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Coordinated Expression and Activity of 3-Hydroxy-3-Methylglutaryl Coenzyme A Synthase and Reductase in the Fat Body of *Blattella germanica* (L.) During Vitellogenesis

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Levels of mRNA for the two 3-hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) synthases, (HMG-S1 and HMG-S2), and for HMG-CoA reductase (HMG-R) of *Blattella germanica* were analyzed in the fat body during the first gonadotrophic cycle. HMG-S2 and HMG-R showed the highest mRNA levels on day 0 and decreased thereafter, whereas HMG-S1, showed faint expression. Western blot using specific antibodies for HMG-S1 and HMG-S2 showed no detectable levels for HMG-S1 but a clear pattern for HMG-S2. Both results point to a very limited role for HMG-CoA synthase-1 in *B. germanica* fat body and suggest that the functional enzyme in this organ is HMG-CoA synthase-2. HMG-CoA reductase and synthase proteins shared a cyclic pattern (maximum levels at day 4 and minimum levels on days 0 and 8), which was coincident with the pattern of activity. The delay between gene transcription and protein synthesis suggests a finely regulated translation mechanism. Moreover, the pattern of mevalonate synthesis parallels that of vitellogenin production, suggesting a coordinate mechanism between the mevalonate pathway and the production of vitellogenin. Copyright © 1996 Published by Elsevier Science Ltd

HMG-CoA synthase HMG-CoA reductase Fat body Gonadotrophic cycle *Blattella germanica*

INTRODUCTION

The mevalonate pathway in vertebrates mainly leads to cholesterol biosynthesis. A finely tuned control mechanism regulates the biosynthesis of mevalonate involving 3-hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) synthase and HMG-CoA reductase in a complex pattern, with transcriptional, post-transcriptional and post-translational mechanisms (Goldstein and Brown, 1990). Insects cannot synthesize cholesterol *de novo* because they lack squalene synthase and lanosterol synthase (Beenakkers *et al.*, 1985). Therefore, the mevalonate pathway leads to other specific isoprenoids such as the juvenile hormones (JH), which are synthesized by the *corpora allata* and play important roles in embryonic development, maintenance of larval form and vitellogenesis (Feyereisen, 1985). Among other end products of the mevalonate pathway, which are essential for the cell, ubiquinone and dolichol are the most prevalent (Silberkang *et al.*, 1983; Sagami and Lennarz, 1987). Dolichol, in its active phosphorylated form, acts as a donor of oligosaccharide residues in the glycosylation of proteins, whereas ubiquinone is involved in the electron transport chain.

Since HMG-CoA synthase and HMG-CoA reductase are considered to be as rate-limiting steps in cholesterol biosynthesis in vertebrates (Goldstein and Brown, 1990), it has been suggested that they could also be rate-limiting enzymes in JH biosynthesis (Feyereisen, 1985). To test this hypothesis, enzyme studies have been carried out in the *corpora allata* of insects (Couillaud and Feyereisen, 1991; Couillaud and Rossignol, 1991). However, little is known about the role of HMG-CoA synthase and HMG-CoA reductase in other relevant physiological processes in insects, such as the maturation of the reproductive system.

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Abbreviations: HMG-CoA, 3-hydroxy-3-methylglutaryl coenzyme A; HMG-S, HMG-CoA synthase; HMG-R, HMG-CoA reductase; JH, Juvenile hormone

We have previously reported the cloning and characterization of HMG-CoA reductase (HMG-R) (Martínez-Gonzalez *et al.*, 1993a), HMG-CoA synthase-1 (HMG-S1) (Martínez-Gonzalez *et al.*, 1993b), and HMG-CoA synthase-2 (HMG-S2) (Buesa *et al.*, 1994) from the cockroach *Blattella germanica*. The expression of these three genes was analyzed throughout development and throughout the ovarian gonadotrophic cycle. In the ovary, the three genes follow complex but coordinated cycles (Buesa *et al.*, 1994). The present paper deals with the expression pattern of the three genes and their cooordinated enzymatic activities in the fat body of *B. germanica* females throughout the first gonadotrophic cycle. The fat body is crucial to insect reproduction, since it provides the ovary with vitellogenin, which is needed for the vitellogenesis and for the development of the embryo (Kunkel and Nordin, 1985). The study of the dynamics of these three enzymes may help us to understand the regulation of fat body activity during vitellogenesis.

