Identification of Telomere Regulating Genes in *Drosophila melanogaster*

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Abstract

Telomeres are the natural ends of linear chromosomes and contribute to the maintenance of chromosome stability. Without the capping effect of telomeres, broken chromosomes can undergo the breakage-fusion-bridge cycle which may cause cancer. In *Drosophila melanogaster*, telomeres are extended by telomere-specific non-LTR retrotransposons which serve as an alternative, yet similar approach to the telomerase. Previously, we described a genetic factor called *Telomere elongation* (*Tel*) on the third chromosome of fruit flies that can enhance telomere elongation. Another telomere-elongating gene was also identified in the similar chromosomal region in *D. melanogaster*. In the present study, we used a bioinformatic approach to identify the genes in this chromosomal region that have the potential to influence chromosomal stability in Drosophila. We hypothesize that the genes that can modulate chromosomal structure or remodeling have the potential to regulate telomere length or structure. We extracted genomic DNA from the strains in which one of these candidate genes is mutated. Using real-time PCR, we are analyzing the telomere length among different mutant strains with the Oregon-R wild-type strain as the control. In addition, to probe whether or not the disruption of these gene candidates causes a structural defect of telomeres, we have performed polytene chromosome staining. The results will help identify the genes that play a role in regulating telomere length and/or structure.

Introduction

Telomeres in fruit flies are extended by the telomere-specific non-LTR retrotransposons, *HeT-A*, *TART* and *TAHRE*.[1]

Previously, we showed:

- The Gaiano-O fly strain has longer telomeres than other wild-type strains, such as Oregon-R.
- A single genetic factor, *Tel,* results in elongated telomere length, increased fusion of chromosomal ends, and a defect involving telomere-binding proteins.

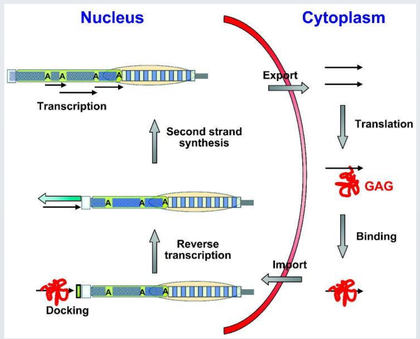

Fig. 1. Schematic of the retrotransposition process of the non-LRT telomere-specific retrotransposons[4].

- *Tel* gene was genetically mapped on chromosome 3, between the genes *stripe (sr)* and *ebony (e)*.[2]

Another telomere-elongating mutation called *E(tc)* was found in the similar region on the chromosome 3 in Drosophila.[3]

Here, we set out to identify telomere length regulating genes in this particular chromosomal region. We **hypothesize** that these genes influence telomere length by regulating chromosomal structure/stability and/or by modulating the telomere-specific retrotransposition process.

The first part of the research is to use a bioinformatic approach to identify the candidate genes that have the potential to regulate telomere length. We are currently conducting molecular genetics experiments, the second part, to screen the identified candidate genes using real-time PCR and polytene chromosome staining and immunostaining.

Materials and Methods

Fruit flies were cultured at 25ºC. Genomic DNA extraction, PCR, real-time PCR, and polytene immunostaining were carried out according to previous publications[2].

Results

1. Investigation and classification of the genes between *sr* and *e* based on their functions

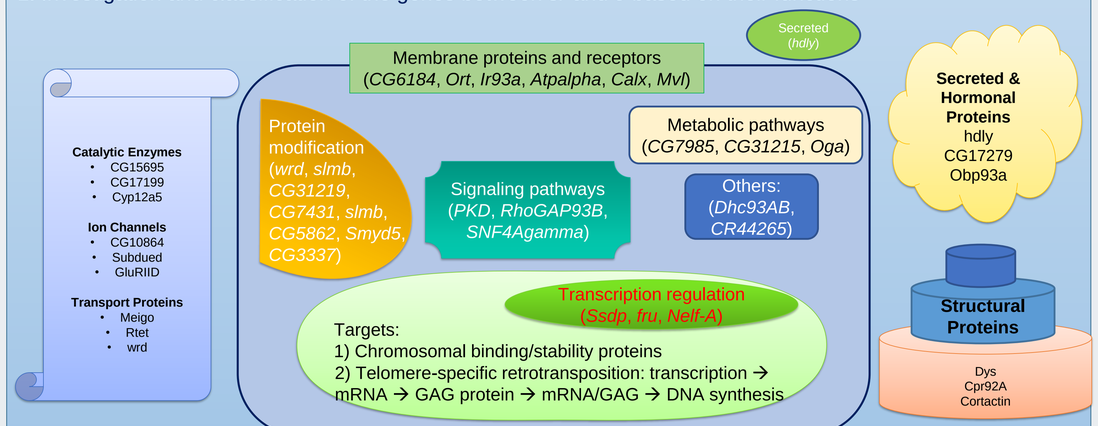

Fig. 2. Identified cellular pathways in which the analyzed genes are involved.

