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Regulation of expression of Vg and establishment of the dorsoventral compartment boundary in the wing imaginal disc by Suppressor of Hairless

Stefan Koelzer, Thomas Klein *

Institute for Genetics, University of Cologne, Zülpicherstr. 47, 50674 Cologne, Germany

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This paper is dedicated to the late Prof. Jose Campos-Ortega.

Abstract

The transcription factor Suppressor of Hairless (Su(H)) belongs to the CSL transcription factor family, which are the main transcriptional effectors of the *Notch*-signaling pathway. Su(H) is the only family member in the *Drosophila* genome and should therefore be the main transcriptional effector of the *Notch* pathway in this species. Despite this fact, in many developmental situations, the phenotype caused by loss of function of *Su(H)* is too weak for a factor that is supposed to mediate most or all aspects of *Notch* signaling. One example is the *Su(H)* mutant phenotype during the development of the wing, which is weaker in comparison to other genes required for *Notch* signaling. Another example is the complete absence of a phenotype upon loss of *Su(H)* function during the formation of the dorsoventral (D/V) compartment boundary, although the *Notch* pathway is required for this process. Recent work has shown that Su(H)/CBF1 has a second function as a transcriptional repressor, in the absence of the activity of the *Notch* pathway. As a repressor, Su(H) acts in a complex together with Hairless (H), which acts as a bridge to recruit the co-repressors Groucho and CtBP, and acts in a *Notch*-independent manner to prevent the transcription of target genes. This raises the possibility that a de-repression of target genes can occur in the case of loss if function of *Su(H)*. Here, we show that the weak phenotype of *Su(H)* mutants during wing development and the absence of a phenotype during formation of the D/V compartment boundary are caused by the concomitant loss of the *Notch*-independent repressor function. This loss of the repressor function of *Su(H)* results in a de-repression of expression of target genes to a different degree in each process. Loss of *Su(H)* function during wing development results in a transient de-repression of expression of the selector gene *vestigial* (*vg*). We show that this residual expression of *vg* is responsible for the weaker mutant phenotype of *Su(H)* in the wing. During the formation of the D/V compartment boundary, de-repression of target genes seems to be sufficiently strong, to compensate the loss of *Su(H)* activity. Thus, de-repression of its target genes obscures the involvement of Su(H) in this process. Furthermore, we provide evidence that Dx does not signal in a *Su(H)*-independent manner as has been suggested previously.
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Keywords: CSL transcription factors; CSL-independent *Notch* signaling; Compartment formation; *Drosophila* wing development; *Vestigial*; Deltex

Introduction

The *Notch*-signaling pathway plays important roles in specifying cell fates in many developmental and pathological processes in multi-cellular animals and humans (reviewed in Artavanis-Tsakonas et al., 1999). *Notch* proteins are type 1 trans-membrane receptors that are activated by ligands of the DSL protein family. In the genome of *Drosophila*, two DSL ligands are present, Serrate (Ser) and Delta (Dl). The binding of these ligands to Notch elicits a sequence of two proteolytic cleavages that release the intracellular domain of Notch (Nintra) into the cytoplasm, from where it travels to the nucleus (reviewed in Kopan, 2002). The two proteolytic cleavages are performed by membrane proteases of the ADAM and Presinillin families. The *Drosophila* ADAM family member Kuzbanian (Kuz) first cleaves Notch in the extra-cellular domain, close to the membrane (Klein, 2002; Lieber et al., 2002). This first cleavage is named S2, and it is the ligand-dependent step. It creates an intermediate that is called NEXT, which is immediately cleaved in the transmembrane domain by the γ-secretase complex that includes Presinillin (Psn) as well as Nicastrin (Nic) to release Nintra

* Corresponding author.
E-mail address: th.klein@uni-koeln.de (T. Klein).

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(S3-cleavage). In the nucleus, Nintra acts together with the sequence specific DNA-binding protein Suppressor of Hairless (Su(H)) to activate the transcription of target genes. Besides these core elements, many additional proteins are involved in regulation of and signal transduction through the *Notch* pathway. One example is Deltex (Dx), which contains a Ring finger motif typical for E3 Ubiqutin ligases and binds to the intracellular domain of Notch (reviewed in Le Borgne et al., 2005). It is involved in signal transduction of the *Notch* signal in some developmental processes such as wing development, possibly in a *Su(H)*-independent pathway (Hori et al., 2004).

