Single N-terminal phosphorylation modulates mutant huntingtin aggregation and toxicity

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Abstract

Huntington's disease (HD) is an incurable neurodegenerative disorder caused by a polyglutamine (polyQ) expansion in the N-terminal region of the huntingtin protein (HTT). The first 17 amino acids of the protein (NT17), immediately preceding the polyQ tract, are a critical functional domain for HTT function and HD pathogenesis. Recent studies suggest that double NT17 phosphorylation at serines 13 and 16 reduces mutant HTT aggregation and toxicity. However, double phosphorylation events are less likely to occur than single phosphorylation events and require overexpression of specific kinases. Understanding the mechanisms involved in mutant HTT aggregation and toxicity mediated by single NT17 phosphorylation may provide promising avenues for the development of simpler and more effective therapeutics. Here, we analyzed the effect of single NT17 phosphorylation events (at Thr3, Ser13 or Ser16) in HTT aggregation and toxicity in cell and *Drosophila* models of HD.

Results

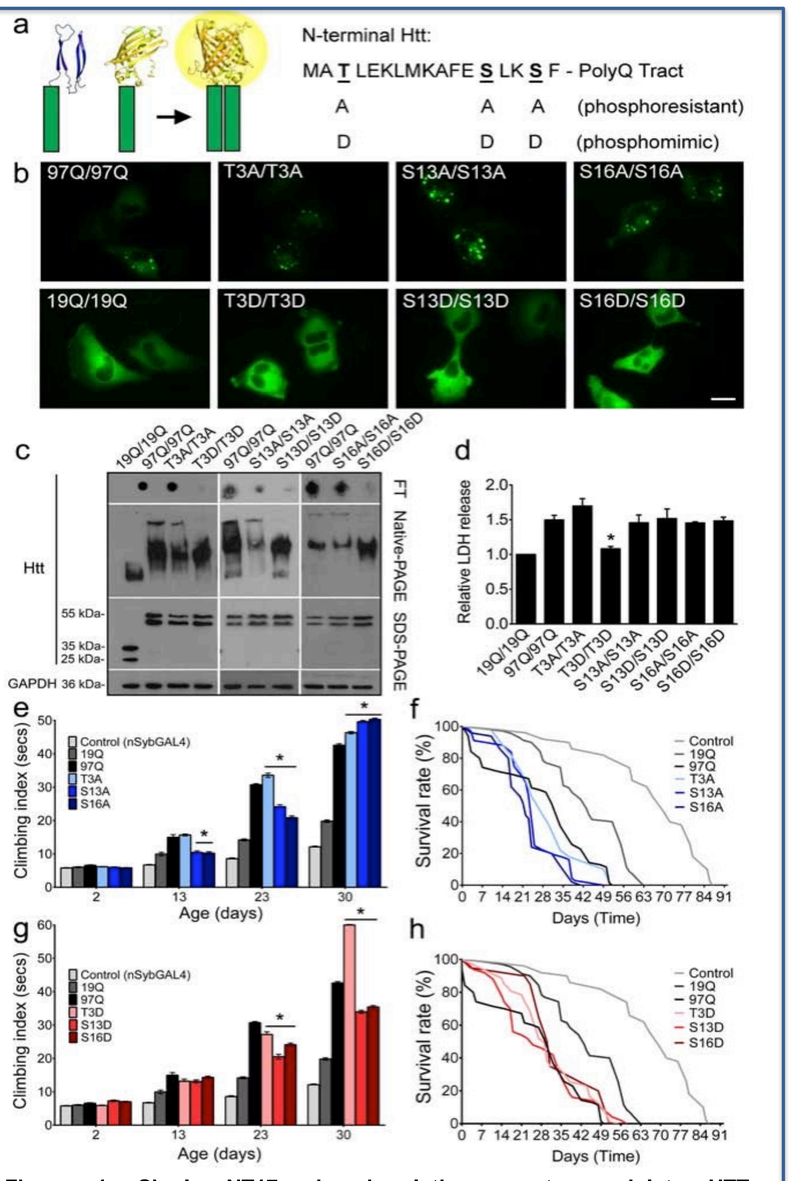

Figure 1. Single NT17 phosphorylation events modulate HTT aggregation but not early-stage oligomerization in mammalian cells. a, HTT (green bars) was fused to two non-fluorescent halves of the Venus protein. When HTT dimerizes, Venus recovers fluorescence. The three NT17 phosphorylatable residues where mutated to mimic phosphorylation or dephosphorylation. **b-c,** Phosphoresistant BiFC pairs showed no overt phenotype, while phosphomimic pairs abolished HTT aggregation in H4 human glioma cells. **d,** only the T3D mutation prevented HTT toxicity. **e-h,** climbing and survival assays in Drosophila expressing HTT in the central nervous system under the nSyb-GAL4. HTT toxicity is age-dependent and more prominent in phosphoresistant mutants. Importantly, T3D mutants were given a climbing index value of 60 in climbing assays at 30 days because flies did not go up at all by the end of the experiment time. *,significant versus 97QHTT, p<0.05.

