ANALYSIS OF THE YOLK PROTEINS IN *DROSOPHILA MELANOGASTER*

Translation in a cell free system and peptide analysis

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1. Introduction

It has been established that the yolk proteins in *Drosophila* are synthesised and secreted by the fat body, transported in the haemolymph, and accumulated in the developing oocytes [1–5]. In this paper we describe experiments which show that YP1, and YP2 are translated in a cell-free system as proteins of the correct molecular weight rather than as larger precisors, furthermore our data indicates that the smallest yolk protein, YP3, may be a post-translationally modified derivative of YP2.

2. Materials and methods

2.1. *Maintenance of stocks*

2.1.1. *Drosophila melanogaster*

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

2.1.2. Isolation and translation of *Drosophila* RNA

Tissues were dissected from 100 *Drosophila* adults and suspended in 0.1 ml ice-cold HMK buffer (40 mM $MgCl_2$, 25 mM KCl, 50 mM Hepes (pH 7.5)). After homogenisation at 4°C the homogenate was deproteinised using the chloroform–phenol method in [6] and then precipitated by addition of NaCl to 0.1 M plus 2.5 vol. ethanol and incubated at −20°C overnight. The RNA precipitate was recovered by centrifugation (30 000 × *g*, 4°C, 30 min), dissolved in 0.1 M NaCl, 0.01 M Hepes (pH 7.5) and precipitated with ethanol. This procedure was repeated once more and then suitable aliquots of each RNA were translated in the wheat germ cell-free system prepared according to [7]. Each 100 μl reaction mixture contained 15 μg RNA and 1.0 mM ATP, 0.2 mM GTP (both neutralised to pH 7 with KOH), 6 mM creatine phosphate, 4 units/ml creatine phosphokinase, 2.0 mM DTT, 10 mM Hepes (pH 7.4), 110 μM each of 19 non-radioactive amino acids, 64 mM KCl, 800 μM spermidine–HCl (neutralised to pH 7.4), 40 μCi [^{35}S]methionine (spec. radioact. 800 Ci/mmol from The Radiochemical Centre, Amersham) and 40 μl wheat germ extract. After incubation for 2.5 h at 25°C the translation products were analysed by immunoprecipitation and/or SDS–acrylamide gel electrophoresis as in [5].

RNA prepared as above was also translated in the New England Nuclear rabbit reticulocyte Rysate translation kit and the products analysed as above.

2.2. *Peptide mapping by limited proteolysis*

Yolk proteins from egg extracts of *D. melanogaster* were separated by SDS–acrylamide gel electrophoresis as in [5] but staining and destaining was according to [8]. Following the protocol in [8] yolk protein-stained bands were cut from the gel and loaded into wells of a 5–20% linear gradient acrylamide gel. After addition of various amounts of *Staphylococcus aureus* V8 protease (see legend to fig.3) electrophoresis was commenced according to [8] at 200 V. When the bromophenol blue tracking dye had moved 2/3rds the distance into the stacking gel the current was turned off for 30 min and then turned on once more

to give 120 V. After overnight electrophoresis the gel was stained and destained according to [5].

3. Results

3.1. *Translation of yolk proteins in the wheat germ cell-free system*

RNA was isolated from ovaries, fat body and gut and translated in the wheat germ cell-free system as in section 2. Analysis of the translation products by SDS–acrylamide gel electrophoresis followed by fluorography revealed that both YP1 and YP2 had been synthesised in response to both fat body and ovary RNA but no polypeptides corresponding to yolk protein were synthesised after addition of gut RNA (fig.1). Immunoprecipitation of translation products prior to SDS–acrylamide gel electrophoresis enriched for putative YP1 and YP2 products as expected (fig.1). The third yolk protein (YP3) observed in vivo and present after incubation of fat body [5] or ovary (unpublished observation) in vitro was not observed as a wheat germ translation product. Presumably YP3 may be an in vivo post-translational modification of YP1 or YP2 or alternatively it is possible that YP3 mRNA is not translated by wheat germ extracts. However the data indicate clearly that both ovary and fat body contain mRNA coding for YP1 and YP2 and therefore that the structural genes for these proteins are active at this developmental stage in both tissues.

To check that the absence of YP3 was not a peculiarity of the wheat germ cell-free translation system RNA from female gut, fat body and ovary and male fat body were translated in the rabbit reticulocyte lysate system. Two major proteins were synthesised by the ovary and female fat body mRNA, of similar molecular weight to the yolk proteins, which precipitated with anti-*Drosophila* yolk antibody. Male fat body and gut made no antibody precipitable proteins.

3.2. *Indications that YP3 is related to YP2*

Immunodiffusion of anti-yolk antibody against eggs or ovary extracts results in two precipitin lines [5] (fig.2) yet the antibody is clearly reactive against all three yolk proteins as shown by SDS–gel electrophoresis of antibody precipitated yolk proteins [5].

