20-hydroxyecdysone stimulates tissue-specific yolk-protein gene transcription in both male and female *Drosophila*

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SUMMARY

The yolk polypeptides of *Drosophila* are normally synthesized in the fat body and ovarian follicle cells of adult females. In response to 20-hydroxyecdysone males synthesize yolk polypeptides. The actual level of yolk polypeptides synthesized in males is not always a direct reflection of the YP-transcripts present. Initially YP-transcripts are efficiently translated into polypeptides whereas later they are not and the YP-transcripts can have a half-life of less than 8 h in males. We suggest that the expression of the genes coding for the yolk polypeptides in males may be regulated at transcriptional and translational levels. Treatment of females with 20-hydroxyecdysone leads to a transient increase in YP-transcript accumulation, but the response is difficult to assess in whole flies due to the high variability in transcript levels during normal development.

Analysing the response to 20-hydroxyecdysone at the level of specific tissues shows that transcript accumulation is dramatically increased in body walls (fat-body cells, epidermis and oenocytes) of both males and females. Gut, Malpighian tubules, testis and ovaries are not affected. Treatment of females with 20-hydroxyecdysone followed by measuring YP-transcript accumulation over the next 24 h in ovaries and body walls separately, confirms that only body walls respond to the hormone. There is an increase in yolk-polypeptide synthesis during the period of increased YP-transcript accumulation in females.

We conclude that the response of the YP-genes to 20-hydroxyecdysone is tissue-, but not sex-specific.

INTRODUCTION

The three yolk polypeptides (YPs) of *Drosophila melanogaster* are synthesized in the follicle cells of the ovary and in the fat body of adult females (Kambysellis, 1977; Hames & Bownes, 1978; Postlethwait & Kashnitz, 1978; Bownes & Hames, 1978; Brennan, Weiner, Goralski & Mahowald, 1982). Adult males do not usually synthesize these polypeptides, however they can be induced to do so by 20-hydroxyecdysone (Postlethwait, Bownes & Jowett, 1980). The response of the males to a single hormone injection or feeding with hormone for 2 h is a short burst of yolk-polypeptide synthesis lasting approximately 24 h, the maximum

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rate of yolk polypeptide synthesis being between 8 h and 14 h after hormone treatment (Bownes, 1982).

When females first emerge YP mRNAs are only present in small amounts and gradually accumulate over the first 24 h of adult life (Barnett & Wensink, 1981). The mRNA available for translation into yolk polypeptides mirrors the total YP-mRNA present indicating that initially the expression of the genes in females operates at the level of transcription. In males, treatment with 20-hydroxyecdysone leads to increased accumulation of YP-transcripts (Shirk, Minoo & Postlethwait, 1983; Shirras & Bownes, unpublished). However the expression of the genes in response to 20-hydroxyecdysone as measured by yolk polypeptide synthesis in vivo, is short-lived (Bownes, 1982). This suggests either that the YP-mRNAs produced are relatively unstable in a male environment, or that controls operate in the male to make the YP-transcripts less readily translated into proteins. In preliminary experiments measuring the hybridization of labelled cloned YP-genes to total RNA, the peak accumulation of YP-transcripts in males occurred at 16 h, not at 8–12 h when the peak of yolk polypeptide synthesis was found to occur. (Shirras & Bownes, unpublished).

Therefore we have investigated the level at which the expression of these three genes is regulated in males by measuring YP-transcript accumulation during a 24 h response to the hormone. The translation of these transcripts into yolk polypeptides by the rabbit reticulocyte lysate cell-free translation system has also been measured. The results indicate that the expression of the genes is modulated in vivo by transcriptional and possibly also post-transcriptional control mechanisms, and that the transcripts are degraded fairly rapidly following induction of the genes by 20-hydroxyecdysone. We then investigated the tissue specificity of the response to hormone. Transcription of the YP-genes only occurs in male carcasses, not in testes, gut or Malpighian tubules. Furthermore the YP-genes in the female body wall are also more actively transcribed, and/or the RNA is made more stable after treatment with 20-hydroxyecdysone. Ovarian transcript levels, however, remain unaffected. 20-hydroxyecdysone therefore seems to affect the expression of the YP-genes in a tissue-specific but not in a sex-limited fashion.

