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Data in Brief

Genome-wide mapping of Painting of fourth on *Drosophila melanogaster* salivary gland polytene chromosomes

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ABSTRACT

The protein Painting of fourth (POF) in *Drosophila melanogaster* specifically targets and stimulates expression output from the heterochromatic 4th chromosome, thereby representing an autosome specific protein [1,2]. Despite the high specificity for chromosome 4 genes, POF is occasionally observed binding to the cytological region 2L:31 in males and females [3] and two loci on the X-chromosome, *PoX1* and *PoX2* only in females [4]. Here we provide a detailed description of the experimental design and analysis of the tiling array data presented by Lundberg and colleagues in G3: Genes, Genomes, Genetics 2013 [4], where the female specific POF binding to *PoX1* and *PoX2* loci on the X chromosome was reported. We show the genome-wide high resolution binding profile of the POF protein where these different POF binding sites are detected. The complete data set is available at http://www.ncbi.nlm.nih.gov/geo/ (accession: GSE45402)

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Specifications [*standardized info for the reader*]	
Organism/cell line/tissue	*Drosophila melanogaster/3rd instar larvae/salivary glands*
Sex	*Males and females*
Sequencer or array type	*Affymetrix GeneChip Drosophila Tiling 2.0R Array*
Data format	*Raw data: CEL files, processed data: CHP file*
Experimental factors	*POF Chromatin immunoprecipitation vs. Input*
Experimental features	*ChIP-chip experiment of POF in salivary gland tissue from Drosophila melanogaster*
Consent	*n/a*
Sample source location	*n/a*

Direct link to deposited data [*provide URL below*]

Deposited data can be found at: http://www.ncbi.nlm.nih.gov/geo/ (accession: GSE45402) http://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE45402.

Experimental design, materials and methods

Chromatin immunoprecipitation

250 Pairs of salivary glands from wild type 3rd instar larvae were dissected in PBS and directly cross-linked in 2% formaldehyde in PBS with 0.1% Triton X-100 for 2 min. The glands were then washed in PBS until all dissections were done and glycine was added to a final concentration of 0.125 M. The salivary glands were washed once with PBS, 1 mM PMSF, protease inhibitor cocktail (Roche) followed by two washes in TBS, 1 mM PMSF and protease inhibitor cocktail. The samples were homogenized in 500 μl Lysis buffer (1% SDS, 10 mM EDTA, 50 mM Tris–HCl [pH 8.1]). To ensure shearing of the cross-linked DNA into 200–1000 bp long fragments, 120 μl glass beads were added prior to sonication for 4 min using a Bioruptor (Diagenode) at high setting (30 s ON, 30 s OFF). The samples were cleared by centrifuging for 10 min at 16,000 *g* at 4 °C. For each ChIP (POF and Mock) and Input, 150 μl of cell lysate was diluted by a factor of ten in ChIP Dilution buffer (0.01% SDS, 1.1% Triton X-100, 1.2 mM EDTA, 16.7 mM Tris–HCl [pH 8.0], 167 mM NaCl), and protein inhibitors were added. To reduce nonspecific background the diluted lysate was pre-cleared by incubation with 60 μl of equilibrated Dynabeads conjugated to Protein A (Dynal) for 30 min at 4 °C with agitation.

For immunoprecipitation, the cleared lysate was incubated with 3 μl of rabbit antibodies raised against full-length POF protein [5] overnight at 4 °C on a rotating platform. No antibodies were added to Mock or Input. The antibody complexes were precipitated by incubation with equilibrated Protein A Dynabeads for 1 h at 4 °C. The beads were

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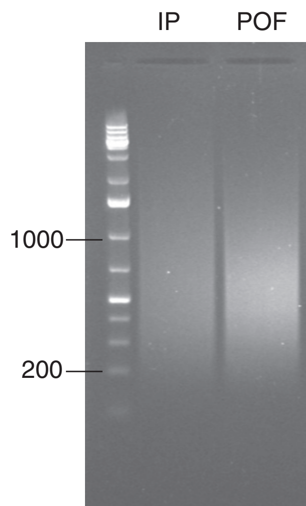

