**pipsqueak**, an early acting member of the posterior group of genes, affects *vasa* level and germ cell-somatic cell interaction in the developing egg chamber

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**INTRODUCTION**

Pattern formation along the anteroposterior axis of the *Drosophila melanogaster* embryo is initiated prior to fertilization. In a process that involves input from both the germ line and the soma, morphogens become localized to the anterior and posterior poles of the developing oocyte (Schüpbach and Wieschaus, 1986a,b; Ruohola et al., 1991; St. Johnston and Nüsslein-Volhard, 1992). Specifically, development of the larval abdomen requires the localization of *nanos* protein to the posterior pole of the *Drosophila* egg (Wang and Lehmann, 1991). The proper localization of *nanos* to the posterior pole is important, as ectopic localization of *nanos* protein to the anterior pole of the oocyte leads to the development of abdominal structures at the anterior (Gavis and Lehmann, 1992). Likewise, development of the germ line requires the localization of as yet unidentified germ line determinants (although a candidate for such a determinant is *germ cell-less*; see Jongens et al., 1992) to the posterior pole.

A number of maternal effect genes have been identified in female sterile screens that affect both abdomen and germ line formation. These genes (among them *oskar* (Lehmann and Nüsslein-Volhard, 1986), *tudor* (Boswell and Mahowald, 1985), *vasa*, *valois* (Schüpbach and Wieschaus, 1986a), *staufen* (St. Johnston et al., 1991), and *mago nashi* (Boswell et al., 1991); for review see Lehmann, 1992), known collectively as posterior group genes of the grandchildless class, all seem to affect the formation of electron dense particles called polar granules, which are thought to consist of both protein and RNA, and which are localized to the posterior pole of mature oocytes and early embryos (Mahowald and Kambysellis, 1980). Other genes have been identified (*cappuccino* and *spire*) whose products are required in the germ line both for the formation of polar granules and for the proper dorsoventral patterning of the egg (Manseau and Schüpbach, 1989). Finally, the products of the neurogenic genes *Notch* and *Delta* are required in the somatic follicle cells for proper anteroposterior patterning in the egg (Ruohola et al., 1991).

Ephrussi and Lehmann (1992) have shown that an early step in posterior patterning involves the localization of the *oskar* mRNA to the posterior pole of the egg. They fused the coding sequence of *oskar* (Ephrussi and Lehmann, 1992) with the anterior localization sequence of *bicoid* (Macdonald and Struhl, 1988). Transgenic females localized the *oskar-bicoid* hybrid mRNA to the anterior of the oocyte; eggs laid by these females developed pole cells and abdominal segments at the anterior end. Under these circumstances, they could distinguish components required solely for *oskar* mRNA localization from those required for later steps. Thus pole cell and abdomen formation at the anterior pole was independent of *cappuccino*, *spire*, and *staufen*, but was still dependent on *vasa* and *tudor*.
The genes that act upstream of oskar are thus thought to be required solely to localize oskar to the posterior pole. One mechanism by which this might occur involves transport of oskar mRNA along microtubules. When a hybrid protein consisting of the plus-end directed microtubule motor domain of kinesin and the enzyme β-galactosidase (kin-lacZ) was expressed in the germ line, this protein was found at the anterior pole of the oocyte (Clark et al., personal communication). The localization of kin-lacZ was dependent on cappuccino and spire and also on the somatically required genes Notch and Delta (ibid.). Thus these genes may act in concert to generate an asymmetric microtubule network in the egg, with minus ends at the anterior of the egg and plus ends at the posterior.

In this paper we present the phenotypic characterization of a previously unidentified maternal effect gene, which we call pipsqueak (psq), whose product appears to be required in the posterior group pathway. The initial P element-associated mutant alleles we identified produce the classic phenotype of a posterior group gene of the grandchildless class. We show that the localization of oskar mRNA to the posterior pole of the oocyte occurs normally in psq mutant ovaries, suggesting that the initial steps in posterior pattern formation occur independently of psq. In contrast, vasa protein can no longer be detected at the posterior pole of the oocyte in psq mutant ovaries.

Additional alleles of psq have been isolated both by imprecise excision of the P element and by further screening of P element insert lines. Females with these alleles lay few or no eggs and exhibit abnormalities or blocks during oogenesis. Interestingly, the range of defects exhibited by the different psq alleles is strikingly similar to the range of defects exhibited by different alleles of the posterior group gene vasa. We show that psq interacts genetically with vasa, and that psq affects the level of vasa protein and vasa mRNA in the developing egg chamber. Based on our findings, we suggest that the alteration in vasa level is a primary but not sole cause of the psq mutant phenotype.

MATERIALS AND METHODS

Stocks

Drosophila melanogaster were raised on standard cornmeal-molasses-yeast-agar medium at 18-25°C. Wild-type flies were of the Oregon-R (OR) strain. Some wild-type flies (yw flies) were marked by the X chromosome marker mutations y and w but have all other chromosomes derived from the OR strain. Balanced stocks of pipsqueak (psq) PlacW insertions at 47B were originally named 21E10, 24B9 and 13B6 and were obtained during the Bier et al. screen (1989). Balanced stocks of psq [P{y}] insertions at 47B, named 2403 and 8109, were provided by Celeste Berg, University of Washington, Seattle, and Allan Spradling, Carnegie Institution of Washington, Baltimore, MD. Balanced stocks of psq HK38, originally provided by Trudi Schüpbach, Princeton University, Princeton, NJ, contained both cappuccino (capu) and psq mutations. The capu mutation was removed from the chromosome by recombination by C. Berg. K2503, a stock containing a germ line expressed kinesin-lacZ on chromosome 3, was provided by Ira Clark and Ed Giniger (Giniger et al., 1993). Most vasa alleles were provided by Trudi Schüpbach. vasa deficiency stocks were provided by Ruth Lehmann, Whitehead Institute, Cambridge, MA and Paul Lasko, McGill University, Montreal, Canada. Stocks used for P element excision were generated by Susan Younger-Shepherd and Ed Grell in our laboratory. Df(2R)27 was provided by Robert Burgess and Tom Schwarz, Stanford University, Stanford, CA.

Antibodies

Anti-vasa antibodies were generated and characterized in this laboratory by Bruce Hay (Hay et al., 1989a, 1990). Anti-germ cell-less antibodies were generated and characterized in this laboratory by Tom Jongens (Jongens et al., 1992). Anti-staufen antibodies were provided by Daniel St. Johnston, University of Cambridge, Cambridge, England (St. Johnston et al., 1991).

DNA probes

Plasmids containing various coding sequences were provided as follows: oskar by Anne Ephrussi, EMBL, Heidelberg, Germany; nanos by Ruth Lehmann; vasa by Bruce Hay; germ cell-less by Tom Jongens; rhomboid by Ethan Bier, University of California, San Diego.