**MATERIALS AND METHODS**

_Maintenance of stocks_

Flies were maintained on standard yeast, cornmeal, sugar and agar medium at 25 °C. The pooled haemolymphs of each set (10–15 flies) were collected into 50 μl Laemmli buffer (Laemmli, 1970). Tissues were dissected in Ringers (Chan & Gehring, 1971), transferred to 50 μl Laemmli buffer, vortexed and the debris separated by centrifugation. 1 μl samples were precipitated with TCA to calculate total incorporation into proteins. The polypeptides were separated by
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polyacrylamide gel electrophoresis as in Bownes, Dempster & Blair (1983) and the gels were prepared for fluorography either by the technique of Bonner & Laskey (1974) or by using 'enlightening' (New England Nuclear).

The resulting autoradiographs were scanned with a densitometer and the peak areas corresponding to yolk polypeptides were calculated. Where comparisons are made between samples, all samples were run on the same gel and the same autoradiograph was scanned in each case. To ensure that this method of scanning gels was reasonably quantitative we compared the total area under all the proteins of an autoradiograph with the total TCA precipitable counts loaded onto a gel. In a series of five samples the ratios of TCA precipitable counts loaded was 1: 1.4: 2.2: 1.2: 0.7 and the ratios of the total areas of the same samples after autoradiography was 1: 1.7: 2.4: 1.4: 0.7. Thus agreement is fairly good and the method would appear to be valid for providing approximate comparisons between proteins in various samples.

Isolation of RNA

RNA was isolated from whole flies and dissected tissues as described by Bownes et al. (1983).

Translation of RNA in a cell-free translation system

Samples of 5 µg of total RNA were translated in the rabbit reticulocyte cell-free translation system, as described by Pelham & Jackson (1976).

Precipitation with anti-YP antibody

Yolk polypeptides were precipitated after translation in the rabbit reticulocyte lysate cell-free translation system as described in Isaac & Bownes (1982).

Preparation of 32P-labelled YP-probes

These were prepared as described by Bownes et al. (1983) using pYP1, pYP2 and pYP3 of Barnett, Pachl, Gergen & Wensink (1980). Probe activities were approximately 10^7 c.p.m./µg DNA.

Specificity of YP-probes

As described by Barnett et al. (1980), we found that the three cloned YP genes pYP1, pYP2, and pYP3 specifically hybridize to the yolk-protein genes and do not hybridize to any other sequences in the Drosophila genome. The experimental conditions used for subsequent hybridization experiments were such that there was no cross-hybridization between pYP1 and pYP3, or pYP2 and pYP3, but that pYP2 and pYP1 showed some cross-hybridization. For example a pYP2 probe hybridized to both pYP2 and pYP1 on nitrocellulose filters showed 10% as much hybridization to pYP1 as to itself. This would be expected from the sequences of these genes (Hovemann & Galler, 1982).
Measurement of YP-RNA levels

Transcripts coding for the yolk polypeptides were measured by the dot hybridization techniques of Thomas (1980). Details of our protocols and methods of quantitation of the resulting autoradiographs are described in Bownes et al. (1983), the only modifications being that filters were hybridized for 40 h and washed finally at 52 °C (two times) rather than 50 °C. The most accurate method is to measure the area x density of the dots on the resulting autoradiographs. This technique is useful for comparing transcript levels between samples, but does not give absolute quantitation of transcripts, nor does it distinguish hnRNA from mRNA. The weak hybridization seen when male RNA is spotted is due to the DNA isolated along with the RNA. A similar signal is seen after alkaline hydrolysis of similar quantities of female RNA, and this signal is removed when the samples are treated with LiCl to remove the DNA. We prefer however to see this weak signal to locate precisely where samples are spotted onto the nitrocellulose.

For Northern blots 10 μg samples of total RNA were separated on formaldehyde gels at 50 V overnight and transferred to nitrocellulose. Hybridization conditions were similar to those used for dot blots. The transcript sizes are 1.59 to 1.61 kb for YP1, 1.59 to 1.61 and 1.66 to 1.68 kb for YP2 (these two transcripts differ at the 3' end) and 1.53–1.55 for YP3 (Hung, Barnett, Woolford & Wensink, 1982). The genes have only small introns, identified by S1 mapping, about 70–80 bp in length and variable length polyA tails (Hung et al. 1982). Thus separation by electrophoresis and hybridization to the pYPl, 2, 3 probe still does not distinguish hnRNA from mRNA, and Northern blots give little more information than the blots used in the majority of these experiments to avoid loss of RNA which occurs in transferring from the gel to nitrocellulose.

