# A cyclase-associated protein regulates actin and cell polarity during *Drosophila* oogenesis and in yeast

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**Background:** A polarised cytoskeleton is required to pattern cellular space, and for many aspects of cell behaviour. While the mechanisms ordering the actin cytoskeleton have been extensively studied in yeast, little is known about the analogous processes in other organisms. We have used *Drosophila* oogenesis as a model genetic system in which to investigate control of cytoskeletal organisation and cell polarity in multicellular eukaryotes.

**Results:** In a screen to identify genes required for *Drosophila* oocyte polarity, we isolated a *Drosophila* homologue of the yeast cyclase-associated protein, CAP. Here we show that CAP preferentially accumulates in the oocyte, where it inhibits actin polymerisation. CAP also has a role in oocyte polarity, as *cap* mutants fail to establish the proper, asymmetric distribution of mRNA determinants within the oocyte. Similarly in yeast, loss of CAP causes analogous polarity defects, altering the distribution of actin filaments and mRNA determinants.

**Conclusions:** This study identifies CAP as a new effector of actin dynamics in *Drosophila*. As CAP controls the spatial distribution of actin filaments and mRNA determinants in both yeast and *Drosophila*, we conclude that CAP has an evolutionarily conserved function in the genesis of eukaryotic cell polarity.

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## Background

Multicellular organisms contain a plethora of cellular forms and functions. In order to pattern an undifferentiated cellular space, symmetry must first be broken, for example by a localised extracellular signal. Then polar filaments are required to communicate this spatial information to all parts of the cell [1]. In multicellular eukaryotes, the precise mechanisms by which cells organise the actin cytoskeleton and generate polarity are poorly understood, in part because of the difficulty of generating mutants that lack important cell-biological functions. To identify such mutations, we conducted a genetic screen in *Drosophila* which relies on the ability to generate mutant germline tissue in a mosaic animal [2].

During *Drosophila* oogenesis, anterior–posterior (A–P) and dorsal–ventral (D–V) axes are established in a process that requires both actin and microtubule cytoskeletons. The dynamic polarity of the developing oocyte is easily visualised by determining the asymmetric localisation of mRNA determinants, for example *bicoid* and *oskar* [3]. At early stages of oogenesis, a single microtubule-organising centre situated at the posterior of the nascent oocyte nucleates a microtubule network which supports the polarised traffic of cellular materials, including mRNAs, from nurse cells into the oocyte [4]. Then at stage 6–8, the oocyte microtubule cytoskeleton is reorganised in response to a signal from the overlying posterior follicle cells [5–7]. The resulting polarised microtubule array is thought to mediate the transport of *bicoid* and *oskar* mRNAs to opposite poles of the oocyte, where they are used to pattern the future A–P axis of the embryo [3]. At late stages, the polar A–P microtubule array is disassembled while microtubules form at the oocyte cortex, driving mixing of the oocyte cytoplasm in a process termed cytoplasmic streaming [3]. While it is well established that microtubules mediate the polar transport of mRNAs during *Drosophila* oogenesis, the role of actin in mRNA localisation is less clear. Mutations or drugs that perturb the actin cytoskeleton disrupt mRNA localisation, however, by inducing premature cytoplasmic streaming [8–10]. Furthermore, at late stages of oogenesis and in the early embryo, tropomyosin, a protein that stabilises actin filaments, is required to correctly localise *oskar* mRNA at the posterior pole [11–13]. Therefore, although actin filaments appear evenly distributed within the wild-type oocyte during establishment of polarity, an intact actin cytoskeleton is required to maintain polarisation of the microtubule array. Then, following disassembly of the A–P-polarised microtubule array, an actin-based structure may be necessary to anchor mRNA determinants at the posterior pole during streaming. Similarly, F-actin is required for the asymmetric distribution of mRNAs in other *Drosophila* tissues, for example neuroblasts [14,15].

In a screen to isolate mutations perturbing the proper organisation of the actin cytoskeleton and oocyte polarity, we identified a *Drosophila* homologue of the cyclase-associated

proteins (CAPs), which we have named *capulet* (*cap*). CAP was first cloned from yeast [16,17], and CAP homologues from a variety of organisms have been shown to associate with adenylate cyclase [18–20], Abl tyrosine kinase [21], and monomeric actin (G-actin) [22–25]. As the domain structure of CAP is similar in yeasts, plants and animals, CAP may have a conserved role, linking signal transduction to reorganisation of the actin cytoskeleton. In this paper we present an analysis of CAP function in the control of actin organisation and cell polarity in both the *Drosophila* germline and in yeast. We show that CAP is a major regulator of actin dynamics in *Drosophila*, and that CAP functions in both animal cells and fungi to organise the polarised distribution of F-actin and mRNAs.

## Results

### Identification of a *Drosophila* homologue of the yeast cyclase-associated protein

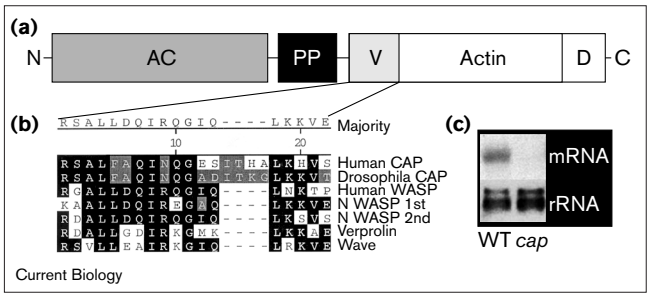
In a mosaic screen to isolate mutations that perturb actin organisation in germline clones we identified a mutation in a novel gene *capulet* (*cap*) (Figure 1a). *cap* was independently identified by two other labs ([26], and Z. Wills *et al.*, unpublished data) and encodes a protein of 424 amino acids (Figure 1a; GenBank accession number AF132566) that is ~50% identical and ~65% similar to both human CAPs [27]. *Drosophila* CAP is represented by a single ~2.4 kb transcript during development, which is absent from homozygous $cap^{10}$ mutant larvae (Figure 1c). CAPs have been shown to inhibit actin polymerisation *in vitro*, by sequestering monomeric actin [22–25]. This actin-binding activity has been mapped to the carboxy-terminal region of CAP [18,23]; however, our analysis identified a 'verprolin homology'-related domain [28] in all CAPs, just carboxy-terminal of the polyproline-rich domain (Figure 1b). In members of the verprolin/WASP family, this motif binds actin monomers *in vitro*, but catalyses actin polymerisation *in vivo* [29,30]. Therefore, in CAP homologues, this region of the protein may be used to facilitate actin binding. As CAP proteins have also been found associated with Abl tyrosine kinase [21] and with adenylate cyclase [18], it is possible that CAP represents an intermediary in these signal transduction cascades, perhaps altering actin dynamics in response to extracellular cues.

