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Blattella germanica Has Two HMG-CoA Synthase Genes

BOTH ARE REGULATED IN THE OVARY DURING THE GONADOTROPHIC CYCLE*

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The isoprenoid pathway leads to various essential non-sterol products in insects. These end products have a crucial role in growth, differentiation, sexual maturation, and reproduction. 3-Hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) synthase (EC 4.1.3.5.) has generally been considered one of the committed steps of the pathway. We had previously reported the cloning of a cytosolic HMG-CoA synthase cDNA in *Blattella germanica*; we have now isolated and characterized a new cDNA clone for HMG-CoA synthase in this insect. Analysis of this 1716-base pair cDNA reveals a deduced protein of 455 residues with a molecular mass of 51,424 Da. The two HMG-CoA synthases have 69% identical amino acid residues, and both lack an N-terminal leader peptide to target the protein into mitochondria. This HMG-CoA synthase cDNA can revert the Chinese hamster ovary-K1-derived cell line, Mev-1, which is a defective mutant for HMG-CoA synthase. Both HMG-CoA synthase genes are expressed differently throughout development. Analysis of adult tissues shows higher expression in ovary and fat body. The expression of HMG-CoA synthase (EC 4.1.3.5.) and reductase (EC 1.1.1.34) genes during the gonadotrophic cycle in *B. germanica* shows that the three genes of the isoprenoid pathway are developmentally regulated in the ovary.

The isoprenoid pathway leads, in vertebrates, to a wide range of final products that are vital for diverse cellular processes such as cholesterol synthesis, glycosylation of proteins, growth control, and synthesis of several hormones. A fine control mechanism regulates the biosynthesis of mevalonate, involving, at least, 3-hydroxy-3-methylglutaryl coenzyme A (HMG-CoA)[1] synthase and HMG-CoA reductase genes and proteins in a complex pattern with transcriptional, post-transcriptional, and post-translational mechanisms (see Ref. 1 for a review).

Insects do not have squalene synthase (farnesyl-diphosphate farnesyl transferase) (EC 2.5.1.21) or lanosterol synthase (EC 5.4.99.7) (2) and, consequently, they cannot produce cholesterol *de novo*, although the mevalonate pathway leads to other specific isoprenoids such as the juvenile hormones (JH), which are synthesized by a pair of retrocerebral glands called *corpora allata*. JH have a crucial role in the maintenance of larval form and also in the maturation of the reproductive system (3).

Since HMG-CoA synthase and HMG-CoA reductase are generally considered as the rate-limiting steps in vertebrate cholesterol biosynthesis (1), it has been suggested that they could also be rate-limiting enzymes in JH biosynthesis (4, 5), and some enzyme studies have been carried out in *Manduca sexta* and *Locusta migratoria corpora allata* (6, 7). The HMG-CoA reductase gene has been characterized in *Drosophila melanogaster* (8) and, more recently, in *Blattella germanica* (9). However, up to now little is known about the role of HMG-CoA synthase, or the mevalonate pathway, in other relevant physiological processes in insects such as the maturation of the reproductive system, studies which have been hampered by the lack of suitable molecular tools. In *B. germanica*, as in many other insects, the development and growth of the organs constituting the internal reproductive system is largely influenced by JH, which regulates the successive reproductive cycles (10, 11). Among these organs, however, the ovary has a pivotal role in general endocrine homeostasis, since, in addition to being a target for JH, it synthesizes other endocrine factors, such as ecdysteroids (12), which seem to influence peripheral tissues, through a cyclic developmental pattern. Further, during development, the ovary itself and, to a lesser extent, the accessory reproductive glands, establish the background against which differentiation processes take place (13).

We have previously reported the cloning and characterization of an HMG-CoA synthase, HMGS-1 (14), from *B. germanica*. We now describe the isolation and characterization of a new cDNA coding for a second cytosolic HMG-CoA synthase in this insect, HMGS-2. We have analyzed its expression pattern throughout the different developmental stages and in different tissues. Furthermore, we show that both genes are coordinately regulated in the ovarian development of this insect. We have also analyzed the HMG-CoA reductase mRNA levels in the ovarian development, as well as the relationship between the respective enzymatic activities.

EXPERIMENTAL PROCEDURES

Materials—The oligonucleotides used in the PCR experiments were synthesized by Operon Technologies. Radioactive compounds were obtained from Amersham Corp. The pCMV-2 eukaryotic expression vector was a gift from Dr. M. Stinsky (Department of Microbiology, School of

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The nucleotide sequence(s) reported in this paper has been submitted to the GenBank™/EMBL Data Bank with accession number(s) X77516.

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[1] The abbreviations used are: HMG-CoA, 3-hydroxy-3-methylglutaryl-coenzyme A; PCR, polymerase chain reaction; OST, synthase oligonucleotide set; JH, juvenile hormone; bp, base pair(s).

