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Tissue-specific autoregulation of *Drosophila suppressor of forked* by alternative poly(A) site utilization leads to accumulation of the suppressor of forked protein in mitotically active cells

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ABSTRACT

The Suppressor of forked protein is the *Drosophila* homolog of the 77K subunit of human cleavage stimulation factor, a complex required for the first step of the mRNA 3′-end-processing reaction. We have shown previously that wild-type *su(f)* function is required for the accumulation of a truncated *su(f)* transcript polyadenylated in intron 4 of the gene. This led us to propose a model in which the Su(f) protein would negatively regulate its own accumulation by stimulating 3′-end formation of this truncated *su(f)* RNA. In this article, we demonstrate this model and show that *su(f)* autoregulation is tissue specific. The Su(f) protein accumulates at a high level in dividing tissues, but not in nondividing tissues. We show that this distribution of the Su(f) protein results from stimulation by Su(f) of the tissue-specific utilization of the *su(f)* intronic poly(A) site, leading to the accumulation of the truncated *su(f)* transcript in nondividing tissues. Utilization of this intronic poly(A) site is affected in a *su(f)* mutant and restored in the mutant with a transgene encoding wild-type Su(f) protein. These data provide an in vivo example of cell-type-specific regulation of a protein level by poly(A) site choice, and confirm the role of Su(f) in regulation of poly(A) site utilization.

Keywords: autoregulation; cleavage stimulation factor; *Drosophila*; mitotic cells; mRNA 3′-end processing

INTRODUCTION

A poly(A) tail is found at the 3′ end of virtually all eukaryotic mRNAs, produced by the mRNA 3′-end-processing reaction. Over the past few years, it has become clear that this reaction can be regulated at various levels, leading to regulation of gene expression (reviewed in Proudfoot, 1996; Colgan & Manley, 1997; Barabino & Keller, 1999; Zhao et al., 1999).

The mRNA 3′-end-processing reaction occurs in two tightly coupled steps: the cleavage of the pre-mRNA at the poly(A) site, followed by the addition of a poly(A) tail to the newly generated 3′ end (reviewed in Wahle & Rüegsegger, 1999; Zhao et al., 1999). In mammals, the poly(A) site is located between the upstream consensus poly(A) signal AAUAAA and a downstream variable GU- or U-rich sequence. These sequences interact with multiprotein complexes: the cleavage and polyadenylation specificity factor (CPSF) and the cleavage stimulation factor (CstF), respectively. CPSF consists of four subunits and binds the AAUAAA through its 160-kDa (160K) and its 30-kDa (30K) subunits, whereas CstF, which consists of three subunits, 77 kDa (77K), 64 kDa (64K), and 50 kDa (50K), binds the GU-rich downstream motif via its 64K subunit. CPSF and CstF bind cooperatively to RNA, promoting the formation of a cleavage complex on the pre-mRNA, which also contains the two cleavage factors CFIm and CFIIm, and the poly(A) polymerase (PAP). Cooperativity of RNA binding by CPSF-CstF results, at least in part, from an interaction between the 160K subunit of CPSF and the 77K subunit of CstF (Murthy & Manley, 1995). The 77K protein also bridges the other two subunits (64K and 50K) of CstF (Takagaki & Manley, 1994), it interacts with itself (Simonelig et al., 1996; Takagaki & Manley, 2000), and it binds to the CTD of RNA polymerase II (McCracken et al., 1997), thus contributing to the cou-

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pling between transcription and 3′-end processing (reviewed in Bentley, 1999).

CstF is thought to play an important role in the regulation of poly(A) site selection. Indeed, poly(A) site efficiency is defined in vitro by the stability of the cleavage complex on the pre-mRNA which, in turn, depends on affinity of CstF for the downstream variable GU-rich elements (Weiss et al., 1991). Consistent with this, several studies have correlated shifts in the choice of poly(A) sites with variations in the level or activity of the 64K subunit of CstF (Mann et al., 1993; Edwalds-Gilbert & Milcarek, 1995; Takagaki et al., 1996; Takagaki & Manley, 1998).

The *suppressor of forked* (*su(f)*) gene of *Drosophila melanogaster* encodes a homolog of the 77K protein of human CstF (Takagaki & Manley, 1994). The two proteins are 56% identical and 69% similar, and homology extends over their entire lengths. The Su(f) protein also shares homology with the yeast RNA14 protein (26% identity and 47% similarity) (Mitchelson et al., 1993), a subunit of yeast cleavage factor I A (CFIA), which is required for the cleavage and polyadenylation reactions in yeast (Minvielle-Sebastia et al., 1994, 1997). These homologies suggest a role for the Su(f) protein in mRNA 3′-end formation. Such a role for *su(f)* is consistent with previous genetic and molecular data that indicate that mutations in *su(f)* affect the utilization of poly(A) sites in genes with inserted transposable elements (reviewed in O'Hare, 1995). We have shown that mutations in *su(f)* also affect the utilization of one of the poly(A) sites within *su(f)* itself (Audibert & Simonelig, 1998). The *su(f)* gene produces three polyadenylated RNAs that differ in the location of their poly(A) sites. Two full-length transcripts of 2.6 kb and 2.9 kb encode the Su(f) protein. A third RNA of 1.3 kb is polyadenylated within intron 4 (Mitchelson et al., 1993). This truncated RNA has no in-frame stop codon, is dispensable for *su(f)* function (Simonelig et al., 1996), and the sequence encoded by intron 4 in this RNA is not conserved in *Drosophila virilis* (Audibert & Simonelig, 1998). This suggests that this RNA could remain untranslated. In addition, accumulation of this truncated RNA requires the wild-type Su(f) protein (Audibert & Simonelig, 1998). These data led us to propose that the 1.3-kb RNA is a by-product of a negative autoregulatory loop in which the Su(f) protein regulates its own level by stimulating 3′-end formation of a truncated transcript. We have also shown that the Su(f) protein does not accumulate at the same levels in all tissues. The protein is detected in mitotically active cells, but not in nondividing cells, at various stages of *Drosophila* development (Audibert et al., 1998). In this article we validate the model of *su(f)* autoregulation by showing that an increased amount of the truncated 1.3-kb *su(f)* RNA correlates with a decreased amount of full-length coding *su(f)* RNAs and with a lack of Su(f) protein accumulation. We also show that this autoregulation is tissue specific and that the accumulation pattern of the Su(f) protein results from this tissue-specific autoregulation.

