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Genetic and Endocrine Regulation of Vitellogenesis in *Drosophila*[1]

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SYNOPSIS. Three yolk polypeptides (YPs) are major constituents of eggs. YPs are synthesized in the fat body and in ovary associated cells, secreted into the blood and sequestered into developing oocytes. YPs are translated as precursors which can be processed to lower molecular weight polypeptides by dog pancreatic microsomes, suggesting signal peptide removal. YP synthesis in both ovary and fat body is stimulated by juvenile hormone (JH), but 20-hydroxyecdysone (20HE) only stimulates YP synthesis in fat body. JH also induces YP sequestration into oocytes. The amount of RNA translatable into YPs increases sevenfold in the first day after eclosion. The increase can be blocked by ligating the abdomen to isolate it from anterior endocrine organs, and it can be restored by injecting these preparations with 20HE. Using electrophoretic variants, the YPs have been genetically mapped to the X chromosome: *Yp1* and *Yp2* are adjacent and *Yp3* is distantly linked. Two mutants have been described which decrease the quantity of a single YP, map near the respective YP structural loci, are *cis*-acting and are not ovary autonomous in transplants. *fs(1)1163* alters the structure of the primary translation product and is hypothesized to alter YP processing and secretion. Although $Yp3^{RI}$ results in no detectable YP3, the mutant genome and mutant RNA contains sequences complimentary to cloned *Yp3* gene. This mutant may result in blocked translation. Further analysis of *Drosophila* vitellogenesis using molecular and classical genetic techniques promises to help us understand how hormones regulate gene activity.

INTRODUCTION

Hormonal control of oogenesis

People invest nearly two decades in providing sustenance and instruction to their offspring, but insects often spend only a few days in equipping their eggs with nutritive reserves and developmental instructions sufficient to sustain life and program development. The process whereby yolk and developmental information are packaged in silkmoth eggs has been shown by Carroll Williams to involve a programmed sequence triggered during metamorphosis by the action of the same endocrine organs which regulate morphogenesis (Williams, 1952). Once this process is triggered, the development of oocytes in the silkmoth is autonomous as illustrated by Williams' classical experiment: A silkmoth abdomen from which the head and thorax had been removed as a diapausing pupa and implanted with prothoracic glands, nevertheless, underwent normal oogenesis. In many insects, however, egg production does not occur as a programmed step in metamorphosis, but rather requires additional hormonal input after adult emergence (for review see Hagedorn and Kunkel, 1979). Since hormone action in development causes changes in gene activity, it is helpful to study an insect whose genetics can be manipulated readily, such as the fruit fly *Drosophila melanogaster*. The following review summarizes our current understanding of the hormonal and genetic regulation of yolk formation, or vitellogenesis, in *Drosophila*. Earlier reviews have appeared on the ultrastructural (King, 1970), genetic (King and Mohler, 1975), and developmental (Kambysellis, 1977) aspects of *Drosophila* oogenesis.

BIOLOGY AND STRUCTURE OF THE YOLK POLYPEPTIDES

Tissue distribution of yolk polypeptides

Yolk polypeptides (YPs) are the major constituents of oviposited eggs and mature oocytes detected after sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) (Gelti-Douka *et al.*, 1973, 1974; Gavin and Williamson, 1975, 1976*a*; Kambysellis, 1977; Bownes and Hames,

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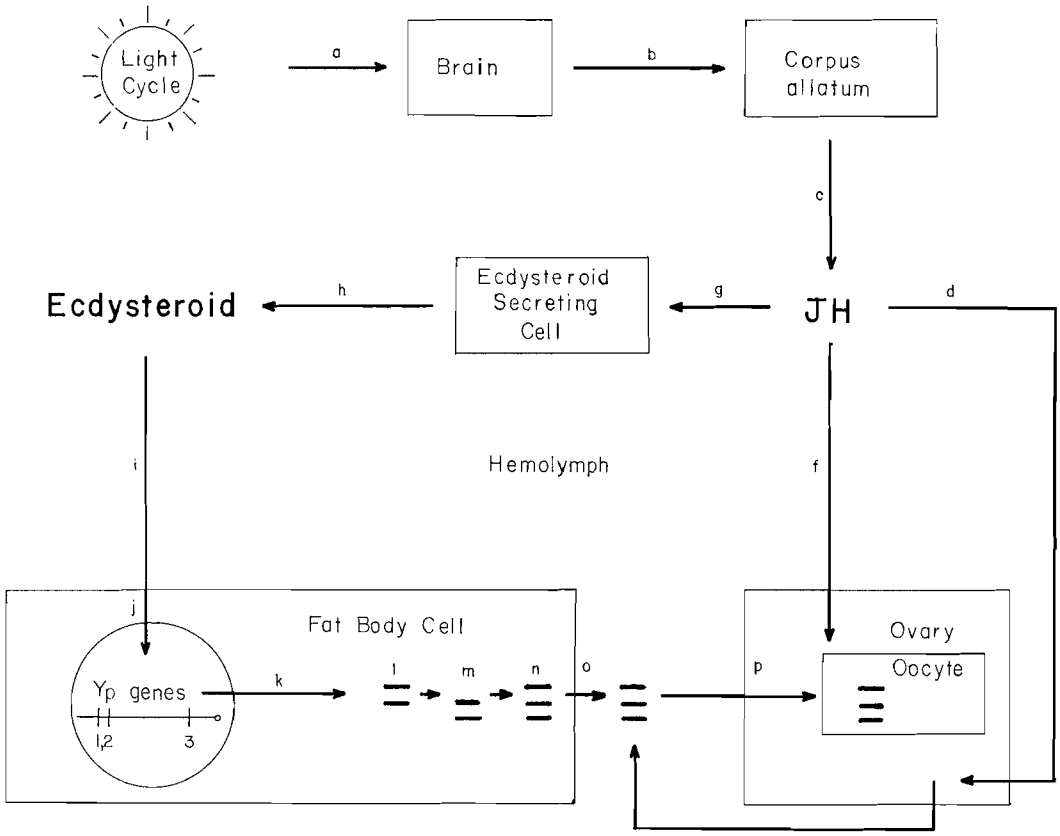

FIG. 1. Proposed endocrine pathway regulating *Drosophila* vitellogenesis. Circadian cues (a) trigger the brain to cause (b) the corpus allatum to release JH (c), which in turn induces (d) YP synthesis and secretion in ovarian cells (e), YP uptake (f) from the hemolymph, and (g) the secretion of an ecdysteroid from an unknown cell type in female abdomens (h) (possibly oenocytes). The ecdysteroid interacts (i) with receptors on and/or in (j) fat body cells to increase (k) the rate of transcription or translation of precursors to the YPs (l), from which a signal peptide is removed (m) and further modifications occur to YP1 (n) prior to secretion into the blood (o) and sequestration (p) into oocytes.

