SHORT NOTE

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# Cuticular protein genes in *Tenebrio molitor* (Coleoptera: Tenebrionidae)

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***Tenebrio molitor*, cuticular protein, juvenile hormone, metamorphosis**

**Abstract.** We have previously isolated from the beetle *Tenebrio molitor*, cDNAs coding for two glycine-rich cuticular proteins named ACP-20, ACP-22 and ACP-17 and an alanin-rich cuticular protein named LPCP-22. The ACP-20, ACP-22, ACP-17 mRNAs are detected by Northern blot and in situ hybridization analysis only in epidermal regions secreting heavily sclerotized cuticle during the pharate adult stage. The LPCP-22 mRNA is detected in most epidermal regions during the secretion of larval and pupal cuticles. Then, its presence is restricted to the epidermal zones secreting intersegmental soft cuticle in the newly ecdysed pupa. This stage- and tissue-specific gene system seems to be a convenient model for studying the regulation of sequential gene expression by ecdysteroids and juvenile hormone.

## *Introduction*

In *Tenebrio*, previous studies (Roberts & Willis, 1980; Lemoine & Delachambre, 1986) showed that the larval and pupal cuticle protein patterns are similar, in contrast to the adult patterns, suggesting that the main changes in epidermal gene expression occur at the pupal-adult transition.

Changes in cuticle structure are correlated with different protein composition and thus with changes in epidermal gene expression. Therefore, cuticular protein genes, regulated in a stage- and tissue-specific manner, should be relevant tools to study how JH and ecdysteroids interact to regulate metamorphosis at molecular level.

Two cDNA encoding adult cuticular proteins named ACP-20 and ACP-22 were first characterized in *Tenebrio* (Bouhin et al., 1992; Charles et al., 1992). Recently we have isolated another cDNA coding for an adult cuticular protein: ACP-17 (Mathelin et al., in press) and a cDNA encoding a larval-pupal cuticular protein named LPCP-22 (Rondot et al., in press).

In this report, the temporal and spatial distributions of the transcripts, their hormonal regulation by JH and the structure features of the deduced primary sequence of ACP-20, ACP-22, ACP-17 and LPCP-22 are compared.

## *Results and Discussion*

### Developmental profiles of the mRNAs during metamorphosis

Epidermal wing RNAs were taken to Northern blot analysis at different steps of metamorphosis using the ACP-20, ACP-22, ACP-17 and LPCP-22 cDNA inserts as probes.

Fig. 1 shows that ACP-20 and ACP-22 expressions were restricted to the period of preecdysial deposition of adult cuticle and were first detected 6 days after pupal ecdysis, indicating that they are coordinaly expressed, whereas ACP-17 mRNA was detected during the period of postecdysial adult cuticle secretion (a low expression was detected three days earlier). LPCP-22 transcript was detected only during the secretion of larval and pupal cuticle.

In order to determine the spatial distribution of ACP-20, ACP-22, ACP-17 and LPCP-22, end-labelled synthetic oligonucleotides were used as probes for in situ hybridization to mRNA integumental cryo-sections. We found that the corresponding mRNAs were present only in epidermal cells secreting cuticle, and absent in other tissues such as muscles and fat body (Table 1). ACP-20, ACP-22 and ACP-17 were

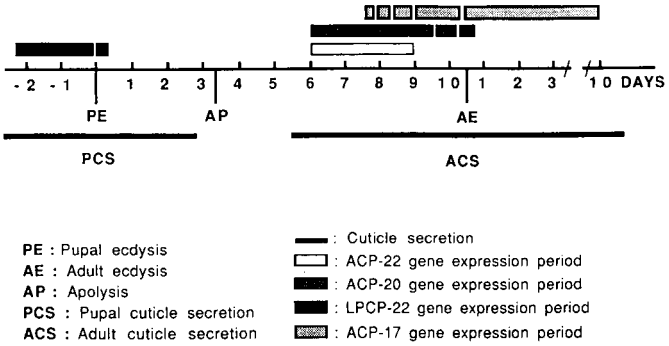


Fig. 1. Schematic representation of ACP-20, ACP-22, ACP-17 and LPCP-22 gene expression periods during metamorphosis.

restricted to epidermis synthesizing hard cuticle: sternal epidermis, legs, elytral upper sheet; in contrast, soft cuticle such as intersegmental membranes and tergites, posterior wings and pleura were unlabelled. During the secretion of pupal cuticle, LPCP-22 was first detected in the sternital, tergital and wing bud epidermis. In newly ecdysed pupae, the expression of LPCP-22 was restricted to the epidermal zones secreting soft cuticle.

