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ORIGINAL ARTICLE

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Specific interactions between *vestigial* and *scalloped* are required to promote wing tissue proliferation in *Drosophila melanogaster*

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Abstract The two genes *vestigial* (*vg*) and *scalloped* (*sd*) are required for wing development in *Drosophila melanogaster*. They present similar patterns of expression in second and third instar wing discs and similar wing mutant phenotypes. *vg* encodes a nuclear protein without any recognized nucleic acid-binding motif. Sd is a transcription factor homologous to the human TEF-1 factor whose promoter activity depends on cell-specific cofactors. We postulate that Vg could be a cofactor of Sd in the wing morphogenetic process and that, together, they could constitute a functional transcription complex. We investigated genetic interactions between the two genes. We show here that *vg* and *sd* co-operate in vivo in a manner dependent on the structure of the Vg protein. We ectopically expressed *vg* in the patch (*ptc*) domains. We show evidence that wing-like outgrowths induced by ectopic expression of *vg* are severely reduced in *vg* or *sd* mutant backgrounds. Accordingly, we demonstrate that *ptc*-GAL4-driven expression of *vg* induces both expressions of the endogenous *vg* and *sd* genes and that the two Vg and Sd proteins have to be produced together to promote wing proliferation. Futhermore, we show an interaction between the two proteins by double hybrid experiments in yeast. Our results therefore support the hypothesis that Sd and Vg directly interact in vivo to form a complex regulating the proliferation of wing tissue.

Key words *Drosophila* wing development · *vestigial* · *scalloped*

Introduction

vestigial (*vg*) mutants of *Drosophila melanogaster* are characterized by severely reduced wings and loss of the wing-margin structures (Lindsley and Grell 1968; Williams and Bell 1988). Weak *vg* mutants display occasional gaps in the wing margin. For extreme alleles, wing and haltere structures can be lost completely, this phenotype being associated with extensive cell death in the wing pouch of the third instar larval imaginal discs (Fristrom 1969; Bownes and Roberts 1981). As for *vg*, *scalloped (sd)* mutants exhibit different wing phenotypes which can vary from only gaps in the wing margin to complete loss of the wing structures (Daniels et al. 1985; Campbell et al. 1991). Strong reduction of the wings is associated with elevated cell death in larval wing discs (Simpson et al. 1981; James and Bryant 1981). In addition, *sd* and *vg* expression patterns appear identical in early and late imaginal wing discs. Both expressions, ubiquitously detectable at a very low level in early second instar wing discs, become elevated throughout the disc and resolve into a well defined stripe of cells along the future wing margin during the early third larval instar. In late third instar imaginal discs, *vg* and *sd* are expressed at high levels in a broad stripe, which includes the primordia of the wing pouch and hinge regions (Campbell et al. 1992; Williams et al. 1993).

Kim et al. (1996) have shown that GAL4-targeted expression of *vg* induces formation of wing-like outgrowths, and they report that ectopic expression of *vg* induces *sd* expression. However, targeted *sd* expression does not induce either wing outgrowths or *vg* expression (Irvine observations in Kim et al. 1996). Activation of *sd* is therefore insufficient to induce wing outgrowths.

The *vg* locus encodes a nuclear protein without any known nucleic acid-binding motif (Williams and Bell 1988; Williams et al. 1991). The Sd protein is a transcription factor with a TEA/ATTS DNA-binding domain. It is homologous to the human transcriptional enhancer factor TEF-1, whose promoter activity is dependent on the presence of cell-specific cofactors (Campbell et al. 1992; Xiao et al. 1991). Postulating that Vg could be a specific cofactor of Sd in the wing morphogenetic process, we have investigated in vivo genetic interactions between *vg* and *sd* and examined interactions between the two proteins in yeast by double-hybrid experiments.

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By using weak alleles of *sd*, a new *vg*^{null} allele and the peculiar *vg*^{79d5} allele, we show that *sd* and *vg* co-operate in the wing morphogenetic process and we present evidence that the genetic interactions between these two genes are dependent on the structure of the Vg protein. The construction of transgenic UAS-*vg* flies allows us to show that ectopic wing-like outgrowths induced by *ptc*-GAL4-driven expression of *vg* is dependent on the activity of *sd* and also on the activity of the endogenous *vg* gene. Furthermore, we demonstrate that ectopic expression of *vg* induces expression of both genes. This explains the incapacity of *sd* alone to promote wing-like outgrowths when it is expressed ectopically and shows that the two proteins are co-required to promote wing tissue proliferation. Accordingly we show that Sd and Vg interact in yeast in the double-hybrid system. Thus, our results support the hypothesis that *vg* and *sd* expression are positively regulated by *vg* activity and that both products directly interact in vivo to form a complex regulating the development and proliferation of wing tissue.

