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Soma-to-Germline Interactions During Drosophila Oogenesis Are Influenced by Dose-Sensitive Interactions Between *cut* and the Genes *cappuccino*, *ovarian tumor* and *agnostic*

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ABSTRACT

The *cut* gene of *Drosophila melanogaster* encodes a homeodomain protein that regulates a soma-to-germline signaling pathway required for proper morphology of germline cells during oogenesis. *cut* is required solely in somatic follicle cells, and when *cut* function is disrupted, membranes separating adjacent nurse cells break down and the structural integrity of the actin cytoskeleton is compromised. To understand the mechanism by which *cut* expression influences germline cell morphology, we determined whether binucleate cells form by defective cytokinesis or by fusion of adjacent cells. Egg chambers produced by *cut*, *cappuccino*, and *chickadee* mutants contained binucleate cells in which ring canal remnants stained with antibodies against Hu-li tai shao and Kelch, two proteins that are added to ring canals after cytokinesis is complete. In addition, defects in egg chamber morphology were observed only in middle to late stages of oogenesis, suggesting that germline cell cytokineses were normal in these mutants. *cut* exhibited dose-sensitive genetic interactions with *cappuccino* but not with *chickadee* or other genes that regulate cytoskeletal function, including *armadillo*, *spaghetti squash*, *quail*, *spire*, *Src64B*, and *Tec29A.* Genomic regions containing genes that cooperate with *cut* were identified by performing a second-site noncomplementing screen using a collection of chromosomal deficiencies. Sixteen regions that interact with *cut* during oogenesis and eight regions that interact during the development of other tissues were identified. Genetic interactions between *cut* and the *ovarian tumor* gene were identified as a result of the screen. In addition, the gene *agnostic* was found to be required during oogenesis, and genetic interactions between *cut* and *agnostic* were revealed. These results demonstrate that a signaling pathway regulating the morphology of germline cells is sensitive to genetic doses of *cut* and the genes *cappuccino*, *ovarian tumor*, and *agnostic.* Since these genes regulate cytoskeletal function and cAMP metabolism, the *cut*-mediated pathway functionally links these elements to preserve the cytoarchitecture of the germline cells.

INTERACTIONS between different cell types play pivotal roles in developmental programs of tissues and organs. Oogenesis in Drosophila provides an ideal model system for understanding these interactions. The events that occur during oogenesis take place in egg chambers that are composed of somatically derived follicle cells surrounding a syncyctial cyst of germline-derived cells (Spradling 1993). Each cyst consists of 16 cells; 15 of these are polyploid nurse cells and the sixteenth is the diploid oocyte. Cells in the cyst are connected by cytoplasmic bridges called ring canals, which result from incomplete cytokineses during the earliest stages of oogenesis. After the germline cell cytokineses are complete, the cyst is encapsulated by an epithelial monolayer of follicle cells. Interactions between the follicle cells and germ cells are crucial to initiate and coordinate the events that are required for oogenesis (Spradling 1993; Ray and Schüpbach 1996).

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Genetic analyses have begun to clarify the many signaling pathways operating between the soma and germline that establish the identity and functions of these cells. The *small ovaries* gene is required in the soma, but influences the differentiation of germline cells (Wayne *et al.* 1995). Somatic mutations in *cut* (Jackson and Blochlinger 1997), *daughterless* (Cummings and Cronmiller 1994), and the neurogenic group of genes (Ruohola *et al.* 1991; Xu *et al.* 1992; Bender *et al.* 1993) disrupt the ability of follicle cells to recognize and encapsulate the germline-derived cells. The *toucan* locus is also required for the follicle cells to encapsulate the germline cyst, but appears to be required in the germline and not the soma (Grammont *et al.* 1997). Germline-to-soma interactions are also important at the posterior of the oocyte, where localized *gurken* RNA yields a TGF-α homologue that signals through the Epidermal growth factor receptor pathway in the follicle cells to establish posterior follicle cell fate. A subsequent signal from these posterior follicle cells back to the oocyte affects Protein kinase A function in the germ cells and initiates a reorganization of the microtubule cytoskeleton that leads to the establishment of the anterior-posterior and

dorsal-ventral axes of the egg chamber and embryo (reviewed in Ray and Schüpbach 1996). Finally, the product of the *bullwinkle* locus is required in a separate germline-to-soma signaling pathway that influences the migration of the follicle cells as they produce the respiratory appendages on the dorsal side of the eggshell (Rittenhouse and Berg 1995).

Other genes not directly involved in signaling between soma and germline are important for the regulation of cytoskeleton function. When these genes are mutated, the structural integrity of individual germline-derived cells is compromised and binucleate nurse cells are produced. These binucleate cells are thought to result either from defective cytokineses of the germline cystoblast cells (*chickadee*, Verheyen and Cooley 1994; *cappuccino*, Manseau *et al.* 1996) or from fusion of adjacent nurse cells after cytokineses are complete (*spaghetti squash*, Wheatly *et al.* 1995; Edwards and Kiehart 1996; *armadillo*, Peifer *et al.* 1993; rho family of small GTPases, Murphy and Montell 1996). Signaling events and the regulation of cytoskeletal function are integrated processes. For example, subcortical actin breaks down at the ring canals when Protein kinase A catalytic subunit (Pka-C1) function is lost in germline clones. This breakdown results in the fusion of adjacent nurse cells into binucleate cells that are morphologically indistinguishable from those seen in the cytoskeleton-associated mutants described above (Lane and Kalderon 1993, 1995). Thus, cAMP-dependent Protein kinase A regulates multiple important cytoskeleton-dependent processes, both actin- and tubulin-based, during oogenesis.

Recently, we described a soma-to-germline signaling pathway that requires the activity of the *cut* gene (Jackson and Blochlinger 1997). Our analyses showed that in the ovary, *cut* RNA and protein expression are restricted to the follicle cells; moreover, *cut* mutant germline clones are phenotypically normal. When *cut* function is lost in the follicle cells, however, germline-derived cysts are mispackaged into egg chambers with abnormal numbers of cells, and the structural organization of oocyte-nurse cell complexes disintegrates, generating binucleate germline-derived cells similar to those described above. To date, *cut* is the only gene known to be required in the follicle cells that when mutated results in binucleate cells. The assembly of egg chambers and the maintenance of germline cell morphology therefore requires the activity of the *cut* gene in the soma, revealing a signaling pathway that influences the morphology and function of the germline-derived cells. In support of this conclusion, *cut* interacts genetically during oogenesis with two genes that influence intercellular communications, *Notch* and Pka-C1 (Jackson and Blochlinger 1997).

The *cut* locus encodes a nuclear homeodomain-containing protein with multiple DNA-binding motifs (Blochlinger *et al.* 1988). Regulation of *cut* expression is complex and is controlled by a large promoter/enhancer region extending 200 kb from the coding region (Jack 1985; Jack and DeLotto 1995). The *cut* gene is expressed in other tissues besides the ovary, and *cut* function is required for the development of all the tissues in which it is expressed. The genetic networks that influence the function of *cut* in these tissues are beginning to be elucidated. Along the wing margin, loss of *cut* function affects the differentiation of sensory bristles and results in loss of wing blade material (Jack *et al.* 1991; Jack and DeLotto 1992; Blochlinger *et al.* 1993). During development of the wing margin, *cut* interacts with many genes, including *Notch*, *Serrate*, *strawberry notch*, *vestigial*, *scalloped*, *mastermind*, and *Chip* (Jack and DeLotto 1992; Morcillo *et al.* 1996; Majumdar *et al.* 1997). *Notch*, *Delta*, and *wingless* interact with *cut* by affecting Cut protein expression along the wing margin (Micchelli *et al.* 1997). In the embryonic and adult peripheral nervous system, *cut* acts as a bimodal switch between external sense organ fate and chordotonal organ fate (Bodmer *et al.* 1987; Blochlinger *et al.* 1990, 1991). In developing poly-innervated external sense organs, the *pox-neuro* gene induces *cut* expression (Vervoort *et al.* 1995). *cut* is also expressed in the developing central nervous system, trachea, and Malpighian tubules and loss of *cut* function affects differentiation of each of these organs (Blochlinger *et al.* 1990, 1993; Liu and Jack 1992). Although several genes act upstream of *cut* in developing tissues, downstream targets of *cut* gene activity are unknown in Drosophila.

Since *cut* appears to determine cell fate in a cell-autonomous manner in other tissues, we were surprised to find that germline cell morphology was influenced by *cut*-mediated events in the follicle cells. Virtually nothing is known about this unique interaction; thus, in this work we analyze the basis of the interactions between these two cell types. First, we examined the mechanism responsible for the morphological defects by determining whether binucleate cells form due to defective cytokinesis or by fusion of adjacent cells. We found that in *cut* mutants, binucleate nurse cells were present only in later stages and contained remnants of ring canals, suggesting that cytokinesis proceeded normally. Surprisingly, and in contrast to current models of *cappuccino* and *chickadee* function, we also found evidence of normal cytokinesis in these mutants. Second, we found that *cut* exhibits dose-sensitive genetic interactions with *cappuccino* but not with *chickadee.* Finally, we took advantage of the dose-sensitive nature of *cut* to identify additional genes in this signaling pathway. We found 16 genomic regions that interact with *cut* during oogenesis and 8 regions required for the development of other tissues. As a result of this screen, we identified novel genetic interactions between *cut* and two genes, *ovarian tumor* and *agnostic.* We previously hypothesized that this unique signaling pathway regulates the activity of cytoskeleton-associated proteins in the germline cells.

