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# Control of the *spineless* antennal enhancer: Direct repression of antennal target genes by Antennapedia

Dianne Duncan, Paula Kiefel, Ian Duncan *

*Department of Biology, Washington University, 1 Brookings Drive, St. Louis, MO 63130, USA*

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ABSTRACT

It is currently thought that antennal target genes are activated in Drosophila by the combined action of *Distal-less*, *homothorax*, and *extradenticle*, and that the Hox gene *Antennapedia* prevents activation of antennal genes in the leg by repressing *homothorax*. To test these ideas, we analyze a 62 bp enhancer from the antennal gene *spineless* that is specific for the third antennal segment. This enhancer is activated by a tripartite complex of Distal-less, Homothorax, and Extradenticle. Surprisingly, Antennapedia represses the enhancer directly, at least in part by competing with Distal-less for binding. We show that Antennapedia is required in the leg only within a proximal ring that coexpresses Distal-less, Homothorax and Extradenticle. We conclude that the function of Antennapedia in the leg is not to repress *homothorax*, as has been suggested, but to directly repress *spineless* and other antennal genes that would otherwise be activated within this ring.



## Introduction

Mutations of several genes in Drosophila cause transformations of antenna toward second leg. The best known of these mutations are dominant gain-of-function alleles of the Hox gene *Antennapedia* (*Antp*), which can cause the antenna to develop as a complete leg. Struhl (1981, 1982a) showed that loss-of-function alleles of *Antp* have the opposite effect, causing transformation of leg structures to antenna, but have no effect on development of the antenna itself. He proposed that *Antp* is normally expressed in the legs but not the antenna, and that its function is to repress the activation of antenna-specific genes in the leg. The gain-of-function alleles were suggested to cause ectopic expression of Antp in the antenna. Molecular studies confirmed that *Antp* is expressed as inferred by Struhl (Frischer et al., 1986). However, until recently, the identities of the antennal genes controlled by *Antp* remained uncertain, as it was not known how antennal identity is specified.

We now know that the identity of most of the antenna is specified by the combined action of homeodomain transcription factors encoded by the *homothorax* (*hth*) and *Distal-less* (*Dll*) genes (Casares and Mann, 1998; Dong et al., 2000). These genes are coexpressed extensively in the antenna, whereas in the leg they are coexpressed in only a narrow proximal ring of cells. Several antennal genes have been shown to be activated independently by combined Hth and Dll expression (Dong et al., 2002). One of the most important of these targets is *spineless* (*ss*), which encodes a bHLH transcription factor homologous to the mammalian dioxin receptor (Duncan et al., 1998). The expression patterns of *Dll*, *hth* and *ss* in the antennal imaginal disc, and an adult antenna are shown in Fig. 1A.

Hth is required for normal identity of the entire antenna, and is expressed throughout the antennal disc in the first and second larval instars. $hth^{-}$ mitotic recombination clones induced at these times transform the entire antenna to a leg-like appendage (Casares and Mann, 1998). Subsequently, Hth expression is lost in the most distal portion of the disc, the primordium of the arista, whose development becomes independent of *hth* (Emmons et al., 2007). Hth is also expressed in the most proximal segments of the leg, where it is required for normal growth and proper formation of segment boundaries (Abu-Shaar and Mann, 1998; Wu and Cohen, 1999; Casares and Mann, 2001). Hth functions as a heterodimer with the homeodomain protein Extradenticle (Exd) (Rieckhof et al., 1997; Pai et al., 1998; Kurant et al., 1998), which is also required for antennal specification and proximal leg development (González-Crespo and Morata, 1995). In addition to these roles, Hth and Exd serve as important cofactors that increase the binding specificity of the Hox proteins (for review see Mann et al., 2009).

*Dll* is required for the development of distal structures in all of the ventral appendages (Cohen et al., 1989). In the antenna, *Dll* is expressed in the primordia of the second (A2), and third (A3) antennal segments and the arista, and this entire expression domain is deleted in $Dll^{-}$ mutants (Cohen and Jürgens, 1989). However, weak alleles of *Dll* cause transformations of antenna toward leg (Sunkel and Whittle, 1987; Dong et al., 2000), suggesting that *Dll* has a role in specifying antennal identity that is distinct from its general role of

* Corresponding author. Fax: +1 314 935 4432.
*E-mail address:* duncan@biology.wustl.edu (I. Duncan).