1977) (See Fig. 2). Of the other organs which have been tested, only hemolymph (Fig. 2) has been shown to contain large quantities of YPs (Gavin and Williamson, 1975; Bownes and Hames, 1977; Kambysellis, 1977; Postlethwait and Kaschnitz, 1978). The yolk peptides, called YP1, YP2, and YP3, have apparent molecular weights of about 47, 46, and 45 thousand daltons, respectively (Bownes and Hames, 1977; Postlethwait and Kaschnitz, 1978; Warren and Mahowald, 1979), and they combine in oocytes to form a native protein of high molecular weight (Gelti-Douka *et al.*, 1974; Gavin and Williamson, 1976*a*).

Peptide mapping studies using staphylococcal V8 protease indicate that each YP is structurally distinct, although the YP1 and YP2 peptide maps are somewhat similar after chymotrypsin digestion (Bownes and Hames, 1978*b*; Warren and Mahowald, 1979; Postlethwait, 1980). YP1 and YP2 also share some immunogenic determinants (Warren and Mahowald, 1979), suggesting that, although the YPs are not related in a precursor/product fashion, they may share a common evolutionary origin. Comparison of closely related *Drosophila* species has shown variability in the number and mobility of YPs in SDS-PAGE, suggesting that the YPs have evolved rapidly (Kambysellis, 1977; Srdić *et al.*, 1978; Bownes, 1980*a*; Postlethwait, 1980).

Origin of ovarian YPs

To test whether ovarian YPs are sequestered from the hemolymph, immature ovaries were transplanted from one stock of flies into hosts which had genetically altered YP electrophoretic mobility, using either different *Drosophila* species (Srdić *et al.*, 1979; Bownes, 1980*a*) or genetic variants of *D. melanogaster* (Postlethwait and Jowett, 1980*a*). When the implanted ovaries matured, they generally contained YPs characteristic of the host genotype rather than their own genotype, proving that the developing oocyte sequesters YPs from the hemolymph (Fig. 1p). Sequestration by the oocyte is accomplished by pinocytosis (Mahowald, 1972; Giorgi and Jacob, 1977).

The source of the YPs in the hemolymph has been studied by both ovary transplantations and organ culture. In the interspecific transplantations cited above, most combinations resulted in YPs of host origin being found in the donor oocytes, showing that a major source of YPs is outside the ovary. Organ culture experiments have proven this source to be the fat body (Gelti-Douka *et al.*, 1973; Hames and Bownes, 1978; Postlethwait and Kaschnitz, 1978) (Fig. 1o). However, some transplantation results suggested that the fat body is not the sole source of YPs. When nonvitellogenic ovaries of *D. melanogaster,* carefully cleaned free of any adhering fat body, were implanted into *D. virilis* the donor oocytes failed to mature unless the host was treated with a juvenile hormone analogue (JHA). When the YPs of the *melanogaster* donor oocytes from hormone treated *virilis* hosts were analzyed, they were found to be characteristic of the donor rather than the host (Postlethwait, unpublished; cited in Srdić *et al.*, 1979), suggesting that cells associated with the ovary can synthesize YPs under these conditions (Fig. 1e). This conclusion was tested further by implanting ovaries into male hosts which were genetically different with respect to YP electrophoretic mobility, using either different *Drosophila* species (Srdić *et al.*, 1979; Bownes, 1980*a*) or YP electrophoretic variants within *D. melanogaster* (Postlethwait and Jowett, 1980*b*). The results showed that oocytes which matured in males contained YPs characteristic of the donor ovary rather than the male host. When ovaries cultured in males and the host male fat body were tested in organ culture, it was found that the ovaries, but not the host male fat body, were capable of incorporating [^{35}S]methionine into newly synthesized YPs (Postlethwait *et al.*, 1980*a*). Since both fat body and ovaries can secrete newly labeled YPs in organ culture, but other organs do not (Bownes, 1980*b*; Bownes and Hames, 1978*b*; Postlethwait and Kaschnitz, 1978) it is concluded that the YPs have a dual origin in *Drosophila.* This situation contrasts with other insects, where only the fat body has been shown to synthesize yolk protein precursors (Hagedorn and Kunkel, 1979). The cells associated with *Drosophila* ovary which synthesize YPs have not yet been identified.

YP biosynthesis

The vitellogenins of many insects and egg laying vertebrates are high molecular weight precursors for smaller peptides found in the yolk (Tata and Smith, 1979; Hagedorn and Kunkel, 1979). To test whether *Drosophila* YPs are synthesized in precursor forms, mRNA was translated in a cell free system. Message from sexually mature females, but not from males, directs the synthesis of two bands comigrating with YP1 and YP2 (Bownes and Hames, 1978*b;* Postlethwait and Kaschnitz, 1978). Warren *et al.* (1979) showed that the upper band contains precursors to YP1 and YP2, and the lower band is a precursor to YP3 (Fig. 1*l*). Post-translational modifications of the two precursor bands initially result in a reduction of the apparent molecular weight by 1,000 daltons (Fig. 1m). To test whether this shift in mobility of YP precursors in SDS-PAGE is related to cleavage of a signal peptide found in most secretory proteins (Blobel and Dobberstein, 1975), we have translated message for YPs in the reticulocyte cell-free translation system in the presence and absence of dog pancreas microsomes, a preparation which contains the signal peptidase (Shields and Blobel, 1978). Our experiments showed (Fig. 3) that in the presence of microsomes, the two primary translate

bands were processed to smaller polypeptides comigrating with YP2 and YP3 (Fig. 1m) (Kaschnitz and Postlethwait, unpublished). Similar results have been obtained by Barnett *et al.* (1980). These results show that a signal peptide is removed from the pre-YPs before secretion, thus verifying the earlier suggestion by Warren *et al.* (1979). Since the cleaved peptide is only about 1,000 daltons—half as big as most signal peptides—it may have a structure that deviates significantly from most signal peptides. We therefore propose to call the extension the "Warren piece," with apologies to Tolstoy.