Materials and methods

Drosophila strains and culture conditions

The *vg*^{BG} (also named *vg*^{1}), *vg*^{79d5} and *sd*^{1} strains came from the Bowling Green stock centre and were initially described in Lindsley and Zimm (1992). The *vg*^{BG} mutation is more extensively described in Zider et al. (1996) and the *vg*^{79d5} mutation in Williams et al. (1990 and 1991).

The *vg*^{83b27} mutant is a no-wing mutant given by John Bell. The *vg*^{83b27} allele deletes an intronic regulatory element required for gene expression in the wing and haltere imaginal discs. It is described in Williams et al. (1990 and 1991).

The *vg*^{null} mutant is a viable mutant which was isolated in our laboratory by targeted P-element mutagenesis. Preliminary molecular analysis shows that the eight exons have been deleted.

The *sd*^{ETX4} strain was a gift of Shelagh Campbell. The *sd*^{ETX4} mutation corresponds to a P *ry*$^{+}$ *lacZ* transposon insertion in the first intron of the *scalloped* gene and is used as an *sd* enhancer-trap strain (Anand et al. 1990; Campbell et al. 1992).

The *wg-lacZ* strain is a *wg* enhancer-trap strain. It was provided by R. Phillips and described in Kassis et al. (1992) and in Phillips and Whittle (1993).

The *ptc*-GAL4 line was obtained from the Bloomington stock centre.

All crosses were performed at 21°C in standard corn medium.

Escherichia coli β-galactosidase assay

Wing discs were dissected from wandering third instar larvae in 0.1 M phosphate-buffered saline (PBS) and fixed for 30 min in 1% p-formaldehyde, 0.2% glutaraldehyde in 0.1 M PBS. After fixation, discs were rinced in 0.1 M PBS and incubated overnight in 2 mM X-gal, 4 mM potassium ferricyanide, 4 mM potassium ferrocyanide, 4 mM magnesium chloride in 0.1 M PBS.

Reverse transcriptase-polymerase chain reaction (RT-PCR) analysis

RNA from dissected imaginal discs was isolated with the Gibco BRL Trizol reagent kit, treated with DNAse and reverse transcribed with an oligo dT and the superscript reverse transcriptase (Gibco BRL) according to the manufacturer's protocol. Of the reaction mixture 10% was amplified using the specific forward and reverse primers for the *rp49* cDNA (5′-tcctaccagcttcaagatgac-3' and 5′-gtgtattccgaccacgttaca-3′), and *vg* cDNA (5′-ttctcttccgattgagcggc-3′ and 5′-tattctgctttgcgatgtgg-3′). The fragment amplified with the *vg* primer corresponds to the sequence of the *vg* cDNA between the nucleotides 358–556. This sequence was lacking in the vg cDNA cloned in the pUAST vector (Zider et al. 1998).

Two hybrid assay

The different plasmids and the methodological procedure used in this study were provided by R. Brent from the Massachusetts General Hospital (MGH). The yeast strain used was EGY48 (MATa, *his3*, *trp1*, *ura3–52*, *leu2*: *pLEU2-LexAop6*).

Plasmid vectors pEG202 and pJG4-5 encoding the LexA DNA-binding domain and the B42-activating domain, respectively, were used to express fusion protein. They contain both the 2 μ replicator and the *HIS3* and the *TRP1* genes, respectively, as selectable markers (Gyuris et al. 1993; Russel et al. 1995). In pJG4-5 vector, expression of the fusion protein is under the control of the *GAL1* promoter which is inductible by galactose. In the pEG202 vector the fusion protein is expressed under the control of the constitutive *ADH* promoter. Two reporter genes were used: (1) an integrated copy of the *LexAop-LEU2* gene in which upstream activating sequences are replaced by six *LexA* operators, and (2) the plasmid pSH18-34 which carries eight *LexA* operators upstream of the *lacZ* gene. pSH18-34 contains the 2 μ replicator and the *URA3* selectable marker.

To screen for protein interactions in the two hybrid system, we used a pEG-*sd*-Δ*TEA* vector which expresses the C-terminus domain of the Sd protein in fusion with the LexA DNA-binding domain and the pJG-*vg* vector which expresses the Vg protein in fusion with the B42-activation domain. The pEG-*sd*-Δ*TEA* vector was constructed by insertion of the *sd* cDNA, which lacks the 212 N-terminal amino acids of the open reading frame, into the pEG202 vector. Indeed preliminary experiments have shown that expression of the N-terminus domain of the Sd protein which contains the TEA DNA-binding domain is toxic in yeast. The pJG-*vg* vector was constructed by insertion of the *vg* cDNA which lacks the seven N-terminal amino acids of the open reading frame, into the pJG vector. Three B42-Vg derivatives were also produced by insertion of the *vg* sequences corresponding to the amino-acids 7–127, 127–276, 276–453, respectively, into the PJG4-5 vector.

