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Correlations between ecdysteroid titre and silk gene expression in the last instar larvae of *Galleria mellonella* (Lepidoptera: Pyralidae)

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Abstract. Developmental profiles of total RNA and four silk protein mRNAs exhibit different correlations with ecdysteroid titre changes in course of the 180 hr-long last larval instar. Total RNA and heavy-chain fibroin mRNA increase gradually until 168 hr and 156 hr, respectively, dropping sharply afterwards. The content of sericin1 mRNA rises for 120 hr, remains high until 168 hr and then drops. The amounts of ligh-chain fibroin mRNA and sericin2 mRNA increase sharply at the start of the instar; the former rises until 168 hr, whereas the latter begins to decline already at 120 hr. The pupation-inducing ecdysteroid surge at 144–168 hr causes a drop in all examined RNA classes. Similar drop is induced precociously with exogenous ecdysterone, while a slow decrease of RNA contents to similar bottom levels occurs when the larvae are ligated behind prothorax.

INTRODUCTION

Studies on the domestic silkworm, *Bombyx mori*, indicated that the development and function of silk glands are controlled by changes in ecdysteroid concentration. As early as in 1942, Fukuda concluded from his surgical experiments that low concentrations of the molting hormone (= ecdysteroids) exert a stimulatory effect on silk production whereas the pupation-inducing surge of the hormone causes the silk glands to degenerate. Recent investigations on the in vitro effects of 20-hydroxyecdysone (20E) on the silk glands of *Galleria mellonella* seemed to confirm this suggestion by showing that within 1 hr of application, the doses of 5 and 50 ng 20E per 1 ml culture medium stimulated, whereas 5,000 ng 20E/ml inhibited the total RNA synthesis (Kodrík & Sehnal, 1994). A decrease of RNA synthesis, but not the stimulation, was also induced by applying different 20E doses to the donor insects 3 days prior to silk gland dissection. Our present paper examines possible role of ecdysteroids in silk gland function by correlating ecdysteroid titre with fluctuations in the silk gland content of total RNA and four dominant mRNAs. We also compare changes in RNA content that occur after ligation with those induced with 20E injections.

MATERIAL AND METHODS

Freshly ecdysed last-instar larvae of *Galleria mellonella* L. (Lepidoptera: Pyralidae) were taken from our laboratory culture and maintained on a semi-artificial diet at 30°C (Sehnal, 1966). Groups of 2–8 larvae provided hemolymph samples for ecdysteroid quantification by radioimmunoassay (Jindra et al., 1994) and simultaneously also samples of silk glands, from which RNA was extracted with the aid of RNA-Clean System (Angewandte Gentechnologie Systeme, Heidelberg). The total amount of extracted

RNA was assessed from the absorbance at 260 nm. Effect of the pupation-inducing ecdysteroid surge on silk gland RNA was examined in a group of larvae that were injected with 20E (4 μg in 2.5 μl 10% ethanol per larva) at 120 hr of the instar. In intact larvae the instar lasted 180 hr.

Two dilution series of total RNA from each silk gland sample were applied on Hybond-N membranes (Amersham) with Bio-Dot applicator (Bio-Rad). The first series began with 10% of gland pair RNA per dot, the second series with 2 μg RNA per dot. These initial amounts were serially diluted in ratio 1 : 4 to obtain 8 gradually declinining RNA quantities. Four cDNA probes described by Žurovec et al. (1992) were used for mRNA detection: 0.5 kb PG1 probe for light-chain fibroin mRNA; 1.3 kb PG2 probe for heavy-chain fibroin mRNA; 0.42 kb MG1 probe for multiple sericin1 mRNA species, and 1.7 kb MG2 probe for both sericin2 mRNAs. The probes were labelled with [α-^{32}P]dATP using the Random Primed Labelling Kit (USB), and hybridized to membranes according to Amersham protocol. Following their exposure to X-ray film, the membranes were cut to squares 6 × 6 mm around each dot and used for scintillation counting. Radioactivity of membrane areas without administered RNA reflected non-specific probe binding and was substracted from values established at the sites of RNA dotting: the resulting figures correspond to the amounts of bound mRNA to which the labelled probed hybridized. The highest value established with each probe was taken as 100% and other values were expressed as its per cent ratio.

