FEBS Letters 586 (2012) 7–12

journal homepage: www.FEBSLetters.org

α-Synuclein modifies huntingtin aggregation in living cells

Federico Herrera [a], Tiago Fleming Outeiro [a,b,c,*]

[a] Cell and Molecular Neuroscience Unit, Instituto de Medicina Molecular, Lisboa, Portugal
[b] Instituto de Fisiologia, Faculdade de Medicina da Universidade de Lisboa, Av. Prof. Egas Moniz, 1649-028 Lisboa, Portugal
[c] Department of Neurodegeneration and Restaurative Research, University Medizin Gottingen, Waldweg 33, 37073 Gottingen, Germany

ARTICLE INFO

Article history:
Received 26 October 2011
Accepted 10 November 2011
Available online 24 November 2011

Edited by Jesus Avila

Keywords:
Huntingtin
α-Synuclein
Co-aggregation
Bimolecular fluorescence complementation assay

ABSTRACT

Several neurodegenerative disorders are characterized by the accumulation of proteinaceous inclusions in the central nervous system. These inclusions are frequently composed of a mixture of aggregation-prone proteins. Here, we used a bimolecular fluorescence complementation assay to study the initial steps of the co-aggregation of huntingtin (Htt) and α-synuclein (α-syn), two aggregation-prone proteins involved in Huntington's disease (HD) and Parkinson's disease (PD), respectively. We found that Htt (exon 1) oligomerized with α-syn and sequestered it in the cytosol. In turn, α-syn increased the number of cells displaying aggregates, decreased the number of aggregates per cell and increased the average size of the aggregates. Our results support the idea that co-aggregation of aggregation-prone proteins can contribute to the histopathology of neurodegenerative disorders.

Structured summary of protein interactions:
Htt and **Htt** physically interact by bimolecular fluorescence complementation (View interaction)
alpha-syn and **Htt** physically interact by bimolecular fluorescence complementation (View interaction)
alpha-syn and **alpha-syn** physically interact by comigration in non-denaturing gel electrophoresis (View interaction)
Htt and **Htt** physically interact by comigration in non-denaturing gel electrophoresis (View interaction)
alpha-syn and **Htt** colocalize by fluorescence microscopy (View Interaction: 1, 2)
alpha-syn and **alpha-syn** physically interact by bimolecular fluorescence complementation (View interaction)
Htt and **alpha-syn** physically interact by comigration in non-denaturing gel electrophoresis (View interaction)

1. Introduction

The presence of amyloid-like proteinaceous inclusions is the common histopathological hallmark of a large group of human diseases known as protein misfolding disorders. Neurodegenerative disorders such as Alzheimer's (AD), Parkinson's (PD) or Huntington's disease (HD) belong to this group of pathologies. The specific location and composition of protein aggregates are characteristic of each disorder. For example, while PD aggregates (Lewy Bodies) consist primarily of α-synuclein (α-syn) protein, the main component of HD aggregates is a very large protein named huntingtin (Htt). The areas of the brain that are initially affected by these aggregates and the clinical symptoms are also different among the different neurodegenerative disorders [Reviewed in [1–3]]. However, growing evidence indicates that the differences between neurodegenerative disorders are not as clear as initially thought and that there is substantial overlap among them.

Neurodegenerative disorders often show mixed features at all levels [4]. Patients with movement disorders, such as PD and HD, frequently develop dementia in intermediate or late stages [5]. Conversely, patients with a primary diagnosis of dementia can show PD-like motor symptoms [6]. The differences between disorders are even more blurred at the cellular and molecular level. For example, α-syn was originally identified as the precursor of the major non-amyloid component of AD plaques [7]. The deposition

Abbreviations: Htt, huntingtin; α-syn, α-synuclein; HD, Huntington's disease; PD, Parkinson's disease; AD, Alzheimer's disease; BiFC, bimolecular fluorescence complementation assay

* Corresponding author at: Department of Neurodegeneration and Restaurative Research, University Medizin Gottingen, Waldweg 33, 37073 Gottingen, Germany. Fax: +351 21 799 9436.
E-mail address: touteiro@gmail.com (T.F. Outeiro).

0014-5793/$36.00
doi:10.1016/j.febslet.2011.11.019

of α-syn has been identified in multiple disorders traditionally characterized as tau-associated pathologies (tauopathies), such as AD, Down syndrome, progressive supranuclear palsy, parkinsonism dementia complex of Guam and frontotemporal dementia [8–11]. Conversely, deposition of tau has been observed in patients with familial and sporadic PD, sporadic dementia with Lewy bodies and multiple system atrophy and animal models of some of these disorders [12–18].

The co-occurrence of aggregation-prone proteins might have important pathological consequences. For example, Tau enhances α-syn aggregation in vitro [15] and in cell cultures [19]. Likewise, α-syn can promote amyloid β peptide aggregation in vivo [20] and, triple transgenic mice for amyloid β peptide, tau and α-syn show an enhancement of all three pathologies [21].

A decade ago, two independent groups showed that Htt inclusions in patients and mouse models of HD are positive for α-syn [22] and that over-expression of α-syn in human cells promotes Htt aggregation [23]. To the best of our knowledge, these are the only reports on the interplay between these two aggregation-prone proteins. In the present work we employed the bimolecular fluorescent complementation (BiFC) assays we recently developed [24,25] to visualize and study further the interaction between Htt and α-syn in living human cells.

