Characterization of *Suppressor of fused*, a Complete Suppressor of the *fused* Segment Polarity Gene of *Drosophila melanogaster*

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ABSTRACT

fused (*fu*) is a maternal effect segment polarity gene of *Drosophila melanogaster*. In addition, *fu* females have tumorous ovaries. Two ethyl methanesulfonate mutageneses were carried out in order to isolate suppressors of the fu phenotype. A new gene, *Suppressor of fused* (*Su(fu)*), was identified. It is located in the 87C8 region of the third chromosome. *Su(fu)* displays a maternal effect and is also expressed later in development. Although $Su(fu)^{LP}$ is a complete loss-of-function mutation, it is homozygous viable and has no phenotype by itself. *Su(fu)* fully suppresses the embryonic and adult phenotypes of *fu* mutants. *Su(fu)* mutations are semidominant and a $Su(fu)^{+}$ duplication has an opposite effect, enhancing the fused phenotype. It is proposed therefore that the $Su(fu)^{+}$ product is involved in the same developmental step as the Fu^{+} kinase. Thus, a new gene interacting with the segment polarity pathway was identified using an indirect approach.

EMBRYONIC development is under genetic control. In *Drosophila melanogaster*, extensive genetic and molecular analyses have allowed characterization of hierarchical interactions between several classes of genes (reviewed in INGHAM 1988). One of these corresponds to the segment polarity genes, which are required within each embryonic segment in order to define specific anteroposterior domains (NÜSSLEIN-VOLHARD and WIESCHAUS 1980). Fourteen such segment polarity genes have been identified so far (reviewed in INGHAM and NAKANO 1990; INGHAM 1991). They form a very particular class of segmentation genes as they encode a large variety of products. Wingless is a secreted protein (RIJSEWIJK *et al.* 1987; VAN DEN HEUVEL *et al.* 1989; GONZÁLEZ *et al.* 1991), Armadillo is the homolog of Plakoglobin, a mammalian protein associated to cell junctions (PEIFER and WIESCHAUS 1990), Patched is a transmembrane protein (HOOPER and SCOTT 1989; NAKANO *et al.* 1989), Fused (PRÉAT *et al.* 1990) and Shaggy (BOUROUIS *et al.* 1990; SEIGFIRED *et al.* 1990) are putative serine/threonine kinases, and Engrailed (FJOSE, MCGINNIS and GEHRING 1985; DESPLAN, THEIS and O'FARRELL 1985), Gooseberry (BOPP *et al.* 1986; BAUMGARTNER *et al.* 1987), and Cubitus-interruptus Dominant (ORENIC *et al.* 1990) are transcriptional regulators which possess specific DNA binding domains. The initial expression of some segment polarity genes is controlled by proteins encoded by the pair-rule genes (HOWARD and INGHAM 1986; DINARDO and O'FARRELL 1987; INGHAM, BAKER and MARTINEZ-ARIAS 1988), which define another class of segmentation genes. After cellularization of the embryo, the expression of segment polarity genes is under the control of segment polarity gene products themselves (MARTINEZ-ARIAS, BAKER and INGHAM 1988; DINARDO *et al.* 1988; HIDALDO and INGHAM 1990; EATON and KORNBERG 1990; HIDALGO 1991; HEEMSKERK *et al.* 1991). Cell-cell interactions are thought to take place during this second phase, but the actual molecular mechanisms involved remain largely unknown. Unlike most other segmentation mutations, segment polarity mutations generally affect both embryonic and adult development. Furthermore, the corresponding phenotypes and the expression patterns can be related in some cases (reviewed in WILKINS and GUBBS 1991), suggesting that segment polarity products have similar functions at different developmental stages.

fused (*fu*) is a maternal effect segment polarity gene located on the *X* chromosome (1-59.5) (NÜSSLEIN-VOLHARD and WIESCHAUS 1980; GERGEN and WIESCHAUS 1986; PERRIMON and MAHOWALD 1987; BUSSON *et al.* 1988) (Figure 1). *fu* embryos derived from *fu* females show a deletion of the posterior part of each thoracic and abdominal segment and, ventrally, a mirror duplication of the anterior part which bears the denticles rows. Abnormal cell death has been shown to occur in the ectoderm and the mesoderm of developing *fu* embryos, which may account for the terminal deletion pattern (MARTINEZ-ARIAS 1985). *fu* embryos derived from heterozygous *fu*/+ females develop normally because of the product (RNA or protein) accumulated in the oocytes, but *fu* adults present several defects due to its lack during metamorphosis

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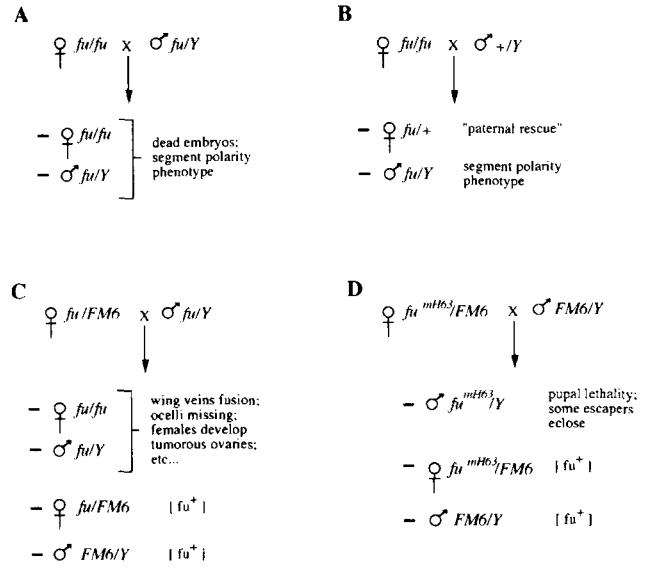

FIGURE 1.—Genetic properties of *fu* mutations. (A) Maternal effect leading to a segment polarity phenotype. (B) Paternal rescue of the maternal effect. This rescue is very efficient in the case of weak *fu* alleles (recovery of adults), but it is low in the case of strong *fu* alleles (slightly rescued segment polarity phenotype) (PERRIMON and MAHOWALD 1987; BUSSON *et al.* 1988). (C) Zygotic effect of weak *fu* alleles. (D) Zygotic effect of strong *fu* alleles (pupal lethals).

(WURST and HANRATTY 1979) and adult life. One of these defects is the fusion of the third and fourth wing veins, which gave its name to the mutation (MORGAN and BRIDGES 1916). This phenotype was reinterpretated by FAUSTO-STERLING (1978) as a lack of the fourth vein and thickening of the third one. This interpretation matches the one of the embryonic phenotype, since in both cases there is failure to form a posterior structure and duplication of an anterior one. In addition, adult *fu* females display reduced fecundity due to several ovarian defects including the appearance of tumorous egg chambers (KING 1959; SMITH and KING 1966). We have cloned and sequenced the *fused* gene (PRÉAT *et al.* 1990). It encodes a putative serine/threonine kinase, which indicates the fundamental role of post-translational modifications in intrasegmental pattern formation.

I have characterized a new gene, *Suppressor of fused*, amorphic forms of which fully suppress all fu phenotypes. Suppression effects have been previously described among segmentation mutations (COULTER and WIESCHAUS 1988; HÜLSKAMP *et al.* 1989; IRISH, LEHMANN and AKAM 1989; LIMBOURG-BOUCHON, BUSSON and LAMOUR-ISNARD 1991; HIDALGO 1991; E. WIESCHAUS, unpublished result cited in INGHAM, TAYLOR and NAKANO 1991), but this is the first report of mutageneses specifically designed to isolate suppressors of an abnormal segmentation phenotype. Genetic properties of *Su(fu)* mutations have been extensively analyzed. The results suggest that the expression of *Su(fu)* is similar to that of *fu*, but that the two gene products have opposite roles throughout development.

