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Interaction between EGFR signaling and DE-cadherin during nervous system morphogenesis

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SUMMARY

Dynamically regulated cell adhesion plays an important role during animal morphogenesis. Here we use the formation of the visual system in *Drosophila* embryos as a model system to investigate the function of the *Drosophila* classic cadherin, DE-cadherin, which is encoded by the *shotgun* (*shg*) gene. The visual system is derived from the optic placode which normally invaginates from the surface ectoderm of the embryo and gives rise to two separate structures, the larval eye (Bolwig's organ) and the optic lobe. The optic placode dissociates and undergoes apoptotic cell death in the absence of DE-cadherin, whereas overexpression of DE-cadherin results in the failure of optic placode cells to invaginate and of Bolwig's organ precursors to separate from the placode. These findings indicate that dynamically regulated levels of DE-cadherin are essential for normal optic placode development. It was shown previously that overexpression of DE-cadherin can disrupt Wingless signaling through titration of Armadillo out of the cytoplasm to the membrane. However, the observed defects are likely the consequence of altered DE-cadherin mediated adhesion rather than a result of compromising Wingless signaling, as overexpression of a DE-cadherin-α-catenin fusion protein, which lacks Armadillo binding sites, causes similar defects as DE-cadherin overexpression. We further studied the genetic interaction between DE-cadherin and the *Drosophila* EGF receptor homolog, EGFR. If EGFR function is eliminated, optic placode defects resemble those following DE-cadherin overexpression, which suggests that loss of EGFR results in an increased adhesion of optic placode cells. An interaction between EGFR and DE-cadherin is further supported by the finding that expression of a constitutively active EGFR enhances the phenotype of a weak *shg* mutation, whereas a mutation in *rhomboid* (*rho*) (an activator of the EGFR ligand Spitz) partially suppresses the *shg* mutant phenotype. Finally, EGFR can be co-immunoprecipitated with anti-DE-cadherin and anti-Armadillo antibodies from embryonic protein extracts. We propose that EGFR signaling plays a role in morphogenesis by modulating cell adhesion.

Key words: EGFR signaling, DE-cadherin, *shotgun*, Morphogenesis, Adhesion, Visual system, *Drosophila melanogaster*

INTRODUCTION

The cadherin superfamily constitutes a diverse class of cell adhesion molecules (Yagi and Takeichi, 2000; Tepass et al., 2000). Expression and experimental studies in vertebrates suggest that cadherins are involved in every step of neural development, from the determination and segregation of neural progenitors, axonal outgrowth and pathfinding, to synapse formation (Redies, 2000). In *Drosophila*, two classic cadherins have been identified, DE-cadherin, encoded by the gene *shotgun* (Oda et al., 1994; Uemura et al., 1996; Tepass et al., 1996), and DN-cadherin (Iwai et al., 1997). DE-cadherin is required for epithelial stability and morphogenesis in the *Drosophila* embryo and plays key roles in cell sorting and cell migration during *Drosophila* oogenesis (Tepass et al., 1996; Godt and Tepass, 1998). As shown for its vertebrate counterparts, DE-cadherin, β-catenin (Armadillo) and α-catenin form a protein complex (the cadherin-catenin complex or CCC) that connects the membrane to the actin cytoskeleton, a linkage that is required for the effectiveness of DE-cadherin mediated adhesion (reviewed by Tepass, 1999). While it is clear that the dynamic regulation of cadherin activity plays a pivotal role during morphogenesis, the mechanisms that modulate cadherin activity are still largely unknown.

In *Drosophila* and vertebrates, there are numerous examples where adhesion among epithelial cells has to be adjusted dynamically to allow for morphogenetic movements, such as ingression, invagination, or cell migration to occur. Here we study DE-cadherin function in the formation of the *Drosophila* optic placodes and their subsequent differentiation. Progenitors of the insect central nervous system, called neuroblasts, typically delaminate from the neurectoderm as individual cells, whereas neighboring cells thaat stay behind in the neurectoderm form epidermal progenitors. Some regions

within the insect neurectoderm, among them the optic placode, develop similarly to the vertebrate neural tube: instead of forming neuroblasts by delamination, these regions form placodes of highly cylindrical cells that invaginate and form internal neuroepithelial vesicles in which cells maintain their apical-basal polarity and junctional complex (Dumstrei et al., 1998). The optic placode splits into two lineages, one that forms the larval eye (Bolwig's organ), and the other that gives rise to the optic lobe. Cells of Bolwig's organ differentiate as sensory neurons that send their axons to the optic lobe (Steller et al., 1987; Green et al., 1993).

Cadherins can be regulated on the level of transcription, which is often seen when transitions between epithelial and mesenchymal cells take place such as neural crest in vertebrates, neuroblast or heart precursors in *Drosophila* (Duband et al., 1995; Tepass et al., 1996; Haag et al., 1999). A more rapid mechanism of controlling cadherin activity is by modifying its coupling to the actin cytoskeleton. Tyrosine phosphorylation of β-catenin may result in a disassembly of the CCC and a consecutive loss in cadherin-mediated adhesion (Aberle et al., 1996; Ozawa and Kemler, 1998; Provost and Rimm, 1999). Several tyrosine kinases and tyrosine phosphatases have been identified that can increase or decrease the degree of phosphorylation of the CCC. In vertebrates, receptor tyrosine kinases, including EGF receptor, were shown to be physically linked to the CCC and to be responsible for CCC phosphorylation (Hoschuetzky et al., 1994; Hazan and Norton, 1998). We speculated that the *Drosophila* EGF receptor homolog, EGFR, may play a similar role in modulating DE-cadherin mediated adhesion during tissue morphogenesis. This aspect of DE-cadherin mediated adhesion had not previously been addressed. It is known that the cytoplasmic pool of the *Drosophila* β-catenin homolog, Armadillo (Arm) is phosphorylated on serine/threonine residues as part of the Wingless pathway (see Peifer et al., 1994); the localization and significance of phosphorylated tyrosine residues in Arm has not been studied.

In this paper we show that finely adjusted DE-cadherin-mediated adhesion is required for normal optic placode morphogenesis. In embryos that lack DE-cadherin, this structure dissociates and undergoes apoptotic cell death. Overexpression of DE-cadherin results in the failure of optic placode cells to invaginate, and of Bolwig's organ precursors to separate from the placode. This phenotype is also observed in embryos that lack the EGFR when the widespread cell death (the most prominent aspect of loss of EGFR) is suppressed, suggesting that compromising EGFR signaling results in an increased adhesion of optic placode cells. This notion is corroborated by the genetic interactions we find between DE-cadherin and EGFR signaling, and by the fact that EGFR forms part of the CCC as shown by co-immunopreciptation from embryonic extracts. These results suggest that EGFR signaling negatively regulates DE-cadherin activity, thereby facilitating the invagination of the optic placodes.