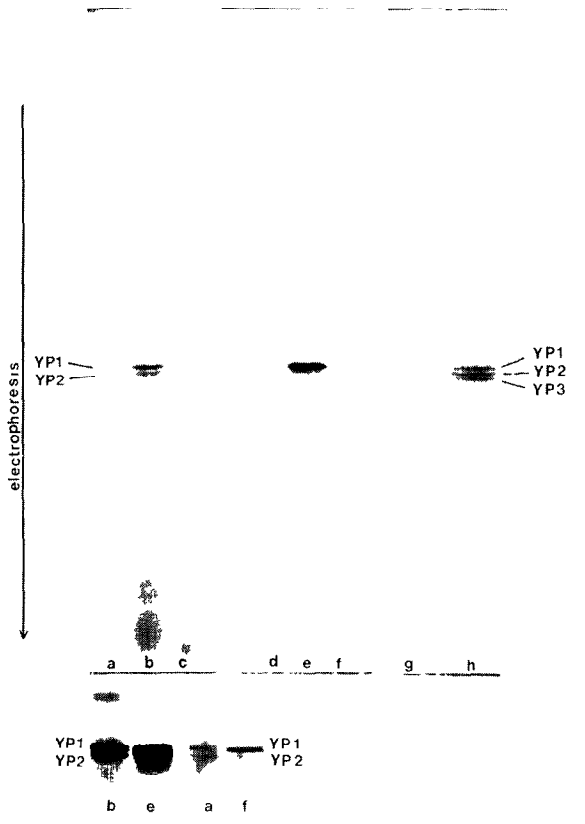

Fig.1. Yolk protein synthesis in response to ovary and fat body RNA. RNA was isolated and translated in the wheat germ cell-free system as in section 2. Translation products were analysed by SDS–acrylamide gel electrophoresis followed by fluorography. (a) Ovary total translation products. (b) Fat body total translation products. (c) Gut total translation products. (d) Gut anti-yolk antibody precipitated products. (e) Fat body anti-yolk antibody precipitated products. (f) Ovary anti-yolk antibody precipitated products. (g) Fat body total translation products. (h) Marker yolk proteins. The insert shows the detail of a long-term exposed fluorograph of yolk proteins in the fat body (tracks b,c) and ovary (tracks a,f).

One way in which these results might be obtained is if two of the proteins were immunologically identical and that one was a derivative of the other. Yolk protein mutant 1163 provides further information on this point. At the restrictive temperature of 29°C the synthesis of YP1 is greatly reduced but YP2 and YP3 accumulation are unaffected [9] as would be expected if YP3 were derived from YP2. Furthermore mutant

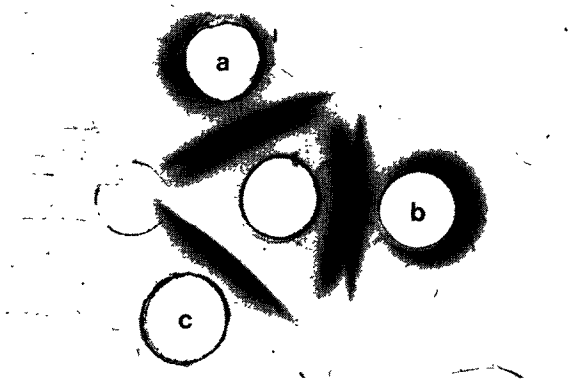

Fig.2. Immunodiffusion of yolk proteins. Anti-yolk antibody was placed in the centre well and challenged with extracts of: Well (a) wild-type ovary; (b) heterozygous 1163/+ ovary from females raised at 29°C; (c) homozygous 1163/1163 ovary from females raised at 29°C (one precipitin line almost deleted).

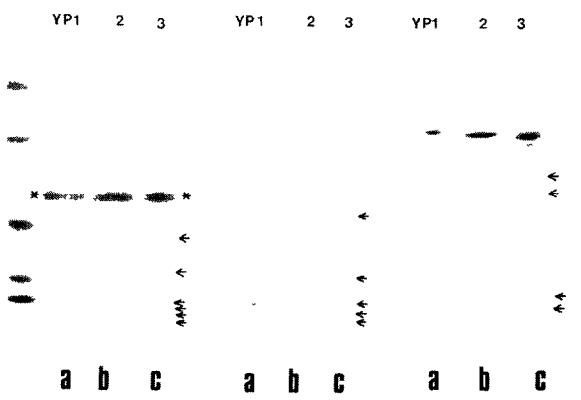

Fig.3. Peptide mapping by limited proteolysis. Yolk proteins were recovered after SDS–acrylamide gel electrophoresis and analysed by limited proteolysis as in section 2. In addition to yolk protein, tracks (a), (b) and (c) contained 5 μg V8 protease, tracks (d), (e) and (f) contained 0.5 μg V8 protease; and tracks (g), (h) and (i) contained 0.05 μg V8 protease. Peptide products in common for YP2 and YP3 are marked with arrows whilst V8 protease is marked with an asterisk. Some arrowed peptides are only present in small amounts and, although clearly visible on the original stained gel, have not been reproduced clearly in the photograph.