This work provides concrete evidence that somatic *cut* expression cooperates with at least two cytoskeleton-regulating genes in the germline cells, presumably by cAMP-mediated events.

MATERIALS AND METHODS

Stocks and crosses: Flies were raised in vials of standard cornmeal-molasses-agar medium at 25° unless stated otherwise. *cut* follicle cell clones were generated using FLP-mediated mitotic recombination as described previously (Jackson and Blochlinger 1997). After heat shock-induced generation of ct^{C145} null clones, adult females recovered for 4–7 days at room temperature, and egg chamber morphology was examined in dissected ovaries as described below.

Interactions with cut *nulls:* Genetic interactions between *cut* null alleles and known autosomal mutations were examined in a doubly heterozygous background by crossing males containing mutations in the gene of interest (*geneX*) to *y w* ct^{C145}/*FM3* or *y w* ct^{DB7}/*FM7c* virgins at 25° in vials. Females doubly heterozygous for *cut* and *geneX* (*e.g.*, *y w* ct^{C145}/+; *geneX*/+) were harvested and kept with males on fresh yeast for several days before ovaries were dissected. Egg chamber morphology was examined by staining with 4′,6-diamidino-2-phenylindole (DAPI) and rhodamine-conjugated phalloidin as described below. First chromosome mutations were examined by crossing *y w* ct^{C145}/y^+ ct^+ *Y* males to *geneX*/*Balancer* virgins and harvesting double heterozygotes (*y w* ct^{C145}/*geneX*).

Interactions with cut *hypomorphs:* To examine the ability of second chromosome mutations to interact with *cut* hypomorphs, males containing the gene of interest (*geneX*) were crossed to *y w* ct^{L188}; *Pin*/*CyO* virgins at 22° (room temperature). *y w* ct^{L188}/*Y*; *geneX*/*CyO* males were then crossed to *y w* ct^{C145}/*FM3* or *y w* ct^{DB7}/*FM7c* virgins at 25° in bottles. *y w* ct^{L188}/*y w* ct^{C145}; *geneX*/+ females were harvested and kept with males on fresh yeast for several days and scored as described above. Third chromosome mutations were analyzed in a similar manner using appropriate balancers. To examine the ability of a strong *armadillo* (*arm*) mutation to interact with *cut* hypomorphs, arm^4 was recombined onto the ct^{C145} chromosome and balanced using standard genetic techniques. Three independent lines (*w* arm^4 ct^{C145}/*FM6*, *ct*) were generated and the presence of arm^4 in each line was verified by examining cuticle phenotypes. Virgins from these stocks were collected and mated to *y w* ct^{L188} males, and *w* arm^4 ct^{C145}/*y w* ct^{L188} females were collected and assayed as described above. Each independent line gave identical results.

$agnostic^{ts3}$ mutants (agn^{ts3}) were raised at 25°; adult females were placed in vials with fresh yeast at 29° for 5 days to examine the phenotype at the restrictive temperature. Similarly, *y w* ct^{C145}/y^+ ct^+ *Y* males were mated to agn^{ts3} females at 25°, *y w* ct^{C145}/agn^{ts3} females were harvested and placed at 29° for 5 days to examine the phenotype. As controls, w^{1118} stocks were raised at 25°, placed at 29° for 5 days, and ovaries were dissected and examined at the same time.

Screen for interactions between *cut* and chromosomal deficiencies: The stock *y w* ct^{C145}/*FM3*, y^+ ct^+ *Y* was used to screen a collection of chromosomal deficiencies. First chromosome deficiencies were provided by the Bloomington stock center; second and third chromosome deficiencies were also obtained from the Bloomington stock center but maintained separately by S. Parkhurst. For most of the first chromosome deficiencies, *Df(1)*/*Balancer* virgins were crossed to *y w* ct^{C145}/y^+ ct^+ *Y* males; for some of the deficiencies, it was necessary to use *y w* ct^{C145}/*FM3* virgins and deficiency chromosome-containing males. For second and third chromosome deficiencies, males containing the deficiency chromosome were crossed to *y w* ct^{C145}/*FM3* virgins. Flies were crossed in vials at 25°; females doubly heterozygous for *y w* ct^{C145} and the deficiency were placed in fresh vials with yeast and w^{1118} or Canton-S males for 2–7 days. The siblings containing *cut* and nondeficiency chromosomes were examined in an identical manner to rule out the possibility that an interacting mutation mapped outside the deficiency. Ovaries were dissected and stained with DAPI and rhodamine-conjugated phalloidin as described below. A total of at least 500 egg chambers at all stages were examined from each mutant combination for morphological defects. For completeness, we scored egg chambers between stages 2 and 11; if we had counted only stages 9–11 (when the binucleate phenotype was most often observed), the penetrance would have been higher. Deficiencies that displayed a phenotype were checked for specific *cut* interactions by crossing each deficiency to *y w* ct^{C145}/*FM3*, *y w* ct^{DB7}/*FM7*, or w^{1118} stocks. Females were harvested, checked for reproducibility of the phenotype, and ovaries were dissected and stained as described below.

Immunocytochemistry: Adult females were placed in vials with fresh yeast and males for several days prior to dissection. Ovaries were hand dissected in PBSE (phosphate-buffered saline plus 1 mm EDTA) and fixed in PBTE (PBSE plus 0.2% Tween-20) plus 4% formaldehyde at room temperature for 20 min. Ovaries were rinsed once with PBTE, and then permeablized for at least 1 hr in PBSE plus 1% Triton X-100. The tissue was rinsed once with PBTE, blocked for at least 1 hr in PBTE plus 5% bovine serum albumin and 0.02% sodium azide, and incubated in primary antibodies in blocking solution overnight at 4°. The ovaries were then washed with four 5-min washes with PBTE, incubated in secondary antibody in PBTE for at least 1 hr at room temperature, washed again, and mounted in PBTE plus 35% glycerol and one drop of Vectashield antiphotobleaching agent (Vector Labs, Burlingame, CA). DAPI (0.2 μg/ml) and rhodamine-conjugated phalloidin (2 units/ml, Molecular Probes, Eugene, OR) were included with secondary antibody-staining solution. Antibody concentrations were as follows: anti-c-Myc 9E10 1:20 (Oncogene Science, Manhassett, NY), anti-Hts-RC 1:10, anti-Kelch 1:1 (both from L. Cooley), and Oregon Green-anti-mouse 1:100 (Molecular Probes). Images were examined and photographed on a Nikon Microphot FXA microscope and slides were scanned with a Microtek ScanTek III flatbed scanner or acquired digitally on a Deltavision deconvoluting microscope (Applied Precision, Issaquah, WA) and processed using nearest neighbor algorithms.

RESULTS

Binucleate cells contain remnants of ring canals: In the germarium, which is found at the anterior tip of the ovary, a germline cystoblast cell divides exactly four times to produce 15 nurse cells and a single oocyte. Cytokinesis is incomplete during these divisions, resulting in a syncytium with channels (ring canals) through which materials are transported or freely pass. Several proteins are added to reinforce the ring canals after the germline cell cytokineses are completed, beginning with a tyrosine-phosphorylated protein(s), followed by filamentous actin and Hu-li tai shao (Hts) protein in region 2 of the germarium and, last, Kelch protein in region 3 of the germarium (Robinson *et al.* 1994). Other proteins are also added to ring canals after

the germline cell divisions are completed, but these proteins have yet to be ordered with respect to ring canal assembly (Cheerio, Robinson *et al.* 1997; Tec29, Guarnieri *et al.* 1998; Roulier *et al.* 1998; Src64B, Dodson *et al.* 1998; Roulier *et al.* 1998). Subcortical filamentous actin marks the boundaries between adjacent cells and in later stages tethers the nucleus to the middle of the cell. These structures can be seen in egg chambers stained with fluorescent phalloidin conjugates (Figure 1). We took advantage of the fact that Hts and Kelch proteins are added to the ring canals after cytokineses are completed to examine the mechanism leading to binucleate cells.

In principle, binucleate germline-derived cells could arise either by normal karyokinesis followed by defective cytokinesis, or by normal, incomplete cytokinesis followed by a subsequent breakdown in structural elements of adjacent cells, resulting in cell fusions. The presence of ring canal remnants in binucleate cells would suggest that the ring canals were assembled properly after the germline cytokineses and subsequently became detached when membrane integrity became compromised. To determine which of the two possible mechanisms was responsible for the phenotype when *cut* expression was lost, egg chambers containing *cut* null (*ct*^{C145}) follicle cell clones were generated by FLP-mediated mitotic recombination and stained with antibodies that recognize ring canal markers (Figure 1). In egg chambers containing *cut* null follicle cells, nurse cells with two nuclei and a spot of filamentous actin-containing material could be found. The binucleate cells could be found at any position and among any of the nurse cells in the egg chamber. A majority of the affected egg chambers contained a single binucleate cell, but occasionally more than one binucleate cell was present (Figure 1b). Most of the time (>90%) these cells were binucleate, but in *cut* clones and in genetic combinations discussed below, more than two nuclei could occasionally be observed within a single cell. In most cases, it was not possible to correlate the location of the follicle cell clone with the binucleate nurse cell, since the follicle cells migrate over the oocyte during the stages when binucleate cells are most frequently observed (stages 9–11). Nevertheless, a clone in which ~25–30% of follicle cells were null for *cut* function was sufficient to produce an egg chamber with binucleate cells, suggesting that an important factor is dose sensitive. It is not possible to observe the effect of larger *cut* clones since larger clones result in cyst-packaging defects (Jackson and Blochlinger 1997).