Fig. 1. Gel electrophoresis of amplified input DNA and POF-ChIP DNA. Approximately 500 ng DNA from input (IP) and POF-ChIP (POF) were separated on a 1.2% agarose gel. GeneRuler™ 1 kb DNA Ladder Plus (Fermentas) was used as reference for fragment size.

washed for 4 min with agitation at 4 °C with the following buffers; once with low salt buffer (0.1% SDS, 1% Triton X-100, 2 mM Tris–HCl [pH 8.0], 150 mM NaCl), once with high salt buffer (0.1% SDS, 1% Triton X-100, 2 mM Tris–HCl [pH 8.0], 500 mM NaCl), once with LiCl-containing buffer (250 mM LiCl, 10 mM Tris–HCl [pH 8.0], 1 mM EDTA, 1% NP-40, 1% sodium deoxycholate), and twice with TE Buffer (10 mM Tris–HCl [pH 8.0], 1 mM EDTA).

The protein/DNA complexes were eluted from the antibody by incubating for 15 min at room temperature in 250 μl Elution buffer (1% SDS, 0.1 M $NaHCO_3$) with rotation. The elution was repeated once and the eluates were combined to a total volume of 500 μl. NaCl was added to a final concentration of 200 mM, and protein/DNA crosslinks were reversed by heating at 65 °C for 4 h. A total of 10 μl of 0.5 M EDTA, 20 μl of 1 M Tris–HCl [pH 6.5], and 1 μl of 20 mg/ml proteinase K were added before an additional incubation at 45 °C for 1 h. The DNA was recovered by phenol/chloroform extraction followed by ethanol precipitation. The immunoprecipitated DNA was then dissolved in 24 μl water.

Amplification

Approximately 100 ng DNA from each ChIP (POF and Mock) and 50 ng Input DNA were used for library preparation followed by a 20-cycle amplification using GenomePlex® Complete Whole Genome Amplification (WGA) Kit (Sigma-Aldrich). The amplified DNA was purified with a QIAquick PCR purification kit (QIAGEN) according to the manufacturer's recommendations. To verify that no amplification bias affected the enrichment profiles, we analyzed the ChIP-DNA/Input-DNA ratio before and after amplification by using real-time PCR as previously described in [6]. Correct size distribution of the amplified DNA samples were confirmed with gel electrophoresis (Fig. 1).

Tiling array

For tiling array analysis, the amplified POF-ChIP and Input DNA were fragmented, labeled and hybridized to an Affymetrix *Drosophila* Genome 2.0 array according to standard Affymetrix protocols. The signal intensity data generated were analyzed with Affymetrix Tiling Analysis Software (v. 1.1.0.2), using 200-bp and 400-bp bandwidth as smoothing parameters and limited to perfect match only. The enrichment profiles were produced as ChIP-DNA/Input-DNA ratios expressed on a log2 scale and analyzed by using Integrated Genome Browser (7.0.1) (Fig. 2A) [7].

Discussion

Here we present a high resolution genome-wide enrichment profile of POF protein in salivary gland tissue. In immunostainings of polytene chromosomes two sites on the X chromosome, *PoX1* and *PoX2*, and cytological region 2L:31 are occasionally found targeted by POF in fully polytenized nuclei [1-4] (Fig. 2B). Even though these sites are only detected in a fraction of all nuclei they are still distinguished in the genome-wide data set. This demonstrates the strength in combining chromosome immunostaining data with genome-wide mapping data such as ChIP-chip or ChIP-seq to distinguish differences in binding strength from occasional binding.

Conflict of interest

The authors hereby declare that no conflict of interest exists.