Medicine, University of Iowa). The Chinese hamster ovary-derived cells, Mev-1, were a gift from Dr. M. Sinensky (Eleanor Roosevelt Institute, Denver, CO). Clone pST-365 contains a partial HMG-CoA synthase cDNA from *B. germanica* obtained by PCR using the degenerate oligonucleotides described below. Clone λBgST1–18 contains the longest HMG-CoA synthase cDNA obtained by screening of the cDNA library from *B. germanica*.

Insects—Specimens of *B. germanica* were taken from a colony reared in the dark at 30 ± 1 °C and 60–70% relative humidity. All the specimens were carefully dated from freshly ecdysed adults. The tissues chosen for study of mRNA levels or ovaries dissected for analysis of enzymatic activities were explanted by standard surgical methods. In the above conditions of insect rearing, the first cycle of ovarian development (previtellogenic growth, vitellogenesis, and chorionation) lasted 7 days. To study mRNA levels during development, RNA was isolated from whole specimens of both sexes in the middle of each larval instar. In the case of embryos, RNA was isolated from 4-, 12-, and 17-day-old ootheca from mated females in which the presence of spermatozoids in the spermatheca had been established.

PCR Conditions—Two sets of degenerate oligonucleotides derived from highly conserved amino acid sequences of the HMG-CoA synthase N-terminal region were used in PCR experiments as previously described (14). OST_1 is a sense 20-mer corresponding to the residues 13–19 of the hamster protein. OST_2 is a reverse 20-mer corresponding to residues 128–134 of the hamster protein. They are as follows: OST_1 5′-TGG CCN AAR GAY GTN GGN AT-3′; OST_2 5′-GCN GTN CCN CCR TAR CAN GC-3′.

RNA Blot Analysis—B. germanica RNA from the whole organism and from different tissues was isolated as described elsewhere (15) with minor modifications. Poly(A)-rich RNA was purified by oligo(dT)-cellulose chromatography according to Aviv and Leder (16). RNA samples were fractionated in 1% agarose/formaldehyde gels, transferred to Nytran-N membranes (Schleicher & Schuell) and UV cross-linked. Hybridizations were carried out as described (17) using either pST-365 or λBgST1–18 as a probe, and washes were performed at 68 °C with 0.2 × SSC and 0.1 × 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 with the mRNA of the other *B. germanica* HMG-CoA synthase previously reported (14). mRNA levels were measured by densitometry of the autoradiograms with a Molecular Dynamics computing densitometer. Densitometry values were corrected using mouse cyclophilin (18) as a constitutive probe. Filters were dehybridized either in water at 100 °C for 10 min or in 50% formamide/6 × SSPE at 70 °C for 2 h (1 × SSPE is 0.15 M NaCl, 10 mM sodium phosphate, pH 7.4, 5 mM Na_2EDTA). mRNA levels of 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.

Construction and Screening of λgt-10 cDNA Libraries—Poly(A)-rich RNA from adult females of *B. germanica* was used to generate oligo(dT)-primed double-stranded cDNA according to Gubler and Hoffman (19). 500,000 plaque-forming units of the library were screened using pST-365 as a probe. The positive phages were purified by two additional rounds of plaque screening and were then amplified.

DNA Sequencing—PCR amplification products and cDNA clones were subcloned into pBluescript vectors (Stratagene) and sequenced by the dideoxynucleotide chain termination method (20) with modified T7 DNA polymerase (Sequenase, U. S. Biochemical Corp.).

Expression of B. germanica λBgST1–18 HMG-CoA Synthase in Mev-1 Cells—Clone λBgST1–18 was digested with *Eco*RI and *Bgl*II. The *Bgl*II site is located in the 3′-untranslated region. The 1601-bp fragment isolated was subcloned into a pCMV-2 expression vector previously linearized with *Eco*RI and *Bgl*II. The new plasmid called pCMVST-1ex was used to perform stable transfection of Mev-1 cells, which are defective Chinese hamster ovary mutants cells for HMG-CoA synthase. Mev-1 cells were cultured in Ham's F-12 medium supplemented with 5% fetal calf serum and 430 μM of mevalonate, and transfections were carried out by the standard calcium phosphate method (21). After transfection, the trypsinized cells were cultured in Ham's F-12 medium supplemented with 5% fetal calf serum but without mevalonate, and the plates were maintained for 8–10 days. By this time, several clones of revertant cells were distinguishable. These revertants were called MEV-1SB.

Assay of Ovarian HMG-CoA Reductase Activity—Ovaries were explanted every day of the gonadotrophic cycle. Individual ovary pairs were prepared with a Dounce homogenizer in 100 μl of TEN buffer (40 mM Tris HCl, pH 7.6, 1 mM EDTA, 150 mM NaCl) and two aliquots of 25 μl were assayed in parallel. In total, four to eight ovary pairs were analyzed for each day of the cycle. HMG-CoA reductase activity was determined in the ovarian tissue by the radiometric method described by Goldstein *et al.* (22). 1 unit of HMG-CoA reductase is defined as the amount of enzyme that converts 1 nmol of HMG-CoA into mevalonate in 1 min at 37 °C.

