THE JOURNAL OF EXPERIMENTAL ZOOLOGY 232:87–98 (1984)

Identification, Synthesis, and Characterization of the Yolk Polypeptides of *Plodia interpunctella*

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ABSTRACT The mature eggs of *Plodia interpunctella* were found to contain four major polypeptides. These yolk polypeptides (YPs) were found to have approximate molecular weights of 153,000 daltons (YP1), 69,000 daltons (YP2), 43,000 daltons (YP3), and 33,000 daltons (YP4) as determined by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE). In addition, we found YP1 was resolved by a 5% polyacrylamide gel into two separate polypeptides of 153,000 and 147,000 daltons. All of the YPs could be labeled in vivo or in vitro with [^{35}S]-methionine. Yolk peptide 1 and YP3 were synthesized by fat body of pharate adult and adult females and secreted into the hemolymph. Yolk peptide 2 and YP4 were synthesized and secreted into incubation medium by ovaries that contained vitellogenic oocytes, but these polypeptides were not found in the hemolymph. Fat bodies of males synthesized and secreted an immunoprecipitable polypeptide similar to YP3 as well as immunoprecipitable polypeptides larger than 200,000 daltons that had no counterparts in the oocytes. Peptide mapping by protease digestion showed each YP to be cleaved into unique fragments, suggesting that no precursor-product relationship exists between the YPs. Ion exchange chromatography and gel permeation chromatography separated the yolk proteins into two groups with approximate molecular weights of 462,000 and 264,000 daltons. By resolving these peaks on SDS-PAGE, it was found that YP1 and YP3 formed the 462,000-dalton yolk protein and YP2 and YP4 formed the 264,000-dalton yolk protein.

In the moths, the yolk proteins arise from precursors synthesized in two separate tissues. The major protein component of the yolk typically comes from vitellogenin synthesized in the fat body (cf. Wyatt and Pan, '78; Engelmann, '79; Hagedorn and Kunkel, '79). During vitellogenesis the fat body secretes a vitellogenin into the hemolymph that is transported to the ovaries and then taken up by the maturing oocytes (Telfer, '54; Pan et al., '69). The hemolymph of *Hyalophora cecropia*, *Bombyx mori*, and *Manduca sexta* contains a vitellogenin with subunit molecular weights of approximately 180,000 daltons and 45,000 daltons (Kunkel and Pan, '76; Izumi et al., '80a; Harnish and White, '82; Imboden and Law, '83). When the fat body of *B. mori* was cultured in vitro, the two vitellogenin subunits were synthesized and secreted into the culture medium (Izumi et al., '80b; Izumi and Tomino, '83). The synthesis of these two polypeptides was shown to be dependent on the presence of two unique mRNAs in the fat body that directed their synthesis in a cell-free translation system (Izumi et al., '80b; Izumi and Tomino, '83). During the later stages of embryogenesis, these polypeptides are utilized by the growing embryo (Irie and Yamashita, '80).

A second group of proteins found in the mature oocytes arises from the follicle cells surrounding the oocytes (Ono et al., '75; Bast and Telfer, '76). The egg-specific protein of *B. mori* (Ono et al., '75) or paravitellogenin of *H. cecropia* (Bast and Telfer, '76) account for about 20% of the total yolk proteins of the oocyte. Although these two proteins appear to have similar functions during embryogenesis, the two polypeptides have slightly different reported molecular weights of approximately 55,000 daltons for egg-specific protein (Irie and Yamashita, '83) and 70,000 daltons for paravitellin (Telfer et al., '81). When taken into the maturing oocyte, these polypeptides are not only homogeneously distributed throughout the protein yolk spheres, but are also laid down in a stratum of vesicles surrounding the protein yolk spheres near the surface of the egg (Telfer and Anderson, '68; Melius and Telfer, '69; Telfer and Smith, '70; Takesue et al., '76). The role of these polypeptides apparently is to meet specific nutrient requirements during development, as suggested in *B. mori*, where the egg-specific protein is consumed preferentially during early embryogenesis (Irie and Yamashita, '80, '83).

These studies were undertaken to characterize the yolk polypeptides (YPs) and their precursors in *Plodia interpunctella*. By utilizing antiserum raised against nonchorionic egg proteins, sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE), and peptide mapping, we describe the presence of four major YPs contained in the mature oocytes of *P. interpunctella*, their biosynthetic origin, and their structural interrelationships. We have found the presence of two YPs that are secreted from the fat body and constitute a protein similar to the major vitellogenin found in the hemolymph of other moths. In addition, we found that ovaries that contained vitellogenic oocytes synthesized and secreted YPs that are incorporated into the oocytes as part of the vitellin.

MATERIALS AND METHODS

Animals and materials

The *P. interpunctella* culture was established from wild adults and reared on an artificial diet (Silhacek and Miller, '72) at 27°C in 60–80% relative humidity and light:dark (LD) 16:8. Under these conditions, adult development took 8 days. As a reference point for adult development, tarsal claw darkening occurred on day 5. L-[^{35}S]-Methionine ([^{35}S]-Met) (specific activity, 1,200 Ci/mmole) was obtained from New England Nuclear. Grace's insect medium was obtained from Grand Island Biological.

Preparation of antiserum against yolk

Freshly laid eggs were homogenized in 150 mM NaCl and 10 mM phosphate (pH 7.2). The homogenate was spun at 12,000 × g, and the supernatant was mixed with an equal volume of Freund's complete adjuvant and injected subcutaneously and intramuscularly into a rabbit. After 1 month, the rabbit was boosted by a subcutaneous injection of antigen in Freund's incomplete adjuvant. Two weeks after each boosting, the rabbit was bled, and the serum was collected and frozen.

Double diffusion test for YPs

The rabbit serum (from here on referred to as anti-Yolk antiserum) was tested for antigenicity towards YPs by the Ouchterlony double diffusion test (Ouchterlony and Nilsson, '73). The immunodiffusion was carried out with a 1% Noble agar gel in 150 mM NaCl and 10 mM phosphate (pH 7.2) at 4 °C. The diffusion was allowed to continue for 4 days to assure complete precipitation of the antigens.