### CAP is required in the germline for proper actin organisation

To characterise the *cap* mutant phenotype in detail, we compared actin filaments in wild-type ovaries and in mosaic egg chambers carrying *cap* germline clones (Figures 2a,b,3a,b). F-actin organisation within the wild-type egg chamber does not appear to change dramatically until stage 10B when actin 'dumping' fibres appear in nurse cells. In *cap* germline clones, however, the distribution of F-actin appears relatively normal during the very early stages of oogenesis, but becomes highly polarised and dynamic as the egg chamber develops. Ectopic F-actin is first seen at stage 5–6 of oogenesis in a dense structure at the posterior pole of the oocyte (Figure 2a). By stages 6–8, ectopic actin filaments appear to shift to the anterior of the oocyte, where they are found close to ring canals (Figure 2a). At stage 10B, dumping fibres can be seen forming on schedule in the nurse cells of *cap* clones (data not shown). Finally, in eggs, extensive filamentous actin structures form close to the cortex (Figure 2c), and ectopic F-actin is visible in the few embryos that are produced (data not shown). Interestingly, the change in the distribution of F-actin, from posterior cortical to anterior vesicle-like structures, mirrors the reorganisation of the microtubule cytoskeleton seen in the wild type during stages 6–8 [31,32]. Repolarisation of the microtubule array is thought to be induced by a signal from posterior follicle cells, dependent upon the prior action of Gurken in the germline [6,7]. Therefore, to determine whether the same signal also affects actin organisation, we reduced Gurken function in the *cap* mutant background. We find that the localisation of ectopic F-actin is unaltered in *gurken cap* double germline clones, so further work will be required to identify the cues responsible for the dynamic distribution of actin aggregates in the *cap* mutant.

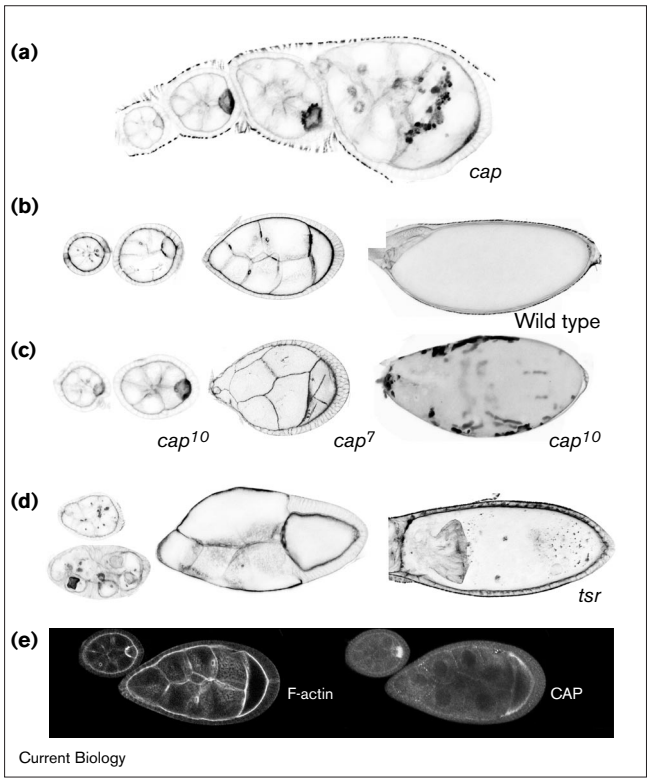
As ectopic actin structures are formed in *cap* mutant egg chambers, other F-actin-rich structures are lost. In particular, cortical F-actin underlying the nurse cell membranes disappears prematurely at stages 8–9 of oogenesis (Figures 2a,3). Therefore CAP may simultaneously inhibit actin polymerisation at some sites and facilitate the formation of F-actin at others. If the pool of actin within the egg chamber is limited, an alternative hypothesis can

**Figure 1**



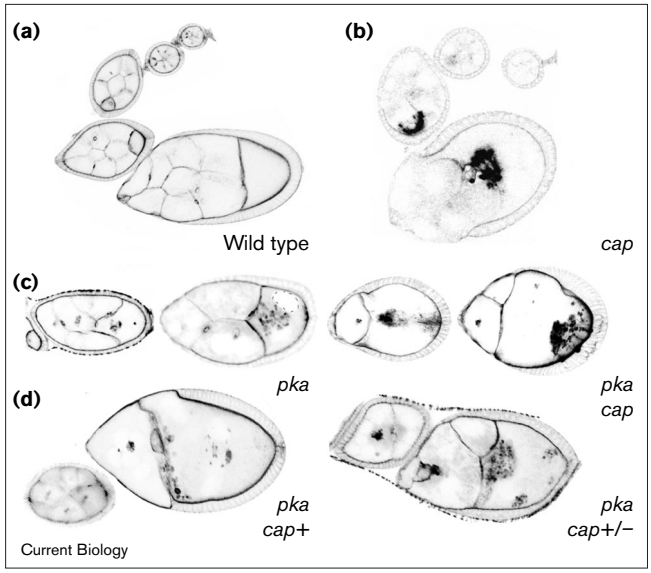

*cap* is homologous to the cyclase-associated proteins. **(a)** We identified *cap* in a screen for mutations that perturb F-actin in germline clones. CAP is ~50% identical and ~65% similar to both human CAPs [27]. CAPs have a conserved structure with an amino-terminal domain (AC) that binds adenylate cyclase in yeast [18], a polyproline-rich domain (PP) which interacts with c-Abl [21], followed by a carboxy-terminal monomeric actin-binding domain [18,23] and a dimerisation domain (D) [43]. **(b)** CAP contains a region found in verprolin (V) family members [28]. This sequence lies outside the defined CAP carboxy-terminal region [18,23,25] (368–524 in yeast). **(c)** A single mRNA species at ~2.4 kb was observed in a developmental northern blot. This was absent from homozygous mutant $cap^{10}$ third instar larvae.

