ELSEVIER

Mechanisms of Development 68 (1997) 179–186

Short communication

cDNA sequence and expression pattern of the *Drosophila melanogaster* PAPS synthetase gene: a new salivary gland marker

Denis Jullien[a], Michèle Crozatier[b], Emmanuel Käs[a,*]

[a]*Laboratoire de Biologie Moléculaire Eucaryote, C.N.R.S. U.P.R. 9006, 118 route de Narbonne, 31062 Toulouse Cedex, France*
[b]*Centre de Biologie du Développement, U.M.R. 5547, Bâtiment 4R3, 118 route de Narbonne, 31062 Toulouse Cedex, France*

Received 23 July 1997; revised version received 22 August 1997; accepted 25 August 1997

Abstract

PAPS synthetase is a bifunctional enzyme containing both ATP sulfurylase and APS kinase activities required for the biosynthesis of PAPS, the sulfate donor in sulfation reactions. Here we report the sequence of the *Drosophila melanogaster* PAPS synthetase, the first gene implicated in the sulfation pathway to be described in that organism, and the characterization of its specificity of expression in embryos. Whole-mount in situ hybridization reveals that *DmPAPSS* is a novel salivary gland marker. At the end of embryogenesis, expression of *DmPAPSS* is also observed at the entry and exit of the gut and the posterior spiracles. We discuss the possibility that the pattern of expression of the *DmPAPSS* gene might reflect a major role for sulfation in mucus biosynthesis at the end of *Drosophila* embryogenesis. © 1997 Elsevier Science Ireland Ltd.

Keywords: PAPS synthetase; APS kinase; ATP sulfurylase; *Drosophila melanogaster*; Salivary glands

1. Introduction

Recent studies have shown that, in mammals, sulfation is a widespread modification for many biologically active molecules. This modification has been implicated in an increasing number of biological processes such as blood clotting, connective tissue formation, metabolism of drugs and toxins, growth factor and hormone action (Leyh, 1993). Sulfation involves the transfer of a sulfuryl group from a sulfate donor to a variety of acceptor molecules that include xenobiotics, proteins, glycoproteins, glycolipids, glycosaminoglycans (heparan sulfate), phenols and steroids.

The sulfate donor in metabolism is adenosine 3′-phosphate 5′-phosphosulfate (PAPS). PAPS biosynthesis involves the sequential action of two enzyme activities: ATP sulfurylase (EC 2.7.7.4), which catalyzes the formation of adenosine 5′-phosphosulfate (APS) from ATP and free sulfate, and APS kinase (EC 2.7.1.25), which subsequently phosphorylates APS to produce PAPS. Whereas the two enzymes are present on separate polypeptide chains in bacteria, fungi, yeast and plants (Cherest et al., 1987; Korch et al., 1991; Leyh et al., 1992; Foster et al., 1994; Jain and Leustek, 1994; Leustek et al., 1994), the recent molecular characterization of APS kinase and ATP sulfurylase activities in the marine worm *U. caupo* and in the mouse shows that both are carried by a single bifunctional enzyme: PAPS synthetase or *PAPSS* (Rosenthal and Leustek, 1995; Li et al., 1995). The fact that a cDNA sequence encoding *PAPSS* has also been identified in the nematode *C. elegans* (Rosenthal and Leustek, 1995) further suggests that the fusion of APS kinase and ATP sulfurylase on a single enzyme is conserved in animals. Sequence comparison between these new sequences and APS kinase and ATP sulfurylase sequences from different sources reveals that the bifunctional enzymes share a conserved organization, with the APS kinase and ATP sulfurylase activities localized to the *N*- and *C*-terminal portions, respectively, of these bifunctional enzymes. It is important to note that APS kinase and ATP sulfurylase amino acid sequences, whether fused in a single protein or encoded by separate genes, are strongly conserved during evolution.

* Corresponding author. Tel.: +33 5 61335959; fax: +33 5 61335886; e-mail: kas@ibcg.biotoul.fr

PII S0925-4773(97)00136-6

While many biological events requiring sulfation have been characterized in detail essentially in mammals, the pattern of expression of ATP sulfurylase-APS kinase activities in a whole organism has never been described. In animals in which *PAPSS* has been isolated, it is not known if the gene is ubiquitously expressed by all the cells of the organism, or if it exhibits a specific pattern of expression. The identification of the *PAPSS*-expressing tissues would help characterize the biological processes for which sulfation is required. In *Drosophila*, an organism of choice to address the question of the role(s) of sulfation during development, very little is known about sulfation and the components of the sulfate activation pathway have not been identified yet.

During screening of an embryonic *Drosophila melanogaster* cDNA library, we isolated a clone encoding a protein which we identified as *PAPSS* on the basis of its very high homology with previously characterized sequences. The isolation of a cDNA encoding the first *Drosophila* enzyme implicated in sulfation encouraged us to characterize this cDNA further, since this offers an opportunity to initiate a careful analysis of the role of sulfation during development. In this study, we report on the *D. melanogaster* PAPS synthetase (*DmPAPSS*) cDNA sequence and the spatio-temporal study of the expression of its gene. We show that, whereas *DmPAPSS* is expressed throughout the *Drosophila* life cycle, it exhibits a strong tissue-specific expression during embryogenesis and larval development, the main transcription site being salivary glands.

2. Results

2.1. Sequence of the DmPAPSS cDNA and comparison with previously identified PAPS synthetase genes

During screening of an embryonic *Drosophila melanogaster* cDNA library for sequence-specific DNA-binding proteins, we isolated a clone designated D7. Preliminary sequence analysis showed that D7 did not encode a DNA-binding protein but rather, was part of a background of false positives. Characterization of the D7 cDNA clone yielded a final sequence of 2469 nucleotides (EMBL Nucleotide Sequence Database accession number Y12861). This sequence contains a single open reading frame that translates into a deduced 629-amino acid sequence with a calculated molecular mass of 71.2 kDa (see Fig. 1). Comparison of the predicted amino acid sequence against the protein sequence databases revealed extensive homologies with PAPS synthetase, ATP sulfurylase and APS kinase sequences from several sources. Alignment of ATP sulfurylase and APS kinase sequences with the D7 amino acid sequence showed a strong homology of the first 200 *N*-terminal amino acids to APS kinase whereas the last 395 *C*-terminal amino acids shared a high degree of similarity with ATP sulfurylase (data not shown).

