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Developmental and Metabolic Regulation of the *Drosophila melanogaster* 3-Hydroxy-3-Methylglutaryl Coenzyme A Reductase

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The enzyme 3-hydroxy-3-methylglutaryl coenzyme A (HMG CoA) reductase in *Drosophila melanogaster* synthesizes mevalonate for the production of nonsterol isoprenoids, which are essential for growth and differentiation. To understand the regulation and developmental role of HMG CoA reductase, we cloned the *D. melanogaster* HMG CoA reductase gene. The nucleotide sequence of the *Drosophila* HMG CoA reductase was determined from genomic and cDNA clones. A 2,748-base-pair open reading frame encoded a polypeptide of 916 amino acids (M_r, 98,165) that was similar to the hamster HMG CoA reductase. The C-terminal region had 56% identical residues and the N-terminal region had 7 potential transmembrane domains with 32 to 60% identical residues. In hamster HMG CoA reductase, the membrane regions were essential for posttranslational regulation. Since the *Drosophila* enzyme is not regulated by sterols, the strong N-terminal similarity was surprising. Two HMG CoA reductase mRNA transcripts, ~3.2 and 4 kilobases, were differentially expressed throughout *Drosophila* development. Mevalonate-fed Schneider cells showed a parallel reduction of both enzyme activity and abundance of the 4-kilobase mRNA transcript.

Cholesterol is important for structural purposes in the membranes of vertebrates, but recent studies have also elucidated its role in regulating the expression of certain genes (5). The rate-controlling enzyme for cholesterol synthesis, 3-hydroxy-3-methylglutaryl coenzyme A (HMG CoA) reductase, is regulated by cholesterol in a complex pattern with both transcriptional (28, 34) and posttranslational (8, 19) mechanisms. As a typical housekeeping gene, the HMG CoA reductase gene is usually transcriptionally active. Its steady-state activity is subject to multivalent feedback suppression (4) by at least two classes of metabolic products derived from its immediate product, mevalonate. Cholesterol, the major mevalonate-derived product of mammalian cells, suppresses both HMG CoA reductase activity and its mRNA levels in cultured cells (10) and rat liver (26). Pretreatment of cells with cholesterol also prevented transcription from the gene in isolated nuclei (28), and the hamster HMG CoA reductase promoter remained cholesterol sensitive when transfected into cultured cells (34). Posttranslational regulation was revealed by the accelerated degradation of the enzyme in response to exogenous cholesterol (8). Thus, cholesterol has regulatory effects in both the nucleus and cytoplasm.

Owing to the rapid and high conversion of mevalonate to sterols in mammals, it has been difficult to assess the sterol-independent regulatory pathway of HMG CoA reductase. Studies of this pathway suggested that the basal regulation was mediated by unidentified nonsterol mevalonate derivatives (16, 47). These and other life-essential isoprenoids such as dolichol or ubiquinone may also participate in processes ranging from growth control (20, 36) to development (6).

To characterize the role of HMG CoA reductase in development and to unravel the regulatory mechanisms of this nonsterol pathway, we chose to study the HMG CoA reductase of *Drosophila melanogaster* as a model system for several reasons. First, *D. melanogaster* has a well-characterized pattern of development, and rich molecular and genetic tools are available. Second, two biochemical aspects of insect metabolism should aid in these studies: (i) insects are incapable of sterol synthesis (21), and (ii) the developmentally important juvenile hormones (JH) are isoprenoid derivatives. Recent studies of insect HMG CoA reductases have revealed a relatively simple regulation (3) and a role in the biogenesis of the JH which facilitate vitellogenesis (35) and possibly spermatogenesis (44). The evidence for potential HMG CoA reductase regulation of JH synthesis has been derived from characterization of isolated corpora allata, the principal site of JH synthesis, of the tobacco hornworm. These studies have shown that JH synthesis can be blocked by mevalonate analogs (37) and competitive inhibitors of HMG CoA reductase (33) but is stimulated 100-fold by the addition of a key JH intermediate derived from mevalonate, farnesenic acid (45). Although the tobacco hornworm evidence suggests that the availability of mevalonate is essential for JH synthesis, studies in the cockroach corpora allata suggest that HMG CoA reductase may not be rate limiting for JH synthesis (18).

Like the mammalian enzyme, the HMG CoA reductase of cultured *Drosophila* cells (Kc and Schneider cells) appears to be a microsomal enzyme whose activity is modulated by the addition of exogenous mevalonate. In contrast to the hamster enzyme, exogenous sterols have no effect upon the *Drosophila* HMG CoA reductase (3, 32, 43) and thus allow a clear separation of the sterol and nonsterol regulatory pathways. Since insects lack the ability to synthesize sterols and their HMG CoA reductase is insensitive to sterol regulation, one might conclude that insects either lost or never acquired the downstream genes necessary for sterol synthesis and the associated sterol feedback system. However, with the diverse and essential roles that isoprenoids (6, 30) and isoprenyl modified proteins (40, 46) play in development, we

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expect to discover common mechanisms underlying the basal mevalonate regulation of both mammalian and *Drosophila* HMG CoA reductases. In this report, we describe the isolation of the *Drosophila* HMG CoA reductase gene and characterize its regulation and developmental expression.

