

The Iroquois homeobox genes function as dorsal selectors in the *Drosophila* head

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This paper is dedicated to the memory of Fernando Jiménez (1950-1999)

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SUMMARY

The Iroquois complex (Iro-C) genes are expressed in the dorsal compartment of the *Drosophila* eye/antenna imaginal disc. Previous work has shown that the Iro-C homeoproteins are essential for establishing a dorsoventral pattern organizing center necessary for eye development. Here we show that, in addition, the Iro-C products are required for the specification of dorsal head structures. In mosaic animals, the removal of the Iro-C transforms the dorsal head capsule into ventral structures, namely, ptilinum, prefrons and suborbital bristles. Moreover, the Iro-C⁻ cells can give rise to an ectopic antenna and maxillary palpus, the main derivatives of the antenna part

of the imaginal disc. These transformations are cell-autonomous, which indicates that the descendants of a dorsal Iro-C⁻ cell can give rise to essentially all the ventral derivatives of the eye/antenna disc. These results support a role of the Iro-C as a dorsal selector in the eye and head capsule. Moreover, they reinforce the idea that developmental cues inherited from the distinct embryonic segments from which the eye/antenna disc originates play a minimal role in the patterning of this disc.

Key words: Iroquois complex, Imaginal eye/antennal disc, Eye, Head, *Drosophila*

INTRODUCTION

The epithelia that give rise to most of the *Drosophila* adult epidermis, the imaginal discs, are subdivided into large territories known as compartments (García-Bellido et al., 1976). Compartments are delimited by fixed, largely straight borders that correspond to cell-lineage restriction boundaries. It is thought that the cells of a compartment have specific cell affinities and, consequently, that cells of adjoining compartments tend to minimize their contacts (reviewed in Blair, 1995). Compartments are defined by selector homeotic genes (García-Bellido, 1975). In the pair of wing imaginal discs, which give rise to the mesothoracic body wall (notum and pleura) and wings, expression of *engrailed* (*en*) and its related gene *invected* defines a posterior compartment (Morata and Lawrence, 1975; Hidalgo, 1994; Guillén et al., 1995; Tabata et al., 1995; Zecca et al., 1995), and that of *apterous* (*ap*) a dorsal compartment (Díaz-Benjumea and Cohen, 1993; Blair et al., 1994). Absence of these selector genes largely transforms posterior or dorsal cells into anterior or ventral cells, respectively, as manifested by the adult structures that these cells give rise to.

Compartment boundaries are prime organizers for patterning. In the wing disc, cellular interactions across the compartment borders mediated by the signaling molecules Hedgehog (Hh; anterior/posterior, AP border) and Serrate and Delta (dorsal/ventral, DV border) activate the expression of Decapentaplegic (Dpp) and Wingless (Wg), respectively, which acting as morphogens organize growth and patterning of the

wing (Lecuit et al., 1996; Nellen et al., 1996; Zecca et al., 1996; Neumann and Cohen, 1997). However, not all organizing centers need be associated to cell-lineage-restriction boundaries. This seems to be the case for the notum/proximal wing hinge organizer defined by the expression of the Iroquois complex (Iro-C) in the presumptive notum (Diez del Corral et al., 1999).

The cells of the wing disc are derived from cells of the embryonic mesothoracic segment. In contrast, the cells of the eye/antenna disc derive from five embryonic cephalic segments and the acron (Jürgens and Hartenstein, 1993). The disc is morphologically organized into two parts, one that gives rise to the eye and most of the surrounding head capsule, and the other that originates the antenna, rostral membrane and maxillary palpus (Fig. 1 and Haynie and Bryant, 1986). Each of these two parts of the disc have been considered as being equivalent to a distinct imaginal disc, known as the eye and the antenna discs (discussed in Morata and Lawrence, 1979). This interpretation arose from the respective homeotic transformations of eye and antenna into wing/notum and leg, thoracic structures that develop from separate dorsal (wing) and ventral (leg) discs. Moreover, comparative anatomy analyses suggested that the eye, antenna and palpus might develop from cells derived from different embryonic territories (acron, antenna and maxillary segments, respectively; reviewed in Younossi-Hartenstein et al., 1993). The alternative interpretation, that eye and antenna discs are a single disc, found support in that (1) no cell-lineage-restriction boundaries were present between eye and antenna, and (2) in

transplantation experiments, a head-eye fragment of the disc could regenerate the ablated antennal part. The cell lineage analyses also showed that a single clone could encompass structures assumed to derive from different embryonic segments (Morata and Lawrence, 1979). This latter finding suggested that, at the eye/antennal disc, the segmental origin of a cell does not necessarily commit it to form structures related to that embryonic segment.

While in the wing the AP compartment subdivision is inherited from the embryo and the DV one is established in the early second instar (García-Bellido et al., 1973; García-Bellido, 1975), the only early cell lineage restriction in the eye/antenna disc derivatives is the DV one that subdivides the head capsule and the eye into two halves (Baker, 1978; Morata and Lawrence, 1979; Fig. 1). Moreover, an AP cell restriction boundary arises late (at the beginning of the third larval instar) and subdivides the antenna, maxillary palpus and neighbouring tissue (Morata and Lawrence, 1978, 1979). The DV boundary constitutes an organizing center essential for eye growth and for the establishment of the DV polarity of the ommatidia (Cho and Choi, 1998; Domínguez and de Celis, 1998; Papayannopoulos et al., 1998). This eye organizer requires the confrontation of *fringe* (*fng*)-expressing (ventral) and non-expressing (dorsal) cells, which, by means of Delta and Serrate, leads to the activation of the receptor Notch in the cells at both sides of the DV boundary. In contrast, growth and patterning of the antenna does not rely on compartment subdivisions, but on cell-cell interactions triggered by the asymmetric expression of *hh*, which induces the activation of *wg* and *dpp* in reciprocally exclusive domains (Díaz-Benjumea et al., 1994; Theisen et al., 1996).

