Spatial control of the actin cytoskeleton in *Drosophila* epithelial cells

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The actin cytoskeleton orders cellular space and transduces many of the forces required for morphogenesis. Here we combine genetics and cell biology to identify genes that control the polarized distribution of actin filaments within the *Drosophila* follicular epithelium. We find that profilin and cofilin regulate actin-filament formation throughout the cell cortex. In contrast, CAP—a *Drosophila* homologue of Adenylyl Cyclase Associated Proteins—functions specifically to limit actin-filament formation catalysed by Ena at apical cell junctions. The Abl tyrosine kinase also collaborates in this process. We therefore propose that CAP, Ena and Abl act in concert to modulate the subcellular distribution of actin filaments in *Drosophila*.

The actin cytoskeleton is a dynamic network of filaments that has a crucial role in cell and tissue morphogenesis[1]. In each cell, distinct actin-filament-based structures control such diverse activities as cytokinesis, polarized intracellular trafficking, adhesion and migration[1–4]. Tailoring F-actin to such a wide array of biological functions requires precise spatial and temporal regulation of actin dynamics, about which little is known.

Extensive biochemical analyses have given us a broad understanding of actin-filament dynamics[5,6] (reviewed in ref. 2) and have identified a host of proteins that modulate multiple steps in the cycle of actin polymerization and depolymerization *in vitro*[2,4,7–10]. For example, Ena/VASP family proteins[11] catalyse actin-filament formation, either by facilitating the nucleation of actin filaments by the Arp complex (reviewed in ref. 10) and/or, like profilin[12,13], by promoting the addition of actin-ATP monomers to the fast-growing 'barbed' end of existing filaments. Meanwhile, other proteins use a variety of mechanisms to inhibit actin-filament formation. *In vitro*, Adenylyl Cyclase Associated Proteins limit polymerization by sequestering monomeric actin[14–17], whereas cofilin/ADF catalyses filament disassembly[18]. However, so far, few studies have analysed the functions of actin-regulatory proteins in the context of an intact Metazoan organism. Here we use the *Drosophila* follicular epithelium as a model genetic system for this purpose.

In *Drosophila*, ~1,000 follicle cells form a single layered epithelium that surrounds each germline cyst (reviewed in refs 19, 20). Early during oogenesis, cells within the epithelium have a cuboidal morphology. As the egg chamber grows, the epithelium migrates posteriorly to cover the oocyte, causing most of the follicle cells to take on a columnar morphology. Then, at late stages, cells within the columnar epithelium change morphology again, extending and flattening to encompass the growing oocyte. The cuboidal/columnar follicular epithelium offers several advantages as a model system

Table 1 Nomenclature and putative gene functions for mutants used in this study

Protein name used	*Drosophila* gene name	Homologues	Putative function	Follicle cell function	References
CAP	*capulet* (*capt*)/*actup* (*acu*)	Adenyl Cyclase Associated Protein/SRV2	Actin monomer sequestration	Limits apical actin filament formation	14, 15, 17, 23, 24
Cofilin	*twinstar* (*tsr*)	Cofilin	Promotes filament disassembly	Limits cortical actin filament formation	18, 31
Profilin	*chicadee* (*chic*)	Profilin	Actin nucleotide exchange Actin monomer sequestration	Promotes cortical actin filament formation	32, 33, 34
Ena	*enabled* (*ena*)	Enabled/Mena/VASP/Evl	Promotes nucleation/ elongation of actin filaments	Promotes local actin filament formation	3, 4, 25, 26, 36
Abl	*ableson* (*abl*)	Ableson tyrosine kinase	Oncogene with nuclear and cytoskeletal functions. Binds F-actin	Controls actin and cell polarity	4, 38
Lgl	*lethal (2) giant larvae* (*l(2)gl*)	Lethal (2) giant larvae	Interacts with Dlg/Scrib	Controls cell polarity and is a tumour suppressor	35
Dlg/Scrib	*discs large/scribble* (*dlg/scrib*)	Discs large/Scribble	PDZ proteins	Controls cell polarity and is a tumour suppressor	35, 37

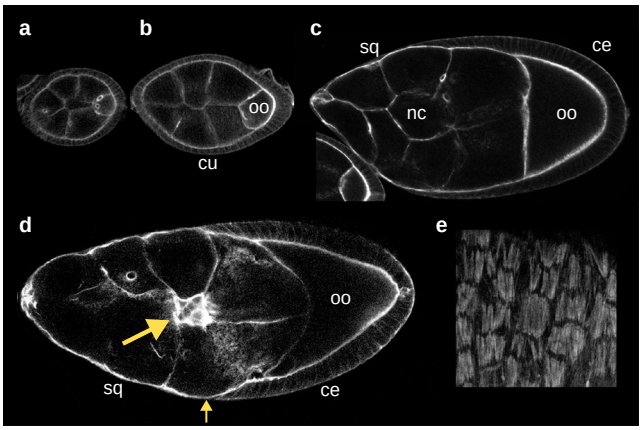

Figure 1 **F-actin staining reveals the cortices of cells in the egg chamber.** **a**, In wild-type egg chambers at different stages of oogenesis, actin filaments were revealed with TRITC-phalloidin. (In all panels the posterior is to the right.) Spherical egg chambers bud off from the germarium and consist of 16 germline cells surrounded by a cuboidal follicle-cell epithelium. F-actin is present throughout the cortex of follicle cells but is concentrated at the apical cell surface, which contacts the germline. **b–d**, As they mature, egg chambers elongate and the uniform cuboidal epithelium changes morphology. At stage 8–9, epithelial cells move posteriorwards to form a columnar epithelium that overlies the oocyte, leaving behind a squamous epithelium supporting the nurse cells. At this time a population of 'border cells' (large arrow) migrates through the nurse-cell cluster towards the oocyte, keeping pace with the moving interface between squamous and columnar follicle cells (small arrow). Nomenclature: cuboidal epithelium (cu), squamous epithelium (sq), columnar epithelium (ce), nurse cell cluster (nc) and oocyte (oo). **e**, At late stages, as the follicle cells extend to encompass the rapidly growing oocyte, parallel actin fibers are observed at the basal surface of follicle cells. These parallel actin bundles lie perpendicular to the anterior–posterior axis of the egg chamber.

with which to study the actin cytoskeleton. First, using the FLP–FRT system, clones of homozygous mutant tissue can be generated within the epithelium, enabling a mosaic genetic analysis of actin filament organization (see Methods)[21]. Second, actin filaments at the cortices of the relatively large, highly polarized cells of the epithelium are easily made visible with tetramethyl rhodamine isothiocyanate (TRITC)-phalloidin. Moreover, in these cells F-actin is concentrated at the apical surface of the epithelium[20] (Fig. 1a–d), permitting the identification of genes that modulate the spatial organization of the *Drosophila* actin cytoskeleton[22].

