

***quemao*, a *Drosophila* Bristle Locus, Encodes Geranylgeranyl Pyrophosphate Synthase**

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ABSTRACT

The *quemao* (*qm*) locus of *Drosophila melanogaster* is characterized by a *P*-element-associated mutant lacking most of the large bristles on the thorax and by several EMS-induced recessive lethals. *quemao* was cloned using a transposon tagging strategy. *P*-element-mediated transformation demonstrated that the cloned *qm* DNA sequence (from the 65F cytological region) rescues the mutant phenotype. A 2.3-kb *qm* transcript was identified by Northern blot analysis by sequencing of the isolated *qm* cDNA clones and by 5' rapid amplification cDNA end (RACE). The predicted amino acid sequence (338 residues) of the coding region of the *qm* transcript shares 42, 31, 13, 20, and 12% identical amino acid sequences with the geranylgeranyl pyrophosphate synthase (GGPPS) of fungi, yeast, plants, archaeobacteria, and eubacteria, respectively. It also contains five highly conserved domains common among all known isoprenyl pyrophosphate synthases. The *P* element associated with the original *qm* mutant is inserted in the 5' untranslated region of the transcript. An EMS-induced *qm* nonsense mutation at the 12th codon leads to recessive lethality at the first larval instar, indicating the essential role of *qm* in the isoprenoid biosynthesis of insects.

THE isoprenoid biosynthetic pathway is the sole source of a variety of compounds with diverse structures and functions, including the sterols (Poulter and Rilling 1981; Goldstein and Brown 1990), ubiquinones (Ashby and Edwards 1990), dolichols (Matsuo *et al.* 1991), carotenoids (Spurgeon and Porter 1981; Sandmann 1991), and precursors for prenylated proteins (Clarke 1992; Schafer and Rine 1992; Omer and Gibbs 1994). Protein prenylation is a post-translational modification by which isoprenoid compounds are covalently attached to cysteine residues at or near the C termini of proteins (Clarke 1992; Casey 1994). Prenylated proteins account for ~2% of total cellular proteins (Epstein *et al.* 1991).

Two types of prenylation are known: farnesylation and geranylgeranylation. The former is catalyzed by the farnesyltransferases (FTase) that add the farnesyl group from farnesyl pyrophosphate (FPP) to the CAAX (where C is cysteine, A is an aliphatic amino acid, and X can be methionine, cysteine, alanine, glutamine, phenylalanine, or serine) motif of the C terminus. Geranylgeranylation is catalyzed by the geranylgeranyltransferases (GGTase) that transfer the geranylgeranyl group from geranylgeranyl pyrophosphate (GGPP) onto the cysteine in the CAAX motif. In most organisms, geranylgera-

nylation is more common than farnesylation (Epstein *et al.* 1991).

FPP and GGPP are intermediates in the diverse isoprenoid biosynthetic pathway (Goldstein and Brown 1990). Although there is evidence that archaeobacterial FPP and GGPP are synthesized by one bifunctional enzyme (Chen and Poulter 1994), in other eubacteria and eukaryotes (fungi, yeast, plants, and mammalian cells) FPP and GGPP are preferentially synthesized by the farnesyl pyrophosphate synthase (FPPS) and geranylgeranyl pyrophosphate synthase (GGPPS), respectively (Sagami *et al.* 1992; Chen *et al.* 1994). The GGPPS locus of *Saccharomyces cerevisiae* was recently cloned. A mutation at this locus suppresses a *bet2-1* mutant (one of the three subunits of GGTase-II) by increasing the intracellular pool of GGPP (Jiang *et al.* 1995). GGPPS null mutant yeast cells show slow vegetative growth at a low temperature; thus, GGPPS is not essential for yeast cells. Although GGPPS genes from prokaryotes, plants, and yeast are well characterized, the gene for this enzyme has not been identified from the animal kingdom. FPPS has been studied in yeast (Anderegg *et al.* 1988; Anderson *et al.* 1989; Schafer and Rine 1992) and mammalian tissues (Hancock *et al.* 1989; Sheares *et al.* 1989; Goldstein and Brown 1990). Less is known of GGPPS and its association with protein prenylation and sterol synthesis in animals.

In this article, we present the first description of *quemao*, a gene in *Drosophila melanogaster*, identified through an analysis that began with a hypomorphic mutant, *qm*¹, that lacks the bristle shafts of macrochaetes.

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We have cloned and characterized this locus and demonstrated through *P*-element-mediated transformation that cloned sequence flanking the original mutation rescues the mutant phenotype. Interestingly, we find that this locus is a homologue of the genes for GGPPS, the first identified in the animal kingdom. The loss of large bristles in *qm* mutants is associated with the *P*-element insertion into the 5' untranslated region of the GGPPS transcript. EMS-induced recessive lethal *qm* mutations indicate that GGPPS is an essential gene in *Drosophila* development.

MATERIALS AND METHODS

Stocks and culture conditions: The gene markers and chromosomes used are described in Lindsley and Zimm (1992). All flies were reared on 10 ml agar-yeast-glucose medium in shell vials at room temperature, unless otherwise specified.

1. *Inbred Samarkand (SAM)*: this inbred stock that contains no *P* elements has been described in detail by Mackay *et al.* (1992).

SAM *h'*: a stock was derived by 20 generations of backcrossing *h'* progeny to the inbred SAM.

C(1)DX, y w f' Y; SAM (SAM attached-*X*): an inbred SAM stock with a compound *X* chromosome (see Lai and Mackay 1993).

2. *CyO/Sp, Sb ry⁵⁰⁶ P[ry⁺ Δ2-3](99B)/TM6, Ubx SAM* (SAM *Cy/Sp Sb Δ2-3/Ubx*): This stock has a stable transposase source for *P*-element transposition. It has been described in detail by Mackay *et al.* (1992).

3. *TM6B, Tb/Sb; SAM* (SAM *Tb/Sb*): the *TM6B* third chromosome balancer contains the dominant marker *Tb*. The *TM6B, Tb* and *Sb* chromosomes were made congenic with inbred SAM (see Mackay *et al.* 1992).

4. *TM6, Ubx/TM3, Sb; SAM* (SAM *Ubx/Sb*): a stock with double third chromosome balancers in the SAM genetic background.

5. *quemao (qm')*: This new mutant was identified from the progeny of a *P*-element mobilization cross between an *M* strain, inbred SAM, and a *P* strain, inbred *Harwich* derivative (see Lai and Mackay 1990). It was mapped to 23.0 cM on the left arm of the third chromosome (M. Jackson and C. Lai, unpublished result). *Que mao*, in Chinese, means "loss of hairs." The original mutant *qm'* lacks all the macrochaetes on the thorax and most of the macrochaetes on the body, including two large bristles on each side of the sternopleural plate, but the sockets remain. The original mutant is spontaneously unstable, and partial revertants are readily obtained. *qm'* homozygotes are viable and fertile.

6. *Df(3L)Hn^{DEB}/TM6B*: This stock was kindly provided by Susan Shepherd (University of California at San Francisco). It has a deletion with breakpoints of 65E5 and 66C on the third chromosome and is maintained over the *TM6B* balancer chromosome.

7. *Df(3L)pb1-X1/TM6B*: This stock was provided by the Bloomington Stock Center, Indiana University. It has a deficiency chromosome with breakpoints of 65F3 and 66B10 and the *TM6B* balancer chromosome.

8. *w¹¹¹⁸*: This is a standard stock routinely used for transformation. *w¹¹¹⁸, qm'* is a stock derived from *w¹¹¹⁸* by introducing the entire third chromosome from the *qm'* stock. *w¹¹¹⁸; Tff/CyO* is a stock with the background from *w¹¹¹⁸* and heterozygous for *Tff* and the balancer *CyO*. *w¹¹¹⁸; TM3/TM6B (w Sb/Tb)* is a double third chromosome balancer stock with the background from *w¹¹¹⁸*.

Generation of revertants from *qm'*: Females of the *P*-element transposase source stock SAM *Cy/Sp, Sb Δ2-3/Ubx* were mass-mated with SAM *CyO; Ubx/qm'* males at 16° to generate viable F₁ flies. *Sb/qm'* F₁ males were then crossed with SAM *Ubx/Sb* females. At the next generation 100 single *Ubx/qm'* males each were mated with 5 SAM *Ubx/Sb* females to breed *Ubx/qm'* males and females. *qm'* alleles in each of these chromosomes are potential revertants of the parental *qm* allele. The heterozygous siblings (*Ubx/qm*) were mated to produce offspring. The non-*Ubx* homozygous progeny were scored for new *quemao* bristle phenotypes. The revertants were maintained over the balancer chromosome *TM6, Ubx*.

