Drosophila Oocyte Polarity and Cytoskeleton Organization Require Regulation of Ik2 Activity by Spn-F and Javelin-Like

Simha Amsalem,a Anna Bakrhat,a Tetsuhisa Otani,b Shigeo Hayashi,b,c Bareket Goldstein,a Uri Abdua

Department of Life Sciences, Ben-Gurion University, Beer-Sheva, Israel; Laboratory for Morphogenetic Signaling, RIKEN Center for Developmental Biology, Kobe, Hyogo, Japan; Department of Biology, Kobe University Graduate School of Science, Kobe, Hyogo, Japan

The Drosophila melanogaster Spn-F, Ik2, and Javelin-like (Jvl) proteins interact to regulate oocyte mRNA localization and cytoskeleton organization. However, the mechanism by which these proteins interact remains unclear. Using antibodies to activated Ik2, we showed that this protein is found at the region of oocyte and follicle cell where microtubule minus ends are enriched. We demonstrate that germ line Ik2 activation is diminished both in jvl and in spn-F mutant ovaries. Structure-function analysis of Spn-F revealed that the C-terminal end is critical for protein function, since it alone was able to rescue spn-F sterility. On the other hand, germ line expression of Spn-F lacking its conserved C-terminal region (Spn-FΔC) phenocopied ik2, leading to production of centralized eggshell and bicaudal embryos. In Spn-FΔC-expressing oocytes, Gurken protein is mislocalized and oskar mRNA and protein localization is disrupted. Expression of Ik2 rescued Spn-FΔC ovarian phenotypes. We found that whereas Spn-F physically interacts with Ik2 and Jvl, Spn-FΔC physically interacts with Ik2 but not with Jvl. Thus, expression of Spn-FΔC, which lacks the Jvl-interacting domain, probably interferes with interaction of Ik2 and Jvl. In summary, our results demonstrate that Spn-F mediates the interaction between Ik2 and Jvl to control Ik2 activity.

During development and cell differentiation, mRNA localization is a crucial step in the regulation of gene expression of many transcripts. Accurate mRNA localization permits precise temporal and spatial regulation of protein production during development in a variety of organisms and cell types. RNA localization has been described in organisms as diverse as yeast and humans and has been observed in many polarized cells, such as oocytes, fibroblasts, or neurons. In Drosophila melanogaster, mRNA localization provides a particularly important mechanism for polar localization of axis-determining factors during oogenesis. The asymmetric localization of mRNA within the developing egg chamber relies on both microtubules (MTs) and actin networks, as well as on motor proteins. Although the organization of MTs and actin during midoogenesis has been revealed, the mechanism that leads to this complex cytoskeleton organization is still not fully understood.

The Drosophila spindle-F (Spn-F), IKKε homologue (Ik2), and novel MT-associated protein Javelin-like (Jvl) together produce a complex of proteins that affect both oogenesis and bristle development (1–4). We and others have shown that females carrying mutations in these genes produce eggs and embryos with polarity defects that arise due to disruptions in cytoskeleton organization and mRNA localization during oocyte development (1, 2, 4). We, moreover, have demonstrated that these three proteins physically interact and that their proper cell localization and function are interdependent (3, 4). In their physical interaction, Ik2 phosphorylates Spn-F, although such phosphorylation does not affect the stability of the protein (4). In addition, ik2 has also been found to be involved in other processes, including spindle organization (5, 6), dendrite pruning (7), bristle MT function (8, 9), F-actin assembly regulation (10, 11), and the shuttling of recycling endosomes during bristle cell elongation (12).

Closer examination of spn-F and ik2 ovarian defects reveals that whereas both mutants share the same defects in terms of cytoskeleton organization, the mutations differ in their effects on mRNA localization. In the mutants, both transport toward the minus end of the MT and the organization of the MTs that surround the oocyte nucleus are strongly affected (1, 2). The spn-F and ik2 mutants also present the same defects in terms of grk mRNA and protein localization. However, while over 90% of the embryos produced by ik2 mutant females are bicaudal (2), this phenotype is only rarely found in spn-F mutant embryos (1). This difference could be attributed to the fact that in ovaries and embryos produced by ik2 mutant females, oskar (osk) mRNA and protein are localized posteriorly and anteriorly, while in spn-F mutant ovaries, osk mRNA and protein localization are not affected. The difference seen in osk mRNA but not in grk mRNA localization defects between spn-F and ik2 mutants raises the question as to which molecular mechanisms control the actions of these proteins.