Results

In weak *sd* mutant backgrounds, the *vg*^{79d5} mutation has a dominant effect

We have used weak hypomorphic alleles of *vestigial* (*vg*^{79d5}) and *scalloped* (*sd*^{ETX4} and *sd*^{1}) to investigate interactions between the two genes (Fig. 1). These three mutations give homozygous adult phenotypes with gaps in the wing margin (Fig. 1A–C). The double homozygous flies *sd*^{ETX4}; *vg*^{79d5} (Fig. 1E) exhibit a significant reduction in wing size and an uplifting of the postscutellar bristles, similar to the phenotype of the flies homozygous for the strong *vg*^{BG} mutation (Fig. 1D), which possess a very low level of wild-type transcript (Zider et al. 1996). A phenotype almost as strong is observed in *sd*^{1}; *vg*^{79d5} homozygous flies (Fig. 1F), showing that the *vg*^{BG}-like phenotype observed with *sd*^{ETX4} is not specific for this *sd* allele. These adult phenotypes are correlated with a strong reduction of both the presumptive wing

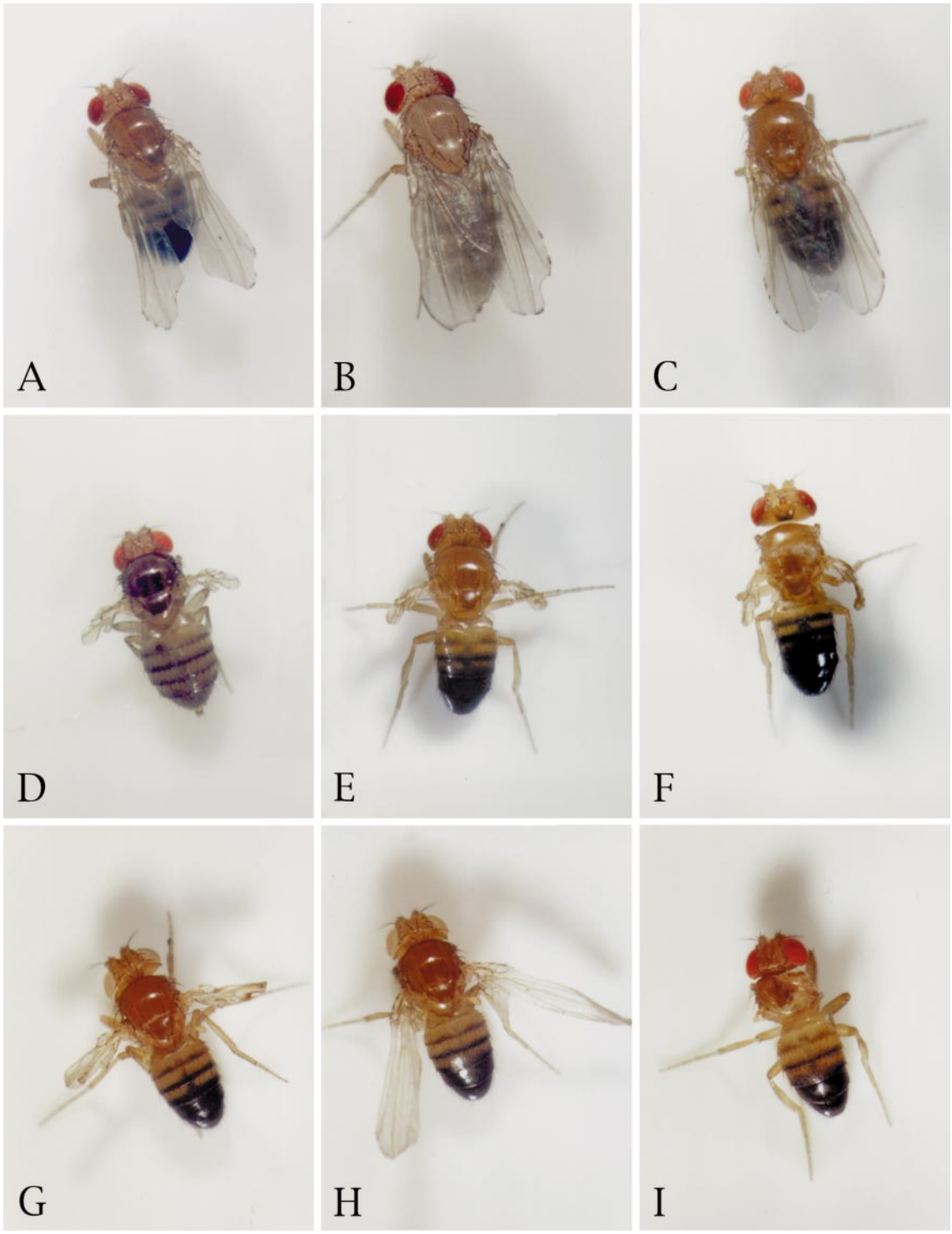

Fig. 1A–I The association between the *vg*^{79d5} mutation and weak *sd* alleles has a dramatic effect on wing development. Mutant strains: *vg*^{79d5} (**A**), respectively *sd*^{ETX4} and *sd*^{1} (**B**, **C**), *vg*^{BG} (**D**) and *vg*^{null} (**I**). Phenotypes resulting from the association between these different mutations are: *sd*^{ETX4}/Y; *vg*^{79d5}/*vg*^{79d5} (**E**), *sd*^{1}/Y; *vg*^{79d5}/*vg*^{79d5} (**F**), *sd*^{ETX4}/Y; *vg*^{79d5}/*vg*$^{+}$ (**G**), *sd*^{ETX4}/Y; *vg*^{null}/*vg*$^{+}$ (**H**). In the hypomorphic *sd* mutant background, the flies homozygous for the weak *vg*^{79d5} allele (**E, F**) have a wing phenotype as strong as that of the *vg*^{BG} homozygous flies (**D**). Note, the phenotype of the *sd*^{ETX4} flies heterozygous for the *vg*^{79d5} allele (**G**) which is almost as strong as that of the homozygous *sd*$^{ETX4;}$ *vg*^{79d5} flies (**E**) whilst the *sd*^{ETX4} flies heterozygous for the *vg*^{null} allele (**H**) do not exhibit such a reduction of the wing

