Pleitropic effects of H3K9 methylation regulate heterochromatin stability.

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We previously demonstrated that *Drosophila* cells lacking H3K9 methylation and RNAi pathway components displayed disorganized nucleoli, ribosomal DNA (rDNA), and satellite DNAs (Peng and Karpen, NCB, in press). Specifically, altered H3K9 dimethylation in heterochromatin (H3K9me2) due to loss of the HMTase Su(var)3-9 or Dicer-2 resulted in substantial increases in the amounts of extrachromosomal repeated DNAs. Ectopic nucleolus formation was suppressed by mutations in Ligase 4. These findings suggest a mechanism for how local chromatin structure can regulate stability of repeated DNAs and nucleolar organization.

The extensive sequence homology inherent to repeated DNAs could generate translocations and other chromosome aberrations in somatic and/or germ cells, if exchange was not repressed by heterochromatic structures. We have recently shown that somatic and meiotic cells from *Su(var)3-9*^{null} mutants exhibit significantly elevated levels of H2AvS137 phosphorylation (γH2Ax in mammalian cells). H2Av phosphorylation is thought to spread from sites of DNA breaks and to recruit repair pathway components, and TUNEL staining confirmed that loss of *Su(var)3-9* results in a significantly increased frequency of DNA breaks. IF analysis of cell cycle stages showed that *Su(var)3-9* imaginal discs have a 7-fold increase in G2 cells, and a 4-fold increase in mitotic cells. These results suggest that viability of *Su(var)3-9* null mutants is ensured by activation of cell cycle checkpoints and amelioration of chromosome defects. Delays in G2 could occur in response to activation of the ATM/ATR repair checkpoint, allowing efficient repair of the elevated levels of breaks, and mitotic delays could occur due to cohesion defects and activation of the Spindle Assembly Checkpoint (SAC). The Drosophila homolog of ATR is encoded by the *mei41* gene; in the absence of radiation or other sources of DNA damage, homozygous *mei41* mutants are viable. We have observed that combining *Su(var)3-9* and *mei41* mutants results in synthetic lethality, demonstrating that *Su(var)3-9* viability is ensured by the G2 repair checkpoint, consistent with the observed G2 delay/arrest. These preliminary results suggest that *Su(var)3-9* and H3K9 methylation are required to ensure general genome stability, in addition to suppressing eccDNA formation.