The neurogenic genes *egghead* and *brainiac* define a novel signaling pathway essential for epithelial morphogenesis during *Drosophila* oogenesis

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SUMMARY

*Notch* (*N*) and other neurogenic genes have been implicated in two fundamental processes, lateral specification of cell fates, and epithelial development. Previous studies have suggested that the neurogenic gene *brainiac* (*brn*) is specifically required for epithelial development (Goode, S., Morgan, M., Liang, Y-P. and Mahowald, A. P. (1996). Development 122, 3863-3879). In this report we show that *egghead* (*egh*), a gene with phenotypes identical to *brn*, encodes for a novel, putative secreted or transmembrane protein. We describe the role of *egh* and *brn* germline function in the morphogenesis of the follicular epithelium from the time it is born through the time that it migrates towards the oocyte late in oogenesis.

By comparing the function of germline *egh* and *brn* to *N* during oogenesis, we have obtained direct evidence for the involvement of Notch in maintenance of the follicle cell epithelium, and the specificity of *brn* and *egh* in epithelial development during oogenesis. The most striking phenotype observed for all three genes is a loss of apical-basal polarity and accumulation of follicular epithelial cells in multiple layers around the oocyte. The spatiotemporal onset of this adenoma-like phenotype correlates with the differential accumulation of *egh* transcripts in the oocyte at stage 4 of oogenesis. In contrast to *N*, we find that *brn* and *egh* are essential for the organization, but not specification, of stalk and polar cells.

The expression patterns and functional requirements of *brn*, *egh*, and *N* lead us to propose that these genes mediate follicular morphogenesis by regulating germline-follicle cell adhesion. This proposal offers explanations for (1) the involvement of *egh* and *brn* in *N*-mediated epithelial development, but not lateral specification, (2) why *brn* and *egh* embryonic neurogenic phenotypes are not as severe as *N* phenotypes, and (3) how *egh* and *brn* influence Egfr-mediated processes. The correlation between the differential expression of *egh* in the oocyte and the differential requirement for *brn*, *egh*, and *N* in maintaining the follicular epithelium around the oocyte, suggests that Egghead is a critical component of a differential oocyte-follicle cell adhesive system.

Key words: Notch, epithelium, cell interaction, morphogenesis, neurogenic genes, oogenesis, signaling pathway, *Drosophila*

INTRODUCTION

Epithelia are one of the universal architectural units used in the development and maintenance of animal form. Epithelial cells are arranged in sheets that have distinct apical-basal polarity. The different shapes of organs develop from the regulated molding of epithelial structure via tissue-specific morphogenetic programs. These programs govern the complex integration of epithelial cell behaviors, including division, rearrangement, shape, shape changes and the recruitment or loss of cells from the epithelium.

As a model to understand epithelial morphogenesis, we are analyzing the simple monolayer follicular epithelium that develops in concert with the differentiating germline during *Drosophila* oogenesis. The follicular epithelium has developmental, morphological, and molecular properties of vertebrate epithelia (Fig. 1). Further, *Drosophila* oogenesis is amenable to genetic analysis, making it an attractive model for identifying molecules involved in epithelium formation. In this report we present the molecular and functional characterization of the gene *egghead* (*egh*), which encodes a novel molecule that is likely to be crucial for the adhesion of the follicular epithelium to germ cells throughout oogenesis.

In both invertebrates and vertebrates, cell adhesion molecules have been shown to play an essential role in epithelial organization (reviewed by Gumbiner et al., 1992). For example, in vertebrates and *Drosophila*, E-cadherin cell adhesion molecules are required for the formation and maintenance of epithelial structure, particularly in epithelia undergoing complex morphogenetic rearrangements (reviewed by Takeichi, 1995; Tepass et al., 1996; Uemura et al., 1996). Key mediators of cadherin function are cytoplasmic α- and β-catenin, which bind to both the cytoplasmic domain of cadherins and to f-actin filaments, providing a crucial link between adhesion and regulation of cell shapes and movements.
A second class of cell surface factors essential for epithelial development are receptor tyrosine kinases and their ligands. For example, vertebrate hepatocyte growth factor and p190MET receptor tyrosine kinase induce chemotactic migration, proliferation, and differentiation of epithelial cells, and elicit epithelial tubule morphogenesis (Naldini et al., 1991). Epidermal growth factor receptor (Egfr) mutant mice suffer from multi-organ failure due to widespread impairment of epithelial development (Miettinen et al., 1995), and mice with reduced levels of one Egfr ligand, transforming growth factor α (TGFα), share some of these developmental abnormalities (Luetteke et al., 1993; Mann et al., 1993). Egfr and grrk TGFα mutant Drosophila show similar abnormalities. Disruption of Egfr in follicle cells or TGFα signals in germ cells results in loss of dorsal-ventral patterned array of follicle cell shapes and impaired development of the follicular epithelium (Schübäch, 1987; Goode et al., 1996). The discontinuities of the monolayer follicular epithelium observed in TGFα or Egfr mutant Drosophila are reminiscent of the extreme thinning observed in stratified epithelia of newborn Egfr mutant mice (Goode et al., 1996; Miettinen et al., 1995).

A third class of molecules essential for epithelial development are encoded by the neurogenic genes. Although intensively studied for their function in lateral specification (reviewed by Artavanis-Tsakonas et al., 1995), the Drosophila neurogenic genes Notch (N), Delta, neuralized, and Enhancer of split are also essential for the development of epithelial characteristics in embryonic tissues in which no obvious cell fate choices are in question. For example, loss of neurogenic function causes invaginating midgut rudiments to prematurely loose their epithelial characteristics during gastrulation; following gastrulation, the transition of midgut rudiments to form the midgut epithelium does not occur (Hartenstein et al., 1992). In Xenopus, expression of an activated form of the N homolog Xotch expands epithelial neural tube tissue and abolishes mesenchymal neural crest cells (Coffman et al., 1993). During Drosophila oogenesis, N and DI are crucial for regulating bipotential cell fate decisions between follicle cells (Ruohola et al., 1991; Xu et al., 1992), but their role in the morphogenesis of the follicular epithelium remains to be investigated.

Previous studies have suggested that the neurogenic gene brainiac (brn) plays a specific role in epithelial development. Although the early embryonic brn phenotype is similar to that of mutants of the neurogenic genes, brn does not appear to be required for several bipotential cell fate decisions that utilize the N signaling cassette (Goode et al., 1996). brn encodes a novel, putative secreted protein that is required in the germline for establishing the follicular epithelium and for determining its dorsal-ventral polarity (Goode et al., 1992; Goode et al., 1996). brn mutations show synergistic genetic interactions with TGFα and Egfr mutations at each step in which these genes are essential in the morphogenesis of the follicular epithelium (see above), consistent with an essential requirement for brn in epithelial development. In this study, we directly characterize the function of brn, and an additional gene with identical phenotypes, egf, in epithelial development during oogenesis, and compare the requirement of brn and egf with the function of N in this process.

