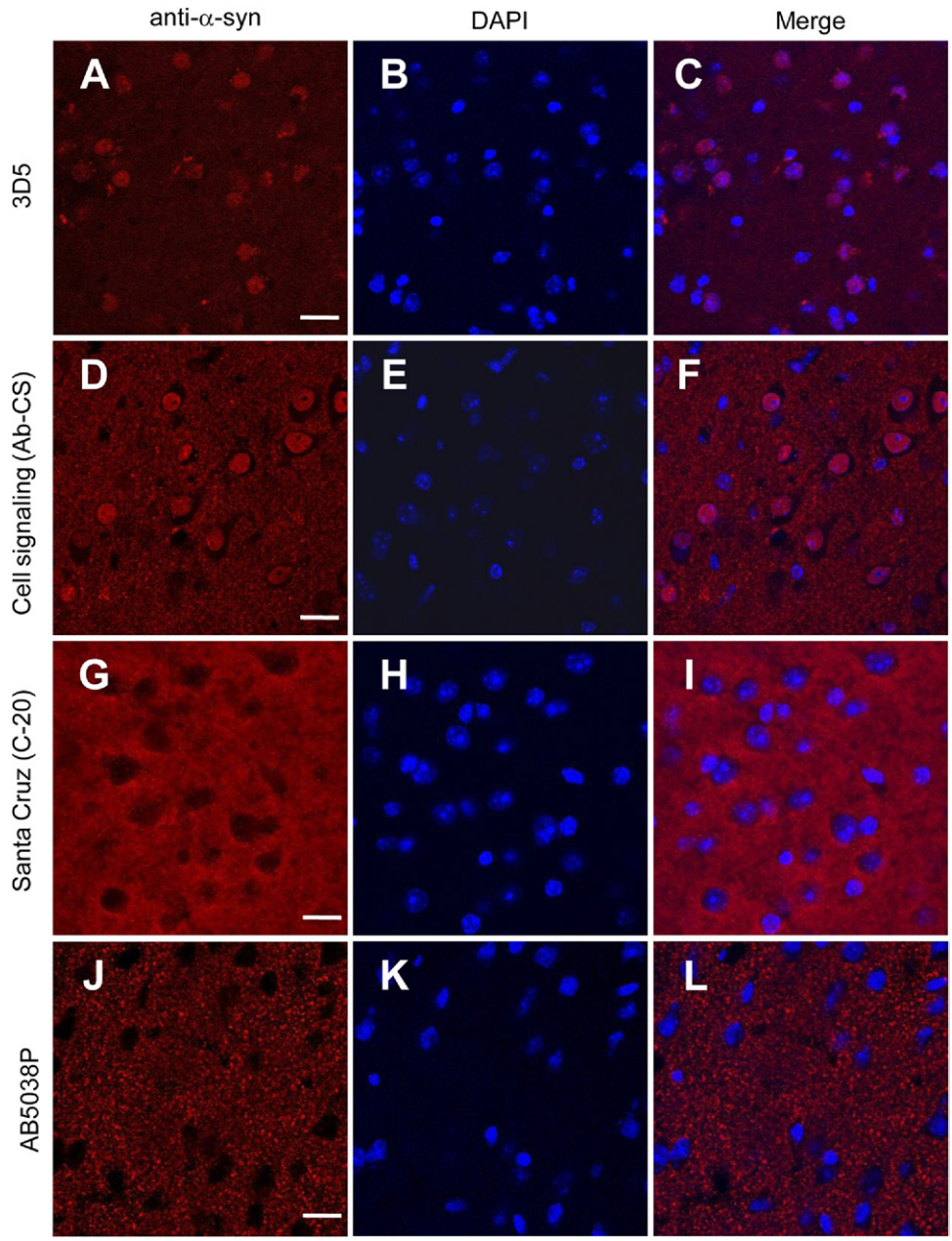

Fig. 1. α-Syn antibodies exhibited different nuclear immunoreactivity in mouse brain neurons. Confocal immunofluorescence images of mouse cerebral cortex stained with four different α-Syn antibodies (A, D, G, J) and counterstained with DAPI to depict the nuclei (B, E, H, K). Intensive nuclear staining was revealed in brain slices probed with both the 3D5 (A, C) and Cell Signaling (D, F) antibodies, but absent in that stained with either the C-20 (G, I) or the AB5038P antibody (J, L). Representative images from one of five independent experiments are shown. Scale bar=20 μm. For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.