Zelda's Effect on Gene Expression in the Early Development of Drosophila Embryo

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Abstract: Zinc-finger protein Zelda (Zld) is thought to play an essential role in the development of early-stage Drosophila embryos, and this paper takes a step further by confirming the Zld's effect on Class I & II gene expression, and exploring the synergistic effects of Zld and other transcription factors in gene expression. The primary methods used in this paper are Zelda-binding pattern and JASPAR analysis results. It was found that Zld played a direct and indirect, decisive and non-decisive role in Class I and II gene, respectively. Besides Zld, this paper spotted that the gap proteins derived from gap genes were an key transcription factor in the expression of the pair-rule genes, a subdivision of the Class II genes. There is also evidence shows that sloppy paired 1 might be the enhancer of sloppy paired 2. On the basis of previous studies, this work studied the effects of Zld and other transcription factors on the expression of different types of genes in more detail. Directions for future research were discussed.

1 INTRODUCTION

In recent years, several studies have explored the critical role of the zinc-finger protein Zelda (Zld) in early embryonic drosophila. (Liang, Nien, Liu, Metzstein, Kirov, Rushlow 2008) confirmed Zld as a key activator in the early zygotic genome; (Nien, Liang, Butcher, Sun, Fu, Gocha, Kirov, Manak, Rushlow 2011) investigated how Zld affects the timing mechanism of the development of early genes (Fu, Nien, Liang, Rushlow 2014); demonstrated that Zld is a predictor of enhancer activity and the co-coordinate to regulate gene expression. However, the synergistic effects of Zld and other transcription factors in the regulation of gene expression in the preliminary stage still need to be further explored.

For narrowing such a gap, this paper will use Zelda-binding patterns and JASPAR analysis results to summarize the effects of Zld on Class I & II genes and explore whether Zld coordinates with other transcription factors to influence the gene expression.

In this paper, we defined the Class I gene as the ubiquitous gene expressed throughout the embryo at an earlier stage of development. Class II gene, also known as patterning gene, is expressed in some regions of embryos in the later stage of development according to a specific pattern.

We utilized Kuk (CG575) and pairing-rule genes Slp 1&2 (CG6738 & CG2939) as Class I and II gene representatives, respectively. Our research showed that in Class I genes, Zld directly binds to the promoter to activate its expression, while in Class II gene, Zld binds to the enhancer to affect certain expression levels. We also spotted other transcription factors, gap proteins, that control Class II genes' expression patterns.

2 METHODS

2.1 Integrated Genome Browser (IGB)

We used Integrated Genome Browser (IGB) to put several experimental data in order based on Drosophila melanogaster genes. Wildtype RNA polymerase expression in Cycle 12 was used as the baseline reference; wildtype RNA polymerase expression in Cycle 13 and RNA polymerase expression in Cycle 13 when Zld knocked out were used to compare the effect of Zld on gene expression; wildtype ChIP-seq of Zld and TAGteam (CAGGTAG) site were used to confirm the Zld binding pattern. This step enables us to obtain a

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preliminary inference about Zld binding patterns toward the selected genes for conducting further steps.

2.2 JASPAR

JASPAR was used to further affirm Zld's effect on gene expression and predict whether other transcription factors were involved in gene expression (JASPAR 2020). We first got the Zld binding peak coordinate from the IGB and used intercept ± 200bp of it as the region to obtain the FASTA-formatted targeted gene sequence in Biotechnology Information (NCBI) (National Center for Biotechnology Information) database. Based on the criteria with a relative profile score threshold of 80%, JASPAR scanned all transcription factors that have the possibility to bind to the given gene sequence and arranges them by correlation.

2.3 FlyBase & Berkeley Drosophila Genome Project (BDGP)

FlyBase and Berkeley Drosophila Genome Project (BDGP) (BDGP 2021) are both information libraries that can provide data to consolidate our hypothesis. FlyBase database contains information about genes and transcription factors, while BDGP contains gene expression images at different stages.