MATERIALS AND METHODS

Stocks and culture: fu^1 is hypomorphic allele recovered as a spontaneous mutation (MORGAN and BRIDGES 1916). The fu^1 stock in this study was marked with the f^{36a} mutation. fu^{mH63} is a strong EMS-induced allele (WURST and HANRATTY 1979; BUSSON *et al.* 1988). $Df(1)fu^{Z4}$ is a 40-kb diepoxybutane-induced deletion (BUSSON *et al.* 1988) which covers the entire *fu* locus (MARIOL, PRÉAT and BOUCHON 1987). Some of the genetic properties of these *fu* alleles are described in BUSSON *et al.* (1988). The Oregon-R and *w* stocks used for the mutageneses had been isogenized for the *X* chromosome in our laboratory and therefore, although not isogenic, the autosomal chromosomes had been generated out of a small pool. The $l(3)SzC^3$, $l(3)SzD^8$, $Df(3R)kar^{Sz11}$ (87C7-8;87E5-6), $Df(3R)kar^{Sz21}$ (87C7;87C8-9) and $Df(3R)kar^{3Q}$ (87B2-4;87C9-D3) mutations are described in GAUSZ *et al.* (1979) and in GAUSZ, AWAD and GYURKOVICS (1980). The third chromosome mutations *se*, *cp*, *e*, *cd* and kar^2, $Tp(3;2)ry^+$ (87C2-3;88C2-3) carrying a $Su(fu)^+$ copy, and the balancer chromosomes *MKRS*, *TM3*, *M5*, *FM3* and *FM6* are described in LINDSLEY and ZIMM (1985, 1987, 1990). Stocks were maintained on a yeast/maize/agar medium (GANS, AUDIT and MASSON 1975). Crosses were made at 23° unless otherwise specified.

Ethyl methanesulfonate (EMS) mutageneses: In the first mutagenesis, wild-type Oregon-R males were fed 25 mM EMS as described by LEWIS and BACHER (1968) and mated to f^{36a} $fu^1/FM3$ virgin females (see Figure 2A) at 20°. f^{36a} $fu^1/Y^*;+^*/+;+^*/+;+^*/+$ single males with a suppressed fused wing phenotype were screened out of the F_1 progeny. The suppressor mutation was further amplified by crossing suppressed F_1 individual to f^{36a} $fu^1/FM3$ females. The expressivity of the vein fusion phenotype is rather constant for a particular *fu* allele raised at a given temperature, so that suppressors only partially rescuing this wing phenotype could be identified. About 1,700 fu^1 males were screened. This mutagenesis yielded the $Su(fu)^{LP}$ allele. The *karmoisin* (*kar*) gene is located next to *Suppressor of fused* in 87C8, and both genes are mutant in the $Su(fu)^{LP}$ strain (see RESULTS). The $Su(fu)^{LP}$ allele could thus be followed using the recessive *kar* phenotype as a marker. This situation turned out to be useful because homozygous *Su(fu)* mutations display no visible phenotype by themselves. Homozygous stocks f^{36a} $fu^1;Su(fu)^{LP}$ and $fu^{mH63};Su(fu)^{LP}$ were generated.

fu^{mH63}/Y males normally die as late pupae (only a few fu^{mH63}/Y escapers eclose, which are recognizable as they are subviable and display an extreme wing phenotype). On the contrary, viable $fu^{mH63}/Y;Su(fu)^{LP}/+$ flies displaying a partially suppressed phenotype can be recovered. In the second mutagenesis, *w* males were fed 25 mM EMS and mated to $fu^{mH63}/FM6$ virgin females (see Figure 2B). Forty bottles containing 20 EMS-treated males and 20 females were generated, and the parents transferred every day for 4 days. Half of the progeny was raised at 23° and half at 25°. Viable $fu^{mH63}/Y^*;+^*/+;+^*/+;+^*/+$ males were screened daily from the F_1 progeny and crossed to attached-*X* females. Males displaying an extreme fu phenotype were never found fertile, and most likely corresponded to fu^{mH63}/Y escapers. The equivalent of about 10,000 fu^{mH63} individuals were screened. One *Su(fu)* allele, $Su(fu)^{12d}$, was isolated. This low mutation rate (10^{-4}) could be due in part to the fact that suppression of the pupal lethality by *Su(fu)* heterozygous mutation is only partial. Other mutations were recovered during this

screen which do not map to the *Su(fu)* locus (T. PRÉAT, unpublished results). As a control, the rate of *X* chromosomes bearing a lethal mutation was estimated. EMS-treated *w/Y* males were crossed to *M5/M5* virgin females. The progeny of individual *w*/M5* females crossed to *M5/Y* males was scored. Out of 122 fertile females, 32 (26%) gave rise to no *w*/Y* males, indicating that the mutagenized *X* chromosome carried at least one lethal mutation.

Genetic localization of *Su(fu)* mutations: Both $Su(fu)^{LP}$ and $Su(fu)^{12d}$ are viable mutations, and, except for the *kar* phenotype displayed by the $Su(fu)^{LP}$ allele, these stocks do not display any recessive phenotype by themselves. As a consequence, it was not possible to prove that these mutations actually affect the same gene by a direct $Su(fu)^{LP}/Su(fu)^{12d}$ complementation analysis. Because the suppressor phenotype itself is semi-dominant, a complementation analysis in a $fu/Y;Su(fu)^{LP}/Su(fu)^{12d}$ combination would not have been conclusive either. In order to address this problem, both *Su(fu)* mutations were localized after recombination with a marked third chromosome in a *fu* background. *M5/+; se cp e cd/TM3* virgin females were crossed to $f^{36a}\ fu^{1}/Y;Su(fu)^{LP}/Su(fu)^{LP}$ males. Virgin $M5/f^{36a}\ fu^{1}$; *se cp e cd/*$Su(fu)^{LP}$ females were recovered and crossed to *se cp e cd/se cp e cd* males. The phenotype of 253 $f^{36a}\ fu^{1}$ males was scored at the F_2 generation for the presence of both the semidominant $Su(fu)^{LP}$ mutation and the various recessive markers. The $Su(fu)^{LP}$ mutation was found to be between *cp* (45.3) and *e* (70.7) at the approximate position 53.1 ± 2.3. As the *kar* gene is at 51.7, this result is consistent with the hypothesis that the viable $Su(fu)^{LP}$ mutation is a small molecular event affecting both the *kar* gene and the *Su(fu)* gene. The $Su(fu)^{12d}$ mutation was localized similarly, and was found to be between *cp* and *e* at 20 ± 1.8 units from *e*. This corresponds to the position 50.7 ± 1.8, which strongly suggests that $Su(fu)^{12d}$ and $Su(fu)^{LP}$ are alleles. A $Su(fu)^{12d}$ *e cd* stock was generated during this experiment.

Analysis of 87C complementation groups: To determine whether any of the previously described 87C complementation groups (GAUSZ *et al.* 1979) interacts with *fu*, $l(3)SzC^{3}/TM3$, $l(3)SzD^{8}/TM3$, and kar^{2}/kar^{2} males were crossed to $f^{36a}\ fu^{1}/FM3$ virgin females, and the fused veins phenotype of the non-TM3 males of the progeny was checked. In addition, $fu^{mH63}/fu^{mH63};Su(fu)^{LP}/Su(fu)^{LP}$ females were crossed to *l(3)SzC/TM3* and *l(3)SzD/TM3* males, and the vein phenotype of the $fu^{mH63}/Y;Su(fu)^{LP}/l(3)Sz$ males was compared to the one of their $fu^{mH63}/Y;Su(fu)^{LP}/TM3$ siblings. The eye-color phenotype observed in $Su(fu)^{LP}/Su(fu)^{LP}$ flies corresponds to a recessive mutation in the *kar* gene since $Su(fu)^{LP}/kar^{2}$ flies display a karmoisin phenotype. The *MKRS* balancer carries the kar^{1} mutation, and $f^{36a}\ fu^{1}/Y;MKRS/+$ were generated to further insure that *kar* mutations have no effect on the fused phenotype. To generate *Su(fu)/Df(3R)* individuals, virgin *Su(fu)/Su(fu)* females were mated to *Df(3R)kar/TM3* males.

