Development 127, 3553-3566 (2000)
Printed in Great Britain © The Company of Biologists Limited 2000
DEV1553



# Two different activities of *Suppressor of Hairless* during wing development in *Drosophila*

**Thomas Klein[1,*], Laurent Seugnet[2], Marc Haenlin[3] and Alfonso Martinez Arias[2]**

[1]Institut für Genetik, Universität zu Köln, Weyertal 121, 50931 Köln, Germany
[2]Department of Zoology, University of Cambridge, Cambridge CB2 3EJ, UK
[3]Centre de Biologie du Developpement, Batiment 4R3, 118, route de Narbonne, 31062 Toulouse, France
*Author for correspondence (e-mail: Th.Klein@uni-koeln.de)

*Accepted 31 May; published on WWW 20 July 2000*

## SUMMARY

**The Notch pathway plays a crucial and universal role in the assignation of cell fates during development. In *Drosophila*, Notch is a transmembrane protein that acts as a receptor of two ligands Serrate and Delta. The current model of Notch signal transduction proposes that Notch is activated upon binding its ligands and that this leads to the cleavage and release of its intracellular domain (also called Nintra). Nintra translocates to the nucleus where it forms a dimeric transcription activator with the Su(H) protein. In contrast with this activation model, experiments with the vertebrate homologue of Su(H), CBF1, suggest that, in vertebrates, Nintra converts CBF1 from a repressor into an activator. Here we have assessed the role of *Su(H)* in Notch signalling during the development of the wing of *Drosophila*. Our results show that, during this process, Su(H) can activate the expression of some Notch target genes and that it can do so without the activation of the Notch pathway or the presence of Nintra. In contrast, the activation of other Notch target genes requires both Su(H) and Nintra, and, in the absence of Nintra, Su(H) acts as a repressor. We also find that the Hairless protein interacts with Notch signalling during wing development and inhibits the activity of Su(H). Our results suggest that, in *Drosophila*, the activation of Su(H) by Notch involve the release of Su(H) from an inhibitory complex, which contains the Hairless protein. After its release Su(H) can activate gene expression in absence of Nintra.**

Key words: *Notch*, Signalling, Wing, *Hairless*, *Su(H)*, *Drosophila*

## INTRODUCTION

The Notch signalling pathway plays a central role during the assignation of cell fates in *Drosophila*. For example, during the selection of neural or muscle precursors from clusters of equivalent cells, the Notch pathway is required to ensure that only some of the cells of the clusters adopt such fates (reviewed in Artavanis-Tsakonas et al., 1999). In other instances, e.g. during wing development, Notch signalling provides an inductive signal essential for the growth and patterning of the wing (Speicher et al., 1994; Couso et al., 1995; Diaz-Benjumea and Cohen, 1995; deCelis et al., 1996a; Doherty et al., 1996; Jönsson and Knust, 1996; Klein and Martinez Arias, 1998a).

The current view of signalling through Notch involves the interaction of three core elements, a DSL signal, a LNG receptor and a CSL transcription factor. In *Drosophila*, two DSL proteins are known, Delta (DL) and Serrate (Ser). Both ligands are single transmembrane proteins with several EGF-like repeats and the DSL domain in their extracellular domain (see Greenwald, 1998; and Artavanis-Tsakonas et al., 1999, for further details). They can activate the only *Drosophila* LNG receptor Notch, which is also a single transmenbrane protein with several EGF repeats in its extracellular domain. In contrast to its ligands, Notch has a large intracellular domain, which includes a region of six CDC 10-like repeats that are essential for signal transduction. There is only one CSL factor in *Drosophila*, Suppressor of Hairless (Su(H)).

The activation of Notch by its ligands results in the cleavage and release of its intracellular domain (Nintra), which, together with the nuclear protein Su(H), generates a bifunctional transcription factor required for target gene regulation in the nucleus (Blaumueller et al., 1997; Pan and Rubin, 1997; Fortini and Artavanis-Tsakonas, 1994; Jarriault et al., 1995; Lecourtouis and Schweisguth, 1998; Struhl and Adachi, 1998, Schroeter et al., 1998). In this complex, Nintra probably acts as the transactivation domain and Su(H) as the DNA-binding domain of the binary factor (Jarriault et al., 1995; Hsieh et al., 1996; Wettstein et al., 1997; Lecourtois and Schweisguth, 1998; Kidd et al., 1998; Struhl and Adachi, 1998). Recent results suggest that the cleavage of Nintra requires the function of the transmembrane protein Presenilin (Psn) (De Stroopper et al., 1999; Struhl and Greenwald, 1999; Ye et al., 1999).

Despite the many similarities that exist between Notch signalling in different species, there appear to be some differences. For example, in the absence of Notch signalling, the vertebrate CSL factor CBF1 seems to act as a suppressor of gene expression (Hsieh et al., 1996; Kao et al., 1998), but suppression of gene expression only applies to Su(H) during

formation of the midline of the embryonic CNS (Morel and Schweisguth, 2000). Further, in *Drosophila*, the product of the *Hairless* (*H*) gene can antagonize the function of Su(H) (Brou et al., 1994; Bang et al., 1995).

Here we have characterized the role of Su(H) in Notch signalling during two processes: the selection of the adult sensory precursors and the development of the wing. Our results show that during neural precursor selection, but not wing development, the activity of *Su(H)* can be mediated by the genes of *E(spl)C* alone. They also show that during wing development, Su(H) can activate expression of a subset of Notch target genes in the absence of the intracellular domain of Notch (Nintra), indicating that Su(H) can activate gene expression alone or in combination with factors other than Nintra. In other instances, Su(H) is capable of repressing gene expression; these targets, however, are activated by a fusion protein between Su(H) and the transcriptional activator VP16, suggesting that in these cases Su(H) acts in concert with Nintra, as a transcription activation domain. The results also suggest that Hairless is a suppressor of Su(H) activity during wing development. Based on our results, we suggest a model in which Su(H) function is activated by Notch through the release of Su(H) from an inhibitory complex containing Hairless.

## MATERIALS AND METHODS

### *Drosophila* stocks

The $ap^{UG035}$ allele is described in Cohen et al. (1992). The *Su(H)* alleles *AR9* and *SF8* are described in Schweisguth and Posakony (1992). $Psn^{B3}$ and $Psn^{I2}$ are null *Presinillin* mutants and were provided by Mark Fortini (Yeh et al., 1999; Lukinova et al., 1999). FRT82B$H^{E31}$ provided by F. Schweisguth is a null mutant described in Schweisguth and Lecourtois (1998). The insertion line A101 labels all sensory precursor in the imaginal discs and is described in Huang et al. (1991).

Expression of *vg* at the DV boundary was detected using the vestigial Boundary Enhancer is described in Williams et al. (1994) and referred to here as *vg*BE (for Boundary Enhancer). The *vg*-quadrant enhancer is described in Kim et al. (1996) and is referred to here as *vg*QE. For the expression of *wg* in the developing discs, we used a *lacZ* insertion in the *wg* gene on a CyO chromosome (Kassis et al, 1992). The *Dl lacZ* line is described in Klein and Martinez Arias (1998b), *E(spl)m8 lacZ* in Lecourtois and Schweisguth (1995). *E(spl)mβCD2* is a gift of S. Bray and described in Dominguez and deCelis (1998).

Ectopic expression of the different genes was achieved through the GAL4/UAS system of Brand and Perrimon (1993). The UAS construct used were the following: UAS-GFP (Yeh et al., 1995), UAS-*Su(H)VP16* (Kidd et al., 1998), UAS-*m8* (Nakao and Campos-Ortega, 1996), and UAS-*m7* (Ligoxygakis et al., 1999). The expression of the different UAS constructs was driven in the imaginal discs with various GAL4 inserts. *patched* Gal4 (*ptc*-Gal4) expresses UAS*X* in a stripe along the AP boundary of the discs (Speicher et al., 1994). In the third instar, *decapentaplegic* Gal4 (*dpp*-Gal4) (Wilder and Perrimon, 1995) is expressed in a similar pattern to *ptc*-Gal4, although the expression is weaker over the ventral side (Klein and Martinez Arias, 1998a). The *klu*-Gal4 is expressed in all territories of bristle development and is described in Klein and Campos-Ortega (1997).

Stocks carrying various GAL4 and UAS combinations in wild type and mutants were generated. All stocks were balanced over the SM6a-TM6b compound balancer, which allowed the identification of larvae of the correct genotype because of the dominant larval marker Tb. Details of the stocks as well as the stocks themselves are available upon request. In the case of *ap* mutations, stocks were established with a CyO balancer carrying a P-*lacZ* insertion in *wg* and the mutant discs were checked for the absence of the *wg* expression pattern. In the case of some experiments involving *ap* mutants, mutant discs were identified by the aberrant morphology of the wing disc and the absence of the CyO *wg-lacZ* balancer.

### Clonal analysis

Clones were induced using a UAS-Flp construct (kindly provided by N. Perrimon; Duffy et al., 1998) activated with *ptc*-Gal4. The Df(1)N81K FRT101 chromosome is a gift from K. Brennan and carries a null allele of *Notch* (Brennan et al., 1997). The y w (Ubq.GFP) (FRT101) chromosome is a gift from N. Perrimon. Both chromosomes are described in Brennan et al. (1999).

### Immunohistochemistry

The following donated antibodies: S. Cohen (anti-Wg), Sean Carroll (anti-Vg), E. Knust (anti-Ser), F. Schweisguth (anti-Su(H)), S. Bray (anti-E(spl) – mAb 323), S. Artavanis-Tsakonas (anti-Notch – C17.9C6). The Cut antibody developed by G. Rubin was obtained from the Developmental Studies Hybridoma Bank developed under the auspices of the NICHD and maintained by the University of Iowa, Department of Biological Sciences, Iowa City, IA 52242.

Staining were performed according to standard protocols. The secondary antibodies were purchased by Jackson Immuno Research Laboratories. Staining were performed according to standard protocols.

### Construction of the UAS-*Su(H)* constructs

The degree of rescue of the *Su(H)* mutant phenotype by the different lines allowed us to select a strong expresser (UAS-*Su(H)II*) and a weak one UAS-*Su(H)III*). Anti-Su(H) stainings revealed that only the second chromosomal construct can be detected slightly above the normal level of expression in third instar wing discs, if expressed with *ptc*-Gal4. UAS-*Su(H)III* produces only very low levels of *Su(H)* expression. However, as described here, these levels seems to be sufficient to provide the full function)

UAS-*Su(H)* construct were made as follows: the 2.8 kb *Hin*dIII-*Eco*RI Su(H) cDNA fragment was inserted into Bluescript KS+ (KS12, Schweisguth and Posakony, 1992) and subcloned in pUAST (Brand and Perrimon, 1993) as a *Xho*I-*Xba*I fragment. Transformant flies bearing this construct were obtained using standard procedures.

## RESULTS

To study the role of Su(H) in Notch signalling in *Drosophila*, we have established transgenic lines carrying the *Su(H)* gene under control of the yeast Gal4 targetted expression system (Brand and Perrimon, 1993; see Materials and Methods). Since *Su(H)* is expressed ubiquitously in all adult tissues (Gho et al., 1996), the expression of *Su(H)* with this system simply leads to an overexpression of the protein and consequently we shall use this term throughout this work. Lines carrying UAS-*Su(H)* were tested for functionality in two ways. First we tried to rescue the defects of *Su(H)* null mutants; secondly, we monitored the effects of overexpression of *Su(H)* on the development of the peripheral nervous system in the wild type (Fig. 1). In the absence of *Su(H)*, the wing primordium is established but does not grow and lacks the definition of a margin (Schweisguth and Posakony, 1992; Couso et al., 1995; Klein and Martinez Arias, 1998a). In the notum, lateral inhibition fails and, as a consequence, there is an excess of neural precursors in each of the proneural clusters (Schweisguth and Posakony, 1992). Expression of a weak UAS-*Su(H)* line (see Materials and

methods) in *Su(H)* mutants rescues both phenotypes (Fig. 1A-H). In one these experiments, we expressed UAS-*Su(H)* under the control of *dpp*-Gal4 and observed a rescue of the size of the wing pouch and of *wg* expression within the domain of *dpp*-Gal4 expression (Fig. 1A-D). Although we expressed *Su(H)* throughout the anterior compartment, the expression of *wg* was restricted to a narrow line of cells in the middle of the pouch, which probably corresponds to the dorsoventral (DV) boundary (Fig. 1C). This pattern is likely to reflect the interactions between the activity of *Notch*, which is restricted to the DV boundary, and *Su(H)* (Klein et al., 1998).