margin and the wing pouch in third instar wing discs that we have observed by using the *sd-lacZ* enhancer-trap properties of the sd^{ETX4} allele and a *wg-lacZ* enhancer-trap mutation (data not shown). The adult wing phenotype of the double mutants and the strong disruption observed in wing disc structures, indicate that *vg* and *sd* cooperate during wing development in the same morphogenetic process.

The vg^{79d5} mutation is recessive. Surprisingly, in an sd^{ETX4} background, the flies heterozygous for the vg^{79d5} allele (Fig. 1G) exhibit a mutant phenotype which is almost as strong as that of the sd^{ETX4} flies homozygous for this allele (Fig. 1E). The same phenomenon is observed in an sd^{1} background (not shown). Moreover, the sd^{ETX4} flies heterozygous for a vg^{null} mutation do not exhibit such a significant reduction of the wing (Fig. 1H), whereas the vg^{null} homozygous mutants have a very extreme phenotype (Fig. 1I). Therefore, in a hypomorphic *sd* background, the vg^{79d5} mutation has a dominant effect which leads to an enhancement of the *sd* mutant phenotype. According to Williams et al. (1990), the vg^{79d5} allele encodes a protein with an internal deletion corresponding to the 5′ end of exon 3 which includes a poly-alanine-rich region, the correct reading frame being preserved. When the expression of *scalloped* is reduced, the presence of the vg^{79d5} allele encoding such a deleted protein is more drastic than the complete loss of one *vg* allele. Therefore, the genetic interactions observed between *vestigial* and *scalloped* are dependent on the structure of the Vg protein.

Formation of wing-like outgrowths by ectopic expression of *vestigial* is dependent on *scalloped* expression

In order to target *vg* expression by the UAS-GAL4 system (Brand and Perrimon 1993), homozygous UAS-*vg* lines were produced (Zider et al. 1998) and males were crossed with homozygous *ptc*-GAL4 females. The majority of the F_1 flies died in the early pupae stage. Flies dying in late pupae stage exhibit extensive wing-like outgrowths (not shown) associated with a disorganization and reduction of the wings which, according to Kim et al. (1996), could be due to a disruption of the wing's morphogenetic process.

