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The *Polycomb* group gene *Posterior Sex Combs* encodes a chromosomal protein

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SUMMARY

The *Posterior Sex Combs* (*Psc*) gene of *Drosophila* has been studied at the molecular level both because it is a *Polycomb* group (*Pc*-G) gene and hence required for the maintenance of segmental determination, and because it is the *Drosophila* homolog of the murine *bmi-1* oncogene. Although genetic interactions indicated that *Psc* functioned as a *Pc*-G gene, the zygotic mutant phenotype of *Psc* showed little evidence of segmental transformations. We have examined mutant embryos derived from a mutant maternal germ line and found a stronger mutant phenotype, indicating that the weak zygotic phenotype of *Psc* is due to maternal rescue. We have found that *Psc* RNA accumulates in developing oocytes and this maternal RNA is presumably responsible for the maternal rescue. We have studied the expression of the *Psc* gene at both the RNA and protein levels. On northern blots, we find evidence for two *Psc* mRNAs and, on western blots, we find evidence for two Psc proteins that are altered either in abundance or size in *Psc* mutants. The Psc protein accumulates in all regions of the embryo and also in many tissues in a variety of developmental stages. In all cases, it is nuclear, as is its mammalian homolog, the bmi-1 protein. On polytene chromosomes, we find Psc at 45 chromosomal loci where two other *Pc*-G proteins are present.

Key words: *Posterior Sex Combs*, *Polycomb* group, chromosomal protein, *Drosophila*, mRNA, *bmi-1*

INTRODUCTION

The *Polycomb* group (*Pc*-G) genes are required for maintaining the anterior restriction on the expression of the homeotic selector genes of the BX-C and Antp-C, and hence for the maintenance of segmental determination during embryonic development (Jürgens, 1985; Karch et al., 1985; Celniker et al., 1990). In the absence of *Pc*-G function, the selector genes are expressed in a relatively unrestricted fashion (Struhl and Akam, 1985; Wedeen et al., 1986; Dura and Ingham, 1988; Jones and Gelbart, 1990; McKeon and Brock, 1991; Simon et al., 1992) resulting in all segments being transformed toward eighth abdominal segment development (Struhl and White, 1985; Busturia and Morata, 1988).

The *Pc*-G genes differ in the relative importance of the maternal and zygotic genomes for embryonic function. *Polycomb* (*Pc*) is primarily zygotically acting and embryos lacking *Pc* function show strong anterior toward posterior segmental transformations (Lawrence et al., 1983). A second *Pc*-G gene, *extra sex combs* (*esc*), is largely maternally acting and embryos derived from an *esc* mother show strong posteriorly directed segmental transformations even if they are heterozygous for an *esc* mutation (Struhl, 1981). Eight additional *Pc*-G genes have been shown to function both maternally and zygotically (Ingham, 1984; Breen and Duncan, 1986; Dura et al., 1988; Phillips and Shearn, 1990; Jones and Gelbart, 1990). The zygotic mutant phenotype for these genes is weak compared to *Pc* as only slight segmental transformations are seen. More severe segmental transformations are seen when the maternal contribution is removed either by generating a mutant maternal germ line via pole cell transplantation (Ingham, 1984; Breen and Duncan, 1986), mitotic recombination in the female germ line (Dura et al., 1988), or by using a temperature sensitive mutant (Jones and Gelbart, 1990; Phillips and Shearn, 1990). We have used mitotic recombination to generate mutant clones in the female germ line for the *Pc*-G gene *Posterior sex combs* (*Psc*) and found evidence both for substantial maternal rescue for *Psc*, and for *Psc* being pleiotropic as are several other *Pc*-G genes (Breen and Duncan, 1986; Dura et al., 1987; Phillips and Shearn, 1990; Smouse et al., 1988).

The sequences of three *Pc*-G genes (*Pc*, *ph*, *Psc*) have been reported (Paro and Hogness, 1991; Deatrick et al., 1991; DeCamillis et al., 1992; Brunk et al., 1991b; van Lohuizen et al., 1991). *Psc* was found to share a 200 amino acid domain with the murine *bmi-1* nuclear oncogene and the neighboring *Drosophila* gene *Suppressor 2 of zeste* (van Lohuizen et al., 1991; Brunk et al., 1991b). Here we describe the developmental expression of *Psc* at both the RNA and protein levels.

It has been proposed that the *Pc*-G genes function to regulate homeotic selector gene expression either by maintaining an inactive chromatin structure (Reuter et al., 1990; Paro, 1990; Gaunt and Singh, 1990; Singh et al., 1991; Messmer et al., 1992) or by acting as negative transcription factors (Duncan and Lewis, 1982; Denell and Freder-

ick, 1983; Struhl, 1983; Struhl and Akam, 1985; Wedeen et al., 1986; Castelli-Gair and Garcia-Bellido, 1990). The protein products of the Pc (Zink and Paro, 1989; Zink et al., 1991; Franke et al., 1992) and ph (DeCamillis et al., 1992) loci are co-localized at about 100 salivary gland polytene chromosome loci (Franke et al., 1992). We have found that the Psc protein is present on salivary gland polytene chromosomes at about 45 loci including its presumed targets the BX-C and Antp-C. Interestingly, almost every location where the Psc protein is located is also a location for the Pc and ph proteins.

MATERIALS AND METHODS

Drosophila stocks and culture

Marker mutations and balancer chromosomes are described in Lindsley and Grell (1968). Psc mutations are described in Table 1 and in more depth elsewhere (Lasko and Pardue, 1988; Brunk et al., 1991a).

Generation of germ-line clones

The stock (no. 513) carrying the *Fs(2)D* chromosome used for the generation of mitotic clones in the female germ line was kindly provided by Trudi Shupbach. *T(1,2) Bld*/ *Fs(2)D* males were crossed to *Gla*/*CyO* virgin females. From this cross, the *Fs(2)D*/*Gla* males were isolated and crossed to Psc^{*}/*CyO* virgin females ($Psc^{Arp.1}$/*CyO* or Psc^{1445}/*CyO*). The progeny were irradiated at 2-3 days of development with 1000 R to induce mitotic recombination in the germ line. Only germ cells that lose the dominant female sterile mutation *Fs(2)D* can produce viable eggs, and these germ line cells are now also homozygous for the *Psc* mutation. The resulting *Fs(2)D*/Psc^{*} virgin females (20-30/vial) were then crossed to Psc^{*}/*CyO* males and the vials examined for the production of larvae. The embryos generated are derived from a mutant maternal germ line and half of these are also zygotically mutant for *Psc*. Embryos were mounted and examined as described previously (Adler et al., 1989, 1991).

Plasmid cDNA library screens

The plasmid cDNA libraries were constructed by Nicholas Brown in the pNB40 vector (Brown and Kafatos, 1988), and were screened using the protocol recommended by Brown and Kafatos.

Nucleic acid isolation and manipulation

The techniques used were standard and details are described elsewhere (Brunk and Adler, 1990; Brunk et al., 1991a). In situ hybridization to tissue sections were done as described in Brunk et al. (1991a).

Induction of fusion proteins

The homology region [HR - see Brunk et al., 1991a) of *Psc* (from *Sal*I (1607)-*Hin*dIII (2566)] was subcloned into the pATH2 vector to make a *TrpE-PscHR* fusion gene. A colony containing a *TrpE-PscHR* plasmid was inoculated into 5 ml of M9 media [M9 salts (Sambrook et al., 1989) to which were added the following 1 mM $MgS0_4$, 0.1 mM $CaCl_2$, 0.2% glucose, 10 μg/ml thiamine B1, 50 μg/ml ampicillin, 5 mg/ml casamino acids, 20 μg/ml tryptophan], grown overnight and this culture used to inoculate 500 ml of M9 media **minus** tryptophan which was grown for 3.5 hours at 33°C. The culture was induced with 2.5 ml of 1 mg/ml indoleacrylic acid in ethanol (IAA) and grown for an additional 4 hours. The samples were spun down, the supernatant removed and the pellets frozen at −20°C overnight.

The fusion protein was isolated according to Benson and Pirrotta (1987) with the following changes. The bacterial pellet was resuspended in 1% culture volume of buffer A (25% sucrose, 0.2 mM EDTA, 40 mM Tris-HCl pH 7.5 and 1 mM dithiothreitol), and the samples were sonicated after incubation with lysozyme and centrifuged at 20,000 revs/minute for 1 hour. The pellet was resuspended in 6 M urea in buffer A (0.5-1% culture volume) and stored on ice for 30 minutes. The sample was centrifuged at 20,000 revs/minute for 30 minutes, and the supernatant removed and stored. The pellet was resuspended in 6 M urea and the procedure repeated. The two 6 M urea supernatants were combined and dialyzed as in Benson and Pirrotta (1987).

We also isolated a β-galactosidase-PscHR fusion protein. 500 ml of LB media containing 50 μg/ml of ampicillin was inoculated with a 5 ml overnight of the β-galactosidase-*PscHR* fusion clone [*Psc* cDNA sequence from *Sal*I (1607)-*Hin*dIII (2566) subcloned into the pUR278 vector] (Rüther and Müller-Hill, 1983), and the culture was grown at 37°C for 2 hours. IPTG was added to a final concentration of 10^{-3} M and the culture was grown for another 2 hours. The protein extract was prepared according to Benson and Pirrotta (1987). The β-galactosidase-PscHR fusion protein was affinity purified using an p-aminophenyl β-D thiogalactopyranoside column (APTG; Sigma A-8648) following the procedure of Germino et al. (1983).

