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Drosophila Ik2, a member of the IκB kinase family, is required for mRNA localization during oogenesis

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In both *Drosophila* and mammals, IκB kinases (IKKs) regulate the activity of Rel/NF-κB transcription factors by targeting their inhibitory partner proteins, IκBs, for degradation. We identified mutations in *ik2*, the gene that encodes one of two *Drosophila* IKKs, and found that the gene is essential for viability. During oogenesis, *ik2* is required in an NF-κB-independent process that is essential for the localization of *oskar* and *gurken* mRNAs; as a result, females that lack *ik2* in the germline produce embryos that are both bicaudal and ventralized. The abnormal RNA localization in *ik2* mutant oocytes can be attributed to defects in the organization of microtubule minus-ends. In addition, both mutant oocytes and mutant escaper adults have abnormalities in the organization of the actin cytoskeleton. These data suggest that this IκB kinase has an NF-κB-independent role in mRNA localization and helps to link microtubule minus-ends to the oocyte cortex, a novel function of the IKK family.

KEY WORDS: *ik2*, IκB kinases, mRNA localization, Oogenesis, Dynein-based transport, *oskar*, *gurken*

INTRODUCTION

Protein kinases of the IκB kinase (IKK) family are known for their roles in innate immune response signaling pathways in both mammals and *Drosophila* (Ghosh and Karin, 2002; Peters and Maniatis, 2001; Silverman and Maniatis, 2001). Mammalian IKKs all have roles in immune responses, but have a variety of targets. IKKα and IKKβ were identified in a protein complex that phosphorylates IκB and targets it for degradation, thereby allowing the nuclear localization and activation of NF-κB transcription factors (DiDonato et al., 1997; Mercurio et al., 1997; Zandi et al., 1997). Gene targeting experiments in the mouse demonstrated that IKKβ, but not IKKα, is required for NF-κB activation by pro-inflammatory stimuli through receptors such as TLR4 (Li et al., 1999a; Li et al., 1999b; Tanaka et al., 1999). IKKα activates the Rel/p52 transcription factor, because it activates proteolytic processing of the p100 precursor of p52 in an IκB-independent process (Senftleben et al., 2001). IKKε and TANK binding kinase 1 (TBK1) are required to phosphorylate and activate the transcription factor Interferon regulatory factor 3 (IRF3) in response to viral infection (Fitzgerald et al., 2003; McWhirter et al., 2004; Sharma et al., 2003). In addition to these immune response functions, IKKα has an NF-κB-independent role in epidermal differentiation and limb development (Hu et al., 1999; Hu et al., 2001; Sil et al., 2004; Takeda et al., 1999).

Dorsoventral patterning of the *Drosophila* embryo relies on the activation of Dorsal, a Rel-family transcription factor, by a signaling pathway that is homologous to mammalian TLR pathways (Anderson, 2000). In response to activation of the receptor Toll, Cactus (the *Drosophila* IκB) is degraded, which allows Dorsal to move to embryonic nuclei and activate genes, such as *twist*, that are required for specification of ventral cell types. Phosphorylation of Cactus is required for its degradation (Fernandez et al., 2001), but the responsible kinase has not been identified. The *Drosophila* genome encodes two members of the IKK family. *DmIkkβ* (*ird5* – FlyBase) is essential for the response to bacterial infection (Lu et al., 2001). *DmIkkβ* is required for proteolytic processing and activation of Relish, a p100-like Rel/ankyrin-repeat protein, like the role of mammalian IKKα in the activation of p100. The function of the second *Drosophila* protein kinase of the IKK family, *ik2* (*IκB kinase-like 2*), has not been characterized, but it was a good candidate to control the phosphorylation and degradation of Cactus.

To test whether *ik2* encodes a Cactus kinase, we characterized the phenotypes caused by loss of *ik2* function. Here, we present data showing that Ik2 is essential for dorsoventral and anteroposterior embryonic patterning of the *Drosophila* embryo. However, Ik2 does not act as a Cactus kinase, but exerts its effects on embryonic patterning through the localization of specific mRNAs during oogenesis. The data indicate that *Drosophila* Ik2 regulates RNA localization through regulation of the cytoskeleton and define a novel function for this protein family.

MATERIALS AND METHODS

DNA sequences of *ik2* alleles

The *l(2)38Ea* complementation group (Kozlova et al., 1998) consists of five alleles and were renamed accordingly: Ea^{36} ($ik2^{1}$), Ea^{41} ($ik2^{2}$), Ea^{46} ($ik2^{3}$), Ea^{47} ($ik2^{4}$), Ea^{66} ($ik2^{5}$). The following mutations in the kinase domain were identified from sequenced *ik2* genomic DNA: $ik2^{1}$ (G250D), $ik2^{2}$ (N69I), $ik2^{3}$ (G19D), $ik2^{4}$ (G109A), $ik2^{5}$ (D160N). $ik2^{alice}$ was recovered in a maternal effect genetic screen (Luschnig et al., 2004), and also had a point mutation in the kinase domain (F297I).

Fly stocks and genetic analyses

The X chromosome FLP recombinase stock $P[ry^{+}$, hsFLP], the second chromosome FRT stock $P[w^{+}$ FRT 40A] (Chou and Perrimon, 1996), $Df(2L)Ketel^{RX32}$ (Erdelyi et al., 1997), $BicD^{1}$ and $BicD^{2}$ (Mohler and Wieschaus, 1986), and the tubulin-Gal4 and daughterless-Gal4 drivers were obtained from the Bloomington Stock Center. The recombinant chromosomes $ik2^{1}$ $P[w^{+}$ FRT 40A] and $ik2^{alice}$ $P[w^{+}$ FRT 40A] were provided by F. Schnorrer and C. Nüsslein-Volhard (Tübingen). The following stocks were also used: Tau-GFP line 2.1 (Micklem et al., 1997); Kinesin-β-galactosidase insertion line KZ503 (Clark et al., 1994); and Nod-β-galactosidase insertion line NZ143.2 (Clark et al., 1997). To induce expression of FLP recombinase, flies were mated for 24 hours, and second instar larvae were heat shocked in a 37°C water bath for two hours on two consecutive days.

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Eggshell and cuticle preparations

To visualize the chorion under the microscope, eggs were washed with 0.7% NaCl and 0.1% Triton X-100, and mounted in Hoyer's medium (Van der Meer, 1977). For cuticle preparations, embryos were collected on apple juice agar plates, washed with 0.7% NaCl and 0.1% Triton X-100, and bleached to remove the chorion. Embryos were fixed for 1 hour at 65°C in 1:4 glycerol:acetic acid and mounted in Hoyer's medium.

Ovarian and embryonic in situ hybridization

Whole-mount ovaries were hybridized with digoxigenin-labeled *grk*, *bcd* and *osk* RNA probes, all described previously (Berleth et al., 1988; Ephrussi et al., 1991; Neuman-Silberberg and Schüpbach, 1993). Ovaries were fixed and stained according to Suter and Steward (Suter and Steward, 1991). Embryonic fixation and hybridization were performed as described previously (Tautz and Pfeifle, 1989). Fluorescent ovarian in situ hybridization to detect *osk* mRNA was performed as described previously (Cha et al., 2002).

Immunohistochemistry

For Twist staining of embryos, 0- to 2-hour embryos were collected, aged 2 hours at 25°C, and fixed with 4% paraformaldehyde. Embryos were rehydrated with 1×PBS and incubated in 0.3% BSA in PBST (1×PBS with 0.3% Triton X-100) for 30 minutes. After overnight incubation at 4°C with primary antibody (in 0.3% BSA in PBST) and washing with PBST, the samples were incubated for 1 hour with biotin goat anti-rabbit secondary antibody (Jackson ImmunoResearch) and signal was amplified using the Vector Elite ABC kit (Vector Laboratories). Rabbit anti-Twist (1:5000) was kindly provided by Siegfried Roth (Cologne).

Ovaries from 24- to 48-hour-old females were dissected and fixed as previously described (Verheyen and Cooley, 1994a). Antibodies were used at the following concentrations: mouse monoclonal P1H4 anti-dynein heavy chain, 1:500 (McGrail and Hays, 1997); anti-β-galactosidase monoclonal antibody, 1:2000 (Promega). For Grk antisera (1:10), ovaries were fixed and stained as described previously (Queenan et al., 1999). For visualization of actin, ovaries were incubated with either FITC-phalloidin or rhodamine-phalloidin (Molecular Probes) for 2 hours. Images were captured using a Leica TCS SP2 confocal microscope system and Leica Confocal Services software (version 2.61).

Electron microscopy

Fly heads were fixed with 4% formaldehyde overnight, then dehydrated with a graded ethanol series. Samples were critical-point dried in CO_2, then sputter coated with 30 nm of gold palladium and examined with a scanning electron microscope.

UAS-*ik2* rescue construct

An *ik2* genomic DNA fragment from 95 bp before the first codon to 350 bp following the stop codon was cloned from Oregon R genomic DNA using the primers 5′-GCTCTAGAGTCACAATCGAGAAGGCGCTT-3′ and 5′-GCTCTAGAGCTCAATGGCGTCGAG-3′, which incorporated an *Xba*I site on both the 5′ and 3′ ends. The resulting 3.1 kb fragment was inserted into the *Xba*I site of the UASp vector to drive maternal expression (Rørth, 1998). The rescue plasmid was injected into *yw* embryos and transgenic lines were selected for expression of the *white* gene.

