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Profilin is required for posterior patterning of the *Drosophila* oocyte

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SUMMARY

We have investigated the role of the actin cytoskeleton during mid-oogenesis and have found that disrupting the actin cytoskeleton with cytochalasin D induces microtubule bundling and microtubule-based cytoplasmic streaming within the oocyte, similar to that which occurs prematurely in *cappuccino* and *spire* mutant oocytes. After examining a number of mutants that affect the actin cytoskeleton, we have found that *chickadee*, which encodes the actin-binding protein, profilin, shares this phenotype. In addition to the microtubule misregulation, mutants in *chickadee* resemble *cappuccino* in that they fail to localize STAUFEN and *oskar* mRNA to the posterior pole of the developing oocyte. Also, a strong allele of *cappuccino* has multinucleate nurse cells, similar to those previously described in *chickadee*. In an independent line of experiments, we have identified profilin as a CAPPUCCINO interactor in a two-hybrid screen for proteins that bind to CAPPUCCINO. This, together with the similarity of mutant phenotypes, suggests that profilin and CAPPUCCINO may interact during development.

Key words: profilin, *cappuccino*, anterior-posterior, *Drosophila* oocyte

INTRODUCTION

Drosophila oogenesis has become a focus for cell biological analyses. The microtubule and microfilament rearrangements that occur during oogenesis have been described in detail and mutants that affect these processes have been identified and characterized. During the later stages of oogenesis, there are two movements that depend on the specialized cytoskeleton of the egg chamber (see for review Knowles and Cooley, 1994). At stage 11, the nurse cells dump their contents into the oocyte through cytoplasmic bridges termed ring canals. This process requires microfilaments since it is inhibitable by cytochalasins (Gutzeit, 1986). Microfilament bundles form in the nurse cells during this process and are apparently required to hold the nurse cell nuclei in place so that they do not obstruct the ring canals and allow the rapid flow of nurse cell cytoplasm into the oocyte. Mutants in *chickadee*, *quail* and *singed* affect actin bundle formation. Profilin, encoded by *chickadee*, is presumably required for the polymerization of the actin filaments that compose the bundles (Cooley et al., 1992), while a villin-related protein encoded by *quail* (Mahajan-Miklos and Cooley, 1994) and a fascin-related protein encoded by *singed* (Bryan et al., 1993; Cant et al., 1994) are thought to be required to cross-link the actin filaments to form the bundles. Two components of the actin-lined ring canals have also been identified – an adducin-like protein encoded by *hu-li tai shao* (Yue and Spradling, 1992) and a protein containing scruin repeats encoded by *kelch* (Xue and Cooley, 1993).

During the microfilament-dependent rapid transfer of cytoplasm from the nurse cells into the oocyte, microtubules bundle within the oocyte (Theurkauf et al., 1992) and microtubule-dependent cytoplasmic streaming, inhibitable by colcemid, occurs within the oocyte (Gutzeit, 1986). It is thought that this movement within the oocyte is necessary to mix the oocyte cytoplasm with the cytoplasm being rapidly added from the nurse cells.

cappuccino (*capu*) and *spire* (*spir*) are required to repress this microtubule-based ooplasmic streaming in the oocyte and to ensure the proper partitioning of molecular determinants within the oocyte. In mutants, the bundling of the microtubules at the cortex of the oocyte and streaming of the oocyte cytoplasm occurs prematurely, by stage 8 of oogenesis (Emmons et al., 1995; Theurkauf, 1994). *capu* and *spir* fail to accumulate components of the polar granules at the posterior pole (Bardsley et al., 1993; Ephrussi et al., 1991; Kim-Ha et al., 1991; Lasko and Ashburner, 1990; Manseau and Schüpbach, 1989; St Johnston et al., 1991; Wang and Lehmann, 1991) and *gurken* mRNA at the oocyte nucleus (Neuman-Silberberg and Schüpbach, 1993). The finding that *capu* and *spir* mutants exhibit premature cytoplasmic streaming within the oocyte led to two models to explain these patterning defects. The premature streaming could sweep away molecular determinants before they are properly anchored and/or the microtubules bundled at the cortex could prevent their proper function in the localization of determinants. Since the strength of the *capu* mutant allele does not correlate with either the speed or the timing of streaming in mutant oocytes, all of the patterning defects can not be explained by the streaming (Emmons et al., 1995). By inducing streaming prematurely in wild-type oocytes with cytochalasin D, we have seen the gradual elimination of STAUFEN from the posterior pole (Emmons et al., 1995), Under these same conditions, *gurken* mRNA remains localized in the dorsal anterior corner. This again suggests that the dorsal-ventral and posterior

patterning defects in *capu* and *spir* might be caused by distinct mechanisms.

Molecular analysis of *capu* indicates that the carboxy terminal half of the protein is closely related to that of formins, which are encoded by the mouse *limb deformity* (*ld*) locus (Emmons et al., 1995). Mutants in the mouse *ld* locus have truncations of the anterior-posterior limb axis resulting in fused digits (Zeller et al., 1989). In addition to these limb patterning defects, *ld* mutants often exhibit renal aplasia. Unfortunately, little is known about the cellular role that *ld* plays during development. Within the region of CAPU that is conserved with the formins is a proline-rich domain and, at a similar distance, a highly conserved domain, known as the FH2 domain, that is shared with a number of other proteins (Castrillon and Wasserman, 1994; Emmons et al., 1995). Although mutants in *capu* affect the distribution of microtubules, some proteins with FH2 and proline-rich domains affect processes that seem more likely to involve the actin cytoskeleton. The most compelling of these are the *S. pombe* gene *CDC12*, mutants in which do not undergo cytokinesis and fail to form the actin contractile ring (F. Chang, personal communication) and the *Drosophila* gene *diaphanous* (*dia*), mutants of which have defects in cytokinesis (Castrillon and Wasserman, 1994).

