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Synthesis of the sulfate donor PAPS in either the *Drosophila* germline or somatic follicle cells can support embryonic dorsal-ventral axis formation

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The establishment of dorsal-ventral (DV) polarity in the *Drosophila* embryo depends upon a localized signal that is generated in the perivitelline space of the egg through the action of a serine proteolytic cascade. Spatial regulation of this pathway is determined by the expression of the *pipe* gene in a subpopulation of ventral follicle cells in the developing egg chamber. The Pipe protein exhibits homology to vertebrate glycosaminoglycan sulfotransferases. In a previous study, we demonstrated that embryonic DV polarity depends upon the sulfotransferase activity of Pipe. Surprisingly, however, our results also indicated that formation of the embryonic DV axis does not require the synthesis of the high-energy sulfate donor, 3′-phosphoadenosine 5′-phosphosulfate (PAPS) in the follicle cells in which Pipe is presumed to function. Here, we resolve this apparent paradox by demonstrating that dorsalized embryos are only produced by egg chambers in which both germline and follicle cells lack PAPS synthetase activity. Thus, PAPS produced either in the germline or in the follicular epithelium can support the requirement for Pipe sulfotransferase activity in embryonic DV patterning. This finding indicates the existence of a conduit for the movement of PAPS between the germline and the follicle cells, which highlights a previously unappreciated mechanism of soma/germline cooperation affecting pattern formation.

KEY WORDS: Egg chamber, Sulfation, Sulfonation, Oogenesis, Dorsoventral, PAPS Synthase, *papss*, *pipe*, *slalom*, *sugarless*, *sulfateless*

INTRODUCTION

Pattern and polarity along the dorsal-ventral (DV) axis of the *Drosophila* embryo depends upon the spatial regulation of a serine protease cascade in the perivitelline space that surrounds the developing embryo (Morisato and Anderson, 1995; Moussian and Roth, 2005; Roth, 2003). This protease cascade leads ultimately to the ventral activation of the Toll receptor, which establishes the orientation of DV polarity in the developing embryo. The spatial parameters of the activated serine protease cascade in the perivitelline space are determined during oogenesis by the pattern of expression of the *pipe* gene (Sen et al., 1998), which is specifically transcribed in the ventral follicle cells of the stage-10 egg chamber. The *pipe* locus encodes up to 11 distinct protein isoforms (Sergeev et al., 2001) (see Flybase), produced by alternative splicing, that all exhibit amino acid similarity to vertebrate heparan sulfate 2-0-sulfotransferase (HSST) and dermatan/chondroitin sulfate 2-0-sulfotransferase (D/CSST) (Kobayashi et al., 1997; Kobayashi et al., 1999). These Golgi-resident proteins mediate the transfer of sulfate to the 2-hydroxyl position of uronic acid residues of glycosaminoglycan (GAG) carbohydrates such as heparin (Hep), heparan sulfate (HS), chondroitin sulfate (CS) and dermatan sulfate (DS). Like these enzymes, Pipe is present in the Golgi (Sen et al., 2000). In previous work we demonstrated that females carrying follicle cell clones that are mutant for genes required for HS and CS/DS GAG formation do not produce dorsalized embryos (Zhu et al., 2005). This, as well as other observations, indicates that HS and CS/DS GAGs do not serve as substrates for Pipe sulfotransferase activity, despite the amino acid similarity between Pipe and vertebrate HSST and D/CSST. However, additional lines of evidence are consistent with the proposal that Pipe does function as a sulfotransferase. For example, dorsalized embryos are produced by females carrying follicle cell clones that are mutant for *slalom* (*sll*), which encodes the Golgi transporter of 3′-phosphoadenosine 5′-phosphosulfate (PAPS) (Kamiyama et al., 2003; Luders et al., 2003), the universal donor in sulfation reactions.