SDS-polyacrylamide gel electrophoresis

The proteins were prepared for electrophoresis by homogenizing the tissues in SDS-sample buffer (Laemmli, '70). The proteins were resolved by SDS-PAGE (0.75 mm thick; 8–15% gradient) (O'Farrell, '75; O'Farrell et al., '77) at 270 V for 18 h. To determine the molecular weights, freshly laid eggs were homogenized in SDS-sample buffer and electrophoresed in SDS-slab gels of 5, 6, 8, or 10% polyacrylamide with protein molecular weight standard (Sigma) side markers. The gels were stained for protein with Coomassie brilliant blue R. The relative mobility of each band was determined with reference to the dye front, and the molecular weights of the YPs were computed from the linear regression generated from the mobilities of the molecular weight standards. Labeled proteins were visualized by autoradiography using Kodak X-Omat AR X-ray film.

The antigens cross-reacting with the anti-Yolk antiserum were identified by SDS-PAGE. The samples were prepared for electrophoresis by homogenizing the tissues in 150 mM NaCl and 10 mM phosphate (pH 7.2) and diluting the 12,000 × g supernatant two times with 150 mM NaCl, 2 mM EDTA and 50 mM Tris/HCl (pH 7.6) (NET) plus 1% Triton-X 100 or diluting the culture medium two times with NET plus 1% Triton-X 100. To this, 0.1-volumes of anti-Yolk antiserum were added, and the mixture was incubated

16 h at 0°C. The antigen/antibody complexes were precipitated by addition of 0.1-volumes of IgGSORB (The Enzyme Center) and continued incubation at 0°C for 2 h more. The precipitates were washed three times with NET and finally dissolved in SDS-sample buffer.

To determine the relative amounts of stained proteins present, negatives of Coomassie blue-stained gels were scanned with a Joyce-Loebel microdensitometer, and the peak areas of the bands were computed with a Zeiss MOP-3.

Peptide mapping of Yps

Peptide mapping of the various proteins was accomplished essentially as described by Cleveland et al. ('77). The [^{35}S]-Met labeled tissue samples were electrophoresed by SDS-PAGE, and the desired bands were cut from the dried gel after autoradiography. The bands were rehydrated with swelling buffer (0.125 M Tris/HCl at pH 6.8, 0.5% SDS, 10% glycerol, and 0.0001% bromophenol blue) in the wells of an 8–15% SDS gel. The proteins contained in each band were digested with 0.05 μg of *Staphylococcus aureas* V8 protease, *Streptomyces griseus* protease, or α-chymotrypsin in 0.125 M Tris/HCl 6.8, 0.1% SDS, and 1 mM EDTA (0.05 mg/ml). The fragments were resolved by SDS-PAGE at 5 mAmp, and after drying the fragments were visualized by autoradiography.

Incorporation of [^{35}S]-Met in vivo and in vitro

Newly eclosed adult females and males were injected with 10 μCi of [^{35}S]-Met in 1 μl Weevers' saline (Weevers, '66). After 6 h at 27°C the moths were bled, and the ovaries and body walls (composed primarily of fat body tissues) were dissected and homogenized. We used body walls instead of fat body to avoid damaging the small amount of fat body while dissecting it away from the cuticle. The tissue preparations were either immunoprecipitated with anti-Yolk antiserum or placed directly in SDS-sample buffer and prepared for SDS-PAGE.

The body walls and ovaries were dissected and washed several times in sterile Weevers' saline. The tissues were then transferred to Grace's insect medium plus [^{35}S]-Met (2 μCi/μl) and incubated in a 95% O_2/5% CO_2 atmosphere at 27°C for 6 h. The tissue homogenates and culture media were either immunoprecipitated with anti-Yolk antiserum or placed directly in SDS-sample buffer and prepared for SDS-PAGE.

Ion exchange column chromatography

The yolk proteins were separated by ion exchange using a DEAE-Sepharose (CL-6B) column (38 cm × 1.5 cm). Eggs were homogenized in 150 mM KCl and 50 mM PO_4 (pH 7.5) at 4°C, and centrifuged at 12,000 × g for 20 min. The supernatant was saturated with ammonium sulfate, and the precipitate was redissolved in homogenization buffer and dialyzed exhaustively against 5 mM KCl and 50 mM PO_4 (pH 7.5). The dialyzed solution was layered on the column in 5 mM KCl and 50 mM phosphate (pH 7.5), and the materials were washed onto the column with the buffer. The salt concentration was increased in steps of 5 mM, 50 mM, 100 mM, 150 mM, 200 mM, and 500 mM KCl. Fractions were collected, and the ultraviolet (UV, 280 nm) absorbing peaks were combined and concentrated for electrophoresis on SDS-PAGE.

Gel permeation chromatography

The yolk proteins were sized by gel permeation using a Sephacryl S-300 column (79 cm × 1.2 cm). A dialyzed ammonium sulfate precipitate of an egg homogenate or the concentrated UV-absorbing peaks from ion exchange chromatography were layered on the column and washed with 150 mM KCl, 50 mM phosphate (pH 7.5), and 0.05% sodium azide. The fractions were collected and the UV- (280 nm) absorbing peaks were combined and concentrated for electrophoresis on SDS-PAGE.

