Brainiac and Fringe Are Similar Pioneer Proteins That Impart Specificity to Notch Signaling during *Drosophila* Development

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Notch (N) has been implicated in a plethora of signaling events in a variety of tissues throughout the *Drosophila* life cycle (for review, see Artavanis-Tsakonas et al. 1995). N receptors are found in animals spanning phylogeny, and mutations in mammalian N genes have been implicated in leukemia, breast cancer, stroke, and dementia (Ellisen et al. 1991; Robbins et al. 1992; Joutel et al. 1996). N receptors have a modular structure. The large extracellular domain consists of 34–36 tandem epidermal growth factor (EGF)-like repeats, at least two of which are known to be essential for binding to N ligands, and three extracellular cysteine-rich Notch/Lin-12 repeats of unknown function. The intracellular domain consists of six tandem Ankyrin repeats that mediate interactions with cytoplasmic proteins and are sufficient for induction of at least some N-mediated cell fate decisions (Lieber et al. 1993; Rebay et al. 1993; Struhl et al. 1993). Functionally, N receptors are involved in more than one kind of signaling event, including lateral specification of cell fates between groups of equivalent cells and induction of cell fates across fields of nonequivalent cells, as well as for the development and maintenance of sheets of polarized epithelial cells (Fig. 1) (Hartenstein et al. 1992; Artavanis-Tsakonas et al. 1995; Goode et al. 1996a). The involvement of N in many different types of interactions between cells and tissues in a multitude of contexts raises the issue of how specificity is generated from these receptors.

Two structurally similar N ligands, Serrate (Ser) and Delta (Dl), regulate N signaling events. Ser and Dl are members of a family of transmembrane molecules that comprise an amino-terminal, extracellular cysteine-rich DSL motif (named after family members *D*elta, *S*errate, and *L*ag-2), a variable number of extracellular EGF repeats, and a small intracellular domain of variable similarity. Abundant genetic evidence indicates that Dl and Ser interact with N, and Dl or Ser expressing cells have been shown to aggregate with N expressing cells. Overexpression of Dl or Ser during development causes phenotypes resembling gain of N function, whereas loss of Dl or Ser causes phenotypes resembling loss of N function. Combined with the finding that both Dl and Ser act non-cell-autonomously, these experiments demonstrate that these molecules act as N ligands (for review, see Artavanis-Tsakonas et al. 1995).

The differential expression of Dl and Ser suggests a means for producing specific N signals via differential, localized activation of N receptor, a hypothesis supported by their differential expression patterns (Fleming et al. 1990; Thomas et al. 1991). The best studied example is in the wing disk, in which Dl and Ser have complementary and distinct roles in defining the wing margin (Fig. 2) (Doherty et al. 1996). Ser is expressed in cells on the dorsal side of the margin and triggers N in ventral cells, whereas Dl is expressed in cells on the ventral side of the margin and triggers N in dorsal cells. N activation by either ligand induces expression of the margin-specific genes *wingless, cut*, and *vestigal* (Couso et al. 1995; Diaz-Benjumea and Cohen 1995; de Celis et al. 1996; Doherty et al. 1996; Neuman and Cohen 1996). In contrast, Ser specifically activates Dl expression in ventral cells, whereas Dl specifically activates Ser in dorsal cells, forming a positive feedback loop (Fig. 2) (Doherty et al. 1996; Panin et al. 1997). If Ser is expressed artificially in dorsal cells, or Dl in ventral cells, they cannot induce expression of the complementary ligand, indicating that Dl and Ser induce tissue-specific responses. Significantly, expression of constitutively active N, which is active independent of the N extracellular domain, can induce Ser in dorsal cells and Dl in ventral cells (Doherty et al. 1996; Panin et al. 1997). This striking result suggests that the tissue-specific effects of Dl and Ser result from direct or parallel modification of N signaling capacity.

In this paper, we summarize recent findings on a novel class of putative secreted factors, Fringe (Frg) and Brainiac (Brn), which may impart specificity to N signaling events by acting upstream or in parallel to N. Brn and Frg are involved in regulating N action in patterning fields of cells, essential for induction of cell fates and for the maintenance of epithelial cell polarity and differentiation, but apparently not for regulating lateral specification decisions. Thus, Brn and Frg appear to be essential for generating qualitatively distinct Notch signals.