RESULTS AND DISCUSSION

Our ecdysteroid measurements revealed a slight titre increase after feeding termination at 96 hr of the last larval instar (Fig. 1). This is consistent with ecdysteroid quantification in whole body extracts (Sehnal et al., 1981) but no such increase was detected in previous analysis of hemolymph ecdysteroids (Sehnal et al., 1988). A sharp rise of ecdysteroids occurs after the completion of cocoon spinning at 144 hr. A peak surpassing 1 μg 20E equiv./ml hemolymph is reached around 156 hr at the stage of mobile prepupae; then the titre drops to nearly zero in insects 4 hr before pupal ecdysis (Jindra et al., 1994).

Galleria last instar larvae spin more or less continuously since a few hours after ecdysis until the start of ecdysteroid rise at 144 hr. The rate of silk output seems to increase in correlation with the body growth, reaching its maximum at the time of cocoon spinning. This pattern of spinning behavior is consistent with the steady increase of silk gland RNA. According to Sehnal et al. (1983) and Michalik et al. (1984), the increase levels off at 144 hr but our data show a rise of total RNA until 156 hr (Fig. 2, solid line). This discrepance may be due to differences in the staging of larvae or in the method of RNA extraction.

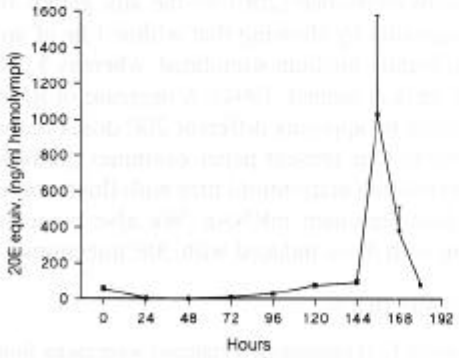

Fig. 1. Titre of hemolymph ecdysteroids in silk gland donors during the last larval instar (0 hr = last larval ecdysis; 180 hr = pupal ecdysis).

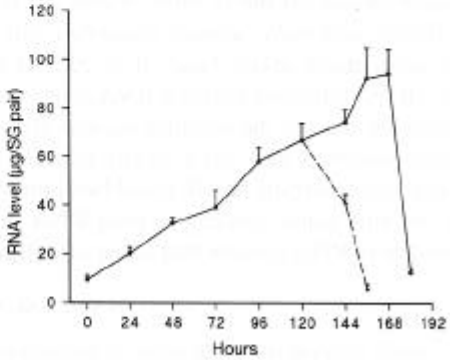

Fig. 2. Changes in total RNA content (μg/gland pair) of silk glands during normal last larval instar (solid line) and after injection of 4 μg 20E at 120 hr of the instar (broken line).