Generation of antibodies

Polyclonal antibodies were raised in rabbits via injection of 1 mg of TrpE-PscHR fusion protein followed by two boosts of 200 μg. An affinity column [0.5 ml Affigel 10 plus 0.5 ml Affigel 15 (BioRad)] was prepared and used to purify the polyclonal antibody as described in Lasko and Ashburner (1990) using the

Table 1. List of mutations

Mutation	Synonym	Comments	Reference*
$Psc^{Arp.1}$	$Arp^{1.DC11}$	Arp^{1} revertant with normal cytology, single gene deletion of *Psc*	1
$Psc^{Arp.2}$	$Arp^{1.Fbf1a}$	Arp^{1} revertant, deletion between the terminal *P* elements of *In(2R)Arp* removing the entire *Psc* region	2
$Su(z)2^{1.b8}$	645.13-35asm	Deletion which removes the entire *Psc* region	2
$Psc^{1.d20}$	678.hhsm	gamma ray-induced translocation which reverts the lethal interaction of Psc^{1} and $Su(z)2^{1}$	3
Psc^{1}	*Psc*	EMS induced mutation in *Psc* which is embryonic lethal and is a dominant suppressor of the $zeste^{1}$-*white* interaction	4,5,6
Psc^{1445}	vr14-45	EMS induced hypomorphic embryonic lethal *Psc* allele	7
Psc^{14P4}	vr14-P4	Hypomorphic *Psc* allele caused by a *P* element insertion	7
Psc^{1433}	vr14-33	EMS induced hypomorphic *Psc* allele	7

*1, Brunk et al. (1991a); 2, Brunk and Adler (1990); 3, kindly provided by C.-T. Wu; 4, Nüsslein-Volhard et al. (1984); 5, Wu (1984); 6, Jürgens (1985); 7, Lasko and Pardue (1988).

APTG-purified β-galactosidase-PscHR fusion protein. Monoclonal antibodies were raised in mice via injection of 100 μg of TrpE-PscHR fusion protein followed by two boosts of 100 μg and hybridomas generated using standard techniques. Five hybridomas were isolated that recognize the Psc protein produced in larvae from an *hsp70-Psc* fusion gene. Two of these were used in the western blot experiments (6E8 and 1F4).

Isolation of *Psc* mutant embryos and larvae for western analysis

$Psc^{Arp.1}/Su(z)2^{1.b8}$ and Psc^{1}/Psc^{1} are embryonic lethal resulting in embryos with severe head defects (Adler et al., 1989) which we were able to use to identify the mutant embryos from their heterozygous and wild-type siblings. Psc^{1445}/Psc^{1445} embryos also die as late embryos but the head defects were less severe. Since these embryos were more difficult to identify, we let them age 24-32 hours before collecting to assure that non-mutant embryos had hatched. Psc^{1433}/Psc^{1433} individuals can survive to pupae at a low frequency. Psc^{1433}/CyO were crossed to *T(2,3)CyO,Tb*/+;*TM3*. The Psc^{1433}/*T(2,3)CyO,Tb* siblings were crossed so that homozygous mutant embryos could be identified by their normal size (i.e not *Tb*).

Western analysis

Proteins were separated on 8% SDS-PAGE gels (Hames and Rickwood, 1981). Important modifications to the standard procedures that enabled us to detect the endogeneous Psc proteins on western blots were to add urea to the gel (4 M final urea concentration) and sample buffer (8 M final urea concentration) as a denaturant in addition to the SDS (1% in gel and 4% in sampling buffer) and the samples were **not** heated. The gels were blotted to Nitrocellulose (Schleicher and Schuell) using the ABN Polyblot semi-dry blotting system. A mix of cell culture supernatents of the monoclonal antibodies 6E8 and 1F4, each diluted 1:5 in PBS + 0.1%Tween 20 + 1%BSA were used. We used the chemiluminescence (ECL) system from Amersham to detect the primary antibody following the procedure recommended. Molecular weights were estimated using Rainbow Markers (Amersham).

Both monoclonal antibodies (6E8 and 1F4) and the affinity purified anti-Psc polyclonal antibody recognize specifically the β-galactosidase-PscHR but not a β-galactosidase-Su(z)2HR fusion protein (Fig. 6D,E). *Su(z)2* is a neighboring and related gene (Brunk et al., 1991b) and the HR sequences of the two proteins are 37% identical.

Antibody staining of tissues

Larvae were dissected in 4% paraformaldehyde/PBS to expose internal tissues. The staining procedure followed that of Suter and Steward (1991). For detection we used the HRP Vectastain ABC kit following the procedure of Bopp et al. (1991). The staining of whole-mount embryos essentially followed the procedure of Bopp et al. (1991) using an ABC kit and DAB substrate (Vectastain).

The staining of salivary gland chromosomes followed the procedure in Zink et al. (1991) with the following changes. The primary antibody incubation (1:50 dilution of affinity purified anti-Psc antibody) went overnight at 4°C and then 1 hour at room temperature. The secondary antibody incubation went for 3-4 hours at room temperature. The DAB step was limited to 1-2 minutes. This signal was enhanced using the silver amplification kit (Amersham) following their protocol. The silver reaction was monitored under the microscope. The slides were stained with Giemsa (1:20) and mounted in 99.5% glycerol for storage and water for examination.

RESULTS

Maternal rescue and the *Psc* null phenotype

To assess the consequences for embryogenesis of a complete lack of *Psc* function, we used mitotic recombination to generate homozygous *Psc* mutant clones in the germ line of *Psc*/*Fs(2)D* females (see Methods). Clones were made for the putative null mutation $Psc^{Arp.1}$ and the hypomorphic allele Psc^{1445} (Lasko and Pardue, 1988; Brunk et al., 1991a). Germ-line-clone-containing females were crossed to males of four different genotypes (vg^{62}/*CyO*, $Psc^{Arp.1}$/

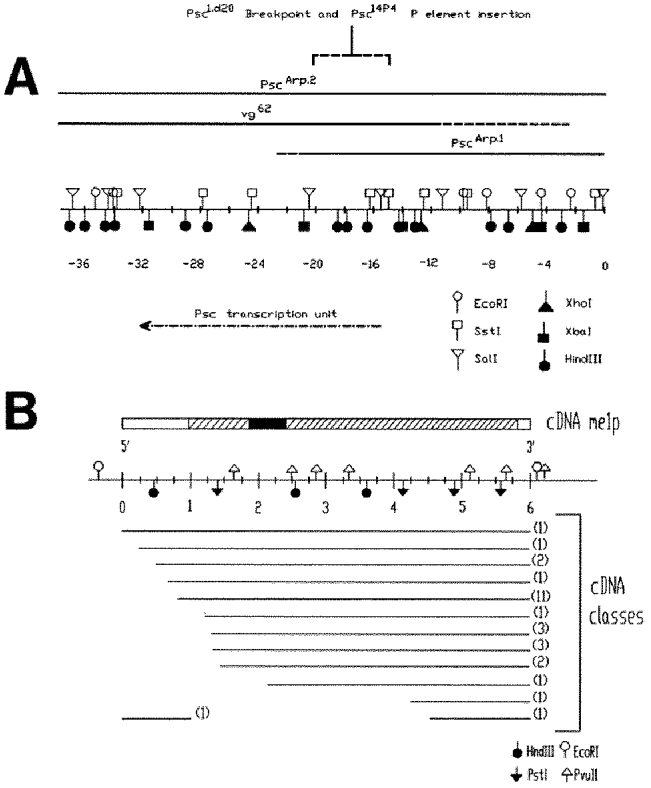

Fig. 1. (A) Molecular map of the *Psc* region. The restriction map was taken from Brunk et al. (1991a). The *Psc* transcription unit represents the minimal primary transcript. Deletions are indicated by lines above the restriction map with dashed lines indicating the interval to which the deletion endpoints have been mapped (Brunk et al., 1991a). Deletions that extend beyond the cloned DNA are shown ending at the end of the restriction map. The fragment to which the breakpoint, $Psc^{1.d20}$, and the P element insertion, Psc^{14P4}, map is indicated by a dashed horizontal line. (B) Summary of the *Psc* molecular data. The restriction map of the longest cDNA, me1p, is indicated in the center of the figure. Above the restriction map is a schematic of the sequence data for me1p. The open boxes code for untranslated regions. The hatched box represents the protein coding region of the *Psc* gene with the solid box indicating the location of the HR, the region of high homology with the neighboring *Su(z)2* gene (Brunk et al., 1991b). The lines below the restriction map indicate the *Psc* cDNA classes. We isolated 29 cDNAs that fall into minimally 13 classes. The classes were determined predominantly by restriction mapping of the cDNAs using the 6 cutters indicated, as well as the 4 cutter *Rsa*I. Limited sequence data was obtained to distinguish the endpoints of some cDNAs. The numbers in parentheses indicate the number of clones isolated for each class.

CyO, $Psc^{Arp.2}$/*CyO*, Psc^{1445}/*CyO*, see Table 1, Fig. 1A). Both vg^{62} and $Psc^{Arp.2}$ are cytologically visible deletions that delete the *Psc* gene (Brunk et al., 1991a).

