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Review

Developmental expression of mRNAs for epidermal and fat body proteins and hormonally regulated transcription factors in the tobacco hornworm, *Manduca sexta*

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ABSTRACT

This paper provides a compilation of diagrammatic representations of the expression profiles of epidermal and fat body mRNAs during the last two larval instars and metamorphosis of the tobacco hornworm, *Manduca sexta*. Included are those encoding insecticyanin, three larval cuticular proteins, dopa decarboxylase, moling, and the juvenile hormone-binding protein JP29 produced by the dorsal abdominal epidermis, and arylphorin and the methionine-rich storage proteins made by the fat body. The mRNA profiles of the ecdysteroid-regulated cascade of transcription factors in the epidermis during the larval molt and the onset of metamorphosis and in the pupal wing during the onset of adult development are also shown. These profiles are accompanied by a brief summary of the current knowledge about the regulation of these mRNAs by ecdysteroids and juvenile hormone based on experimental manipulations, both *in vivo* and *in vitro*.

Contents

1. Introduction

The tobacco hornworm, *Manduca sexta,* has been the "laboratory rat" of insect endocrinology during the past 40 years. Its relatively large size and the ease of timing developmental and endocrine events made it an ideal subject for study. We have utilized these characteristics to study the regulation of several epidermal and fat body proteins, both *in vivo* and *in vitro*, first at the tissue and cellular level, then at the molecular level. This compilation of the diagrammatic representations of the developmental profiles of the mRNAs of these various proteins and of the hormonally regulated transcription factors that in turn regulate them was sparked by Judy Willis who has appreciated and used them for her lectures and feels that they should all be in an easily accessible place for all to partake. Therefore, we will briefly review a few salient points about each protein and the transcription factors and provide references for the relevant studies for those who want more detail.

These mRNA profiles are for larval dorsal abdominal epidermis, pupal wings, or fat body isolated at defined times during development. They are based on dot blot hybridization of total RNA except for the fat body mRNAs which were analyzed by in solution hybridization assays and the E74 and the E75C and E75D isoforms which were analyzed by northern hybridization. Relative levels can be compared only for a single mRNA in a particular tissue. Our review of each protein and its regulation is based on

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hormonal and other experimental manipulations, both *in vivo* and on isolated tissues *in vitro*.

2. Epidermal proteins

The epidermis produces the overlying cuticle during both the ecdysteroid-induced molt and the intermolt feeding phase when the larva is feeding and growing. During a larval molt the epidermis makes a new larval cuticle due to the presence of juvenile hormone (JH) at the onset of the rise of ecdysteroid (see Fig. 1; Riddiford, 1994), then at metamorphosis a polymorphic epidermis such as found in Lepidoptera must switch to a program of pupal differentiation. In *Manduca* the general body epidermis becomes committed to pupal differentiation by the small prewandering peak of ecdysteroids in the absence of JH, but does not express that differentiation until the much larger prepupal surge of ecdysteroids (Riddiford, 1978; see Fig. 1). Once pupally committed, the epidermis can no longer express larval-specific proteins, either in the presence or absence of JH.

Insecticyanin (INS) is a blue, biliverdin-containing protein found in granules in *Manduca* larval epidermis and in the hemolymph (Cherbas, 1973; Goodman et al., 1985). The two isoelectric forms of INS (INS-a and INS-b) produced by the epidermis are encoded by two different genes (Riddiford et al., 1990; Li and Riddiford, 1992). Both isoelectric forms are stored in the epidermis, but only INS-b is secreted into the hemolymph. Small amounts of both mRNAs are also found in the larval fat body in the 4th instar, but only INS-b mRNA is found there in the final instar (Li and Riddiford, 1994). The mRNAs are up-regulated during the feeding periods and down-regulated temporarily during the larval molt and terminally at the onset of metamorphosis (Riddiford et al., 1990) (Fig. 1). In both cases, 20-hydroxyecdysone (20E) *in vitro* caused the down-regulation. At the onset of metamorphosis, INS-a was found to be more sensitive to 20E *in vitro* than INS-b, hence accounting for the differential down-regulation seen *in vivo* (Fig. 1) (Li et al., 1995).

