Proceedings of the 3rd International Conference on Biological Engineering and Medical Science
DOI: 10.54254/2753-8818/23/20231084

Investigating the role of Elba3 in drosophila embryonic development

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Abstract. Elba (erythroid-myeloid lymphatic binding protein associated) is a transcription factor in fruit flies that plays a crucial role in regulating gene expression, particularly during embryonic development. Elba3, which acts as a transcription factor protein, exhibits significant involvement in the control of gene expression throughout embryogenesis and has been demonstrated to be essential for proper embryo development and central nervous system formation. In this work, by using CRISPR-Cas9 technology's results, I have been able to study the effects of manipulating the Elba3 gene in Drosophila embryos, providing insights into the function of Elba3 in the regulation of gene expression during embryonic development and Elba3 and Zelda are related to each other in a few different ways.

Keywords: Elba3, Drosophila, Embryo, CHRISPR-CAS9, Zelda

1. Introduction

Drosophila is a valuable model organism for figuring out various biological mechanisms and processes, including aging, behavior, and disease. Its short lifespan and ease of experimental manipulation make it an ideal subject for these studies. Additionally, the highly conserved genome of Drosophila with that of humans makes it a valuable tool in understanding human biology [1].

Elba3, as the assembly of active Elba complexes by interacting with the N-terminal domains of *Bsg25A(Elba1)* and *Elba2*, is required for chromatin domain boundary function. There are multiple methods to probe the gene's effect in Drosophila's embryos; in this work, I will use data analysis to discuss the role of *Elba3* in Drosophila embryonic development, especially in the early stages.

Zelda (Zld) is a transcriptional activator that can bind to CAGGTAG. It allows many different factors to gain access, thus helping them establish the other tissues of the embryo—for example, muscle and nerves [2].

Transcription factors such as *Zelda* and *Elba3* have different effects on early embryonic development in Drosophila. Any dysregulation or mutations in these genes can lead to developmental defects or diseases later. Therefore, comprehending their functions and regulatory pathways is essential for basic science and potential therapeutic interventions.

Recent technological advancements have enabled researchers to study gene expression at a more precise resolution during embryonic development. This has provided new insights into the complex interactions between different cell types and signaling pathways involved in early development. These

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findings may have implications for understanding normal development and regenerative medicine and tissue engineering.

The Drosophila remains an important model organism for studying genetics and developmental biology due to its simplicity, versatility, and relevance to the human genome.

2. Litereture review

2.1. Elba3 expression is upregulated in 0-4 hours' embryos

The expression of *Elba3* transcript reaches its peak during the blastocyst stage (2-4 hours) of embryogenesis and then diminishes. It is observed during oogenesis but not in embryos aged 0–2 hours. Additionally, it shows low expression levels in 22-24 hours of seeds and adult-male fruit flies (Figure 1). Several studies have indicated an upregulation of *Elba3* expression in embryos aged 0-4 hours, suggesting its potential significance for early embryonic development. For example, a study utilized microarray analysis to compare the transcriptomes of embryos at different developmental stages and found that *Elba3* was one of the genes significantly upregulated in embryos aged 0-4 hours compared to later stages [1]. These findings imply that *Elba3* may be crucial during this critical period.

Furthermore, another study employed in situ hybridization techniques to visualize the patterns of *Elba3* expression in embryos. The results demonstrated predominant localization of *Elba3* within the nuclei and cytoplasm of embryos aged 0-4 hours [3]. This localization suggests that Elba3 might regulate gene expression or essential cellular processes for early embryonic development.

Although the precise function of Elba3 during these early stages remains unclear, its dynamic expression pattern indicates its importance for proper embryo formation and subsequent development. Further investigations are necessary to elucidate the specific molecular mechanisms underlying the role played by *Elba3* in early embryogenesis.

In conclusion, multiple studies have provided evidence supporting an increase in the expression level, specifically within a few hours after fertilization. These findings emphasize its potential significance for early embryonic development and call for further exploration into understanding its exact functions and regulatory roles during this critical period.