**Figure 2**




In *cap* germline clones, F-actin accumulates in the oocyte, where CAP is concentrated in the wild type. **(a)** The ovaries of females carrying *cap* mutant germline clones were stained with TRITC–phalloidin to visualise F-actin. In *cap* germline clones, actin accumulates in the oocyte at stage 5–6 of oogenesis. Actin filaments then shift at stages 6–8 to the oocyte–nurse cell boundary. Subsequently, F-actin is lost from nurse cell cortices as germ cells begin to fuse (see also Figure 3). **(b)** A wild-type ovariole is shown for comparison. Following germ-cell differentiation, the level of F-actin appears slightly higher within the oocyte at the posterior of the egg chamber (to the right in all panels). **(c)** Ectopic actin filaments first form at the posterior of the oocyte at stage 5–6 of oogenesis, in both $cap^{10}$ and $cap^{1}$ germline clones. In $cap^{7}$ mutant tissue, F-actin cables form in the oocyte at a similar time, only to disappear at stage 8–9. Actin aggregates later accumulate at the cortex of *cap* mutant eggs. **(d)** We compared the distribution of F-actin accumulation in the *cap* mutant with that seen in *twinstar* germline clones (using either $tsr^{2}$ and $tsr^{1}$ alleles). At early stages of oogenesis, actin filaments are seen in clumps throughout the *twinstar* mutant egg chamber, while the morphology of mutant tissue often appears very disrupted. Later in oogenesis, F-actin is present at highest levels at the cortices of the nurse cells and oocyte, and in aggregates and filamentous structures within *tsr* mutant eggs. **(e)** A polyclonal anti-CAP peptide antibody was generated and used to stain wild-type ovaries. The staining was not seen with pre-immune sera and was confirmed using an independent antibody generated against the whole protein (Wills *et al.*, in preparation). CAP is present at low levels throughout the egg chamber but, following germ cell differentiation, the protein accumulates preferentially at the posterior of the oocyte (compare F-actin and CAP staining), and at the oocyte cortex in later egg chambers.

**Figure 3**




Protein kinase A (PKA) and CAP have related functions in the germline. **(a)** TRITC-labelled phalloidin was used to visualise F-actin within wild-type egg chambers. **(b)** Aggregates of actin filaments accumulate in *cap* mutant ovaries, whereas F-actin is lost from nurse cell cortices. This later phenotype may cause the cell fusion events observed, allowing occasional nurse cell nuclei to enter the oocyte (indicated by broken line). **(c)** *pka* and *pka cap* double germline clones were stained to visualise F-actin. *pka* mutants both lose nurse cell cortical actin [49,50] (leading to cell fusion, nurse-cell nuclei indicated by a broken line) and accumulate ectopic actin structures within the oocyte, close to the nurse cell–oocyte interface. The double mutant has an exaggerated phenotype, with complete fusion of the germline and ectopic actin aggregates at the posterior pole of the syncytium. This implies a related function for CAP and PKA in the germline. *cap* and *pka* clones exhibit distinct phenotypes in other tissues, however, which are not accentuated in the double mutant (data not shown). **(d)** Loss of one copy of CAP enhances a *pka* mutant phenotype.

be imagined, in which actin filaments are lost from nurse cell cortices to compensate for the formation of actin aggregates within the oocyte. In order to test whether the actin cytoskeleton is similarly polarised in other mutants that have excess accumulation of F-actin, we analysed *twinstar* germline clones. *twinstar* inhibits actin filament formation *in vivo* and encodes the *Drosophila* homologue of an actin-severing protein, cofilin [33]. We find that although ectopic actin filaments form in *twinstar* germline clones, as in the *cap* mutant, ectopic actin aggregates form at sites throughout the early *twinstar* mutant egg chamber (Figure 2d). Therefore, CAP has the specific function of inhibiting actin polymerisation within the oocyte.

As CAP inhibits actin polymerisation within the oocyte, but not in nurse cells, we generated an antibody to *Drosophila* CAP to see if this localised function is reflected in the wild-type distribution of the protein. This antibody is specific, as it recognises CAP in tissue extracts (~45 kilodaltons (kD)) (data not shown). CAP is present throughout the follicle cells and in the germline, but at early stages of oogenesis the protein preferentially

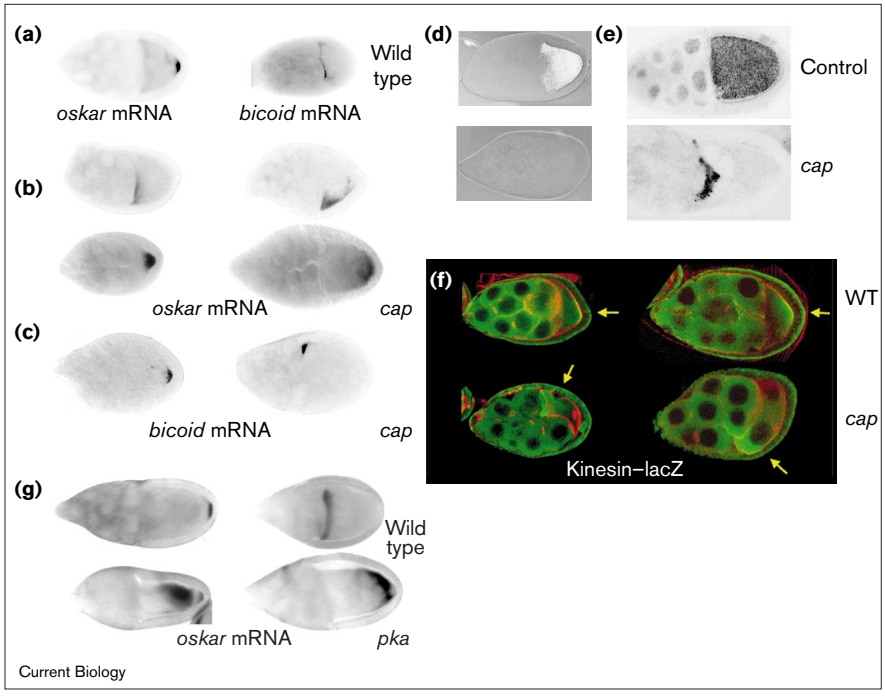
accumulates in the oocyte (Figure 2e). Later in oogenesis, CAP appears to be localised at the oocyte cortex. Thus, CAP is concentrated in the oocyte, where it functions to inhibit actin accumulation.