We find that egf encodes a novel, putative secreted or transmembrane protein, egf expression in the germline correlates with morphogenesis of the follicular epithelium throughout oogenesis. In the absence of egf or brn function, formation of the follicular epithelium is less efficient, frequently resulting in a failure to individuate germline cysts. These genes are also essential for maintaining the epithelial characteristics of the follicular epithelium once it is formed. When brn or egf germline function is disrupted, follicle cells lose their apical-basal polarity and accumulate in multiple layers, particularly around the oocyte. Further, egf and brn are essential for the efficient migration and maintenance of border cells and main body epithelial cells when they move towards the oocyte during the late phases of oogenesis. These results suggest that egf and brn are germline components of a differential oocyte-follicle cell adhesive system.

Strikingly, we find that neither brn nor egf is essential for regulating the bipotential cell fate decisions that N has previously been implicated in during oogenesis. In contrast, we find that in addition to its role in cell fate specification, N is also essential for maintaining the epithelial characteristics of the follicular epithelium. Since brn egf double mutant animals display phenotypes no more severe than either mutant alone, we suggest that these genes function in a novel signaling pathway specific for epithelial morphogenesis. We propose that Brainiac and Egghead, originating from the germline, collaborate with Notch on the apical surface of follicle cells to mediate germline-follicle cell adhesion, and discuss our results in this context to explain how these genes may influence Egfr mediated processes.

**MATERIALS AND METHODS**

**Genetics**

All egf mutations used in this study are described in Table 1. brn mutations are described by Goode et al. (1992). Most brn and egf mutations were recombined with FRT101, and germline clone (GLC) analysis was completed as described by Chou and Perrimon (1992). For egf mutations egf62d18 and egf65B2, GLC analysis was completed using X-irradiation as described by Perrimon et al. (1989). Except for experiments involving conditional mutations, all experiments were completed at 25°C on standard Drosophila medium. Nps1 phenotypes were analyzed following a shift of females to 32°C for 36 hours. ovoDel (Del) is described by Oliver et al. (1987).

**Histology and microscopy**

For all stainings except with tPS1 integrin sera, ovaries were fixed for 20 minutes in 4% ultrapure, methanol-free, EM-grade formaldehyde (Polysciences, Inc.) in PBS, rinsed in PBS, then teased apart into ovarioles. F-actin stainings were completed as described by Goode et al. (1996). Antibody and propidium iodide staining were completed as described by Orsulic and Peifer (1994). α-PS1-integrin stainings were completed on unfixed, non-permeabilized tissue as described by Brower et al. (1984).

For double stainings with 4.1 and phosphotyrosine (PT) sera, tissue was first incubated with monoclonal 4.1 (Feihon et al., 1994) and a rhodamine-conjugated secondary, then incubated with anti-PT monoclonal antibody directly conjugated to a fluorescein fluorophore (US Biochemical) at a concentration of 1:100. All tissues were analyzed with a conventional epifluorescence, or a Biorad laser scanning confocal microscope, attached to a Zeiss Axiophot microscope.

Embryonic Hoyer’s mounts, sections, and HRP-antibody stainings were completed as described by Goode et al. (1992).

**Follicle cell division patterns**

Mitotic follicle cells were scored using f-actin and DNA stained with Hoechst 33258. For double stainings with 4.1 and phosphotyrosine (PT) sera, ovaries were fixed in 4% ultrapure methanol-free, EM-grade formaldehyde and sections were completed as described by Goode et al. (1996).
RESULTS

egghead phenotypes are similar to brainiac
To account for both the neurogenic and ovarian defects associated with zw-f mutations (Table 1; Robbins, 1983; Perrimon et al., 1989), we have renamed the gene egghead (egh). Females with germ line clones (GLCs) of egh mutations have phenotypes similar to brn. They lay a few eggs with fused dorsal appendages and the embryos have an expanded nervous system (this study; Perrimon et al., 1989; Goode et al., 1992). Only a few eggs are laid because early oogenesis is blocked due to the development of egg chambers containing multiple oocyte-nurse cell complexes.

egh and brn do not appear to be required for regulating germ cell division
Wild-type (WT) oogenesis is described in detail in Fig. 1. Since previous results indicated that brn is required in the germ line for formation of the follicular epithelium, we characterized markers that would distinguish germ cells from follicle cells, and reveal the epithelial characteristics of the follicular epithelium. Phosphotyrosinase (PT) substrates have been shown to be enriched in epithelial cell junctions and sites of cell contact in vertebral epithelial cells (Maher et al., 1985; Takata and Singer, 1988), and we find a similar distribution of these substrates in follicular epithelial cells (Fig. 1, turquoise). In addition, PT substrates are localized in the cortex of germ cells (this study), and in specialized structures that interconnect germ cells, called ring canals (Robinson et al., 1994). In contrast, membrane protein 4.1 is specifically localized in follicle cells (Fig. 1).

GLCs of mutations in either brn or egh produce egg chambers containing more than one oocyte-nurse cell complex (Fig. 2A,B; Table 2). To rule out the possibility that egh mutant egg chambers result from supernumerary germ cell divisions, we stained egg chambers with PT and analyzed the ring canal pattern of oocytes as previously described for brn (Goode et al., 1992). As for WT and brn, oocytes in egh egg chambers have only 4 ring canals (Fig. 2B), indicating that egh does not inhibit germ cell divisions.

A second class of egg chambers observed less frequently in brn and egh mutant ovaries contain one oocyte with less than 15 nurse cells, or contain more than one oocyte, but without the normal complement of nurse cells (Fig. 2C; Table 2). These egg chambers may result from a deficit of germ cell division, but this does not seem likely since brn and egh transcripts do

<table>
<thead>
<tr>
<th>Allele</th>
<th>Mutagen</th>
<th>Lethal phase</th>
<th>References</th>
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<td>9PP4</td>
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<td>pharate adult</td>
<td>Perrimon et al., 1989</td>
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<td>EMS</td>
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<td>63s4</td>
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<tr>
<td>GA107</td>
<td>X-ray</td>
<td>pupal</td>
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*Experiments were conducted at 25°C. All alleles fail to complement each other and have similar maternal effect neurogenic and ovarian phenotypes.

tissues. Confocal microscopy was used to obtain a complete z-series at 1 μm intervals for each egg chamber analyzed. Since egg chambers have an ellipsoid shape, each z-section is an ellipse (Fig. 1), with A/P length of each ellipse decreasing away from the egg chamber’s center. To determine the position of dividing cells from the posterior pole, the position of each cell from the posterior in a given z-section, as well as the length of the z-section, was established. All measurements were obtained at 1 μm resolution using a scale bar tool (Biorad software package).

The center of each egg chamber is the z-section(s) with the longest A/P axis. For dividing cells in this plane, the distance from the posterior is equal to the measured distance. For dividing cells above and below the center plane, the distance from the posterior must be adjusted to take account of the ellipsoid’s curvature. The following formula was used: actual distance from posterior = measured distance + (A/P length of center z-section – A/P length of the z-section including the dividing cell)/2.

Molecular biology
Two egh cDNAs were cloned from the 0- to 4-hour embryonic cDNA library described by Brown and Kafatos (1988) using a 2.5 kb EcoRI fragment as probe. These cDNAs and the 18XS genomic fragment were sequenced by standard methods and the sequence analyzed using the GCG package (Genetics Computer Group, Wisconsin, 1994). Kyte-Doolittle hydropathy analysis was completed with the DNASTAR program.

