Development 118, 1191-1207 (1993)
Printed in Great Britain © The Company of Biologists Limited 1993

A role for the *Drosophila* segment polarity gene *armadillo* in cell adhesion and cytoskeletal integrity during oogenesis

Mark Peifer[1,*], Sandra Orsulic[1], Dari Sweeton[2] and Eric Wieschaus[2]

1Department of Biology, CB#3280, University of North Carolina, Chapel Hill NC 27599-3280, USA
2Department of Molecular Biology, Princeton University, Princeton, NJ 08544-1014, USA

*Author for correspondence

SUMMARY

The epithelial sheet is a structural unit common to many tissues. Its organization appears to depend on the function of the multi-protein complexes that form adherens junctions. Elegant cell biological experiments have provided support for hypotheses explaining the function of adherens junctions and of their components. These systems, however, lack the ability to test function within an entire organism during development. The realization that the product of the *Drosophila* segment polarity gene *armadillo* is related to the vertebrate adhesive junction components plakoglobin and β-catenin led to the suggestion that *armadillo* might provide a genetic handle to study adhesive junction structure and function. An examination of the potential function of Armadillo in cell-cell adhesive junctions was initiated using the *Drosophila* ovary as the model system. We examined the distribution of Armadillo in the *Drosophila* ovary and demonstrated that this localization often parallels the location of cell-cell adhesive junctions. The consequences of removing *armadillo* function from the germ-line cells of the ovary were also examined. Germ-line *armadillo* mutations appear to disrupt processes requiring cell adhesion and integrity of the actin cytoskeleton, consistent with a role for Armadillo in cell-cell adhesive junctions. We have also used *armadillo* mutations to examine the effects on ovarian development of altering the stereotyped cell arrangements of the ovary. The implications of these results for the role of adhesive junctions during development are discussed.

Key words: *armadillo*, β-catenin, *Drosophila*, oogenesis, cytoskeleton, cell adhesion

INTRODUCTION

All multicellular organisms face the challenge of creating organized tissues out of individual cells. One common solution is to organize cells into a single-cell-thick epithelial sheet. To create this organized epithelium, cells must accomplish three goals: (1) they must adhere to each other, (2) they must know that they are adhered to each other and organize themselves accordingly and (3) they must coordinate their actions by linking their cytoskeletons. A complex membrane organelle, the adherens junction, is thought to accomplish many of these functions.

Molecular components of the adherens junction have begun to be identified (reviewed in Magee and Buxton, 1991). The central junctional component is a transmembrane cell adhesion molecule of the cadherin family. The cadherin extracellular domain is thought to mediate homophyllic adhesion and to receive the signal that neighboring cells are present. This signal is thought to be conveyed across the membrane to the cytoplasmic domain, nucleating formation of a multi-protein complex (Ozawa et al., 1989) including the vinculin-related α-catenin (Nagafuchi et al., 1991; Herrenknecht et al., 1991) and the recently cloned β-catenin (McCrea et al., 1991).

Functional studies of these molecules have begun to confirm hypotheses of adherens junction function. Cloned cadherins, when transfected into non-adherent cells, can confer both cell adhesion and also other more complex properties of junctions (Nagafuchi et al., 1987; Nose et al., 1988; McNeill et al., 1990). Likewise, cells lacking α-catenin are non-adherent; both cell adhesion and the ability to form epithelial-like structures can be conferred by α-catenin transfection (Hirano et al., 1992). However, this sort of experimental systems has not been developed to test β-catenin function nor do these systems allow one to test the function of these molecules in the context of the entire organism.

Realization that the *Drosophila* segment polarity gene *armadillo* (gene, *arm*; protein, Armadillo) is closely related in sequence to the vertebrate adhesive junction components plakoglobin and β-catenin (Peifer and Wieschaus, 1990; Franke et al., 1989; McCrea et al., 1991) provided an avenue to address questions of the functions of β-catenin/plakoglobin and of adhesive junctions during development. While

raising the possibility that Armadillo may play a role in *Drosophila* adhesive junctions, sequence identities alone do not prove functional similarity. Further, similarity both to vertebrate plakoglobin (Cowin et al., 1986), which is active in desmosomes that organize the intermediate filament cytoskeleton, and to vertebrate β-catenin, which is a component of adherens junction that organizes the actin cytoskeleton, left open the question of which type of junction might contain Armadillo. We desired to carry out a test of both Armadillo's cell biological localization and its functional characteristics to determine whether it plays a role in cell adhesion and other functions suggested for adhesive junctions.

Previous analysis of *arm* mutations revealed a role in pattern formation during embryogenesis and imaginal disc development (Wieschaus et al., 1984; Peifer et al., 1991). The segment polarity phenotype of *arm* mutants results from a block in the *wingless* intercellular signaling pathway. *wingless* signaling is critical for appropriate cell fate choice by cells within epithelial sheets giving rise to both embryonic and adult segments (reviewed in Ingham and Martinez-Arias, 1992; Peifer and Bejsovec, 1992), and this signaling pathway is particularly sensitive to reductions in *arm* function.

While this analysis revealed that adhesive junctions may be required for certain intercellular signaling pathways, it also raised the question of why *arm* mutations do not have drastic effects on either cell adhesion or the cytoskeleton. At least part of the answer is that embryos mutant for even the strongest *arm* alleles contain residual wild-type Armadillo (Riggleman et al., 1990), resulting from maternal contribution of *arm* product to the egg (Wieschaus and Noell, 1986). The phenotype of null mutants, therefore, is the result of a reduction in the amount of Armadillo rather than its total removal. The Armadillo detectable in mutant embryos (Riggleman et al., 1990; unpublished data) may provide enough *arm* function for the postulated adhesive and cytoskeletal roles of junctions. In contrast, *wingless* signaling seems to be sensitive to more subtle reductions in *arm* function.

Examination of the cellular effects of totally eliminating *arm* function required a different approach. In *Drosophila*, one can produce homozygous clones of mutant tissue in the female germ line. Ovaries derived from germ-line clones of null *arm* alleles lack *arm* function completely, providing an opportunity to examine whether Armadillo is required for cell adhesion and cytoskeletal anchoring in the ovary, as is predicted for its mammalian counterparts. There is no germ-line contribution of *wingless* to oogenesis (Baker, 1988), simplifying analysis of *arm*'s role in the ovary.

It was known that females with germ cells homozygous for strong *arm* alleles are blocked in oogenesis, producing small eggs that are not laid (Wieschaus and Noell, 1986). We extended this examination of *arm*'s function in the egg chamber, a well-defined set of interacting germ-line and somatic cells. The expression pattern of Armadillo was characterized and revealed features consistent with a role for Armadillo in cell-cell adhesive junctions as well as some unexpected patterns of localization. The expression pattern was compared with the ovary's junctional morphology as revealed by electron microscopy, demonstrating co-localization of Armadillo and cell-cell adhesive junctions resembling vertebrate adherens junctions. Finally, the phenotype of ovaries with germ cells lacking *arm* function was examined at the level of cellular morphology, and with probes revealing the actin cytoskeleton and the anterior-posterior pattern of the ovary. Ovaries with mutant germ cells show a wide array of defects, affecting cell position, cell shape and the actin cytoskeleton. Together these results suggest a possible role for *arm* in both cell adhesion and cytoskeletal structure.

MATERIALS AND METHODS

Fly stocks

Wild-type controls were either *Canton* S or w^1 (Lindsley and Zimm, 1992). The isolation of arm^{YD35}, arm^{XK22} and arm^{XP33} is described in Wieschaus et al. (1984), $arm^{H8.6}$ in Klingensmith et al. (1989), arm^{2a9} in Peifer and Wieschaus (1990), and arm^{25B} in Zusman et al. (1985). The embryonic phenotypes of *arm* mutants are described in Peifer and Wieschaus (1990). All of these lines of *arm* are viable and fertile in the presence of a small duplication of the X chromosome, and arm^{YD35}, $arm^{H8.6}$ and arm^{2a9} can be completely rescued by an arm^+ transgene, suggesting that no other mutations on these chromosomes are responsible for the phenotypes observed. $chickadee^{WC57}$ is described in Schüpbach and Wieschaus (1991) and in Cooley et al. (1992). The stocks used to produce germ-line clones are described in Chou and Perrimon (1992).

Generation of homozygous germ-line clones

Flies were raised on standard Drosophila media in a 25°C incubator. Germ-line clones were produced using the yeast recombinase-based FLP-DTS system, described in Chou and Perrimon (1992). A cross was done between the following flies: *Fs (1) ovo*^{D1} *FRT101 / Y ; hs-flp-F38* males X *arm FRT101 / FM7* females. *FRT 101* is an insertion of the FLP site-specific recombination site near the base of the X chromosome, proximal to both *arm* and the dominant female-sterile, *Fs (1) ovo*^{D1}. *hs-flp-F38* is an autosomal insertion of the FLP recombinase under control of the heat-shock promoter. Eggs laid by the females of this cross were collected in bottles overnight and then the parents transferred to a new bottle. Recombinase was induced by heat shocking second or third instar larvae at 37°C in air flow incubator for 3-5 hours. This heat shock stimulates production of FLP recombinase, leading to site-specific mitotic recombination between the two *FRT 101* sites on homologous chromosomes in a subset of the cells in animals of the genotype *Fs (1) ovo*^{D1} *FRT101 / arm FRT101*. This recombination event leads to production of clones either homozygous for *arm* or for the dominant female sterile. After heat shock, the bottles were returned to the 25°C incubator to continue development. Flies of the appropriate genotype, *Fs (1) ovo*^{D1} *FRT101 / arm FRT101* were dissected 3-20 days after eclosing. Because of dominant female-sterile mutation *Fs (1) ovo*^{D1}, only flies possessing germ-line clones homozygous for *arm* develop ovaries.

Whole-mount immunocytochemistry

The procedure used for samples in Figs 1 and 4 was a modification of Carroll and White (1989). Ovaries were dissected in Ringer's solution and fixed 30 minutes in 1% formaldehyde in PEM/NP-40 (0.1 M Pipes pH 6.9, 2 mM $MgSO_4$, 1 mM EDTA, 1% NP40) at room temperature. The fixative was removed by rinsing once with antibody wash (50 mM Tris pH 7.4, 150 mM NaCl, 0.5% NP-40, 1 mg/ml BSA) and blocked for 1-8 hours at 4°C in antibody block (the same as antibody wash except for 5 mg/ml BSA). The primary antibody, anti-Armadillo N2 polyclonal

serum (Riggleman et al., 1990), was applied as 1:200 dilution in antibody wash and incubated with ovaries overnight at 4°C. After four changes of antibody wash in 90 minutes, ovaries were incubated for 2 hours at room temperature with secondary antibody labeled with rhodamine (Boehringer Mannheim). The secondary antibody had been preabsorbed against ovaries overnight at 4°C and diluted to 1:500 in antibody wash before use. After incubation with secondary antibody ovaries were washed with 3-4 changes of antibody wash for 1 hour at room temperature. Phalloidin was used to visualize membrane-associated actin in germ-line and somatic cells in ovaries. For double labeling with phalloidin, after incubation with antibodies and washing, ovaries were then stained with FITC-labeled phalloidin (Molecular Probes) in PBS for 20 minutes at room temperature and washed three times for 5 minutes in antibody wash. Both single and double-labeled ovaries were mounted in Aqua-polymount (Polysciences, Inc.). Immunofluorescence in Fig. 2 was done on ovaries heat-fixed as in Miller et al. (1989) and then processed as above.

Electron microscopy

Ovaries were dissected in Ringers solution and fixed for 3 hours in 1% osmium tetraoxide, 1% KFeCN, 1% KCrO in 0.05 M calcium cacodylate, pH 6.9. They were then stained in 2% uranyl acetate for 6-10 hours, dehydrated in acetone and propylene oxide, and embedded in Embed 812. After 0.05-0.06 μm sections were cut on an LKB ultramicrotome, the sections were stained with lead citrate and examined on a JOEL 100C TEM.

Propidium iodide staining

For staining nurse cell and follicle cell nuclei with propidium iodide, ovaries were treated with RNase A in PBS (400 μg/ml) for 2 hours at room temperature and then rinsed three times with PBS. The ovaries were then incubated in 10 μg/ml propidium iodide solution in PBS and washed three times for 5 minutes in PBS. Actin staining was done as described above.

