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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A R T I C L E  I N F O

Article history:
Received 11 June 2014
Received in revised form 16 June 2014
Accepted 17 June 2014
Available online 25 June 2014

Keywords:
Sine oculis
ChIP-seq
Drosophila
Eye
Development

A B S T R A C T

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.

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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].

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.

http://dx.doi.org/10.1016/j.gdata.2014.06.016
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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].

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 ChIP lysis buffer (same composition as ChIP lysis buffer but with 0.2% NP-40). A 50 μL aliquot of the chromatin was run on a 1% agarose gel in order to test successful shearing, indicated by a ~1.5 kb fragment. The majority of the chromatin was run on a 1% agarose gel in order to test successful shearing, indicated by a ~1.5 kb fragment. Most (84.7%) of the peaks fully overlapped an annotated transcription start site (TSS).

We used w118 *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.

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.

Acknowledgements

This work was funded by the Retina Research Foundation (RRF), the National Eye Institute (NEI) grant R01 EY011232 and the NEI/NIH Core Grant for Vision Research EY-002520.

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