

# Developmental Regulation of Yolk Protein Gene Expression in *Anastrepha suspensa*

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A partial cDNA clone for the 48,000 dalton yolk polypeptide gene from *Anastrepha suspensa* was isolated from a cDNA expression library using a yolk polypeptide antibody probe and hybridization to the *Drosophila melanogaster* yolk protein 1 gene. The sequenced DNA has greatest homology to the yolk protein genes from *Ceratitis capitata*, *D. melanogaster*, and *Calliphora erythrocephala* and, similar to these genes, shares amino acid sequence domains with those from lipases. RNA hybridization studies indicated that the yolk protein gene expression is completely female-specific and limited to the ovaries, without apparent regulation by 20-hydroxyecdysone or juvenile hormone. This is in contrast to an earlier study which suggested, based on immunological probes, that a very low level of yolk protein synthesis occurred in fat body and was not sex-specific. Arch. Insect Biochem. Physiol. 36:25–35, 1997. © 1997 Wiley-Liss, Inc.\*

**Key words:** vitellogenin; vitellogenesis; insect ovaries; 20-hydroxyecdysone; juvenile hormone

## INTRODUCTION

Insect vitellogenesis has been defined as the production of vitellogenin by the fat body which is then secreted into the hemolymph, with its ultimate sequestration as vitellin by the oocytes (Hagedorn and Kunkel, 1979). Almost all insects follow this paradigm, though for many, primarily the higher Diptera, the ovarian follicular epithelium also provides a source for the same proteins (Brennan et al., 1981; Jowett and Postlethwait, 1980). While the adult fat body is the major source of yolk protein (YP\*) in most of these dipterans,

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for one insect described thus far, the stable fly *Stomoxys calcitrans*, the ovary been shown to be the sole source of YP (Houseman and Morrison, 1986; Chen et al., 1987). Similarly, immunological studies in the Caribbean fruit fly, *Anastrepha suspensa*, indicated that YP was almost completely derived from the ovary, but a relatively small amount of YP was detected in the hemolymph which was not sex-specific (Handler and Shirk, 1988). This observation, however, was based on the use of polyclonal antisera raised against a gel-purified yolk polypeptide having a molecular mass of 48,000 daltons. Thus, the possibility remained that the 48 kdalton antigenic protein found in both the male and female hemolymph was in fact a non-sex-specific contaminant having the same mobility properties as YP. Given that the fat body is the sole or primary source of YP for almost all insects, it is important to critically determine which species have only an ovarian source of YP, so that the strategies resulting in insect vitellogenesis can be fully appreciated.

For most insects, either 20-hydroxyecdysone (20-HE) or juvenile hormone (JH), or both, have been found to stimulate YP synthesis (see Izumi et al., 1994). In *D. melanogaster*, synthesis from the fat body is promoted by both 20-HE and JH, while the ovarian synthesis is only affected by JH (Handler and Postlethwait, 1978; Jowett and Postlethwait, 1980). In a preliminary protein synthesis study, we failed to promote YP synthesis in *A. suspensa* with either hormone (Handler and Shirk, 1987). If YP synthesis in *A. suspensa* is limited to the ovaries, then a lack of 20-HE regulation might not be unexpected. This needs to be critically evaluated, as well as the possibility that JH may be important to ovarian synthesis.

It has been recognized that yolk proteins exhibit evolutionary conservation in their structure, with commonalities present throughout the Insecta (Harnish and White, 1982). The highest levels of homology have been observed among dipteran YP, having a common size group including 44–50 kdalton yolk polypeptides. These yolk proteins share common antigenicity as well, which is supported by direct molecular comparisons. The *D. melanogaster* YP1 and *A. suspensa* YP cross-react to each other's antisera (Handler and Shirk, 1988), and the *D. melanogaster* YP and *C. capitata* vitellogenin share high DNA and amino acid sequence homology (Rina and Savakis, 1991). This homology includes similarities to binding domains found in lipoprotein and triacylglycerol lipases (Terpstra and AB, 1988; Bownes, 1992).

To more critically test both the tissue and sex specificity of YP synthesis in *A. suspensa*, as well as to initiate a molecular comparison of the YP gene to those in other insects, we isolated and sequenced a partial YP cDNA. Its use as a probe in hybridization studies indicates that YP gene expression in *A. suspensa* is completely female-specific and limited to ovarian tissue, without apparent hormonal regulation.