Alignments of D7 with the three *PAPSS* sequences already characterized reveal that the protein encoded by the *Drosophila* cDNA is highly similar to *PAPSS*, with 62.8%, 61.3% and 54% amino-acid identity between the *Drosophila* protein and *U. caupo*, *M. musculus* and *C. elegans* sequences, respectively (Fig. 1). The two putative functional motifs that correspond to an ATP-GTP binding motif (P-loop) and a possible binding site for PAPS that is conserved in APS kinase (Satishchandran et al., 1992), previously described in *U. caupo* (Rosenthal and Leustek, 1995), in the mouse (Li et al., 1995) and also present in the *C. elegans PAPSS*, are found in the *N*-terminal domain of the protein encoded by our cDNA. The *C*-terminal domain contains the conserved PP-motif found in all ATP-sulfurylase sequences (Bork and Koonin, 1994). Based on the high amino-acid sequence homology and the conservation of functional motifs, we conclude that we have isolated the cDNA from the *Drosophila melanogaster* PAPS synthetase gene, and have called it *DmPAPSS*.

2.2. DmPAPSS is encoded by a single-copy number gene which is expressed throughout the Drosophila life cycle

In *U. caupo* and in the mouse, *PAPSS* is encoded by a single-copy gene (Li et al., 1995; Rosenthal and Leustek, 1995). We asked whether this was the case in *Drosophila* as well. In situ hybridization of a *DmPAPSS* cDNA probe to salivary gland polytene chromosomes yielded a single signal at the cytological position 76C on the left arm of the third chromosome (data not shown). Southern-blot analysis of *D. melanogaster* genomic DNA digested with several restriction enzymes and hybridized to the same probe resulted in the detection of single bands (data not shown). This simple pattern was also detected when hybridization was performed at lower stringency. Taken together, these results suggest that *DmPAPSS* sequences are present as a single copy in the *Drosophila melanogaster* genome.

To determine both the size and the temporal expression pattern of *DmPAPSS* transcripts, we performed a developmental Northern blot analysis using a fragment of the cDNA as a probe. A single mRNA species of approximately 2.4 kb was detected at all stages of the fly life cycle, albeit at variable levels (Fig. 2). This size suggests that the 2469-bp cDNA insert that we isolated is full-length. *DmPAPSS* expression was clearly highest in embryos and pupae, whereas lower levels of transcripts were detected in L2 and L3 larvae (see Section 2.3). Because zygotic transcription only begins 2 hours after fertilization in *D. melanogaster*, the signal observed in the 0–2 hour embryos shows a maternal contribution of *DmPAPSS* mRNA.

2.3. DmPAPSS exhibits a strong spatial specificity of expression

The temporally ubiquitous expression of *DmPAPSS* might mask a spatially localized expression. To study a