Embryos and adults preparations: Embryos were mounted in Hoyer's for cuticle examination as described in VAN DER MEER (1977) and observed under a Zeiss microscope on a dark field. In cases where fu^{1}/fu^{1} females were crossed to fu^{1}/Y males in various *Su(fu)* backgrounds, both unhatched developed embryos and first instar larvae were collected. This procedure avoided bias in the statistical analysis of the suppression of the segment polarity phenotype (embryos which hatch probably display a less severe mutant phenotype). To quantify the efficiency of the suppression of the embryonic fu phenotype, 200 embryos were collected from each cross. Unhatched developped embryos (UD) and hatched embryos (H) were counted after 3 days at 23°, and the H/UD + H ratio calculated. Wings were mounted in Euparal following storage in 100% ethanol. Ovaries were mounted and stained with fuchsin as described in ZALOKAR and ERK (1977).

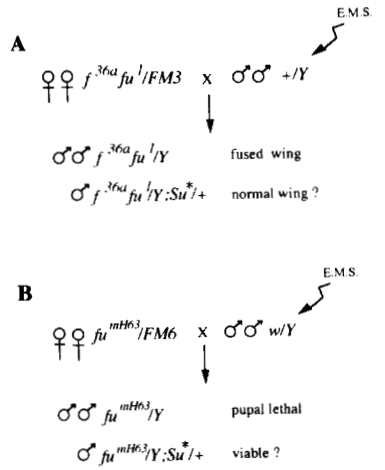

FIGURE 2.—Mutagenesis schemes used to isolate fu phenotype suppressors. (A) EMS-treated males were crossed to heterozygous *fu* females. Genotypically *fu* males with a suppressed fused wing phenotype were screened *en masse* in the F_1 progeny (only the relevant genotypes are shown). Presence of the *forked* (*f*) bristles marker ensured that males displaying a normal phenotype actually carried a *fu* chromosome. (B) EMS-treated males were crossed to females heterozygous for the pupal lethal mutation fu^{mH63}. Viable *fu* males were screened *en masse* in the F_1 progeny. Suppression of their fused wing phenotype was monitored.

RESULTS

Isolation of *Su(fu)* mutations: With the aim of identifying new genes involved in the segment polarity pathway, two EMS mutageneses were carried out in order to isolate suppressors of *fu* mutant phenotypes (Figure 2 and MATERIALS AND METHODS). The suppression effect was not identified directly on the segment polarity phenotype of *fu* embryos, but on *fu* adult phenotypes. Both mutageneses allowed to isolate *en masse* partially rescued *fu/Y;Su/+* individuals bearing a semidominant autosomal suppressor. This would not have been possible with an embryonic screen, because a partial rescue of the segment polarity phenotype generally does not allow to get a viable adult. In the first mutagenesis, *fu* flies with a suppressed wing phenotype were screened (Figure 2A). This mutagenesis led to the isolation of the $Su(fu)^{LP}$ allele. In the second mutagenesis, suppression of the pupal lethality of the fu^{mH63} allele was screened (Figure 2B). It allowed to get the $Su(fu)^{12d}$ allele.

***Su(fu)* is located in the 87C8 band of the third chromosome:** The $Su(fu)^{LP}$ and $Su(fu)^{12d}$ mutations were localized using recombination with a marked third chromosome (see MATERIALS AND METHODS). As deletions covering the 87C region do suppress the fused phenoytpe (see below), precise localization of *Su(fu)* could be achieved by testing the suppression of the fused vein phenotype using deficiencies in a *fu/*

TABLE 1

Complementation pattern of 87C mutations

Mutation	Complementation group with chromosomal localization[a]				
	87C6	87C7	87C8		87C9
	l(3)SzB	*l(3)SzC*	*kar*	*Su(fu)*	*l(3)SzD*
$l(3)SzC^3$	+	−	+	+	+
kar^1	+	+	−	+	+
kar^2	+	+	−	+	+
$Su(fu)^{12d}$	+	+	+	−	+
$Su(fu)^{LP}$	+	+	−	−	+
$l(3)SzD^N$	+	+	+	+	−
$Df(3R)kar^{Sz11}$	+	+	−	−	−
$Df(3R)kar^{Sz21}$	+	−	−	−	+
$Df(3R)kar^{3Q}$	−	−	−	−	−

A "+" sign indicates that the mutation complements alleles of the corresponding group in a trans combination. In the case of *Su(fu)* however, it indicates that the heterozygous mutation does not suppress the fused phenotype (see MATERIALS AND METHODS). No standard complementation analysis can be performed with *Su(fu)* as it is a cryptic mutation (see text).

[a] The respective position of *kar* and *Su(fu)* is not yet determined.

Y;Df(3R)/+ combination (Figure 3D). *Su(fu)* was found to be included in $Df(3R)kar^{3Q}$ as well as in $Df(3R)kar^{Sz11}$ and $Df(3R)kar^{Sz21}$. Their common deleted region corresponds to the 87C8 band of the third chromosome (GAUSZ *et al.* 1979). GAUSZ and collaborators (1979) saturated the 87C region in a search for viable and lethal mutations. After EMS mutagenesis, they identified 52 mutations corresponding to 5 complementation groups–*l(3)SzA*, *l(3)SzB*, *l(3)SzC*, *kar* and *l(3)SzD*–with a minimum of 6 alleles per group (Table 1). I have brought additional data to this study as regards to *Su(fu)* (Table 1). No previously known complementation group was found to be associated with a Su(fu) phenotype, confirming that *Su(fu)* is a newly characterized gene. In particular, *kar* mutations do not affect the fused phenotype.

***Su(fu)* mutations correspond to a loss of function:** As deficiencies covering the 87C region suppress the fused phenotype (Figure 3D), *Su(fu)* mutants are due to a loss of function. Moreover, the partially suppressed wing phenotype is identical in $fu^{mH63}/Y;Su(fu)^{LP}/+$ and in $fu^{mH63}/Y;Df(3R)kar^{3Q}/+$ flies (Figure 3, D and E), defining $Su(fu)^{LP}$ as an amorphic allele (no activity). The $Su(fu)^{12d}$ allele behaves as a strong hypomorph (strongly reduced activity) because $fu^{mH63}/Y;Su(fu)^{12d}/Su(fu)^{12d}$ flies display a very weak fused veins phenotype (Figure 3G), whereas $fu^{mH63}/Y;Su(fu)^{LP}/Su(fu)^{LP}$ individuals are normal.

***Su(fu)* mutations are viable and display no visible phenotype:** *Su(fu)* mutations were screened as heterozygotes in a hemizygous *fu* background. To analyze the phenotype of *Su(fu)/Su(fu)* individuals independently of the *fu* mutation itself–which could affect this phenotype–stocks bearing the *Su(fu)* mutations in a fu^+ background were generated. *Su(fu)* mutations are viable and *Su(fu)/Su(fu)* adults display no visible phenotype (Figure 3B, Figure 4B). The $Su(fu)^{LP}/Df(3R)kar^{3Q}$, $Df(3R)kar^{Sz21}/Df(3R)kar^{Sz11}$, $Su(fu)^{12d}/Su(fu)^{LP}$ and $Su(fu)^{t12d}/Df(3R)kar^{3Q}$ combinations are also viable and the flies display no obvious phenotype (except for the *kar* phenotype itself in the case of the first two combinations). Thus the amorphic *Su(fu)* mutation is cryptic.