To better understand the function of these genes in mRNA localization and cytoskeleton organization during development, structure-function analysis of Spn-F protein was conducted. We show that the Spn-F protein may act as a mediator between Ik2 and Jvl to regulate Ik2 activity. Thus, our results provide a new perspective on the function of these proteins in pattern formation of the Drosophila egg and embryo, demonstrating that Spn-F and Jvl act on the core Ik2 function to augment the activity of this complex.

MATERIALS AND METHODS

Drosophila stocks. Oregon-R served as a wild-type control. The following mutants and transgenic flies were used: spn-F1, Df(3R)Ill-e (1), jvl1, Df(3R)Ill-e (1), jvl1,
RESULTS

Activation of Ik2 phosphorylation is dependent on the spn-F and javelin-like genes. To study the activation pattern of Ik2 during oogenesis, we used antibodies raised against Ik2 phosphorylated on serine 175 (pIkKε) (12). We found that in egg chambers from stage 5 to 6, pIkKε is found throughout the oocyte, with higher accumulation seen at the posterior end (Fig. 1A). Later on, after the oocyte nucleus migrates to the dorsal-anterior corner of the oocyte, pIkKε is found at the anterior ring, with higher accumulation seen in the vicinity of the nucleus (Fig. 1B) and in a punctate pattern in nurse cells (Fig. 1B). We also noticed that pIkKε is present on the apical side of the follicle cells (Fig. 1B′ to B′′). Thus, we found that pIkKε accumulates at MT minus-end-rich regions both in the oocyte and in follicle cells. Next, we studied the localization pattern of pIkKε in spn-F and jvl mutant ovaries and found that whereas in spn-F mutants, anti-pIkKε antibody staining of the oocyte and nurse cells was abolished (Fig. 1C and D), in jvl mutants, no such pIkKε staining was detected in the oocyte but the staining was still seen in the nurse cells (Fig. 1D). Also, in both the spn-F (Fig. 1D′) and jvl (Fig. 1F′) mutants, anti-pIkKε antibody staining was still evident on the apical side of the follicle cells. Thus, our results suggest that both spn-F and jvl are required for Ik2 function in the germ line but not in somatic follicle cells.

Next, we studied whether the absence of anti-pIkKε antibody staining in the germ lines of spn-F and jvl mutants could arise due to defects in Ik2 protein stabilization. Using antibodies directed against the Ik2 protein (10), we thus compared the levels of Ik2 protein in ovarian extracts from wild-type flies and from spn-F (Fig. 1G) and jvl (Fig. 1H) mutants. We found that in the ovarian extracts of both mutants, the level of Ik2 protein was similar to that in the wild-type ovarian extract. Thus, our results show that spn-F and jvl are required for Ik2 activation but not for its stabilization in the germ line.

Expression of C-terminally truncated Spn-F affects the anteroposterior and dorsoventral axes. In this study, we sought to understand how Spn-F affects Ik2 activity by conducting structure-function analysis of the Spn-F protein. Our search for conserved regions of Spn-F revealed the presence of two coiled-coil domains, the first extending from amino acid residue 32 to 114 and the second from residue 210 to 243. We had previously shown that the C-terminal end (but not the N-terminal end) of Spn-F is crucial for interaction with Ik2 (4). To investigate the functional importance of these Spn-F domains, we tested the functions of several mutant Spn-F transgenes deleted of sequences encoding different domains of the protein. Examination of multispecies spn-F protein sequences aligned by ClustalX showed that the two coiled-coil domains are conserved in all species considered. Using this alignment, an additional C-terminal conserved region spanning from amino acid 285 to the end of the protein sequence was found (data not shown). Three deletion constructs were thus generated in plasmid pUASP to yield truncated versions of Spn-F N-terminally tagged with GFP. The first variant encodes the N-terminal region of Spn-F (residues 1 to 162, here termed Spn-F-

Coimmunoprecipitation and colocalization assay. S2 cells expressing constructs as described in Results were treated with lysis buffer, and immunocomplexes were recovered using GFP-Trap_A (Chromotek) according to the manufacturer’s instructions. To detect interactions between proteins, Western blotting with anti-mCherry or anti-Ikk antibodies was performed. To detect protein localization patterns, mCherry-Jvl, Myc-tagged Ik2, GFP-Spn-F, and GFP-Spn-FΔC constructs were used. For Ik2 detection, primary mouse anti-α-Myc antibodies (1:150; Santa Cruz Biotechnology) were used. Goat anti-mouse Alexa Fluor 633-labeled secondary antibodies (Molecular Probes) were used at a dilution of 1:100.

