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Advances in Invertebrate Reproduction 4
M. Porchet, J.-C. Andries and A. Dhainaut editors

REGULATION OF VITELLOGENESIS IN DROSOPHILA

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INTRODUCTION

Provisioning the egg with yolk requires substantial biosynthetic activity during reproduction of invertebrates. Yolk deposition, or vitellogenesis, involves production and storage of glycogen, lipids, and specific yolk proteins called vitellogenins. Understanding regulation of vitellogenin synthesis is a central problem in invertebrate reproduction and can be achieved by learning how genes act to control vitellogenesis. In Drosophila melanogaster, female fat body cells and ovarian follicle cells synthesize and secrete three yolk polypeptides (YPs) which are then sequestered into the developing oocyte to form yolk granules (see for review 1,2).

The work presented here investigated three questions: (1) Is the decision to transcribe Yp genes made autonomously by individual male and female fat body cells, or does a sex-specific humoral factor govern Yp gene expression? (2) What does the sequence of the Yp3 gene and its protein suggest about the regulation of vitellogenesis? And (3) What nucleotide sequences are responsible for the expression of Yp1 in the adult female fat body? The results showed that each fat body cell autonomously decides to express Yps depending upon its sexual genotype, and suggested that nucleotide sequences (cis-acting regulatory elements) located within a few hundred base pairs of each Yp gene control Yp expression in fat body cells. We conclude that when correct hormonal conditions are present, female-specific factors within cells interact with cis-acting regulatory elements to enhance transcription of Yp genes, and to allow yolk to be formed so that the fly can reproduce.

RESULTS

Is Yp Gene Expression Cell Autonomous?

Hormones regulated Yp expression in Drosophila: Juvenile hormone (JH) causes ovarian follicles to mature to a YP synthesizing stage (3-5), and the insect steroid hormone 20-hydroxyecdysone (20HE) stimulates the accumulation of transcripts from Yp genes both in female abdomens isolated from the endocrine organs of the head and thorax (3-6) and in intact females (7). Males do not

normally transcribe Yp genes, but will express Yps if injected with high doses of 20HE (4,7-9). These data suggest the hypothesis that males do not express Yps because they lack sufficient 20HE. However, adult female Drosophila have only about twice the concentration of 20HE found in males (10,11). We wondered whether a difference in hormone concentration is sufficient to cause the sex difference in Yp expression (the humoral hypothesis), or whether an autonomous program driven by sex differentiation genes within individual fat body cells is responsible for the sexual dimorphism in Yp gene expression (the autonomous hypothesis). We tested these two hypotheses by examining Yp expression in individual male or female fat body cells contained within the same animal, bathed by the same hemolymph, and hence exposed to identical concentrations of any circulating humoral factors.

Mosaic flies containing both male and female cells (gynandromorphs) develop after loss of a ring-X chromosome and can be easily recognized. The problem was to determine whether individual fat body cells in a sex mosaic all have the same amount of Yp messenger RNA as predicted by the humoral hypothesis -- i.e., all express Yps like female cells, or all fail to express like male cells, or all express at an intermediate level -- or alternatively if some of the cells in gynandromorphs express Yp genes like female cells and others like male cells within the same mosaic animal as predicted by the autonomous hypothesis.

To determine Yp expression in individual cells, we hybridized radiolabeled copies of the Yp1 gene to Yp1 mRNA in tissue sections of gynandromorphs, females, and males, all on the same slide (12,13). Silver grain densities were measured by a computer-assisted imaging system to determine the relative area of a cell covered by silver grains. Figure 1 shows one typical representative among ten gynandromorphs so far tested. Control female fat body cells had an average of about 24% of their area covered by silver grains, while control male cells had an average of less than 1%. In contrast, the gynandromorph contained two populations of fat body cells -- those with many grains and those with few grains. The results clashed with the predictions of the humoral hypothesis, but were upheld expectations of the autonomous hypothesis. We conclude that each fat body cell determines its own sexual phenotype with respect to Yp expression independent of circulating factors. Thus, while circulating concentrations like 20HE may differ in male and female flies, this difference does not account for the difference in Yp expression. Rather, female cells must be much more sensitive to the hormone, perhaps because of sexual differences in an ecdysone receptor (10), which could explain why high doses of 20HE must be used before male fat body cells transcribe Yp genes.

