# Investigations into the effects of epigenetic factors in the pathomechanism of Huntington's disease in a *Drosophila* model

**Summary of Ph.D. thesis**

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## Introduction

Huntington's disease (HD) is a fatal, dominantly inherited, late-onset, progressive neurodegenerative disorder, for which there is no available cure or effective treatment. The human *Huntingtin* (*HTT*) gene is 210 kb long and consists of 67 exons. In the first exon of *HTT* there is an unstable polymorphic CAG trinucleotide (encoding for glutamine, Q) repeat, which, if expands over a certain repeat length causes gain of function mutation. In healthy individuals the number of CAG repeat units are between 9 and 36. If the length of the CAG repeat exceeds 36 repeat units, the resulting elongated polyglutamine domain changes the properties of the Huntingtin (Htt) protein and induces the complex pathogenesis of HD. Longer repeats cause earlier disease onset and more severe symptoms.

The Huntingtin protein is mainly localized to the cytoplasm, but incorrect proteolytic cleavage of mutant Htt (mHtt) results in short N-terminal fragments that can be imported to the nucleus. A main part of the multifaceted pathomechanism of HD is the dysregulation of gene expression, which partially caused by disturbed modification of histones. CREB-binding protein (CBP) and PCAF histone acetyltransferases, among others, bind to mHtt proteins, which leads to incorrect histone acetylation in the HD. In *Drosophila* and mouse HD models some inhibitors of histone deacetylases (HDACs) were shown to ameliorate the symptoms of HD previously. However, it is still not elucidated that the acetylation state of which lysine residues of which histone proteins might play a crucial role in the alleviating effect of broad spectrum HDAC inhibitors.

In the experiments that form the basis of my Ph.D. thesis our aims were to study the functions of the histone H4 specific *Drosophila* Hat1 histone acetyltransferase and to examine the potential involvement of Hat1 and specific H4 / His4r acetylation states in protheopathic stress conditions caused by mutant Huntingtin. The function of Hat1 has not yet been characterized in connection with the Huntington's disease. This enzyme is mainly localized in the cytoplasm and acetylates the 5th and 12th lysine residues (K) of nascent histone H4. The resulting H4-K5K12 diacetylation is a universal histone mark in eukaryotic organisms. Canonical H4 and the His4r variant histone proteins have identical amino acid sequences but the regulation of their expression is different. We hypothesized, that similarly to the case of histone H3 and its variant H3.3, canonical H4 might be replaced by His4r at sites of active gene expression. Based on this assumption we decided to study those lysines of His4r, which can be acetylated to find epigenetic marks that are involved in the pathomechanism of Huntington's disease.

For our experiments we used a *Drosophila* model of HD that was proven to be appropriate to study the cellular aspects of HD pathogenesis. In this model expression of an N-terminal fragment of mHtt leads to effects similar to those characteristic for the human disease: mutant Huntingtin protein forms intracellular aggregates in the brain of flies, cause neurodegeneration, movement and circadian rhythm defects, and reduced lifespan.

## Aims

In the project part of analysis of *Drosophila* Hat1 and examination of its role in connection with Huntington's disease our specific aims were:

- Generate a *Hat1* deletion mutant *Drosophila melanogaster* strain and analyze the histone acetylation targets of the Hat1 enzyme.
- Examine, whether the histone post-translational modifications made by Hat1 are necessary for the nuclear import of histone H4.
- Determine the effects of Hat1 on gene expression.
- Characterize the phenotypic effects of the Hat1 deletion and analyze effects of the partial loss of Hat1 on mutant Huntingtin induced phenotypes.

In the project part of analysis of the role of acetylation mimetic His4r variant histone proteins in connection with Huntington's disease our specific aims were:

- Generate transgenic flies that express various acetylation state mimetic His4r variant histone proteins and validate their sequence and expression.
- Examine the effects of neuronal expression of different acetylation state mimetic His4r proteins on viability and neuronal degeneration.

- Investigate the effects of neuronal expression of different acetylation state mimetic His4r variant histone proteins in the *Drosophila* model of Huntington's disease.
- Analyze the connection between the results of loss of Hat1 and the neuronal expression of His4r transgenes in the *Drosophila* model of Huntington's disease.

## Methods

- Generation of *Hat1* deletions by P-element remobilization.
- Identification of *Hat1* deletions using PCR.
- Analysis of the viability, longevity and fecundity of *Hat1* mutants.
- Spectrophotometric measurement of eye pigment concentration.
- Monitoring circadian rhythm and daily motor activity.
- Examination of the effects of heat stress.
- *In vitro* mutagenesis of *His4r*.
- Generation of *His4r* transgenic flies.
- Verification of the substitutions in the His4r transgene by DNA sequence analysis.
- Examination of the effects of the neuronal His4r transgenes expression for viability.
- Viability and longevity analysis of mHtt expressing flies.
- Investigation of neurodegeneration by pseudopupil assay.
- Testing mRNA expression levels with RT-qPCR.
- RNA sequencing based trascriptome analysis.
- Western blot experiments.
- Immunostaining of larval salivary glands.

