Genetic analysis of vitellogenesis in
*Drosophila melanogaster*: the identification of a
temperature-sensitive mutation affecting
one of the yolk proteins

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SUMMARY

A number of female sterile mutations on the first and third chromosomes of *Drosophila melanogaster* have been screened for defects in the yolk proteins using polyacrylamide gel electrophoresis. Two new mutants were identified. 6m45 accumulates all three yolk proteins (YP1, YP2 and YP3) in the haemolymph but they are all absent from the ovaries suggesting it is a yolk-protein-uptake mutant. In contrast, 1163 is a temperature-sensitive mutation with a large reduction in the quantity of YP1 in the haemolymph and ovaries at 29 °C. Both mutants are autonomous in ovary transplant experiments.

INTRODUCTION

Vitellogenesis has been studied in insects for many years and is an ideal system for study at the biochemical level, since large quantities of yolk proteins are synthesized in the fat body and transported via the haemolymph to the ovary during adult life (Koeppe & Ofengand, 1976; Hagedorn, 1974; Chen, Couble, DeLucca & Wyatt, 1976; Gelti-Douka, Gingeras & Kambysellis, 1974; Gavin & Williamson, 1976a, Hames & Bownes, 1978).

There are three yolk proteins in *Drosophila melanogaster* with molecular weights around 45000 (Gavin & Williamson, 1976a, Bownes & Hames, 1977; Kambysellis 1977). If we are to understand the control of vitellogenesis, then *Drosophila* has many advantages because of the ease of screening for mutations affecting the different steps involved in the development of a normal vitellogenic ovary. The classes of mutants which might be useful are of three types. (1) Those which do not synthesize any yolk proteins. (2) Those which synthesize the yolk proteins but fail to deposit them in the ovary to a satisfactory extent. This class would include both mutations with defective ovaries and those with an abnormal

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hormonal environment in the female. Mutations of this kind have been analysed by Kambysellis (1977), Postlethwait & Weiser (1973) and Gavin & Williamson (1976b). (3) Mutations affecting the synthesis, structure, or uptake of individual yolk proteins.

We would expect mutations of type 1 and 2 to be either non-vitellogenic or show very little accumulation of yolk. However, mutants in class 3 may well be vitellogenic and may even lay eggs. For this reason we screened non-vitellogenic mutants, mutants which accumulated late-staged oocytes and those which laid eggs that failed to hatch.

In this paper we describe the phenotype of a mutant in class 2 and one in class 3 which we have identified. The latter is the first mutation affecting one of the yolk proteins individually which has been described.

MATERIALS AND METHODS

Maintenance of stocks

Stocks of Drosophila melanogaster were maintained at 18 °C on a standard yeast, agar, sugar and cornmeal medium. The following mutants were tested for defects in the yolk proteins: oc (ocelliless); 6m45, 6m203 and 6m271 (3rd chromosome female steriles kindly supplied by A. Garen and isolated by Rice, 1973); 1163, 288, 148, 125, 473, 180, 1304, 273, 1268, 456, 1059, 384, 147, 59, 321, 120, 508, 371, 117, 1561, 336 (1st chromosome female steriles isolated by Gans, Audit & Masson (1975) and kindly supplied by M. Gans). All stocks were maintained as balanced heterozygotes; 3rd chromosome mutants were balanced over TM1, and 1st chromosome mutants over FM3.

Mutants were reared and matured at both 18 °C and 29 °C for screening and the haemolymph and ovaries were collected from eight-to ten homozygotes. The samples were sonicated in Laemmli buffer (Laemmli, 1970), heated at 90 °C for 15 min and run on polyacrylamide gradient gels as described previously (Bownes & Hames, 1977; Hames & Bownes, 1978).

Morphology of mutants identified

Ovaries from 6m45 and 1163 females were observed using Nomarski interference optics and after feulgen staining. Eggs from 1163 homozygous females were collected, dechorionated and observed under the compound microscope.

Ovary transplants

Ovaries were dissected from freshly emerged females in Drosophila Ringers (Chan & Gehring, 1971) and implanted into host females aged 3 days. After 3–4 days on standard medium the hosts were dissected. The host and donor ovaries were each checked for morphology, then these ovaries along with the haemolymph were prepared for gel electrophoresis.
Immunodiffusion

Mutant 1163 ovaries were also tested against anti-yolk antibody reactive against all three proteins by immunodiffusion. The technique was described previously by Hames & Bownes (1978).

RESULTS

Screen for yolk protein mutants

The procedures used to isolate the female sterile mutants we screened can be found in Rice (1973) and Gans et al. (1975). We analysed not only mutants which failed to lay eggs but also a series of mutants which laid eggs with a flaccid appearance and failed to hatch. Most of the mutants had all three yolk proteins (YP1, YP2 and YP3) present in both the haemolymph and ovary (Fig. 3, tracks a and b). Two mutants were identified as defective in the yolk proteins. Mutant 1163 showed a reduction in YP1 at 29 °C (Fig. 1) and mutant 6m45 showed an accumulation of yolk proteins in the haemolymph but no yolk proteins were found in the ovaries (Fig. 2).