***Su(fu)* amorphic mutation fully suppresses the segment polarity phenotype of *fu* embryos:** *fu* embryos produced by *fu* females (Figure 1A) die and display a segment polarity phenotype (Figure 4C): the posterior part of each thoracic and abdominal segment is deleted, and the denticle rows are mirror-image duplicated. In order to analyze the suppression of this phenotype by the *Suppressor of fused* mutation, $fu^1/fu^1;Su(fu)^{LP}/Su(fu)^{LP}$ females were generated and crossed to $fu^1/Y;Su(fu)^{LP}/Su(fu)^{LP}$ males. $fu^1;Su(fu)^{LP}$ embryos produced by these females display a normal segmentation with no denticle duplication (Figure 4D). The penetrance and expressivity of this suppression are total as even a partial "fused" phenotype was never observed in $fu^1;Su(fu)^{LP}$ embryos. These *fu;Su(fu)* embryos hatch and eventually give rise to normal adults. Thus, although the mutagenesis screens were based on adult phenotype suppression, they identified a suppressor which also fully suppresses the embryonic segment polarity phenotype.

***Su(fu)* amorphic mutation fully suppresses the phenotypes of *fu* adults:** *fu* embryos produced by heterozygous *fu/+* females have a normal embryonic development due to the maternal expression of fu^+ (Figure 1C). But *fu* adults grown from these embryos display several anomalies due the lack of Fu^+ product during metamorphosis (WURST and HANRATTY 1979) and adult life. When homozygous, *Su(fu)* fully suppresses these various defects.

Su(fu) suppresses the wing phenotype: One of these adult phenotypes is the abnormal wing vein pattern. *Su(fu)* fully suppresses this phenotype, as $fu^{mH63}/Y;Su(fu)^{LP}/Su(fu)^{LP}$ flies have normal wings (Figure 3F).

Su(fu) suppresses the tumorous ovarian phenotype: fu/fu females demonstrate reduced fecundity due to several defects, including tumorous egg chambers (KING 1959) (Figure 5B). These tumors originate from an anarchic division of egg chamber cells. Eight-day-old fu^1 females raised at 25° have about 50% of tumorous egg chambers (SMITH and KING 1966), and one chamber may contain as many as 10,000 cells (KING 1970). This phenotype is fully suppressed by *Suppressor of fused.* $fu^1/fu^1;Su(fu)^{LP}/Su(fu)^{LP}$ females have normal ovaries, and no tumorous chamber was found out of several hundred observed (Figure 5C). In this respect *Su(fu)* mutations can be considered as antioncogenic.

Su(fu) suppresses the pupal lethality of strong fu alleles: fu alleles belong to two molecular classes (PRÉAT *et al.*

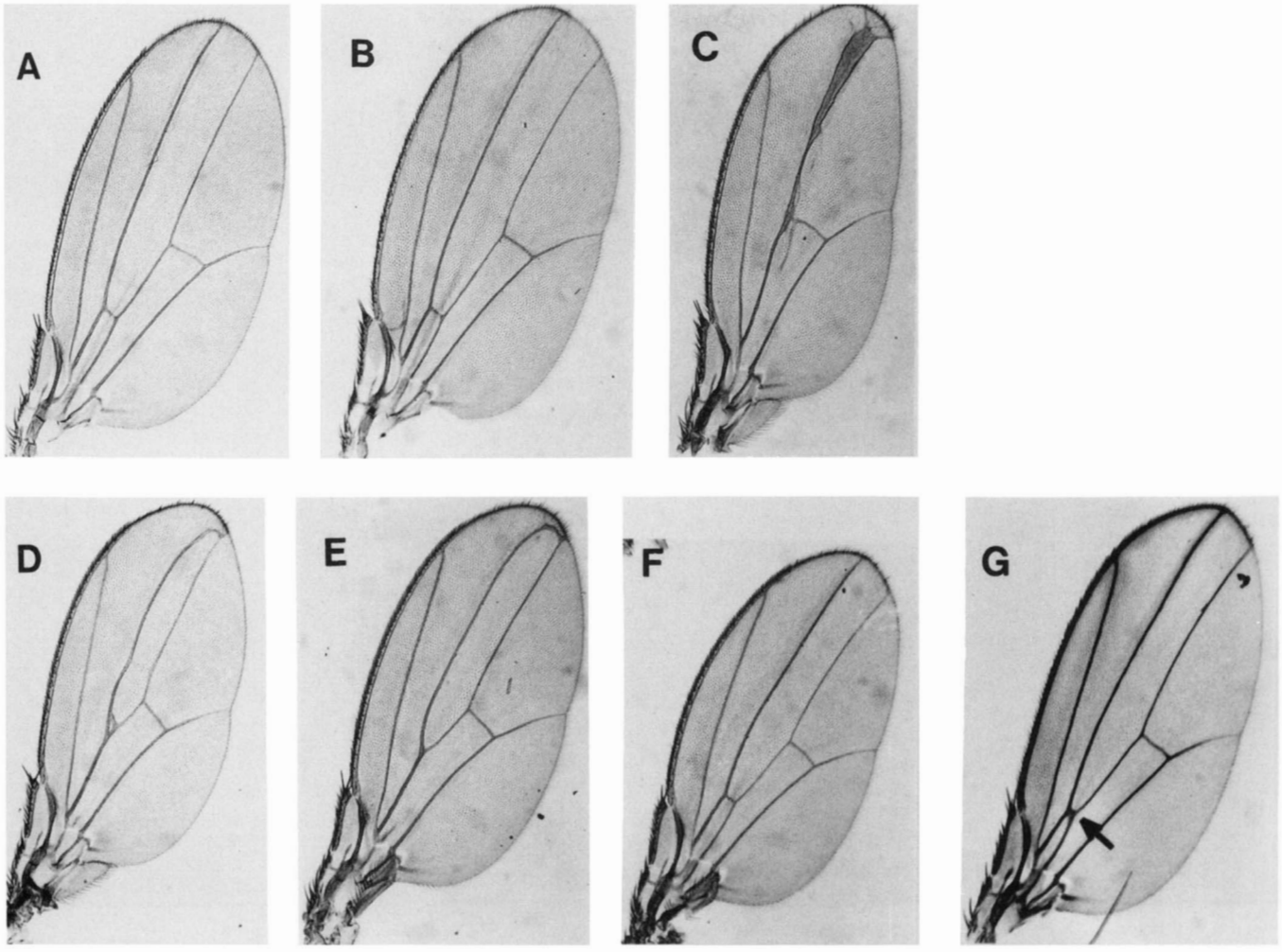

FIGURE 3.—Suppression of the fused wing phenotype by *Suppressor of fused*. (A) Wing of a wild-type fly. There are five longitudinal veins (LV). LV1 which runs along the margin is on the left side. The anteroposterior boundary lies between LV3 and LV4. (B) Wing of $Su(fu)^{LP}/Su(fu)^{LP}$ fly. This wing cannot be differentiated from wild type. (C) Wing of a fu^{mH63}/Y escaper displaying an extreme fused phenotype (LV4 is entirely missing). (D) Wing of a $fu^{mH63}/Y;Df(3R)kar^{3Q}/+$ male, showing a partially suppressed phenotype. (E) Wing of a $fu^{mH63}/Y;Su(fu)^{LP}/+$ male. Note that suppression by the heterozygous $Su(fu)^{LP}$ mutation is as efficient as that due to $Df(3R)kar^{3Q}$. (F) Complete suppression of the extreme fused phenotype by the homozygous $Su(fu)^{LP}$ mutation. This $fu^{mH63}/Y;Su(fu)^{LP}/Su(fu)^{LP}$ wing is normal. (G) Wing of a $fu^{mH63}/Y;Su(fu)^{12d}/Su(fu)^{12d}$ fly. The arrow indicates the very weak fused phenotype still displayed by these adults. The dark color pigmentation is due to the presence of the *ebony* marker on this $Su(fu)^{12d}$ chromosome.