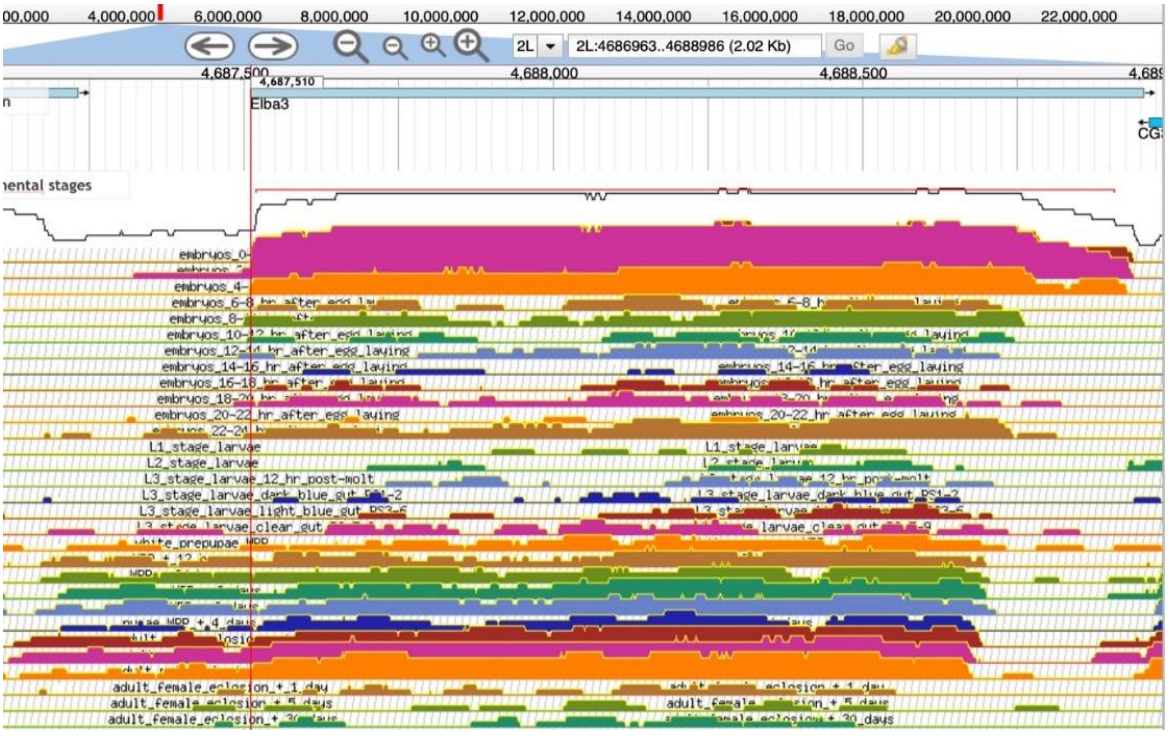

Figure 1. Developmental stages from FlyBase.

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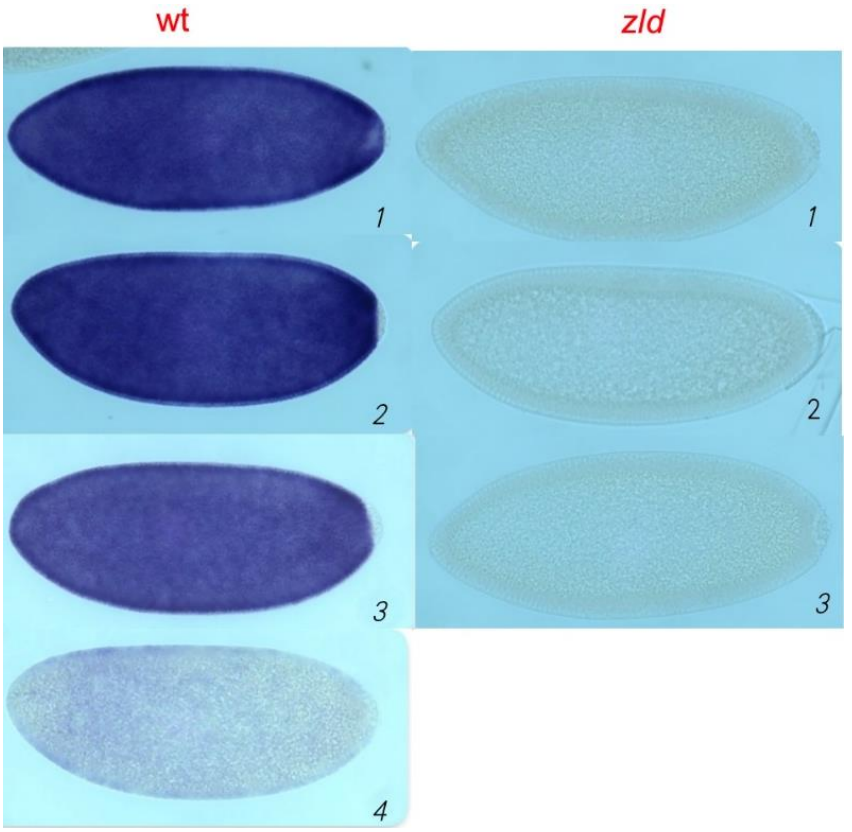

Figure 2. Expression pattern graphs of Elba3 in WT and Zld-mutant embryos (Done by Y.M.)

