EXPRESSION OF THE FUNCTIONAL ECDYSONE RECEPTORS DURING DEVELOPMENT OF *Drosophila melanogaster*

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In *Drosophila*, the steroid hormone ecdysone triggers the key regulatory cascades controlling the coordinated changes in the developmental pathway of molting and metamorphosis. Ecdysone action is mediated by heterodimer consisting of the ecdysone receptor(EcR) and *ultraspiracle* proteins (USP). In this study, the transcripts of functional ecdysone receptors during development were analyzed by using a RT-PCR assay. The transcripts of *usp* and *EcR* isoforms were detected in all developmental stages. *usp* transcripts were detected with nearly equal amount in all developmental stages. Each EcR isoform have independent and quite distinct spatial and temporal expression patterns during development(Table 1). In general, the larval tissues contain more EcR-B1 than EcR-A, while imaginal discs contain much more of EcR-A than EcR-B1. This result suggest that differential combination of ecdysone isoforms with USP control the hormonally regulated aspect of developmental dision in different tissues.

INTRODUCTION

The metamorphosis of holometabolous insects which leads to the dramatic reorganization of the entire body plan, offer a simple system in which to study the hormonal regulation of development. In *Drosophila melanogaster*, divergent morphogentic pathways are initiated at the end of larval development in response to the steroid hormone 20-hydroxyecdysone(20-HE). Nearly all larval tissues are histolyzed, the imaginal discs and abdominal histoblasts differentiate into adult cuticular structure while clusters of imaginal cells form the internal organs that replace their larval counterparts (Robertson, 1936 ; Bodenstein, 1965). These differences in the metamorphic response of imaginal tissues to 20-HE, as well as differences in the degenerative response of larval tissues to this steroid, evoke a fundamental question about metamorphosis. *Drosophila EcR* gene encodes three protein isoforms(EcR-A, EcR-B1 and EcR-B2) that posses the same DNA- and hormone-binding domains but are distinguished by different N-terminal regions (Koelle et al., 1991 ; Talbot et al., 1993). EcR proteins by themselves are not active ecdysone receptors ; rather they are activated by forming heterodimers with USP, another member of the steroid recepotor superfamily encoded by the *Drosophila* gene *ultraspiracle*(*usp*, Koelle, 1992 ; Yao et al., 1992 ; Koelle et al., 1993 ; Thomas et al., 1993). EcR isoform distribution do not, therefore, necessarily represent distributions of active receptors. All three EcR isforms form active receptors when combined with USP(Koelle, 1992 ; Koelle et al., 1993). In order to understand the ecdysone action during development, qualitative and quantitative changes of functional ecdysone receptor transcripts were investigated in individual tissues by using a RT-PCR assay.

MATERIALS AND METHODS

A wild-type Canton-S strain were raised on standard medium. Embryos were collected at two hour intervals after egg laying. For larval and adult tissues were dissected in Ringer's solution. A homozygous mutant strain for *l*(3)*ecd-1ts*(Garen et al., 1977) was reared on a standard medium at the permissive temperature, 18℃. To analyze the effects of prolonged upshift to 29℃ that elicit an ecdysteroid deficiency in mutant, the cultures were transferred from 18℃ to 29℃ nine days after egg laying and sacrified 3 days after the upshift. For *in vitro* experiments, mutant larvae was reared at 29℃ continuously until the onset of wandering during the third larval instar. At this time, the tissues were dissected and incubated in Ringer's containg 1.8×10^{-6} M 20-hydroxyecdysone. Total RNA was extracted by the method of Huet et al.(1993). The RNA was recovered by centrifugation and resuspended in distilled water. To calculate the amounts of RNA, the absorbances at 260nm and 280nm were measuerd. As primers we used 20-mer oligonucleotides having a GC content as close to 50% as possible, preferably placed in different exons. This allow to distinguish between a band derived mRNA and a second band that may derived from either pre-mRNA or contaminating DNA. The first strand of cDNA was synthesized using RT-PCR Kit(Clontech) according to manufacturer's protocol. PCR was performed for 35 cycles

with 45 seconds denaturation at 94℃ and 45 seconds annealing at 60℃, followed by 2 minutes at 72℃. The resulting PCR products were analyzed on 2% agarose gel.