RESULTS

Alteration of the Su(f) protein accumulation pattern in *su(f)* mutants

We have shown previously that the poly(A) site in intron 4 of *su(f)*, the utilization of which results in the production of a *su(f)*-truncated 1.3-kb transcript, is particularly sensitive to *su(f)* activity. The amount of this truncated RNA strongly decreases in temperature-sensitive *su(f)* mutants at the restrictive temperature, compared to wild type (Audibert & Simonelig, 1998). We have proposed that the Su(f) protein stimulates 3′-end formation of this truncated 1.3-kb RNA, which would be produced at the expense of the full-length *su(f)* mRNAs to down regulate the amount of the Su(f) protein. As a first step in confirming this model, we determined whether the accumulation pattern of the Su(f) protein is altered when *su(f)* function is affected. We used the $su(f)^{ts67g}$ mutant, a temperature-sensitive lethal allele that encodes a protein with a single substitution in the N-terminal region encoded by exon 3 (K. Elliott, C. Williams, K. O'Hare, and M. Simonelig, unpubl. data). $su(f)^{ts67g}$ is phenotypically wild type at 18 °C, it survives and shows a *su(f)* phenotype at 25 °C and 27 °C as it suppresses the $forked^{1}$ phenotype, and it is lethal at the third instar larval stage with larvae showing very small imaginal discs and an atrophied brain when raised at 29 °C (Dudick et al., 1974; Audibert & Simonelig, 1999).

In the wild type, the Su(f) protein accumulates in mitotically active cells in ovaries and in larvae (Audibert et al., 1998). Immunostaining of third instar larvae with an anti-Su(f) antibody shows that the Su(f) protein is detected ubiquitously in imaginal discs (e.g., wing disc, Fig. 1A), except in the eye disc, where the protein is present anteriorly to the morphogenetic furrow and up to the second wave of mitoses, where cells are dividing (Audibert et al., 1998) (Fig. 1C). The Su(f) protein is not detected in the part of the eye disc posterior to the morphogenetic furrow that contains postmitotic differentiated cells (Audibert et al., 1998) (Fig. 1C), and in Malpighian tubules that are mitotically inactive at this stage (Skaer, 1989) (Fig. 1E). We analyzed the accumulation of the Su(f) protein in the $su(f)^{ts67g}$ mutant at the restrictive temperature of 27 °C. Immunostaining of $su(f)^{ts67g}$ mutant larvae raised at 27 °C revealed that the Su(f) protein is present ubiquitously, both in dividing and nondividing tissues (Fig. 1B,D,F). These data demonstrate that the presence of the wild-type Su(f) protein represses its own accumulation in mitotically inactive tissues and are consistent with the proposed model of *su(f)* autoregulation. In addition, they reveal

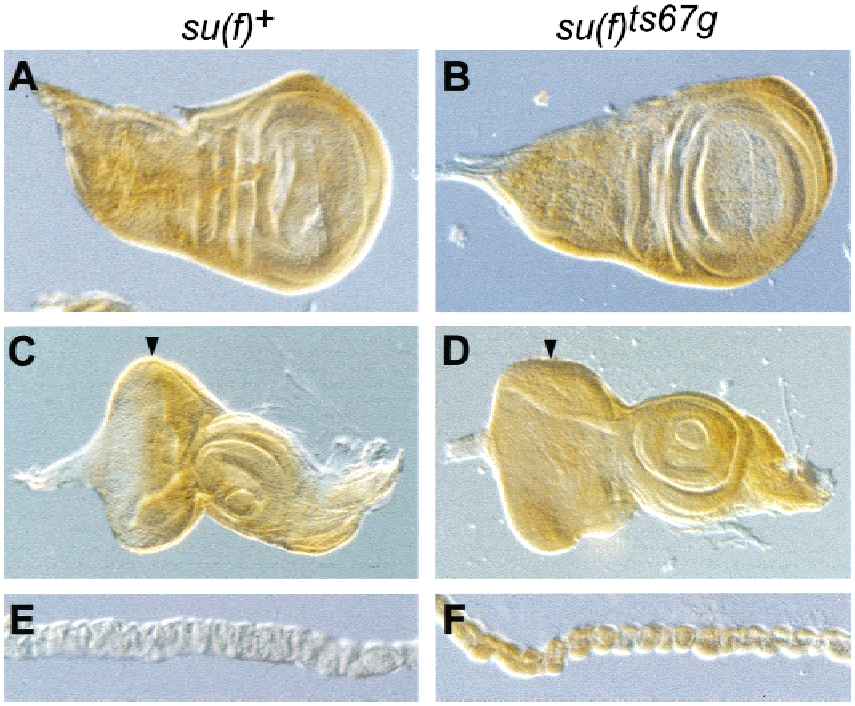
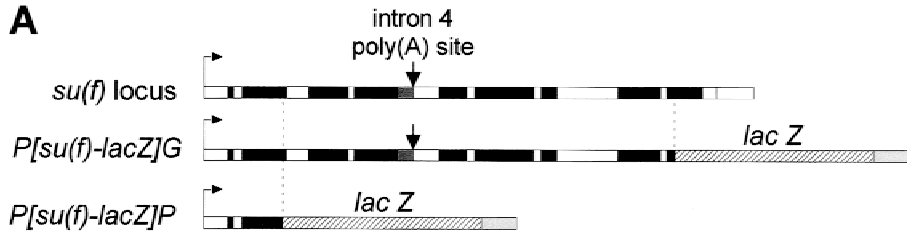


FIGURE 1. Detection of the Su(f) protein in third instar larvae, in the wild type and in the *su(f)*^{ts67g} mutant. Staining was with the purified anti-Su(f) antibody (Audibert et al., 1998). **A**,**B**: Wing imaginal discs; **C**,**D:** eye-antennal imaginal discs; **E**,**F:** Malpighian tubules. **A**,**C**,**E:** Wild-type larvae raised at 27 °C; **B**,**D**,**F:** *su(f)*^{ts67g} larvae raised at 27 °C. In **C** and **D** posterior of the discs is to the left; arrowheads indicate the morphogenetic furrow; accumulation of the Su(f) protein in wild type **C** is observed up to the second wave of mitoses, a few rows of cells posterior to the morphogenetic furrow (Audibert et al., 1998).



