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Interallelic Complementation at the *suppressor of forked* Locus of Drosophila Reveals Complementation Between Suppressor of forked Proteins Mutated in Different Regions

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ABSTRACT

The Su(f) protein of *Drosophila melanogaster* shares extensive homologies with proteins from yeast (RNA14) and man (77 kD subunit of cleavage stimulation factor) that are required for 3′ end processing of mRNA. These homologies suggest that *su(f)* is involved in mRNA 3′ end formation and that some aspects of this process are conserved throughout eukaryotes. We have investigated the genetic and molecular complexity of the *su(f)* locus. The *su(f)* gene is transcribed to produce three RNAs and could encode two proteins. Using constructs that contain different parts of the locus, we show that only the larger predicted gene product of 84 kD is required for the wild-type function of *su(f)*. Some lethal alleles of *su(f)* complement to produce viable combinations. The structures of complementing and noncomplementing *su(f)* alleles indicate that 84-kD Su(f) proteins mutated in different domains can act in combination for partial *su(f)* function. Our results suggest protein-protein interaction between or within wild-type Su(f) molecules.

DIFFERENTIAL RNA processing mechanisms have been shown, in many cases, to be responsible for the regulation of developmental processes. For example, the entire sex determination pathway of Drosophila is regulated at the level of sex specific splicing (for reviews, see BAKER 1989; HODGKIN 1989; STEINMANN-ZWICKY *et al.* 1990). In Drosophila, genes involved in mRNA metabolism have been identified by their effect upon mutations caused by insertion of transposable elements within noncoding sequences of genes (RUTLEDGE *et al.* 1988). In this type of mutation, the insertion results in an abnormal transcription pattern of the gene, and this transcription pattern as well as the phenotype of the mutant can be further altered by mutations in other unlinked genes (reviewed by SMITH and CORCES 1991). These genetic interactions have been very useful in identifying genes involved in critical steps of RNA processing such as *suppressor of white-apricot* [$su(w^a)$] and *suppressor of sable* [*su(s)*]. Mutations at $su(w^a)$ suppress the phenotype of the $white^{apricot}$ allele of *white* where a *copia* retrotransposon is inserted in the second intron of the *white* gene (LEVIS *et al.* 1984). The $su(w^a)$ gene encodes one of the few genetically characterized splicing regulators. It regulates its own expression by repressing accumulation of the fully spliced $su(w^a)$ mRNAs (ZACHAR *et al.* 1987, 1994). Mutations at *su(s)* suppress the phenotype of mutations due to insertion of transposable elements within 5′ untranslated regions of *vermillon* and *yellow* (FRIDELL *et al.* 1990; GEYER *et al.* 1991). Su(s) protein is also believed to have a role in splicing and has been suggested to act by preventing splicing complexes from forming on poor 5′ splice sites (FRIDELL and SEARLES 1994).

The *suppressor of forked* [*su(f)*] locus was identified from its interaction with the $forked^1$ (f^1) mutation where a *gypsy* retrotransposon is inserted in an intron of the *forked* (*f*) gene (HOOVER *et al.* 1993; ISHIMARU and SAIGO 1993). The $su(f)^1$ mutation suppresses the forked bristle phenotype of f^1, so the bristles appear wild type in the stock $f^1su(f)^1$ (WHITTINGHILL 1937). ISHIMARU and SAIGO (1993) have shown that in f^1, very few wild-type *f* transcripts are made and that truncated transcripts terminating within the 5′ LTR of *gypsy* accumulate. In the double mutant $f^1su(f)^1$, the abundance of the wild-type *f* transcripts is strongly increased, which correlates with the suppression of the forked bristle phenotype. This shows that the Su(f) protein is involved in some step of mRNA metabolism.

The Su(f) protein is homologous to the RNA14 protein of *Saccharomyces cerevisiae* (MITCHELSON *et al.* 1993). The homology extends over the entire length of the RNA14 protein with 26% of residues being identical and 47% similar between the two proteins. RNA14 has recently been shown to be involved in polyadenylation of mRNA (MINVIELLE-SEBASTIA *et al.* 1994). Another recent study has described the high homology (56% identity, 69% similarity) between the Su(f) protein and a human 77-kD protein also known to play a role in poly-

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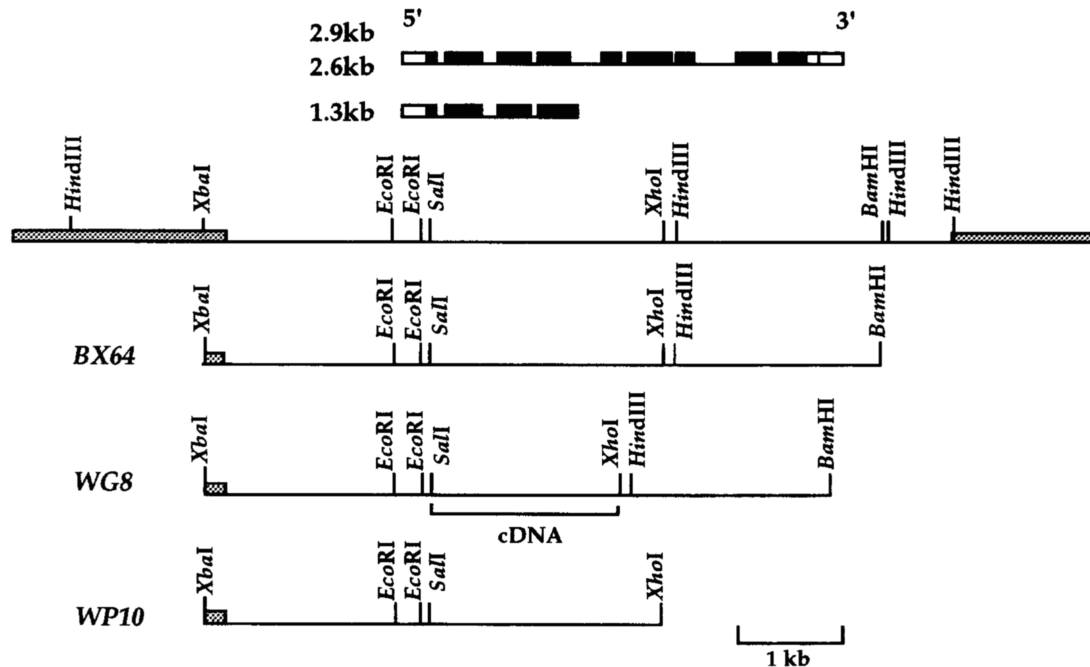

FIGURE 1.—Constructs with different parts of the *su(f)* locus. A map of the locus is depicted. Below are indicated the restriction fragments cloned into pW8 to produce the different constructs. For *WG8*, the genomic fragment exchanged for the corresponding cDNA fragment is noted. Thin line corresponds to unique DNA, grey boxes are repeated DNA. At the top are shown polyadenylated transcripts produced by *su(f)*. Thin lines are introns, black boxes are coding sequences, open boxes are untranslated parts of exons.

adenylation (TAKAGAKI and MANLEY 1994). These homologies suggest that *su(f)* is also involved in mRNA 3′ end formation and that some aspects of 3′ end processing are conserved from yeast, through Drosophila to man.

The *su(f)* gene is complex at both the molecular and genetic levels. It produces three RNAs (Figure 1) resulting from utilization of alternative polyadenylation sites (MITCHELSON *et al.* 1993). The 1.3-kb mRNA has its 3′ end within intron 4 and could encode a protein of ~39 kD. The 2.6- and 2.9-kb *su(f)* mRNAs encode an 84-kD protein. While $su(f)^{1}$ is a viable allele, null alleles of *su(f)* are lethal at the end of first instar larvae (PERRIMON *et al.* 1989; LINDSLEY and ZIMM 1992). However, it has long been known that some lethal alleles of *su(f)* complement and produce viable heteroallelic combinations (see below).

To investigate the basis for the genetic complexity of *su(f)*, we tested the requirement for each putative Su(f) protein in the wild-type function of *su(f)* and showed that only the 84-kD protein is required. We have also mapped the molecular lesions of complementing and noncomplementing *su(f)* alleles. Their structures suggest that alleles that complement encode close to wild-type proteins, whereas alleles unable to complement any other *su(f)* allele encode no, or very small, polypeptides. We propose that complementation between lethal alleles of *su(f)* results from interaction between molecules of 84-kD Su(f) protein mutated in different domains.