In our previous study, we also tested whether the expression of *papss* (also known as *Paps* – Flybase), the gene encoding PAPS synthetase (Jullien et al., 1997), is required in the follicle cell layer to support the formation of embryonic DV polarity (Zhu et al., 2005). Surprisingly, we did not detect embryos with DV defects among the progeny of females carrying follicle cell clones mutant for *papss*. This finding was paradoxical, given that the requirement for *sll* expression demonstrated a need for PAPS to be transported into the Golgi of the follicle cells. To explain this result, we proposed that PAPS is transported between the germline and the follicle cells, perhaps through gap junctions that are known to exist between follicle cells and the oocyte (Bohrmann and Haas-Assenbaum, 1993; Giorgi and Postlethwait, 1985; Goldberg et al., 2004; Waksmonski and Woodruff, 2002). In the work reported here, we have tested this hypothesis by generating females with egg chambers in which both the germline and clones of follicle cells were mutant for *papss*. These females produced dorsalized embryos, indicating that PAPS is required in the ovary for embryonic DV patterning, consistent with Pipe's role as a sulfotransferase. In addition, we show that dorsalized embryos are not generated by females carrying both germline and follicle cell clones mutant for the GAG synthesis-related genes *sugarless* (*sgl*) (Binari et al., 1997; Häcker et al., 1997; Haerry et al., 1997) or *sulfateless* (*sfl*) (Lin et al., 1999). These findings definitively rule out HS and CS/DS GAGs as substrates for Pipe. The results reported here also demonstrate that although biological

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sulfation in the follicle cells is crucial for the production of embryos with normal DV patterning, it is apparently dispensible for the viability of follicle cell themselves. Our finding that *papss* mutant follicle cells can be rescued by a wild-type germline is consistent with a model in which PAPS can move between the germline and the follicle cells. This represents a novel mechanism for maintaining homeostasis in an embryonic patterning pathway.

MATERIALS AND METHODS

Genetics

The wild-type stock was Oregon R. The $papss^2$ (Zhu et al., 2005), sll^{7E18} (Luders et al., 2003), sgl^{08310} (Häcker et al., 1997) and sfl^{03844} (Lin et al., 1999) mutations were carried on a chromosome bearing an insertion of P{w[+mW.hs]=FRT(w[hs])}2A at chromosomal interval 79D (FRT^{79D}). An insertion of the *D. melanogaster* variant H2A.F/Z-class histone fused to green fluorescent protein (hGFP) (Clarkson and Saint, 1999) on 3L of the FRT^{79D} chromosome was used to identify mutant clones in the ovary. An insertion of P{w[+mC]=*ovoD*1-18} on 3L ($P[ovo^{D1}]$) of the FRT^{79D} chromosome was used to select for embryos derived from germline clones (Chou et al., 1993). FLP expression was induced by heat shock from the *hsFLP1* insertion (Golic and Lindquist, 1989), and from an insertion of *UAS-FLP* by the Gal4 enhancer trap insertion *e22c-GAL4* (Duffy et al., 1998). Larvae carrying *hsFLP1* were heat shocked for 1 hour at 37°C on two consecutive days during the second and third larval instar stages.

Identification and characterization of follicles and embryos from mosaic females

Follicles

Six days after eclosion, females were placed in yeasted vials for one day, then their ovaries were dissected and fixed in 4% paraformaldehyde in PBS and heptane for 15 minutes, followed by extensive washing in PBT (PBS+0.1% Tween 20). Ovaries were stained for 5 minutes with 0.2 μg/ml DAPI in PBT followed by three 5-minute washes in PBT. Stained ovaries were mounted in a 1:1 mix of glycerol:PBS and photographed using a Zeiss Axioscope II microscope outfitted with a Zeiss axiocam digital camera. Mutant clones were identified by the absence of GFP fluorescence in the nuclei.

Embryos

Six days following eclosion, mosaic females were mated to wild-type males and placed in an egglay collection tube. After an additional 48 hours, embryos were collected on yeasted apple juice agar plates (Wieschaus and Nüsslein-Volhard, 1986) that were changed every 24 hours. Cuticle preparations (van der Meer, 1977) were made from embryos that were allowed to complete development. Dorsalization of cuticles was classified according to Roth et al. (Roth et al., 1991). Cellular blastoderm-stage embryos were subjected to antibody staining (Macdonald and Struhl, 1986) using anti-Twist rabbit polyclonal antibody (1:5000) (Roth et al., 1989) and a biotinylated goat anti-rabbit secondary antibody (1:500) that was visualized with avidin-HRP complex (Vector Laboratories).

