**Distal-less** and *homothorax* regulate multiple targets to pattern the *Drosophila* antenna

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**SUMMARY**

The *Drosophila* antenna is a highly derived appendage required for a variety of sensory functions including olfaction and audition. To investigate how this complex structure is patterned, we examine the specific functions of genes required for antenna development. The nuclear factors, *Homothorax*, Distal-less and Spineless, are each required for particular aspects of antennal fate. Coexpression of *Homothorax*, necessary for nuclear localization of its ubiquitously expressed partner *Extradenticle*, with Distal-less is required to establish antenna fate. Here we test which antenna patterning genes are targets of *Homothorax*, Distal-less and/or Spineless. We report that the antennal expression of *dachshund*, *atonal*, *spalt*, and *cut* requires *Homothorax* and/or Distal-less, but not Spineless. We conclude that Distal-less and *Homothorax* specify antenna fates via regulation of multiple genes. We also report for the first time phenotypic consequences of losing either *dachshund* or *spalt* and *spalt-related* from the antenna. We find that *dachshund* and *spalt/spalt-related* are essential for proper joint formation between particular antennal segments. Furthermore, the *spalt/spalt-related* null antennae are defective in hearing. Hearing defects are also associated with the human diseases Split Hand/Split Foot Malformation and Townes-Brocks Syndrome, which are linked to human homologs of *Distal-less* and *spalt*, respectively. We therefore propose that there are significant genetic similarities between the auditory organs of humans and flies.

Key words: Distal-less, extradenticle, homothorax, atonal, cut, dachshund, spalt, spalt-related, spineless, Antenna, Audition, *Drosophila*, Townes-Brocks syndrome, Split Hand/Split Foot Malformation

**INTRODUCTION**

The adult fruit fly antenna consists of six segments. From proximal to distal, these are called a1, a2, a3, a4, a5 and arista (a6; Fig. 1A). The different segments of the antenna serve distinct sets of functions including hygro-sensation (arista), thermosensation (a3), olfaction (a3), and audition (arista and a2) (Carlson, 1996; Eberl, 1999; Goepfert and Robert, 2001; Sayeed and Benzer, 1996). Although the various functions of the antenna have been studied in detail, there is relatively little known about the genetic hierarchy that governs antenna formation. We are investigating what genes are involved and how they are involved in patterning the *Drosophila* antenna to understand how particular peripheral sensory structures are generated along the proximodistal (PD) axis of the antenna.

In the antenna, as in other appendages, the process of pattern formation requires both limb fate information and PD information. The homeodomain transcription factor-encoding genes, *Distal-less* (*Dll*) and *homothorax* (*hth*), provide both. Losing the function of either *Dll* or *hth*, results in deletions of the distal and proximal domains, respectively (Casares and Mann, 1998; Cohen and Jurgens, 1989; Pai et al., 1998). In addition, coexpression of *Dll* and *hth* is required for the specification of antenna fate (Dong et al., 2000). Losing the function of either gene results in antenna to leg transformation (Fig. 1B,C) (Casares and Mann, 1998; Cohen and Jurgens, 1989; Dong et al., 2000; Sato, 1984; Sunkel and Whittle, 1987).

One might expect the targets of *Dll* or *hth* for PD patterning to be expressed in both the antenna and the leg. Consistent with this, the *Dll* targets, *bric a brac* (*bab*), *aristaless* (*al*), and *BarH1/BarH2*, are expressed and required in both the distal antenna and leg (Campbell and Tomlinson, 1998; Godt et al., 1993; Kojima et al., 2000; Schneitz et al., 1993). In contrast, the targets of *Dll* and *hth* involved in antenna specification would be predicted to have antenna-specific patterns of expression. Consistent with this, *spalt* (*sal*), a known *Dll* and *hth* target (Dong et al., 2000), and *spalt-related* (*salr*; adjacent and homologous genes with similar expression patterns) are expressed in identical circular patterns in the antennal disc (Barrio et al., 1999; Wagner-Bernholz et al., 1991) and only expressed at low levels in the leg imaginal disc, proximal to the presumptive leg (Fig. 3G). Another target is a *bHLH-PAS* encoding gene, *spineless* (*ss*), a homolog of the mammalian dioxin receptor gene (Duncan et al., 1998). As with *Dll* and *hth* loss-of-function mutants, loss of *ss* also results in antenna to leg transformations (Balkaschina, 1929; Burgess and Duncan, 1990; Struhl, 1982) (Fig. 1D). *Dll* is required for the antennal expression of *ss* (Duncan et al., 1998). Also, loss of
hth in the antenna results in loss of ss expression (Ian Duncan, personal communication). This, in conjunction with the ss transformation phenotype, raised the possibility that ss is a primary target through which Dll and hth effect antenna specification. That both Dll and hth mutants exhibit PD defects in the antenna in addition to transformation phenotypes, while ss leads primarily to transformation, indicated that Dll and hth almost certainly have functions that are not mediated by ss. We therefore focussed on genes with antenna-specific patterns of expression and tested whether these were regulated by ss. We demonstrate here that there are multiple targets of both Dll and Hth in the antenna whose activation is independent of Ss.

