The Effect of the Transcription Factors Zelda, Dorsal, and Bicoid on the Pattern Formation of Drosophile Melanogaster during the Early Development

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Abstract: The insect Drosophila melanogaster refers to the common species fruit fly. Many researches have been done extensively on its marvelous early embryonic development, particularly during nuclear cycle 1-14, to better understand the transcriptional mechanism behind it. Three well-acknowledged transcription factors have been discovered by many scientists more than a decade ago, and they suggested that these transcription factors, along with some others, are of vital importance to the decision making upon how a gene is expressed and where it is expressed. In this study, I did some online researches, together with some reliable tools, to examine the role of these transcription factors in regulating the gene expression and pattern formation of fruit fly. I found out that, not surprisingly, these transcription factors have a profound and decisive impact on the development.

1 INTRODUCTION

During the early development of Drosophila, the gene expression of the embryo is dominated completely by the mother. The mother decides which gene to be expressed and puts those maternally-expressed gene products into the embryo and allows the zygotic gene expression to happen. The zygote then starts to express some of the genes at about one hour of development, and a lot of the genes that are expressed turn out to be ubiquitous. Then at about three hours of development, the egg is filled with those ubiquitous genes that are expressed just about everywhere. But sooner after this stage, the egg starts to have what we call the patterning genes, because they are expressed in certain patterns.

In this review, I focused on the effect of zelda, dorsal, and bicoid, which are the three most important transcription factors, on the early embryonic development of Drosophila, in particular their regulations on a single gene, and multiple genes that give rise to specified pattern formations.

1.1 Overview of the Maternal-To-Zygotic Passway, MZT

The fertilized egg of Drosophila starts its development very differently from you and me. Instead of cell division, the egg starts with rapid division of nucleus. When the number of nucleus in the embryo is large enough, they migrate to the surface of the egg and start the formation of *cellular blastoderm,* where the plasma membrane starts to grow inward and encapsulates those nucleus to form cells. In the earliest stages of development, the zygotic genome is generally inactive, with the embryo's molecular processes driven by proteins, RNAs and other substances packed into the egg by the mother (Eisen, 2011). Later on, the embryo passes through a stage during which developmental control is handed from maternally provided gene products to those synthesized from the zygotic genome, known as MZT (Liang, Nien, [...], and Rushlow 2008). This complex yet fascinating process has been studied extensively by many scientists and college professors. Christine Rushlow (Alberts, Johnson, Lewis at al. 2002), a professor at NYU, together with some other scientists, discovered that many of the early genes in Drosophila share a cis-regulatory heptamer motifs, CAGGTAG

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and related sequences, collectively referred to as TAGteam sites raised the possibility that dedicated transcription factor could interact with these sites to activate transcription. The protein that binds to the site is known as Zelda (the zinc-finger protein). Rushlow (Alberts, Johnson, Lewis at al. 2002) and her colleagues suggested that Zelda has an important role in the activation of the early zygotic genome and may also be responsible for regulating maternal RNA degradation during MZT.

Although the process of maternal regulation seems to be short, it is crucial because it puts the necessary gene products to the zygote, such as zelda, bicoid, and dorsal, allowing for the zygotic gene expression and specified pattern formation. Unlike zygotic mutation, the mutation of maternally-expressed genes could be fatal to the embryo. The mutant of maternally-expressed gene bicoid, for example, will result in a headless larva (Carrell, O'Connell, Jacobsen, Pomeroy, Hayes, Reeves 2017). Table 1 and 2 shows the two types of mutation.

Table 1: Maternal mutation Maternally required genes.

Parents	Offspring
M/+♂ × M/+♀	M/M, M/+,+/+ all normal
M/M♂	M/M, M/+ all normal
+/+, M/+ or M/M ♂ × M/M♀	M/+, M/M all mutant phenotype

Table 2: Zygotical mutation Zygotically required genes.