RESULTS AND DISCUSSION

To test our hypothesis that *papss* mutant follicle cells can be non-autonomously rescued by wild-type germline cells, it was necessary to generate females carrying mosaic egg chambers in which both germline and follicle cell clones were mutant for *papss*. In these follicles, the germline would be incapable of providing PAPS to the follicle cell layer. Thus, if PAPS were required for Pipe activity, the embryos resulting from these follicles would exhibit dorsalized phenotypes. We first determined the feasibility of generating and detecting egg chambers carrying both follicle cell and germline clones using FLP/FRT-mediated recombination (Golic and Lindquist, 1989). To mark the clones, the FRT^{79D} chromosome was carried in trans to another FRT^{79D} chromosome with an insertion of the *D. melanogaster* variant H2A.F/Z-class histone fused to green fluorescent protein (hGFP) (Clarkson and Saint, 1999). Following recombination, clones of cells homozygous for the unmarked FRT^{79D} chromosome were identified by their lack of fluorescence. To achieve a high frequency of clones in the follicle cell layer, females carried the *e22c-GAL4* enhancer trap insertion and a UAS-FLP transgene on the second chromosome (Duffy et al., 1998). To generate germline clones, FLP expression was induced in response to heat shock using the *hsFLP1* insertion (Golic and Lindquist, 1989), which places FLP under *hsp70* promoter transcriptional control.

In females of the genotype *e22c-GAL4, UAS-FLP/+; hGFP,* FRT^{79D}/FRT^{79D}, in which FLP was expressed only in the somatic cells, 76% of stage 9-10 egg chambers exhibited at least one mitotic clone in the follicular epithelium (Table 1). When larval heat shock was used to induce FLP expression in *hsFLP1/+; hGFP,* FRT^{79D}/FRT^{79D} females, 10 out of 760 (1.3%) of the stage 9-10 egg chambers examined carried FRT^{79D}/FRT^{79D} germline cells (Table 1). In the dissected ovaries of *hsFLP1/+; e22c-GAL4, UAS-FLP/+; hGFP,* FRT^{79}/FRT^{79D}, we identified 8 out of 660 (1.2%) egg chambers in which an oocyte derived from a germline clone was surrounded by an epithelium containing a clone of FRT^{79D}/FRT^{79D} follicle cells (Table 1). Thus, although they are relatively rare, it is possible to generate and identify follicles containing both germline and somatic cell clones.

We next determined if it is possible for follicle cell clones that are mutant for *papss* to survive in an egg chamber in which the germline also lacks *papss* expression. We generated females of the genotype *hsFLP1/+; e22c-GAL4, UAS-FLP/+; hGFP,* FRT^{79D}/$papss^2$, FRT^{79D}. When the two sources of FLP were used simultaneously to induce somatic and germline recombination, we observed that 6 out of 920 (0.7%) stage 9-10 egg chambers had coincident germline and follicle cell clones that were mutant for *papss* (Table 1) (Fig. 1E,F). This indicated that mutant follicle cells could survive without rescue by a wild-type germline, and suggested that a very small percentage of the embryos produced by these females would be derived from follicles simultaneously carrying germline and somatic mutant clones. Consistent with this prediction, when the embryos produced by *hsFLP1/+; e22c-GAL4, UAS-FLP/+; hGFP,* FRT^{79D}/$papss^2$, FRT^{79D} females were examined, 10 out of 795 (1.3%) cuticles exhibited phenotypes that ranged from weak to complete dorsalization (Table 2). This result indicates that *papss* expression, and therefore PAPS synthesis, is required in the ovary for DV patterning of the embryo. The percentage of dorsalized embryos corresponded well with the percentage of follicles that carried simultaneous germline and follicle cell clones. This implies that embryonic patterning is only affected in follicles in which the mutant follicle cells are paired with a mutant germline. This is consistent with our proposal that in other follicles containing *papss* mutant follicle cells, the presence of a wild-type oocyte can rescue the embryonic phenotype.