When mutant egg chambers were stained with antibodies against Hts proteins, the bright actin-containing spot observed in binucleate cells also reacted with Hts antibodies. In these egg chambers, ring canals at various stages of degeneration were observed, from free-floating, detached, round ring canals to dense spots that stained with antibodies against actin and Hts. Identical results were obtained using Kelch antibodies (data not shown). Binucleate cells that lacked Hts or Kelch immunoreactivity or actin-containing spots were never observed. The total number of normal ring canals and ring canal remnants in egg chambers with binucleate

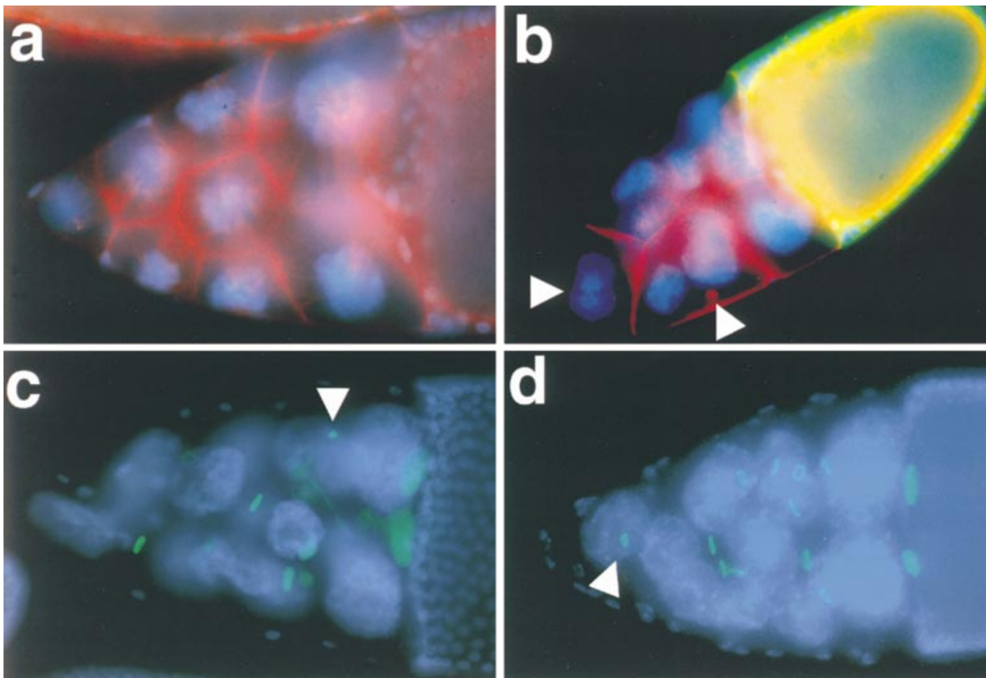

Figure 1.—Binucleate cells produced by *cut* follicle cell clones contain remnants of ring canals. (a) Wild-type stage 10 egg chamber. Note the position of the nurse cell nuclei (DAPI stained, blue), subcortical actin marking the boundaries of individualized nurse cells, and nuclear-anchoring actin filaments (rhodamine-conjugated phalloidin stained, red). (b) Stage 10 egg chamber containing homozygous *cut* follicle cell clones. Two binucleate nurse cells with actin-containing spots are indicated with arrowheads. Homozygous *cut* clones, marked with a c-Myc epitope tag (green), are over the oocyte and outside the plane of focus. (c and d) Egg chambers with homozygous *cut* clones have misshapen ring canal remnants that stain with Hu-li tai shao antibodies (green). Two closely apposed nuclei in a binucleate cell can be observed in d. Although the ring canals in unaffected nurse cells are normal and rounded, some of these are outside the plane of focus and appear as diffuse patches of green staining.

nurse cells was 15, suggesting that there was no net change in the number of cystoblast cytokineses. Finally, binucleate cells were not observed prior to stage 5 in *cut* clones and were observable in stage 10 egg chambers as soon as 4 days after clonal induction. These results are consistent with a model in which the binucleate cells produced by *cut* follicle cell clones result from the postmitotic fusions of adjacent nurse cells.

We employed a similar analysis of ring canal markers to determine if binucleate cells present in *cappuccino* (*capu*) female sterile mutants arise by defective cytokinesis or subsequent nurse cell fusions (Figure 2). In *capu*^{RK12} and *capu*^{G7} homozygous mutant egg chambers, all of the binucleate cells contained fragments of ring canals that reacted with Hts (Figure 2a) and Kelch antibodies (data not shown). As in the *cut* somatic clones, binucleate cells were not detectable in the germarium or early stages, suggesting that binucleate cells in these mutants also arise by fusion of adjacent cells. Since *chickadee* (*chic*) produces similar phenotypes and interacts genetically with *capu*, we also analyzed the binucleate cell phenotype in *chic* mutants. Binucleate cells produced by *chic*^{7886} (Figure 2b) or *chic*^{1320} (data not shown) mutants also contained remnants of ring canals that displayed Hts and Kelch immunoreactivity (Figure 2b and data not shown). Although all of the binucleate cells produced by *cut* and *capu* mutants contained ring canal remnants, rare *chic* binucleate cells (<1%) exhibited no detectable ring canal remnants. *chic* mutants therefore may produce binucleate cells one of two ways: most commonly, by fusion of adjacent cells, or rarely, by defective cytokinesis. Alternatively, all binucleate cells arise by fusion, but loss of *chickadee* function accelerates the rate of ring canal degradation. Germline clones of Pka-C1 mutations also undergo fusion of adjacent nurse cells, resulting in binucleate cells with Hts- and Kelch-containing ring canal remnants (Lane and Kalderon 1993).

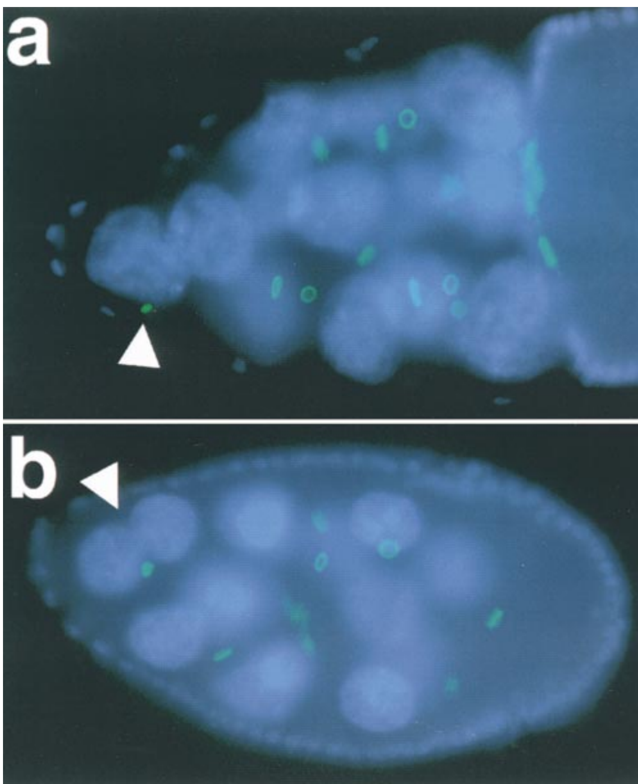

Figure 2.—Binucleate cells produced by *chickadee* and *cappuccino* homozygous females also contain remnants of ring canals. (a) Stage 10 egg chamber from *capu*^{RK12} homozygous female. The cell with two closely juxtaposed nurse cell nuclei (DAPI stained, blue) contains a dense spot of ring canal remnant that reacts with Hu-li tai shao antibodies (green staining, arrowhead). (b) Stage 9 egg chamber from *chic*^{7886} homozygous female. The ring canal remnant in the cell with two nuclei also stains with Hu-li tai shao antibodies (arrowhead). Again, note that the ring canals in unaffected nurse cells have a normal appearance.