The phenotypes scored ranged from embryos that made little or no cuticle to those that hatched, appeared normal, and grew to adulthood. We have placed the embryos into five phenotypic classes. Class 1 embryos showed the most severe phenotypes with some arresting prior to secreting cuticle. Most class 1 embryos were largely covered by a thin, relatively uniform cuticle with the only distinctive structures coming from the posterior end of a normal embryo. We typically found evidence of posterior spiracle differentiation, most notably Filzkörper material. Occasionally, we also found anal sensilla and Fell hairs. These pigmented hairs are found at the dorsal base of the tail. These three structures were found at both normal and ectopic locations (Fig. 2A,B). The tuft of ventral setae posterior to the eighth abdominal segment (A8) also often appeared well differentiated (Fig. 2C). In contrast to the structures mentioned above, this was not seen at ectopic locations. It is notable that the three structures found at ectopic locations are derived from A8 while the tuft is derived from the unsegmented telson (Jürgens, 1987). Thus, the ectopic structures presumably represent transformations of more anterior segments to an A8 fate. The cuticle in other body regions showed evidence of segmentation

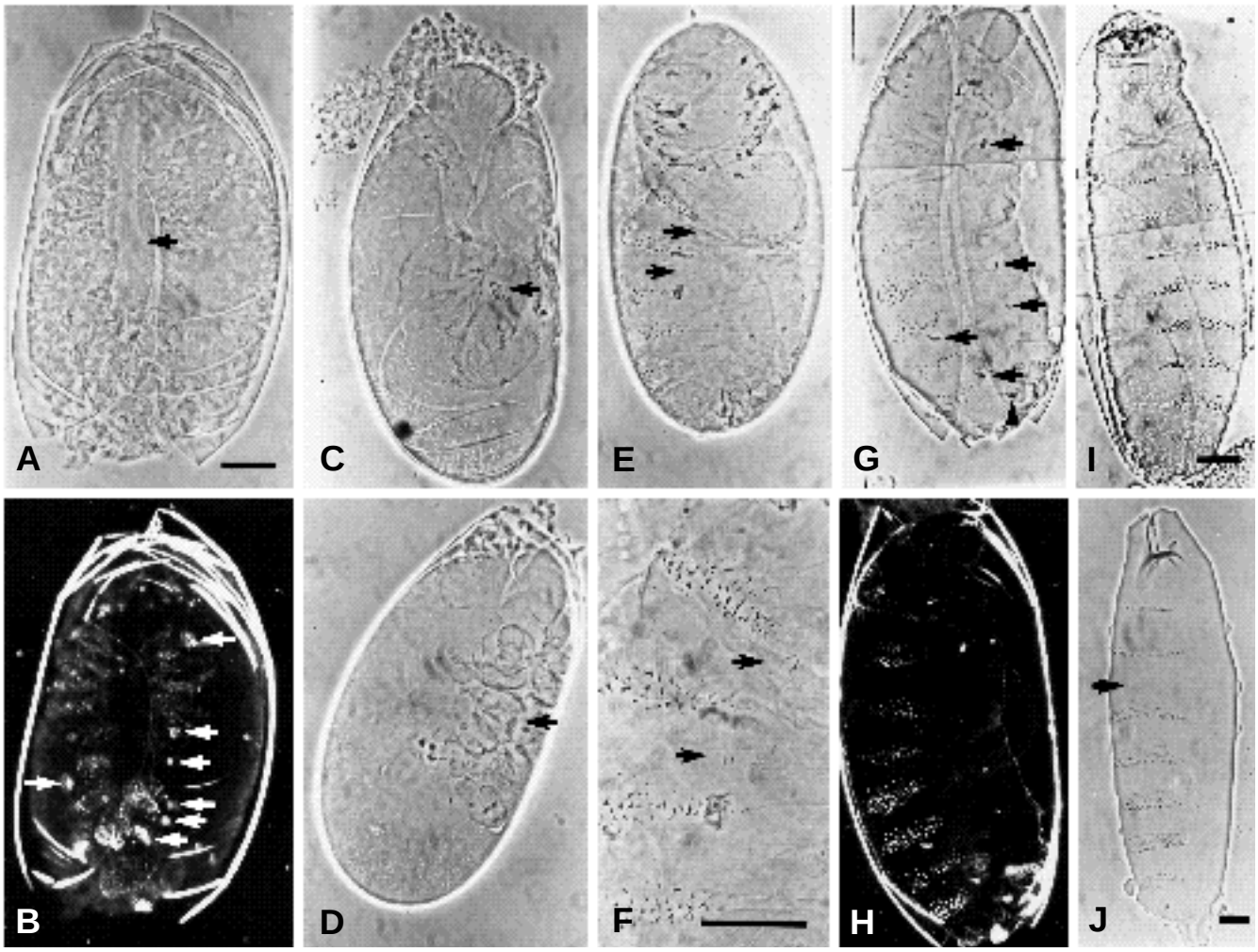

Fig. 2. Phenotypes of embryos generated from mothers with *Psc* mutant maternal germ lines. (A) Cuticle from a class 1 germ line clone embryo. Note the dorsal midline gap (arrow). (B) Dark-field of the same embryo as in A showing the presence of ectopic Filzkörper material (arrows). (C) Cuticle from a class 1 embryo showing the well-differentiated A9 setae tuft (arrow). (D) Cuticle from a class 1 embryo. Note the dorsal midline gap (arrow). (E) Cuticle from a class 2 embryo showing the twisted phenotype. The arrows indicate the presence of ectopic Fell hairs. (F) A higher magnification of the region of the embryo in E that has the ectopic Fell hairs (arrows). (G) Cuticle from a class 2 embryo showing the transformation of more anterior segments toward the A8 segment. Class 2 embryos often show ectopic Filzkörper in more anterior locations (arrows). One normal Filzkörper is at the posterior end of the embryo in the focal plane (arrowhead). (H) Dark-field image of the same embryo as in G showing the dramatic transformation of ventral setae belts toward the A8 ventral setae belt. Note the more rectangular shape of the setae belts. (I) Cuticle from a class 3 embryo showing a characteristic zygotic *Psc* phenotype. The most obvious phenotype is the head defect, a consequence of improper head involution. In addition, there is some midline erosion of the ventral setae belts. (J) Cuticle from a class 4 larva that developed nearly normally but which exhibits loss of the third abdominal setae belt (arrow). Bar represents 50 μm.

(folds), but usually no bands of ventral setae. There typically was a gap or discontinuity at the dorsal midline and the embryos were often 'curled' up (Fig. 2A,B,D). Examination of small populations of both living and fixed embryos at early developmental stages revealed no evidence for abnormalities prior to early germband retraction. Soon after that stage very abnormal embryos were seen. While further studies will be needed to identify completely the abnormalities, it appeared that there was a failure of dorsal closure, a gross failure in head involution and, sometimes, a failure to complete germband retraction. Although we did not see any morphological abnormalities prior to germband retraction, we did find evidence for subtle abnormalities. In several immunostaining experiments, precellular blastoderm embryos derived from *Psc*$^{Arp.1}$ germ line clones swelled during processing (Fig. 8K). This was never seen with embryos derived from other types of mothers. Embryos derived from mothers carrying a *Psc*$^{Arp.1}$ germ line clone also appeared more fragile than typical embryos at the germband extension and retraction stages. Many of these fell apart during standard processing procedures that did not effect wild-type embryos. These observations suggest abnormalities in structural components.

Class 2 embryos elaborated substantial well differentiated cuticle, but they were often twisted, and the abnormalities in ventral setae belt shape and head morphology were more severe than in *Psc* zygotic embryos (Fig. 2E,G,H). The ventral setae belts appeared more rectangular in shape (Fig. 2G,H) suggesting posteriorly directed transformations toward A8 differentiation. Ectopic Filzkörper material was found providing further evidence of posteriorly directed transformations (Fig. 2G,H). This was typically seen in A7, in A6, and, occasionally, in more anterior segments. The presence of Filzkörper material at ectopic locations has also been reported for several other *Pc*-G genes (Struhl, 1983; Lawrence et al., 1983; Ingham, 1984; Dura et al., 1988). We also saw evidence for ectopic anal sensilla and ectopic Fell hairs in a variety of segments. The ectopic Fell hairs were occasionally in ventral or ventrolateral locations indicating a ventral toward dorsal transformation (Fig. 2F). Finally, there were frequently holes in the cuticle, suggesting either cell death or transformation of epidermal cells to an alternative fate. Some class 1 embryos showed small regions of cuticle typical of a class 2 embryo.

Class 3 embryos spanned the range of phenotypes seen for *Psc* zygotic embryos (Fig. 2I; Jürgens, 1985; Adler et al., 1989, 1991). The shape of the ventral setae belts was relatively normal and no ectopic Filzkörper material or Fell hairs were present. Head defects were the most obvious phenotype, ranging from slight to dramatic failure of head involution. Class 4 embryos were characterized by minor segmental defects, but otherwise they appeared normal and often hatched. Most often there was a partial or complete loss of the third abdominal setae belt (Fig. 2J). The class 5 embryos appeared normal with most giving rise to fertile adults.

Females with *Psc*$^{Arp.1}$ (null allele) homozygous germ line clones were crossed to males carrying either *Psc* null or hypomorphic mutations. The phenotypes of the resulting embryos were very similar when the germ-line-clone-containing females were crossed to males carrying any of three *Psc* null mutations (*Psc*$^{Arp.1}$/*CyO*, *Psc*$^{Arp.2}$/*CyO*, or *vg62*/*CyO*; Table 2). Approximately half the embryos fell into class 1 or 2, with most being in class 1. We suggest that these embryos carried a paternally derived mutant *Psc* gene, and hence lacked both maternal and zygotic *Psc* function. The remainder of the embryos fell into classes 3, 4 and 5, with the majority being class 5. We suggest that these embryos carried a paternally derived wild-type *Psc* gene (on the *CyO* chromosome), which rescued the lack of *Psc* maternal function. Indeed, all of the class 5 embryos that developed into adults have carried the *Psc*$^{+}$ (*CyO*) chromosome. When females carrying the *Psc*$^{Arp.1}$ homozygous germ line clone were crossed to males carrying the hypomorphic *Psc*^{1445} allele, the phenotypic distribution was shifted to the less severe classes of embryos (Table 2). While about half the embryos remained in class 1 and 2 most now fell into class 2 instead of class 1.

Females with *Psc*^{1445} (hypomorphic allele) homozygous germ line clones were crossed to *Psc*$^{Arp.1}$/*CyO* and *Psc*^{1445}/*CyO* males. The smaller number of progeny obtained in these experiments reflected the smaller scale of the experiments and not that this genotype gave germ line clones at a lower frequency or that the females carrying such clones had lower fecundity. The phenotypes of embryos from these crosses were less severe than those

Table 2. Summary of phenotypic distribution of germ-line-clone-derived embryos

Cross to generate mutant embryos	Class 1	2	3	4	5
Psc$^{Arp.1}$/*FS(2)D* × *vg62*/*CyO*	48 (39%)	18 (15%)	18 (15%)	11 (9%)	27 (22%)
**Psc*$^{Arp.1}$/*FS(2)D* × *Psc*$^{Arp.2}$/*CyO*	28 (37%)	4 (5%)	8 (11%)	10 (13%)	25 (33%)
**Psc*$^{Arp.1}$/*FS(2)D* × *Psc*$^{Arp.2}$/*CyO*	84 (41%)	10 (5%)	38 (19%)	3 (1%)	70 (34%)
Psc$^{Arp.1}$/*FS(2)D* × *Psc*$^{Arp.1}$/*CyO*	24 (39%)	5 (8%)	9 (15%)	2 (3%)	21 (34%)
Psc$^{Arp.1}$/*FS(2)D* × *Psc*^{1445}/*CyO*1	9 (16%)	23 (41%)	1 (2%)	12 (21%)	11 (20%)
Psc^{1445}/*FS(2)D* × *Psc*$^{Arp.1}$/*CyO*	0 (0%)	7 (41%)	1 (6%)	0 (0%)	9 (53%)
Psc^{1445}/*FS(2)D* × *Psc*^{1445}/*CyO*	0 (0%)	12 (67%)	1 (6%)	0 (0%)	5 (28%)

*These are two independent experiments.

