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CHROMATIN STRUCTURE OF TELOMERIC piRNA CLUSTERS IN DROSOPHILA GERMLINE

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Telomeric and subtelomeric regions contain potent Piwi interacting RNA (piRNA)-producing regions in *Drosophila melanogaster*. The telomeres of *Drosophila* are maintained as a result of the retrotransposition of specialized telomeric non-long terminal repeat retrotransposons, *TART, HeT-A* and *TAHRE*. Telomeric piRNAs were shown to be involved in the telomere length control and assembly of the telomere protection complex in the *Drosophila* germline. It is still unclear whether all telomeric sequences are equally efficient in the piRNA production. Chromatin structure of telomeric piRNA clusters located at different chromosomes was not yet analyzed. Transgenic constructs inserted in different sites of telomeric regions were chosen as the unique marks, which allowed us to monitor piRNA production from the repetitive telomeric sequences.

Ovarian small RNA library sequencing and their subsequent bioinformatic analysis have shown that transgenes inserted in retrotransposon arrays of 2nd and 3^{d} chromosomes produce predominantly piRNAs whereas transgene located at the telomere of 4th chromosome produces mainly siRNAs in *Drosophila* ovaries. Chromatin immunoprecipitation analysis (ChIP) revealed accumulation of trimethylated histone H3 lysine 9 (H3K9me3) at all studied telomeric transgenes irrespective of their location within telomeres. This chromatin mark is recognized by heterochromatic protein 1 (HP1) and its homologues. The germline-specific HP1 paralog Rhino is enriched at transgenes generating mainly piRNAs. These transgenes are located within retrotransposon array or telomere associated region (TAS). HP1 was shown to be enriched at siRNA-producing telomeric regions, such as a transegene located at chromosome 4. We suggest that the 4th chromosome embedded within HP1-enriched chromocentre provides conditions for the establishment of siRNA production at telomeres. Finally, we observed that insulator of *gypsy* transposon (Su(Hw)) located within telomeric transgenes prevents piRNA production from the locus suggesting that insulator proteins can disrupt transcription of piRNA clusters.

B CHROMOSOMES IN *APODEMUS FLAVICOLLIS*

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In genus *Apodemus*, the presence of B chromosome has been confirmed in six out of twenty-two species. One of them is yellow-necked field mouse *Apodemus flavicollis.* The presence of B chromosomes has been confirmed in almost all studied populations of *A. flavicollis*

in Serbia in a wide range of frequencies (0,07- 0,63). Five B chromosomes per animal was the highest number of Bs recorded in our analyzed populations.

These extra chromosomes in *A. flavicollis* are euchromatic and acrocentric, and by size and distribution of G- and C-bands can be sorted into the group of five smallest chromosomes in karyotype.

The absence of Bs accumulation in male meiosis, no significant difference in the mean number of scars and embryos between females with and without Bs, as well as the absence of significant difference in the presence of Bs among six age categories, speaks in favor of heterotic model of their maintenance, contrary to the model of parasitic behavior of these additional genomic elements.

In general, the number of Bs carriers is higher in environment that is not optimal for this species which indicates that those individuals have higher fitness. Seasonal variation of Bs frequencies has been recorded, in such a manner that highest frequencies are present before and after winter, with stable frequency fluctuations during successive years. Different frequency of Bs carriers is related to adaptive differentiation to diverse habitats mediated by environmental variables that directly and/or indirectly influence population dynamics of *A. flavicollis*. Changes in frequency follow up increase in population density in circumstances without food and space competition. Positive correlation between the frequency of Bs carriers and the average number of sub zero days, as well as with altitude increase, has been recorded.

Considering phenotypic effects, higher level of morphological integration of mandible in animals with Bs in *A. flavicollis* has been found. Furthermore, studies of the effects of Bs on nonmetric cranial traits, as well as on three components of cranial variability confirmed that Bs does not disturb developmental homeostasis in their carriers. Moreover, Bs play a significant role in structuring cranial variation.

Negative correlation between the expression of Tgf-β gene and the presence of Bs was also observed. This could be of great importance for B chromosome which has to pass through different mitotic and meiotic check points, and Tgf-β gene, through cell cycle regulation, influences development and homeostasis.

The existence of specific DNA profiles and differential expressions of three genes in the presence of Bs, testify about interaction of Bs with the rest of the genome. Absence of C-KIT exon 1 in more than one copy in specimens with 1, 2 and 3B chromosomes excludes the possibility that this gene is present on Bs. On the other hand, presence of S18 gene copy has been indicated on some Bs.

Considering the results of population studies, euchromatic structure of Bs and molecular studies that have been conducted so far, we can say that the contribution of Bs to overall genetic

diversity in this species is in sight, but there is still need for more specific evidence such as precise determination of Bs molecular structure, origin and function.

INT6 IS REQUIRED FOR PROPER MICROTUBULE DYNAMICS AT *DROSOPHILA* KINETOCHORES

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Our past RNAi-based screens identified several mitotic genes inclusive of *int6,* which encodes a component of the translation initiation complex that also interacts with both the 26S proteasome and the COP9 signalosome. Previous studies have shown that *Int6* is a proto-oncogene implicated in various types of cancer, but did not define its precise biological activity. We found that RNAi-mediated depletion of Int6 in *Drosophila* S2 cells, results in several mitotic defects including particularly short spindles, persistent SAC activity, delayed metaphase-anaphase transition and defective chromosome segregation. FRAP analysis suggested that Int6 enhances the turnover of microtubule (MT) plus-end at kinetochores without affecting the minus end dynamics at the spindle poles. This altered MT dynamics is due to an accumulation at kinetochores of Klp67A, a *Drosophila* kinesin-8 protein with MT-depolymerizing activity. In *int6* RNAi cells, Klp67A is less ubiquitinylated than in control cells, suggesting the Int6 controls ubiquitin-mediated degradation of Klp67A at kinetochores. Consistent with these results, we found that Klp67A overexpression in S2 cells mimics the *Int6* loss-of-function phenotype, although with milder effects. Collectively, our results suggest that Int6 mediates proper Klp67A turnover during *Drosophila* mitosis; it remains to be established whether Int6 regulates the turnover of other mitotic proteins. We also addressed the mitotic function of the highly conserved Int6 homologue in human cells. RNAi-mediated depletion of INT6/EIF3E in HeLa cells led to frequent multipolar spindles and massive chromosome missegregation. We are now exploring the possibility that these phenotypes are caused by failure to degrade mitotic proteins, particularly KIF18A, the human homologue of Klp67A.

CHROMOSOME MONO-ORIENTATION: MECHANISM AND RAMIFICATIONS

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I will illustrate, using high resolution time-lapse video phase-contrast and DIC light microscopy, the mono-orientation of chromosomes during the early stages of spindle assembly in