***cut* interacts with *cappuccino*, a gene that regulates cytoskeletal function:** Binucleate cells with a phenotype similar to that observed in *cut* follicle cell clones have been observed in egg chambers produced by flies carrying mutations in other genes, including those that are associated with cytoskeletal, cell adhesion, and cell-signaling functions. To determine whether these phenotypic similarities reflected functional interactions, we determined whether mutations in these genes produced a binucleate cell phenotype in either a doubly heterozygous genetic background, or in a background of hypomorphic combinations of *cut* alleles (*cut* hypomorphs; Jackson and Blochlinger 1997). We found that double heterozygotes containing strong alleles of *capu* (*capu*^{RK12} and *capu*^{G7}; Manseau and Schüpbach 1989; Emmons *et al.* 1995) and null alleles of *cut* (*ct*/+; *capu*/+) produce binucleate cells (Figure 3 and Table 1). We used two different embryonic lethal *cut* null alleles for our analyses: *ct*^{C145}, an EMS-induced allele that produces no detectable Cut protein on immunoblots and *ct*^{DB7}, a diepoxibutane-induced allele that makes a truncated, non-nuclear Cut protein (Blochlinger *et al.* 1990). When doubly heterozygous with strong *capu* alleles, each *cut* allele produced binucleate cells with a similar frequency, demonstrating that the observed genetic interaction is not restricted to specific alleles. As in the *cut* clones, a majority (>50%) of affected egg chambers had a single binucleate cell, but occasionally more than one binucleate cell could be observed and these cells could be found anywhere among the nurse cells. The binucleate cells formed by *ct*/+; *capu*/+ heterozygotes were not observed prior to stage 5 of oogenesis and contained anti-Hts and anti-Kelch immunoreactive remnants of ring canals (Figure 3), suggesting that, like *cut* follicle cell clones and *capu* and *chic* homozygous mutant ovaries, they arose by fusion of adjacent cells rather than defective cytokineses.

Although a weaker *capu* allele (*capu*^{HK3}) did not interact with null alleles of *cut* in a doubly heterozygous

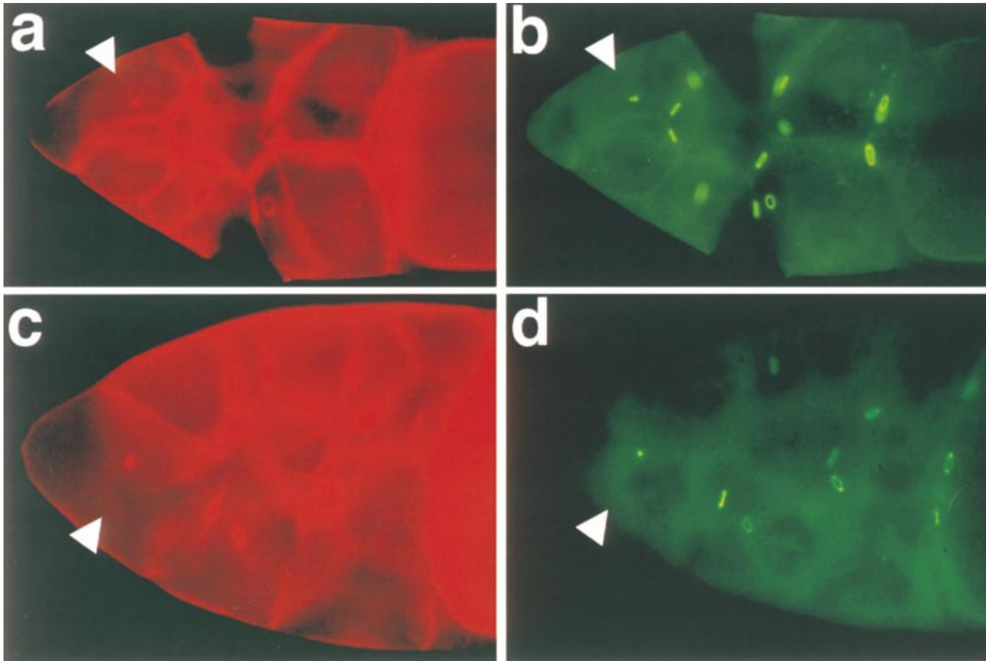

Figure 3.—*cut* and *cappuccino* interact during oogenesis to produce binucleate cells. Stage 10 egg chambers from $ct^{C145}/+$; $capu^{RK12}/+$ doubly heterozygous females have binucleate cells (arrowheads) with ring canal remnants that contain filamentous actin (a and c, stained with rhodamine phalloidin) and also stain with Hu-li tai shao antibodies (b) and Kelch antibodies (d). The same egg chambers are shown in a and b and in c and d. The apparent membrane staining in b is bleed-through from the rhodamine staining.

background, it did enhance the frequency of binucleate cell production in *cut* hypomorphs (Table 1, compare $ct^{C145}/+$; $capu^{HK3}/+$ to ct^{L188}/ct^{C145}; $capu^{HK3}/+$ and compare $ct^{DB7}/+$; $capu^{HK3}/+$ to ct^{L188}/ct^{DB7}; $capu^{HK3}/+$). These results demonstrate that the genetic interaction is sensitive to the levels of activities of *ct* and *capu* gene products. We also tested *chickadee* (Verheyen and Cooley 1994; Manseau *et al.* 1996), *spaghetti-squash* (Wheatly *et al.* 1995; Edwards and Kiehart 1996), *armadillo* (Peifer *et al.* 1993), *Tec29A* (Guarnieri *et al.* 1998; Roulier *et al.* 1998) and *Src64B* (Dodson *et al.* 1998; Guarnieri *et al.* 1998), each of which produces binucleate cells when homozygous in a wild-type *cut* background (*e.g.*, $+/+$; *chic*/*chic*). None interacted with *cut* to produce binucleate cells, either in double heterozygotes or as enhancers of *cut* hypomorphs (*e.g.*, $ct^{C145}/+$; *chic*/+ or ct^{L188}/ct^{C145}; *chic*/+). In addition, several other genes that either affect cytoskeletal function during oogenesis (*quail*, Mahajan-Miklos and Cooley 1994; *spire*, Manseau and Schüpbach 1989) or signaling between the germline and soma ($Egfr^{top}$, Price *et al.* 1989) did not interact with *cut* either in double heterozygotes or as enhancers of *cut* hypomorphs.

Null alleles of *cut* exhibit dose-sensitive interactions with specific genomic regions: The observation that *cut* and *capu* exhibit dose-sensitive genetic interactions suggested that other components of the *cut*-mediated soma-to-germline signaling pathway may also be sensitive to genetic dose. To begin to identify other genes that interact with *cut*, a collection of Drosophila chromosomal deficiencies was utilized in an F_1 screen for second-site noncomplementing loci that interact with a *cut* loss-of-function mutation. This collection of 157 genomic deletions with defined chromosomal breakpoints provides an opportunity to assay ~70% of the euchromatic genome for dose-sensitive genetic interactions with *cut.* Double heterozygotes of the null allele ct^{C145} and each deficiency were scored for lethal interactions, defects in adult morphology, and female sterility. Ovaries of double heterozygotes were examined for the presence of binucleate cells after staining with DAPI and rhodamine phalloidin. To verify that observed genetic interactions were specific for the *cut* mutation, deficiencies that interacted with ct^{C145} were also assayed with a different *cut* null allele (ct^{DB7}) and with w^{1118} as a negative control. Out of 157 first, second, and third chromosome deficiencies screened, 19 deficiencies defining a minimum of 16 regions interacted with *cut* to give binucleate cells (Figure 4 and Table 2). The penetrance of the binucleate cell phenotype varied from ~1–5% of all egg chambers to ~50% of all egg chambers. Eight deficiencies interacted with *cut* in other developmental pathways.

Each interacting deficiency was inspected for known mutations that map to the same region (FlyBase, http://flybase.bio.indiana.edu). We reasoned that genes known to cooperate with *cut* in other tissues or genes involved in cAMP/PKA-mediated signaling, cytoskeletal regulation, or cell adhesion would be good candidates for interacting genes in the ovary. Regions containing genes that met these criteria were subjected to more detailed analyses as described below. Some of the deficiencies that interacted with *cut* in other pathways in our screen confirmed genetic interactions that have been observed previously. A few regions either did not have obvious candidate genes according to our criteria or did not have mutations available in candidate genes. Although these genomic regions are of interest, our initial efforts concentrated on regions for which muta-