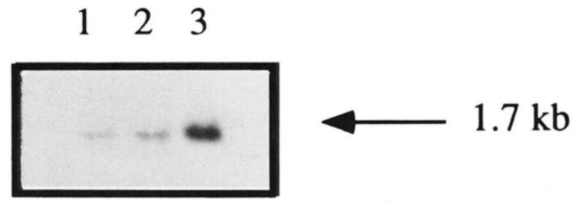

FIG. 1. **mRNA levels of *B. germanica* HMG-CoA synthase-2 using the amplified product as a probe.** Poly(A)-rich RNA (5 μg/lane) from sixth instar larvae (*lane 1*), from isolated heads (*lane 2*), and from whole bodies of 6-day-old adult females (*lane 3*) were run in 1% agarose-formaldehyde gel and transferred to a Nytran-N membrane (Schleicher & Schuell) and hybridized against pST-365. Washes were carried out at 68 °C in 0.2 × SSC, 0.1% SDS. RNA markers (Promega) were used to estimate transcript size.

Assay of HMG-CoA Synthase Activity—Three pooled ovary pairs from each day of the gonadotrophic cycle were processed as above, and two aliquots of 50 μl were assayed. For each day of the cycle, three different pools were measured. HMG-CoA synthase activity was determined in the ovarian tissue by the radiometric method described by Clinkenbeard *et al.* (23), as modified by Gil *et al.* (24) except using 5-fold higher specific radioactivity. 1 unit of HMG-CoA synthase is defined as the amount of enzyme that catalyzes the formation of 1 μmol of HMG-CoA in 1 min at 37 °C.

RESULTS

Isolation of a Second cDNA for HMG-CoA Synthase (HMGS-2) from B. germanica—The analysis of the primary structures of rat mitochondrial (25) and cytosolic HMG-CoA synthases from hamster (24) and rat (26) revealed several highly conserved sequences in the N-terminal region containing the catalytical domain. Two of these motifs were selected to derive oligonucleotides to be used as primers in PCR experiments. Following this approach, a cDNA encoding for an HMG-CoA synthase in *B. germanica* was first obtained, as we have previously reported (14). However, the PCR amplification method yielded a new cDNA fragment (365 bp), which is slightly longer than that previously described. Sequence analysis of this amplification product showed an open reading frame of 121 amino acids with high identity (72%) to the other *B. germanica* HMG-CoA synthase.

Analysis of *B. germanica* mRNA in isolated heads, whole adult bodies, and last instar larvae using this cDNA as a probe revealed a transcript size (1.7 kilobases), which was similar to the HMGS-1 previously reported (Fig. 1). Furthermore, this analysis showed that the expression of this new HMG-CoA synthase gene (HMGS-2) was higher in adults than in sixth instar larvae, in contrast with data obtained for HMGS-1 gene. However, both HMGS genes showed higher expression in whole body than in isolated heads.

In a screening of a λgt-10 cDNA library from whole adult females, several positive clones were isolated and characterized by restriction mapping. After subcloning into pBluescript, clone λBgST1–18, which contained the longest insert, was sequenced to reveal an insert of 1716 bp (Fig. 2) in close agreement with the mRNA size estimated by Northern blot analysis (1.7 kilobases). This sequence showed, after the first ATG, an open reading frame of 1365 bp, which codes for a protein of 455 amino acids, with a predicted molecular mass of 51,424 Da and a theoretical isoelectric point of 5.33. The 5′- and 3′-untranslated regions were 96 and 255 bp, respectively. Two putative polyadenylation sites were found at positions 1543 and 1587.

Comparison with Other HMG-CoA Synthases—Alignments of HMGS-2 with cytosolic HMG-CoA synthases of rat (26), ham-