3 RESULTS & DISCUSSION

3.1 Class I Genes (Ubiquitous Genes)

Kugelkern (kuk, CG5175). "[kuk] encodes a nuclear envelope protein required for nuclear elongation during cellularization." (FlyBase Homepage) And it can be drawn from Figure 1 that kuk is a typical class I gene as it is expressed throughout the whole embryo.

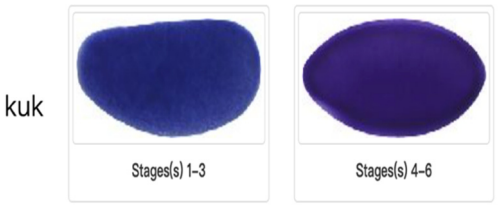
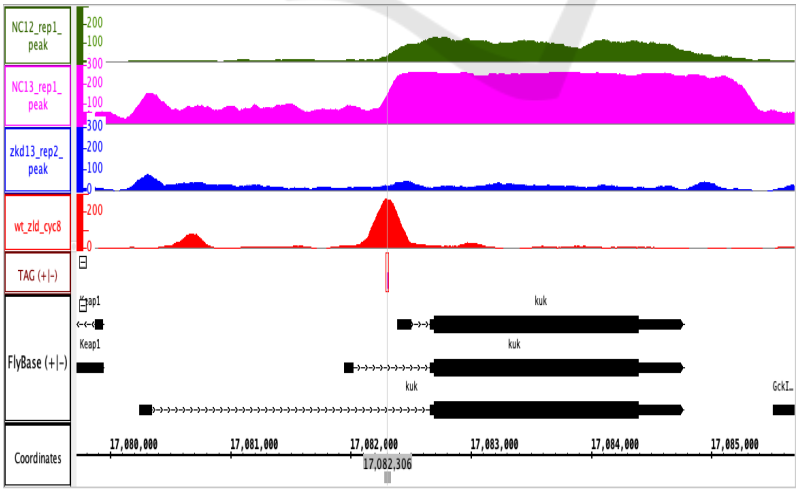


Figure 1: Expression Pattern Image for kuk from BDGP.

As shown in Figure 2, the highest Zld binding peak in the 4th track corresponds to the TAGteam site (CAGGTAG) in the 5th track. We further used JASPAR to scan ±200bp near this site, as shown in table 1, confirming that there was indeed a strong Zld binding site with a high score of 13.82. Then, by comparing the kuk expression with or without Zld, we see that a considerable amount of RNA expression is demonstrated in wildtype case (the second track), while the expression level witnesses a huge decrease when Zld was knocked out (the third track). Such a phenomenon suggests that Zelda is a key determinator for the expression of kuk.



Note. RNA polymerase expression in Cycle 12 (the first green track); wildtype RNA polymerase expression in Cycle 13 (the second pink track); RNA polymerase expression in Cycle 13 when Zld knocked out (the third blue track); wildtype ChIP-seq of Zld (4th red track) and TAGteam site (5th brown track). The previous four tracks are on the same scale from 0 to 300.

Figure 2: Snapshot of Gene Kuk From IGB.

Table 1 JASPAR –kuk–vfl–Analysis Result

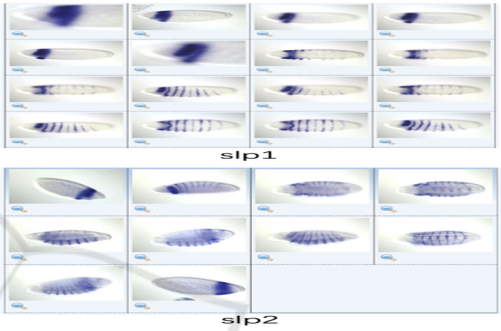
Matrix ID	Name	Score	Relative score	Sequence ID	Start	End	Strand	Predicted sequence
MA1462.1	vfl	13.8206	0.993095999606	NT_033777.3:17082105-17082505	197	208	+	CGGCAGGTAGAT
MA1462.1	vfl	12.0672	0.958080363668	NT_033777.3:17082105-17082505	221	232	-	TTGCAGGTACGT

Note. Vfl, as known as Zld; The higher the score, the higher the affinity that the transcription factors bind to the gene sequence.