A transformation construct with the egh open reading frame behind the heat shock promoter was made by introducing BglII and XbaI restriction sites at the 5¢ and 3¢ ends of the egh ORF, respectively, using PCR. This product was then cloned into pCaSpeR-hs (Thummel program).

The numbers in the table are percentages based on the total number of egg chambers scored (number scored). *OO, oocyte. †Oocyte detached or misplaced, but not found at anterior pole. ‡NS, not scored. §The number in parenthesis is the actual number with 1 oocyte, which accounts for the percentages in the next two columns.

Table 1. egh alleles

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In situ hybridization
In situ hybridizations were completed as described by Goode et al. (1996), using an antisense RNA probe generated from the T7 promoter in egh cDNA 4e (described in the Results).
Fig. 1. Morphogenesis of the wild-type follicular epithelium is organized along the anterior-posterior axis of egg chambers starting early in oogenesis. The figure shows a composite of confocal images from an ovariole of an adult Drosophila female. The ovariole is double-stained with sera to phosphotyrosine (PT), which recognizes both germ and follicle cell tissue (white/turquoise), and protein 4.1 (Fehon et al., 1994), which is specifically localized in follicle cells (pink/red). The exclusion of PT and 4.1 staining from germ and follicle cell nuclei and late stage oocytes is evident as false black color.

As seen in the stage 6 (s6) follicle, the germline is composed of 15 polyploid nurse cells located anteriorly (large nuclei, only 6 revealed in this section), and a diploid oocyte at the posterior (arrow). PT substrates, but not 4.1, are localized to the cortex of all 16 germ cells (turquoise), and in specialized donut-shaped structures, called ring canals (obvious on the oocyte and some nurse cells), that interconnect the germ cells to form a syncytium. 4.1 is specifically localized in follicle cells (red). PT colocalizes with actin on the apical surface of follicle cells that abut the germ line, in zonulae adherens that interconnect apical membranes of follicle cells (green arrowheads), and in lateral follicle cell membranes.

Follicle cells (red) are associated with dividing germ cells (turquoise) as cysts are produced in gerarium region I (cf. Mahowald and Strassheim, 1970). Once cysts have matured to 16 cells at the posterior of region I, follicle cells ensheathe the egg chamber at the beginning of region IIa (see also Fig. 3A). PT begins to accumulate in follicle cells at the posterior of cysts (white, arrowheads; this study), and in ring canals (Robinson et al., 1994). PT staining in posterior follicle cells is the first manifestation of anterior-posterior polarity, and is dramatic by the time egg chambers span the width of the gerarium in region IIb (blue arrow), when ring canals are aligned at the anterior of the egg chamber (yellow arrow), and the oocyte begins to differentiate.

Following these primary organizational events along the A/P axis, germ cells elongate first posteriorly, then anteriorly, to produce a spherical s1 egg chamber. The oocyte is positioned at the posterior. Simultaneous follicle cell division produces an epithelium of approximately 80 cells. Apical accumulation of PT substrates in all follicle cells marks the establishment of the follicular epithelium, while strong PT staining persists in polar and intercalating follicle cells that will form a stalk to connect successive egg chambers (white arrow). The egg chamber is ready to begin growth in the vitellarium.

The spheroidal s2 egg chamber has a simple, monolayer, cuboidal follicular epithelium. The egg chamber elongates to an ellipsoid between s3 and s4. At s6, when follicle cells stop dividing, the epithelium has grown to approximately 1200 cells. Between s6 and s8, oocyte-associated nurse cells protract anteriorly, as the oocyte flattens across posterior follicle cells. By s8, columnar follicle cells associated with the oocyte show a clear posterior to anterior height gradient. At s9, most main-body epithelial cells migrate towards the oocyte in concert with a few mesenchymal-like, PT-rich, border cells that break from the epithelium to move through the center of the egg chamber (blue arrow).

not appear to be expressed in germ cells when they are dividing (Figs 1, 11A,B; Goode et al., 1996). Further, hu-li tai shao (hts) mutations specifically block germ cell divisions, but in contrast to brn and egh GLC females, hts females never produce egg chambers with more than one oocyte-nurse cell complex (Yue and Spradling, 1992), indicating that completion of germ cell division is not requisite for individuating germ cell cysts. Rather, egg chambers from egh and brn GLCs that contain too few germ cells probably result from splitting of cysts in the gerarium, since these egg chambers are frequently arranged in complementary patterns, with sister cells of comparable size (Fig. 2C). We provide direct evidence for this hypothesis in the next section.

eygh and brn are required for formation of the follicular epithelium

The analysis of brn and egh GLC egg chambers presented in the previous section indicated that these genes are not required for regulating germ cell divisions. To determine the primary defect in these mutants, we directly analyzed the process of egg chamber formation using confocal microscopy to visualize the distribution of α-spectrin (Byers et al., 1987). α-spectrin has previously been localized in a branching structure that runs throughout germ cell cysts, called the fusome (Lin et al., 1994; Fig. 3A). In addition, we find that α-spectrin recognizes follicle cell processes as follicle cells begin to surround and envelop newly formed 16-cell cysts (Fig. 3A). Follicle cell processes extend from cell bodies of prefollicle cells that line the gerarium at the junction of regions IIa/b. These cells may be epithelial stem cells, or stem cell daughters, as stem cells have been localized to the IIa/b junction (Margolis and Spradling, 1995; Fig. 3A). Germ cell processes are absolutely essential for the extension of follicle cell processes and formation of a monolayer follicular epithelium, since these processes fail to occur in germaria that lack germ cells (Fig. 3A,B).

Likewise, in brn and egh GLC animals, follicle cells fail to extend processes, leading to a failure of germ cells to rearrange like WT cyst cells in region IIa (Fig. 3C). Thus, as previously suggested, brn and egh appear to disrupt a germline to follicle
Fig. 2. brn and egh do not regulate germ cell division. (A-C) Analysis germ cells in egh mutant egg chambers. (A) An ovariole from an egh female stained with DAPI reveals nuclei of germ and follicle cells. Each egg chamber in the gerarium (arrows, v) has supernumerary nurse cells (large nuclei) and oocytes (not visible); (g) gerarium. (B) An egg chamber from an egh female stained with propidium iodide and PT to reveal nuclei (red), and ring canals (green). On the left, two sections through the same egg chamber reveal all three oocytes (diploid nuclei, arrows). A complete z-series through each oocyte is shown on the right. As for WT, each oocyte contains four ring canals (numbered). (C) Complementing egg chambers from an egh GLC female stained with PT. The posterior egg chamber has one oocyte and 12 nurse cells, while the anterior egg chamber contains 3 complementary nurse cells, giving the WT oocyte to nurse cell ratio of 1:15.

cell signaling process essential for individuating germ cell cysts. Another possibility is that brn and egh are required in germ cells for cyst cell rearrangement, and that cyst cell reorganization drives follicle cell extension. We do not feel this is likely since (1) mutant germ cells undergo a large degree of normal differentiation (Figs. 2, 4), and (2) both genes are required in the germ line at later stages for the maintenance of the follicular epithelium (described below). Our results do not rule out the possibility that cyst cell reorganization and follicle cell extension is a cooperative process requiring continual signaling between these tissues.