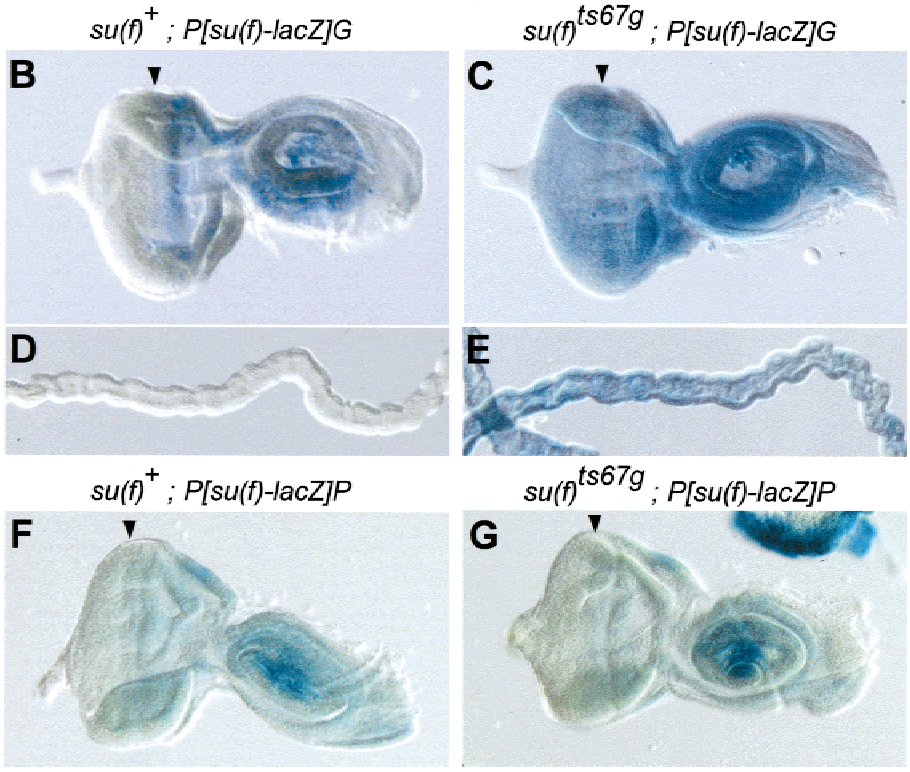


FIGURE 2. Expression pattern of *P[su(f)-lacZ]G* and of *P[su(f)-lacZ]P* in the wild type and in *su(f)*^{ts67g} third instar larvae. **A:** The *su(f)* locus is depicted. Black boxes are coding sequences, open boxes are noncoding sequences. The gray box is the part of intron 4 incorporated in the truncated 1.3-kb *su(f)* RNA. In *P[su(f)-lacZ]G*, the *lacZ* gene is fused in frame to *su(f)* exon 9; in *P[su(f)-lacZ]P*, the *lacZ* gene is fused in frame to *su(f)* exon 2. The pale gray box in both transgenes is the SV40 small t 3′ UTR. **B**–**G**: X-Gal staining of third instar eye-antennal imaginal discs and Malpighian tubules. **B**,**C**,**F**,**G:** eye-antennal discs. Posterior is to the left. Arrowheads indicate the morphogenetic furrow. **D**,**E:** Malpighian tubules. Wild type (**B**,**D**,**F**) and *su(f)*^{ts67g} (**C**,**E**,**G**) larvae were raised at 27 °C.

that this autoregulation is tissue specific, and therefore suggest a tissue-specific utilization of the *su(f)* intronic poly(A) site.

Control of the Su(f) accumulation pattern by *su(f)* requires *su(f)* introns

To ask whether the repression of Su(f) accumulation in nondividing tissues results from the formation in these tissues of a *su(f)* transcript truncated in intron 4, we analyzed the effect of truncating *su(f)* transcripts upstream of intron 4. We first made use of a *su(f)-lacZ* reporter, *P[su(f)-lacZ]G*, in which the *lacZ* gene of *Escherichia coli* is fused in frame in the last exon of *su(f)* (Audibert et al., 1998) (Fig. 2A). This reporter contains all introns of *su(f)*. It does not rescue the $su(f)^{ts67g}$ mutant; therefore, it allows us to uncouple the study of *su(f)* expression from that of regulation by *su(f).* In the wild type, the Su(f)-β-galactosidase fusion protein encoded by *P[su(f)-lacZ]G* accumulates as the Su(f) protein in mitotic tissues and not in nondividing tissues (Audibert et al., 1998) (Fig. 2B,D). In $su(f)^{ts67g}$ mutant larvae raised at the restrictive temperature, the Su(f)-β-galactosidase protein was detected both in dividing and in nondividing tissues (Fig. 2C,E). The same result was obtained using the viable allele $su(f)^{1}$ (data not shown). We next constructed another *su(f)-lacZ* transgene in which the *lacZ* gene is fused in frame in exon 2 of *su(f)* (Fig. 2A). This *P[su(f)-lacZ]P* construct has the same 5′ sequence upstream of the *su(f)* coding region as *P[su(f)-lacZ]G*, but it lacks *su(f)* sequences downstream of exon 2. We used three independent transformant lines and found that the expression level of *P[su(f)-lacZ]P* is extremely low. We analyzed its expression pattern in the eye-antennal disc of third instar larvae where a weak expression can be detected. The Su(f)-β-galactosidase protein encoded by *P[su(f)-lacZ]P* is detected at a low level in the anterior part of the eye disc (Fig. 2F). This suggests a transcriptional tissue-specific regulation of *P[su(f)-lacZ]P* expression. However, the very low overall level of expression of *P[su(f)-lacZ]P* indicates that the contribution of such transcriptional regulation to *su(f)* endogenous expression is likely to be minimal. This transcriptional regulation does not depend on *su(f)* function, as the expression pattern of *P[su(f)-lacZ]P* is not altered in $su(f)^{ts67g}$ mutant larvae (Fig. 2G). In contrast, the expression level of the transgene *P[su(f)-lacZ]G,* which contains all *su(f)* introns, is higher, and the lack of accumulation of the fusion protein encoded by this transgene in nondividing tissues is dependent on *su(f)* function. This indicates that regulation by *su(f)* activity requires *su(f)* introns and is in agreement with a model predicting that, in nondividing tissues, the Su(f) protein promotes the utilization of a poly(A) site in *su(f)* intron 4, thus preventing the accumulation of the Su(f) protein at a high level.