Parents	Offspring
M/+♂ × M/+♀	M/+, +/+ normal M/M mutant phenotype

2 METHOD AND RESULT

More recent studies aimed at the interaction between Zelda as well as other important transcription factors in the formation of specified patterns during the early embryonic development, particularly with some of the maternally-expressed genes, such as bicoid and dorsal. Those genes that are expressed in patterns are collectively referred to as patterning genes. It is the fly Drosophila melanogaster, more than any other organism, that has transformed our understanding of how genes govern the patterning of the body. The anatomy of Drosophila is more complex than that of C. elegans, with more than 100 times as many cells, and it shows more obvious parallels with our own body structure (Rushlow, Colosimo, Kirov 2001). Therefore, to understand Drosophila is to initiate our deep knowledge towards gene regulation, and dig into its secret ever more.

According to the 2008 paper by Christine Rushlow et al. (Zehra, Thomas 2016), the major burst of activity occurs during 2 to 3h of development when the embryo is undergoing cellular blastoderm formation. Many genes contain TAGteam in their upstream regulatory region including direct targets of bicoid and dorsal. Christine Rushlow et al. (Zehra, Kornberg 2016) performed a yeast one-hybrid screen and gel shift on zen, a gene that requires TAGteam for the early formation. What they found out was the site CAGGTAG, which had the strongest affinity for zelda. They then generated deletion alleles of zelda by imprecise excision on gene CG12701(the gene that translates zelda protein), and found abnormal body formation in the embryo. Through a IGB test, I then examined the Zelda binding peaks at the promoter and enhancer sites of zen during nuclear cycle 8 of embryo, and RNA polymerase binding peaks on the gene during both nuclear cycle 13 wild type and zelda knock-down. As shown in figure 1, there is a high peak of zelda binding right at the start of transcription, and several base pairs away at the enhancer region. The blue lines below represent the TAGteam, CAGGTAG. During NC13 WT, there are several peaks of RNA polymerase binding peaks, suggesting the proceeding of transcription. Whereas during zkd13, all the peaks are gone. This result further indicates the importance of zelda and corresponds with the study by Christine Rushlow et al. (Zehra, Thomas 2016).

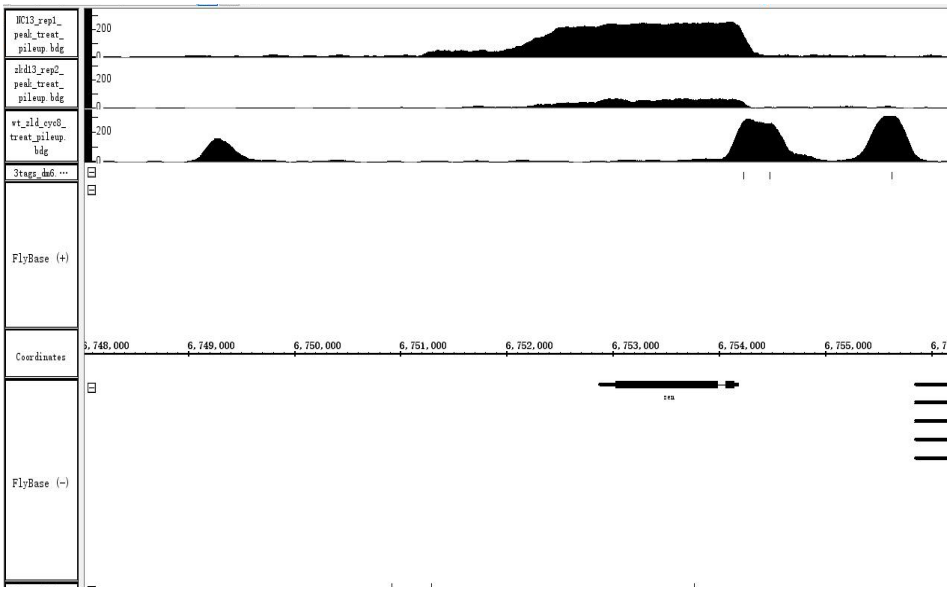

Figure 1: The IGB analysis on zen.

Figure 2: The transcriptional pattern of zen during the early development.