In situ hybridization to the polytene chromosomes: Larvae were raised at 18°. The technique of *in situ* hybridization was based on the method described by Shrimpton *et al.* (1986). The DNA probes were labeled with biotinylated dATP (bio-7-dATP; GIBCO BRL, Gaithersburg, MD) by nick translation. Hybridization was detected using the VECTASTAIN ABC kit (Vector Laboratories, Burlingame, CA) and visualized with horseradish peroxidase/3,3'-diaminobenzidine. To detect the presence of *P* elements, 0.8-kb *HindIII/HindIII* fragments of plasmid pπ25.1 (O'Hare and Rubin 1983) or the whole plasmid was used as the DNA probe, unless otherwise specified.

Southern blot hybridization: Genomic DNA was isolated and purified from adult flies in a CsCl gradient (Bingham *et al.* 1981) or by phenol extraction (Jowett 1986). The procedures for digestion, electrophoresis, and blotting were essentially the same as described by Sambrook *et al.* (1989). DNA probes were labeled with ³²P using a random priming kit from Boehringer Mannheim (Indianapolis). DNA Blots were hybridized with labeled DNA probes at 57° in 1% bovine serum albumin (BSA), 1 mM EDTA (pH 7.2), 0.5 M NaPi (pH 7.2), 7% sodium dodecyl sulfate (SDS) hybridization solution (Church and Gilbert 1984; Kreitman and Aguadé 1986).

Northern blot hybridization: Total RNA and poly(A)⁺ RNA from embryos, larvae, pupae, or adults was extracted using the QIAGEN (Venlo, The Netherlands) RNA isolation kit and was electrophoresed on formaldehyde-agarose gels and blotted onto Nytran (Schleicher & Schuell, Keene, NH), as described in Sambrook *et al.* (1989). DNA probe labeling and hybridization were performed in the same way as Southern blot hybridization.

Genomic DNA and cDNA library and screening: DNA was isolated from *qm⁸⁰*, a homozygous partial revertant of *qm'*, and partially digested with *Sau* 3A. The mildly digested DNA was ligated into *Bam*HI/*Eco*RI completely digested λGEM12 vector. The ligation was packaged using the Packagene extract from Promega (Madison, WI). A λDASH SAM genomic DNA library was constructed by a similar procedure. The cDNA library of the embryos (1–24 hr) came originally from Bruce Hamilton (Palazzolo *et al.* 1990; Hamilton *et al.* 1991). Genomic DNA and cDNA libraries were screened using DNA probes labeled with ³²P as described by Sambrook *et al.* (1989). GGPPS cDNA clones were identified using a DNA fragment amplified from the 3.4 O/O region with primers 1.0H2 (5'-CTAATGCTTCAAAGTATATTAAGCTTG-3') and 1.6H/H2 (5'-GAACTTGCCCTCCGTCAGTC-3').

P-element-mediated transformation: The 11.5-kb fragment (from the right-most *Xba*I site to 0.4 kb 3' of the left-most *Bam*HI; see Figure 2) of the putative *qm* clone isolated from the SAM genomic DNA library was subcloned into the *Xba*I site of the pCaSpeR transformation vector (Pirrotta 1988) to make the construct *P{w, CSX11.5}*. This vector is transcriptionally nonautonomous and contains a functional *w⁺* gene, allowing the use of pigmented eyes as a marker of transformation. Homozygous *w¹¹¹⁸* embryos were coinjected with this construct and the plasmid P[ry(Δ2-3)], a source of *P*-element transposase (Spradling and Rubin 1982; Laski *et al.* 1986).

G₀ progeny were mated with *w¹¹¹⁸* flies. G₁ *w⁺* progeny were selected and individually crossed with both *w¹¹¹⁸*; *TM3/TM6B* and *w¹¹¹⁸*; *Tft/CyO* flies if they were males, or to one of them if females. Some of the G₂ larvae were dissected to prepare polytene chromosomes, and the location of construct insert (s) was determined using *in situ* hybridization. The copy numbers of the constructs were determined by Southern blot analysis. Transformant lines containing a single insertion of the construct *P{w, CSX11.5}* were maintained either as *CyO* heterozygotes for insertions on the second chromosomes (selected each generation) or as hemizygous males for insertions on the *X* chromosome (with compound *X* females). Insertions on the third chromosome were avoided.

Rescue of *qm* mutations: *w¹¹¹⁸/y; +/+*; *qm² h¹* males were mated to virgin females, *w¹¹¹⁸ P{w, CSX11.5}/ w¹¹¹⁸; +/+*; *TM3(Sb)/+* for the *X*-linked insertion of *P{w, CSX11.5}* and *w¹¹¹⁸/w¹¹¹⁸*; *CyO/P{w, CSX11.5}*; *TM3(Sb)/+* in the case of the second chromosome insertion (*P{w, CSX11.5}*). Pair matings among *Sb (TM3)*, non-*Cy* F₁ individuals with pigmented eyes yielded distinguishable F₂ *h¹* progeny: homozygous for *w¹¹¹⁸*; *qm² h¹* and with either pigmented eyes (bearing the *P{w, CSX11.5}*) or without (lacking the *P{w, CSX11.5}*). The association of the *qm⁺* phenotype with pigmented eyes is evidence of rescue. Similar crosses tested the rescue of the normally lethal hemizygote *qm^{L144}* by *P{w, CSX11.5}*.

5' and 3' cDNA rapid amplification cDNA end (RACE) method: 5' RACE reactions were performed using Marathon cDNA Amplification Kit from CLONTECH (Palo Alto, CA; Chenchik *et al.* 1995). First-strand cDNA was reverse-transcribed from mRNA of the embryos or pupae using an oligo(dT) primer (provided with the kit) or antisense primer 1.6H/H6 (5' -GCTTTTGGGTGCGAACCGCATGGATTACC-3') in the *qm* locus. The second-strand cDNA was synthesized from the first-strand cDNA, then ligated to the adapter sequence (provided with kit). The 5' end cDNA sequence was amplified by PCR using the nested primers (1.6H/H2) and the anchor primer. The PCR product was cloned into the pCRII vector for sequencing using the TA Cloning Kit from Invitrogen (San Diego).

Induction of *qm* mutations using EMS: Three-day-old SAM *h¹* male flies were fed with 25 mM EMS (methanesulfonic acid ethyl ester) in 1% sucrose solution for 24 hr at room temperature. After the treated flies were kept in 40 ml agar-yeast-glucose medium in a quarter pint milk bottle for 24 hr, they were mated to *w¹¹¹⁸*, *qm²* virgin females for 3 days, then mated females were transferred into a new food bottle after the males were discarded. F₁ flies with the *quemao* bristle phenotype were collected and mated to SAM *Tb/Sb* flies for further characterization.

Larva collecting and mounting: Embryos were collected on grape-juice corn meal media at 25°, then were allowed to hatch at room temperature (22–23°). Larvae of different stages were collected and washed thoroughly with water before dissecting and mounting on slides and coverslips with Hoyer's media (van der Meer 1977).

Heteroduplex analysis: This technique was used to detect point mutations in EMS-induced *qm* lethal mutants. DNA was isolated from heterozygotes of the *qm* lethal mutant and the wild-type progenitor chromosome (the SAM chromosome). DNA fragments that range from 100 to 1000 bp and cover the exons and some of the introns of the *qm* locus were amplified by PCR using appropriate primers. PCR products were denatured at 95° for 3 min, then were cooled down slowly to 37° before running in 0.5× MDE gel (from J. T. Baker Inc., Phillipsburg, NJ). The gel was then visualized by staining with ethidium bromide. Primers 3.8S/S4 (5'-CTAACCTATCGA TAGAACATCGACTTGC-3') and 95T3 (5'-GCAGAATCTG GATAGCG-3') were used to identify a point mutation in *qm^{L144}*,

and 1.6H/H3 (5'-CATTCGCCTGATGCAGCTGTTTCAG-3') and 1.6H/H (5'-GATTGATCTGGTTCGACTGC-3') were used to pinpoint the ATG insertion of *qm^{L5.1}*.

DNA sequencing: Most of the DNA sequence reported here was determined on an ABI 377 sequencer utilizing the manufacturer's recommended reagents and protocols (Perkin-Elmer Corp., Norwalk, CT).