Yeast two-hybrid assay. Yeast two-hybrid analysis was performed using the yeast two-hybrid phagemid vector kit ( Stratagene), following the manufacturer’s instructions. The pAD-Spn-F and truncated plasmids were used as bait, while a plasmid encoding full-length Jvl protein was used as prey.

In situ hybridization. RNA in situ hybridization on ovaries and embryos was carried out as described previously (1, 20).

β-Galactosidase and antibody staining. β-Galactosidase staining of ovaries was performed as described by Peretz et al. (18), with the exception that the ovaries were incubated in X-Gal (5-bromo-4-chloro-3-indolyl-β-D-galactopyranoside) stock solution at room temperature. Antibody staining of ovaries was performed as described previously (4). The following primary antibodies were used: mouse anti-Grk (1:10; clone 1D12) (21), rabbit anti-Oskar (1:3,000) (22), mouse anti-α-tubulin (1:100; Sigma), and rabbit anti-pIKK (10). Goat anti-mouse Cy3- or Cy2-labeled secondary antibodies and goat anti-rabbit Cy3- or Cy2-labeled secondary antibodies (Jackson Immunoresearch) were used at a dilution of 1:100. For α-tubulin staining, ovaries were kept at room temperature after fixation to prevent MT depolymerization. The dyes Oregon green 488 and Alexa Fluor 568 phalloidin (1:250; Molecular Probes) were used. All pictures were imaged on an Olympus FV1000 laser-scanning confocal microscope.

Western blot analysis. Dissected ovaries were ground in Laemmli sample buffer (10 µl per ovary), and the protein extracts were boiled for 5 min and loaded onto a 10% SDS-PAGE gel. Following electrophoresis, proteins were transferred to nitrocellulose membranes for 1 h at 300 mA. The nitrocellulose membranes were blocked by incubation in TTBS (0.2 M Tris-HCl [pH 7.5], 1.5 M NaCl, 9 mM Tween 20) containing 2.5% nonfat dry milk for 30 min at room temperature followed by incubation either for 1 h with anti-α-tubulin (1:1,000; Sigma) or overnight with anti-Ikk (1:20) (10) primary antibodies. The membranes were washed in TTBS and incubated for 30 min with horseradish peroxidase (HRP)-labeled anti-mouse antibodies (1:2,000; Amersham). Antibody labeling was visualized in a Fujifilm LAS300 imager using an ECL detection kit (Biological Industries).

Expression of C-terminally truncated Spn-F affects the anteroposterior and dorsoventral axes. In this study, we sought to understand how Spn-F affects Ik2 activity by conducting structure-function analysis of the Spn-F protein. Our search for conserved regions of Spn-F revealed the presence of two coiled-coil domains, the first extending from amino acid residue 32 to 114 and the second from residue 210 to 243. We had previously shown that the C-terminal end (but not the N-terminal end) of Spn-F is crucial for interaction with Ik2 (4). To investigate the functional importance of these Spn-F domains, we tested the functions of several mutant Spn-F transgenes deleted of sequences encoding different domains of the protein. Examination of multispecies spn-F protein sequences aligned by ClustalX showed that the two coiled-coil domains are conserved in all species considered. Using this alignment, an additional C-terminal conserved region spanning from amino acid 285 to the end of the protein sequence was found (data not shown). Three deletion constructs were thus generated in plasmid pUASP to yield truncated versions of Spn-F N-terminally tagged with GFP. The first variant encodes the N-terminal region of Spn-F (residues 1 to 162, here termed Spn-F-