In situ hybridizations

Whole-mount in situ hybridizations of ovaries were performed according to Tautz and Pfeifle (1989) with the modifications of Suter and Steward (1991). DNA fragments used for hybridization are *orb* (oo18 RNA-binding; described in Lantz et al., 1992) and *oskar* (Ephrussi et al., 1991 and Kim-Ha et al., 1991). Hybridization probes labeled with digoxigenin were prepared by random priming using the Genius digoxigenin labeling kit (Boehringer Mannheim). Ovaries were mounted on microscopic slides in Aqua-polymount (Polysciences, Inc.).

RESULTS

The sequence similarity between the vertebrate cell-cell adhesive junction molecules β-catenin and plakoglobin and the product of the *Drosophila* segment polarity gene *armadillo (arm)* (Peifer and Wieschaus, 1990; McCrea et al., 1991) raised the possibility that *arm* might provide a genetic model both for the functions of β-catenin/plakoglobin and the role of adhesive junctions during development. We have examined the expression and function of Armadillo in the *Drosophila* ovary, to see if these are consistent with a role for Armadillo in cell-cell adhesive junctions and to test some of the hypotheses for the functions of adhesive junctions in a developing organism.

Fig. 1A summarizes *Drosophila* oogenesis (for details see Mahowald and Kambysellis, 1980; Spradling, 1993). An ovary is composed of a set of ovarioles containing both germ-line and somatic cells. A single ovariole is an egg assembly line; within each ovariole are a series of egg chambers at progressively more advanced stages of oogenesis. Fig. 1A depicts an idealized ovariole. Oogenesis begins at its anterior end within the germarium. At the anterior germarium tip, germ-line stem cells divide to produce both more stem cells and cystoblasts. Four cystoblast divisions with incomplete cytokinesis produce 16 interconnected cells: one cell becomes the oocyte while the other 15 are nurse cells, producing RNA, protein and other components for transport into the oocyte. At the posterior end of the germarium, each group of 16 germ cells is enclosed in an epithelial sheet of somatic follicle cells and pinched off, forming an egg chamber. Within each egg chamber, the oocyte is always in the posterior position, while the 15 nurse cells are at the anterior end. As egg chambers mature, they move distally through the ovariole, expanding dramatically in size. In addition, oocytes grow in size relative to nurse cells, which transport macromolecules into the oocyte through the connecting cytoplasmic bridges. This continues at a relatively uniform rate until late oogenesis when the remaining nurse cell contents are rapidly dumped into the oocyte and the nurse cells degenerate. At about the same time that 'dumping' begins, the follicle cell epithelium retracts from the nurse cells and a subset of follicle cells grows inward, separating the oocyte from the nurse cells. Another follicle cell subset, the border cells, migrate from the anterior end of the egg chamber to the anterior end of the oocyte.

Armadillo accumulation occurs in or near cell-cell adhesive junctions

In most cell types previously examined, Armadillo accumulates in a polarized fashion at the cell surface (Riggleman et al., 1990; Peifer and Wieschaus, 1990). In mid- to late-stage egg chambers, Armadillo accumulates on the surface of the somatic follicle cells in a polarized fashion, while being found more uniformly on the surface of the germ-line oocyte and nurse cells (Riggleman et al., 1990; Peifer and Wieschaus, 1990). We have used anti-Armadillo antibody (Riggleman et al., 1990) and confocal microscopy to extend this analysis, and have compared Armadillo localization with the position of cell-cell junctions as assayed by electron microscopy.

Within each egg chamber, the follicle cells form a fairly typical polarized epithelium surrounding the developing germ cells (see Mahowald and Kambysellis, 1980), with an external basal lamina and apical ends with microvilli that interdigitate with processes from the nurse cells and oocyte. The follicle cell epithelium provides a simple comparison to vertebrate epithelia. Vertebrate adherens junctions form at the apical end of the lateral surface where two epithelial cells are in contact. This is the region of the follicle cells where Armadillo accumulation is most pronounced (Figs 1,2).

Even within the germarium, at the earliest stages in follicle cell envelopment of germ cells, the localized pattern of Armadillo accumulation in follicle cells is apparent (Fig. 1B-C). In each follicle cell, Armadillo accumulates heavily on the lateral cell surface near the apical end abutting the germ cells while accumulating less heavily on the rest of the follicle cell-follicle cell interface. The heaviest Armadillo accumulation is in a band around the follicle cell near the

interface between the lateral and apical surfaces (e.g. Figs 1E, 2A), appearing as a dot in optical cross section. Armadillo localization was also examined in ovaries fixed via an alternate procedure developed to examine the distribution of cytoskeletal proteins (Miller et al., 1989). In ovaries (Fig. 2A,C,F,G) and embryos (unpublished data), this fixation procedure allows loosely bound Armadillo protein to wash away. What remains, presumably that most

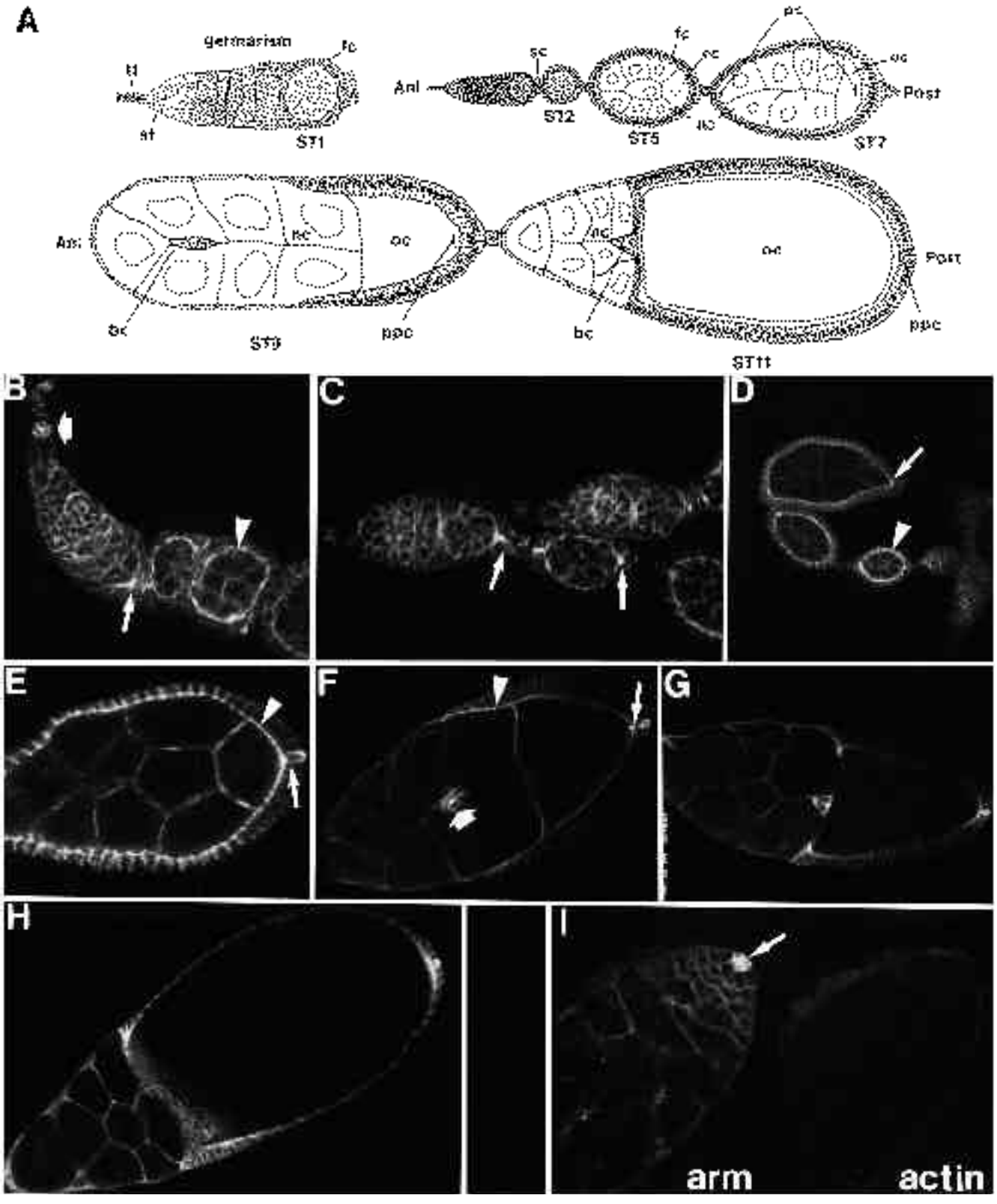

Fig. 1

tightly bound, is the Armadillo localized at the lateral/apical interface. The amount of Armadillo within follicle cells decreases slightly as development proceeds. The results of Western blots comparing wild-type ovaries with ovaries lacking Armadillo in the germ line (see below) suggest that the majority of Armadillo in the ovary is in the follicle cells (data not shown).

To examine coincidence between the position of junctions of the adherens-type morphology and Armadillo localization, electron microscopy was used to identify cell-cell adhesive junctions in follicle cells. At the boundary between the lateral and apical follicle cell surfaces, apparent cell-cell adhesive junctions are found that are morphologically similar to vertebrate adherens-type junctions (Fig. 2B). These junctions are present throughout follicle cell development and their position coincides well with Armadillo localization within follicle cells. Other types of cell-cell junctions (see Eichenberger-Glinz, 1979, for *Drosophila* junction types) such as gap junctions and septate junctions (that may correspond to vertebrate tight junctions) are also seen at different positions along the follicle cell-follicle cell interface.

Armadillo is also expressed in the germ-line nurse cells and oocyte. Armadillo accumulation in germ cells is first seen at the anterior tip of the germarium, staining quite intensely what may be stem cells (Fig. 1B). Similar intense staining is also seen at the anterior tip of the testis (Fig. 1I). Armadillo is expressed throughout the development of the cystocytes, and in the earliest nurse cells and oocytes. As in the follicle cells, Armadillo accumulates near or at the cell surface of germ cells but, in contrast to follicle cells, Armadillo in germ cells is not concentrated at any position along the cell-cell interface. In optical sections that graze the cell surface, Armadillo staining resolves into an irregular meshwork. In cross section, the meshwork appears as a punctate pattern along the entire cell interface, similar to accumulation along the lateral cell surface of follicle cells (Fig. 2C). Regions of intense staining are spaced at about 1 μm. This relatively non-localized expression of Armadillo on the germ-line cell surface is found throughout their development (Fig. 1D-H). Serial optical sections through late stage egg chambers suggest that protein accumulation is heaviest where the nurse cell-nurse cell junction abuts the overlying follicle cells. Armadillo also accumulates in the cortical region of the oocyte.

The germ-line nurse cells and oocyte do not form a traditional epithelium, but rather are connected at least in part by cytoplasmic bridges known as ring canals. These interruptions in the plasma membrane are strongly stained by phalloidin (see below), revealing their high actin content, but contain little if any Armadillo. When the nurse cell-oocyte complex is examined by electron microscopy, the cell membranes are very closely apposed along their lengths (Fig. 2D,E), except where interrupted by ring canals. There are few clearly defined junctions of any type in the germline. Adherens-type junctions with well-organized cytoplasmic complexes could not be identified. Areas along the cell-cell boundaries with some electron-dense material between the membranes, weakly resembling septate junctions but lacking the characteristic septa of these junctions, alternate with less distinct regions of membrane contact (Fig. 2E). Evidence below suggests that Armadillo is involved in cell-cell adhesion in germ cells, so its localization in germ cells may reflect the apparently more diffuse rather than localized regions of cell-cell contact. There are vertebrate cell types in which cadherin expression exhibits a similar diffuse distribution (Salomon et al., 1992), and β-catenin can be found all along the cell-cell junction in certain cultured cells (Peifer et al., 1992). Likewise a diffuse distribution of plakoglobin is seen in some vertebrate cell types (Franke et al., 1987).