Treatment with hormone

Adults aged 3 days were lightly etherized and either injected with 0.2 μl of \(10^{-2}\ \text{m-20-hydroxyecdysone in Ringers (Chan & Gehring, 1971), or alternatively they were fed on a sugar solution containing }10^{-3}\ \text{m-20-hydroxyecdysone for 2 h at 25 °C. Control flies for feeding experiments were fed on the sugar solution for a similar period of time. They were subsequently maintained on normal medium at 25 °C.}

All experiments were performed at different times, on different fly populations, and the transcript levels were measured using different probes. This was done to avoid biasing results because of the characteristics of particular populations of flies. Nonetheless experimental protocol, rearing conditions, method, site and volume of injection, and feeding methods were always repeated as exactly as possible. In some experiments RNA from the same batch of induced males was analysed by dot hybridization (Thomas, 1980) and in the cell-free translation system. The same groups of induced males were also used to label
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their haemolymph yolk polypeptides with [35S]methionine before RNA extraction in some experiments.

In experiments looking at individual tissues, these were dissected into Ringers at the end of the experiment and transferred immediately into RNA extraction buffer. They were either extracted immediately or frozen rapidly in liquid nitrogen and stored at —70°C until the RNA was extracted.

RESULTS AND DISCUSSION

Protein synthesis in vivo

We measured the synthesis of yolk polypeptides and their secretion into the haemolymph by injecting 3-day-old adults with [35S]methionine and collecting the haemolymph 2 h later for analysis by SDS polyacrylamide gel electrophoresis and fluorography (some of the results are presented in Table 1). Our previous studies showed that the peak of the yolk-polypeptide synthesis in males which have been stimulated with 20-hydroxyecdysone, occurred at about 12 h after hormone treatment, but that this varied between the populations. Yolk-polypeptide synthesis was subsequently reduced, and sometimes undetected, 24 h after treatment (Bownes, 1982). The degree of induction of the yolk polypeptides varies between experiments. Sometimes the yolk polypeptides represent only 12–14% of the total newly synthesized haemolymph proteins, yet in other experiments they can reach 35–40%. The method of hormone treatment contributes to this, feeding with the hormone (10^-3 M) generally giving a

Table 1. YP-synthesis and YP-transcript accumulation in males treated with 20-hydroxyecdysone compared to wild-type females

<table>
<thead>
<tr>
<th>Experiment</th>
<th>Time after treatment in hours</th>
<th>% female control level</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. Males injected with 20-hydroxyecdysone</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>4</td>
<td>32</td>
</tr>
<tr>
<td></td>
<td>8</td>
<td>63</td>
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<td>20</td>
<td>51</td>
</tr>
<tr>
<td></td>
<td>24</td>
<td>48</td>
</tr>
<tr>
<td>2. Males injected with 20-hydroxyecdysone</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>12</td>
<td>44</td>
</tr>
<tr>
<td></td>
<td>24</td>
<td>13</td>
</tr>
<tr>
<td>3. Males injected with 20-hydroxyecdysone</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>12</td>
<td>44</td>
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<td></td>
<td>24</td>
<td>15</td>
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* Corrected for background in untreated male samples.
larger induction than injecting it (0.2 µl 10⁻² M per fly). Females also show variation in the rate at which they synthesize yolk polypeptides. In 3-day-old females 43–64 % of the total [³⁵S]methionine-labelled haemolymph proteins in a 2 h period were yolk polypeptides. This variability is probably related to many factors including the nutritional condition of the population, time of day, etc., these factors will vary despite maintaining flies under essentially identical culture conditions.

Expressing YP-induction in males in terms of the level of YP-expression in females of the same age from the same population in the same vial (this comparison should avoid, as much as is technically possible, variabilities due to differences between cultures) we obtain values of from 30 % to 100 %. Males have only half the number of genes coding for yolk polypeptides in each cell due to their X-chromosome location, so the observed yolk polypeptide synthesis represents a substantial induction of yolk polypeptide gene expression in male tissue.

