Interrogating the role of splicing factors in Huntington's Disease

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Introduction

Huntington's disease (HD) is a fatal neurodegenerative disorder caused by the expansion of a salient region within the huntingtin protein (htt). Given the wide range of cellular interactions involving htt, pathogenesis is attributed to both disruption of numerous cellular and metabolic pathways, as well as toxic effects, most notably a propensity of mutant htt to misfold and aggregate[3].
Following transcription of DNA to mRNA, nascent mRNA undergoes several stages of processing prior to translation into a functional protein. One such modification is splicing, the process by which mRNA is cut and reassembled in various combinations to facilitate spatial and temporal protein changes. The vast majority of genes undergo splicing, and approximately 15% of genetic diseases are now thought to affect mRNA splicing[2, 5] Defects in this level of processing have been attributed to several neurological diseases, though the role of such defects in HD pathogenesis is unclear[4]. Recently work conducted in yeast, identified several splicing genes that suppressed mutant htt toxicity when overexpressed. Here we interrogate these candidate genes in mammalian cell lines to elucidate the mechanism(s) underlying this protection.

Toxicity

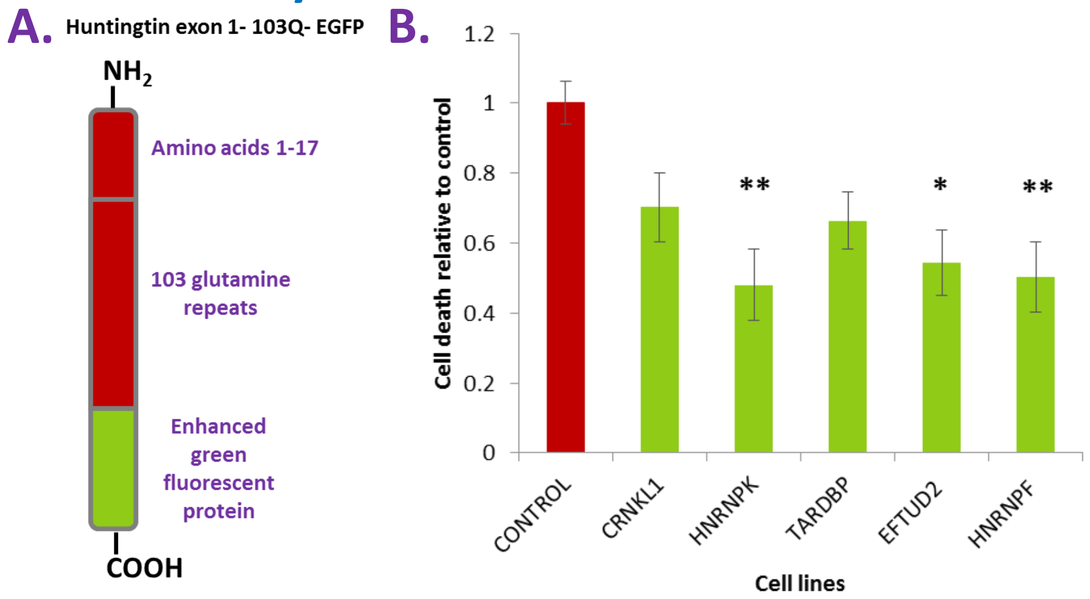
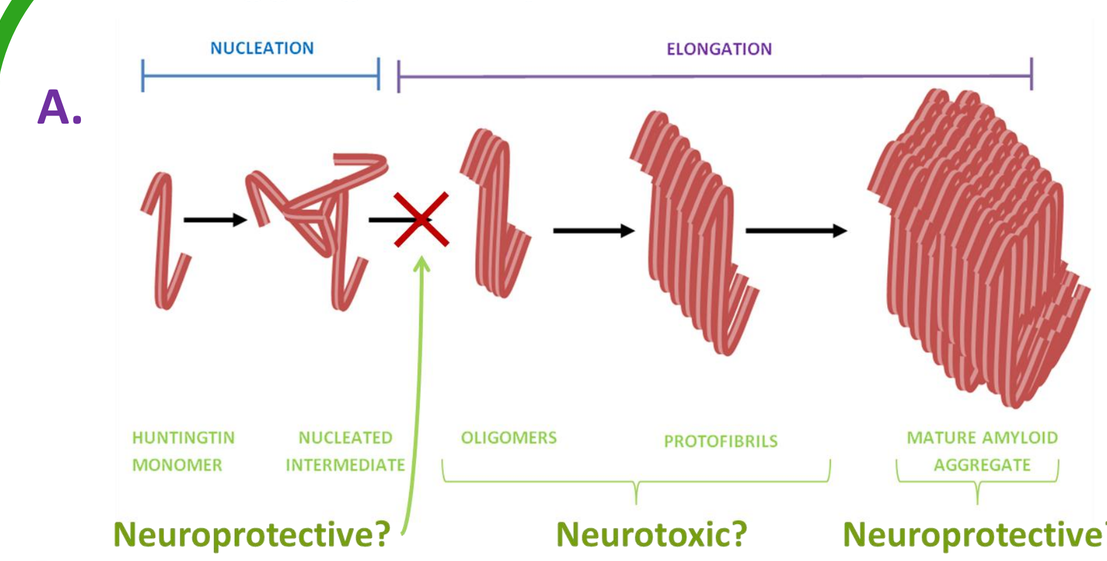
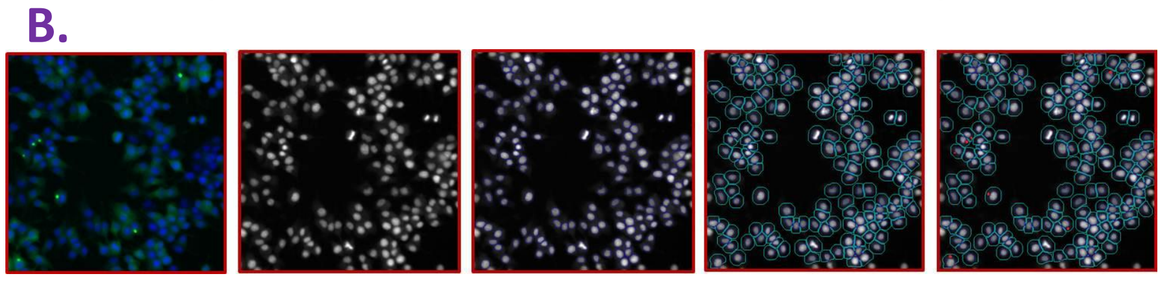