Fig. 1. Armadillo localization during oogenesis. (A) Normal oogenesis (derived in part from Ruohola et al., 1991). A single ovariole is pictured. At its proximal end is the germarium (enlarged at top left), containing the somatic terminal filament cells (tf), and the germ-line stem cells (st). Cystocyte divisions produce 16 cells connected by cytoplasmic bridges (stage 1-ST1) - one cell becomes the oocyte (oc) and15 become nurse cells (nc). Nurse cell-oocyte clusters become surrounded by somatic follicle cells (fc), and pinch off from the germarium as egg chambers connected by stalk cells (sc). Egg chambers move distally, growing in size and maturing. The oocyte is always at the posterior (post) end. By stage 5 (ST5), the oocyte (distinguishable by distinctive nuclear morphology) is enlarged relative to nurse cells. Follicle cells at an egg chamber's anterior (ant) and posterior ends become specialized as polar follicle cells (pc). Anterior polar follicle cells (=border cells (bc)) migrate inward to oocyte's anterior end, while posterior polar follicle cells (ppc) undergo characteristic shape changes. From stages 7 (ST7) to 11 (ST11), the oocyte grows at the expense of the nurse cells. (B-H) Confocal microscope images of wild-type ovaries stained with antibodies to Armadillo. (B,C) Germarium and early egg chambers. Note intensely stained cells at the anterior tip of the germarium (fat arrow), intense staining at poles of earliest egg chambers (thin arrows) and polarization of staining within follicle cells (arrowhead). (D) Stages 3-6 egg chambers. (E) Stage 7 egg chamber. (F) Stage 9 egg chamber. Note in all strong polarization of Armadillo accumulation within the follicle cells, leading to intense Armadillo staining at interface between lateral and apical cell surfaces (arrowheads). Note also differential staining in both posterior polar follicle cells (thin arrows), and anterior polar follicle cells (fat arrow). (G) Stage 10 egg chamber. (H) Stage 11 egg chamber. (I) Confocal image of wild-type testis double labeled with antibody to Armadillo and with phalloidin to reveal actin. Note prominent accumulation of Armadillo in cells at the proximal tip of testis.

Armadillo protein accumulates differently in certain follicle cell subsets

Specific follicle cell subsets accumulate Armadillo in unusual patterns. Follicle cells at the anterior and posterior ends of each egg chamber show more intense Armadillo staining. These 'polar follicle cells' differ from their neighbors both in morphology and gene expression (Brower et al., 1981; Ruohola et al., 1991). Differences in Armadillo expression in polar follicle cells are visible in the germarium (Fig. 1B-C) and this difference continues throughout oogenesis (Fig. 1D-H). By stage 8, two to three cells at the oocyte's posterior end, the posterior polar follicle cells, accumulate Armadillo all along their lateral cell interfaces, with even heavier accumulation at their apical ends where they abut the oocyte (Figs 1F-H, 2G). When posterior polar follicle cells round up during late oogenesis, the differential staining becomes even more pronounced. The neighbors of

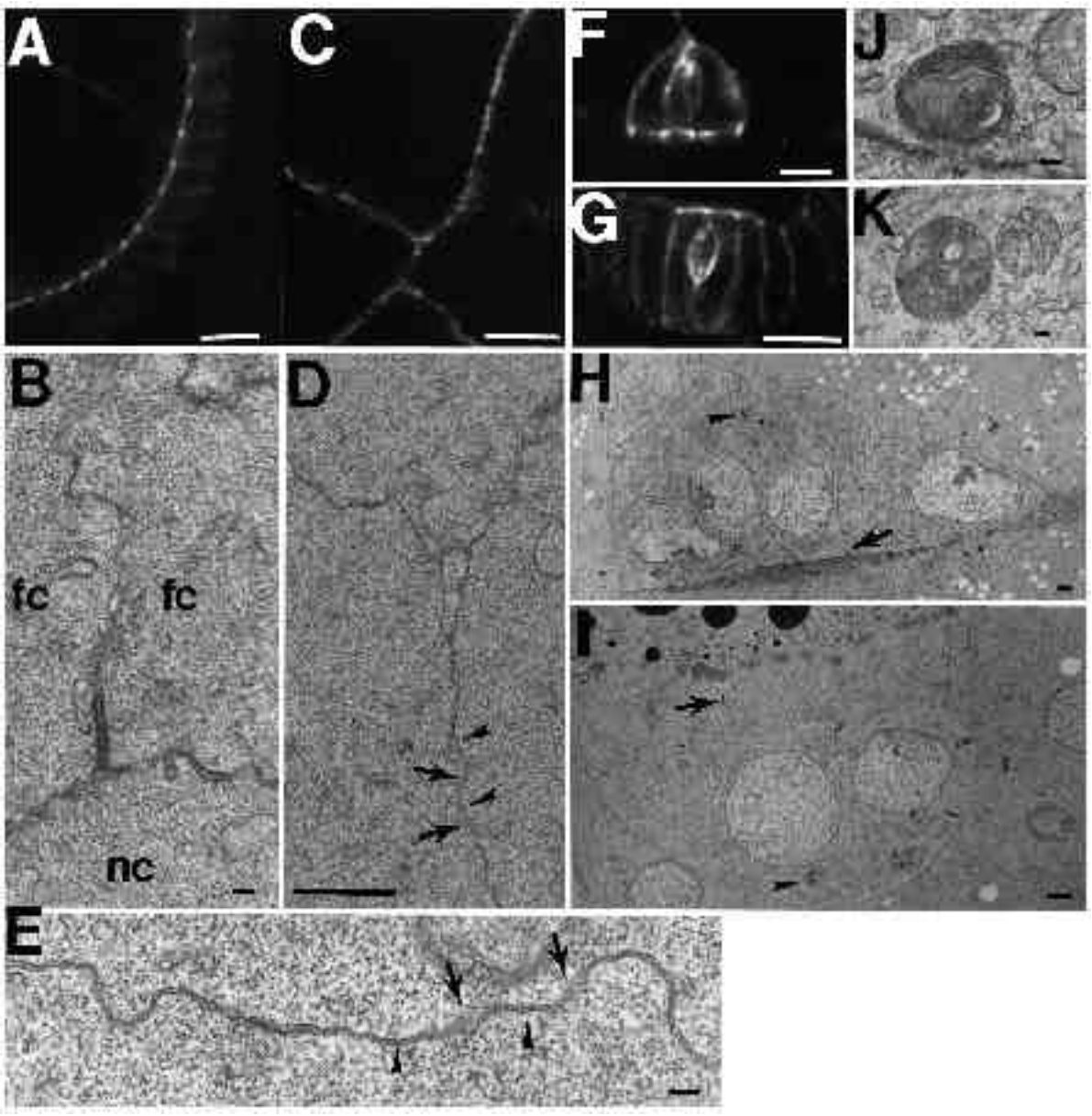

Fig. 2. Armadillo localizes to regions of cell-cell adhesion. Armadillo localization in wild-type ovaries, determined via antibody staining and confocal microscopy (A,C,F,G) was compared with cell-cell adhesive junctions, visualised via electron microscopy (B,D,E,H-K). This Figure displays heat-fixed tissue; heat-fixation preserves only the most tightly bound Armadillo. (A) Armadillo in follicle cells is highly enriched in a band surrounding the apical end of the lateral surface (appears as a dot in cross-section). (B) Close up of apical ends of two follicle cells (fc) abutting a nurse cell (nc) showing structure resembling an adherens junction. (C) In contrast, at germ cell-germ cell interfaces, Armadillo is distributed all along apposing surfaces, and is organized into a network that appears punctate in cross-section. (D,E; closeup). At germ cell-germ cell interfaces, cells are tightly apposed along their entire lengths, and there are no structures resembling adherens junctions. Instead, alternating regions of discrete (arrowheads) and fuzzy (arrows) membrane interfaces are seen. (F,G) Armadillo localization in the anterior (F) and posterior (G) polar follicle cells differs from that of the typical follicle cell. (F) When the anterior polar follicle cells reach oocyte's anterior end, Armadillo becomes highly concentrated in apical end of a cell's lateral surfaces, where it contacts oocyte (at bottom in this view). There is also prominent Armadillo staining along the lateral membranes of these cells where they contact each other. (G) Late in oogenesis, posterior polar follicle cells become round and retract from oocyte. Armadillo is enriched all along their surfaces. Neighboring follicle cells also accumulate more than typical amounts of Armadillo, both in adherens junctions and along the follicle cell-oocyte interface (at top in this view). (H,I) Electron micrographs of anterior (H) and posterior (I) polar follicle cells equivalent to those in F and G. Note structures resembling adherens junctions in some places where Armadillo staining is especially prominent (arrows). (J,K) Lammellar (J) and multi-vesicular bodies (K), structures that are greatly enriched in anterior and posterior polar follicle cells compared to their neighbors. Multi-vesicular bodies are also seen in H and I (arrowheads). Some regions of heavy Armadillo accumulation in F and G could represent staining of these multi-vesicular bodies. Scale bars indicate the following sizes: A,C,F and G, 10 μm. D,H,I, 1 μm. B,E,J,K, 0.1 μm.

posterior polar follicle cells also accumulate more Armadillo than average follicle cells (Figs 1H, 2G). Anterior polar follicle cells form at least a subset of the border cells that delaminate from the follicle cell epithelium during oogenesis stage 9 and migrate between the nurse cells to the oocyte's anterior end. During and after migration, they accumulate more Armadillo than other follicle cells (Figs 1F-G, 2F).

We examined the morphology of both anterior and posterior polar follicle cells at the electron microscopic level (Fig. 2H,I). While these two groups of cells share features with other follicle cells, both have peculiar morphological properties. They change shape from columnar to pyramidal to round, unlike their neighbors, which remain columnar or flatten. While all follicle cells contain many