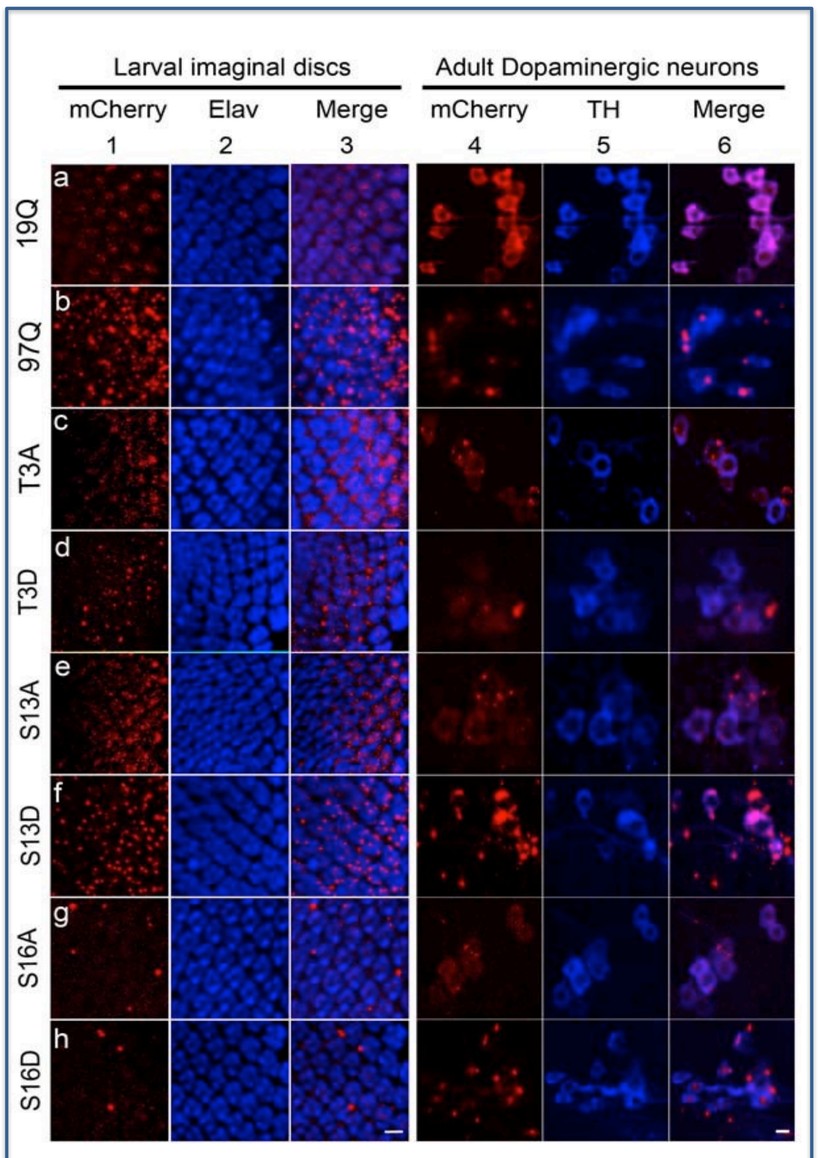

Figure 2. The role of single NT17 phosphorylation/dephosphorylation events on HTT aggregation in flies. Confocal pictures of larval eye imaginal discs (columns 1-3) and adult brain dopaminergic neurons (columns 4-6) in flies expressing 97QHTT exon 1 under the control of GMR-GAL4 or TH-GAL4, respectively. HTT is shown in red, and photoreceptors (columns 2-3, anti-Elav) or dopaminergic cell cytoplasm (columns 5-6, anti-TH) are shown in blue. Any mutation in the NT17 phosphorylatable residues reduced HTT aggregation in larvae (columns 1-3), with the exception of S13D, which shows no change versus non-mutated 97QHTT (row f). In adults, most phosphomutants inhibited aggregation, but S13D enhanced aggregation (row f) and S16D showed no change in aggregation (row h). Additionally, S16D enhanced nuclear localization of HTT aggregates (row h, column 6).

