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Application of a *Drosophila* model to study Huntington's disease pathogenesis *in vivo*

PhD Thesis

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2004.

INTRODUCTION

At least nine hereditary neurodegenerative disorders are caused by the expansion of an unstable endogenous CAG repeat in the coding regions of otherwise unrelated disease genes. This class of disorders includes Huntington's disease, spinal and bulbar muscular atrophy, dentatorubral-pallidoluysian atrophy and several types of spinocerebellar ataxias. Dominant inheritance, late-onset progressive pathology and aggregate formation are characteristic features of these disorders. The expanded trinucleotide repeats translate to uninterrupted arrays of glutamine residues in the corresponding proteins. Polyglutamine expansions over a certain repeat number are pathologic and cause disease.

Expanded polyQ domains are thought to cause structural changes in the affected proteins, leading to aberrant protein interactions such as those that allow formation of extra- and intranuclear aggregates. Beside self-association, polyQ domains are also able to mediate direct interactions with other proteins, inhibit their activities and sequester them to aggregates. These interactions interfere with the proper functioning of several cellular mechanisms. Some of these affected mechanisms were identified during the past few years, including protein turnover, transcriptional regulation, Ca^{2+} homeostasis, mitochondrial functions, apoptosis and intracellular trafficking. In order to to analyze the molecular pathomechanisms underlying polyglutamine diseases *in vivo* we developed a transgenic *Drosophila melanogaster* model system of Huntington's Disease. The model system is based on the neuron specific expression of a pathogenic fragment of

huntingtin and polyglutamine transgenes in flies. We have shown that the transgenic system is an effective means for *in vivo* screening for both genetic and chemical agents.

Histone acetyltransferases (HATs) have been found to interact with polyQ peptides. HATs play an important role in the establishment of the proper transcriptional activity and fine tuning of several cellular processes. To establish the correct acetylation pattern the proper balance of HATs and histone deacetylases (HDACs), the opposing enzyme group, is necessary. Based on the observation that polyQ containing proteins interact with the catalytic domain of HAT enzymes and reduce their enzymatic activity *in vitro* we hypothesized that unbalanced acetylation activity might be one of the components of polyglutamine pathogenesis. Using our *Drosophila* model system we were able to prove the *in vivo* relevance of this interaction. Our work demonstrated that the distorted protein acetylation balance is one of the key factors of polyglutamine pathogenesis and pointed to HDAC inhibitor compounds as candidate drugs for effective treatment.

AIMS

1. Characterization of transgenic *Drosophila* stocks expressing polyglutamine or *htt* transgenes under the control of the bipartite UAS/GAL4 expression system. Determine which transgenic stock combinations have sensitive phenotypes to reflect changes in polyQ pathology.

2. Optimize fly culturing conditions for feeding experiments.

3. Test the ability of the *Drosophila* model system to respond to factors affecting polyglutamine pathology by challenging it with relevant chemical and genetic agents.

4. Investigate whether genetic restoration of the acetylation balance suppresses polyglutamine induced pathology *in vivo*.

5. Investigate the effects of HDAC inhibitor compounds on polyQ pathology *in vivo*.

6. Determine whether the influence of acetylation balance on polyQ pathology are due to additional effects of several affected proteins or whether a few loci account for the bulk of the effects observed.

MATERIALS AND METHODS

Expression of polyglutamine containing proteins in transgenic flies using the bipartite UAS/GAL4 expression system.

Compound feeding and genetic interaction tests.

Pseudopupil analysis of the adult retina.

Western analysis of larval brain extracts.

Mapping of deletion by genomic PCR.

Statistical analysis.

RESULTS

1. Several transgenic *Drosophila* lines expressing *Q48*, *Q108*, *Q50Httexon1* or *Q93Httexon1* transgenes under the control of UAS were generated in our lab previously. Neuron specific expressions of the UAS construct were directed by transgenes expressing GAL4 in the nervous system. Our goal was to select those lines that are appropriate to reflect changes in polyQ pathogenesis. As the transactivation activity of GAL4 is increased at higher temperatures we tested the viability of UAS lines in combination with neuron specific GAL4 drivers at several culturing temperatures. Three lines (a *Q48*, a *Q108* and a *Q93Httexon1* line) that exhibit severe semilethal phenotypes and are highly sensitive to the expression levels of transgenes were selected for further experiments.

