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# REGULATION OF VITELLOGENESIS IN DROSOPHILA[1]

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ABSTRACT Female fat body in Drosophila makes yolk polypeptides (YPs) but male fat body does not. We have tested whether hormonal factors are sufficient to trigger yolk polypeptide gene (Yp) expression. The quantity of Yp1 transcript was assayed by in situ hybridization to individual cells of gynandromorphs, mosaics of genetically male cells and genetically female cells. If hormones are sufficient, then all fat body cells in a gynandromorph should have the same level of expression of the Yp gene. We found, however, that some cells within a gynandromorph expressed a female level, while other adjacent cells had a male level of Yp transcript. We conclude that Yp expression is not regulated solely by circulating hormonal factors. To determine the effect of sex determination genes on the expression of Yps, we performed in situ hybridization to Yp transcripts in intersex (ix) and doublesex-dominant ($dsx^D$) mutant flies. We found that genetic female ix intersexes had reduced quantities of Yp1 transcript compared to heterozygous female controls, and genetic female $dsx^D$ intersexes had male levels. We conclude that sex determination genes establish cell sex, and hence whether individual cells can express Yps, and that hormones then allow phenotypically female cells to transcribe the genes.

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## INTRODUCTION

The two sexes make very different contributions to the next generation. While males donate a haploid nucleus and a trigger for development, females contribute not only a haploid nucleus, but also developmental information and stored nutrition in the form of yolk. An elaborate system of genetic controls ensures that the behavior, anatomy, and macromolecular synthesis of the two sexes are specialized to facilitate these two very different functions (1). A central question in biology is how genetic controls act to regulate sex. A useful marker for female sexual development is the production of yolk protein. Yolk polypeptides (YPs) in Drosophila females are synthesized by ovarian follicle cells and by fat body cells and secreted into the surroundings (for review, see (2,3). The developing oocyte sequesters YPs to form yolk. In contrast to female fat body, male fat body cells do not normally make yolk polypeptides even though the cells are cytologically indistinguishable in the two sexes. To help determine the mechanisms of sex differentiation, the work discussed here focuses on the question: What mechanisms cause female fat body cells to make YPs and prevents male fat body cells from producing YPs? To answer this general question we first review surgical experiments investigating the hormonal regulation of YP synthesis; second, we use sex mosaics (gynandromorphs) to ask whether these humoral factors are sufficient to evoke YP production; and third, we test the effect of the sex determination mutants ix and $dsx^D$ on the expression of the yolk genes. The results suggest that 20-hydroxyecdysone and the cell-autonomous action of sex determination genes are both necessary factors for a female level of Yp expression, although neither factor alone is sufficient to ensure proper expression of Yp genes.

## RESULTS

### What Humoral Factors Regulate Yp Gene Expression?

To determine if Yp expression can be controlled by factors circulating in the blood, fat body and ovarian follicle cells in the abdomen were separated from the endocrine glands present in the head and thorax of female

flies. These isolated abdomens were either left untreated or treated with the insect steroid hormone 20-hydroxyecdysone or an analogue of juvenile hormone. The results (4,5,6) showed that 20-hydroxyecdysone stimulated Yp expression in the fat body, and that juvenile hormone analogue stimulated Yp expression in the ovary. In addition, juvenile hormone was necessary to allow YPs to be taken into the oocyte.

These results suggested the hypothesis that male fat body cells do not make YPs because of a sex specific difference in hormone titers. This sex-specific-hormone proposition was tested in two ways. First, males were treated with hormones and their hemolymph was assayed for YPs, or their fat body cells were tested for transcripts from Yp genes (7,8,9). The results showed that male Drosophila could accumulate Yp transcript and YPs if given enough 20-hydroxyecdysone, but they failed to respond to juvenile hormone. For a strong response, however, males required about 100 times as much hormone as did isolated female abdomens, and the response lasted only a few days. Thus, these results showed that the same hormone that stimulates Yp genes in female fat body can also act on fat body cells in males. The results suggested that fat body cells in males do not make YPs either because they are less responsive to 20-hydroxyecdysone than fat body cells in females, or that they have lower levels of circulating hormone than females.

