The *Suppressor of fused* Gene Encodes a Novel PEST Protein Involved in Drosophila Segment Polarity Establishment

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ABSTRACT

Suppressor of fused, Su(fu), was identified as a semi-dominant suppressor of the putative serine/threonine kinase encoded by the segment polarity gene *fused* in *Drosophila melanogaster*. The amorphic *Su(fu)* mutation is viable, shows a maternal effect and displays no phenotype by itself. *Su(fu)* mutations are often found associated to *karmoisin (kar)* mutations but two complementation groups can be clearly identified. By using a differential hybridization screening method, we have cloned the *Su(fu)* region and identified chromosomal rearrangements associated with *Su(fu)* mutations. Two classes of cDNAs with similar developmental patterns, including a maternal contribution, are detectable in the region. Transformation experiments clearly assigned the $Su(fu)^+$ function to one of these transcription units while the other one can be most likely assigned to the kar^+ function. Surprisingly the 5′ end of the *kar* RNA mapped within the 3′ untranslated region of the *Su(fu)* transcribed sequence. The *Su(fu)* gene encodes a 53-kD protein, which contains a PEST sequence and shows no significant homologies with known proteins. Genetic analysis shows that proper development requires a fine tuning of the genetic doses of *fu* and *Su(fu)* both maternally and zygotically. These results, together with previous genetic and molecular data, suggest that *fused* and *Suppressor of fused* could act through a competitive posttraductionnal modification of a common target in the hedgehog signaling pathway.

THE segmentation process in Drosophila embryo leads to the formation of a set of repeated organized metameres along the antero posterior axis. This process begins with the deposition of maternal products in the oocyte, which define anteroposterior and dorsoventral axes. Then a cascade of gene regulation occurs and leads to the activation of pair rule genes, which in turn are believed to activate a set of segmentation polarity genes (NÜSSLEIN and WIESCHAUS 1980; AKAM 1987; INGHAM 1988; INGHAM and NAKANO 1990; HOOPER and SCOTT 1992). At the blastoderm stage, the segmentation pattern may be visualized by 14 repeated bands of *engrailed* (*en*) and *wingless* (*wg*) expression, which define the parasegment boundary (DINARDO *et al.* 1985; FJÓSE *et al.* 1985; INGHAM *et al.* 1985; KORNBERG *et al.* 1985; BAKER 1987, 1988; VAN DEN HEUVEL *et al.* 1989). During the morphogenetic movements of the germ band, the segmentation pattern is further refined to establish and stabilize the positional information inside all the embryonic cells. This process is believed to occur through cell-cell interactions, and several genes have been shown to be involved in this phase of development. For instance, the proper expression of *en* at the end of germ band retraction requires the presence of *wg, patched* (*ptc*), *naked* (*nkd*), *zeste white 3* (*zw3*), *armadillo* (*arm*), *porcupine* (*porc*), *dishevelled* (*dsh*), *smooth* (*smo*), *costal2* (*cos2*), *Cubitus interruptus* (Ci^D), *gooseberry* (*gsb*), *fused* (*fu*) and *hedgehog* (*hh*) while *en, ptc, nkd, porc, dsh, smo, hh, cos2,* Ci^D, *gsb* and *fu* are necessary to the proper *wg* expression (PERRIMON and MAHOWALD 1987; MARTINEZ-ARIAS *et al.* 1988; DINARDO *et al.* 1988; RIGGLEMAN *et al.* 1990; HIDALGO 1991; LIMBOURG-BOUCHON *et al.* 1991; PEIFER *et al.* 1991; FORBES *et al.* 1993; VAN DEN HEUVEL *et al.* 1993; KLINGENSMITH *et al.* 1994; NOORDERMEER *et al.* 1994; SIEGFRIED *et al.* 1994).

The molecular cloning of some of these genes has further strengthened the picture of their involvement in cell-cell signaling. The wingless and hedgehog protein are secreted (VAN DEN HEUVEL *et al.* 1989; GONZALEZ *et al.* 1991; TAYLOR *et al.* 1993; TABATA and KORNBERG 1994), while *hedgehog, patched* and *armadillo* have been shown to contain cell membrane proteins motifs (NAKANO *et al.* 1989; RIGGLEMAN *et al.* 1989; PEIFER and WIESCHAUS 1990; MOHLER and VANI 1992; TAYLOR *et al.* 1993) and *zeste white 3* and *fused* encode serine threonine kinases (BOUROUIS *et al.* 1990; PRÉAT *et al.* 1990; SIEGFRIED *et al.* 1990; THÉROND *et al.* 1993). In spite of this important molecular knowledge, a description of

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the signal transduction pathways is still elusive. The analysis of pair wise combinations of segmentation genes is a powerful tool to understand this regulation cascade. Until now it has been used mainly to reveal epistatic relationships between already known segment polarity mutants (LIMBOURG-BOUCHON *et al.* 1991; BEJSOVEC and WIESCHAUS 1993; FORBES *et al.* 1993; NORDEMEER *et al.* 1994; SIEGFRIED *et al.* 1994). Another approach is the search of suppressors of already known mutants which has been very successful to understand regulatory pathways in yeast. Thus a dominant suppressor of the *fused* gene, *Suppressor of fused* [*Su(fu)*] has been recently identified (PRÉAT 1992; PRÉAT *et al.* 1993) that presents unusual characteristics. First of all the amorphic *Su(fu)* mutation is viable without visible defect. Second, in the absence of the *Su(fu)* product all the phenotypes associated with mutations of the *fused* gene are suppressed: the segment polarity phenotype of embryos derived from fu^- females, the fused wing phenotype of fu^- adult flies and the ovarian tumor phenotype seen in fu^- females. This implies that both genes can be eliminated in the Drosophila genome without any apparent consequences for the flies and questions for the role of *fused* and *Suppressor of fused* in Drosophila.

Interactions between *fused* alleles and a null allele of *Suppressor of fused* allowed for the first time three distinct types of *fused* alleles to be distinguished. For class 0 and class I mutations, the fused phenotypes are suppressed and normal, and viable adults can be recovered from $fu^O;Su(fu)^-$ or $fu^I;Su(fu)^-$ females. This suppression is dominant because $fu^O;Su(fu)^-/+$ or $fu^I;Su(fu)^-/+$ flies are partially rescued for their fused wing phenotype. For class II mutations, the fused phenotypes are also suppressed in a $Su(fu)^-$ background but new phenotypes are observed: $fu^{II};Su(fu)^-$ embryos derived from $fu^{II};Su(fu)^-$ females are lethal and present a phenotype similar to that of *costal2- (cos2)* (GRAU and SIMPSON 1987) embryos. Moreover $fu^{II};Su(fu)^-/+$ males are lethal in pupa, and the wings of dissected pharates show a cos2 Epaulette phenotype (while the fused wings phenotype is suppressed). The class I alleles can complement the *fu* class II alleles for their lethality and their cos2 like phenotypes in a $Su(fu)^-$ background while the class 0 alleles cannot.

The *fused* gene encodes a putative serine threonine kinase with a catalytic domain located at the NH2 terminal part of the protein being uniformly distributed in the embryo (PRÉAT *et al.* 1990; THÉROND *et al.* 1993). Sequence data suggest that the phenotype displayed by the class 2 *fused* mutants might be associated with the lack of the COOH part of the Fu protein corresponding to a putative regulatory domain (PRÉAT *et al.* 1993). In view of these results, it has been proposed that the Fu^+ kinase is a posterior inhibitor of $Cos2^+$ while $Su(fu)^+$ is an activator of $Cos2^+$ (PRÉAT *et al.* 1993).

In this paper, we report the molecular analysis and the expression pattern of the *Su(fu)* gene. The *Su(fu)* gene is present uniformly in the embryo, as observed so far for all the segment polarity genes having a maternal contribution. The *Su(fu)* protein contains a PEST sequence indicative of fast protein turnover and shows no significant homologies with known proteins. Furthermore our genetic data show that proper development requires a fine tuning of the genetic doses of *fu* and *Su(fu)* in the embryo. Recently proposed models will be discussed in view of our results.

