cappuccino, a Drosophila maternal effect gene required for polarity of the egg and embryo, is related to the vertebrate limb deformity locus

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We report the molecular isolation of cappuccino (capu), a gene required for localization of molecular determinants within the developing Drosophila oocyte. The carboxy-terminal half of the capu protein is closely related to that of the vertebrate limb deformity locus, which is known to function in polarity determination in the developing vertebrate limb. In addition, capu shares both a proline-rich region and a 70-amino-acid domain with a number of other genes, two of which also function in pattern formation, the Saccharomyces cerevisiae BNI gene and the Aspergillus FigA gene. We also show that capu mutant oocytes have abnormal microtubule distributions and premature microtubule-based cytoplasmic streaming within the oocyte, but that neither the speed nor the timing of the cytoplasmic streaming correlates with the strength of the mutant allele. This suggests that the premature cytoplasmic streaming in capu mutant oocytes does not suffice to explain the patterning defects. By inducing cytoplasmic streaming in wild-type oocytes during mid-oogenesis, we show that premature cytoplasmic streaming can displace staufen protein from the posterior pole, but not gurken mRNA from around the oocyte nucleus.

[Key Words: Drosophila; cappuccino; pattern formation; cytoplasmic streaming; maternal effect; oogenesis; formins]

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The developing Drosophila oocyte is an excellent model system for studying the establishment of polarity within a single cell because the embryonic anterior–posterior and dorsal–ventral axes are initially organized within the oocyte. Anterior–posterior axis formation of the larva involves three pathways within the oocyte: one that establishes the anterior end, one at the posterior end to establish the abdomen and pole cells, and one at both termini of the oocyte to establish the ends [for reviews, see Manseau and Schüpbach 1989a and Nüsslein-Volhard et al. 1987]. The larval anterior is established during oogenesis through localization of the bicoid mRNA to the anterior end of the oocyte [Berleth et al. 1988; Driever and Nüsslein-Volhard 1988a,b]. Formation of the abdomen and pole cells requires localization of a large number of molecular determinants to the posterior pole of the developing oocyte (vasa protein, Hay et al. 1988; Lasko and Ashburner 1990; askar mRNA and protein, Ephrusso et al. 1991; Kim-Ha et al. 1991; Smith et al. 1992; staufen protein, St. Johnston et al. 1991; tudor protein, Bardsey et al. 1993; nanos mRNA and protein, Wang and Lehmann 1991; Ephrusi and Lehmann 1992; Smith et al. 1992; germ cell-less mRNA, Jongens et al. 1994]. The termini of the larva are marked during oogenesis by a signaling process between the oocyte and the surrounding epithelium of follicle cells [Stevens et al. 1990; Savant-Bhonsale and Montell 1993].

The first known step in dorsal–ventral axis formation is the localization of the oocyte nucleus to the dorsal anterior corner of the oocyte. gurken mRNA, which encodes a transforming growth factor-α (TGF-α)-like protein, is then localized adjacent to the oocyte nucleus [Neuman-Silberberg and Schüpbach 1993]. Presumably, the encoded gurken protein is at a higher level on the dorsal side of the oocyte where it is thought to serve as a ligand for the EGF-receptor (encoded by torpedo) [Price et al. 1989; Schejter and Shilo 1989] in the surrounding epithelium of follicle cells. This signaling process initiates a cascade of differential activities in the follicle cells on the dorsal and ventral sides of the oocyte. This information in the follicle cells is eventually communicated back to the oocyte or its derivative, the egg, to establish the dorsal–ventral axis of the developing embryo [Stein et al. 1991].

cappuccino (capu) and spire (spir) are unusual among maternal effect genes involved in pattern formation in Drosophila in that they affect both anterior–posterior
In oogenesis, components of the polar granules and dorsal–ventral axis formation are regulated in two separate pathways. The posterior pole phenotype is a consequence of molecular determinants previously localized within the developing oocyte. For instance, capu and spir share two small domains of similarity to the vertebrate limb deformity locus. An allelic series suggests that the microtubule cytoskeleton is misregulated, resulting in premature microtubule bundling at the cortex of the oocyte and premature microtubule-dependent cytoplasmic streaming within the oocyte. Careful analysis of an allelic series suggests that the premature streaming alone cannot explain all of the patterning defects. In support of this argument, we show that the induction of premature, microtubule-based streaming in wild-type oocytes does not sweep away all previously localized molecular determinants.

