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ORIGINAL PAPER

Effects of heat and UV radiation on the mobilization of transposon *mariner-Mos1*

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Abstract There are many complex interactions between transposable elements (TEs) and host genomes. Environmental changes that induce stressful conditions help to contribute for increasing complexity of these interactions. The transposon *mariner-Mos1* increases its mobilization under mild heat stress. It has putative heat shock elements (HSEs), which are probably activated by heat shock factors (HSFs). Ultraviolet radiation (UVC) is a stressor that has been suggested as able to activate heat shock protein genes (*Hsp*). In this study, we test the hypothesis that if UVC induces *Hsp* expression, as heat does, it could also promote *mariner-Mos1* transposition and mobilization. The *Drosophila simulans white-peach* is a mutant lineage that indicates the *mariner-Mos1* transposition phenotypically through the formation of mosaic eyes. This lineage was exposed to UVC or mild heat stress (28 °C) in order to evaluate the induction of *mariner-Mos1* expression by RT-qPCR, as well as the *mariner-Mos1* mobilization activity based on the count number of red spots in the eyes. The effects of both treatments on the developmental time of flies and cell cycle progression were also investigated. Both the analysis of eyes and *mariner-Mos1* gene expression indicate that UVC radiation has no effect in *mariner-Mos1* transposition, although heat increases the expression and mobilization of this TE soon after the treatment. However, the expression of *Hsp70* gene increased after 24 h of UVC exposure, suggesting different pathway of activation. These results showed that heat promotes *mariner-Mos1* mobilization, although UVC does not induce the expression or mobilization of this TE.

Keywords Transposable elements · Mechanisms of transposition · *Drosophila simulans white-peach* · Temperature · UV radiation

Introduction

Transposable elements (TEs) are DNA sequences with the ability to move from one chromosomal location to another in the genome. They are widely distributed among the majority of studied organisms and can correspond to a greater portion of their genomes, as much as 45 % in humans or 50 to 90 % in certain grass genomes (Feschotte and Pritham 2007; Pritham 2009). TEs show huge sequence diversity and are classified in a taxonomic system based on their transposition mechanism (Wicker et al. 2007).

The biological consequence of TE mobilization is their mutagenic effect, ranging from small nucleotide changes to chromosome rearrangements and epigenetic modifications. In general, TE mobilization is detrimental, but from an evolutionary perspective, they are a formidable resource of genetic variability to feed evolution (Hua-Van et al. 2011). To minimize the unfavorable effects of TEs, their "host" genomes developed mechanisms such as RNAi and epigenetic silencing through DNA methylation or heterochromatinization (Yamanaka et al. 2013; Creasey et al. 2014). Many TEs mobilize only in germ cells, and for a long time, this was the main

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focus of TE research. However, evidence on the importance of somatic mobilization is now accumulating (Kazazian 2011), indicating its involvement in genomic instability related to cancer (Helman et al. 2014), aging (De Cecco et al. 2013), and neurodegenerative diseases (Li et al. 2013).

Stressors act as activators of transposition of many TEs (Capy et al. 2000; Guerreiro 2011). The *mariner-Mos1* transposon is a good genetic tool to investigate how a transposition mechanism is induced under stressful conditions (Guerreiro 2011). Chakrani et al. (1993) have shown the effect of increased temperature upon *mariner-Mos1* mobilization experimentally. This study also compared the 5′ terminal inverted repeats (TIR) sequence of *mariner-Mos1* with the promoter sequences of four heat shock protein (*Hsp*) genes, finding homology among them. A homology of 57 % was found between a 14-bp stretch of *mariner-Mos1* and the *Hsp70* gene. These observations suggest that *mariner-Mos1* TIR contains functional heat shock elements (HSEs) activated by heat shock factors (HSFs). The heat shock proteins (HSPs) are the best characterized and conserved set of polypeptides that respond to thermal stress (Lindquist and Craig 1988). The HSPs are also implicated in the cell cycle regulation, in resistance to stress-induced apoptosis or necrotic cell death, and in antioxidative defense (Helmbrecht et al. 2000; Takayama et al. 2003; Mosser et al. 1997; Buzzard et al. 1998).

