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The *Drosophila* gene *fs(2)cup* interacts with *otu* to define a cytoplasmic pathway required for the structure and function of germ-line chromosomes

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SUMMARY

The Drosophila *ovarian tumor* gene *(otu)* encodes cytoplasmic proteins that are required in germ-line cells for cyst formation, nurse cell chromosome structure and egg maturation. We have analyzed a gene, *fs(2)cup*, that participates in many of the same processes and interacts with *otu* genetically. Both nurse cell and oocyte chromosomes require *cup* to attain a normal morphology. In addition, the gene is needed for the oocyte to grow normally by taking up materials transported from the nurse cells. The gene encodes a 1132-amino-acid protein containing a putative membrane-spanning domain. Cup protein (but not *cup* RNA) is transported selectively into the oocyte in germarial cysts, like the p104 Otu protein. It is strongly associated with large structures in the cytoplasm and perinuclear region of nurse cells and, like Otu, moves to the periphery of these cells in stages 9-10. Moreover, *cup* mutations dominantly disrupt meiotic chromosome segregation. We propose that *cup*, *otu* and another interacting gene, *fs(2)B*, take part in a common cytoplasmic pathway with multiple functions during oogenesis.

Key words: *Drosophila*, *ovarian tumor* gene, *otu*, *cup*, chromosome, germ line, nurse cell, egg maturation, oocyte

INTRODUCTION

Eggs are produced rapidly in the adult *Drosophila* ovary due to the activity of specialized accessory cells (reviewed in Spradling, 1993). In the germarium located at the tip of each ovariole (Fig. 1a), germ-line cells containing a special cytoplasmic structure, known as the fusome, divide synchronously four times to found germ-line cysts containing 16 interconnected cells. 15 of the cells differentiate as nurse cells, which synthesize and transport products required for the development of the remaining cell, the synthetically inactive oocyte (Fig. 1a). In addition, follicle cells surround growing 16-cell cysts while they still reside within the germarium. Eventually, cysts and their associated follicle cells bud off as discrete units called egg chambers, each separated from adjacent chambers by follicular stalks.

Germ-line cell chromosomes become highly adapted for the specialized functions they perform during oogenesis. Oocyte chromosomes undergo synapsis and recombination during the early stages of cyst development. Then the nucleus condenses into a synthetically inactive karyosome around stage 3 (see Fig. 1a for stages), and remains in meiotic prophase until just before the oocyte matures. Nurse cell chromosomes also undergo visible changes in structure as egg chambers develop (Hsu and Hansen, 1953; King, 1970; Hammond and Laird, 1985). The banded polytene chromosomes characteristic of young nurse cells progressively disperse, and large, dispersed nucleoli form. These changes in nurse cell chromosome organization have been postulated to facilitate the high synthetic levels of ribosomes and other components required for rapid oocyte formation (Spradling, 1993).

Several genes are required for normal nurse cell chromosome development and to sustain normal oocyte growth, including *fs(2)B* (King et al., 1957), *supressor of Hairy-wing (su(Hw)*, Klug et al., 1968), *ovarian tumor (otu*, King et al., 1981), *D-elg* (Schultz et al., 1993) and *string-of pearls* (Crampton and Laski, 1994). The role played by *otu* is particularly interesting, since nurse cells bearing certain *otu* alleles frequently develop large polytene chromosomes (King and Storto, 1988) and are unable to support a normal rate of oocyte growth.

The *otu* gene has long been thought to play a key role in cyst development and growth based on the analysis of three classes of alleles (Storto and King, 1988). Germ-line cells are absent in quiescent (QUI) alleles, but overproliferate to form benign tumors in oncogenic (ONC) alleles. In differentiating (DIF) alleles, nurse cells display abnormal chromosome morphology, fail to growth normally and do not fully transfer their contents to the oocyte. *otu* encodes a 104 kDa cytoplasmic protein sufficient for gene function as well as a 98 kDa isoform of uncertain function (Rodesch et al., 1995; Sass et al., 1995). At least some germ cells in *otu* tumors fail to undergo proper female sex determination (Pauli et al., 1993), but it remains unclear whether this is a direct effect (Bae et al., 1994; Horabin et al., 1995). *Otu* has also been proposed to act via the fusome (King and Storto, 1988), the cytoskeleton (Sass et al., 1995) and in association with microtubules (Tirronen et al., 1995).

We have characterized a new gene, *fs(2)cup*, that affects many of the same processes as *otu*. *cup* encodes a cytoplasmic

protein that is expressed in a similar pattern to Otu p104, and interacts genetically with the p104-specific *otu*^{11} allele. Our results imply that these genes participate in a specialized cytoplasmic pathway required for egg chamber formation and growth. We speculate that it may involve the trafficking of microtubule-associated vesicles.

MATERIALS AND METHODS

Drosophila stocks and crosses

Flies were raised on standard food at 22-25°C except as indicated. Genetic markers and balancer chromosomes are described in Flybase (1993). *cup*^{1355}, *cup*^{4506} and *cup*^{6890} were recovered in a single-*P* element mutagenesis screen (Karpen and Spradling, 1992). Derivatives of *cup*^{4506} and *cup*^{1355} were generated by inducing transposition of the *P[lacZ, ry]* element in the male germ line. EMS-induced alleles of *cup* were provided by T. Schüpbach. Alleles of *otu* (see King and Storto, 1988) were obtained from A. Mahowald and from the Bloomington Stock Center. Strains bearing *fs(2)B* were kindly provided by the Bowling Green Stock Center. For non-disjunction tests, progeny stocks were backcrossed to parental *otu* and *cup* chromosomes to confirm their genotypes.

Microscopy

Whole ovaries were dissected, fixed and mounted in 50% glycerol containing 1 μg/ml DAPI as described previously (Lin and Spradling, 1993). Relative egg chamber volumes were estimated by using the program NIH Image.

Molecular analysis

Genomic DNA flanking the insertion in *cup*^{4506} was isolated by plasmid rescue and used to probe a genomic library (Maniatis et al., 1978). cDNAs were isolated from an ovarian library (Stroumbakis et al., 1994). In situ hybridization of digoxigenin-labeled sense and antisense cDNA probes was carried out using the Genius labeling kit (Boehringer) by the method of Tautz and Peifle with modifications (Suter and Steward, 1991).

Antibody production and western analysis

Bacterially expressed Cup protein for immunization was produced using the 6× his tag system (QIAExpress, Qiagen). Two regions of the predicted Cup protein (cDNA nucleotides 758-1655 and 1655-2729) were independently fused in frame to N-terminal histidine tags. Recombinant protein from bacterial lysates affinity-purified over a Ni-NTA column (Qiagen) was used as immunogen. Polyclonal rat antisera were produced by HRP, Inc. Ovary extracts from wild-type and mutant females run on a 7% SDS-PAGE gel were blotted to nitrocellulose for western analysis. Primary anti-Cup antibodies were detected using secondary horseradish peroxidase-conjugated goat anti-rat IgG (Amersham) and chemiluminescent staining (ECL, Amersham). Antibody staining of ovaries was carried out as described in Lin et al. (1994).

RESULTS

Identification of *fs(2)cup*, a gene that disrupts nurse cell chromosome structure

Changes in chromatin structure that accompany wild-type nurse cell development are readily observed following DAPI staining of whole ovarioles (Fig. 1b). Nurse cells initially contain banded polytene chromosomes that can be resolved by squashing (Hsu and Hansen, 1953). During stages 4-5 (Fig. 1b,