\*Abbreviations used: AsYP = *Anastrepha suspensa* yolk polypeptide; CcVg1G = *Ceratitis capitata* vitellogenin 1-gamma; DmYP1 = *Drosophila melanogaster* yolk polypeptide 1; JH = juvenile hormone; JHA = juvenile hormone analog; SDS = sodium dodecyl sulfate; YP = yolk protein; 20-HE = 20-hydroxyecdysone.

## MATERIALS AND METHODS

### Insect Rearing and Hormonal Treatments

*A. suspensa* larvae were reared on a wheat germ–yeast–glucose diet at 27°C with wandering larvae placed on moist vermiculite for pupation until adult emergence. Adults were maintained on a yeast–sucrose diet at 24°C. Abdomens of 2.5-day-old females were isolated with a nylon ligature between the thorax and abdomen with the thorax and head immediately cut away. The isolated abdomens were kept on moist filter paper within a sealed petri dish at 24°C. Six-day-old males or isolated abdomens 2 days after ligation were treated with hormone. Approximately 2  $\mu$ l of 20-hydroxyecdysone (Rohto Pharm, Osaka, Japan) dissolved in 10% ethanol was injected into intact or isolated abdomens with a micropipet. We presume an approximate twofold dilution of injected hormone within the hemocoel. A juvenile hormone analog (JHA), methoprene (kindly provided by Zoecon Corp., Palo Alto, CA), was dissolved in acetone, with approximately 1  $\mu$ l topically applied to the abdomen with a micropipet. Six hours after treatment, the treated samples were frozen at –90°C until prepared for RNA extraction.

### cDNA Library Construction

A lambda gt11 cDNA expression library was constructed from poly A<sup>+</sup> RNA from *A. suspensa* ovaries using standard protocols (see Sambrook et al., 1989). RNA was extracted from the ovaries of 4–5-day-old females by the method of Chirgwin et al. (1979), with poly A<sup>+</sup> RNA isolated by binding to and elution from oligo (dT) cellulose. The poly A<sup>+</sup> RNA was reverse-transcribed with first-strand synthesis monitored by <sup>32</sup>P-CTP incorporation. The cDNA was methylated and blunted and the termini ligated to phosphorylated *Eco*RI linkers with subsequent ligation into lambda gt11 bacteriophage (Stratagene, La Jolla, CA) according to Huynh et al. (1985).

### Library Screening

The lambda gt11 expression library was plated onto Y1090 bacteria, and fusion protein synthesis was induced by overlaying the plaques with isopropylthio- $\beta$ -D-galactoside impregnated filters. The filters were then reacted to *A. suspensa* 48 kdalton yolk polypeptide polyclonal antibody (Handler and Shirk, 1988), with subsequent second antibody reaction to horseradish peroxidase–linked goat anti-rabbit IgG. Positively reacting bacteriophage were grown and rescreened with *D. melanogaster* YP1 (DmYP1) (Hoveman et al., 1981) radiolabeled probe. The DNA from positive bacteriophage was purified and digested with *Eco*RI and subcloned into pUC19. The pUCAsYP plasmid subclones were sequenced on both strands with the initial use of the M13 priming sites. Complete sequential sequencing was performed on one clone using internal primer sites. Sequence alignments were performed using GeneWorks 2.3 software (Intelligenetics, Mountain View, CA).

### RNA Extraction and Hybridization

RNA for hybridization studies was extracted from whole insects or dissected tissues from four to six insects by homogenization in TriZOL reagent

(Gibco BRL, Gaithersburg, MD) according to the manufacturer's specifications. RNA was solubilized in 60  $\mu$ l of formaldehyde/formamide buffer, and indicated volumes were separated on formaldehyde gels. The electrophoresed RNA was blotted to nylon filters, UV-irradiated, and hybridized to gel-purified AsYP cDNA probe radiolabeled with [ $^{32}$ P]-dCTP by random priming (Gibco BRL). Hybridizations were performed in 0.25 M phosphate buffer (pH 7.2), 1% BSA, 7% SDS at 65°C with an initial wash in 2X SSC, 0.5% SDS at room temperature and two washes in 1X SSC, 0.1% SDS at 60°C for 30 min. RNA samples and markers were visualized by methylene blue staining of the blot. Autoradiography was performed by exposure on Kodak X-Omat film at -90°C.