Cuticle preparations

Procedure was essentially as described by Wieschaus and Nüsslein-Volhard (1986). Embryos were aged at least 4 hours prior to dechorionation with 50% bleach. To determine percentage viability, dechorionated embryos were transferred to Petri dishes containing water and allowed to terminal differentiate. Then all hatched larvae and unhatched eggs were mounted in Hoyers:lactic acid 1:1 and heated to 50-65°C to clear.

P element excision

We used A2-3 (Robertson et al., 1988) integrated into the genome as a source of P element transposase. yw; fs[w+]CyO virgin females were mated to y[w+]; BcE[Fsc]; Sb Δ2-3/TM6 males, yw; fs[w+]CyO; Sb Δ2-3/+ males were collected. These males had a variegated red eye phenotype, indicating that the w+ containing P element was actively transposing. Single males were mated to yw; CyO/Sco or yw; CyO/Pin88 virgins. White eyed, non Sco, non Sb males were collected. These males should have lost the w+ P element from the chromosome. Single males were mated to yw; CyO/Pin88 virgins to generate balanced stocks.

Genetic mapping

al dp b pr cn px sp or stw cn sca sp marker chromosomes were used to map the female sterile mutation by recombination according to standard procedures. The mutation mapped near 2-60 on the genetic map. Df(2R)17 and Df(2R)27 extend from 47A to about 47C (Schwarz, personal communication). No psq allele complements these deficiencies, consistent with the chromosomal location of the P element.

Plasmid rescue

DNA flanking the P element was isolated by digestion of homozgyous fs[w+] by DNA with either EcoRI or XbaI, ligation and transformation as described (Pirrotta, 1986). We have performed plasmid rescue with DNA from the P element containing lines 21E10 and 24B9. Restriction mapping of flanking DNA as well as genomic Southern analysis of wild-type and mutant DNA suggest that the two inserts are within a few hundred base pairs of each other. We have used for all further analysis the 21E10 insert, which we have renamed ppsqP1. Approximately 7 kb of DNA on one side (the XbaI plasmid rescue) and 2 kb of DNA on the other side (the EcoRI plasmid rescue) of the P element was isolated.

In situ hybridization to salivary chromosomes

Salivary chromosomes isolated from larvae containing the P element insertion were hybridized with biotin or digoxigenin-labeled P element probe, and wild-type chromosomes were hybridized with DNA flanking the P element, which was obtained
by plasmid rescue (see above) using the enzyme XbaI. Both probes hybridized to region 47B on the right arm of the second chromosome (data not shown).

**Molecular analysis of mutations**

Wild type, P element allele, and P element excision allele DNA was analyzed on genomic Southern using both P element and flanking genomic DNA as probe. By this analysis, we found that psqP1 is a simple insertion of the P lacW element and psqP1–30 is a 3.3 kb chromosomal deletion (in the region of chromosomal DNA isolated by plasmid rescue with XbaI) and a partial P element deletion (data not shown).

**Northern blots**

Northers were performed according to Jongens et al. (1992). Total or poly(A)⁺ RNA was resolved on 1% agarose-formaldehyde gels as in Vaessen et al. (1987). Gels were transferred to Hybond-N⁺, crosslinked using a Stratalinker, and then prehybridized and hybridized in 50% formamide, 5× SSC, 1× Denhardt’s, 20 mM phosphate, pH 7, 100 µg/ml salmon sperm DNA at 42°C. Blots were washed twice in 1× SSC, 0.5% SDS at 42°C for 5 minutes and twice in 0.1× SSC, 0.5% SDS at 65°C for 15-30 minutes prior to exposure to X-ray film.

**Whole-mount in situ hybridization with digoxigenin probes**

This procedure was performed essentially according to Tautz (Tautz and Pfeifle, 1989) for embryos and was modified as follows for stainings of whole ovaries. Ovaries were dissected in Robb’s buffer (Theurkauf et al., 1992) or in EB (125 mM NaCl, 5 mM KCl), were transferred to microfuge tubes, and were fixed 1 hour in fresh 4% formaldehyde (Ted Pella, Inc) in phosphate-buffered saline (PBS, 130 mM NaCl, 7 mM Na₂HPO₄, 2H₂O, 3 mM NaH₂PO₄, 2H₂O) with 5% DMSO at room temperature. Ovaries were then washed five times with PBS and then dehydrated through an ethanol series into 100% ethanol and stored at ~20°C. Hybridization was then essentially as described, with a 1 hour proteinase K treatment with 50 µg/ml proteinase K, and a 30 minutes postfixation in 5% formaldehyde in PBS + 0.1% Tween-20. Ovaries were dissected into ovarioles either after staining if mounted in glycerol or just prior to staining if mounted in Permount. If Permount was used, samples were dehydrated through ethanol and toluene prior to mounting.

**Whole-mount immunocytochemistry of ovaries**

Ovaries were dissected and fixed as for in situ hybridization. Ovaries were washed in PBS + 0.1% Triton X-100 (PBT) and then extracted overnight in PBS + 1% Triton X-100. Ovaries were washed in PBT and then blocked with PBT + 10% normal goat serum (PBT Block) and incubated overnight; then samples were washed three times for 30 minutes each in PBT. For diaminobenzidine (DAB) stainings, secondary antibody conjugated to horseradish peroxidase was diluted into microfuge tubes and incubated overnight. Samples were then rinsed three times for 30 minutes each (or longer) each in PBT. In the third wash, ovaries were dissected into ovarioles or egg chambers and transferred to 24-well plates. Samples were then rinsed three times in 0.12 M Tris-HCl, pH 7.6 and once with 0.12 M Tris-HCl, pH 7.6, containing 0.5 mg/ml DAB. Samples were stained in 0.12 M Tris-HCl, pH 7.6, containing 0.5 mg/ml DAB and 0.006-0.03% hydrogen peroxide. Staining reactions were stopped with 95% ethanol. After two rinses in ethanol, samples were transferred back to microfuge tubes, rinsed in 100% ethanol, rinsed in xylene, and mounted in Permount.

For fluorescent stainings, secondary antibody conjugated to either Texas Red or Fluorescein (Jackson Laboratories) was used. Samples were kept dark except during the dissection into ovarioles.

All samples were incubated in 0.5 µg/ml DAPI (Sigma) to visualize DNA. The procedure was essentially as above except that samples were transferred from PBT to PBS after dissection into ovarioles and were mounted in glycerol containing 2% n-propyl gallate to reduce photobleaching (Giloh and Sedat, 1982) and 0.1× PBS.

