Development 116, 177-192 (1992)
Printed in Great Britain © The Company of Biologists Limited 1992

The neurogenic locus *brainiac* cooperates with the *Drosophila* EGF receptor to establish the ovarian follicle and to determine its dorsal-ventral polarity

SCOTT GOODE, DAVID WRIGHT and ANTHONY P. MAHOWALD

University of Chicago, Department of Molecular Genetics and Cell Biology, Cummings Life Science Center, 920 East 58th Street, Chicago, IL 60637, USA

Summary

We have characterized the function of a new neurogenic locus, *brainiac* (*brn*), during oogenesis. Homozygous *brn* females lay eggs with fused dorsal appendages, a phenotype associated with *torpedo* (*top*) alleles of the *Drosophila* EGF receptor (DER) locus. By constructing double mutant females for both *brn* and *top*, we have found that *brn* is required for determining the dorsal-ventral polarity of the ovarian follicle. However, embryos from mature *brn* eggs develop a neurogenic phenotype which can be zygotically rescued if a wild-type sperm fertilizes the egg. This is the first instance of a *Drosophila* gene required for determination of dorsal-ventral follicle cell fates that is not required for determination of embryonic dorsal-ventral cell fates. The temperature-sensitive period for *brn* dorsal-ventral patterning begins at the inception of vitellogenesis.

The interaction between *brn* and DER is also required for at least two earlier follicle cell activities which are necessary to establish the ovarian follicle. Prefollicular cells fail to migrate between each oocyte/nurse cell complex, resulting in follicles with multiple sets of oocytes and nurse cells. *brn* and DER function is also required for establishing and/or maintaining a continuous follicular epithelium around each oocyte/nurse cell complex. These *brn* functions as well as the *brn* requirement for determination of dorsal-ventral polarity appear to be genetically separable functions of the *brn* locus. Genetic mosaic experiments show that *brn* is required in the germline during these processes whereas the DER is required in the follicle cells. We propose that *brn* may be part of a germline signaling pathway differentially regulating successive DER-dependent follicle cell activities of migration, division and/or adhesion and determination during oogenesis. These experiments indicate that *brn* is required in both tyrosine kinase and neurogenic intercellular signaling pathways. Moreover, the functions of *brn* in oogenesis are distinct from those of *Notch* and *Delta*, two other neurogenic loci that are known to be required for follicular development.

Key words: *brainiac*, *Drosophila* EGF receptor (DER), oogenesis, neurogenic, ovarian follicle, dorsal-ventral polarity, germline, soma, intercellular communication.

Introduction

Oogenesis involves direct interaction between germline cells and follicle cells for the successful development of fertile eggs (see Raven, 1961; McLaren and Wylie, 1983). In insects, these interactions take place within elongated tubes known as ovarioles. From the proximal end of the *Drosophila melanogaster* ovariole, where oogenesis begins with the formation of the egg chamber in the germarium, through the vitellarium where eggs mature, to the distal end of the ovariole, where eggs pass into the oviduct on their way to being laid, there are 12-13 increasingly mature egg chambers (King, 1970).

Egg chamber formation begins at the proximal tip of the germarium, where an oogonial stem cell divides to produce another oogonial stem cell and a cystoblast. The cystoblast undergoes four divisions with incomplete cytokinesis to give rise to a cluster of sixteen cystocytes interconnected by specializations called ring canals (Brown and King, 1964). Follicle cells (FCs) migrate in from the periphery of the germarium and completely surround each newly formed 16-cell cyst. The FCs along with the cyst form an egg chamber, or follicle (Koch and King, 1966). Newly formed follicles are displaced to the distal end of the germarium (by new cystocyte clusters formed more proximally), where the future oocyte becomes located at the posterior tip of the follicle. The remaining 15 daughter cells of the cystoblast differentiate as nurse cells (NCs), which provide most of the RNA and protein synthetic functions required for oogenesis (Koch and King, 1966). As the egg chamber leaves the germarium and enters the vitellarium, the FCs divide (Chandley, 1966) and change shape, forming a continuous,

one-cell thick cuboidal epithelium that completely surrounds the egg chamber.

The newly formed egg chamber remains connected at its proximal end to the germarium and at its distal end to next older follicle by a one-cell thick stalk of 5-7 stalk cells. The follicle undergoes a phase of development in which both NCs and oocyte increase in volume. The follicular sheath increases from approximately 80 to 1100 cells during this growth phase (King and Vanoucek, 1960), maintaining a continuous follicular epithelium around the expanding egg chamber. Subsequently, at the inception of vitellogenesis, most of the FCs migrate posteriorly around the enlarging oocyte, leaving a thin layer of squamous FCs surrounding the NCs (King and Vanoucek, 1960). The cells overlying the rapidly growing oocyte become columnar. A clear dorsal-ventral asymmetry emerges in both the follicular epithelium and oocyte by this stage, as the oocyte nucleus comes to lie at the future anterior-dorsal edge of the oocyte, and the FCs on the dorsal side of the egg chamber become taller than those on the ventral side. After completion of these growth and polarization phases, most of the contents of the nurse cells (except the polyploid nuclei) flow into the oocyte, the vitelline membrane (or first layer of the eggshell) is completed, and the FCs switch their synthetic functions to the production of the outer eggshell or chorion (see King, 1970 and Mahowald and Kambysellis, 1980, for reviews of *Drosophila* oogenesis).

Interactions between germline cells and FCs have been shown to be involved in establishing the polarity of the ovarian follicle. Thus the *dicephalic* gene, which functions in locating the oocyte to the posterior end of the follicle (Lohs-Schardin, 1982), is required in both germline and somatic cells of the ovary (Frey and Gutzeit, 1986). The establishment of the dorsal-ventral polarity of the growing egg chamber requires germline, e.g. *K10* and *gurken* (*grk*), and FC functions, e.g. *torpedo* (*top*). In the absence of *K10*, the oocyte and eggshell become dorsalized, whereas in the absence of either *grk* or *top*, both the eggshell and the embryo become ventralized (Wieschaus et al., 1978; Schüpbach, 1987). *top* mutations are alleles of *faint little ball* (*flb*), which has been shown to encode the *Drosophila* homolog of the EGF receptor (abbreviated DER, Schejter and Shilo, 1989; Price et al., 1989; Clifford and Schüpbach, 1989), a molecule known to play diverse roles in intercellular communication in vertebrates (for reviews see Hunter and Cooper, 1985; Yarden and Ullrich, 1988).

An influential role for intercellular communication is also apparent during insect neurogenesis. Cell ablation studies in the grasshopper (Doe and Goodman, 1985) have suggested that direct interactions between ectodermal cells occur in the early embryo, whereby neuroblasts (NBs) inhibit adjacent ectodermal cells from assuming a neuroblast fate. Inhibition of the NB fate is required to allow development of epidermoblasts, the precursors of the epidermis. In *Drosophila melanogaster*, the inhibitory signal appears to be mediated by a set of loci termed neurogenic (Hartenstein and Campos-Ortega, 1984; Lehmann et al., 1983). In the absence of neurogenic gene function, most or all of the cells within the neurogenic ectoderm adopt the NB fate. Two neurogenic loci, *Notch*(*N*) and *Delta*(*Dl*), encode large transmembrane proteins with EGF repeats (Wharton et al., 1985; Kidd et al., 1986; Vaessin et al., 1987). Two other neurogenic loci, *pecanex* and *big brain*, also encode large transmembrane proteins (La Bonne et al., 1989; Rao et al., 1990). One transcript within the *E(spl)* gene, another neurogenic locus, codes for a G-like protein subunit (Hartley et al., 1988), possibly involved in intracellular signal transfer. These molecular findings are consistent with a role for these loci in the production of a neuroblast inhibitory signal (reviewed by Campos-Ortega and Knust, 1990).

Neurogenic gene function has also been shown to be required in oogenesis. *N* and *Dl* appear to be required somatically for interaction between FCs leading to the development of specialized groups of FCs at the anterior and posterior poles of the egg chamber (Ruohola et al., 1991). The proper development of these FCs appears to be required for establishing the anterior-posterior polarity of the oocyte (Ruohola et al., 1991). In contrast to *N* and *Dl*, we show that *brainiac* (*brn*), a new neurogenic locus, is required for interaction between the germline and FCs. These interactions are necessary for the determination of the dorsal-ventral polarity of the follicle. In addition, our studies of *brn* demonstrate that interactions between germline and FCs begin very early in oogenesis to establish/maintain the ovarian follicle. *brn* acts in the germline in cooperation with somatically functioning DER to establish individual NC-oocyte complexes, maintain the follicular epithelium, and determine the dorsal-ventral polarity of the ovarian follicle. These are separable germline functions of the *brn* locus that appear to be needed for successive FC activities during oogenesis: migration, maintenance and dorsal-ventral cell fate determination. We propose that *brn* is necessary for the integrity of a germline signaling pathway differentially regulating DER-dependent FC activities throughout oogenesis.

Materials and methods

Genetics

The wild-type strain was Oregon R (OrR). $brn^{fs.107}$, previously *fs(1)A107*, and $Fs(1)ovo^{D1}$ were obtained in screens for female steriles (Gans et al., 1975). $brn^{l.6P6}$, previously *l(1)6P6*, was obtained in a screen for larval/pupal lethal mutations (Perrimon et al., 1989). A *y w* $brn^{l.6P6}$ chromosome, obtained as a recombinant with OrR, was used in most experiments. *fs(1)K10* is described by Wieschaus et al. (1978). top^{1} and top^{CJ} are described by Schüpbach (1987) and Clifford and Schüpbach (1989). grk^{WG41}, grk^{HK36}, and grk^{HG21} are described by Schüpbach (1987). All *top* and *grk* chromosomes are marked with *cn bw*. $Df(1)rb^{1}$, $Df(1)rb^{33}$ and $Df(1)rb^{46}$ are described by Banga et al. (1986). $Df(1)rb^{1}$ probably removes *brn* completely since it also removes two loci on either side of *brn*, *male-diplolethal* (*mdl*) and *mei-9* (Banga et al., 1986; Oliver et al., 1988). *Df(1)GA102* and $Dp(1;1)w^{+64b13}$ are described by Craymer and Roy (1980). *rugose* (*rg*) and *echinus* (*ec*) were obtained from the Mid America *Drosophila* stock center, and are described by Lindsley and Grell (1968).

$brn^{fs.107}$ was mapped between polytene chromosome bands 3F7,8 and 4A3,6 because it is complemented by $Df(1)rb^{46}$ = *Df(1)4A3,6- 4C6-7* and *Df(1)GA102 = Df(1)3D5; 3F7- 8*, whereas $Df(1)rb^{33}$ *=Df(1)3F3-4; 4C15-16* fails to complement the neurogenic and fused dorsal appendage phenotypes associated with this

allele. We are not able to deficiency map *brn*$^{l.6P6}$ because the *brn* locus lies immediately adjacent to the *mdl* locus. We therefore meiotically mapped *brn*$^{l.6P6}$ relative to *ec* (5.5) and *rg* (11.0). We obtained 11 out of 1972 *y w ec*$^{+}$ *brn*$^{l.6P6+}$ progeny from *y w brn*$^{l.6P6}$/*ec* females. We obtained 93/1761 *y*$^{+}$ *w*$^{+}$ *brn*$^{l.6P6+}$ *rg*$^{+}$ progeny from *y w brn*$^{l.6P6}$/*rg* females. All 104 of these recombinants were back crossed to the *y w brn*$^{l.6P6}$ chromosome, in order to determine that the fused dorsal appendage, neurogenic and fused chamber phenotypes mapped to the same locus as *brn*$^{l.6P6}$. All females were fully fertile and did not lay brown eggs or eggs with FDAs, indicating that all four phenotypes map to the same locus. Based on *echinus* recombinants, *brn*$^{l.6P6}$ maps to meiotic position 6.1; based on *rugose* recombinants, *brn*$^{l.6P6}$ maps to position 5.7. We assign *brn*$^{l.6P6}$ to position 5.9 on the meiotic map, which correlates with the chromosome band location of *brn*$^{fs.107}$. In addition, we obtained *Dp(1;2)w*$^{+64b13}$ in which the *diplolethal* locus was mutated by X-irradiation (Helen Salz, unpublished). This duplication complements the *brn*$^{fs.107}$ fused dorsal appendage and neurogenic phenotypes as well as the *brn*$^{l.6P6}$ lethality. With these mapping data, combined with the fact that *brn*$^{fs.107}$/*brn*$^{l.6P6}$ females lay eggs with fused dorsal appendages which develop neurogenic phenotypes, we conclude that *brn*$^{fs.107}$ and *brn*$^{l.6P6}$ are alleles of the same locus.

