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Dpp and Hh signaling in the *Drosophila* embryonic eye field

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SUMMARY

We have analyzed the function of the Decapentaplegic (Dpp) and Hedgehog (Hh) signaling pathways in partitioning the dorsal head neurectoderm of the *Drosophila* embryo. This region, referred to as the anterior brain/eye anlage, gives rise to both the visual system and the protocerebrum. The anlage splits up into three main domains: the head midline ectoderm, protocerebral neurectoderm and visual primordium. Similar to their vertebrate counterparts, Hh and Dpp play an important role in the partitioning of the anterior brain/eye anlage. Dpp is secreted in the dorsal midline of the head. Lowering Dpp levels (in *dpp* heterozygotes or hypomorphic alleles) results in a 'cyclops' phenotype, where mid-dorsal head epidermis is transformed into dorsolateral structures, i.e. eye/optic lobe tissue, which causes a continuous visual primordium across the dorsal midline. Absence of Dpp results in the transformation of both dorsomedial and dorsolateral structures into brain neuroblasts. Regulatory genes that are required for eye/optic lobe fate, including *sine oculis* (*so*) and *eyes absent* (*eya*), are turned on in their respective domains by Dpp. The gene *zerknuellt* (*zen*), which is expressed in response to peak levels of Dpp in the dorsal midline, secondarily represses *so* and *eya* in the dorsomedial domain. Hh and its receptor/inhibitor, Patched (Ptc), are expressed in a transverse stripe along the posterior boundary of the eye field. As reported previously, Hh triggers the expression of determinants for larval eye (*atonal*) and adult eye (*eyeless*) in those cells of the eye field that are close to the Hh source. Eya and So, which are induced by Dpp, are epistatic to the Hh signal. Loss of Ptc, as well as overexpression of Hh, results in the ectopic induction of larval eye tissue in the dorsal midline (cyclopia). We discuss the similarities between vertebrate systems and *Drosophila* with regard to the fate map of the anterior brain/eye anlage, and its partitioning by Dpp and Hh signaling.

Key words: Dpp signaling, Hh signaling, *Drosophila*, Brain, Eye, Optic lobe

INTRODUCTION

The eye of vertebrates derives from the eye field, an unpaired anlage that is located in the anterior part of the neural plate (Adelmann, 1936; Li et al., 1997; Bernier et al., 2000). Together with the anlagen of the dorsal forebrain, midbrain, olfactory system and pituitary, the eye field forms the anterior neural plate, a neurectodermal domain that differs in its molecular properties from the posterior neural plate that gives rise to hindbrain and spinal cord. Molecularly, the anterior neural plate is characterized by the overlapping expression of several regulatory genes, including *Otx1* and *Otx*2 (Simeone et al., 1993; Kablar et al., 1996), and *Tlx* (Yu et al., 1994; Hollemann et al., 1998). Hox genes, which provide the posterior part of the neural primordium with specific anteroposterior 'identities', are not expressed in the anterior brain/eye anlage (Holland and Graham, 1995). The eye field, which forms the central part of the larger anterior brain/eye anlage, gives rise not only to the eyes, but also to the optic stalk and hypothalamus (Fig. 1A). Initially, regulators of eye fate, including Six3 and Six6 and Rx are expressed in the entire eye field (Oliver et al., 1995; Bernier et al., 2000; Zhou et al., 2000; Mathers et al., 1997). Cell-cell interactions, which were ultimately triggered by a signal derived from the prechordal plate or, in mouse, from the extra-embryonic endoderm, are required to partition the eye field into its different domains (Pera and Kessel, 1997; Thomas and Beddington, 1996). At the time of neurulation, a bilateral eye primordium has become distinct from hypothalamic primordium. As the neural tube closes, both regions become further subdivided into smaller partitions. The eye primordium differentiates into the optic stalk, pigment epithelium and neural retina.

Some of the molecular pathways that control the partitioning and morphogenesis of the eye field have been identified, but many details are still elusive. Signals of the BMP family are initially expressed in the ectoderm and inhibit the formation of the neural plate (Wilson and Hemmati-Brivanlou, 1995). Signals derived from the organizer, among them Chordin, Noggin, Cerberus and Shh, relieve this inhibition and, at the same time, begin to partition the emerging eye field (Piccolo et al., 1996; Zimmermann et al., 1996; Li et al., 1997). For example, graded activity of Sonic hedgehog (Shh) is crucially involved in specifying a separate hypothalamus from eye; absence of Shh results in the conversion of the entire eye field into an unpaired eye, manifesting itself as cyclopia (Chiang et al., 1996; Goodrich and Scott, 1998). Specific regulatory

genes, such as *Pax2* (expressed in the optic stalk) and *Pax6* (in the retina) are under control of Shh (Macdonald et al., 1995). bone morphogenetic proteins (BMPs), in what may be considered a second phase of action, are released from the dorsal neural tube and are required for dorsal cell fates in the spinal cord, brain and eye (Liem et al., 1995; Furuta et al., 1997; Dudley et al., 1995). In conjunction with Shh, ventrally released BMP7 is also required for hypothalamus fate (Dale et al., 1999).

Previous studies by us and others have revealed that the ectodermal domain that gives rise to the anterior brain and visual system in *Drosophila* are laid out in a manner that bears strong resemblance to the topology of the anterior neural plate in vertebrates (summarized in Fig. 1). Whereas the ventral nerve cord and basal part of the brain (deuterocerebrum, tritocerebrum) are derived from the segmented ventral neurectoderm, the precursors of the anterior brain (protocerebrum) and visual system map to a dorsal position within the head of the embryo. As in vertebrates, the anlagen of the eye and optic lobe come from a mid-dorsal position and only secondarily migrates laterally away from the midline (Green et al., 1993; Dumstrei et al., 1998; Namba and Minden, 1999). The pars intercerebralis, which, similar to the vertebrate hypothalamus, constitutes the neuroendocrine 'compartment' of the insect brain (Mobbs, 1985), also maps to a medial part of the protocerebral neurectoderm (Younossi-Hartenstein et al., 1996; Zacharias et al., 1993). The *Drosophila Otx* and *Tlx* homologs, *orthodenticle* (*otd*; *oc* – FlyBase) and *tailless* (*tll*) are expressed in a nearly overlapping pattern in the anterior brain anlage (Hirth et al., 1995; Younossi-Hartenstein et al., 1997). Expression of the *Six3/6* homolog, *sine oculis* (*so*), defines an unpaired dorsomedial domain that includes all components of the visual system, including larval and adult eye, as well as optic lobe, and thereby serves as a marker for the eye field (Cheyette et al., 1994; Daniel et al., 1999).

The conservation in anterior brain and eye field topology prompts the question of whether the signaling pathways that specify different fates within this domain also play a similar role in *Drosophila*. The *Drosophila* homologs of Shh and BMP4, Hh and Dpp, act as morphogens in numerous different larval and embryonic contexts, notably dorsoventral patterning of the trunk region of the germ band, anteroposterior patterning within individual segments, and cross-germ layer signaling during mesoderm and endoderm cell fate specification (Raftery and Sutherland, 1999; Goodrich and Scott, 1998). Hh and Dpp have been studied in great detail in the development of the compound eye, where they are required for the initiation of ommatidial cell determination (Ma et al., 1993; Heberlein et al., 1993). We have analyzed the role of Dpp and Hh in the embryonic head neurectoderm, a structure that is topologically much more similar to the vertebrate eye field than the eye disc is. Thus, the *Drosophila* eye disc develops from a small group of 'set aside cells' derived from the embryonic eye field (Fig. 1). The disc gives rise to photoreceptors and support cells only, not to any component of the central nervous system. By contrast, the embryonic eye field, like its vertebrate counterpart, is the origin of the entire visual system (photoreceptors and neurons) and anterior brain. Results pertaining to the function of the Hh and Dpp gradients in partitioning the embryonic eye field may be more readily comparable with corresponding processes in the vertebrate head.

Our data show that Dpp is expressed at the blastoderm stage in the dorsal head. High levels of Dpp are required in the dorsal head epidermis. Reduction of Dpp function results in a 'cyclops' phenotype where mid-dorsal head epidermis is transformed into dorsolateral structures, i.e. eye/optic lobe tissue. Absence of Dpp causes the transformation of both head epidermis and visual structures into protocerebral neuroblasts. Regulatory genes that are required for eye/optic lobe fate, including *sine oculis* (*so*) and *eyes absent* (*eya*; *cli* – FlyBase), are regulated in their respective domains by Dpp. Hh and its receptor/inhibitor, Patched (Ptc) are expressed in a transverse stripe along the posterior boundary of the eye field. Hh triggers the expression of determinants for larval eye (*atonal*) and adult eye (*eyeless*) in those cells of the eye field that are close to the Hh source. Eya and So, induced by Dpp, are epistatic to the Hh signal. Loss of Ptc, as well as overexpression of Hh, results in the ectopic induction of larval eye tissue in the dorsal midline (cyclops). We discuss the similarities between vertebrate systems and *Drosophila* in regard to the fate map of the eye field and the role of Dpp and Hh in this structure.

