The Biochemical Characterization of Drosophila Telomere Capping Proteins and Their Potential Role in Speciation

Ph. D. thesis summary

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2017
Szeged

Introduction

The most important molecular functions of a cell are usually performed by highly conserved proteins. Any mutation in the essential sequences of these could compromise the survival of the organism. However, in some cases the cells are seemingly tempting fate; they fulfil important functions by fast evolving proteins. In order to resolve this contradiction, we assume that these proteins may have an additional function; they contribute to species formation.

A fast evolving protein complex in Drosophila is the hypothetical terminin complex, which is responsible for the protection of the ends of linear chromosomes, the telomeres. Telomeres prevent the chromosome ends from being detected as DNA double stranded breaks and also protect the coding regions from degradation. In most organisms, this protective function is performed by the highly conserved shelterin protein complex. However, in Drosophila there are no homologues of shelterin proteins. Instead a similar, so far less characterized complex, the terminin fulfils this role of chromosome end maintenance.

Intriguingly, the members of terminin complex are fast evolving proteins. Their evolutionary speed and important cellular function make these proteins good candidates for post-zygotic barriers.

Terminin is believed to consist of HOAP, HipHop, Ver and DTL/Moi protein subunits. HP1 is generally regarded as the fifth subunit of the putative complex, though it is not strictly terminin-specific. While other terminin proteins localize only at chromosome ends, HP1 plays a role at non-telomeric regions as well. Furthermore, HP1 is evolutionary highly conserved, while other terminin proteins manifest an accelerated rate of evolution. Deletion of the HP1 gene or any other terminin protein results in telomere fusions.

Physical interactions between terminin proteins have been demonstrated *in vitro*. According to these findings Ver interacts with DTL and HOAP, DTL interacts with Ver, HOAP and HP1. HipHop interacts with HP1 and HOAP, but it does not interact with Ver or DTL.

The available data about terminin proteins strongly suggest the existence of a shelterin like telomere capping

complex in Drosophila; however, terminin is not an experimentally proven entity, and no biochemical studies have been performed to investigate its assembly and action in detail.

Aims

We performed *in silico* analysis to study the evolutional rate of terminin proteins in order to determine whether their interacting domains show accelerated evolution.

We expressed and purified terminin components in bacteria to study and biochemically characterize terminin complex assembly.

To investigate whether the terminin complex plays role in speciation, we studied interspecies hybrid terminin subcomplex formation and function.

Finally, we intended to compare the canonical and Drosophila telomeres concerning complex formation and function in the light of our findings.

Methods we used extensively

- multiple alignment of protein coding regions
- examination of synonym and non-synonym mutations in order to calculate the rate of evolution
- protein structure predictions
- construction of mono- and polycistronic expression vectors by classical cloning methods
- heterologous protein expression
- tricin-polyacrylamide gel electrophoresis
- western blot
- peptide mass fingerprinting
- heparin-Sepharose chromatography
- immunoaffinity chromatography
- size-exclusion chromatography
- DNA affinity pull-down
- bio-layer interferometry

Results

1. In order to study terminin proteins' potential role in speciation, we used bioinformatics and biochemical approaches. To get a detailed view on the evolutionary rates of these proteins, first we collected and analysed the homologues sequences of the terminin proteins from 21 Drosophila species. By comparing them we found that the interacting domains of these proteins show accelerated evolution, which supports our hypothesis about their possible role in speciation. To investigate further, we created homology-based structure models. However, among the fast evolving terminin proteins only one, Ver resulted a reliable model. We evaluated the model of Ver by assessing the link between structure and amino acid conservation rates. We have predicted the conserved amino acids that have a role in the formation of specific structure motif, DNA binding and nuclear localization. I also found two conserved surfaces that may have a role in protein-protein interaction, although the roles of these surfaces are obscure. The structure model provided a lot of information about Ver. However, our hypothesis about

species formation and terminin proteins is still need to be tested.

