MECHANISMS OF DEVELOPMENT 125 (2008) 411–420

available at www.sciencedirect.com

ScienceDirect

journal homepage: www.elsevier.com/locate/modo

The role of 20-hydroxyecdysone and juvenile hormone in pupal commitment of the epidermis of the silkworm, *Bombyx mori*

Daisuke Muramatsu[a], *Terunori Kinjoh*[a,b], *Tetsuro Shinoda*[c], *Kiyoshi Hiruma*[a,b,*]

[a]*Faculty of Agriculture and Life Sciences, Hirosaki University, Hirosaki 036-8561, Japan*
[b]*Graduate School of Agricultural Sciences, Iwate University, Morioka 020-8550, Japan*
[c]*National Institute of Agrobiological Sciences, Tsukuba, Ibaraki 305-8634, Japan*

ARTICLE INFO

Article history:
Received 14 January 2008
Received in revised form
2 February 2008
Accepted 5 February 2008
Available online 12 February 2008

Keywords:
Commitment
Broad
Epidermis
Metamorphosis
Ecdysone
Juvenile hormone
Bombyx mori

ABSTRACT

During the pupal metamorphosis in insects, cellular commitment for pupal differentiation must precede before its differentiation. The pupal commitment of *Bombyx mori* epidermis occurred from day 3 to day 6 last (5th) instar larvae in response to the gradual increase in ecdysteroid titer in the presence of a small amount of juvenile hormone (JH). Yet the concealed preparatory process of the commitment had begun in the newly synthesized 5th instar larval epidermis (∼6 h before the ecdysis) as a competence phase, in which pupal commitment *in vitro* was induced by 20-hydroxyecdysone (20E) but inhibited by JH. This competence phase continued until day 2 5th instar, and the decrease and increase in cellular sensitivity to JH and 20E, respectively, occurred gradually during this period. In early day 3, autonomous pupal commitment began *in vitro* and 20E stimulated the commitment, but JH could only partially prevent the commitment in both cases. This apparent reversible to irreversible transition ended in early day 6 by the completion of pupal commitment, when the cells completely lost their sensitivity to JH and no longer expressed the larval cuticle protein gene 30. The expression of the transcription factor, *broad*, closely followed the commitment, so that we could use this gene expression as a molecular marker for pupal commitment. These results indicate that exposure to 20E and loss of the sensitivity of the epidermal cells to JH are required for the completion of pupal commitment, and suggest that the unusually long process over 3 days could be due to the presence of the detectable JH during the commitment.

1. Introduction

During the metamorphosis of arthropods, the developmental dynamics of cell specification must occur, i.e., cellular events have to switch from one state to another before the overt changes. To study this mechanism, one of the best available systems is the epidermis of the holometabolous insects which undergoes a larval–pupal transformation. When larvae develop toward the pupal stage, cellular commitment to pupal differentiation must take place before the actual differentiation, and this process is a necessary step for the completion of their life cycle through metamorphosis.

The insect developmental trajectory is coordinated primarily by two hormones: ecdysteroid and juvenile hormone (JH). When both ecdysteroid and JH are present, larval ecdysis is induced (see a review, Hiruma, 2003). Yet after ecdysis to

---

[*] *Corresponding author.* Tel.: +81 172 39 3819.
E-mail address: hiruma@cc.hirosaki-u.ac.jp (K. Hiruma).

0925-4773/$ - see front matter © 2008 Elsevier Ireland Ltd. All rights reserved.
doi:10.1016/j.mod.2008.02.001

the last instar larvae in *Manduca sexta*, the high JH titer in the hemolymph declines and becomes undetectable by day 3 (Baker et al., 1987) followed by a small ecdysteroid peak in the absence of JH (Wolfgang and Riddiford, 1986), which causes larval–pupal commitment of the epidermis. Thereby, this small peak of ecdysteroid is called "commitment peak". But actual pupal differentiation does not occur until the exposure to the molting surge of ecdysteroid during the prepupal stage (Riddiford, 1976a, b, 1978). Once pupally committed, the epidermal cells lose the ability to synthesize a new larval cuticle (Riddiford, 1982). A similar commitment mechanism in the epidermis is also found in *Mamestra brassicae* (Tsutsumiuchi et al., 1989). However, the occurrence of pupal commitment of lepidopteran wing discs is much earlier than the epidermis. In *Bombyx mori* wing discs, pupal commitment occurs within 16 h after the last larval ecdysis, and loss of the sensitivity of the wing disc cells to JH is the primary factor for the pupal commitment (Obara et al., 2002; Koyama et al., 2004b). A similar early commitment of wing discs also has been observed in *M. sexta* (Zhou et al., 1998) and *Precis coenia* (Kremen and Nijhout, 1989).