MATERIALS AND METHODS

Drosophila stocks and genetics: All *su(f)* alleles used in this study are described by LINDSLEY and ZIMM (1992) except $su(f)^{16\text{-}185}$ (SCHALET and LEFEVRE 1973). The original names of *su(f)* alleles have been used throughout and alleles called *l(1)x* have been changed to $su(f)^{x}$. For example, *l(1)R-9-18* is $su(f)^{R\text{-}9\text{-}18}$. Drosophila stocks containing *su(f)* alleles that we have used are: $su(f)^{L26}/FM6$, $su(f)^{16\text{-}185}/FM6$, $w\ m\ f\ su(f)^{MS252}/FM6$, $w\ m\ f\ su(f)^{MS97}/FM6$, $In(1)\ sc^{4L}\ sc^{8R}$, $y\ sc^{4}\ sc^{8}\ w^{a}\ Tu\ su(f)^{S1}/FM6$, $su(f)^{D13}/FM6$, $su(f)^{S2}/FM6$, $In(1)\ sc^{4L}\ sc^{8R}$, $y\ sc^{4}\ sc^{8}\ w^{a}\ f^{X}\ su(f)^{X1}/FM6$, $y\ ct^{6}\ v\ f\ su(f)^{X2}/FM6$, $y^{2}\ v\ f\ su(f)^{3DES}/FM6$, $y\ ct^{6}\ v\ f\ car\ su(f)^{R\text{-}9\text{-}18}/FM6$, $y\ v\ f\ su(f)^{ts726}$, $y^{2}\ w^{a}\ f\ su(f)^{ts726}/FM7b$, $y\ w\ f\ su(f)^{madts}$, $f^{1}\ su(f)^{madts}$ and $f^{1}\ su(f)^{1}$. Details of balancers, other special chromosomes and other genes are given by LINDSLEY and ZIMM (1992). To test the complementation between *su(f)* alleles, we used the $B^{S}Y$ chromosome, which contains a translocation of chromosome *X* covering the *su(f)* region (LINDSLEY and ZIMM 1992). Females $su(f)^{lethal1}/balancer$ were crossed with males $su(f)^{lethal2}/B^{S}Y$ and resulting $su(f)^{lethal1}/su(f)^{lethal2}$ females were scored. The stock w^{1118} was used for transformation. Transformant stocks were established by crossing to balancer strains in the w^{1118} background. Unless specified otherwise, the rescue of *su(f)* alleles by transgenes was determined with a single copy of the construct. Each primary transformant line and each secondary insertion line made by mobilization with $\Delta 2\text{-}3$(99B) (ROBERTSON *et al.* 1988) were analyzed by DNA blotting. We found a single nonrearranged insertion in every line except for *WG8.2*, which contains three inserts at least one of which is not rearranged, and for *WP10.13*, *WP10.17*, *WP10.29* and *WP10.44*. These four last lines result from mobilization of *WP10.1* and contain the primary insertion *WP10.1* as well as another nonrearranged insertion on chromosome *III*.

Transformation: *P*-element mediated transformation was carried out as described by RUBIN and SPRADLING (1982). All

TABLE 1

Complementation pattern between *su(f)* lethal alleles at 25°

	L26	*16-185*	*MS252*	*MS97*	*S1*	*D13*	*S2*	*X1*	*3DES*	*X2*	*R-9-18*
L26	−	ND	−	−	−	−	ND	−	−	−	−
16-185		−	−	−	ND	−	−	ND	ND	ND	−
MS252			−	−	−	ND	−	ND	−	−	−
MS97				−	−	−	−	−	−	−	−
S1					−	−	ND	ND	−	−	−
D13						−	−	−	−	−	−
S2							−	−	−	−	+
X1								−	−	−	+
3DES									−	−	+
X2										−	+
R-9-18											−

−, lethal combination; +, viable combination; ND, not determined.

constructs were in the pW8 vector (KLEMENZ *et al.* 1987). Construct DNA (500 μg/ml) with 250 μg/ml of the helper plasmid pUChsΠΔ2-3 were injected into w^{1118} embryos.

Molecular biology: Plasmid construction and other routine molecular biology techniques were carried out as described by SAMBROOK *et al.* (1989). RNA blots were as reported by MITCHELSON *et al.* (1993) except that total RNA was extracted using RNAzol (Bioprobe Systems). PCR amplifications were carried out as recommended by the manufacturer (Perkin Elmer-Cetus). Oligonucleotides used for PCR were: 5'CCGCATACTGGTACTGAATTAATATC (−539 to −513), 5'CGATGACACTATCGCAGTTACATCC (242 to 266), 5'CCATCCAAATAAAGCCAATG (509 to 490), 5'GTGAGTGTTATACGCAGAGTTTGC (1304 to 1327), 5'CTGGTTTTAAGTTGGAATTTAGAAAGAAC (1361 to 1333), 5'GTAGGAACACACATATTGAATATG (2158 to 2181), 5'CTGGAGTATTAAATTTCGATGGTATC (2222 to 2197), 5'GTCTTACTTTAATCACCTCGTTCTGC (3222 to 3247), 5'CCCACCTTGTTCAGTATAATGCCC (3278 to 3255), 5'GTGAGTGTCCCTGATTGAAATTCTC (3551 to 3575), 5'CGTACTTGTATCCACGATCCAGTG (3696 to 3673), 5'GCATGAAGGCAAATTCCCAAGCAGC (3988 to 3963), 5'CTGAGGTAATGGAGGAGTGCTTC (4341 to 4319); coordinates indicated within brackets are from MITCHELSON *et al.* (1993). PCR products were purified from low melting gel and either cloned into M13 vectors or directly sequenced. DNA sequencing was by the dideoxy chain-termination method (SANGER *et al.* 1977) using sequenase (U.S. Biochemical).

RESULTS

Genetic interactions between *su(f)* alleles: A complex pattern of complementation has been described for a particular allele of *su(f)*, $su(f)^{R\text{-}9\text{-}18}$ (SCHALET 1972). At 25°, $su(f)^{R\text{-}9\text{-}18}$ homozygotes die at pupariation, but at 18°, a very small proportion of them eclose showing thread-like, pale yellow bristles and hairs and wrinkled wings. $su(f)^{R\text{-}9\text{-}18}$ suppresses the phenotype of f^1 at 18°, and it is allelic to $su(f)^1$ since in females of the genotype $f^1\ su(f)^{R\text{-}9\text{-}18}/f^1\ su(f)^{1,}$ the f^1 phenotype is suppressed. Nevertheless, at 25° $su(f)^{R\text{-}9\text{-}18}$ complements for viability some *su(f)* alleles that are lethal as homozygotes at all temperatures. The complementation pattern for viability at 25° in a large number of combinations of 11 alleles of *su(f)* has been studied (SCHALET 1972; SCHALET and LEFEVRE 1973; A. SCHALET, unpublished results and this study) and is summarized in Table 1. This shows that the allele $su(f)^{R\text{-}9\text{-}18}$ complements four lethal alleles of *su(f)* for viability but not six others and except for $su(f)^{R\text{-}9\text{-}18}$, all other alleles fail to complement one another at 25°.

To determine if $su(f)^{R\text{-}9\text{-}18}$ is the only *su(f)* allele able to complement other *su(f)* lethal alleles, we carried out complementation analysis with two other temperature-sensitive lethal alleles, $su(f)^{madts}$ and $su(f)^{ts726}$. Four temperature-sensitive lethal alleles of *su(f)* with similar mutant phenotypes have been described, $su(f)^{madts}$ (JÜRGENS and GATEFF 1979), $su(f)^{ts67g}$ (DUDICK *et al.* 1974), $su(f)^{ts76a}$ (WILSON 1980) and $su(f)^{ts726}$ (RUSSEL 1974). Homozygotes of these alleles are phenotypically wild type at 18°, they survive and suppress the f^1 phenotype at intermediate temperatures and are lethal at 29°. We studied the complementation pattern for viability at 30° of the 10 lethal alleles of *su(f)* used in earlier studies with $su(f)^{R\text{-}9\text{-}18}$, $su(f)^{madts}$ and $su(f)^{ts726}$ (Table 2).

TABLE 2

Complementation pattern between lethal alleles and temperature-sensitive lethal alleles of *su(f)* at 30°

	R-9-18	*madts*	*ts726*
L26	−	−	−
16-185	−	−	−
MS252	−	−	ND
MS97	−	−	−
S1	−	−	−
D13	−	−	−
S2	+	+	+
X1	+	+	+
3DES	+	+	+
X2	−	−	−
R-9-18	−	+	+
madts		−	−
ts726			−

−, lethal combination; +, viable combination; ND, not determined.