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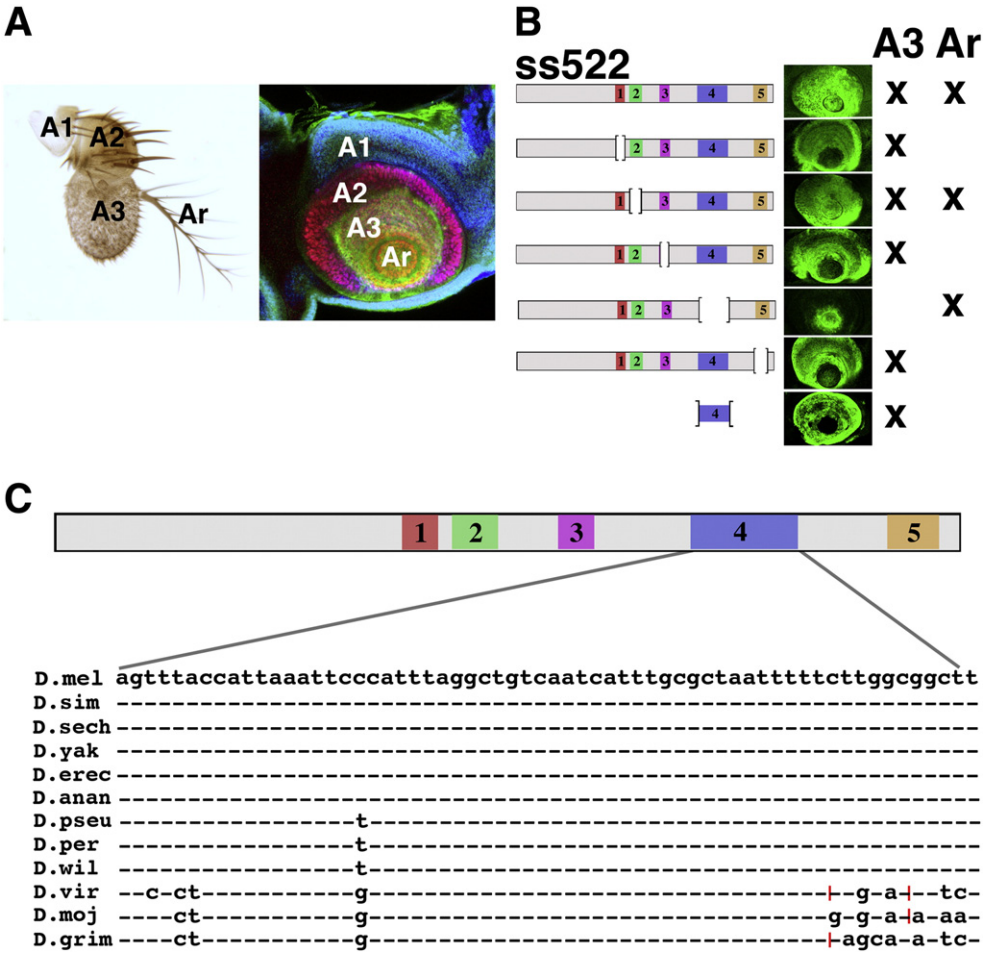


**Fig. 1.** (A) Left: A wild-type adult antenna. The first (A1), second (A2), and third (A3) antennal segments and the arista (Ar) are indicated. Right: A mature antennal disc stained for Hth (blue), Dll (red), and the *ss* reporter *B6.9/lacZ* (Emmons et al., 2007) (green). Hth is expressed in the primordia of A1, A2, and A3; Dll is expressed in A2, A3, and the arista; and *ss* is expressed in A3 and the arista. (B) Five conserved domains within *ss522* and their deletion derivatives are indicated. The antennal expression each drives *in vivo* is shown to the right. (C) Conservation of the sequence of domain 4 in 12 Drosophila species; dashes indicate identity, red hatch marks indicate 3 bp insertions relative to the *D. melanogaster* sequence.

specifying distal limb structures. Dong et al. (2000) proposed that Dll acts in concert with Hth (and presumably also Exd) to define antennal identity. This proposal is supported by the effects of $hth^{-}$ and $Dll^{-}$ alleles on the expression of antenna-specific target genes and by the effects of combined ectopic expression of Hth and Dll (Duncan et al., 1998; Dong et al., 2000, 2002; Emmons et al., 2007).

Many of the identity functions of Hth and Dll in the distal antenna are executed by the target gene *ss* (Dong et al., 2002; Emmons et al., 2007), which is expressed in the primordia of A3 and the arista. In $ss^{-}$ mutants, A3 lacks all olfactory sensilla, and the arista is transformed to distal leg (Struhl, 1982b; Duncan et al., 1998). In previous work (Emmons et al., 2007), we identified the antennal enhancer from *ss* and showed that its expression depends upon Dll and Hth, and that it is repressed by ectopically expressed Antp. The enhancer is also repressed in A2 by the homeodomain protein Cut (Blochlinger et al., 1988).

In this report, we address two major unresolved questions. First, how are inputs from Dll, Hth, and Exd integrated at antennal target genes? To date, no antennal enhancers have been characterized at the molecular level, so the mechanism of action of these factors has remained uncertain. Second, how does Antp repress antennal identity in the leg? Based on the finding that $Antp^{-}$ clones in the leg sometimes show ectopic distal expression of Hth, Casares and Mann (1998) proposed that the primary function of Antp is to repress *hth* in the distal leg, which then prevents activation of antennal target genes. Although this view is widely accepted, it has not been subject to direct test.

To address these questions, we focused our attention on the antennal enhancer of *ss*. We identify a 62 bp subregion of this enhancer that drives expression specifically in A3. Like the full antennal enhancer, the A3 enhancer requires Dll, Hth, and Exd for expression. All three of these factors interact directly with the enhancer. The binding of Dll shows strong cooperativity with Hth and Exd, indicating that these proteins bind as a complex. This Dll/Hth/Exd tripartite binding suggests that Dll behaves much like a Hox protein in specifying antennal identity. Surprisingly, we find that Antp also interacts directly with the A3 enhancer. Antp binds cooperatively with Hth and Exd, and represses the enhancer at least in part by competing with Dll for binding.

Our finding that Antp interacts directly with the A3 enhancer led us to reexamine the role of *Antp* in leg development. We find that the A3 enhancer is sometimes activated within $Antp^{-}$ clones in the leg, consistent with the transformation to antenna that such clones can cause. However, this activation occurs only within a narrow ring of cells in the proximal leg that coexpresses Dll, Hth, and Exd (Wu and Cohen, 1999). Subsequently, some of the $Antp^{-}$ cells in which the A3 enhancer has been activated begin to express Ss, Cut, and other antennal markers, indicating a transformation to antenna. Importantly, we find that expression of Hth and Dll in the proximal ring is unaffected in $Antp^{-}$ clones, indicating that Antp does not block antennal development in the leg by repressing *hth*, as has been thought. Rather, we conclude that the main, and perhaps sole, function of Antp in the leg imaginal disc is the direct repression of