Our screen also identified a mutation in the catalytic subunit of protein kinase A (PKA). Therefore, we compared *pka* and *cap* mutant phenotypes in the *Drosophila* germline. Like the cap mutant, *pka* germline clones lose nurse cell cortical actin, while simulataneously accumulating ectopic actin structures (Figure 3c). In addition, the *pka* mutant phenotype is sensitive to the dosage of CAP, and actin defects are dramatically enhanced in *pka cap* double germline clones (Figure 3c,d). These data suggest that PKA and CAP functionally cooperate in the germline to control actin organisation.

### CAP is required for oocyte polarity

In *cap* germline clones, F-actin accumulates in a highly polarised fashion within the egg chamber and oocyte. Thus, we investigated whether loss of CAP perturbs other aspects of normal polarity, including the asymmetric localisation of mRNAs within the oocyte. We examined the distribution of *bicoid* and *oskar* mRNAs, which localise to anterior and posterior poles of the oocyte, respectively (Figure 4a). We find that although *oskar* mRNA is concentrated in one region of the oocyte in over 90% of egg chambers, *oskar* mRNA is mislocalised in 76% of stage 8–10 *cap* germline clone egg chambers ($n = 184$). Moreover, in 28% of cases, *oskar* transcripts are localised to the anterior or lateral part of the oocyte (Figure 4b). In addition, in 64% of stage-10 egg chambers that maintain correct overall polarity, *oskar* mRNA has a diffuse distribution and is not tightly focused at the posterior pole (Figure 4b bottom right panel). We also examined the localisation of *bicoid* transcripts ($n = 184$). *bicoid* mRNA accumulates at an aberrant site in 65% of *cap* mutant egg chambers (Figure 4c), and is localised to the posterior pole in 36% of stage 8–10 egg chambers. Thus, *cap* germline clones display two related mRNA polarity defects. First,

**Figure 4**

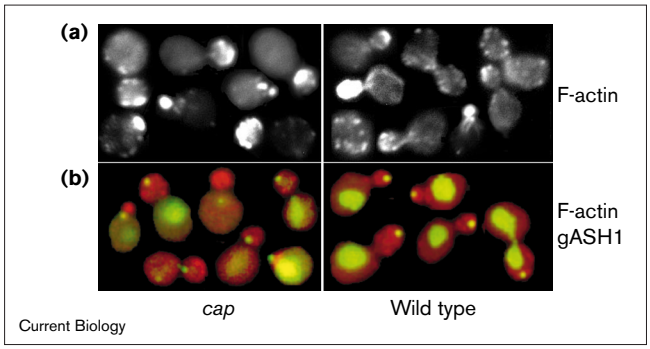



CAP is required for proper oocyte polarity. Wild-type and *cap* egg chambers were stained for *oskar* mRNA to assess cell polarity. **(a)** In the wild type, *oskar* RNA is tightly localised to the posterior of the oocyte, while *bicoid* mRNA localises to the anterior margin. **(b)** In over 90% of *cap* germline clones, *oskar* mRNA accumulates in a discrete region of the oocyte. *oskar* RNA appears aberrantly localised in 76% of mutant egg chambers, however, and in 28% of cases is concentrated in anterior or lateral regions of the oocyte. In addition, in 64% of stage-10 egg chambers that properly concentrate *oskar* mRNA in the posterior region of the oocyte, *oskar* transcripts appear to diffuse away from the pole ($n = 184$). **(c)** In 65% of *cap* mutant egg chambers, *bicoid* mRNA is mislocalised, and in 50% of egg chambers appears concentrated at an alternative site, for example the lateral, central or posterior region of the oocyte ($n = 184$). **(d,e)** Yolk particles were visualised in fixed tissue under (d) light microscopy or (e) in live ovarioles by autofluorescence. Yolk accumulates in the oocytes of control egg chambers which lack profilin function (in which premature cytoplasmic streaming occurs [8]), and in wild-type egg chambers (data not shown), but is not seen in the oocytes of *cap* mutant egg chambers. (e) In live *cap* mutant egg chambers, autofluorescent yolk particles accumulate at the nurse cell–oocyte boundary and are not properly transported into the oocyte. These particles appear abnormally large in the mutant. In stage 7–9 *cap* mutant egg chambers, yolk particles do not appear to exhibit the movements characteristic of premature cytoplasmic streaming [10] (data not shown). **(f)** F-actin (red) and β-galactosidase (green) were visualised in wild-type and *cap* mutant egg chambers expressing kinesin–lacZ. At this stage, kinesin–lacZ is transported to the posterior pole of the wild-type oocyte (arrows). In many *cap* mutant egg chambers, kinesin–lacZ is concentrated at the anterior cortex, often at a site where the oocyte membrane seems to force its way into the nurse cell cluster. **(g)** *pka* germline clones exhibit mRNA polarity defects similar to those observed in *cap* mutant egg chambers. *oskar* transcripts are misplaced in almost 50% of egg chambers lacking PKA activity (mRNA accumulates at a central position within the oocyte in fewer than 10% of cases). In late egg chambers, a diffuse gradient of *oskar* mRNA is occasionally observed at the posterior of the oocyte. Also, yolk fails to form within the oocyte of many *pka* mutant egg chambers (data not shown). These defects resemble those seen in *cap* germline clones, implying that *pka* and *cap* mutant phenotypes have a common aetiology.

although oocytes are able to concentrate *oskar* and *bicoid* mRNAs locally within the oocyte, they appear unable to coordinate mRNA polarity with the morphological polarity of the egg chamber. Second, in the majority of egg chambers in which *oskar* mRNA is correctly transported to the posterior pole of the oocyte, *oskar* message is not tightly localised at the cortex.