RESULTS

Characterization of the yolk polypeptides

The proteins from homogenates of mature eggs were resolved by SDS-PAGE (8–15% gradient), and stained with Coomassie blue. Figure 1 shows the presence of four major polypeptides that we have designated YP1, YP2, YP3, and YP4 in descending molecular weight order. These four polypeptides were immunoprecipitable by the anti-Yolk antiserum. The hemolymph proteins and immunoprecipitable hemolymph proteins of both females and males were also electrophoresed. YP1 and YP3 were found to be present in the hemolymph of adult females, which was confirmed by immunoprecipitation Fig. 1c,d). The immunoprecipitates of adult male hem-

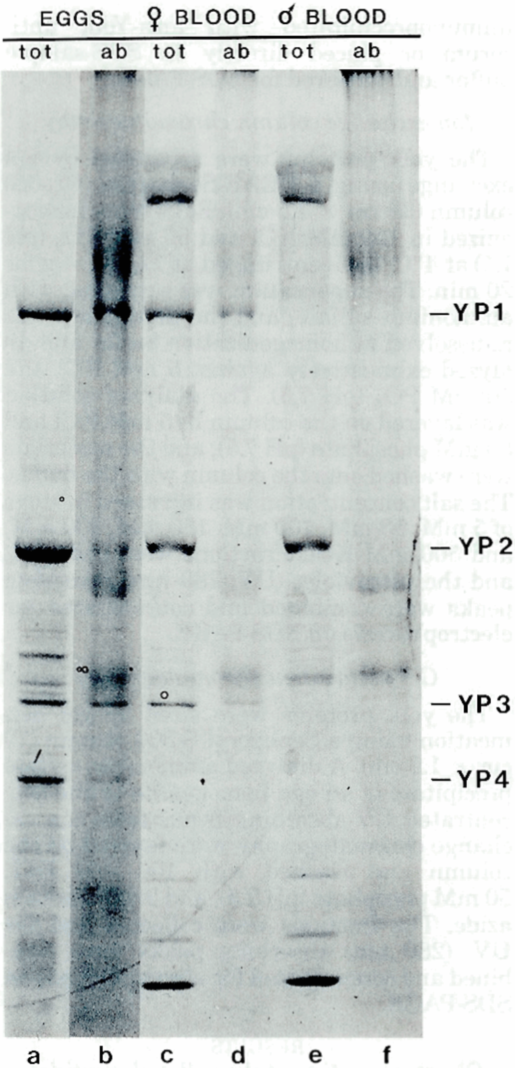


Fig. 1. Sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) (8–15% gradient) resolving the total polypeptides (a, c, e) and anti-Yolk antiserum immunoprecipitable polypeptides (b, d, f) of eggs (a, b), adult female hemolymph (c, d), and adult male hemolymph (e, f). tot, total proteins; ab, anti-Yolk antiserum-precipitated proteins. Lanes b, d, and f were overexposed to show the proteins present. The proteins appearing in lane f are from the antiserum.

olymph (Fig. 1e,f) did not contain significant quantities of any of the four YPs, although there were hemolymph-specific polypeptides that were common in both females and males.

The molecular weights of the major egg polypeptides were determined by SDS-PAGE on gels of 5, 6, 8, or 10% polyacrylamide.

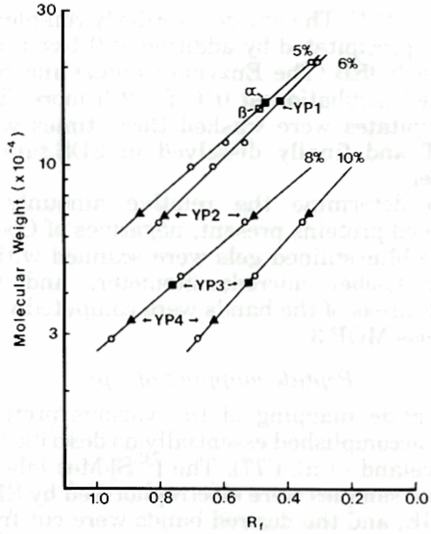


Fig. 2. Determination of the molecular weights of the *Plodia* YPs. The molecular weight standards were co-electrophoresed with the YPs on 5, 6, 8, and 10% SDS-PAGE. The following proteins were used as standards: rabbit myosin (205,000 daltons), α-galactosidase (116,000 daltons), phosphorylase b (97,400 daltons), bovine serum albumin (66,000 daltons), ovalbumin (45,000 daltons), carbonic anhydrase (29,000 daltons). The relative mobilities were determined by comparing the migration of the proteins with the migration of the dye front. The regression lines are as follows: 5% Y = 5.58 - 0.86X; 6% Y = 5.60 - 0.98X; 8% Y = 5.27 - 0.85X; 10% Y = 5.2 - 1.08X.

Figure 2 shows the regression line for each of the gels. Four major polypeptides were found in the egg proteins, and they had approximate molecular weights of 153,000, 69,000, 43,000, and 33,000 daltons for YP1, YP2, YP3 and YP4 respectively. When electrophoresed on 5% gels, the YP1 band resolved into two separate polypeptide bands of 153,000 daltons (YP1α) and 147,000 daltons (YP1β).

To determine the apparent quantities of YPs present in the yolk, negatives of the Coomassie blue-stained gels were scanned and the peak areas determined. YP1 and YP2 accounted for approximately 40% of the stained material on the gel. The other two YPs represented about 20% of the total stained material. Thus, the major YPs comprise nearly 60% of the total Coomassie blue-stained polypeptides found in the eggs. When compared to each other, the YPs were present in a ratio of 35% YP1:32% YP2:13% YP3:20% YP4.

Tissue specific biosynthesis of YPs

Newly eclosed adult females and males were injected with [^{35}S]-Met and incubated for 6 h. The various tissue preparations were either directly electrophoresed or immunoprecipitated with anti-Yolk antiserum and then resolved by SDS-PAGE. All four of the YPs found in the oocytes had been labeled during the incubation, including the α and β forms of YP1 (Fig. 3). However, the body wall tissues of the adult females contained only YP1 and YP3, as did the tissues and medium of fat body incubated in vitro. The presence of these two YPs in the hemolymph of adult females was also demonstrated. An immunoprecipitable polypeptide with a molecular weight identical to that of YP3 was also found to be present in the adult male body walls and hemolymph. Interestingly, the male also contains polypeptides of molecular weights greater than 200,000 daltons that