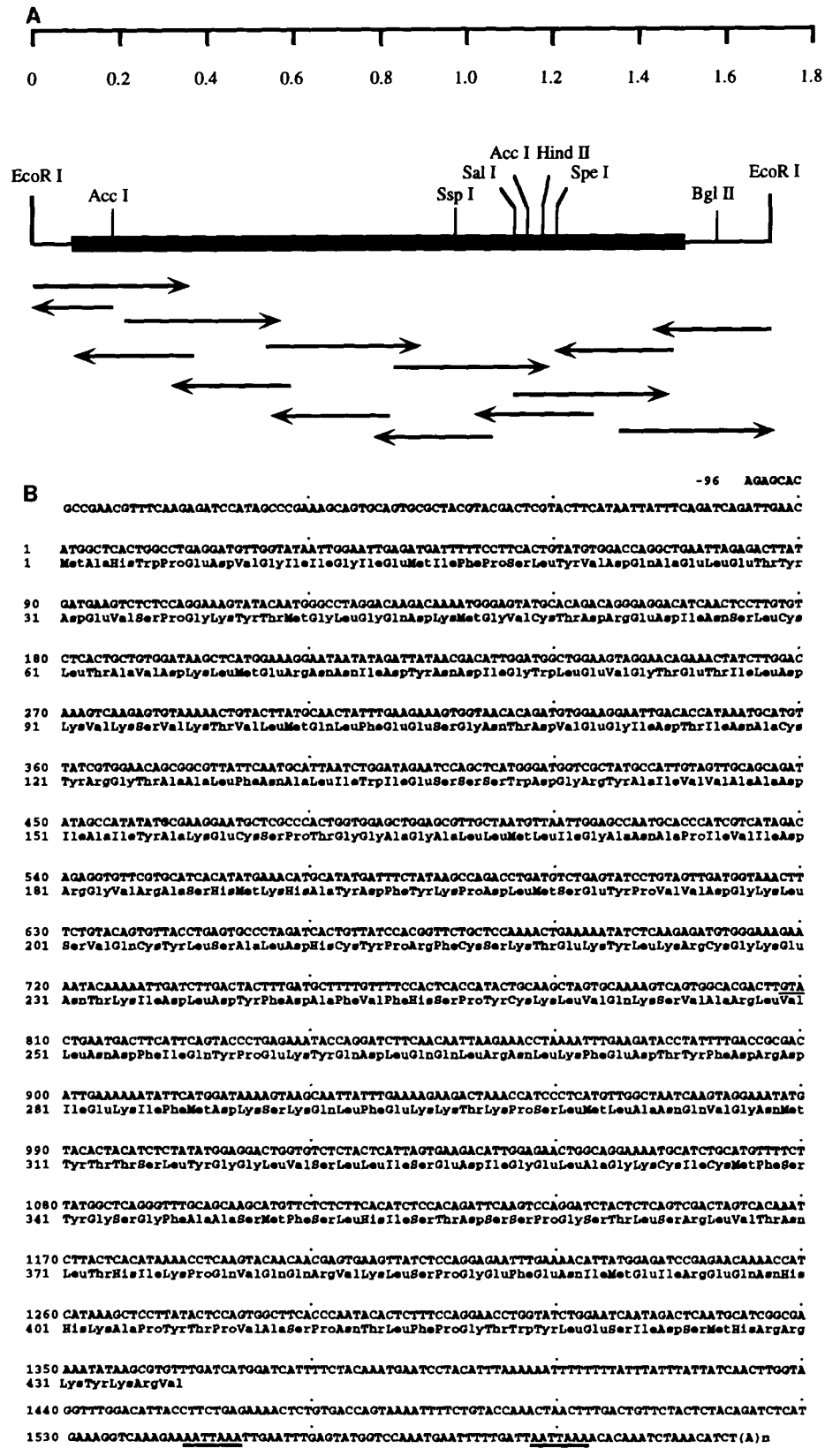

FIG. 2. **Restriction endonuclease map (*A*) and nucleotide and deduced amino acid sequences of the cDNA encoding *B. germanica* HMG-CoA synthase-2 (*B*).** *A*, *solid bars* indicate the coding sequence. Scale at the *top* is in kilobases. *Arrows* indicate the start site, direction, and strand sequenced. *B*, *numbers* indicate nucleotide and amino acid positions. The putative polyadenylation sites are *underlined*.

ster (24), chicken (27), and humans (28) as well as with rat mitochondrial HMG-CoA synthase (25) and HMGS-1 of *B. germanica* (14) were obtained using the CLUSTAL program (29). The alignment (not shown) revealed an extensive homology, which, in terms of identity of residues, ranged from 70% with HMGS-1 of *B. germanica* to about 60% with the cytosolic vertebrate proteins and 55% with the rat mitochondrial protein. Glycines and prolines, which are scattered along the sequence, appear to be highly conserved. The level of conservation of cysteine residues is also high, and it is worth noting that HMGS-2, like all the other HMG-CoA synthases, has a conserved cysteine in relative position 120. This residue is involved