Here we show that CAP, a *Drosophila* homologue of Adenylyl Cyclase Associated Proteins[23,24], controls the level and distribution of actin filaments within cells of the follicular epithelium. Our analysis shows that CAP acts in opposition to Ena[11,25–27] to limit the extent of actin-filament formation at apical adherens junctions, which link adjacent epithelial cells. The Abl tyrosine kinase, a CAP-binding protein[28] that antagonizes Ena's function in neurons[25,29,30], seems to collaborate in this process. Thus we have identified a set of genes, CAP, Ena and Abl, that seem to function together to modulate the level and distribution of actin filaments in polarized *Drosophila* cells.

Results

CAP controls the spatial organization of the actin cytoskeleton. Cells within the follicle-cell epithelium have a simple polarized actin cytoskeleton, with microfilaments concentrated at apical adherens junctions (Fig. 1a–d)[20]. This asymmetric distribution of F-actin is maintained through the pronounced epithelial movements that occur during the development of an egg. Midway through this process, as the epithelium begins to migrate, there is an increase in the level of F-actin in border cells, a group of motile follicle cells that migrate through the germline towards the oocyte (Fig. 1d, large arrow). Then, as the columnar epithelium expands to surround the egg at late stages of oogenesis, pronounced parallel actin bundles are observed at the basal cell surface of follicle cells (Fig. 1e). To identify genes regulating the spatial organization of F-actin we examined actin filaments in mutant cell clones within the follicular epithelium[21] (*Drosophila* gene nomenclature is explained in Table 1).

We began our analysis by exploring the function of CAP in follicle cells. CAP was recently identified as a gene that limits actin polymerization in the eye imaginal disc and in the *Drosophila* oocyte[23,24]. Although large *capt*-null follicle-cell clones can be generated without disrupting the shape of the epithelium, *capt* mutant cells exhibit profound defects in their actin cytoarchitecture. Strikingly, ectopic F-actin accumulates at the apical surface of *capt* clones, close to the germline (Fig. 2a–c). By comparison, actin filaments at lateral and basal cortices usually remain unaffected. On occasion, however, F-actin is also lost from the basal surface of *capt* follicle-cell clones within mature egg chambers (Fig. 2c). In apical sections through *capt* mutant cells, actin filaments are first observed accumulating at sites of cell–cell contact (Fig. 2d). Because this is where adherens junctions are situated in the wild type[20], it suggests that junctional material might nucleate new F-actin synthesis in the *capt* mutant. To test the specificity of the *capt* mutant phenotype we also analysed actin organization in *twinstar* (*tsr*) mutant cells. Because *tsr* encodes the actin-filament-severing protein cofilin[31], F-actin accumulates in *tsr* mutant cells, as it does in the *capt* mutant. In *tsr* mutant follicle cells, ectopic actin filaments form at apical, basal and lateral cortices (Fig. 2e, f), although F-actin accumulation often seems more pronounced at the basal cell surface (Fig. 2e). Frequently, *tsr* mutant follicle cells also exhibit an altered columnar morphology (Fig. 2f). These data suggest that cofilin functions throughout the cell cortex to catalyse the disassembly of actin filaments, whereas in contrast CAP functions in a polarized manner to regulate apical actin accumulation. The polar actin organization seen in *capt* mutant follicle cells might result from an asymmetric distribution of the CAP protein. However, we found with the use of immunofluorescence that the protein is evenly distributed within the cytoplasm of wild-type follicle cells (Fig. 2g, h). Therefore it seems possible that the loss of CAP might trigger apical actin filament formation indirectly, by deregulating the function of another, asymmetrically localized, protein that modulates actin filament formation.

In vitro, CAP has been shown to inhibit actin polymerization by sequestering actin monomers[14–17], mirroring its role *in vivo*, where it limits actin-filament formation[23,24]. Profilin, encoded by the *chicadee* (*chic*) gene[32], is another well-characterized monomeric actin-binding protein that can serve to promote or to inhibit actin polymerization (reviewed in ref. 33). To determine which of these biochemical activities dominate in follicle cells, we examined the phenotype of *chic*-null mutant clones. In *chic* mutant columnar follicle cells, levels of F-actin seem markedly decreased at all cortices, although actin filaments are lost preferentially from the basal cortex (Fig. 3b). Thus, profilin promotes actin polymerization in follicle cells as it does in the *Drosophila* germline and in imaginal discs[24,34]. To test whether profilin is also required for the formation of apical actin aggregates in *capt* mutant cells, we looked at actin filaments in *chic capt* double-mutant cells (Fig. 3c; compare with Fig. 3a, b). In clones of the double mutant, ectopic actin filaments are not formed, a result consistent with observations made by Benlali *et al.* in the eye[24]. Thus, profilin is required for actin-filament formation at the cortex of wild-type follicle cells and for the synthesis of apical F-actin aggregates in the *capt* mutant. Interestingly, *chic capt* double-mutant clones exhibit an additional morphological phenotype not seen in clones of either single mutant. Double-mutant cells lose their columnar morphology and collapse, forming a thin squamous-like