2. To be able to use the transgenic model system as a reliable screening tool we had to optimize the conditions of culturing. We chose 27°C as culturing temperature because the phenotypes of the transgenic lines were most severe and yet modifiable at this temperature. We tested two kinds of culturing media by investigating how can they support the development of wild type flies. We found one of them (named Heidi food) providing better and more reliable results, thus we used this media for the consequent experiments. Finally, we determined the highest applicable dose of DMSO, an organic solvent, we planned to use as a drug vehicle in chemical feeding experiments. We found that 0.5% is the highest dose of DMSO that do not exhibit toxic effects at any developmental

stages.

3. Before setting up experiments using the *Drosophila* model we tested its ability to respond to factors influencing polyglutamine pathology. In order to validate our model we challenged it with Congo red, an inhibitor of aggregate formation. We have found that Congo red rescued both viability and progressive neurodegeneration of polyglutamine expressing flies. We also tested the effects of partial loss of *C-terminal Binding Protein*, a genetic enhancer of polyQ phenotypes, and got similar results than those described previously in a transgenic SCA1 model. Thus, the model system was proved to be capable of indicating conditions affecting polyQ pathology.

4. Among others, HATs are targets of polyglutamine proteins. These enzymes modify the activity of several important proteins and have a fundamental role in the regulation of transcriptional activity. Studies showing that polyglutamine proteins bind to HAT proteins and inhibit their activity *in vitro* urged us to investigate the *in vivo* relevance of this interaction. We hypothesized that if inhibition of HAT activity has a significant role in polyQ pathology then reducing the histone deacetylase activity will relieve the symptoms. To test this hypothesis we reduced HDAC activity by introducing a loss of function allele of the HDAC co-factor, *Sin3A*. We have found that partial reduction of *Sin3A* activity suppressed the phenotypes elicited by *Q93Httexon1* expression. This result indicates that altered protein acetylation plays a major role in polyQ pathomechanism and that restoration of the disturbed

acetylation balance suppresses symptoms.

5. To rule out the possibility that the effects described above are due to some specific effects of *Sin3A* rather than to the restoration of the acetylation balance we monitored the effects of feeding HDAC inhibitor drugs with transgenic flies. We administered non-toxic doses of butyrate and suberoylanilide hydroxamic acid to flies expressing expanded polyQ peptides or the first exon of *Htt* with expanded polyQ domain. We found that both compounds suppressed the phenotypes of transgenic flies expressing a polyglutamine domain either alone or embedded in a disease gene context. Thus, feeding experiments supported the hypothesis of disturbed acetylation balance and identified HDAC inhibitors as the first compounds ameliorating polyQ symptoms *in vivo*.

6. Having seen that disturbed acetylation balance has a major role in polyQ pathogenesis we were interested to see whether the effects of HATs and HDACs on pathology are the result of many small incremental effects or the dysfunction of one critical HAT enzyme is responsible for the majority of effects. To investigate this, we set up genetic interaction screens. We tested several deficiencies for the absence of the genes of interest if mutant alleles were not available. Interaction crosses with histone acetyltransferases showed that various HAT enzymes classified to distinct protein families are enhancers of the neurodegenerative phenotype. Similarly, loss of HDAC enzymes with distinct mechanism of catalysis rescued polyQ phenotypes. These results suggest that the cellular toxicity observed in polyglutamine expressing flies is

at least partially due to impairment of the general acetylation balance rather than the dysfunction of few key factors.

RELATED PUBLICATIONS

Steffan, J. S., **Bodai, L.**, Pallos, J., Poelman, M., McCampbell, A., Apostol, B. L., Kazantsev, A., Schmidt, E., Zhu, Y. Z., Greenwald, M., Kurokawa, R., Housman, D. E., Jackson, G. R., Marsh, J. L., Thompson, L. M. (2001). Histone deacetylase inhibitors arrest polyglutamine-dependent neurodegeneration in *Drosophila*. Nature 413, 739-743.
IF: 30.4

Apostol, B. L., Kazantsev, A., Raffioni, S., Illes, K., Pallos, J., **Bodai, L.**, Slepko, N., Bear, J. E., Gertler, F. B., Hersch, S., Housman, D. E., Marsh, J. L., Thompson, L. M. (2003). A cell-based assay for aggregation inhibitors as therapeutics of polyglutamine-repeat disease and validation in *Drosophila*. Proc Natl Acad Sci U S A 100, 5950-5955.
IF: 10.7

Bodai, L., Pallos, J., Thompson, L. M., and Marsh, J. L. (2003). Altered protein acetylation in polyglutamine diseases. Curr Med Chem 10, 2577-2587.
IF: 5.8

RELATED PATENT

Steffan, J. S., Thompson, L. M., Marsh, J. L., **Bodai, L.**, Pallos, J. (14.11.2002) Method for treating neurodegenerative, psychiatric and other disorders with deacetylase inhibitors
International publication number: WO 02/090534 A1