UV radiation is a stressor able to increase *Hsp* gene expression in human skin cells and fish tissues during embryonic stages (Trautinger 2001; Vehniäinen et al. 2012). Furthermore, UVC promoted transposition and excision of the *Tc1/mariner* superfamily of fungus *Aspergillus oryzae* (Ogasawara et al. 2009). UVC radiation damages DNA molecules, which is the major cellular chromophore of UVC light. This absorption generates lesions known as DNA photoproducts, in which the most common are cyclobutane pyrimidine dimers (CPDs) and (6–4) pyrimidine-pyrimidone photoproducts (6–4PPs) (Ravanat et al. 2001; Schuch et al. 2013). The presence of these lesions drastically alters the metabolic processes in DNA, since they represent a physical block to both the replication and transcription machinery (De Santis et al. 2002; Costa et al. 2003). As a result, a cell cycle arrest in the G1 phase is observed because the cells are unable to progress through S phase, thus triggering cell death (Ortolan and Menck 2013).

Considering that *mariner-Mos1* element (i) is activated by heat and (ii) has putative HSE and that the UVC radiation can induce cell stress, in this study, we tested the hypotheses that heat and UVC would increase *mariner-Mos1* expression and that UVC could induce *mariner-Mos1* transposition, as heat does. For testing these hypotheses, we used *Drosophila simulans* strains containing a specific mutation called *white-peach*.

The *D. simulans white-peach* strain is an interesting model for investigating the transposition mechanism because it is an excellent system allowing to quantify the somatic mobilization and is easily manipulated in vivo (Medhora et al. 1991). The *white-peach* strain has a defective copy of *mariner-Mos1* inserted in the promoter region of the *white* gene. The *white* gene encodes an enzyme involved in the production of red pigmentation in the eyes of wild flies. This insertion results in the eye color becoming *white-peach* instead of red (Jacobson and Hartl 1985; Jacobson et al. 1986). Furthermore, this defective copy of *mariner-Mos1* (nonautonomous) can be mobilized *in trans* by the transposase enzyme synthesized from an autonomous copy of *mariner-Mos1*. This transposition occurs during the eyes' development, and the *mariner-Mos1* mobilization generates a new phenotype: *white-peach* eyes with red spots (mosaic) (Capy et al. 1992).

Therefore, we investigated whether UVC and the mild heat stress would induce *Hsp* gene expression in the lineage *D. simulans white-peach*. Moreover, if UVC can induce *Hsp* genes, it is possible that it could also promote *mariner-Mos1* activation, transcription, and mobilization, since this element has a putative promoter sequence homologous to the *Hsp* promoter.

Materials and methods

Drosophila strains

In this study, we used a *D. simulans white-peach* isoline called *Dswp test*, which has active copies of the *mariner-Mos1* element. This isoline was produced by crossing a *D. simulans white-peach* female (which has no active *mariner-Mos1*) (Bryan et al. 1990) with wild-type *D. simulans* males collected in Brasília, Brazil (which has active *mariner-Mos1* elements). Furthermore, in the F2 generation, an isoline was established expressing the mosaic eye phenotype. This isoline presents a basal rate of *mariner-Mos1* activity, which was confirmed by the presence of red spots in the eyes and one active copy of *mariner-Mos1* in the genome, as estimated by qPCR. For the experimental procedures performed in this work, we chose the second larvae instar since the first is very sensitive to manipulation (influencing larval survival) and the third is too late to evaluate the transposition activity with reliable fidelity.

Estimation of transposition rate

To estimate the *mariner-Mos1* transposition rate under variable stress conditions, we quantified the red spots in the mosaic eyes of adult flies that were submitted to stress during the second larval stage. Each spot is interpreted as one transposition event of *mariner-Mos1*, and the individuals were classified in different levels related to the number of observed spots: level 0=without spots, level 1=one to four spots, level 2=five to ten spots, and level 3=more than ten spots (Chakrani et al.

1993; Jardim and Loreto 2011). Twenty-five larvae were submitted to each stress treatment, in three replicates, for three independent experiments. The same number of control larvae was maintained at 20 °C, and the adults were quantified for spots in their eyes.

Exposure to UVC radiation

In order to estimate a sub-lethal dose and perform the other procedures with UVC, first, a larvae group was submitted to different UVC doses one under in which the survival rate was similar to control. The larvae were irradiated with 10, 25, 50, 75, and 100 J/m^2 generated by a UVC germicide lamp (Sanyo G-light, 15 W). The UVC measurements were performed with a portable radiometer (EKO UV Monitor MS-211-1, Japan). For this procedure, groups of 25 second-instar larvae were collected manually and exposed to UVC light in Petri dishes containing 200 μl of phosphate-buffered saline (1× PBS).