Germ-line-clone-containing females of two different genotypic classes were generated (*Psc*$^{Arp.1}$/*Fs(2)D* and *Psc*^{1445}/*Fs(2)D* females). The embryos were classified into five phenotypic classes (see results). The number of embryos in each class is given. In addition, the percentage that this represents is given in parentheses. Class 1 and 2 embryos were derived from a mutant maternal germ line and were presumably homozygous mutant. We consider class 1 embryos to represent the *Psc* null phenotype. We used chi square analysis (Graphpad Instat statistical program) to determine whether the number of null embryos generated is dependant on the paternal genotype. Chi square analysis indicates that there was no significant difference in the proportion of class 1 embryos from *Psc*$^{Arp.1}$/*Fs(2)D* germ line clone females crossed to males carrying any of the three *Psc* deletions [*vg62*/*CyO*, *Psc*$^{Arp.2}$/*CyO*, or *Psc*$^{Arp.1}$/*CyO* (P=0.955)]. On the contrary, when *Fs(2)D*/*Psc*$^{Arp.1}$ germ line clone females were crossed to males carrying the hypomorphic *Psc* allele, *Psc*^{1445}, there was a significant shift in the distribution of embryos. There were proportionately fewer class 1 embryos (P=0.015). The distributions for embryos derived from *Psc*^{1445} germ line clones could not be examined statistically because the samples were too small.

derived from mothers with $Psc^{Arp.1}$ homozygous germ line clones (Table 2). None of the embryos fell into class 1. About half the embryos fell into class 2. These embryos likely carried a paternally derived mutant *Psc* gene. Most of the remaining embryos fell into class 5 and were presumably rescued by a paternally derived Psc^{+} gene (on the *CyO* chromosome). Indeed, all embryos that reached adulthood carried the *CyO* chromosome.

Abd-B protein is misexpressed in *Psc* germ line clone embryos

Abd-B is the predominant homeotic gene expressed in the A8 segment and it is required for the development of this segment (Lewis, 1978; Struhl and White, 1985; Busturia and Morata, 1988; Celniker et al., 1989; Delorenzi and Bienz, 1990). Since mutant embryos derived from a mutant maternal germ line (class 1 and 2) show an obvious transformation of more anterior segments toward eighth abdominal segment differentiation, we examined the expression pattern of the *Abd-B* protein in these embryos. We did not see any embryos with grossly abnormal accumulation of *Abd-B* protein until mid-germband retraction. At this time about half the embryos showed a dramatic anterior expansion of the region where *Abd-B* protein was found (Fig. 3A,B), consistent with the segmental transformations seen in cuticle preparations of similar embryos. Since the number of embryos examined was small, we cannot be confident that *Abd-B* expression is not altered at earlier stages.

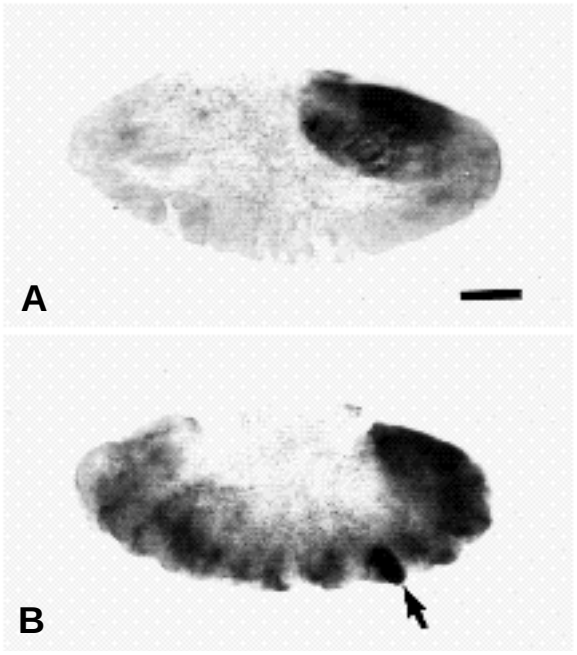

Fig. 3. Abd-B antibody staining of *Psc* germ line clone embryos. (A) An embryo derived from a mother with a mutant germ line showing relatively normal Abd-B protein distribution. This could be a paternally rescued embryo, or it could be a *Psc* homozygous mutant embryo not yet showing derepression of the *Abd-B* gene. (B) An embryo derived from a mother with a mutant germ line showing mis-expression of the Abd-B protein. It is now expressed in all segments. The dark-staining spot marked by an arrow is a piece of dirt and does not represent a high local staining for Abd-B protein. Bar represents 50 μm.

Characterization of Psc cDNA clones

The *Psc* region was previously cloned (Brunk and Adler, 1990) and DNA alterations associated with two *Psc* mutations ($Psc^{1.d20}$ and Psc^{14P4}) mapped to a restriction fragment at −16 to −20 Kb on the genomic map (Fig. 1A; Brunk et al., 1991a). Using DNA from this region as a probe, we isolated four independent cDNAs, the longest being 6 kb (called me1p). Using fragments from me1p as a probe, we subsequently isolated 25 additional clones. Restriction mapping of these clones indicated that they represented a minimum of 13 independent clones, all but one having the same 3′ end (Fig. 1B). The one unusual clone contained only sequences from the 5′ most kb of me1p. Its 3′ end started at an oligo(A) run that corresponded to an AAAAA sequence present in me1p. This atypical clone presumably arose from internal priming during reverse transcription. Based on restriction mapping, all of the other cDNA clones appeared to be derived from the same mRNA (Fig. 1B). Several of these clones were shown by sequence analysis to have the same 3′ end that corresponded to a genomically encoded oligo(A) stretch. Thus, these clones also appear to have resulted from internal priming during reverse transcription. The 5′ most fragment of cDNA me1p mapped to the interval −16 to −20 on the genomic map (Fig. 1A) and the 3′ most fragment mapped to the interval −28 to −32 on the genomic map (Fig. 1A) indicating that *Psc* is transcribed from distal to proximal on the chromosome.

Temporal expression pattern of *Psc* mRNA

On northern blots, we found two bands of hybridization to our *Psc* cDNA clones. These two large messages (6.3 and 6.7 kb) were found throughout development, being most abundant in 12-24 hour embryos and least abundant in third instar larvae (Fig. 4). In an attempt to determine what the difference was between the two *Psc* mRNAs, we probed northern blots with small probes derived from different regions of me1p (Fig. 1B). Probes derived from all regions of me1p hybridized to both bands (data not shown).

Spatial expression pattern of *Psc* mRNA

The spatial expression of *Psc* was examined by in situ hybridization to frozen tissue sections. In situ analysis of third instar larvae showed that *Psc* mRNA was found in the CNS (Fig. 5A,B), being most abundant in the brain and present at a slightly lower level in the ventral nerve cord. *Psc* mRNA was also found at a lower level in all regions of all of the imaginal discs (Fig. 5A,B). *Psc* mRNA was found localized in the brain, epidermis and nerve cord in pupae 24 hours after white prepupae formation. In adult females, *Psc* RNA was primarily localized in the ovaries (Fig. 5C,D), where it accumulated in developing oocytes.

Two *Psc* proteins are found on western blots

We raised both polyclonal and monoclonal antibodies against a Psc fusion protein (see Methods). On western blots, we primarily used a pool of two monoclonal antibodies. This reagent recognized two proteins in all developmental stages although the relative intensity of the bands

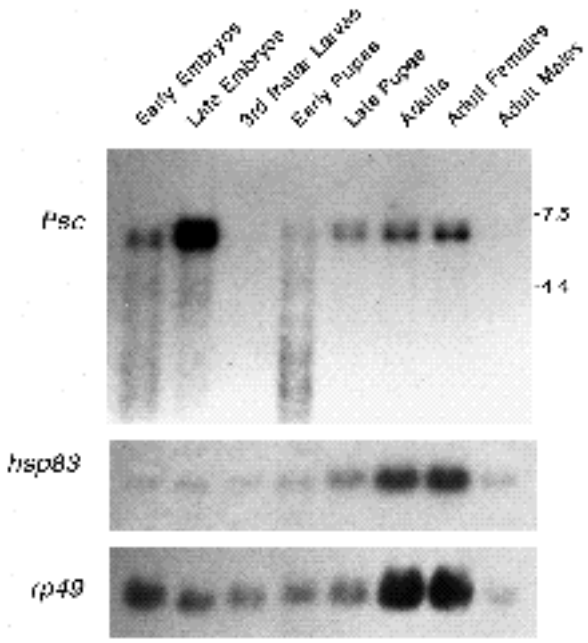

Fig. 4. *Psc* northern analyses. A developmental northern showing that *Psc* codes for two messages (6.7 and 6.3 kb) and that the mRNA is most abundant in late embryos (12-24 hour) and least abundant in third instar larvae (a very faint hybridization signal can be seen on the original film). The blot probed for *Psc* was additionally probed for *hsp83* and *rp49*, two constitutive genes, to determine relative loading of mRNA in each lane.

was not always consistent. This may reflect slight variations in developmental stage or physiological state. In the early embryo, the larger protein was consistently more abundant (Fig. 6A,B). The cDNA (me1p) sequence of *Psc* predicts a molecular mass of 170,000 for the Psc protein (Brunk et al., 1991b; van Lohuizen et al., 1991). The estimated sizes from the western blot analysis are 190,000 and 180,000 which is slightly higher than predicted. This difference could reflect post-translational modification or the proteins migrating aberrantly due to an unusual amino acid content. Psc protein induced in embryos or larvae from a heat-shock promoter-*Psc* cDNA transgene (whose coding region was derived from me1p) co-migrated with the larger of the two endogenous protein bands (Fig. 6C). As a control for specificity, we compared extracts of embryos heterozygous for two *Psc* deletions [$Psc^{Arp.1}/Su(z)2^{1.b8}$ mutant embryos derived from crossing $Psc^{Arp.1}/CyO$ females and $Su(z)2^{1.b8}$/+ males (see Table 1)] with a pool of their recently hatched wild-type and heterozygous siblings. This developmental stage was chosen because we expected that by this time most if not all of the Psc protein encoded by maternal mRNA would have turned over. A dramatic reduction in the intensity of staining of both bands was seen in the mutant extract (Fig. 7A,B; lanes 1,2). Only limited western blot experiments were done with our polyclonal antibody, but these gave results consistent with those obtained with the monoclonal antibodies.