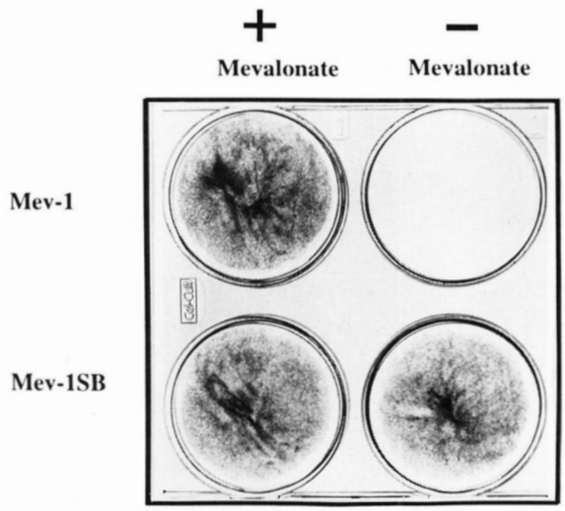

FIG. 3. **Validation of HMGS-2 clone as HMG-CoA synthase.** A 1601-bp fragment of HMGS-2 containing the whole coding region was subcloned into pCMV-2 eukaryotic expression vector. Mev-1, an HMG-CoA synthase mutant cell line, was transfected with this construction and with the plasmid control. HMGS-2 was able to revert the mutant phenotypically as a result of a stable transfection. This new line, Mev-1SB, was now able to grow in the absence of mevalonate, whereas Mev-1 transfected with control plasmid died in a few hours. Cells were stained with 0.1% methylene blue in 50% methanol.

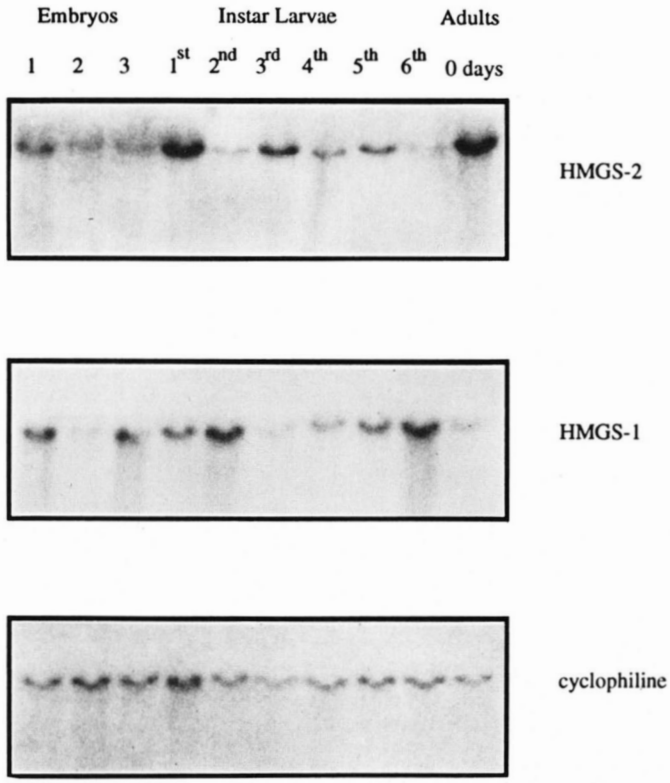

FIG. 4. **Analysis of *B. germanica* HMG-CoA synthase-2 mRNA levels throughout development.** Total RNA (20 μg) from 4-, 12-, and 17-day-old embryos (*lanes 1–3*), from sixth instar larvae (*lanes 4–9*), and from recently emerged whole adult female (*lane 11*) were run in 1% agarose-formaldehyde gel, transferred to a Nytran-N membrane (Schleicher & Schuell), and hybridized against λBgST1–18 insert as a probe. Washes were carried out at 68 °C in 0.2 × SSC, 0.1% SDS. RNA markers (Promega) were used to estimate transcript size. The membrane was also probed against HMGS-1 (see Ref. 14) and against mouse cyclophilin as a constitutive probe.

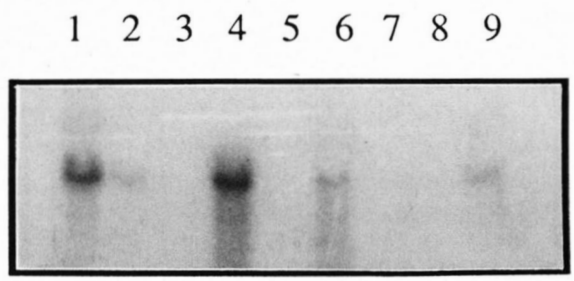

FIG. 5. **Analysis of *B. germanica* HMG-CoA synthase-2 mRNA levels in different adult tissues.** Total RNA (8 μg) from 2-day-old female fat body (*lane 1*), testes (*lane 2*), 6-day-old female fat body (*lane 3*), 2-day-old female ovary (*lane 4*), 2-day-old male accessory gland (*lane 5*), 2-day-old female left colleterial gland (*lane 6*), muscle (*lane 7*), gut (*lane 8*), and brain (*lane 9*) were run in 1% agarose-formaldehyde gel and transferred to a Nytran-N membrane (Schleicher & Schuell) and hybridized against λBgST1–18 insert as a probe. Washes were carried out at 68 °C in 0.2 × SSC, 0.1% SDS. RNA markers (Promega) were used to estimate transcript size.

in the acetylation of the enzyme (30). HMGS-2, like HMGS-1, is shorter than the vertebrate proteins, lacking 11 and 49–50 residues in the N- and C-terminal regions, respectively.

