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Specification and Differentiation of Antennal Identity in *Drosophila*

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The antenna and legs of *Drosophila* are considered homologous structures because it is believed that they would have evolved from a common ancestral appendage. Although the primordia for the leg and antenna, the leg and antenna imaginal discs are similar in shape and structure in the adult they develop and differentiate into distinct structures and perform different functions, such as olfaction and hearing in the case of antenna and locomotion in the case of legs. Recently, new insights have been gained about the process of antennal development and identity. Here, I describe some of these genes and the regulatory interactions among them, which lead to antenna development and identity in *Drosophila.*

Key Words : Antenna development, *Drosophila, hth, Dll, ss, ct, dan, danr*

Introduction

The fruit fly *Drosophila melanogaster* is a holometabolous insect that undergoes complete metamorphosis, which involves embryonic, three larval and a pupal stage before it emerges into an adult fly. One interesting fact about this mode of life stages is that it does not represent gradual changes towards adulthood but each stage is a distinct polymorphic form of the same organism. During the pupal stage of development, the tissues, which make the larval body, disintegrate and are replaced by the adult structures. These precursors of the adult appendages are specified very early during the embryonic stage by the coordinated action of the signalling molecules *wingless (wg*) and *decapentaplegic* (*dpp*) and are termed imaginal cells. These cells are set-aside during the embryogenesis, they proliferate during the larval stages and form the specialized adult precursors known as imaginal discs, which differentiate during the pupal stage to give rise to the adult structures (Cohen 1993). Out of the 19 imaginal discs in *Drosophila*, which form the different parts of the adult body, there are three pairs of leg imaginal discs and a pair of eye-antennal discs. These give rise to the three pairs of the adult legs, eyes and the antennae, respectively. The leg and antenna discs are considered homologous because both are similar in structure, possess almost identical segments corresponding to each other and differentiate by the identical method with concentric circular folds (Postlethwait & Schneidermann 1971). The antenna includes antennal segments 1-3 (A1-A3) and the arista while the leg possesses coxa, trochanter, femur, tibia, tarsal segments 1-5 and the claws at the tip (figure 1C, D). The fact that these discs have very similar shape and structure at the disc level but develop and differentiate to form morphologically distinct structures with specific functions in the adult fly (figure1 A, B) suggests that distinct mechanisms, controlled by different sets of genes, exist for the development and differentiation of the leg and antenna. Recently, insights have been gained about the mechanisms and the genes which are specific for antennal development and differentiation.

Morphogens and Ventral Appendage Determination

For a long time, it was believed that the difference in development and differentiation of antenna and leg could be explained by the way two groups of molecules, namely the morphogens and the homeotic selector genes, are expressed in these discs. Morphogens are secretory-signalling molecules, which act in a concentration-dependent manner along the anterio-posterior, dorso-ventral and proximo-distal axes of the different appendages. Depending upon their difference in concentration, they are said

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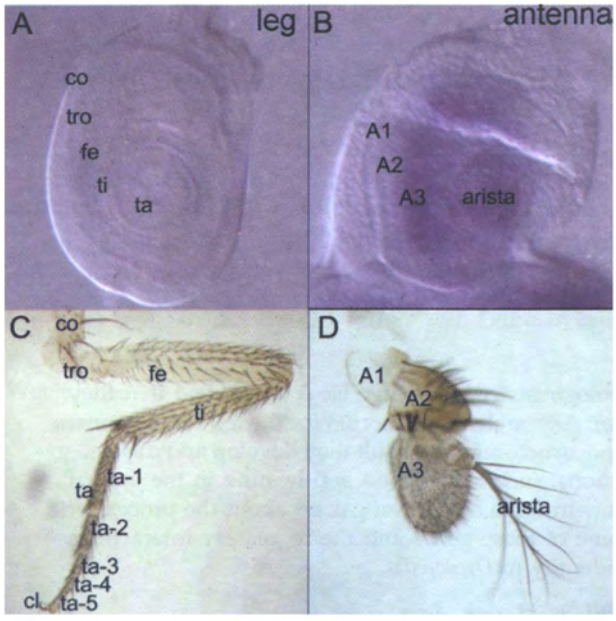

Figure: 1 (A) Leg imaginal disc; **(B)** antenna imaginal disc depicting the similar shape and structure with concentric arrangement of the segments (co- coxa, tro- trochanter, fe- femur, ti-tibia, ta-tarsus, A1- 1st antennal segment, A2- 2nd antennal segment, and A3- 3rd antennal segment); **(C)** Adult wild type leg (ta1-5- 1st to 5th tarsal segments, cl- claw); **(D)** Adult wild type antenna.