A test to help distinguish between these possibilites is the assay of ecdysteroid titers in adult males and females. Two such studies (10,11) found little difference between males and females, and a third investigation reported about 20 fold excess of ecdysteroids in females (12). But even if a sex specific difference in ecdysteroid concentration exists, neither the experiments with hormone-treated males nor the assays of ecdysteroid content can prove that a hormonal difference actually causes the difference in gene expression. Is the sex specificity of Yp expression caused by any possible difference in hormones between the sexes?

Does a Sex Specific Humoral Factor Regulate Yp Expression?

One hypothesis for regulation of Yp genes is that females possess a humoral activator of the Yps and/or males have a humoral repressor. The alternative possibility is that Yp genes in each cell are autonomously regulated by factors intrinsic to the genetic sex of the individual cell. The humoral hypothesis predicts that when male and female fat body cells are exposed to the same humoral environment, they will express the Yps to equivalent levels -- cells of both sexual genotypes will have a female level of Yp expression in a female hormonal environment, but a male level if bathed by male humoral factors. The hypothesis predicts an intermediate level of Yp expression if the humoral environment were a mixture of male and female humoral factors. The alternative cell-autonomous hypothesis predicts that male cells exposed to female hormones will nonetheless fail to express the Yp genes.

To test these predictions, we examined the level of Yp gene expression in individual fat body cells of animals that were part male and part female -- sex mosaics, or gynandromorphs. Since the normal female genotype is two X-chromosomes and two sets of autosomes, and the male genotype is only one X and two sets of autosomes, gynandromorphs arise when a nucleus in an early female embryo loses an X-chromosome. Such chromosome loss creates two populations of cells: some genetically female with two X-chromosomes, and some genetically male, with a single X. A special ring-X-chromosome facilitates gynandromorph construction since it spontaneously eliminates at high frequency from some early cleavage nuclei. Two-X and one-X cells can be distinguished in a gynandromorph's cuticle due to recessive marker mutations (yellow and split bristles and white eyes) carried on the non-ring-X-chromosome that remains in cells with a single X (13).

The level of Yp expression in sex mosaics was determined by in situ hybridization (14,15) to messenger RNA in fat body cells. Sex mosaics whose abdominal cuticle was part male and part female were placed between an XY male fly and an XX female fly as negative and positive controls and tissue sections were prepared. Sections were then hybridized to nick-translated probe homologous to Drosophila's Yp1 gene (16). The results (Fig. 1) showed that fat body cells in male controls had few silver grains, but