Accumulation of the 1.3-kb truncated RNA in specific tissues correlates with the lack of Su(f) protein

We directly quantified the different species of *su(f)* transcripts in dividing and nondividing tissues using reverse transcription-PCR (RT-PCR). Two sets of oligonucleotides were used: set 1 allows the amplification of a fragment specific to the 1.3-kb transcript spanning exons 3 and 4, and set 2 allows the amplification of a fragment specific to the full-length 2.6-kb and 2.9-kb mRNAs, which spans exons 8 and 9 (Fig. 3A). Wing imaginal discs and Malpighian tubules of third instar larvae were used as dividing and nondividing tissues, respectively. Total RNA was prepared from both tissues, reverse transcribed with a $(dT)_{20}$ primer, and PCR amplified with both sets of primers in the same reaction. Aliquots of amplified DNA were taken during the PCR reaction at every second cycle to follow the exponential accumulation of the amplified fragments and to ensure that the reaction was not saturated. Amplified DNA fragments were visualized by Southern hybridization, quantified by PhosphorImager and the ratios of the 1.3-kb truncated RNA to the full-length 2.6-kb and 2.9-kb RNAs were calculated. An example of the experiment is shown in Figure 3B, and the quantification is in Figure 3C. In wild-type Malpighian tubules, where the Su(f) protein does not accumulate (Fig. 1E), the amount of the 1.3-kb truncated RNA is similar to that of the full-length RNAs (Fig. 3B, lane 1) (ratio 1.3 kb/(2.6 kb + 2.9 kb) ~ 1; Fig. 3C). In contrast, in wild-type wing discs where the Su(f) protein accumulates (Fig. 1A), this truncated RNA is about five times less abundant than the full-length RNAs (Fig. 3B, lane 2) (ratio 1.3 kb/(2.6 kb + 2.9 kb) ~ 0.2; Fig. 3C). In $su(f)^{ts67g}$ mutant larvae at 27 °C, the large amount of the 1.3-kb truncated RNA in Malpighian tubules is not maintained. This amount is similar in Malpighian tubules and in wing discs, and it is again about five times weaker than the amount of the full-length transcripts (Fig. 3B, lanes 3 and 4) (ratio 1.3 kb/(2.6 kb + 2.9 kb) ~ 0.2; Fig. 3C). This decrease of the 1.3-kb RNA amount in Malpighian tubules correlates with the accumulation of the Su(f) protein in this tissue in $su(f)^{ts67g}$ larvae (Fig. 1F). In $su(f)^{ts67g}$ mutant larvae raised at 27 °C and shifted for 24 h to 29 °C, the amount of the 1.3-kb RNA decreases again by a factor of 2 compared to that obtained in $su(f)^{ts67g}$ larvae at 27 °C, both in Malpighian tubules and in wing discs (Fig. 3B, lanes 5 and 6) (ratio 1.3 kb/(2.6 kb + 2.9 kb) ~ 0. 1; Fig. 3C). These results show that the 1.3-kb truncated RNA accumulates in nondividing tissues where the Su(f) protein is not detected. This accumulation of the 1.3-kb RNA requires *su(f)* wild-type function. This is in agreement with a tissue-specific autoregulation of *su(f).* In nondividing tissues,

