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Ectopic activation of *torpedo/Egfr*, a *Drosophila* receptor tyrosine kinase, dorsalizes both the eggshell and the embryo

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SUMMARY

The *Drosophila* gene *torpedo/Egfr* (*top/Egfr*) encodes a homolog of the vertebrate Epidermal Growth Factor receptor. This receptor is required several times during the life cycle of the fly for the transmisson of developmental cues. During oogenesis, Top/Egfr activation is required for the establishment of the dorsal/ventral axis of the egg and the embryo. To examine how ectopic Top/Egfr activation affects cell fate determination, we constructed an activated version of the protein. Expression of this activated form (λtop) in the follicle cells of the ovary induces dorsal cell fates in both the follicular epithelium and the embryo. Different levels of expression resulted in different dorsal follicle cell fates. These dorsal cell fates were expanded in the anterior, but not the posterior, of the egg, even in cases where all the follicle cells covering the oocyte expressed λtop. The expression of genes known to respond to *top/Egfr* activation, *argos* (*aos*), *kekkon1* (*kek 1*) and *rhomboid* (*rho*), was also expanded in the presence of the λtop construct. When λtop was expressed in all the follicle cells covering the oocyte, *kek 1* and *argos* expression was induced in follicle cells all along the anterior/posterior axis of the egg chamber. In contrast, *rho* RNA expression was only activated in the anterior of the egg chamber. These data indicate that the response to Top/Egfr signaling is regulated by an anterior/posterior prepattern in the follicle cells.

Expression of λtop in the entire follicular epithelium resulted in an embryo dorsalized along the entire anterior/posterior axis. Expression of λtop in anterior or posterior subpopulations of follicle cells resulted in regionally autonomous dorsalization of the embryos. This result indicates that subpopulations of follicle cells along the anterior/posterior axis can respond to Top/Egfr activation independently of one another.

Key words: *Drosophila*, oogenesis, receptor tyrosine kinase, *top/Egfr*, *argos*, *kekkon1*, *rhomboid*

INTRODUCTION

One of the basic questions in developmental biology is how extracellular signals are received and interpreted by cells to determine different cell fates. Molecules required for this communication process include external ligands and their cognate cell surface receptors such as receptor tyrosine kinases (RTKs). Signaling through receptor tyrosine kinases is conserved from invertebrates to mammals (Schlessinger and Ullrich, 1992; Fantl et al., 1993). While components of the signal transduction cascade are similar, the cellular response to RTK signaling can vary; the cell receiving the signal may divide or differentiate. *Drosophila* oogenesis provides a powerful model system in which to study how activation of a receptor tyrosine kinase, *torpedo/Egfr* (*top/Egfr*), results in the determination of cell fates.

The *Drosophila* gene *top/Egfr* encodes a homolog of the vertebrate EGF receptor (Livneh et al., 1985; Wadsworth et al., 1985; Price et al., 1989). Like other members of this RTK family, *top/Egfr* has an extracellular ligand-binding domain, one transmembrane pass and an intracellular tyrosine kinase domain. Top/Egfr activity is necessary for the development of embryonic tissues, imaginal discs and the egg (Price et al., 1989; Schejter and Shilo, 1989). During *Drosophila* oogenesis, the activity of Top/Egfr is necessary for the establishment of the anterior/posterior and dorsal/ventral axes of both the eggshell and the embryo that will develop within (Schüpbach, 1987; Gonzalez-Reyes et al., 1995; Roth et al., 1995). The presumed ovarian ligand for *top/Egfr* is *gurken*, which belongs to the TGF-α family of signaling molecules (Neuman-Silberberg and Schüpbach, 1993).

Upon binding of an extracellular ligand, the RTKs dimerize and phosphorylate each other (Schlessinger and Ullrich, 1992; Fantl et al., 1993). A number of components acting downstream of RTKs have been elucidated by a combination of biochemical and genetic methods. In both vertebrate and invertebrate systems, the Ras pathway is responsible for transmission of the signals from several receptor tyrosine kinases. Members of the Ras pathway have been shown to act downstream of *top/Egfr* in oogenesis during the process of axis formation (reviewed in Duffy and Perrimon, 1996; Ray and Schüpbach, 1996).

The major body axes of the egg and the future embryo are determined during oogenesis by a system of intercellular communication. The egg chamber, which will produce a mature egg, consists of three different cell types. The oocyte and its sister support cells, the nurse cells, are derived from the germline. These cells are surrounded by an epithelial layer of

somatically derived follicle cells. Experiments involving pole cell transplantation revealed that *gurken* is required in the germline, while *top/Egfr* function is required in the follicle cells (Schüpbach, 1987). Loss-of-function mutations in either the ligand or receptor results in the ventralization of eggshells and embryos. Additionally, strong loss-of-function mutations also result in the duplication of anterior follicle cell fates at the posterior of the egg chamber and cause defects in the A/P polarity of the oocyte microtubule network (Gonzalez-Reyes et al., 1995; Roth et al., 1995).

Establishment of the dorsal/ventral axis is believed to be a consequence of localized activation of Top/Egfr. This receptor is expressed in all the follicle cells that cover the oocyte (Kammermeyer and Wadsworth, 1987; Schweitzer, R., Zak, N. and B-Z. Shilo, personal communication). In mid-oogenesis, the ligand Gurken is found tightly localized to the future dorsal anterior area of the oocyte, around the oocyte nucleus (Neuman-Silberberg and Schüpbach, 1993). Spatially restricted activation of Top/Egfr in the follicle cells that lie over the dorsal anterior patch of Gurken is believed to induce these follicle cells to assume dorsal cell fates and secrete the appropriate structures for the dorsal anterior eggshell. After receiving patterning instructions from the germ line, the follicle cells will eventually regulate the production of a new signal that activates the embryonic dorsal/ventral patterning system (Morisato and Anderson, 1995). Production of this new signal is inhibited by Top/Egfr activity in the dorsal follicle cells. Based on this model, we predict that ectopic activation of Top/Egfr will dorsalize the egg and embryo. This prediction is supported by the demonstration that females with four extra copies of *gurken*, which accumulate *gurken* RNA around the entire anterior of the oocyte, produce dorsalized eggs and embryos (Neuman-Silberberg and Schüpbach, 1994).

To get a better understanding of which cell fates are determined when Top/Egfr is activated ectopically during oogenesis, we wanted to obtain an activated form of Top/Egfr. No gain-of-function alleles of *top/Egfr* have been isolated in screens for mutants that affect oogenesis, presumably because such a mutation would be embryonic lethal. In this report, we describe a system to activate *top/Egfr* ectopically in the fly. First, we constructed an active form of *top/Egfr*, by fusion with a heterologous dimerization domain, similar to a previously reported activated form of the *Drosophila* receptor *breathless* (Lee et al., 1996). Then we controlled the expression of this construct in the follicle cells with the UAS/Gal4 system (Brand and Perrimon, 1993).