Brn REQUIREMENT IN N SIGNALING EVENTS

Phenotypes associated with loss of *brn* and *N* function during oogenesis and early embryogenesis have revealed that Brn is crucial for a subset of processes in which N

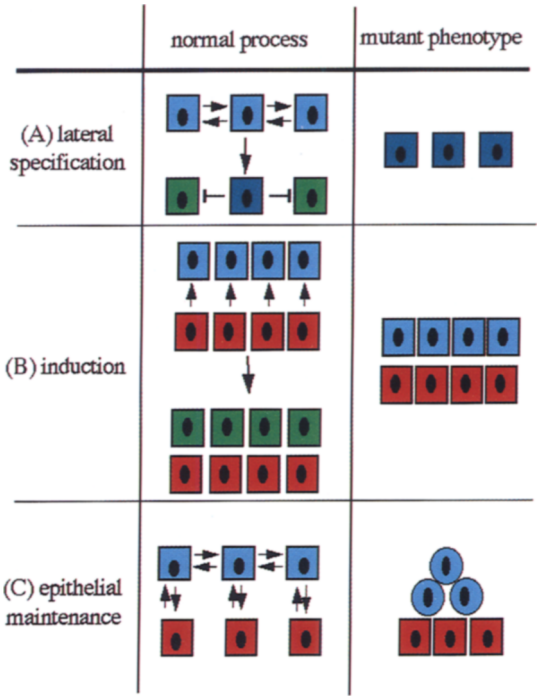

Figure 1. Patterning processes regulated by N receptors. There are three types of patterning processes: lateral specification, induction, and epithelial maintenance, regulated by N receptors. Small arrows indicate D1 or Ser signals that impinge on N receptors. (*A*) Lateral specification. N signals act between individual cells of equal potential (*blue cells*). Through a presumed stochastic process, one cell adopts an alternative fate (*purple cell*). This cell then sends an inhibitory signal to adjacent cells, restricting them from adopting the same fate (*green cells*). In the absence of N (mutant phenotype), all cells adopt the same fate. (*B*) Induction. N signals act between fields of cells of nonequivalent potential. One block of cells (*red*) sends a signal to another (*blue*), switching their fate (*green cells*). Signals may also pass from the receiving cells back to the sending cells, changing the phenotype of the sending cells, as in the wing disk (Doherty et al. 1996; see text). In the absence of N signals (mutant phenotype), cells do not switch their fate. (*C*) Epithelial maintenance. N signals act between cells of nonequivalent potential and perhaps cells of equivalent potential. Without these signals (mutant phenotype), cells fail to develop and/or lose their polarized epithelial morphology and/or cannot complete the morphogenetic transitions essential for the development and maintenance of epithelial sheets. As during the process of induction, cells receiving N signals may send signals back to the sending cells, altering their morphology.