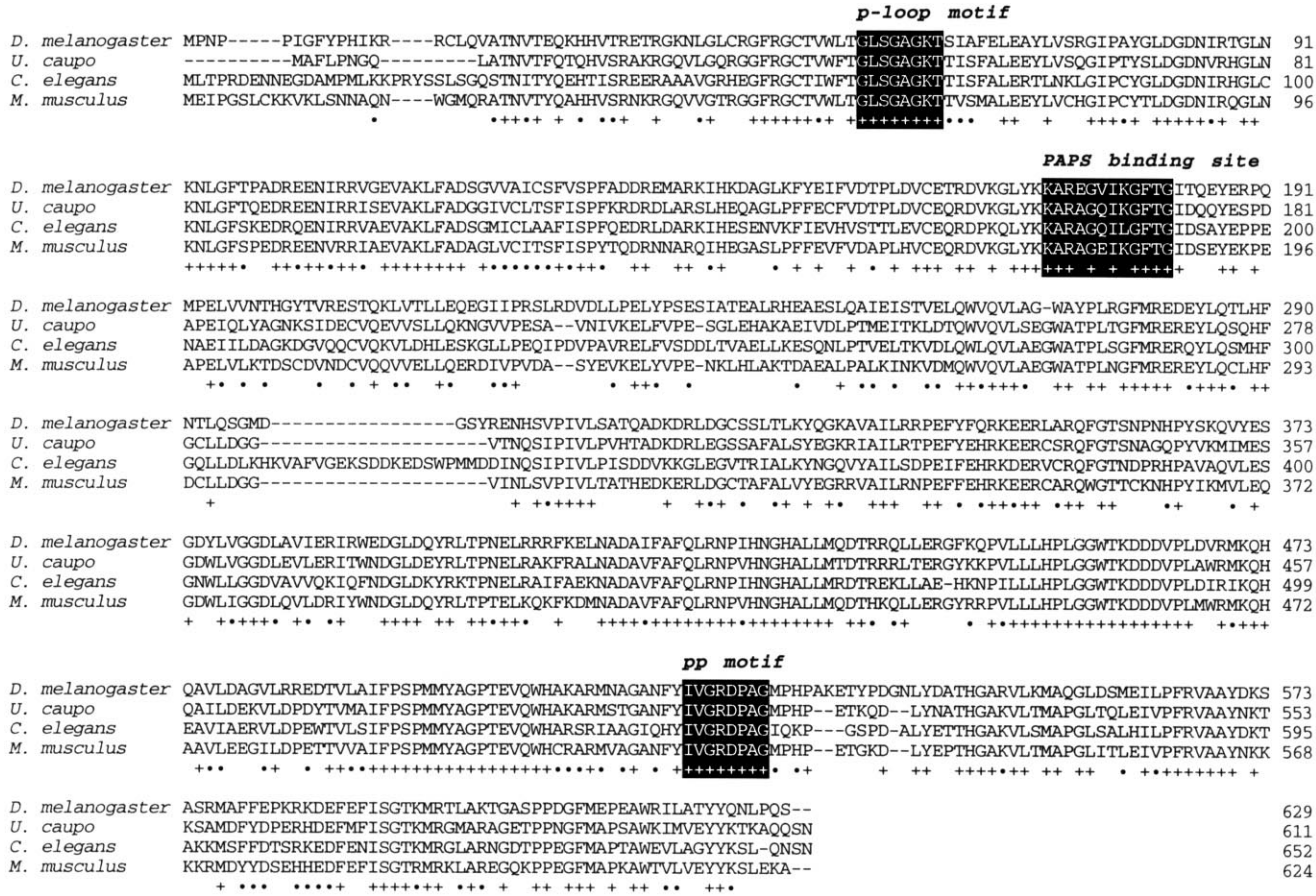

Fig. 1. *DmPAPSS* amino acid sequence and multiple alignment with *PAPSS* sequences from *U. caupo*, mouse and *C. elegans*. The amino acids strictly conserved among the four sequences are marked by plus signs while conservative substitutions are indicated by dots below the sequence. Conserved putative functional motifs are boxed and include the P-loop, the PAPS binding site and the PP motif. The peptide sequences aligned to *DmPAPSS* were obtained by translation of the following GenBank entries: *U. caupo* (L39001), *M. musculus* (U34883) and *C. elegans* (Z68880).

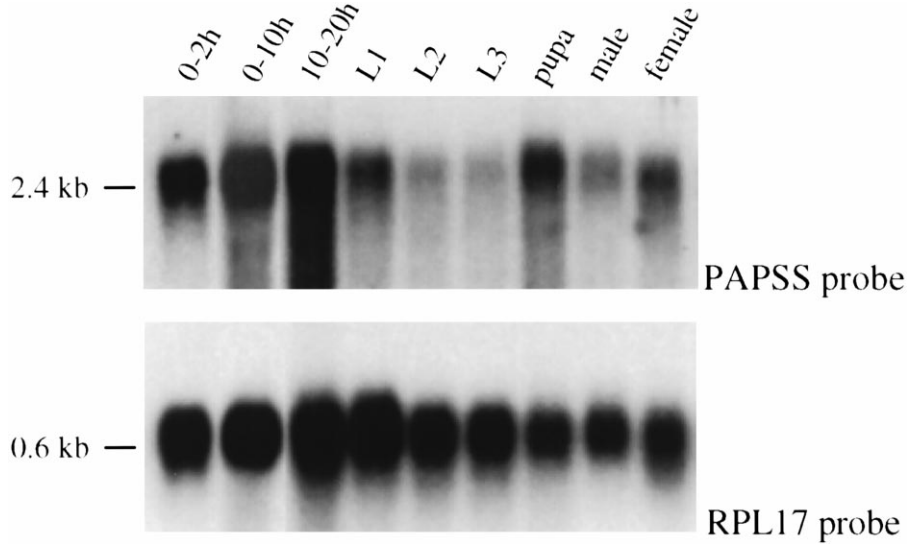

Fig. 2. Northern blot analysis of the temporal expression pattern of *DmPAPSS* during the *Drosophila* life cycle. Poly(A)$^+$ RNA samples isolated from whole organisms at the indicated stages and from adult males and female flies were separated on a 1% agarose-formaldehyde gel and hybridized to a 1.6 kb HincII-EcoRI ^{32}P-labeled *PAPSS* cDNA probe (upper panel). To control for differences in the amount of RNA loaded in each lane, the filter was rehybridized with a probe for the rpL17A ribosomal protein gene (Noselli and Vincent, 1992) which is expressed at a roughly constant level throughout development (lower panel). The size of the transcripts detected by the probes used is indicated to the left of each gel.

possible tissue-specificity in the expression of the *DmPAPSS* gene, we performed whole-mount in situ hybridizations on embryos, using the whole cDNA as a probe. Accumulation of *DmPAPSS* transcripts was clearly detected in preblastoderm and blastoderm embryos (Fig. 3A), confirming the maternal contribution, as already suggested by Northern-blot analysis (see Fig. 2). These maternal transcripts were no longer detected at the beginning of gastrulation (data not shown). Zygotic expression of *DmPAPSS* begins at the onset of germ band retraction (Fig. 3B) and was found strongly restricted to two ventrolateral subsets of cells which were identified, on the basis of position and morphology, as the salivary gland placodes (Fig. 3C,D). Since salivary gland morphogenesis begins at the onset of germ band retraction (stage 11), *DmPAPSS* is thus expressed very early in salivary gland development. This strict specificity of expression in salivary glands is lost in stage-13 embryos where transcription of *DmPAPSS* is also observed in the posterior spiracles (Fig. 3C). At the end of embryogenesis (stages 16–17), the pattern of expression of *DmPAPSS* transcripts is further extended to the anal pads and to the atrium (Fig. 3D,E,F).