2.2. Elba3 expression is downregulated in Zld-mutant embryos.

For *Elba3*, the scale used in IGB was 0-300 for Poly II Chip and Poly I Chip, except for zkd 13. The subtype of Elba3 was not shown in the IGB results (Figure 3). *Elba3* expresses low in *Zld* Chip (Figure3). Two *Zld* peaks were shown at *CG11929* ang the gene gap between *CG11929* and *Elba3* (Figure 4). In addition to *Elba3*, there were three adjacent genes, *Bsg25A(Elba1)*, *CG11929* and *CG3251* (Figure 4).

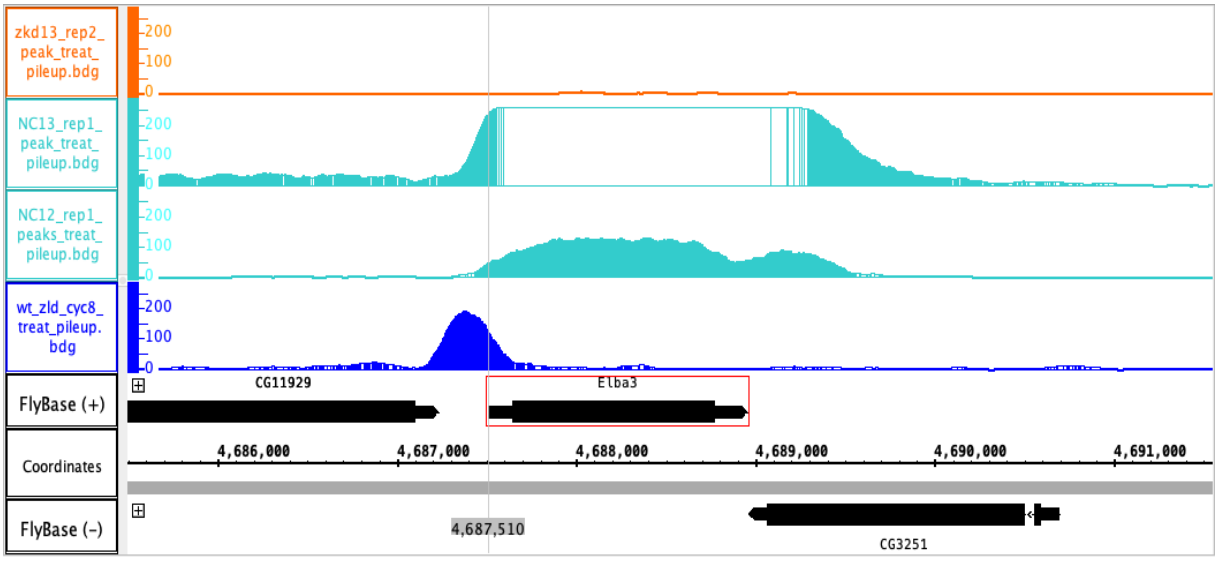

Figure 3. IGB results for *Elba3*(zoom in).

