pitkin^{D}, a Novel Gain-of-Function Enhancer of Position-Effect Variegation, Affects Chromatin Regulation During Oogenesis and Early Embryogenesis in Drosophila

Steffi Kuhfittig,* János Szabad,† Gunnar Schotta,* Jan Hoffmann,* Endre Máthé†,[1] and Gunter Reuter*

**Institute of Genetics, Martin Luther University, D-06120 Halle, Germany and* †*Department of Biology, University of Szeged, H-6720 Szeged, Hungary*

Manuscript received January 14, 2000
Accepted for publication November 10, 2000

ABSTRACT

The vast majority of the >100 modifier genes of position-effect variegation (PEV) in Drosophila have been identified genetically as haplo-insufficient loci. Here, we describe *pitkin*^{Dominant} (*ptn*^{D}), a gain-of-function enhancer mutation of PEV. Its exceptionally strong enhancer effect is evident as elevated spreading of heterochromatin-induced gene silencing along euchromatic regions in variegating rearrangements. The *ptn*^{D} mutation causes ectopic binding of the SU(VAR)3-9 heterochromatin protein at many euchromatic sites and, unlike other modifiers of PEV, it also affects stable position effects. Specifically, it induces silencing of *white*$^{+}$ transgenes inserted at a wide variety of euchromatic sites. *ptn*^{D} is associated with dominant female sterility. +/+ embryos produced by *ptn*^{D}/+ females mated with wild-type males die at the end of embryogenesis, whereas the *ptn*^{D}/+ sibling embryos arrest development at cleavage cycle 1–3, due to a combined effect of maternally provided mutant product and an early zygotic lethal effect of *ptn*^{D}. This is the earliest zygotic effect of a mutation so far reported in Drosophila. Germ-line mosaics show that *ptn*$^{+}$ function is required for normal development in the female germ line. These results, together with effects on PEV and *white*$^{+}$ transgenes, are consistent with the hypothesis that the *ptn* gene plays an important role in chromatin regulation during development of the female germ line and in early embryogenesis.

DIFFERENTIAL gene expression during development comprises establishment and maintenance of defined expression patterns. These epigenetic patterns of gene expression depend on alternative self-perpetuating higher-order chromatin states, which are achieved through balanced activities of repressing and actuating chromatin functions.

Genes encoding functions controlling higher-order chromatin structure in Drosophila have been identified in screens for dominant suppressor and enhancer mutations of position-effect variegation. In position-effect variegation (PEV), euchromatic regions become transcriptionally silenced after their relocation into a new intimate vicinity of pericentric heterochromatin. The >400 dominant PEV modifier mutations isolated (Reuter and Wolfe 1981; Sinclair *et al.* 1983; Wustmann *et al.* 1989; Dorn *et al.* 1993) have provided an enriched source for the identification of new genes affecting gene transcription and chromatin regulation (Grigliatti 1991; Reuter and Spierer 1992; Weiler and Wakimoto 1995). Molecular analysis of several of these PEV modifier genes in several cases proved involvement in various aspects of chromatin regulation. These loci encode heterochromatin-associated proteins like HP1 (James and Elgin 1986; Eissenberg *et al.* 1990, 1992), SU(VAR)3-7 (Reuter *et al.* 1990; Cléard *et al.* 1997), and SU(VAR)3-9 (Tschiersch *et al.* 1994; Schotta and Reuter 2000), or chromatin proteins, which are also involved in transcriptional control of euchromatic loci like MODULO (Krejci *et al.* 1989; Perrin *et al.* 1998), MOD(MDG4) (Dorn *et al.* 1993; Gerasimova *et al.* 1995; Büchner *et al.* 2000), E2F (Seum *et al.* 1996), and the *E(var)* gene *hel*, which encodes a DEAD-box helicase (Eberl *et al.* 1997). The *E(Pc)* gene suppresses PEV and controls expression of homeotic and other genes (Sinclair *et al.* 1998). Other modifier loci encode enzymatic functions that affect modification of chromosomal proteins, *e.g.*, *Su(var)3-6*, which encodes protein phosphatase PP1 (Baksa *et al.* 1993), or *Su(var)2-1*, which affects histone H4 deacetylation (Dorn *et al.* 1986) and histone deacetylase RPD3 mutations (DeRubertis *et al.* 1996; Mottus *et al.* 2000).

Almost all of the dominant modifiers of PEV mutations were isolated on the basis of their suppressor or enhancer effect on *white* gene variegation associated with the *In(1)w*^{m4} rearrangement. The *w*^{m4} phenotype is very sensitive for the detection and genetic analysis of dominant PEV modifier mutations (Reuter *et al.* 1985).

Corresponding author: Gunter Reuter, Institute of Genetics, Martin Luther University, D-06120 Halle, Weinbergweg 10, Germany. E-mail: reuter@genetik.uni-halle.de

[1]*Present address:* Department of Genetics, University of Cambridge, Cambridge CB2 3EH, United Kingdom.

In a certain number of eye primordial cells, heterochromatin spreads into neighboring euchromatin and the *white* locus in w^{m4} becomes silenced by acquiring a more condensed and less accessible chromatin structure (Reuter and Szidonya 1983; Boivin and Dura 1998). It is likely that modification of chromatin structure in the euchromatic *white* gene region and/or flanking heterochromatin leads to suppression or enhancement of *white* variegation in w^{m4}. Enhancer mutations result in excess heterochromatization, preventing expression of the $white^{+}$ gene in almost all of the eye primordial cells whereas suppressor mutations allow $white^{+}$ gene expression in almost all eye cells.

Most *E(var)* and *Su(var)* mutations are loss-of-function type mutations and the corresponding loci represent haplo-insufficient genes; a deletion for such a locus displays a dominant modifier effect on PEV indicating that two wild-type gene copies are required for normal function (Reuter and Spierer 1992). The haplo-dependent effect of PEV modifier genes implies that *E(var)* products are required for open chromatin conformation, whereas the products of *Su(var)* genes appear to be involved in chromatin condensation. However, not only juxtaposition of euchromatic and heterochromatic regions but also abnormal nuclear position of a gene or its inclusion into repeated arrays may cause gene silencing (*cf.* Henikoff 1997; Weiler and Wakimoto 1998). To understand whether spreading of chromatin status, nuclear compartmentalization, or chromosome pairing represent nonmutually exclusive mechanisms for gene silencing in PEV, further understanding of the biochemical structure and molecular regulation of heterochromatic silencing complexes is required. In these studies gain-of-function type PEV modifier mutations like $pitkin^{Dominant}$ (ptn^{D}) might represent useful tools for genetic dissection of the unknown regulatory components of heterochromatic silencing complexes.

This article provides a combined genetic and developmental analysis of ptn^{D}, a gain-of-function type PEV enhancer mutation of Drosophila. Strong enhancement of PEV by ptn^{D} is accompanied by elevated spreading of heterochromatin-induced gene inactivation into euchromatic regions in all tested PEV rearrangements. In addition to its strong PEV enhancer effect, ptn^{D} induces repression of several *mini-white* transgenes located within euchromatin, which appears to be correlated with ectopic chromosomal distribution of heterochromatin protein SU(VAR)3-9.

ptn^{D} also induces dominant female sterility through a combination of maternal effect lethality and a zygotic lethal effect as early as cleavage cycle 1–3, which is accompanied by excess condensation of cleavage nuclei. Complete degeneration of egg primordia associated with condensation of nurse cell nuclei is found in $ptn^{D}/Df\ ptn^{-}$, ptn^{D}/ptn, ptn^{D}/ptn^{D}, and ptn/ptn females, indicating a requirement of the *ptn* gene during differentiation of female germ-line cells.

Our results imply a dominant negative nature of the ptn^{D} mutation; *i.e.*, the ptn^{D}-encoded mutant gene product impedes the activities of the maternally provided wild-type counterpart at early embryogenesis. These data underline the importance of gain-of-function mutations for the study of chromatin regulation during germline development and early embryogenesis.

MATERIALS AND METHODS

Origin and maintenance of ptn^{D}: The ptn^{D} was isolated as a dominant enhancer of PEV after X-ray mutagenesis. It is named after Pitkin, the principal figure of Robert Ascher's movie *A Stitch in Time*, who caused perplexing confusions by overdoing almost everything. The ptn^{D} mutation is maintained by crossing w^{m4h}/w^{m4h}; $Sco/T(2;3)ap^{Xa}$ + $In(2)Cy$, $Cy\ Su(var)3\text{-}9^{s}/+$ females (red eyes) with w^{m4h}/Y; $Sco/T(2;3)ap^{Xa}$ + $In(2)Cy$, $Cy\ Su(var)3\text{-}9^{s}/ptn^{D}$ males (white eyes). The ptn^{D} enhancer effect is epistatic to $Su(var)3\text{-}9^{s}$, a spontaneous suppressor mutation on $T(2;3)ap^{Xa}$. In every generation the $Sco\ Cy\ ap^{Xa}$ red-eyed females are mated with white-eyed $Sco\ Cy\ ap^{Xa}\ ptn^{D}$ sibling males. For descriptions of chromosomes and marker mutations, see Lindsley and Zimm (1992) and FlyBase (2000). The ptn^{D} mutation, like other third chromosomal dominant female-sterile (*Fs*) mutations, is also maintained in a self-propagating system in which $ptn^{D}/TM3$, *Sb Ser* males are mated with *TM3, Sb Ser*/*T(1;3)OR60* females (Erdélyi and Szabad 1989).

Analysis of PEV: The PEV modifier effect of ptn^{D} was studied in eight different PEV rearrangements: $In(1)w^{m4h}$, $In(1)w^{m51b}$, $In(1)rst^{3}$, $T(1;2)N^{264h\text{-}10}$, $T(1;4)w^{258\text{-}21}$, $T(1;4)N^{a8}$, $In(1)sc^{8}$, and $In(1)sc^{S1}$. These rearrangements differ with respect to the block of heterochromatin-inducing PEV and the region of euchromatin affected (Figure 1). In studies with w^{m4h}, w^{m51b}, rst^{3}, or sc^{8}, homozygous females were mated with *w*/Y; ptn^{D}/Sb males and $ptn^{D}/+$ offspring were compared with their *Sb*/+ control siblings. In $N^{264\text{-}10}$, $w^{258\text{-}21}$, N^{a8}, and sc^{S1} crosses, heterozygous *FM6*, $y^{31d}\ sc^{8}\ dm\ B$ females were used for the analysis. PEV for the genes *roughest* (*rst*, rough eyes), *vertical* (*vt*, missing dorsal thoracic bristles), *scute* (*sc*, missing scutellar bristles), and *Notch* (*N*, notched wings) was quantified by determining the proportion of flies showing expression of each mutant phenotype (penetrance). Variegation for *white* was quantified by counting the proportion of offspring with white variegated eyes or by red eye pigment measurements (Reuter and Wolff 1981). PEV for essential X chromosomal genes, whose inactivation leads to lethality in hemizygous condition, was quantified by determination of relative viability between $ptn^{D}/+$ and *Sb*/+ sibling males (Table 1).

Effects of ptn^{D} on *mini-white* gene expression in *P-lacW* insertions: With the exception of chromosome regions 66–68, an otherwise random selection of 62 third-chromosomal *P-lacW* insertions isolated by Deak *et al.* (1997) was tested for the effect of ptn^{D} on *mini-white* gene expression (Figure 2). *w*; *P-lacW*/*TM3* females were crossed to w^{m4h}/Y; $ptn^{D}/TM3$ males and eye color phenotypes compared by visual inspection of ~50 *w*/Y; $ptn^{D}/P\text{-}lacW$ and 50 *w*/Y; *P-lacW*/*TM3* sibling males of equivalent age. *w*/Y; *P-lacW*/*TM3* control males of similar age are uniform in phenotype.

Polytene chromosome analysis: Chromosomal distribution of SU(VAR)3-9-enhanced green fluorescent protein (EGFP) fusion protein (Schotta and Reuter 2000) was studied in polytene chromosomes of +/pP{GS[ry^{+}, hs(Su(var)3-9cDNA)EGFP]} and ptn^{D}/pP{GS[ry^{+}, hs(*Su(var)3-9* cDNA)EGFP]} third instar larvae after 15 min heat-shock treatment. +/pP{GS[ry^{+}, hs(*Su(var)3-9* cDNA)EGFP]} larvae were received after a cross of

pP{GS[ry^+, hs(*Su(var)3-9* cDNA)EGFP]} homozygous females with wild-type males whereas the ptn^D/pP{GS[ry^+, hs(*Su(var)3-9* cDNA)EGFP]} larvae were selected after a cross of pP{GS[ry^+, hs(*Su(var)3-9* cDNA)EGFP]} homozygous females with ptn^D/T(2;3)*CyO* green fluorescent protein (GFP)-*TM3* GFP males. The T(2;3)*CyO* GFP-*TM3* GFP green balancer is described in Rudolph *et al.* (1999).

Preparation of polytene chromosomes was performed as described by Silver and Elgin (1978), with the following modifications: salivary glands of third instar larvae were dissected in PBS (130 mM NaCl, 7 mM Na_2HPO_4, 3 mM NaH_2PO_4), fixed for 10 min, and squashed in 45% acetic acid/2% formaldehyde. Chromosomes were incubated with mouse monoclonal α-GFP antibody (1:25 dilution; CLONTECH, Palo Alto, CA) at 4° overnight, followed by incubation with a secondary FITC-conjugated goat α-mouse antibody (1:25 dilution; Jackson ImmunoResearch Laboratories, West Grove, PA) for 2 hr at 37°. DNA was stained with propidium iodide. The preparations were mounted in Vectashield medium and examined with a confocal laser scanning microscope (LSM 510; Carl Zeiss, Thornwood, NY).