λBgST1–18 cDNA Corresponds to HMG-CoA Synthase—To validate λBgST1–18 as HMG-CoA synthase, a 1601-bp *Eco*RI/*Bgl*II fragment containing the 5′-untranslated region, the whole coding region, and 140 bp of 3′-untranslated region was subcloned into pCMV-2 eukaryotic expression vector. In this vector, the termination signal is provided by the 3′-untranslated region of human growth hormone gene, which contributes about 400 bp to the fusion transcript. The transfection of this cDNA in a Chinese hamster ovary-derived cell line, Mev-1, defective mutant for HMG-CoA synthase activity, produced colonies able to grow in the absence of mevalonate as a result of a stable transfection. When Mev-1 were transfected only with the plasmid control and transferred to a medium without mevalonate, all the cells died in a few hours (Fig. 3). The presence of the fusion transcript HMGS2-human growth hormone was assessed in Mev-1SB by Northern blot analysis (data not shown). This result, together with the alignments discussed above, was an indication that the cDNA cloned was indeed that corresponding to HMG-CoA synthase.

Developmental Pattern and Tissue Expression of B. germanica HMGS-2—The expression of HMGS-2 mRNA throughout *B. germanica* development was analyzed using λBgST1–18 cDNA as a probe (Fig. 4). HMGS-2 mRNA levels were higher in early embryos (4 days) than in 12- or 17-day-old embryos. In addition, the highest HMGS-2 mRNA levels were detected in first and third instar larvae, whereas the levels remained low in the other instar larvae and increased again after the imaginal molt. Northern blot of different tissues showed a high expression of HMGS-2 in previtellogenic ovary and fat body, whereas the mRNA levels were very low in brain, testes, and left colleterial glands. No expression was found in muscle, male accessory glands, nor gut. (Fig. 5).

HMG-CoA Synthase and Reductase Activity in B. germanica Ovary—HMG-CoA synthase and HMG-CoA reductase activities showed a marked cycle during the ovarian gonadotrophic cycle (Fig. 6). HMG-CoA synthase activity showed steady, low levels in days 0–3 with a basal activity of 5–10 pmol of HMG-CoA/ovary equivalent and minute and, thereafter, there was a marked rise to maximal levels on day 6 of about 90 pmol of HMG-CoA/ovary equivalent and minute. On day 7, the HMG-CoA synthase activity fell again, reaching the basal levels on days 8–10. On the other hand, HMG-CoA reductase activity showed low levels during the first days of the gonadotrophic cycle, with basal levels of 1–2 nmol of mevalonate/ovary equivalent and minute. On day 5, the HMG-CoA reductase activity rose, reaching a maximum on day 7, with levels of about 10 nmol of mevalonate/ovary equivalent and minute. The measurement corresponding to days 8–10 refer to ovaries that hav-

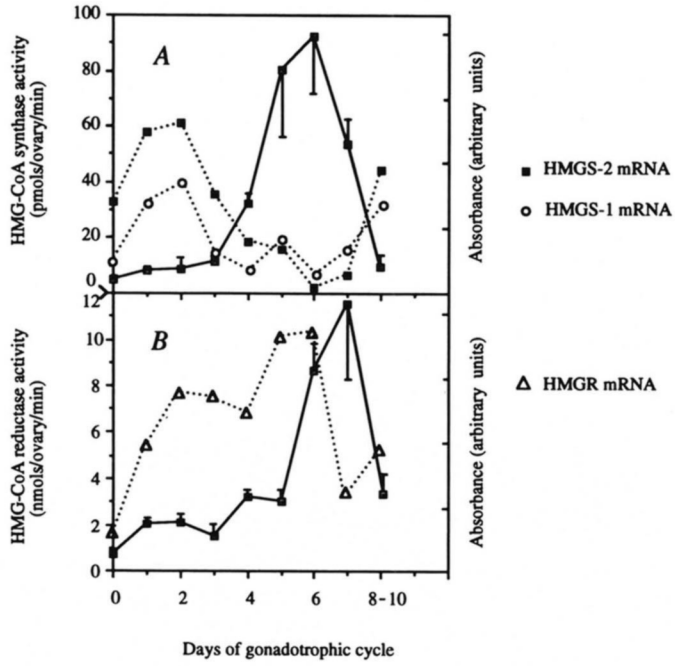

FIG. 6. **HMG-CoA synthase and HMG-CoA reductase activities in *B. germanica* ovary.** Surgically extracted ovaries from each day of the gonadotrophic cycle were assayed for HMG-CoA synthase activity (*A*). The levels are indicated in microunits (pmol)/ovary equivalent/min. HMG-CoA reductase activity levels (*B*) are indicated in units (nmol)/ovary equivalent/min. *Vertical bars* show the variance (σ_n).

