The role of alternative linker histone BigH1 during *Drosophila* early development

Ph.D. thesis summary

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INTRODUCTION

The early stage of embryogenesis is a crtitical time in the life of every metazoan species, as this period is crucial for the formation of the morphological basis of the adult animal. The beginning of this process is defined and controlled by maternal RNA and protein products, including histone proteins which are essential for the establishment of chromatin structure. In animals, chromatin organization typically requires an alternative H1 linker histone during the early stages of development, called an oocyte- and/or early embryo-specific linker histone. Most metazoan species possess several other H1 variants, which makes researching the functional differences between linker histones exceedingly difficult, as oftentimes functional redundancy can be observed among these proteins.

Drosophila melanogaster is an ideal model organism for studying the role of linker histone variants, as it possesses only two H1 proteins: somatic canonical H1, and its only variant, called BigH1. In 2013, BigH1 was descibred as an embryo-specific linker histone, present in a large quantity in the developing embryo before cellularization, after which its levels decline and somatic H1 takes its place. BigH1 is retained in the primordial germ cells of the embryonic tissue, and can be detected in the germline of the ovaries (in the oocyte and accompanying nurse cells) and testes (in germline stem cells and differentiated spermatocytes) of adult animals.

The exact role of BigH1 during early embryogenesis is still unknown. The aim of my work was to identify the functional differences between somatic H1 and BigH1, and to determine how these differences make BigH1 a more appropriate linker histone for the organization of early embryonic chromatin structure. My main goal was to study the distinctions between chromatin structure assembled

with H1 and BigH1, for which we set the analysis of chromatin accessibility, nucleosome and chromatosome stability and the comparison of changes in gene expression in the presence of H1 and BigH1 as our objectives.

RESEARCH METHODS

Sample collection:

- collection and preparation (dechorionation and freezing) of *Drosophila melanogaster* embryos

Molecular biology methods:

- construction of plasmids containing BigH1 mutant alleles using classic cloning techniques and SLIC reaction
- isolation of plasmids using commercial kit and alkaline lysis
- chemical transformation of bacteria
- protein and chromatin preparation from embryos
- Tricine-SDS-PAGE and Western blot
- MNase assay on embryonic chromatin
- RNA isolation and DNase treatment
- end-point PCR, RT-PCR and qPCR
- QINESIn assay for measuring nucleosome stability

Bioinformatics:

- using online databases and tools
- designing plasmid constructs (SnapGene)
- primer design (SnapGene)
- image editing (Adobe Illustrator)
- image analysis (ImageJ)
- statistical analysis (GraphPad)
- analysis of qPCR data (Microsoft Excel)
- multiple sequence alignment (UGENE)

DISCUSSION OF RESULTS

1. Establishment of mutant *Drosophila* lines

In our group, we have created plasmids and, with their help, *Drosophila* lines in which the coding sequence of the endogenous *BigH1* gene is partially (BigH1-H1 chimeras) or completely (*HHH*) replaced with the coding sequence of the somatic *H1* gene. Using these *Drosophila* lines, we can functionally compare the BigH1 and H1 proteins, and also assess the individual role of BigH1's domains.

To extend this experimental setup, I have created a plasmid which we used to generate a *Drosophila* line which does not express the BigH1 protein (*NULL*). This mutant can be used to research the phenotypical changes upon BigH1 deletion.

I have also created a plasmid which was used to establish a *Drosophila* line in which the BigH1 protein cannot be phosphorylated at S287, S288, S299 and S331 amino acids (*P0*). In the future, this mutant can be used to study the role of BigH1 phosphorylation during *Drosophila* early development.

By creating various *Drosophila* mutant lines, we have established an experimental setup which can be used to assess the role of the BigH1 alternative linker histone *in vivo*.

2. BigH1-H1 chimeras and somatic H1 can substitute BigH1 in early embryonic chromatin under optimal conditions

We have determined that our BigH1 mutant *Drosophila* lines are completely viable at 25 °C.

Using Western blot, I have demonstarted that the BigH1-H1 chimeric proteins and somatic H1 are expressed in the mutant *Drosophila* lines during early embryogenesis similarly to wild type BigH1, and show the appropriate size. This proves that BigH1-H1 chimeras and full-length

H1 are capable of substituting wild type BigH1 at 25 °C during embryogenesis.

3. **The N-terminal domain of BigH1 is required for proper protein expression**

   Using Western blot, I have demonstarted that protein levels produced from alleles in which the N-terminal region of BigH1 is replaced with that of H1 (H1-NTD) are significantly lower compared to other chimeras and the wild type. I have determined that in the *HHH* mutant embryos, where H1 completely replaces BigH1, lower protein level is a result of lower RNA level compared to the wild type BigH1 RNA.

4. **The type of linker histone influences the gene expression program**

   I have examined whether BigH1 plays a role in transposon silencing, as a germline- and early embryo-specific linker histone. I have found that from a total of fourteen, the RNA level of eight transposable elements show significant differences in embryos in which BigH1 is completely replaced with H1, compared to the wild type. These changes (up- or downregulation) are independent from the tissue-specificity (somatic, germline-specific or intermedier) of the analysed transposons. These results suggest that the gene expression program differs in the presence of BigH1 and H1.