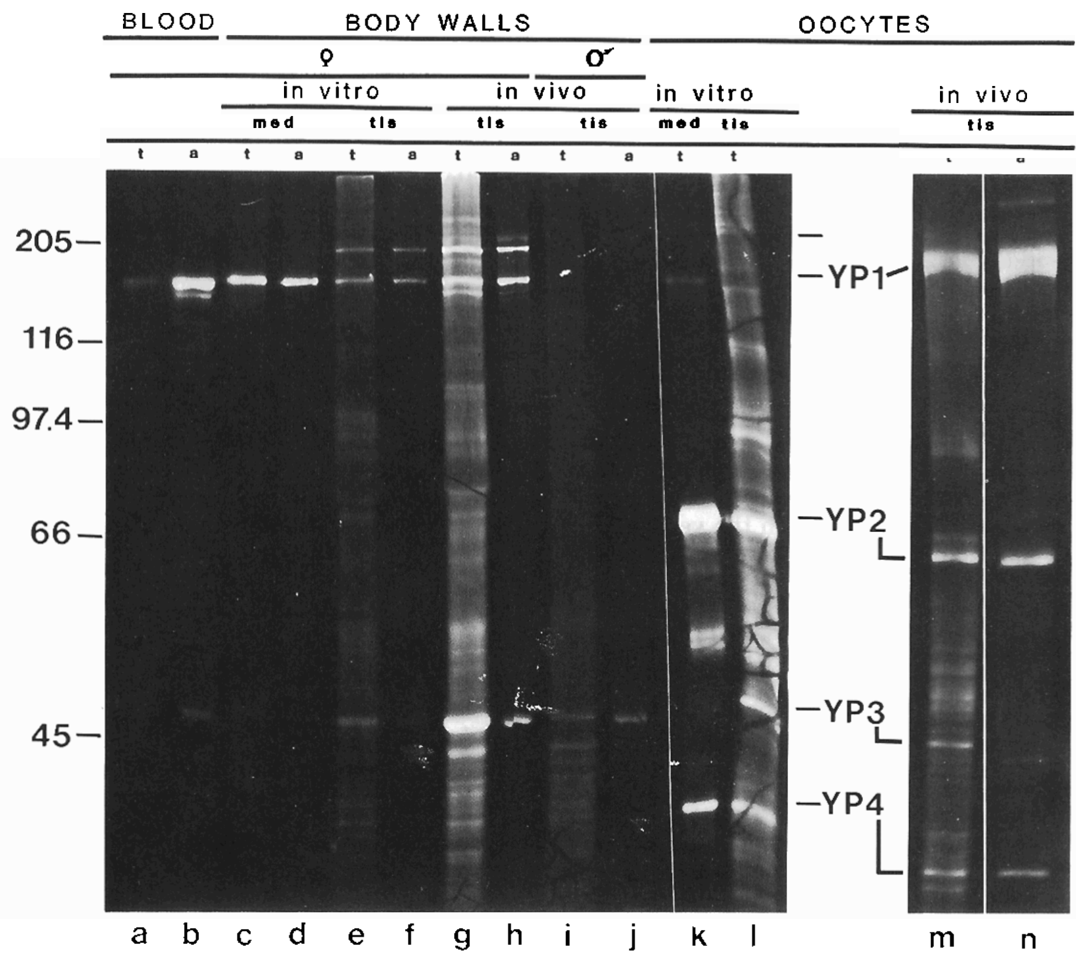


Fig. 3. Autoradiogram of [^{35}S]-methionine-labeled polypeptides from adult female and male *Plodia*. Total polypeptides: lanes a, c, e, g, i, k, l, m; anti-Yolk antiserum immunoprecipitable polypeptides: lanes b, d, f, h, j, n; female hemolymph: a, b; female body walls (incubation medium): c, d; female body walls (incubation tissues): e, f; female body walls (in vivo tissues): g, h; male body walls (in vivo tissues): i, j; oocytes (incubation medium): k; oocytes (incubation tissues): l; oocytes (in vivo tissues): m, n. The molecular weight markers (×10 daltons) are designated on the left. Equal quantities of trichloroacetic acid-precipitable radioactive labeled material were added to each lane. med, medium; tis, tissue; t, total proteins; a, anti-Yolk-precipitated proteins.

cross-react with the anti-Yolk antiserum but that are not apparent in the ovary homogenates.

To determine the site of biosynthesis of the YPs, various organs of pharate adults were dissected and incubated in vitro with [^{35}S]-Met to label the newly synthesized polypeptides. Ovaries and body walls of pharate adult females and body walls of pharate adult males both 8 days after pupation were dissected and incubated in Grace's insect culture medium. The media and tissue preparations were resolved by 8–15% SDS-PAGE. The medium of pharate adult female body walls contained immunoprecipitable polypeptides equivalent to YP1 and YP3, as did the homogenates of the incubated tissues (Fig. 4). YP2 and YP4, however, were only found in the medium from cultured ovaries and in the ovary tissue homogenates. The body walls of the males were again found to synthesize and secrete an immunoprecipitable polypeptide with a mobility equivalent to YP3. The male body walls also secreted polypeptides larger than 200,000 daltons that were immunoprecipitable by the anti-Yolk antiserum. The significance of this cross-reactivity is not known.

Partial peptide mapping of the YPs

To determine the structural relationships of the YPs, partial protease digests of the YPs were made and resolved on SDS-PAGE. Each of the YPs had individual fragment patterns when treated with the various proteases (Fig. 5, lanes a–d). This suggests that each of the YPs originated from a unique polypeptide, and that none of the YPs were generated in a precursor-product relationship from any other YP. When the peptide maps of the in vitro labeled forms of each of the polypeptides were compared with the in vivo forms, there appeared to be structural identity between the products from incubations in vitro and those found in situ. It should be noted, however, that the peptide maps of the YP1α and YP1β polypeptides appeared to be identical in all protease digestions (Fig. 5, lanes h–k). These two YPs may represent differing forms of the same polypeptide that result from specific variations in the posttranslational modification pathways or products of two closely related genes.

Characterization of the native yolk proteins

A dialyzed ammonium sulfate precipitate of homogenized eggs was layered on a DEAE-Sepharose column, and the proteins were eluted with increasing steps of KCl buffer. The fractions containing the UV absorbing peaks for each salt step were combined and concentrated for SDS-PAGE. The Coomassie blue-stained proteins contained in each of the combined fractions are shown in Figure 6. Both YP2 and YP4 eluted together in the 5-mM KCl peak, whereas the majority of YP1 and YP3 eluted in the 200-mM KCl peak.