Figure 1. Overexpression of several splicing genes modulates cell death in neuronal cells expressing mutant htt. A. The PC12 neuronal cell model[1] expresses a truncated form of the htt protein, consisting of amino acids 1 to 17, followed by 103 glutamine residues, conjugated to a green fluorescent protein. **B.** Splicing genes were stably expressed in neuronal cells along with the mutant htt construct, and suppression subsequently determined with the use of a cell death assay. These results were normalised to the cells expressing only the htt constuct. Overexpression of five splicing genes was found to suppress toxicity (*P<0.05, **P<0.01)

Aggregation dynamics





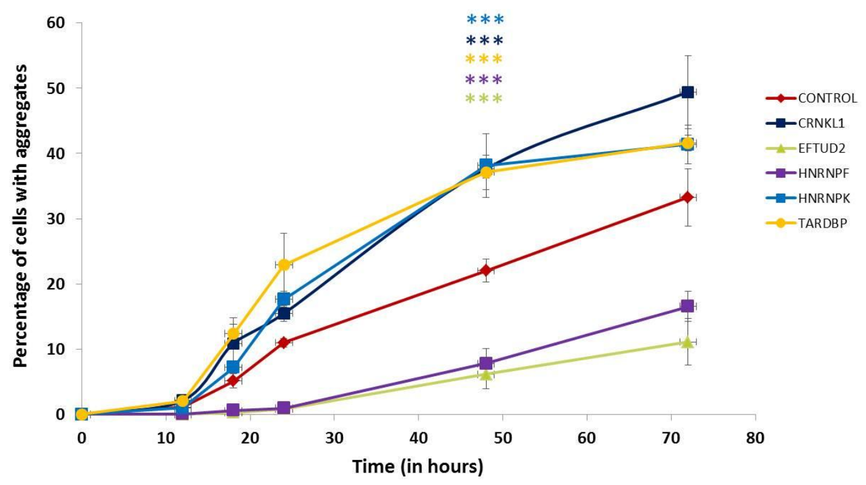


Figure 3. Splicing factors modulate aggregation dynamics in neuronal cell model A. Huntingtin aggregation is believed in a series of stages, in which monomers come together to form nucleated intermediates. This in turn undergoes elongation to form the putatively neurotoxic intermediates, oligomers and protofibrils. Further elongation leads to the formation of mature aggregates, believed to be neuroprotective. **B.** An automated cell analysis software (Cellomics) was used to examine aggregation dynamics. The bioapplication identifies the nucleus of the cell with the aid of a blue DNA binding dye, Hoechst 33342, and extrapolates this region to define the cell body. The bioapplication subsequently analyses the green flourescently tagged huntingtin within this given area **C.** Overexpression of CRNKL1, HNRNPK and TARDBP appeared to increased the level of aggregate formation, while overexpression of EFTUD2 and HNRNPF significantly reduced formation of these aggregate species (***P<0.001).