Mutations in several actin-related genes disrupt mRNA localisation, by inducing microtubule-based cytoplasmic streaming [8–10]. To test whether loss of CAP also disrupts the distribution of mRNAs by inducing premature cytoplasmic streaming, we looked at the movement of yolk particles within *cap* mutant egg chambers [10]. Interestingly, yolk often fails to form in the oocyte in *cap* germline clones (Figure 4d). Instead, the analysis of yolk autofluorescence in live *cap* mutant egg chambers reveals abnormal yolk particles accumulating at the nurse cell–oocyte boundary (Figure 4e). Therefore CAP may be required for a relatively late step in the formation of yolk granules and/or for the directional transport of yolk into the oocyte. Moreover, in a time-lapse analysis of yolk particles in stage 7–9 *cap* mutant egg chambers, we did not observe the movements characteristic of cytoplasmic streaming (data not shown), This is not unexpected because streaming disrupts mRNA localisation completely [8], whereas *cap* mutant oocytes accumulate mRNA determinants at discrete sites. Alternatively, the mRNA and yolk localisation defects observed in the *cap* mutant could result from a misoriented microtubule array. To test this hypothesis we used kinesin–lacZ (kin–lacZ, a fusion between β-galactosidase and a plus-end-directed microtubule motor [34]) to assay microtubule polarity in *cap* germline clones. In the wild type, kin–lacZ translocates to the posterior pole of stage 8–9 oocytes [34]. Within *cap* germline clones, kin–lacZ often becomes concentrated at specific but aberrant sites in the oocyte, indicating that microtubules are polarised but misaligned in the absence of CAP (Figure 4f). Interestingly, in cases where kin–lacZ is found at the anterior cortex, this altered microtubule polarity is accompanied by a change in morphology of the *cap* mutant oocyte, which appears to invade the nurse cell cluster (Figure 4f). This may in turn contribute to the fusion of nurse cells and oocyte observed in the mutant. Finally, in later egg chambers, kin–lacZ appears delocalised, as it does in the wild type following the onset of cytoplasmic streaming. In conclusion, early defects in *oskar* and *bicoid* localisation in the *cap* mutant are likely to reflect underlying defects in the microtubule cytoskeleton. Interestingly, *pka* germline clones exhibit mRNA polarity and yolk defects like those of the *cap* mutant (Figure 4g, and data not shown). These data support the notion that CAP and PKA have related germline functions.

**Figure 5**




CAP is required in yeast for control of the actin cytoskeleton and for proper cell polarity. **(a)** Actin was visualised in cells lacking the *cap* gene using TRITC–phalloidin. *capΔ* cells exhibit morphological and F-actin defects. **(b)** *cap* mutant and wild-type yeast expressing MS2–GFP together with *ASH1* mRNA with MS2-binding sites amass a single GFP-labelled mRNA particle (g*ASH1* [42]). g*ASH1* is found at the bud tip in 90% of wild-type cells. g*ASH1* enters the bud in 85% of *capΔ* cells, but in the majority of cases it fails to localise at the bud tip. g*ASH1* movement within the bud was monitored in live wild-type and *capΔ* cells. In 10 sec intervals, visible changes in g*ASH1* position were noted and averaged. In films of wild-type cells, g*ASH1* remained at the bud tip 75% of the time, but in the *cap* mutant, g*ASH1* was present at the bud tip in only 19% of frames (data not shown).

### CAP is required for yeast cell polarity and control of the actin cytoskeleton

To investigate whether CAP has an evolutionarily conserved function to control the spatial organisation of F-actin and mRNAs, we turned to *Saccharomyces cerevisiae* (budding yeast), where F-actin structures and a mRNA determinant, *ASH1*, are asymmetrically localised within the bud [35]. In yeast, in contrast to *Drosophila* oogenesis, microfilament and microtubule cyoskeletons function independently [36], and polarity is organised primarily by actin filaments, simplifying the analysis.

Yeast cells deleted for *cap* (*capΔ*) exhibit several morphological defects [37]. Mutant cells vary in size and shape when compared to the wild type (Figure 5a,b). This reflects unpolarised growth, probably arising from actin-related defects in vesicle targeting [38]. *capΔ* cells also exhibit a disorganised actin cytoskeleton (Figure 5a). The majority of *cap* mutant cells, however, are still able to generate a polar actin organisation, with filaments concentrated in the bud. Moreover, the wild-type F-actin distribution appears accentuated in many *capΔ* cells, implying that, in yeast, CAP prevents hyperpolarisation of the actin cytoskeleton, as it does in *Drosophila cap* germline clones.

