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Data in Brief

Nucleosomal chromatin in the mature sperm of *Drosophila melanogaster*

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ABSTRACT

During spermiogenesis in mammals and many other vertebrate classes, histone-containing nucleosomes are replaced by protamine toroids, which can repackage chromatin at a 10 to 20-fold higher density than in a typical somatic nucleus. However, recent evidence suggests that sperm of many species, including human and mouse retain a small compartment of nucleosomal chromatin, particularly near genes important for embryogenesis. As in mammals, spermiogenesis in the fruit fly, *Drosophila melanogaster* has also been shown to undergo a programmed substitution of nucleosomes with protamine-like proteins. Using chromatin immunoprecipitation (ChIP) and whole-genome tiling array hybridization (ChIP-chip), supported by immunocytochemical evidence, we show that in a manner analogous to nucleosomal chromatin retention in mammalian spermatozoa, distinct domains packaged by the canonical histones H2A, H2B, H3 and H4 are present in the fly sperm nucleus. We also find evidence for the retention of nucleosomes with specific histone H3 trimethylation marks characteristic of chromatin repression (H3K9me3, H3K27me3) and active transcription (H3K36me3).
Raw and processed data from the experiments are available at GEO, accession GSE52165.

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Specifications	
Organism/cell line/tissue	Spermatozoa isolated from *Drosophila melanogaster* strain w1118 transgenic for either H2Av-mRFP1 or protamine-eGFP [1].
Sex	Male
Sequencer or array type	NimbleGen 2.1M *Drosophila melanogaster* whole-genome tiling arrays (Roche 05542308001). Slides were scanned at 5 μm resolution using a GenePix Axon scanner.
Data format	Raw data: ChIP and input DNAs in pair format; processed data: SOFT, MINiML and TXT.
Experimental factors	Experimental: genomic DNA obtained by chromatin immunoprecipitation vs. total genomic DNA; control: genomic DNA obtained by non-specific antibody precipitation vs. total genomic DNA.
Experimental features	Equal quantities of experimental and control DNAs were hybridized to the arrays using total genomic DNA as a reference.
Consent	*NA*
Sample source location	*NA*

1. Direct link to deposited data

http://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE52165.

2. Experimental design, materials and methods

2.1. Fly strains used in this study

Two different transgenic *D. melanogaster* w1118 strains were used; one carrying H2Av tagged with red fluorescent protein (H2Av-mRFP1 [1,2] and the other carrying protamine tagged with green fluorescent protein (protamine-eGFP; [3]).

2.2. Isolation of spermatozoa

To avoid any somatic cell contamination, large, mature, young males with full seminal vesicles were selected as the source of sperm. Males were anaesthetized with CO_2, placed on a clean microscope slide, immersed in a drop of phosphate buffered saline (PBS) and dissected using fine forceps. The accessory glands were separated from the testes, which were then transferred onto a new slide. One end of the seminal vesicle was anchored with forceps and pressure was gently applied with a second pair of forceps to force out the sperm, which were collected as a filamentous bundle in a freezing medium (Dulbecco's Modified Eagle Medium (DMEM; GIBCO containing 10% dimethylsulfoxide (DMSO) and 20% fetal bovine serum) and stored at −80 °C after snap-freezing on dry ice. Random samples of sperm were stained by DAPI and examined by fluorescence microscopy to assess the quality of the sperm isolation and confirm somatic cell removal.

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2.3. Chromatin immunoprecipitation (ChIP)