In addition to a failure to extend processes, follicle cells in brn or egh GLC germaria display a number of unusual behaviors. Follicle cells sometimes appear organized as an epithelial sheet within the gerarium (Fig. 3F), perhaps resulting from the aberrant movement of pre-follicle epithelial cells. This interpretation could explain how egg chambers containing multiple oocyte-nurse cell complexes emerge from the gerarium. Further, follicle cells sometimes appear to have migrated in between germ cells, not around them, perhaps splitting them in two (Fig. 3D,E). Alternatively, inappropriate interactions between follicle cells and germ cells may stretch cysts, pulling them apart (Fig. 3C). Both of these mechanisms may contribute to the production of complementary cysts (Fig. 2C).

egh and brn are required for maintaining the follicular epithelium

After egg chambers exit the gerarium, brn and egh are required in the germline for maintaining the follicular epithelium. In brn and egh GLC egg chambers, follicle cells frequently lose their cuboidal or columnar shape and exit the epithelium around the oocyte and its adjacent nurse cells (Table 3, Fig. 4A-D). Follicle cells have clearly lost polarity, since alpha-spectrin is localized on lateral membranes of follicular cells in WT egg chambers (Fig. 4A), whereas in brn and egh GLC egg chambers, alpha-spectrin accumulates ectopically or around the circumference of follicle cells (Fig. 4B). A surface view of mutant epithelia reveals that whereas WT follicle cells have hexagonal shapes arranged as an isotropic honeycomb array, follicle cells in brn and egh GLC egg chambers can be quadrilateral or pentagonal and arranged anisotropically (Fig. 4C). Further, follicle cells which are exiting the follicular epithelium have a rounded, apolar, mesenchymal-like shape compared to the polygonal architecture of follicle cells in the main body follicular epithelium (Fig. 4C).

Exiting follicle cells are observed in brn and egh GLC egg chambers containing a single oocyte and 15 nurse cells, suggesting that the phenotype is not linked to a problem in epithelium formation (see previous section). This hypothesis is further supported by determination of the earliest stage at which follicle cells exit the epithelium. Follicle cells exit the

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**Table 3. Stage specificity of dislocated cells**

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<th>Genotype</th>
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<td>2-3</td>
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<tr>
<td>brn^h107 (25°C)</td>
<td>126</td>
<td>0</td>
</tr>
<tr>
<td>brn^h107 (18°C)</td>
<td>139</td>
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</tr>
<tr>
<td>brn^h496</td>
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<tr>
<td>egh^DF944 brn^h496</td>
<td>215</td>
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</table>

*Only egg chambers having a single nurse cell-oocyte complex were scored. For brn^h107, this was essentially every egg chamber (see text). For all other mutations, dislocated cells associated with egg chambers having multiple oocyte-nurse cell complexes are not included in this table. An egg chamber was scored positive if at least one dislocated cell was observed.

†All of these egg chambers were s7 (none were s6).
which abuts the apical surface of the follicular epithelium. We focused on the apical membrane domain of follicle cells since follicle cells. We analyzed their involvement in inhibiting follicle cell divisions in maintaining the monolayer follicular epithelium, we formed in the germarium.

egh brnfs.107 morphogenetic transitions of follicle cell division. In s7 defects associated with single follicle cells, independent of the germarium two 16-cell cysts are recognized by the accumulation of oocyte of mutant egg chambers compared to WT, correlating with the dramatic propensity of follicle cells to exit in this region (Figs. 4B, 5D). This analysis does not distinguish between two alternatives for how brn and egh might inhibit follicle cell division:

Role of brn and egh in inhibiting follicle cell division

Since follicle cells accumulate in multiple layers around the oocyte of mutant egg chambers, we sought to determine if follicle cell divisions occur more frequently in this region of the epithelium. We compared numbers of mitotic follicle cells as a function of position along the anterior-posterior axis of WT and eghGA107 GLC egg chambers. We found approximately 80% more mitotic follicle cells around the oocyte of mutant egg chambers compared to WT, correlating with the dramatic propensity of follicle cells to exit in this region (Figs. 4B, 5D). This analysis does not distinguish between two alternatives for how brn and egh might inhibit follicle cell division:

(1) these genes might inhibit follicle cell division as well as maintaining the polarity of follicle cells, or
(2) these genes might only be required for maintaining follicle cell polarity, with increased follicle cell divisions reflecting a
requirement to replace exiting cells. Based on the following observations, we favor the second hypothesis.

As in WT, we never observe follicle cells dividing in s7 to s10 brn and egh GLC egg chambers, suggesting that these genes do not play a role in prohibiting follicle cell divisions during the late phases of oogenesis. In contrast, discslarge does appear to play such a role, since in discslarge mutant animals mitotic follicle cells are observed after s6 (S. G., unpublished). Further, in animals harboring the weak mutation brn<sup>6-107</sup>, follicle cells never exit the epithelium before s7, when follicle cells have normally stopped dividing (Table 3). Likewise, in strong egh and brn mutant backgrounds follicle cells rarely accumulate in more than a single layer before s7, yet cells can accumulate in multiple layers by s10 (Fig. 4D). The most discernible requirement for brn and egh in regulating follicle cell divisions correlates with their role in maintaining follicle cell polarity. In egh and brn GLC egg chambers, follicle cells sometimes divide perpendicular to, instead of within the plane of the follicular epithelium, resulting in at least one sister cell ending up outside the epithelium (Fig. 5A-C).

**egh and brn are required for induction of dorsal follicle cell fates, but not specification of polar/stalk cell fates**

N signals participate in a lateral specification mechanism that regulates the ratio of polar precursor to stalk cells as egg chambers exit the gerarium, as well as the ratio of polar to ‘flanking cells’ during later stages of oogenesis (Ruohola et al., 1991). Using FasIII as a marker for polar cells (Ruohola et al., 1991), and a new marker, α-PS1-integrin, as a marker for stalk cells (Fig. 6A,B), we confirmed the misappropriate development of these cells in N<sup>ts1</sup> animals (Fig. 6C-E). In contrast, we do not observe supernumerary polar cells in brn and egh GLC egg chambers (Fig. 6HJ). Further, although stalks separate brn and egh GLC egg chambers, they are typically disorganized because the cells making up the stalks are rounded compared to WT disc-shaped cells (Fig. 6F,G1).

brn signals have been shown to be essential in the germline at stages 6-7 of oogenesis for establishing dorsal follicle cells (Goode et al., 1992, 1996), and egh is differentially expressed in the oocyte at this time (see below, Fig. 11). As for brn animals, we observe that egh GLC animals lay eggs with fused dorsal appendages, suggesting a shift of dorsal follicle cell fates to more dorsal-lateral fates (Fig. 7A,B). We confirmed this by analyzing the expression of an enhancer trap inserted in the *kekkon-1* gene (*kek-1*; Musacchio and Perrimon, 1996), which is expressed in a broad dorsal to ventral gradient in dorsal follicle cells at s10 (Fig. 7C). In egh GLC egg chambers, *kek-1* expression is significantly eliminated in dorsal-lateral follicle cells, consistent with a shift of ventral-lateral cells to more dorsal positions (Fig. 7D). This alteration in gene expression occurs independently of whether follicle cells have exited the epithelium (not shown).