MATERIALS AND METHODS

Materials. We obtained [γ-^{32}P]ATP from ICN Pharmaceuticals, Inc. (Irvine, Calif.), [α-^{32}P]ATP from Amersham Corp. (Arlington Heights, Ill.), DL-3-hydroxy-3-methyl-[3-^{14}C]glutaryl coenzyme A from New England Nuclear Corp. (Boston, Mass.); T4 DNA polymerase from International Biotechnology (New Haven, Conn.); T4 polynucleotide kinase, Sequenase, and the Klenow fragment of *Escherichia coli* DNA polymerase from US Biochemical (Cleveland, Ohio); and T4 ligase and restriction enzymes from Pharmacia Fine Chemicals (Piscataway, N.J.); Boehringer Mannheim Biochemicals (Indianapolis, Ind.), and Bethesda Research Laboratories (Gaithersburg, Md.). Oligonucleotides were provided by the Biomolecular Resource Center at the University of California at San Francisco and Elutips were from Schleicher & Schuell Co. (Keene, N.H.).

Screening of genomic *Drosophila* library. Replicate nitrocellulose filters from a λ Charon 4A library (2.4×10^5 phage or ~10 genome equivalents) containing *Drosophila* DNA inserts (31) were hybridized with an oligonucleotide probe derived from a region conserved in both *Saccharomyces cerevisiae* and hamster HMG CoA reductases (1)—corresponding to residues 685 through 699 of the hamster sequence Asn-Tyr-Cys-Thr-Asp-Lys-Lys-Pro-Ala-Ala-Ile-Asn-Trp-Ile-Glu. The single most commonly used *Drosophila* codons (C. Zucker, [University of California, San Diego], personal communication) were used to design an antisense probe, and where a clear preference for one codon was not evident an inosine residue was inserted (Thr, ACI; Asp, GAI; Pro, CCI; Ala, GCI) as follows: 5′-CTCGATCCAGT TGATIGCIGCIGGCTTCTTITCIGTGCAGTAGTT-3′. The oligonucleotide probe was labeled by incubating 50 nmol of the purified 45-mer with 1 mCi of [γ-^{32}P]ATP and 10 U of T4 polynucleotide kinase in 70 mM Tris hydrochloride (pH 7.5)–10 mM $MgCl_2$–5 mM dithiothreitol for 1 h at 37°C. The labeled probe was purified by adsorption to an Elutip column to a specific activity of $\sim 1 \times 10^9$ to 2×10^9 cpm/μg.

Screening of λ gt-10 cDNA libraries. Fragments of a positive genomic clone were used to isolate cDNA clones from a λ gt-10 cDNA library (obtained from L. Kauvar, University of California, San Francisco) prepared from late embryonic mRNA. Hybridization probes were prepared by the random primer method (17). A radiolabeled 294-base-pair (bp) *Hin*dIII-*Sac*I fragment was used to isolate clones in a more N-terminal direction, whereas a 290-bp *Eco*RI-*Bam*HI fragment was used to isolate clones in the C-terminal direction. The conditions for screening λ gt-10 bacteriophage have been described (12).

DNA sequencing. Fragments of genomic and cDNA clones were subcloned into M13mp18 and M13mp19 vectors (50). A family of nested deletions of the p42.1 cDNA was generated by the method of Dale et al. (11). Sequencing by the dideoxy-chain termination method (41) with appropriate DNA primers was performed for both DNA strands on buffer gradient gels (2) often containing 20 or 40% (vol/vol) formamide to minimize secondary structure.

Expression of *Drosophila* HMG CoA reductase in *E. coli*. The 775-bp *Sal*I-*Bam*HI fragment of λ Dred-2 and the 550-bp *Bam*HI-*Asp*718 fragment of p10.2 were cloned into a pGEM4 vector (Promega Biotec Co., Madison, Wis.). The intermediate vector, SBA1, was fused to the 321-bp *Pst*I-*Sal*I fragment of p42.1. The resulting vector, pCA1, contained all the sequences of the potential catalytic region of the HMG CoA reductase. A blunt-ended *tac* promoter (14) was inserted into an upstream *Hin*dIII site in the vector designated as pRCA1. An initiator methionine and a proline residue were attached to the catalytic region as a consequence of the reading frame established by the polylinker region. This vector was grown in either JM105 or XL-1 Blue cells and induced with 100 μM isopropyl thiogalactoside.

Cell culture. Schneider cells were seeded at 2×10^5 cells per 75-cm^2 flask in 10% (vol/vol) fetal calf serum in Schneider medium at 22°C. The cells were fed every 3 days until day 6, when cultures received normal medium or medium supplemented with 10 mM mevalonate. The following day, the cells were collected by centrifugation, and RNA was isolated by a modification of the guanidinium thiocyanate method (10). Total RNA was denatured with 5 mM methyl mercury and subjected to electrophoresis as previously described (10).

Measurement of HMG CoA reductase. HMG CoA reductase activity from detergent-solubilized cell extracts was performed as previously described (4). Protein concentration was measured by a modification of the procedure of Lowry et al. (27).

