Segmental Polarity in *Drosophila melanogaster*: Genetic Dissection of *fused* in a *Suppressor of fused* Background Reveals Interaction With *costal-2*

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ABSTRACT

fused (*fu*) is a segment polarity gene that encodes a putative serine/threonine kinase. A complete suppressor of the embryonic and adult phenotypes of *fu* mutants, *Suppressor of fused* (*Su(fu)*), was previously described. The amorphic *Su(fu)* mutation is viable and displays no phenotype by itself. We have used this suppressor as a tool to perform a genetic dissection of the *fu* gene. Analysis of the interaction between *Su(fu)* and 33 *fu* alleles shows that they belong to three different classes. Defects due to class I *fu* alleles are fully suppressed by *Su(fu)*. Class II *fu* alleles lead to a new segment polarity phenotype in interaction with *Su(fu)*. This phenotype corresponds to embryonic and adult anomalies similar to those displayed by the segment polarity mutant *costal-2* (*cos-2*). Class II alleles are recessive to class I alleles in a *fu[I]/fu[II];Su(fu)/Su(fu)* combination. Class 0 alleles, like class I alleles, confer a normal segmentation phenotype in interaction with *Su(fu)*. However class II alleles are dominant over class 0 alleles in a *fu[0]/fu[II];Su(fu)/Su(fu)* combination. Alleles of class I and II correspond to small molecular events, which may leave part of the Fu protein intact. On the contrary, class 0 alleles correspond to large deletions. Several class I and class II *fu* mutations have been mapped, and three mutant alleles were sequenced. These data suggest that class I mutations affect the catalytic domain of the putative Fu kinase and leave the carboxy terminal domain intact, whereas predicted class II proteins have an abnormal carboxy terminal domain. *Su(fu)* enhances the cos-2 phenotype and *cos-2* mutations interact with *fu* in a way similar to *Su(fu)*. All together these results suggest that a close relationship might exist between *fu*, *Su(fu)* and *cos-2* throughout development. We thus propose a model where the Fu^+ kinase is a posterior inhibitor of Costal-2^+ while $Su(fu)^+$ is an activator of Costal-2^+. The expression pattern of *wingless* and *engrailed* in *fu* and *fu;Su(fu)* embryos is in accordance with this interpretation.

SEGMENT polarity genes are required for the antero-posterior definition of intrasegmental patterns (NÜSSLEIN-VOLHARD and WIESCHAUS 1980). Segment polarity products are believed to participate in the establishment of positional information, which allows a cell or a group of cells to localize itself within the segment (reviewed in MARTINEZ-ARIAS 1989, WILKINS and GUBB 1991). Nine segment polarity genes have been cloned and sequenced so far. Analysis of their function and expression strongly suggests that they encode proteins involved in cell-to-cell communication processes after the blastoderm stage (reviewed in INGHAM 1991, HOOPER and SCOTT 1992, PEIFER and BEJSOVEC 1992). However little is known about the direct interactions taking place during these signal transduction cascades, in part because several products remain to be characterized.

fused (*fu*) embryos derived from *fu* females present a deletion of the posterior part of the segments and mirror-image duplication of the anterior part. When produced by *fu*/+ females *fu* embryos develop normally, but *fu* adults display several anomalies such as abnormal wing veins and tumorous ovaries. The *fu* gene encodes a putative serine/threonine kinase (PRÉAT *et al.* 1990) and the *fu* transcript is distributed evenly in the embryo (THÉROND *et al.* 1993), suggesting that post-translational modifications may play a key role in intrasegmental pattern specification. Several proteins encoded by developmental genes are known to be phosphorylated by ser/thr kinases, but so far the substrate of the Fu kinase is not known. The upstream regulation of the Fu kinase is also not understood. The putative Fu protein contains all the amino-acids conserved in ser/thr kinases, sharing up to 30% amino-acid identity within the catalytic domain (PRÉAT *et al.* 1990), but it has not been possible to place Fu within a specific class of ser/thr kinases.

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In particular, Fu shares no additional similarity to any of the Drosophila putative kinases so far identified (reviewed in SIEGFRIED, AMBROSIO and PERRIMON 1990), and Fu is *not* the homolog of the cdc2 kinase.

A complete suppressor of the phenotype of *fu* embryos and adults, *Suppressor of fused* (*Su(fu)*), was isolated and described (PRÉAT 1992). *Su(fu)* mutations correspond to a loss of function, are semidominant and display a maternal effect. The amorphic *Su(fu)* mutation is viable and has no phenotype by itself. *Su(fu)* fully suppresses all fu phenotypes, suggesting that *fu* and *Su(fu)* are engaged in a same process.

In an attempt to gain information about the role and the regulation of the Fu^+ kinase, we have analyzed the behavior of 33 *fu* alleles in a *Su(fu)* background. This study leads to a new and qualitative classification of *fu* alleles. The molecular bases underlying this classification were investigated. The results shed light on the functional organization of the *fu* gene, and provide important insights into possible interactions occurring between the Fu^+ kinase and some other segment polarity products.

MATERIALS AND METHODS

Stocks and culture: $Su(fu)^{LP}$ and $Su(fu)^{12d}$ are EMS-induced mutations previously described (PRÉAT 1992). The $Su(fu)^{LP}$ allele behaves as an amorphic mutation. The $Su(fu)^{LP}$ strain is also mutant for the adjacent eye-color gene *karmoisin* (*kar*). Thirty-one *fused* alleles are listed in Table 1 with references. Genetic properties of these *fu* alleles are described in BUSSON *et al.* (1988), except for fu^{1PP7}, fu^{9P2} and fu^{2P} (PERRIMON and MAHOWALD 1987), and the fu^{RX} alleles, which are X-ray induced mutations isolated in our laboratory over a fu^1 allele (D. BUSSON, C. LAMOUR-ISNARD, B. LIMBOURG-BOUCHON, M.-C. MARIOL and T. PRÉAT, unpublished data). The fu^1 and fu^A mutations we used in this study as reference alleles were marked with f^{36a}. $Df(1)fu^{Z4}$ is a 40-kb DEB-induced deletion which removes all *fu* sequences (PRÉAT *et al.* 1990). $Df(1)fu^{P1}$ was recovered during a PM mutagenesis by R. HOLMGREN (cited in MARIOL, PRÉAT and LIMBOURG-BOUCHON 1987). This strain contains no transposable element inserted in *fu*, but corresponds to a large deficiency breaking in the 5′ region of the gene (PRÉAT *et al.* 1990; THÉROND, MASTRIPPOLITO and TRICOIRE 1992). An $ovo^{D1},v/Y;Su(fu)^{LP}/Su(fu)^{LP}$ × $C(1)DX,y\ f/Y;Su(fu)^{LP}/Su(fu)^{LP}$ stock was generated for germ line mitotic recombination experiments. $Df(3R)kar^{Sz11}$ and $Df(3R)kar^{3Q}$ are described in GAUSZ *et al.* (1979) and in GAUSZ, AWAD and GYURKOVICS (1980). The *costal-2* (*cos-2*) mutations $cos\text{-}2^3$, $cos\text{-}2^7$ and $cos\text{-}2^{V1}$ (GRAU and SIMPSON 1987; SIMPSON and GRAU 1987) were kindly provided by PATRICIA SIMPSON. $cos\text{-}2^3$ is a strong embryonic lethal allele. $cos\text{-}2^7$ is a hypomorphic allele leading to partial pupal lethality. Escapers display a strong cos-2 wing phenotype. $cos\text{-}2^3$ and $cos\text{-}2^7$ are on a *cn bw sp* chromosome. $cos\text{-}2^{V1}$ is a viable wild-type isoallele showing no phenotype by itself, but leading to a cos-2 phenotype in interaction with dominant *Cos-1* mutations (GRAU and SIMPSON 1987; SIMPSON and GRAU 1987). $cos\text{-}2^{V1}$ is on a *b pr cn bw* chromosome. The *patched* (*ptc*) mutation ptc^{JN108} is a strong EMS-induced mutation (HIDALGO and INGHAM 1990). Stocks were maintained on a yeast/maize/agar medium (GANS, AUDIT and MASSON 1975). Crosses were made at 23° unless otherwise specified.

Definition of the *fu* allele' classes at the adult stage: *fu[x]/Balancer* virgin females were crossed in vials to $Su(fu)^{LP}/Su(fu)^{LP}$ males, generating $fu[x]/Y;Su(fu)^{LP}/+$ males. For some *fu* alleles (named class I alleles), adults with a suppressed *fu* wing phenotype were recovered. These males might have received a wild-type *X* chromosome from their father, for example, if some of the *fu[x]/Balancer* females had carried a *Y* chromosome. To rule out this possibility, putative $fu[I]/Y;Su(fu)^{LP}/+$ males were crossed to *C(1)DX,y f* females. *fu[I]/Y* males displaying a regular fused phenotype were recovered in the progeny of all crosses, confirming that their fathers actually carried a *fu* mutation, which was suppressed by *Su(fu)*. In the cases of class II *fu* alleles, $fu[x]/Y;Su(fu)^{LP}/+$ males were found to die as pupae. Escapers were obtained in some of the crosses, allowing direct observation of the phenotype of these flies. The phenotype of non-escaping $fu[II]/Y;Su(fu)^{LP}/+$ males was checked by dissecting out pharate adults from the pupae cases.

Analysis of the interaction between the various classes of *fu* alleles in a *Su(fu)* background was performed by crossing *fu[x]/Y* males to $f^{36a}\ fu^A/FM6;Su(fu)^{LP}/Su(fu)^{LP}$ and to $f^{36a}\ fu^1/f^{36a}\ fu^1;Su(fu)^{LP}/Su(fu)^{LP}$ females, *fu[x]* representing one of the alleles listed in Table 1. However, in the case of pupal lethal alleles fu^{1PP7}, fu^{9P2} and fu^{mH63}, which belong to class I—and are therefore viable in interaction with *Su(fu)*—$fu[pupal\ lethal]/Y;Su(fu)^{LP}/Su(fu)^{LP}$ males were crossed to $f^{36a}\ fu^1/FM3$ and $f^{36a}\ fu^A/FM6$ females. Parents were removed from the vials after a few days so that the progeny could develop under uncrowded conditions. $fu^1/fu[x];Su(fu)/+$ and $fu^A/fu[x];Su(fu)/+$ flies were scored in the progeny and their phenotype checked. Studies over *fu* deficiencies were performed by crossing $Df(1)fu^{Z4}/FM6;Su(fu)^{LP}/Su(fu)^{LP}$ and $Df(1)fu^{P1}/FM6;Su(fu)^{LP}/Su(fu)^{LP}$ females to $f^{36a}\ fu^A/Y$ males.

To analyze the interaction with *cos-2*, *fu/Balancer* females were crossed to $cos\text{-}2^3/CyO$ males. A $fu^1/FM3;cos\text{-}2^3/CyO$ stock and a $cos\text{-}2^3/CyO;Su(fu)^{LP}/TM3$ stock were generated. Wings were mounted in Euparal after storage in 100% ethanol. Other body parts were mounted in Euparal after KOH digestion.

