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Identification and Analysis of the Major Yolk Polypeptide From the Caribbean Fruit Fly, *Anastrepha suspensa* (Loew)

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A single major yolk polypeptide (YP) having a molecular mass of approximately 48,000 daltons (Da), was identified in the ovaries and oviposited eggs of the Caribbean fruit fly, *Anastrepha suspensa*. The polypeptide was partially purified from oviposited eggs using gel permeation and ion-exchange chromatography. Analysis of YP synthesis in vivo and in tissues cultured in vitro indicated that the ovary was the major site of synthesis with very low levels of YP derived from the adult fat body. Using a monospecific polyclonal antiserum to 48 kDa YP in an immunoblot assay, low levels of vitellogenin were found in female hemolymph; slightly lower levels of an immunoreactive 48-kDa polypeptide were detectable in male hemolymph. Although YP synthesis was detectable within 12 h after eclosion, the major increase in YP accumulation occurred at 3–4 days posteclosion coincident with the initiation of observable yolk deposition. The physical characteristics of YP from *A. suspensa* were similar to YPs from other dipterans in terms of molecular mass and antigenicity, yet the tissue- and sex-specific regulation of the YP differed from other dipterans as well as most other insects.

Key words: yolk protein, vitellogenesis, tephritids

INTRODUCTION

Insect yolk proteins are of significant biological importance because they are generally the most abundant proteins in adult females, indicating a high level of gene activity, and because they have an important role during embryogenesis [1]. They are of additional genetic interest since expression of

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the YP* genes is usually highly regulated with respect to temporal, spatial, hormonal, and sex specificities. Analyses of insect vitellogenesis have elucidated important developmental processes as well as revealed mechanisms that may be used to manipulate the reproductive capacity of economically important insects. Additionally, important physiological and evolutionary relationships have been demonstrated through comparative studies that have revealed specific trends in the structural differences between the YPs themselves, as well as differences in regulation of YP synthesis [2].

The size and number of the polypeptide subunits comprising yolk protein varies among insect species, although their molecular masses can generally be associated with particular groups of species [2]. In higher Diptera, the YPs are generally within the 40,000–54,000 dalton range, and their number varies from one to five [3,4]. While this variability may be due in part to differential posttranslational modification of a single polypeptide, it is more likely that there is a separate transcriptional unit for each YP subunit as found in *Drosophila melanogaster* [5–8].

There is also variation in the temporal and tissue-specific regulation of YP gene expression. Initiation of YP synthesis is generally limited to the adult stage, although the precise timing may vary from the mid-pharate adult stage as in several lepidopterans [9–11] to several days after adult eclosion in response to feeding, as in anatogenous mosquitos [12,13]. Alternatively, vitellogenesis may be regulated by the biological clock that controls eclosion behavior as in *D. melanogaster* [14,15]. The source of the major YP precursor, vitellogenin, in most insects is the adult fat body. YP is synthesized and secreted into the hemolymph by this tissue [1], although in several dipteran species, synthesis of homologous YPs occurs at varying degrees in ovarian tissue [3,16,17]. This was most directly demonstrated in *D. melanogaster* where the follicular epithelium was found to contribute between one-third to one-half the total YP accumulated in the oocytes [18, 19].

Perhaps the most invariant aspect of YP production is the sex-specific regulation. Usually YP synthesis by the fat body is universally female-limited, and as found in *D. melanogaster*, the genetic mechanisms regulating female sex-determination are the primary control mechanisms of YP gene expression [20]. Nevertheless, YPs have been found in varying, though generally small amounts, in the male hemolymph of a few insects [21–23], and YP synthesis has been experimentally stimulated in males by hormonal treatment [24,25], indicating that the genetic and cellular mechanisms for YP gene expression are functional in both sexes.

In this report we extend the study of insect vitellogenesis by identifying the major YP in the Caribbean fruit fly, *Anastrepha suspensa*, and by characterizing the regulation of its synthesis in terms of temporal-, tissue-, and sex-specificities. This analysis serves to widen our perspective of insect vitellogenesis, reveals unusual regulatory specificities for a YP gene, and provides

*Abbreviations: Da = dalton; SDS-PAGE = sodium dodecylsulfate-polyacrylamide gel electrophoresis; YP = yolk polypeptide.

information useful for controlling the reproduction of an economically important insect.

MATERIALS AND METHODS

Animal and Sample Preparation

Animals and oviposited eggs were obtained from a laboratory strain of *A. suspensa* reared on a corncob-grit diet at 29°C. Animals were mass-reared, and eggs were collected after overnight egg-laying. Tissue samples were dissected, and in some cases cultured in vitro, in insect Ringers solution [26]. Hemolymph was collected from the thorax of decapitated insects in a drawn-out calibrated microcapillary. Tissues or hemolymph were taken from 10 animals unless otherwise noted. For protein synthesis studies, [^{35}S]methionine (> 1,000 Ci/mmol; Du Pont NEN, Boston, MA) was dissolved in Ringers and either injected into the posterior of the abdomen (2μCi/animal) or added to the incubation medium (15 μCi/culture) for 3 h.

Gel Electrophoresis

One-dimensional 10% or 9–12% linear gradient SDS-PAGE was performed according to O'Farrell [27] with modifications and sample preparation as described previously [20]. Gels were stained with Coomassie blue and dried, and those with radioactive samples were autoradiographed for periods ranging from 12 to 48 h using Kodak X-Omat AR film. Autoradiograms were scanned using an Ultroscan XL laser densitometer (LKB, Gaithersburg, MD), and the molecular mass and protein quantitation estimates were computed with the LKB 2400 GelScan software. Two-dimensional gels were performed according to O'Farrell [27] using a pH 3–10 ion-gradient in the first isoelectric-focusing dimension. The second dimension was a 10% SDS-PAGE.