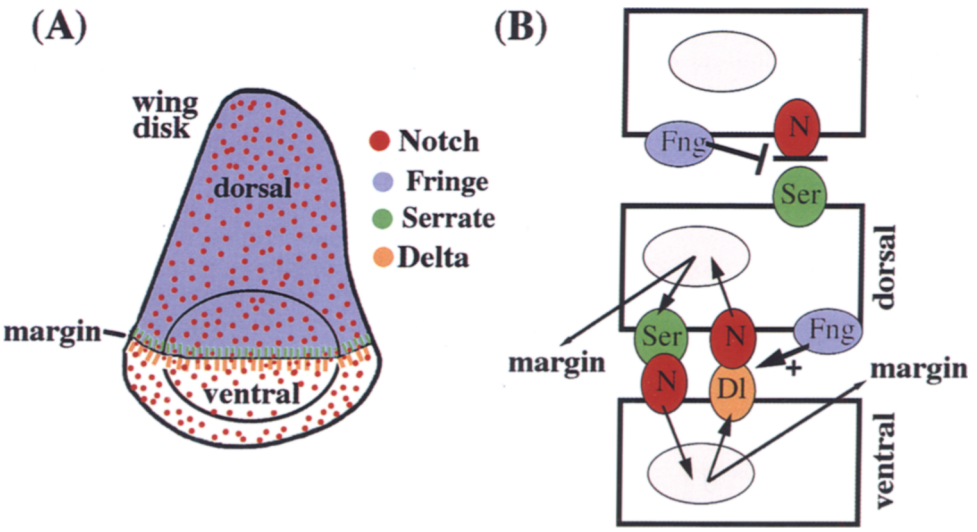

Figure 2. Patterning the wing margin. (*A*) Schematic of a developing wing disk, the tissue that differentiates into an adult wing. The margin is the interface at which dorsal and ventral compartments meet. The distribution of key signaling molecules that pattern the margin is indicated. Ser and Fng are expressed in the dorsal compartment, D1 is expressed in the ventral compartment, and N is expressed throughout the wing disk. Ser expression is induced, and wing margin is formed, wherever Frg expressing cells juxtapose Frg nonexpressing cells, at the margin in wild-type animals, or at ectopic sites under experimentally constructed conditions (Irvine and Wieschaus 1994; Kim et al. 1995). (Adapted from Doherty et al. 1996 and Panin et al. 1997.) (*B*) A model for Frg action. N activation by either D1 or Ser triggers spatially restricted expression of the margin organizing genes *wingless, vestigal,* and *cut* in both dorsal and ventral margin cells (not shown; Couso et al. 1995; Diaz-Benjumea and Cohen 1995; de Celis et al. 1996; Doherty et al. 1996; Neuman and Cohen 1996). Expression of D1 and Ser is maintained at the margin by a positive feedback loop, in which N activation by D1 in dorsal cells induces expression of Ser, and N activation by Ser in ventral cells induces D1 (Doherty et al. 1996; Panin et al. 1997). Frg acts to position this feedback loop apparently through a cell autonomous mechanism by which Frg potentiates activation of N by D1 (arrow), and blocks N activation by Ser (inhibitory symbol; described in detail in Panin et al. 1997). (Adapted from Doherty et al. 1996 and Panin et al. 1997.)

signaling is needed during early development. Brn, like N, is required for the segregation of neural precursor cells from epidermal precursor cells during early embryogenesis, as indicated by the *brn* maternal-effect "neurogenic" phenotype (Perrimon et al. 1989; Goode et al. 1992). This phenotype is similar to zygotic phenotypes associated with *N* mutant animals, as well as other "neurogenic" mutants (Lehmann et al. 1983; Goode et al. 1992). Although the *brn* and *N* embryonic neurogenic phenotypes have not been compared in detail, it is clear that the *brn* phenotype is not as severe as complete loss of *N* function (Goode et al. 1992). This consideration suggests that Brn may participate only in a subset of N signaling processes, and this idea has been substantiated by comparing *brn* and *N* phenotypes in other tissues.

For example, during pupal metamorphosis, N signaling is crucial for lateral specification of epidermal versus sensory organ precursor cell fates (see Fig. 1). Absence of N of D1, or most neurogenic genes within the pupal ectoderm, but not Brn, causes flies to have a bald phenotype because of hypertrophy of neuronal cells at the expense of epidermal cells (for review, see Posakony 1994). Likewise, during egg chamber morphogenesis, absence of N or D1 signals causes too many polar cells to accumulate at the expense of polar flanking cells, resulting from defective Notch signaling in the specification of polar versus polar-flanking cell fates (Fig. 2) (Ruohola et al. 1991). Loss of Brn has no consequence on these decisions (Fig. 2) (Goode et al. 1996a). Brn is involved in a second, apparently separate, N signaling event essential for main-