1163, kept at the restrictive temperature, yields an immunodiffusion pattern in which one precipitin line is almost deleted (fig.2) suggesting that the remaining precipitin line may involve YP2 and YP3 as immunologically cross-reactive proteins. Peptide mapping using limited proteolysis with *Staphylococcus aureus* V8 protease by the method in [8] confirms that YP2 and YP3 are related, producing several common peptides under a variety of protease digestion conditions (fig.3). However, some large peptide products are unique to YP3 indicating that YP3 is not simply a cleaved derivative of YP2 but either represents a substantially modified form of YP2 or is a separate gene product which has substantial sequence homology with YP2 allowing immunological crossreactivity between YP2 and YP3. The peptide map of YP1 is almost completely different from YP2 and YP3 indicating that these proteins are largely unrelated.

4. Discussion

It is surprising that RNA from the ovary of *Drosophila* led to the synthesis of YP1 and YP2. The cytological evidence is conflicting, some data argues for synthesis within the ovary [10–12], yet other papers indicate pinocytotic uptake from the haemolymph [12–13]. Synthesis within the ovary is in agreement with our findings that isolated ovaries cultured in vitro were able to synthesise all three yolk proteins in large quantities. Thus it seems that the ovary of *Drosophila,* as well as the fat body, may be a site of yolk protein synthesis. It should be noted, however, that small amounts of fat body are attached to the ovary and although it is unlikely that they could account for the large quantities of protein synthesised in vitro or the large amount of yolk protein mRNA present in the ovaries, the possibility that this fat body is 'superactive' cannot at the moment be eliminated.

It is of great interest that using the wheat germ cell-free system we only observe synthesis of YP1 and YP2. This may be because YP3 is a separate gene product whose mRNA is non-translatable by the wheat germ extract although the ability of this system to translate most eukaryotic mRNAs renders this unlikely. Alternatively YP3 may be translated into a larger precursor molecule which the wheat

germ extract is unable to process or, finally, be a post-translational derivative of YP2. Attempts to mimic the post-translational processing which each of these latter two schemes requires, by addition of fat body extracts to wheat germ translation products, have failed to either result in a diminution of the amount of YP2 or an appearance of YP3 (unpublished observations). However it is unlikely that a larger precursor of YP3 is normally produced since none has ever been observed in vivo [5] nor are any of the larger polypeptides synthesised by wheat germ extracts enriched by anti-yolk antibody precipitation (fig.1).

Our peptide sequencing and immunological studies indicate a significant sequence homology between YP2 and YP3 suggesting that either YP3 is derived from YP2 by a modification process which we are presently unable to duplicate in vitro or YP3 and YP2 are indeed separate gene products with a close evolutionary relationship. The presence of some large peptide fragments after proteolysis of YP3 which are lacking in YP2 indicates that if the former is correct the modification is more complex than a simple cleavage process. Distinction between these alternative schemes for the origin of YP3 would be facilitated by the acquisition of mutants deficient in YP2 production and this is now being attempted.

Acknowledgements

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References

[1] Gelti-Douka, H. T., Gingeras, M. P. and Kambysellis, M. P. (1974) J. Exp. Zool. 187, 167–172.
[2] Gavin, J. A. and Williamson, J. H. (1976) J. Insect Physiol. 22, 1457–1460.
[3] Kambysellis, M. P. (1977) Am. Zool. 19, 535–550.
[4] Bownes, M. and Hames, B. D. (1977) J. Exp. Zool. 200, 149–156.
[5] Hames, B. D. and Bownes, M. (1978) J. Insect Biochem. (in press).
[6] Perry, R. P., La Torre, J., Kelley, D. E. and Greenberg, J. R. (1972) Biochim. Biophys. Acta 256, 220–226.
[7] Marcu, K. and Dudock, B. (1974) Nucleic Acids Res. 1, 1385–1398.
[8] Cleveland, D. W., Fischer, S. G., Kirschner, M. W. and Laemmli, U. K. (1977) J. Biol. Chem. 252, 1102–1106.
[9] Bownes, M. and Hames, B. D. (1978) J. Embryol. Exp. Morphol. (in press)
[10] Cummings, M. R. and King, R. C. (1970) J. Morph. 130, 467–468.
[11] Cohn, R. H. and Brown, E. H. (1968) Dros. Inf. Serv. 43, 117–118.
[12] Mahowald, A. P. (1972) J. Morph. 137, 29–48.
[13] Giorgi, F. and Jacob, J. (1977) J. Embryol. Exp. Morph. 38, 125–138.