For each ChIP replicate, 10^6 frozen spermatozoa were thawed at room temperature, washed in cold PBS and fixed in 100 µl 1% formaldehyde in PBS at room temperature for 10 min. Crosslinking was quenched by the addition of 400 µl 0.125 M glycine in PBS and incubation at 4 °C for 5 min. Cells were washed twice in 500 µl ice-cold PBS and incubated for 1 h at room temperature in 500 µl lysis buffer A (10 mM DTT, 10 mM HEPES pH 8, 10 mM EDTA, 0.5 mM EGTA, 0.25% Triton X-100, 10 mM DTT, 0.5% (w/v) SDS and protease inhibitors [10 mg/ml aprotinin, 5 mg/ml leupeptin, and 0.5 mM PMSF]). The DTT was quenched by the addition of N-ethylmaleimide to a final concentration of 5 mM and the cells were resuspended in 100 µl lysis buffer B (10 mM HEPES pH 8, 200 mM NaCl, 0.5 mM EGTA, 1 mM EDTA, 0.01% Triton X-100 and protease inhibitors), pelleted and resuspended in 100 µl IP buffer (25 mM Tris pH 8, 150 mM NaCl, 2 mM EDTA, 1%Triton X-100 and 7.5% glycerol) with 0.25% SDS and protease inhibitors. Chromatin was sheared to approximately 500 bp fragments by sonication on ice using a MEM Soniprep 150 sonicator at 6 micron amplitude for 5 cycles of 20 second sonication and 40 second cooling. Lysates were diluted in four volumes I of P buffer and cleared by centrifugation. For each IP, a 10 µl suspension of Protein G Dynabeads (pre-absorbed with BSA and herring sperm DNA) was incubated in 500 µl 100 mM sodium phosphate pH 8, 0.5% BSA, containing a 5 µg ChIP-grade antibody (or 5 µg goat anti-rabbit IgG antibody for mock IPs) at 4 °C with rotation for 2 h. The Dynabeads were washed in IP buffer and resuspended in 490 µl cleared lysate along with 25 µg sonicated herring sperm DNA. Reactions were incubated at 4 °C with rotation for 4 h. Immune complexes were recovered, suspended in 500 µl buffer 1 (20 mM Tris–HCl pH 8, and 150 mM NaCl, 2 mM EDTA, 1% Triton X-100, 0.1% SDS and protease inhibitors) and incubated on ice for 10 min before being washed twice in 500 µl buffer 2 (20 mM Tris–HCl pH 8, 500 mM NaCl, 2 mM EDTA, 1% Triton X-100, 0.1% SDS and protease inhibitors) for 10 min at 4 °C with rotation and once in 500 µl buffer 3 (10 mM Tris–HCl pH 8, 0.25 M LiCl, 1% sodium deoxycholate, 1 mM EDTA, and 1% NP40) for 10 min at 4 °C with rotation. Finally, the beads were washed twice in ice-cold TNE buffer (10 mM Tris pH 8, 1 mM EDTA, 50 mM NaCl). Chromatin was eluted from the beads in 50 µl 1% SDS, 0.1 M $NaHCO_3$ pH 10.1 at room temperature with rotation for 15 min. Eluates were transferred to fresh non-stick tubes, then the procedure was repeated and the eluates were pooled. To each pooled eluate, 4 µl 5 M NaCl and 5 µl 0.2 M EDTA were added and eluates were incubated overnight at 55 °C. The pH of the eluates was adjusted to 8.3 by the addition of 4 µl 2 M Tris pH 6.5. Residual RNAs were removed by the addition of 1 µl 10 mg/ml RNase A followed by incubation at 37 °C for 30 min, and proteins were removed by the addition of 1 µl 10 mg/ml proteinase K and incubation at 55 C for 4 h. Input fly DNA was extracted from the sonicated chromatin of 2×10^6 spermatozoa in 200 µl IP buffer by the addition of 10 µl 3 M sodium acetate pH 7 and 10 µl 10% SDS, followed by overnight incubation at 55 °C. Residual RNAs and proteins were removed as before. DNAs were recovered using a Genelute™ PCR Clean-Up kit (Sigma-Aldrich) and quantified using a PicoGreen dsDNA Quant-iT™ kit (Invitrogen) according to the manufacturers' instructions. DNAs of input, IP and mock samples were amplified in two steps; firstly, DNA was amplified using a GenomePlex Single Cell™ kit (WGA4; Sigma-Aldrich), and secondly, the DNA was re-amplified using a GenomePlex Re-amplification™ kit (WGA3; Sigma-Aldrich) according to the manufacturer's instructions.

Table 1
Antibodies used for ChIP.

Antibody	Type	Supplier cat #
Anti-histone H3	Rabbit polyclonal; primary	Cell signaling #2650
Anti-histone H4	Rabbit polyclonal; primary	Abcam ab10158
Anti-H3K9me3	Rabbit polyclonal; primary	Abcam ab8898
Anti-H3K27me3	Mouse monoclonal; primary	Abcam ab6002
Anti-H3K36me3	Rabbit polyclonal; primary	Abcam ab9050
Anti-rabbit IgG	Goat polyclonal; secondary	Thermo Scientific 31210

Table 2
Spearman correlations for probe intensities.

Correlations	S	rho	p-Value
H3 vs H4	1.27E+17	0.925717	$<2.2e-16$
H3 vs H3 + H4	2.18E+17	0.872715	$<2.2e-16$
H4 vs H3 + H4	2.19E+17	0.872294	$<2.2e-16$
H3K9me3 Cy5 vs H3K9me3 Cy3	2.13E+17	0.876137	$<2.2e-16$
H3K27me3 Cy5 vs H3K27me3 Cy3	4.16E+17	0.757616	$<2.2e-16$
H3K36me3 Cy5 vs H3K36me3 Cy3	4.10E+17	0.761541	$<2.2e-16$

2.4. Microarray hybridization

Three independent immunoprecipitates (IP) obtained with anti-H3 and anti-H4 antibodies and two each obtained with anti-H3K9me3, anti-H3K27me3 and anti-H3K36me3 antibodies (Table 1) were carried out. In addition, two independent mock IPs were used to correct for non-specific binding of chromatin to IgG. Total genomic DNA isolated from fly sperm was used as an input reference in each hybridization. DNAs were processed and labelled according to protocols (http://www.flychip.org.uk/protocols/chip/NimblegenBioPrime.php) recommended by Nimblegen and hybridized to NimbleGen 2.1M *Drosophila melanogaster* whole-genome tiling arrays (Roche 05542308001) at the Flychip facility (www.flychip.org.uk). Slides were scanned at 5 µm resolution using a GenePix Axon scanner and raw data were exported using NimbleScan software.