To determine the molecular weights of the yolk proteins, each of the two peaks from ion-exchange was passed over a Sephacryl S-300 column. Native molecular weight standards were cochromatographed to establish the molecular weight range. Figure 7 shows the regression line for the molecular weight standards and the elution points for the yolk proteins. The yolk proteins eluted in two major peaks at approximate molecular weights of 462,000 and 264,000 daltons (Fig. 8). When the proteins of these peaks were resolved on SDS-PAGE, the 462,000-dalton peak was found to be composed of YP1 and YP3 (Fig. 6, lane k). The 264,000-dalton peak contained YP2 and YP4. Thus, the yolk proteins appear to be composed of polypeptide groups that are organized on the basis of their site of synthesis.

DISCUSSION

The mature eggs of *P. interpunctella* contained four polypeptides that comprised the bulk of the proteinacious yolk. Approximately 60% of the yolk proteins detectable by Coomassie blue staining of SDS-PAGE was contributed by the four YPs. Together, YP1 and YP2 accounted for nearly 70% of these four polypeptides. Of the four YPs, only YP1 and YP3 were found in the hemolymph of vitellogenic adult females.

We found that the YPs were produced by two tissues: the fat body and the ovaries that contained vitellogenic oocytes. YP1 and YP3 were synthesized and secreted by the fat body into the hemolymph of the vitellogenic females. The role of the fat body of *P. interpunctella* in producing vitellogenin appears to be similar to that in the other moths such as *H. cecropia* (Kunkel and Pan, '76; Harnish and White, '82) or *B. mori* (Izumi and Tomino, '83) where the fat body synthesizes and secretes two polypeptides: one large polypeptide of approximately 180,00 daltons and one small polypeptide of approximately 45,000 daltons. In *P. interpunctella*, the higher molecular weight polypeptide, YP1, has a slightly smaller molecular weight (153,000 daltons) than that found in the other moths

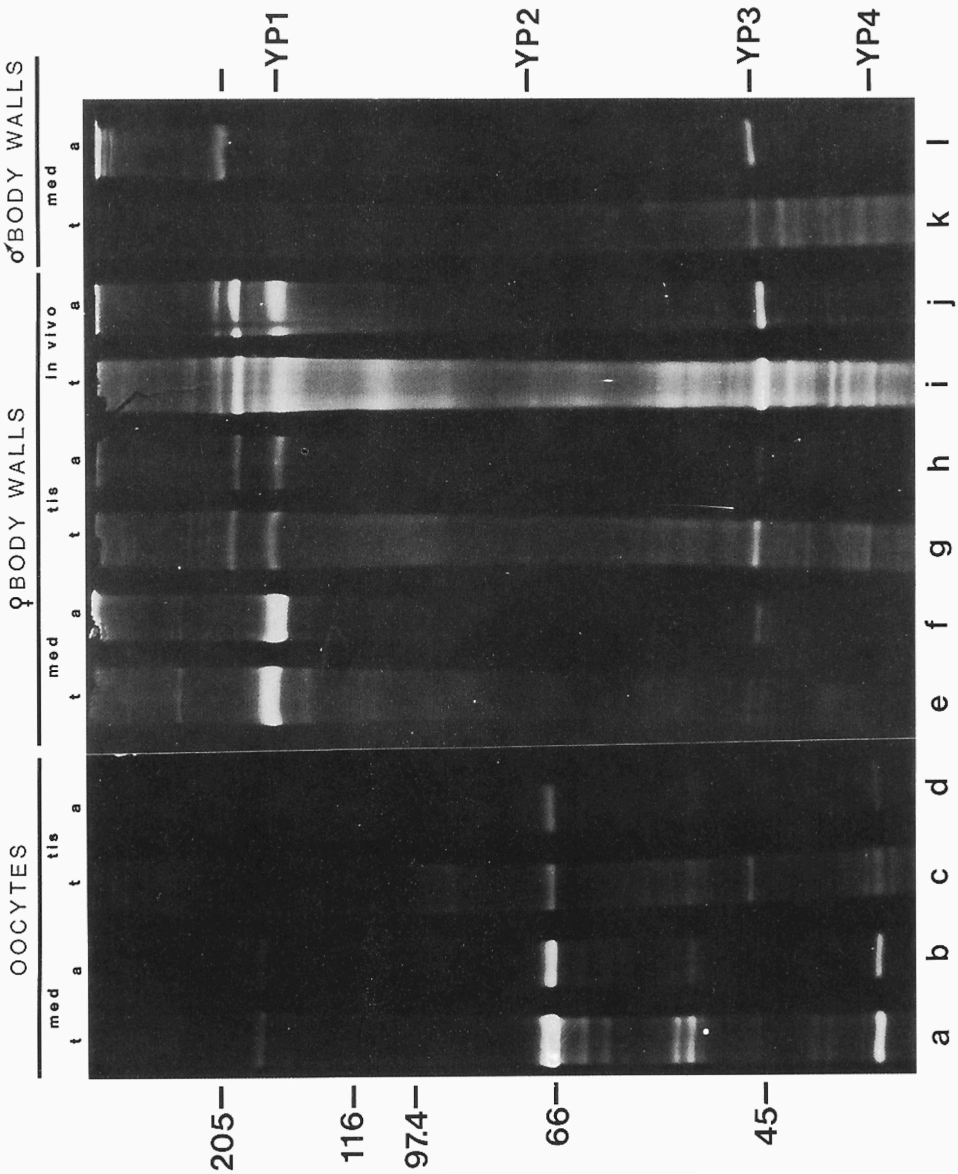


Fig. 4. The YPs secreted from tissues of pharate adult female and male *Plodia* incubated in vitro. Total polypeptides: lanes a, c, e, g, i, k; anti-Yolk antiserum immunoprecipitable polypeptides: lanes b, d, f, h, j, l; oocytes (incubation medium): a, b; oocytes (incubation tissues): c, d; female body walls (incubation medium): e, f; female body walls (incubation tissues): g, h; female body walls (in vivo tissues): i, j; male body walls (incubation medium): k, l. The molecular weight markers ($\times 10^{-3}$ daltons) are designated on the left. Equal quantities of trichloroacetic acid-precipitable radiolabeled material were added to each lane. med, medium; tis, tissue; t, total proteins; a, anti-Yolk antiserum precipitated proteins.