MATERIALS AND METHODS

Drosophila stocks: Flies were kept at 20° on standard medium as described in GANS *et al.* (1975). The strains fu^1, fu^A, $Su(fu)^{12D}$ and $Su(fu)^{1P}$ used in this study were described previously (BUSSON *et al.* 1988; PRÉAT 1992). $Su(fu)^A$ and $Su(fu)^E$ were obtained from an X-ray-induced mutagenesis for fused wings phenotype suppression in fu^1 males and were originally described as partial suppressors (BUSSON *et al.* 1988). The $Su(fu)^A$ and $Su(fu)^{1P}$ strains are also mutant for the recessive eye color mutation *karmoisin (kar)*. fu^1 and fu^A are hypomorphic alleles; $Su(fu)^{1P}$ is considered as amorphic (PRÉAT 1992). The $Df(3R)kar^{SZ11}$ and $Df(3R)kar^{SZ21}$ strains are described in GAUSZ *et al.* (1979) and GAUSZ *et al.* (1980). *MKRS* is a balancer of the third chromosome bearing a *kar* mutation (LINDSEY and ZIMM 1990).

Germ line transformation: The 9.2-kb *Eco*RI-*Bam*HI fragment, the 11.3-kb *Eco*RI-*Sal*I fragment and the 4.5-kb *Sal*I fragments corresponding to coordinates (4.0–13.2), (4.0–15.3), (10.5–15.3) in the map of Figure 2, were cloned respectively into the pW6 vector (KLEMENZ *et al.* 1987), which contains the $white^+$ gene as a selective marker for transgenic lines. The resulting plasmids (SUFU000, SUFU100 and SUFU200, respectively) were injected into the $w^{1118};P(;gD2–3);Sb/TM6$ *UbX* host line (ROBERTSON *et al.* 1988) under standard conditions (SPRADLING and RUBIN 1982).

The surviving adults were mated to *w* flies, and their progeny examined for $white^+$ activity. Stocks were established from independent transformant lines, and the insertions transferred using appropriate crosses into the $Su(fu)^{1P}$ strains.

Isolation of cDNA clones: To isolate additional cDNAs, screening of distinct libraries were simultaneously performed. First, 1.4 × 104 recombinant λ gt10 phages from cDNA libraries made to 3–12-hr embryonic mRNA, provided by T. KORNBERG, were screened using a 1.1-kb *Hin*dIII fragment of the *Su(fu)* cDNA. A 1.6-kb cDNA, designed as CSU-B, was recovered. A plasmid pNB40 library made to imaginal discs mRNA and provided by N. BROWN, was also screened in parallele using either the same ^{32}P-labeled probe, or the entire *Su(fu)* cDNA. As in the first screen, only a 1.6-kb cDNA was obtained from this plasmid library. This clone was later determined by sequencing to be a cDNA beginning 9 bp upstream the CSU-A cDNA, previously isolated from embryonic libraries.

DNA sequencing and computer analysis: To sequence the genomic DNA, a 1.6-kb *Hin*dIII-*Eco*RI restriction fragment of the CSUA cDNA clone was subcloned into bluescript KS + (Stratagene), partially deleted by exonuclease III digestion, and sequenced on both strands by the dideoxy chain termination method (SANGER *et al.* 1977) using the sequenase system (U.S. Biochemical Corp.). Using synthetic oligonucleotides primers, a genomic fragment covering the CSUA cDNA and the 5′ end of the CSUE cDNA were also sequenced on one strand to confirm the abscence of introns and the position of the two cDNAs, by the dideoxy chain termination method (SANGER *et al.* 1977). DNA sequences were compiled using the UWGCG: University of Wisconsin Genetic Corporation Group software (DEVEREUX *et al.* 1984). The deduced amino acid sequence was compared with the HBRF data base using the program FASTA (PEARSON and LIPMAN 1988).

Northern blot analysis: Total RNA was extracted from Oregon-R staged embryos, larvae, pupae and adults, by using the hot phenol/SDS method, modified by SATO *et al.* (1982). Once extracted, total RNA was precipitated, recovered by centrifugation and dissolved in sterile distilled water. Poly(A)+ RNA was prepared by oligo(dT) cellulose chromatography (SAMBROOK *et al.* 1989) and performed according to the manufacturers' procedures (Pharmacia RNA purification kit). Ten milligrams of poly(A)+ RNA from various stages was fractioned on 6% formaldehyde-1% agarose gels and blotted overnight onto nylon membranes (Pall, Biodyne B, Amersham). Hybridization was performed overnight at 65°, in a [Na2HPO4, 0.25 M; EDTA, 1 mM; 7% SDS; 1% BSA] buffer, with cDNA ^{32}P-labeled probes prepared from two purified inserts: a 0.95-kb *Eco*RI-*Hin*dIII *Su(fu)* fragment, specific for the *Su(fu)* region and a 5.1-kb *Bgl*II-*Kpn*I fragment of the *fused* cDNA (THÉROND *et al.* 1993), covering the entire fused sequence. The blots were washed twice in [Na_2HPO_4, 20 mM; SDS 1%; EDTA, 1 mM] at 65°, for 5 min each time.

Whole-mount *in situ* hybridization: Whole-mount *in situ* hybridization were performed according to TAUTZ and PFEIFLE (1989) with some modifications provided by U. WALDORF (Biozentrum, Basel). Embryos were collected and fixed for 20 min at room temperature in a two-phase mixture consisting of heptane-[10% formaldehyde; phosphate buffered saline (PBS); 50 mM EGTA]. Once devitellinized in methanol/heptane mixture, embryos were rinsed several times in methanol, then in ethanol and stocked in ethanol at −20°. After a second fixation in a PBS-0.1%, Tween20, 5% formaldehyde solution, embryos were treated with proteinase K (50 mg/ml) for 5 min, their pretreatment being stopped with glycine 2 mg/ml. Once repostfixed in a [PBS-0.1% Tween20–5% formaldehyde] buffer, embryos were hybridized overnight at 70°. The cDNA restriction fragment used were prepared using the digoxigenin DNA labeling kit from Boehringer Mannheim, following the manufacturer's instructions.

RESULTS

Differential hybridization screening allows the molecular cloning of the *Su(fu)* gene: Previous genetic studies have shown that the *Su(fu)* gene is included in the *Df(3R)kar*^{SZ11} and *Df(3R)kar*^{SZ21} deficiencies (PRÉAT 1992). Their deleted region common to *Df(3R)kar*^{SZ11} and *Df(3R)kar*^{SZ21} corresponds to the 87C8 band of the third chromosome. These deficiencies complement each other for lethality, which allowed us to clone the genomic DNA absent in both of them by differential hybridization screening as depicted on Figure 1. A lambda genomic library was screened with DNA extracted from the DY313 YAC of a YAC Drosophila library (AJIOKA *et al.* 1991), which was mapped to the 87C;87D bands of the third chromosome. Forty-one phages were isolated and their DNA dotted on membranes after denaturation. The membranes were successively hybridized with ^{32}P-labeled genomic DNA from flies of genotype *Df(3R)kar*^{SZ11}/*Df(3R)kar*^{SZ21} and, after stripping off this first probe, with labeled genomic DNA from wild-type flies. At both steps the membranes were imaged with the SOFI radioimager (LEBLANC *et al.* 1988; MASTRIPPOLITO *et al.* 1991) and the resulting hybridization signals quantified for each phage *i*, giving the values N^i_{df} and N^i_{wt}, respectively.

We expected the ratio $R_i = N^i_{df}/N^i_{wt}$ to be smaller for phages spanning the DNA region common to the deficiencies than for phages located outside this region, as the genomic DNA of *Df(3R)kar*^{SZ11}/*Df(3R)kar*^{SZ21} flies does not contain this DNA. Therefore we selected phages with ratio $R_i < 0.3*Rm$, where Rm denotes the mean value of the R_i ratios. Six phages were selected and provided three independent genomic clones. One of these did not cross hybridize with the other positive clones and appears to be located outside the region uncovered by the two deficiencies: it thus appeared to be a false positive. On the contrary, the two other clones (P34, P25) cross hybridize and span a 20.9-kb genomic region (Figure 2). By Southern analysis, the *Df(3R)kar*^{SZ11} break point was located in a 1.1-kb fragment included in both clones that also appeared to be absent in the *Df(3R)kar*^{SZ21} deficiency. By screening a cosmid library with the P25 clone, a further 2.1-kb DNA fragment (coordinates 13.2–15.3 in map of Figure 2) was recovered and Southern analysis revealed that it contains the *Df(3R)kar*^{SZ21} break point.