The observation that the actin cytoskeleton is defective in mutants in some of the FH2 domain-containing genes to which *capu* is related led us to investigate the role of the actin cytoskeleton during the time that *capu* and *spir* are thought to function. We found that treatment with cytochalasin D, a microfilament depolymerizing drug, during mid-oogenesis induces microtubule bundling at the cortex of the oocyte and premature microtubule-based cytoplasmic streaming. Further investigation revealed that mutants in profilin, an actin-binding protein thought to regulate actin polymerization, share these microtubule phenotypes, as well as patterning phenotypes, with *capu* and *spir*. An independent project to identify proteins that directly interact with CAPU via the yeast two-hybrid system yielded profilin as a frequent positive. Together, these results suggest that CAPU and profilin may physically interact during development.

MATERIALS AND METHODS

Time-lapse video microscopy

Time-lapse video microscopy was performed as described (Emmons et al., 1995). To show the effect of a drug on streaming, chambers were filmed for 20-30 minutes. Then the coverslip was lifted and excess solution was removed, drug was added and filming was resumed. Colchicine was initially dissolved in DMSO at 20 mg/ml. It was further diluted before use to 20 μg/ml in Robb's Saline. Controls were performed by adding Robb's Saline containing 0.1% DMSO. Cytochalasin D was dissolved in DMSO at 10 mg/ml and then diluted to 10 μg/ml in Robb's Saline before use. The following genotypes were filmed: for *chic*-$chic^{221}/chic^{1320}$, $chic^{1320}/chic^{1320}$, $chic^{221}/chic^{4612}$, $chic^{4612}/chic^{4612}$; for *quail*-$quail^{HM14}/quail^{HM14}$, for *singed*-$singed^{x2}/singed^{x2}$.

Immunocytochemistry

Immunocytochemistry of egg chambers was as described in Emmons et al. (1995). Microtubules were stained using an anti-α-tubulin antibody (α4a1) at a 1:10 dilution. STAUFEN antibody (generously provided by Daniel St. Johnston) was used at a concentration of 1:1000. Confocal microscope analysis was done using a Leica Confocal Microscope. All sections shown are 1 μm unless otherwise noted.

Morphological analyses

Chorions were prepared for analysis as described in Wieschaus and Nüsslein-Volhard (1986). Whole-mount tissue in situ hybridizations were performed basically as in Tautz and Pfeifle (1989) using digoxygenin-labeled RNA probes. The *gurken* probe was made from cDNA clone 1.7 (Neuman-Silberberg and Schüpbach, 1993). Egg chambers were stained with fluorescein-phalloidin and DAPI by dissecting in Robb's saline, fixing in 8% formaldehyde in PBS for 1 hour, washing in PBS for 2× 30 minutes and then incubating overnight in fluorescein-phalloidin dissolved in PBST (PBS, 0.1% Triton X-100) at 1 U per 100 ul. Excess phalloidin was removed by washing for 2× 30 minutes in PBST. DAPI was added to the last wash solution immediately prior to mounting in glycerol.

Interaction trap testing

The *chic* cDNA was amplified from a plasmid containing the DROCHICKB transcript (generously provided by Lynn Cooley, accession # M84529; Cooley et al., 1992) by PCR using primers that added an *Eco*RI site at position −38 relative to the ATG and a *Xho*I site at position +101 relative to the stop codon. This fragment was then cloned into the interaction trap prey vector pJG4-5 (marked with TRP1+) and the bait vector pEG202 (marked with HIS3+). The *capu* cDNA between the *Bcl*I site at position 192 (Emmons et al., 1995) (accession # U34258) (about 200 bases 5′ to the start codon) and the *Not*I site 3′ to the cDNA in the carrier bluescript vector was cloned into the interaction trap bait vector pEG202 (marked with HIS3+) between the *Bam*I and *Not*I sites. The insert frames of pJG4-5-*chic* and pEG262-*capu* were checked by sequencing over the protein fusion boundaries. These plasmids were introduced into yeast strain EGY48 along with the *lacZ* reporter plasmid pSH18-34 (marked with URA3+). The transformants were selected on -ura -his -trp dextrose plates, a number of individual colonies were picked and replicated onto -ura -his -trp dextrose, -ura -his -trp -leu dextrose and -ura -his -trp -leu galactose plates along with yeast containing two separate bait constructs other than the CAPU bait described above. These were RFMH-1 (Golemis et al., 1994) and a fusion with the cytoplasmic domain of the *Drosophila* β integrin (Tom Bunch, personal communication). Subsequently, pEG202-*capu* was cut with *Xho*I to liberate an in frame fragment of *capu* containing the entire coding region. This was then cloned into pJG4-5. pEG202-*chic* and pJG4-5-*capu* were then co-transformed into EGY48 and tested for an interaction. The dynamin bait plasmid contains amino acids 15-821, including the first 85 amino acids of the 100 amino acid proline-rich region (Viswanathan Raghuram and Mani Ramaswami, personal communication). All interaction trap vectors (pJG4-5, pEG202 and pSH18-34), the yeast strain EGY48 and yeast manipulations are as described in Finley and Brent (1995).