EXPERIMENTAL PROCEDURES

Materials

Radioactive compounds were obtained from Amersham. Guanidine thiocyanate, Acetyl-CoA, HMG-CoA and acetoacetyl-CoA were from Sigma (St Louis, MO). The Bradford reagent was from BioRad. UM-BGE-1 was a *B. germanica* cell line kindly provided by Dr T. J. Kurtti (Department of Entomology, University of Minnesota, MN). The pET prokaryotic expression vectors and the *Escherichia coli* strains for their expression were a gift from Dr F.W. Studier (Biology Department, Brookhaven National Laboratory, Upton, NY).

Insects

Adult females of *B. germanica* were taken from a colony reared in the dark at 30±1°C and 60–70% relative humidity. All specimens were carefully dated from freshly ecdysed adults, by measuring the basal oocyte length. In these rearing conditions, the first gonadotrophic cycle in adult females lasted 7 days. Fat bodies dissected to determine mRNA levels and enzyme activities were explanted by standard surgical methods, taking care to obtain the same proportion of tissue per specimen each time (ca 90% of the abdominal fat body).

RNA blot analysis

Fat body RNA was isolated as described by Chirgwin *et al.* (1979) with minor modifications. The fat bodies of 3–5 adult females were pooled for each determination, and three determinations were carried out on each day of the gonadotrophic cycle. 9 μg of RNA from each sample was fractionated in 1% agarose/formaldehyde gels, transferred to Nytran-N membranes (Schleicher and Schuell) and UV cross-linked. Hybridizations were carried out as described by Sambrook *et al.* (1989) and full-length cDNAs of the corresponding three genes (HMG-S1, HMG-S2 and HMG-R) (Martínez-Gonzalez *et al.*, 1993a,b; Buesa *et al.*, 1994) were used as probes. Washes were performed at 68° C with 0.2 ××SSC and 0.2 ××SDS (1 ××SSC is 0.15 M NaCl, 0.015 M Na citrate, pH 7.0). In these conditions we established that there was no cross hybridization between HMG-S1 and HMG-S2. mRNA levels were measured by densitometry of the autoradiograms with a Molecular Dynamics computing densitometer. Densitometry values were corrected using corn H4 histone RNA (Chauvet *et al.*, 1991) as a constitutive probe. Filters were dehybridized in 50% formamide/6 ××SSPE at 68°C for 1 h (1 ××SSPE is 0.15 M NaCl, 10 mM sodium phosphate, pH 7.4, 5 mM Na_2EDTA). mRNA levels of the two HMG-CoA synthases and HMG-CoA reductase were analyzed throughout the gonadotrophic cycle from two sets of samples, and at least three different determinations were carried out for each gene.

Expression of B. germanica *HMG-S1 and HMG-S2 in* E. coli *cells*

The coding region of the HMG-S1 and HMG-S2 cDNA for *B. germanica* was modified in the 5′ and 3′ flanking regions by introducing *Nco*I and *Bam*HI sites through PCR with modified primers. Thereafter, the cassette containing only the entire coding region of each HMG-CoA synthase was obtained by digestion with *Nco*I and *Bam*HI restriction enzymes and subcloned in the prokaryotic expression vector pET-8c. A summary of the main steps is presented in Fig. 3(A). The inductions were carried out in *E. coli* BL21DE3 pLys S cells with 1 mM IPTG. After induction, cells at 2.5 optical density were collected by centrifugation and sonicated, and the supernatant was analyzed in SDS-PAGE gels. The appearance of a protein of the expected size (50 and 52 kDa, respectively) and HMG-CoA synthase activity were assessed in each induction experiment.

Western blot analysis

Individual fat body samples were processed with a Douncer homogenizer in 100 μl of a buffer composed of 40 mM K_2HPO_4 pH 7.2, 30 mM EDTA, 50 mM KCl, 100 mM phenyl methyl sulfonyl fluoride (PMSF), 0.25% (v/v) Triton X-100 and 10 mM dithiothreitol. 10 μl of the protein extract, corresponding to 20–60 μg of total protein, was electrophoresed in 10% SDS-PAGE gels and transferred to cellulose nitrate membrane. Immunoblotting was carried out as described by Beisiegel *et al.* (1982) with minor modifications. For the HMG-S1 immunoblotting the antibody against *B. germanica* HMG-CoA synthase-1 was used. This antibody was obtained by injecting rabbits with a peptide corresponding to the amino acid sequence 267–282 (RASEEERTTKYSSLEA) of the *B. germanica* HMG-S1, coupled to keyhole limpet hemocyanin. This peptide is clearly different (only four identical amino acids) from its equivalent counterpart of the *B. germanica* HMG-S2. For HMG-S2 immunoblotting, the antibody elicited in