YP Purification

YP purification followed procedures as described previously [11,28]. One gram of newly oviposited eggs was washed and homogenized in phosphate-buffered saline (50 mM $NaPO_4$, 150 mM KCl, 5 mM EDTA, 0.02% NaN_3, pH 7.6) plus 1 mM phenylmethylsulfonyl fluoride. The crude homogenate was precipitated in 75% ammonium sulfate, and the redissolved precipitate was resolved by Sephacryl S-300 gel permeation chromatography (column dimensions: 95 × 2.5 cm) eluted with phosphate-buffered saline into 7.5-ml fractions that was monitored continuously at 280 nm. The presence of YP in specific fractions was confirmed by SDS-PAGE, and fractions containing the highest levels of YP were pooled and concentrated by dialysis against polyethylene glycol. The combined S-300 YP fractions were further resolved by ion-exchange chromatography on DEAE Sepharose C1-6B (column dimensions: 40 × 2.5 cm) eluted with a linear gradient of 50–500 mM KCl in phosphate-buffered saline continuously monitored at 280 nm. The presence of YP in specific fractions was determined by SDS-PAGE.

YP Antiserum Preparation and Immunoblots

DEAE purified YP was resolved by preparative 10% SDS-PAGE and detected by soaking the gel in 1 M KCl. The YP band was cut out, and

approximately 1 mg of protein was emulsified thoroughly in Freund's complete adjuvant and injected subcutaneously and intramuscularly into rabbits. Four weeks later the rabbits were boosted with 0.5 mg of the protein in Freund's incomplete adjuvant. The specificity of the serum was determined by binding to YPs electroblotted to nitrocellulose. Immunoblots were prepared according to Towbin et al. [29] using crude or purified YP samples resolved on SDS-PAGE. The proteins were electroblotted to nitrocellulose (BA-85; Schleicher and Schuell, Keene, NH) in transfer buffer (25 mM Tris, pH 8.3, 192 mM glycine, 20% methanol) at 20 V for 12 h using a Transblot cell (Bio-Rad, Richmond, CA). After the transfer, the electroblot was blocked with 3% gelatin in 20 mM Tris, pH 7.5, and 500 mM NaCl. The binding specificity of the serum was determined by reacting the electroblot with YP antiserum and visualizing the bands with an Immun-Blot color assay (Bio-Rad) using a horseradish peroxidase-linked goat anti-rabbit IgG as the second antibody.

RESULTS

YP Identification and Isolation

A single polypeptide was identified as the major constituent of proteinaceous yolk in oviposited eggs and mature ovaries of the Caribbean fruit fly, *A. suspensa*. The predominant polypeptide observed on denaturing one-dimensional SDS-PAGE had an electrophoretic mobility corresponding to a molecular mass of approximately 48,000 Da (Fig. 1). On two-dimensional gel electrophoresis (Fig. 2), the 48-kDA polypeptide from vitellogenic ovaries exhibited a charge heterogeneity that is most likely due to differential post-translational modification of a single polypeptide.

Since the fat body is usually the major or sole source of vitellogenin in most insects, the 48-kDa YP or other major sex-specific YP precursor was expected to be observed as a major constituent of hemolymph proteins in mature vitellogenic females. However, resolution of proteins by SDS-PAGE from 5 μl hemolymph of 3–4-day newly vitellogenic females indicated the lack of a detectable 48-kDa polypeptide (Fig. 1f). A 48-kDa polypeptide was detectable in 10 μl hemolymph from older 5–6-day females, and, interestingly, a comigrating polypeptide was also observed in 10 μl of hemolymph from sibling males, but at a lower relative concentration (Fig. 3). This observation suggested the possibility, addressed further on, that the tissue and/or sex-specific synthesis of YP in this species is different from other insects. The SDS-PAGE revealed various polypeptides other than the 48-kDA YP that comigrated in samples from the hemolymph, ovaries, and oviposited eggs, but the majority were either not sex-specific or only minor constituents of the yolk from oocytes. Although some of these polypeptides may be defined as minor YPs, the remainder of this analysis will focus on the major 48-kDa polypeptide.

The YP was purified from the crude egg homogenate using ammonium sulfate precipitation followed by gel permeation column chromatography on Sephacryl S-300 and ion-exchange chromatography on DEAE-Sepharose as described in Materials and Methods. The ammonium sulfate precipitated egg