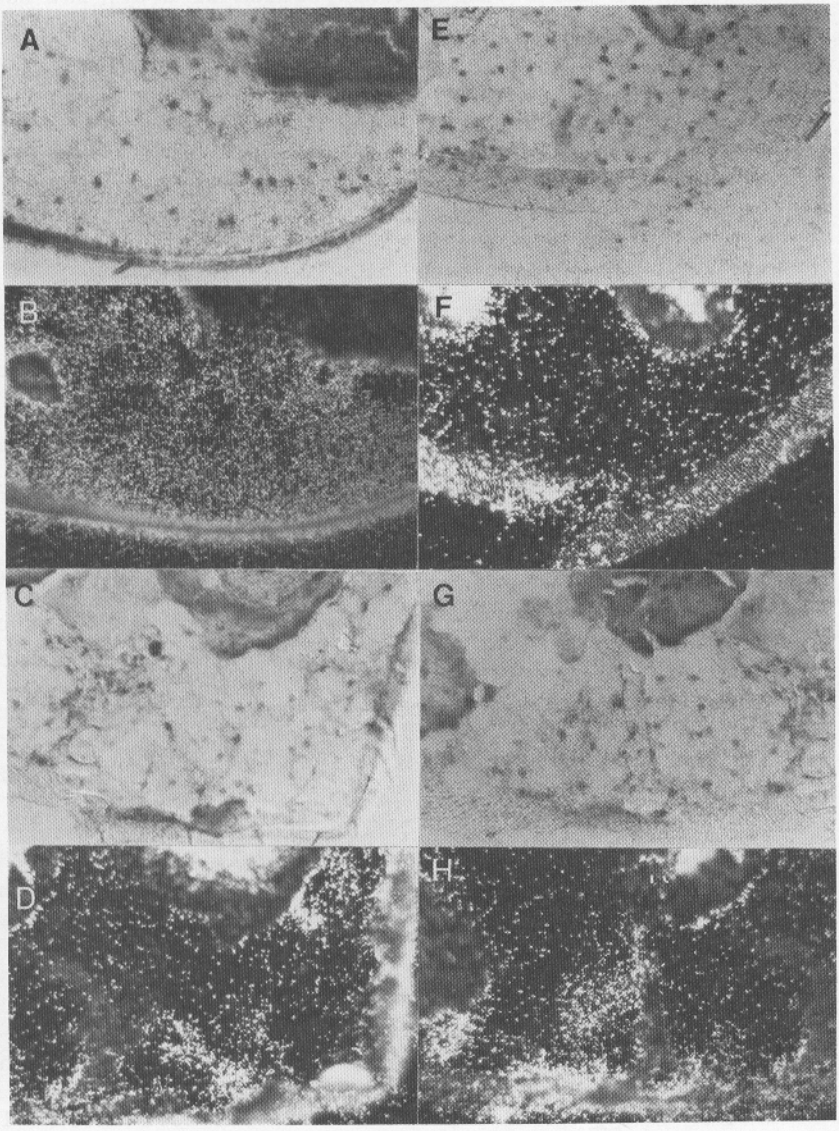


Fig. 1. Sex mosaics are also mosaic for Yp1 expression. A & B, Normal female. C & D, Gynandromorph. E & F, Normal male. G & H, Gynandromorph. A,C,E and G, bright field. B,D,F and H, dark field. Sections were hybridized to Yp1 probe.

female controls had a substantial portion of every fat body cell covered with silver grains (17), Some of the gynandromorphs examined, however, were not homogeneous -- they were mosaic for Yp expression, having some cells with a male level of expression and other cells with a female level. In these gynandromorphs, the demarcation between female-level and male-level expression was dramatic and adjacent cells could usually be unambiguously categorized as containing male or female amounts of Yp transcript. Thus, these sex mosaics were also mosaic for Yp expression.

The results show that individual fat body cells in sex mosaics autonomously accumulate either a male or a female amount of Yp transcript. Cell-autonomous differentiation of sex combs and genital structures is well documented in Drosophila (18,19) but this is the first time cell autonomy in gynandromorphs has been demonstrated at the level of a specific gene transcript. Since the same hemolymph bathed all of the fat body cells in each individual gynandromorph, there can be no circulating factors that alone are sufficient to activate or repress Yp genes in Drosophila fat body cells. We suggest from this result that each individual cell responds to 20-hydroxyecdysone or other circulating regulatory factors in a fashion consonant with its own sexual genotype.

The gynandromorphs were chosen so that half of the abdominal epidermis of each sex mosaic was genetically male and the other half female, but because fat body is mesodermal in origin and not closely related to the adult epidermis (20), there was no direct correlation between the expression of Yp genes in fat body cells and the sex of the epidermal cells that lie above. To determine the genetic sex of each fat body cell independent of its Yp phenotype, we stained the slides with Hőchst 33258, a fluorescent dye that binds DNA in a way that makes one-X cells distinguishable from two-X cells in mosaics (21). In fluorescence optics, the silver grains were invisible, and so we could score the sex of fat body nuclei independently of the silver grains that represent Yp transcript. The results showed that nuclei scored as one-X were surrounded by cytoplasm with the male level of Yp transcript, but that nuclei scored as two-X had female levels. The finding that neighboring cells in sex mosaics can contain amounts of Yp message appropriate for sexually opposite genotypes unequivocally rules out the hypothesis that the sex-specific expression of Drosophila's

Yp genes is generated by sex differences in blood borne hormones. Elimination of the humoral hypothesis leaves open the possibility that sex specific expression of Yp genes is generated by sex-determining genetic factors inherent to each individual fat body cell. So we next turned our attention to Yp transcripts in sex determination mutants.

