ISSN print: 1224-5984
ISSN online: 2248-3942

Rom Biotechnol Lett. 2019; 24(2): 324-331
doi: 10.25083/rbl/24.2/324.331

Received for publication, September, 20, 2018
Accepted, January, 5, 2019

Original paper

Hypomorphic alleles of gammaCop gene from Drosophila melanogaster display an unexpected expression pattern in mutant eggs, testes and embryos

ALEXANDRU AL. ECOVOIU[1], MARIAN GRAUR[2], ATTILA CRISTIAN RATIU[1]*

[1]University of Bucharest, Faculty of Biology, Department of Genetics, Intrarea Portocalelor Street, No. 1-3, 060101, Bucharest, Romania
[2]MedLife Genetics, Calea Grivitei Street, No. 365, 010719, Bucharest, Romania

Abstract

We report an unusual overexpression pattern of the hypomorphic alleles γCop^{11a}, γCop^{14a} and γCop^{16b} of gammaCop gene from Drosophila melanogaster. Each allele contains a distinct short remnant of the P{lacW} transposon located in 5'UTR. Repeated crosses between $\gamma Cop^{14a}/\gamma Cop^{14a}$ females and $\gamma Cop^{11a}/\gamma Cop^{11a}$ or $\gamma Cop^{16b}/\gamma Cop^{16b}$ males proved to be sterile, but the reciprocal ones are fertile.

The most severe allele is γCop^{14a} since a $\gamma Cop^{14a}/\gamma Cop^{14a}$ line cannot be obtained. Remarkably, qRT-PCR performed on mutant eggs, testes and embryos reveals that γCop^{11a}, γCop^{14a} and γCop^{16b} are overexpressed, which is counterintuitive for hypomorphic alleles. Moreover, the expression scale overlaps the hierarchy of the allelic phenotype severity.

We found that the folding ΔG values of the mutant 5'UTRs are very low as compared to the folding ΔG value of the wild-type 5'UTR. This thermodynamic context may account for the uncommon expression pattern of the hypomorphic alleles described herein.

Keywords Drosophila melanogaster; gammaCop; hypomorphs; gene expression; qRT-PCR

To cite this article: ECOVOIU AA, GRAUR M, RATIU AC. Hypomorphic alleles of gammaCop gene from Drosophila melanogaster display an unexpected expression pattern in mutant eggs, testes and embryos. *Rom Biotechnol Lett*. 2019; 24(2): 324-331. DOI: 10.25083/rbl/24.2/324.331

✉ *Corresponding author: ATTILA CRISTIAN RATIU, University of Bucharest, Faculty of Biology, Department of Genetics, Intrarea Portocalelor Street, No. 1-3, 060101, Bucharest, Romania
E-mail: attila.ratiu@bio.unibuc.ro

Introduction

Former studies revealed that *gammaCop* (*γCop*) localized on chromosome 3R of *Drosophila melanogaster* is an essential gene since lethal *γCop* alleles were described (JAYARAM & al. [1]). Not surprisingly, *γCop* is highly conserved among eukaryotes and its biological function is related to membrane and protein trafficking within the secretory apparatus (FlyBase gene report, *https://flybase.org/*), which explains why the gene product is required for viability. On the other hand, *in situ* hybridization data reveal that *γCop* transcripts are present in the later stages of oogenesis, in testis and in the early embryo (GRIEDER & al. [2]; JAYARAM & al. [1]). These data suggest a potential maternal/parental effect of *γCop* that supposedly sustains at least the first stages of embryogenesis. Previously, we proved that a *P{lacW}* transposon symbolized as $P\{lacW\}\gamma Cop^{S057302}$ (DEÁK & al. [3]) is inserted in the 5'UTR region of *γCop* (GenBank accession number AJ492220) and is responsible for embryo lethality. The mutant phenotype is revertible by precise excision of the transposon (ECOVOIU & al. [4]). In a genetics endeavor described elsewhere (ECOVOIU & al. [5]), we constructed three excisional alleles by imprecise mobilization of $P\{lacW\}\gamma Cop^{S057302}$. The respective alleles are hypomorphic for the viability phenotype and are symbolized as γCop^{11a}, γCop^{14a} and γCop^{16b} (GenBank accession numbers DQ279401, DQ279402, and, respectively, DQ279403). The bioinformatics analysis revealed that their 5'UTRs harbor very short but different molecular remnants of *P{lacW}*. None of the alleles complement lethality of $P\{lacW\}\gamma Cop^{S057302}$, but we noticed that $\gamma Cop^{11a}/\gamma Cop^{11a}$, $\gamma Cop^{14a}/\gamma Cop^{14a}$ and $\gamma Cop^{16b}/\gamma Cop^{16b}$ homozygous adults are viable and conditionally fertile (ECOVOIU & al. [6]).