The homeoproteins Araucan (Ara), Caupolican (Caup) and Mirror (Mirr), encoded in the *Iroquois* gene complex (Iro-C; Gómez-Skarmeta et al., 1996; McNeill et al., 1997), play a fundamental role in establishing this DV organizing center (Cho and Choi, 1998; Domínguez and de Celis, 1998; Papayannopoulos et al., 1998; Cavodeassi et al., 1999). At least from late first larval instar, the three proteins accumulate in the dorsal half of the eye disc and restrict *fng* expression to its ventral domain. Generalized expression of any Iro-C homeoprotein in the eye disc eliminates *fng* expression, abolishes Notch activation along the DV boundary and blocks eye differentiation (Domínguez and de Celis, 1998). Conversely, clones of cells lacking Iro-C function within the dorsal domain, or expressing Iro-C genes ectopically in the ventral domain, generate new *fng* expression borders that give rise to ectopic organizers and ectopic eyes (Cavodeassi et al., 1999). Moreover, the Iro-C appears to confer to dorsal cells specific affinity properties, since dorsal Iro-C⁻ clones near the DV border segregate into the ventral compartment (Cavodeassi et al., 1999). These data suggest that the Iro-C genes act as dorsal selectors for the eye disc. However, the ability of Iro-C proteins to specify dorsal as distinct from ventral structures has remained unclear since the dorsal and ventral halves of the eye are composed of the same elements.

Here we show that the Iro-C homeoproteins are required for the specification of dorsal head structures. The removal of these proteins in mosaic animals transforms the dorsal head capsule into ventral structures, namely, ptilinum, prefrons and suborbital bristles. Moreover, the Iro-C⁻ cells can autonomously give rise to a complete antenna disc, which

differentiates an ectopic antenna and maxillary palpus. The fact that the descendants of a dorsal Iro-C⁻ cell can give rise to elements that have been assumed to derive from different embryonic segments strongly supports the idea that the eye/antenna disc develops as an integrated unit, although with different patterning mechanisms in the eye and the antenna parts.

MATERIALS AND METHODS

Fly stocks

Df(3L)iro^{DFM3}, a deficiency for *ara*, *caup* and the promoter for *mirr*, and *UAS-caup* are described in Diez del Corral et al. (1999). *dpp-lacZ* (*dpp^{P10638}*), *Dll-lacZ*, *wg-lacZ*, *dpp^{disk}-Gal4*, *ey-Gal4*, *Dll-Gal4*, *Actin>y⁺>Gal4*, *UAS-ara* and *UAS-mirr* are described in FlyBase (<http://gin.ebi.ac.uk:7081>).

Mosaic analysis

Heat-shock induction of *FLP* expression (Xu and Rubin, 1993) was performed either at 24-48 hours after egg laying (AEL), 48-72 hours AEL or 72-96 hours AEL for 1 hour at 37°C. Staining with anti-Myc or anti-β-galactosidase antibodies revealed mutant clones. For induction of *NMyc* expression, late third instar larvae were subjected to a second heat shock (1 hour at 37°C). Genotypes of the larvae analysed were:

y w hsFLP122; mwh Df(3L)iro^{DFM3} FRT80B/ hsNMyc (or *tubulin-lacZ*) *FRT80B*

y w hsFLP122; wg-lacZ/+; mwh Df(3L)iro^{DFM3} FRT80B/ hsNMyc *FRT80B*

y w hsFLP122; Dll-lacZ/+; mwh Df(3L)iro^{DFM3} FRT80B/ hsNMyc *FRT80B*

y w hsFLP122; dpp-lacZ/+; mwh Df(3L)iro^{DFM3} FRT80B/ hsNMyc *FRT80B*

Misexpression experiments

Clones of cells overexpressing *ara* or *caup* were obtained by heat-treating *y hsFLP122; UAS-ara* (or *UAS-caup*)/*Actin>y⁺>Gal4*, *UAS-lacZ* larvae (at 24-48 hours AEL) 7 minutes at 37°C. *UAS-ara*, *UAS-caup*, or *UAS-mirr* alone or in pairwise combinations were crossed to *dpp^{disk}-Gal4* or *ey-Gal4* at 25 or 29°C. Overexpression of *UAS-ara* using *Dll-Gal4* driver was lethal in larva.

Histochemistry

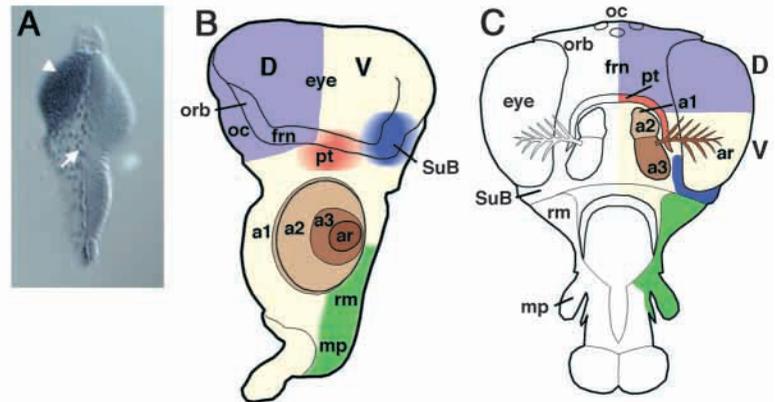
Imaginal discs were dissected and stained as described (Cavodeassi et al., 1999). Primary antibodies were: rabbit anti-β-galactosidase (Cappel), mouse anti-β-galactosidase (Promega), mouse anti-Myc (BabCo), rat anti-Caup (Diez del Corral et al., 1999) and rabbit anti-Homothorax (a gift from H. Sun). Secondary antibodies were from Jackson and Amersham. Images were collected in a Zeiss LS310 confocal microscope. Scanning electron microscopy was performed as described (Cavodeassi et al., 1999).