to bring in diversity by the dosedependent activation of different target genes (for review see Lawrence & Struhl 1996, Neumann & Cohen 1997). In *Drosophila* the development and elaboration of ventral structures along the proximodistal axis are comparable. It had been suggested to be determined by the action of the secretory signalling molecule *hehdehog (hh)*, which signals from the posterior compartment and induce the secondary signalling molecules *wg* in the ventral compartment and *dpp* in the dorsal compartment (Struhl & Basler 1993, Basler & Struhl 1994, Diaz-Benjumea et al. 1994, Lecuit & Cohen 1997). It had been suggested that by negatively regulating each other's expression, *wg* and *dpp* form a concentration gradient along the proximo-distal axis, which independently activate different target genes *Distalless* (*Dll*) and *dacshund* (*dac*) expression (Brook & Cohen 1996, Jiang & Struhl, 1996, Heslip et al. 1997). *Dll* is expressed in the distal domain, which is the high threshold region for both the morphogens, Wg and Dpp, and *dac* is expressed proximal to *Dll* where the concentration is low (Brook & Cohen 1996, Lecuit & Cohen 1997). Thus, the expression of morphogens is crucial for the determination of the appendage primordia and as mentioned, the signalling molecules Wg and Dpp play the crucial role in the development of ventral appendages. Interestingly, the expression pattern of these two morphogens is similar between the leg and antenna imaginal discs (figure 2A, B). This led to the idea that most of the process of leg and antenna development is similar and the distinctive nature of these appendages should be set up at the later stage.

Homeotic Selector Gene *Antp* Specifies Leg Identity

This view is further supported by the role played by the second group of molecules, which are products of the homeotic selector genes, the latter are said to bring in morphological identity along the antero-posterior axis by their expression in the embryos (Lewis 1978) and act as switches governing the choice between alternative fates. During the development and differentiation of adult structures they act on the precursor cells and select or specify them to a particular developmental pathway. Thus, by the expression or absence of expression of a particular homeotic selector gene, these cells can develop into a particular appendage. In *Drosophila,* the homeotic genes are found in two complexes namely the *Antennapedia* complex and the *Bithorax* complex. The homeotic gene *Antennapedia (Antp)*

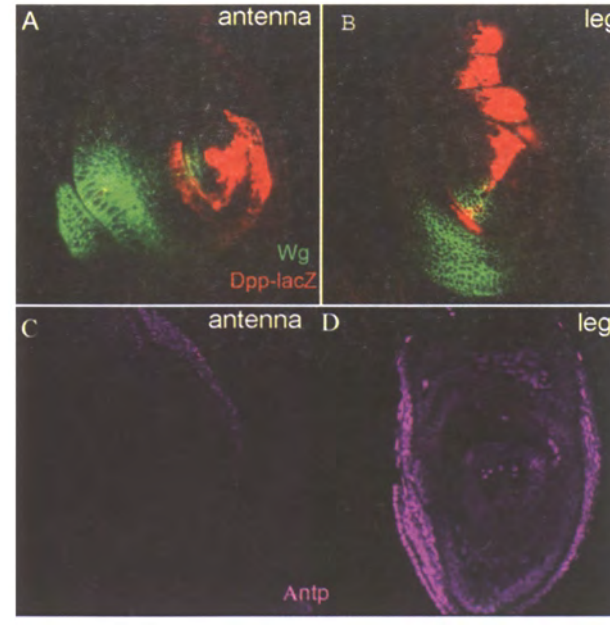

Figure 2 The secretory signalling molecules *wg* and *dpp* are expressed in similar pattern both in the antenna **(A)** and leg **(B)** imaginal discs. The leg specific homeotic selector gene *Antp* is expressed only in the leg imaginal disc **(D)** and is not expressed in the antenna imaginal disc **(C).**

of the Antennapedia complex has been shown to be expressed only in the leg while the homologous structure antenna develops without any input from homeotic genes (Gehring 1966, Kaufman et al. 1990, figure 2C, D). These studies clearly demonstrate coordination between the effects of hoemotic selector genes and the activities of the signalling molecules such as Wg and Dpp so that correct identity of the appendages is set up during the normal course of development. Thus, the simple model for the leg versus antenna development is the common primordia determination by the coordinated action of the signalling molecules and the selector gene action by *Antp* in the leg primordial cells resulting in leg.