Analysis of embryonic phenotypes: Eggs of ptn^D/+ females do not hatch. Embryonic phenotypes were analyzed as described in Wieschaus and Nüsslein-Volhard (1986) and Jürgens *et al.* (1986). Genotypic differentiation between ptn^D/+ and +/+ embryos produced by ptn^D/+ females crossed to +/+ males was achieved by crossing *y*/*y*; ptn^D/y^+ *TM3, Sb Ser* females to *y*/Y; +/+ males. Head skeletons of *y*/*y* (or *y*/Y); ptn^D/+ larvae are yellow and those of the *y*/*y* (or *y*/Y); y^+ *TM3* (ptn^+)/+ larvae are dark due to y^+.

In crosses of ptn^D/+ females with ptn^D/+ males, three different embryonic phenotypes are observed (Figure 4). Genotype determination of embryos was performed by PCR analysis after a cross of ptn^D/*TM3, Ser* P{*w* [+m]UAS:GFP} females with ptn^D/P[*w*+ HS-lacZ](65E) *Sb* males. The *TM3, Ser* P{*w* [+m]UAS:GFP} and P[*w*+ HS-lacZ](65E) chromosomes are described in (Rudolph *et al.* (1999) and Lu *et al.* (1996), respectively. Embryos were dechorionated with sodium hypochloride, transferred into PBST (PBS plus 0.2% Tween 20), briefly fixed in methanol followed by washing four times in PBST, rehydrated for 1.5 hr in PBST, 4′6-diamidino-2-phenylindole (DAPI) stained, briefly washed in PBST, and inspected under a UV microscope. Very early arrested, late cleavage arrested, and gastrulated embryos were individually collected. From each type, five embryos were collected and DNA preparation was performed according to Gloor and Engels (1992). Two primer sets were used for PCR detection of wild-type (wt)GFP and lacZ, respectively (wtGFP forward 5′-AGTGGAGAGGGTGAAGGTGATG and wtGFP reverse 5′-AAGGGCAGATTGTGTGGACAGG; lacZ forward 5′-TGA CCTGAGCGCATTTTTAC and lacZ reverse 5′-GCAGCAG ACCATTTTCAATCC). The size of the expected fragments is 534 bp for wtGFP and 505 bp for lacZ. In *TM3, Ser* P{*w* [+m]UAS:GFP}/ptn^D (paternal) embryos the wtGFP-specific fragment is amplified whereas from P[*w*+ HS-lacZ](65E) *Sb*/ptn^D (maternal) embryos the lacZ-specific fragment is received. Amplification of both the GFP- and lacZ-specific fragments identifies the *TM3, Ser* P{*w* [+m]UAS:GFP}/P[*w*+ HS-lacZ](65E) *Sb* = ptn^+/ptn^+ embryos.

Early cleavage defects in embryos derived from ptn^D/+ females were analyzed in 0- to 1-hr-old DAPI-stained embryos with a Zeiss fluorescent microscope, or, after staining with propidium iodide, immunostaining of tubulin (YL1/2 rat monoclonal anti-tubulin; Serva), and of the nuclear lamina (T47 anti-lamin monoclonal antibody; Amersham, Arlington Heights, IL; Paddy *et al.* 1996), with the confocal laser scanning microscope. The specimens were incubated in the primary antibodies overnight (4°). The secondary antibodies (Jackson Laboratories, Avondale, PA) were applied for 2 hr at room temperature. Specimens were mounted in 85% glycerol containing 2.5% *n*-propyl gallate. Optical sections were generated in a Zeiss 410 or Zeiss LSM510 confocal laser scanning microscope.

Mapping of the *pitkin* locus: For crossover mapping of ptn^D we made use of the polygenic modifiers present in the *ru cu ca* inbred strain (Erdélyi and Szabad 1989). Some of the w^{m4h}/+; *ru cu ca*/ptn^D females gave rise to a few offspring. Recombinant offspring were identified after a backcross to w^{m4h}/Y; *ru cu ca*/*ru cu ca* males. According to the genetic map position determined, transheterozygotes of ptn^D with different *Dp(3;3)S2a* chromosomes (Craymer 1984) covering region 64C–71D were studied for rescue of dominant female sterility (Figure 6). Further refinement of the map position has been achieved by overlapping deficiencies within region 67–68 and *P*-element-induced *ptn* mutations by testing for the no egg phenotype, a diagnostic feature for ptn^D/ptn^- transheterozygous females (Figure 7).

Reversion of ptn^D and *P*-element-induced *pitkin* mutations: ptn^D is a gain-of-function mutation whose female sterility and dominant PEV enhancement phenotypes are revertible using X rays and *P*-element hybrid dysgenesis, respectively. X-ray-induced revertants were isolated after irradiating ptn^D/*TM3, Sb Ser* males with 4000 R of X rays. These males were crossed to *TM3, Ser*/*TM1* females and revertants were selected as fertile ptn^{DrX}/*TM3, Ser* or ptn^{DrX}/*TM1* females where *rX* denotes the X-ray-induced revertant chromosomes (*TM3, Sb Ser*/*TM3, Ser* and *TM3, Sb Ser*/*TM1* are lethal). For isolation of *P*-element hybrid dysgenesis-induced revertants, *P* cytotype *In(3LR)CxD*/*TM3, Ser* females were crossed to ptn^D/*TM3, Sb Ser* males. The F_1 ptn^D/*CxD* males were backcrossed to M cytotype w^{m4h}; *TM3, Sb Ser*/*Pr Dr* females. The w^{m4h}/Y; *TM3, Sb Ser*/ptn^D male offspring were then crossed to w^{m4h}; *In(2L)Cy*+*In(2R)Cy, Cy* cn^2 sp^2/*T(2;3)*ap^{Xa}, ap^{Xa} *Su(var)2-1*^{01}/*Sb* females. *T(2;3)*ap^{Xa}, ap^{Xa} *Su(var)2-1*^{01} male offspring were inspected for reversion of the strong enhancer effect of ptn^D (red-pigmented instead of white eyes).

After screening of the *P-lacW* insertion collection of Deak *et al.* (1997) for recessive *ptn* alleles, the two *P-lacW*-induced $ptn^{P890/4}$ and $ptn^{P893/2}$ mutations were identified on the basis of their no egg phenotype over ptn^D. The *P-lacW* element carries the w^+ marker gene and excisions of the element can be selected by loss of w^+ function. Revertant analysis was performed with both $ptn^{P890/4}$ and $ptn^{P893/2}$ by selection of w^- offspring in a cross of *w*/*w*; *TM3*/*TM6* females to *w*/Y; $ptn^{P893/2}$ or $ptn^{P890/4}$/*TM3*, Δ*2-3* males. The *TM3,*ry^{RK} *Sb e* P[(ry^+)Δ*2-3*] (99B) balancer chromosome contains a stable source of transposase that is efficient in *P*-element remobilization (Reuter *et al.* 1993).

Ovarian phenotypes: Homozygous ptn^D/ptn^D females were recovered at a very low frequency in crosses of ptn^D/*In(3LR)CxD* females to ptn^D/*TM3* males. The ptn^D/*CxD* females for this cross were generated by a series of crosses with a *ru cu ca* multiply marked stock, which was shown to contain modifiers that reduce the penetrance of several dominant female-sterile mutations (Erdélyi and Szabad 1989). The *ptn* gene is uncovered by *Df(3L)AC1* (see results). The two *P-lacW* inserts $ptn^{P890/4}$ and $ptn^{P893/2}$ (Figure 7) represent recessive *ptn* mutations. The ptn^D/Df ptn^- and *ptn*/*Df* ptn^- females were generated by crossing *Df(3L)AC1*/*TM3* females with ptn^D/*TM3*, $ptn^{P890/4}$/*TM3* or $ptn^{P893/2}$/*TM3* males, respectively. Ovaries of 7- to 10-day-old females were dissected, fixed, and analyzed as described previously (Theurkauf and Hawley 1992). Staging of egg primordia is according to Spradling (1993).

Germ-line mosaics: +/+ female germ-line clones in ptn^D/+ females were generated by X-irradiation of w^{m4h}/w^{m4h}; ptn^D/*ve h th* adult females with 1500 R (150 kV; 0.5-mm Al filter; 1000 R/min). According to Erdélyi and Szabad (1989), this X-ray

treatment produces +/+ germ-line cells in ~10% of the irradiated females. To test whether ptn^D-free germ-line cells give rise to offspring, irradiated females were crossed to w^{m4h}/Y; *ve h th/ve h th* males and monitored for offspring production over a period of 15 days.

Germ-line chimeras: Three types of germ-line chimeras were constructed through transplantation of pole cells (Ilmensee 1973; Van Deusen 1977). (1) Pole cells of donor embryos produced by a cross of w^{m4h}/w^{m4h}*; ve h th/ve h th* females with w^{m4h}/Y; ptn^D/*TM3* males were transplanted into host embryos that originated from a cross of wild-type females with ovo^{D1}/Y males. ovo^{D1} is a dominant female-sterile mutation that alters function of the germ line without affecting the soma (Busson *et al.* 1983; Komitopoulou *et al.* 1983). ovo^{D1}/+ females' offspring, which might have received ptn^D/*ve h th* or *ve h th/TM3* pole cells, were mated with w^{m4h}/Y; +/+ males. (2) Pole cells from embryos produced by a cross of *fs(1)K10/ClB* females with *fs(1)K10 w*/Y males were transplanted into host embryos produced by cross of w^{m4h}/w^{m4h}*; ve h th/ve h th* females with w^{m4h}/Y; ptn^D/*TM3* males. Eclosing females were mated with *fs(1)K10 w*/Y males. *fs(1)K10* is an egg-shaped marker mutation in which the mutant phenotype depends on the genotype of the germ-line cells (Wieschaus *et al.* 1978). (3) Pole cells from donor embryos produced by *y w*/*y w*; $ptn^{P890/4}$/*TM6b, Tb* females crossed to *y w*/Y; $ptn^{P893/2}$/*TM3, Sb Ser* males were transplanted into host embryos derived from a cross of wild-type females with ovo^{D1}/Y males. Eclosing females were mated with *y w*/Y males.

Follicle cell mosaic analysis: To determine the effect of the *ptn* gene on follicle cell development we generated homozygous $ptn^{P893/2}$ follicle cell clones by X-ray-induced mitotic recombination in $ptn^{P893/2}$/*Fs(3)Apc* females. *Fs(3)Apc* is a dominant female-sterile mutation that only disrupts follicle cell function without interfering with function of germ-line cells (Erdélyi and Szabad 1989). For induction of *ptn* homozygous follicle cells, *Fs(3)Apc*/$ptn^{P893/2}$ heterozygous females were irradiated with 1500 R X rays (150 kV, 0.5-mm Al filter, 1000 R/min) as early third instar larvae or adults and afterward tested for offspring production. Whether *ptn* homozygous follicle cells support egg development can be determined by comparing offspring production frequencies between $ptn^{P893/2}$/*Fs(3)Apc* and +/*Fs(3)Apc* (control) females.

RESULTS

***ptn*^{D} is the strongest known enhancer mutation of PEV:** The ptn^D mutation was isolated after X-ray mutagenesis of wild-type chromosomes on the basis of its very strong PEV enhancer effect on *white* variegation in w^{m4h}. The mutation was formerly described as *E-var(3)2*^{01} (Reuter *et al.* 1985). Normally only variegation of the white gene is observed in w^{m4h} flies and enhancement of PEV is evident by the more frequent inactivation of the white gene and by inactivation of genes located more distally from the breakpoint. w^{m4h}/Y; ptn^D/+ males express a complete *w* and *rst* eye mutant phenotype, show variegation for *vt*, and inactivation of essential genes more distally located from the breakpoint is indicated by their reduced viability (Table 1, Figure 1). In w^{m4h}; ptn^D/+ females *Notch* variegation becomes visible. Reversion of variegating rearrangements is accompanied by changes in the overall structure of the rearrangement and the amount and type of heterochromatin at the variegating breakpoint. To assay the general strong enhancer capability of ptn^D, we tested in an earlier study its effect on 52 w^+ revertant chromosomes of w^{m4h} (Reuter *et al.* 1985). ptn^D induced white variegation in 50 out of the 52 w^+ revertant chromosomes. Enhancement of PEV by ptn^D in w^{m4h} is also visible in Malpighian tubules. Regularly, in w^{m4h} larvae only about one to two white cells in Malpighian tubules are found whereas large white sectors are seen in w^{m4h}; ptn^D/+ larvae (data not shown).

Strong enhancement of PEV by ptn^D is also observed for all of the other analyzed rearrangements (Figure 1, Table 1). The different rearrangements juxtapose various euchromatic regions to different blocks of heterochromatin (Figure 1). rst^3 flies usually show only *rst* variegation and infrequently they exhibit variegation of *vt*. In rst^3/Y males, ptn^D induces complete inactivation of *rst*, strong variegation of *vt*, and the significant reduction of viability of these males indicates inactivation of more distally located essential genes (Figure 1, Table 1). In rst^3/w; ptn^D/+ females *w* and *N* variegation appears. The enhancer effect of ptn^D on white variegation in w^{m51b} was quantified by red eye pigment measurements. w^{m51b}/Y; ptn^D/+ males show strong *w* mottling and variegate for *rst* and *vt*, which is never found in control genotypes (Table 1). $T(1;2)N^{264\text{-}10}$ variegates weakly for *N*, but not for *w*. In contrast, all $T(1;2)N^{264\text{-}10}/w$; ptn^D/+ females show *w* variegation and *N* variegation is strongly enhanced (Table 1, row 4). In $T(1;4)w^{m258\text{-}21}$ the ptn^D mutation results in a strong *N* mutant phenotype in all females and *w* variegation is strongly elevated as shown by red eye pigment measurement (Table 1, row 5). Significant enhancement of *w* variegation is found in $T(1;2)N^{a8}/w$; ptn^D/+ females (Table 1, row 6). ptn^D also enhances variegation of the *sc* gene in sc^8 as indicated by reduction of the mean number of scutellar bristles (Table 1, row 7, column 6). At low temperature of development inactivation of essential genes distal to *sc* in sc^8 is indicated by reduced viability of sc^8/Y; ptn^D/+ males (Table 1, row 7, column 5). There is no significant inactivation of essential genes at the proximal breakpoint of sc^{S1} (Baker 1971). ptn^D significantly enhances inactivation of essential genes at this breakpoint, as indicated by the marked reduction of sc^{S1}/Y male viability (Table 1, row 8). In all the PEV rearrangements studied ptn^D strongly promotes spreading of heterochromatin-induced gene inactivation along euchromatic regions and loci more distant from the breakpoint become subjected to gene silencing (Figure 1).