Splicing efficiency

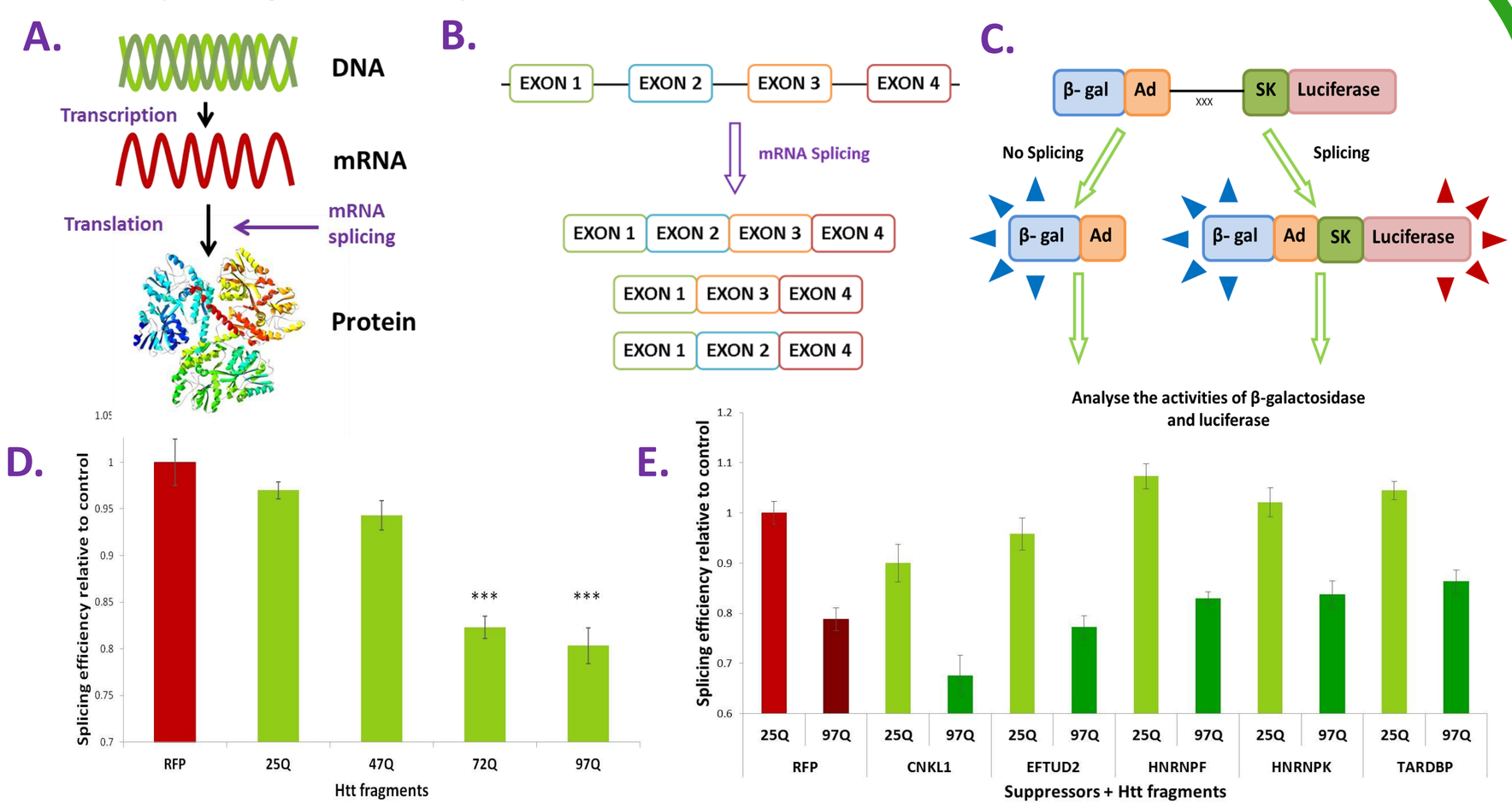


Figure 2. Expression of mutant htt impairs splicing in mammalian cells. A. The central cellular dogma, DNA is transcribed into messenger RNA, which is subsequently translated into a protein. mRNA splicing is necessary to modify immature mRNA to form mature transcripts . **B.** mRNA splicing acts upon immature mRNA, containing non-coding regions (introns), and ligates protein coding regions (exons) together to form a continuous transcript. This process can also facilitate the formation of multiple mature transcripts from a single immature mRNA , through exon "skipping". **C.** The splicing efficiency assay utilises a construct encoding β-galactosidase (β-gal) and luciferase which are separated by an intron flanked by recombinant fragments encoding adenovirus (Ad) and skeletal muscle isoforms of human tropomyosin (SK). These fragments contain three in-frame translational stop signals (xxx), which prevent luciferase expression when the construct is inadequately spliced. Splicing efficiency can be determined by analysing the activity of luciferase in relation to the constitutively expressed β -galactosidase activity(Nasim and Eperon, 2006). **D.** Lentiviral constructs expressing htt fragments with different polyglutamine lengths were transiently transfected into mammalian cells, alongside the splicing efficiency construct. Results were subsequently normalised to cells transfected with an control plasmid. The 25Q and 47Q constructs do not alter mRNA splicing, while the 72Q and 97Q fragments both reduce splicing efficiency (***P<0.001). E**.