When expressed in the follicle cells, activated *top/Egfr* dorsalizes both the eggshell and the embryo. Increased expression of activated *top/Egfr* results in increased degrees of dorsalization. Differences in the response to Top/Egfr activation, as indicated by molecular markers and chorion phenotype, revealed that the follicular epithelium contains a prepattern along the A/P axis. We also observed a correlation between the location of the activated *top/Egfr* in the follicle cells and the resulting dorsalization of the embryo.

MATERIALS AND METHODS

DNA constructs and germline transformation

The activated *top/Egfr* construct (λtop) was modeled after the λbtl construct, a gift from Denise Montell. A 0.75 kb *Not*I-*Xmn*I fragment containing 0.6 kb λ DNA and 0.15 kb of *breathless*, including the transmembrane domain, was ligated to a 1.9 kb *Nco*I(partial)-*Xho*I genomic fragment containing the 3′ end of *top/Egfr*. The fusion junction occurs 14 amino acids after the transmembrane domain of Top/Egfr (Livneh et al., 1985). This construct was inserted into the *Not*I site of pUAST (Brand and Perrimon, 1993) and used for P-element-mediated germline transformation (Spradling, 1986). The DNA was injected into *yw* embryos at a concentration of 500 ng/μl. The helper plasmid was pTurbo (gift from Paul Schedl), injected at 100 ng/μl. Four transgenic lines were obtained from 64 adult G_os. All lines produce dorsalized phenotypes when expressed in the ovary, ranging from weak to strong dorsalization when tested with the Gal4 line CY2. The λtop line used in this study was 4.2, which mapped to the first chromosome. This insertion gave a strongly dorsalized phenotype.

Fly stocks

All flies were cultured in standard agar cornmeal medium at room temperature. Crosses with the Gal4 lines were done at 21.6˚C, except where noted in text or figure legends. The Gal4 lines 55B and T155 were a gift from Norbert Perrimon (Brand and Perrimon, 1994; Harrison et al., 1995). The Gal4 lines CY2, CU1 and E4 were generated by remobilizing one of the original Gal4 inserts. The *gurken* allele used was grk^{E12} (Neuman-Silberberg and Schüpbach, 1993).

Histological staining techniques

Digoxigenin-labeled RNA probes were made using the DIG RNA Labeling Kit (Boehringer Mannheim) according to the manufacturer's instructions. For the lambda probe, a 0.75 kb *Not*I-*Xmn*I fragment from the λbtl construct was used. For *aos* (Freeman et al., 1992) and *kek1* (Musacchio, 1996), the cDNAs were used to make riboprobes. The *rho* probe (Bier et al., 1990) was a gift from Siegfried Roth. Ovaries were dissected in Ringers and the ovarioles were partially separated. The ovaries were fixed in 4% paraformaldehyde in PBS, 10% DMSO and 3 volumes heptane for 20 minutes at room temperature. In situ hybridizations were a modified version of Tautz and Pfeifle (1989) using 55˚C as the hybridization temperature.

Antibody staining on embryos was a modification of Ashburner (1989) with PBS/0.2% Tween used for washes. Embryos were blocked for 1 hour in 10% BSA/PBST and incubated overnight at 4˚C with Twist antibody, a gift from Siegfried Roth (Roth et al., 1989), used at 1:2000. Subsequent washes were done in BNT (1 M NaCl, 3% normal goat serum, 1% BSA, 0.2% Tween in PBS) until HRP detection with biotinylated anti-rabbit antibody and the Vectastain ABC kit (Vector Laboratories).

β-galactosidase staining was performed as described in Ashburner on ovaries fixed in 2.5% glutaraldehyde/PBS for 15 minutes.

Microscopic examination techniques

Scanning electron microscopy was performed at the Princeton Materials Institute on a Philips XL30 FEG-SEM. Whole flies were fixed in 70% ethanol overnight and dehydrated through a series of 10 minute washes in 95% ethanol, 100% ethanol and acetone. Eggs were fixed in 65˚C 1% Triton X-100/PBS for 2 seconds, put on ice 1 minute and transferred to methanol. The samples were dried with TMS (Tetramethylsilane-Ted Pella) for 10 minutes in a capped tube. The TMS was allowed to evaporate, samples were mounted on studs with carbon tape disks and sputter coated with platinum for 70 minutes.

For microscopic examination of chorions and wings, samples were washed in water and mounted in Hoyers mounting medium. To examine embryonic cuticles, eggs were dechorionated and mounted in Hoyers:Lactic Acid 7:3 (Wieschaus and Nüsslein-Volhard, 1986).

RESULTS

Construction of an activated allele of *torpedo/Egfr*

Axis formation in *Drosophila* depends on intercellular signaling

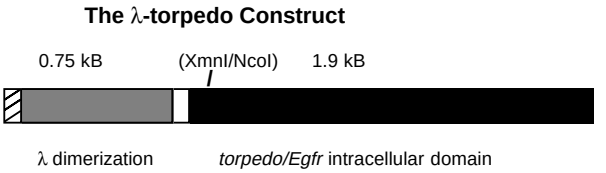

Fig. 1. The activated *torpedo/Egfr* construct. Diagram of the λtop fusion protein, in which the extracellular domain of Torpedo/Egfr was replaced with the dimerization domain of the λ repressor. The signal peptide (hatched), λ (gray) and transmembrane (white) domains are derived from the λbtl construct (Lee et al., 1996). The Top/Egfr intracellular kinase domain (black) begins 14 amino acids after the transmembrane region, at the NcoI site of *top/Egfr*.

between the germline and the follicle cells during oogenesis (reviewed in Ray and Schüpbach, 1996). The *Drosophila* homolog of the Epidermal Growth Factor Receptor, encoded by the *top/Egfr* gene, plays a central role in these processes. Genetic analysis of *top/Egfr* loss-of-function mutants has revealed that *top/Egfr* activation is required in the follicle cells for axis formation. In order to study how ectopic *top/Egfr* activation affects dorsoventral axis formation of the eggshell and the embryo, we constitutively activated Top/Egfr in the ovary and analyzed the response of the follicle cells by molecular and genetic methods. This analysis required the construction of an activated form of *top/Egfr*.

