Transcripttiters of ecdysteroid receptor components vary between tissues and stages during *Drosophila* development

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Abstract

In *Drosophila*, the ecdysteroids trigger the key regulatory cascades controlling the coordinated changes in the developmental pathway of molting and metamorphosis. Ecdysone action is mediated by a heterodimer consisting of the three ecdysone receptor (EcR) isoforms and ultraspiracle proteins (USP). Heterodimers of these proteins bind to ecdysone response element and ecdysone to modulate gene transcription. In this study, we developed competitive RT-PCR assays to quantify the transcripts of functional ecdysone receptor components in individual tissues and whole animals. The relatively small amount of variation of usp transcripts in the different tissues suggests that this gene does not perform a spatially restricted function in the late third instar wandering larvae. In contrast, EcR isoforms are expressed in more tissue-restricted patterns in the late third instar wandering larvae. EcR-B1 is expressed at higher levels in larval tissues that are fated for histolysis, whereas EcR-A predominates in the imaginal discs. This result supports the hypothesis that particular metamorphic responses require particular EcR isoforms. The transcript levels of functional ecdysone receptor components fluctuate dramatically during development, suggesting that regulation of the transcriptional and posttranscriptional levels of these genes plays some role in ecdysteroid response during *Drosophila* development.

Introduction

In insects, ecdysteroids, particularly 20-hydroxyecdy-sone (20-HE), are the key steroid hormones. 20-HE plays a central role in orchestrating development during molting and metamorphosis. The multivalent actions of 20-HE are thought to be mediated by the ecdysone receptor (EcR) complex, which when activated by its ligands triggers a cascade of transcription factors that direct the molting process (reviewed in Andres and Thummel, 1992; Henrich and Brown, 1995; Cherbas and Cherbas, 1996; Thummel, 1996). The EcR identified from Drosophila is a member of the nuclear receptor superfamily and has three isoforms (EcR-A, EcR-B1 and EcR-B2) with common DNA- and hormone-binding domains, but different N-terminal regions due to the use of different promoters and alternative splicing (Koelle et al., 1991; Talbot et al, 1993). To form a functional receptor, EcR needs to heterodimerize with Ultraspiracle (USP), the *Drosophila* homologue of the vertebrate retinoid X receptor (Yao et al., 1992, 1993; Thomas et al., 1993).

In *Drosophila*, the metamorphic responses of tissues to ecdysteroids are correlated with the expression patterns of EcR isoforms (Talbot et al., 1993). The EcRA isoform predominates in the imaginal discs, imaginal rings, two sets of specialized larval cells that postpone their deaths to provide late metamorphic functions (Talbot et al., 1993), and type II larval neurons of the central nervous system (Robinow et al., 1993; Truman et al., 1994). In contrast, the EcR-B1 isoform predominates in the other larval tissues and in the imaginal histoblasts that form the abdominal epithelium and the midgut of the adult (Talbot et al., 1993). Since the abundance of each EcR isoform differs in tissues showing different metamorphic responses to ecdysteroids (e.g., imaginal discs versus larval tissues), it is suggested that the different metamorphic responses

require different combinations of the EcR isoforms (Talbot et al., 1993). The quantitative difference between the functional ecdysone receptor complexes in vivo may arise through EcR/USP-associated proteins or cofactors in addition to their ligands (Thummel, 1995; Jones and Sharp, 1997). Information about the exact transcript levels of functional ecdysone receptors is helpful for understanding the complexity of the ecdysone response in *Drosophila*. In this study, we developed a competitive RT-PCR assay to quantify the transcripts of functional ecdysone receptor components. We showed that transcript titers of the ecdysteroid receptor components vary between tissues and stages during Drosophila development.

Materials and methods

Drosophila culture and collection of staging animals

A wild-type Canton-S strain of *Drosophila* melanogaster was raised at 25C on standard medium containing cornmeal, sugar, yeast, and agar.

RNA purification

Larvae and adults were dissected in insect Ringers. All samples were frozen in liquid nitrogen, and stored at 70C until required for RNA extraction. Total RNA was extracted by using the RNAgents Total RNA Isolation System (Promega) according to the manufacturer's protocol The RNA was recovered by centrifugation and resuspended in distilled water. Dilutions were made in distilled water and stored at −70℃ as necessary

The RT-PCR protocol

The primers were designed to have a GC content as close to 50% as possible and were preferably located in different exons. The primer sets were placed so that the products were in the size range of 300-700 base pairs (bp) to ensure efficient PCR amplification. The first strand of cDNA was synthesized using the Advantage-RT-for-PCR Kit (Clontech) according to the manufacturer's protocol. Competitive RT-PCR was performed using the PCR MIMIC Construction Kit (Clontech). PCR was performed using the cDNA template in a DNA Thermal Cycler. The resulting PCR products were analyzed on 2% agarose gels, and quantified using the BioDocII gel video system (Biometra).