Yolk polypeptide transcript accumulation in hormone-treated male flies

During the hybridization of labelled cloned YP-gene probes to RNA isolated from induced males and immobilized on DBM paper, we observed that the peak of yolk polypeptide transcript accumulation was at around 14–16 h after hormone induction (Shirras & Bownes, unpublished). This suggested that the peak of yolk polypeptide synthesis occurred before the peak of yolk polypeptide transcript accumulation. However, because of the possibility of variability between groups of flies, we could not be sure that, had we measured the synthesis in vivo of YP's in flies whose peak transcript accumulation occurred at 14–16 h, the same population would not have shown a 14–16 h peak in yolk polypeptide synthesis.

We decided, therefore, to perform an experiment using the same group of flies measuring protein synthesis in vivo and YP-transcript levels in the whole flies, to look at the relationship between transcription and translation of the yolk polypeptide genes.

3-day-old males were treated with 10⁻² M-20-hydroxyecdysone. Yolk polypeptides synthesized and secreted into the haemolymph during a 2 h labelling period.
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Fig. 1

In vivo protein synthesis

Transcript accumulation

In vitro protein synthesis

Fig. 1
were measured. Their rate of synthesis was highest at 12 h (84% of the female level) and, in this experiment, was still relatively high at 24 h (52% of the female level). The degree of protein synthesis is shown in Fig. 1A. Transcript levels were measured using the technique of Thomas (1980); dotting 5 μg samples of RNA onto nitrocellulose, hybridizing with a mixed probe containing equimolar quantities of labelled cloned YP1, 2 and 3 genes, and exposing the filters to X-ray film. The area x density of the dot is proportional to the amount of YP-transcript present under the conditions we use (Bownes et al. 1983). The results are recorded in Fig. 1B. The peak of transcript accumulation was at 16 h (100% of female level), and fell by 24 h (54% female level). In this group of flies, the peak of yolk polypeptide synthesis preceded the peak of YP-transcript accumulation, which suggests that post-transcriptional control mechanisms may regulate expression of the YP-genes as well as factors at the transcriptional level. It is interesting that the accumulated transcript levels dropped by 50% between 16 and 24 h after induction, suggesting a half-life for the male YP-transcripts of not more than 8 h. We do not know if further transcription of the genes occurs during this period. If so, then the half-life may be shorter still.

RNA from induced males and control females was translated in the rabbit reticulocyte lysate cell-free translation system. As shown in Fig. 1C the synthesis of yolk polypeptides follows the general trend of the total transcripts present, but the male YP-transcripts were in all cases translated considerably less well in vitro than were the female YP-transcripts. This is not true of all other male messages since general stimulation of protein synthesis in the cell free system was from 19 000–52 000 c.p.m. /μl of lysate stimulated with male RNA, and 27 000 c.p.m. /μl of lysate stimulated with female RNA. The curves for cell-free translation product follow the shape of the transcript-accumulation curves, and do not follow the curve for yolk polypeptide synthesis in vivo, at least at the early time points. This suggests that the factor regulating in vivo rates of translation does not lie within the structure of the RNA.

RNA isolated from males after hormone treatment was analysed by Northerns and, as can be seen in Fig. 2, YP-transcripts were always of similar size to those observed in females. There is also no signal in male samples before hormone treatment, and the lack of unusual-sized transcripts suggests that there are no major differences in the transcripts induced in males compared to those normally present in females. We would not, however, detect small differences in processing or transcript size by this method.