RESULTS

Phenotype of *quemao* mutants: The original *qm¹* mutant is unstable, which is a common feature of mutations derived from a stock carrying active *P* elements. *qm¹* is characterized by a lack of all or most of macrochaetes of the thorax, sternopleura, and head. The typical bristle phenotype of *qm* mutants is shown in Figure 1, A and B. Scanning electron microscopy (Figure 1C) reveals that the bristle shaft is usually lost or defective. Occasionally, a short bristle shaft can be found, but a single socket is always retained (Figure 1C). The pattern of bristle loss of partial revertants (see next section) and more severe derivative mutants of *qm¹* form a hypomorphic series: the least severe lack the two large bristles on the sternopleural plates, more severe mutants additionally lack the macrochaetes on the thorax, and finally, the most severe mutants also lack bristles on the head, on the dorsal abdomen, and the sex-comb teeth. Three *qm¹*-derived mutants described here are the following:

qm²: a stock derived spontaneously from *qm¹* that has a more severe bristle loss than *qm¹* (see Figure 1, A and B). Its phenotype is stable. Homozygotes are viable and fertile (*qm² h¹* is derived from recombination of *qm²* with *h¹*).

qm⁸⁹: a partial revertant of *qm¹* is wild type except for the absence of the two large bristles on each side of the sternopleural plates. Female bristle loss is more penetrant than that in males. The homozygote is viable and fertile. It retains a *P* element inserted at 65F.

qm⁵⁷: an extreme derivative of the *qm¹* locus that lacks all the macrochaetes on the thorax and head, and most of macrochaetes on the rest of the body. Males lack all or most of the sex-comb teeth (Figure 1D). Homozygous females are viable and fertile, whereas the males are viable, but sterile.

The bristle defect can be assessed by dissecting before the adult flies emerge from the pupa, indicating that loss of the bristle shaft occurs before eclosion of the adults. Sexual dimorphism in bristle loss was also observed among the partial revertants (see next section). Typically, *qm* females have more severe bristle loss than males. *qm* mutants have little effect on the microchaetes (see Figure 1).

Generation of revertants and deficiency mapping: Analysis of *in situ* hybridization to the polytene chromosomes from the original *qm* mutant indicated that there are four sites of *P*-element insertion (65A, 65F, 66A, and 68B) near the map position of *qm* (23.0 cM). To

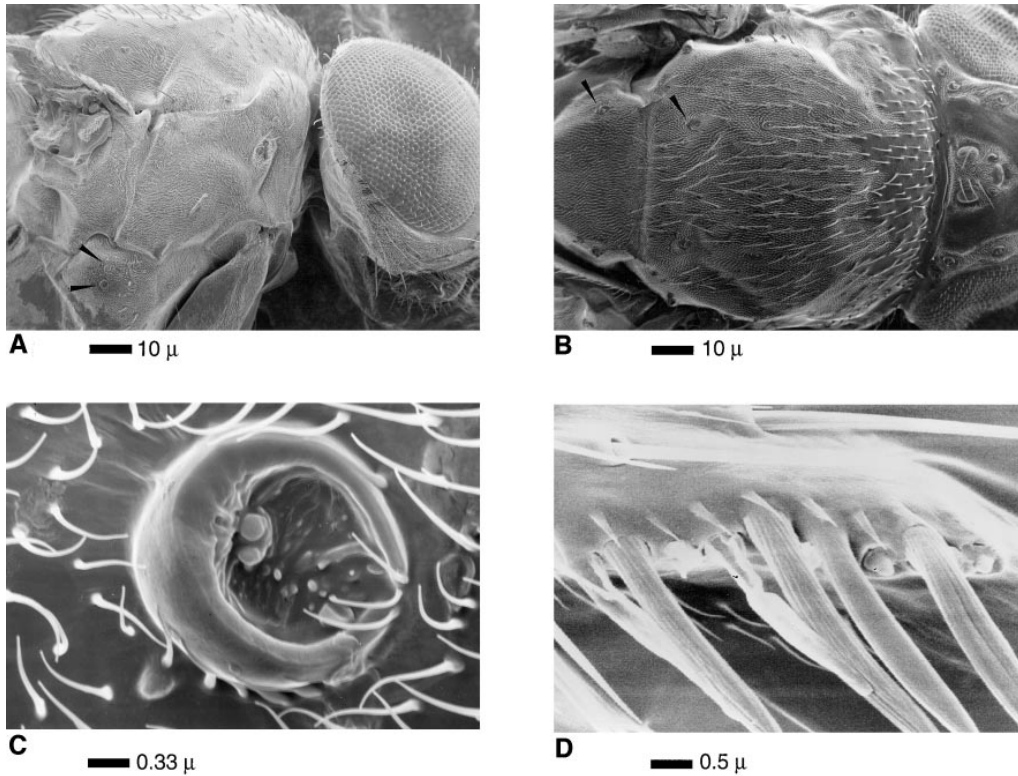


Figure 1.—Scanning electronic micrographs of bristle defects caused by *qm* mutations. (A) The side view of *qm*² thorax and head: two large defective sternopleural bristles are indicated by small arrows. (B) The dorsal view of *qm*² thorax: the macrochaetes are lost, indicated by the arrows, and their sockets are retained and the microchaetes are wild type. (C) An enlarged view of the socket lacking the bristle shaft. (D) Defects in the sexcomb teeth of *qm*⁵⁷: some teeth appear normal, and others are shriveled or absent.

determine which *P*-element insertion might be associated with the *qm* mutation, SAM *Sb* Δ 2-3/*Ubx* males were crossed to the *qm*¹ females in an attempt to mobilize that *P* element and generate revertants. A total of 20 partial (toward the wild type) and three complete revertants were identified among 100 chromosomes. *In situ* hybridization analysis of these revertant lines using the p π 25.1 (O'Hare and Rubin 1983) as the probe showed that the three complete revertants lost the *P* element inserted at the chromosomal band 65F, whereas the original mutant and other partial revertants still retain *P*-element sequences at 65F. The other three *P* elements near 65F did not show such a pattern of loss in the various revertants and derivatives. Furthermore, we crossed *qm*² (a severe and stable derivative of *qm*¹) to two deficiencies, *Df*(3L)*Hn*^{DEB}/*TM6B* and *Df*(3L)*pb1-X1*/*TM6B* (breakpoints at 65E5; 66C and 65F3; 66B10, respectively). The complementation pattern placed the *quema* locus in the chromosomal region of 65E5; 65F3. These two lines of cytological evidence indicate that the *P* element inserted at the position 65F causes the *qm* mutation.

Cloning of the *qm* locus: A genomic DNA library was made from a partial revertant, *qm*⁸⁹, as this stock has the *P*-element insertion of interest (at 65F) and the least number of other *P*-element insertions (nine copies, data not shown) in its genome. The DNA library was screened using the 0.8-kb *Hind*III/*Hind*III fragment of the *P* element (O'Hare and Rubin 1983). Eleven positive clones were initially isolated and tested individually by *in situ*

hybridization to the polytene chromosomes of SAM, the wild-type strain without *P* elements. One of these clones was found to hybridize to cytological band 65F. However, this clone also hybridized lightly to \sim 100 additional sites, suggesting that this clone contained repeated, dispersed DNA sequences. It was characterized using restriction enzymes (*Bam*HI, *Eco*RI, *Hind*III, *Sac*I, *Xho*I, and *Xba*I) and Southern blot analysis. This phage clone contains a 15.2-kb genomic fragment in which a *P*-element insertion was identified (see the map in Figure 2A). This 15.2-kb segment was divided into several fragments, each of which was labeled as a probe to hybridize to a genomic DNA blot from SAM, to determine the parts containing the repetitive DNA sequences. The unique sequence flanking the *P* element includes an \sim 9.5 kb (between the *Sac*I sites at positions -7.4 and $+2.2$; Figure 2A). Analysis of Southern blots of genomic DNA of *qm*⁸⁹ flies using the unique subclones as probes indicates that the genomic DNA segment in the phage clone is indeed derived from a single contiguous region from *qm*⁸⁹ flies (data not shown). To isolate the wild-type *qm* DNA sequence, a 5.5-kb O/O fragment (Figure 2) was used as a probe to screen the λ Dash SAM genomic DNA library. Several clones were isolated and characterized; the restriction map of a 24-kb region is given in Figure 2. The unique sequence 5.5-kb S/S fragment was utilized as a probe for *in situ* hybridization to the polytene chromosomes of SAM. A single band at position 65F was observed (see Figure 3). Furthermore, genomic Southern blot analysis using the 5.5-kb S/S