**X-Gal staining of ovaries**

Ovaries were dissected as for in situ hybridization and fixed for 7 minutes in 2.5% glutaraldehyde in PBS. After three rinses in PBS, ovaries were incubated at 37°C overnight in 0.2% X-Gal in 10 mM sodium phosphate, pH 7.2, 3.1 mM Kt[Fe(II)[CN]₆], 3.1 mM Kt[Fe(III)[CN]₆], 150 mM NaCl, 1 mM MgCl₂. Then, after three rinses in PBS, ovaries were postfixed in 2.5% glutaraldehyde in PBS for 30 minutes, rinsed in PBS and dissected and mounted in 80% glycerol.

**Whole-mount immunocytochemistry of embryos**

Embryos were dechorionated in 50% bleach and then fixed for 20 minutes in 4 ml 4% formaldehyde in PBS and 5 ml heptane in a glass scintillation vial. The fixative was removed, 5 ml methanol was added, and the samples were vigorously agitated for 1 minute to remove the vitelline membrane. The heptane layer and most of the methanol was removed and the embryos were rinsed twice more in methanol, transferred to microfuge tubes, and then rinsed three times for 30 minutes each in PBT. Samples were blocked for several hours in PBT + 10% normal goat serum and then incubated with primary and horseradish peroxidase-conjugated secondary antibody, and stained with DAB as for ovaries. Some embryos were stained with fluorescent secondary antibody and also counterstained with DAPI to visualize DNA.

**Western blots**

Wild-type or mutant ovaries were dissected into Robbs buffer and transferred to 1.5 ml microfuge tubes; then, most of the buffer was removed and the tubes were frozen in liquid N₂ and stored at −80°C. Ovaries were thawed into basic Laemmli sample buffer (3.5% lauryl sulfate, 14% glycerol, 120 mM Tris base, 8 mM EDTA, 0.12 M DTT), homogenized, and boiled for 5 minutes. Protein from between 0.05 and 50 ovaries was loaded per lane and resolved by SDS-PAGE on 10-15% gradient gels. Gels were transferred to nitrocellulose for 1-2 Amp-hour in SDS containing transfer buffer. Nitrocellulose blots were blocked for 1 hour in 5% milk in 10 mM Tris-HCl, pH 7.5, 150 mM NaCl, 0.05% Tween-20 (TST-milk) and then incubated overnight at 4°C in TST-milk containing rabbit anti-vasa serum diluted 1:5000. Blots were rinsed three times for 30 minutes each in TST and then incubated for 4-4 hours in TST-milk containing HRP-conjugated donkey anti-rabbit IgG diluted 1:10,000. After three rinses in TST, blots were incubated in ECL (Amersham) substrate and exposed to X-ray film.

**RESULTS**

*pipsqueak, a new posterior group gene*

In a screen of P element insert lines (Bier et al., 1989) for mutations causing a female sterile phenotype, we identified two lines, 21E10 and 24B9, which exhibited a posterior group defect. Females homozygous for either of these insertions, or transheterozygous for each of these insertions, laid eggs which differentiated and secreted a larval cuticle. About 99% of eggs laid by mutant females less than 5 days old did not hatch, and the larval cuticle was abnormal. Fig. 1A shows a wild-type cuticle and Fig. 1B shows a representative cuticle derived from a mutant female. We found a range of abdominal segmentation defects, extending from...
loss or fusion of a subset of abdominal segments to complete loss of abdominal segments. The remaining eggs hatched into larvae and developed into viable albeit often sterile adults. Specifically, 70% of the adult offspring of mutant females developed somatic gonadal structures but did not make germ cells. Females more than 7 days old laid eggs which never hatched and which 90% of the time developed into larvae completely missing abdominal segments. In addition, abdominal segments were missing. In the larva shown in B, all the abdominal segments appear fused. In the most extreme case (not shown), we fail to find any abdominal segments. The variability in abdominal segmentation defects depicted here was observed in a single isogenic line; the severity of the defects appeared to worsen as the females aged. (C,D) Cellular blastoderm stage embryos stained with the DNA dye DAPI. Anterior is to the left and dorsal is up. (C) Wild type. The position of the pole cells is marked with an arrow. (D) Mutant. No pole cells can be seen in the mutant embryo (the posterior pole is marked with an arrow).

In addition to the abdominal segmentation defects, 21E10 and 24B9 homozygous females produced embryos that in greater than 90% of the embryos examined failed to form pole cells. Fig. 1C shows a wild-type embryo at the cellular blastoderm stage stained with the DNA dye DAPI, and Fig. 1D shows a similarly staged embryo laid by a mutant female (which will subsequently be referred to as mutant embryo). The pole cells, which can be seen at the posterior pole of a wild-type embryo (indicated by the arrow), are missing in the mutant embryo. Thus this posterior group defect falls into the grandchildless class, and can be grouped together with oskar, staufen, valois, vasa, tudor and mago nashi, all of which affect the assembly or maintenance of polar granules.

We mapped the P element inserts in both female sterile lines to chromosome 2. In order to determine whether we had obtained mutant alleles of previously isolated posterior group genes, we performed complementation tests with known posterior group genes on chromosome 2. We found that the P element insert lines complemented stauD3,
tud\textsuperscript{WC8}, vasa\textsuperscript{PD23}, and val\textsuperscript{PE}, but did not complement each other. From these results, we concluded that the P element inserts define a new posterior group gene, which we named \textit{pipsqueak} (psq), and named the mutant alleles in P element insert lines 21E10 \textit{psq}\textsuperscript{P1}, and in 24B9 \textit{psq}\textsuperscript{P4}, respectively.

We excised the P element genetically using \Delta2-3 transposase. 85\% (112 out of 132) of our viable excision lines were fertile, confirming that in each line the mutant phenotype was caused by the P element. In the course of the excision experiments we generated two additional alleles, \textit{psq}\textsuperscript{X1-30} and \textit{psq}\textsuperscript{X1-36}, which exhibited defects during oogenesis (see below).

The P elements in \textit{psq}\textsuperscript{P1} and \textit{psq}\textsuperscript{P4} map to 47B1-2 (data not shown). Genetic recombination experiments linked the posterior group defect with the \textit{w}\textsuperscript{+} marker of the P inserts, and gave them a genetic map position of 2-60. An X-ray induced deficiency, \textit{Df(2R)27}, which extends from 47A to 47C was obtained from Tom Schwarz. We found that females transheterozygous for \textit{psq}\textsuperscript{P1} and \textit{Df(2R)27} were sterile, further confirming the map position of the mutation. These transheterozygous females laid at most 10\% the

\textbf{Fig. 2.} \textit{nanos} and \textit{vasa} staining of embryos laid by wild-type and \textit{psq}\textsuperscript{P1} females. (A-C) \textit{nanos} mRNA staining by whole-mount in situ hybridization; (D-H) \textit{vasa} staining by immunohistochemistry with 46F11, a monoclonal antibody which recognizes the \textit{vasa} antigen. Although we could detect localized \textit{nanos} mRNA (A) and \textit{vasa} protein (D,E) in wild-type embryos, we often failed to detect localized staining of either \textit{nanos} mRNA (B) or \textit{vasa} protein (F,H) in mutant embryos. Occasionally we saw a small amount of localized staining (C,G). Furthermore, we failed to find pole cells (pc) in blastoderm embryos (F) or within the posterior midgut (pm, H), where pole cells can be found in wild-type embryos (D). All embryos are oriented anterior to the left, dorsal up, with the exception of those in B and F, where the orientation is arbitrary.

