THREE GENES FOR THREE YOLK PROTEINS IN *DROSOPHILA MELANOGASTER*

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1. Introduction

The three yolk proteins in *Drosophila melanogaster* [1] can be labelled with [^{35}S]methionine during their synthesis either 'in vitro' or 'in vivo' [2,3]. It has been shown that mRNA isolated from the fat body and ovaries of *Drosophila* can be translated in the wheat germ cell free system into YP1 and YP2, yet YP3 was not synthesised [3,4]. Consequently it was not known whether the wheat germ simply failed to translate the mRNA for YP3 or if perhaps YP3 was derived from YP1, YP2, or a larger precursor. We found no evidence of a precursor, and furthermore peptide analysis and the presence of YP3 in a mutant with reduced quantities of YP1 in the haemolymph and ovary [5] indicated that YP3 is either a modification of YP2 or is translated from a separate mRNA. In this paper short labelling periods are used both 'in vivo' and 'in vitro' to indicate that is in unlikely that YP3 is a derivative of another protein.

2. Materials and methods

2.1. *Maintenance of stocks*

Drosophila melanogaster Oregon R stocks were maintained on a cornmeal, yeast, sugar and agar medium at 25°C.

2.2. *'In vivo' labelling of yolk proteins*

Groups of 5 females were etherised, lined up on double-sided sticky tape, and injected with 0.3 μl [^{35}S]methionine (7.7 mCi/ml) and left for 2, 5, 10, 15, 30 or 60 min. The haemolymph, fat body and ovary of each female was then collected. For the very short pulses of 2 min and 5 min the females could be used for only one type of tissue since it took a finite amount of time to inject and collect the tissues. The haemolymph was placed directly in Laemmli buffer [6]; the ovaries and fat bodies were dissected in *Drosophila* Ringers [7], transferred to Laemmli buffer, whirlimixed to break the cells, then frozen. Each series of injections and subsequent analyses was done several times, and survival was 100% in all cases.

2.3. *'In vitro' labelling of yolk proteins*

Groups of 5 females were dissected and their ovaries and fat bodies cultured in Chan and Gehring's Ringers [7] (50 μl) and 5 μl [^{35}S]methionine (7.7 mCi/ml) for 10, 30 and 60 min. The cells and medium were separated by centrifugation; Laemmli buffer was added to both fractions which were whirlimixed and frozen. Each timed culture was done several times.

2.4. *Pulse/chase experiments*

[^{35}S]Methionine was injected into sets of 5 females and after 10 min the ovaries and fat bodies were transferred to Chan and Gehring's medium for 3 h in the absence of labelled methionine. The cells and medium were separated, Laemmli buffer was added and after whirlimixing the cells were frozen.

2.5. *Analysis of samples*

The samples were heated to 90°C for 15 min and then analysed by SDS gradient acrylamide gel electrophoresis as in [2]. The gels were stained for protein, destained and photographed. Subsequently they were prepared for fluorography [8]. Gels were dried and pressed against Kodarex X-ray film at −60°C for

varying periods of time before developing the film. The resulting fluorographs were also photographed.

3. Results

3.1. *'In vivo' labelling of yolk proteins*

Haemolymph, fat bodies and ovaries were collected and analysed for the presence of labelled yolk proteins (2 min, 5 min, 10 min, 15 min, 30 min, 60 min) after injection of [^{35}S]methionine, according to the schedule detailed above. Yolk proteins 2 and 3 were labelled in the fat body after 2 min and YP1, YP2 and YP3 after 5 min (fig.1). The proteins in the haemolymph labelled a little more slowly; they were faintly visible after 10 min and strongly so after 15 min or more (fig.1). The ovary became very quickly and heavily labelled, but YP1, YP2 and YP3 do not appear as major proteins even after 60 min. In all cases YP2 and YP3 became labelled at exactly the same time, and all three proteins appear labelled in the haemolymph simultaneously. After 15, 30 and 60 min of labelling there is an equal intensity in YP1, YP2 and YP3 in the fat body, but in the haemolymph there is more label in YP1 and YP3 than in YP2.

3.2. *'In vitro' labelling of yolk proteins*

After the fat bodies and ovaries were labelled 'in vitro' for 10, 30 and 60 min, the cells and medium were analyzed separately for the presence of the 3 yolk proteins (fig.2). After 30 min culture they appeared as 3 labelled bands in the medium from both the ovary and fat body; YP1, YP2 and YP3 were labelled simultaneously. Again YP2 and YP3 appeared before YP1 in the fat body cells and were labelled after 10 min. The ovary was intensely labelled throughout. As can be seen in the fluorograph (fig.2) the cells are extremely active with proteins of all molecular weights