To visualise cell polarity in yeast, we used *ASH1* mRNA as a reporter. In yeast, *ASH1* mRNA is a determinant of cell differentiation and is asymmetrically localised by myosin motors tracking along polar actin cables. Upon cell division, the daughter cell derived from the bud inherits *ASH1* message, preventing it from switching mating type [39–41]. Therefore, using green fluorescent protein (GFP) to label

*ASH1* mRNA, (g*ASH1* [42]; see Materials and methods), dynamic cell polarity can be visualised (Figure 5b). In the wild type, *ASH1* mRNA is assembled into a single particle, which is transported into the bud and rapidly localised to the bud tip (g*ASH1* is found at the tip in ~95% of small buds). Similarly, *capΔ* cells amass a g*ASH1* particle, which enters the bud in 85% of cases, demonstrating the presence of actin cables in the mutant. In half of these cells, however, g*ASH1* is not found at the bud tip. To assess whether this reflects a defect in mRNA movement or in recognition of the bud tip, g*ASH1* was followed in the buds of living wild-type and *capΔ* cells using time-lapse confocal microscopy (see Materials and methods). In the wild type, g*ASH1* is observed at the bud tip in 75% of frames, moving 18% of the time between snapshots taken at 10 second intervals. In contrast, g*ASH1* is only present at the bud tip 19% of the time in the *cap* mutant, changing position between 44% of consecutive frames. Therefore *ASH1* mRNA moves excessively in the mutant, and does not become properly anchored. Thus, in yeast, as in the *Drosophila* oocyte, CAP is not required to establish asymmetries in the distribution of F-actin and mRNAs, but is necessary to define or stabilise wild-type cell polarity.

## Discussion

### CAP controls actin dynamics in *Drosophila*

CAP proteins from a variety of organisms have been shown to inhibit actin polymerisation *in vitro*, by sequestering monomeric actin [22–25,43]. Here we describe the isolation and functional analysis of a *Drosophila* CAP homologue. We identified CAP in a screen for mutants that perturb proper F-actin organisation and egg morphology in germline clones and present an analysis of the *cap* mutant phenotype, comparing the effects of loss of CAP in *Drosophila* and *S. cerevisiae*. This analysis shows that CAP has a conserved function, regulating actin dynamics and cell polarity in both *Drosophila* and yeast.

In the egg chamber of *cap* germline clones, the actin cytoskeleton is strikingly altered, leading to the accumulation of ectopic actin-rich structures in the oocyte, where CAP protein is normally concentrated in the wild type. We can conclude that CAP is a major regulator of actin dynamics in *Drosophila*, and that CAP is likely to function to inhibit actin polymerisation *in vivo*, as it does *in vitro*. A striking feature of the *cap* phenotype is the accumulation of actin filaments at polar sites within the egg chamber. This cannot be explained by differences in the monomeric actin pool in nurse cells versus the oocyte, as G-actin, as measured by DNaseI staining, is equally distributed within the egg chamber (data not shown), as is profilin [44]. This distribution of actin filaments is peculiar to the *cap* mutant, because F-actin accumulates at sites throughout the egg chamber in *twinstar* germline clones. Thus, CAP inhibits actin filament formation at specific cellular sites, possibly in response to signalling events.

In yeast and *Dictyostelium*, cells that lack CAP exhibit clear defects in the control of actin dynamics [25,37]. In addition, CAP localises to cortical actin patches in yeast and to the leading edge of migrating *Dictyostelium* cells, where actin is most dynamic [25,37]. Although these data clearly implicate CAP in the control of the actin cytoskeleton, they do not reveal the precise nature of the actin defect in the mutant. Therefore, in the light of our observations in the *Drosophila* germline, we decided to look again at actin organisation in the yeast *cap* mutant. In agreement with previous reports [37,45], we find that the actin cytoskeleton is perturbed in yeast cells lacking CAP function. We find, however, that the wild-type asymmetric F-actin distribution appears accentuated in many *cap* mutant yeast cells. In addition, *cap* mutant cells maintain the capacity to reorganise their actin cytoskeleton in response to an extracellular pheromone cue (data not shown). Therefore, in both yeast *capΔ* cells and in *Drosophila cap* germline clones, the actin cytoskeleton is disrupted in such a way that ectopic actin filaments form in regions of the cell where CAP and F-actin are concentrated in the wild type, in the yeast bud and in the *Drosophila* oocyte (see Figures 2a,b,5a). This leads us to conclude that CAP has a conserved role in modulation of the distribution of actin filaments. So, by altering CAP activity, cells may be able to alter actin dynamics differently at distinct cellular locations.

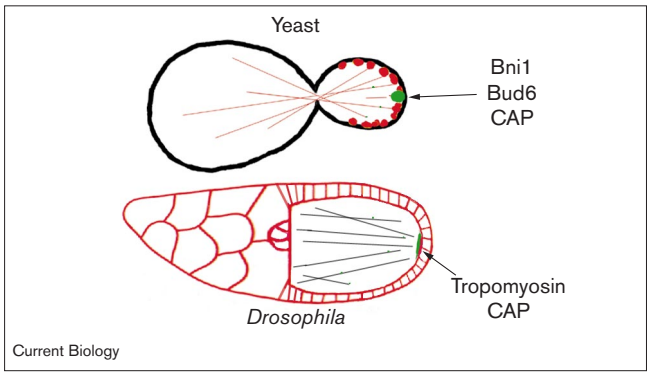
In both yeast and multicellular eukaryotes, the actin cytoskeleton responds to cell signalling events. Therefore it is interesting to note that homologues of *Drosophila* CAP have been shown to interact physically with an Abl tyrosine kinase and adenylate cyclase [18,21]. These latter proteins transduce extracellular cues, in a way that is not fully understood, to remodel the actin cytoskeleton within the growth cones of migrating neurons to facilitate axon guidance [46,47]. Thus, CAP may constitute part of the machinery that reorganises the actin cytoskeleton in response to these signals in neurons and in other polarised cells (Z. Wills *et al.*, unpublished data). Interestingly, our screen also identified the catalytic subunit of protein kinase A (PKA), which acts downstream of adenylate cyclase, as a gene required for proper actin organisation and oocyte polarity [48–50]. As yeast, *Hydra* and human CAPs have been shown to facilitate the activation of adenylate cyclase [17,20,27], CAP and PKA may be elements of a conserved signal transduction pathway. The phenotypic similarities shared by *cap* and *pka* germline clones suggest that CAP and PKA act together in the *Drosophila* female germline. Given this interaction, CAP could be a substrate for PKA, or could facilitate the activation of adenylate cyclase upstream of PKA. Alternatively, because a reduction in both CAP and PKA activity leads to a more severe phenotype, the two genes may act in parallel pathways. CAP and PKA are, however, unlikely to be essential components in a common signal transduction pathway in

*Drosophila* because we find no evidence for related CAP and PKA functions in somatic tissues (data not shown).