***ptn*^{D} represses *mini-white* gene expression at euchromatic insertion sites and causes ectopic distribution of heterochromatin protein SU(VAR)3-9:** We have analyzed the effect of ptn^D on *mini-white* gene expression in 61 *P-lacW* elements inserted at random sites within the third chromosome (Figure 2). In 23 of the 61 inserts studied (~38%), *mini-white* gene expression was strongly reduced in ptn^D/*P-lacW* males. In only one case (*P-lacW 1018/1*), a variegated expression of the *mini-white* gene

TABLE 1

Enhancer effect of *ptn*^{D} on position-effect variegation

Rearrangement	Genotype[a]		No. of flies	Viability (%)[b]	Position-effect variegation[c]			
	Sex chromosomes	Autosomes			White variegation in % of eyes (red eye pigments)	Roughest variegation (% of eyes)	Vertical variegation (% of flies)	Notch variegation (% of flies)
In(1)w^{m4h}	*w*^{m4h}/*w*^{m4h}	+/*TM3*	155		100	0	0	0
		ptn^{D}/+	135		all *w*$^{-}$	98.8	6.1	1.2
	w^{m4h}/Y	+/*TM3*	123		100	0	0	0
		ptn^{D}/+	46	37.4	all *w*$^{-}$	100	26.1	—
In(1)rst^{3}	*rst*^{3}/*w*	+/*TM3*	304		—	—	—	0
		ptn^{D}/+	306		100	—	—	0.3
	rst^{3}/Y	+/*TM3*	314		*w*$^{+}$	100	1.0	—
		ptn^{D}/+	274	87.3	*w*$^{+}$	100	58.0	—
In(1)w^{m51b}	*w*^{m51b}/*w*	+/*TM3*	228		100 (43.1)	—	—	0
		ptn^{D}/+	207		100 (5.1)	—	—	0
	w^{m51b}/Y	+/*TM3*	213		100 (93.3)	0	0	—
		ptn^{D}/+	197	92.5	100 (15.9)	79.7	16.2	—
T(1;2)N$^{264-10}$	*N*$^{264-10}$/*w*	+/*TM3*	83		*w*$^{+}$	—	—	2.4
		ptn^{D}/+	84		100	—	—	30.9
T(1;2)w$^{m258-21}$	*w*$^{m258-21}$/*w*	+/*TM3*	119		100 (104.1)	—	—	85.9
		ptn^{D}/+	149		100 (31.3)	—	—	100
T(1;2)N^{a8}	*N*^{a8}/*w*	+/*TM3*	135		100 (7.7)	—	—	Notch^{-}
		ptn^{D}/+	139		100 (3.6)			Notch^{-}
					Variegation for scute[d]			
In(1)sc^{8}	*sc*^{8}/Y (25°)	+/*TM3*	185		2.88			
		ptn^{D}/+	133	71.9	0.71			
	sc^{8}/Y (18°)	+/*TM3*	186		3.89			
		ptn^{D}/+	33	17.1	1.20			
In(1)sc^{S1}	*sc*^{S1}/Y (25°)	+/*TM3*	106					
		ptn^{D}/+	58	54.7				
	sc^{S1}/Y (18°)	+/*TM3*	92					
		ptn^{D}/+	15	16.3				

[a] Homozygous *w*^{m4h} females were crossed to *w*^{m4h}/Y; *ptn*^{D}/*TM3*, *Sb* males. Otherwise the homozygous (*rst*^{3}, *w*^{m51b}, *sc*^{8}) or heterozygous (*N*$^{264-10}$, *w*$^{m258-21}$, *N*^{8a}, *sc*^{S1}) females were crossed to *w*/Y; *ptn*^{D}/*TM3*, *Sb* males. PEV in +/*TM3*, *Sb* control and *ptn*^{D}/+ sibling genotypes was compared.

[b] Percentage of control. Unless otherwise indicated, the fly cultures developed at 25°.

[c] —, phenotypically not detectable inactivation of the gene (due to recessive phenotype or lethality). Red eye pigments in percentage of wild-type Canton-S flies are shown.

[d] Mean number of scutellar bristles.

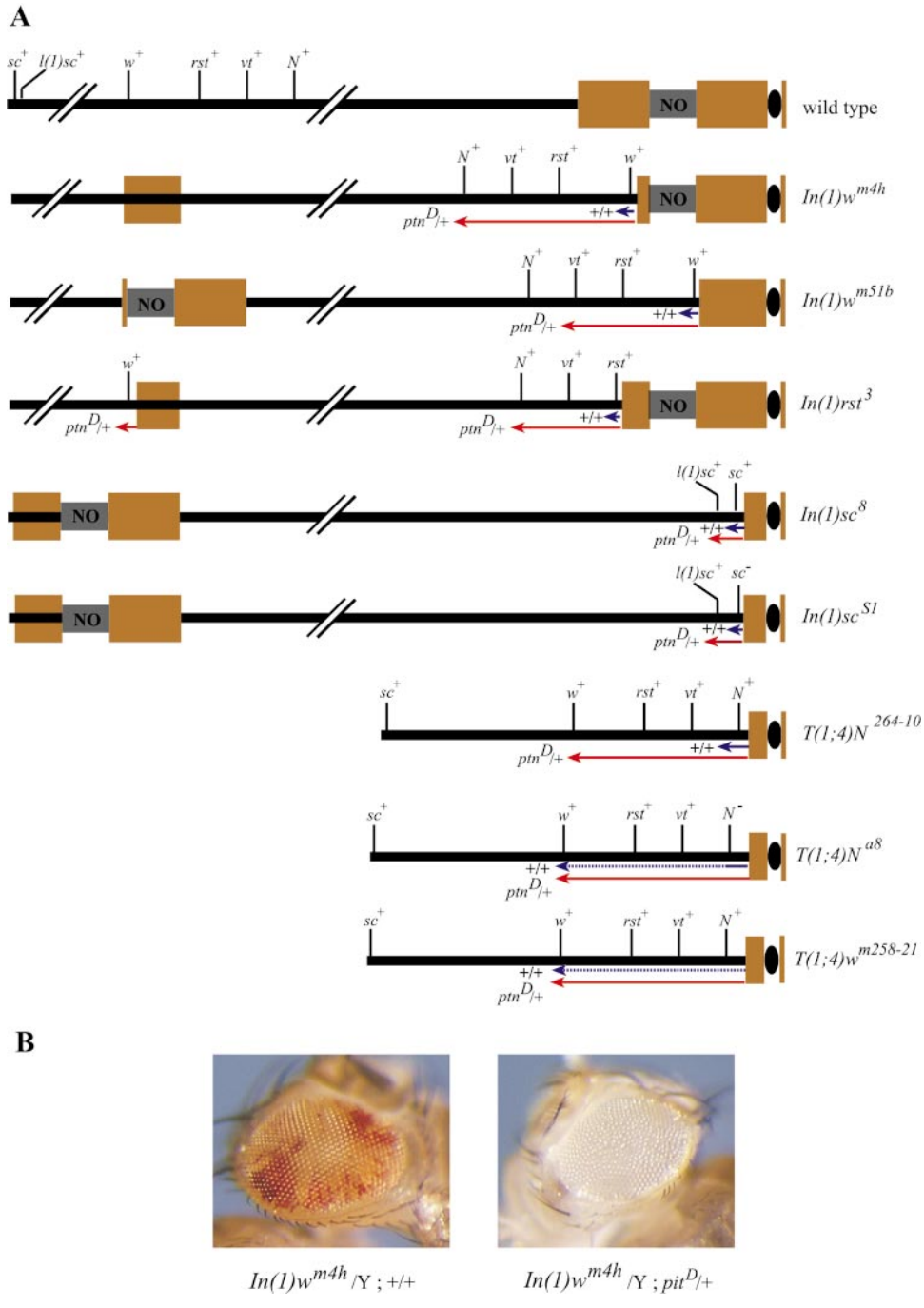

FIGURE 1.—*ptn*^{D} increases the spreading of inactivation in PEV rearrangements. (A) Schematic representation of eight different chromosomal rearrangements that juxtapose different blocks of constitutive heterochromatin (thick bars) to different segments of euchromatin (thin lines) and induce PEV for the genes shown. Arrows illustrate the direction of the heterochromatin-induced gene silencing. In *T(1;4)N*^{a8} and *T(1;4)w*$^{m258\text{-}21}$ the dotted blue lines indicate a significantly lower frequency of variegation in +/+ as compared to *ptn*^{D}/+ (red lines). (B) Photographic representation of the enhancer effect of *ptn*^{D} on *w*^{m4}. Variegated expression of the *white*$^+$ gene, associated with the *w*^{m4h} inversion, leads to the formation of red-white spotted eyes. The strong enhancer effect of *ptn*^{D} causes complete inactivation of the *white* and *roughest* genes.

was observed. These results suggest an effect of *ptn*^{D} on both heterochromatic and euchromatic regions. In almost all of the *P-lacW* inserts tested, *mini-white* gene expression is already repressed in the control and *ptn*^{D} might enhance stable position effects at certain euchromatic regions. Alternatively, the effects of *ptn*^{D} on euchromatic *mini-white* gene insertions could be caused by a mechanism related to heterochromatin-induced gene silencing in PEV. To test this hypothesis, we studied chromosomal distribution of heterochromatin protein SU(VAR)3-9 in salivary gland giant chromosomes of wild-type and *ptn*^{D}/+ larvae (Figure 3). In wild type the SU(VAR)3-9EGFP protein is almost exclusively associated with chromocenter heterochromatin and the fourth chromosome (SCHOTTA and REUTER 2000; Figure 3A). In contrast to this, >100 ectopic SU(VAR)3-9EGFP binding sites along all chromosome arms are detected in salivary gland chromosomes of *ptn*^{D}/+ larvae (Figure 3B). An identical ectopic distribution of SU(VAR)3-9EGFP is also found in unfixed salivary gland nuclei (Figure 3D). Most of the ectopic sites correlate with heavily stained bands. Furthermore, all of the affected *mini-white* gene insertions are located at chromosomal sites where ectopic SU(VAR)3-9EGFP is detected.

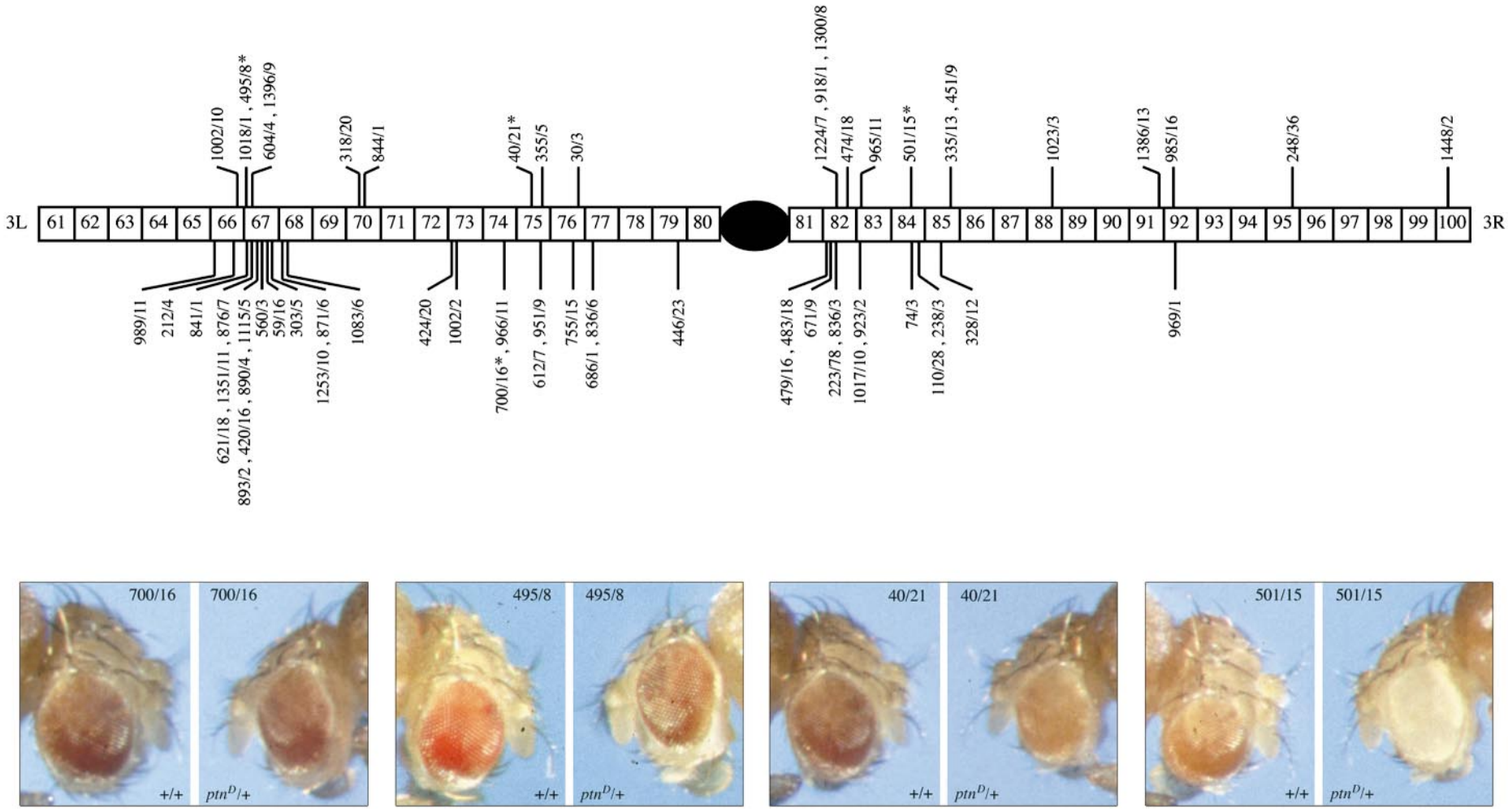

FIGURE 2.—*ptn*^{D} reduces the expression of the *mini-white* gene in *P-lacW* inserts. Expression of *mini-white* gene is significantly reduced in the inserts shown above the schematic cytological map. Examples are shown for three different lines (*495/8*, *40/21*, and *501/15*; control *700/16* with no effect). *ptn*^{D} has no effects *on mini-white* gene expression in the inserts shown below the map.

