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Effect of 20-Hydroxyecdysone on Yolk Protein Synthesis in *Drosophila* sp. (*robusta* Species Group)

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20-hydroxyecdysone (20-HE) seems to be related in the regulation of vitellogenesis in *Drosophila* sp. (*robusta* species group). Although yolk proteins (YPs) synthesis does not occur at a high rate in fat body cells of one day-old female after eclosion, application of 20-HE to isolated abdomens deprived of anterior endocrine glands stimulated the synthesis and secretion of YPs into the hemolymph. An injection of 0.3 μl of a 10^{-6} M 20-HE was sufficient to promote synthesis and secretion of YPs in isolated abdomens. The response of isolated abdomens to hormones was first detected between 2 hr and 3 hr after treatment of 10^{-3} M 20-HE. Transcript analysis showed that the effect of 20-HE on yolk protein synthesis was mediated at the level of transcription.

KEY WORDS: *Drosophila*, Yolk proteins, 20-hydroxyecdysone

The process of vitellogenesis is a major event in oogenesis in a number of different insects. In *D. grimshawi*, the three yolk proteins (YP1, YP2, and YP3) are encoded by single copy genes (*yp1*, *yp2*, *yp3*) on the X chromosome (Barnett *et al.*, 1980; Hovemann *et al.*, 1981; Riddel *et al.*, 1981; Hotzopolous and Kambysellis, 1987). These genes are normally expressed only in female producing YPs throughout adulthood by two tissues; the adult fat body cells and the ovarian follicular epithelium (Gutzeit, 1980; Brennan *et al.*, 1982). The YPs made in the fat body are secreted as vitellogenin into the hemolymph (Bownes and Hames, 1978), from which they are sequestered by maturing oocytes (Mahowold, 1972).

In *D. melanogaster*, the expression of YP genes is regulated by not only the sex-determination genes *transformer* (*tra*), *transformer-2* (*tra-2*), *doublesex* (*dsx*), and *intersex* (*ix*), but also the two insect hormones, 20-hydroxyecdysone and juvenile hormone (Handler and Postlethwait, 1978; Postlethwait and Handler, 1979; Bownes and Nothiger, 1981; Belote *et al.*, 1985; Burtis *et al.*, 1991.

Although the YP gene system of *D. melanogaster* have been extensively studied, little is known about the other species of *Drosophila*. Privious studies have shown that the YPs in *Drosophila* sp. (robusta species group) are consisted of three distinctive polypeptides (Kim *et al.*, 1992). Here, we have studied the effect of 20-hydroxyecdysone on the YP synthesis in this species.

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Materials and Methods

Maintenance of flies

Laboratory stocks of *Drosophila* sp. (*robusta* species group; Cheju strain) were maintained on yeasted medium containing sugar, commeal, agar

and yeast on a 12 hr light-12 hr dark cycle at 25°C.

Hormone treatment

To deprive of anterior endocrine glands, abdomens were isolated by ligating and cutting between the thorax and first abdominal segment from one day-old female flies after eclosion. Ten abdomens were injected with approximately 0.3 μl of 20-hydroxecysone (20-HE, Sigma) dissolved in 10% ethanol-Ringer solution, and 10% ethanol-Ringer solution as control.

After hormone treatment, hemolymph proteins were labelled by injecting the abdomens with 1 μ Ci of [^{35}S]-methionine (Amersham) dissolved in Ringer solution. After incubation for 2 hr at 25°C, the hemolymph was collected and solubilized in SDS sample buffer (Laemmli, 1970).

Electrophoresis of proteins

Sodium dodecyl sulphate (SDS)-polyacrylamide gel electrophoresis was performed on 10% or 8-15% gradient slab gel, according to Laemeli (1970). For the fluorography, the gels were impregnated with diphenyl oxazole (PPO), dried and exposed to Kodak XR film, as described by Laskey and Mills (1975).

Prepration of RNA

Total RNAs were isolated by homogenizing the flies in RNA extraction buffer (7 M urea, 2% SDS, 0.35 M NaCl, 1 mM Tris-HCl, pH 8.0). The homogenate was extracted with phenol and chloroform and was ethanol precipitated. To remove DNA the resulting precipitate was washed with 2 M LiCl, re-precipitated and resuspended in distilled water.

Transcript analysis

D. melanogaster YP genes in cloned in plasmid were a kind gift from Dr. M. Bowenes (Univ. of Edinburgh, Edinburgh). One μg of an equimolar mixture of pGem YP1, pGem YP2 was labelled with [α-^{32}P] dCTP (Amersham, 3000 Ci/mmol) to approx. 10^9 dpm/μg using the Pharmacia's oligolabelling kit.