These results show that the genes encoding ACP-20, ACP-22, ACP-17, LPCP-22 are developmentally regulated and that their expressions are stage- and tissue-specific.

TABLE 1. Comparison of the results obtained in hybridization in situ. Transcript localization: present (+), absent (–).

| | ACP-20 | ACP-22 | LPCP-22 | ACP-17 |
|---|---|---|---|---|
| muscles | – | – | – | – |
| fat body | – | – | – | – |
| epidermis (ep): | | | | |
| tracheal ep | – | – | – | – |
| sternal ep | + | + | + | + |
| legs ep | + | + | + | + |
| wings ep | + | + | + | + |
| intersegmental ep | – | – | + | – |
| tergites ep | – | – | + | – |
| pleura ep | – | – | + | – |

Hormonal regulation by juvenile hormone

In *Tenebrio*, several studies demonstrated a period of sensitivity to juvenile hormone analogues (JHAs) from the pupal ecdysis to pupal-adult apolysis (Geyer et al., 1968; Socha & Sehnal, 1972). Newly ecdysed pupae fail to develop into adult after topical applications of the JHA methoprene, which trigger the development of supernumerary pupae 6 days later. We found that under these conditions (Table 2), the expression of ACP-20 and ACP-22 fails to occur whereas LPCP-22 is expressed during the synthesis of the supernumerary cuticle.

TABLE 2. Comparison of ACP-20, ACP-22, ACP-17 and LPCP-22 expressions after application of JHA to newly ecdysed pupae. Presence (+) or absence (–) of the transcript.

| | Controls | Treated |
|---|---|---|
| ACP-20 | + | – |
| ACP-22 | + | – |
| LPCP-22 | – | + |

So, methoprene prevents accumulation of the adult-specific ACP-20 and ACP-22 transcripts, suggesting a negative control of these genes by the juvenile hormone, whereas it triggers a supernumerary expression of LPCP-22, suggesting a positive control.

cDNA sequences and deduced primary sequences of proteins

The complete sequences of the cDNAs were established by cDNA and mRNA (for the 5' region) sequencing. As shown in Table 3, ACP-20, ACP-22, ACP-17 and LPCP-22 have common features in the length of their mRNA with a very close nucleotides number. An interesting common feature of ACP-20, ACP-22 and ACP-17 is the presence of a high percentage of glycine residues which reflects the over-representation of glycine in some hard cuticle proteins (Apple & Fristrom, 1991) as well as in other structural proteins such as cytokeratins (Steinert et al., 1983) or plant cell wall (Kiruma et al., 1976). The amino acid composition of ACP-17 further exhibits a high percentage of alanine (20%) which is also the main amino-acid of LPCP-22. A repeated alanine-rich motif is found in other cuticular proteins of *Locusta migratoria* (Andersen, 1993).

TABLE 3. Comparison of cDNA and deduced primary sequence structure of ACP-20, ACP-22, ACP-17 and LPCP-22 (nt: nucleotides).

| cDNA sequence: | | | |
|---|---|---|---|
| | mRNA size | 5' untranslated region | open reading frame |
| ACP-20 | 786 nt | 45 nt | 624 nt |
| ACP-22 | 741 nt | 42 nt | 597 nt |
| ACP-17 | 783 nt | 40 nt | 561 nt |
| LPCP-22 | 726 nt | 30 nt | 636 nt |
| Deduced primary sequence: | | | |
| | Amino acids quantities | molecular mass | major amino acid |
| ACP-20 | 208 | 18.4 kDa | glycine 29% |
| ACP-22 | 199 | 20.7 kDa | glycine 27% |
| ACP-17 | 185 | 16.4 kDa | glycine 28% |
| LPCP-22 | 212 | 20.99 kDa | alanine 31% |