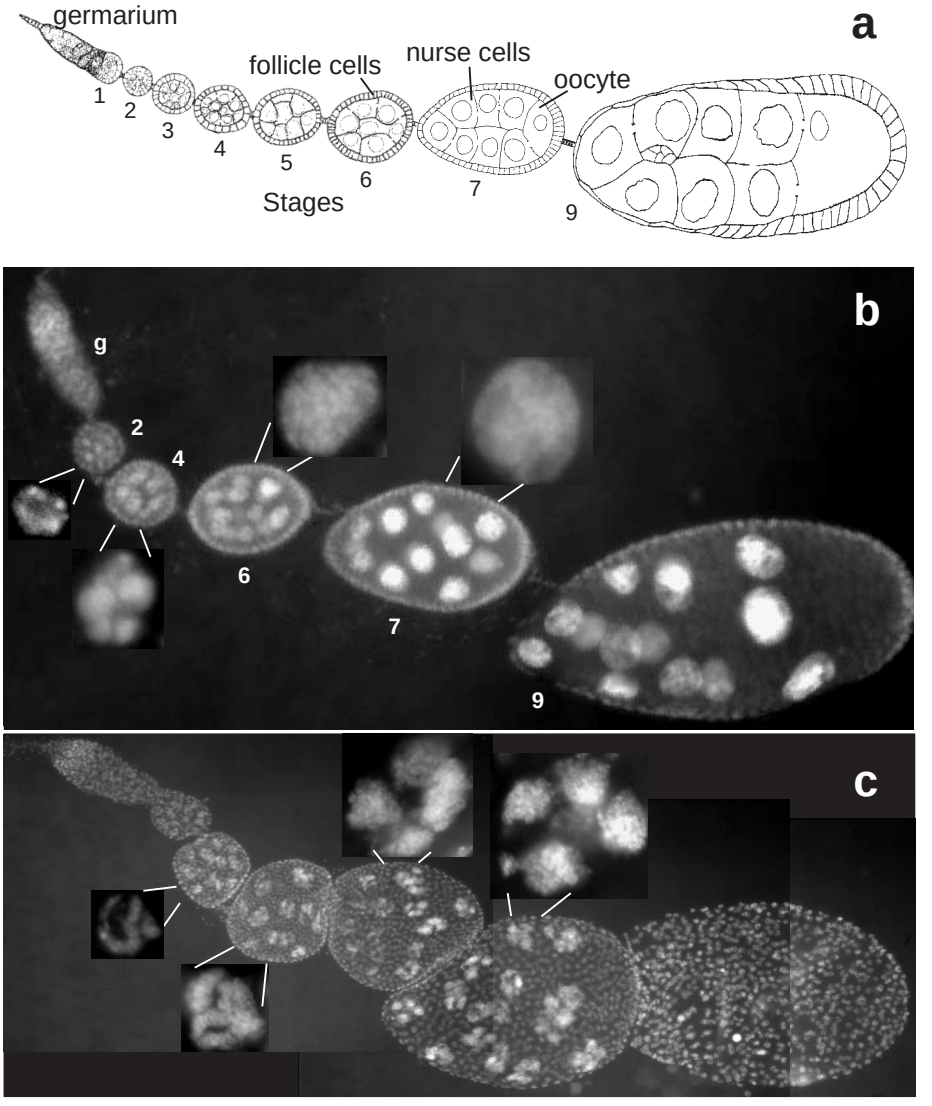

Fig. 1. Nurse cell nuclear morphology of wild-type and *cup* ovaries. Single ovarioles are shown from wild-type (b) and *cup*^{1} (c) females at equal magnification. For comparison, a schematic drawing of a wild-type ovariole (adapted from King, 1970) is shown in (a). The germarium (g) and major cell types are shown; numbers indicate specific stages of egg chamber development. The 15 large nurse cell nuclei are readily visible in the interior of the chambers, surrounded by the small follicle cell nuclei; the oocyte nucleus is not usually detectable. In b and c, one nurse cell nucleus from several chambers of increasing age is enlarged in the insets to show detail. Note that *cup*^{1} nurse cells never progress beyond a morphological stage characteristic of stage 4 wild-type chambers.

2nd chamber), banding is lost and five large blobs of chromatin become apparent, that presumably correspond to individual chromosome arms (King, 1970). Subsequently, the individual chromatin masses dissociate further into an indistinct jumble of oligotene fibers. Because several genes that are important for cyst formation and egg chamber growth are known to disrupt this progression, we screened a collection of female sterile single-*P* element insertion mutations (Karpen and Spradling, 1992) for strains with effects on nurse cell chromosomes.

One mutation in the collection, *fs(2)04506*, blocked nurse cell chromosomal development. The insertion in *fs(2)04506* was mapped to polytene band 27B by in situ hybridization. Complementation analysis with other genes in this region revealed that *fs(2)04506* is allelic to a previously described female sterile gene, *fs(2)cup* (Schüpbach and Wieschaus, 1991). In most *cup* alleles nurse cell chromosomes fail to decondense completely so that individual chromosomal masses remain distinct (Fig. 1c). There were no discernable effects of the mutation on the nuclear morphology of somatic cells; larval salivary gland polytene chromosomes appeared normal, as did ovarian follicle cell nuclei.

22 alleles of *cup* are described in Table 1. 18 of these were generated in the EMS screen of Schüpbach and Wieschaus (1991). Two additional *P* element-induced female sterile lines that also contain insertions in the 27B region, *fs(2)01355* and *fs(2)06890*, were shown by appropriate crosses to be allelic to *cup*. Mobilization of the P elements in cup^{4506} and cup^{1355} produced many fertile revertants, indicating that the *P* insertions in these strains were responsible for the *cup* phenotype. In addition, imprecise excision resulted in alleles that retained female sterility, of which one allele, cup^{R4}, showed a stronger effect on oogenesis. None of the excision derivatives affected viability or male fertility.

Effects of *cup* alleles on oogenesis

The *cup* alleles can be grouped into three general classes based on their stage of arrest (Table 1). Class 1, the largest set of alleles, causes egg chambers to arrest prior to vitellogenesis. The strongest class I alleles produce enlarged and misshapen germaria from which egg chambers do not always bud normally (Fig. 2a). Frequently two or more chambers remain tightly associated, and the nurse cells fail to grow past early stages. Commonly, as in cup^{4506}, egg chambers appear almost normal until stage 5, fail to decondense nurse cell chromatin, and subsequently degenerate by the time they reach the size of stage 7-8 egg chambers.

Egg chambers from class II females grow larger than class I chambers, taking up yolk and sometimes supporting follicle cell migration. However, nurse cell nuclei display abnormal chromatin configurations that can be easily visualized due to their large size. Some class II alleles retain the five-blob morphology characteristic of normal stage 5 egg chambers and of class I alleles (Fig. 2b), but others appear to contain 8-16 small polytene chromosomes in place of each blob (Fig. 2c,f). The exact borders of the domains are not sufficiently distinct to determine the exact number of these 'sub-polytene' chromosomes, however, and the chromosomal organization of individual nuclei frequently varies within a single egg chamber.

Females from the weakest group of *cup* alleles, class III, produce defective mature eggs characteristically shaped like cups. Egg chambers that will develop into cup-shaped eggs have normal proportions during early stages, but during stages 9 and 10 their oocytes reach only 1/4 to 1/2 the size of corresponding wild-type oocytes, and occupy no more than 33% instead of 50% of the egg chamber. The dwarf oocytes are not caused by a reduction in growth per se; indeed, some *cup* egg chambers become larger than wild type prior to nurse cell breakdown, despite their small oocytes, and contain extra follicle cells (Fig. 2i). The decreased size of the *cup* oocyte relative to its nurse cells suggests that the transport of materials from the nurse cells into the oocyte is reduced during stages 9 and 10. Nurse cell chromatin also remains abnormally condensed except in cup^{1355} and cup^{6890}, which have normal chromatin morphology.

The cup-shaped chorions characteristic of class III alleles result from changes in follicle cell migration that may be caused indirectly by the undersized oocytes. Normally, at stage 9 most of the follicle cells migrate posteriorly onto the oocyte surface (Fig. 2g). Later, some of these cells migrate centripetally

Table 1. Properties of selected *cup* alleles

Class	Allele	Stage of arrest	Nurse cell nuclei	Similar alleles
I	R4[c]	S5	Little DNA	8[b], 17[b]
	4506[a]	S5-6	Like normal S5	3[b], 6[b], 22[b], 33[b]
	20[b]	S5-6	Tight condensation	
	16[b]	S6-7	5-blob, variable	28[b]
II	13[b]	S7-8	5-blob, variable ploidy	
	15[b]	S7-8	Decondensed, variable	31[b]
	32[b]	S10	Condensed oligotene, high ploidy	
	4[b]	S10	Polytene, high ploidy	
	1[b]	S9-12	5-blob	
III	1355[a]	S14	Normal	6890[a], 26[b]
	24[b]	S14	Variable condensation	
	21[b]	S14, weakly fertile	Variable condensation	

Cup alleles are grouped into three major classes, as described in the text.

Sources: a, Karpen and Spradling (1992); b, Schüpbach and Wieschaus (1986); c, this study.

The order of the alleles reflects severity, as indicated by the oldest stage typically reached by egg chambers prior to degenerating. The morphology of nurse cell nuclear chromatin in the oldest chambers is listed.

Similar alleles: other *cup* alleles whose phenotype does not appear significantly different from the described prototype allele.

between the nurse cells and the oocyte, where they will eventually secrete the anterior end of the eggshell (Fig. 2h). *cup* oocytes may be too small to accommodate all the migrating follicle cells; many cells are observed to remain in contact with the four posterior nurse cells (Fig. 2i). A centripetal migration never occurs. Eventually, a chorion layer is secreted over the oocyte and posterior nurse cells by the overlying follicle cells, leaving the anterior end open, like a cup (Fig. 2j). Dorsal appendage-secreting follicle cells remain near their site of induction by the oocyte nucleus.

With a few possible exceptions, *cup* alleles behave as a continuous allelic series. Crosses using representative alleles from each phenotypic class produced progeny whose phenotype was intermediate between the parents, suggesting that these individual alleles produce different quantitative levels of *cup* function. For example, when a class I allele causing early arrest (cup^{R4}) was crossed to a class III allele (cup^{1355}), the ovarian arrest and chromosomal phenotype of the transheterozygote resembled that seen in class II chambers (Fig. 2f). Moreover, when stronger alleles from within class I and class III are used to make the combinations, egg chambers arrest growth earlier than when weaker alleles are combined.

Two alleles appeared to be distinctive. cup^{4} (class II) chambers frequently contain large banded polytene chromosomes in their nurse cells (Fig. 2e). These chambers develop as far as stage 9 or 10 and the chromosomes achieve high ploidy levels. This behavior is similar to several *otu* alleles that produce polytene chromosomes of sufficient quality for cytological analysis (King and Storto, 1988). cup^{20} (class I) chromosomes also remain tightly condensed, as in stage 4, with some evidence of a banding pattern. However, chromosomal organization within the nucleus is unique; the individual chromosomes traverse a uniform spiral path around the inside of the nuclear membrane (Fig. 2d). These phenotypes are not observed in other *cup* alleles or allelic combinations and may reveal an additional function of the Cup protein. The lack of a deficiency for the 27B region prevented further tests of the nature of these alleles.