**egh and brn are required for concerted border cell-main body epithelium migration**

Polar cells at the anterior of the egg chamber are part of a larger group of cells, termed border cells, that break from the main body epithelium (MBE, i.e. that portion of the epithelium that maintains a cuboidal/columnar phenotype and ends up covering the oocyte), become mesenchymal-like, and migrate through the center of the egg chamber at s9 of oogenesis (Fig. 1). slow border cells (*slbo*) is specifically expressed in border cells starting at s8 of oogenesis (Fig. 8A; Montell et al., 1992). Although follicle cells at the posterior of brn and egh mutant egg chambers become mesenchymal-like (Fig. 4C), they do not express *slbo*, indicating that they have not adopted a border cell fate (Fig. 8C).

Instead, analysis of *slbo* expression at s8-s10 in these mutants reveals that the migration of the MBE and border cells is not always concerted. The MBE frequently move ahead of, and completes its migration well before, the migration of the border cells (Fig. 8B,D,E). The shape of the migrating epithelium is sometimes unusual (Figs 3, 8D). Further, border cells frequently split apart before or during their migration (Fig. 8E,F), and sometimes remain with the thin squamous follicle cell population that covers nurse cells (Fig. 8F). Once the MBE has completed its migration, *slbo* expression initiates at the anterior and posterior of the oocyte, but expression appears weaker compared to that in WT (Fig. 8B,F). All of these defects are independent of whether multiple layers of follicle cells accumulate around the posterior of the oocyte, and are consistent with a requirement for brn, egh, and N in regulating the relative epithelial state of the follicular epithelium during a dramatic morphogenetic reorganization.

**N is also required for the development and maintenance of the follicular epithelium**

The results presented thus far indicate that egh and brn are essential for the development and maintenance of epithelial structure, a function previously assigned to N and other neurogenic genes based on embryonic patterning defects (Hartenstein et al., 1992). Like *brn* and *egh* mutant animals, animals mutant for *N* frequently develop egg chambers containing multiple oocyte-nurse cell complexes (Xu et al., 1992), and *N* is required in the gerarium for formation of the follicular epithelium (S. G., data not shown). By s1 of oogenesis, N is localized on the apical and lateral surface of all follicular epithelial cells in a pattern indistinguishable from PT and f-actin (Fig. 4E; Xu et al., 1992). We analyzed s5 to 10 *N<sup>ts1</sup>* mutant egg chambers and found epithelial defects similar to those described for *brn* and *egh* (Fig. 4F). While Notch is expressed at low levels in the germline, these epithelial defects are not found in *N* GLC animals, indicating that as for its role in specifying stalk cells versus polar cells (Fig. 6), *N* is required in follicle cells for maintenance of the follicular epithelium. Further, *N<sup>ns1</sup>* egg chambers having multilayered epithelia may or may not have supernumerary polar cells (described above, not shown), indicating that *N* has separable functions in both cell fate specification and epithelial maintenance (see Discussion).

**Neurogenesis in egh and brn mutant animals**

We obtained additional evidence that egh and brn are not identical to N by comparing the neurogenic defects associated with these mutants. Although several egh alleles cause lethality as pharate adults (Table 1), like *brn<sup>6-107</sup>*, these animals never have ectopic bristles like other neurogenic mutants (Goode, 1994; not shown). Further, as shown above, *N<sup>ns1</sup>* animals develop extreme hyperplasia of polar cells during oogenesis, yet only develop a weak embryonic neurogenic phenotype (not shown). In contrast, egh and brn do not develop hyperplasia of
polar cells (Fig. 6), yet strong alleles of both mutants produce a differentiated maternal effect neurogenic phenotype that is intermediate in strength, indistinguishable from intermediate strength mutants of the neurogenic genes \( N \), \( Dl \), \( E(spl) \), or \( neu \) (Fig. 9; Goode et al., 1992; Lehmann et al., 1983). The phenotype is characterized by the absence of ventral and cephalic epidermal cells (Fig. 9A-B), a hypertrophy of the nervous system in corresponding territories (Fig. 9D-F), and a failure of the midgut epithelium to achieve a folded pattern like WT (Fig. 9B,E). In contrast, animals mutant for strong \( N \) alleles have a severe embryonic phenotype characterized by a very small patch of dorsal cuticle and extremely hypertrophied nervous system (Lehmann et al., 1983).

**egh brr** double mutants display phenotypes no more severe than either mutant alone

One explanation for the difference in the severity of the neurogenic phenotypes between \( brn \), \( egh \), and \( N \), consistent with our results, is that \( N \) is required for both cell fate specification as well as epithelial development, whereas \( brn \) and \( egh \) are strictly required for epithelial development. Alternatively, \( egh \) and \( brn \) may be functionally redundant for cell fate specification. We constructed \( egh brn \) double mutant animals to distinguish these possibilities. We found that double mutant animals do not display ovarian or embryonic phenotypes any more severe than either mutant alone (Fig. 9C, Table 4; data not shown), suggesting that these genes are not functionally redundant, but function in a common pathway.

**egh encodes a novel, putative secreted or transmembrane protein**

A genomic walk across the \( egh \) region, and characterization of a rearrangement in the putative \( egh \) gene, has been reported (Williams et al., 1995). We established that sequences in the
Fig. 5. Follicle cell division in WT and mutant egg chambers. (A) Cross section through a WT follicular epithelium stained with phalloidin (green), which recognizes actin cortices, and with propidium iodide (red), which recognizes follicle cell DNA. Follicle cell divisions take place in the plane of the epithelium. Dividing cells are distinguished by enriched membranous f-actin and heightened propidium iodide staining (due to DNA condensation). Four dividing cells at four cell cycle stages are evident (p, prophase; m, metaphase; a, anaphase; t, telophase). (B) A dividing follicle cell in telophase at the anterior pole of a s4 egg chamber. Curvature of the follicular epithelium at the poles of the egg chamber results in a component of the follicle cell division axis being perpendicular to the plane of the epithelium. (C) Occasionally, in contrast to WT, follicle cells divide perpendicular to the plane of the follicular epithelium in brn and egh mutant egg chambers. (D) Comparison of cell division frequency along the A/P axis of WT and mutant egg chambers with the position at which cells exit the monolayer follicular epithelium. The points plotted on the graphs were obtained by totaling the number of dividing cells between positions 0-20, 21-40, 41-60, 61-80, and 81-100, and plotting the total for each interval at positions 10, 30, 50, 70, and 90 on the graph, respectively. Red line; cell divisions along the A/P axis of WT s4-6 egg chambers occur at about equal frequency. Green line: the relative frequency of cell divisions in egh mutant egg chambers is approximately 80% greater around the oocyte compared to WT. Purple line: almost all follicle cells exit the epithelium of egh mutant egg chambers around the oocyte.

putative egh region (Fig. 10A) hybridize to a 3.7 kb transcript from 0-2 hour embryos, consistent with this region coding for a maternally expressed gene (not shown). To determine if this transcript corresponds to egh, we attempted to rescue egh mutations by transformation with a 7 kb genomic fragment, 18XS (Fig. 10A). 18XS rescues egh mutations, but does not complement mutations in a gene immediately proximal, KLP 3A (Fig. 10A).