Crosses were performed between sd^{+}; UAS-*vg* males and sd^{ETX4}; *ptc*-GAL4 females. Compared to the previous homozygous sd^{+} females, more sd^{ETX4} heterozygous females were recovered, which died in late pupae stage. They exhibit ectopic wing-like outgrowths at the posterior of the head capsule with a very strong deformation of the eye (Fig. 2A,B), this phenotype being however less extensive compared to sd^{+} females. These ectopic wing-like outgrowths are severely reduced for the sd^{ETX4} hemizygous males, and only a slight deformation in the posterior part of the eye is observed (Fig. 2C,D). Moreover, the adults are able to emerge from the pupae, although they die within a few hours. These results show

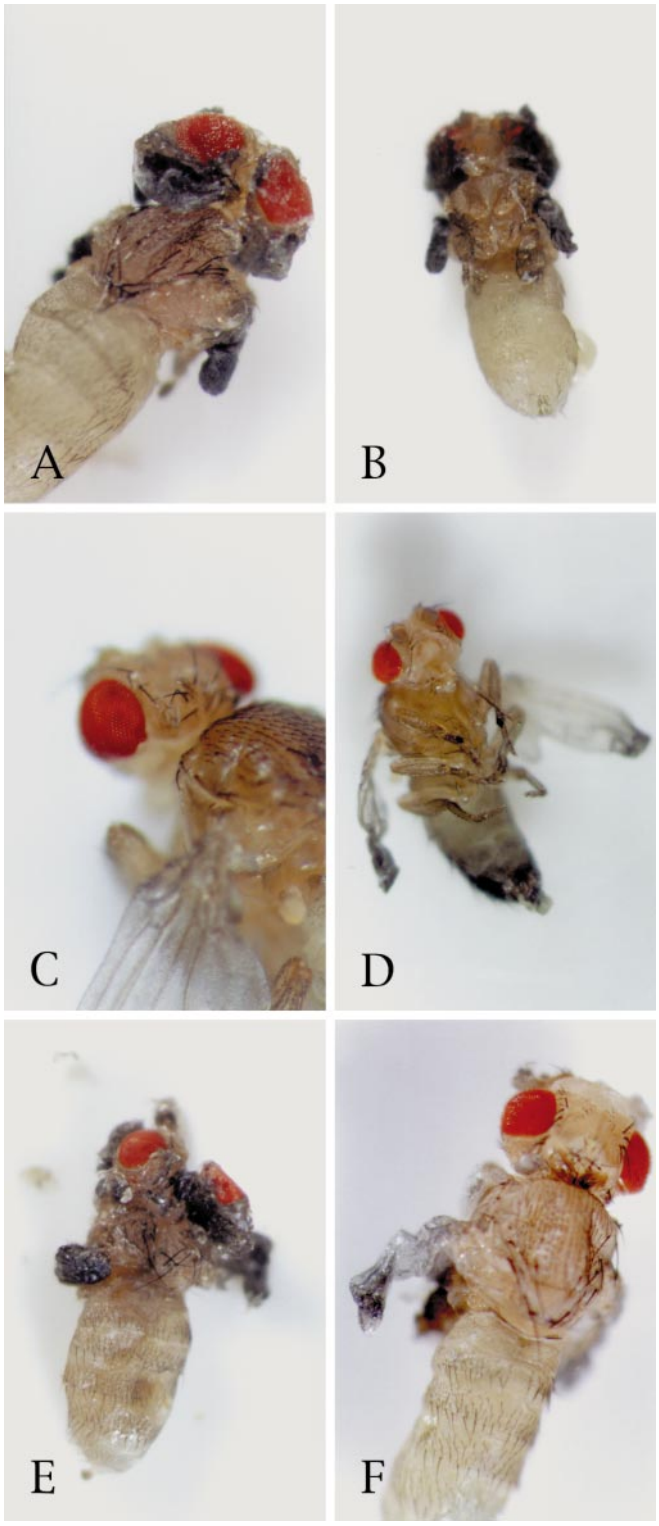

Fig. 2A–F Development of wing-like outgrowths resulting from ectopic expression of UAS-*vg* driven by *ptc*-GAL4. Comparison between sd^{ETX4}/sd^{+}; *ptc*-GAL4/UAS-*vg* females (**A, B**), and sd^{ETX4}/Y; *ptc*-GAL4/UAS-*vg* males (**C, D**) shows that the ability of *vg* to promote wing-like proliferation depends on the activity of *scalloped*. Comparison between vg^{+}/vg^{BG}; *ptc*-GAL4/UAS-*vg* female (**E**), and vg^{BG}/vg^{BG}; *ptc*-GAL4/UAS-*vg* females (**F**) shows that the ability of *vg* to promote wing-like proliferation depends on the activity of the endogenous *vestigial* gene

