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From Mad Cows to Neurotic Yeast: Novel Molecular Approaches to Understand Neurodegeneration

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Aging is the major known risk factor for Alzheimer's disease (AD) and Parkinson's disease (PD), but genetic deffects have been associated with familial cases. Huntington's disease (HD) is a purely genetic neurodegenerative disorder, where mutations in the *IT15* gene, encoding for the protein huntingtin, determine the development of the disease. The Prion diseases differ from these other disorders because they can also have infections origin [1-3].

A common hallmark to many neurodegenerative diseases is the presence of proteinacious inclusions inside neuronal populations, which are selectivelly affected in each disorder. Neurofibrillary tangles made of the tau protein in AD, Lewy bodies made of α-synuclein in PD, and huntingtin inclusions in HD are but a few examples of protein aggregates deposited inside neurons. Whether inclusions are themselves toxic or actually cytoprotective is still under current debate, but it is widely accepted that protein misfolding and oligomerization are central molecular events in these diseases.

Molecular genetic approaches using different model organisms, from yeast to mammalian cell culture and mouse models, coupled with advanced microscopy techniques resulted in a detailed characterization of the pathways and events involved in cytotoxicity. The engineering of green fluorescent protein (GFP) has yielded a variety of reagents for the direct visualization of the molecular events in living cells. Through the use of fusion proteins, and the manipulation of protein expression levels, we gained insight into the biology of mammalian prions through the study of their yeast relatives, which constitute a novel agent for the transmission of phenotypic information [4].

Using the budding yeast *Saccharomyces cereviseae* as a 'living test tube' we were able to distinguish between membrane-bound and aggregated forms of α-synuclein, in a yeast model of PD [5]. Powerful yeast genetic screens enabled us to identify several molecular pathways as playing central roles in the toxicity induced by α-synuclein in yeast. Genes involved in intracellular trafficking, lipid metabolism, and oxidative stress, were among the most highly represented categories.

Imaging techniques, such as Fluorescence Lifetime Imaging Microscopy (FLIM), bimolecular fluorescence complementation (BiFC) and multi-photon in vivo imaging, are powerful tools in the study of protein-protein interactions associated with protein deposition in disease. We are applying a variety of tools to unravel the molecular basis of neurological disorders associated with protein misfolding, with the goal of developing novel avenues for therapeutic intervention.

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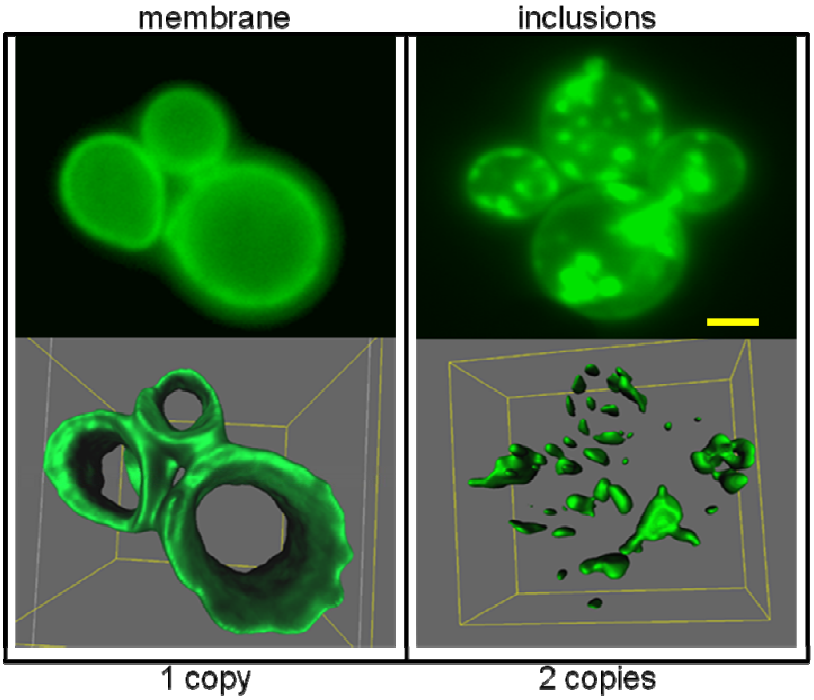

Figure 1. Intracellular distribution of α-synuclein in yeast cells. Low levels of expression (1 copy of the fusion α-synuclein-GFP) result in membrane localization, whereas the introduction of a second copy of the same gene (2 copies) leads to the formation of intracellular inclusions. Scale bar, 1μm.

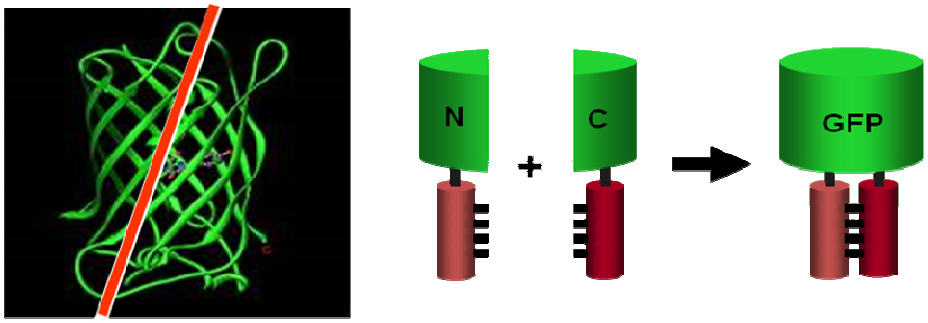

Figure 2. Bimolecular fluorescence complementation assay (BiFC). Each of the two halves of GFP are fused to the proteins of interest and co-expressed in the same cell. Each fusion protein, by itself, is non-fluorescent. If the proteins of interest interact they bring the two halves of GFP in close proximity enabling the reconstitution of the fluorophore and emission of fluorescence.

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