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Data in Brief

Genome-wide DNA binding pattern of the homeodomain transcription factor Sine oculis (So) in the developing eye of *Drosophila melanogaster*

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ABSTRACT

The eye of the fruit fly *Drosophila melanogaster* provides a highly tractable genetic model system for the study of animal development, and many genes that regulate *Drosophila* eye formation have homologs implicated in human development and disease. Among these is the homeobox gene *sine oculis* (*so*), which encodes a homeodomain transcription factor (TF) that is both necessary for eye development and sufficient to reprogram a subset of cells outside the normal eye field toward an eye fate. We have performed a genome-wide analysis of So binding to DNA prepared from developing *Drosophila* eye tissue in order to identify candidate direct targets of So-mediated transcriptional regulation, as described in our recent article [20]. The data are available from NCBI Gene Expression Omnibus (GEO) with the accession number GSE52943. Here we describe the methods, data analysis, and quality control of our So ChIP-seq dataset.

Specifications	
Organism/cell line/tissue	*Drosophila melanogaster* eye–antennal imaginal disc
Strain(s)	w^{1118}
Sequence or array type	Illumina Genome Analyzer IIx
Data format	FASTQ and WIG
Experimental features	ChIP-seq
Consent	N/A

Direct link to deposited data

Deposited data are available from the following link: http://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE52943.

Experimental design, materials and methods

Tissue source

The external structures of the adult *Drosophila* head arise from a larval precursor structure known as the eye–antennal imaginal disc. The eye–antennal imaginal disc resolves into morphologically distinct eye and antennal portions during the second larval instar stage, with the anterior part fated to become the antenna, and the posterior part fated to give rise to the compound eye of the adult; both the eye and antennal discs also contribute to the adult head capsule [1]. During the first and second instar, the eye disc consists of undifferentiated, proliferating cells. At the onset of the third and final instar, a constriction known as the morphogenetic furrow forms at the posterior margin of the eye disc and then gradually sweeps across the eye disc toward the anterior margin [2]. As the furrow advances, cells anterior to it undergo cell cycle arrest, followed by the onset of retinal differentiation as cells enter the furrow [3,4]. During late larval and subsequent pupal stages, cells become progressively recruited to become photoreceptors, lens-secreting cone cells, pigment cells, and bristles of the adult compound eye [4].

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The dynamic nature of retinal development is reflected in the expression pattern of Sine oculis (So), which is necessary for eye differentiation [5]. Expression of the *so-lacZ* reporter allele can be detected in the eye–antennal disc as early as the first larval instar [6]. During the second instar, So expression is confined to the eye portion of the eye–antennal disc, being strongest near the posterior margin [5]. As the furrow progresses across the eye disc during the third instar, So continues to be expressed in a band of cells anterior to the furrow, as well as in the differentiating cells posterior to the furrow [5]. We harvested the eye–antennal discs from wandering third instar larvae, a stage at which the majority of the cells in the eye disc have passed through the morphogenetic furrow and begun to differentiate. The majority of the cells in the eye disc express So at this stage [5].

We used w^{1118} *Drosophila melanogaster* larvae, which are homozygous for a loss-of-function mutation in the *white* (*w*) gene. The *w* gene is required for pigmentation of the adult eye [7]. Aside from the *w* mutation, the larvae used were not known to have homozygous mutations in any genes affecting the eye, and the eyes of the adult flies of this strain are morphologically normal.

Sample preparation

ChIP sample preparation was similar to the method previously described [8]. We dissected eye–antennal disc complexes including mouth hooks, but not brains, from wandering third instar larvae in phosphate buffered saline (PBS). The antennal disc does not express So [5], and hence its inclusion in the ChIP sample would not be expected to influence the So ChIP-seq profile. A total of 400 eye–antennal disc complexes (800 discs) were used for each biological replicate. The discs were transferred into 500 μL S2 media on ice for <30 min, fixed by adding 20.25 μL 37% formaldehyde and incubating at room temperature for 15 min, and quenched with 25 μL 2.5 M glycine, followed by 5 min incubation on ice. The discs were washed 3× in PBS and placed on ice. Following the dissection and fixation, 200 disc pairs were combined in a 1.5 mL microcentrifuge tube with 600 μL ChIP lysis buffer (50 mM K-Hepes [pH 7.8 adjusted with KOH], 140 mM NaCl, 1 mM EGTA, 1 mM EDTA, 1% Triton X-100, 0.1% Na-deoxycholate) with a Mini EDTA-free protease tablet (Roche) (1 tablet/10 mL buffer). Two tubes with 200 disc pairs each were processed per replicate. The discs were ground with a nuclease-free pestle, and passed 10× through a 25-gauge needle and 10× through a 27-gauge needle. The homogenized discs were incubated 20 min at 4 °C on a nutator.