RESULTS

Drosophila ik2 is essential for viability

We identified a gene on the *Drosophila* second chromosome, *ik2*, as a member of the IKK family. The closest mammalian homologs of *Drosophila* Ik2 are IKKε and TANK binding kinase 1 (TBK1), which are 60-61% identical to Ik2 in the kinase domain and 51% identical across the entire protein; by contrast, Ik2 is only 28% identical to the other *Drosophila* IKK, DmIkkβ. A saturation mutagenesis experiment had identified lethal complementation groups in polytene chromosome region 38E (Kozlova et al., 1998), the region that includes the *ik2* gene. We identified missense mutations in the *ik2* kinase domain in all five alleles of the *l(2)38Ea* complementation group. A sixth allele, $ik2^{alice}$, identified in a genetic mosaic screen for maternal effect mutations (Luschnig et al., 2004), had a missense mutation in the C-terminal end of the kinase domain. All six *ik2* alleles caused recessive lethality, and the majority of the mutants died as first instar larvae. At low temperature and in uncrowded culture conditions, rare escaper adults (<1%) were observed, but they died shortly after eclosion.

Loss of *ik2* in the female germline results in bicaudal and ventralized embryos

If *ik2* encoded the Cactus kinase, embryos produced by females that lack *ik2* would not be able to degrade Cactus, so Dorsal would not enter embryonic nuclei to activate genes required for ventral cell fate specification and the embryos would be dorsalized. Because *ik2* mutations were lethal, we used FRT/FLP recombination combined with the ovo^{D} dominant female-sterile mutation to generate mutant clones in the female germline (Chou and Perrimon, 1992). More than 95% of the embryos laid by $ik2^{alice}$ and $ik2^{1}$ mutant females did not hatch; however, larval cuticle preparations showed that none of the embryos were dorsalized. Instead, the majority of embryos produced by $ik2^{alice}$ and $ik2^{1}$ mutants had a bicaudal phenotype, ranging from headless embryos (Fig. 1B) to embryos with a duplicated abdomen in place of the head and thorax (Fig. 1C). In addition to this anteroposterior patterning defect, a large number of embryos from both $ik2^{alice}$ (Fig. 2B) and $ik2^{1}$ (Fig. 2C) germline clones had expanded ventral cuticular structures, the opposite of the expected phenotype. Some embryos were both ventralized and bicaudal, with expanded ventral denticle bands and filzkörper (a posterior structure) in both the tail and the anterior of the embryo. Both *ik2* alleles produced bicaudal and ventralized embryos, but 89% (n=190) of the embryos produced by $ik2^{alice}$ mutant females were bicaudal with no apparent dorsoventral abnormalities, whereas only 47% (n=110) of embryos produced by $ik2^{1}$ mutant females were bicaudal, and the remainder of the embryos appeared to be too ventralized to score for ectopic posterior cuticular structures. We observed a similar range of phenotypes in embryos produced by $ik2^{2}$, $ik2^{3}$ and $ik2^{5}$ females with mutant germline clones.

The *ik2* bicaudal phenotype is the result of ectopic localization of *oskar* mRNA during oogenesis

The anteroposterior pattern of the embryo depends on the localization of maternal mRNAs. The *bicoid* (*bcd*) mRNA is localized to the anterior end of the egg, and specifies anterior cell fates including the head and thorax (Berleth et al., 1988). *nanos* mRNA is localized at the posterior pole of the egg, a process that depends on prior posterior localization of *oskar* (*osk*) mRNA, and specifies the pattern of the abdomen (Nüsslein-Volhard et al., 1987; Ephrussi et al., 1991).

The best-characterized mutations that lead to a high frequency of bicaudal embryos are gain-of-function alleles of the *Bicaudal C* (*BicC*) and *Bicaudal D* (*BicD*) genes (Mohler and Wieschaus, 1986; Suter and Steward, 1991; Wharton and Struhl, 1989; Schüpbach and Wieschaus, 1991; Mahone et al., 1995; Castagnetti and Ephrussi, 2003). Bicaudal embryos produced by *BicC* and *BicD* females have ectopic anterior *nanos* and *oskar* mRNA, leading to duplicated posterior structures (Mahone et al., 1995; Ephrussi et al., 1991). We examined the localization of these key patterning mRNAs in embryos produced by *ik2* germline clones to determine whether the cause of the *ik2* bicaudal phenotype was similar to that of the *BicC* and *BicD* mutants.

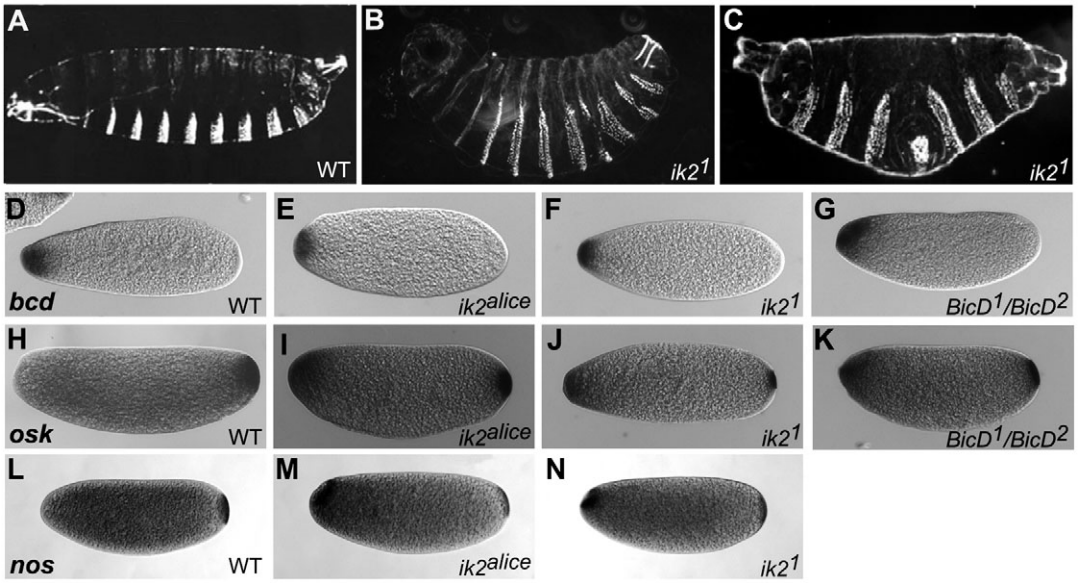

Fig. 1. Defects in anteroposterior embryonic patterning caused by loss of *ik2* in the female germline. (**A-C**) Dark-field views of larval cuticle preparations at the end of embryogenesis from (A) wild-type and (B,C) bicaudal embryos from an $ik2^{1}$ germline clone female. (**D-G**) *bicoid* mRNA is localized to the anterior pole of wild-type embryos (D), $ik2^{alice}$ (E) and $ik2^{1}$ (F) mutant germline-derived embryos, and $BicD^{1}/BicD^{2}$ (G) embryos. (**H-K**) *oskar* mRNA localization at the posterior pole of a wild-type embryo (H), and at both the anterior and posterior poles of embryos produced by an $ik2^{alice}$ germline clone female (I), an $ik2^{1}$ germline clone female (J), and a $BicD^{1}/BicD^{2}$ mutant female (K). (**L-N**) *nanos* mRNA localization at the posterior pole of a wild-type embryo (L), and at both the anterior and posterior poles of embryos produced by an $ik2^{alice}$ germline clone female (M), and an $ik2^{1}$ germline clone female (N). Anterior to the left.

In wild-type embryos prior to gastrulation, *bcd* mRNA is localized to the anterior pole (Fig. 1D) (Berleth et al., 1988) and *osk* mRNA is found exclusively at the posterior pole (Fig. 1H) (Ephrussi et al., 1991). *bcd* mRNA was localized normally in all $ik2^{alice}$ (Fig. 1E) and $ik2^{1}$ (Fig. 1F) mutant embryos examined, similar to $BicD^{1}/BicD^{2}$ mutants (Fig. 1G). By contrast, *oskar* mRNA was present at both the anterior and posterior poles in 100% of the embryos produced by $ik2^{alice}$ (Fig. 1I) and $ik2^{1}$ (Fig. 1J) mutant germline clone females, and 100% of the embryos produced by $BicD^{1}/BicD^{2}$ mutant females (Fig. 1K). Thus even though a bicaudal phenotype could not be detected in all cuticle preparations of the ventralized class of embryos, all embryos produced by $ik2^{alice}$ and $ik2^{1}$ mutant females had anteriorly localized *oskar* mRNA. While gain-of-function *BicC* and *BicD* alleles produce bicaudal embryos at high frequency, *ik2* is the first locus to be described where loss of function causes a completely penetrant bicaudal phenotype.