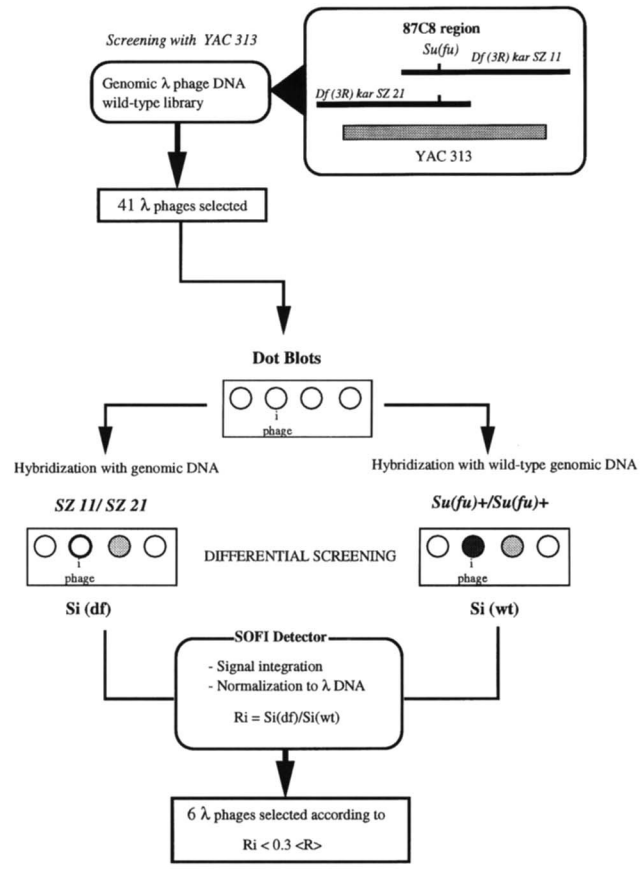

FIGURE 1.—Strategy of *Su(fu)* cloning by differential hybridization screening. Schematic map of the *Su(fu)* region as deduced from genetic data. The *Su(fu)* function is lost in flies bearing either a *Df(3)kar*^{SZ11} or a *Df(3)kar*^{SZ21} third chromosome. The overlapping region of the two deficiencies is included in the YAC313. After selection of 41 phages hybridizing with the YAC313 we looked at a lost of signal on dotted phages when hybridized with genomic DNA from double heterozygotes for the third chromosome deficiencies compared with hybridization with genomic DNA from wild type flies. Normalization with lambda DNA hybridization ensures the correction for the different amounts of DNA in the dots.

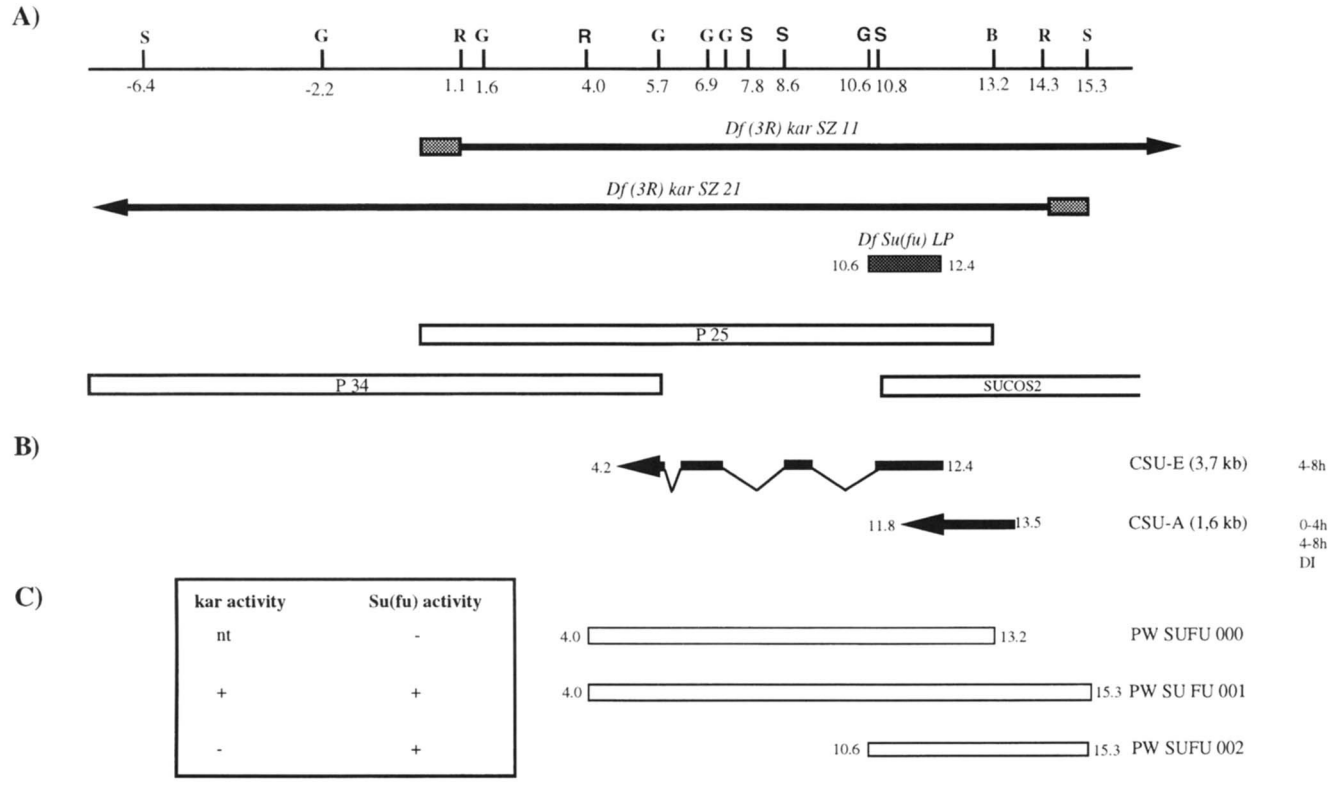

FIGURE 2.—Molecular map of the *Su(fu)* locus and transformation experiments. (A) Isolated 23-kb region and its restriction map (R, *Eco*RI; S, *Sal*I; G, *Bgl*II; B, *BamH*I). The phages P25 and P34 were isolated by differential screening hybridization as described in Figure 1 and the SUCOS2 cosmid was recovered by screening of a cosmid library with phages fragments. The break points of the $Df(3)kar^{SZ11}$ and $Df(3)kar^{SZ21}$ deficiencies are indicated. The $Su(fu)^{LP}$ allele was mapped as a small deletion between coordinates 10.6 and 12.4. (B) Two types of cDNAs were recovered after cDNA library screening and were mapped by hybridization with genomic probes or on genomic blots. The CSUE cDNA contains at least three introns depicted as dotted lines. No intron is present in CSUA. (C) Three genomic DNA fragments were used to test the phenotypic rescue of *Su(fu)* or *kar* in transformation experiments. The 4.5-kb *Sal* fragment of SUFU200, which includes the CSUA cDNA, is able to provide a full $Su(fu)^{+}$ activity.

In different screens for suppressors of the *fused* mutant phenotype, different *Su(fu)* alleles were isolated: $Su(fu)^{A}$ is a lethal mutation, presumably amorphic for *Su(fu)*, $Su(fu)^{E}$ and $Su(fu)^{I}$ are viable alleles, $Su(fu)^{12d}$ is a strong hypomorph while $Su(fu)^{LP}$ behaves as an amorphic viable allele. We tried to map these alleles in the region deleted in the *Df(3R)kar* strains by Southern genomic analysis. We were unable to map the $Su(fu)^{12D}$, $Su(fu)^{E}$, $Su(fu)^{I}$ alleles. The $Su(fu)^{A}$ allele appeared to be associated with a large deficiency deleting the entire cloned region. The amorphic $Su(fu)^{LP}$ allele was found to be associated to a small 1.5-kb deletion located at the distal end of the P25 clone (coordinates 10.6–13.2 in map of Figure 2).

