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Differential expression of the adhesion molecule Echinoid drives epithelial morphogenesis in *Drosophila*

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Epithelial morphogenesis requires cell movements and cell shape changes coordinated by modulation of the actin cytoskeleton. We identify a role for Echinoid (Ed), an immunoglobulin domain-containing cell-adhesion molecule, in the generation of a contractile actomyosin cable required for epithelial morphogenesis in both the *Drosophila* ovarian follicular epithelium and embryo. Analysis of *ed* mutant follicle cell clones indicates that the juxtaposition of wild-type and *ed* mutant cells is sufficient to trigger actomyosin cable formation. Moreover, in wild-type ovaries and embryos, specific epithelial domains lack detectable Ed, thus creating endogenous interfaces between cells with and without Ed; these interfaces display the same contractile characteristics as the ectopic Ed expression borders generated by *ed* mutant clones. In the ovary, such an interface lies between the two cell types of the dorsal appendage primordia. In the embryo, Ed is absent from the amnioserosa during dorsal closure, generating an Ed expression border with the lateral epidermis that coincides with the actomyosin cable present at this interface. In both cases, *ed* mutant epithelia exhibit loss of this contractile structure and subsequent defects in morphogenesis. We propose that local modulation of the cytoskeleton at Ed expression borders may represent a general mechanism for promoting epithelial morphogenesis.

KEY WORDS: Echinoid, Epithelial morphogenesis, Actomyosin cable, Follicular epithelium, Dorsal closure, Epithelial tube

INTRODUCTION

The morphogenesis of diverse epithelia during development derives from coordinated remodeling of cell shape and intercellular interactions that drives cell movements and reorganization (Pilot and Lecuit, 2005; Schöck and Perrimon, 2002b). These changes typically arise from modulation of the actin cytoskeleton. Distinct cell behaviors are associated with specific cell types, but how the determination of cell fate engages the subcellular mechanisms that drive morphogenesis is not well understood. We have used the *Drosophila* ovary and embryo as model systems to study how interactions between cell types lead to local changes in the cytoskeleton that mediate epithelial morphogenesis.

In the ovary, the follicular epithelium surrounds individual cysts of germline cells, each of which gives rise to a single egg (Spradling, 1993). Late in oogenesis, the follicle cells secrete the eggshell, which exhibits pronounced asymmetries produced by specialized follicle cell domains (Berg, 2005; Dobens and Raftery, 2000). The most prominent features are the two appendages that project from the dorsal anterior region of the eggshell (Fig. 1A). The follicle cell primordia that produce these appendages are specified in mid-oogenesis and flank the dorsal anterior midline of the epithelium. Subsequently, the two cell types that comprise the primordium undergo a series of coordinated cell shape changes that remodel the flat primordia into epithelial tubes that then extend anteriorly. Secretion of chorion into the lumen of each tube produces the appendages (Dorman et al., 2004; Ward and Berg, 2005). The signals that specify the fate and position of the appendage primordia are well understood (Berg, 2005; Nilson and Schüpbach, 1999; Roth, 2003), but the changes in cell shape and organization that occur during the morphogenesis of these primordia have only recently been described in detail (Dorman et al., 2004; Ward and Berg, 2005). Mutations that disrupt appendage morphology downstream of primordia specification have been identified, and thus may affect factors that contribute specifically to tube formation (Berg, 2005), but the molecular mechanisms that control these morphogenetic movements remain unknown.

By contrast, the cytoskeletal forces driving epithelial movements during *Drosophila* embryonic dorsal closure have been well characterized. Dorsal closure occurs when two lateral epidermal sheets move dorsally over the extra-embryonic amnioserosa and converge at the dorsal midline, sealing the dorsal side of the embryo. These movements are driven by multiple forces, including tissue-specific changes in the shape of individual cells as well as the tension generated by a supracellular contractile actin cable that arises at the interface of the lateral epidermis and amnioserosa (Kiehart et al., 2000; Jacinto et al., 2002; Martin and Parkhurst, 2004). Differential activity of the Jun N-terminal kinase pathway between the epidermis and amnioserosa is involved in generating these forces (Reed et al., 2001), but how this difference in activity produces a local effect on the cytoskeleton at the interface between these tissues is not understood.

We demonstrate that Echinoid (Ed), a cell-adhesion molecule and adherens junction component (Bai et al., 2001; Islam et al., 2003; Spencer and Cagan, 2003; Wei et al., 2005) is required for the epithelial sheet movements that occur during appendage primordia morphogenesis and dorsal closure. Specifically, we find that the juxtaposition of cells expressing and lacking Ed induces the assembly of a contractile actomyosin cable at their interface. We initially identified a mutant allele of *ed*, based on the smooth borders exhibited by homozygous mutant follicle cell clones, and found that the apical clone border displays morphological and molecular characteristics of a contractile actomyosin cable. Strikingly, we demonstrate that Ed is absent from some cell types during development, generating endogenous interfaces between cells with and without Ed; these Ed expression borders display contractile

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features identical to those of *ed* mutant clones. In wild-type ovaries, Ed is absent from one of the two cell types of the appendage primordium, generating an Ed expression border within the primordium. In the embryo, Ed becomes undetectable in the amnioserosa prior to dorsal closure, resulting in an Ed expression border that coincides with the well-characterized contractile actomyosin cable between these tissues. In both cases, elimination of Ed results in the absence of the contractile structure and in defective morphogenesis. Taken together, these data suggest that differential Ed expression between cell types induces the formation of a contractile actomyosin cable at their interface. These observations may identify a general morphogenetic mechanism that converts a difference in protein expression into a local effect on the cytoskeleton.

MATERIALS AND METHODS

Drosophila strains

The ed^{F72} allele was isolated in a genetic screen of mosaic females bearing *P{neoFRT}40A* chromosomes that had been mutagenized with ethyl methanesulfonate. Other strains used were $dec\text{-}1^{VA28}$; *P{dec-1⁺}2L P{neoFRT}40A* (Nilson and Schüpbach, 1998), ed^{lF20} *P{neoFRT}40A* (gift of J.-C. Hsu), *rho-lacZ* R1.1 line (Ip et al., 1992), *w; al dp P{NM}31E P{neoFRT}40A*, and *y w P{hsFLP}122; P{NM}31E P{neoFRT}40A*, sqh-GFP-moesin (SGMCA) (gift of D. Kiehart).

Mapping and identification of the F72 mutation

The F72 mutation was mapped through meiotic recombination between the parental strains *w; al dp P{NM}31E P{neoFRT}40A* and *w;* ed^{F72} *P{neoFRT}40A* using single nucleotide polymorphisms as molecular markers, as described previously (Berger et al., 2001; Hoskins et al., 2001; Martin et al., 2001). After mapping the F72 phenotype to an interval containing *ed*, all nine *ed* exons, their splice sites and ~1 kb of flanking genomic DNA were amplified by PCR, in fragments of ~800 bp, and sequenced. The only difference between the F72 chromosome and the parental chromosome was an A to T substitution at position 1043 of the transcript, generating a premature stop at codon 205 of the *ed* open reading frame. The ed^{F72} allele is therefore predicted to encode a protein truncated in the second of seven immunoglobulin-like domains predicted by the ExPASy ScanProsite proteomics algorithm.

