

## Introduction

*Drosophila* Trimethylguanosine Synthase 1 is encoded by the *dtl* gene, from which a single mRNA is transcribed that codes two ORFs. DTL is coded by the first ORF, while TGS1 is translated from the second ORF. The TGS1 protein has a role in the 2,2,7-trimethylguanosine modification of snRNA and snoRNA molecules. These RNA species play an important role in rRNA modification, splicing, and RNA maturing. These study claims, that these RNA modification contribute in the egg development process.

## Results

To examine the function of the *dtl* gene, our group created two null mutant alleles: d192, which removes the upstream ORF and the first part of the downstream ORF, and d189, which lacks the promoter region (Fig. 1.). In the following experiments, I used d189/d192 as a background. We created four p-element constructs, three of them expressed only one ORF (pCasper-TGS1, pCasper-DTL, pUAS-TGS1), the fourth contains the original *dtl* gene (pCasper-ORF1-2, Fig. 1.).

**Δ TGS1:** Actin-Gal4 driver, pUAS-TGS1, pCasper-DTL.  
**Independent DTL and TGS1 rescue:** pCasper-TGS1, pCasper-DTL.  
**Dtl rescue:** pCasper-ORF1-2

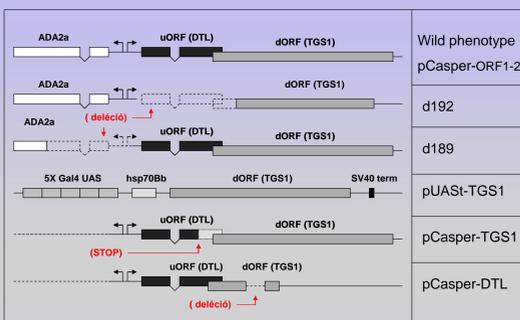
In the absence of TGS1 in the germline, female sterility is observed, only a small portion of the eggs develop to adulthood (Fig. 2.). The average number of the laid eggs decreased (Fig. 3.), resulted from the reduction of ovarioles (Fig. 4.), delayed egg development (Fig. 9.) and increased apoptosis (Fig 10-11.).

The morphology of the eggs produced by the TGS1 null mutant germ line was changed, the dorsal appendages were shorter and the average length of eggs decreased (Fig. 7-8.).

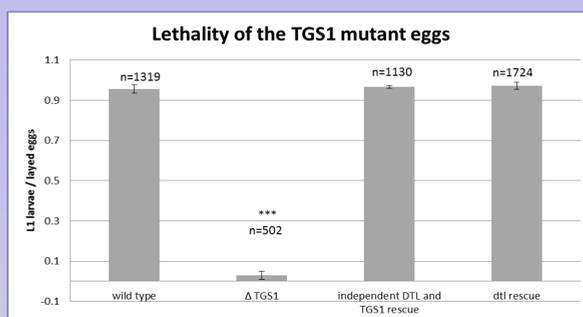
While in the 4th stage of the egg chamber development chromosomes of the nurse cells condense, in the next developmental stage a chromosome dispersion occurs. This process happened very rarely in TGS1 null egg chambers, and a lobular chromosome structure remained in the following developmental stages (Fig 5-6.).

In ovarioles of TGS1 null animals the number of egg chambers representing different stages of development greatly reduced and elevated level of apoptosis could be observed at stage 8. (Fig. 10.)

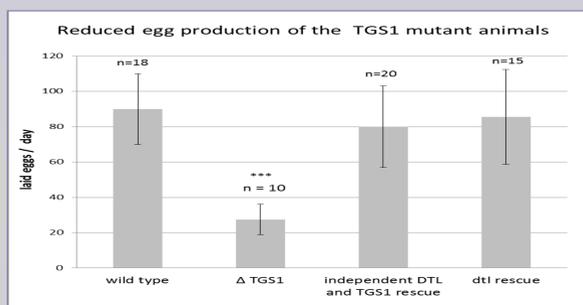
These observations reveal that the proliferation of germ cells could be decreased in the absence of TGS1, the egg filling mechanism could be damaged probably due to the altered chromosome structures of nurse cells resulting from the lack of TMG-cap modifications. In the case of insufficient egg filling at 8th stage, starvation induced apoptosis was occurred.



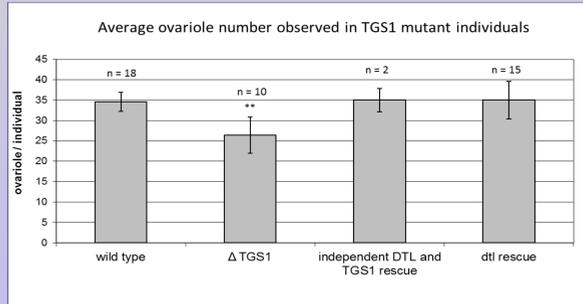
**Fig. 1.** The nomenclature and the schematic illustration of the genetic backgrounds and used p-element constructs.