2.5. Data analysis

Experimental and mock data were processed, quality assessed and analyzed using the Bioconductor packages Ringo [4] and GenomicRanges [5]. Data were normalized by variance-stabilizing normalization [6] and smoothed by computing the median ratios of ChIP/input signals for probes contained within sliding 800 bp windows. Correlations between unprocessed probe signal intensities are shown in Table 2 and separated Cy3 (input genomic DNA) and Cy5 (ChIP DNA) density distributions obtained for the three core histone ChIP experiments are shown in Fig. 1. Plotted smoothed $\log_2$(ChIP/input) values obtained with anti-histone_H3 and anti-histone_H4 antibodies across chromosome 3L are shown in Fig. 2.

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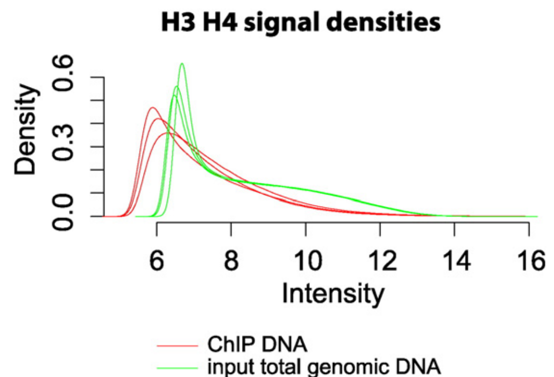

Fig. 1. Density plots of signal intensities ($\log_2$, X-axis) against probe expression values (density, Y-axis) for anti-H3 and anti-H4 ChIP.

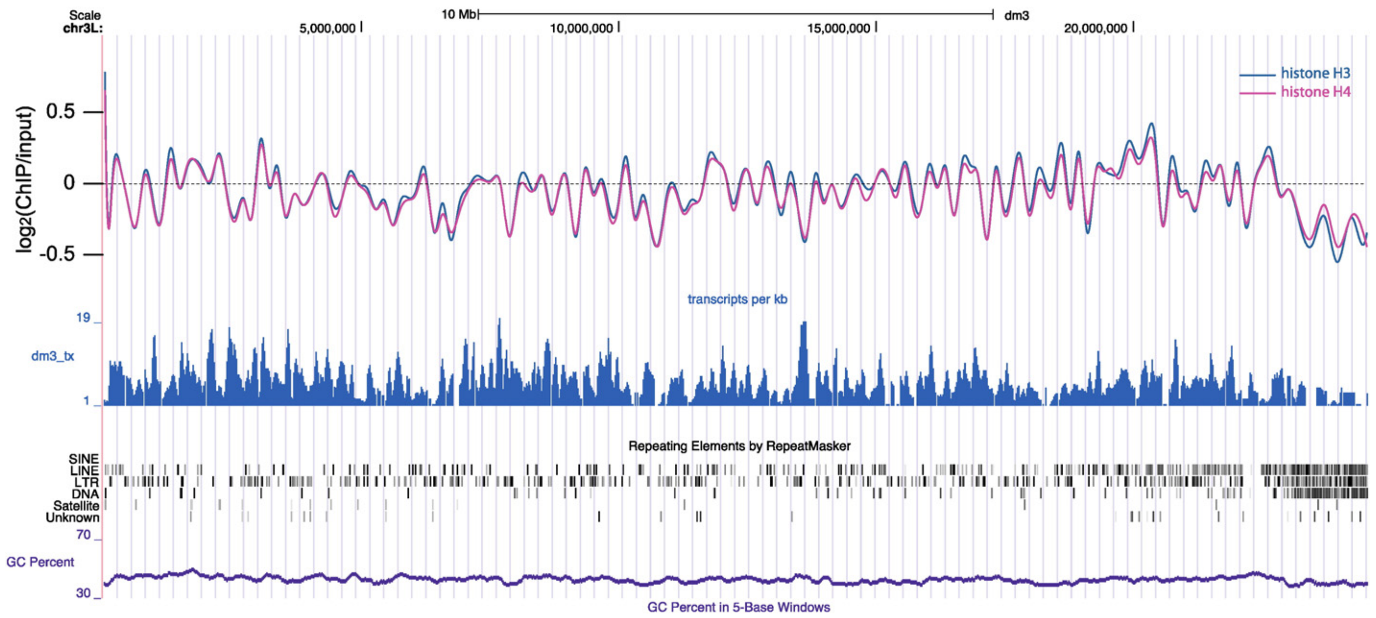

Fig. 2. Smoothed spline plot of $\log_2$(ChIP/input) data for the anti-H3 and anti-H4 ChIP experiments across chromosome 3L.

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