For analysis of maternal effect phenotypes, homozygous females were taken from balanced stocks. Heterozygous females were obtained from appropriate crosses. Double mutant females were taken from *brn*$^{fs.107}$/*FM3*; *top*/*CyO* stocks. Germline clones were induced by X-irradiation (1200 rads) of first instar larvae obtained from the following crosses: (a) *brn*$^{l.6P6}$/*FM7* × *Fs(1)ovo*^{D1}/Y, (b) *brn*$^{fs.107}$/*FM3*;*top*^{CJ}/*CyO* × *Fs(1)ovo*^{D1}/Y;*top*^{CJ} and (c) *brn*$^{l.6P6}$/*FM7*;*top*^{CJ}/*CyO* × *Fs(1)ovo*^{D1}/Y;*top*^{CJ}. Informative females were identified by scoring appropriate markers, and females carrying germline clones (GLCs) were identified as described below. These females are referred to as (a) *brn*$^{l.6P6}$GLC, (b) *brn*$^{fs.107}$GLC;*top*^{CJ} and (c) *brn*$^{l.6P6}$GLC;*top*^{CJ}, respectively, in the text.

brn$^{fs.107}$;*top*/+ as well as *brn*$^{fs.107}$/+;*top* flies were analyzed as controls for *brn*$^{fs.107}$;*top* double mutant experiments. Females of both genotypes are highly fecund and never lay eggs having stronger ventralization than fusion of dorsal appendages, although the penetrance and expressivity of the FDA phenotype is increased in both cases (data not shown). Some ovarioles from these control females, nevertheless, contain chambers with more than one NC-oocyte complex. 6%, 4% and 6% of ovarioles from *brn*$^{fs.107}$;*top*^{CJ}/+, *brn*$^{fs.107}$/+;*top*^{1} and *brn*$^{fs.107}$/+;*top*^{CJ} females, respectively, have at least one fused chamber. Discontinuities of the follicular epithelium are never observed in control egg chambers. In contrast, most egg chambers in every ovariole from double mutant females are abnormal. For germline clone experiments, *brn*$^{fs.107}$GLC;*top*^{CJ}/*CyO* females were analyzed as controls, and were found to have aberrant egg chambers at frequencies comparable to control females described above.

For the zygotic rescue experiment, *brn*$^{fs.107}$ females were mated to wild-type males, and embryos were collected for 5-6 hours. A set number of embryos was then transferred to another plate and, after aging for 30 hours, eggs that did not hatch were transferred to another agar plate and allowed to incubate at 25°C for 24 hours. The number of embryos that hatched was taken as the number of starting eggs minus the number of eggs transferred. Brown eggs (having melanized cuticle) were scored as not zygotically rescued, while white eggs, presumably unfertilized, were ignored.

Characterization of embryonic phenotypes

Eggs were collected and aged at 25°C on yeasted agar-molasses plates.

Hoyer's mounts of embryonic cuticles were prepared as described by Wieschaus and Nüsslein-Volhard (1986) and viewed using phase contrast microscopy. Cuticles were scored under phase contrast optics as intermediate or weak according to the criteria of Lehmann et al. (1983). If an embryo had any ventral cuticle remaining, it was scored as weak.

Drosophila embryonic nervous systems were stained with horse radish peroxidase (HRP) (Jan and Jan, 1982) according to the procedure of Fredieu and Mahowald (1989). Polyclonal antisera to HRP (Organon Teknika) were purified by HRP affinity chromatography, and detected with a FITC-conjugated secondary antibody. Embryos were mounted in 1:1 glycerol:ethanol and viewed by epifluorescence.

For histological preparations, embryos were removed from the vitelline membrane as for antibody staining and then placed overnight at 4°C in the fixative of Kalt and Tandler (1971). After dehydrating through an ethanol series, embryos were infiltrated with JB4 plastic overnight at 4°C, and then embedded in plastic. Serial 4-micron sections were stained with 1% methylene blue in a 1:1 mixture of 1% boric acid:70% ethanol, and viewed in bright field optics.

Characterization of egg phenotypes

Eggs were collected at 18°C or 25°C on yeasted agar-molasses plates. All eggs in Tables 2 and 3 were scored using a dissecting scope. Follicle cell imprints on double mutant eggs were analyzed by phase contrast microscopy of Hoyer mounted eggs (prepared as described for embryos).

Phenocritical period for brn *dorsal-ventral patterning activity*

brn$^{fs.107}$ flies were grown at either 18°C or 25°C and females collected over a two day period. Eggs were collected for 3-4 days at 12-24 hour intervals. Flies were shifted to either 18°C or 25°C and eggs were collected at intervals as designated in Fig. 3. All eggs laid following the shift were scored. Six bottles (40 females/bottle) were used per experiment. Results were consistent between bottles.

Characterization of oogenesis phenotypes

All experimental ovaries were dissected at 1-1.5 days post eclosion, to avoid analyzing secondary phenotypes. For example, egg chambers are commonly observed fusing within the vitellarium of 3-day old double mutant females, but we do not observe these events in 1- to 1.5-day old females. However, chambers having supernumerary NC-oocyte complexes as well as discontinuities in the follicular epithelium are observed in newly eclosed *brn*$^{fs.107}$; *top* double mutant females. Since 1- to 1.5-day old females do not lay eggs, ovaries in germline clone experiments that had ovarioles with egg chambers developed past stage 3-4 (the point at which *Fs(1)ovo*^{D1} blocks oogenesis [Busson et al., 1983]) were scored as containing germline clones. In most females, only one of the two ovaries contains a clone, and the contralateral ovary can be used as a control. Egg chambers in ovarioles from such contralateral controls do not have supernumerary oocyte NC complexes or follicular epithelium discontinuities as do the experimental ovaries.

For Hoechst staining, ovaries were dissected in *Drosophila* Ringers solution and then stained in 1% Hoechst # 33528 dye (obtained from Sigma, prepared in *Drosophila* Ringers) for 10 minutes, rinsed in Ringers, then fixed for 5 minutes in acetone. Ovaries were then mounted on a slide in 60% glycerol, the ovarioles teased apart using tungsten needles, and examined by epifluorescence. Oocyte nuclei were distinguished from NC nuclei by size, and then confirmed by differential interference contrast (DIC) microscopy. NC and oocyte numbers were scored by focusing through the egg chamber. In all cases, the numbers were con-

firmed by rescoring. For chambers containing 16 to approximately 40 cystocytes, counts were typically repeated once, and the repeated count typically fell within +/− 1 of the original count. Sometimes counts were repeated more than this, depending on the difficulty of the sample. For chambers containing >40 cystocytes, counts were repeated 3-4 times, with counts falling within +/− 2 of the noted count.

Ring canals were stained with phalloidin according to a modification of Warn et al. (1985). After dissecting ovaries in Ringer's, they were fixed in 4% formaldehyde in phosphate-buffered saline (PBS) for 30 minutes, then extracted with acetone for 5 minutes at −20°C before incubating in rhodamine-phalloidin (0.16 μg/ml PBS) for an hour. Ovaries were then mounted on a slide in 60% glycerol and the ovarioles teased apart. Ring canals were revealed using epifluorescence as above. For confocal microscopy, a Zeiss confocal laser scan microscope was used (courtesy of Dr. T. Karr) to produce a 3-D reconstruction of phalloidin-stained egg chambers.

Hoechst-stained preparations were analyzed for holes in the follicular epithelium. Since acetone is a harsh fixative and can cause tissue distortion, we confirmed this phenotype by viewing both living tissue as well as glutaraldehyde-fixed tissues using DIC microscopy. Following fixation in 2% glutaraldehyde (in PBS) for 10 minutes, ovaries were removed, washed and the ovarioles were teased apart with tungsten needles. Ovarioles were either mounted in Aquamount (Lerner) for temporary preparations, or dehydrated through an ethanol series, cleared in xylene and mounted in Permount for permanent preparations. The percentage of chambers having holes was determined using Hoechst-stained preparations only.

Ovaries were fixed and then stained with Fas III monoclonal antibody as described by Ruohola et al. (1991), except that anti-Fas III was used at 1:5 dilution with a FITC- conjugated antibody. Ovarioles were teased apart and mounted in 60% glycerol, and fluorescently labelled follicle cells were examined using epifluorescence as above.

Construction of genetic mosaics by pole cell transplantation

Embryonic pole cells were transplanted by the method of M. Zalokar and P. Santamaria as described by Lehmann and Nüsslein-Volhard (1987), with minor differences. Cloroxed hosts and donors were stage-selected by posterior clearing to mid-late cellular blastoderm during agar alignment and mounted on double stick tape (3M #666). Hosts were desiccated over silica gel for a fixed time, empirically giving about 5% leaking eggs. Injections were performed under Halocarbon Product's Series 700 oil and the embryos allowed to hatch on plain agar under Series 27 oil in a humid chamber. Processing, injection and hatching were carried out at 17°C, then hatchings were transferred *en masse* to standard vials. Isolated females were mated on eclosion to $brn^{fs.107}$ males, observed for egg laying, and dissected after 5 days.

Results

The *brainiac* (*brn*) locus maps between polytene bands 3F7,8 and 4A3,6 and to meiotic map position 1-5.9 (see Materials and methods). The maternal effect phenotypes described in this paper are based on analysis of two partial loss-of-function alleles of the *brn* locus, $brn^{fs.107}$ and $brn^{l.6P6}$. $brn^{fs.107}$ females are viable, whereas $brn^{l.6P6}$ animals die as pharate adults. Analysis of the maternal effect phenotypes associated with $brn^{l.6P6}$ was accomplished either by induction of germline clones (GLCs) in females heterozygous for $brn^{l.6P6}$ (referred to as $brn^{l.6P6}$ GLC females, see Materials and methods), or by analysis of eggs derived from females doubly heterozygous for $brn^{fs.107}/brn^{l.6P6}$.

brainiac *is required zygotically for the segregation of neuroblasts and epidermoblasts*

brainiac females produce embryos with epidermal hypoplasia (Fig. 1A, B) and neural hyperplasia (Fig. 1C, D) (Perrimon et al., 1986, 1989; our results). As for other neurogenic mutants (Lehmann et al., 1983), this phenotype appears to arise during hours 4-7 of embryogenesis due to an excessive commitment of ectodermal cells to the NB lineage (Fig.1E, F). The strongest *brn* phenotype resembles the "intermediate" level (as classified by Lehmann et al., 1983) of neurogenic transformation.

brn embryos are zygotically rescuable. Whereas 2% of embryos hatch when $brn^{fs.107}$ females are mated to $brn^{fs.107}$ males (N=328), 51% hatch (N=607) when $brn^{fs.107}$ mothers are mated to wild-type males (embryos from $brn^{fs.107}/brn^{l.6P6}$, $brn^{fs.107}/Df(1)rb^{1}$, and $brn^{l.6P6}$ GLC females are also zygotically rescuable, to a large but undetermined extent). As for the well-characterized zygotic neurogenic loci, *brainiac* gene activity is thus required zygotically to prevent all cells of the neurogenic region from differentiating as NBs. The $brn^{l.6P6}$ allele shows greater reduction in the neurogenic component of gene activity than $brn^{fs.107}$, the former behaving as a complete loss of gene activity (Table 1).

brainiac *is required for dorsal-ventral patterning the ovarian follicle*

The chorion of the *Drosophila* egg is secreted by the FCs during the final stages of oogenesis and contains several polarized structures that reflect the asymmetries arising during the earlier phases of development (King, 1970; Mahowald and Kambysellis, 1980; Wieschaus, 1979). For example, the respiratory appendages identify the anterior-dorsal surface (Fig. 2A) of the egg. The chorions produced by *brn* females have dorsal appendages located at the normal anterior position, but fused along the dorsal midline (Fig. 2). The fused dorsal appendage (FDA) phenotype is similar to the phenotype associated with weak alleles of two loci known to be required for the determination of the dorsal-ventral axis of the embryo and eggshell, *torpedo* (*top)* and *gurken* (*grk*) (Fig. 2; Schüpbach, 1987). Stronger alleles of these loci are associated with a complete loss of the dorsal appendages. These phenotypes have been interpreted as resulting from a "ventralization" of FC fates, such that FCs located on the dorsal side of the egg have adopted the fates normally adopted by FCs located more ventrally. Zygotes developing within ventralized *grk* and *top* eggshells show a corresponding increase in embryo ventralization with greater loss of maternal gene function, developing greater amounts of ventrally derived mesoderm with loss of dorsal ectoderm (Schüpbach, 1987).