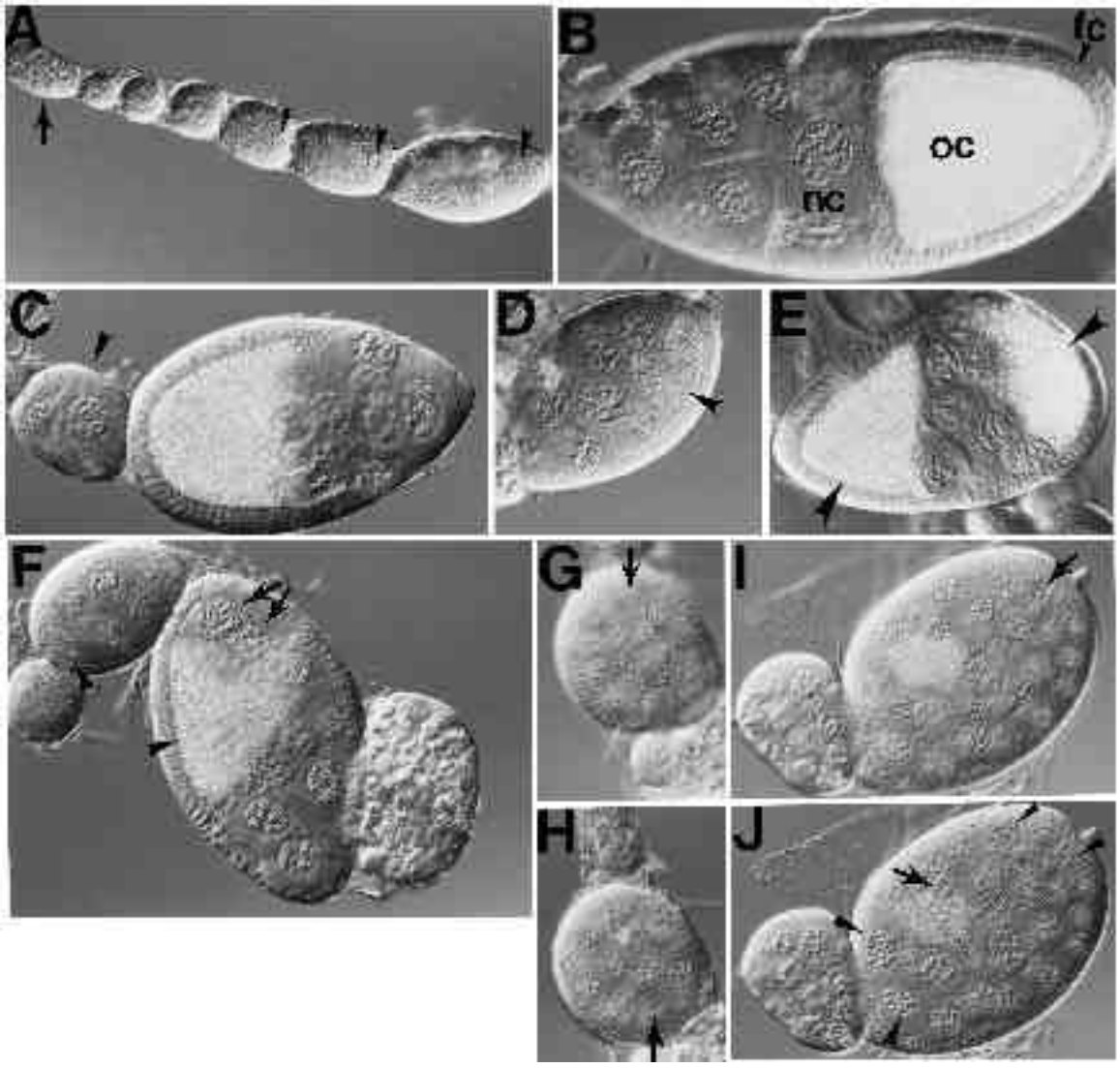

Fig. 3. Germ-line *arm* mutations disrupt cell shape and cell arrangement. Ovaries were dissected from wild-type or *arm* germ-line mutant females, fixed and examined by DIC microscopy. (A,B) Wild-type ovarioles contain a germarium (arrow in A) and egg chambers at increasingly later stages of development. An egg chamber= somatic follicle cells (fc) surrounding 15 nurse cells (nc) and an oocyte (oc). At all stages, the oocyte (arrowheads in A) is at posterior end of egg chamber. At late stages, each nurse cell is regular in shape and contains a single polyploid nucleus. The oocyte-nurse cell boundary is straight or nearly so. (C-J) Egg chambers in which germ line is mutant for arm^{YD35}. Oocyte is often displaced from its normal posterior location, nurse cells and oocyte are often very irregular in shape and some nurse cells seem to have fused, resulting in multinucleate nurse cells. At a low frequency, fused egg chambers are seen with two oocytes and about twice as many nurse cells as normal, often accompanied by small egg chambers with too few nurse cells. (C) At right is an egg chamber with anterior rather than posterior oocyte. Despite this, its morphology is relatively normal. Adjacent egg chamber has fewer nurse cells than normal (arrow). (D) Egg chamber with oocyte (arrowhead) in lateral position. (E) Oocyte (arrowheads) is a very abnormal shape, extending from posterior end to the anterior end of egg chamber. (F) Lateral or anterior oocyte (arrowheads). Note multinucleate nurse cell (arrows). (G,H) Egg chamber with two oocytes (arrows) and about twice as many nurse cells. (I,J) Another egg chamber with two oocytes (arrows) and extra nurse cells. This appears to result from fusion of two egg chambers - one oocyte and its associated nurse cells are at a later developmental stage than others as evidenced by differences in yolk deposition and size of nurse cell nuclei (arrowheads). Note adjacent egg chamber with fewer than normal nurse cells.

membrane-bound vesicles, a much larger proportion of the volume of the polar follicle cells is taken up by vesicles. Unlike other follicle cells, many of the vesicles in polar follicle cells are reminiscent of multivesicular bodies (Fig. 2K), while others, the lammellar bodies, contain tightly packed layers of membrane (Fig. 2J). While it is difficult to determine at this level of resolution, it is possible that some of the regions of the polar follicle cells that accumulate high levels of Armadillo could represent multivesicular bodies derived from the plasma membrane (e.g. compare Fig. 2G,I).

Gap, septate and adherens junctions are frequent in polar follicle cells. Posterior polar cells have adherens-type junctions at their apical ends abutting the oocyte, a region

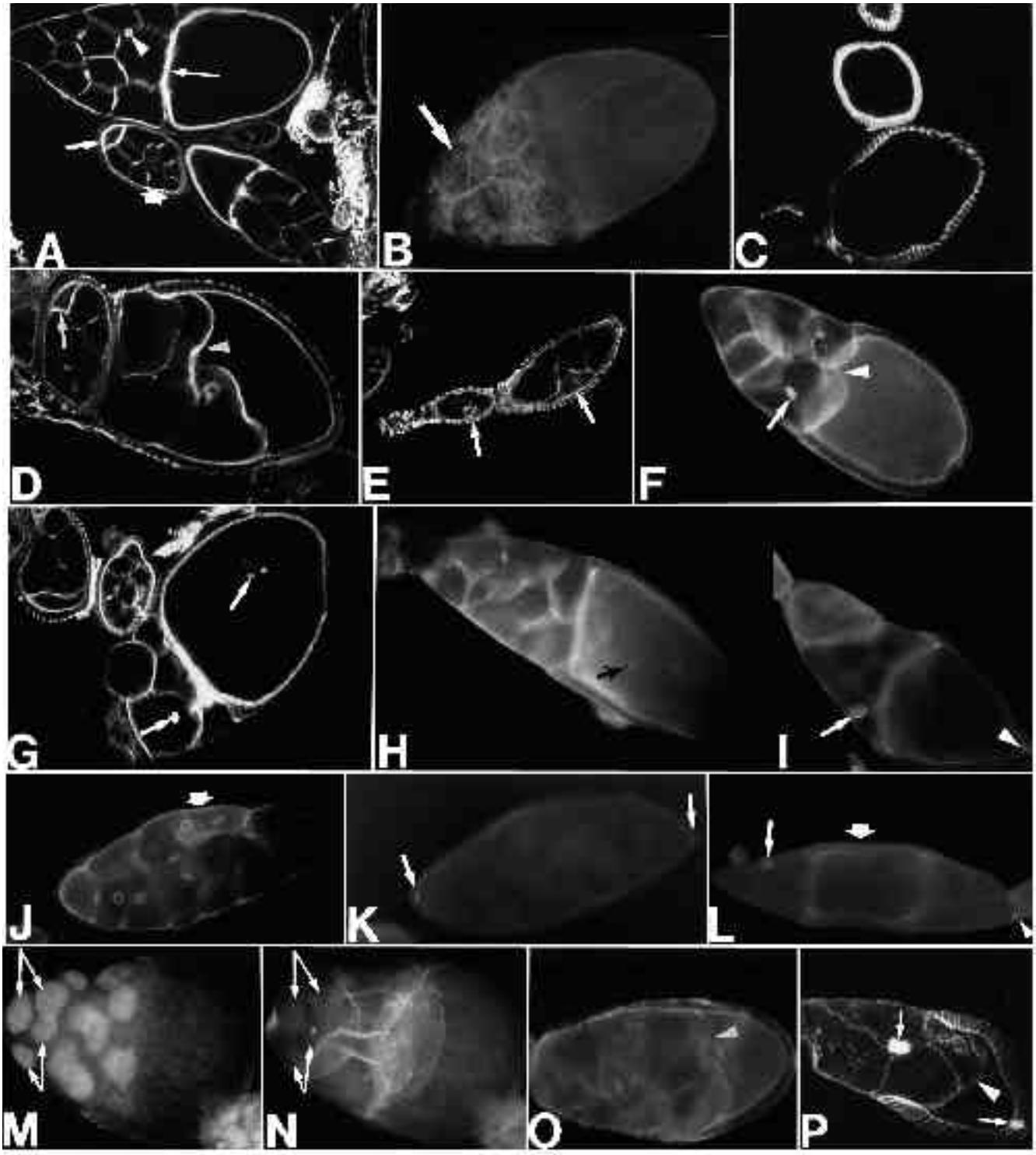

Fig. 4

of striking Armadillo accumulation (Fig. 2G,I). Border cells, however, do not have adherens junctions in this region, despite very intense Armadillo staining there. They do, however, have smaller electron-dense regions of the membrane in the position where adherens junctions would normally be found. Septate junctions are also common in this region (Fig. 2F,H). The lateral membranes of both types of polar follicle cells are electron dense in comparison to lateral membranes of typical follicle cells, and occasional small junctional structures can be discerned there. A higher density of these apparent smaller junctions may account for the difference in staining between polar cells and their neighbors.

arm is required to maintain germ-cell shape and architecture

Hypothesized roles of cell-cell adhesive junctions include mediating cell adhesion and anchoring the actin cytoskeleton, leading one to expect mutations in putative junctional components such as Armadillo to disrupt these processes. The ovary's germ cells are arranged in defined relationships to each other suggesting regulated intercellular adhesion and they have regular shapes that presumably require support of the cortical actin cytoskeleton. This cytoskeleton is also thought to be required for cell shape changes accompanying transfer of nurse cell contents to the oocyte. The ovary is therefore a reasonable tissue in which to test proposed roles of Armadillo and adhesive junctions.

arm is required in the germ-line cells for proper oogenesis (Wieschaus and Noell, 1986). When clones of *arm* mutant tissue are made in an otherwise arm^{+} animal, and such clones populate the germ line, mutant cells undergo the initial stages of oogenesis, but these germ cells produce only small eggs that are never laid. Several genes have this phenotype - they constitute the 'dumpless' class, in which the nurse cells fail to transfer their contents to the oocyte during the late stages of oogenesis (Schüpbach and Wieschaus, 1991). The dumpless phenotype has been proposed in some cases to be a result of alterations in the actin cytoskeleton (Cooley et al., 1992).

We examined ovaries in which the germ line is homozygous for *arm* mutations of various strengths. Germ-line clones were produced using the high-efficiency system of Golic (1991) and Chou and Perimmon (1992) that utilizes site-specific recombination catalyzed by the yeast *flp* recombinase. Chromosomes were constructed that have a site-specific recombination site for the *flp* recombinase at the base of the X-chromosome, and also carry either an *arm* mutation or the germ-line-dependent dominant female-sterile mutation $Fs(1)\ ovo^{D}$. The *flp* recombinase is expressed from another transgene under control of the heat-shock promoter. Crosses were done to produce flies heterozygous for the *arm* mutation on one X-chromosome and $Fs(1)\ ovo^{D}$ on the other X chromosome. Since *arm* is recessive, these flies are arm^{+} but they totally lack ovaries, due to the dominant female-sterile mutation. If these flies are heat shocked during larval development, the *flp* recombinase induces mitotic recombination, leading to clones of cells homozygous for *arm* and lacking the dominant female-sterile mutation. If such a clone is induced in the germ line, since it lacks the dominant female-sterile mutation, it can result in the production of an ovary, which also contain germ cells homozygous mutant for *arm* — any ovaries found in such a female thus lack Armadillo in the germ line. This system results in a high frequency of clones; if females are dissected after such a heat shock more than 70% have ovaries derived from *arm* mutant clones.

This allowed generation of ovaries that contain germ cells mutant for *arm*. Ovarian cellular morphology was examined using DIC optics and fluorescent probes for actin and DNA. All revealed profound alterations in cellular morphology in ovaries with *arm* mutant germ cells. The strongest mutations, arm^{YD35} and arm^{2a9} completely lack *arm* function (Peifer and Wieschaus, 1990). arm^{YD35} makes no detectable protein in embryos (Peifer and Wieschaus, 1990) or in arm^{YD35} mutant germ-line cells (Fig. 4C). arm^{+} somatic follicle cells stain normally while the staining of arm^{YD35} mutant nurse cells and oocyte is completely lost. Polarization of the follicle cell epithelium does not require *arm* function within the germ line.