Several mutations, although they were not defective in the yolk proteins, showed differences in one of two other major proteins found in the haemolymph of wild-type females (hm1 and hm2). Mutants 59, 336 and 384 showed an accumulation of this protein and mutant 6m271 showed two proteins either side of the band and 288 showed two protein bands running in the position of hm1 (Fig. 3, tracks c, d and e). As all these mutants are female steriles this protein may well be involved in normal oogenesis. There is not a major protein of the same molecular weight in the ovaries. Investigation of the role of this protein in oogenesis may well be facilitated by these mutants.

Mutant 6m45

Mutant 6m45 showed an accumulation of yolk proteins in the haemolymph and no yolk proteins in the ovaries at both 18 °C and 29 °C (Fig. 2), thus it is not temperature-sensitive for this defect. Heterozygotes 6m45/TM1 females were normal and laid viable eggs.

Feulgen-stained whole mounts of ovaries from homozygous 6m45 females showed no signs of vitellogenesis. The early egg chambers were present and the nurse and follicle cells appear normal until stage 7 (King, 1970). There was some degeneration of egg chambers and abnormal nurse cells were observed in a few of the stage-7 oocytes.

Ovary transplants between 6m45 homozygotes and OrR homozygotes were performed. 6m45 ovaries in a wild-type host still failed to show any morphological signs of vitellogenesis and no yolk proteins were observed in polyacrylamide gels. The host ovary and haemolymph contained all three yolk proteins. When wild-type ovaries were injected into 6m45 hosts they took up the yolk
proteins and accumulated stage-14 oocytes which were shown to contain all three proteins by polyacrylamide gel electrophoresis. The 6m45 host ovaries remained rudimentary and had no yolk proteins present. We can conclude then that the defect in 6m45 lies within the ovary itself and it cannot be cured by a wild-type environment at this stage of development. The presence of a wild-type ovary is also unable to cause the 6m45 ovary to function. It may, however, be possible to rescue this mutant phenotype by larval gonad or pole cell transplants.

**Mutant 1163**

This mutation is a temperature-sensitive female sterile. At 18 °C the heterozygous Fm³/1163 females lay some eggs which hatch and others which fail to hatch but at 29 °C these heterozygotes are totally sterile, laying only eggs which fail to hatch. The homozygous females, 1163/1163, lay eggs which fail to hatch at both temperatures indicating that 1163 shows a dominant female sterile effect at 29 °C and a recessive female sterile effect at 18 °C.

Feulgen-stained whole mutants and living mounts of the 1163/1163 homozygotes at both 18 °C and 29 °C showed that the oocytes do accumulate yolk. However, there are some decaying egg chambers present. The stage-14 oocytes, which tend to be accumulated, appear abnormal. The yolk is very patchy compared to the wild-type. Although some eggs are laid by these females they are flaccid and there are folds in the vitelline membrane.

They are extremely fragile and collapse very easily if touched. Once dechorinated they are extremely abnormal and appear to lack a normal vitelline membrane. No development was observed in these eggs.

The heterozygous Fm³/1163 female ovaries are mostly normal in appearance at 18 °C and lay turgid eggs which hatch and a few flaccid eggs which fail to develop. At 29 °C the ovaries show a tendency to accumulate stage-14 oocytes. There is no decay of egg chambers, but the stage-14 oocytes are flaccid, although the yolk granules appear normal. The eggs laid are flaccid and fail to develop. When

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**FIGURE 1**

Analysis of 1163 using polyacrylamide gels.

(a) Eggs laid by 1163/1163 females at 29 °C.
(b) Ovary of 1163/Fm³ females reared and matured at 29 °C.
(c) Haemolymph of 1163/Fm³ females reared and matured at 29 °C.
(d) Ovary of 1163/1163 female reared and matured at 18 °C.
(e) Haemolymph of 1163/1163 female reared and matured at 18 °C.
(f) Ovary of 1163/1163 female reared and matured at 18 °C and matured at 29 °C.
(g) Haemolymph of 1163/1163 female reared at 18 °C and matured at 29 °C.
(h) Ovary of 1163/1163 female reared and matured at 29 °C.
(i) Haemolymph of 1163/1163 female reared and matured at 29 °C.

Tracks (a), (h) and (i) showed reduced amounts of YP1. Inserts show details of yolk proteins in tracks (a) and (b), (f), (g), (h) and (i).
Fig. 2. Analysis of 6m45 using polyacrylamide gels. (a) Ovary of 6m45 female at 18 °C. (b) Haemolymph of 6m45 at 18 °C. (c) Ovary of 6m45 female at 29 °C. (d) Haemolymph of 6m45 female at 29 °C. Tracks (b) and (d) show an accumulation of YP1, YP2 and YP3.

Fm³/1163 females reared at 29 °C are moved to 18 °C they still lay eggs which fail to hatch after 1 week. Those reared at 18 °C and moved to 29 °C still laid some fertile eggs after 4 days, but thereafter laid large numbers of eggs which failed to hatch.