## How Do Sex Determination Genes Regulate Yolk Polypeptide Genes?

Since the humoral environment is insufficient to dictate the level of expression of Yp genes in fat body cells, the determining factor must be the sexual genotype of the individual cell. Sexual genotype is initially determined by the ratio of X-chromosomes to autosomes, and then relayed down a cascade of sex determination genes including Sex lethal, transformer, transformer-2, intersex, and doublesex to the sex differentiation genes like the Yps that actually encode the proteins that make females look and function differently from males (1,22). Some alleles of these genes cause sexually ambiguous genotypes which lead to mutant animals that display mixtures of male and female phenotypes. We showed earlier that some of these mutants have yolk polypeptide quantities in their blood that are inconsistent with their X-to-autosome ratio (7). Experimentally manipulating a conditional allele of tra-2 showed that normal activity of this gene is necessary for Yp expression (23). In our next series of experiments we wanted to examine Yp expression in sex determination mutants at the level of the individual cell.

The hypothesis to be tested in these experiments was advanced by (24) working on intersexes in the moth Solenobia and revived by (25) studying sexually ambiguous genotypes of Drosophila. The basic suggestion is that cells have strict alternative sexual forms into which they may differentiate. According to this idea, individual cells may be either fully male or fully female, but not intermediate. This hypothesis -- the one-cell-one-sex hypothesis -- suggests that in sexually ambiguous genotypes (intersexes), individual cells independently select either the male or the female developmental pathway at some time in development and then pass that decision to their clonal descendents, causing the animal as a whole to show a mixture of male and female characteristics. (Note the difference between gynandromorphs

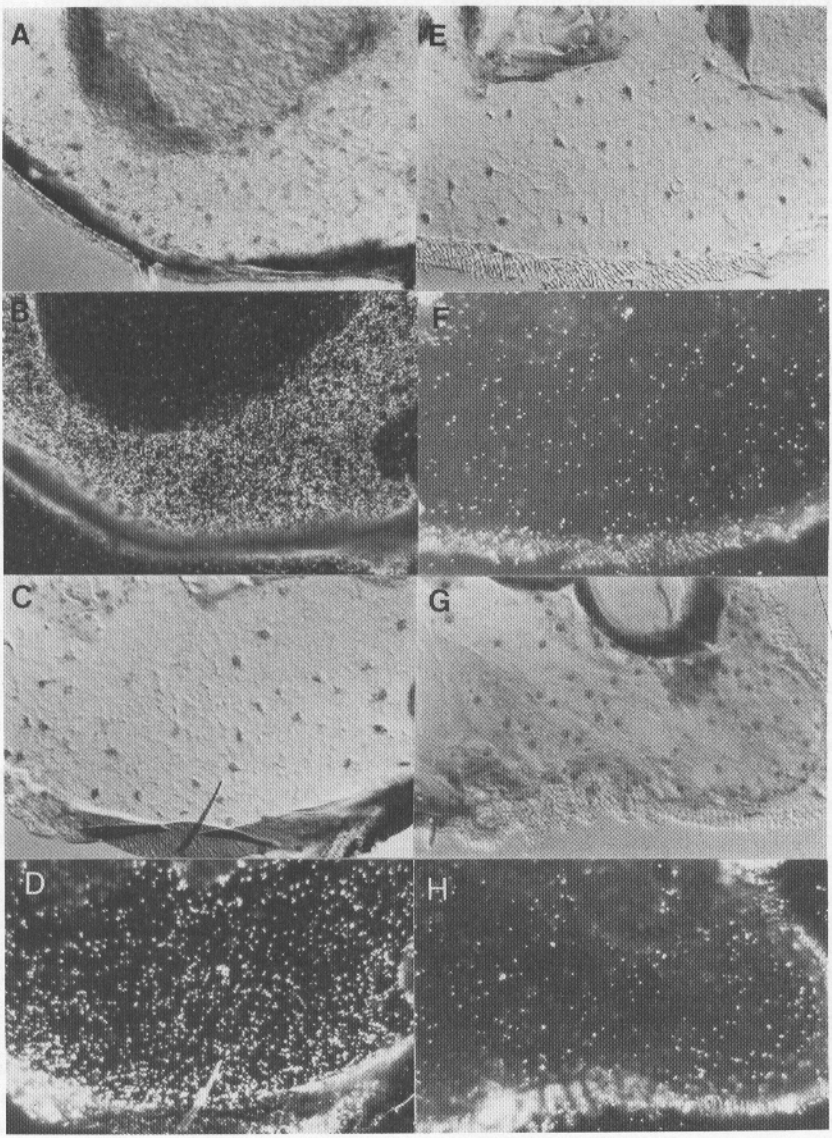


Fig. 2. The wild allele of intersex increases Yp1 expression. A & B, XX;ix/ix$^{+}$ control female. C & D, XX;ix/ix mutant female. E & F, XY;ix/ix$^{+}$ control male. G & H, XY;ix/ix mutant male. A,C,E and G, bright field. B,D,F and H, dark field. Sections were hybridized to Yp1 probe.