3.2 Class 2 Genes (Patterning Genes)

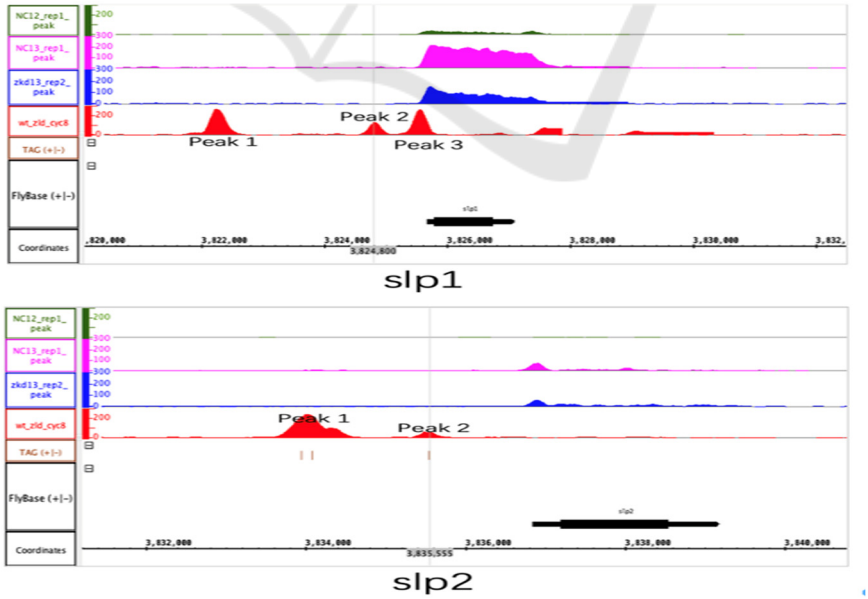
This research selected sloppy paired 1 (slp1) & sloppy paired 2 (slp2) / CG16738 & CG2939 as the Class 2 gene for analysis because they accord with the Class 2 gene's criterion of being expressed in a certain area of the embryo at a later stage of development (demonstrated in Figure 3). Figure 3 also reveals the similar expression pattern that slp1 and slp2 share, that is, start from the head and gradually extend to the whole embryo in strips with intervals. It shows the slp1 and slp2's role in the process of establishing body segments as pair-rule genes.

By comparing the second and third tracks of Figure 4, we found that after Zld was knocked out, the gene expression of slp1 experienced a moderate decline, while the gene expression of slp2 did not change significantly. In the fourth column, several Zld binding peaks in this gene segment were displayed, and JASPAR analysis confirmed that Zld indeed has high scores in these peaks both in slp1 & slp2 (Table 2). Therefore, we speculated that Zld could play an influential but not decisive role in the expression of Class 2 genes, and other transcription factors are of more significant impact on their expression.



Note. Drosophila embryos are in the developmental stage 4-6.

Figure 3: Expression Pattern Image for slp1 & slp2 from BDGP.



Note. Track contents are the same as Figure 2.

Figure 4: Snapshot of gene slp1 & slp 2 from IGB.

Table 2: JASPAR -slp1 & slp 2 -vfl- Analysis Results *Note.* The number in the Peak column stands for Zld binding peak in the 4th track of Figure 3.