Two overlapping cDNAs are present in the *Su(fu)* region: Genomic DNA probes of the P25 clone were used to screen cDNAs libraries made from 0–4-, 4–8- and 6–24-hr embryos and from imaginal disks (BROWN and KAFATOS 1988). Six cDNAs were recovered and were analyzed by restriction mapping and hybridization to the cloned genomic DNA (Figure 2). They appeared to belong to two classes: the CSU A type cDNAs were recovered in 0–4-hr and 4–8-hr embryonic libraries and in the imaginal disk library; they are 1.6 kb long and hybridize to genomic DNA in the region 11.8–13.5 kb of the map; restriction mapping suggest that they do not contain any intron. The CSU E type cDNAs were recovered in 4–8-h embryonic libraries; they are 3.7 kb long and hybridize to genomic DNA in the region 4.2–12.4 kb of the map; at least three introns are present in this transcription unit, as revealed by restriction mapping analysis.

The two cDNAs cross hybridize, and sequence analysis reveals that they share a 470-bp DNA fragment at the 3′ end of the CSU A cDNA (corresponding to the 5′ end of the CSU E cDNA; Figure 3). Partial sequence of the CSU E cDNA did not reveal any open reading frame with good codon usage before the ATG codon located at coordinate 1515 of the CSU A sequence. Because the open reading frame of CSU A ends at coordinate 1418, we concluded that no common protein sequence is generated by these two transcription units.

As a matter of fact, genetic data suggested the existence of two closely linked genes in the region. Indeed it has been noticed that the *Su(fu)* mutations are frequently associated with a karmoisin (*kar*) phenotype (PRÉAT 1992). Nevertheless there is not a strict correlation between these two phenotypes because some *Su(fu)* mutations [$Su(fu)^{12D}$, $Su(fu)^{E}$, $Su(fu)^{I}$] are not associated to a kar phenotype and inversely. The exis-

tence of a region common to the two transcription units described above explains nicely these genetic observations if each complementation group corresponds to one transcription unit.

A 4.5-kb genomic fragment including the small cDNA rescues the *Su(fu)* phenotype: Southern analysis of the mutants did not definitively assign one transcription unit to the *Su(fu)* gene because only one allele was mapped, $Su(fu)^{LP}$, which actually corresponds to a 1.5-kb deficiency deleting a region common to the two transcription units. Therefore we turned to phenotypic rescue to check the ability of the cDNAs to bring the *Su(fu)* wild-type function. We used the semidominant effect of the $Su(fu)^{LP}$ mutation on the class I (fu^1) and class II (fu^A) fused alleles: indeed $fu^1/Y;Su(fu)^{LP}/Su(fu)^+$ flies bearing one dose of the wild-type *Su(fu)* gene display a partially suppressed wing phenotype while $fu^1/Y;Su(fu)^+/Su(fu)^+$ flies bearing two doses of wild-type *Su(fu)* display a fused wing phenotype. In addition $fu^A/Y;Su(fu)^{LP}/Su(fu)^+$ flies bearing one dose of the wild-type *Su(fu)* gene are lethal as pharate adults, and display a cos2 wing phenotype, while $fu^A/Y;Su(fu)^+/Su(fu)^+$ flies bearing two doses of wild-type *Su(fu)* gene are viable and display a fused wing phenotype (PRÉAT 1992; PRÉAT *et al.* 1993).

Three fragments were injected that include either the CSU E cDNA (PwSUFU 000) or the CSU A cDNA (PwSUFU 200) or both (PwSUFU 100) and 17, 5 and 7 transformed lines, respectively, were recovered (Figure 2). To assess the presence of a *Su(fu)*+ function in the transformed lines, transgenic males of these lines were crossed with females of the $fu^1/FM6;Su(fu)^{LP}/Su(fu)^{LP}$ or $fu^A/FM6;Su(fu)^{LP}/Su(fu)^{LP}$ genotypes and the phenotype of the male progeny bearing the *fu* mutant allele and a PwSUFU insert was examined [males of genotype $fu^-/Y;PwSUFU/+;Su(fu)^{LP}/Su(fu)^+$]. With both PwSUFU 100 and PwSUFU 200 transformed lines, irrespective of the fused allele used, viable males were recovered with wing phenotypes similar to fu^1 or fu^A males, respectively. Therefore the PwSUFU 200 transposon, which includes the CSU A cDNA but not the CSU E cDNA, is able to fully compensate the decreased amount of the *Su(fu)* product in the $fu^-/Y;Su(fu)^{LP}/Su(fu)^+$ males. In contrast, with the PwSUFU 000 transformed lines, no fu^A males were recovered in the second cross, and the fu^1 males recovered in the first cross were partially rescued for their fused wing phenotype, consistent with the lack of $Su(fu)^+$ function in the insert.

Therefore we postulated that the CSU A transcription unit corresponds to the *Su(fu)* gene while the CSU E transcription unit corresponds to the *kar* gene. We tested this last assumption by crossing *PwSUFU/+;MKRS/+* flies with $Su(fu)^{LP}/Su(fu)^{LP}$ flies. As both the *MKRS* and the $Su(fu)^{LP}$ chromosomes bear a kar^- mutation, we expected half the progeny of this cross [flies of genotype $PwSUFU/+;MKRS/Su(fu)^{LP}$] to be phenotypically kar^- unless the transposon carries the kar^+ activity. As a matter of fact, only kar^+ flies were recovered when the SUFU 100 transposon is present, which indicates the presence of the kar^+ function in the insert. In contrast, kar^- flies were recovered in the presence of the SUFU 200 transposon, consistent with the lack of the kar^+ function in the insert.

In conclusion, the *Su(fu)*+ activity is fully contained in a 4.5-kb DNA fragment that does not contain the kar activity and the *Su(fu)* gene can be identified as the CSU A transcription unit.

The *Su(fu)* gene encodes a 53-kD protein with a PEST sequence: The CSU A cDNA, the 5′ end of the CSU E cDNA and the corresponding genomic DNA were sequenced (Figure 3). As suspected from restriction mapping analysis, the CSU A cDNA is colinear to genomic DNA. An extra G is present at the 5′ end of the cDNA; this could either be a cloning artifact or indicate mRNA capping. An independent cDNA CSUDI2, obtained by screening an imaginal disc cDNA library, begins 9 bp upstream the 5′ end of the CSUA cDNA and presents also an extra G at its 5′ end. Two ATG codons are present close to the beginning of the CSUA cDNA (at nucleotides 14 and 182) from which ORFs 1405 and 1237 nucleotides long could be read. Two observations suggest that the first traduction initiation site is used: first of all a good codon bias usage for Drosophila genes is observed just downstream this first ATG. Secondly, a *Su(fu)* homologue gene has been cloned in *D. virilis* and presents a high degree of homology with its *D. melanogaster* counterpart, which begins at nucleotide 25 (12 bp downstream the first ATG) and ends at nucleotide 1488 of the sequence of Figure 3. A poly A trail is present at the 3′ extremity of the cDNA beginning at nucleotide 1577, but the usual polyadenylation consensus site AATAAA is not found upstream. An AATATA sequence at nucleotide 1553 could represent an unusual polyadenylation signal. The CSU E cDNA begins at nucleotide 1107 and is colinear with CSU A DNA. The partial sequence of this cDNA indicates no significant ORF before an ATG present at position 1515.

Conceptual traduction of the CSU A cDNA yielded a protein of 468 amino acids with a molecular weight of 53 kD. Hydropathy analysis and basic or acidic region analysis of the protein did not reveal any noticeable feature. From a search in protein sequence database, no significant homology was found with any known protein. The only significant feature of this protein is the existence of a PEST sequence between nucleotides 935–994. These sequences have been implicated in fast protein degradation (ROGERS *et al.* 1986; TYERS *et al.* 1992).