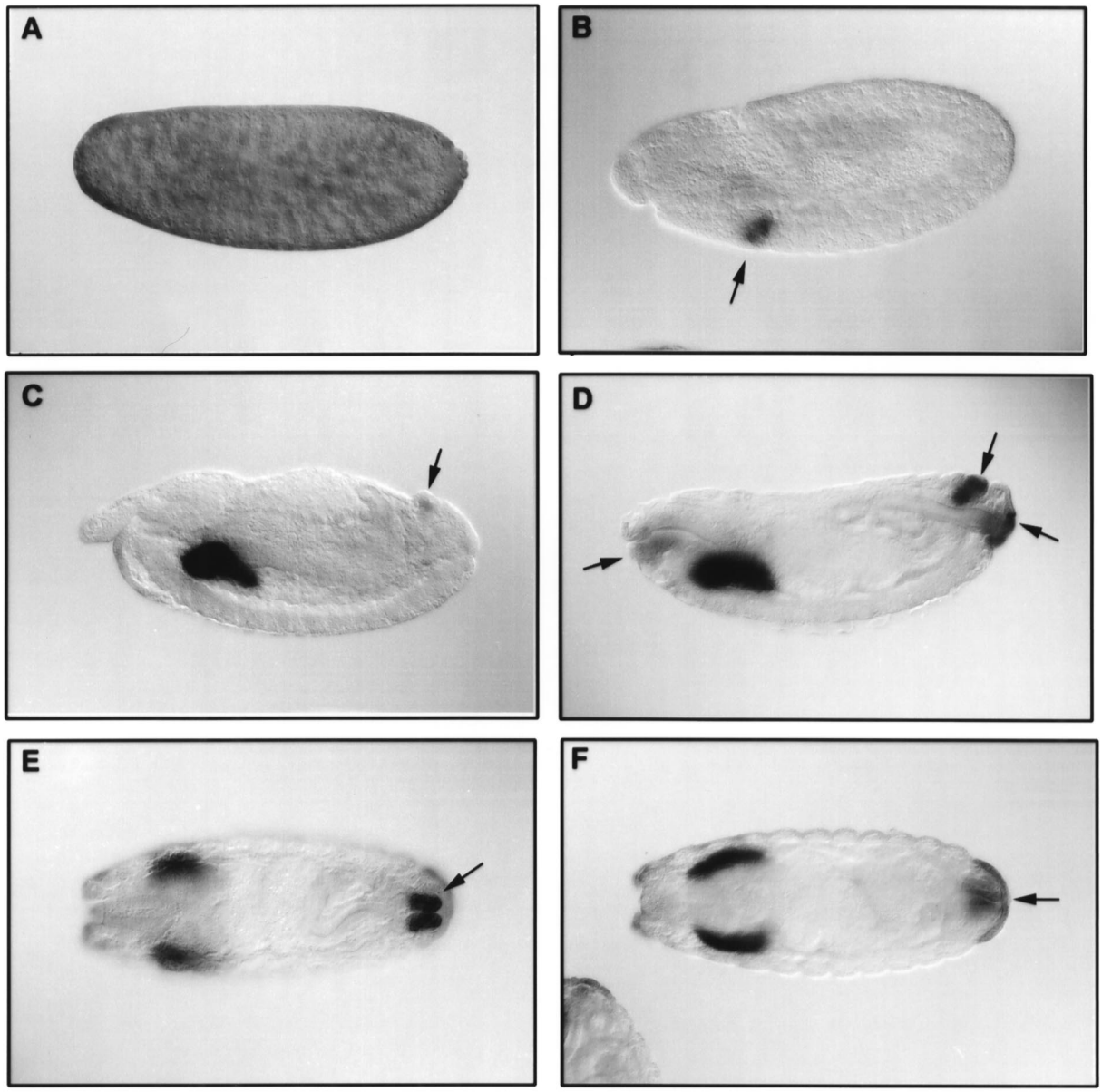

Fig. 3. In situ hybridization to *DmPAPSS* transcripts in wild-type *Drosophila* embryos. All embryos are oriented with the anterior and dorsal sides at the left and top of each panel, respectively. Panels E and F show dorsal and ventral views, respectively. Regions of *PAPSS* transcription are indicated by arrows. Panel A: embryo at nuclear cycle 10, showing the strong accumulation of maternal *DmPAPSS* transcripts throughout the embryo. Panel B: activation of *DmPAPSS* zygotic expression in a stage-11 embryo. Panel C: stage-13 embryo showing strong expression in the salivary glands and the onset of transcription in the posterior spiracles. Panel D: a stage-16 embryo, showing expression of *DmPAPSS* transcripts in the posterior spiracles, in the anal pads and the atrium, in addition to the salivary glands. Panel E: dorsal view of a stage-16 embryo showing *DmPAPSS* expression in the posterior spiracles. Panel F: ventral view of a stage-16 embryo showing expression in the anal pads.

To investigate whether *DmPAPSS* expression in salivary glands was maintained later in development, we performed in situ hybridizations on third instar-larvae imaginal discs, central nervous system and salivary glands. As was the case in embryos, the probe hybridized to the salivary glands (Fig. 4A) but we did not detect transcription of the gene in imaginal discs or in the central nervous system (data not shown). This spatial restriction is in agreement with the reduced *DmPAPSS* mRNA levels we observed in larvae (see Fig. 2). Closer observation of the signal detected in larval salivary glands shows that the fat-body cells do not express *DmPAPSS* (Fig. 4B). *DmPAPSS* expression thus appears to be restricted to the secretory cells of the salivary gland, as only very weak levels of transcription, if any, can be detected in the anterior-most salivary duct cells (Fig. 4A). Expression is similarly restricted to the secretory cells in embryonic salivary glands as, in older embryos, no signal is detected in the salivary duct, which is located between the anterior part of the salivary glands and the pharynx (Fig. 3D–F). These results demonstrate that *DmPAPSS* exhibits a specific pattern of expression in embryos as well as in third-instar larvae, suggesting that it is continuously expressed in the salivary glands from stage 11 to the end of larval development.