Psc mutations alter the size or abundance of the *Psc* proteins

Besides examining extracts of embryos heterozygous for two *Psc* deletions (see above), we also examined by western blot analysis protein extracts made from embryos or pupae homozygous for three other *Psc* mutations. Two of these alleles (Psc^1 and Psc^{1445}) result in death as late embryos with a distinctive head defect (Jürgens, 1985; Adler et al., 1989), which we used to identify homozygous late embryos. Psc^1 is a complex EMS-induced allele (Jürgens, 1985), which for different phenotypes is antimorphic, null or hypomorphic (Adler et al., 1989). Extracts of Psc^1 homozygotes show a loss of the two normal Psc proteins recognized by our monoclonal antibodies and their replacement by two proteins of 74,000 and 66,000 (Fig. 7A; lanes 4,5). These two bands are actually each doublets. These proteins appeared more abundant than the wild-type Psc proteins, but it is possible that this is simply a consequence

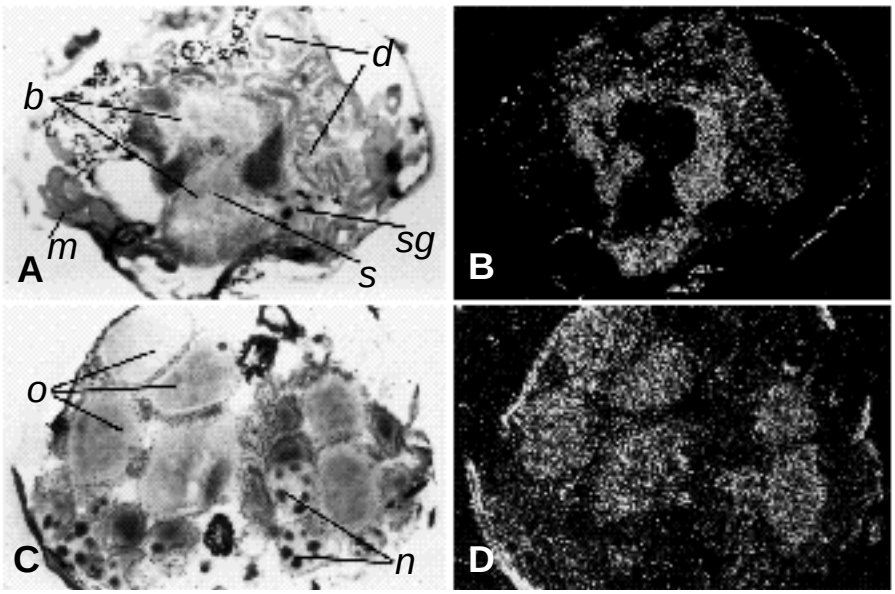

Fig. 5. *Psc* in situ hybridization analysis. (A) Bright-field and (B) dark-field image of an anterior cross section through a third instar larva showing *Psc* RNA expression in the brain (b) and imaginal discs (d). There is no RNA localization in the muscles (m), subesophogeal ganglion (s) or salivary glands (sg). (C) Bright-field and (D) dark-field images of a cross section through the abdomen of an Oregon R adult female showing the localization of the *Psc* RNA in the developing oocytes (o) and the nurse cells (n).

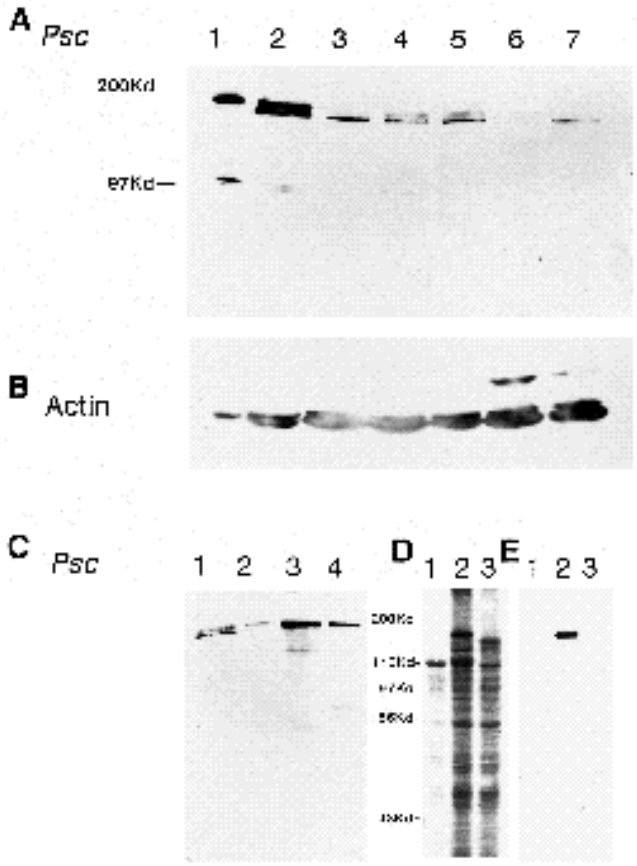

Fig. 6. Developmental western analysis of Psc protein expression. (A) A developmental western incubated with a pool of two monoclonal antibodies (6E8 and 1F4) and (B) the same western blot incubated later with an anti-actin monoclonal antibody (Amersham) as a control for protein loading. The protein extracts loaded in each lane in A and B are: lane 1, 0-4 hour embryos; lane 2, 12-24 hour embryos; lane 3, third instar larvae; lane 4, 0-24 hour pupae; lane 5, 0-96 hour pupae; lane 6, adult females; lane 7, adult males. We have seen protein in adult females in another experiment. The smaller bands seen in the embryo samples are due to cross reaction with the secondary antibody. In B, lanes 6 and 7 a protein larger than actin was detected. The identity of this protein is not known, and this presumed cross reaction was not seen in other experiments where adult samples were run. (C) western of heat-shock-induced proteins from a heat-shock promoter-*Psc* cDNA transgene. Lane 1 contains a protein extract from 12-24 hour OreR embryos. Lane 2 contains a protein extract from 0-4 hour embryos carring the *Psc* transgene subjected to a 1 hour heat shock with no recovery. Lane 3 contains a protein extract from 12-24 hour embryos carrying the *Psc* transgene subjected to a 1 hour heat shock with no recovery. Lane 4 contains a protein extract from third instar larvae carrying the *Psc* transgene subjected to 30 minute heat shock and 1 hour recovery. These samples were not adjusted for protein concentration. (D) Coomassie and (E) western analysis of fusion protein samples to test specificity of the monoclonal antibody 6E8 (see Methods). D and E were independent experiments with 1/5 to 1/10 of the proteins in D being loaded in E. Lane 1 contains protein extract from induced bacteria containing the pUR278 vector. Lane 2 contains protein extract from induced bacteria containing the pUR278-PscHR fusion protein construct. Lane 3 contains protein extract from induced bacteria containing the pUR278-Su(z)2HR fusion protein construct. This western demonstrated the specificity of this monoclonal antibody for the Psc fusion protein. We obtained similar results for the monoclonal 1F4 and the polyclonal antibody.

of more efficient transfer to nitrocellulose for the smaller proteins. Extracts made from embryos homozygous for the strong hypomorphic EMS-induced allele Psc^{1445} contained dramatically reduced amounts of wild-type-sized proteins (Fig. 7A,B; lanes 3,4). Weak hypomorphic *Psc* alleles frequently survive to form pupae. We examined extracts made from pupae homozygous for the EMS-induced hypomorphic allele, Psc^{1433} (Fig. 7A,B; lanes 6,7). We found that extracts of Psc^{1433} larvae and pupae had lost the wild-type Psc protein, and instead a smaller protein of 145,000 was recognized by our monoclonal antibodies. Again, the mutant protein was more abundant than wild type. The significance of this is unclear.