MATERIALS AND METHODS

Fly stocks

Oregon R flies were used as the wild-type stock. The following mutations, which are described by Lindsley and Zimm (Lindsley and Zimm, 1992) if not otherwise indicated, were used in this study: arm^{1} (Bloomington Stock Center); arm^{S14} (Orsulic and Peifer, 1996) (kindly provided by Dr M. Peifer); $Egfr^{f5}$ (flb^{2G31}; kindly provided by Dr U. Banerjee); $Egfr^{f1}$ (flb^{If26}; provided by the Umea stock center); *df (3L) H99*. (kindly provided by Dr J. Lengyel); hs-M*E-cadherin*^{4b} (a gift from A. Wodarz); rho^{M3} (kindly provided by Dr E. Bier); shg^{IH} and $shg^{P34\text{-}1}$ (Tepass et al., 1996). The following driver lines and UAS constructs (Brand and Perrimon, 1993) were used: *da*-Gal4 (kindly provided by Dr J. Campos-Ortega); UAS-Activated *EGFR* (Queenan et al., 1997); UAS-*DE-cadherin*^{5,9} (Sanson et al., 1996). UAS-*DE-cad-α-catenin* (P. Niewiadomska and U. T. unpublished work). Egg collections were done 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 prior to labeling with antibody, following the standard procedure (Ashburner, 1989). Embryos stained with anti-Armadillo antibody were heat fixed (Miller et al., 1989). This method greatly emphasizes the signal at the adherens junction. Anti β-galoctosidase antibody (Sigma) was used at 1:1000. A monoclonal antibody against Armadillo, which labels the zonula adherens junction (Peifer, 1993) (provided by the Developmental Studies Hybridoma Bank), was used at 1:100. Anti-phosphohistone H3 antibody, which labels dividing cells, (available from Upstate Biotechnology) was used at 1:500. A monoclonal antibody against Crumbs protein that labels apical membranes of ectodermal tissues (Tepass et al., 1990) (kindly provided by Dr E. Knust) was used at 1:20. Antibody against Coracle, which marks the septate junctions was used at 1:250 (Fehon et al., 1994). Antibody against EGFR (Zak et al., 1990) (kindly provided by Dr B. Shilo) was used at 1:200. A commercial monoclonal antibody against activated MAPK (dp-ERK) (available through Sigma), used at 1:200, was used to visualize the embryo domains in which the Ras signaling pathway is activated (Gabay et al., 1997a; Gabay et al., 1997b). A monoclonal antibody that recognizes the Fasciclin ll protein (Fasll) (Grenningloh et al., 1991) (kindly provided by Dr C. Goodman) which labels subsets of neuronal precursors, among them part of the optic lobe, larval eye and dorsomedial brain was used at 1:100. Fasciclin lll antibody (Faslll) (Patel et al., 1987) (provided by the Developmental Studies Hybridoma Bank) labels the basolateral surface of ectodermal tissue was used at 1:100. The monoclonal antibody mAb22C10 (Zirpursky et al., 1984) (provided by the Developmental Studies Hybridoma Bank) which labels all neurons was used at 1:200. Anti DE-cadherin antibody was used at 1:20 (kindly provided by Dr T. Uemura) (Uemura et al., 1996). Confocal images were taken on a Biorad MRC 1024ES miroscope using Biorad Lasersharp version 3.2 software.

In situ hybridization

Digoxigenin-labeled DNA probe was prepared following manufacturer's instructions (Genius kit; Boehringer) using a full-length cDNA clone of the *rpr* gene (White et al., 1994) (kindly provided by Dr K. White). Embryos were dechorionated and fixed in PBS containing 5% formaldehyde and 50 mM EGTA and stored in ethanol. In situ hybridizations to whole-mount embryos were carried out according to the protocol of Tautz and Pfeifle (Tautz and Pfeifle, 1989). Embryos were dehydrated in ethanol and embedded in epon.

Temperature shift experiments

$Egfr^{f1}$ embryos were collected for 2 hours at 25°C, and shifted to 31°C at 3, 4, 5 and 6 hours post-fertilization. Embryos remained at 31°C for 2 hours and were then allowed to develop at 22°C until stage 16 of embryogenesis, at which time they were fixed for subsequent staining. Completion of embryogenesis takes 42 hours at 18°C, 16

hours at 29°C, and 22 hours at 25°C. To compensate for timing differentials, ratios of these hours were used.

Scanning electron microsocopy (SEM)

Wild-type and *Egfr*^{f5} embryos were fixed and devitellinized using the same protocol as for immunohistochemistry. They were then dehydrated in ethanol and washed twice for 10 minutes in Hexamethyldisilazane (available from Ted Pella Inc.). SEM images were made on a Hitachi model # S-2460N at 15 kV. To identify *Egfr*^{f5} homozygous mutants a second chromosome balancer containing a P element with a *ftz*-lacZ promoter fusion construct was used. The *Egfr*^{f5} line was stained with anti β-galoctosidase antibody and homozygous mutant embryos were selected and processed as stated above.

Immunoprecipitation and immunoblotting

0- to 15-hour old embryos were collected and dechorianted with bleach for 3 minutes. 0.1 ml of embryo were homogenized with 0.2 ml of lysis buffer (50 mM Tris-HCl, pH 7.5, 150 mM NaCl, 1% NP40, 0.5% sodium deoxycholate, 1 mM DTT, 1 mM PMSF). Lysate was centrifuged at 13,000 ***g*** at 4°C for 10 minutes. The supernatant was pre-absorbed with 50 μl protein A-agarose beads (Amersham Corp) at 4°C for 30 minutes with gentle agitation to eliminate non-specific binding of the proteins to the beads. Protein A-agarose beads were separated from the lysate by centrifugation for 1 minute at 13,000 ***g*** at 4°C. Anti-DE-cadherin (1:100) or anti-Armadillo/β-catenin (1:50) or anti-EGFR (1:50) was incubated with the lysate for 1 hour at 4°C with gentle agitation. 25 μl of protein A-agarose beads were subsequently added to each lysate/antibody mixture and incubated over night at 4°C with gentle agitation. The immune complex was pelleted by centrifugation at 3,000 ***g*** at 4°C for 1 minute. The complex was washed 2 times with low stringency (50 mM Tris-HCl, pH 7.5, 500 mM NaCl, 0.1% NP40) and high stringency (50 mM Tris-HCl, pH 7.5, 0.1% NP40) buffers. The proteins were eluted by boiling the beads with 20 μl SDS-PAGE buffer for 10 minutes.

Eluted proteins were separated on 8% SDS-PAGE gel and electroblotted to PDF membrane (Bio-Rad). The blots were blocked with PBS containing 5% non fat milk and 0.3% Tween 20 (Sigma). The membranes were incubated with anti-DE-cadherin, anti-Armadillo, anti-EGFR and anti-BP106 (Patel et. al., 1987) (provided by the Developmental Studies Hybridoma Bank) antibodies at 1:1000, 1:500 1:200 and 1:300 dilution followed by peroxidase-conjugated antibody at 1:2000 dilution. After washing with buffer containing PBS and Tween 20, protein bands were visualized with ECL detection kit (Amersham Corp). Immunoprecipitation experiments were independently performed three times to confirm the results.

RESULTS

Distribution of DE-cadherin and EGFR during visual system morphogenesis

The head ectoderm of early *Drosophila* embryos is subdivided into several domains that realize different morphogenetic programs (Fig. 1). The embryonic eye field (Chang et al., 2001) is the posterior-medial region of the procephalic neurectoderm that gives rise to the visual system, which includes the larval eye (Bolwig's organ) and adult eye, as well as the optic lobe (Fig. 1E). Around gastrulation, cells of the eye field undergo a convergent extension directed laterally (Fig. 1A,B). Shortly afterwards these cells form two morphologically visible placodes, one on either side of the embryo. (Fig. 1C). These optic placodes sink inside and become the optic lobe primordia, epithelial double layers attached to the posterior surface of the brain (Fig. 1D,F) (Green et al., 1993). The optic placode of a stage 12-13 embryo is V-shaped, with the anterior