rabbit anti-rat cytosolic HMG-CoA synthase, previously prepared in this laboratory (Royo *et al.*, 1991) was used. Rat peptide 1 (Royo *et al.*, 1991) was very similar to its counterpart of *B. germanica* HMG-S2 (11 identical amino acids out of 15). Although this peptide 1 was also similar to *B. germanica* HMG-S1 (13 identical amino acids out of 15), Western blot experiments revealed that it did not cross-react with HMG-S1. The antibodies anti-HMG-CoA reductase used for the immunoblotting experiments, were (a set of polyclonal antibodies) elicited against the catalytic moiety of the rat HMG-CoA reductase (Haro *et al.*, 1990). The specificity of these antibodies was determined by immuno-inactivation. Three different Western blot analyses were carried out for each experiment.

Assay of HMG-CoA reductase activity

Individual fat body was homogenized with a Douncer homogenizer in 100 μl of a buffer composed of 100 mM sucrose, 40 mM K_2HPO_4 pH 7.2, 30 mM EDTA, 50 mM KCl, 100 mM PMSF, 0.25% (v/v) Triton X-100 and 10 mM DTT. Two aliquots were assayed in parallel and before the assay the protein content was determined by the method of Bradford (1976). HMG-CoA reductase activity was determined in the fat body tissue by the radiometric method described by Goldstein *et al.* (1983). The assay was performed with 100 μg of total fat body protein for 10 min; in these conditions the assay was linear and the substrate consumed was less than 5%. 1 unit of HMG-CoA reductase is defined as the amount of enzyme that converts 1 nmol HMG-CoA into mevalonate in 1 min at 37°C.

Assay of HMG-CoA synthase activity

Samples of the three fat bodies collected on each day of the gonadotrophic cycle were processed as above, and two aliquots of 25 μl were assayed. HMG-CoA synthase activity was determined by the radiometric method described by Clinkenbeard *et al.* (1975), as modified by Gil *et al.* (1986), using a specific radioactivity of 20 mCi/mmol; the concentrations of acetyl-CoA and acetoacetyl-CoA were 200 μM and 20 μM, respectively. 1 unit of HMG-CoA synthase is defined as the amount of enzyme that catalyses the formation of 1 μmol HMG-CoA in 1 min at 37°C. The aforementioned considerations of linearity apply to this assay.

Determination of the content of vitellogenin in the fat body

Vitellogenin was quantified by the ELISA procedure using antiserum against vitellogenin-vitellin, as described elsewhere (Martín *et al.*, 1995), using secondary peroxidase labeling which was developed with 3,3', 5,5',-tetramethylbenzidine. The absorbance was read at 450 nm with a Titertek Multiscan Plus MKII spectrophotometer.

RESULTS

HMG-CoA synthase-1, HMG-CoA synthase-2 and HMG-CoA reductase mRNA levels in the fat body

The determination of HMG-S1 mRNAs showed a smear with more intense bands at 1.7 kb (the size expected for the full-length mRNA) and 0.5 kb (Fig. 1). The mRNA levels of the two bands were undetectable on day 0, increased very slightly just after imaginal ecdysis, on days 1 and 2, and then were almost undetectable from days 3–8. To characterize the 0.5 kb band, the Northern blots were again hybridized with a DNA fragment corresponding to the first 350 bp of the HMG-S1 cDNA 5' region and a picture identical to that shown in Fig. 1 was obtained. Therefore, it was deduced that the 0.5 kb band corresponded to the 5' region of the mature mRNA. It is hard to conclude that the 0.5 kb mRNA observed could be functional, because the truncated protein coded by this mRNA fragment probably does not contain the catalytic site, which is located within the amino acid 121 (Martínez-Gonzalez *et al.*, 1993b).