A drawback of the experimental design described above is that it did not permit us to definitively demonstrate that the dorsalized embryos we observed were derived from follicles containing coincident germline and somatic *papss* mutant clones. The hGFP marker enabled the detection of egg chambers carrying both types of clones in dissected ovaries, but it did not allow us to identify directly the embryos that were derived from such follicles. To overcome this limitation, we incorporated the dominant female-sterile technique (Chou and Perrimon, 1993) to select for eggs derived from *papss* mutant germline clones. In these females, the $papss^2$, FRT^{79D} chromosome was carried in trans to $P[ovo^{D1}]$, FRT^{79D}, which carries a transgenic insertion of the $P[ovo^{D1}]$ allele. Follicles in which the germline is either heterozygous or

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Table 1. Observed frequencies of egg chambers containing mutant mitotic clones in the follicle cell layer, the germline, or both, following induction of FLP expression

Mutation	Follicle cell clones *n*/total counted (%)	Germline clones *n*/total counted (%)	Follicle and germline clones *n*/total counted (%)
Wild-type control	464/612 (76)	10/760 (1.3)	8/660 (1.2)
papss	312/504 (64)	8/868 (0.9)	6/920 (0.7)
sgl	415/596 (70)	6/678 (0.9)	5/710 (0.7)
sfl	405/601 (67)	8/918 (0.9)	6/692 (0.9)

papss, *sgl* and *sfl* mutant alleles were carried on an FRT^{79D}-bearing chromosome. An FRT^{79D}-bearing chromosome carrying wild-type alleles of these three genes served as the wild-type control. The frequency of follicle cell clones was determined in females expressing FLP under the control of the *e22c-GAL4* enhancer-trap element. Germline clone frequencies were determined in females expressing FLP under the control of the *hsFLP1* transgenic insertion. The frequency of egg chambers carrying both follicle cell and germline clones was determined in the ovaries of females expressing FLP under the control of both of these elements.

homozygous for $P[ovo^{D1}]$ degenerate and produce no mature eggs (Oliver et al., 1987). Thus, the only eggs that are laid by these females must be derived from homozygous $papss^2$ germline clones. Our previous results demonstrated that 76% of the follicles carry somatic clones when FLP expression is driven by *e22c-GAL4*. Therefore, we expected the *hsFLP1/+; e22c-GAL4, UAS-FLP/+;* $P[ovo^{D1}]$, $FRT^{79D}/papss^2$, FRT^{79D} females, which were heat shocked as larvae, to produce many eggs that formed in an egg chamber containing a follicle cell clone. The embryos produced by these females were collected, allowed to complete embryogenesis, and then cuticle preparations were made. Of 2736 embryonic cuticles examined, 800 (29%) exhibited a dorsalized phenotype, including 81 that showed the completely dorsalized D0 cuticular phenotype (Table 2) (Fig. 2H). To confirm our assessment of the cuticular phenotype with a molecular marker, we stained blastoderm-stage embryos with an antibody against Twist (Thisse et al., 1988), a marker for mesoderm, which is the most ventral pattern element along the embryonic DV axis. Many of the embryos produced by these females exhibited either complete elimination (not shown) or partial disruption of Twist expression (Fig. 2G). By contrast, the wild-type pattern of Twist staining was observed in embryos derived from *papss* mutant germline clones that presumably developed in a follicular epithelium in which tissue-specific FRT-mediated recombination had not been induced (Fig. 2E). As reported previously, disruption of Twist staining was also seen in the progeny of females carrying *sll* mutant follicle cell clones (Fig. 2C) (Luders et al., 2003). Thus, the phenotypic consequence of depleting PAPS is equivalent to eliminating *sll* expression in the follicle cells. This is consistent with the idea that sulfotransferase activity in the follicle cells is crucial for DV patterning of the embryo.