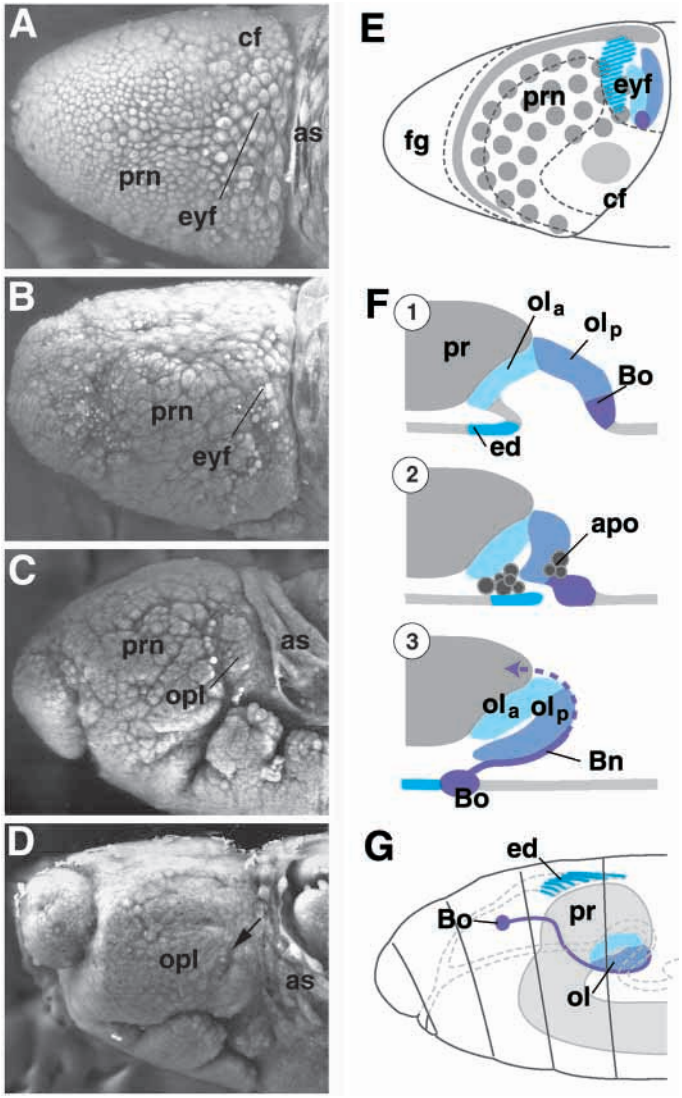

Fig. 1. Morphogenesis of the *Drosophila* embryonic visual system. (A-D) SEM photos of embryonic head at stage 7 (gastrulation; A), stage 9 (early extended germ band; B); stage 11 (late extended germ band; C) and stage 12 (germ band retraction; D). In all SEM anterior is to the left. Orientation in A is dorsal, whereas B,C and D are lateral views with dorsal up. The migrating eye field (eyf in A,B) and optic placode (opl in C,D) are distinguishable by surface morphology. (E) Fate map of the eye field. Schematic representation of stage 7 embryonic head, lateral view. Approximate position of anlagen are indicated (Bo, larval eye; ed, adult eye disc; ol_a, anterior lip of optic lobe, which gives rise to inner optic anlage; ol_p posterior lip of optic lobe, which gives rise to outer optic anlage). (F) Morphogenesis of the optic placode. Schematic representation of the invaginating optic placode at three successive timepoints. (G) Visual system of late embryo. Other abbreviations: as, amnioserosa; Bn, Bolwig's nerve; cf, cephalic furrow; fg, foregut; prn, protocerebral neurectoderm.

leg of the V representing the anterior lip, which later forms the inner anlage of the optic lobe, and the posterior leg forming the posterior lip, later forming the outer anlage. As the invagination deepens and the two lips 'sink' inside the embryo, ectodermal cells that earlier surrounded the perimeter of the optic placode approach each other and eventually form a closed

epidermal cover. Abundant cell death accompanies the closing of the head epidermis over the optic lobe anlage, and the subsequent separation of this anlage from the epidermis (Nassif et al., 1998) (Fig. 1F). A small number of cells that originally formed part of the posterior lip of the optic placode remain integrated in the head epidermis and form the larval eye or Bolwig's organ. As these cells move away from the optic lobe anlage their basal ends become drawn out and form axons that constitute the Bolwig's nerve (Fig. 1G).

DE-cadherin is expressed throughout the ectoderm including the eye field and its epithelial derivatives (Fig. 2A). One would expect that normal optic lobe development requires modulation of DE-cadherin activity to allow, for example, the segregation of the invaginating optic placodes from the surrounding ectoderm. As cell culture studies have indicated that the mammalian EGF receptor can disrupt cadherin-based adhesion (Hazan and Norton, 1998), we were curious to see whether EGFR is expressed in the visual system to allow for such a possibility in *Drosophila* as well. EGFR is expressed in a complex and dynamic pattern that closely parallels the pattern of double-phosphorylated ERK (dpERK) expression, indicating activation of the MAP kinase signaling pathway (Gabay et al., 1997a; Gabay et al., 1997b). During stage 7 both *rho* (an activator of EGFR signaling) and dpERK are expressed in two stripes in the head ectoderm (Fig. 2C,D). The expression of dpERK in these two stripes is the result of EGFR activity (Gabay et al., 1997a; Gabay et al., 1997b; Dumstrei et al., 1998). The anterior stripe corresponds to part of the head midline, while the posterior stripe reaches into the eye field as demonstrated by double labeling the preparation in Fig. 2D with GFP expressed in a *sine oculis* pattern (Chang et al., 2001). *Sine oculis* is the earliest known marker for the eye field. Distribution of dpERK in the two stripes becomes patchy during stage 10. At the same time, the posterior stripe widens dorsally to overlap with part of the optic lobe placode (Fig. 2E). Finally, at the late extended germ band stage and during germ band retraction (stages 11 and 12), dpERK becomes restricted to the optic lobe placodes and cells of the dorsal head midline (Fig. 2F). This expression pattern demonstrates that EGFR activation accompanies the determination, morphogenesis and differentiation of the embryonic visual system.

On the subcellular level, EGFR is expressed diffusely on the membrane of epithelial cells and neuroblasts (Fig. 2B red). EGFR overlaps with Armadillo, the *Drosophila* β-catenin homolog, which is an integral component of the cadherin-catenin complex (Fig. 2B yellow). Like DE-cadherin, Armadillo is concentrated strongly in the apically located zonula adherens (2B green, arrows) but is also found at lower levels in the entire lateral membranes.

A second type of junction, called a septate junction, develops in *Drosophila* epithelial cells at a slightly later stage than the zonula adherens (Tepass and Hartenstein, 1994). Septate junctions have been implicated in maintaining epithelial stability (Woods et al., 1997). The Coracle protein forms part