The size of the HMG-S2 mRNA (1.7 Kb), was equal to that expected from the cDNA, with maximal expression at the beginning of the cycle (Fig. 1). Densitometry showed that HMG-S2 mRNA levels were five times the lowest level of the cycle on days 0, 1 and 2. They then decreased on day 3 to a minimum on day 4,

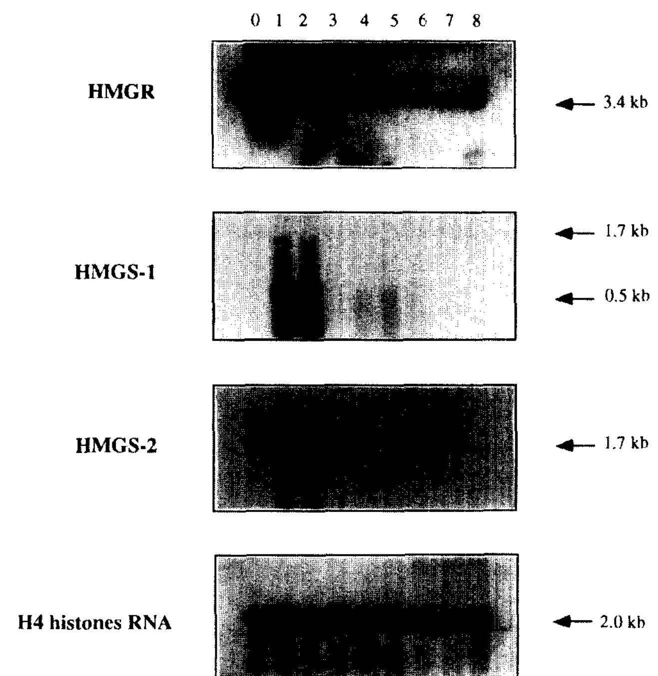

FIGURE 1. Analysis of *B. germanica* HMG-CoA reductase (HMGR) and the two HMG-CoA synthases (HMGS-1, HMGS-2) mRNA levels in the fat body throughout the first gonadotrophic cycle. A blot with total RNA obtained on various days of the gonadotrophic cycle was hybridized with full length cDNAs of the corresponding three genes (HMG-S1, HMG-S2 and HMG-R) used as probes. The membranes were also hybridized against corn H4 histone RNA (Chauvet *et al.*, 1991) as a constitutive probe. The molecular masses of the bands are indicated with arrows. The days of the gonadotrophic cycle (0–8) are indicated above.

which was maintained until the last day of the cycle (day 8, the first day of ootheca transport).

HMG-CoA reductase mRNA levels (Fig. 1), were maximal just after imaginal ecdysis (day 0 of the gonadotrophic cycle) and decreased until day 4; then, they increased again until the formation of the ootheca (day 8).

Validation of the HMG-CoA reductase and HMG-CoA synthase antibodies

To confirm that the anti-rat HMG-CoA reductase antibodies (Haro *et al.*, 1990) reacted with epitopes of the cockroach enzyme, we carried out immunotitration experiments of this enzymatic activity. Aliquots of a *B. germanica* cell line UM-BGE-1 extract (see Material and Methods) were incubated with the rat antibodies or with a γ-globulin fraction of the pre-immune rabbit serum. As shown in Fig. 2, increasing amounts of the antibodies reduced the HMG-CoA reductase activity from the extract in a dose-dependent manner.

To validate the functionality of antibodies against HMG-S1 and HMG-S2, we expressed high amounts of these proteins in *E. coli.* To this end, we introduced the open reading frame of the cDNA of the two genes in pET-8c expression vectors, as summarized in Fig. 3(A). Extracts of *E. coli* cells that expressed HMG-S1 and HMG-S2 showed a clear induction in SDS-PAGE gels of a protein of the expected molecular mass (about 50 kDa for HMG-S1 and about 52 kDa for HMG-S2) [Fig. 3(B)], Then the SDS-PAGE gels were immunoblotted with anti-HMG-S1 and anti-rat cytosolic HMG-CoA synthase [Peptide 1, (P1)] antibodies, respectively. Anti-HMG-S1 antibody recognized only the HMG-S1 protein [Fig. 4(A)], whereas P1 antibody recognized only the HMG-S2 protein [Fig. 4(B)].