Results

Genetic fine mapping of capu

Previous genetic analysis had mapped capu to the polytene chromosomal region 24C3.4–24D3.4 (Manseau and Schüpbach 1989b). This localization is based on complementation analysis with deficiencies that break in the region of capu. To narrow the location of capu within this region, the gene was mapped genetically with respect to a white+ [w+] transposable element in polytene chromosome region 24D1.2 [P[w+]]. To do this, we screened 23,000 recombinant chromosomes for recombination events between the P[w+] element and a mutant capu allele, capuEE and identified 17 females carrying P[w+]-capu recombinant chromosomes [see Fig. 1]. To determine whether capu lies proximal or distal to the P[w+] element, we used a restriction fragment length polymorphism (RFLP) proximal to the capu region in polytene chromosome region 26 [RFLPA and RFLPA], as a distant flanking marker. If capu lies distal to the P[w+] element, then recombinant chromosomes bearing both capu and the P[w+] element would carry RFLPA. Alternatively, if capu lies proximal to the P[w+] element, then these recombinant chromosomes would carry RFLPA. Southern blots of genomic DNA isolated from single recombinant flies were analyzed, and of the two recombinant chromosomes examined, both were found to carry RFLPA, indicating that capu lies proximal to the P[w+] element.

A chromosomal walk through this region was initiated from clones flanking the P[w+] element [generously provided by Tulle Hazelrigg, Columbia University, New York]. To locate the capu gene within the 100-kb chromosomal walk shown in Figure 1C, the P[w+]-capu recombinant chromosomes were analyzed for RFLPs found within the walk. Thirteen P[w+]-capu recombinants carried a capu chromosome RFLP [RFLPB] identified by phase clone AZ3M-6A, while one recombinant carried the P[w+] RFLP [RFLPB]. Because the distance between the P[w+] cbr element and the RFLP identified by AZ3M-6A was known to be ~38 kb, this suggested that capu lies ~3 kb [38 kb/13 recombinants = 3 kb/recombinant] proximal to RFLPB [see Fig. 1B,C].

Identification of the capu transcription unit

The RFLP mapping indicated that the lesion in capuEE lies ~3 kb proximal to RFLPB. This suggested that at least a portion of the capu transcription unit would lie in

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Figure 1. (A) RFLP mapping of capu. To further localize the capu gene within the cytological region 24C3.4–24D3.4, we mapped capu with respect to a P[w+] element that was inserted in 24D1.2 (see text). (B,C) The capu region proximal to the P[w+] element. (B) To locate the capu gene within the 100-kb chromosomal walk shown in C, P[w+]-capu recombinant chromosomes were analyzed for RFLPs found within the walk (see text). (C) Molecular map of the chromosomal walk through the capu region. The restriction map shows EcoRI sites throughout the region. The arrow above the restriction map represents the capu transcription unit.

(A) RFLP analysis of the chromosomal walk extending up to 23 kb proximal to capu. (B) To locate the capu gene within the 100-kb chromosomal walk shown in C, P[w+]-capu recombinant chromosomes were analyzed for RFLPs found within the walk (see text). (C) Molecular map of the chromosomal walk through the capu region. The restriction map shows EcoRI sites throughout the region. The arrow above the restriction map represents the capu transcription unit.

We examined the expression pattern of the capu transcription unit during oogenesis with in situ hybridization of DIG-labeled RNA probes made from the capu cDNA clone [see Fig. 2]. The earliest accumulated mRNA is in region 2 of the germarium. The mRNA is present in the nurse cells of stage 1–13 egg chambers. In stages 4–9, we also see staining of the oocyte nucleus. In addition, there is a low level of staining in the follicle cells, first evident by stage 4 and continuing through stage 11. This mRNA distribution is consistent with the earliest known phenotype for capu, the lack of posterior localization of staufen protein at stage 8 of oogenesis [St. Johnston et al. 1991] and with the germ-line requirement for capu identified in mosaics [Manseau and Schüpbach 1989b]. There is no phenotype known to result from lack of expression of capu in the follicle cells of the egg chamber.

We also examined the distribution of the capu mRNA during other stages of development using Northern analysis [see Fig. 3]. The 4.0-kb message is present throughout embryogenesis but is absent by the first larval instar. It reappears during the third larval instar and is present in ovarectomized females and in adult males. A slightly larger transcript of ~4.3 kb is also present in these later stages of development. There is no known phenotype for capu corresponding to the expression outside of the ovary in females or to the expression in adult males.
capu is highly related to the vertebrate limb deformity locus and shares small domains of similarity with a number of other proteins