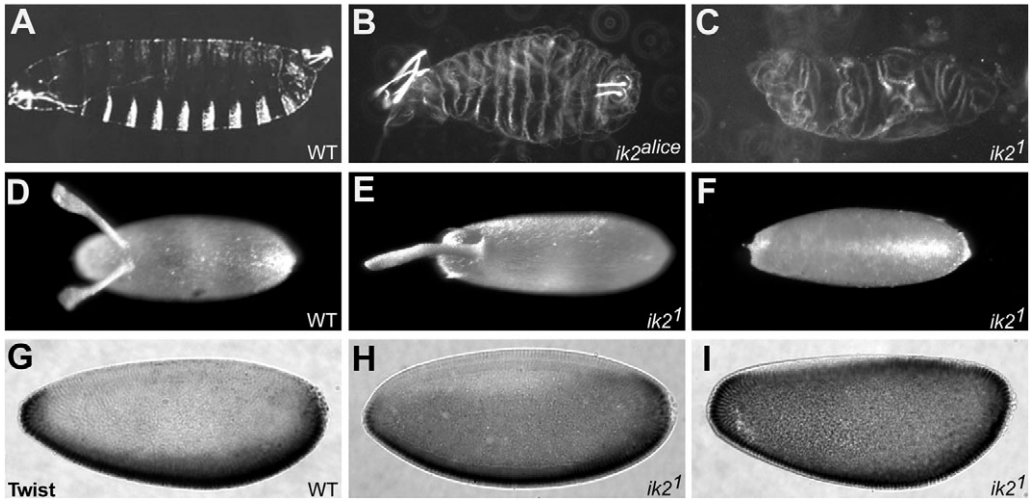

Fig. 2. Defects in dorsoventral patterning caused by loss of *ik2* in the female germline. (**A-C**) Dark-field views of larval cuticle preparations at the end of embryogenesis from (A) wild type, (B) a weakly ventralized embryo from an $ik2^{alice}$ germline clone female and (C) a ventralized embryo from an $ik2^{1}$ germline clone female. (**D-F**) Eggshells produced by (D) wild type, (E) an $ik2^{alice}$ germline clone female and (F) an $ik2^{1}$ germline clone female. Wild-type eggshells have two dorsal chorionic appendages, whereas the eggs in E and F have a single or no dorsal appendages, respectively. (**G-I**) Twist expression. Twist is expressed in the ventral 25% of cells of the wild-type blastoderm embryo (G), but is expressed in an expanded domain in embryos produced by $ik2^{1}$ germline clone females (H,I). Anterior to the left; dorsal is up in G-I.

In embryos produced by *BicD* mutant females, ectopic anterior *oskar* is sufficient to recruit the posterior determinant *nanos* to the anterior pole to specify abdominal development (Ephrussi et al., 1991). We found ectopic *nanos* mRNA at the anterior pole of all $ik2^{alice}$ (Fig. 1M) and $ik2^{1}$ (Fig. 1N) mutants, which indicated that *oskar* localized at the anterior of *ik2* embryos was sufficient to mislocalize *nanos* to the anterior pole.

The *bcd* and *osk* mRNAs are transcribed in the nurse cells, transported into the oocyte and targeted to the anterior and posterior ends of the oocyte during stages 9-10 of oogenesis (Schnorrer et al., 2000; St Johnston et al., 1989; Ephrussi et al., 1991). In *BicD* mutant females, *osk* mRNA is transported efficiently from the nurse cells into the oocyte, but accumulates at both the anterior and posterior poles of the oocyte (Ephrussi et al., 1991). We examined *osk* mRNA in both $BicD^{1}/BicD^{2}$ (data not shown) and *ik2* mutant oocytes using a probe conjugated directly to fluorescein. At a stage when *osk* mRNA was localized tightly to the posterior pole of wild-type oocytes (Fig. 3A), *osk* mRNA was present in all regions of the *ik2* mutant oocyte cortex (Fig. 3B,C) and was enriched at both the anterior and posterior poles.

While *osk* mRNA is being transported to the posterior pole of wild-type oocytes, the message is translationally repressed, ensuring that the protein is only active once it reaches its correct subcellular location (Kim-Ha et al., 1995; Markussen et al., 1995; Rongo et al., 1995). Females that carry mutations in *BicC* produce bicaudal embryos as a result of ectopic anterior *osk* mRNA and premature translation of Osk at ectopic sites in the oocyte (Mahone et al., 1995; Saffman et al., 1998). In *ik2* oocytes, Osk protein was localized exclusively at the posterior of the oocyte at stage 9 and was not detectable at earlier stages (data not shown). Thus the ectopic localization of *osk* mRNA in *ik2* mutant oocytes, and not premature translation, is the cause of the *ik2* bicaudal phenotype.

The *bcd* mRNA is transported from the nurse cells to the oocyte and then restricted to the anterior of the developing oocyte by stages 8-9 of oogenesis (Berleth et al., 1988; St Johnston et al., 1989). The *bcd* transcript was tightly localized to the anterior cortex of the oocyte in most *ik2* egg chambers. However, in approximately 20% of $ik2^{1}$ mutant egg chambers the expression domain of *bcd* mRNA was expanded toward the posterior of the oocyte (data not shown), a pattern that has been seen when dynein-mediated RNA transport is disrupted (Duncan and Warrior, 2002; Januschke et al., 2002; Schnorrer et al., 2000).

The ventralized phenotype of *ik2* embryos is the result of the failure of *gurken* mRNA localization in the oocyte

More than half of the embryos produced by $ik2^{1}$ germline clone females had a ventralized cuticle pattern, with a range of phenotypes that included expanded and disorganized ventral denticle belts, ventral holes and a reduced dorsal cuticle. To determine whether the cuticle defects reflected an early disruption of dorsoventral patterning, we examined the expression of Twist, a direct target of the Rel protein Dorsal. At the cellular blastoderm stage, Twist is expressed in cells in the ventral 25% of the embryo (Fig. 2G). Consistent with the ventralized cuticle pattern, the Twist expression domain expanded to more dorsal positions in the majority of *ik2* mutant embryos (Fig. 2H,I). The Twist domain was expanded in 61% of $ik2^{alice}$ germline clone embryos (n=62). Ninety-four percent of $ik2^{1}$ mutant embryos (n=106) had an expanded Twist domain, and Twist was often expressed in cells around the entire dorsoventral circumference of the embryo, similar to the strong ventralized phenotype caused by the strongest constitutively active *Toll* allele (Schneider et al., 1991).

Establishment of the dorsoventral pattern of the embryo is dependent on two sequential signaling pathways, the Gurken/Egf receptor pathway, which acts during oogenesis, and the embryonic Toll-Dorsal signaling pathway (Morisato and Anderson, 1995). Loss of the Tgfα ligand Gurken (Grk) or its receptor, the *Drosophila* Egf receptor (Egfr) causes ventralization of both the embryo and the surrounding eggshell (Schüpbach, 1987). The eggshell, which is made by the somatic follicle cells that surround the developing oocyte, has a clear dorsoventral polarity, marked by a pair of dorsal appendages at a dorsoanterior position on the eggshell (Fig. 2D). The majority of eggs produced by $ik2^{1}$ germline clones had a single, fused dorsal appendage (Fig. 2E), and some lacked dorsal appendages altogether (Fig. 2F), such as *grk* or *Egfr* mutants. Because both the embryos and the eggshells produced by *ik2* mutants were ventralized, like *grk* or *Egfr* mutants, it seemed likely that *ik2* affected the Grk/Egfr pathway.

By stage 9 of oogenesis, *grk* mRNA is localized as a cap around the oocyte nucleus, in the dorsoanterior corner of the oocyte (Fig. 3D) (Neuman-Silberberg and Schüpbach, 1993). Grk protein is translated adjacent to the oocyte and signals to the Egfr on nearby follicle cells to instruct those cells to adopt a dorsal cell fate, which sets up the dorsoventral axis of the eggshell and embryo. In the majority of the $ik2^{alice}$ oocytes (Fig. 3E) and in all $ik2^{1}$ oocytes (Fig.

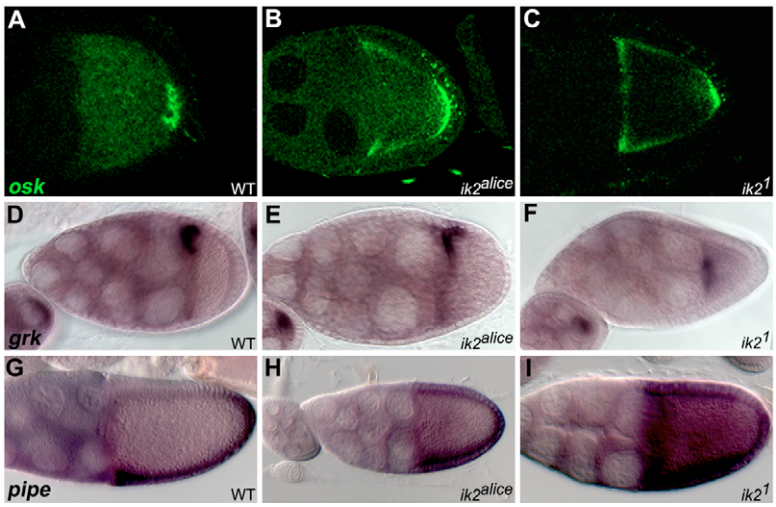

Fig. 3. Defects in mRNA localization and expression during oogenesis caused by loss of *ik2* in the female germline. (**A-C**) Fluorescent in situ hybridization shows that *osk* mRNA is localized to the posterior pole of a stage 9 wild-type oocyte (A), but is also found at the lateral cortex of an $ik2^{alice}$ oocyte (B), and at the anterior and lateral cortex of an $ik2^{1}$ oocyte (C). (**D-F**) In situ hybridization shows that *grk* mRNA is localized to the dorsoanterior corner of the wild-type oocyte at stage 8-9 (D), but is also found along the anterior border of an $ik2^{alice}$ oocyte (E), and is not enriched dorsally in an $ik2^{1}$ oocyte (F). (**G-I**) *pipe* mRNA is expressed in the follicle cells on the ventral side of the wild-type stage 10 egg chamber (G), but can also be detected in dorsal follicle cells of an $ik2^{alice}$ egg chamber (H), and is expressed at high levels both ventrally and dorsally in an $ik2^{1}$ egg chamber (I). *pipe* and *grk* in situ hybridizations were performed in parallel with wild-type controls, and all samples were developed for equal lengths of time. Anterior to the left, dorsal up.