The role of the Antp as a genetic switch is further supported by the fact that several gains of function mutations of *Antp*, which ectopically express Antp in the antenna, result in antenna to leg transformation (Gehring 1966, Struhl 1981). The same antenna to leg transformation is also shown by way of genetic manipulations (Zeng et al. 1993). Further, the absence of normal expression of *Antp* in the legs leads to the reverse leg to antenna transformation and, as expected, loss of *Antp* had no phenotype in the antenna (Struhl 1981). This led to the suggestion that the antenna phenotype may be the default state (Struhl 1981). It is also suggested that the way by which *Antp* imposes leg identity may be by repressing those genes, which are important for the normal development and differentiation of antennal identity (Casares & Mann 1998). The suggestion that the antenna is the ground state for ventral appendage development gained further support by the transformations seen in the beetle *Tribolium* in the absence of the homeotic genes (Stuart et al. 1991). In the absence of the normal homeotic gene expression all the legs were transformed into antenna, favouring the possibility that the leg development is imposed on the antennal default state by the action of homeotic genes.

How Does the Antenna Develop?

Although homeotic input is not needed for antennal development, other homeo-domain containing genes such as *homothorax (hth*) (Casares & Mann 1998, Pai et al.1998), *Distal-less* (*Dll*) (Cohen & Jurgens 1989, Cohen et al. 1989, Gorfinkiel et al. 1997), *spineless-aristapedia* (*ss*) (Struhl 1982, Burgess & Duncan 1990, Duncan et al. 1998), *distal antenna* (*dan*) and *distal antenna related* (*danr*) (Emerald et al. 2003) have been shown to play a role in antennal development based on loss of function and ectopic expression studies. Ectopic expression of these genes in other parts of the body was shown to induce antenna development; and loss of their function resulted in antenna to leg transformation. This suggested that antennal development also involves specific regulatory interactions. The fact that both antenna and leg appendages develop as a result of distinct regulatory interactions gained further support by the analysis of ground state for ventral appendages (Casares & Mann 2001). Removal of the *Antp* the leg specific homeotic selector gene and the antenna specific gene *hth* resulted in development of structures similar to leg suggesting the common building block of ventral appendage is leg like (Cassares & Mann 2001). This result along with what is seen in the case of *Tribolium*, suggests that the antenna and leg developments involve distinct genes and regulatory interactions as discussed in the following.

Homothorax

The gene *hth* encodes a transcription factor with two functionally important domains which are conserved among plants, fungi and animals: (1) an atypical three aminoacid loop extension (TALE) class of homeodomain in the C-terminus and (2) an N-terminal domain termed as the Homothorax-Meis (HM) domain (Burglin & Aspock 1999). In embryo, Hth is first detected in the cytoplasm at the gastrula stage. At the germ band extended stage of the embryogenesis, it starts to accumulate in the nucleus (Rieckhof et al. 1997). Hth has been shown to function by promoting the nuclear localization of another homeodomain protein, Extradenticle (Exd), a cofactor for various other homeodomain proteins (Pai et al. 1998). In the imaginal discs, the correlation between expression of *hth* and nuclear localization of *exd* occurs both in the leg and the antenna (Rieckhof et al.1997). In the absence of *hth,* Exd is localized to the cytoplasm and is non-functional. Hth associates physically with Exd by the HM domain and promotes its nuclear localization, which is essential for the functional specificity of Exd (Rieckhof et al. 1997). Cell culture studies showed

that this localization can be exerted in an hour and can happen even in the absence of a functional homeodomain of Hth (Abu-Shaar & Mann 1998). There is also some evidence for Exd regulating Hth post-transcriptionally. In the absence of Exd, the Hth protein is lost in the clones. However, the lac^Z expression under the *hth* promoter is unaffected suggesting Exd regulates Hth post-transcriptionally, probably by altering its stability (Pai et al. 1998, Rieckhof et al. 1997, Kurant et al. 1998).

Exd is the co-factor for *Hox* gene products and forms a heterodimer with different hox proteins (Ryoo & Mann 1999). Hox gene products regulate their downstream target genes by binding to their DNA. The general way by which *hth, exd* and *Hox* function is by forming a trimeric complex involving Hth, Exd and Hox proteins that regulate their downstream targets (Ryoo & Mann 1999). For this function, Exd has to be in the nucleus. For Exd to be in the nucleus it has to bind Hth and thus the trimeric complex is formed that regulates their downstream targets. However, the binding of Hth to the target genes along with Exd and Hox proteins has been shown to be cell type specific and context dependent (Berthelsen et al. 1999, Ryoo et al. 1999).