Starting from these observations, we performed a functional analysis of *γCop* in order to inquire its involvement in fertility of *D. melanogaster*. The functional analysis was completed with several qRT-PCR experiments revealing unexpectedly conflicting results obtained for eggs, testis and embryos derived from $\gamma Cop^{11a}/\gamma Cop^{11a}$, $\gamma Cop^{14a}/\gamma Cop^{14a}$ and $\gamma Cop^{16b}/\gamma Cop^{16b}$ individuals. We present a scenario intended to interpret the discrepancy between genetics and molecular data.

Materials and Methods

Genetic analysis – *Drosophila melanogaster* stocks harboring the *γCop* mutant alleles (γCop^{11a}, γCop^{14a} and γCop^{16b}) were maintained at 18°C and were balanced over TM3, Sb Ser e or over TM6, Tb Hu e chromosomes, which harbor obvious dominant phenotypic markers. For the experiments involving embryos, the mutant strains were balanced over TM3, Ser GFP in order to select homozygous embryos against GFP marker. As a reference strain we used *Oregon*. Genetic crosses were performed at both 18°C and 25°C.

Molecular biology analysis – The relative expression of *γCop* alleles was quantified in adult testes, unfertilized eggs (hereafter called eggs) and embryos. Total RNA was extracted from two biological replicates, each consisting of approximately 10 adult individuals for the quantifications involving testes and eggs, and of roughly 120 embryos, respectively. Whenever necessary, flies were collected under CO_2 anesthesia and readily dissected in DEPC treated water. For RNA extraction from embryos, the female adults were removed from plates after eggs laying and embryos were aged for about 24 hours on the medium surface. The total RNA extraction was performed using NucleoSpin-RNA XS kit (Macherey-Nagel), according to manufacturer's instructions. For some total RNA samples, a secondary treatment with DNase (Promega) was performed when considered appropriate. cDNA was generated using 500 ng or 1000 ng of whole RNA and Reverse Transcription System (Promega). The reverse transcription (RT) reaction corresponding to a 1000 ng of total RNA input consisted of 2 µl 10X RT Buffer, 4 µl $MgCl_2$ 25 mM, 2 µl dNTP mixture 10 mM, 0.5 µl RNase Inhibitor Protein 40 U/µl, 1 µl Random Primers 500 µg/ml, 15 U of AMV Reverse Transcriptase, in a final volume of 20 µl. When the RNA input was different from 1000 ng, the RT reaction was accordingly adjusted. Reactions were incubated on a PalmCyclerPCR (Corbett) at 25°C for 10 min, 42°C for 15 min, then 95°C for 5 min, followed by a short 5 min cooling cycle at 4°C. RT-PCR analysis was performed for *γCop* target gene using *RpL32* housekeeping gene as a referential. *γCop* specific primers were designed with *FastPCR* software (KALENDAR & al. [7]), and have the following sequences: 5'TGGTCGTGCAGGCCATTTGC3' (forward) and 5'CTCGCACAGATGCGACAAACC3' (reverse), which allow to obtain a 191 base pairs (bp) amplicon. *RpL32* specific primers are described in the literature (FIUMERA & al. [8]) and determine a 195 bp amplicon. Each biological replicate was assesed in three technical replicates. Every 25 µl of PCR reaction consisted of 60 ng cDNA, 12.5 µl 2X SYBR Green PCR Master Mix (Fermentas), 0.16 µM forward primer, 0.16 µM reverse primer, and completed with DNase free-water (Fermentas). SYBR Green PCR

assays for all specific alleles were performed on cDNA samples in 96-well optical plates on an ABI Prism 7500. The PCR protocol consisted in one cycle at 95°C for 10 min, 40 cycles for 30 sec at 95°C, 30 sec at 57°C, 31 sec at 72°C, followed by a dissociation step; data collection was performed during the 72°C extension step.