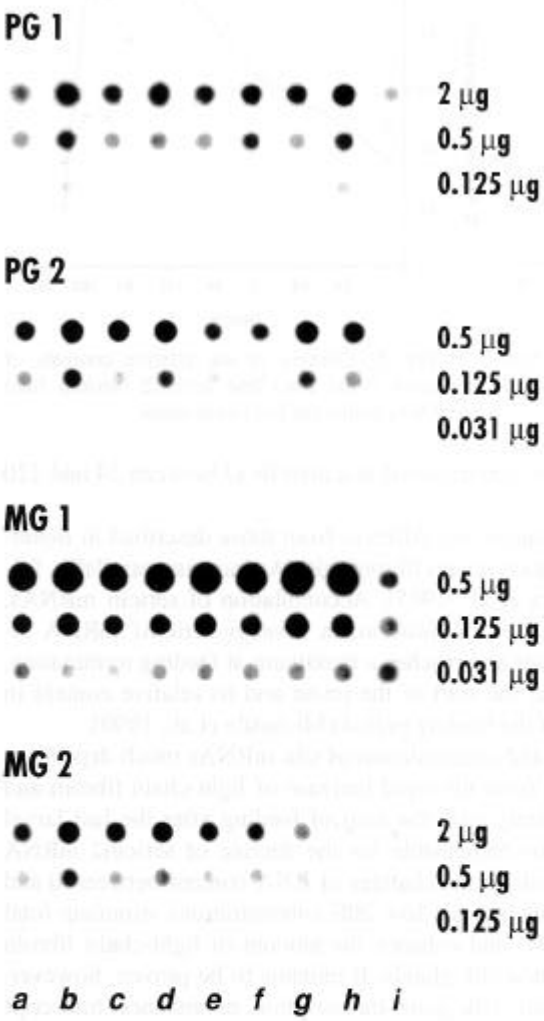

Fig. 3. Dot blot hybridization of indicated amounts (μg/dot) of silk gland RNA with probes for light-chain fibroin (PG1), heavy-chain fibroin (PG2), sericin1 (MG1) and sericin2 (MG2). Age of silk gland donors in hr since the last larval ecdysis: 0 (a), 24 (b), 48 (c), 72 (d), 120 (e), 144 (f), 156 (g), 168 (h) and 174 (i).

The drop of silk gland RNA after 168 hr is obviously caused by the pupation-inducing surge of ecdysteroids. When the ecdysteroid peak was mimicked by injecting younger larvae with 4 μg 20E/spec., these larvae ceased moving and within 36 hr they produced a new cuticle, while the old cuticle was digested by the molting fluid. Even though the new cuticle was not perfectly pupal, the effect of injection can be regarded as artificial induction of precocious pupal molt. The content of silk gland RNA decreased in the injected larvae (Fig. 2, dashed line) in similar manner as it normally does after 168 hr of the last instar.

The proportion of specific mRNAs in the total RNA was assessed by dot blot hybridization. The dot blots of equal amounts of total RNA indicated that the contents of studied mRNAs fluctuate in course of the instar in different ways but the patterns of their changes could not be assessed (Fig. 3). Somewhat better results were obtained when silk gland aliquotes of total RNA were dotted (data not shown), but only scintillation measurements of the dot areas yielded reliable semi-quantitative data (Figs 4 and 5).

Heavy-chain fibroin mRNA (Fig. 4, broken line) and sericin1 mRNA (Fig. 5, solid line) accumulate during the instar somewhat similarly as the total RNA (Fig. 2). Heavy-chain fibroin mRNA grows gradually until 156 hr, whereas sericin1 mRNA reaches a maximum already at 120 hr but remains high until 168 hr. The contents of light-chain mRNA (Fig. 4, solid line) and sericin2 mRNA (Fig. 5, broken line) are characterized by a rapid increase at the start of the instar. Light-chain fibroin mRNA shows another steep rise between 156 and 168 hr and

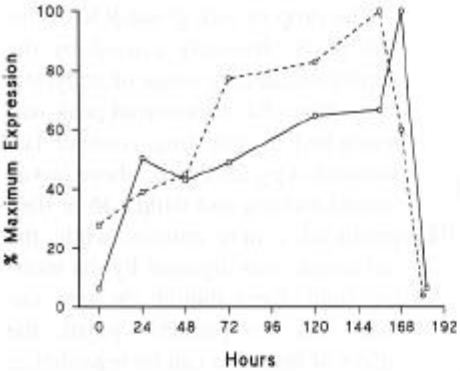

Fig. 4. Changes in the relative contents of light-chain (solid line) and heavy-chain (broken line) fibroins during the last larval instar.