RESULTS

Treatment with cytochalasin D induces microtubule-based cytoplasmic streaming and bundling of the microtubules

To determine what effect disrupting the actin cytoskeleton would have during mid-oogenesis, we treated wild-type stage 8 egg chambers with cytochalasin D, a drug that inhibits microfilament function. Much to our surprise, treatment with cytochalasin D-induced cytoplasmic streaming in wild-type egg chambers at stage 8. The induction of streaming is rapid (less than 5 minutes) and exhibits characteristics of that seen

in *capu*: it is microtubule-based (inhibitable by colchicine) and has approximately the same speed (0.1 μm/sec) as the streaming in *capu* mutant oocytes (Emmons et al., 1995).

Since microtubule bundling at the cortex of the oocyte accompanies ooplasmic streaming in wild-type (Theurkauf et al., 1992) and premature streaming in *capu* and *spir* (Emmons et al., 1995; Theurkauf, 1994), we examined the microtubule distribution in cytochalasin D-treated egg chambers. In chambers that have been treated for 10 minutes with cytochalasin D, we observe abnormal microtubule distributions similar to those seen at the time of streaming in wild-type and during premature streaming in *capu* and *spir* mutant oocytes. In both cases, long and thick anti-tubulin-staining fibers are wrapped around the cortex of the oocyte (see Fig. 1).

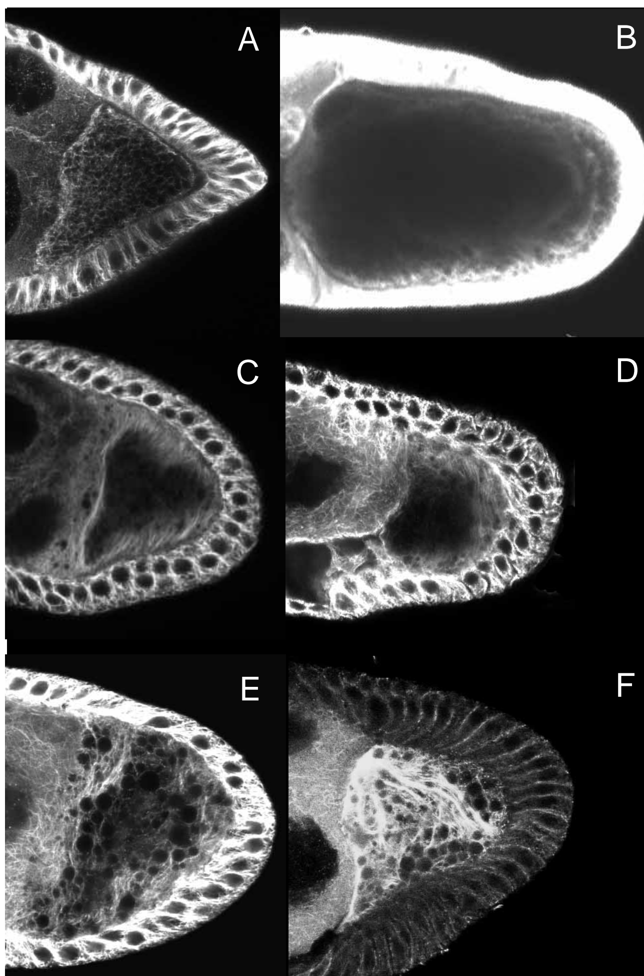

Fig. 1. Disruption of the actin cytoskeleton induces abnormal microtubule distributions. Microtubule distribution in wild-type egg chambers of (A) a stage 8 oocyte and (B) a stage 10 oocyte. Microtubules are bundled at the cortex of stage 8 oocytes in (C) a *capu* mutant chamber ($capu^{EE}/capu^{EE}$), (D) a wild-type chamber treated with cytochalasin D for 15 minutes, (E) a *chic* mutant chamber ($chic^{1320}/chic^{1320}$) and (F) a $chic^{1320}/chic^{221}$ egg chamber. Note the bundling of the microtubules at the cortex of the oocyte in B-F. The microtubule distribution is more variable in *chic*, with long tubulin-staining fibers being found throughout the oocyte. The *chic* microtubule phenotype more closely resembles *capu* in the stronger allelic combination in F.

Mutants in profilin exhibit premature bundling of the microtubules and premature microtubule-based streaming

Because cytochalasin D treatment induces cytoplasmic streaming in the oocyte, it is possible that mutants affecting the actin cytoskeleton might also do so. For this reason, we used time-lapse video microscopy to examine stage 8 egg chambers from females mutant for *chickadee*, known to encode profilin (Cooley et al., 1992), for *quail*, known to encode villin (Mahajan-Miklos and Cooley, 1994), and for *singed*, known to encode fascin (Bryan et al., 1993; Cant et al., 1994). Stage 8 *chickadee* (*chic*) egg chambers exhibit premature cytoplasmic streaming, while *quail* and *singed* do not. The frequency of oocytes that display premature cytoplasmic streaming correlates with the strength of the *chic* mutant allele (see Table 1). This does not appear to be the case with *capu*, as even the weakest alleles exhibit a very high frequency of streaming (data not shown). The $chic^{1320}$ allele has dramatically reduced expression of profilin in the germline, but expression in the follicle cells appears normal (Verheyen and Cooley, 1994). This allele shows a strong streaming phenotype, indicating that it is loss of profilin in the oocyte-nurse cell complex that is responsible for the premature cytoplasmic streaming. While the apparent higher sensitivity of the streaming phenotype to the dose of CAPU compared to the dose of profilin may be significant, it may simply be that weak alleles of *capu* have a less obvious mutant phenotype and thus are missed in mutant screens.