Besides the peptide cleavage discussed above, YP1 was found to undergo a second processing event (Warren *et al.*, 1979). After cleavage of the Warren piece, YP1 undergoes an alteration which decreases its mobility in SDS-PAGE by an amount corresponding to about a thousand daltons (Fig. 1n). The nature of this change is yet to be determined.

ENDOCRINE REGULATION OF VITELLOGENESIS

Control of vitellogenesis

When a fly ecloses there are no yolky oocytes in the ovary, and YPs in the hemolymph are barely detectable, although YP synthesis has already begun (Postlethwait and Kaschnitz, 1978) (Fig. 2). The hormonal events initiating vitellogenesis are synchronized not with respect to the completion of adult development, but rather with respect to eclosion (Handler and Postlethwait, 1977), an event programmed by photoperiodic cues (Fig. 1a) (Pittendrigh, 1954). The photoperiodic signals are apparently mediated by the brain (Fig. 1b), since decapitation before 10 min after eclosion prohibits vitellogenesis (Handler and Postlethwait, 1977). Removal of both the head and thorax from the abdomen before 16 hr after eclosion prevents vitellogenesis (Handler and Postlethwait, 1977), suggesting that a thoracic organ mediates the effect of the brain. Since a juvenile hormone analogue (JHA) can overcome both decapitation and abdominal isolation, and since either surgical (Bouletreau-Merle, 1974) or chemical (Landers and Happ, 1980) removal of the corpus allatum (the source of juvenile hormone in flies [Wigglesworth, 1964]) inhibits vitellogenesis, the brain probably acts (Fig. 1b) to trigger juvenile hormone release from the corpus allatum (Fig. 1c), which in turn stimulates vitellogenesis.

Control of YP synthesis

During the first 24 hr after eclosion, the rate of incorporation of [^{35}S]methionine into YPs increases by a factor of at least six (Jowett and Postlethwait, 1980), and YP concentration increases to almost a third of the hemolymph protein (Gavin and Williamson, 1976*a*). Abdominal isolation at eclosion inhibits the increase in the rate of YP labeling (Handler and Postlethwait, 1978; Postlethwait and Handler, 1979; Jowett and Postlethwait, 1980). This inhibition can be overcome by topical treatments with JHA or injections of 20-hydroxyecdysone (20HE). The final concentration of 20HE required is about 5×10^{-7} *M* (Postlethwait and Handler, 1979; Jowett and Postlethwait, 1980), which is near to physiological concentrations (Garen *et al.*, 1977; Hodgetts *et al.*, 1977). The detection of an increase in YP labeling within 2 hr after hormone treatment (Jowett and Postlethwait, 1980) suggests that transcription or translation are altered by the hormone rather than cell growth. We have tested whether the increase in YP synthesis which occurs in the first day after eclosion is associated with a change in the levels of translatable YP mRNA (Fig. 3) (Postlethwait and Kaschnitz, unpublished). Densitometric scans of the autoradiographs normalized to the amount of total RNA translated show that the translatable YP mRNA increased 7.6-fold in the first day after eclosion. Isolating the abdomens from the anterior endocrine organs at eclosion blocks this increase as shown by comparing the amount of translatable YP mRNA in 24-hr-old isolated abdomens with that in freshly eclosed flies (Fig. 3, isolated abdomen translatable YP: freshly eclosed fly translatable YP = 0.96). If abdomens isolated at eclosion are treated with 10^{-4} *M* 20HE at 18 hr and then extracted for RNA after 24 hr, there is a specific increase in the amount of translat-