Cloning the *cup* gene

Genomic DNA flanking the cup^{4506} insertion was isolated by plasmid rescue and used as an entry point to the *cup* locus. Within a 30 kb genomic region surrounding the insertion site, northern analysis identified a 4.1 kb poly(A)-containing RNA that is detected only in the ovary and in 0-2 hour embryos, but not in later embryos or other tissues (Fig. 3b). The 4.1 kb RNA is severely reduced in the ovaries of *cup* mutant females (Fig. 3b). In the testes, the 4.1 kb transcript is absent, but two male-specific transcripts of 4.3 and 3.8 kb are detected. An apparently full-length 4.1 kb cDNA was isolated from an ovarian cDNA library and used to map the position of the transcript. The cup^{1355} insertion is located within the untranslated region of the first exon, while the cup^{4506} and cup^{6890} insertions map within introns located further downstream. The location of all three *cup* insertions inside the transcription unit of the 4.1kb RNA argues strongly that it corresponds to *cup*.

Sequence analysis of the 4.1 kb cDNA predicted a protein of 1132 amino acids (Fig. 3c). The proline-rich N-terminal third contains a potential membrane-spanning domain

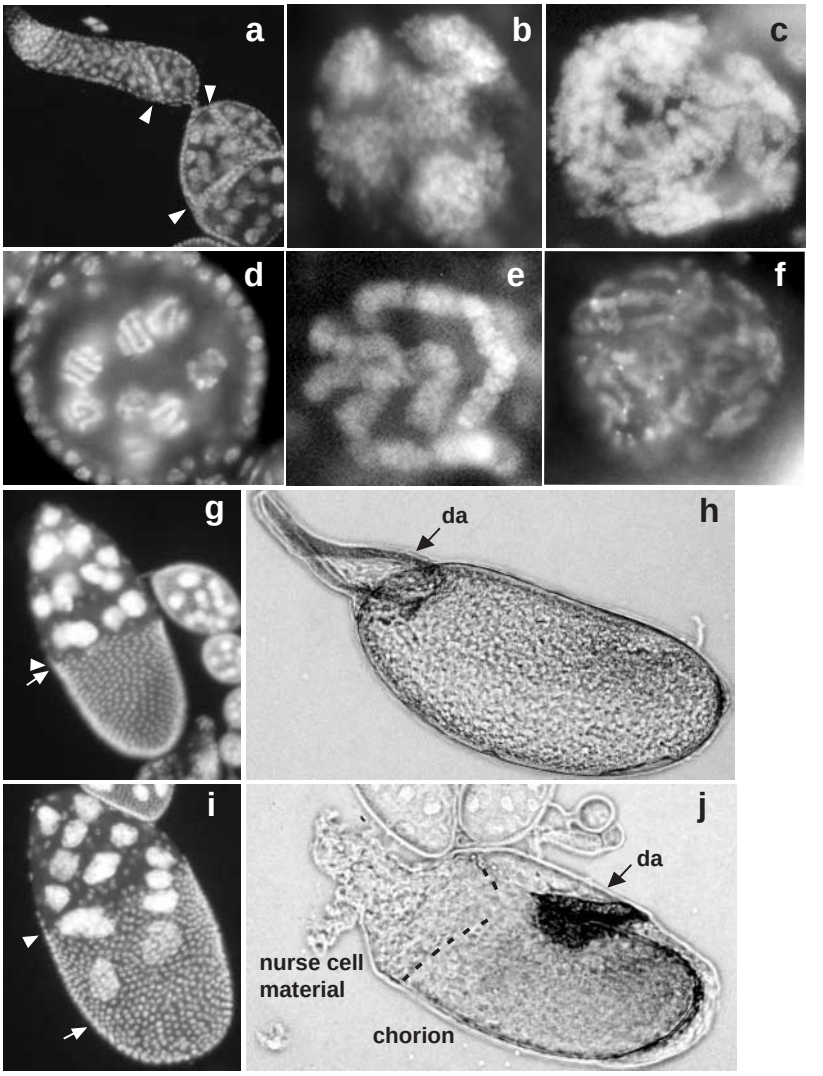

Fig. 2. Phenotypes of the *cup* alleles. DAPI-staining of nurse cell nuclei from *cup* mutant females. (a) cup^{31}germarium with unbudded chambers. (b) cup^{1} nucleus from stage 8 chamber, displaying stage 5 nuclear arrest morphology. (c) cup^{32} nucleus from stage 9 chamber, containing small condensed oligotene chromosomes. (d) cup^{20}, stage 5 chamber, with tighly condensed 'spiral' chromosomes. (e) cup^{4} nucleus, stage 9-10, with distinct polytene arms. (f) stage 9 nucleus from cup^{R4}/cup^{1355} transheterozygote. Formation of the cup-shaped chorion (g-j); 200× magnification. (g) wild-type egg chamber, stage 10a; arrow marks the anterior margin of the migrating follicle cells, arrowhead indicates the postion of the nurse cell/oocyte boundary. (h) wild-type mature egg (stage 14), with arrow indicating dorsal appendages (da). (i) cup^{1355} egg chamber, stage 10a. The arrow marks the anterior margin of the migrating follicle cells, the arrowhead indicates the position of the nurse cell/oocyte boundary. (j) cup^{1355} mature egg chamber, with open chorion; arrow indicates fused, mis-oriented dorsal appendages (da); dashed line marks the anterior margin of the chorion. Anterior is toward the top of the panel in g and i, to the left in h and j.

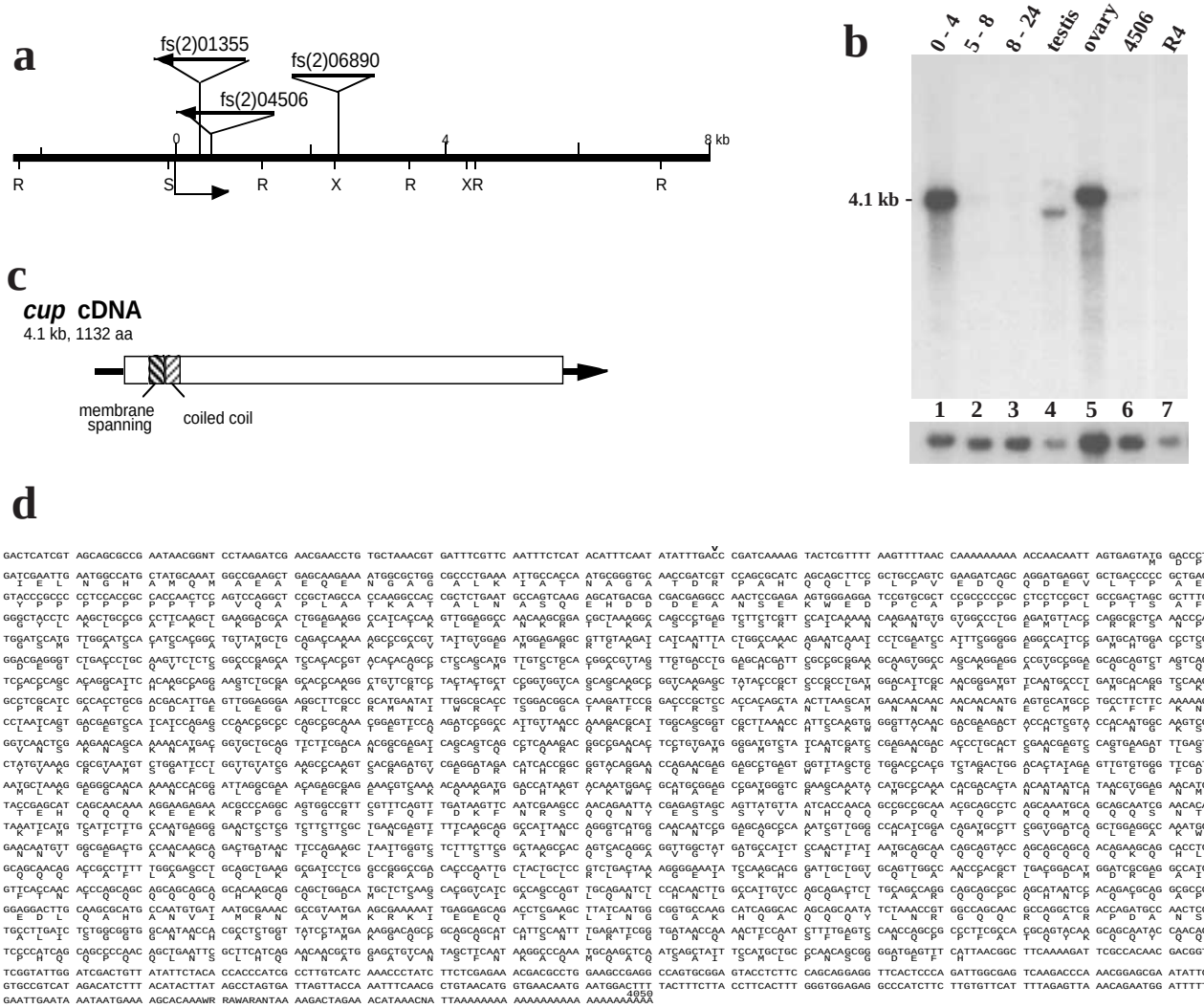

Fig. 3. Molecular structure of *cup*. (a) A restriction map of the 11 kb genomic region that includes the *cup* locus and P element insertion sites is shown. The 5′ end of the 4.1 kb cDNA is indicated by an arrow. The cup^{1355} insertion lies within untranslated exon 1; the cup^{4506} and cup^{6890} insertions are presumed to reside within introns since their flanks do not match the cDNA sequence, but the exact number and position of introns was not fully determined. (b) Northern blot of poly(A)$^+$ RNA hybridized with *cup* cDNA; tubulin reprobe is shown below. Lanes: 1, 0-4 hour embryos; 2, 5-8 hour embryos; 3, 8-24 hour embryos; 4, wild-type testes; 5, wild-type ovaries; 6, cup^{4506} ovaries; 7, cup^{R4} ovaries. (c) Schematic map of the *cup* cDNA. (d) Nucleotide and amino acid sequence of the 4.1 kb cDNA. The position of the cup^{1355} insertion is indicated by a triangle.