Germ line recombination and antibody staining: Germ line mitotic recombinants were selected using the ovo^{D1} dominant sterile mutation (BUSSON *et al.* 1983; PERRIMON and GANS 1983). $f^{36a}\ fu^A/FM6;Su(fu)^{LP}/Su(fu)^{LP}$ virgin females were crossed to $ovo^{D1},v/Y;Su(fu)^{LP}/Su(fu)^{LP}$ males, and transferred daily to new bottles. At 36 ± 12 hr after oviposition at 25°, first instar larvae were irradiated with 1000 rad of X-rays as described in PERRIMON and GANS (1983). Upon emergence, F_1 females were allowed to mate with their $FM6/Y;Su(fu)^{LP}/Su(fu)^{LP}$ brothers. These $f^{36a}\ fu^A/ovo^{D1},v;Su(fu)^{LP}/Su(fu)^{LP}$ females did not lay eggs, unless mitotic recombination had occurred in their germ line during irradiation and led to an ovo^+ clone (see PERRIMON and GANS 1983 for more details). In most cases this recombination event gave rise to a $f^{36a}\ fu^A/f^{36a}\ fu^A;Su(fu)^{LP}/Su(fu)^{LP}$ germ line clone. Egg-laying females were identified and their progeny studied individually. Unhatched embryos were dechorionated and mounted in Hoyers for cuticle examination as described by VAN DER MEER (1977). The frequency of $f^{36a}\ fu^A/ovo^{D1},v;Su(fu)^{LP}/Su(fu)^{LP}$ females carrying a germ line clone was of 3%. The same scheme was used with $Df(1)fu^{Z4}$, and the same frequency of clones was observed. In both experiments most of the clones were small, containing only a few embryos.

The same protocol was used to produced embryos for staining experiments except that $f^{36a}\ fu^A/ovo^{D1},v;Su(fu)^{LP}/$

Su(fu)^{LP} virgin females were crossed to *w fu*^{A}/*Y* males—rather than to *FM6/Y;Su(fu)*^{LP}/*Su(fu)*^{LP} males—to ensure that all embryos of the progeny had an identical genotype. Egg-laying females were pooled for embryo collection. Some embryos were allowed to develop and their mutant cuticular phenotype was checked. Antibody reactions were performed as described by ASHBURNER (1989). The monoclonal En antibody was generously provided by THOMAS KORNBERG, and the polyclonal Wg antibody was generously provided by MARCEL VAN DEN HEUVEL.

Molecular analysis of *fu* mutations: The *fu* mutations were localized by Southern analysis using acrylamide gels as described by PRÉAT (1990). For the sequence analysis, mutant alleles were amplified using the polymerase chain reaction (PCR) (SAIKI *et al.* 1985). The oligonucleotides fuCD51 (GTACCGAAGCCGAGTTCCCACCTC, position 801) and fuGE32 (ATCGTTCTCGATGGGCGGAC, position 2589) were used to amplify the region bearing the *fu*^{62} and *fu*^{M1} mutations, and fuCD52 (AGTGGTAATCACATCCCAATCCGC, position 861) and fuCD31 (CTCGATGGGCGGACTTTGGG, position 2583) were used to amplify *fu*^{mH63}. The PCR products were then digested by *Bam*H1 (position 940) and *Kpn*1 (position 1432) and cloned into the bluescript plasmid vector. The fragments were sequenced using double stranded DNA. In the case of *fu*^{62} and *fu*^{M1} two independent clones were sequenced.

RESULTS

The interaction between *Su(fu)* and *fu* is general but reveals qualitative allele specificity: As a first step, interaction between *Su(fu)* and 31 *fu* alleles was studied by looking at *fu[x]/Y;Su(fu)/+* flies derived from a *fu[x]/Balancer* × *Su(fu)*^{LP}/*Su(fu)*^{LP} cross. It allowed classification of these 31 *fu* alleles into two groups (Table 1). For the seven *fu* alleles of class I, *fu[I]/Y;Su(fu)*^{LP}/+ flies display a partially suppressed fu phenotype. For the 24 class II alleles, *fu[II]/Y;Su(fu)*^{LP}/+ individuals die as pupae, while these 24 alleles allow viability by themselves. This classification could not be deduced from the phenotypes conferred by these alleles in a *Su(fu)*$^{+}$ background. The two alleles *fu*^{1} and *fu*^{mH63} were used to isolate the suppressor mutations (PRÉAT 1992), and they have been extensively studied in interaction with *Su(fu)*. All the defects due to these class I alleles are fully suppressed by *Su(fu)* (Figure 1d and Figure 2d). Flies from *fu[I];Su(fu)* stocks are viable and normal.

In Drosophila, many suppressors that display an allele-specific rescue are in fact specific for mutations due to the insertion of a particular transposable element (reviewed in KUBLI 1986). The *fu-Su(fu)* interaction is different since *fu* alleles listed in Table 1 were analyzed molecularly, and none of them corresponds to the insertion of a transposable element (this study; PRÉAT *et al.* 1990). Rather, the observation that all *fu* alleles interact with *Su(fu)* suggests that the two gene products are parts of a same pathway. Previous Southern analyses showed that *fu* mutations belong to two molecular classes (PRÉAT *et al.* 1990). The first category corresponds to large deficiencies including several genes, and the second category to small events leaving part of the *fu* gene intact. Both class I and class II *fu* alleles listed in Table 1 belong to the latter molecular class.

TABLE 1

Classification of 31 *fu* alleles in a *Su(fu)* background

Allele	Class	Origin	Chromosome	Reference[b]
fu^{1}	I	Spontaneous	Unknown	1
fu^{S}	II	Spontaneous	M56i	2
fu^{59}	II	Spontaneous	Unknown	3
fu^{mimi}	II	PM	Oregon-R	4
fu^{ecn}	I	Unknown	Unknown	5
fu^{62}	I	X-ray	Unknown	6
fu^{A}	II	X-ray	w	7
fu^{RX1}	II	X-ray	car	8
fu^{RX2}	II	X-ray	car	8
fu^{RX7}	II	X-ray	car	8
fu^{RX11}	II	X-ray	car	8
fu^{RX13}	II	X-ray	car	8
fu^{RX15}	II	X-ray	car	8
fu^{RX16}	II	X-ray	car	8
fu^{mH63}	I	EMS	Canton-S	9
fu^{9P2}	I	EMS	Unknown	10
fu^{1PP7}	I	EMS	Unknown	10
fu^{2P}	II[a]	EMS	Unknown	10
fu^{new}	II	DEB	Oregon-R	7
fu^{MC2}	II	DEB	Oregon-R	7
fu^{JB3}	I	DEB	Oregon-R	7
fu^{Y1}	II	DEB	Oregon-R	7
fu^{W3}	II	DEB	Oregon-R	7
fu^{L4}	II	DEB	Oregon-R	7
fu^{M1}	II	DEB	Oregon-R	7
fu^{C10}	II	DEB	Oregon-R	7
fu^{J3}	II	DEB	Oregon-R	7
fu^{DB3}	II	DEB	Oregon-R	7
fu^{DB4}	II	DEB	Oregon-R	7
fu^{DB11}	II	DEB	Oregon-R	7
fu^{G3}	II	DEB	Oregon-R	7

[a] Displays a weak class II phenotype.

[b] 1, MORGAN and BRIDGES (1916); 2, isolated by A. SCHALET, cited in BUSSON *et al.* (1988); 3, isolated by R. F. GRELL, cited in SMITH and KING (1966); 4, isolated by M. GANS, cited in BUSSON *et al.* (1988); 5, isolated by E. WIESCHAUS, cited in BUSSON *et al.* (1988); 6, isolated by M. J. FAHMY, cited in SMITH and KING (1966); 7, BUSSON *et al.* (1988); 8, D. BUSSON, C. LAMOUR-ISNARD, B. LIMBOURG-BOUCHON, M.-C. MARIOL and T. PRÉAT, unpublished data; 9, WURST and HANRATTY (1979); 10, PERRIMON and MAHOWALD (1987).

The classification of *fu* alleles does not depend on the *Su(fu)* allele used. For example, *fu*^{1}/*Y;Su(fu)*^{LP}/+ flies display a partially suppressed wing phenotype as do *fu*^{1}/*Y;Su(fu)*^{12d}/+, *fu*^{1}/*Y;Df(3R)kar*^{3Q}/+ and *fu*^{1}/*Y;Df(3R)kar*^{Sz11}/+ flies, while *fu*^{A}/*Y;Su(fu)*^{LP}/+, *fu*^{A}/*Y;Su(fu)*^{12d}/+, *fu*^{A}*;Df(3R)kar*^{3Q}/+ and *fu*^{A}/*Y;Df(3R)kar*^{Sz11}/+ individuals all die in pupae. *Su(fu)* alleles behave identically although they were induced in different backgrounds, which rules out the possibility that the class II phenotype could result from an interaction between *fu* and some unidentified autosomal mutation instead of *Su(fu)* itself. *Su(fu)* mutations correspond to loss of function (PRÉAT 1992), as con-

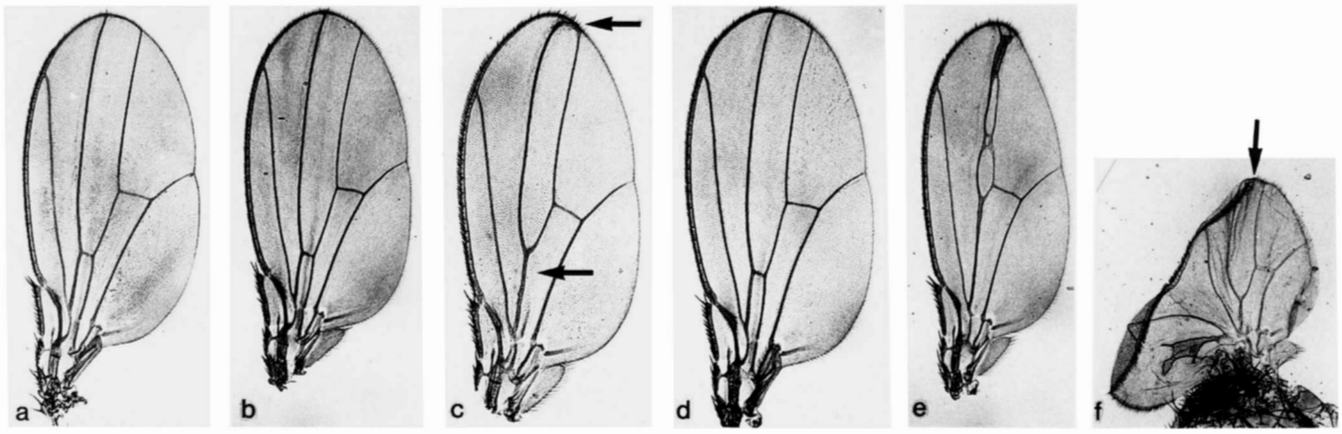

FIGURE 1.—Wing phenotypes conferred by class I and class II *fu* alleles in a *Su(fu)* background. (a) Wing of a wild-type fly. There are five longitudinal veins (LV). LV1 runs along the margin. (b) Wing of a $Su(fu)^{LP}/Su(fu)^{LP}$ fly displaying normal vein pattern. (c) Wing of a fu^{1}/Y fly. Arrows outline the anomalies of LV3 and LV4. (d) Wing of a $fu^{1}/Y;Su(fu)^{LP}/Su(fu)^{LP}$ fly. The fused phenotype displayed by class I allele is fully suppressed by $Su(fu)^{LP}$. (e) Wing of a fu^{Y1}/Y fly. (f) Wing of a $fu^{Y1}/Y;Su(fu)^{LP}/+$ fly, which bears an anterior duplication including LV1, LV2 and LV3. The arrow indicates the partially suppressed fused phenotype of the original LV3 and LV4 (compare with (e)). Note the size reduction along the proximo-distal axis.

firmed by the observation that *fu* alleles interact identically with EMS-induced *Su(fu)* mutations and with deficiencies covering the 87C region.