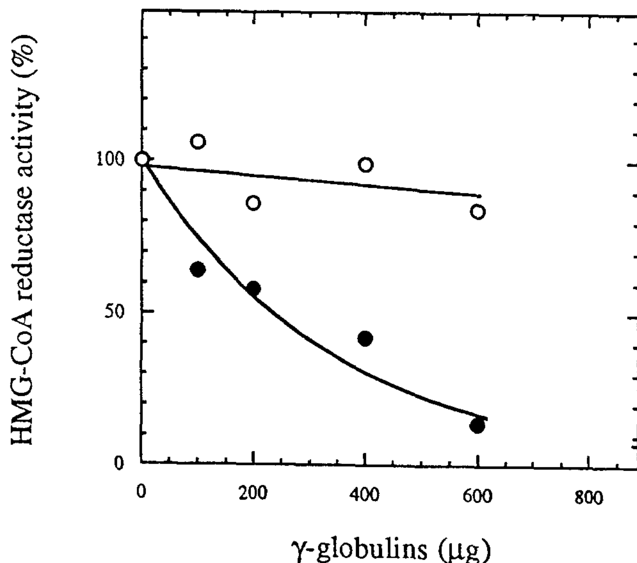

FIGURE 2. Immunotitration of fat body HMG-CoA reductase activity. UM-BGE-1 cells were incubated for 24 h with 0.2 mM compactin. Then, 180 μg of cell extracts was incubated for 30 min at 37°C with different amounts of immune (closed circles) or non immune (open circles) γ-globulin fraction. After incubation, the enzymatic activity was assayed in duplicate; the activity is expressed as a percentage of the value obtained in the absence of γ-globulin (22.3 pmol mevalonate/min xxmg protein)

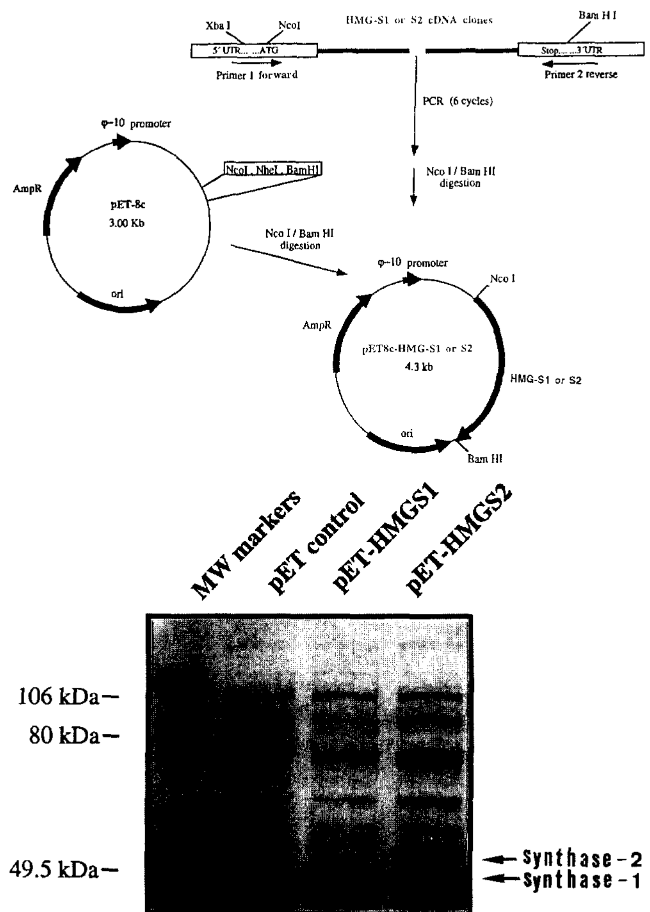

FIGURE 3. (**A**) Strategy for cloning the coding region of HMG-S1 and HMG-S2 in the expression vector pET3c. The plasmids containing cDNAs for HMG-S1 and HMG-S2, (Martínez-Gonzalez *et al.*, 1993b; Buesa *et al.*, 1994) were amplified by PCR with appropriate primers to yield two new *Nco*I and *Bam*HI sites in the 5′ and 3′ flanking regions of their cDNAs. After digestion with these enzymes, the resulting cassettes containing only the coding region were subcloned. Plasmids are drawn in circles with the relevant elements, and restriction sites are indicated. (**B**) The expression plasmids were induced in *E. coli* strain BL21DE3 pLys S with 1 mM IPTG. The performance of the inductions was assessed by SDS-PAGE of crude protein extracts (10 μg) stained with Coomassie blue. Lane 1, molecular weight markers (BioRad); lane 2, cells transformed with plasmid control; lane 3, cells transformed with plasmid pET3c-HMG-S1; lane 4, cells transformed with plasmid pET3c-HMG-S2. The arrows indicate the expressed proteins corresponding respectively to HMG-S1 and HMG-S2 (lanes 3 and 4)