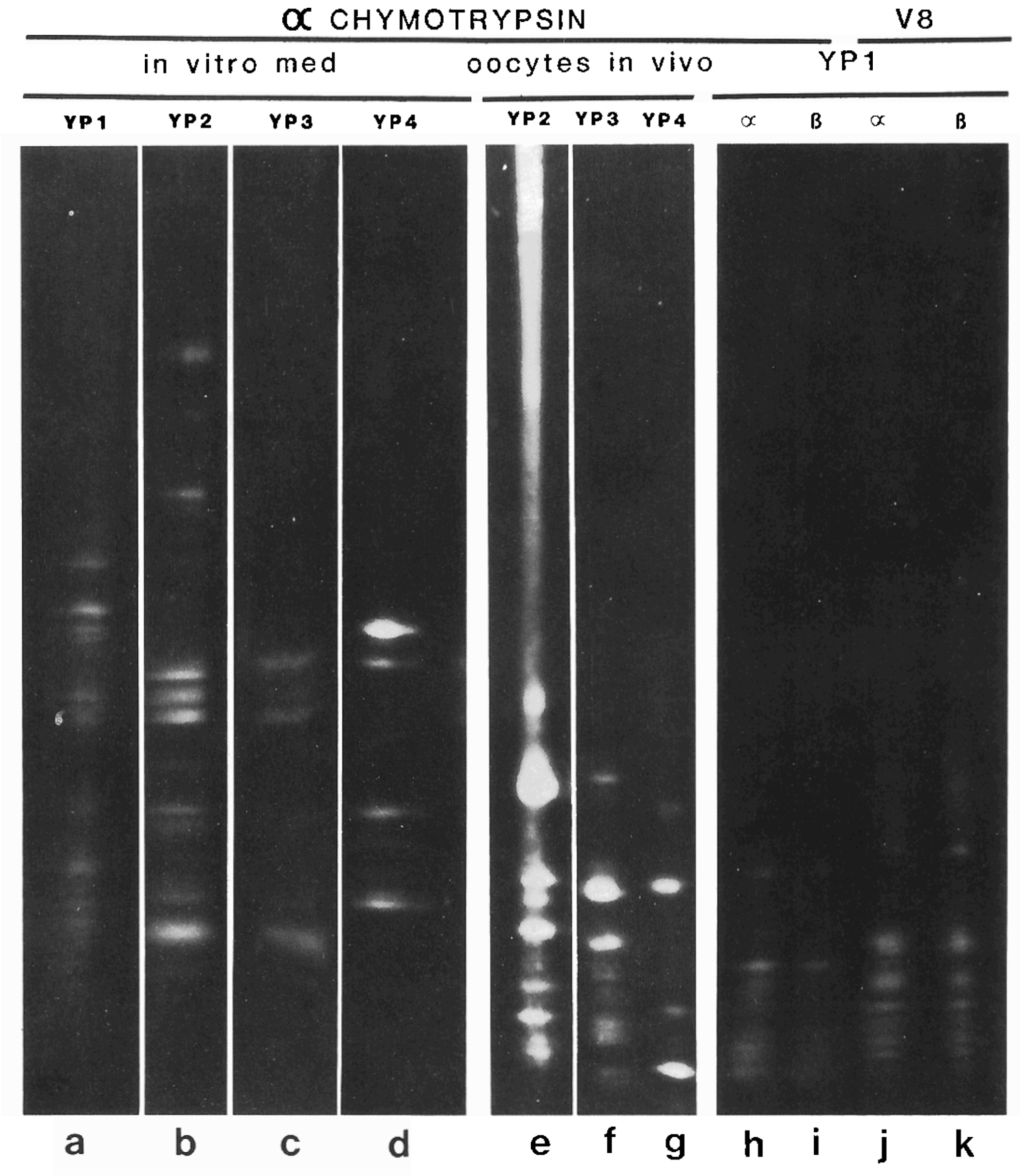
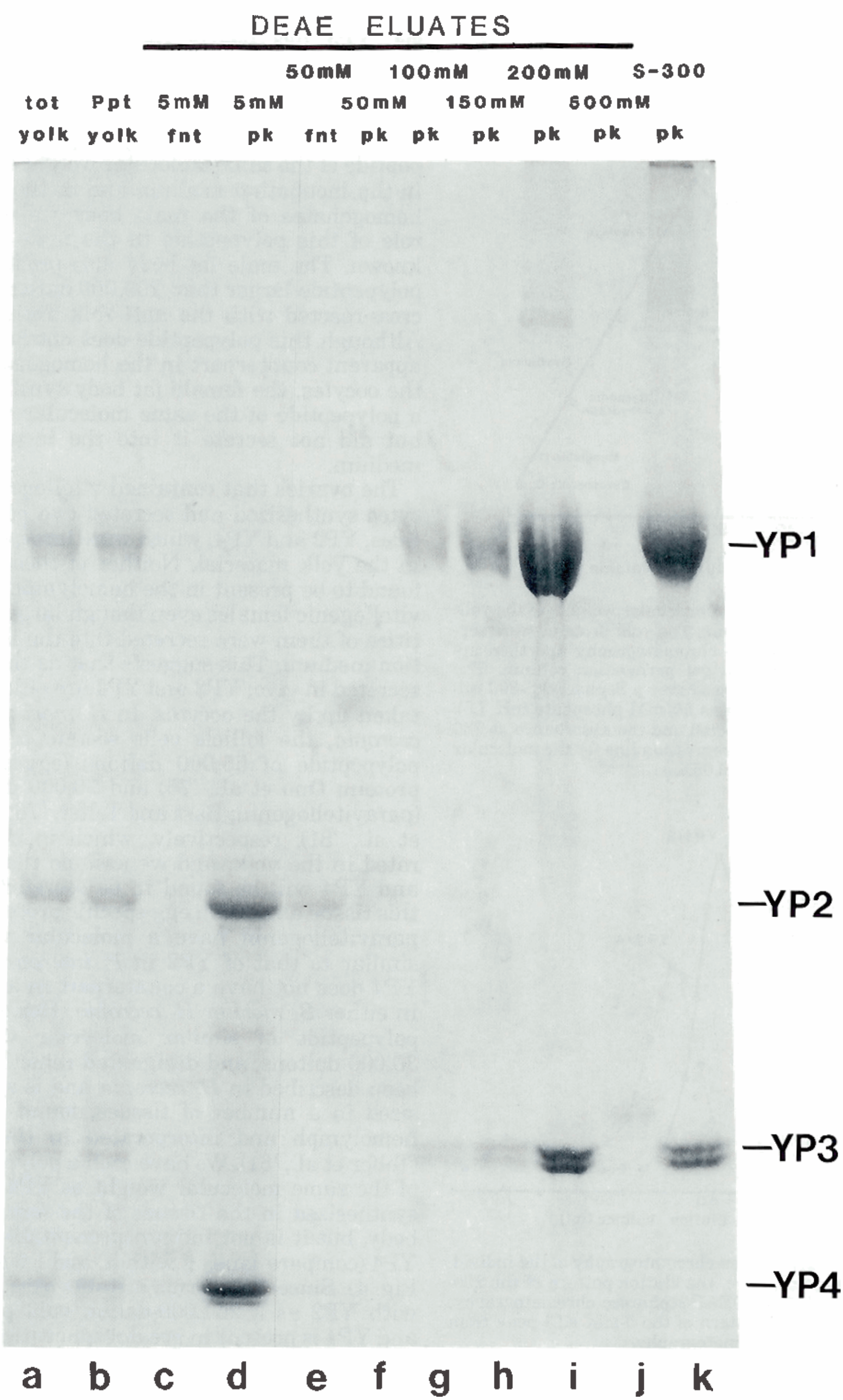