DNA sequencing of the capu cDNA clone revealed the presence of a 1058-amino-acid open reading frame (ORF) that is predicted to encode a 114-kD protein [see Fig. 4A]. Comparison of the DNA sequence of the capu cDNA clone with sequences in GenBank by use of BLASTX (Altschul et al. 1990) indicated a striking similarity between the carboxy-terminal 503 amino acids of capu and the formins, encoded by the Id locus of mouse and chicken (Woychil et al. 1990; Jackson-Grusby et al. 1992; Trumpf et al. 1992). By use of an alignment generated by BESTFIT [Devereux et al. 1984], the carboxy-terminal portion of the capu protein is 40% identical and 60% similar to the mouse or chicken formins [see Fig. 4C]. In addition, there is a 71-amino-acid region within the carboxy-terminal domain that is highly conserved both with the formins and with a number of other proteins [see Fig. 4B,C], including the Saccharomyces cerevisiae bud neck involvement 1 (BNII) [J. Pringle, pers. comm.], Aspergillus FigA [forced expression inhibition of growth [Aspergillus]] [Marhoul and Adams 1995], a Drosophila gene known as diaphanous (dia) (Castrillon and Wasserman 1994), two Schizosaccharomyces pombe genes called CDC12 (F. Chang, pers. comm.) and fusl (Petersen et al. 1995), a genetically undefined S. cerevisiae ORF in GenBank, an Arabidopsis EST, a human EST, and a rice EST. This domain of similarity was independently identified by ourselves and S. Wasserman and has been named the FH2 domain [Castrillon and Wasserman 1994].

The carboxy-terminal portions of capu and of the vertebrate formins are preceded by a proline rich region of ~162 amino acids. This proline-rich region (also known as the FH1 domain [Castrillon and Wasserman 1994]) is present with approximately the same spacing from the FH2 domain in BNII, FigA, dia, CDC12, and the yeast ORF [see Fig. 4B]. The proline-rich region in mouse formin has been demonstrated to be capable of acting as a binding site for a Src homology 3 (SH3) domain [Morrisey et al. 1993; Sparks et al. 1994; Yu et al. 1994]. On the basis of randomizations with BESTFIT, the amino-terminal 484 amino acids of capu do not appear similar to those of the mouse or chicken formins, nor does there appear to be any significant similarity between capu and BNII, FigA, CDC12, or the yeast ORF outside of the FH2 domain and the proline-rich region. We do, however, find significant similarity between capu and dia in the region between the proline-rich region and the FH2 domain [see Fig. 4B]. Finally, we would like to point out that the formins, BNII, FigA, CDC12, dia, fus, and the yeast ORF have regions that are predicted by the algorithm of

**Figure 2.** Distribution of the capu transcript in ovaries. Tissue in situ hybridizations were performed as described [Tautz and Pfeifle 1989] with a nonrepetitive probe made from a 1-kb EcoRI–NotI fragment at the 3' end [A,B], and a 0.9-kb EcoRI fragment at the 5' end of the cDNA (C,D). Similar staining patterns were observed for each probe. (A) Expression in the germarium in region 2. (B) Expression in the follicle and nurse cells of a stage 10 egg chamber. Note the basal position of expression in the follicle cells. (C) mRNA staining pattern from germarium through stage 8 egg chambers. (D) Stage 9 egg chamber showing staining of oocyte nucleus.

**Figure 3.** Distribution of the capu transcript during development. Poly(A)+ mRNA (5 µg) from ovaries, 0–4 hr, 4–8 hr, 8–12 hr, and 12–24 hr embryos, first, second, and third larval instars, adult males, and females, from which the ovaries were removed, was electrophoresed, transferred to nylon membrane, and hybridized with a 0.9-kb EcoRI fragment probe from the 5' end of the 4.3-kb capu cDNA clone [A], and a plasmid containing the ribosomal protein 49 gene [RP49] [B]. The 4.0-kb capu message found in ovaries, throughout embryogenesis and in third larval instars and the 4.3-kb capu message found in adult males and ovarectomized adult females are indicated. The RP49 probe was included to indicate whether equivalent amounts of mRNA were loaded on the gel.
Lupas et al. (1991) to form coiled-coils, but capu does not.

To provide further confirmation that we have identified the capu gene and to identify regions of the protein that are functionally important, we have sequenced the region carboxy-terminal to position 2200 (-47% of the protein coding region) from genomic DNA of the 8 mutant alleles (capu<sup>7</sup>, capu<sup>2f</sup>, capu<sup>RK</sup>, capu<sup>38</sup>, capu<sup>L2</sup>, capu<sup>2F</sup>, and capu<sup>3871</sup>) and compared it to the respective parental chromosomes. capu<sup>RK</sup>, capu<sup>2F</sup>, and capu<sup>HR</sup> are all missense mutations outside of the FH2 domain, but at positions conserved with the mutant alleles.