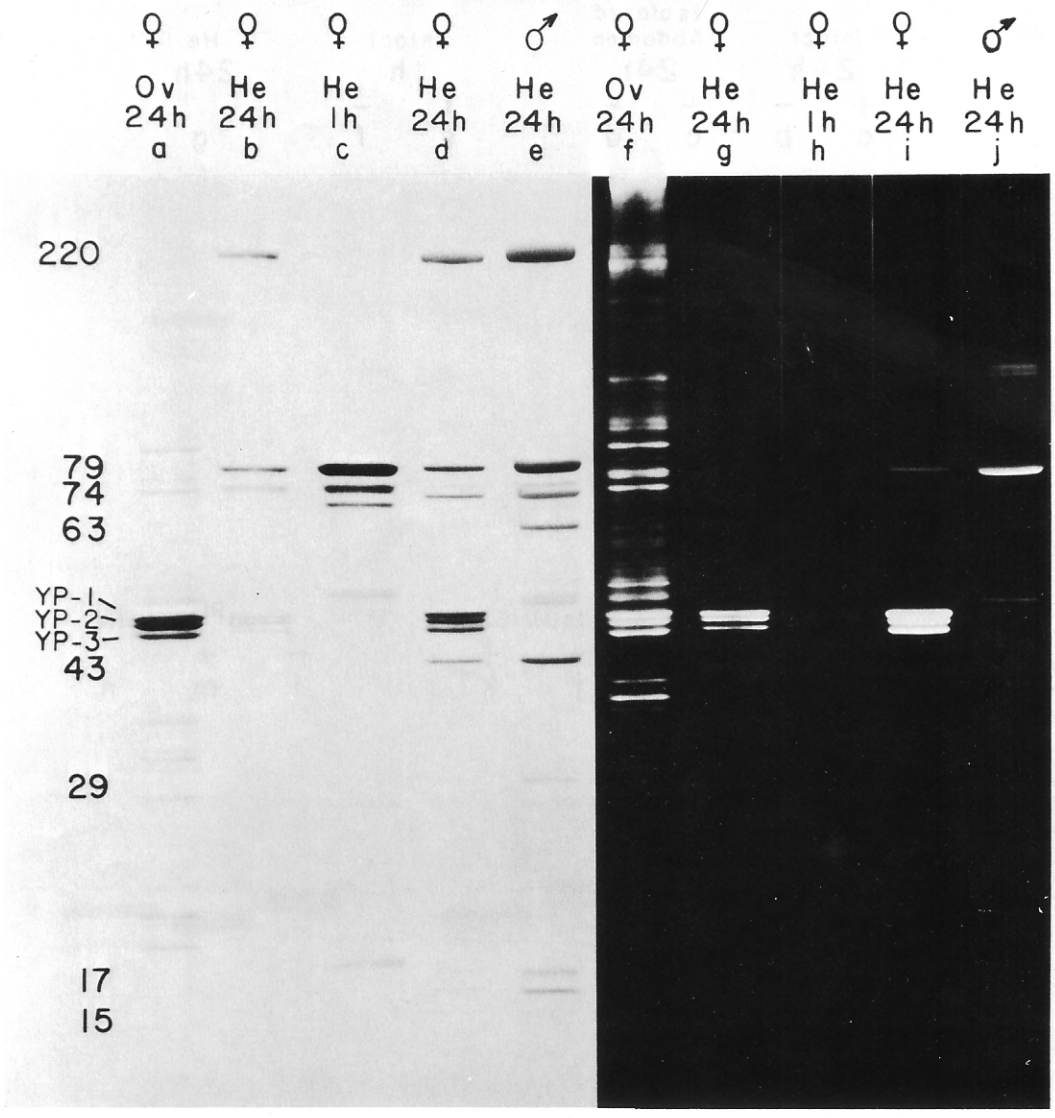

FIG. 2. Synthesis of *Drosophila* YPs. The most prominent bands from ovaries (a) after SDS-PAGE stained with coomassie blue are the YPs. The YPs are also prominent in the blood of females 24-hr-old (b and d), although they are minor species in blood from 2-hr-old females (c) and are not detectable in males (e). Autofluorography shows that labeling of YPs over a two hour incubation period with [^{3}H]valine is about six times as great in 24-hr-old (g) as 2-hr-old females (h).

able RNA coding for polypeptides which comigrate with the YP precursors and a 79,000 dalton polypeptide (Fig. 6) (Minoo and Postlethwait, unpublished). The 79,000 band also becomes heavily labeled with [^{35}S]methionine in the hemolymph of isolated abdomens treated with 20HE (Jowett and Postlethwait, 1980). From these data we conclude that hormonal stimulation of YP synthesis is due to an increase in the amount of stable translatable YP mRNA. Vertebrate steroid hormones have also been shown to act by increasing the rate at which stable mRNA appears in cells, probably by increasing the rate of transcription (Rosen and O'Malley, 1975; Tata and