We examined the microtubule distribution in $chic^{1320}$ mutant egg chambers and found abnormal microtubule distributions similar to those seen at the time of streaming in wild-type, in premature streaming in *capu* and *spir* mutant oocytes and in cytochalasin D-treated egg chambers. We see long and thick anti-tubulin-staining fibers. In contrast to what we have seen in *capu* and *spir*, this phenotype is somewhat variable, with longer than normal fibers sometimes being found in more central regions of the oocyte cytoplasm instead of being restricted to the oocyte cortex (see Fig. 1E). Similar to what we observed when examining the cytoplasmic streaming phenotype, we find that in stronger *chic* allelic combinations such as $chic^{1320}/chic^{221}$ ($chic^{221}$ is a null mutation (Verheyen and Cooley, 1994)), the microtubule phenotype more closely resembles that of *capu* (see Fig. 1F).

chic mutants exhibit patterning defects similar to *capu*

Since *chic* shares the misorganization of the microtubules and the premature microtubule-based streaming phenotype with *capu*, we investigated whether *chic* mutant oocytes also exhibit

Table 1. Streaming in profilin mutants

Allelic combination	Alive but not streaming	Streaming
$chic^{4612}/chic^{4612}$	67% (*n*=6)	33% (3)
$chic^{4612}/chic^{221}$	50% (5)	50% (5)
$chic^{1320}/chic^{1320}$	20% (2)	80% (8)
$chic^{1320}/chic^{221}$	20% (1)	80% (4)

$chic^{4612}$ is a weak allele, $chic^{1320}$ is a strong, female sterile allele and $chic^{221}$ is a lethal allele (Verheyen and Cooley, 1994; Cooley et al., 1992) .

dorsal-ventral and anterior-posterior patterning defects. In the anterior-posterior axis, STAUFEN and *oskar* mRNA do not localize to the posterior pole in *capu* oocytes (Ephrussi et al., 1991; Kim-Ha et al., 1991; St Johnston et al., 1991). Using an anti-STAUFEN antibody, we examined the distribution of STAUFEN in *chic* mutant oocytes and found that STAUFEN-staining is not tightly localized to the posterior pole, but is instead found distributed throughout the cytoplasm of the oocyte. Even at stage 8, when STAUFEN is first localized to the posterior pole in wild-type, there is no localized STAUFEN at the posterior pole in *chic* mutant oocytes. This distribution is similar to that found in *capu* (see Fig. 2A-C). *Oskar* mRNA is first localized to the posterior pole of the oocyte in stage 8. We examined the distribution of *oskar* mRNA in stage 8 *chic* mutant chambers and found that it resembles that seen in *capu*. In *chic*, *oskar* mRNA is found throught the oocyte cytoplasm and is not found localized at the posterior pole (see Fig. 2D-F). Both *oskar* mRNA and STAUFEN localization appear normal before stage 8 (data not shown). We examined *bicoid* mRNA distribution in both *capu* and *chic* by whole-mount in situ hybridization and found that, in both cases, the pattern at stage 10 resembles that in wild-type (see Fig. 2G-I).

gurken mRNA is normally found tightly localized to the dorsal-anterior corner of the oocyte, but in *capu* is found in a ring along the anterior end of the oocyte (Neuman-Silberberg and Schüpbach, 1993). This results in dorsalization of the egg and embryo (Manseau and Schüpbach, 1989). Eggs laid by *chic* mutant females are small (Schüpbach and Wieschaus, 1991), presumably because the actin filaments that are required to hold the nurse cell nuclei in place are absent, resulting in the nurse cell nuclei blocking the ring canals and preventing the rapid flow of nurse cell cytoplasm into the oocyte during stage 11 of oogenesis (Cooley et al., 1992). Examination of *chic* egg shells reveals that, in addition to being small, they sometimes have fused dorsal appendages (see Fig. 2L-N), suggestive of weak dorsal-ventral patterning defects (Manseau and Schüpbach, 1989; Schüpbach, 1987). *Gurken* mRNA distribution is not convincingly altered in *chic* egg chambers (see Fig. 2J,K), but this is not surprising for a subtle dorsal-ventral phenotype. Confirming that the chorion abnormalities in *chic* are the result of dorsal-ventral patterning defects, we found that *chic* enhances the dorsal-ventral phenotype of *capu* in two separate double mutant combinations. Approximately 6% of the eggs from $capu^{G7}/capu^{2F}$ and 30% of those from $chic^{1320}/chic^{1320}$ have fused dorsal appendages while approximately 76% of those from $capu^{G7}chic^{1320}/capu^{2F}chic^{1320}$ have dorsal-ventral eggshell defects. Similarly, $capu^{2F}chic^{1320}/capu^{2F}chic^{1320}$ produce 70% eggs with dorsal-ventral type defects, while

