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Regulation of the *Drosophila* distal antennal determinant *spineless*

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Abstract

The transformation of antenna to leg is a classical model for understanding segmental fate decisions in *Drosophila*. The *spineless* (*ss*) gene encodes a bHLH-PAS transcription factor that plays a key role in specifying the identity of distal antennal segments. In this report, we identify the antennal disc enhancer of *ss* and then use enhancer-*lacZ* reporters to work out how *ss* antennal expression is regulated. The antennal determinants *Distal-less* (*Dll*) and *homothorax* (*hth*) are key activators of the antennal enhancer. *Dll* is required continuously and, when present at elevated levels, can activate the enhancer in regions devoid of *hth* expression. In contrast, *homothorax* (*hth*) is required only transiently both for activation of the enhancer and for specification of the aristal portion of the antenna. The antennal enhancer is repressed by *cut*, which determines its proximal limit of expression, and by ectopic Antennapedia (Antp). Repression by Antp is not mediated by *hth*, suggesting that *ss* may be a direct target of Antp. Finally, we show that ss^{+} is not a purely passive target of its regulators: ss^{+} partially represses *hth* in the third antennal segment and lies upstream of *Dll* in the development of the maxillary palp primordia.

Keywords: *spineless*; *aristapedia*; Dioxin receptor; Aryl hydrocarbon receptor; *Distal-less*; *homothorax*; Antenna; *Drosophila*; *cut*; *Antp*

Introduction

How the identity of the antenna is specified in *Drosophila* was for many years a mystery. It was known that the Hox genes are not involved since the anterior limit of their expression in the body lies just posterior to the antennal segment. In the last few years, it has become clear that at least three genes play key roles in specifying antennal identity: *homothorax* (*hth*) and *Distal-less* (*Dll*), which encode homeodomain proteins, and *spineless* (*ss*), which encodes a bHLH-PAS protein. The most important of these is *hth*. Mitotic recombination clones homozygous for hth^{-} alleles can transform the entire antenna to a limb that is leg in identity, although not form (Casares and Mann, 1998, 2001). Consistent with this transformation, *hth* is expressed throughout the antennal primordium in the first and second larval instars. However, during the late second or early third instar, *hth* is repressed in the region of the antennal disc that gives rise to the arista and down-regulated in the next most proximal region, which gives rise to the third antennal segment (A3) (Fig. 1D). In leg discs, *hth* undergoes a more extreme distal repression, so that it comes to be expressed only in the most proximal segments (coxa and trochanter), where it serves to distinguish these segments from more distal ones (Abu-Shaar and Mann, 1998; Wu and Cohen, 1999). Hth functions as a heterodimer with the homeodomain protein Extradenticle (Exd), which is also required for antennal identity and normal proximo-distal subdivision of the leg (González-Crespo and Morata, 1995; Rieckhof et al., 1997; Kurant et al., 1998; Pai et al., 1998).

The *Dll* gene is expressed in the distal portions of all of the ventral appendages, and loss-of-function alleles of *Dll* cause deletions of distal structures in these appendages. In the antenna, *Dll* is expressed in A2, A3 and the arista (Fig. 1E), and this entire expression domain is deleted in Dll^{-} mutants (Cohen and Jürgens, 1989). However, some weak alleles of *Dll* cause transformations of distal antennal structures toward leg, suggesting that Dll^{+} has a role in specifying distal antennal identity that is distinct from its requirement for distal limb development (Sunkel and Whittle, 1987; Dong et al., 2000). Since *Dll* is expressed in the distal portions of all the ventral appendages, this function must depend upon interaction with some other factor that is differentially expressed in the legs and

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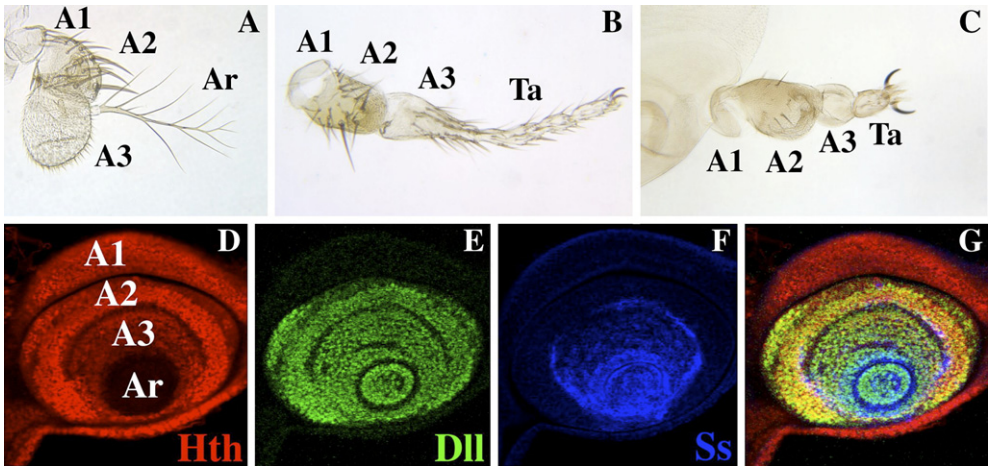