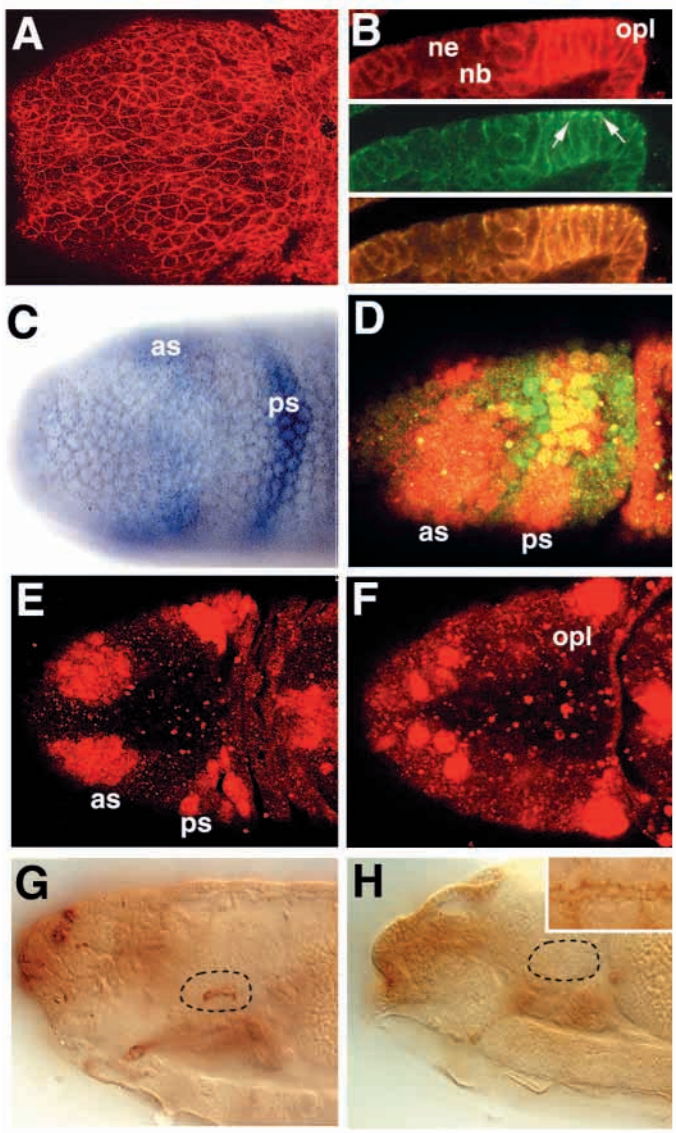

Fig. 2. Pattern of expression of DE-cadherin and EGFR in the embryonic head. (A) Anti-DE-cadherin labeling of stage 11 embryo, dorsal view. Confocal section of head epidermis close to apical surface, showing concentration of DE-cadherin in zonula adherens of epithelial cells. (B) Anti-EGFR (red) and anti-Armadillo (green) labeling of stage 11 embryo, dorsal view. Note diffuse expression of EGFR in membrane of epithelial cells and neuroblasts, and apical concentration of Armadillo (arrows). Merged EGFR and Armadillo staining is shown in yellow. (C) In situ probe showing expression of *rho*, an activator of the EGFR signal, in two stripes (as, anterior stripe; ps, posterior stripe) in stage 7 embryo, dorsal view. (D-F) Pattern of EGFR activity, visualized by antibody against phosphorylated MAPK (dpERK; red). At stage 7 (D; dorsal view), EGFR is active in two stripes, corresponding to the anterior (as) and posterior stripe (ps) of *rho* expression (see C). The preparation was double labeled by GFP expressed in the *sine oculis* pattern by an *so*-Gal4 driver (green). *Sine oculis* is the earliest marker for the eye field (ef). Note partial overlap between eye field and posterior stripe of ERK activity (yellow). During later stages (E, stage 9; F, stage 11) posterior stripe of ERK activity stretches laterally and stays in spatial overlap with the optic lobe placode (opl). (G) Anti-Crumbs staining of stage-15 wild-type embryo, lateral view. Anti-Crumbs labels the apical membrane of the optic lobe (indicated by dashed outline). (H) Anti-Coracle labeling of wild-type embryo, same stage and view as in G. Anti-Coracle is a marker for the septate junctions. In the optic lobe (outlined by dashed lines) septate junctions are absent. Insert in H shows anti-Coracle-labeled septate junctions in the dorsal tracheae.

of the septate junctional complex (Fehon et al., 1994), and an antibody against Coracle serves as a sensitive marker for this junction. Applying this marker to embryos of different stages we found that all ectodermally derived epithelia express Coracle, except for the optic lobe and the invaginations that form the stomatogastric nervous system (Fig. 2H). Accordingly, no septate junctions have been reported in previous electron microscopic investigations of these tissues (Green et al., 1993; Tepass and Hartenstein, 1994). The reliance on adherens junctions alone may make the optic lobe (and stomatogastric nervous system, not considered here) more susceptible to changes in the stability of these junctions, as those described below resulting from manipulations of DE-cadherin and EGFR.

Loss and gain of DE-cadherin function alter visual system morphogenesis

Both loss and overexpression of DE-cadherin affect the maintenance and morphogenesis of the visual system. Using antibody markers against neural and epidermal cells, we find a loss of epithelial integrity and an increase in cell death in the head epidermis, resulting in a partial or full exposure of the brain in late *shg* mutant embryos (Fig. 3B,D). Similar defects are seen in the optic placodes and the invagination, which in wild-type moves this placode inside the embryo, fails to take place, leaving cells at the outer surface. The defects in visual system morphogenesis in *shg* mutant embryos are probably due to the loss of epithelial tissue structure and increased apoptosis.

Overexpression of DE-cadherin was facilitated by the Gal4/UAS system using the strong ubiquitous driver *daughterless* (*da*)-Gal4 (Brand and Perrimon, 1993). All cells of the optic lobe are highly columnar in *da*-Gal4;UAS-*DE-cadherin*^{5,9} embryos. The optic lobe placodes invaginate incompletely or not at all (Fig. 3E,F). The most characteristic defect is the 'non-disjunction' of Bolwig's organ and optic lobe. Cells of Bolwig's organ stay in contact with the optic lobe and remain epithelial, instead of developing dendrites and axons. Despite their epithelial structure, these cells express neural antigens such as 22C10 (data not shown). We ascribe the abnormal shape, lack of invagination and non-disjunction of the optic placodes to the inability of cells to dynamically down regulate the strength of the CCC to allow for normal morphogenesis.

Armadillo acts in cell adhesion and not Wg signaling in the embryonic development of the visual system

Armadillo (Arm), the *Drosophila* homolog of β-catenin, is an effector of Wingless (Wg) signaling and a core component of the CCC (reviewed by Tepass, 1999; Peifer and Polakis, 2000). A decrease or increase in the levels of DE-cadherin are capable of modulating Wg signaling through altering the levels of cytoplasmic Armadillo (Orsulic and Peifer, 1996; Sanson et al., 1996). Moreover, embryos that lack Wg display severe defects in head morphogenesis that also affect the visual system (data not shown). Thus, loss or gain of DE-cadherin could result in visual system defects not only by changing cell adhesion but also by changing Wg signaling activity. To differentiate between these two effects we analyzed embryos that lack Arm function in cell adhesion but not Wg signaling.

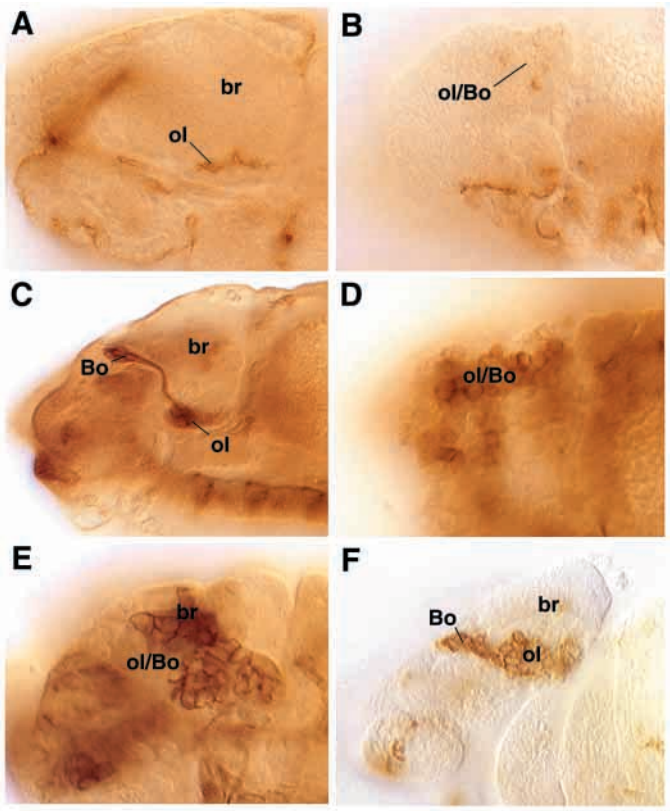

Fig. 3. Defects in the optic placode following DE-cadherin loss of function and overexpression. (A,B) Anti-Crumbs, labeling apical membrane of ectodermal cells. Stage 15, lateral view of wild-type embryo (A) and *shg*^{IH} mutant (B). Note invaginated optic lobe (ol) attached to basal brain surface (br) in wild-type. In the mutant, the optic placode does not invaginate and remains at the surface. Placode cells lose contact and dissociate, as evidenced by reduced and patchy Crumbs expression. (C,D) Anti-FasII, labeling Bolwig's organ (Bo) and posterior lip of optic lobe (ol). Stage 15, lateral view of wild-type (C) and *shg*^{IH} mutant (D). In wild type, Bolwig's organ has separated from optic lobe and differentiated as photoreceptor neurons with axons staying attached to optic lobe. In *shg* mutant, cells of optic lobe and Bolwig's organ still express the marker FasII but do not differentiate structurally. (E,F) Anti-FasII, stage 15, lateral view of embryo in which (E) heatshock mouse E-cadherin (hs-M*E-cadherin*^{4b}) and (F) *Drosophila* full length DE-cadherin (UAS-*DE-cadherin*^{5,9}) construct is expressed by *da*-Gal4. Cells of optic placode maintain their epithelial shape but do not invaginate. Bolwig's organ does not separate from optic lobe.