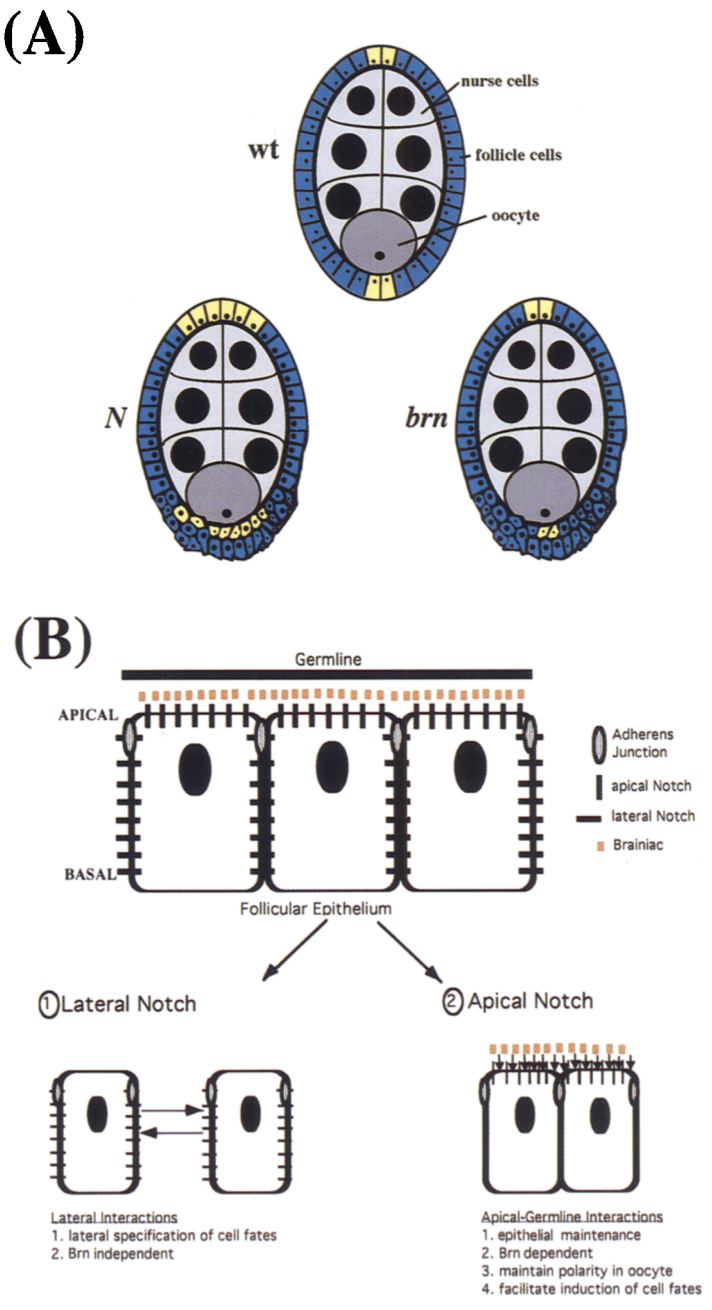

Figure 3. Roles of Brn and N in patterning the follicular epithelium. (*A*) Wild-type egg chambers comprise germ cells and follicle cells. There are two types of germ cells, the oocyte, and 15 nurse cells. Two polar follicle cells are found at the anterior and posterior poles of the egg chamber (*yellow cells*). N is expressed throughout the follicular epithelium. Brn is expressed in germ cells. In *N* mutant egg chambers, but not *brn* mutant egg chambers, too many polar cells segregate. This phenotype led to the model that N is required in a lateral specification process that ensures segregation of two polar cells from neighboring polar-flanking cells (see Fig. 1A; Ruohola et al. 1991). In *N* and *brn* mutant egg chambers, follicle cells lose polarity and accumulate in several layers specifically around the oocyte. This phenotype has led to the model that both N and Brn are required for maintaining the integrity of the follicular epithelium around the oocyte (Goode et al. 1996a). (*B*) A model to account for Brn and N action during oogenesis. The upper portion of the figure shows the distribution of N and presumptive distribution of Brn (Goode et al. 1996a,b). We propose that lateral N has distinct function(s) from apical N (below). (*1*) N expression in lateral membranes is likely to be required for mediating lateral specification of polar versus flanking cells. (*2*) N expression on the apical surface of follicle cells is likely to be essential for epithelial maintenance. The focus of Brn action between germ cells and follicle cells provides an explanation for the specificity of Brn's collaboration with N in epithelial maintenance. (Adapted from Goode et al. 1996.)