Fig. 1. Antennae from wild-type and *ss* mutant adults, and expression of Hth, Dll and the *ss* antennal reporter B6.9 in a mature antennal disc. (A) A wild-type antenna. The first (A1), second (A2) and third (A3) antennal segments and the arista (Ar) are indicated. (B) Antenna from an ss^{a}-type mutant ($ss^{D114.7}/Df(3R)ss^{D114.4}$). Note that distal A3 and the arista are transformed to an almost complete set of tarsi (Ta). (C) Antenna from an *ss* null mutant ($ss^{D115.7}/Df(3R)ss^{D114.4}$). Apart from a reduction in bristle size, A1 and A2 are basically normal; A3 is composed of naked cuticle, and the arista is reduced to a fifth tarsal segment (Ta) with claws. Panels D–G show a late third instar antennal disc triply labeled for Hth (red), Dll (green) and the *ss* antennal reporter B6.9 (blue). The primordia of A1, A2, A3 and Ar are indicated. Note that Hth (red) is present at a lower level in A3 than in A1 and A2 and is absent in the aristal region (D), Dll (green) is expressed in A2 and more distally (E), and the B6.9 *ss* antennal reporter (blue) is expressed in A3 and more distally (F). (G) Merging of panels D–F.

antennae. Dong et al. (2000) have suggested that this factor is Hth and that the identity of A2, A3 and the arista is defined by the combined expression of *hth* and *Dll*. This idea is supported by the effects of hth^{-} and Dll^{-} alleles on the expression of antenna-specific genes and by the finding that combined ectopic expression of Hth and Dll can cause transformations to antenna (Dong et al., 2000, 2002).

The *ss* gene is the *Drosophila* homolog of the mammalian aryl hydrocarbon receptor (AHR), also known as the dioxin receptor (for a review, see Schmidt and Bradfield, 1996). In the antenna, *ss* is expressed continuously in A3 and more distal segments (Duncan et al., 1998) (Fig. 1F) and is required for these segments to develop with antennal identity (Struhl, 1982; Burgess and Duncan, 1990; Duncan et al., 1998). In ss^{-} mutants, A3 develops with almost no specialization and consists of naked cuticle, while the region distal to A3 develops as a fifth tarsal segment with claws. Ectopic expression of *ss* can induce ectopic distal antennal structures, indicating that *ss* is an antennal determinant (Duncan et al., 1998). ss^{+} is also required for development of tarsal segments 1–4 in the legs and is expressed transiently in a ring in the tarsal regions of the leg discs. Consistent with the model of Dong et al. (2000), *ss* expression in the antenna requires both Dll^{+} and hth^{+} (Duncan et al., 1998; this report). Thus, ss^{+} lies downstream of these genes and likely executes many of their functions in specifying distal antennal identity.

In this report, we use *lacZ* reporters to identify the *cis*-regulatory elements responsible for driving *ss* expression in embryos and imaginal discs. We identify enhancers responsible for almost all aspects of *ss* expression. At least three distinct antennal enhancers are present; we focus on the enhancer responsible for expression in the larval antennal disc. We analyze the activity of this enhancer in clones homozygous for null alleles of other genes involved in limb development. We find that the antennal disc enhancer is positively regulated by *Dll* and *hth*; *Dll* is required continuously for activation, whereas *hth* is required only early in development. Clones expressing Dll ectopically can activate the enhancer in the absence of Hth expression, suggesting that Dll is its primary activator. The enhancer is repressed by *cut*, which defines the proximal limit of its activity. The antennal disc enhancer is also repressed by ectopic expression of Antennapedia (Antp). Repression by Antp can occur within clones induced long after the requirement for *hth* has passed, suggesting that *ss* is an independent target of Antp responsible for mediating transformations of distal antenna to leg. We also identify an *ss* enhancer that drives expression in a ring in the tarsal primordia of the legs and in the distal antenna. The latter expression likely accounts for the development of a full set of tarsal segments in the transformed antennae of *ss* mutants lacking only the antennal enhancer. Finally, we show that *ss* is not a purely passive target of its regulators; *ss* down-regulates *hth* expression in A3 and lies upstream of *Dll* in the development of the maxillary palps and perhaps also the bract cells of the legs.

Materials and methods

Restriction fragments from *ss* λ clones (Duncan et al., 1998) were subcloned into the enhancer-tester vector *pCaSpeR-hs43-βgal* (Thummel and Pirrotta, 1992) and germ-line transformants recovered by standard methods. Two independent insertions were recovered for each of the EX6.5 and EX4.3 fragments; three or more independent insertions were analyzed for the remaining fragments.

Dissection of B6.9 and EX6.5: the B6.9 subfragments E1.6, E1.9, E2.0 and EX1.9 were generated by *Eco*RI or *Eco*RI and *Xba*I digestion of λ clones from *ss* (Duncan et al., 1998). S4.9 was generated by digesting B6.9 with *Spe*I. Four subclones of E2.0, of 522, 542, 531 and 554 bp, were generated by PCR; these were cloned into *pCR2.1* (Invitrogen), verified by sequencing and then transferred to *pCaSpeR-hs43-βgal*. The EX6.5 fragment was subdivided by complete or partial digestion by *Pst*I (see Fig. S2).