MATERIALS AND METHODS

Fly stocks

Oregon R flies were used as the wild-type stock. The following mutations were used in this study: dpp^{Hin46}, dpp^{E87}, hh^{21}, hh^{ts2}, *hs-hh AN14*, ptc^{IN108}, sog^{s6} and zen^{4} (Bloomington Stock Center); br^{M68};sog^{YS06} double mutant and scw^{s12} (kindly provided by Dr S. Roth); a *PlacZ* insertion in *sine oculis* (*so-lacZ*) (Cheyette et al., 1994). The following driver lines and UAS constructs were used: *da*-Gal4 (kindly provided by Dr J. Campos-Ortega), *hs*-Gal4 (kindly provided by Dr J. Merriam) and UAS-*dpp* (Bloomington Stock Center). Eggs were collected on yeasted apple juice agar plates. Embryonic stages are given according to Campos-Ortega and Hartenstein (Campos-Ortega and Hartenstein, 1997).

Immunohistochemistry

Embryos were dechorionated and fixed in 4% formaldehyde containing PT (1% PBS, 0.3% Triton X-100) with heptane. Embryos were then devitellinized in methanol and stored in ethanol before labeling with antibody, following the standard procedure (Ashburner, 1989). Expression of β-galactosidase (β-gal) in enhancer trap lines was detected with a monoclonal anti-β-galactosidase antibody (Sigma) at a dilution of 1:1000. A monoclonal anti-FasII (Grenningloh et al., 1991) antibody was used at a 1:1000 dilution to detect FasII (Fas2 – FlyBase). Monoclonal antibody mAb22C10 (Zipursky et al., 1984) (Iowa Hybridoma Bank) was used at a dilution of 1:200, and monoclonal antibody 1SN (kindly provided by Dr P. Chambon) was used at a 1:100 dilution to detect Snail. A polyclonal anti-pMAD 'PS1' antibody (kindly provided by Dr T. Tabata) (Aurelio and Cohen, 2000) was used at a 1:1000 dilution to detect phosphorylated Mad, and a polyclonal antibody AbN (kindly provided by Dr T. Kornberg) (Aza-Blanc et al., 1997) was used at a 1:1000 dilution to detect Ci.

In situ hybridization

Embryos were dechorionated and fixed in phosphate-buffered saline (PBS) containing 5% formaldehyde and 50 mM EGTA and stored in ethanol. They were then treated with xylene and fixed for a second time in PBS containing 0.1% Tween-20 and 5% formaldehyde. The embryos were then hybridized with probes synthesized using

digoxigenin-labeled UTP (Boehringer) according to standard protocol (Tautz and Pfeifle, 1989). Embryos labeled with DNA probes were treated with Proteinase K (50 μg/ml) followed by glycine (2 mg/ml) before hybridization. The digoxigenin-labeled probes described below were hybridized to fixed embryos in buffer containing 50% formamide at 55°C. Anti-digoxigenin antibody (Boehringer) was used according to the manufacturer's instructions to detect hybridized probe, after which the embryos were dehydrated in ethanol and embedded in epon. Alternatively embryos were washed and incubated with a second antibody for immunohistochemistry after in situ hybridization.

Probes

cDNA containing *otd* (Finkelstein and Perrimon, 1990) (kindly provided by Dr J. Lengyel) was linearized with *Kpn*I and used as template to make the *otd* RNA probe. cDNA containing *dpp* (St Johnston and Gelbart, 1987) (kindly provided by Dr E. Bier) was linearized with *Hin*dIII and used as template to make the *dpp* RNA probe. A *sog* RNA probe was kindly provided by Dr A. Courey. cDNA containing *br* (Jazwinska et al., 1999) (kindly provided by Dr S. Roth) was linearized with *Eco*RI and used as template to make the *br* RNA probe. pKS-race (Rusch and Levine, 1997) (kindly provided by Dr M. Levine) was digested with *Hin*dIII and used as template to make the *race* RNA probe. pBS:eya I (Bonini et al., 1993) (kindly provided by Dr F. Pignoni) plasmid linearized with *Sal*I and used as template to make the *eya* RNA probe. cDNA containing intermediate neuroblasts defective (*ind*) (Weiss et al., 1998) (kindly provided by Dr M. Scott) was linearized with *Hin*dIII and used as template to make the *ind* RNA probe. tc1 pBS containing *tll* (Pignoni et al., 1990; Steingrímsson et al., 1991), was digested with *Eco*RI to make DNA probe and linearized with *Sal*I to make the RNA probe. Plasmid pBS-pF3k (Cheyette et al., 1994) linearized with *Bam*HI and used as template to synthesize the *so* RNA probe. cDNA containing *hh* (Lee et al., 1994) (kindly provided by Dr J. Lengyel) was linearized with *Kpn*I and used as template to make the *hh* RNA probe. pBSK-*ptc* (Goodrich and Scott, 1998) (kindly provided by Dr J. Hooper) was linearized with *Hin*dIII and used as template to make the *ptc* RNA probe. cDNA containing *ey* (Quiring et al., 1994) (kindly provided by Dr F. Pignoni) was linearized with *Sal*I and used as template to make the *ey* RNA probe.

Temperature shift experiments

hs-GAL4; UAS-*dpp*, *da*-GAL4; UAS-*dpp* and *hs-hh AN14* embryos were collected for 2 hours at 25°C, and shifted to 37°C at 2, 4 and 6 hours post-fertilization. Embryos were heatshocked for 2 hours and allowed to develop at 25°C until stage 16 of embryogenesis, at which time, they were fixed for subsequent staining. hh^{ts2} embryos were collected for 2 hours at either 18°C (for upshift experiments) or 29°C (for downshift experiments), and shifted either up or down at 2, 4, 6 and 8 hours post-fertilization. Completion of embryogenesis takes 42 hours at 18°C, 16 hours at 29°C and 22 hours at 25°C. To compensate for timing differential, a ratio of these hours were used.

RESULTS

Topology of the *Drosophila* head

At the onset of gastrulation, the anlage that gives rise the anterior brain (protocerebrum) and the eye, roughly defined by the expression of *otd*, extends from the cephalic furrow to the anlage of the foregut (Hirth et al., 1995; Younossi-Hartenstein et al., 1997) (Fig. 2A,B). In the dorsoventral axis, the anlage crosses the dorsal midline; laterally it reaches to ~50% of egg diameter where it is bounded by the ventral neurectoderm. During gastrulation and germband elongation, the anlage splits up into different components that can be recognized morphologically and with the help of molecular markers. Three main domains, the head midline ectoderm, protocerebral neurectoderm and the visual primordium, can be distinguished.

Head midline ectoderm

A narrow strip straddling the dorsal midline gives rise to the medial portion of the head epidermis. In the acephalic larva, these cells (and most other cells of the head epidermis) are folded inside the animal to form the dorsal pouch (Younossi-Hartenstein et al., 1993) (Fig. 2D).

Protocerebral neurectoderm

The lateral part of the head neurectoderm produces the neuroblasts that form the central protocerebrum, the major compartment of the insect brain that includes associative centers such as the mushroom bodies and central complex. A narrow domain within the dorsomedial protocerebrum is the anlage of the so-called pars intercerebralis (Zacharias et al., 1993; Younossi-Hartenstein et al., 1996), which contains clusters of neuroendocrine cells producing various neuropeptides (Mobbs, 1985; Taghert, 1999) (Fig. 2A,E). The neuroendocrine neurons project their axons in a peripheral nerve that leaves the brain and reaches the corpora cardiaca (arrow in Fig. 2E), a neurohemal organ located close to the heart (Hartenstein et al., 1994) (Fig. 2D,E). The pars intercerebralis-corpora cardiaca system is highly reminiscent of the vertebrate hypothalamus-pituitary axis, and this similarity extends to the embryonic origin of the corpora cardiaca. Thus, the corpora cardiaca arise as invaginations from the foregut. Their embryonic origin has been well documented in *Manduca sexta* (Copenhaver and Taghert, 1991); in *Drosophila*, we have evidence that the corpora cardiaca, along with precursors of the stomatogastric (i.e. autonomic) nervous system, also invaginate from the foregut (Fig. 2F).

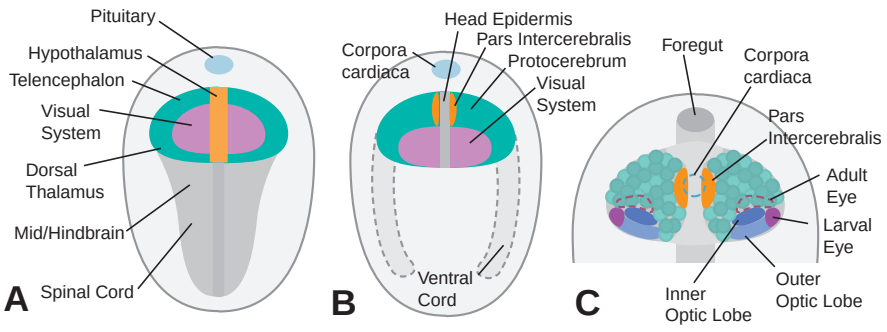

Fig. 1. Topology of the anterior brain/eye anlage (green) and eye field (magenta) in vertebrates (A) and *Drosophila* (B,C). (A,B) The fate map of the head structures before neurulation (dorsal view). Map positions of main neural structures of the head are indicated. (C) The progenitors of the *Drosophila* brain and visual system at a later stage when the visual primordium has split into larval and adult eye, and inner/outer optic lobe.