In another set of experiments, we used *klu*-Gal4 to express UAS-*Su(H)* in the proneural clusters of *Su(H)* mutants (see Fig. 1H; Materials and methods), and observed a strong suppression of the *Su(H)* mutant phenotypes both in neurogenesis and wing development (Fig. 1E-H). The rescue of the wing pouch is interesting, since *klu*-Gal4 is expressed only as a narrow stripe along the DV boundary (Klein and Campos-Ortega, 1997), and indicates that, for the development of the wing, the activity of *Su(H)* is only required at the DV boundary.

The observation that UAS-*Su(H)* can provide lateral inhibition function in *Su(H)* mutants led us to test if overexpression of *Su(H)* in the wild type can increase lateral inhibition. Overexpression of UAS-*Su(H)* with *klu*-Gal4 results in the loss of nearly all neural precursors and derived bristles in the notum and the wing margin of wild-type flies (data not shown). This loss of bristles is not due to a loss of proneural gene expression since Achaete is initially expressed in the notum of these discs (data not shown), and therefore must reflect an excess of lateral inhibition.

Altogether these results indicate that our *Su(H)* construct is able to mediate all aspects of *Su(H)* activity.

### Members of the *E(spl)C* affect the function of *Su(H)* during lateral inhibition

A variety of genetic results have suggested that the function of *Su(H)* during lateral inhibition is mediated by genes of the *Enhancer of split complex* (*E(spl)C*) (Jennings et al., 1994; Nakao and Campos-Ortega, 1996; Lecourtois and Schweisguth, 1995). To test whether or not this is the case, we have made use of the fact that the expression of *klu*-Gal4 is independent of *Su(H)* (see above) and thus drives expression of two members of the *E(spl)C*, *m8* and *m7*, in *Su(H)* mutant wing discs (Fig. 2). Expression of either construct alone leads to a strong suppression of the neurogenic phenotype caused by loss of *Su(H)* function, but is unable to correct the defects of wing development and patterning of this mutant (data not shown). This result demonstrates that, as suspected from loss-of-function experiments, the expression of a single gene of the *E(spl)C* is sufficient to mediate the full activity of *Su(H)* during selection of neural precursors in the adult PNS (deCelis et al., 1996b; Heitzler et al., 1996). They also show that the bHLH proteins of the *E(spl)C* do not mediate the activity of *Su(H)* during the development of the wing blade (see also deCelis et al., 1996a).

### Overexpression of Nintra and *Su(H)* lead to differential gene activation during wing development

Notch signalling relies on interactions between the intracellular domain of Notch and Su(H) (reviewed in Artavanis-Tsakonas

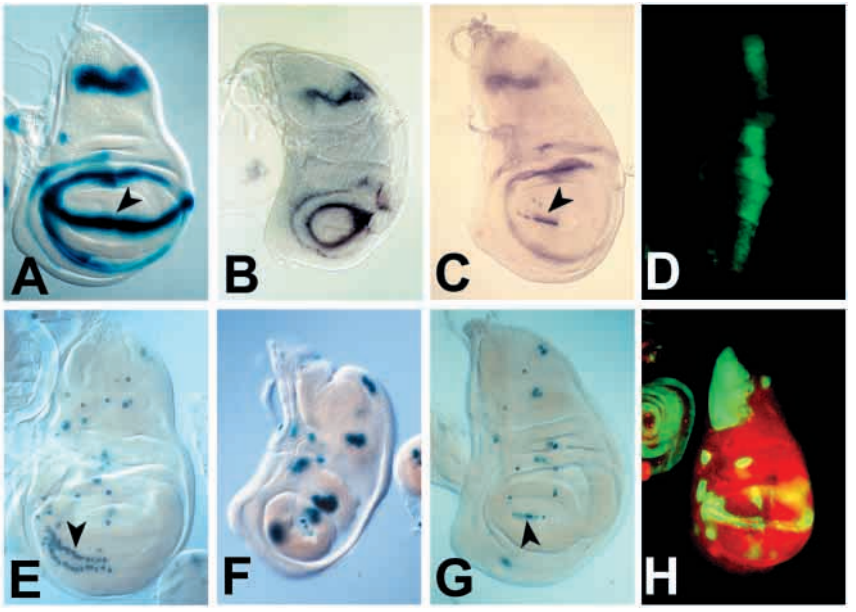


**Fig. 1**. Rescue of the defects of loss of *Su(H)* function ($Su(H)^{AR9}/Su(H)^{SF8}$) by expression of the third chromosomal UAS-*Su(H)*. The effects of expression of this line in the wild type are very weak and the level of expression is similar to that of the endogenous Su(H) (see Materials and methods). (A) Expression of *wg* in a late third instar wild-type wing disc. *wg* is expressed in two concentric rings in the hinge region and along the DV boundary, which becomes the margin (arrowhead). (B) The DV boundary expression is lost in *Su(H)* and the diameter of the circular domains are reduced in *Su(H)* mutant discs, indicating the loss of the wing margin and most of the wing pouch in these mutants. (C) Expression of the weak third chromosomal UAS-*Su(H)* with *dpp*-Gal4 in the *Su(H)* mutant wing discs recovers the margin expression of *wg* (arrowhead) at the DV boundary in the region of *Su(H)* overexpression and the diameter of the circular domains of expression in the hinge is increased, indicating the recovery of the wing pouch. Note that the margin expression is recovered only at its normal place at the DV boundary, although *Su(H)* is overexpressed in a stripe throughout the blade. This indicates that, in addition to *Su(H)*, the activity of Notch (which is restricted to the DV boundary) is required for the activation of *wg* expression. (D) Expression pattern of *dpp*-Gal4, revealed by the fluorescence of a UAS-GFP construct. *dpp*-Gal4 is expressed in a stripe along the AP boundary throughout the disc. (E) A101 staining in a late third instar wild-type wing disc revealing the SMCs (Huang et al., 1991). (F) Arrowhead points to the row of SMCs along the wing margin A101 staining in a *Su(H)* mutant disc. Instead of single cells as in the wild type, clusters of cells are stained indicating the neurogenic state. The two rows of SMCs along the margin are lost due to the lack of margin formation. (G) Expression of the weak UAS-*Su(H)* with *klu*-Gal4 rescues the neurogenic phenotype of the *Su(H)* mutants. Note the partial recovery of the SMCs along the DV boundary, indicating the recovery of the margin in these mutants (arrowhead, compare with F). The rescue of the defects of *Su(H)* mutant wing discs by expression of UAS-*Su(H)* indicates the full function of the UAS-*Su(H)* constructs. (H) Double staining of a wild-type wing disc with A101(red) and *klu*-Gal4 UAS-GFP (green). The double staining reveals that the expression domain of *klu*-Gal4 includes all regions of bristle formation.

et al., 1999) and it has been suggested that the stoichiometry of this interaction is an important element of this activity (Kidd et al., 1998). To test this in vivo, we have compared the abilities of Nintra and Su(H) on their own to elicit gene expression in a wild-type context. In these experiments, we have focused on the developing wing pouch and have monitored the expression of several targets of Notch signalling. Some of them, for example, *cut*, *wg*, *Dl*, *Ser*, the *vg*-boundary enhancer (*vg*BE) and *E(spl)m8*, are activated by Notch/Su(H) activity and one, the *vg*-quadrant enhancer (*vg*QE), is suppressed (Couso et al., 1995; Diaz-Benjumea and Cohen, 1995; Doherty et al., 1996; Kim et al., 1996; deCelis et al., 1996b; Klein and Martinez Arias, 1999). The results are summarized in Figs 2 and 3.

Surprisingly, overexpression of *Su(H)* on its own mimics only some of the effects of Nintra and can elicit ectopic expression of the members of the *E(spl)C* detected by mAb 323 (Jennings et al., 1994; Fig. 2C,D), *Dl*, *Ser* and *vg*BE (and Vg, data not shown, see Fig. 5J), also suppress the activity of the *vg*QE (Figs 2L-O, 3A-E).

We notice that, in these experiments, the overexpression of *Su(H)* induces ectopic *Ser* expression only in the dorsal half (Fig. 3B), an effect also produced by Nintra (Klein and Martinez Arias, 1998b). This effect suggests that the differences between dorsal and ventral cells are due not to the differential distribution of Fringe (Panin et al., 1997), but to intrinsic differences, which might have been inherited from the embryo (Klein and Martinez Arias, 1998b). We further observe that the overexpression of Su(H) elicits the typical overgrowth of the imaginal disc also observed with UAS-Nintra or UAS-*Dl*.

In contrast to UAS-Nintra or UAS-*Dl*, which activate Notch signalling constitutively, UAS-*Su(H)* it is not able to ectopically activate the expression of *cut*, *wg*, *E(spl)m8* and *E(spl)mβ* in the wing pouch (Fig. 2A,B,E,F,G-I,J,K). This could be interpreted as a result of different thresholds of the Nintra/Su(H) activity required for the activation of the expression of different target genes. However, in the case of *wg* and *E(spl)m8*, overexpression of *Su(H)* in the wild type does not induce their expression, even when it is expressed at high temperature (29°C, Fig. 2H), at which the Gal4 system is highly active, or with other strongly activating Gal4 lines such as *dpp*-Gal4 or *sd*-Gal4 (Fig. 2H; data not shown). Furthermore, in the case of *E(spl)m8*, *E(spl)mβ* and *cut* (*cut-lacZ* and Cut protein), UAS *Su(H)* not only fails to induce gene expression, but actively represses it (even at high expression levels, see Fig. 2B,F,H,I). These results argue against the