1990). The first class corresponds to large rearrangements, the smallest one, $Df(1)fu^{Z4}$, being a 40kb deletion which includes several transcription units beside *fu* (MARIOL, PRÉAT and BOUCHON 1987). $Df(1)fu^{Z4}/Y$ males produced by heterozygous $Df(1)fu^{Z4}/FM6$ females die as first instar larvae (BUSSON *et al.* 1988). The second molecular class corresponds to events that only affect the *fu* transcription unit, and which, so far, were found to be smaller than 40 bp. The strongest *fu* alleles of this second class lead to a late pupal lethality (WURST and HANRATTY 1979; PERRIMON and MAHOWALD 1987) (Figure 1D), while weaker *fu* alleles are viable and display only maternal effect lethality. *Su(fu)* mutations suppress the fu pupal lethality. For example, the $fu^{mH63};Su(fu)^{LP}$ stock shows no significant pupal lethality. These flies are viable, fertile, and display no fu phenotype (Figure 3F). The pupal lethality of the fu^{1PP7} allele is also suppressed.

***Su(fu)* amorphic mutation does not suppress the larval lethality due to $Df(1)fu^{Z4}$:** Because of the lack of *fu* deletions of size intermediate between 40 bp and 40 kb, the lethal stage resulting from a mutation which would remove all *fu* sequences–but these sequences only–is not known. It was unclear wether the larval lethality of $Df(1)fu^{Z4}/Y$ males was due to the absence of the *fu* gene itself, or to another gene included in this deficiency. Studying the effect of *Su(fu)* on this phenotype allowed examination of this problem. Unlike the pupal lethality due to the fu^{mH63} allele, the larval lethality due to $Df(1)fu^{Z4}$ is not suppressed by *Su(fu)*: $Df(1)fu^{Z4}/FM6;Su(fu)^{LP}/Su(fu)^{LP}$ females crossed to $FM6/Y;Su(fu)^{LP}/Su(fu)^{LP}$ males generate $Df(1)fu^{Z4}/Y;Su(fu)^{LP}/Su(fu)^{LP}$ individuals which die as larvae, despite the presence of the homozygous $Su(fu)^{LP}$ muta-

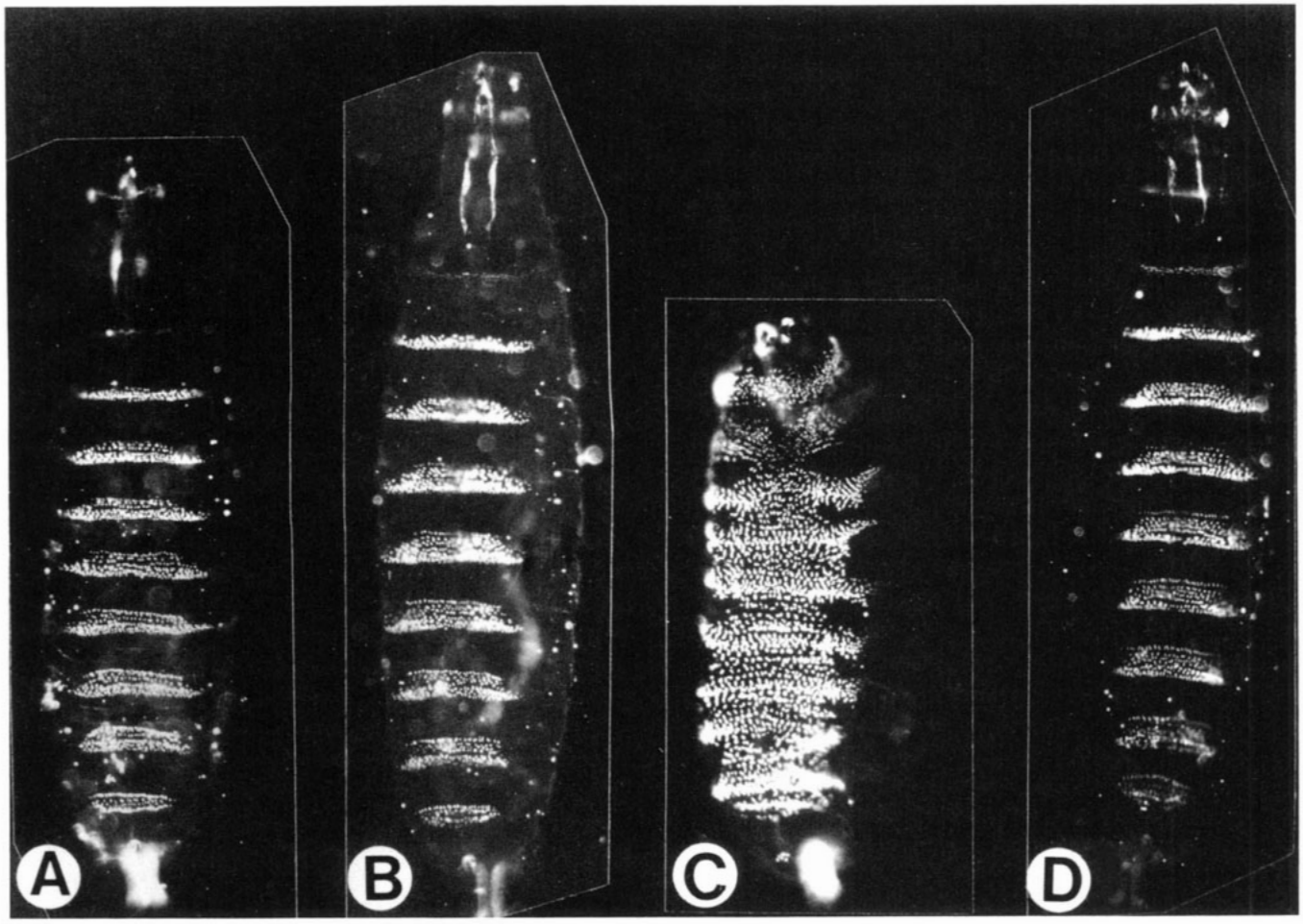

FIGURE 4.—Suppression of the segment polarity phenotype of *fu* embryos by *Suppressor of fused*. (A) Ventral view of a wild-type embryo. Each thoracic and abdominal segment bears denticle belts in the anterior region. Denticles appear white on this dark field image (eight abdominal belts are prominent). (B) Homozygous $Su(fu)^{LP}/Su(fu)^{LP}$ embryo displaying normal pattern. (C) fu^1 embryo. Each denticle belt is duplicated in a mirror image and the naked cuticle is absent. Note the reduced size of this embryo. (D) $fu^1;Su(fu)^{LP}$ embryo. The segment polarity fu phenotype is fully suppressed. There is no sign of denticle duplication, and the size of the embryo is normal suggesting that no pattern is deleted either. These embryos hatch and develop into normal adults.

tion. As all the other *fu* phenotypes are suppressed by *Su(fu)*–including the segment polarity phenotype of $Df(1)fu^{Z4}$ itself (T. PRÉAT, unpublished result)–this result strongly suggests that the larval lethality of $Df(1)fu^{Z4}$ is not due to *fu*, but to an adjacent gene included in the deficiency.

Maternal and zygotic effects of *Su(fu)* on the segment polarity phenotype of *fu* embryos: The segment polarity gene class includes maternal effect genes and genes expressed from the embryonic genome (reviewed in Ingham and Nakano 1990). In order to study the expression of *Su(fu)*, and also to further analyze the *fu-Su(fu)* interaction, suppression of the phenotype of *fu* embryos in various *Su(fu)* context was assessed. The segment polarity phenotype of *fu* embryos is determined by the *fu/fu* genotype of their mothers. But, in *fu*/+ embryos derived from a *fu/fu* × +/*Y* cross, the lack of maternal product can be partially compensated by early zygotic expression of the fu^+ allele ("paternal rescue") (Figure 1B). The following observations indicate that, like *fu*, *Su(fu)* is a maternal effect gene. But, unlike *fu*, *Su(fu)* is not "paternally rescued"–although it is very likely also expressed in the embryo.