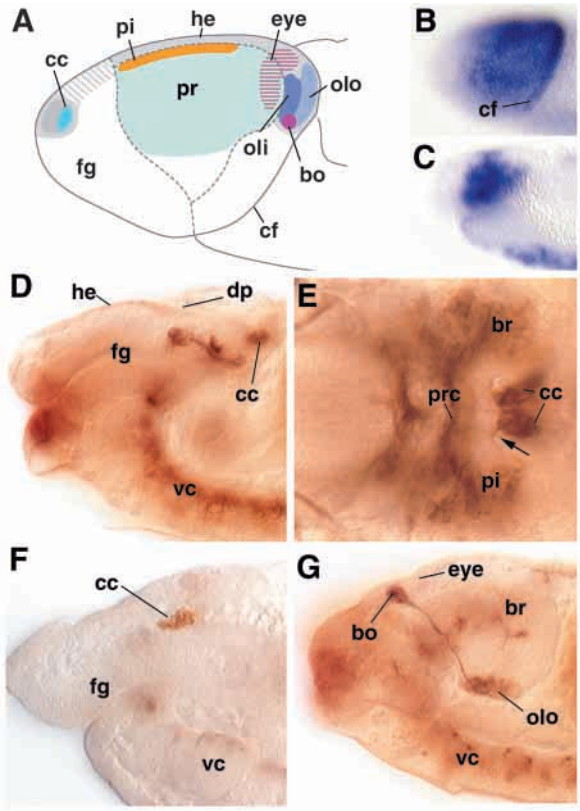

Fig. 2. The *Drosophila* anterior brain/eye anlage and its derivatives. (A) Map of the anterior brain/eye anlage around gastrulation (lateral view, anterior towards the left, dorsal upwards). Positions of various head derivatives and landmark structures are indicated (bo, larval eye (Bolwig's organ); cc, corpora cardiaca; cf, cephalic furrow; eye, adult eye; fg, foregut; he, dorsal head epidermis; oli, inner optic lobe (lobula complex); olo, outer optic lobe (medulla and lamina); pr, protocerebral neurectoderm). (B,C) Expression of *orthodenticle* (*otd*) at the onset of gastrulation (stage 6, B) and at the extended germband stage (stage 11, C; both panels show whole-mount in situ hybridization using an *otd* cDNA probe). Note widespread early expression of *otd* in the entire anterior brain/eye anlage. Later expression becomes restricted to the protocerebrum. (D,E) Lateral view (D) and dorsal view (E) of stage 15 embryo labeled with anti-FasII, which visualizes founder tracts of the brain (br) and ventral nerve cord (vc), as well as the corpora cardiaca (cc) and thin nerves (arrow in E) connecting the corpora cardiaca with the pars intercerebralis (pi) of the brain. Dorsal head epidermis (he) is in the process of involuting to form the dorsal pouch (dp). (F) Stage 12 embryo, lateral view, expressing *lacZ* under the control of the *glass* promoter in the precursors of the corpora cardiaca (cc). These cells have just separated from the dorsal aspect of the foregut primordium (fg). (G) Lateral view of stage 15 embryo labeled with anti-FasII. In this more lateral plane of focus, the larval eye (bo) and outer optic lobe (olo) are visible. Small placode dorsal of the larval eye represents the primordium of the adult eye (eye).

Visual primordium

The visual primordium, defined molecularly by the expression of *so*, is wedged in between the midline ectoderm and the protocerebral neurectoderm in the posterior head (Fig. 2A). During gastrulation and germband extension, cells of the visual primordium move laterally and are subdivided into the larval and adult eye primordia and the inner and outer optic lobe (Fig. 2A) (Green et al., 1993; Cheyette et al., 1994; Daniel et al., 1999). The optic lobe and larval eye form a triangular placode that invaginates. The posterior lip of this invagination, marked by the expression of FasII, represents the primordium of the lamina and medulla ('outer optic lobe'; Fig. 2A,G); the anterior lip, marked by expressing the homeobox gene *ind* (not shown), gives rise to the lobula complex ('inner optic lobe') (Fig. 2A). The larval eye, or Bolwig's organ, labeled by FasII and mAb22C10, develops at the lateralmost tip of the optic lobe placode. The cells that will become the eye imaginal disc (adult eye) are anterior and dorsal to the optic lobe placode (Daniel et al., 1999) and can be recognized by the expression of *eyeless* (*ey*; see Fig. 9E).

Expression of Dpp and its antagonists Brk and Sog in the embryonic head

Dpp expression and function were followed using an in situ RNA probe and an antibody against phosphorylated MAD protein (anti-pMAD), respectively. The patterns revealed by both markers in the embryonic head matched closely, supporting the notion that *dpp* itself is a target of Dpp signaling. Dpp is expressed at the blastoderm stage in the entire dorsal half of the trunk and head of the embryo (Fig. 3A). Subsequently, the level of *dpp* and pMAD is elevated in a narrower dorsomedial stripe that includes the eye field (Fig. 3B,C). With the onset of gastrulation throughout the early extended germband stage (stages 7-10), *dpp* disappears from most of the head, except for an anterior domain in the anlage of the foregut, and a narrow posterior domain bordering the visual primordium posteriorly. This domain is contiguous with a *dpp*-expressing domain in the dorsal ectoderm of the trunk (Fig. 3D). During the late extended germband (stage 11) there appears a mid-dorsal domain of *dpp* expression in the posterior head, overlapping with prospective head epidermis. In addition, laterally, *dpp* appears in a small discrete spot in the antennal segment, right adjacent to the visual primordium. (Fig. 3E). Based on this expression pattern, we anticipate that the distribution of the Dpp protein in the head may be complex, and may shift during development from a dorsoventral gradient (early phase) over a posteroanterior gradient (intermediate phase) to a local point source (late phase).

In the trunk, the effect of Dpp is inhibited in the ventral ectoderm by the Chordin homolog Sog (Francois et al., 1994) and the transcriptional repressor Brk (Jazwinska et al., 1999). As the spatial control of the Dpp gradient in the head is likely to be influenced by the same players, we investigated the expression pattern of these genes in the embryonic head. At the blastoderm stage, *sog* and *brk* are expressed in the ventral half of the embryo along the entire anteroposterior axis (Fig. 3F). During gastrulation, expression in the head gradually spreads dorsally (Fig. 3G). At the extended germband stage *sog* and *brk* expression at a low level covers the protocerebral neurectoderm (Fig. 3H,I). *sog* disappears from the head during stage 11, while *brk* is on somewhat longer (data not shown). Note that the dorsal expression of *sog* and *brk* comes on later than the downturn of Dpp, which is complete with the onset of gastrulation (compare Fig. 3D,G). This suggests that the repression of *dpp* in the dorsal head is effected by factors in addition to Sog and Brk. Support for this hypothesis comes from the observation that in *brk; sog* double mutants, *dpp*

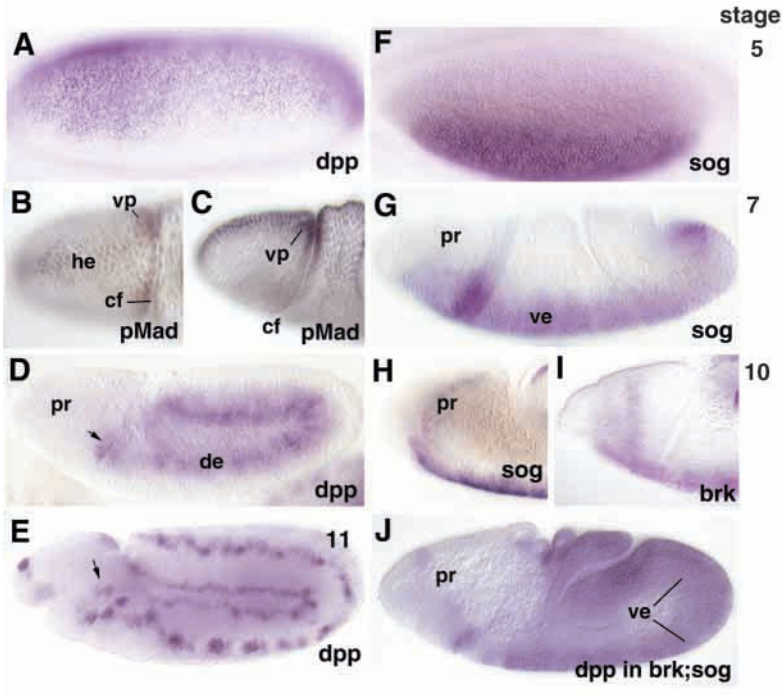

Fig. 3. Expression of *dpp* and its antagonists, *sog* and *brk*, in the head region of the early embryo. All panels show lateral view of embryo whole-mounts labeled by antibody or in situ hybridization with the probes of the corresponding genes (indicated in at bottom right). Stages are indicated at the right. (A) Blastoderm stage. *dpp* expression in dorsal region of trunk and head. (B,C) Gastrulation. Antibody against pMAD shows activation of Dpp pathway in the visual primordium (vp). B, dorsal view; C, lateral view. (D) Late gastrulation and early extended germ band. *dpp* expression has ceased in most of the head, except posterior rim of visual primordium (arrow). (E) Late extended germband. Dpp is expressed in small discrete spots in the dorsal midline and anterior to the larval eye (arrow). (F) Expression of *sog* in the ventral blastoderm of the trunk and head region. (G) Around gastrulation, *sog* expression expands from the ventral ectoderm (ve) into the domain of the protocerebrum (pr). (H,I) Low level expression of *sog* and *brk* in the stage 10 protocerebral neurectoderm. (J) In a *sog; brk* double mutant, expression of *dpp* is expanded into the entire ventral neurectoderm (ve; compare this image with G), but not the protocerebral neurectoderm (pr). cf, cephalic furrow; de, dorsal trunk ectoderm.

expression does not expand into the protocerebral ectoderm, although it does cover most of the ventral ectoderm (Fig. 3H).