2. For the biochemical characterization of the terminin complex I constructed multiple expression plasmids and produced terminin subunits in bacteria. Ver, DTL, HOAP and HP1 was expressed in high levels, but HipHop expression was consistently low, and despite various attempts, which included alterations in construct designs, conditions of induction and choices of host cells and as well trials of co-expression with other terminin proteins, we could not achieve notable expression. However, interaction data of terminin proteins suggest that a terminin complex may form without HipHop.

3. The insolubility of Ver and DTL proteins during protein expression also delayed our attempt to reconstitute of the terminin complex. However, when we co-expressed these proteins with their interacting partners, their solubility increased.

4. We subjected the lysate of cells that co-expressed four heterologous proteins (HOAP, HP1, Ver and DTL/Moi) in soluble form to chromatography on heparin-Sepharose column. We found that Ver together with

DTL/Moi, and similarly HOAP together with HP1 eluted in different fractions, but no holo-complex was formed. The increasing salt concentration that we applied during the elution of our proteins might explain the lack of the terminin holo-complex formation, therefore we subjected peak fractions from the heparin-Sepharose matrix to gel filtration column at low salt concentration. The gel filtration also revealed the existence of two terminin sub-complexes, the Ver-DTL and the HOAP-HP1 (and HipHop) sub-complexes. Our findings are in accordance with the literature since Ver and DTL are responsible for the inhibition of the single stranded, while HOAP, HP1 and HipHop are responsible for the inhibition of double stranded break repairs.

5. In order to investigate Ver and DTL role in speciation, we reconstituted *Drosophila melanogaster* Ver-DTL sub-complexes by co-expressing the subunits in bacteria and performing purification as described above. We also created hybrid sub-complex by the replacement of Ver with its orthologue from the closely related *Drosophila yakuba*. We demonstrated both by gel filtration and immunoaffinity chromatography that Ver

from *D. yakuba* and DTL from *D. melanogaster* could form a stable hybrid complex, despite the differences in their sequences.

6. We examined the DNA binding properties of the sub-complex and the hybrid sub-complex, and we found that both complexes bind single stranded DNA higher affinity than double stranded DNA. Therefore no loss of function was detected in the case of the hybrid complex.

The formation of the hybrid complex suggests that the interaction between Ver and DTL may form through conserved surfaces. In this case, the accelerated evolution of amino acids does not affect the function of the protein, therefore it is unlikely that Ver and DTL have a role in species formation. However, our finding does not completely disprove our hypothesis about terminin and species formation.

Although our theorem about the terminin role in species formation remains unproven, we have successfully isolated two terminin sub-complexes. We have further studied the Ver-DTL heterodimer and its molecular

function. This sub-complex also gave an opportunity to examine and solve the contradiction between the fast evolution and the important function of terminin proteins. We suppose that for the interactions between fast evolving proteins only a few amino acids are needed to be conserved, such as the amino acids that contribute to structure formation and the amino acids that form the interacting surfaces, while all the other parts of the molecule could change freely. In this case, fast evolution is not compromising the molecular function of the protein.

Publications

MTMT number: 10029430

Publication related to the Ph.D. thesis:

Cross-Species Interaction between Rapidly Evolving Telomere-Specific *Drosophila* Proteins

Balázs Vedelek, András Blastyák, Imre M. Boros

Research Article | published 13 Nov 2015 | PLOS ONE (2015 Impact Factor 3.057)

http://dx.doi.org/10.1371/journal.pone.0142771

Other publication:

mRNA Levels of Related *Abcb* Genes Change Opposite to Each Other upon Histone Deacetylase Inhibition in Drug-Resistant Rat Hepatoma Cells

Ádám Sike, Enikő Nagy, Balázs Vedelek, Dávid Pusztai, Péter Szerémy, Anikó Venetianer, Imre M. Boros

Research Article | published 07 Jan 2014 | PLOS ONE (2014/2015 Impact Factor 3.234)

http://dx.doi.org/10.1371/journal.pone.0084915

Total impact factor: 6,291