Fig. 4. (See facing page for B, C, and legend.)
**Figure 4.** Sequence analysis of *capu*. (A) Primary sequence of the *capu* cDNA clone (GenBank accession no. U34258). The predicted 114-kD protein encoded by the 1058-amino-acid ORF is shown below the DNA sequence. The protein consists of 3 domains: the amino-terminal 484 amino acids, which are not conserved; the proline-rich region, which is underlined; and the carboxy-terminal 503 amino acids, which are conserved with the vertebrate *Id* proteins (see Fig. B and C). The asterisks indicate the FH2 domain. DNA and amino acid changes identified in mutant alleles are noted. (B) Known intervening sequences. All intervening sequences have been identified 3' to position 2200 by genomic sequencing. Additional intervening sequences may be present 5' to 2200. (B) Line drawing showing the conserved spacing between the proline-rich and FH2 domains. The small solid rectangles represent the proline-rich and FH2 domains, whereas the hatched rectangles indicate the region of similarity between *capu* and the *formins* and the stippled rectangle indicates the region of similarity between *capu* and *dia* (25% identity, 46% similarity outside of the FH2 and proline-rich domains). Only partial sequence for FigA is available. The percent sequence identity and similarity in the FH2 domain between *capu* and the other FH2 domain containing proteins is indicated. (C) Multiple alignment (GCG Pileup) of the similar domains of *capu*, mouse *formin IV* (Jackson-Grusby et al. 1992), *Drosophila* *dia* (Castrillon and Wasserman 1994), *S. cerevisiae* BNII (J. Pringle, pers. comm.), S. *pombe* CDC12 (F. Chang, pers. comm.), *Aspergillus* FigA (Marhoul and Adams 1995), S. *pombe* fusl (GenBank accession no. L37838; Petersen et al. 1995), an unnamed *S. cerevisiae* ORF (GenBank accession no. 238059, NCBI gi:557764), a human EST (GenBank accession no. R39757), a rice EST (GenBank accession no. D24760), and an *Arabidopsis* EST (GENBANK accession no. R30345). While the entire carboxy-terminal regions of *capu* and the formins are shown, only the FH2 domains of the other proteins are shown. Amino acid changes found in *capu* mutant alleles are marked. There is no significant alignment between the *formins* and *dia* in the carboxyl domain outside of the proline-rich and FH2 regions. We do, however, see a significant alignment between the formins and *dia* in the carboxyl domain outside of the proline-rich and FH2 regions, but the similarity is substantially lower (41% identity, 60% similarity between *capu* and formin IV vs. 20% identity, 45% similarity between *dia* and formin IV). Where a majority of the sequences are identical, they are shaded in black. Where a majority are similar to each other, they are shaded in gray. Similar is defined as a similarity >0.5 in the normalized Dayhoff matrix used by UWGCG (Gribskov and Burgess 1986).
rather than loss of a specific component of the pathway.

capu egg chambers display abnormal microtubule distributions and premature cytoplasmic streaming

Because capu (and spir) affect both the dorsal-ventral and anterior-posterior axes, we suggested that the mutants might affect the cytoskeleton (Manseau and Schüpbach 1989b). To examine the microtubule cytoskeleton, mutant and wild-type ovaries were labeled by indirect immunofluorescence with an antibody directed against α-tubulin. Abnormal microtubule distributions were seen in mutant stage 8 and 9 egg chambers (see Fig. 6). Long and thick immunofluorescently labeled tubulin fibers are seen wrapping around the cortex of mutant oocytes that are not seen in similarly staged wild-type oocytes (see also Theurkauf 1994).

This phenotype is reminiscent of that seen in yeast when α- and β-tubulin are overexpressed—unusual structures containing microtubules are seen around the cortex of the cell (Burke et al. 1989; Bollag et al. 1990). It is difficult to assess the levels of tubulin in mutant and wild-type egg chambers because the configuration of the microtubules is different in these two genotypes. For this reason, we examined the tubulin levels in mutant and wild-type egg chambers by use of immunoblots. Both α- and β-tubulin are found in equivalent levels in mutant and wild-type egg chambers (data not shown), suggesting that the reason for the novel microtubule distribution is not high levels of tubulin in mutant egg chambers. The possibility still exists, however, that tubulin levels are normal within the egg chamber as a whole, but are elevated within the oocyte.

Because the abnormal distribution of microtubules in mutant stage 8 egg chambers resembles that seen in stage 10 wild-type oocytes, Theurkauf examined the behavior of capu mutant egg chambers and found that they undergo premature cytoplasmic streaming within the oocyte (W.E. Theurkauf, pers. comm.). We have confirmed this finding and have also seen this phenotype in spir mutant egg chambers. The premature streaming within the oocyte is inhabitable by colchicine, indicating that it, like that at stage 10 in wild-type oocytes, is microtubule based.