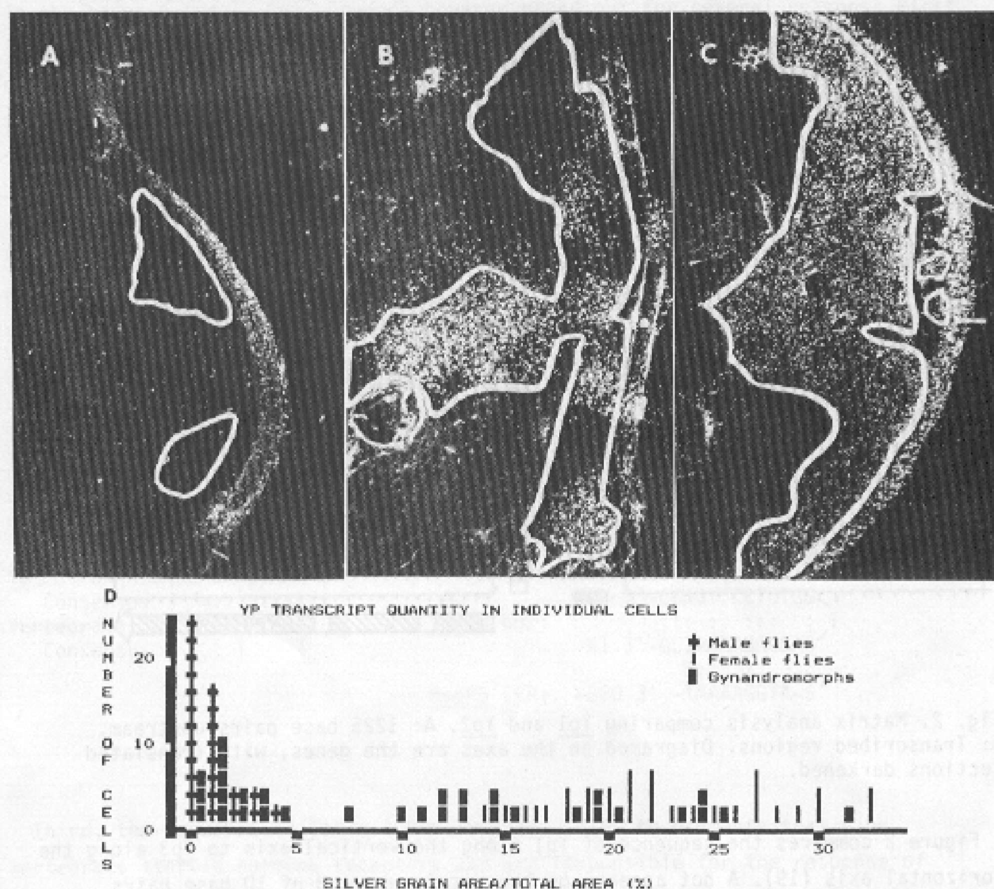

Fig. 1. Yp expression in individual fat body cells. A: Male. G: Gynandromorph. C. Female. D. Silver grain density per cell. (.) Male. (+) Gynandromorph. (o) Female.

Conserved Elements among the Yps: the Structure of the Yp3 Gene.

The sex mosaic experiments showed that a fat body cell's sexual genotype determines whether or not it will express Yp. Therefore, some signal communicates a fat body cell's genetic sex to the Yp genes, which interpret that signal to promote or prevent Yp transcription. Judging from mechanisms of gene regulation in prokaryotes (14), signal transduction probably occurs when a sex specific protein binds to a nearby cis-acting regulatory element, which governs Yp transcription. Since the three Yp genes are coordinately expressed in fat

body cells, they all might be expected to have copies of the same cis-acting regulatory element nearby. Any conserved sequence becomes a candidate for a regulatory element. To identify such elements, we determined the sequence of 4282 base pairs that include Yp3, and compared it to Yp1 and Yp2 (15-18).

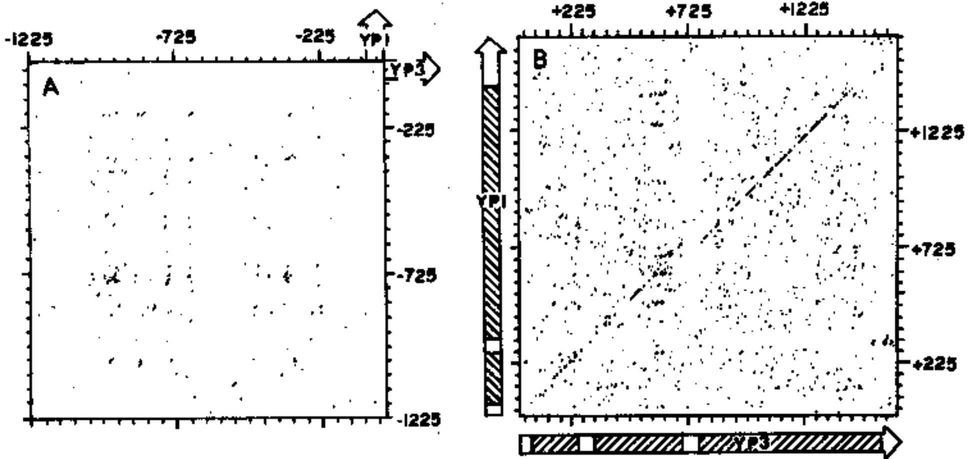

Fig. 2. Matrix analysis comparing Yp1 and Yp2. A: 1225 base pairs upstream. B: Transcribed regions. Diagramed on the axes are the genes, with translated sections darkened.