In a first approach, we attempted to activate *top/Egfr* by truncation of the extracellular domain. This modification mimics constitutively active forms of the EGF receptor family and has been successfully used to activate *sevenless*, a RTK that acts in the eye (Basler et al., 1991). While a transgene containing our truncated form of Top/Egfr was expressed in the ovary, we were unable to generate any phenotypes consistent with activation of the kinase (data not shown). We therefore turned to another method of activation, forced dimerization.

Receptor dimerization and transphosphorylation is the first step in a kinase cascade that ultimately results changes in gene expression. A heterologous dimerization domain from the bacteriophage lambda repressor protein has recently been used to make a constitutively activated form of *breathless*, a *Drosophila* RTK (Lee et al., 1996). We fused this lambda dimerizaton domain to the *top/Egfr* intracellular kinase domain to produce a similarly activated form of Top/Egfr. We refer to this fusion protein as λtop (Fig. 1).

Because of the potential lethality of a constitutively activated *top/Egfr*, the UAS/Gal4 system (Brand and Perrimon, 1993) was used to control expression of λtop. The λtop fusion transgene was cloned behind a promoter activated by the yeast transcriptional activator Gal4 and transformed into flies. One line, 4.2, was chosen for further study. In the absence of the Gal4 protein, the λtop transgene is transcriptionally silent (data not shown). Flies that contain UAS-λtop are then crossed to flies expressing Gal4 in a tissue-specific manner, resulting in tissue specific expression of activated *top/Egfr*.

Expression of the λtop transgene results in phenotypes predicted for activated Top/Egfr

The loss-of-function alleles of *top/Egfr* affect the embryo, eye, wing and ovary (Clifford and Schüpbach, 1989; Schejter and Shilo, 1989). We used lines that express Gal4 in these tissues to drive the λtop transgene insertion 4.2 and analyzed the resulting phenotypes. When λtop is expressed early in development, the embryos die, confirming the suspected lethality of the λtop construct (data not shown). Expression of λtop in the imaginal discs and ovaries results in phenotypes consistent with activation of Top/Egfr. The wild-type eye consists of a smooth, regular array of ommatidia (Fig. 2A). Top/Egfr is required in the eye for both proliferation of the disc cells and for the differentiation of the cell types in the ommatidial cluster (Baker and Rubin, 1992; Freeman, 1997), with loss-of-function leading to smaller, rough eyes. When λtop is expressed in the eye using the Gal4 line T155, some ommatidia are enlarged and irregular (Fig. 2B). The wild-type wing contains five longitudinal veins and two crossveins (Fig. 2C). Whereas a reduction of *top/Egfr* signaling causes a loss of wing vein

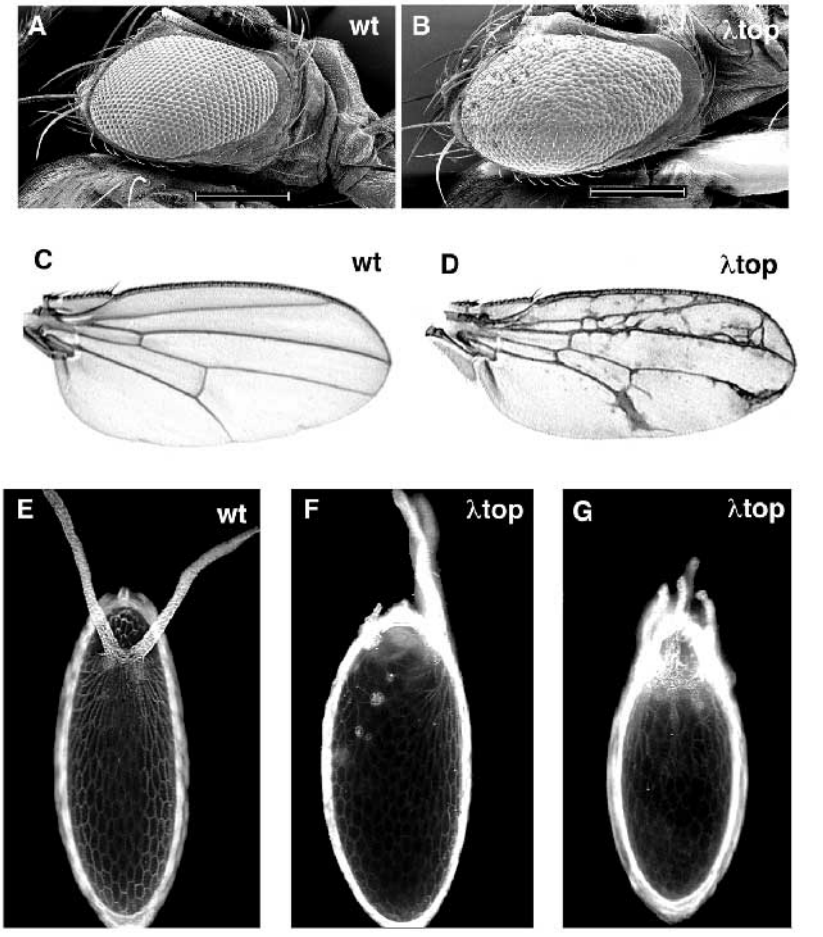

Fig. 2. Expression of the λtop construct causes *top/Egfr* gain-of-function phenotypes. (A) Wild-type eye (bar is 200 μm); (B) λtop;T155 eye, 20˚C; (C) Wild-type wing; (D) λtop; CY2 wing, 25˚C; (E) Wild-type egg, dorsal view, anterior is up; (F, G) λtop; T155 at 18 and 25˚C.

material (Clifford and Schüpbach, 1989; Diaz-Benjumea and Garcia-Bellido, 1990), the presence of the λtop transgene driven by the Gal4 line CY2 results in extra wing veins (Fig. 2D). This phenotype also occurs when activated Ras pathway components are expressed in the wing disc (Brand and Perrimon, 1993; Brunner et al., 1994).

The eggshell is secreted by the follicle cells during oogenesis. Specialized groups of follicle cells on the dorsal side of the egg chamber will form the two dorsal respiratory appendages (Fig. 2E). In the ovary, partial loss-of-function alleles of *top/Egfr* cause a reduction of the population of dorsal follicle cells, resulting in a loss of appendage material (Schüpbach, 1987). When λtop is expressed in the ovary with the Gal4 line T155, a mass of dorsal appendage material forms around the anterior circumference of the egg (Fig. 2G). From these data, we conclude that expression of the λtop fusion protein results in phenotypes consistent with Top/Egfr activation.

The activated Top/Egfr phenotypes were more severe at higher temperatures. This temperature sensitivity is frequently observed with phenotypes induced by Gal4 lines, and is presumably due to increased transcriptional activity of Gal4 at higher temperature. When females carrying λtop, driven by Gal4 line T155, were tested at a lower temperature, the amount of ectopic dorsal appendage material was reduced (Fig. 2F). Extra wing vein production resulting from λtop expression was also reduced at lower temperatures (data not shown).