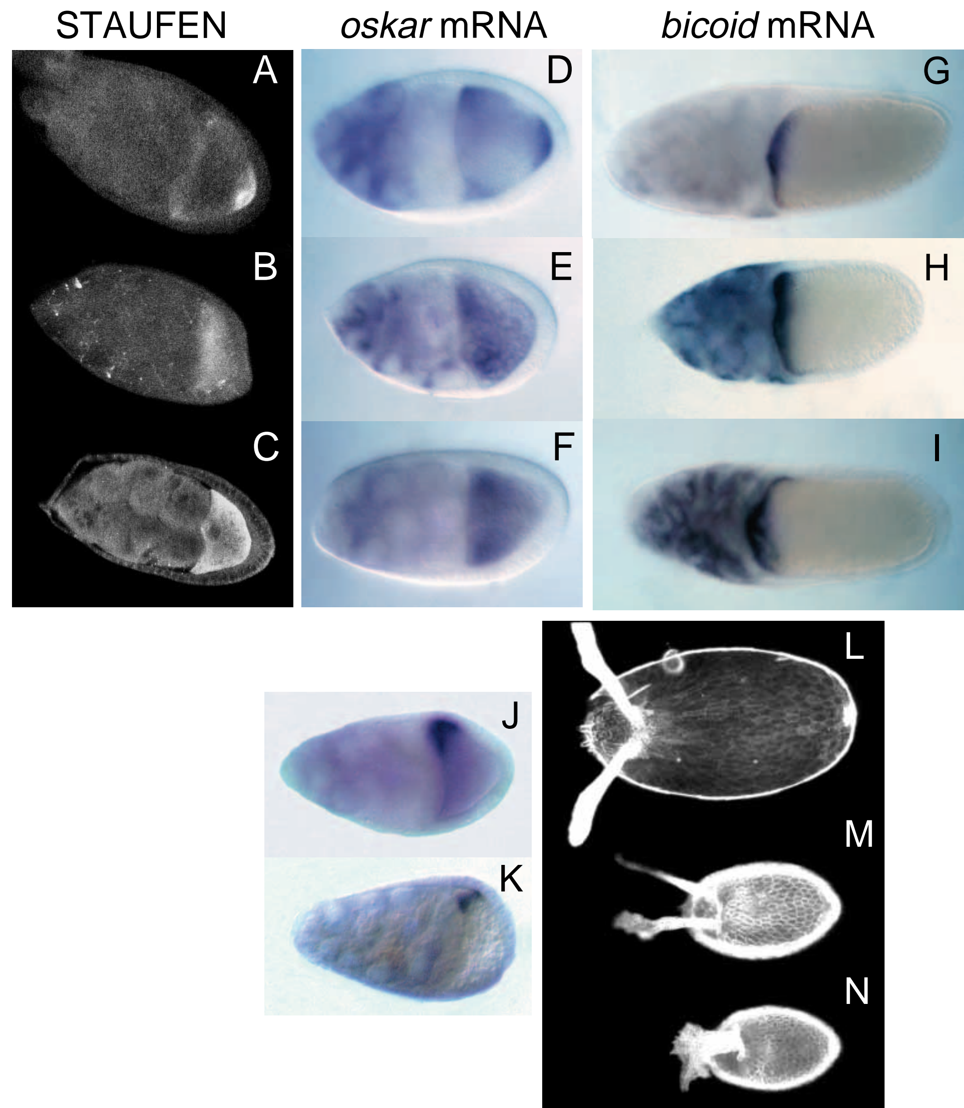

Fig. 2. Patterning defects in *chic* mutant oocytes. (A-C) Confocal image of STAUFEN localization in stage 8 egg chambers. (A) Wild-type: STAUFEN is tightly localized to the posterior cortex of the oocyte. (B) $chic^{1320}/chic^{1320}$ and (C) $capu^{EE}/capu^{EE}$ mutant oocytes: STAUFEN is found throughout the oocyte. (D-F) Similar to STAUFEN, *oskar* mRNA does not localize to the posterior pole in *chic* and *capu*. *oskar* mRNA distribution in stage 9 (D) wild-type, (E)$chic^{1320}/chic^{1320}$ and (F) $capu^{EE}/capu^{EE}$. (G-I) *bicoid* mRNA distribution resembles wild-type in *chic* and *capu*. *bicoid* mRNA distribution in stage 10 (G) wild-type, (H)$chic^{1320}/chic^{1320}$ and (I) $capu^{EE}/capu^{EE}$. (J,K) *gurken* mRNA distribution is relatively normal in *chic*. (J, wild-type; K, $chic^{1320}/chic^{1320}$). (L-N) Eggshells from $chic^{1320}$ mutant mothers exhibit dorsal-ventral patterning defects. (L, wild-type egg; M and N, egg from $chic^{1320}$ mutant females). Note the two separate respiratory appendages typical of a wild-type dorsal-ventral pattern in L and M. (N) The *chic* egg has fused respiratory appendages characteristic of eggs with dorsal-ventral defects.

capu^{2F} by itself produces only 2%. In addition, a significant portion of the eggs laid by the *capu chic* females are considerably larger than those produced by *chic*. This allows us to separate the effects of small egg size from dorsal-ventral perturbations. Since these larger eggs often have dorsalized eggshells not seen in *capu*^{2F}/*capu*^{2F} or *capu*^{G7}/*capu*^{2F}, but typical of stronger allelic combinations of *capu*, *chic* is enhancing the dorsal-ventral defects of *capu* and is likely, therefore, to affect dorsal-ventral patterning on its own.

Mislocalization of STAUFEN to the posterior pole correlates with abnormal microtubules

To more closely examine the correlation between microtubule bundling and mislocalization of posterior determinants, we stained egg chambers homozygous for a weak allele of *chic*, *chic*^{4612}, with both anti-α-tubulin and anti-STAUFEN. In *chic*^{4612}, many of the chambers have normal localization of STAUFEN to the posterior pole. We found that chambers with no STAUFEN at the posterior pole always had abnormal distributions of microtubules, while those with normal STAUFEN localization had relatively normal microtubules (see Fig. 3). We also noticed an interesting complementary pattern between the tubulin-staining regions and the STAUFEN-staining region. In the case of *chic*, the lack of localization of STAUFEN to the posterior pole correlates with abnormal microtubules.

Thus, in addition to the premature microtubule-based streaming and bundling of the microtubules at the cortex of the oocyte, mutants in *chic* also block localization to the posterior pole at the same point as *capu* and *spir,* upstream of STAUFEN and *oskar* mRNA localization. This similarity in mutant phenotypes suggests that *chic* is affecting the same process as *capu* and *spir*.