Embryos that do not express *arm* zygotically display a strong Wg loss-of-function phenotype, rather than dramatic defects in epithelial integrity that is typical for *shg* mutants. This led to the suggestion that maternally provided Arm is sufficient for the adhesion function of this molecule (Cox et al., 1996). We investigated the *arm* mutant defects in the embryonic visual system more closely in *arm* mutant embryos that express the *arm*^{s14} construct, which is active in Wg signaling but not adhesion (Orsulic and Peifer, 1996). Such embryos show no *wg* phenotype in the cuticle. However, they exhibited defects in the ventral epidermis and the head that resembled a weak to moderate *shg* mutant phenotype. In particular, there were foci of cell death in and around the optic placodes. This structure initially formed, but lost its epithelial

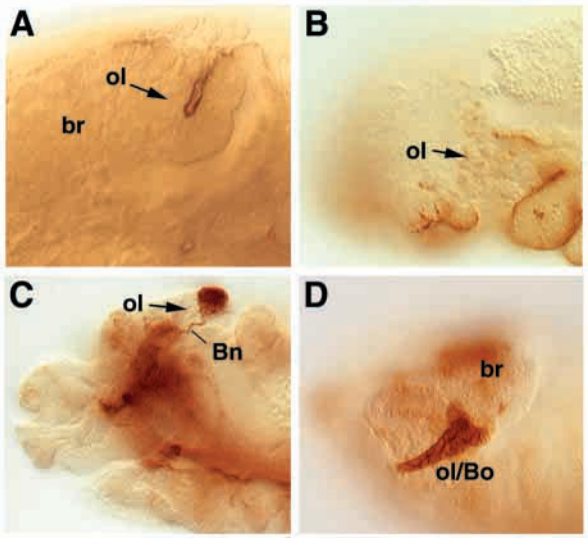

Fig. 4. Role of β-catenin in optic placode morphogenesis. (A) Invaginating optic placode of stage-13 wild-type embryo, labeled with anti-Crumbs. (B,C) Rudimentary optic placode of *arm*^{1}; *arm*^{s14} embryo (B) stage 13 labeled with anti-Crumbs and (C) stage 16 using anti-Fasll to label optic lobe (ol) and Bolwig's nerve (Bn). The *arm*^{s14} is a deletion construct of β-catenin, which lack the α-catenin binding domain. *Arm*^{s14} is able to carry out Wg signaling function but not DE-cadherin-related function. Optic placode cells have dissociated into small, disjunct vesicles that fail to invaginate. (D) Anti-Fasll, stage 16, showing lack of separation of optic lobe and Bolwig's organ in embryos expressing the fusion protein UAS-DE-cad-α-catenin under the control of *da*-Gal4. Other abbreviations: bo, Bolwig's organ; br, brain.

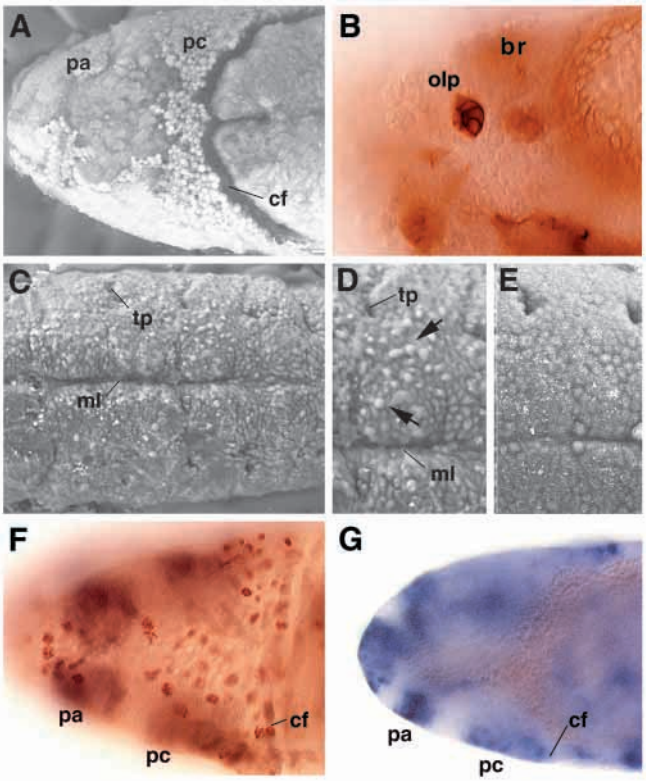

Fig. 5. Cell death in the neurectoderm of EGFR loss-of-function embryos. (A) SEM of head of stage 11 *Egfr*^{f5} mutant, dorsal view, anterior to the left. Note domains of cell death in anterior head (pa) and posterior head (pc). The posterior domain covers the optic placode. (B) In stage-14 *Egfr*^{f5} mutant the brain (br) is exposed and only a small rudiment of optic placode is left (olp; labeled with anti-FasII; compare to Fig. 3C). (C,D) SEM of stage-11 embryos, ventral view, showing cell death in ventral neurectoderm of *Egfr*^{f5} mutant embryos. Cell death, indicated by light, round cell fragments (arrows in D), occurs scattered throughout ventral neurectoderm of stage 10/11 embryo. (E) Wild-type neurectoderm for comparison. (F,G) Comparison of EGFR activation with 'pre-apoptotic domains' in dorsal head of stage 10 embryo. (F) Domains of EGFR activation are visualized by anti-dpERK antibody (black staining), embryo is also labeled with Anti-phosphohistone H3 (brown staining). (G) Pre-apoptotic domains are labeled by probe against *reaper*. Note presence of anterior and posterior band of labeling (pa, pc). Other abbreviations: cf, cephalic furrow; ml, midline; tp, tracheal pit.

structure and failed to invaginate in late embryos (Fig. 4B,C). Furthermore, we overexpressed a fusion protein in which the cytoplasmic tail of DE-cadherin was replaced by a truncated α-catenin protein (DE-cad-α-catenin) lacking the N-terminal domain. As this construct lacks Arm binding sites it does not titer out cytoplasmic Arm to block Wg signaling. *da*-Gal4*;* UAS-*DE-cad-α-catenin* embryos display similar visual system defects as *da*-Gal4*;* UAS-*DE-cadherin*^{5,9} embryos (Fig. 4D). Expression of DE-cad-α-catenin in a *shg* null background partially rescues the *shg* loss-of-function phenotype to the same extent that a full lenght DE-cadherin construct rescues the *shg* loss-of-function phenotype (data not shown). This shows that the fusion construct is able to interact normally with the actin cytoskeleton to support cell adhesion.These results indicate that the visual system phenotypes in *shg* and *arm* mutant embryos and those resulting from DE-cadherin overexpression are caused by preventing dynamic changes in cell adhesion needed during visual system morphogenesis.

EGFR functions in the procephalic neurectoderm

EGFR functions first and foremost in epithelial cell maintenance. In embryos carrying an *Egfr* null allele, *Egfr*^{f5}, cells that normally contain the EGFR and dpERK undergo apoptosis (Dumstrei et al., 1998). Cell death becomes first apparent during late stage 10 at the anterior and posterior part of the procephalic neurectoderm, in the domains overlapping with the anterior and posterior stripe of EGFR/dpERK (Fig. 5A). Apoptosis will eventually remove a major portion of cells of the head epidermis, resulting in an exposed brain (Fig. 5B). The cells of the optic placodes die later. At stage 13, a large part of an abnormally shaped posterior lip is still present, but by late stages it has almost disappeared (Fig. 5B). The delayed apoptosis of optic placode versus head epidermis matches the distribution pattern of dpERK (see above). Loss of *Egfr* also results in cell death in the ventral neurectoderm of the trunk (Fig. 5C,D). From late stage 10, round cell fragments can be observed throughout the ventromedial neuroectoderm.

The analysis of the expression of the cell death gene *reaper* (*rpr*) (White et al., 1994) in wild-type and *Egfr* mutant embryos revealed the interesting observation that the spatial extent of *rpr* expression is largely normal in *Egfr* mutants (data not shown). In a previous study we had shown that *rpr* appears in large 'pre-apoptotic domains' from within which only a

certain number of cells die during normal development (Nassif et al., 1998). Some of the pre-apoptotic domains overlap spatially and temporally with the domains containing dpERK (Fig. 5F,G). This pattern supports the idea that *rpr* expression indicates the potential to undergo apoptosis in all cells of the pre-apoptotic domains, but that simultaneous activation of the EGFR pathway can rescue the majority of pre-apoptotic cells. In an *Egfr* mutant, all pre-apoptotic cells die, but the extent of pre-apoptotic domains themselves is unchanged.