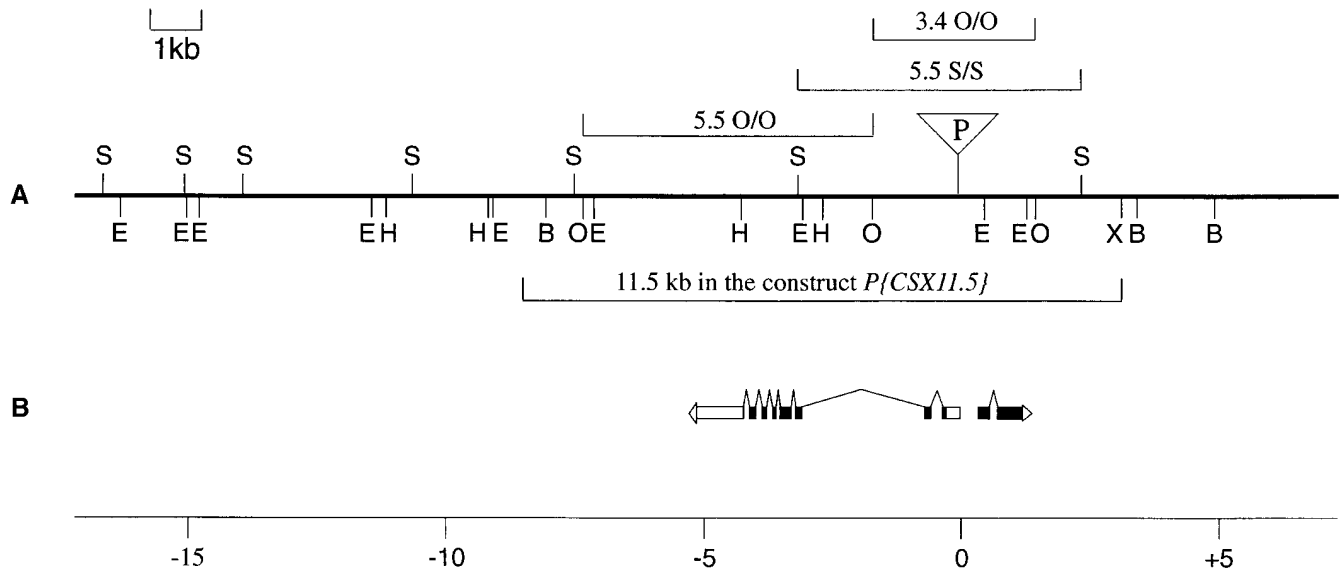


Figure 2.—(A) The map of the *qm* locus. The restriction map of the 24-kb region including the *qm* locus is summarized based on four phage clones from *qm⁸⁹* and SAM genomic DNA libraries. B, *Bam*HI; E, *Eco*RI; O, *Xho*I; S, *Sac*I; X, *Xba*I. The position of an apparently full-length *P*element insertion in *qm⁸⁹* is shown by an inverted triangle containing “P.” The 3.4 O/O fragment was used to screen the cDNA library, which led to identification of the *SRP19* transcript, and the 5.5 S/S fragment was used for Southern blot hybridization (see Figure 4), whereas the 5.5 O/O fragment was used as a probe for *in situ* hybridization (see Figure 3) to SAM polytene chromosome. The 11.5-kb fragment was used to construct CSX11.5 (see text). (B) The *qm* (left) and the *SRP19* (right) transcripts: the large, solid bars are putative coding regions; the large, open bars are untranslated regions; and the thin lines are introns. The directions of transcription are indicated with open arrows.

fragment as a probe indicates (Figure 4) that the wild-type revertants have lost the *P* element, but the partial revertants retain the insertion. Southern blot analysis of the *Df(3L)Hr^{DEB}* chromosome heterozygous with SAM or *qm⁸⁹* (using the 3.4 O/O fragment as probe; data not shown) demonstrates that the *Df(3L)Hr^{DEB}* chromosome lacks the 3.4 O/O region. These results support the conclusion that the *qm¹* mutation was caused by the insertion of the *P* element at position 65F.

Transformation and mutation rescue experiment: The *P* element associated with *qm¹* is inserted in a 9.5-kb segment of unique sequence DNA flanked on each end by repetitive sequences. We proposed that the *qm* locus was located within this 9.5-kb region. To deter-

mine whether this 9.5-kb fragment indeed contained the *qm* gene, the 11.5-kb fragment extending from the *Xba*I site to the *Bam*HI site (including the 9.5 kb unique fragment) was cloned into a *P*element-mediated transformation vector, pCaSpeR (Pirrotta 1988). This construct, P{w, CSX11.5}, was coinjected with the helper plasmid Δ2-3 into embryos of the stock *w¹¹¹⁸*, prior to the formation of pole cells (Spradling and Rubin 1982). Eight independent transformants were identified. To localize where the P{w, CSX11.5} inserted, polytene chromosome preparations from the larvae (G2) of each transformed line were analyzed by *in situ* hybridization using the unique sequence 5.5-kb S/S fragment as a probe. Among them, three transformants harbor a single construct inserted at different chromosomal positions: P{w, CSX11.5}8F at cytological position 8F on the X chromosome, and P{w, CSX11.5}47A and P{w, CSX11.5}42C on the second chromosome. Southern blot analysis indicated that each of these carries a single insertion of P{w, CSX11.5} (data not shown).

To test whether the construct P{w, CSX11.5} contains the functional *qm* gene, flies from these three transformants were crossed as described in the materials and methods. For each of the three insertions, the *h¹* homozygotes with pigmented eyes (transformants) exhibited the *qm⁺* phenotype, and white eye *h¹/h¹* flies exhibited the characteristic *qm²* phenotype. The results indicate that the construct P{w, CSX11.5} indeed contains sufficient *qm* gene sequences to rescue *qm²*.

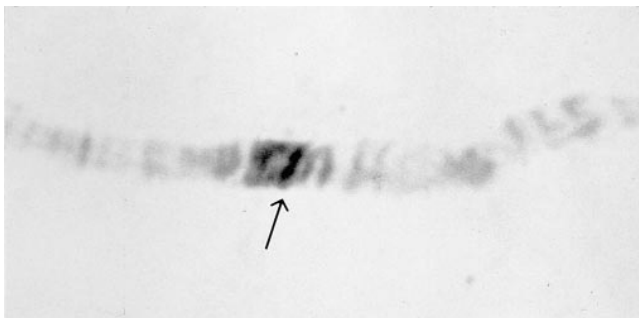


Figure 3.—*In situ* hybridization of the 5.5 S/S segment (Figure 2) to chromosome band 65F of the SAM polytene chromosome is visualized as the darker band (arrow).

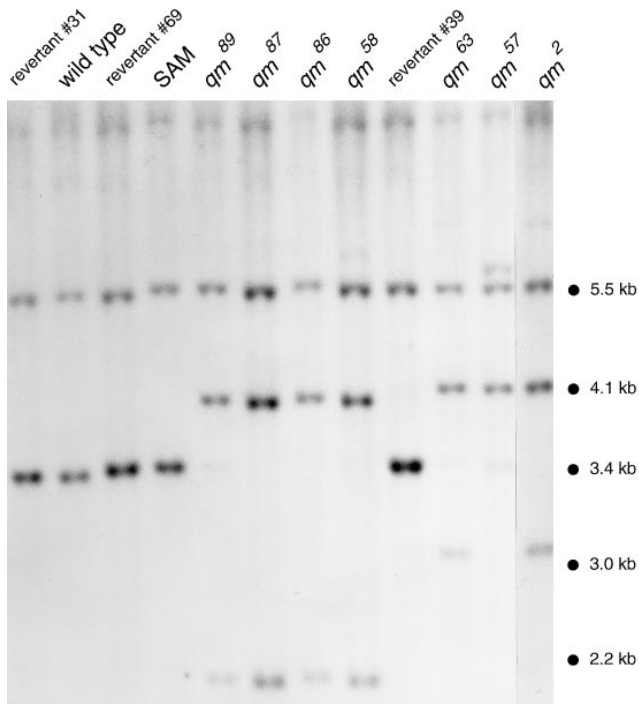


Figure 4.—Genomic Southern blot analysis of the *P*-element insertions at *qm*. *Xho*I-digested genomic DNAs of the wild type and *qm* mutant flies were probed with the 5.5 S/S fragment from the wild-type *qm* locus. Lanes 1, 3, and 9 are independent complete revertants (#31, #69, and #39) associated with the complete excision of the *P* element from *qm*¹. Lane 2 is a wild-type stock with a *qm*⁺ allele derived from the same cross from which *qm*¹ emerged. Lane 4 is wild-type stock SAM. Lanes 5–8 are partial revertants (*qm*⁸⁹, *qm*⁸⁷, *qm*⁸⁶, and *qm*⁵⁸); all retain a *P* element. Lanes 10–12 are partial revertants (*qm*⁶³, *qm*⁵⁷, *qm*²) that lack the 2.2-kb band, compared with the previous partial revertants (lanes 5–8), but retain the 5.5 kb plus new bands. These may be due to a second *P* element inserted next to the previous *P* element. The variable size of these fragments is due to differences in the structures of the parental-inserted *P* elements. In lanes 5 (*qm*⁸⁹) and 10 (*qm*⁵⁷) there is a light 3.4-kb band, which is likely due to the wild-type revertants in these unstable stocks.