\textit{psq}\textsuperscript{X1-30} and \textit{psq}\textsuperscript{X1-36}, which exhibited defects during oogenesis (see below).
number of eggs laid by $psq^{P1}$ homozygotes, suggesting that $psq^{P1}$ is not a null mutation (see below).

**Position of *pipsqueak* in the posterior group pathway**

The posterior group genes have been ordered into a pathway in which different gene products are sequentially localized to the posterior pole of the developing oocyte (Ephrussi et al., 1991; St. Johnston et al., 1991; Hay et al., 1988b; Lasko and Ashburner, 1988; Golumbeski et al., 1991; Wang and Lehmann, 1991; Barker et al., 1992; Macdonald, 1992). This ultimately results in the localization of the gene products required for abdominal segment formation (*nanos*; Wang and Lehmann, 1991, and *pumilio*; Barker et al., 1992), and also in the localization of the gene products required for pole cell formation (Ephrussi et al., 1991; Ephrussi and Lehmann, 1992; Jongens et al., 1992). We wondered if and where *pipsqueak* fits into this pathway. To answer these questions, we stained wild-type and *psq* mutant ovaries with a number of probes for gene products of the posterior group.

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**Fig. 3. ** *pipsqueak* acts between *oskar* and *vasa* in the posterior group pathway. (A) *oskar* staining in a mature $psq^{X1-30}$ oocyte; (B) X-Gal staining in a stage 9 $psq^{X1-30}$ egg chamber containing a kinesin-*lacZ* P element on the 3rd chromosome; (C) *vasa* staining in a stage 10 $psq^{X1-30}$ egg chamber; (D) *vasa* staining in a stage 10 wild-type egg chamber. We found that *oskar* mRNA (A) and kinesin-*lacZ* protein (B) were localized in mutant egg chambers. In contrast, *vasa* protein (C) was not localized. Under identical staining conditions, we were able to detect localized *vasa* at this stage in wild-type egg chambers (D). Note however that the majority of *vasa* protein at this stage is found in the nurse cell cytoplasm. ncn, nurse cell nucleus; o, oocyte; fc, follicle cell. All egg chambers are oriented anterior to the left.
We started at the end of the pathway by looking at the localization of nanos mRNA in embryos laid by psq\(^{P1}\) homozygous females. Consistent with the variability in the abdominal segmentation defects in the larvae, we found variable amounts of nanos mRNA localized to the posterior pole of the egg. In greater than 90% of embryos examined, we could not find any localized nanos mRNA (Fig. 2B); in the remaining embryos we found a narrow band of nanos mRNA at the posterior pole (Fig. 2C). nanos localization in wild-type embryos is shown for comparison in Fig. 2A.

We used the vasa antigen as a marker for polar granules and pole cells (vasa staining in wild-type embryos is shown in Fig. 2D,E). In embryos laid by homozygous psq\(^{P1}\) females, greater than 90% of the time we failed to detect any localized vasa protein (Fig. 2F); in the remaining embryos, we detected a narrow band of vasa protein at the posterior pole (Fig. 2G). Thus the vasa protein distribution in these embryos was similar to nanos mRNA distribution.

During gastrulation, pole cells migrate within the posterior midgut invagination into the interior of the embryo. Comparison of vasa staining in mutant (Fig. 2H) and wild-type (Fig. 2D) embryos at the germ band extended...
stage show quite clearly the lack of pole cells in the mutant embryo.

Given that nanos mRNA and vasa protein were not localized properly to the posterior pole in eggs laid by psq mutant females, we wondered whether the initial stages of posterior pattern formation occurred normally. The first gene products known to be localized to the posterior pole are the mRNA product of the gene oskar (Ephrussi et al., 1991) and the protein product of the gene staufen (St. Johnston et al., 1991). These products are localized at stages 8-9 of oogenesis (see King, 1970, and Mahowald and Kambyssellis, 1980, for staging). Localization of both components may depend on the presence of an organized cytoskeleton, since a kinesin-lacZ fusion protein (consisting of the motor domain of kinesin fused to β-galactosidase), which apparently marks the plus ends of microtubules (Ginger et al., 1993), also localizes to the posterior pole throughout oogenesis; in contrast, kinesin-lacZ localization is lost by stage 10B, suggesting both that the microtubule distribution changes at Stage 10B and that previously localized oskar mRNA and staufen protein are maintained at the posterior pole by a microtubule-independent mechanism.

Fig. 3 shows oskar in situ hybridization in psqX1-30 mutant ovaries (Fig. 3A) and X-Gal staining (Fig. 3B) in psqX1-30 mutant ovaries containing a kinesin-lacZ reporter gene. We found both components to be appropriately localized to the posterior pole. Thus we conclude that neither the initial microtubule array nor the posterior anchor is disturbed in the pipsqueak mutant egg chambers. Staufen protein was also localized normally in psqX1-30 mutant ovaries (data not shown).

Subsequent to the localization of oskar mRNA and staufen protein to the posterior pole, the protein product of the posterior group gene vasa becomes localized (Hay et al., 1988b; Lasko and Ashburner, 1988). When we stained psqX1-30 egg chambers with anti-vasa antibody (Hay et al., 1988b), we failed to detect posteriorly localized vasa protein (Fig. 3C), although we could detect localized protein in our wild-type control egg chambers (Fig. 3D). Thus we can position psq between oskar and vasa in the posterior pathway.

**pipsqueak mutant ovaries contain decreased levels of vasa protein and vasa mRNA**

In addition to a loss of vasa protein from the posterior pole of psqX1-30 oocytes, we found a reduction in the cytoplasmic level of vasa protein in psqX1-30 nurse cells. This reduction in vasa protein level is seen throughout oogenesis. Fig. 4A shows egg chambers from a wild-type female stained for vasa protein. We found vasa protein throughout the nurse cell cytoplasm. In contrast, in psqX1-30 egg chambers, cytoplasmic vasa was limited to a tight ring around the nurse cell nucleus (Fig. 4B,C).

In order to quantitate these findings, we dissected ovaries and prepared them for western blot analysis. Fig. 5A compares vasa protein levels in wild-type (lanes b, c) and psqX1-30 (lane a) ovaries. Lanes a and c contain roughly the same amount of total ovarian protein, while lane b contains one tenth the protein of the other two lanes. We estimate from these results that the amount of vasa protein present in psqX1-30 mutant ovaries is decreased to approximately 1% that found in wild-type ovaries. Vasa protein in psqP1 ovaries is decreased on average to approximately 50% that found in wild-type ovaries (data not shown).

