

Short Communication

tantalus, a potential link between *Notch* signalling and chromatin-remodelling complexes

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Abstract The *tantalus* (*tan*) gene encodes a protein that interacts specifically with the Polycomb/trithorax group protein Additional sex combs (ASX). Both loss-of-function and gain-of-function mutations in *tan* cause tissue-specific defects in the eyes, wing veins and bristles of adult flies. As these defects are also typical for components of the *Notch* (*N*) signalling pathway, we wished to determine if TAN interacts with this pathway. Through careful examination of ectopic *tan* phenotypes, we find that TAN specifically disrupts all three major processes associated with the *N* signalling pathway (boundary formation, lateral inhibition, and lineage decisions). Furthermore, ectopic *tan* expression abolishes expression of two *N* target genes, *wingless* (*wg*) and *cut*, at the dorsal-ventral boundary of the wing. An interaction between *tan* and *N* was also observed using a genetic assay that previously detected interactions between *tan* and *Asx*. The previously observed ability of TAN to move between the cytoplasm and nucleus, and to associate with DNA, provides a potential mechanism for TAN to respond to *N* signalling.

Keywords Tantalus - Notch - *wingless* - *Drosophila* - *Asx*

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Introduction

The *tantalus* (*tan*) gene was identified in a yeast two-hybrid screen designed to uncover interactors for the Polycomb and trithorax group (Pc-G and trx-G) protein Additional sex combs (ASX; Dietrich et al. [2001](#)). The Pc-G and trx-G protein complexes act to maintain states of transcription during

development in a wide range of organisms (reviewed in Orlando [2003](#)). ASX is ubiquitously expressed, but has tissue-specific functions, which led to the postulation that it most likely requires the cooperation of tissue-specific cofactors. ASX and TAN were shown to interact directly *in vitro* as well as *in vivo* and to co-localize to approximately 35 of the 66 TAN polytene chromosome binding sites. Mutants of *Asx* and *tan* were also shown to interact genetically during bristle formation. Although *tan* is expressed throughout embryogenesis and larval development, higher levels of expression are observed in some tissues (e.g. the morphogenetic furrow of the eye disc; Dietrich et al. [2001](#)). Flies homozygous for *tan* null mutations exhibit a variety of sensory organ defects including roughened eyes, bristle loss and duplication and minor defects in distal wing vein formation, but are otherwise viable and fertile. These results led to the proposal that TAN acts as a tissue-specific cofactor for ASX function during sensory organ development. However, its relatively ubiquitous expression left it unclear as to why TAN function appears to be limited to this relatively small subset of tissues. Functional redundancy is a likely possibility.

Defects in the development of eyes, bristles and wings in *tan* mutants are characteristic of *Notch* (*N*) signalling pathway defects, raising the possibility that *tan* function may be involved in *N* signalling. The *N* pathway is required for cell-to-cell communication during a large number of developmental processes/pathways (reviewed in Kadesch [2000](#)). In general, *N* signalling commences when the *N* receptor is bound by one of its extracellular ligands (Delta or Serrate), which causes release of the intracellular domain of N. The intracellular domain then translocates to the nucleus where it binds to the transcription factor Suppressor of Hairless [Su(H)], leading to the activation of *N* downstream target genes such as *wingless* (*wg*), *cut* and the *Enhancer of Split Complex* [*E(spl)-C*]. The *N* signalling pathway also contains several regulators of *N* activity that can act either by modifying the *N* protein post-translationally or by binding directly to cytoplasmic *N* to regulate its activity (Kadesch [2000](#)).