Downloaded from http://mcb.asm.org on June 29, 2017 by guest
N), encompassing the first coiled-coil domain. The second construct encodes the C-terminal domain of Spn-F (residues 165 to 364, here termed Spn-F-C) that includes the second coiled-coil domain of the protein. Finally, the third construct encodes a version of Spn-F that includes the two coiled-coil domains but lacks the 84 C-terminal residues (i.e., residues 1 to 280, here termed Spn-F/H9004C) (Fig. 2A). Transgenic flies expressing each of the truncated proteins under the control of the UAS/Gal4 system were created. Three independent transgenic lines were tested for the expression of each of the constructs. All lines showed the same pattern, as described below. To drive the expression of the different Spn-F-encoding constructs, we used mat\alpha\alpha\alpha\alpha\alpha\alpha\alpha\alpha\alpha\alpha\alpha\alpha\alpha\alpha\alpha\alpha\alpha\alpha\alpha\alpha\alpha Tub-GAL4-VP16, a GAL4-VP16 fusion protein expressed under the control of the alphaTub67C promoter (Bloomington stock number 7062 or Df, deficiency.

FIG 1 spn-F and javelin-like (jvl) are required for Ik2 activation. (A to F) Confocal images of egg chambers stained with antibodies against phosphorylated Ik2, pIkK (red), and actin (green). In all figures, the egg chamber posterior is to the right. (A and B) Wild type (WT); (C and D) spn-F mutant; (E and F) jvl hemizygous. (A, C, and E) Stage 6 egg chambers; (B, D, and F) stage 7 to 8 egg chambers. (A) pIkK shows posterior accumulation in the oocyte. (B) pIkK is localized to the anterior end of the oocyte and close to the oocyte nucleus. In nurse cells, pIkK is found in a punctuate pattern (arrows). (B' to B'') pIkK is also found at the apical side of follicle cells. (C to D') pIkK is not detected in the spn-F germ line but is found in follicle cells (arrow in panel D'). (E to F') pIkK is not detected in jvl mutants oocytes but is found in nurse cells (arrows in panel F) and follicle cells (arrow in panel F'). (G and H) Western blot analysis of Ik2 levels in ovaries. Ik2 levels in ovarian extracts from wild-type and mutant flies were detected using antibodies against this protein. The level of acetylated tubulin served as a loading control. Mutations in spn-F and jvl do not affect the level of Ik2 protein.

FIG 2 Expression of Spn-FΔC leads to the appearance of ventralized eggshell and bicaudal embryos. (A) Schematic presentation of Spn-F protein domains and deletion constructs used to make transgenic flies. CC1, coiled coil 1; CC2, coiled coil 2; CT, conserved C terminus. (B) Western blot analysis of ovarian extract from flies expressing either GFP-Spn-FΔC, GFP-Spn-F-C, or GFP-Spn-F-N. The level of actin served as a loading control. (C to F) Eggshells from flies expressing Spn-FΔC; 8% of the eggshells had a wild-type appearance (C), 13% had a weakly ventralized eggshell with fused appendage (D), 76% had a ventralized eggshell with one appendage (E), and 3% had a strongly ventralized eggshell with no dorsal appendages (F). (G) Wild-type embryo. (H) Ninety-eight percent of embryos from flies expressing Spn-FΔC had a bicaudal phenotype. In all figures, the Gal4 that was used was P(mat\alpha\alpha\alpha\alpha\alpha\alpha\alpha\alpha\alpha\alpha\alpha\alpha\alpha\alpha\alpha\alpha\alpha\alpha\alpha\alpha\alpha\alpha mat\alpha\alpha\alpha\alpha\alpha\alpha\alpha\alpha\alpha\alpha\alpha\alpha\alpha\alpha\alpha\alpha\alpha\alpha\alpha\alpha\alpha Tub4-GAL4-VP16).
These Gal4 drivers lead to higher protein expression when starting from stage 5 to 6 egg chambers. First, we tested the ability of each of the constructs to rescue spn-F mutant female sterility. We found that neither spn-F-N nor spn-F/H9004C germ line expression rescued spn-F female sterility. On the other hand, 95% of the eggs (n/H11005 236) laid by females expressing spn-F-C in the spn-F mutant background hatched, demonstrating that the C-terminal half of Spn-F is sufficient for Spn-F function. Interestingly, we noticed that whereas germ line expression of spn-F-N and spn-F-C in a wild-type background had no effect on female fertility, expression of spn-F/H9004C led to complete female sterility. Closer examination revealed that 92% (n = 1,036) of eggs laid by females expressing spn-F/H9004C in the germ line produced ventralized eggshells (Fig. 2B to E). Furthermore, we found that expression of spn-FΔC severely affected the anterior-posterior axis of the embryos. Ninety-eight percent (n = 126) of the embryos produced by females expressing spn-FΔC showed a strong bicaudal phenotype, with several abdominal segments appearing in mirror image symmetry, and Filzkopfer and telson at both ends (Fig. 2G). The same results were obtained with nontagged constructs, demonstrating that the defects seen with spn-FΔC flies are not due to fusion of the GFP tag to the protein.