RESULTS

Iro-C⁻ clones transform the dorsal head capsule into ventral structures

At least as early as the late first instar or early second instar, the Iro-C homeobox genes are expressed in the dorsal region of the eye disc, which gives rise to the dorsal half of the eye and to the dorsal cephalic capsule (Cavodeassi et al., 1999; Fig. 1). These genes are also expressed in the dorsal part of the peripodial membrane (Fig. 1A). We have analyzed the function of the Iro-C in the formation of the cephalic capsule using the

Fig. 1. Eye/antenna imaginal disc: accumulation of Ara/Caup protein and fate map. (A) Late second instar disc showing Ara/Caup accumulation in the dorsal part of the disc (arrowhead) and in the dorsal half of the peripodial membrane (arrow). Note the straight borders of the Ara/Caup domains of accumulation. (B,C) Fate map of the eye/antenna disc according to Haynie and Bryant (1986). The respective fates of different regions of the disc are shown by similarly coloured adult structures. The domain of expression of Iro-C is marked in magenta and corresponds to the dorsal part of the disc (McNeill et al., 1997; Domínguez and de Celis, 1998; Papayannopoulos et al., 1998; Cavodeassi et al., 1999). a1, a2, a3, first, second and third antennal segments; ar, arista; D, dorsal domain; frn, frons; mp, maxillary palpus; oc, ocelli; orb, supraorbital bristles; pt, ptilinum; rm, rostral membrane; V, ventral domain; SuB, suborbital bristles.



lethal allele *iro*^{DFM3}, a deficiency of *ara*, *caup* and the promoter region of *mirr* (Gómez-Skarmeta et al., 1996; Diez del Corral et al., 1999). Mitotic recombination clones homozygous for this deficiency and induced during the first larval instar (24-48 hours AEL) showed conspicuous transformations of the dorsal head capsule (Fig. 2). The most common transformation was a dorsal enlargement of the eye, at the expense of the head capsule, or the appearance of ectopic eyes on this capsule (Fig. 2A,B,D,E). This phenotype has already been reported (Cavodeassi et al., 1999) and is caused by the creation of an ectopic eye organizer at the interface between Iro-expressing and non-expressing cells (see Introduction). Many of the clones that gave rise to ectopic or enlarged eyes also showed additional transformations of the dorsal cuticle into ventral structures, namely, ptilinum, suborbital bristles and prefrons; they also included ectopic antenna and maxillary palpus (Fig. 2A-D,G,I). The ectopic structures always appeared as mirror images of the extant ones with a conserved DV arrangement. This caused the ventralmost ectopic structure, the maxillary palpus, to arise on the back of the head (Fig. 2A). Moreover, the ectopic structures generated by the clones could be arranged in the above named series, so that the clones displaying a given ventral structure generally displayed those structures preceding it in the series (Fig. 2I). Note also that the appearance of ectopic structures was accompanied by the removal of characteristic dorsal capsule elements like orbital and ocellar bristles and ocelli (Fig. 2A-D).

Transformations of head capsule were also observed that were not associated with ectopic or enlarged eyes and restricted to one or two types of ventral structures (ectopic ptilina and prefrons). Such transformations were the most common in later-induced clones (48-72 and 72-96 hours AEL; Fig. 2I). These late clones could also give rise to suborbital bristles or to the most proximal part of the antenna. Iro-C⁻ clones in the antenna disc or the ventral region of the eye disc had a wild-type phenotype (not shown and Cavodeassi et al., 1999).

The cuticular marker *mwh*, which labels trichomes, could be discerned only in prefrons, antenna, rostral membrane and maxillary palpus. It showed that these ectopic ventral structures were exclusively composed by mutant Iro-C⁻ tissue (Fig. 2A,B,D). Non-autonomous effects were seen in the wild-type dorsal head cuticle located in between the extant and an ectopic eye, which displayed duplicate sets of supraorbital bristles in mirror-image arrangements (Fig. 2E), and in the ectopic eyes

themselves, which were composed of mutant and wild-type tissue (see Cavodeassi et al., 1999).

Iro-C⁻ clones in the eye/antenna imaginal disc

Iro-C⁻ clones induced during the first larval instar (24-48 hours AEL) were examined in the eye/antenna disc. Clones could be grouped into two categories. (1) Clones where the general morphology of the disc was not affected and their sizes were roughly similar to those of their twin wild-type clones (Figs 3B, 4B,C). In the dorsal part of the eye disc, these clones had smooth contours probably due to the apposition of Iro-C-expressing and non-expressing cells (Cavodeassi et al., 1999; Diez del Corral et al., 1999). (2) Clones that displayed extra proliferation and formed large outgrowths of mutant tissue (Figs 3C, 4D-F). These clones arose from the anterior/dorsal region of the eye disc and always reached to the disc margin. In the most extreme cases, and with the help of appropriate markers (i.e. Wg), the outgrowths could be recognized as complete or near complete duplications of the ventral parts of the eye disc and the antenna disc (Fig. 3C). Clones of this class were those that could give rise to the extensive duplications of eye and ventral structures described above.

Iro-C represses *Distal-less* but does not affect *homothorax*

We examined the expression of genes required to make antenna in the Iro-C⁻ clones, such as the homeobox gene *Distal-less* (*Dll*) (Cohen et al., 1989; Gorfinkiel et al., 1997). Using *Dll-lacZ*, we found that *Dll* expression started at the early third instar and was confined to a small group of cells in the centre of the antennal primordium (Fig. 5B). Later expression of *Dll* encompassed all the antennal segments with the exception of the most proximal one (Gorfinkiel et al., 1997). In the non-overproliferating Iro-C⁻ clones, we found that *Dll* could be activated (Fig. 4B), although this only occurred in clones located at the dorsal/posterior quadrant of the eye disc (Fig. 4A,B). In the antennal primordium, activation of *Dll* depends on simultaneous signaling by Dpp and Wg (Díaz-Benjumea et al., 1994; Theisen et al., 1996). *wg* was expressed by most of the cells of dorsal Iro-C⁻ clones (Fig. 3B and Cavodeassi et al., 1999). Many of these clones also had Dpp, which could be provided by either the morphogenetic furrow or, in clones that were near the disc margin, by a source of Dpp induced by the ectopic eye