### The role of CAP and actin in *Drosophila* and oocyte polarity

In existing mutants known to perturb the germline actin cytoskeleton, oocyte polarity is either unaffected or completely disrupted. We therefore investigated whether oocyte polarity was altered in the *cap* mutant by examining the localisation of both *bicoid* and *oskar* mRNAs. When compared to other known mutants, *cap* germline clones exhibit novel mRNA polarity defects (although similar defects are exhibited by *pka* null germline clones). First, *cap* mutant oocytes are able to localise mRNAs to discrete areas within the oocyte, but the sites of mRNA deposition do not respect the existing morphological axes of the egg chamber. Second, in the majority of stage-10 egg chambers with the correct polarity, *oskar* mRNA is observed in a shallow gradient, as if diffusing away from the cortex at the posterior pole. Thus, CAP seems to be required, both to coordinate mRNA localisation with the axial polarity of the egg chamber, and to tether mRNAs to the cortex. Because microtubules are thought to mediate the transport of mRNAs to opposite poles of the oocyte in the wild type, the defect in oocyte axial polarity in the *cap* mutant may result from defects in the underlying microtubule cytoskeleton. As expected, we find that *cap* germline clones frequently contain a misoriented microtubule array, with plus ends focused at the anterior cortex. This altered microtubule polarity is therefore probably responsible for the mislocalisation of *oskar* and *bicoid* mRNAs at early stages of oogenesis. Finally, at later stages, following disassembly of the polar microtubule array, an actin-based structure at the posterior pole of the *Drosophila* oocyte, dependent on CAP and tropomyosin [11], may act as a tether to hold *oskar* mRNA at the cortex (Figure 6).

**Figure 6**




A model comparing mRNA polarity in a budding yeast cell and in the *Drosophila* oocyte. mRNA determinants (green) are asymmetrically localised in *S. cerevisiae* cells and in *Drosophila* oocytes. In both organisms, filaments (microtubules or actin filaments) oriented along the polar axis of the cell transport mRNA determinants to the poles, where they become tethered at the cell cortex. In the *Drosophila* oocyte, loss of CAP causes a misalignment of the mRNA transport machinery and a failure to capture mRNAs at the correct cortical site. Similarly, in yeast, CAP is required to define a fixed cortical site for mRNA localisation. Therefore, we propose that in both organisms a cortical F-actin-based structure (shown in red), organised by CAP and a complex of functionally related proteins [11,52,53], acts as a stable reference point to orient polar transport, for example by correctly aligning the oocyte microtubule array. This cortical marker then provides a point of anchorage once cargo has been transported to the cell pole.

Budding yeast cells, like the *Drosophila* oocyte, also use directional transport to localise an mRNA determinant, *ASH1*, to a polar site at the cell cortex [39,40]. In yeast, we find that CAP is required for the localisation of *ASH1* mRNA. Labeled g*ASH1* mRNA is found in the bud in the majority of *cap* mutant cells, where it remains highly motile, failing to become anchored at the bud tip. Thus, in both yeast and *Drosophila cap* mutants, overall polarity is disrupted even though individual mRNA determinants are concentrated within discrete regions of the cell. As CAP binds monomeric actin directly and is required for the proper distribution of F-actin in yeast and *Drosophila*, the primary defect in the absence of CAP is likely to be a change in actin organisation. We propose that CAP is required, in both organisms, to establish an actin-based spatial reference point at the cell cortex (Figure 6). This would serve to correctly align a polar microfilament or microtubule array, defining the cell's axis of polarity and allowing mRNAs and other cargo to be transported unidirectionally to the cell poles. Finally, at the pole, an analogous actin-based structure may be used to tether mRNAs to a fixed plasma membrane site (Figure 6).

## Conclusions

We have identified a major regulator of the actin cytoskeleton in flies. We show that CAP functions in *Drosophila* to inhibit actin polymerisation, possibly by sequestering monomeric actin, an activity exhibited by CAP homologues *in vitro*. As CAP protein preferentially accumulates in the oocyte, where it is required to limit the formation of F-actin, CAP is likely to have an important role in control of the spatial organisation of actin filaments within the egg chamber. We also find that CAP is required for the correct asymmetric distribution of mRNA determinants in both the *Drosophila* oocyte and yeast. Therefore, our work indicates that, through its effects on actin dynamics, CAP may be important for the genesis of cell polarity in eukaryotes.

## Materials and methods

### *Screen*

We conducted a mosaic screen for zygotic lethal mutations that perturb oogenesis in germline clones. For the efficient generation of homozygous mutant germlines, we used the Flp-DFS method described by Chou and Perrimon [2]. Six thousand mutations ([48], and N.P. *et al.*,

unpublished results) on the X, 2L, 2R, 3L and 3R chromosomes, were screened. Of these, approximately 300 (5%) failed to complete oogenesis [48]. Ovaries from females carrying homozygous mutant germline tissue were visually screened, with the help of David Bilder and Scott Goode, using TRITC-labelled phalloidin to visualise actin filaments, DAPI to stain DNA and Normarski optics to visualise yolk. Two P-element-induced mutations clearly affected both egg chamber polarity and the actin cytoskeleton. One of them identified a null mutation (*pka-C1*$^{01272}$) in the *pka-C1* gene, which has previously been shown to be required for actin organisation and mRNA polarity within the oocyte [49,50], and the other one, *l(2)06995* (*cap*$^{1}$), identified the gene that we have named *capulet*.