Expression of C-terminally truncated Spn-F affects Gurken (Grk) protein localization. Dorsal-ventral polarity defects can be attributed to disruptions in the grk-Egfr signaling pathway. Since specific expression of C-terminally truncated Spn-F protein in the germ line led to ventralized egg production, we examined the localization and expression of grk RNA and Grk protein in these ovaries. In situ hybridization analysis with a grk probe was performed in female flies expressing Spn-FΔC and wild-type ovaries. We found that similar to the case for wild-type ovaries (arrow in Fig. 3A) (23–25), ovaries expressing spn-F/H9004C showed no effect on grk mRNA localization, with grk mRNA being detected as a cap around the oocyte nucleus in the anterior-dorsal corner of the oocyte (arrow in Fig. 3B). Next, we tested the localization pattern of Grk protein and found that expression of spn-F/H9004C in the germ line led to a profound effect on Grk localization throughout oocyte development. In wild-type ovaries from stage 7 onward, Grk is restricted to the anterior-dorsal corner of the oocyte (arrow in Fig. 3C), much as seen in terms of grk mRNA localization. In stage 7 (data not shown) to stage 9 (Fig. 3D, arrow) egg chambers from Spn-F/H9004C-expressing females, Grk protein was localized to abnormally large punctae close to the oocyte. These results indicate that the dorsal-ventral defects in Spn-F/H9004C-expressing eggs are due to mislocalization of Grk protein.

Secreted and microfilament-related proteins are localized to ectopic actin cages in oocytes from flies expressing spn-FΔC. (A and B) In situ grk mRNA localization in stage 9 egg chambers. (A) Wild type; (B) Spn-FΔC. grk mRNA is found at the anterior-dorsal corner of the oocyte (arrows). (C to F) Confocal images of egg chambers stained with antibodies against Grk (white) and actin (red). (C) Stage 9 wild-type egg chamber; (D to F) stage 9 egg chamber from flies expressing Spn-FΔC. In the wild type, Grk protein is localized to the dorsal-anterior corner of the oocyte (arrow in panel C); however, in egg chambers from flies expressing Spn-FΔC, Grk protein is localized to ectopic actin clumps (arrows in panels D, E, and F). (G to J) Confocal images of egg chambers stained with antibodies against spectrin (white) and actin (red). (G) Stage 9 wild-type egg chamber; (H to J) stage 9 egg chamber from flies expressing Spn-FΔC. Spectrin protein is also localized to ectopic actin clumps (arrows in panels H to J).

FIG 3 Secreted and microfilament-related proteins are localized to ectopic actin cages in oocytes from flies expressing spn-FΔC. (A and B) In situ grk mRNA localization in stage 9 egg chambers. (A) Wild type; (B) Spn-FΔC. grk mRNA is found at the anterior-dorsal corner of the oocyte (arrows). (C to F) Confocal images of egg chambers stained with antibodies against Grk (white) and actin (red). (C) Stage 9 wild-type egg chamber; (D to F) stage 9 egg chamber from flies expressing Spn-FΔC. In the wild type, Grk protein is localized to the dorsal-anterior corner of the oocyte (arrow in panel C); however, in egg chambers from flies expressing Spn-FΔC, Grk protein is localized to ectopic actin clumps (arrows in panels D, E, and F). (G to J) Confocal images of egg chambers stained with antibodies against spectrin (white) and actin (red). (G) Stage 9 wild-type egg chamber; (H to J) stage 9 egg chamber from flies expressing Spn-FΔC. Spectrin protein is also localized to ectopic actin clumps (arrows in panels H to J).
in comparison to wild-type oocytes, where Osk protein is found as osk mRNA was found both at the posterior and anterior poles (Fig. 4B). Analysis of Osk protein localization revealed that and/or abnormally accumulated in the middle of the oocyte (arrows in Fig. 4C). In wild-type ovaries,过剩 mRNAs to the anterior and posterior poles of the oocyte, mRNAs to the anterior and posterior poles of the oocyte, as described above, 98% of the eggs from females expressing Spn-FΔC produced bicaudal embryos. We initially examined bcd mRNA localization and found that in Spn-FΔC-expressing egg chambers, bcd mRNA was localized to the anterior ring of the oocyte, similarly to that in wild-type flies (data not shown). Next, we examined the localization pattern of osk mRNA and protein in ovaries and eggs from Spn-FΔC-expressing females. In wild-type ovaries, osk mRNA was tightly localized to the posterior pole of the oocyte (Fig. 4A). However, in 47% of stage 9 or 10 egg chambers (n = 36) from Spn-FΔC-expressing females, osk mRNA was found both at the posterior and anterior poles and/or abnormally accumulated in the middle of the oocyte (arrows in Fig. 4B). Analysis of Osk protein localization revealed that in comparison to wild-type oocytes, where Osk protein is found as a tight crescent at the oocyte posterior end (Fig. 4C), in Spn-FΔC-expressing stage 10 oocytes, Osk protein was distributed more diffusely (Fig. 4D).