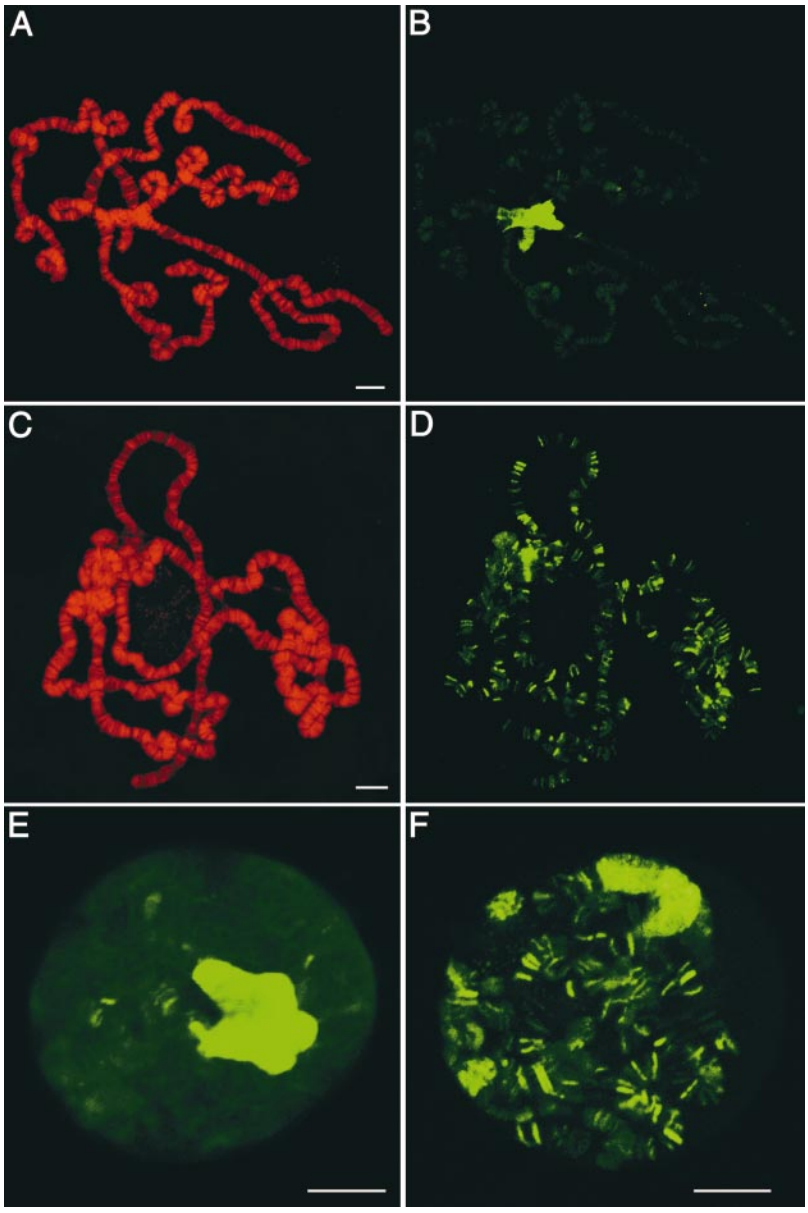

FIGURE 3.—*ptn*^{D} induces recruitment of heterochromatin protein SU(VAR)3-9 to many ectopic sites along all euchromatic chromosome regions. Immunolocalization of SU(VAR)3-9EGFP fusion protein expressed under the control of the hsp70 promoter in salivary gland giant chromosomes of wild-type (A and B) and *ptn*^{D}/+ (C and D) larvae is shown. Chromosomes were stained with propidium iodide (A and C) and an anti-GFP antibody (B and D). In the control (B), SU(VAR)3-9EGFP is associated with chromocenter heterochromatin and the fourth chromosome whereas in *ptn*^{D}/+ (D) >100 reproducible additional binding sites of SU(VAR)3-9EGFP over all chromosome arms are detected. Similar ectopic distribution of SU(VAR)3-9EGFP is also seen in unfixed salivary gland nuclei inspected with a confocal microcope for EGFP fluorescence. (E) Control SU(VAR)3-9EGFP/+ ; +/+ and (F) SU(VAR)3-9EGFP/+ ; *ptn*^{D}/+. Bars, 10 μm.

Our results indicate that *ptn*^{D}-induced repression of euchromatic *mini-white* insertions is caused by ectopic binding of heterochromatin protein complexes. *ptn*^{D} is the first modifier of PEV mutations that is shown to induce ectopic distribution of a heterochromatin-associated protein.

***ptn*^{D} induces dominant female sterility through maternal-effect and zygotic lethality:** *ptn*^{D}/+ females exhibit wild-type fecundity and all of their eggs are fertilized (data not shown). However, larvae do not hatch from the eggs. When *ptn*^{D}/+ females are mated with wild-type (+/+) males, two types of embryos can be differentiated after DAPI staining (Figure 4). In ~50%, development is arrested shortly after initiation of cleavage divisions. Developmental arrest of these embryos was studied after 2-hr aging of eggs collected for a 1-hr period. DAPI staining revealed that most did not initiate cleavage or were arrested around cleavage cycle 1–3 (88.5%; 54/61) and a maximum of six small nuclei that appear abnormal in chromatin structure were visible (Figure 6). Only 11.5% (7/61) reached cleavage cycles 4–10. The other 50% of the embryos developed to the larval stage of differentiation. They had defective cephalopharyngeal head skeletons (Figure 5) and did not hatch. To determine the genotype of the two types of embryos, we crossed *y*/*y*; *ptn*^{D}/*y*$^{+}$*TM3* females with *y*/Y; +/+ males (all *TM3* chromosomes are *ptn*$^{+}$; *cf.* MATERIALS AND METHODS). All embryos with an abnormal head skeleton were yellow^{+} in phenotype and hence they inherited *y*$^{+}$*TM3* from the *ptn*^{D}/*y*$^{+}$*TM3* mother. Therefore, lethality of the +/+ embryos derived from *ptn*^{D}/+ mothers must be due to maternally provided *ptn*^{D} mutant gene product. Zygotes in which development is arrested shortly after fertilization are thus *ptn*^{D}/+ and received the *ptn*^{D} allele from their *ptn*^{D}/+ mothers. Therefore, the presence and early expression of the *ptn*^{D}

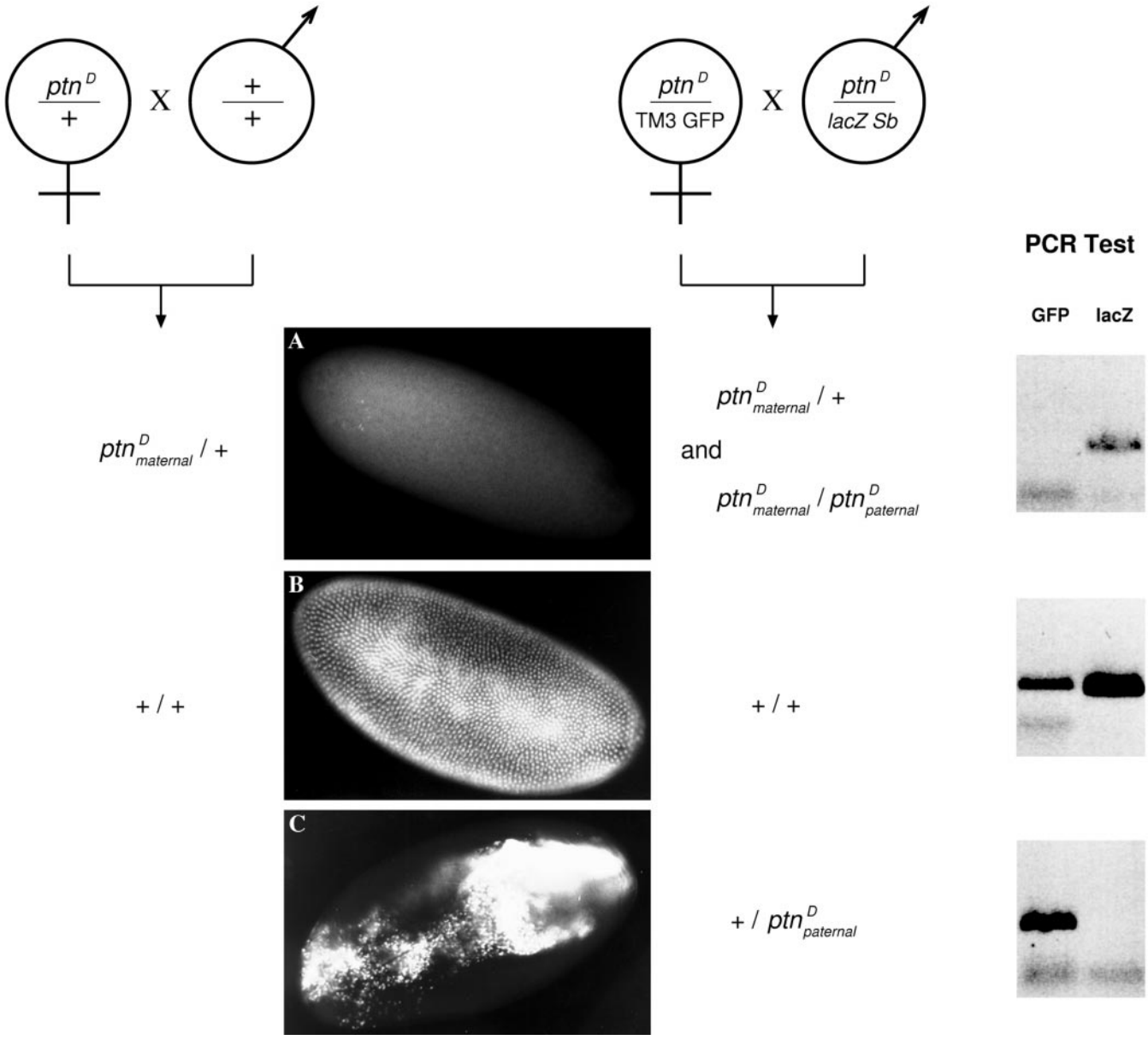

FIGURE 4.—Embryonic phenotypes associated with ptn^D. $ptn^D/+$ females crossed with +/+ wild-type males produce two types of embryos at a 1:1 ratio: (A) The chromatin appears highly condensed after one to three cleavage divisions in the ptn^D(maternal)/+ embryos leading to termination of embryogenesis. (B) The +/+ embryos reach the blastoderm stage and stop development at the end of embryogenesis (*cf.* Figure 5). (C) A third phenotype is observed in ~25% of embryos from the matings of $ptn^D/+$ females and $ptn^D/+$ males. Chromatin of late cleavage nuclei becomes highly condensed in these $+/ptn^D$(paternal) embryos. Determination of embryonic genotypes was performed after a cross of ptn^D/TM3 GFP females with ptn^D/lacZ Sb males by PCR analysis. Amplification of the lacZ- or GFP-specific DNA fragments allowed differentiation between ptn^D(maternal)/+ and $+/ptn^D$(paternal) embryos. All embryos were DAPI stained. (Magnification ×100.)

allele leads to an early arrest of embryogenesis in the $ptn^D/+$ zygotes (Figures 4 and 6).

Three types of embryos develop from $ptn^D/+$ females crossed to $ptn^D/+$ males (Figure 4). Cleavage divisions did not initiate in ~50% of the zygotes, and ~25% of the embryos had abnormal head skeleton development (+/+ zygotes). Each of the remaining 25% of the embryos possessed several hundred brightly DAPI-fluorescent small nuclei that are irregularly distributed (Figures 4 and 6). In these embryos, cleavage initiates but the nuclei never populate the entire cortex and no blastoderm is formed. Genotypes of the different classes of embryos were determined by PCR after a cross of $ptn^D/TM3$, GFP females with ptn^D/lacZ *Sb* males (*cf.* MATERIALS AND METHODS and Figure 4). PCR amplification of a GFP or lacZ-specific DNA fragment allowed differentiation between ptn^D (maternal)/+ (*TM3*, GFP) and ptn^D (paternal)/+ (lacZ *Sb*) embryos, respectively (Figure 4).