Generation of Ed antiserum

The cDNA RE66591 (*Drosophila* Genome Resources Center) was used as template to amplify by PCR the fragment encoding the C-terminal domain of Ed, which was then cloned in frame into the pGEX2T-His_6 vector (gift of S. Gunderson). After expression in *E. coli* BL21 cells, the recombinant protein was purified by selection for the His_6 tag and used to immunize rats.

Mitotic recombination

Mitotic follicle cell clones were induced in females of the genotype *y w P{hsFLP}122; P{NM}31E P{neoFRT}40A/ed P{neoFRT}40A* by incubating pupae at 37°C for 1 hour on three consecutive days. Prior to dissection, well-fed mosaic females were incubated at 37°C for 80 minutes to induce expression of N-myc (NM) clone marker (Xu and Rubin, 1993). Germline clones homozygous for either ed^{F72} *or* ed^{lF20} were generated and imaged as described previously (Chou and Perrimon, 1996; Schöck and Perrimon, 2002a).

Immunohistochemistry

Fixation and staining of ovaries and embryos was performed as described previously (Van Buskirk and Schüpbach, 2002; Wieschaus and Nusslein-Volhard, 1986). Antibodies used were anti-Ed (1:1000 for ovaries, 1:10,000 for embryos), anti-c-Myc supernatant 9E10 (1:100, Developmental Studies Hybridoma Bank (DSHB)), anti-phospho-myosin light chain 1 Ser19 (1:250, Cell Signaling Technology), anti-DE-cadherin DCAD2 supernatant (1:100, DSHB), anti-Arm N2 7A1 supernatant (1:100 for ovaries; 1:500 for embryos, DSHB), anti-β-galactosidase 40a1 supernatant (1:50, DSHB), anti-Broad Core supernatant (1:50, DSHB), anti-Enabled 5G2 (1:500, DSHB) and anti-phosphotyrosine (1:200, Upstate Cell Signaling Solutions). All secondary antibodies (Molecular Probes) were pre-blocked against ovaries and used at a final concentration of 1:500 for 2 hours at room temperature or 1:1000 overnight at 4°C. For F-actin labeling, tissues were incubated for 2 hours at room temperature with 0.5 U/ml of Alexa Fluor 546 phalloidin (dried of methanol; Molecular Probes).

RESULTS

Follicle cell clones homozygous for a novel mutation exhibit smooth borders

In a genetic screen for defects associated with follicle cell clones, we recovered a mutation, initially designated F72, with a novel effect on the organization of the imprints on the eggshell surface. The pattern of these imprints reflects the organization of the cells in the follicular epithelium, which secretes the eggshell and degenerates before the egg is laid (Fig. 1A). Eggs produced by females bearing mitotic follicle cell clones homozygous for this mutation display subsets of eggshell imprints organized into groups with smooth borders (Fig. 1B). When the F72 mutant clones were marked with the *defective chorion 1* (*dec-1*) marker, which confers a distinct appearance on the eggshell secreted by the mutant cells (Hawley and Waring, 1988; Nilson and Schüpbach, 1998; Wieschaus et al., 1981), the *dec-1*-marked imprints were contained exclusively within the smooth borders, indicating that these borders occur at the interface of imprints produced by mutant and non-mutant cells (Fig. 1C).

Consistent with the mosaic eggshell phenotype, clones of homozygous mutant follicle cells exhibit smooth borders with adjacent heterozygous or homozygous wild-type cells. Interfaces between mutant cells within the clone, however, appear normal (Fig. 1E,E′). Interestingly, the smooth clone border is detectable only at

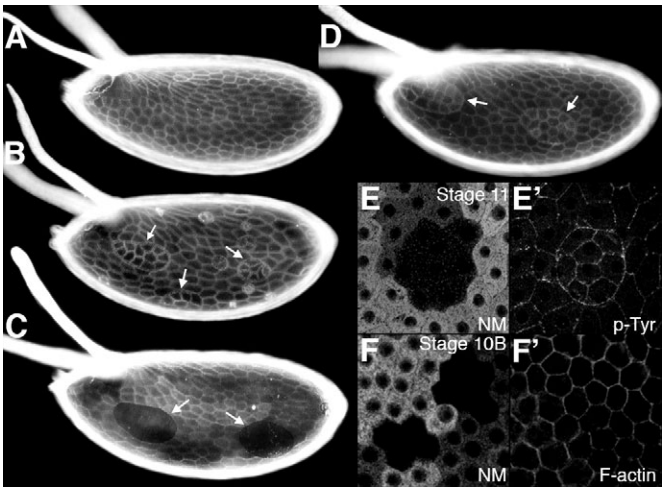

Fig. 1. F72 homozygous follicle cell clones produce groups of eggshell imprints with a smooth border. In all figures, anterior is towards the left and dorsal is towards the top, unless otherwise indicated. (**A**) Wild-type egg. (**B**) Egg from an F72 mosaic female. Groups of surface imprints exhibit smooth borders (arrows). (**C**) Egg from a female with homozygous F72 clones marked with the *dec-1* eggshell marker (arrows). (**D**) Egg from an ed^{lF20} mosaic female. (E,E′) Clone of F72 homozygous follicle cells in a stage 11 egg chamber. (**E**) Clone is marked by the absence of the NM clone marker (basal confocal section); heterozygous and homozygous wild type cells are also visible. (**E′**) Anti-phosphotyrosine (p-Tyr) staining, apical confocal section. The apical clone border is smooth. (**F**,**F′**) Rhodamine-phalloidin staining to visualize filamentous actin (F-actin). F72 mutant clones at stage 10B (F, basal confocal section) do not exhibit a smooth border (F′, apical confocal section).

the apical side of the epithelium, while the basal aspect of the clone displays no obvious phenotype (compare Fig. 1E with 1E′). This mosaic phenotype also exhibits a surprising temporal profile. The smooth clone border phenotype is completely penetrant in early stage egg chambers (data not shown) but, during stage 10 of mid-oogenesis, the border of F72 mutant clones becomes indistinguishable from adjacent intercellular interfaces (Fig. 1F,F′). The disappearance of the phenotype is transient, however, and by stage 11 the marked smoothness of the clone border is again readily detectable and completely penetrant (Fig. 1E,E′), and persists for the remainder of oogenesis.