One predicted result of expressing λtop is that forced dimerization would allow ligand-independent activation of the receptor. We tested whether the λtop transgene is able to signal in the absence of ligand by crossing the λtop;T155 combination into a *gurken* mutant background. The resulting eggs, while still retaining the double micropyle, show ectopic dorsal appendage material (Fig. 3A,B). This indicates that, even in the absence of ligand, the activated *top/Egfr* construct induces the follicle cells to assume a dorsal cell fate. Since the Gal4 lines express relatively late during oogenesis, earlier defects caused by the absence of Gurken, such as the double micropyle, cannot be rescued by the transgene.

To test if the dorsalization generated by activated *top/Egfr* is specific to this contruct, we expressed another activated RTK in the follicle cells using the Gal4 line CY2. Expression of λbtl, an activated form of the *Drosophila* Fibroblast Growth Factor receptor homolog, also dorsalizes the eggshell and the embryo. In contrast to the strong dorsalization produced by the λtop construct expressed with this Gal4 line (Fig. 4I), the effect of λbtl on the eggshell is weak and variable, with 83% of the eggs showing an expansion of dorsal structures (Fig. 3C). The resulting embryos are also variably dorsalized, with the most dorsalized class losing the ventral denticle bands but retaining the dorsolateral Filzkorpers (Fig. 3D). These results demonstrate that activating another RTK is sufficient to induce dorsal cell fates in the ovary. The weaker effect may be due to a lower level of λbtl expression produced by this particular construct. Alternatively, the activated *btl* may only partially substitute for *Top/Egfr* activity.

Expression of λtop in subpopulations of follicle cells

Several Gal4-expressing lines were used to drive expression of the activated *top/Egfr* transgene in different regions of the ovarian follicle cell epithelium. In order to obtain a representation of the expression pattern of the λtop transgene, a riboprobe to the lambda portion of the transgene was used to examine the expression pattern of λtop by in situ hybridization. Expression patterns of Gal4 in these lines were also characterized by β-galactosidase staining in ovaries of females carrying a UAS-LacZ transgene (Fig. 4).

The Gal4 line 55B (Brand and Perrimon, 1994) induces expression λtop in the anterior portion of the egg chamber over the developing oocyte, beginning at stage 8 (Fig. 4A). Since β-gal is stable over several hours, the UAS-LacZ pattern presumably represents the sum of Gal4 expression in the follicle cells over a longer time period as compared to the λtop RNA (Fig. 4B). Females expressing λtop in this pattern lay eggs that are mildly dorsalized (Fig. 4C). 43% of their eggs display an increase in the dorsal midline space between the appendages or ectopic dorsal appendage material. The Gal4 line T155 (Harrison et al., 1995) expresses λtop all over the follicle cell epithelium beginning at stage 9 (Fig. 4D). The UAS-LacZ pattern indicates that most follicle cells express Gal4, but in a somewhat patchy manner, with higher expression at the anterior (Fig. 4E). 100% of the resulting eggs have extra dorsal appendage material around the anterior circumference (Fig. 4F). The Gal4 line CY2 expresses the λtop transgene in all the follicle cells covering the oocyte from stage 8 with a similar UAS-LacZ staining pattern (Fig. 4G,H). In females carrying this transgene, 100% of the eggs have no dorsal appendages (Fig. 4I). While loss of dorsal appendage material might be interpreted as an expansion of ventral cell fates, the observed phenotype is different from the ventralized eggshell, and

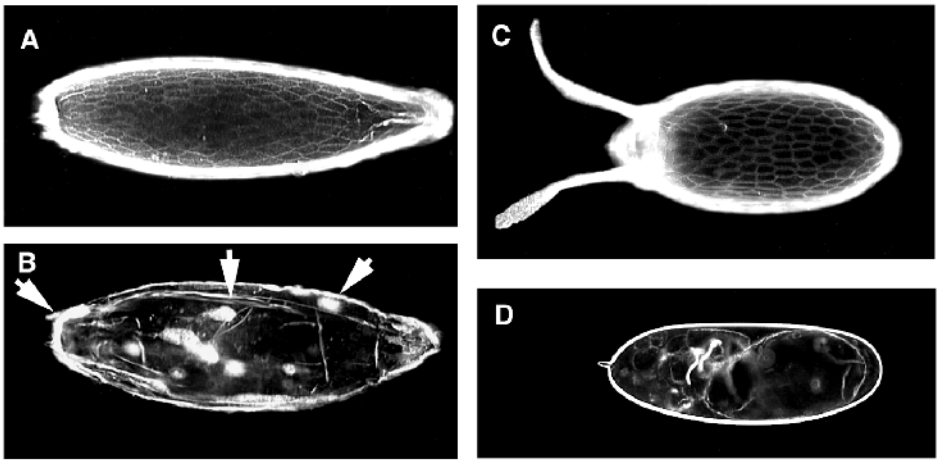

Fig. 3. (A,B) Expression of λtop in gurken mutant background causes ligand-independent production of appendage material. Eggs produced at 20˚C from females of genotype (A) grk^{E12}/grk^{E12}. (B) λtop; grk^{E12}/grk^{E12}; T155. Arrows point to ectopic dorsal appendage material. (C,D) Expression of activated *breathless* (λbtl) dorsalizes both the eggshell and the embryo. Eggshell (C) and embryonic cuticle (D) from λbtl;CY2 female.