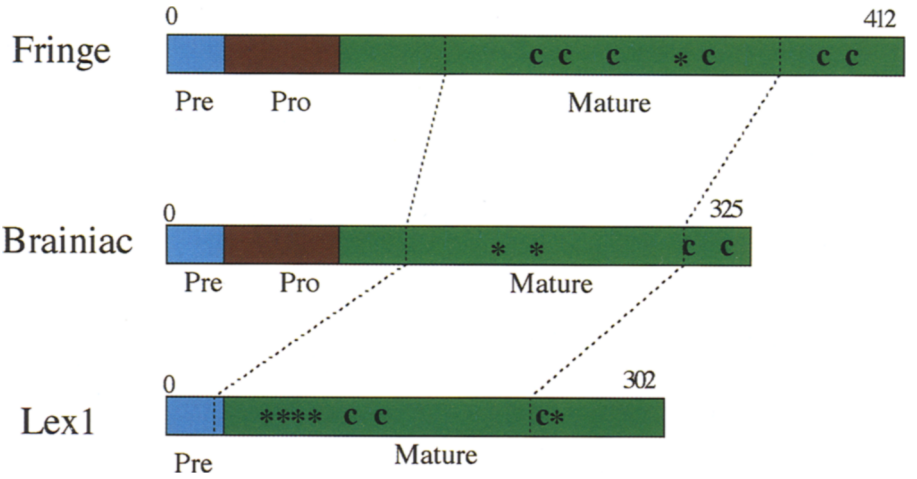

Figure 4. Structure of Frg, Brn, and Lex1 molecules. Frg and Brn share the same presumptive structure, a preregion (the signal peptide) suggestive of secretion, a proregion ending with a dibasic site for proteolytic processing, and a mature region. The central regions of Brn and Frg show limited similarity to Lex1 (*dotted lines*), a glycosyltransferase of the parasitic bacterium *Haemophilus influenzae* (Yuan et al. 1997). Unlike Frg and Brn, Lex1 apparently has no proregion. Neither Frg nor Brn has been shown to be a glycosyltransferase. The presumptive secreted nature of Frg has been shown in vivo (Panin et al. 1997), whereas a *Xenopus* Frg-like molecule, lunatic Frg, has been shown to undergo processing in a manner consistent with the suggested pre-pro structure (Wu et al. 1996). There is no direct evidence yet that Brn is a secreted molecule or that either Frg or Brn has glycosyltransferase activities. Putative Frg, Brn, and Lex1 glycosylation sites are shown (*). A striking difference between Frg and Brn and Lex1 structures is the number of cysteine residues (c). Frg contains six cysteine residues within the proregion of the molecule. Although these cysteines are not arranged in the cysteine knot pattern characteristic of classic cytokines, the pattern of cysteines is conserved in *Xenopus* and human Frg molecules (Wu et al. 1996), suggesting that they are likely to be crucial for determining Frg tertiary structure, perhaps by forming an atypical cysteine knot (Irvine and Wieschaus 1994). Although Brn has only two cysteine residues and Lex1 has only three cysteine residues, we do not believe that this makes it less likely that Brn serves as a signaling factor, because considerable heterogeneity has been described in chemokine cysteine residue patterns (Mackay 1997). Brn shares with Frg and Lex1 a predicted alternating arrangement of α-helices and β-strands suggestive of α/β folding within the central portion of each molecule (*dotted lines*; Yuan et al. 1997).

taining the apical-basal polarity of follicular epithelial cells (Goode et al. 1996a). In *brn* and *N* mutant animals, epithelial cells accumulate in multiple layers around the oocyte (Fig. 2) (Goode et al. 1996a).

How is Brn specificity in N signaling events during oogenesis achieved? Specificity may be accomplished, at least in part, by differential activation of N on the apical surface of follicle cells by Brn, expressed in germ cells (Fig. 3). N is expressed on both apical and lateral follicle cell surfaces. On the lateral surface of follicle cells, N can mediate lateral specification decisions, and on the apical surface, N can participate with germ line Brn in maintaining the follicular epithelium (Fig. 3) (Goode et al. 1996a). Because Brn is not expressed on lateral cell membranes, where the signals responsible for cell fate determination are generated, it apparently has no role in these decisions. How does Brn, a putative secreted factor (Fig. 4) with no similarity to membrane-spanning N ligands Dl and Ser, modulate N signaling? Currently, we can only speculate, but studies of Frg suggest that Brn may participate in N signaling by modulating N-ligand interactions.

Frg REQUIREMENT IN N SIGNALING EVENTS

Like Brn, Frg is essential for viability and is required in many different signaling events during adult metamorphosis to pattern eyes, wings, and legs (Irvine and Wieschaus 1994; Kim et al. 1995). Intensive focus has been placed on the role of Frg in wing development, where it is essential for positioning the D1-Ser/N feedback loop that establishes the wing blade (see Fig. 2) (see introduction; Irvine and Wieschaus 1994; Kim et al. 1995; Panin et al. 1997). Frg is expressed with Ser strictly in dorsal cells (see Fig. 2). Loss of Frg or Ser or D1 causes a "notched" wing phenotype similar to that found in hemizygous *N* mutant animals (Irvine and Wieschaus 1994; Kim et al. 1995). Mosaic analyses have shown that whenever Frg expressing and nonexpressing cells are juxtaposed, margin formation and wing growth occur in a pattern identical to expression of constitutively activated N signals (Irvine and Wieschaus 1994). Frg establishes a sharp wing margin boundary apparently by inhibiting the ability of N to respond to Ser in dorsal cells while potentiating the ability of N to respond to Dl in dorsal cells (see Fig. 2) (Panin et al. 1997). A similar mechanism for Frg action is likely to be crucial for patterning the apical ectodermal ridge, the apparent homologous organizer to the invertebrate wing margin in vertebrates limbs (Concepción et al. 1997; Laufer et al. 1997).

Loss of Frg activity in the wing and thorax apparently has no effect on N decisions in the lateral specification of thoracic or margin bristles, even though Frg signals appear to impinge on the field of cells in which these N decisions are taking place (Irvine and Wieshaus 1994). Thus, Fng is similar to Brn in that it appears to be specifically required in N inductive events that pattern non-

equivalent fields of cells but is not required for lateral specification between individual cells within equivalence groups. As noted in the previous section, the specificity of Brn action may be accounted for by the fact that Brn signals are restricted to only one surface on cells in which N is expressed. A similar mode of specificity for Frg action has not been proposed and will have to await a detailed analysis of Frg and N localization and function in the imaginal wing epithelium.