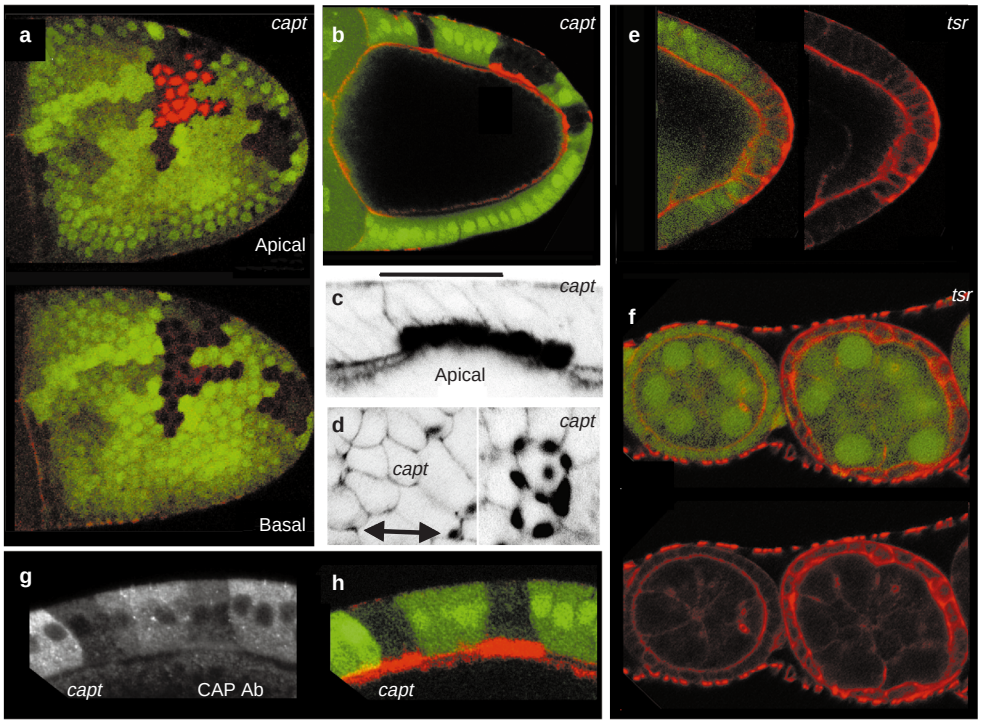

Figure 2 **CAP regulates actin dynamics differently at opposite poles of an epithelial cell. a**, Ectopic F-actin is observed in apical sections through a *capt* follicle-cell clone. F-actin was revealed in apical and basal sections with TRITC-phalloidin (red), and mutant clones were detected by the absence of GFP. Because the egg chamber is curved, F-actin is seen only in those cells whose apical cortex lies within the plane of the section. **b**, **c**, Ectopic actin is clearly seen at the apical surface of all *capt* mutant cells in a cross section. **c**, In addition, cells occasionally lose F-actin from the basal cortex. Homozygous mutant cells are marked with a line to denote the absence of GFP and F-actin is shown in black. **d**, Within early egg chambers, ectopic actin filaments are first observed in apical sections at adherens junctions linking adjacent *capt* mutant cells. Actin filaments might therefore be nucleated at these sites in the *capt* mutant (for example, see arrows). **e**, **f**, In *tsr* mutant follicle-cell clones, actin filaments form close to the membrane at apical, basal and lateral sites. (Compare GFP-marked wild-type tissue with *tsr* mutant tissue). **g**, **h**, CAP is uniformly distributed in wild-type follicle cells (as detected by immunofluorescence with an anti-CAP antibody (Ab)) but is lost from *capt* mutant clones (marked by the absence of GFP in **h**).

layer of cells (Fig. 3d, e). The loss of CAP therefore perturbs cell architecture in the *chic* mutant, even though a corresponding change in the level of cortical F-actin is not observed (Fig. 3c–e). Moreover, the *chic capt* double-mutant phenotype shows that an ordered actin cytoskeleton is likely to be crucial for the proper morphogenesis of the columnar epithelium.

Epithelial polarity is maintained in *capt* mutant cells. Our initial analysis of CAP in the *Drosophila* germline showed that this protein is required for proper oocyte polarity[23]. To determine whether CAP is also required for the establishment and/or maintenance of follicle-cell apical–basal polarity, we tested the localization of microtubules, Crumbs, α-spectrin and β-spectrin in *capt* clones. With these markers, we find that *capt* mutant follicle cells retain many aspects of their wild-type epithelial polarity despite the profound change in actin organization (Fig. 3f–i). Thus, it is possible that apical actin-filament formation in *capt* mutant clones is localized by the action of the cell's apical–basal targeting machinery. Alternatively, proximity to the germline could define the site of preferential apical actin-filament formation in a *capt* mutant cell. To distinguish between these two possibilities we generated clones within the columnar epithelium that lacked both *capt* and *lethal(2)giant larvae* (*l(2)gl)* gene functions (because *l(2)gl* is required cell-autonomously for the maintenance of epithelial polarity)[35]. In *capt l(2)gl* double-mutant cells, F-actin is frequently seen accumulating at a single, randomly positioned site within the cell (Fig. 3j, k). Thus, although components of the apical–basal targeting machinery are not required to limit actin-filament formation to a single site, they are necessary to target actin-filament formation to the apical surface of *capt* mutant cells. These data led us to postulate the existence of a protein that is targeted to apical junctions and that promotes local actin-filament formation after the loss of CAP. As a potential candidate we turned to Ena.

Ena is an important regulator of epithelial F-actin organization in *Drosophila.* Ena family members are thought to be key regulators of actin-filament dynamics[8,9,26]. In mammalian cells they promote actin-filament formation and are localized at adherens junctions and focal contacts[3,36]. To determine whether *Drosophila* Ena shares similar properties, we analysed the distribution and function of Ena within follicle cells. In early egg chambers, Ena protein is concentrated at the apical cell cortex of wild-type follicle cells (Fig 4a–d). Subsequently, as the epithelium begins to migrate, the level of Ena increases, particularly within posterior follicle cells and in motile border cells (data not shown). At this stage the protein remains localized together with F-actin at the apical surface of the epithelium (Fig 4e–g). However, Ena is also observed in punctate cytoplasmic structures that lack coincident actin filaments (Fig. 4e–g). These structures are present in follicle cells throughout oogenesis but become more striking as the egg chamber develops. Finally, at late stages of oogenesis, Ena protein becomes concentrated at the basal surface of the follicular epithelium, flanking stress-fibre-like actin filaments (Fig. 4h). To locate Ena more precisely, we also compared the distribution of Ena with that of Armadillo (Arm) (Fig. 4i–k). Arm is the *Drosophila* β-catenin homologue and is localized at apical adherens junctions interconnecting cells within the