To determine whether the premature streaming is likely to be the cause of the patterning defects in capu, we have carefully analyzed the speed and timing of streaming in a capu mutant allelic series. We see no significant difference in the speed of streaming between
weak \((capu^{2F}, <5\% \text{ abnormal eggshells})\), moderate \((capu^{RR}, 40\%-70\% \text{ abnormal eggshells})\), and strong alleles \((capu^{G7}, >75\% \text{ abnormal eggshells}; capu^{EE}, 60\%-90\% \text{ abnormal eggshells})\) at stage 8 of oogenesis [see Fig. 7]. Nor do we see a difference in the speed of streaming between a strong allele that dorsalizes, \(capu^{EE}\), and a strong allele that weakly ventralizes, \(capu^{G7}\). Thus, the speed of streaming does not correlate with the strength of the mutant allele [as shown by the percentage of eggshells exhibiting dorsal–ventral defects]. The dorsal–ventral defects in \(capu\) mutant offspring are thought to result from the mislocalization of \(gurken\) mRNA at stage 8 of oogenesis (see Fig. 7). Two distinct models can be invoked to explain the lack of properly localized molecular determinants in \(capu\) and \(spir\). The first is that after determinants are localized to their proper intracellular position during stages 8 and 9 of oogenesis, they must be anchored to the cytoskeleton to be stable to the cytoplasmic streaming that happens during stage 10. In this model, the premature cytoplasmic streaming during stages 8 and 9 in \(capu\) and \(spir\) mutant oocytes happens before this anchoring step and thus sweeps away molecular determinants. In the second model, it is the misorganization of the microtubules, required for subcellular targeting of determinants, that is responsible for the localization defects. Cytochalasin D treatment induces premature, microtubule-dependent cytoplasmic streaming within the oocYTE of approximately the same speed as that seen in \(capu\) [0.1 \(\mu\text{m/sec}\)]. This induced streaming is accompanied by microtubule bundling around the cortex of the oocyte [J. Calley, H. Phan, S. Emmons, and L. Manseau, in prep]. This ability to induce cytoplasmic streaming provided us with a unique opportunity to distinguish between these models.

To determine whether streaming induced with cytochalasin D results in the mislocalization of molecular determinants similar to what is seen in \(capu\) and \(spir\), we examined the distribution of determinants in egg chambers that were treated with cytochalasin D for 15 or 30 min and then fixed. \(Staufen\) protein is one of the earliest components to localize to the posterior pole in wild-type oocytes, but does not localize to the posterior pole in \(capu\) and \(spir\) mutant oocytes [St. Johnston et al. 1991]. In the dorsal–ventral axis, \(gurken\) mRNA is localized in wild-type oocytes specifically to the dorsal–anterior corner near the oocyte nucleus in stage 8 egg chambers, whereas in \(capu\) and \(spir\) mutant oocytes, \(gurken\) mRNA is found along the entire anterior end of the oocyte [Neuman-Silberberg and Schüpbach 1993]. We induced streaming in wild-type egg chambers by immersing oocytes, but not dorsal–ventral determinants

**Figure 7.** Speed of cytoplasmic streaming in wild-type and \(capu\) mutant oocytes. The speed of streaming at stage 8 of oogenesis is shown for two wild-type strains: \((cn\ bw)\ cn\ bw/cn\ bw, n = 12\) and \((OrR)\ Oregon\ R, n = 13\), for \(capu\) mutant alleles: strong alleles: \((G7)\ capu^{G7}/capu^{G7}, n = 9\) and \((EE)\ capu^{EE}/capu^{EE}, n = 9\); moderate alleles \((RK)\ capu^{RK}/capu^{RK}, n = 10\); and weak alleles \((2F)\ capu^{2F}/capu^{2F}, n = 11\). By Tukey’s test of multiple comparisons [SAS Proc GLM] the speeds of streaming in the \(capu\) mutant genotypes are indistinguishable from each other and are distinguishable from the parental strain \(cn\ bw\) at the 0.05 level. All are also distinguishable from the wild-type strain \(Oregon\ R\) except for \(capu^{EE}\). Oregon R and \(cn\ bw\) are not distinguishable from each other. The error bars represent the S.E.

**Premature ooplastic streaming displaces posterior but not dorsal–ventral determinants**

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plasmic streaming. Protein in stage 8 oocytes of untreated wild type
Figure 8. Distribution of determinants after 30 min of cytoplasmic streaming. [A,B] The distribution of gurken mRNA in
stage 8 oocytes of untreated wild-type oocytes (A), and wild-type
oocytes treated with cytochalasin D for 30 min [B]. After 30 min of
cytoplasmic streaming, the gurken mRNA staining is indistin-
guishable from wild type. (C,D) The distribution of staufen protein in stage 8 oocytes of untreated wild type (C) and wild-
type treated with cytochalasin D for 30 min [D]. After 30 min of
cytoplasmic streaming, the staufen protein no longer localized
to the posterior pole.