The resulting chromatin was sonicated with a Branson Digital Sonifier 250 using the following settings: 15% amplitude, 15 s (0.9 s on/0.2 s off), 15 rounds, and 2 min rest between rounds. A 5 μL aliquot of the chromatin was run on a 1% agarose gel in order to test successful shearing, indicated by a ~1.5–4 kb DNA smear. The rest of the chromatin sample was centrifuged for 10 min, 13,200 rpm at 4 °C to remove cell debris, and the supernatant from the two tubes (containing chromatin from 200 disc pairs each) was combined in a single siliconized tube. We set aside 10 μL of the supernatant at −20 °C as input sample. We precleared the remaining sample by incubating at 3 h at 4 °C on a nutator with 40 μL nProtein A Sepharose 4 Fast Flow bead slurry (GE Healthcare, previously washed 3× with PBS and 1× with ChIP lysis buffer). Following the incubation, the beads were removed by centrifuging for 1 min, 5000 rpm at 4 °C, and the supernatant was split into two equal samples, experimental and control. 1:500 polyclonal guinea pig anti-So antibody (gift from Ilaria Rebay; [8]) was added to the experimental sample, and the two samples were incubated overnight at 4 °C on a nutator. In parallel, 60 μL washed bead slurry was incubated overnight at 4 °C on a nutator in blocking solution (30 μL 100× Bovine Serum Albumin [NEB], 13 μL 10 μg/μL denatured salmon sperm DNA, 500 μL ChIP lysis buffer).

Following the overnight incubation, the blocked beads were centrifuged to remove the supernatant, and resuspended in 30 μL ChIP lysis buffer to make 1:1 bead:buffer slurry. 20 μL bead slurry was added to each chromatin sample, followed by 3–4 h incubation at 4 °C on a nutator. After a brief centrifugation to remove the supernatant, each bead sample was washed 3× with ChIP lysis buffer, 1× with high salt ChIP lysis buffer (same composition as ChIP lysis buffer but with 500 mM NaCl), and 1× with TE (each wash was 1 mL, for 5 min at 4 °C on a nutator). We resuspended each bead sample in 150 μL TE/SDS (10 mM Tris pH 8.0, 1 mM EDTA, 1% SDS), and added 140 μL TE/SDS to the 10 μL input sample. The chromatin was eluted from the beads by a 10 min incubation in a 65 °C water bath, with brief vortexing every 2 min. The samples were centrifuged for 1 min, 14,000 rpm at room temperature. The supernatant was transferred to new tubes that were sealed with Parafilm and incubated 5 h to overnight in a 65 °C water bath to reverse crosslinks. We extracted the DNA with a QIAquick PCR Purification Kit (Qiagen), eluting the DNA in 30 μL Elution Buffer (10 mM Tris–Cl, pH 8.5).

The purified DNA was tested using qPCR with primers flanking a previously identified So-binding site 3′ of the *atonal* gene (*3′ato*), as well as control primers flanking a site not expected to bind So in the eye. If qPCR showed >4-fold enrichment in the experimental (+anti-So) sample relative to the negative control (−anti-So) sample, we proceeded with Illumina library preparation following manufacturer's instructions. Briefly, ~10 ng of DNA was end-repaired using polynucleotide kinase and Klenow. The 5′ ends of the DNA fragments were phosphorylated and a single adenine base was added to the 3′ ends using Klenow exonuclease. Illumina Y-shaped index adaptors were ligated to the repaired ends, and the DNA fragments were PCR amplified for 21 cycles. Fragments in the 200–500 bp range were isolated by gel purification. The libraries were quantified using the PicoGreen fluorescence assay and their size distributions were determined by the Agilent 2100 Bioanalyzer.

The library was tested again by qPCR to ensure >4-fold enrichment prior to sequencing.

Data analysis

The libraries of two biological replicates were sequenced using the Illumina Genome Analyzer IIx and a total of 21.9 million 35-bp single-end reads were generated, including 12.4 million from the first replicate and 9.5 million from the second replicate. In order to maximize our power in downstream data analysis, the reads from two biological replicates were combined, and the combined reads were mapped to the *D. melanogaster* reference genome (dm5) using Eland software. Approximately 4.74 million reads were mapped to the dm5 genome. Among them, about 3.4 million reads were uniquely mapped. There were a total of over 19 million reads for control sample. Among them, 6.2 million reads were mapped to dm5 genome and 5.7 million reads were unique.

Peaks were called from the mapped reads using Model-based Analysis of ChIP-Seq (MACS) [9]. As default settings, peaks with less than 3-fold enrichment or with $P > 10^{-5}$ were filtered out. A total of 7566 peaks were then obtained and annotated using an in-house bioinformatics tool (a Perl script, available upon request). The median width of the resulting peaks is ~1 kb. Most (84.7%) of the peaks fully or partially overlap an annotated *Drosophila* gene, with 52.4% of all peaks being <1 kb from an annotated transcription start site (TSS).

Discussion

The So transcription factor is a necessary regulator of *Drosophila* eye development, and its homologs have been implicated in cancer and developmental disorders in human patients [10–14]. We have recently presented a genome-wide profile of So binding to chromatin in developing *Drosophila* eye discs [2]. Our data set shows So DNA-binding enrichment at enhancers previously shown to require So-mediated regulation in the developing eye [15–19], as well as So binding to or near genes that function in multiple aspects of eye development. The

data suggest that a broad spectrum of genes may be regulated by So during eye development and is expected to expand our understanding of the genetic basis of eye formation.

Conflict of interest

The authors state that there are no conflicts of interest.

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