Distal-less

The gene *Dll* codes for a homeodomain containing transcription factor and functions by regulation of its downstream targets by binding to their DNA (Cohen et al. 1989). The homeodomain is conserved between Dll and its mammalian counterparts, the Dlx proteins (reviewed in Panganiban & Rubenstein 2002). Apart from the homeodomain they also show some similarity outside such as the Proline rich domains both upstream and downstream of the homeodomain. Proline rich domains have been implicated in functions such as oligomerization and transcription activation (Tanaka & Herr 1990). In embryos, *Dll* is essential for the formation of rudimentary larval limbs (Cohen & Jurgens 1989). In the adults *Dll* has been shown to exert two important but independent functions. One is its functional role in the elaboration of the proximo-distal axis of all distal limb structures (Cohen et al. 1989). It has also been suggested that this function of *Dll* is conserved through the animal kingdom (Panganiban et al. 1994, Panganiban et al. 1995). Supporting this role is the graded lack of distal segments of ventral appendages with increasing severity of hetero-allelic combinations of *Dll* mutants (Cohen et al. 1989, Dong et al. 2000). The need of *Dll* for the normal development of distal cells is further supported by the fact that the clone of cells lacking *Dll*, segregate and move towards the proximal domain (Wu & Cohen 1999). On the other hand, ectopic expression of *Dll* can induce ectopic proximo-distal axis in other places of the body (Gorfinkiel et al. 1997). The other function of Dll is its role in establishing antenna versus leg identity (Cohen et al. 1989, Dong et al. 2000, Emerald et al. 2003). In the absence of *Dll* the antenna is transformed into leg and when ectopically expressed with *hth* it can induce distal antenna structures at different places of the body (Dong et al. 2000).

Spineless

The gene *spineless* (*ss)* is the homologue of the mammalian *Aryl hydrocarbon receptor* (*Ahr*) which has been shown to play important roles in the transcriptional regulation of genes involved in metabolizing aryl hydrocarbons (reviewed in Crews 1998). The gene *ss* encodes a basic-helix-loop-helix-PAS domain containing transcription factor. It plays three important functions during the normal development of the fly. It is needed for the development of antennal structures and in its absence, the distal antenna gets transformed to the distal second thoracic leg. The distal tarsal segments of all the legs were fused and also there was general reduction in the length of all the bristles. The role of *ss* as the primary determinant of distal antennal identity is further favored by the observation that its ectopic expression gives rise to complete duplication of antenna from the rostral membrane (Duncan et al. 1998). This is very interesting because under normal conditions, rostral membrane does not produce any appendage like structures in the adult. Moreover, it can also induce transformation of the distal leg to distal antenna specifically (Duncan et al. 1998). Ss is expressed in the leg and antenna in the second instar stage, but is gradually lost from the leg while it is maintained in the antenna. In the 3rd instar antenna disc, *ss* is expressed from the third antennal segment to the

distal end (Duncan et al. 1998). The functional characterization of Ahr, the mammalian homologue of *ss*, showed that upon activation it is translocated to the nucleus and forms a hetero-dimer with another b-HLH-PAS domain protein, Aryl hydrocarbon receptor nuclear translocator (Arnt). The Ahr:Arnt dimer has been shown to bind DNA and activate target genes. Similarly, by using yeast two-hybrid assay *ss* has been shown to function by forming a heterodimer with the aryl hydrocarbon nuclear translocator homologue, Tongo (Tgo) in *Drosophila* (Emmons et al. 1999). Favouring this further, loss of function of *tgo* also results in antenna to leg transformation (Emmons et al. 1999).

Dll and hth are Expressed Differentially in the Leg and the Antenna

The fact that the leg and antennal discs possess very similar initial developmental potential and can change identity into one another based on the presence or absence of specific genes suggests that the regulation of specific genes in specific domains determines the identity of these structures. The specific domains of expression become extremely important because of the fact that the same set of genes are expressed both in the leg and antenna. The genes *hth* and *Dll* are expressed in the leg as well as in the antennal discs, but their expression domains differ in the two discs. In the leg discs, *hth* is expressed in the proximal most segments, coxa and trochanter (Abu-Shaar & Mann 1998, Casares & Mann 1998, Wu & Cohen 2000), while it is expressed in the three proximal antennal segments (A1-A3) (Pai et al. 1998) (figure 3A, C). In the antennal disc, *Dll* is expressed from the second antennal segment to the distal tip (Cohen & Jürgenes 1989, Gorfinkiel et al. 1997) while in the leg, it is expressed in an outer ring between the femur and trochanter and an inner circle from the tibia to the distal tip (Abu-Shaar & Mann 1998, Wu & Cohen 1999) (figure 3A, C). Thus *hth* and *Dll* co-express in a domain of second and third segments, which is specific to the antenna. In contrast to this in the leg there is almost no overlap in the expression of these two genes. Instead a third domain which is attributable to the action of the nuclear protein Dac is formed and maintained by the regulatory interactions between the genes *teashirt (tsh), hth and Dll* (Wu & Cohen 2000). In