The Ct values were collected at a threshold of 0.019004 and a 3-10 or 3-15 baseline. For the relative mRNA quantitivation step we used the $2^{-\Delta\Delta Ct}$ method (LIVAK and SCHMITTGEN [9]). The statistical analysis was performed with two-tailed Student's *t*-test using $2^{-\Delta Ct}$ values and GraphPad Prism 5.04 software (GraphPad Software, La Jolla California USA, *www.graphpad.com*). For the grafics showing the logarithmated fold-changes (FC), data are expressed as mean ± SEM.

Bioinformatics analysis – The nucleotide sequences standing for the 5'UTRs of wild-type and transcript variants of mutant *γCop* alleles were investigated within RNAfold web server (*http://rna.tbi.univie.ac.at/cgi-bin/RNAfold.cgi*) in order to predict their secondary structures and corresponding folding ΔG values (minimum free energy – MFE). We used the implicit fold algorithms and basic options, including RNA energy parameters calculations according to the Turner model.

Results

Genetic analysis – Consecutive to generation of γCop^{11a}, γCop^{14a}, and γCop^{16b} mutant alleles, we repeatedly performed complementation tests (at least a few tens of descendants were analyzed for each complementation test) which allowed us to conclude that none of these alleles complement the lethality of $P\{lacW\}\gamma Cop^{S057302}$. Nevertheless, in the next generations of the complementation tests we found viable $\gamma Cop^{11a}/\gamma Cop^{11a}$, $\gamma Cop^{14a}/\gamma Cop^{14a}$ or $\gamma Cop^{16b}/\gamma Cop^{16b}$ individuals of both sexes. In an attempt to stabilize homozygous lines, we remarked that this achievement is possible only for $\gamma Cop^{11a}/\gamma Cop^{11a}$ and $\gamma Cop^{16b}/\gamma Cop^{16b}$ lines, which are hard to be kept alive for more than a few generations, especially the $\gamma Cop^{11a}/\gamma Cop^{11a}$ line. Appropriate crosses were repeatedly performed in an attempt to obtain a $\gamma Cop^{14a}/\gamma Cop^{14a}$ homozygous line. We noticed that, in spite of a normal mating behavior of males and females, and of consistent eggs laying, crosses between $\gamma Cop^{14a}/\gamma Cop^{14a}$ parents are always sterile, at both 18°C and at 25°C temperature (room-temperature – RT).

Jayaram *et al*. (2008) describe the deficiency $\gamma Cop^{\Delta 114}$, an excisional null allele of *γCop*. The authors report the transgenic $\gamma Cop^{\Delta 114}/\gamma Cop^{S057302}$ embryos as being defective for tracheal tubes enclosure and only the expression of a wild-type copy of *γCop* in trans-heterozygous embryos rescues their lethality. Such data rank both $\gamma Cop^{\Delta 114}$ and $\gamma Cop^{S057302}$ as null alleles, which is in accordance with our genetics data, since complementation tests revealed that $\gamma Cop^{\Delta 114}$ do not complement neither $\gamma Cop^{S057302}$ nor γCop^{14a} alleles.

We performed tens of different crosses in order to analyze the role of *γCop* in the fertility phenotype of *D. melanogaster* (raw counting data are available upon request). We concluded that $\gamma Cop^{14a}/\gamma Cop^{14a}$ females are fertile (adult flies emerge in F_1) only when mated with males having at least a wild-type copy of *γCop* gene. Alternatively, $\gamma Cop^{14a}/\gamma Cop^{14a}$ descendants are always viable and healthy when they result from $\gamma Cop^{14a}/+$ females mated with $\gamma Cop^{14a}/\gamma Cop^{14a}$, reflecting the maternal effect of *γCop*. The results are summarized in table 1 and led us to conclude that γCop^{11a}, γCop^{14a}, and γCop^{16b} alleles are hypomorphs for viability and fertility.

Crosses of $\gamma Cop^{14a}/\gamma Cop^{14a}$ females with either $\gamma Cop^{11a}/\gamma Cop^{11a}$, $\gamma Cop^{14a}/\gamma Cop^{14a}$ or $\gamma Cop^{16b}/\gamma Cop^{16b}$ males are sterile at both 18°C and RT. Rare escapers pertaining to all of the postembryo developmental stages were found at RT and their incidence is slightly enhanced at 18°C, suggesting that lowering of the temperature reduces the phenotypic severity. Conversely, the reciprocal crosses between $\gamma Cop^{11a}/\gamma Cop^{11a}$ or $\gamma Cop^{16b}/\gamma Cop^{16b}$ females and any kind of homozygous *γCop* mutant males are fertile even at the stringent RT condition, pointing again to the prevalent importance of the maternal specific contribution. Not surprisingly, the homozygous mutant females for any of the hypomorphic alleles exhibit normal fertility when mated with wild-type males and *vice versa*.