$fu^1/Y;Su(fu)^{LP}/+$ and $fu^1/fu^1;Su(fu)^{LP}/+$ embryos derived from a $fu^1/fu^1;Su(fu)^{LP}/Su(fu)^{LP} \times fu^1/Y;+/+$ cross display a normal segmentation pattern (Figure 6A) and give rise to adults. On the contrary, embryos with the same genotype but derived from the reciprocal cross $fu^1/fu^1;+/+ \times fu^1/Y;Su(fu)^{LP}/Su(fu)^{LP}$ die as embryos with a mutant fu phenotype (Figure 6B). Thus, for *Su(fu)* as for *fu*, the phenotype of the embryos depends on the genotype of their mothers. This suggests that $Su(fu)^+$ is expressed in the ovaries.

The complete lack of maternal $Su(fu)^+$ product in $fu^1/fu^1;Su(fu)^{LP}/+$ and $fu^1/Y;Su(fu)^{LP}/+$ embryos derived from a $fu^1/fu^1;Su(fu)^{LP}/Su(fu)^{LP} \times fu^1/Y;+/+$ cross insures a complete suppression of the fu segment

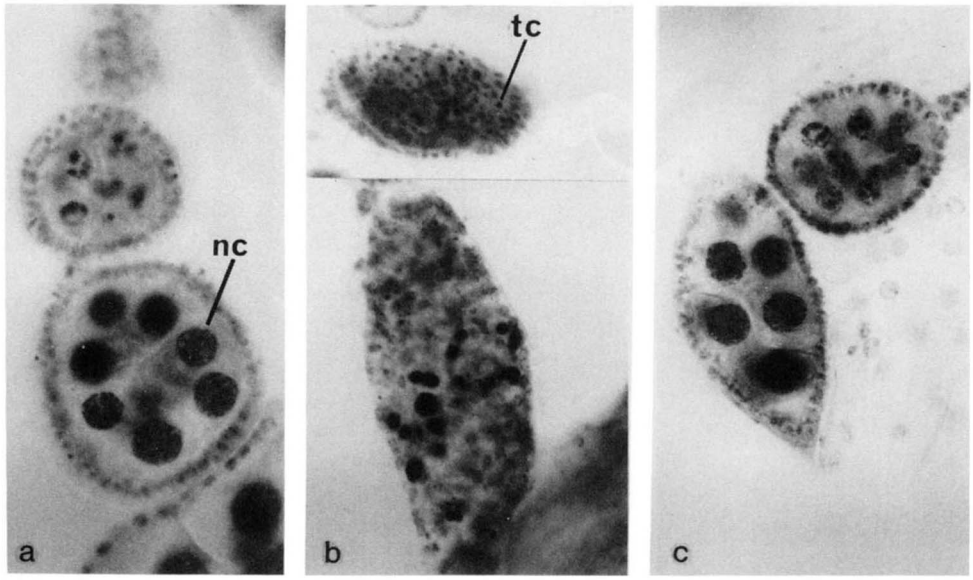

FIGURE 5.—Suppression of the tumorous ovaries phenotype by *Suppressor of fused*. (A) Wild-type egg chambers at different developmental stages. The nuclei are stained with fuchsin. The large cells are nurse cells (nc). (B) Tumorous egg-chambers from fu^1/fu^1 females. These chambers are made of hundred of tumorous cells (tc). (C) Egg-chamber from $fu^1;Su(fu)^{LP}$ females. No tumors are observed.

polarity phenotype, despite the presence of a $Su(fu)^+$ copy in the embryo: the maternal effect of the *Su(fu)* mutation is not "paternally rescued" by $Su(fu)^+$. It could mean either that the $Su(fu)^+$ gene is not expressed in the embryo, or that the expression of a single $Su(fu)^+$ embryonic copy cannot compensate for the complete lack of maternal product. I mentioned that $fu^1/fu^1;Su(fu)^{LP}/+$ and $fu^1/Y;Su(fu)^{LP}/+$ embryos derived from a $fu^1/fu^1;+/+ \times fu^1/Y;Su(fu)^{LP}/Su(fu)^{LP}$ cross die with a segment polarity phenotype (Figure 6B). Actually, their phenotype is on average weaker than the one displayed by fu^1/fu^1 and fu^1/Y embryos (Figure 4C). Also, 5% of the embryos derived from a $fu^1/fu^1;+/+ \times fu^1/Y;Su(fu)^{LP}/Su(fu)^{LP}$ cross were found to hatch (Table 2), whereas fu^1 embryos never do. The absence of one zygotic $Su(fu)^+$ copy allows a partial suppression of the embryonic fu phenotype, despite the presence of the maternal $Su(fu)^+$ product. *Su(fu)* presents both a maternal and a zygotic suppression effect on *fu* embryos phenotype. In the wild type, some functional $Su(fu)^+$ product is made in the embryo. Therefore, the fact that the maternal effect of the *Su(fu)* mutation is not affected by the zygotic expression of a single $Su(fu)^+$ allele in embryos derived from a $fu^1/fu^1;Su(fu)^{LP}/Su(fu)^{LP} \times fu^1/Y;+/+$ cross–as shown by the complete suppression of the segment polarity fu phenotype–is probably due to the fact that the amount of zygotic $Su(fu)^+$ product remains below the required threshold in these embryos.

Zygotic effect of *Su(fu)* on the phenotype of *fu* adults: Genetic properties of *fu* indicate that it is expressed in the female germ line and in the embryo, but also later for adult development (FAUSTO-STERLING 1978; WURST and HANRATTY 1979). Unlike the suppression of the embryonic phenotype, the suppression of the phenotype of *fu* adults does not depend on the *Su(fu)* genotype of their mother, but only on the lack of zygotic $Su(fu)^+$ product. $fu^1/Y;Su(fu)^{LP}/+$ males derived from a $fu^1/FM6;+/+ \times +/Y;Su(fu)^{LP}/Su(fu)^{LP}$ cross display the same partially suppressed phenotype as the males of the same genotype derived from the reciprocal cross $fu^1/FM6;Su(fu)^{LP}/Su(fu)^{LP} \times +/Y;+/+$ (not shown).

***Su(fu)* is semidominant:** The two mutageneses reported here allowed recovery of semidominant or dominant *fu* suppressors, as the mutations were screened as heterozygotes in a *fu* background. The $Su(fu)^{LP}$ mutation, which behaves as an amorphic allele, is semidominant. For example, $fu^{mH63}/Y;Su(fu)^{LP}/+$ flies display a partially suppressed wing phenotype while $fu^{mH63}/Y;Su(fu)^{LP}/Su(fu)^{LP}$ wings are wild type (Figure 3). A semidominant effect is also observed in the case of the maternal suppression of the fu segment polarity phenotype, since embryos derived from a $fu^1/fu^1;Su(fu)^{LP}/+ \times fu^1/Y$ cross display a partially suppressed segment polarity phenotype (Figure 6C), whereas embryos derived from a $fu^1/fu^1;Su(fu)^{LP}/Su(fu)^{LP} \times fu^1/Y$ cross are normal (Figure 6A).

The fused phenotype is enhanced by a $Su(fu)^+$ duplication: *Su(fu)* loss-of-function mutations suppress the fu phenotype. The effect of overexpression of the $Su(fu)^+$ function was studied by generating *fu* flies bearing three copies of the $Su(fu)^+$ gene. The fused vein phenotype of $fu^1/Y;Tp(3,2)ry^+,Su(fu)^+/+;+/+$ flies is more severe than that of fu^1/Y flies (Figure 7). Thus, increasing the expression of $Su(fu)^+$ leads to a stronger fu mutant phenotype. The Fu and Su(fu) functions are tightly correlated since the strength of the fu phenotype is directly related to the number of $Su(fu)^+$ copies in the genome.