N-dependent signalling is required during three types of developmental processes: boundary formation, lateral inhibition, and lineage decisions (reviewed in Bray [1998](#); see description below). As the loss-of-function phenotypes for *tan* are weak, we have used the Gal4-mediated ectopic expression system to show that ectopic *tan* specifically affects each of these *N*-dependent processes during the formation of adult structures, consistent with the *tan*-like mutant phenotypes. To confirm a transcriptional effect on the *N* signalling pathway, we analysed *wg* and *cut* expression and found that ectopic *tan* expression at the dorsal-ventral (D/V) boundary in the wing abolishes expression of both genes at this location. Furthermore, we show that *tan* and *N* interact genetically using the same assay used previously to identify a genetic interaction between *Asx* and *tan*, supporting the idea of a link between the functions of all three genes. We propose that TAN may link the *N* signalling cascade to the Pc-G and trx-G family of transcriptional regulators.

Materials and methods

Ectopic expression and genetic analysis

All *tan* lines and constructs used here have been described previously (Dietrich et al. [2001](#)), and Gal4 driver lines used are described in Flybase (<http://flystocks.bio.indiana.edu/gal4.htm>). The loss-of-function allele *N*⁸ and the hypomorphic allele *N*^{55e11} (described in Flybase) were crossed to either *tan*² homozygotes or P[*tan*];*tan*² homozygotes and the number of flies with four or fewer interocellar bristles were counted. The *tan*² allele was created by P-element mutagenesis and has 266 of the 299 encoded TAN amino acids deleted (Dietrich et al. [2001](#)). P[*tan*] is a transgenic copy of the full-length

tan gene under the control of its own promoter, which was previously shown to be sufficient for rescue of homozygous *tan*² flies (Dietrich et al. [2001](#)).

In-situ hybridization

Flies were raised on standard yeast-sucrose-agar medium. Third instar larvae were collected, dissected in RNase-free 1× PBS and probed with digoxigenin UTP- (Boehringer Mannheim) labelled single strand anti-sense *wg* and *cut* RNA as described (Dietrich et al. [2001](#)).

Results and discussion

Ectopic *tan* expression disrupts *N* signalling during boundary formation, lateral inhibition, and lineage decisions

The null *tan*² allele causes sensory-specific defects, but does not result in lethality (Dietrich et al. [2001](#)). Similar sensory defects and non-lethality are typical for several members of the *N* signalling cascade. For example, mutations in the *Suppressor of deltex* gene (Mazaleyrat et al. [2003](#)) and genes within the *E(spl)-C* (Ligoxygakis et al. [1999](#)) also do not cause lethality when null, due possibly to functional redundancy. They do, however, exhibit dramatic sensory organ defects when ectopically expressed (Ligoxygakis et al. [1999](#); Mazaleyrat et al. [2003](#)). To test whether *tan* plays a similar role in general *N* signalling, the Gal4-UAS system was used to express *tan* in tissues where the three basic *N*-dependent processes are well characterized.

Boundary formation

Boundary signalling by the *N* pathway occurs between non-equivalent groups of cells and is inductive in that *N*-expressing cells at the boundary act as an organiser to direct cell fate decisions on either side of the boundary (reviewed in Bray [1998](#)). This activity has been best studied at the wing margin where *N* expression is limited to cells between the future dorsal and ventral wing surfaces. When this signalling is compromised, notches begin to appear in the adult wing. Figure [1b](#) shows that ectopic *tan* expression (using C96-GAL4) along the wing margin, where *N* is expressed, also causes notching of the wing. Since *N* expression was not affected by ectopic *tan* (data not shown), we looked to see if genes regulated by *N* signalling were affected. Two genes frequently used to monitor *N* signalling, *wg* and *cut* (Klein et al. [2000](#)), were tested for altered expression at the wing margin. Figure [1d](#) shows that *wg* expression along the D/V wing disk boundary is specifically repressed when *tan* is ectopically expressed along the D/V boundary. Expression in the wing pouch (where the C96 driver is not expressed) remained normal (Fig. [1c](#), d). Figure [1f](#) shows the effect on *cut* expression when TAN is expressed perpendicularly along the A/P boundary using a *patched*-Gal4 driver. In this case, *cut* expression is repressed where the A/P and D/V boundaries intersect. Thus, *N* signalling is disrupted wherever *tan* expression is up-regulated, resulting in local notching of the wing margin.