Identification of the *qm* transcript: Two approaches have been taken to identify potential *qm* transcripts within the 11.5-kb *qm* rescue construct. First, DNA fragments flanking the site of the *P*-element insertion were hybridized to a poly(A)⁺ RNA blot from early pupae of SAM and the mutant *qm*². Any transcript exhibiting a difference between the mutant and wild type was considered a candidate transcript from the *qm* locus. The second and more fruitful approach was to first determine DNA sequences of the 9.5-kb unique sequence segment in the rescue construct. The genomic DNA sequence was analyzed for potential reading frames and transcripts using the GENE FINDER programs (Solovyev 1995). All positive reading frames were translated into amino acid sequences, then compared with the GenBank protein data base sequences. By the first method, an obvious 0.7-kb transcript on the right side of the

P-element insertion (Figure 2B) was identified. The corresponding cDNA was identified and sequenced from a pupal cDNA library. A GenBank search revealed this transcript to be a homologue of the 19 kDa signal recognition particle protein (*SRP19*) gene (Lai and Langley 1997). The second approach enabled us to identify another transcript on the left side of the *P* element (see Figure 2B). This second transcript extends over 5 kb of genomic DNA sequences to the left side of the *P*-element insertion (Figure 2B). The predicted amino acid sequence, used to search GenBank, shares 50% identity with the geranylgeranyl pyrophosphate synthase (GGPPS) protein sequence of *Neurospora crassa* (Carattoli *et al.* 1991). Two cDNA clones were isolated (one 2.0 kb and the other 2.6 kb) using part of the predicted conserved coding region (Chen and Poulter 1994), the fragment including domain II-V (222-712; see Figures 5 and 7) amplified with primers 1.0H2 and 1.6H/H2 (see materials and methods) as a probe to screen ~100,000 plaques of an embryonic cDNA library.

To determine which of these two transcription units might correspond to *qm*, we induced several *qm* mutants using EMS as a mutagen. DNA sequence analysis of six recessive *qm* mutants revealed no mutation within the SRP19 coding region. This result suggested that the homologue of SRP19 is not *qm*. For two of the EMS-induced recessive lethal *qm* mutants, the coding region and several introns of the GGPPS-related transcription unit were screened for mutations using a heteroduplex method and DNA sequencing. One lethal mutation, *qm*^{L14.4} (see below), is associated with a point mutation at the 12th codon of the putative coding region; the codon AAA (lysine) in the wild type is mutated to the stop codon, TAA in this mutant (Figure 5). A second EMS-induced recessive lethal *qm* mutation *qm*^{L5.1} has an insertion of ATG in the middle of the sixth intron (and reduced transcript levels; see below). These results indicate that the identified homologue of GGPPS is the *qm* gene.

The transcription start site of the GGPPS homologue was identified from the 5' end sequence of a RACE product (see materials and methods) based on two antisense primers (1.6H/H2 and 1.6H/H6) and the poly(A)⁺ RNA of 1–24-hr SAM embryos. Nine independent RACE clones have been identified and sequenced (materials and methods), and 225 bp of 5' end sequence have been identified upstream of the 5' end of the 2.0-kb cDNA clone. However, after careful examination, the 2.6-kb cDNA clone appears to be an artifact of cDNA library construction. Thus, the sequence included in the 225 bp 5' end and the 2.0 kb cDNA represents the transcript of the *qm* locus (see Figures 2 and 5), GenBank accession number AF049659. The position of the *P* element in *qm*¹ and its derivatives (inserted in the 5' untranslated region of this transcript; see Figure 5) is also consistent with this homologue of GGPPS being the *qm* gene.

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-295 CAATATGCAGGCCCGGAAGAACATCAATACAGTCGTTTTTGGCTCTTTCCGCTGGTGTGA
-235 ATTGATTGTGAGTTGCGCGGTAACCGGAAAACCAATCGACGAGCGTGGCCGAGCAATTC
-175 TAGTCACGGCGTGTGGGAAGCCAAAGCGGTAACAAAAGACAGCCCGCACCGCAGTGAC
-115 CCCCCTCCATCAACACCAACACCACCCCGCCGCTCTATCAAAAACAACAAAAGCACA
-55 CACGCAAGGCCACGGCGGAGCAGCAACAAGAAAAACAACAACCGCGCAACAACATGGGA
ME
6 AGAACTGAACATCATATTACAGAAGACCAGAGATAAATCAACGCAAAGGAGCAGGATGA
E L N I I L Q K T K D K S T Q K E O D E
66 GATTCTGCTGCAGCCCTTTACATACATACAACAGATTCTCGCAAGCAATTCGCTCTGA
I L L Q P F T Y I Q Q I P G K Q F R S E
126 GTTGGCCCTTGGCCCTTCAATCACTGGTTGCTATACCGGGCGAAAAGTTGGCGCAGATCGG
L A L A F N H W L L I R G E K L A Q I G
186 AGACATTGTGCAGATGCTGCACAATTCAGTTTGCCTCATTTGATGATATTGAAGACAATTC
D I V Q M L H N S S L L I D D I E D N S
246 GATCCCTGCAGAGGTGTGCCCGTGGCGCATTCCTACTACGGCGTGGCCAGCACCATAAA
I L R R G V P V A H S I Y G V A S T I N
306 TGGCGCAACTATGCACCTTTCTTGGCGCTGGAGAAGGTGCAGCAGTGGATCATCCGGA
A A N Y A L F L A L E K V Q Q L D H F P E
366 GGCTACCAAGTGTACACCGAACAAATGGCTGGAGCTGCACCGTGGACAGGGCATGGAGAT
A T K V Y T E Q L L E L H R G Q G M E I
426 CTATTGGCCGACAGCTTCCAGTCCCATCCGAGTCCGATACAAAGCTGATGACTGCTGCG
Y W R D S F T C P S E S D Y K L M T V R
486 CAAAACCTGGCGCCCTTTATGCTGGCCATTCGCTTATGCAAGTGTTCAGCTCCAAACA
K T G G L F M L A I R L M Q L P S S N K
546 GGAGGACTTTCGAAGTTGACGGCTATATTGGCCGTACTTTCAGATACCGCAGGACTA
E D Y S K L T A I L G L Y F Q I R D D Y
606 TTGCAATCTGAGTCTGAAAGAGTACACGGAGAACAAGAGCTTCGCGAGGACTTTCAGGGA
C N L S L K E Y T E N K S F A E D L T E
666 GGGCAAGTTCGGCTTCGCGGTAATCCGCTGGCTTCGCACCCAAAAGCAGGATAAAGAGT
G K F G F P V I H A V R T Q K Q D K Q V
726 TCTACACATATTAGCCAGCCAGCAGCAATTCAGGTTCAAGAACTACTGCATCAACCTT
L H I L R Q R T H D I E V K K Y C I T L
786 GTTGGAAAAGCTGGGAGCTTTCAGTATACACCAAGGTTCTGGAGTCCGCTCGACCCAGA
L E K L G S F Q Y T R K V L E S L D A E
846 GGCTCGGTCGAGGCTGGCTCTGCTGAGTACCAATCGTACATGGACCCGCTGCTCAACA
A R S E V A R I G S N P Y M D R L N N K
906 GCTGCTGCTGGGAAGACGAGCGATAGCCAGCAGTACAGCAGTCAAGCAGATCAATCA
L L S W K T S D S A S I T Q S N Q I N H
966 CAACAACGCTCACGACCGCCCAACCAAGCAACCGTTCATTTCAATTCAGATTCAGAGCCAAA
N N V S R S S P N Q N T F N C N *
1026 GAGCAAAAAGAGGGAGCAGTGTGAATTCGGAACCGCAGAGCCCTTTTGGCCGCAATCC
1086 GGGACAGCAGCAGCCAGCAGCCAGCCGACCCCTGGCTGCTCAGTGGCCGCTGTTGGG
1146 TTTCCCTGCAGAGCTGAAGATATTCCTGAGATTTATGTTACCTTTCGCAACCGGAAAAGC
1206 CCACAGAAACCCCTACCTTACTGTCAACCCAAACAGAGCGATGAGAACGGAAATAGGA
1266 GGAGGATAGGATTTGGATTGTAGTAGTGAATCGGATGAAATGGGATGATAACAAACCAA
1326 CAGCAGCCTTACAGAAAATTTGTAAGCCAAAAGTAAATGATATAGCCAGCTTATTCGT
1386 TGAGCTAGCAAGAGCCATATTACAGCTTTTCAAGAAATTTGTTTTTTACTTTTACATT
1446 TTACTTTTACTTTTAAATTTAGTTTTGCTTCAAAGTTACGTTAGTTAGTTAGCTGCTTTAG
1506 TTTCCCGAGTTTTAGTTTTGCCCCAACGAATGGGAGAGGGGGGGTGGGGATCAAGCAAT
1566 CTCGACAGCATTACTGAATATAGAAATGCAATTTTTCAGCTCTTTGATTTCTAAGTGC
1626 TGTTTCGATTCGTAACCTCAATTCCTCAAGCAATTTTCAATTTCTAAGTGCATTTTATC
1686 AAATCTAATCACTCCGAACCTCAATGTGCCTTCCCTTATTCGAGCTTTTCCATTTTAAAT
1746 TGTATACAAATGGGATTCGATACATACATATGCTATTTAAGCAAAATTTGTTA
1806 ATGTTTTATGCGACACTATAAATACACCAAAATGAACGCAACTATATATTTGAATA
1866 ATGATAATGATGCACCTGCACGGCAGGTTTTTTCACATTCGCCACCTTAAAAA
1926 AA
    