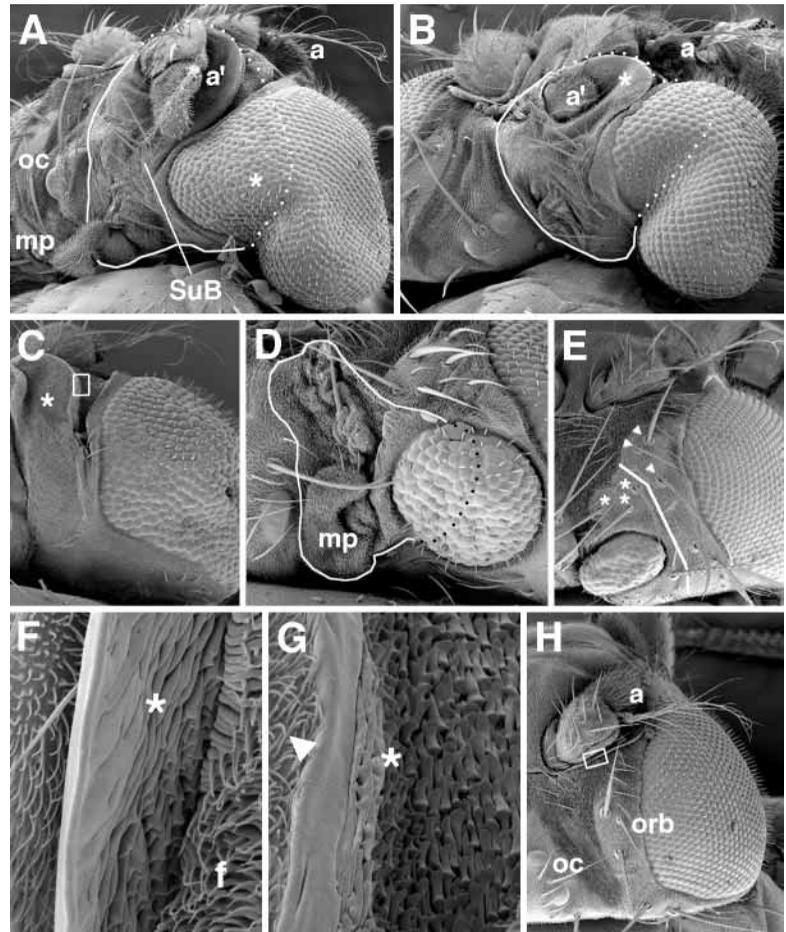


Fig. 2. Iro-C⁻ clones give rise to ventral structures. Solid lines demarcate clearly visible clone borders. Dotted lines show approximate clone borders. All heads are shown with posterior down. A wild-type head is shown in H. (A) Complete set of ventral ectopic structures associated to an Iro-C⁻ clone which also causes enlargement of the eye (asterisk). The enlarged eye territory adjacent to ventral ectopic structures was composed of Iro-C⁻ cells. This was visualized with a *w* marker (not shown; see Cavodeassi et al., 1999). (B) Clone with less extensive transformation. Only proximal segments of the ectopic antenna have developed (a'). Asterisk, ectopic prefrons. (C) Ectopic ptilinum forms an invaginating fold along the dorsal border of the eye. Area within rectangle is shown at higher magnification in G. Asterisk, ectopic prefrons. (D) High magnification of a clone showing the autonomous nature of the dorsal-to-ventral transformation. All visible ventral ectopic structures display the *mwh* marker. Additional ectopic structures, including antenna, are invaginated within the head, as detected by light microscopy. (E) Ectopic eye associated with an Iro-C⁻ clone showing non-autonomous development of ectopic supraorbital bristles (asterisks). Arrowheads, extant bristles; solid line, symmetry axis between ectopic and extant orbital elements. (F,G) High magnification of wild-type and ectopic ptilinum from H and C, respectively, showing typical squamous cuticles (asterisks). Arrowhead, ectopic (*mwh*) prefrons. (H) Dorsal view of a wild-type head. Images shown in A-E were taken from a slightly more posterior angle. Rectangle marks the wild-type ptilinum shown at larger magnification in F. a and a', extant and ectopic antenna; f, wild-type frons; mp, ectopic maxillary palpus; oc, ocellus; orb, supraorbital bristles; SuB, ectopic suborbital bristles. (I) Quantification of recognizable ectopic structures associated with Iro-C⁻ clones induced at the indicated developmental times. n, number of clones. For each developmental period, structures associated with only three clones could not be unambiguously recognized. Asterisks indicate that only truncated antennae were generated. An, antenna; Ey, eye; Pt, maxillary palpus; Pf, prefrons; Pt, ptilinum; SuB, suborbital bristles.

24-48h							48-72h							72-96h			
n	Ey	Pt	SuB	Pf	An	MP	n	Ey	Pt	SuB	Pf	An	MP	n	Ey	Pt	SuB
12	—	—	—	—	—	—	1	—	—	—	—	—	—	1	—	—	—
5	—	—	—	—	—	—	1	—	—	—	—	—	—	45	—	—	—
4	—	—	—	—	—	—	2	—	—	—	—	—	—	2	—	—	—
7	—	—	—	—	—	—	10	—	—	—	—	—	—	9	—	—	—
11	—	—	—	—	—	—	13	—	—	—	—	—	—				
35	—	—	—	—	—	—	21	—	—	—	—	—	—				
14	—	—	—	—	—	—	2	—	—	—	—	—	—				
2	—	—	—	—	—	—	1	—	—	—	—	—	—				
							1	—	—	—	—	—	—				

organizer associated with the clone (Fig. 3D, and Cavodeassi et al., 1999). Thus, the clone cells were exposed to both Dpp and Wg, which in combination should activate *Dll*.

Iro-C⁻ cells at the dorsal/anterior region, which are also exposed to Wg and Dpp (Fig. 3B, D), probably failed to activate *Dll* because they were located within the domain of expression of *homothorax* (*hth*; Fig. 5A and Pai et al., 1998). The removal of the Iro-C did not appreciably modify the expression of this gene (Fig. 4C, D). The presence of Hth might impair the activation of *Dll* since, in the leg disc, this protein, by driving the nuclear localization of Extradenticle (a cofactor of many homeoproteins; Rieckhof et al., 1997; Pai et al., 1998), appears to reduce the sensitivity of cells to Wg and Dpp signaling (Wu and Cohen, 1999). Thus, at the dorsal eye disc, Iro-C and *hth* probably cooperate to repress *Dll*.