## Summary of the results

1. For our experiments we generated *Hat1* mutant and *UAS-His4r* transgenic *Drosophila melanogaster* strains. The *Hat1*$^{\Delta 57}$ amorphic mutant was generated by P-element remobilization that resulted in the deletion of almost the whole coding sequence of the gene including the HAT domain. For experimental control we generated a precise revertant line in which the gene was restored after remobilization of the transposon. We also generated transgenic *Drosophila* strains that carry inducible *His4r* histone transgenes that are labeled with a C-terminal FLAG-tag and contain substitutions in their N-terminal tail regions that mimic the acetylated (lysine (K) to glutamine (Q) change) or unacetylated/unmodified (lysine to arginine (R) change) forms of specific lysine residues (K5, K8, K12, K16). For experimental control we made a transgenic line that carries a wild-type (not modified) *His4r* gene. We also made a transgenic line with K5 and K12 double substitutions to arginine (K5R-K12R) to imitate a condition in which the target lysine residues of the Hat1 histone acetyltransferase cannot be acetylated.
2. We showed that the *Hat1* mutant and the neuronally expressed *His4r* transgenes are viable and fertile, although loss of *Hat1* caused mild sub-lethality.
3. We found that the expression of *His4r* transgene variants did not differ at the mRNA level but their protein levels in neurons depended on the specific substitution. His4r proteins with K to Q substitutions that mimic the acetylated state were present at lower

levels in the case of every position than K to R substitutions that mimic unacetylated/unmodified state.

4. Using fluorescence microscopy analysis we showed that the cellular localization of the various modified His4r proteins was alike, all variants showed nuclear localization.
5. We showed that neuronal expression of the various *His4r* point mutant transgenes did not cause neurodegeneration or reduced viability.
6. We showed that in *Hat1* mutant embryos H4K5 and K12 acetylation was lost indicating that Hat1 acetylates these lysine residues. This activity seems to be specific for these positions as the acetylation state of H4K8 did not change. Furthermore, lack of Hat1 reduced the acetylation level of the H3K18 residue, that presumably an indirect effect according to previously published data.
7. We proved that the universal cytoplasmic H4-K5K12 diacetylation is not necessary for the nuclear transport of H4 in *Drosophila*. Using immunofluorescence confocal microscopy we found that both transgenic wild-type His4r in *Hat1* null background and transgenic His4r-K5RK12R proteins in wild-type background showed correct nuclear localization.
8. We showed that loss of *Hat1* has no remarkable effects on heat stress resistance or circadian activity and rhythm of flies.
9. Using position effect variegation analysis we found that Hat1 may play a role in the formation or maintenance of closed chromatin structure.

10. By RNA-seq transcriptome analysis we found that the expression of more than 2000 genes changed significantly in 6-12 hours old *Hat1* null embryos. 2/3 of the genes showing altered expression levels were upregulated, while 1/3 were downregulated. The majority of the observed transcriptional alterations were presumably the consequence of a developmental delay. However, dysregulated transcription in 10% of the upregulated genes and 30% of the downregulated genes could not be explained by this phenomena. Most of the upregulated genes in this group play roles in developmental and translational regulation while the majority of the downregulated genes encode factors that participate in metabolism.
11. We found that contrary to the previously examined nuclear histone acetyltransferase enzymes reduced levels of the mainly cytoplasmic Hat1 ameliorated neurodegeneration in a transgenic model of Huntington's disease. As we previously found that Hat1 might play a role in the formation of closed chromatin, it is conceivable that a more relaxed chromatin structure caused by reduced levels of Hat1 is the reason for the reduced symptoms.
12. By the analysis of UAS-His4r epigenetic mark mimetic substitution mutants we found that co-expression of wild-type His4r transgene along with mHtt reduced lifespan compared to the lifespan of contol flies expressing mHtt only. We found that none of the mHtt – $His4r^{mimic}$ co-expressing categories had improved viability compared to mHtt expressing control flies. Both His4r K12Q and K12R modified transgenes were lethal if co-expressed

with mHtt. In general, in the case of each tested His4r lysine residue (except for K12 modifications that gave no viable offspring) flies co-expressing unacetylated lysine mimetic (K→R) His4r transgenes with mHtt had lower viability than flies co-expressing acetylation mimetic (K→Q) His4r transgenes with mHtt. Flies co-expressing mHtt and His4r-K8Q or His4r-K16Q transgenes had better viability and improved longevity compared to flies co-expressing mHtt with wild-type His4r. In neurodegeneration tests we found that the K5Q, K16Q and K16R modification also had a positive effect on the survival of photoreceptor neurons.

13. We showed that His4r-K5Q and His4r-K16Q proteins had lower protein levels, therefore the reason for the observed positive effects on HD phenotypes in the case of these substitutions is most likely the lower level of His4r protein. Based on protein expression data and the results of the neurodegeneration assays acetylation mimetic His4r-K8Q and the unacetylated mimetic His4r-K16R transgenes had a significant, protein level independent positive effect on the pathomechanisms induced by mutant Htt.

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## List of publications

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### The 2 publications for the doctoral procedure:

1. Varga, Júlia; Korbai, Szabina; Neller, Alexandra; Zsindely, Nóra; Bodai, László. Hat1 acetylates histone H4 and modulates the transcriptional program in Drosophila embryogenesis. Scientific Reports 9(1): 17973 (2019)
2. Varga, Júlia; Dér, Nikolett Petra; Zsindely, Nóra; Bodai, László. Green tea infusion alleviates neurodegeneration induced by mutant Huntingtin in Drosophila. Nutritional Neuroscience, 23(3):183-189. (2020)

**Peer-reviewed publications** [Total impact factors: 7.961]

*Publication related to the thesis:*

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