Adult phenotype conferred by class II *fu* alleles in interaction with *Su(fu)*: $fu[II]/Y;Su(fu)^{LP}/+$ individuals die as late pupae but some escapers emerge. These flies display a range of severe defects affecting the head and thoracic structures. For example, the wings bear a mirror-image duplication, which lies anteriorly and can include the first three longitudinal veins (Figure 1f). The proximo-distal axis is also affected as the wing is about 50% shorter. No structure is missing. Other appendages such as legs and halteres display similar defects: duplication of structures in the anterior compartment, and shortening along the proximal-distal axis (not shown). This phenotype is similar to the one conferred by some viable *costal-2* alleles (WHITTLE 1976; SIMPSON and GRAU 1987). Homozygous $fu^{A}/Y;Su(fu)^{LP}/Su(fu)^{LP}$ individuals die as early pupae. They are more severely affected than $fu^{A}/Y;Su(fu)^{LP}/+$ heterozygotes, showing that *Su(fu)* acts as a semidominant mutation with respect to the class II phenotype, just as it does for the suppression of the fu phenotype (PRÉAT 1992).

The adult fused phenotype itself is suppressed for class II alleles as it is for class I alleles. Comparison of the veins of fu^{Y1}/Y and $fu^{Y1}/Y;Su(fu)^{LP}/+$ flies shows that the fused phenotype is partially suppressed by the heterozygous *Su(fu)* mutation, despite the occurrence of the new mutant phenotype. The suppression is only partial in *fu[II]/Y;Su(fu)/+* flies, which suggests that, as in the case of class I alleles, *Su(fu)* acts as a semidominant mutation with respect to the suppression of the fused vein phenotype. However, it is impossible to look at the wings of *fu[II]/Y;Su(fu)/Su(fu)* individuals—which would be expected to display no vein fusion—as these die as early pupae.

Segment polarity phenotype of *fu[II];Su(fu)* embryos: The adult phenotype previously described corresponds to a zygotic interaction between class II *fu* alleles and *Su(fu)*. As both *fu* and *Su(fu)* present a maternal effect (PRÉAT 1992), the interaction between *Su(fu)* and class II *fu* alleles was further analyzed at the embryonic stage. Because *fu[II]/fu[II];Su(fu)/Su(fu)* females die as early pupae their progeny could not be analyzed directly. Germ line mitotic recombination was used to produce *fu[II];Su(fu)* embryos derived from a *fu[II];Su(fu)* female germ line (see MATERIALS AND METHODS). These *fu[II];Su(fu)* embryos die and display a segment polarity phenotype different from that of *fu* embryos (Figure 2f). Each abdominal segment shows deletion of the last rows of denticles and mirror-image duplication of anterior rows, apparently accompanied by duplication of the segment boundary. Thoracic denticle belts are absent. This phenotype is similar to that of *costal-2* embryos (GRAU and SIMPSON 1987). Thus, the interaction between *fu[II]* alleles and *Su(fu)* leads to a phenotype resembling the one of *cos-2* at both embryonic and adult stages. This is a surprising observation since *fu* mutations by themselves do not affect the same part of the segments as *cos-2*. The phenotype of *cos-2* mutants is like that of another segment polarity mutant, *patched*, except that the most anterior denticle row is not duplicated in *cos-2* segments whereas it is in *ptc*. In *fu[II];Su(fu)* embryos, like in *cos-2* embryos, the anterior row is not duplicated. However, the two phenotypes are slightly different since in *cos-2* mutants the denticle belts of the anterior abdominal segments are narrowed (GRAU and SIMPSON 1987), which has not been observed in *fu[II];Su(fu)* embryos.

In *fu* embryos, the naked part of each thoracic and abdominal segment is absent and there is a mirror-

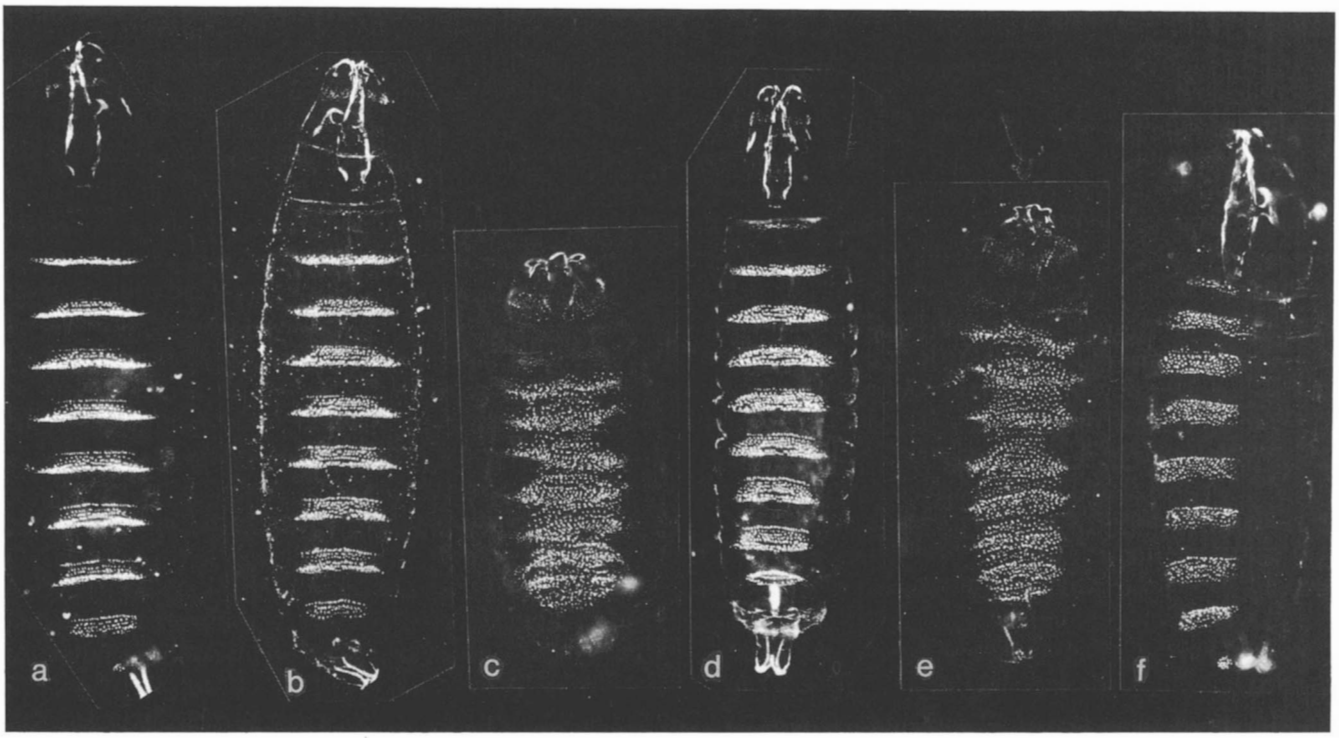

FIGURE 2.—Embryonic phenotype conferred by class I and class II *fu* alleles in a *Su(fu)* background. (a) Wild-type embryo (ventral view). The denticle belts are located in the anterior region of each thoracic and abdominal segment. (b) $Su(fu)^{LP}/Su(fu)^{LP}$ embryo displaying normal segmentation. (c) fu^{1} embryo. In each segment the denticle rows are duplicated in a mirror-image and the naked region is absent. (d) $fu^{1};Su(fu)^{LP}/Su(fu)^{LP}$ embryo. Class I alleles lead to a wild-type phenotype in interaction with *Su(fu)*. These embryos hatch and develop into normal adults. (e) fu^{A} embryo. The phenotype of class II alleles is not distinguishable from the one conferred by class I alleles in a $Su(fu)^{+}$ background. (f) $fu^{A};Su(fu)^{LP}/Su(fu)^{LP}$ embryo. The most posterior denticle rows are deleted and replaced by a duplication of the anterior rows (excluding the most anterior row). The posterior region of the segments is normal.

image duplication of the denticle rows, whereas in *fu[II];Su(fu)* embryos the posterior part of the segments is naked and presents no sign of denticle duplication. The embryonic fu phenotype due to class II alleles is suppressed by *Su(fu)* as it is for class I alleles.

***costal-2* interacts with *fu* and *Su(fu)*:** As some *fu* alleles display a cos-2-like phenotype in interaction with *Su(fu)*, we investigated possible interactions occurring between *fu* and *cos-2* itself. In a first step $fu/Y;cos\text{-}2^{3}/+$ individuals were generated. In the case of the class I alleles fu^{1}, fu^{mH63} and fu^{JB3}, these flies are viable and display no cos-2 phenotype. Class I *fu* alleles do not enhance the cos-2 phenotype but they do not suppress this phenotype. For example $fu^{1};cos\text{-}2^{7}/cos\text{-}2^{7}$ flies bearing the semiviable allele $cos\text{-}2^{7}$ display a cos-2 phenotype similar to that of $cos\text{-}2^{7}/cos\text{-}2^{7}$ flies (not shown). In the case of the class II alleles fu^{A}, fu^{M1}, fu^{RX2} and fu^{RX15}, $fu/Y;cos\text{-}2^{3}/+$ individuals die as late pupae and display an extreme adult cos-2 phenotype (Figure 3) (while $cos\text{-}2^{3}/+$ flies themselves are normal). The same classification of *fu* alleles is thus observed in interaction with *cos-2* and *Su(fu)*.

Interestingly, the heterozygous $cos\text{-}2^{3}$ mutation partially suppresses the fu vein phenotype (Figure 3A), just as does *Su(fu)*. To further analyze the suppression of the fu syndrome by *cos-2*, we crossed $fu^{1}/fu^{1};cos\text{-}2^{3}/+$ females to $fu^{1}/Y;cos\text{-}2^{3}/+$ males. Embryos of the progeny display a partially suppressed fu phenotype, some of the segments showing no denticle duplication at all (Figure 3E). Embryos homozygous for strong *cos-2* mutations and derived from heterozygous *cos-2*/+ females die, but they display an essentially normal segmentation (GRAU and SIMPSON 1987). The segment polarity cos-2 phenotype is displayed only by embryos derived from a *cos-2/cos-2* female germline. As shown here a decrease in the amount of Cos-2 product leads to a partial suppression of the embryonic fu phenotype. A more complete suppression of the embryonic fu phenotype would probably be observed in the absence of any Cos-2 product. Unlike *cos-2*, *ptc* mutations do not suppress the fu phenotype. For example, the segments of $fu^{1};ptc^{IN109}/ptc^{IN108}$ or $fu^{A};ptc^{IN108}/ptc^{IN108}$ embryos show no naked cuticle despite the lack of Patched product (not shown). This result outlines the specificity of the interaction between *fu* and *cos-2*.