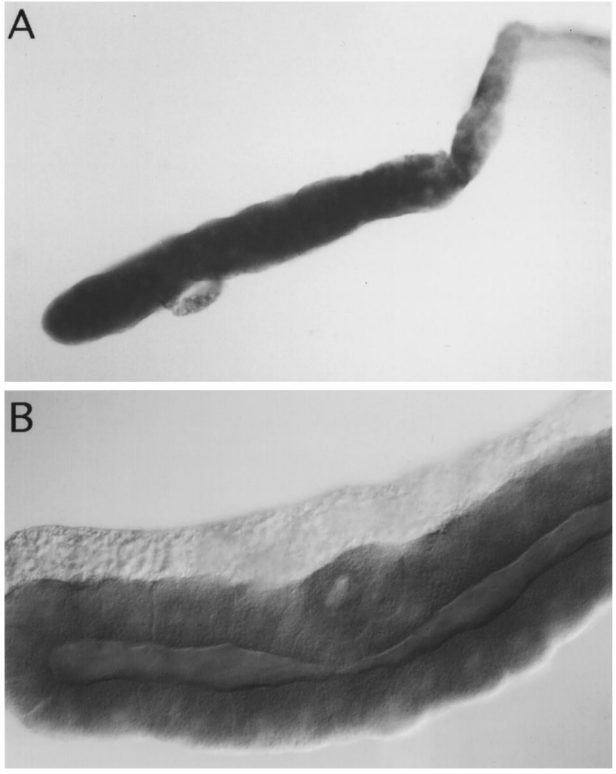

Fig. 4. In situ hybridization to *DmPAPSS* transcripts in third-instar larvae salivary glands. Glands are positioned with their anterior side to the right of each panel. Panel A shows the accumulation of *PAPSS* transcripts in the salivary gland, while no signal is detected in the anterior-most cells of the salivary duct. Similarly, the lack of signal in fat-body cells is clearly evident in the dissected salivary gland shown in Panel B.

2.4. DmPAPSS expression in salivary glands is regulated by Sex combs reduced

Salivary glands arise during embryogenesis in parasegment 2 (PS2) from two ventrolateral subsets of cells expressing *Sex combs reduced* (*Scr*), (Panzer et al., 1992). *Scr* plays a pivotal role in the formation of the salivary glands, which do not develop in loss-of-function *Scr* mutants (Panzer et al., 1992). In addition, all the salivary gland markers whose transcriptional regulation has been analyzed are dependent on *Scr* activity (Andrew et al., 1994). Therefore, in Scr^- mutant backgrounds, expression of salivary gland-specific genes is lost in PS2. To determine whether *DmPAPSS* behaves as a salivary gland marker, we analyzed its expression in Scr^- embryos. No *DmPAPSS* transcripts could be detected in the region of PS2 where salivary glands normally form (Fig. 5B; compare with the control wild-type embryo shown in Fig. 5A). However, transcripts were still detected in the spiracles, the anal pads and in the atrium (Fig. 5B), suggesting that *Scr* does not control the expression of *DmPAPSS* in these structures.

When Scr protein is ubiquitously expressed, using an *Scr* cDNA under the control of the *hsp70* promoter (HS-SCR), supernumerary salivary gland placodes are induced and new salivary glands develop in parasegments 0 and 1 in the same dorsal-ventral position as the normal salivary glands (Andrew et al., 1994). As a consequence, salivary gland markers are also expressed in parasegments anterior to PS2. Fig. 5C shows that this is also true for the *PAPSS* gene, as ectopic expression of SCR protein leads to the accumulation of *DmPAPSS* transcripts in PS0–1 in addition to PS2 in heat-shocked HS-SCR embryos.

Expression of the *DmPAPSS* gene was further examined under more drastic conditions of ectopic salivary gland development using animals homozygous for loss-of-function mutations of *teashirt* (*tsh*) and carrying an inducible HS-SCR construct. The tsh gene is responsible for the negative regulation of the development of salivary glands in parasegments 3–13 (Andrew et al., 1994). As expected, in heat shocked HS-SCR/tsh^- embryos, *DmPAPSS* transcription was detected in PS 0–2 but also in PS3–13 (Fig. 5D). These results show that regulation of *DmPAPSS* is similar to that described for other salivary gland markers, as its expression is tightly linked to the development of salivary glands induced by *Scr*. The fact that *DmPAPSS* behaves like a salivary gland marker also confirms the identity of the ventro-lateral expression site we detect in early embryos with the salivary glands.

3. Discussion

We have isolated a cDNA encoding the *Drosophila melanogaster PAPSS* gene, the first gene involved in sulfur metabolism to be described in that organism. This cDNA encodes a bifunctional protein, *DmPAPSS*, that contains