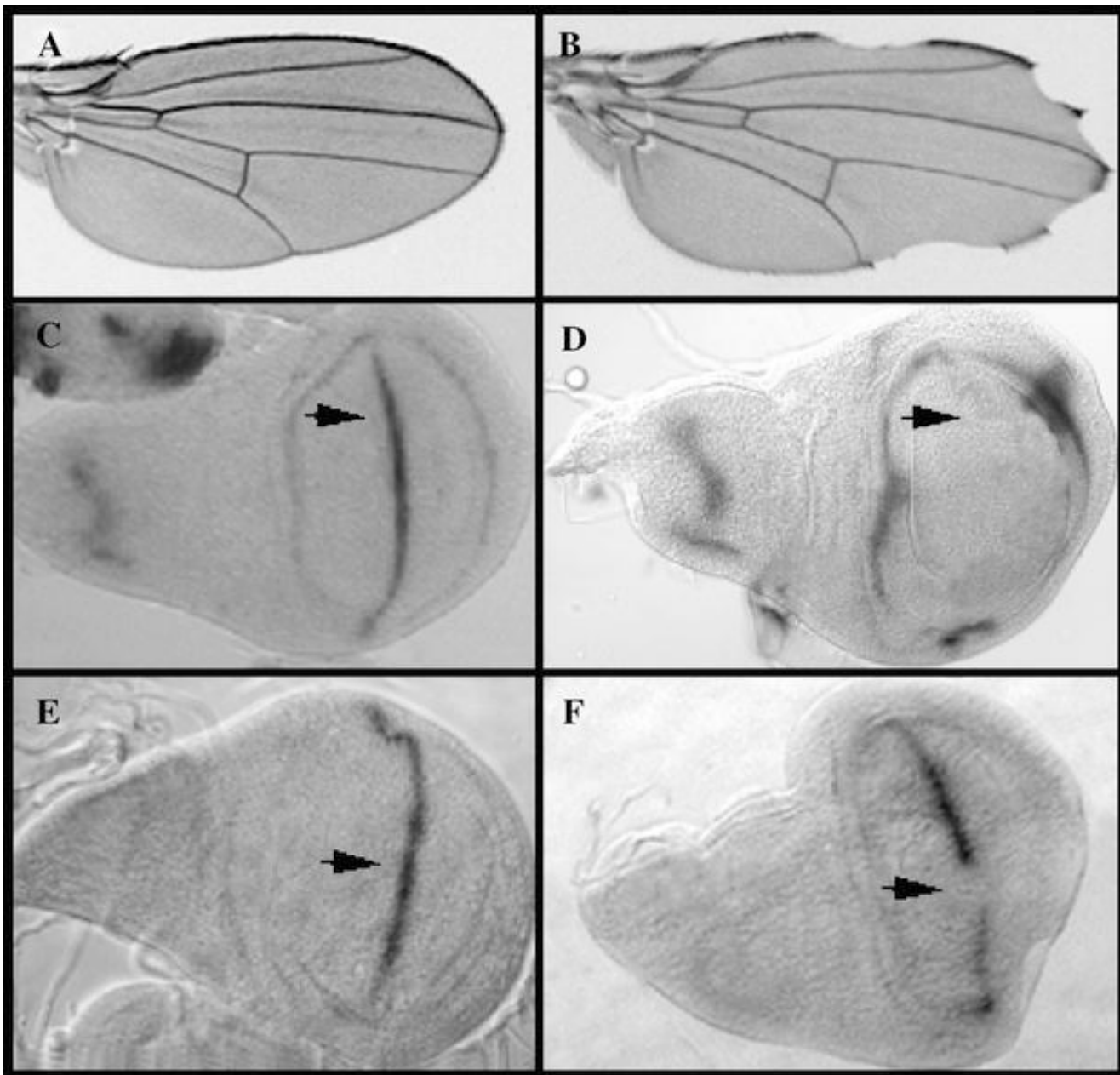


Fig. 1 Effects of ectopic *tantulus* (*tan*) expression at the wing margin. **a** A wild-type wing and **b** a C96-Gal4::UAS-*tan* wing. Note the notching in (**b**). **c** *wingless* (*wg*) expression in a wild-type wing imaginal disc and **d** in a C96-Gal4::UAS-*tan* wing imaginal disc. Note in **d** that *wg* expression is specifically inhibited at the D/V boundary (arrows). **e** *cut* expression in a wild-type wing imaginal disc (arrow) and **f** the gap in *cut* expression (arrow) in a *patched*-Gal4::UAS-*tan* wing imaginal disc. *Patched* is expressed along the A/P boundary and *cut* expression is lost at the intersection of the A/P and D/V boundaries

N boundary signalling activity is also required at the boundary of the leg tarsals for joint formation, with loss of *N* activity leading to fusion of leg segments (de Celis et al. 1998) and duplication of sex combs (Mishra et al. 2001). To see if *tan* plays a role in this signalling process, we expressed *tan* at the tarsal leg boundary using a *hairy*-Gal4 driver (Carroll and Whyte 1989). Although *hairy*-mediated *tan* expression was often pupal-lethal, flies could be dissected from their pupal cases. The majority of these had legs with missing joints and only four tarsal segments (Fig. 2a, b). Duplication of the sex combs was also frequently observed (Fig. 2a, b), as has previously been observed when *Suppressor of deltex* is ectopically expressed (Mazaleyrat et al. 2003). As with the wing phenotypes, these results show that TAN has the ability to inhibit *N* signalling during boundary foundation.