Antibody staining of tissues

We used our polyclonal anti-Psc antibody to examine the tissue distribution and subcellular localization of the Psc protein. In all tissues where detected, the Psc protein is nuclear. As a control for the specificity of the antibody, we have stained 10-14 hour embryos derived from crossing $Psc^{Arp.1}/CyO$ females and $Su(z)2^{1.b8}/+$ males (and vice

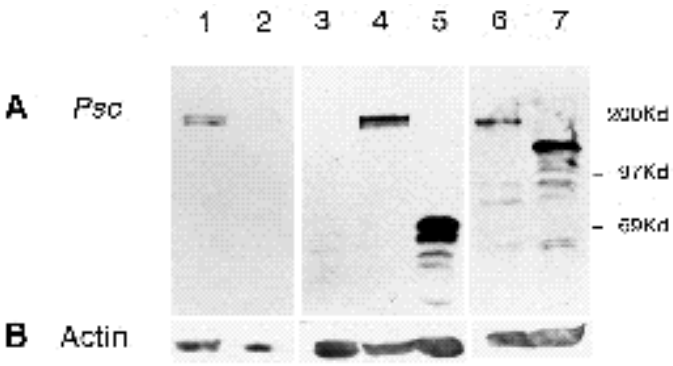

Fig. 7. Western analysis of *Psc* mutant extracts. (A) Westerns incubated with Psc monoclonals (6E8 and 1F4) and (B) the same westerns incubated with the anti-actin monoclonal antibody as a control for protein loading. Lanes 1/2, lanes 3/4/5 and lanes 6/7 are derived from three independent western blots. Lane 1 contains a protein extract from the hatched siblings and lane 2 contains a protein extract of mutant embryos heterozygous for two *Psc* deletions ($Psc^{Arp.1}/Su(z)2^{1.b8}$). Lane 3 contains a protein extract from Psc^{1445}/Psc^{1445} mutant embryos and lane 4 contains a protein extract from OreR 15-18 hour embryos. On overexposed blots containing protein from $Psc^{Arp.1}/Su(z)2^{1.b8}$ (lane 2) or Psc^{1445} (lane 3) embryos, we see what appear to be the two Psc protein bands, although it is hard to be certain that the signals are not due to non-specific bacground. These signals are indistinguishable, which is somewhat surprising since $Psc^{Arp.1}$ is a null mutation and Psc^{1445} is a strong hypomorphic mutation (see Methods). Lane 5 contains a protein extract from Psc^{1}/Psc^{1} mutant embryos. We see the loss of the wild-type bands in lane 4 and the appearance of two smaller bands (74,000 and 66,000). These two bands are actually doublets. The light, smaller bands in lane 5 are presumably degradation products of the mutant proteins. Lane 6 contains a protein extract from 0-48 hour OreR pupae and lane 7 contains a protein extract from 0-48 hour Psc^{1433}/Psc^{1433} mutant pupae. We see the loss of the wild-type-sized Psc protein and the appearance of a smaller protein (145,000).

versa). One quarter of the progeny of this cross will lack a functional *Psc* gene and, by these developmental stages, we expected that Psc protein translated from the maternally derived *Psc* mRNA should be greatly reduced in abundance. Approximately one quarter of the embryos in such preparations showed little or no staining (Fig. 8J). This demonstrated the specificity of the antibody.

We found nuclear staining of Psc as early as the 16 nuclei stage. During nuclear multiplication, the protein is present in nuclei in all regions of the embryo (Fig. 8A,B) and it remains associated with condensed chromosomes during mitosis (Fig. 8F). There is a dramatic decrease in the amount of protein in nuclei during cellularization (Fig. 9A). Staining is strong and relatively even across all syncitial blastoderm nuclei (Fig. 9B). In the embryo shown, the cell membranes have not yet begun to grow in (Fig. 9C). As

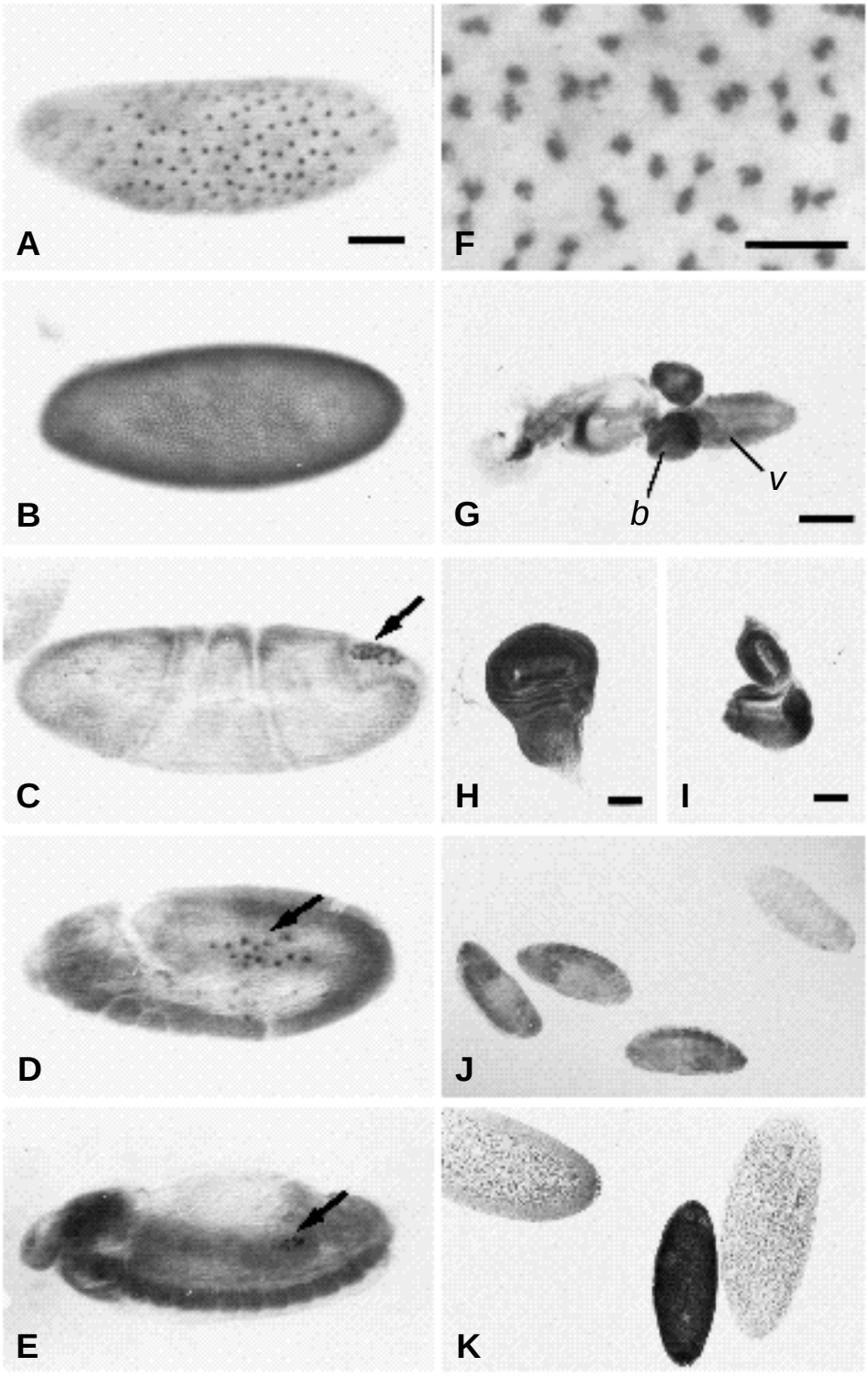

Fig. 8. Developmental expression of the Psc protein. For A-E anterior is to the left. (A) A late nuclear multiplication stage embryo. Psc protein is present in all somatic nuclei. (B) A cellular blastoderm embryo. Psc protein level is still high in all nuclei. It is present in pole cells at the posterior end (not in focal plane). (C) A gastrulating embryo. Psc protein level has decreased in somatic cells but remains high in the pole cells (arrow). (D) A germband-extended embryo. Psc protein level remains high in pole cells which are being internalized (arrow). (E) A stage 14 embryo. Psc protein is present at a higher level in the embryonic CNS with continued staining of the pole cells (arrow). (F) A nuclear multiplication stage embryo showing that the Psc protein remains associated with condensed chromosomes during mitosis. (G) A dissected 1st instar larva shows staining predominately in the CNS [higher in the brain (b) than the ventral ganglia (v)]. The tissue to the left of the brain is what remained of the head following dissection. (H) Wing disc and (I) eye-antennal disc from a dissected third instar larva show Psc protein localization in all nuclei. The white spot in H is a hole due to damage incurred during dissection. (J) A DIC image of a control experiment (*Su(z)2*$^{1.b8}$/+ X *Psc*$^{Arp.1}$/*CyO*) showing staining of 12-16 hour embryos. From this cross, one quarter of the embryos are expected to be homozygous mutant for *Psc*. The embryo that is unstained is presumed to be homozygous mutant for *Psc*. (K) A modulation contrast image of embryos showing the severe swelling that we often saw when staining embryos derived from the mutant germ line clone. The darkly staining embryo is an OreR control showing Psc staining. The two light embryos are derived from the germ line clone and show little Psc staining which is presumably background staining. Bars represents 50 μm except in F where the bar represents 25 μm.