We carefully analyzed embryos from crosses between $\gamma Cop^{14a}/\gamma Cop^{14a}$ parents and find out that, basically, most of them complete embryogenesis. Seldom, a few escapers of the next development stages emerge. More than 1.800 embryos were counted from crosses maintained at 18°C, but only four apathetic larvae were found. For the similar crosses kept at RT, from more than 1.300 embryos counted, no larvae escapers were found, suggesting that RT is a more stringent condition for $\gamma Cop^{14a}/\gamma Cop^{14a}$ homozygous survival.

Table 1. The outcome of the crosses involving different *γCop* genotypes. The wild-type allele (+) is harboured by a TM balancer chromosome in all heterozygous genotypes, while +/+ genotype is particular to *Oregon* individuals.

***γCop* genotypes**		**Outcome of the cross**
Female	**Male**	
$\gamma Cop^{14a}/\gamma Cop^{14a}$	$\gamma Cop^{14a}/\gamma Cop^{14a}$	Sterile
	$\gamma Cop^{11a}/\gamma Cop^{11a}$	Sterile
	$\gamma Cop^{16b}/\gamma Cop^{16b}$	Sterile
	γCop^{14a}/+	Semisterile
	γCop^{11a}/+	Semisterile
	γCop^{16b}/+	Partially fertile
	+/+	Fertile
γCop^{14a}/+	$\gamma Cop^{14a}/\gamma Cop^{14a}$	Fertile
$\gamma Cop^{11a}/\gamma Cop^{11a}$	$\gamma Cop^{14a}/\gamma Cop^{14a}$	Fertile
	$\gamma Cop^{11a}/\gamma Cop^{11a}$	Fertile
	$\gamma Cop^{16b}/\gamma Cop^{16b}$	Fertile
$\gamma Cop^{16b}/\gamma Cop^{16b}$	$\gamma Cop^{14a}/\gamma Cop^{14a}$	Fertile
	$\gamma Cop^{11a}/\gamma Cop^{11a}$	Fertile
	$\gamma Cop^{16b}/\gamma Cop^{16b}$	Fertile

Molecular biology analysis – The relative expression of *γCop* mutant alleles in testes, eggs and embryos of $\gamma Cop^{11a}/\gamma Cop^{11a}$, $\gamma Cop^{14a}/\gamma Cop^{14a}$, and $\gamma Cop^{16b}/\gamma Cop^{16b}$ individuals were estimated as compared to wild-type *γCop* allele from *Oregon* by qRT-PCR. The results revealed that the hypomorphic alleles are overexpressed in all of the analyzed biological sample (Figure 1).

The overexpression of γCop^{14a} has the highest relative value in all considered biological samples, followed by the relative levels of γCop^{11a} and γCop^{16b}. The resulting hierarchy matches the functional severity gradient of the respective alleles. The FC values corresponding to each mutant allele do not vary considerably when different biological sample types were inquired (Table 2). The highest level of overexpression was detected in testes of $\gamma Cop^{14a}/\gamma Cop^{14a}$.

