# Stopping Protein Trouble 4 Good

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When things go wrong on the genetic or molecular level, changes that are not observable to the naked eye often become detrimental and ultimately lead to the onset of a particular disease. However, it is interesting to note that while many diseases may have similar pathologies, such as toxic protein accumulation, or similar symptoms, such as impaired movement, every disease has a unique underlying cause. Huntington's Disease (HD) is a neurodegenerative disease caused by lengthy CAG trinucleotide repeats in the protein coding region for polyglutamine also known as polyQ. While the huntingtin gene normally has 8-25 CAG repeats, a mutant can have 36 or more CAG repeats. Taking this into consideration, it makes sense that the mutant produces more protein which then aggregates or clumps causing cellular dysfunction. An interesting area to consider is a mechanism that can reduce long polyQ production in HD. In a recent study, scientists looked at transcription: a cellular process that makes RNA from a DNA template so that proteins can later be made from RNA through the process of translation. Scientists analyzed how transcription

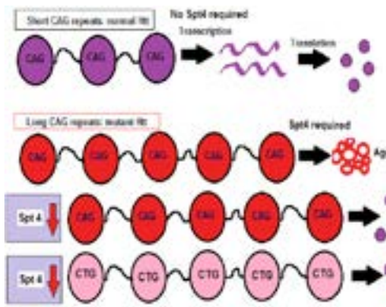


**Figure 1: Chia-Rung Lu et al. reveal that short CAG repeats do not require Spt4 for transcription. However, in long CAG repeats, the Spt4 transcription elongation factor aids in transcription which ultimately allows for mutant protein to be translated. If Spt4 is reduced or deleted in the long CAG repeats, protein activity can be restored. It is interesting to note that if Spt4 is deleted in non CAG repeats such as CTG, proteins can also regain some function.**

can be disrupted to reduce long polyQ expression (*2, 5*) in the study.

Chia-Rung Lu et al. (2012) identified that proteins coded by long CAG trinucleotide repeats present in HD and many other neurodegenerative diseases are dysfunctional. The authors demonstrated this by inserting a short section of polyQ repeats (25Q) and a long section of polyQ repeats (97Q) into Ade2 protein of yeast cells to observe whether the colonies could change color from red to white. If the colonies changed to white, this would indicate that they are capable of converting p-ribosylamino imidazole (AIR) to p-ribosylamino imidazole carboxylate (CAIR) and are functional. From the 180,000 colonies, only 200 colonies changed from red to white. The 97Q repeat colonies remained red revealing that they were dysfunctional.

In order to target long polyQ expression, the authors suggested disrupting the mechanism of transcription. The authors observed the role of Spt4, a transcription elongation factor present in yeast, and Supt4h, a human ortholog transcription factor. In earlier studies, it was found that the "Spt4-Spt5 complex is an essential RNA polymerase II elongation factor" and "spt4 mutation causes a modest decrease in growth and rRNA synthesis rates" (Guo et al., 2008). When Spt4 was deleted and an analysis of the cellular 97Q and 25Q protein expression and aggregation was conducted through an assay, there was a reduction of polyQ production for 97Q but not for 25Q. It was previously established that the "roles for gene transcription intermediates in an aggregation pathway as toxic species...are not yet proved" (Ross, 2002, p. 822). While there may have been uncertainties about the exact role of spt4 earlier through the deletion of Spt4 in this study, the 2012 study revealed that spt4 deletion leads to disruption of transcription and ultimately accomplishing the goal: a decrease in protein accumulation.

Although protein production was decreased for 97Q when Spt4 was deleted, the protein levels remained relatively constant in the assay for the 27Q revealing an important characteristic of Spt4. The expansion mutation is strongly dependent upon the repeat length. Similarly, a characteristic of Spt4 is that it is selective for long repeat regions (Pearson & Sinden, 1998). The expansion mutation and transcription factor is dependent upon length. This is groundbreaking because not only does this finding provide a potential therapy to prevent expression of long polyQ in HD, but it also demonstrates that deleting Spt4 will not have negative consequences on short repeats of other nucleotides, however, this is not completely verified at this point.

Amongst CAG repeats, there *are* other lengthy non-CAG expansions such as CUG, CTG, CGG, and CCUG that are characteristic of diseases. In various cases "the repeat expansions confer toxicity...to the encoded proteins" (Wojciechowska & Krzyzosiak, 2011, p. 1). In the study, Chia-Rung Lu et al. reveal that Spt4 deletion can also reduce protein accumulation in lengthy non-CAG repeats such as CTG. This finding was made after Spt4 was deleted in a long CTG repeat and the deletion resulted in decreased protein production in the assay. The discovery also relates to how only the long repeat was affected while the short repeat of CTG had a relatively consistent amount of protein. This is an important finding because it provides a pathway to eliminate toxic proteins in other diseases that are encoded by long trinucleotide repeats.

While it is great to observe the effects of Spt4 in order to understand how to eliminate toxic protein accumulation in humans, Supt4h (the human ortholog) still needs to be considered. Again, Chia-Rung Lu et al. found that the length of the CAG trinucleotide repeat matters. In the 7Q repeats, Supt4h deletion lead to little change in the protein amount in the assay while in the 81Q repeats, the amount of protein was reduced. Overall, in order to eliminate the mutant protein expression, the transcription elongation factor can be removed to impair transcription and decrease mutant protein levels.

Along with the existing techniques involving single-stranded RNA to reduce protein expression, Chia-Rung Lu et al. provide a revolutionary approach of using a transcription elongation factor to fight the destruction that it aids in producing. Through this study, various characteristics of the transcription elongation factor became clearer. Though it has been established that non-lengthy repeats will not be impacted through the deletion of Spt4, it will be beneficial to further test this. Furthermore, as seen in earlier studies, chemical modification and mismatches of single-stranded RNAs can enhance selectivity of single-stranded RNA to ultimately aid in removing unwanted protein. It is possible that in future studies modification of Spt4 and Supt4h can also improve selectivity and reduce protein aggregation even further.

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**References**

Guo, M., Xu, F., Yamada, J., Egelhofer, T., Gao, Y., Hartzog, G. A.,...Niu, L. (2008). Core structure of the yeast spt4-spt5 complex: a conserved module for regulation of transcription elongation. *Structure, 16*(11), 1649-1658.

Liu, C. R., Chang, C. R., Chern, Y., Wang, T. H., Hsieh, W. C., Shen, W. C.,...Cheng, T. H. (2012). Spt4 is selectively required for transcription of extended trinucleotide repeats. *Cell, 148*(4), 690-701.

Miller, V. M., Xia, H., Marrs, G. L., Gouvion, C. M., Lee, G., Davidson, B. L., & Paulson, H. L. (2003). Allele-specific silencing of dominant disease genes. *Proceedings of the National Academy of Sciences, 100*(12), 7195-7200.

Pearson, C. E., & Sinden, R. R. (1998). Trinucleotide repeat DNA structures: dynamic mutations from dynamic DNA. *Current Opinion in Structural Biology, 8*(3), 321-330.

Rondón, A. G., García-Rubio, M., González-Barrera, S., & Aguilera, A. (2003). Molecular evidence for a positive role of Spt4 in transcription elongation. *The EMBO Journal, 22*(3), 612-620.

Ross, C. A. (2002). Polyglutamine pathogenesis: emergence of unifying mechanisms for Huntington's disease and related disorders. *Neuron, 35*(5), 819-822.

Wojciechowska, M., & Krzyzosiak, W. J. (2011). Cellular toxicity of expanded RNA repeats: Focus on RNA foci. *Human Molecular Genetics, 20*(19), 3811-3821.

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