Discussion
capu is highly related to the vertebrate formins
Capu is most closely related to the products of the Id locus, known as the formins, being 40% identical and
60% similar over the 503-amino-acid carboxyl terminus of the protein. The amino terminus of the capu protein
does not appear to be related to that of the formins. This
is not surprising, as the formins have strongly divergent
amino termini encoded by a group of alternatively
spliced messages. In particular, one splice product of the mouse Id locus, formin IV, produces a protein with an
acidic amino terminus [pI = 4.5], whereas the pI of the
amino termini of the other three isoforms is basic
[pI = 9.8] [Jackson-Grusby et al. 1992]. The amino termi-

nus of capu most closely resembles that of formin IV in
this respect, having a pI of 5.27. The variation in the
amino terminus of this gene family suggests that the
amino- and carboxy-terminal regions of the protein are
probably separate functional domains with perhaps only
the carboxy-terminal function being conserved between
capu and the vertebrate formins.

The Id locus, like capu, functions in polarity. Mutants in the mouse Id locus have truncations of the anterior–
posterior limb axis resulting in fusions of digits, indicating
that the gene functions in limb patterning [Zeller et al. 1989]. The morphology and packing of the cells of the
apical ectodermal ridge (AER) is abnormal in Id mutant
animals, suggesting that the AER is defective either in
differentiation or in organization [Zeller et al. 1989]. The
Id locus is expressed at a fivefold higher level in the
ectoderm than in the neighboring mesenchyme. Taken
together with the fact that the anterior–posterior axis
defects in Id are similar to those seen when portions of the
AER are removed, this suggests that the AER is the
primary focus of the defects in Id anterior–posterior axis
formation [Zeller et al. 1989]. In addition to limb defects,
Id mutants often exhibit renal aplasia [Trumpp et al.
1992].

Chick formin has been shown to be localized in a
punctate pattern in the nucleus and to be distributed
throughout the cytoplasm during mitosis [Trumpp et al.
1992]. The nuclear localization of the chick formin
[Trumpp et al. 1992] and the binding of mouse formin IV
to DNA–cellulose in crude nuclear extracts [Vogt et al.
1993] has led to the suggestion that the protein might
function as a transcription factor, although the lack of in
vivo localization to the chromosomes [Trumpp et al.
1992] casts doubt on this hypothesis. A second possible
nuclear function—in splicing—has been partially dis-
credited because the protein does not colocalize with at
least some spliceosomes [Trumpp et al. 1992].

The in vitro demonstration of an SH3 domain-binding
region in mouse formin suggests a role for the formins in
protein–protein interactions during signal transduction
or in the cytoskeleton [Gout et al. 1993; Mayer and Balti-
more 1994; see references in Musacchio et al. 1992;
Pawson and Gish 1992]. The exact function of SH3 do-
main–ligand interaction is not well-defined. In a number
of enzymes, the SH3 domains play a role in regulation of
enzymatic activity [Gout et al. 1993; Mayer and Balti-
more 1994; Pleiman et al. 1994]. They are also thought to
provide specificity to protein–protein interactions [Gout
et al. 1993; Ren et al. 1993; Lim et al. 1994]. In at least
some cases, the SH3 domain can target molecules to a
particular subcellular location. For instance, the SH3 do-
main of phospholipase Cγ is sufficient for targeting to
stress fibers, whereas that of GRB2 targets to membrane
ruffles [Bar-Sagi et al. 1993].

The FH2 domain containing family
All of the FH2 domain-containing proteins for which we
have sufficient sequence, also contain a proline-rich re-

region. In addition to capu and the formins, two of these
also appear to function in polarity. The S. cerevisiae gene
BN11 was identified by mutants that are synthetically
lethal with mutants in CDC12, one of the bud neck fil-
ament proteins [J. Pringle, pers. comm.]. Mutants in
BN11 result in random bud-site selection in diploids dur-
ing bipolar budding, suggesting a role in cell polarity in
yeast. Mutants in Aspergillus FigA result in fat, highly
branched hyphae and abnormal hyphal tips [Marhoul and
Adams 1995].

Two members of this group play a role in cytokinesis.
Mutants in diaphanous (dia) have binucleate cells indicative
of defects in cytokinesis during spermatogenesis,
during oogenesis in the follicle cells, during imaginal
disc development, and in neuroblasts from the larval
central nervous system [Castrillon and Wasserman
1994]. Mutants in the S. pombe gene CDC12 do not un-
dergo cytokinesis and fail to form the actin contractile
ring [F. Chang, pers. comm.]. The final gene, S. pombe
fus1, functions in conjugation–conjugation tubes meet,
but the intervening cell walls do not dissolve [Bresch et
al. 1968].
It is intriguing that all of the proteins identified as containing the FH2 domain also contain a proline-rich domain with approximately the same spacing. This suggests that these two domains function together in some way. Because the proline-rich region of the formins has been demonstrated to be capable of acting as a binding site for SH3 domains in vitro (Ren et al. 1993), it seems likely that the proline-rich regions in the remaining FH2 domain-containing proteins are acting similarly. In most of the FH2 domain-containing proteins, the proline-rich region is quite extensive, being much larger than that required to function in SH3 domain binding in vitro (Ren et al. 1993; Sparks et al. 1994; Yu et al. 1994). Perhaps these extensive proline-rich regions are serving some additional purpose besides SH3 binding. Proline-rich regions are known to sometimes function as hinge regions between protein domains as has been seen in vinculin (Sakai and Shibata 1985), although the significance of this is unclear.