RESULTS

Isolation of the *Drosophila* HMG CoA reductase gene. The strategy for isolation of the *Drosophila* HMG CoA reductase gene was based upon the assumption that an enzyme central to intermediary metabolism should be conserved in evolution. Initial attempts at its isolation by cross-hybridization at reduced stringency with nonoverlapping fragments of the hamster cDNA (9) resulted in the isolation of several clones whose identity could not be verified by sequence analysis. In contrast, a strategy employing a synthetic oligonucleotide probe (see Materials and Methods) designed from a region conserved in both *S. cerevisiae* and hamster HMG CoA reductase genes was successful. From a screen of a *D. melanogaster* genomic library, a single clone, λ Dred-2, was identified by the 45-mer oligonucleotide probe (Fig. 1). Genomic blots containing *Drosophila* DNA were hybridized with the oligonucleotide probe and identified restriction fragments identical in size to genomic fragments hybridized with a 1,030-bp *Bam*HI-*Pst*I probe from λ Dred-2 (data not shown). These data confirmed the *Drosophila* origin of the clone.

Preliminary sequence analysis of a genomic subclone identified the conserved region specified by the oligonucleotide probe and revealed potential amino acid sequences flanking this region that were similar to the hamster sequence. This finding suggested that we had isolated a portion of the *Drosophila* HMG CoA reductase gene. To determine whether this region encoded a mRNA, a 1,030-bp *Bam*HI-*Pst*I genomic fragment was hybridized to poly(A)$^+$ mRNA prepared from various stages of *Drosophila* development (Fig. 2). Hybridization was detected in all developmental stages including the earliest collection point (0- to 2-h embryos), but this 4-kb mRNA may have been of maternal origin. The 4-kb transcript increased in relative abundance in late embryos and was expressed at relatively low levels in larvae, pupae, and adults as compared with levels of actin mRNAs. A ~3.2-kilobase (kb) mRNA was detected at relatively low levels in third-instar larvae and increased in

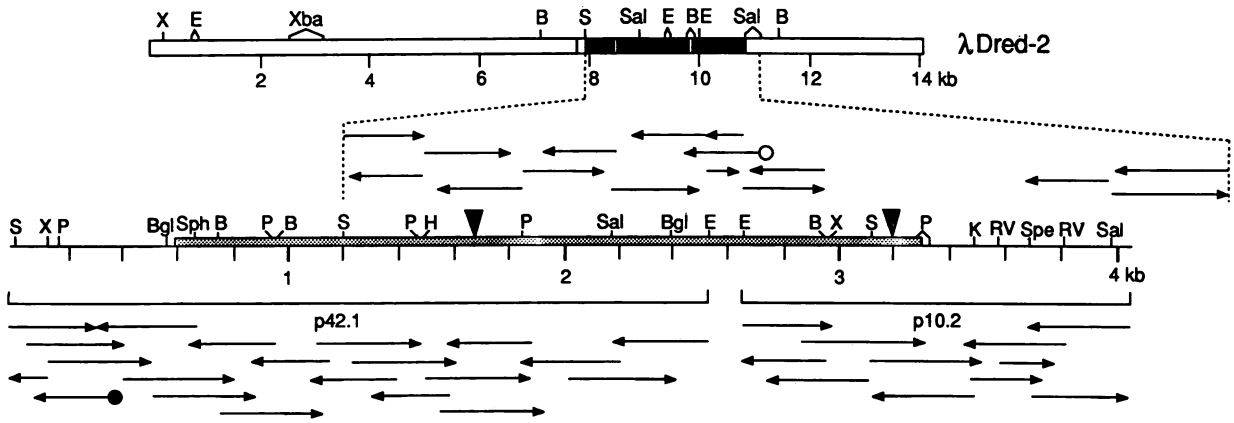

FIG. 1. Strategy for isolation of a *Drosophila* HMG CoA reductase clone and sequencing of two cDNA clones and a genomic subclone. λ Dred-2 was isolated from a genomic library by using an oligonucleotide probe representing a conserved region present in both *S. cerevisiae* and hamster HMG CoA reductases. Eight positive phage were found in the primary screen, but only one phage, λ Dred-2, survived the tertiary screen. The solid region in the upper line represents the region of amino acid similarity identified by the oligonucleotide. This region contains three exons; one of these overlaps the two cDNA clones (p42.1 and p10.2) shown below. The locations of the two introns are indicated by the inverted triangle above the cDNA map. Arrows between the genomic and cDNA maps indicate sequences from the genomic subclone. Arrows below the cDNA map indicate sequences from the cDNA clones. The cross-hatched area in the cDNA map corresponds to the open reading frame of 2,748 bp, and the flanking line represents untranslated regions. The universal M13 primer was used for all sequencing except for the 45-mer oligonucleotide which led to the isolation of λ Dred-2 (○) and a special oligonucleotide primer used to span a gap (●). Abbreviations: B, *Bam*HI; Bg, *Bgl*II; E, *Eco*RI; H, *Hin*dIII; K, *Kpn*I; P, *Pst*I; RV, *Eco*RV; S, *Sac*I; Sal, *Sal*I; Spe, *Spe*I; X, *Xho*I; and Xb, *Xba*I.

pupae and adults to a level equivalent to that of the 4-kb transcript. Taken together, the evidence suggests that one or both poly(A)$^+$ mRNAs are transcribed from a gene that encodes a functional product.