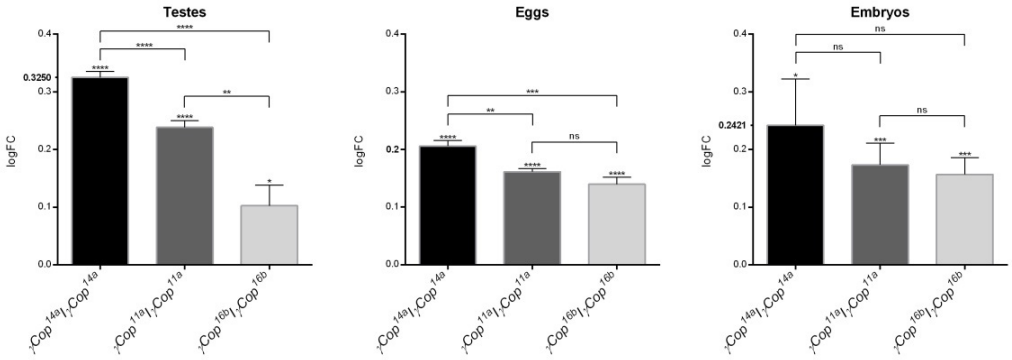

Figure 1. Relative expression levels of the *γCop* alleles corresponding to the genotypes indicated under the x-axes. The highest values of logarithmated FC values, characteristic for γCop^{14a} allele regardless of the biological sample (testes, eggs or embryos), are depicted with bolded numbers. Statistical significance of the overexpression levels was calculated relative to the wild-type *γCop* allele (indicated above expression bars) and between the hypomorphic alleles (shown above the horizontal lines). **** stands for $p < 0.0001$, *** stands for $p < 0.001$, ** stands for $p < 0.01$, * stands for $p < 0.05$, and ns indicates that the differences are not statistically significant.

Table 2. The comparative FC values of γCop^{14a}, γCop^{11a} and γCop^{16b} alleles relative to wild-type *γCop*. FC values of γCop^{14a} allele are bolded.

***γCop* allele**	**FC values**		
	Testes	**Eggs**	**Embryos**
γCop^{11a}	1.73	1.45	1.49
γCop^{14a}	**2.11**	**1.61**	**1.75**
γCop^{16b}	1.27	1.38	1.43

Bioinformatics analysis – Consecutive to the imprecise excision of $P\{lacW\}\gamma Cop^{S057302}$, very short molecular remnants of *P{lacW}* are detected in 5'UTR of *γCop* gene, ranging from 30 to 39 nucleotides (table 3).

Table 3. Nucleotide sequences of the *P{lacW}* remnants of γCop^{11a}, γCop^{14a}, and γCop^{16b} mutant alleles.

***γCop* allele**	***P{lacW}* remnants (5'-3' orientation)**
γCop^{11a}	catgatgaaataacatcatgttatttcatcatg
γCop^{14a}	catgatgaaataacatgttatttcatcatg
γCop^{16b}	catgatgaaataataataataatatgttatttcatcatg

Since these remnants are basically fragments from inverted repeats of *P{lacW}* transposon, they fold in hairpin structures that affect the MFE of the 5'UTR (Figure 2).

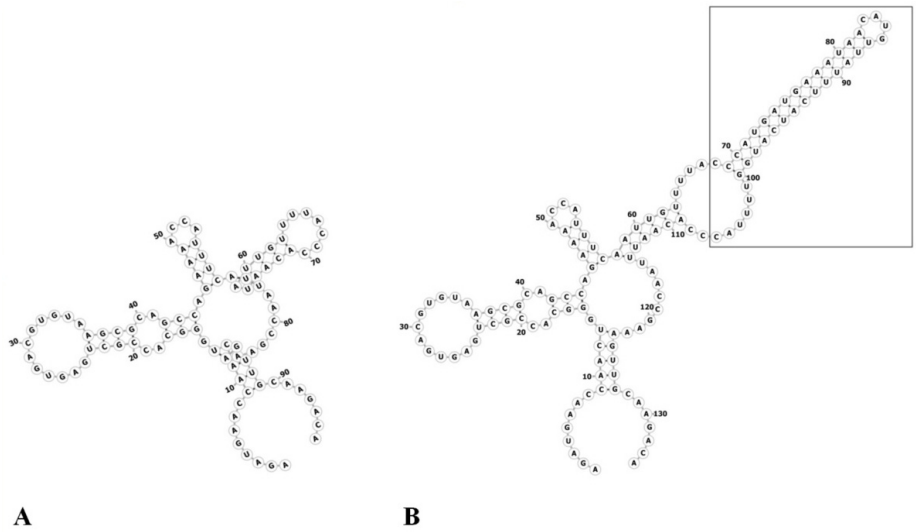

Figure 2. Secondary structures of the 5'UTR corresponding to the RA, RC and RD isoforms of wild-type *γCop* (A) and γCop^{14a} (B). In the area of the rectangle is shown the hairpin structure formed by the *P{lacW}* remnant specific to γCop^{14a} allele (images generated by the RNAfold web server).