Protein levels of HMG-CoA synthase-1, HMG-CoA synthase-2 and HMG-CoA reductase in the fat body

When *B. germanica* fat body extracts sampled on each day of the gonadotropic cycle were immunoblotted against anti-HMG-S1 antibody, no band of the expected molecular weight (50 kD) appeared [Fig. 4(C)]. When anti-rat cytosolic HMG-CoA synthase (P1 peptide) antibodies were used, a 51 kD band was observed, which corresponded to the expected molecular weight of the HMG-S2 protein [Fig. 4(D)]. Quantification of three

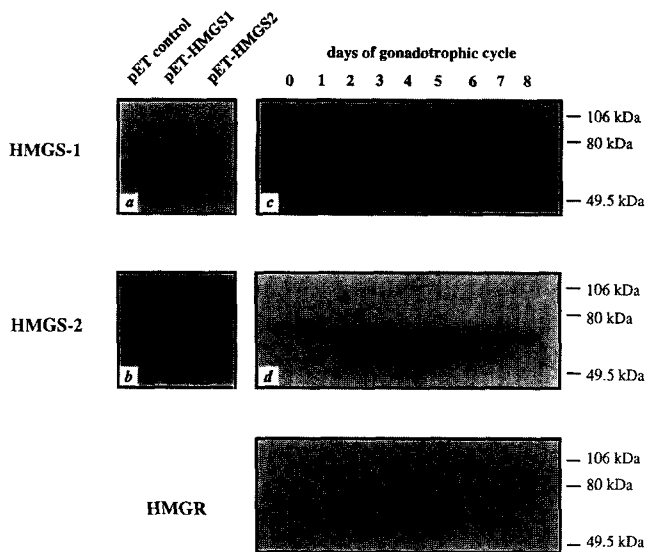

FIGURE 4. Analysis of the protein levels of the two *B. germanica* HMG-CoA synthases and HMG-CoA reductase in the fat body throughout the first gonadotrophic cycle. Crude protein extracts of HMG-S1 and HMG-S2 overexpressed in *E. coli*, and *E. coli* harboring the expression plasmid were fractionated in SDS/PAGE. The gel was immunoblotted as described by Beisiegel *et al.* (1982) against either antibody anti HMG-CoA synthase-1 (panel a) or antibody anti rat cytosolic HMG-CoA synthase (Royo *et al.*, 1991) (panel b). Individual fat body protein extracts, were electrophoresed and immunoblotted against either antibody anti HMG-CoA synthase-1 (panel c) or antibody anti rat cytosolic HMG-CoA synthase (panel d). Anti rat HMG-CoA reductase antibodies (Haro *et al.*, 1990) were used to detect HMG-R protein levels in the insect fat body (panel e).

independent Western blots showed that HMG-S2 protein levels showed a marked cycle, with a maximum on day 4 and minima on days 0 and 8 (Fig. 5). Rat HMG-CoA reductase antibodies were able to immunodetect two proteins of molecular masses of 58 kD and 66 kD from the fat body [Fig. 4(E)], neither of which, however, corresponds to the molecular weight of the native form deduced from the cDNA sequence (93.1 kDa). We assume that they are both proteolytic fragments of the native *B. germanica* HMG-CoA reductase due to: (i) the well documented proteolysis of mammalian native HMG-CoA reductase (Liscum *et al.*, 1985; Edwards *et al.*, 1980), whose fragments of 62 kDa and 53 kDa show enzymatic properties; (ii) the capacity of the rat antibody to immunoinactivate *B. germanica* HMG-CoA reductase activity (Fig. 4); and (iii) the good correlation between the immunodetected proteins and the HMG-CoA reductase activity during the gonadotrophic cycle (see below). The two proteolytic fragments of HMG-CoA reductase detected show analogous expression patterns throughout the gonadotrophic cycle. The photodensitometric analysis of both proteins together is shown in Fig. 5(C).

HMG-CoA synthase and HMG-CoA reductase activities in the fat body

HMG-CoA synthase activity showed a cyclic pattern in the fat body during the first gonadotrophic cycle. The activity increased from day 0 to day 4, when it showed a maximum of 131 pmol of HMG-CoA/fat body ××min [Fig. 5(C)]. Then the activity decreased to a minimum on day 6, and remained low until the end of the cycle. HMG-CoA reductase activity also showed a marked pattern during the gonadotrophic cycle. The activity increased from day 0 to a peak on day 4 (3 pmol mevalonate/fat body ××min), and then decreased steadily to a minimum (0.4 pmol mevalonate/fat body ××min) on day 8 [see Fig. 5(C)].