Brn AND Frg SHOW LIMITED SIMILARITY AND BELONG TO A LARGE GENE FAMILY THAT MAY INCLUDE GLYCOSYLTRANSFERASES

Molecular characterization of Brn and Frg revealed that they share the same presumptive structure, a preregion (the signal peptide) suggestive of secretion, a proregion ending with a dibasic site for proteolytic processing, and a mature region that will be functionally active after cleavage from the precursor protein (see Fig. 2). Biochemical experiments have confirmed this structure for a *Xenopus* Frg homolog (Wu et al. 1996). The action of *Drosophila* Brn and Frg on cells adjacent to those in which they are expressed (described above) is consistent with their putative secreted structure.

Similarity between Brn and Frg pioneer proteins is fairly weak and is not detected using standard search algorithms, but emerges when conservation patterns gleaned by comparing Brn and Frg-like signaling molecules from *Caenorhabditis elegans* to humans are used to execute very sensitive motif and profile searches (Bork and Gibson 1996; Yuan et al. 1997). The Frg and Brn alignments predicted from these comparisons reveals an alternating arrangement of α-helices and β-strands suggestive of an α/β folding type for the central portion of each subfamily, similar to that of prokaryotic and eukaryotic glycosyltransferases (Yuan et al. 1997). Within a region spanning 150–180 amino acids, Brn and Frg show greatest similarity to Lex1 (Fig. 4), a glycosyltransferase found in the parasitic bacterium *Haemophilus influenzae* that is essential for the biosynthesis of its extracellular lipopolysaccharides (Yuan et al. 1997). The most conserved regions are also the hallmarks of the putative glycosyltransferase superfamily.

In contrast to Brn and Frg, glycosyltransferases do not appear to have a proregion that would serve as a target for proteolytic processing (Fig. 4). The proregion may have been added to Brn and Egh during evolution to impart an additional target for developmental regulation, sensitive to the action of proteolytic cascades. Brn or Frg may be glycosyltransferases that modulate N-ligand interactions by regulating accessibility of ligand to receptor or trigger N (or another receptor) activity by altering conformation of the receptor via carbohydrate modification, but there is currently no direct evidence that Brn or Frg have glycosyltransferase activity. Alternatively, Brn and Frg may be descendants of glycosyltransferases that have lost enzymatic activity and trigger receptor action in a manner similar to that of more classic cytokines.

Egh MAY FACILITATE Brn ACTIVITY

Although Brn is expressed in all germ cells throughout oogenesis, *brn* phenotypes are manifested specifically around the oocyte during mid-oogenesis, suggesting a high degree of spatiotemporal specificity to Brn signaling action (Goode et al. 1996a). Many hypotheses can be created to explain this anomaly. Differential expression of Egghead (Egh), a neurogenic mutant with phenotypes indistinguishable from Brn mutant phenotypes, offers a clue (Goode et al. 1996a). Egh becomes expressed in the oocyte precisely at the time and place that *brn* and *egh* epithelial defects become manifest (Fig. 5). Egh is a putative membrane protein of 457 amino acids, with a homo-