and intersexes: gynandromorphs are mosaic animals that contain some cells with an authentic male genotype and some with a genuine female genotype; in contrast, intersexes are animals that contain a genetically homogeneous population of cells all of which have the same sexually ambiguous genotype.)

We can now test the one-cell-one-sex hypothesis in Drosophila intersexes at the level of gene transcript since in situ hybridization can detect a male versus female phenotype in individual fat body cells (Fig. 1). The hypothesis would predict that fat body cells in intersex mutants would have either a fully female amount of Yp transcript or a fully male lack of transcript, but not an intermediate level.

One type of sexually ambiguous genotype occurs in flies that are homozygous for the intersex mutation (ix) and have two X chromosomes (genetic females). These intersexes show irregular genitalia that are a mixture of male and female parts, no sexcombs, and abdominal pigmentation that is a mixture of the normal male and female forms. Homozygous ix flies with one X chromosome (genetic males) look and act as normal males. To determine whether all cells in sexually ambiguous ix flies make the same level of Yp transcript, we hybridized Yp1 probe to tissue sections of XX and XY flies that were homozygous for ix, and to their phenotypically normal heterozygous siblings. Fig. 2 shows that fat body cells in intersexual XX;ix/ix animals have less Yp transcript than in heterozygous control XX;ix/ix$^{+}$ females, but that XY males homozygous or heterozygous for ix both show the absence of Yp transcript that is characteristic of males.

As Fig. 2 shows, the distribution of label over fat body cells in XX;ix/ix intersexes was uniformly reduced: each cell had accumulated an amount of Yp transcript that is intermediate between the normal male and female levels found in the sibling controls on the same section. This result contradicts the predictions of the one-cell-one-sex hypothesis. Thus, the ix gene does not appear to canalize cells into one sexual category or the other, but seems to govern sex in a graded fashion.