Large outgrowths resulting from Iro-C⁻ clones encompassed

hth-expressing cells in their anterior part (Fig. 4D) and displayed ectopic *Dll* expression in the posterior part (Fig. 4E,F). In some outgrowths, the domains of expression of *hth* and *Dll* were adjacent to each other (Fig. 4E) whereas, in more developed outgrowths, these domains partially overlapped (Fig. 4F). These differences in the patterns of expression seemed to recapitulate successive stages of development of the wild-type antennal primordium, since initially the *hth* and *Dll* domains were complementary (Fig. 5B, B') and only later they partially overlapped (Casares and Mann, 1998). In summary, the results suggest that, upon removal of Iro-C in dorsal cells, the cells become exposed to Wg and Dpp, and this and the absence of Iro-C products promote *Dll* activation. The interaction of *Dll* with *hth*, a gene with an antenna-selector function (Casares and Mann, 1998), should allow the growth of an ectopic antenna.

Overexpression of Iro-C genes prevents development of ventral structures

We examined the effect of ectopic expression of Iro-C genes in the eye/antenna disc with the help of the Gal4 system (Brand and Perrimon, 1993). By using the drivers *ey-Gal4* (Hauck et al., 1999) and *dpp^{disk1}-Gal4* (Staehling-Hampton et al., 1994), which respectively promote generalized or margin-bound expression in the early eye disc, we overexpressed *UAS-ara* (Gómez-Skarmeta et al., 1996) and confirmed that eye development was blocked and that the remaining suborbital bristles resembled supraorbital bristles (Domínguez and de Celis, 1998; Fig. 6A and results not shown). This suggests a ventral-to-dorsal transformation of the suborbital cuticle. Similar results were obtained by overexpressing either *UAS-caup*, *UAS-mirr* or a combination of both (not shown). Using the *dpp^{disk1}-Gal4* driver, the antenna was truncated, lacking the arista (Fig. 6A). This phenotype might be due to a repression of *Dll* by the overexpression of the Iro-C gene.

UAS-ara was also overexpressed in flip-out Gal4 clones (Struhl and Basler, 1993). Most of these clones were incompatible with viability and few individuals reached the pharate state. When the clones included the anlagen for the maxillary palpus and rostral membrane, these structures were absent or reduced (Fig. 6B) and the remaining head structures were correspondingly displaced (not shown). The removal of ventral structures was probably not due to a toxic effect of the large amounts of overexpressed Ara (several times higher than the endogenous accumulation; Diez del Corral et al., 1999 and results not shown), since dorsal clones did not interfere with head capsule development (not shown). Moreover, some ventral clones were associated with cuticle vesicles (Fig. 6B), which indicated that cells that expressed *UAS-ara* had a different affinity from the neighbouring non-expressing wild-type cells. This suggests that expression of *UAS-ara* modified their identity. The scarcity of individuals with overexpressing clones impeded our assessment of their effect on other ventral structures.

DISCUSSION

During development of the *Drosophila* eye, the homeodomain proteins of the Iro-C complex are expressed in the dorsal half of the eye primordium, thereby restricting expression of *fng* to the ventral half. The apposition of *fng*-expressing with non-expressing cells across this DV compartment boundary leads to *N* activation, which ensures growth and patterning of the eye (Cho and Choi, 1998; Domínguez and de Celis, 1998; Papayannopoulos et al., 1998; Cavodeassi et al., 1999). In this paper, we show a further function of the Iro-C proteins in the development of the eye-antennal imaginal disc: the specification of dorsal head capsule.

Iro-C specifies dorsal head structures

It has been suggested that the Iro homeodomain proteins act as dorsal selectors in the eye disc (Cavodeassi et al., 1999). Indeed, they appear to confer a differential affinity to dorsal cells with respect to ventral cells (Cavodeassi et al., 1999), a property probably responsible for the separation of these cell populations into different lineage compartments (Baker, 1978; Morata and Lawrence, 1978, 1979). As for other compartment

selector genes (*en* or *ap*, see Introduction), their asymmetric distribution at the compartment border ultimately results in the formation of an organising centre that patterns the tissue on the two sides of this border (Cho and Choi, 1998; Domínguez and de Celis, 1998; Papayannopoulos et al., 1998; Cavodeassi et al., 1999). We now find that the Iro-C fulfils another expected property of dorsal selector genes, namely, the ability to specify dorsal as opposed to ventral fates. The removal of Iro-C products leads to unambiguous dorsal-to-ventral transformations. Thus, Iro-C⁻ clones that are not associated with extensive overgrowths show the replacement of dorsal head capsule by ventral structures, most frequently the ptilinum. Iro-C⁻ clones associated with markedly increased cell proliferation give rise to several ventral structures, even to a full complement of them (ptilinum, suborbital bristles and prefrons), and to an antenna and maxillary palpus. Moreover, these transformations appear to be cell autonomous. These findings thus support a selector role for the Iro-C genes in the dorsal eye and dorsal head capsule.

In contrast, when Iro-C genes are overexpressed in the presumptive ventral head territory, no clear ventral-to-dorsal transformations have been observed. Only suborbital bristles may be transformed into supraorbital-like bristles (see also Domínguez and de Celis, 1998). Otherwise, overexpressions either remove ventral structures, like the maxillary palpus or the rostral membrane, and/or cause the appearance of cuticular vesicles. Although this is suggestive of a change of fate, the acquired fate remains undetermined. In the wing disc, the Iro-C is necessary for notum specification, since its removal transforms notum cells into wing hinge cells (Diez del Corral et al., 1999). And similarly to the eye/antenna disc, Iro-C genes ectopically expressed in wing cells do not cause the reverse transformation. The Iro homeodomains are closely related to those of the Pbx/Meis proteins (Gómez-Skarmeta et al., 1996; McNeill et al., 1997), which act as cofactors of many HOX proteins (Mann and Affolter, 1998, review). Moreover, the Iro proteins have additional domains that probably mediate protein-protein interactions (Gómez-Skarmeta et al., 1996; McNeill et al., 1997). Thus, they probably act as transcription factors in multimeric complexes. Their absence would impair the function of these complexes, thereby causing the dorsal-to-ventral transformations, but their ectopic expression in ventral cells would not reproduce their normal function if other members of the complexes were unavailable in these cells. Hence, the reciprocal transformations would not be accomplished.