A strong *capu* allele exhibits cytokinesis defects similar to those seen in *chic*

Having noticed *capu*-like phenotypes in *chic*, we turned to examine *capu* for phenotypes previously observed in *chic*. Because *chic* egg chambers have multinucleate nurse cells indicative of cytokinesis defects, we have examined strong *capu* alleles to see if they also exhibit cytokinesis defects. Ovaries were stained with both fluorescein-phalloidin, which stains the actin cortex of the cells and DAPI to visualize the nuclei. We have found multinucleate nurse cells in 85% of *capu*^{G7}/*capu*^{G7} egg chambers (see Fig. 4), but not in *capu*^{EE} or *capu*^{3871} chambers. *capu*^{G7} is also unusual in that it is the only allele of *capu* that results in a ventralized phenotype – all other strong alleles result in dorsalization. To determine whether the multinucleate nurse cells result from a lesion in *capu* and not from a second site on the same chromosome, we have examined egg chambers from *capu*^{G7} combined with other *capu* alleles. We found that chambers from *capu*^{G7}/Df do not have multi-nucleate nurse cells, but chambers from *capu*^{G7} in combination with the hypomorphic alleles *capu*^{HK} and *capu*^{RK} do. This confirms that mutants in *capu* can produce cytokinesis defects and suggests that, in *capu*^{G7}, this mutant phenotype is a recessive gain of function since it is stronger in homozygotes and transheterozygotes than in hemizygotes. Whether the cytokinesis defects in *capu*^{G7} result from it interfering with a process in which CAPU normally participates or one in which it does not usually play a role is unknown.

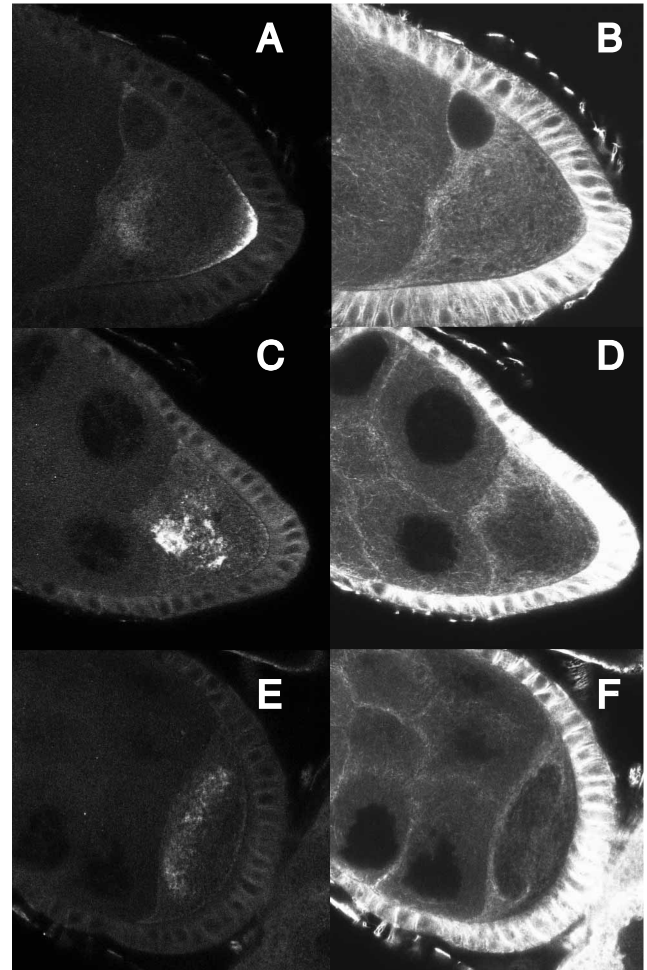

Fig. 3. Lack of STAUFEN localization correlates with microtubule abnormalities in *chic*. Chambers are stained with both STAUFEN and α-tubulin. Confocal images of (A,C,E) STAUFEN distribution and (B,D,F) α-tubulin distribution in *chic*^{4612}/*chic*^{4612}. When STAUFEN is localized to the posterior pole as in A, the microtubules appear relatively normal (B). (C,E) When STAUFEN is not properly localized, the microtubules appear abnormal (D,F).

CAPU interacts directly with profilin

To identify proteins that interact with CAPU, we have used the yeast Interaction Trap system (Finley and Brent, 1995; Gyuris et al., 1993) to screen an ovarian cDNA 'prey' library with a CAPU 'bait' plasmid. The CAPU bait plasmid used consists of the entire *capu* coding region fused to a LexA DNA-binding domain. Profilin was a frequent positive identified in this screen. The induction of both the CAPU bait and the profilin prey reconstitutes a functional transcriptional activation activity and enables the transcription of two separate reporters. We have also seen this interaction using the CAPU bait plasmid and a profilin prey construct that we have made (see Fig. 5) and when the orientation of the bait and prey are reversed such that a profilin-LexA bait plasmid and CAPU prey construct are used. CAPU contains a 162 amino acid proline-rich region (Emmons et al., 1995) and profilin is known to bind poly-proline (Tanaka, 1985). To address whether this interaction is at all specific for CAPU, we tested and found that

a profilin prey plasmid would not activate transcription in the interaction trap system with a bait construct containing the proline-rich region of dynamin (van der Bliek and Meyerowitz, 1991) (data not shown). Together, these results suggests that CAPU and profilin are capable of direct protein-protein interaction. To ask whether CAPU and profilin form a stable complex, we have attempted but have not yet succeeded in co-immunoprecipitating CAPU and profilin from ovaries and from yeast carrying the two-hybrid fusions.