3F), *grk* mRNA was localized to the anterior margin of the oocyte but was never concentrated dorsally. The *pipe* gene, which is required to activate the Toll ligand, is expressed only in ventral follicle cells, as the result of repression by Egfr signaling in dorsal follicle cells (Sen et al., 1998). During stages 9-10 of oogenesis, *pipe* is expressed in ventral follicle cells, corresponding to the future ventral side of the embryo where the Toll-Dorsal signaling pathway will be activated (Fig. 3G). By contrast, *pipe* mRNA was expressed in both ventral and dorsal follicle cells in the majority of the $ik2^1$ egg chambers analyzed (Fig. 3H,I). Thus, the absence of a source of *grk* mRNA at the dorsoanterior corner of *ik2* mutant oocytes prevents the specification of dorsal follicle cells and causes ventralization of the eggshell and embryo.

To confirm that the anteroposterior and dorsoventral defects observed in *ik2* mutants are the result of a genetic requirement for *ik2* in oogenesis only, we also performed an epistasis analysis of *pipe*. Females that are homozygous for recessive mutations in *pipe* produce dorsalized embryos (Fig. 4C), as the Toll ligand is never activated and ventral cell fates are never specified (Sen et al., 1998). We examined the embryos produced by mothers that carry germline clones of $ik2^1$ in a $pipe^1/pipe^1$ and a $pipe^1/pipe^2$ mutant background. A hundred percent of the embryos examined were dorsalized in a manner similar to *pipe* mutants (Fig. 4D), suggesting that *ik2* affects dorsoventral patterning at a step upstream of the Toll-Dorsal pathway.

Abnormal minus-end directed microtubule transport in *ik2* oocytes

Localization of mRNAs to the correct position within the oocyte depends on microtubules and microtubule motors (Brendza et al., 2000; MacDougall et al., 2003; Pokrywka and Stephenson, 1991; Pokrywka and Stephenson, 1995). Although recent re-examinations of the oocyte microtubule cytoskeleton have demonstrated that the bulk oocyte microtubules are non-polar (Cha et al., 2001; Cha et al., 2002), it has been proposed that subsets of microtubules are used by microtubule motors to localize mRNAs to specific subcellular regions (MacDougall et al., 2003; Januschke et al., 2006). Localization of *osk* mRNA to the posterior pole depends on kinesin-based transport, whereas *grk* mRNA localization to the anterior dorsal corner depends on dynein-based transport. We therefore evaluated the organization of the microtubule cytoskeleton and microtubule motors in *ik2* mutants.

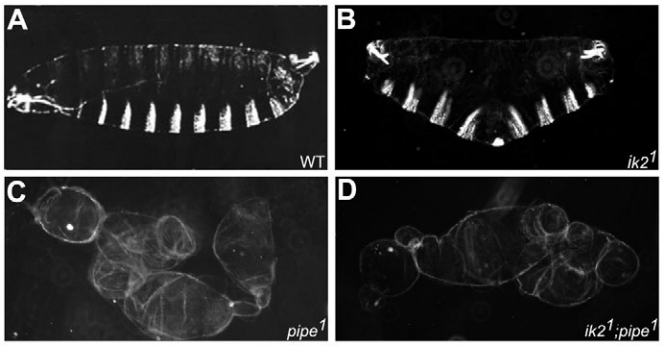

Fig. 4. Epistasis analysis with *pipe* reveals that *ik2* acts upstream of the Toll-Dorsal pathway during oogenesis. (**A-D**) Dark-field views of larval cuticle preparations at the end of embryogenesis from (A) wild type, (B) a bicaudal embryo from an $ik2^1$ germline clone female, (C) a dorsalized embryo from a $pipe^1$ mutant female and (D) a dorsalized embryo from a $ik2^1$; $pipe^1$ double homozygous mutant female.

Examination of a *Drosophila* Tau-GFP reporter (Micklem et al., 1997), which decorates all microtubules, revealed that the microtubule cytoskeleton is abnormal in *ik2* mutants. In wild-type stage 9 oocytes (Fig. 5A) fixed to preserve the microtubule cytoskeleton, Tau-GFP highlighted the non-polar microtubules in the cytoplasm, but was excluded from the oocyte nucleus. In stage 9 *ik2* mutant egg chambers (Fig. 5B), we observed staining throughout the ooplasm, but there were abnormal aggregates of Tau-GFP at the circumference of the nucleus, which suggested that a subpopulation of microtubules was disrupted in the mutant.

After stage 7 of oogenesis, the plus-ends of microtubules are concentrated at the posterior end of the oocyte, and kinesin, the plus-end directed motor, is enriched at the posterior. In wild-type stage 9 egg chambers, the *Kinesin Heavy Chain-β-galactosidase* fusion (*KHC-β-lacZ*) transgene was localized to the posterior pole of the oocyte (Fig. 5C) (Clark et al., 1994). At stage 8-9, $ik2^{alice}$ (Fig. 5D) and $ik2^1$ (data not shown) mutant egg chambers were indistinguishable from wild type. This indicates that microtubule plus-ends are correctly concentrated at the posterior of the oocyte in *ik2* mutants at the time when the kinesin motor is actively transporting *osk* mRNA to the posterior pole.

During mid-oogenesis, Dynein heavy chain (Dhc) accumulates at the posterior of the oocyte (Fig. 5E) (McGrail and Hays, 1997), where it presumably is stored to allow repeated rounds of minus end-directed transport. In $ik2^{alice}$, $ik2^1$ and $BicD^1/BicD^2$ mutant egg chambers, Dhc was distributed along the lateral cortex of the oocyte (Fig. 5F and data not shown) and was sometimes enriched in the dorsal and ventral corners of the anterior margin.

Although the plus-ends of the microtubules appeared normal in *ik2* mutants, the position of the minus-ends was not. In most cells, microtubule minus-ends are anchored in a microtubule organizing centre (MTOC) at the centrosome, whereas the minus-ends in the *Drosophila* oocyte are distributed along the anterior and lateral cortex, and are enriched in the area around the oocyte nucleus (Theurkauf et al., 1992; Cha et al., 2002). A transgene that encodes a fusion protein of the motor-like domain of Nod, a kinesin-related protein, with the coiled-coil domain of KHC and β-galactosidase has been used in previous studies as a marker of microtubule minus-ends (Clark et al., 1997). Although Nod preferentially binds microtubule plus-ends in vivo (Matthies et al., 2001; Cui et al., 2005), the Nod-β-gal transgene reliably localizes to the oocyte microtubule minus-ends, probably as a result of sequences outside of the Nod motor-like domain. At stage 8-10 in wild type, Nod-β-gal is localized to the anterior margin of the oocyte and is enriched in the dorsoanterior corner adjacent to the oocyte nucleus (Fig. 5G). Nod-β-gal was not detected at the anterior of stage 8-9 $ik2^1$ oocytes (Fig. 5H), and instead was present at low levels at the posterior and lateral cortex; at later stages, Nod-β-gal could not be detected in $ik2^1$ oocytes (Fig. 5J). Thus, microtubule minus-ends are not properly distributed at the anterior of *ik2* mutant oocytes during mid-oogenesis.

ik2 mutations also disrupt the actin cytoskeleton

In addition to the microtubule abnormalities in *ik2* mutant oocytes, all of the rare *ik2* escaper adults had abnormal bristles with a morphology that suggested defects in the actin cytoskeleton (Fig. 6). Bristles are constructed with rings of membrane-attached, cross-linked actin bundles (Tilney et al., 1995; Tilney et al., 1996). At high magnifications, the actin footprints along the length of the bristle shaft in *ik2* escaper adult eyes (Fig. 6F) appeared less organized than those in wild-type bristles (Fig. 6E). Loss of activity of specific actin-associated proteins, including Profilin (*chickadee*) (Verheyen and Cooley, 1994b) and the β-subunit of capping protein (Hopmann

