THE SYNTHESIS OF *DROSOPHILA MELANOGASTER* VITELLOGENINS *IN VIVO*, IN CULTURE, AND IN A CELL-FREE TRANSLATION SYSTEM

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1. Introduction

The vitellogenins of vertebrates [1] and most insects [2] are proteins which are synthesized by the liver or the fat body, secreted into the blood or hemolymph, sequestered by the oocytes to form yolk, and finally consumed as a food source by the embryo. In both vertebrates [1] and some insects [3–5] the synthesis of these proteins is under hormonal control. We have started an investigation of vitellogenin synthesis in *Drosophila melanogaster* in order to study the hormonal regulation of gene activity. Three vitellogenins or yolk proteins have been shown [6] in *Drosophila* hemolymph and ovaries of mature females with est. mol. wt ~47 500, 45 700 and 44 700. Here we report work on the synthesis of *Drosophila* vitellogenins in vivo, in organ culture, and in the wheat germ cell-free translation system. We show that vitellogenins are rapidly synthesized and secreted by female fat body and that RNA from this tissue directs the synthesis of proteins with apparent molecular weights identical to 2 of the 3 vitellogenins detected in vivo.

2. Materials and methods

An Oregon R stock of *Drosophila melanogaster* was cultured under standard conditions. For in vivo studies, 0.9 μCi [^{35}S]methionine (780 Ci/mmol, The Radiochemical Centre, Amersham) or 0.4 μCi [^{3}H]valine (33 Ci/mmol) in 0.5 μl *Drosophila* Ringers [7] were injected into each fly. At the time given in the text, hemolymph was collected in a capillary, ovaries were dissected, and the samples prepared for electrophoresis. For organ culture experiments, fat body-enriched tissue was obtained by using female abdomens from which the gut, reproductive apparatus, and malpighian tubules were removed. This body wall preparation consisted of fat body contaminated by a smaller amount of epidermis. In a typical experiment, 10 body walls were labeled in culture for a 2 h period in 10 μl Ringers containing 20 μCi [^{3}H]-leucine (51 Ci/mmol). Electrophoretic separation of proteins was performed in SDS–polyacrylamide gels (5–20%) by the Laemmli method [8]. The dried gels were subjected to either autoradiography or fluorography [9]. For the cell-free translation studies, total RNA was extracted as in [10] from male or female flies (in batches of 700–1000 animals), or from the body wall preparations above (400 female flies). Poly(A)-containing RNA was prepared from the whole body extracts by fractionation on oligo(dT)-cellulose (Collaborative Research, Inc.) [11]. A cell-free system from wheat germ was used for the translation of the RNA preparations [12] in the presence of 15 μCi [^{35}S]methionine/assay.

3. Results

3.1. *Synthesis of vitellogenins in vivo*

Hemolymph collected from mature adult females (fig.1B,D) contains 3 major protein species, termed YP-1, YP-2 and YP-3 [6] which comigrate with the 3 yolk proteins from the ovaries (fig.1A). These 3 yolk proteins present in the ovaries and hemolymph

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of mature females are, however, lacking from the hemolymph of both immature females (fig.1C) and mature males (fig.1E).

After injection of radioactive amino acids, labeled vitellogenins can be detected in the hemolymph. The relative rate of vitellogenin synthesis can be judged by comparing fig.1B,D–G, I. The hemolymph of day 1 females contains, besides vitellogenins, several