Suppressor of fused and *costal-2* interact similarly with *fused*. In addition, *Su(fu)* strongly increases the effect of *cos-2* mutations, which suggest that the two genes cooperate. For example $cos\text{-}2^{3}/+;Su(fu)^{LP}/Su(fu)^{LP}$ individuals die as larvae, whereas both $cos\text{-}2^{3}/+$ and $Su(fu)^{LP}/Su(fu)^{LP}$ flies are viable and display

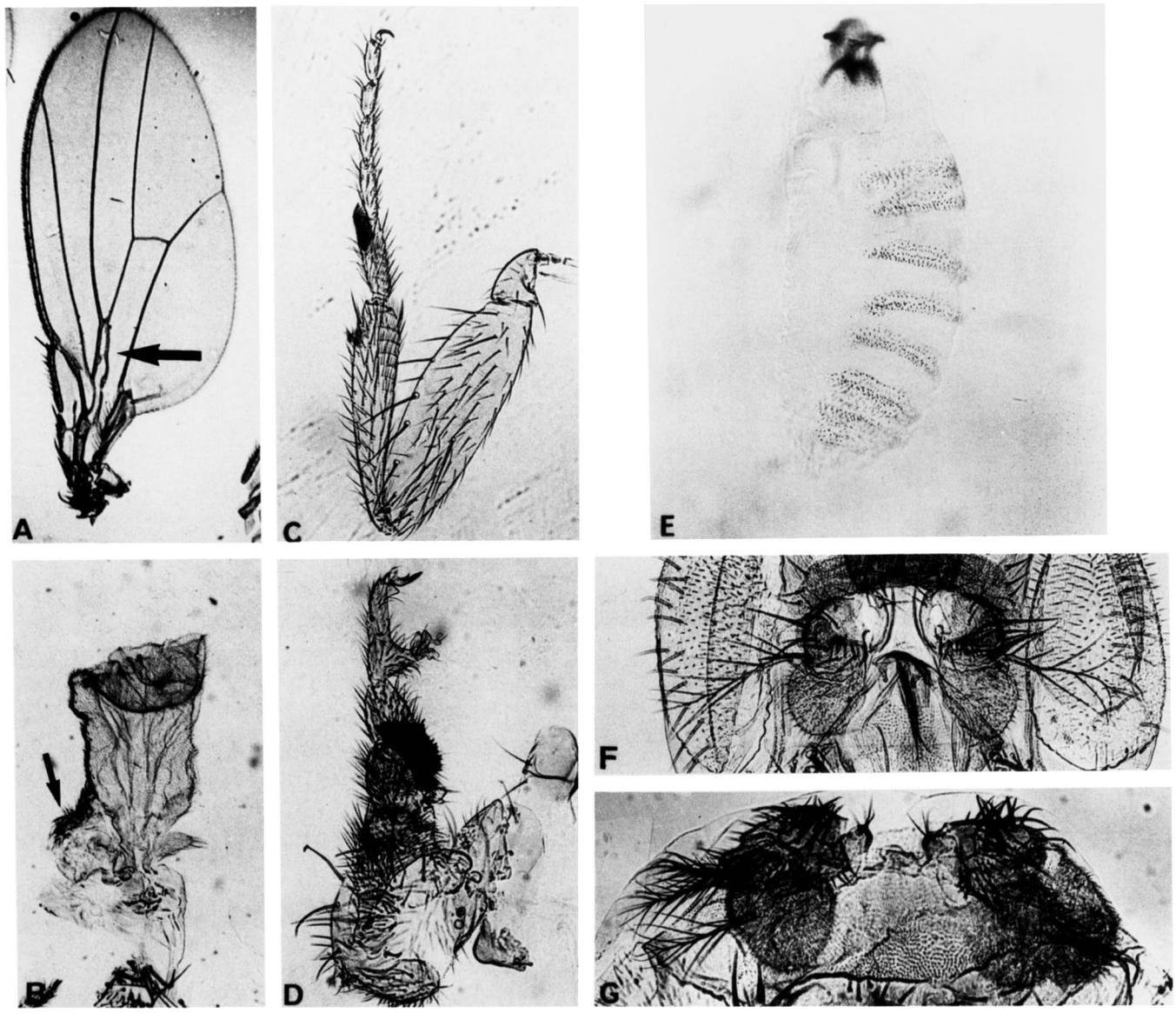

FIGURE 3.—Interaction between *costal-2* and *fused*. (A) Wing of a $fu^1/Y;cos\text{-}2^3/+$ male displaying a partially suppressed fu phenotype (arrow). (B) Wing of a $fu^A/Y;cos\text{-}2^3/+$ pharate adult showing a duplication of the costa (arrow) characteristic of *cos-2* mutants. $cos\text{-}2^3/+$ individuals are normal (not shown). (C) First leg of a fu^A/Y male. (D) First leg of a $fu^A/Y;cos\text{-}2^3/+$ male showing an extreme cos-2 phenotype. Note the overgrowth of the sex combs, which are an anterior structure. (E) Embryo derived from a $fu^1/fu^1;cos\text{-}2^3/+ \times fu^1/Y;cos\text{-}2^3/+$ cross displaying a partially suppressed fu segment polarity phenotype. (F) Antennae of a fu^A/Y male. (G) Abnormal antennae of a $fu^A/Y;cos\text{-}2^3/+$ male with duplicated aristae.

no mutant phenotype. Similarly, $cos\text{-}2^{V1}/cos\text{-}2^{V1};Su(fu)^{LP}/Su(fu)^{LP}$ individuals derived from a $cos\text{-}2^{V1}/Cyo;Su(fu)^{LP}/TM3$ stock also die as larvae. Like $cos\text{-}2^3/cos\text{-}2^3$ individuals derived from heterozygous $cos\text{-}2^3/+$ females, these larvae display no obvious abnormal cuticular phenotype.

The phenotype of $Df(1)fu^{Z4}$ embryos is suppressed by *Su(fu)*: The *fu* alleles listed in Table 1 correspond to small molecular events, which do not remove the entire *fu* gene. To understand more about the cause of the class II phenotype, it was important to analyze the interaction between a complete deletion of the *fu* locus and *Su(fu)*. The smallest of these deficiencies, $Df(1)fu^{Z4}$, is 40 kb long and includes several other genes besides *fu* (MARIOL, PRÉAT and LIMBOURG-BOUCHON 1987). The absence of one of these genes is responsible for the larval lethality of $Df(1)fu^{Z4}$ individuals (BUSSON *et al.* 1988; PRÉAT 1992). As a consequence, it is not possible to analyze directly the suppression by *Su(fu)* of the adult fu phenotype due to $Df(1)fu^{Z4}$. However, suppression of the segment polarity phenotype conferred by this deficiency could be examined by producing germinal clones (see MATERIALS AND METHODS). $Df(1)fu^{Z4}/Y;Su(fu)^{LP}/Su(fu)^{LP}$ embryos derived from a $Df(1)fu^{Z4}/Df(1)fu^{Z4};Su(fu)^{LP}/Su(fu)^{LP}$ female germ line have normal segments (Figure 4). *Su(fu)* suppresses the segment polarity phenotype conferred by a complete *fu* deficiency. There-

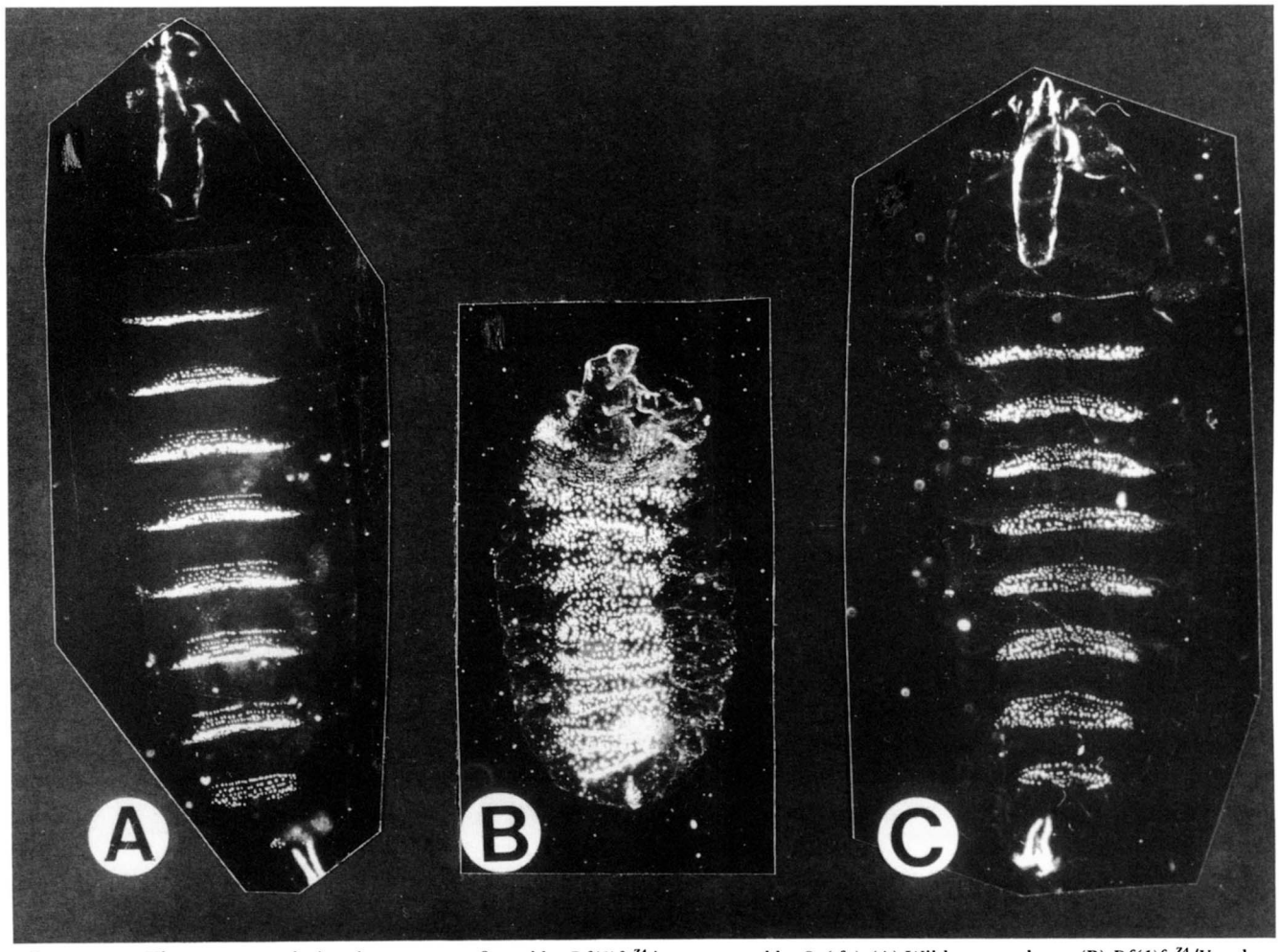

FIGURE 4.—The segment polarity phenotype conferred by $Df(1)fu^{Z4}$ is suppressed by *Su(fu)*. (A) Wild-type embryo. (B) $Df(1)fu^{Z4}/Y$ embryo displaying an extreme segment polarity fu phenotype. (C) $Df(1)fu^{Z4};Su(fu)^{LP}/Su(fu)^{LP}$ embryo showing suppressed fu phenotype.

fore the cos-2-like phenotype of *fu[II];Su(fu)* embryos can be considered as resulting from an abnormal activity of mutant Fu[II] products, which is revealed in a *Su(fu)* context.