In particular, we report that nuclear factors, dachshund (dac), atonal (ato), sal and cut (ct) are antenna-specific targets of Dll and/or hth. In addition, through phenotypic analyses, we identify novel functions for dac in the antenna and for sal and salr in both antenna and eye. Furthermore although ss is required for antennal specification, the antennal expression of dac, ato, sal, and ct is independent of ss. The work we present here defines distinct roles for Dll, hth and ss in antenna specification. With a greater understanding of how the antenna is specified and its PD axis is subdivided, we can now begin to explain how key sensory structures are both fated and positioned during antennal development.

**MATERIALS AND METHODS**

**Fly strains and genetic manipulations**

The following fly strains were employed: (1) DllGAL4/T(2;3) SM6a; TM6B; (2) Dll3/T(2;3) SM6a; TM6B; (3) FRT42D DllSA1 (Gorlinski et al., 1997); (4) y w FLPase; FRT82B Mi(3)121 πM1/ TM3; Ser; (5) w; FRT82B hhP2/TM6B, Tb, Hu (Pai et al., 1998); (6) Df(3R)ss D114.4/TM6 (Ian Duncan); (7) ssD114.9/TM6 (Ian Duncan); (8) ssD115.7/TM6 (Ian Duncan); (9) w; dac4 FRT40A/T(2;3) SM6a; TM6B; (10) w; dac3 FRT40A/T(2;3) SM6a; TM6B; (11) dac-

**Fig. 1.** Dll, hth and ss mutants all exhibit antenna to leg transformations. (A) Wild-type antenna. (B) Dll3/Dll7 hypomorphic antenna in which distal a3 and the arista are transformed toward leg. (C) A large hthP2 clone in the antenna results in transformation of a1 to arista into leg structures. (D) ss null antenna of genotype Df(3R)ss D114.4/ ss D114.9. a1-a5, antennal segments 1-5: ar, arista.

**Fig. 2.** ss is regulated by Dll and hth. (A) Wild type antenna disc showing Hth protein (green) and Dll protein (red). (B) ss null antenna disc of genotype Df(3R)ss D114.4/ ss D114.9 in which Hth and Dll expression is normal. (C) Wild-type expression of ss in a late third instar leg disc. (D) Expression of ss is induced in the distal leg (arrow) by ectopic expression of Hth. (E) Wild-type expression of ss in a late third instar wing disc. (F) Expression of ss is induced in the wing pouch (arrows) by ectopic expression of Hth.

lacZ/T(2;3) SM6a; TM6B (Graeme Mardon); (12) ato1 (Jarman et al., 1994; Jarman et al., 1995); (13) sal16 FRT40A (Ethan Bier); (14) salFCK-25/T(2;3) SM6a; TM6B (Rosa Barrio); (15) w; Df(2L)32FP-5 FRT40A/T(2;3) SM6a; TM6B (Rosa Barrio et al., 1999); (16) dpp-GAL4 (A.3)/TM6B (Morimura et al., 1996); (17) w; UAS-GFP-hth8/TM6B, Tb, Hu (Casares and Mann, 1998); (18) y hs-FLPase; FRT82B 2pM; (19) y w ey-FLPase GMR-lacZ; FRT40A; and (20) y w ey-FLPase GMR-lacZ; FRT42D.