RESULTS AND DISCUSSION

The expression patterns of functional ecdysone receptors during *Drosophila* life cycle were analyzed at all developmental stage using RT-PCR. The ribosomal protein 49(*rp49*) served as a standard for RNA extraction and the subsequent RT-PCR assay. *usp* transcripts were detected with nearly equal amount in all developmental stages (embryogenesis, all larval stages, pupal stage, adult) investigated (Figure 1). This result is consistent with previous report that USP play pleiotropic function during various developmental stages. Phenotypic analysis of *usp* mutant flies has revealed that *usp* is required in development stages and multiple tissue during the Drosophila life cycle. *usp* has been shown to be essential for embryogenesis, larval development, pupation, and other development events (Oro et al., 1992). Also, in the profile of *usp* transcription and translation assay, *usp* expression is not confined to developmental periods and cell types associated specifically with major ecdysteroid-induced events. USP is expressed in many tissues throughout development with fluctuations in mRNA and protein levels(Henrich et al., 1994). EcR isoforms also are expressed simultaneously with *usp* in all developmental stages. The expression of EcR-A was maintained in high level during embryogenesis, decreased during larval stage, and increased after prepupal stage. While EcR-B1 was expressed with higher level during larval stages comparing to EcR-A. This result suggests that the functional ecdysone receptor are ubiquitous throughout *Drosophila* life cycle, but their expression level is regulated according to specific developmental stage. Especially, the expression levels of each EcR isoform were different during life cycle. These differential expression of EcR isoforms resulted in the ecdysone receptor complexes consisting of the different combination of EcR isoforms(EcR-A and EcR-B1) and USP. Each EcR isoform have independent and quite distinct spatial and temporal expression patterns during development(Table 1). We showed that the larval tissues contain more EcR-B1 than EcR-A, while imaginal discs contain much more of EcR-A than EcR-B1. Now, we are quantifying the functional ecdysone receptor isoforms by using competitive RT-PCR in individual tissues during development.

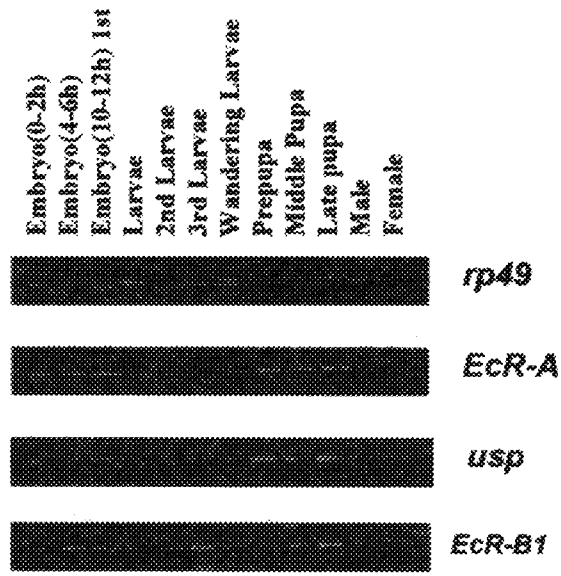

Fig.1. Analysis of transcripts of functional ecdysone receptor complex during development. RT-PCR products were separated on 2% agarose gel.

Table 1. Summary of the functional ecdysone receptor expression in various tissues during development

Developmental stage	Tissue	Functional ecdysone receptor			
		usp	EcR-A	EcR-B1	EcR-B2
Embryo	0-2 hr	+ +	+ + +	+ +	+ + +
	4-6 hr	+ +	+ +	+ +	+ + +
	10-12 hr	+ + +	+ +	+ + +	+ + +
Late 3rd Larvae	BrVg	+ + +	+ +	+	+ +
	WD	+ +	+ + +	+	+
	EAD	+ +	+ +	+	+
	SG	+	+	+ +	+ +
	EP	+ + +	+	+ + +	+
Adult	Ovary	+ + +	+ + +	+	+
	Testis	+ + +	+	+	+

REFERENCES

1. Henrich, V. C., A. A. Szekely., S. J. Kim., N. E. Brown., C. Antoniewski., M. A. Hayden., J-A. Lepesant., and L. I. Gilbert (1994). Dev. Biol. : 165. 38-52.

2. Oro, A. E., M. McKeown., and R. M. Evans (1992) Development 115 : 449-462.

3. Talbot, W. S., E. A. Swyryd., and D. S. Hogness, (1993) Cell 75 : 307-320.