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# Transformation of eye to antenna by misexpression of a single gene

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ABSTRACT

In *Drosophila*, the eye and antenna originate from a single epithelium termed the eye-antennal imaginal disc. Illumination of the mechanisms that subdivide this epithelium into eye and antenna would enhance our understanding of the mechanisms that restrict stem cell fate. We show here that Dip3, a transcription factor required for eye development, alters fate determination when misexpressed in the early eye-antennal disc, and have taken advantage of this observation to gain new insight into the mechanisms controlling the eye-antennal switch. *Dip3* misexpression yields extra antennae by two distinct mechanisms: the splitting of the antennal field into multiple antennal domains (antennal duplication), and the transformation of the eye disc to an antennal fate. Antennal duplication requires Dip3-induced under proliferation of the eye disc and concurrent over proliferation of the antennal disc. While previous studies have shown that overgrowth of the antennal disc can lead to antennal duplication, our results show that overgrowth is not sufficient for antennal duplication, which may require additional signals perhaps from the eye disc. Eye-to-antennal transformation appears to result from the combination of antennal selector gene activation, eye determination gene repression, and cell cycle perturbation in the eye disc. Both antennal duplication and eye-to-antennal transformation are suppressed by the expression of genes that drive the cell cycle providing support for tight coupling of cell fate determination and cell cycle control. The finding that this transformation occurs only in the eye disc, and not in other imaginal discs, suggests a close developmental and therefore evolutionary relationship between eyes and antennae.



## 1. Introduction

In *Drosophila melanogaster*, the eye and antenna originate from a cluster of ~23 cells set aside during embryonic development. During the three larval instars, this cell cluster proliferates continuously and organizes into an epithelial sac termed the eye-antennal imaginal disc. During late larval and pupal development, the anterior lobe of this epithelium (the antennal disc) gives rise to the antenna, while the posterior lobe (the eye disc) gives rise to the eye. The eye or antennal identity of these domains is not determined until mid or late second larval instar with the restricted expression of genes such as *eyeless* (*ey*) in the eye disc and *cut* (*ct*) in the antennal disc (Garcia-Bellido and Merriam, 1969;

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Kenyon et al., 2003; Kumar and Moses, 2001; Postlethwait and Schneiderman, 1971).

During the mid to late second larval instar, the components of the retinal determination gene network (RDGN), including *eyeless* (*ey*), *twin of eyeless* (*toy*), *eyes absent* (*eya*), *sine oculis* (*so*), and *dachshund* (*dac*), are first co-expressed in the eye field (Kenyon et al., 2003; Kumar and Moses, 2001). Each RDGN gene encodes a conserved transcription factor that is required for normal retinal development (Bonini et al., 1993; Cheyette et al., 1994; Mardon et al., 1994; Quiring et al., 1994). Overexpression of these genes individually or in combination in other imaginal discs including the antennal, leg, wing, genital, and haltere discs can induce ectopic eye development, but only in the presence of the products of all the other RDGN genes. The mechanisms that control RDGN expression are complex. While *toy* appears to act first, a myriad of cross-regulatory and feedback interactions allow these factors to enhance each other's ability to induce ectopic eyes (Bonini et al., 1997; Chen et al., 1997; Halder et al., 1995; Pappu and Mardon, 2002; Pignoni et al., 1997; Shen and Mardon, 1997).

Antennal determination is thought to require *homothorax* (*hth*), *extradenticle* (*exd*), and *Distal-less* (*Dll*). Loss-of-function mutations in any one of these genes leads to antenna-to-leg transformation (Casares and Mann, 1998; Cohen et al., 1989; Pai et al., 1998; Sunkel and Whittle, 1987), while ectopic expression of either *hth* or *Dll* produces ectopic antennae in the head, leg, wing, or genitals, but only in the presence of the product of the other gene (Casares and Mann, 1998; Dong et al., 2000; Gorfinkiel et al., 1997). Analysis of the interactions among these genes and their products reveals that Hth is required for nuclear localization of Exd, and only in the presence of Hth can nuclear Exd produce ectopic antennae. Dll and Hth, on the other hand, function cooperatively and in parallel to regulate normal antennal development.