The *Su(fu)* gene is expressed ubiquitously throughout embryonic development: Northern blot analysis was performed on poly-(A+) selected RNA derived from different developmental stages, using a 1.1-kb *Hin*dIII cDNA *Su(fu)* fragment. The resulting pattern shows that the *Su(fu)* gene is expressed throughout development as a unique 1.6-kb transcript, temporally

A)

B)

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-281   taa aca gat gta aat ata cca cgc aag aat act cat act gac tgg tgg tgg ttt aag cta
-221   tat gat ata tat ttt cac tgt ttt tct cta cat ttt aaa aat aag cag aaa aaa gta agt
-161   agg taa taa tta tta taa aaa agt ttt gaa aat aaa taa tgg ttt taa aag aaa ctc atg
-101   taa gtt tcc att gtc aaa tcc gca ctt tac atg gca gta ttt tta ttc atg gat att caa
                                              G--> CSUDI  G--> CSUA
-41    gcg gtg gta ttc cgc aac gtt cca act gag gtc ata ctg atc aaa ctg gat gaa ATG GCC
1                                                                          met ala
20     GAG GCG AAT TTG GAC AAA AAA CCT GAG GTG AAG CCT CCG CCG GGC CTT AAG GCC ATC ATA
3      glu ala asn leu asp lys lys pro glu val lys pro pro pro gly leu lys ala ile ile
80     GAC CAC CTC GGG CAG GTG TAC CCC AAC CAG CCG AAC CCG CTG CAG GTG ACC ACA CTG CTC
23     asp his leu gly gln val tyr pro asn gln pro asn pro leu gln val thr thr leu leu
140    AAG TAC TGG CTG GGC GGT CAG GAT CCG TTG GAC TAC ATT AGC ATG TAC AAG TTT CCC GGA
43     lys tyr trp leu gly gly gln asp pro leu asp tyr ile ser met tyr lys phe pro gly
200    GAT GTG GAT AGG AAC GTT CCG CCG CAC TGG CAC TAC ATT AGT TTC GGA CTC AGC GAC CTA
63     asp val asp arg asn val pro pro his trp his tyr ile ser phe gly leu ser asp leu
260    CAC GGG GAC GAG CGA GTC CAC TTG CGC GAG GAG GGC GTC ACT CGG TCT GGC ATG GGG TTC
83     his gly asp glu arg val his leu arg glu glu gly val thr arg ser gly met gly phe
320    GAG TTG ACC TTT CGC TTG GCC AAA ACA GAA ATA GAG CTA AAG CAG CAG ATT GAG AAC CCG
103    glu leu thr phe arg leu ala lys thr glu ile glu leu lys gln gln ile glu asn pro
380    GAA AAG CCC CAA AGA CCG CCC ACC TGG CCG GCG AAC CTG TTG CAG GCC ATC GGA CGC TAC
123    glu lys pro gln arg pro pro thr trp pro ala asn leu leu gln ala ile gly arg tyr
440    TGT TTT CAA ACA GGC AAC GGA TTG TGC TTC GGA GAC AAC ATC CCG TGG CGC AAG AGT CTG
143    cys phe gln thr gly asn gly leu cys phe gly asp asn ile pro trp arg lys ser leu
500    GAT GGC AGT ACC ACT TCC AAG CTA CAG AAC CTG CTC GTC GCC CAG GAC CCG CAG TTG GGC
163    asp gly ser thr thr ser lys leu gln asn leu leu val ala gln asp pro gln leu gly
560    TGC ATC GAC ACT CCC ACG GGC ACG GTG GAC TTC TGC CAG ATC GTC GGC GTC TTT GAC GAC
183    cys ile asp thr pro thr gly thr val asp phe cys gln ile val gly val phe asp asp
620    GAA CTT GAG CAG GCA TCG CGT TGG AAC GGG CGC GGT GTG CTC AAC TTC CTT CGC CAG GAT
203    glu leu glu gln ala ser arg trp asn gly arg gly val leu asn phe leu arg gln asp
680    ATG CAA ACT GGC GGT GAT TGG CTG GTG ACT AAC ATG GAT CGC CAG ATG AGC GTC TTC GAA
223    met gln thr gly gly asp trp leu val thr asn met asp arg gln met ser val phe glu
740    CTG TTT CCC GAA ACG CTG CTC AAC CTA CAG GAC GAT CTG GAG AAG CAG GGT TCC GAT CTG
243    leu phe pro glu thr leu leu asn leu gln asp asp leu glu lys gln gly ser asp leu
800    GCC GGC GTT AAT GCA GAT TTT ACG TTC CGT GAG CTG AAA CCT ACC AAG GAG GTG AAG GAG
263    ala gly val asn ala asp phe thr phe arg glu leu lys pro thr lys glu val lys glu
860    GAG GTG GAT TTC CAG GCG CTG AGC GAG AAG TGC GCC AAC GAC GAA AAC AAT CGG CAG CTG
283    glu val asp phe gln ala leu ser glu lys cys ala asn asp glu asn asn arg gln leu
920    ACG GAT ACT CAA ATG AAA CGC GAG GAA CCA AGC TTT CCA CAA TCC ATG TCG ATG AGC AGC
303    thr asp thr gln met lys arg glu glu pro ser phe pro gln ser met ser met ser ser
980    AAT TCG CTG CAC AAG TCC TGT CCC CTG GAC TTT CAA GCG CAG GCA CCA AAC TGC ATT TCA
323    asn ser leu his lys ser cys pro leu asp phe gln ala gln ala pro asn cys ile ser
1040   CTG GAC GGT ATT GAG ATT ACT CTC GCA CCT GGT GTG GCC AAG TAC CTC CTA CTG GCC ATC
343    leu asp gly ile glu ile thr leu ala pro gly val ala lys tyr leu leu leu ala ile
                 ----> CSUE
1100   AAG GAT CGC ATC CGA CAT GGG CGC CAC TTC ACC TTC AAG GCT CAG CAT CTG GCG CTC ACT
363    lys asp arg ile arg his gly arg his phe thr phe lys ala gln his leu ala leu thr
1160   TTG GTG GCG GAA TCC GTC ACC GGC TCG GCG GTG ACA GTG AAC GAA CCG TAC GGC GTG CTG
383    leu val ala glu ser val thr gly ser ala val thr val asn glu pro tyr gly val leu
1220   GGC TAC TGG ATT CAG GTC CTG ATT CCC GAT GAA CTG GTG CCG CGC CTG ATG GAA GAC TTC
403    gly tyr trp ile gln val leu ile pro asp glu leu val pro arg leu met glu asp phe
1280   TGC AGC GCG GGC CTA GAC GAG AAA TGC GAG CCC AAG GAG CGA CTG GAG CTC GAG TGG CCC
423    cys ser ala gly leu asp glu lys cys glu pro lys glu arg leu glu leu glu trp pro
1340   GAC AAG AAT CTG AAG CTG ATC ATC GAC CAG CCG GAA CCT GTG CTG CCC ATG TCG CTC GAC
443    asp lys asn leu lys leu ile ile asp gln pro glu pro val leu pro met ser leu asp
1400   GCT GCT CCT CTG AAA ATG tga tgg tgt cca ttg gtt agc tag tag tta ttt aaa tta atc
463    ala ala pro leu lys met OPA
1460   gaa ttg aat ctt cca cta acg agt aag ttt tac att tat cat ctc atc taa cac aat gca
1520   cgt ggc ctt tgc tcc tcg ccg gag tcc agt caa aat ata caa cac ata cta tac ccg aaa
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regulated (Figure 4D). The *Su(fu)* message appears to be accumulated in adult females and early embryos, whereas it decreases to lower expression levels during larval stages. This observation is in accordance to previous genetic analysis (PRÉAT *et al.* 1993) demonstrating the existence of a maternal component of *Su(fu)* gene. The $Su(fu)^{LP}$ allele, which behaves as an amorphic mutation, is associated to a 1.5-kb small deletion. In $Su(fu)^{LP}$ homozygous mutant adults, we still observed a *Su(fu)* transcript but, as expected, its size is modified down to 1.3 kb.

To compare the *Su(fu)* expression profile with that of the *fused* gene, the same blot was also probed to a *fused* cDNA probe containing the entire *fused* coding region. Those two expression patterns are both depicted in Figure 4D. The *fused* gene encodes a unique 3.2-kb trancript (THÉROND *et al.* 1993) displaying, like *Su(fu)*, a maternal expression in adult females and in early embryos. The developmental profile of the two gene is very similar but the *fused* transcript seems somehow less abundantly expressed during development than *Su(fu)* is.

Whole-mount *in situ* hybridization experiments revealed that the *Su(fu)* transcript is uniformly distributed throughout the embryo from preblastoderm to shortened germ band stages (Figure 4, A–C). Compared with *fused* expression, the *Su(fu)* gene appears to be transcribed at higher levels. Unlike *fused,* it does not disappear at shortened germ band stage (Figure 4C).

