学位論文の要旨

Abstract of Thesis

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学位論文題目 Title of Thesis（学位論文題目が英語の場合は和訳を付記）

Analysis of control mechanism of the pupal development and eclosion timing by transcription factors FTZ-F1 and Blimp-1 in *Drosophila melanogaster*

(キイロショウジョウバエの転写因子 FTZ-F1 と Blimp-1 による蛹発生および蛹化タイミングの制御機構の解析)

学位論文の要旨 Abstract of Thesis

During the development in *Drosophila*, six morphologically distinct developmental events, hatching of embryo, two larval molts, puparium formation, pupation, and eclosion of adult, occur at relatively precise time. These events are regulated by an insect hormone, ecdysone. Transcription factor FTZ-F1 is a member of the nuclear hormone receptor, induced after ecdysone pulse, expressed transiently in almost all organs during the development, and plays important roles during embryogenesis, larval ecdysis, and early metamorphic stages. However, its expression pattern, regulation mechanism, and functions during the pupal period are still unknown.

To know the function of FTZ-F1 during the pupal stage, its expression pattern was analyzed by Western blotting. The results revealed that the expression of βFTZ-F1 was increased at the mid-pupal period, reached a high level during the late pupal stage, and declined just before eclosion, and that the decline timing in female was earlier than that in male, consistent with the difference of the eclosion timing. By immunohistological analysis in the late pupal stage, FTZ-F1 was detected in the nuclei in most cells, except that cytoplasmic localization was observed in oogonia and follicle cells of the ovary. Because the βFTZ-F1 expression level starts to increase at the time when the ecdysteroid titer decreased to a low level, I expected that βFTZ-F1 is induced in whole body after the decline of ecdysteroid level. To examine this possibility, I injected 20-hydroxyecdysone at different doses into female pupae at different time points and measured

the expression level of βFTZ-F1 6 hours later by Western blotting. The increase of βFTZ-F1expression level was inhibited by injection of 20-hydroxyecdysone at high dose, confirming that the expression of βFTZ-F1 is dependent on decline of ecdysteroid level. Because it has been shown that Blimp-1, an ecdysone inducible transcriptional repressor, binds to the promoter region of the *ftz-f1* gene and plays an important role in determining the precise timing of *ftz-f1* expression during the prepupal period, I examined developmental changes in *ftz-f1* and *blimp-1* mRNA levels during the pupal stage by RT-PCR. The result showed that *ftz-f1* mRNA level was increased after disappearance of *blimp-1* transcript, suggesting that the *ftz-f1* gene is also regulated by Blimp-1 during the pupal stage. Next, *blimp-1* was overexpressed in whole body during the pupal stage using *GAL4/UAS* system, and the effect on βFTZ-F1 expression was detected by Western blotting. I found that βFTZ-F1 expression was severely repressed in animals carrying *tub-GAL4>UAS-blimp-1*, while in control animals, the expression level started to increase at the mid-pupal stage. Moreover, *blimp-1* overexpression in whole body in the animals carrying *tub-GAL4>UAS-blimp-1* inhibited pupal development and eclosion. To further explore the effect of *blimp-1* overexpression on βFTZ-F1 expression in whole body, I induced Blimp-1 under control of heat shock promoter using *hs-blimp-1* line and the expression level of βFTZ-F1 was examined by Western blotting during the mid- to late pupal stage. As expected, significant delaying in the expression level of βFTZ-F1 as well as in pupal development were observed, although the same heat treatment did not cause any effect on βFTZ-F1 expression or pupal development in control animals. In contrast, *blimp-1* knockdown in whole body during the pupal stage using *arm-GAL4>UAS-blimp-1 RNAi*, which can express *dsblimp-1* RNA in whole body, induced premature expression of βFTZ-F1 and advancing of pupal development and eclosion timing. Taken together, I concluded that Blimp-1 also acts as a repressor for the *ftz-f1* gene at the pupal stage and plays an important role in pupal development including eclosion.

The observed correlation between the timing of the pupal development and the expression timing of βFTZ-F1 in *blimp-1* knockdown or overexpressed animals prompted me to examine the function of βFTZ-F1 for the determination of the developmental timing during the pupal period. When βFTZ-F1 was prematurely expressed by heat shock in the *hs-βFTZ-F1* line, which can induce βFTZ-F1 under control of heat shock promoter, advancing of pupal development was observed. In contrast, knockdown of *ftz-f1* by using *tub-GAL4>UAS-ftz-f1 RNAi*, which can express *dsftz-f1* RNA in whole body, or by using *hs-FTZ-F1 RNAi* line, which can express *dsftz-f1* RNA under control of heat shock promoter, induced delaying of pupal

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development. From these results, I concluded that βFTZ-F1 plays an important role in determination of the developmental timing during pupal development.

On the other hand, I expected that the decline of βFTZ-F1 is important for determination of the eclosion timing because I found that the expression level of βFTZ-F1 was declined just before eclosion. To examine this possibility, pupae of the *hs-βFTZ-F1* line were heat shocked at the end of the pupal stage to induce βFTZ-F1 and the eclosion timing was observed. As expected, the eclosion timing in βFTZ-F1 induced animals was delayed about 4 hours compared to control animals. In contrast, around 6 hours advancing of the eclosion timing was observed in *ftz-f1* knockdown animals by using *GAL4/UAS* system, and around 5 hours advancing of the eclosion behavior by using *hs-FTZ-F1 RNAi* line was observed compared to control animals. Taken all together, I concluded that the eclosion timing is dependent on the decline of βFTZ-F1 expression level.

Next, I examined effects of *blimp-1* and *ftz-f1* knockdown in specific organs during the pupal period by using *GAL4/UAS* system. Defects in eye, wing, antenna, and leg development were observed when *blimp-1* or *ftz-f1* was knocked down in these organs during the pupal stage. Interestingly, I found that *blimp-1* knockdown in fat body induced premature expression of βFTZ-F1 and advancing of pupal development and eclosion timing. Moreover, *ftz-f1* knockdown in fat body during the pupal stage induced advancing of eclosion timing, confirming that the decline of βFTZ-F1 in fat body controls eclosion timing. These results indicate that the fat body is an important organ in which the *ftz-f1* is regulated by Blimp-1 and βFTZ-F1 determines the timing of pupal development and eclosion.