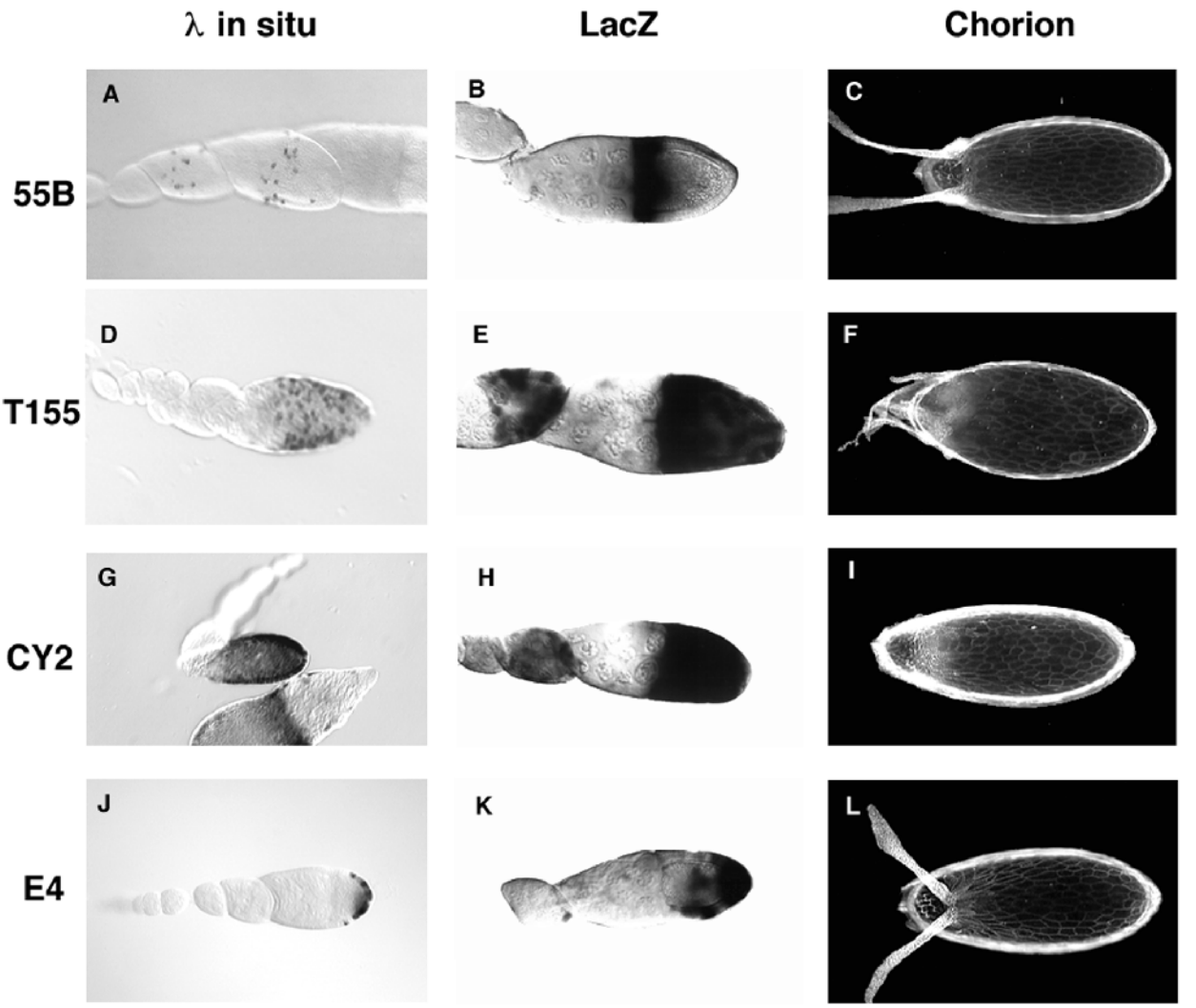

Fig. 4. Expression of λtop in follicle cells generates dorsalized eggs. In situ hybridizations (A,D,G,J) with a riboprobe to the λ portion of the construct. β-gal staining pattern (B,E,H,K) of the Gal 4 lines. Chorion phenotypes (C,F,I,L) from females expressing λtop. Gal4 lines (A-C) 55B, (D-F) T155, (G-I) CY2 and (J-L) E4.

appears to be an expansion of dorsal midline fate that now surrounds the entire anterior circumference of the egg. Examination of these eggs by phase and by scanning electron microscopy revealed that the entire anterior surface of the egg was covered by operculum-like material, a dorsal structure (Fig. 5). The Gal4 line E4 expresses the λtop transgene and UAS-LacZ in a posterior patch of follicle cells from stages 9-11 (Fig. 4J,K). Females expressing this pattern of activated Top/Egfr lay eggs that look wild type (Fig. 4L).

In summary, we obtained dorsalized eggshells similar to those obtained when the activated UAS-Draf^{gof}, a positive effector of *top/Egfr* signaling, is expressed in the follicle cells (Brand and Perrimon, 1994). The strength of the expression pattern of the λtop transgene, as measured by in situ analysis, correlates with amount of dorsalization observed in the resulting eggshell. We also observed that, in cases where the λtop transgene was expressed in follicle cells situated over the entire oocyte, such as Gal4 lines T155, CY2 and CU1(data not shown), only the anterior region of the eggshell produced extra dorsal appendage or operculum material. The Gal4 line E4, which expresses λtop only in the posterior follicle cells of the stage 9-11 egg chamber, results in eggs that lack any ectopic appendage material and look wild type. These results demonstrate that an anterior/posterior prepattern is present in the follicular epithelium at the time of Top/Egfr activation and only the anterior follicle cells of the egg chamber are capable of producing appendages and operculum material in response to activated *top/Egfr*.

Activated Top/Egfr expands the expression of *rho*, *kek 1* and *aos*

The response of the follicle cells to signaling by the activated *top/Egfr* construct was also analyzed at the molecular level. We performed in situ hybridization experiments on ovaries using probes that detect transcripts of genes known to be regulated by *top/Egfr* activity. As expected from the eggshell pattern, we detected an expansion in the expression pattern of several dorsally expressed genes. These patterns of expansion could be classified into two categories: expansion along the entire anterior/posterior axis and expansion limited to the anterior of the egg chamber.

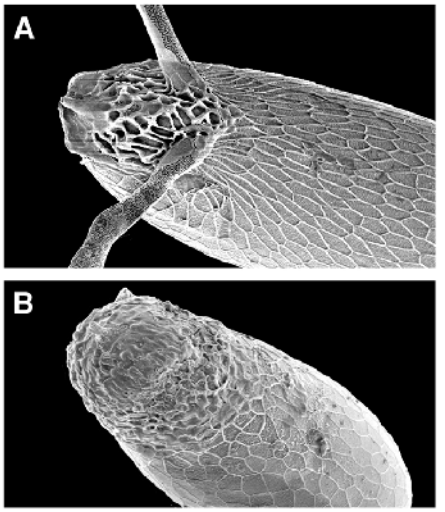

Fig. 5. Expression of λtop with Gal4 line CY2 causes anterior follicle cells to secrete operculum-like material. Scanning electron micrographs of eggs. (A) Wild type; (B) λtop; CY2.