Sequence of the *Drosophila* HMG CoA reductase. To determine the primary structure of the encoded protein, fragments of subcloned genomic DNA were used as hybridization probes to isolate clones from a late embryonic *Drosophila* cDNA library which, as revealed by Northern analysis, contained the highest level of HMG CoA reductase transcripts. The composite sequence of 3,973 bp from two cDNA clones and a 1.75-kbp *Bam*HI-*Sac*I genomic fragment yielded an open reading frame of 2,748 bp that is capable of encoding a 916-amino-acid protein (M_r, 98,165) flanked by 572 and 653 bp of 5′ and 3′ untranslated regions, respectively (Fig. 3). The deduced translational initiator codon was flanked by sequences that conformed to both the general eucaryotic consensus sequence CC [G/A] CCATGG (22) and to the *Drosophila* consensus sequence ANN[C/A]A[A/C] [A/C]ATGN (7). The 5′ untranslated region had two upstream AUG sequences flanked by the first 3 of the 6 in-frame stop codons (positions −191, −266, −272, −324, −367, and −386) and 13 stop codons in the other two frames. No significant similarity between either of the untranslated regions to the corresponding hamster sequences was found. A canonical polyadenylation site (AAUAAA) 24 bp upstream of the poly(A)$^+$ region was found (nucleotide 3353, Fig. 3).

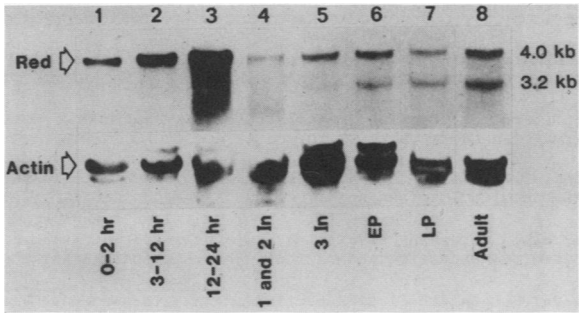

FIG. 2. Developmental expression of *Drosophila* HMG CoA reductase mRNA transcripts. A blot with poly(A)$^+$ mRNA from various stages in *Drosophila* development was hybridized with the 1,030-bp *Bam*HI-*Pst*I fragment of λ Dred-2 for 24 h at 42°C in 50% (vol/vol) deionized formamide–4× SSC (1× SSC is 0.15 M NaCl plus 0.015 M sodium citrate)–10 mM EDTA–0.4% (wt/vol) sodium dodecyl sulfate–2× BFP–0.1 μg of sheared and sonicated calf thymus DNA per ml. (1× BFP is 0.02% [wt/vol] Ficoll, 0.02% [wt/vol] polyvinylpyrrolidone, and 0.02% [wt/vol] bovine serum albumin.) The filter was washed in 2× SSC–0.4% sodium dodecyl sulfate for 10 min at room temperature and at 52°C for 30 min in 0.2× SSC–0.4% sodium dodecyl sulfate. After exposure to film for 20 h, the blot was stripped with 0.05 M NaOH–0.5 M NaCl for 20 min at 20°C, neutralized with 4× SSC, and exposed to film for 2 days to confirm removal of the first probe. The blot was rehybridized with a *Drosophila* actin probe for 20 h, washed and exposed to film for 12 h. Lanes: 1 through 3, embryos at 0 to 2, 3 to 12, and 12 to 24 h, respectively; 4, first- and second-instar larvae; 5, third-instar larvae; 6, early pupae; 7, late pupae; 8, adults. The positions of 18S and 28S rRNA and actin bands were used to estimate size.

Analysis of the deduced amino acid sequence. The deduced amino acid sequence predicts an uncleaved leader peptide as was found in the hamster sequence (9). Analysis of the DNA sequence specifying the region conserved in both the hamster and *S. cerevisiae* enzymes revealed a similar sequence but with four mismatches (double-underlined sequence, Fig. 3). One of the mismatches resulted from the use of an alternate codon (Ile: ATC→ATT), whereas the remaining three mismatches represent divergence from the predicted amino acid sequence of the conserved region (Tyr→Phe, Thr→Cys, and Glu→Lys).

The methods of Kyte and Doolittle (23) were applied to the deduced amino acid sequence predicted a structure (see Fig. 5) similar to that of the hamster reductase (9, 25) that connects a hydrophobic N-terminal region, capable of spanning a membrane seven times, with a hydrophilic C-terminal region. Two potential glycosylation sites (residues 339 and