2. Examples of the identified candidate genes that are involved in chromosome structure remodeling

Directly Related Genes		
Gene	**Location**	**Function of Gene Product**
Moi	18,255,520..18,257,677	Chromosome binding (telomere capping)
Ino80	19,368,995..19,403,153	Chromatin remodeling
bon	20,593,139..20,612,540	Chromatin Binding , Ubiquitin Transferase
Stat92E	20,535,323..20,552,311	Histone Binding, Transcriptional activator
Sirt2	20,328,465..20,330,915	Histone Deacetylase activity, Maintaining chromatin structure at telomeres
CG10887	20,114,838..20,117,139	Transcription elongation from RNA polymerase II promoter and histone modification

3. PCR was conducted to amplify the actin gene and HeT-A retrotransposon from the extracted fly genomic DNA

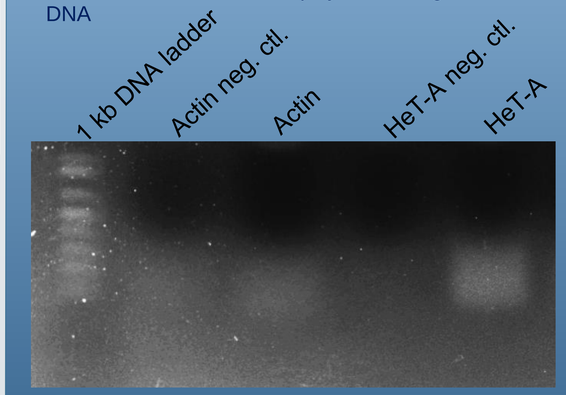

Fig. 3. The genomic DNA sample extracted from the wild-type flies was amplified for the actin gene and HeT-A retrotransposon.

4. Real-time PCR:

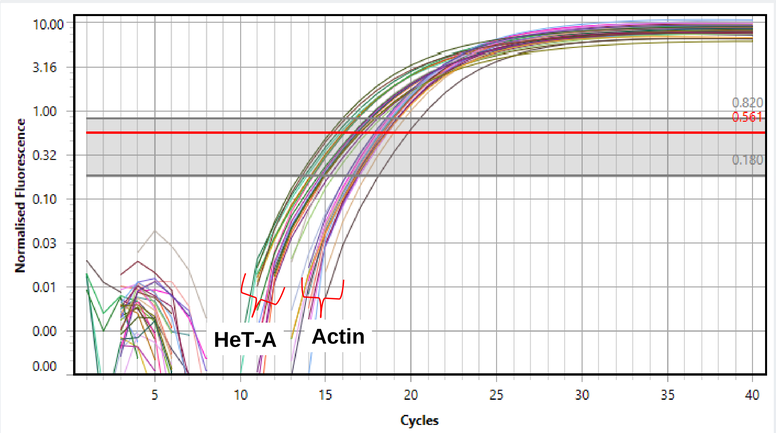

Fig. 4. Real-time PCR was conducted using 100 ng of the extracted fly genomic DNA to amplify HeT-A sequence and the Actin gene sequence.

5. Polytene chromosome staining

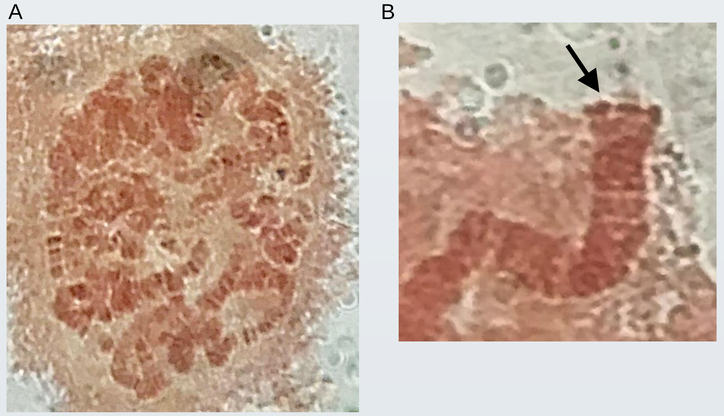

Fig. 5. The salivary glands were removed from the wild-type larvae and stained with the aceto-orcein staining solution. Left panel: 40x; Right panel: 100x. Arrow: Telomere.

Conclusion

- All the genes within the selected range (between *sr* and *e*) were studied, analyzed, and categorized for their potential to regulate telomere length.
- We have extracted fly genomic DNA and established the PCR methods to amplify HeT-A retrotransposon sequence with the actin gene as a reference.
- The real-time PCR method has been established to probe the copy numbers.
- We have established the polytene chromosome staining to analyze the potential defects at telomeres.
- **Ongoing experiments:**
  - Real-time PCR to measure the HeT-A copy numbers in various mutants using the wild-type as the control
  - Polytene staining to probe chromosomal fusion in various mutants
  - Polytene immunostaining to detect abnormal telomere structures

References

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