The role of Dpp signaling in head patterning

Loss, reduction and overexpression of Dpp in the head ectoderm results in a phenotype that can be most easily interpreted by assuming that similar to what has been postulated for the trunk, there is a graded requirement for Dpp at dorsomedial and dorsolateral levels. Reduction of Dpp function, as seen in the *dpp* hypomorph dpp^{E87}, or embryos lacking *sog*, results in a highly characteristic 'cyclops' phenotype. The dorsal epidermis that normally forms the dorsal pouch is absent, as evidenced by the loss of expression of the gene *race* that normally appears in the amnioserosa and dorsomedial head epidermis (Rusch and Levine, 1997) (Fig. 4A,B). Head epidermis is replaced by ectopic optic lobe and larval eye tissue (Fig. 4C-F) which are exposed at the surface because head involution fails to occur. The pattern of protocerebral neuroblasts, visualized by anti-Sna antibody, is unchanged in dpp^{E87} (data not shown), unlike the situation in *dpp*-null embryos where neuroblast levels are strongly increased (see below). These findings imply that, similar to the amnioserosa of the trunk, the epidermal midline ectoderm of the head requires the highest levels of Dpp. Reduction of Dpp results in the transformation of the midline to dorsolateral structures that, in the head, are represented by the visual primordium.

A different and much more severe phenotype results from the total absence of Dpp. As in *dpp* hypomorphs, head midline epidermis does not form; however, instead of dorsolateral fates replacing the head midline fates, both midline and dorsolateral regions exhibit characteristics of lateral neurectoderm. Optic lobe and Bolwig's organ are absent (Fig. 5B). Neuroblasts are formed in realms of the head midline and visual system (Fig. 5D). To what dorsoventral level does the fate of the ectopic neural tissue correspond? The neurectoderm of the head gives rise to neuroblasts at ventrolateral levels (tritocerebrum and deuterocerebrum), as well as dorsolateral levels (protocerebrum). Based on the expression pattern of the markers *ey*, FasII and *ind*, we conclude that the ectopic neuroblasts in dpp^{-} embryos appear to be of dorsolateral provenance. Thus, *ind* is normally expressed in the stage 9 wild-type embryo in a small dorsolateral cluster that gives rise to several protocerebral neuroblasts, as well as the anterior lip of the optic lobe (Fig. 5E). In dpp^{-}, *ind*-expressing cells are displaced to the dorsal midline (Fig. 5F).

We used the ubiquitously expressed driver line *daughterless* (*da*)-Gal4 to express UAS-*dpp*. This Gal4 line is not expressed in the blastoderm but comes on with gastrulation. Correspondingly, the resulting changes in cell fate in the head and trunk were relatively mild and can be best described in terms of a ubiquitously raised base level of Dpp, superimposed on the regular gradient of endogenous Dpp. Mid-dorsal structures, including the amnioserosa and head epidermis, were much wider than in wild type (Fig. 5H-J). Dorsolateral structures, including the visual primordium, are relatively normal in size and shape, but are shifted to lateral or ventrolateral levels (Fig. 5J). Ventral tissues are partially missing (not shown).

Overexpression of *dpp* by using the heat-inducible driver line *hs*-Gal4 resulted in a phenotype very similar to the one described for *da*-Gal4-driven UAS-*dpp*. Applying 2 hour heat pulses at different stages of development support the idea that the phenocritical period of Dpp action is around the onset of gastrulation. Thus, a high number of embryos heat pulsed between 3 and 5 hours post fertilization showed the characteristic dorsalization phenotype described above. Later heat pulses had no effect on head patterning.

Effect of Dpp on early gene expression patterns in the head

The above described phenotypic effects observed in mid- and

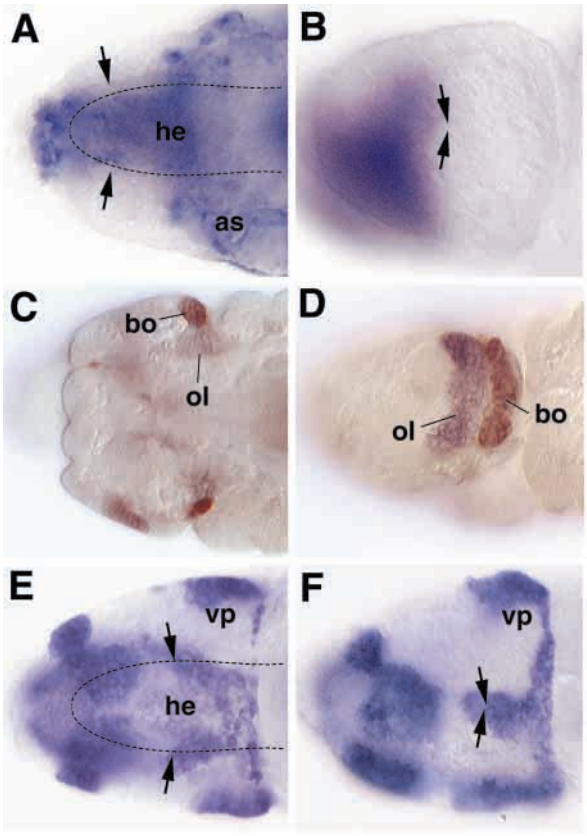

Fig. 4. Phenotypic effects of reduction in Dpp function. (A,B) Dorsal view of stage 11 wild-type (A) and *dpp* hypomorph, dpp^{E87} (B), embryo labeled with a cDNA probe for the *race* gene, a downstream target of Dpp expressed in the amnioserosa (as) and dorsal head epidermis (he). Note absence of *race* (arrows) in the *dpp* hypomorph (B). (C,D) Dorsal view of stage 13 embryo (C, wild type; D, dpp^{E87}) in which outer optic lobe (ol) and larval eye (bo) are labeled by anti-Fas II (violet) and mAb22C10 (brown), respectively. In the *dpp* hypomorph, the dorsal midline has been transformed into visual primordium, resulting in an unpaired median larval eye and optic lobe (cyclops). (E,F) Dorsal view of stage 10 wild-type (E) and dpp^{E87} (F) labeled with probe for the *eya* gene. *eya* is expressed in a complex pattern in the anterior protocerebrum, visual primordium (vp) and, at lower level, dorsal head epidermis (he; outlined by arrows and a broken line). The head epidermis is missing in dpp^{E87} (arrows)

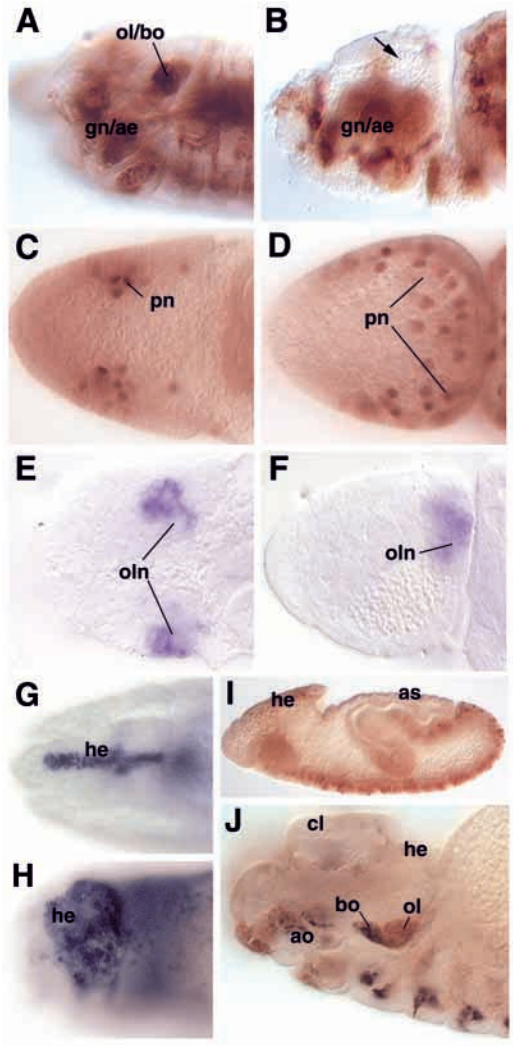

Fig. 5. Phenotypic effects of loss of Dpp function and *da*-Gal4-directed overexpression of *dpp*. (A,B) Lateral view of stage 13 wild-type (A) and *dpp*-null (B) embryos labeled with anti-FasII. Note absence of optic lobe and larval eye (ol/bo in A) in the mutant (arrow in B). (C,D) Dorsal view of stage 9 wild-type (C) and *dpp*-null (D) embryos labeled with anti-Snail, which recognizes protocerebral neuroblasts (pn). In the mutant, dorsal head ectoderm is 'invaded' by protocerebral neuroblasts. (E,F) Dorsal view of stage 11 wild-type (E) and *dpp*-null (F) embryos labeled with a probe for the *ind* gene that is expressed in the anterior lip of the optic lobe and a group of neuroblasts delaminating from this domain (oln). In the wild type, the oln neuroblasts occupy a lateral position; in the mutant, they appear dorsomedially. (G,H) Dorsal view of stage 15 wild-type (G) and *da*-Gal4; UAS-*dpp* embryos (H) labeled with probe for *race*. Note widening of dorsomedial strip of *race*-positive head epidermis (he). (I) Lateral view of stage 10 *da*-Gal4; UAS-*dpp* embryo, showing expansion of amnioserosa (as). (J) Lateral view of stage 14 *da*-Gal4; UAS-*dpp* embryo labeled with anti-FasII (brown) and mAb22C10 (purple) to visualize larval eye (bo) and optic lobe (ol). Both structures are of normal size but displaced ventrally. Epidermis of clypeolabrum (cl) and dorsomedial head (he) has expanded. ao, antennal organ; gn/ae, gnathal segments and anterior endoderm.