TABLE 1

Genetic interactions between *cut* and selected genes

First chromosome	Second/third chromosome	Binucleate cells?
+/+	+/+	−
y w ct^{C145}/+	+/+	−
y w ct^{C145}/*y w ct*^{L188}	+/+	+
y w ct^{C145}/*y sqh*^{1}	+/+	−
y w ct^{C145}/*y sqh*^{2}	+/+	−
y w ct^{C145}/*w arm*^{1}	+/+	−
y w ct^{C145}/*w arm*^{4}	+/+	−
w arm^{4} *ct*^{C145}/*y w ct*^{L188}	+/+	+
+/+	*capu*^{HK3}/*capu*^{HK3}	−
+/+	*capu*^{RK12}/*capu*^{RK12}	++
+/+	*capu*^{HK3}/+	−
+/+	*capu*^{RK12}/+	−
+/+	*capu*^{G7}/*capu*^{G7}	++
y w ct^{C145}/+	*capu*^{HK3}/+	−
y w ct^{C145}/+	*capu*^{RK12}/+	+
y w ct^{C145}/+	*capu*^{G7}/+	+
y w ct^{C145}/*y w ct*^{L188}	*capu*^{HK3}/+	+
y w ct^{C145}/*y w ct*^{L188}	*capu*^{RK12}/+	++
y w ct^{DB7}/+	+/+	−
y w ct^{DB7}/+	*capu*^{HK3}/+	−
y w ct^{DB7}/+	*capu*^{RK12}/+	+
y w ct^{DB7}/*y w ct*^{L188}	*capu*^{HK3}/+	++
+/+	*chic*^{7886}/*chic*^{7886}	+
+/+	*chic*^{7886}/+	−
y w ct^{C145}/+	*chic*^{7886}/+	−
y w ct^{C145}/+	*chic*^{1320}/+	−
y w ct^{C145}/+	*chic*^{k13321}/+	−
y w ct^{C145}/*y w ct*^{L188}	*chic*^{7886}/+	−
y w ct^{C145}/*y w ct*^{L188}	*chic*^{k13321}/+	+
y w ct^{DB7}/*y w ct*^{L188}	*chic*^{k13321}/+	+
y w ct^{C145}/+	*spir*^{RP48}/+	−
y w ct^{C145}/+	*spir*^{HP10}/+	−
y w ct^{C145}/*y w ct*^{L188}	*spir*^{RP48}/+	±
y w ct^{C145}/*y w ct*^{L188}	*spir*^{HP10}/+	−
y w ct^{C145}/+	*Src64B*$^{\Delta 17}$/+	−
y w ct^{C145}/*y w ct*^{L188}	*Src64B*$^{\Delta 17}$/+	±
y w ct^{C145}/+	*qua*^{HM14}/+	−
y w ct^{C145}/*y w ct*^{L188}	*qua*^{HM14}/+	±
y w ct^{C145}/+	*Egfr*^{topC0}/+	−
y w ct^{C145}/*y w ct*^{L188}	*Egfr*^{topC0}/+	+
y w ct^{C145}/+	*Tec29A*$^{l(2)k00206}$/+	−
y w ct^{C145}/*y w ct*^{L188}	*Tec29A*$^{l(2)k00206}$/+	+

++, 20–50% of all stage 2–11 egg chambers have binucleate cells; +, 5–20% have binucleate cells; ±, 1–5% have binucleate cells; −, <1% have binucleate cells.

tions were available and that exhibited a high penetrance of the binucleate cell phenotype.

Regions that are known to interact with *cut*: Two deficiencies were identified that exhibited loss of wing margin material in double heterozygotes. *Df(1)N-8* removes the *Notch* locus and, although small wing margin gaps were present in all flies that carried this deficiency, the wing margin notches appeared to be deeper and more frequent in flies doubly heterozygous for *cut* and *Df(1)N-8.* Similarly, *Df(1)sd72b* removes the *scalloped* gene, and double heterozygotes of this deficiency and *cut* exhibited wing notches as well, but to a smaller extent than observed with *Df(1)N-8.* Neither of these deficiencies interacted with *cut* to produce binucleate cells in ovaries. Previous studies showed that both *N* and *sd* interact with *cut* in wing margin development (Jack and DeLotto 1992), confirming that known genetic interactions can be detected in this screen. Finally, *Df(2L)30C* deletes the Pka-C1 locus, but binucleate cells were infrequent (∼1%) in double heterozygotes of *cut* and this deficiency. These observations are consistent with our previous results, however, since genetic interactions between a lethal *P*-element insertion in Pka-C1 (Pka-C1$^{l(2)01272}$) and *cut* were not observed in double heterozygotes. Rather, Pka-C1$^{l(2)01272}$/+ dominantly enhanced the frequency of binucleate cell formation in *cut* hypomorphs (Jackson and Blochlinger 1997).

Several regions when heterozygous with *cut* are haploinsufficient during embryogenesis: Three regions were lethal when doubly heterozygous with *cut.* A fourth deficiency, which deleted *cut*, was also lethal. The 11D1;11D2 region demonstrated a lethality that was highly penetrant (3% of expected + *Df*/*ct* + females survived), and this lethality was genetically separable from the *agnostic* locus (Figure 6 and discussed below). *Df(3R)P14* (90C2;91A1-2) exhibited a lethal interaction with *cut* (6% of expected *ct*/+; *Df*/+ flies survived). The 97A;98A2 region uncovered by *Df(3R)Tl-P* was partially lethal; 38% of expected females of the double heterozygous class eclosed. Although the developmental defects of the lethal classes were not characterized further, no dead larvae or pharate adults were observed, suggesting that each interaction resulted in embryonic lethality. Adult escapers of each of the lethal classes produced egg chambers with binucleate cells, but no other defects in adult morphology or fertility were observed.

Two regions failed to give a reproducible phenotype when uncovered by different stocks. *Df(1)A209* (20A;20F) produced shortened and tapered bristles when heterozygous with *cut.* Two other deficiencies that uncover the same region (*Df(1)HF396*, 18E1;20 and *Df(1)DCB1-35b*, 19F1;20E-F) failed to produce the same phenotype. Since the proximal breakpoints of these deficiencies are not well defined, the interacting gene may have resided in a unique region of 20F. Alternatively, another mutation existed elsewhere on the X chromosome that may have interacted with *cut* to pro-

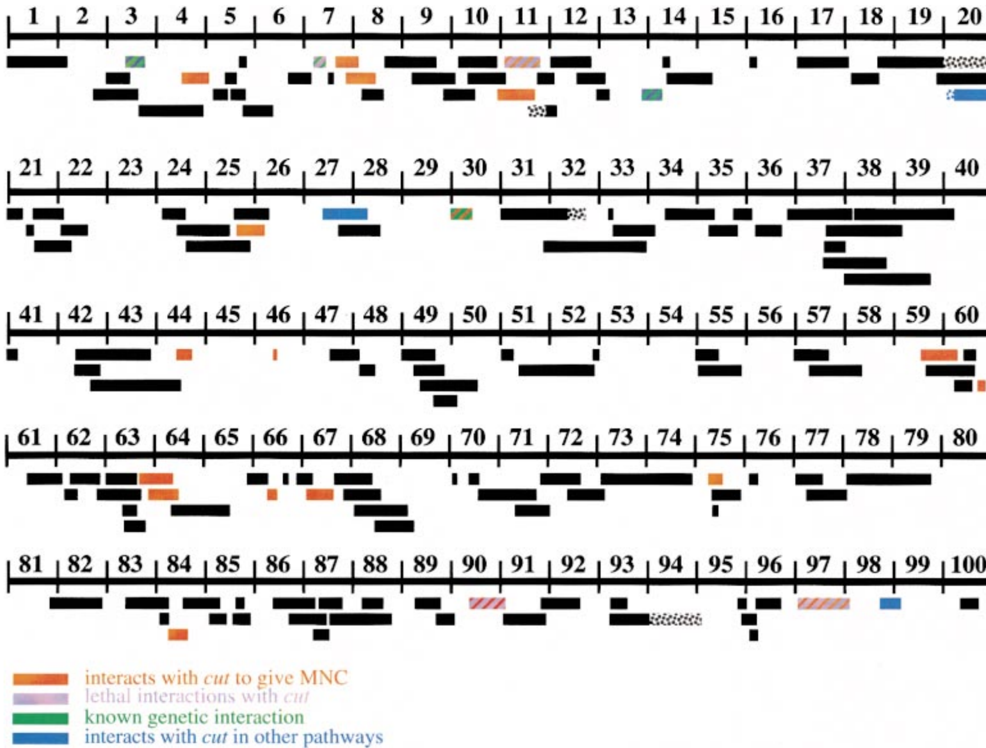

Figure 4.—Genomic map of deficiencies that produce phenotypes when doubly heterozygous with *cut.* The relative positions of all deficiencies used in the screen are shown. Deficiencies defined by black bars showed no interaction as double heterozygotes with *cut*, those denoted by red bars interacted to give binucleate cells, those indicated by purple bars were lethal as double heterozygotes, those defined by blue bars interacted with *cut* in other pathways, and those denoted by green bars uncovered a locus known to interact with *cut.* Hatching indicates more than one characteristic was associated with the interacting deficiency. Stippling indicates that the breakpoints are not well defined.

duce this phenotype. *Df(2L)GpdhA* (25D7;26A9) produced binucleate cells when heterozygous with *cut*, but *Df(2L)cl-h3* (25D2;26B2-5) deleted a larger region and failed to produce binucleate cells. It is possible that one of the assigned breakpoints of these deficiencies is inaccurate. Alternatively, an interacting mutation residing elsewhere on the chromosome might account for the observed interaction of *cut* with *Df(2L)GpdhA.* For these reasons we conclude that *cut* does not interact specifically with *Df(2L)GpdhA.* Of note, *chickadee* is contained in the region uncovered by *Df(2L)GpdhA.*

Finally, a deficiency uncovering the *cappuccino* gene failed to interact with *cut* in our screen. No binucleate cells were observed in egg chambers from females doubly heterozygous for a *cut* null allele and *Df(2L)ed1*, a deficiency that fails to complement the female sterility defects associated with several *capu* alleles (Manseau and Schüpbach 1989). Each of the *capu* alleles used in our studies are EMS-induced missense mutations that map to different domains of the *capu* coding sequence (Emmons *et al.* 1995), raising the possibility that they are gain-of-function rather than loss-of-function alleles. No null mutations in *capu* have been identified. Our conclusion is consistent with the dose-sensitive nature of the $ct^{null}/+$; $capu^{strong}/+$ *vs.* *cut* hypomorphs; $capu^{weak}/+$ interactions.