## RESULTS AND DISCUSSION

### cDNA Analysis and Comparisons

Previous protein synthesis studies indicated that YP synthesis begins in ovarian tissue at 3–4 days after eclosion (Handler and Shirk, 1988). RNA used for lambda gt11 cDNA library construction was therefore extracted from ovaries dissected from 3–5-day-old females. Positive cDNA subclones were identified initially by their expression and antigenic response to polyclonal YP antibody. Since it was previously recognized, and confirmed in this study, that the AsYP antisera may not be completely YP-specific, positively reacting subclones were rescreened with *D. melanogaster* YP1 (DmYP1) probe (Hoveman et al., 1981). The relationship of the *A. suspensa* YP to DmYP1 was previously demonstrated by cross-reactivity of YP from each species to both of the YP antisera (Handler and Shirk, 1988) and inferred by the homology of DmYP1 to vitellogenin genes from another tephritid fruit fly, *Ceratitis capitata* (Rina and Savakis, 1991).

Three positive subclones were identified, with restriction digest analysis and initial sequencing indicating the pUCAsYP-2 (now referred to as pAsYP) as having the greatest amount of 5' end sequence. Complete sequencing resulted in a 1,232 bp sequence including a poly A<sup>+</sup> tail and having a continuous uninterrupted open reading frame of 1,125 bp (Fig. 1). A polyadenylation signal sequence is present at nucleotides 1,188–1,193. This was considered to be a partial cDNA due to the conceptual translation of the sequence yielding a 41 kdalton polypeptide, which is smaller than the purified 48 kdalton polypeptide. Comparisons to homologous YP DNA and amino acid sequences from *D. melanogaster* (DmYP1) and *C. capitata* (vitellogenin 1-gamma; CcVg1G) are also consistent with a partial AsYP cDNA, with overlapping amino acid sequences beginning downstream from both respective start sites at approximately amino acid 64 (Fig. 2). Importantly, the level of sequence identity confirms that the *A. suspensa* cDNA encodes a yolk polypeptide. The amino acid sequence identity between AsYP and CcVg1G in the overlapping sequence is 76%, with 55% identity in the overlapping sequence between AsYP and DmYP1.

Consistent with these direct comparisons, a BLAST search (Devereux et al., 1984) indicated greatest amino acid sequence similarities to the *C. capitata*

CCCGTGGAGGAAGGCCAACAGGAAATTCAGAAAAATTATCACCTATCCCAAATCAACCAT	60
P V E E G Q Q E I Q K N Y H L S Q I N H	
GCTCTGCAGCCATCATTGTTCCAAGTCCCAGTAATGTGCCCGTAGTCTTGATGAAACCC	120
A L Q P S F V P S P S N V P V V L M K P	
AATGGTCAACCTGAGCGCACCAATCTCAATAACTTGGCTGAAGCCGCAAAGCAACAGCGC	180
N G Q P E R T N L N N L A E A A K Q Q R	
AACTTTGGCAATCAGGAAGTTACCATTTTCATCACTGGCTTGCCACAAACCAGCCAAGCA	240
N F G N Q E V T I F I T G L P Q T S Q A	
GTGCGAAAAGGCCAACAGAAATAGTTTCAGGCGTACATGCAACGTTATTATGGTCAACAA	300
V A K A N K K L V Q A Y M Q R Y Y G Q Q	
CAACCAATGGACATCAACAGCAAGGAATATGATTATGGCAGCATTAGTGGTAACAAAATT	360
Q P M D I N S K E Y D Y G S I S G N K I	
TCCTCTTCAGCGAAGAAGACAATGATTCAAGCAAGAATCCAAGACCCACTCGAGGAGAC	420
S S S S E E D N D S S K N P R P T R G D	
CTTGTTCATCAACTTAGGCGCCACATTGACCGACATGAAACGCTATGCCCTCCTCGAT	480
L V I I N L G A T L T D M K R Y A L L D	
GTAGACGAAACCGGTAATGATTGGCAAAGCTATTGTTCAATTGACCAACGAAGTTGAT	540
V D E T G K M I G K A I V Q L T N E V D	
GTGCCAGAAGAAATCATTACATTGTTGCCCAGGGTATTGCTGCTCAGGTTGCCGGACCC	600
V P E E I I H I V A Q G I A A Q V A G P	
GCCGCACGTGAATACAAACGTTGACTGGTCATAAAATCCGCCGTATCACAGCGTTGGAT	660
A A R E Y K R L T G H K I R R I T A L D	
CCCTCTAAGATTTACGCCAAGAACAATGAAATGATAACTGGCTTAACCCGTGGAGATGCT	720
P S K I Y A K N N E M I T G L T R G D A	
GATTTTCGTGATGCCATCCATACCTCGACTTGTGGCATGGGAACACATGAACGCGTCGGT	780
D F V D A I H T S T C G M G T H E R V G	
GATGTCGACTTCTACGTTAATGGTCCCGGATCAATTGCCCTGGCGCTAACAAATGTGGTT	840
D V D F Y V N G P G S I A P G A N N V V	
GAAGCATCTATGCGTGCTACACGTTACTTCGCCGAGACGGTGCGCCAGGAAATGAGGCC	900
E A S M R A T R Y F A E T V R P G N E A	
AACTTCCCCGCTCTCGCCGCAACTCTCTGAGCCAATACGAAAATAACGAAGGCACTGGA	960
N F P A L A A N S L S Q Y E N N E G T G	
AAACGCGCCTACATGGGTATTGCCACCGATTTGATTTGGAAGGTGACTACGTTCTGAAG	1020
K R A Y M G I A T D F D L E G D Y V L K	
GCTAACGCCAAGAGTCCATTCCGCAAGAGCTCTCCCGCCCAACAGCAAAACGCTTACCAC	1080
A N A K S P F G K S S P A Q Q Q N A Y H	
CGTCAACACAATACATGGAAAAACAGCAACCCCTTGAACATGAAGTAACAACTAACTAT	1140
R Q H N T W K N S N P L N M K X	
CCATCCTATCTTTTCCACACATAATTTTGTAACTTTGAAAAA <u>AAAAA</u> AGGATTA	1200
TTTTTGTATGTA <u>AAAAAAAAAAAAAAAAA</u> AG	1232