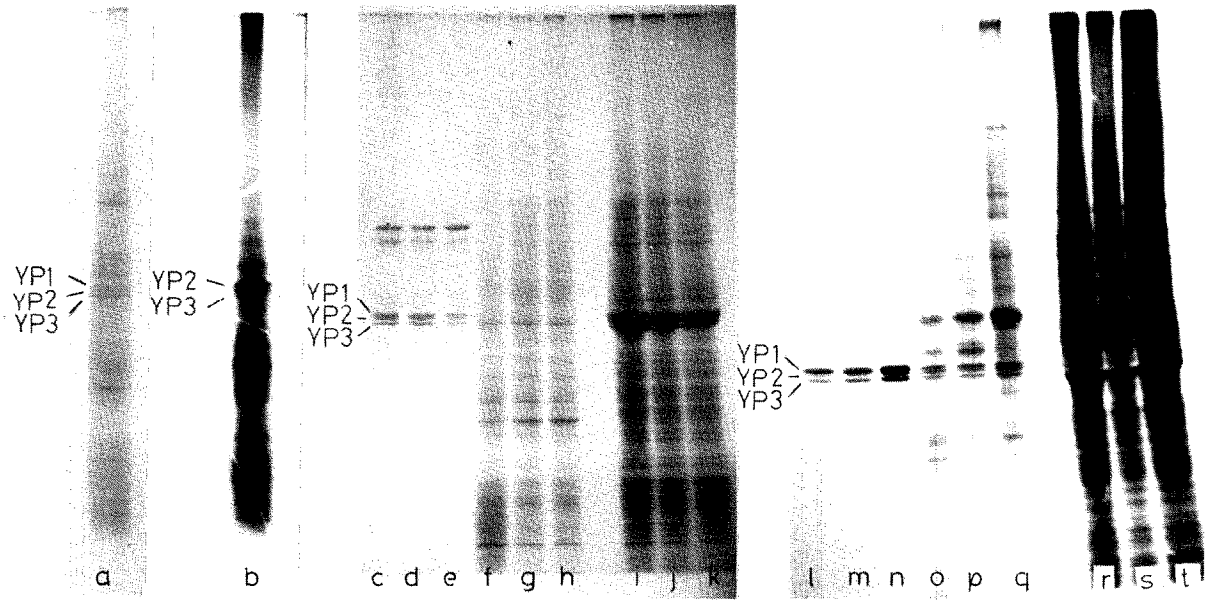

Fig.1. 'In vivo' labelling of yolk proteins. (a) Fat body after 2 min in the presence of [^{35}S]methionine stained for protein. (b) Autoradiograph of track a. YP2 and YP3 are present, and were identified by alignment with a control female haemolymph track. Tracks c–k are stained for protein and 1, m are their autoradiographs. Tracks c–e and l–n represent the haemolymph after 15 min (c, l); 30 min (d, m); and 60 min (d, n); in the presence of [^{35}S]methionine. In all cases YP1, YP2 and YP3 are actively being synthesised. Tracks f–h and o–q represent the fat body after 15 min (f, o); 30 min (g, p); and 60 min (g, q); in the presence of [^{35}S]methionine. Again YP1, YP2 and YP3 are being actively synthesised. Tracks i–k and r–t represent the ovaries after 15 min (i, r); 30 min (j, s); and 60 min (k, t); in the presence of [^{35}S]methionine. YP1, YP2 and YP3 are clearly major proteins in the stained gel but are not major labelled proteins in the ovary fluorograph.

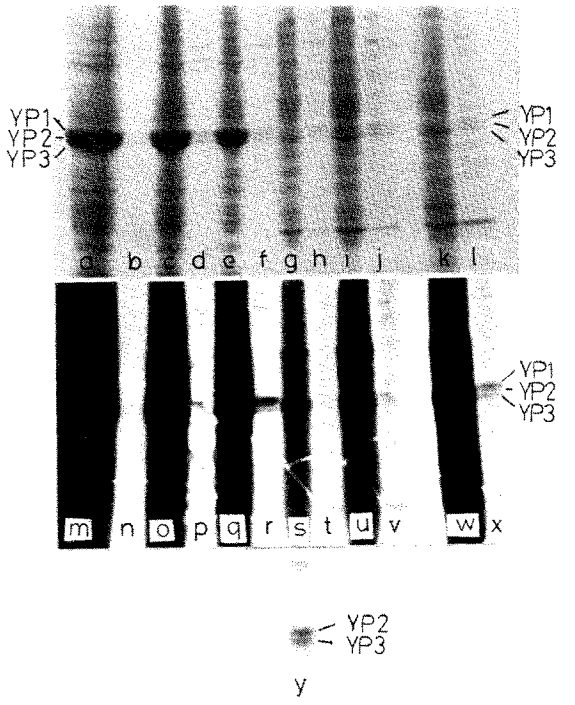

Fig.2. 'In vitro' labelling of yolk proteins. Tracks a–l are stained for protein and tracks m–x are their fluorographs. In each case there is the tissue sample next to the medium in which it was cultured. Tracks a–f and m–r are ovaries cultured in the presence of [^{35}S]methionine for 10 min, 30 min and 60 min from left to right. Tracks f–l and s–x are fat bodies cultured in the presence of [^{35}S]methionine for 10 min, 30 min and 60 min from left to right. Labelled yolk proteins appear selectively in the medium after 30 min and all 3 proteins appear simultaneously. Track y is the less exposed fluorograph of tracks a, s to show that YP2 and YP3 appear in the cells before YP1. In all cases only the central portion of the gel containing the yolk proteins is shown.

being synthesised leading to heavy labelling throughout these tracks on the gel; however, the tracks containing the culture medium show that the yolk proteins are specifically secreted into the culture medium.

