Misfolding and aggregation of polyQ-extended androgen receptor: Structural biology and pathology aspects

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Spinal bulbar muscular atrophy (SBMA, also known as Kennedy's disease) is one of the so-called polyQ diseases, a group of late onset progressive neurodegenerative disorders that also includes Huntingdon's and several spinocerebellar ataxias. The common feature of this group is protein misfolding and aggregation associated with an abnormally elongated CAG repeat sequence in a specific gene. The proteins involved are different in each disease of the group and appear to be unrelated other than in the presence of a polyglutamine tract coded by the variable length CAG repeat. In the case of SBMA, the protein in question is the androgen receptor (AR). Healthy individuals have an AR with a 9-36 residue polyglutamine tract near the N-terminus, while in SBMA patients this is extended to 38-62 residues. A certain amount of aggregation is still observed in AR with normal polyQ tract length, but apparently this is not sufficient to cause disease symptoms. The disease occurs only in males because it is strictly dependent on the presence of androgen.

The present work is a structural biology investigation of pathological misfolding and aggregation process in polyQ-extended AR, including the role of post-translational modifications such as phosphorylation. Insights at the molecular level are expected to contribute to the elucidation of the mechanism of pathology by exposing correlations between toxicology and structure, as well as providing tools for analyzing the effects of potential modifiers of pathology.

In AFM investigations, we have observed significant differences in the morphology of purified aggregates of native and polyQ extended AR, with and without addition of androgen. Intriguingly, mutant AR with no polyQ stretch but with two phosphorylation sites deleted display the same aggregate morphology as polyQ extended AR. This appears to explain the pathological phenotype caused by deletion of these phosphorylation sites in a in a *Drosophila* model, as observed in previous work of the Cato group[1]. The data also imply that a cyclization mechanism absent in polyQ AR may prevent the formation of large aggregates in native AR.

In complementary FTIR spectroscopy studies we are pursuing three lines of investigation: spectroscopy of cultured cells expressing the native, CAG repeat extended or phosphorylation site mutant AR genes, with or without addition of androgen, in order to identify spectral signals characteristic of misfolding and aggregation in vivo; spectroscopy of isolated aggregates in vitro, in particular in order to correlate secondary structure motifs with the observed morphological differences; and time-resolved spectroscopy of the misfolding process in purified AR, in order to obtain accurate misfolded vs. native folded difference spectra containing detailed information on the structural properties of the misfolded product.

References:

[1] S. F. Funderburk, L. Shatkina, S. Mink, Q. Weis, S. Weg-Remers, A. C. B. Cato, *Neurobiol. Aging* (in press)