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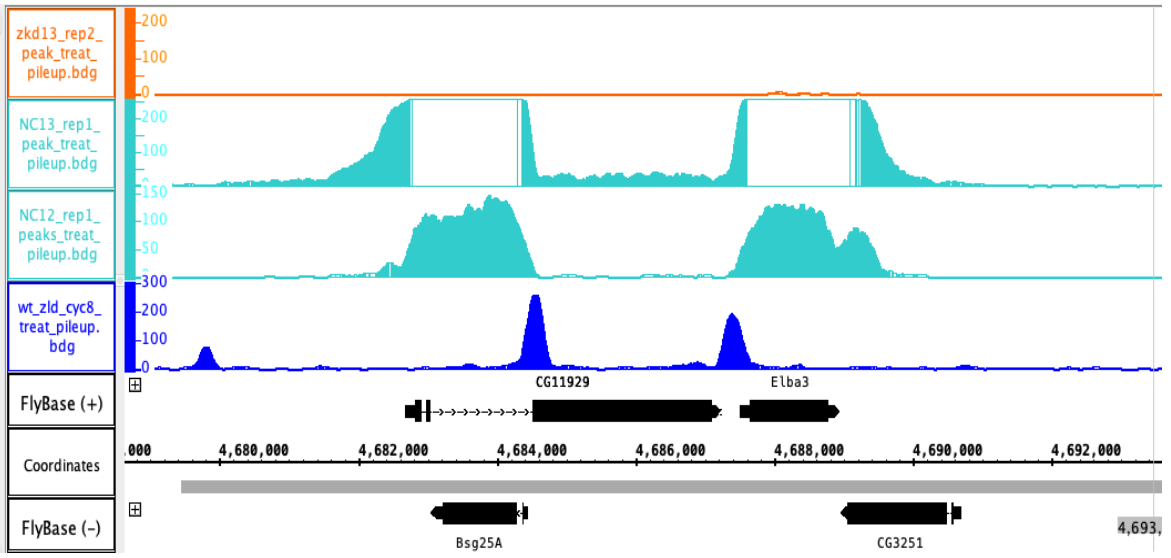

Figure 4. IGB result for *Elba3, Bsg25A(Elba1), CG11929* and *CG3251* (zoom out).

2.3. Elba3 and Zelda work together in a regulatory feedback mechanism.

Elba3 lacks DNA-binding activity and functions as an adaptor protein. [4] *Elba3* is an inhibitory transcription factor that binds to a specific DNA sequence called the *Elba3* site found in the promoters and enhancers of target genes. *Elba3* inhibits the expression of these genes by preventing other transcription factors, including *Zelda*, from binding to their regulatory regions. This inhibition is essential for the embryo's normal development, ensuring that specific genes are expressed at the right time and place. The *Elba3-Zelda* feedback mechanism occurs because *Elba3* represses the expression of *Zelda*, whereas *Zelda* promotes the expression of *Elba3*.

3. Discussion

In addition to its role in early embryonic development, *Elba3* has also been found to play a crucial role in the later stages of Drosophila embryogenesis. Studies have shown that the spatiotemporal regulation of *Elba3* expression suggests its involvement in specific developmental processes and tissues.

A critical aspect of *Elba3*'s function is its nuclear localization, which indicates that it may act as a transcriptional regulator. This suggests that *Elba3* could be involved in controlling the expression of genes essential for proper cell movement, morphogenesis, and pattern formation during embryonic development. Furthermore, experiments with *Elba3* knockout embryos have provided further evidence for its functional significance. These embryos exhibit defects in proembryo formation, embryonic band extension, and segmentation. These findings strongly suggest that *Elba3* is essential for early developmental processes and plays a critical role in later stages of embryo development.

Interestingly, studies have revealed an interaction between *Elba3* and *Zelda* during Drosophila development. It has been observed that *Elba3* acts as a negative regulator of *Zelda* by interacting with the promoter regions of several target genes involved in embryonic patterning and segmentation. By repressing their transcription, *Elba3* helps maintain proper gene expression patterns during development. On the other hand, *Zelda* functions as a positive regulator of *Elba3* expression, forming a feedback loop with it to regulate gene expression throughout different stages of Drosophila development. This intricate regulatory mechanism highlights the importance of maintaining precise control over gene expression patterns for normal embryo development.

Studies have shown that *Elba3*, a protein found in Drosophila, plays a crucial role as a regulator of *Zelda* during the development of this species [5]. *Elba3* interacts with the promoter regions of several target genes involved in embryonic patterning and segmentation. By binding to these promoter regions, *Elba3* effectively represses their transcription, thereby controlling the expression levels of these

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essential developmental genes. Interestingly, there is also a reciprocal relationship between *Zelda* and *Elba3*. While *Elba3* acts as a negative regulator of *Zelda*'s activity, it has been discovered that *Zelda* functions as a positive regulator for the expression of *Elba3* itself. This forms an intricate feedback mechanism between these two proteins to tune gene expression during Drosophila development. The interaction between *Zelda* and *Elba3* highlights the complexity and precision required for proper embryonic patterning and segmentation in Drosophila. The delicate balance maintained by this regulatory mechanism ensures that genes involved in these critical processes are expressed at appropriate levels at specific stages of development.