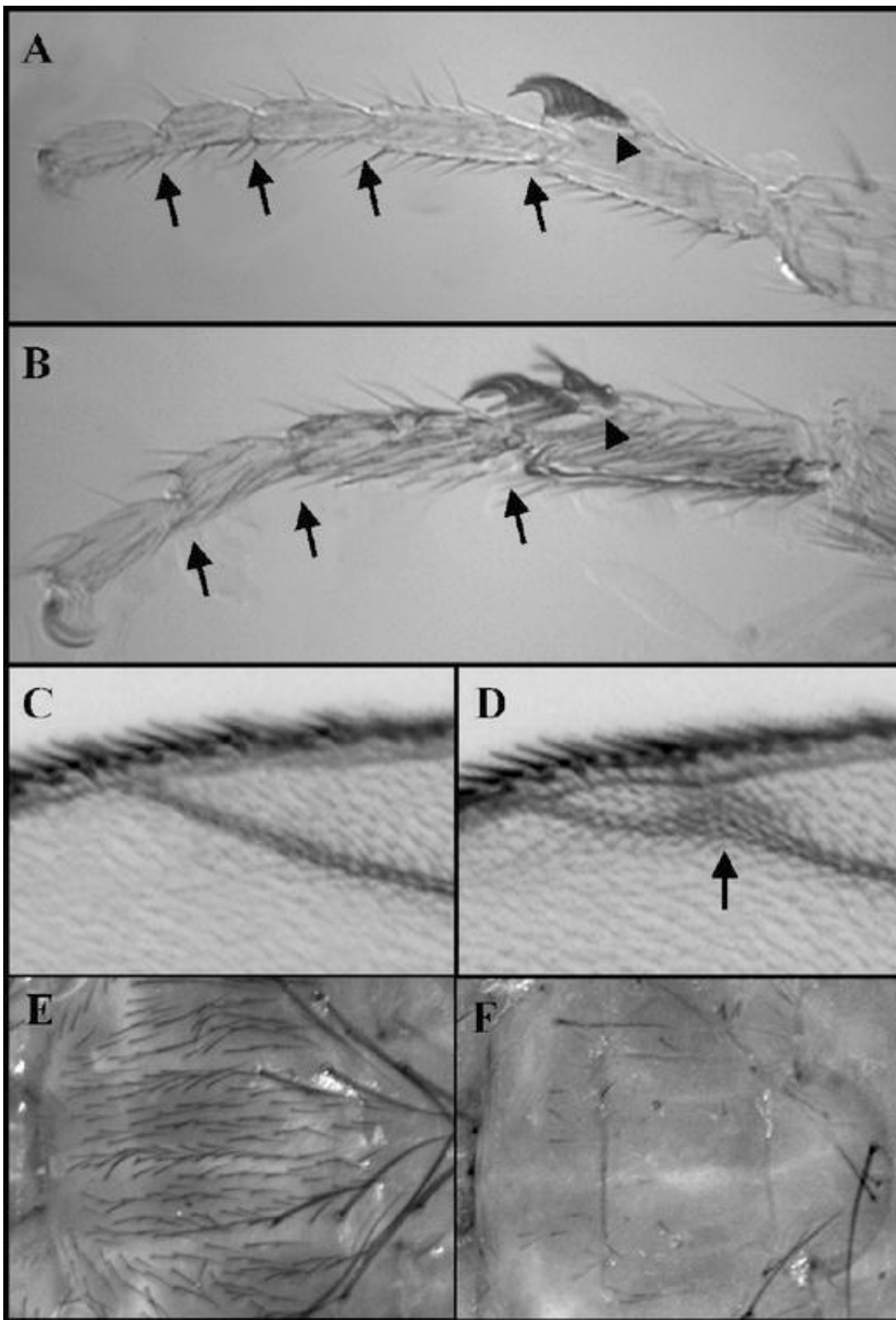


Fig. 2 *tantalus* ectopic expression affects the three *Notch*-dependent signalling processes. **a, b** Effects on boundary formation. Wild-type (**a**) and *hairy-Gal4::UAS-tan* (**b**) legs with boundaries (*arrows*) and sex combs (*arrowhead*) marked. One of the boundaries is missing in the *hairy-Gal4::UAS-tan* leg and sex combs are duplicated. **c, d** Effects on lateral inhibition. Wild-type (**c**) and *hairy-Gal4::UAS-tan* (**d**) second wing veins. Extra vein material is present in the *hairy-Gal4::UAS-tan* wing (*arrow*). **e, f** Effects on lineage decisions. Wild-type (**e**) and *scabrous-Gal4::UAS-tan* (**f**) dorsal thoraxes. The majority of both the macro- and microchaetae are deleted when *tan* is ectopically expressed

Lateral inhibition

To determine if *tan* can also affect lateral inhibition, we analysed wing vein formation following ectopic *tan* expression. Initially, the wing is divided into inter-vein and pro-vein territories. The pro-vein territory is approximately seven to eight cells wide and uses the *N* pathway to produce the vein proper, which ends up being two to three cells wide (de Celis [1998](#)). Due to earlier patterning events, cells in the middle of the pro-vein region express relatively high levels of Delta ligand (DL), while cells at the edge of the pro-vein region express higher levels of *N*. When DL binds to *N* in adjacent cells, a feedback mechanism begins that amplifies the relative differences in DL and *N* expression. This polarized signalling forms and maintains the boundary between the vein proper and pro-vein regions. If *N* activity is reduced, this process of lateral inhibition fails to occur normally, and the wing vein regions remain abnormally wide.

As *hairy* is also expressed in the pro-vein region of veins 2 and 3 in the wing (Carroll and Whyte [1989](#)), we used a *hairy*-Gal4 driver to determine if *tan* expression in this region has an effect on vein formation. In contrast to *tan* mutants, which exhibit losses of distal vein material (Dietrich et al. [2001](#)), ectopic *tan* expression results in the production of extra vein material in distal regions (Fig. [2c, d](#)). This result is also consistent with increased expression of *tan* impacting negatively on *N* signalling. However, to fully interpret these results, it will be necessary to determine the exact time and position that *tan* exerts its function.