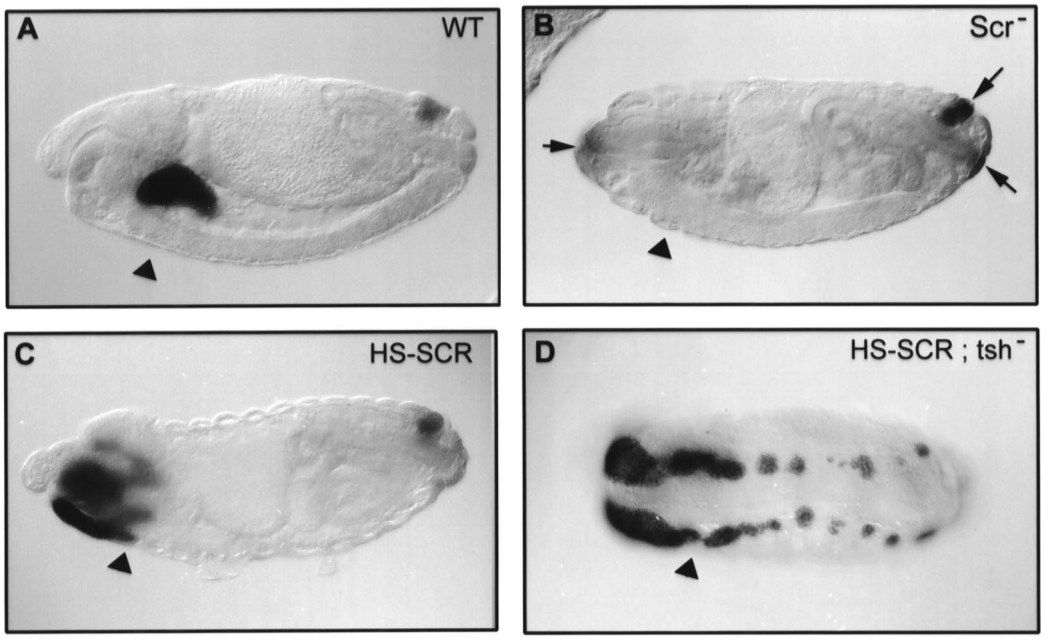

Fig. 5. In situ hybridization to *DmPAPSS* transcripts in *Scr* and *tsh* mutant contexts. Embryos are positioned with their anterior and dorsal sides at the left and top of each panel, respectively. Bold arrowheads indicate the normal *PAPSS* expression derived from PS2. A wild-type stage 16 control embryo is shown in Panel A. Panel B: *Scr*$^-$ embryos do not develop salivary glands, and as a consequence do not show any transcription in PS2; transcripts are still detected in the posterior spiracles, the anal pads and the atrium (arrows). Panel C: heat shocked HS-SCR embryos develop supernumerary salivary glands in PS0–1 and exhibit ectopic expression of *DmPAPSS* in the same parasegments. Panel D: heat-shocked HS-SCR/*tsh*$^-$ embryos develop extra salivary glands in PS0–1 and PS3–13 and show ectopic expression of *DmPAPSS* in the same parasegments.

both of the enzyme activities, ATP sulfurylase and APS kinase, required for the synthesis of PAPS, the activated sulfate donor in sulfation pathways. This is to our knowledge the fourth PAPS synthetase to be identified, following the characterization of the *U. caupo* (Rosenthal and Leustek, 1995) and mouse (Li et al., 1995) *PAPSS* genes and of homologous sequences in *C. elegans* (Rosenthal and Leustek, 1995). The fusion of both enzyme activities in a single polypeptide chain in animals, as opposed to procaryotes, unicellular eucaryotes and plants, where both ATP sulfurylase and APS kinase are contained on separate proteins (Leyh, 1993), is confirmed again with the characterization of *PAPSS* in *Drosophila* and might correspond to an evolutionary trend toward a more efficient biosynthesis of the activated sulfate donor.

To our knowledge, the different steps leading to sulfation of acceptor molecules constitute a cell-autonomous process. The detailed characterization of the expression pattern of *DmPAPSS* should thus provide a tool to investigate when and where sulfation is required during *Drosophila* development. We have addressed the question of the spatio-temporal expression pattern of *DmPAPSS* during *Drosophila* embryogenesis by performing whole-mount in situ hybridizations. A major result of this study is that *DmPAPSS* constitutes a new salivary gland marker. We show that *DmPAPSS* is strongly expressed in the salivary glands from the beginning of their morphogenesis to the end of embryogenesis. Moreover, *DmPAPSS* transcripts are clearly detected in the salivary glands of third-instar larvae, suggesting that *PAPSS* expression continues in the salivary glands throughout larval development.

Scr is necessary and sufficient to initiate the program of salivary gland morphogenesis in *Drosophila*. To confirm that *DmPAPSS* constitutes a new salivary gland marker, we investigated its expression in *Scr*$^-$ mutant embryos in which no salivary glands develop and in HS-SCR or HS-SCR/*tsh*$^-$ embryos, where development of supernumerary salivary glands is induced. As already described for other salivary gland markers (Andrew et al., 1994), we found a strict correlation between salivary gland development induced by *Scr* and the expression of *DmPAPSS* in those developing glands. While these results show that *DmPAPSS* is a bona fide salivary gland marker whose embryonic expression is at least in part under the control of *Scr*, we do not know at present whether this control is direct or not. Another likely candidate for direct control of *DmPAPSS* expression is the region-specific *fork head* (*fkh*) homeotic gene (Weigel et al., 1989). *fkh*, whose expression is regulated by *Scr* in embryonic salivary glands, plays a crucial role in their morphogenesis (Panzer et al., 1992). *fkh* protein immunolocalizes to the 76C–D region of the third chromosome, the cytological location of *DmPAPSS* (V. Mach, pers. commun.), suggesting that it could directly control the expression of *PAPSS* in the salivary glands, at least during late stages of development: salivary gland markers whose expression is dependent on *fkh* in the developing embryo have not been described yet.

It is important to note that *DmPAPSS* expression is not

confined to the salivary glands in the *Drosophila* embryo. During late stages of embryogenesis, *DmPAPSS* is also expressed in the posterior spiracles, in the anal pads and, at lower levels, in the atrium (Fig. 3), even in Scr^- mutants. This pattern of expression leads to the question of the possible role(s) of sulfation at this stage of *Drosophila* development. In mammals, mucus and saliva are known to play a very important role in the protection of epithelial tissues (Nieuw Amerongen et al., 1995). Their biosynthesis requires *PAPSS* activity, as the most characteristic and prominent components of mucous secretions are mucins, highly sulfated *O*-glycoproteins. Insect mucins remain poorly characterized (Kramerov et al., 1996). However, it is tempting to speculate that, consistent with the expression pattern we observe, at the end of embryogenesis but also during larval development, mucus needs to be produced not only by the salivary glands, but at the relatively exposed entry and exit points of the gut and of the tracheal system as well. At any rate, our hypothesis that *DmPAPSS* might be involved in the biosynthesis of saliva and mucus fits well with the recent molecular characterization of other salivary glands markers: many of the genes expressed beginning at the onset of salivary gland invagination or soon after, appear to be involved in glycosylation or secretion (S. Beckendorf, pers. commun.). The salivary gland thus is prepared for its secretory role very quickly after its initial determination.