In order to determine whether the effect on vasa protein level can be accounted for by an effect on vasa mRNA level, we compared vasa mRNA levels by northern blot analysis, and also visualized vasa mRNA in psq mutant ovaries by whole-mount in situ hybridization. Fig. 5B shows the northern blot. We failed to detect vasa transcript by this method in any of the psq alleles tested (compare lanes b-d with a). However, we were able to detect residual vasa mRNA in psqX1-30 egg chambers by whole-mount in situ hybridization (Fig. 4E), although the level was significantly lower than in wild-type (Fig. 4D) or psqP1 (not shown) egg chambers. These results are consistent with the presence of a low level of vasa protein in psqX1-30 mutant ovaries. We conclude from these experiments that psq mutations affect the level of vasa protein primarily by affecting the transcription or the stability of vasa mRNA.

**pipsqueak vasa double mutants are blocked in oogenesis**

Similar to psqP1 homozygous females, vasaPD homozygous females lay eggs which exhibit a posterior group defect. vasaPD is a hypomorphic allele; vasa protein can be detected...
in vasa<sup>PD</sup> homozygous females primarily in the germarium and early vitellarium (Hay et al., 1990, Lasko and Ashburner, 1990). Given that psq affects the level of vasa mRNA in the ovary, one might predict that the double mutant combination would have even lower levels of vasa protein in the ovary, leading to a phenotype more closely resembling that of a vasa null allele.

We generated psq<sup>P1</sup> vasa<sup>PD</sup> double mutants by recombination. We found that psq<sup>P1</sup> vasa<sup>PD</sup> double mutant females, in contrast to single mutant females, did not lay eggs. In order to determine whether there was a specific block in oogenesis, we dissected ovaries from single and double mutant females, and stained them with the DNA dye DAPI (Fig. 6); a wild-type ovariule is shown for comparison in Fig. 6A. Although we found vitellogenic egg chambers in virtually every psq<sup>P1</sup> (Fig. 6B) and vasa<sup>PD</sup> (Fig. 6C) ovariule, we found vitellogenic egg chambers in fewer than 5% of double mutant ovaries (Fig. 6D,E). This block is similar to the one found in vasa deficiency females (Fig. 6F).

Some psq<sup>P1</sup> alleles exhibit oogenesis defects

Given the early oogenesis phenotype of the psq<sup>P1</sup> vasa<sup>PD</sup> double mutant, we wondered, first, whether psq is absolutely required for vasa expression, and, second, whether psq affects genes besides vasa. In the first case, we would expect to obtain psq alleles that have phenotypes more closely resembling those of a vasa null mutation, and in the second case, we would expect to find phenotypes that are not observed in vasa mutations.

We have obtained multiple psq alleles which are blocked earlier in oogenesis. In addition to the P element excision lines psq<sup>X1-30</sup> and psq<sup>X1-36</sup>, which have regions (3.5 kb or less) adjacent to the P element deleted, we obtained a PlacW insert called 13B6 and three P[<sup>ry</sup>]<sup>+</sup> inserts called psq<sup>2043</sup>, psq<sup>8109</sup>, and psq<sup>0115</sup>. We have not analyzed the psq<sup>0115</sup> mutant phenotype, but summarize the phenotypes of the other psq alleles in Table 1, and compare them to those of a vasa null mutation.

Some psq<sup>P1</sup> egg chambers contain supernumerary germ cells

psq<sup>X1-30</sup> and psq<sup>X1-36</sup> females lay fewer than 10% of the eggs laid by wild-type or psq<sup>P1</sup> females; approximately 70% of these eggs are collapsed or short. About 10-30% of these eggs have dorsal appendages which are fused, either just at the base or along the entire length of the appendage (data not shown). Consistent with this, preliminary experiments suggest that the pattern of rhomboid mRNA (Bier et al., 1990) expression, which has been shown to be both sufficient and necessary for dorsal pattern in the egg shell (Ruohola-Baker et al., 1993), is narrowed in psq<sup>X1-30</sup> egg chambers (data not shown).

Because the number of eggs laid by psq<sup>X1-30</sup> and psq<sup>X1-36</sup> females is small, we looked for additional defects in oogenesis. In order to visualize these defects, we dissected ovaries from mutant females and stained them with DAPI; a wild-type egg chamber is shown for comparison in Fig. 7A. We found that oogenesis proceeded normally in most egg chambers from psq<sup>P1</sup> females (Fig. 7B); however, in approximately 1% of egg chambers, we found more than the normal number of 16 germ cells (marked as sgc). In psq<sup>X1-30</sup> (Fig. 7C) and psq<sup>X1-36</sup> (Fig. 7D) ovaries, the number of egg chambers containing such supernumerary germ cells increased to between 10 and 50%, suggesting that psq<sup>X1-30</sup> and psq<sup>X1-36</sup> are stronger alleles than psq<sup>P1</sup>. Furthermore, we found that the germaria from these females were enlarged (compare Fig. 7A and D, for example), and that follicle cells seemed to migrate well into the germarium and to surround nurse cells of different sizes and thus presumably different ages (Fig. 7D). We found that psq<sup>P1</sup> vasa<sup>PD</sup> double mutant ovaries also contained egg chambers with supernumerary germ cells (Fig. 6D, E), at a frequency comparable to that found in psq<sup>P1</sup> ovaries.

There are a number of possible explanations for the supernumerary germ cell phenotype in psq<sup>P1</sup>. In normal development, a germ line precursor divides four times with incomplete cytokinesis to generate a cluster of 16 cells, and follicle cells surround this cluster in order to separate it from other clusters, thereby generating an egg chamber. In principle, supernumerary germ cells could result from abnormal divisions of the germ line precursor or from abnormal behavior of the follicle cells. It could also result from a failure of the 16-cell cyst, surrounded by follicle cells, to leave the germarium at the proper time, which then leads to fusion of 16-cell cysts.

Both germ line-dependent and somatic cell-dependent mutations have been identified which cause egg chambers with supernumerary germ cells to form. These include Notch and Delta, which are required in the soma (Ruohola et al., 1991), and brainiac (Goode et al., 1992), which is required in the germ line. Also, females containing the vasa<sup>PD</sup> allele have been reported to develop egg chambers with supernumerary germ cells (Lasko and Ashburner, 1990).

The follicle cells that migrate between germ cell clusters and then intercalate to separate the stage 2 egg chamber from the germarium have been shown to be enriched in fasciclin III (fasc III). In Notch and Delta mutant ovaries (Ruohola et al., 1991), the domain of fasc III expression was found to be expanded. In brainiac egg chambers (Goode and Mahowald, 1992), no fasc III staining cells migrated inward to form the ‘pinch’. Fasc III staining provides a marker for the cell type presumably involved in egg chamber formation; however, fasc III is not itself required for the process, as a null mutation is completely viable and fertile (Elkins et al., 1990).