$su(f)^{R\text{-}9\text{-}18}$ shows identical complementation patterns at 25° and at 30° except with the lethal allele $su(f)^{X2}$, which complements $su(f)^{R\text{-}9\text{-}18}$ for viability at 25° but not at 30°. This result has already been described (SCHALET 1972). $su(f)^{madts}$ and $su(f)^{ts726}$ both present the same complementation pattern as $su(f)^{R\text{-}9\text{-}18}$. They both complement three lethal alleles of $su(f)$ [$su(f)^{S2}$, $su(f)^{X1}$ and $su(f)^{3DES}$] among the 10 alleles tested. In addition, $su(f)^{R\text{-}9\text{-}18}$ complements both $su(f)^{madts}$ and $su(f)^{ts726}$ at 30°. Finally, $su(f)^{madts}$ and $su(f)^{ts726}$ do not complement one another. From these patterns of complementation, five different groups can be defined: (I) $su(f)^{L26}$, $su(f)^{16\text{-}185}$, $su(f)^{MS252}$, $su(f)^{MS97}$, $su(f)^{S1}$ and $su(f)^{D13}$, which do not complement any $su(f)$ alleles; (II) $su(f)^{S2}$, $su(f)^{X1}$ and $su(f)^{3DES}$, which complement $su(f)^{R\text{-}9\text{-}18}$, $su(f)^{madts}$ and $su(f)^{ts726}$; (III) $su(f)^{X2}$, which complements $su(f)^{R\text{-}9\text{-}18}$ at 25° only; (IV) $su(f)^{madts}$ and $su(f)^{ts726}$; and (V) $su(f)^{R\text{-}9\text{-}18}$.

Complementation at the *su(f)* locus is independent of the allelic state of the *zeste* gene: Interallelic complementation has been described at other loci in Drosophila, including *white* (BABU and BHAT 1980), *decapentaplegic* (GELBART and WU 1982) and *yellow* (GEYER *et al.* 1990). In all these cases, complementation is dependent upon alignment of the two alleles resulting from pairing of the two homologous chromosomes. The phenotypes of certain allelic combinations are affected by rearrangements that disrupt chromosome pairing and by mutations of the *zeste* (*z*) locus. Hypomorphic alleles of *z* have a similar effect to that of chromosomal rearrangements and eliminate complementation between alleles of the same gene. To determine whether interallelic complementation at the $su(f)$ locus depends upon *z*, we studied the effect of two different *z* alleles, z^a and z^1 on the viable combination $su(f)^{3DES}/su(f)^{R\text{-}9\text{-}18}$. z^a is a hypomorphic allele of *z* and z^1 is a neomorphic allele, but both can affect interallelic complementation (GELBART and WU 1982). Various *z su(f)* recombinants were made and used to show that $z^a\ su(f)^{3DES}/z^a\ su(f)^{R\text{-}9\text{-}18}$ and $z^1\ su(f)^{3DES}/z^1\ su(f)^{R\text{-}9\text{-}18}$ are as viable as $z^+\ su(f)^{3DES}/z^+\ su(f)^{R\text{-}9\text{-}18}$. Thus, the allelic state of the *z* gene has no effect on complementation at the $su(f)$ locus. This suggests that this complementation could be independent of chromosome pairing.

Transformation experiments prove that the 84-kD Su(f) protein is sufficient for the wild-type *su(f)* function: The $su(f)$ gene (Figure 1) is transcribed to produce three polyadenylated RNAs that have different 3′ ends (MITCHELSON *et al.* 1993). Both the 2.9- and 2.6-kb RNAs encode an 84-kD protein. The 3′ end of the 1.3-kb $su(f)$ RNA is within intron 4 of the gene. This RNA has no in frame stop codon and would encode a protein with the same 313 N-terminal amino acids as the 84-kD protein, then 37 amino acids encoded by intron 4 followed by a C-terminal poly-lysine tract encoded by poly(A).

Part of the complex complementation pattern described at the $su(f)$ locus (Tables 1 and 2) could result from a requirement for both proteins in $su(f)$ function. A $su(f)$ lethal allele altered in the coding sequence specific to the 84-kD protein might complement another $su(f)$ lethal allele altered in the region specific to a protein encoded by the 1.3-kb RNA. We therefore made $su(f)$ transgenes to test the requirement for these two putative proteins in the wild-type function of $su(f)$ (Figure 1). *BX64* contains a 6.4-kb *Xba*I-*Bam*HI genomic fragment covering the whole $su(f)$ locus, and we have already reported that this construct rescues all $su(f)$ mutant phenotypes (MITCHELSON *et al.* 1993). *WG8* also contains the whole locus, but a fragment from exon 1 to exon 6 has been replaced by the corresponding cDNA fragment. This construct lacks the first five introns of the gene and is thus unable to produce the 1.3-kb RNA that includes part of intron 4. It can encode the 84-kD Su(f) protein. *WP10* contains an *Xba*I-*Xho*I genomic restriction fragment from the 5′ region of $su(f)$ up to exon 6. *WP10* cannot encode the 84-kD protein, but should produce the protein encoded by the 1.3-kb RNA. The three constructs were introduced into flies by *P*-element mediated transformation. Additional insertions were generated by transposing primary insertions using a stable source of *P* transposase (ROBERTSON *et al.* 1988). In this way, we recovered two primary and two secondary insertions of *BX64*, four primary and three secondary insertions of *WG8* and two primary and four secondary *WP10* insertions (Figure 2). We have confirmed by RNA blotting that *WG8* does not, and that *WP10* does, produce the 1.3-kb mRNA (Figure 3).

The primary insertions were tested for rescue of two $su(f)$ mutant phenotypes: lethality and the suppression of the f^1 bristle phenotype. Transformants with the transgene on an autosome were crossed with seven $su(f)$ alleles representing all five groups defined earlier and with the stock $f^1\ su(f)^1$ (Table 3). Both *BX64* primary transformants rescued all lethal $su(f)$ alleles as well as the $su(f)^1$ allele since $f^1\ su(f)^1;BX64$ flies had forked bristles. The secondary insertions of *BX64* were shown to rescue $su(f)^{L26}$, a deletion of $su(f)$. These results indicate that in the four insertion sites tested, the *BX64* transgene provides fully wild-type function of $su(f)$. One primary *WG8* transformant (*WG8.4*) was able to rescue all lethal alleles for viability and the suppression of the bristle phenotype of f^1 by $su(f)^1$. The other two primary autosomal *WG8* transformants showed only a very small effect in combination with the $su(f)^{R\text{-}9\text{-}18}$ allele at 18°. The survival of $su(f)^{R\text{-}9\text{-}18}/Y;WG8.2/+$ and $su(f)^{R\text{-}9\text{-}18}/Y;WG8.3/+$ males at 18° was better than that of $su(f)^{R\text{-}9\text{-}18}/Y$ males (Table 3). Two of the three secondary *WG8* insertions rescued the lethal alleles, $su(f)^{3DES}$ and $su(f)^{L26}$, and the remaining primary insertion on the *X* chromosome (*WG8.1*) was viable when recombined with $su(f)^{L26}$ (Figure 2). In one case, an insertion that rescued (*WG8.4.15*) was recovered after transposition from a site where the construct

	Transformant and mobilized lines	Rescue of *su(f)* mutant phenotypes	Number of lines which rescue/ Total number of lines
BX64	*BX64.2*	+	4/4
	BX64.1	+	
	→ *BX64.11*	+	
	→ *BX64.12*	+	
WG8	*WG8.1*	+	4/7
	WG8.2	-	
	WG8.3	-	
	WG8.4	+	
	→ *WG8.4.38*	+	
	→ *WG8.4.14*	-	
	→ → *WG8.4.15*	+	
WP10	*WP10.2*	-	0/6
	WP10.1	-	
	→ *WP10.13*	-	
	→ *WP10.17*	-	
	→ *WP10.29*	-	
	→ *WP10.44*	-	

FIGURE 2.—Rescue of *su(f)* lethality with three constructs containing various parts of the *su(f)* locus, located at different places in the genome. Arrows indicate insertions obtained from mobilization of another insertion. +, rescue; −, no rescue.

did not rescue (*WG8.4.14*). This shows that the *WG8* transgene is able to provide wild-type *su(f)* function, but only in some chromosomal locations.

Neither primary *WP10* transformants rescued any *su(f)* phenotype (Table 3), and all four secondary *WP10* insertions did not rescue the lethality of $su(f)^{3DES}$, $su(f)^{L26}$ or $su(f)^{R\text{-}9\text{-}18}$ (Figure 2). Furthermore, the failure of *WG8.3*, *WP10.1* and *WP10.2* to rescue *su(f)* mutants is not dose dependent. Even when homozygous for these autosomal insertions, $su(f)^{L26}$ or $su(f)^{R\text{-}9\text{-}18}$ were not rescued at 25°.