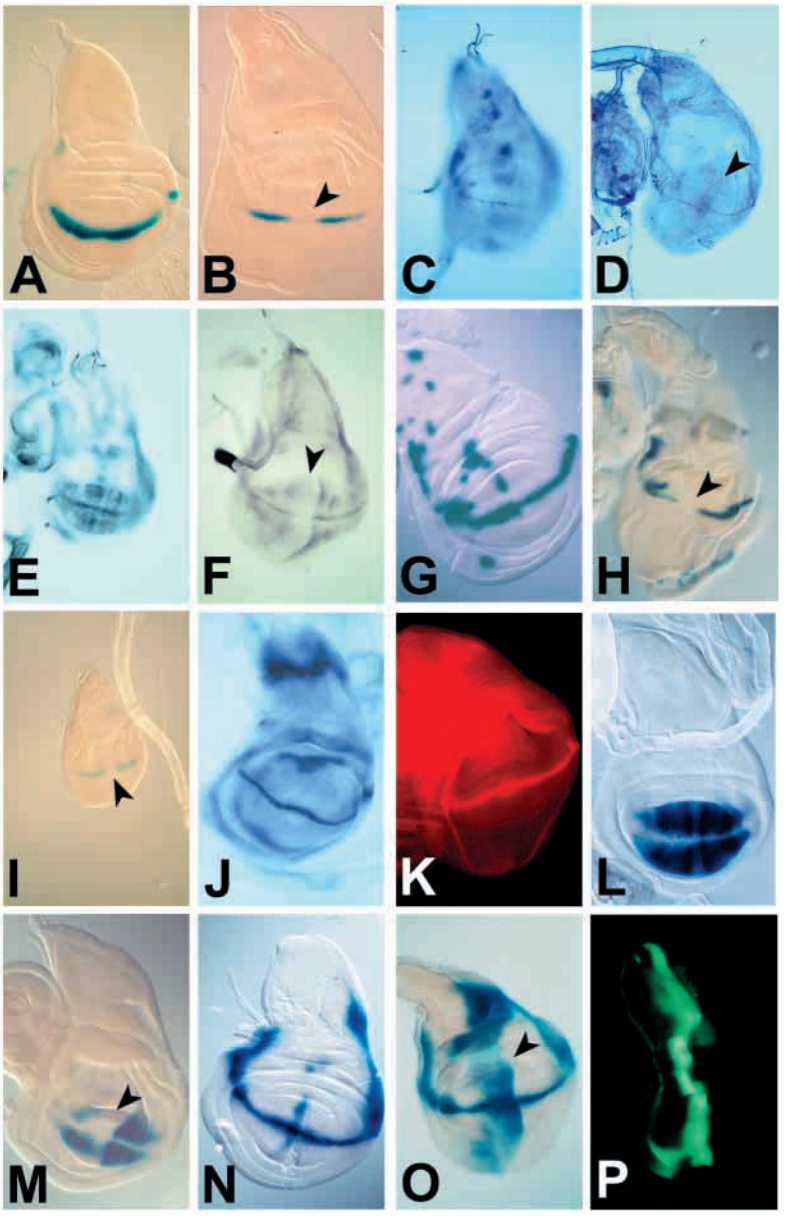


**Fig. 2**. The effects of overexpression of UAS-*Su(H)* on the expression of Notch target genes. UAS-*Su(H)* is expressed with *ptc*-Gal4 (see P) except in M where it is activated with *dpp*-Gal4 (see Fig. 1D). Both Gal4 lines are expressed in a stripe along the AP compartment boundary throughout the wing area. All discs are from late third instar larvae. (A,C,E,G,J,L,N) The normal expression pattern of *cut* $^{HZ-1}$, mAB 323, *mβ* CD2, *E(spl)m8* lacZ, *wg*, *vg*QE and *vg*BE, respectively. (B,D,F,H,K,M,O) The expression pattern of *cut* $^{HZ-1}$, mAb323, *E(spl)mβCD2*, *E(spl)m8 lacZ*, *wg*, *vg*QE and *vg*BE when UAS-*Su(H)* is overexpressed. The expression of the *vg*QE, *cut*, *E(spl) mβ CD2* and *E(spl)m8* is suppressed upon *Su(H)* expression, indicated by the arrowhead in each picture. Monitoring the expression of Cut with an anti-Cut antibody confirms the result obtained with *cut*$^{HZ-1}$ (data not shown). In contrast, the expression of *wg* is not affected (K) and that of the *vg*BE and as well as expression of *E(spl)C* genes detected by mAb323 is ectopically activated. (H) Expression of UAS-*Su(H)* is achieved at 29°C indicating that the suppression of *m8* expression occur also at very high expression levels of UAS-*Su(H)* expression. The same suppression is observed if the experiment is performed at 22°C suggesting that it is not a matter of levels of activity. (I) *E(spl)m8* expression in an early third instar disc where *Su(H)* is overexpressed. The suppression of its expression is already visible during this stage, indicating that the suppressive effect is not mediated by the downregulation of the expression of proneural genes, which are required for the correct expression of *E(spl)m8* during neurogenesis at later stages of wing development (Bailey and Posakony, 1995). Note the overproliferation of the discs caused by *Su(H)* overexpression, which is especially obvious in H. For further information see text.

possibility that the levels of *Su(H)* overexpression are not sufficient to activate the expression of certain target genes.

One simple explanation for the suppression of expression of some genes by Su(H) is that Su(H) might 'squelch' other limiting proteins from promoters and therefore leads to their inactivation (Gill and Ptashne, 1988). Since it is not known if, for example, *cut* is a direct target of Notch signalling, it is possible that Su(H) squelches a protein from its promoter, which leads to loss of *cut* expression. However, squelching has so far only been observed for promoters that do not have a binding site for the overexpressed transcription factor (Gill and Ptashne, 1988). In the case of the *vg*BE and the *E(spl)m8* promoter, it has been shown that Su(H) is required for their activation by direct binding to target sites in their control regions (Lecourtois and Schweisguth, 1995; Kim et al., 1996). Despite this requirement, *vg*BE expression is activated, whereas that of *E(spl)m8* is suppressed. Therefore, these data suggest that the differences observed in the effects of UAS-*Su(H)* and UAS-Nintra are qualitative and that Su(H) cannot activate certain promoters in the absence of Notch signalling. They further suggest that the genes whose expression is activated through Su(H) overexpression are activated by Su(H) alone. The results therefore raise the possibility that Su(H) can activate expression of some genes without Notch activation.

### Su(H) is required for the activation of all target genes of Notch signalling during wing development

The observation that some target genes of Notch signalling, e.g. *wg*, are not activated by overexpression of *Su(H)* alone, but are activated by ectopic expression of *Dl* or Nintra prompted us to test whether Notch activity might induce expression of some of these genes in the absence of *Su(H)*. Evidence for such a *Su(H)*-independent Notch pathway has recently accumulated (see e.g. Artavanis-Tsakonas et al., 1999)

It has been reported that expression of a Nintra construct under the control of the heat-shock promoter is not sufficient to rescue the defects occurring in *Su(H)* mutant discs (Bailey and Posakony, 1995). However, in these experiments, *Notch* activity is induced by a relatively short heat shock and therefore might decay before eliciting some morphological changes in the *Su(H)* mutants. Further, in this experiment, only the expression of *E(spl)* genes was monitored and not wing formation. For this reason, we repeated this experiment providing sustained expression of UAS-*Dl* and UAS-Nintra in *Su(H)* mutant discs with *dpp*-Gal4, whose expression is independent of *Su(H)* (see above and Fig. 1C). In these experiments, we never observed any effect of *Notch* activation in the absence of *Su(H)* (Fig. 4A-C). This is not likely to be due to low levels of expression since, in *ap* mutants, the same combinations are sufficient to induce a wing pouch with a margin (Klein and Martinez Arias, 1998b).

One caveat of this experiment is that Su(H) is required for the activation of the pouch-determining gene *vg* early in development (Kim et al., 1996; see above) and genes like *wg* are activated by Notch only in the pouch, under the influence of *vg* (Klein and Martinez Arias, 1998a, 1999). Therefore, it is possible that the absence of *vg* in *Su(H)* mutants obscures any possible effect of *Notch* activity in these mutants. To account for this, we coexpressed UAS-*Ser* and UAS-*vg* in *Su(H)* mutant discs. In these experiments, we also found no significant activation of *wg* expression or any significant change in the morphology of the discs (Fig. 4D,E). This failure is not due to an inefficiency of Ser to activate Notch, since its expression in *ap* mutant discs in the same way leads to a good rescue of the wing pouch and margin (Klein and Martinez Arias, 1998b). Therefore, we conclude that Su(H) is absolutely required for the activation of all Notch target genes during wing development. However, for the expression of some target genes, like *wg*, *cut* and *E(spl)m8* and *mβ*, it is not sufficient.

### A chimeric Su(H)VP16 protein can mimic Nintra

The ability of Nintra to activate genes like *wg*, *cut*, *E(spl)m8* and *mβ* suggests that Nintra is required for the activation of these genes in addition to Su(H). It is likely that in these cases Nintra is acting as a transactivation domain for Su(H), as has been proposed before (Jarriault et al., 1995; Hsieh et al., 1996; Wettstein et al., 1997; Kidd et al., 1998). One prediction of this model is that Su(H) could activate expression of these targets, if a transactivation domain is provided to it. Therefore we expressed a *Su(H)* construct bearing the viral VP16 transactivation domain (Kidd et al., 1998) in the wild type and monitored the expression of various Notch targets. We found that this *Su(H)-VP16* construct is able to ectopically activate the expression of *wg*, *cut* and the *vg*BE in a similar manner as Nintra (Fig. 4F-H). Furthermore, the expression of the *vg*QE is suppressed by this construct as when *Su(H)* is overexpressed (Fig. 4I), suggesting that this effect is mediated through a factor activated by Su(H). The effects of *Su(H)-VP16* expression on *cut* expression are especially interesting, because on its own Su(H) represses the expression of this gene. Adult wings, where *Su(H)-VP16* is expressed show ectopic margin structures, similar to these induced by Nintra (data not shown).

Altogether these results suggest that Su(H)-VP16 can mimic the activity of Nintra and further support our conclusion that Su(H) is required for the expression of all targets of Notch signalling during wing development. They also show that, in cases like *wg* and *cut*, Su(H) is not sufficient to activate

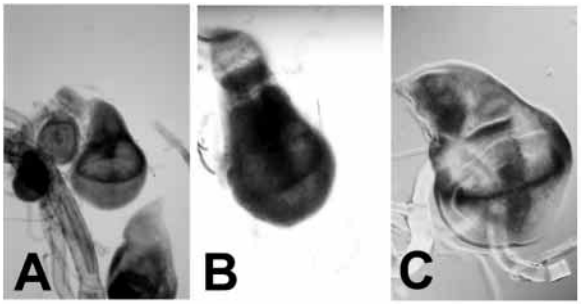


**Fig. 3**. Effects of *Su(H)* overexpression. (A-C) Overexpression is achieved with *ptc*-Gal4. (A) Normal expression of *Ser*. (B) The ectopic activation of *Ser* by *Su(H)* overexpression is restricted to the dorsal half of the wing disc, indicating the existence of intrinsic differences between dorsal and ventral cells of the wing blade (see text). (C) *Dl* expression is ectopically activated upon *Su(H)* overexpression in both dorsal and ventral cells. The normal expression of *Dl* in early third instar discs is restricted to the DV boundary, very similar to the *vg*BE (Klein and Martinez Arias, 1998b). The expression of *wg* is not ectopically activated if UAS-*Su(H)* is activated with other Gal4 lines such as *dpp*-Gal4 or *sd*-Gal4 (data not shown).

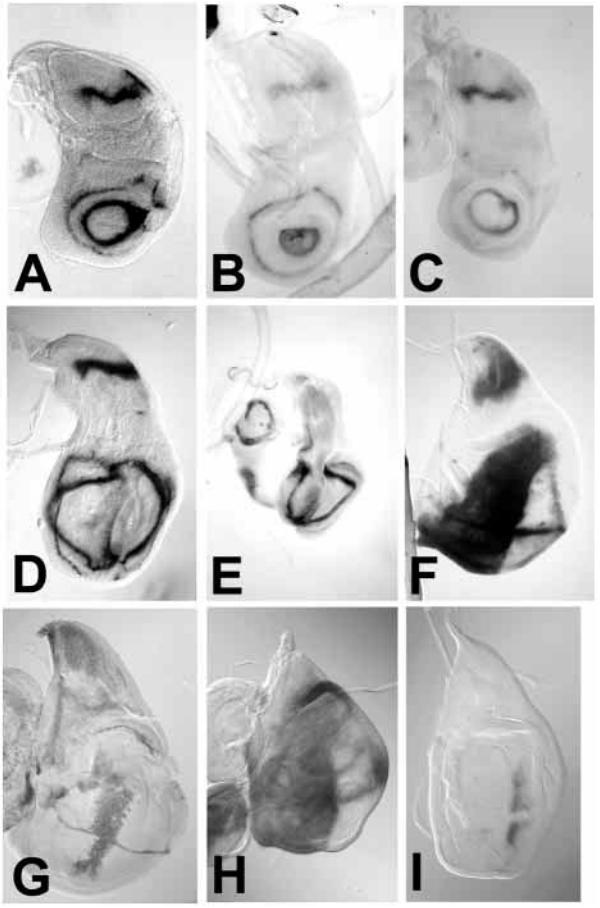


**Fig. 4**. Expression of UAS-Nintra and UAS-*Dl* in *Su(H)* $^{AR9}$*/Su(H)* $^{SF8}$ mutant wing discs with *dpp*-Gal4. *dpp*-Gal4 expression is independent of *Su(H)* activity as shown in Fig. 1. *wg* expression was detected by antibody staining in B,C,E and by in situ hybridization in A,D. (A) *Su(H)* mutant phenotype for comparison. The expression of *wg* along the DV boundary is lost and the diameter of the circular expression domains in the hinge is strongly reduced as a consequence of the loss of the wing pouch. Expression of UAS-Nintra (B) or UAS-*Dl* (C) in the mutant discs does not lead to any change in the phenotype, as well as expression of UAS-*Ser* (Klein et al., 1997). (D) Expression of UAS-*vg* in *Su(H)* mutant discs. The diameter of the circular domains is strongly increased as a result of the induction of the pouch fate in the center. This phenotype is not changed if UAS-*Ser* and UAS-*vg* are coexpressed, indicating that, even in the presence of *vg* activity, the activation of *Notch* does not lead to activation of *wg* expression in the absence of *Su(H)* activity. This result shows that, although it is not sufficient (see above), Su(H) is necessary for activation of genes like *wg*. (F-I) In contrast to *Su(H)* overexpression, expression of UAS-*Su(H)VP16* with *dpp*-Gal4 during normal development leads to the ectopic activation of the *wg* (F) and *cut* (G). It further ectopically activates the expression of the *vg*BE (H) and suppresses the activity of the *vg*QE (I) as does *Su(H)* overexpression. These results suggest that Su(H)-VP16 can elicit all effects of Nintra. For further information see text.

expression and seems to require transactivation domain, probably Nintra, as a partner.