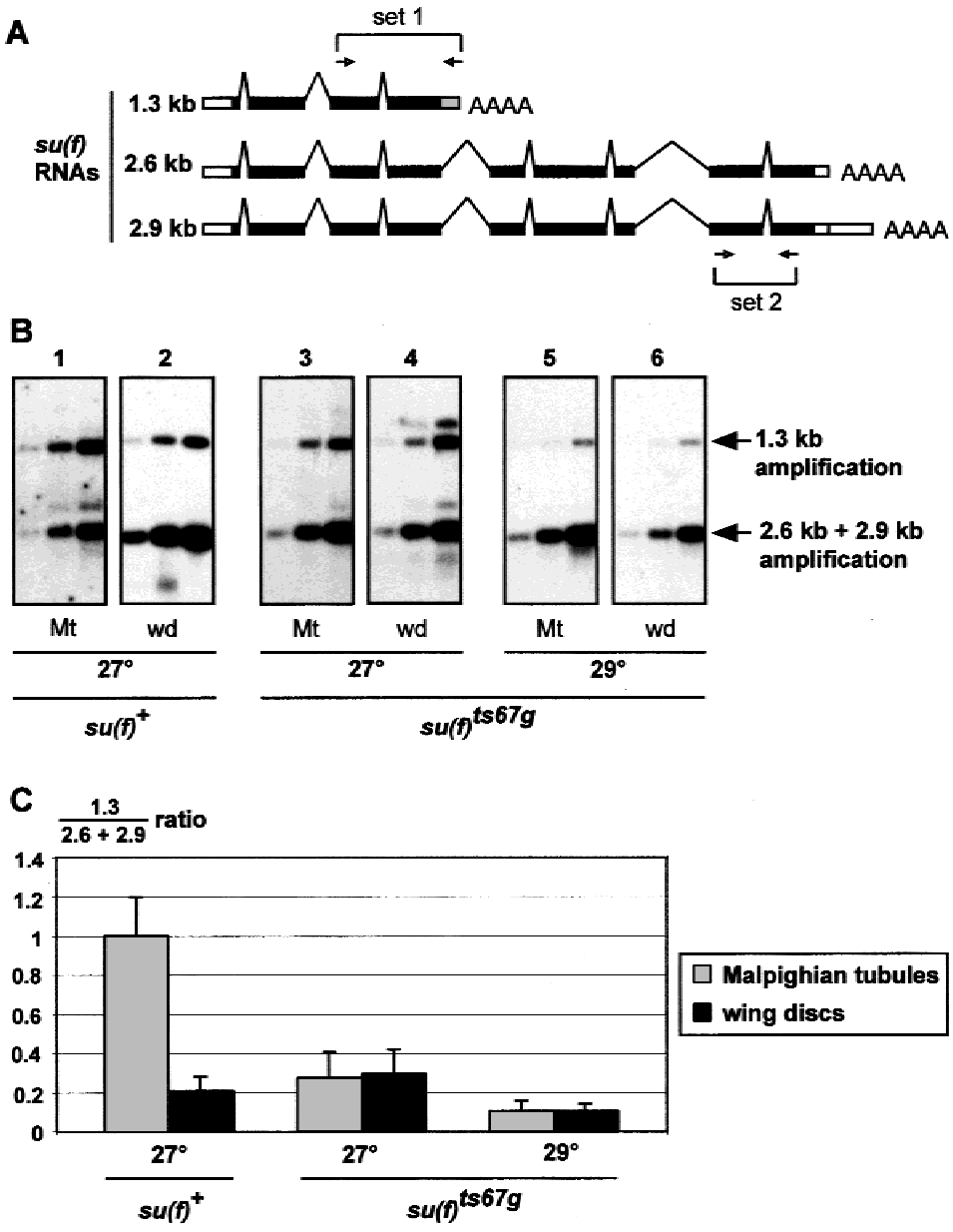


FIGURE 3. Quantification of the truncated and of the full-length *su(f)* RNAs in dividing and nondividing tissues by RT-PCR. **A**: The *su(f)* RNAs are depicted. Black boxes are coding sequences, open boxes are noncoding sequences. The gray box is the part of intron 4 incorporated in the truncated *su(f)* RNA. Arrows indicate the position of the primers. **B**: Southern blots of RT-PCR from Malpighian tubules (Mt) and wing imaginal discs (wd) of third instar larvae, hybridized with a full-length *su(f)* cDNA. Three consecutive samples of the same PCR are shown. Lanes 1 and 2: wild-type larvae were raised at 27 °C. Lanes 3 and 4: $su(f)^{ts67g}$ larvae were raised at 27 °C. Lanes 5 and 6: $su(f)^{ts67g}$ larvae were raised at 27 °C and shifted to 29 °C for 24 h. **C**: Quantification of RT-PCR was performed by using PhosphorImager scanning (Molecular Dynamics) and the ImageQuant program (Molecular Dynamics). The mean value of the 1.3 kb/(2.6 kb + 2.9 kb) ratio was calculated from four samples of two to four independent experiments. Error bars designate ± standard error.

the Su(f) protein appears to be involved in efficient utilization of the *su(f)* intronic poly(A) site, leading to the formation of a truncated RNA and to a low amount of the Su(f) protein. This *su(f)* autoregulation is less efficient in dividing tissues where the amount of the truncated *su(f)* RNA is low and the Su(f) protein accumulates.

Ubiquitous expression of wild-type Su(f) protein restores tissue-specific *su(f)* regulation by utilization of the intronic poly(A) site

As a first step in understanding the tissue specificity of *su(f)* autoregulation, we asked whether the tissue specificity would be maintained when the Su(f) protein is

expressed at the same level in dividing and in nondividing tissues. We used the UAS/Gal4 system (Brand & Perrimon, 1993) to overexpress the Su(f) protein ubiquitously and determined whether this overexpression restores tissue-specific *su(f)* regulation in a *su(f)* mutant. A *UAS-su(f)* transgene that contains a full-length *su(f)* cDNA in the pUAST vector (Brand & Perrimon, 1993) was constructed (Audibert et al., 1998) and expression of this transgene was driven by the *da-Gal4* activator (Wodarz et al., 1995), which directs a ubiquitous expression of the Gal4 protein during *Drosophila* development. For all three *UAS-su(f)* transformants we have tested, expression of the transgene driven by *da-Gal4* was able to rescue the lethality of the $su(f)^{ts67g}$ mutant at 29 °C. We also assayed the effect of such an overexpression of *su(f)* in the wild type and found that expression of *UAS-su(f)* driven by *da-Gal4* can cause up to 70% pupal lethality, depending on the transformant line tested. This indicates that misregulation of *su(f)* is detrimental.

To analyze the effect of ubiquitous production of the Su(f) protein on *su(f)* tissue-specific regulation, we used the *P[su(f)-lacZ]G* reporter. This transgene encodes a Su(f)-β-galactosidase protein that accumulates in dividing tissues. In wild-type eye-antennal imaginal discs, this protein is detected anteriorly to the morphogenetic furrow and up to the second wave of mitoses (Fig. 2B). In $su(f)^{ts67g}$ mutant larvae, the Su(f)-β-galactosidase protein accumulates both in the posterior part of the disc where cells do not divide, and in the anterior part (Fig. 2C, 4A). As we have shown that in the $su(f)^{ts67g}$ mutant, tissue-specific accumulation of the truncated 1.3-kb RNA is not maintained (Fig. 3B), we propose that ubiquitous expression of the *P[su(f)-lacZ]G* transgene in $su(f)^{ts67g}$ eye-antennal discs results from a decreased utilization of the poly(A) site in intron 4 of the transgene, when *su(f)* function is altered. When the Su(f) wild-type protein is provided ubiquitously in $su(f)^{ts67g}$ eye discs, using *UAS-su(f)* and the *da-Gal4* activator, a wild-type expression pattern of the *P[su(f)-lacZ]G* construct is restored (Fig. 4B), probably as a result of poly(A) site utilization in intron 4 of *P[su(f)-lacZ]G* in nondividing cells.