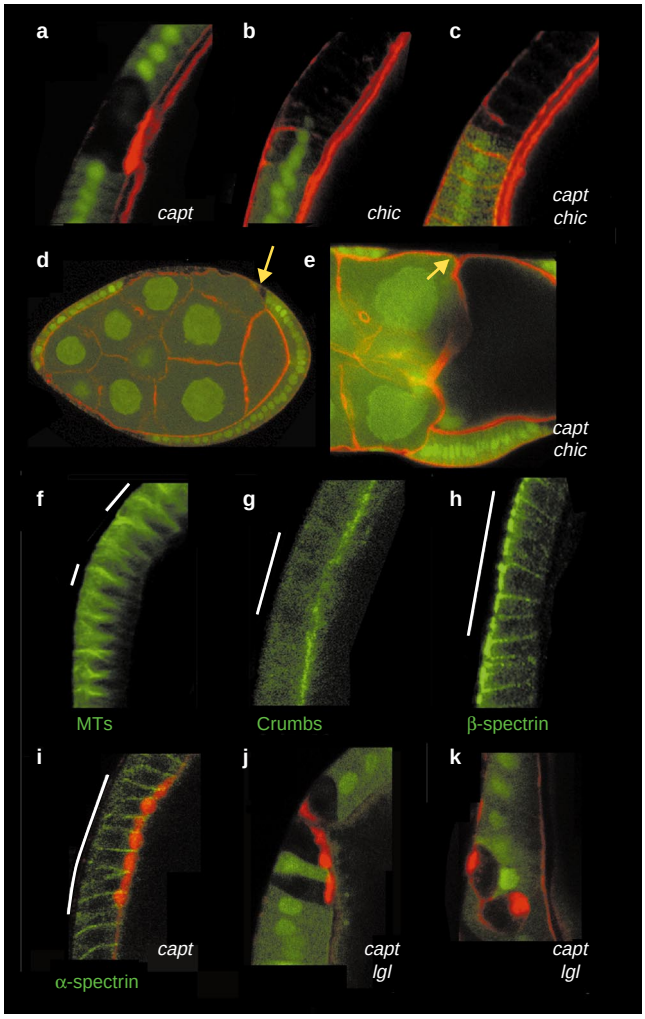

Figure 3 **A genetic analysis of the *capt* F-actin phenotype. a**, **b**, *capt* and *chic* have opposing activities. F-actin was revealed with TRITC-phalloidin (red). *capt* (**a**) and *chic* (**b**) follicle-cell clones were detected by the absence of GFP. *chic* mutant clones exhibit decreased levels of F-actin at cell cortices. Basal actin filaments are lost preferentially. **c**, *capt chic* double-mutant cells do not accumulate ectopic actin filaments. Clones seem to have levels of F-actin that are similar to those in *chic* mutant cells. **d**, **e**, *capt chic* double-mutant clones also exhibit severe morphological defects. Cells are unable to maintain their wild-type columnar morphology. Mutant clones therefore interfere with the migration of wild-type portions of the epithelium (**e**, arrow indicates a very thin, flat clone that blocks migration of the wild-type epithelium). However, *capt chic* mutant cells maintain extensive contacts with adjacent wild-type cells (arrow in **d**). **f–l**, The subcellular distributions of microtubules (MTs), Crumbs, β-spectrin and α-spectrin remain relatively unaffected by a loss of CAP. Proteins were revealed by immunolocalization in mosaic egg chambers (green); F-actin is shown in red in **i**. *capt* mutant tissue is marked by white lines. Although properly localized, Crumbs protein seems to have a more diffuse distribution in *capt* clones (**g**), implying some disturbance at the apical cell surface (as observed in Figs 2c and 3a). **j**, **k**, *l(2)gl capt* double-mutant clones accumulate F-actin at a single randomly positioned cortical site.

epithelium[20]. We find that Ena and Arm localize together apically in wild-type follicle cells (Fig. 4k). Thus, through much of oogenesis, Ena is concentrated together with F-actin and Arm at follicle-cell adherens junctions, the site of ectopic actin-filament formation in *capt* mutant cells (Fig. 2d).

As a test of Ena function, we generated follicular *ena* clones (Fig 4l–n). Cells homozygous for hypomorphic *ena* alleles (ena^{210}, ena^{23}) lose cortical actin filaments from apical, basal (Fig. 4l, m) and lateral sites (Fig. 4n). However, whereas *chic* clones preferentially lose basal actin filaments, *ena* mutant cells also exhibit a marked decrease in the amounts of apical F-actin. This might reflect the fact that Ena is concentrated at apical junctions in the wild type, whereas profilin has a broader cellular distribution[34]. We conclude that Ena facilitates actin-filament formation in *Drosophila*, much as it does in mammalian cells[3].

In mammalian cells, the overexpression of Ena homologues is able to induce ectopic actin-filament formation[26]. To determine whether Ena can promote excessive actin-filament formation in *Drosophila*, we overexpressed Ena in follicle cells (Fig. 4o) and, in a separate experiment, in the germline (Fig. 4p). In both cell types we find that Ena is able to induce the formation of novel F-actin structures at sites where Ena aggregates accumulate. Thus, Ena is likely to be a critical determinant of the subcellular distribution of actin filaments within a cell.

Given Ena's important role in the control of epithelial actin organization, we generated *ena capt* double-mutant clones to test whether Ena is also required for the formation of apical actin aggregates in the *capt* mutant (Fig. 4q–t). Because *ena* and *capt* genes are located on opposite arms of chromosome II, this is an experimental challenge (see Methods). However, we recombined the mutants onto a double FRT chromosome to generate double-mutant cells that were identified by the absence of green fluorescent protein (GFP) and the loss of CAP (Fig. 4q, r) or Ena protein (Fig. 4s, t). F-actin aggregates do not form in the double mutant lacking both Ena and CAP functions, and *ena capt* clones frequently lose actin filaments (Fig. 4q, t). This result places Ena genetically downstream of CAP. Thus, like profilin, Ena is required for the synthesis or nucleation of actin filaments at adherens junctions in the *capt* mutant.