The *Su(fu)* gene interact with fused in a dose-dependent manner: Using transformed lines containing a *Su(fu)* transposon (SF lines), we investigated the influence of *Su(fu)* dose number on the viability and the phenotype of the fu^- males. Two *fu* alleles were studied, fu^1 and fu^A, which belong to the class I and class II of *fused* alleles, respectively (PRÉAT *et al.* 1993). A complete analysis was carried out with strains bearing the SF107, SF133, SF140 transposons located on the *X*, second and third chromosome, respectively, but similar results were obtained with other lines (data not shown). The results depicted in Table 1 and Figure 5 show that when the number of $Su(fu)^+$ doses in the fu^- male progeny is greater than two, its viability decreases, irrespectively of the class of the fused allele. This decrease of viability is correlated with an enhancement of the fused wing phenotype (Figure 5). The decrease of viability is more pronounced when the transgene is provided by the mother: fu^1, *SF107/Y;SF140,*$Su(fu)^+/Su(fu)^+$ males derived from fu^1, *SF107/FM7* females show a weaker viability and a stronger fused wing phenotype than the fu^1/*Y;SF133/+;SF140,* $Su(fu)^+/Su(fu)^+$ males derived from $fu^1/FM7$ females, although they both contain four doses of $Su(fu)^+$. This result is fully consistent with the maternal effect, already described for the *Su(fu)* gene (PRÉAT 1992). However we can not exclude that this phenotypic difference could result from differences of $Su(fu)^+$ gene expression in the two transposons SF107 and SF133, because of position effects. We do not favor this hypothesis because the two transposons are indistinguishable for their $Su(fu)^-$ rescue of $fu^-;Su(fu)^{LP}/+$ males in the functionnal tests described above and because this effect can be reproduced with other combinations of *Su(fu)* transformed lines (data not shown).

The viability of the fu^A flies, but not of fu^1 ones, is also affected when the *Su(fu)*+ activity decreases, corresponding to one or zero *Su(fu)*+ doses (Table 1). As already described (PRÉAT 1992), the decrease of viability was found associated with a new phenotype of anterior wing duplication similar to the phenotype observed in *cos2* mutants.

DISCUSSION

Differential hybridization screening method offers a powerful way of gene cloning in nonrepeated genomes: We have shown in this paper that the genetic information collected on the *Su(fu)* gene allowed us to clone this gene by differential hybridization screening much faster than with conventional methods. Indeed, this cloning procedure required only a two-step hybridization procedure to isolate a small region of interest including our gene from a large genomic region (here a 280-kb region spanned by a YAC). This contrasts with the tedious work of phage digestion, Southern blotting and multihybridization procedures conventionally required to identify chromosomal rearrangements breakpoints.

The differential hybridization screening procedure requires flies of different genotypes to generate complex genomic probes presenting a difference in sequence dose number for the region of interest. These complex probes are then hybridized on a set of clones, and the differences between normalized hybridization signals allows the selection of the clones. Previously, the use of such complex probes with different gene dosage has been used sucessfully to map accurately deficiencies breakpoints independently of the size of the region analyzed (THÉROND *et al.* 1992). To our knowledge, it is the first time that it is used directly for gene cloning.

The selectivity of the method is higher when high ratio of gene sequences dosage are used. For the *Su(fu)* gene cloning, this procedure was facilitated by the exis-

FIGURE 3.—Molecular organization and sequence of the *Su(fu)* locus. (A) Depictions of the two overlapping cDNAs detected in hybridizing cDNA libraries with a fragment providing full rescue of the *Su(fu)* phenotype. The ORFs are shown as open boxes and the PEST sequence observed in the CSUA cDNA is figured as a shaded box. (B) Sequence of the CSUA cDNA with 280 bp of upstream genomic sequence. Base substitutions because of polymorphism were observed in the sequencing of a genomic clone (A, G, G and A being present at coordinates 55, 460, 928 and 954, respectively) leading to one conservative change (Phe314 to Tyr) in the protein sequence. The beginning of the CSUA, CSUE and CSUDI2 cDNAs are indicated. The *Su(fu)* protein contains a PEST sequence between coordinates 935 and 994 that is underlined.

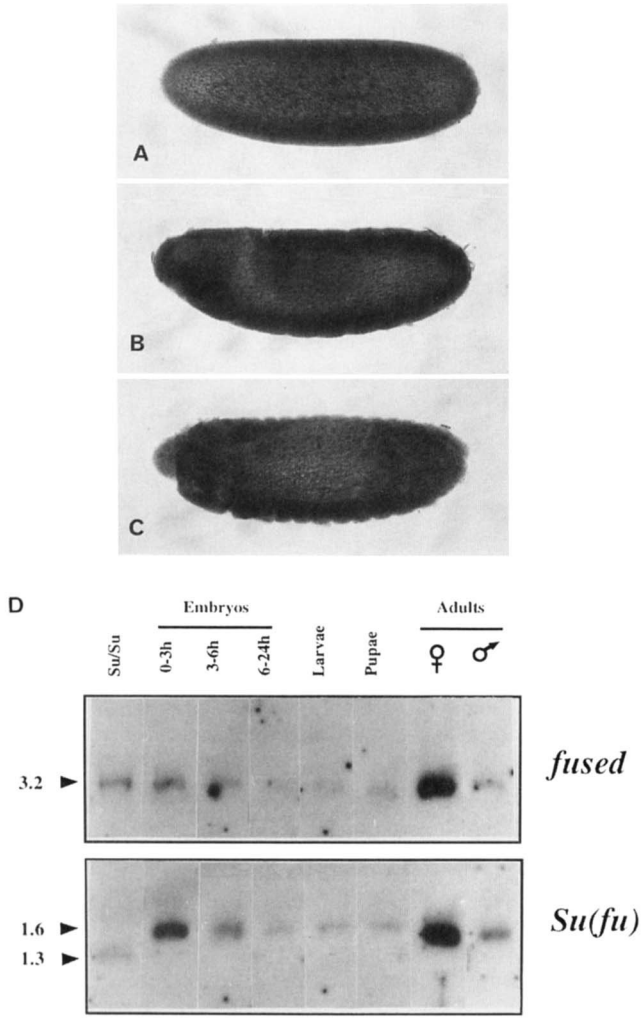


FIGURE 4.—Embryonic spatial and developmental profile expression of *Suppressor of fused*. Three contiguous nonoverlapping genomic *Su(fu)* probes were used to performe whole-mount *in situ* hybridizations on various stages wild-type embryos: a 0.8-kb *Hin*dIII-*Bam*HI fragment covering the 5′ region of the *Su(fu)* gene (data shown here), a 0.8-kb *Bam*HI-*Hin*dIII fragment of the coding region and a 0.9-kb *Hin*dIII-*Pvu*II fragment containing the 3′ part of the gene. In all cases, similar results were obtained. The *Su(fu)* transcript is uniformly distributed and detected from preblastoderm to shortened germ band stage. (A) Cellular blastoderm embryo. Staining is observed in the peripheral cells. (B) Elongated germ band embryo displaying an uniform staining in all embryonic cells. (C) Shortened germ band embryo. Unlike *fused* transcript fading at this developmental stage, overall *Su(fu)* expression still remains. Anterior is to the left. Dorsal region is up. (D) Ten micrograms of poly(A+)-selected RNA derived from different developmental stages were subjected to electrophoresis on a 6% formaldehyde-1% agarose gel, blotted onto nylon membrane and probed with a 1.1-kb *Hin*dIII fragment from the *Su(fu)* cDNA (bottom). A 1.6-kb transcript is expressed throughout development, appearing to fall to lower levels during larval stages. The *Su(fu)* transcript is shortened to 1.3 kb in $Su(fu)^{LP}$ homozygous mutant adults. The blot was also probed with the *fu* cDNA (top). As previously described (THÉROND *et al.* 1993), *fused* encodes a unique 3.2-kb transcript, displaying a similar temporal pattern as *Su(fu)* during development. Time points and lengths of the transcripts are respectively indicated on the top and left of the panels.

tence of two deficiencies deleting the gene and complementing each other: because genomic probes generated from *trans* heterozygotes flies are devoided of the sequences deleted in both deficiencies, the selectivity was good. From 40 initial phages, seven were selected after the first screen and only two turned out to be false positives. When less favorable situations are encountered (for instance a ratio of 2:1 sequence dose number between the probes), it will be highly desirable to do redundant hybridization for each clone to minimize the false positives.

Two main features limit this method: first, the signal-to-noise ratio is poor. This is because of the use of complex probes in which the clone homologous sequences represent only a small portion of the probe sequences. This may limit this method to genomes of small or intermediate size. In any case, careful attention must be paid to noise minimization. Second, this method is not usable when several copies of the clone sequences are present in the genome, as, in this case, the hybridization ratio will not reflect the ratio expected from genetic dosage. In particular it is clear that this method can not be used for highly repeated genomes. In spite of these restrictions, we think that it could be a valuable method for accelerating the cloning steps in many cases: for instance, it could allow the detection of small X-ray-induced deletions not detected cytologically.