We sequenced the genomic rescue fragment 18XS and isolated and sequenced egh cDNAs. CDNA 7 appears to be derived from a 2.2 kb egh transcript because it starts immediately downstream of a TATA box in genomic sequence and contains a complete ORF with good codon usage (Fig. 10B). cDNA 4e is collinear with CDNA 7 starting at 4e base pair 771, contains a complete ORF, and appears to correspond to a 3.7 kb egh transcript. Although the first 930 base pairs of this cDNA are not found in 18XS genomic rescue DNA, CDNA 4e is probably derived from an authentic egh transcript (see Fig. 10B).

Considering the complexity of the egh gene, we wanted to determine if we had isolated all protein coding sequences essential for egh function. We constructed a chimeric HSegh transgene by placing the entire putative egh ORF of CDNA 7 (also present in cDNA 4e) under the control of the heat-inducible hsp70 promoter (see Materials and Methods). The basal expression level of HSegh is sufficient to rescue the lethality and fertility of partial loss-of-function egh mutations, confirming that these sequences correspond to egh. Additionally, we sequenced the egh56e mutation and found that it deletes egh protein coding sequences starting in an intron at the 3’ end of the egh gene (Fig. 10A,B).

The only open reading frame with good codon usage in either egh cDNAs 7 or 4e, or in the corresponding 4.5 kb of genomic DNA, predicts a 52x10^3 M_1 protein product (Fig. 10B). The protein is 60% identical to a predicted C. elegans protein of identical length, but unknown function (Fig. 10C; Wilson et al., 1994). Examination of both sequences reveals two notable features: a putative signal sequence and at least one putative transmembrane domain (Fig. 10B-D). Our best prediction of the primary structure of the egh protein is indicated in Fig. 10E. Without further characterization, we cannot rule out other structures for the predicted egh protein product, but the likelihood that egh encodes for a secreted or transmembrane molecule is in accordance with the non-autonomy of egh function during oogenesis (described above).

Table 4. Penetrance of egh, brn, and egh brn embryonic neurogenic phenotypes

<table>
<thead>
<tr>
<th>Genotype*</th>
<th>Number scored</th>
<th>% Weak neurogenic</th>
<th>% Intermediate neurogenic</th>
<th>% Strong neurogenic</th>
</tr>
</thead>
<tbody>
<tr>
<td>brn^2.107</td>
<td>252</td>
<td>16</td>
<td>83</td>
<td>1</td>
</tr>
<tr>
<td>brn^7.109</td>
<td>116</td>
<td>12</td>
<td>87</td>
<td>1</td>
</tr>
<tr>
<td>egh^DP444</td>
<td>102</td>
<td>15</td>
<td>83</td>
<td>2</td>
</tr>
<tr>
<td>egh^GA107</td>
<td>99</td>
<td>11</td>
<td>88</td>
<td>1</td>
</tr>
<tr>
<td>egh^DP444 brn^p.107</td>
<td>108</td>
<td>10</td>
<td>89</td>
<td>1</td>
</tr>
<tr>
<td>egh^GA107 brn^p.107</td>
<td>125</td>
<td>14</td>
<td>82</td>
<td>4</td>
</tr>
<tr>
<td>egh^DP444 brn^p.107</td>
<td>91</td>
<td>12</td>
<td>87</td>
<td>1</td>
</tr>
</tbody>
</table>

*All embryos were derived from germline clones of the indicated mutation, except for brn^2.107, for which embryos were derived from homozygous females. For analysis of embryonic neurogenic phenotypes (Table 4), all females were crossed to OR/Y males, and hatching, zygotically rescued embryos were discarded. Hoyer’s mounts of brown eggs were scored for the strength of the neurogenic phenotype according to the following designations: weak, at least two denticle belts on the ventral surface, intermediate, dorsal cuticle without ventral denticle belts, filzkörper and pharyngeal cuticle intact, strong, dorsal patch of cuticle, no pharyngeal cuticle, filzkörper absent. Most embryos in the weak class represent partial zygotically rescued embryos. Essentially no embryos with a strong N-like phenotype are observed for any of the indicated genotypes. Embryos scored in the strong class meet the criteria described above, but do not look exactly like strong N mutants (not shown), and may result from secondary development defects such as failed germ band retraction.

egh and brn are essential for epithelial morphogenesis 3871
Accumulation of *egh* transcripts in germ tissue correlates with *egh* and *brn* requirements in the morphogenesis of the follicular epithelium

Accumulation of *egh* transcripts in germ tissue correlates with *brn* and *egh* requirements in the morphogenesis of the follicular epithelium. Like *brn*, *egh* is first expressed in germ cells as prefollicle cells ensheath newly formed cysts at the junction of germarium region IIA/b, when both genes are essential for forming the follicular epithelium (Figs. 11A,B; 1; 3A; Goode et al., 1996). *egh* expression appears to increase in germ cells as egg chambers span the width of the germarium (Figs. 11B; 1; 3A), and expression continues at uniform levels in all germ cells as the egg chamber assumes a spherical shape through s3 of oogenesis (Figs. 11; 1). At s4, when egg chambers transform from a spherical to an ellipsoidal shape, *egh* transcripts differentially accumulate in the oocyte (Figs. 11C; 1), corresponding to the time that *egh* and *brn* become essential for maintaining the follicular epithelium around the oocyte. Differential *egh* expression persists through s9 of oogenesis, as (1) the egg chamber undergoes further extension along the A/P axis, (2) follicle cells begin to accumulate and achieve a columnar shape around the oocyte (Figs. 11D; 1), and (3) the follicular epithelium migrates towards the oocyte (Figs. 11E; 1). We have shown that *brn* and *egh* are required throughout these stages for maintaining and helping to achieve normal migration and activation of gene expression within the follicular epithelium. At s10a of oogenesis, follicle cells have completed their migration, and *egh* expression decreases dramatically in the...
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DISCUSSION

egh and brn appear to be components of a novel signaling pathway essential for epithelial development and maintenance

In this study we describe the molecular characterization of egh and extend the functional analysis of brn, egh, and N during oogenesis. We find that egh encodes a novel, putative secreted or transmembrane protein. The spatiotemporal pattern of increasing egh expression in germ cells suggests Egghead is a critical component of a germline signaling pathway, organized as a cascade of morphogenetic activity, which helps to coordinate the growth and differentiation of germ cells with morphogenesis of follicular epithelium. Several observations suggest that egh and brn function in a common pathway specific for the formation and maintenance of epithelial structure: (1) the expression of both genes is restricted to the germline (this study, Goode et al., 1996); (2) they are required...
identically in the germline for differentiation of the follicular epithelium; (3) neither gene appears to be essential for 
mediated cell fate specification during oogenesis, and (4) egh 
bro double mutant animals do not show phenotypes any more 
severe than either mutant alone.