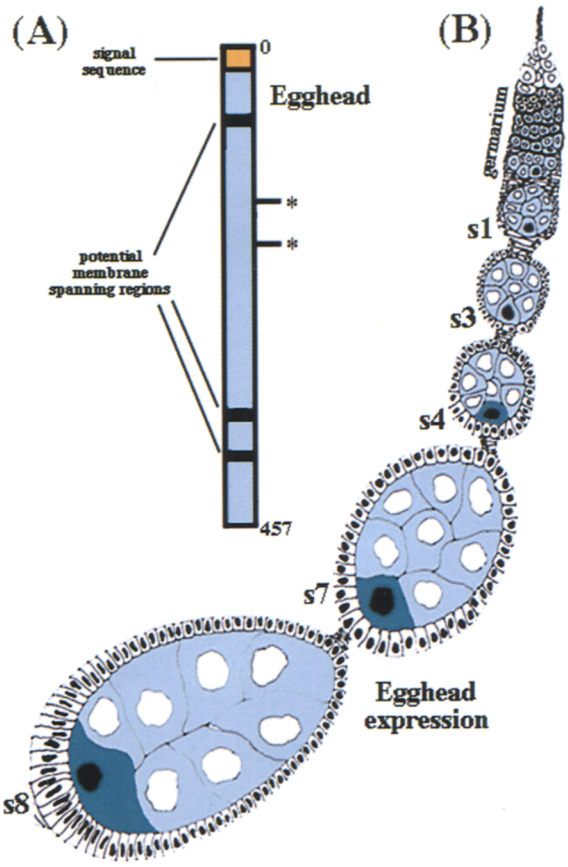

Figure 5. Egh structure and expression. (*A*) Predicted structure of the Egh protein (Goode et al. 1996a). Database searches indicate that Egh does not show similarity to proteins of known function, but Egh homologs have been identified in animals from *C. elegans* to humans. Egh has a putative signal sequence at the amino terminus and several putative transmembrane spanning regions. Egh thus appears to be a multipass membrane protein. Putative glycosylation sites (*) are consistent with this hypothesis. (*B*) Expression of Egh mRNA during oogenesis. Egh is expressed in all germ cells starting very early when the egg chamber is born in the germarium. At stage 4 (s4), Egh becomes differentially localized in the oocyte, corresponding to the time that epithelial defects first appear around the oocyte in animals harboring *brn* and *egh* mutant germ cells (Goode et al. 1996a).

log identified in *C. elegans* (Goode et al. 1996a). Egh is likely to participate directly in Brn signaling processes since *brn, egh* double-mutant animals do not display phenotypes any more severe than either mutant alone (Goode et al. 1996a). Egh may directly or indirectly bind to secreted Brn, limiting Brn diffusion, and/or increasing Brn activity, and/or modifying the structure of Brn. Additional factors similar to Egh have not been identified in the Frg pathway.

Brn AND Egh ARE REQUIRED FOR DORSAL VENTRAL PATTERNING

In addition to maintaining the apical-basal polarity of follicular epithelial cells, Brn and Egh are essential for dorsoventral (D/V) patterning during oogenesis. Brn and Egh are expressed in the oocyte during the time that D/V polarity of the egg is established, and eggs laid by both *brn* and *egh* mutant females have D/V defects, as indicated by a shift of morphological and molecular markers from dorsolateral to more dorsal positions on the egg shell and follicular epithelium (Goode et al. 1992, 1996a,b; Goode 1994). Temperature-shift experiments indicate that the requirement for Brn in D/V patterning is temporally separable from the requirement for Brn in maintaining the apical-basal polarity of the follicular epithelial cells (Goode et al. 1992). As described below, the involvement of Brn in D/V patterning is likely to occur via modulation of EGF receptor (Egfr) signals, the primary signals responsible for D/V patterning during oogenesis.

Grk, a transforming growth factor-α (TGF-α) homolog, is expressed on the dorsal side of the oocyte and triggers the *Drosophila* Egfr in dorsal follicle cells. In the absence of TGF-α or Egfr signals, all follicle cells assume a ventral cell fate, expression of dorsal follicle cell markers is completely abolished, and the oocyte also loses D/V polarity as indicated by the transformation of dorsal embryonic cell fates to more ventral cell fates in eggs laid by *grk* and Egfr mutant females (for review, see Ray and Schüpbach 1996). Brn phenotypes are subtle by comparison and have no consequence on D/V patterning in the early embryo.

To address the possibility that Brn acts by modulating Egfr signals, we asked whether TGF-α or Egfr signals are sensitive to reduction in Brn signals (Fig. 6). We approached this genetically, by looking for dominant and synergistic interactions between *brn* and *grk* or *Egfr*. We found defects in the D/V pattern of the eggshell in *brn*/+; *grk*/+ or *brn*/+; *Egfr*/+ doubly heterozygous animals and synergistic phenotypes in *brn*; *grk* and *brn*; *Egfr* and *N*; *grk* and *N*; *Egfr* double-mutant animals (Goode et al. 1992, 1996a,b; Goode 1994). These results strongly suggest that Brn signals significantly overlap with Grk signals. It will be of interest to examine further the interaction between Egfr and N signals in order to characterize at what levels these signaling pathways interact (Fig. 6).

SUMMARY AND FUTURE DIRECTIONS

In this review, we have made a simple comparison of functional parallels and molecular similarities between Brn and Frg. Analysis of Brn function in egg chambers suggests that Brn specificity in patterning fields of cells versus lateral specification is achieved by restricting Brn cooperation with N to the apical surface of follicle cells. Misexpression of Brn in the follicular epithelium, where it would have access to the N signaling process on the lateral surface of follicle cells, might be expected to interfere with N signaling processes, but this has not been