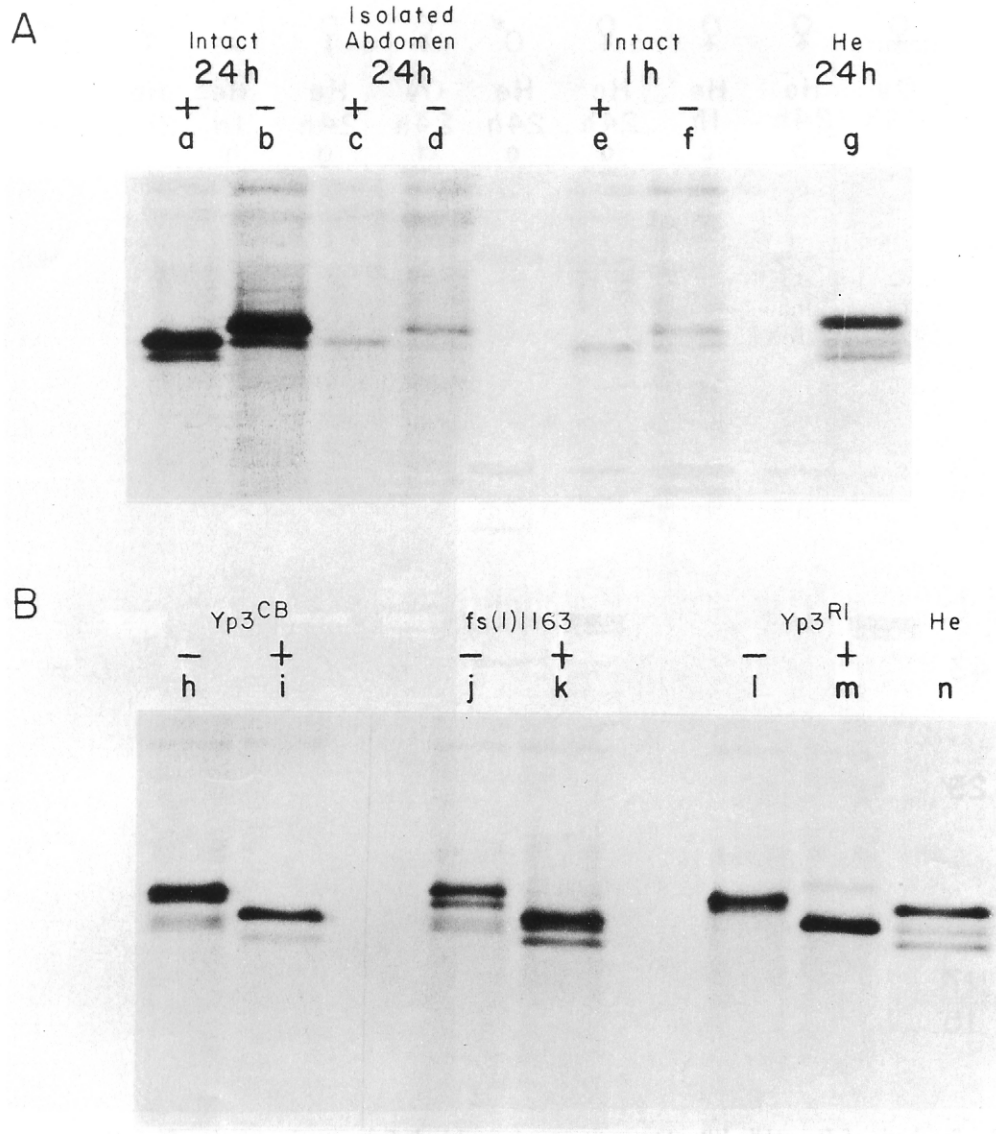

FIG. 3. Translation of mutant and wild type RNAs in the reticulocyte cell free translation system in the presence (+) and absence (−) of dog pancreas microsomes. (A) Developmental regulation of the levels of translatable YP message in Oregon R wild type females. RNA from 24-hr-old female abdomens (a and b) translates into two bands which are precipitable with anti-YP serum and migrate approximately with YP1 and YP2 (g). Microsomes (a) process the primary translates (b) to lower molecular weight products. One-hour-old abdomens (f and e) contain $^1/_7$th as much translatable message as mature flies, and this developmental increase is prevented by isolating the abdomens from anterior endocrine glands at eclosion and extracting RNA 24-hr later (c and d). (B) Translates from a Coos Bay wild stock (h and i) are compared to translates from *fs(1)1163* message, which shows abnormal migration of one band, presumably YP1, and RI, which shows absence of any translatable YP3.

Smith, 1979; McKnight and Palmiter, 1979).

The interaction of JH and 20HE have been studied in mutants which apparently block the availability of either JH (*ap*^{4}, Postlethwait and Weiser, 1973; Gavin and Williamson, 1976*b*; Postlethwait and Handler, 1978; Postlethwait and Jones, 1978; Wilson, 1980) or ecdysteroids (*ecd*^{1}, Garen *et al.*, 1977). Postlethwait and Jowett (1980*b*) found that YP labeling was not diminished in *ecd*^{1} females, but was reduced 80% to 90% in *ap*^{4} females. In the double homozygote labeling was less than 2% of the double heterozygous control. This result confirms the experiments on wild type flies and suggests that in the absence of either JH or 20HE, YP synthesis can occur, but when both hormones are lacking, YP synthesis is blocked.

The finding that two hormones stimulate YP synthesis and two organs synthesize YPs raises the hypothesis that each hormone acts on a different organ. This was tested by treating isolated abdomens with neither, either, or both of the hormones, and after incubation testing whether the fat body or ovary was able to secrete newly labeled YPs during organ culture in the presence of [^{35}S] methionine. These experiments showed that the ovary responded to only JHA (Fig. 1d) while the fat body responded to both JHA and 20HE (Jowett and Postlethwait, 1980). These results are consistent with the explanation that JH acts directly on the ovary, but acts only indirectly on the fat body by provoking the release of an ecdysteroid from some abdominal tissue. Hormonal induction of YP synthesis by naive tissues in organ culture would help to resolve the ambiguities which have arisen from the use of intact animals, but these experiments have yet to be successful.

The relationship of the two hormones was further studied by testing whether they could stimulate YP synthesis in males (Postlethwait *et al.*, 1980*a*). Males treated with 20HE, but not JHA responded by secreting into the blood newly labeled polypeptides which comigrated with YPs and had peptide maps identical to authentic YPs (Postlethwait *et al.*, 1980*a*; Jowett and Postlethwait, 1980). This suggests that 20HE may be the proximal cause for YP synthesis in the fat body (Fig. 1i) and that JHA acts only by causing a female abdominal tissue (Fig. 1g) to secrete an ecdysteroid (Fig. 1h). Although in several insects, including *Drosophila,* the ovary has been found to contain or produce an ecdysteroid (Hagedorn *et al.*, 1975; Legay *et al.*, 1976; Lagueux *et al.*, 1977; Garen *et al.*, 1977; Goltzene *et al.*, 1978; Hsiao and Hsiao, 1979), it seems unlikely that the ovary is the source of the fat body stimulating factor since: (1) ovaries implanted into males do not induce YP synthesis by the male fat body but rather synthesize YPs themselves (Srdić *et al.*, 1979; Bownes, 1980*a*; Postlethwait *et al.*, 1980*a*; Postlethwait and Jowett, 1980*b*); (2) ovariectomy at eclosion does not prevent an increase in the rate of YP labeling, whereas isolating the abdomen from anterior endocrine organs does (Postlethwait *et al.*, 1980*b*); (3) a mutant with no germ cells and only a few abnormal ovarian mesodermal cells nevertheless produces YPs at normal rates (Postlethwait *et al.*, 1980*b*). Although it is unlikely that the ovaries provide a necessary link in stimulating YP synthesis by the fat body, abdominal oenocytes, which lie scattered among the fat cells of *Drosophila* (Rizki, 1978), have been shown to make 20HE in *Tenebrio* (Romer, 1971; Romer *et al.*, 1974). These cells may be the source of the fat body stimulating factor in *Drosophila.*

Control of YP sequestration

The uptake of YPs into oocytes begins several hours after YP synthesis has begun, suggesting that the two processes are independently controlled. The block to both YP synthesis and YP sequestration is overcome by JHA in isolated abdomens (Fig. 1f) but 20HE reverses only the lack of YP synthesis (Postlethwait and Handler, 1979; Jowett and Postlethwait, 1980). These results have been confirmed by studying flies of different genotypes. In some interspecific ovary transplants, JHA is necessary to cause yolk deposition in the implanted ovary (Srdić *et al.*, 1979; Bownes, 1980*a*, *b*). Genotypes with apparent defects in JH

secretion accumulate some YPs in the blood, but fail to deposit yolk unless treated with JHA or implanted with corpora allata (Postlethwait and Weiser, 1973; Kambysellis and Heed, 1974; Gavin and Williamson, 1976*b*; Postlethwait *et al.*, 1976; Kambysellis and Craddock, 1976; Kambysellis, 1977; Postlethwait and Handler, 1978).