late-stage mutant embryos indicate that dorsal epidermal and visual system fates, in particular those of the posterior optic lobe and larval eye, are not expressed in *dpp* loss of function. It is likely that these abnormalities are the result of changes in early head gene expression. This was followed in detail by assaying the expression of several regulatory genes known to be required for the normal development of the visual primordium, including *otd* (Finkelstein and Perrimon, 1990), *tll* (Pignoni et al., 1990), *so* (Cheyette et al., 1994) and *eya* (Bonini et al., 1993) in *dpp*-null mutants (Fig. 6).

otd

otd is normally expressed in a wide domain that spans the dorsal midline and encompasses the entire dorsal head ectoderm. In normal development, its expression is turned off in the head midline (the head epidermis precursors) and in the part of the visual primordium forming the posterior optic lobe and larval eye (Fig. 6A). In *dpp* mutants, expression persists in the entire dorsal head ectoderm until stage 11 (Fig. 6B).

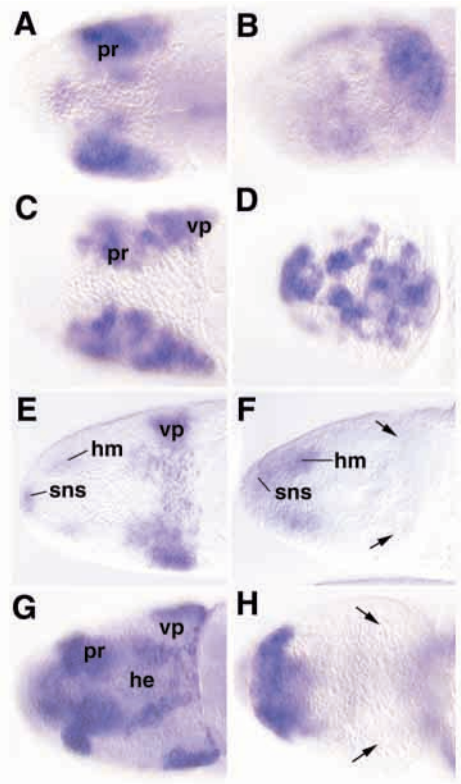

Fig. 6. Dpp controls the expression of the head gap genes *otd* (A,B) and *tll* (C,D), as well as the early eye genes *so* (E,F) and *eya* (G,H). All panels show dorsal view of stage 10 embryos labeled with cDNA probes for the corresponding genes. (A,C,E,G) Wild-type controls; (B,D,F,H) *dpp*-null embryos. Both *otd* and *tll*, normally excluded from the dorsal midline (A,C), are expressed in the dorsal midline in *dpp* mutants (B,D). Expression of both *so* and *eya* in the visual primordium (vp) is abolished in *dpp* mutants (arrows in F,H), although anterior head expression is preserved.

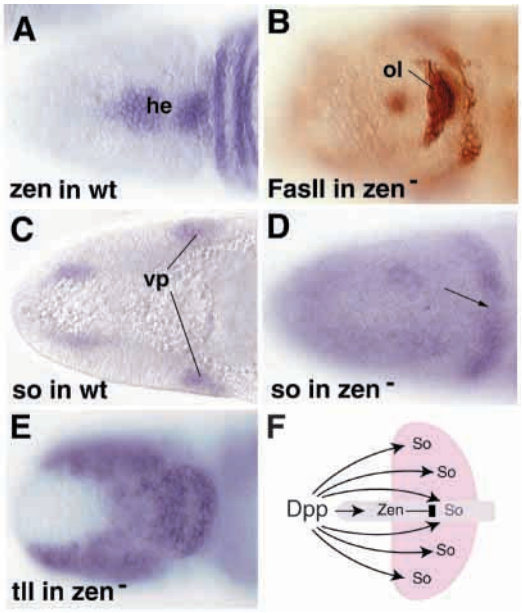

Fig. 7. Requirement of *zerknuellt* (*zen*) during head patterning. (A) Dorsal view of stage 6/7 embryo labeled by in situ hybridization, showing expression of *zen* in primordium of dorsal head epidermis (he). (B) Dorsal view of stage 15 *zen* mutant embryo labeled with anti-FasII, showing cyclopic optic lobe (ol). (C,D) Expression of *so* in the dorsal head of a stage 11 wild-type (C) and *zen* mutant (D) embryo. Note high level of *so* in dorsal midline (arrow) in the mutant, compared with wild type where *so* labels the laterally migrated visual primordia (vp). (E) Dorsal view of stage 10 *zen* mutant embryo labeled with in situ probe against *tll*, showing persistence of *tll* in dorsal midline (compare with Fig. 6, showing wild-type expression of *tll*). (F) Model illustrating the role of Zen as repressor of early regulatory genes (exemplified by *so*) in the dorsal midline.

Expression then becomes patchy as many cells undergo apoptotic cell death.

tll

tll appears in the protocerebral ectoderm, including the head midline ectoderm. Only later does expression spread to cover part of the visual primordium. In embryos that lack Dpp, expression is expanded from the beginning to include the entire dorsal head. As for *otd*, expression also persists in the head midline ectoderm (Fig. 6D).

so

so is expressed in a transverse stripe spanning the dorsal midline (Fig. 6E). This unpaired domain defines the eye field. Around gastrulation, *so* expression ceases in the dorsal midline and becomes restricted to the bilateral visual primordia. In addition to the visual system, *so* appears in the anlage of the stomatogastric nervous system (SNS) and head mesoderm. In a *dpp*-null fly, *so* is never expressed in the anlage of the visual system (Fig. 6F), although expression in the SNS and head mesoderm is unchanged.

eya

eya is normally expressed in a complex pattern that essentially consists of three domains located in the anlage of the SNS, the anterior protocerebrum and the anlage of the visual system (Fig. 6G). In *dpp*-null embryos, *eya* expression in the primordia of the visual system and SNS is absent from the beginning (Fig. 6H). The anterior protocerebral expression is narrowed.

The observed downregulation of head gap genes and early eye genes in the dorsal midline is an indirect effect of Dpp mediated by the Dpp target *zerknuellt* (*zen*). Previous studies have demonstrated that high levels of Dpp in the dorsal midline upregulate and focus the expression of *zen* in the amnioserosa and, further anteriorly, in the dorsomedial head epidermis (Rusch and Levine, 1997). An RNA in situ probe revealed expression of zen in the early eye field of a stage 5-7 embryo (Fig. 7A). Assaying the expression of head gap and early eye genes in a *zen*-null mutant background demonstrates that Zen acts as a repressor of these genes. Whereas in wild type, after an initial unpaired expression straddling the dorsal midline, *tll*, *so* and *eya* are turned off in the dorsal midline, they continue to be expressed in this domain in a *zen* mutant (Fig. 7D,E). At later stages, lack of *zen* results in a cyclops phenotype (Fig. 7B).

Hh signaling in the *Drosophila* embryonic head

Hh is expressed in metameric stripes that coincide with the posterior compartment of each segment. In the head, *hh*

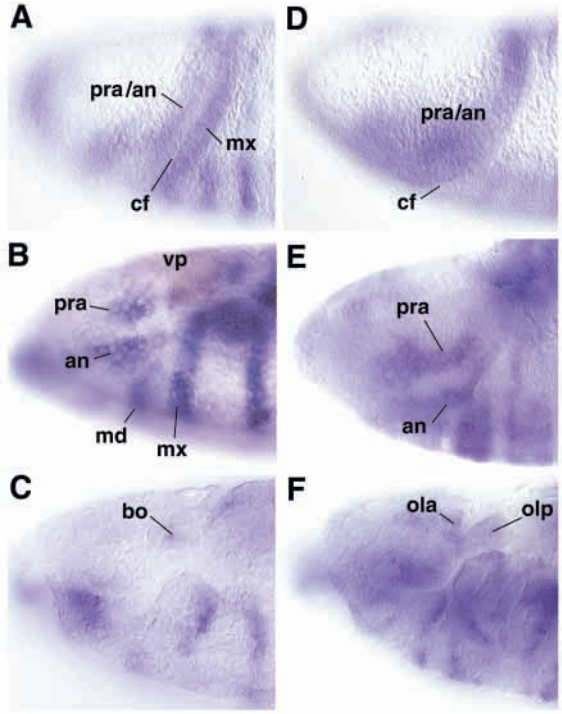

Fig. 8. Expression of *hh* and *ptc* in the embryonic head. All panels show lateral views of embryos labeled with a cDNA probe for *hh* (left column; A-C) and *ptc* (right column; D-F). Embryo in B also expressed a *so-lac*Z construct in the visual primordium (brown, vp). (A,D) Stage 7; (B,E) stage 9; (C,F) stage 11. an, antennal stripe; bo, larval eye; cf, cephalic furrow; md, mandibular stripe; mx, maxillary stripe; ola, anterior lip of optic lobe invagination; olp, posterior lip of optic lobe invagination; pra, pre-antennal stripe; vp, visual primordium.