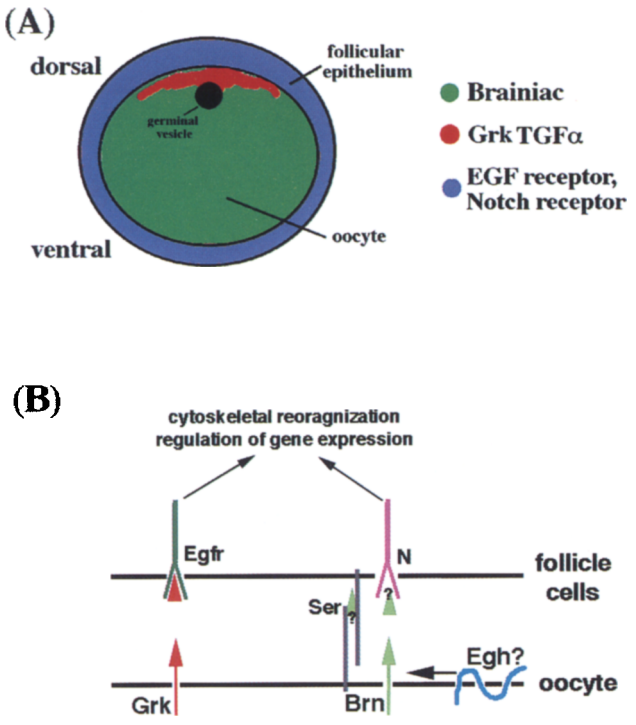

Figure 6. Expression and cooperativity of Brn and Grk in D/V patterning. (*A*) mRNA expression of Brn, Grk, Egfr, and protein expression of N in and around the oocyte from stages 8–9 of oogenesis (Xu et al. 1992; Goode et al. 1996b; Ray and Schüpbach 1996). Grk TGF-α is expressed on the dorsal side of the oocyte, where it triggers EGFr in dorsal follicle cells. Brn is expressed throughout the oocyte and may interact with the N signaling machinery in all follicle cells or with a yet unknown signaling system in a parallel pathway. (*B*) The relationship between Brn/N signals and Grk/Egfr signals is unclear, but an abundance of genetic evidence indicates that they interact at some level within follicle cells (see text; Goode et al. 1992, 1996a,b). D1 does not appear to be expressed in the oocyte or oocyte-associated follicle cells throughout the time that D/V pattern is established (Bender et al. 1993). The expression of Ser (?) has not been reported. It is not clear whether putative secreted Brn interacts with Ser, N, or some other extracellular factor(s) (?), but our current evidence indicates that Brn somehow regulates N signals. On the basis of results from genetic interaction experiments (see text), it seems likely that signals generated by N overlap with those generated by Egfr, at either a common cytoskeletal structure, a nuclear factor, or some component of the signal transduction machinery responsible for altering gene expression and rearranging the cytoskeleton.

tested. Furthermore, it will be of interest to test, as we propose for Brn, whether the specificity of Frg in patterning the wing disk relies on restricting Frg activity to a particular epithelial cell surface. We currently have no molecular knowledge of how Brn participates in the N signaling process. The apparent requirement of Frg to inhibit activation of N by Ser, and to potentiate activation of N by Dl, suggests avenues for investigation.

If conclusions drawn from analysis of Brn function during oogenesis can be applied to Brn function in early embryos, then we might expect to find that Brn is required for maintaining the epithelial integrity of the neurogenic ectoderm during neuroblast segregation but not for lateral specification between cells within the ectoderm (Goode et al. 1996a,b). Interestingly, N expression in mesodermal cells dramatically rescues the epidermal phenotype of *N* mutant embryos, which may imply that in addition to being required between "equivalent" ectodermal cells to ensure proper segregation of neural precursor cells, N acts between distinct tissue layers (Baker and Schubiger 1996). We find this hypothesis attractive because it suggests an alternative means by which Brn might be involved in regulating the segregation of neural precursor cells within the neurogenic ectoderm and is consistent with the tenet that Frg and Brn are specifically involved in N processes of induction and/or maintenance between nonequivalent fields of cells.

Brn and Frg are the first secreted factors to be implicated in N signaling processes. We would like to know whether Brn and Frg are diffusible factors and if their secretion is crucial for establishing their activity, perhaps by analyzing the functional consequences of expressing forms of the molecules that remain tethered to the membrane. Further understanding of Brn and Frg in N induction and maintenance processes will also depend on determining whether they act as glycosyltransferases or by a distinct mechanism to influence N-ligand interactions. It will also be important to understand how Brn and Frg differ in function, and to what degree, if any, they can substitute for each others' function. The proposal that N serves as a multifunctional receptor by using its multitude of EGF repeats to bind distinct ligands (Rebay et al. 1991) suggests the possibility that Brn and Frg might bind N either directly or through a distinct protein. Alternatively, Brn and Frg might modify the glycosylation state of N or another receptor to influence their ability to be activated by distinct ligands. Further analysis of Egh will be essential to demonstrate whether this protein is part of the Brn signaling process and whether Egh or an Egh homolog acts in the Frg signaling pathway.

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