A growing body of evidence points to the important roles played by vertebrate mucins in cellular communication, cell-cell and cell-extracellular matrix recognition and in differentiation (Devine and McKenzie, 1992). More generally, the diversity of cellular functions requiring sulfation suggests that, in *Drosophila*, PAPS biosynthesis is likely to be required for several distinct biological processes during the development and the adult life of the fly. For example, a surface mucin associated with hemocytes and implicated in the immune response has been recently identified in *Drosophila* (Theopold et al., 1996). In addition, as there is no evidence for secretory events occurring at the preblastoderm and blastoderm stages, the maternal contribution of *DmPAPSS* transcripts in *Drosophila*, which has been observed in *U. caupo* as well (Rosenthal and Leustek, 1995), further suggests that sulfation is also implicated in processes other than secretion.

PAPS synthetase is but one component of a pathway likewise requiring a sulfate transporter that acts upstream of the enzyme, and a PAPS transporter and sulfotransferases that act downstream. These essential components of the sulfation pathway, which remain poorly characterized at the molecular level, would be expected to exhibit the same expression pattern as *DmPAPSS*. The cloning of the *Drosophila PAPSS* gene, its genetic analysis and the further characterization of the expression pattern of the gene during the life cycle of the fly, should make it possible to identify in more detail the nature of the biological processes that require sulfation.

4. Experimental procedures

4.1. Drosophila stocks and culture

An Oregon-R lab stock was used as wild type. Flies were grown at 25°C on standard cornmeal-glucose-yeast medium. The mutant alleles Scr^4 and tsh^8 used in this study are null mutations and are described by (Lindsley and Zimm, 1992). The line bearing a fusion of the inducible *hsp70* promoter to the *Scr* structural gene (HS-SCR) and the Scr^4 null allele strain were obtained from D. Cribbs (Centre de Biologie du Développement, Toulouse, France). The tsh^8 line was obtained from S. Kerridge (Laboratoire de Génétique, Université de Marseille, Marseille, France). To examine the expression of *DmPAPSS* as a function of HS-SCR expression in a wild-type or mutant tsh context, embryos were collected for 4 h, aged for 1 h and heat-shocked at 37°C for 45 min (Andrew et al., 1994). Embryos were then aged for 12 h at 25°C.

4.2. cDNA isolation and sequence analysis

cDNA clone D7 was isolated from a 9 to 12-h embryonic *Drosophila melanogaster* λgt11 cDNA library (Zinn et al., 1988). Phage D7 contained a 2.5-kb EcoRI cDNA insert which was subcloned into pUC18. The techniques used for λgt11 and DNA fragment manipulation and for transformation of *E. coli* were as described by (Sambrook et al., 1989). Lambda DNA purification was performed using the Qiagen Lambda Kit. The 2469-bp *PAPSS* cDNA was sequenced by the dideoxy method using the ALF DNA sequencer (Pharmacia) or the T7 sequencing kit (Pharmacia Biotech). Uncertainties in the gel patterns were resolved by sequencing both strands. Database searches were carried out using BLASTP programs (Altschul et al., 1990). The multiple alignment program CLUSTAL (Higgins and Sharp, 1988) was used to align the *DmPAPSS*-deduced amino acid sequence with the *PAPSS* amino acid sequences of *U. caupo*, *C. elegans* and mouse.

4.3. Genomic Southern-blot and developmental Northern analysis

Samples containing 10 μg of genomic DNA isolated from adult flies were digested overnight with BamHI, BglII, EcoRI, PstI and XbaI. DNA fragments were fractionated on a 1% agarose gel and electroblotted onto a nylon membrane (Hybond N^+; Amersham). Messenger RNA was purified from each developmental stage shown in Fig. 2 using the Straight A's mRNA Isolation System (Novagen). Samples containing 2 μg of poly$(A)^+$-RNA were separated on a 1% agarose/formaldehyde gel and transferred onto a Hybond N^+ filter. The DNA and the RNA blots were prehybridized in 3× SSPE, 5× Denhardt's, 1% SDS, and 25 μg/ml salmon sperm DNA at 65°C for 2 h and hybridized to the ^{32}P-labeled 2.5 kb whole cDNA or to a 1.6-kb HincII-EcoRI

fragment from the whole cDNA, respectively. Hybridizations were performed for 12 h at the same temperature. The filter was washed twice with 2× SSPE, 0.1% SDS at room temperature, twice in 1× SSPE, 0.1% SDS at 65°C and twice with 0.1× SSPE, 0.1% SDS at 65°C. Filters were exposed for 5 h at −70°C using intensifying screens.

4.4. In situ hybridization

In situ hybridizations to whole-mount embryos and imaginal discs were performed using a digoxigenin-labeled full-length 2.5-kb *PAPSS* cDNA fragment (Genius kit, Boehringer Mannheim) according to (Tautz and Pfeifle, 1989) and (Sturtevant et al., 1993). Salivary gland polytene chromosome squashes from third-instar larvae were hybridized to the same digoxigenin-labeled probe. Hybridization and detection of the probe were performed according to (de Frutos et al., 1990).

Acknowledgements

We thank Drs. David Cribbs and Alain Vincent for advice, materials and use of facilities as well as for constructive comments. We also thank Drs. Caroline Monod and Leonora Poljak for suggesting improvements to the manuscript. This work was funded by grants to E.K. from the Centre National de la Recherche Scientifique (CNRS), the Association pour la Recherche sur le Cancer (ARC, grant No. 2066), the Fondation pour la Recherche Médicale (FRM) and the Région Midi-Pyrénées. Denis Jullien was supported by pre-doctoral fellowships from the Ministère de l'Enseignement Supérieur et de la Recherche (MESR) and from the Association pour la Recherche sur le Cancer (ARC).

References

Altschul, S.F., Gish, W., Miller, W., Myers, E.W., Lipman, D.J., 1990. Basic local alignment search tool. J. Mol. Biol. 215, 403–410.