For dot blots, Hybond membrane were washed in water, equilibrated with 20 × SSC, and dried. RNA samples (5 μg) in 5 μl distilled water were spotted onto the Hybond membrane, dried and baked for 2 hr at 80°C in a vacuum oven. Northern blots were made by transfering RNA from a 1% agarose denaturing gel onto Hybond membrane by wet blotting with 20 × SSC (Sambrook *et al.*, 1989). The blots were prehybridized for 8-20 hr at 37°C in hybrid buffer (50% formamide, 20 × SSPE, 2 × Dehardt's reagent, 0.1% SDS). Hybridization was in the same solution, at the same temperature and in the presence of denatured probe and 250 μg/ml salmon sperm DNA. Membranes were washed three times, for 10 min each at room temperature in 2 × SSC, 0.1% SDS. They were further washed, twice, for 20 min at 55° in 0.1 × SSC, 0.1% SDS and then dried and set up against X-ray film for autoradiography.

Results and Discussion

To determine the developmental schedule for yolk protein (YP) synthesis in normal female of *Drosophila* sp. (*robusta* species group), flies were injected with [^{35}S]-methionine at defined periods after eclosion. Two hours later the heamolymph was collected and prepared for electrophoresis. At eclosion, incorporation of label into YPs was barely detectable. The rate of label incorporation increased considerably from the three day after eclosion, but not equally in each YP (Fig. 1). The rate of label incorporation into YPs was variable from 12% to 40% of total hemolymph proteins during the nine days after echlosion. These fluctuations of YP levels suggest that the regulation of YP synthesis may operate by feedback of YP hemolymph levels.

Thus, to investigate the effect of 20-HE on YP synthesis, female abdomen isolated within first day after eclosion were aged for 24 hr to allow edogenous levels of hormone to decline, and then treated with various concentration of 20-HE. All concentrations tested of 20-HE caused a stimulation of label incorporation into the YPs (Fig. 2). The amount of label incorporation increased with the hormone concentration, the label incorporation in response to 20-HE was characteristically YP1 > YP2 ≃ YP3 (Fig. 3). An increase in amount of freshly labeled YPs into the hemolymph was first detected at 2 hr after hor-

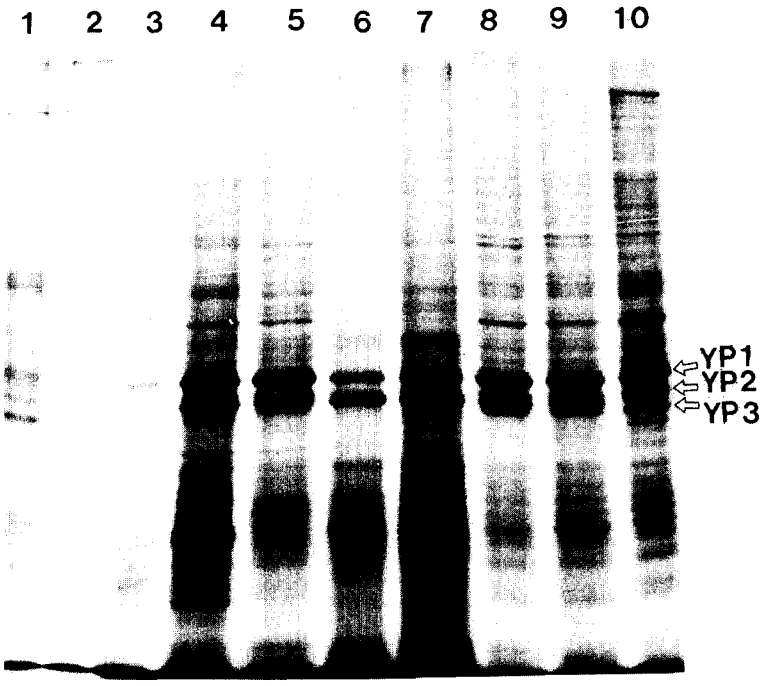

Fig. 1. Fluorogram of hemolymph proteins derived from normal female development of *Drosophila* sp. (*robusta* group). At various day after eclosion, the newly synthesized hemolymph proteins were analyzed as described in Materials and Methods. Lane 1, 30 min-old; 2, 1 day-old; 3, 2 days-old; 4, 3 days-old; 5, 4 days-old; 6, 5 days-old; 7, 6 days-old; 8, 7 days-old; 9, 8 days-old; 10, 9 days-old female after eclosion.

mone treatment (Fig. 4). The short time between the addition of hormone and the stimulation of YP synthesis in *D.* sp. (*robusta* species group) compares quite favorably with the results of *D. melanogaster* (Postlethwait and Handler, 1979; Jowett and Postlethwait, 1980, 1981).

