



Data in Brief

Stimulation of the *Drosophila* immune system alters genome-wide nucleosome occupancy

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ABSTRACT

In eukaryotes, nucleosomes participate in all DNA-templated events by regulating access to the underlying DNA sequence. However, nucleosome dynamics during a genome response have not been well characterized [1,2]. We stimulated *Drosophila* S2 cells with heat-killed Gram-negative bacteria *Salmonella typhimurium*, and mapped genome-wide nucleosome occupancy at high temporal resolution by MNase-seq using Illumina HiSeq 2500. We show widespread nucleosome occupancy change in S2 cells during the immune response, with the significant nucleosomal loss occurring at 4 h after stimulation. Data have been deposited to the Gene Expression Omnibus (GEO) database repository with the dataset identifier GSE64507.

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Specifications

Organism/cell line/tissue	<i>Drosophila melanogaster</i> S2 cell line
Sex	N/A
Sequencer or array type	Illumina HiSeq 2500
Data format	fastq and bed files (raw and processed)
Experimental factors	S2 cells were stimulated with heat-killed <i>Salmonella typhimurium</i> at a final concentration of 10^7 cells/ml
Experimental features	Genome-wide nucleosome occupancy was measured by MNase-seq at 0 h, 30 min, 1 h and 4 h post <i>S. typhimurium</i> stimulation
Consent	N/A
Sample source location	N/A

serum (Life Technologies). For bacterial stimulation, heat-killed *S. typhimurium* was added to S2 cells at a final concentration of 10^7 cells/ml. At 0, 30 min, 1 h, and 4 h after stimulation, 3×10^7 S2 cells were harvested each time point, and cross-linked by incubating with 1% formaldehyde at room temperature for 10 min. To stop the protein-DNA cross-links, 125 mM glycine was added. Nuclei were isolated by re-suspending cells in nuclei isolation buffer (0.3 M sucrose, 2 mM MgOAc₂, 1 mM CaCl₂, 1% Nonidet P-40, 10 mM HEPES, pH 7.6) and spinning at 800 g for 10 min. Two biological replicates with independent growth and treatment were processed at each time point.

Direct link to deposited data

Illumina Hi-Seq 2500 data is deposited at the NCBI Gene Expression Omnibus (GEO) database under GEO Series and is available at the following link: <http://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE64507>.

Experimental design, materials and methods

Cell growth and bacterial stimulation

S2 cells were maintained at 28 °C in Schneider's *Drosophila* medium (Invitrogen), supplemented with 10% heat-inactivated fetal bovine

MNase cleavage, isolation and purification of mononucleosomal DNA

S2 nuclei were digested with 40 U/ml micrococcal nuclease (MNase) (Worthington Biochemical Corp) in MNase cleavage buffer (4 mM CaCl₂, 25 mM KCl, 4 mM MgCl₂, 12.5% glycerol, 50 mM HEPES, pH 7.4) at 37 °C for 5 min. The reactions were stopped with 50 mM EDTA. RNA was removed by treating samples with 100 µg/ml RNase A (Thermo Scientific) at 55 °C for 10 min. Samples were incubated at 65 °C over night with 0.2 µg/µl proteinase K (Worthington Biochemical Corp) and 1% sodium dodecyl sulfate to remove the cross-links. DNA was purified by phenol-chloroform extraction and ethanol precipitation. To isolate mononucleosomal DNA, the DNA samples were resolved on 2% agarose gel. The area of the gel containing mononucleosomal DNA (200 bps and below) was excised and purified by the freeze-squeeze method [1–3].

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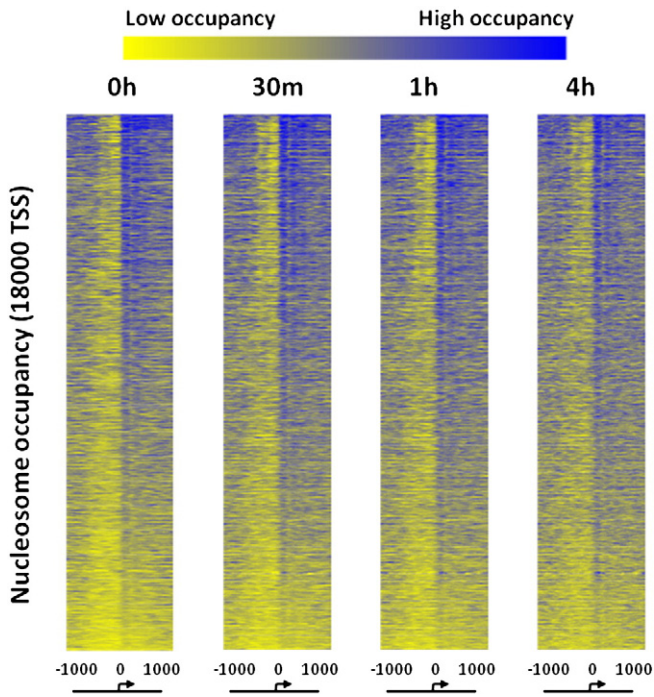


Fig. 1. *S. typhimurium* stimulation leads to genome-wide nucleosomal loss in S2 cells. Heatmaps representing nucleosome occupancy are shown for each time point at each TSS in the fly genome (~18,000 genes). In the 0 h heatmap, loci were sorted by maximum occupancy across 1000 bp surrounding the TSS (arrow) for all genes. This sort order was maintained in all other heatmaps for direct comparison. Blue represents areas with high occupancy; yellow indicates low occupancy.

Mononucleosomal DNA library preparation and Illumina sequencing

Libraries of mononucleosomal DNA were prepared using NEBNext® Ultra™ DNA Library Prep Kit for Illumina® (NEB #E7370S/L), starting with 30 ng of mononucleosomal DNA for each reaction. The samples were end-prepared, adaptor-ligated and indexed (NEB #E7335S/L, NEB #E7500S/L) according to the manufacturer's instructions, then purified with AMPure XP beads (Beckman Coulter, Inc. #A63881) without size selection. The quantity and quality of the library were checked

with Qubit 2.0 Fluorometer (Life Sciences) and Agilent 2100 Bioanalyzer (Agilent Technologies). Libraries were quantified using KAPA Library Quantification (KK824). Resulting libraries were plated using the Illumina cBot and run on the Illumina HiSeq 2500 platform configured for 50 bp paired-end reads.

Data analysis

Raw read pairs were mapped to the *Drosophila* reference genome UCSC.dm3. Nucleosome occupancy was calculated by reads per million that mapped to the fly genome with bedtools 2.17. Analyses of nucleosome occupancy were performed in R environment using our lab-developed software package: RAGE.R. Data were uploaded to the UCSC genome browser for further analysis (<http://genome.ucsc.edu>).

Detection of nucleosome occupancy dynamics during the immune response

We created heatmaps for each time point by plotting the nucleosome occupancy of all fly genes and centering the genes at the TSS. We found a significant loss of nucleosome occupancy at the 4 h time point post *S. typhimurium* stimulation (K-S test 2.2×10^{-16}) (Fig. 1). These results suggest that widespread nucleosome loss may be an instrumental part of genome regulation during the immune response to *S. typhimurium*.

Acknowledgments

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