In contrast to the effect on early neurogenesis, $brn^{fs.107}$ appears to represent a greater loss of *brn* gene activity for dorsal appendage patterning than $brn^{l.6P6}$ (Tables 1 and 2), suggesting that the *brn* chorion and embryo phenotypes are independent. This idea is substantiated by *brn* embryos

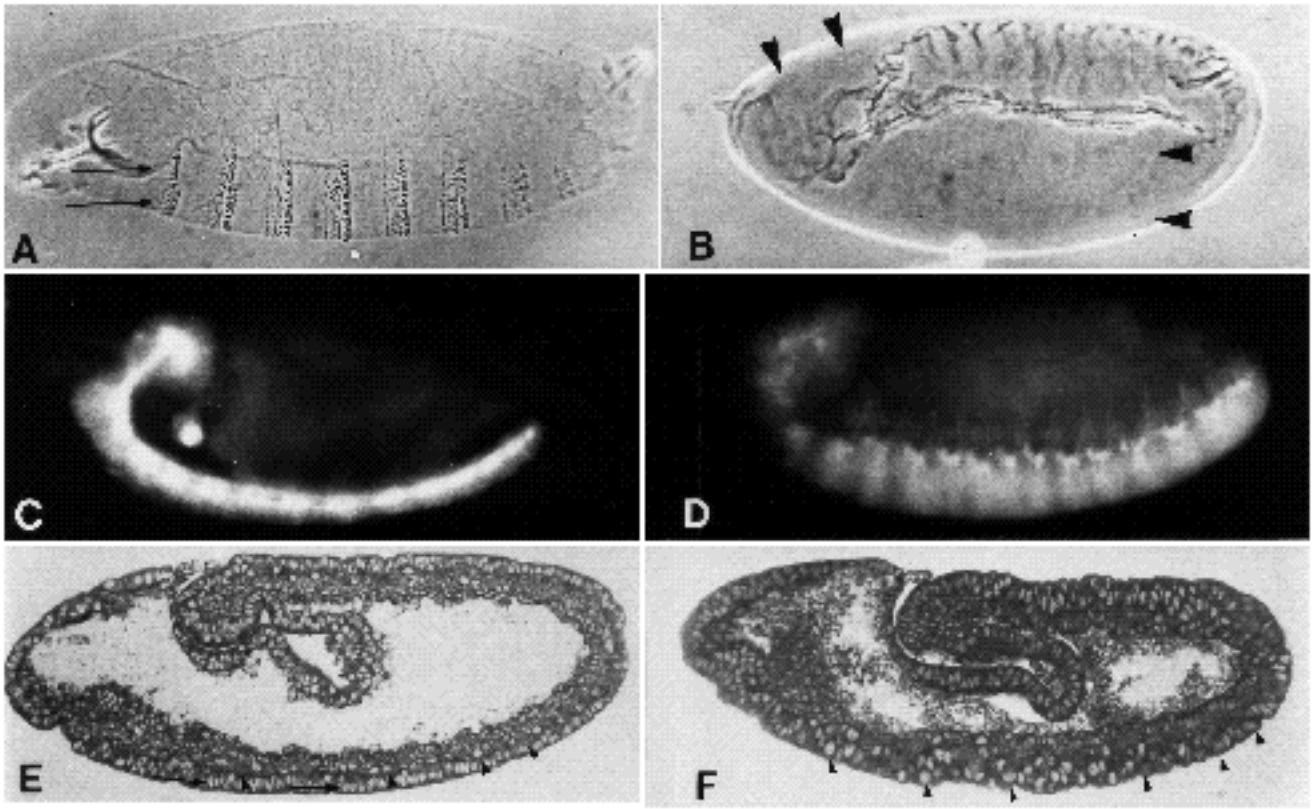

Fig. 1. *brainiac* is a neurogenic mutant. All embryos are shown anterior left and ventral down. Lactic acid (A) and Hoyer's (B) mounts of a wild-type embryo removed from the egg coverings (A), and of an embryo from a $brn^{fs.107}/brn^{l.6P6}$ female (X $brn^{fs.107}$/Y) within the vitelline membrane (B). Ventral (demarcated by denticle belts on the wild-type embryo, arrows), ventrolateral and cephalic cuticle (arrow-heads) is missing in the *brn* mutant embryo. (C) The embryonic nervous system is revealed in this 10-12 hour wild-type embryo stained with anti-HRP antibody. The embryo from the $brn^{fs.107}/brn^{fs.107}$ female (X $brn^{fs.107}$/Y) shown in D has a hypertrophied nervous system, in the same region of the embryo that suffers loss of epidermally secreted cuticle shown in B. Large, round, presumptive neuroblasts (arrowheads) can be observed forming and already segregated into the 5-7 hour wild-type embryo shown in E. The 5-7 hour embryo from the $brn^{fs.107}/brn^{fs.107}$ female (X $brn^{fs.107}$/Y) shown in F contains supernumerary neuroblasts (arrowheads) along the surface of the embryo where more columnar presumptive epidermoblasts are found in the wild-type embryo (arrows). The dorsal patch of cuticle in *brn* mutant embryos (B) has no denticle belts or disruption of Filzkorper structures typical of ventralized embryos, e.g. from females carrying loss of function *torpedo* or gain of function *easter* mutations. In addition, mutant embryos undergo normal gastrulation and germband elongation (F), further indicating that *brn* embryos have normal dorsal-ventral polarity. All embryos from females reared at 25°C. Embryos reared at 18°C show no differences in cuticle phenotype.

showing no evidence of a shift in dorsal-ventral polarity (Fig.1) and being zygotically rescuable (see previous section). In contrast, the embryonic ventralization phenotype of *top* and *grk* correlates with the chorion phenotype and is strictly dependent on the genotype of the mother. Further, the ventralized FDA phenotype associated with top^{1} and top^{CJ} homozygous females is increased in severity in $top^{1}/Df(2)top^{3F18}$ or $top^{CJ}/Df(2)top^{3F18}$ hemizygous females, which lay severely ventralized eggs completely lacking dorsal appendages (Clifford and Schüpbach, 1989). In contrast, neither homozygous $brn^{fs.107}$ nor hemizygous $brn^{fs.107}/Df(1)rb^{1}$ females (18°C) lay more severely ventralized eggs.

Does the *brn* FDA phenotype represent a defect in dorsal-ventral patterning or another developmental process? This question was addressed by constructing females doubly mutant for $brn^{fs.107}$ and *top*. *top* is required for the dorsal-ventral patterning of the ovarian follicle; if *brn* is required likewise, then *top* function should depend on *brn* function, and females double mutant for *brn* and weak *top* alleles should have more strongly ventralized eggs. Most of the eggs laid by double mutant $brn^{fs.107}$; *top* females completely lack dorsal appendages (Fig. 2D; Table 3), have a more rounded dorsal side than wild-type eggs, and have FC imprints around their circumference characteristic of the ventral surface of the wild-type egg (data not shown). As demonstrated in genetic mosaic experiments (Table 6), *brn* is required in the germline for determination of FC fates.

The FDA phenotype of $brn^{fs.107}$ eggs shows a cold temperature sensitivity (Table 2) which is first detected at stages 6-7 of oogenesis (Fig. 3). This is the point at which the egg chamber first begins overt polarization. This phenocritical period is consistent with a role for *brn* in determining the dorsal-ventral axis of the ovarian follicle.

brainiac *and* torpedo *are required for the initial formation of the follicle in the germarium*

$brn^{l.6P6}$ GLC females lay only 3-7 eggs over their lifetime

Table 1. brn$^{l.6P6}$ *shows greater loss of gene activity than* brn$^{fs.107}$ *for early neurogenesis*

Genotype of females crossed to *brn*$^{fs.107}$ males	Level of neurogenic transformation		
	% intermediate	% weak	% hatch
brn$^{fs.107}$/*brn*$^{fs.107}$ (N= 252)	80	18	2
brn$^{fs.107}$/*brn*$^{l.6P6}$ (N= 201)	99	1	0
brn$^{fs.107}$/*Df(1)rb*^{1} (N= 276)	99	1	0

Embryos are scored as intermediate or weak based on cuticle phenotypes as defined by Lehmann et al. (1983). Loss offunction associated with *brn*$^{l.6P6}$ is equivalent to the presumptive complete loss of *brn* function associated with *Df(1)rb*^{1}. Experiments were performed at 25°C.

(Perrimon et al.,1989, and this report). Similarly, *brn*$^{fs.107}$; *top* double mutant females lay only a few or no eggs (Table 3), in contrast to females mutant for either *brn*$^{fs.107}$ or *top*, which lay hundreds of eggs. Although double mutant females show a reduction in viability (Tables 4 and 5), reduced egg production is not caused by this reduced viability. The poor fecundity of these females must be due to a specific ovarian interaction of *brn* and *top*, since the same ovarian phenotype is obtained from *brn*$^{fs.107}$; *top*^{CJ} double mutant females and from *brn*$^{fs.107}$GLC; *top*^{CJ} females, in which only the germ cells, and not the whole animal, are mutant for *brn*$^{fs.107}$.

The cause of the reduced fecundity of doubly mutant *brn*$^{fs.107}$GLC; *top*^{CJ} females appears to be the same as that found in *brn*$^{l.6P6}$GLC females. Most follicles (see below) have more than one oocyte and more than 15 NCs. These follicles are formed within the germarium (Fig. 4E, F and below) and can proceed to late stages of oogenesis (Fig. 4G, H). Occasionally such chambers will proceed with chorion synthesis and form an "egg" in the shape of a ball, lacking overt polarity, presumably because competition for polarity among several oocytes is balanced. In most cases, however, such chambers degenerate around stages 9-11. Development of normal egg chambers depends on *brn* function in the germline, since in these germline clone experiments only the germ cells are homozygous mutant for *brn* (there is a normal copy of *brn* in the FCs).

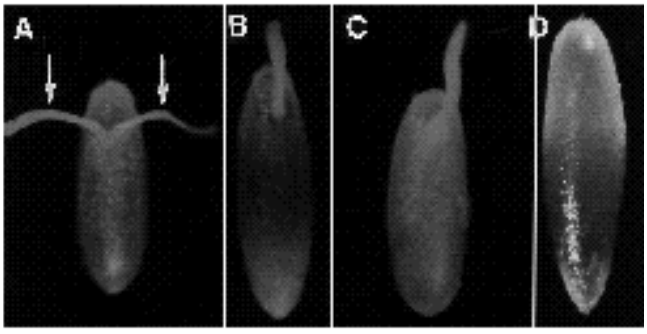

Fig. 2. *brainiac* is required for dorsal-ventral patterning of the ovarian follicle. The two dorsal appendages of the wild-type egg (A, arrows) demarcate the dorsolateral surface of the egg. The dorsal appendages have been shifted to the dorsal midline of *top*^{1} (B) and *brn*$^{fs.107}$ (C) eggs, resulting in a fused dorsal appendage phenotype. This indicates that follicle cells along the dorsal midline have assumed fates of follicle cells located more ventrally. The *brn*$^{fs.107}$; *top*^{1} egg shown in D has completely lost the dorsal appendages and has other features of the completely ventralized phenotype associated with strong *top* and *gurken* mutants (see text). This indicates that *top* dorsal-ventral patterning activity depends on *brn* gene activity, and thus that *brn* is required for dorsal-ventral patterning of the ovarian follicle. Note that *top*^{1} eggs (B) have pointed anterior and posterior ends similar to completely ventralized eggs (D), whereas *brn*$^{fs.107}$ eggs (C) retain the rounded anterior and posterior characteristic of wildtype eggs (A). Eggs photographed using a dissecting microscope.

Table 2. *For dorsal appendage patterning* brn$^{fs.107}$ *is cold sensitive and shows greater loss of gene activity than* brn$^{l.6P6}$

Genotype	fused > 50%	fused<50%	not fused
	18/25°C	18/25°C	18/25°C
brn$^{fs.107}$/*brn*$^{fs.107}$ (N= 288/103)	93/15	6/70	1/15
brn$^{fs.107}$/*brn*$^{l.6P6}$ (N=265/321)	85/8	15/73	0/19
brn$^{fs.107}$/*Df(1)rb*^{1} (N=266/390)	94/16	5/67	1/17

Numbers represent percentage of eggs judged by eye to be fused 50% to complete (measuring along the length of the appendage, from the base of the egg), less than 50%, or not fused, resembling wild-type. Loss of function associated with *brn*$^{fs.107}$ is equivalent to the presumptive complete loss of gene function associated with *Df(1)rb*^{1}.

The synergistic interaction between *brn* and *top* is not allele-specific. Flies doubly mutant for *brn*$^{fs.107}$ and three *top* allele combinations (cf. Table 3) display similar phenotypes. Further, *brn*$^{l.6P6}$ GLC; *top*^{CJ} ovaries have a strikingly enhanced phenotype compared to *brn*$^{l.6P6}$ GLC ovaries, showing in some instances chambers with more than 100 NCs (Fig. 4D). We compared the phenotypes of these different allelic combinations by scoring the frequency with which chambers are found with different numbers of NCs and oocytes (Fig. 5). These results show that these different allele combinations fall into a series of decreasing severity: *brn*$^{l.6P6}$ GLC; *top*^{CJ} > *brn*$^{fs.107}$GLC; *top*^{CJ} > *brn*$^{l.6P6}$ GLC.

Table 3. *Nearly all eggs formed by* brn*;* top *double mutant females are completely ventralized*

Genotype	% with fused dorsal appendages	% completely ventralized
brn$^{fs.107}$	85[1]	0
top^{1}, or *top*^{1}/*top*^{CJ} or *top*^{CJ} (N> 500)	100	0
brn$^{fs.107}$;*top*^{1} (N= 105)	8	92
brn$^{fs.107}$;*top*^{1}/*top*^{CJ} (N= 111)	3	97
brn$^{fs.107}$;*top*^{CJ}	0	do not lay eggs[2]

Eggs were scored as completely ventralized if they completely lacked dorsal appendages. This phenotype corresponds in severity to intermediate and strong ventralization as designated by Schupbach (1987). Most such "completely ventralized" eggs were judged to be in the strong class.

[1] The remaining 15% are wild type in appearance, see Table 2.

[2] Occasionally a mature, completely ventralized, egg is formed within these females. Most eggs that form, however, are grossly deformed. Most ovarioles do not contain chorion-secreting follicles. For the other *brn*;*top* double mutant females, only 5-10 eggs are laid.