Transdetermination, a process whereby already determined imaginal disc cells change fate to that of another disc, has been observed in many *Drosophila* imaginal discs, giving rise, for example, to eye-to-wing, wing-to-leg, leg-to-antenna, and antenna-to-wing transformations (Maves and Schubiger, 2003). A hallmark of transdetermination is the "transdetermination weak point", a small cell cluster in each imaginal disc that has a high probability of changing fate in response to fragmentation of the disc through the weakpoint or misexpression of the Wnt-family signaling protein Wingless (Wg) in the weakpoint. Cell proliferation has an essential role in this process and cells about to undergo transdetermination exhibit a distinct cell cycle profile that is not seen in normal development (Sustar and Schubiger, 2005).

Since the eye and antenna originate from the same cell population and are specified relatively late in development, it is perhaps not surprising that an antenna can be regenerated from *in vivo* culture of an eye disc (Gehring and Schubiger, 1975; Schubiger and Alpert, 1975). However, neither mechanical disc fragmentation followed by regeneration nor over-expression of *wg*, the two treatments that induce other forms of transdetermination, induce eye-to-antenna transdetermination (Maves and Schubiger, 2003). Furthermore, the conversion of the eye disc to an antennal fate by misexpression of antennal determination genes such as *exd*, *Dll*, or *hth* has not been previously demonstrated.

In this study we show that misexpression of *Dip3*, which encodes a MADF/BESS domain family transcription factor required for cell type specification during late eye development (Bhaskar and Courey, 2002; unpublished data), perturbs the eye-antennal decision. By pursuing this observation, we have gained new insight into the mechanisms that control this switch. Expression of *Dip3* in the early eye-antennal disc leads to both eye-to-antenna transformation, in which the eye disc gives rise to one or more partial or complete antennae, as well as antennal duplication, in which the antennal disc gives rise to two or more antennae. Both of the phenotypes may result in part from perturbation of the cell cycle, since expression of cell cycle genes prevents their appearance. Antennal duplication occurs when cell cycle perturbation leads to under-proliferation of the eye disc and concurrent over-proliferation and splitting of the antennal disc, while eye-to-antenna transformation results from cell cycle perturbation along with down-regulation of retinal determination genes and concurrent up-regulation of antennal determination genes in the eye disc. These findings provide support for the idea that cell fate determination is intimately coupled to the cell cycle. Furthermore, the ability of Dip3 to reprogram the eye disc, but not other discs, to an antennal fate implies a close relationship between these two sense organs.

## 2. Results

### 2.1. *Dip3 misexpression results in antennal duplication and eye-to-antenna transformation*

In a screen for genes that perturb eye development when misexpressed, we randomly integrated a UAS/promoter-containing P-element (Brand and Perrimon, 1993; Rorth, 1996) into the genome. An insertion immediately upstream of the *Dip3* coding region was found to result in the appearance of extra antennae when combined with the *ey*-Gal4 driver (Fig. 1). Several lines of evidence (see below) lead us to conclude that these extra antennae are of two distinct origins: some result from antennal duplication, while others result from eye-to-antenna transformation. In antennal duplication (Fig. 1B), the extra antennae arise from over-proliferation and splitting of the antennal disc into multiple domains, each of which gives rise to an antenna. In this case the extra antennae are located anterior to the antennal foramen (dashed line), where antennae are normally found. In eye-to-antenna transformation, the extra antennae arise from the transformed eye disc and are therefore located posterior to the antennal foramen (Fig. 1C), where eyes are normally found.

In previous cases where extra antennae were initially thought to arise from eye-to-antenna transformation, subsequent analysis showed that they were more likely to be the result of antennal duplication (Kenyon et al., 2003). Evidence that the extra antennae observed in *ey>Dip3* flies do, in some cases, result from the transformation of eye tissue to an antennal fate comes from our observation of partial eye-to-antenna transformations. In mild to moderate partial transformations, the eye consists exclusively of ommatidial units, but bulges out or forms a rod-shaped structure (Fig. 1D and E), suggesting that although eye tissue identity is intact, the eye is assuming a