Abl cooperates with CAP to control the level and localization of F-actin. Given that the Abl tyrosine kinase binds mammalian CAP[28] and antagonizes the function of Ena in *Drosophila*[9,25], we tested whether Abl, like CAP and Ena, might have a role in the control of F-actin organization in the follicular epithelium. Clonal analysis reveals that loss of Abl causes subtle defects in F-actin organization. In abl^4 mutant cells, apical actin filaments are often mislocalized, appearing at elevated levels at lateral cell cortices (Fig. 5a, arrows). In addition, *abl* clones exhibit severe defects in epithelial architecture (Fig. 5a–c), with mutant tissue forming a multilayered epithelium close to the posterior pole of the egg chamber (Fig. 5a, c) and, to a smaller extent, at the anterior pole (Fig. 5b). A similar phenotype has been described in *dlg/l(2)gl/scrib* mosaic egg chambers[35,37]. Because these genes are required for proper epithelial cell polarity[35], Abl might also regulate the polarity of follicle cells. As a further perturbation of Abl function we used the heat-shock Gal4 driver to express the protein at high levels within the follicular epithelium. Like the *abl* loss of function, high-level overexpression of Abl also perturbs epithelial architecture, leading to the formation of multiple layers of cells at the posterior pole of the egg chamber (Fig. 5d). Thus Abl functions to modulate follicle-cell F-actin organization and cell polarity and must be tightly regulated in follicle cells to maintain proper epithelial character.

To test genetically for an interaction between Abl and CAP, we expressed Abl at more moderate levels (using the T155 Gal4 driver; see Methods) in egg chambers containing *capt* mutant clones. Although a modest overexpression of Abl has little visible effect on wild-type follicle cells (Fig. 5f, g; GFP-marked cells, and data not shown), an increase in Abl expression in *capt* mutant follicle cells has profound effects (Fig. 5f, g; compare with Fig. 5e). Increased levels of Abl protein alter both the level and distribution of actin filaments in *capt* mutant cells. The formation of large localized F-actin aggregates seems to be suppressed and actin filaments often become more widely distributed around the cell cortex (Fig. 5f, g), as observed in *abl* mutant clones. In addition, in a minority of egg

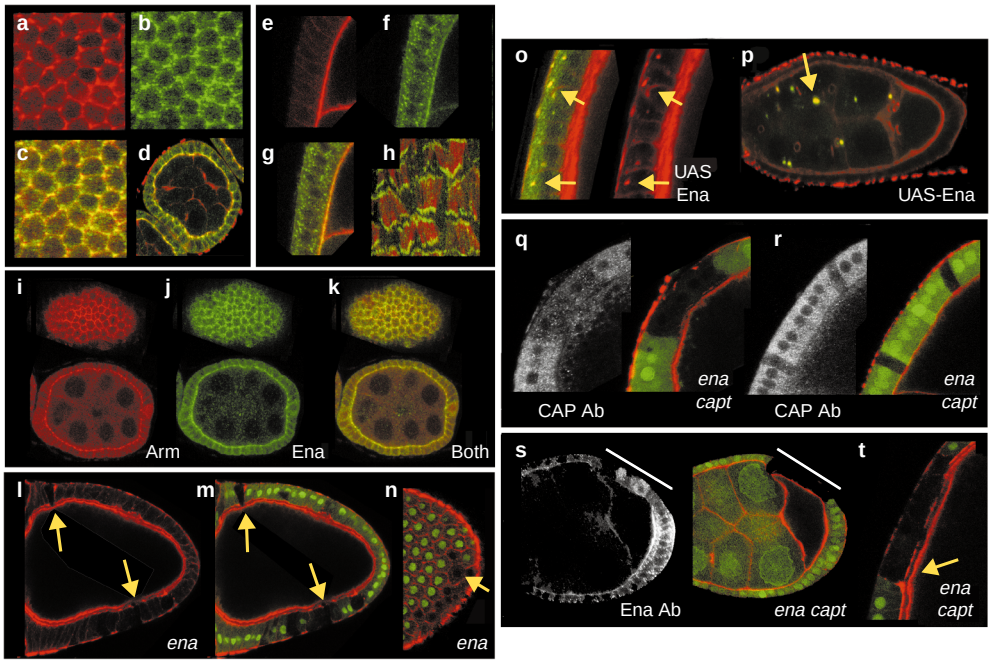

Figure 4 **Ena is a key regulator of the epithelial actin cytoskeleton. a**–**d**, F-actin (red) and Ena (green) localize together (yellow), primarily at the apical cell cortex of wild-type follicle cells in apical section (**a**–**c**) and cross section (**d**) through an early-stage egg chamber. **e**–**g**, Ena expression is upregulated during mid-oogenesis. At this stage F-actin (red) and Ena (green) remain localized together at the apical cell surface, but Ena is also seen in punctate cytoplasmic structures that lack F-actin. **h**, Ena (green) protein flanks parallel actin filaments (red) at the basal surface of the mature follicular epithelium, in a section parallel to the plane of the epithelium. **i**–**k**, Arm (red) and Ena (green) localize together at the apical cell cortex in two sections through an egg chamber. **l**–**n**, *ena* mutant follicle cells lose cortical F-actin. Actin filaments are lost from apical, basal (arrows in **l** and **m**) and lateral cortices (arrow in **n**) of *ena*[23] mutant cells. **o**, **p**, Overexpression of Ena in the follicular cell layer (with the CY2 Gal4 driver) and in the germ line (with the V32 Gal4 driver) induces the formation of punctate cytoplasmic actin-filament-based structures (red) associated with clumps of Ena (green). Arrows highlight Ena/F-actin aggregates (yellow). **q**–**t**, *ena capt* double-mutant cells were generated and identified with GFP to mark loss of one chromosome and immunofluorescence to mark the loss of the other (CAP in **q** and **r**, Ena in **s** and **t**; see Methods). In *ena capt* double-mutant cells, ectopic actin filaments (red) do not form and F-actin levels often seem decreased, as observed in *ena* mutant cells. The F-actin occasionally observed accumulating within *ena capt* mutant cells (arrow in **t**) might be due to the relative timing of loss of CAP and Ena. Ab, antibody.

chambers, the combination of Abl overexpression and loss of CAP causes a profound disruption of epithelial morphology (data not shown). This genetic interaction between *capt* and Abl implies that the two genes have related functions in the control of epithelial F-actin organization.