***cut* does not interact with *Src64B* during oogenesis:** Two overlapping deficiencies on the third chromosome, *Df(3L)GN50* and *Df(3L)GN24*, interacted with *cut* to produce binucleate cells. Although *Src64B* mutations produce binucleate cells (Dodson *et al.* 1998), and this locus is uncovered by the overlap of these two deficiencies, we found no interactions between *cut* and a *Src64B* mutation, either as double heterozygotes or enhancers of *cut* hypomorphs (Table 1). In addition, no binucleate cells were seen in egg chambers produced by flies doubly heterozygous for *cut* and *Df(3L)10H* (64B10-64B12; 64C5-64C9), a smaller deficiency that removes the *Src64B* locus. Therefore, a locus residing in 63F4-64B10, included in the overlap in the interacting deficiencies but excluded from *Df(3L)10H*, interacts with *cut* to produce a binucleate cell phenotype.

***cut* interacts with the *ovarian tumor* gene to produce binucleate cells:** *Df(1)RA2* and *Df(1)KA14* overlap in the 7F1-8A5 region of the first chromosome and both deficiencies produced binucleate cells when doubly heterozygous with *cut.* One potential interacting gene of the minimum of 112 genes contained within this overlap (FlyBase) is *ovarian tumor* (*otu*, 7F1). *otu* mutations produce a range of phenotypes during oogenesis (King *et al.* 1986; King and Storto 1988), including agametic ovaries (quiescent alleles), tumorous egg chambers (oncogenic alleles), and nurse cell dumping defects or arrest at late stages (differentiating alleles). Recent studies suggest that these phenotypes result from misregulation of actin cytoskeleton function at multiple stages of oogenesis (Rodesch *et al.* 1997). *otu* therefore met our criteria as a potential interacting gene. To determine whether *otu* interacts with *cut*, females doubly heterozygous for *cut* and the strong alleles $otu^{P\Delta1}$ (a *P*-element excision allele that is null for *otu*; Geyer *et al.* 1993) or otu^{11} (an allele of the tumorous class; Steinhauer and

TABLE 2

Deficiency kit summary

Stock no.	Name	Cytology	Strength	Genes	Phenotype
		Deficiencies that produce binucleate cells with cut			
944	*Df(1)JC70*	4C15;5A2	+		
950	*Df(1)RA2*	7D10;8A5	++		
951	*Df(1)KA14*	7F1;8C6	++	***ovarian tumor***	
962	*Df(1)N105*	10F7;11D1	++		
964	*Df(1)JA26*	11A1;11D-E	++	***agnostic***	
1128	*Df(2L)GpdhA*	25D7;26A9	+		
3702	*Df(2L)30C*	29F7;30C5	±	***Pka-C1***	
3643	*Df(2R)44CE*	44C4;44E4	+		
1545	*Df(2R)eve1.27*	46C3;46C11	+		
1682	*Df(2R)or-BR6*	59D5;60B8	++		
2471	*Df(2R)M-c33a*	60E2;60E12	+		
3687	*Df(3L)GN50*	63E1;64B17	++		
3686	*Df(3L)GN24*	63F4;64C15	++	*Src64B*[a]	
1541	*Df(3L)66C-G28*	66B7;66C10	+		
997	*Df(3L)AC1*	67A2;67D13	±		
2607	*Df(3L)W4*	75B10;75C2	+		
1842	*Df(3R)Antp17*	84B1;84D12	±		
3010	*Df(3R)P14*	90C2;91A1-2	±		
1910	*Df(3R)Tl-P*	97A;98A2	±		
		Deficiencies that are lethal with *cut*			
3221	*Df(1)ct4b1*	7B2;7C4	++	***cut***	Lethal
964	*Df(1)JA26*	11A1;11D-E	++		
3010	*Df(1)P14*	90C2;91A1-2	++		
1910	*Df(1)Tl-P*	97A;98A2	+		Partial lethality
		Deficiencies that produce other phenotypes with *cut*			
729	*Df(1)N-8*	3C2;3E4	++	***Notch***	Wing margin notches
3347	*Df(1)sd72b*	13F1;14B1	+	***scalloped***	Wing margin notches
3714	*Df(1)A209*	20A;20F	++		Bristles tapered and short
1357	*Df(2L)J136-H52*	27C2;28B4	+		Wing margin bristle loss and notching
430	*Df(3R)3450*	98E3;99A8	++		Malpighian tubules malformed

++, 20–50% of all stage 2–11 egg chambers have at least one binucleate cell; +, 5–20% have at least one binucleate cell; ±, 1–5% have at least one binucleate cell. For the deficiencies producing lethality or other phenotypes, + (10–33%) and ++ (33–100%) indicate penetrance of the phenotype. Underlining indicates overlapping deficiencies. Boldface indicates interacting loci.

[a] Mutation tested for interactions, but failed to interact as double heterozygote.

Kalfayan 1992) were examined for ovariole morphology. Binucleate cells were observed when either allele was doubly heterozygous with *cut* (Figure 5). As in other mutant combinations discussed above, a majority of the affected egg chambers contained a single binucleate nurse cell positioned anywhere in the chamber, but occasionally two or sometimes three binucleate cells could be observed in a single egg chamber. Binucleate cells were not observed, however, when *otu*-mutant chromosomes were placed *in trans* with the w^{1118} chromosome, indicating that heterozygosity in *otu* alone is insufficient to produce binucleate cells. Other oogenic defects associated with loss of *otu* function, such as tumorous or dumpless egg chambers, were not observed in double heterozygotes. These results demonstrate that *cut* and *otu* cooperate during oogenesis to affect membrane integrity of the germ cells. Current models of *otu* function suggest that this interaction may influence the function of elements associated with the actin cytoskeleton.

***cut* interacts with the *agnostic* gene to produce binucleate cells:** *Df(1)N105*, uncovering the region from 10F7 to 11D1, interacted with *cut* to produce binucleate cells. A neighboring deficiency, *Df(1)JA26* (11A1;11D-E), was lethal when doubly heterozygous with *cut.* The basis for the lethality has not yet been explored. Nevertheless, rare escapers of the *Df(1)JA26/cut* class that survived the lethal phase produced egg chambers that contained binucleate cells. This result suggests that an interacting locus is contained within the overlapping region of these two deficiencies. Smaller deficiencies in this region were viable in combination with *cut* and resulted in binucleate cells (Figure 6). The proximal breakpoint of *Df(1)JA26* is not well defined, but *Df(1)N12* (11D1-2;11F1-2) did

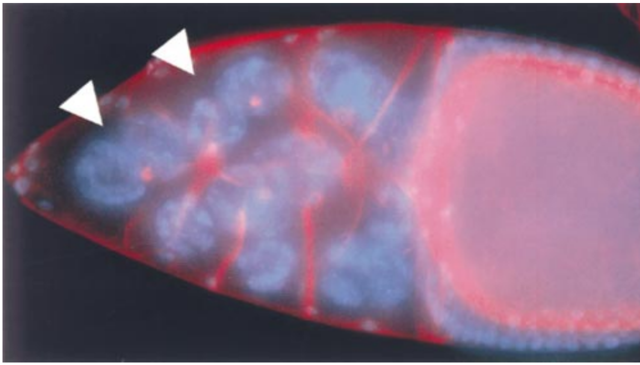

Figure 5.—*cut* interacts with *ovarian tumor* to produce binucleate nurse cells. Stage 10 egg chamber from *y w* ct^{C145}/*y* $otu^{P\Delta 1}$ female stained with DAPI (blue) and rhodamine-phalloidin (red). Two binucleate cells with actin-containing ring canal remnants are indicated with arrowheads.

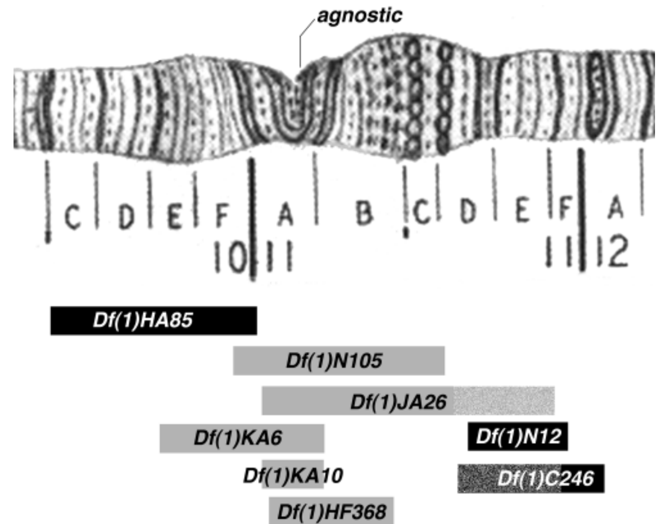

Figure 6.—Positions of deficiencies used to identify *cut* interactions with *agnostic.* Deficiencies that produced a binucleate cell phenotype are indicated with gray bars; noninteracting deficiencies are shown in black. Stippled regions of *Df(1)JA26* and *Df(1)C246* indicate that the breakpoints of these deficiencies are not well defined. The relative position of the *agnostic* gene (11A8) is shown. A second gene, distinct from *agnostic*, is lethal when heterozygous with *cut* and resides in the 11D1-2 region.