Andrew, D.J., Horner, M.A., Petitt, M.G., Smolik, S.M., Scott, M.P., 1994. Setting limits on homeotic gene function: restraint of *Sex combs reduced* activity by *teashirt* and other homeotic genes. EMBO J. 13, 1132–1144.

Bork, P., Koonin, E.V., 1994. A P-loop-like motif in a widespread ATP pyrophosphatase domain: implications for the evolution of sequence motifs and enzyme activity. Proteins 20, 347–355.

Cherest, H., Kerjan, P., Surdin-Kerjan, Y., 1987. The Saccharomyces cerevisiae MET3 gene: nucleotide sequence and relationship of the 5' noncoding region to that of MET25. Mol. Gen. Genet. 210, 307–313.

de Frutos, R., Kimura, K.I., Peterson, K., 1990. In situ hybridization of *Drosophila* polytene chromosomes with digoxigenin-dUTP labeled probes. Methods Molec. Cell. Biol. 2, 32–36.

Devine, P.L., McKenzie, I.F., 1992. Mucins: structure, function, and associations with malignancy. BioEssays 14, 619–625.

Foster, B.A., Thomas, S.M., Mahr, J.A., Renosto, F., Patel, H.C., Segel, I.H., 1994. Cloning and sequencing of ATP sulfurylase from Penicillium chrysogenum. Identification of a likely allosteric domain. J. Biol. Chem. 269, 19777–19786.

Higgins, D.G., Sharp, P.M., 1988. CLUSTAL: a package for performing multiple sequence alignment on a microcomputer. Gene 73, 237–244.

Jain, A., Leustek, T., 1994. A cDNA clone for 5′-adenylylphosphosulfate kinase from Arabidopsis thaliana. Plant Physiol. 105, 771–772.

Korch, C., Mountain, H.A., Bystrom, A.S., 1991. Cloning, nucleotide sequence, and regulation of MET14, the gene encoding the APS kinase of Saccharomyces cerevisiae. Mol. Gen. Genet. 229, 96–108.

Kramerov, A.A., Arbatsky, N.P., Rozovsky, Y.M., Mikhaleva, E.A., Polesskaya, O.O., Gvozdev, V.A., Shibaev, V.N., 1996. Mucin-type glycoprotein from *Drosophila melanogaster* embryonic cells: characterization of carbohydrate component. FEBS Lett. 378, 213–218.

Leustek, T., Murillo, M., Cervantes, M., 1994. Cloning of a cDNA encoding ATP sulfurylase from Arabidopsis thaliana by functional expression in Saccharomyces cerevisiae. Plant Physiol. 105, 897–902.

Leyh, T.S., 1993. The physical biochemistry and molecular genetics of sulfate activation. Crit. Rev. Biochem. Mol. Biol. 28, 515–542.

Leyh, T.S., Vogt, T.F., Suo, Y., 1992. The DNA sequence of the sulfate activation locus from Escherichia coli K-12. J. Biol. Chem. 267, 10405–10410.

Li, H., Deyrup, A., Mensch, J. Jr., Domowicz, M., Konstantinidis, A.K., Schwartz, N.B., 1995. The isolation and characterization of cDNA encoding the mouse bifunctional ATP sulfurylase-adenosine 5'-phosphosulfate kinase. J. Biol. Chem. 270, 29453–29459.

Lindsley, D.L., Zimm, G.G., 1992. The genome of *Drosophila melanogaster*. San Diego Academic Press, San Diego.

Nieuw Amerongen, A.V., Bolscher, J.G., Veerman, E.C., 1995. Salivary mucins: protective functions in relation to their diversity. Glycobiology 5, 733–740.

Noselli, S., Vincent, A., 1992. The *Drosophila melanogaster* ribosomal protein L17A-encoding gene. Gene 118, 273–278.

Panzer, S., Weigel, D., Beckendorf, S.K., 1992. Organogenesis in *Drosophila melanogaster*: embryonic salivary gland determination is controlled by homeotic and dorsoventral patterning genes. Development 114, 49–57.

Rosenthal, E., Leustek, T., 1995. A multifunctional Urechis caupo protein, PAPS synthetase, has both ATP sulfurylase and APS kinase activities. Gene 165, 243–248.

Sambrook, J., Frisch, E.F., Maniatis, T., 1989. Molecular cloning: a laboratory manual, second ed. Cold Spring Harbor Laboratory Press, New York.

Satishchandran, C., Hickman, Y.N., Markham, G.D., 1992. Characterization of the phosphorylated enzyme intermediate formed in the adenosine 5′-phosphosulfate kinase reaction. Biochemistry 31, 11684–11688.

Sturtevant, M.A., Roark, M., Bier, E., 1993. The *Drosophila* rhomboid gene mediates the localized formation of wing veins and interacts genetically with components of the EGF-R signaling pathway. Genes Dev. 7, 961–973.

Tautz, D., Pfeifle, C., 1989. A non-radioactive in situ hybridization method for the localization of specific RNAs in *Drosophila* embryos reveals translational control of the segmentation gene hunchback. Chromosoma 98, 81–89.

Theopold, U., Samakovlis, C., Erdjument-Bromage, H., Dillon, N., Axelsson, B., Schmidt, O., Tempst, P., Hultmark, D., 1996. Helix pomatia lectin, an inducer of *Drosophila* immune response, binds to hemomucin, a novel surface mucin. J. Biol. Chem. 271, 12708–12715.

Weigel, D., Jurgens, G., Kuttner, F., Seifert, E., Jäckle, H., 1989. The homeotic gene *fork head* encodes a nuclear protein and is expressed in the terminal regions of the *Drosophila* embryo. Cell 57, 645–658.

Zinn, K., McAllister, L., Goodman, C.S., 1988. Sequence analysis and neuronal expression of fasciclin I in grasshopper and *Drosophila*. Cell 53, 577–587.