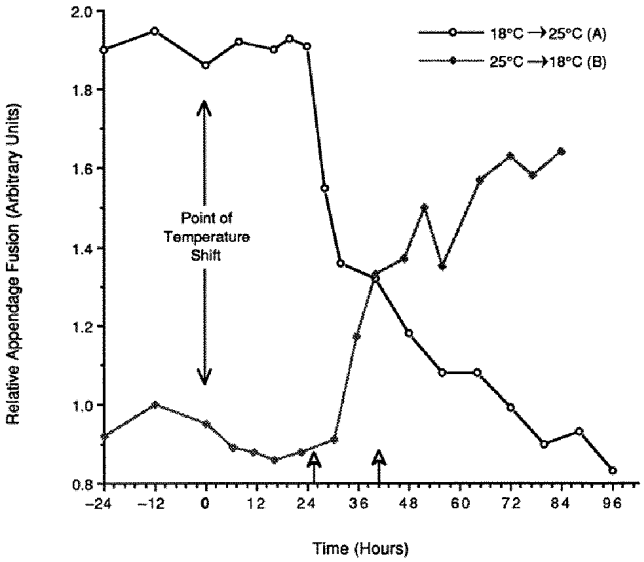

Fig. 3. Phenocritical period for *brainiac* dorsal-ventral patterning activity. *brn*$^{fs.107}$ females were reared at 18°C or 25°C and shifted to 25°C (A) or 18°C (B), respectively. Eggs were scored as in Table 2 and assigned values such that fused > 50% = 2, fused < 50% = 1, and not fused = 0. The relative appendage fusion for a given time point was calculated by summing the values for the 100-300 eggs scored (for each time point), and dividing by the total number of eggs. When reared continuously at 18°C the average value is approximately 1.9 and at 25°C the average value is approximately 0.9. We make the assumption that as for embryogenesis, oogenesis takes place twice as fast at 25°C versus 18°C. Time values for (B) were thus halved to compensate for slower development. We assign the phenocritical period to between 26 and 41 hours post shift (open-headed arrows). The decrease in slope for both shifts at about 60 hours is presumably due to retention of eggs by some ovarioles. Using the data of David and Merle (1968), 26 to 41 hours previous to egg laying corresponds to approximately stages 6-7 of oogenesis, when the egg chamber first begins to polarize following the early vitellarium growth phase.

brainiac *and* torpedo *are required for the proper gathering of FCs around 16-cell cysts*

We consider two manners in which follicles containing supernumerary oocytes and NCs might originate in the germarium. Egg chambers with supernumerary cystocytes could result from aberrant patterns of cystocyte division as postulated for ovarian tumor mutations (King et al., 1957). On the other hand, these chambers might result from the failure of FCs to gather around and correctly separate individual 16-cell cysts. Both of these mechanisms depend on *brn* function in the germline, since the results presented in the previous section were obtained in germline clone experiments in which only the germ cells, but not the remaining cells of the animal, were homozygous mutant for *brn.*

Follicles with supernumerary NCs and oocytes might be produced by additional cystoblast divisions, producing follicles with multiples of 16 cells. A number of observations make this unlikely. First, the cells of a wild-type 16-cell cluster remain interconnected via ring canals remaining after each incomplete cystocyte division (Brown and King, 1964). The oocyte and one of the NCs have four ring canals.

Table 4. *Relative viability of* brn$^{fs.107}$ *and* top^{CJ} *flies*

Genotype	Relative viability
brn$^{fs.107}$	98
brn$^{fs.107}$*/In(1) FM3*	102
top^{CJ}	94
top^{CJ}*/ In(2LR)CyO*	106

Numbers represent percent of expected assuming Mendelian segregation from the crosses *brn*$^{fs.107}$*/In(1)FM3* × *brn*$^{fs.107}$*/Y* and *top*^{CJ}*/In(2LR)CyO* × *top*^{CJ}*/top*^{CJ}.

Extra rounds of cell division would be expected to give rise to oocytes and NCs having 5, 6 and 7 ring canals. We have stained egg chambers with phalloidin to observe the actin ring of the canals (Warn et al., 1985) and we do not observe oocytes or NCs with more than 4 canals. Further, all cystocytes have at least one ring canal. This contrasts with the ovarian tumor phenotype, which is thought to result from the over-proliferation of cystocytes (King et al., 1957). In these mutants, most cystocytes undergo complete division (Johnson and King, 1972; King and Riley, 1982), and do not have ring canals interconnecting adjacent cystocytes. Further, the supernumerary oocytes found in the tumorous egg chambers of *benign gonial cell neoplasm* females remain clustered together at the normal posterior position yet fail to take up yolk (Gutzeit and Strauss, 1989), in contrast to the varied distributions of the vitellogenic oocytes we observe (e.g. Fig. 4G, H). Second, in follicles recently derived from the germarium in which there is no size differential between NCs, the ring canal pattern clearly establishes the separate origin of chambers (Fig. 6). In this instance, the size of the ring canals for the posterior oocyte establishes its greater age (cf. Koch and King, 1969). Third, within follicles having extra sets of NCs and oocytes, the NC clusters are clearly of different sizes, suggesting that they are at different levels of ploidy, and are thus derived from cystoblasts with different birthdates. In the most extreme case found in a *brn*$^{l.6P6}$ GLC; *top*^{CJ} female, a clear gradient in NC size can be seen from apparent germarial cystocytes at the proximal end of the egg chamber to apparent stage 10-type polyploid NCs at the distal end of the egg chamber (Fig. 4D). A similar distribution of NC sizes can be seen distributed between more egg chambers in ovarioles from *brn*$^{fs.107}$ GLC;*top*^{CJ} and *brn*$^{l.6P6}$ GLC females shown in Fig. 4. Since no FCs are observed between clus-

Table 5. *Relative viability of* brn$^{fs.107}$*;*top^{CJ} *double mutant flies*

Genotype	Relative viability
brn$^{fs.107}$*/In(1) FM3;top*^{CJ}*/In(2LR) CyO*	96
brn$^{fs.107}$*;top*^{CJ}*/In(2LR)CyO*	92
brn$^{fs.107}$*/In(1) FM3;top*^{CJ}	92
brn$^{fs.107}$*;top*^{CJ}	22

Numbers represent percent of expected based on Table 4 controls from the cross *brn*$^{fs.107}$*/In(1)FM3;top*^{CJ}*/In(2LR)CyO* × *brn*$^{fs.107}$*/Y ;top*^{CJ}*/top*^{CJ}. Flies doubly mutant for *brn*$^{fs.107}$ and other *top* allele combinations are also subviable.

ters of NCs, these chambers are most easily explained by the inclusion of multiple 16-cell cysts, each derived from a single cystoblast, within one follicular epithelium. These results suggest that mutant egg chambers result from the failure of the FCs to separate adjacent 16-cell cysts.

The number of supernumerary NCs within a given egg chamber is not always a multiple of 15. We frequently observe, however, that the total number of NCs in two adjacent chambers is a multiple of 15. For example, in *brn*$^{l.6P6}$ GLC females, one chamber had 17 NCs and an oocyte and the next chamber had 13 NCs and an oocyte. In another instance, one chamber had 35 NCs and 2 oocytes, while an adjacent chamber had 25 NC and 2 oocytes. In both of these cases, the combined NC to oocyte ratio between pairs of chambers is 15:1. A cluster of NCs in such a chamber containing more NCs than 15 X (no. of oocytes) appears to be approximately the size of the NCs in the neighboring egg chamber with fewer NCs than 15 X (no. of oocytes). This complementarity between chambers suggests that some cystocytes somehow become separated from their sister cells and are included within adjacent follicles.

Alternatively, complementary chambers might originate by incomplete cystocyte division and subsequent enclosure of multiple incomplete cysts within one egg chamber. This type of mechanism would predict that the average NC:oocyte ratio among several chambers would be less than the normal 15. The total number of NCs and oocytes for 160 different chambers was calculated. The average NC:oocyte ratio was 14.6 for 106 chambers from *brn*$^{l.6P6}$ GLC females, 14.7 for 24 chambers from *brn*$^{fs.107}$ GLC; *top*^{CJ} females, and 15.1 for 30 chambers from *brn*$^{l.6P6}$ GLC; *top*^{CJ} females. These findings are consistent with normal patterns of cystocyte division.

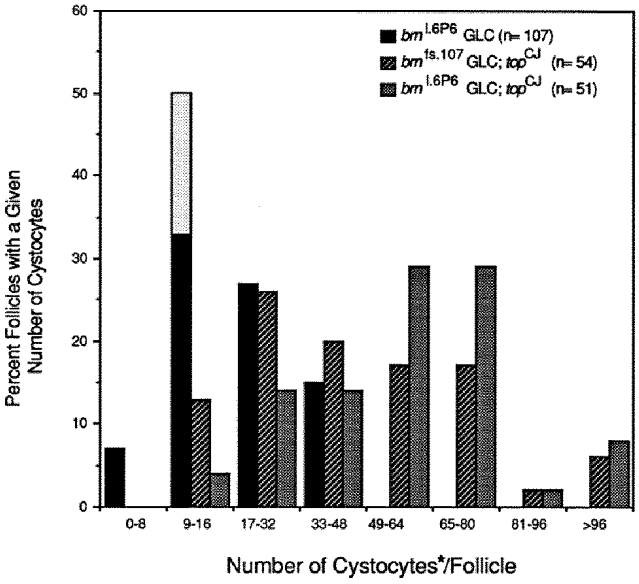

Fig. 5. Comparison of chamber size for *brainiac* and *torpedo*^{CJ} mutant gene combinations. Average egg chamber size from mutant females increases such that *brn*$^{l.6P6}$ GLC; *top*^{CJ} > *brn*$^{fs.107}$ GLC; *top*^{CJ} > *brn*$^{l.6P6}$ GLC. The *brn*$^{l.6P6}$ GLC 9-16 cystocyte bar is divided to indicate the number of chambers containing 15 nurse cells and one oocyte (dark shading, approximately 66% of the 9-16 class) versus those with 9-15 cystocytes (light shading), which may or may not contain an oocyte. *The number of cystocytes equals the number of nurse cells plus the number of oocytes scored for a given chamber.

Fig. 4. *brainiac* and the *Drosophila* EGF receptor are required to establish the ovarian follicle. Tissues are stained with Hoechst and the nurse cell nuclei (large, polyploid nuclei), oocyte nuclei (small nuclei not visible in most of the egg chambers shown; arrowheads in (B)) and follicle cell nuclei are revealed with flourescence microscopy. (A) Ovarioles from a *brn*$^{fs.107}$ female. As for *top*^{1}, *top*^{CJ} or *top*^{1}/*top*^{CJ} females, egg chambers (arrows) within the vitellarium are completely normal, consistent with the high fecundity of females of all four genotypes. Bar, 50 μm. (B) All ovarioles from *brn*$^{l.6P6}$ germ-line clone(GLC) females have aberrant egg chambers containing supernumerary oocytes and nurse cells. The arrow points to the germarium. (C) All ovarioles from *brn*$^{fs.107}$GLC;*top*^{CJ} females resemble those from *brn*$^{l.6P6}$GLC females (B). In addition, egg chambers are formed with large discontinuities in the follicular epithelium (arrowheads). (D) Egg chambers containing over six presumptive nurse cell-oocyte complexes can occupy essentially the whole ovariole of *brn*$^{l.6P6}$GLC;*top*^{CJ} females. The egg chamber forming here appears to be continuous with, or just pinching from the germarium. Note the graded increase in nurse cell nuclear size from the proximal to distal end of this egg chamber, indicating the earlier birthdate of oocyte-nurse cell complexes found more distally. In contrast to chambers found within *brn*$^{fs.107}$GLC;*top*^{CJ} ovarioles(C), egg chambers as large as those shown here do not have discontinuities within the follicular epithelium. Note that follicle cells are not found within egg chambers from *brn*$^{l.6P6}$GLC or double mutant females. (E and F) *brn*$^{l.6P6}$GLC germarium enlarged 5 times from B. An egg chamber is pinching from the germarium (arrows) containing over 40 cystocytes. Similar formations are found for double mutant combinations (also see Fig. 7). Bar, 5 μm. (G and H) Stage 10 egg chamber from a *brn*$^{fs.107}$GLC;*top*^{CJ} female. This egg chamber has five oocytes (G, arrowheads) that are able to take up yolk and approximately 75 nurse cells. In addition, these oocytes are able to attract migratory follicle cells (H, arrowheads). Note the starred oocyte (G) "pulling" follicle cells toward it (H). The cap of follicle cells surrounding the oocyte at the bottom of the figure is out of focus. Experiments were performed at 25°C. Ovarioles were dissected from 1- to 1½-day old females.

