Annu. Rev. Entomol. 2005. 50:181–99
doi: 10.1146/annurev.ento.50.071803.130356

First published online as a Review in Advance on August 5, 2004

THE MEVALONATE PATHWAY AND THE SYNTHESIS OF JUVENILE HORMONE IN INSECTS

Xavier Bellés, David Martín, and Maria-Dolors Piulachs
Department of Physiology and Molecular Biodiversity, Institut de Biologia Molecular de Barcelona (CSIC), Jordi Girona 18, 08034 Barcelona, Spain;
email: xbragr@ibmb.csic.es; dmcagr@ibmb.csic.es; mdpagr@ibmb.csic.es

Key Words cholesterol, SREBP, biosynthesis, *Blattella*, *Ips*, HMG-R

■ **Abstract** The mevalonate pathway in insects has two important peculiarities, the absence of the sterol branch and the synthesis of juvenile hormone (JH), that may have influenced the mechanisms of regulation. The data available on these mechanisms indicate that cholesterol does not play a regulatory role and that JH modulates transcript levels of a number of genes of the mevalonate pathway or can influence the translatability and/or stability of the transcripts themselves. These data suggest that the mevalonate pathway in insects can best be interpreted in terms of coordinated regulation, in which regulators act in parallel to a number of enzymes, as occurs in the cholesterol-driven pathway in vertebrates.

INTRODUCTION

The mevalonate pathway is of current interest because one of its final products is cholesterol, which in addition to its role in maintaining cell membranes also has implications for human cardiovascular diseases. Therefore, most research on this pathway has been based on vertebrate models, resulting in such achievements as the identification of the role of cholesterol as a key regulator (24) and the discovery of the sterol regulatory element-binding proteins (SREBP) pathway (29, 41).

Insects, however, are a special case because they do not synthesize cholesterol de novo (13). Moreover, they produce juvenile hormones (JHs), unique sesquiterpenoid hormones that regulate embryonic development, repress metamorphosis, and induce vitellogenin synthesis and pheromone production in most insect species (38). To date, six forms of JH have been fully characterized from a chemical and physiological point of view (1a, 46). JH I and II were identified in Lepidoptera for the first time, and JH III is the most widespread JH in insects. JH 0 and 4-methyl-JH I seem exclusive to Lepidoptera. Finally, Diptera produce the 6,7-epoxide of JH III, JHB3. The peculiarities of JHs suggest that the regulatory mechanisms of the mevalonate pathway in insects are different from those of vertebrates. However, these regulatory mechanisms have remained elusive. One of the most

recent reviews on the insect mevalonate pathway (46) underlines the challenge that insects pose in this respect. The absence of appropriate molecular tools has been a drawback in the past years, but the cloning of the emblematic 3-hydroxy-3-methylglutaryl coenzyme A reductase in the fruit fly, *Drosophila melanogaster* (22), opened the door to a new strategy. Since then, other enzymes of the mevalonate and JH pathway have been cloned and sequenced, and the SREBP pathway in *D. melanogaster* has been characterized (18). Although the answer to the question how do insects, in general, regulate the mevalonate pathway is not yet available, this review seeks to show that the road toward such an answer is now much better paved.

THE MEVALONATE PATHWAY IN INSECTS

The mevalonate pathway is a ramified metabolic route based on reductive polymerization of acetyl-CoA, which leads to a great diversity of isoprenoid compounds (Figure 1). These include cholesterol, which is involved in maintaining membrane structure; the heme A and ubiquinone, which participate in electron transport across the membrane; dolichol, which is necessary for glycoprotein carrier synthesis; and isopentenyl adenine, which is present in certain transfer RNAs. In addition, isoprenoids from the mevalonate pathway are involved in the prenylation of membrane-bound proteins that play an important role in cell signaling and carcinogenesis, such as $p21^{ras}$, which is encoded by *ras* oncogenes and proto-oncogenes. Final products of the pathway also include hormonal messengers, such as cytokinins and phytoalexins in plants, steroid hormones in mammals (8), and defensive secretions, pheromones, and JH in insects (48) (Figure 1).

Because of its important functions in the cell membrane, and its key regulatory role, cholesterol has become the most emblematic compound of the mevalonate pathway (23, 24). However, insects and other arthropods do not synthesize cholesterol de novo (13) because they lack the genes encoding squalene synthase and other subsequent enzymes of the sterol branch (45). Another peculiarity of the mevalonate pathway in insects is the capacity to synthesize JHs, which involves special homoisoprenoid intermediates and enzymes in the steps after the formation of farnesyl diphosphate, although the preceding steps, from acetyl-CoA to farnesyl diphosphate, are identical (and regulated by the same enzymes) to those of cholesterol-producing organisms (Figure 1). Among the insects, lepidopterans also generate and use propionyl-CoA in a parallel homomevalonate pathway, thus yielding special JH homologs, such as JH I, JH II, JH 0, and 4-methyl JH (1a, 46).

THE ENZYMES: STRUCTURAL DATA

Data available on the enzymes involved in the mevalonate pathway in insects are copious (see, e.g., References 46 and 48) and could not be summarized in a reasonable space. Therefore, we deal only with structural aspects of those

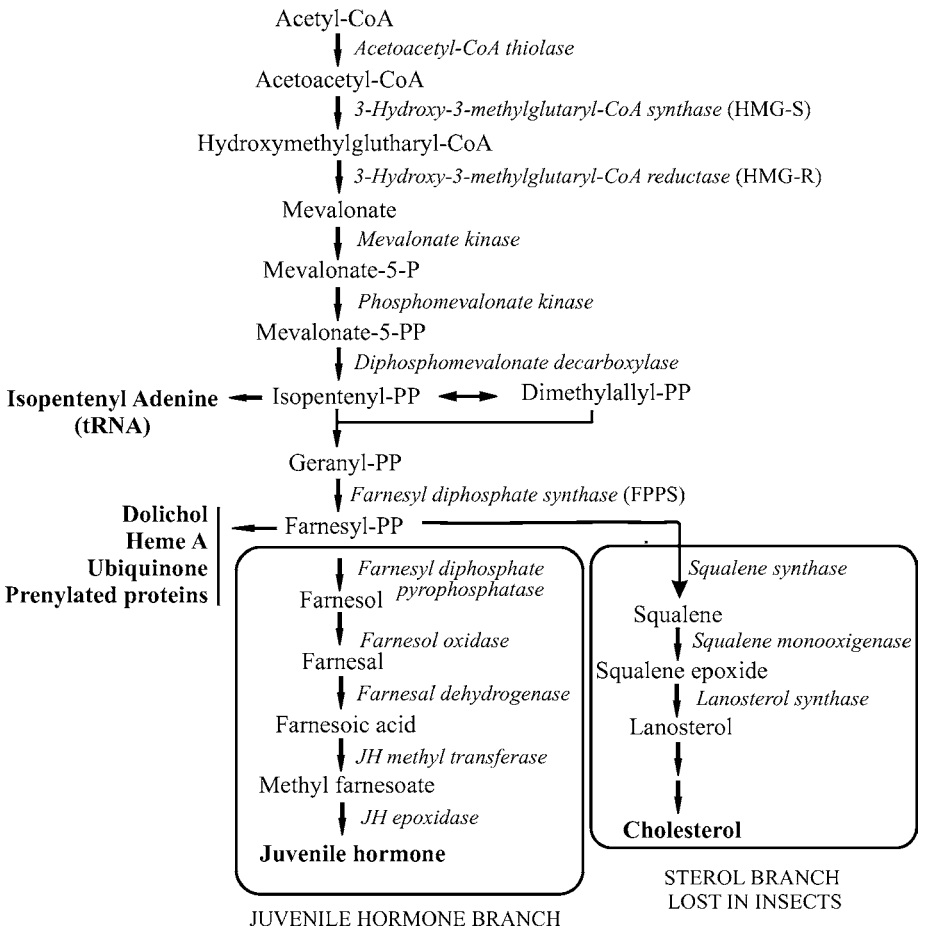

Figure 1 Flux diagram of the mevalonate pathway and JH biosynthesis in insects. Other important final products and the final steps leading to cholesterol in vertebrates are also indicated.

enzymes that have been molecularly characterized, according to the order followed in Figure 1.

