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MACROAUTOPHAGY AND THE PROTEASOME ARE DIFFERENTLY INVOLVED IN THE DEGRADATION OF ALPHA-SYNUCLEIN WILD TYPE AND MUTATED A30P IN AN *IN VITRO* INDUCIBLE MODEL (PC12/TETON)

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Abstract—Many data suggest that alpha synuclein (α-syn) aggregation is involved in Parkinson's disease (PD) neurotoxicity and is accelerated by the pathogenetic point mutation A30P. The triplication of α-syn gene has been linked to early-onset familial PD, suggesting that the cellular dosage of α-syn is an important modulator of its toxicity. To verify this point, we developed an inducible model of α-syn expression (both wild type [WT] and mutated A30P) in rat PC12/TetOn cells. At low expression level, both α-syn(WT) and (A30P) did not aggregate, were not toxic, and displayed a protective action against oxidative stress triggered by hydrogen peroxide (H_2O_2). By increasing α-syn expression, its antioxidant function was no longer detectable as for the A30P form, but again no aggregation and cell death were present both for the WT and the mutated protein. To clarify why α-syn did not accumulate at high expression level, we inhibited macroautophagy by 3-methyladenine (3-MA) and the proteasome by MG132. In presence of 3-MA, α-syn(WT) accumulated, A11 anti-oligomer antibody-positive aggregates were detectable, and cell toxicity was evident, while proteasome inhibition did not increase α-syn(WT) accumulation. Macroautophagy or proteasome inhibition slightly increased α-syn(A30P) toxicity, with no detectable aggregation. This model can provide useful details about α-syn function, aggregation, and degradation pathways.

Key words: alpha-synuclein, protein aggregation, macroautophagy, proteasome, oxidative stress, Parkinson's disease.

Parkinson's disease (PD) is a neurodegenerative disorder characterized by the degeneration of the dopaminergic neurons of the *substantia nigra pars compacta*. This event correlates with a deficit of dopamine in the *striatum* that is at the basis of PD clinical features, including bradykinesia, resting tremor, rigidity, and postural instability (Fahn, 2003). From the neuropathologic point of view, PD is characterized in almost all forms by proteinaceous intracytoplasmic inclusion bodies called Lewy bodies (LB) and by the presence of abnormally shaped neurites named Lewy neurites (LN) (Dauer and Przedborski, 2003). The etiopathogenesis of PD is probably multifactorial, including both environmental and genetic factors (Di Monte, 2003; Gasser, 2009; Warner and Schapira, 2003). Several genes (α-syn, parkin, UCH-L1, DJ-1, LRRK2, PINK-1, and NR4A2) have been linked to genetic cases of PD (Bekris et al., 2010; Hashimoto et al., 2003; Giasson and Lee, 2003; Cookson, 2003). Alpha-syn is the principal component of LB and LN (Fahn, 2003), and three autosomal dominant missense mutations (an Ala to Pro substitution at codon 30 [A30P], an Ala to Thr substitution at codon 53 [A53T] and a Glu to Lys [E46K] at codon 46) have been linked to familial PD (Polymeropoulos et al., 1997; Krüger et al., 1998; Zarranz et al., 2004). The available data about α-syn physiological functions deal with presynaptic plasticity, dopamine trafficking and homeostasis, exocytotic vesicle interaction, regulation of monoamine transporters, and chaperone-like activity (Burré et al., 2010; Scott et al., 2010; Garcia-Reitböck et al., 2010; Lykkebo and Jensen, 2002; Wersinger et al., 2003; Oaks and Sidhu, 2011; Osterova et al., 1999). A30P and A53T mutations affect the ability of α-syn to modulate dopamine vesicle trafficking, modify its interaction with cellular membranes (Saha et al., 2004; Jensen et al., 1998), and increase its natural propensity to aggregate (Anderson et al., 2010; Volles and Lansbury, 2003; el-Agnaf and Irvine, 2002; Conway et al., 2000).

The description of a family with early-onset PD that showed a triplication of a chromosomic region containing α-syn gene suggested that the cellular dosage of the protein is critical for PD etiopathogenesis (Singleton et al., 2003; Uversky and Eliezer, 2009). Moreover, many putative triggers of idiopathic PD (mitochondrial complex I inhibitors, environmental toxins, oxidative stress, or proteasome impairment) cause α-syn modifications that are sufficient to alter its intracellular concentration, leading to aggregation and related toxicity (Riedel et al., 2011; Esteves et al., 2011; Xie et al., 2010; Sherer et al., 2003; Norris et al., 2003; Ischiropoulos and Beckman, 2003). From the other side, the existence of a neuroprotective pathway mediated by native α-syn is supported by some evidence (Jin et al., 2011; Lee et al., 2010; Jensen et al., 2003;

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Abbreviations: CMA, chaperone-mediated autophagy; DOXY, doxycycline; FCS, fetal calf serum; HS, horse serum; LB, Lewy bodies; LDH, lactate dehydrogenase release to lactate dehydrogenase; LN, Lewy neurites; PD, Parkinson's disease; UPS, ubiquitin-proteasome system.