Inspection of egg chambers derived from these and weaker mutants revealed a wide variety of defects (Fig. 3). In a wild-type ovariole, the oocyte always lies at the posterior end of the egg chamber (Fig. 3A,B). Wild-type nurse cells are quite regular in size and shape, and abut the

Fig. 4. *arm* mutations affect organization of the actin cytoskeleton. Egg chambers stained with fluorescently-labeled phalloidin to detect filamentous f-actin, or for immunofluorescence with anti-Armadillo antibodies. (A) Actin in wild-type egg chambers. The cortical actin cytoskeleton surrounds germ cells and accumulates at the apical surface of the follicle cells (fat arrow). Note ring canals (arrowhead) and the especially heavy actin accumulation around oocyte (thin arrows). (B) At stage 10, prominent cytoplasmic actin filaments appear (arrow), coincident with final transfer of nurse cell contents into egg. This occurs normally in ovaries like this with $arm^{H8.6}$ mutant germ cells. (C) Ovary derived from arm^{YD35} germ-line clone stained for Armadillo. arm^{+} follicle cells have normal Armadillo accumulation, while Armadillo expression in the germ line is abolished. (D,H) Actin in ovaries derived from *arm* mutant germ-line clones (arm^{YD35} — D,H; arm^{XK22} — E,F,G.) (D,E) Gross alterations in cell shape (arrowhead) and mis-positioning of oocytes (arrows). (F) Mild perturbation of nurse cell-oocyte border (arrowhead) and inclusion of actin (arrow) (G-H) Actin inclusions (white arrows) and free floating ring canals (black arrow). (I) Even in mutant egg chambers, polar follicle cells accumulate Armadillo in a different way from their neighbors (arrow and arrowhead). However, at times anterior polar follicle cells become lost during migration (arrow). (J-K) arm^{XK22} egg chamber, double-labeled to detect actin (J) or Armadillo (K). While oocyte is on the side of the egg chamber (fat arrow), the polar follicle cells remain in appropriate anterior and posterior positions and continue to accumulate more Armadillo than their neighbors (thin arrows). (L) arm^{YD35} egg chamber with oocyte in the middle. Posterior polar follicle cells are correctly located and behaving correctly (arrowhead), while border cells have migrated incorrectly (thin arrow). The other follicle cells retracted from nurse cells and remain columnar over oocyte (fat arrow). (M,N) arm^{YD35} egg chamber double-labeled to detect nuclei (M) and actin (N). Nuclei are clumped rather than separated by cortical actin cytoskeleton. (O,P) $chickadee^{WC57}$ mutant egg chambers, labeled to reveal actin (O) or Armadillo (P). Note disrupted nurse cell-oocyte boundaries (arrowheads) and the appropriate localization of Armadillo in the polar follicle cells (arrows).

oocyte at a relatively straight boundary, especially during late stages. These invariant features of the wild-type egg chamber are grossly altered in *arm* mutants. The oocyte's position within the egg chamber is no longer fixed in *arm* mutants. In a mild mutant phenotype, nurse cells protrude into the oocyte. In a more extreme manifestation, the oocyte is displaced from its posterior position and may be found on the side or even at the anterior end of the egg chamber (Fig. 3C-F). Alteration in oocyte position can occur quite early during development. There are also striking changes in the shape of both nurse cells and oocyte. The oocyte can assume an extremely irregular shape, extending from one end of the egg chamber to the other (Fig. 3E), and nurse cells become quite irregular in shape and size (Fig. 3C-F). The nurse cell nuclei, which are normally positioned roughly in the center of each cell, seem to become untethered and often end up in apparently adjacent clusters (Fig. 3F).

It is worth noting, however, that despite dramatic defects, many aspects of oocyte development occur relatively normally. Even when the oocyte is mis-positioned, it enlarges relative to the nurse cells, and follicle cells retract from nurse cells and become columnar atop the oocyte (Fig. 3C). Development continues to the point at which rapid dumping of nurse cell contents into the oocyte would begin. This process is blocked in both strong and moderate mutations, resulting in the dumpless phenotype previously observed. Very late stage egg chambers have small eggs abutting residual nurse cells, long after the stage when nurse cells degenerate in wild-type ovaries. Females mutant for the moderate allele arm^{XP33} do lay eggs. Many of these eggs are flaccid, however, perhaps because residual nurse cells prevent proper closure of the egg shell at the anterior end.

Differences in phenotypic severity between alleles precisely paralleled their severity in embryos (Peifer and Wieschaus, 1990). At one end of the spectrum, when germ cells are homozygous for the null mutation arm^{YD35}, 49% of egg chambers had a misplaced oocyte (n=397). In contrast, when germ cells are homozygous for the weakest mutation $arm^{H8.6}$, oogenesis is normal, but reduction in maternal contribution of *arm* to the egg enhances the embryonic phenotype (Klingensmith et al., 1989; Peifer et al., 1991). One other phenotype, egg chambers with abnormal numbers of germ cells (Fig. 3G-J), is seen in the most severe genotypes, but at a lower frequency (1-5% of egg chambers). Some egg chambers have excessive numbers of germ cells. While precise counts were not done, these egg chambers contain about twice the normal number of nurse cells and also contain two oocytes. Often, these chambers are found adjacent to egg chambers with too few germ cells. These egg chambers likely result from the fusion of two oocyte-nurse cell complexes, as they generally contain two oocyte-nurse cell complexes at different stages of development. This may result from errors in follicle cell packaging of nurse cell-oocyte complexes into egg chambers.

arm mutations disrupt the actin cytoskeleton

Vertebrate adherens-type junctions are thought to anchor the actin cytoskeleton. To examine the cytoskeleton, rhodamine-labeled phalloidin was used to reveal filamentous actin. During wild-type oogenesis, actin is abundant in the cortical region of the germ cells (Fig. 4A) and is particularly abundant at the nurse cell-oocyte boundary, allowing identification of the oocyte quite early in oogenesis. Late in oogenesis, during the rapid phase of transfer of nurse cell contents to the oocyte, a dramatic network of cytoplasmic actin filaments can be observed in nurse cells (Fig. 4B). The most prominent actin structures in germ cells are the ring canals (Fig. 4A), cytoplasmic bridges between nurse cells and between nurse cells and oocyte.

The cortical actin cytoskeleton, cytoplasmic actin filaments and ring canals are all disrupted by *arm* mutations. Phalloidin-labeling reiterates the defects seen in DIC optics. Egg chambers have misplaced oocytes and abnormally shaped cells (Fig. 4). However, phalloidin-labeling also reveals two other defects directly affecting the actin cytoskeleton. In severe mutations, nuclei are mispositioned within nurse cells (Fig. 4M,N), forming clumps that are not separated by cortical actin staining. This may result from cell membrane breakdown between nurse cells, or the remaining membrane may be depleted of actin. Severe mutations also have a second defect in actin accumulation. Large aggregates of actin are seen within nurse cells and less frequently within the oocyte (Fig. 4F,G). Some aggregates may be derived from degenerating ring canals (Fig. 4H).

As other genes have a superficially similar 'dumpless' phenotype, we compared the best characterized of these, *chickadee*, to *arm*. $chickadee^{WC57}$ mutants are similar in phenotype to moderate and weak *arm* mutants (Cooley et al., 1992; Fig. 4O-P) showing occasional minor oocyte displacement (Fig. 4O), breakdown in the nurse cell/oocyte boundary and occasional apparent nurse cell fusion. However, the oocyte usually remains at the distal end of the egg chamber and actin aggregates are not seen. Armadillo staining in *chickadee* mutant ovaries is roughly normal (Fig. 4P).

Potential interaction between the oocyte and the polar follicle cells

Oocyte displacement permitted examination of the relationship between the oocyte and follicle cells that normally overlie it. Of particular interest are the polar follicle cells, normally abutting the oocyte's anterior and posterior ends. Communication between polar follicle cells and the oocyte may be necessary for each to assemble specialized structures that anchor critical molecules involved in determination of the embryo's anterior and posterior ends. By combining phalloidin staining, to reveal the oocyte's position by its increased actin accumulation, and Armadillo staining, to reveal the polar follicle cells, we were able to ask if alteration in oocyte position alters the position or Armadillo staining pattern of polar follicle cells. Egg chambers mutant for the strong mutations arm^{YD35} and arm^{XK22} were examined. In most cases, posterior polar follicle cells remained at the posterior end of the egg chamber, despite oocyte displacement (Fig. 4K-L). Occasionally, both groups of polar follicle cells migrated toward the oocyte, as border cells normally do. While in many mutant egg chambers the border cells complete their normal migration through the nurse cell cluster to the anterior end of the oocyte, in some egg chambers border cell migration is perturbed, resulting in border cells at the egg chamber periphery (Fig. 4I,L).

The effects of germ-line *arm* mutations on ovarian anterior-posterior polarity

arm plays an important role in setting up anterior-posterior pattern within each segment during embryogenesis and adult pattern formation. We examined whether *arm* is required for anterior-posterior patterning during oogenesis. The embryo has a detailed anterior-posterior and dorsal-ventral pattern initiated by maternal cues. If fertilized eggs were laid by females carrying germ-line clones of particular *arm* mutations, the resulting embryos were examined to

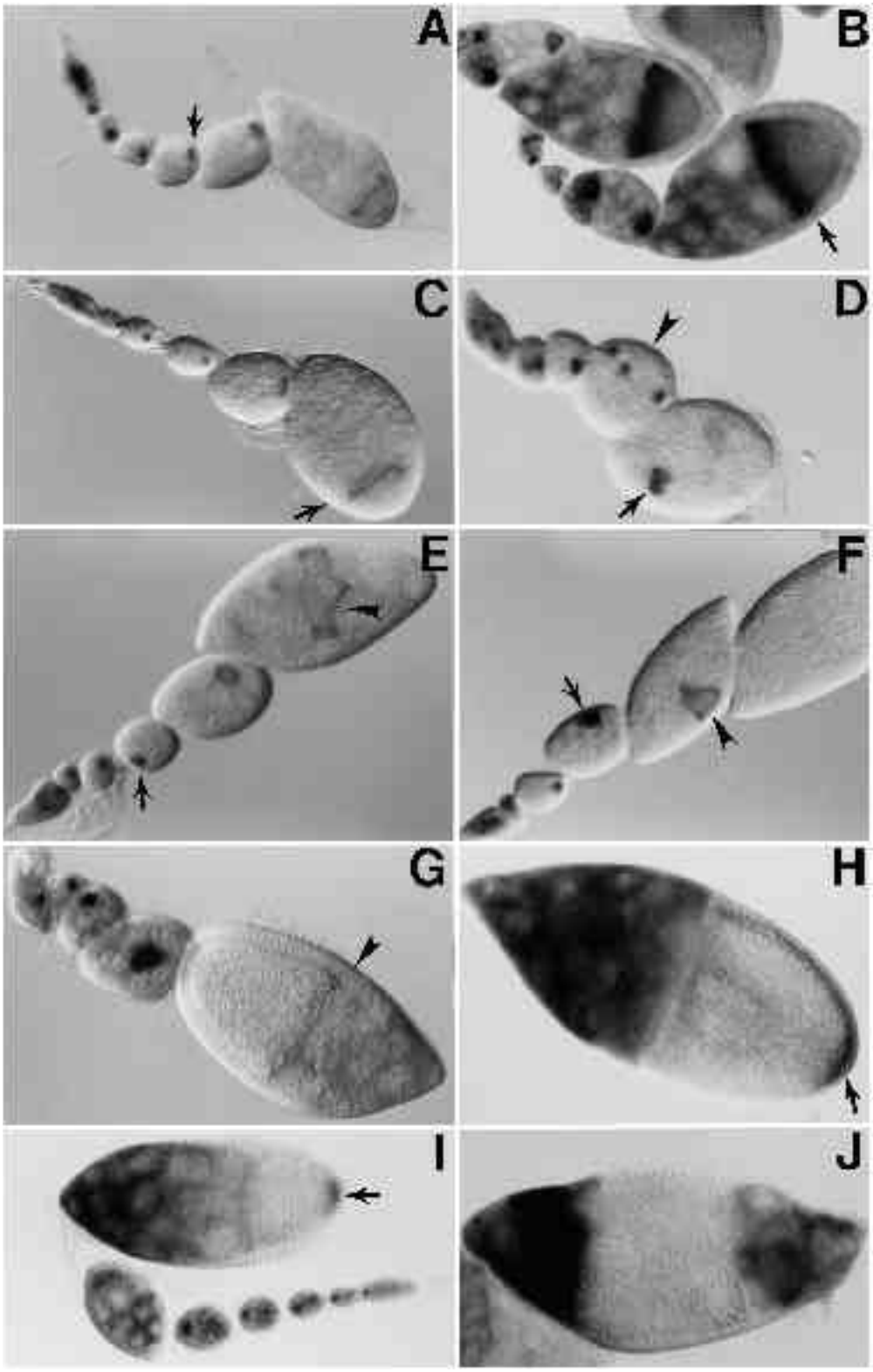

Fig. 5. Anterior-posterior polarity in ovaries with *arm* mutant germ cells. Whole-mount ovary preparations hybridized with probes to detect *orb* (A-G) or *oskar* (H-J) RNAs. (A) In wild-type egg chambers, *orb* RNA is first localized to posterior end of oocyte (e.g. arrow). (B) $arm^{H8.6}$ germ-line clone, second phase of *orb* RNA accumulation (re-localization to oocyte's anterior end) occurs correctly in this weak allele. (C-G) arm^{YD35} germ-line clones. *orb* localization can be relatively normal (C), although oocyte is often mis-localized (arrows in D-F). Egg chamber with more than one oocyte (arrowhead in D). Arrowheads in E and F show abnormal *orb* localization seen when oocyte is in middle of egg chamber. (G) An oocyte localized at anterior end, opposite the normal localization, with relatively normal, though inverted pattern of *orb* accumulation (arrowhead). (H) Wild-type accumulation of *oskar* RNA. (I,J) *oskar* in arm^{YD35} mutant egg chambers. (I) *oskar* RNA localization is relatively normal when the oocyte is appropriately positioned. (J) When oocyte is in middle, *oskar* RNA accumulates in both sets of nurse cells but is not localized within oocyte.

determine whether pattern was appropriately established. The weak/moderate alleles $arm^{H8.6}$, arm^{25B} and arm^{XP33} all lay eggs, and many of these eggs begin development (some arm^{XP33} eggs are flaccid). At least some of the eggs fertilized by a sperm carrying the arm^{+} gene complete development normally and live to be fertile adults. Thus, in weak alleles, there are no irreversible defects in polarity. Embryos that do not receive an arm^{+} gene from their father have an extreme segment polarity phenotype (similar to that of $arm^{H8.6}$— Klingensmith et al., 1989; Peifer et al., 1991), but do not seem to have any other defects in anterior-posterior or dorsal-ventral polarity.