The *γCop* gene has four isoforms, symbolized *γCop*-RA, *γCop*-RB, *γCop*-RC, and *γCop*-RD. These transcripts have two alternative 5'UTRs. The short 5'UTR has 96 nucleotides and is reported in FlyBase for the alternative transcripts *γCop*-RA, *γCop*-RC, and *γCop*-RD (Figure 2). For wild-type short 5'UTR of *γCop*, the folding ΔG of 5'UTR is -14.40 kcal/mol, but for the hypomorphic alleles, the ΔG is much lower (ΔG = -31.20 kcal/mol for γCop^{14a}, ΔG = -35.10 kcal/mol for γCop^{11a}, and ΔG = -33.00 kcal/mol for γCop^{16b}). A similar situation is evident when analyzing the long 5'UTR of *γCop*-RB transcript, where ΔG values of -66.90 kcal/mol, -85.00 kcal/mol, -88.90 kcal/mol, and -86.80 kcal/mol are computed for wild-type, γCop^{14a}, γCop^{11a}, and γCop^{16b} alleles. The values of the MFE for the optimal secondary structures were calculated with RNAfold web server (HOFACKER [10]). Although the folding ΔG values hierarchy do not perfectly match the one revealed by qRT-PCR data, it may be noticed the evident decrease of ΔG caused by the remnants of *P{lacW}*. These thermodynamic data suggests that the mRNAs encoded by the respective alleles are more stable (strongly folded) and therefore more eligible for degradation (RINGNER and KROUGH [11]).

Discussion

According to functional genetic tests, Muller classifies alleles (morphs) as amorphic, hypomorphic, hypermorphic and neomorphic (MULLER [12]) therefore molecular basis of their mutant phenotypes may be only presumed. If transcriptional levels are affected, it makes sense to consider amorphic alleles as transcriptionally nulls, hypermorphs as upregulated ones and hypomorphs as being downregulated when compared to the wild-type. If *m* is a functional hypermorphic allele, then *m*/null heterozygous should present an almost normal phenotype, but if *m* is a hypomorphic allele, then *m*/null should display a more severe phenotype as compared to *m*/*m* homozygous. According to our tests, $\gamma Cop^{11a}/\gamma Cop^{11a}$, $\gamma Cop^{14a}/\gamma Cop^{14a}$ and $\gamma Cop^{16b}/\gamma Cop^{16b}$ are viable and conditionally fertile, but when the alleles are in heterozygous condition with $\gamma Cop^{S057302}$ or $\gamma Cop^{\Delta 114}$ null alleles, the respective genotypes determine lethality. Since a single dose of γCop^{11a}, γCop^{14a} or γCop^{16b} is not enough for survival it results that they behave as hypomorphic alleles at least for lethality.

From our genetic analysis experiments it results that relative to $\gamma Cop^{14a}/\gamma Cop^{14a}$, the $\gamma Cop^{11a}/\gamma Cop^{11a}$ and $\gamma Cop^{16b}/\gamma Cop^{16b}$ females have a milder sterility phenotype, most probably because they succeed to deliver a required threshold amount of the *γCop* transcript to their eggs. Based on our functional genetic analysis we concluded that the phenotype severity within this polyallelic series is $\gamma Cop^{14a} > \gamma Cop^{11a} > \gamma Cop^{16b}$, where γCop^{14a} is the most severe allele. Such a conditional sterility may be explained by assuming that *γCop* is both a maternal and a paternal effect gene, meaning that it is differently supplied by both parents. The conditional infertility of the some of the described crosses may be caused by an insufficient delivery of maternal *γCop* transcript in oocytes corroborated with a low level of the specific male contribution. Indeed, the reciprocal crosses were fertile, revealing that the maternal contribution of *γCop* product is more important than the paternal one, so the mutant phenotype determined by each of the hypomorphic alleles has more severe functional consequences in females compared to males.