## Su(H) can activate Notch target gene expression and induce wing development in the absence of Notch

The results presented above suggest that Su(H) can activate gene expression in the absence of Notch activity. To test this further, we first overexpressed UAS-*Su(H)* in wing discs that lack *Notch* signalling, either because of the absence of ligands for Notch, as in an *ap* mutant (Couso et al., 1995; Klein and Martinez Arias, 1998b; Klein et al., 1998), or because of a failure to process Notch effectively, as in mutants for *Presenilin* (*Psn*) (Struhl and Greenwald, 1999; Ye et al., 1999). In both cases, expression of *Su(H)* under the control of *dpp*-Gal4 elicits the development of a wing pouch as defined by the growth of the tissue and the activity of the two enhancers of *vg*, *vg*QE and *vg*BE or *vg* expression itself (Fig. 5A-J). However, in contrast to the effects of Nintra, Ser or Dl, Su(H) does not induce expression of *wg* in these wings (Fig. 6I, data not shown). In *Psn* mutant discs, where *Su(H)* is activated, *vg* expression is induced in the wing area, a trait that is not observed in *Psn* mutant discs (Fig. 5J; Ye et al., 1999).

In agreement with Ye et al. (1999), we also find that expression of Nintra with *dpp*-Gal4 leads to a rescue of the wing pouch and margin in *Psn* mutant wing imaginal discs, but expression of UAS-*Dl* does not (Fig. 5H, data not shown).

A similar rescue is observed when Su(H) is expressed in *Ser* mutant wing discs where Notch signalling is initially weaker and decays during early wing development (data not shown, Klein et al., 1998b).

Although these experiments support the conclusion that Su(H) is able to activate gene expression in the absence of effective activation or processing of Notch, it is still possible that small amounts of Nintra might be present in the different mutants due to a spontaneous cleavage of Notch. These minute amounts could be sufficient to activate gene expression in association with abundant Su(H) and elicit the observed effects in the mutant situations. To rule out this possibility, we induced *Notch* mutant clones in the domain where UAS-Su(H) is expressed and monitored the expression of the *vg*BE (Fig. 6). The *vg*BE contain a functional Su(H)-binding site and represents a direct target of *Notch/Su(H)* signalling in the wing pouch (Kim et al., 1996). We find that Su(H) is able to ectopically activate the expression of the *vg*BE and induce strong proliferation in the absence of Notch as in wild-type cells (Fig. 6A-F). Antibody stainings with an antibody directed against the intracellular domain of Notch (antibody C17.9C6, Fehon et al., 1990) show that the clones lack Notch protein (data not shown). As a control, we induced *Notch* mutant clones in wild-type wing discs, which confirmed the requirement for Notch in the activity of the *vg*BE (data not shown).

In summary, these results clearly show that Su(H) is able to activate gene expression in the absence of Notch function and without the intracellular domain of Notch.

## Hairless is an antagonist of Su(H) during wing development

Since Su(H) is expressed ubiquitously and continuously (Gho et al., 1996), the fact that Su(H) can promote transcription without the presence of Nintra suggests that the activity of Su(H) must be suppressed in the absence of Notch activation during normal development. One possible mechanism for this is the binding of an inhibitory factor. A candidate for this function is the Hairless (H) protein, which has been shown to interact with Su(H) and antagonize its DNA-binding activity (Brou et al., 1994). Furthermore, several reports show that H

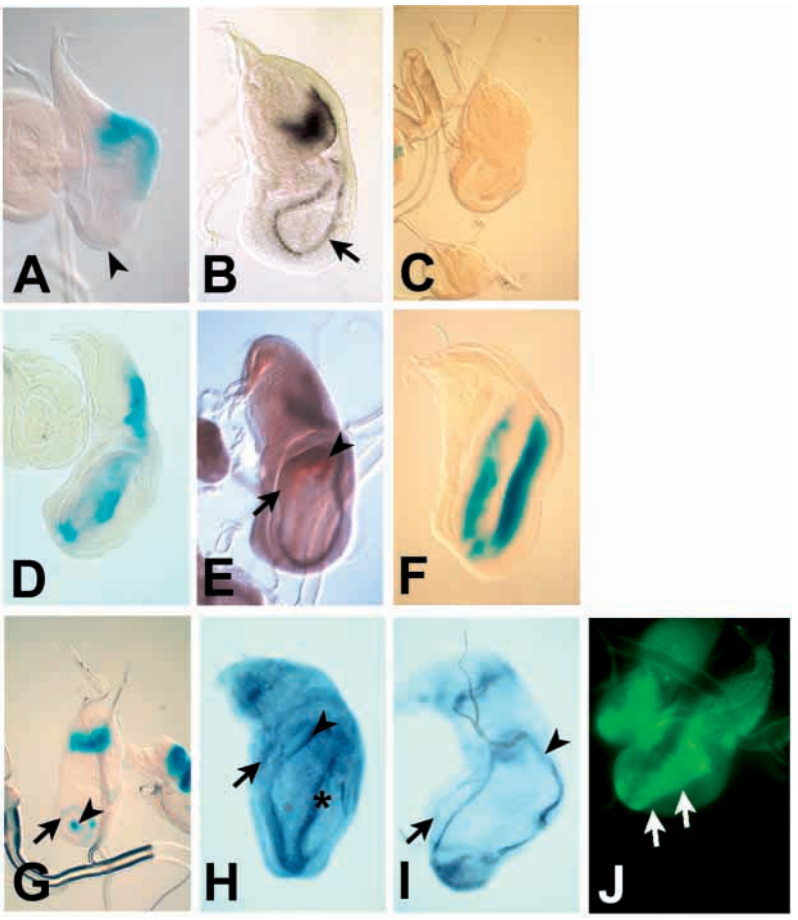


**Fig. 5.** Expression of UAS-*Su(H)* and UAS-Nintra in $ap^{UG035}$ and $Psn^{B3}/Psn^{I2}$ mutant wing discs by *dpp*-Gal4 (D-F) or *ptc*-Gal4 (H-J). (A-C) Expression of *vg*BE (A), *wg* (B) and *vg*QE (C) in a late third instar wild $ap^{UG035}$ mutant wing imaginal disc. Expression of the *vg*BE is lost in the region where the remnant of the wings are developing (arrowhead in A). (B) *wg* expression in *ap* mutant wing discs is reduced to a small circular ring of expression, which labels the residual proximal hinge region (arrow in B). Compare with the wild-type expression shown in Fig. 2J. (C) *ap* mutant wing disc carrying the *vg*QE. The enhancer is not activated if *ap* function is lost. (D) Expression of UAS-*Su(H)* in *ap* mutant leads to a recovery of the expression of the *vg*BE in the wing region where UAS-*Su(H)* is expressed. (E) Double staining of the same genotype as in D with *wg* (blue) and β-gal antibody (brown) is revealing the expression of the *vg*QE. *vg*QE (arrowhead) is activated in the region of residual *wg* expression (arrow). Furthermore, the circular domain of *wg* expression is strongly increased. The activation of *vg* and the increase of the circular expression domain of *wg* indicates the induction of a wing pouch, which normally is not present in *ap* mutant discs. However, as expected from experiments presented above, UAS-*Su(H)* is not capable of induction of *wg* expression within the developing pouch and therefore the pouch develops in the region of residual *wg* expression as revealed by the expression of the *vg*QE. (F) Expression of UAS-Nintra in *ap* mutant wing discs also activates the *vg*QE, but in a larger domain that follows the expression of Nintra. This difference can be explained by the ability of UAS-Nintra to activate *wg* expression in the developing wing pouch. Therefore, the *vg*QE is activated in the flanking region of UAS-Nintra expression. Within the actual domain of *Notch* activity, the activity of *vg*QE is suppressed as expected from our results presented in Fig. 2. For further details of the regulation of the *vg*QE, see also Kim et al. (1996) and Klein and Martinez Arias (1999). (G-J) Expression of UAS-Nintra (H) and UAS-*Su(H)* (I,J) in *Psn* mutant wing discs. (G) *wg* expression in a *Psn* mutant late third instar wing disc revealed by a P-*lacZ* insertion in *wg*. The inner circular domain of expression is reduced to a point (arrowhead), indicating the loss of all fates distal to it, such as wing pouch and margin. The diameter of the outer (proximal) ring is strongly reduced. Note that the phenotype of loss of *Psn* is stronger than that of *Su(H)* (compare with Fig. 4A). (H) Expression of Nintra results in a dramatic increase of the diameter of the circular domains of *wg* expression as well as induction of *wg* expression in the center of the induced pouch (asterix). The phenotype indicates the rescue of the wing pouch and the induction of a margin, which is determined by the activity of *wg* in the pouch (Couso et al., 1994). In contrast to Nintra, expression of UAS-Dl does not lead to a recovery of the pouch and *wg* expression, confirming the conclusion of Ye et al. (1999) (data not shown). (I) Expression of UAS-*Su(H)* in *Psn* mutant wing discs leads to a comparable increase of the diameter of the circular domains of *wg* expression but, in contrast to UAS-Nintra, not to the induction of *wg* expression within the induced pouch. As seen in J, Vg is expressed in the center of the mutant wing discs as detected by anti-Vg antibody staining (arrows). *Psn* mutant discs are normally devoid of Vg expression (Ye et al., 1999). (G-I)Arrow, outer ring; arrowhead, inner ring of *wg* expression in the hinge region of the wing.

antagonizes Notch signalling during adult PNS development (Bang et al., 1991; Bang and Posakony, 1992; reviewed in Posakony, 1994; Bang et al., 1995). To test whether H is an antagonist of Su(H) also during wing development, we first induced *H* mutant clones in the wing pouch and asked wether genes dependent only on Su(H) activity are expressed in these clones. If H regulates the activity of Su(H), the removal of H might lead to the activation of Su(H) and result in the expression of its targets, e.g. the *vg*BE and *Ser*. We find that both are ectopically activated in *H* mutant clones (Fig. 7A-G). The ectopic expression of the *vg*BE in the clones varies and is strongest near the DV boundary (Fig. 7B,C). This graded expression is possibly due to the requirement of a diffusible factor coming from the DV boundary. One candidate for this is Wg, which seems to be required for the proper expression of the *vg*BE (Klein and Martinez Arias, 1999; Zhang and Carthew, 1998). The cells in the *H* mutant clones do not express *cut* or *wg*, which are dependent on the presence of Nintra (Fig. 7H,I), suggesting that Notch is not activated in these clones. The loss of H function seems to elicit Su(H)-dependent target gene expression in the wing pouch, a region probably devoid of *Notch* activity. This suggests that the inactivation of H is sufficient to activate Su(H). To test further this conclusion, we looked at whether the activity of the *vg*BE is maintained in *H* mutant wing pouches if Notch is concomitantly removed. For this, we induced *Notch* mutant clones in *H* mutant wing discs (Fig. 8). In *H* mutant wing pouches, weak ubiquitous expression of the *vg*BE is observed throughout the whole area of the wing (Fig. 8A), confirming the clonal analysis described above. *vg*BE is also active in several *Notch* mutant clones near the DV and anteroposterior (AP) boundary (Fig. 8A-F), but the activity is not maintained in all clones. One explanation for this

might be again the requirement of other so far unidentified factors enamating from the two compartment boundaries. In agreement with this, the *vg*BE enhancer has a late expression domain along the AP boundary, suggesting an input from these areas for its proper expression. However, as seen in Fig. 8G-I, this domain is also dependent on Notch during normal development. The removal of the Su(H)-binding site in the enhancer leads to the loss of all expression domains in the wing pouch, suggesting that Su(H) is required (Kim et al., 1996). Therefore, the fact that the cells of several mutant clones do express the *vg*BE suggest that the *vg*BE can be activated in the complete absence of *Notch* activity and that the inactivation of H is sufficient to activate Su(H). In agreement with Neumann and Cohen (1996), we never found any activation of the *vg*BE in *Notch* mutant clones induced in wild-type wing pouches (see Fig. 8G-I; insert in Fig. 8I), suggesting that during wild-type development, the activity of Notch is required to activate the *vg*BE. Hence, Notch probably activates Su(H) through inactivation of H.