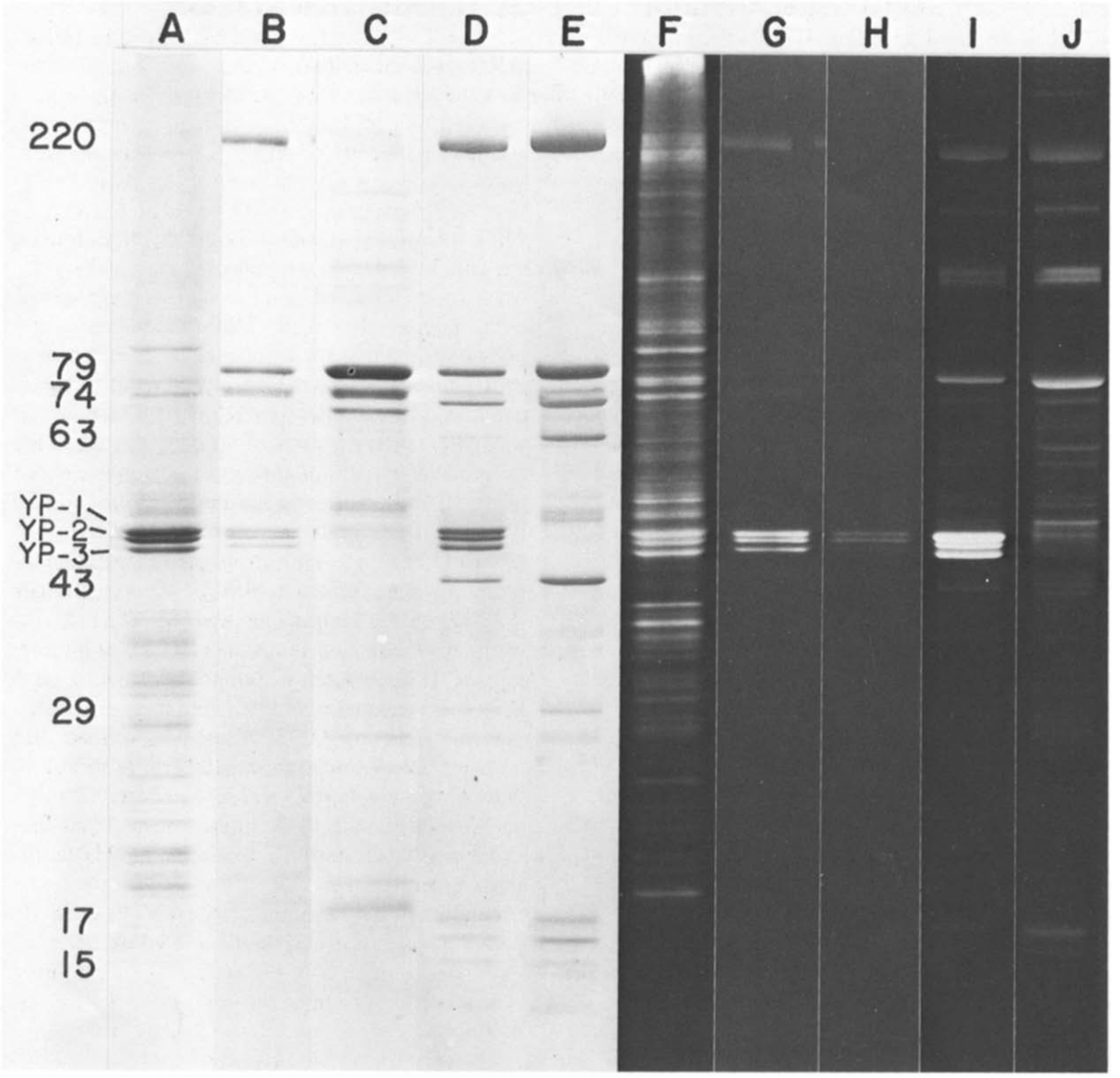

Fig.1. Identification of *Drosophila* vitellogenins by polyacrylamide gel electrophoresis. Samples A,F: 4 ovaries from 24 h females labeled 2 h in vivo with [^{3}H]valine. Samples B,G: hemolymph from ten 24 h females labeled 2 h in vivo with [^{3}H]valine. Samples C,H: hemolymph from ten 2 h females labeled 2 h in vivo with [^{3}H]valine. Samples D,I: hemolymph from 24 h females labeled 2 h in vivo with [^{35}S]methionine. Samples E,J: hemolymph from ten 24 h males labeled 2 h in vivo with [^{35}S]methionine. A–E are Coomassie blue-stained gels and F–J are autoradiograms or fluorograms, respectively. Numbers along the left side indicate approximate molecular weight of hemolymph proteins in daltons, × 10^3. Vitellogenins are marked as YP (yolk proteins).

other proteins present in equal or greater amounts (fig.1B,D). However, 2 h after injection of radioactive amino acids much more label was incorporated into the vitellogenins than into the other hemolymph components (fig.1G,I). Synthesis of labeled vitellogenins was low in newly eclosed females and was limited to YP-1 and YP-2 (fig.1H). Vitellogenin synthesis was not detectable in males (fig.1J). It is assumed that newly synthesized vitellogenin molecules are rapidly removed from the hemolymph and appear in the ovaries. In fact it could be shown in these experiments that 2 h after the injection of [^{3}H]valine the yolk proteins in the ovary were heavily labeled (fig.1F).

3.2. *Synthesis of vitellogenins in organ culture*

Vitellogenin has been shown [13] to arise from the fat body of several species of *Drosophila*, but their analysis could not resolve the 3 yolk protein species. We have cultured fat body attached to body walls in vitro to check whether all 3 yolk proteins are synthesized and secreted into the medium. Figure 2F shows that cultured body walls did in fact secrete all three vitellogenins. Labeled vitellogenins were detected only in the medium, and not in fat body cells (fig.2F,G), which suggests that these proteins are not stored to any extent.