In situ and microarray data reveal specific upregulation in accessory glands, testes and ovaries, suggesting the involvement of *γCop* in fertility (GRIEDER & al. [2]; CHINTAPALLI & al. [13]). According to *FlyAtlas* (*www.flyatlas.org*), the highest specific transcription rate is present in the accessory glands, where a relative upregulation enrichment value of 4.7 was detected (CHINTAPALLI & al. [13]). Our qRT-PCR results revealed that the mutant *γCop* alleles are significantly overexpressed in specific types of tissues relative to the wild-type allele, which is generally unexpected for hypomorphic alleles. More surprisingly, the overexpression hierarchy mimics the phenotype severity scale inferred from the functional assays, since the most overexpressed allele is γCop^{14a}. The differences among the FC levels of γCop^{11a}, γCop^{14a} and γCop^{16b} transcripts are statistically significant in testes, but not between γCop^{11a} and γCop^{16b} in the eggs. In embryos, we detected statistically significant differences only between each of the hypomorphs and wild-type alleles (Figure 1). Nevertheless, the genetic analysis always revealed biologically significant differences among phenotypes determined by the γCop^{11a}, γCop^{14a} and γCop^{16b} alleles. The qRT-PCR data obtained for testes and the genetic analysis results suggest a paternal contribution of *γCop* product, aside from the maternal and zygotic effects of *γCop*.

Microarray experiments performed in our laboratory on $\gamma Cop^{14a}/\gamma Cop^{14a}$ males revealed that γCop^{14a} is 2.45-folds overexpressed relative to the wild-type allele (GEO/NCBI accession number GSE80084), a result very similar with those obtained by qRT-PCR. Moreover, the overexpression of γCop^{14a} is detected even when $\gamma Cop^{14a}/\gamma Cop^{14a}$ and *Oregon* males are comparatively analysed consecutive to experimental infection with *Pseudomonas aeruginosa*. Although the overexpression of a hypomorphic allele is uncommon, a similar situation was reported for the hypomorphic allele of *FRIGIDA* gene of

Arabidopsis thaliana, involved in the regulation of flowering (FENG & al. [14]).

Using the RNAfold web server, we performed a bioinformatics analysis intended to assess the impact of the specific *P{lacW}* remnants over the secondary structure and MFE of 5'UTRs of γCop^{11a}, γCop^{14a}, γCop^{16b} relative to the wild-type allele. We noticed that the folding ΔG values of the mutant alleles are considerably lower than the folding ΔG value of wild-type *γCop*, therefore their packing is more stable and they could be more probable targets for specific degradation (RINGNER and KROUGH [11]). Interestingly, studies performed on yeast (DORI-BACHASH & al. [15]), as well as on mice (SUN & al. [16]), demonstrated that there is a cis-coupling between elevated transcripts degradation and higher levels of transcription, determined by mutations in the untranslated gene regions. This adaptive mechanism could explain the apparent contradiction unveiled by our study, where functional hypomorphic alleles are transcriptionally overexpressed. The specific molecular remnants present in the 5'UTRs of γCop^{11a}, γCop^{14a}, and γCop^{16b} alleles may determine a biased degradation of the specific transcripts, which in turn could signal for a compensatory enhancement of the transcription rate. Additionally, the mRNA secondary structures such as hairpins, particularly those located at the 5' end of the transcript and upstream to the AUG codon, may impair the rates of translation. Depending on the location and folding stability of the hairpin structures, the mRNA association to ribosomal small subunits can be severely compromised and affects the translation processes (KOZAK [17]; STUDER and JOSEPH [18]). The effects of mRNA secondary structures over translation have been reported in bacteria (STUDER and JOSEPH [18]), maize (WANG and WESSLER [19]), *D. melanogaster* (HESS and DUNCAN [20]), and mammalian cells (KOZAK [17]).

Conclusions

Our study was focused on γCop^{11a}, γCop^{14a} or γCop^{16b} hypomorphic alleles of *γCop*, a gene affecting the fertility phenotype of *D. melanogaster*. Their qRT-PCR profiles are unexpected, since all of these hypomorphic alleles are overexpressed relative to wild-type allele. Bioinformatics analysis revealed that different short molecular remnants of transposon *P{lacW}* are present in the mutant 5'UTRs. The molecular footprints significantly decrease the folding ΔG values of the mutant 5'UTRs, which probably account for the overexpression pattern of γCop^{11a}, γCop^{14a} and γCop^{16b} alleles. The experimental results suggest that care should be taken when hypomorphs are considered by default as transcriptionally downregulated alleles. Based on our genetic analysis and molecular data, we suggest that *γCop* is also a paternal effect gene in *D. melanogaster*.

Acknowledgements

This work was financially supported by the Romania's CNCSIS-A project, No. 279/2003, CNCSIS-A project, No. 1675/2007, CNCSIS-Ideas project, No. 147/2007, and CNCSIS-Ideas project, No. 154/2011.

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