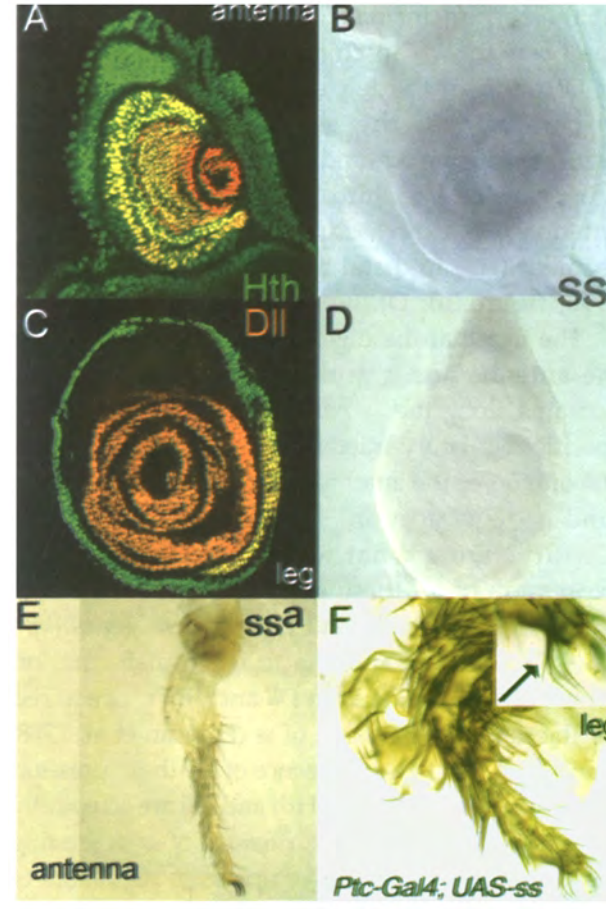

Figure 3 Expression patterns of *hth* and *Dll* in the antenna **(A)** and leg; **(C)** Co-expression of *hth* and *Dll* in the antenna (yellow) leads to stable expression of *ss* in the antenna imaginal disc; **(B)** while the non overlapping expression of them in the leg results in the loss of *ss* expression in the late 3rd instar leg disc; **(D) (E)** Transformation of antenna to leg results in the absence of normal expression of *ss* while its ectopic expression induces leg to antenna transformation; **(F)** (insert: higher magnification showing the arista in the distal end of the leg).

most of the proximal leg Hth and Tsh are expressed in an overlapping fashion although Hth expression extends further distally (Wu & Cohen 2000). Hth limits the proximal domain of Dac expression while Tsh limits the proximal Dll domain. In turn Dac can repress both Tsh and Hth if expressed in their endogenous domains (Wu & Cohen 2000).

ss is Maintained only in the Antenna

Thus there is co-expression of *Hth* and *Dll* only in the antenna (Wu & Coehn 2000, Dong et al. 2001). Because of this although initially *ss* starts to express in identical domains in both the leg and antenna at the second instar stage, it is not maintained in the leg discs (Duncan et al. 1998).

At the second instar stage the expression of *ss* has been shown to coincide with the presence of *Dll* both in the leg and antenna. But as the disc develops, the *ss* expression is down regulated in the leg and is absent in the leg disc proper at the late third instar stage while in the antenna it is expressed from the third antennal segment (A3) to distal tip (arista) (Duncan et al. 1998, figure 3B, D).

The fact that these genes are expressed both in the antenna and leg imaginal discs in specific domains along the proximo-distal axis shows that specific regulatory interactions are involved in the elaboration of the antennal identity. If it is so, what kind of regulatory interactions lead to antenna identity? From what we know so far, ectopic expression of all these genes can induce ectopic antenna development and loss of these genes (*hth, Dll, ss*) results in antenna to leg transformation (figure 3E). Moreover both *Dll* and *Hth* are required for the normal expression of *ss* (Duncan et al. 1998, Dong et al. 2002). In the absence of *Dll* the expression of *ss* is also lost and when Hth and Dll are ectopically expressed there is ectopic expression of *ss* suggesting these two genes are positive upstream regulators of *ss* (Duncan et al. 1998, Chu et al. 2002). This also explains the way by which these three genes interact in establishing the antennal identity. When there is co-expression of *hth* and *Dll* there is ectopic expression of *ss* and there is ectopic distal antenna formation. *ss* is needed for the antenna development and can do it alone when ectopically expressed (Duncan et al. 1998, figure 3F). Thus, *ss* is a downstream target of *Dll* and *hth* and the way by which these genes promote distal antenna formation is by regulating expression of *ss*.