The eye/antenna disc develops as an integrated unit

The eye-antenna disc has a complex origin. Its cells, which appear to be derived from five embryonic segments and the acron, fuse into the disc primordium (Jürgens and Hartenstein, 1993). Thus, in principle, cells derived from different segments might inherit specific cues that would commit them to their respective developmental fates. We now find that cells that would ordinarily give rise to dorsal head capsule, on losing the Iro-C products, give rise to ventral eye plus ventral head and appendages thought to be derived from different segments. This suggests that the origin of each cell within the disc primordium does not irreversibly commit it to a specific fate. Hence, the eye/antenna disc, as with the wing and leg discs, largely develops as an integrated unity despite its

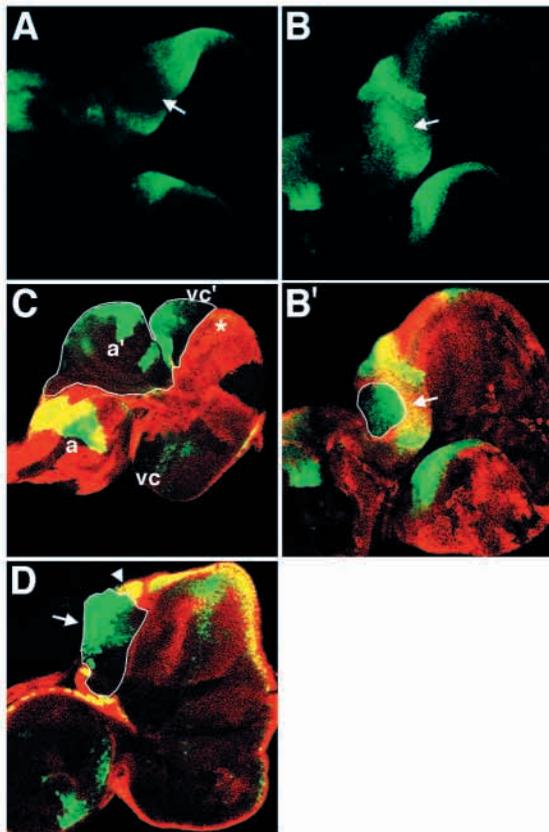


Fig. 3. Upregulation of *wg-lacZ* and *dpp-lacZ* associated with *Iro-C⁻* clones. Late third instar eye/antennal discs were stained with anti- β -galactosidase (green) and with anti-Myc (red) antibodies. In this and other figures, discs are oriented with dorsal to the top and posterior to the right. *Iro-C⁻* clones are revealed by the absence of Myc staining (outlined in white). (A) In wild-type eye discs, *wg-lacZ* expression is restricted to territories of the dorsal and ventral cephalic capsule. Note the absence of expression in the ocellar region (arrow). (B,B') *Iro-C⁻* clones at the ocellar region upregulate *wg-lacZ* (arrow). Green channel image is shown in B. (C) *Iro-C⁻* clone that has given rise to a large outgrowth. *wg-lacZ* expression labels the ectopic prospective antenna (a') and ventral capsule (vc'). a, extant antenna; vc, extant ventral capsule (most of *wg-lacZ* staining is out of the focal plane). Note that the wild-type twin clone (asterisk, strong red) is much smaller than the *Iro-C⁻* clone, which indicates an increased proliferation of *Iro-C⁻* cells. (D) *dpp-lacZ* is ectopically expressed (green channel) by *Iro-C⁻* cells close to the margin of the disc (arrow) and by adjacent wild-type cells (arrowhead; see also Cavodeassi et al., 1999).

multisegmental origin. In support of this interpretation, transplantation experiments showed that a head-eye fragment of the disc can regenerate the ablated antennal part (summarized in Morata and Lawrence, 1979). Moreover, cell lineage analyses have shown that a single *Minute⁺* clone can include the maxillary palpus, antenna and ventral part of the eye (Baker, 1978; Morata and Lawrence, 1979), precisely those structures that can be generated by *Iro-C⁻* clones.

The *Iro* expression border as a pattern organizer

Interactions between *Iro-C*-expressing and non-expressing cells at the border of a dorsal *Iro-C⁻* clone create an ectopic organising center which, depending on its position with respect