Expression of the genes *kek 1* and *aos* expanded along the entire anterior/posterior axis. The gene *kek 1* encodes a transmembrane protein with immunoglobulin and leucine-rich repeat motifs. In the ovary, *kek 1* is expressed in a dorsal anterior patch of follicle cells at stage 9 and is positively regulated by the *top/Egfr* pathway (Musacchio and Perrimon, 1996, Fig. 6A). When the activated *top/Egfr* construct is expressed in the entire epithelium of follicle cells that cover the oocyte, using the Gal4 line CY2, *kek 1* RNA expression in the follicle cells expands to cover the entire oocyte (Fig. 6B). The gene *aos* encodes a secreted protein with a cysteine-rich region similar to an EGF motif (Freeman et al., 1992). In the embryo, *aos* is expressed in response to activation of *top/Egfr* (Golembo et al., 1996). Using a riboprobe for *aos*, we found that *aos* is also expressed in the ovaries. *aos* expression is first visible at stage 7 where it covers the posterior of the egg chamber. The *aos* staining disappears at stage 9, and returns at stage 10A, labelling the dorsal centripetal follicle cells. At stage 10B, a faint dorsal patch appears, as well as staining in the border cells. The dorsal patch refines to a very narrow T-shaped patch in the anterior over the dorsal midline (Fig. 6C). *aos* is also expressed in the follicle cells at posterior pole beginning at stage 12. In females expressing λtop under the control of the Gal4 line CY2, the expression of *aos* is expanded in the follicular epithelium. Whereas *kek 1* showed uniform expression with the Gal 4 line CY2, *aos* showed consistent higher expression at the anterior end, with patchy expression in the remaining follicle cells which cover the oocyte (Fig. 6D). These data demonstrate that λtop is sufficient to activate transcription of *kek 1* and *aos* in all regions of the epithelium which covers the oocyte.

The expression of *rho*, a novel transmembrane protein (Bier et al., 1990), was also expanded in the presence of the λtop transgene, but only in the anterior follicle cells of the egg chamber. *rho* expression in the ovary, in response to *top/Egfr* signaling, begins in a dorsal anterior patch, which resolves into two dorsolateral stripes (Ruohola-Baker et al., 1993, Fig. 6E). Expression of λtop with the Gal4 line CY2 resulted ectopic expression of *rho*. In contrast to the results obtained with *kek1* and *aos*, the expansion of *rho* was restricted to the anterior of the egg chamber (Fig. 6F). In addition, when λtop was expressed in the posterior of the egg chamber using the Gal4 line E4, no ectopic *rho* expression was detected (data not shown). There are two possible explanations for this result. The first is that *top/Egfr* activation is not sufficient to activate *rho* in the posterior follicle cells, and some other factor or signal is needed that is only present in anterior follicle cells. The second possibility is that *rho* expression is repressed in the posterior follicle cell population, and activation of Top/Egfr is not sufficient to overcome this repression. In addition to the production of dorsal appendage material only in the anterior of the egg, the limitation of *rho* expansion to the anterior follicle cells is further evidence for an anterior/posterior pattern pre-existing within the follicle cells.

Expression of λtop in the follicle cells dorsalizes the embryo

In addition to its role in establishing the dorsal characteristics of the eggshell, the activity of *top/Egfr* is also needed on the dorsal side of the egg chamber to restrict a ventralizing patterning process that affects the embryo. As a result, loss of *top/Egfr* function results in embryos that are ventralized (Schüpbach, 1987; Morisato and Anderson, 1995). Ectopic activation of Top/Egfr in the follicle cells is predicted to have a dorsalizing effect on the embryo. Examination of the embryonic cuticles of eggs from females carrying λtop in combination with various Gal4 lines revealed that these embryos are dorsalized. The embryos from these females were

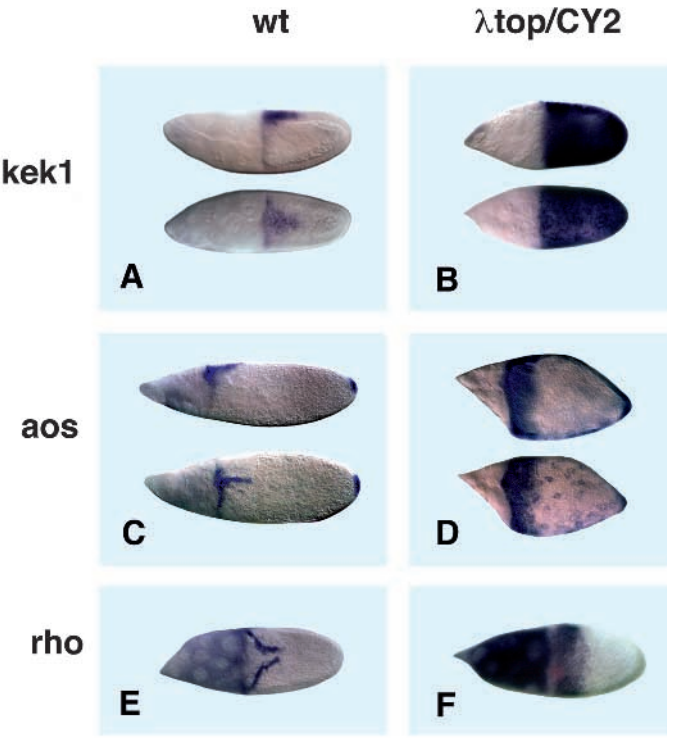

Fig. 6. Expression of λtop with Gal4 line CY2 causes ectopic *kek 1*, *aos* and *rho* expression. Whole-mount in situ hybridization on wild-type (A,C,E) and λtop;CY2 (B,D,F) ovaries with probes for *kek 1* (A,B), *aos* (C,D) and *rho* (E,F). (A,C) Top is lateral view, bottom is dorsal view. (B,D) Two focal planes are shown.

also stained with Twist antibody in order to visualize their dorsal/ventral pattern at an earlier stage. In the wild-type embryo, Twist expression is localized to a band approximately ten cells wide on the ventral side of the blastoderm (Fig. 7A). As shown in Fig. 7, the expression of λtop in the follicle cells results in the loss of Twist expression, i.e. dorsalization, of the embryo (Fig. 7C,E,G,I). When λtop was expressed in all the follicle cells with the Gal4 line CY2, loss of Twist occurred all along the anterior/posterior axis, with some staining still seen in the termini of the embryo (Fig. 7C). The cuticle of these embryos was very strongly dorsalized (Fig. 7D). In embryos produced by females carrying λtop expressed via the Gal4 line CU1, which has an expression pattern very similar to Gal4 line T155, Twist staining was still lost along the ventral side of the embryo, although the staining at the termini was more pronounced (Fig. 7E). The embryonic cuticles were strongly dorsalized (Fig. 6F). When λtop was expressed in the follicle cells over the anterior of the developing oocyte using the Gal4 line 55B, loss of Twist staining occurred only in the anterior (Fig. 7G). In the cuticles, the anterior region of the embryo was dorsalized, whereas the posterior region appeared normal (Fig. 7H). Conversely, when λtop was expressed in the posterior of the egg chamber using the Gal4 line E4, loss of Twist staining was limited to the posterior (Fig. 7I). Examination of the corresponding cuticle phenotypes also demonstrated that dorsalization was restricted to the posterior (Fig. 7J). These data show that activation of *top/Egfr* in the follicle cells is sufficient to dorsalize the embryo. The dorsalization is dependent on the location of the follicle cells expressing the activated *top/Egfr*, suggesting that each region of activated follicle cells is able to affect the embryo in an autonomous manner.