We stained germaria with Fasciclin III (FasIII) antibody (Patel et al., 1987). In wild-type germaria, FasIII-positive FCs (Fig. 7A) can be observed to line the walls of and to span across the germarium (Brower et al., 1981; Ruohola et al., 1991), separating individual cysts. In *brn*$^{l.6P6}$ GLC; *top*^{CJ} females, Fas III-positive FCs are found to be lining the walls of the germarium in regions 2 and 3, but in 69% of germaria (N=30) FCs fail to migrate within the germarium (Fig. 7C). For the remaining 31%, a single band of FCs can be observed to span the germarium, enclosing multiple sets of cystocytes (Fig. 7B). FCs are always found in germaria from *brn*$^{fs.107}$GLC; *top*^{CJ} females. *brn*$^{l.6P6}$ GLC; *top*^{CJ} females, which have the most severe phenotype in terms of the number of NC-oocyte complexes per egg chamber (Fig. 5), also have the most severe disruption of FC migration, suggesting that the primary reason follicles are formed with supernumerary NC-oocyte complexes is a

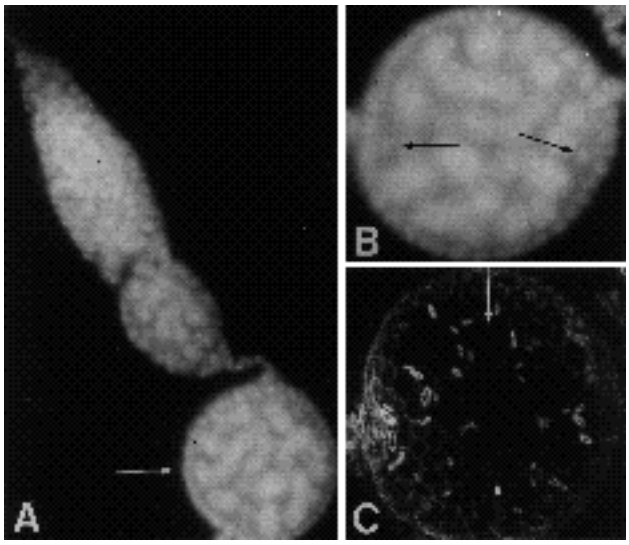

Fig. 6. Egg chambers containing supernumerary oocyte-nurse cell complexes appear to be derived from multiple cystoblasts. The Hoechst-stained *brn*$^{fs.107}$;*top*^{CJ}egg chamber (A, arrow) newly pinched from the germarium contains two oocyte-nurse cell complexes. Arrows point to oocyte nuclei in B, which is an enlargement of the egg chamber shown in A. This chamber was also stained with phalloidin and serial confocal images were combined to reveal the ring canals that interconnect the nurse cells with the oocyte (C). The cells within the middle of this egg chamber (arrows) are not connected by ring canals, indicating that the two nurse cell-oocyte complexes are separate from each other. Further, the four ring canals associated with the distal oocyte on the left are larger than the four ring canals associated with the oocyte on the right, indicating that the oocyte on the left is older and is thus derived from a separate cystoblast than the oocyte on the right.

failure of FCs to respond to germline signals necessary for enveloping each 16-cell cyst.

brainiac *and* torpedo *are required for formation of a continuous follicular epithelium*

In addition to supernumerary NC-oocyte complexes, egg chambers from *brn*$^{fs.107}$ GLC; *top*^{CJ} females frequently (66%, N=195) have discontinuous follicular epithelia (Fig. 4C). These discontinuities are judged to range from as large as one third of the surface of the follicle to as small as 7-8 cells. The phenotype is not allele specific for *top*, because *brn*$^{fs.107}$; *top*^{1} (45%, N=190) as well as *brn*$^{fs.107}$; *top*^{1}/*top*^{CJ} (% not determined) females develop egg chambers with the same phenotype. The phenotype is allele-specific for *brn*, however, as neither *brn*$^{l.6P6}$ GLC nor *brn*$^{l.6P6}$ GLC; *top*^{CJ} females develop similar phenotypes. Thus, the normal packaging of NC-oocyte complexes and development of a complete follicular epithelium appear to be separate functions of the *brn* locus.

Since the phenotype is apparent in *brn*$^{fs.107}$GLC; *top*^{CJ} females, in which somatic cells have a wild-type copy of *brn*, the formation of a continuous follicular epithelium appears to be a specific germline-dependent function of the *brn* locus. Discontinuities clearly do not arise as a result of overstretching of the follicular epithelium in unusually large

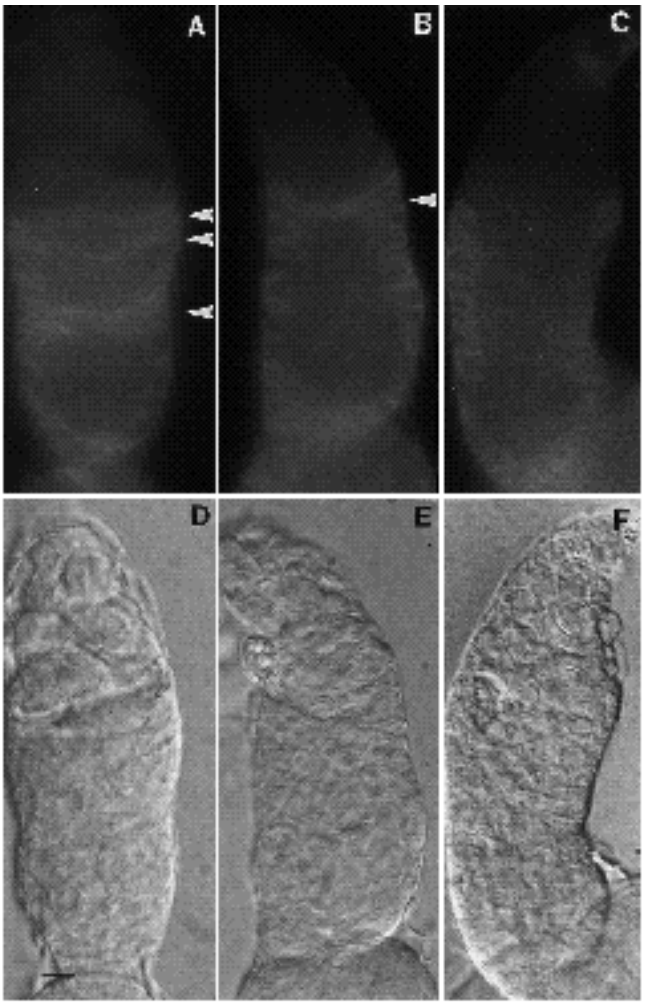

Fig. 7. *brainiac* and *torpedo* are required for follicle cell migration into the germarium. Germarial follicle cells revealed with anti-FasIII antibody (A-C) with companion differential interference contrast images below (D-F). Three bands of follicle cells can be seen to have migrated into the wild-type germarium shown in A (arrowheads). In the *brn*$^{l.6P6}$GLC; *top*^{CJ} germaria shown in (B) and (C) follicle cells still line the walls of each germarium, but in the germarium shown in B only one band of follicle cells has migrated in (arrowhead), forming an unusually large egg chamber. This follicle would presumably pinch from the germarium to form a large egg chamber like those shown in Fig. 4. In most *brn*$^{l.6P6}$ GLC; *top*^{CJ} germaria the follicle cells completely fail to migrate as in C. Bar, 10 μm.

chambers (containing multiple NC-oocyte complexes), because egg chambers of *brn*$^{l.6P6}$ GLC; *top*^{CJ} females as large as those shown in Fig. 4D do not develop holes. Even normal sized chambers from *brn*$^{fs.107}$GLC; *top*^{CJ} females containing single NC-oocyte complexes may possess holes. As many as 4 consecutive chambers, each having a hole, can be observed within the vitellarium of one day old females. Such configurations may contribute to the fusion of adjacent chambers observed in older females.

Significantly, we also find that *gurken* females that lay completely ventralized eggs (*grk*^{WG41}, *grk*^{WG41}/*grk*^{HK36}, or *grk*^{HG21}, data not shown) also have egg chambers with holes

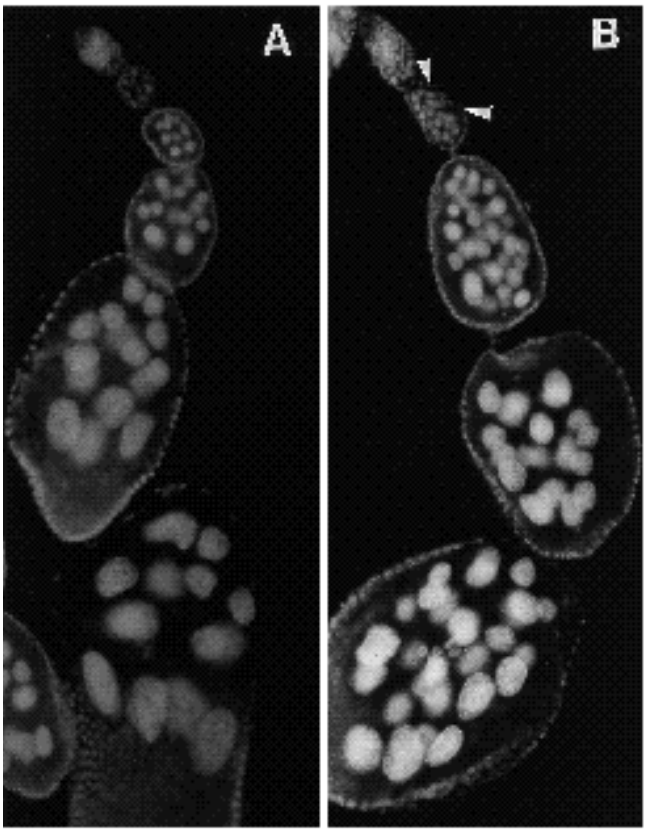

Fig. 8. *top* is required in the follicle cells during the early phases of oogenesis. (A) Hoechst-stained ovariole from a female in which all somatic cells (including the follicle cells) are homozygous mutant *top*^{CJ}. This female's germline is heterozygous for *brn*$^{fs.107}$. The egg chambers are completely normal. Ovarioles from females in which the follicle cells are heterozygous for *top*^{CJ} and the germline is either heterozygous or homozygous mutant for *brn*$^{fs.107}$ appear the same (see Table 6). (B) When the follicle cells are homozygous mutant for *top*^{CJ} and the germline is homozygous mutant for *brn*$^{fs.107}$, egg chambers containing supernumerary nurse cell-oocyte complexes and discontinuous follicular epithelia are formed. These females also produce a few completely ventralized eggs (not shown). The result is the same for double mutant or germline clone females, confirming the germline requirement for *brn* function, and demonstrating the somatic requirement for DER function during the early phases of oogenesis.

Table 6. top *is required in the follicle cells during the early phases of oogenesis*

Number and genotype of surviving host females injected[a]	Number of mosaic females	Number and fecundity of mosaic females, phenotype of eggs and ovarioles, and genotype germ cells[b]
58 *top*^{CJ}/*CyO*	23	9, highly fecund, "weakly fused" *brn* shaped eggs[c], normal ovarioles, *brn*$^{fs.107}$/*In(1)FM3*
		14, highly fecund, "strongly, fused" *brn* shaped eggs[d], normal ovarioles, *brn*$^{fs.107}$
27 *top*^{CJ}	8	6, highly fecund, "strongly fused" *top* shaped eggs[e], normal ovarioles, *brn*$^{fs.107}$/*In(1)FM3*
		3[f], not fecund, completely ventralized *top*-like eggs, all mosaic ovarioles contain aberrant egg chambers, *brn*$^{fs.107}$

Females were constructed that had follicle cells mutant for *top*^{CJ} (*top*^{CJ} host females) and germ cells mutant for *brn*$^{fs.107}$. Follicle cells heterozygous mutant for *top* (*top*^{CJ}/*In(2LR)CyO* host females) and germ cells heterozygous mutant for *brn* (*brn*$^{fs.107}$/*In(1)FM3*) serve as controls. See Fig. 8 for typical ovarioles from mosaic females. Mosaic ovarioles were detected as in experimental procedures.

[a]Genotypes of donor parents were *top*^{CJ}/*In(2LR)CyO* × *Fs(1)ovo*^{D1}/*Y*; *top*^{CJ}. The presence of the dominant female sterile mutation *Fs(1)ovo*^{D1} (Busson et al., 1983) in the host germline means that all eggs produced by mosaic females derive from implanted germ cells. Genotype in the table is given only with respect to *top*. All host females were mated to *brn*$^{fs.107}$ males.

[b]Genotype of donor parents was *brn*$^{fs.107}$/*In(1)FM3* × *brn*$^{fs.107}$/*Y*. Genotype of transplanted pole cells (the embryonic stem cells of the adult germline) was inferred from genetic markers displayed by adult progeny for *brn*$^{fs.107}$/*In(1)FM3* pole cells transplanted into *top*^{CJ}/*In(2LR)CyO* females. For remaining donor-host pairs, the genotype of transplanted pole cells was inferred from the eggs' phenotype, since progeny were unobtainable.

[c]The designation "weakly fused" means that most eggs were either wild-type or had weak fused dorsal appendages, while the remaining eggs had strongly fused appendages. Degree of fusion designations are as described in Table 2. Fertilized eggs hatch embryos (they do not give neurogenic or ventralized embryos). The same result is obtained for *brn*$^{fs.107}$/+; *top*^{CJ}/+ females × *brn*$^{fs.107}$/*Y*. *brn*$^{fs.107}$/*In(1)FM3* progeny were obtained from all 9 of the *top*^{CJ}/*In(2LR)CyO* females.

[d]The designation "strongly fused" means that most eggs were strongly fused, while the remaining eggs were weakly fused or wild-type, as designated in Table 2. All fertilized eggs develop neurogenic embryos. The same result is obtained for *brn*$^{fs.107}$;*top*^{CJ}/+ females crossed to *brn*$^{fs.107}$/*Y*.

[e]Fused dorsal appendage *top* eggs can be distinguished from fused dorsal appendage *brn* eggs based on shape, as shown in Fig. 2. These eggs also develop ventralized embryos, like those from *top*^{CJ} females (Schupbach, 1987).

[f]One of these 3 females was obtained from a preliminary experiment in which the genotype of the donor parents was *brn*$^{fs.107}$ × *brn*$^{fs.107}$/*Y*. The genotype of all the donor pole cells was thus known to be *brn*$^{fs.107}$. This female had the same ovarian phenotypes as the other two females derived from *brn*$^{fs.107}$/*In(1)FM3* × *brn*$^{fs.107}$/*Y* donorparents, confirming the inferred genotype of the donor pole cells.

in the follicular epithelium similar to those in Fig. 4C. Thus, three loci required for the determination of the dorsal fate of the FCs are also required for the formation of the follicular epithelium. In contrast to these loci, neither *fs(1)K10*, which is required for determination of ventral FC fates, nor doubly mutant *fs(1)K10, brn*$^{fs.107}$ females develop incomplete follicular epithelia.