The F72 mutation generates a nonsense mutation in the *ed* gene

Meiotic mapping using single nucleotide polymorphisms as molecular markers located the F72 mosaic phenotype to a small interval containing exons 3, 4 and 5 of a single gene, *ed* (data not shown). Sequencing revealed a single nucleotide substitution, which generates a premature termination at codon 205 of the *ed* open reading frame. The identification of this nonsense mutation, together with the mapping of the phenotype to the same small interval, suggests that F72 is allelic to *ed*. Moreover, the F72 mutation fails to complement ed^{lF20}, an independently isolated *ed* allele that contains a premature termination at codon 63 (de Belle et al., 1993; Escudero et al., 2003). Follicle cell clones homozygous for ed^{lF20} exhibit a smooth border phenotype indistinguishable in all respects from F72 mutant clones (Fig. 1D; data not shown). Together these data confirm that the phenotype is the result of the mutation in *ed*.

ed encodes a 1332 amino acid transmembrane protein with seven immunoglobulin (Ig) domains, a fibronectin type III domain and a cytoplasmic tail with a PDZ-binding domain (see Materials and methods) (Bai et al., 2001; Wei et al., 2005). The ed^{F72} allele is predicted to encode an Ed protein that is truncated in the second Ig domain, thus lacking most of the extracellular domain, as well as the transmembrane and intracellular domains, and therefore unlikely to retain Ed function. The Ed extracellular domain resembles that of Ig-type cell-adhesion molecules, and recently Ed has been reported to be a component of adherens junctions (Wei et al., 2005). The molecular nature of Ed is therefore consistent with the *ed* mosaic phenotype, and suggests that the absence of this molecule from the cell surface affects the interaction between wild-type and mutant follicle cells.

ed clone borders induce assembly of a contractile actomyosin cable

Our initial observations of *ed* mutant follicle cell clones revealed that the apical clone circumference is markedly reduced relative to the basal circumference (compare Fig. 1E with 1E′ and Fig. 2A with 2A′; Fig. 2B,B′). In addition, filamentous actin (F-actin) appears enriched at the apical interface between wild-type and *ed* mutant cells (Fig. 2A,A′), but not at the basal interface (see Fig. S1 in the supplementary material). Together with the reduced apical circumference, this observation suggested the presence of a contractile actin cable at the clone border. Consistent with this hypothesis, the active, phosphorylated form of the light chain of non-muscle myosin II (p-MLC) is also enriched at the apical clone border (Fig. 2C,C′), suggesting that non-muscle myosin II is activated at the interface between wild-type and *ed* mutant cells (Sellers, 1991; Trybus, 1991). Closer inspection of the clone border resolves two ‘rings’ of p-MLC immunoreactivity (Fig. 2D,D′), raising the possibility that one contractile structure assembles within the *ed* mutant cells at the clone border and another in the adjacent wild-type cells. Identical mosaic phenotypes were observed with both ed^{F72} and ed^{lF20}.

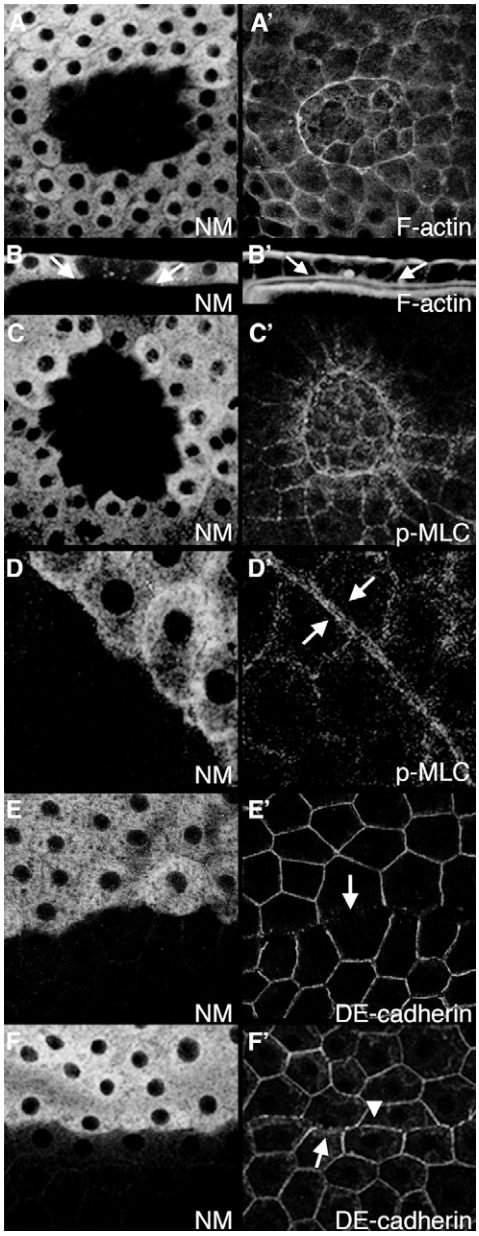

Fig. 2. *ed* clone borders induce the formation of a contractile actin cable and reduction in adherens junction components. (**A**) ed^{F72} follicle cell clone (lack of NM marker, basal confocal section). (A′) Staining with fluorescently-labeled phalloidin (apical confocal section) reveals enriched F-actin at the clone border and reduced apical circumference. (**B**,**B′**) Cross-section of an ed^{F72} mutant clone (B) stained with fluorescently-labeled phalloidin (B′) illustrates the apical constriction of the clone border (arrows, apical is towards the bottom). (**C**) ed^{F72} follicle cell clone (basal confocal section). (C′) Increased p-MLC immunoreactivity (apical confocal section) is detected at the clone border. (**D**,**D′**) Enlargement of an ed^{F72} clone border. Two parallel lines of p-MLC immunoreactivity can be resolved. (**E**,**E′**) Stage 12. Some ed^{F72} mutant clones exhibit severely reduced or discontinuous DE-cad immunoreactivity at the clone border (arrow). (**F**,**F′**) Stage 12. At ed^{F72} mutant clone borders with a milder DE-Cad defect, DE-Cad is occasionally absent (arrow) but often discontinuous or unaffected (arrowhead).

The reduced circumference and enrichment of F-actin and p-MLC at the apical *ed* clone border are characteristic of the supracellular contractile actomyosin structures that mediate the epithelial movements observed in processes such as wound healing and embryonic epithelial closure (Bement, 2002; Martin and Parkhurst, 2004). We propose that the juxtaposition of wild-type and *ed* mutant cells is sufficient to trigger the assembly of such a structure at their interface, resulting in a contractile force at the border that generates the apically constricted smooth circumference of *ed* mutant follicle cell clones. This phenotype is similar to that reported for *ed* mutant clones in the wing imaginal disc epithelium (Wei et al., 2005). However, although *ed* mutant cells in the wing disc required a genetic growth advantage to recover sufficient clones for analysis, *ed* mutant follicle cells display no detectable defects in growth or viability. This difference may reflect tissue specificity in the requirement for *ed*, or a difference in the *ed* mutant chromosome studied in the wing.