Lineage decisions

During bristle formation each sensory organ precursor (SOP) cell divides to create four major cell types, two of which become externally visible as the shaft and socket (reviewed in Posakony [1994](#)). SOP differentiation is first restricted by *N* signalling to a single cell. This prevents expression of basic helix-loop-helix (bHLH) proneural genes in all but the future SOP cell. Following this, lineage decisions occur during division of the SOP and SOP daughter cells through *N* signalling activity. Loss of *N* signalling during SOP differentiation results in a transformation of all four SOP daughter cells into neurons. In contrast, early *N* hyperactivity tends to cause a loss of all SOP cells through suppression of the proneural genes.

We previously demonstrated that loss of *tan* function results in modest bristle defects limited to the adult abdomen, head and eye. These defects include reduction in bristle size and loss of bristles (sometimes leaving only the socket), suggesting that *tan* plays an important role in *N* signalling during SOP differentiation (Dietrich et al. [2001](#)). To further explore this role, we used a *scabrous*-Gal4 (*sca*-Gal4) driver to express *tan* in sensory organs. The *sca*-Gal4 line is expressed early in cells surrounding the SOP cell and was previously used in a gain-of-function screen to search for genes involved in sensory organ formation (Abdelilah-Seyfried et al. [2000](#)). Only 2.3% of the lines screened in that study (2,293 lines tested) caused a total or partial loss of the external and internal support cells. Among these were several genes previously known to have important functions during SOP differentiation (e.g. *extra macrochaetae*, *escargot*). These results suggest that relevant interacting genes are readily identified using this assay, and that false positives are minimal.

The expression of *tan* under *sca*-Gal4 control resulted in a dramatic loss of most bristles on the adult thorax (Fig. [2e, f](#)), consistent with a normal role for TAN in sensory bristle formation. Use of the A101 enhancer trap line, which expresses *lacZ* under control of the *neuralised* gene (Bellen et al. [1989](#)), showed that the majority of sensory cells in the notum are missing (data not shown), suggesting that the

bristle defects observed occur at the time of SOP differentiation.

Genetic interaction between *tan* and *Notch*

Homozygous *tan* mutants display a loss of sensory bristles in the interocellar region of the head. This phenotype is genetically enhanced by mutations in the *Asx* gene (Dietrich et al. [2001](#)). In wild-type adults, there are usually eight bristles in this area, although this varies, with around 3% of adults displaying five bristles and no adults displaying fewer than four bristles. While *tan*² heterozygous adults are normal, 40% of *tan*² homozygotes have only five interocellar bristles, and 35% have four or fewer bristles (75% total with five or fewer bristles). To look for a genetic interaction with *N*, we counted the number of flies in *trans*-heterozygotes with four or fewer bristles (to minimize the chances of detecting a false interaction) (Table [1](#)).

Table 1 *tantalus* and *Notch* interact genetically during interocellar bristle specification. The percentage of flies with four or fewer interocellar bristles is shown with the number of flies counted *in parentheses*. Both *N* alleles show a strong genetic interaction in *N* and *tan*² *trans*-heterozygotes (*column 2*). This interaction is strongly rescued by the addition of the P[*tan*] construct (*column 3*). *tan*² heterozygotes do not display the interocellar bristle phenotype

Mutant allele	<i>N</i> /+; <i>tan</i> ² /+	<i>N</i> /+;P[<i>tan</i>]/+; <i>tan</i> ² /+
<i>N</i> ⁸	74.7% (75)	16.3% (104)
<i>N</i> ^{55e11}	26.7% (90)	4.1% (73)

The null *N*⁸ allele, when *trans*-heterozygous with the *tan*² allele, displayed a strong reduction in the number of interocellar bristles (74.7% with four or fewer bristles). The hypomorphic *N*^{55e11} allele also showed a strong reduction in bristle number (26.7% with four or fewer bristles). Importantly, this effect could be alleviated by providing a single copy of a *tan* rescue construct (Table [1](#), and see [Materials and methods](#)). The values of 16.3% (*N*⁸) and 4.1% (*N*^{55e11}) seen in the rescued lines suggest that *N* alleles on their own have significant effects on interocellar bristle formation (the rescued lines have, in effect, two wild-type copies of *tan*). This synergism between *N* and *tan* heterozygous alleles is once again consistent with a functional connection between the two gene products.