DISCUSSION

Forced dimerization constitutively activates Torpedo/Egfr

During *Drosophila* oogenesis, the major axes of both the eggshell and the future embryo are determined by a series of processes that require intercellular communication. The receptor tyrosine kinase *top/Egfr* is necessary for signaling between the germline and the soma. In order to study how Top/Egfr activation affects the determination of cell fates in the ovary, we constructed an activated form of this receptor. Studies of mammalian RTKs indicate that the first step of their activation process is dimerization upon ligand binding and transphosphorylation (Schlessinger and Ullrich, 1992; Fantl et al., 1993). Genetic evidence that *top/Egfr* may also act in this way was provided by the analysis of interallelic complementation (Clifford and Schüpbach, 1994; Raz et al., 1991). There are several examples of RTKs that have been activated by fusion with protein-protein interaction domains. For instance, in mammalian systems, activated forms of the Ret RTK are produced when chromosomal rearrangements result in fusion of the Ret catalytic domain with a dimerization motif (reviewed in Hunter, 1997). Based on the work by Lee et al. (1996), we used a dimerization domain derived from the lambda repressor to create a transmembrane fusion protein that contains the Top/Egfr intracellular catalytic domain. When the λtop fusion protein is expressed in the fly, under UAS/Gal4 control, the phenotypes predicted for activation of Top/Egfr are observed in the eye, wing and ovary.

During *Drosophila* oogenesis, *top/Egfr* is expressed in the epithelium of follicle cells that surrounds the developing oocyte, and acts in axis determination (for review see Ray and Schüpbach, 1996). In mid-oogenesis, the dorsal localization of its ligand, Gurken, is thought to activate Top/Egfr in a restricted manner on the dorsal side. This activation leads to the assumption of dorsal fates by the follicle cells and the production of appropriate eggshell structures for this region: the dorsal midline, operculum and appendages. In addition, the signal transduced by Top/Egfr regulates the production of a later signal that determines the dorsal/ventral polarity of the embryo (reviewed in Morisato and Anderson, 1995). To test whether the activation of *top/Egfr* is sufficient to regulate both these processes, we ectopically expressed λtop in the follicle cells of the ovary and analyzed the response in both the eggshell and embryo.

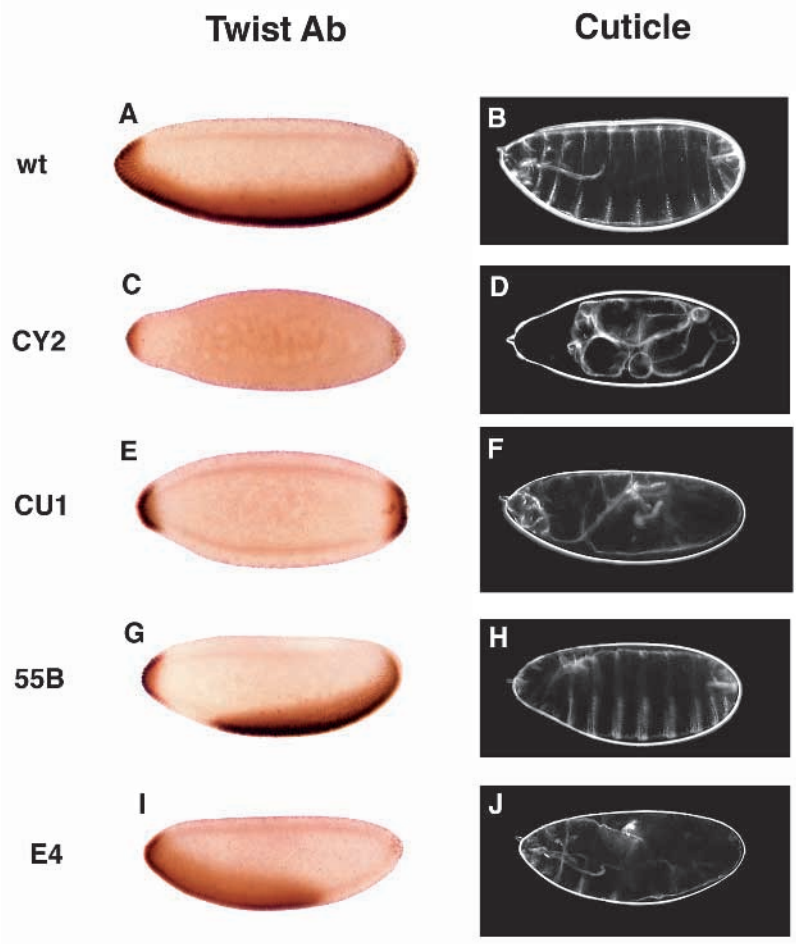

Fig. 7. Expression of λtop in follicle cells dorsalizes the embryo. (A,B) Wild-type controls. (A,C,E,G,I) Twist antibody staining of blastoderm embryos. (B,D,F,H,J) Embryonic cuticles from females carrying λtop and Gal4 line. (C,D) CY2; (E,F) CU1; (G,H) 55B; (I,J) E4. (Anterior to the left).

Different levels of activated Top/Egfr result in different follicle cell fates

Among the anterior follicle cells, a minimum of three cell fates can be distinguished by their contribution to the final eggshell morphology. The most dorsal cells produce the midline/operculum region, dorsolateral cells secrete the respiratory appendages and the ventral cells contribute to the main body of the eggshell. Activation of *top/Egfr* ectopically in the anterior follicle cells causes dorsalization of the eggshell. Our results demonstrate that increasing expression of the λtop construct correlates with follicle cells assuming more dorsal cell fates. For example, low levels of λtop expression, using the Gal4 line 55B, generate dorsalized phenotypes that range from occasional extra patches of dorsal appendage material to expansion of the dorsal midline between the appendages. The Gal4 lines T155 and CU1, expressing λtop more strongly, result in a phenotype in which most of the anterior follicle cells assume the dorsolateral or appendage producing fate. In CY2, the most strongly expressing Gal4 line used in our study, the anterior circumference of the eggs showed the most dorsal cell fate, the midline/operculum region. In addition, different λtop insertions, when tested with the same Gal4 line, showed this same range of dorsalized phenotypes. Finally, increasing the temperature, and presumably the levels of λtop expression, results in a more dorsalized eggshell phenotype. Taken together, our results demonstrate that the levels of activated receptor expressed can determine which of the three fates is acquired by the anterior follicle cells.