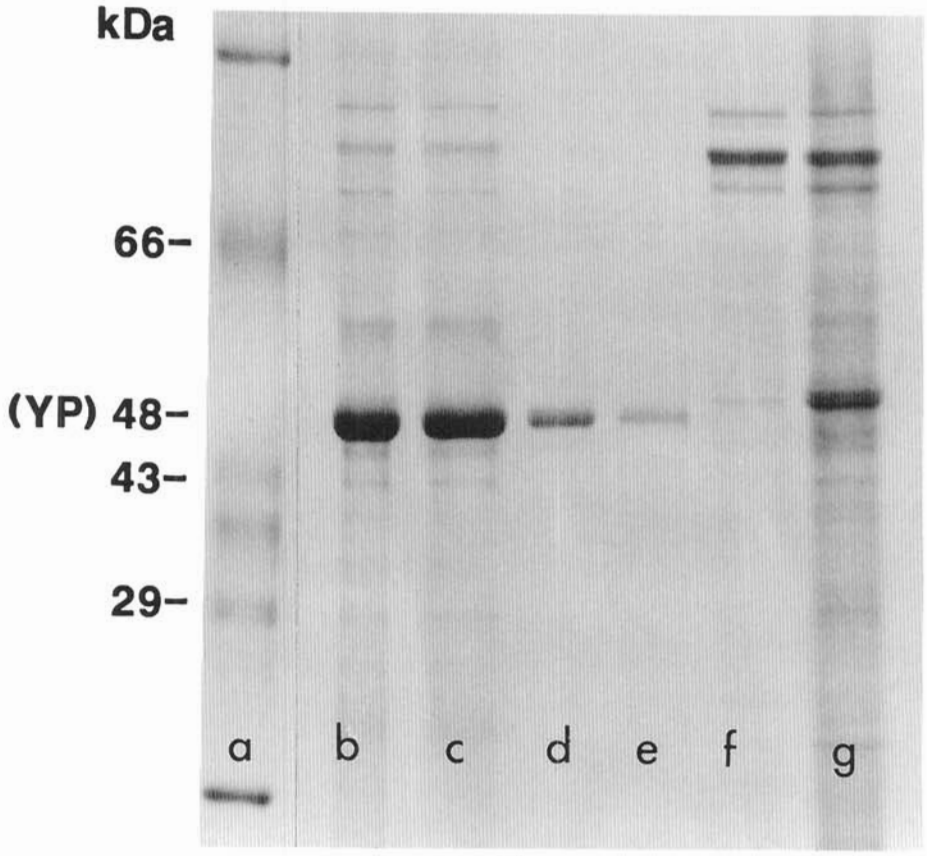


Fig. 1. Resolution and identification of YP on 10% SDS-PAGE stained for protein with Coomassie Blue. Lane designations: Molecular mass standards, YP designated at 48 kDa by densitometric analysis **(a)**; soluble proteins from oviposited eggs **(b)**; ammonium sulfate precipitated egg proteins **(c)**; combined YP fractions from S-300 **(d)**; combined YP fractions from DEAE **(e)**; 5 μl hemolymph from 3 to 4-day males **(f)**; 5 μl from 3 to 4-day females **(g)**.

proteins were resolved by S-300 chromatography into several A_{280} peaks, with the YP eluting exclusively in the peak fractions 32–37 (Fig. 4) as verified by SDS-PAGE (Fig. 1d). The YP-containing fractions were combined, concentrated, and resolved further by ion-exchange chromatography. YP was eluted from the DEAE column from approximately 200–300 mM KCl as represented by the broad A_{280} peak (Fig. 5A). The approximate molar concentration of KCl was determined by measuring the refractive index of each fraction. The relative purity of the combined YP eluted from the ion-exchange column was determined by SDS-PAGE (Fig. 5B) and by immunoblotting (see Fig. 6A), which demonstrated that the final YP preparation was devoid of contaminating proteins. This preparation is, nevertheless, considered to be a partial purification.

Immunological Identity of YP

Polyclonal antiserum against the ion-exchange purified YP was raised in rabbits as described. Specificity of the antiserum was determined by Western

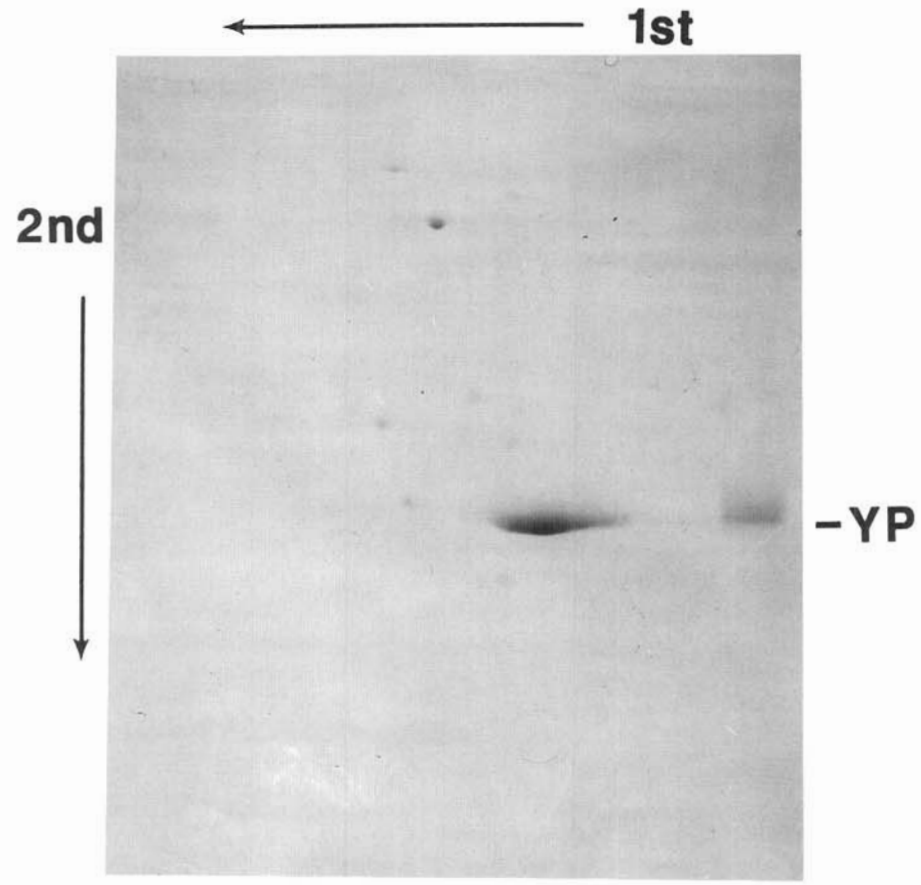


Fig. 2. Resolution of YP from vitellogenic ovarian polypeptides on two-dimensional gel electrophoresis. The directions of the isoelectrofocusing first dimension and the SDS-PAGE second dimension are indicated by the arrows. YP denotes DEAE purified marker YP run on the second dimension.