With the purpose of evaluating if UVC is able to activate *mariner-Mos1* transposase transcription or induce *mariner-Mos1* mobilization, groups of larvae were irradiated with the chosen UVC sub-lethal dose and were submitted to different manipulations and analyses (three independent experiments): (i) a larvae group was maintained in culture medium until becoming adult flies in order to analyze the developmental time and carry out the phenotypic analysis; (ii) the total messenger RNA (mRNA) was extracted from other larvae groups, 6 and 24 h after stress, to analyze the expression of *mariner-Mos1*, *Hsp*70, and reference genes, by RT-qPCR; and (iii) the third group had the cells dissociated out of larvae tissues and submitted to flow cytometry, 48 h after the UVC irradiation. The control group samples to all procedures were manipulated in the same manner, but the UV lamps were not turned on. These results were statistically discriminated by one-way ANOVA followed by Dunnett's test.

Mild heat stress

The temperature of 28 °C was used as a mild heat stress. This temperature was used as a stressor agent in a previous work (Jardim and Loreto 2011). A group of 25 second-instar larvae was maintained in culture medium while another group was kept at 28 °C, until becoming adult flies in the sense of analyzing the developmental time and carrying out phenotypic analysis. For gene expression analyses, other larvae groups were submitted at 28 °C for different periods of time: 6 or 24 h. The molecular procedures, mRNA extraction, were performed soon after treatment. Twenty-five larvae were collected for both treatments (three independent experiments). For flow cytometry, other larvae groups were maintained at 28 °C for 48 h and the cells dissociated out of larvae tissues. The control group samples to all procedures were manipulated in the same manner, but the temperature was maintained at 20 °C. These results were statistically discriminated by one-way ANOVA followed by Dunnett's test.

Estimation of *mariner-Mos1* copy number

For the estimation of *mariner-Mos1* copy number in the *Dswp-test* strain, qPCR of a unique copy reference gene (Ribosomal protein L17-RPL17) was performed and compared with the *mariner-Mos1* amplification. DNA from 20 flies was extracted individually using the protocol described in Oliveira et al. (2009), quantified in a NanoDrop 2000 spectrophotometer (Thermo Scientific, USA), and diluted to a concentration of 3 ng/μl. Each qPCR reaction was performed in a 20-μl volume containing 20 pmol of each *primer*, 5 mM of dNTPs, 1× PCR buffer, 2.5 mM $MgCl_2$, 1× SYBR Green (Molecular Probes, USA), 0.5 U of Platinum Taq DNA polymerase (Invitrogen, USA), and 15 ng of DNA. The primers used are described in Supplementary Table 1. The *mariner-Mos1* copy number was estimated by the $2^{-\Delta\Delta Ct}$ method (Livak and Schmittgen 2001) using the Ct values obtained in the ECO™ Real-Time PCR System (Illumina, USA). The estimated *mariner-Mos1* copy number in the *Dswp-test* strain was 1.84 (±0.83) copies. As all individuals have a copy of the inactive *peach mariner-Mos1* in the *white* gene, we can conclude that in this strain, the majority of individuals have one copy of an active element and a few have either none or two copies.

RNA extraction and cDNA synthesis

Total RNA was extracted from larvae with TRIzol® reagent (Invitrogen, CA, USA). The quality of the RNA samples was assessed by 1 % agarose gel electrophoresis and quantified using a NanoDrop 2000 spectrophotometer (Thermo Scientific, USA). Afterward, the samples were treated with DNaseI (Promega, USA) to eliminate possible DNA contamination. The complementary DNA (cDNA) synthesis was performed with M-MLV reverse transcriptase enzyme (Invitrogen, CA, USA) and oligo-dT primers.

mariner-Mos1 gene expression

To evaluate the putative activation of transcriptions of *mariner-Mos1* transposase gene by stress, the *Hsp70* gene was used as a positive marker, since this gene is expressed under different stressful conditions (Sørensen et al. 2005; Trautinger 2001). We used the RT-qPCR method, performed on a StepOnePlus™ Real-Time PCR System (Applied Biosystems, USA). The reaction was carried out in a final volume of 20 μl containing 10 μl of diluted cDNA (1:100), 0.25 U Platinum Taq DNA Polymerase (Invitrogen, CA, USA), 1× PCR reaction buffer, 3 mM $MgCl_2$, 25 μM dNTPs,

0.2 μM of each reverse and forward primer, and 1× SYBR Green (Molecular Probes, USA).