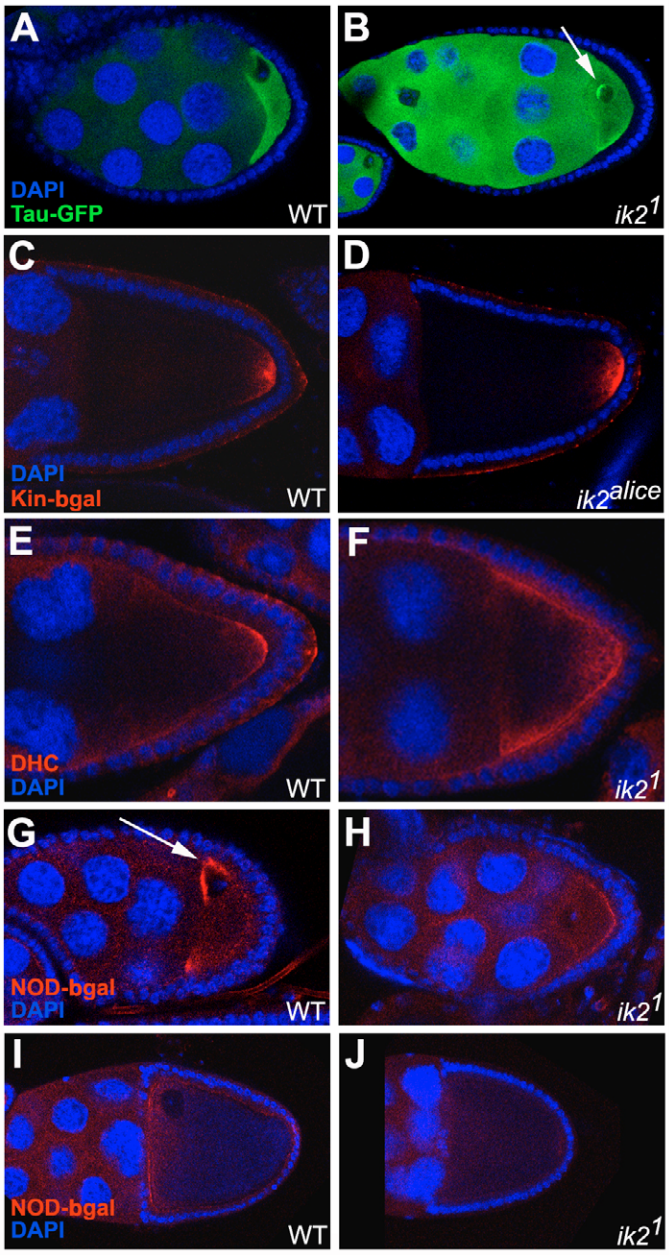

Fig. 5. Effects of the loss of *ik2* on microtubule organization in the oocyte. (**A**,**B**) Expression of Tau-GFP in wild-type (A) and $ik2^{1}$ (B) stage 7-8 egg chambers. An abnormal enrichment of Tau-GFP is seen around the oocyte nucleus in $ik2^{1}$ (arrow). Tau-GFP is also seen at higher levels in the nurse cells of the mutant than in wild type. (**C**,**D**) Localization of Kinesin-β-gal, as assayed by antibodies to β-galactosidase, in wild-type (C) and $ik2^{alice}$ (D) stage 9 oocytes, shows that the plus-ends of microtubules in $ik2^{alice}$ show the normal posterior enrichment. (**E**,**F**) The localization of dynein heavy chain (DHC) in wild-type (E) and $ik2^{1}$ (F) stage 9 oocytes; DHC is seen throughout the lateral cortex of $ik2^{1}$ oocytes, in contrast to its posterior localization in wild type. (**G-J**) Nod-β-gal as assayed with antibodies to β-galactosidase in wild-type (G,I) and $ik2^{1}$ (H,J) oocytes. In contrast to the dorsal anterior localization of Nod-β-gal in wild-type stage 8 oocytes (G, arrow), Nod-β-gal is ectopically localized at the posterior and lateral cortex of the $ik2^{1}$ (H) oocyte. By stage 10, Nod-β-gal was not detected in the $ik2^{1}$ oocyte (J), whereas it was localized at the anterior of the wild-type oocyte (I). Nuclei are stained with DAPI (blue). Anterior to the left, dorsal up.

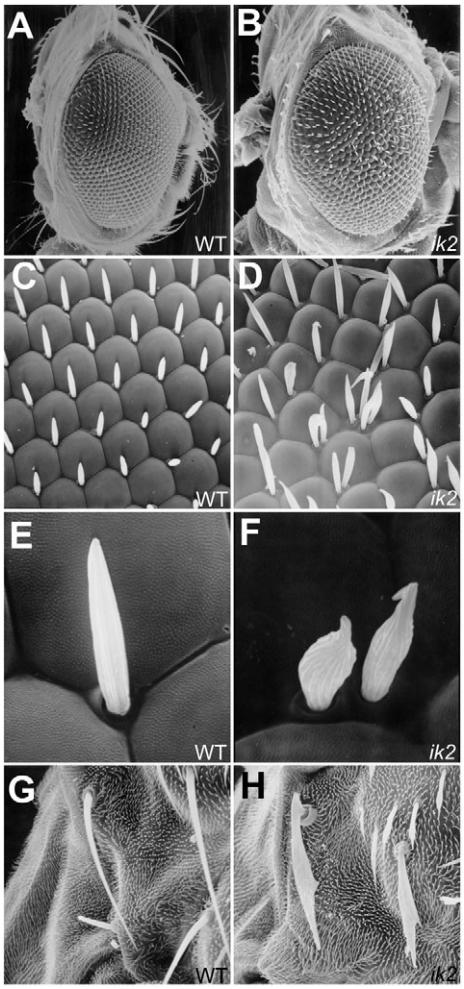

Fig. 6. Abnormal bristles in *ik2* adult escapers, analyzed by scanning electron microscopy. (**A-F**) Bristles in the eye. The ommatidia of the wild-type compound eye are arranged in an orderly pattern (A), whereas the compound eyes of *ik2* escaper adults (B) appear rough. At higher magnification, the interommatidial bristles can be seen at alternating vertices of wild type (C), but *ik2* bristles are often duplicated at a single vertex (D). The wild-type interommatidial bristle is a long, tapered, actin-based structure (E), whereas the *ik2* interommatidial bristles are misshapen and kinked (F). (**G**,**H**) Humeral bristles from wild type (G) and from an $ik2^{1}$ homozygous adult escaper (H).

et al., 1996), causes abnormal bristles similar to those seen in *ik2* mutants. All of the *ik2* allelic combinations analyzed, as well as the alleles in trans to the deficiency $Df(2L)Ketel^{RX32}$, which removes the *ik2* genomic region, displayed similar bristle phenotypes. Expression of a UASp-*ik2* transgene under the direction of either the ubiquitous *tubulin-Gal4* or *daughterless-Gal4* driver rescued both the viability and bristle abnormalities of *ik2* allelic combinations completely (data not shown), which confirmed that these phenotypes are caused by loss of *ik2*.

We also examined the actin cytoskeleton in oocytes derived from females containing *ik2* germline clones. Beginning at stage 7, we identified ectopic sites of actin polymerization in the ooplasm in $ik2^{1}$ (Fig. 7G) and $ik2^{alice}$ (data not shown) mutant egg chambers. Grk protein colocalizes with the oocyte cortical F-actin at stages 7-8 (Neuman-Silberberg and Schüpbach, 1996), and we observed that

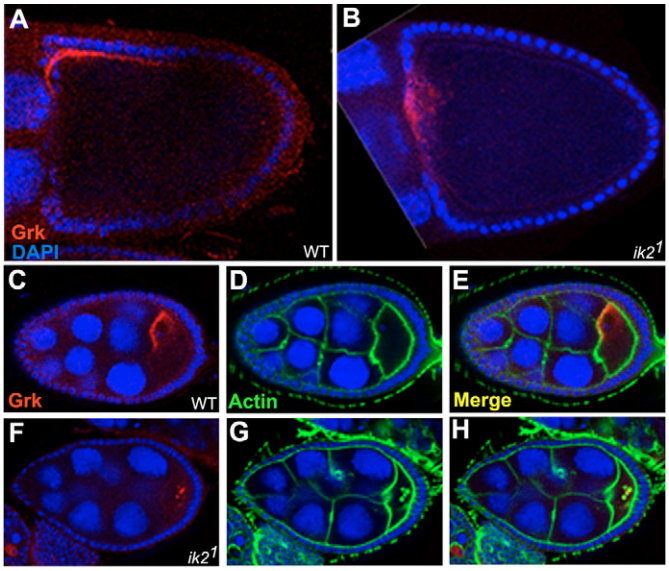

Fig. 7. Ectopic F-actin colocalizes with ectopic Gurken protein in *ik2* mutant oocytes. (**A**,**B**) Grk protein (red) is localized in the dorsoanterior region of the wild-type stage 10 oocyte (A), but is found at the anterior of the *ik2*^{1} oocyte (B). (**C-E**) Grk (red; C) colocalizes with F-actin, visualized by phalloidin staining (green; D) in a wild-type stage 8/9 egg chamber; a merged image is shown in E. (**F-H**) In some *ik2*^{1} germline clones, Grk (red) is present in aggregates (F) that colocalize with F-actin (green; G); a merged image is shown in H. Nuclei are stained with DAPI (blue). Anterior to the left, dorsal up.