Dll, hth, and sal/salr null clones were generated using the FLP/FRT system (Xu and Rubin, 1993). Animals of the genotype: y hs-FLPase; FRT82B 2pM/FRT82B hhP2 were heatshocked at 37°C for 1 hour at 48-72 hours after egg laying (AEL) and examined in mid- to late-third instar. Dll and sal/salr null clones were generated using ey-FLPase (Newsome et al., 2000). The genotypes of the larvae and adults examined were: y w ey-FLPase GMR-lacZ; FRT42D 2pM/FRT42D DllSA1 and y w ey-FLPase GMR-lacZ; FRT40A/Df(2L)32FP-5 FRT40A. In addition to Dll null clones, strong Dll hypomorphic antennae of the genotype DllGAL4/Dll7 were examined. These alleles were balanced over T(2;3) SM6a; TM6B, Hu, Tb. Dll mutant larvae therefore could be identified by lack of a Tb phenotype.

The ss null genotype examined was Df(3R)ss D114.4/ ss D114.9. Strong ss hypomorphs of the genotype Df(3R)ss D114.4/ ss D115.7 also were examined and exhibited similar phenotypes. dac null animals were
generated by crossing w; dac<sup>1</sup> FRT40A/T(2;3) SM6a; TM6B with w; dac<sup>2</sup> FRT40A/T(2;3) SM6a; TM6B. Null animals were selected on the basis of the absence of the Tubby phenotype. The ato nulls tested were homozygous for ato<sup>l</sup>, which is homozygous viable.

In addition to sal<sup>1</sup>/sal<sup>r</sup> null clones, two additional genotypes lacking sal and sal<sup>r</sup> in the antenna also were examined: sal<sup>FCK-25</sup>sal<sup>FCK-25</sup> and sal<sup>FCK-25</sup>Df(2L)32FP-5 FRT40A. sal<sup>FCK-25</sup> flies have a small deletion of sal/sal regulatory sequences that results in the loss of both sal and sal<sup>r</sup> expression in the antenna (Barrio et al., 1999). Df(2L)32FP-5 is a deletion that removes both the sal and sal<sup>r</sup> genes (Barrio et al., 1999). Since both sal<sup>FCK-25</sup> and Df(2L)32FP-5 FRT40A were balanced over T(2;3) SM6a; TM6B, sal<sup>r</sup> mutants could be identified by lack of the Tubby phenotype.

Ectopic expression of hth was induced using the GAL4/UAS binary system (Brand and Perrimon, 1993). dpp-GAL4 was used to activate UAS-GFP-hth along the anterior-posterior compartment boundary of the developing imaginal discs.

In situ hybridization and immunohistochemistry

In situ hybridization was carried out as previously described (Jiang et al., 1991; Tautz and Pfeifle, 1989). Antibody stainings and immunohistochemistry also were carried out as described previously (Halder et al., 1998). Antibodies used were: rabbit anti-Hth (Pai et al., 1998), rabbit anti-Dll (Panganiban et al., 1995), mouse anti-Dll (Vachon et al., 1992), rat anti-Sal (Barrio et al., 1999), rabbit anti-Atonal (Jarman et al., 1995), mouse anti-Cut and mouse anti-Dac (both from the University of Iowa Developmental Studies Hybridoma Bank). Secondary antibodies coupled to Cy2, Cy3, and Cy5 were obtained from Jackson ImmunoResearch. Imaging was carried out on a BioRad MRC1024 confocal microscope and a Zeiss Axioplan microscope equipped with an Axiocam.

RESULTS

**spineless acts downstream of both Distal-less and homothorax**

As with Dll and hth loss-of-function mutants, loss of ss also results in antenna to leg transformations (Fig. 1D) (Balkaschina, 1929; Burgess and Duncan, 1990; Struhl, 1982). This led us to investigate the genetic relationship among these genes. The expression of both Dll and hth appears relatively normal in the ss null antennal disc (Fig. 2A,B). We therefore conclude that ss is not required for either the activation or the maintenance of Dll or hth expression in the antenna. It has been reported that Dll is required for the antennal expression of ss (Duncan et al., 1998). To test whether Hth is also required to activate antennal ss expression, we examined the effect of ectopic hth. We find that ectopic Hth where Dll is expressed, for example in the wing pouch and leg disc, can activate ss expression (Fig. 2C-F). Conversely, loss of hth in the antenna results in loss of ss expression (Ian Duncan, personal communication). Taken together, these results indicate that ss functions downstream of both Dll and hth in the antenna. This led us to test whether ss is a primary target through which Dll and hth function in the antenna.