***Su(fu)* restores a wild-type expression pattern of *engrailed* and *wingless* in *fu[I];Su(fu)* embryos:** Suppression of the fu segment polarity phenotype could be due either to an early compensation of the lack of Fu product, or to the activation of a secondary pathway that acts on later events. Studying the expression of other segment polarity genes in *fu;Su(fu)* embryos helped addressing this question. We have shown previously that the expression of *engrailed* (*en*) and *wingless* (*wg*) was abnormal in *fu* embryos (LIMBOURG-BOUCHON, BUSSON and LAMOUR-ISNARD 1991). At the end of the extended germ band stage *wg* expression starts to disappear from epidermal cells, and no Wg protein can be detected in *fu* embryos' epidermis from germ band retraction (Figure 5B). At this stage the expression of *en* appears irregular (Figure 5H). This patchy pattern might be due either to an abnormal regulation of *en* or to the death of expressing cells, since extensive cell death occurs in *fu* embryos at this stage (MARTINEZ-ARIAS 1985). In *fu[I];Su(fu)* embryos the expression of both *en* and *wg* is entirely normal (Figure 5C, I). The fact that early events associated with a *fu* genotype are fully corrected by *Su(fu)* is in accordance with the idea that both genes are involved in the very same process. The observation that *fu[I];Su(fu)* individuals are viable and normal also supports this interpretation.

Expression of *en* and *wg* in *fu[II];Su(fu)* embryos is similar to their expression in *cos-2* mutants: To determine whether the same expression changes were observed in *fu[II];Su(fu)* and in *cos-2* mutants, we monitored the distribution of Wg and En proteins during the development of $fu^{A};Su(fu)^{LP}/+$ embryos derived from a $fu^{A}/fu^{A};Su(fu)^{LP}/Su(fu)^{LP}$ female germline (see MATERIALS AND METHODS). At the end of the extended germ band stage, the *wg* stripes are broadened in $fu^{A};Su(fu)^{LP}/+$ embryos (Figure 5E). The expression of *en* appears normal at all stages in these embryos (Figure 5K). These patterns are indeed similar to the one associated with *cos-2* mutations (A.

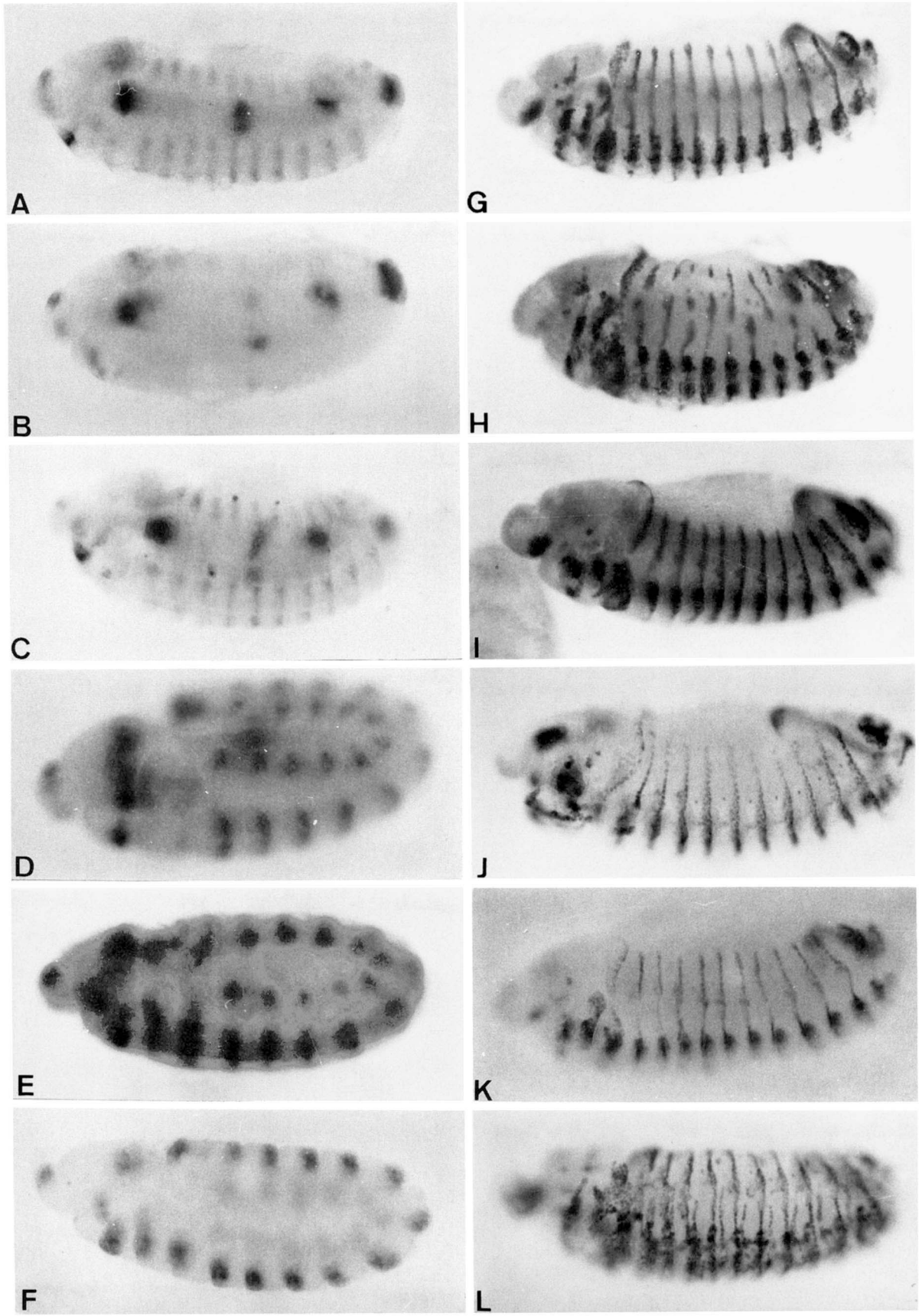

FIGURE 5.—Expression of *wingless* and *engrailed* in *fu;Su(fu)* embryos. (A–F) Expression of the Wingless protein. (A) Homozygous $Su(fu)^{LP}$ embryo during germ band shortening. The expression is normal. (B) fu^1 embryo. Wg expression disappears from the epidermis. Staining remains in the foregut, the hindgut and the anal region. fu^1 is a class I allele. A similar pattern is observed in *fu[II]* embryos (not shown). (C) $fu^1;Su(fu)^{LP}$ embryo. The suppressor restores a normal expression of Wg. (D) Wild-type embryo at extended germ band. (E) $fu^4;Su(fu)^{LP}/+$

FORBES and P. INGHAM, unpublished data). Extension of the *wg* domain is also observed in *ptc* mutants (MARTINEZ-ARIAS, BAKER and INGHAM 1988; see Figure 5F). However, *ptc* embryos also display an ectopic band of *en* expression, which is not observed in *fu[II];Su(fu)* embryos. Also, two parasegmental grooves per metameric unit are observed in *ptc* embryos (MARTINEZ-ARIAS, BAKER and INGHAM 1988), but not in *cos-2* embryos (A. FORBES and P. INGHAM, unpublished data) nor in *fu[II];Su(fu)* embryos. These results support the idea that the terminal costal-2-like phenotype of *fu[II];Su(fu)* embryos arises from the same initial events than that occurring in *costal-2* embryos.

Class II *fu* alleles are recessive to fu^+ in a *Su(fu)* background: In a $fu^A/FM6 \times fu^+/Y;Su(fu)^{LP}/Su(fu)^{LP}$ cross, $fu^A/Y;Su(fu)^{LP}/+$ males of the progeny die as pupae with a cos-2-like phenotype, whereas their $fu^A/fu^+;Su(fu)^{LP}/+$ sisters are viable and phenotypically wild-type (not shown). Furthermore, $fu^A/FM6;Su(fu)^{LP}/Su(fu)^{LP}$ adult females are also normal. The class II phenotype is recessive to fu^+. The Fu^+ product must thus prevent the abnormal activity of Fu[II] products from being expressed. Considering their interaction with *Su(fu)*, class II *fu* alleles must therefore be regarded as antimorphic (new function that is offset in the presence of the Fu^+ function).

However, one could assume that the cos-2-like phenotype is not due to an interaction between *Su(fu)* and *fu* itself, but between *Su(fu)* and a recessive mutation of the *X* chromosome present before the induction of the *fu[II]* mutations. In this hypothesis, the normal phenotype of $fu[II]/FM6;Su(fu)^{LP}/Su(fu)^{LP}$ flies would result from the presence of a wild-type copy of this other gene on the FM6 chromosome. Several arguments rule out this possibility. First, the original wild-type strain used to induce DEB *fu* mutations was isogenic for the *X* chromosome (BUSSON *et al.* 1988). Most, but not all, of the *fu* alleles recovered during these mutageneses are lethal in interaction with *Su(fu)* (Table 1), but the original strain does not display any lethality when crossed to *Su(fu)*. Furthermore, this strain rescues the class II phenotype in a $fu^A/fu^+;Su(fu)^{LP}/+$ combination. Second, several $P[fu^+]$-transformed lines have been produced (PRÉAT *et al.* 1990; THÉROND *et al.* 1993). These $P[fu^+]$ insertions fully rescue the class II phenotype in a $fu^A/Y;P[fu^+]/+;Su(fu)/+$ combination. Finally, *fu[II]* alleles are dominant over a complete *fu* deficiency in a *Su(fu)* context: $fu^A/Df(1)fu^{Z4};Su(fu)^{LP}/+$ individuals display the class II phenotype, whereas $Df(1)fu^{Z4}$ itself does not lead to a cos-2-like phenotype in interaction with *Su(fu)* (Figure 4). The class II phenotype described here is therefore always associated with a *fu[II]* genotype.