As this group of flies fell towards one of the extremes of variation between populations, in that it showed a very high peak of yolk polypeptide synthesis and still quite substantial synthesis at 24 h, we repeated the experiment twice, measuring transcript levels and protein synthesis in vivo, using fewer time points. In both populations yolk polypeptide synthesis was much reduced by 24 h (Table 1) and at 12 h, male yolk polypeptide synthesis in vivo represented a smaller proportion of the control female level than did transcript accumulation.
Fig. 2. Northern analysis of RNA extracted from whole male flies induced to produce YP-transcripts with 20-hydroxyecdysone. RNA was separated on formaldehyde gels, transferred to nitrocellulose and hybridized to pYP1, pYP2, and pYP3. The resulting autoradiograph is shown. (a) Control; (b) 0 h after hormone treatment; (c) 4 h after hormone treatment; (d) 8 h after hormone treatment; (e) 12 h after hormone treatment; (f) 16 h after hormone treatment; (g) 20 h after hormone treatment; (h) 24 h after hormone treatment.
Transcript levels reached 64% and 67% of female levels by 12 h, while yolk polypeptide synthesis reached 44% and 40% respectively. Yolk polypeptide synthesis in vivo was therefore 69% and 60% of the predicted levels for the number of transcripts available in males compared to females. In the first experiment at 24 h the relationship between protein synthesis in vivo and transcript levels showed no discrepancy, but in the latter two experiments there was only a 35% and 25% comparative efficiency of translation (see Table 1). Furthermore at 4, 8 and 12 h in experiment 1 the male RNA seems to be translated into protein more efficiently than the female RNA. Thus there may well be translational controls in flies operating at various points after hormone induction, since the level of protein synthesis in the males, late in the cycle of induction, is often lower than one would predict on the basis of the number of transcripts present and their translation into yolk polypeptides in females. In the second and third experiments the reduction in transcript accumulation was much slower than in the first experiment but yolk polypeptide synthesis in vivo fell more rapidly. Presumably YP-gene expression results from a combination of transcriptional control of the YP-genes, mRNA turnover, and the rate of translation of the YP-mRNAs. Regulation at each of these levels appears to operate at some point during hormonal induction, although it is possible that the inefficient translation into yolk polypeptides several hours after hormone treatment could result from altered amino acid uptake and be a non-specific effect of the hormone rather than a specific mechanism to inhibit YP translation in males.

In feeding experiments we had previously observed very high levels of induction, but yolk-polypeptide synthesis was still low by 24 h. We measured YP-transcript levels after feeding 20-hydroxyecdysone to males for 2 h at times 2 h, 6 h, 18 h and 24 h. At 2 and 6 h transcript accumulation was less than 2% female levels, but by 18 h it had reached 60% and by 24 h it had fallen to 3%. Thus virtually all of the transcripts were degraded in a 6 h period.

The arguments for there being post-transcriptional control of these genes rely upon measured rates of secretion of yolk polypeptide into the haemolymph being an accurate reflection of the rates of yolk polypeptide synthesis in vivo. We therefore investigated the incorporation of $^{[35S]}$methionine into protein in male fat bodies. Proteins co-migrating with the yolk polypeptides are abundant but, as in females, we could see no obvious signs of yolk polypeptides. Thus there appears to be no accumulation of proteins in the fat body. To confirm this, we precipitated fat-body-synthesized yolk polypeptides with anti-YP antiserum and found only the very low levels of yolk polypeptides normally present in female fat body. These results show that yolk-polypeptides are not accumulated in the fat body, however the results of $^{[35S]}$methionine incorporation in yolk polypeptides may still not precisely reflect yolk-protein synthesis in the fat body. There could be changes in the methionine pool available for translation during the course of the experiment. For example, as the hormone treatment induces the synthesis of a new subset of proteins there will be different amino acid
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Fig. 3. YP-transcript accumulation in hormone-treated females. A) Groups of females were fed with $10^{-3}$ M-20-hydroxyecdysone for 4 h. Subsequently RNA was isolated from the whole flies and YP-transcripts measured by dot-hybridization. B) Groups of females were fed continuously on $10^{-3}$ M-20-hydroxyecdysone for 24 h. The graphs show the area $\times$ density of the dot plotted against time after hormone treatment ceased. The points show the mean from three to four separate experiments using flies from two different populations ± standard deviation.
requirements in the fat body. This may affect the uptake of the additionally
injected, labelled methionine and cause artefacts in our results. Thus it is still
possible that the apparent inhibition of translation of YP-transcripts could be a
non-specific effect.

**Yolk-polypeptide-transcript accumulation in hormone-treated female flies**

Since male YP-genes clearly become actively transcribed in the presence of
high doses of 20-hydroxyecdysone we asked whether females also responded in
a similar way. The results of feeding 20-hydroxyecdysone to females for 2 h, then
measuring YP-transcription accumulation as described earlier for males is shown in
Fig. 3A. There does appear to be a slight increase in transcript accumulation,
though it is difficult to judge how significant this is against the variability of YP
mRNA levels in adult females. Continuous feeding of 20-hydroxyecdysone also
shows an increase in transcript levels soon after hormone treatment commences
(Fig. 3B).