The *Notch*-signaling pathway plays a pivotal role during the establishment of the proximo-distal axis of the wing and the establishment of the dorsoventral compartment boundary (D/V boundary) (reviewed in Dahmann and Basler, 1999; Klein, 2001). It mediates the interactions between dorsal and ventral cells at the D/V boundary that lead to the expression of genes that are essential for establishment and patterning of the proximo-distal axis. The dorsal cell fate is defined by the activity of the Apterous (Ap) selector protein, which in addition controls the activity of the Notch pathway through the activation of expression of Ser and the Glycosyltransferase Fringe (Fng). Fng modifies the Notch receptor so that Ser can only signal to ventral and Dl to dorsal cells (Haines and Irvine, 2003). As a consequence, the activity of the pathway is restricted to a small stripe of cells along the D/V boundary. There, it induces transcription of genes essential for wing development and patterning of the proximo-distal axis (P/D axis), chief among them *vestigial* (*vg*) and *wingless* (*wg*) (reviewed in Klein, 2001). *vg* encodes a nuclear protein that forms a dimeric transcription factor with the TEA-domain DNA binding protein Scalloped (Sd) (Halder et al., 1998). Previous studies have revealed that the expression of target genes is activated by Su(H). Activation of *vg* has been studied in some details (Kim et al., 1997a,b). Its transcription is initiated through the activation of the *vestigial* boundary enhancer (*vg*BE). This enhancer contains a single Su(H) DNA binding site that is essential for its activity. Nevertheless, the mutant phenotype of *Su(H)* described in the literature is significantly weaker than that of *vg* null mutants and that of other genes required for the signal transduction in the *Notch* pathway. This discrepancy could argue for the existence of another, *Su(H)*-independent signaling mechanism. The existence of such a pathway has been suggested several times, although the evidence remains weak (reviewed in Mumm and Kopan, 2000).

However, the interpretation of the *Su(H)* mutant phenotype during wing development is hampered by the fact that the strength of the alleles of *Su(H)* analyzed in previous studies is not clear. Hence, it is possible that the weaker phenotype is caused by a residual activity of *Su(H)* (Gho et al., 1996).

The interactions between *ap*-expressing and non-expressing cells, mediated by the *Notch* pathway, are also required for the formation of the dorsoventral (D/V) compartment boundary (reviewed in Klein, 2001). This boundary prevents the mixing between dorsal and ventral cell populations. How the segregation of these two cell populations is achieved is not understood, but an attractive explanation is that both populations have differential adhesive properties. Because of these adhesive differences, the cells from each lineage try to minimize their contact with cells from the other lineage (reviewed in Dahmann and Basler, 1999). Although previous work showed that *Notch* signaling is required for the formation of this boundary, it also provided evidence that Su(H) is not (Miccheli and Blair, 1999). This has led to the conclusion that either a *Su(H)*-independent mechanism of signal transduction mediates the activity of the pathway or a transcriptional response to the Notch signal is not required.

Work on the function of the vertebrate homologue of Su(H), CBF-1, in cell culture and studies of the interaction of CBF-1 with the viral protein EBNA2, especially in the laboratory of D. Hayward, suggested that CBF-1 has a second function as a repressor of transcription in the absence of *Notch* signaling (reviewed in Lai, 2002). More recently, it has been shown that, in *Drosophila*, Su(H) interacts with Hairless (H) and the co-repressor proteins Groucho and dCtBP to repress transcription (Barolo et al., 2002). This raises the possibility of de-repression of expression of target genes in *Su(H)* mutants that could result in a weaker phenotype than observed for mutants of other genes required for Notch signal transduction (Koelzer and Klein, 2003; Morel and Schweisguth, 2000).

Here, we have analyzed the phenotype caused by homozygosity of a null allele of *Su(H)* (Morel and Schweisguth, 2000), during wing development. We confirmed that during pattern formation, the mutant phenotype is weaker than expected and found that this is caused by the loss of the repressor function of Su(H). The loss of *Su(H)* function results in a transient de-repression of expression of the selector gene *vestigial* (*vg*), mediated by a weak and transient activation of one of its enhancers, the *vestigial* boundary enhancer (*vg*BE). Furthermore, we show that Su(H) is involved in the formation of the D/V compartment boundary, despite previous reports on the contrary. This involvement is obscured by the de-repression of expression of the target genes that allow the process to occur in the absence of Su(H) function. In summary, the data reveal that the weaker phenotype of *Su(H)* mutants during wing development can be explained by the dual function of Su(H) and does not provide evidence for the existence of a *Su(H)*-independent signal transduction mechanism. Furthermore, we show that Dx does not signal in a *Su(H)*-independent manner during wing development as suggested previously.

Materials and methods

Fly strains

The following alleles were used in this work: $Su(H)^{\Delta 47}$ P(B)FRT40A (Morel and Schweisguth, 2000), Psn^{C1} (Struhl and Greenwald, 1999), Psn^{I2} (Ye et al., 1999), nic^{A7} (Hu et al., 2002), kuz^{1405}, kuz^{1403} (Sotillos et al., 1997), $Df(1)N^{81K}$ FRT101 (Brennan et al., 1997); ap^{UG035} and *ap-lacZ* (ap^{rK568}) (Cohen et al., 1992), $Su(H)^{Sf8}$ and H^{E31} (Lecourtois and Schweisguth, 1995); vg^{83b27R} and the *vg*BE (Williams et al., 1994).

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