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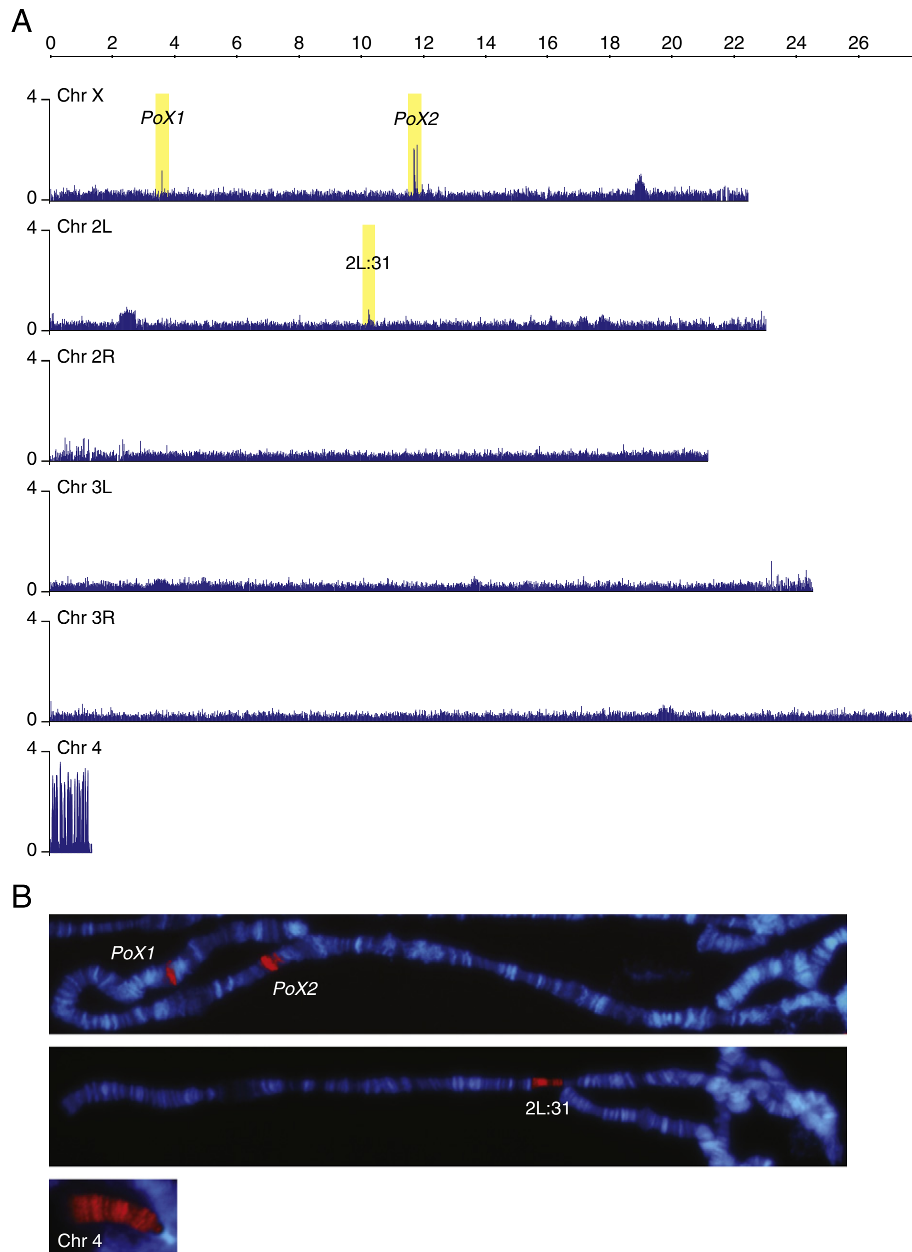

Fig. 2. POF binding profiles for all chromosomes. (A) The tiling array results are computed as the ratio between the POF-ChIP value and the value of the corresponding input DNA. The plots show the mean enrichment ratios obtained using a bandwidth of 400 bp. Numbers on the *x*-axis denote chromosomal position for each chromosome arm in Mb. The *y*-axis shows the POF enrichment as the log2-ratio. The peaks within the yellow boxes correspond to *PoX1* and *PoX2* on the X chromosome and 2L:31 on chromosome 2L. (B) Immunostaining of polytene chromosomes. In addition to binding on the 4th chromosome (Chr4), POF (red) occasionally binds to *PoX1* and *PoX2* on the X-chromosome and cytological region 2L:31. DNA is counterstained with DAPI (blue).