Figure 2 compares the sequence of Yp1 along the vertical axis to Yp3 along the horizontal axis (19). A dot appears on the matrix where 8 of 10 base pairs correspond at the indicated place in the two sequences. If a series of dots appears along a diagonal, a substantial region of the two genes are identical. The analysis shows that large regions of homology exist between Yp1 and Yp3. Homology is strongest in regions that are translated into protein, but weak or non-existent in the introns, in the 5' untranslated leader and in the 3' untranslated trailer. The conserved translated sequences suggest the origin of Yps by gene duplication. Sequence conservation may be maintained due to a necessity to retain certain amino acid sequences for proper secretion, processing, transport, sequestration, and utilization of the YPs during development.

To identify conserved elements that might play a role in Yp expression, we parameters used demanded less stringent matching (14/21 base pairs), few homologies appeared. We examined by hand the sequences identified by each computer-generated dot, and found that most dots indicated regions simply rich

in the bases A and T. One sequence however stood out for several reasons: First, it was present in each gene in multiple copies between about 100 and about 400 base pairs upstream of all three Yps (Table 1 [A]). Second, two of these sequences were accompanied by an adjacent inverted repeat (Table 1, [B]), a structure typical of DNA sequences that bind regulatory proteins (14).

TABLE I

A CONSERVED SEQUENCE AMONG THE YP GENES

[A]

Gene	Locus	Sequence
Yp3	-265	ATaAG.AGAACTAAA
Yp3	-164	ATCAGCAGAACgAAA
Yp3	-75	gTCAGCAGAAaatcg
Yp2	-184	AaaAGggGAACgAAA
Yp2	-161	cTCAtCAGAAgTgtt
Yp1	-439	tTaAGCAGAAgaAAA
Yp1	-290	ATCAGCgGAACTAcA
Yp1	-254	ATCAGgCgGgAgcAg
Yp1	-113	cTCAGCAcAAgTgAc
Yp Consensus		5'-ATCAGCAGAACTAAA-3'
		:::: : :
Vertebrate Consensus		AGAAACAGA T T

[B]

Gene	Locus	Sequence
hsp23 ECR:	-200	5'-ATGGCAGAT3'
		:::::
Yp1 H box:	-254	5'-ATCAGGCGGCAGCAG
		:: : ::::::
	-225	3'-TACTGAATCGTCGTG
		: :: ::
hsp23 ECR:	-220	3'-TAAAAGGTA-5'
hsp23 ECR:	-228	5'-ATTTTCCAT-3'
		: :: : :
Yp3 H box:	-87	5'-CGACTTCCTGTGG
		::: :: ::: : T
	-61	3'-GCTAAAAGACGAC
		::::::
hsp23 ECR:	-220	3' -TAAAAGGTA-5'

Third, the sequence in Table 1 has homology (6/9) to elements that bind vertebrate steroid hormone receptors and are responsible for the response of certain vertebrate genes to their hormones (20,21); for convenience we call this sequence the H-box. Finally, the H-box has homology (7/10) to ECR, a DNA element implicated in the ecdysteroid control of a small heat shock gene hsp23 (22). For these reasons, we predict that the H-box will prove to be important for the 20HE-induced expression of Yp genes in Drosophila fat body cells.

What Nucleotide Sequences Are Required for Yp Expression?

The preceding work commends the following model for Yp gene expression: cell autonomous genetic sex determination mechanisms provide a regulatory protein which interacts with available 20HE and binds to a cis-acting regulatory region (perhaps the H-box) upstream from each Yp gene to promote transcription. If the regulatory protein stimulates transcription in females, then when we delete the cis-acting regulatory element, the regulatory protein can not bind the DNA, and the adjacent Yp gene can not be transcribed. By systematically deleting regions adjacent to a Yp gene and testing Yp expression of each deletion mutant, we should be able to identify existing regulatory elements.