The process of commitment is divided into two stages, specification and determination (Harrison, 1933; Slack, 1991; Gilbert, 2006), and the former is characterized as a reversible but the latter is an irreversible state. In *M. sexta* dorsal epidermis, pupal commitment occurs in the sequence of the acquisition of competence to form pupal cuticle, the loss of responsiveness to JH, and then the loss of competence to form larval cuticle (Riddiford, 1978). In *B. mori* wing discs, Koyama et al. (2004b) also divided the commitment process into three different phases: initiation (reversible), progression (irreversible to reversible), and completion (irreversible). In both cases, these processes are completed in about 24 h.

In *B. mori*, the JH titer declines after the last larval ecdysis, but unlike in *M. sexta* (Fain and Riddiford, 1975; Baker et al., 1987), JH in the hemolymph is never wiped out completely (Niimi and Sakurai, 1997; Plantevin et al., 1987). In addition, there is no particular commitment peak of ecdysteroid detected, although the titer gradually increases from day 2 onward (Sakurai et al., 1998). Since JH has to be completely disappeared from the hemolymph to induce the commitment peak of ecdysteroid (Browder et al., 2001), the hormonal control underlying the epidermal commitment in *B. mori* seems to be different from that in *M. sexta*.

Here we show that larval–pupal commitment of *B. mori* epidermis occurs in response to the gradual increase in ecdysteroid titer even in the presence of a small amount of JH in the hemolymph. The competence phase, which has acquired the competence to form pupal cuticle, unexpectedly begins as early as newly formed 5th instar integument which is even before the ecdysis, but JH can prevent this process. Pupal commitment then begins to occur in day 3 5th instar larvae, and completes 3 days later. During this commitment phase, loss of the sensitivity of the cell responsiveness to JH occurs, and larval cuticle gene expression ceases. The commitment process is much longer than that found in *M. sexta* epidermis (Riddiford, 1978), which is probably due to the presence of JH during the process.

2. Results

2.1. *Change of pupal commitment of the dorsal abdominal epidermis in vivo*

Implantation of tissues into the penultimate (4th) instar larvae before the initiation of the molt to the 5th instar forces the implants to molt in the presence of both ecdysteroid and JH; therefore the state of commitment of the implants can be assessed (Riddiford, 1976a). When pieces of the dorsal integument from day 0 to day 7 5th instar *B. mori* larvae were implanted into day 1 4th instar larvae, the implants formed larval, larval–pupal intermediate and pupal cuticle when they were dissected out on day 1 5th instar stadium (Fig. 1). Implantation of day 0, 1, and 2 epidermis produced only larval cuticle without any trace of pupal cuticle. Yet early day 3 to day 4 epidermis produced very small patches of pupal cuticle among the larval cuticle, indicating the beginning of pupal commitment of the epidermis (Fig. 2b). After the early day 5, the pupal patches in the implants expanded, and all the epidermis produced >90% pupal cuticle by the early day 6, when spinneret pigmentation occurred. Differences between the segments were not observed (data not shown). These results indicate that pupal commitment of epidermis begins in very early day 3 5th instar larvae and progresses slowly for next 24 h. The commitment then rapidly progresses from early day 4 to early day 6.

The expression of the transcription factor, *broad*, corresponds with the pupal commitment of the *M. sexta* epidermis and wing discs (Zhou et al., 1998; Zhou and Riddiford, 2001), and specifies pupal development (Zhou and Riddiford, 2002). Therefore, we examined whether or not *broad* expression correlated with the pupal commitment of the *B. mori* epidermis. Fig. 2c showed that the expression of *broad* mirrored the pupal

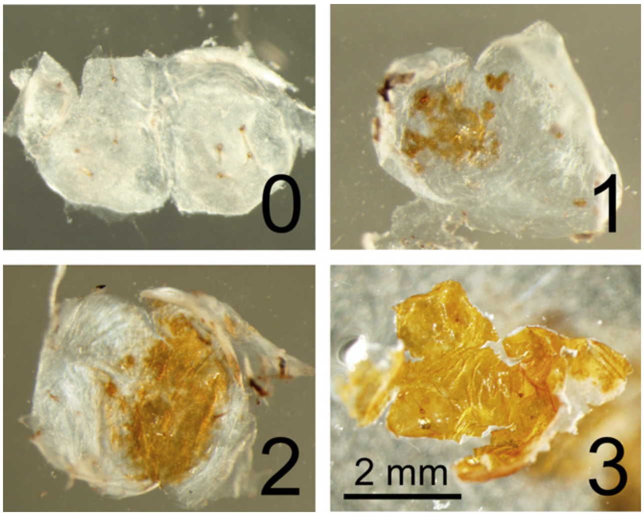

Fig. 1 – Scoring system for the pupal commitment of the *B. mori* epidermis assessed by the implantation assay. Score 0, cysts forming larval cuticle without any sign of pupal cuticle; Score 1, cysts forming <30% pupal cuticle; Score 2, cysts with 30–90% pupal cuticle; Score 3, cysts with >90% pupal cuticle.