DISCUSSION

The search for suppressor mutations has long been recognized as a fruitful approach for identifying new

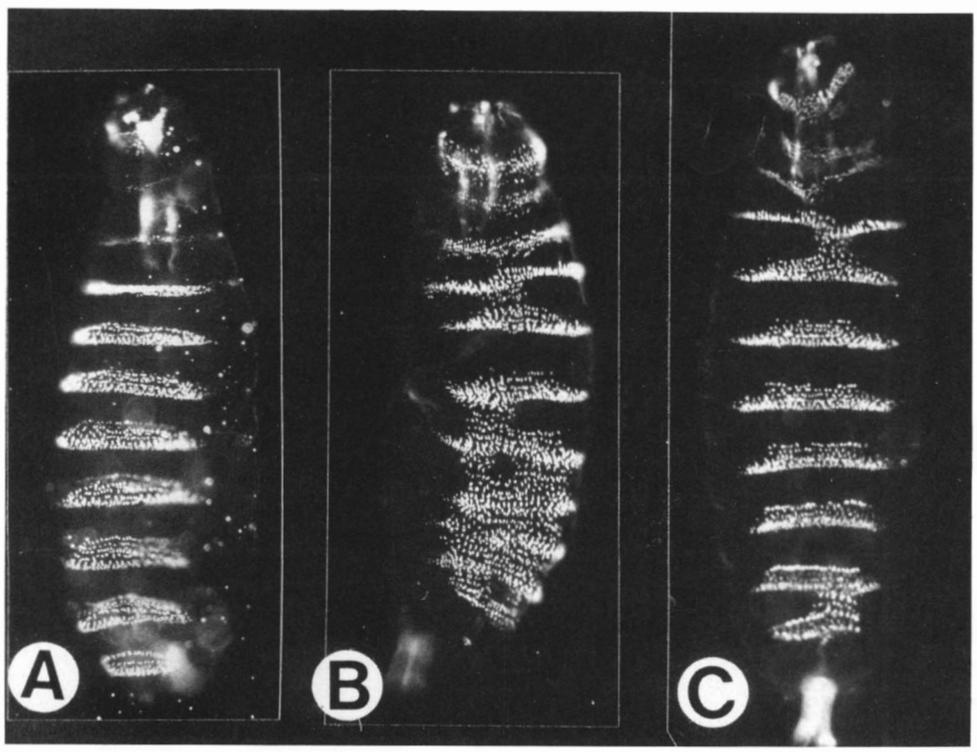

FIGURE 6.—Maternal and zygotic effects displayed by *Su(fu)*. (A) Embryo derived from a $fu^1/fu^1;Su(fu)^{LP}/Su(fu)^{LP} \times fu^1/Y;+/+$ cross. The segments are normal. These embryos hatch and eventually give rise to adults. (B) Embryo derived from the reciprocical cross $fu^1/fu^1;+/+ \times fu^1/Y;Su(fu)^{LP}/Su(fu)^{LP}$. Most of the segments bear duplicated denticles. However, the segment polarity phenotype is weaker than that of fu^1 embryos (Figure 4C), and 5% of these embryos were found able to hatch. (C) Embryo derived from a $fu^1/fu^1;Su(fu)^{LP}/+ \times fu^1/Y$ cross. Maternal suppression by the heterozygous $Su(fu)^{LP}$ mutation is not complete since some segments still display an abnormal pattern. About 35% of these embryos hatch but most individuals do not reach the adult stage as they die as larvae or early pupae.

TABLE 2

Genetic properties of *Suppressor of fused*

Parental genotypes	Genotype of offspring	Phenotype of offspring	Hatched embryos
fu/fu;Su(fu)/Su(fu) × *fu/Y;Su(fu)/Su(fu)*	–*fu/fu;Su(fu)/Su(fu)* –*fu/Y;Su(fu)/Su(fu)*	–Complete suppression of the segment polarity fu phenotype –Complete suppression of the adult fu phenotype	98%
fu/fu;Su(fu)/Su(fu) × *fu/Y;+/+*	–*fu/fu;Su(fu)/+* –*fu/Y;Su(fu)/+*	–Complete suppression of the segment polarity fu phenotype –Partial suppression of the adult fu phenotype	96%
fu/fu;+/+ × *fu/Y;Su(fu)/Su(fu)*	–*fu/fu;Su(fu)/+* –*fu/Y;Su(fu)/+*	Partial suppression of the segment polarity fu phenotype	5%
fu/fu;Su(fu)/+ × *fu/Y;+/+*	–*fu/fu;Su(fu)/+* –*fu/Y;Su(fu)/+* –*fu/fu;+/+ fu/Y;+/+*	Partial suppression of the segment polarity fu phenotype (the suppression is always stronger than the one observed in the previous cross)	35%
fu/FM6;+/+ × *fu/Y;Su(fu)/Su(fu)*	–*fu/fu;Su(fu)/+*[a] –*fu/Y;Su(fu)/+*	Partial suppression of the adult fu phenotype	N/A
fu/FM6;Su(fu)/Su(fu) × *fu/Y;+/+*	–*fu/fu;Su(fu)/+*[a] –*fu/Y;Su(fu)/+*	Partial suppresson of the adult fu phenotype (similar to the one observed in the previous cross)	N/A
fu/FM6;Su(fu)/Su(fu) × *fu/Y;Su(fu)/Su(fu)*	–*fu/fu;Su(fu)/Su(fu)*[a] –*fu/Y;Su(fu)/Su(fu)*	Complete rescue of the adult fu phenotype	N/A
fu/FM6 × *+/Y;Tp(3,2)Su(fu)⁺/+*	–*fu/Y;Tp(3,2)Su(fu)⁺/+*[a]	More severe adult fu phenotype	N/A

[a] Only the relevant genotypes are shown.