**The relationship between microtubule distribution, cytoplasmic streaming, and patterning**

It seems unlikely that the premature cytoplasmic streaming in capu is sufficient to explain all of the patterning defects. We have extended the observations of Theurkauf (1994) by analyzing streaming in weak, moderate, and strong alleles of capu and see no correlation between the speed or the timing of streaming and the strength of the mutant capu allele. The dorsal–ventral defects in capu are easier to score than the anterior–posterior ones and thus, have been used to rank the mutant alleles (Manseau and Schüpbach 1989; L. Manseau and J. Calley, unpubl.). Thus, the lack of correlation between the dorsal–ventral defects of the mutant alleles and the speed or timing of streaming cannot be explained and suggests that the premature streaming is not the cause of these patterning defects. The same may be true of the posterior defects, but measurement difficulties make this less clear.

That the induction of streaming with cytochalasin D does not displace gurken mRNA from the oocyte nucleus, indicates that as soon as gurken mRNA is positioned during stages 8 and 9 of oogenesis, it is stable to cytoplasmic streaming. This suggests that the lack of localization of gurken mRNA in capu and spir mutant oocytes is not likely to result from being swept away by premature cytoplasmic streaming. An alternative explanation is that mislocalization results from the microtubules being bundled at the cortex of the oocyte so that they are unable to transport gurken mRNA to its normal intracellular location. As it is currently unknown whether microtubules are required for the localization of gurken mRNA, some other transport system responsible for gurken mRNA localization to the oocyte nucleus may also be disrupted.

In the anterior–posterior axis, the lack of pole cells in capu is completely penetrant, but the extent of abdominal segmentation defects appears to correlate with the strength of the mutant allele (Manseau and Schüpbach 1989b). This, together with our findings that there is no correlation between the streaming and the strength of the mutant allele, suggests that premature streaming in capu may not explain the posterior defects. It would be easy to miss subtle differences in the speed of streaming between alleles, however, and there are difficulties in quantitating the extent of the posterior defects [which are masked by the dorsal–ventral defects]. In addition, the finding that cytochalasin-induced streaming does displace staufen protein from the posterior pole supports the model that premature streaming in capu does result in posterior defects. Pokrywka and Stephenson (1995) report that cytochalasin D treatment does not affect oskar mRNA localization at the posterior pole. We do not believe, however, that these results conflict with ours because the concentration of cytochalasin D used in their study does not induce premature cytoplasmic streaming (L. Manseau, unpubl.).

The observation that capu egg chambers have abnormal distributions of microtubules and premature microtubule-based cytoplasmic streaming suggests it is likely that capu is either directly or indirectly regulating the cytoskeleton. Continued molecular analysis of capu, including protein localization within the oocyte–nurse cell complex should provide clues to the role that this gene family plays in pattern formation. In addition, ongoing molecular analysis of spir, a gene with the same phenotype as capu, should provide further insight into the role capu and related genes play in polarity establishment.

**Materials and methods**

**RFLP mapping of capu**

Females homozygous for w and for a w+–carrying P element in 24D1.2 were mated to w/Y capu<sup>EE</sup>/SM6.b Roi males. In the F<sub>1</sub> females carrying the P[w<sup>+</sup>1] and capu<sup>EE</sup> were mated with w/Y capu<sup>EE</sup>/SM6.b Roi males. In the F<sub>2</sub> P[w<sup>+</sup>1] females who had mated with their brothers were placed in groups of 50 into plastic cups and their eggs collected on apple juice agar plates. To identify females carrying P[w<sup>+</sup>1]–capu recombinant chromosomes, the plates were examined for the presence of eggs with the capu phenotype. Once such eggs were identified, the females were segregated and their eggs examined to identify the individual P[w<sup>+</sup>1]–capu recombinant female. We screened 23,000 chromosomes for recombination events between the P[w<sup>+</sup>1] and a mutant capu allele, capu<sup>y</sup>, and identified 17 females carrying P[w<sup>+</sup>1]–capu recombinant chromosomes. Southern blots of genomic DNA isolated from single recombinant flies (Jowett 1986) were analyzed with DIG-labeled probes as described in the Genius System User’s Guide for Filter Hybridization (Boehringer Mannheim).

**Northern blot analysis**

Poly(A)<sup>+</sup> mRNA was isolated according to the procedure of Jowett (1986) and Northern blots were prepared as in Sambrook et al. (1989). Each lane contained 5 μg of Poly(A)<sup>+</sup> mRNA. Blots were probed using DIG-labeled probes as described in the Genius System User’s Guide for Filter Hybridization (Boehringer Mannheim). The probe for the Northern blot of mutant RNA was made from the 1-kb EcoRI–NotI fragment at the 3' end of
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the gene. The probe for the developmental Northern was made from the 0.9-kb EcoRI fragment from the extreme 5’ end of the gene.