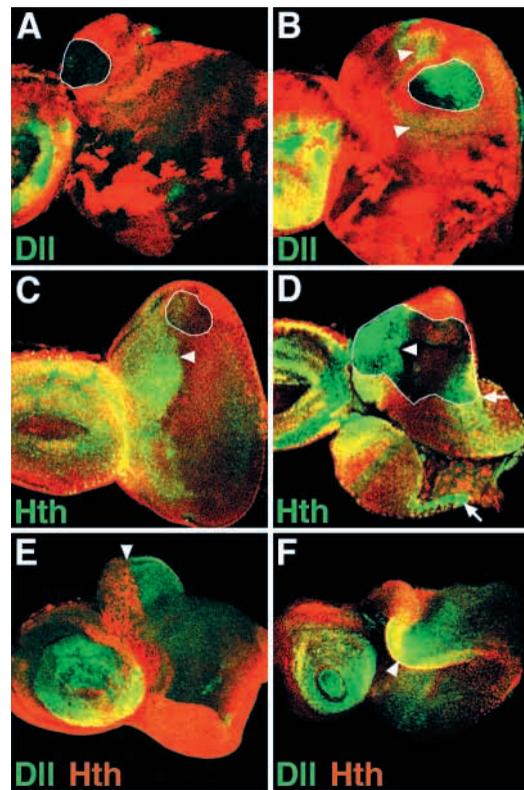


Fig. 4. *Dll* and *hth* expression in *Iro-C⁻* clones. Late third instar eye/antennal discs are labeled as indicated in the respective panels. Clones, revealed by absence of Myc staining (red, A-D), are outlined in white. (A) Anterior clone lacking *Dll-lacZ* expression. (B) More posterior clone in which only the posteriormost cells express *Dll-lacZ*. This gene is expressed by all the cells of clones entirely located in the posterior/dorsal region (not shown). Note that the presence of the *Iro-C⁻* clone non-autonomously induced a weak expression of *Dll-lacZ* in adjacent wild-type tissue (arrowheads). A similar non-autonomous activation of *Dll-lacZ* associated to *Dll*-overexpressing clones has been found in the leg disc (Gorfinkiel et al., 1997). (C,D) *Hth* accumulation remains in the anterior region of the eye disc, both in non-overproliferating (C) and overproliferating (D) *Iro-C⁻* clones (arrowheads). *Hth* is also present in differentiating ommatidia (arrows in D). (E,F) Outgrowths presumably associated to *Iro-C⁻* clones show activation of *Dll-lacZ* that abuts to the domain of *Hth* accumulation (E, arrowhead), or that partially overlaps with it (F, arrowhead).

to the extant eye field, either enlarges the extant eye or promotes the development of an ectopic eye (Cavodeassi et al., 1999). In both cases, the ectopic organizer, similar to the extant eye organizer, promotes cell proliferation and patterns the ectopic eye tissue, which is composed of wild-type cells at one side of the boundary and mutant cells at the other side (Fig. 7). The patterning effect of the ectopic organizer is manifested by the opposite polarity of many rows of ommatidia on both sides of the boundary. Our observations suggest that both the extant and the ectopic organising centers have effects that extend beyond the eye field into the adjacent cephalic capsule. This is noticeable in the dorsal capsule that lies in between the extant and the ectopic eye. Here, wild-type cells give rise to mirror-image duplications of supraorbital bristles. The suborbital capsule, given its disposition with respect to the DV organizer,

i.e., symmetric to that of the dorsal capsule, may be similarly patterned, although no data are yet available to support this suggestion. Note, however, that the apparent removal of the extant DV organizer, by means of overexpression of *UAS-ara*

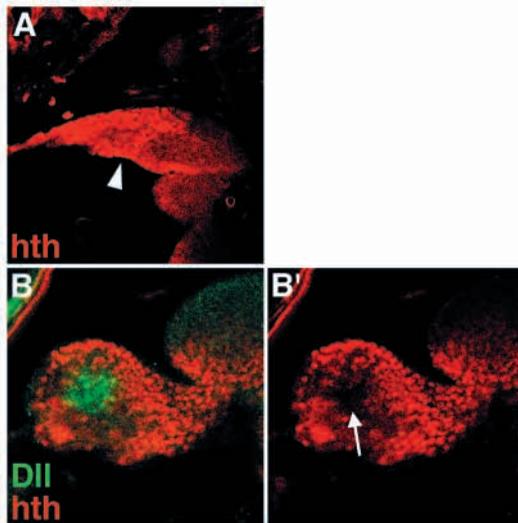


Fig. 5. Early expression of *Dll-lacZ* and *hth* in the eye/antenna disc. (A) Early second instar eye/antenna disc showing accumulation of Hth in the anterior part of the disc (arrowhead). (B,B') Antennal part of an early third instar disc showing *Dll-lacZ* expression (green) in a central domain of minimal accumulation of Hth (red; arrow in B').

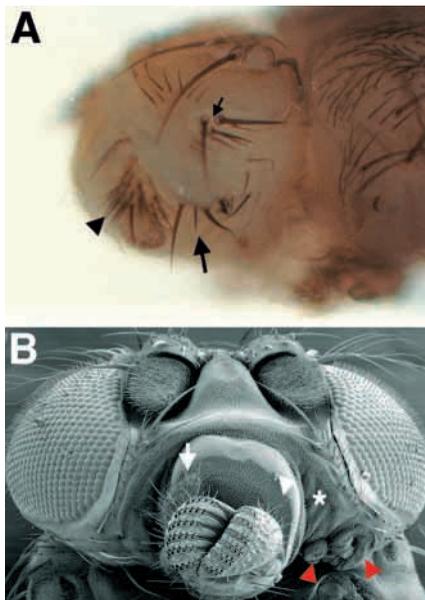


Fig. 6. Ectopic expression of *UAS-ara* prevents formation of ventral structures. (A) *UAS-ara* expression driven by *dpp-Gal4*. Eye development is abolished, antenna is truncated (arista is missing, arrowhead), most suborbital bristles are removed and those remaining (large arrow) resemble supraorbital bristles (small arrow). Note that they have lost the forward orientation typical of suborbital bristles. (B) Loss of maxillary palpus (arrowhead, compare with contralateral palpus, arrow), partial removal of rostral membrane (asterisk) and development of segregating cuticular vesicles (red arrowheads) associated with an *UAS-ara* overexpressing clone marked by γ and detected by light microscopy.

(driven by *ey-GAL4*), obliterates the eye but it only slightly affects the development of the head capsule (Cho and Choi, 1998; Domínguez and de Celis, 1998; Papayannopoulos et al., 1998). While we cannot provide a clear explanation for this observation, the possibility remains that the overexpression of *UAS-ara* does not completely suppress DV organizing activity (i.e., *ey-Gal4* may not drive expression sufficiently early and at the appropriate levels).