Two overlapping transcription units correspond respectively to the *Su(fu)* and the *kar* genes: Two chromosomal defects associated with the *Su(fu)* mutations were located in the region cloned by differential hybridization screening and two major cDNAs were isolated from this region. An intriguing feature was the overlapping of the two transcription units: the two cDNAs are transcribed in the same direction and exhibit a common part of 470 nucleotides. To our knowledge, the initiation of transcription within the transcribed region of an upstream gene has been previously described in Drosophila in only two cases: the *actin 5C* overlapping gene (RAO *et al.* 1988) and the *janB* gene (YANICOSTAS *et al.* 1989), which initiate their transcription inside the *actin 5C* and the *janA* transcribed region, respectively. In both cases no genetic information on the role of both transcription units is available. For the *Su(fu)* region, the significance of this overlap is quite uncertain because transformation experiments followed by phenotypic rescue clearly assigned the *Su(fu)* gene to one of these transcription units and the *kar* gene to the other one. This confirms genetic results suggesting a close proximity of the two genes but no identity, because some *Su(fu)* alleles are also mutant for *kar* while others alleles complement all the *kar* mutations (PRÉAT 1992).

The two transcription units present the same expression pattern: a maternal component observed in early embryos, strong expression in gastrulating embryos and weaker expression in later stages. This result is in

TABLE 1

Viability of fu^- males in different *Su(fu)* dose number context

Females	Males	Males offspring genotype	*Su(fu)* dose number	No. of embryos	Viability fu^- males
$fu^1/FM7$	$Su(fu)^{LP}$	fu^1/Y; $Su(fu)^{LP}/Su(fu)^+$	1	153	0.55
	w	fu^1/Y; $Su(fu)^+/Su(fu)^+$	2	284	0.71
	SF140	fu^1/Y; *SF140*,$Su(fu)^+/Su(fu)^+$	3	377	0.45
	SF133;SF140	fu^1/Y;*SF133/+*; *SF140*, $Su(fu)^+/Su(fu)^+$	4	191	0.23
fu^1,*SF107/FM7*	$Su(fu)^{LP}$	fu^1,*SF107/Y*; $Su(fu)^{LP}/Su(fu)^+$	2	187	0.48
	w	fu^1,*SF107/Y*; $Su(fu)^+/Su(fu)^+$	3	262	0.27
	SF140	fu^1,*SF107/Y*; *SF140*,$Su(fu)^+/Su(fu)^+$	4	77	0.11
	SF133;SF140	fu^1,*SF107/Y*; *SF133/+*; *SF140*, $Su(fu)^+/Su(fu)^+$	5	153	0.00
$fu^A/FM3$	$Su(fu)^{LP}$	fu^A/Y; $Su(fu)^{LP}/Su(fu)^+$	1	141	0.00
	w	fu^A/Y; $Su(fu)^+/Su(fu)^+$	2	287	0.69
	SF140	fu^A/Y; *SF140*,$Su(fu)^+/Su(fu)^+$	3	228	0.66
	SF133;SF140	fu^A/Y;*SF133/+*; *SF140*, $Su(fu)^+/Su(fu)^+$	4	210	0.35
fu^A,*SF107/FM7*	$Su(fu)^{LP}$	fu^A,*SF107/Y*; $Su(fu)^{LP}/Su(fu)^+$	2	180	0.69
	w	fu^A,*SF107/Y*; $Su(fu)^+/Su(fu)^+$	3	177	0.00
	SF140	fu^A,*SF107/Y*; *SF140*,$Su(fu)^+/Su(fu)^+$	4	192	0.00
	SF133;SF140	fu^A,*SF107/Y*;*SF133/+*; *SF140*, $Su(fu)^+/Su(fu)^+$	5	377	0.00

To avoid the problem of the difference of viability of the *fused* alleles used and of the lethal *X* balancers which do not allow a direct comparison between males, the viability was calculated for each cross as 2 $N_{fu}/N_{females}$ where N_{fu} denotes the number of fu^- adult males and $N_{females}$ the total number of adult females. The dose number of *Su(fu)* was varied by using either the $Su(fu)^{LP}$ amorphic allele or lines containing transposons bearing a $Su(fu)^+$ activity. The line *SF140* is homozygote for a transposon on the third chromosome while the line *MSF22* is homozygote for transposons on the second *(SF133)* and third chromosome *(SF140)*. fu^-, *SF107* lines were obtained by recombination between fu^- lines and lines bearing the *SF107* transposon located on the *X* chromosome, which has also a $Su(fu)^+$ activity.

agreement with the genetic data obtained previously for the *Su(fu)* gene, because it has been shown that this gene has a maternal component and that it can suppress both embryonic and adult defects associated with the *fu* gene (PRÉAT 1992). On the other hand, this expression pattern was unexpected for the *kar* transcription unit, because only phenotypic defects in the adult have been assigned to *kar* mutations. The significance of this observation is still unclear.

The *Su(fu)* gene encodes a novel protein containing a PEST sequence that interferes with the action of the fused protein in a dose-dependent manner: Transformation experiments demonstrated that the *Su(fu)* gene interacts with the *fu* gene in a dose-dependent manner: increasing the number of doses of the $Su(fu)^+$ gene enhances the fused phenotype of fu^- flies, independently of the type of *fu* allele. On the other hand, decreasing the number of $Su(fu)^+$ copies leads to lost of

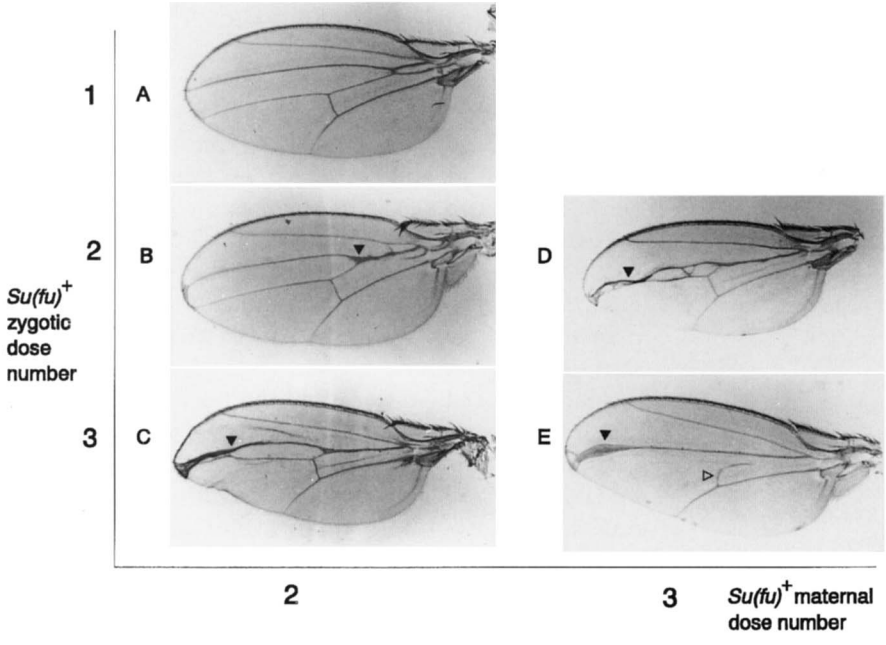
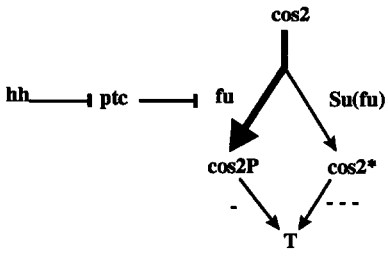


FIGURE 5.—Effects of the dose number of the *Su(fu)* gene on adults fused wing phenotype. The dose number was varied by using $fu^1/FM7$ (A–C) or fu^1, *SF107/FM7* (D and E) females crossed with wild-type (B and E), $Su(fu)^{LP}/Su(fu)^{LP}$ (A and D) or *SF140*, $Su(fu)^+/SF140$, $Su(fu)^+$ males (C) allowing the *Su(fu)* dose number to be varied both from its maternal contribution and its zygotic contribution [*SF107* and *SF140*, $Su(fu)^+$ denotes respectively *X* and third chromosomes including one copy of a transposon bearing a $Su(fu)^+$ activity;cb. Wings were mounted from males of genotypes fu^1/Y;$Su(fu)^{LP}/Su(fu)^+$ (A), fu^1/Y (B), fu^1/Y;*SF140*, $Su(fu)^+/Su(fu)^+$ (C), fu^1, *SF107/Y*;$Su(fu)^{LP}/Su(fu)^+$ (D), fu^1, *SF107/Y*;$Su(fu)^+/Su(fu)^+$ (E). Notice the increase of the severity of fused wing phenotype (arrowheads) both when the zygotic dose number of *Su(fu)* increases and when the maternal dose number of *Su(fu)* increases.