While it is true that the co-expression of the *hth* and *Dll* genes can induce antennal identity, it is also true that they are not required for each other's expression (Dong et al. 2000). Moreover, although the expression patterns of *hth* and *Dll* overlap in the second and third antennal segments, their target gene *ss*, through which they promote distal antenna identity, is expressed only from the third antennal segment (Duncan et al. 1998). This raises an important question? How is that the *ss* expression domain, which is different from *hth* and *Dll* overlap, generated and maintained? One possibility is that some other genes playing important roles in the development and elaboration of antennal identity exist. This also leaves the question open about how exactly these different genes are regulated so that the definite domains are formed and maintained leading to correct antennal identity.

Cut

The presence of a different domain of *ss*, compared to those of *Dll* and *hth,* suggests the action of another gene specific for the proximal domain. If this is true such a gene will be expressed in a non-overlapping domain with *ss* and may down regulate *ss* in the distal domain if

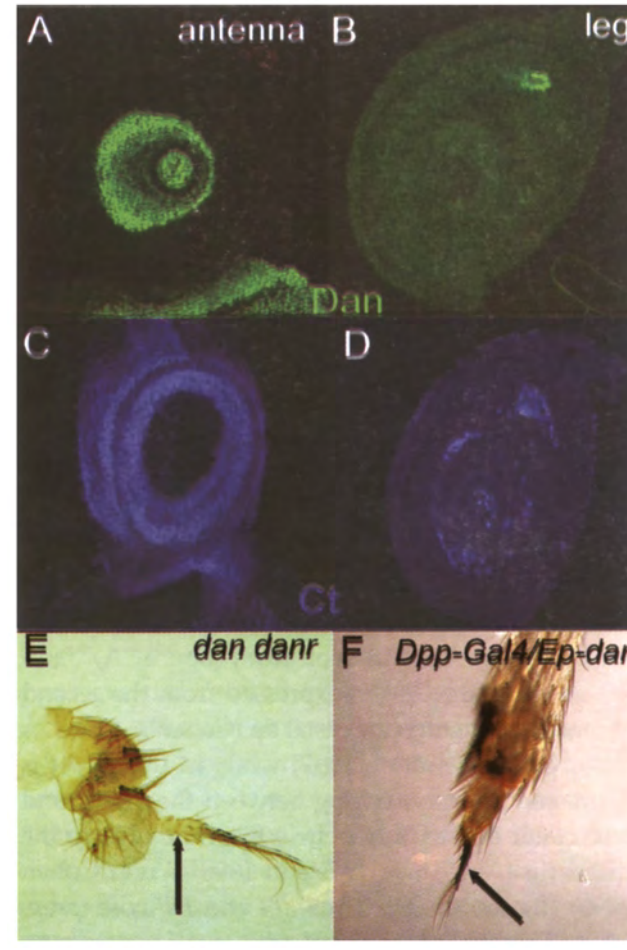

Figure 4 (A) *dan* is expressed from the 3rd antennal segment to the distal end and is not expressed in the leg imaginal disc; **(B)** The gene *ct* is expressed in the proximal two antennal segments; **(C)** and is not expressed in a specific pattern in the leg imaginal disc except in some cells of neuronal origin; **(D)**. **(E)** Absence of *dan* and *danr* results in partial antenna to leg transformation (arrow) while ectopic expression induces leg to distal antenna transformation (F, arrow).

ectopically expressed. It may be hypothesized that the result of such ectopic expression will be antenna to leg transformation, which is the loss of function phenotype of *ss* (figure 3E). *ct* is one such gene which has been shown to induce distal antenna to leg transformation when ectopically expressed (Johnston et al. 1998). *Ct* is another homeodomain containing transcription factor, which is expressed in the proximal 1st and 2nd antennal segment and is not expressed in the distal domains (Dong et al. 2002, Emerald et al. 2003, figure 4C). It is also not expressed in a specific pattern in the leg except some of the neuronal precursor cells (figure 4D). The domain of *ct* expression is important because it is complementary to the domain of *ss* and when ectopically expressed in the distal domain, it induces distal antenna to leg transformation. It also induces ectopic expression of the leg specific homeotic gene *Antp* in the distal domain, which may be responsible for the antenna to leg transformation (Johnston et al. 1998).