Taken together, these data indicate several conclusions. *WG8* encodes the 84-kD Su(f) protein only, and this protein is sufficient for the wild-type function of *su(f)*. It is able to rescue the lethality of a complete deletion of the *su(f)* locus, $su(f)^{L26}$, and it also rescues the suppression of the f^{1} phenotype. The failure of some *WG8* insertions to rescue *su(f)* mutants appears to be due to a strong position effect when the first five introns of *su(f)* are missing. This position effect is not seen in *BX64* transformants, suggesting that an enhancer necessary for the proper expression of *su(f)* may be located in the first five introns of the gene. *WP10* insertions show no indication of providing any *su(f)* function. They do not rescue any *su(f)* allele, and they do not complement *WG8* transformants that fail to rescue *su(f)* phenotypes. Males of the genotypes $su(f)^{L26}/Y;WG8.3/+;WP10.1/+$, $su(f)^{L26}/Y;WG8.3/+;WP10.2/+$, $su(f)^{R\text{-}9\text{-}18}/Y;WG8.3/+;WP10.1/+$ or $su(f)^{R\text{-}9\text{-}18}/Y;WG8.3/+;WP10.2/+$ do not survive. Our results strongly suggest that a putative protein encoded by the 1.3-kb RNA is dispensable for the *su(f)* function. Given the unusual structure of this RNA in lacking a stop codon, it is possible that there is no such protein. These results also clearly demonstrate that the complementation pattern obtained with the various *su(f)* alleles does not result from a requirement for two different Su(f) proteins.

Molecular mapping of *su(f)* mutants: The structures of the 11 lethal *su(f)* alleles described in Table 1 and the temperature-sensitive *su(f)* alleles, $su(f)^{mad\text{-}ts}$, $su(f)^{ts67g}$, $su(f)^{ts76a}$ and $su(f)^{ts726}$ were initially investigated by DNA blotting (data not shown). The results of this analysis are summarized in Figure 4A. Eight of the 11 lethal alleles showed differences from wild-type DNA. $su(f)^{MS252}$ and $su(f)^{MS97}$ have *P*-element insertions within exon 2 of *su(f)* at positions 615 and 682, respectively, (MITCHELSON *et al.* 1993) while in $su(f)^{S2}$, there is a *Doc* element insertion in the last exon of the gene at position 3659 (DRIVER *et al.* 1989; MITCHELSON *et al.* 1993). Nucleotide positions are from the DNA sequence of MITCHELSON *et al.* (1993). In $su(f)^{L26}$, there is a deletion of 8.5 kb including the whole *su(f)* locus and 1.5 kb of repeated DNA flanking the gene

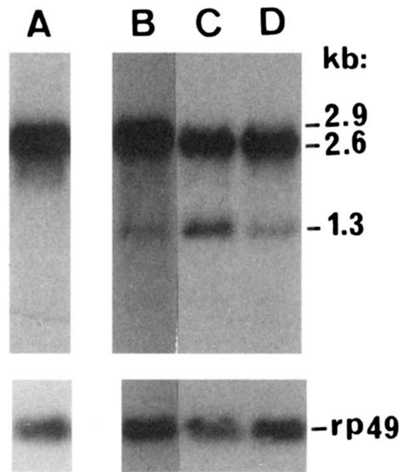

FIGURE 3.—Production of *su(f)* mRNAs from *WG8* and *WP10* constructs. 5 μg of poly(A)$^+$ RNA from adult females were loaded per lane. The Northern blot was hybridized to a single-stranded DNA probe corresponding to a cDNA of the 1.3-kb *su(f)* RNA (MITCHELSON *et al.* 1993). The filter was then reprobed with the *rp49* clone (VASLET *et al.* 1980) as a loading control. (A) w^{1118} $su(f)^{L26}$;*WG8.4*, (B) w^{1118} $su(f)^{L26}$; *WG8.4*;*WP10.1*, (C) w^{1118};*WP10.2*, (D) w^{1118}. In lanes A and B, *su(f)* mRNAs are produced exclusively by *WG8.4* and *WP10.1* insertions, since the allele $su(f)^{L26}$ is a complete deletion of the *su(f)* locus (see text). Note that *WG8.4*, as expected, does not produce the 1.3-kb mRNA (lane A) and that, in contrast, *WP10.1* does (lane B). In lanes C and D, *su(f)* mRNAs are produced by the *su(f)* locus and by the *WP10.2* insertion. Note the increase in the level of the 1.3-kb mRNA when *WP10.2* is present in the genome (lane C).

(M. TUDOR and K. O'HARE, unpublished data). In $su(f)^{16-185}$, there is either a large insertion or an inversion with one breakpoint within *su(f)*; it was not characterized further. The last three alleles, $su(f)^{S1}$, $su(f)^{X1}$ and $su(f)^{X2}$ showed small differences from wild type. Mutant specific fragments were amplified by the PCR and their DNA sequences were determined. In $su(f)^{S1}$, there is an insertion of the most 3′ 222 bp of the *I* factor (FAWCETT *et al.* 1986) terminated by one TAA triplet followed by four repetitions of the sequence TAAA. The complete insertion is 241 bp and it is located at the end of exon 3 (position 1302) (Figure 4A). Figure 4B shows that the *I* element has inserted in inverse orientation with respect to *su(f)* transcription and that this insertion has duplicated a 14-bp target sequence. In $su(f)^{X1}$, 149 bp of the last exon (from position 3822 to 3970) are deleted (Figures 4A and 5). In addition, Figure 5 shows that in $su(f)^{X1}$, 7 bp located at the 3′ break point of this small deletion are duplicated. Finally, $su(f)^{X2}$ corresponds to a 1110-bp deletion (from position 379 to 1488, Figure 5) that starts within exon 1 and includes exon 2, exon 3 and the 5′ half of exon 4 (Figure 4A).

DNA from $su(f)^{D13}$, $su(f)^{3DES}$, $su(f)^{R\text{-}9\text{-}18}$ and from the four temperature-sensitive *su(f)* alleles showed no difference from wild-type DNA in DNA blots. We have determined the nucleotide changes for the mutations in $su(f)^{3DES}$ and $su(f)^{R\text{-}9\text{-}18}$. DNA was made from flies of the genotype *WG8.1* $su(f)^{L26}/su(f)^{3DES}$ and used to amplify a fragment specific to $su(f)^{3DES}$, from intron 5 to downstream of the 3′ untranslated region of *su(f)*. We sequenced 0.8 kb of this fragment (from position 3350 to 4150) and found 1-bp difference from the wild-type sequence, at the end of intron 8 (Figure 4A). This difference is at position 3606 and changes the AG consensus dinucleotide (MOUNT 1982) at the 3′ splice site of intron 8 to TG (Figure 5). To determine whether this difference is responsible for the $su(f)^{3DES}$ mutant phenotype, we checked the presence of the wild-type A at position 3606 in six different wild-type stocks since the parental stock for $su(f)^{3DES}$ has not been described in the literature. The difference found in $su(f)^{3DES}$ disrupts a *Pvu*II restriction site (Figure 5). We therefore amplified the corresponding region from the six wild-type stocks and digested the amplified DNA with *Pvu*II. In contrast to $su(f)^{3DES}$, all six stocks have the wild-type *Pvu*II site. Moreover, in an independent analysis, LANGLEY *et al.* (1993) showed that this *Pvu*II site is present

TABLE 3

Rescue of *su(f)* alleles with three constructs containing various parts of the *su(f)* locus

	L26		*S2*		*X1*		*3DES*		*X2*		*R-9-18*		*madts*		*1*	
	18°	25°	18°	25°	18°	25°	18°	25°	18°	25°	18°	25°	25°	30°	18°	25°
BX64.1	ND	+	ND	+	ND	+	ND	+	ND	+	ND	+	ND	+	ND	+
BX64.2	ND	+	ND	+	ND	+	ND	+	ND	+	ND	+	ND	+	ND	+
WG8.2	–	–	–	–	–	–	ND	–	–	–	+[a]	–	ND	ND	–	–
WG8.3	–	–	–	–	–	–	ND	–	–	–	+[a]	–	–	–	–	–
WG8.4	+	+	+	+	+	+	+	+	+	+	+	+	+	+	ND	+
WP10.1	–	–	ND	–	ND	–	ND	–	–	–	–	–	–	–	–	–
WP10.2	–	–	ND	–	ND	–	ND	–	–	–	–	–	–	–	–	–

–, lethal for $su(f)^{L26}$, $su(f)^{S2}$, $su(f)^{X1}$, $su(f)^{3DES}$, $su(f)^{X2}$, $su(f)^{R\text{-}9\text{-}18}$, and lethal for $su(f)^{madts}$ at 30°, f^+ phenotype for $su(f)^{madts}$ at 25° and for $su(f)^{1}$; +, viable for $su(f)^{L26}$ and $su(f)^{S2}$ and viable with a *f* phenotype for $su(f)^{X1}$, $su(f)^{3DES}$, $su(f)^{X2}$, $su(f)^{R\text{-}9\text{-}18}$, $su(f)^{madts}$ and $su(f)^{1}$ (see MATERIALS AND METHODS for details of the *su(f)* stocks); ND, not determined.