The genetic and surgical experiments cited above suggest the conclusion that YP sequestration is controlled by JH but not 20HE. Nevertheless, those experiments do not prove that JH acts directly on the ovary, since whole animals or isolated abdomens were used. Giorgi (1979) has overcome that criticism by studying protein sequestration in oocytes during organ culture. He has found that pinocytosis in vitellogenic oocytes occurs only in the presence of JHA. This leads to the conclusion that JH acts directly on ovaries to stimulate YP sequestration (Fig. 1f).

Hypothesis

The data summarized in the previous sections suggest the hypothesis outlined in the legend to Figure 1. Several steps of the hypothetical scheme are unproven. (1) The cells in the brain responsible for the head signal have not been identified, nor is it known whether this signal is neural or humoral. (2) It is not proven that JH acts indirectly and 20HE directly on the fat body, since in all experiments whole animals or entire abdomens were used. (3) The identity of the postulated JH sensitive ecdysteroid secreting cells is in doubt. (4) The developmental schedule for JH and ecdysteroid titers has yet to be determined, as well as whether a causal relationship between JH and ecdysteroid titers exists. (5) The cells in the ovary which secrete YPs have not been identified. (6) The role of ecdysteroid in the ovary has yet to be elucidated. Besides these questions regarding the endocrine network, there remain more fundamental molecular problems of hormone action. How does 20HE act on a molecular level to increase the rate of YP synthesis and secretion? What happens between i and o in Figure 1? How does JH act on the follicle to promote YP sequestration? The answers to these questions are presently being pursued using both molecular cloning techniques and classical genetic and biochemical approaches.

GENETICS OF THE YPS

Genetic localization of the YPs

The advantage of *Drosophila* for investigating the mechanism of hormone action is that mutant genes can be used to perturb the system in ways that facilitate analysis. In order for this approach to be successful, the genes coding for the YPs must be identified. This allows a rational approach for the screening and isolation of mutations specifically affecting the structure or regulation of these polypeptides.

A screen of over two hundred wild and laboratory strains of *D. melanogaster* uncovered fertile electrophoretic variants for each of the YPs (Postlethwait and Jowett, 1980*a*, *b*). The YPs in the blood of each variant migrate with those in the variant ovary, proving that the mutants do not affect a post-translational modification occurring after YP sequestration. None of the variant genes affect more than one YP, indicating that each YP comes from a distinct gene (See also Bownes, 1979). Since these mobility variants are codominant and the quantity of polypeptide formed is dependent on gene dose, they probably represent the structural genes. The variants were all mapped to the X chromosome: *Yp1* and *Yp2* (the genes for the polypeptides YP1 and YP2) to locus 29 and *Yp3* to locus 44. Cytogenetic localization using existing duplications and deletions indicated that YP1 and YP2 reside in bands 9A1 to 9B1, and YP3 in bands 12A6 to 12D3.

The extensive collections of female sterile mutants generated by Gans *et al.* (1975) and Mohler (1977) include a number located near the *Yp* genes. These are currently being examined to test whether any affect yolk polypeptide structure or regulation.

YP1 and YP2 share several properties besides their genetic proximity (Postlethwait and Jowett, 1980*a*, *b*; Barnett *et al.*, 1980). (1) They have structural and antigenic characters in common (Warren and Mahowald, 1979); (2) they are both stimulated by JHA to a greater degree than

YP3 (Jowett and Postlethwait, 1980; Postlethwait and Jowett, 1980*b*); (3) they are synthesized earlier in development than YP3 (Postlethwait and Kaschnitz, 1978); and (4) they are utilized faster in embryogenesis than YP3 Bownes and Hames, 1977). These similarities suggest a common origin and regulation of the two genes.

Cloned Yp genes

Barnett *et al.* (1980) have recovered from a library of random *D. melanogaster* DNA fragments clones complementary to female, but not male, cDNA. These female specific clones were shown to contain sequences complimentary to the *Yp* genes since in hybridizations the clones selected only mRNAs which could be translated into the YPs. One class of clones they found contained the sequence for *Yp3* and the other contained those for both *Yp1* and *Yp2*. The restriction map shows only 800 base pairs between the *Yp1* and *Yp2* complementary areas proving that these two genes lie very close to each other. Quantitative investigations of the *Yp* genes showed that the *Drosophila* haploid genome contains only one copy of each gene. Radiolabeled *Yp* clones hybridized to salivary polytene chromosomes at 8F to 9A for *Yp1* and *Yp2*, and at 12B–C for *Yp3*, which are the same chromosomal locations shown to contain the *Yp* genes by standard genetic analysis. Since *Yp1* and *Yp2* are distantly located from *Yp3*, coordinate control of all three genes by 20HE must involve transcription initation at no fewer than two different sites.

Quantitative YP mutants

Models of gene regulation (*e.g.*, Britten and Davidson, 1969) suggest that regulation of transcription rates may be affected by sites closely linked to the structural genes which code for the regulated protein. Mutations at these sites might be expected to act only on the allele to which they are linked (be *cis*-acting) and to have altered rates of polypeptide synthesis. Several mutants have recently been found which have altered quantities of a single YP, and are thus candidates for *cis*-acting regulatory mutants.

