CLAMP and Zelda Synergistically Regulate Transcription during *Drosophila* Zygotic Genome Activation

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Abstract

In *Drosophila*, the zygotic genome activation (ZGA) protein Zelda (ZLD) is known to function as a pioneer transcription factor (TF) to activate early transcription. However, many genomic loci remain active in the absence of ZLD. Therefore, we hypothesized that other TFs that have not yet been identified also regulate early transcription. Chromatin-linked adaptor for Male-specific lethal (MSL) proteins (CLAMP) regulates dosage compensation in males but is also required for embryos to progress through ZGA. However, the functional relationship between CLAMP and Zelda, two key early TFs, has not been investigated.

Here, we depleted maternally deposited ZLD or CLAMP and used ChIP-seq to define their relationship before (0-2hr) and after ZGA (2-4hr) in early *Drosophila* embryos. We found synergistic binding between CLAMP and ZLD at promoters during ZGA. In contrast, CLAMP and ZLD bind independently at intronic binding sites. Depletion of maternally deposited CLAMP reduces ZLD binding both before and after MZT. However, depletion of maternally-deposited ZLD only influences CLAMP binding before MZT. These observations suggest that ZLD is required more prior to MZT, while CLAMP is required both prior and post-ZGA to modulate ZLD occupancy. Moreover, ZLD and CLAMP regulate the transcription level of genes at dependent binding sites through clusters of motifs. Taken together, our results reveal a novel function of CLAMP as a transcription regulator at promoters to activate transcription during ZGA.

KEYWORDS WORDS Maternal to zygotic transition, zygotic genome activation, CLAMP, Zelda, *Drosophila* embryo

Introduction

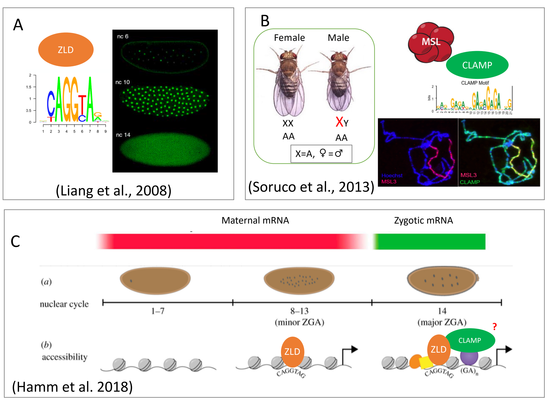
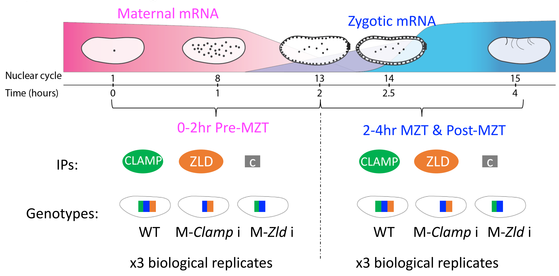


Figure 1. Pioneer factors drive genomic activation in early embryos
A. Zelda (ZLD) is expressed in early embryos to regulate transcription during ZGA.
B. Dosage compensation regulator CLAMP binds to GA-rich motifs.
C. Genomic loci that remain active in the absence of ZLD are enriched with (GA)s.

Materials and Methods

Experimental design



- A maternal triple driver (MTD-GAL4) line was crossed with a Transgenic RNAi Project (TRiP) *Clamp* RNAi line or a TRiP Zld RNAi line. The MTD-GAL4 line alone was used as the control line in our study.
- ChIP-seq experiments were performed to measure CLAMP and ZLD protein binding pre-MZT (0-2hr) and post-MZT (2-4hrs).
- Maternal CLAMP or ZLD was depleted by RNA interference (RNAi) to determine the functional interaction between these two essential TFs.

Results

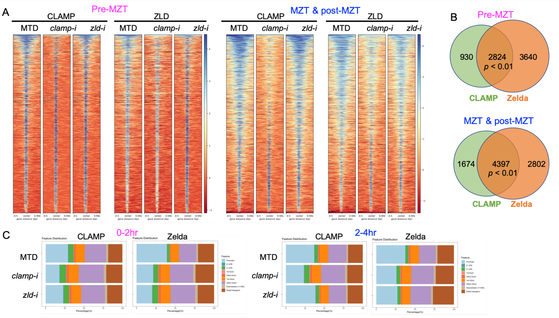


Figure 2. CLAMP and ZLD exhibit synergistic binding to promoters during MZT.
A. Depletion of CLAMP reduces ZLD binding both before and after MZT. However, depletion of ZLD only influences CLAMP binding before MZT.
B. CLAMP and ZLD peaks showed a significant overlap ($p < 0.01$) at both time points.
C. CLAMP and ZLD facilitates binding to promoters but not introns.