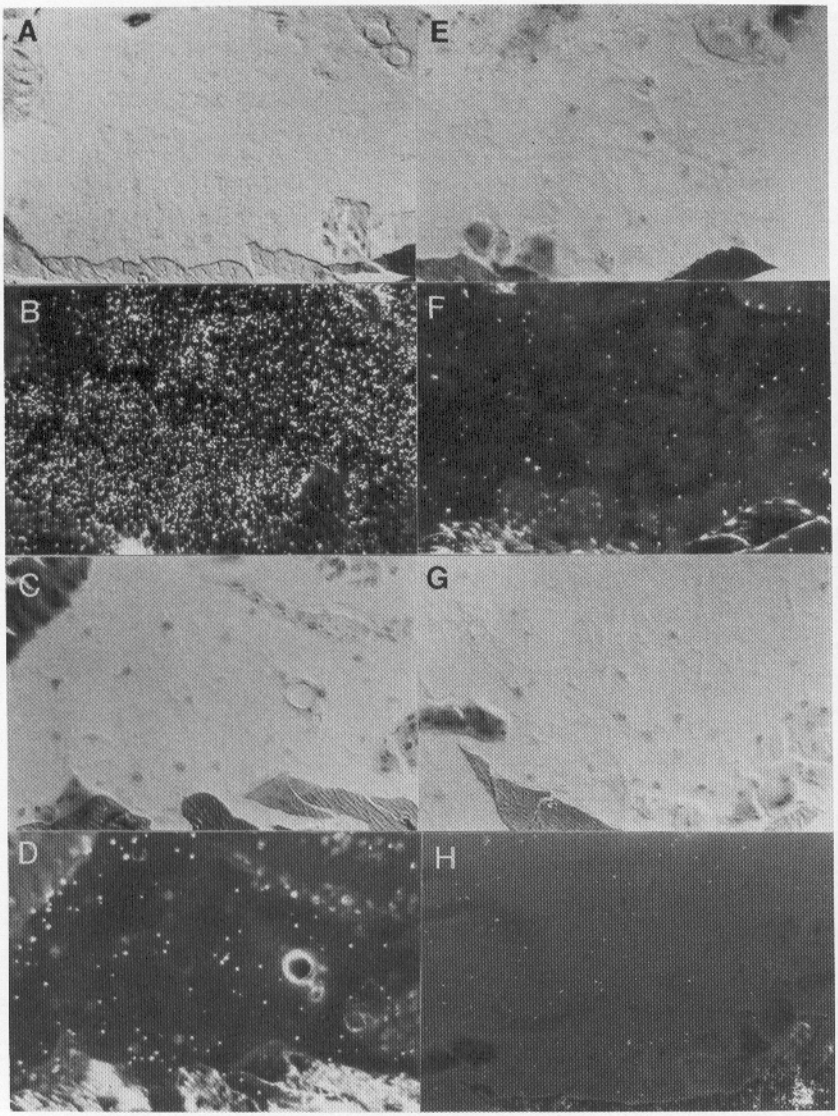


Fig. 3. The dominant dsx$^{D}$ allele blocks expression of Yp1. A & B, XX;dsx$^{+}$/dsx$^{+}$ control female. C & D, XX;dsx$^{+}$/dsx$^{D}$ mutant female. E & F, XY;dsx$^{+}$/dsx$^{+}$ control male. G & H, XY;dsx$^{+}$/dsx$^{D}$ mutant male. A,C,E and G, bright field. B,D,F and H, dark field. Sections were hybridized to Yp1 probe.

Since fat body cells that are homozygous XX;ix/ix contain less Yp transcript than heterozygous XX;ix/ix$^+$ cells, the ix$^+$ allele must be necessary for full expression of Yp genes. These data suggest that the ix$^+$ allele controls the presence of an activator for Yp gene expression. Whether this putative activator controls the level of some circulating factor like 20-hydroxyecdysone that normally stimulates Yp expression in females, or whether the proposed activator acts autonomously in fat body cells is unknown.

The second sexually ambiguous genotype we examined was doublesex-dominant (dsx$^D$). XX flies heterozygous for dsx$^D$ have an intersexual phenotype similar to ix except that the tip of the abdomen and the genitalia show a more complete male phenotype. XY flies heterozygous for dsx$^D$ are phenotypically normal males. When XX;dsx$^D$/dsx$^+$ flies were examined by in situ hybridization and compared to their normal homozygous dsx$^+$/dsx$^+$ siblings (Fig. 3), they were found to lack Yp1 transcript as do control males. This result shows that the dominant dsx$^D$ allele in some way inhibits the expression of the Yp genes even in the presence of the normal dsx$^+$ allele. One possible cause for this behavior would be if dsx$^D$ caused a repressor of Yp gene function to appear, or removed a Yp activator. The dsx$^D$ allele probably causes a constitutive expression of the male determining mode of the dsx locus (28) and in the male mode dsx represses female development.

## DISCUSSION

The experiments discussed here show that, while 20-hydroxyecdysone can stimulate the expression of Yp genes in both male and female fat body cells, the same humoral environment can allow individual cells in a gynandromorph to express opposite sexual phenotypes. We propose that genetically female cells respond readily to the Yp stimulating action of circulating factors, but genetically male cells are relatively refractory to these substances.

The experiments with sex determination mutations showed that full expression of the Yp genes requires the activity of ix$^+$ and the absence of dsx$^D$. These results are consistent with a Yp activator produced by the ix$^+$ allele and a Yp repressor controlled by the dsx$^D$ allele. How might these putative activators and repressors act on Yp genes? We have

little data to guide speculations, but one possibility is that sex determination genes cause male fat body cells to have very low quantities of the ecdysone receptor (10). Since the ecdysone receptor is a nuclear protein (26,27, 28) a female fat body cell in a gynandromorph would be expected to have high levels of hormone receptors and thus express high levels of Yp transcript, when female levels of the hormone were present in the fly's hemolymph. Likewise, a genetically male fat body cell would not be able to produce Yp transcript at high level even in a female humoral environment due to its lack of ecdysone receptor. A male cell with few ecdysone receptors might require very high levels of ecdysone to increase its activity of the Yp genes.