The Route to Mevalonate

The gene encoding acetoacetyl-CoA thiolase (EC 2.3.1.9) has been identified in the genome of *D. melanogaster* and the mosquito *Anopheles gambiae* and in an EST of the scolytid beetle *Ips pini* (19a, 30a) (Table 1). In vertebrates, acetoacetyl-CoA thiolase is functional as a tetramer of identical subunits and has two cysteine residues at the active sites (21), which are conserved in insects.

TABLE 1 Species in which genomic or cDNA sequences for enzymes of the mevalonate pathway and juvenile hormone synthesis are available

Enzyme	Species	Accession number	Reference
Acetoacetyl-CoA thiolase	*Drosophila melanogaster*	NM_13,2186.1	
	Anopheles gambiae	XP_30,9320.1	
	Ips pini	CB407884	(19a, 30a)
3-Hydroxy-3-methylglutaryl CoA synthase (HMG-S)	*Dendroctonus jeffreyi*	AAF 89,580	(57)
	Blattella germanica	P544870	(6)
	D. melanogaster	NP_52,4711	
	A. gambiae	XP_31,5872	
	Bombyx mori	br—1362	
	I. pini	CB408343	(19a, 30a)
3-Hydroxy-3-methylglutaryl CoA reductase (HMG-R)	*D. melanogaster*	P14773	(22)
	B. germanica	P54960	(36)
	I. pini	AF304440	(19a, 26, 30a)
	Ips paraconfusus	AF071750	(56)
	D. jeffreyi	AF159136	(55)
	Anthonomus grandis	AF162705	
	Agrotis ipsilon	O76819	(19)
	Apis mellifera	BI503396	
	A. gambiae	XP_30,7890.1	
Mevalonate kinase	*D. melanogaster*	NM_17,6158	
	A. gambiae	XP_31,9701	
Phosphomevalonate kinase	*D. melanogaster*	AE003663	
	A. gambiae	XP_31,0779.1	
Diphosphomevalonate decarboxylase	*D. melanogaster*	NP_ 57,3068.3	
	A. gambiae	XP_30,7373.1	
	I. pini	CB407651	(19a, 30a)
Isopentenyl diphosphate isomerase	*D. melanogaster*	NP_ 65,0962.1	
	A. gambiae	XP_32,1388.1	
	I. pini	CB407826	(19a, 30a)
Geranyl diphosphate synthase	*I. pini*	CB407488	(19a, 30a)
Farnesyl diphosphate synthase (FPPS)	*A. ipsilon*	AJ009962	(12)
	D. melanogaster	AJ009963	
		AF132554	
	B. mori	BAB69490	
	A. gambiae	XP_30,8653	
	I. pini	CB408189	(19a, 30a)
JH methyl transferase	*B. mori*	AB113578	(49)
	Manduca sexta	AAF16712	
	D. melanogaster	AB113579	
	A. gambiae	XP_31,4173	
	Callosobruchus maculatus	CB_37,7599	
	A. mellifera	BI946505	
JH expoxidase	*Diploptera punctata*	AY509244	(28)
	A. gambiae	XP_31,5677	

3-hydroxy-3-methylglutaryl-CoA synthase (HMG-S) (EC 2.3.3.10, formerly 4.1.3.5) has been cloned in the cockroach *Blattella germanica* (6, 35) and in the scolytid beetle *Dendroctonus jeffreyi* (57) (Table 1). It has also been identified in an EST of *I. pini* (19a, 30a). In *B. germanica* two enzymes that are 78% similar have been reported, HMG-S-1 (35) and HMG-S-2 (6) (Table 1). The gene encoding HMG-S-1 has no introns, and expression studies and phylogenetic analysis suggest that it is a functional retrogene derived from HMG-S-2 by retrotransposition (6, 7, 9). In this review, when mentioning HMG-S from *B. germanica*, we refer to HMG-S-2 unless stated otherwise. The gene encoding HMG-S can be found in the genome of *D. melanogaster*, *A. gambiae*, and the lepidopteran *Bombyx mori* (Table 1). HMG-S has a characteristic cysteine in the catalytic domain that is conserved in insect sequences at position 120. HMG-S of insects does not show any recognizable N-terminal targeting sequence to mitochondria, which suggests that the enzyme is cytosolic in insects (6, 57).

3-Hydroxy-3-methylglutaryl-CoA reductase (HMG-R) (EC 1.1.1.34) has been cloned from *D. melanogaster* (22); *B. germanica* (36); the beetles *I. pini* (26), *Ips paraconfusus* (56), *D. jeffreyi* (55), and *Anthonomus grandis* (partial sequence); and the moth *Agrotis ipsilon* (19) (Table 1). The gene encoding HMG-R can be found in the genome databases of *D. melanogaster*, *A. gambiae*, and the hymenopteran *Apis mellifera* (Table 1). HMG-R is a membrane-bound enzyme with an N-terminal membrane anchor that contains a variable number of transmembrane-spanning domains, followed by a linker region, and a C-terminal catalytic domain (39). The catalytic domain is remarkably well conserved, especially in the distribution of cysteine residues and in the occurrence of a histidine that is conserved in all HMG-R characterized to date, including those of prokaryotes (4).

From Mevalonate to Farnesyl Diphosphate

Data on mevalonate kinase (EC 2.7.1.36), phosphomevalonate kinase (EC 2.7.4.2), diphosphomevalonate decarboxylase (EC 4.1.1.33), and isopentenyl diphosphate isomerase (EC 5.3.3.2) in insects come from the corresponding genes found in *D. melanogaster* and *A. gambiae* genomes (Table 1). While other insect enzymes of the mevalonate pathway are between 40% and 60% identical to the vertebrate orthologs, the mevalonate kinases of *D. melanogaster* and *A. gambiae* are only 30% similar. In the case of phosphomevalonate kinase, two types have been described, one corresponding to the higher eukaryotes, including insects, and the other corresponding to plants and fungi (30). Diphosphomevalonate decarboxylase has been identified in an EST of *I. pini* (19a, 30a).

Data on the structure of isopentenyl diphosphate isomerase can be found in the genome database of *D. melanogaster* and *A. gambiae*, and in an EST of *I. pini* (19a, 30a) (Table 1). Geranyl diphosphate synthase has also been identified in an EST of *I. pini* (30a) (Table 1).

Farnesyl diphosphate synthase (FPPS) (EC 2.5.10) has been cloned in *A. ipsilon* (12), and its gene is present in *D. melanogaster*, *A. gambiae*, and *B. mori* genomes, and it has been identified in an EST of *I. pini* (19a, 30a) (Table 1).

FPPS from insects, the vertebrates *Homo sapiens*, *Mus musculus*, and *Rattus norvegicus*, and the plant *Arabidopsis thaliana* have an N-terminal extension that ranges from 113 (*A. gambiae*) to 65 (*H. sapiens*) amino acids in length. These extensions are enriched in basic, polar, and hydrophobic residues; contain a number of tetrapeptides that fit the consensus sequence RXXS, which is currently reported as a mitochondrial targeting cleavage motif (59); and may form a positively charged amphiphilic α-helix. These features suggest that the N-terminal extensions of FPPS contain a mitochondrial transit peptide, a hypothesis that has been confirmed for *D. melanogaster*, *H. sapiens* (D. Martín, M.-D. Piulachs, N. Cunillera, A. Ferrer & X. Bellés, unpublished data), and *A. ipsilon* (see below).