## *Conclusions*

We isolated three cDNAs encoding adult specific cuticular proteins, present in the sclerotized cuticle (ACP-20, ACP-22, ACP-17), and a cDNA encoding a larval-pupal cuticular protein LPCP-22. ACP-20, ACP-22 and ACP-17 are expressed by epidermal cells and at different stages of the development than LPCP-22. The absence of ACP-20 and ACP-22 transcripts and the supernumerary expression of LPCP-22 in the epidermis of JHA-treated animals demonstrates a different sensitivity of these gene to the juvenile hormone. It's likely that the encoded proteins play a role in determining the physical and chemical properties of the cuticle. Identified genes provide a good example of sequential gene expression may be used as a convenient model for studying the regulation of gene expression by hormones. An analysis of cis and trans factors involved of the specific expression of these four genes has been undertaken. In parallel, several members of the steroids receptors superfamily are under investigation.

## REFERENCES

ANDERSEN J.S., ANDERSEN S.O., HOPJUR P. & ROEPSTORFF P. 1993: Primary structure of a 14-kDa basic structural protein (Lm 76) from the cuticle of the migratory locust, Locusta migratoria. *Insect Biochem. Molec. Biol.* **23**: 391–402.

APPLE R.T. & FRISTROM J.W. 1991: 20-hydroxyecdysone is required for, and negatively regulates, transcription of Drosophila pupal cuticle protein genes. *Dev. Biol.* **146**: 569–582.

BOUHIN H., CHARLES J.P., QUENNEDEY B., COURRENT A. & DELACHAMBRE J. 1992: Characterization of a cDNA clone encoding a glycine-rich cuticular protein of Tenebrio molitor: developmental expression and effect of a juvenile hormone analog. *Insect Molec. Biol.* **1**: 53–62.

CHARLES J.P., BOUHIN H., QUENNEDEY B., COURRENT A. & DELACHAMBRE J. 1992: cDNA cloning and deduced amino acid sequence of a major, glycine-rich cuticular protein from the coleopteran Tenebrio molitor. Temporal and spatial distribution of the transcript during metamorphosis. *Eur. J. Biochem.* **206**: 813–819.

GEYER A., HERDA G. & SCHMIALEK P. 1968: Beeinflüssung der Metamorphose von Tenebrio molitor durch Juvenilhormonewirksame Isoprenoids. *Acta Entomol. Bohemoslov.* **65**: 161–165.

KIRUMA S., LIPKE H. & STROUT H.V. 1976: Peptidochitodextrines of Sarcophaga bullata: nonidendity of limit glycopeptides from larval and puparial cuticle. *Insect Biochem.* **6**: 65–77.

LEMOINE A. & DELACHAMBRE J. 1986: A water-soluble protein specific to the adult cuticle in Tenebrio. Its use as a marker of a new programme expressed by epidermal cells. *Insect Biochem.* **16**: 483–489.

MATHELIN J., BOUHIN H., QUENNEDEY B., COURRENT A. & DELACHAMBRE J. Identification and sequence of a cDNA clone encoding a glycin-rich cuticular protein in Tenebrio molitor. Expression pattern of its mRNA during metamorphosis. *Gene* (in press).

ROBERTS P.E. & WILLIS J.H. 1980: Effects of juvenile hormone, ecdysterone, actinomycine D, and mitomycin C on the cuticular proteins of Tenebrio molitor. *J. Embryol. Exp. Morphol.* **56**: 107–123.

SOCHA R. & SEHNAL F. 1972: Inhibition of adult development in Tenebrio molitor by insect, hormones and antibiotics. *J. Insect. Physiol.* **18**: 317–337.

STEINERT P.M., RICE R.H., ROOP D.R., TRUS B.L. & STEVEN A.C. 1983: Complete amino acid sequence of a mouse epidermal keratin subunit and implication for the structure of intermediate filaments. *Nature* **302**: 794–800.