The RT-qPCR amplification parameters were as follows: 95 °C for 7 min, followed by 40 cycles of 94 °C for 15 s, 60 °C for 15 s, and 72 °C for 20 s. Normalization input was performed with reference genes (GPDH, RPL17q2, and EF1). The primer sequences are described in Supplementary Table 1. The *Hsp70* primers amplify only the *Hsp70* genes Aa and Ba and do not anneal in their cognates. The relative gene expression was based on $2^{-\Delta\Delta cq}$ (Livak and Schmittgen 2001) using the Cq values. Furthermore, the efficiency of the PCR reactions was calculated using LinRegPCR (Ruijter et al. 2009) software and varied between 1.85 and 1.91 (±0.03). The relative expression indices were compared at each treatment time (6 and 24 h) with the respective control using one-way ANOVA followed by Dunnett's test.

Cell cycle analyses

To carry out the cell cycle analysis, the larvae of second instar were treated with UVC light (25 J/m^2) and maintained for 48 h at 20 °C or heat-shocked (28 °C for 48 h). As control, other larvae were maintained for the whole time at 20 °C. After each treatment, the samples were washed with 10 % bleach for 1 min and rinsed three times with 1× PBS. Then, 30 larvae were stretched out using tweezers and their internal contents were removed and maintained in 1× PBS solution supplemented with 10 % fetal bovine serum (FBS). Next, the tissue was broken up as much as possible by pipetting it up and down for 5 min. Afterward, the samples were centrifuged for 10 min at 1500 rpm. The supernatant was discarded, and 100 μl of trypsin/EDTA solution 250 mg/l was added for 5 min. Subsequently, 1 ml of 1× PBS (10 % FBS) solution was added to inactivate the trypsin and the suspension was centrifuged again. The cell pellet was fixed with 70 % ethanol and maintained at −20 °C. The dissociated cells were resuspended in 500 μl of 1× PBS solution, and after 5 min of decantation, the supernatant was transferred to a new tube (this step is required to clean the sample in order to avoid obstruction of the cytometer). Then, 200 μl of a solution containing 200 μg/ml of RNase A (Macherey-Nagel, Germany), 20 μg/ml of propidium iodide, 0.1 % Triton X-100, and 1× PBS was added in the supernatant. The samples were submitted to flow cytometry analysis in a BD Accuri C6 cytometer (BD Biosciences USA). Statistical analysis was performed by one-way ANOVA followed by Dunnett's test.

Results

Determination of sub-lethal UVC dose to be applied

To evaluate if the damage caused by UVC promotes transposition of the *mariner-Mos1* element, we firstly determined a sub-lethal dose to be applied in all other procedures performed in this work. As can be seen in Fig. 1, a UVC dose of 25 J/m^2 showed a mean survival rate similar to that observed in control and with a dose of 10 J/m^2. However, exposure to higher UVC doses (50, 75, and 100 J/m^2) decreased the survival rate considerably, as expected.

mariner-Mos1 gene expression under mild heat and UVC stress

Here, we tested if UVC and mild heat stress could increase the transcriptions of *mariner-Mos1*. A significant increase in the expression of *Hsp70* occurred when the larvae were exposed to 28 °C for 6 and 24 h (Fig. 2). This corresponded to about an eightfold increase in comparison to the control larvae ($p<0.05$). Curiously, for the UV treatment, the increased expression of *Hsp70* only occurred 24 h after irradiation ($p<0.05$). With regard to the *mariner-Mos1* transposase gene, this result suggests that 28 °C induces a rapid increase in *mariner-Mos1* expression, since its expression level after 6 h of incubation at 28 °C was higher in comparison with control and remains high for the next 24 h. The relative expression of *mariner-Mos1* at 28 °C was still about threefold higher than that observed in the control samples (20 °C) ($p<0.05$). On the other hand, after the UV treatment, the *mariner-Mos1* expression was basal and similar to that observed in the control, regardless of the time of analysis. This result clearly shows that UVC did not induce transcription of the *mariner-Mos1* transposase gene, although it can activate expression of the *Hsp70* gene as a late response.