Female flies within first day after eclosion were injected with 10^{-3} M of 20-HE, and 10% ethanol in Ringer's solution as control. At 6 hr after hormone treatment, RNA was extracted from equal numbers of flies and YP transcripts were identified by probing dot and Northern blots. The treatment of 20-HE produced detectable YP transcripts (Fig. 5) homologous to YP transcripts of *D. melanogaster.* Thus, this result showed that the effects of 20-HE on YP synthesis was mediated at the level of transcription of the YP genes in female fat bodies and ovarian follicles of *D.* sp. (*robusta* species group).

In *D. melanogaster,* although 20-HE and juvenile hormone can cause synthesis and secretion of YPs in fat bodies and ovarian follicles of female flies, juvenile hormone cause its sequenstration into oocytes (Postlethwait and Handler, 1979; Jowett and Postlethwait, 1981). We observed that application of juvenile hormone III into the immature females caused to be increase the vitellogenic oocytes in ovaries of *D.* sp. (data not shown). Thus, it seems likely that juvenile hormone stimulate YP uptake in many insects.

However, we are still unkown of the mechanism whereby the normal sex-specific synthesis of YPs in fat body cells is acheived. Male and female adults of *D. melanogaster* have almost identical levels of 20-HE (Handler, 1982; Bownes *et al.,* 1984). Thus, it is suggested that the sex determination genes (*intersex, transformer, transformer*-2, and *doublesex*) themselves rather than any sexually dimorphic levels of hormones cause the YP genes to be transcribed in females and to be repressed in males (Baker and Ridge, 1980; Postlethwait *et al.,* 1980; Bownes and Nothiger, 1981; Belote *et al.,* 1985). Using P element mediated transformation, it is reported that the sex genes

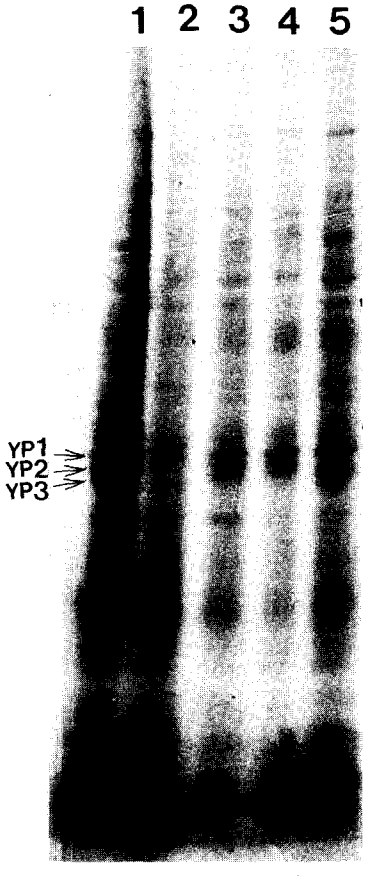

Fig. 2. Fluorographic analysis of the electrophoresed hemolymph from isolated abdomens treated with different concentrations of 20-hydroxyecdysone. For each treatment, 10 abdomens isolated at eclosion and aged for 24 hr were treated with 0.3 μl of 20-HE per abdomen for 6 hr, then injected with 1 μCi/abdomen of [^{35}S]methionine. The newly synthesized hemolymph proteins were analyzed as described in Materials and Methods. Lane 1, 10^{-3} M; 2, control (10% Ethanol/Ringer's sol.); 3, 10^{-4} M; 4, 10^{-5} M; 5, 10^{-6} M of 20-hydroxyecdysone.

and 20-HE are independently or synergistically at different site for the fat body and ovarian expression of the YPs (Garabedian *et al.*, 1985; Bownes *et al.*, 1987; Shirras and Bownes, 1987; Burtis *et al.*, 1991).

It remains to be established just what significance the ecdysteroid-induced synthesis of YPs has with respect to the normal functioning of the YP genes in *D.* sp. (*robusta* species group). However, by isolation from this species YP genes,

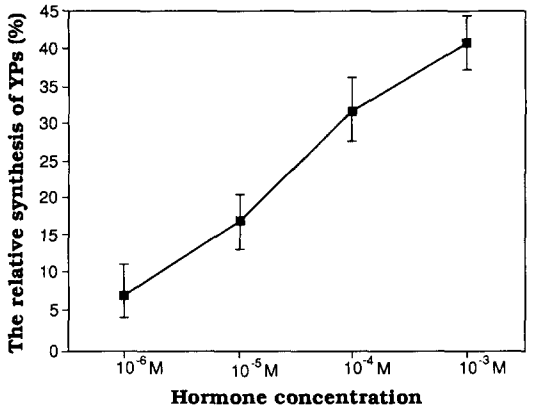

Fig. 3. Quantification of the dose-response to 20-hydroxyecdysone. Flurographs of the experiment in Fig. 2 were scanned using microdensitometer and the label incorporation estimated by measuring the peak areas of the YP bands among the total hemolymph proteins. Each point represents the mean of three separate experiments. The error bar represents standard deviation.