egh and brn are clearly required for the formation of the fol-
licular epithelium since in egh and brn GLC animals follicle 
cells fail to extend processes in region I/IIa of the germarium, 
where germline cysts are initially encapsulated. Significantly, 
we obtained evidence that mutant egg chambers containing
Fig. 10. Molecular characterization of egh. (A) Molecular map of the egh region. The position, relative size, direction of transcription, and intron structure of egh transcripts are represented by cDNAs 4e and 7 (arrows). KLP3A lies immediately proximal (Williams et al., 1995). DNA fragment 18XS (gray box) rescues egh but not KLP3A mutations. egh63e4 is a deletion that breaks in the indicated intron (Williams et al., 1995), and removes the 5' end of the egh gene, leaving 3/5ths of egh protein coding sequences intact. Restriction sites: B, BamH1; R, EcoRI; Sc, Scal; S, SstI and X, Xhol. (B) egh sequence. Upper case nucleotide sequence is found in both genomic DNA and cDNA 4e. Lower case sequence is restricted to genomic DNA. The first 930 base pairs (bps; not shown) of egh sequence originate outside the 18XS fragment and have not been mapped on the genomic walk. This sequence appears to be derived from an independent exon(s) based on the observations that, (1) bp 931 of cDNA 4e is adjacent to a putative CAG splice acceptor site at genomic position 563, and (2) cDNA 4e detects a transcript of equivalent size on northern blots (not shown). cDNA 7 is collinear with cDNA 4e starting at position 771, and appears to represent an independent transcript based on the presence of a 5' TATA box in genomic sequence (underlined, position 727). All three sequences contain a presumptive AATAAA polyadenylation signal (underlined). The only open reading frame (ORF) with good codon usage in c- or genomic DNA is represented by the predicted Egghead protein sequence. These ORF sequences are sufficient for egh function (see text), which is consistent with the ability of genomic fragment 18XS to rescue egh mutations (see above). The open reading frame is interrupted by a single intron starting at position 1852. egh63e4 is a deletion which removes DNA starting within this intron at position 1963. Egghead is predicted to have 457 amino acids and a relative molecular mass of 52×103 without posttranslational modification. The first 22 amino acids (underlined) are extremely hydrophobic (see D) and conform to the rules established for signal sequences (von Heine, 1985). The protein has two potential N-linked glycosylation sites (NXS, underlined). cDNA 4e sequence can obtained via GenBank accession number U15602; genomic sequence, U21218. (C) The predicted Egghead protein sequence is 60% identical to a C. elegans protein of identical length, but unknown function (Wilson et al., 1994). (D) Hydropathy plots of the Drosophila (bold) and C. elegans (shaded) Egghead proteins. Both proteins have a conserved signal sequence (SS; B). Arrows indicate presumptive membrane spanning domains, inferred from the content of hydrophobic residues flanked by positively charged amino acids, and the conservation of hydrophobicity between the two proteins (threshold = 1.3). (E) Schematic representation of a potential Egghead protein. The presumptive signal sequence is indicated by a gray box, putative transmembrane domains are indicated by black boxes, and potential glycosylation sites are indicated by asterisks.

Female germ cells diverging from a 15:1 nurse cell to oocyte ratio arise from (1) the inappropriate stretching and breaking of unencapsulated cysts, and/or (2) the inappropriate migration of follicle cells between, instead of along the outside of germ cell cysts (Fig. 3C-E). These observations are consistent with the complementarity observed between adjacent egg chambers in the vitellarium (Fig. 2C), but do not exclude the possibility that brn and egh may regulate germ cell divisions. The observation that hts mutants never produce egg chambers containing more than a single oocyte-nurse cell complex suggests that if brn and egh do play a role in organizing germ cell divisions, they do so as a cooperative process with cyst encapsulation.

Our results provide compelling evidence for a role of neurogenic genes in epithelial maintenance, distinct from epithelium formation and cell fate specification. We found that cells start to exit the follicular epithelium and lose apical-basal polarity well after the epithelium is formed, at s4 for the strongest brn and egh mutant alleles. In this context, it is worth noting that Notch has been suggested to act as a tumor suppressor gene in both vertebrates and invertebrates (Ellisen et al., 1991; Watson et al., 1994). Our results are consistent with a specific role for egh, brn, and N in tumor suppression, since

Fig. 11. mRNA expression of egh during oogenesis. WT ovarian tissue (g, gerarium; v, vitellarium). Tissue in B was stained twice as long as tissue in other panels. (A,B) Germarium expression. egh expression appears to be initiated at the junction of gerarium region I/IIa (arrowheads). Germ cell expression is evident by accumulation in germ cell cytoplasm (B, arrows); follicle cell bodies, located at the periphery, do not stain (B, **; see also Fig. 6A). (A,C,D,F) egh is expressed uniformly in all germ cells until s4 (C, s4), when transcripts differentially accumulate in the oocyte (arrow). egh continues to be expressed at uniform levels in nurse cells, but differentially in the oocyte until s9 of oogenesis. Restriction of egh transcripts to germ cells is clear in (A). Apparent faint follicle cell staining (C,D,F) results from optical diffraction in images captured at high magnification, as can be seen in (E), a s8 control egg chamber stained with egh sense strand probe. The absence of follicular phenotypes in follicle cell clones of egh mutations further supports that this staining is an artifactual (not shown). (G) egh expression is tremendously reduced in the oocyte while transcription increases dramatically in nurse cells.
Follicle cells appear to over proliferate in these mutants, and the follicular epithelium acquires an early onset adenoma-like phenotype with a spatial-temporal pattern that directly correlates with the differential expression of egh in the oocyte.

Most significantly, loss of structure in the follicular epithelium appears to be independent of a role for brn, egh, or N in cell fate specification. Neither brn or egh are required for regulating polar versus stalk cell fate decisions, and although cells which have exited the follicular epithelium are mesenchymal-like, they have not adopted a mesenchymal border cell fate. Further, while polar cell fates are shifted in only about half of Nts1 mutant egg chambers (Ruohola et al., 1991; this study), many N mutant egg chambers that suffer multilayered epithelia do not have ectopic polar cells (not shown), supporting the hypothesis that cell fate specification and epithelial maintenance are separate functions of N.

To account for the requirement of Notch in both cell fate specification and epithelial development, and the specificity of brn and egh in epithelial development, we suggest a model for Brainiac, Egghead, and Notch action during oogenesis (see Discussion). Notch is distributed on both lateral and apical follicle cell membranes, and therefore has the potential to interact with molecules in the germline, as well as with signals from neighboring follicle cells. Lateral specification of polar versus stalk cells appears to depend on Notch signals acting between follicle cells, and therefore presumably lateral expressed Notch. In contrast, we propose that brainiac and egghead germ line functions mediate epithelial development in collaboration with apically expressed Notch.

Based on the following considerations, we propose that germline Brainiac and Egghead and apically expressed Notch function in epithelial development by mediating germline-follicle cell adhesion; (1) Notch acts as a multifunctional receptor and has been implicated in cell adhesion (Cagan and Ready, 1989; Fehon et al., 1990; Rebay et al., 1991), (2) Brainiac is a putative secreted protein, and Egghead is a putative secreted or transmembrane protein; secreted proteins have not been implicated in lateral specification processes, but...
have extensively been implicated in cell adhesion and epithelial development, and (3) follicle cells appear to detach from the cell in which egh is expressed at highest levels, the oocyte.