We next tested whether the degree of endogenous Su(H) activation that results from the removal of H is sufficient to elicit a biological effect. To assay this, we asked whether or not removal of *H* activity can induce *Su(H)*-dependent development of the pouch in wing discs in which Notch signalling is absent, such as *ap* and *Psn* mutant wing discs. We find that loss of *H* function rescues the loss of wing development of *ap* mutants (Figs 9A-H, 11): whereas *ap* mutants have no wing pouch (Ng et al., 1996; Klein and Martinez Arias, 1998b, Fig. 11A), *ap,H* double mutants have large wing pouches with no margin structures (Figs 9D, 10D). The enlarged pouch of the double mutant discs expresses *spalt* (*sal*) and the two *vg* reporters, *vg*QE and *vg*BE, all of which are expressed specifically in the wing pouch in a Notch/Su(H)-dependent manner and are not expressed in *ap* mutants (Fig. 9A-D,H). In contrast, no *wg* expression is induced in these double mutant discs (Fig. 9D), suggesting that the observed rescue is likely to be due to the activation of Su(H) in the double mutants. This is strongly supported by the fact that *Su(H),H* double mutants exhibit a small wing rudiment identical to that of *Su(H)* mutants (compare Fig. 10I with Fig. 4A). Expression of UAS-*vg* by *dpp*-Gal4 in *ap* mutant discs can recover the pouch-specific expression domain of *sal* (Fig. 9J, Klein et al., 1998a), suggesting that the activation of *vg* expression by Su(H) is responsible for the recovered *sal* expression in the *ap,H* double mutant wing discs. Similar to overexpression of UAS-*Su(H)* in *ap* mutant wing discs, the pouch in *ap,H* mutant discs develops near the residual *wg* expression in the remaining hinge (compare Figs 5E and 9C, arrowheads). As expected from the analysis of the wing discs, the pharate adult *ap; H* double mutants have large wing pouches, which are devoid of any margin like structure such as innervated bristles (Fig. 10D).

We further examined the effects of removing *H* on wing development in *Psn* mutants. As in the case of *ap*, loss of function of *H* effects a strong rescue of the wing pouch in the *Psn,H* mutant discs in comparison to the *Psn* mutant discs (Figs 5G, 9K,L). However, in this case, the morphology of the discs is more like wild type (Fig. 9L) and, in contrast to *ap,H* mutant discs, the pouch develops at its normal place (arrowhead in Fig. 9L). Closer monitoring of double mutant discs reveals some expression of *wg* and the *vg*BE along the DV boundary (Fig. 9K,L). This suggests that, in contrast to the situation of *ap* mutants, in *Psn* mutants, there is some activation of Notch and it seems that the lack of *H* activity can enhance this residual signalling of Notch at the DV boundary. This is remarkable considering that the wing phenotype caused by the loss of *Psn* is stronger than that caused by loss of *Su(H)* function (compare Fig. 4A and 5G).

Taken together, our results provide further evidence for a positive transcriptional activity of Su(H). They further show that H is an antagonist of Su(H) during early wing

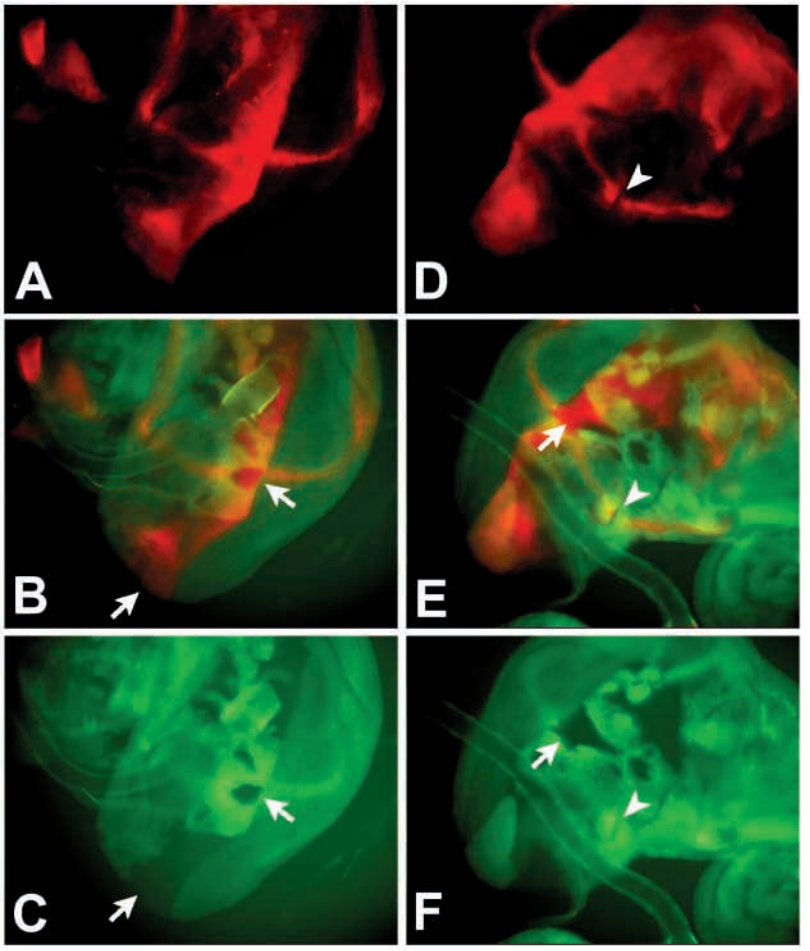


**Fig. 6**. Su(H)-dependent activation of the *vg*BE in Notch mutant clones. Clones of Df(1) *Notch*$^{81K}$ were induced by UAS-Flp expressed by *ptc*-Gal4. Df(1) *Notch*$^{81K}$ is a null allele of *Notch* (Brennan et al., 1997). Clones are recognized by the absence of GFP fluorescence. Concomitantly UAS-*Su(H)* is expressed in these discs with the ptc-Gal4. (A-C,D-F) Two examples of wing discs are shown. The genotype of these discs is: Df(1)*N*$^{81K}$ FRT101/Ubiquitin GFP FRT 101; *ptc*-Gal4/UAS-*Su(H); vg*BE/UAS-Flp. (A,D) The overexpression of Su(H) leads to the ectopic activation of the *vg*BE in the *ptc* expression domain. (C,F) The clonal areas are revealed by the loss of the GFP fluorescence and some are highlighted by the arrows. (B,E) Composite of A,C (B) and D,F (E), respectively. The arrows highlight the Notch mutant clones. The composites reveal that, within the clones, Su(H) is still able to activate the expression of the *vg*BE. This result shows that Su(H) can activate gene expression in the absence of Notch and without Nintra.

development and that it suppresses the activity of Su(H) in the absence of Notch signalling. The results also suggest that the inactivation of *H* is sufficient to activate Su(H) and that the activity of Notch is required to inactivate H during normal development.

## DISCUSSION

Here we have assessed the role of Su(H) during early wing development and shown that it is required for all aspects of Notch signalling in this process. This is important to know in the light of an increasing number of reports of the existence of an Su(H)-independent Notch signalling event (Nofziger et al., 1999; Matsuno et al., 1997; Wang et al., 1997; Brennan et al., 1999; Zecchini et al., 1999). Our results show that this pathway does not operate during early development of the wing. We have observed that Su(H) can activate expression of some Notch target genes in the absence of Notch signalling and Nintra, and that the activity of Su(H) is antagonized by H during wing development as during lateral inhibition. Furthermore, we have shown that expression of Su(H) suppresses some of the Notch target genes in absence of Notch activation, providing evidence that Su(H), like its mammalian counterpart, can act as a repressor of transcription.

Our results shed a new light on some aspects of Notch signalling, which we discuss below.

### Activation of Su(H)

Our observation that Su(H) can activate transcription of target genes in the absence of Notch suggests the existence of a mechanism that impedes this activity in vivo. Analysis of the interactions between H and Su(H) suggests that H is part of this mechanism. We observe that clones of *H* mutant cells express genes that can be activated by Su(H) without Notch activity and that the loss of *H* activity in *ap* mutant discs leads to a formation of a wing pouch, in a Su(H)-dependent manner. The activation of some Notch target genes in *H* clones is important, since the clonal analysis presented here shows that it occurs even in the absence of *Notch* activity. Therefore, the loss of *H* activity seems to be sufficient to activate Su(H). Our clonal analysis shows that removal of *H* leads to the activation of Su(H)-dependent gene expression in the absence of Notch. This indicates that the inactivation of *H* leads to the activation of Su(H). Furthermore, our analysis reveals that the activation of the *vg*BE requires the activity of Notch during wild-type

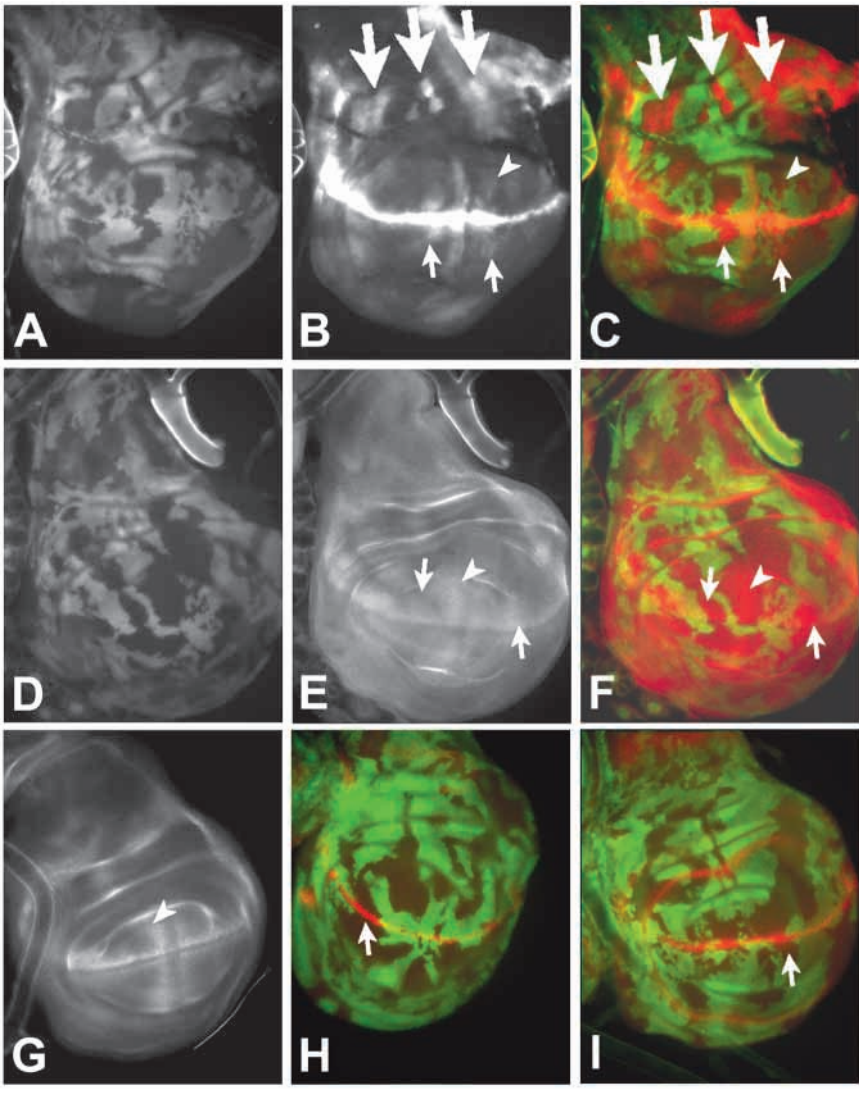


**Fig. 7**. Loss of *H* activity leads to the activation of expression of genes that are solely dependent on Su(H) in the wing pouch. Clones of $H^{E31}$ indicated by the loss of GFP fluorescence. $H^{E31}$ is a null allele described in Schweisguth and Lecortouis (1998). (A-C) Expression of the *vg*BE. (A) Clones labeled by loss of GFP staining. (B) The expression of the *vg*BE. Smaller arrows, region of ectopic activation of the *vg*BE; arrowhead, a clone that does not touch the DV boundary. Note that the ectopic activation gets weaker with distance from the DV boundary. Large arrows, regions of ectopic activation in the hinge (compare with Fig. 2N). (C) Composite of A and B reveals that the ectopic expression of the *vg*BE falls within the clonal area. Arrowhead, the same clone as in B, showing *vg*BE expression which does not touching the DV boundary. This excludes the possibility that the ectopic activation of the *vg*BE occurs through a spreading of expression from the DV boundary. (D-F) Expression of *Ser* in *H* mutant clones. (D) The clones are marked by the absence of GFP. (E) Expression of *Ser* expands in certain regions (arrows, arrowhead). Compare with the normal *Ser* expression (G). (F) The composite of D and E reveals that this expansion of *Ser* expression occurs within the clones. Arrows and arrowhead indicate the same regions as in E. (G) *Ser* expression in a wild-type wing disc. (H,I) The loss of H in clones does not ectopically activate genes, which require Nintra in addition to Su(H), such as *cut* (H) or *wg* (I). The arrow indicates areas where mutant clones include the DV boundary. In no case, we observe ectopic activation of expression of these genes. The data suggest that the loss of H leads to the activation of Su(H), even in areas devoid of *N* activity.

