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A ROLE FOR THE *DROSOPHILA* HISTONE VARIANT H2AV IN MITOTIC CHROMOSOME SEGREGATION

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We found that mutations in the H2Av gene, which encodes the Drosophila H2A variant, impair chromatin compaction of pericentric chromosome regions and lead to irregular chromosome segregation during mitotic divisions of larval neuroblasts. Immunostaining with tubulin, DNA and centrosomal proteins revealed that the most straightforward phenotype elicited by H2Av-depleted mutant cells is the presence of apparent anaphase-looking bipolar spindles (20%; n= 125), with chromosomes not connected to the spindle poles by bundles of kinetochore microtubules (MTs). In addition, mutant chromosomes were not able to congress to the equator of the cell spindle, failed to separate in sister chromatids and appeared to segregate randomly to the poles. This was also confirmed by immunolocalization experiments using kinetochore markers such as Cenp-C, Cid and Hec1/Ndc80, which appeared localized on mutant chromosomes always as two twin spots. We have also found that most of the irregular mitotic figures (85%, n= 45) exhibited high levels of Cyclin B with respect to anaphase control cells, in which Cyclin B was almost absent (3%, n= 55). This indicates that all anaphase-looking bipolar spindles with scattered chromosomes are indeed in a metaphase-like status (ana/metaphase-like figures). Consistent with these findings, we observed that checkpoint proteins ZW10 and BubR1 remained strongly localized at centromeres of mutant chromosomes, whereas in wild-type ana/telophase figures both proteins are mostly absent from segregating chromosomes. Moreover, in the mutant ana/metaphase-like figures ZW10 failed to stream towards the cell poles, as occurs in normal metaphases, suggesting that depletion of H2Av causes defective microtubule attachment to the kinetochore. Finally, MT regrowth experiments after cold exposure revealed that mutations in *H2aV* inhibit kinetochore-driven MT growth. As a consequence Dgt6, an Augmin component that plays a pivotal role in K-fibers formation, is strongly reduced in mutant neuroblasts.

Taken together our results suggest that H2Av might be required for the regulation of mitotic chromosome segregation in *D. melanogaster*. Furthermore, our data highlight an unanticipated role of this specific histone variant in controlling the interactions between kinetochore and k-fibers.