Localization of Ena and Abl in *capt* mutant cells. Finally, having found genetic evidence to suggest that Ena and Abl cooperate with CAP in the control of epithelial F-actin, we examined the distribution of Ena and Abl proteins in *capt* mutant follicle cells. First we looked at Ena protein. In the *capt* mutant, Ena's distribution is altered so that the majority of the protein becomes localized with apical actin filaments (Fig. 6a, b). Thus Ena is found tightly associated with apical F-actin both in the wild type and in *capt* mutant cells. In contrast, significant amounts of Ena are not observed at the apical surface of *capt chic* double-mutant cells, in which apical F-actin aggregates are not formed (Fig. 6c). Therefore, both in the wild type and in various mutants, the amount of Ena present at adherens junctions closely parallels the level of apical F-actin. We also examined the localization of Abl in the wild type and in *capt* mutant tissue (Fig. 6d, e). Abl, like CAP, seems to have a diffuse staining pattern within wild-type follicle cells. However, in *capt* clones, Abl protein becomes concentrated at the apical cell surface, partly localizing with Ena (Fig. 6e). Thus, a loss of CAP leads to a change in localization of both Ena and Abl. Because these proteins act together with CAP to control the spatial organization of the follicular actin cytoskeleton, their altered distribution is likely to contribute to the generation of the marked *capt* mutant phenotype.

Discussion

Although many proteins have been identified that regulate actin-filament dynamics, it is not known how cells generate an ordered network of actin filaments. Here we use *Drosophila* genetics and the follicular epithelium to characterize how various actin-binding proteins act to regulate the spatial organization of F-actin. Our results show that actin dynamics are regulated by distinct mechanisms within different domains of a polarized epithelial cell. CAP, Ena and Abl seem to modulate apical actin-filament formation, whereas cofilin and profilin seem to have a more global function, regulating cortical actin-filament dynamics throughout the cell. Moreover, the accumulation of F-actin at apical, basal and lateral sites in *tsr* mutant follicle cells and the loss of cortical actin filaments in *chic* mutant cells indicates that cortical actin filaments are turned over continuously throughout the cell. This being so, it is striking that F-actin becomes so highly polarized in the *capt* mutant.

In vitro, CAP has been shown to inhibit actin polymerization[14–17], which is consistent with its role in limiting epithelial actin-filament formation[23,24]. In such assays, CAP seems to block actin polymerization by sequestering actin monomers[14–17]. However, CAP protein and monomeric actin (data not shown) seem evenly distributed within the follicular epithelium. Because the loss of a uniformly distributed, actin-monomer sequestering protein would not be expected to result in the polarized accumulation of F-actin, we considered it possible that CAP might function by inhibiting the activity of a protein at apical adherens junctions that promotes

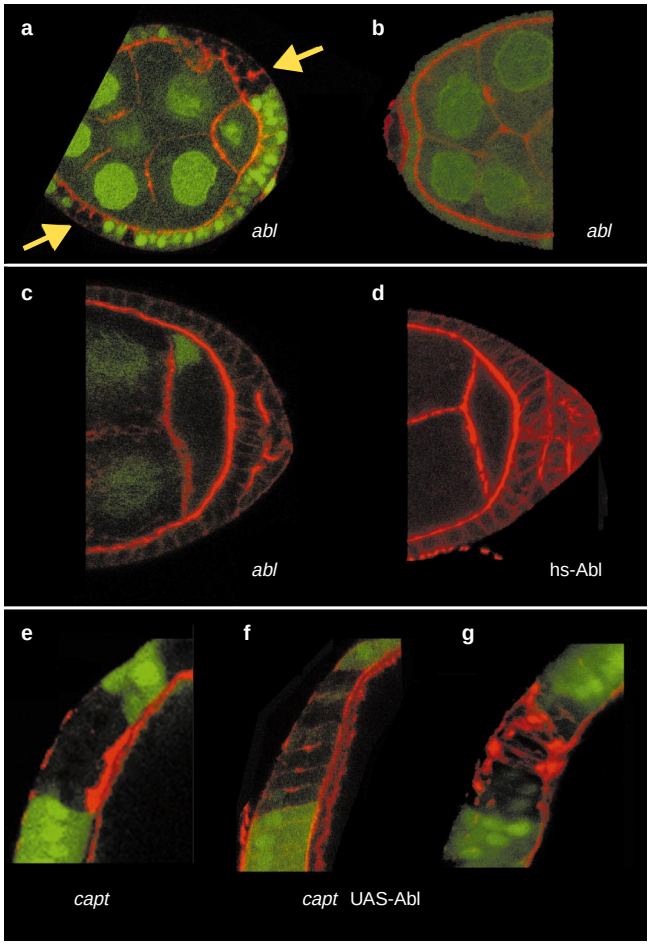

Figure 5 **Abl controls actin and cell polarity in follicle cells. a**, The actin cytoskeleton is moderately disorganized in *abl*[4] mutant clones (marked by a loss of GFP). F-actin (red) is frequently seen at ectopic sites, particularly at lateral cell surfaces (arrows). **b**, **c**, This phenotype seems to worsen as the egg chamber develops, leading to disruption of the epithelial sheet and the formation of multiple cell layers at anterior (**b**) and posterior (**c**) poles of the egg chamber. **d**, Like the *abl* loss of function, high-level Abl expression also leads to multi-layering of the epithelium. UAS-Abl was expressed in the follicular epithelium with hs-Gal4. **e**–**g**, Moderate Abl overexpression (from the T155-Gal4 driver) perturbs cell morphology and alters the level and distribution of F-actin in *capt* mosaic egg chambers, but has little effect on wild-type tissue (GFP-positive cells) (**f**, **g** (compare with **e**). In addition, ectopic Abl can markedly perturb the morphology of the entire follicular epithelium (data not shown).