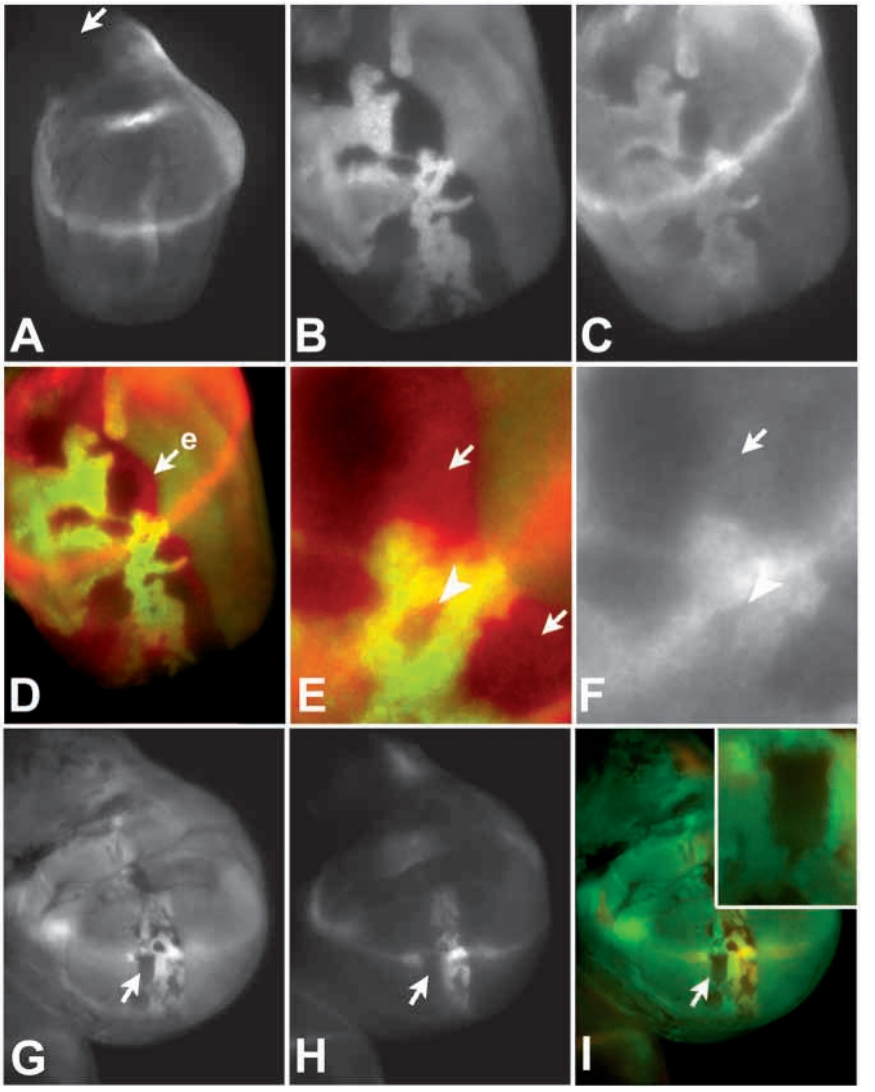


**Fig. 8**. Clonal analysis of a *Notch* loss-of-function mutant in $H^{E31}$ mutant wing pouches. Clones were induced in the same way as in Fig. 6. (A) The activity of *vg*BE in a $H^{E31}$ mutant wing disc. *vg*BE is weakly active throughout the whole area of the wing. Arrow points to the notum, where the activity is absent. (B) Notch mutant clones in a $H^{E31}$ mutant wing pouch visible by the loss of fluorescence. (C) Expression of the *vg*BE in the same disc as shown in B. (D) Composite of B and C reveals that in several Notch mutant clones the vgBE (in red) is expressed in certain areas of the clones, labelled by the absence of the green fluorescence (arrow). (E) Magnification of the area labelled 'e' (D). The arrows indicate two clones where the *vg*BE (in red) is expressed in the region of the clone near the DV boundary. The arrowhead points to a clone near the DV boundary. (F) Magnification of the same area as in E is showing only the expression of the *vg*BE in this region. Arrowhead highlights the same region as in E. The comparison reveals that the expression of the *vg*BE in the *Notch* clone is weaker but not abolished. (G,H) Induction of Notch mutant clones in wild-type wing pouches as in A-F. (G) Clones revealed by the absence of green fluorescence. (H) The red channel shows the expression of the vgBE. (I) Composite of G and H. The arrow in G,H point to a large clone at the DV boundary. The area of this clone is enlarged in the insert in I. No expression of the *vg*BE (red) is found in the clonal area (loss of green), suggesting that Notch is required to activate the *vg*BE in wild-type discs.

development (Neumann and Cohen, 1996; see above), suggesting that Notch activation causes the inactivation of *H* and that this inactivation result in the activation of Su(H) (Fig. 11). The behavior of *H* mutant cells is not compatible with a simple function of H in defining a threshold for Notch signalling activity as has been proposed (Bang et al., 1995). If H only defines a simple threshold, one would expect that its removal in regions devoid of Notch activity would not lead to activation of Notch target genes. H can associate with Su(H) and this association prevents Su(H) from binding to its DNA target site in vitro experiments (Brou et al., 1995). This suggests that H might inactivate Su(H) through a physical association in vivo and that the activation of Su(H) occurs through its release from this inhibitory complex. The above discussed results further strongly suggest that this release might be mediated by Nintra, since it can activate the expression of all Notch-dependent genes. How Nintra achieves this is at present not clear and will require further biochemical experiments. Two observations, however, support our conclusion. First, it has been reported that Nintra can physically associate with H in vitro (Wang et al., 1997). Secondly, we have shown here that, in *Psn* mutants, loss of function of *H*, as well as expression of Nintra but not overexpression of *Su(H)*, leads to the expression of *wg*, a target of Notch signalling that requires Nintra and Su(H). This suggests that the small amounts of Nintra that exist in a *Psn* mutant cannot interact with Su(H), even when there is abundant Su(H). In contrast, elimination of *H* can stimulate the not so abundant endogenous Su(H) to interact with these small amounts. Both results are in agreement with the suggested mode of Su(H) activation by Notch signalling through inactivation of *H* by Nintra and raise the possibility that Nintra inactivates *H* by direct physical interactions.

### Regulation of target gene expression by Su(H)

Our results show that overexpression of Su(H) leads to three different responses: (1) activation, as is the case for *vg*, some *E(spl)* genes, *Dl* and *Ser*; (2) inactivation, as shown for *cut* and *E(spl)m8*; or (3) no effect, as is the case for *wg*. This differential behavior is, at least in some cases, a consequence of direct binding of Su(H) to the promoters: The *vg*BE as well as the *E(spl)* genes contain Su(H)-binding sites to which Su(H) binds in vitro experiments and which are necessary for their activation in vivo (Bailey and Posakony, 1995; Lecortouis and

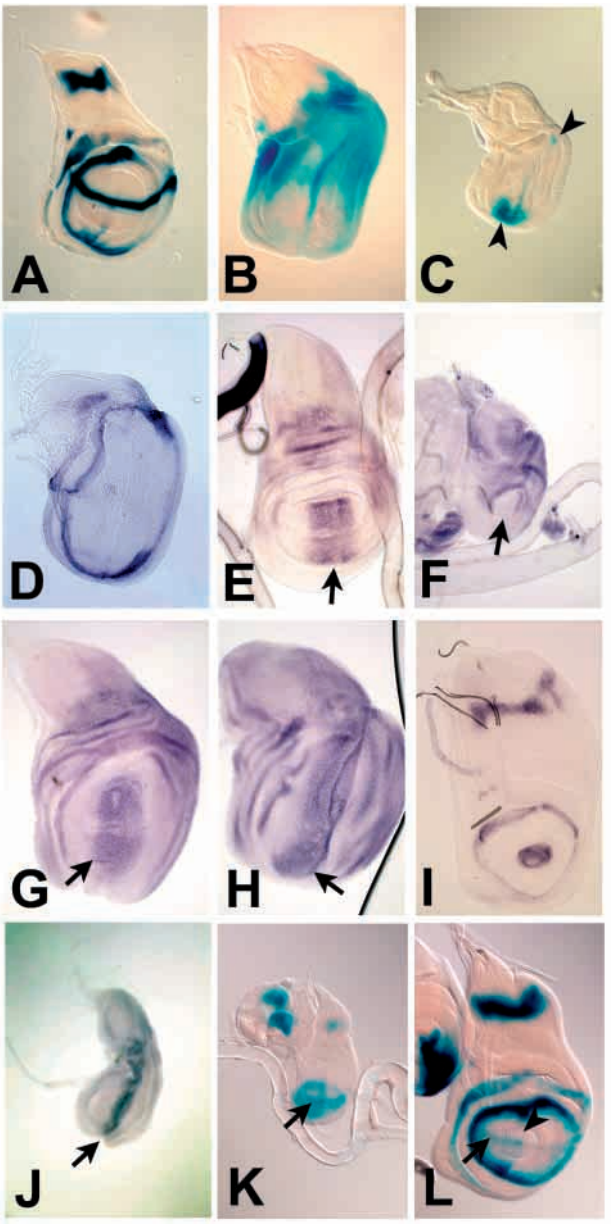


**Fig. 9**. Analysis of the $ap^{UG035}$; $H^{E31}$ and $Psn^{B3}$ $H^{E31}$/$Psn^{I2}$ $H^{E31}$ mutant phenotypes in the wing imaginal disc. (A) *wg* expression in a late third instar wing imaginal disc. (B) Expression of the *vg*BE in a $ap^{UG035}$; $H^{E31}$ mutant wing disc. Strong expression is observable in a stripe running in a DV direction. Weak staining is seen on both sides of this stripe. Expression of this enhancer is normally absent in the wing region in *ap* mutant discs (Klein and Martinez Arias, 1998a; data not shown). (C) Expression of the *vg*QE in $ap^{UG035}$; $H^{E31}$mutant discs is recovered at the dorsal and ventral edges of the wing area near the residual *wg* expression domain (compare with Fig. 5E). As shown in Fig. 5C, the *vg*QE is usually not active in *ap* mutant wing discs. (D) The diameter of the circular hinge domain of *wg* expression is strongly increased in the double mutants. Note that the expression is still missing in the recovered pouch suggesting a margin has not formed as expected if only *Su(H)* is activated. The reactivation of *vg* expression in the $ap^{UG035}$; $H^{E31}$ double mutant discs indicate that the wing pouch fate has been recovered. (E) Wild-type *sal* expression in a late third instar wig disc. *sal* is expressed in a broad stripe in the middle of the wing pouch (arrow). (F) This domain is missing in *ap* mutants as a consequence of the loss of the wing pouch (arrow). (G) A $H^{E31}$ mutant wing disc showing expression of *sal* in the wing pouch similar to wild type (arrow). (H) In the $ap^{UG035}$; $H^{E31}$ mutant wing discs, the *sal* expression is recovered (arrow), which further indicates the recovery of the wing pouch. (I) *wg* expression in a $Su(H)^{SF8}$/$Su(H)^{AR9}$; $H^{E31}$ double mutant wing imaginal disc is the same as in $Su(H)^{SF8}$/$Su(H)^{AR9}$ discs (compare with Fig. 4A), indicating that the *Su(H)* phenotype is epistatic over that of *H*. The epistatic relationship suggests that the phenotype caused by loss of *H* is mediated by *Su(H)* activity and that therefore the rescue of the wing pouch observed in the $ap^{UG035}$; $H^{E31}$ mutants wing discs is due to an activation of *Su(H)*. (J) Expression of UAS-*vg* in an *ap* mutant wing disc can recover the expression domain of *Sal* (arrow). This suggests that the observed recovery of *Sal* expression in $ap^{UG035}$; $H^{E31}$ is due to the activation of *vg* expression (see B,C) by Su(H). The lack of activation of *wg* expression in the double mutants further supports this genetic sequence, since, as shown above, *Su(H)* activity is not sufficient for activation of *wg* expression. (K) *wg* expression in an early third instar $H^{E31}Psn^{B3}$/$H^{E31}$ $Psn^{I2}$ mutant wing disc. See Fig. 6 for a comparison with the *Psn* mutant phenotype. Arrow points to the recovered expression at the DV boundary. (L) Late third instar disc of the same genotype as in K. The rescue of the *Psn* mutant phenotype is now obvious through the enlargement of the inner ring of *wg* expression in the hinge, the presence of a small round wing pouch (arrowhead), and a weak expression of *wg* along the DV boundary (arrow). The residual pouch has, in contrast to the $ap^{UG035}$; $H^{E31}$ situation, developed at its normal location (arrowhead), suggesting that, in this case, the early development is normal. This is confirmed by the normal expression of *wg* along the DV boundary in early third instar $Psn^{B3}$/$Psn^{I2}$; $H^{E31}$ double mutant discs shown in K. Therefore, in contrast to the *ap; H* double mutant, *Psn* mutants still have residual activity of *Notch*, which is sufficient to drive early wing development in a sensitized background. Note that the wing phenotype of *Psn* mutant disc is more severe than that of *Su(H)* mutants (compare Fig. 4A with Fig. 5G).