As shown by PCR analysis, the genotype of embryos that die after cleavage is + (maternal)/ptn^D (paternal) (Figure 4). Cleavage nuclei of these embryos appear

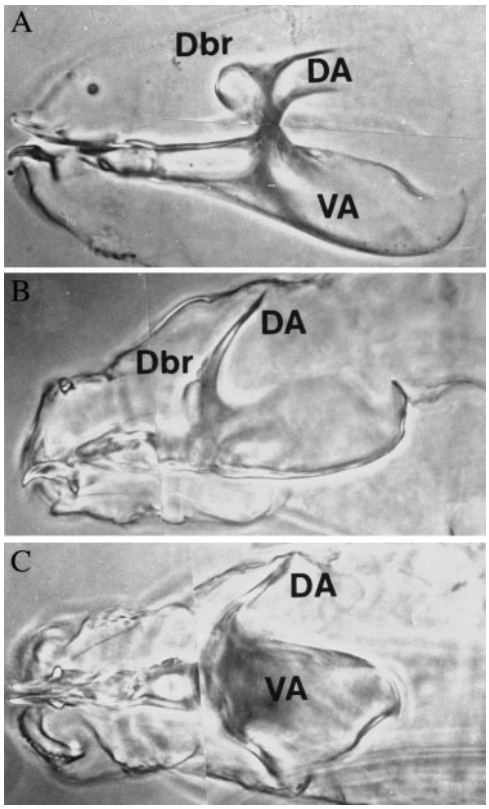

FIGURE 5.—Head skeleton of late +/+ embryos produced by ptn^D/+ females crossed to +/+ wild type males. (A) Wild-type control embryos. (B and C) Genotypically +/+ embryo of ptn^D/+ mothers. Note the abnormal cephalopharyngeal skeleton in B and C: the dorsal arms (DA) bend dorsally and outward. The dorsal bridge (Dbr) is reduced and the ventral arm (VA) is distorted. The distorted head skeleton is due to the maternally provided ptn^D mutant gene product. These embryos fail to hatch. (Magnification ×750.)

strongly condensed and chromatin is frequently fragmented (Figures 4 and 6). This might be due to additive/synergistic effects of the maternally and paternally derived mutant ptn^D gene products. In accordance with this suggestion, all embryos produced by +/+ females that were crossed to ptn^D/+ males develop normally.

The *pitkin* gene is located in the 67C3-5 cytological region: A few offspring develop from ~1% of the ptn^D/*rucuca* females (*cf.* MATERIALS AND METHODS). The recombinant offspring from a test cross between w^{m4h}/w^{m4h}; ptn^D/*ru cu ca* females and w^{m4h}/Y; *ru cu ca* /*ru cu ca* males allowed crossover mapping of the *ptn* locus. All recombinant chromosomes were tested for dominant female sterility and enhancement of PEV. ptn^D is located within the interval delineated by the *hairy* (*h*) and the *thread* (*th*) marker mutations (Figure 6). The 66 recombinants isolated (52 *h* ptn^D + and 14 + ptn^D *th*) divided the *h–th* interval at a 52/14 ratio. Taking the position of the *h* and *th* loci as reference points (26.5 and 43.2 cM, respectively; LINDSLEY and ZIMM 1992) the *ptn* is located at ~39 cM. The dominant female sterility and the dominant PEV enhancer effect of ptn^D were not separated by any of the recombinants, suggesting that the two phenotypes stem from the same mutation.

Genetic map position 39 cM corresponds to the 66D(*h*)–72B(*th*) cytological region (LINDSLEY and ZIMM 1992; Figure 7). We combined ptn^D with a series of *Dp(3;3)S2a* chromosomes (CRAYMER 1984) and tested for rescue of ptn^D mutant phenotypes (Figure 7). Larvae hatched and developed to adulthood from 38 and 18% of the eggs deposited by ptn^D/*Dp(3;3)S2a2* and ptn^D/*Dp(3;3)S2a8* females, respectively. None of the other duplications showed a rescue effect on ptn^D-associated dominant female sterility. This partial rescue of dominant female sterility in ptn^D/+/*Dp* ptn^+ females shows that ptn^D resides within the 66D; 67D–E chromosome segment and indicates an antimorphic nature of the ptn^D mutation. This result also implies an involvement of both the normal and the ptn^D-encoded mutant gene products in the same process. Comparison of white mottling between w^{m4h}/Y; ptn^D/+ and w^{m4h}/Y; ptn^D/+/*Dp* ptn^+ males did not reveal a significant effect of the duplications on the dominant PEV enhancer effect of ptn^D.

Complementation analysis with *Df(3L)* chromosomes indicated that *Df(3L)AC1* uncovers the *ptn* locus. ptn^D/*Df(3L)AC1* (= ptn^D/*Df* ptn^-) females do not deposit eggs, suggesting a location of the *ptn* gene within 67B–67D. All the egg primordia in ptn^D/*Df(3L)AC1* degenerate prior to vitellogenesis and excess chromatin condensation is apparent in nurse cell nuclei (Figure 8C). A comparable phenotype is found in egg primordia of ptn^D/ptn^D females (Figure 8).

We have tested all of the *P-lacW* inserts in the 67B–67D region from the DEAK *et al.* (1997) collection of third chromosomal *P*-element-induced mutations for allelism with ptn^D. Two of the *P*-induced mutations ($ptn^{P890/4}$ and $ptn^{P893/2}$) failed to complement ptn^D. The $ptn^D/ptn^{P\text{-}lacW}$ females do not produce vitellogenic egg primordia, and as in ptn^D/ptn^D and ptn^D/*Df* ptn^- females, chromatin of nurse cell nuclei also appears to be more condensed in $ptn^{P893/2}/ptn^D$ and $ptn^{P890/4}/ptn^{P893/2}$ egg primordia (Figure 8, E and F). In these egg primordia propidium iodide does not stain nurse cell cytoplasm, indicating an unusually low amount of RNA (Figure 8, B, C, and F). It remains to be determined whether this indicates repression of gene activity in nurse cell nuclei. Neither of the $ptn^{P\text{-}lacW}$ alleles causes dominant or recessive enhancement of PEV. *In situ* hybridization analysis revealed that the two *P*-element insertions are located in region 67C3-5 (DEAK *et al.* 1997), narrowing the cytogenetic location of the *ptn* locus further.

Reversion analysis of ptn^D: Both X-ray- and *P*-induced revertants of ptn^D were isolated in this study. The X-ray revertants were isolated on the basis of loss of dominant female sterility whereas the *P* revertants were isolated by reversion of PEV enhancement. The two kinds of revertants are designated as ptn^{DrX} and ptn^{DrP}, respectively. From 4728 X-ray-treated ptn^D chromosomes, 11 carried a ptn^{DrX} allele. None of the ptn^{DrX} chromosomes

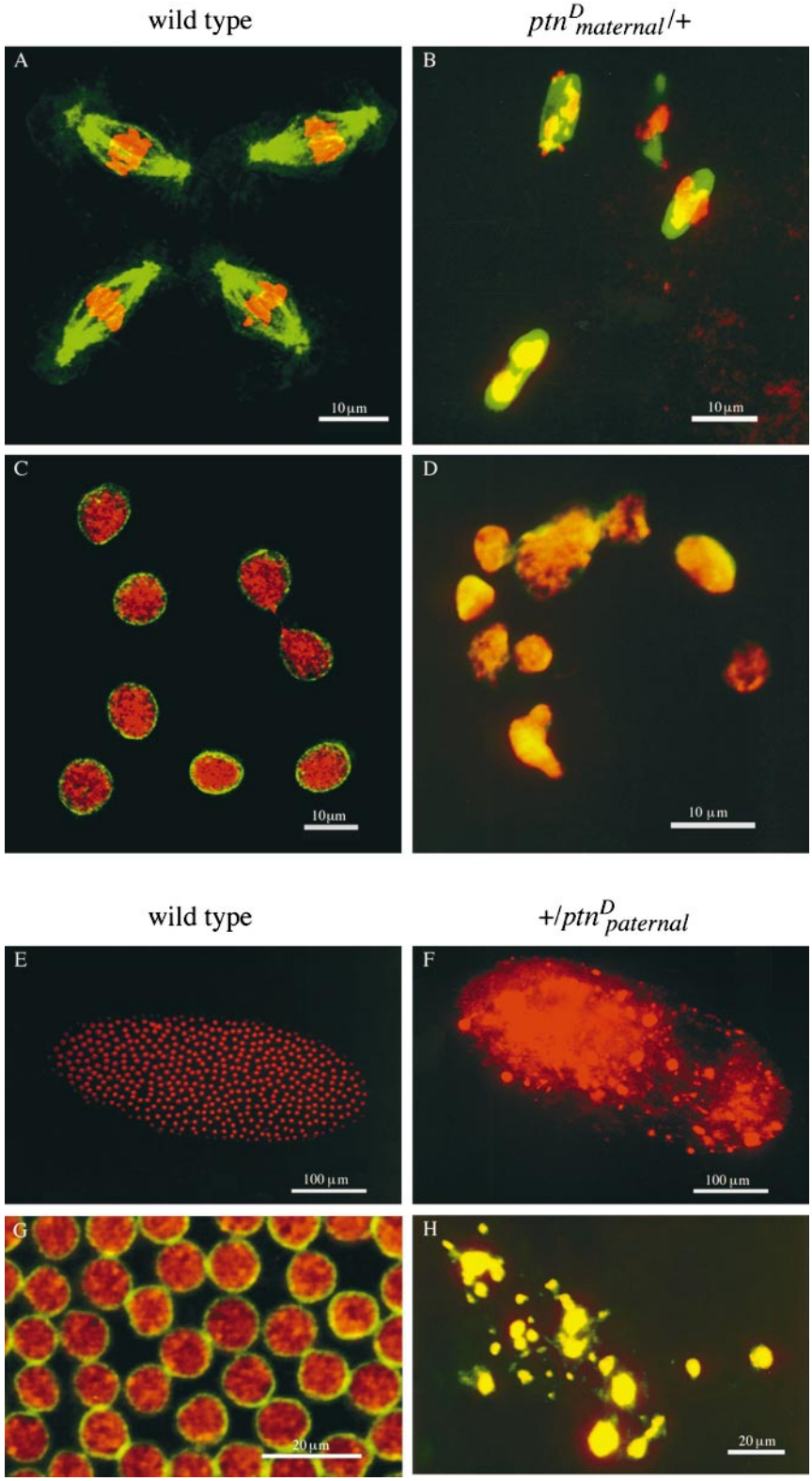

FIGURE 6.—*ptn*^{D} affects structure of early cleavage nuclei. Wild-type embryos (A, C, E, and G), *ptn*^{D}(maternal)/+ (B and D) and +/*ptn*^{D}(paternal) embryos (F and H; *cf.* Figure 4) are shown. In the optical sections, DNA is stained with propidium iodide and labeled red. Nuclear lamina (C, D, G, and H) and tubulin (A and B) appear green. *ptn*^{D}(maternal)/+ embryos show chromatin fragmentation and chromatin bridges during early cleavage (B) and the irregularly sized interphase nuclei appear condensed (D). The +/*ptn*^{D}(paternal) embryos (F and H) show clumps of strongly condensed and fragmented chromatin. Nuclear lamina in wild-type nuclei surrounds chromatin (C and G) whereas it appears disintegrated and is no longer clearly separated from chromatin (yellow staining) in *ptn*^{D}/+ nuclei (D and H). Note the different levels of magnification as labeled in the bars.

enhances PEV in heterozygous condition, showing that *ptn*^{D}-associated dominant female sterility and the enhancement of PEV revert concomitantly; this suggests that the two phenotypes stem from the same mutation. On the other hand, two *ptn*^{DrP} alleles were recovered from among 20,000 chromosomes tested for a loss of the PEV enhancer effects of *ptn*^{D}. The *ptn*^{DrP} alleles do not induce dominant female sterility. The fact that *ptn*^{D} is revertible by these two mutagenic agents supports our contention about its gain-of-function nature. Altogether 9 of the isolated revertant chromosomes are homozygous viable and fertile and only 4 are recessive lethal.

Revertant analysis of the insertional *ptn*$^{P\text{-}lacW}$ alleles: We found that for both *ptn*$^{P\text{-}lacW}$ alleles, precise excis-

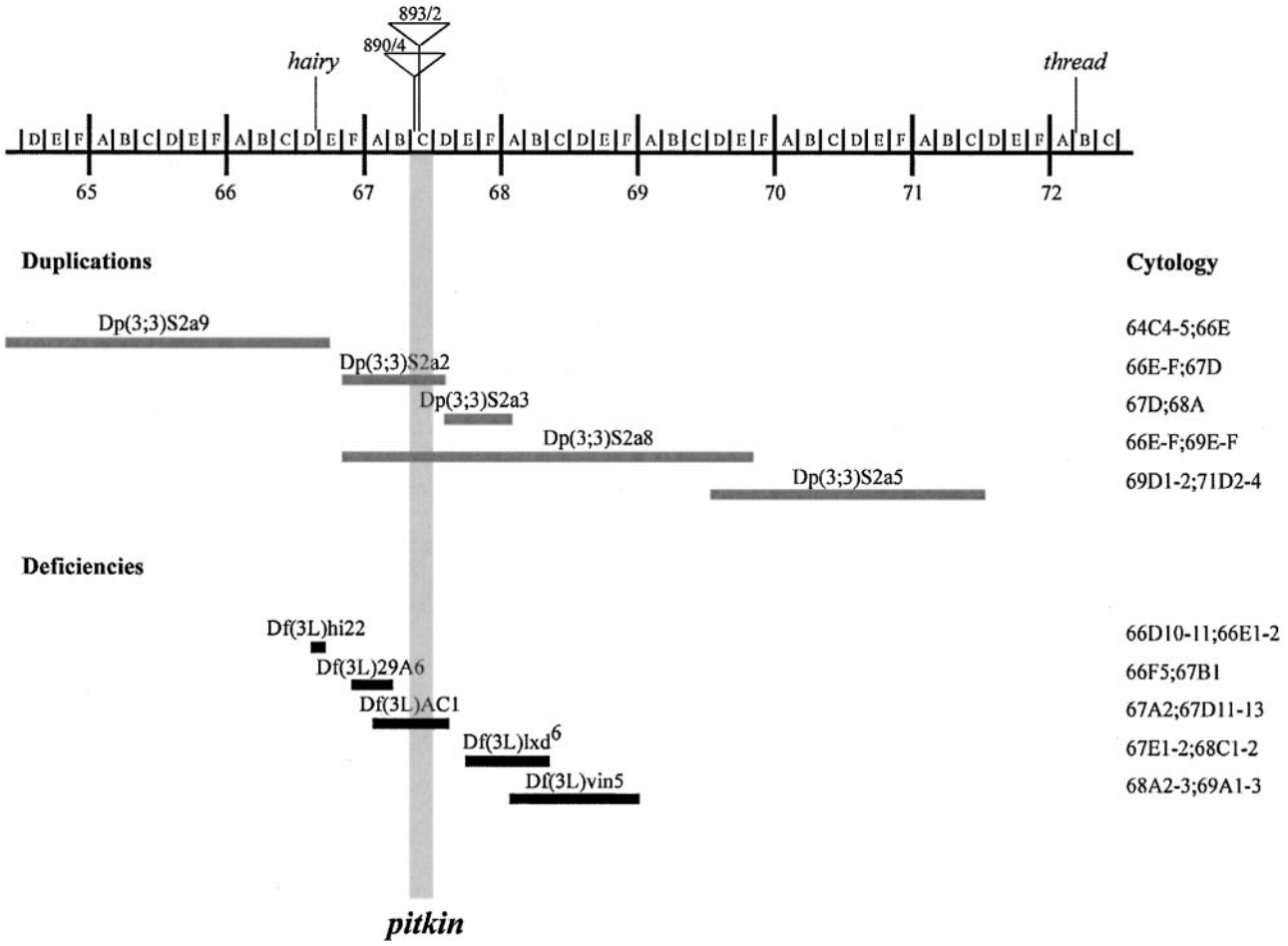

FIGURE 7.—Cytogenetic mapping of the *ptn* gene in region 67C. Breakpoints of duplications [*Dp(3;3)S2a*] and deficiencies [*Df(3L)*] used are indicated at the right. The two *P-lacW* insertions at region 67C3-5 represent recessive *ptn* mutations and showed that the *ptn* locus resides in the 67C3-5 cytological region.