immunoblot analysis, which demonstrated a highly specific immunoreactivity to the 48-kDa YP from ovaries, soluble egg proteins, and purified YP (Fig. 6A). Minor immunoreaction to smaller polypeptide components was observed only in samples containing possible proteolytic cleavage products of YP. The YP-antiserum was used to examine more critically whether the 48-kDa polypeptide in the hemolymph of females and males comigrating with YP shares antigenic homology with YP. Equal amounts of hemolymph from both 5–6-day males and females were immunoblotted and reacted with YP-antiserum. The blot demonstrated that the 48-kDa polypeptide present in the hemolymph of both sexes shares antigenic immunoreactivity with YP (Fig. 6Ad–e) and, we conclude, is most likely YP. Although YP was present in the hemolymph of both sexes, the YP was at a relatively lower concentration in the hemolymph of males than in females.

As a test of the structural relatedness between *A. suspensa* and *D. melanogaster* YPs, YP specific antisera from each species was cross-reacted with the

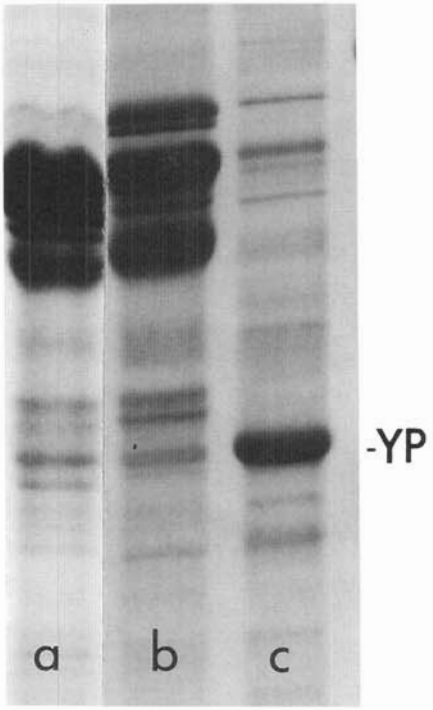


Fig. 3. Polypeptides from hemolymph and eggs resolved on 9–12% SDS-PAGE stained for protein with Coomassie blue. Lane designation: 10 μl hemolymph from 5 to 6-day males **(a);** 10 μl hemolymph from 5 to 6-day females **(b);** solubilized oviposited eggs **(c).**

electroblotted YPs of the heterologous species. Interestingly, all three YPs (45, 46, and 47 kDa) from *D. melanogaster* ovaries displayed significant antigenic cross-reactivity against the YP-antiserum from *A. suspensa* (Fig. 6Ah), although by inspection this heterologous cross-reaction appears highly specific but not as strong when compared with the immunoreaction to similar quantities of YP from *A. suspensa* ovaries (Fig. 6Ag); no immuno-cross-reactivity was observed for any other polypeptides from the eggs of *D. melanogaster*. Conversely, the YP-antiserum from *D. melanogaster* cross-reacted with vitellogenic ovaries and purified YP from *A. suspensa* (Fig. 6B); again, immuno-cross-reactivity was not observed for any polypeptides other than YP. These mutual cross-reactivities to heterologous antisera demonstrate that the YPs of these species share considerable structural homology that can be exploited in further comparisons.

Site of YP Synthesis

For some dipteran species, YP production occurs in the ovaries as well as in the fat body with both sites contributing significantly to the overall accumulation of YP in the oocyte. However, the minor accumulation of YP in the hemolymph of female *A. suspensa* (Figs. 1 and 3) suggested the possibility that the fat body was not the major site of YP synthesis. To determine the site(s) of YP synthesis, protein synthesis was assayed in hemolymph and

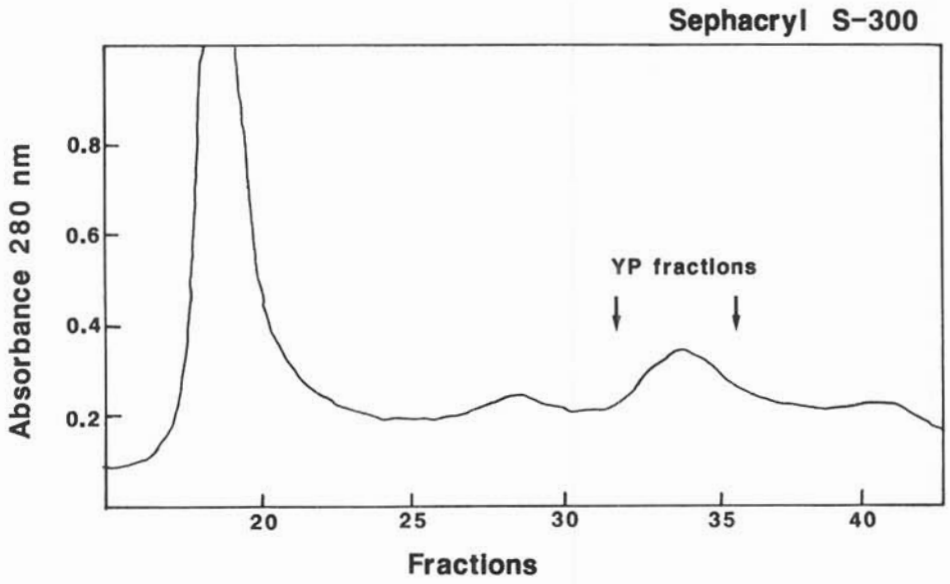


Fig. 4. Elution profile of ammonium sulfate precipitated YPs from gel permeation (absorbance measured at 280 nm). Each fraction represented 7.5 ml, and YP containing fractions were identified by SDS-PAGE.