(residues 116-136) that lies adjacent to a small predicted coiled coil region (residues 135-159); no signal sequence was detected. The remainder of the protein contains multiple asparagine- and glutamine-rich regions (Fig. 3d). Database searches using BLAST and FASTA detected no strong homology to any previously identified genes.

Searches with two pattern-based programs, BLOCKS (Henikoff and Henikoff, 1994) and FASTA-PAT (Ladunga et al., 1996), detected weak similarities between Cup and several proteins involved in vesicle trafficking and/or microtubule binding. The BLOCKS program found a match between Cup and the vertebrate microtubule-associated protein MAP1B that was better than expected in 99% of comparisons between the randomized sequences. The strongest similarity is found between amino acids 190-212 of Cup and amino acids 2446-2468 that form the COOH terminus of MAP1B. The FASTA-PAT program matched Cup to a pattern derived from the yeast Uso1 protein and the related vertebrate p115 gene (Sapperstein et al., 1995) with a probability value given as 10^{-29}. (Simple FASTA alignment of Cup and Uso1 shows that approximately 14% of residues are identical over the whole protein.) Both Uso1p and p115 are known to be involved in the reassembly of ER and Golgi vesicles following mitosis. Scores lower than 10^{-26} were also recorded for three other proteins: gp360 macrogolgin, a Golgi-associated protein (Seelig et al., 1994), nestin, a nuclear intermediate filament protein (Kachinsky et al., 1994), and the CLIP170 vesicle-microtubule linker protein (Pierre et al., 1992). The significance of these similarities remains uncertain.

Expression of *cup* gene products

To learn where *cup* is transcribed during oogenesis, the 4.1 kb

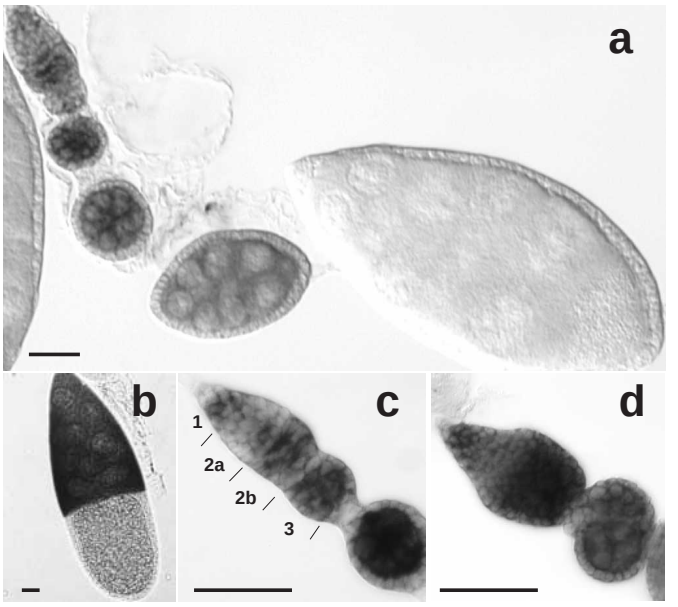

Fig. 4. Expression of*cup* RNA in ovaries. In situ hybridization of antisense-labelled *cup* cDNA to whole ovaries. (a) Wild-type ovariole, through stage 9. (b) Stage 10b, wild-type ovariole. (c) Germarium of wild-type ovariole; stages of germarial development are indicated. (d) Germarium of cup^{R4} ovariole. Scale bars, 20 μm.

cDNA was hybridized in situ to wild-type ovaries. *cup* RNA is present in germ-line cells throughout pre-vitellogenic development, but is not detected in the somatic follicle cells (Fig. 4a). The RNA is present in region 1 of the germarium, where it is detectable in stem cells, cystoblasts and dividing cysts (Fig. 4c). Steady state RNA levels decrease in region 2a, rise again in region 2b, and peak around stage 3 or 4. Subsequently, RNA levels decline and reach undetectable levels by stage 8. A second round of expression begins during stage 10 (Fig. 4b) and continues through stage 14. The transcript is not differentially localized within the germ line at any stage. None of the strong alleles abolished all *cup* RNA expression (Fig. 4d and data not shown).

Polyclonal antibodies were raised against two independent domains of the Cup protein. Antisera against both domains produce similar results when used to analyze ovarian extracts on western blots. Both sera detect a single predominant band of approximately 150 kDa that is abundant in wild-type ovaries but greatly reduced in *cup* mutant females (Fig. 5a). This is similar in size to the 130 kDa product predicted by the 4.1 kb cDNA. A second smaller band of 75 kDa reacts more weakly and variably with all *cup* sera tested, but this protein is unaffected by mutants and is presumed to be encoded by a separate locus. Testes contain very little of the 150 kDa protein; the anti-Cup antibodies instead detect bands of approximately 180, 110 and 45 kDa (Fig. 5a). This suggests that the testis-specific *cup* RNAs (Fig. 3b) encode non-identical but antigenically related Cup proteins.

The protein products of the EMS- and P-derived *cup* alleles were examined by western analysis. All but one of the *cup* alleles affect the quantity, rather than size of the 150 kDa protein, indicating that these alleles are not the products of premature termination. None of the strong *cup* alleles completely eliminates all immunoreactive protein (Fig. 5a, lanes 3-7 and data not shown), suggesting that null alleles have not been recovered. The level of Cup protein detected in these experiments correlates well with the genetic strength of the allele in question: weak alleles such as cup^{1355} and cup^{6890} (Fig. 5a, lanes 4 and 5) produce much more of the 150 kDa Cup protein than strong alleles such as cup^{16} and cup^{20} (Fig. 5a, lanes 6 and 7). One allele, cup^{1}, produced a smaller Cup protein of about 135 kDa (Fig. 5b), further confirming that the gene analyzed corresponds to *cup*.

Immunolocalization of Cup protein

Immunofluorescent antibody staining was used to determine the expression pattern and subcellular localization of the Cup protein. Beginning with the stem cells, Cup protein is found in the cytoplasm of all germ-line cells (Fig. 6a,b), but was never detected inside either nurse cell or oocyte nuclei. Staining of early embryos confirmed that Cup protein is maternally deposited in the egg (Fig. 6g). The protein is abundant and uniformly distributed in the cytoplasm of all cleavage stage embryos through stage 9, after which signal intensity declines. Staining is effectively absent by gastrulation, and remains so throughout the remainder of embryogenesis.

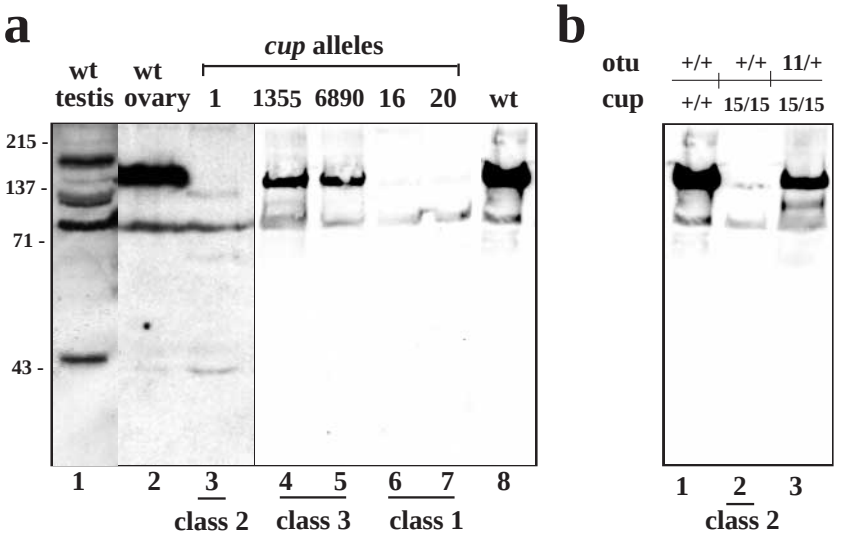

Fig. 5. Western analysis of protein extracts using antisera raised against amino acids 201-499 of the Cup protein. Vitellogenic egg chambers (stage 8 and older) were removed to minimize stage-specific protein contribution; equivalent amounts of tissue were loaded on each lane and loading equivalence was confirmed by staining total protein with Ponceau S. Migration of molecular mass markers is indicated at left. (a) Abundance of Cup protein in wild-type and *cup* mutant tissue. Lanes: 1, wild-type testes; 2, wild-type ovaries, previtellogenic stages; 3-7: ovaries from females homozygous for *cup* alleles. Lane 3, cup^{1} produces a smaller protein; 4, cup^{1355}; 5, cup^{6890}; 6, cup^{16}; 7,cup^{20}; 8, wild type. An 80 kDa band of undetermined origin is sporadically detected in every tissue and genotype examined; signal intensity varies with individual sera, suggesting that it represents a cross-reactive protein. (b) Homozygous *cup* mutant ovaries with one or two doses of otu. Lanes: 1, wild type; 2, cup^{15}; 3, otu^{11}/+; cup^{15}/cup^{15}.