We have previously shown that DV patterning is normal in embryos derived from egg chambers containing ventral follicle cell clones mutant for *sgl* (Zhu et al., 2005), which encodes *Drosophila* UDP-glucose dehydrogenase (Binari et al., 1997; Häcker et al., 1997; Haerry et al., 1997). This finding led us to conclude that HS and CS/DS GAGs could not be the target of Pipe activity, because the product of Sgl enzymatic activity, UDP-gluronic acid (UDP-GlcA), is required for their synthesis. Like PAPS, however, UDP-GlcA is a small molecule (577 Da) that could potentially move between the oocyte and follicle cells via gap junctions. Our finding that PAPS can be supplied to *papss* mutant follicle cells from a wild-type oocyte raised the possibility that in our previous study, follicle cell clones mutant for *sgl* were non-autonomously rescued by the transfer of UDP-GlcA from the germline. Indeed, observations made in the developing wing disc are consistent with this possibility. In this tissue, Wingless protein acts as a morphogen that forms a concentration gradient through a restricted diffusion mechanism. Wingless diffusion requires the function of the HS proteoglycans Dally and Dally-like; cells that lack either of these two proteins impede the movement of Wingless (Han et al., 2005). Although UDP-glucose dehydrogenase is required for HS synthesis, cells mutant for *sgl* do not interfere with Wingless diffusion (Strigini and Cohen, 2000). This discrepancy could be explained if the UDP-GlcA required for the formation of the HS chains in Dally and Dally-like were supplied to the *sgl* mutant cells by nearby wild-type cells. Therefore, to rule out a similar mechanism and definitively test the requirement for HS and CS/DS GAGs in embryonic DV axis formation, we generated females that simultaneously carried

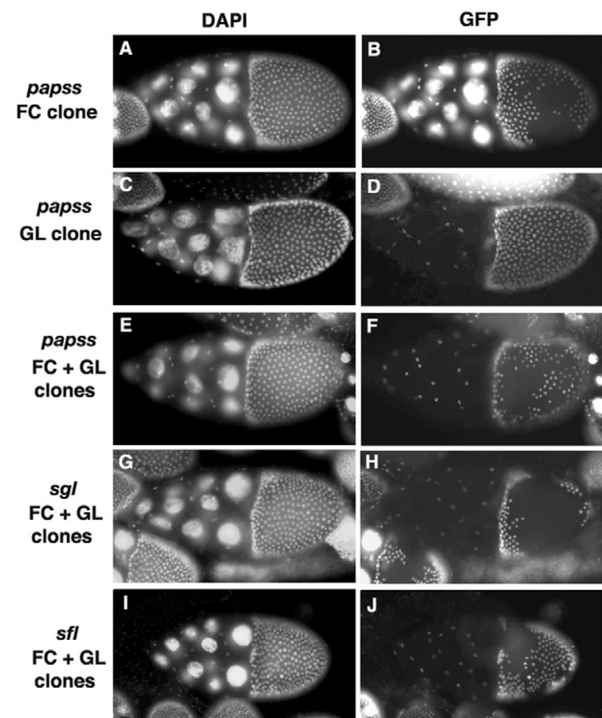

Fig. 1. Follicle cell clones mutant for *papss*, *sgl* or *sfl* are viable even when the germline is also homozygous for the same mutation. Follicle cell and germline nuclei in stage-10 egg chambers visualized by DAPI (A,C,E,G,I) and GFP (B,D,F,H,J) fluorescence. Nuclei of mutant cells lack the GFP marker but still exhibit DAPI fluorescence. (**A**,**B**) Egg chamber from *e22c-GAL4, UAS-FLP/+; hGFP* $FRT^{79D}/papss^2$ FRT^{79D} female containing *papss* mutant cells in the follicular epithelium. (**C**,**D**) Follicle from *hsFLP1/+; hGFP* $FRT^{79D}/papss^2$ FRT^{79D} female with *papss* mutant germline cells. (**E-J**) Follicles carrying both follicle cell and germline mutant clones from females of the following genotypes: (E,F) *hsFLP1/+; e22c-GAL4, UAS-FLP/+; hGFP* $FRT^{79D}/papss^2$ FRT^{79D}; (G,H) *hsFLP1/+; e22c-GAL4, UAS-FLP/+; hGFP* FRT^{79D}/sgl^{08310} FRT^{79D}; (I,J) *hsFLP1/+; e22c-GAL4*, *UAS-FLP/+; hGFP* FRT^{79D}/sfl^{03844} FRT^{79D}.