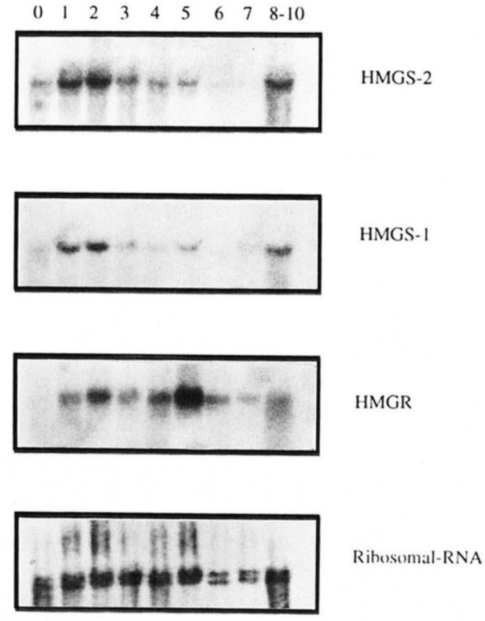

FIG. 7. **HMGS-1, HMGS-2, and HMGR mRNA levels in *B. germanica* ovary.** Total RNA (10 μg) from surgically extracted ovaries from each day of the gonadotrophic cycle were run in 1% agarose-formaldehyde gel, transferred to a Nytran-N membrane (Schleicher & Schuell), and hybridized against λBgST1–18 insert, HMGS-1, and HMG-CoA reductase cDNA of *B. germanica* as probes. Washes were carried out at 68 °C in 0.2 × SSC, 0.1% SDS. RNA markers (Promega) were used to estimate transcript size.

ing completed the first gonadotrophic cycle undergo a subsequent cycle with new basal oocytes.

HMGS-1, HMGS-2, and HMG-CoA Reductase mRNA Levels in B. germanica Ovary—To analyze the mRNA levels, 15–30 ovary pairs from each day of the gonadotrophic cycle were processed to obtain total RNA. Levels of HMGS-2 mRNA increased just after imaginal ecdysis and were maximal on days 1 and 2, then decreased steadily until they were almost undetectable on days 6 and 7, and increased again on days 8–10 (a representative experiment is shown in Fig. 7). A similar profile was obtained for HMGS-1. On the other hand, HMG-CoA reductase mRNA levels showed a rise on days 1 and 2, but, thereafter, instead of decreasing, they remained stable on days 3 and 4, increased again to reach maximal expression on days 5 and 6, and decreased sharply on day 7. The histone mRNA levels were also checked as a marker of DNA division using as a probe a 328-bp *Bam*HI/*Hin*dIII fragment of corn H4 histone (31). At moderate astringency conditions, the *B. germanica* ovary histones hybridized well with this probe and showed a fairly constant expression after day 1 (data not shown).

DISCUSSION

The PCR approach to the isolation of *B. germanica* HMG-CoA synthase resulted in the amplification of an additional HMG-CoA synthase cDNA (pST-365), which is different from that previously reported, HMGS-1 (14). In insects, the organs that apparently present the highest specific activity for both HMG-CoA synthase and HMG-CoA reductase enzymes are the *corpora allata* (32), which are located in the head. However, the expression of this new HMG-CoA synthase, HMGS-2, in isolated heads was, similarly to HMGS-1, lower than in the whole body, indicating a poor mass contribution of *corpora allata* RNAs to the head pool. Because the HMGS-2 mRNA levels in adults were higher than those of HMGS-1, a new cDNA library was constructed from adult females. In this library, pST-365 was successfully used to isolate full-length cDNA clones of this second gene, HMGS-2.

HMGS-2 and HMGS-1 proteins share extensive conservation with almost 70% of identical amino acid residues. This conservation is also high with the other known HMG-CoA synthases, and most of the conserved amino acid residues in the six known HMG-CoA synthases are also conserved in HMGS-2, including the catalytic cysteine in position 120. The recovery of the mutant cells Mev-1 by transfection with the isolated cDNA demonstrates that HMGS-2 shows HMG-CoA synthase activity. Thus *B. germanica* seems to have two different genes coding for this activity. We have considered the possibility that one of these proteins could be a mitochondrial enzyme. There is no consensus sequence for targeting to mitochondria, but neither HMGS-2 nor HMGS-1 shares the common transient N-terminal targeting presequences features (33). This strongly suggests that both enzymes present in *B. germanica* are cytosolic. This is the first report of two cytosolic HMG-CoA synthases in an animal system.

In early embryos (4-day-old), HMGS-1 and HMGS-2 showed a similar high expression. In this stage, the *B. germanica* embryo is already well segmented in preparation for dorsal closure and full organogenesis (34). In *Drosophila* (8) and also in *B. germanica* (9), the highest HMG-CoA reductase mRNA levels were also found in early embryos. Coordinated expression of HMG-CoA reductase, dolichol production, and glycoproteins biosynthesis has been postulated in sea urchin development, which may have a crucial role in the pattern development, since tunycamicine treatments cause failure in the formation of the gastrula, and this can be reverted with treatments with dolichol (35). Thus, considering the HMG-CoA reductase expression in *B. germanica* early embryos, a similar function for both HMG-CoA synthases could be postulated in this species. However, when the development pattern advances, this coordinate high expression disappears, and the HMG-CoA synthases seem to be differentially regulated in the later embryo stages.