The Formation of Juvenile Hormone

No structural data are available on farnesyl diphosphate pyrophosphatase, farnesol oxidase, or farnesal dehydrogenase. The conversion of farnesol to farnesal was believed to be catalyzed by a nicotinamide-dependent dehydrogenase (46), but recent studies in the lepidopteran *Manduca sexta* suggest that it is a metal- or flavin-dependent oxidase (51).

In orthopteroid insects, esterification of farnesoic acid occurs before epoxidation, yielding JH. By contrast, in lepidopterans epoxidation precedes esterification, which is under developmental control, and the corresponding JH methyl transferase vanishes during metamorphosis (50). The first JH methyl transferase was cloned from *B. mori* (49) (Table 1) and, using the *B. mori* sequence as a template, orthologs have been found in *D. melanogaster* and *A. gambiae* (Table 1). In *M. sexta*, a previously described odorant-binding protein (43) shows the structural features of a JH methyl transferase (49) (Table 1). We have found new JH methyl transferase orthologs in EST databases of *A. mellifera* and the beetle *Callosobruchus maculatus* (Table 1). JH methyl transferase transfers a methyl group of *S*-adenosyl-L-methionine (SAM) to farnesoic acid or epoxyfarnesoic acid. Thus, it is not strange that the amino acid sequences of the insect JH methyl transferase contain a conserved SAM-binding motif typical of the family of SAM-dependent methyl transferases. Orthologs of insect JH methyl transferases from a number of crustacean species are known (25, 44).

More recently, a specific JH epoxidase has been cloned and characterized from the corpora allata (CA) of the cockroach *Diploptera punctata* (28) (Table 1). It belongs to the large superfamily of cytochrome P450 proteins (19b) and epoxidizes methyl farnesoate with high regio- and stereoselectivity. A clear ortholog of this JH epoxidase has been identified in the genome of *A. gambiae* (Table 1), whereas structurally related sequences have been found in *B. mori* and *D. melanogaster* (28).

ENZYME ACTION: SITES AND PATTERNS

Data related to the functions and physiology of the mevalonate pathway enzymes have been selected for processes involving JH. The most obvious process is the biosynthesis of JH itself, but we have also dealt with JH-dependent processes such

as the synthesis of vitellogenin in the fat body, oogenesis, embryogenesis, and the production of isoprenoid pheromones in the midgut of scolytids. A final section on the localization of the enzymes in different subcellular compartments has also been included given the interest of this aspect from a functional and physiological point of view.

Juvenile Hormone Synthesis in the Corpora Allata

Biochemical information implicates the mevalonate pathway in the synthesis of JH (46), including data from studies on the production of JH by the CA, which showed that addition of precursors such as mevalonate, farnesol, or farnesoic acid increased JH production, whereas the use of HMG-R inhibitors such as compactin or mevilonin decreased it, thus indicating that isoprenoid flux modulates the rates of JH synthesis (46).

Subsequently, developmental profiles of enzymatic activity in the CA were obtained for HMG-S and HMG-R in the adult female of *D. punctata* (14, 20), for HMG-R in the adult female of the locust *Locusta migratoria* (15), and for JH acid methyl transferase in last larval instar of *M. sexta* (50). Molecular studies have led to the detection of HMG-R mRNA in the CA of *A. ipsilon* (19) and *B. germanica* (J.L. Maestro & X. Bellés, unpublished results), and FPPS mRNA in the CA of *A. ipsilon* (12). In *B. mori*, mRNA levels of JH methyl transferase have been monitored in the CA of last larval instars (49).

Developmental patterns reveal a parallelism between the profile of enzyme mRNA levels or enzymatic activity and that of JH production, although the decline of JH often precedes enzyme decline by one or two days. This happens, for example, when HMG-S and HMG-R activity is measured in the CA of *D. punctata* (14, 20) (Figure 2). In terms of mRNA, developmental patterns for JH methyl transferase in *B. mori* indicate that levels are high and constant in antepenultimate and penultimate instars and then decrease in the last larval instar, this decrease occurring one day after the switch from producing JH to producing JH acid in the CA (49). The data suggest that enzyme levels are not responsible for the sudden decline in JH production observed at the end of the CA activity cycle. Rather, data suggest that JH acts regulating enzyme activity in the second half of the enzyme cycle. The first transcript expression data on the recently described JH epoxidase of *D. punctata* indicate that it is selectively expressed in the CA, at least at the time of maximal JH production (28).

Vitellogenesis in the Fat Body

One of the most important functions of the female fat body in the reproductive cycle is the production of vitellogenins and their release into the hemolymph, from which they are incorporated into developing oocytes (2). In the fat body of *B. germanica*, HMG-R and HMG-S exhibit coordinated expression and activity during the vitellogenic cycle (10). The mRNA levels of both enzymes are high after adult emergence and then decline progressively until oviposition. Conversely,

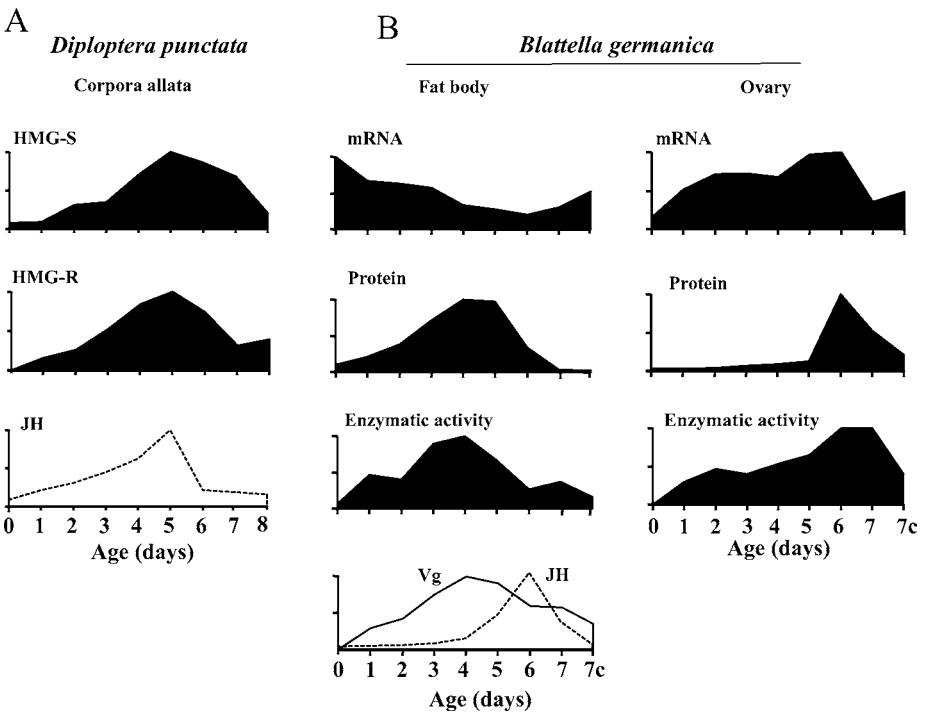

Figure 2 (*A*) Patterns of enzymatic activity of HMG-S and HMG-R, and dynamics of JH biosynthesis rates in the CA of adult females of *D. punctata* during the first gonadotrophic cycle (data based on References 14, 20). (*B*) Patterns of mRNA, protein, and enzymatic activity of HMG-R in the fat body and ovary of adult females of *B. germanica* during the first gonadotrophic cycle; patterns of vitellogenin (Vg) and JH are also indicated (data based on References 2, 6, 10, 60, 61). Data from *B. germanica* have been normalized for a typical first gonadotrophic cycle of seven days (plus 7c, when chorionation takes place). To compare the patterns in the same scale, units in ordinates are arbitrary but proportional to reported values.

protein levels and enzymatic activity increase as mRNA levels decrease, peak at the middle of the reproductive cycle, and decrease thereafter (10). The protein and enzymatic activity profiles of both enzymes are analogous to that of vitellogenin production in the fat body (32) (Figure 2).