ion results in reversion of the *ptn* mutant phenotype. $ptn^{P\text{-}lacW}$ excisions were selected by loss of the *mini-white* marker gene. In the analysis of $ptn^{P893/2}$ a total of 11 revertant chromosomes were isolated. A short flanking genomic region was cloned by inverse PCR. Consecutive analysis of revertant chromosomes revealed a second defective *P* element ~500 bp next to the *P-lacW* element (Figure 9). PCR analysis using a primer pair complementary to sequences from the genomic region flanking *P-lacW* and the 3′ *P*-repeat yielded two different-sized fragments for the revertant chromosomes. In the $ptn^{P893/2}$ revertants *2*, *3*, *4*, *5*, *8*, *10*, and *11*, a fragment of ~0.8 kb was generated, indicating excision of the *P-lacW* element. In these chromosomes the primers amplified the region between the flanking genomic sequence and the 3′ *P*-repeat of the adjacent defective element. PCR analysis of the $ptn^{P893/2}$ revertants 1, 6, 7, and 9 yielded a 300-bp fragment in all cases, indicating the presence of the 3′ *P*-repeat of the *P-lacW* element (Figure 9). These revertants therefore contain internal deletions within *P-lacW* affecting the *mini-white* marker gene. All revertants that represent excisions of *P-lacW* complement ptn^{D}, whereas those that represent internal deletions within *P-lacW* do not complement ptn^{D} and show an ovarian phenotype identical to $ptn^{D}/Dfptn^{-}$ females. In the analysis of the $ptn^{P890/4}$ allele, a total of 69 w^{-} revertants were isolated. Of these, 22 are recessive female sterile, 41 are homozygous female fertile, and 6 are recessive lethal. With respect to the sterile lines, the homozygous females either did not lay any eggs or deposited only a few eggs that did not develop further. We propose that all of the *ptn* alleles that produce no eggs are amorphs for the *ptn* gene function.

Germ-line mosaics and chimeras show the gain-of-function nature of ptn^{D} as well as an essential function of the *ptn* gene in female germ-line cells: To study whether the ptn^{D}-associated dominant female sterility is the consequence of an altered function of germ-line or somatic cells we analyzed germ-line mosaics. First, mitotic recombination was induced in w^{m4h}/w^{m4h}; $ptn^{D}/ve\ h\ th$ and w^{m4h}/w^{m4h}; $ptn^{D}/se\ ss\ e\ ro$ adult females for the generation of ptn^{D}-free +/+ germ-line cells. If ptn^{D} alters the function of germ-line cells, offspring are expected to derive from the +/+ germ-line cells generated by

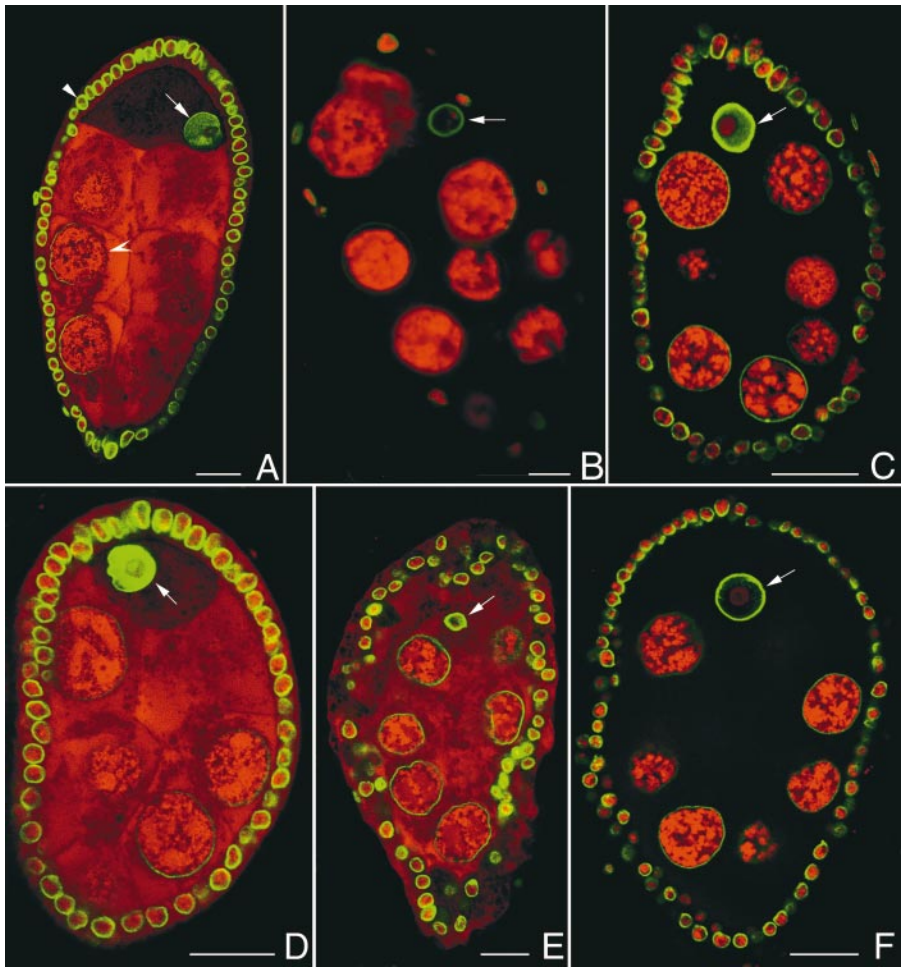

FIGURE 8.—Effects of *ptn* mutations on ovarian development. Optical section of stage 7–8 egg primordia of wild type (A), ptn^{D}/ptn^{D} (B), $ptn^{D}/Df(3L)AC1$ (C), $ptn^{P890/4}/ptn^{D}$ (D), $ptn^{P893/2}/ptn^{D}$ (E), and $ptn^{P893/2}/ptn^{P890/4}$ (F) females. DNA and RNA are stained red and nuclear lamina appears green. Arrows point to oocyte nuclei, the arrowhead in A points to the follicle cells surrounding the egg chamber, and one nurse cell nucleus is indicated with a sharp arrowhead. In ptn^{D}/ptn^{D} (B), $ptn^{D}/Df(3L)AC1$ (C), $ptn^{P893/2}/ptn^{D}$ (E), and $ptn^{P893/2}/ptn^{P890/4}$ (F) mutant egg chambers, the egg cell nucleus develops abnormally. Nuclear lamins become concentrated around the nuclear envelope of the egg cell nucleus and are no longer uniformly distributed as in wild type. Within nurse cell nuclei clumps of condensed chromatin are found frequently (B, C, E, and F). In mutant egg chambers of ptn^{D}/ptn^{D} (B), $ptn^{D}/Df(3L)AC1$ (C), and $ptn^{P893/2}/ptn^{P890/4}$ (F) females, no RNA staining in nurse cell cytoplasm is detected. Furthermore, the number of follicle cells is reduced (B, C, and F) and their arrangement appears abnormal in $ptn^{P893/2}/ptn^{D}$ (E). Bar, 10 μm.

mitotic recombination. Only 2 (0.1%) of the 2029 $ptn^{D}/ve\ h\ th$ and $ptn^{D}/se\ ss\ e\ ro$ irradiated adult females gave rise to one offspring each. Based on the frequency of mitotic recombination, ~200 of the 2029 $ptn^{D}/+$ females were expected to carry ptn^{D}-free germ-line clones (ERDÉLYI and SZABAD 1989). This striking difference between the expected and observed frequencies may be explained by suggesting either that the ptn^{D}-induced sterility originates from altered function of the somatic cells or that ptn^{D} alters function of the female germ line and an extensive perdurance of the ptn^{D} mutant product prevents development of +/+ germ line clones. The latter explanation supports the contention that the ptn^{D} allele has gain-of-function properties. Perdurance of the mutant gene product was already described for a large fraction of dominant female-sterile mutations isolated by ERDÉLYI and SZABAD (1989) and SZABAD *et al.* (1989). To distinguish between a possible perdurance effect or a nongerm-line autonomous function of ptn^{D}, we constructed two types of germ-line chimeras through pole cell transplantation. First, $ptn^{D}/+$ pole cells were transplanted into $ovo^{D}/+$ host embryos. Six chimeras carried $ptn^{D}/+$ germ-line cells (Table 2A). Typical dead embryos with abnormal head skeleton developed in ~50% of their eggs. The other 50% of the eggs showed very early arrest typical for the $ptn^{D}/+$ mutant phenotype. Therefore, ptn^{D}-related female sterility is germ-line dependent. This is further supported by the results of an experiment where nonmutant pole cells were implanted into $ptn^{D}/+$ host embryos (Table 2B). The three chimeras with nonmutant germ-line cells and $ptn^{D}/+$ soma each gave rise to offspring originating from the implanted germ-line cells in addition to their eggs from which no larvae hatched. To further characterize the function of the *ptn* gene in female germ-line and follicle cells, we constructed germ-line chimeras in which normal follicle cells surround *ptn/ptn* germ-line cells (Table 2C). In these studies, the *P*-element-induced recessive female-sterile $ptn^{P890/4}$ and $ptn^{P893/2}$ mutations were used (MATERIALS AND METHODS). Each of the five germ-line chimeras deposited only very few eggs throughout the 2-wk test period. There was no indication of embryonic development, showing that the normal function of the *ptn* gene is required in the germ-line components of the egg primordia.

The possible role of the *ptn* gene in follicle cells was studied following induction of *ptn/ptn* homozygous follicle cell clones in *ptn/Fs(3)Apc* females. After X-ray-induced mitotic recombination in larvae and adults, both the *ptn/Apc* females and the +/*Apc* control females

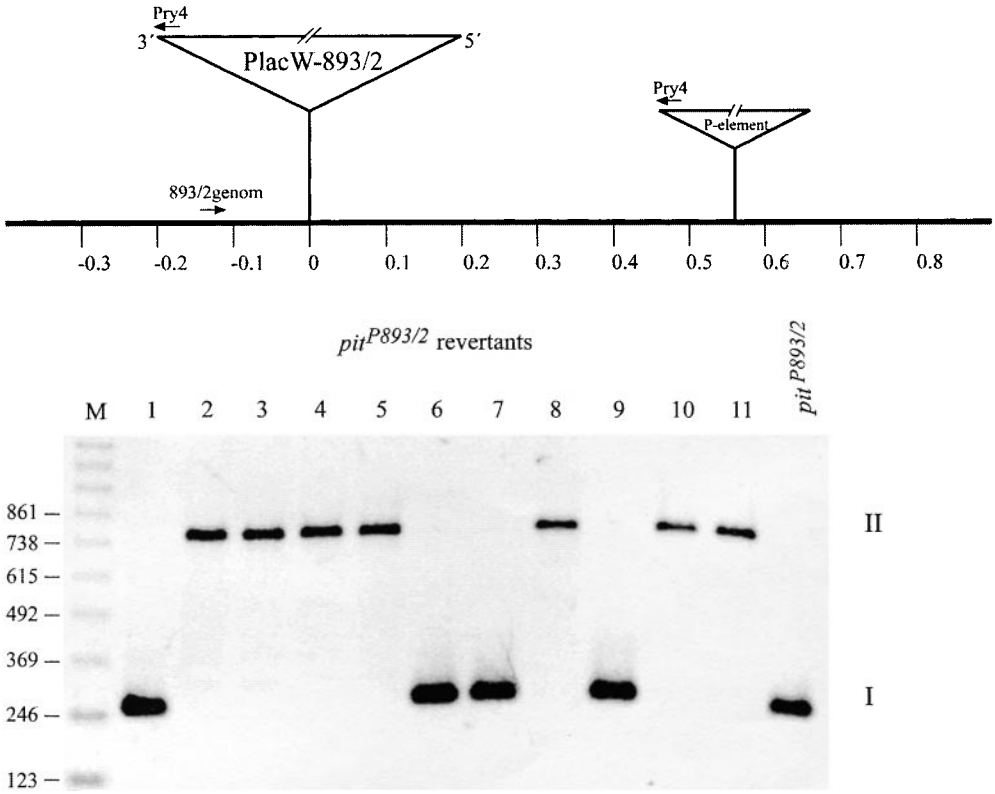

FIGURE 9.—Reversion analysis of $ptn^{P893/2}$. (Top) Organization of the genomic region and (bottom) PCR analysis of revertant chromosomes. Primer 893/2genom: 5′-CAA CGC TCT CTT AAC CTG TC-3′. In revertants due to complete excisions of *P-lacW* an ~800-bp fragment is amplified between 893/2genom and the 3′ *P*-repeat sequences of a second defective element.

produced offspring at similar rates (Table 3), showing that the *ptn* gene is not required for normal follicle cell function. Our analysis of the germ-line and follicle cell mosaics shows that the ptn^D-induced sterility is due to an altered function of female germ-line cells. Our results also provide strong evidence for our contention of a gain-of-function nature for the ptn^D allele, its expression in the female germ line, and induction of embryonic lethality by the maternally contributed ptn^D-encoded mutant gene product.