[a] The viability of $su(f)^{R\text{-}9\text{-}18}$ is slightly increased.

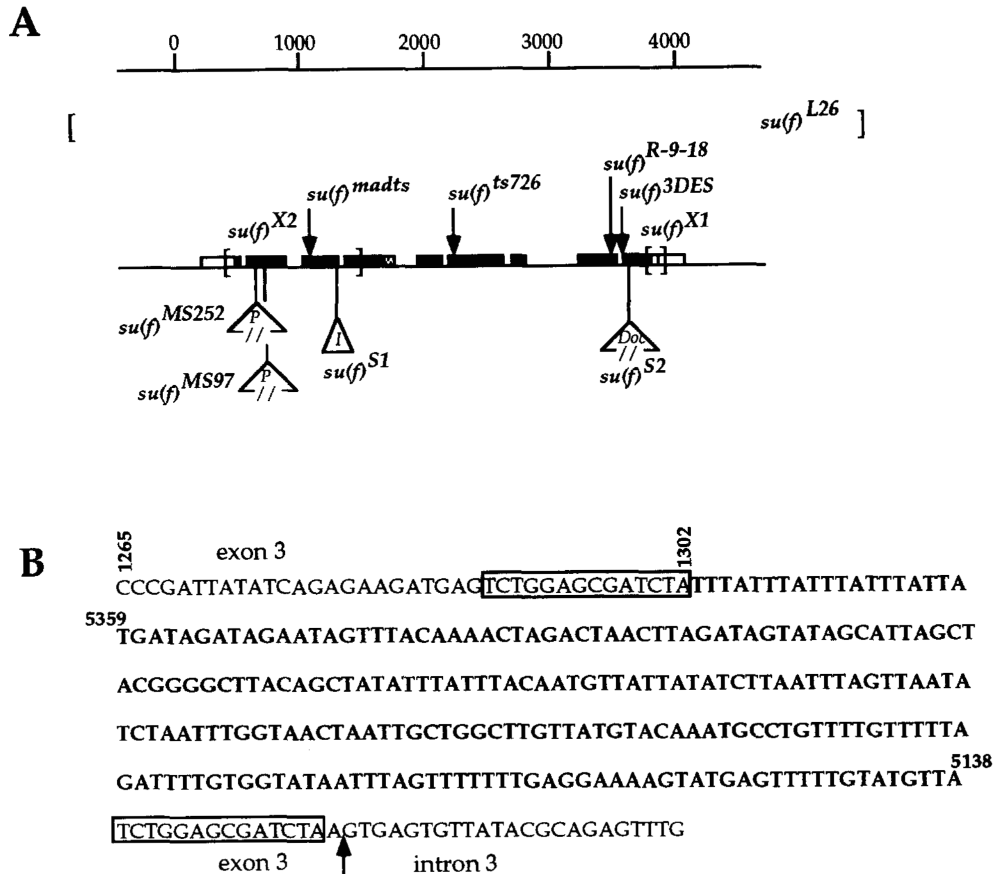

FIGURE 4.—Map of *su(f)* mutants. (A) The *su(f)* locus is depicted. Black boxes are coding sequences, open boxes are noncoding parts of exons, stippled box is the part of intron 4 incorporated within the 1.3-kb *su(f)* mRNA, and thin lines between exons are introns. Brackets represent the location of break points of deficiencies. Arrows indicate location of point mutations. Insertions are represented by small triangles. (B) The sequence of the $su(f)^{S1}$ mutant in the region of the *I* element insertion point is shown. Nucleotides from the *I* factor are in bold. The *I* element is inserted in inverse orientation with regard to *su(f)*; coordinates for the insertion (written horizontally) are those of the *I* factor (FAWCETT *et al.* 1986). The 14-bp target sequence is boxed. Arrow indicates the 5′ splice site of *su(f)* intron 3. Coordinates for the *su(f)* locus are from MITCHELSON *et al.* (1993).

in 64 lines generated from natural populations. These data indicate that the difference found in the allele $su(f)^{3DES}$ at position 3606 is the cause for the $su(f)^{3DES}$ mutant phenotype. $su(f)^{R\text{-}9\text{-}18}$ individuals die as pupae at 25° and survive very poorly at 18°. DNA from selected male $su(f)^{R\text{-}9\text{-}18}/Y$ larvae was prepared and used to amplify fragments to cover the whole *su(f)* locus. A total of 3.4 kb was sequenced (from positions −540 to 2223 and 3221 to 3988) and only 1-bp difference from the wild-type sequence was found in exon 8 (Figure 4A). Figure 5 indicates that in the $su(f)^{R\text{-}9\text{-}18}$ mutant, nucleotide 3490 is changed from G to A, which results in a change in the protein sequence from glycine to arginine. To check that this difference is the cause for the $su(f)^{R\text{-}9\text{-}18}$ mutant phenotype, we also amplified and sequenced the corresponding region from three different wild-type strains and from the stock *l(1)R-9-28/FM6* (LIFSCHYTZ and FALK 1969). The mutant *l(1)R-9-28* was obtained in the same screen as $su(f)^{R\text{-}9\text{-}18}$ and on the same parental chromosome, which is no longer available. Thus, the *l(1)R-9-28* mutant chromosome can be considered as having a parental version of the *su(f)* locus for $su(f)^{R\text{-}9\text{-}18}$. All four stocks have the wild-type G at position 3490. This shows that the single difference found in the allele $su(f)^{R\text{-}9\text{-}18}$ is indeed responsible for the mutant phenotype. The DNA sequences of the entire *su(f)* locus from the four temperature-sensitive alleles have also been determined and in each case, there was a single base pair difference from the wild-type sequence (K. ELLIOTT, K. O'HARE and M. SIMONELIG, unpublished observations). The fact that the level of DNA sequence polymorphism at the *su(f)* locus is extremely low has already been reported and results from the hitchhiking effect in a region of very low recombination (LANGLEY *et al.* 1993). In $su(f)^{madts}$, the substitution is within exon 3, while in $su(f)^{ts726}$, it is within exon 6 (Figure 4A). For both mutants, the single base pair difference results in a change of one amino acid in the 84-kD protein sequence.

***su(f)* alleles that complement one another encode an almost complete 84-kD protein:** The interallelic complementation pattern described in Tables 1 and 2 defines five groups of *su(f)* alleles. Group I contains the lethal alleles $su(f)^{L26}$, $su(f)^{16\text{-}185}$, $su(f)^{MS252}$, $su(f)^{MS97}$,