**Which tissues are induced to transcribe the YP-genes by 20-hydroxyecdysone?**

The above results clearly show that males can transcribe the YP-genes in
response to 20-hydroxyecdysone and that perhaps transcription increases in
females. However, this requires unphysiological doses of hormone to be injected
into the flies, and although it may well be rapidly metabolized and excreted, the
high doses do seem to be needed to activate the genes. We therefore asked
whether YP-transcripts appear in just the fat body of males, or whether they also
appeared in the testes, gut and Malpighian tubules (i.e. non-specifically in all
tissues). We have previously shown that in females YP-transcripts are present in
body walls and ovaries, but not in the gut and Malpighian tubules (Bownes
et al. 1983).

Females and males were injected with 20-hydroxyecdysone and 12 h later RNA
was extracted from the carcass, i.e. the head, thorax and abdominal body wall (this
contains fat body cells, epidermal cells and oenocytes); the gut and Malpighian
tubules, and the gonads. YP-transcript levels were established using the dot blot
method. The results (one set of which is shown in Fig. 4) indicate that only the male
and female carcass, and the female gonad preparations of RNA contained YP-
transcripts. Other tissues in males and females did not respond to the presence of
20-hydroxyecdysone by transcribing the YP-genes. Thus the hormonal induction
in males shows the same tissue specificity for YP-gene transcription as is normally
observed in untreated females, and high doses of 20-hydroxyecdysone do not in-
duce YP-gene expression in the gut and Malpighian tubules of females. It was also
observed that treatment of females with 20-hydroxyecdysone stimulated YP-
transcript accumulation in the body wall preparations, but not in the ovaries. This
was reproducible in two repeats of the experiments and suggested that in females
the YP-genes were responsive to 20-hydroxyecdysone only in the body walls, thus
the response is similar in males and females.
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Fig. 4. Tissue-specificity of hormone induction. Dot blot of RNA from various tissues hybridized against a YP1, YP2 YP3 probe. (a) untreated ♀ (3-7); (b) untreated ♀ carcass (7-9); (c) untreated ♀ ovary (3-4); (d) 20-hydroxyecdysone-treated ♀ ovary (3-2); (e) 20-hydroxyecdysone-treated ♀ carcass (18-1); (f) untreated ♀ gut and Malpighian tubules (0-07); (g) 20-hydroxyecdysone-treated ♀ gut and Malpighian tubules (0-16); (h) 20-hydroxyecdysone-treated ♂ carcass (2-75); (i) 20-hydroxyecdysone-treated ♂ testis and accessory glands (0-00); (j) 20-hydroxyecdysone-treated ♂ gut and Malpighian tubules (0-00); (k) untreated whole ♂ (0-05). Number in brackets is area × density of the dot.
**Yolk-polypeptide transcript accumulation in hormone-treated female tissues**

It seemed possible that since the response to 20-hydroxyecdysone was limited to the body walls of females that ovarian levels of transcripts had obscured the response to hormone in the female time course shown above. We therefore repeated these experiments feeding females with 20-hydroxyecdysone for 2 h, but separated the ovary and carcass before measuring YP-transcript levels after various times. The results in Fig. 5, show that there is a dramatic increase in YP-transcript accumulation compared to the controls, and that indeed the response is tissue limited.

![Graph showing YP-transcript accumulation](image)

**Fig. 5.** YP-transcript accumulation in ovaries and body walls of hormone-treated females. Flies from the same population were fed either on 20-hydroxyecdysone or sugar for 2 h then transferred to normal medium. At various times the ovaries and body walls were isolated, RNA separated and YP-transcript levels measured. The graph shows the area × density of dot plotted against time after ceasing feeding with 20-hydroxyecdysone. ▲—▲ control body wall; △—△ 20-hydroxyecdysone-treated body wall; •—• control ovary; ○—○ 20-hydroxyecdysone-treated ovary.
The increased transcript levels are observed as soon as the feeding period ends (i.e. 2 h), thus there is a rapid response to the hormone. We cannot determine from these experiments whether this is due to increased transcription or preventing turnover of mRNAs already present, but since there is a rapid two-fold increase in transcript levels, new transcription seems likely to be involved. YP transcript levels remained higher than controls for 24 h. The quantity of YP transcripts present did not fall by a half during the experiment, thus the half-life of the YP-mRNA or the rate of transcription may exceed that in males. However, due to the background of high YP transcript levels, it is unclear whether the induced transcripts will have the same stability as those present normally. Experiments labelling YP-RNA in vivo directly will be necessary to calculate the half-life of YP-mRNA in females. These experiments are difficult due to the problems of radioactively labelling a specific mRNA with tritium to a sufficient degree to detect it reliably. It is not clear why levels of YP transcripts fall during this experiment in the control population of flies, but it may be associated with the period spent feeding on sugar rather than on complete medium.