**spalt, dachshund, atonal, cut and spineless have antenna-specific patterns of expression**

There are only a few genes expressed in either the antenna or the leg but not in both. Among these are sal and sal<sup>r</sup>, which are identically expressed in a ring pattern in presumptive a2 (Barrio et al., 1999), but are detected at low levels only in leg imaginal disc cells that contribute to the body wall and not to the leg itself (Fig. 3C,G).

In contrast, there are other genes expressed in both antenna and leg precursors that have distinct patterns in the two appendages. Among these are dac, ato, ct and ss (Fig. 3A,B,D,E,F,H) (Duncan et al., 1998; Jarman et al., 1995; Mardon et al., 1994). The domain of dac expression in the antenna (a3) is much smaller than in the leg where it is expressed in multiple segments (Fig. 3D,H) (Dong et al., 2001). The function of dac in antennal development has not been described previously.

The bHLH transcription factor encoding gene, ato, is expressed in a ring in presumptive a2, but restricted to small spots in the dorsal leg disc (Fig. 3B,F) (Jarman et al., 1995). ato is required for the formation of most chordotonal organs in the fly (Jarman et al., 1993; Jarman et al., 1995). In the antenna, ato is required for formation of Johnston’s organ (JO) (Jarman et al., 1995), a complex sense organ composed of a large number of chordotonal organs that is used for sensing acoustic vibrations transmitted from the arista through a3 (Eberl, 1999; Goepfert and Robert, 2001).

ct, which is required for differentiation of external sensory (ES) class neurons (Bodmer et al., 1987), is expressed throughout the presumptive proximal antenna (a1 and a2) and head capsule but is expressed in small clusters of cells throughout the leg disc (Fig. 3A,E) (Blochlinger et al., 1993).

ss is expressed in a circular pattern in the antenna covering the presumptive a2 through the arista (Duncan et al., 1998). In the leg disc, ss is transiently expressed in a ring pattern in the presumptive tarsal region (Duncan et al., 1998) and subsequently becomes restricted to leg bristle precursors (Fig. 2C) (Duncan et al., 1998). Consistent with the ss expression domain, cuticular defects in ss null mutants can be found from a2 through the arista. These include the elongation of a2, loss of olfactory sensilla from a3, and transformation of a4, a5, and arista to tarsal segments (Fig. 1D) (Balkaschina, 1929; Burgess and Duncan, 1990; Duncan et al., 1998; Struhl, 1982).

The large differences in the expression patterns of

![Fig. 3](image-url)  

**Fig. 3.** cut, ato, sal and dac are differentially expressed in antenna and leg discs. Wild-type expression of cut (A,E), ato (B,F), sal (C,G) and dac (D,H) in wild-type antenna (A-D) and leg (E-F) discs.
these genes between the antenna and the leg led us ask whether these differences are due to differential regulation by antenna-determining genes such as *Dil* and *hth*. To test whether *Dil* or *hth* are responsible for the antenna-specific expression patterns of these genes, we examined the effects on their patterns in *Dil* and *hth* loss-of-function mutants. We also tested whether *Dil* and *hth* are regulating their antenna-specific targets via *ss* by examining their expression in *ss* null antennal discs.

**The role and regulation of dachshund in the antenna**

In contrast to the leg, in the antenna *dac* expression is restricted primarily to a single segment (a3; Fig. 3D,H) (Dong et al., 2001; Mardon et al., 1994). However we can detect trace levels of Dac in areas of the antennal disc immediately distal and proximal to a3 (Fig. 3D and not shown). Because no antennal phenotypes have been reported for loss-of-function *dac* mutants, it was unclear whether *dac* plays a role in patterning this appendage. In transheterozygous *dac* null mutants (*dac<sup>4</sup>/dac<sup>4</sup>), we observe a fusion of the a5 segment with the arista and a reduction in the width of the a5 segment (Fig. 4A,B). This fusion phenotype is similar to what is observed in *dac* hypomorphic and null legs (Mardon et al., 1994). However, unlike the leg phenotype, we find no obvious reductions in length or loss of segments in the *dac* mutant antenna. In addition, we only observe this antennal phenotype in *dac* null animals but not in strong hypomorphic combinations such as *dac<sup>lacZ/Dac<sup>4</sup></sup>*. Therefore, high levels of Dac are probably not necessary for *dac* function in the antenna.

