

## Identification of $\alpha$ -synuclein in the CNS of lower vertebrates by two novel monoclonal antibodies: first immunohistochemical and western blot evidence

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$\alpha$ -Synuclein ( $\alpha$ -Syn) is a 140 aa protein which is highly expressed in the CNS of different vertebrates and linked to Parkinson's disease (PD) in mammals. We have examined the localization of  $\alpha$ -Syn in the CNS of zebrafish (*Danio rerio*), a widely used model species for PD, the carp (*Cyprinus carpio*) and the African clawed frog (*Xenopus laevis*) through immunohistochemistry and western blot (WB) by using two new monoclonal antibodies (2E3 and 3D5) against  $\alpha$ -Syn. In mammals, both antibodies labeled nerve fibers and 3D5 also localized  $\alpha$ -Syn within the neuronal nuclei. Conversely to mammals, no nuclear staining was detectable in fish and amphibian neuronal bodies by 3D5. Moreover, the anatomical distribution of the protein is markedly different. In fact, in mammals  $\alpha$ -Syn is ubiquitarily distributed in the brain and the spinal cord, whereas in lower vertebrates only few brain regions display a positive staining. In accordance with literature data, these regions provide the amount of dopamine and catecholamines to the brain. Therefore, our results suggest that in lower vertebrates  $\alpha$ -Syn is exclusively linked to catecholaminergic and dopaminergic systems and it may represent a protein linked to synaptic vesicles, whereas in mammals it becomes an invariant and structural component of distinct neuronal subtypes assuming a new subcellular localization. WB analysis performed on the CNS homogenates by 2E3 antibody revealed an intense band at 19 kDa. However, the 3D5 antibody stained only faint bands in the same homogenates. These results confirm the presence of  $\alpha$ -Syn in the CNS of lower vertebrates analyzed. Our observation suggest that the role of  $\alpha$ -Syn in the neuronal physiology could improve, during the evolution of vertebrates and phylogenetic approach could represent a novel method to understand the physio-pahological role of  $\alpha$ -Syn in neurological diseases.

### Key words

Alpha-Synuclein, *Danio rerio*, *Xenopus laevis*, Immunohistochemistry, Western blot, Parkinson disease