Gene	Matrix ID	Name	Score	Relative score	Sequence ID	Start	End	Strand	Predicted sequence	Peak
Slp2	MA1462.1	vfl	13.2734	0.982167314844	NT_033779.5:3833900-3834100	176	187	+	CGGCAGGTAGCG	1
Slp1	MA1462.1	vfl	12.2371	0.961472452634	NT_033779.5:3825455-3825655	79	90	-	CATCAGGTAGTT	3
Slp1	MA1462.1	vfl	12.0538	0.957812957086	NT_033779.5:3822050-3822450	175	186	-	CTTCAGGTAGTG	1
Slp1	MA1462.1	vfl	11.035	0.937467450787	NT_033779.5:3822050-3822450	144	155	-	ATCCAGGTAAGA	1
Slp2	MA1462.1	vfl	10.8576	0.933924242155	NT_033779.5:3835455-3835655	83	94	+	GCTCAGGTAAAA	2
Slp1	MA1462.1	vfl	10.4841	0.926465843882	NT_033779.5:3824600-3825000	185	196	-	ACTCAGGTAATC	2
Slp2	MA1462.1	vfl	10.0659	0.918115258717	NT_033779.5:3833900-3834100	40	51	+	GCGTAGGTAGGA	1

Table 3: JASPAR -slp1 & slp 2 -gap genes- Analysis Results.

Gene	Matrix ID	Name	Score	Relative score	Sequence ID	Start	End	Strand	Predicted sequence	Peak
Slp2	MA0452.2	Kr	14.616	0.937574067021	NT_033779.5:3833900-3834100	149	162	+	CTTAACCCCTTCAG	1
Slp1	MA0452.2	Kr	14.5752	0.93695773075	NT_033779.5:3824600-3825000	172	185	+	TTTAACCCCTTCGG	2
Slp1	MA0452.1	Kr	13.7451	0.966483513258	NT_033779.5:3824600-3825000	174	184	-	CGAAGGGGTTA	2
Slp2	MA0452.2	Kr	13.5852	0.922015290106	NT_033779.5:3833900-3834100	1	14	+	CTTAACTCTTTCGA	1
Slp1	MA0049.1	hb	12.8235	1.0000000052	NT_033779.5:3822050-3822450	388	397	+	GCATAAAAAA	1
Slp2	MA0452.1	Kr	12.4301	0.933338080611	NT_033779.5:3833900-3834100	151	161	-	TGAAGGGGTTA	1
Slp2	MA0452.1	Kr	11.79	0.917205123065	NT_033779.5:3833900-3834100	3	13	-	CGAAAGAGTTA	1
Slp1	MA0459.1	tll	11.6933	0.887199901339	NT_033779.5:3822050-3822450	205	214	+	AAAAGTGAAA	1
Slp2	MA0049.1	hb	11.2386	0.951168571865	NT_033779.5:3835455-3835655	68	77	-	TCATAAAAAA	2
Slp1	MA0452.2	Kr	10.833	0.880472403084	NT_033779.5:3824600-3825000	71	84	-	GGCAATCCTTTTGG	2

In addition, it was also found from JASPAR analysis (Table 3) that both slp1 and slp2's peaks have high scores of gap protein, e.g., Kruppel (Kr), Hunchback (hb), and Tailless (tll). According to Griffiths et al., Kr and hb both are regulators, but repressor and activator, respectively, jointly control the expression of the pair-rule gene. Their differences in concentration at the embryo's position control each pair-rule stripe formation (Griffiths, Doebley, Peichel, Wassarman 2020). Our data reaffirm the above findings and identify one more gap protein, tll, as the regulator for forming embryonic stripe formation.

Other than gap genes, another high score gene repeated shows up in the peak of slp1 from JASPAR analysis results (Table 4), namely defective proventriculus (dve), which is considered as a transcriptional repressor that involves in

developmental patterning (FlyBase Homepage). We speculate that dve has the same function as the Kr to control the slp1 gene expression. The data presented in this paper support such a view, but specifically, how dve influences the embryonic stripe formation remains to be determined by further studies.