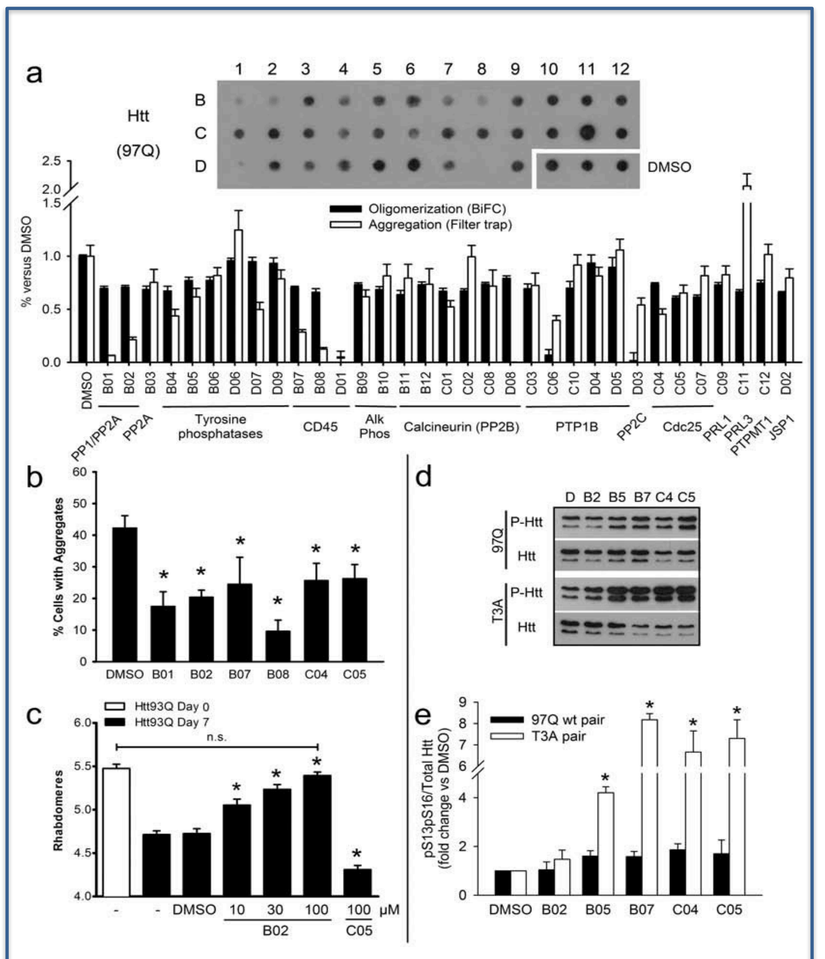

Figure 5. Protein phosphatases regulate HTT phosphorylation, oligomerization, aggregation and toxicity. a, Representative filter trap of protein extracts from cells transfected with 97QHTT BiFC constructs and treated protein phosphatase inhibitors. The graph shows oligomerization levels as determined by flow cytometry (black bars) and aggregation levels as determined by optic density of filter trap dots (white bars). Phosphatase inhibitors were named by their position in the commercial 96-well plate (Enzo Life Sciences) for the sake of brevity, but their common names and concentrations are specified in Supplementary Table I. **b,** Selected inhibitors also reduced the percentage of H4 cells with large HTT inclusions. **c,** a PP1/PP2A inhibitor (B02) prevented HTT toxicity in flies in a dose-dependent manner, while a cdc25 inhibitor enhanced it (C05), as determined by the pseudopupil analysis. **d-e,** selected inhibitors did not modify significantly HTT S13/S16 phosphorylation in normal conditions, but they enhanced it when a T3A mutation was present. The T3D mutation completely blocked S13/S16 phosphorylation. **d,** representative films from western blots with antibodies against total HTT and pS13/pS16 HTT in the presence of selected phosphatase inhibitors. **e,** densitometric analysis of western blots. *, significant versus DMSO, p<0.05.

Main findings

- Single N-terminal phosphorylation completely abolished mutant HTT aggregation in living cells;
- Thr3 phosphorylation seems to play a dominant role in mutant HTT aggregation *in vitro* and *in vivo;*
- Single N-terminal dephosphorylation potentiated mutant HTT toxicity in flies;
- Specific protein phosphatases regulate HTT phosphorylation, aggregation and toxicity *in vitro* and *in vivo.*

Conclusions

Our findings suggest that phosphorylation of the HTT N-terminal region plays a critical role in HD pathogenesis, and support the targeting of specific NT17 phosphorylatable sites and protein phosphatases for HD therapy.

Acknowledgements

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