### Drosophila *stocks*

We used three cap alleles in this study: *cap*$^{l(2)06995}$ (*cap*$^{1}$) *FRT40A/CyO, cap*$^{l(2)k01217}$ (*cap*$^{7}$) *FRT40A/CyO* and *cap*$^{10}$ *FRT40A/CyO* (this study). Unless specifically stated we used the *cap*$^{10}$ allele. The stock, *hs-CAP/hs-CAP; cap*$^{10}$ *FRT40A/CyO* was used to rescue the mutant phenotype. In Figure 4 we used a transheterozygote combination of *profilin* alleles to generate a hypomorphic egg chamber phenotype: *chic*$^{05205a}$/*chic*$^{1}$ [9]. The *gurken*$^{HK}$ allele was recombined onto *cap*$^{7}$ and *cap*$^{10}$ chromosomes with FRT40A, to generate *gurken cap* double germline clones. We were able to phenocopy the *pka* null by expressing *UAS–pka-R1* (from D. Kalderon) in the germline using the V32 Gal4 line (from D. St Johnston). Genetic interactions between *cap* and *pka* were tested in the presence or absence of a *cap*$^{7}$ allele, and a *pka cap FRT40A* recombinant was used to generate the double germline clone. Kin–lacZ was used to assess microtubule polarity in the *cap* mutant [34].

### *Molecular biology*

P-element flanking sequences were rescued by transforming religated genomic DNA into *Escherichia coli*. A small portion of this sequence showed homology to CAPs from other organisms [27]. A similar rescue fragment was isolated by Berkeley Drosophila Genome Project (BDGP) [51]. A representative cDNA, LD18894, was obtained from BDGP, and we isolated others by hybridisation to an embryonic library (gift from Nick Brown). LD18894 was shown to map to 21F (Dm0065) on a P1 grid (Genome Systems). cDNAs and regions from Dm0065 were sequenced to determine the full extent of the gene. The lethality associated with *cap*$^{1}$ was reverted by precise excision, indicating that the P element was associated with lethality, and a null allele, *cap*$^{10}$, was generated by imprecise excision. An allele containing a P-element insertion, *cap*$^{k01217}$, called *cap*$^{7}$ in this paper, had a similar phenotype in clones. The ~2 kb cDNA, LD18894, was cloned into a pCaSpeR-hs vector and introduced into flies, where it was able to rescue *cap* mutant animals to adulthood following daily heat shock at 37°C throughout development. A polyclonal rabbit antibody was generated to a CAP peptide sequence, from amino acids 225–241 (QCB). This antibody and not the pre-immune serum, detected a ~45 kD protein on a gel and stained wild-type tissue.

### *Hybridisation analysis*

For northern analyses, RNA was isolated from ovaries, a mixed collection of embryos, larvae, and from individual *cap*$^{10}$ larvae, using Trizol (Gibco-BRL). Approximately 5 μg of RNA was denatured in 1× MOPS, 5% formaldehyde and 50% de-ionised formamide with ethidium bromide, and loaded on a formaldehyde gel in 1× MOPS. The gel was transferred overnight in 10× SSC onto a nylon membrane. Hybridisation was carried out in 1 M NaCl, 1% SDS, 10% dextran sulphate at 60°C. rDNA was used as a loading control. A probe was generated from the LD18894 cDNA.

### *Tissue staining*

Flies were fed yeast for 2 days before the isolation of ovaries. For antibody, TRITC–phalloidin and DAPI staining, tissue was fixed in 4% formaldehyde in PBS for 20 min. Tissue was then pre-washed in 1% Triton X-100 for 4 h at room temperature, blocked in 10% BSA with 5% normal horse serum in PBS, 0.1% Triton X-100 (PBT) and incubated overnight at 4°C in anti-CAP rabbit antibody or pre-immune serum at 1:2000, and with anti-β-galactosidase antibody at 1:1000 (Cappel). After extensive washing in PBT, fluorescently labelled secondary antibody was added. Tissue was stained for 30 min with TRITC–phalloidin (Molecular Probes) and DAPI (1μg/ml), before mounting in antifade and glycerol. A polar CAP signal was not seen in *cap* germline clone egg chambers. For *in situ* hybridisation, formaldehyde-fixed tissue was dehydrated overnight in 100% methanol at –20°C. Ovaries were then rehydrated in steps in PBT and equilibrated in 50% formamide, 10× SSC, 0.2 mg/ml ssDNA, 0.1 mg/ml heparin at pH 5.0. Tissue was prehybridised for 2 h at 70°C before the addition of the prewarmed digoxigenin-labelled RNA probe (made using a Boehringer-Mannheim kit from *oskar* or *bicoid* DNAs, a gift from T. Schupbach). Tissue was incubated overnight at 70°C and washed extensively in PBT, before adding precleared anti-digoxigenin antibody (Boehringer-Mannheim) at 1:5000 for 2 h. Tissue was again washed and the signal developed using NBT and BCIP (X-phosphate) in 0.1 M NaCl, 50 mM $MgCl_2$, 0.1 M Tris pH 9.5, 0.1% Tween20. For the visualisation of live tissue, ovaries were dissected in injection oil, and placed between a coverslip and a membrane (YSI) and viewed by confocal microscopy using a green laser.

### *Yeast strains, plasmids and methods*

The following yeast strains were used: MATa *cap/srv2Δ2::HIS3*, *leu2-3*, *hisΔ200*, *ura3-52* (from D. Drubin) and *MATa leu2*, *ura3* (from P. Silver). Cells were simultaneously transformed with the plasmids GFP–MS2 (pG14–MS2–GFP/LEU) and YEP195–LacZ–MS2–ASH1-3′UTR (from P. Chartrand [42]), under selection for uracil and leucine. Cells were then grown to mid-log phase at 30°C in synthetic medium supplemented with 2% raffinose. Expression from the *GAL1* promoter was subsequently induced by the addition of 2% galactose for 3 h. In some cases a nuclear GFP signal was visible due to the nuclear localisation signal within MS2. Media were supplemented with 10 μM α-factor for 3 h to induce pheromone arrest. Cells were fixed for 2 h at room temperature in 5% formaldehyde, washed, stained with TRITC–phalloidin, and visualised on polylysine-coated slides. Live cells were visualised by confocal microscopy (Leica) at room temperature. Films of individual cells were made, taking ~1 sec snapshots every 10 sec for 90 sec. One hundred timepoints were taken for each strain and marked movements between frames were noted and the mean motion calculated.

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