Other features of anterior-posterior polarity are revealed by egg morphology, as the eggshell has anterior-posterior and dorsal-ventral asymmetries. Females with germ lines homozygous for extreme *arm* alleles do not lay eggs. The eggs in their ovaries are small and retain residual nurse cells at their anterior end. These eggs are, however, surrounded by eggshells with normally positioned dorsal/anterior chorion appendages and with a micropyle at the anterior end, suggesting that at least some aspects of anterior-posterior and dorsal-ventral polarity are retained. This relatively normal appearance of the eggs is somewhat surprising in view of the severe disruptions in cell shape and arrangement of germ cells documented above. It may be that egg chambers with the most severe defects abort during the oogenesis process; degenerating egg chambers are seen in these mutants.

To examine anterior-posterior patterning earlier in oogenesis, molecular probes revealing molecules with anterior-posterior asymmetries in localization within the egg chamber were used. We focused on RNAs encoded by the *orb* and *oskar* genes; their distribution in wild-type and germ-line mutant ovaries was visualized by in situ hybridization (Tautz and Pfeifle, 1989). Wild-type patterns of accumulation of these RNAs (*orb* — Lantz et al., 1992; *oskar* — Ephrussi et al., 1991 and Kim-Ha et al., 1991) are briefly reviewed in Fig. 5. *orb* RNA distribution during oogenesis can be divided into three phases. During stages 1-7, *orb* RNA is concentrated in the oocyte where it accumulates preferentially towards the posterior end (Fig. 5A). In stages 8-10, the pattern of *orb* RNA localization changes dramatically; *orb* RNA accumulates near the oocyte's anterior end, at the border with the nurse cells (Fig. 5B). In stages 10-14, *orb* RNA is strongly detected in nurse cells while weak hybridization is detected throughout the oocyte. *oskar* RNA is localized specifically to the oocyte (Fig. 5I) and, by stage 8, becomes localized to the oocyte's posterior pole (Fig. 5H).

In weak *arm* mutants ($arm^{H8.6}$) that complete oogenesis normally, *orb* RNA localization is normal (Fig. 5B). Early events of *orb* localization are also relatively normal in ovaries with germ cells mutant for strong alleles (e.g. arm^{YD35}). *orb* RNA becomes localized to a single cell that we presume is the oocyte (Fig. 5C-G), but at times this cell is mis-positioned within the egg chamber (Fig. 5D). Mislocalization occurs as early as stage 3 or 4. In egg chambers with arm^{YD35} mutantgerm cells that do not show oocyte mislocalization, *orb* RNA localization is essentially the same as in wild-type (Fig. 5C). However, if the oocyte is mis-posi-

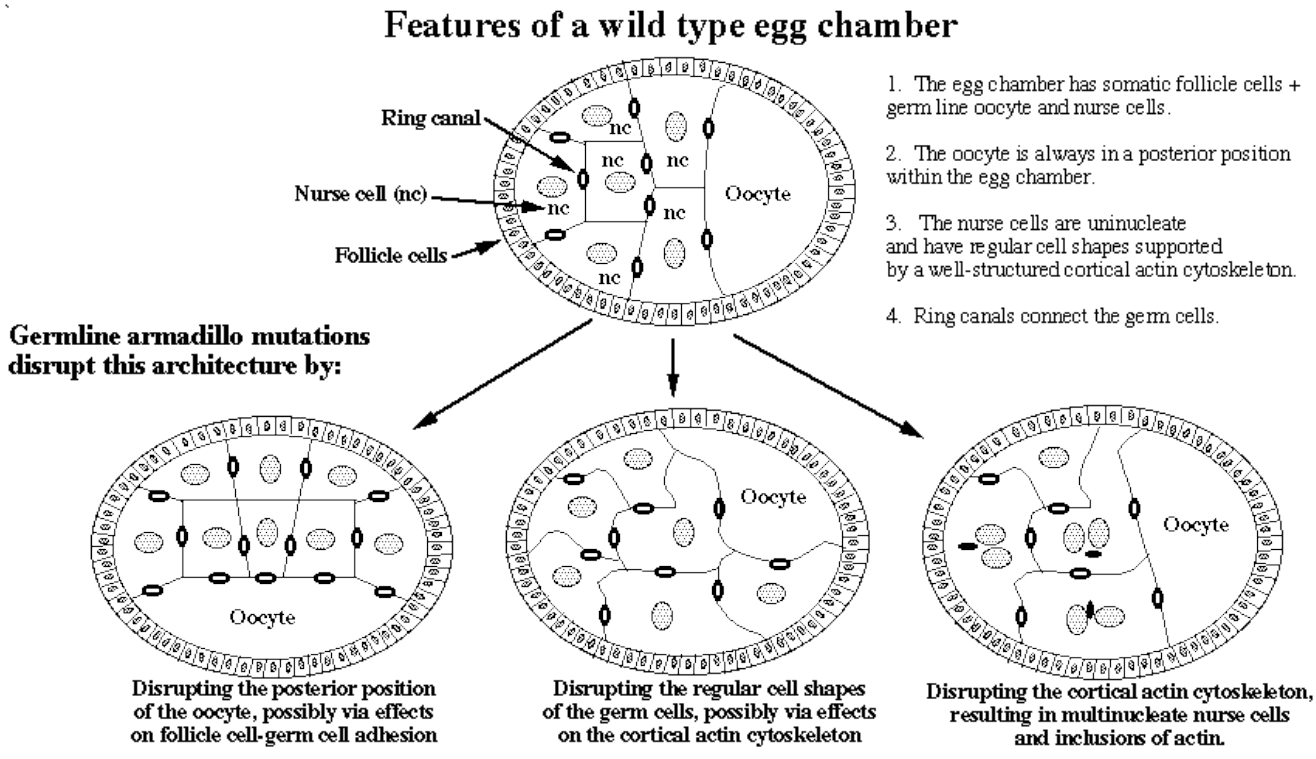

Fig. 6. The effects of *arm* mutations on oogenesis can be explained by a role for Armadillo in cell-cell adhesive junctions similar to vertebrate adherens junctions.

tioned to the middle of the egg chamber, later stages of *orb* RNA localization are abnormal. By stage 8, when *orb* RNA would normally be re-localized to the nurse cell/oocyte border, this change does not occur. Instead, *orb* RNA is spread out around the oocyte circumference, or it accumulates seemingly randomly at one or two edges of the oocyte (Fig. 5E, F). In rare cases where the oocyte is at the opposite end of the egg chamber from its normal location, *orb* RNA hybridization is relatively normal, but its anterior-posterior polarity is reversed (Fig. 5G). *arm* mutations have similar effects on *oskar* RNA localization. *oskar* localization appears normal in weak mutations. In egg chambers with arm^{YD35} mutant germ cells, *oskar* RNA localization is relatively normal when the oocyte is at the posterior pole of the egg chamber (Fig. 5I). In egg chambers with the oocyte in the middle, however, re-localization of *oskar* RNA to the posterior pole of the oocyte does not occur. *oskar* RNA instead randomly accumulates around the oocyte circumference. In stage 10 egg chambers with the oocyte in the middle of the egg chamber, *oskar* RNA accumulates in nurse cells at the both sides of the oocyte (Fig. 5J).

DISCUSSION

The *Drosophila* segment polarity genes encode components of cell-cell signaling systems required to establish cell fate within embryonic segments (reviewed by Ingham and Martinez-Arias, 1992; Peifer and Bejsovec, 1992). *wingless* encodes a key cell-cell signal and segment polarity genes like *armadillo (arm)* that share the *wingless* phenotype may encode components of its signal transduction pathway. Given this hypothesis, Armadillo's molecular identity was surprising. Armadillo is the homolog of the vertebrate adhesive junction proteins β-catenin and plakoglobin (Peifer and Wieschaus, 1990; McCrea et al., 1991), suggesting that Armadillo may play a role in adhesive junctions in *Drosophila*, and that such junctions might be important for the transmission of intercellular signals.

Adherens junctions have been proposed to help assemble and maintain epithelial cell sheets by mediating cell adhesion, communication and cytoskeletal integration. Adherens junction multi-protein complexes are organized around cell adhesion molecules of the cadherin family (reviewed in Magee and Buxton, 1991). Cadherin extracellular domains interact to mediate adhesion (reviewed by Takeichi, 1991) while their intracellular domains organize α-, β-, and γ-catenins into a protein complex (reviewed by Kemler and Ozawa, 1989). α-catenin is a vinculin relative (Nagafuchi et al., 1991; Herrenknecht et al., 1991) and β-catenin an Armadillo homolog (McCrea et al., 1991); γ-catenin is unidentified, but may be plakoglobin, the other Armadillo homolog (Peifer et al., 1992; Knudsen and Wheelock, 1992). The cadherin-catenin complex is thought to mediate signaling and anchoring of the actin cytoskeleton.

Armadillo is localized to cell-cell junctions in the *Drosophila* ovary

To test the hypothesis that Armadillo plays a role in cell-cell junctions and to assess the function of these junctions within a developing animal, the expression and function of Armadillo in the *Drosophila* ovary was examined. Armadillo has properties consistent with a role in cell-cell adhesive junctions. In many cell types, Armadillo is enriched in the region of the membrane (Riggleman et al., 1990; Peifer and Wieschaus, 1990). Its distribution in the follicle cell epithelium is especially instructive. Armadillo is highly enriched at the junction between the apical and lateral surfaces of the follicle cells, in apparently discrete structures (Fig. 1D,E). Armadillo in these structures is tightly bound, as this distribution pattern is exaggerated by fixing ovaries by an alternate procedure that washes away weakly bound Armadillo (Fig. 2A). In the location where Armadillo distribution is enriched, there are structures highly reminiscent in morphology to vertebrate adherens junctions (Fig. 2B). These results strongly support a junctional localization of Armadillo in the follicle cell epithelium and are similar to results obtained in other tissues (Peifer, 1993).

Armadillo distribution in germ cells differs from that in follicle cells. In germ cells, Armadillo is more uniformly distributed along the cell-cell interface. An inspection of cell-cell boundaries by electron microscopy revealed that germ cells lack well-defined adherens junctions. In this cell type, we suspect that functions provided by adherens junctions in epithelia are served instead by more diffusely distributed adhesive junction complexes. In fact, while cadherins are enriched in adherens junctions in many vertebrate cell types, they are also found all along the cell-cell boundary. There are cell types in which cadherins are located diffusely along the entire cell surface (e.g. Salomon et al., 1992). β-catenin can have a similar, more diffuse localization in some cell types (Peifer et al., 1992). It seems likely that these more diffusely organized cell-cell junctions are similar in function to adherens junctions; as we discuss below, the experiments described here suggest that Armadillo may mediate cell adhesion and cytoskeletal anchoring in germ cells lacking well-defined adherens junctions.

arm mutations appear to disrupt cell adhesion and the actin cytoskeleton

Cell biologists have begun to test proposed functions of vertebrate adherens junction proteins. In tissue culture cells, both cadherin and α-catenin are required for adhesion and other proposed adherens junctions functions (Nagafuchi et al., 1987; Nose et al., 1988; Nagafuchi and Takeichi, 1989; McNeill et al., 1990; Ozawa et al., 1990; Hirano et al., 1992), supporting the hypothesis that adherens junctions mediate formation of an epithelium. β-catenin's role remains untested, however, and these approaches cannot test the role of junctions in the development of organs and organisms.