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Table 2. The proportion of dorsalized embryos produced by females in which follicle cell and germline clones mutant for *papss*, *sgl* or *sfl* are being generated

Third-chromosome genotype of females	DV phenotypes of progeny embryos				
	DO	D1	D2	D3	Wild type
papss FRT^{79D}/*hGFP* FRT^{79D}	2	2	3	3	785
sgl FRT^{79D}/*hGFP* FRT^{79D}	0	0	0	0	754
sfl FRT^{79D}/*hGFP* FRT^{79D}	0	0	0	0	670
papss FRT^{79D}/*P[ovo^{D1}]* FRT^{79D}	81	166	210	343	1936
sgl FRT^{79D}/*P[ovo^{D1}]* FRT^{79D}	0	0	0	0	1485
sfl FRT^{79D}/*P[ovo^{D1}]* FRT^{79D}	0	0	0	0	1310

FLP was expressed under the combined control of the *e22c-GAL4* enhancer-trap element and *hsFLP1* in females carrying mutant alleles of *papss*, *sgl* or *sfl* on an FRT^{79D}-bearing chromosome. In one set of experiments, a transgene bearing the dominant female-sterile marker *P[ovo^{D1}]* was present on the FRT^{79D} chromosome carried in trans to the mutation-bearing chromosome to ensure that all embryos produced were derived from egg chambers with a mutant germline. Embryos from females in which clones were being generated were examined for dorsalization in cuticle preparations and classified according to Roth et al. (Roth et al., 1991) as follows: D0 embryos are completely dorsalized, lacking all lateral and ventral pattern elements; D1 embryos carry the dorsolaterally-derived tracheal structures, Filzkörper; D2 embryos carry Filzkörper and ventral denticle bands of reduced width; D3 embryos carry Filzkörper and ventral denticle bands of normal width and are either twisted at a point along the anterior-posterior axis or exhibit a tail-up or U-shaped phenotype.

somatic and germline *sgl* mutant clones, as described above for *papss* (Table 1; Fig. 1G,H). In contrast to our finding with *papss*, none of the 2239 embryos derived from *sgl* mosaic females exhibited a dorsalized phenotype, even among the 1485 embryos from females in which *P[ovo^{D1}]* was used to select for embryos derived from *sgl* germline clones (Table 2). This finding conclusively demonstrates that the function of Pipe in embryonic DV patterning does not require the presence of HS or CS/DS GAGs in the follicle cells.

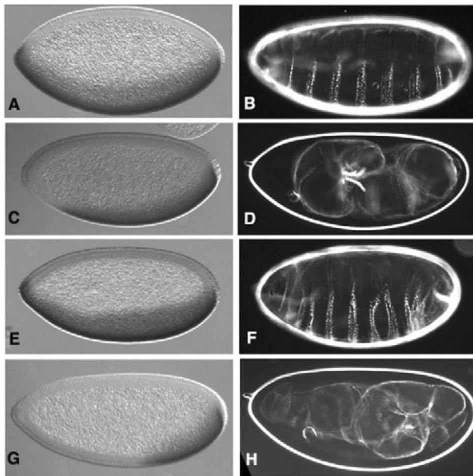

Fig. 2. Egg chambers that contain both follicle cell and germline clones mutant for *papss* produce dorsalized embryos. (A,C,E,G) Whole-mount stainings of syncytial blastoderm embryos with anti-Twist antibody. (B,D,F,H) Cuticle preparations of developed embryos. (**A**,**B**) Embryos from wild-type mothers. (**C**,**D**) Embryos from *e22c-GAL4, UAS-FLP/+;* FRT^{82B} *hGFP*/FRT^{82B} *sll^{7E18}*mother carrying homozygous *sll* mutant follicle cell clones. Anterior Twist expression is disrupted (C) and the cuticle is dorsalized (D). (**E**,**F**) Embryos from *hsFLP1/+; P[ovo^{D1}]* FRT^{79D}/*papss^2* FRT^{79D} mothers mated to wild-type males. Loss of *papss* from the germline alone does not affect Twist expression (E) or DV pattern formation of the embryo (F). Paternally-rescued embryos exhibit weak segmentation defects (F), similar to paternally-rescued embryos derived from *sgl* mutant germline clones (Binari et al., 1997; Perrimon et al., 1996). (**G**,**H**) Embryos from *hsFLP1/+; e22c-GAL4, UAS-FLP/+; P[ovo^{D1}]* FRT^{79D}/*papss^2* FRT^{79D} mothers mated to wild-type males. Loss of *papss* function in both the germline and the follicular epithelium disrupts Twist expression (G) and results in embryos that lack ventral and lateral pattern elements (H).