ovaries in vivo or in adult fat body and ovaries during in vitro culture. Tissues were assayed from previtellogenic (3-day), early vitellogenic (4–5-day), and vitellogenic (6-day) females as determined by the stage of oocyte maturation. After a 3-h incubation with radiolabeled [^{35}S]methionine, newly synthesized proteins in tissues and media were resolved by SDS-PAGE, and the autoradiograms were quantified by densitometric analysis. Samples incubated in vivo showed a lack of newly synthesized YP in previtellogenic ovaries or hemolymph (Fig. 7A,D), but by early vitellogenesis, newly synthesized YP was detectable in the ovaries but not in the hemolymph (Fig. 7B,E). In fully vitellogenic 6-day females, newly synthesized YP in the hemolymph represented 9% of the total labeled proteins (Fig. 7F), while YP synthesis in the ovaries comprised 26% of total labeled proteins (Fig. 7C).

The results from labeling in vivo indicated that either hemolymph proteins, presumably synthesized by the fat body, were rapidly sequestered by the ovary or the major site of YP synthesis was the ovary itself. These possibilities were clarified by culturing the two organs in vitro. When ovaries were cultured, YP synthesis was detected at high levels from all three stages (Fig. 7G–L), although the synthetic rate increased in the older ovaries. As observed for ovarian cultures in other insects [11,13], the culture media from all three stages contained almost exclusively YP (Fig. 7G,I,K). Cultured abdominal walls containing large amounts of attached fat body also exhibited considerable total protein synthesis, but the 48-kDa polypeptide was produced at relatively low levels (less than 1% total synthesis) in early and fully vitellogenic stages (Fig. 7M–P). Unlike the ovaries, the fat body secreted an array of polypeptides into the medium, and the YP was only barely detectable.

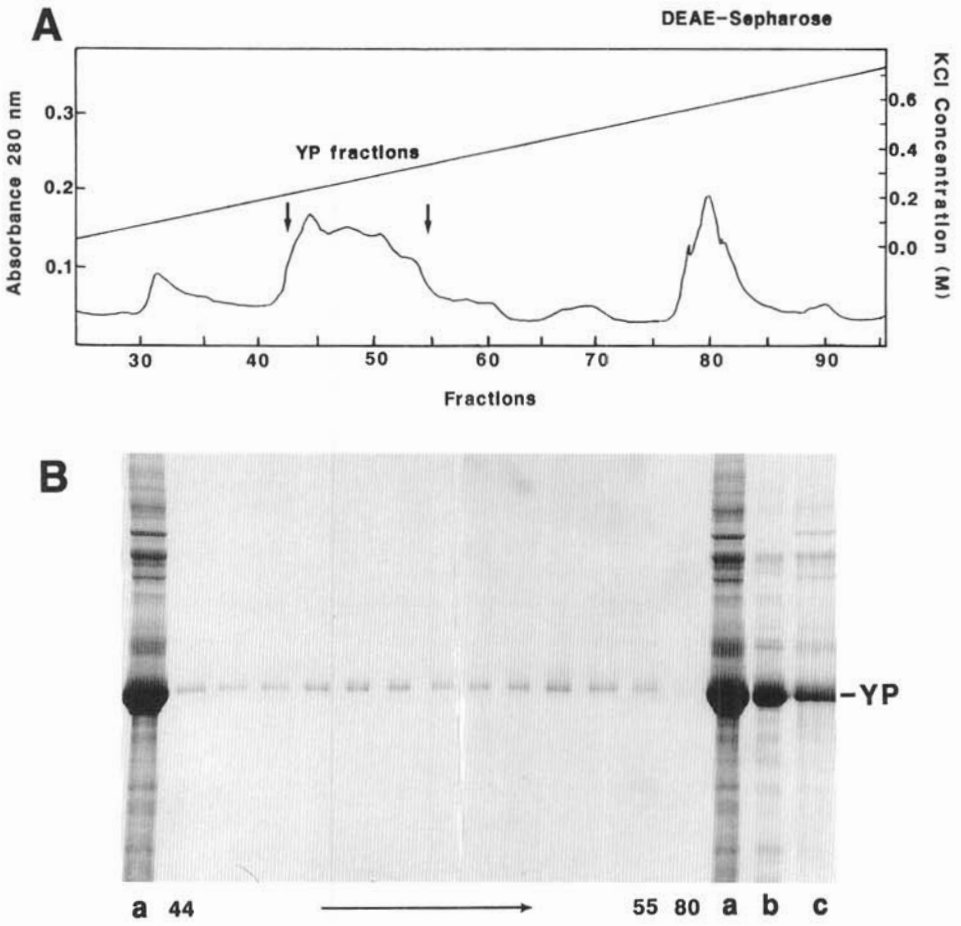


Fig. 5. **A,** Elution profile of S-300 YP fractions from DEAE ion-exchange chromatography (absorbance measured at 280 nm). Proteins were eluted by a continuous linear KCl gradient as illustrated in upper portion of the figure. Each fraction represented 7.5 ml, and YP-containing fractions were identified by SDS-PAGE. **B,** Resolution of purified YP by 9–12% SDS-PAGE. Fractions 44–55 were compared with the 280-nm absorbance peak fraction 80 and polypeptides from ovaries **(a),** solubilized eggs **(b),** and ammonium sulfate precipitated egg proteins **(c).**