5. **Chromatin structure on nucleosomal level is similar in the presence of BigH1 and H1**

   According to experiments done in our laboratory, nuclei are significantly larger in early embryos where BigH1 is replaced with somatic H1, compared to the wild type. We believed that this is a result of changes in chromatin structure.

   To test this I have performed MNase assay, and determined from the results that nuclear DNA is longer in BigH1-containing nucleosomes

with 5 bp on average. From this I concluded that as BigH1 is a larger linker histone than somatic H1, it protects a larger area from MNase digestion.

However, linker DNA size was unchanged in the presence of BigH1 and H1, suggesting that nucleosome density is similar.

Regarding the ratio of mono- and multinucleosomal DNA, I have not detected significant differences between BigH1 and H1-containing chromatin, indicating that chromatin accessibility is similar irrespective of linker histone type, and is not the reason behind larger nuclear size in the presence of somatic H1.

6. Nucleosomes are more stable in the presence of BigH1

For the analysis of nucleosome stability, I have optimized to assays, QINESIn and a Western blot based method, for use in *Drosophila* embryos. Both techniques are based on the salt treatment of chromatin and the measurement of core histone elution upon treatment. Using these methods, I have determined how strongly the H2A, H3 and H4 core histones are associated with chromatin.

I have found that the analysed histone proteins are more difficult to remove from wild type chromatin, where the linker histone is BigH1, compared to chromatin formed in the presence of somatic H1.

I have concluded that nucleosomes show greater stability in the presence of BigH1, compared to nucleosomed formed with somatic H1.

7. BigH1's binding to nucleosomes is weaker

I have analysed how strongly the BigH1 and somatic H1 proteins associate with chromatin, and I have determined that although nucleosomes formed in the presence of BigH1 are more stable, BigH1 itself is more easily removed from chromatin than H1.

This suggests that BigH1's binding to nucleosomes is weaker than somatic H1's, indicating that BigH1 is a more mobile linker histone.

Further experiments done in our laboratory support that the reason behind BigH1's weaker nucleosome binding capabilities is the shortening of a DNA-binding loop in the globular domain. Other experiments suggested that BigH1's increased mobility compared to H1 allows more dynamic nucleosome exchange during DNA replication.

8. **BigH1 is a less conserved linker histone compared to somatic H1**

   By collecting and comparing protein sequence data from twelve *Drosophila* species, I have determined that the orthologues of BigH1 can be found in the examined species. By comparing sequence data from eight related species, I have concluded that the most conserved domain of BigH1 is the central globular domain, however greater similarity can only be observed between closely related species. In contrast, somatic H1 is extremely conserved in all analysed species. By comparing H1 and BigH1 sequences, I have determined that the similarity by sequence alignment between the two linker histones is less than 30 %.

SUMMARY

Results presented in the dissertation are important elements of the research project in our work group. Our results provide evidence that BigH1 is a more mobile linker histone which binds to nucleosomes more weakly. Therefore BigH1 allows a more dynamic nucleosome exchange and DNA replication, and by this rapid nuclear cycles during the early stages of *Drosophila* embryogenesis, when the zygotic genome is transcriptionally silent.

LIST OF PUBLICATIONS

MTMT ID: 10062451

Publication which the dissertation is based on:

Henn, László* ; **Szabó, Anikó*** ; Imre, László ; Román, Ádám ; Ábrahám, Andrea ; Vedelek, Balázs ; Nánási, Péter ; Boros, Imre M. Alternative linker histone permits fast paced nuclear divisions in early Drosophila embryo. NUCLEIC ACIDS RESEARCH 48 : 16 pp. 9007-9018. (2020)

IF: 11,501

*joint first authors

Other publications:

Huliák, Ildikó ; Bodai, László ; Czepán, Mátyás ; Kovács, Dávid ; **Szabó, Anikó** ; Tiszlavicz, László ; Lázár, György ; Rakonczay, Zoltán ; Hegyi, Péter ; Boros, Imre Miklós et al. Genetic, epigenetic and transcriptional comparison of esophagus tumor-associated and adjacent normal myofibroblasts. ONCOLOGY REPORTS 41 : 2 pp. 839-852. , 14 p. (2019)

IF: 3,417

Carbonell A, Henn L, Pérez-Roldán J, Tamirisa S, **Szabó A**, Boros IM, et al. In response to Li et al.: Linker histones function in Drosophila embryogenesis. 2020 Mar 21;001529. DOI: 10.1101/2020.03.21.001529

SUPERVISOR'S DECLARATION

I, **Prof. Dr. Imre Miklós Boros, Dr. Dániel László Henn**, the undersigned, hereby declare as the supervisor of the candidate (Anikó Szabó) that the doctoral dissertation is her own work completed under my supervision and it conforms to the formal and content requirements of the Doctoral School of Biology (Univesity of Szeged).

Szeged, 2020.november 7.

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