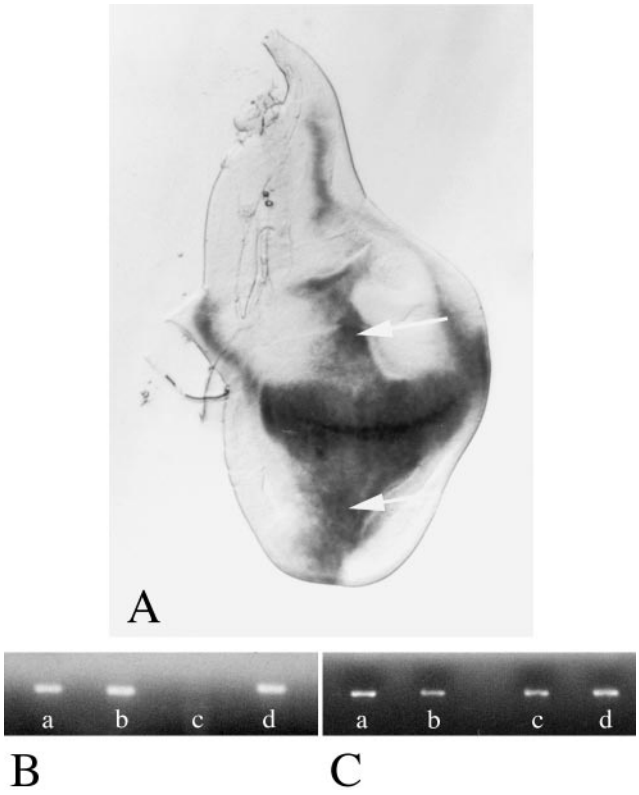

Fig. 3A–C *ptc*-GAL4-driven expression of *vg* induces both expression of *sd* and the endogenous *vg* gene. **A** Expression of the *sd-lacZ* reporter gene revealed by β-galactosidase assay in sd^{ETX4}/sd^{+}; *ptc*-GAL4/UAS-*vg* third instar wing discs. The *sd* reporter gene is expressed in a stripe corresponding to the wing margin and in the wing pouch. In addition, *sd* expression is observed in the *ptc* domain (marked by arrows). This shows that the expression of *vg* induces the expression of *sd*. **B** Transcription of the endogenous *vg* gene revealed by RT-PCR experiments in third instar wing or eye-antennal discs: *a* wild-type CantonS wing discs, *b ptc*-GAL4/UAS-*vg* wing discs, *c* wild-type CantonS eye-antennal discs and *d ptc*-GAL4/UAS-*vg* eye-antennal discs. **C** Transcription of the *RP49* gene as control in the same extracts. Note the expression of the endogenous *vg* gene in eye-antennal discs when the *vg* transgene is expressed in the *ptc* domain (*d*) whilst it does not occur in the wild-type background (*c*). This shows that *vg* is implicated in the regulation of its own expression

that the weak sd^{ETX4} mutation greatly reduces the ability of *vg* to promote wing-like outgrowths. We have checked that this was not the result of some influence of the sd^{ETX4} mutation on *ptc*-driven expression (not shown). The development of wing tissue induced by ectopic expression of *vg* is therefore dependent on the level of *sd* expression.

Expression of the *sd-lacZ* reporter gene was examined in third instar wing discs of sd^{ETX4}/sd^{+}; UAS-*vg*/*ptc*-GAL4 females (Fig. 3A). As expected, the *sd* reporter gene is expressed in the wing pouch and in a stripe corresponding to the wing margin (Campbell et al. 1992). In addition, *sd* expression is observed in the *ptc* domain. Therefore, *ptc*-GAL4-driven expression of *vg* induces expression of the *sd* gene.

Ectopic expression of the *vestigial* transgene induces ectopic expression of the endogenous *vestigial* gene

The effect of *vg* ectopic expression was analysed in flies heterozygous or homozygous for *vg* alleles. We have used three alleles: vg^{BG}, vg^{83b27} and vg^{null}. For all, we made the same observation: wing-like outgrowths were severely reduced in the homozygous flies compared to the heterozygous ones. An example is given in Fig. 2E and F for the vg^{BG} allele. These results reveal that the ability of the *vg* transgene to induce formation of ectopic wing proliferation depends on the activity of the endogenous *vg* alleles and suggest that the *vg* transgene induces the expression of the endogenous *vg* gene.

This was confirmed by analysing *vg* transcripts in RT-PCR experiments: the *vg* cDNA present in our UAS-*vg* transgene lacks the first 800 nucleotides of the 5′ untranslated sequence. Appropriate primers (see Materials and methods) only allowed us to reveal transcription of the endogenous gene (Fig. 3B), the *RP49* gene being used as a control (Fig. 3C). In normal conditions, the endogenous *vg* gene is not transcribed in the eye-antennal discs (Fig. 3B c), whereas its transcription occurs when expression of the *vg* transgene is driven by *ptc*-GAL4 (Fig. 3B d). We can therefore conclude that *vg* activates its own transcription.