The subcellular location and distribution of Cup protein within 16-cell cysts underwent marked changes during the course of oogenesis. Cup protein accumulates preferentially in the future oocyte within 16-cell cysts of the germarium. This enrichment begins very early: localization to a single cell is detectable in region 2a, prior to overt differentiation of the oocyte (Fig. 6a). Unlike most oocyte-enriched proteins such as Bic-D or Oskar, no corresponding enrichment of *cup* mRNA was observed (Fig. 4a). Cup protein may be selectively transported from the nurse cells and/or differentially translated or stabilized within the oocyte. The retention of relatively high levels of Cup protein in the nurse cells suggests that the protein may function in both cell types.

Cup protein continues to be selectively enriched in the oocyte until approximately stage 8, a time when egg chamber microtubules are extensively reorganized (Theurkauf et al., 1992). During stage 9 the nurse cells and oocyte contain similar amounts of Cup protein (Fig. 6d), and by stage 10 most of the protein lies in the nurse cells. Prior to stage 8 Cup forms a cap at the posterior of the oocyte; later much smaller amounts of protein are found at the oocyte surface and also persist in a small cap at the posterior.

The behavior of Cup protein in the nurse cells is particularly interesting. Cup accumulates almost exclusively in large aggregates, whose location varies as egg chambers develop (Fig. 6c,d). In early chambers, and especially around stage 4, Cup aggregates are found predominantly around the periphery of the nurse cell nuclei (Fig. 6b,c). After stage 4, Cup leaves the nuclear membrane and becomes dispersed throughout the nurse cell cytoplasm in large aggregates that eventually move toward the cellular periphery. By stage 10, Cup protein is localized almost exclusively in particulate structures along the subcortical surface of the nurse cells (Fig. 6e,f). The movement of Cup protein away from the nucleus corresponded in time to the sharp reduction in *cup* mRNA levels after stage 4, suggesting that a decrease in the rate of Cup synthesis might play a role in these changes.

Cup mutations drastically reduce the level of protein detectable by immunofluorescence microscopy using anti-Cup antibodies (data not shown). This was true even in the germarium, where the effects of mutations on the steady state level of *cup* RNA were weakest. In females homozygous for cup^1, the one allele for which a physical change in the protein has been confirmed, residual protein was still localized to the oocyte (Fig. 6h).

cup interacts with *otu* early in development

To further analyze the role played by *cup* in oogenesis, we

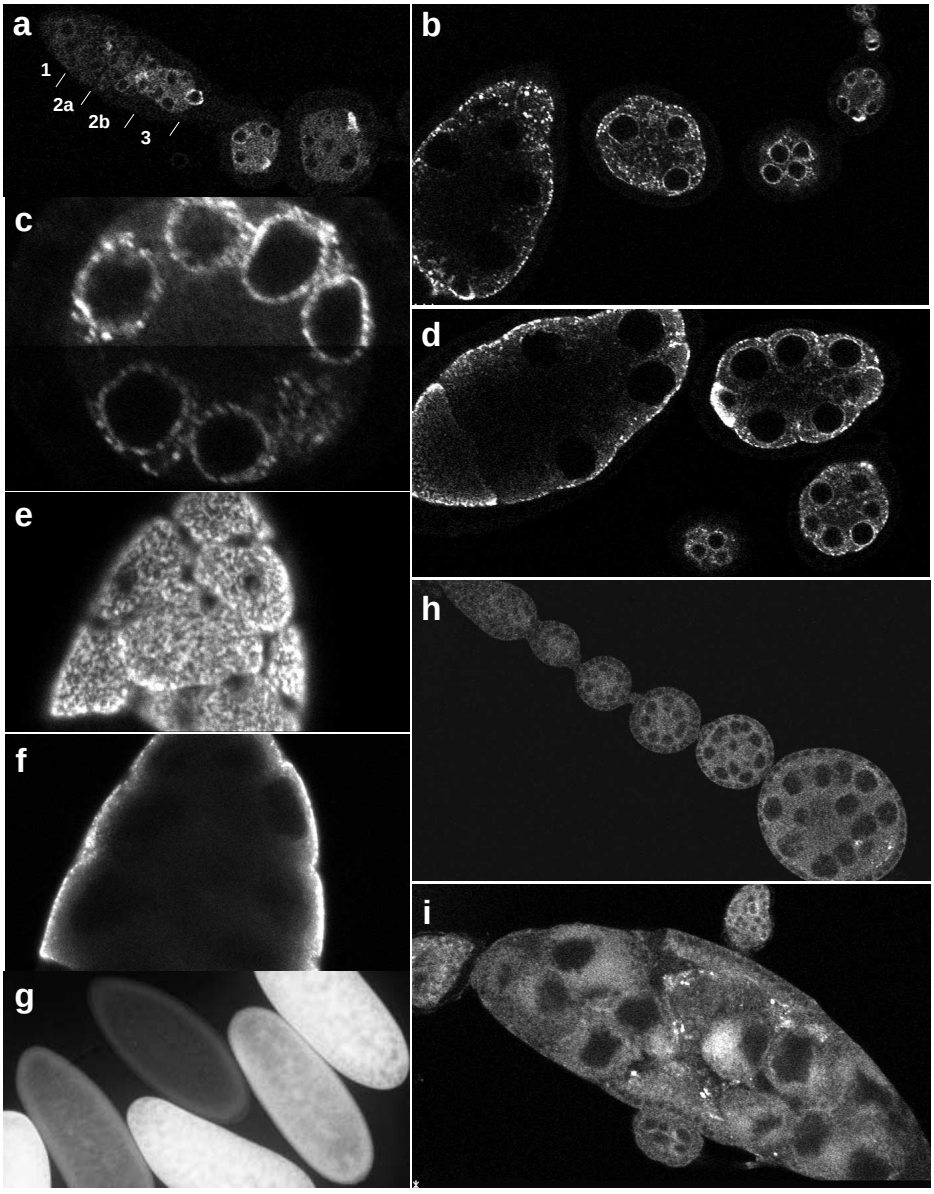

Fig. 6. Expression and localization of the Cup protein. Confocal microscopy of fluorescein-stained anti-Cup antibody in ovaries and embryos. (a and b) Germarium and early egg chambers: enrichment of the protein can be seen in the presumptive oocyte as early as region 2a. (c) Higher magnification of a stage 4 egg chamber, showing the ring of Cup aggregates around the nurse cell nuclei. (d) Stages 5, 7 and 9: the loss of oocyte enrichment and clearing of Cup protein from the internal regions of the nurse cells can be seen. (e) Stage 10b nurse cells, optically sectioned along the surface of the chamber. (f) Same chamber as in e, medial optical section. (g) Wild-type cleavage stage embryos, showing loss of Cup staining during blastoderm formation. (h) cup^1 ovariole. A small amount of Cup protein remains visible in the oocyte, in large aggregates. (i) Dicephalic egg chamber from otu^{11}/+; cup^4/cup^4ovariole, showing Cup protein enriched in the centrally located oocyte. The higher apparant background staining in h and i is an artifact of microsopy conditions used to maximize detection of low levels of Cup protein.

looked for genetic interactions between *cup* and previously described genes with similar effects on development. Several observations suggested that *cup* and *otu* might interact. Late arresting alleles of *otu* affect condensation of the nurse cell chromosomes and partially prevent nurse cells from transferring their contents into the oocyte. Moreover, the distribution of Otu p98 and p104 parallel Cup expression: p104 becomes enriched in early oocytes and both proteins move toward the periphery of the nurse cells during stages 9-10 (Sass et al., 1995).

Our experiments identified a strong genetic interaction between *cup* and *otu*. otu^{11} is a member of the ONC class of *otu* alleles; it is temperature-sensitive and phenotypically variable, producing ovarioles with both tumorous germaria and polytenized late chambers that arrest around stage 12 (Fig. 7a). The point mutation responsible for this phenotype is located in the differentially spliced exon 6a and affects only the 104 kDa isoform (Steinhauer et al., 1989). Four *cup* alleles, with arrest stages ranging from stage 5 to stage 10, were tested in combination with otu^{11}.