mariner-Mos1 transposition rate under mild heat and UVC stress

Phenotypic analysis of the adult flies' eyes was performed after the larvae treatment with UV dose of 25 J/m^2 and maintained at 20 °C or by keeping the larvae at 28 °C, until becoming adult flies. The estimation of *mariner-Mos1* transposition frequency was achieved by quantifying the red spots in the eyes (Fig. 3). UVC-treated flies showed similar numbers of red spots as observed in nonirradiated control samples (0 and 1–4, respectively), suggesting that UVC does not induce *mariner-Mos1* transposition, because the mosaic phenotype could not be observed. However, when the larvae were submitted to the mild heat treatment at 28 °C, the result was the opposite. Most of the individuals were distributed between 5–10 (32 %) and +10 (60 %) spot levels. These data showed that heat was able to increase the *mariner-Mos1* mobilization

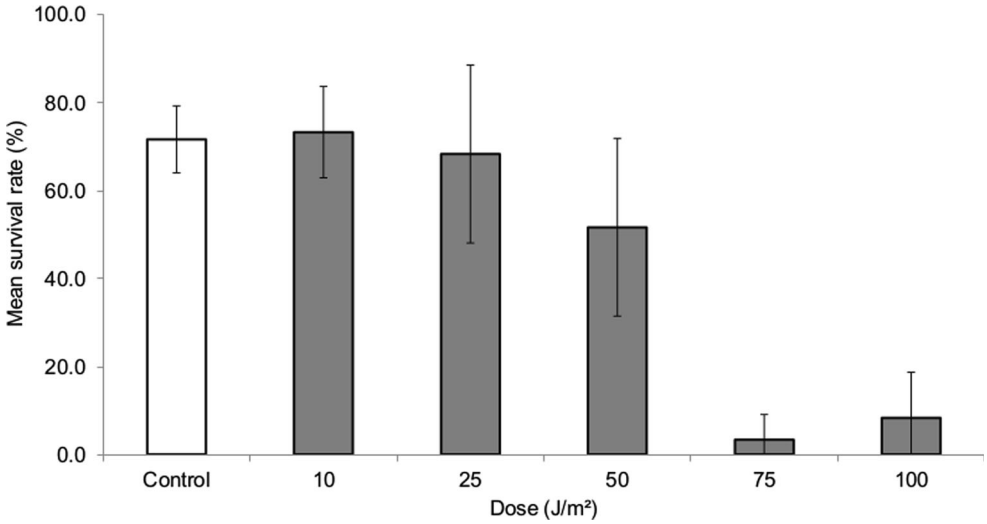

Fig. 1 Survival rate of larvae after UVC treatments. Average survival rate (percentage) and standard deviation from three independent experiments. The larvae of second instar were treated with different doses of UVC. The dose of 25 J/m^2 presented a survival superior to 50 % and was chosen to perform the other procedures with UVC

frequency in the *Dswp-test* strain, despite UVC failed to activate it.

Developmental time and cell cycle progression after stress conditions

During the phenotypic analysis, it was observed that the developmental time of treated samples was different in relation to the control ones. Then, we registered the days that second-instar larvae took to become adult flies. The larvae that were maintained at 20 °C required 10–14 days to complete their development, whereas those kept at 28 °C completed it in only 8 days. In contrast, UVC treatment resulted in delayed development, since UV-exposed larvae took 15 to 17 days to become adult flies.

Flow cytometry analyses were then performed to investigate if the effects caused by these stressors on the developmental time could be related to cell cycle progression. For this purpose, larvae that were previously treated with UVC or mild heat stress for 48 h had their cells separated to be submitted to flow cytometry. The proportion of cells in each phase of cell cycle is shown in Fig. 4. The cell proportion in the UV+20 °C was bigger in the phases G1/S than control sample, indicating an arrest as consequence of UV-induced DNA damage. On the other hand, the mild heat stress (28 °C) resulted in an accumulation of cells in the G2/M.