Schweisguth, 1995; Kim et al., 1996; Nellesen et al., 1999). Despite that, they react differently towards *Su(H)* overexpression. Since *E(spl)m8*, which is suppressed by Su(H) overexpression, can be activated by expression of Su(H)VP16 or Nintra, we conclude that Nintra is required in addition to Su(H) to activate *E(spl)m8* expression. Our results suggest that, in this case, Nintra probably acts as an activation domain of a dimeric transcription factor containing Su(H), as has been proposed (Jarriault et al., 1995; Hsieh et al., 1996; Wettstein et al., 1997; Lecourtois and Schweisguth, 1998; Kidd et al., 1998; Struhl and Adachi, 1998; Schroeter et al., 1998). From this, it follows that Nintra might have two function during a Notch signalling event: first it inactivates H, which leads to the release of Su(H) and then, in some instances, it provides the transactivation domain for free Su(H) to activate the expression of target genes (Fig. 11).

Flies carrying reporter *lacZ* constructs with up to 12 Su(H)-binding sites do not display any activity in the wing disc (Go et al., 1998). This suggests that Su(H) (even in association with Nintra) is not sufficient to activate transcription and requires other collaborating factors. It further suggests that, even in promoters that can be activated by Su(H) in the absence of Nintra, Su(H) probably interacts with other factors to promote gene expression. This is confirmed by a study of the *vg*BE. Although the Su(H)-binding site is absolutely necessary for its activity, other sites are equally important (Kim et al., 1997; Klein and Martinez Arias, 1999). So far the factors that bind

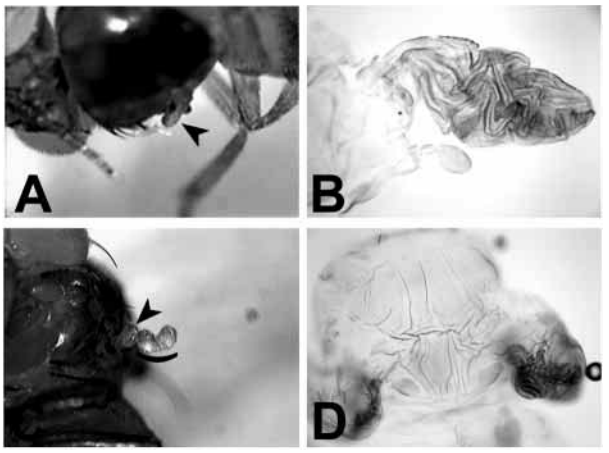


**Fig. 10**. Phenotype of $ap^{UG035}$; $H^{E31}$ double mutant pharate adult flies. (A) The wings of $ap^{UG035}$ mutant flies are reduced to a small stump of proximal hinge tissue (arrowhead). (B) $H^{E31}$ pharate adults have normal looking wings with bristle defects at the anterior margin. (C) The $ap^{UG035}$ phenotype can already be weakly rescued if one copy of *H* is reduced (genotype $ap^{UG035}$; $H^{E31}$/+) and, as a consequence, the stump is longer with a small winglet developing at its tip (bracket). (D) In a $ap^{UG035}$; $H^{E31}$ double mutant pharate, large wing pouches are developing, which are devoid of any margin. The lack of a wing margin is predicted from the results of the analysis of the double mutant wing imaginal discs described in Fig.9.

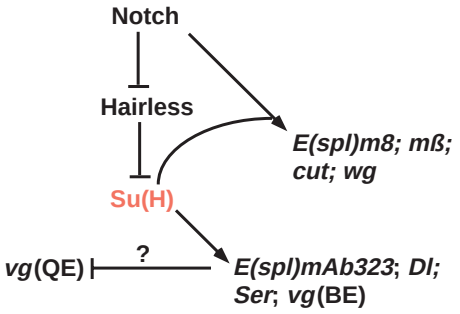


**Fig. 11.** Formal relation deduced from the presented experiments. The activation of Notch leads to the suppression of the activity of Hairless and thereby relieves the inhibition imposed on Su(H). The result of this double-negative mechanism is the activation of Su(H), which can then activate *vg*, *Dl*, *Ser* and some genes of the *E(spl)C* and suppress the *vg*QE without further help of Notch. In contrast, the genes, *wg*, *cut*, *E(spl)m8* or *E(spl) mβ*, require the combined activity of Su(H) and Nintra for their induction of expression.

to these sites are not identified. The dependence of Su(H) on these others factors is probably the reason for the differential expression of Notch target genes in *H* and *H/N* mutant clones that we have observed.

Recently it has been shown that Su(H) acts as a suppressor of *sim*-transcription during the formation of the midline cells in the embryonic central nervous system of *Drosophila* (Morel and Schweisguth, 2000). This observation provided the first evidence that Su(H), like its mammalian counterpart CBF1, can act as a suppressor of transcription. The inactivation of the *cut* and *E(spl)m8* expression in absence of Nintra suggests that Su(H) can act as a suppressor of gene expression also during adult development and provides further evidence for a suppressing activity of Su(H). However, we show here that this suppression is context dependent and not a general feature of Su(H). This context dependency might also exist for CBF1, since only the reaction of a small number of genes towards its activity has been tested so far and it is possible that some target genes can be activated by CBF1 in the absence of Nintra in a similar way, as we have here shown for Su(H). In summary, these results suggest that the consequence of the binding of Su(H) to a promoter is dependent on its local architecture and, therefore, Su(H) can at the same time activate and suppress gene expression, like many other transcription factors, such as the mammalian WT1 gene (see e.g. Little et al., 1999).

The removal of both the maternal and zygotic expression of H during embryogenesis seems to have no consequence for the embryo (Schweisguth and Lecourtois, 1998). Since the overactivation of Notch/Su(H) signalling during embryogenesis has deleterious consequences (Struhl et al., 1993, Rebay et al., 1993; Lieber et al., 1993), this observation contradicts our conclusion that H is required to inactivate Su(H). However, the context dependency and differential reaction of the target genes observed during wing development offer several explanations for this discrepancy, without having to postulate an unknown factor, which can functionally replace H. First, it is likely that the interacting factors, which are required for gene expression in concert with Su(H), are different during embryogenesis and this could modulate the responsiveness of the target promoters. This conclusion is supported by the observation that the genes of *E(spl)C*, although probably all require Su(H) for their expression, are all very similar expressed in the embryo, but their expression pattern in the wing imaginal disc is very different (Campos-Ortega, 1993; deCelis et al., 1996a; Nellesen et al., 1999). Another explanation is that the target promoters of Su(H) during embryogenesis might be all of the type, which require the additional activity of Nintra. Therefore they would stay inactive even in the presence of free Su(H) until Notch is activated.

Thanks to A. Lüschnig, S. Cohen, S. Carroll, J. deCelis, E. Knust, T. Lieber, M. Young, S. Bray, F. Schweisguth, K. Tamura, M. Fortini, N. Perrimon and J. A. Campos-Ortega for supplying material crucial for this work. We would further like to thank F. Schweisguth for his critical comments on the manuscript. This work was supported by the Wellcome Trust (T. K. and A. M. A.), The Deutsche Forschungs-Gemeinschaft (T. K.) and Ligue Nationale contre le Cancer, Association pour la Recherche contre le Cancer (ARC) (L. S. and M. H.).

## REFERENCES

**Artavanis-Tsakonas, S., Rand, M. D. and Lake, R. L.** (1999). *Notch* signaling: cell fate control and signal integration in Ddevelopment. *Science* **284**, 770-776

**Bailey, A. M. and Posakony, J. W.** (1995). Suppressor of Hairless directly activates transcription of *Enhancer of Split Complex* genes in response to *Notch* receptor activity. *Gen. Dev.* **9**, 2609- 2622.

**Bang, A. G., Hartenstein, V. and Posakony, J. W.** (1991). *Hairless* is required for the development of adult sensory organ precursor cells in *Drosophila*. *Development* **111**, 89- 104

**Bang, A. G. and Posakony, J. W.** (1992). The *Drosophila* gene *Hairless* encodes a novel basic protein that controls alternative cell fates in adult sensory organ development. *Gen. Dev*. **6**, 1752-1769

**Bang, A. G., Bailey, A. M. and Posakony, J. W.** (1995). *Hairless* promotes stable commitment to the sensory organ precursor cell fate by negatively

regulating the activity of the *Notch* signaling pathway. *Dev. Biol*. **172**, 479-494

**Blaumueller, C. M., Qi, H., Zagouras, P. and Artavanis-Tsakonas, S.** (1997). Intracellular cleavage of Notch leads to a heterodimeric receptor on the plasma membrane. *Cell* **90**, 281- 291

**Brand, A. and Perrimon, N.** (1993). Targeted expression as a means of altering cell fates and generating dominant phenotypes. *Development* **118**, 401-415

**Brennan, K., Tateson, R., Lewis, K. and Martinez Arias, A.** (1997). A functional Analysis of *Notch* Mutations in *Drosophila*. *Genetics* **147**, 177-188

**Brennan, K., Tateson, R., Lieber, T., Couso, J. P., Zecchini, V. and Martinez Arias, A.** (1999). The *Abruptex* mutations of *Notch* disrupt the establishment of proneural clusters in *Drosophila*. *Dev. Biol*. **216**, 230-242

**Brou, C., Logeat, F., Lecoutois, M., Vandekerckhove, J., Kourilsky, P., Schweisguth, F. and Israel, A.** (1994). Inhibition of the DNA-binding activity of *Drosophila* Supressor of Hairless and of its human homolog, KBF2/RBP-jk, by direct protein-protein interaction with *Drosophila* Hairless. *Genes Dev.* **8**, 2491- 2503

**Campos-Ortega, J. A.** (1993). Early neurogenesis in *Drosophila melanogaster*. In *The Development of Drosophila melanogaster* Vol. II. (ed. M. Bate and A. Martinez Arias). pp. 1091-1129 Cold Spring Harbor: Cold Spring Harbor Press.

**Couso, J. P., Knust, E. and Martinez Arias, A.** (1995). *Serrate* and *wingless* cooperate to induce *vestigial* gene expression and wing formation in *Drosophila*. *Curr. Biol.* **5**, 1437-1448

**deCelis, J., Garcia-Bellido, A. And Bray, S.** (1996a). Activation and function of *Notch* at the dorsal-ventral boundary of the wing imaginal disc. *Development* **122**, 359-369

**deCelis, J. F., deCelis, J., Ligoxygakis, P., Preiss, A., Delidakis, C. and Bray, S.** (1996b). Functional relationships between *Notch*, *Su(H)* and the bHLH genes of the *E(spl)* complex: the *E(spl)* genes mediate only a subset of *Notch* activities during imaginal development. *Development* **122**, 2719-2728

**Cohen, B., McGuffin, M. E., Pfeifle, C. Segal, D. and Cohen, S. M.** (1992). *apterous*: a gene required for imaginal disc development in *Drosophila* encodes a member of the LIM family of developmental regulatory proteins. *Genes Dev*. **6**, 715-729

**de Strooper, B., Annaert, W., Cupers, P., Saftig, P., Craessaerts, K., Mumm, J. S., Schroeter, E. H., Schrijvers, V., Wolfes, M. S., Ray, W. J., Goate, A. and Kopan, R.** (1999). A presinilin-1-dependenty-secretase-like protease mediates release of *Notch* intracellular domain. *Nature* **398**, 518-522

**Diaz-Benjumea, F. J. and Cohen, S. M.** (1995). *Serrate* signals through *Notch* to establish a *wingless*-dependent organizer at the dorsal/ventral compartment boundary of the *Drosophila* wing. *Development* **121**, 4215-4225

**Doherty, D., Feger, G., Younger-Sheperd, S., Jan, L. Y. and Jan, Y. N.** (1996). *Delta* is a ventral to dorsal signal complementary to *Serrate*, another *Notch* ligand, in *Drosophila* wing formation. *Genes Dev*. **10**, 421-434

**Dominguez, M. and deCelis, J. F.** (1998). A dorsal/ventral boundary established by Notch controls growth and polarity in the Drosophila eye. *Nature* **386**, 276-278

**Duffy J. B., Harrison D. A. and Perrimon N.** (1998). Identifying loci required for follicular patterning using directed mosaics. *Development* **125**, 2263-2271

**Fehon, R. G., Kooh, P. J., Rebay, I., Regan, C. L., Xu, T., Muskavitch, M. A. and Artavanis-Tsakonas, S.** (1990). Molecular interactions between the protein products of the neurogenic loci Notch and Delta, two EGF-homologues of *Drosophila*. *Cell* **61**, 523-534

**Fortini, M. E. and Artavanis-Tsakonas, S.** (1994). The *Suppressor of Hairless* protein participates in *Notch* receptor signalling. *Cell* **79**, 273-282

**Greenwald, I.** (1998). Lin-12/Notch signaling: lessons from worms and flies. *Genes Dev.* **12**, 1751-1762

**Gho, M., Lecourtois, M., Geraud, G., Posakony, J. W. and Schweisguth, F.** (1996). Subcellular localization of Suppressor of Hairless in *Drosophila* sense organ cells during *Notch* signalling. *Development* **122**, 1673- 1682

**Gill, G. and Ptashne, M.** (1988). Negative effect of the transcriptional activator GAL4. *Nature* **334**, 721-724

**Go, M. J., Eastman, D. S. and Artavanis-Tsakonas, S.** (1998). Cell proliferation control by Notch signaling in *Drosophila* development. *Development* **125**, 2031-2040.