Because vitellogenins are glycosylated proteins, a functional link between isoprenoid synthesis and vitellogenesis may be the production of dolichol, which is a carrier for oligosaccharide components for vitellogenin glycosylation. Following this reasoning, Martín et al. demonstrated (33) that fluvastatin, a potent inhibitor of HMG-R, reduced the release of vitellogenin from fat bodies of *B. germanica* incubated in vitro. The authors (33) also showed that allatostatins, peptides that

impair the first step(s) of the mevalonate pathway in insects (52), inhibited vitellogenin release from the fat body, whereas mevalonate reversed this effect. Other studies have shown that fluvastatin and compactin inhibit HMG-R in the fat body of *B. germanica*, in terms of both protein and enzymatic activity levels, which results in reduced concentration of vitellin in the insect ovaries (60).

Oogenesis

The first studies on tissue expression indicated that HMG-R and HMG-S in *B. germanica* (35, 36) are highly expressed in the adult ovary. Further studies on the first reproductive cycle showed that enzymatic activity of HMG-R and HMG-S is maximal some two days before oviposition, whereas mRNA levels are minimal (6). Protein levels of HMG-R are also maximal before oviposition (61) (Figure 2).

These patterns had suggested that some product of the mevalonate pathway might play a role in choriogenesis, which starts one day before oviposition (61). However, inhibition of the ovarian preoviposition peak of HMG-R with fluvastatin did not impair chorionation in *B. germanica*. However, egg hatching was severely affected by fluvastatin treatment, which suggests that the inhibitor was incorporated into the oocytes of treated females and elicited the effects on the eggs laid (61). It is clear from these studies that HMG-R, while not influencing choriogenesis, is essential for embryogenesis. Indeed, the physiological sense of the high expression of HMG-R toward the end of oogenesis may be the accumulation of the enzyme and its corresponding mRNA into the maturing oocyte for further use in early embryogenesis.

Embryogenesis

Expression of HMG-R and HMG-S in embryos of the cockroach *B. germanica* has been studied. The first experiments showed that mRNA levels for both enzymes are high after oviposition, low in mid-embryogenesis, and mid-range before egg hatching (6, 36, 61). Conversely, protein levels were high during the first three or four days of embryogenesis, and then decreased abruptly, whereas enzyme activity showed an acute peak on day 3. Treatment of adult females with fluvastatin in late vitellogenesis reduced the peak of HMG-R activity on day 3 and blocked embryogenesis (61). These findings suggest that HMG-R, or some product of the mevalonate pathway, plays an essential role in early embryogenesis, coinciding with germ band formation, the extension of the appendage rudiments, and the segmentation of cephalic and thoracic regions.

In *D. melanogaster*, maternal HMG-R is required for early embryonic development (40) and for guiding primordial germ cells to the somatic gonad (58), which explains that HMG-R mutant embryos are inviable. Mutants for FPPS and geranylgeranyl diphosphate synthase (*fpps* and *quemao*, respectively) show phenotypes similar to those of HMG-R mutants (45). Furthermore, mutants for geranylgeranyl transferase type I (an enzyme necessary for protein prenylation of protein G, Ras,

RhoB, and Rac) exhibit defects in cell migration equivalent to those described in HMG-R mutants (45). Therefore, prenylation of proteins appears to be a key process for germ cell migration in *D. melanogaster* embryogenesis. It was also suggested (45) that HMG-R activity is involved not only in the regulation of the prenylation of certain proteins, in organelle trafficking, or in cell migration, but also in regulating control gene expression. Indeed, flies carrying large homozygous mutant clones for HMG-R in the imaginal discs die as pupae and exhibit patterning alterations encompassed by subexpression of *engrailed* and overexpression of *wingless*, two important patterning gene markers (45).

Isoprenoid Pheromone Biosynthesis in the Midgut

Another important process in insects involving isoprenoid biosynthesis is the production of terpenoid aggregation pheromones in bark beetles, which has recently been reviewed (48). Initial studies in scolytids (*Ips* spp.), using compactin and precursors (acetate and mevalonate) of the mevalonate pathway, suggested that monoterpenoid pheromones are produced de novo via the classical mevalonate pathway (53). Over the past five years, the use of molecular techniques has shown that scolytids synthesize isoprenoid-derived aggregation pheromones (ipsenol, ipsdienol, and frontalin) through the classical mevalonate pathway. The species most studied are *I. paraconfusus*, *I. pini*, and *D. jeffreyi*, in which HMG-R and HMG-S have been cloned and characterized. Expression analysis and in situ hybridization studies have shown that HMG-R is highly expressed in specialized cells of the male anterior midgut, where monoterpenoid aggregation pheromones are synthesized de novo (37a).

Cell Compartmentalization

At least three different subcellular compartments (cytoplasm, peroxisomes, and mitochondria) participate in the mevalonate biosynthetic pathway in mammals (31). The occurrence of compartment-specific genes is a way to organize such a compartmentalization, as occurs in rat mitochondrial and cytosolic HMG-S, which are encoded by two different genes (1). Alternatively, the enzyme may be encoded by a single gene, but mechanisms for transporting it to different subcellular compartments are required.

In general, most enzymes of the mevalonate pathway in insects seem to act in the cytoplasm, as seen for HMG-S in *L. migratoria* (15). However, an example of organelle translocation has been found in *D. melanogaster*, in which the FPPS gene encodes two different proteins, one located in the cytoplasm and the other presenting an N-terminal mitochondrial signal peptide that conveys the enzyme to mitochondria (D. Martín, M.-D. Piulachs, N. Cunillera, A. Ferrer & X. Bellés, unpublished data) (Figure 3). The FPPS of *A. ipsilon* also possesses an N-terminal extension with the features of a mitochondrial transit peptide (12). Following a previously described methodology of complementation assays on the WSR mutant strain of *Saccharomyces cerevisiae* (17), we have shown that this N-terminal

extension of *A. ipsilon* also contains a transit peptide that conveys a passenger protein into mitochondria (Figure 3).

REGULATION

Unveiling the regulatory mechanisms of the mevalonate pathway and the synthesis of JH in insects is a major challenge in the field. To date, most of the approaches addressed to study these mechanisms have been inspired by the experience with vertebrates. These approaches have often given negative, but in any event informative, results. Conversely, other studies have shown that JH itself is a key regulatory element of the pathway, at least in selected insect models and processes.

Cholesterol Does Not Regulate the Mevalonate Pathway in Insects

The diversity of potential products of the mevalonate pathway may be indicative of the existence of finely tuned regulatory mechanisms. The absence of such precise control would result in a failure to fulfill the temporal, spatial, and quantitative restrictions placed upon isoprenoid synthesis and the deleterious accumulation of potentially toxic compounds. Data obtained in vertebrates pointed to cholesterol as a key negative regulator of the pathway (24), which inspired the first contributions on the regulation of the mevalonate pathway in insects. A set of detailed experiments carried out by Watson's team (5, 27) on the *Drosophila* cell line Kc demonstrated that HMG-R was not affected by exogenous sterols. These results suggested that, in contrast with vertebrates, the activity of HMG-R in insects was not regulated by a sterol-mediated negative feedback. From these data an intriguing question arose: Have the insects lost an ancestral ability to regulate the mevalonate pathway through sterols? The answer came with the complete characterization of the SREBP pathway in the fruit fly.