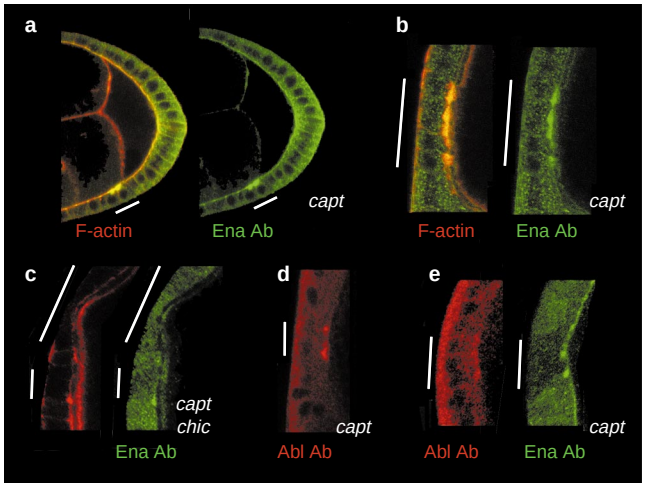

Figure 6 **Ena and Abl localize together with ectopic actin filaments in *capt* mutant cells. a**, **b**, Ena (green) localizes with apical F-actin aggregates (red) in *capt* mutant cells, denoted by the white line. **c**, F-actin (red) and Ena (green) do not accumulate at the apical cortex of *chic capt* double-mutant cells, implying that F-actin (or profilin itself) recruits Ena to the apical domain of the cell in the *capt* mutant. **d**, Abl (red) seems diffuse in wild-type follicle cells, but accumulates at the apical domain of *capt* mutant cells, denoted by the white bar. **e**, In *capt* mutant cells, a subpopulation of the Abl protein (red) becomes localized with Ena (green) at the apical cell cortex. Ab, antibody.

local actin-filament formation. Given Ena's ability to promote F-actin-filament assembly in mammalian cells[9,26,36], we considered Ena as a potential target of CAP activity. Our cell-biological and genetic analysis of Ena within the *Drosophila* follicular epithelium supports this idea. Ena is concentrated together with F-actin at apical adherens junctions, and the level of Ena protein closely parallels the extent of local actin-filament formation. These results indicate that Ena has an important role in dictating the spatial organization of the actin cytoskeleton in *Drosophila* follicle cells. Moreover, we find that Ena is required for the synthesis of apical actin aggregates in the *capt* mutant, which adds strength to the hypothesis that CAP might inhibit apical actin-filament formation catalysed by Ena. However, it is important to note that Ena is also present in cytoplasmic aggregates that lack concomitant F-actin and that in the wild type Ena localizes with F-actin primarily at the apical cell cortex (Fig. 4). The presence of Ena is therefore not sufficient to induce local actin polymerization, and its ability to catalyse actin-filament formation might be augmented at adherens junctions. Finally, homologues of Ena have been shown to localize to focal contacts and adherens junctions in mammalian epithelial cells in culture[3], suggesting that Ena might have an evolutionarily conserved function to control actin-filament formation at these sites.

Although profilin binds Ena[11] and is required for F-actin formation within the wild type and in *capt* mutant follicle-cell clones, profilin seems to differ from Ena in two respects. First, we find that the overexpression of profilin has little effect on the level or distribution of F-actin (data not shown). Second, profilin protein is not localized within follicle cells[34]. Therefore profilin might have a general function within cells, facilitating actin-filament formation throughout, whereas Ena catalyses actin-filament formation at specific subcellular sites.

Like CAP and Ena, Abl is also required for proper F-actin organization within the follicle-cell epithelium. Interestingly, Abl also exhibits a cell-polarity phenotype reminiscent of that seen in *l(2)gl/dlg/scrib* mutants[35]. Furthermore, the activity of Abl has a potent effect on the organization of F-actin in follicle cells lacking CAP. This is different from that observed in *capt l(2)gl* mutant cells because excess Abl generates more diffusely localized ectopic F-actin (compare Fig. 5f, g with Fig. 3j, k). The synergistic interaction between CAP and Abl suggests that these two proteins act in the same pathway. However, because Abl alters the organization of actin within *capt* mutant cells, Abl and CAP are unlikely to be components in a simple linear signalling cascade. One possibility is that Abl modulates the integrity of adherens junctions, where Ena and CAP seem to act. In support of the idea that CAP and Abl have related functions, mammalian homologues of CAP and Abl have been shown to interact physically, indicating that this relationship might be conserved in mammals[28]. Moreover, actin aggregates reminiscent of those seen in the fly *capt* mutant cells are formed in the neurons of *abl* mutant mice[38]. Thus Abl might modulate actin-filament formation in

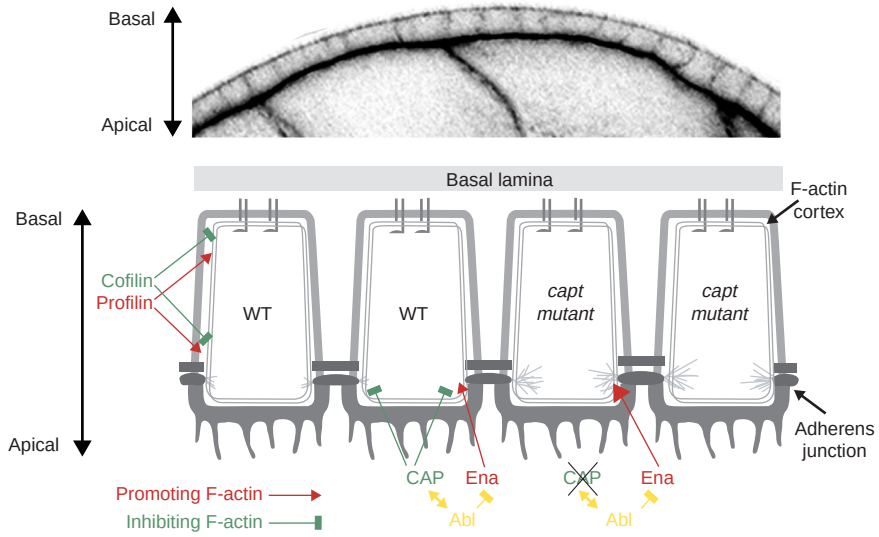

Figure 7 **A model for the regulation of F-actin organization in the follicular epithelium.** We propose this model to explain the role of CAP, Ena, Abl, profilin and cofilin in the control of the spatial organization of F-actin in cells of the follicular epithelium. CAP limits actin-filament formation at cell–cell junctions, at a site determined by the machinery controlling epithelial polarity. At early and intermediate stages of oogenesis, Ena is concentrated at apical junctions, where it is required for actin-filament formation. In the absence of CAP (compare labelled wild-type (WT) with *capt* mutant cells) the regulation of Ena is compromised, leading to excess local actin-filament formation. F-actin accumulating at apical junctions then recruits more Ena[3,36] from the cytoplasm (together with Abl), promoting further apical actin-filament formation. Thus, the loss of CAP initiates an explosive cycle of local actin-filament formation and Ena recruitment, culminating in the formation of vast amounts of F-actin within the apical domain of the cell. In contrast, profilin (encoded by *chic*) and cofilin (encoded by *tsr*) have less polar functions modulating the level of actin-filament formation at the cortex throughout the cell.

multiple organisms and cell types, perhaps by cooperating with Ena and CAP.