Grk protein colocalized with the ectopic actin in affected *ik2* egg chambers (Fig. 7H). Thus, both the actin cytoskeleton and the anchoring of microtubule minus-ends are disrupted in *ik2* mutants.

DISCUSSION

Contrary to the prediction based on its sequence, *ik2* does not act as a Cactus/IκB kinase in the *Drosophila* embryo. The embryonic ventralization caused by loss of *ik2* is the opposite of the phenotype predicted for a Cactus kinase, and all effects of *ik2* on the dorsoventral pattern of the embryo can be explained by a loss of activity of the Grk/Egfr pathway during oogenesis. Embryos that lack maternal activity of both *Drosophila* IKKs, Ik2 and DmIkkβ, are ventralized and are indistinguishable from *ik2* single mutants (data not shown), which rules out the possibility that the *Drosophila* IKKs act in both the Grk/Egfr and Toll pathways, and indicates that an unidentified kinase of another family is required to target Cactus for degradation. Additional experiments will be required to test whether *ik2* plays other roles in the immune response.

We found that instead of playing a role in Cactus degradation, *Drosophila ik2* is required for the localization of specific mRNAs during oogenesis. Both the actin and microtubule cytoskeletons are disrupted in *ik2* mutants, and defects in microtubule-based transport are sufficient to account for the defects in mRNA localization seen in *ik2* mutants. Because *Drosophila ik2* is specifically required for organization of the oocyte cytoskeleton, our results raise the possibility that some of the NF-κB-independent roles of the mammalian IKKs may act through the cytoskeleton.

The embryonic patterning defects caused by the loss of *ik2* function are due to the failure to transport all *osk* mRNA to the posterior pole of *ik2* mutant oocytes, which leads to bicaudal embryos, and failure to localize *grk* mRNA to the dorsal anterior of the oocyte, which leads to ventralized embryos. Loss of *ik2* has a milder effect on *bcd* mRNA localization; *bcd* is correctly localized in most oocytes, but is not tightly restricted to the anterior pole in a minority of cases.

Many lines of evidence indicate that *osk* localization to the posterior pole depends on kinesin and *gurken* localization to the dorsoanterior corner depends on dynein (Brendza et al., 2000; Cha et al., 2002; MacDougall et al., 2003). However, the kinesin and dynein motors in the oocyte are interdependent. For example, posterior localization of dynein (Fig. 5) and the anterodorsal localization of *gurken* are both disrupted in *Khc* mutants (Brendza et al., 2002), and hypomorphic *Dhc* mutants have a reduced amount of *Khc*-β-gal at the posterior pole (McGrail and Hays, 1997). Both motor systems are at least partially functional in *ik2* mutants: most *oskar* is localized to the posterior pole of the oocyte (a kinesin-dependent process) and *grk* mRNA is localized anteriorly (a dynein-dependent process).

Several lines of evidence suggest that the RNA localization defects seen in *ik2* oocytes are associated with defects in a subset of dynein-mediated, minus-end-directed transport processes. The movement of *grk* mRNA to the dorsoanterior corner of the oocyte depends on two sequential dynein-based movements: *grk* mRNA moves first to the anterior of the oocyte along microtubules with plus-ends at the posterior pole and minus-ends at the anterior, and then moves dorsally on microtubules with minus-ends that form a cage around the oocyte nucleus (MacDougall et al., 2003). The dorsal movement of *grk* mRNA is specifically blocked in *ik2* mutants, which would be consistent with a failure in this dynein-based movement. Restriction of *bcd* mRNA to the anterior margin of the oocyte, which is disrupted in some *ik2* oocytes, depends on the *swallow* gene product, which binds dynein light chain (Schnorrer et al., 2000). Overexpression of dynamitin disrupts dynein function and causes changes to the localization of *grk* and *bcd* mRNA that are similar to the phenotype of *ik2* oocytes (Duncan and Warrior, 2002; Januschke et al., 2002). In addition, *BicD* mutations produce a maternal effect phenotype similar to that of *ik2*. BicD is part of a protein complex with dynein light chain in early oocytes, neuroblasts and the early embryo (Bullock and Ish-Horowicz, 2001; Hughes et al., 2004; Navarro et al., 2004), and has been proposed to link cargo to microtubules in both *Drosophila* and mammalian cells (Hoogenraad et al., 2003; Matanis et al., 2002).

Although this evidence links *ik2* to a dynein transport system, the most penetrant phenotype in *ik2* mutants is *osk* mislocalization and subsequent production of bicaudal embryos, a kinesin-dependent process. However, loss of *ik2* function, like the *BicD* gain-of-function mutations, does not eliminate kinesin function, because the majority of *osk* mRNA accumulates at the posterior pole. Because the kinesin and dynein motors in the oocyte are interdependent, *osk* mRNA mislocalization could be caused by a decreased kinesin activity that is secondary to dynein disruption.

In addition to defects in minus-end-directed transport, the organization of the microtubules is also perturbed in *ik2* oocytes (Fig. 5). The plus-ends of microtubules are localized correctly to the posterior pole of the oocyte. However, there are abnormal aggregates of microtubules around the oocyte nucleus, where a population of microtubule minus-ends is normally anchored, and the microtubule minus-end marker, Nod-β-gal, is not localized at the anterior of the oocyte. These defects suggest that abnormal organization of microtubule minus-ends during mid-oogenesis could be the basis of the defect in minus-end-directed transport.

The adult bristles and ovaries of *ik2* mutants also displayed abnormalities in the actin cytoskeleton. The bristle defects are nearly identical to those caused by mutations in actin-associated

proteins (Hopmann et al., 1996; Verheyen and Cooley, 1994b), or to bristles that were treated with F-actin-inhibitors (Tilney et al., 2000). Bristles contain a central core of microtubules, but mutations in the dynein heavy chain gene *Dhc64C* or treatment with drugs that disrupt microtubule dynamics do not cause bristle phenotypes like the thick, branched bristles seen in *ik2* mutants (Gepner et al., 1996; Tilney et al., 2000) (data not shown). The actin cytoskeleton of the oocyte is also disrupted in *ik2* mutants, with ectopic sites of actin polymerization in the ooplasm (Fig. 7). These actin defects are distinct from those caused by mutations that affect nurse cell ring canal actin (Hudson and Cooley, 2002), which suggests that actin organization is not globally disrupted in *ik2* mutants and that the actin defects are restricted to the oocyte cortex.

Recent data have defined two sets of microtubules in the oocyte that are both nucleated from minus-ends at the centrosome associated with the oocyte nucleus; one set remains associated with the oocyte nucleus, whereas the remaining microtubules shift their minus-ends from the oocyte to the cortex (Januschke et al., 2006). It was suggested that translocation of the minus-ends of the latter set of microtubules to the cortex could depend on actin and motor proteins (Januschke et al., 2006). Our data suggest that anchoring of microtubule minus-ends to the oocyte cortex depends upon an Ik2-dependent interaction of microtubule minus-ends with the F-actin network, analogous to the interaction of microtubule plus-ends with the actin cytoskeleton through microtubule tip proteins (Gundersen et al., 2004).

The phenotypes of *ik2* in the ovary and adult bristles are very similar to those caused by mutations in *spn-F* (Abdu et al., 2006). Like *ik2* mutations, null mutations in *spn-F* affect the localization of *osk* and *grk* mRNAs during oogenesis, and cause bicaudal and ventralized embryos. *spn-F* mutant oocytes have ectopic sites of F-actin polymerization, and *spn-F* bristles are similar to *ik2* mutant bristles. Ik2 and Spn-F have been shown to interact in a yeast two-hybrid screen (Giot et al., 2003), which suggests that these proteins can form a complex. Spn-F associates specifically with microtubule minus-ends (Abdu et al., 2006). We therefore propose that Ik2 and Spn-F act together to regulate interactions between the minus-ends of microtubules and the actin-rich cortex.

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References

Abdu, U., Bar, D. and Schüpbach, T. (2006). *spn-F* encodes a novel protein that affects oocyte patterning and bristle morphology in *Drosophila*. *Development* **133**, 1477-1484.

Anderson, K. V. (2000). Toll signaling pathways in the innate immune response. *Curr. Opin. Immunol.* **12**, 13-19.

Berleth, T., Burri, M., Thoma, G., Bopp, D., Richstein, S., Frigerio, G., Noll, M. and Nusslein-Volhard, C. (1988). The role of localization of *bicoid* RNA in organizing the anterior pattern of the *Drosophila* embryo. *EMBO J.* **7**, 1749-1756.

Brendza, R. P., Serbus, L. R., Duffy, J. B. and Saxton, W. M. (2000). A function for kinesin I in the posterior transport of *oskar* mRNA and Staufen protein. *Science* **289**, 2120-2122.

Brendza, R. P., Serbus, L. R., Saxton, W. M. and Duffy, J. B. (2002). Posterior localization of dynein and dorsal-ventral axis formation depend on kinesin in *Drosophila* oocytes. *Curr. Biol.* **12**, 1541-1545.

Bullock, S. L. and Ish-Horowicz, D. (2001). Conserved signals and machinery for RNA transport in *Drosophila* oogenesis and embryogenesis. *Nature* **414**, 611-616.

