N-terminal phosphorylation of Hungtintin exon 1: liquid-liquid phase separation and aggresome formation in mammalian cells

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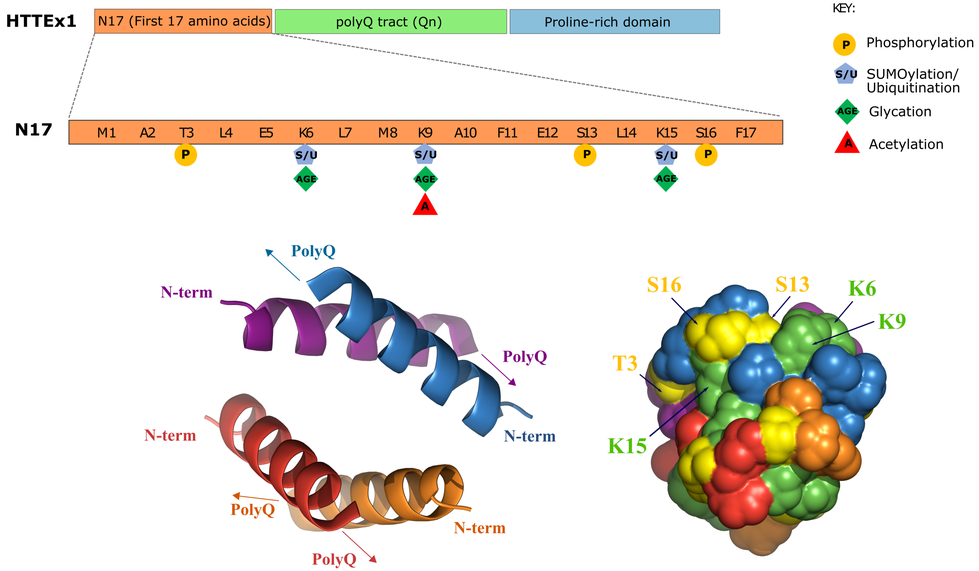
Background

An extended polyglutamine **(polyQ) tract >35** glutamines in the first exon of huntingtin (**HttEx1**) is the cause of **Huntington disease** (HD), a neurodegenerative disorder characterized by the presence of huntingtin inclusions in the striatum. HttEx1 is an intrinsically disordered region with a strong tendency to aggregate. Flanking the polyQ tract, HttEx1 contains a sequence of 17 amino acids (N17) at the N-terminal region and by a proline-rich domain (PRD) of 51 amino acids at the C-terminal region (Fig. 1). The N17 domain is susceptible to phosphorylation at the T3, S13 or S16 residues, and their phosphorylation state regulates HttEx1 aggregation and toxicity.

Figure 1. Sequence and structure of the N-terminal domain of huntingtin



Results

HttEx1 aggregates have been described and classified according to their size, morphology and dynamics. We have characterized them by means of a microscopy approach on living cells, combined with image analysis software. HttEx1 forms a **fibrillar aggresome** in the perinuclear region of the cells and **liquid-like condensates by liquid-liquid phase separation (LLPS)**, as a previous step to the formation of **amyloid-like fibrils (Fig. 2)**.

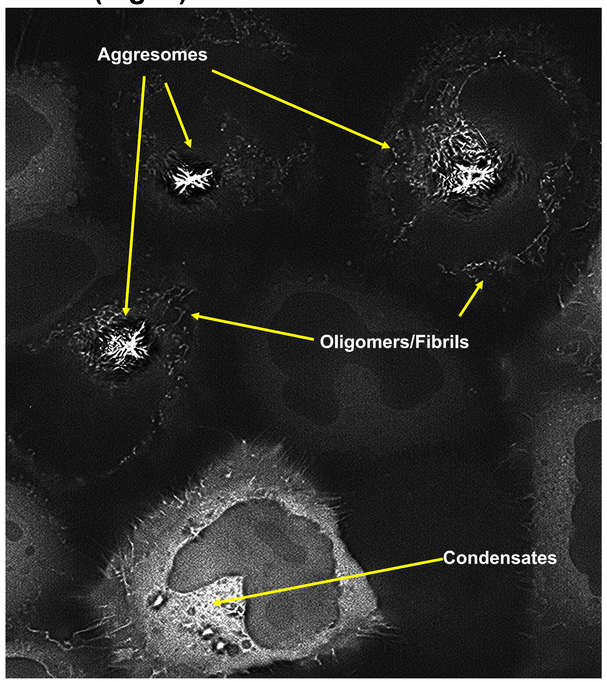


Figure 2. Types of HttEx1 aggregates in living cells

The length of the **polyQ tract** has a significant influence in **aggresome** formation, a phenomenon that is driven by the quality control system of the cell. In contrast, **phosphorylation on the N17 region** of HttEx1 has a greater effect on liquid-liquid phase separation and dynamics of the condensates. Our results show that different structural factors of the protein drive the branched process of Httex1 aggregation in live cells.

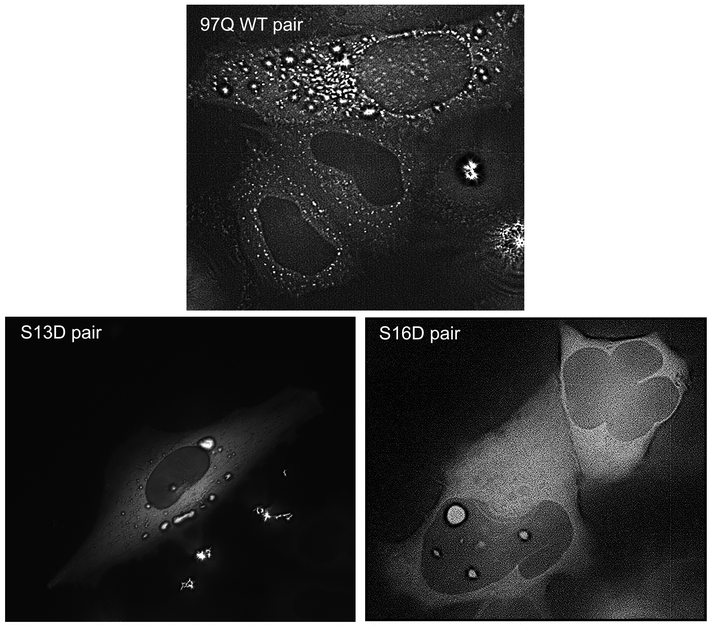


Figure 3. Mimicking N-terminal phosphorylation produces Liquid-Liquid Phase separation