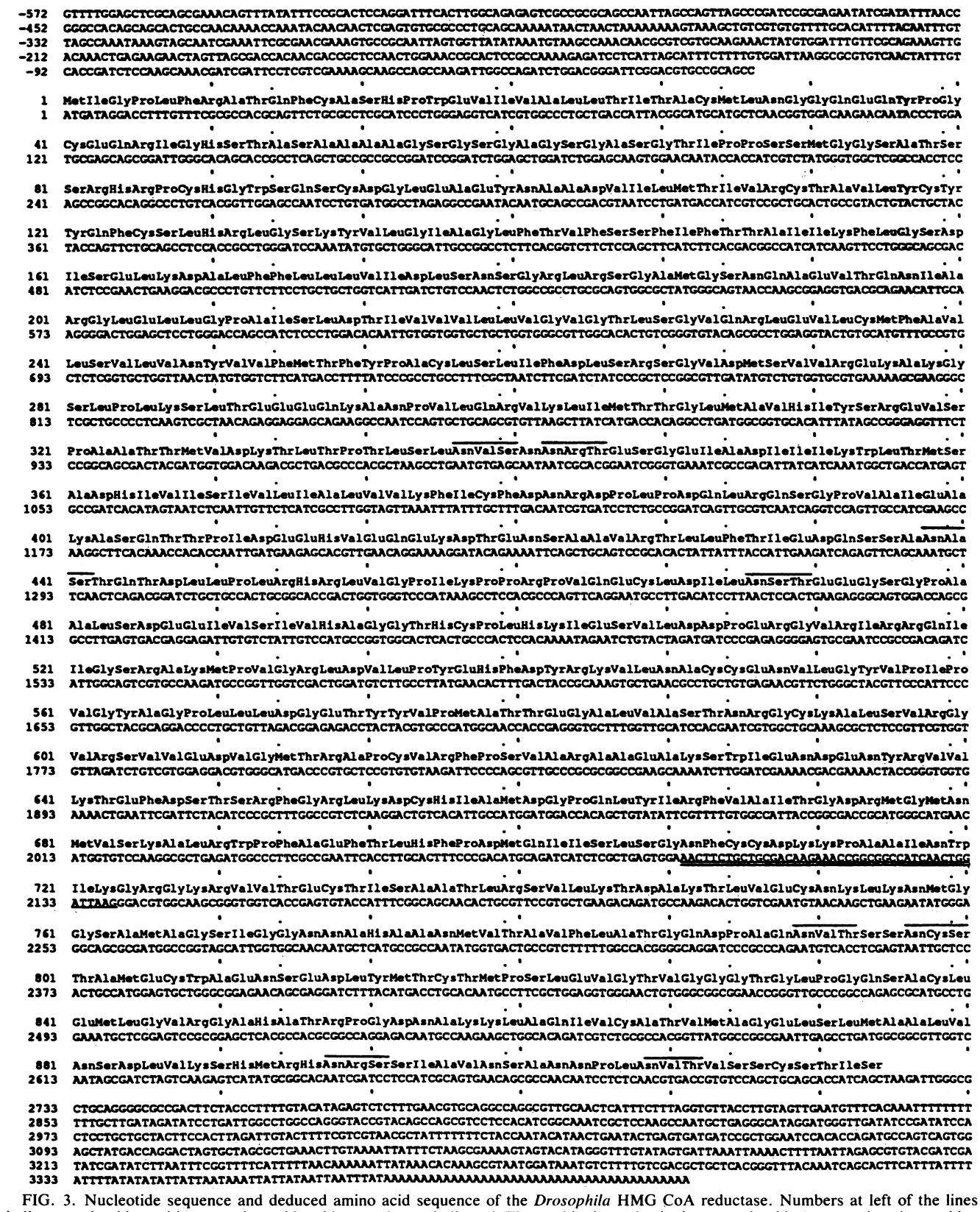

FIG. 3. Nucleotide sequence and deduced amino acid sequence of the *Drosophila* HMG CoA reductase. Numbers at left of the lines indicate nucleotide position or amino acid residue number as indicated. The methionine at beginning at nucleotide 1 was assigned as residue 1 due to the presence of stop codons in alternate reading frames, the similarity of flanking sequences to translation start site consensus sequences (7, 26), and the extensive similarity between the *Drosophila* and hamster sequences (see Fig. 4). The double-underlined sequence indicates the region corresponding to the 45-mer oligonucleotide used to isolate the genomic clone. Potential sites for N-linked glycosylation (Asn-X-Ser/Thr) are underlined.