In the *otu*; *cup* doubly homozygous combinations, neither gene is epistatic to the other. Instead, the double mutant combinations produce defects that are much more severe than either homozygote alone. For example, cup^{15} is an intermediate allele that arrests around stage 9 (Fig. 7b). Doubly homozygous otu^{11}; cup^{15} females contain ovarioles that are either severely tumorous, with no budded chambers (Fig. 7c), or appear completely agametic. This interaction, seen with all four tested *cup* alleles (cup^{4506}, cup^{32}, cup^{15} and cup^{4}), resembles a strong enhancement of the *otu* phenotype and suggests that *cup*, which is expressed during these stages (Fig. 6a), has an *otu*-related function during early oogenesis.

Further evidence for an interaction between *cup* and *otu* is provided by the phenotype of females homozygous for *cup* but heterozygous for *otu*. Although heterozygosity for *cup* did not affect the phenotype of otu^{11} homozygotes, a single copy of otu^{11} had a strong dominant effect on three of the four *cup* alleles tested. This effect was most striking for cup^{15}. In the presence of one copy of otu^{11}, the fertility of females homozygous for cup^{15} is restored. Rescue is partial, decreasing with age; some eggs laid have flaccid chorions and do not hatch. The effect of otu^{11}/+ on cup^{15} fertility could be reversed by raising the flies at 18°, the permissive temperature for otu^{11}. Under these conditions, otu^{11} /FM6; cup^{15}/cup^{15} females were sterile with ovaries resembling cup^{15} alone.

In addition to rescuing the fertility of cup^{15}, otu^{11} affects the nuclear morphology of *cup* nurse cells. cup^{15} nuclei are heterogeneous and irregular (Table 1); addition of otu^{11}/+ caused nurse cell chromosomes to resemble class II nuclei (Fig. 7d), demonstrating that fertility of these females can be rescued without fully restoring chromatin morphology. Two other alleles, cup^{4} and cup^{32}, while still sterile, also showed a strong phenotypic improvement in the presence of one dose of otu^{11}. For both alleles the reduced dose of *otu* supported chamber growth to a later developmental stage with more uniform chromatin morphologies (data not shown). The ability of a reduced dose of *otu* to rescue an intermediate *cup* allele suggests that the balance of these gene products is important.

The addition of one copy of otu^{11} to *cup* homozygotes produced an additional effect not seen in *cup* alleles alone: many of the egg chambers in otu^{11}/+; *cup*/*cup* ovaries were dicephalic, with nurse cells positioned on both ends of a centrally located oocyte (Fig. 7d). The effect on egg chamber polarity varied depending upon the strength of the *cup* allele. In the combination otu^{11}/+; cup^{32}/cup^{32}, 80% (36/45) of stage 9 or older egg chambers were dicephalic. Substitution of cup^{4} or cup^{15} for cup^{32} produced dicephalic chambers at a frequency of 41% (24/58) and 18% (19/106), respectively. Dicephalic chambers were never observed in any of the *cup* alleles (<0.1% for cup^{32}, <1% for other alleles) or in *otu* heterozygotes (<1%). Immunofluorescent staining of dicephalic chambers indicates that Cup protein is enriched in the centrally located oocytes (Fig. 6i), suggesting that the disruption of normal chamber polarity does not affect the ability of Cup protein to preferentially accumulate in the oocyte.

We carried out western blotting and immunofluorescence labeling to determine if the suppression of *cup* sterility by otu^{11} involved a restoration of Cup protein. Cup^{15} ovaries with one copy of otu^{11} produce much more Cup than cup^{15} ovaries wild type for *otu*. (Fig. 5b: lanes 2 and 3). Similar results were obtained for otu^{11} and cup^{4} (data not shown). Much of the

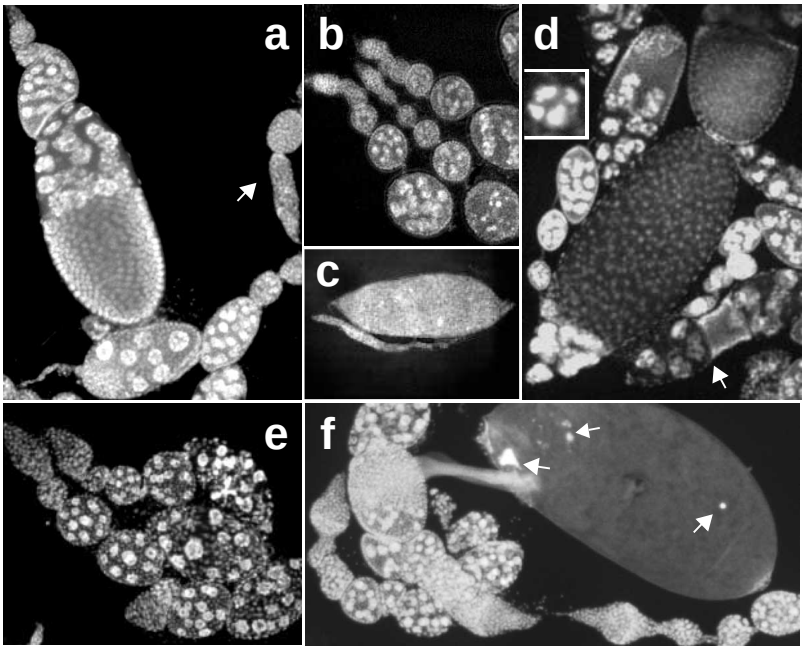

Fig. 7. *cup* interacts with*otu* and *fs(2)B*. (a) Ovarioles from otu^{11} homozygote; arrow indicates tumorous germarium. (b) cup^{15} homozygote; chamber degeneration occurs before stage 9. (c) otu^{11}; cup^{15} double homozygote. Most germ-line cells appear undifferentiated, a few chambers are visible with small polytene nurse cells. (d) Ovarioles from fertile *otu*/+; cup^{15}/cup^{15} females. Chamber growth and follicle cell migration is near normal, but nuclear morphology is abnormal, with chromosomes condensed in stage 5 or oligotene arrest morphologies (inset). Arrow indicates dicephalic egg chamber. (e) ovarioles from *fs(2)B*/*fs(2)B* homozygote. (f) ovarioles from *fs(2)B*/*fs(2)B; cup*^{15}/+ female. Arrows, abnormal chromatin masses in mature egg. All flies were grown at 25°.

protein in these egg chambers is found in large aggregates in the oocyte (Fig. 6i). In both examples protein levels remain below wild type, and a novel, smaller band is detected that may represent an abnormal form of Cup. Thus *otu*^{11} partially restores *cup* function by increasing the amount and possibly altering the structure of Cup products.

cup interacts with *fs(2)B*

fs(2)B is another female sterile mutation that arrests egg chamber development and produces nurse cells with polytene chromosomes. *fs(2)B* is a dominant modifier of *otu* (King et al., 1981), suggesting that these genes are related in function. We tested *fs(2)B* for interaction with *cup* alleles. In a *cup*$^{+}$ background, *fs(2)B* homozygotes arrest early. Egg chambers develop to stage 14 only rarely and such eggs are not laid (Fig. 7e). By contrast, when the females are also heterozygous for *cup*^{4}, *fs(2)B* homozygotes are weakly fertile, with an average of one viable embryo per female (n=19). Most of the mature egg chambers produced are not laid; although these resemble wild type and are covered with an apparently normal chorion, they contain abnormal DNA masses within the oocyte (Fig. 7f). *fs(2)B* females are also weakly rescued to fertility by the addition of one copy of another class II allele, *cup*^{13}, but not by the strong class I allele, *cup*^{04506}. In all three cases ovarian development progresses further than in *fs(2)B* homozygotes and the frequency of tumor formation is greatly reduced, but oogenesis remains abnormal with defects resembling those seen in *fs(2)B* alone (Fig. 7f). Despite the relatively weak suppression by *cup* of the *fs(2)B* oogenesis defects, the production of viable progeny suggests that the interaction is significant. These results, in addition to the previous evidence that some *fs(2)B* and *otu* alleles interact in their effects on nurse cell chromosome structure, strongly suggest that all three genes affect common processes and may lie in a single pathway.

Cup mutations disrupt meiotic chromosome segregation

In the course of studying the interactions between *cup* and *otu*, another effect of *cup* mutations became apparent. Females of the genotype *otu*^{11}/FM6; *cup*^{15}/CyO were observed to produce a high frequency of exceptional progeny displaying non-Mendelian inheritance of *X* chromosomal markers. The production of patroclinous sons, (sterile XO males with paternally derived *X* chromosome markers), as well as matroclinous daughters (presumptive *XXY* females with maternal markers), suggests a high rate of failure of normal *X* chromosome disjunction during meiosis in the mother.

Meiotic non-disjunction in these females could result from the reduction in *cup*, *otu* or both. In addition, achiasmatic balancer chromosomes contribute to chromosome loss by preventing the recombination events that physically link the homologues during meiotic prophase I. To determine the relative contribution of these factors in causing nondisjunction, we crossed *otu*^{11}/FM6; *cup*^{15}/CyO females to *y*; *Sco*/*CyO* males. Independent segregation of the *otu*, *cup* and balancer chromosomes results in six classes of related females, which were then tested for fidelity of *X* chromosome transmission. The results are summarized in Table 2. These data show that in the presence of two balancer chromosomes, *cup* has a dominant effect on meiotic *X* chromosome segregation (Table 2) that is independent of *otu*. Similar results were obtained when *cup*^{4} was substituted for *cup*^{15}.