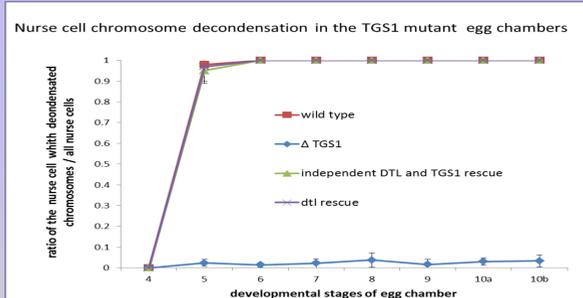
**Fig. 2.** The TGS1 null eggs show developmental failure, compared to the wild type and the rescued animals. (Standard Deviation, ... : p < 0.001)



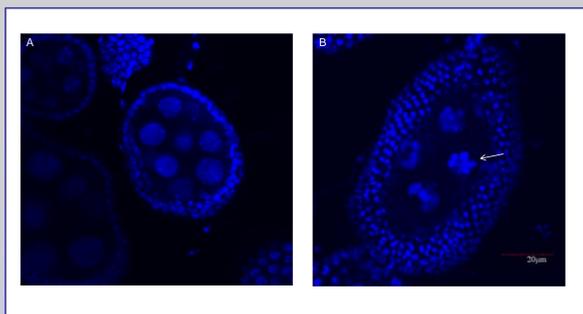
**Fig. 3.** Decreased egg production observed in TGS1 null ovaries, compared to the wild type and the rescued animals. (Standard Deviation, ... : p < 0.001)



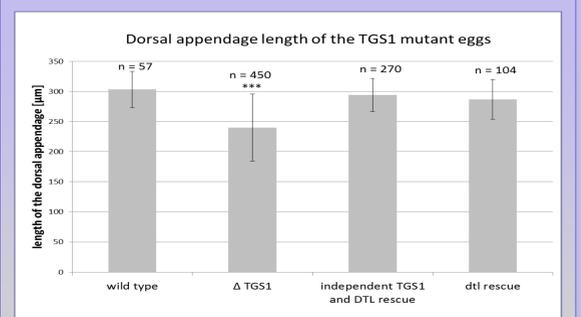
**Fig. 4.** The number of the ovarioles in TGS1 null eggs decrease, compared to the wild type and the rescued animals. (Standard Deviation, ... : p < 0.01)



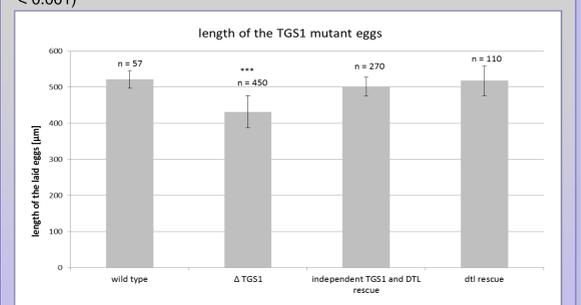
**Fig. 5.** In TGS1 absent nurse cells, significant decrease of chromosome decondensation can be observed, compared to the wild type and the rescued animals.



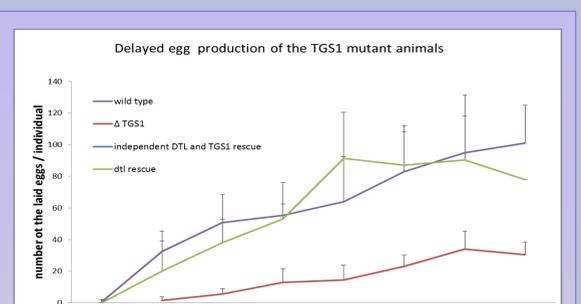
**Fig. 6.** Chromosome decondensation defect can be observed in TGS1 absent nurse cells, which remains in all developmental stages in the ovary. (A) wild type (B) TGS1 mutant animals stained with DAPI. Arrowhead shows aberrant nurse cell chromosomes at older than stage 4.



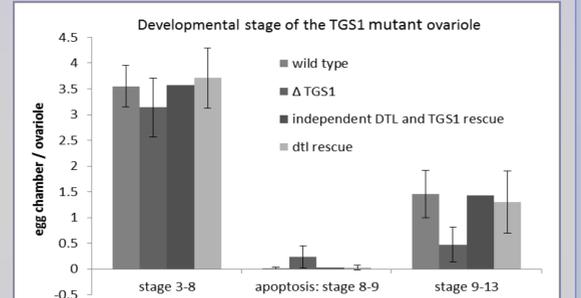
**Fig. 7.** The length of the dorsal appendage in TGS1 null eggs is significant decreased, compared to the wild type and the rescued animals. (Standard Deviation, ... : p < 0.001)



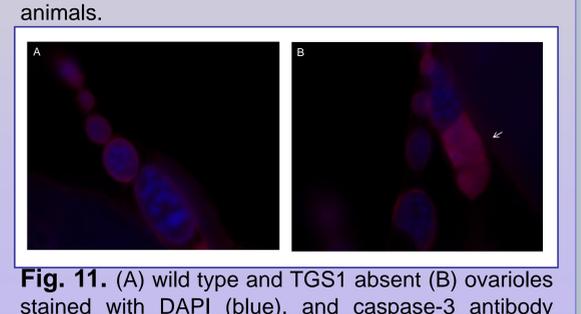
**Fig. 8.** The length of the TGS1 null eggs is significant decreased, compared to the wild type and the rescued animals. (Standard Deviation, ... : p < 0.001)



**Fig. 9.** Delayed egg production can be observed in TGS1 null nurse cells, compared to the wild type and the rescued animals.



**Fig. 10.** Reduced number of every developmental stages could be observed in TGS1 mutant ovarioles, and increased apoptosis was observed at around stage 8-9, compared to the wild type and the rescued animals.



**Fig. 11.** (A) wild type and TGS1 absent (B) ovarioles stained with DAPI (blue), and caspase-3 antibody (red). Arrowhead shows apoptosis at stage 8.

## Discussion

Our observations suggest that the trimethylguanosine modification of RNAs plays a crucial role in the process of polytene chromosome dispersion and the oogenesis in *Drosophila*. The fact, that in rare occasions fertile eggs can be observed, suggests that this modification is not essential in oogenesis, but significantly contribute to this process.