Fig. 5(A) and (B) show the graph of the mRNA and protein levels of both HMG-S2 and HMG-CoA reductase from the fat body of *B. germanica* estimated from Figs 1 and 4. The patterns of both proteins are similar to each other and to those of the enzymatic activities [Fig. 5(C)]. However, the mRNA pattern is different, showing a minimum on day 4, when the enzymatic activity and protein was maximum.

Figure 5(C) also shows the vitellogenin pattern throughout the gonadotrophic cycle. A close correspondence is observed between this pattern and those of protein and enzymatic activities of both HMG-S-2 and HMG-CoA reductase.

DISCUSSION

As occurs in the cockroach ovary (Buesa *et al.*, 1994), mevalonate production in the fat body of *B. germanica* shows a cyclic pattern during the gonadotrophic period. However, in contrast to what was observed in the ovary, the two HMG-CoA synthases do not follow the same expression pattern. In the fat body, HMG-S1 protein is absent and mRNA levels of the appropriate molecular mass are very low. The fact that HMG-S1 exhibits a pool of different sized mRNAs (from 1.7 kb to very small species), whereas it remains unaltered in other tissues, is intriguing. This smear only appears when the filter is hybridized with the HMG-S1 probe, whereas the same membranes stripped and hybridized against either HMG-S2 or HMG-CoA reductase reveal single bands of the expected size, which suggests that an artefactual explanation may be ruled out. The energy predicted for the 3′ region of the two HMGS mRNAs (−68 kcal and −53 kcal, respectively, for HMGS-1 and HMG-S2) indicates a similar theoretical stability for the two messengers. Therefore, the instability of HMG-S1 in the fat body could be due to the lack of specific proteins which would interact with the HMG-S1 cytoplasmic polyadenylation signal (see below). These observations, together with the failure to detect HMG-S1 protein, suggest that HMG-S1 is not functional in the fat body of *B. germanica*.

HMG-S2 mRNA levels in fat body are maximal on days 0–2. However, the maxima of the corresponding HMG-S2 protein were found on days 3–5. Thus, in the fat body there is a marked delay between the transcriptional activation of HMG-S2 gene and the translation to protein, as occurs in the ovary (Buesa *et al.*, 1994). The