We directly tested whether the Su(f) wild-type protein restores poly(A) site utilization in intron 4 of endogenous *su(f)* in the $su(f)^{ts67g}$ mutant, using Northern blots. $su(f)^{ts67g}$ shows a strong decrease in the amount of the truncated *su(f)* RNA at restrictive temperatures. We determined whether this truncated transcript would reappear in the $su(f)^{ts67g}$ mutant when the Su(f) wild-type protein is provided ubiquitously with the UAS/Gal4 system. In $su(f)^{ts67g}$ adult males at the restrictive temperature (29 °C), the amount of the 1.3-kb RNA is very low (Fig. 4C, lanes 2 and 3), compared to the wild type (Fig. 4C, lane 1). When the Su(f) protein is provided ubiquitously in the mutant at the restrictive temperature, an increase in the amount of the 1.3-kb truncated RNA is observed (Fig. 4C, lane 4). The same rescue was obtained in mutant larvae and pupae at 25 °C (data not shown). This indicates that wild-type Su(f) protein is required for utilization of the poly(A) site in *su(f)* intron 4.

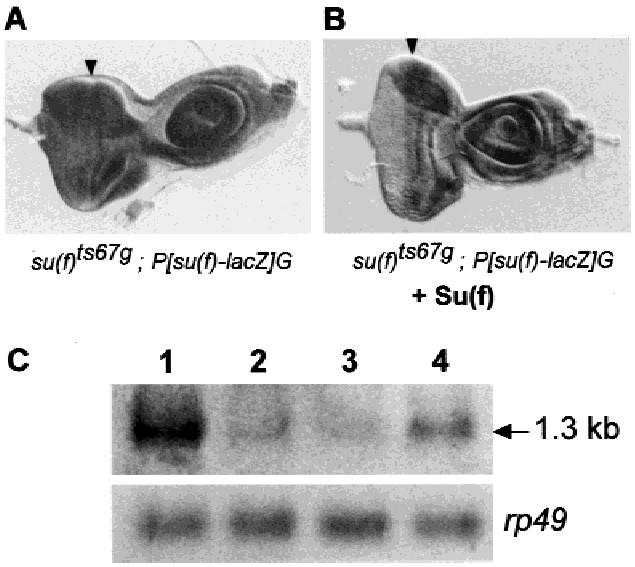


FIGURE 4. Rescue with Su(f) of *P[su(f)-lacZ]G* expression pattern and of utilization of the *su(f)* intronic poly(A) site, in the $su(f)^{ts67g}$ mutant. **A**,**B**: X-Gal staining of third instar eye-antennal imaginal discs. Posterior of the discs is to the left. Arrowheads indicate the morphogenetic furrow. Larvae were raised at 27 °C. **A**: $su(f)^{ts67g}$*/Y; da-Gal4 P[su(f)-lacZ]G/+*. **B**: $su(f)^{ts67g}$*/Y; da-Gal4 P[su(f)-lacZ]G/UAS-su(f)*. **C**: RNA blot with poly(A)$^+$ RNA from males raised at 25 °C and shifted for 4 days to 29 °C. Hybridization was with an RNA probe specific to the 1.3-kb RNA, which does not reveal the *UAS-su(f)* transgene. This probe is complementary to 88 nt located in the part of intron 4 incorporated in the 1.3-kb RNA. The blot was reprobed with the *rp49* clone as a loading control. Lane 1: $su(f)^{+}$*/Y*. Lane 2: $su(f)^{ts67g}$*/Y*. Lane 3: $su(f)^{ts67g}$*/Y; da-Gal4/+*. Lane 4: $su(f)^{ts67g}$*/Y; da-Gal4/UAS-su(f)*.

Taken together, these results show that tissue-specific utilization of the intronic poly(A) site is maintained when the Su(f) protein is provided ubiquitously. This suggests that the tissue specificity does not rely solely on the Su(f) protein.

DISCUSSION

In this article, we present a new example of a protein, the *Drosophila* Su(f) protein, whose level is regulated in a cell-type-specific manner by poly(A) site choice. As has been described in several cases in mammals (see below), regulation of poly(A) site choice in the *su(f)* gene depends on CstF activity. It is becoming clear that the 3′-end-processing reaction as described in mammals is well conserved in *Drosophila*. Indeed, all the proteins required for the reaction in mammals and the sequences of which are known, have their homologs in this species. This includes the three subunits of CstF as well as the four subunits of CPSF, two subunits of

CFIm, the poly(A) polymerase, and the poly(A) binding protein II (Takagaki & Manley, 1994; Bai & Tolias, 1998; Salinas et al., 1998; Benoit et al., 1999; Hatton et al., 2000; Berkeley *Drosophila* genome project). The overall homology between mammalian and *Drosophila* proteins is between 44% and 63% identity depending on the protein analyzed. Further support for functional conservation of the 3′-end processing reaction is provided by our previous demonstration that *Drosophila* poly(A) polymerase and *Drosophila* poly(A)-binding protein II have the same function as their mammalian homologs in in vitro reconstitution assays (Benoit et al., 1999; F. Juge, M. Simonelig, C. Temme, and E. Wahle, unpubl. results). Moreover, we have shown that the *Drosophila* Su(f) protein can be replaced in vivo by chimeric proteins between Su(f) and its human homolog 77K (B. Benoit, F. Juge and M. Simonelig, unpubl. results). Taken together these data strongly suggest that a CstF complex exists in *Drosophila* with a function similar to that of mammalian CstF.