Fig. 1. The *A. suspensa* YP cDNA partial sequence with conceptual translation given underneath. The polyadenylation signal site is underlined. The GenBank accession number is U88976.

vitellogenin 1 gene ( $P_{BLASTP} = 1.4e-190$  [Rina and Savakis, 1991]), the *Calliphora erythrocephala* yolk protein 3 gene ( $P_{BLASTP} = 1.4e-149$  [Martinez and Bownes, 1994]), the *D. melanogaster* yolk protein 1 gene ( $P_{BLASTP} = 1.1e-145$  [Hoveman et al., 1981]), and the other YP genes from these species. The ho-

AsYP	-----PVEEGQQEIQKNYHLSQINHALQPSHFVP	28
CcVg1G	MNPLKIFCF LALVI AVASANKHGKNDKDNAGPNSLKPTDWLSVEELQSM TAI DDITLQQL ENMSV EDAERKIEKIYHLSQINHAL EPSYVVP	90
DmYP1	MNPMRVL SLLA-CLAVAALAKPNGRMDNSV NQALKPSQWLSGSQLEAIPALDDFTIERLENMNL ERGAELLQQVYHLSQIHNVEPNYVVP	89
Consensus	MNP.....LA-..AVA...K.....DN.....LKP..WLS...L....A.DD.T...LENM.VE.G...IQK.YHLSQINHAL EPSYVVP	90
AsYP	SPSNV FV LMKPNCQPERTNLINLAEAAKQRFNFGNCEVTIIFITGLPQTSQAVAKANKKLVQAYMORYYQ000PMDINSKEYDYGSISGN	118
CcVg1G	SPSNV FV LMKPNCQSQTNHNELVEAAKQRFNFGDEEVTIIFITGLPQTSQAVL KANKKLVQAYMORYYQ000PINGN-KDYDYGSSQGN	179
DmYP1	SGIQVMP--KPNGDKTVAPLNEMIQRLKQKCNFGED EVTIIFITGLPQTSQAVKATRKLVQAYMORYYQ000RQHGKNGNDYQDQSNE	177
Consensus	SPSNV FV LMKPNCQ...TNLNEL.EAAKQQ.NFG..EVTIIFITGLPQTS.QV.KANKKLVQAYMORYYQ000P..GN.K.YDYG.S.SGN	180
AsYP	K---ISSSEED-NDSSKNRPRTHGDLVLIINLGATLITDMKRYALLDVEITGKMIGKAIIVOLTNEVDMP EETIHIVAGGIAAVVAGAAARE	204
CcVg1G	Q---GATSSSEEDYSEWKNQKSTGNLVIINLGSTLITNMKRFAALLDVEITGNMIGKTLVOLTNEVDMPQEI IHHIVAGCIGAVVAGAAAGRQ	266
DmYP1	QRKNQRTSSEEDYSEEVKNAKTQSGDIIVIDLGSKLNTYBRYAMLDIEKTGAKIGKWIIVQMVNELDMPFDTIHLICQNVGAVVAGAAAQE	267
Consensus	Q---.TSSEEDYSES.KN.K.T.GDLVLIINLGSTLIT.MKRYALLDVE.ITG.MIGK.IVOLTNEVDMP.EIHHIVAG.IGAVVAGAAARE	270
AsYP	YKRLTGHKLRRITALDPSKIYAKNNEMITGLTRGDALFVDIAHTSTCGMGTHERVGDVDFYNGPGSIAPGANNVVEASMRATRYFAETV	294
CcVg1G	YKRLTGHLRRITALDPSKIFAKNRNALTGLARGDAEFVDIAHTSTCGMGTQRVGDVDFYNGPASTAPGTNNVVEASMRATRYFAESL	356
DmYP1	FTRLTGHKLRRITCLDPSKIMAKSKNTLTGLARGDAEFVDIAHTSVYGMGTPIRSGDVDFYNGPAAGVPGASNVVEAAMRATRYFAESV	357
Consensus	YKRLTGHKLRRITALDPSKI.AKN.N.LTGLARGDAEFVDIAHTSTCGMGT..R.VGDVDFYNGPAS.APGANNVVEASMRATRYFAESV	360