Adherens junctions are destabilized at the border of *ed* mutant clones

Because the smooth border of *ed* mutant follicle cell clones suggested possible differential adhesion with the neighboring wild-type cells (Lawrence, 1997; Dahmann and Basler, 1999; Tepass et al., 2002), we determined whether *ed* mutant clones exhibit altered levels of the cell-adhesion molecule DE-cadherin (DE-cad). At stage 10, when the smooth border phenotype is not detectable, the level and distribution of DE-cad appeared normal at the border of 38/38 *ed* mutant clones examined (data not shown). After stage 11, DE-cad immunoreactivity was strongly reduced or absent at the clone border in 6/20 clones observed (Fig. 2E,E′). This effect was less dramatic in 11/20 clones (Fig. 2F,F′), where DE-cad at individual interfaces along a single clone border appeared either undetectable (Fig. 2F′, arrow), wild type or discontinuous (Fig. 2F′, arrowhead). In 3/20 clones, there was no detectable effect. Levels of Armadillo (Arm), the *Drosophila* homolog of β-catenin and an intracellular component of adherens junctions, were similarly affected (data not shown). In both cases, the degree of disruption did not correlate with clone size or position, and was variable even between egg chambers of the same stage. These data indicate that the juxtaposition of wild-type and *ed* mutant cells can affect the distribution of DE-cad and Arm. However, given the variability of this effect, it remains unclear whether alteration of adherens junction components is the cause of the *ed* smooth border phenotype.

Ed exhibits a dynamic expression pattern in the follicular epithelium

To visualize the distribution of Ed in the follicular epithelium, we generated an antiserum against the Ed intracellular domain. We detected no immunoreactivity in *ed* mutant follicle cells, confirming the specificity of the antiserum (Fig. 3A,A′). In individual follicle cells, Ed levels appear highest apically with lower levels detectable in lateral membranes (Fig. 3B, inset), resembling the distribution of DE-cad and Arm and consistent with recent evidence implicating Ed as an adherens junction component (Wei et al., 2005). As reported previously, Ed immunoreactivity is either discontinuous (Fig. 3A, arrowhead) or absent (Fig. 3A, arrow) from the surrounding wild-type cells at the interface with *ed* mutant cells, consistent with previous observations that Ed molecules on adjacent cells can interact homophilically (Islam et al., 2003; Spencer and Cagan, 2003).

Ed exhibits a spatially and temporally dynamic expression pattern in wild-type ovaries. In early stages of oogenesis, similar levels of Ed are detectable in all follicle cells (Fig. 3B, left), but begin to decline by stage 8 (Fig. 3B, middle). By early stage 10B, little or no Ed is detectable above background levels (Fig. 3B, right). Interestingly, the absence of detectable Ed at this stage coincides with the transient disappearance of the *ed* mosaic phenotype, supporting the hypothesis that the smooth border of *ed* mutant clones is triggered by the juxtaposition of cells with and without Ed; presumably *ed* mutant clones do not exhibit a smooth border at this stage (see Fig. 1F,F′) because Ed is also absent from the surrounding wild-type cells.

Also consistent with the temporal profile of the *ed* mosaic phenotype, Ed immunoreactivity reappears in late stage 10B and persists throughout the remainder of oogenesis. In late stage 10B,

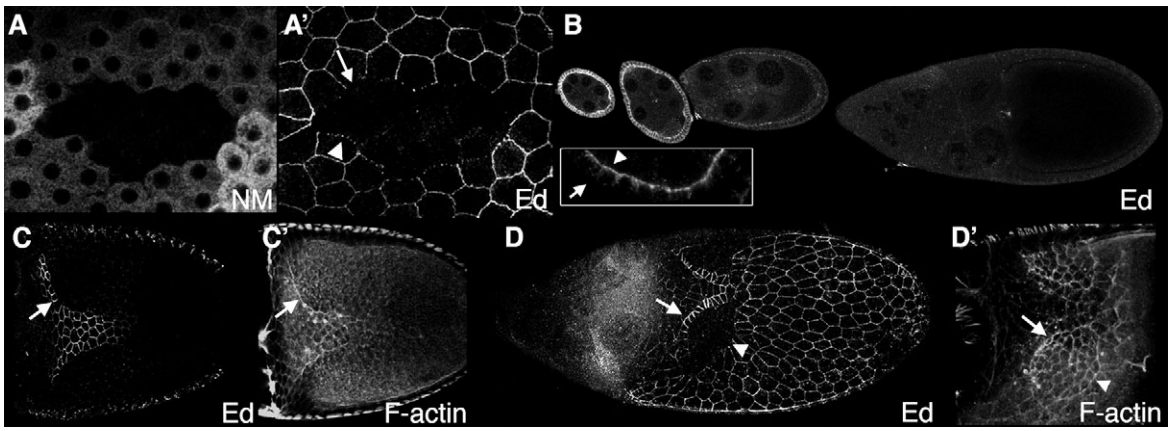

Fig. 3. Ed exhibits a spatially and temporally dynamic distribution during oogenesis. (**A**) Homozygous ed^{F72} follicle cell clone (lack of NM marker, basal confocal section). (**A′**) Ed immunoreactivity is absent from the *ed* mutant cells and absent (arrow) or discontinuous (arrowhead) from wild-type cells at the clone border (apical confocal section). (**B**) Ed is enriched at the apical (germline-facing) side of the tissue (inset; apical is indicated with an arrowhead, basal with an arrow) at early stages (left) then becomes undetectable by early stage 10B (right). (**C**) Late stage 10B (dorsal view). Ed is detected at the dorsal midline of the main body follicle cells (only the posterior half of the egg chamber is shown). (**C′**) Same egg chamber as in C, labeled with fluorescently-labeled phalloidin. A smooth interface coincides with the endogenous Ed expression border (arrow). (**D**) Stage 11 (dorsolateral view). Ed is absent from two dorsolateral groups of follicle cells. (**D′**) Same egg chamber as in D (dorsal anterior portion), labeled with fluorescently-labeled phalloidin. A smooth interface (arrow, arrowhead) corresponds to the endogenous Ed expression border.

Ed is present in a T-shaped pattern along the dorsal anterior midline of the epithelium (Fig. 3C). Later, beginning in stage 11, Ed is present in all main body follicle cells except for two dorsolateral populations that exhibit no detectable Ed (Fig. 3D). The border of an *ed* mutant clone is not smooth when it falls within one of these two populations, providing functional evidence for the absence of Ed (data not shown). During stage 12, Ed becomes detectable in this domain (data not shown).

The absence of Ed from specific follicle cell populations generates endogenous interfaces between cells with and without Ed, which we refer to as Ed expression borders, that resemble the ectopic interfaces generated by *ed* mutant clones. Indeed, we found that these endogenous Ed expression borders are smooth and exhibit the same apical enrichment of F-actin and p-MLC associated with *ed* mutant clones (Fig. 3C′,D′; data not shown). At later stages, however, the enrichment of actin at this endogenous border appears less pronounced, owing to increased F-actin levels in the individual cells within this domain (Fig. 3D′). These data further support the hypothesis that juxtaposition of cells with and without Ed results in formation of a contractile actin cable at their interface. Moreover, the spatial and temporal regulation of the appearance of this Ed expression border suggested that it may have a developmental function.

