SUPPLEMENTAL METHODS

PCR and Southern blot analysis

Genomic DNA was isolated from S2 cells, BG3 cells, and Oregon R flies according to standard protocols (Sambrook and Russell 2001). The borders of four H3K9me2-enriched domains were examined: Chr2L: 14,707,500-14,973,000; Chr2R: 9,480,000 - 9,683,000; Chr3R: 1,689,000 - 1,823,000; and Chr3R: 2,286,000 - 2,472,000. For the PCR assay, fragments of 4kb to 5kb were amplified using KlenTaqLA according to recommendations in (Barnes 2003) with the following primers: Chr2L_14707500 F - tag cca acg ctt cca act ct and R - cac cta ggc acc acc ctc aa; Chr2L_14973000 F - cta gcg acg agg gaa aag tg and R - cga cac ata cag gag cg ga; Chr2R_948000 F - aat taa acg aac acc gcg ac and R - ata cag tgg cca aat cct gc; Chr2R_9683000 F - cag ctc aag tgt cac gga aa and R - ttg tag aat gca gag tgc gg; Chr3R_1689000 F - ttg tga acg gca cac att tt and R - tat tga gcg ccc aga aga gt; Chr3R_1823000 F - gag acg ctg gag acc aga ac and R - cta atc cag aat gcc gag cc; Chr3R_2286000 F - aac atg gcc tac cag ttt cg and R - gtg cct ggt tta tgg agc at; Chr3R_2472000 F - cgt cga taa acg cca aaa at and R - ggg tt tgt ctt cgg tgt gt. For Southern blot analysis, 20µg of genomic DNA from S2 cells, BG3 cells, and Oregon R flies were digested with *Bam*HI, *Eco*RI, and *Hind*III according to the manufacturer's recommendations (Fermentas or New England Biolabs). Standard protocols were used to prepare Southern blots using the alkaline transfer method, to produce radioactive probes by random priming, and to carry out hybridizations and washes (Sambrook and Russell 2001). Signal was detected by a phosphoimager (BIORAD).

Mass spectrometry

For mass spectrometry, Drosophila histones were isolated from S2 cells by acid extraction. The cell nuclei were prepared according to (Risau et al. 1983) and partially depleted of non-histone proteins by sequential extraction with buffered 350mM and 600mM NaCl solutions. The histones were then extracted from the nuclei by solubilization in 0.2M sulfuric acid. Human histones were isolated from HeLa S3 cells cultured in K8R10 SILAC medium according to

(Thomas et al. 2006). Growth in SILAC medium imparts stable isotope labels into all proteins that can later be resolved by mass spectrometry (Ong et al. 2002).

H3K9 methylation

To compare the abundance of H3K9me2 and H3K9me3 in Drosophila and human cells histones from both species were separated by HPLC as described (Thomas et al. 2006). The H3 from the two species were mixed 1:1 as determined by HPLC trace area and H3 was further purified by SDS-PAGE. The H3 band was treated with acetic anhydride to acetylate all non-dimethylated or non-trimethylated lysines (Garcia et al. 2007). The band was subject to tryptic digestion and analyzed on an Orbitrap XL mass spectrometer. Peptides containing to K9me2 and K9me3 from Drosophila (light) and human (heavy) histones were targeted for MS/MS and areas under the curves of extracted ion chromatograms for the peptides from each species were used to determine the ratio of the modifications between humans and drosophila. All data were normalized to an unmodified peptide spanning residues 54-63 of H3. To validate the specificity of anti-H3K27me3 (Ab6002) antibody, the immunoprecipitated and input H3 was purified by SDS-PAGE, in-gel propionylated and digested with trypsin. The H3 K27-40 peptides were analyzed as described above.

H3K27me3 antibody validation

To validate the specificity of anti-H3K27me3 (Ab6002) antibody, acid extracted core Drosophila histones were subjected to immunoprecipitation under conditions employed for ChIP. The input and immunoprecipitated materials were separated by SDS-PAGE and the gel bands corresponding to histone H3 were cut out. After sequential in-gel propionlyation and tryptic digestion the abundance of variously modified species of histone H3 peptide 27-40 in each sample was quantitatively measured by targeted LC-MS/MS. The enrichment of different peptide species after immunoprecipitation was calculated as a ratio between areas under the curves of extracted ion chromatograms for the corresponding peptide species in immunoprecipitated and input materials. The ratios between average enrichment of H3K27me3 species and that of H3K27me1, H3K27me2 and unmodified species are shown.

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