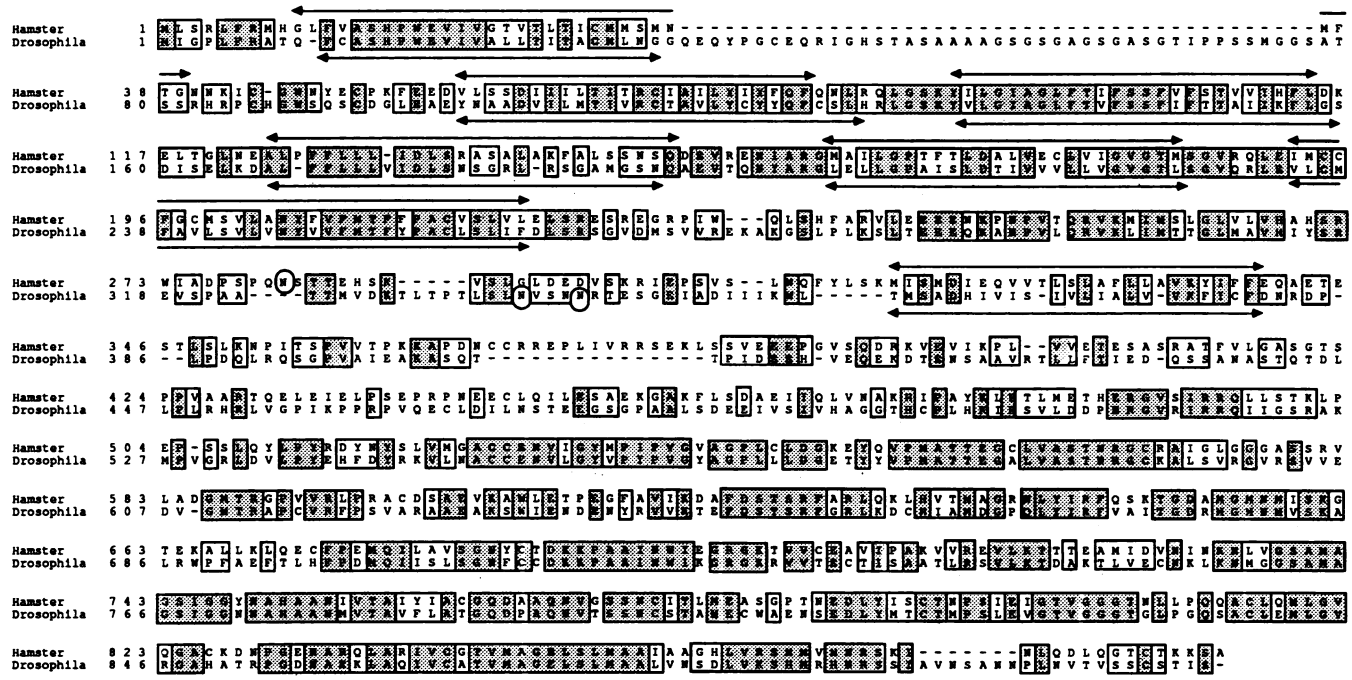

FIG. 4. Alignment of HMG CoA reductase sequences. The deduced amino acid sequence of the *D. melanogaster* HMG CoA reductase sequence was aligned with the Chinese hamster ovary HMG CoA reductase (9) initially by the dFastp program (48). Gaps were inserted to maximize conservative substitutions according to the Dayhoff et al. relative mutability tables (13). Potential membrane-spanning regions of the hamster enzyme are overlined, and those of the *Drosophila* enzyme are underlined. Potential glycosylation sites in the loop between the sixth and seventh membrane regions are circled. Boxed residues indicate conserved and identical residues (shaded). The single-letter designation for amino acids is used: A, alanine; C, cysteine; D, aspartic acid; E, glutamic acid; F, phenylalanine; G, glycine; H, histidine; I, isoleucine; K, lysine; L, leucine; M, methionine; N, asparagine; P, proline; Q, glutamine; R, arginine; S, serine; T, threonine; V, valine; W, tryptophane; and Y, tyrosine. The numbers at left of the sequences indicate residue numbers.

343) were found in the segment separating the sixth and seventh potential transmembrane domains of the *Drosophila* HMG CoA reductase; these positions are near the hamster protein's glycosylation site (residue 281, Fig. 4). Other potential glycosylation sites (residues 439, 471, 793, 798, 892, and 906) are located in a region analogous to the catalytic domain of the hamster enzyme, which had unused glycosylation sites owing to its cytoplasmic location (24). In analogy to the hamster HMG CoA reductase sequence, the linker region (residues 381 through 516) joining the N- and C-terminal regions of the *Drosophila* HMG CoA reductase included "PEST" sequences found in many proteins with rapid turnover times (39). Residues 403 through 411 (SQTT PIDEE) are enriched in Pro, Glu, Ser, and Thr residues. This segment was also flanked by charged residues and contains the sequence PXE/D, which is often present in short-lived proteins (39).

Comparison to hamster HMG CoA reductase. Similarities between the hamster and *Drosophila* HMG CoA reductases were apparent after inserting gaps to optimize the sequence alignment (Fig. 4), which was initially determined by the dFastp program (48). Due to the evolutionary distance between *D. melanogaster* and rodents, the probability of acceptable mutation tables of Dayhoff et al. (13) for related proteins were used to assign probable conservative substitutions. The similarity between the *Drosophila* and hamster enzymes ranged from 32 to 60% identical and 23 to 40% conserved amino acids for the seven potential transmembrane domains of 22 to 33 residues in length. The hydrophilic loops joining five of seven adjacent transmembrane regions (Fig. 4 and 5) were also similar. When the probability of acceptable mutation (13) values of the hamster and *Drosophila* HMG CoA reductases were averaged over 15 residues, the structural conservation of membrane regions 1, 2, 3, 5, and 6 identified by the hydrophobicity plot was apparent (Fig. 5). Similarly, the C-terminal region had 57% identical and 19% conserved amino acids in a region that corresponded to the hamster enzyme's catalytic (residues 495 through 825) domain (9, 24, 25). A weak similarity to the catalytic region of the hamster enzyme was found at the nucleotide sequence level, but no significant similarities in the nucleotide sequence of the N-terminal region were found (data not shown). The most divergent region was the linker segment separating the N- and C-terminal regions (Fig. 5). Residues 381 through 516 had 16% identical and 23% conserved amino acids when compared with residues 340 through 494 of the hamster enzyme.

Validation of the putative *Drosophila* HMG CoA reductase. To confirm the identity of the putative *Drosophila* HMG CoA reductase gene, we fused the predicted catalytic domain (residues 424 through 916) to the *tac* promoter (14) in a bacterial expression vector. HMG CoA reductase activity was induced 3- to 150-fold by isopropyl thiogalactoside in five experiments. Fractionation studies often revealed more activity in the derived fractions than the levels present in total (lanes 9, 10, and 11, Fig. 6), suggesting that interfering inhibitors may have been present. In contrast to noninduced cells, a large proportion of enzyme activity was insoluble in induced cells fractionated by centrifugation at a low speed or at 100,000 × *g* (data not shown). Neither low- nor high-speed