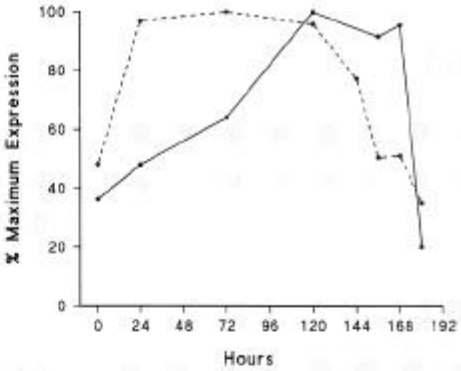

Fig. 5. Changes in the relative contents of sericin1 (solid line) and sericin2 (broken line) mRNAs during the last larval instar.

then a sharp drop, while sericin2 mRNA is maintained at a high level between 24 and 120 hr and then gradually declines.

Developmental patterns of mRNA changes are different from those described in *Bombyx mori*. In this species, the light- and heavy-chain fibroin mRNAs increase parallelly, following an exponential S-curve (Kimura et al., 1985). Accumulation of sericin mRNAs, however, somewhat resembles the situation in *Galleria*: in *Bombyx* sericin1 mRNA increases slowly at the start of the last instar and reaches a maximum at feeding termination, whereas sericin2 mRNA rises rapidly at the start of the instar and its relative content in total RNA declines in the second half of the feeding period (Michaille et al., 1990).

It is likely that the transcription rate and accumulation of silk mRNAs much depend on the supply of nutrients. This is evident from the rapid increase of light-chain fibroin and sericin2 mRNAs that occurs concomitantly with the start of feeding after the last larval ecdysis. Termination of feeding may be responsible for the decline of sericin2 mRNA after 120 hr. Possible role of ecdysteroids in the changes of RNA content between 0 and 144 hr is indicated by observations showing that low 20E concentrations stimulate total RNA synthesis (Kodrík & Sehnal, 1994) and enhance the amount of light-chain fibroin mRNA (Grzelak et al., 1993) in explanted silk glands. It remains to be proven, however, whether low ecdysteroid titres stimulate silk gene transcription or enhance transcript stability.

Ecdysteroid peak at 156 hr is associated with a drop of heavy-chain fibroin mRNA and within 12 hr it is followed by the decline of all mRNAs we examined (Figs 4 and 5). This depletion of mRNAs is obviously a part of silk gland response to increased ecdysteroid titre. In *Bombyx mori*, a dramatic decline in fibroin and sericin mRNAs was elicited by injecting the larvae with 20E (Tripoulas & Samols, 1986) or by adding 20E to the culture of silk gland in vitro (Maekawa et al., 1984).

The decline of silk gland RNA prior to pupal ecdysis might be regarded as a sign of the forthcoming organ histolysis, which is the ultimate silk gland response to the pupation-inducing ecdysteroid surge. An induction of silk gland degradation by high 20E doses was shown both in situ and in vitro (Chinzei, 1975; Kodrík & Sehnal, 1994). It must be noted, however, that RNA declines to similarly low levels also in the persisting silk glands of

ligated larvae (Table 1) which contain no significant amounts of ecdysteroids (Rohner & Meyer, 1980). It is likely that the decline of RNA and the functional regression of silk glands are primarily due to the cessation of transcription that is caused either by lack of nutrients or by high ecdysteroid titre. This is linked to degradation of cellular organelles of proteosynthesis that also ensues both in the starving and pupating larvae (Blaes et al., 1980). On the other hand, the involution of silk glands is a specific response to high ecdysteroid concentration acting in the absence of the juvenile hormone; silk gland apoptosis sets in at higher RNA levels than those found in the well-preserved silk glands of ligated larvae.

TABLE 1. Contents of silk gland RNA in ligated larvae.

Larval age at ligation	μg total RNA/gland pair after ligation				
	0 hr	24 hr	48 hr	96 hr	144 hr
36 hr	32 ± 3	16 ± 7	9 ± 2	7 ± 3	4 ± 0.6
120 hr	81 ± 7	55 ± 9	25 ± 7	20 ± 3	5 ± 0.1

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