A)



B)

GENOTYPE	major cos2 protein	T activity	Phenotype
WT	cos2 P	* * *	WT
fu	cos2 *	*	fu
Su(fu)^{LP}	cos2 P	* * *	WT
cos2	none	* * * * * *	cos2
fu^{1} ; Su(fu)^{LP}	cos2	* * *	WT
fu^{A} ; Su(fu)^{LP}	cos2-fu^{A}	* * * * * *	cos2
fu^{1}/fu^{A} ; Su(fu)^{LP}	cos2	* * *	WT

FIGURE 6.—Putative model of the interaction between *fused, Suppressor of fused* and *costal2*. (A) The Fu protein activity is inhibited by a Ptc function under the negative control of the Hedgehog signal. The Cos2 protein can be modified through its interaction with either Fu (which produces a Cos2P modified protein) or *Su(fu)* (which produces a Cos2* modified protein). The modified proteins then control the activity of a putative target T in opposite ways: Cos2P stabilize the T activity to an intermediate level while Cos2* decreases the T activity. In the wild-type embryos, the modification by the fused protein is dominant compared with the modification by the *Su(fu)* protein (thick arrow), thus stabilizing the T activity to its normal value. This activity is modified when the Fu, *Su(fu)* or Cos2 proteins are mutated, resulting in mutant phenotypes as depicted in (B). (B) In the wild-type embryo, the interaction of the Cos2 protein would occur preferentially with the Fu protein and leads to a major phosphorylated form Cos2P and thus to a proper and normal development. In a *fused*-mutant, the phosphorylation would not occur and the interaction of Cos2 with *Su(fu)* would lead to a modified form of the protein Cos2*, a reduced T activity (*) and to the segment polarity fused phenotype. In the *cos2*-mutant, the abscence of Cos2 would lead to an increase of the T activity (*****) and to the cos2 segment polarity phenotype whenever Fu or *Su(fu)* are present or not. The suppression of the embryonic defects in class 0 or class 1 *fused* mutants when the *Su(fu)* protein is absent would indicate that the non phosphorylated form of Cos2 has the same activity as the phosphorylated form and can provide a proper T activity (***). Thus the fused phenotype would not result from the absence of phosphorylation but from the interaction of Cos2 with *Su(fu)*. On the other hand, the cos2-like phenotype observed in class 2 *fused* mutants when the *Su(fu)* protein is absent would indicate that the class 2 fused proteins are able to modify the Cos2 protein (may be by irreversible binding) in such a way that the normal Cos2 function is inhibited, as in *cos2*-mutants, providing an increase of T activity (*****). The presence of class 1 fused proteins would remove this inhibition as revealed by the dominance of *fuI* over *fuII* observed in the two types of alleles in a *Su(fu)*-background. Thus normal level of T activity (***) would be restored.

viability of *fu*$^{-}$ flies, only for class II *fu* alleles, which is associated to a new phenotype closely resembling the phenotype seen with *cos2* mutations. These results are quite surprising because we observed no defects in *fu*$^{+}$ flies when the dose number of *Su(fu)*$^{+}$ is varied from 0 to 6 (data not shown). Therefore it appears that in a *fu*$^{-}$ genetic context, and only in this case, the dose number of *Su(fu)* is critical for a proper embryonic development: depending on the dose number of *fu* and *Su(fu)* and the type of *fused* allele, switches in the developmental pathway lead either to *cos2* like defects or *wg* like defects.

The observation that both types of alleles are affected in the same way (fused type phenotype) when the *Su(fu)* dose number is increased contrasts with the involvement of only class II alleles in viability reduction when the *Su(fu)* dose number is decreased. Nevertheless it should be stressed that, for both types of alleles, the fused phenotypes (embryonic cuticle and wing) are suppressed when the *Su(fu)* dose number is decreased, the cos2 like phenotype thus appearing as an extra phenotype linked to the particular structure of the class II *fu* alleles. So our genetic analysis emphasizes the involvement of *fu* and *Su(fu)* genes in a similar process and point out the importance of dose equilibrium between these two genes to provide a normal development.

The fused protein is a serine threonine kinase that has been postulated to interfere with *wg* signaling (LIMBOURG-BOUCHON *et al.* 1991). Sequence analysis and expression pattern in the embryos do not provide direct information on the role of *Su(fu)* in the segmentation process. The only significant feature of the protein is the existence of a PEST sequence that is thought to be present in unstable proteins (ROGERS *et al.* 1986). This may indicate a fast turnover of the *Su(fu)* protein itself; alternatively the *Su(fu)* protein could interact with other proteins and induce their degradation through the PEST sequence. We examine now how these features fit our present knowledge of the role of *fu* and *Su(fu)* in the establishment of segment polarity in Drosophila embryos.

Competition of *fused* and *Suppressor* of fused in embryonic development: On the basis of genetic epistasis analyses, it has proposed that *fused* is a component of the Hedgehog signaling pathway, where it would regulate the activity of the *Ci*^{D} gene (LIMBOURG-BOUCHON *et al.* 1992; FORBES *et al.* 1993; INGHAM 1993). The fused protein would act antagonistically to Cos2 and its function would be repressed by the Ptc activity. The Hedgehog signal would either inhibit the Ptc activity or act in parallel to Ptc. On the other hand, PRÉAT *et al.* (1993) described genetic interactions between *fu, Su(fu)* and *cos2* mutant alleles and proposed that the *Su(fu)*+ protein would activate the costal2+ function whereas the fused kinase would inhibit this activation in the posterior part (naked cuticle) of the segment. We have shown here the existence of a genetic dosage depen-

dence between *fu* and *Su(fu)* genes. This observation strongly supports the hypothesis of competitive role performed by the *fu* and *Su(fu)* genes.

To take into account the whole genetic and molecular data, including the existence of different classes of *fused* alleles, we propose the following modifications of this model: (1) the cellular fate in the *wingless* competent domain could be determined by the intracellular activity of an hypothetical T protein, negatively controlled by the Cos2 product, and (2) the fused and Suppressor of fused proteins would modify in a competitive way the T protein activity, either directly, or indirectly by acting on the Cos2 product (Figure 6). At this step, several scenarios can be envisaged, differing in two aspects: the *fu* and *Su(fu)* target (T or Cos2) and the position of the Hh-Ptc signal in this network. One of them, presented in Figure 6, integrates the main features of the model of PRÉAT *et al.* (1993), being somehow simpler, as it releases from some additional regulating elements, previously postulated (PRÉAT *et al.* 1993).

In all cases, the main features of the model, the modulation of the T activity by a competitive post translational modification of the protein, remains basically unchanged. It is likely that the fused protein would act by phosphorylating the putative common target, because it has been shown to be highly homologous to serine threonine kinases. The role of *Su(fu)* is more elusive. The existence of a PEST sequence in its coding sequence suggests a possible mode of action for this protein: by binding with its target, it could influence its activity, which would be critical for the normal specification of the posterior part of the segment. Such a regulation of a protein activity by its binding with a PEST ligand has been demonstrated for the complex *cdc28-Cln3* in the control of cell division in yeast (TYERS *et al.* 1992).

The wingless protein disappears in *fused*$^{-}$ mutants whereas it extends in *cos2* or fu^{A};$Su(fu)^{LP}$ mutants (cited in PRÉAT *et al.* 1993). It is thus tempting to speculate that T could be directly related to wg production. As the *CiD* gene has been shown to be part of the Hedgehog signaling pathway and to be implicated in the regulation of *wg* and *ptc* expression (FORBES *et al.* 1993), it is an obvious candidate for being the target whose expression is modulated by Fu and *Su(fu)*. Experiments are in progress to test this hypothesis.

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