The YP-genes, then, respond to 20-hydroxyecdysone in one tissue, but not in another tissue where they are normally expressed in adult females. One of several possibilities is that the ovarian follicle cells lack the appropriate hormone receptors, but that ecdysone receptors are present in one or more of the cell types found in the body wall preparations. It does suggest that the regulation of expression of the YP-genes may differ in these two tissues. This finding is in agreement with Jowett & Postlethwait (1980) who found that when isolated abdomens were treated with 20-hydroxyecdysone, only the body walls resumed YP synthesis.

**Yolk-polypeptide synthesis in hormone-treated females**

We measured yolk polypeptide synthesis by labelling with $[^{35}\text{S}]$methionine in vivo at various points after hormone treatments. The results shown in Fig. 6 show only a slight increase in the hormone-treated flies in the ratio of newly synthesized yolk polypeptides compared to total haemolymph polypeptides at 12 and 24 h, but at 12 h there is a distinct accumulation of yolk-polypeptides in the fat body cells (YP1 and YP2, can be clearly seen accumulating above a protein which comigrates with YP3 in Fig. 6), thus indicating that the new transcripts are translated into proteins, and that they mostly remain in the fat body cells rather than being secreted into the haemolymph.

This shows that in females, rates of YP-gene transcription and translation are not normally maximal. The increased yolk polypeptide synthesis in the females treated with ecdysone did not result simply from increased rates of protein synthesis. Table 2 shows the incorporation of $[^{35}\text{S}]$methionine into TCA-precipitable counts, and it can be seen that although the incorporation is variable as might be expected when haemolymph is collected by capillary action, there is no trend towards increased translation in the ecdysone-treated flies. There are
Fig. 6
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Table 2. Protein synthesis in hormone-treated females

<table>
<thead>
<tr>
<th>Experiment</th>
<th>T.C.A. precipitated counts/female</th>
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<tbody>
<tr>
<td></td>
<td>haemolymph</td>
<td>body wall</td>
</tr>
<tr>
<td>0 h + 20HE control</td>
<td>4450</td>
<td>236810</td>
</tr>
<tr>
<td>12 h + 20HE control</td>
<td>9700</td>
<td>208450</td>
</tr>
<tr>
<td>24 h + 20HE control</td>
<td>2690</td>
<td>178200</td>
</tr>
</tbody>
</table>

| 20HE, 20-hydroxyecdysone. |

one or two other peptides, indicated in Fig. 6, that also seem to be present in greater quantities in the hormone-treated flies, yet others do not change. The increased YP-synthesis therefore appears to be part of a specific response to the hormone.

**GENERAL DISCUSSION**

The results presented in this paper suggest that, when the YP-genes are expressed in males following treatment with 20-hydroxyecdysone, the actual level of gene expression, as determined by the synthesis of yolk polypeptides may be modulated by post-transcriptional as well as transcriptional controls. One problem we encounter with this type of experiment is the large variability in YP-transcript accumulation and rates of yolk polypeptide synthesis between groups of flies. This is true of untreated females and hormone-induced males and means that quantitation is difficult and we are only able to discover the general features involved in regulating YP-gene expression. It will prove interesting in the future to look at the expression of the three genes individually to determine if they are co-ordinately regulated.