3.3. *Pulse/chase experiment*

The yolk proteins were labelled 'in vivo' for 10 min, the time when YP1, YP2 and YP3 first appear in the haemolymph. The fat bodies, presumably containing labelled yolk proteins, were then dissected out and transferred to unlabelled medium. There they continued to function, and the gel stained for protein showed that the yolk proteins were secreted into the medium. The fluorograph indicated that YP1, YP2 and YP3 were still all labelled, showing that there was no transfer of label from one protein to another, as might be expected if YP3 were derived from YP2 (fig.3).

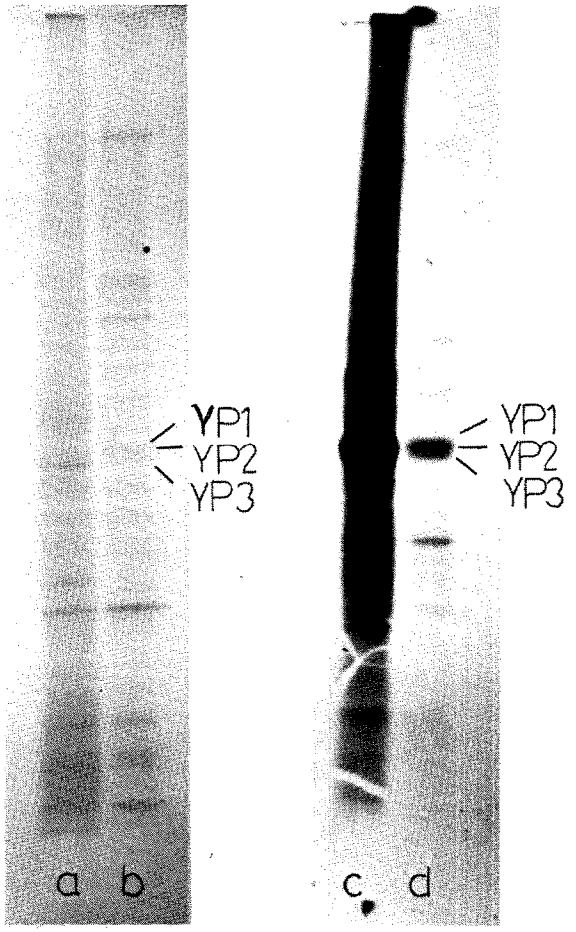

Fig.3. Pulse/chase experiment. (a) Protein stained track of fat body labelled for 10 min 'in vivo' with [^{35}S]methionine, then cultured for 180 min in unlabelled medium 'in vitro'. (b) Culture medium from (a). (c, d) Autoradiographs of a and b, respectively, showing that there is no shift of label into YP3.

4. Discussion

Both 'in vivo' and 'in vitro' labelling showed that the yolk proteins within the fat body were labelled first, and that YP2 and YP3 were labelled simultaneously and before YP1. It is of some interest that the ratios of YP1 : YP2 : YP3 are 1 : 1 : 1 in the fat body cells, but upon secretion into the medium the amount of label in YP1 and YP3 is greater than in YP2. This observation could reflect differences in the methionine content of the 3 proteins, or differences in the rates of synthesis and secretion of the 3 proteins. These differences are not so obvious in the relative amounts of proteins secreted into the medium 'in vitro' by the ovaries and fat body. The proteins also appear more slowly 'in vitro', suggesting that the culture conditions do not allow synthesis of the yolk proteins to occur at normal physiological rates.

In order for YP3 to be derived from YP2 (it could not derive from YP1 since it is present as a labelled protein before YP1) it would take a finite period of time to translate YP2 and for it to be enzymatically cleaved into YP3. YP2 and YP3 are, however, labelled at the same time. In *Locusta migratoria* [9] a precursor to the yolk proteins is synthesised, and labelled amino acids appear first in proteins of 260 000 mol. wt after 10 min, then move to proteins of 140 000–52 000 mol. wt after a cold chase of 30–60 min. We observed no such movement of label with our pulse/chase experiment, even after 3 h in unlabelled medium. Thus it seems unlikely that YP3 is derived from YP2, and we saw no signs of a precursor. All these data together suggest that each yolk protein is coded for by a separate mRNA, and that there are 3 genes in *Drosophila melanogaster* for the 3 yolk proteins.

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