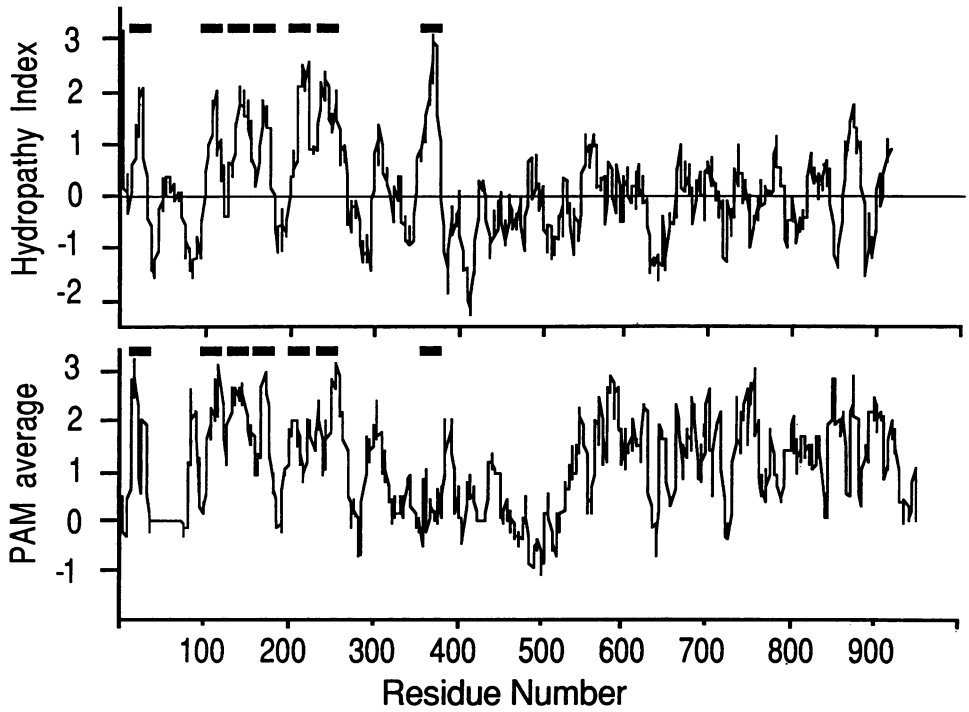

FIG. 5. Hydropathy index and Dayhoff similarity analysis. The hydropathy index of the predicted *Drosophila* HMG CoA reductase amino acid sequence was calculated over an average of 15 residues by the method of Kyte and Doolittle (23). Values of >0 indicate hydrophobic regions. Overlined bars indicate probable membrane spanning regions. A running average of similarity over 15 residues was calculated by using the probability of acceptable mutation (PAM) values of Dayhoff (13). Values of >0 indicate sequence conservation, whereas values of <0 indicate divergence.

pellets could be solubilized with detergents (unpublished observations). Since none of the potential membrane regions identified by the hydropathicity plot were included in this construct, the insoluble activity may have resulted from nonspecific aggregation of aberrantly folded proteins (49). Alternatively, there may be other regions capable of interacting with membranes not predicted by the hydropathy plot.

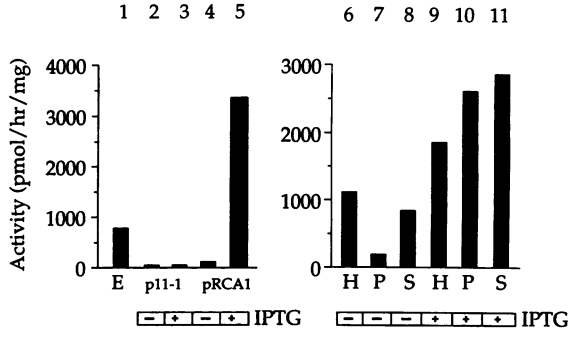

FIG. 6. Expression of *Drosophila* HMG CoA reductase in *E. coli*. The catalytic region of the *Drosophila* HMG CoA reductase was placed under the control of the *tac* promoter (see Materials and Methods) and expressed in pRCA1 cells. Logarithmically growing cells were induced with 100 μM isopropyl thiogalactoside for 5 h. "E" represents a *D. melanogaster* embryonic extract. p11-1 cells contain a plasmid encoding a chimeric β-galactosidase-HMG CoA reductase protein without HMG CoA reductase activity. Fractionation studies of the bacteria were performed in cells induced for 2 h by freeze-thaw, sonication, and centrifugation at 15,000 × *g* for 15 min. Samples of the homogenate (H), pellet (P), and supernatant (S) were assayed in duplicate for HMG CoA reductase activity. Total proteins in cell fractions were 3.95, 1.69, 2.1, 4.4, 1.82, and 2.06 mg, respectively, for lanes 6 through 11. No activity was found in bacteria lacking plasmids or in cells containing the vector without an insert (data not shown).

Since the activities of both *Drosophila* and hamster HMG CoA reductases were suppressed by exogenous mevalonate (10), we asked whether the insect mRNA was down regulated by mevalonate in a manner analogous to the mammalian mRNA (10). Thus, Schneider cells were grown in the absence or presence of 10 mM mevalonate, and mRNA was prepared for hybridization analysis. Growth in the presence of mevalonate resulted in the reduced abundance, ninefold as determined by densitometry, of a specific 4-kb HMG CoA reductase transcript (Fig. 7). These values matched the eightfold suppression of HMG CoA reductase activity. In four experiments, the fold suppression of mRNA levels varied from two- to ninefold with a five- to ninefold suppression of enzyme activity. The source of this variability is still under investigation. Taken together, the evidence strongly suggests that we have indeed isolated the *Drosophila* HMG CoA reductase gene.