Such differential regulation is observed in larval development. This is especially evident in the last four instar larvae in which HMGS-2 mRNA levels show a steady decrease, whereas

HMGS-1 mRNA levels undergo a steady increase. This situation is inverted following the imaginal ecdysis, when HMGS-1 mRNA levels fall, whereas HMGS-2 mRNA levels rise. These data suggest a kind of complementation between the HMG-CoA synthases during larval development. HMGS-1 may be related with the processes of organogenesis of secondary sexual structures preceding metamorphosis, whereas the reactivation of HMGS-2 after the imaginal molt may be related with sexual maturation.

The analysis of different adult organs showed that the highest levels of HMGS-2 mRNA were found in previtellogenic ovary and fat body, while lower levels were detected in testis, brain, and left colleterial glands. No expression was found in gut, male accessory glands, nor in muscle. In contrast with the expression throughout development, the expression of the two HMG-CoA synthases was very similar in adult tissues. We previously reported no expression of HMGS-1 in adult fat body of day 6, and, in this stage, a similar poor expression was found for HMGS-2 mRNA, but when previtellogenic fat body (days 1 and 2) was analyzed, a clear expression of HMGS-1 was also found (data not shown). This suggest that fat body could undergo a cyclic modulation of the mevalonate pathway, as occurs in the ovary, and we shall discuss below.

The mevalonate pathway has been extensively correlated with the cell cycle and DNA division in many systems (36, 37), and it is also well known that in some insects the ovary can undergo polyploidy throughout its development (38, 39). As histone H4 mRNA is known to be preferentially expressed in young tissues with high mitotic activity (31), we analyzed the H4 expression in *B. germanica* ovary as a marker of DNA division. However, the histone levels detected in the ovary of *B. germanica* was stable and high from day 1. This result contrasts with the regulation of both HMG-CoA synthases and HMG-CoA reductase in this organ, which suggests that only a small part of the mevalonate flux is responsible for the plausible polyploidy of the *B. germanica* ovary. Therefore, a role for the mevalonate pathway mainly related with sexual maturation of the insect should be considered.

The ovarian expression of the three genes HMGS-1, HMGS-2, and HMG-CoA reductase have been analyzed in detail. There is a striking delay between the increase in mRNA levels and the rise of the respective enzymatic activities of 48–72 h for both HMG-CoA synthases and 24–48 h for HMG-CoA reductase. This phenomenon of mRNA storage has been described extensively (40), especially in growing oocytes in which a large fraction of mRNA synthesized is not used for immediate translation but is dormant or masked and stored for future translation. Most stored messages are activated at various times during oocyte maturation and early embryonic development when little or no mRNA is produced. A mechanism involving the rate of deadenylation and the occurrence of specific sequences in the 3′-untranslated region has been proposed (41). These specific sequences, which are located at a variable 5′ distance to the polyadenylation signal, are known as cytoplasmic polyadenylation signal and have a consensus sequence UUUUUAU. Both HMG-CoA synthases show putative cytoplasmic polyadenylation signals close to the polyadenylation signal, whereas HMG-CoA reductase has a putative cytoplasmic polyadenylation signal at position 3005, *i.e.* 223 nucleotides upstream of the polyadenylation signals. Thus, in the regulation of mevalonate production, the asynchronism between mRNA and enzymatic activity could be considered as forming part of the general program of oocyte development with its specific temporal pattern. This pattern results in high activities of the enzymes involved in the synthesis of mevalonic acid in the later moments of the oocyte maturation, when vitellogenesis is most active and the follicle cells prepare for choriogenesis (42). The importance of glycosylation processes during chorion formation has been reported in other insects (43, 44), in the cattle tick (45), and in *Schistosoma mansoni* (46), and it can be postulated that an substantial part of the mevalonate flux is directed to the dolichol involved in glycosylation of chorion proteins.

On the other hand, HMG-CoA reductase activity is delayed about 24 h with respect to the maximal HMG-CoA synthase activity. This, together with the fact that HMG-CoA synthase is a much slower enzyme than HMG-CoA reductase, suggests that the limiting step in the mevalonate pathway in the ovary could be the availability of HMG-CoA in the tissue. A similar model has been proposed in rat hepatocytes (47) and in rat liver in experiments *in vivo* (48).

The existence of two different genes for HMG-CoA synthase and the temporally well established pattern of activation of the mevalonate pathway in the maturation of *B. germanica* ovary highlight the importance of this pathway in insect reproduction. Our work represents a preliminary approach to understanding the molecular events involved in the sexual maturation of insects. However, further studies are necessary to elucidate the fine mechanisms involved in the endocrine and molecular control of this pathway in the insect ovary.

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