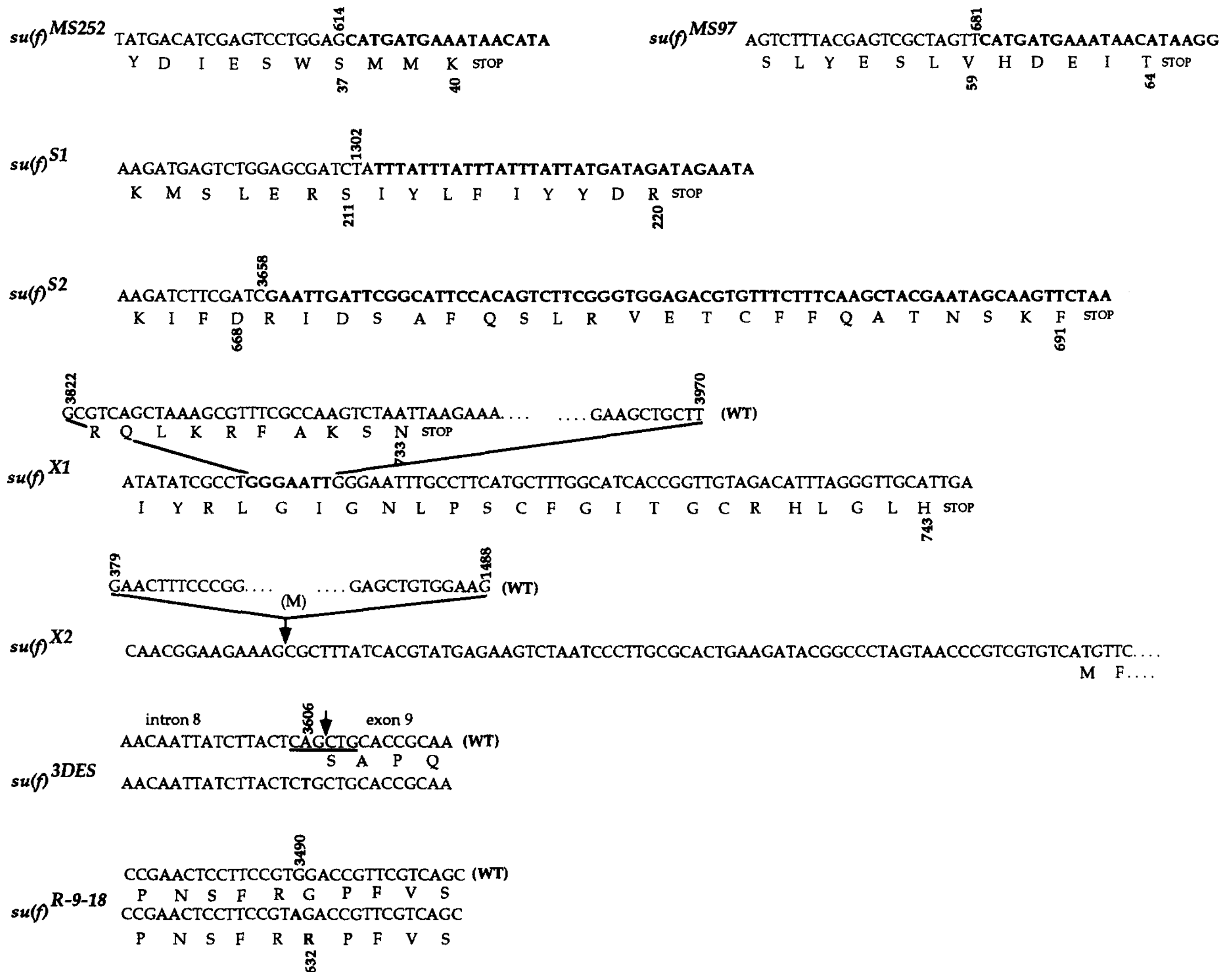

FIGURE 5.—Coding capacity for the *su(f)* mutants. For each mutant, the nucleotide and deduced amino acid mutant sequences are indicated. For mutations due to deletion and for point mutations, the wild-type sequences (WT) are written above the mutant sequences. Nucleotides from the insertions, from the duplication [in $su(f)^{X1}$] and mutant nucleotides in point mutations are in bold. For $su(f)^{3DES}$, the arrow indicates the end of intron 8 and the *Pvu*II site is underlined. Coordinates are from MITCHELSON *et al.* (1993).

$su(f)^{S1}$ and $su(f)^{D13}$, which do not complement any other *su(f)* allele. Group II contains the lethal alleles $su(f)^{S2}$, $su(f)^{X1}$ and $su(f)^{3DES}$, which complement the lethality of $su(f)^{R\text{-}9\text{-}18}$ and of the temperature-sensitive alleles, $su(f)^{madts}$ and $su(f)^{ts726}$ at 30°. Group III corresponds to the lethal allele $su(f)^{X2}$, which complements $su(f)^{R\text{-}9\text{-}18}$ at 25° only. $su(f)^{R\text{-}9\text{-}18}$ and the alleles $su(f)^{madts}$ and $su(f)^{ts726}$, represent respectively the two last groups that complement one another, in addition to complementing group II.

Three alleles from group I, $su(f)^{MS252}$, $su(f)^{MS97}$ and $su(f)^{S1}$ have insertions into the very 5′ part of the gene (Figure 4A). The putative proteins encoded by these mutants would be very small (40 amino acids, 64 amino acids and 220 amino acids, respectively, Figure 5). A fourth member of this group, $su(f)^{L26}$, is a complete deletion of the gene. In contrast, all group II alleles, $su(f)^{S2}$, $su(f)^{X1}$ and $su(f)^{3DES}$ have alterations in the very 3′ end of the gene. Assuming that transcription and translation in $su(f)^{S2}$ continued into the insertion, only the C-terminal 65 residues of the 84-kD Su(f) protein would be substituted by 23 residues encoded by the *Doc* transposon (Figure 5). The small deletion in $su(f)^{X1}$ includes the stop codon of the 84-kD Su(f) protein and the 3′ end of the 2.6-kb mRNA. However, the 3′ end for the 2.9-kb *su(f)* mRNA remains and the deleted mRNA would encode a protein where the 10 C-terminal residues of the 84-kD Su(f) protein would be substituted by 20 amino acids encoded by the 3′ untranslated region (Figure 5). In the $su(f)^{3DES}$ allele, the point mutation in the 3′ splice site of intron 8 should prevent wild-type splicing of this intron. We do not know whether this intron remains unspliced in the $su(f)^{3DES}$ mutant or whether a cryptic 3′ splice site is used, but in any

case, the protein encoded by $su(f)^{3DES}$ would lack a maximum of 82 C-terminal residues of the wild-type Su(f) protein, encoded by the last exon. The lethality of these three alleles indicates that the C-terminal part of the Su(f) protein is important. These mutants do complement $su(f)^{R\text{-}9\text{-}18}$, $su(f)^{madts}$ and $su(f)^{ts726}$ where 84-kD proteins altered at a single site should be produced. These results strongly suggest that interallelic complementation results from complementation between complete and/or close-to-complete 84-kD Su(f) proteins that are altered in different regions.

$su(f)^{X2}$ is interesting since it complements $su(f)^{R\text{-}9\text{-}18}$ at 25° but not at 30°. This temperature sensitivity indicates that this *trans*-heterozygote combination is less viable than the others. In contrast to $su(f)^{S2}$, $su(f)^{X1}$ and $su(f)^{3DES}$, Figure 5 shows that $su(f)^{X2}$ cannot encode a nearly complete Su(f) protein. This allele has a 1110-bp deletion from exon 1 to exon 4 that includes the initiation codon of the Su(f) protein, but not the transcription start site. As we hypothesize that for complementation both alleles produce proteins altered in different ways, we suggest that a protein is produced in $su(f)^{X2}$ that is able to act with a protein encoded by $su(f)^{R\text{-}9\text{-}18}$ and so permit the *trans*-heterozygote to survive. Assuming that transcription occurs in $su(f)^{X2}$ to produce an RNA where exon 1 has been fused to exon 4 and the remaining introns are spliced as in wild type, the first in frame methionine corresponds to residue 278 within exon 4 (Figure 5). This putative $Su(f)^{X2}$ protein would correspond to the 456 C-terminal amino acids of the 733 amino acids Su(f) protein.

DISCUSSION

The 84-kD Su(f) protein is sufficient for the wild-type function of *su(f)*: The *su(f)* locus is complex and is transcribed to produce three RNAs that could encode two different proteins. The three RNAs (2.9, 2.6 and 1.3 kb) arise from utilization of alternative polyadenylation sites and from alternative splicing of intron 4. Polyadenylation within intron 4 generates the 1.3-kb mRNA.

We have shown that the 84-kD Su(f) protein is sufficient for the *su(f)* function as assayed by two phenotypes: viability and restoration of the f^1 bristle phenotype. Transformants containing a construct encoding only the 84-kD Su(f) protein can rescue a complete deficiency of the *su(f)* locus. Flies of the genotype $su(f)^{L26}$;*WG8.4* are perfectly viable at all temperatures. Such transformants also rescue the suppressed bristle phenotype of f^1 $su(f)^1$ flies, *i.e.*, f^1 $su(f)^1$;*WG8.4* flies have mutant forked bristles. Our results show that the 1.3-kb mRNA is dispensable for the wild-type function of *su(f)*. We do not know if this RNA is translated. Although it has the features necessary for translation, it is possible that a regulatory process prevents its translation. This is the case for $su(w^a)$ where RNAs that are not fully spliced are retained in the nucleus (ZACHAR *et al.* 1994).

Genetic complementation between *su(f)* alleles can be explained by complementation at the protein level: We have examined a role for the Zeste protein in the interallelic complementation at the *su(f)* locus. For some loci in Drosophila, interallelic complementation is supposed to result from interaction between an intact regulatory sequence on one chromosome and an intact transcription unit on the other paired chromosome. This interaction can be altered by disruption of chromosome pairing or by mutations at the *z* locus (JACK and JUDD 1979; GELBART and WU 1982; GEYER *et al.* 1990; LEISERSON *et al.* 1994). We have shown that interallelic complementation at the *su(f)* locus is independent of mutations in the *z* gene. This suggests that this complementation would not result from interaction between regulatory sequences on one chromosome and a transcription unit on the homologous chromosome.