The *top/Egfr* signaling process has been ectopically activated in other experiments by mislocalization of Gurken, by expression of activated Raf and Ras, and by overexpression of Rhomboid. The phenotype most often observed is an expansion of the dorsolateral appendage fate (Ruohola-Baker et al., 1993; Brand and Perrimon, 1994; Neuman-Silberberg and Schüpbach, 1994). In contrast, with λtop and our strongest expressing Gal4 line, we observed the dorsal midline/operculum cell fate expanded all around the anterior circumference of the egg. It is possible that the earlier ectopic activation experiments did not achieve a high enough level of signaling activity to promote dorsal midline/operculum cell fates. Alternatively, the activity of the gene *aos* may explain the difference in phenotypes. We have shown that *aos*, a negative regulator of *top/Egfr* signaling, is expressed in response to activation of *top/Egfr* in the midline cells. *aos* encodes a secreted factor that can negatively regulate receptor activity in cells adjacent to the *aos*-expressing cells (Freeman, 1997; Schweitzer and Shilo, 1997)**.** Therefore, *aos*, and possibly other negative factors, could lead to secondary modulations or to a slight decrease in the response of follicle cells from the dorsal midline to the dorsolateral fates. In the earlier experiments, ectopic activation of *top/Egfr* signaling may lead to an expansion of *aos* expression, which in turn would result in expansion of dorsal appendage fates. However, the activated λtop used in our experiments lacks a ligand-binding domain and, presumably, would not be downregulated by *aos*. Receptor activity would remain unmodulated and induce dorsal midline fates in all anterior follicle cells.

Target genes differ in their response to uniform activation of Top/Egfr in the follicle cells

Expression patterns of genes known to respond to *gurken/torpedo* signaling were analyzed by in situ hybridization. The three populations of follicle cells in the anterior of the developing egg chamber express different combinations of downstream genes. The dorsal midline cells express *rho, kek1, pointed* and *aos* (Ruohola-Baker et al., 1993; Morimoto et al., 1996; Musacchio and Perrimon, 1996; this work). The dorsolateral cells express *rho* and *kek1*. The ventral follicle cells are distinguished by the expression of CF2 (Hsu et al., 1996) Among the target genes tested, some show a very direct response to *top/Egfr* activation, others appear to require additional factors.

We expressed λtop in all of the follicle cells that cover the oocyte from stage 8 onward with the Gal4 line CY2. Differences in the anterior/posterior response to activated *top/Egfr* were seen in the resulting expression patterns. *kek1* expression recapitulated the expression pattern of λtop and represents the simplest response to ectopic *top/Egfr* signaling. *aos*, while also expressed along the entire anterior/posterior axis, showed a more complicated expression pattern. Although expression of λtop was uniform in the follicle cells by in situ analysis, the in situ hybridization to *aos* revealed a strong activation in the anterior follicle cells, but a very patchy pattern in more posterior follicle cells. The expression pattern of *rho* was also expanded in the anterior follicle cells, indicating that the gene does respond to Top/Egfr activation. However, the response was restricted to the anterior follicle cells, even when λtop was expressed in the entire follicular epithelium. Both the patchy expression of *aos* and the lack of expression of *rho* demonstrate that the follicle cells contain an anterior/posterior prepattern. The *rho* promoter, and to a lesser extent, the *aos* promoter, appear to require some factor(s) supplied by the anterior/posterior system in addition to activation of Top/Egfr to be effectively transcribed. One candidate molecule for the regulation of this prepattern is the TGF-β-like protein, *dpp*. The expression of *dpp* in the anterior follicle cells of the egg chamber, and mutant and ectopic expression phenotypes (Twombly et al., 1996) suggest a role for *dpp* in this anterior/posterior patterning . As a final result of these differential requirements for both Top/Egfr activation and anterior/posterior factors, production of operculum and appendage material is restricted to the anterior region of the egg.

Anterior and posterior groups of follicle cells respond to *top/Egfr* activation independently of one another to pattern the embryo

At any one time during oogenesis, there is only a small group of follicle cells in contact with the region of the oocyte where high amounts of Gurken protein are present. Several of the known response genes are seen expressed in an anterior dorsal patch, and only the anterior of the eggshell produced ectopic dorsal structures, raising the possiblity that only the anterior follicle cells are responding to *gurken/torpedo* signaling. However, we found that posterior follicle cells respond to *top/Egfr* signaling and pattern the embryo in a local manner, as was shown by examination of the dorsal/ventral pattern of the embryos from females expressing λtop. The predicted embryonic phenotype from activation of Top/Egfr is dorsalization. Examination of embryonic cuticles and Twist-expression pattern verified this prediction; the embryos were dorsalized in response to activated *top/Egfr*. By confining the expression of λtop to subpopulations of follicle cells, we dis-

covered that localized activation of Top/Egfr in a region of follicle cells can locally affect the embryo. For example, if λtop is expressed in the anterior follicle cells with the Gal4 line 55B, the anterior of the embryo is dorsalized whereas the posterior follicle cells are still able to confer the correct dorsal/ventral pattern to the embryo. Conversely, when the posterior of the embryo is dorsalized with the Gal4 line E4, the anterior can still regulate the embryonic dorsal/ventral pattern normally. Therefore, both the anterior and the posterior follicle cells respond directly to Top/Egfr signaling. This result is consistent with a model where activation of Top/Egfr occurs all along the anterior/posterior axis to regulate the signal back to the embryo. Since Gurken protein is present as a localized patch in the anterior of the oocyte, the dorsal follicle cells may be activated as they migrate over this patch of Gurken. Our observation rules out a model of a dorsal anterior point source of Top/Egfr activation affecting the entire embryo by posterior propagation through secondary signals. A point source model would predict that expressing λtop in the critical region would affect the entire embryo, whereas expression outside of the critical domain would not affect embryonic pattern. This was, however, not observed.

In conclusion, we have used λtop to examine how ectopic activation of *top/Egfr* affects cell fate determination and the resulting dorsoventral patterning of the eggshell and embryo. Expression of λtop dorsalizes both the eggshell and the embryo, with more severe dorsalization seen with increasing expression of λtop. The restriction of ectopic appendage material and *rho* expression to the anterior revealed a prepattern in the follicular epithelium along the A/P axis. In addition, we have shown that activating Top/Egfr in subpopulations of follicle cells dorsalizes the embryo in a locally restricted manner.

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