not interact with *cut* at all. This region therefore appears to contain two interacting loci. One produces binucleate cells and is defined by the overlap of all the deficiencies tested (11A2;11A8), and a second is in the 11D1;11D2 region that is lethal in combination with null alleles of *cut.*

A scan of the 74 known mutations that map to the 11A2;11A8 region (FlyBase) for loci that met our criteria for *cut*-interacting genes suggested the *agnostic* gene (*agn*, 11A8) as a likely candidate. *agnostic* is thought to regulate the activity of adenylyl cyclases and phosphodiesterases indirectly by encoding a calmodulin-inhibiting protein (Peresleni *et al.* 1997). A temperature sensitive-lethal allele of *agn* was obtained (agn^{ts3}), and the requirement for this gene during oogenesis was characterized at the restrictive temperature. In addition, we asked whether the *agn* mutation produced binucleate cells at the restrictive temperature when doubly heterozygous with *cut.*

After 5 days at 29°, ovaries from agn^{ts3} homozygous females exhibited striking defects that were completely penetrant (Figure 7). In stage 5–6 egg chambers, the follicle cell epithelium became gapped. The follicle cells at this stage also appeared more elongated and less cuboidal. No squamous follicle cells (stretch cells) could be found associated with the gaps in the epithelium. In later stages, the follicle cell epithelium was absent, and binucleate germline-derived cells could be observed. Rarely, single egg chambers contained large nurse cells in which three or more nuclei were present. The morphology of these late-stage egg chambers was similar to that observed in *cut* hypomorphs (Jackson and Blochlinger 1997). Although w^{1118} females were unaffected at 29° for 5 days, agn^{ts3} females stopped laying eggs under these conditions. Defects in oogenesis were observable after only 2 days at the restrictive temperature.

When ct^{C145}/agn^{ts3} adult females were placed at 29° for 5 days, egg chambers were produced with binucleate cells (1–5% of all egg chambers). These egg chambers were indistinguishable in morphology from those produced by *cut* clones and in other double heterozygous combinations described above. The frequency at which binucleate cells were observed in ct^{C145}/agn^{ts3} females was less than that observed with the deficiencies (20–50% of all egg chambers contained at least one binucleate cell). This difference in expressivity probably reflects the fact that agn^{ts3} is not a null allele. Alternatively, two interacting genes could reside in the region uncovered by the overlapping deficiencies. Notably, no defects were observed when either agn^{ts3} homozygotes or ct^{C145}/agn^{ts3} double heterozygotes were maintained at the permissive temperature of 25°; ovaries were mostly wild type with the exception of rare binucleate cells observed in agn^{ts3} homozygous females. These results demonstrate that the *agnostic* gene product is required during oogenesis and that *agnostic* cooperates with *cut* during oogenesis to affect egg chamber morphology.

DISCUSSION

In this study, we used two approaches to understand the mechanism by which somatic expression of the homeodomain protein *cut* affects the morphology of the germline cells. First, using a cytological approach, we found that the binucleate nurse cells produced by *cut*, *cappuccino*, and *chickadee* mutants probably arise by fusion of adjacent cells, rather than by defective cytokineses of the cystoblast cells. Second, using a genetic approach, we found that *cut* exhibits dose-sensitive inter-

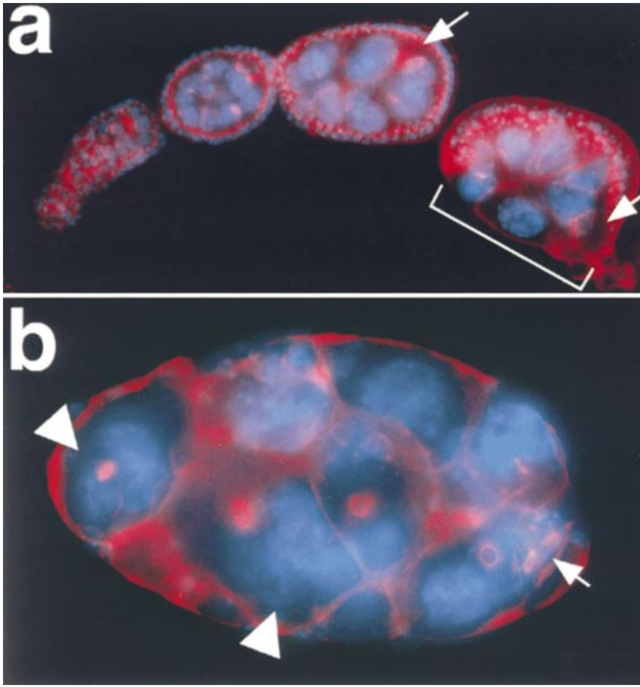

Figure 7.—*agnostic* function is required during oogenesis. Egg chambers from *agnostic*^{ts3} females placed at the restrictive temperature for 5 days, stained with DAPI (blue) and rhodamine phalloidin (red). (a) Germarium (far left) and early-staged egg chambers. The stage 5–6 egg chamber at the center is elongated, and the oocyte is displaced to the side of the chamber. In the posteriormost egg chamber (right), the follicle cell epithelium is gapped (bracket), and remaining follicle cells on the other side of the chamber appear elongated. The oocyte is indicated with an arrow. (b) Later-staged egg chamber; the exact stage could not be determined. Note the lack of a follicle cell epithelium and disorganization of the nurse cells. Two binucleate nurse cells with ring canal remnants are indicated with arrowheads. The oocyte, which has failed to take up any material from the nurse cells, is indicated with an arrow.

actions with specific genes and regions of the genome, some of which include genes involved in cAMP-mediated signaling or cytoskeletal function. These results are consistent with our hypothesis that *cut* affects cAMP-mediated events that ultimately influence the function of the cytoskeleton of the germline-derived cells.

Binucleate cells result from fusions of adjacent germline-derived cells: The maintenance of germline cytoskeletal membrane integrity is a complex process, since mutations in several genes produce a binucleate cell phenotype. Many of these genes encode cytoskeleton-associated proteins and their regulation is therefore important for preventing binucleate cell formation. The molecular mechanism by which disrupting cytoskeletal function results in binucleate cells, however, is not yet clear. Work from other investigators suggested that binucleate cells observed in *chic* and *capu* mutants resulted from cytokinesis defects. For example, *chickadee* encodes a Drosophila homolog of Profilin (Verheyen and Cooley 1994), which regulates cytokinesis in a number of systems (Edmatsu *et al.* 1992; Balasubrimanian *et al.* 1994; Haugwitz *et al.* 1994; Verheyen and Cooley 1994; Giansanti *et al.* 1998). In addition, genetic and biochemical interactions have been detected between Profilins and Formin homology domain-containing proteins (such as Capu) in yeasts and in flies, implicating the Formin homology domain proteins in regulating actin cytoskeleton function (Manseau *et al.* 1996; Evangelista *et al.* 1997); moreover, some Formin proteins are required for cytokinesis (Castrillon and Wasserman 1994; Imamura *et al.* 1997). Thus, the connection between these proteins, the actin cytoskeleton, and cytokinesis appears to be reasonable. This hypothesis is complicated, however, by the observation that microtubule function is also affected by mutations in the *capu* gene (Manseau *et al.* 1996). This result raises the possibility that misregulation of microtubule function may contribute to binucleate cell production in *capu*-mutant egg chambers.

Our results suggest that defective cytokineses are not responsible for the binucleate cell phenotype in *cut*, *capu*, and *chic* mutants. First, binucleate cells were not present in any mutant combination prior to stage 5 of oogenesis. Second, stage 10 egg chambers with *cut* follicle cell clones exhibited binucleate nurse cells as soon as 4 days after clonal induction. Since 16-cell cysts require 5 days to mature to stage 10, and single cystoblast cells require 7 days (King 1970), binucleate cells in stage 10 egg chambers must have arisen after the divisions were completed. Third, remnants of ring canals stain with Hts and Kelch antibodies, two proteins that are added to ring canals after the germline cell divisions are completed. Finally, the total number of normal ring canals and ring canal remnants was 15, suggesting that cytokineses proceeded normally. Taken together, these results are consistent with a model in which binucleate cells form by fusion of adjacent cells after cytokineses are complete. Presumably, these fusions result from the disruption of cytoskeletal functions other than those necessary for cytokinesis. Alternatively, sufficient product is available to ensure proper cytokinesis, but this level of activity may be insufficient to maintain the integrity of the membrane/cytoskeletal junctions during the considerable growth of the maturing egg chamber. In addition, our results do not rule out the formal albeit unlikely possibility that ring canal-like structures, containing filamentous actin, Hts, and Kelch proteins, form spontaneously when cytokinesis is defective. Finally, we have not observed a noticeable loss in fecundity in binucleate cell-producing mutant combinations. This result suggests that loss of membrane integrity between adjacent cells does not dramatically affect the functions of the nurse cells in the syncytium, provided the disruptions are not severe (*e.g.*, as in *agnostic* mutants).

