Identification of Telomere Regulating Genes in *Drosophila melanogaster*

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ABSTRACT

Telomeres contribute to the maintenance of chromosome stability. In *Drosophila melanogaster*, telomeres are composed of and extended by non-LTR retrotransposons. Previously, a genetic factor called *Telomere elongation* (*Tel*) was discovered on the third chromosome of fruit flies that can enhance telomere elongation. Another telomere-elongating gene was also identified in this chromosomal region. In the present study, we used a bioinformatic approach to identify the genes in this chromosomal region that have been indicated to influence chromosomal stability. We hypothesized that the genes that can modulate chromosomal structure or remodeling have the potential to regulate the telomere length or structure. We extracted genomic DNA from various mutant strains of these candidate genes. Using real-time PCR, we have analyzed the telomere length among different mutant strains. In addition, to probe whether disruption of these gene candidates causes a structural defect of telomeres, we are performing polytene chromosome staining. Currently, we have identified two genes, *CG6026* and *Ino80*, whose mutation can lead to elongated telomeres while other tested candidate genes do not affect the telomere length. This research can help understand the molecular regulatory mechanisms of telomere elongation and structure in fruit flies, which in turn may shed light on the mechanisms of alternative lengthening of telomeres in human cancer cells.

INTRODUCTION

- Telomeres in fruit flies are extended by the telomere-specific non-LTR retrotransposons, *HeT-A*, *TART* and *TAHRE*. [1]
- Previously
  - A genetic factor, *Tel*, which results in elongated telomere length and increased fusion of chromosomal ends, has been mapped to a region on chromosome 3, between the genes *stripe* (*sr*) and *ebony* (*e*).[2]
  - Another telomere-elongating mutation called *E(tc)* was found to fall in this region except for the *sr* end being extended to *Stubble* (*Sb*).[3]
- We set out to identify telomere length regulating genes in this particular chromosomal region.

MATERIALS AND METHODS

Fruit flies were purchased from Carolina Biological and Bloomington Drosophila Stock Center (BDSC) and cultured at 25ºC. Genomic DNA extraction, PCR, real-time PCR, and polytene chromosome staining were carried out according to previous publications[2].

RESULTS

- Thirty-one candidate genes were identified to be involved in chromosome structure remodeling.

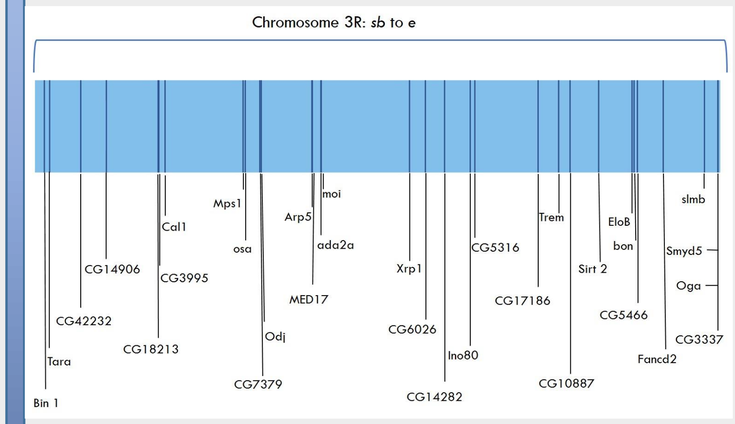

Fig. 1. Thirty-one candidate genes selected from more than 500 genes between *sb* and *e* on chromosome 3R.

- Real-time PCR experiments were done for a set of mutant fly strains with Oregon-R as the wild-type control and the *Tel* mutant strain as the positive control.

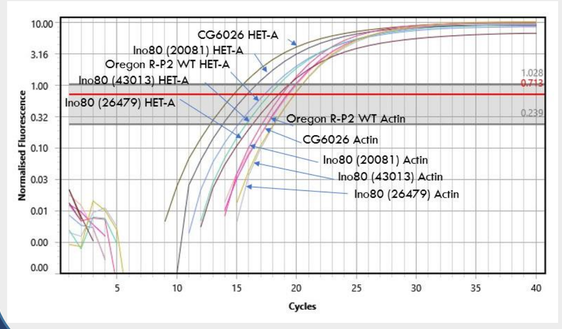

Fig. 3. A representative qPCR result.

- PCR to amplify *HeT-A* and *Actin* specifically.

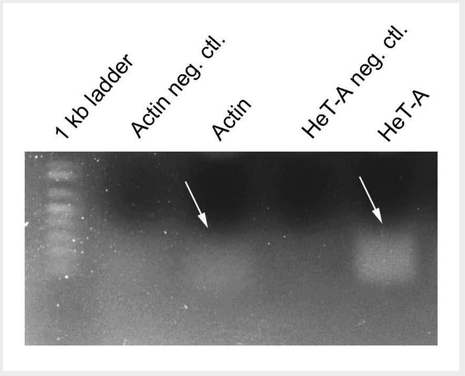

Fig. 2. PCR gel electrophoresis to amplify specific sequences of the *HeT-A* retrotransposon and *Actin* gene.

- A *CG6026* mutant strain has longer telomeres.

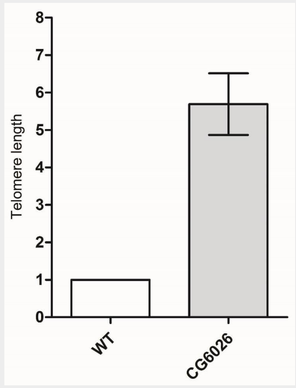

Fig. 4. A *CG6026* mutant strain has longer telomeres compared to those of the wild-type strain Oregon-R. (N = 5; Error bar: SEM; T test, $p < 0.01$)

- Three *Ino80* mutant strains potentially have longer telomeres.

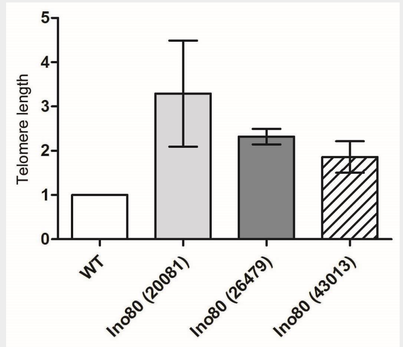

Fig. 5. Three *Ino80* mutant strains show different levels of elongated telomeres. (N = 5; Error bar: SEM)

- Polytene chromosome staining has been conducted to probe telomere fusion and other possible structural defects in mutant strains.

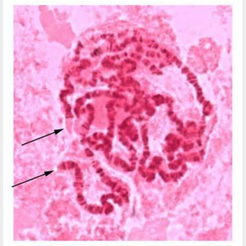

Fig. 6. A representative image of polytene chromosome staining in the wild-type strain Oregon-R. (Arrows: examples of telomere ends.)

CONCLUSIONS

- We have identified two genes, *CG6026* and *Ino80*, that may play a role in telomere elongation in *D. melanogaster*.
- Polytene chromosome staining and immunostaining can help unravel the molecular mechanisms of *CG6026* and *Ino80*.

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