Bownes and Hames (1978*a*) found that *fs(1)1163*, a female sterile mutation discovered by Gans *et al.* (1975), contains less YP1 than normal in both the hemolymph and the ovary. The mutation maps near the *Yp1* locus (M. Gans, personal communcation) and females heterozygous for *fs(1)1163* and *Df(1)C52*, a deletion of the *Yp1* gene, are sterile (Postlethwait and Jowett, 1980*b*). Since a precursor to mature YP1 comigrates with YP2 (Warren *et al.*, 1979), it is possible that the *fs(1)1163* defect results in some of the secreted YP1 comigrating with YP2. Therefore, we compared peptide maps of YPs from hemolympyhs of mutant and normal flies (Fig. 4), and found that no YP1 peptide fragments in *fs(1)1163* comigrated with YP2. In females heterozygous for the $Yp1^{LC}$ allele, which produces normal amounts of an electrophoretically fast form, and *fs(1)1163*, which produces very low quantities of an electrophoretically standard form, we found normal levels of $YP1^{LC}$, but low levels of the standard *YP1* (Fig. 4). This experiment shows that *fs(1)1163* is *cis*-acting: it does not exert its affect on the quantity of YP1 via a factor diffusible in the cell such as an altered processing enzyme. The ovaries of *fs(1)1163* females are able to sequester YPs normally, as demonstrated by transplanting ovaries between *fs(1)1163* females and females homozygous for an electrophoretic variant of *Yp1* (Fig. 4), thus extending and confirming the results of Bownes and Hames (1978*a*). As a first step to test whether *fs(1)1163* affects the level of Yp1 mRNA, we have translated total RNA from homozygous *fs(1)1163* flies (Kaschnitz and Postlethwait, unpublished) and have found an altered mobility pattern of immunoprecipitable YPs (Fig. 3), although quantities were about normal. This result suggests that the YP1 primary translation product in *fs(1)1163* is structurally abnormal. Such a structural abnormality could alter post-translational processing events (Warren *et al.*, 1979) so that secretion of YP1 is reduced, leading to decreased amounts of YP1 in the hemolymph and ovary. Although at this point *fs(1)1163* does not seem to affect hormonally contolled transcription, further study of this mutant may

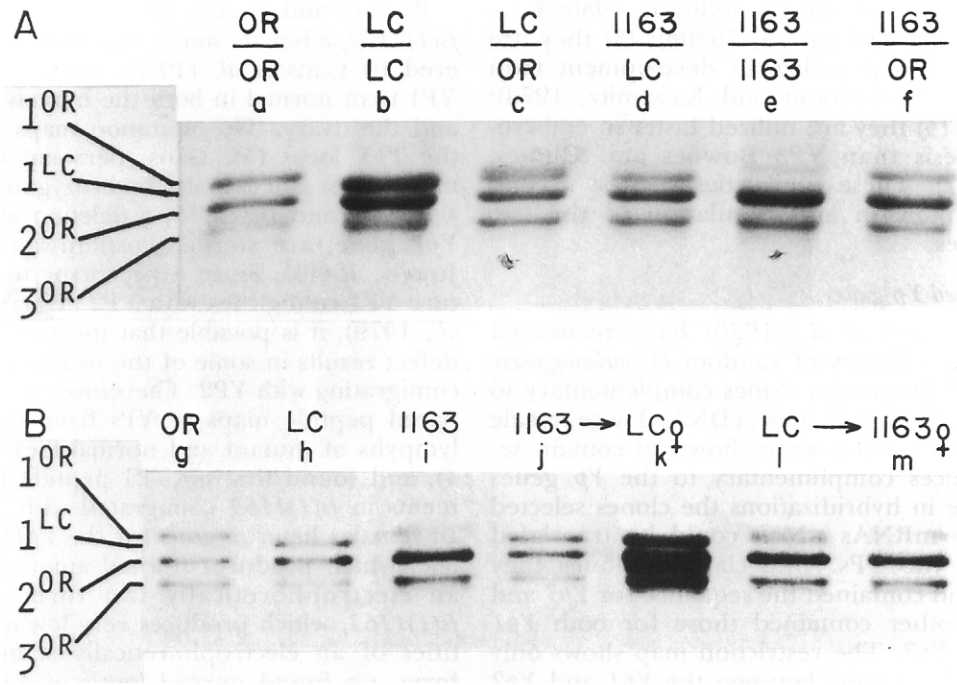

FIG. 4. The mutation *fs(1)1163* is *cis*-acting. A. YP1 from the standard wild stock OR (a) migrates more slowly in SDS-PAGE than LC (b). Both slow and fast YP1 are seen in the LC/OR heterozygote (c). Homozygous *fs(1)1163* flies (e) possess YP1 of slow mobility and low quantity. *LC/fs(1)1163* heterozygotes (d) have low amounts of slow YP1 but normal quantities of fast YP1. B. Ovaries from *fs(1)1163* flies (i) implanted into an LC host (h) sequester rapidly migrating LC YP1 (j) from the host (k).

help to elucidate some of the mechanisms regulating the process of polypeptide secretion.

We have extracted from a natural population of flies collected by Dr. Laurie-Ahlberg another candidate for a *cis*-acting regulatory variant. *RI* has no detectable YP3 in either the hemolymph or ovary. Peptide mapping showed that $YP3^{RI}$ is not comigrating with YP1 or YP2 (Fig. 5). Since *RI* females are not sterile, YP3 must not be required for fertility. The genetic factor responsible for the *RI* phenotype maps very near to the *Yp3* locus: among 49 recombinants between *v* (33.0) and *f* (56.7), none separated *RI* from *g* (locus 44.4) (Postlethwait, unpublished). Since the *Yp3* gene has not been separated from *g* among 79 recombinants between *v* and *f* (Postlethwait and Jowett, 1980*a*), *RI* must map very close to *Yp3*. In heterozygotes between *RI* and an electrophoretic variant of *Yp3*, *RI* was found to be *cis*-acting. After translation of RNA extracted from *RI* females in the reticulocyte cell free system (Kaschnitz and Postlethwait, unpublished), we found no peptides which migrated as a precursor to YP3 (Fig. 3), and so conclude that no translatable YP3 message exists in *RI* females. Next we tested whether RI was a deletion of the *Yp3* gene (Shirk and Postlethwait, unpublished), by separating restriction enzyme digested wild type and *RI* DNA on agarose gels, blotting the gels onto nitrocellulose paper (Southern, 1975), and probing the paper with labeled DNA prepared from the cloned *Yp3* gene (Barnett *et al.*, 1980). The results showed that sequences homologous to YP3 are present in the *RI* genome. We have also tested whether RNA transcripts complementary to the *Yp3* gene are present in *RI* by separating poly(A) RNA from wild type and *RI* flies on agarose gels, transferring the RNA to DBM paper, and probing with labeled