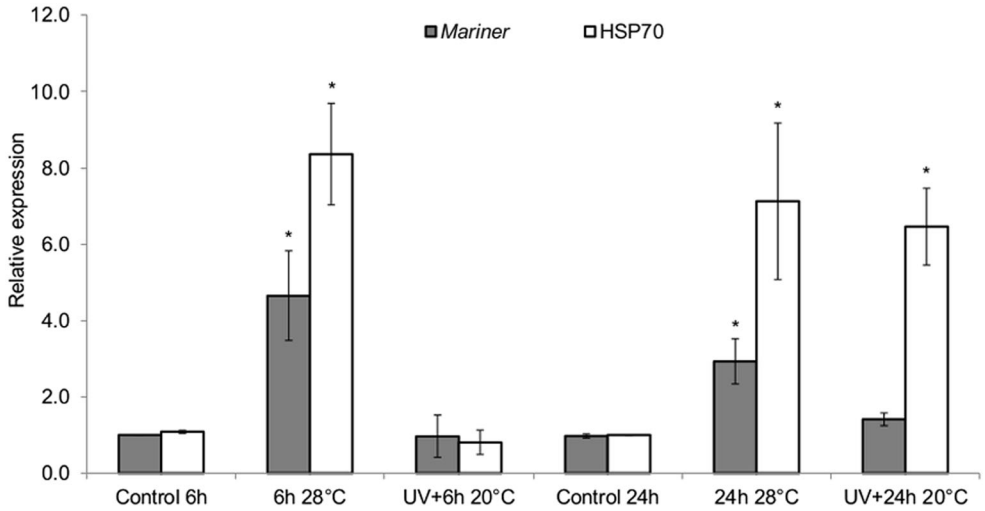

Fig. 2 Relative expression of *mariner-Mos1* and *Hsp70* genes after heat and UVC treatments. Average and standard deviation from three independent experiments. The input normalization was performed with the reference genes GPDH, RPL17q2, and EF1 genes. The larvae (second instar) were maintained at 28 °C or irradiated (25 J/m^2) and maintained at 20 °C for 6 or 24 h. After RNA extraction, cDNA and expression protocols were performed. One-way ANOVA followed by Dunnett's test, which compare the treatments with the respective control ($p<0.05$)

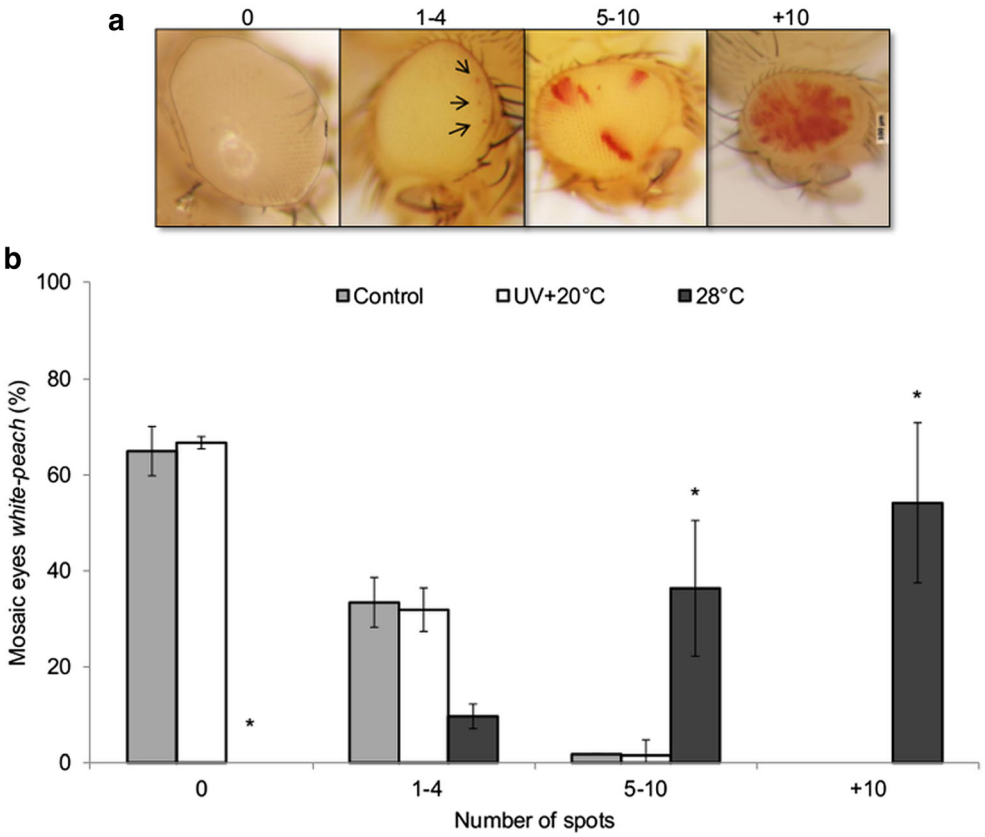

Fig. 3 Frequency of red spots in the eyes of adult flies after treatments. **a** Eyes with and without spots were classified into levels related to the number of red spots: level 0=without spots, level 1=one to four spots, level 2=five to ten spots, and level 3=more than ten spots. **b** Average percentage of each level of mosaic eyes and standard deviation from three independent experiments. The larvae (second instar) were maintained at 28 °C or irradiated (25 J/m^2) and maintained at 20 °C until becoming adult flies. One-way ANOVA followed by Dunnett's test, which compare the treatments with the respective control ($p<0.05$)