Initiation of YP synthesis

The radiolabeling of YP in vivo demonstrated the initial presence of low amounts of YP in the ovaries of females 4–5 days after eclosion, while in vitro culture analysis showed YP synthesis in ovaries as early as 3 days, which was prior to any observable yolk deposition. To determine when YP first appears in ovaries, an immunoblot analysis, which is highly sensitive and specific, was performed on ovaries dissected from 12-h to 5-day females. Figure 8 shows a detectable, yet very low level of YP in 12-h ovaries, with low but increasingly higher levels at 1–3 days. A large increase in YP accumulation occurs between 3 and 4 days, continuing through day 5. At these latter times, yolk is first observable in maturing oocytes. These data are

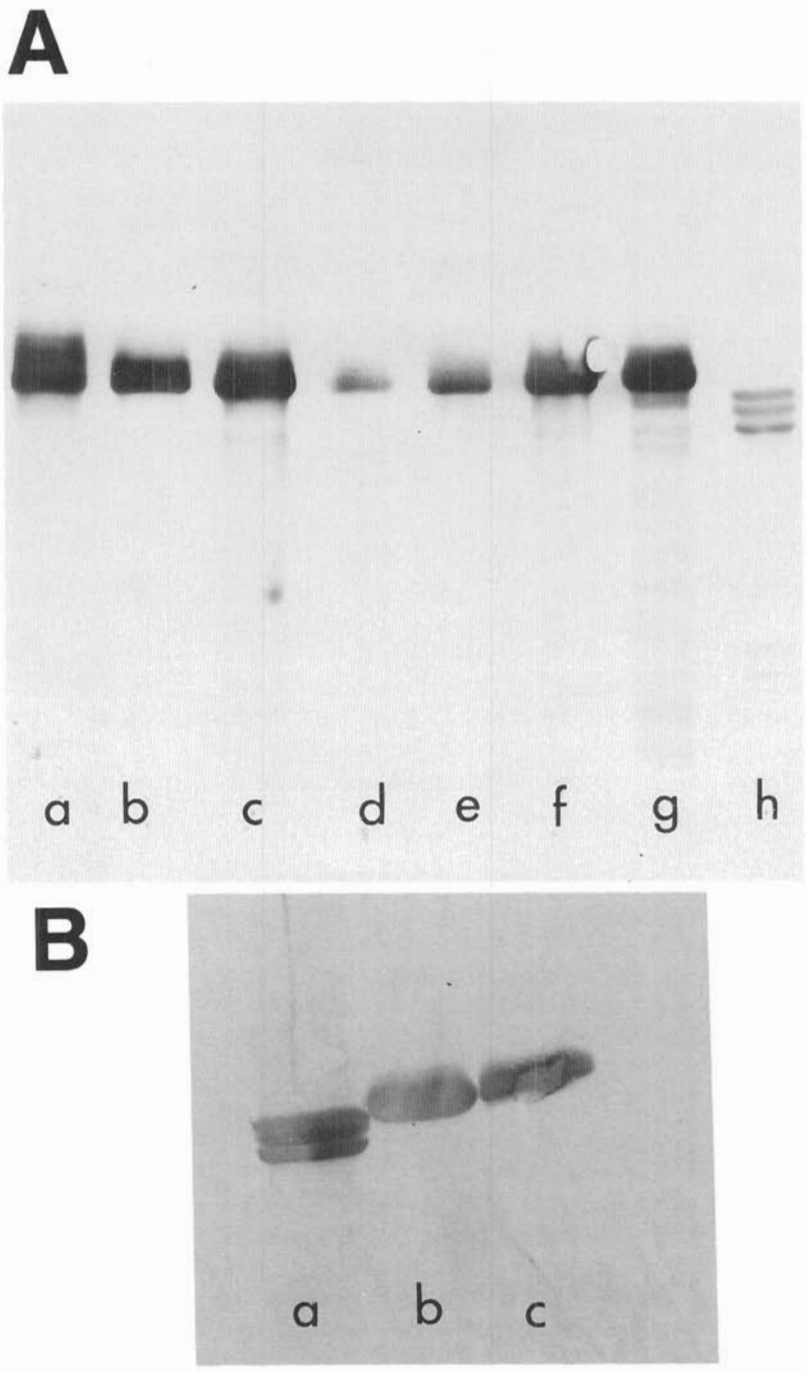
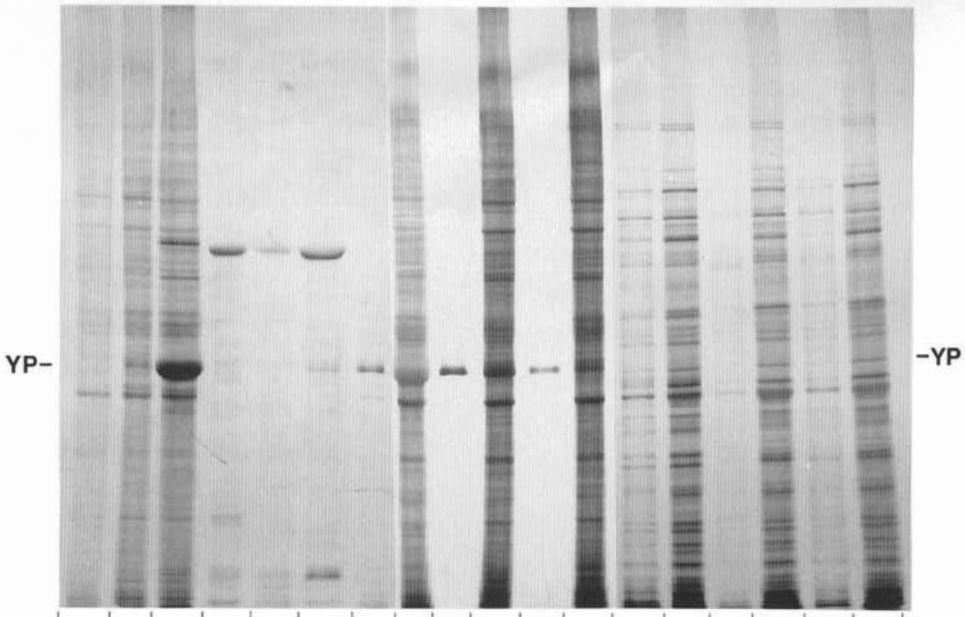