But how does zen obtain its unique transcriptional pattern? Figure 2 shows a set of pictures taken from BDGP. It clearly shows the pattern of zen during the nuclear cycle 4-6 of embryonic development, which is occupied in the amnioserosa, dorsal ectoderm, lateral ectoderm region. Zelda is translated by the gene CG12701, a ubiquitous gene that switches off at cycle 14, before cellularization (Gilbert 2000). As mentioned previously, the expression of zen is controlled by zelda extensively, with zelda mutation comes no body formation in the transcriptional region. However, zelda is a ubiquitously expressed protein, whereas the expression of zen is only limited to the amnioserosa, dorsal ectoderm, lateral ectoderm

of the embryo, so there must be something else that is repressing zen during the development. This something turns out to be one of the most important maternally-expressed genes: dorsal (DI). The dorsal gradient in the blastoderm embryo, through modeling and experimental studies, was shown to be caused by Cactus complex. This gradient is very important in the early development. Where dorsal concentration is highest, it switches on twi and sna, these two genes code for transcription factor that represses brk, and sog in the middle region of the embryo, where the concentration of dorsal is significantly lower than the bottom region but high enough to represses decapentaplegic(dpp) and zen in the upper region, where the dorsal concentration is zero. Brk and sog are also responsible for repressing dpp and zen. Dpp is a gene belonging to the *TGF-β superfamily*, which gives rise to a series of signal translocation passway all the way down to the specific target genes. Dpp regulates the gene zen via the receptor-regulated Mad protein. Table 3 represents a JASPER result (note that I also highlighted zelda and dorsal, and the CAGGTAG sequence, to further suggest the importance of zelda) which I found out that the transcription factor Mad binds very close to the enhancer site of zen--it is only about 50 base pairs away and have a relative high binding score, suggesting that dpp is responsible for the regulation of zen because Mad helps translocating its signal to the gene zen. Furthermore, from the figure we can see many dorsal (represented by symbol dl) binding sites, suggesting that dorsal is repressing zen extensively in the middle region of the embryo.

Table 3: The JASPER result of zen at the enhancer site(range selected: 200 base pair).

Name	Score	Relative Score	Start	End	Strand *	Predicted sequence
cad	14.3542	0.9660256	124	134	+	GATCATAAAAC
cad	14.009	0.9614392	82	92	+	TGCCATAAAAT
Stat92E	12.951	0.8955322	24	38	-	AAGATTTTCGGGAAA
vfl	12.8612	0.9739354	162	173	-	TTTCAGGTAGGT
Mad	11.9366	0.9061524	244	258	+	GGGGCGCCGCCAGGC
fkh	11.8268	0.9307487	153	163	+	TGTTTATTCAC
twi	11.7513	0.9311181	71	81	+	TCACACATGCC
dl	11.0919	0.8878104	19	30	+	CTGGTTTTCCCG
Hsf	10.9239	0.8929197	23	34	+	TTTTCCCGAAAA
slp1	10.8339	0.9150343	5	15	-	CTGTTTTCGTT
dl(var.2)	10.644	0.8938483	19	28	+	CTGGTTTTCC
bcd	10.5191	1	396	401	-	TAATCC
cad	10.4822	1	57	63	+	TTTATTG
dl	10.3655	0.8695819	342	353	+	TGGGTTTCTCCC
hsf	10.3373	0.8803307	297	308	+	AAATCCAGAAGT
* "+ " stands for up-strand, "- " stands for down-strand						

The patterning genes are yet more fabulous than you probably think. A large-scale genetic screen has shown that many genes during the early development can be classified into four categories, which are, respectively, egg-polarity genes, gap genes, pair-rule genes, and segment polarity genes. The highest concentration of bicoid is located at the anterior of the embryo, and gradually fades off towards the posterior. Bicoid is encoded by a *maternal effect gene* that produces mRNAs placed in certain regions of the embryo. Consequently, bicoid is classified as an egg-polarity gene because it is expressed in the anterior of the embryo. This special patterning must have a reason for its existence.

Bicoid is responsible for the formation of the head because it is mostly concentrated at the anterior of the embryo. And as mentioned previously, the mutation of bicoid can bring death to the larva even before its birth because the larva will not form a head. But what keeps the bicoid in its limited region? In 1988, Christiane Nüsslein-Volhard (who later won the noble prize for her discovery of the anterior-posterior polarity of early development of Drosophila) found out that two genes, *exuperantia* and *swallow,* are responsible for repressing bicoid, and with the mutation of these two genes comes the diffusion of bicoid further to the posterior region.