The SREBP Pathway in Insects

Sterol-dependent regulation in mammals is accomplished through the action of the SREBPs and the SREBP pathway (29, 41). The SREBPs insert into the membrane of the endoplasmic reticulum, where they form a complex with another protein called SCAP (SREBP cleavage-activating protein). When cells lack lipids, the SCAP transports the SREBPs to the Golgi apparatus, where they are cleaved by two proteases named S1P and S2P. This releases a fragment that migrates to the nucleus and activates the expression of genes encoding enzymes involved in cholesterol and fatty acid synthesis. Excess cholesterol and fatty acids impair the proteolytic release of SREBPs from Golgi membranes, while the SCAP plays the role of sterol and fatty acid sensor. Relevant to this review is that mammals have two main SREBP isoforms and pathways: SREBP-1c, which is involved in regulating fatty acids, triacylglycerides, and phospholipids, and SREBP-2, which is restricted

to regulating genes involved in cholesterol homeostasis. Another isoform, SREBP-1a, is a general transcriptional activator for all SREBP-responsive genes (29, 41).

Do insects have a homologous system of regulation? Recent work by Goldstein, Brown, and colleagues (18, 47) has shown that *D. melanogaster* encodes all the members of this pathway, including a single SREBP protein (dSREBP) as well as the processing proteins and proteases (dSCAP, dS1P, and dS2P) (18). However, dSREBP controls the enzymes involved in fatty acid synthesis, not those of the mevalonate pathway, as demonstrated by experiments of RNAi silencing dSREBP and dSCAP in *Drosophila* S2 cells. Results showed that four enzymes of fatty acid biosynthesis (acetyl-CoA synthase, acetyl-CoA carboxylase, fatty acid synthase, and fatty acyl-CoA synthase) were downregulated, while transcripts of the enzymes of the mevalonate pathway were not affected (47). Indeed, processing of dSREBP is halted by palmitate and its derivative phosphoethanolamine, not by an excess of cholesterol or unsaturated fatty acids. These results indicate the insect SREBP pathway would be homologous to the SREBP-1c system of mammals, and that *D. melanogaster* does not have the pathway equivalent to the mammalian SREBP-2, which specifically regulates cholesterol. A single SREBP pathway in the mosquito *A. gambiae* has also been found (41).

Regulation Through Juvenile Hormone

The physiological importance of JH suggests that it may be a candidate to regulate the mevalonate pathway in insects, at least in some groups and processes. In scolytids (48), biochemical analysis demonstrated that JH regulates isoprenoid pheromone production de novo in the midgut of *I. pini* males (54). Further analysis revealed that JH increases mRNA levels of HMG-R and HMG-S in *I. paraconfusus*, *I. pini*, and *D. jeffreyi*, although there were differences among these species in terms of dose dependency and timing of induction (55, 56). JH activated HMG-R more strongly (8-fold in *I. pini* and 30-fold in *D. jeffreyi*) than HMG-S (4-fold in *D. jeffreyi*) (55, 57). Moreover, JH elicited a modest induction of geranyl diphosphate synthase expression in male *I. pini* (48). A recent study on *I. pini* using quantitative real-time PCR examined feeding-induced changes in gene expression of seven mevalonate pathway genes: acetoacetyl-CoA thiolase, HMG-S, HMG-R, diphosphomevalonate decarboxylase, isopentenyl diphosphate isomerase, geranyl diphosphate synthase, and FPPS (30a). After feeding, expression in all seven genes increased in males, but only the first five genes increased in females. This suggests that feeding stimulates JH biosynthesis in the CA, which, in turn, stimulates the enzymes involved in pheromone production in the anterior midgut of males. In addition, these results support the hypothesis that the regulatory effect is exerted through coordinated regulation of the enzyme genes of the mevalonate pathway necessary for pheromone biosynthesis (30a).

The role of JH in regulation of the mevalonate pathway has also been investigated in the cockroach *B. germanica*. Here also the work has focused on HMG-R and HMG-S. The analysis of the mRNA and protein levels as well as their enzymatic activities in fat bodies and ovaries of adult females (Figure 2) showed

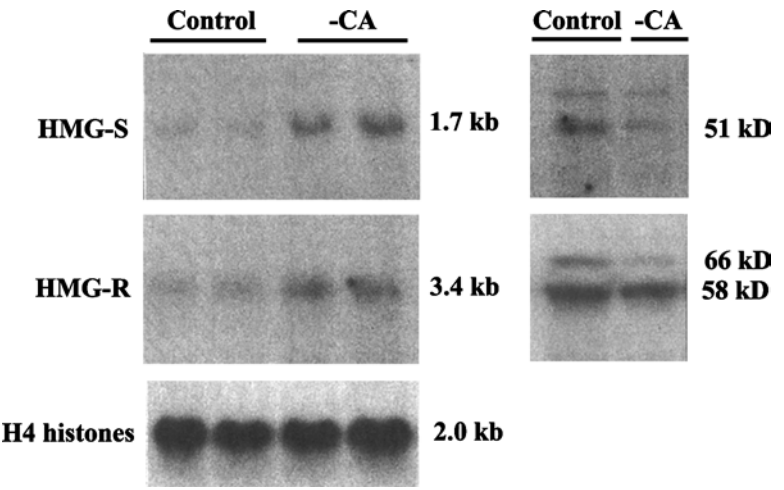

Figure 4 Northern blot (*left*) and Western blot (*right*) analyses of HMG-S and HMG-R in the fat body from control and allatectomized (-CA) females of the cockroach *B. germanica*. Insects were allatectomized after the imaginal molt, and analyses were carried out on day 4. The molecular masses of the mRNA or protein are indicated on the right of the corresponding gel. In Western blot analysis of HMG-R, the antibody immunodetects two proteins with molecular masses of 58 kDa and 66 kDa that are proteolytic fragments of the *B. germanica* enzyme (data based on Reference 11).

no obvious correlation between these levels and JH production (3, 6, 10), while JH applied to last larval instar females had no effect on HMG-R mRNA levels in the fat body (16). Moreover, mRNA levels of HMG-R and HMG-S are already high at the imaginal molt in the fat body, which suggests that high transcription rates occur at the end of the last nymphal instar, when JH is virtually absent (J. Cruz, D. Martín & X. Bellés, unpublished results). However, comparison of protein with mRNA levels reveals that translation takes place between days 1 and 4 of imaginal life, when JH production starts to increase (3, 10) (Figure 2). This suggests that JH may regulate the translatability and/or the stability of the enzyme transcripts. Females allatectomized at adult emergence and studied four days later showed twofold-higher levels of HMG-R and HMG-S mRNAs, whereas the corresponding protein levels were about half those measured in intact females (11) (Figure 4), which is consistent with the above hypothesis.

IN SEARCH OF REGULARITIES

Enzymes, Patterns, and Regulation by Juvenile Hormone

The structural studies of the enzymes of the mevalonate pathway in insects indicate that they have no special features and are homologous to the corresponding

enzymes of other metazoans. Those operating in the JH branch are restricted to arthropods and offer excellent opportunities not only to study the specific regulation of this peculiar branch, but also to explore them as targets for which to design and screen selective insecticides (28). Expression studies on a number of enzymes, especially HMG-S and HMG-R, in a variety of models have revealed a diversity of patterns, showing specificities in terms of time, tissue, and physiological process.