Fig. 6. **A,** Immunoblot of egg and hemolymph proteins resolved by 9–12% SDS-PAGE reacted with *A. suspensa* YP-antibody. Lane designations: 100 μg combined DEAE-YP-containing fractions **(a);** 100 μg combined S-300-YP-containing fractions **(b);** 100 μg solubilized egg proteins **(c,f);** 10 μl hemolymph from 5 to 6-day males **(d);** 10 μl hemolymph from 5 to 6-day females **(e);** 100 μg *A. suspensa* ovary proteins **(g);** 100 μg *D. melanogaster* ovary proteins **(h). B,** Immunoblot of egg proteins resolved by 9–12% SDS-PAGE reacted with *D. melanogaster* YP-antibody. Lane designations: *D. melanogaster* ovary proteins **(a);** *A. suspensa* ovary proteins **(b);** S-300-YP-containing fractions from *A. suspensa* **(c).**



	A	B	C	D	E	F	G	H	I	J	K	L	M	N	O	P	Q	R
%	0	7	26	0	0	9	40	11	84	10	71	6	1	1	0	1	0	0

Fig. 7. Protein synthesis in tissues in vivo or cultured in vitro from 3-day previtellogenic (PV), 4–5-day early vitellogenic (EV), and 6-day vitellogenic (V) females. Figure shows an autoradiogram of [^{35}S]methionine-labeled polypeptides resolved by 9–12% SDS-PAGE. Lane designations: in vivo ovaries from PV **(A)**, EV **(B)**, and V **(C)** females and hemolymph from PV **(D)**, EV **(E)**, and V **(F)** females. In vitro culture media and ovaries, respectively, from V **(G,H)**, EV **(I,J)**, and PV **(K,L)** females. In vitro culture media and abdominal walls, respectively, from V **(M,N)**, EV **(O,P)**, and PV **(Q,R)** females. Label incorporation into newly synthesized YP as a percentage of label incorporation into total proteins is given beneath each lane (%). Percentages were determined by densitometric scanning of the autoradiograms. This analysis allows a comparison of relative rates of YP synthesis between subgrouped samples. YP mobility was determined by comparison with coelectrophoresed unlabeled purified YP markers.

consistent with the appearance of radiolabeled YP from ovaries of the same age. In addition, it is clear that YP was resolved as only a single 48-kDa polypeptide in this experiment. Although the existence of other distinct YPs with identical molecular masses has not been ruled out, another YP with a similar but different molecular mass, possibly obscured in previous experiments, was not apparent in this analysis where YP was detected at low concentrations.

DISCUSSION

YP Identification and Site of Synthesis

The major constituent of proteinaceous yolk in eggs of *A. suspensa* was identified by SDS-PAGE as a single 48,000-Da polypeptide that was subsequently purified by gel permeation and DEAE chromatography. The molec-

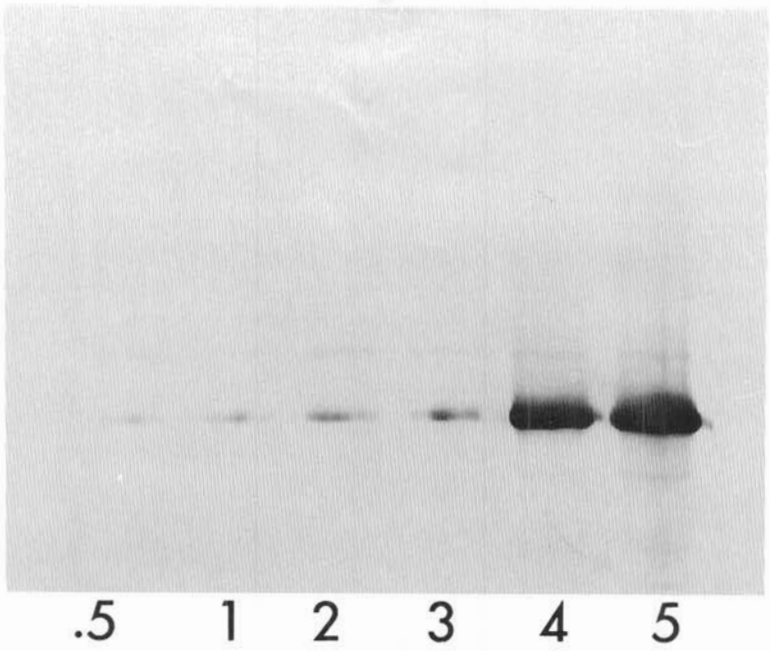


Fig. 8. Immunoblot of ovarian proteins resolved on 9–12% SDS-PAGE and reacted with *A. suspensa* YP-antibody. Ovaries were dissected from 0.5- to 5-day-old females as designated. The proteins from six pairs of ovaries were loaded per lane.

ular mass of the 48-kDa YP from *A. suspensa* is quite comparable to the 46- to 49-kDa vitellin subunits found in the tephritid flies *Ceratitis capitata* [16] and *Dacus oleae* [30] as well as various drosophilids [3]. Consistent with two-dimensional gel electrophoresis analysis of YP in these dipterans, the *A. suspensa* YP shows charge heterogeneity. This may indicate differential post-translational modifications of a single polypeptide as observed in *D. melanogaster* [31] or additional YPs with identical molecular masses. This can be more definitively resolved by a comparative molecular analysis of several YP DNA genomic clones.