Castagnetti, S. and Ephrussi, A. (2003). Orb and a long poly(A) tail are required for efficient *oskar* translation at the posterior pole of the *Drosophila* oocyte. *Development* **130**, 835-843.

Cha, B. J., Koppetsch, B. S. and Theurkauf, W. E. (2001). In vivo analysis of *Drosophila bicoid* mRNA localization reveals a novel microtubule-dependent axis specification pathway. *Cell* **106**, 35-46.

Cha, B. J., Serbus, L. R., Koppetsch, B. S. and Theurkauf, W. E. (2002). Kinesin I-dependent cortical exclusion restricts pole plasm to the oocyte posterior. *Nat. Cell Biol.* **4**, 592-598.

Chou, T. B. and Perrimon, N. (1992). Use of a yeast site-specific recombinase to produce female germline chimeras in *Drosophila*. *Genetics* **131**, 643-653.

Chou, T. B. and Perrimon, N. (1996). The autosomal FLP-DFS technique for generating germline mosaics in *Drosophila melanogaster*. *Genetics* **144**, 1673-1679.

Clark, I., Giniger, E., Ruohola-Baker, H., Jan, L. Y. and Jan, Y. N. (1994). Transient posterior localization of a kinesin fusion protein reflects anteroposterior polarity of the *Drosophila* oocyte. *Curr. Biol.* **4**, 289-300.

Clark, I. E., Jan, L. Y. and Jan, Y. N. (1997). Reciprocal localization of Nod and kinesin fusion proteins indicates microtubule polarity in the *Drosophila* oocyte, epithelium, neuron and muscle. *Development* **124**, 461-470.

Cui, W., Sproul, L. R., Gustafson, S. M., Matthies, H. J. G., Gilbert, S. P. and Hawley, R. S. (2005). *Drosophila* Nod protein binds preferentially to the plus ends of microtubules and promotes microtubule polymerization in vivo. *Mol. Biol. Cell* **16**, 5400-5409.

DiDonato, J. A., Hayakawa, M., Rothwarf, D. M., Zandi, E. and Karin, M. (1997). A cytokine responsive IκB kinase that activates the transcription factor NF-κB. *Nature* **388**, 548-554.

Duncan, J. E. and Warrior, R. (2002). The cytoplasmic dynein and kinesin motors have interdependent roles in patterning the *Drosophila* oocyte. *Curr. Biol.* **12**, 1982-1991.

Ephrussi, A., Dickinson, L. K. and Lehmann, R. (1991). *oskar* organizes the germ plasm and directs localization of the posterior determinant *nanos*. *Cell* **66**, 37-50.

Erdelyi, M., Mathe, E. and Szabad, J. (1997). Genetic and developmental analysis of mutant Ketel alleles that identify the *Drosophila* importin-beta homologue. *Acta Biol. Hung.* **48**, 323-338.

Fernandez, N. Q., Grosshans, J., Goltz, J. S. and Stein, D. (2001). Separable and redundant regulatory determinants in Cactus mediate its dorsal group dependent degradation. *Development* **128**, 2963-2974.

Fitzgerald, K. A., McWhirter, S. M., Faia, K. L., Rowe, D. C., Latz, E., Golenbock, D. T., Coyle, A. J., Liao, S. M. and Maniatis, T. (2003). IKKε and TBK1 are essential components of the IRF3 signaling pathway. *Nat. Immunol.* **4**, 491-496.

Gepner, J., Li, M., Ludmann, S., Kortas, C., Boylan, K., Iyadurai, S. J., McGrail, M. and Hays, T. S. (1996). Cytoplasmic dynein function is essential in *Drosophila melanogaster*. *Genetics* **142**, 865-878.

Ghosh, S. and Karin, M. (2002). Missing pieces in the NF-κB puzzle. *Cell* **109**, S81-S96.

Giot, L., Bader, J. S., Brouwer, C., Chaudhuri, A., Kuang, B., Li, Y., Hao, Y. L., Ooi, C. E., Godwin, B., Vitols, E. et al. (2003). A protein interaction map of *Drosophila melanogaster*. *Science* **302**, 1727-1736.

Gundersen, G. G., Gomes, E. R. and Wen, Y. (2004). Cortical control of microtubule stability and polarization. *Curr. Opin. Cell Biol.* **16**, 106-112.

Hoogenraad, C. C., Wulf, P., Schiefermeier, N., Stepanova, T., Galjart, N., Small, J. V., Grosveld, F., de Zeeuw, C. I. and Akhmanova, A. (2003). Bicaudal D induces selective dynein-mediated microtubule minus end-directed transport. *EMBO J.* **22**, 6004-6015.

Hopmann, R., Cooper, J. A. and Miller, K. G. (1996). Actin organization, bristle morphology, and viability are affected by actin capping protein mutations in *Drosophila*. *J. Cell Biol.* **133**, 1293-1305.

Hu, Y., Baud, V., Delhase, M., Zhang, P., Deerinck, T., Ellisman, M., Johnson, R. and Karin, M. (1999). Abnormal morphogenesis but intact IKK activation in mice lacking the IKKα subunit of IκB kinase. *Science* **284**, 316-320.

Hu, Y., Baud, V., Oga, T., Kim, K. I., Yoshida, K. and Karin, M. (2001). IKKα controls formation of the epidermis independently of NF-κB. *Nature* **410**, 710-714.

Hudson, A. M. and Cooley, L. (2002). Understanding the function of actin-binding proteins through genetic analysis of *Drosophila* oogenesis. *Ann. Rev. Genet.* **36**, 455-488.

Hughes, J. R., Bullock, S. L. and Ish-Horowicz, D. (2004). Inscuteable mRNA localization is dynein-dependent and regulates apicobasal polarity and spindle length in *Drosophila* neuroblasts. *Curr. Biol.* **14**, 1950-1956.

Januschke, J., Gervais, L., Dass, S., Kaltschmidt, J. A., Lopez-Schier, H., St Johnston, D., Brand, A. H., Roth, S. and Guichet, A. (2002). Polar transport in the *Drosophila* oocyte requires Dynein and Kinesin I cooperation. *Curr. Biol.* **12**, 1971-1981.

Januschke, J., Gervais, L., Gillent, L., Keryer, G., Bornens, M. and Guichet, A.