The early occurring cell death in the neurectoderm prevents other aspects of EGFR function being evaluated. To investigate the *Egfr* phenotype in the absence of cell death we studied *Egfr* mutant embryos that also were homozygous for *Df(3L)H99*, which uncovers several genes required for cell death (White et al., 1994). In double mutants, all cells of the head epidermis and optic lobe placode are preserved and express a normal array of markers. These markers include the regulatory genes *tailless* (*tll*) and *orthodenticle* (*otd*/*oc, ocelliless*), both widely expressed in the procephalic neurectoderm (Hirth et al., 1995; Younossi-Hartenstein et al., 1997), as well as antibodies for structural proteins such as Crumbs (apical cell membranes) (Tepass et al., 1990), FasIII (basolateral cell membranes) (Patel et al., 1987), 22C10 (Zipursky et al., 1984), and Armadillo (zonula adherens) (Peifer, 1993). The most conspicuous aspect of the *Egfr;Df(3L)H99* phenotype is the optic lobe defect (Fig. 6A,B). Invagination of the placode does not take place, leaving it exposed at the surface of the embryo. Furthermore, the cells that would normally separate from the placode and become larval photoreceptors, the Bolwig's organ, remain part of the placode. Although they display a typical epithelial phenotype and are structurally indistinguishable from the surrounding optic lobe cells, these cells express specific neuronal markers such as 22C10 (Fig. 6C). In some cases, outgrowth of short, axon-like processes can be observed. The optic lobe placode defects resulting from loss of EGFR function resembles in every aspect the defects caused by overexpression of DE-cadherin.

We also used the temperature sensitive allele, $Egfr^{f1}$, to eliminate EGFR function at discrete time intervals, and thus circumvent the early role of EGFR in epithelial cell maintenance. Analysis of $Egfr^{f1}$ mutant embryos after 2-hour long heat pulses (31°C) delivered at defined developmental time points supports the role of EGFR in cell maintenance, adhesion and differentiation. Heat pulses between 3 and 5 hours result in a reduced size of brain and head epidermis, and an increase in apoptotic cell death (Fig. 6D). No significant effect on morphogenesis and differentiation of the visual system was prevalent. Heat pulses from 5-7 hours had less severe brain defects, but caused the Bolwig's organ-optic lobe non-disjunction phenotype in a large number of cases (Fig. 6E). The number of cells of optic lobe and Bolwig's organ was not altered. Late heat pulse (7-10 hours) caused defects in the size of the visual system, in particular of Bolwig's organ (Fig. 6F), confirming a late differentiative/maintenance role of EGFR in this structure.

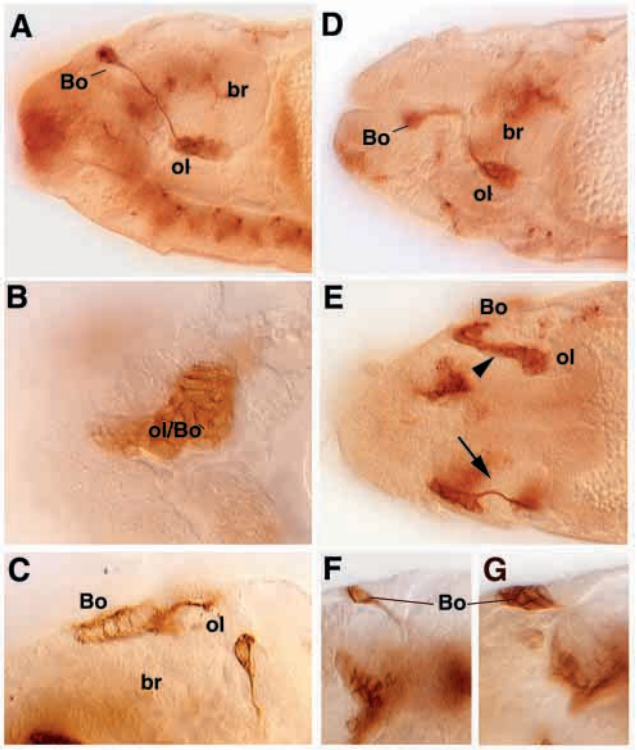

Fig. 6. Hyperadhesive phenotype in optic placode following reduction of EGFR function. (A) Anti-FasII labeling of stage-15 wild-type embryo, lateral view. Optic placode has invaginated and split into Bolwig's organ (Bo) and optic lobe (ol), the latter attached to the brain (br). (B) In embryo lacking EGFR ($Egfr^{f5}$) and carrying deficiency H99, which inhibits cell death, optic placode fails to invaginate and Bolwig's organ remains attached to optic lobe ('non-disjunction'). (C) Labeling of Bolwig's organ with mab22C10 demonstrates that photoreceptor neurons remain arranged in a layered array, rather than transforming into spindle shaped cells as in wild type (compare with A). (D-F) Defects in visual system following application of 2-hour heat pulses (3°C) to EGFR temperature sensitive allele ($Egfr^{f1}$). All panels show heads of stage-15 embryos, labeled with anti-FasII antibody. Heat pulses between 3 and 6 hours (D) cause defects in head development, but leave the visual system intact. (E) Heat pulses around the time of optic placode invagination and disjunction (6-8 hours) result in non-disjunction phenotype of Bolwig's organ and optic lobe (arrowhead), although usually milder than phenotype observed in *Egfr* null. Frequently phenotype is asymmetric: in the embryo shown in dorsal view, non-disjunction is prominent on right side, but absent from left side (arrow). (F) Heat pulses from 8-10 hours do not affect optic placode morphogenesis, but causes reduction in Bolwig's neurons. Note small size of Bolwig's organ, compared with wild type shown in G.

Genetic interactions between components of the EGFR signaling pathway and DE-cadherin

The similar character of the EGFR loss-of-function and the DE-cadherin gain-of-function phenotypes suggest that EGFR signaling may negatively regulate DE-cadherin activity. To test this hypothesis, we looked for genetic interactions between EGFR signaling and DE-cadherin. As all available *shg* mutant alleles have severe head defects, that include loss of optic lobes, we utilized another phenotype of the *shg* mutant embryos namely the cuticle defect that results from the loss of epithelial integrity of epidermal cells, as a genetic assay (Tepass et al., 1996; Uemura et al., 1996). Embryos mutant for the hypomorphic allele $shg^{P34\text{-}1}$, typically have minor defects in their ventral cuticle (Fig. 7B). A small fraction of these embryos have a more severe phenotype resembling that caused by a *shg* null allele, where most of the ventral cuticle is missing.

To address the question whether ectopic activation of EGFR can enhance the $shg^{P34\text{-}1}$ cuticle phenotype we studied $shg^{P34\text{-}1}$ homozygous embryos in which a constitutive active form of EGFR was expressed with *da*-Gal4. Among these embryos, 27% (n=117) lacked most of the ventral cuticle compared to sibling $shg^{P34\text{-}1}$ control embryos, in which 12% (n=117) had this severe phenotype (Fig. 7C,E). Moreover, to test if a reduction of EGFR signaling in a $shg^{P34\text{-}1}$ mutant background results in a rescue of the $shg^{P34\text{-}1}$ phenotype, we utilized a *rho* null allele, rho^{M3}. Among $shg^{P34\text{-}1}$; rho^{M3} homozygous double mutants, we detected no embryos with the severe cuticle phenotype (n=83), whereas 8% (n=83) of sibling $shg^{P34\text{-}1}$ control embryos had no or little ventral cuticle (Fig. 7 D,E) left. A χ^2 test showed that the observed differences in the number of embryos with a severe cuticle phenotype are highly significant (see Fig. 7 legend). These results indicate that changes in the level of EGFR signaling modulate DE-cadherin mediated adhesion, and are consistent with the assumption that EGFR activity negatively regulates adhesion. However, the relatively minor change in phenotypic strength observed in the double mutants suggests that EGFR is only one out of several factors that modulate the activity of the CCC.