In an effort to identify the vitellogenin precursor of the *A. suspensa* YP, a minor 48-kDa polypeptide component of the adult female hemolymph was found, while no other polypeptide fit the criteria of a vitellogenin as a predominant female-specific hemolymph protein. This suggested either that vitellogenin was removed from the hemolymph very rapidly by maturing oocytes or that the fat body was not a major site of synthesis in this species. By examining the biosynthetic products of the fat body and ovaries either in vivo or when placed in vitro tissue culture, it was clearly demonstrated that the ovary is quantitatively the major source of the 48-kDa YP found in the oocyte. The observation that the ovary secretes primarily YP into the culture medium suggests that vitellogenin found in the hemolymph of vitellogenic females may be derived from the ovary, in addition to the fat body. While the presence of YP in the culture medium may be the result of leakage or damage to the cultured oocytes, the pre- and early vitellogenic ovaries are

not easily disrupted, and the majority of other polypeptides are conserved within the ovary.

Until recently, insect YP precursors were thought to be derived solely from the fat body of the adult female [1]. The first indication that insect ovaries were also capable of YP synthesis resulted from interspecific ovarian transplants in *Drosophila* [3] and, more directly, by the finding that *D. melanogaster* YP genes were transcribed in the ovarian follicular epithelium, as well as in the fat body [18]. Since then, YP synthesis has been observed in the ovaries of other dipterans including the Mediterranean fruit fly, *Ceratitis capitata* [16], the stable fly, *Stomoxys calcitrans* [17], and the fleshfly, *Sarcophaga bullata* [32]. Interestingly, similar to the Caribbean fruit fly, YP synthesis is almost totally restricted to the ovaries of the stable fly [17], while in the more closely related tephritid fly, *C. capitata*, significant levels of YP synthesis occur in both the ovaries and the fat body [16]. Although ovarian synthesis of the major YPs has thus far been limited to the higher dipterans, there is no clear evidence that the propensity to utilize this tissue as a source of YP is an evolutionary characteristic of any related group of flies. This will be clarified as the tissue-specific synthesis of VP is examined in more insects and molecular probes allow a more definitive site specific and quantitative assessment of YP gene expression during development.

Initiation of Vitellogenesis

YP synthesis first became detectable in vivo by 4–5 days after eclosion, which was coincident with the first observable deposition of yolk in oocytes. However, the presence of low YP antigen levels in ovaries was detected as early as 12 h after adult eclosion, which did not increase appreciably until 3 days after eclosion. Both increased levels of synthesis and observable YP occurred on days 3–5. The ovarian cultures showed that the rate of YP synthesis is dependent upon the developmental age of the ovaries and that the major increase in YP synthesis occurs coincident with the initiation of yolk deposition. The initiation of YP synthesis near the time of eclosion is consistent with similar findings in other dipterans including *D. melanogaster* [14] and *C. capitata* [16]. The processes controlling the initiation of YP synthesis were not examined in this study, but it is apparent that adult feeding is not required to trigger YP synthesis.

Sex-specificity of YP Synthesis

Immunoreactive protein corresponding to the 48-kDa YP in oocytes was shown to be present at low levels in the hemolymph of both adult females and males. Although this result requires confirmation by measuring YP gene transcript production in both sexes, we conclude that YP production is not strictly sex-specifically regulated in this species. Vitellogenin has generally been defined as a female-specific protein [1], although immunological analyses in a few insects distantly related to *A. suspensa* have revealed low levels of antigenically identical or similar proteins in males [21–23]. However, since these immunological studies were limited by the specificity of the probes and methods of detection, these results also require further confirmation.

The observation that almost all YP in *A. suspensa* is ovarian-derived, with low equivalent YP levels in the hemolymph of both sexes, suggests that the female-specific regulation of YP synthesis is a function of sex-specific tissue development. This is in contrast to YP production in *D. melanogaster*, where, although the ovaries contribute at least one-third of the total YP [18], the major contribution of YP by the fat body is autonomously regulated by the genetic mechanisms that control sex-determination [20,24]. In *D. melanogaster*, YP gene transcription does not normally occur in males [8,33], and the ability of the female fat body to support YP transcription is dependent upon specific genes acting in a female mode [20]. These data together suggest differing mechanisms regulating the sex-specific expression of YP genes in *D. melanogaster* and *A. suspensa*.

Antigenic Homology of YPs Between Species

The YPs of most higher dipterans have been found to share similar molecular masses that are different from those of other insects, and this similarity in size presumably represents a conservation of structure within the group. To test for conserved YP structure between *A. suspensa* and *D. melanogaster*, antisera raised to the YPs of each species were cross-reacted with the YPs of the heterologous species. Both antisera specifically recognized the YPs of the opposite species in immunoblots of ovarian proteins. The antibody to the purified 48-kDa YP from *A. suspensa* specifically recognized all three YPs from *D. melanogaster*. Conversely, antibody to the soluble egg proteins of *D. melanogaster* specifically recognized the 48-kDa YP of *A. suspensa*. The antigenic cross-reactivity and to a lesser degree the similarity in molecular mass between the YPs of these two species is consistent with an evolutionary conservation of YP structure among the higher dipterans. The conservation of YP structures and specifically with *Drosophila* YP has allowed *Drosophila* cloned YP DNA to be used as a probe to select for putative cloned YP DNA from a *Ceratitis* genomic library [34]. Based upon the shared antigenic homologies of the YPs demonstrated here, we are encouraged to utilize cloned *Drosophila* YP genes as probes to isolate the YP gene from *A. suspensa*. Having the cloned YP gene will ultimately allow a more detailed examination of the regulatory interactions resulting in YP synthesis and ovarian maturation in the Caribbean fruit fly.

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