Egg chamber establishment and polarization require brn *in the germline and* top *in the soma*

Phenotypes developed by *brn*$^{l.6P6}$ GLC as well as *brn*$^{fs.107}$ GLC; *top*^{CJ} females indicate that *brn* gene function is required in the germline for FC migration, formation/maintenance of the follicular epithelium, and determination of dorsal-ventral FC fates. These results, however, leave open the question as to whether the DER is required in the germline and/or the soma for early oogenesis. Considering that the DER has been shown to be required in the FCs for

dorsal-ventral patterning (Schüpbach, 1987), and that growth factors and their receptors are required for cell migration and cell division in vertebrates (Grotendorst et al., 1981; Blay and Brown, 1985; Postlethwaite et al., 1987; Yarden and Ullrich, 1988; White, 1990), we considered it an attractive possibility that the DER is required in the FCs for their migration into the germarium and for their proliferation over the egg chamber during the early growth phase.

We constructed mosaic females by pole cell transplantation (Illmensee, 1973) in which the germ cells are mutant for $brn^{fs.107}$, while the surrounding host, including the somatically derived FCs, is mutant for top^{CJ}. These females have normal *brn* function in the FCs and normal DER function in the germline. As shown in Fig. 8 and Table 6, these females develop egg chambers having multiple NC-oocyte complexes and discontinuous follicular epithelia, demonstrating the somatic (presumably FC) requirement for *top* function during the early phases of oogenesis.

Discussion

brn *requirement for dorsal-ventral FC fate determination is separable from* brn *requirement for neuroblast segregation and follicle establishment*

All "maternal" neurogenic loci, like *brn*, show some degree of zygotic rescuability (Shannon, 1972; Perrimon et al., 1984, 1989). All "zygotic" neurogenic loci, with the exception of *big brain*, show a maternal effect (Jimenez and Campos-Ortega, 1982; Campos-Ortega, 1985). The distinction between "maternal" and "zygotic" neurogenic loci appears to be a reflection of the degree to which a given neurogenic gene product is contributed by the mother, not a reflection of an intrinsic developmental mechanism as, for example, distinguishes the maternal dorsal-ventral loci from the zygotic dorsal-ventral loci (Simpson, 1983). Indeed, the zygotic rescuability of *brn* function strongly indicates that the *brn* requirement for dorsal-ventral FC fate determination is independent of the *brn* requirement in the early embryo. The *brn* requirement for dorsal-ventral FC fate determination occurs at the inception of vitellogenesis, many hours before zygotic genome activation. Further, *brn* embryos show no indications of altered gastrulation as seen in mutants for *top, grk, fs(1)K10, cappuccino* and *spire*, which are required maternally for determination of FC dorsal-ventral fates (Wieschaus et al., 1978; Schüpbach, 1987; Manseau and Schüpbach, 1989).

Thus, *brn* appears to be unique among loci required for dorsal-ventral patterning during oogenesis. The DER is required in the FCs and *grk, fs(1)K10, cappuccino* and *spire* are required in the germline for the determination of both follicle and embryonic dorsal-ventral cell fates (Wieschaus et al., 1978; Schüpbach, 1987; Manseau and Schüpbach, 1989). *nudel*, *pipe* and *windbeutel* are required in the soma (presumably the FCs) for the determination of embryonic, but not FC fates (Stein et al., 1991), demonstrating that a flow of information from soma to germline exists which can be separated from FC fate determination. *brn*, on the other hand, is required in the germline for the determination of follicle, but not embryonic, dorsal-ventral cell fates, demonstrating that a flow of information from germline to FCs exists which can be separated from determination of embryonic cell fates. Consistent with this notion, the overall shape of *brn* oocytes is not altered as are *top* and *grk* oocytes (see Fig. 2).

nudel, *pipe* and *windbeutel* appear to be components of a FC signaling pathway required for production of a ligand that specifies embryonic dorsal-ventral cell fates (Stein et al., 1991). The *Toll* gene product, which is evenly distributed in the membrane surrounding the embryo, appears to encode the receptor for the ligand (Hashimoto et al., 1988; Stein et al., 1991). The DER is expressed continuously during oogenesis on the surface of the FCs (and not in the germline) , including the period during which the egg chamber has obtained dorsal-ventral asymmetry (R. Schweitzer, N. Zak, and B. Shilo, personal communication). Further, the genetic mosaic experiments presented in Table 6 demonstrate that the dorsal-ventral pattern activity, which requires DER activity in the follicle cells, is also dependent on germline functions, at least one of which is *brn*. Thus, the DER appears to be a receptor for (a) germline signal(s) (cf. Manseau and Schüpbach, 1989).

We have not used null alleles of either *brn* or *torpedo* in these studies, and it is therefore not possible to deduce whether *brn* and the DER act in the same or a parallel genetic pathway, i.e. to decide whether *brn* might be a component of a germline signaling pathway required for DER dorsal-ventral patterning activity. Although our results do not rule out that *brn* and the DER may be in parallel pathways, the mosaic studies clearly show that the DER depends on germline functions, presumably a ligand(s), to dorsal-ventral pattern the follicle. Acting in a parallel pathway to the DER, *brn* may be required in a more general fashion for reception of DER signals. For example, *brn* may be required for proper contact/adhesion between germline and follicle cells necessary to ensure proper germline signaling for follicle cell dependent DER function.

Both the chorion and embryonic phenotypes associated with $brn^{fs.107}$ and $brn^{l.6P6}$ are not as strong as the strongest phenotypes associated with prototypical ventralizing or neurogenic loci. Since both $brn^{fs.107}$ and $brn^{l.6P6}$ behave like deficiencies, or a complete loss of gene function, for dorsal-ventral patterning and neurogenesis, respectively, a hypothetical null *brn* allele would not be associated with stronger phenotypes. *brn* function may be redundant, either at the gene family level, or at the level of gene pathways (Rykowski et al., 1981; Ferguson and Horvitz, 1989). Functional redundancy is a common feature associated with growth factors and their receptors (cf. Nicola and Metcalf, 1991).

The $brn^{fs.107}$ temperature-sensitive period for dorsal-ventral patterning occurs during stages 6-7 of oogenesis, well after the follicle has been established. This temperature-sensitive period is consistent with the period in oogenesis in which the oocyte nucleus has been found to be required for dorsal-ventral patterning, stages 6-9 (Montel et al., 1991). This indicates that the *brn* gene product is needed at the time dorsal-ventral patterning takes place during oogenesis, and thus separates this requirement for *brn* gene function from the requirement to establish the egg chamber. In contrast to $brn^{l.6P6}$, $brn^{fs.107}$ retains the early *brn* oogenesis

functions. However, when somatic DER function is reduced simultaneously, germline *brn*$^{fs.107}$ function is revealed as inadequate for packaging 16-cell cysts and forming a complete follicular epithelium. This synergistic phenotype demonstrates that intercellular communication between germline cells and somatic follicle cells starts very early in oogenesis.

Somatic DER function depends on germline brn *function for regulating at least two separable FC functions needed to establish the ovarian follicle*

Our data indicate that egg chambers containing multiple NC-oocyte complexes result from a failure of FCs to migrate into the germarium and enclose individual 16-cell cysts. Pole cell transplantation experiments demonstrate that germline *brn* function is required with somatic DER function for the enclosure of individual cysts. Although the level of *brn* and *top* activity retained by either *brn*$^{fs.107}$ or *top*^{CJ} mutations is sufficient for formation of individual follicles, germline *brn*$^{fs.107}$ activity is not sufficient for normal follicle cell activities when DER function is simultaneously reduced in the FCs. This synergistic phenotype resembles that found for *brn*$^{l.6P6}$GLC females (in the presence of normal DER follicle cell function); thus, the level of *brn* activity retained by *brn*$^{l.6P6}$ is not sufficient for follicle formation, presumably due to disruption of a signal from the germline to the follicle cells. This notion is strengthened by the finding that reduction of *top* function dramatically enhances the *brn*$^{l.6P6}$GLC phenotype. The expression of the DER on the surface of FCs and not in the germline (R. Schweitzer, N. Zak, and B. Shilo, personal communication), and the involvement of tyrosine kinase receptors and their growth factors in regulating cell migration in vertebrates (Grotendorst et al., 1981; Blay and Brown, 1985; Postlethwaite et al., 1987; White, 1990) are consistent with the idea that *brn* and the DER are necessary for the integrity of an intercellular signaling pathway required for FC migration.

Both the discovery of complementary sets of NCs in adjacent follicles, and the finding of an average NC-oocyte ratio among a large number of mutant egg chambers of approximately 15, suggest that abnormal numbers of NCs are not due to faulty cystocyte divisions. Normal packaging of 16-cell cysts by the FCs may be needed for the stabilization of the cyst. Koch and King (1969) found that prior to FC enclosure cystocytes are more loosely attached than the FC-enveloped germarial cystocytes, which form a very compact mass. The normal FC enclosure may be required to prevent stretching and breakage of the ring canal interconnections between cystocytes. On the other hand, the FCs may play a more active role in breaking cysts apart. Mahowald and Strassheim (1970) found that FCs always migrate around, and not between cystocytes. Further, Koch and King (1966) found that invading mesodermal cells apply as much of their surface as possible to interconnected cystocytes. If FCs are unable to recognize putative specialized properties of the cystocytes, or if such specialized properties are abnormally distributed over the cystocyte surfaces, then FCs might migrate between, instead of around, the cystocyte clusters.

Discontinuity of the FC epithelium is a novel phenotype not previously described in *Drosophila* mutants affecting oogenesis. The discontinuities associated with *brn*, *top* and *grk* mutations do not appear to be due to FC death, because pycnotic FC nuclei are only observed late when the whole chamber is necrosing. Discontinuities are already evident on some chambers as they leave the germarium (Fig. 8), so that it is reasonable to assume that the cause of the discontinuities is due to a defect evident already in the germarium. One interpretation is that in some instances the invading follicular epithelium failed to complete the envelopment of a follicle, perhaps because of the absence of an essential adhesive property in some cyst cells. Since the holes persist and may also arise during the early growth phase of the egg chamber, this adhesive property appears to be required even during the subsequent growth stages of oogenesis.

An alternate explanation for the origin of the follicular discontinuities is a failure of sufficient cell division, beginning already in the germarium. The predominant activity of the FCs as the egg chamber leaves the germarium (Chandley, 1966) and during the early growth phases of the egg chamber (King and Vanoucek, 1960) is mitosis: the FCs increase from 10-20 in number to approximately 1100 cells. Without the normal number of follicle cells, a gap might arise in the follicular epithelium. Since epithelia can ordinarily stretch over large surfaces, as happens in stage 10 when the follicle cells forms a squamous epithelium over the nurse chamber, this explanation is less likely. However, the presence of DER on the surface of all FCs during the early egg chamber growth phase (R. Schweitzer, N. Zak, and B. Shilo, personal communication) and the known role of tyrosine kinase receptors in regulating cell division in vertebrates (Yarden and Ullrich, 1988) are consistent with this explanation. These hypotheses are not mutually exclusive. Proper FC adherence to the germline may be needed for successful engagement of mitotic signals, or successful completion of mitosis might be needed to trigger follicle cell adhesion functions, or the phenotype might arise due to a simultaneous disruption of both processes.

The association of discontinuous follicular epithelia with egg chambers from *brn*$^{fs.107}$; *top* double mutant females, but not egg chambers from either *brn*$^{l.6P6}$GLC or *brn*$^{l.6P6}$GLC; *top*^{CJ} females suggests that these are separable functions of the *brn* locus. This indicates that *brn* and the DER are not only needed for signals within the germarium responsible for follicle cell migration, but are also required for the adherence and/or division of the follicle cells to and/or around the 16-cell cyst. *brn* and the DER would presumably be needed for these adherence and/or division functions throughout oogenesis. That the DER is expressed on the apical surface of all follicle cells throughout oogenesis (R. Schweitzer, N. Zak, and B. Shilo, personal communication) is consistent with this proposition.

Overlapping requirements for intercellular communication functions in oogenesis and neurogenesis

Mutations in three other neurogenic loci show significant phenotypes in both oogenesis and neurogenesis. Mutation of another late zygotic lethal locus with a neurogenic maternal effect, *zw4*, is associated with the development of egg chambers having "tumorous-like overgrowth of NCs" (Perrimon et al., 1989). *N* and *Dl* encode large transmembrane

proteins with EGF repeats (Wharton et al., 1985; Kidd et al., 1986; Vaessin et al., 1987), although neither has been shown to interact with the DER. Both, however, are required in subpopulations of FCs (Ruohola et al., 1991). Reduction of either function causes a hypertrophy of polar precursor and polar follicle cells and a corresponding hypotrophy of stalk and flanking follicle cells, respectively, comparable to the hypertrophy of NBs at the expense of epidermoblasts during neurogenesis (Ruohola et al., 1991). These findings suggest that *N* and *Dl* are required for lateral inhibition between follicle cells, and thus appear to have parallel roles in neurogenesis and oogenesis. This similarity has led to the suggestion that *N* and *Dl* as well as other neurogenic loci may act as a "cassette" to produce the intercellular signalling required for lateral inhibition in more than one developmental context (Ruohola et al., 1991).