DISCUSSION

We have investigated the role of the actin cytoskeleton during mid-oogenesis and found that it plays a role in posterior localization of determinants. This effect of the actin cytoskeleton on posterior pattern seems to involve the microtubule cytoskeleton. Our finding that *capu*, known to regulate behavior of the microtubules during development, shares a number of phenotypes with *chic*, a known regulator of the actin cytoskeleton, again suggests that the microtubule and microfilament cytoskeletons within the egg chamber are connected either directly or indirectly and suggests that *capu* and *chic* affect the same process.

Role of the actin cytoskeleton in posterior patterning

Our evidence suggests that within the egg chamber the actin cytoskeleton is required for maintaining a normal microtubule cytoskeleton and this in turn is required for patterning. First, treatment with cytochalasin D rapidly induces microtubule-based cytoplasmic streaming. This induced streaming is capable of sweeping STAUFEN from the posterior pole (Emmons et al., 1995). Treatment with doses of cytochalasin D that do not induce streaming do not result in mislocalization of posterior determinants (Pokrywka and Stephenson, 1995). The second, and perhaps more convincing, line of evidence is that *chic* mutants have defects in anterior-posterior patterning. STAUFEN is never found correctly localized to the posterior pole in strong alleles of *chic*. It appears that misregulation of the microtubule cytoskeleton is likely to be responsible for the posterior patterning defects: in a weak *chic* allele, the lack of localization of STAUFEN to the posterior pole is correlated with abnormal microtubule distributions.

There are other examples of disruption of microfilaments affecting mRNA localization, but in none of those cases do the microtubules seem to be affected. Using cytochalasin, it has been shown that microfilaments are required for proper localization of actin mRNA in fibroblasts and the microtubules appear unaffected by the drug treatment (Sundell and Singer, 1991). In *Xenopus* oocytes, cytochalasin B disrupts anchoring of Vg1 mRNA at the vegetal cortex but, again, the microtubule array appears unaffected (Yisraeli et al., 1990). Finally, in *Drosophila*, tropomyosin mutants also have defects in posterior localization of *oskar* mRNA, but the microtubule distributions are normal (Erdelyi et al., 1995).

Relationship between the actin and tubulin cytoskeletons

That disruption of the actin cytoskeleton either with cytochalasin D or with *chic* mutants induces microtubule-based streaming in the *Drosophila* oocyte suggests that the two cytoskeletons are functionally connected. Both the cytochalasin D-induced streaming and the streaming in *chic* mutants display characteristics of streaming seen both in wild-type stage 10 oocytes and in stage 8 *capu* and *spir* mutant oocytes: they are microtubule-based and concomitant with the appearance of microtubule fibers around the cortex of the oocyte. This suggests that some aspect of the actin cytoskeleton normally represses microtubule-based streaming within the oocyte. Because the egg chamber contains three different cell types, the oocyte, the nurse cells and the follicle cells, it is

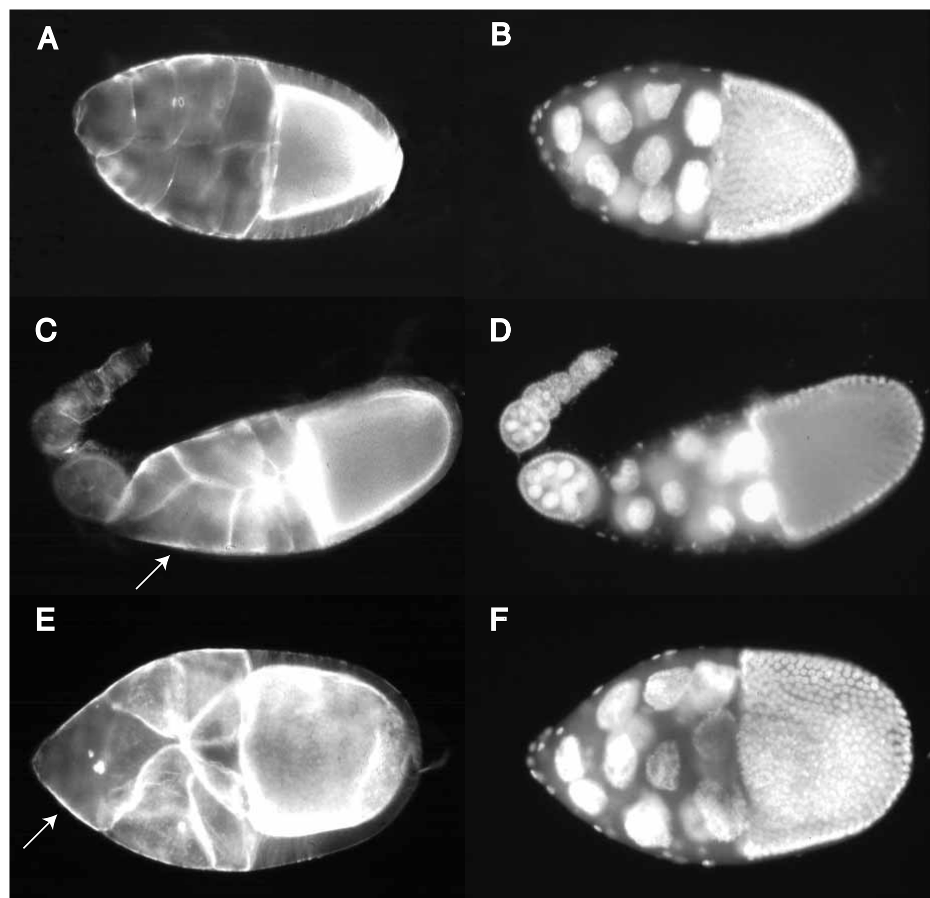

Fig. 4. *capu* and *chic* share cytokinesis defects in the nurse cells. Stage 10 egg chambers are stained with (A,C,E) fluorescein-phalloidin to visualize filamentous actin and (B,D,F) DAPI to visualize DNA. (A,B) Wild-type: there are 15 nurse cells with each cell containing a single nucleus. (C,D)$chic^{1320}/chic^{1320}$ and (E,F)$capu^{G7}/capu^{G7}$: chambers with multinucleate nurse cells resulting from failure to properly cytokinese are apparent (see arrows).