2. Materials and methods

2.1. Yeast strains, plasmids and growth conditions

We used *Saccharomyces cerevisiae* W303 strain (*MATa can1-100 his3-11, 15 leu2-3, 112 trp1-1 ura3-1 ade2-1*) under standard growth conditions. Cells were transformed with lithium acetate with p426GPD-a-synuclein-YFP and p423GPD-huntingtin-CFP constructs [26,27]. Cells were maintained in SD-URA-HIS solid medium and were grown up to mid log phase for epifluorescence microscopy analysis.

2.2. Cell cultures and plasmids

Human H4 glioma cells (ATCC HTB-148, LGC Standards, Barcelona, Spain) were maintained and seeded as previously described [24]. The development of wild-type (25Q) and mutant (103Q) Htt-Venus BiFC plasmids was described in detail elsewhere [24]. α-Synuclein-Venus BiFC plasmids were a kind gift of Dr. Pamela J. McLean (Department of Neurology, Alzheimer's Disease Research Unit, Massachusetts General Hospital, MA, USA). Cells were transfected with the different combinations of plasmids using Fugene 6 transfection reagent (Roche diagnostics, Mannheim, Germany) 16–24 h after seeding. Samples were collected or analyzed 24 h after transfection unless otherwise indicated. Toxicity assays were carried out as described previously [24].

2.3. Flow cytometry

Cells were collected by trypsinization (5 min at 37 °C), washed once with PBS, and fixed in 1% (w/v) paraformaldehyde in PBS for 10 min at room temperature. Samples were analyzed by means of a FACSCalibur flow cytometer (Beckton Dickinson, Franklin Lakes, NJ, USA). Ten thousand cells were analyzed per group. Graphics and data analysis were carried out by means of the FlowJo software (Tree Star Inc., Ahsland, OR, USA).

2.4. Immunocytochemistry and microscopy

Living cells were analyzed directly by widefield fluorescence microscopy (BiFC experiments, Figs. 2 and 3) or processed for immunocytochemistry as described previously [28] (Fig. 1). The following antibodies were used: Htt (1:100, Millipore, Billerica, MA, USA), α-syn (1:100, Cell Signaling, Danvers, MA, USA), and Alexa fluor 488- and 568-conjugated secondary antibodies (1:1000, Invitrogen Technologies, Carlsbad, CA, USA).

Cells were visualized, and pictures were taken, by means of an Axiovert 200 M widefield fluorescence microscope equipped with a CCD camera (Carl Zeiss MicroImaging GmbH, Germany). Images were analyzed and prepared for publication by means of the ImageJ free software (http://rsbweb.nih.gov/ij/).

2.5. Immunoblotting

Proteins were extracted by scrapping cells directly from the plates into lysis buffer. For denaturalizing conditions, the lysis buffer was 0.1% Triton X-100, 150 mM NaCl, 50 mM Tris pH 7.4 and a protease inhibitor cocktail tablet (Roche diagnostics, Mannheim, Germany). For native conditions, the lysis buffer was 50 mM Tris–HCl pH 7.4, 175 mM NaCl, 5 mM EDTA and a protease inhibitor cocktail tablet (Roche diagnostics, Mannheim, Germany). Cells were then sonicated and centrifuged at 10 000×g for 10 min at 4 °C. Supernatants were collected and the protein concentration was quantified by the Bradford assay (Bio-rad, Hercules, CA,

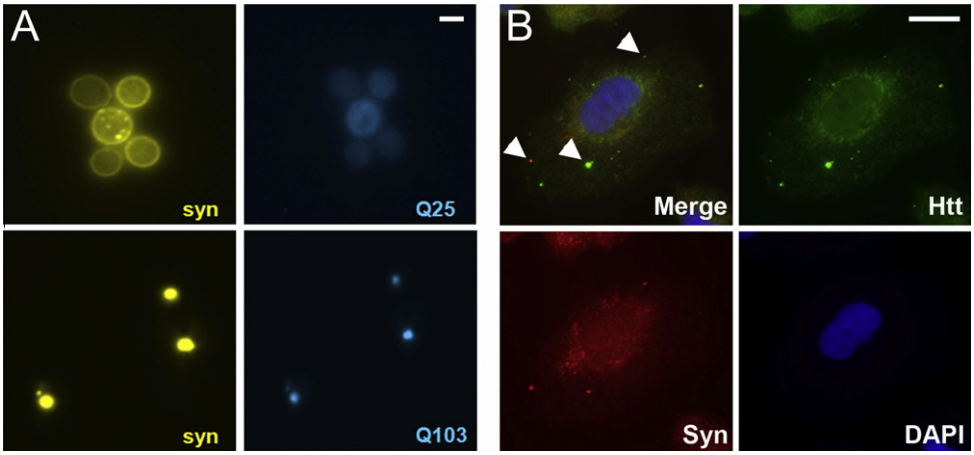

Fig. 1. α-Syn co-localizes with Htt aggregates. (A) Yeast cells were co-transfected with α-syn-YFP (in yellow) and wild type (25Q) or mutant (103Q) Htt exon 1 (in blue). Scale bar 2 μm. (B) H4 human glioma cells co-transfected with 103QHtt exon 1 tagged with full length EGFP (in green) and non-tagged α-syn (in red). In both cases, there is co-localization of α-syn and mutant Htt in aggregates (white arrowheads). Scale bar 20 μm.