(2006). The centrosome-nucleus complex and microtubule organization in the *Drosophila* oocyte. *Development* **133**, 129-139.
Kim-Ha, J., Kerr, K. and Macdonald, P. M. (1995). Translational regulation of *oskar* mRNA by Bruno, an ovarian RNA-binding protein, is essential. *Cell* **81**, 403-412.
Kozlova, T., Pokholkova, G. V., Tzertzinis, G., Sutherland, J. D., Zhimulev, I. F. and Kafatos, F. C. (1998). *Drosophila* hormone receptor 38 functions in metamorphosis: a role in adult cuticle formation. *Genetics* **149**, 1465-1475.
Li, Q., Van Antwerp, D., Mercurio, F., Lee, K. F. and Verma, I. M. (1999a). Severe liver degeneration in mice lacking the IκB kinase 2 gene. *Science* **284**, 321-325.
Li, Z. W., Chu, W., Hu, Y., Delhase, M., Deerinck, T., Ellisman, M., Johnson, R. and Karin, M. (1999b). The IKKβ subunit of IκB kinase (IKK) is essential for nuclear factor κB activation and prevention of apoptosis. *J. Exp. Med.* **189**, 1839-1845.
Lu, Y., Wu, L. P. and Anderson, K. V. (2001). The antibacterial arm of the *Drosophila* innate immune response requires an IκB kinase. *Genes Dev.* **15**, 104-110.
Luschnig, S., Moussian, B., Krauss, J., Desjeux, I., Perkovic, J. and Nusslein-Volhard, C. (2004). An F1 genetic screen for maternal-effect mutations affecting embryonic pattern formation in *Drosophila melanogaster*. *Genetics* **167**, 325-342.
MacDougall, N., Clark, A., MacDougall, E. and Davis, I. (2003). *Drosophila gurken* (TGFalpha) mRNA localizes as particles that move within the oocyte in two dynein-dependent steps. *Dev. Cell* **4**, 307-319.
Mahone, M., Saffman, E. E. and Lasko, P. F. (1995). Localized *Bicaudal-C* RNA encodes a protein containing a KH domain, the RNA binding motif of FMR1. *EMBO J.* **14**, 2043-2055.
Markussen, F. H., Michon, A. M., Breitwieser, W. and Ephrussi, A. (1995). Translational control of oskar generates short OSK, the isoform that induces pole plasma assembly. *Development* **121**, 3723-3732.
Matanis, T., Akhmanova, A., Wulf, P., Del Nery, E., Weide, T., Stepanova, T., Galjart, N., Grosveld, F., Goud, B., De Zeeuw, C. I. et al. (2002). Bicaudal-D regulates COPI-independent Golgi-ER transport by recruiting the dynein-dynactin motor complex. *Nat. Cell Biol.* **4**, 986-992.
Matthies, H. J. G., Baskin, R. J. and Hawley, R. S. (2001). Orphan kinesin NOD lacks motile properties but does possess a microtubule-stimulated ATPase activity. *Mol. Biol. Cell* **12**, 4000-4012.
McGrail, M. and Hays, T. S. (1997). The microtubule motor cytoplasmic dynein is required for spindle orientation during germline cell divisions and oocyte differentiation in *Drosophila*. *Development* **124**, 2409-2419.
McWhirter, S. M., Fitzgerald, K. A., Rosains, J., Rowe, D. C., Golenbock, D. T. and Maniatis, T. (2004). IFN-regulatory factor 3-dependent gene expression is defective in Tbk1-deficient mouse embryonic fibroblasts. *Proc. Natl. Acad. Sci. USA* **101**, 233-238.
Mercurio, F., Zhu, H., Murray, B. W., Shevchenko, A., Bennett, B. L., Li, J., Young, D. B., Barbosa, M., Mann, M., Manning, A. et al. (1997). IKK-1 and IKK-2: cytokine-activated IκB kinases essential for NF-κB activation. *Science* **278**, 860-866.
Micklem, D. R., Dasgupta, R., Elliott, H., Gergely, F., Davidson, C., Brand, A., Gonzalez-Reyes, A. and St Johnston, D. (1997). The *mago nashi* gene is required for the polarisation of the oocyte and the formation of perpendicular axes in *Drosophila*. *Curr. Biol.* **7**, 468-478.
Mohler, J. and Wieschaus, E. F. (1986). Dominant maternal-effect mutations of *Drosophila melanogaster* causing the production of double-abdomen embryos. *Genetics* **112**, 803-822.
Morisato, D. and Anderson, K. V. (1995). Signaling pathways that establish the dorsal-ventral pattern of the *Drosophila* embryo. *Annu. Rev. Genet.* **29**, 371-399.
Navarro, C., Puthalakath, H., Adams, J. M., Strasser, A. and Lehmann, R. (2004). Egalitarian binds dynein light chain to establish oocyte polarity and maintain oocyte fate. *Nat. Cell Biol.* **6**, 427-435.
Neuman-Silberberg, F. S. and Schüpbach, T. (1993). The *Drosophila* dorsoventral patterning gene *gurken* produces a dorsally localized RNA and encodes a TGFα-like protein. *Cell* **75**, 165-174.
Neuman-Silberberg, F. S. and Schüpbach, T. (1996). The *Drosophila* TGFα-like protein Gurken: expression and cellular localization during *Drosophila* oogenesis. *Mech. Dev.* **59**, 105-113.
Nüsslein-Volhard, C., Frohnhofer, H. G. and Lehmann, R. (1987). Determination of anteroposterior polarity in *Drosophila*. *Science* **238**, 1675-1681.
Peters, R. T. and Maniatis, T. (2001). A new family of IKK-related kinases may function as IκB kinase kinases. *Biochim. Biophys. Acta* **1471**, M57-M62.
Pokrywka, N. J. and Stephenson, E. C. (1991). Microtubules mediate the localization of *bicoid* RNA during *Drosophila* oogenesis. *Development* **113**, 55-66.
Pokrywka, N. J. and Stephenson, E. C. (1995). Microtubules are a general component of mRNA localization systems in *Drosophila* oocytes. *Dev. Biol.* **167**, 363-370.
Queenan, A. M., Barcelo, G., Van Buskirk, C. and Schüpbach, T. (1999). The transmembrane region of Gurken is not required for biological activity, but is necessary for transport to the oocyte membrane in *Drosophila*. *Mech. Dev.* **89**, 35-42.
Rongo, C., Gavis, E. R. and Lehmann, R. (1995). Localization of *oskar* RNA regulates oskar translation and requires Oskar protein. *Development* **121**, 2737-2746.
Rørth, P. (1998). Gal4 in the *Drosophila* female germline. *Mech. Dev.* **78**, 113-118.
Saffman, E. E., Styhler, S., Rother, K., Li, W., Richard, S. and Lasko, P. (1998). Premature translation of *oskar* in oocytes lacking the RNA-binding protein Bicaudal-C. *Mol. Cell. Biol.* **18**, 4855-4862.
Schneider, D. S., Hudson, K. L., Lin, T. Y. and Anderson, K. V. (1991). Dominant and recessive mutations define functional domains of *Toll*, a transmembrane protein required for dorsal-ventral polarity in the *Drosophila* embryo. *Genes Dev.* **5**, 797-807.
Schnorrer, F., Bohmann, K. and Nusslein-Volhard, C. (2000). The molecular motor dynein is involved in targeting *swallow* and *bicoid* RNA to the anterior pole of *Drosophila* oocytes. *Nat. Cell Biol.* **2**, 185-190.
Schüpbach, T. (1987). Germ line and soma cooperate during oogenesis to establish the dorsoventral pattern of egg shell and embryo in *Drosophila melanogaster*. *Cell* **49**, 699-707.
Schüpbach, T. and Wieschaus, E. (1991). Female sterile mutations on the second chromosome of *Drosophila melanogaster*. II. Mutations blocking oogenesis or altering egg morphology. *Genetics* **129**, 1119-1136.
Sen, J., Goltz, J. S., Stevens, L. and Stein, D. (1998). Spatially restricted expression of *pipe* in the *Drosophila* egg chamber defines embryonic dorsal-ventral polarity. *Cell* **95**, 471-481.
Senftleben, U., Cao, Y., Xiao, G., Greten, F. R., Krahn, G., Bonizzi, G., Chen, Y., Hu, Y., Fong, A., Sun, S. C. et al. (2001). Activation by IKKa of a second, evolutionary conserved, NF-kB signaling pathway. *Science* **293**, 1495-1499.
Sharma, S., tenOever, B. R., Grandvaux, N., Zhou, G. P., Lin, R. and Hiscott, J. (2003). Triggering the interferon antiviral response through an IKK-related pathway. *Science* **300**, 1148-1151.
Sil, A. K., Maeda, S., Sano, Y., Roop, D. R. and Karin, M. (2004). IκB kinase-alpha acts in the epidermis to control skeletal and craniofacial morphogenesis. *Nature* **428**, 660-664.
Silverman, N. and Maniatis, T. (2001). NF-κB signaling pathways in mammalian and insect innate immunity. *Genes Dev.* **15**, 2321-2342.
St Johnston, D., Driever, W., Berleth, T., Richstein, S. and Nüsslein-Volhard, C. (1989). Multiple steps in the localization of *bicoid* RNA to the anterior pole of the *Drosophila* oocyte. *Development* **107 Suppl**, 13-19.
Suter, B. and Steward, R. (1991). Requirement for phosphorylation and localization of the Bicaudal-D protein in *Drosophila* oocyte differentiation. *Cell* **67**, 917-926.
Takeda, K., Takeuchi, O., Tsujimura, T., Itami, S., Adachi, O., Kawai, T., Sanjo, H., Yoshikawa, K., Terada, N. and Akira, S. (1999). Limb and skin abnormalities in mice lacking IKKα. *Science* **284**, 313-316.
Tanaka, M., Fuentes, M. E., Yamaguchi, K., Durnin, M. H., Dalrymple, S. A., Hardy, K. L. and Goeddel, D. V. (1999). Embryonic lethality, liver degeneration, and impaired NF-κB activation in IKK-β-deficient mice. *Immunity* **10**, 421-429.
Tautz, D. and Pfeifle, C. (1989). A non-radioactive in situ hybridization method for the localization of specific RNAs in *Drosophila* embryos reveals translational control of the segmentation gene hunchback. *Chromosoma* **98**, 81-85.
Theurkauf, W. E., Smiley, S., Wong, M. L. and Alberts, B. M. (1992). Reorganization of the cytoskeleton during *Drosophila* oogenesis: implications for axis specification and intercellular transport. *Development* **115**, 923-936.
Tilney, L. G., Tilney, M. S. and Guild, G. M. (1995). F-actin bundles in *Drosophila* bristles. I. Two-filament cross-links are involved in bundling. *J. Cell Biol.* **130**, 629-638.
Tilney, L. G., Connelly, P. S., Smith, S. and Guild, G. M. (1996). F-actin bundles in *Drosophila* bristles are assembled from modules composed of short filaments. *J. Cell Biol.* **135**, 1291-1308.
Tilney, L. G., Connelly, P. S., Vranich, K. A., Shaw, M. K. and Guild, G. M. (2000). Actin filaments and microtubules play different roles during bristle elongation in *Drosophila*. *J. Cell Sci.* **113**, 1255-1265.
Van der Meer, J. M. (1977). Optical clean and permanent whole mount preparation for phase-contrast microscopy of cuticular structures of insect larvae. *Drosophila Information Service* **52**, 160.
Verheyen, E. and Cooley, L. (1994a). Looking at oogenesis. *Methods Cell Biol.* **44**, 545-561.
Verheyen, E. M. and Cooley, L. (1994b). Profilin mutations disrupt multiple actin-dependent processes during *Drosophila* development. *Development* **120**, 717-728.
Wharton, R. P. and Struhl, G. (1989). Structure of the *Drosophila BicaudalD* protein and its role in localizing the posterior determinant *nanos*. *Cell* **59**, 881-892.
Zandi, E., Rothwarf, D. M., Delhase, M., Hayakawa, M. and Karin, M. (1997). The IκB kinase complex (IKK) contains two kinase subunits, IKKα and IKKβ, necessary for NF-κB phosphorylation and NF-κB activation. *Cell* **91**, 243-252.