Whole-mount tissue in situ hybridizations

Whole-mount tissue in situ hybridizations were performed basically as in Tautz and Pfeifle [1989] with DIG-labeled RNA probes. Probes from both the 0.9-kb EcoRI fragment at the 5’ end and the 1-kb EcoRI-NotI fragment at the 3’ end of the capu gene were used. The gurken probe was made from cDNA clone 1.7 [Neuman-Silberberg and Schüpbach 1993].

DNA sequencing

DNA sequencing was done by use of a Sequenase kit (U.S. Biochemical). DNA sequencing of mutant alleles was performed on DNA that was PCR amplified from genomic DNA of homozygous mutant alleles and then treated with shrimp alkaline phosphatase and exonuclease I as in the Sequenase PCR Product Sequencing Kit (U.S. Biochemical). Mutant alleles were sequenced in the region between position 2185 and 3754 in the DNA sequence.

Morphological analyses

Cuticles and chorions were prepared for analysis as described in Wieschaus and Nüsslein-Volhard [1986].

Immunocytochemistry

Wild-type and mutant ovaries were dissected in Ehrussi and Beadle’s Ringer’s solution and fixed for 10 min with vigorous shaking in a 5:1:5 ratio of heptane, 37% formaldehyde, PEM (0.1 M PIPES, 1 mM MgCl₂, 1 mM EGTA at pH 6.9). The aqueous phase was removed and an equal volume of 90% methanol: 10% 500 pg/ml and then rehydrated through a methanol series (70% MeOH, 50% MeOH, and 30% MeOH in PBS) for 10 min each wash and then finally washed in PBS for 10 min. Ovaries were blocked for 2 hr in 0.1% fish gelatin, 0.8% BSA, and 0.03% Tween 20 in PBS. Ovaries were then incubated at 4°C for ~48 hr with primary antibody diluted in blocking solution with 0.1% Triton-X 100. The anti-a-tubulin antibody (4a41) was used at a dilution of 1:10. Excess primary antibody was then removed by four 30-min washes with blocking solution at room temperature. The anti-staufen antibody (provided by Daniel St. Johnston, Welcome/CRC Institute, Cambridge, UK) was used at a dilution of 1:10. Ovaries were then incubated with cy3 (1:100)-labeled secondary antibody diluted in blocking solution for 18 hr at 4°C. Excess secondary antibody was removed by three 30-min washes with blocking solution at room temperature. Ovaries were dissected and mounted in a 0.2% solution of n-propylgallate in glycerol for viewing.

Immunoblots

To assay the levels of tubulin in mutant versus wild-type egg chambers, 50 stage 8 or 9 egg chambers were hand dissected from capu⁷⁷/capu⁷⁷, capu⁷⁵/capu⁷⁵, and wild-type Oregon R females. The egg chambers were then lysed in protein sample buffer at 95°C for 5 min. The samples were split in half and the duplicate samples were loaded on a 10% polyacrylamide-SDS gel. After electrophoretic separation, one set of lanes was stained with Coomassie Blue to assure that the samples were loaded equivalently. Samples on the other half of the gel were transferred to Immobilon-P membrane (Millipore Corp.) [Towbin et al. 1979] and then probed with antibody raised against Drosophila α-tubulin (4a1). Antibodies were visualized by chemiluminescence with the Renaissance system made by DuPont NEN. The anti-α-tubulin antibody was then removed from the membrane by washing in 1% SDS for 10 min at 50°C. The blot was then reprobed for β-tubulin levels with anti-β-tubulin antibody (Sigma T 4026) and visualized as above.

Time-lapse video microscopy

Egg chambers were mounted in Robb’s saline solution under a coverslip that was supported by pieces of coverslips. The chambers were viewed on a Zeiss Axiosplan microscope and recorded with a Sanyo VCB-3524 CCD camera and a Toshiba KV-6300A time lapse video recorder at the rate of one frame every 4 sec. To measure the speed of streaming, this was projected on a screen at 60 frames per second (240×1). With a Sharp QA-1050 LCD Computer Projection Panel, the image of a moving ball produced by a Macintosh computer program (written by J. Colley; available upon request) was projected onto the same screen. After calibration, the direction and speed of the moving ball was modified until it visually matched the speed of the moving cytoplasm. The speed of the moving ball was then recorded as the streaming speed. To show the effect of a drug on streaming, chambers were filmed for 20–30 min. Then the coverslip was lifted and excess solution was removed. Finally, the 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 solution. Controls were performed by adding Robb’s saline solution 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 solution before use.

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