Vg and Sd interact in yeast

We examined the protein interactions between Vg and Sd by using a double-hybrid interaction test (Gyuris et al. 1993; Russel et al. 1995). The EGY48 yeast strain was co-transformed with the pJG-*vg* vector which encodes the fusion protein Vg-B42, and with the pEG*sd*-ΔTEA vector, which encodes the fusion protein SdΔTEA-LexA (see Materials and methods). In this latter fusion protein, we used the C-terminal domain of the Sd protein (amino acids 212–440), because the N-terminal domain, which includes the TEA DNA-binding domain was toxic in yeast (data not shown). Transformants were analysed for leucine auxotrophy and β-galactosidase reporter gene activity. Figure 4 shows that co-expression of the Vg-B42 and SdΔTEA-LexA proteins in yeast leads to a significative activation of the two reporter genes *lacZ* and *leu2* as observed with the positive control [pEG-*Ci*/pJG-*Su(fu)*] This interaction was specific since we did not observe any activation of the *lacZ* reporter gene when SdΔTEA-lexA was co-expressed with an unrelated B42-derivative (B42-βGT). Furthermore, no activation of the reporter gene was observed when Vg-B42 was co-expressed with an unrelated LexA derivative (LexA-rab3). These experiments demonstrate that the two proteins Vg and Sd are able to interact in yeast in the same protein complex.

To determine which region of Vg is implicated in the formation of this complex, we used three Vg-B42 derivatives. A positive result was obtained with the C-terminal

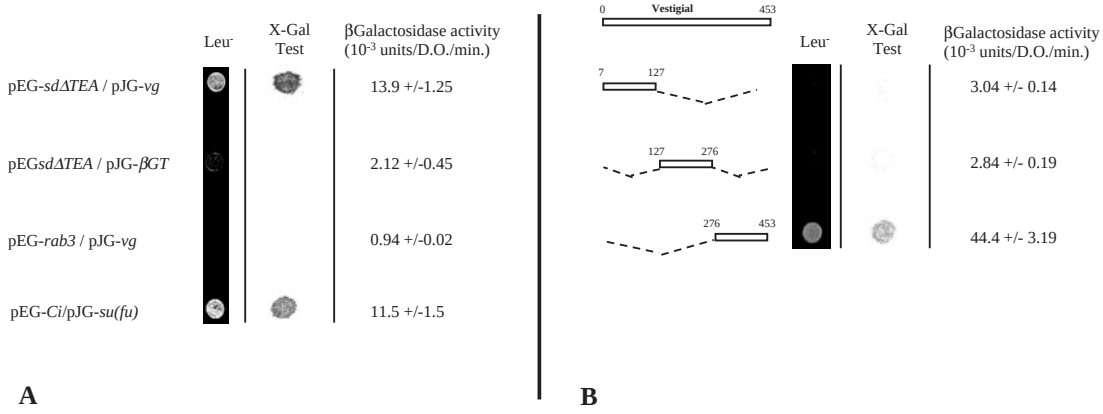

Fig. 4A, B Vg and Sd interactions are revealed by double hybrid experiments in yeast. **A** The reporter strain EGY48 was co-transformed with pEG-*sdΔTEA* and pJG-*vg* and analysed for leucine auxotrophy and β-galactosidase activity. Growth in the absence of leucine and significative β-galactosidase activity indicate interaction between the two proteins. The *blue colour* in X-gal test developed within 15 min and the growth on media lacking leucine was observed after 48 h at 30°C. The activation of the reporter genes observed when yeast were co-transformed with pEG-*sdΔTEA* and pJG-*vg* was as strong as that observed with pEG-*Ci*/pJG-*Su(fu)* [Ci and Su(fu) are two proteins known to interact (Monnier et al. 1998) and are used as a positive control in our test]. This interaction was specific since no interaction was observed when EGY48 was co-transformed with pEG-*sdΔTEA* and pJG-β*GT* or with pEG-*rab3* and pJG-*vg*. **B** To determine which region of the Vg protein interacts with the Sd protein, the three Vg-B42 derivatives, expressing the indicated sequence of the Vg protein, were co-transformed with the pEG-*sdΔTEA* vector in the reporter strain EGY48 and analysed for leucine auxotrophy and β-galactosidase activity

domain of the Vg protein (amino acids 273–453). No interaction occurred with the two other Vg-B42 derivatives that correspond to the N-terminal and to the central domains of the Vg protein. This demonstrates that the C-terminal region of the Vg protein contains the domain involved in the formation of the complex. As amino acids deleted in the Vg^{79d5} protein are not part of the C-terminal region, the Vg^{79d5} protein possesses this domain.

