Biosynthesi and regulation of two vitellogenins in the fat body and ovaries of *Ceratitis capitata* (Diptera)

Maria D. Rina and Anastassios C. Mintzas

Division of Genetics, Cell and Developmental Biology, Department of Biology, University of Patras, Patras, Greece

**Summary.** In adult female *Ceratitis capitata* both fat body and ovaries synthesize two vitellogenins (Vg-1 and Vg-2) with the same molecular masses as the respective vitellins of the eggs. Furthermore, both tissues contain two abundant mRNAs which yield, in a cell-free system, two previtellogenin polypeptides with molecular masses approximately 1,000 daltons higher than the mature Vgs. In vivo and in vitro studies during development suggested co-ordinate synthesis of Vg-1 and Vg-2 in each tissue. Also, at least in the fat body, Vg synthesis appears to be regulated at the level of transcription. Although both Vg-1 and Vg-2 are synthesized in the fat body and ovaries synchronously, their relative synthetic rates differ in the two tissues. The ratio of Vg-1 to Vg-2 synthesis in the fat body is approximately twice the respective ratio in the ovaries. Adult *C. capitata* males do not synthesize Vgs; however, they do so after treatment with 20-hydroxyecdysone.

**Key words:** *Ceratitis capitata* – Vitellin – Vitellogenin

**Introduction**

The production of eggs in insects involves massive synthesis of specific proteins (vitellogenins, Vgs) in the fat body of the mature females. After secretion in the haemolymph, the Vgs are taken up by the developing oocytes and deposited, sometimes in slightly modified forms, as vitellins (Vns), the major proteins of the yolk (Wyatt and Pan 1978; Hagedorn and Kunkel 1979; Engelmann 1979; Kunkel and Nordin 1983). In addition to its physiological importance for reproduction, insect vitellogenesis also provides a favourable system for the study of mechanisms of hormone-controlled gene expression. In several species, juvenile hormone controls Vg synthesis while in others both juvenile hormone and ecdysone are involved (Wyatt and Pan 1978; Engelmann 1979; Postlethwait and Shirk 1981; Huybrechts and De Loof 1982; Briers and Huybrechts 1984). In higher Diptera, Vgs are composed of similar polypeptides with molecular masses between 44,000 and 50,000 daltons and are very similar if not identical to the respective Vns (Warren and Mahowald 1979; Mintzas and Kambysellis 1982; Harnish and White 1982; Fourney et al. 1982; Adams and Filippi 1983; De Bianchi et al. 1985). The Vgs of higher Diptera are immunologically related (Huybrechts and De Loof 1982) and ecdysone seems to play an important role in their synthesis by the fat body (Postlethwait and Shirk 1981; Huybrechts and De Loof 1982; Briers and Huybrechts 1984). Several studies in *Drosophila melanogaster* have demonstrated that ovaries also synthesize significant amounts of Vgs (Bownes and Hames 1978; Srdic et al. 1979; Bownes 1979; Postlethwait et al. 1980; Brennan et al. 1982). Vg synthesis by the ovaries has also been reported for two other higher Diptera, *Calliphora erythrocephala* and *Musca domestica* (Fourney et al. 1982; De Bianchi et al. 1985).

In the Mediterranean fruit fly *Ceratitis capitata*, we have isolated and characterized two Vns with similar immunological and biochemical properties (Rina and Mintzas 1987). Both proteins appear to be homotetramers consisting of 46,000 and 49,000 dalton subunits respectively and show no apparent differences from the Vgs in the haemolymph. In the present work, the synthesis of *C. capitata* vitellogenins, in both the fat body and ovaries, has been studied in vivo as well as in vitro in tissue culture and a cell-free system. Furthermore, the developmental profile of Vg synthesis was determined by in vivo labelling and immunoprecipitation and was compared with the respective developmental profiles of translatable Vg mRNAs in the fat body and ovaries. Evidence that Vg synthesis in the fat body is regulated by 20-hydroxyecdysone is also presented.

**Materials and methods**

**Preparation of tissue extracts and haemolymph.** A laboratory stock of *C. capitata* (Mintzas et al. 1983) was used throughout these studies. Adult flies were synchronized at eclosion, within a period of 20 min, and then maintained on a 50% yeast-50% sucrose medium in plastic vials at 25°C. Haemolymph was collected by centrifugation of punctured flies as previously described for larval (Mintzas and Rebutsicas 1984). Whole body homogenates were obtained by homogenizing ten flies in 100 μl Robb's medium (Robb 1969) at 4°C. The homogenates were centrifuged in an Eppendorf centrifuge for 5 min at 4°C twice and the final supernatants were stored at −80°C. Fat bodies and ovaries were isolated by dissection in Robb's medium, washed several times and the tissue extracts were prepared as above. For fat bodies, abdominal body walls containing epidermis, muscle and cuticle were used.