We previously observed that if Dac levels are elevated in the antenna, expression of *Dll* and *hth* is repressed and medial leg structures are induced (Dong et al., 2001). Therefore if Dac levels are too high, antenna development is compromised. Because *bab* mutants exhibit phenotypes similar to those of *dac*, and *dac* regulates *bab* expression in the antenna (Chu et al., 2002; Godt et al., 1993), we think it likely that antennal *dac* function is mediated via its regulation of *bab*.

We have also found that the antennal *dac* expression domain expands in *Dll* hypomorphs and in *hth* null clones (Dong et al., 2001). This expansion of *dac* expression in *Dll* and *hth* mutant antennae resembles the leg pattern of *dac* expression. In contrast, in the *ss* null antenna, there appears to be neither expansion nor reduction of *dac* expression (Fig. 6G). The only detectable difference in the *ss* null antennal disc is overgrowth in the central (distal) area such that the ring of *dac* expression has a larger radius (Figs 2B and 6G). This correlates with the transformation phenotype of the *ss* null arista into a tarsus, which is a larger structure. Since the expression of *dac* relative to other genes appears normal in *ss* null antennae, we do not think *ss* regulates *dac*.

**Regulation of atonal in the antenna**

The expression of *ato* is required for the formation of the JO (Jarman et al., 1995). The JO is a structure unique to the antenna and is required to sense sound vibrations transmitted from the arista (Eberl, 1999; Goeptfert and Robert, 2001). A circular outline of the a2/a3 joint, to which the JO is attached, can be seen in an optical section through the interior of the a2 cuticle (Figs 5A, 6B). This outline is lost in the *ato* null antennae (Fig. 5B). *ato* function is generally associated with neuronal differentiation (Jarman et al., 1993; Jarman et al., 1995), so it is interesting that we observe cuticular defects associated with *ato* null antennae. It may be that formation of the JO is required for the normal morphology of the a2/a3 joint. The circular outline of the a2/a3 joint is lost in *hth* and *Dll* loss-of-function mutants (Fig. 1B,C), but is present in *ss* null mutants (Figs 1D, 5C). Consistent with this, the antennal expression of *ato* is lost in *hth* null clones (Fig. 5E,F) and in *Dll* hypomorphs (Fig. 5D), but persists in the *ss* null antenna discs (Fig. 5E). Thus although *ss* null mutants exhibit cuticular defects in a2 and a3 (Fig. 1D), the a2/a3 joint to which the JO is attached is present (Fig. 5C). We note that the *Dll* hypomorphic combination used, *Dll<sup>Gal4</sup>/Dll<sup>3</sup>* does not lead to loss of a2 (Fig. 7D). Thus the absence of *ato* expression in these antennae is not due to death of the cells that would normally express it.

**spalt and spalt-related are required for normal development of the second antennal segment**

*sal* and *salr* have similar sequences and are identically -
We have also observed a rough-eye phenotype in clones that are correlated with their expression domains in the antennal disc. This supports the view that (Barrio et al., 1999), exhibit cuticular defects in the antenna (Fig. 6A). The circular outline of the a2/a3 joint, to which the chordotonal organs of the JO attach, is defective in Df(2L)32FP-5 clones (Fig. 6B,C) and lost in salFCK-25/Df(2L)32FP-5 mutant antennae (P. D. S., S. Todi, D. Eberl and G. P., unpublished). Furthermore, a3 is unable to rotate in a2. The same antenna phenotypes are observed in salFCK-25 homozygous flies (not shown). However, these phenotypes are not observed in sal null clones generated using a sal16 FRT40A chromosome or in salFCK-25/sal16 transheterozygous antennae (not shown), that do not express sal but do express salr in the antenna (Barrio et al., 1999). Together, the loss of the a2/a3 joint and the loss of the freedom of rotation of a3 in a2 indicate that sal/salr null antennae are defective in hearing and implicate both sal and salr in normal development of the auditory organ.