Discussion

The two genes *vg* and *sd* are required for wing development. The Sd protein is a transcription factor with a TEA/ATTS DNA-binding domain, homologous to the human transcription enhancer factor TEF-1, whose promoter activity is dependent on the presence of cell-specific cofactors (Campbell et al. 1992; Williams et al. 1991). Vg is a nuclear protein without any known nucleic acid-binding motif (Williams and Bell 1988; Williams et al. 1991). We postulated that Vg could be a co-factor of Sd in the wing morphogenetic process. We show here that ectopic *ptc*-GAL4-driven expression of *vg* induces expression of both the endogenous *sd* and *vg* genes and we present evidence that the activities of the two genes are required for the development of wing-like outgrowths. Ectopic expression of *sd* induces neither such outgrowths nor the expression of *vg* (Irvine observations in Kim et al. 1996). This means that co-expression of *vg* and *sd* is necessary in this process. We have demonstrated that Vg and Sd are able to directly interact in yeast in double hybrid experiments. It can thus be expected that such interaction between the two proteins does occur in vivo within a complex regulating wing tissue proliferation.

The double mutants for weak *sd* and *vg* alleles exhibit a strong wing mutant phenotype. This shows that the two genes co-operate during wing development in the same morphogenetic process. Surprisingly, in an sd^{ETX4} background, the vg^{79d5} heterozygous flies exhibit a reduction of wing size more extensive than the reduction observed for the vg^{null} heterozygous ones. So, in an *sd* hypomorphic background, the vg^{79d5} mutation has a dominant effect which leads to enhancement of the sd^{ETX4} phenotype. The vg^{79d5} allele produces a transcript which is internally deleted for the 5′ end of exon 3 and is recovered at the same level as the wild transcript. It encodes a Vg^{79d5} protein with an internal deletion which includes a polyalanine region, the wild-type reading frame being preserved (Williams et al. 1990). The vg^{null} mutant used lacks any *vg* sequences.

We have demonstrated that the Vg^{79d5} protein does not lack the domain required for the formation of an Sd-Vg activating complex in yeast. To explain the effect resulting from the presence of the vg^{79d5} allele, compared to the loss of one *vg* allele in an *sd* hypomorphic background, we suggest that the Vg^{79d5} proteins can trap the Sd proteins into proteic complexes less active than the wild complexes but either more stable or with more affinity to the DNA targets. In these conditions, in heterozygous vg^{79d5} flies, more abnormal complexes will bind to the DNA compared to full active ones whilst in heterozygous vg^{null} genotypes, all complexes binding to the DNA will have normal activities. In *sd* hypomorphic mutants, we can expect that less Sd proteins are produced. Therefore less Vg-Sd complexes will be formed so that the resulting activity may become limiting, giving the weak wing mutant phenotype observed in sd^{ETX4}; vg^{+} background. In sd^{ETX4}; vg^{79d5}/ vg^{+} flies, the formation of less active complexes will lead to a reduction of the resulting activity and to an increase of the mutant pheno-

type. We have shown that *vg* regulates both its own expression and that of *sd* during the wing proliferation process. Otherwise, ectopic expression of *sd* under a heat-shock promoter induces *sd-lacZ* reporter gene activity in third-instar wing discs, this induction not being ubiquitous but restricted mainly to the wing-blade region of the discs (Deshpande et al. 1997). This suggests that the *sd* locus is autoregulated but that *sd* is not sufficient to drive its own activity and requires tissue-specific factors. Since *vg* is specifically expressed in the wing-blade region, we propose that Vg is one of these factors and that the Vg-Sd complexes are involved in the regulation of *sd* expression during the wing morphogenetic process. So, in sd^{ETX4}; vg^{79d5}/ vg^{+} flies, the reduction of the amount of Vg-Sd active complexes and of the resulting activity will lead to a diminution of the expression of *sd*. The amount of active complexes, as for the expression of *sd*, will progressively decrease and become insufficient giving an adult phenotype as strong as that of the homozygous vg^{BG} flies. We have shown that *ptc*-GAL4-driven expression of *vg* induces *sd* expression in regions of third instar wing discs where *sd* is not normaly expressed. We dont know if *vg* alone is able to initiate *sd* expression in the *ptc* domain or if *vg* requires the Sd protein. Indeed, both proteins are ubiquitously expressed at a low level throughout the wing disc in early second instar larvae (Williams et al. 1993). If Vg interacts with Sd to regulate *sd* expression, ectopic expression of *sd* in the *ptc* domain observed in third instar larvae could result from a process initiated at an earlier stage when *ptc*-GAL4-driven expression of *vg* occurs in a region of the wing disc where *sd* is yet to be expressed.

Our results support the hypothesis that the two proteins Vg and Sd directly interact in vivo to form a complex which could act as an active transcription factor positively regulating wing proliferation. The role of *vg* in the Vg-Sd complex is currently being tested in our laboratory.

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