From the point of view of key regulators, experiments with cholesterol treatment and studies of the SREBP pathway of *D. melanogaster* have led to the conclusion that sterols do not regulate the mevalonate pathway in insects, as otherwise expected, given the absence of the cholesterol branch. Research also focused on JH, working on two different models: the production of pheromones in male scolytids, and the synthesis of JH and other processes related to cockroach female reproduction. Results suggest that JH can exert a pleiotropic role upon regulation of the mevalonate pathway, acting in some tissues, stages, and associated physiological processes as a transcriptional activator (pheromone biosynthesis in scolytids; 48, 30a), and also modulating the translatability and/or stability of the transcripts themselves (processes related to reproduction in cockroaches; 11). Often, data have shown that JH can act in parallel with a number of enzymes in the pathway, which suggests that the mevalonate pathway in insects can best be interpreted in terms of coordinated regulation and metabolic control analysis (23, 30a), rather than in terms of a key regulatory step(s).

The SREBP Pathway: Evolutionary Speculations

Data from animal genomes indicate that vertebrates have two SREBP homologs, and hence two SREBP pathways, whereas invertebrates have only one (41). This is strengthened by the fact that invertebrates (at least Arthropoda, Cephalopoda, Scaphopoda, Lamellibranchia, Nematoda, Plathyelminthes, and Cnidaria; 42) do not synthesize sterols de novo, in contrast to vertebrates (41). Although functional data in invertebrates are limited to *D. melanogaster* and *Caenorhabditis elegans*, in these organisms the SREBP pathway is involved in fatty acid regulation (18, 37, 47), as in the mammalian SREBP-1c pathway, which suggests that the invertebrate SREBP pathway is homologous to vertebrate SREBP-1c. Indeed, the similarity between the DNA-binding domains of dSREBP and human SREBP-1c is higher (83%) than that between the two human isoforms (80%) (41).

From an evolutionary point of view, the data indicate that the ancestral function of the SREBP pathway may have been the maintenance of the cell membrane by regulating fatty acid flux. Then, the single ancestral *SREBP* gene became duplicated in the lineage that led to chordates, and this second copy may have been coopted to perform the more specific role of regulating cholesterol (47). On the other hand, invertebrates had lost the sterol branch of the mevalonate pathway leading to cholesterol, thus becoming sterol auxotrophs. In insects in particular, quite paradoxically, this may have helped develop finely tuned mechanisms to regulate ecdysteroids from dietary sterols, and JH from farnesyl diphosphate.

After truncation of the mevalonate pathway, farnesyl diphosphate may have become the key branch point of the mevalonate pathway in insects, capable of diverting carbon flow specifically to the biosynthesis of JH and other critical compounds (such as dolichol and ubiquinone). Hence, farnesyl diphosphate may be considered a regulatory point, perhaps the key negative regulator of the mevalonate pathway. In this sense, it is worth remembering that the enzyme FPPS can be translocated to mitochondria, which offers more versatile possibilities of regulation by compartmentalization. Final or intermediate compounds of the lateral branches may, in turn, be specific regulators of their own branch.

FUTURE DIRECTIONS

Although considerable information on the mevalonate pathway has been obtained during the past decade, the absence of good "genetics" in most insect species used has precluded better knowledge of the regulation of the pathway or even of the composition of the pathway itself. In addition, the genome sequence of these species was unavailable, and furthermore, genetic transformation of them was undeveloped. Future research should be directed toward completing the molecular characterization of the enzymes involved in the different branches of the pathway (especially in those leading to JH), understanding the regulatory network controlling the pathway, and finding new functions for the products of the pathway in insects.

The achievement of these goals can be accelerated by following the genomic approaches recently developed, for example, using microarray experiments to carry out rapid, precise, and exhaustive analyses of thousands of genes in a wide variety of insect models. Using these approaches, the molecular dissection of their 5′ and 3′ flanking regions to characterize *cis*-sequences and *trans*-acting factors involved in their precise regulation, would be a reasonable objective in terms of time and effort, and would unravel the regulatory processes acting at different points in the pathway (19a, 30a). In addition, the use of new techniques of protein silencing such as RNAi would allow a way to surpass the limitations imposed by the difficulty of using transgenesis in nondrosophilid insects. RNAi may unveil the involvement of the mevalonate pathway in new processes, for example, related to development, as we have recently seen in recent and fascinating studies carried out on fruit fly embryos.

ACKNOWLEDGMENTS

The Dirección General de Investigación Científica y Técnica (Spain) and the Comissió Interdepartmental de Recerca i Innovació Tecnològica (Catalonia) have provided sustained financial support for our research line "The reproductive biology in cockroaches," which generated most of the results on the German cockroach herein reviewed. Thanks are due to Gary J. Blomquist, Anna-Gilg Young, Matthew Ginzel, and René Feyereisen, who critically reviewed the manuscript.

The *Annual Review of Entomology* is online at http://ento.annualreviews.org

LITERATURE CITED

1. Ayte J, Gil-Gomez G, Haro D, Marrero PF, Hegardt FG. 1990. Rat mitochondrial and cytosolic 3-hydroxy-3-methylglutaryl-CoA synthases are encoded by two different genes. *Proc. Natl. Acad. Sci. USA* 87:3874–78

1a. Baker FC, Reuter CC, Tsai LW, Brindle PA, Richard DS, et al. 1990. Use of microderivatization techniques in combination with thin-layer chromatography, liquid chromatography and gas chromatography-mass spectrometry for investigation of juvenile hormones and related compounds. In *Chromatography and Isolation of Insect Hormones and Pheromones*, ed. AR McCaffery, ID Wilson, pp. 19–31. New York: Plenum

2. Bellés X. 2004. Vitellogenesis directed by juvenile hormone. In *Reproductive Biology of Invertebrates*, Vol. 12, Part B: *Progress in Vitellogenesis*, ed. A Raikhel, TW Sappington, pp. 157–98. Enfield, NH/Plymouth, UK: Sci. Publ.

3. Bellés X, Casas J, Messeguer A, Piulachs MD. 1987. In vitro biosynthesis of JH III by the corpora allata of *Blattella germanica* (L.) adult females. *Insect Biochem.* 17:1007–10

4. Bochar DA, Stauffacher CV, Rodwell VW. 1999. Sequence comparisons reveal two classes of 3-hydroxy-3-methylglutaryl coenzyme A reductase. *Mol. Genet. Metab.* 66:122–27

5. Brown K, Havel CM, Watson JA. 1983. Isoprene synthesis in isolated embryonic *Drosophila* cells. II. Regulation of 3-hydroxy-3-methylglutaryl coenzyme A reductase activity. *J. Biol. Chem.* 258: 8512–18

6. Buesa C, Martínez-Gonzalez J, Casals N, Haro D, Piulachs MD, et al. 1994. *Blattella germanica* has two HMG-CoA synthase genes. Both are regulated in the ovary during the gonadotrophic cycle. *J. Biol. Chem.* 269:11707–13

7. Cabañó J, Buesa C, Hegardt FG, Marrero PF. 1997. Catalytic properties of recombinant 3-hydroxy-3-methylglutaryl coenzyme A synthase-1 from *Blattella germanica*. *Insect Biochem. Mol. Biol.* 27: 499–505

8. Cane DE. 1999. Isoprenoid biosynthesis: overview. In *Comprehensive Natural Products Chemistry*, ed. O Meth-Cohn, pp. 1–13. Amsterdam: Elsevier

9. Casals N, Buesa C, Marrero PF, Bellés X, Hegardt FG. 2001. 3-Hydroxy-3-methylglutaryl coenzyme A synthase-1 of *Blattella germanica* has structural and functional features of an active retrogene. *Insect Biochem. Mol. Biol.* 31:425–33

10. Casals N, Buesa C, Piulachs MD, Cabañó J, Marrero PF, et al. 1996. Coordinated expression and activity of 3-hydroxy-3-methylglutaryl coenzyme A synthase and reductase in the fat body of *Blattella germanica* (L.) during vitellogenesis. *Insect Biochem. Mol. Biol.* 26:837–43