Class II *fu* alleles are recessive to class I *fu* alleles in a *Su(fu)* background: No complementation between *fu* alleles has ever been observed (SMITH and KING 1966; WURST and HANRATTY 1979). In particular, we have checked that alleles belonging to both classes lead to a regular fused vein phenotype in a *fu[I]/fu[I]*, *fu[II]/fu[II]* or *fu[I]/fu[II]* combination (not shown). Because fu^+ is dominant over *fu[II]* alleles in a *Su(fu)* context, it was interesting to know whether some of the *fu[I]* alleles could display the same effect despite the fact that they do not encode a normal product. Using class I (fu^1) and class II (fu^A) reference alleles, systematic analyses were carried out by generating $fu^1/fu[x];Su(fu)/+$ and $fu^A/fu[x];Su(fu)/+$ females (*fu[x]* represents any allele listed in Table 1, which belongs either to class I or to class II). The results of these analyses yield several conclusions: (i) $fu^1/fu[I];Su(fu)^{LP}/+$ flies display a class I phenotype as expected. (ii) $fu^A/fu[II];Su(fu)^{LP}/+$ flies display the class II phenotype. No complementation between any class II allele and the class II allele fu^A was found in a *Su(fu)* context. (iii) $fu^A/fu[I];Su(fu)^{LP}/+$ and $fu^1/fu[II];Su(fu)^{LP}/+$ flies display a class I phenotype, showing that class I alleles are dominant over class II alleles in a *Su(fu)* context. Although class I alleles by themselves lead to a mutant fused phenotype in a $Su(fu)^+$ background, they seem to encode proteins that are able to offset the antimorphic effect of Fu[II] products in a *Su(fu)* context—as does fu^+. The observation that none of the class I alleles corresponds to a complete deletion of the *fu* coding sequence is in accordance with this interpretation. Although $Df(1)fu^{Z4}$ does not confer a cos-2-like phenotype in interaction with *Su(fu)*, it is recessive to class II alleles in a $fu[II]/Df(1)fu^{Z4};Su(fu)^{LP}/+$ combination. $Df(1)fu^{P1}$ also is recessive to *fu[II]* alleles. Thus, large deficiencies affecting the *fu* gene belong to a class of mutants different from class I. This new class actually represents amorphic *fu* mutations (complete loss of function) and was called class 0.

Previous genetic studies concluded that pupal lethal alleles fu^{9P2}, fu^{1PP7} (PERRIMON and MAHOWALD 1987) and fu^{mH63} (WURST and HANRATTY 1979; BUSSON *et al.* 1988) were amorphic mutations. Analysis of their behavior in a *Su(fu)* background indicates that this is not the case since, unlike $Df(1)fu^{Z4}$, these three alleles

embryo derived from a $fu^A/fu^A;Su(fu)^{LP}/Su(fu)^{LP}$ female germ line. The Wg stripes are broadened. (F) ptc^{IN108}/ptc^{IN108} embryo showing enlarged expression domain. (G–L) Expression of the Engrailed protein. (G) $Su(fu)^{LP}$ embryo during germ band shortening showing normal expression. (H) fu^1 embryo. The En stripes are interrupted, leading to a patchy pattern. (I) $fu^1;Su(fu)^{LP}$ embryo. *Su(fu)* suppresses the abnormal expression pattern of En. (J) Wild-type embryo. (K) $fu^A;Su(fu)^{LP}/+$ embryo derived from a $fu^A/fu^A;Su(fu)^{LP}/Su(fu)^{LP}$ female germ line. The expression of En is normal. In particular no extra stripe is observed. (L) ptc^{IN108}/ptc^{IN108} embryo. In each parasegment an additional domain of expression of En is formed.

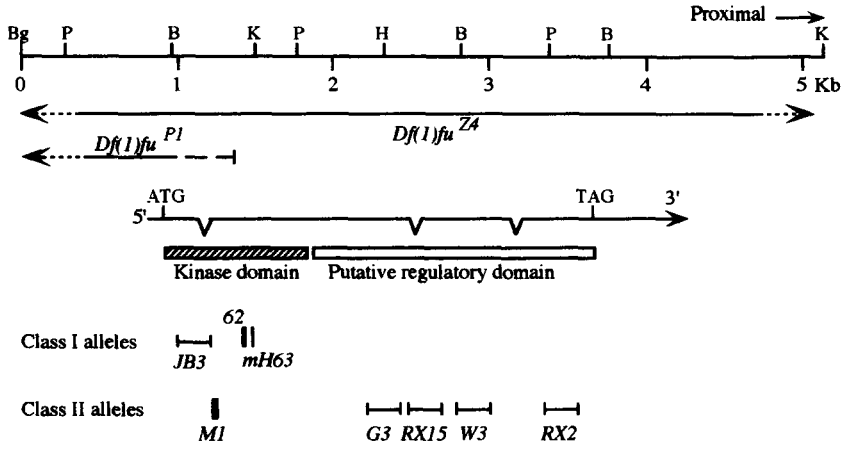

FIGURE 6.—Localization of class I and class II *fu* mutations. The 5′ region encoding the catalytic domain of the putative Fu kinase corresponds to about one-third of the coding region. Class I mutations reside in 5′ whereas most class II mutations are in 3′. The mutations that have been sequenced are represented by vertical bars. In the other cases the smallest fragment known to contain the mutation is represented. The restriction sites are symbolized as follow: B: *Bam*HI; Bg: *Bgl*II; H: *Hin*dIII; K: *Kpn*I; P: *Pvu*II.

can rescue the cos-2-like phenotype due to class II alleles. Thus, despite the fact their function seems to be fully abolished in a *Su(fu)*$^+$ background, the pupal lethal alleles isolated so far are not amorphic (class 0) *fu* mutations but actually belong to class I. A true amorphic *fu* mutation must (i) lead to an extreme fu phenotype in a *Su(fu)*$^+$ background (pupal lethality; extreme maternal effect), (ii) lead to a wild-type phenotype in interaction with *Su(fu)* and (iii) be recessive over class II *fu* alleles in a *Su(fu)* background.

Molecular analysis of class I and class II *fu* alleles: With the hope of understanding the molecular bases underlying the classification of *fu* alleles, we studied the distribution of some class I and class II mutations by performing sensitive Southern analyses (PRÉAT 1990). All three class I mutations analyzed map within the 5′ region of the gene, which encodes the catalytic domain (Figure 6). Of five class II mutations, four map in the 3′ region of the gene, and one in the 5′ region (fu^{M1}). These results indicated that class I mutations were in 5′, whereas class II mutations were more likely to reside in 3′. However, the fact that one of the class II mutation did affect the same region as class I mutations was puzzling.

To solve this issue, three alleles affecting the 5′ region were sequenced (see MATERIALS AND METHODS). The class I allele fu^{62} corresponds to a 9-bp deletion (Figure 7). Three amino-acids of the catalytic domain are deleted, and the carboxy terminal domain of the predicted protein is unaffected. The class I allele fu^{mH63} corresponds to a single base substitution, which changes an alanine–highly conserved among ser/thr kinases (HANKS, QUINN and HUNTER 1988)–into a threonine. The class II allele fu^{M1} corresponds to a 28-bp deletion, and therefore it affects the open reading frame. It leads to a small predicted protein of 118 amino acids that lacks the normal carboxy terminal domain of the Fu kinase. These molecular results suggest that class I mutations affect the region encoding the catalytic domain *and* do not change the open reading frame. On the contrary, class II alleles affect the carboxy domain of the protein, either directly, or indirectly by changing the open reading frame in the 5′ region. These postulates will need to be confirmed, for example, by *in vitro* mutagenesis. At the moment, however, they can account for many of the genetic properties of the three classes of *fu* mutants.

DISCUSSION

Su(fu) suppresses the phenotype of embryos carrying a 40-kb *fu* deficiency, and therefore this suppressor does not reactivate the *fu* locus. *Su(fu)* does not act only on the final phenotype of *fu* embryos since it suppresses the earliest observed events associated with the lack of Fu$^+$ product. All *fu* alleles interact with *Su(fu)*, but three different classes of phenotypes are observed (Table 2). Class I alleles lead to a entirely wild-type phenotype in the presence of a homozygous *Su(fu)* mutation. The fu phenotype due to class II alleles is also suppressed by *Su(fu)*, but they lead to a new segment polarity phenotype (while *Su(fu)* individuals by themselves are normal). This class II phenotype resembles the one displayed by the segment polarity mutant *costal-2*, both in the embryo and in the adult. This new qualitative classification of *fu* alleles, once combined with molecular data, provides important information about the organization of the *fu* gene. It also brings clues about possible interaction involving the Fu kinase and the Cos-2 product.

Functional organization of the *fu* gene: Most *fu* mutations that leave part of the *fu* gene intact belong to class II. These class II alleles behave as antimorphic mutations as they display a new function revealed in a *Su(fu)* background but are recessive to the fu^+ allele. It is surprising that so many alleles encode products that seem to have acquired a new function, as that kind of mutation is expected to be rare. Furthermore, the phenotype of *fu[II];Su(fu)* individuals is very similar for all class II *fu* alleles. These observations suggest that class II alleles actually correspond to selective loss-of-function mutations, rather than to actual gain-

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      /.....................................domain 1.....................
901 acc ATG AAC CGC TAC GCG GTA AGC TCG CTG GTG GGG CAA GGA TCC TTC GGG TGC GTA TAC
      met asn arg tyr ala val ser ser leu val gly gln gly ser phe gly cys val tyr

    ................/.....................domain 2.....................
961 AAG GCG ACA CGC AAG GAC GAC AGC AAG GTG GTG GCC ATC AAA GTG ATC TCC AAG gtg agt
    lys ala thr arg lys asp asp ser lys val val ala ile lys val ile ser lys

1021 ggg gcg ggc cag gtg ata aag caa caa gtc cat aca act agt tca cac cat att cat gtt

    ....................................../...........domain 3.....................
1081 ctg cag CGC GGA AGA GCC ACG AAA GAG CTG AAG AAT TTG CGC AGG GAG TGC GAC ATT CAG
           arg gly arg ala thr lys glu leu lys asn leu arg arg glu cys asp ile gln

    ............/.........................domain 4....................../.......
1141 GCC CGG CTG AAG CAT CCG CAC GTC ATC GAG ATG ATC GAG TCC TTC GAG TCG AAG ACG GAC
     ala arg leu lys his pro his val ile glu met ile glu ser phe glu ser lys thr asp

    ....................................domain 5.............................../
                                        M1 (Δ)
1201 CTT TTC GTG GTC ACT GAG TTC GCG CTG ATG GAC CTG CAC CGC TAC CTG TCC TAC AAT GGA
     leu phe val val thr glu phe ala leu met asp leu his arg tyr leu ser tyr asn gly
                         Ø  Ø  Ø  Ø  Ø  Ø  Ø  Ø  Ø  thr cys pro thr met glu

    ....................................domain 6.....................................
1261 GCC ATG GGC GAG GAG CCG GCA CGT CGG GTG ACC GGG CAT CTG GTG TCC GCT CTG TAC TAC
     ala met gly glu glu pro ala arg arg val thr gly his leu val ser ala leu tyr tyr
      pro trp ala arg ser arg his val gly stop