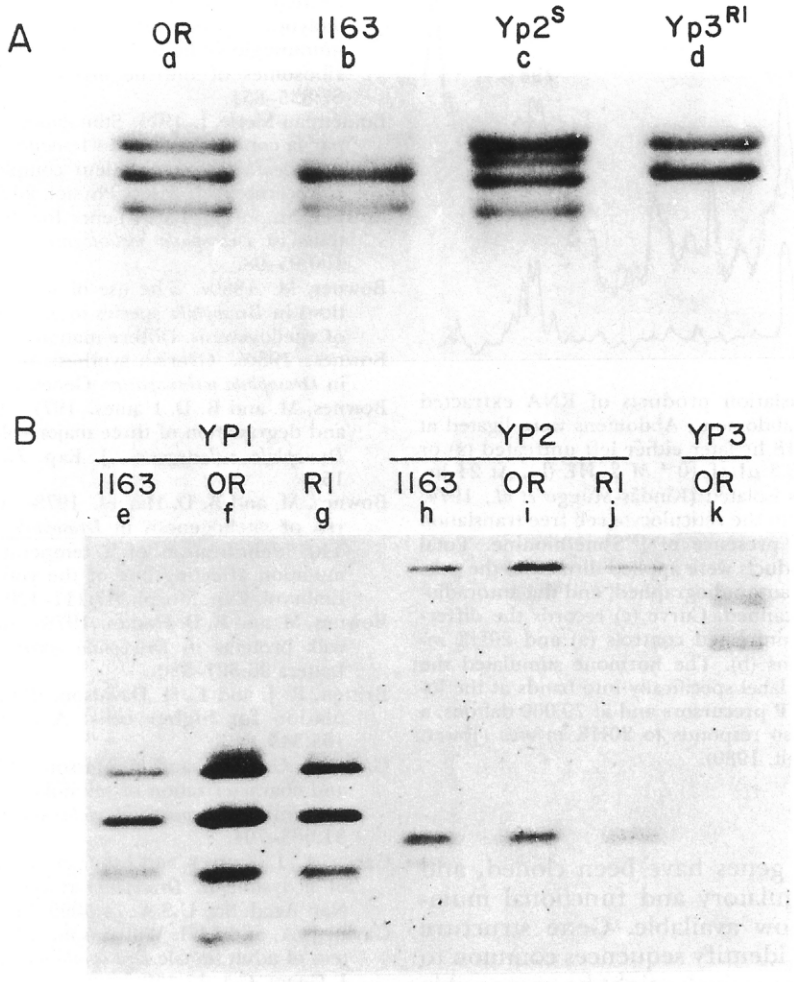

Fig. 5. Variant polypeptides do not comigrate with other YPs. A. Ovarian phenotypes of variants. *fs(1)1163* ovaries (b) contain less YP1 than normal (a), and *RI* ovaries contain no detectable YPs (d). Ovaries in a *D. simulans* heterozygote for a YP2 of slow mobility and low quantity and a YP2 of fast mobility and high quantity (c). B. Peptide mapping of YPs from variant flies. No peptide fragments of YP1 (f) are present in the YP2 band of *fs(1)1163* (h), and no peptides from YP3 (k) are present in the YP2 band of *fs(1)1163* (h), and no peptides from YP3 (k) are present in YP2 from RI (j).

DNA obtained from the cloned *Yp3* genes (Shirk and Postlethwait, unpublished). The results showed that transcripts which hybridize to the *Yp3* gene probe are present in normal quantities in *RI* females. We conclude that the *RI* mutation alters a post-transcriptional step in *Yp* gene expression. *RI* could be a nonsense mutant, or it could alter other sequences necessary for proper translation. Further work is rquired to identify the molecular defect in this mutant.

Prospects

Vitellogenesis in *Drosophila* provides a rare opportunity for the investigation of hormone action at a variety of levels. The hormonal regulators are identified, the structural genes are localized, mRNA has been isolated and translated in a cell free

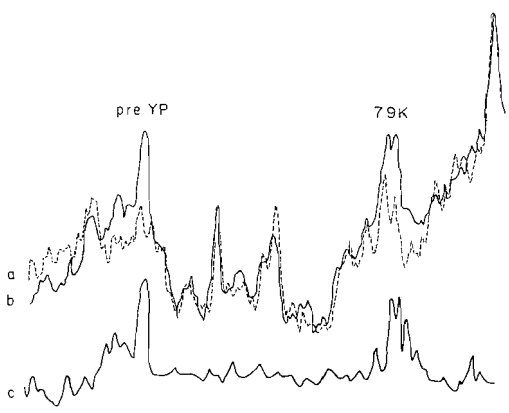

FIG. 6. Translation products of RNA extracted from isolated abdomens. Abdomens were ligated at eclosion, and 18 hr later either left untreated (a) or injected with 0.3 μl of 10^{-4} *M* 20HE (b). At 24 hr, total RNA was isolated (Kindås-Mügge *et al.*, 1974) and translated in the reticulocyte cell free translation system in the presence of [^{35}S]methionine. Total translation products were applied directly to the gels. The gels were autoradiographed, and the autoradiographs were scanned. Curve (c) records the difference between untreated controls (a) and 20HE injected abdomens (b). The hormone stimulated the appearance of label specifically into bands at the location of the YP precursors and at 79,000 daltons, a band which also responds to 20HE *in vivo* (Jowett and Postlethwait, 1980).

system, the genes have been cloned, and putative regulatory and functional mutations are now available. Gene structure studies may identify sequences common to all three genes which might be responsible for their coordinate control. An analysis of *Yp* clones mutated *in vitro* and transcribed in a cell free system (Sakonju *et al.*, 1980) may provide clear evidence regarding the location and nature of sequences necessary for transcription. The rare opportunity exists here also to compare *in vitro* results to the molecular phenotype and gene sequence of regulatory mutants produced *in vivo*. If hormonal induction of the *Yp* genes could be obtained in a cell free system, then dissection of that system would help reveal the regulatory steps involved in endocrine control of gene activity.

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