The most extreme phenotype resulting from *Iro-C⁻* clones, i.e., the formation of ectopic ventral capsule, antenna and palpus, is associated to an ectopic eye field. This might indicate that the ectopic DV eye organizer would also be responsible for the patterning of these structures. However, a more

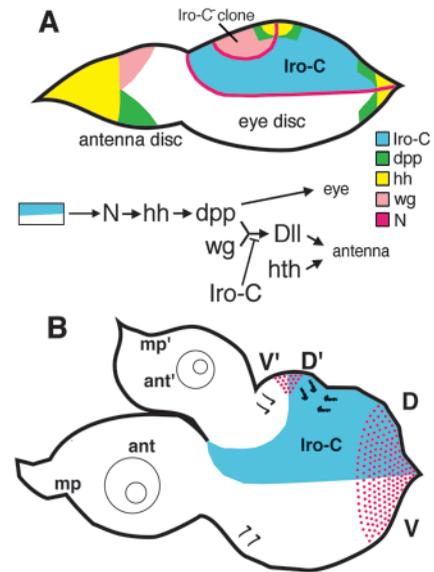


Fig. 7. Summary of signals that permit cells of dorsal *Iro-C⁻* clones to generate ventral derivatives at the eye/antenna disc. (A) As previously indicated (Cavodeassi et al., 1999), *fng* is derepressed in a dorsal *Iro-C⁻* clone, which leads to the activation of N at the boundary of *Iro-C⁺* and *Iro-C⁻* cells (red). This in turn would promote expression of *hh* at the posterior intersection of this boundary with the disc margin (yellow). Hh activates *dpp* transcription in nearby wild-type and *Iro-C⁻* cells (green). Hh activates *dpp* transcription in nearby wild-type and *Iro-C⁻* cells (green). *wg* is derepressed within the clone (pink). The presence of both Dpp and Wg triggers cell proliferation and the activation of *Dll*, which, together with Hth, promotes development of an antenna. *Dll* is not activated in wild-type eye disc cells exposed to both Dpp and Wg due to repression by the *Iro-C*. Extensive cell proliferation and development of ventral structures only occurs in clones abutting to a critical region of the disc margin. In these clones, *dpp* is activated only in the vicinity of the posterior intersection of the border of the *Iro-C⁻* clone with the disc margin, the region from which the ectopic eye field will develop. More posterior clones give rise to only ectopic eye fields, while more anterior clones proliferate less extensively and do not differentiate eye. (B) Fate map of a late third instar disc with an idealized complete duplication caused by the presence of an extensively proliferating *Iro-C⁻* clone. Such disc would give rise to a head similar to that shown in Fig. 2A, albeit with a duplicated eye as in Fig. 2E. Note that the pattern organizing properties of the *Iro-C* border would be restricted to the eye and surrounding orbital cuticle. ant, presumptive antenna; mp, presumptive maxillary palpus; mp', ant', presumptive ectopic territories are indicated with primes; small red dots, ectopic and extant developing ommatidia; thick and thin ticks, supraorbital and suborbital bristles, respectively.

plausible alternative explanation is offered by the molecular mechanisms thought to control growth and patterning of the antenna disc. Development of the antenna depends on the establishment of two reciprocally exclusive domains of expression of *wg* and *dpp* (Díaz-Benjumea et al., 1994; Theisen et al., 1996). Both genes are activated by Hh, which accumulates at least from the late first instar in the posterior half of the antenna disc (Cavodeassi et al., 1999). The accumulation of both Wg and Dpp in the center of the antennal primordium activates transcription of *Dll* which, in conjunction with Hth, promotes growth of the disc and development of the antenna (Díaz-Benjumea et al., 1994; Gorfinkiel et al., 1997; Lecuit and Cohen, 1997; Casares and Mann, 1998). This combination of factors is reproduced within the dorsal Iro-C⁻ clones (Cavodeassi et al., 1999; and Fig. 7). Thus, *wg* is upregulated within the clone, the Iro-C border induces *dpp* expression and together Wg and Dpp would activate *Dll* and cell proliferation. Indeed, *Dll* was activated and this occurred preferentially in the posterior part of the proliferating clones, possibly due to the higher concentration of Dpp and/or the absence of Hth in this region. Hence, these clones express *hth* and *Dll* in adjacent domains, a situation that resembles the complementary distribution of these proteins in the early antenna disc (Fig. 5B). According to this scenario, the growth of the ectopic antenna would be associated with the generation of an ectopic eye because N activation at the ectopic DV boundary upregulates *dpp* expression (Fig. 7). The fact that the Iro-C⁻ clones also give rise to the remaining antenna disc derivatives (i.e., rostral membrane, maxillary palpus, etc.) further suggests that the cell interactions mediated by Wg and Dpp are sufficient to pattern the whole antenna disc. Indeed, *Dll* is also required for development of the maxillary palpus, although its expression is delayed to the pupal stage (Cohen and Jürgens, 1989). In summary, the DV organizer is paramount for growth and patterning of the eye and the surrounding head capsule, while the antenna would develop independently from this organizer and require the absence of Iro-C and the presence of Hth, Wg and Dpp.

Iro-C in the head and mesothorax

It is of interest to compare the functions of the Iro-C in the development of the head and mesothorax. In the precursors of the mesothorax, the wing and second leg imaginal discs, early Iro-C expression is restricted to the dorsalmost region of the wing disc, which will give rise to the notum. Removal of this early expression in Iro-C⁻ clones transforms the notum into proximal wing hinge (Diez del Corral et al., 1999). This has a correlate in the eye disc, as Iro-C is expressed early in the dorsalmost part of the disc and it is required for development of its dorsal structures. Moreover, interactions between Iro-C-expressing and non-expressing cells at the notum/hinge border and at the DV eye compartment border establish organising centers that help pattern the neighbouring tissue. (Note, however, that the notum/hinge border does not coincide with a restriction of cell lineage; Diez del Corral et al., 1999.) Thus, the early function of the Iro-C genes appears to specify dorsal body wall in both the head and the mesothorax. In both cases, the Iro-C genes may be repressing genetic functions that allow cells to give rise to appendages (wing, antenna and maxillary palpus). We have shown that Iro-C appears to repress *Dll*, and thereby antennal development, in the dorsal part of the eye

disc, a region that early in development expresses *wg* and receives Dpp from the disc margin. Consistent with this repressing function, the Iro-C genes are not expressed in the antenna disc, nor are they expressed in the leg discs (except for a late expression in the prospective tibia; Gómez-Skarmeta, 1995), and their overexpression in either the antenna or the leg disc truncates the corresponding appendage (R. Diez del Corral and J. M., unpublished, and this work). It should be of interest to examine whether the Iro-C genes have a similar dorsal specifying function in other parts of the fly's body wall.

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