** Overexpression constructs were transfected in mammalian cells alongside the splicing efficiency construct and either the 25Q or 97Q, however overexpression of our suppressors failed to alleviate the splicing efficiency deficit attributed to the longer polyQ construct.

Conclusions

- We observe a polyglutamine length dependent change in mRNA splicing efficiency.
- Given the wide range of genes within the human genome that undergo mRNA splicing, it is possible that correction of any deficits in this process may provide a new avenue for therapeutic intervention in HD.
- Overexpression of several splicing genes reduces mutant htt toxicity, though the mechanism by which these genes convey suppression appears to be related to aggregation dynamics and independent of mRNA splicing.

References
[1]APOSTOL, B.L., et al, 2003.. *Proceedings of the National Academy of Sciences of the United States of America,* **100**(10), pp. 5950-5955.
[2]CÁCERES, J.F. and KORNBLIHTT, A.R., 2002. *Trends in Genetics,* **18**(4), pp. 186-193.
[3]LANDLES, C. and BATES, G.P., 2004. Fourth in molecular medicine review series. *EMBO reports,* **5**(10), pp. 958-963.
[4]LICATALOSI, D.D. and DARNELL, R.B., 2006. *Neuron,* **52**(1), pp. 93-101.
[5]JOHNSON, J.M., et al., 2003. *Science,* **302**(5653), pp. 2141-2144.
[6]NASIM, M.T. and EPERON, I.C., 2006. *Nature Protocols,* **1**(2), pp. 1022-1028.