AsYP	RPGNEANFPAL AANSLQYENNEG GKRAYMGIATDFDLEGDYILKVNPKSPFGKSSPAQCNAYHRQFN TWKNSNPLNMK-	375
CcVg1G	RPGNERNFPAVAANSLQYENNEG GKRAYMGIATDFDLEGDYILKVNPKSPFGKSAQAQRHYHGUFHQSWKSGKNQNX	438
DmYP1	RPGNERSFPAVFANSLQYKQNDGFGKRAYMGIATDFDLEGDYILQVNP KSPFGRNAPAQKQSSYHGUFHQA WNTNQD SKDYQ	439
Consensus	RPGNERNFPAVAANSL.QYENNEG.GKRAYMGIATDFDLEGDYILKVNPKSPFGKSAQAQK..YHG.HQ.WK.....N...	442

Fig. 2. Amino acid sequence alignment of the conceptual translations of the *A. suspensa* YP cDNA (AsYP), *C. capitata* vitellogenin 1-gamma gene (CcVg1G) (Rina and Savakis, 1991) (X54661), and the *D. melanogaster* YP1 gene (DmYP1) (Hoveman et al., 1981) (V00248). Consensus sequences are boxed and shown below the alignment. Introduced gaps are shown by dashes.

mologies in these sequences to phospholipase domains is well documented (Terpstra and AB, 1988; Bownes, 1992), and succeeding high-scoring similarities to AsYP were almost exclusively to lipoprotein lipases and triacylglycerol lipases. Although this was not unexpected, it is interesting that, beyond the dipteran YP genes noted, no other insect YP genes had significant similarity to AsYP. A further YP molecular analysis and comparisons will be completed upon isolation of genomic clones, which is in progress.

#### Developmental and Tissue Specificity of YP RNA Accumulation

The developmental, tissue, and sex specificity of AsYP gene expression was tested by Northern hybridization using radiolabeled AsYP cDNA as probe to male and female whole animal RNA samples and RNA from specific body segments and tissues. Figure 3 shows that YP RNA having a length of approximately 1.3–1.4 kb was only detectable in 3–6-day-old females, in 6-day female abdomens containing ovaries, and in 6-day ovaries isolated by dissection. Male samples contained twice the amount of total RNA on a per organism basis relative to female samples, yet, after extended autoradiographic exposures for this blot (data not shown) and the blot shown in Figure 4, hybridization was not detectable. Similarly, female samples containing fat body (female abdominal wall, head, and thorax) in the absence of ovaries did not exhibit YP RNA.