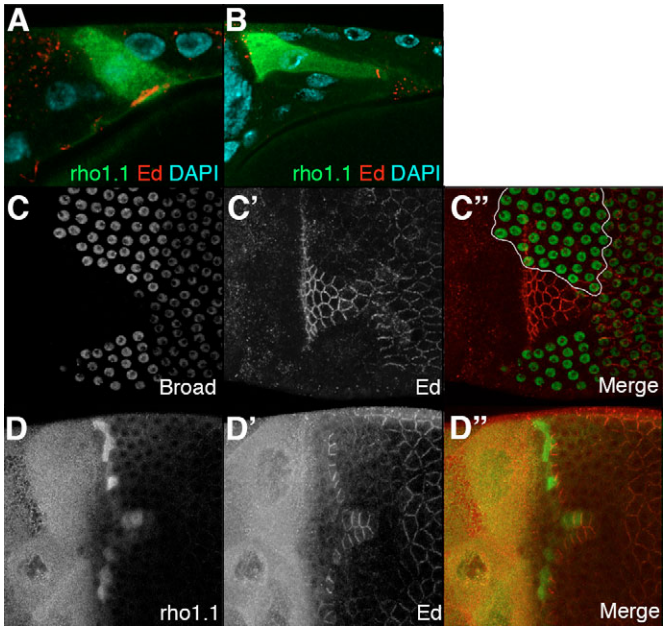

Fig. 4. Absence of Ed from the roof cells generates an endogenous Ed expression border. (**A**,**B**) Cross-sections (lateral view) through the dorsal anterior follicular epithelium at early (A) and late (B) stage 11. Ed (red) is detected in cells expressing the *rho-lacZ* floor cell marker (green). The floor cell shown in A has begun to elongate posteriorly; this elongation is more pronounced by the stage shown in B. Nuclei are shown in blue. (**C-C″**) Stage 11 (dorsal view). (C) High Broad levels mark the roof cell nuclei. (C′) Ed is present in all follicle cells except for two dorsolateral domains. (C″) Merge. Ed (red) is absent from the roof cells (green); one roof cell domain is outlined. (**D-D″**) Stage 11 (dorsal view). The two L-shaped floor cell domains (D, basal confocal section) align with the limit of the Ed domain (D′, apical confocal section). (D″) Merge of Ed (red) and *rho-lacZ* (green). The patterns in merged images (C″,D″) are slightly out of register owing to the different planes of the images and the onset of morphogenesis (see A).

Ed is absent from the roof cells of the appendage primordia

The dorsal-anterior Ed expression pattern suggested a correlation between Ed expression and the specification or behavior of the dorsal appendage primordia, which are located in the same region of the epithelium. Each primordium consists of two cell types, which are specified coordinately during midoogenesis (Dorman et al., 2004; Ward and Berg, 2005). The majority of the cells in the primordium will form the roof of the appendage-producing tube, while the cells in a single 'L'-shaped row at the anterior and medial edges of the primordium will form the tube floor. The roof cells are distinguished by high nuclear levels of the Broad protein, a zinc-finger transcription factor (Bayer et al., 1996; Deng and Bownes, 1997; DiBello et al., 1991; Tzolovsky et al., 1999), while the floor cells express a *lacZ* reporter driven by a region of the *rhomboid* promoter (*rho-lacZ*) (Dorman et al., 2004; Ip et al., 1992; Ward and Berg, 2005). The *rho-lacZ* marker is detectable throughout the floor cell cytoplasm and thus also highlights the changes in floor cell shape that occur during appendage morphogenesis (Fig. 4A,B).

We found that the follicle cell domains that lack Ed expression (see Fig. 3D) coincide precisely with the two roof cell populations, which express high levels of Broad (Fig. 4C-C″). In addition, visualization of the *rho-lacZ* floor cell marker revealed that Ed is present apically in the adjacent floor cells (Fig. 4A,B,D-D″). The border of the domain lacking Ed therefore aligns precisely with the roof/floor interface, which has been observed previously to be smooth (Ward and Berg, 2005). Our data show that an endogenous Ed expression border corresponds to a contractile interface between the two cell types that populate the appendage primordium.

The dorsoventral pattern of follicle cell fates, including both the appendage primordia and the dorsal midline, is established through dorsally localized activation of the *Drosophila* epidermal growth factor receptor (Egfr) pathway (Berg, 2005; Nilson and Schüpbach, 1999; Roth, 2003), and Ed has been reported to downregulate Egfr signaling in the eye imaginal disc (Bai et al., 2001; Rawlins et al., 2003b; Spencer and Cagan, 2003). However, large *ed* mutant follicle cell clones encompassing the dorsal midline and one or both appendage primordia (n=6) have no detectable effect on the pattern of Broad expression (data not shown). This observation indicates that Ed functions downstream of cell fate determination and does not regulate Egfr signaling in this patterning process.

ed is required for morphogenesis of the appendage primordia

The contractile features of endogenous and ectopic Ed expression borders suggested a potential function for the endogenous Ed expression border in appendage tube morphogenesis. We first asked whether differential Ed expression is required for the smooth border of the roof cell domain. In wild-type stage 11 epithelia, a smooth border is detectable along the entire circumference of the roof cell domain, both at the roof cell/floor cell interface (see Fig. 3D′, arrow) and at the posterior of the roof cell domain (see Fig. 3D′, arrowhead). When the endogenous Ed expression border was eliminated at this stage by a large *ed* mutant follicle cell clone (Fig. 5A,A′), the smooth border at the posterior of the roof cell domain was abolished in 7/7 primordia recovered (Fig. 5A″, arrowhead). Some delineation between the roof and floor cell populations was still visible, but this interface was not smooth (Fig. 5A″, arrow).