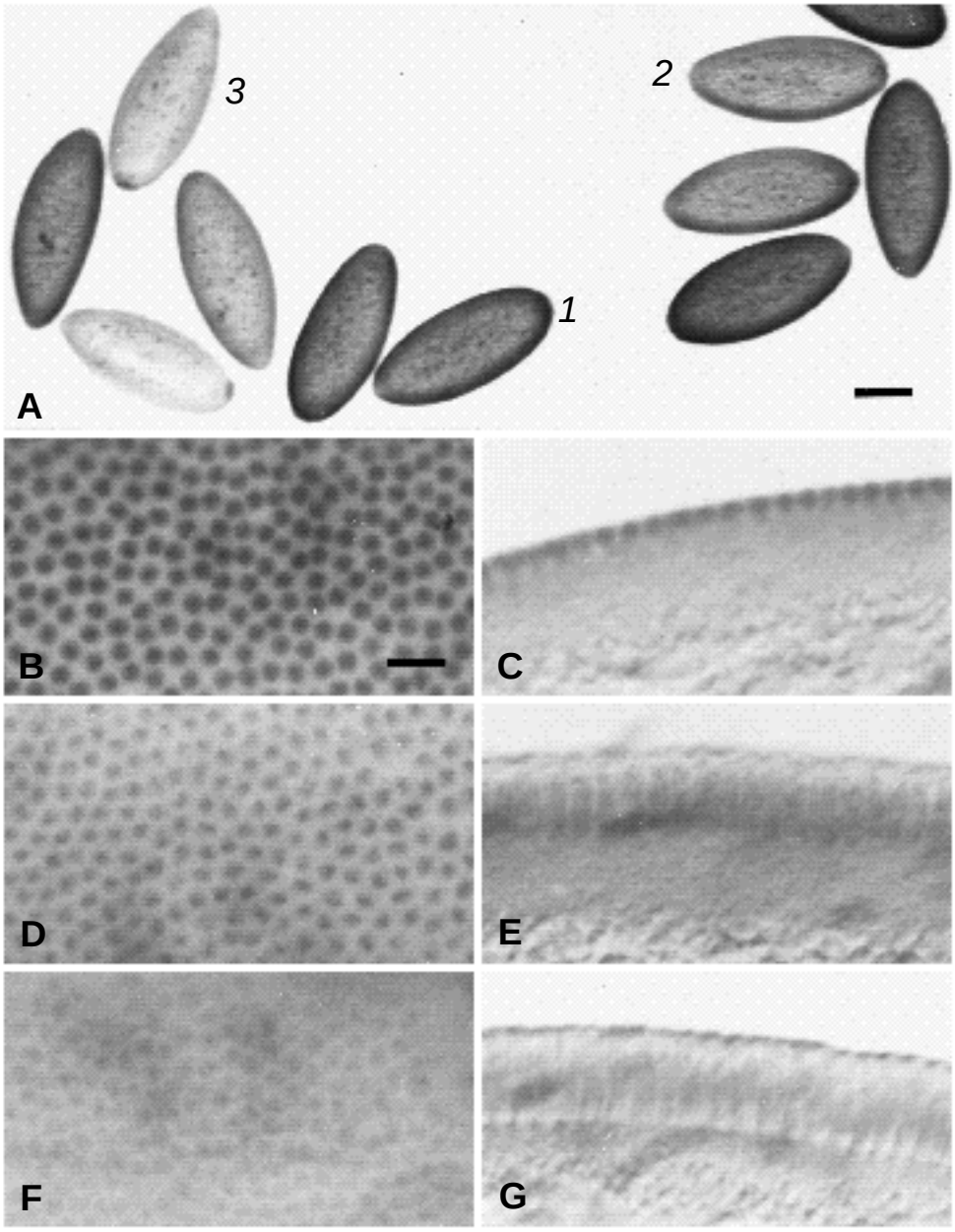

Fig. 9. Rapid decrease in Psc protein expression during cellularization. (A) A bright-field image showing embryos at various stages of cellularization. Embryo 1 shows a high level of Psc antibody staining prior to cellularization. Embryo 2 is beginning to undergo cellularizaton and shows an intermediate level of Psc protein expression. Embryo 3 has finished cellularization and shows a very low level of Psc protein expression, except in the pole cells where it remains high. (B-G) These are higher magnification images showing Psc staining levels as cellularization proceeds. (B) A higher magnification bright-field image of embryo 1 showing the Psc staining level and (C) a modulation contrast image of this embryo showing that the cell membranes have not yet begun to grow in when Psc protein expression is greatest. (D) A higher magnification bright-field image of embryo 2 showing Psc protein level and (E) a modulation contrast image of same embryo showing that Psc antibody staining decreases as membranes start to form. (F) A higher magnification bright-field image of embryo 3 showing a very low level of Psc staining and (G) a modulation contrast image of the same embryo showing that cellularization is complete. In A, bar represents 100 μm, in B 10 μm.

the inward growth of the cell membranes begins (Fig. 9E), the level of staining decreases (Fig. 9D) and the nuclear staining appears less uniform across individual nuclei. By the completion of cellularization (Fig. 9G), staining is greatly reduced (Fig. 9F). During gastrulation, the level of Psc staining remained low but significant (Fig. 8C,D). Later in embryonic development, the level of staining increased again being strongest in the brain and ventral ganglia (Fig. 8E). Besides staining somatic cell nuclei, our antibody also stained pole cell nuclei darkly from the time they budded off through their migration to the gonad during mid-embryogenesis (Fig. 8C-E).

We continued to see Psc antibody staining in many cell types during postembryonic development. There was strong staining of the brain in first instar larvae (Fig. 8G) and slightly weaker staining in the ventral ganglia at this time. In third instar larvae, the Psc protein was found in nuclei in all regions of all imaginal discs (Fig. 8H,I). The staining of the CNS was erratic, being strongest in regions where the tissue was damaged during dissection. We suspect that this was due to a failure of the ABC reagent to penetrate the intact CNS. Besides the diploid larval cells that our in situ hybridization experiments predicted would contain the Psc protein, a variety of larval polytene cell nuclei also stained, including salivary gland nuclei described below. Ovaries showed no significant staining, suggesting that maternally derived mRNA but not protein is deposited in the oocyte.

The Psc protein is chromatin associated

We immunostained salivary gland polytene chromosomes (Fig. 10) and found that the Psc protein is present at about

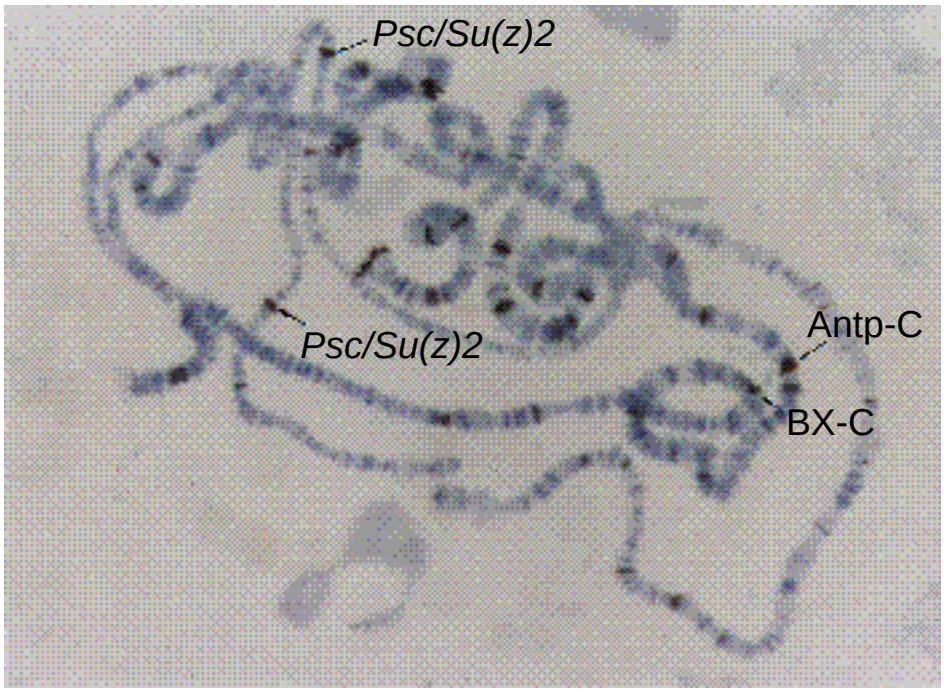

Fig. 10. Immunolocalization of Psc protein to specific loci on salivary gland chromosomes. The Psc antibody recognized about 45 loci on salivary gland polytene chromosomes. One of the strongest sites is the *Psc/Su(z)2* region itself. In this preparation, 2R has become asynapsed. Other strong sites include the BX-C and Antp-C, predicted targets of *Psc.* A summary of the sites is given in Table 3.

45 discrete loci (Table 3). The sites differ greatly as to their strength of staining. The darkest stained bands in Fig. 10 were far out of the linear staining range. At early times in the staining reaction only a few (3-4) very strong sites were typically seen. As the time of staining increased, other strong staining sites were found. One of the strongest sites was 49EF, which is the location of the *Psc* gene. Also among the strong staining sites were the BX-C and Antp-C. Because the *Psc* mutant phenotype includes an anterior-to-posterior segmental transformation, these sites are predicted to be targets of the Psc protein. The weak staining sites were only detected in 'good' preparations with long staining times (≈5× longer than the strongest sites). All of the reported weak sites were seen in at least a moderate fraction of our well-stained chromosomes (>30%), thus we think these are likely to be bona fide sites for the localization of Psc protein. Almost all of the Psc binding sites are also sites where the Pc and ph proteins are found (Table 3; Zink and Paro, 1989; Zink et al., 1991; DeCamillis et al., 1992; Franke et al., 1992).

DISCUSSION

Maternal rescue and the *Pc*-G genes

Although previously believed to be a homogeneous class of genes whose function is to maintain patterns of homeotic gene expression, the *Pc*-G genes are now proving to have more varied functions. The one function common to all members is the maintenance of homeotic gene expression. Mutations in *Pc*-G genes result in an anterior-to-posterior transformation. Often these effects are quite modest in embryos mutant for one gene, but for several of these loci (*Psc*, *Sce*, *Scm*, *Asx*, *Pcl* and *l(4)29*) embryos that are mutant for two of these genes display a far more severe transformation indicating that these genes function synergistically (Jürgens, 1985; Adler et al., 1991). It had previously been shown for all of these loci except *Psc* that maternal rescue was responsible for the relatively weak zygotic phenotype (Ingham, 1984; Breen and Duncan, 1986; Dura et al., 1988; Phillips and Shearn, 1990; Jones and Gelbart, 1990). We have now shown that this is also true for *Psc.* We have further shown that *Psc* RNA accumulates in developing oocytes, although Psc protein does not, and it is likely that this maternally deposited RNA is responsible for the maternal rescue. The zygotic mutant phenotype of *Psc* is presumably due to cells progressively becoming depleted of Psc protein due to diluting out or turning over maternally derived mRNA and/or the protein translated from this RNA. This is an attractive hypothesis for the other *Pc*-G genes that also show maternal rescue. The dramatic synergism seen in double mutants would then be due to cells simultaneously becoming depleted for two of these proteins. One molecular hypothesis to explain the synergism would be that the products of these genes associate to form a complex, and that the probability of forming such a complex is proportional to the product of the concentrations of each individual protein species. Tests of such hypotheses await the development of probes for protein products of the other *Pc*-G genes that display the dramatic synergistic interactions. A similar model was proposed previously for both the *Pc*-G in general and for Suppressors and Enhancers of Position Effect Variegation (Locke et al., 1988; Tartof et al., 1989; Paro, 1990). As is discussed in more detail below, the overlap in the location of the protein products of three *Pc*-G genes in salivary gland chromosomes is consistent with some variants of these models, as is the recent finding of a protein complex that contains the Pc and ph proteins (Franke et al., 1992).