3.3. *Synthesis of vitellogenins in a cell-free system*

Having investigated the synthesis of vitellogenins in vivo and in culture, we were ready to test the ability of fly RNA to direct the synthesis of yolk proteins in a cell-free translation system. Figure 2 (C,D) shows the proteins that were translated in vitro under the direction of poly(A)-containing RNA obtained from complete females or males, respectively. As can be seen, most of the products formed by translating female or male mRNA corresponded to each other. However, there is a striking difference in the vitellogenin region. Only with female mRNA, two bands with mobilities equivalent to those of YP-1 and YP-2 were made. A band corresponding to YP-3 was missing in both instances. A few of the radioactive proteins translated from whole body female or male mRNA were also present in ovaries labeled in vivo. This indicates that *Drosophila* mRNAs are translated with a high degree of fidelity in the wheat germ system.

Since vitellogenins are synthesized and secreted rapidly by the fat body (as shown above), we isolated RNA from female body walls and tested it in the cell-free system. Figure 2E shows that, except for a low molecular weight band that also occurs in controls with no fly RNA added (fig.2H), species with mobilities corresponding to YP-1 and YP-2 are more intensely labeled than any other band. As was found with whole female mRNA, the translation of body wall mRMA also yields very little product at the location of YP-3. The fidelity of the wheat germ translation system is again demonstrated by the synthesis of a protein of ~60 000 mol. wt which is also heavily labeled in intact fat body cells (fig.2G).

4. Discussion

The results of these experiments show that the 3 vitellogenin species are the most rapidly labeled proteins to appear in the hemolymph of mature females, but that the synthesis and secretion of vitellogenins occur at a low rate in freshly eclosed females and probably not at all in males. The rapid increase in rate of vitellogenin synthesis shown here to take place after eclosion can be blocked if abdomens of young females are isolated from the head and thorax. Vitellogenin synthesis reappears if these preparations are treated with either ecdysterone or juvenile hormone [5].

The 3 species of vitellogenin are also major secretory products of fat body maintained in organ culture. Since labeled vitellogenin can only be detected in the medium but not in the cells, it can be concluded that vitellogenin is not stored, but secreted soon after synthesis.

Finally, it is demonstrated that the translation of either poly(A)-containing RNA from whole females or total RNA from body walls in a cell-free system yields products that migrate upon electrophoresis as vitellogenins. But to our surprise, 2 rather than 3 radioactive components comigrating with authentic vitellogenins were detected. The translation of poly(A)-containing RNA from male flies yielded many components with mobilities and intensities equal to those obtained from female RNA, and yet little product appeared at the vitellogenin position. This result is consistent with the observation that males do not syn-