Bicoid also activates the adjacent gene *hunchback*, a zygotic gene responsible for the formation of thorax, as bicoid is developing (the hunchback concentration appears at about 2 hours of development). Hunchback is considered as a gap gene because it is in gap with another gene (information not shown). Bicoid also turns on the genes *giant* and *Krüppel*, which are yet another two examples of zygotic gap genes (for more information about bicoid, see *Bicoid gradient formation and function in the Drosophila pre-syncytial blastoderm*, by Zehra Ali-Murthy and Thomas B Kornberg, 2016). And all these above-mentioned genes have an impact on the formation of pair-rule genes. For example, the stripe gene eve is activated by bicoid and hunchback, whereas Krüppel and giant represses it, keeping it limited in the stripe region.

3 CONCLUSION

The transcription factor zelda plays an important role in the embryonic development of fruit fly. It is first translated by the maternal gene and later replaced by the zygotic ones. The mutation of maternal gene translating zelda is fatal because the embryo lacks the transcription factor zelda to regulate the gene expression. The greatest affinity for zelda is CAGGTAG, and it is shown to appear on both promoter and enhancer sites of many pre-celluar genes. And the lack of binding on either of these two sites can bring to the non-transcription of the gene and the abnormal body formation.

Dorsal is a maternally-expressed gene. It establishes a gradient where it is mostly concentrated at the bottom of the embryo and none at the top of the embryo. This gradient helps establish the specified transcriptional pattern, because it both activates and represses genes, and these genes that are activated or repressed also activate and repress each other, limiting each other in the specific region.

Similar to dorsal, bicoid also activates and represses certain genes and establishes the specified transcriptional pattern. It establishes the gradient where it is mostly concentrated at the anterior region, and gradually fades off towards the posterior region. Unlike dorsal, bicoid has a more profound effect because it is the premise for the later formation of gap genes, pair-rule genes, and segment polarity genes since these genes that are activated or repressed by bicoid also interact with each other in certain ways(I did not talk about segment polarity genes because it happens in the late stage of development).

Besides the information covered in this paper, there are many other aspects of Drosophila that are also being studied by scientists extensively. Consequently, understanding Drosophila is a big giant in the field of biology, and will no doubt receive more attention in the future.

REFERENCES

Alberts B, Johnson A, Lewis J, at al. (2002). Drosophila and the Molecular Genetics of Pattern Formation: Genesis of the Body Plan. https://www.ncbi.nlm.nih.gov/books/NBK26818

Carrell, S. N., O'Connell, M. D., Jacobsen, T., Pomeroy, A. E., Hayes, S. M. and Reeves, G. T. (2017). A facilitated diffusion mechanism establishes the Drosophila Dorsal gradient. https://journals.biologists.com/dev/artical/144/23/4450/19267

Eisen M., (2011). Zelda (the coolest transcription factor ever) is a master regulator of embryonic adolescence. https://www,michaeleisen.org/blog/?P=617

Gilbert SF. (2000). The origins of Anterior-posterior Polarity. https://www.ncbi.nlm.nih.gov/books/NBK9983

Liang H., Nien C.Y., [...], and Rushlow C. (2008). The zinc-finger protein Zelda is a key activator of the early zygotic genome in Drosophila. https://pubmed.ncbi.nlm.nih.gov/18931655/

Rushlow C., Pamela F. Colosimo, and Kirov. N. (2001). Transcriptional regulation of the Drosophila gene zen by competing Smad and Brinker inputs. genesdev.cshlp.org/content/15/3/340

Tadros W., Howard D. Lipshitz. (2009). The maternal-to-zygotic transition: a play in two acts. https://journals.biologists.com/dev/article/136/18/3033/65348/The-maternal-to-zygotic-transition-a-play-in-two

Zehra Ali-Murthy, Thomas B Kornberg. (2016). Bicoid gradient formation and function in the Drosophila pre-syncytial blastoderm. https://elifesciences.org/articles/26811