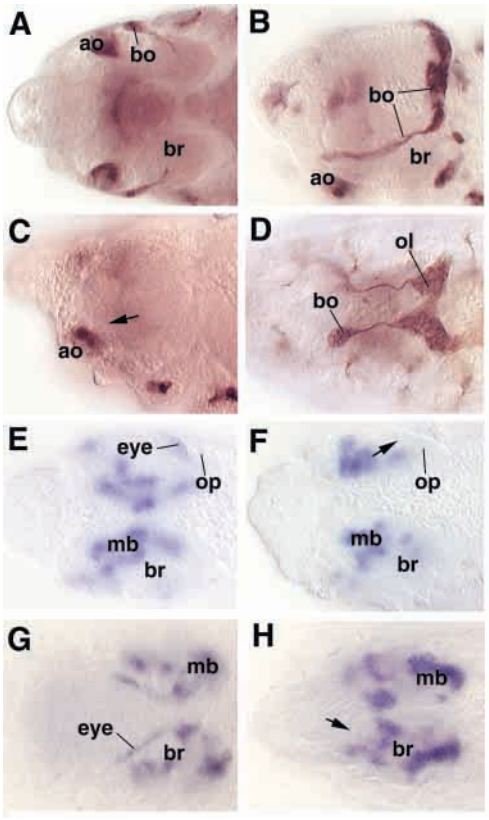

Fig. 9. Phenotypic effects of loss and overactivity of Hh signaling in the embryonic head. (A) Dorsal view of stage 15 wild-type embryo labeled with mAb 22C10 to visualize larval eye (bo). (B) *ptc*-null embryo at same stage and orientation as A. Note cyclopic pattern and increased size of larval eye (bo). (C) Lateral view of stage 15 *hh* mutant embryo labeled with mAb22C10. The larval eye, normally dorso-posterior of the antennal organ (ao) is absent (arrow). (D) Dorsal view of stage 16 embryo in which a *hs-hh* construct was activated during 3-5 hours of development. Larval eye and optic lobe are labeled with anti-FasII. Early overexpression of *hh* results in cyclopic optic lobe.(E, F) Dorsal view of stage 12 wild-type (E) and *hh*-null (F) embryos labeled with an *eyeless* probe. In wild type, *ey* expression begins at this stage in the primordium of the adult eye (eye) which is right in front of the optic placode (op). Note absence of *ey* expression in the mutant (arrow in F). (G,H) Dorsal view of stage 16 wild-type (G) and *hh*-null (H) embryos. Beside mushroom body (mb) and other neural foci, *ey* is expressed in the lateral rim of the dorsal pouch which represents the eye primordium (eye). This expression domain is absent in the mutant (arrow in H). br, brain.

expression in the stage 5-7 embryo forms a wide stripe in front of the cephalic furrow. This stripe, that crosses the dorsal midline, includes the future antennal segment and posterior part of the visual anlage (Fig. 8A). As germ band extension proceeds, *hh* expression disappears from the dorsal midline and two separate bands are parceled out (antennal stripe, pre-antennal or occular stripe; Fig. 8B) (Suzuki and Saigo, 2000). The pre-antennal stripe overlaps with the lateral boundary of the visual primordium (visualized in Fig. 8B by the expression of a *so-lacZ* construct). Towards the late extended germband stage, the Hh head domain decreases in size and expression level. During stage 11 and early 12, only a small cluster of cells corresponding to the precursors of the larval eye located laterally in the visual primordium remain *hh* positive (Fig. 8C).

Hh signaling is negatively regulated by Ptc, a membrane linked protein that, by binding to Hh ligand, becomes inactivated in cells receiving high levels of Hh (Goodrich and Scott, 1998). Ptc expression in the head resembles *hh* expression at an early stage (stage 5-7; Fig. 8D). A wide antennal/pre-antennal stripe traverses the head in front of the cephalic furrow. During germband extension, this domain splits up into two stripes (Fig. 8E). At the late extended germ band stage, *ptc* remains expressed in a large domain that corresponds to the anterior optic lobe (Fig. 8F).

Loss of *hh* results in a strong reduction of the head midline epidermis, a reduction in the size of the brain and optic lobe, and the total absence of the larval and adult eye primordium (Fig. 9C,F,H). The requirement of Hh for the formation of the larval eye has been recently reported by Suzuki and Saigo (Suzuki and Saigo, 2000). Our temperature-sensitive shift experiments of hh^{ts2} embryos indicate that the phenocritical period for Hh function in Bolwig's organ development is between 4 and 7 hours. Aside from the larval eye, the primordium of the compound eye, which is marked from stage 12 onward by the expression of *eyeless* (*ey*), is also affected by the loss of *hh*. Heatshock induced overexpression of *hh*, as well as loss of *ptc*, causes an increase in larval eye neurons and optic lobe precursors (Fig. 9B,D). Interestingly, ectopic Hh activity is able to induce optic lobe and Bolwig's organ tissue in the

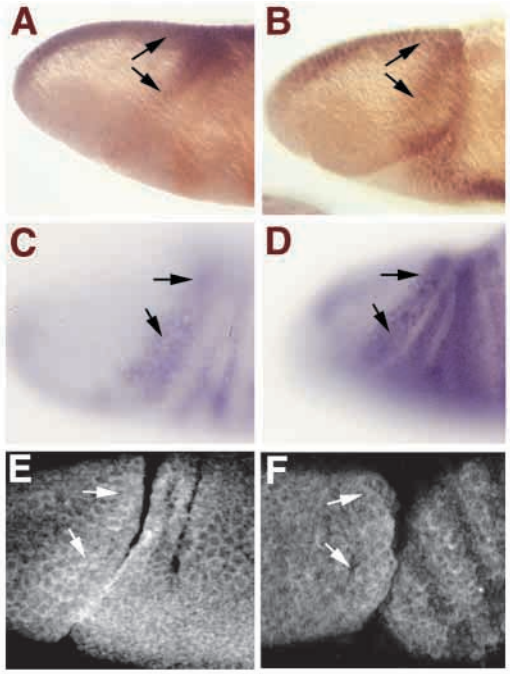

Fig. 10. (A,B) Expression of pMAD (purple) in visual primordium (arrows) of stage 7 wild-type (A; note brown Ftz stripes caused by the presence of *ftz-lacZ* on balancer chromosome) and *hh*-null (B) embryos. (C,D) Whole-mount in situ hybridization of stage 7 embryos, showing expression of *hh* (C) and *ptc* (D) in visual primordium (arrows) of *dpp*-null mutant. (E,F) Expression of Ci in eye primordium (arrows) of stage 7 wild-type (E) and *dpp*-null (F) embryos.

head midline and thereby generate a cyclops phenotype similar to the condition described above for partial reduction of *dpp*. Applying heatshocks at different times of development indicates that the phenocritical period for the Hh induced cyclops is early, between 2.5 and 5 hours. Thus, heat pulses administered during this time caused fusion of the optic lobe and, at a lower frequency, larval eye without increasing the number of optic lobe and larval eye cells significantly. By contrast, later heat pulses (after 5 hours) led to larval eye/optic lobe hyperplasia but no concomitant cyclops phenotype (data not shown).

The intersection of Dpp and Hh signaling in the head

The finding that both loss of Hh and Dpp cause the absence of visual structures, and ectopic expression of Hh and partial loss of Dpp cause transformation of head midline epidermis into visual primordium, begs the question of how the two signaling pathways interact. In *Drosophila* compound eye development, *hh* expression is required to turn on *dpp* expression (Heberlein et al., 1993). To establish whether a regulatory relationship exists between Hh and Dpp signaling, we looked for the expression of *dpp* and pMAD in the background of *hh* loss of function, as well as *hh*, *ptc* and Cubitus interruptus (Ci) expression in the background of *dpp* loss of function. In a recent study (Dorfman and Shilo, 2001), it has been shown that cells in which Dpp signaling is activated can be visualized by an antibody against phosphorylated MAD (pMAD) protein. Dpp RNA expression (not shown) and pMAD are normal in a stage 5-9 *hh*-null background (Fig. 10B), indicating that Hh is not required to activate Dpp signaling in the embryonic head.

The expression of *hh* and *ptc* is normal in early embryos mutant for *dpp* (Fig. 10C,D). As *ptc* is a downstream target of Hh signaling (Forbes et al., 1993; Goodrich et al., 1996; Marigo et al., 1996; Méthot and Basler, 2001), this result strongly suggests that Dpp signaling is not required to activate the Hh cascade. To show more directly whether this cascade is interrupted, we used the antibody AbN, which recognizes both the full-length Ci protein and the cleaved repressor form (Ci75) (Aza-Blanc et al., 1997) in the background of a *dpp*-null mutation. According to the present model (McMahon, 2000; Aza-Blanc and Kornberg, 1999), Hh function consists of preventing the cleavage of the Ci protein to generate the repressor form, which is able to enter the nucleus and inhibit transcription of target genes such as *ato* and/or *hh*. In a mutation of Ci that produces only the repressor form or in eye clones that lack *hh*, a higher level of Ci can be detected in the cells (Suzuki and Saigo, 2000; Domínguez, 1999). In *dpp*-null embryos, cytoplasmic Ci signal in the visual primordium of stage 7 embryos is at the same level as in wild type (Fig. 10E,F), indicating that Dpp is not required for Hh signal to go through. However, it should be conceded that it is very difficult to quantify, in embryonic tissues as opposed to cultured cells, expression levels using the Ci antibodies available, which leaves open the possibility that Dpp might have a quantitative effect of on the strength of the Hh signal reaching the nucleus.