Bringing these data together, we envisage the model in Fig. 7 to explain the aetiology of the pronounced *capt* mutant phenotype. Normally, in the columnar epithelium, Ena protein is concentrated at adherens junctions, where it promotes local F-actin synthesis. This activity of Ena is counterbalanced by CAP, which limits the amount of apical F-actin. Excess F-actin therefore forms at apical junctions in *capt* mutant cells. This newly formed apical F-actin is able to recruit additional molecules of Ena from the cytoplasm, because Ena binds microfilaments[3,36], which leads to further actin-filament formation. This explains why, in the absence of apical F-actin aggregates, Ena does not become concentrated at the apical cortex of cells in the *capt chic* mutant. Thus, the loss of CAP initiates an explosive cycle of local actin polymerization and Ena recruitment at adherens junctions, culminating in the striking polar actin aggregates observed in *capt* mutant cells. We speculate that within wild-type epithelial cells, controlled autocatalytic cycles of actin-filament formation of this type might help to limit the accumulation of actin filaments to a single site within a cell. For instance, during the formation of a *Drosophila* wing hair, a similar process might be required to generate a single bundle of actin filaments at the apical cortex of the epithelium[39].

In *Drosophila*, Ena and Abl are thought to be part of a signalling pathway that changes local actin polymerization within the growth cones of neurons to guide axonal pathfinding. However, it has not been possible so far to analyse the cytoskeletal consequences of perturbations in Ena and Abl function directly within *Drosophila* neurons[4]. The easily visible, asymmetric actin cytoskeleton in follicle cells has allowed us to define cell-biological roles for these actin-regulatory genes. In these cells, CAP, Ena and Abl modulate actin-filament assembly at specific subcellular sites, probably by altering local actin dynamics. Thus, by analogy, these proteins might alter the site of actin-filament formation in response to signalling in neurons. If so, it will be interesting to determine whether similar signals impinge on this putative CAP/Ena/Abl pathway in neurons and in epithelia. Finally, given the fact that CAP, Ena and Abl control actin cytoskeletal organization in multiple tissues[4,23,24] and in different organisms[3,9,26,38], these genes might have a conserved function, acting together to control the distribution of actin filaments in many other types of polarized animal cell. □

Methods

Genetics.

The following mutations were used: $capt^{10}$, $chic^{05205a}$, $l(2)gl^{4}$, ena^{210}, ena^{23}, tsr^{1} and tsr^{2} and abl^{4}. ($capt^{10}$, $chic^{05205a}$, $l(2)gl^{4}$ are null mutations; ena^{210}, ena^{23}, tsr^{1} and tsr^{2} are hypomorphic mutations). To generate mosaics, mutations were recombined with the appropriate FRT elements (*FRT40A* for 2L, *FRTG13* for 2R, and *FRT2A* for 3L (as explained in ref. 40)). Mosaics were generated as described previously by using the X-linked hs-FLP insertion *FLP 22* (ref. 21). Mutant clones were detected by the loss of a GFP marker, *Ubi-GFP*, distal to *FRT40A*, *FRTG13* or *FRT2A* (from D. Bilder). To generate follicle clones by using hs-FLP , a 1-h 37 °C heat shock was administered to adult flies on three consecutive days, and flies were then left to recover for ~1 day. *ena capt* double-mutant clones were generated in *hs-FLP 22; $capt^{10}$ FRT40A FRTG13 ena/Ubi-GFP FRT40A FRTG13* flies and separately in *hs-FLP 22; $capt^{10}$ FRT40A FRTG13 ena/FRT40A FRTG13 Ubi-GFP* flies. Loss of both *capt* and *ena* was detected by the loss of staining for both GFP and Ena in the first instance and by the loss of GFP and CAP protein in the second. Hypomorphic *ena* alleles were used and mutant clones were monitored by the redistribution of Ena and by a decrease in the level of the protein. Most clones seemed to be homozygous for both mutant loci. Occasional *ena capt* mutant cells were seen with ectopic apical actin filaments (Fig. 4t). This is expected because the amount of F-actin formed depends on the temporal order of loss of CAP and Ena. V32 and CY2 GAL4 drivers (from D. St Johnston and T. Schupbach, respectively) were used to drive the expression of UAS-Ena (from D. Van Vactor) in the germline and follicle cells, respectively. Although this is a UAST construct[41], Ena is well expressed within the germline. UAS-*Abl* (from D. Van Vactor) was expressed in *capt* mutant clones with the *T155-Gal4* driver[21]. To enhance T155-Gal4 expression, flies were grown at 29 °C.

Tissue analysis.

Egg chambers were stained with fluorescein isothiocyanate (FITC)/TRITC-phalloidin (Molecular Probes) before mounting in anti-fade and glycerol (Molecular Probes). The following antibodies were used: anti-Ena (gift from M. Hoffmann and D. Van Vactor), anti-β-spectrin (gift from D. Brenton), anti-α-spectrin (DSHB Iowa), anti-microtubule (Sigma), anti-Arm (DSHB Iowa), anti-Abl (gift from M. Hoffmann and D. Van Vactor), anti-Crumbs (gift from E. Knust) and anti-CAP (see ref. 31). Antibodies were used at various dilutions and incubated with tissue overnight in 0.5% BSA, 5% NHS PBT. FITC-, TRITC- or Cy5-labelled secondary antibody was used at a dilution of 1:250 (Molecular Probes). G-actin was assayed with labelled DNase I staining (Molecular Probes). For co-localization

experiments, channels were analysed separately. Actin was detected with the green channel to prevent bleed-through of signals between channels. Images were subsequently overlaid in false colours with Adobe Photoshop.

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