In addition to ruling out HS and CS/DS GAGs as substrates for the Pipe sulfotransferase, the experiments described above indicate that GAG synthesis is not required for either follicle cell viability or egg formation. We wanted to confirm these results, at least with respect to HS, using the *sfl* mutation, which in contrast to *papss* and *sgl* would not be expected to be subject to non-autonomous rescue. *sfl* encodes N-deacetylase/N-sulfotransferase (Lin et al., 1999), an enzyme that mediates the deacetylation and sulfation of the N-acetyl group on N-acetylglucosamine residues of HS. Sfl is an integral membrane protein that acts on GAG monosaccharide units in the lumen of the Golgi. Its product, sulfated HS, is unlikely to pass freely between cells. In a previous study, we demonstrated that it is possible to generate females carrying follicle cell clones mutant for *sfl* and that they do not produce dorsalized embryos (Zhu et al., 2005). Here, we found that it was possible to generate females with coincident germline and follicle cell clones mutant for *sfl* (Table 1) (Fig. 1I,J). As expected, none of the 1980 embryos produced by *sfl* mosaic females, 1310 of which derived from females with an *sfl* mutant germline, exhibited a dorsalized phenotype (Table 2). Thus, the complete lack of HS GAG synthesis does not impede the establishment of DV polarity in the embryo, nor does it affect the viability of either the follicle cells or the germline, or the patterning of the egg chamber.

Despite Pipe's similarity to glycosaminoglycan uronic acid-specific 2-0 sulfotransferases, the findings presented here conclusively demonstrate that uronic acid-containing GAGS such as HS and CS/DS do not play an essential role in the establishment of embryonic DV polarity. Our results do support, however, previous evidence suggesting that Pipe is acting as a sulfotransferase (Zhu et al., 2005), as PAPS must be available to the follicle cells for DV pattern formation to occur normally. Although our data rule out the possibility that Pipe acts upon HS or CS/DS GAGs, we consider it likely that Pipe acts on an alternate type of glycoprotein or glycolipid-associated carbohydrate.

Our ability to generate egg chambers with simultaneous follicle cell and germline clones mutant for *sgl* provides evidence for the surprising conclusion that uronic acid-containing carbohydrates such as HS, CS and DS, as well modification of other molecules by glucuronidation (King et al., 2000), are dispensible for the viability and growth of the oocyte and follicle cells. Even more strikingly, our results from females carrying both *papss* mutant germline and follicle cells indicate that biological sulfation itself is not essential for follicle cell viability or oocyte maturation. Indeed, the only requirement for PAPS in the egg chamber that was detected in our experiments was in embryonic DV patterning, which presumably reflects the function of PAPS in the sulfation of the Pipe target.

Although PAPS is required for the function of Pipe, our results demonstrate that PAPS need not be synthesized in the Pipe-expressing ventral follicle cells as it can be supplied to them from the neighboring germline cells. PAPS is a highly polar molecule that is unlikely to diffuse freely across lipid membranes. We consider it likely that in the ovary, PAPS molecules travel through the gap junctions that are known to exist between the oocyte and follicle cell layer (Bohrmann and Haas-Assenbaum, 1993; Giorgi and Postlethwait, 1985; Waksmonski and Woodruff, 2002). PAPS (507 Da) is sufficiently small to pass through the gap junctions, which allow passage of molecules of approximately 1 kDa (Goldberg et al., 2004). The gap junctions of insects and other invertebrates are composed of protein subunits called innexins (Phelan, 2005), for which eight genes have been identified in *Drosophila* (Stebbings et al., 2002). The gonads of flies lacking Innexin 4, encoded by *zero population growth* (also known as *inx4*), contain small numbers of early germline cells but lack more mature stages (Tazuke et al., 2002), indicating that communication via gap junctions is required for gametogenesis. During the course of oogenesis, the 15 polytene nurse cells provide the developing oocyte with various metabolites, and the ability of the follicle cells to receive PAPS from the oocyte provides evidence for a mechanism by which the follicle cells benefit from the robust synthetic capacity of the nurse cells. Thus, in addition to a potential influence of gap junctional communication on embryonic DV patterning, our findings raise the possibility that communication between germline and soma contributes to the homeostasis of other metabolites within the egg chamber. Finally, it is worth noting that the ability of other small metabolites to move from cell to cell should also be considered as a factor in other developmental contexts.

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