We stained psq<sup>X1-30</sup> egg chambers with anti-fasc III antibody. We found multiple patches of fasc III staining cells (Fig. 8A; patches of fasc III-enriched cells are marked by brackets) in egg chambers containing supernumerary germ cells. This is similar to what is found in Notch and Delta egg chambers (Ruohola et al., 1991).

The transcript of the posterior group gene oskar becomes enriched in oocytes very early during oogenesis (Ephrussi et al., 1991) and thus provides a convenient marker for oocyte formation. We stained psq<sup>X1-30</sup> ovaries with a probe specific for osk mRNA (Fig. 8B-D) and found that supernumerary egg chambers contained multiple osk-staining cells (marked by brackets), suggesting that multiple oocytes have at least initiated development within these egg chambers. We also found egg chambers in which
multiple cells within an egg chamber take up yolk (Fig. 8E), suggesting that they continue development as oocytes.

**Nurse cell DNA in some pipsqueak mutants remains condensed throughout their development**

Nurse cell DNA is highly polyploid (each nurse cell reaches a ploidy of either 512 or 1024). Through stage 4 of oogenesis, the DNA copies remain somatically paired, giving a characteristic lobed pattern in DAPI-stained preparations (Figs 6A, 7A; Spradling, 1992). This pairing is lost at stage 5, and the DNA appears more dispersed throughout the nucleus (Figs 6A, 7A; seen as diffuse DAPI staining). Egg chambers from *vasa* deficiency ovaries seem to successfully undergo the polytene to polyploid transition, as they contain nurse cell nuclei with dispersed DNA (Fig. 6F).

In contrast, egg chambers from *psq*13B6 (Fig. 7E) or from *psq*13B6/*psq*8109 (Fig. 7F) ovaries, as well as from *psq*X1-30 (Fig. 7C) and *psq*X1-36 (not shown) ovaries, contain nurse cell nuclei in which the DNA continues to appear lobed and condensed, even though other markers, such as the shape of the egg chamber, or the presence of yolk in the oocyte (Fig. 7C, F), suggest that the egg chambers have advanced past stage 4.

**psq**13B6 egg chambers are blocked prior to vitellogenesis

*psq*13B6 females resemble *vasa* deficiency females in that greater than 90% of egg chambers never enter vitellogenesis (Fig. 7E; compare with Fig. 6F). Interestingly, we failed to detect *vasa* transcript in *psq*13B6 egg chambers by in situ hybridization analysis (Fig. 4F), even when the staining reaction was allowed to proceed long enough to visualize *vasa* DNA within the polyploid nurse cell nuclei.
Fig. 7. Oogenesis defects in pipsqueak alleles. Ovaries were stained with DAPI to visualize DNA. (A) Wild type. A typical ovariole is shown (see also 6A). A stack of somatic cells called terminal filament cells is found at the anterior of each ovariole. Immediately posterior to this is the germarium (germ), where stem cell divisions and egg chamber formation occur. A cluster of 16 interconnected germ cells is surrounded by a layer of somatic follicle cells (fc). Each egg chamber is separated by stalk cells. Early in oogenesis, the nurse cell DNA is condensed (ncn toward the left of the figure); later in oogenesis, the DNA appears diffuse (ncn, towards the right of the figure). (B) pqsP1. Occasionally egg chambers are found that contain more than the normal number of germ cells. Such an egg chamber is shown here (marked sgc). (C) pqsX1-30. An egg chamber containing supernumerary germ cells and at least one oocyte (o) is shown. (D) pqsX1-36. Shown here is a germarium, at the identical magnification to that shown in A. It is significantly larger than wild type, and contains multiple germ cell clusters and follicle cells, suggesting a defect in egg chamber formation. (E) pqsX1-36. We only rarely found vitellogenic egg chambers. Furthermore, the nurse cell DNA appeared condensed at all stages. One nurse cell nucleus is marked. (F) pqsX1-36/psqsX1109. We found vitellogenic egg chambers (one is marked) with condensed nurse cell DNA. (G) pqsX1-30/Df2R27. Note the egg chamber developing in an otherwise rudimentary ovary. Note also the stacks of cells at the anterior of the ovary (marked tfc?), which should be compared to the terminal filament cells marked in Figs 6 and 8. These stacks appear much longer than wild type. Note also the large cells within the body of the ovary (marked gc?), whose identity remains undetermined.
**Table 1. pipsqueak alleles and their phenotypes**

<table>
<thead>
<tr>
<th>Mutagen type</th>
<th>Allele name</th>
<th>Embryonic phenotype</th>
<th>Oogenesis phenotype</th>
<th>vasa expression</th>
</tr>
</thead>
<tbody>
<tr>
<td>EMS</td>
<td>psqHK38</td>
<td>Egg shell normal, cuticle has abdominal deletions</td>
<td>n.d.</td>
<td>n.d.</td>
</tr>
<tr>
<td>PlacW insertion</td>
<td>21E10-psqP1</td>
<td>Egg shell normal, cuticle has abdominal deletions, no pole cells</td>
<td>Occasional egg chambers with supernumerary germ cells</td>
<td>RNA and protein decreased (protein level 50% wild type)</td>
</tr>
<tr>
<td></td>
<td>psq13B6</td>
<td>Same as psqP1</td>
<td>n.d.</td>
<td>Same as psqP1</td>
</tr>
<tr>
<td></td>
<td>psqP1</td>
<td>No eggs</td>
<td>Oogenesis block around Stage 6, nurse cell DNA remains condensed</td>
<td>RNA not detectable by in situ hybridization</td>
</tr>
<tr>
<td></td>
<td>psq403</td>
<td>Few collapsed eggs with fused dorsal appendages</td>
<td>Nurse cell DNA remains condensed</td>
<td>Protein level down (by immunohistochemistry); RNA level n.d.</td>
</tr>
<tr>
<td></td>
<td>psq8109</td>
<td>Semilethal as homozygote although viable over deficiency; no eggs</td>
<td>Rudimentary ovary, almost agamic</td>
<td>There are a few vasa staining cells in ovary</td>
</tr>
<tr>
<td>A2-3 excision from psqP1</td>
<td>psqX1-30</td>
<td>Few collapsed eggs, egg shell often has fused dorsal appendages</td>
<td>Egg chambers contain supernumerary germ cells; nurse cell DNA remains condensed</td>
<td>RNA and protein level decreased (protein level 1% wild type).</td>
</tr>
<tr>
<td></td>
<td>psqX1-36</td>
<td>Similar to psqX1-30</td>
<td>Similar to psqX1-30</td>
<td>n.d.</td>
</tr>
<tr>
<td>A2-3 excision from 2403</td>
<td>psqBL34u</td>
<td>Lethal line, but lethality is genetically separable from psq</td>
<td>n.a.</td>
<td>n.a.</td>
</tr>
<tr>
<td></td>
<td>psqR34a</td>
<td>Same as psqP1</td>
<td>n.d.</td>
<td>n.d.</td>
</tr>
<tr>
<td>X-ray</td>
<td>Df(2R)17</td>
<td>Lethal, deficiency extends from 47A-47C</td>
<td>n.a.</td>
<td>n.a.</td>
</tr>
<tr>
<td></td>
<td>Df(2R)27</td>
<td>Lethal, deficiency extends from 47A-47C, pole cells scatter in homozygotes</td>
<td>n.a.</td>
<td>n.a.</td>
</tr>
</tbody>
</table>