The developmental time course agrees, in general, with the immunological detection of YP which reaches high levels between days 3 and 4 after eclosion (Handler and Shirk, 1988) and with that observed in *C. capitata* (Rina and Mintzas, 1988). The previous biochemical study in *A. suspensa* had concluded that YP synthesis is not strictly female-limited and that low levels of fat body-derived vitellogenin may exist in both males and females (Handler and Shirk, 1988). It was recognized, however, that these conclusions were based on immunological probes that might not be totally specific and that confirmation required more highly specific molecular analysis. The present data, indeed, argue more convincingly for a completely female-limited and ovarian-specific synthesis of *A. suspensa* YP. In this context, *A. suspensa* would be only the second insect thus far recognized as having YP synthesis limited to the ovaries, with no contribution from the adult fat body. The first insect discovered to have ovarian-specific YP synthesis is the stable fly, *Stomoxys calcitrans*, though this was based on biochemical tests (Houseman and Morrison, 1986; Chen et al., 1987). In contrast, other tephritid species in which vitellogenesis has been investigated, including *Dacus oleae* (Levedakou and Sekeris, 1987; Zongza and Dimitriadis, 1988) and *C. capitata* (Rina and Mintzas, 1988), all exhibit YP synthesis in the fat body as well as in the ovaries.

#### Hormonal Regulation of RNA Accumulation

For one of the tephritid species, *C. capitata*, it was demonstrated that vitellogenin synthesis is stimulated in males by extremely high concentrations of 20-HE ( $10^{-2}$  M), presumably in the fat body, which is consistent with the hormonal regulation of YP synthesis in many insect species, including males as well as females (Huybrechts and De Loof, 1982; Shirk et al., 1983). Previously we were unable to observe significant increases in YP synthesis

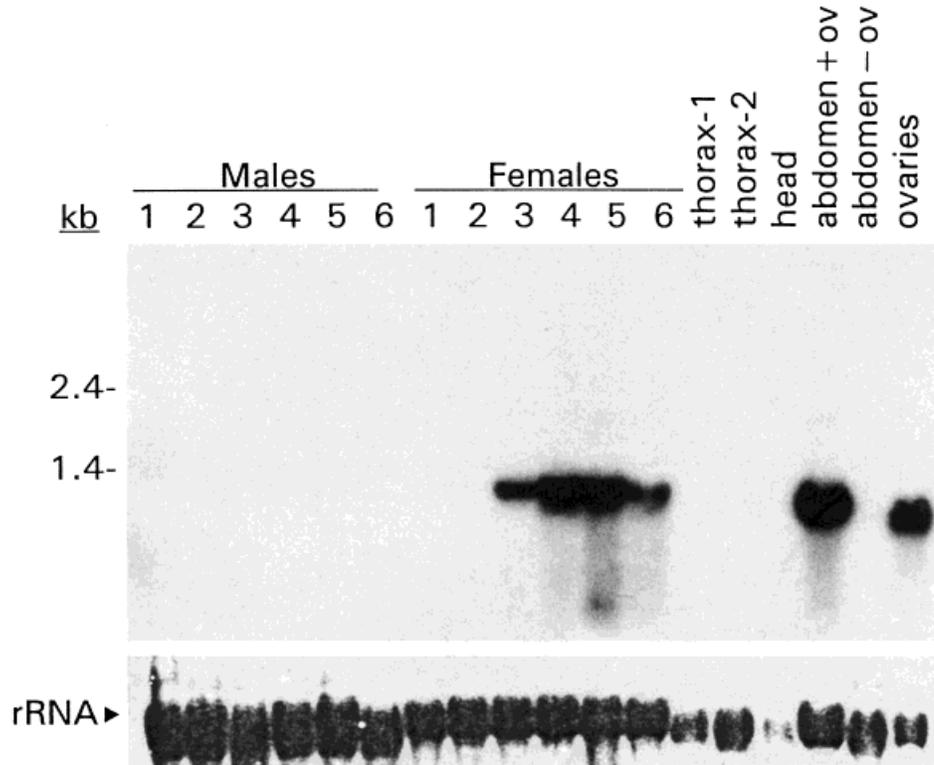


Fig. 3. Northern blot RNA hybridization of whole *A. suspensa* male and female samples of indicated ages (days after adult emergence) and dissected body regions and tissues from 6 day females. These included thoraces, heads, and abdomens with ovaries from the same females and dissected abdomens without ovaries and the dissected ovaries from the same females. RNA was extracted from whole insects or tissues from four individuals, with one-fourth of the total sample loaded for males and the female thorax-2 sample. All other lanes were loaded with one-eighth of the total sample. Hybridization was performed with radiolabeled AsYP cDNA. The lower panel shows methylene blue staining of ribosomal RNA on the same filter previous to hybridization as a control for the amounts of RNA loaded.