DISCUSSION

Comparison of the mammalian (9, 29), partial *S. cerevisiae* sequence (1), and *Drosophila* HMG CoA reductases reveals extensive conservation in the C-terminal regions. Since this domain encodes the probable catalytic site, the structural conservation of this domain reflects its essential function. However, it is not clear why the membrane-spanning regions of both the mammalian and *Drosophila* HMG CoA reductase are so similar. In the case of the hamster HMG CoA reductase, turnover of the enzyme in response to sterols

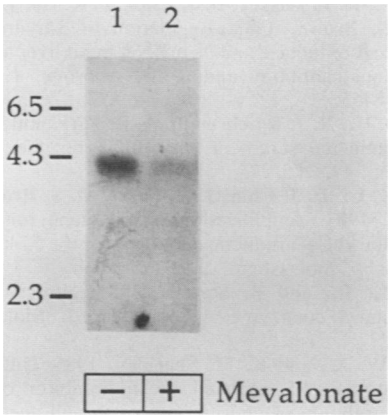

FIG. 7. Effect of mevalonate upon HMG CoA reductase mRNA abundance in Schneider cells. Schneider cells were grown for 20 h in the absence or presence of 10 mM mevalonate, and RNA was prepared from the cells as described in Materials and Methods. The RNA was transferred to Zetaprobe membranes and hybridized with ^{32}P-labeled p42.1. Lanes: 1, no mevalonate, 240 μg of RNA; 2, 10 mM mevalonate, 220 μg of RNA. λ DNA digested with *Hin*dIII was similarly denatured and run in a lane for size markers. The HMG CoA reductase values were 640 and 82 pmol/h per mg, respectively, for cells grown in the absence and presence of 10 mM mevalonate.

required the presence of these membrane-spanning regions (residues 10 through 342) (19). What possible role can the conserved hydrophobic regions present in the *Drosophila* enzyme play? Like all arthropods, *D. melanogaster* cannot synthesize sterols (21); hence it is unlikely that this structural similarity was maintained to provide sensitivity to cholesterol-mediated degradation of the *Drosophila* HMG CoA reductase. Previous work (3, 43) confirmed by our studies (unpublished observations) has shown that exogenous sterols fail to suppress the HMG CoA reductase activity of cultured *Drosophila* cells. Alternatively, the conserved N-terminal regions of *Drosophila*, hamster (9), and human (29) HMG CoA reductases may instead reflect structural features for targeting the enzyme into the smooth endoplasmic reticulum, the primary location of the hamster HMG CoA reductase. Expression of HMG CoA reductase in heterologous cell lines may allow a functional test of this structural conservation.

An interesting possibility for the role of these conserved hydrophobic domains can be inferred from several hormone receptors which also have seven transmembrane domains that may interact with small molecules in a functional manner (15). Similarly, the seven membrane-spanning regions of the hamster enzyme (19) were required for sterol-triggered degradation. Thus, the seven conserved transmembrane HMG CoA reductase regions of the *Drosophila* enzyme may be essential for recognizing specific mevalonate derivatives or their binding proteins.

We do not know the extent of sequence identity between the 3.2-kb mRNA and the 4-kb mRNA whose sequence is reported. The difference in size may result from shorter 3′ untranslated regions, alternate splicing of 5′ untranslated regions, or even alternate exon usage. Primer extension studies and primer-extended cDNAs suggest at least two alternate transcription initiation sites (unpublished observations). We have not ruled out the presence of multiple promoters, but the two mRNAs are likely to be transcribed from a single *Drosophila* HMG CoA reductase gene, since (i) a single hybridization site was found on the third chromosome at 95A and (ii) hybridization of genomic DNA blots with cDNA fragments has revealed a simple set of bands (unpublished observations). By comparison, the hamster HMG CoA reductase also exhibited two transcripts derived from a single gene which had multiple polyadenylation sites (9) and a complex pattern of alternate splicing in the 5′ untranslated region (38).

On the basis of studies performed on the tobacco hornworm, it has been suggested that HMG CoA reductase might be the control point for the synthesis of JH (33), which vary over 1,000-fold throughout *Drosophila* development (44). HMG CoA reductase mRNA expression was not enhanced in postfeeding larvae and adults—during periods of high JH expression (44). If HMG CoA reductase expression in the corpora allata accounted for less than 0.1% of the total, then a 1,000-fold induction in HMG CoA reductase could still elude detection. Our results are consistent with recent observations (18) suggesting that HMG CoA reductase was not rate limiting for JH expression. However, the *Drosophila* HMG CoA reductase is amenable to mevalonate suppression (3), whereas the cockroach enzyme is not (18). This difference in feedback regulation might account for the lack of correlation of JH levels and HMG CoA reductase activity in the latter organism. Consequently, the role of HMG CoA reductase in the modulation of *Drosophila* JH synthesis remains to be elucidated.

The abundant HMG CoA reductase mRNA expression found in 12- to 24-h embryos may reflect an even larger metabolic demand for isoprenyl derivatives other than JH. Since several polypeptides have been found posttranslationally modified by mevalonate-derived isoprenes (40, 42, 46), it is tempting to speculate that some of these polypeptides are responsible for mediating mevalonate-dependent effects upon cell growth (20, 36), cellular differentiation (30), or the direction of gastrulation (6). We hope that the physiological role(s) and identities of these compounds may be elucidated by studying flies with aberrant or constitutive HMG CoA reductase expression.

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