Paternal rescue of the *Psc* maternal phenotype

Besides the maternal rescue of the zygotic *Psc* phenotype,

Table 3. Summary of the sites of Psc protein localization on salivary gland chromosomes

location		*Pc* site	*ph* site
(A) Major binding sites of *Psc* protein			
4B			
5A		5A	5A
8A	*Su(Cbx)*	8A	8A
8B		{8B}	8B
14B		(14A)	14B
21A*	*Su(Pc)*	21AB	21A
25EF	*En(var)26A*	25EF	25EF
32F	*Su(var)2-9*	32EF	32EF
33F		33F	33F
35EF	*Su(var)206*		
36AC	*dl, Bic-D*	(36B)	36AC
37AB		[37A]	[37B]
		[37B]	[37B]
48A	*en,inv, En(Pc)*	48A	48A
49EF*	*Psc, Su(z)2*	49E4-7	49EF
56C		56BC	56C
61C		61C1,2	61C
69D*		69D1,2	69D
82E		82E1	82E
84AB*	*Antp-C*	84A4,5-B1,2	84AB
89E	*Bx-C*	89E	89E
90E	*Su(var)302*	90E	90E
93E		93E1-4	93E
94E	*Su(var)3-11*	[94E]	94E
100A			
100B			
(B)Minor sites (weak and variable immunostaining)			
2CD	*ph*	2D1,4	2D
7B		7B	7B
9A		{9A}	9A
16DE		[16D]	16D
17AB			17A
22A	*Su(var)326*	22A	22A
22BC		(22C)	22B
24A	*odd*	24A	24A
051A	*Asx*	51A	51A
58C			
59F		59EF	59F
60E	*Kr, gsb*	60E1	60E
65ED		65D	65D
69C		69C1	69C
70D		[70DE]	70DE
84D	*Su(var)3-4*	84D1,2	84D
84F85A	*Pox-meso*	84F1,2	84F
86C	*Su(var)3-14*	86C1,2	86C
88A	*Su(var)330*	88A1,2	88A
89A			
102C		102C1	102AC

The Psc sites are compared to those of Pc and ph. The Pc sites were reported in Zink and Paro (1989).

* refers to the strongest Psc sites. {} refers to those binding sites reported by DeCamillis et al. (1992) as being previously identified Pc targets. () refers to binding sites reported by Zink and Paro (1989) but DeCamillis et al. (1992) believes they were inaccurately mapped. [] refers to those binding sites that were reported by DeCamillis et al. (1992) to be newly identified targets of Pc. The ph binding sites were reported in DeCamillis et al. (1992).

there is paternal rescue of the *Psc* maternal phenotype. The failure to see any phenotypic consequences in those rescued embryos that reached adulthood suggests that either the zygotic *Psc* gene is expressed during the nuclear multiplication stage or that the Psc protein does not have an essential function in early embryogenesis. Preliminary results on the immunostaining of rescued embryos indicates that Psc protein is present at early times, which suggests that the paternally derived *Psc* gene can be expressed during the nuclear multiplication stage.

Two *Psc* mRNAs and proteins

A variety of molecular and genetic arguments make it very likely that the collection of cDNA clones isolated and described in this paper are products of the *Psc* gene. Extensive genetic studies have identified only the *Psc* gene in the chromosomal region shown in Fig. 1A, and two *Psc* mutations map to the 4 kb restriction fragment that encodes several exons included in me1p (unpublished). It was this restriction fragment that was used as a probe in our initial screening of cDNA libraries. Furthermore, *Psc* mutations alter the two proteins detected by antibodies directed against a fusion protein made from one of the cDNA clones.

Northern blots of poly(A)+ RNA show two bands of hybridization to *Psc* probes that differ by 400 bp in length. The presence of two bands of *Psc* RNA was not detected by van Lohuizen et al. (1991). We suspect that our electrophoresis conditions provided better resolution of these mRNAs. There appears to be only slight developmental variation in the relative abundance of the two forms. All of the cDNA clones that we isolated had restriction maps consistent with them having been derived from the same species of RNA. Considering the relative abundance of the two mRNAs and the number of cDNA clones isolated, this is surprising and suggests that the difference(s) between the two forms of the mRNA is located in a region not well represented in our cDNA collection (e.g. the 5′ or 3′ ends). We found two bands of Psc protein in our western blots. These could represent two proteins of different primary sequence, perhaps encoded by the two mRNAs detected in the northern blot experiments. Alternatively, these could be due to postranslational modification of a single protein species. Finding only one Psc protein band induced from a *hs-Psc* gene [even many hours after induction (E. Sharp and P. N. Adler, unpublished)] leads us to favor the model where the two mRNAs encode two distinct proteins. For the smaller proteins found in Psc^{1} mutants, we were able to resolve doublets. These could represent post-translational modifications of the Psc proteins that we could not resolve for the much larger wild-type proteins. Definitive conclusions regarding the structural differences between the two *Psc* mRNAs and proteins will require additional experimental approaches.

Two Psc mutations result in smaller proteins

Via western blot analysis we found that the Psc^{1} and Psc^{1433} alleles resulted in the loss of the wild-type-sized Psc proteins and the production of smaller proteins recognized by the anti-Psc monoclonal antibodies. There are several possible mechanisms to explain the smaller proteins. For example, the mutations could result in an altered full-length protein that is then cleaved to yield the smaller versions detected on the western blots. However, we think the most likely explanation is that these two mutations are nonsense mutations that result in truncated primary translation products.

The expression pattern of *Psc*

Psc protein is present at a similar concentration in somatic nuclei in all regions of the embryos until germband extension. This is not surprising since a lack of *Psc* gene activity affects development over most of the embryo. It is also consistent with the pattern for the product of a *Pc*-G gene, which is expected to be active in all segments. The higher accumulation of Psc protein in the CNS in mid and late embryos is also not surprising considering that *Psc* has been found to be required for normal CNS development in embryos (D. Smouse, C. T. Wu, N. Perrimon, personal communication).

Three unexpected results were obtained from our immunostaining of early embryos. One was the high level of Psc protein found in the pole cells throughout embryonic development. The second was that the developmental stage with the highest level of staining with the anti-Psc antibody would be nuclear multiplication, as the *Pc*-G genes do not have a known function at this stage. Furthermore, there is no obvious sign of developmental abnormalities at cellular blastoderm associated with a loss of *Psc* function. It is possible that *Psc* functions to regulate gene expression during nuclear multiplication, but that the consequences of misregulation are not obvious until later stages (e.g. mid-germband retraction). The third unexpected result is that the level of Psc staining decreases very rapidly during cellularization in the early embryo. We suspect that this represents a degradation of existing Psc protein, although we cannot rule out a change in accessibility to the antibody.

Pc-G genes encode chromosomal proteins

The Pc and ph proteins are present at identical loci on salivary gland polytene chromosomes by immunohistochemistry (Zink and Paro, 1989; Zink et al., 1991; DeCamillis et al., 1992; Franke et al., 1992). The targets of these genes include homeotic genes, several genes that suppress or enhance position-effect variegation (PEV), as well as other *Pc*-G genes. We have found the Psc protein present at about 45 loci on polytene chromosomes and almost all of the loci where the Psc protein is found are also locations of ph and Pc. Forty five sites is fewer than is seen for Pc or Ph and must be considered a minimum estimate, as we put a high priority on keeping the background staining as low as possible. It is thus possible that the true number of Psc sites is larger, but that we did not allow the staining reactions to go long enough to detect low abundance sites. If so, then perhaps all Pc and Ph protein sites also contain Psc. Finding that essentially all of the Psc binding sites are also Pc and ph sites is in some ways paradoxical, as the *Psc* mutant phenotype is quite different from and more extensive than that of *Pc*. The only phenotypes associated with *Pc* mutations in embryos are segmental transformations (Lawrence et al., 1983). Thus one would expect that there are genes (and perhaps cells) that require *Psc* but not *Pc* function for normal regulation. There are several possible explanations for the apparent paradox that the Pc protein is found at all sites where the Psc protein is located, although *Psc* likely has targets that *Pc* does not. One is that the salivary gland is unusual. While *Psc* has functions that do not overlap those of *Pc* in some tissues and developmental stages, this may not be the case in the salivary gland cells. Alternatively, the assay may be misleading us. The presence of one or more of the *Pc*-G proteins at some loci may not be of functional significance. Neither of these explanations are however, particularly satisfying. A similar set of explanations have been proposed to explain the similarly paradoxical association of the ph and Pc proteins (Franke et al., 1992). It is, however, hard to ignore the striking coincidence in chromosomal locations where the three proteins are found. This is consistent with the possibility that the Psc protein is part of the recently described complex that contains the Pc and ph proteins (Franke et al., 1992). However, the resolution of the immunostaining technique is perhaps only 50-100 kb. Finer mapping of the binding sites for Psc and their correlation with the binding sites for Pc and ph will require examining transformed segments of DNA as has been done for Pc and ph (Zink et al., 1991; DeCamillis et al., 1992). The *Pc*-G proteins could directly bind DNA or be involved in protein-protein or protein-nuclear scaffolding interactions. The Pc protein does not bind DNA in vitro (Paro, 1990), thus it is likely to bind to chromosomal locations via interaction with other proteins. We have not yet obtained any compelling evidence for direct and specific binding of the Psc protein to DNA, however, we note that it shares a conserved protein domain with the product of the murine *mel-18* gene (Brunk et al., 1991b, van Lohuizen et al., 1991). The mel-18 protein has been shown to bind DNA in vitro and a conserved putative metal binding domain within the larger domain was shown to be essential for this binding activity (Tagawa et al., 1991). Perhaps the Psc protein serves as a linker between DNA and one or more other *Pc*-G proteins. Our finding that the Psc protein is a locus-specific chromosomal protein suggests that the other members of this homology group also may be localized in a similar fashion.

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