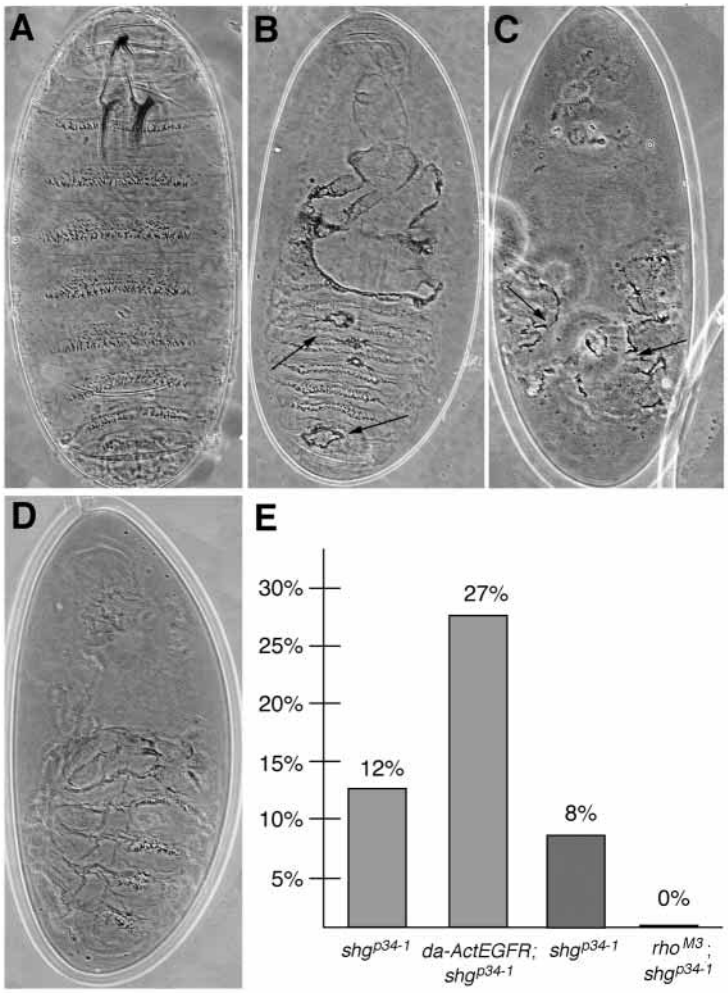

Fig. 7. Genetic interaction between components of EGFR signaling pathway and DE-cadherin. (A-D) Cuticle preparations from wild-type and experimental embryos, all ventral views. (A) Wild-type embryo. (B) $Shg^{P34\text{-}1}$ homozygous embryos, head cuticle is missing but ventral trunk cuticle has relatively minor defects such as small holes in the cuticle (arrows). 12% of these cuticles have a more severe phenotype in which the entire ventral cuticle is missing, n=117. C: *Da*-GAL4; UAS-Activated *EGFR;* $shg^{P34\text{–}1}/shg^{P34\text{–}1}$, 27% of these embryos show lack of ventral cuticle, P=0.001, n=117, (arrows). (D) $shg^{P34\text{–}1}/shg^{P34\text{–}1}$;$rho^{M3}/rho^{M3}$. Among these double mutants, no embryos were observed that lacked the entire ventral cuticle. 8% of $shg^{P34\text{–}1}/shg^{P34\text{–}1}$ sibling embryos from this cross, lacked ventral cuticle, P=0.01, n=83. (E) Histogram of data.

EGFR forms part of the CCC in *Drosophila* embryo

In vertebrates, there is direct evidence that EGFR binds to the CCC. Upon EGF stimulation, activated EGFR phosphorylates β-catenin resulting in dissociation of Armadillo from E-cadherin, thus weakening cell adhesion (Hazan and Norton, 1998).

Results reported above show that EGFR and DE-cadherin are expressed in an overlapping pattern and interact genetically in *Drosophila* embryo. To establish a direct link between EGFR and the CCC, proteins from 0- to 15-hour old embryos were extracted, and immunoprecipitation (IP) using antibodies against DE-cadherin, Armadillo and EGFR were preformed. The same antibodies were used to probe western blots, which contained the IP products. The IP results indicate that EGFR forms a complex with the CCC, consistent with our genetic data. EGFR is detected in both anti-DE-cadherin and anti-Armadillo IP products (Fig. 8A,B). Inversely, IP using anti-EGFR antibody confirms the interaction between EGFR and CCC (Fig. 8C). To verify the specificity of the EGFR-CCC interaction, the membrane protein neurotactin (Patel et al., 1987) was used on western blots containing anti-DE-cadherin and anti-Armadillo IP products. As indicated in Fig. 8D, neurotactin does not associate with the CCC. We obtained the same result with numerous other membrane and secreted proteins, including the Wingless protein (not shown).

DISCUSSION

The cellular changes constituting morphogenesis are executed by structural molecules involved with adhesion and cytoskeletal structure. The actin based cytoskeleton, rho/racGTPases, as well as the cadherin-catenin complex have been implicated in epithelial folding and convergent-extension (Sullivan and Theurkauf, 1995; Lu and Settleman, 1998; Tepass, 1999), although a detailed model placing functional interconnections between the different molecules has not yet materialized. The present paper demonstrates that a finely adjusted level of DE-cadherin is required for optic placode morphogenesis, and that β-catenin, as well as EGFR signaling, is involved in this process. Reduction in DE-cadherin results in dissociation of the placode around the time when it normally invaginates, suggesting that the forces exerted on the epithelial sheet while folding may disrupt cell contacts. A similar phenotype was described for other epithelial invaginations, including the Malpighian tubules and stomatogastric nervous

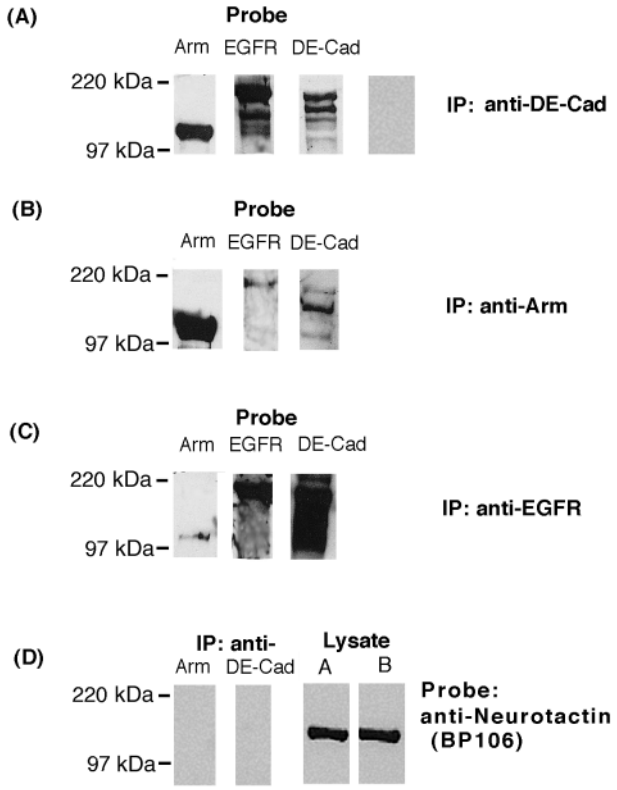

Fig. 8. EGFR is co-immunoprecipitated with DE-cadherin and Armadillo in the *Drosophila* embryo. (A-C) Co-immunoprecipitation of CCC-associated proteins using anti-DE-cadherin (DE-cad) antibody (A), anti-Armadillo (Arm) antibody (B), and anti-EGFR (C). (Left column) Blots were probed with anti-Armadillo antibody. (Center) Blots were probed with anti-EGFR antibody. (Right) Blots were probed with anti-DE-cadherin antibody. Owing to the different sources of the antibodies, it was not possible to normalize the amount of antibody used in conducting the CoIP experiments. Thus, the quantity of proteins pulled down using one antibody differs from that obtained with another antibody. (D) Western blot probed with anti-neurotactin (BP106), documenting the absence of neurotactin in anti-DE-cad and anti-Arm IP products. Western blot contains anti-Arm and Anti-DE-cad IP products (left two lanes) and embryo lysate (right two lanes; A: lysate used for anti-Arm IP; B: lysate used for anti-DE-cad IP). In the lanes loaded with embryo lysate, a band labeled by anti-neurotactin antibody proves that neurotactin is present in the lysate.

system (Tepass et al., 1996; Uemura et al., 1996). As shown in this study, abolishing Armadillo/β-catenin function results in a similar, if somewhat weaker phenotype. If DE-cadherin is overexpressed, invagination is also impaired. Cells stay together in a placode-like formation (as would be expected from "hyperadhesive" epithelial cells), but do not noticeably constrict apically. It should be noted that the interpretation of this failure of optic placode cells to constrict is complicated by the accompanying increase in cell death in surrounding head epidermal cells. This phenomenon, in addition to a direct effect of an increased amount of DE-cadherin in the optic placode cells, could be part of the pathology responsible for the non-invagination phenotype. By contrast, the non-disjunction of optic lobe and larval eye is likely to be a rather direct consequence of an increased amount of DE-cadherin expression. Interestingly, other adhesion systems, notably the *Drosophila* N-CAM homolog FasII, are also involved in optic lobe-larval eye separation. Thus, a recent study by Holmes and Heilig (Holmes and Heilig, 1999) demonstrated that the down regulation of FasII by the 'anti-adhesion' molecule Beaten path is also required for normal larval eye morphogenesis.