The three cuticular cDNAs whose regulation was analyzed in detail were all found in larval epidermis, but each was regulated differently. All of the encoded proteins contain the Rebers–Riddiford RR1 Consensus sequence that is important in binding chitin (Rebers and Riddiford, 1988; Andersen et al., 1995; Rebers and Willis, 2001; Willis et al., 2005). Larval cuticle protein 14 (LCP14; 14 kDa) (Rebers and Riddiford, 1988) is a protein specific to larval flexible cuticle and its mRNA is expressed in all epidermal cells except for the trichogen and tormogen cells (LMR, unpublished) during the feeding stages. It is regulated similarly to INS, turning off temporarily in response to 20E at the molt and permanently in response to 20E in the absence of JH at the time of pupal commitment (Fig. 1) (Rebers and Riddiford, 1988; Hiruma et al., 1991). LCP14.6 (14.6 kDa) mRNA is expressed in a similar manner to that of LCP14 in the larva (Fig. 1) (Riddiford et al., 1986). In contrast to LCP14, it is not larval-specific. Instead it becomes spatially restricted to the flexible intersegmental membranes of the pupa and even more restricted to the muscle attachment sites in that membrane in the adult (Rebers et al., 1997). The mRNAs for the 16/17 kDa family of cuticular proteins (LCP16/17) appear in response to low ecdysteroids in the absence of JH on the penultimate day (day 2) of feeding in the final instar and are shut off by the larger prewandering ecdysteroid peak the following day (Wolfgang and Riddiford, 1986; Horodyski and Riddiford, 1989). The appearance of these proteins coincides with the thinning of the cuticular lamellae and a consequent increase in flexural stiffness of the cuticle (Wolfgang and Riddiford, 1987). They like LCP14 are not found in the pupal or adult cuticle (Horodyski and Riddiford, 1989).

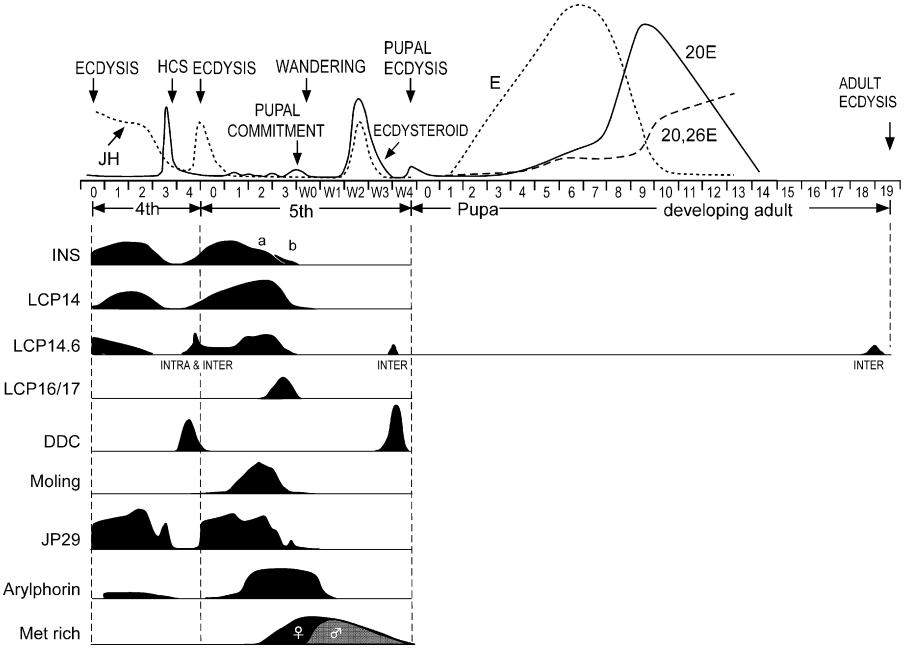

Fig. 1. Diagrammatic representations of developmental profiles of the mRNAs for insecticyanin (INS), the larval cuticle proteins (LCP) LCP14, LCP14.6, and LCP16/17, Moling, the 29 kDa JH-binding protein (JP29), and dopa decarboxylase (DDC) in the dorsal abdominal epidermis and the two storage proteins, arylphorin and the methionine (Met)-rich protein, in the fat body during the final two larval instars of the tobacco hornworm, *Manduca sexta*, including the prepupal period [1–4 days after the onset of wandering behavior (W1–W4)]. See the text for the details about each mRNA and its regulation by ecdysteroids and juvenile hormone (JH). The ecdysteroid titers in the hemolymph for the 4th instar molt (Langelan et al., 2000), the 5th larval intermolt (Wolfgang and Riddiford, 1986), and the pupal molt (Hiruma et al., 1999) are based on radioimmunoassay (RIA) analysis. The titers of specific ecdysteroids for the onset of the adult molt are based on RIA analysis of HPLC-purified hemolymph (Warren and Gilbert, 1986). The JH titers in the hemolymph are based on the *black* larval assay for the 4th larval instar (Fain and Riddiford, 1975) and on the gas chromatographic–mass spectrometric analysis of JHs I and II in the final instar and prepupal period (Baker et al., 1987). E, ecdysone; 20E, 20-hydroxyecdysone; 20,26E, 20,26-dihydroxyecdysone; HCS, head capsule slippage during the larval molt; intra & inter, intra- and intersegmental expression.