Since ato is expressed within a subset of the sal/salr domain and is activated later than sal and hth in the antenna, we tested to see whetherDll and hth activate ato via sal/salr. We find no detectable reduction of ato expression in a2 either in Df(2L)32FP-5 clones (not shown) or in salFCK-25/Df(2L)32FP-5 transheterozygous animals (Fig. 6D,E). This allelic combination lacks detectable sal and salr expression in the antenna, but retains sal and salr expression in the eye (Fig. 6D,E). The normal expression of ato in the antennae of these mutants suggests that the activation of ato expression by Dll and hth is independent of sal/salr. Antennal sal/salr expression is also unaffected in a2 null imaginal discs (Fig. 6F). Therefore, sal/salr and ato are required in parallel for development of antennae that are functional in audition.

We have shown previously that Dll and hth are required for the expression of sal in the antenna (Dong et al., 2000). We report here that sal expression does not appear to be affected in ss null antenna (Fig. 6G). The fact that Ss is not required for the expression of either ato or sal in a2 is consistent with our observation that the a2/a3 joint is still present in the ss null antenna (Fig. 1D, Fig. 5C).

cut expression in the antennal disc requires hth, but not Dll

Expression of the homeodomain transcription factor encoded by ct almost completely fills the hth expression domain of the third instar antennal disc (Fig. 7A,A′). In contrast, the ct and hth expression patterns in the leg disc are distinct from one another (Fig. 3E and not shown). This made ct a strong antenna-specific candidate target for Hth. The antennal expression of ct is lost in hth null clones (Fig. 7B,B′) indicating that ct is indeed downstream of hth. To test whether the a2 expression of ct also requires Dll, we examined ct expression in Dll mutants. ct expression is not reduced in Dll null clones or in Dll hypomorphs (Fig. 7C,C′,C″,E). Therefore, although Dll and hth are both required for antennal fate, ct is an antenna-specific target of Hth activation that is independent of Dll. As with other antenna-specific targets of Dll and Hth, ct expression is also not lost in ss null antenna (Fig. 7F).

expressed in the antennal imaginal disc in presumptive a2 (Barrio et al., 1999). However, functions for sal and salr in the antenna have not yet been described. To investigate whether sal and/or salr are required for normal antenna development, we examined clones null either for sal alone or for both sal and salr in the adult head. Clones null for only sal in the antenna have no obvious cuticular phenotypes (not shown). However Df(2L)32FP-5 clones, which are null for both sal and salr (Barrio et al., 1999), exhibit cuticular defects in the antenna (Fig. 6A-C). This supports the view that sal and salr have some redundant functions. The areas affected in the mutants are correlated with their expression domains in the antennal disc. We have also observed a rough-eye phenotype in clones that are null for both sal and salr but not for sal alone (data not shown) (Mollereau et al., 2001). a2 normally forms a cup, in which a3 sits and must rotate along the PD axis, to transmit sound vibrations from the arista (Goepfert and Robert, 2001). An overall reduction in a2 is along the PD axis, to transmit sound vibrations from the arista (Mollereau et al., 2001).
DISCUSSION

Distal-less and homothorax regulate multiple targets

Dll and hth have dual functions in the Drosophila antenna. They are required for the specification of the distal versus proximal domains, respectively, and for the specification of antenna fate. We identify multiple targets of Dll and hth during antennal development (Fig. 8A). These targets, which have expression patterns unique to the antenna, include dac, ato, sal, ct and ss (Fig. 8C). Because ss mutants also exhibit transformations of the antenna toward leg, and ss is a Dll target, it was thought that the antenna specification functions of Dll and hth might occur only via ss. Contrary to this, none of the other four genes regulated by Dll and hth examined here are
activated via ss. We conclude that Dll and hth regulate multiple independent targets during Drosophila antennal development.

The roles of Distal-less, homothorax and spineless in homeotic specification of the antenna

By examining the expression domains, the mutant phenotypes, the genetic interactions and the downstream targets of Dll, hth and ss, we can start to understand the different roles that these homeotic genes are playing in antenna specification. During imaginal disc development, the expression of Dll and ss is found from a2, a3, a4, a5 and arista. Expression of hth is dynamic and retracts from the distal-most segments by late third instar, but hth is expressed and cell-autonomously required throughout the antenna from a1 through to the arista (Casares and Mann, 1998).