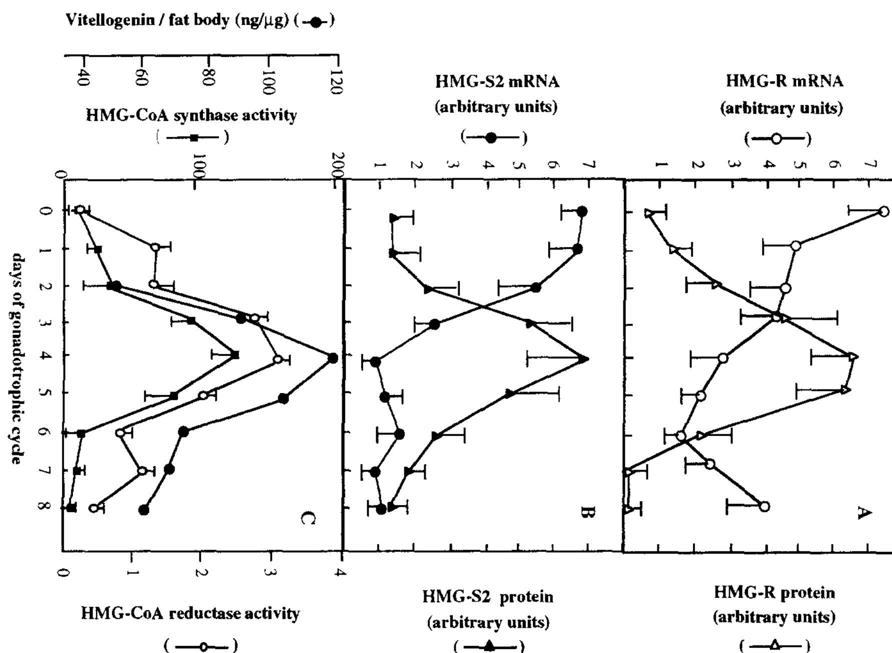

FIGURE 5. (**A**) and (**B**) Quantitative comparison of HMG-R, HMG-S2 mRNA levels and HMG-R, HMG-S2 protein levels throughout the first gonadotrophic cycle. Densitometry of the autoradiograms of Figs 1 and 4 was carried out with a Molecular Dynamics computing densitometer. Densitometry values for mRNA were corrected using corn H4 histone RNA (Chauvet *et al.*, 1991) as a constitutive probe. The values represented are the mean of three Western or Northern blot experiments. (**C**) HMG-CoA synthase and HMG-CoA reductase activities and vitellogenin content in *B. germanica* fat body. Fat bodies extracted on each day of the gonadotrophic cycle were assayed for HMG-CoA synthase and HMG-CoA reductase activities. The levels are indicated in μUnits (pmols) /fat body equivalent ×xmin (left). HMG-CoA reductase activity levels are indicated in μUnits (pmol)/fat body/min (right). Vertical bars show the SEM (n=8). The fat body levels of vitellogenin (Martín *et al.*, 1995) are shown in filled circles.

pattern of HMG-CoA synthase activity coincides with that of the corresponding protein, which suggests that the regulation of HMG-CoA production relies mainly on the stability of the messenger and its translation. Since HMG-S1 protein is not detected in the fat body, it appears that HMG-S2 is responsible for the HMG-CoA synthase activity in this organ.

A similar picture to HMG-S2 was observed for HMG-CoA reductase: mRNA levels were maximal on day 0, whereas maximal protein levels and enzymatic activity appeared 4 days later. This suggest that the mechanism of regulation of mevalonate production relies mainly on the translation step. Since most apparent increases in HMG-CoA reductase activity and protein occurred on days 3, 4 and 5, when the mRNA levels had decreased, it appears that only some of the mRNA detected in the first days of the cycle takes part in the mevalonate cycle of the adult fat body. The remaining mRNA may be a surplus from the late larval stage, which is not translated.

The phenomenon of mRNA storage previously reported for these genes in cockroach ovary (Buesa *et al.*, 1994) and now found in the fat body, has been described in other instances (Bachvarova, 1992). In growing oocytes, a large fraction of the mRNA synthesized is not processed, but remains dormant or masked and is stored for future translation. To explain this phenomenon, a mechanism involving the rate of deadenylation and the occurrence of specific sequences, like the cytoplasmic polyadenylation signal (UUUUUAU), to which specific proteins would interact, has been proposed (Kwon and Hecht, 1993). It is worth noting that HMG-S1, HMG-S2 and HMG-CoA reductase mRNAs contain these putative cytoplasmic polyadenylation signals.

The mechanism by which both enzymes are regulated in the fat body during the reproductive cycle remains to be determined. HMG-R and HMG-S2 genes are already highly expressed at imaginal ecdysis, which indicates that transcription occurs towards the end of the last larval instar, when the JH levels are low, which induces metamorphosis, and ecdysteroids are high, which induces the molt. Conversely, translation takes place in the first days of imaginal life, around day 3, just when JH production rates begin to increase (Bellés *et al.*, 1987). Therefore, it is tempting to consider that the hormonal milieu at the end of the last larval instar may induce the transcription of both genes. In this context, it is worth remembering that JH has been reported to regulate the transcription and mRNA stability of genes related with metamorphosis in other insects (Hiruma *et al.*, 1991; Jones *et al.*, 1993; Shemshedini *et al.*, 1990).

The regulation of mevalonate synthesis in the fat body during the gonadotrophic cycle has a parallel in vitellogenesis. The pattern of vitellogenin content in the fat body and its release in the hemolymph during the first gonadotrophic cycle [Fig. 5(C)] (Martín *et al.*, 1995) coincides with those of HMG-CoA synthase and reductase activi-

ties, which suggests the involvement of the mevalonate pathway in vitellogenesis. Vitellogenin is produced by the fat body and released to the hemolymph to be incorporated by the growing oocytes. The massive production of vitellogenin takes place from days 1–6 with a maximum on day 4 [Fig. 5(C)], when mevalonate synthesis is maximal. Since vitellogenins are highly glycosylated (7% in *B. germanica*), principal by mannose residues (Kunkel *et al.*, 1981; Beenakkers *et al.*, 1985), the mevalonate pathway could play a role in the glycosylation of vitellogenins, since it is mediated by dolichol, one of the end products of the pathway.

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