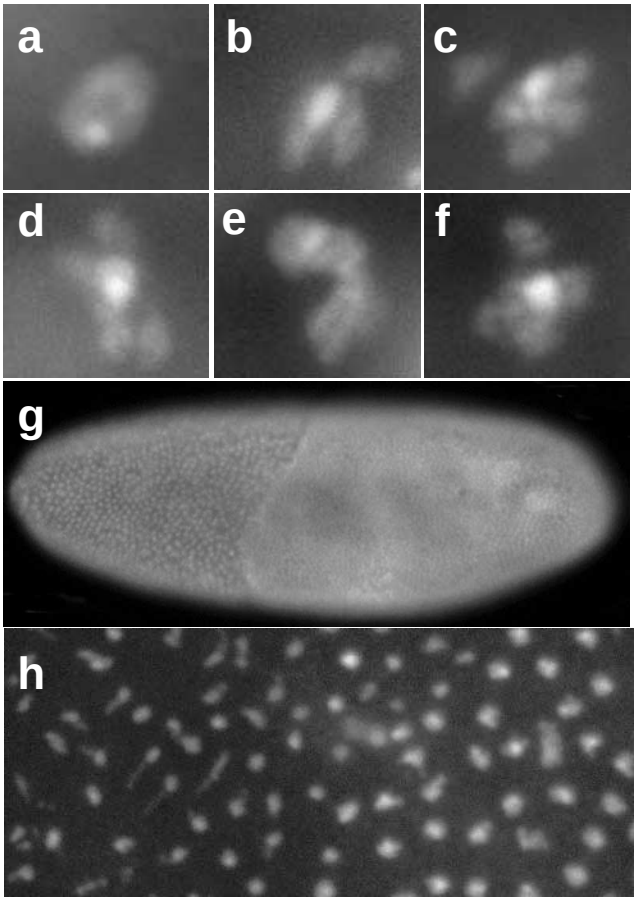

Fig. 8. Cup affects the structure and disjunction of oocyte chromosomes. (a-f) DAPI-stained nuclei from wild-type and *cup*^{1} oocytes, stage 5 egg chambers. (a) Oocyte nucleus from wild-type female, with chromosomes condensed into a karyosome. (b-f) *cup*^{1} oocyte nuclei with chromosome arms radiating from DAPI-bright center. (g) DAPI image of blastoderm embryo, progeny of *cup*^{21} female, with abnormally condensed nuclei; (h) same as g, 100× magnification.

Because *cup* affected the segregation of the germ-line chromosomes, we examined the appearance of the oocyte nucleus during oogenesis for any abnormalities. In wild-type females the oocyte arrests in meiotic prophase and condenses into a small karyosome during stage 3 (Fig. 8a). In contrast, females homozygous for strong or intermediate *cup* alleles produce eggs with abnormal karyosomes. Instead of condensing completely, the chromosomes appear to be organized about a central chromocenter (Fig. 8b-f). Often, five chromosome arms that appear to be double radiate outward from the central DAPI-bright center. Sometimes the chromatin separates into two or more groups (Fig. 8e and data not shown). These observations indicate that *cup* is required for the proper structure of all the germ-line chromosomes and its action is not limited to cells with polytene chromosomes.

Maternally provided *cup* is essential for embryonic development

Immunohistochemical staining demonstrated that *cup* protein is strongly expressed in stage 10 and that high levels are present in preblastoderm embryos (Fig. 6g), suggesting that maternally

Table 2. X chromosome non-disjunction in *cup* females

Line	Maternal genotype (crossed to *FM6* males)	X chromosome genotype of progeny: Females: Expected: *y otu^{11} f/FM6* and *y/FM6*	Females: Expected	Females: Matroclinous: *y otu^{11} f/y*	Males: Expected: *y otu^{11} f*	Males: Expected: *y f^{+}*	Males: Patroclinous: *FM6*
1	*y otu^{11} f/y*; *cup^{15}/CyO*	175 (52%)		0	70 (21%)	91 (27%)	0
2	*y otu^{11} f/y*; *cup^{15}/Sco*	131 (58%)		0	43 (19%)	51 (23%)	0
3	*y otu^{11} f/y*; *Sco/CyO*	84 (54%)		0	26 (17%)	45 (29%)	0
	(crossed to y+ males)	*y/y+*	*FM6/y^{+}*	*y/FM6*	*y*	*FM6*	*y^{+}*
4	*y/FM6*; *cup^{15}/CyO*	96 (32%)	98 (33%)	7 (2%)	45 (15%)	40 (13%)	14 (5%)
5	*y/FM6*; *cup^{15}/Sco*	119 (33%)	99 (27%)	0	94 (26%)	53 (15%)	0
6	*y/FM6*; *ScO/CyO*	31 (31%)	30 (30%)	0	21 (21%)	17 (17%)	0
	(crossed to y males)	*y/y^{+}*	*FM6/y*	*y^{+}/FM6*	*y^{+}*	*FM6*	*y*
7	*y^{+}/FM6*; *cup^{4}/CyO*	108 (30%)	80 (23%)	12 (3.4%)	87 (25%)	59 (17%)	9 (2.5%)
8	*y^{+}/FM6*; *cup^{4}/Sco*	72 (34%)	52 (25%)	1 (0.5%)	56 (26%)	31 (15%)	0
9	*y^{+}/FM6*; *Sco/CyO*	53 (25%)	66 (32%)	1 (0.5%)	61 (29%)	26 (13%)	1 (0.5%)

Females homozygous for *cup^{15}* or *cup^{4}* were tested for X chromosome disjunction by crossing to *FM6* or *y* males, and scoring the number of expected, matroclinous and patroclinous progeny. *FM6* and *CyO* are multiply rearranged balancer chromosomes, while *y*, *y^{+}*, *Sco* and the chromosomes carrying the *cup* and *otu* alleles are unrearranged. Presumptive genotypes of progeny classes are presented as deduced from visible markers; patroclinous male progeny were confirmed to be sterile. All viable progeny were scored for each cross. Because of the variation in viability, data are also expressed as a percentage of the total yield (in parentheses).

To control for background genotype, all the tested females were generated from two crosses: (1), *y otu^{11} f/FM6*; *cup^{15}/CyO* × *y/Y*; *Sco/CyO* or (2) *y otu^{11} f/FM6*; *cup^{4} /CyO* × *y/Y*; *Sco/CyO*. Lines 1-6 were generated from cross 1, and lines 7-9 from cross 2.

loaded Cup protein functions in early embryos. To look for a maternal role of *cup* in embryonic mitosis, we examined the offspring of homozygous *cup* females for defects in cleavage stage nuclear division. Although most *cup* alleles do not produce any progeny, a small number of eggs are laid by *cup^{21}* females. Rarely, these eggs underwent development and could be studied. In addition, we also examined the embryos developing from fertile *otu^{11}*/FM6; *cup^{15}/cup^{15}* females. Although more viable than the *cup^{21}* progeny, these embryos display a similar pattern of abnormalities (see below).

The cleavage stage progeny of both types of female display a wide range of aberrant phenotypes. Embryonic nuclei appear condensed, irregularly spaced, and sometimes stain more weakly with DAPI than wild type (Fig. 8g,h). More than 50% of the embryos from *cup^{21}* females contained abnormal nuclei within a single domain comprising approximately half of the embryo, and separated from phenotypically normal nuclei by a distinct boundary (Fig. 8g). This pattern suggests that a chromosomal defect was produced during the first zygotic division and propagated by one of the two daughter nuclei. These morphological abnormalities are subsequently transmitted over many nuclear generations and persist through blastoderm formation, beyond which embryos usually do not continue development. Embryos that had successfully completed gastrulation displayed no further chromosomal defects, suggesting that the maternal effect of *cup* is limited to the earliest stages of development. Of the rare surviving offspring of *cup^{21}* mothers, *cup* heterozygotes and homozygotes were equally viable, indicating that the paternal *cup* contribution did not influence embryonic survival for this allele.

DISCUSSION

We have characterized a gene, *fs(2)cup*, that nurse cells require to maintain their normal chromatin structure, and that is needed in the oocyte for meiotic chromosome structure and segregation. The demonstration that *cup* interacts with *otu* and *fs(2)B* suggests that these genes participate in a pathway or process that exerts a major effect on chromosomal behavior during oogenesis. However, Cup protein, like Otu, is found in the cytoplasm so its effects on chromosome structure are likely indirect. *cup* and *otu* also affect other processes during oogenesis, such as growth of the oocyte during mid oogenesis, envelopment of the egg chamber by follicle cells, and polarization of the cyst along the anterior-posterior axis of the ovariole. It is therefore likely that the *cup-otu* pathway is not specific for chromosome structure and function, but is more generally required for germ line growth and development.

Role of *cup* and *otu* in chromosome structure

In polytene chromosomes as many as several thousand individual chromatin strands remain precisely aligned along their lengths despite continuing replication and gene activity. Many larval and adult tissues in *Drosophila* become polytene as they differentiate and most appear on cytological grounds to maintain this state for the remainder of their lives. In nurse cells the precise association of individual polytene strands becomes weakened and reorganized. The exact structure of the remodeled chromosomes remains uncertain, but they are likely to retain a significant degree of intra-strand pairing (Hammond and Laird, 1985). Both *cup* and *otu* are required for nurse cell chromosome reorganization. Nurse cells mutant for *otu* typically develop simple polytene chromosomes (King and Storto, 1988), while in *cup* mutants nurse cell chromosomes assume a variety of forms that may represent intermediates in the reorganization process. Further study of the *cup* pathway should make it easier to reconstruct the normal processes of chromosome remodeling and deduce the functional importance of these changes.