**In vivo and in vitro radiolabelling of proteins.** For in vivo radiolabelling, flies were injected with 0.2 μl [35S] methio-
nine (10 mCi/ml, Amersham-England). After 2 h haemolymph and tissue homogenates were prepared as described above and stored at −80°C. For tissue culture experiments, isolated fat bodies and ovaries were incubated in Grace’s tissue culture medium (five tissues/50 μl) for 2 h at 25°C. Each culture had 20 μCi [35S] methionine added. At the end of each incubation the culture medium was collected and the remaining tissue was treated as above. Radiolabelled samples were analysed by sodium dodecyl sulphate (SDS) electrophoresis and immunoprecipitation as described below.

In vitro translation. Total RNA from fat bodies and ovaries was isolated as previously described for larval fat body (Mintzas et al. 1983). The extracted RNA was translated in the mRNA-dependent reticulocyte lysate system of Amersham. The incubation mixtures contained 8 μl lysate, 0.5 μl [35S] methionine (10 mCi/ml) and 1.5 μl RNA (3 μg/ml). The mixtures were incubated at 30°C for 1 h and the in vitro translation products were analysed by SDS-electrophoresis and immunoprecipitation as described below.

Gel electrophoresis and autoradiography. Sodium dodecyl sulphate-polyacrylamide gel electrophoresis (SDS-PAGE) was performed on 9% polyacrylamide slabs gels according to Laemmli (1970). After staining with Coomassie brilliant blue, the gels were dried and autoradiographed using Kodak RP-X-Omat X-ray films. For quantification of the polypeptide bands, the autoradiographs were scanned at 580 nm in an ISCO-1130 gel scanner and the area under each peak was determined planimetrically.

Immunoprecipitation. A 1:1 mixture of rabbit antisera prepared against purified Vn-I and Vn-2 (Rina and Mintzas 1987) was used in all the experiments. Immunoprecipitation was performed in Eppendorf tubes as follows. Radiolabelled samples were dialyzed in 100 μl immunoprecipitation buffer (IB) consisting of 20 mM sodium phosphate, pH 7.2, 0.15 M NaCl, 1% Triton X-100, 2% sodium deoxycholate and 10 mM cold methionine. Antisera (8 μl) were also dialyzed in 100 μl IB. The two solutions were centrifuged in an Eppendorf centrifuge for 10 min to remove any undissolved materials and the supernatants were combined. Following incubation at 37°C for 30 min, an equivalent amount of cold Vns was added to the incubation mixture and it was allowed to stand overnight at 4°C. The immunoprecipitates were collected by centrifugation, and washed three times with IB. The antigen-antibody complexes were dissolved with 100 μl 0.1 N NaOH and then were mixed with 5 ml Insta-Gel (Packard) and measured in a liquid scintillation counter. Equal samples were subjected to trichloroacetic acid (TCA) precipitation to determine radioactivity in total proteins. The relative amounts of newly synthesized Vgs were determined by comparing the radioactivity in the immunoprecipitates with the respective radioactivity in the TCA precipitates. For electrophoretic analysis, immunoprecipitates were directly dissolved in SDS-sample buffer (Laemmli 1970).

Electrophoresis of RNA and hybridization on nitrocellulose filters. Total fat body RNA was extracted by the LiCl/urea method of Richards et al. (1983). Fractionation of 10 μg RNA was carried out by electrophoresis in 2.2 M formaldehyde-1.4% agarose gel. After electrophoresis, the RNA bands were transferred to a nitrocellulose filter with 25 mM sodium phosphate, pH 7.2 and hybridized with a radioactive DNA probe (10⁶ cpm/μg), produced by nick translation according to Maniatis et al. (1982). This probe was obtained from a Vg genomic DNA clone of Ceratitis capitata (unpublished work). Prehybridization and hybridization was performed in 0.5 M sodium phosphate, pH 7.2, 7% SDS, 1% bovine serum albumin (BSA) at 65°C. After hybridization, the filter was washed with a preheated at 65°C buffer [40 mM sodium phosphate, pH 7.2, 1 mM ethylenediamine tetraacetate (EDTA), 5% SDS] at 25°C and then exposed to X-ray film with an intensifying screen at −80°C.

Results

Synthesis of vitellogenins in fat body and ovaries

Vg synthesis in C. capitata was first studied by in vivo radiolabelling. Mature females and males, 3 day old, were injected with [35S] methionine and 2 h later fat body extracts and haemolymph were prepared and analysed on SDS gels. Representative autoradiographs of these gels are shown in Fig. 1 A. Two polypeptides with molecular masses of 49,000 and 46,000 daltons, the molecular weights of mature Vg and Vn polypeptides (Rina and Mintzas 1987),