Taken together, our findings suggest that no direct interaction exists between Hh and Dpp signaling, and that the antagonistic effect of Hh and Dpp on the formation of visual structures is most probably based upon an indirect interaction between the two signaling pathways that involves the expression of the eye genes *so* and *eya* (see Discussion).

DISCUSSION

The role of Dpp and Hh in the embryonic eye field

Our results suggest that, similar to its expression in the trunk, Dpp forms a gradient that traverses the anterior brain/eye field from dorsal to ventral. In the trunk, Dpp is restricted by the maternal morphogen Dorsal to the dorsal half of the embryo. Ventrally, the Dorsal morphogen turns on the *Chordin* homolog *sog*, as well as a transcriptional repressor of Dpp-activated genes, *brinker* (*brk*) (Francois et al., 1994; Biehs et al., 1996; Jazwinska et al., 1999; Minami et al., 1999; Raftery and Sutherland, 1999). Highest levels of Dpp at a mid-dorsal level turn on or stabilize target genes such as *zen*, which commit cells to amnioserosa fate (Rusch and Levine, 1997). Moderate Dpp levels activate *pannier* and other targets that specify dorsolateral fates (non-neural epidermis, tracheae). A second BMP homolog, Screw, is required with Dpp for mid-dorsal fates (Arora et al., 1994). The activity of *sog* and *brk* inhibits Dpp and Screw in the ventral ectoderm, thereby allowing the expression of proneural genes and the subsequent neuralization in this domain. Paradoxically, Sog potentiates Dpp function mid-dorsally (Ashe and Levine, 1999).

In the head region, highest levels of Dpp are required to promote mid dorsal fates (head epidermis, analogous to amnioserosa in the trunk). The activation of *screw* is involved

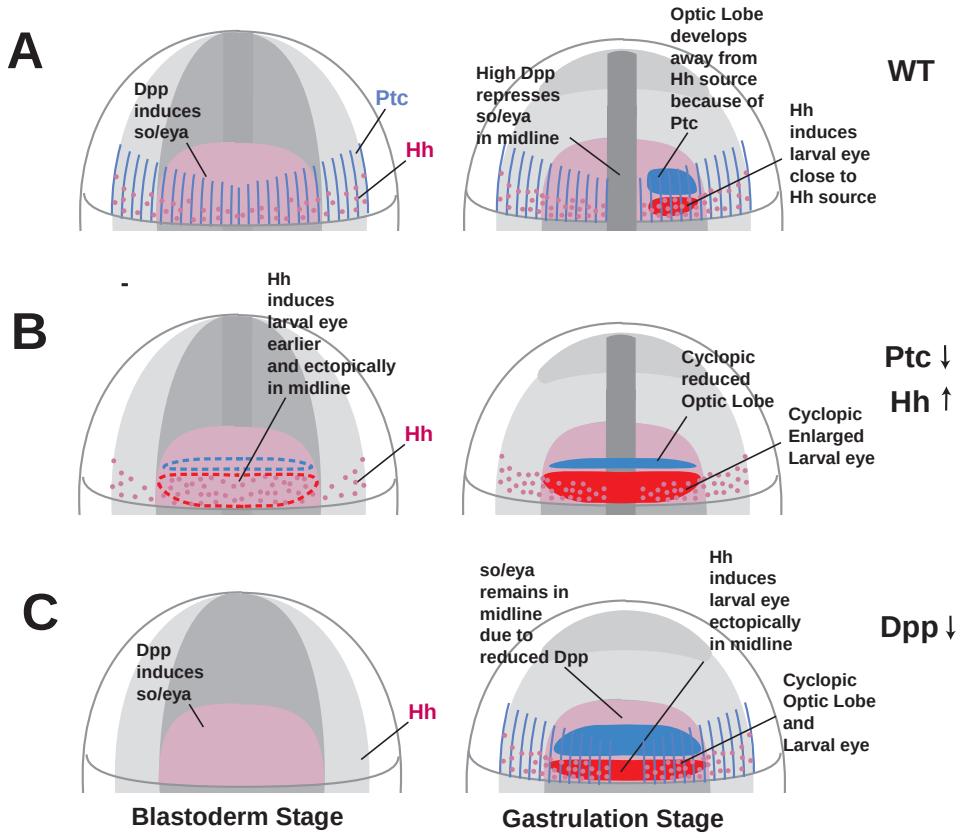

Fig. 11. Model of the function of Dpp and Hh signaling in the *Drosophila* embryonic head, explaining the mechanism that results in the development of a cyclops phenotype in different genetic backgrounds. Each row (A-C) shows dorsal view of head at blastoderm stage (left panel) and after gastrulation (right panel). (A) Wild type; (B) loss of Ptc or overexpression of Hh; and (C) reduction of Dpp. For details, see Discussion.

in this process, similar to its role in the dorsomedial trunk (T. C. and V. H., unpublished). Intermediate Dpp levels promote dorsolateral fates (visual primordium). Low levels of Dpp are reached in the protocerebral neurectoderm and are permissive for the formation of protocerebral neuroblasts. Several of the regulatory genes expressed in the anterior brain and eye field may be direct targets of Dpp signaling. Our findings show that *so*, *eya* and *omb* are activated by Dpp in the visual primordium. These regulatory genes initiate the fate of visual structures, in particular larval eye and outer optic lobe. It has recently been shown that *eya* and *so* are also targets of Dpp signaling in the eye imaginal disc (Curtiss and Mlodzik, 2000).

The secondary restriction of *so* (and other genes with bilateral expression domains developing from unpaired domains, including *tll* and *otd*) is effected by the Dpp target *zen* in the dorsal midline. This homeobox gene is expressed as a response to peak levels of Dpp in the dorsal midline, including amnioserosa and, in the head of the embryo, in the dorsomedial head epidermis primordium (Rusch and Levine, 1997) (this study). We show that loss of *zen*, similar to reduction of Dpp, results in the absence of amnioserosa and head epidermis, and a cyclops phenotype. Scanning electron microscopy of *zen* mutants (Wakimoto et al., 1984) has demonstrated the absence of an optic lobe invagination and has led the authors to believe erroneously that the optic lobe itself is deleted. However, the formation of a cyclopic, mid-dorsal optic lobe also results in the absence of an invagination visible at the surface, which explains why this phenotype was overlooked.

In concurrence with the recent report by Suzuki and Saigo (Suzuki and Saigo, 2000) our data show that Hh is positively required for the visual system. Loss of this gene causes the absence of the larval eye, as well as the adult eye primordium. This phenotype is reminiscent of the later requirement of Hh for the initiation of cell differentiation in the larval eye imaginal disc (Ma et al., 1993). Increased expression of Hh, as well as absence of the inhibitor of Hh function, Ptc, results in a cyclops phenotype. Given that mutants in both Dpp and Hh signaling cause abnormalities in the embryonic visual system, the possibility exists that both pathways interact. However, the findings of this study provide no evidence for this possibility. Expression of *ptc* and *hh* are normal in early *dpp*-null embryo, and the cytoplasmic localization of Ci, an indicator of Hh signaling, was also present in a *dpp* null. Likewise, expression of *dpp* and pMAD was undisturbed in a Hh-null embryo.

In view of these results, we speculate that the interaction between Dpp and Hh is indirect and requires the function of *so*, *eya* and possibly other 'early eye genes': According to this model (Fig. 11), Dpp activates *so* and *eya* in the eye field. Slightly later, expression of *so* and *eya* is lost dorsomedially, owing due to repression by Zen at this level. In a second step, the expression of Hh (which comes on later than Dpp) triggers larval eye fate in cells close to the Hh source. The response of a cell to Hh, that is, its expression of *ato*, depends on its previously expressing so and *eya*. Finally, Ptc inhibits the range of Hh action, similar to its alleged function in the trunk and imaginal discs.

The tenets of our model explain the phenotypes resulting from manipulating Dpp, Hh and Ptc expression:

(1) In wild type, Hh can activate larval eye only in cells expressing *so* and *eya*. No larval eye develops in the dorsal midline because *so* is down regulated in this region rapidly, and Hh 'has no opportunity' to overcome the *ptc* mediated inhibition and induce visual system at an early stage when *so* is still present in the dorsal midline.

(2) In *ptc*$^{-}$, Hh is able to induce larval eye fate in the dorsal midline because it is not inhibited at the early stage when *so* is still expressed dorsomedially.