The second possibility is that sex determination genes regulate the appearance in fat body nuclei of DNA binding proteins, possibly sex-specific transcription factors, that promote or prohibit Yp gene expression. The identification of sequence elements upstream from the Yp1 gene that are necessary and sufficient to cause female, but not male, fat body cells to express the Yps (29,17) provides an opportunity to test this hypothesis for the action of sex determination genes on Yp expression.

In summary, our current view of Yp gene regulation is that the X-to-autosome ratio directs sex determination genes to establish sexual identity in a cell-autonomous fashion. A part of that identity for fat body cells would be sex-specific levels of certain nuclear proteins, like the ecdysone receptor and/or specific transcription factors. If such sexually differentiated cells are exposed to a normal female level of hormone, female cells respond by transcribing Yp genes but male cells can respond only if given substantially higher doses of the hormone due to their lower level of hormone receptor or different configuration of transcription factors. In this view, both 20-hydroxyecdysone and the female mode of dsx are necessary for Yp transcription, but neither is sufficient. Further experiments will test this model.

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REFERENCES


1. Baker B, Ridge KA (1980). Genetics 94:383.
2. Postlethwait J, Giorgi F (1985). Vitellogenesis: Insects. In: L.W. Browder (eds): "Developmental Biology: A Comprehensive Synthesis," Plenum Press, p 85.
3. Bownes M (1986). Ann Rev Entomol 31:507.
4. Handler AM, Postlethwait JH (1978). J Exp Zool 296:247.
5. Postlethwait J, Handler AM (1979). J Insect Physiol 25:455.
6. Jowett T, Postlethwait JH (1980). Devel Biol 80:225.
7. Postlethwait JH, Bownes M, Jowett T (1980). Develop Biol 79:379.
8. Bownes M, Blair M, Kozma R, Dempster M (1983). J Embryol Exp Morphol 78:249.
9. Shirk PD, Minoo P, Postlethwait JH (1983). Proc Natl Acad Sci USA 80:186.
10. Handler AM. (1982) Develop Biol 89:225.
11. Bownes M, Dübendorfer A, Smith T (1984). J Insect Physiol 30:823.
12. Schwartz MB, Kelly TJ, Imberski RB, Rubenstein EC (1985). J Insect Physiol 31:947.
13. Hotta Y, Benzer S (1972). Nature 240:527.
14. Hafen E, Levine M, Garber, RL, Gehring W (1983). EMBO J 2:617.
15. Akam ME, Martinez-Arias A (1985). EMBO J 4:1689.
16. Barnett T, Pachl C, Gergen JP, Wensink PC (1980). Cell 21:729.
17. Postlethwait J, Kunert C, Parker J, Widders-Ellis D, Yan Y (1987). Regulation of Vitellogenesis in Drosophila. In Porchet and Strombi (eds): "Advances in Invertebrate Reproduction," Vol 4, Elsevier Press, p 23.
18. Morgan TH. (1916). Proc Soc Exp Biol Med 11:171.
19. Sanchez L, Nöthiger R (1982). Wilh Rouxs Arch Devel Biol 191:212.
20. Lawrence PA, Johnston, P (1986). J Embryol Exp Morph 91:251.
21. Zusman SB, Wieschaus E (1987). Genetics 115:730.
22. Nöthiger R, Steinmann-Zwicky M (1985). Trends in Genetics 1:209.
23. Belote JM, Handler AM, Wolfner MF, Livak KJ, (1985). Cell 40:339.
24. Seiler J. (1951). Rev Suisse Zool 58:489.
25. Stern C (1966). Rev Suisse Zool 73:339.
26. Yund MA, King PS, Fristrom JW (1978). Proc Nat Acad Sci 75:6039.

27. Landon TM, Sage BA, Seeler BJ, O'Connor JD (1987). Submitted.
28. Sage BA, Tanis MA, and O'Connor JD (1982). J Biol Chem 257:6373.
29. Garabedian MJ, Shepherd BM, Wensink PC (1986). Cell 45:859.(1986).