    ..........................................................................
1321 CTG CAT TCA AAC CGC ATC CTC CAC CGG GAT CTC AAA CCG CAA AAC GTC CTG CTC GAC AAG
     leu his ser asn arg ile leu his arg asp leu lys pro gln asn val leu leu asp lys

    ........./.....................................domain 7......................./
                62 (Δ)                          mH63 (G=A)
1381 AAC ATG CAC GCG AAA CTC TGC GAC TTT GGA CTG GCC CGC AAC ATG ACC CTG GGT ACC CAC
     asn met his ala lys leu cys asp phe gly leu ala arg asn met thr leu gly thr his
                  Ø  Ø  Ø                        thr
1441 etc...
```

FIGURE 7.—Sequence of three mutant alleles affecting the catalytic domain. The upper line represents the wild-type sequence (PRÉAT *et al.* 1990). fu^{M1} corresponds to 28-bp deletion (Δ). The missing amino acids are indicated (Ø) as well as the sequence of the new predicted protein. fu^{62} is a 9-bp deletion. fu^{mH63} is a single base pair substitution. This mutation affects a *Hae*III restriction site, which has been observed by Southern analysis (not shown). The catalytic domain of all protein kinases has been divided into 11 domains by HANKS, QUINN and HUNTER (1988). The first seven domains are shown here. The coding phase starts at nucleotide 904 (0 being at an upstream *Bgl*II site). The first intron is indicated by lower-case letters.

TABLE 2

Properties of the three classes of *fu* alleles

Class	Phenotype in a *Su(fu)*$^+$ background	Phenotype in a *Su(fu)* background	Phenotype over *fu[II]* in *Su(fu)* background	Mutation
fu^+	+	+	+	None
fu[0]	fu[a]	+[a]	costal-2-like	Large deficiencies[b,c]
fu[I]	fu	+	+	-Small events (7/31) -carboxy terminal region intact?
fu[II]	fu	costal-2-like	costal-2-like	-Small events (24/31) -carboxy terminal region affected?

[a] *fu[0]* alleles are lethal because they are deficiencies that include other genes, so the embryonic phenotype was determined in germ line mitotic clones.

[b] In theory some small molecular event could disrupt completely the *fu* gene and therefore lead to a class 0 phenotype, but no such allele was identified so far.

[c] The search for *fu* alleles by non complementation over the fu^1 allele (BUSSON *et al.* 1988) yielded about half of large deficiencies and half of small molecular events.

of-function mutations. Partial qualitative loss of function would confer new properties to the mutant protein. Several small *fu* mutations have been localized, and three mutant alleles were sequenced. These data suggest that class I mutations leave the carboxy terminal domain of the predicted Fu protein intact, whereas this domain is either missing or abnormal in class II mutants. The amino terminal portion of the putative Fu kinase corresponds to the catalytic domain, so the carboxy terminal domain could be the regulatory domain of the protein. Class II proteins would no longer be regulated.

The very high rate of class II *fu* alleles among the mutations that do not remove the entire *fu* gene is explained as follow: the region encoding the catalytic domain covers about one-third of the *fu* gene, so the regulatory domain may include up to two-thirds of the gene (Figure 6). All *fu* mutations affecting the

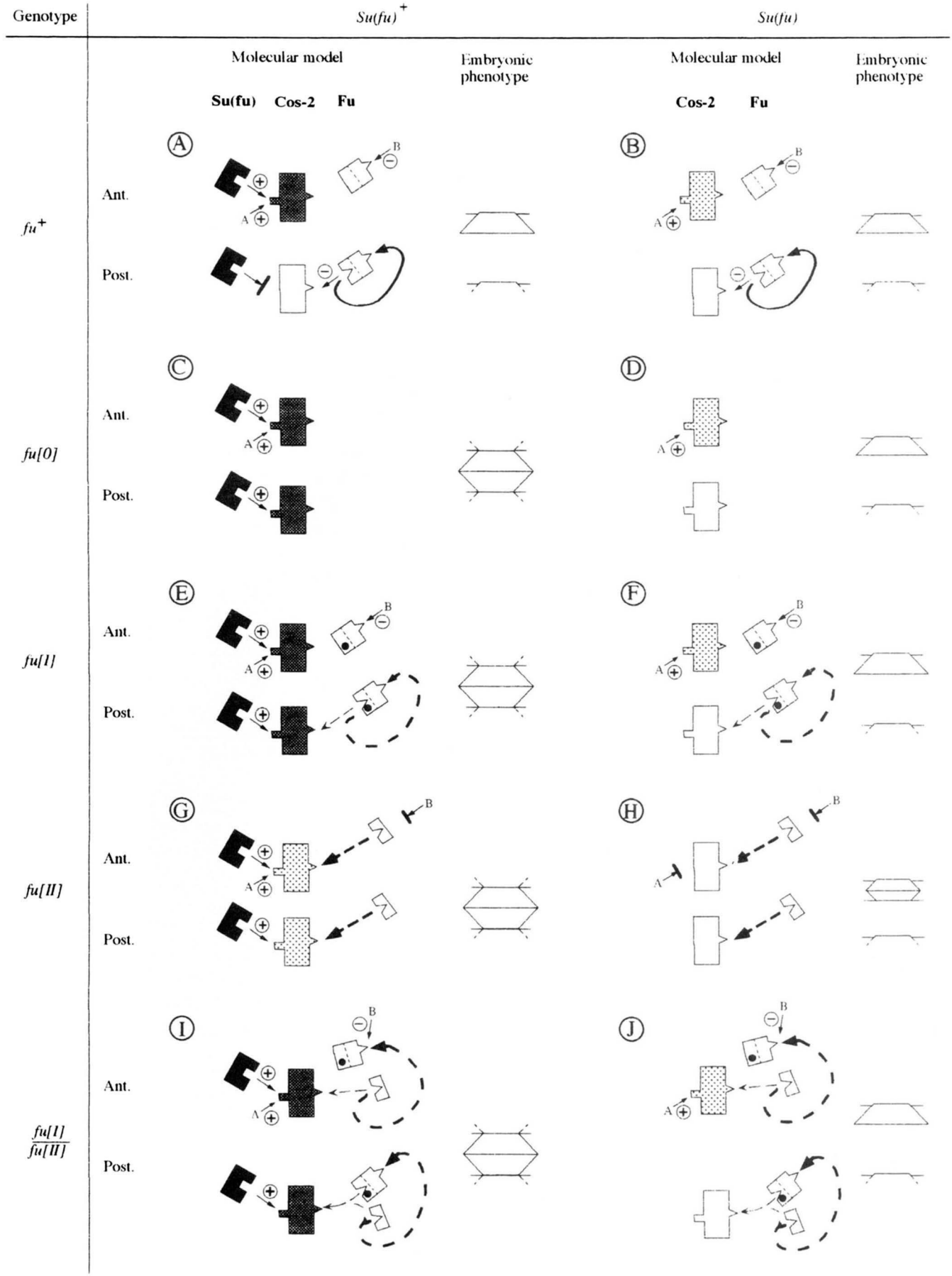

FIGURE 8.—Molecular model. (A) Wild-type embryo. Interactions occurring within the anterior part and the posterior part of each segment are shown. The three proteins Su(fu)$^+$, Cos-2$^+$ and Fu$^+$ are present within the entire segment, but post-translational modifications regulate

second region of the gene (*i.e.*, about two-thirds, if there is no distribution bias along the gene), together with mutations affecting the first region and generating a frame-shift (*i.e.*, two-thirds of about one-third, if nucleotide substitutions are omitted) are expected to affect the putative regulatory domain, and thus lead to a class II phenotype. This corresponds to a 89% theoretical rate of class II alleles among the mutations that leave part of the *fu* gene intact, which is close to the actual rate of 77% (24/31). Class I alleles are indeed expected to be rare if they correspond to mutations that affect the catalytic domain and do not generate a frameshift. In theory a small deletion could create a frameshift at the very beginning of the *fu* gene, thus leading to a totally inactive product instead of a class II product. So far no *fu* allele corresponding to a small deletion was found to belong to class 0. This could signify that the amino acids directly involved in the kinase-substrate interaction lie at the beginning of the Fu protein, corresponding to part of the subdomains III–V for which no function can be assigned so far (see HANKS, QUINN and HUNTER 1988 for subdivision of the kinase domain).

A network of interactions between *fu*, *Su(fu)* and *cos-2*: Class II *fu* alleles lead to a cos-2-like phenotype in interaction with *Su(fu)*. Several results suggest that this phenotype indeed arises from an inhibition of the Cos-2 function. First, *fu[II]* alleles enhance the phenotype of *cos-2* mutants. Second, *Su(fu)* mutations also strongly increase the effect of *cos-2* mutations, suggesting that *Su(fu)*$^+$ and *cos-2*$^+$ cooperate. It is striking that the double mutant *cos-2*^{V1};*Su(fu)*^{LP} is lethal at the larval stage when each mutation is not only viable but confers no phenotype by itself. Third, the expression of *en* and *wg* is similar in *fu[II];Su(fu)* and in *cos-2* embryos. If these antimorphic *fu[II]* alleles are actually able to inhibit the expression of the Cos-2 function it might be because the Fu$^+$ kinase recognizes the Cos-2$^+$ protein as one of its substrates. The results described here can thus be interpreted as if the Fu$^+$ kinase would inhibit Cos-2$^+$ in the posterior part of the segments, while Su(fu)$^+$ would activate Cos-2$^+$ (see model in Figure 8). The observation that both *Su(fu)* and *cos-2* mutations suppress the fu phenotype is in agreement with these hypotheses. The Su(fu)$^+$ function is very sensitive to any decrease in the amount of Su(fu)$^+$ protein (PRÉAT 1992), and therefore it has been proposed that Su(fu)$^+$ is not an enzyme but instead interacts with another protein in a stoichiometric way. The Su(fu)$^+$ protein might thus form an active complex in interaction with the Cos-2$^+$ protein. Direct analysis of the Cos-2 protein will be required to test our model, but cloning of the *cos-2* gene has not been reported yet.

All three genes *fu* (NÜSSLEIN-VOLHARD and WIESCHAUS 1980), *Su(fu)* (PRÉAT 1992) and *cos-2* (GRAU and SIMPSON 1987) display a major maternal effect. Therefore, the initial products (mRNA or protein) are probably distributed ubiquitously within segments, as it is for *fu* (THÉROND *et al.* 1993). However, mutations in these genes affect specific parts of the segments, presumably because some of the wild-type products involved in these interactions are active only in defined domains. A combination of mechanisms could explain how Fu$^+$ and Su(fu)$^+$ may have antagonistic effects and control distinct intrasegmental domains. In the posterior region, the Fu$^+$ kinase might inhibit both Cos-2$^+$ and Su(fu)$^+$. Consequently, an active Su(fu)$^+$ product would be present only in the anterior region. Alternatively, the Cos-2$^+$ protein might be modified structurally after phosphorylation by Fu$^+$ (or Fu$^+$ might phosphorylate directly the site normally recognized by Su(fu)$^+$). As a result, although an active Su(fu)$^+$ product would be present in the entire segment, it would not be able to activate Cos-2$^+$ in the posterior domain (Figure 8A). In the anterior

their activity. Anteriorly, Su(fu)$^+$ activates Cos-2$^+$ which controls determination of the posterior denticle rows together with some adjacent naked cuticle. An anterior inhibitor (named "B") recognizes the regulatory carboxy terminal domain of the Fu$^+$ kinase. Posteriorly, the Fu$^+$ kinase inactivates the Cos-2$^+$ protein. This phosphorylation leads to a structural change of Cos-2$^+$, therefore preventing its recognition by Su(fu)$^+$. The Fu$^+$ kinase interacts with itself (this interaction is only schematically represented as being intramolecular). The Fu$^+$ kinase has a stronger affinity for the Fu$^+$ regulatory domain than for the Cos-2$^+$ protein. The color of the Cos-2$^+$ protein symbolizes the level of activity (dark grey: strong activity; light grey: partial activity; white: strongly reduced or no activity). Note that a partial activity can correspond to a mixed population of fully active and completely inactive proteins. (B) *Su(fu)* embryo. Absence of the Su(fu)$^+$ activator is partially compensated for by an anterior activator of Cos-2$^+$ (named "A"). This activator might be the Patched product. The terminal embryonic phenotype is normal. (C) *fu[0]* embryo. In the absence of the Fu$^+$ inhibitor Su(fu)$^+$ can recognize and activate Cos-2$^+$ posteriorly as well. This ectopic expression of the Cos-2$^+$ function initiates the mirror-image duplication of the denticle belt (fu phenotype). (D) *fu[0];Su(fu)* embryo. The posterior Cos-2$^+$ protein is not activated in the absence of Su(fu)$^+$. *Su(fu)* mutations suppress the fu phenotype. (E) *fu[I]* embryo. Mutant Fu[I] proteins cannot phosphorylate their substrates, but they are still able to interact with both Cos-2$^+$ and themselves posteriorly (dashes). Anteriorly Fu[I] proteins are normally inhibited because they possess the regulatory domain. (F) *fu[I];Su(fu)* embryo. In the absence of Su(fu)$^+$, the Cos-2$^+$ protein is not activated posteriorly. (G) *fu[II]* embryo. Truncated Fu[II] proteins can bind the Cos-2$^+$ protein in the entire segment, and this interaction is strong because Fu[II] proteins are not a target for themselves. Despite this inhibition Cos-2$^+$ can still be activated by Su(fu)$^+$ and "A." (H) *fu[II];Su(fu)* embryo. In the absence of Su(fu)$^+$ the Cos-2$^+$ protein is strongly inhibited after its constitutive binding by Fu[II] proteins. This effect cannot be compensated for by the anterior activator, and therefore *fu[II];Su(fu)* embryos display a cos-2-like phenotype. (I) *fu[I]/fu[II]* individuals display a regular fu phenotype. No complementation occurs between class I and class II *fu* alleles. Fu[II] proteins interact preferentially with Fu[I] proteins. (J) *fu[I]/fu[II];Su(fu)* embryo. Fu[I] proteins titrate the abnormal Fu[II] proteins. This releases the inhibition of Cos-2$^+$, which can be activated anteriorly.