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Figure 5.—Molecular structure and sequence of the *qm* locus. The total sequence (2223 bp) of *qm* transcript is derived from the 2.0-kb cDNA clone and the 225-bp RACE product. The putative *qm* translational start site is indicated by the underlined ATG, and the stop codon by *. The position of the *P*-element insertion in the 5' end untranslated region is depicted by a large, solid inverted triangle at -264. The six intron positions are marked by six small open reverted triangles after the positions of the 66th, 221st, 366th, 627th, 730th, and 858th nucleotides. The nonsense mutation correlated with *qm*^{L14.1} is identified at the 34th nucleotide by the bolded A.

Molecular structure of the *qm* locus: Alignment of the *qm* transcript with the corresponding genomic DNA sequence (GenBank accession number AF049658) shows that the *qm* locus is composed of seven exons and six introns. The molecular structure of the *qm* locus is given in Figure 5. The *qm* transcript contains a single open reading frame of 1014 bp, a 295-bp 5' untranslated region, and a long 760-bp 3' untranslated region. The putative start codon (ATG) is characterized by a 5' flanking sequence (AACA), which is similar to the *Drosophila* consensus sequence (C/A AA A/C) before the start codon (Cavener 1987). The predicted *qm* protein is 338 amino acids and has a predicted molecular weight

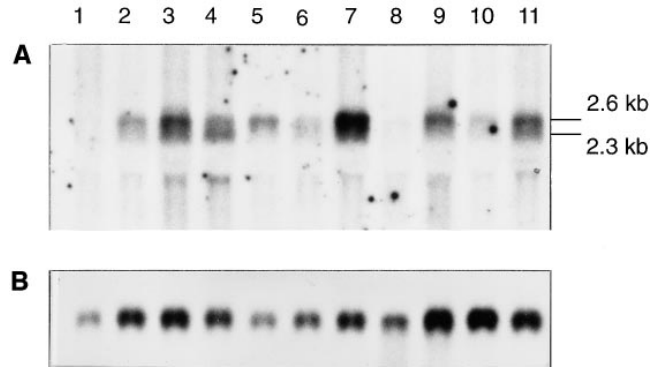


Figure 6.—Northern blot analysis of *qm* transcription. (A) Each lane contains ~5–8 μg of total RNA from different developmental stages of *Drosophila*. The RNA blot was probed with the 2.0-kb cDNA clone labeled with ³²P, then exposed to X-ray film for 2 wk. Lane 1 RNA is from adults of *qm*^{15.1} hemizygous for the deficiency chromosome *Df(3L)HnDEB*. Lanes 2 and 3 are from adults, lanes 4 and 5 are from pupae, lanes 6 and 7 are from third instar larvae, lanes 8 and 9 are from first instar larvae, lanes 10 and 11 are from embryos, of *qm*² and SAM, respectively. Two distinct transcripts, 2.6 kb and 2.3 kb, are indicated on the blot. (B) The same RNA blot (after stripping of the probe) was hybridized with the Rpl132 coding sequence (also known as *rp49*; O'Connell and Rosbash 1984), then exposed to X-ray films for 2 hr. It reflects the relative amount of total mRNA loaded into each lane.

of 38,859 Daltons, with a pI of 6.5. Introns 1, 2, 3, 4, 5, 6 are 187 bp, 2251 bp, 53 bp, 67 bp, 73 bp, and 133 bp long, respectively.

RNA blot analysis: To analyze the temporal pattern of *qm* expression and potential differential expression in mutant and wild-type individuals, Northern blots of stage-specific total RNA from SAM and *qm*² homozygotes were hybridized with the 2.0-kb *qm* cDNA clone (Figure 6). There are two distinct species of transcripts: ~2.3 kb and 2.6 kb. These two transcripts were also confirmed in another Northern blot analysis (data not shown). The 2.3-kb transcript corresponds approximately to the size of the 2.0-kb cDNA clone isolated from the embryonic cDNA library plus the 225 bp of 5' end sequences. Both transcripts are expressed in all developmental stages. With reference to the amount of total RNA loaded in each lane, it is obvious that the mutant *qm*² caused by the *P*-element insertion shows consistently less *qm* mRNA expression than does the wild type for all stages, except for pupal stage, where the relative levels of *qm* expression is less obvious. However, there is no detectable difference in the size of the *qm* transcript between the mutant and wild type. This suggests that the *P* element inserted in the 5' untranslated region causes a reduction in *qm* expression but may not alter the size of the *qm* transcript (presumably beginning within the *P* element). The EMS-induced mutant *qm*^{15.1} adults have negligible *qm* mRNA expression when hemizygous (see Figure 6). The levels of *qm* mRNA expression in wild-type individuals are much lower than that of

SRP19 (Lai and Langley 1997) and ribosomal protein 49 (Figure 6). This is also consistent with the low abundance of *qm* positive cDNA clones (2/100,000 plaques) in the cDNA library we screened.

The *qm* gene is essential in *Drosophila*: Although *qm¹* and its derivatives that carry a *P*-element insert in the 5' untranslated region are viable and fertile, most of the EMS-induced *qm* alleles are recessive lethal and die at larval or pupal stages. The six EMS-induced recessive lethal *qm* mutants fail to complement each other and the deficiency *Df(3L)Hnr^{DEB}* as well as *qm²*. A seventh EMS-induced mutant, *qm^{15.1}*, is viable over *Df(3L)Hnr^{DEB}* (males are sterile), and *qm^{15.1}* homozygotes are lethal. These seven *qm* recessive lethals show the same level of bristle loss as *qm²* homozygotes when heterozygous with *qm²*. *qm^{L14.4}* homozygotes and hemizygotes (over the deficiency *Df(3L)Hnr^{DEB}* chromosome) die as larvae 5 days after hatching. Their mouth parts remain as those of first instar larva, and their body size is much reduced compared with wild-type larvae of the same age. However, there is no detectable abnormality in the external larval cuticle (data not shown). Most homozygous *qm^{15.1}* animals die at the pupal stage, although occasionally some homozygous males survive to emerge as adults. The dead pupae or adult males have underdeveloped and abnormal abdomens (fewer bristles and misshapen cuticles). However, hemizygous females *qm^{15.1}/Df(3L)Hnr^{DEB}* are viable and fertile, whereas hemizygous males are viable but sterile. The viable hemizygous *qm^{15.1}* males and females show a bristle phenotype similar to that of *qm⁵⁷*, indicating that the bristle phenotype is not limited to *qm¹* and its derivatives. Hemizygotes of the recessive lethal *qm^{L14.4}* are rescued by the *P{w, CSX11.5}* construct and show no mutant bristle phenotypes (data not shown).