In contrast to this view, *brn* has distinct functions in oogenesis from *N* and *Dl*. *brn* is required in the germline for interactions between the germline and surrounding FCs, necessary for development and maintenance of the follicular epithelium around individual cysts as well as for determination of dorsal-ventral follicle cell fates. None of these functions is shared by *N* and *Dl*. Possibly only a subset of the genes involved in the lateral inhibition by neuroblasts play a similar role in the development of ovarian polar cells. The failure of a deletion of the neurogenic locus *pecanex* to disrupt oogenesis (LaBonne et al., 1989) is consistent with this notion. We propose that *N* and *Dl* may utilize other signaling pathways for effecting inhibition between FCs. Comparison and contrast of the manner in which neurogenic functions are used during oogenesis and neurogenesis should aid in understanding the role these functions play for both processes.

brn appears to be required for normal tyrosine kinase function during oogenesis, a biochemical function not associated with any of the six cloned neurogenic loci. Our preliminary experiments indicate that *brn* is in the same genetic pathway as *Notch* and other neurogenic loci. The neurogenic phenotypes associated with mutations in all of these loci (with the exception of the neurogenic gene *big brain*, which is not in the same genetic pathway) are suppressed

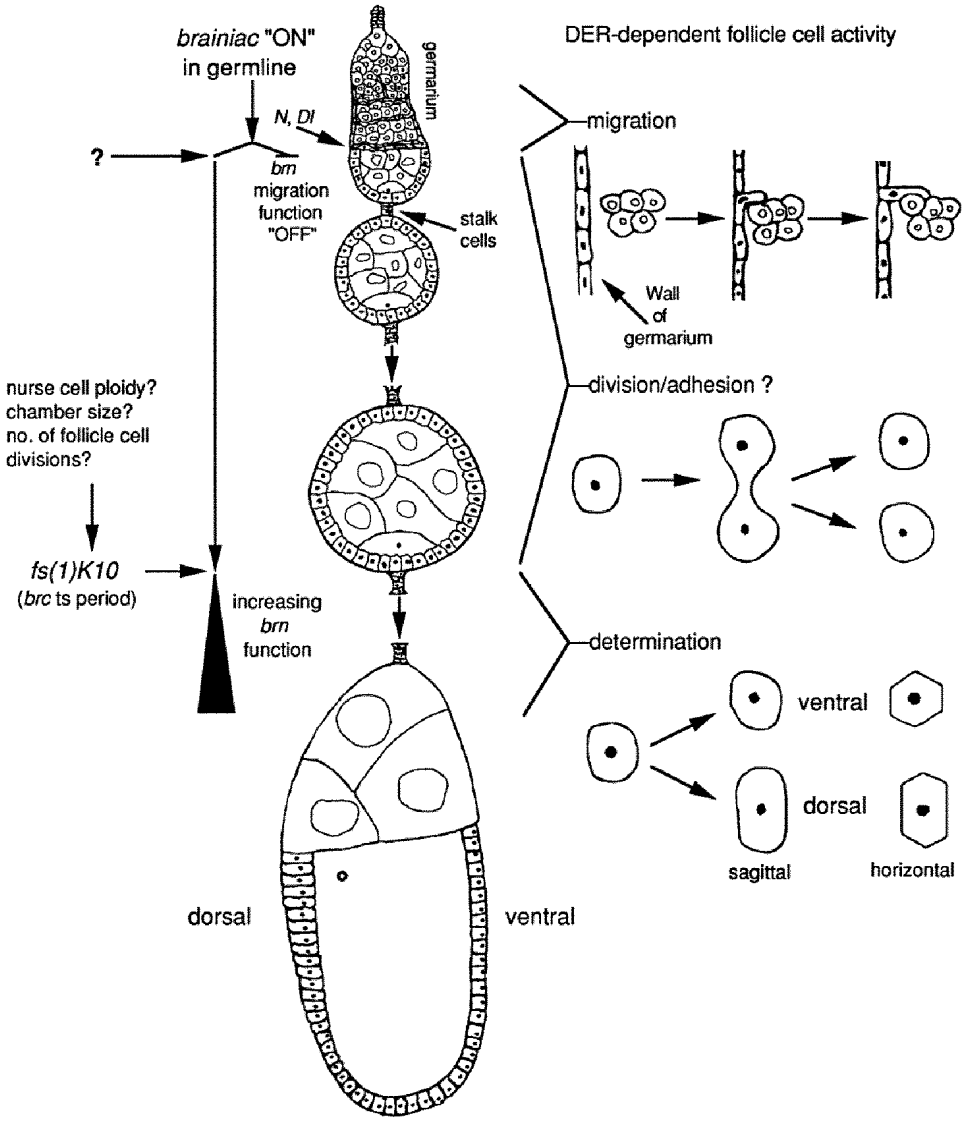

Fig. 9. A model for *brn* and DER function during oogenesis. Normal oogenesis is depicted in the center, beginning with the germarium, followed by three egg chambers of increasing development. Follicle cells are depicted by solid nuclei and germline cells are depicted by the large, hollow nurse cell nuclei and the small oocyte nucleus at the posterior of each egg chamber. Our data support a model in which *brn* and the DER are required for at least three successive follicle cell functions, depicted on the right: first, (pre)follicle cell migration to surround individual 16-cell cysts; second, follicle cell division and/or adhesion to establish and/or maintain a continuous follicular epithelium; third, dorsal-ventral cell fate determination to establish the dorsal-ventral polarity of the follicle (sagittal and horizontal are in reference to the egg axis). As discussed in the text and depicted on the left, we propose that the separability of these *brn* functions means that they can be differentially regulated.

by mutations in the proneural gene *daughterless* (Brand and Campos-Ortega, 1988). Likewise, *daughterless* mutations suppress the *brn* neurogenic phenotype (S. G., unpublished data).

brainiac *is required for the integrity of a germline signal received by the FCs through the DER*

top function is almost certainly required only in the FCs throughout oogenesis. Our genetic mosaic experiments show that *top* function is required in the FCs during the early phases of oogenesis, consistent with the observed expression of DER only on the surface of the FCs and not in the germline. *brn* is clearly a germline function, although the genetic mosaic studies do not rule out the possibility that *brn* function is also required in the FCs. These genetic mosaic experiments, however, suggest that the DER is dependent on (a) germline signal(s) throughout oogenesis (Fig. 9).

We propose that, in the germarium, both the migration and division and/or adhesion functions of *brn* are "ON" in the germline and are required for a signal to the (pre)follicle cells received by the DER. Prefollicle cell division is observed in this region of the germarium and appears to be required to replenish migratory follicle cells (Johnson and King, 1972). At the posterior of the germarium, *brn* migration function is turned "OFF" by an unknown mechanism (**?**, Fig.9). Interestingly, this appears to be the time at which the neurogenic loci *Notch* and *Delta* are required for the development of the stalk cells that connect egg chambers throughout oogenesis (Ruohola et al., 1991). *brn* division and/or adhesion function remains "ON" for follicle cell division and/or adhesion around the growing egg chamber at the posterior of the germarium and throughout the early growth phase.

At the end of the early growth phase, when the egg chamber begins to polarize, the *brn* gene product is needed for dorsal-ventral patterning, as determined by the $brn^{fs.107}$ temperature-sensitive period. *fs(1)K10*, required for determination of ventral FC fates, is expressed at approximately the same time (Haenlin et al., 1987). In addition to the finding that neither *fs(1)K10* nor *fs(1)K10*, $brn^{fs.107}$ females develop discontinuous follicular epithelia, it appears that *fs(1)K10* is specifically required for dorsal-ventral patterning. We suggest that *fs(1)K10* may modify continuously required *brn*, *grk* and DER function at the end of the early growth phase, altering their function from a cell division and/or adhesive mode to a cell determination mode (Fig. 9). This idea would be consistent with the model for dorsal-ventral patterning gene function proposed by Manseau and Schüpbach (1989), in which *fs(1)K10* is required upstream of *top* and *grk* for the initial polarization of the oocyte, as well as with our suggestion that *brn* is required upstream of the DER as part of a signaling pathway. If *fs(1)K10* expression is turned on according to an intrinsic developmental clock, such as NC ploidy or number of FC divisions (Fig. 9), this would explain how dorsal-ventral polarity is always established at the proper time, in an environment of continuously acting functions and variable rates of egg chamber development. At least part of the switch may involve increasing the level of activity of these continuously acting functions (thickening line, Fig. 9), since we observe that all females laying completely ventralized eggs (*brn*; *top* double mutants, *grk*) also develop discontinuous follicular epithelia. Females, laying less severely ventralized eggs having fused dorsal appendages (*brn*, *top*), never develop these discontinuities, suggesting that more of these functions are required for cell fate determination than for the proposed follicle cell division. The apparent complexity of *brn* and DER activity in regulating follicle cell behavior during *Drosophila* oogenesis is reminiscent of complexities described for regulation of cell behavior by growth factors and their receptors in other developmental systems (e.g. see Noble et al., 1988; Raff et al., 1988; Flanagan and Leder, 1990; Flanagan et al., 1991).

Neurogenic loci are all thought to be required for the specific neuroblast to epidermoblast inhibitory signal (Lehmann et al., 1983). Most of these loci appear to be required for the transmission of the signal rather than the intracellular response (Technau and Campos-Ortega, 1987). Similarly, at least half of the known loci required to determine ventral embryonic cell fates appear to act upstream of the putative *Toll* receptor (Stein et al., 1991). Our studies of *brn* also indicate a considerable complexity in the signals provided to the FCs, even though these signals are apparently received via "the same" DER function. Much of the current focus in understanding the function of tyrosine kinase receptors centers on finding phosphorylated molecules utilized in transferring surface signals to the nucleus (Yarden and Ullrich, 1988). Continued study of *brn* should illuminate how signals can be regulated to give the varied set of intracellular responses needed for regulation of varied cellular processes.

The early phases of this work were completed in the Department of Genetics at Case Western Reserve University. We thank Brian Oliver and Peter Harte for reading and commenting on the manuscript and for sharing their knowledge and interest throughout this project. Thanks to Charlie Rudin for reading and commenting on the manuscript. We acknowledge John Fredieu for help with embryo antibody staining. We thank Helen Salz for the dpl^{-} *Dp(1;2)*w^{+64B13} stock prior to publication. We thank Trudi Schüpbach for sending *top* and *grk* stocks, and thank Jim Price and Robert Clifford for helpful discussions concerning *brn* and *brn-top* interactions. Thanks to Hannele Ruohola for sending and discussing the Fas III antibody. We are indebted to Tim Karr for generating confocal images and sharing his knowledge of confocal microscopy. We thank Jim Shapiro for taking pictures of *Drosophila* eggs. We thank Gerry Grofman for help with making figures. We thank Macintosh guru Dr. Mark D. Garfinkel for help with the computer. This work has been supported by a grant from the American Cancer Society (A.P.M.) and NIH fellowships (#T32 HD7104 and #T32 GM07197) to S.G.

References

Banga, S. S., Bloomquist, B. T., Brodberg, R. K., Pye, Q. N., Larrive, D. C., Mason, J. M., Boyd, J. B. and Pak, W. L. (1986). Cytogenetic characterization of the 4BC region on the X chromosome of *Drosophila melanogaster*: localization of the *mei-9*, *norpA* and *omb* genes. *Chromosoma* **93**, 341-346.

Blay, J. and Brown, K. D. (1985). Epidermal growth factor promotes the chemotactic migration of cultured rat intestinal epithelial cells. *J. Cell. Phys.* **124**, 107-112.

Brand, M. and Campos-Ortega, J. A. (1988). Two groups of interrelated genes regulate early neurogenesis in *Drosophila melanogaster*. *Roux's Arch. Dev. Biol.* **197**, 457-470.

Brower, D. L., Smith, R. J. and Wilcox, M. (1981). Differentiation within the gonads of *Drosophila* revealed by immunofluorescence. *J. Embryol. exp. Morphol.* **63**, 233-242.

Brown, E. H. and King, R. C. (1964). Studies on the events resulting in the formation of an egg chamber in *Drosophila melanogaster*. *Growth* **28**, 41-81.

Busson, D., Gans, M., Komitopoulou K. and Masson, M. (1983). Genetic analysis of three dominant female-sterile mutations located on the X-chromosome of *Drosophilamelanogaster*. *Genetics* **105**, 309-325.

Campos-Ortega, J. A. (1985). Genetics of early neurogenesis in *Drosophilamelanogaster*. *Trends Neurosci.* **8**, 245-250.

Campos-Ortega, J. A. and Knust, E. (1990). Genetics of early neurogenesis in *Drosophila melanogaster. Annu. Rev. Genet.* **24**, 387-407.

Clifford, R. J. and Schüpbach, T. (1989). Coordinately and differentially mutable activities of *torpedo*, the *Drosophila melanogaster* homolog of the vertebrate EGF receptor gene. *Genetics* **123**, 771-787.

Chandley, A. C. (1966). Studies on oogenesis in *Drosophilamelanogaster* with ^{3}H-thymidine label. *Expl. Cell Res.* **44**, 201-215.

Craymer, L. and Roy, E. (1980). New mutants: *Drosophilamelanogaster*. *Dros. Inf. Serv.* **55**, 200-204.

David, J. and Merle, J. (1968). A re-evaluation of the duration of egg chamber stages in oogenesis of *Drosophilamelanogaster*. *Dros. Inf. Serv.* **43**, 122-123.

Doe, C. Q. and Goodman, C. S. (1985). Early events in insect neurogenesis II. The role of cell interactions and cell lineage in the determination of neuronal precursor cells. *Dev. Biol.* **111**, 206-219.

Ellisen, L. W., Bird, J., West, D. C., Soreng, A. L., Reynolds, T. C., Smith, S.D. and Sklar, J. (1991). *TAN-1*, the human homolog of the *Drosophila Notch* gene, is broken by chromosomal translocations in T lymphoblastic neoplasms. *Cell* **66**, 649-661.