This has been confirmed by the mapping of *su(f)* mutants. We have determined the molecular lesions of *su(f)* alleles that show interallelic complementation and have no evidence that any of these mutations affect regulatory sequences. All our results suggest that the interallelic complementation at *su(f)* results from complementation at the protein level. Three lethal alleles, $su(f)^{MS252}$, $su(f)^{MS97}$ and $su(f)^{S1}$, which do not complement any other *su(f)* allele, have insertions in the 5′ end of the gene and could only encode small polypeptides (40–220 residues). In contrast, all *su(f)* alleles that fall into groups that do exhibit some degree of complementation are potentially able to encode proteins close to the wild-type 84-kD Su(f) protein. Three of them, $su(f)^{madts}$, $su(f)^{ts726}$ and $su(f)^{R\text{-}9\text{-}18}$, are point mutants that encode Su(f) proteins altered at only one amino acid. $su(f)^{S2}$, $su(f)^{X1}$ and $su(f)^{3DES}$ complement these three *su(f)* point mutants and have lesions that affect the last exon of the gene. Proteins encoded by these alleles would lack only the C-terminal 65 amino acids [in $su(f)^{S2}$], 10 amino acids [in $su(f)^{X1}$] and 82 amino acids [in $su(f)^{3DES}$] of the 84-kD Su(f) protein. Finally, it is possible that in $su(f)^{X2}$, which is able to complement $su(f)^{R\text{-}9\text{-}18}$ at 25° only, the deletion might result in production of a protein corresponding to the C-terminal two-thirds of the 84-kD Su(f) protein. Complementation for viability occurs between lethal *su(f)* alleles able to encode mutant Su(f) proteins altered in different regions.

Several models can be proposed for complementation between Su(f) protein molecules. In a first model, *su(f)* would encode a single polypeptide with several independent functions. Complementation would occur between alleles that affect different functions. Such complementation has been described for two genes in Drosophila, the *rudimentary* (FREUND *et al.* 1986) and the *Gart* loci (HENIKOFF 1986; HENIKOFF *et al.* 1986), which encode several enzyme activities involved in pyrimidine and in purine biosynthesis respectively. In a second model, interallelic complementation supposes

protein-protein interaction between several monomers of the same protein. Complementation may result from the production of a partly functional hybrid complex formed from different mutant derivatives of the same protein (CRICK and ORGEL 1964). This phenomenon has been demonstrated for some loci in Drosophila (GELBART *et al.* 1976; PELLICIA and COUPER 1984).

Although the first model cannot be ruled out, we favor the second one. When interallelic complementation results from different mutant proteins altered in different independent functions, a fully wild-type function can be restored. In contrast, when interallelic complementation results from protein-protein interaction, the hybrid complex formed from different mutant monomers is usually less than fully active (PELLICIA and COUPER 1984). This is precisely what occurs at *su(f)*. For each complementing combination of lethal alleles of *su(f)*, there is sufficient activity for viability, but flies of such combinations that are homozygous for f^1 always present a suppressed bristle phenotype, diagnostic of reduced *su(f)* activity. Moreover, the hypothesis of an interaction between mutant Su(f) proteins is reinforced by the fact that complementing combinations involve temperature-sensitive mutants that may encode Su(f) proteins with altered conformations. In these combinations, heterodimeric Su(f) complexes could be partly functional because their conformation would be more compatible with activity than that of homodimeric temperature-sensitive mutant complexes.

In this hypothesis, the allele $su(f)^{X2}$ is informative since it is potentially able to encode a protein corresponding to the C-terminal two-thirds of the wild-type Su(f) 84-kD protein. As it complements the point mutant $su(f)^{R\text{-}9\text{-}18}$ at 25° to produce a viable combination, this suggests that the most N-terminal region of the protein (residues 1–277) is dispensable, at least for one molecule, in the interaction between Su(f) proteins.

Role of Su(f) protein: The Su(f) protein has been shown to be homologous to a human protein involved in mRNA 3′ end formation. In higher eukaryotes, this process requires several complexes, one of which has been called cleavage stimulation factor (CstF) (for reviews, see WAHLE and KELLER 1992; KELLER 1995; MANLEY 1995). CstF has a role in the cleavage of pre-mRNA, but is dispensable *in vitro* for the addition of poly(A) to the newly generated 3′ end. In HeLa cells, CstF has subunits of 77, 64 and 50 kD (TAKAGAKI *et al.* 1990). The 64-kD (TAKAGAKI *et al.* 1991) and 50-kD (TAKAGAKI and MANLEY 1992) proteins have been shown to contain, respectively, a RNP-type RNA binding domain, which binds pre-mRNA, and transducin repeats, which could be involved in protein-protein or in protein-nucleic acid interactions (DURONIO *et al.* 1992). The 77-kD subunit of CstF makes contact with the 64- and 50-kD subunits (TAKAGAKI and MANLEY 1994). The 77- and the 84-kD Su(f) proteins are homologous over their entire length and the degree of homology, 56.2% identity and 69.4% similarity (TAKAGAKI and MANLEY 1994), suggests functional conservation. Su(f) could thus be part of a complex homologous to human CstF and indeed, a homologue of the 64-kD subunit of human CstF has been cloned from Drosophila (TAKAGAKI and MANLEY 1994).

In addition, a role for *su(f)* in mRNA 3′ end formation is in good agreement with the phenotypes of viable *su(f)* alleles. The suppression of the f^1 phenotype in f^1 $su(f)^1$ individuals is correlated with an increase in the level of wild-type spliced *f* transcripts that could result from reduced polyadenylation within *gypsy* (ISHIMARU and SAIGO 1993). A similar effect of *su(f)* on the balance between truncated mRNA polyadenylated within *gypsy* and read-through RNA has been reported in another study where *gypsy* is inserted in an intron of the *hsp82* gene (DORSETT *et al.* 1989).

Our study indicates that mutant Su(f) proteins can act in combination to provide partly functional Su(f) activity. They may do this by interacting with each other, which would suggest an interaction between wild-type Su(f) proteins. It is possible that for some aspect of wild-type *su(f)* function, at least two Su(f) molecules must interact. CstF purifies as a heterotrimeric complex containing a single 77-kD polypeptide (TAKAGAKI *et al.* 1990). However, formation of higher forms *in vivo*, perhaps for assembly in the cytoplasm or translocation into the nucleus, cannot be excluded.

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LITERATURE CITED

BABU, P., and S. G. BHAT, 1980 Effect of *zeste* on *white* complementation, pp. 35–44 in *Development and Neurobiology of Drosophila*, edited by O. SIDDIQI, P. BABU, L. M. HALL and J. C. HALL. Plenum Press, New York.

BAKER, B. S., 1989 Sex in flies: the splice of life. Nature **340:** 521–524.

CRICK, F. H. C., and L. E. ORGEL, 1964 The theory on inter-allelic complementation. J. Mol. Biol. **8:** 161–165.

DORSETT, D., G. A. VIGLIANTI, B. J. RUTLEDGE and M. MESELSON, 1989 Alteration of the *hsp82* gene expression by the gypsy transposon and suppressor genes in *Drosophila melanogaster*. Genes Dev. **3:** 454–468.

DRIVER, A., S. F. LACEY, T. E. CULLINGFORD, A. MITCHELSON and K. O'HARE, 1989 Structural analysis of Doc transposable elements inserted in *white* and *suppressor of forked* loci of *Drosophila melanogaster*. Mol. Gen. Genet. **220:** 49–52.

DUDICK, M. E., T. R. F. WRIGHT and L.-L. BROTHERS, 1974 The developmental genetics of the temperature-sensitive lethal allele of the *suppressor-of-forked*, $l(1)su(f)^{ts67g}$, in *Drosophila melanogaster*. Genetics **76:** 487–510.

DURONIO, R. J., J. I. GORDON and M. S. BOGUSKI, 1992 Comparative analysis of the β Transducin family with identification of several new members including *PWP1*, a nonessential gene of *Saccharomyces cerevisiae* that is divergently transcribed from *NMT1*. Proteins **13:** 41–56.

FAWCETT, D. H., C. K. LISTER, E. KELLET and D. J. FINNEGAN, 1986 Transposable elements controlling I-R hybrid dysgenesis in *D. melanogaster* are similar to *mammalian* LINEs. Cell **47:** 1007–1015.

FREUND, J. N., W. VERGIS, P. SCHEDL and B. P. JARRY, 1986 Molecular organization of the *rudimentary* gene of *Drosophila melanogaster*. J. Mol. Biol. **189:** 25–36.

FRIDELL, R. A., and L. L. SEARLES, 1994 Evidence for a role of the *Drosophila melanogaster suppressor of sable* gene in the pre-mRNA splicing pathway. Mol. Cell. Biol. **14:** 859–867.

FRIDELL, R. A., A.-M. PRET and L. L. SEARLES, 1990 A retrotransposon 412 insertion within an exon of the *Drosophila melanogaster vermilion* gene is spliced from the precursor RNA. Genes Dev. **4:** 559–565.

GELBART, W. M., and C.-T. WU, 1982 Interactions of zeste mutations with loci exhibiting transvection effects in *Drosophila melanogaster*. Genetics **102:** 179–189.

GELBART, W. M., M. MCCARRON and A. CHOVNICK, 1976 Extension of the limits of the XDH structural element in *Drosophila melanogaster*. Genetics **84:** 211–232.