region some additional post-transcriptional or post-translational regulation may occur in the cellularized embryo. For example, a segment polarity gene expressed in the zygote might encode a protein that inactivates Fu^+ in the anterior domain, allowing $Su(fu)^+$ to activate Cos-2^+. More generally, such post-translational interactions might represent a major way of regulation for the segment polarity genes expressed maternally.

The segment polarity phenotype due to a complete *fu* deletion is suppressed by *Su(fu)*. In the absence of the Fu^+ kinase, $Su(fu)^+$ would recognize and activate Cos-2^+ posteriorly as well as anteriorly, thus leading to the terminal phenotype of *fu* embryos (Figure 8C). In the absence of both Fu^+ and $Su(fu)^+$, Cos-2^+ would not be activated posteriorly and the fu phenotype would thus be suppressed (Figure 8D). The observation that *cos-2* mutations suppress the fu phenotype supports this scheme. In the absence of the Cos-2^+ product, the expression of *wg* extends anteriorly at the end of germ band extension (A. FORBES and P. INGHAM, unpublished data). Cos-2^+ thus behaves as a repressor of *wg*. The observation that *wg* expression disappears in *fu* embryos at the end of germ band extension (LIMBOURG-BOUCHON, BUSSON and LAMOUR-ISNARD 1991) is in agreement with the hypothesis that the Cos-2^+ function is expressed in the *wg* domain in these embryos. The duplication in *fu* embryos covers more than just the domain controlled by *cos-2*. Ectopic expression of the Cos-2^+ function might in turn induce the expression of some other segment polarity function. For example, it has been shown that *naked* mutations suppress the segment polarity fu phenotype (LIMBOURG-BOUCHON, BUSSON and LAMOUR-ISNARD 1991). This observation suggests that $naked^+$ is also involved in generating *fu* embryo phenotype.

Homozygous *Su(fu)* individuals are viable and display no apparent anomalies. However, *Su(fu)* mutations strongly enhance the effect of *cos-2* mutations, which supports the idea that $Su(fu)^+$ is a an anterior activator of Cos-2^+. One way to explain the fact that *Su(fu)* mutations are cryptic is to hypothesize the existence of another activator of Cos-2^+, which could partially compensate for the lack of $Su(fu)^+$ product (Figure 8B). The effect of this activator should be restricted to the anterior region of the segment since only the anterior Cos-2^+ protein should be active in the absence of Fu^+ and $Su(fu)^+$ (Figure 8D). This anterior activator of Cos-2^+ might correspond to the Patched protein.

Class II *fu* alleles lead to a cos-2-like phenotype in a *Su(fu)* background and class I alleles—unlike class 0 alleles—can rescue this phenotype in a *fu[I]/fu[II];Su(fu)/Su(fu)* combination. It is likely that class I and class II proteins do not act through some remaining kinase activity for two reasons. First these proteins are not able to fulfill the normal function of the Fu^+ kinase. Second, some mutations from each class have been shown to dramatically affect the catalytic domain of the kinase. Consequently, the genetic interactions described here might involve nonenzymatic protein-protein interactions. However, properties of the mutant products provide useful hints about the activity of the wild-type kinase.

It is striking that mutant Fu[II] products affect, in a *Su(fu)* background, part of the segments where the Fu^+ function itself is not required. It might be because truncated Fu[II] proteins lack the regulatory domain required anteriorly for inhibition of the Fu^+ kinase. The cos-2-like phenotype of *fu[II];Su(fu)* individuals would thus be due to abnormal anterior inhibition of the Cos-2^+ protein after constitutive binding by unregulated Fu[II] proteins (Figure 8H).

Class I *fu* alleles confer a wild-type phenotype in a *Su(fu)* background, and they do not enhance the effect of *cos-2* mutations. Predicted class I proteins possess the regulatory domain, so they would not interact abnormally with Cos-2^+ in the anterior region (Figure 8E, F). Rescue of the class II phenotype by *fu[I]* alleles would be due to a direct interaction between truncated Fu[II] proteins and the carboxy terminal domain of Fu[I] proteins (Figure 8J). Existence of such direct interaction between the two classes of mutant proteins would strongly suggest that the Fu^+ kinase interact with itself in wild-type individuals. Several ser/thr kinases are known to directly regulate their own activity (reviewed in KREBS 1986). In the case of the Fu^+ protein, such interaction could indeed involve autophosphorylation, or simply contact between the catalytic domain and the carboxy terminal domain. The possible role of such Fu^+ autoregulation remains unclear at the moment.

We postulated the existence of two products in the anterior region: a second activator of Cos-2^+ to explain the fact that *Su(fu)* and *fu[0];Su(fu)* individuals are normal, and an inhibitor of Fu^+. One could assume that actually these two activities are controlled by a unique function that reinforces Cos-2^+ anteriorly and thus impedes its inhibition by the Fu^+ kinase. However, two observations suggest that the Fu^+ kinase is indeed turned off in the anterior region. First, if some active Fu^+ product was present in the anterior region, one should be able to detect a partial inhibition of Cos-2^+. For example, the phenotype of viable *cos-2* mutants should be partially rescued in the absence of the Fu^+ kinase. This was not observed since *fu[I]* alleles do not affect the phenotype of $cos\text{-}2^7$ mutants. Second, class I proteins can rescue the antimorphic effect of class II proteins presumably because they titrate truncated Fu[II] proteins. If class I proteins were not turned off anteriorly, they would have to titrate both themselves and class II proteins in *fu[I]/*

fu[II];Su(fu)/Su(fu) individuals. Since there would be twice as many catalytic domains compared with regulatory domains, at least half of the Fu proteins would be available for binding Cos-2$^+$. Rescue of the class II phenotype by *fu[I]* alleles would then be incomplete, which is not the observed situation.

A prediction of our model is that activation by Su(fu)$^+$ of posterior Cos-2$^+$ product should be only partial in *fu[II]* mutants, because Fu[II] proteins tend to bind Cos-2$^+$ constitutively (Figure 8G). As the postulated role of the Fu$^+$ kinase is to phosphorylate and thus inhibit Cos-2$^+$ posteriorly (Figure 8A), it implies surprisingly that mutant Fu[II] proteins should partially mimic the effect of the wild-type Fu$^+$ product. Consequently, *fu[II]* mutants should not display an extreme fu phenotype, even though Fu[II] proteins may have a fully abolished kinase activity. The strongest *fu* mutations lead to pupal lethality. Indeed, although class I alleles are three times less frequent than class II alleles, all three pupal lethal alleles belong to class I.

Origin of the wing fu phenotype: Although the adult fu wing phenotype and the embryonic segment polarity phenotype were never compared as for their cause, several observations suggest that similar mechanisms actually generate both defects. First, FAUSTO-STERLING (1978) showed that the wing phenotype is better described as thickening of the third vein and nonformation of the fourth vein, rather than as fusion of V3 and V4. The anteroposterior boundary lies between V3 and V4 (GARCIA-BELLIDO, RIPOLL and MORATA 1973). Therefore, as in the case of the segment polarity phenotype, the wing fu phenotype would correspond to the duplication of an anterior structure and deletion of a posterior one. Second, all *fu* alleles confer both the embryonic phenotype and the wing phenotype, and there is a good correlation between the strength of these two phenotypes (BUSSON *et al.* 1988). Third, the *Su(fu)^{LP}* mutation fully suppresses both phenotypes. Fourth, class II *fu* alleles lead to a cos-2-like phenotype in interaction with *Su(fu)* in the embryonic segments as well as in the wing. Consequently, both the segment polarity phenotype and the adult wing phenotype of *fu* individuals would arise from a unique cause, namely the ectopic posterior expression of the Cos-2$^+$ function in the absence of the Fu$^+$ inhibitor and under the effect of the Su(fu)$^+$ activator. The observation that *costal-2* mutations can suppress both fu phenotypes supports this interpretation.

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