The EMS allele was originally identified on a cappuccino (capuHkh38) chromosome which failed to complement psq2403. The chromosome was found to have a second lesion at pipsqueak. PlacW insertion alleles were obtained in a screen carried out by Bier et al. (1989). P[ry+] insertion alleles were obtained in a screen carried out in Allan Spradling’s laboratory (see Spradling, 1992). psqRV34a was derived from psqRL34a by recombination. X-ray deficiencies were obtained in a screen carried out in Tom Schwarz’s laboratory. pipsqueak mutants exhibited a range of phenotypes. psqP1 and psqHkh38 females laid eggs with posterior group defects. psq13B6 females developed ovaries in which egg chambers rarely progressed beyond Stage 6 before becoming necrotic. psq13B6 in trans to psqP1 resembles homozygous psqP1 in its posterior group defects. Females homozygous for the remaining alleles laid few or no eggs. psq2403 females laid very few eggs, which almost always had a single dorsal appendage along the dorsal midline. psq8109 females laid no eggs; ovaries were rudimentary. In general, we found that transheterozygous combinations were weaker in phenotype than either allele as a homozygote. For example, psqHkh38 resembles psqP1 in that homozygous females lay eggs which do not hatch. In trans to psqP1, females lay eggs that either hatch into larvae or develop abdominal defects. The larvae which hatch are often sterile. This allelic combination thus resembles tud2 in its grandchildless phenotype (Boswell et al. 1985). Females transheterozygous for psq2403 and psq8109 laid many eggs which often have only a single dorsal appendage along the dorsal midline. Females transheterozygous for psqP1 and either psq2403 or psq8109 laid normal looking eggs with posterior defects. An excision line of psq2403, called psqRV34a, exhibits a posterior group defect as a homozygote, but is grandchildless in trans to psqP1. psq8109 is a semi-lethal line. psq8109 in trans to psq13B6 was blocked in oogenesis, but oocytes became vitellogenic (Fig. 7F). Occasionally stage 10 and even mature eggs lacking dorsal appendage material were found. The DNA within the nurse cells remained condensed throughout development. Such interallelic complementation has been previously described, for example at the white, Ultrabithorax, and decapentaplegic loci. In these cases, the complementation has been shown to be dependent on self-association of a DNA binding protein called zeste (Bickel and Pirrotta, 1990). While we have not investigated the role of zeste in the complementation shown here, we note that zeste protein is expressed in ovaries and that a zeste binding site has been found on salivary gland chromosomes near 47B (Pirrotta et al., 1988; Rastelli et al., 1993). n.d., not determined; n.a., not applicable.

**pipsqueak alleles in trans to a deficiency exhibit a rudimentary ovary phenotype**

We crossed psqP1, psq13B6, psqX1-30, psq2403 and psq8109 to Df(2R)27. All transheterozygous combinations were sterile. psqP1/Df(2R)27 females laid at most 10% the number of eggs laid by wild-type females; these eggs were small or collapsed, and had short, thin, or fused dorsal appendages; other combinations laid no eggs. In all combinations, some females contained rudimentary ovaries with no apparent organization into ovarioles. For example, 10 out of 12 psqX1-30/Df(2R)27 ovary pairs examined had a phenotype similar to the one shown in Fig 7G. The ovary on the right contains a single egg chamber, which seems not to be associated with an ovariole. Cells are found stacked at the anterior end of the ovary (marked ‘tfc’), which we presume to be terminal filament cells (for comparison with terminal filament cells in wild-type ovarioles, see Figs 6A and 7A), but these cells did not seem to be associated with germ cells.
pipsqueak, a new posterior group gene

(large cells within the ovary that may be germ cells are marked ‘gc?’). We have not determined whether germ line stem cells are lost during development, or whether they are present in appropriate numbers but do not organize into ovarioles or do not divide to produce cystoblasts. The rudimentary ovary phenotype is not observed in vasa deficiency ovaries, suggesting that psq plays additional roles early in oogenesis.

Summary of pipsqueak mutant phenotypes

To summarize our studies of the pipsqueak mutant phenotype, we have isolated a number of alleles and have ordered them into a hypomorphic series. The weakest alleles (psq\(^{P1}\), psq\(^{P4}\)) reduce the level of vasa mRNA, resulting in the absence of vasa protein from the posterior pole of the oocyte and embryo and, concomitantly, in posterior group defects. Stronger alleles cause more significant reductions in

Fig. 8. pipsqueak egg chambers contain patches of fasc III positive follicle cells and multiple oocytes. (A) A psq\(^{XI-30}\) egg chamber stained with a monoclonal antibody against fasc III. Fasc III enriched cells are marked with brackets. (B-D) oskar mRNA staining in psq\(^{XI-30}\) ovaries; (E) Egg chamber from psq\(^{P1}\) ovary. Areas that stain are marked by brackets. We found numerous egg chambers that contained multiple oskar staining cells, although in many cases only a single cell would take up yolk. Occasionally, as is shown in E, we would find egg chambers with two yolk containing cells (marked as o).
vasa level, resulting in earlier blocks in oogenesis. psq<sup>13B6</sup> egg chambers contain no detectable vasa transcript and, like vasa deficiency egg chambers, rarely enter vitellogenesis.

psq alleles also exhibit phenotypes not seen in a vasa deficiency, suggesting additional roles for the psq locus. Some alleles (psq<sup>13</sup>, psq<sup>X1-30</sup>, psq<sup>X1-36</sup>) seem to disrupt egg chamber formation, leading to a supernumerary germ cell phenotype. Some alleles (psq<sup>13B6</sup>, psq<sup>X1-30</sup>, psq<sup>X1-36</sup>) also alter the morphology of the nurse cell nucleus, apparently blocking the polytene to polyploid transition. Finally, in trans to a deficiency, psq causes a rudimentary ovary phenotype, suggesting that germ cells are either lost or fail to produce cystoblasts during development.