Distal Antenna and Distal Antenna Related

Although *ss* and *ct* are expressed in non-overlapping domains, *ss* is expressed both in the leg and antennal imaginal discs at an early stage. Further the adult leg and antenna are distinct and carry different sensory structures to perform specific functions. This suggests that there must be still other genes, with specific roles in antenna or leg disc. We have recently identified a novel family of genes *distal antenna (dan)* and *distal antenna related (danr)* which are specific for the antenna (Emerald et al. 2003). It was suggested that the genes *dan* and *danr* are required for the normal development and differentiation of the distal antennal segments and may be playing a role in the elaboration of antennal identity based on the following observations. (1) They are mainly expressed in the eye-antennal disc and in the antennal disc from the third antennal segment to distal end and is not expressed in other discs (figure 4A, B); (2) Absence of normal Dan and Danr expression results in defects in antennal development and partial antenna to leg transformation of distal antennal structures (figure 4E); (3) Ectopic expression of these genes in the leg imaginal discs induces leg to antenna transformation (figure 4F). Based on the regulatory interaction between *dan, danr* other genes known to be involved in antennal development it was shown that they act downstream of ss in the regulatory hierachy involved in the normal development and diffeentiation of antennal identity in *Drosophila.* Ss, by its positive regulation, triggers the expression of antenna specific genes *dan* and *danr* and regulates antennal *identity.*

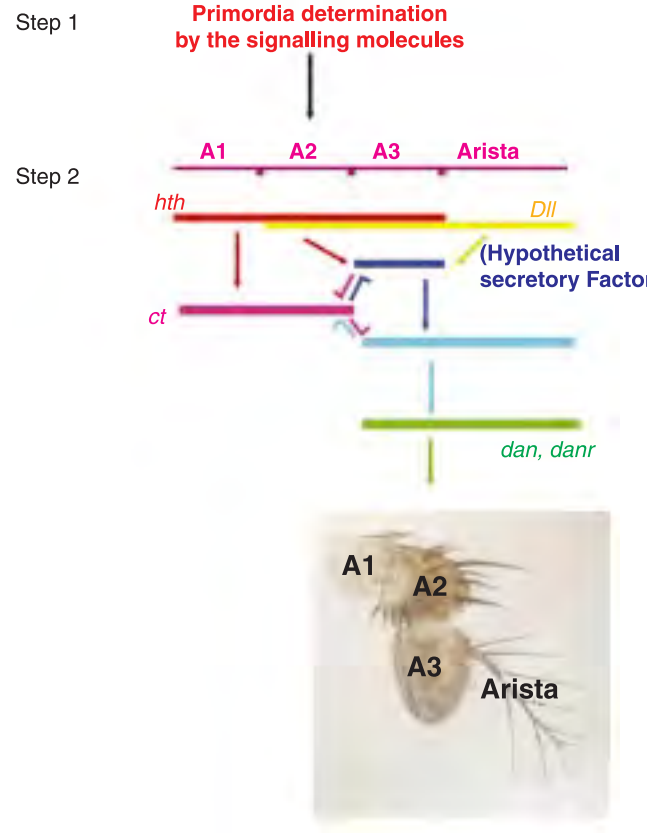

Figure 5 A cartoon of the regulatory hierarchy involved in the antennal development and identity after the primordia are set up (step 1). The genes *hth* and *Dll* act independently in their respective proximo-distal expression domains and are not required for each other's expression (step 2). Hth also positively regulates *ct* in the proximal first and second antennal segments. At the same time *ct* is independent of *Dll* expression in the second antennal segment even though they overlap in that segment. At least one additional secretory signalling molecule, positively regulated by both *Dll* and *hth*, is hypothesized (blue) to exist in the 3rd antennal segment; it is further hypothesized that this molecule in turn regulates *ss,* which is expressed from the third antennal segment to the distal tip of the antenna. Ct, which is expressed in the proximal two antennal segments, may negatively regulate this molecule and thus can limit the proximal boundary of Ss to the 3rd antennal segment. In the next step as a response to Ss, the *dan* and *danr* genes are co-expressed from the third antennal segment to the distal end resulting in antennal identity (arrows indicate positive regulation and lines with end bars indicate negative regulation).

dan and danr are Responsible for the Dll and hth Mediated Antennal Development and are Downstream Targets of ss

There is a positive correlation between ectopic expression or loss of function of genes like *hth*, *Dll* and *ss* and the expression of *Dan* and *Danr.* Ectopic expression of any of these in leg coincides with the ectopic expression of *Dan* and *Danr* and eventual leg to antenna transformation. Their expression is lost when *hth*, *Dll* or *ss* genes are absent, a condition resulting in antenna to leg transformation. This suggests that normal expression of this novel family of genes is required for the antennal development. The fact that *Dan* and *Danr* are present in the antennal disc and not in leg and can impose antennal identity when ectopically expressed in the leg, points out that in the absence of a homeotic input, a distinct regulatory mechanism orchestrated by *hth* and *Dll* genes, and mediated through *ss*, *dan* and *danr* exists for the development and elaboration of antennal identity (Emerald et al. 2003; figure 5).