Study of the *Drosophila arm* gene supplements these cell biological studies, providing a genetic approach to function of the Armadillo/β-catenin/plakoglobin family and to the role of adhesive junctions during development. *arm* is critical for cells to receive and interpret *wingless* intercellular signal (Peifer et al., 1991). This connection between adhesive junctions and *wingless* signaling was unexpected, but these experiments revealed an even more surprising

finding, namely that mutations in the apparent homolog of a key junctional component had no dramatic effects on cell adhesion or on organization of the cytoskeleton. The failure of null *arm* mutations to affect these properties likely has a simple explanation. The mother puts a large store of *arm* into the egg, and thus even embryos homozygous for null *arm* mutations do not totally lack Armadillo (Riggleman et al., 1990). The embryonic *arm* phenotype, disruption of *wingless* signaling, results from reduction in functional *arm* rather than its absence. Adhesive and cytoskeletal functions of Armadillo-containing junctions may be less sensitive to reductions in *arm* function. Analysis of *arm* function during adult development supports this possibility; while *arm* is required for *wingless* signaling in certain cells, it is also required more generally in all cells to mediate some other cellular property (Peifer et al., 1991).

To assess this hypothesis required examination of the consequences of eliminating *arm* function. In *Drosophila*, one can remove the maternal contribution of a gene product by making a mosaic female in which the germ line is homozygous mutant. Reduction of *arm* maternal contribution (by making the germ line homozygous for weak *arm* alleles) increases the severity of disruption of *wingless* signaling in the progeny (Klingensmith et al., 1989; Peifer et al., 1991; these results). Eliminating maternal *arm* disrupts oogenesis (Wieschaus and Noell, 1986). While this prevents examination of embryonic effects of complete removal of *arm* function, it provides the possibility of examining ovaries with germ cells lacking *arm* function. As *wingless* is not required in the germ line for oogenesis (Baker, 1988), one can examine Armadillo's general cellular functions without complicating effects on *wingless*.

Ovaries with homozygous mutant germ cells show a complex set of defects, many if not all of which can be explained by a model in which removal of *arm* disrupts function of cell-cell adhesive junctions (summarized in Fig. 6). An egg chamber provides an excellent model to study both cell interactions and integrity of the cytoskeleton. It contains 15 nurse cells and an oocyte in intimate contact with each other, joined by cytoplasmic bridges. These germline cells are surrounded by and interact with an epithelial layer of somatic follicle cells, and the entire package is enclosed in a muscular tube that propels developing egg chambers toward the oviduct. This tube exerts substantial force and, in the face of this constriction, egg chambers presumably retain their normal morphology via connections between their constituent cells and via the supporting actin cytoskeleton within each cell.

One function that cell-cell adhesive junctions must mediate is adhesion. This function seems to be disrupted by mutations in *arm.* While the oocyte and nurse cells are mutant for *arm* in the germ-line clones, the somatic follicle cells are wild-type; follicle cell-follicle cell interactions appear unaffected. The most striking defect seen in *arm* mutant egg chambers — the mis-positioning of the normally posteriorly located oocyte — is accommodated by a model in which the normal arrangement of the germ cells within the follicular epithelium is maintained via cell-cell contacts between germ and follicle cells. This cell adhesion is apparently disrupted by *arm* mutations. Alterations in normal interactions between germ and follicle cells may also explain the occasional mis-packaging of fewer or more than the normal complement of germ cells within a single follicle cell epithelium (Fig. 3G-J) and also may result in the frequent mistakes made by border cells as they attempt to migrate between *arm* mutant nurse cells toward the anterior end of the oocyte (Fig. 4I,L).

Another function ascribed to cell-cell junctions, particularly of the adherens type, is to anchor the actin cytoskeleton. The alterations in cell shape and possible breakdown of nurse cell membranes in mutant egg chambers may be explained by disruption of the cortical actin cytoskeleton (Fig. 4), which likely functions in part to allow cells to resist pressures exerted upon them by muscular contractions. Breakdown of the actin cytoskeleton is not entirely hypothetical - in mutant egg chambers large inclusions of actin can be seen. In addition, the actin-containing ring canals sometimes become detached from the membrane and cortical actin cytoskeleton, and float free in the cytoplasm.

The terminal phenotype of *arm* germ-line mutations, production of small eggs, is also explained by this model. It has been proposed that the final phase of 'dumping' nurse cell contents into the egg requires the actin cytoskeleton. *arm* is one of several 'dumpless' mutants (Schüpbach and Wieschaus, 1991). Another, *chickadee*, encodes the *Drosophila* profilin homolog (Cooley et al., 1992). While *chickadee* has a less severe phenotype than *arm* (Fig. 4O,P), all *chickadee* alleles examined reduce rather than eliminate profilin function (Cooley et al., 1992). Other 'dumpless' mutants might also encode cytoskeletal components or the machinery that anchors it to the membrane.

Armadillo is an adherens junction component

The sequence similarity between Armadillo and two different vertebrate adhesive junction components, plakoglobin and β-catenin, raised the question of which is the 'true' homolog of Armadillo. A three-way sequence comparison does not provide a definitive answer. While Armadillo is significantly more similar to β-catenin (71%) than to plakoglobin (63%), particularly in the N-terminal domain (Peifer et al., 1992), this difference alone is not great enough to implicate Armadillo as a component of adherens-type rather than desmosomal junctions in *Drosophila.*

In the vertebrate, adherens junctions and desmosomes differ both in their molecular components and in their functions; adherens junctions anchor the actin cytoskeleton, while desmosomes anchor the intermediate filament cytoskeleton. The data presented here provide strong circumstantial evidence that Armadillo is enriched in adherens-type junctions and required for maintenance of the actin cytoskeleton. These data, together with evidence that Armadillo often co-localizes with actin (Riggleman et al., 1990; Peifer and Wieschaus, 1990), support a model in which Armadillo-containing junctions anchor the actin cytoskeleton, as adherens junctions do in vertebrates. This model is further substantiated by experiments demonstrating that Armadillo is part of a multi-protein complex similar to the vertebrate adherens junction (Peifer, 1993). This complex includes Armadillo, a 150 kD glycoprotein, and the *Drosophila* homolog of alpha-catenin (Peifer, 1993; Oda et al., 1993). Armadillo is thus a component of an adherens-like junction in *Drosophila*. However, the existence of both β-

catenin and plakoglobin in the vertebrate adherens junction complex (Cowin et al., 1986; Peifer et al., 1992; Knudsen and Wheelock, 1992) complicates the picture, rendering it difficult to conclude definitively which molecule, if either, is Armadillo's 'true' homolog. The existence of cytoplasmic intermediate filaments in *Drosophila*, and thus of junctions mediating their anchorage, remains an area of some disagreement. Thus, a distinct *Drosophila* plakoglobin homolog may exist, or desmosomes may not occur in *Drosophila*.

Cell-cell interactions and patterning during oogenesis

The ovary and eggs produced by it have genetically determined anterior-posterior and dorsal-ventral patterns (Nüsslein-Volhard et al., 1987; Manseau and Schüpbach, 1989). *arm* mutations dramatically affect pattern formation during embryonic and adult development, effects that seem specific for *wingless* intercellular signaling, not affecting other cell-cell signaling processes. *wingless* is not required in the germ line for patterning during oogenesis (Baker, 1988) and it was of interest to see if mutations in *arm* result in defects in patterning during oogenesis, independent of the *wingless* system.

To assess *arm*'s potential role in pattern formation during oogenesis, we assayed effects of germ-line *arm* mutations on patterning the embryo and eggshell. In addition, even if *arm* mutations do not directly affect patterning during oogenesis, they result in rearrangement of the ovary's stereotyped architecture, allowing one to ask questions about the role of certain cell juxtapositions in patterning. Cell-cell signaling between follicle cells and oocyte is important for patterning egg and embryo (e.g. Schüpbach, 1987; Stevens et al., 1990). Strong *arm* mutations disrupt normal positioning of the oocyte within the follicle cell epithelium and thus alter these interactions. To assess subtle effects of cellular rearrangements on patterning, we used molecular markers, *orb* and *oskar* RNAs, which are asymmetrically distributed in the egg, and examined effects of rearrangements in cell positions on the polar follicle cells normally positioned at the egg chamber's anterior and posterior ends.

While even the weakest *arm* mutations disrupt embryonic and adult pattern (Peifer et al., 1991), germ-line clones of weak and moderate *arm* mutations have no noticeable effects on egg pattern. Reductions in maternal *arm* contribution lead to more severe embryonic segment polarity defects, but do not cause defects in other patterning systems active in oogenesis or embryogenesis. While ovaries with germ cells mutant for null alleles like arm^{YD35} do not produce fertilizable eggs, the eggs that they produce have a normal anterior-posterior and dorsal-ventral pattern in their eggshell. Egg chambers mutant for null mutations of *arm* can produce a quite normal pattern. If the oocyte in mutant egg chambers is positioned at approximately its appropriate posterior position, then *orb* and *oskar* RNAs are localized normally and the posterior and anterior polar follicle cells are in their normal positions and have their normal phenotypes. These results support a hypothesis in which Armadillo plays no direct role in patterning during oogenesis; the defects discussed below are more likely due to indirect effects of rearrangements in normal intercellular architecture.

Germ-cell differentiation into 15 nurse cells and one oocyte is unaffected by *arm* mutations, as is initial specification of anterior and posterior polar follicle cells. The first disruptions are seen at stage 3 or 4 when oocytes in some egg chambers are not at the posterior end. The mechanism of oocyte differentiation remains unclear. The observation that the oocyte is always at the egg chamber's posterior end in contact with posterior polar cells led to the hypothesis that signals from posterior follicle cells might trigger oocyte differentiation (King, 1970). In many *arm* mutant egg chambers, the oocyte is not in contact with posterior follicle cells. Since mis-localized oocytes can be detected very early in oogenesis (Fig. 5D-F), those oocytes have either never been in close contact with posterior polar cells or were in contact only briefly, yet these oocytes differentiate relatively normally. Continuous contact between oocyte and polar follicle cells is not required for oocyte differentiation.

Proper cell arrangement is important for RNA localization. Disruptions in patterning occur in cases in which the oocyte is in the middle of the egg chamber; in these egg chambers, *orb* and *oskar* RNAs are localized randomly or fail to be localized. Contact with posterior polar follicle cells, for example, may be important for differentiation of the oocyte's posterior pole. While egg chambers with an oocyte in the middle could be those with the most severe disruptions of the actin cytoskeleton, we believe this less likely, because in contrast to the results just described, in egg chambers with an oocyte localized opposite to its normal location the oocyte develops relatively normally, but with an opposite polarity (Fig. 5G). Cell rearrangements affecting *orb* and *oskar* localization have few apparent consequences on polar follicle cell differentiation, suggesting that continuing contact between the oocyte and posterior polar follicle cells is not required for many aspects of polar follicle cell differentiation (Fig. 4K,L). This investigation of polar follicle cells may miss subtle effects, however. Late differentiation events of polar follicle cells may be influenced by oocyte contact. Normally, anterior polar follicle cells (not initially in contact with the oocyte) become border cells and migrate to the anterior end of the oocyte. At times when the oocyte is in the middle of the egg chamber, both sets of polar follicle cells began this migration. Oocyte contact may influence the decision to be either border cells or posterior follicle cells.

Most models of cadherin-mediated cell adhesion propose homophyllic interaction between cadherin molecules on opposing cells. In the ovary, Armadillo is found at the cell surface of follicle cells and germ cells. At boundaries between follicle cells, it is localized in the region of both cells where adherens type-junctions are found, consistent with a homophyllic interaction. If Armadillo localization is an indication, interactions among germ cells and those between follicle cells and germ cells occur in less-well-defined structures. Elimination of *arm* function in one member of a pair of cells interferes with some cell behaviors and not others. For example, border cells begin their migration between nurse cells but often get lost. Likewise, at the follicle cell-germ cell border, interactions required to maintain the oocyte at the posterior end of the egg chamber

are disrupted, while those required to allow follicle cells to retract from nurse cells are not (Fig. 4L). Homophyllic interactions between cadherin-catenin complexes may be required for certain cell interactions but not for others.