One important issue is whether this model can be extended to other tissues. As we noted in the results, the intermediate strength of the brn, egh, and brn egh embryonic neurogenic phenotypes, and the apparent lack of a requirement for these genes in cell fate specifications in the pupal ectoderm is consistent with the specificity of action we have described during oogenesis. One puzzle is that brn and egh appear to be required for cell fate specification in the embryonic ectoderm (Fig. 9). We think that the apparent difference between brn and egh function in oogenesis versus early neurogenesis is likely to reflect the distinct structures of the simple monolayer follicular epithelium versus the more complex and dynamic arrangement of cells in the process of cell fate determination within the neurogenic ectoderm (cf. Uemura et al., 1996; Tepass et al., 1996). Thus, we suggest that a loss of epithelial structure within the neurogenic ectoderm, similar to what we have described for the follicular epithelium, would likely have dramatic consequences on signaling processes regulating cell fate specification. A more instructive model for brn and egh function in the neurogenic ectoderm is that they help to couple the dynamic rearrangement of neuroblasts and epidermoblasts with fate specification. Significantly, not all neurogenic genes have been implicated in epithelial development (Hartenstein et al., 1992), raising the possibility that some neurogenic genes are specifically required for lateral specification. The observation of exiting, apolar follicle cells during mid-oogenesis, before the MBE migrate towards the oocyte, suggests that cell adhesion of the follicular epithelium to the oocyte. Adhesion function has previously been shown to be required for establishing polarity of the cytoskeleton in the oocyte. Alternatively, morphogen localization may primarily depend on the Egfr signaling pathway, which is facilitated by neurogenic adhesion function. Loss of brn or egh function during oogenesis results in a ventralized chorion phenotype that is more severe than eliminating half of grk TGFβ molecules from the system (Goode et al., 1996; Fig. 7; unpublished observations). These hypotheses are not mutually exclusive.

Disrupting the function of members of the Egfr signaling process leads to discontinuities of the follicular epithelium, in which follicle cells covering nurse cells, but not the oocyte, are missing due to failed migration and/or division (Goode et al., 1996). Based on these findings, it has been suggested that the oocyte has a specialized adhesive system that distinguishes it from the nurse cells. The observations that follicle cells show a propensity to detach from the oocyte rather than nurse cells in brn, egh and N egg chambers starting at s4 and continuing through s9 of oogenesis (Figs 4, 5; Table 3), and that egh is differentially expressed in the oocyte throughout this time, suggests that brn, egh, and N are components of at least one follicular-epithelial cell adhesive system during stages 4 to 9 of oogenesis, and that egh is a critical component.

Differential adhesion to the oocyte and maintenance of the follicular epithelium is likely to be crucial for the efficiency of at least two parallel, perhaps interconnected, sets of processes that depend on germline-follicle cell interactions. The first set of processes include the changes in follicle cell shapes that occur concomitantly with their gradual accumulation around the oocyte during mid-oogenesis, before the MBE migrate towards the oocyte in concert with border cells at s9 (Fig. 1). The observation of exiting, apolar follicle cells during mid-oogenesis, and the failure of border cells to migrate in concert with the MBE indicates that brn, egh and N are essential for both processes. The breaking of border cells from the MBE, and the migration of the MBE towards the oocyte are both likely to require tight regulation of the relative epithelial state of follicle cells (see Introduction), and differential adhesion/attraction to the oocyte (Lee et al., 1996), and brn and egh appear to influence both of these processes (Fig. 8). Strikingly, the MBE appears to migrate too fast in brn and egh GLC egg chambers, suggesting that these genes may be needed to inhibit the migration process, a particularly intriguing possibility considering the role of the genes in inhibiting the segregation (migration) of neuroblasts during early neurogenesis.

A second set of processes that appear to depend on oocyte-follicle cell adhesion are inductive events mediated by the Egfr signaling process. Egfr signals on the apical surface of follicle cells are induced by germline grk TGFβ signals to both form the follicular epithelium (Goode et al., 1996), and to establish anterior-posterior and dorsal-ventral pattern within the epithelium, which is used for localization of morphogens in and around the oocyte (Roth et al., 1995). Likewise, N neurogenic function has previously been shown to be required for localization of morphogens at the anterior and posterior poles of the egg chamber (Ruohola et al., 1991), and we observe delayed or mislocalized oskar at the posterior pole of brn and egh mutant egg chambers (not shown). Our current analysis does not allow us to decide the hierarchical relationship between neurogenic and Egfr signaling in controlling these processes (if one exists). One possibility is that the Egfr pathway is essential for establishing and maintaining posterior cell fates, while neurogenic gene function acts subsequently to mediate adhesion of the follicular epithelium to the oocyte. Adhesion function appears to be essential for maintaining the apical-basal polarity of follicle cells, which in turn may be necessary for establishing polarity of the cytoskeleton in the oocyte. Alternatively, morphogen localization may primarily depend on the Egfr signaling pathway, which is facilitated by neurogenic adhesion function. Loss of brn or egh function during oogenesis results in a ventralized chorion phenotype that is more severe than eliminating half of grk TGFβ molecules from the system (Goode et al., 1996; Fig. 7; unpublished observations). These hypotheses are not mutually exclusive.

Functional tests are consistent with the interdependence of the neurogenic and Egfr signaling processes. brn, egh, and N mutations show dominant and synergistic interactions with mutations in components of the Egfr signaling process, for both dorsal-ventral patterning and induction of cell proliferation and/or migration (Goode et al., 1992, 1996; Goode, 1994; not shown). In contrast, like brn egh animals, N brn double mutant animals do not show phenotypes any more severe than either mutant alone during oogenesis (Goode, 1994), consistent with the close functional relationship that we have documented between these genes in this study.

These observations are consistent with a plethora of studies linking adhesion, maintenance of cell polarity, and execution of inductive signals. For example, in budding yeast, proteins essential for the development and maintenance of cell polarity organize a large complex of proteins, including actin, cdc24, ste20 and Cla4 protein kinases, as well as MAP kinases at sites of polarized growth (reviewed by Drubin and Nelson, 1996). In C. elegans, lin-7 encodes a membrane associated DHR-containing protein that is essential for maintaining the localization of the Egfr, and loss of lin-7 function results in inefficient Egfr signaling, and Egfr mutant phenotypes in most animals

egh and brn are essential for epithelial morphogenesis
In vertebrate cells, integrins are essential for the organization of a protein scaffold for the assembly of a signaling network that includes focal adhesion kinase and components of the Ras pathway, and GTP-binding proteins (reviewed by Clark and Brugge, 1995). In these studies, we have shown a clear disruption in cell polarity, loss of apical membrane integrity, and presumably adhesion in \textit{brn}, \textit{N}, and \textit{egh} mutants; interestingly, disruption of spectrin function results in the accumulation of follicle cells in multiple layers similar to that seen in \textit{brn}, \textit{egh}, and \textit{N} mutants (Deng et al., 1995), suggesting a close functional relationship between Brn/Egh and Notch adhesion signals and regulated cytoskeletal processes. It will be interesting in future studies to determine if the apparent interdependence of neurogenic and Egr signaling processes is at the level of organization and interdependence of intracellular signaling complexes within the apical cortex of follicle cells, synergistic action in the activation of transcription of new genes, a combination of these processes, or even more complex scenarios.

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