DISCUSSION

ptn^D represents the first gain-of-function type enhancer of PEV described in Drosophila. Almost all modifier mutations of PEV represent loss-of-function type alleles and only recently gain-of-function type suppressor mutations were identified (CLEGG *et al.* 1998; MOTTUS *et al.* 2000). In addition to its strong dominant enhancer effect on PEV, ptn^D causes dominant female sterility. It affects female germ-line development, early embryogenesis, enhances PEV, induces ectopic *white* gene silencing in somatic cells, and causes ectopic association of heterochromatin protein SU(VAR)3-9 with numerous euchromatic sites. Furthermore, genetic analysis shows that *ptn* represents a novel PEV modifier gene. The known PEV modifier genes are characterized by a dosage-dependent effect on PEV (HENIKOFF 1979; REUTER and SZIDONYA 1983; TARTOF *et al.* 1984; WUSTMANN *et al.* 1989). They manifest a haplo-dependent effect either as suppressors or enhancers of PEV; *i.e.*, a 50% reduction of the wild-type gene product results in a dominant phenotype. In contrast, neither a deletion of the *ptn* gene nor any of the hypomorphic and loss-of-function *ptn* alleles have dominant effects on PEV. Several of the classical PEV modifier genes express triplo-dependent effects; these fall into two groups, haplo-suppressor with a triplo-dependent enhancer effect and haplo-enhancers with a triplo-dependent suppressor effect (WUSTMANN *et al.* 1989). Three of the former genes have been molecularly characterized and all were shown to encode heterochromatin-associated proteins. These genes are *Su(var)2-5*, which encodes HP1 (JAMES and ELGIN 1986; EISSENBERG *et al.* 1992), *Su(var)3-7*, which codes for a protein with multiple zinc fingers (REUTER *et al.* 1990; CLÉARD *et al.* 1997), and *Su(var)3-9*, a gene that encodes a protein containing the chromo and SET domains, two evolutionarily conserved motifs also found in many other chromatin proteins (TSCHIERSCH *et al.* 1994; JENUWEIN *et al.* 1998; SCHOTTA and REUTER 2000). Overproduction of these proteins results in strong enhancement of PEV. Duplications covering the *ptn* gene do not show any effect on PEV. Therefore, the *ptn* gene does not belong to any of the different groups of PEV modifier loci described to date and the various effects of ptn^D are clearly due to its gain-of-function nature. This is supported by the following three findings:

TABLE 2

Features of *ptn^D^*/+ and *ptn*/*ptn* germ-line chimeras

Genotype of embryos		Types and nos. of germ-line chimeras
Donor	Host	
A. *ptn^D^*/+ germ-line cells surrounded by normal soma		
*ptn^D^*/*ve h th*	*ovo^D1^*/+	*ptn^D^*/*ve h th*: 6
ve h th/*TM3*	*ovo^D1^*/+	*ve h th*/*TM3*: 10
B. Normal germ-line cells surrounded by *ptn^D^*/+ soma[a]		
+/+	*ptn^D^*/*ve h th*	*ptn^D^*/*ve h th*: 3[b]
+/+	*ve h th*/*TM3*	*ve h th*/*TM3*: 9[c]
C. *ptn*/*ptn* germ-line cells surrounded by normal soma		
*ptn^P893/2^*/*TM6b*	*ovo^D1^*/+	6
*ptn^P890/4^*/*TM3*	*ovo^D1^*/+	2
TM3/*TM6b*	*ovo^D1^*/+	4
*ptn^P893/2^*/*ptn^P890/4^*	*ovo^D1^*/+	5[d]

In A the donor and in B the host embryos derived from a cross between *w^m4h^*/*w^m4h^*; *ve h th*/*ve h th* females and *w^m4h^*/*Y*; *ptn^D^*/*TM3* males. Pole cells of the donor embryos in A and C were transplanted into host embryos that derived from a cross of wild-type (Canton-S) females and *ovo^D1^*/Y males. In C donor embryos derived from a cross of *y w*/*y w*; *ptn^P890/4^*/*TM6b, Tb* females with *y w*/Y; *ptn^P893/2^*/*TM3, Sb Ser* males. Pole cells of the donor embryos were transplanted into host embryos that derived from a cross of wild-type females with *ovo^D1^*/Y males. Eclosing females were mated with *y w*/Y males.

[a] The donor embryos in B derived from a cross of *fs(1)K10 w*/*ClB* females with *fs(1)K10 w*/Y males. Their genotypes are *fs(1)K10 w*/*fs(1)K10 w* and *ClB*/*fs(1)K10 w*.

[b] All embryos received *K10 w*/*ClB* pole cells from which progeny developed. Eggs of the *ptn^D^*/*ve h th* host females did not hatch.

[c] Besides their own eggs six of the *ve h th*/*TM3* host females deposited *K10* eggs and three produced offspring from *K10 w*/*ClB* pole cells (besides their own progeny).

[d] Chimeras with *ptn^P893/2^*/*ptn^P890/4^* germ-line cells did not lay eggs. Their egg primordia were similar to those that develop in *ptn^D^*/*Df(3L)AC1* females.

1. In contrast to the situation for haplo-insufficient *E(var)* mutations, the PEV enhancer effect of *ptn^D^* is not rescued in flies that carry an extra copy of the normal *ptn* gene as in *ptn^D^*/+/+ triploids (data not shown) or in *ptn^D^*/+/*Dp ptn^+^* flies.
2. The *ptn^D^* PEV enhancer effect and the *ptn^D^*-associated dominant female sterility can be reverted by elimination of the function of the *ptn^D^* allele. Concomitant reversion of both mutant phenotypes suggests that they originate from the same mutation.
3. As shown by the analysis of different types of mosaics, the *ptn^D^*-induced dominant female sterility is caused by an altered function of the gene in female germ-line cells.

The gain-of-function nature of *ptn^D^* becomes apparent following induction of +/+ germ-line clones in *ptn^D^*/+ females by X-ray-induced mitotic recombination. The +/+ germ-line clones only appear at a very low frequency. Behavior of these +/+ clones is best explained by a perdurance of the *ptn^D^* mutant gene product. Similar behavior of the +/+ germ-line clones was reported previously for many other dominant female-sterile mutations (Erdélyi and Szabad 1989; Szabad *et al.* 1989).

Our genetic data show that the *ptn* gene product functions during female germ-line development and early embryogenesis. All the effects of *ptn^D^* can best be explained by suggesting an antimorphic nature of the *ptn^D^* mutant gene product. It appears to strongly reduce the activity of its normal maternally provided and zygotically synthesized counterpart. The two different phenotypes produced by *ptn^D^*/+ females indicate a substantial maternal contribution as well as a very early zygotic activity of the *ptn* gene. A *ptn* deficiency heterozygote does not display such an effect probably because there is sufficient maternally provided and zygotically produced wild-type product in early embryos. The very early arrest of cleavage in *ptn^D^*/+ embryos derived from the cross

TABLE 3

The effect of *ptn^P893/2^* on homozygous mutant follicle cell clones

Irradiation	Genotype of females	Females tested[a]	Mosaics	Frequency of mosaicism (%)	Offspring	Offspring production[b]
None	+/*Apc*	226	1	0.4	1	0.12×10^{-5}
None	*ptn^P893/2^*/*Apc*	218	1	0.5	1	0.14×10^{-5}
Larvae	+/*Apc*	359	30	8.3	43	2.6×10^{-5}
Larvae	*ptn^P893/2^*/*Apc*	600	60	10.0	91	1.9×10^{-5}
Adults	+/*Apc*	241	17	5.0	9	0.9×10^{-5}
Adults	*ptn^P893/2^*/*Apc*	398	20	5.0	40	1.7×10^{-5}

*ptn^P893/2^*/*Fs(3)Apc* and control +/*Fs(3)Apc* larvae or adults were irradiated with 1500 R of X rays for induction of *ptn^P893/2^*/*ptn^P893/2^* (+/+ in the control) follicle cell clones through mitotic recombination. Both the *Fs(3)Apc* mutation and the *ptn* gene are located on chromosome arm 3L.

[a] All females were tested for a minimum of 14 days.

[b] Offspring/female/day.

of $ptn^D/+$ females to $+/+$ males is likely caused by the antimorphic effects of both the maternally provided and the zygotically produced pit^D mutant product. Viability of $+/ptn^D$ (paternal) embryos produced by $+/+$ females crossed to $ptn^D/+$ males can therefore be explained by the presence of a sufficient amount of maternal ptn^+ gene product. These embryos survive but the early effect of ptn^D on chromatin regulation becomes visible by its strong enhancement of PEV and ectopic silencing of *mini-white* gene inserts as well as induction of ectopic SU(VAR)3-9 binding at >100 sites along all chromosomes. An early zygotic activity of the paternally inherited ptn^D allele is also indicated by lethality of $+/ptn^D$ (paternal) embryos derived from a cross of $ptn^D/+$ females with $ptn^D/+$ males. In all these genotypes, the amount of *ptn* wild-type product might be reduced far below 50% by the antimorphic nature of the ptn^D mutant product. Homozygotes for the recessive *ptn* mutation produced by a cross of *ptn*/+ females with *ptn*/+ or *ptn*/*ptn* males do not show a dominant enhancer effect on PEV because the maternally provided wild-type gene product is sufficient for normal cleavage and chromatin assembly.

The ptn^D causes ectopic association of heterochromatin protein SU(VAR)3-9 with numerous sites along all euchromatic parts of the Drosophila chromosome complement. Rea *et al.* (2000) showed a site-specific histone H3 methyltransferase activity of mammalian SU(VAR)3-9 proteins. Overexpression of *Su(var)3-9* and *SUV39H1* enhances heterochromatin-induced gene silencing in Drosophila and mammalian cell lines, respectively (Tschiersch *et al.* 1994; Firestein *et al.* 2000). All these data predict a key role of the SU(VAR)3-9 protein in assembly of higher-order chromatin structure and gene silencing in heterochromatin. The ptn^D mutation might cause nucleation of heterochromatin-like structures at ectopic SU(VAR)3-9 binding sites. Therefore, silencing of *mini-white* gene inserts observed in $ptn^D/+$ flies might be caused by formation of ectopic heterochromatin. An influence of ptn^D on higher-order chromatin structure in heterochromatic regions is indicated by strong enhancement of PEV. According to this model, the ptn^D mutation might interfere with both chromosomal distribution as well as architecture and function of heterochromatin protein complexes. Embryonic lethality induced by ptn^D might also be caused by its possible regulatory effect on distribution and function of heterochromatin protein complexes. Additive effects of maternally provided and zygotically produced ptn^D mutant product could result in extensive heterochromatization all over the genome. Such a model would also explain the abnormal morphological structure of cleavage nuclei in $ptn^D/+$ embryos and of nurse cell nuclei in egg chambers of mutant heterozygous and homozygous females.

Alternatively, the *ptn* gene-encoded product might be an abundant chromatin protein associated with eu- and heterochromatic regions and its loss could result in increased accessibility of chromatin for components inducing compaction and gene silencing. As a consequence ptn^D might affect normal transition of cleavage chromatin into chromatin of somatic cells with its subdivision into eu- and heterochromatin. If the *ptn* gene product represents a factor required to maintain an open chromatin conformation, its absence could result in excess chromatin condensation because activities of chromatin condensing factors are no longer balanced. Such an explanation is also consistent with our data showing an early zygotic lethal effect of ptn^D. Complete elimination of the *ptn* gene causes an arrest of development during female germ-line differentiation and its function in early embryonic development can be revealed only by the help of specific types of mutant alleles.

The ptn^D-encoded antimorphic mutant gene product might reduce the activity of the ptn^+ allele through formation of nonfunctional dimers or by competition for a common interaction partner for heteromer formation. This type of heteromer disruption has been shown in studies of other dominant female-sterile mutations. The mutant tub67C α-tubulin molecules encoded by the $Tomaj^D$ dominant female-sterile alleles disrupt microtubule formation (Mathé *et al.* 1998) and the importin-β molecules encoded by the $Ketel^D$ dominant female-sterile mutations compete with normal importin-β counterparts for interaction with importin-α (J. Szabad, unpublished data).

Processes of chromatin transition at the end of cleavage, when the cell cycle is prolonged (Edgar and O'Farrell 1989), H1 histones are incorporated into chromatin (Ner and Travers 1994), and heterochromatin is formed (*cf.* Foe *et al.* 1993), are only poorly understood.

The *ptn* gene appears to represent a candidate gene involved in control of euchromatin-heterochromatin balance. The gain-of-function type ptn^D mutation induces strong enhancement of heterochromatin-induced gene silencing in PEV as well as transgene repression within euchromatic regions. It could act in either a structural or regulatory fashion to influence global processes of chromatin regulation during early development. Exceptional gain-of-function alleles such as ptn^D will serve as useful tools for molecular analysis of these processes. This study provides the first step toward the molecular definition of *ptn* function in the control of chromatin regulation during development of female germ-line cells, the formation of cleavage nuclei chromatin, and its transition into higher-order chromatin structures typical for somatic cells.

We are grateful to our colleagues Drs. B. Wakimoto, M. Erdélyi, J. Szidonya, S. Pimpinelli, and R. Dorn for discussions and their critical comments on the manuscript. We thank Drs. Y. Grünbaum and H. Saumweber for the anti-lamin antibody sample. The article greatly benefited from the referees' comments on a previous version of the manuscript. This work was supported by grants from Deutsche

Forschungsgemeinschaft (Re911/2-2) to G.R. and from OTKA 992 to J.S.