We also deduced the relationship between slp1 and slp2 through IGB graphic and JASPAR analysis. Firstly, from the zoom-out snapshot of the two genes (Figure 4), we found that slp1 and slp2 appeared in pairs, and slp1 appeared earlier than slp2. Second, slp1 protein shows high scores in both peaks of slp2 (Table 5). Both phenomena are suggesting that slp1 is an enhancer of slp2. However, this is only speculation based on the data. A control experiment should be carried out to compare the expression of slp2 with or knocking out slp1 to determine the role of slp1 in slp2's expression.

Table 4 JASPAR - slp 1 - dve- Analysis Results

Matrix ID	Name	Score	Relative score	Sequence ID	Start	End	Strand	Predicted sequence	Peak
MA0915.1	dve	12.1441	0.985360610986	NT_033779.5:3824600-3825000	313	320	-	CTAATCCC	2
MA0915.1	dve	11.6975	0.975487844496	NT_033779.5:3824600-3825000	270	277	+	ATAATCCC	2
MA0915.1	dve	11.3993	0.968895149107	NT_033779.5:3824600-3825000	183	190	-	GTAATCCG	2

Table 5 JASPAR - slp 2 - slp1- Analysis Results

Matrix ID	Name	Score	Relative score	Sequence ID	Start	End	Strand	Predicted sequence	Peak
MA0458.1	slp1	10.4209	0.904717113268	NT_033779.5:3833900-3834100	84	94	-	CTGTTTACATG	1
MA0458.1	slp1	11.992	0.943959392058	NT_033779.5:3835455-3835655	181	191	-	TTGTTTTCACA	2

4 CONCLUSION

In this study, kuk, slp1 and slp2 were used as representatives of class 1 and class 2 genes to investigate the role of Zld in regulating the expression of different types of genes. Specifically, Zld plays a decisive role in the expression of class 1 gene, as when Zld being knocked out, the expression of kuk will be greatly reduced; The effect of Zld on class 2 gene is not direct and definite because the expression of slp1 and slp2 don't witness such significant decrease after same procedure. These findings further confirm that zin-finger protein Zld plays an important role in drosophila embryonic development.

At the same time, this study comes across two tentative conclusions for further researches to confirm. First, several transcription factors (i.e. Dve, Kr, Hb, etc.) were identified that might collaborate with Zld to control the expression of pair-rule gene. Secondly, slp 1 is a potential enhancer of slp2. The data in this paper support these hypothesis, but uniquely designed experiments are needed to validate.

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REFERENCES

BDGP. (2021). Patterns of gene expression in Drosophila embryogenesis. Berkeley Drosophila Genome Project. https://insitu.fruitfly.org/cgi-bin/ex/insitu.pl.

FlyBase. (n.d.). FlyBase homepage. FlyBase Homepage. https://flybase.org/.

Fu, S., Nien, C.-Y., Liang, H.-L., Rushlow, C. (2014). Co-activation of microRNAs by Zelda is essential for EARLY drosophila development. Development, 141(10), 2108–2118.

Griffiths, A., Doebley, J., Peichel, C., Wassarman, D. (2020). Introduction to genetic analysis. W.H. Freeman and Company Macmillan Learning, New York.

JASPAR 2020. http://jaspar.genereg.net/.

Liang, H.-L., Nien, C.-Y., Liu, H.-Y., Metzstein, M. M., Kirov, N., Rushlow, C. (2008). The zinc-finger protein Zelda is a key activator of the early zygotic genome in Drosophila. Nature, 456(7220), 400–403.

Nien, C.-Y., Liang, H.-L., Butcher, S., Sun, Y., Fu, S., Gocha, T., Kirov, N., Manak, J. R., Rushlow, C. (2011). Temporal coordination of gene networks by Zelda in the EARLY Drosophila Embryo. PLoS Genetics, 7(10).

U.S. National Library of Medicine. (n.d.). National center for Biotechnology Information. National Center for Biotechnology Information. https://www.ncbi.nlm.nih.gov/.229.