difficult to know whether or not it is the actin cytoskeleton within the oocyte that is responsible for suppressing microtubule-based streaming. While cytochalasin D treatment would certainly affect the actin cytoskeleton in all three cell types, the *chic* mutant that we have used dramatically reduces profilin expression only in the nurse cell-oocyte complex (Verheyen and Cooley, 1994), indicating that the actin cytoskeleton in either the nurse cells or the oocyte is regulating the oocyte microtubule cytoskeleton.

Profilin can act either to depolymerize or polymerize actin filaments (for reviews see Carlier and Pantaloni, 1994; Theriot and Mitchison, 1993). There is also evidence implicating profilin in the PLCγ signaling cascade (mediated by binding to PIP_2) and the RAS signaling pathway (Goldschmidt-Clermont et al., 1990; Vojtek et al., 1991) suggesting that profilin may either mediate cytoskeletal responses to external signals and/or modulate incoming signals to reflect the state of the cytoskeleton (reviewed in Machesky and Pollard, 1993; Sohn and Goldschmidt-Clermont, 1994; Theriot and Mitchison, 1993). In vitro studies indicate that cytochalasins cap the barbed ends of actin filaments, reduce polymerization at the pointed ends of filaments and convert ATP-actin to ADP-actin, all of which result in depolymerization of actin filaments (Sampath and Pollard, 1991). Within the cell, it is possible that various actin-binding proteins might alter the effect of cytochalasin D treatment, but it seems likely that the net result is depolymerization of filaments. Thus, in *chic* mutants, it is likely to be the loss of profilin's promotion of actin polymerization that is important in inducing premature microtubule-based cytoplasmic streaming and bundling of the microtubules.

We suggest two basic models for the regulatory interaction between the actin and microtubule cytoskeletons. We need only consider models involving the oocyte-nurse cell complex, since we see the microtubule phenotypes in a *chic* mutant allele that has dramatically reduced expression only in these cells. The first model is that there is a direct physical interaction between the two cytoskeletons such that a change in the actin cytoskeleton enables bundling of the microtubules at the cortex of the oocyte. Microtubules could be tethered to the microfilament cytoskeleton such that depolymerization of the actin would enable bundling of the microtubules at the cortex. Alternatively, the polymerized actin cortex might mask sites for initiation of microtubule bundling. A second class of models involves signaling between the two cytoskeletons, such that depolymerization of the actin cytoskeleton releases a signal for bundling of the microtubules. This signal could be transmitted from the nurse cells to the oocyte or could be released within the oocyte.

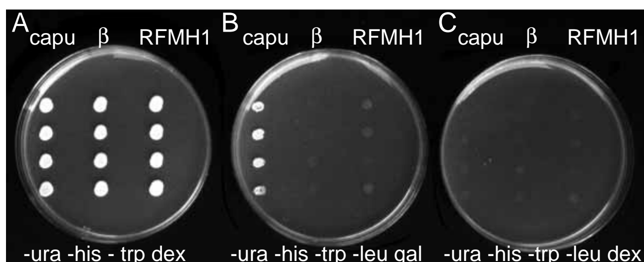

Fig. 5. Co-expression of CAPU and profilin activates transcription in the Interaction Trap System. We replicated onto each plate four independent transformants of the CAPU test bait (leftmost column), a β-integrin-negative control (Tom Bunch, unpublished results) (middle column), and a *bicoid*-bait-negative control RFMH1 (Golemis et al., 1994) (rightmost column), all of which are expressed constitutively. (A) Positive control for growth of all twelve yeast cultures. The presence of the *lacZ* reporter plasmid (Ura^+), the bait plasmid (His^+) and the prey plasmid (Trp^+) have been selected. (B) Expression of the bait fusions has been induced (with galactose). The presence of profilin and CAPU fusions in the cell activates transcription of LEU2 allowing growth on –leu media, but profilin with either the *bicoid* or β integrin bait fusion proteins does not allow growth. (C) Profilin expression is not induced (dextrose instead of galactose). The CAPU fusion alone is not sufficient to support growth. A filter lift assay for β-galactosidase activity (Breeden and Nasmyth, 1985) shows similar results in that β-galactosidase activity is present if and only if both CAPU and profilin fusions are present [data not shown].

Profilin may interact directly with CAPU during development

We have provided genetic evidence suggesting that CAPU and profilin act in the same process. *capu* and *chic* share a number of mutant phenotypes including premature microtubule-based streaming and premature microtubule bundling within the oocyte. In addition, *capu* and *chic* disrupt posterior localization at the same point in the posterior localization pathway, upstream of both STAUFEN and *oskar* mRNA localization. Finally, a strong *capu* allele shares cytokinesis defects with *chic*. These results suggest that *capu* and *chic* affect the same pathway and imply a role for *capu* in regulating or responding to the state of the actin cytoskeleton.

That CAPU and profilin interact in the two-hybrid system suggests a model whereby CAPU and profilin may affect the same process through a direct physical interaction. Although we have failed to co-immunoprecipitate profilin and CAPU from ovaries or yeast, this may be because the interaction is too transient to be detected by immunoprecipitation. While the combination of a similar mutant phenotype and a two-hybrid interaction is suggestive that CAPU and profilin interact directly in vivo, this awaits confirmation.

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