Results

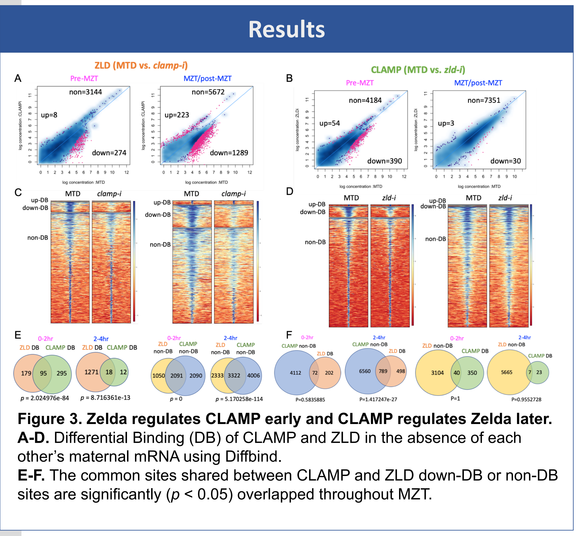


Figure 3. Zelda regulates CLAMP early and CLAMP regulates Zelda later.
A-D. Differential Binding (DB) of CLAMP and ZLD in the absence of each other's maternal mRNA using Diffbind.
E-F. The common sites shared between CLAMP and ZLD down-DB or non-DB sites are significantly ($p < 0.05$) overlapped throughout MZT.

Results

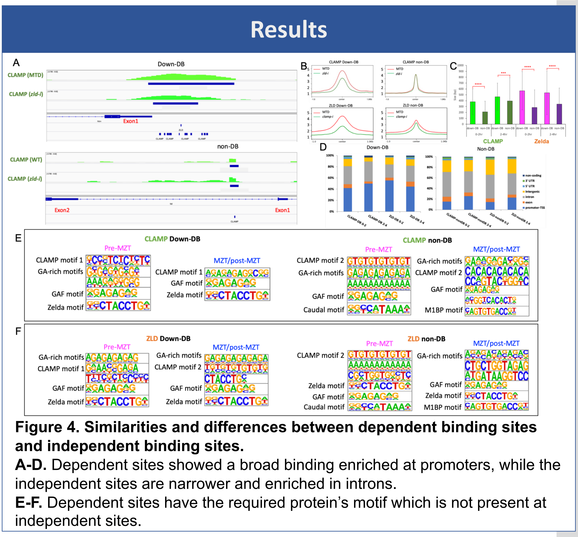


Figure 4. Similarities and differences between dependent binding sites and independent binding sites.
A-D. Dependent sites showed a broad binding enriched at promoters, while the independent sites are narrower and enriched in introns.
E-F. Dependent sites have the required protein's motif which is not present at independent sites.

Results

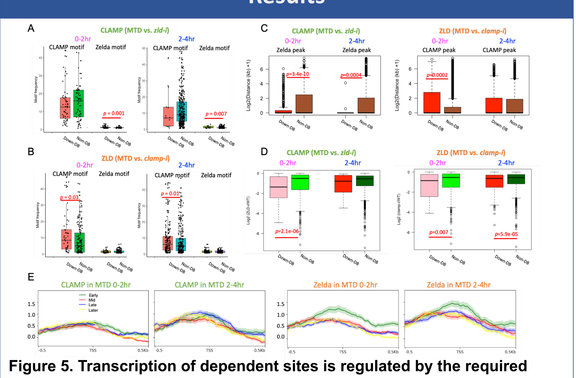
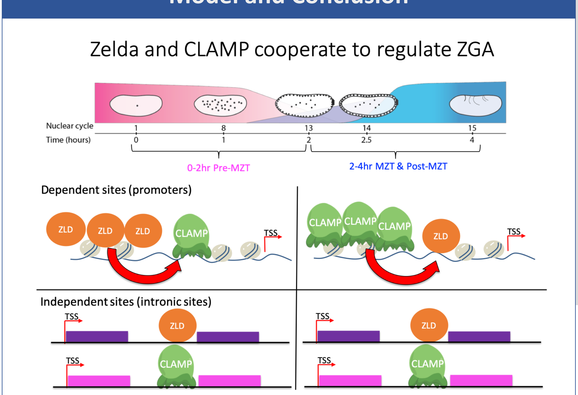


Figure 5. Transcription of dependent sites is regulated by the required protein via their motifs.
A-B. The number of binding motifs for the required protein is much higher at the dependent sites than independent sites for both TFs.
C. ZLD regulates CLAMP binding from a short distance and CLAMP regulates ZLD through both long and short range interactions.
D-E. ZLD and CLAMP regulate the transcription level of genes at dependent sites in a synergistic manner.

Model and Conclusion



Model and Conclusions

- CLAMP and ZLD bind synergistically to promoters during ZGA.
- In contrast, CLAMP and ZLD bind independently at intronic binding sites.
- Clusters of ZLD and CLAMP binding sites regulate the transcription level of genes at dependent sites.

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This work was supported by NIH grant F32GM109663 and K99HD092625 to Dr. Leila Rieder and R35GM126994 to Dr. Erica Larschan.

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