**Heitzler, P., Bourouis, M., Ruel, L., Carteret, C. and Simpson, P.** (1996). Genes of the *Enhancer of split* and *achaete-scute* complexes are required for a regulatory loop between *Notch* and *Delta* during lateral signalling in *Drosophila*. *Development* **122**, 161- 171

**Hsieh, J. J., Henkel, T., Salmon, P., Robey, E., Peterson, M. G. and Hayward, S. D.** (1996). Truncated mammalien Notch1 activates CBF1/RBPJk-repressed genes by a mechanism resembling that of Epstein-Barr virus EBNA2. *Mol. Cell. Biol*. **16**, 952-959

**Huang, F., Dambly-Chaudiere, C and Ghysen, A.** (1991). Emergence of sense organs in the wing disc of *Drosophila*. *Development* **111**, 1087-1096

**Jarriault, S., Brou, C., Logeat, F., Schroeter, E. H., Kopan, R. and Israel, A.** (1995). Signalling downstream of activated mammalien *Notch*. *Nature* **377**, 355- 358

**Jennings, B., Preiss, A., Delidakis, C. and Bray, S.** (1994). The *Notch* signalling pathway is required for *Enhancer of split* bHLH protein expression during neurogenesis in the *Drosophila* embryo. *Development* **120**, 3537- 3548

**Jönsson, F. and Knust, E.** (1996). Distinct functions of the *Drosophila* genes *Serrate* and *Delta* revealed by ectopic expression during wing development. *Dev. Genes Evol. 206*, 91-101

**Kassis, J.A., Noll, E., Van Sickle, E., Odenwald, W. and Perrimon, N.** (1992). Altering the insertional specificity of a *Drosophila* transposable element. *Proc. Nat. Acd. Sci. USA* **89**, 1919-1923.

**Kao, H. Y., Ordentlich, P., Koyano-Nakagawa, N., Tang, Z., Downes, M., Kintnere, C., Evans, R. M. and Kadesch, T.** (1998). A histone deacetylase corepressor complex regulates the Notch signal transduction pathway. *Genes Dev.* **12**, 2269-2277

**Kidd, S., Lieber, T. and Young, M. W.** (1998). Ligand-induced cleavage and regulation of nuclear entry of *Notch* in *Drosophila melanogaster* embryos. *Gen. Dev.* **12**, 3728-3740

**Kim, J., Sebring, A., Esch, J., Kraus, M. E., Vorwerk, K., Magee, J. und Carroll, S. B.** (1996). Integration of positional signals and regulation of wing formation and identity by *Drosophila vestigial* gene. *Nature* **382**, 133-138

**Kim, J., Magee, J. and Carroll, S. B.** (1997). Intercompartmental signalling and the regulation of vestigial expression at the dorsoventral boundary of the developing *Drosophila* wing. *Cold Spring Harbor Symposium Quant. Biol.* **62**, 283-291

**Klein, T. and Campos-Ortega, J. A.** (1997). *klumpfuss*. a *Drosophila* gene encoding a member of the EGR family of transcription factors, is involved in bristle and leg development. *Development* **124**, 3123-3134

**Klein, T., Brennan, K. and Martinez Arias, A.** (1997). An intrinsic dominant negative activity of *Serrate* that is modulated during wing development in *Drosophila*. *Dev. Biol*. **189**, 123-134

**Klein, T. and Martinez Arias, A.** (1998a). Different spatial and temporal interactions between *Notch*, *wingless* and *vestigial* specify proximal and distal pattern elements of the wing in *Drosophila*. *Dev. Biol*. **194**, 196-212

**Klein and Martinez Arias, A.** (1998b). Interactions among *Delta*, *Serrate* and *Fringe* modulate *Notch* activity during *Drosophila* wing development. *Development* **125**, 2951-2962

**Klein, T. and Martinez Arias, A.** (1999). The Vestigial gene product provides a molecular context for the interpretation of signals during the development of the wing in *Drosophila*. *Development* **126**, 913- 925

**Lecourtois, M. and Schweisguth, F.** (1995). The neurogenic *suppressor of hairless* DNA-binding protein mediates the transcriptional activation of the *enhancer of split complex* genes triggered by *Notch* signalling. *Genes Dev.* **9**, 2598-2608

Lecourtois, M. and Schweisguth, F. (1998). Indirect evidence for *Delta*-dependent intracellular processing of *Notch* in *Drosophila* embryos. *Curr. Biol.* **8**, 771-774

**Lieber, T., Kidd, S., Alcamo, E., Corbin, V. And Young, M. W.** (1993). Antineurogenic phenotypes induced by truncated *Notch* proteins indicate a role in signal transduction and may point to a novel function of *Notch* in the nuclei. *Genes Dev*. **7**, 1949-1965

**Ligoxygakis, P., Bray, S. J., Apidianakis, Y. and Delidakis, C.** (1999). Ectopic expression of individual *E(spl)* genes has differential effects on different cell fate decisions and underscores the biphasic requirement for *Notch* activity in wing margin establishment in *Drosophila*. *Development* **126**, 2205-2214

**Little, M., Holmes, G. and Walsh, P.** (1999). WT1: what has the last decade told us? *BioEssays* **21**, 191-202

**Lukinova, N. I., Roussakova, V. V. and Fortini, M. E.** (1999). Genetic characterization of cytological region 77A-D harboring the *Presinillin* gene of *Drosophila melanogaster*. *Genetics* **8**, 525-529

**Matsuno, K., Go, M. J., Sun, X., Eastman, D. S. and Artavanis-Tsakonas, S.** (1997). Supressor of Hairless-independent events in Notch signaling imply novel pathway elements. *Development* **124**, 4265-4273

**Morel, V. and Schweisguth, F.** (2000). Repression of Su(H) and activation by Notch are required to define a single row of *single-minded* expressing cells in the *Drosophila* embryo. *Genes Dev*. **14**, 377-388

**Nakao, K. and Campos-Ortega, J. A.** (1996). Persistent expression of Genes of *the Enhancer of split* Complex suppresses neural development in *Drosophila*. *Neuron* **16**, 275-286

**Nellesen, D. T., Lai, E. C. and Posakony, J. W.** (1999). Discrete enhancer elements mediate selective responsiveness of Enhancer of split Complex genes to common transcriptional enhancers. *Dev. Biol*. **312**, 33-53

**Neumann, C. J. and Cohen, S. M.** (1996). A hirarchy of cross-regulation involving *Notch*, *wingless*, and *cut* organizes the dorsal/ventral axis of the *Drosophila* wing. *Development* **122**, 609-612

**Ng, M., Diaz-Benjumea, F., Vincent, J. P., Wu, J. and Cohen, S. M.** (1996). Specification of the wing by localized expression of *wingless* protein. *Nature* **381**, 316-318

**Nofziger, d., Miyamoto, A., Lyons, K. M. and Weinmaster, G.** (1999). Notch signaling imposes two distinct blocks in the differentiation of C2C12 myoblasts. *Development* **126**, 1689-1702

**Pan, D. and Rubin, G. M.** (1997). Kuzbanian controls proteolytic processing of Notch and mediates lateral inhibition during *Drosophila* and vertebrate neurogenesis. *Cell* **90**, 271-280

**Panin, V. M., Papayannopolos, V., Wilson, R. and Irvine, K. D.** (1997). *fringe* modulates Notch-ligand interactions. *Nature* **387**, 908-912

**Posakony, J. W.** (1994). Nature versus Nurture: asymmetric Cell Divisions in *Drosophila* Bristle Development. *Cell* **76**, 415-418

**Rebay, I., Fehon, R. G. and Artavanis-Tsakonas, S.** (1993). Specific truncation of *Drosophila* Notch define dominant activated and dominant negative forms of the receptor. *Cell* **74**, 319-329

**Schroeter, E. H., Kisslinger, J. A. and Kopan, R.** (1998). *Notch-1* signalling requires ligand-induced proteolytic release of intracellular domain. *Nature* **393**, 382-386

**Schweisguth, F. and Posakony, J. W.** (1992). *Suppressor of Hairless*, the *Drosophila* homolog of the mouse recombination signal-binding protein gene, controls sensory organ cell fates. *Cell* **69**, 1199-1212

**Schweisguth, F. and Lecourtois, M.** (1998). The activity of *Drosophila Hairless* is required in pupae but not in embryos to inhibit notch signal transduction. *Dev. Genes Evol*. **208**, 19–27

**Speicher, S. A., Thomas, U., Hinz, U. and Knust, E.** (1994). The *Serrate* locus of *Drosophila* and its role in morphogenesis of the wing imaginal discs: control of cell proliferation. *Development* **120**, 535-544

**Struhl, G., Fitzgerald, K. and Greenwald, I.** (1993). Intrinsic activity of the Lin-12 and *Notch* intracellular domains in vivo. *Cell* **74**, 331-345

**Struhl, G. and Adachi, A.** (1998). Nuclear access and action of *Notch* in vivo. *Cell* **93**, 649-660

**Struhl, G. and Greenwald, I.** (1999). Presinilin is required for activity and nuclear access of *Notch* in *Drosophila*. *Nature* **398**, 522-525

**Tautz, D. and Pfeiffle, C.** (1989). A non-radioactive in situ hybridization method for the localization of specific RNAs in *Drosophila* embryos reveals translational control of the segmentation gene *hunchback*. *Chromosoma* **98**, 81-85

**Wang, S., Younger-Sheperd, S., Jan, L. Y. and Jan, Y. N.** (1997). Only a subset of the binary cell fate decisions mediated by Numb/Notch signaling in *Drosophila* sensory organ lineage requires *Suppressor of Hairless*. *Development* **124**, 4435-4446

**Wettstein, D. A., Turner, D. L. and Kintner, C.** (1997). The Xenopus homolog of *Drosophila Suppressor of Hairless* mediates *Notch* signaling during primary neurogenesis. *Development*, **124**, 693- 702

**Wilder, B. and Perrimon, N.** (1995). Dual functions of *wingless* in the leg imaginal disc of Drosophila. *Development* **121**, 477-488

**Williams, J. A., Paddock, S. W., Vorwerk, K. and Carroll, S. B.** (1994). Organization of wing formation and induction of a wing patterning gene at the dorsal/ ventral compartment boundary. *Nature* **368**, 299-305

**Ye, Y., Lukinova, N. and Fortini, M.** (1999). Neurogenic phenotypes and altered *Notch* processing in *Drosophila Presinilin* mutants. *Nature* **398**, 525-529

**Yeh, E., Gustafson, K. and Boulianne, G.** (1995). Green Fluorescent protein as a vital marker and reporter of gene expression in *Drosophila*. *Proc. Natn. Acad. Sci. USA* **92**, 7036-7040

**Zhang, J. and Carthew, R. W.** (1998). Interactions between Wingless and Dfz2 during Drosophila wing development. *Development* **125**, 3075-3085

**Zecchini, V., Brennan, K. and Martinez Arias, A.** (1999). An Activity of Notch regulates JNK signalling and affects dorsal closure in *Drosophila*. *Curr. Biol*. **9**, 460-469