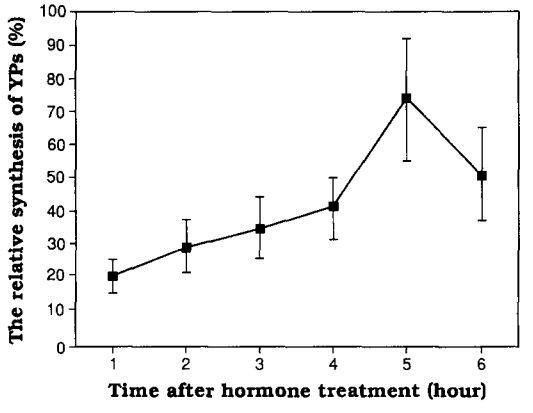

Fig. 4. Time course of the response of isolated abdomens to 20-hydroxyecdysone. A group of ten abdomens isolated from 1 day-old female after eclosion and aged for 24 hr was treated with 0.3 μl 10^{-3} M/abdomen of 20-hydroxyecdysone, and analysezed as described in Materials and Methods. The resulting fluorographs of gels were canned by microdensitometer and the label incorporation estimated by measuring the peak areas of the YP bands among the total hemolymph proteins. Each point represents the mean of three separate experiments. The error bar represents standard deviation.

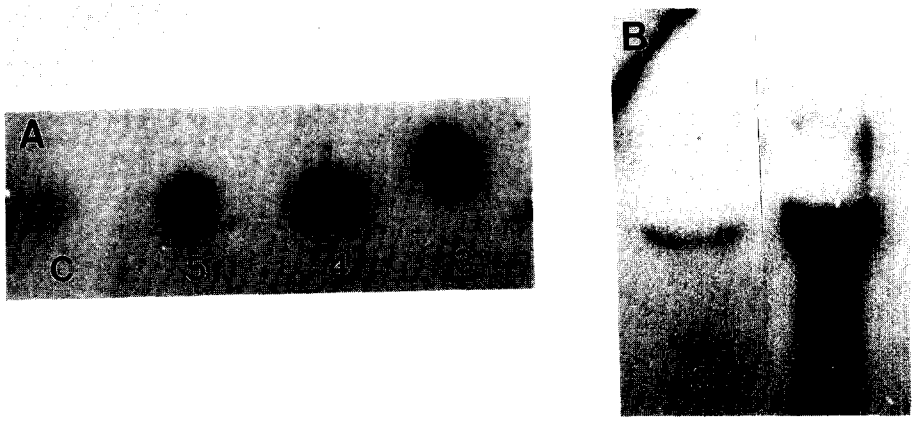

Fig. 5. Dot blot (A) and Northern blot (B) showing the induction of YP-homologous trnscripts in immature females of *D.* sp. (*robusta* species group) after 20-hydroxyecdysone. The blots were hybridized against a mixed YP1/2 probe of *D. melanogaster* as described in Materials and Methods. C, control (10% Ethanol/ringer's sol.); 3, 10^{-3} M; 4, 10^{-4} M; 5, 10^{-5} M of 20-hydroxyecdysone.

sequencing them, and then comparing the DNA sequences flanking the genes with those of *D. melanogaster,* it may be possible to identify conserved sequences important for the responses of these genes.

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제주먹초파리에서 난황 단백질 합성에 미치는 20-Hydroxyecdysone의 영향

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제주먹초파리의 난황형성 과정의 조절에는 20-hydroxyecdsone(20-HE)도 일부 관여한다. 부화후 1일된 암컷 성체에서 난황 단백질의 합성은 미약하다. 그러나 전장의 내분비선을 제거한 복부에 20-HE을 주입하면 난황 단백질의 합성과 hemolymp로 분비가 촉진된다. 분리된 복부에서 난황 단백질 합성과 분비의 촉진효과는 10^{-6} M 농도의 호르몬 처리로 충족되었으며, 10^{-3} M 농도로 호르몬을 처리했을 때 호르몬의 효과는 처리후 2-3시간에 처음 검출되었다. 그리고 전사체의 분석 결과는 20-HE는 전사수준에서 난황단백질의 합성을 촉진시키는 것으로 사료되었다.