Further research into the molecular mechanisms underlying this regulatory network will provide valuable insights into how organisms control gene expression during development. Understanding such intricate interactions can shed light on Drosophila biology and potentially contribute to our knowledge about similar processes in other organisms, including humans. In conclusion, studies have revealed that while *Elba3* negatively regulates *Zelda*'s activity by repressing target gene transcription during Drosophila development, a reciprocal relationship exists where *Zelda* positively handles the expression of its negative regulator - forming an essential feedback loop for precise control.

Materials and Methods

3.1. Integrated genome browser (IGB)

In the study, IGB is used to show biological patterns of *Elba3*, *Bsg25A(Elba1)*, *CG11929*, and *CG3251*, including related *Zld* peaks, adjacent genes to the target gene, isoforms of the target gene, and transcription start site (TSS).

3.2. FlyBase

FlyBase is an open database providing information on the Drosophila genome. In this study, FlyBase provides DNA or protein sequence, gene or mutant name, or terms from several ontologies that capture functional, phenotypic, and anatomical data of Elba3, Elba1, and *CG11929*.

3.3. SnapGene

This study uses SnapGene to intuitively edit and manipulate *Elba3* DNA sequences, such as insert, delete, replace, reverse complement, and combine base pairs to perform specific DNA sequence editing. SnapGene automatically identifies promoters, terminators, and coding regions and provides *Elba3* DNA-to-RNA-to-protein transcripts and translation results. SnapGene helps to design and optimize *Elba3* DNA cloning experiments by selecting appropriate restriction sites, primer design, and target DNA sequences for cloning fragment construction and operation.

4. Chop Chop

CHOPCHOP is a network tool for selecting target sites for CRISPR/Cas9, CRISPR/Cpf1, CRISPR/Cas13 or NICKASE/TALEN-directed mutagenesis. This study uses CHOPCHOP to design gene knockout sgRNA for *Elba3*.

4.1. Methods

To investigate the expression pattern of *Elba3* during embryonic development, I used Drosophila's embryonic pictures made by immunofluorescence staining with an *Elba3*-specific antibody and confocal microscopy to analyze the localization of *Elba3* in developing embryos. Download the gene sequence of *Elba3* on Fly Base and import it into Snap Gene. Amplify the *Elba3* gene in IGB, download the gene sequence, find the transcriptional start site, return to Snap Gene, and mark it as plus one. Then, use the sequence lookup function of Snap Gene to find sequences CAGGTAG and TATAA.I also designed an experiment to probe deeper.

Hypothesis:

If *Elba3* is essential for embryonic development, disrupting its expression will negatively affect the outcome of Drosophila embryos.

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Materials:
- Drosophila melanogaster embryos
- CRISPR/Cas9 gene editing system
- Elba3 guide RNA
- Control guide RNA
- Microinjection equipment
- Incubator

Steps:

1. Design and synthesize a guide RNA targeting the *Elba3* gene using the CRISPR/Cas9 gene editing system.
2. Inject a mix of *Elba3* guide RNA and the Cas9 protein into Drosophila melanogaster embryos at the syncytial blastoderm stage, targeting the maternal *Elba3* mRNA.
3. Inject a control guide RNA and the Cas9 protein into a separate group of embryos at the syncytial blastoderm stage.
4. Incubate both groups of embryos at optimal growth conditions and monitor their development until the late stages of embryogenesis.
5. Observe and compare the developmental progress of the experimental group (injected with *Elba3* guide RNA) and the control group (injected with a control guide RNA).

5. Results:

If *Elba3* is essential for embryonic development, disrupting its expression will negatively affect the outcome of Drosophila embryos. The experimental group may exhibit reduced viability and developmental abnormalities compared to the control group, indicating that *Elba3* is critical for normal embryonic development.

To investigate the binding sites of *Elba3* with *Zelda* during embryonic development, I used gene knockout sgRNA (AAAAGTATGGAGTTTACGG) to mutant DNA sequence CAGGTA to CTACATA. (Figure 5) Gene knockdown or knockout experiments can be performed to determine the effects of loss of *Elba3* or *Zelda* on development and to investigate whether there is a functional interaction between the two proteins. Alternatively, transgenic overexpression of *Elba3* and *Zelda* in specific cell types or developmental stages can be used to probe the effects of altered expression levels on embryonic development.

Figure 5. Two primers for in vitro mutagenesis

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