The Dll mutant phenotypes indicate that Dll is required both for the distal limb development and for antenna fate. Dll hypomorphs exhibit distal limb deletions as well as antenna to leg transformation. The transformation phenotypes of hypomorphic Dll antennae can be observed from a2 through to the arista. In these mutants, hth expression is not lost or detectably reduced. Thus medial leg structures can develop in the presence of Hth. This suggests that although loss of hth from the distal and medial leg, via Antennapedia-mediated repression, occurs during normal leg development, loss of Hth is not essential for leg differentiation. It also suggests that the requirement for Antennapedia in normal leg development is not only to regulate hth.

Since ss is not required to activate antenna-specific expression of genes such as sal/salr and ato that are involved in antenna differentiation, the question arises as to what ss does do in the antenna. As described below, ss represses tarsus and tarsal claw organ formation in the antenna. Since loss of ss also leads to loss of olfactory sensilla on a3, ss probably potentiates the formation of these sensilla, either cooperating with or mediating Dll and hth activities in a3. Similarly, since ectopic expression of ss elsewhere in the body can lead to the formation of ectopic aristae, ss may also cooperate with or mediate Dll and hth activities in arista differentiation.

We demonstrate here that sal and salr, like ato, are required for normal auditory functions. Since both Dll and hth are required for the antennal expression of ato (this work) and sal (Dong et al., 2000), Dll and hth mutant antennae are also hearing defective. In contrast, ss null antennae exhibit normal expression of both ato and sal and normal morphology of the a2/a3 joint, leading us to think that ss mutants are likely to be functional in audition.

Homeotic genes act via repression as well as potentiation of tissue fates

We have shown here that homeotic genes, Dll and hth, regulate multiple targets during antennal development. These targets function in specifying antenna structures and/or in repressing leg development (Fig. 8B). For example, the ss mutant phenotype suggests that it represses leg tarsal differentiation. But ss is also required for the formation of olfactory sensory sensilla normally found in a3. Although Dll and hth repress distal leg development via activation of ss, their repression of medial leg development appears to be, at least in part, independent of ss. Instead, this is achieved via their regulation of the medial leg gene, dac, to a narrower domain of expression with lower levels in the antenna as compared to the leg. sal/salr and ato are required for proper differentiation of a2. However, no transformation phenotypes are associated with the sal/salr and ato null antenna. This indicates that while sal/salr and ato are required to make particular antenna-specific structures, they do not appear to repress leg fates. Therefore homeotic genes such as Dll and hth repress the elaboration of other tissue fates in addition to activating genes required for the differentiation of particular tissues.

The roles of atonal and spalt/spalt-related in Drosophila audition

In third instar imaginal discs, coexpression of Dll and Hth activates sal/salr and ato in a2 where they, in turn, are needed for JO development. The expression of ato is required for the formation of the JO (Jarman et al., 1995) and the a2/a3 joint to which it is attached. Although sal and salr are not required for the expression of ato, the a2/a3 joint is lost in the sal/salr null antenna (this work). We expect this leads to improper formation of the JO, although it is also possible that defects in a2/a3 joint formation preclude JO differentiation. In addition, because sal is not lost in ato null antennae, we conclude that sal/salr and ato are required in parallel for proper formation of the JO. Furthermore, in the sal/salr null antenna, a3 cannot freely rotate within a2. This rotation is necessary for transmission of sound vibrations from the arista to the JO. Taken together, these findings implicate sal/salr in Drosophila audition. Interestingly, mutations associated with the human homolog of sal, SALL1 cause the human autosomal dominant developmental disorder, Townes-Brocks Syndrome (TBS) (Kohlhase et al., 1998). Auditory defects are characteristic of TBS (Monteiro de Pina-Neta, 1984). Auditory defects are also associated with the human genetic disorder, Split Hand/Split Foot Malformation (SHFM) (Ignatius et al., 1996; Raas-Rothschild et al., 1989), and the SHFM1 locus is linked to the Dll homologs, DLX5 and DLX6 (Crackower et al., 1996; Scherer et al., 1994). The sensorineural hearing defects associated with the Distal-less and spalt genes in both Drosophila and Homo sapiens, in conjunction with a recent finding that atonal functions in mouse as well as fly audition (Bermingham et al., 1999; Eberl et al., 2000; Jarman et al., 1993; Jarman et al., 1995), lead us to propose that insect and vertebrate hearing share a common evolutionary origin. We anticipate that further developmental genetic dissection of the Drosophila auditory system will provide additional insights into human ear development and suggest that Drosophila could provide a useful model system for studying both TBS and SHFM.

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