Heterozygous females show a normal yolk profile using polyacrylamide gel
Fig. 3. (a) Mutant haemolymph with normal distribution of yolk proteins and haemolymph proteins (hm 1, hm 2). (b) Mutant ovary with normal distribution of yolk proteins. (c) Haemolymph of mutant 288 at 18 °C. Two bands can be seen at the position of hm 1. (d) Ovary of 288 at 18 °C. (e) Haemolymph of mutant 59 at 18 °C. There is an accumulation of hm 1.
electrophoresis in the haemolymph, ovaries and eggs at both temperatures. The homozygotes, however, when reared at 29 °C show a reduction in the amount of YP1 deposited in the ovaries and eggs, and the haemolymph shows a small reduction in YP3 (Fig. 1). To show this effect the homozygotes must be both raised and matured at 29 °C. If they are raised at 18 °C, and subsequently matured at either 18 °C or 29 °C the yolk proteins are normal. If 1163/1163 females raised at 29 °C are moved to 18 °C they still show the defective yolk protein profile.

When an immunodiffusion experiment is set up against antiyolk antibody to YP1, YP2 and YP3, then there are two precipitin lines against the ovaries of wild-type flies and against the heterozygous Fm³/1163 ovaries. Homozygous 1163/1163 ovaries from flies reared at 29 °C show a considerable reduction in one of these precipitin lines (Fig. 4). This again suggests that one of the yolk proteins is considerably reduced in the mutant.

Ovary transplants were made between homozygous 1163/1163 females reared at 29 °C and OrR females at 29 °C. When 1163 ovaries were implanted into a wild-type host the haemolymph and ovaries of the host maintained their normal yolk protein profile, and the donor ovaries also contained all three proteins in

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**Fig. 4. Immunodiffusion of antiyolk antibody against ovaries from:**
(a) Oregon R females – two precipitin lines.
(b) Fm³/1163 females at 29 °C – two precipitin lines.
(c) 1163/1163 females at 29 °C – one precipitin line is considerably reduced.
normal proportions. Thus the 1163/1163 ovaries can take up all of the yolk proteins when they are present in the haemolymph. When transplants of OrR ovaries into 1163/1163 females were made, the host ovaries and haemolymph still showed a reduction of YPl; the presence of a wild-type ovary did not cause YPl to be produced at normal levels. The donor ovaries also showed a reduction in YPl. As with mutant 6m45 it may be possible to rescue this mutation with either larval gonad or pole cell transplants.

**DISCUSSION**

Some yolk protein mutants which have been investigated previously are cured when juvenile hormone (JH) is applied to the abdomen (Kambysellis, 1977; Postlethwait & Weiser, 1973; King & Mohler, 1975; Gavin & Williamson, 1976). Most of these mutants are vitellogenic and a small quantity of yolk is found in the oocytes, although eggs are not laid. Yolk proteins accumulate in the haemolymph of these mutants, e.g. apt4 (Postlethwait & Weiser, 1973; Gavin & Williamson, 1976) and fs(2)A18, fs(3)A1 and fs(3)A16 (Kambysellis, 1977). The yolk found in the ovaries may not in fact be due to a leaky uptake of proteins from the haemolymph in these mutants since the ovary itself is able to synthesize yolk proteins (unpublished observation) and these proteins may result from ovarian activity. This possibility is now being investigated using *in vitro* tissue culture techniques.

Mutant 6m45 is similar in phenotype to fs(2)A17 isolated by Bakken (1973). fs(2)A17 is non-vitellogenic and cannot be rescued by the addition of JH (Kambysellis, 1977; King & Mohler, 1975). The yolk protein profile of fs(2)A17 is similar to 6m45 with YP1, YP2 and YP3 accumulating in the haemolymph and being absent from the ovary (Bownes, unpublished observation). Experiments implanting fs(2)A17 into wild-type females also revealed that like 6m45 they were unable to pick up the three yolk proteins even in a wild-type environment (Bownes, unpublished observation). Both these mutants then contain defective adult ovaries which fail to undergo vitellogenesis.

The most interesting mutant we have identified (1163) has a temperature-sensitive effect on the quantity of YP1 and was isolated by Gans et al. (1975). The female sterility maps at approximately 21 cross-over units on the X chromosome (Gans, personal communication). We are in the process of analysing whether the yolk-protein defect can be separated from the female-sterility effect by recombination; however, this procedure is difficult due to the dominant female sterility of 1163 at 29 °C. This will establish if there are two mutations present in this chromosome or if both effects are the result of one mutation. The isolation of mutations affecting the individual yolk proteins will not only assist us in understanding the control of the synthesis of these proteins but may also help us to prepare antibodies against the individual proteins which will be an essential tool to allow us to dissect the molecular basis of vitellogenesis.
Hopefully we will be able to find mutants affecting all the steps in vitellogenesis and eventually dissect the genetic and molecular control of this essential developmental process in *Drosophila*.

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**REFERENCES**


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