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As a first experiment we needed to test whether a 5000 base pair fragment containing Yp1 and Yp2 (23) included all cis-acting regulatory elements necessary for correct expression, so we inserted it into the P transposable genetic element. We established lines of flies carrying the transposon and its introduced Yp DNA (24), and tested them for expression of the introduced Yp alleles using northern blots (25). We found (26) that both introduced Yp genes were expressed normally, proving that this 5000 base pairs contains all sequences needed in cis for proper Yp gene regulation.

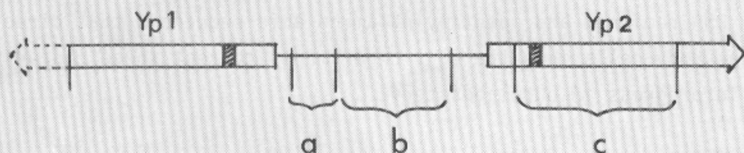
The next step was to delete DNA sequences and test for Yp expression. Transcripts from the introduced Yp1 gene were distinguished from those of the native gene by a radiolabelled probe specific for the read-through transcript of the introduced gene, and transcripts from the introduced Yp2 should differ from transcripts of the native gene in size due to deletion C. No transcripts of Yp2 appeared in any construct containing deletion C (Fig. 3). We conclude that this deletion removes either DNA sequences necessary for Yp2 transcription or sequences required for processing or stability of the message. While deletion C had no effect on the expression of Yp1, deletion A eliminated the accumulation of Yp1 transcripts in both the ovary and the fat body. Deletion B on the other hand did not alter the tissue-specific expression of Yp1. We conclude that the 257 base pairs from 89 to 346 base pairs upstream of Yp1 contains all elements necessary for expression of Yp1 in the fat body and ovary.

These results have recently been extended by Garabedian et al., (27,28) who have shown that a 125 base pair fragment contained within our 257 base pair fragment is sufficient to cause a heterologous promotor to be expressed in the female fat body. We compared the sequence of this 125 base pairs to the upstream portion of Yp3 and found that the only conserved sequence was the H-box inverted repeat shown in Table 1[B]. Since the corresponding H-box in Yp3 appears from 61 to 87 base pairs upstream, we predict that this element will be shown to be an important factor in the regulation of the Yps. Current experiments are testing this prediction.

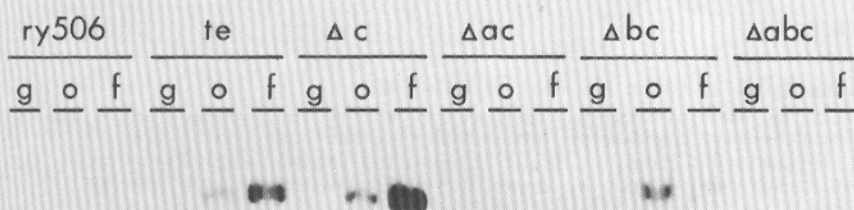
CONCLUSIONS

These experiments suggest a working model for vitellogenesis in Drosophila. The number of X chromosomes and the action of sex differentiation genes (29) launches a sex-specific developmental program autonomous to each individual cell. A part of the female program for a fat body cell includes the production of a regulatory protein, perhaps the ecdysone receptor, that can monitor 20HE concentrations and communicate that assessment to the Yp genes by binding to a cis-acting regulatory element, perhaps the H-box, to promote Yp transcription.

Constructs



Results



Conclusions

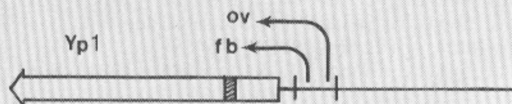


Fig. 3. Deletions identify DNA segments containing cis-acting regulatory elements. Constructs: Yp map. Results: RNA from ry506 untransformed flies; te, flies transformed with intact Yp2 and Yp1 in which the dotted region was replaced by vector sequences used as the probe. The other Stocks are deleted for the indicated regions.

Yp1 and Yp2 may be controlled by the same H-box working in two different directions. After transcription of the Yp genes and translation of YP proteins, conserved amino acid sequences within the proteins direct their secretion, transport, sequestration, packaging into yolk granules, and ultimately their utilization by the developing embryo.

The main utility of this model is to initiate experiments designed to test its tenets. Chief among these include confirming that the H-box is a sequence that can cause a closely linked gene to be expressed in female fat body; testing whether female-specific regulatory proteins exist that bind specifically to Yp regulatory DNA regions; and identifying amino acid sequences that direct the YPs from the fat body cells into the egg. Experiments to test these factors are currently in progress in our laboratory.

ACKNOWLEDGEMENTS

This work was supported by grants from the National Institutes of Health, Monsanto Corporation, and the American Heart Association.

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