Conclusions

We previously observed that null mutations in *tan* cause modest defects in a number of sensory organs, similar to the minimal defects caused by mutations in many genes that function in the *N* signalling pathway. Consistent with the similarity of these phenotypes, the results provided here support a close link between TAN function and *N* signalling. Interactions were observed in a number of different tissues and processes controlled by *N* signalling.

The limited loss-of-function *tan* phenotype, and the stronger, more widespread phenotypes caused by ectopic expression, are similar to those found with other components of the *N* signalling pathway (Ligoxygakis et al. [1999](#); Mazaleyrat et al. [2003](#)). A loss-of-function *Suppressor of deltex* allele, for instance, displays only a weak wing vein gap phenotype that is similar to gain-of-function mutations in *Notch* (Mazaleyrat et al. [2003](#)). However, ectopic expression of *Suppressor of deltex* lead to more severe phenotypes such as fusions of the leg tarsals, as seen with ectopically expressed *tan*. The authors identified a feedback loop, and suggest that the robustness of this feedback, along with other

forms of redundancy, are responsible for the weak phenotypes seen in *Suppressor of deltex* mutants. Indeed, gene redundancy appears to be a general and important property among many of the *N* signalling pathway components.

Unlike most other *N* pathway genes, it appears unlikely that *tan* is a downstream transcriptional target of *N* signalling since *tan* expression is more or less ubiquitous. This probability is supported by the lack of high affinity binding sites for the *Suppressor of Hairless* protein, the effector of transcriptional regulation by the *N* signalling pathway, in the vicinity of the *tan* gene (data not shown). However, to unequivocally verify that *tan* is not a transcriptional target of *N* signalling, it will be important to analyse *tan* gene expression in greater detail. Although we also cannot rule out the possibility that TAN is acting in a parallel pathway, the ability of TAN to specifically interfere with the three *N*-related processes described here (boundary formation, lateral inhibition, and lineage decisions), to negatively regulate two *N* target genes and to genetically interact with *N* suggests that TAN is more directly involved in *N* signalling.

While the data shown here are consistent in their implication of a *N* and TAN interaction, they differ in the types of relationship implied. The genetic enhancement experiments tended to indicate a positive interaction, while most of the ectopic expression assays implied a negative interaction. There are several possible explanations for these seemingly conflicting results. One possibility is that ectopic expression of TAN, superimposed upon ubiquitous expression of the endogenous gene, may result in the sequestering of *N* signalling components into incomplete or non-productive complexes (i.e. a dominant-negative effect). A second possible explanation is the complexity of most *N*-regulated processes, which typically involve a number of sequential events. Loss-of-function and gain-of-function scenarios could well affect these processes at different steps, at different times and in different ways. A third explanation, also stemming from the complexity of the *N* signalling process, is that compensating effects within the pathway may respond differently to gain or loss of TAN function. For example, it has previously been shown that *E(spl)-C* genes, which are normally up-regulated by *N* signalling, can be up-regulated in *Su(H)* mutant backgrounds (reviewed in Lai [2004](#)). This is because *E(spl)-C* gene expression can be activated by both the Su(H) and bHLH proteins, and bHLH expression is up-regulated in the absence of Su(H) expression. Clearly, a full understanding of the role(s) of TAN during these steps will require further analyses at the temporal and cellular level.