***qm* is a homologue of GGPPS:** Comparison of the predicted *qm* protein with the GGPPS of *Neurospora crassa*, *Saccharomyces cerevisiae*, *Arabidopsis thaliana*, *Methanobacterium thermoautotrophicum*, and *Streptomyces griseus* reveals 42, 31, 13, 20, and 12% identity, respectively (Figure 7). The QM protein also has five conserved domains (Figure 7) shared by other isoprenyl pyrophosphate synthases (Chen and Poulter 1994). It has a similar molecular weight to those of isoprenyl pyrophosphate synthases from yeast, *Arabidopsis*, and archaeobacteria and is predicted to be ~1000 Daltons smaller than those of eubacteria and fungi. A phylogenetic tree (Figure 8) constructed from the isoprenyl pyrophosphate synthase amino acid sequences of 15 taxa reveals that GGPPS enzymes fall into two major groups. *Drosophila*, yeast, and fungal GGPPSs are distinctly clustered together. The eukaryotic FPPSs are also clustered, and archaeobacterial, eubacterial, and higher plant (a presumed transfer from a plastid genome) GGPPSs and FPPSs are in a separate group. This analysis supports the hypothesis that FPPS and GGPPS evolved as distinct

genes with separate functions, although the situation in both bacterial lineages is less clear.

DISCUSSION

Using *P*-element tagging and EMS mutagenesis, we have characterized and cloned the *Drosophila* locus, *quemao*. Recessive mutations at *quemao* (*qm*) can cause loss of the shaft of large bristles, lethality, or male sterility. A *P* element inserted at 65F on the left arm of the third chromosome in the original *qm¹* mutant was found to be associated with the bristle phenotype. Twenty-four kilobases of genomic DNA flanking the *P*-element insertion were cloned and characterized. *P*-element-mediated transformation demonstrated that the 11.5-kb segment of the *qm* DNA sequence (flanking the site of the *P* element) rescues the mutant phenotypes. Within the rescue construct, we identified two transcription units that encode distinct proteins. One protein shares a significant amount of identity with the 19-kD signal recognition particle protein SRP19 (Lai and Langley 1997). The protein apparently encoded in the second transcription unit shows significant amino acid sequence similarity to geranylgeranyl pyrophosphate synthase (GGPPS). The *P* element associated with *qm¹* is inserted in the 5' untranslated region of the *qm* GGPPS transcript (and in several of its derivatives). This insertion is responsible for reductions in levels of the GGPPS transcript in these *qm* mutants. The similar size of this *qm¹* mRNA to that encoded by *qm⁺* suggests that the *qm¹* transcript may begin within the portion of the *P* element proximal to the GGPPS transcription unit. Analysis of the EMS-induced *qm* recessive lethal *qm^{L14.4}* reveals it to be a nonsense mutation in the GGPPS coding region. These results indicate that *qm* is an essential *Drosophila* gene that appears to encode GGPPS, an enzyme in the isoprenoid synthetic pathway. This is the first GGPPS-encoding gene identified in the animal kingdom (a partial human cDNA sequence with significant similarity to GGPPS is in GenBank, accession number H60499).

Isoprenyl pyrophosphate synthases are essential enzymes that catalyze the basic chain elongation process in the isoprenoid biosynthetic pathway. Isoprenoids are the building blocks of diverse structural components in all cellular organisms. GGPPS not only catalyzes the formation of geranylgeranyl (C20) pyrophosphate (GGPP) from isopentenyl pyrophosphate and dimethylallyl pyrophosphate, geranyl pyrophosphate, or farnesyl pyrophosphate, but it also may synthesize farnesyl pyrophosphate from dimethylallyl pyrophosphate or geranyl pyrophosphate (Sagami *et al.* 1992). GGPP and FPP are the most common isoprenoid substrates of protein prenylation. They are transferred specifically by geranylgeranyl transferase and farnesyl transferase to many proteins. GGPP and FPP are also intermediates in many other biosynthetic pathways. We anticipate that a null mutation at a nonredundant GGPPS locus would result

<i>Dro.mel.</i> (34)	PGKQFRSELA ---LAFNHWLLI PGEKLAQIGDIVQMLHNS SLLI DDIE -- DNSILRRG	87
<i>Neu.cra.</i> (139)	FGKDIRSQMV ---KAFDAWLDV PSESLEVI TKVIS MLHTASLLV DDVE -- DNSVLRRG	192
<i>Sac.cer.</i> (33)	FGKNFRNLNI ---VQINRV MNLPKDQLAIVSQIV ELLHNS SLLI DDIE -- DNAPLRRG	86
<i>Ara.tha.</i> (113)	GGRVVRPVL ---CIAACELV GGEESTAMPARCAVE EMIHTMSLI HDDLPCMDNDDLRRG	168
<i>Met.the.</i> (41)	GKKIRPSL ---ALLSCEAV GGNPEDAAGVAAAI ELIHTFSLI HDDI -- MDDDEMRRG	94
<i>Str.gri.</i> (81)	GGRMRPRLLW WGM RSCG --AAETAAAL R L GVAL ELIQSCAL I HDDV -- MDRSRLRRG	135
		* * * * *	
		I	II
<i>Dro.mel.</i>		VPVAHSI ----- YGVASTINA ANYAL FLALEK VQQLD HP EA-----TKV Y TE Q LL E -----	133
<i>Neu.cra.</i>		FPVAHSI ----- FGIPQ TINTSN YVYFYALQ ELQ LK LN P KA-----V S IF S E L LN-----	238
<i>Sac.cer.</i>		QTTSHLI ----- FGVP STINTAN YMYFRAMQ LV S QL T TK E PLY H N L IT I F N E L IN-----	137
<i>Ara.tha.</i>		KPTNHKV ----- FGED VAV L AGD ALLS F S FE HL ASAT SSD V S P V R V RA V GE L A K AI G	222
<i>Met.the.</i>		EPSVHVI ----- WG EP M IL AG D V L F SK A FE A V I R NG D S E----- R V K D A L A V V D S C V	143
<i>Str.gri.</i>		KPAMHIG LAER AG LS P D S ER G S D FG SA AV L AG D L A L V W A----- D D T V A E T V L S A A Q R R R I G A L W R A M R	200
<i>Dro.mel.</i>		--- LHRGQ GM E I Y WR D S F T--- C P S E S D Y K L M T V R KT G G L F M L A I R L M Q L F S--- S N K E D --- Y S K L T A I L G L	194
<i>Neu.cra.</i>		--- LHRGQ GM D L F WR D T L T--- C P T E D D Y L E M V S N KT G G L F R L G I K L M Q A E S--- R S P V D --- C V P L V N I I G L	299
<i>Sac.cer.</i>		--- LHRGQ GL D I Y WR D F L PE I I P T Q E M Y L N M V M N KT G G L F R L T L R L M E A L S--- P S S H H G H S L V P F I N L L G I	203
<i>Ara.tha.</i>		TEGLVAGQ V V D I --- S SE G L D L N D V GLE H LE F I H L H KT A A L L E A S A V L G A I V G G G S D D E I E R L R K F A R C I G L	291
<i>Met.the.</i>		K --- I C E G Q A L D M--- G F E E--- R L D V T E D E Y M E M I Y--- K KT A A L I A A A T K A G A I M G G A S E R E V E A L E D Y G K F I G L	208
<i>Str.gri.</i>		TE --- M V A G Q Y L D L--- R --- G Q L S G G S S V A Q A L R T A C L S A S Y S V E R P L A I G A A L A G A D D R T T D A L R S A G R C A G I	267
		* * * * *	
		III	IV
<i>Dro.mel.</i>		YFQIRDDY C N L S L K E Y T E N K S F A E D L T E G K F G F P V I H A V R T Q K----- Q D K Q V L H I L R Q R T H D I E V K K-	257
<i>Neu.cra.</i>		IFQIAD D Y H N L W N R E Y T A N K G M C E D L T E G K F S F P V I H S I R S N P----- S N M Q L L N I L K Q K T G D E E V K R-	362
<i>Sac.cer.</i>		IYQIRDDY L N L K D F Q M S E K G F A E D I T E G K L S F P I V H A L N F T K T K G Q T E Q H N E I L R I L L L R T S D K D I K L -	272
<i>Ara.tha.</i>		LFQVDD I L D V T K S S K E L G K T A G D L I A D K L T Y P K I M G L----- K S R E-----	335
<i>Met.the.</i>		AFQIHDDY L D V S D E E S L G K P V G S D I A E G K M T L M V V K A L E----- E A S E D R R E R L I S I L G S G D----- E G	268
<i>Str.gri.</i>		AFQLRDD L L G V F G D P A L T G K P S G D D I R E G K S T Y L L A V A R N----- S A E A S G D E Q A L A V L G R A T G N A D L T E E	333
		* * * * *	
		V	
<i>Dro.mel.</i>		--- Y C I T L L E --- K L G S F Q Y T R K V L E S L D A E A R S (53)	338
<i>Neu.cra.</i>		--- Y A V A Y M E --- S T G S F E Y T R K V I K V L V D R A R Q (38)	428
<i>Sac.cer.</i>		--- K L I Q I L E F D T N S L A Y T K N F I N Q L V N M I K N (34)	335
<i>Ara.tha.</i>		----- F A E K L N----- R E A R D (25)	371
<i>Met.the.</i>		S V A E A I E I F E--- R Y G A T Q Y A H E V A L D Y V R M A K E (26)	325
<i>Str.gri.</i>		D L A D V R G V L E--- V T G A R A H V E R K A E H L R D H A V R (61)	425