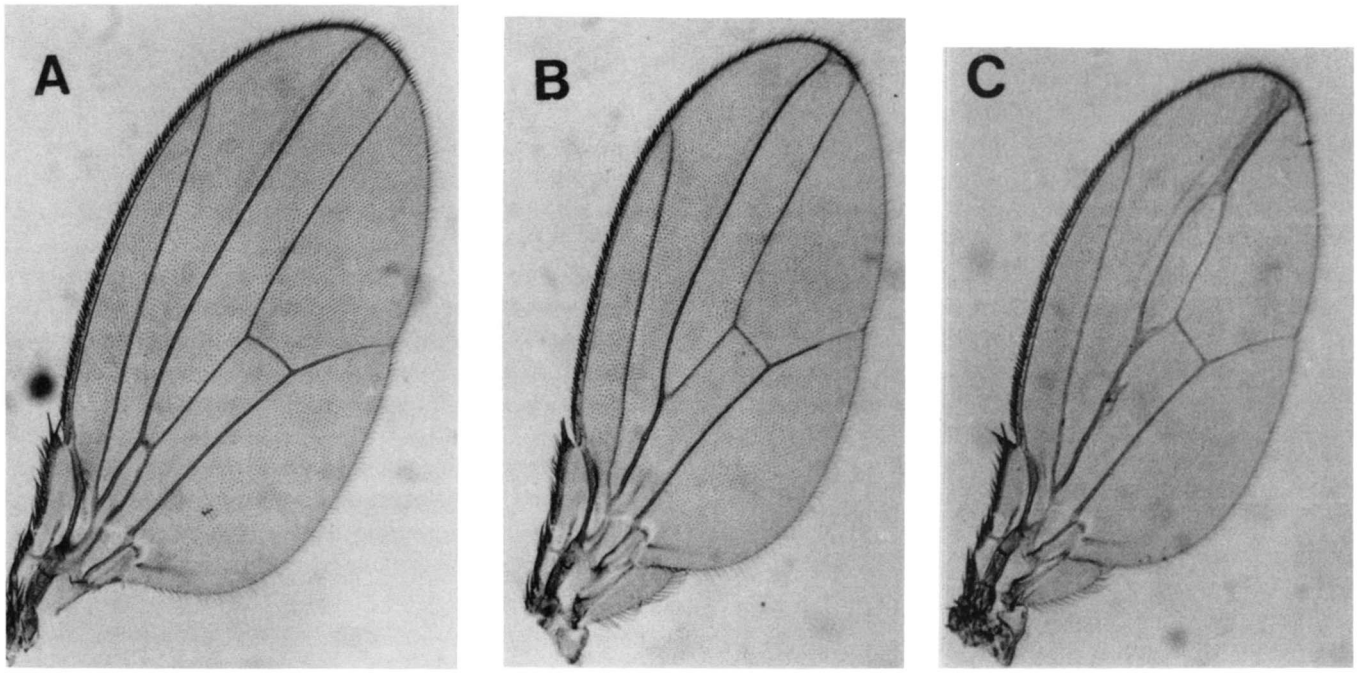

FIGURE 7.—A *Su(fu)*$^+$ duplication increases the fused phenotype. (A) Wing of *fu*^{1}/*Y;Su(fu)*^{LP}/+ individual. (B) Wing of *fu*^{1}/*Y* individual. *fu*^{1} is a hypomorphic allele since wings display a weak fused phenotype (only the proximal portion of LV4 is missing). (C) Wing of *fu*^{1}/*Y;Tp(3,2)ry*$^+$,*Su(fu)*$^+$/+;+/+ fly. The fused phenotype is severely increased. LV4 is almost completely deleted and LV3 is thickened.

genes involved in a given pathway. At the outset of this study however, there was no description of a mutageneses specifically performed in order to isolate suppressors of segment polarity phenotypes. I report here the characterization of a new gene, *Suppressor of fused*, located in the 87C8 region of the third chromosome. *Su(fu)* mutations were isolated after EMS mutageneses, and screened based on the suppression of *fu* adult phenotype. They correspond to loss-of-function mutations; the amorphic mutation is homozygous viable and *Su(fu)/Su(fu)* flies do not display any obvious phenotype.

When produced by *fu/fu* mothers, *fu* embryos die and display a segment polarity phenotype. When produced by *fu*/+ females *fu* embryos develop normally, but *fu* adults display several anomalies (abnormal wing veins, tumorous ovaries. . .). *Su(fu)* fully suppresses both embryonic and adult fu phenotypes (Table 2). It is striking that *fu;Su(fu)* flies are wild-type and give rise to normal progeny. The fact that all *fu* defects are suppressed by *Su(fu)* suggests that the Fu$^+$ product is involved in a single molecular process occurring at different stages during development, despite the pleiotropic effect of the *fu* mutations. Three independent observations support this interpretation. First all *fu* alleles lead to the segment polarity phenotype, the wing phenotype, and the ovarian phenotype (SMITH and KING 1966; BUSSON *et al.* 1988). Second, as I mentioned before, both segment polarity phenotype and wing phenotype can be described as lack of a posterior structure and duplication of an anterior one, and there is a good correlation between the strength of these two phenotypes conferred by various *fu* alleles (BUSSON *et al.* 1988). Third, the various adult defects behaved similarly during temperature shift experiments (WURST and HANRATTY 1979).

Maternal and zygotic effects of *Su(fu)* are dosage sensitive: The screens described here allowed recovery of dominant or semidominant mutations. *Su(fu)* alleles belong to the latter group: when heterozygous, the *Su(fu)*^{LP} amorphic allele only partially suppresses the wing phenotype as well as the segment polarity phenotype, but suppression is complete when the *Su(fu)*^{LP} mutation is homozygous. Interestingly, a *Su(fu)*$^+$ duplication has an effect opposite to that of *Su(fu)* loss-of-function mutations, as it enhances the fu phenotype. This outlines the close and antagonistic relationship between *fu* and *Su(fu)*.

The Su(fu)$^+$ function, as observed in a *fu* background, is very sensitive to any decrease in the amount of Su(fu)$^+$ product. *Su(fu)* presents both a maternal and a zygotic suppression effect on the fu segment polarity phenotype. A partial lack of any of the maternal or zygotic *Su(fu)*$^+$ products affects the phenotype of *fu* embryos. Also, the total lack of maternal Su(fu)$^+$ product cannot be compensated for by the zygotic expression of a single *Su(fu)*$^+$ copy. This situation differs from the Fu function itself, which is less sensitive to a decrease of the amount of Fu$^+$ product. First, *fu* is a recessive mutation. Second, *fu* embryos display a segment polarity phenotype only if their mothers are *fu/fu*. The complete lack of *fu* zygotic

expression alone does not lead to even a partial segment polarity phenotype. Third, *fu* presents a paternal rescue since the zygotic expression of the fu^+ gene is able to partially compensate for the lack of maternal product. One explanation could be that, unlike Fu, Su(fu) is not an enzyme but instead interacts with another protein in a stoichiometric manner. Thus, any decrease in the amount of Su(fu) product would affect the activity of this other protein.

Interaction between fu^+ and $Su(fu)^+$; inhibition and competition models: *Su(fu)* interacts with all *fu* alleles and, in particular, it suppresses the segment polarity phenotype of the 40-kb deficiency $Df(1)fu^{Z4}$ (T. PRÉAT, unpublished results). Thus, *Su(fu)* mutations do not act by reactivating the expression of the *fu* locus itself. In this respect *Su(fu)* differs from most other suppressors known in Drosophila, which interact with specific alleles that correspond to the insertion of transposable elements (reviewed in KUBLI 1986). In these cases, and unlike the *fu-Su(fu)* interaction, the suppression effect is related to the nature of the mutation itself rather than to the function of the suppressed gene. Two different models–at least–allow interpretation of the suppression of the fu phenotype by *Su(fu)*. In the first one (inhibition model), the main function of the Fu^+ ser/thr kinase in wild-type individuals would be to inactivate the $Su(fu)^+$ protein. In the absence of Fu^+ product the $Su(fu)^+$ function would be overexpressed, leading to a fu phenotype. If no $Su(fu)^+$ product is synthesized, the presence of the Fu^+ product would no longer be required and therefore *fu;Su(fu)* individuals would be normal. In an alternative model (competition model), the Fu^+ and $Su(fu)^+$ products have antagonistic effects and act competitively. The Fu^+ ser/thr protein kinase and the $Su(fu)^+$ protein would have a common substrate, but their actions would have opposite effects on the activity of this third protein. The absence of both Fu^+ and $Su(fu)^+$ would result in a close to normal balance status.

Of course, the interaction between the Fu^+ kinase and $Su(fu)^+$ could involve some intermediate. For example in the inhibition model, another protein kinase could be activated after phosphorylation by the Fu^+ kinase and inactivate the $Su(fu)^+$ protein. Also, the Fu^+ and $Su(fu)^+$ functions could be expressed in different parts of the segments and interact thanks to some other segment polarity products involved in cell-cell communication. However, the observation that the suppression of the *fu* syndrome by *Su(fu)* is total suggests that Fu^+ and $Su(fu)^+$ are directly involved in the same molecular process. Cloning of the $Su(fu)^+$ gene is underway, together with the study of the expression of some other segment polarity genes in *fu;Su(fu)* embryos. These results should shed light on the molecular mechanisms involved during the *fu-Su(fu)* interaction.

Su(fu) mutations are viable and do not have any visible phenotype of their own. This raises the question of the role of this gene during development. Indirectly the same question arises for *fu* itself, since *fu;Su(fu)* individuals have normal embryonic and adult development in the absence of Fu^+ product. One explanation could be that $Su(fu)^+$ is a segment polarity gene whose function is partially duplicated. The fu^+ gene may have been selected to limit spatially or temporally the expression of $Su(fu)^+$. Also, *Su(fu)* and/or *fu;Su(fu)* individuals may have a subtle phenotype which has not yet been observed. Thus, they may have a decreased fitness compared to $fu^+;Su(fu)^+$ individuals, which might explain why both genes were maintained throughout evolution.

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