The most interesting aspect of our analyses of *tan* and *N* signalling is the potential link between Pc-G/trx-G gene regulation and the *N* signalling pathway. Although the effects of the Pc-G and trx-G complexes have been most extensively analysed for the homeotic genes, it is clear from mutational analyses and the number of sites on polytene chromosomes bound by different family members, that Pc-G and trx-G complexes must regulate other genes as well. Our results suggest that, at some level, TAN in conjunction with ASX could interact with *N* signalling components to modulate Pc-G/trx-G function. Interestingly, we previously demonstrated that ectopically expressed TAN was capable of cycling between the cytoplasm and nucleus, raising the possibility that TAN entry into the nucleus could be associated, either directly or indirectly, with *N* signalling. The coupling of TAN entry into the nucleus with *N* signalling could help explain the paradox of a ubiquitous expression pattern but specific sensory functions for *tan*.

TAN possesses inherent DNA binding ability (Dietrich et al. [2001](#)), and once localised to the nucleus it is possible that TAN could be recruited to specific targets of *N* signalling. A subsequent interaction between TAN and ASX would make a link to the Pc-G and trx-G proteins. There is currently no evidence to suggest that TAN and *N* signalling are always linked. TAN may cooperate with *N* only during specific signalling events. It will be important to assess whether TAN nuclear localisation is altered in cells where *N* signalling has been activated to determine if these events are functionally coupled.

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References

Abdelilah-Seyfried S, Chan YM, Zeng C, Justice NJ, Younger-Shepherd S, Sharp LE, Barbel S, Meadows SA, Jan LY, Jan YN (2000) A gain-of-function screen for genes that affect the development of the *Drosophila* adult external sensory organ. *Genetics* 155:733–752

Bellen HJ, O’Kane CJ, Wilson C, Grossniklaus U, Pearson RK, Gehring WJ (1989) P-element-mediated enhancer detection: a versatile method to study development in *Drosophila*. *Genes Dev* 3:1288–1300



Bray S (1998) Notch signalling in *Drosophila*: three ways to use a pathway. *Semin Cell Dev Biol* 9:591–597



Carroll SB, Whyte JS (1989) The role of the *hairy* gene during *Drosophila* morphogenesis: stripes in imaginal discs. *Genes Dev* 3:905–916

de Celis (1998) Positioning and differentiation of veins in the *Drosophila* wing. *Int J Dev Biol* 42:335–343

de Celis JF, Tyler DM, de Celis J, Bray SJ (1998) Notch signalling mediates segmentation of the *Drosophila* leg. *Development* 125:4617–4626

Dietrich BH, Moore J, Kyba M, dosSantos G, McCloskey F, Milne TA, Brock HW, Krause HM (2001) Tantalus, a novel ASX-interacting protein with tissue-specific functions. *Dev Biol* 234:441–453



Kadesch T (2000) Notch signalling: a dance of proteins changing partners. *Exp Cell Res* 260:1–8



Klein T, Seugnet L, Haenlin M, Martinez-Arias A (2000) Two different activities of Suppressor of Hairless during wing development in *Drosophila*. *Development* 127:3553–3566

Lai EC (2004) Notch signaling: control of cell communication and cell fate. *Development* 131:965–973



Ligoxygakis P, Bray SJ, Apidianakis Y, 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

Mazaleyrat SL, Fostier M, Wilkin MB, Aslam H, Evans DA, Cornell M, Baron M (2003) Down-regulation of Notch target gene expression by *suppressor of deltex*. *Dev Biol* 255:363–372



Mishra A, Agrawal N, Banerjee S, Sardesai D, Dalal JS, Bhojwani J, Sinha P (2001) Spatial regulation of DELTA expression mediates NOTCH signalling for segmentation of *Drosophila* legs. *Mech Dev* 105:115–127



Orlando V (2003) Polycomb, epigenomes, and control of cell identity. *Cell* 112:599–606



Posakony JW (1994) Nature versus nurture: asymmetric cell divisions in *Drosophila* bristle development. *Cell* 76:415–418