The general pattern which emerges is that after hormone treatment yolk polypeptide synthesis begins in males as transcripts appear. At first they are...
translated into yolk polypeptides very efficiently, compared to translation of the transcripts present in females. Shirk et al. (1983) measured transcript accumulation and yolk polypeptide synthesis at just one time point (8h) after 20-hydroxyecdysone treatment. Their data also show that at this time the transcripts present are translated efficiently into yolk polypeptides. Following this we find that translation into protein often becomes inefficient in males in relation to the transcripts accumulated, the time at which this occurs is very variable between groups of flies. Furthermore the total YP-transcripts present begins to drop by 24 h, thus the mRNA must be degraded fairly rapidly. From our experiments, measuring transcript levels after hormonal stimulation of females, we cannot deduce the half-life of the YP-mRNA since the natural state is that transcripts are present. We do not know, therefore, whether there are post-transcriptional controls in females, but we have detected them in dsxD intersexual flies (Bownes et al. 1983). At present we are trying to in vivo label YP-mRNA so we can measure directly when the genes are being transcribed in females. A further point which we observe is that male YP-transcripts translate very poorly in a cell-free translation system compared to female YP-transcripts. Shirk et al. (1983) translated YP-RNA isolated from males 8 h after hormone induction and also observed a poor translation efficiency compared to female YP-RNA. The reason for this is unclear and it does not seem to help to explain the post-transcriptional controls we observe in vivo in males, because this poor translation in vitro occurs at all points after hormone treatment, not just at the times when we have evidence for translational controls. Although the possible translational controls are difficult to interpret conclusively due to the variability between populations of flies, and possible artefacts resulting from changes in amino acid pools, we can be sure that the YP-transcripts induced by 20-hydroxyecdysone in males are relatively unstable.

One of our most interesting findings is that even though we are possibly using unphysiologically high doses of 20-hydroxyecdysone to induce yolk polypeptide synthesis the response is tissue specific. The genes are not transcribed in gut, Malpighian tubules, testes or accessory glands, but only in the carcass which contains the fat body in males. In females, similarly, 20-hydroxyecdysone treatment increases transcript accumulation only in the body walls. It does not even affect transcription in the ovary where the YP-genes are normally expressed (Bownes & Hames, 1978; Brennen et al. 1982; Bownes, 1982). Thus it seems possible that some cell-types in body walls carry the appropriate ecdysteroid receptors for hormone-induction of YP-gene expression but that other cells in the adult do not. Whether induction uses normal ecdysteroid receptors or not is unclear, the fact that males and females have similar concentrations of 20-hydroxyecdysone (Handler, 1982, Smith & Bownes unpublished) suggests that normally the amount of ecdysone/receptor complex formed in male fat bodies is insufficient to induce transcription of the YP-genes. We cannot be sure from our results that 20 hydroxyecdysone does not generally stimulate transcription
and as a result of this we see more YP-transcripts, but there are several reasons why this is unlikely.

Firstly, when we look at the profiles of protein synthesis in the presence and absence of 20-hydroxyecdysone, only a few bands are affected, suggesting a differential response to the hormone although, of course, some of this specificity could reside at a post-transcriptional level. Secondly, we do not see increased YP-transcripts in ovarian cells, only in the body walls when females are treated and thirdly, we do not induce transcription of the YP-genes in all cells. It is still possible though that transcription is generally increased in just those cell types with appropriate receptors, although the patterns of protein synthesis argue against this. Ideally, we need to measure transcription of a gene that is expressed in all cells at all developmental stages and is not hormonally regulated as a control. Experiments to select such a gene are in progress.

Why is it then that male fat body cells do not normally express these genes, but female cells do. Clearly both sexes have the correct machinery to respond to 20-hydroxyecdysone. Male and female adults have almost identical levels of total ecdysteroids (Hodgetts, Sage & O'Connor, 1977; Handler, 1982; Bownes & Dubendorfer in prep.), suggesting that the control of expression of these genes resides in the fat body cells themselves, probably some process ultimately under the control of the sex genes dsx, tra, tra-3, and ix (Baker & Ridge, 1980; Postlethwait et al. 1980; Bownes & Nothiger, 1981; Bownes et al. 1983). Nonetheless with high doses of hormone these sex differences can be transiently overcome. One possible model for the regulation of expression of the YP-genes is that the tissue specificity of the response lies in the availability of the appropriate hormone receptors and that the sex specificity is controlled by the sex genes, perhaps by altering the conformation of the YP-genes in the chromatin or nuclear matrix, and hence their availability to low levels of hormone/receptor complex. The 20-hydroxyecdysone could act upon other genes in the fat body which indirectly activate the YP-genes. Alternatively the added 20-hydroxyecdysone may act on another tissue in the adult which then stimulates the fat body to transcribe the YP-genes.

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REFERENCES


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