One limitation of this examination is that we have only examined egg chambers in which the germ line is mutant for *arm*. Current technology makes it relatively easy to produce and identify clones of homozygous mutant tissue in the germ line (especially for genes on the X chromosome), but does not offer a simple means of identifying mutant clones of somatic follicle cells. In the future, we hope to tackle this more difficult task, to reveal more about the role of Armadillo and adhesive junctions in cell interactions during *Drosophila* oogenesis, and thus to contribute to an understanding of the more general question of the roles of these junctions during development and differentiation.

We are very grateful to T.-B. Chou and N. Perrimon for providing the *flp* recombinase stocks and advice on the germ-line clone procedure before publication, without which this study would have been impossible. We would also like to thank V. Lantz and B. Suter for providing DNA probes and advice on in situ hybridizations, P. Pukilla for advice on the use of propidium iodide and Susan Whitfield for superb photographic assistance. We are grateful to L. Cooley and members of the Peifer, Wieschaus, Searles and Schüpbach labs for helpful discussions, and C. A. McCormick, G. Sass, L. Searles and our reviewers for helpful comments on the manuscript. This work was supported by grants from the NIH to M. P. and E. W., and by a grant from the Searle Scholars Program to M. P.

REFERENCES

Baker, N. E. (1988). Embryonic and imaginal requirements for *wingless*, a segment polarity gene in *Drosophila*. *Dev. Biol.* **125**, 96-108.

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

Carroll, S. B. and White, J. S. (1989). The role of the *hairy* gene during *Drosophila* morphogenesis: stripes in imaginal discs. *Genes Dev.* **3**, 906-916.

Chou, T.-B. and Perrimon, N. (1992). Use of a yeast site-specific recombinase to produce female germline chimeras in Drosophila. *Genetics* **131**, 643-653.

Cooley, L., Verheyen, E. and Ayers, K. (1992). *chickadee* encodes a profilin required for intracellular cytoplasm transport during *Drosophila* oogenesis. *Cell* **69**, 173-184.

Cowin, P., Kapprell, H.-P., Franke, W. W., Tamkun, J. and Hynes, R. O. (1986). Plakoglobin: A protein common to different kinds of intercellular junctions. *Cell* **46**, 1063-1073.

Eichenberger-Glinz, S. (1979). Intercellular junctions during development and in tissue cultures of *Drosophila melanogaster* : An electron microscopic study. *Wilhelm Roux's Arch Dev. Biol.* **186**, 333-349.

Ephrussi, A., Dickinson, L. K. and Lehmann, R. (1991). *oskar* RNA organizes the germ plasm and directs localization of the posterior determinant *nanos*. *Cell* **66**, 37-50.

Franke, W. W., Kapprell, H.-P. and Cowin, P. (1987). Plakoglobin is a component of the subplasmalemmal coat of lens cells. *Eur. J. Cell Biol.* **43**, 301-315.

Franke, W. W., Goldschmidt, M. D., Zimbelmann, R., Mueller, H. M., Schiller, D. L. and Cowin, P. (1989). Molecular cloning and amino acid sequence of human plakoglobin, the common junctional plaque protein. *Proc. Nat. Acad. Sci. USA* **86**, 4027-4031.

Golic, K. G. (1991). Site-specific recombination between homologous chromosomes in *Drosophila*. *Science* **252**, 958-961.

Herrenknecht, K., Ozawa, M., Eckerskorn, C., Lottspeich, F. and Kemler, R. (1991). The uvomorulin-anchorage protein α-catenin is a vinculin homologue. *Proc. Nat. Acad. Sci. USA* **88**, 9156-9160.

Hirano, S., Kimoto, N., Shimoyama, Y., Hirohashi, S. and Takeichi, M. (1992). Identification of a neural α-catenin as a key regulator of cadherin function and multicellular organization. *Cell* **70**, 293-301.

Ingham, P. W. and Martinez-Arias, A. (1992). Boundaries and fields in early embryos. *Cell* **68**, 221-235.

Kemler, R. and Ozawa, M. (1989). Uvomorulin-catenin complex: Cytoplasmic anchorage of a Ca^{2+}-dependent cell adhesion molecule. *Bioessays* **11**, 88-91.

Kim-Ha, J., Smith, J. L. and Macdonald, P. M. (1991). *oskar* mRNA is localized to the posterior pole of the *Drosophila* oocyte. *Cell* **66**, 23-35.

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

Klingensmith, J., Noll, E. and Perrimon, N. (1989). The segment polarity phenotype of *Drosophila* involves differential tendencies toward transformation and cell death. *Dev. Biol.* **134**, 130-145.

Knudsen, K. A. and Wheelock, M. J. (1992). Plakoglobin, or an 83 kD homologue distinct from β-catenin, interacts with E-cadherin and N-cadherin. *J. Cell Biol.* **118**, 671-679.

Lantz, V., Ambrosio, L. and Schedl, P. (1992). The *Drosophila orb* gene is predicted to encode sex-specific germline RNA-binding proteins and has localized transcripts in ovaries and early embryos. *Development* **115**, 78-88.

Lindsley, D. L. and Zimm, G. G. (1992). *The Genome of* Drosophila melanogaster. San Diego, CA: Academic Press.

Magee, A. I. and Buxton, R. S. (1991). Transmembrane molecular assemblies regulated by the greater cadherin family. *Curr. Opin. in Cell Biol.* **3**, 854-861.

Mahowald, A. P. and Kambysellis, M. P. (1980). Oogenesis. In *The Genetics and Biology of* Drosophila, Vol. 2D. (M. Ashburner, ed.) pp. 141-224. New York: Academic Press.

Manseau, L. and Schüpbach, T. (1989). The egg came first of course. *Trends Genet.* **5**, 400-405.

McCrea, P., Turck, C. and Gumbiner, B. (1991). A homolog of *Drosophila armadillo* (plakoglobin) associated with the cytoplasmic tail of E-cadherin. *Science* **25**, 1359-1361.

McNeill, H., Ozawa, M., Kemler, R. and Nelson, W. J. (1990). Novel function of the cell adhesion molecule uvomorulin as an inducer of cell surface polarity. *Cell* **62**, 309-316.

Miller, K. G., Field, C. M. and Alberts, B. M. (1989). Actin-binding proteins from *Drosophila* embryos: a complex network of interacting proteins detected by F-actin affinity chromatagraphy. *J. Cell Biol.* **109**, 2963-2975.

Nagafuchi, A. and Takeichi, M. (1989). Transmembrane control of cadherin-mediated cell adhesion: a 94 kDa protein functionally associated with a specific region of the cytoplasmic domain of E-cadherin. *Cell Regulation* **1**, 37-44.

Nagafuchi, A., Shirayoshi, Y., Okazaki, K., Yasuda, K. and Takeichi, M. (1987). Transformation of cell adhesion properties by exogenously introduced E-cadherin cDNA. *Nature* **329**, 341-343.

Nagafuchi, A., Takeichi, M. and Tsukita, S. (1991). The 102 kd cadherin-associated protein: similarity to vinculin and post-transcriptional regulation of expression. *Cell* **65**, 849-857.

Nose, A., Nagafuchi, A. and Takeichi, M.(1988). Expressed recombinant cadherins mediate cell sorting in model systems. *Cell* **54**, 993-1001.

Nüsslein-Volhard, C., Frohnhöfer, H.G. and Lehmann, R. (1987). Determination of anterior-posterior polarity in *Drosophila*. *Science* **238**, 1675-1681.

Oda, H., Uemura, T., Shiomi, K., Nagafuchi, A., Tsukita, S. and Takeichi, M. (1993). Identification of a *Drosophila* homologue of alpha-catenin and its association with *armadillo* protein. *J. Cell Biol*., in press.

Ozawa, M., Baribault, H. and Kemler, R. (1989). The cytoplasmic domain of the cell adhesion molecule uvomorulin associates with three independent proteins structurally related in different species. *EMBO J.* **8**, 1711-1717.

Ozawa, M., Ringwald, M. and Kemler, R. (1990). Uvomorulin-catenin complex formation is regulated by a specific domain in the cytoplasmic region of the cell adhesion molecule. *Proc. Nat. Acad. Sci USA* **87**, 4246-4250.

Peifer, M. (1993). The product of the *Drosophila* segment polarity gene *armadillo* is part of a multi-protein complex resembling the vertebrate adherens junction. *J. Cell Sci*., in press.

Peifer, M. and Bejsovec, A. (1992). Knowing your neighbors; cell

interactions determine intrasegmental pattern in *Drosophila*. *Trends Genet.* **8**, 243-248.

Peifer, M. and Wieschaus, E. (1990). The segment polarity gene *armadillo* encodes a functionally modular protein that is the *Drosophila* homolog of human plakoglobin. *Cell* **63**, 1167-1178.

Peifer, M., Rauskolb, C., Williams, M., Riggleman, B. and Wieschaus, E. (1991). The segment polarity gene *armadillo* affects the *wingless* signaling pathway in both embryonic and adult pattern formation. *Development* **111**, 1028-1043.

Peifer, M., McCrea, P. D., Green, K. J., Wieschaus, E. and Gumbiner, B. M. (1992). The vertebrate adhesive junction proteins β-catenin and plakoglobin and the *Drosophila* segment polarity gene *armadillo* form a multigene family with similar properties. *J. Cell Biol.* **118**, 681-691.

Riggleman, B., Schedl, P. and Wieschaus, E. (1990). Spatial expression of the *Drosophila* segment polarity gene *armadillo* is post-transcriptionally regulated by *wingless*. *Cell* **63**, 549-560.

Ruohola, H., Bremer, K. A., Baker, D., Swedlow, J. R., Jan, L. Y. and Jan, Y. N. (1991). Role of the neurogenic genes in establishment of follicle cell fate and oocyte polarity during oogenesis in *Drosophila*. *Cell* **66**, 433-449.

Salomon, D., Ayalon, O., Patel-King, R., Hynes, R. O. and Geiger, B. (1992). Extrajunctional distribution of N-cadherin in cultured human endothelial cells. *J. Cell Sci.* **102**, 7-17.

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

Schüpbach, T and Wieschaus, E. (1991). Female sterile mutations on the second chromosome of *Drosophila melanogaster*. II. Mutations blocking oogenesis or altering egg morphology. *Genetics* **129**, 1119-1136.

Spradling, A. (1993). Developmental genetics of oogenesis. In Drosophila *Development* (ed. M. Bate and A. Martinez-Arias). Cold Spring Harbor, New York: Cold Spring Harbor Labs.

Stevens, L. M., Frohhöfer, H. G., Klingler, M. and Nüsslein-Volhard, C. (1990). Localized requirement for *torso-like* expression in follicle cells for development of terminal anlagen of the *Drosophila* embryo. *Nature* **346**, 660-663.

Suter, B. and Steward, R. (1991). The role of the *Bicaudal-D* protein and its phosphorylation in *Drosophila* oocyte differentiation. *Cell* **67**, 917-926.

Takeichi, M. (1991). Cadherin cell adhesion receptors as a morphogenetic regulator. *Science* **251**, 1451-1455.

Tautz, D. and Pfeifle, C. (1989). A non-radioactive in situ hybridization method for the localization of specific RNAs in *Drosophila* embryos reveals translational control of the segmentation gene *hunchback*. *Chromosoma* **98**, 81-85.

Wieschaus, E. and Noell, E. (1986). Specificity of embryonic lethal mutations in *Drosophila* analyzed in germline clones. *Roux's Arch. Dev. Biol.* **195**, 63-73.

Wieschaus, E., Nusslein-Volhard, C. and Jurgens, G. (1984). Mutations affecting the pattern of the larval cuticle in *Drosophila melanogaster* : zygotic loci on the X-chromosome and the fourth chromosome. *Wilhelm Roux's Arch. Dev. Biol.* **193**, 296-307.

Zusman, S., Coulter, D. and Gergen, J. P. (1985). Lethal mutations induced in the proximal X-chromosome of *Drosophila melanogaster* using P-M hybrid dysgenesis. *Dros. Inf. Serv.* **61**, 217-218.

(Accepted 6 May 1993)