Overexpression of DE-cadherin or the DE-cad-α-catenin fusion construct causes a dramatic change in optic lobe morphogenesis, without causing much disruption in other epithelia (K. D., unpublished). We speculate that this enhanced sensitivity of optic lobe cells towards an increased level of DE-cadherin may be in part due to the fact that adherens junctions form the only means of contact between optic lobe cells. In other epithelia, such as epidermis, trachaeae and hindgut, septate junctions form by far the more prominent junctional complex. Septate junctions have been implicated in epithelial stability from a number of genetic studies (Woods et al., 1997) (reviewed by Tepass et al., 2001). One could surmise that embryonic epithelia, as they enter the phase of differentiation during mid-embryogenesis, construct septate junctions that add to the adherens junctions developed at an earlier stage. This additional junctional complex makes late epithelia more resistant to changes in cadherins, a notion supported by the finding that blocking cadherins (by applying calcium chelators, or tyrosine kinase inhibitors) in early embryos up to stage 10 leads to a break down of epithelia, whereas it has only a small effect in later stages (F. W., unpublished). The optic lobe, which does not differentiate but gives rise to a population of neuroblasts later dring the larval period, does not form septate junctions, which could account for its strong reliance on normally functioning adherens junctions.

Expression of a fusion construct, DE-cad-α-catenin, in which the cytoplasmic domain of DE-cadherin is replaced by a truncated α-catenin, thereby preventing a reduction in the cytoplasmic pool of Arm, results in a similar phenotype as overexpressing full length DE-cadherin. This finding lends support to the notion that dissociation of the CCC may not occur at the interface between DE-cadherin and Arm or Arm and α-catenin. If one were to assume that dissociation occurred between any components of the CCC, one would expect a stronger phenotype, given that by overexpressing the fusion construct one not only increases the amount of DE-cadherin molecules interconnecting cells, but also the stability with which they are coupled to the cytoskeleton. Biochemical studies in vertebrates (Ozawa et al., 1998; Takahashi et al., 1997; Tsukatani et al., 1997) and our own analysis (F. W. and V. H., unpublished) also show that phosphorylation of the CCC does not result in increased dissociation of Arm or α-catenin from the CCC, suggesting that the dissociation occurs distal of α-catenin.

The strength of the CCC and other structural molecules driving morphogenesis has to be controlled in a complex spatiotemporal pattern. Numerous widely conserved signaling pathways have been implicated in this process. In vertebrate embryos, mutations of the Wnt, Shh and BMP signaling pathways result in impressive examples which tissues and organs show defects in morphogenesis (Chiang et al., 1996; Furuta et al., 1997; Goodrich and Scott, 1998). Furthermore, it became clear that frequently signaling proteins affect fundamental cellular behaviors, such as proliferation, motility, adhesiveness and survival. This prompted the hypothesis that

in many developmental scenarios, the 'proximal' effect of receiving a signal could be a change in morphogenetic behavior (Moon et al., 1993a; Moon et al., 1993b; Ungar et al., 1995; Ainsworth et al., 2000; Chuong et al., 2000). The discovery that one of the Wnt signal transducers, β-catenin, leads a 'double life' as a structural component of the cadherin-catenin complex, fueled the idea that Wnt signal could directly exert an effect on the adhesiveness on the cell, an idea that is supported by cell culture experiments (Bradly et al., 1993; Hinck et al., 1994). However, genetic studies demonstrated that in *Drosophila*, the roles of β-catenin as a signaling transducer and a CCC component seem to be quite separate. Although it is clear that the cytoplasmic and membrane bound β-catenin pools are in a steady state, binding of more β-catenin to the membrane, by overexpression of DE-cadherin, reduces the cytoplasmic pool resulting in a *wg* minus phenotype (Sanson et al., 1996). However, Wnt/Wg signaling seems to have no effect on the amount of membrane bound β-catenin (Peifer et al., 1994). Thus, in *Drosophila*, it appears that DE-cadherin mediated adhesion, at least under experimental conditions, interferes with Wnt/Wg signaling by competing for β-catenin but Wnt/Wg signaling may not have a direct effect on adhesion mediated by the CCC.

Our findings suggest that another signaling pathway, the EGFR pathway, is involved in modulating cadherin-mediated adhesion and thereby controlling morphogenesis. In a previous paper (Daniel et al., 1999), we showed that EGFR, similar to its function in the developing compound eye, is activated in the precursors of the larval eye and adjacent optic lobe at a stage preceding optic lobe invagination and larval eye separation. The ligand for EGFR is Spitz, which is activated by Rhomboid.

In a small subset of larval eye precursors (the 'Bolwig's organ founders'). As shown by Dumstrei et al. (Dumstrei et al., 1998), Daniel et al. (Daniel et al., 1999) and in the present paper, loss of EGFR signaling results primarily in cell death, lending further support to the view that EGFR signaling functions generally in the ectoderm and its derivatives to maintain cell viability. Recent studies in *Drosophila* indicate that MAPK directly controls the expression and protein stability of the cell death regulator, Hid (W; Wrinkled) (Kurada and White, 1998; Bergmann et al., 1998). If cell death is prohibited by a deficiency of the *reaper*-complex, cells of the optic placode and most other embryonic cells that undergo apoptosis in EGFR loss of function survive. Both optic lobe and Bolwig's organ express several of their normal differentiation markers, but show a characteristic 'hyperadhesive phenotype', consisting in the failure of optic Iobe invagination and Bolwig's organ separation. Based on the similarity of this phenotype to that one resulting from DE-cadherin overexpression, and the genetic interaction between *Egfr* and *DE-cadherin* mutants in the ventral ectoderm, we propose that EGFR activation is required in normal development to phosphorylate the CCC and thereby allows optic lobe invagination and Bolwig's organ separation to occur. This would be in line with experimental results obtained in vertebrate cell culture studies, which demonstrated that drug- or EGFR-induced phosphorylation of the CCC leads to dissociation between CCC and cytoskeleton. Recent findings have shown that another phosphorylation event, mediated by the rho/rac GTPases, also effects adhesion by dissociating α-catenin from the CCC (Kaibuchi et al., 1999).

Co-IP data indicates that EGFR is linked to the CCC in *Drosophila* as well. This implies that the effect of EGFR on DE-cadherin mediated adhesion could be a direct one that occurs at the cell membrane and does not involve MAPK signal transduction to the nucleus. It has been shown in a number of vertebrates cell culture systems that tyrosine phosphorylation of β-catenin results in a disassembly of the CCC complex and a consecutive loss in cadherin-mediated adhesion (Aberle et al., 1996; Hazan and Norton, 1998). Phenotypically, this results, among others, in increased invasiveness of tumor cell lines (Behrens et al., 1993; Birchmeier, 1995; Noë et al., 1999), neuronal and growth cone motility (Lanier et al., 2000), or inhibition of blastomere compaction (Ohsugi et al., 1999). Several tyrosine kinases and phosphatases have been identified that can increase or decrease the degree of phosphorylation of the CCC. For example, v-src transfected into cultured cells phosphorylates β-catenin and causes cells to dissociate, round up, and become more motile (Behrens et al., 1993). EGFR also phosphorylates the CCC and forms an integral part of this complex (Hoschuetzky et al., 1994). This opens up the possibility that growth factors, with their widespread expression and biological activity in the developing embryo, may exert part of their effect on cell behavior by modulating, in a rather direct way, cell adhesion at the membrane. Such a mechanism would account for the 'rapid mode' of control of adhesion molecules. Systems such as the optic placode of the *Drosophila* embryo, where matters of different cell fates are decided at the same time when morphogenetic movements change the arrangement and shape of the cells involved, constitute favorable paradigms to address how signaling systems control both processes.

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