To determine whether the endogenous Ed expression border is required for the remodeling of the appendage primordia into epithelial tubes, we analyzed *ed* mosaic egg chambers at later stages, when the tubes are being formed. In mosaic epithelia with one

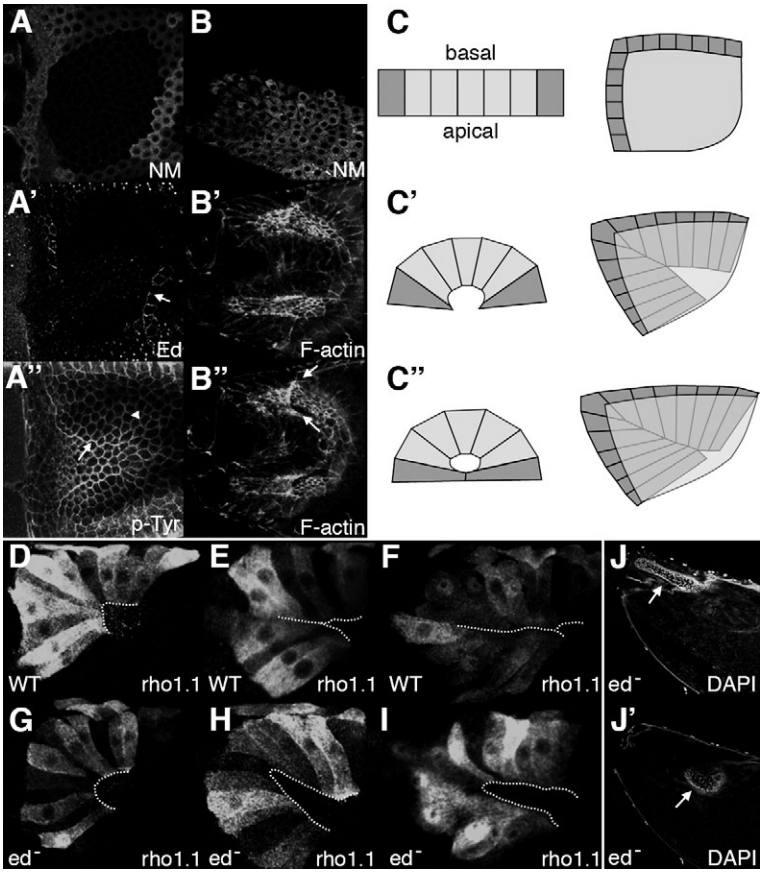

Fig. 5. Ed is required for tube floor closure during appendage morphogenesis. (**A**) Stage 11 mosaic egg chamber (dorsal view) with a large *ed* mutant clone (lack of NM marker) that includes much of both appendage primordia. (**A′**) Ed expression is detectable in the posterior non-mutant follicle cells (arrow), indicating that this egg chamber is of a stage at which Ed would be expressed in all follicle cells except the presumptive roof cells (see Fig. 3D). (**A″**) Anti-phosphotyrosine (p-Tyr) staining. The interface between the roof and floor cell domains is morphologically distinguishable but not smooth (arrow), and the posterior border of the roof cell domain (arrowhead) is not distinguishable. (**B**) Stage 12 egg chamber (dorsal view) with one wild-type (bottom) and one mutant (top, lack of NM marker) appendage primordium. (**B′**,**B″**) Staining with fluorescently-labeled phalloidin, basal section (B′), apical confocal section (B″). The opening of the mutant tube appears wider (arrows). (**C-C″**) Diagram of a single appendage primordium at successive stages of tube formation. Both cross-section (left) and surface views (right; anterior towards the left, dorsal towards the top) are shown. The presumptive roof cells (light gray) are flanked anteriorly and medially by a single row of floor cells (dark gray). To emphasize the floor cell movements, roof cells are not delineated individually in the surface views. (**D-I**) Appendage primordia expressing the *rho-lacZ* floor cell marker. (D-F) Wild-type appendage primordia. (D) Primordium at onset of tube extension phase (floor cell domain aligned with oocyte/nurse cell margin). The floor cells have elongated and the apices nearest the 'hinge' between the anterior and medial domains have met. (E) Tube extension phase (floor cell domain overlaps nurse cells). The tube floor is nearly fully closed. (F) A later stage than E. The tube floor is closed. (G-I) *ed* mutant primordia. (G) Same stage as D. The floor cell apices have not met. (H) Tube extension stage, comparable with E. The floor cell apices have not met; the tube floor remains open. (I) Later stage than H. The tube floor remains open. (**J**,**J′**) Two sides of a single stage 14 egg chamber in which all follicle cells are mutant for *ed*. Autofluorescence and DAPI staining reveal the eggshell structure and nuclei of the follicular epithelium, respectively. The dorsal appendages are severely reduced (arrow).

mutant (Fig. 5B, top) and one wild-type primordium (Fig. 5B, bottom), the nascent *ed* mutant tube was shorter (Fig. 5B′) and exhibited a wider opening (Fig. 5B″, arrows) than the wild-type tube, suggesting a defect in tube morphogenesis in the absence of an Ed expression border (n=3).

We further characterized this tube defect using the *rho-lacZ* reporter to visualize the floor cell movements that occur during tube formation. In wild-type primordia, at the onset of tube morphogenesis the floor cells elongate and the apices of the anterior and medial floor cell domains begin to approach each other, moving underneath the roof cells, which constrict apically at this stage (Fig. 5C,C′; see Fig. 4A,B). The floor cell apices meet first at the intersection (or hinge) between the anterior and medial domains and progressively converge, closing the tube floor (Fig. 5C′,C″,D-F). As floor closure nears completion, the wedge-shaped floor cell domain (Fig. 5D) adopts a more rounded appearance (Fig. 5E,F) (Dorman et al., 2004), and moves anteriorly. By the time the floor cell domain begins to overlap the nurse cell cluster, the tube floor is closed. The completed tube then continues to extends anteriorly.

In all phases of tube morphogenesis, the floor cells of both wild-type (Fig. 5D-F) and *ed* mutant (Fig. 5G-I) primordia elongate to a similar degree and project their apices toward the future tube floor midline, indicating that this change in floor cell shape is Ed independent. However, tube floor closure is defective in the absence of Ed. Prior to the tube extension phase, floor closure was partially complete in 8/38 wild-type primordia but in 0/19 *ed* mutant primordia (Fig. 5D,G). During tube extension, the tube floor was closed in 37/37 wild-type primordia observed (Fig. 5E,F). However, in *ed* mutant primordia, the tube floor remained open in 16/17 cases observed (Fig. 5H,I), indicating that the presence of Ed is required for proper tube floor closure.

This tube formation defect results in abnormal eggshell appendages. Appendage morphology can be visualized in egg chambers at stage 14; this is the final stage of oogenesis, when egg chambers have completed dorsal appendage formation. We examined stage 14 egg chambers from *ed* mosaic females, focusing on those that retained an intact follicular epithelium and lacked detectable Ed. Of 14 *ed* mutant appendages recovered, 12 were severely reduced in length or had failed to extend from the main body of the eggshell (Fig. 5J,J′). These mutant appendage phenotypes confirm that the defects in floor closure observed in the absence of *ed* result in improperly formed epithelial tubes.