Figure 7.—Alignment of the predicted *qm* protein with GGPPS of *Neurospora crassa* “*Neu.cra.*” (Carattoli *et al.* 1991), *Saccharomyces cerevisiae* “*Sac.cer.*” (Jiang *et al.* 1995), *Arabidopsos thaliana* “*Ara.tha.*” (Bartley and Scolnik 1994), *Methanobacterium thermoautotrophicum* “*Met.the.*” (Chen and Poulter 1994), and *Streptomyces griseus* “*Str.gri.*” (GenBank accession number L37405). The alignments were performed using CLUSTAL W (Thompson *et al.* 1994). The numbers of omitted amino acid residues at both C and N termini are shown in parentheses. Deletions are indicated by “-”. Five conserved domains (I, II, III, IV, V) are indicated by underlining. The consensus sequences (4 out of 6) are in boldface, and residues conserved across all species are indicated by “*.”

in lethality due to malfunction of prenylated signaling proteins such as *Drosophila Ras1*, a homologue of human Ras. Indeed, the β subunit of GGTase-I was recently identified in *Drosophila melanogaster* (β GGT1; Therrien *et al.* 1995) based on a mutation that suppresses a dominant allele of *Ras1*. We show here that an EMS-induced nonsense mutation at the 12th codon of *quemao* leads to lethality in the first larval instar, indicating that *qm* is an essential gene. The lethality does not occur during embryonic development, suggesting that the embryonic survival of *qm* recessive lethal homozygotes is due to a substantial maternal contribution. The lethality caused by a nonsense mutation at the GGPPS coding region in *Drosophila* contrasts with the observation that GGPPS mutants (*BTS1*) in yeast are viable with a decreased rate

of vegetative growth (Jiang *et al.* 1995). The nonessential nature of GGPPS in yeast may be explained by the presence of other isoprenoid pyrophosphate synthases, such as farnesyl or hexaprenyl pyrophosphate synthases, that may produce small amounts of GGPP, allowing the cell to survive in the absence of specific GGPPS. In *Drosophila*, Southern blot analysis and *in situ* hybridization analysis to salivary gland chromosomes are consistent with a single GGPPS coding sequence in the *Drosophila* genome.

qm mutations caused by *P*-element insertion in the 5' untranslated region lack the bristle shafts of macrochaetes. Similarly, hemizygotes of the EMS-induced recessive mutant *qm*^{15.1} lack shafts of macrochaetes and exhibit strongly reduced mRNA levels (see Figure 6)

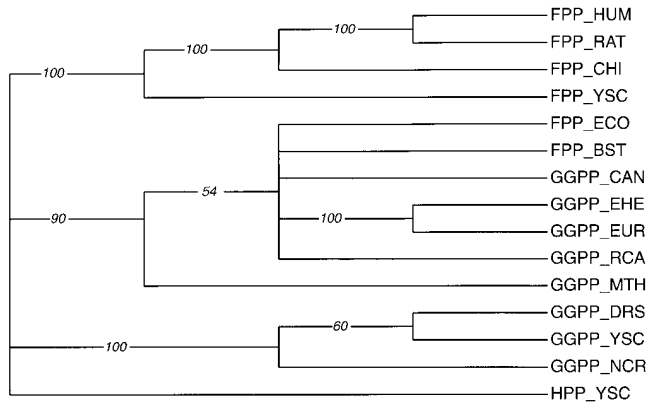


Figure 8.—The phylogenetic relationships among isoprenyl pyrophosphate synthases (FPP–, FPP synthase; GGPP–, GGPP synthase; HPP–, hexaprenyl pyrophosphate synthase; FPP–HUM, *Homo sapiens*; FPP–RAT, *Rattus rattus*; FPP–CHI, *Gallus gallus*; FPP–YSC, *Saccharomyces cerevisiae*; FPP–ECO, *Escherichia coli*; FPP–BST, *Bacillus stearothermophilus*; GGPP–CAN, *Capsicum annuum*; GGPP–EHE, *Erwinia herbicola*; GGPP–EUR, *Erwinia uredovora*; GGPP–RCA, *Rhodobacter capsulatus*; GGPP–MTN, *Methanobacterium thermoautotrophicum*; GGPP–DRS, *Drosophila melanogaster*; GGPP–YSC, *Saccharomyces cerevisiae*; GGPP–NCR, *Neurospora crassa*; HPP–YSC, *Saccharomyces cerevisiae*). This representative collection of sequences is that used in Chen *et al.* (1994) augmented by the yeast GGPPS (Jiang *et al.* 1995) and the QM sequence reported here. These amino acid sequences were aligned using CLUSTAL W (Thompson *et al.* 1994). This “50% majority-rule consensus tree” is derived from an analysis using the program PAUP (Swofford 1993); parameters: number of bootstraps = 100, *heuristic search*. Only those bifurcations present in more than 50% of the bootstrap samples are represented. The numbers on individual branches indicate how many bootstrap analyses (out of 100) include that clade.

and a three nucleotide (ATG) insertion in the intron 6. We did not detect transcripts of altered lengths in *Pelement*-associated mutants, but we did observe a moderate reduction of transcript level in embryos, larvae, pupae, and adults. These observations are most easily interpreted as evidence that levels of GGPPS required are higher during macrochaete development. Macrochaete development is more sensitive to limitations in protein synthesis, as evidenced by many minute mutations, which exhibit poorly formed macrochaetes as heterozygous (Ashburner 1989). At present, there is no reason to suspect that GGPP is involved in protein synthesis, but the extensive and unusual morphological (thus synthetic) elongation of the single cell that forms the macrochaete shaft might be limited by the supply of GGPP for protein prenylation or other unknown isoprenoid components of bristles.

The comparison of *Drosophila* GGPPS amino acid sequences with those of fungi, yeast, plants, eubacteria, and archaeobacteria (see Figure 7) indicates that the five previously recognized conserved regions (Chen *et al.* 1994) are clearly present in the *Drosophila* homologue. The inclusion of the *Drosophila* GGPPS (and several

other recent additions) further supports the hypothesis that all prenyl pyrophosphate synthases share a common ancestor (James *et al.* 1978; Bajaj and Blundell 1984; Chothia and Lesk 1986). Based on an alignment of 13 isoprenyl pyrophosphate synthases, Chen *et al.* (1994) proposed that the earliest divergence separated the short-chain and long-chain synthases (*e.g.*, hexaprenyl pyrophosphate synthase; see Figure 7). The proposed second divergence occurred when short-chain synthases further diverged into two clusters: one of eubacterial, plant, and archaeobacterial proteins, another of fungal and animal enzymes. The additional information incorporated into the phylogenetic analysis presented in Figure 8 sheds new light on the early divergence of this small and ancient gene family. In contrast to the analysis in Chen *et al.* (1994), the *Neurospora* GGPPS sequence now groups with those of *Drosophila* and yeast. The FPPS enzymes of eukaryotes also form a clear group. Interestingly, the archaeobacterial GGPPS enzymes appear to form a group with the eubacterial GGPPSs and FGGSSs. This isoprenyl pyrophosphate synthase gene family may contribute more insights (as sequences accumulate) to the analyses of the earliest branching among eukaryotic lineages.

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