Results and discussion

The precise differences in transcript levels of ecdysone receptor components were quantified by competitive RT-PCR (Figure 1). The transcript levels of usp and EcR were quantified in tissues from late third instar wandering larvae, the stage at which the metamorphic ecdysone pulse reaches a maximum, and adult flies. In the late third instar wandering larvae, usp transcripts were detected in relatively similar amounts (about 0.25 attomoles) in the salivary gland, fat body, and Malpighian tubules, while there were twice as many usp transcripts in the wing discs and brain ventral ganglion complex (Fig. 2A). In contrast, the total amount of EcR transcripts using primers common to all isoforms varied between tissues at this stage. The highest levels of total EcR transcripts were detected in the gut (about 25 attomoles); moderate levels (about 6 attomoles) were seen in the salivary gland and Malpighian tubule; and low levels in the fat body, wing discs, and brain ventral ganglion complex (Fig. 2A). In adult flies, 10 times more usp transcripts were detected in the ovary than in the male reproductive organs (Fig. 2A) and 20 times more total EcR transcripts were detected in the ovary than in the male reproductive organs (Fig. 2A).

Fig. 2B shows the relative abundance of the two EcR isoform transcripts. While both EcR isoform transcripts were detected in all the tissues assayed, tissues that strongly expressed EcR-A generally expressed EcR-B1 weakly and vice-versa. Apparently, the EcR-A and EcR-B1 isoforms are expressed in roughly complementary amounts in the late third instar wandering larvae, the stage at which the ecdysone pulse reaches a maximum. The larval tissues expressed high levels of EcRB1 transcripts and low levels of EcR-A transcripts. Figure 2B shows that this is

the case for larval brain, ventral ganglion complexes, salivary glands, fat bodies, gut, and Malpighian tubules, while the imaginal wing discs strongly express EcR-A transcripts and weakly express EcR-B1 transcripts. EcR-A transcripts predominate over EcR-B1 transcripts in adult female ovaries and the male reproductive organs (Fig. 2B).

The relatively small amount of variation in the number of usp transcripts in both larval and imaginal tissues in the late third instar wandering larvae, suggests that this gene does not perform a spatially restricted function during this period. In contrast, the EcR isoforms are expressed in more tissue-restricted patterns in the late third instar wandering larvae. EcR-B1 is expressed at higher levels in larval tissues that are fated for histolysis, whereas EcR-A predominates in the imaginal discs.

Fig. 3A shows the developmental profiles of usp and total EcR transcripts quantified by competitive RT-PCR. The amounts of usp and total EcR transcripts fluctuated considerably during the life cycle. The highest levels of usp and total EcR transcripts were expressed in the early embryo and adult females (Fig 3A). Interestingly, in the late third instar larvae and adult females, there are more total EcR transcripts than usp transcripts. However, it should be noted that both usp and total EcR transcripts were detected at all stages to varying degrees. Fig. 3B shows the relative abundance of the two EcR isoform transcripts. EcR-B1 transcripts predominate over EcR-A transcripts from late embryogenesis to late in the third instar larval stage. This result can be predicted from the observation that the polyploid and polytene nuclei of larval tissues generally express EcR-B1 transcripts much more strongly than EcR-A transcripts (Fig. 3B). In the early embryo, prepupae, and adult, EcR-A transcripts generally predominate over EcR-B1 transcripts. The early embryonic expression of EcR-A suggest that it may be maternal mRNA transcribed during oogenesis, since EcR-A transcripts predominate over EcR-B1 in the ovaries (Fig. 3B).

Our results clearly show that there are tissue-specific and developmental-specific differences in transcript levels of functional ecdysone receptor components. The expression of EcR and USP proteins analyzed by Western blots (Koelle et al., 1991; Talbot et al., 1993; Henrich et al., 1994) showed some complex patterns during development. Our results combined with these suggest that the transcriptional and posttranscriptional regulation of these genes play a number of roles in the ecdysteroid response during *Drosophila* development.

References

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