Fig. 5. Partial peptide maps of the YPs. Lanes a–i were digested with α-chymotrypsin and lanes j and k were digested with *S. aureus* V8 protease. Lanes a–d and h–k were polypeptides secreted into incubation medium. Lanes e–g were polypeptides from in vivo labeled oocytes. Lane designation: a, YP1 from fat body; b, YP2 from oocytes; c, YP3 from oocytes; d, YP4 from oocytes; e, YP2 from oocytes; f, YP3 from oocytes; g, YP4 from oocytes; h, YP1α from fat body; i, YP1β from fat body; j, YP1α from fat body; k, YP1β from fat body.

Fig. 6. SDS-PAGE of the yolk proteins separated by ion exchange column chromatography. Lane designation: a, crude egg homogenate; b, ammonium sulfate precipitate of egg homogenate; c, 5-mM KCl front; d, 5-mM KCl peak; e, 50-mM KCl front; f, 50-mM peak; g, 100-mM KCl peak; h, 150-mM KCl peak; i, 200-mM KCl peak; j, 500-mM peak; k, 462,000-dalton peak from Sephacryl S-300 chromatography of 200 mM KCl peak (see Fig. 8). tot, total; Ppt, precipitate; fnt, front; pk, peak.


DEAE ELUATES
50mM
100mM
200mM
S-300
tot yolk
Ppt yolk
5mM fnt
5mM pk
fnt
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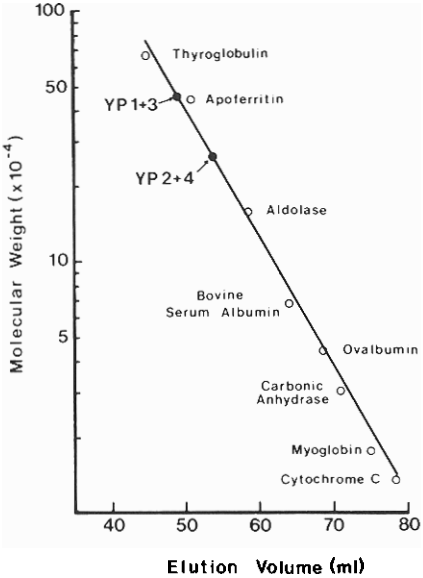

Fig. 7. Determination of molecular weights of the yolk proteins by gel permeation. The yolk proteins were separated by ion exchange chromatography and then applied separately to the gel permeation column. The native proteins were passed over a Sephacryl S-300 column in 150 mM KCl plus 50 mM phosphate (pH 7.5). The fractions were collected and the absorbance at 280 nm was measured. The regression line for the molecular weights was Y = 8.32 - 0.05X.

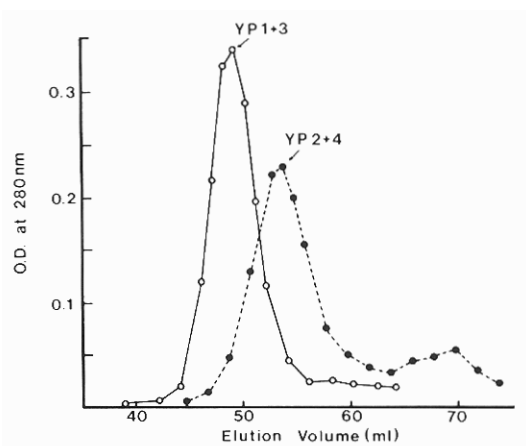

Fig. 8. Gel permeation chromatography of the individual yolk proteins. ∘——∘, the elution pattern of the 200-mM KCl peak from DEAE-sepharose chromatography; •- - - -•, the elution pattern of the 5-mM KCl peak from DEAE-sepharose chromatography.

and is the major proteinacious product secreted from the fat body during vitellogenesis. YP3 has nearly the same molecular weight as its counterpart in *H. cecropia* and *B. mori*, and is produced in smaller quantities than is YP1. It is interesting to note that we found an immunoprecipitable polypeptide of the same molecular weight as YP3 in the incubation medium and in the tissue homogenates of the male body walls. The role of this polypeptide in the male is not known. The male fat body also produced a polypeptide larger than 200,000 daltons that cross-reacted with the anti-Yolk antiserum. Although this polypeptide does not have an apparent counterpart in the homogenates of the oocytes, the female fat body synthesized a polypeptide of the same molecular weight but did not secrete it into the incubation medium.