LITERATURE CITED

Baker, W. K., 1971 Evidence for position-effect suppression of the ribosomal RNA cistrons in *Drosophila melanogaster*. Proc. Natl. Acad. Sci. USA **68:** 2472–2476.

Baksa, K., H. Morawietz, V. Dombradi, M. Axton, H. Taubertet *et al.*, 1993 Mutations in the protein phosphatase 1 gene at 87B can differentially affect suppression of position-effect variegation and mitosis in *Drosophila melanogaster*. Genetics **135:** 117–125.

Boivin, A., and J.-M. Dura, 1998 In vivo chromatin accessibility correlates *with* gene silencing in Drosophila. Genetics **150:** 1539–1549.

Büchner, K., P. Roth, G. Schotta, V. Krauss, H. Saumweber *et al.*, 2000 Genetic and molecular complexity of the PEV modifier *mod(mdg4)* in Drosophila. Genetics **155:** 141–157.

Busson, D., M. Gans, K. Komitopoulou and M. Mason, 1983 Genetic analysis of three dominant female-sterile mutations located on the X-chromosome of *Drosophila melanogaster*. Genetics **105:** 309–325.

Cléard, F., M. Delattre and P. Spierer, 1997 SU(VAR)3-7, a Drosophila heterochromatin-associated protein and companion of HP1 in the genomic silencing of position-effect variegation. EMBO J. **16:** 5280–5288.

Clegg, N. J, B. M. Honda, I. P. Whitehead, T. A. Grigliatti, B. Wakimoto *et al.*, 1998 Suppressors of position-effect variegation in Drosophila melanogaster affect expression of the heterochromatic gene light in the absence of a chromosome rearrangement. Genome **41:** 495–503.

Craymer, L., 1984 New mutants. Dros. Inf. Serv. **60:** 234–236.

Deak, P., M. M. Omar, R. D. C. Saunders, M. Pal, O. Komonyi *et al.*, 1997 P-element insertion alleles of essential genes on the third chromosome of *Drosophila melanogaster*: correlation of physical and cytogenetic maps in chromosomal region 86E–87F. Genetics **147:** 1697–1722.

DeRubertis, F., D. Kadosh, S. Henchoz, D. Pauli, G. Reuter *et al.*, 1996 The histone deacetylase RPD3 counteracts genomic silencing in Drosophila and yeast. Nature **384:** 589–591.

Dorn, R., S. Heymann, R. Lindigkeit and G. Reuter, 1986 Suppressor mutation of position-effect variegation in *Drosophila melanogaster* affecting chromatin properties. Chromosoma **93:** 398–403.

Dorn, R., V. Krauss, G. Reuter and H. Saumweber, 1993 The enhancer of position-effect variegation of Drosophila, *E(var)3-93D*, codes for a chromatin protein containing a conserved domain common to several transcription regulators. Proc. Natl. Acad. Sci. USA **90:** 11376–11380.

Eberl, D. F., L. J. Lorenz, M. B. Melnik, V. Sood, P. Lasko *et al.*, 1997 A new enhancer of position-effect variegation in Drosophila encodes a putative RNA helicase that binds chromosomes is regulated by the cell cycle. Genetics **146:** 951–963.

Edgar, B. A., and P. H. O'Farrell, 1989 Genetic control of cell division patterns in the Drosophila embryo. Cell **57:** 177–187.

Eissenberg, J. C., T. C. James, D. M. Foster-Hartnett, T. Hartnett, V. Ngan *et al.*, 1990 Mutation in a heterochromatin-specific chromosomal protein is associated with suppression of position-effect variegation in *Drosophila melanogaster*. Proc. Natl. Acad. Sci. USA **87:** 9923–9927.

Eissenberg, J. C., G. D. Morris, G. Reuter and T. Hartnett, 1992 The heterochromatin-associated protein HP-1 is an essential protein in Drosophila with dosage-dependent effects on position-effect variegation. Genetics **131:** 345–352.

Erdélyi, M., and J. Szabad, 1989 Isolation and characterization of dominant female sterile mutations of *Drosophila melanogaster*. I. Mutations on the third chromosome. Genetics **122:** 111–127.

Firestein, R., X. Cui, P. Huie and M. L. Cleary, 2000 Set domain-dependent regulation of transcriptional silencing and growth control by SUV39H, a mammalian ortholog of Drosophila Su(var)3-9. Mol. Cell. Biol. **20:** 4900–4909.

FlyBase, 2000 The Drosophila database. Available from World Wide Web at the URLs http://morgan/harvard.edu and http://www.ebi.ac.uk/flybase/.

Foe, V. E., G. M. Odell and B. A. Edgar, 1993 Mitosis and morphogenesis in the Drosophila embryo: point and counterpoint, pp. 149–300 in *The Development of Drosophila melanogaster*, edited by M. Bate and A. Martinez Arias. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY.

Gerasimova, T. I., D. A. Gdula, D. V. Gerasimov, O. Simonova and V. G. Corces, 1995 A Drosophila protein that imparts directionality on a chromatin insulator is an enhancer of position-effect variegation. Cell **82:** 587–597.

Gloor, G., and W. Engels, 1992 Single-fly DNA preps for PCR. Dros. Inf. Serv. **71:** 148–149.

Grigliatti, T., 1991 Position-effect variegation—an assay for nonhistone chromosomal proteins and chromatin assembly and modifying factors. Methods Cell Biol. **35:** 587–627.

Henikoff, S., 1979 Position-effects and variegation enhancers in an autosomal region of *Drosophila melanogaster*. Genetics **93:** 105–115.

Henikoff, S., 1997 Nuclear organization and gene expression: homologous pairing and long-range interactions. Curr. Opin. Cell Biol. **9:** 388–395.

Ilmensee, K., 1973 The potentialities of transplanted early gastrula nuclei of *D. melanogaster*. Production of their imago descendants by germ line transplantation. Wilhelm Roux Arch. **171:** 331–343.

James, T. C., and S. C. R. Elgin, 1986 Identification of nonhistone chromosomal protein associated with heterochromatin in Drosophila and its gene. Mol. Cell. Biol. **6:** 3862–3872.

Jenuwein, T., G. Laible, R. Dorn and G. Reuter, 1998 SET domain proteins modulate chromatin domains in eu- and heterochromatin. Cell. Mol. Life Sci. **54:** 80–93.

Jürgens, G., R. Lehmann, M. Schardin and C. Nüsslein-Volhard, 1986 Segmental organisation of the head in the embryo of *Drosophila melanogaster*. Roux's Arch. Dev. Biol. **195:** 359–377.

Krejci, E., V. Garzino, C. Mary, N. Bennani and J. Pradel, 1989 Modulo, a new maternally expressed Drosophila gene encodes a DNA-binding protein with distinct acidic and basic regions. Nucleic Acids Res. **17:** 8101–8115.

Komitopoulou, K., M. Gans, L. Margaritis and F. C. Kafatos, 1983 Isolation and characterization of sex-linked female-sterile mutants in *Drosophila melanogaster* with special attention to eggshell mutants. Genetics **105:** 897–920.

Lindsley, D. L., and G. G. Zimm, 1992 *The Genome of Drosophila melanogaster*. Academic Press, San Diego/London.

Lu, B. Y., C. P. Bishop and J. C. Eissenberg, 1996 Developmental timing and tissue specificity of heterochromatin-mediated silencing. EMBO J. **15:** 1323–1332.

Mathé, E., I. Boros, K. Josvay, K. Li, J. Puro *et al.*, 1998 The tomaj mutant alleles of α-*Tubulin67C* reveal a requirement for the encoded maternal specific tubulin isoform in the sperm aster, the cleavage spindle apparatus and neurogenesis during embryonic development in Drosophila. J. Cell Sci. **111:** 887–896.

Mottus, R., R. Sobel and T. A. Grigliatti, 2000 Mutational analysis of a histone deacetylase in *Drosophila melanogaster*: missense mutations suppress gene silencing associated with position effect variegation. Genetics **154:** 657–668.

Ner, S. S., and A. Travers, 1994 HMG-D, the *Drosophila melanogaster* homologue of HMG 1 protein, is associated with early embryonic chromatin in the absence of histone H1. EMBO J. **13:** 1817–1822.

Paddy, M. R., H. Saumweber, D. A. Agard and J. W. Sedat, 1996 Time-resolved, in vivo studies of mitotic spindle formation and nuclear lamina breakdown in Drosophila early embryos. J. Cell Sci. **109:** 591–607.

Perrin, L., O. Demakova, L. Fanti, S. Kallenbach, S. Saingery *et al.*, 1998 Dynamics of the sub-nuclear distribution of Modulo and the regulation of position-effect variegation by the nucleolus in Drosophila. J. Cell Sci. **111:** 2753–2761.

Rea, S., F. Eisenhaber, D. O'Carroll, B. D. Strahl, Z-W. Sun *et al.*, 2000 Regulation of chromatin structure by site-specific histone H3 methyltransferases. Nature **406:** 593–599.

Reuter, G., and P. Spierer, 1992 Position effect variegation and chromatin proteins. BioEssays **14:** 605–612.

Reuter, G., and J. Szidonya, 1983 Cytogenetic analysis of variegation suppressors and a dominant temperature sensitive lethal in region 23–26 of 2L in *Drosophila melanogaster*. Chromosoma **88:** 277–285.

Reuter, G., and I. Wolff, 1981 Isolation of dominant suppressor mutations for position-effect variegation. Mol. Gen. Genet. **182:** 516–519.

Reuter, G., I. Wolff and B. Friede, 1985 Functional properties

of the heterochromatic sequences inducing w^{m4} position-effect variegation in *Drosophila melanogaster*. Chromosoma **93:** 132–139.

Reuter, G., M. Giarre, J. Farah, J. Gausz, A. Spierer *et al.*, 1990 Dependence of position-effect variegation in Drosophila on dose of a gene encoding an unusual zinc-finger protein. Nature **344:** 219–223.

Reuter, G., G. Hoffman, R. Dorn and H. Saumweber, 1993 Construction and characterization of *TM3* balancer carrying P[(ry^+)Δ2-3] as a stable transposase source. Dros. Info. Serv. **72:** 78–79.

Rudolph, T., B. Lu, T. Westphal, J. Szidonya, J. Eissenberg *et al.*, 1999 New type of CyO and TM3 green balancers. Dros. Inf. Serv. **78:** 99–100.

Schotta, G., and G. Reuter, 2000 Controlled expression of tagged proteins in Drosophila using a new modular P-element vector system. Mol. Gen. Genet. **262:** 916–920.

Seum, C., A. Spierer, D. Pauli, J. Szidonya, G. Reuter *et al.*, 1996 Position-effect variegation in Drosophila depends on the dose of the gene encoding the E2F transcriptional activator and cell cycle regulator. Development **122:** 1949–1956.

Silver, L. M., and S. C. R. Elgin, 1978 Production and characterization of antisera against three individual NHC proteins: a case of a generally distributed NHC protein. Chromosoma **68:** 101–114.

Sinclair, D. A. R., R. C. Mottus and T. A. Grigliatti, 1983 Genes which suppress position effect variegation in *Drosophila melanogaster* are clustered. Mol. Gen. Genet. **191:** 326–333.

Sinclair, D. A. R., N. J. Clegg, A. Antonchuk, T. A. Milne, K. Stankunas *et al.*, 1998 Enhancer of Polycomb is a suppressor of position-effect variegation in *Drosophila melanogaster*. Genetics **148:** 211–220.

Spradling, A. C., 1993 Developmental genetics of oogenesis, pp. 1–70 in *The Development of Drosophila melanogaster*, edited by M. Bate and A. Martinez Arias. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY.

Szabad, J., M. Erdélyi, G. Hoffmann, J. Szidonya and T. R. F. Wright, 1989 Isolation and characterization of dominant female sterile mutations of *Drosophila melanogaster*. II. Mutations on the second chromosome. Genetics **122:** 823–835.

Tartof, K. D., C. Hobbs and M. Jones, 1984 A structural basis for variegating position effects. Cell **37:** 869–878.

Theurkauf, W. E., and R. S. Hawley, 1992 Meiotic spindle assembly in Drosophila females: behaviour of nonexchange chromosomes and the effects of mutations in the nod kinesin-like protein. J. Cell Biol. **166:** 1167–1180.

Tschiersch, B., A. Hofmann, V. Krauss, R. Dorn, G. Korge *et al.*, 1994 The protein encoded by the Drosophila position-effect variegation suppressor gene *Su(var)3-9* combines domains of antagonistic regulators of homeotic gene complexes. EMBO J. **13:** 3822–3831.

Van Deusen, E. B., 1977 Sex determination in germ line chimeras in *Drosophila melanogaster*. J. Embryol. Exp. Morphol. **37:** 173–185.

Weiler, K. S., and B. T. Wakimoto, 1995 Heterochromatin and gene expression in Drosophila. Annu. Rev. Genet. **29:** 577–605.

Weiler, K. S., and B. T. Wakimoto, 1998 Chromosome rearrangements induce both variegated and reduced, uniform expression of heterochromatic genes in a development-specific manner. Genetics **149:** 1451–1464.

Wieschaus, E., and C. Nüsslein-Volhard, 1986 Looking at embryos, pp. 199–227 in *Drosophila: A Practical Approach*, edited by D. B. Roberts. IRL Press, Oxford.

Wieschaus, E., J. L. Marsh and W. T. Gehring, 1978 *fs(1)K10* a germ line-dependent female sterile mutation causing abnormal chorion morphology in *Drosophila melanogaster*. Wilhelm Roux Arch. Entwicklungsmech. Org. **184:** 75–82.

Wustmann, G., J. Szydonya, H. Taubert and G. Reuter, 1989 The genetics of position-effect variegation modifying loci in *Drosophila melanogaster*. Mol. Gen. Genet. **217:** 520–527.

Communicating editor: T. C. Kaufman