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CHARACTERIZATION OF α-SYNUCLEIN AND HISTONE H1 INTERACTION AND ITS POTENTIAL ROLE AS THERAPEUTIC TARGET IN TREATMENT OF PARKINSON'S DISEASE

INTRODUCTION. Parkinson's disease is the **second most important neurodegenerative** disease affecting 1% of the population over 60 years old. PD hallmarks are **dopaminergic neurons loss** and the presence of fibrillar α-synuclein, known as **Lewy's bodies** (LB), on surviving cells. Previous studies has revealed that **histone H1** interacts with **α-synuclein** accelerating its fibrillation. It has been seen as well that in neurodegenerating conditions histone H1 overexpression does not change while its localization does, senile plaques present in brains with Alzheimer's disease are immunoreactive for histone H1. What is more nuclear localization of histone H1 causes neurotoxicity but on the other hand its sequestration on the cytosol is neuroprotective. All those points make us realize that histone H1 must have an important role not just in PD but also in other neurodegenerative diseases.

DETERMINE THE ROLE OF HISTONE H1 IN THE CINETICS OF α-SYNUCLEIN FIBRILATION

OBJECTIVES

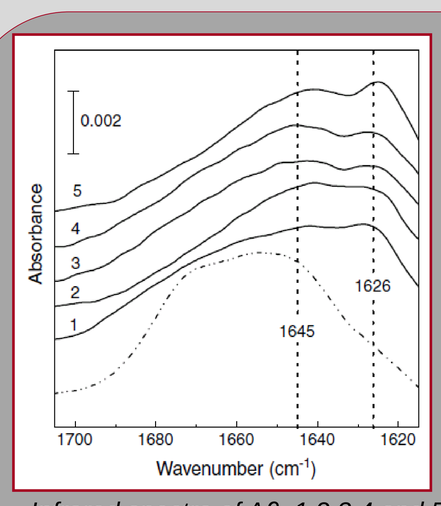
1. To study the role of histone H1 phosphorylation, methylation and acetylation in the α-synuclein fibrillation rate.
2. To study the nature of the interaction between the two proteins.
3. To determine if fibrillar α-synuclein is more sensitive to proteolysis than the complex α-synuclein/histone-H1

METHODOLOGY

We will perform qualitative and quantitative assays in order to obtain brand new information about the role of histone H1 in PD due its interaction with α-synuclein. How?

- *In vitro* assays using human α-synuclein expressed in *Escherichia coli.*
- *In vivo* assays using α-synuclein purified from an animal model.
- α-synuclein- histone H1 **complex formation** in vitro will be tested using **ThT fluorescence**, **FTIR spectroscopy** and **native PAGE**.
- The nature of the interaction between the two proteins will be assayed in vivo changing **medium composition** and its effect will be tested by **electron microscopy**, **ThT fluorescence** and **native PAGE**.
- In vitro **proteolithic assay** is going to be done using **kallikrein-6** and the result will be assayed by **electron microscopy** and **FTIR spectroscopy**

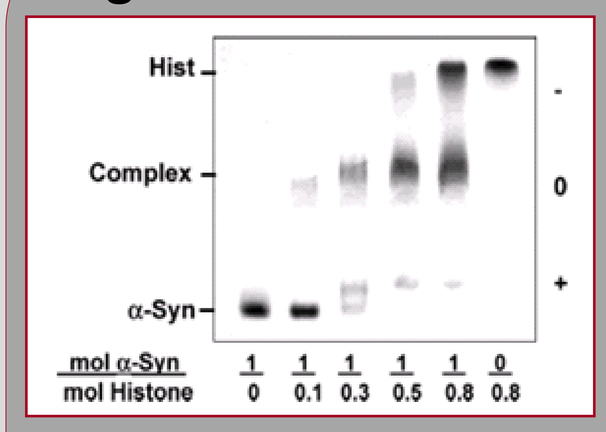
Figure 1: FTIR spectroscopy



Infrared spectra of Aβ. 1,2,3,4 and 5 correspond to different times of oligomeritzation. (Benseny-Cases, Cócera, & Cladera, 2007)

FTIR is used to study secondary structure. It's possible to distinguish between two regions of the spectra: a band around 1626 cm-1 (corresponding to ordered structures) and a band at 1645 cm-1 indicative of unordered and helical structures. The ratio of *A1626/A1645* is used to monitor β structure formation

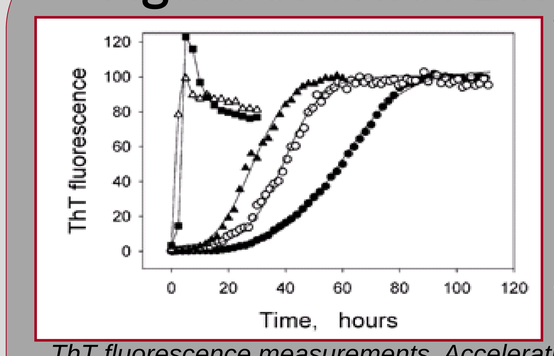
Figure 2:Native PAGE



Native Page of human recombinant α-synuclein with increasing concentrations of histone H1.(Goers et al., 2003)

Histone H1 and α-synuclein has opposite net charge, in a native PAGE they will run in opposite directions while its complex (without net charge) will stay on the middle of the gel. The study of the bands from the gel will help us to analyze complex formation.

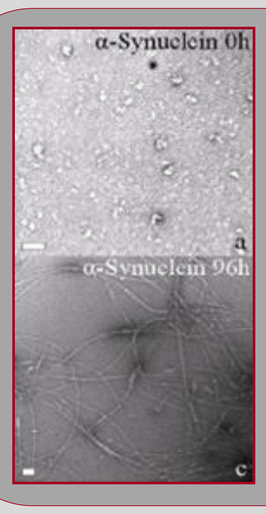
Figure 3: THIOFLAVIN T FLUORESCENCE



ThT fluorescence measurements. Acceleration of α-synuclein fibrilation with increasing concentrations of histone H1. (Goers et al., 2003)

Thiflavin T (ThT) is a molecule that increase its fluorescence when it is localized on a fibrilar structure. Mesuring ThT fluorescence we will monitoraize α-synuclein fibrillation.

Figure 4: ELECTRON MICROSCOPY



With this technique we will evaluate if the introduction of histones H1 on the fibrils induce any drastic morphologic change and also how the fibrils are affected by proteolysis.

α-synuclein and aged α-synuclein were visualized using electron microscopy. Aged α-synuclein show fibril formation. (Duce et al., 2006)

EXPECTED RESULTS

- Histone H1 and histone H1 with posttranslational modifications are expected to increase α-synuclein aggregation rate, been the effect higher in phosphorylated histone H1.
- α-synuclein-histone H1 complex should be less sensitive to proteolysis due fibrill α-synuclein stabilization thanks to histone H1 protective effect.
- Histone H1 and α-synuclein has opposite net charge it is believed that their interaction is electrostatic so salty medium will affect complex formation.
- Considering the role of histone H1 in many neurodegenerative disease we believe that characterizing α-synuclein and histone H1 interaction could lead to the development of brand new therapies that will reduce dopaminergic neuronal loss.

Web page development to microfunding the project

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