(3) Heatshock-induced Hh expression at an early stage (stage 5; around 3 hours) has the same effect, overcoming the *ptc*-mediated inhibition and inducing larval eye dorsomedially

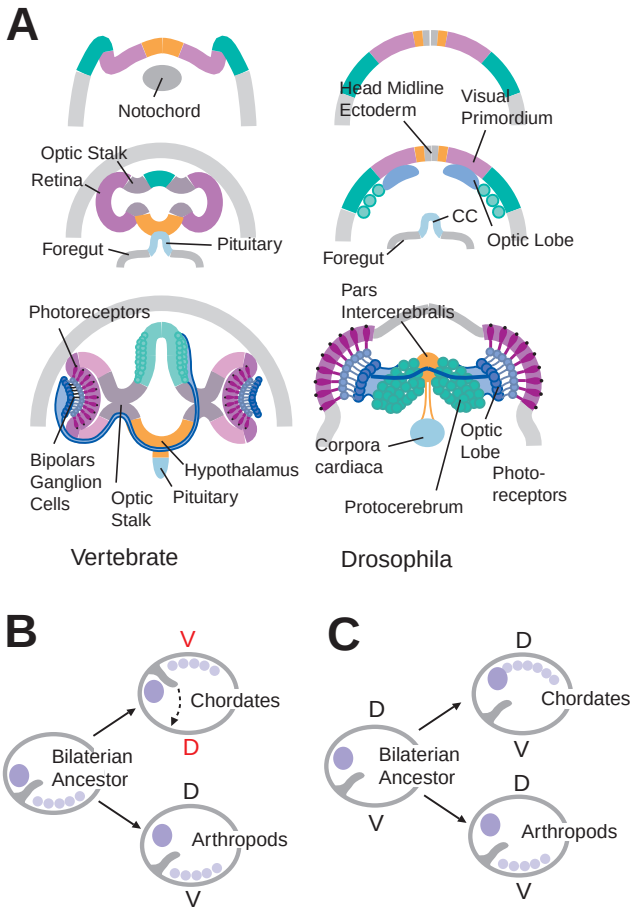

Fig. 12. (A) Comparison of eye morphogenesis in a vertebrate (left column) and insect (right column). All panels represent schematic cross-sections of the head region of the embryo. First row shows anterior brain/eye anlage before neurulation. The second and third row depict the derivatives of this anlage after neurulation and in the mature organism, respectively. For details, see Discussion. (B) Evolution of chordate body plan by dorsoventral axis reversal. According to this hypothesis, bilaterian ancestor had a ventral nervous system. (C) Evolution without axis reversal. This alternative hypothesis suggests that ancestor had only anterior brain. Separate lines of evolution added a trunk nervous system dorsally (chordates) or ventrally (arthropods).

(4) If the level of Dpp is reduced (in *dpp* null heterozygote, or *dpp* hypomorph), *so* and *eya* are stably expressed in the dorsal midline, as *zen*, which normally inhibits the early eye genes, is not expressed. As a result Hh can induce larval eye dorsomedially.

(5) In the cyclops phenotype that results from reduction of Dpp, the visual primordium develops as a double crested placode that spans the dorsal midline. In this placode, the posterior crest is formed by larval eye cells, in line with the tenet that Hh induces larval eye fate in the cells next to the Hh source (posteriorly). The anterior crest, which is further away from the Hh source, constitutes posterior optic lobe (see Fig. 4D).

(6) In the cyclops phenotype induced by loss of Ptc or overexpression of Hh, larval eye cells are increased in number, compared with the Dpp reduction induced cyclops. At the same time, posterior optic lobe cells are reduced in number.

Conserved origins and genes: the topology of the anterior brain anlage and eye field in flies and vertebrates

The topology in which different derivatives of the anterior brain anlage are laid out in the early embryo exhibits considerable similarity. To appreciate this similarity, one needs to remember that the neurectoderm of insects does not invaginate (Fig. 12A). As a result, early embryonic tissues located in the dorsal midline (i.e. the head midline ectoderm) of the fly embryo remain where they are, i.e. mid-dorsally, whereas in vertebrates, they form the ventral midline of the neural tube. This inverse topology may explain in part why dorsomedial structures in *Drosophila* share several functional and molecular similarities with the ventral forebrain in vertebrates. For example, both give rise to neuroendocrine centers (the pars intercerebralis of the insect brain, hypothalamus of vertebrates). In both vertebrates and insects, cells that start out as epithelial placodes in the foregut anlage anteriorly adjacent to the eye field, form neurohemal structures (anterior pituitary in vertebrates, corpora cardiaca in insects) (Copenhaver and Taghert, 1991) (this study) that become innervated by the neuroendocrine neurons derived from the midventral/mid-dorsal brain. The topological similarity between the eye field in *Drosophila* and vertebrates extends to the location of the eye. In both systems, the eye maps close to the midline and genetic manipulations affecting the midline result in the fusion of the eyes (cyclopia) (Chiang et al., 1996; Pera and Kessel, 1997).

The dorsal location of the eye field and protocerebral neurectoderm in *Drosophila*, as well as all extant arthropods, is not easy to reconcile with the hypothesis that the chordate body plan is derived from a arthropod/annelid-like ancestor whose dorsoventral axis is reversed (Arendt and Nuebler-Jung, 1996), although it does not directly contradict this idea. Thus, eye field and protocerebral ectoderm of ancestral arthropods might have actually occupied a ventral position in front of the stomodeum, and subsequently shifted dorsally (Fig. 11B). However, given that no comparative-structural or fossil evidence exists for such a shift, an alternative hypothesis can be offered: the CNS of the ancestor of chordates (deuterostomes) and arthropods/annelids (protostomes) may have been restricted to the head of the animal where also sensory receptors (eyes, statocysts, chemoreceptors) are concentrated (Fig. 11C). In support of this view, nerve cells in many groups of platyhelminths, in particular Acoels (considered as the sister group of bilaterians according to recent molecular-phylogeneitc data), are exclusively derived from the anterior pole of the embryo (N. Ramachandra, R. Gates, P. Ladurer, D. Jacobs and V. H., unpublished; Younossi-Hartenstein et al., 2000; Younossi-Hartenstein and Hartenstein, 2000a; Younossi-Hartenstein and Hartenstein, 2000b). From this primitive anterior ganglion of the bilaterian ancestor, the protocerebrum/eye field of present day bilaterians is directly derived, with no change in dorsoventral axis. In the trunk region, which originally lacked central neurons, a central nervous system was ‘added’ during evolution following different patterns. In the line leading to higher protostomes, ganglia located ventrally were added, whereas a dorsal trunk neurectoderm formed in chordates.

Irrespective of which of the two aforementioned hypotheses regarding topology of the neural fate map will turn out to be

correct, the high degree of conservation of signaling pathways and regulatory genes controlling the patterning of the fate map in *Drosophila* and vertebrates emphasizes how 'close' the body plans manifested during early embryogenesis still are. Dpp/BMP and Hh/Shh signaling are centrally involved in head patterning in both systems, and could have exerted this role already in the bilaterian ancestor. However, it is also true that the impact of Dpp and Hh signaling on midline and eye structures seems very different in chordates and arthropods, which makes the independent recruitment of the two signaling pathways into head patterning in these phyla a distinct possibility. In chordates, loss of Hh results in a cyclops phenotype and holoprosencephaly, as high levels of Hh are required for hypothalamus and optic stalk. Hh positively regulates Pax2, a regulatory gene expressed in and required for the optic stalk (Ekker et al., 1995; MacDonald et al., 1995; Hallonet et al., 1999). In the *Drosophila* embryo, excess function of Hh causes cyclopia. Moreover, Hh has a positive effect on the Pax6 homolog, *eyeless*; *ey* expression requires the presence of the Hh signal.

The effect of BMPs/Dpp on early eye formation maybe more similar than the role of Shh/Hh signaling. In *Drosophila*, both at the early embryonic and larval stage, Dpp promotes eye formation and differentiation (Heberlein et al., 1993) (this study). Vertebrate BMPs are expressed in the dorsal neural tube and are required for dorsal cell fates in the spinal cord, brain and eye (Liem et al., 1995; Furuta et al., 1997; Dudley et al., 1995). In mouse, BMP2, BMP4, BMP5, BMP6, BMP6 and BMP7 are expressed in the dorsal telencephalon, a region that gives rise to the choroid plexus and dorsomedial walls of the cerebral cortex (hippocampus) and diencephalon. At a later stage, BMP7 is also expressed in the retina. Mice homozygous for BMP2 and BMP4 die long before fate changes in the forebrain can be scored (Zhang and Bradley, 1996). BMP7 homozygotes show a late embryonic phenotype that includes degeneration of the retina (Dudley et al., 1995).

When comparing the expression pattern of conserved regulatory genes, such as *otd*, *tll*, *so* and many others (Arendt and Nuebler-Jung, 1996; Hartenstein and Reh, 2001) in anterior brain and eye development of fruit flies and vertebrates, one is also struck by the high number of similarities. These similarities indicate that the bilaterian ancestor might have possessed a head in which photoreceptors, various brain structures and neuroendocrine cells were laid out in a way reminiscent of the one found in present day taxa. This obviously does not imply the existence of complex organs, such as the eye, pituitary or brain structures. What it does imply is that the bilaterian ancestor had an anterior 'neurectoderm' in which clusters of cells with the basic properties of photoreceptors, pigment cells, neuroendocrine cells or central neurons were positioned in a pattern reminiscent of the modern pattern formed by the progenitors of these structures in different animals. During evolution, these cell types diversified further and became shaped by morphogenetic movements into more complex organs. For example, in the chordates (including urochordates and cephalochordates), the anterior neurectoderm invaginated to form a tube that included all cells with the fate of photoreceptors, pigment cells and target neurons. In vertebrates these cells then evaginated as the optic cup, induced lens and other structures from the outer ectoderm and formed an eye. In the evolutionary line leading to arthropods, cells with the fate of photoreceptors and pigment cells were separated at an early developmental stage from cells destined to become optic target neurons. The former remained in the outer ectoderm and became organized into a compound eye, while the latter delaminated along with other neural stem cells to form the brain. The stage is set for comparative studies of eye morphogenesis and gene expression that will elucidate in more detail how a simple visual system changed into the various types of eyes we can observe in extant animal groups.

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