The ovaries that contained vitellogenic oocytes synthesized and secreted two polypeptides, YP2 and YP4, which were incorporated in the yolk material. Neither of these were found to be present in the hemolymph of the vitellogenic females even though large quantities of them were secreted into the incubation medium. This suggests that as they are secreted in vivo, YP2 and YP4 are efficiently taken up by the oocytes. In *B. mori* and *H. cecropia,* the follicle cells secrete a single polypeptide of 55,000 daltons (egg-specific protein; Ono et al., ’75) and 70,000 daltons (paravitellogenin; Bast and Telfer, ’76; Telfer et al., ’81), respectively, which is incorporated in the yolk, and we assume that YP2 and YP4 will be found to be secreted from this tissue also. Both egg-specific protein and paravitellogenin have a molecular weight similar to that of YP2 in *P. interpunctella.* YP4 does not have a counterpart in any YP in either *B. mori* or *H. cecropia.* However, a polypeptide of similar molecular weight, 30,000 daltons, and designated reluctin, has been described in *H. cecropia* and is synthesized in a number of tissues, found in the hemolymph and incorporated in the yolk (Telfer et al., ’81). We have seen a polypeptide of the same molecular weight as YP4 being synthesized in the tissues of the female fat body, but it is not immunoprecipitable as is YP4 (compare lanes g with h, and i with j in Fig. 4). Since YP4 forms a stable association with YP2 as a 264,000-dalton yolk protein and YP4 is present in greater quantities than is YP3, YP4 should be considered a legitimate YP.

Partial peptide mapping of the YPs showed that each of the major YPs is a unique polypeptide (compare lanes a,b,c, and d in Fig. 5). Comparison of the two forms of YP1 showed they have the same fragment pattern, which indicates that they may represent modified forms of the same polypeptide (compare lane h with i, and lane j with k in Fig. 5). This heterogeneity in the size of the larger polypeptide secreted by the fat body has also been described for *B. mori* (Izumii and Tomino, '83) and *M. sexta* (Imboden and Law, '83). However, very little postsecretional and postuptake modifications of the polypeptide chains of the YPs were apparent since the peptide maps of the YPs secreted from the tissues into incubation medium and those of the YPs found sequestered in the oocytes showed no variation for any of the YPs (data not shown).

Separation of the native yolk proteins from the mature eggs of *P. interpunctella* showed the presence of two major proteins with molecular weights of approximately 462,000 daltons and 264,000 daltons. After purification and separation of the two proteins by ammonium sulfate precipitation, ion exchange chromatography and gel permeation chromatography, SDS-PAGE of the 462,000-dalton peak demonstrated that the protein is similar to the vitellins described in *H. cecropia* and *B. mori*. In *H. cecropia*, vitellin is a 450,000–500,000-dalton protein (Kunkel and Pan, '76; Harnish and White, '82) composed of subunits of either 120,000 plus 43,000–44,000 daltons (Kunkel and Pan, '76; Telfer et al., '81) or 180,000 plus 47,000 daltons (Harnish and White, '82). In *B. mori*, vitellin was found to be a 440,000-dalton protein with subunits of 180,000 and 42,000 daltons (Izumi et al., '80a). The three moths described above all have vitellins two times the size of that described in *M. sexta*, which has a vitellin of 260,000 daltons but with subunits of similar sizes, at 180,000 and 50,000 daltons (Mundall and Law, '79; Imboden and Law, '83).

The 264,000-dalton protein that we found in the yolk of *P. interpunctella* was an association between YP2 and YP4, which has not been described in any of the other moths. The stability of this association has not been fully explored, but appears to be of some significance since it survived ammonium sulfate precipitation, ion exchange chromatography, and gel permeation chromatography. The formation of such an association by polypeptides produced in the ovaries is most closely represented in *B. mori* where the egg-specific protein has a molecular weight of 125,000 daltons and is composed of a single subunit of 55,000 daltons (Irie and Yamashita, '83). The paravitellogenin of *H. cecropia* does not appear to form any higher associations (Telfer et al., '81).

The mature eggs of *P. interpunctella* contain four YPs that are secreted from either the fat body or the ovaries. These four YPs form associations constituting the two yolk proteins found in the oocytes. The role of each of these proteins during embryogenesis is not known, but it is likely to be similar to those ascribed to the yolk proteins found in other lepidopterans. It should be noted that in these moths the polypeptides secreted from the follicle cells may play a much more important role in embryogenesis than the classically considered vitellogenin produced by the fat body. When ovaries were transplanted into castrated male *B. mori* larvae, mature eggs lacking fat body produced vitellogenin were found after metamorphosis (Yamashita and Irie, '80). These eggs appeared to be sufficiently complete since normal larvae were raised when the eggs were stimulated to undergo artificial parthenogenesis. Further, the egg-specific protein secreted from the follicle cells of *B. mori* is preferentially utilized during early embryogenesis (Irie and Yamashita, '83). These findings suggest that the proteins secreted from the follicle cells are the major nutrient source for the growing embryo. Considering this information, we find significance in the fact that the polypeptides synthesized by the ovaries contribute 50% of the YP material to the yolk in the oocytes of *P. interpunctella*.

ACKNOWLEDGMENTS

This work was supported by grants from the Department of Defense (No. DAAG 29-81-K-0124), to V.J.B., the Medical Research Foundation of Oregon (to P.D.S.) and the NIH Biomedical Research Support Grant (No. RR07079), to O.S.U.

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