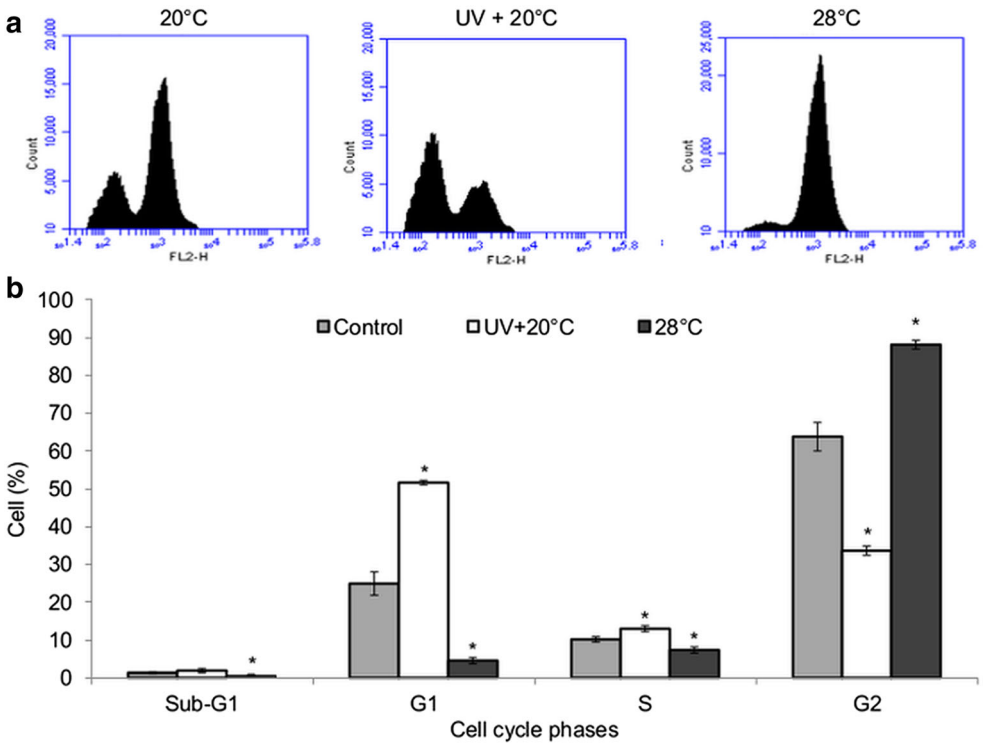

Fig. 4 Effect of mild heat and UVC radiation on cell cycle progression. **a** Histogram generated by a BD Accuri C6 cytometer. **b** Average percentage and standard deviation from three independent experiments showing the proportion of cells in the sub-G1, G1, S, and G2 phases after each treatment. One-way ANOVA followed by Dunnett's test, which compare the treatments with the respective control ($p<0.05$). The larvae (second instar) were maintained at 28 °C or irradiated (25 J/m^2) and maintained at 20 °C for 48 h; after, the cell dissociation protocol was performed and the cells were analyzed

Discussion

As TEs represent a substantial portion of many genomes, with many having potentially active elements, an open question that remains unanswered in functional genomics is how the mechanisms of silencing and activation of TEs work (Hua-Van et al. 2011). Activation by stress factors possesses a special relevance, mainly in somatic mobilization of TEs, since it can be source of deleterious mutations related to cancer, aging, and neurodegenerative diseases (Kazazian 2011). However, not only detrimental effects have been suggested for somatic mobilization of TEs. Some evidence suggests that this process could be involved in phenotypic plasticity (Micale et al. 2012; Iyengar et al. 2014). In the present study, our main focus was to investigate the action of two different stressors on the activation mechanism for somatic mobilization of the *mariner-Mos1* element.

In vivo testing using strains with autonomous TEs is sometimes problematic due to the mobile nature of these sequences, which can increase or decrease the copy number during the experimental period, thus interfering with the interpretation of the results. Therefore, we constructed a strain containing only one copy of the active element. As suggested by our qPCR copy number estimation, our strain presents many individuals with a single active copy of *mariner-Mos1*, but some polymorphism is still maintained. However, this polymorphism did not interfere with the results since the variations observed between replicates were small.