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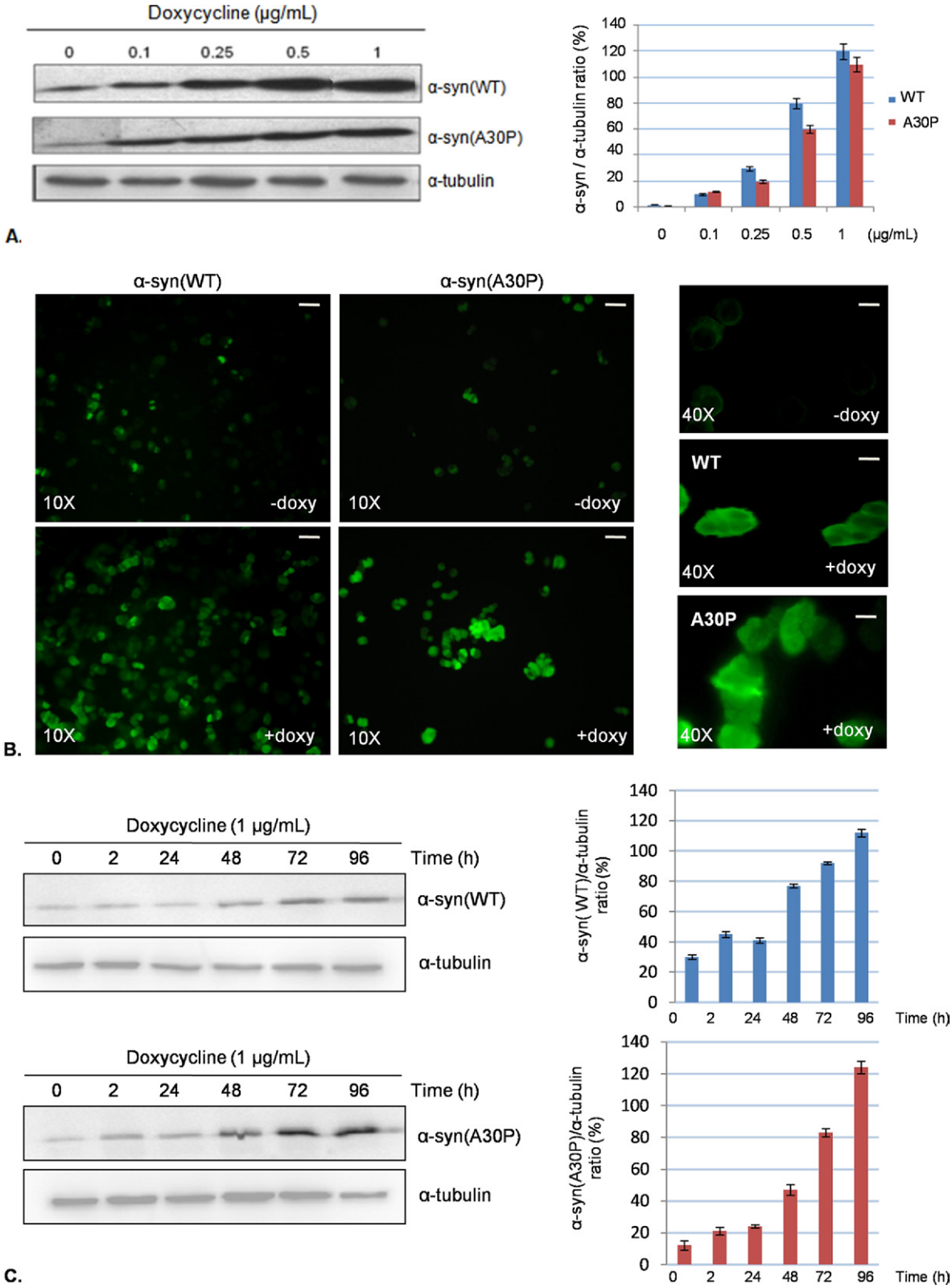

Fig. 1. Development of an inducible α-syn expression system in PC12 cells. (A) Representative Western blot showing human α-syn-inducible expression. PC12 cells were transfected with a tetracycline responsive plasmid (pBI-G) carrying human α-syn(WT or A30P) full-length cDNAs as described in methods. The dynamic range of α-syn expression was tested by addition of the antibiotic doxycycline (doxy.) for 96 h to culture medium in a dose ranging between 0.1 and 1 μg/ml. The bar graph (*right*) is the densitometric analysis of three independent Western blot experiments, using