Other investigators have also hypothesized that binucleate cells arise by fusion of adjacent cells. Disrupting

actin cytoskeleton function by expressing dominant negative RhoL or dominant negative and constitutively active Cdc42 mutant proteins in the germline during oogenesis also resulted in binucleate nurse cells (Murphy and Montell 1996). The authors hypothesized that these binucleate cells resulted from fusions rather than cytokinesis defects. Although our screen detected interactions between *cut* and a deficiency that deletes the *RhoL* gene (*Df(3L)AC1*), the lack of *RhoL* mutations limits further analyses. A deficiency that deletes the Cdc42 gene was not present in our collection and mutations in this locus (Fehon *et al.* 1997) have not been tested for genetic interactions. Loss of *armadillo* (β-catenin) or *spaghetti squash* (regulatory light chain of nonmuscle myosin) function during oogenesis also produces binucleate cells (Peifer *et al.* 1993; Wheatly *et al.* 1995; Edwards and Kiehart 1996). In *arm* and *sqh* mutants, binucleate cells were observed only by constructing germline clones (*arm*) or using heat shock-induced rescue of loss-of-function alleles (*sqh*). Although it was hypothesized that binucleate cells were produced by nurse cell fusions, these investigators did not examine ring canal markers and so it remains a possibility that defective cytokinesis is responsible for the phenotype in these mutants. Nevertheless, we failed to detect interactions between these genes either by looking specifically with known alleles or in the screen. Similarly, mutations in *cheerio* (a ring canal component) also produce binucleate cells (Robinson *et al.* 1997), but we did not detect an interaction with a deficiency that removes the *cheerio* gene. It is possible that all these genes function in the process that regulates nurse cell/oocyte structural integrity during the middle stages of oogenesis, but their products are too abundant to reveal a role in this process using our methods.

***cut* interacts with the *cappuccino* and *ovarian tumor* genes during oogenesis:** Because *cut* is expressed and required only in the follicle cells, its influence on germline cell morphology must be mediated across the soma-germline boundary. Our results demonstrate that *capu* and *otu*, which are both required in the germline (Wieschaus *et al.* 1981; Perrimon and Gans 1983; Manseau and Schüpbach 1989; Steinhauer *et al.* 1989; Emmons *et al.* 1995; Sass *et al.* 1995; Tirronen *et al.* 1995), interact genetically with *cut* and may facilitate *cut*-mediated events originating in the soma. Although *cut* is a transcription factor, the clear separation of cell types in which these genes are expressed suggests that *cut* does not regulate the transcription of *capu* or *otu* directly by binding to their promoters and/or enhancers. Rather, we propose a multistep model in which *cut* activity in the follicle cells first directs expression of a gene or set of genes that regulates adhesion or signaling between the somatic and germline cells. This soma-to-germline interaction then influences cAMP-dependent function in the germline cells. The activity of Capu and Otu is in turn regulated by these cAMP-mediated events, perhaps by post-translational modifications or by alterations in the subcellular localization of one or both of these proteins. Finally, the regulation of Capu and Otu by cAMP results in altered cytoskeletal function. This hypothesis makes several testable predictions that are currently under investigation. Since it is not yet known if *agn* is required in the germline cells or follicle cells, we cannot rule out the possibility that *cut* influences *agn* levels directly by regulating *agn* transcription in the follicle cells. A less favorable model is that *capu*, *otu*, and/or *agn* function genetically upstream of *cut*, and loss of germline function of these genes influences *cut* activity in the follicle cells. At some point in this model, however, *cut* activity in the follicle cells must influence the function of the germline cytoskeleton, since loss of *cut* function in the follicle cells is sufficient to produce binucleate germline cells.

The observed genetic interactions between *cut* and *otu* are consistent with our model that *cut*-mediated events disrupt the function of the germline cytoskeleton. Recently, Rodesch *et al.* (1997) showed that actin cytoskeleton function was disrupted in *otu* mutants; they hypothesized that this defect was the underlying cause for the various otu phenotypes. Although *otu* has been cloned and antibodies have been raised against the protein, the gene's sequence (Steinhauer *et al.* 1989) and the uniform distribution of Otu protein within the germplasm (Steinhauer and Kalfayan 1992; Sass *et al.* 1995) give no clues as to how Otu is affecting the function of the cytoskeleton. Nevertheless, our findings suggest that *otu* regulates cytoskeleton function in response to signaling events that occur after the cystoblast cell divisions are completed and egg chambers leave the germarium. Finally, one of the otu mutant phenotypes is the production of tumorous egg chambers filled with extra germline-derived cells. We did not observe egg chambers that were tumorous or that contained extra germline cell nuclei in *cut/otu* double heterozygotes, suggesting that *cut* and *otu* do not interact in the germarium to regulate germline cell divisions.

***agnostic* is required during oogenesis:** We have provided clear evidence that *agnostic* is required during oogenesis. Loss of *agnostic* function affects the morphology of the follicle cell epithelium and, because follicle cells are missing in late-stage egg chambers, may influence the survival of the follicle cells. In addition, loss of *agnostic* affects the morphology of the germline-derived cells. It is not known whether *agnostic* is required in the follicle cells, germline cells, or both. Signaling between these two cell layers occurs throughout oogenesis and models can be hypothesized in which loss of *agnostic* function in one cell type affects the function and morphology of the other cell type. Nevertheless, *agnostic* is thought to be involved in cAMP metabolism (Sharagina *et al.* 1997). Since the requirement for Protein kinase A is restricted to the germline cells (Lane and

Kalderon 1993, 1995), it is tempting to speculate that minimally, *agnostic* is required in the germline cells.

Irrespective of the cell type in which *agnostic* is required, both adenylyl cyclase and phosphodiesterase enzymatic activity are altered in *agnostic* mutants (Savvateeva 1985; Sharagina *et al.* 1997). These results raise the possibility that *cut* function impinges on the activity of either or both of these enzymes. In Drosophila, mutations in a 3′,5′-cyclic nucleotide phosphodiesterase gene (*dunce*) and an adenylyl cyclase gene (*rutabaga*) have been isolated (Dudai *et al.* 1976; Aceves-Pina *et al.* 1983). Interestingly, some *dunce* alleles are female sterile, revealing a role for *dunce* during oogenesis (Bellen *et al.* 1987). Deficiencies uncovering *dunce* and *rutabaga* failed to interact with *cut* in our screen, however, and no morphological defects were observed in the ovaries of double heterozygotes of *cut* and specific mutant alleles of *dunce* or *rutabaga.* Thus, *cut* does not appear to interact individually with either *dunce* or *rutabaga* in the same dose-sensitive manner as *agnostic.* There are three other adenylyl cyclase genes identified in Drosophila that may be regulated by *agnostic* and/or *cut* (Levin *et al.* 1992). Finally, it is interesting to note that both adenylyl cyclase and phosphodiesterase activity are increased in *agnostic* mutants (Savvateeva 1985), suggesting that the role of this gene in regulating cAMP levels is complex.

At least 24 genes show genetic dose-sensitive interactions with *cut*: A strategy in which the genetic dose of *cut* and genes contained in genomic deficiencies were halved resulted in the identification of 16 regions that produced binucleate cells, 3 that were lethal, and 5 that produced other defects when heterozygous with *cut.* Some of these regions produced multiple phenotypes, suggesting a minimum of 24 genes that are sensitive to genetic dose in a *cut* heterozygous background. There are likely to be more *cut*-interacting loci, however, than those identified by our screen. First, *cut*-interacting genes that are abundant or stable would be difficult to detect by our strategy. For example, *cut* and *wingless* are known to interact during wing development (Micchelli *et al.* 1997); nevertheless, we did not see wing margin defects when *cut* was heterozygous with a deficiency uncovering *wingless.* Second, the phenotypes that we examined were limited to lethality, visible defects in adult cuticle morphology, and oogenesis. Examination of other tissues or stages of development using more stringent criteria may have revealed other interacting regions. For example, *pox-neuro* interacts with *cut* during establishment of certain external sense (es) organ neurons (Vervoort *et al.* 1995), but in our screen, no embryonic lethality or visible adult peripheral nervous system defects were observed in double heterozygotes of *cut* and a deficiency that removes *pox-neuro* (*Df(2R)Jp1*). Interactions between these genes and others in es organ lineage establishment may have been detected if we had specifically examined embryos for es organ morphology. Finally, the chromosomal deficiency kit does not cover the entire genome, and regions not present in the collection may contain additional interacting genes. Although our strategy may not have uncovered all *cut*-interacting loci, it was successful in identifying a number of regions that are important in the soma-to-germline signaling pathway essential for germline membrane integrity and ring canal maintenance. Since very little is known about this pathway, these regions will be the basis for identifying genes essential for this process and for characterizing interactions between pathway members.

Our previous results led us to propose a model in which Cut function in the follicle cells during oogenesis directs expression of a gene or set of genes whose products transduce a signal across the soma-germline boundary and ultimately influence the morphology or function of the germline cytoskeleton (Jackson and Blochlinger 1997). Results presented in this study provide further insights into this process by demonstrating that this signaling pathway is sensitive to genetic doses of *cut* and the genes *cappuccino*, *ovarian tumor*, and *agnostic.* The fact that these genes regulate cytoskeletal function and cAMP metabolism allows us to formulate hypotheses that are starting points for further molecular studies. Moreover, other genomic regions contain genes presumably also involved in regulating or responding to this pathway, including genes that may be direct targets of *cut* during oogenesis. By pinpointing and analyzing genes contained in these regions, we can understand better how signaling pathways influence the morphology and function of cytoskeletal elements.

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