Differential Ed expression promotes embryonic dorsal closure

The morphogenetic movements of the floor cells are reminiscent of those observed during embryonic dorsal closure, where the lateral epidermal sheets of the embryo move dorsally and ultimately fuse at the embryonic dorsal midline, covering the extra-embryonic amnioserosa (Jacinto et al., 2002; Martin and Parkhurst, 2004). Interestingly, the leading edge cells of the lateral epidermis assemble a supracellular contractile actomyosin cable that provides one of the forces driving epithelial closure (Young et al., 1993; Hutson et al., 2003; Kiehart et al., 2000). Given the contractile

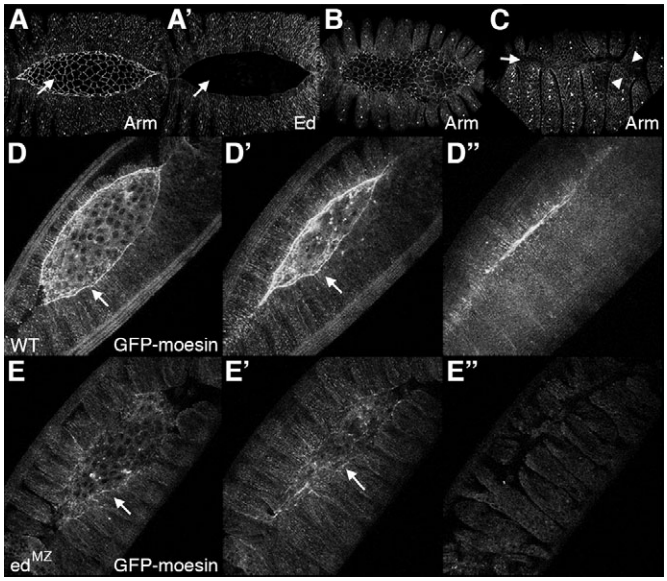

Fig. 6. Ed is required for dorsal closure. (**A**,**A′**) Stage 14 embryo, dorsal view, zippering phase of dorsal closure (Jacinto et al., 2002). (A) Arm immunoreactivity highlights the amnioserosa (arrow) and surrounding lateral epidermis. (A′) Ed immunoreactivity is absent from the amnioserosa (arrow). (**B**,**C**) Arm staining of ed^{MZ} mutant embryos at the zippering phase (B) and termination phase (C) of dorsal closure. There are gaps along the dorsal midline (arrow) and misaligned segments (arrowheads). (**D-D″**) Successive images of a live wild-type embryo expressing GFP-moesin during zippering (D,D′) and termination (D″). Accumulation of GFP-moesin at the leading edge highlights the contractile cable (D,D′, arrows). (**E-E″**) Live imaging of dorsal closure in an ed^{MZ} embryo expressing GFP-moesin. GFP-moesin does not accumulate at the leading edge (E,E′, arrows), and the gaps and segments are misaligned in the termination phase (E″).

nature of endogenous and ectopic Ed expression borders in the follicular epithelium, we asked whether the actomyosin cable between the amnioserosa and lateral epidermis is also associated with differential Ed expression. We found that during dorsal closure Ed is present in the lateral epidermis but undetectable in the amnioserosa (Fig. 6A,A′), generating an endogenous Ed expression border at the interface of these two cell types.

To determine whether this differential Ed expression is necessary for the generation of the actomyosin cable and for dorsal closure, we generated embryos lacking both maternal and zygotic contributions of *ed* (ed^{MZ}). Fixed ed^{MZ} embryos exhibit apparent irregularities in the progression of the leading edge during dorsal closure stages (14/14; Fig. 6B), as well as gaps and segment misalignments at the dorsal midline in later stage embryos (14/14; Fig. 6C).

We also used time-lapse confocal microscopy to image live wild-type and ed^{MZ} embryos expressing a transgene encoding the actin binding fragment of *Drosophila* moesin fused to GFP (GFP-moesin), a well-characterized marker that labels F-actin (Edwards et al., 1997). In wild-type embryos during dorsal closure, this marker highlights the actin cable at the interface of the lateral epidermis and amnioserosa (Fig. 6D,D′, arrows) and allows visualization of the progressive dorsal movement of the epidermis in live embryos (Fig. 6D-D″). In contrast to wild-type embryos, ed^{MZ} embryos during dorsal closure stages fail to exhibit pronounced accumulation of GFP-moesin at the leading edge of the lateral epidermis (Fig. 6E,E′, arrows), suggesting that the actomyosin cable fails to assemble. Moreover, in these mutant embryos, the dorsal epidermis appears to buckle towards the amnioserosa, suggesting that a lack of tension prevents the formation of a taut interface with the amnioserosa (Grevengoed et al., 2001). As development proceeds, these embryos exhibit defective dorsal closure. The dorsal movement of the lateral epidermis is delayed compared with wild-type embryos, and discontinuities and puckering at the dorsal midline and misalignment of opposing segments are ultimately observed. These data support the hypothesis that the Ed expression border is required for assembly of the supracellular actomyosin cable, and that the absence of this structure leads to defective morphogenesis.

DISCUSSION

Ed expression borders assemble a contractile actomyosin structure that mediates epithelial morphogenesis

We demonstrate that the borders of *ed* mutant follicle cell clones display a reduced apical circumference and apical enrichment of F-actin and p-MLC, suggesting that the juxtaposition of follicle cells with and without Ed is sufficient to trigger the assembly of an apical actomyosin cable at their interface. Based on these observations, we propose that the smooth, constricted border of *ed* mutant clones is the result of a contractile force generated by this structure. Consistent with this interpretation, *ed* clone borders do not exhibit this phenotype if the adjacent wild-type cells, owing to their position or developmental stage, also lack Ed. Thus, the generation of this contractile structure is a result of an interface between cells with and without Ed, rather than the loss of Ed per se.

The apical constriction associated with the loss of Ed appears to be restricted to the Ed expression boundary itself; individual *ed* mutant follicle cells that do not contact the clone border do not display pronounced apical constriction. Although the apical circumference of follicle cells in the interior of *ed* mutant clones occasionally appears reduced (see Fig. 2A′,C′), this effect is not observed in larger clones (see Fig. 2E′,F′). The reduction of apical circumference observed in individual *ed* mutant cells may therefore be a secondary consequence of the contractile force generated at the clone border, rather than a direct effect of the absence of Ed.

Although a smooth border has been reported previously for *ed* mutant clones in the wing imaginal disc (Wei et al., 2005), our data are the first to reveal a developmentally regulated absence of Ed in specific cell types associated with epithelial sheet movements. We show that Ed is absent from the presumptive roof cells of the appendage primordia prior to tube morphogenesis, and from the embryonic amnioserosa prior to dorsal closure. In both cases, the resulting endogenous Ed expression borders are smooth and display features of a contractile actomyosin cable, and loss of Ed results in defects in epithelial closure. Because generation of ectopic Ed expression borders is sufficient to generate a smooth contractile intercellular interface, we interpret these defects as a result of the elimination of the endogenous Ed expression borders between these tissues. We propose that the juxtaposition of cells with and without Ed at these endogenous interfaces induces local contractility of the actin cytoskeleton that in turn drives the convergence of opposing epithelial domains during morphogenesis.