It has been shown that though *hth* and *Dll* genes are co-expressed in the 2nd and 3rd antennal segments they do not have any effect on each other's expression (Dong et al. 2000). This suggests that *hth* and *Dll* regulate *dan* and *danr* expression independently of one another. This may be the reason for the absence of ectopic antenna when only one is ectopically expressed in the leg in contrast to when both *hth* and *Dll* are co expressed (Dong et al. 2000). In the leg disc, *hth* and *Dll* down regulate each other's expression (Wu & Cohen 1999, 2000) so that there is eventual loss of either *hth* or *Dll* and no *dan* and *danr* expression. This results in absence of leg to antenna transformation. In contrast when they are co-expressed, the condition in the antenna disc is mimicked, resulting in a continuous expression of *ss.* Consequently *dan* and *danr* are expressed and ectopic antenna develops.

Establishment of a Distinct Proximodistal Domain is needed for the Antennal Identity

If it is true that both leg and antenna start from the same ground state, then how are distinct structures formed at the end? The information available so far suggests that the formation of distinct proximal and distal domains mediated by *ss* and *ct* along the proximo-distal axis is the key to antennal identity. The proximal domain, which includes the first two segments of the antenna, is demarcated by the expression of *ct*. Ectopic expression of Ss in the proximal Ct domain negatively regulates *ct* expression and in the absence of Ct, Dan is ectopically expressed in the 2nd antennal segment; this suggests that negative regulation maintains the distinct proximal and distal domains (Emerald et al. 2003). However it was reported that in the absence of ss, Ct expression was unaltered (Dong et al. 2002). One reason for this may be because under normal conditions in the leg, Ct is not expressed in any specific pattern so it is difficult to evaluate. Moreover, in the absence of an antibody to *ss* a definite assessment is difficult. The distal domain, which includes the third antennal segment to the distal end of the arista, is mainly under the control of *ss*. Any change in the expression of these genes either by genetic or other means changes the identity of antenna (Duncan et al. 1998, Johnston et al. 1998). The stable expression of *ss* in the distal domain, which is mediated by the co-expression of *hth* and *Dll*, leads to the expression of *dan* and *danr* in this domain, which makes it different from the leg leading to antennal identity. In the leg, the mutual repression of *hth* and *Dll* mediated by *dac* prevents their co-expression and so *ss* is transient (Duncan et al. 1998, Dong et al. 2001) and thus ensuing absence of *dan* and *danr* expression resulting in leg fate.

Unresolved Issues

Although we have gained new insights into the mechanism of antenna development there are still very important questions to be addressed. One is the non-autonomous transformation seen in the absence of *hth*. As mentioned above, one of the important determinants of antenna, *hth* is expressed in the proximal domain in the antenna. But in the absence of *hth,* the transformation is seen in the distal domain where it is not expressed at the 3rd instar stage. Although there is some evidence of early expression of *hth* in the distal domain, the basis for this non-autonomous transformation remains to be understood.

Although it is true that co-expression of Dll and Hth is necessary for the stable expression of *ss*, *hth* and *Dll* are co-expressed only in the 2nd and 3rd antennal segments, Ss is present in the distal domain as well eventhough *hth* is not expressed. The mechanism of Ss expression in the distal domain is not understood.

A possible mechanim to explain the non-autonomous transformation of the distal domain in the absence of *hth* and the stable expression of *ss* in this domain is to hypothesize the existence of an as yet unknown secretory molecule, which responds to Hth and Dll from the 3rd antennal segment and maintains expression of *ss* in a concentration dependent manner in the distal domain. If this molecule is positively regulated by both Hth and Dll and is secretory, it will form a gradient in both directions from the 3rd antennal segment. In the proximal segments it may be negatively regulated by Ct so that the proximal domain of *ss* is limited to 3rd antennal segment. But when *hth* or *Dll* is absent, depending upon the severity, it will result in variable loss of this secretory molecule and eventual loss of Ss, finally resulting in antenna to leg transformation. On the other hand because of its secretory nature it will able to maintain Ss in the distal domain. The gradual transformation of antenna to leg in the absence of both *hth* and *ss*, supports such a possibility (figure 5).

Recently EGFR signaling pathway has been implicated in the proximodistal patterning of the leg (Campbell 2002, Galindo et al. 2002). It is possible EGFR signalling may have a comparable role in the antenna. It will be of interest to verify the expression domain of these in the antenna and to verify is it that they are expressed in a differential way in the antenna than the leg. If it is so do they respond to both Hth and Dll as opposed to what is seen in the leg is remains to be seen.

Taken together, it appears that there are still unknown molecules, which play important roles in the antenna development and identity. It is important that these additional molecules are identified and analyzed in detail. With the availability of genome sequence of *Drosophila* and advancement of techniques we hope this will happen in the near future.

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