11. Casals N, Martín D, Buesa C, Piulachs MD, Hegardt FG, Bellés X. 1997. Expression and activity of 3-hydroxy-3-methylglutaryl-CoA synthase and reductase in the fat body of ovariectomized and allatectomized *Blattella germanica*. *Physiol. Entomol.* 22:6–12

12. Castillo-Gracia M, Couillaud F. 1999. Molecular cloning and tissue expression of an insect farnesyl diphosphate synthase. *Eur. J. Biochem.* 262:365–70

13. Clark AJ, Bloch K. 1959. The absence of sterol synthesis in insects. *J. Biol. Chem.* 234:2578–82

14. Couillaud F, Feyereisen R. 1991. Assay of HMG-CoA synthase in *Diploptera punctata* corpora allata. *Insect Biochem.* 21:131–35

15. Couillaud F, Rossignol F. 1991. Characterization and regulation of HMG-CoA synthase in *Locusta migratoria* corpora allata. *Arch. Insect Biochem. Physiol.* 18: 273–83
16. Cruz J, Martín D, Pascual N, Maestro JL, Piulachs MD, Bellés X. 2003. Quantity does matter. Juvenile hormone and the onset of vitellogenesis in the German cockroach. *Insect Biochem. Mol. Biol.* 33:1219–25
17. Cunillera N, Boronat A, Ferrer A. 1997. The *Arabidopsis thaliana* FPS1 gene generates a novel mRNA that encodes a mitochondrial farnesyl-diphosphate synthase isoform. *J. Biol. Chem.* 272:15381–88
18. Dobrosotskaya IY, Seegmiller AC, Brown MS, Goldstein JL, Rawson RB. 2002. Regulation of SREBP processing and membrane lipid production by phospholipids in *Drosophila*. *Science* 296:879–83
19. Duportets L, Bellés X, Rossignol F, Couillaud F. 2000. Molecular cloning and structural analysis of 3-hydroxy-3-methylglutaryl coenzyme A reductase of the moth *Agrotis ipsilon*. *Insect Mol. Biol.* 9:385–92
19a. Eigenheer AL, Keeling CI, Young S, Tittiger C. 2003. Comparison of gene representation in midguts from two phytophagous insects, *Bombyx mori* and *Ips pini*, using expressed sequence tags. *Gene* 316:127–36
19b. Feyereisen R. 1999. Insect P450 enzymes. *Annu. Rev. Entomol.* 44:507–33
20. Feyereisen R, Farnsworth DE. 1987. Characterization and regulation of HMG-CoA reductase during a cycle of juvenile hormone synthesis. *Mol. Cell. Endocrinol.* 53:227–38
21. Gehring U, Harris JI. 1970. The active site cysteines of thiolase. *Eur. J. Biochem.* 16:492–98
22. Gertler FB, Chiu CY, Richter-Mann L, Chin DJ. 1988. Developmental and metabolic regulation of the *Drosophila melanogaster* 3-hydroxy-3-methylglutaryl coenzyme A reductase. *Mol. Cell. Biol.* 8:2713–21
23. Gibbons GE. 2003. Regulation of fatty acid and cholesterol synthesis: cooperation or competition? *Progr. Lipid Res.* 42:479–97
24. Goldstein JL, Brown MS. 1990. Regulation of the mevalonate pathway. *Nature* 343:425–30
25. Gunawardene YI, Tobe SS, Bendena WG, Chow BK, Yagi KJ, Chan SM. 2002. Function and cellular localization of farnesoic acid *O*-methyltransferase (FAMeT) in the shrimp, *Metapenaeus ensis*. *Eur. J. Biochem.* 269:3587–95
26. Hall GM, Tittiger C, Andrews GL, Mastick GS, Kuenzli M, et al. 2002. Midgut tissue of male pine engraver, *Ips pini*, synthesizes monoterpenoid pheromone component ipsdienol de novo. *Naturwissenschaften* 89:79–83
27. Havel C, Rector ER 2nd, Watson JA. 1986. Isopentenoid synthesis in isolated embryonic *Drosophila* cells. Possible regulation of 3-hydroxy-3-methylglutaryl coenzyme A reductase activity by shunted mevalonate carbon. *J. Biol. Chem.* 261:10150–56
28. Helvig C, Koener JF, Unnithan GC, Feyereisen R. 2004. CYP15A1, the cytochrome P450 that catalyzes epoxidation of methyl farnesoate to juvenile hormone III in cockroach corpora allata. *Proc. Natl. Acad. Sci. USA* 101:4024–29
29. Horton JD. 2002. Sterol regulatory element-binding proteins: transcriptional activators of lipid synthesis. *Biochem. Soc. Trans.* 30:1091–95
30. Houten SM, Waterham HR. 2001. Nonorthologous gene displacement of phosphomevalonate kinase. *Mol. Genet. Metab.* 72:273–76
30a. Keeling CI, Blomquist GJ, Tittiger C. 2004. Coordinated gene expression for pheromone biosynthesis in the pine engraver beetle, *Ips pini* (Coleoptera: Scolytidae). *Naturwissenschaften* 91:324–28

31. Kovacs WJ, Olivier LM, Krisans SK. 2002. Central role of peroxisomes in isoprenoid biosynthesis. *Progr. Lipid Res.* 41:369–91
32. Martín D, Piulachs MD, Bellés X. 1995. Patterns of haemolymph vitellogenin and ovarian vitellin in the German cockroach, and the role of juvenile hormone. *Physiol. Entomol.* 20:59–65
33. Martín D, Piulachs MD, Bellés X. 1996. Inhibition of vitellogenin production by allatostatin in the German cockroach. *Mol. Cell. Endocrinol.* 121:191–96
34. Deleted in proof
35. Martínez-Gonzalez J, Buesa C, Piulachs MD, Bellés X, Hegardt FG. 1993. 3-Hydroxy-3-methylglutaryl-coenzyme-A synthase from *Blattella germanica*. Cloning, expression, developmental pattern and tissue expression. *Eur. J. Biochem.* 217:691–99
36. Martínez-Gonzalez J, Buesa C, Piulachs MD, Bellés X, Hegardt FG. 1993. Molecular cloning, developmental pattern and tissue expression of 3-hydroxy-3-methylglutaryl coenzyme A reductase of the cockroach *Blattella germanica*. *Eur. J. Biochem.* 213:233–41
37. McKay RM, McKay JP, Avery L, Graff JM. 2003. *C. elegans*: a model for exploring the genetics of fat storage. *Dev. Cell* 4:131–42
37a. Nardi JB, Young AG, Ujhelyi E, Tittiger C, Lehane MJ, Blomquist GJ. 2002. Specialization of midgut cells for synthesis of male isoprenoid pheromone components in two scolytid beetles, *Dendroctonus jeffreyi* and *Ips pini*. *Tissue Cell* 34:221–31
38. Nijhout HF. 1994. *Insect Hormones*. Princeton, NJ: Princeton Univ. Press. 267 pp.
39. Olender EH, Simon RD. 1992. The intracellular targeting and membrane topology of 3-hydroxy-3-methylglutaryl-CoA reductase. *J. Biol. Chem.* 267:4223–35
40. Perrimon N, Lanjuin A, Arnold C, Noll E. 1996. Zygotic lethal mutations with maternal effect phenotypes in *Drosophila melanogaster*. II. Loci on the second and third chromosomes identified by P-element-induced mutations. *Genetics* 144:1681–92
41. Rawson RB. 2003. The SREBP pathway—insights from Insigs and insects. *Nat. Rev. Mol. Cell Biol.* 4:631–40
42. Rees HH. 1984. Biosynthesis of steroids: comparative aspects. *Nova Acta Leopoldina* 56:267–95
43. Robertson HM, Martos R, Sears CR, Todres EZ, Walden KK, Nardi JB. 1999. Diversity of odourant binding proteins revealed by an expressed sequence tag project on male *Manduca sexta* moth antennae. *Insect Mol. Biol.* 8:501–18
44. Ruddell CJ, Wainwright G, Geffen A, White MR, Webster SG, Rees HH. 2003. Cloning, characterization, and developmental expression of a putative farnesoic acid *O*-methyl transferase in the female edible crab *Cancer pagurus*. *Biol. Bull.* 205:308–18
45. Santos AC, Lehmann R. 2004. Isoprenoids control germ cell migration downstream of HMGCoA reductase. *Dev. Cell* 6:283–93
46. Schooley DA, Baker FC. 1985. Juvenile hormone biosynthesis. In *Comprehensive Insect Physiology, Biochemistry and Pharmacology*, ed. LI Gilbert, 7:363–89. Oxford: Pergamon
47. Seegmiller AC, Dobrosotskaya I, Goldstein JL, Ho YK, Brown MS, Rawson RB. 2002. The SREBP pathway in *Drosophila*: regulation by palmitate, not sterols. *Dev. Cell* 2:229–38
48. Seybold SJ, Tittiger C. 2003. Biochemistry and molecular biology of de novo isoprenoid pheromone production in the Scolytidae. *Annu. Rev. Entomol.* 48:425–53
49. Shinoda T, Itoyama K. 2003. Juvenile hormone acid methyltransferase: a key regulatory enzyme for insect metamorphosis. *Proc. Natl. Acad. Sci. USA* 100:11986–91
50. Sparagana SP, Bhaskaran G, Dahm KH,