in *A. suspensa* after using varying concentrations of either 20-HE or JHA in females (Handler and Shirk, 1987; A.M. Handler, unpublished). As shown in Figure 4, repetition of these experiments in intact males and isolated female abdomens with either hormone failed to show stimulation of YP RNA accumulation. Only trace amounts of RNA were detectable in isolated abdomens that were untreated or treated with 20-HE or JHA and only after extended autoradiographic exposure (6 day exposure compared to 1 day in Fig. 3). Since no YP RNA was detectable from females at the time of ligation, the RNA probably occurred constitutively as an unregulated accumulation after ligation, which was possibly limited by a lack of nutrition. Unlike *C. capitata*, 20-HE had no positive influence in males. While it is difficult to control for negative results, these experiments used protocols that have consistently stimulated YP synthesis in *D. melanogaster* (Handler and Postlethwait, 1978;

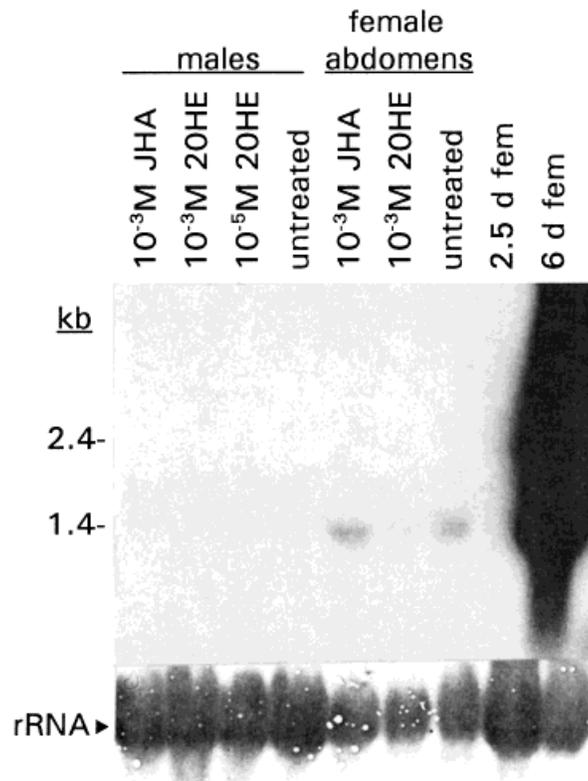


Fig. 4. Northern blot RNA hybridization of *A. suspensa* adult males or isolated female abdomens treated with hormone. Male samples were whole intact 6-day-old flies untreated or treated with indicated hormones. Female abdomens were isolated at 2.5 days after eclosion and were untreated or treated with indicated hormones. Sibling females of those ligated were left intact with RNA extracted at the time of abdomen ligation (2.5 days) and the time of isolated abdomen extraction (6 days). Hybridization was performed with radiolabeled AsYP cDNA. The lower panel shows methylene blue staining of ribosomal RNA on the same filter previous to hybridization as a control for the amounts of RNA loaded.

Postlethwait and Handler, 1979). If either hormone actually has an effect on YP synthesis, it may possibly depend on specific concentrations, developmental periods, or nutrition. They clearly do not have an apparent regulatory function as demonstrated in most other insects.

Since one or both of these hormones usually promotes YP synthesis (see Izumi et al., 1994), it is conceivable that the lack of hormonal regulation in *A. suspensa* is due to a lack of fat body YP expression. In several dipterans the effect of 20-HE appears limited to the fat body (Huybrechts and De Loof, 1982), and in *D. melanogaster* the fat body responds to both 20-HE and JH. Ovaries cultured *in vivo*, however, respond only to JH (Jowett and Postlethwait, 1980). Although a possible influence in JH in *A. suspensa* may be revealed by further study, in particular *in vitro* culture of ovaries, it may be assumed that the lack of 20-HE activity is due to nonresponsive YP genes in

the fat body. The relationship between the tissue specificity of YP synthesis and its hormonal regulation in insects awaits further study in the stable fly and the identification and analysis of other species having YP gene expression limited to their ovaries.

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