We previously proposed a model in which the Su(f) protein, one subunit of *Drosophila* CstF, would regulate its own amount by stimulating the formation of a truncated *su(f)* transcript. This was based on Northern blots showing that, in *su(f)* mutants, the accumulation of the truncated *su(f)* RNA decreases (Audibert & Simonelig, 1998). Our present data validate this model because they correlate a large amount of the 1.3-kb truncated RNA with a lack of Su(f) protein accumulation, and a low amount of this truncated RNA (five times less than the full-length transcripts) with an accumulation of the Su(f) protein. This regulation requires wild-type Su(f) protein; in the *su(f)*^{ts67g} mutant, the level of the *su(f)*-truncated RNA strongly decreases and this correlates with accumulation of the mutant Su(f) protein. In addition, a higher level of the truncated RNA is restored in this mutant when the Su(f) wild-type protein is provided. These data indicate that Su(f) is involved in the regulated utilization of *su(f)* intronic poly(A) site. We also show that this negative autoregulation is tissue specific and that it is responsible for the accumulation pattern of the Su(f) protein: a high amount of Su(f) protein in dividing tissues and a lack of accumulation of the protein in nondividing tissues (Fig. 5). In *su(f)*^{ts67g}, this accumulation pattern is not maintained and the Su(f) mutant protein accumulates at the same level in dividing and nondividing tissues, which is correlated with a low amount of the truncated RNA in both tissues. Our results also demonstrate a function of *su(f)* in nondividing tissues and reveals that a low level of the Su(f) protein is present in nondividing tissues although it is not detectable with anti-Su(f) antibodies (Audibert et al., 1998; Fig. 1C,E). This basal level of Su(f) protein would ensure basal mRNA 3′-end processing in these tissues.

The molecular basis for the tissue specificity of *su(f)* autoregulation, in other words, why *su(f)* autoregulation is more efficient in nondividing tissues than in dividing tissues, is not understood. However, we have found that when the Su(f) protein is provided ubiquitously under the control of the *da-Gal4* activator, a wild-

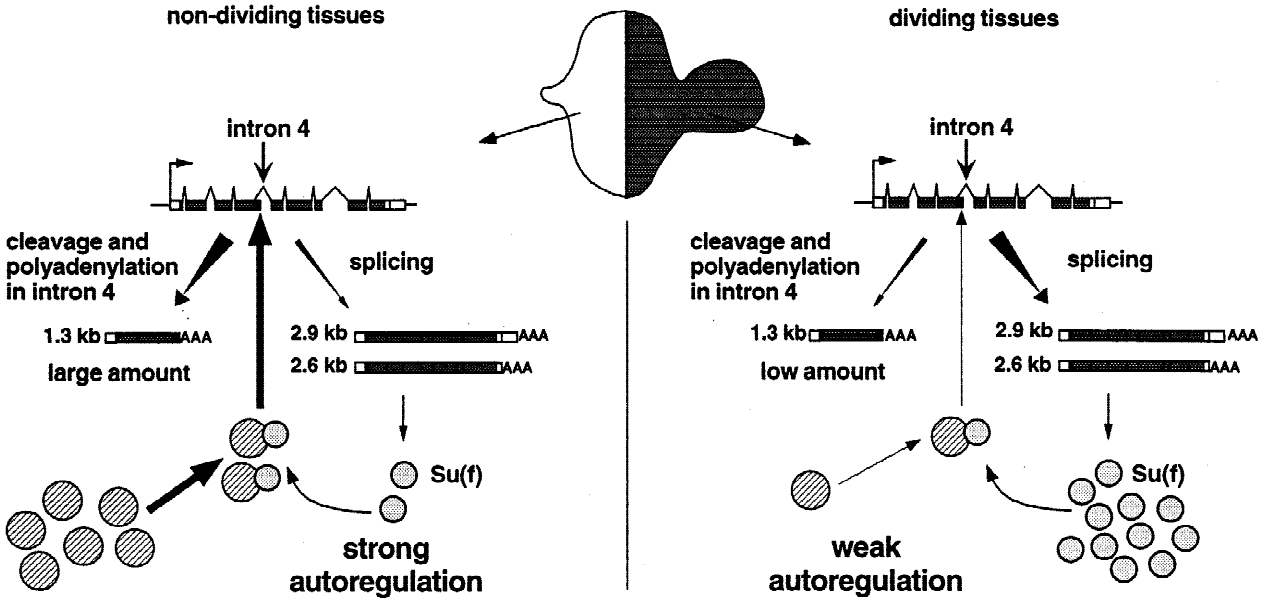


FIGURE 5. Model for tissue-specific *su(f)* autoregulation. The Su(f) protein regulates its own level by promoting the utilization of poly(A) site in *su(f)* intron 4, which leads to the formation of a truncated transcript. In dividing tissues, this autoregulation is weak, possibly because of the lack or the low amount of another component essential for Su(f) activity on the *su(f)* intronic poly(A) site. This allows Su(f) accumulation. In nondividing tissues, *su(f)* autoregulation is strong, possibly as a result of a high level of the protein required for efficient Su(f) activity on the *su(f)* intronic poly(A) site. This results in the synthesis of a very low level of Su(f) protein.

type expression pattern of the *P[su(f)-lacZ]G* reporter is restored in the *su(f)^{ts67g}* mutant. Therefore, in that experiment, accumulation of the Su(f)-β-galactosidase protein is repressed in nondividing tissues but not in dividing tissues although the Su(f) protein is present at the same level in both tissues. This suggests that this repression in nondividing tissues depends on another protein that stimulates utilization of the poly(A) site in intron 4 (Fig. 5). This protein would induce a modification of Su(f) activity either via a direct posttranslational modification of Su(f) or by interacting with Su(f). Such a Su(f)-interacting protein could be a specific protein or a component of the general 3′-end processing machinery. An obvious candidate is the 64K subunit of CstF known to interact with the 77K subunit in human (Takagaki & Manley, 1994). Variations in the level of this 64K subunit contribute to the shift in poly(A) site selection in the immunoglobulin M heavy-chain locus, during B cell differentiation (Takagaki et al., 1996; Takagaki & Manley, 1998).