Riddle V. 1984. Juvenile hormone production, juvenile hormone esterase, and juvenile hormone acid methyltransferase in corpora allata of *Manduca sexta*. *J. Exp. Zool.* 230:309–13

51. Sperry AE, Sen SE. 2001. Farnesol oxidation in insects: evidence that the biosynthesis of insect juvenile hormone is mediated by a specific alcohol oxidase. *Insect Biochem. Mol. Biol.* 31:171–78
52. Sutherland TD, Feyereisen R. 1996. Target of cockroach allatostatin in the pathway of juvenile hormone biosynthesis. *Mol. Cell. Endocrinol.* 120:115–23
53. Tillman JA, Seybold SJ, Jurenka RA, Blomquist GJ. 1999. Insect pheromones—an overview of biosynthesis and endocrine regulation. *Insect Biochem. Mol. Biol.* 29:481–514
54. Tillmann JA, Holbrook GL, Dallara PL, Schal C, Wood DL, et al. 1998. Endocrine regulation of de novo aggregation pheromone biosynthesis in the pine engraver, *Ips pini* (Say) (Coleoptera, Scolytidae). *Insect Biochem. Mol. Biol.* 28:705–15
55. Tittiger C, Barkawi LS, Bengoa CS, Blomquist GJ, Seybold SJ. 2003. Structure and juvenile hormone-mediated regulation of the HMG-CoA reductase gene from the Jeffrey pine beetle, *Dendroctonus jeffreyi*. *Mol. Cell. Endocrinol.* 199: 11–21
56. Tittiger C, Blomquist GJ, Ivarsson P, Borgeson CE, Seybold SJ. 1999. Juvenile hormone regulation of HMG-R gene expression in the bark beetle *Ips paraconfusus* (Coleoptera: Scolytidae): implications for male aggregation pheromone biosynthesis. *Cell. Mol. Life Sci.* 55:121–27
57. Tittiger C, O'Keeffe C, Bengoa CS, Barkawi LS, Seybold SJ, Blomquist GJ. 2000. Isolation and endocrine regulation of an HMG-CoA synthase cDNA from the male Jeffrey pine beetle, *Dendroctonus jeffreyi* (Coleoptera: Scolytidae). *Insect Biochem. Mol. Biol.* 30:1203–11
58. Van Doren M, Broihier HT, Moore LA, Lehmann R. 1998. HMG-CoA reductase guides migrating primordial germ cells. *Nature* 396:466–69
59. von Heijne G, Steppuhn J, Herrmann RG. 1989. Domain structure of mitochondrial and chloroplast targeting peptides. *Eur. J. Biochem.* 180:535–45
60. Zapata R, Martín D, Piulachs MD, Bellés X. 2002. Effects of hypocholesterolaemic agents on the expression and activity of 3-hydroxy-3-methylglutaryl-CoA reductase in the fat body of the German cockroach. *Arch. Insect Biochem. Physiol.* 49:177–86
61. Zapata R, Piulachs M, Bellés X. 2002. Ovarian 3-hydroxy-3-methylglutaryl-CoA reductase in *Blattella germanica* (L.): pattern of expression and critical role in embryogenesis. *J. Insect Physiol.* 48:675–81

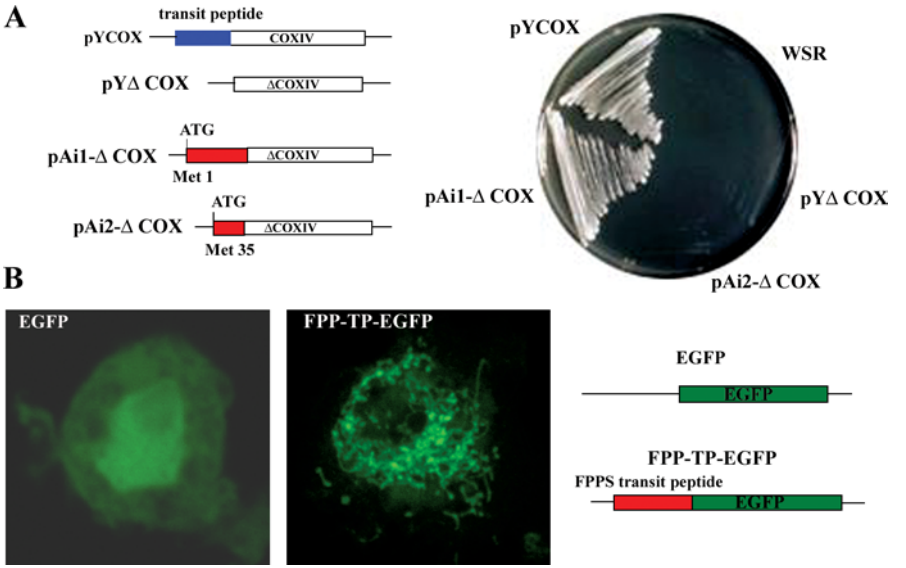

Figure 3 (*A*) Functional complementation of the yeast strain WSR with the N-terminal extension of *A. ipsilon* FPPS. WSR is a CoxIV-deficient strain unable to grow in a medium having glycerol as energy source (17). Plasmids used (*left*) were pYCOX (contains the wild-type *COXIV* gene, including its own mitochondrial transit peptide), pYΔCOX (as pYCOX, but without the transit peptide), pAi1-ΔCOX (contains the region encoding the complete N-terminal extension of *A. ipsilon* FPPS, from Met-1 forward, ligated in front of the partially deleted *COXIV* gene from yeast), and pAi2-ΔCOX (equivalent to pAi1-dCOX, but contains the region coding from Met-35 forward). Complementation analysis (*right*) shows that only the plasmid pAi1-ΔCOX complemented the respiratory function of WSR. (*B*) Functional analysis of the putative mitochondrial transit peptide of the *D. melanogaster* FPPS. Plasmids used (*right*) were FPP-TP-EGFP (contains the *D. melanogaster* FPPS putative transit peptide fused to EGFP) and EGFP (contains EGFP alone). Confocal microscopy images (*left*) of *Drosophila* S2 cells transfected with EGFP show a diffuse pattern, whereas those transfected with FPP-TP-EGFP show a network-like pattern virtually identical to that obtained with mitochondrial-specific staining (MitoTracker).