Ferguson, E.L.and Horvitz, H.R. (1989). The multivulva phenotype of certain *Caenorhabditis elegans* mutants results from defects in two functionally redundant pathways. *Genetics* **123**, 199-121.

Flanagan, J. G. and Leder, P. (1990). The *kit* ligand: a cell surface molecule altered in steel mutant fibroblasts. *Cell* **63**, 185-194.

Flanagan, J. G., Chan, D. C. and Leder, P. (1991). Transmembrane form of the *kit* ligand growth factor is determined by alternative splicing and is missing in the *Sl*^{d} mutant. *Cell* **64**, 1025-1035.

Fredieu, J. R. and Mahowald, A. P. (1989). Glial interactions with neurons during *Drosophila* embryogenesis. *Development* **106**, 739-748.

Frey, A. and Gutzeit, H. (1986). Follicle cells and germline cells both affect polarity in *dicephalic* chimeric follicles of *Drosophila*. *Wilhelm Roux's Arch. Dev. Biol.* **195**, 527-531.

Gans, M., Audit, C. and Masson, M. (1975). Isolation and characterization of sex-linked female sterile mutants in *Drosophila melanogaster*. *Genetics* **81**, 683-704.

Grotendorst, G. R., Seppa, H. E. J., Kleinman, H. K. and Martin, G. R. (1981). Attatchment of smooth muscle cells to collagen and their migration toward platelet-derived growth factor. *Proc. Natl. Acad. Sci. USA* **78**, 3669-3672.

Gutzeit, H. O. and Strauss, A. (1989). Follicle cell development is partly independent of germ-line differentiation in *Drosophila* oogenesis. *Wilhelm Roux's Arch. Dev. Biol.* **198**, 185-190.

Haenlin, M., Roos, C., Cassah, A. and Mohier, E. (1987). Oocyte-specific transcription of *fs(1)K10* : a *Drosophila* gene affecting dorsal-ventral developmental polarity. *EMBO J.* **6**, 801-807.

Hartenstein, V. and Campos-Ortega, J.A. (1984). Early neurogenesis in wild-type *Drosophila melanogaster*. *Roux's Arch. Dev. Biol.* **193**, 308-325.

Hartley, D. A., Preiss, A. and Artavanis-Tsakonas, S. (1988). A deduced gene product from the *Drosophila* neurogenic locus, *Enhancer of split*, shows homology to mammalian G-protein ß-subunit. *Cell* **55**, 785-795.

Hashimoto, C., Hudson, K. L. and Anderson, K. V. (1988). The *Toll* gene of *Drosophila*, required for dorsal-ventral embryonic polarity, appears to encode a transmembrane protein. *Cell* **52**, 269-279.

Hunter, T. and Cooper, J. A. (1985). Protein-tyrosine kinases. *Annu. Rev. Biochem.* **54**, 897-930.

Illmensee, K. (1973). The potentialities of transplanted early gastrula nuclei of *Drosophila melanogaster*. Production of their imago descendants by germ-line transplantation. *Wilhelm Roux's Arch. Dev. Biol.* **171**, 331-343.

Jan, L. Y. and Jan, Y. N. (1982). Antibodies to horseradish peroxidase as specific neuronal markers in *Drosophila* and in grasshopper embryos. *Proc. Natl. Acad. Sci. USA* **79**, 2700-2704.

Jimenez, G. and Campos-Ortega, J. A. (1982). Maternal effects of zygotic mutants affecting early neurogenesis in *Drosophila*. *Wilhelm Roux's Arch. Dev. Biol.* **191**, 191-201.

Johnson, J. H. and King, R. C. (1972). Studies on *fes*, a mutation affecting cystocyte cytokinesis, in *Drosophilamelanogaster*. *Biol. Bull.* **143**, 525-**547.**

Kalt, M. R. and Tandler, B. (1971). A study of fixation of early amphibian embryos for electron microscopy. *Ultrastruct. Res.* **36**, 633-645.

Kidd, S., Kelley, M. R. and Young, M. W. (1986). Sequence of the *Notch* locus of *Drosophilamelanogaster*: Relationship of the encoded protein to mammalian clotting and growth factors. *Mol. Cell. Biol.* **6**, 3094-3108.

King, R. C. (1970). *Ovarian development in* Drosophila melanogaster. New York, Academic Press.

King, R. C., Burnett, R. G. and Staley, N. A. (1957). Oogenesis in adult *Drosophila melanogaster*. IV. Hereditary ovarian tumors. *Growth* **21**, 239-261.

King, R. C. and Riley, S. F. (1982). Ovarian pathologies generated by various alleles of the *otu* locus in *Drosophila melanogaster*. *Dev. Genetics* **3**, 69-89.

King, R. C. and Vanoucek, E. G. (1960). Oogenesis in adult *Drosophila melanogaster*. X. Studies on the behavior of the follicle cells. *Growth* **24**, 333-338.

Koch, E. A. and King, R. C. (1966). The origin and early differentiation of the egg chamber of *Drosophilamelanogaster*. *J. Morphol.* **119**, 283-304.

Koch, E. A. and King, R. C. (1969). Further studies on the ring canal system of the ovarian cystocytes of *Drosophila melanogaster. Z. Zellforsch.* **102**, 129-152.

LaBonne, S. G., Sunitha, I. and Mahowald, A. P. (1989). Molecular genetics of *pecanex*, a maternal-effect neurogenic locus of *Drosophila melanogaster* that potentially encodes a large transmembrane protein. *Dev. Biol.* **136**, 1-16.

Lehmann, R., Jimeniz, F., Dietrich, U. and Campos-Ortega, J. A. (1983). On the phenotype and development of mutants of early neurogenesis in *Drosophila melanogaster*. *Wilhelm Roux's Arch. Dev. Biol.* **192**, 62-74.

Lehmann, R. and Nüsslein-Volhard, C. (1987). *hunchback*, a gene required for segmentation of an anterior and posterior region of the *Drosophila* embryo. *Dev. Biol.* **119**, 402-417.

Lindsley, D. L. and Grell, E. H. (1967). Genetic variations of *Drosophila melanogaster*. *Carnegie Inst. Wash. Publ.* No. 627, Washington, D.C.

Lohs-Schardin, M. (1982). *Dicephalic* - A *Drosophila* mutant affecting polarity in follicle organization and embryonic patterning. *Wilhelm Roux's Arch. Dev. Biol.* **191**, 28-36.

Mahowald, A. P. and Strassheim, J. M. (1970). Intercellular migration of centrioles in the germarium of *Drosophilamelanogaster*. *J. Cell Biol.* **45**, 306-320.

Mahowald, A. P. and Kambysellis, M. P. (1980) Oogenesis. In *Genetics and Biology of* Drosophila, 2nd Vol. (ed. M. Ashburner and T.R.F. Wright), pp. 141-224, New York Academic Press.

Manseau, L. J. and Schüpbach, T. (1989). *cappuccino* and *spire*: two unique maternal-effect loci required for both the anteroposterior and dorsoventral patterns of the *Drosophila* embryo. *Genes Dev.* **3**, 1437-1452.

McLaren, A. and Wylie, C. C. (1983). *Current Problems in Germ Cell Differentiation*. Cambridge: Cambridge University Press.

Montel, D. M., Haig, K., and Spradling, A. C. (1991). Laser ablation studies of the role of the *Drosophila* oocyte nucleus in pattern formation. *Science* **254**, 290-293.

Nicola, N. A. and Metcalf, D. (1991). Subunit promiscuity among hemopoietic growth factor receptors. *Cell* **67**, 1-4.

Noble, M., Murray, K., Strootbant, P., Waterfield, M. D. and Riddle, P. (1988). Platelet-derived growth factor promotes division and motility and inhibits premature differentiation of the oligodendrocyte/type-2 astrocyte progenitor cell. *Nature* **333**, 560-562.

Oliver, B., Perrimon, N. and Mahowald, A. P. (1988). Genetic evidence that the *sans fille* locus is involved in *Drosophila* sex determination. *Genetics* **120**, 159-171.

Patel, N. H., Snow, P. M. and Goodman, C. S. (1987). Characterization and cloning of fasciclin III: a glycoprotein expressed on a subset of neurons and axon pathways in *Drosophila*. *Cell* **48**, 975-988.

Perrimon, N., Engstrom, L. and Mahowald, A. P. (1984). Developmental

genetics of the 2E-F region of the *Drosophila* X chromosome: a region rich in "developmentally important" genes. *Genetics* **108**, 559-572.

Perrimon, N., Mohler, D., Engstrom, L. and Mahowald, A. P. (1986). X-linked female-sterile loci in *Drosophila melanogaster*. *Genetics* **113**, 695-712.

Perrimon, N., Engstrom, L. and Mahowald, A. P. (1989). Zygotic lethals with specific maternal effect phenotypes in *Drosophila melanogaster*. I. Loci on the X chromosome. *Genetics* **121**, 333-352.

Postlethwaite, A. E., Keski-Oja, J., Moses, H. L. and Kang, A. H. (1987). Stimulation of the chemotactic migration of human fibroblasts by transforming growth factor ß. *J. Exp. Medicine* **165**, 251-256.

Price, J. V., Clifford, R. J. and Schüpbach, T. (1989). The maternal ventralizing locus *torpedo* is allelic to *faint little ball*, an embryonic lethal, and encodes the *Drosophila* EGF receptor homolog. *Cell* **56**, 1085-1092.

Raff, M. C., Lillien, L. E., Richardson, W. D., Burne, J. F. and Noble, M. D. (1988). Platelet-derived growth factor from astrocytes drives the clock that times oligodendrocyte develoment in culture. *Nature* **333**, 562-565.

Rao, Y., Jan, L.Y. and Jan, Y.N. (1990). Similarity of the product of the *Drosophila* neurogeneic gene *big brain* to transmembrane channel proteins. *Nature* **345**, 163-167.

Raven, P. (1961). *Oogenesis: the Storage of Developmental Information.* London: Pergamon Press.

Ruohola, H., Bremer, K. A., Baker, D., Swedlow, J. R., Jan, L.Y. and Jan, Y. N. (1991). Role of neurogenic genes in establishment of follicle cell fate and oocyte polarity during oogenesis in *Drosophila*. *Cell* **66**, 1-20.

Rykowski, M., Wallis, J. W., Choe, J. and Grunstein, M. (1981). Histone H2B subtypes are dispensable during the yeast cell cycle. *Cell* **25**, 477-487.

Schejter, E. D. and Shilo, B. Z. (1989). The *Drosophila* EGF receptor homolog (DER) gene is allelic to *faint little ball*, a locus essential for embryonic development. *Cell* **56**, 1093-1104.

Schüpbach, T. (1987). Germ line and soma cooperate during oogenesis to establish the dorsoventral pattern of egg shell and embryo in *Drosophila melanogaster*. *Cell* **49,** 699-707.

Shannon, M. P. (1972). Characterization of the female sterile mutant *almondex* of *Drosophila melanogaster*. *Genetica* **43**, 244-256.

Simpson, P. (1983). Maternal-zygotic gene interactions during formation of the dorsalventral pattern in *Drosophila* embryos. *Genetics* **105**, 615-632.

Stein, D., Roth, S., Vogelsang, E. and Nüsslein-Volhard, C. (1991). The polarity of the dorsal-ventral axis in the *Drosophila* embryo is defined by an extracellular signal. *Cell* **65**, 725-735.

Technau, G. M. and Campos-Ortega, J. A. (1987). Cell autonomy of expression of neurogenic genes of *Drosophila melanogaster*. *Proc. Natl. Acad. Sci. USA* **84**, 4500-4504.

Vaessin, H., Bremer, K. A., Knust, E. and Campos-Ortega, J. A. (1987). The neurogenic gene *Delta* of *Drosophila melanogaster* is expressed in neurogenic territories and encodes a putative transmembrane protein with EGF-like repeats. *EMBO J.* **6**, 3431-3440.

Warn,W. M., Gutzeit, H. O., Smith, L. and Warn, A. (1985). F-actin rings are associated with the ring canals of the *Drosophila* egg chamber. *Expl. Cell Res.* **157**, 355-363.

Wharton, K. A., Jonansen, K. M., Xu, T. and Artavanis-Tsakonas, S. (1985). Nucleotide sequence from the neurogenic locus *Notch* implies a gene product that shares homology with proteins containing EGF-like repeats. *Cell* **43**, 567-581.

Wieschaus, E. and Nüsslein-Volhard, C. (1986). Looking at embryos. In Drosophila: *A Practical Approach*, (ed. D. B. Roberts), pp. 199-227, Washington, D.C.: IRL Press.

Wieschaus, E., Marsh, J. L. and Gehring, W. (1978). *fs(1)K10*, a germline-dependent female sterile mutation causing abnormal chorion morphology in *Drosophila melanogaster*. *Wilhelm Roux's Arch. Dev. Biol.* **184**, 75-82.

Wieschaus, E. (1979). *fs(1)K10*, a female sterile mutation altering the pattern of both the egg coverings and the resultant embryos in *Drosophila*. In *Cell Lineage, Stem Cell and Cell Differentiation* (ed. N. LeDouarin), pp.291-302. Elsevier/North-Holland Biomedical Press, New York.

Yarden, Y. and Ullrich, A. (1988). Growth factor receptor kinases. *Annu. Rev. Biochem.* **57**, 443- 478.

(Accepted 30 May 1992)