GEYER, P. K., M. M. GREEN and V. G. CORCES, 1990 Tissue-specific transcriptional enhancers may act in *trans* on the gene located in the homologous chromosome: the molecular basis of transvection in *Drosophila*. EMBO J. **9:** 2247–2256.

GEYER, P. K., A. J. CHIEN, V. G. CORCES and M. M. GREEN, 1991 Mutations in the *su(s)* gene affect RNA processing in *Drosophila melanogaster*. Proc. Natl. Acad. Sci. USA **88:** 7116–7120.

HENIKOFF, S., 1986 The *Saccharomyces cerevisiae ADE5,7* protein is homologous to overlapping *Drosophila melanogaster Gart* polypeptides. J. Mol. Biol. **190:** 519–528.

HENIKOFF, S., M. A. KEENE, J. S. SLOAN, J. BLESKAN, R. HARDS *et al.*, 1986 Multiple purine pathway enzyme activities are encoded at a single genetic locus in *Drosophila*. Proc. Natl. Acad. Sci. USA **83:** 720–724.

HODGKIN, J., 1989 Drosophila sex determination: A cascade of regulated splicing. Cell **56:** 905–906.

HOOVER, K. H., A. J. CHIEN and V. G. CORCES, 1993 Effects of transposable elements on the expression of the *forked* gene of *Drosophila melanogaster*. Genetics **135:** 507–526.

ISHIMARU, S., and K. SAIGO, 1993 The *Drosophila forked* gene encodes two major RNAs, which, in *gypsy* or *springer* insertion mutants, are partially or completely truncated within the 5′-LTR of the inserted retrotransposon. Mol. Gen. Genet. **241:** 647–656.

JACK, J. W., and B. H. JUDD, 1979 Allelic pairing and gene regulation: a model for the zeste-white interaction in *Drosophila melanogaster*. Proc. Natl. Acad. Sci. USA **76:** 1368–1372.

JÜRGENS, G., and E. GATEFF, 1979 Pattern specification in imaginal discs of *Drosophila melanogaster*. Wilhem Roux's Arch. Dev. Biol. **186:** 1–25.

KELLER, W., 1995 No end yet to messenger RNA 3′ processing! Cell **81:** 829–832.

KLEMENZ, R., U. WEBER and W. J. GEHRING, 1987 The *white* gene as a marker in a new P-element vector for gene transfer in *Drosophila*. Nucleic Acids Res. **15:** 3947–3959.

LANGLEY, C. H., J. MACDONALD, N. MIYASHITA and M. AGUADÉ, 1993 Lack of correlation between interspecific divergence and intraspecific polymorphism at the suppressor of forked region in *Drosophila melanogaster* and *Drosophila simulans*. Proc.Natl. Acad. Sci. USA **90:** 1800–1803.

LEISERSON, W. M., N. M. BONINI and S. BENZER, 1994 Transvection at the *eyes absent* gene of Drosophila. Genetics **138:** 1171–1179.

LEVIS, R., K. O'HARE and G. M. RUBIN, 1984 Effects of transposable element insertions on RNA encoded by the *white* gene of *Drosophila*. Cell **38:** 471–481.

LIFSCHYTZ, E., and R. FALK, 1969 Fine structure analysis of a chromosome segment in *Drosophila melanogaster*. Analysis of ethyl methanesulphonate-induced lethals. Mutat. Res. **8:** 147–155.

LINDSLEY, D. L., and G. G. ZIMM, 1992 *The genome of Drosophila melanogaster*. Academic Press, San Diego, CA.

MANLEY, J. L., 1995 A complex protein assembly catalyzes polyadenylation of mRNA precursors. Curr. Opin. Genet. Dev. **5:** 222–228.

MINVIELLE-SEBASTIA, L., P. J. PREKER and W. KELLER, 1994 RNA14 and RNA15 proteins as components of a yeast pre-mRNA 3′-end processing factor. Science **266:** 1702–1705.

MITCHELSON, A., M. SIMONELIG, C. WILLIAMS and K. O'HARE, 1993 Homology with *S. cerevisiae* RNA 14 suggests that phenotypic suppression in *Drosophila* by *suppressor of forked* occurs at the level of RNA stability. Genes Dev. **7:** 241–249.

MOUNT, S. M., 1982 A catalogue of splice junction sequences. Nucleic Acids Res. **10:** 459–472.

PELLICIA, J. G., and D. G. COUPER, 1984 Intra-genic complementation at the Adh locus. Dros. Inf. Ser. **60:** 160–162.

PERRIMON, N., D. SMOUSE and G. L. G. MIKLOS, 1989 Developmental genetics of loci at the base of the X chromosome of *Drosophila melanogaster*. Genetics **121:** 313–331.

ROBERTSON, H. M., C. R. PRESTON, R. W. PHILLIS, D. M. JOHNSON-SCHLITZ, W. K. BENZ *et al.*, 1988 A stable genomic source of *P* element transposase in *Drosophila melanogaster*. Genetics **118:** 461–470.

RUBIN, G. M., and A. C. SPRADLING, 1982 Genetic transformation of *Drosophila* with transposable element vectors. Science **218:** 348–353.

RUSSEL, M. A., 1974 Pattern formation in the imaginal discs of a temperature-sensitive cell-lethal mutant of *Drosophila melanogaster*. Dev. Biol. **40:** 24–39.

RUTLEDGE, B. J., M. A. MORTIN, E. SCHWARZ, D. THIERRY-MIEG and M. MESELSON, 1988 Genetic interactions of modifier genes and modifiable alleles in *D. melanogaster*. Genetics **119:** 391–397.

SAMBROOK, J., E. F. FRITSCH and T. MANIATIS, 1989 *Molecular Cloning: A Laboratory Manual.* Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY.

SANGER, F., S. NICKLER and A. R. COULSON, 1977 DNA sequencing with chain terminating inhibitors. Proc. Natl. Acad. Sci. USA **74:** 5463–5476.

SCHALET, A., 1972 Report of A. Schalet. Dros. Inf. Ser. **49:** 37.

SCHALET, A., and G. J. LEFEVRE, 1973 The localisation of "ordinary" sex-linked genes in Section 20 of the polytene X chromosome of *Drosophila melanogaster*. Chromosoma **44:** 183–202.

SMITH, P. A., and V. G. CORCES, 1991 *Drosophila* transposable elements: mechanisms of mutagenesis and interactions with the host genome. Adv. Genet. **29:** 229–301.

STEINMANN-ZWICKY, M., H. AMREIN and R. NÖTHIGER, 1990 Genetic control of sex determination in *Drosophila*. Adv. Genet. **27:** 189–230.

TAKAGAKI, Y., and J. L. MANLEY, 1992 A human polyadenylation factor is a G-protein β subunit homologue. J. Biol. Chem. **267:** 23471–23474.

TAKAGAKI, Y., and J. L. MANLEY, 1994 A polyadenylation factor subunit is the human homologue of the *Drosophila* suppressor of forked protein. Nature **372:** 471–474.

TAKAGAKI, Y., J. L. MANLEY, C. C. MACDONALD, J. WILUSZ and T. SHENK 1990 A multisubunit factor, CstF, is required for polyadenylation of mammalian pre-mRNAs. Genes Dev. **4:** 2112–2120.

TAKAGAKI, Y., C. C. MACDONALD, T. SHENK and J. L. MANLEY, 1991 The 64kD polyadenylation factor contains an RNP-type RNA binding domain and unusual auxilliary motifs. Proc. Natl. Acad. Sci. USA **89:** 1403–1407.

VASLET, C. A., P. O'CONNEL, M. IZQUIERDO and M. ROSBACH, 1980 Isolation and mapping of a cloned ribosomal protein gene of *Drosophila melanogaster*. Nature **285:** 674–676.

WAHLE, E., and W. KELLER, 1992 The biochemistry of 3′ end cleavage and polyadenylation of mRNA precursors. Annu. Rev. Biochem. **61:** 419–440.

WHITTINGHILL, M., 1937 Report of M. Whittinghill. Dros. Inf. Ser. **8:** 11–13.

WILSON, T. G., 1980 Studies on the female-sterile phenotype of *l(1)su(f)ts76a*, a temperature-sensitive allele of the *suppressor-of-forked* mutation in *Drosophila melanogaster*. J. Embryol. Exp. Morphol. **55:** 247–256.

ZACHAR, Z., T.-B. CHOU and P. M. BINGHAM, 1987 Evidence that a regulatory gene autoregulates its own splicing. EMBO J. **6:** 4105–4111.

ZACHAR, Z., T.-B. CHOU, J. KRAMER, I. P. MIMS and P. M. BINGHAM, 1994 Analysis of autoregulation at the level of pre-mRNA splicing of the *suppressor-of-white-apricot* gene in Drosophila. Genetics **137:** 139–150.

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