At least some of Cup's actions appear to be on higher order

chromatin packing, as suggested by the specific chromosome-nuclear membrane associations observed in the cup^{20} allele. The failure of the karyosphere within the oocyte to compact fully is also evidence of altered chromatin packing. In *cup* germinal vesicles, individual chromosome arms appear to be associated together in their centromeric regions, and frequently appear double. Karyosome condensation and heterochromatin-mediated association of homologous chromosomes are thought to be important for the disjunction of non-exchange chromosomes (Dernburg et al., 1996; Hawley et al., 1993). The loosening of the normal association may allow some non-exchange chromosomes to break free (Fig. 8c), leading to their failure to segregate with a partner.

cup and *otu* affect oocyte growth

In addition to disrupting chromosome organization, class III *cup* alleles affect the relative growth of the oocyte compared to the nurse cells during vitellogenesis. Oocyte growth depends on the influx of materials from several external sources. Nurse cells synthesize and transport components through the ring canals, while follicle cells and the fat body produce yolk proteins that are taken up at the oocyte surface. The growth defects of *cup* oocytes are probably not related to defects in yolk protein uptake, because mutations in the vitellogenin receptor that completely block yolk uptake do not prevent the oocyte from attaining its normal size (see Schonbaum et al., 1995). Instead, *cup* is more likely to interfere with transport from the nurse cells. The microtubule cytoskeleton is extensively reorganized after stage 7, and a variety of evidence suggests that microtubule-independent transport systems increase in importance during the final growth stages of oogenesis (reviewed by Theurkauf, 1994).

The large aggregates of Cup protein observed in nurse cells may play a role in oocyte transport. The transport of some specific egg components, including *bicoid (bcd)* mRNA, is dependent on the gene *exuperantia (exu*, St Johnston et al., 1989). Exu protein is found in nurse cell cytoplasm in large aggregates that have been proposed to transport *bcd* mRNA along microtubules to the oocyte (Wang and Hazelrigg, 1994). Materials en route to the oocyte via this pathway may first move to the periphery of the nurse cells, because *bcd* RNA is seen to accumulate near nurse cell plasma membranes in an *exu*-dependent manner (St Johnston et al., 1989). However, Exu protein does not localize near the surface of the nurse cells in stages 9-10, and the eggs laid by *exu* females are not cup-shaped. Mutations in a small group of female sterile genes that includes *Bicaudal-C* (Mahone et al., 1995) and *Bicaudal-D* (Swan and Suter, 1996) more closely resemble *cup*'s effect on oocyte growth (Schüpbach and Wieschaus, 1991). Both genes are involved in localizing morphogenic determinants within the oocyte. Whether *cup* participates in any of these pathways will require further study.

Role of the *cup-otu* pathway in early germ-line development

The *cup-otu* pathway is also likely to act early in oogenesis prior to the establishment of individual egg chambers. Disruption of *otu* activity causes germ-line cells to be lost (QUI alleles) or to overproliferate (ONC alleles), leading to tumor formation. Cup protein is expressed in forming cysts and may even be found in stem cells. The fact that *cup* mutations enhance tumor formation in otu^{11} suggests that both proteins function during the earliest stages of cyst differentiation. The strongest *cup* mutations recovered, in contrast, cause only a relatively minor increase in the number of cells in region 2a. However, none of the *cup* alleles tested was null, so lethality or partial redundancy may have prevented the recovery of female sterile *cup* alleles with stronger and earlier-acting effects.

One early process affected by *cup* and *otu* is egg chamber polarity. In the lens-shaped cysts of region 2b, the oocyte is located near the middle of the cyst but must move to a position at the posterior prior to egg chamber budding. When this process fails a dicephalic egg chamber is produced whose nurse cells are located at both ends of the egg. Signals received by the oocyte from an unidentified subpopulation of somatic cells are thought to be important for correct oocyte positioning. The fact that nearly all the egg chambers produced by $otu^{11}/+$; cup^{32} females are dicephalic suggests that these oocytes cannot efficiently receive the polarization signal. The defects several class I alleles display in the budding of individual egg chambers may also result from a failure of signaling with somatic cells (Fig. 2a).

Previously, *otu* has been implicated in one the major early events of germ-line development: sex determination (Bopp et al., 1993; Pauli et al., 1993). More recent data suggest that the effects of *otu* on sex determination are indirect (Bae et al., 1994; Horabin et al., 1995). We found no evidence that *cup* functions in sex determination; ovaries from *cup* females showed no indication of germ-line sex transformation, and antibody staining for Sxl protein appears normal in *cup* mutants (L. N. K., unpublished results).

Nature of the Cup-Otu interaction

Cup and *otu* appear to function in a similar manner in organizing nurse cell chromosome structure, but some other aspects of their phenotypes differ. Both proteins are distributed in a similar manner during oogenesis, suggesting that they are located in close proximity or even in direct contact. The *otu* allele we analyzed, otu^{11}, contains a point mutation in the differentially spliced exon that is specific for Otu p104, an isoform that can provide complete *otu* function, but does not affect the other Otu isoform, Otu p98. The mutant p104 protein is temperature sensitive and retains partial function. The genetic interactions between *cup* and otu^{11} suggest that the relative ratios of Cup and Otu proteins, rather than their absolute levels, are important. Lowering the dose of *otu* increases the amount of Cup protein present in *cup* homozygous ovaries, suggesting that the presence of excess Otu reduces the synthesis of Cup protein or alters its stability. However, additional alleles of *otu* will need to be examined, including null alleles, before this can be concluded with confidence. Preliminary experiments reveal that the *otu* null mutation $otu^{P\Delta 1}$ has an effect on *cup* mutations similar to otu^{11} (L. N. K., unpublished). The lack of epistasis between *cup* and *otu* and the enhanced tumorigenicity of the double mutants suggests that these genes may act together or in parallel.

The *cup-otu* pathway may involve microtubule-based transport

Despite the diversity of effects resulting from perturbations in *cup* function, many of them are known to involve microtubules.

The distribution and relocalization of Cup protein during egg chamber maturation suggest that it is directly or indirectly associated with microtubules, at least during previtellogenic stages. In early stages Cup accumulates at the oocyte posterior, as expected if its movement was associated with a minus-end directed motor (Clark et al., 1994; Theurkauf et al., 1992). Cup does not appear to accumulate in the oocyte after the dispersal of the MTOC at stage 7. Less is known about the organization of the microtubules in late-stage nurse cells; they are found concentrated around the nuclei (Gutzeit, 1986), but their polarity is uncertain. We found a weak similarity between a small domain within Cup and Map1B, while Tironnen et al. (1995) described a potential microtubule-binding domain in Otu. In addition, the proteins in the pattern database that are most closely related to Cup are involved in vesicle transport and fusion; at least one of these, CLIP-170, has been demonstrated to bind microtubules (Pierre et al., 1992). Our identification of a potential membrane-spanning domain in Cup is consistent with a role in vesicle-mediated transport.

An association with microtubules is also supported by the similar effects exerted by *cup* and *nod* on meiotic chromosome segregation. *Nod* encodes a kinesin-related microtubule motor protein required for the proper disjunction of non-recombinant chromosomes. In the presence of multiple balancers *nod*, like *cup*, exerts a dominant effect on meiotic disjunction (Carpenter, 1973). *nod* has been shown to mediate the association between the centromeric heterochromatin and the microtubules of the meiotic spindle (Afshar et al., 1995; Murphy and Karpen, 1995). The ability of *cup* to disrupt chromosome segregation suggests that *cup* may also affect the ability of chromosomes to associate with the meiotic spindle.

Association of Cup with the nuclear membrane

Interactions between chromosomes and the nuclear membrane are likely to be important for chromosome structure. Cup is closely associated with the nuclear membrane during the stages when chromosomes are most condensed, and may indirectly influence chromatin structure by acting in a pathway that affects the nuclear membrane. Profound changes may take place in early nurse cells that are required to reorganize nurse cell chromosome structure during this period. The failure to make these changes would explain why nurse cell chromosomes remain arrested with structures characteristic of early stages. This is particularly true in the case of cup^{20}, in which the chromosomes spiral around the nurse cell nuclei in close association with the nuclear membranes (Fig. 2d).

Defective nuclear membranes could explain other effects of *cup* and *otu* mutations. For example, Otu and Cup proteins accumulated selectively in the oocyte may alter its nuclear membrane to allow it to respond appropriately to somatic signals for oocyte polarization. Still later, these proteins could act directly or via imported effectors to influence the condensation of the karyosome. Eggs in many species accumulate excess nuclear membrane constituents, including nuclear pore complexes, and store them in the cytoplasm. These materials often form at the nuclear periphery, where they assemble into large organelles known as annulate lamellae. Such stored components might correspond to the large particulate material observed in the nurse cell cytoplasm. Although annulate lamellae have been described in late *Drosophila* egg chambers and near the nurse cell nuclei in mid-vitellogenic stages (Mahowald, 1971), their distribution throughout oogenesis is not known with sufficient precision to compare with the location of Cup protein.

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