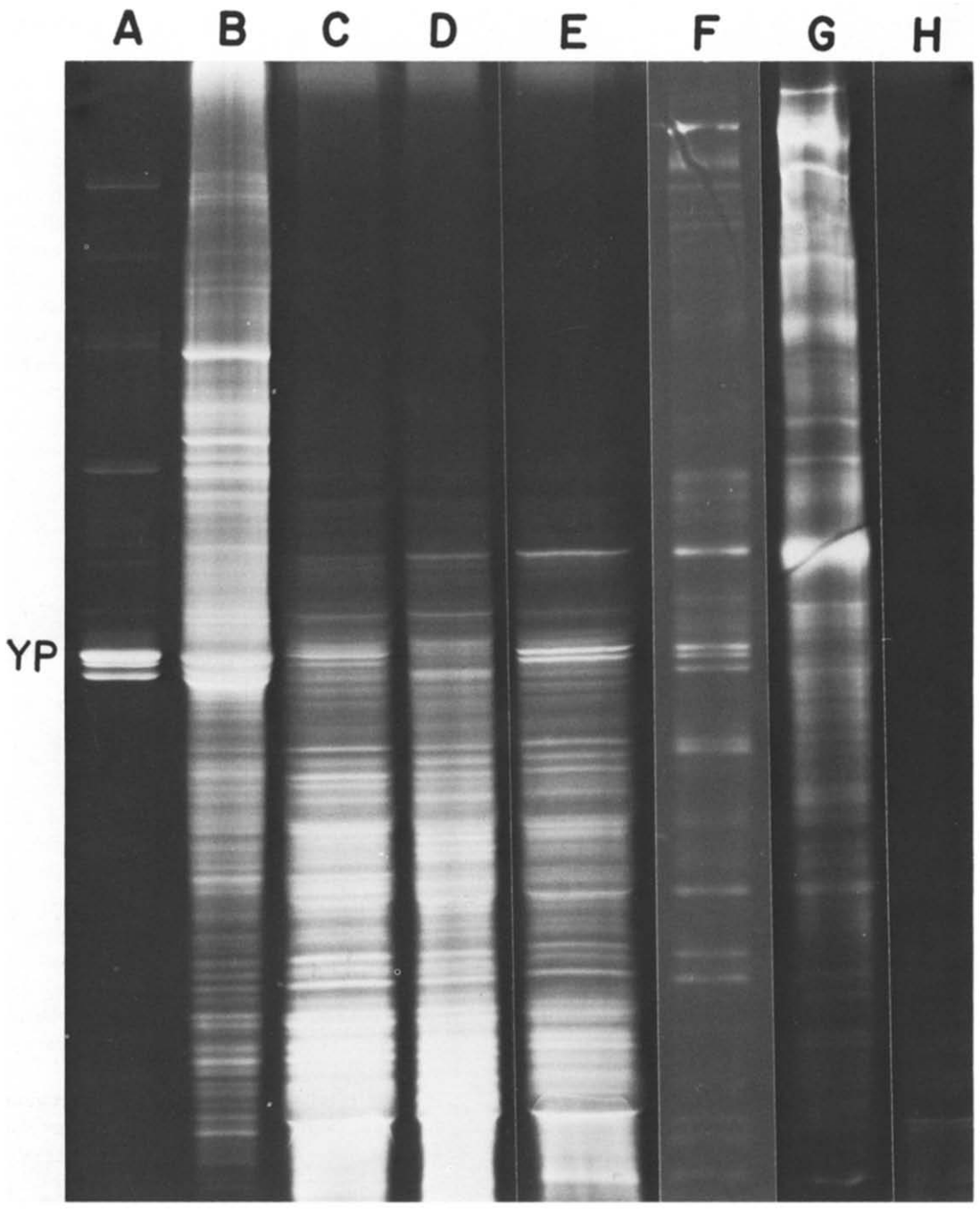

Fig.2. Electrophoretic analysis of *Drosophila* proteins synthesized in culture and in the cell-free translation system. Lanes A and B are references containing hemolymph (A) and ovaries (B) from mature female flies labeled in vivo for 2 h. Sample C: translate of poly(A)-containing whole female RNA. Sample D: translate of poly(A)-containing whole male RNA. Sample E: translate of total body wall RNA. Sample F: medium from body walls in organ culture. Sample G: body wall cells labeled in organ culture. Sample H: endogenous translation of the wheat germ system with no fly RNA added. YP indicates the vitellogenins. All samples were labeled with [^{35}S]methionine, except for the body wall preparations (lanes F,G) which were labeled with [^{3}H]leucine. The cell-free translation system contained 0.05–0.1 A_{260} units of poly(A)-containing RNA or 0.5 A_{260} units of total fat body RNA/assay.

thesize and secrete vitellogenins into the hemolymph.

Two main points of interest emerge from these observations:

1. Products with mobilities of only the 2 larger species of vitellogenin are observed among the cell-free translation products, while one corresponding to the smallest is either absent or made in much lower quantities. This would occur if the mRNA for YP-3 were read very poorly in the wheat germ system. However, since so many other in vitro translation products comigrate with *Drosophila* proteins made by ovaries in vivo, we consider this a remote possibility. Alternatively, the smallest vitellogenin may be derived from a precursor polypeptide with a mobility different from that of the product found in the hemolymph. This seems more likely since it is well known that the cell-free translation system from wheat germ does not catalyze post-translational cleavages of precursor proteins.
2. Mobilities of 2 of the major products synthesized in the cell-free system exactly coincide with those of the 2 larger vitellogenins. Since these are secreted proteins, the cell-free translation product would be expected to contain the amino-terminal extension found for a large number of other secreted proteins, including 1 insect polypeptide [14]. These presecretory polypeptides are 2000–3000 daltons larger than their final product, a difference which would have been easily detected on our gels. Although other interpretations are possible, this raises the intriguing possibility that these 2 *Drosophila* vitellogenins are not synthesized via a presecretory precursor. This situation has so far been demonstrated for only 1 secretory protein, hen ovalbumin [15]. A structural comparison of vitellogenins made in vivo and in the cell-free system should allow us to decide whether a similar situation exists for these *Drosophila* proteins.

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