It is already known that heat shock activates the transposition of *copia*-like retrotransposons and *412* in *Drosophila* genomes (Junakovic et al. 1986; Strand and McDonald 1985; Ratner et al. 1992; Vasilyeva et al. 1999). More recently, it was reported that the retrotransposon MAGGY was induced by heat shock in *Magnaporthe grisea* and *Magnaporthe oryzae* (Chadha and Sharma 2014; Ikeda et al. 2001). For the *mariner-Mos1* element, Giraud and Capy (1996) have shown that temperature is involved in the regulation of somatic transposition in natural populations of *D. simulans*, and Chakrani et al. (1993) showed similar results in a controlled laboratory experiment. In addition, it was demonstrated that *mariner-Mos1* has a sequence homologous to the *hsp* promoters, thus suggesting a possible co-activation of both *mariner-Mos1 and hsp* genes possibly by HSF.

Some studies have indicated that UV radiation can mobilize TEs (Kuan et al. 1991; Eichenbaum and Livneh 1998; Qüesta et al. 2010, 2013; Myakishev et al. 2008; Morales et al 2003). The only reported mobilization in the *Tc1/mariner* superfamily is that of Ogasawara et al. (2009) for the fungus *A. oryzae.* In addition, it has already been demonstrated that UV is able to increase *Hsp* expression in human skin cells (Trautinger 2001), in fish (Vehniäinen et al. 2012), and in sea urchin (Bonaventura et al. 2006), thereby suggesting a possible activation of *mariner-Mos1* mobilization as well.

In this work, we tested the hypothesis that *mariner-Mos1* could be co-activated with *Hsp70* genes as a result of the stress caused by mild heat and UVC radiation. Corroborating the previous studies, the results presented in this work reinforce the activation of *mariner-Mos1* by mild heat, showing that the transposase is upregulated at 28 °C (Fig. 2). In addition, the *Hsp70* gene is also upregulated at 28 °C (Fig. 2) and the frequency of *mariner-Mos1* mobilization is high under this condition (Fig. 3). *Hsp* genes have conserved *cis* sequences called heat shock elements (HSEs). We have reanalyzed the 5′UTR region of *mariner-Mos1* element and found a new HSE in addition to that previously described by Chakrani et al. (1993) (Supplementary Figs. 1S and 2S). Together, these results suggest that *mariner* can be co-activated with *Hsp* genes, possibly due to the action of heat shock factor (HSFs).

On the other hand, although UVC activated the *Hsp70* gene, it did not induce activation of the *mariner-Mos1* transposase (Fig. 2). Furthermore, flies subjected to UVC treatment showed similar numbers of red spots in the eyes of adult flies as observed in the nonirradiated control (Fig. 3), confirming that it indeed did not induce *mariner-Mos1* transposition. One aspect that should be highlighted is that the pattern of *Hsp70* activation was different between the mild heat stress and UVC exposure. With mild heat stress, the activation was rapid (6 h) after the treatment, whereas it occurred much later (24 h) after UVC exposition. It is possible that UV-treated cells need to arrive at this time point before activation of the *Hsp70* gene can occur, using a different pathway of activation from the one that is used by *mariner-Mos1*. Although mammals and plants have four different genes for heat shock factors (HSFs), invertebrates have only one (Åkerfelt et al. 2010). However, even having only one HSF, in invertebrates, this factor can interact with wide range of other biotic and abiotic factors. So, heat and UV could induce differently HSF to activate *hsp70* and *mariner-Mos1*.

Additionally, it is already known that mild heat accelerates cell cycle progression, thus facilitating cell growth and differentiation (Park et al. 2005). In contrast, UVC induces a blockage of the DNA replication fork and causes an arrest in cell cycle progression (Song 2005). As shown by the cell cycle and developmental time analyses, UV promotes an arrest in the cell cycle in the G1 phase (Fig. 4), possibly due to the generation of DNA lesions that need to be repaired and the developmental time that is delayed. On the other hand, 28 °C resulted in an accumulation of cells in the G2 phase and it accelerated the developmental time of the treated flies.

We can conclude that mild heat promotes somatic mobilization of the *mariner-Mos1* transposon, increasing transcription of the transposase gene, while UV radiation promotes neither this transcription nor this mobilization. Both stressors activate the *Hsp70* gene, but with different patterns.

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