**DISCUSSION**

**Position of pipsqueak in the posterior group pathway**

In a screen of P element induced mutations, we identified a new member of the posterior group of genes, which we have named pipsqueak. We have used a number of molecular probes to position psq in the posterior group pathway. We found that in psq mutants, osk mRNA was enriched in the oocyte early in oogenesis and was localized to the posterior pole and maintained there later in oogenesis. Similarly, staufen protein and kinesin-lacZ protein were localized to the posterior pole. We conclude from these results that the early stages of pole plasm assembly, i.e., the establishment of an asymmetric microtubule network and the localization of oskar mRNA and staufen protein, do not require the psq gene product.

In contrast, we found that vasa protein was not localized to the posterior pole of the egg or oocyte and that nanos mRNA was not localized to the posterior pole of the egg. From these experiments, we inferred that psq acts between osk and vasa, and that psq is required for vasa protein transport, synthesis, or stability.

**pipsqueak controls the level of vasa mRNA**

In order to distinguish among these possibilities, we looked for changes in the level of vasa protein and vasa mRNA in psq mutant ovaries. We found that vasa protein was decreased to approximately 1% of wild-type levels in psq<sup>X1-30</sup> mutant ovaries. Similarly, vasa mRNA levels were decreased in psq mutant ovaries. We were unable to detect vasa mRNA by northern analysis in any of the psq mutants we tested. However, by in situ hybridization, we were able to detect vasa mRNA in pre-stage 10 egg chambers in psq<sup>p1</sup> ovaries (not shown), and even some vasa mRNA in psq<sup>X1-30</sup> ovaries. In wild-type ovaries, most of the vasa transcript is present in late stage egg chambers, which may explain the difference between our northern and in situ hybridization analysis. In any case, the presence of vasa mRNA in psq mutants is consistent with the presence of vasa protein in these mutants: in psq<sup>X1-30</sup> ovaries, the amount of vasa protein is significant when compared to vasa deficiency ovaries (data not shown), suggesting that at least some vasa mRNA is present. The levels of other maternal transcripts, such as bicaudal, nanos or germ cell-less mRNAs, were not detectably decreased in psq mutant egg chambers, as assessed by in situ hybridization studies (not shown).

Because of the effects on vasa mRNA levels, it seems reasonable to hypothesize that pipsqueak either encodes a transcription factor that interacts directly with the vasa promoter or regulates the activity of such a factor. Indeed, sequence analysis of a putative pipsqueak cDNA (V. S., L. Y. J., and Y. N. J., unpublished data) reveals homology to a group of proteins, among them broad (DiBello et al., 1991) and tramtrack (Harrison and Travers, 1990; Read and Manley, 1992), which encode Zn-finger-containing transcription factors.

In this model, the level of the psq protein in psq<sup>P1</sup> ovaries would be sufficient to allow early but not late vasa transcription. In stronger psq alleles, the level of psq protein would be even lower, leading to further decreases in vasa mRNA and protein and defects earlier in oogenesis.

In vasa<sup>PD</sup> ovaries, vasa protein can be detected only early in oogenesis. The allele has been sequenced and encodes an unaltered protein (Liang, L., Diehl-Jones, W., and Lasko, P., personal communication), suggesting that the allele is a vasa promoter mutation. It seems possible that the vasa<sup>PD</sup> promoter has a lower affinity for psq protein, so that even at wild-type psq levels, vasa mRNA is no longer apparent late in oogenesis. Alternatively, the vasa<sup>PD</sup> promoter may fail to bind a transcription factor that interacts cooperatively with the psq protein.

In either case, we would predict that a psq<sup>P1</sup> vasa<sup>PD</sup> double mutant ovary would show stronger effects on vasa expression. Indeed, the phenotype of the double mutant is similar to the phenotype of a vasa deficiency ovary. Furthermore, we were able to detect very little vasa protein in the cytoplasm of double mutant egg chambers even at the earliest stages of oogenesis (not shown).

**pipsqueak may have additional target genes**

We think it likely that the posterior group phenotype and some of the early oogenesis defects of psq are the result of the effect of psq on vasa gene expression. However, there are enough differences in psq and vasa mutant phenotypes to suggest that psq acts on genes besides vasa.

First, although females homozygous for strong vasa alleles and females homozygous for strong psq alleles both lay eggs with fused dorsal appendages, in the case of vasa, the eggs are long and torpedo-shaped and in the case of psq the eggs are short. Second, vasa deficiency ovaries do not contain egg chambers with supernumerary germ cells, as found in ovaries of strong psq alleles. Third, unlike vasa mutants, psq alleles show blocks in the polytene to polyploid transition that occurs at stages 4-5. Finally, vasa deficiency ovaries proceed through the early stages of oogenesis and are only blocked during vitellogenesis. In contrast, certain psq alleles give a rudimentary ovary phenotype either as homozygotes or when crossed to a deficiency for the psq locus.

We have studied the rudimentary ovary phenotype of psq<sup>810</sup> homozygous females by staining ovaries with the anti-vasa antibody (data not shown), which stains germ cells. We found vasa-positive cells in the ovaries, but often these cells were not associated with follicle cells or organized into ovarioles. Similarly, in other psq alleles in
trans to a psq deficiency, we could observe wild-type looking somatic cells at the anterior of the ovary, but little or no organization of the ovary into ovarioles (Fig. 7G).

Germ cells and somatic cells associate with each other several times during development. The germ cells migrate into the somatic ovary at the end of germ band shortening (stage 15); 2-3 germ cells associate with a cluster of terminal filament cells to generate the anterior portion of each ovariole; and follicle cells migrate around the 16-germ cell cluster to create an egg chamber. psq mutants are clearly defective in at least the latter two of these processes. It is tempting to speculate that psq may regulate the synthesis of a ligand or receptor that is required for germ cell–somatic cell interactions. We may be able to identify this molecule by looking for additional psq target genes.

We especially thank Bruce Hay, who initially identified the female sterile P element lines among the Bier et al. collection, and Bill Sullivan and Bill Theurkauf, who assisted in the characterization of these lines. We thank Allan Spradling, Haifan Lin, and Celeste Berg for supplying stocks of P[fly] pipsqueak alleles, Ira Clark for the kinesin-lacZ transformatant line used in this work, Paul Lasko for stocks and for sharing unpublished information about mutant vasa alleles, Tom Schwarz for the deficiency stocks, and Ruth Lehmann for stocks and for early discussions about maternal effect genes and oogenesis. We thank Anne Ephrussi, Ruth Lehmann, Daniel St. Johnston, Ethan Bier and Bruce Hay for gifts of antibodies and probes. We thank members of the Jan lab, especially Ed Gingier, Ira Clark, Susan Younger-Shepherd, Karen Blochlinger, and Michelle Rhyu, for helpful discussions throughout the course of this work, and Ira Clark and Helen Doyle for critical reading of the manuscript. We greatly thank Sandra Barbel Rastelli, L., Chan, C. S. and Pirrotta, V. for technical assistance and figure preparation, and Larry Ackerman and William Walantus for photographic assistance.

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