Ed does not appear to play a role, however, in the generation of the actin-rich smooth interface observed at the boundary between dorsal and ventral compartments of the wing imaginal disc (Major and Irvine, 2005). Differential expression of Ed between dorsal and ventral compartments is not detected, and *ed* mutant clones in either compartment exhibit smooth borders (Rawlins et al., 2003a; Wei et

al., 2005). Therefore, despite a general morphological similarity, differential Ed expression does not appear to play a role at this epithelial boundary.

Multiple forces contribute to morphogenesis

Although our data demonstrate that differential Ed expression generates a contractile interface that is required for proper appendage tube formation and dorsal closure, other forces also contribute to these processes. The involvement of multiple forces is best understood for dorsal closure where, in addition to the contractile actin cable at the epidermis/amnioserosa interface, apical constriction of the individual amnioserosa cells also drives the movement of the leading edge, particularly in the initial stages of the process. In later stages, interactions between filopodia of opposing leading edge cells also contribute to the completion of closure (Jacinto et al., 2002; Kiehart et al., 2000). Consistent with the involvement of multiple forces, the lateral epidermal edges do ultimately approach the dorsal midline in ed^{MZ} embryos, suggesting that the elimination of the Ed expression border specifically disrupts the actin cable, while the other forces remain functional.

The cell movements and shape changes associated with the morphogenesis of the appendage primordia appear very similar to those observed in dorsal closure. In addition to the convergence of opposing floor cell domains to form the tube floor, the individual roof cells constrict apically (Dorman et al., 2004), similar to the amnioserosa cells. This roof cell behavior is probably a consequence of roof cell fate determination rather than the absence of Ed, as *ed* mutant cells outside of this domain do not exhibit this same pronounced reduction in apical circumference. Presumably the epithelial groove generated by the coordinated apical constriction of the roof cells, together with the elongation of the floor cells, can generate the rudimentary tubes that give rise to the severely shortened and malformed appendages observed in the absence of floor closure in *ed* mutant primordia.

Given the proposed role of Ed as a homophilic adhesion molecule (Islam et al., 2003; Rawlins et al., 2003a; Spencer and Cagan, 2003), selective affinity may also contribute to morphogenesis. For example, as the anterior and medial floor cells elongate towards the midline of the primordium, preferential affinity for the opposing floor cells, which also express Ed, over the roof cells, which lack Ed, may favor floor cell association. In dorsal closure, Ed-mediated interactions between opposing leading edge cells could play a similar role. It is also possible that differential Ed expression may have a dual function, contributing to morphogenesis through generation of both a contractile interface and differential affinity between cell types.

ed mutant follicle cells do not undergo premature cell death

In irradiated cultured epithelia, a smooth contractile interface has been observed between apoptotic epithelial cells and their neighbors, suggesting that active extrusion of dying cells preserves the integrity of the epithelium (Rosenblatt et al., 2001). This effect resembles the *ed* mosaic phenotype, but the presence on the eggshell surface of imprints produced by *ed* mutant cells indicates that these cells do not die before the secretion of eggshell at the end of oogenesis. Moreover, *ed* mutant clones are not detectably smaller than their associated twin spots and we detect no evidence of DNA fragmentation or the active form of the proapoptotic enzyme caspase 3 in *ed* mutant follicle cells (data not shown), confirming that the contractile border of *ed* mutant clones is not induced by premature cell death.

Ed expression borders affect adherens junction components

We have observed reduced levels and altered distribution of DE-cad and Arm at the border between cells with and without Ed. By contrast, the distribution and level of DE-cad and Arm at the interfaces between *ed* mutant follicle cells within a clone appear normal. This observation demonstrates that, although recent evidence suggests that Ed is a component of adherens junctions (Wei et al., 2005), Ed is not generally required for adherens junction stability.

A border effect on adherens junction components has also been reported in *ed* mutant clones in the wing disc epithelium, where it has been proposed to play a causative role in the generation of a smooth clone border by mediating cell sorting (Wei et al., 2005). However, at the border of *ed* mutant follicle cell clones, this effect is frequently mild and occasionally undetectable, whereas the contractile phenotype is completely penetrant. This difference could suggest that a functionally relevant alteration in adherens junction distribution is only occasionally reflected by diminished immunoreactivity. Alternatively, this effect on adherens junction components could be instead a consequence of contraction of the actin cable assembled at the Ed expression border. Indeed, an actomyosin-based contractile force has been proposed to be capable of disrupting adherens junctions (Bertet et al., 2004; Sahai and Marshall, 2002). However, we have not observed a disruption of adherens junction components at endogenous Ed expression borders (data not shown), raising the possibility that this effect is not involved in Ed expression border function.

Local effect of Ed expression borders on the actin cytoskeleton

How an Ed expression border induces the local assembly of a contractile actin cable remains unclear. A potential connection between Ed and the actin cytoskeleton is suggested by the reported interaction between Ed and Canoe (Cno), which is homologous to mammalian Afadin and contains a actin filament binding domain, suggesting that Ed may function as a Nectin, the Afadin binding partner (Takai and Nakanishi, 2003; Wei et al., 2005). However, in *ed* mosaic wing imaginal discs, Cno distribution is altered throughout *ed* mutant clones, not just at the border (Wei et al., 2005). This observation does not exclude a role for Cno in Ed function but, because this effect on Cno is not restricted to the clone border, it alone cannot explain the localized effect on the actin cytoskeleton. Interestingly, an interaction with Ed does not appear to be strictly required for proper membrane localization of Cno, as Ed is lost from the amnioserosa during dorsal closure (see Fig. 8E′) while Cno remains detectable (Boettner et al., 2003; Takahashi et al., 1998).

An obvious distinguishing feature of Ed expression borders is the absence of Ed from the apposing face of the Ed-expressing population, presumably owing to the absence of trans homophilic interactions. The mechanism that removes or redistributes Ed from this interface, rather than the absence of Ed itself, might therefore mediate the border-specific effect on the actin cytoskeleton. If the machinery that removes Ed, e.g. through endocytosis, is not completely specific, such a model could also account for altered levels of DE-cad and Arm at these interfaces. Alternatively, the absence of homophilic interactions across Ed expression borders could favor the interaction of Ed with other factors, which could in turn mediate border specific effects.

Conclusions

Our data demonstrate a novel role for Ed in epithelial morphogenesis. In both the follicular epithelium and the embryo, the juxtaposition of cells with and without Ed is sufficient to trigger local assembly of a contractile actin cable, and the defective epithelial closure observed in the absence of Ed suggests that this structure drives cell movements. This work identifies an Ed expression border as a functional entity in this process, and demonstrates the existence of a mechanism that functions downstream of cell fate determination to convert a difference in protein expression between two cell types into a local effect on the cytoskeleton that drives epithelial movements and mediates morphogenesis. We propose that differential Ed expression between two cell populations may represent a general mechanism for regulating tissue movements.

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Supplementary material

Supplementary material for this article is available at
http://dev.biologists.org/cgi/content/full/132/16/3255/DC1

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