Autoregulation of *su(f)* appears to be conserved in another *Drosophila* species, *D. virilis* (Audibert & Simonelig, 1998), as the structure of the *su(f)* gene as well as sequences downstream of the intronic poly(A) site, including the GU-rich motif, are conserved in this species. Interestingly, such autoregulation has been proposed to occur for the yeast homolog of *su(f),* the *RNA14* gene (Mandart, 1998). The *RNA14* gene also produces truncated RNAs, whose accumulation requires wild-type *RNA14* function. The fact that autoregulation of this gene is conserved from yeast to *Drosophila* suggests that the level of this protein must be tightly regulated. Consistent with this, overexpression of *su(f)* in *Drosophila* using the *UAS/Gal4* system can lead to lethality.

In mammals, several examples document the fact that variations in the general cleavage factor CstF participate in the regulation of poly(A) site choice. In most cases, the 64K subunit of CstF has been found to be responsible for these variations. Variations in the activity of 64K have been reported to occur during the shift in poly(A) site choice in adenovirus (Mann et al., 1993). An increase in the 64K level participates in the switch of poly(A) site utilization in the immunoglobulin M heavy-chain locus during B cell maturation (Takagaki et al., 1996; Takagaki & Manley, 1998). The 64K subunit level has also been shown to increase in cultured cells induced to proliferate (Martincic et al., 1998), and recently a new form of the 64K protein has been described in mouse (Wallace et al., 1999); this form is specific to testis and brain and has been proposed to replace the 64K form during some steps of spermatogenesis. Variations in the level of the 77K subunit have also recently been reported in cultured keratinocytes induced to differentiate (Terhune et al., 1999). Decreased levels of 77K and 64K in these differentiated cells are thought to contribute to the utilization of more distal poly(A) sites in human papillomavirus type 31. This variation of 77K level reported in cultured cells is consistent with the tissue-specific variations in the level of the Su(f) protein in *Drosophila*. In addition, our data indicate that, in *Drosophila*, the Su(f)/77K protein participates in the tissue-specific regulation of poly(A) site utilization. Taken together, these data suggest that in vivo, variations in the levels or activities of both the 64K and 77K subunits concur to regulate CstF activity in a cell-type-specific manner leading to regulation of poly(A) site utilization.

MATERIALS AND METHODS

Drosophila stocks and germline transformation

The Gal4 driver line was *P[GAL4-da.G32]* (*da-Gal4*), which mediates ubiquitous expression (Wodarz et al., 1995). *P*-element transformation was carried out as described (Rubin & Spradling, 1982). Construct DNA (500 μg/mL) with 250 μg/mL of the helper plasmid, pUChsΠΔ2-3, were injected into *w^{1118}* embryos. *To generate the P[su(f)-lacZ]P* transgene, the *P[su(f)-lacZ]G* plasmid (Audibert et al., 1998) was digested with *Bam*HI, partially digested with *Sca*I, and religated after filling-in of the *Bam*HI site using Klenow. The *su(f)-lacZ* junction was checked by DNA sequencing.

RNA blots and quantitative RT-PCR

RNA blots were performed as reported (Simonelig et al., 1996). For each RT-PCR, RNA was prepared from 10 to 15 wing imaginal discs and from Malpighian tubules of 10 to 15 third instar larvae dissected in PBS. Tissues were transferred to 150 μL of RNA B (Bioprobe Systems) and RNA, prepared as recommended by the manufacturer, was dissolved in 10 μL of DEPC-treated water. This RNA was treated with 10–15 U of RNase free-DNase I (Boehringer Mannheim) in 20 μL of 1× PCR Buffer (Perkin Elmer-Cetus), for 30 min at 37 °C. RNA was treated with phenol-chloroform, precipitated, and dissolved in 10 μL of DEPC-treated water. A volume of 2–5 μL of this RNA was put in a 20-μL reaction with 1× RT buffer (Boehringer Mannheim), 1 mM dNTPs, and 50 pmol of oligo-$(dT)_{20}$. After denaturation for 5 min at 65 °C, 10 U of avian myoblastoma virus-reverse transcriptase (AMV-RT; Boehringer Mannheim) were added for 2 h at 41 °C. Five microliters of this reaction were put in a 50-μL PCR reaction (30 cycles of 1 min at 94 °C, 1 min at 55 °C, 2 min at 72 °C) with 1× PCR Buffer II (Perkin Elmer-Cetus), 50 pmol of each primer (set 1: 5′-CTCGTTCAGCATCTGGCAGG and 5′-CACTACAGGAA CGCAGAGAATAGG; set 2: 5′-CAAAGCCAGAACACCGGA GAGGTCG and 5′-CGTACTTGTATCCAACGATCCAGTG), 0.2 mM dNTPs, 1 mM $MgCl_2$, and 2.5 U of Ampli-Taq DNA Polymerase (Perkin Elmer-Cetus), which were added after a denaturating step of 3 min at 94 °C. Aliquots of 6 μL of the PCR reaction were taken at cycles 18, 20, 22, 24, 26, 28, and 30, and loaded in 2% agarose electrophoresis gel. Southern blots were performed as described (Sambrook et al., 1989) and hybridized with a probe corresponding to a full-length *su(f)* cDNA (cK22; Mitchelson et al., 1993). Quantification was performed by using PhosphorImager scanning (Molec-

ular Dynamics) and the ImageQuant program (Molecular Dynamics). Each experiment was repeated two to four times.

Histochemistry and antibody staining

Staining by 0.1% 5-bromo-4-chloro-3-indolyl-β-D-galactopyranoside (X-Gal) was performed as previously described (Audibert et al., 1998), except for the *P[su(f)-lacZ]P* transformant lines, which were treated as follows. Third instar larvae were dissected in PBS, fixed 10 min in 1% glutaraldehyde, washed in PBS, and stained in 0.1% X-Gal, 3.5 mM $K_4Fe(Cn)_6$, 3.5 mM $K_3Fe(Cn)_6$, 1 mM $MgCl_2$, and 0.3% Triton X-100 in PBS for 48 h at 37 °C. These preparations were mounted in 50% glycerol, 50% ethanol. Antibody staining was performed as described (Audibert et al., 1998).

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