To characterize the localization pattern of Osk protein in oocytes at later stages of oogenesis, we used an osk-lacZ construct as a reporter of osk mRNA translation since most antibodies fail to penetrate at these stages (16). We found that in mature eggs produced by wild-type females, Osk-LacZ protein localized exclusively to the posterior pole (Fig. 4E). However, in eggs from Spn-FΔC-expressing females, Osk-LacZ protein accumulated at the anterior end (Fig. 4F, arrow) and was seen at high levels throughout the egg, in addition to its normal localization to the posterior pole, suggesting that anteriorly localized osk mRNA in Spn-FΔC-expressing egg chambers is translated only in the mature egg and does not undergo premature translation during oogenesis. Next, we analyzed osk mRNA localization in early embryos and found that in wild-type embryos, osk transcripts are concentrated at the posterior of the embryo, as well as being expressed at low levels throughout the embryo (Fig. 4H). In 90% of the embryos (n = 65) produced by Spn-FΔC-expressing females, high osk mRNA levels were found at the posterior pole and all over the embryo, in contrast to the case for the wild type, and osk mRNA also slightly accumulated at the anterior end of the embryo (arrow in Fig. 4I).

Microtubule polarity defects in oocytes from flies expressing Spn-FΔC. We next examined the integrity of the microtubule network, as revealed by α-tubulin antibody staining, and found that expression of Spn-FΔC affects MT organization in midstage egg chambers. In stage 9 egg chambers, oocyte MTs are organized in a gradient, with higher accumulation observed at the anterior cortex of the oocyte (arrow in Fig. 5A). In oocytes from females expressing Spn-FΔC, a significant reduction in α-tubulin levels along the anterior cortex was observed (arrow in Fig. 5B).

To investigate the polarity and functionality of the MT network, we tested transport to MT plus and minus ends, using the cortical surface (Fig. 3C), the ectopic F-actin network is juxtaposed to the oocyte nucleus (arrow in Fig. 3E).

Ectopic actin clumps were also observed in trailer hitch (tra) and Bic-C mutants, where it was shown that other microfilament-related and secreted proteins also associate with the ectopic actin cages (26, 27). We saw that in flies expressing Spn-FΔC, the secreted protein Grk is colocalized with the abnormal actin clumps in oocytes (arrow in Fig. 3F). Moreover, we found that spectrin, a microfilament-related protein, is also associated with the actin clumps (Fig. 3G to J). Thus, our results show that both secreted proteins, such as Grk, and the microfilament-related protein spectrin colocalized to the ectopic actin network in oocytes from flies expressing Spn-FΔC.
Microtubule organization and function are affected upon Spn-FΔC expression. (A and B) Confocal images of egg chambers stained with antibodies against tubulin (red). Stage 9 egg chambers were from wild-type flies (A) and flies expressing Spn-FΔC (B). The anterior-to-posterior tubulin gradient detected in the wild type (arrow in panel A) is abolished upon Spn-FΔC expression (arrow in panel B). (C to H) β-Galactosidase staining of Nod β-GAL (C and D) and kinesin β-GAL (E to H). (C and E) Wild-type egg chambers; and (B and F to H) Spn-FΔC egg chambers. In flies expressing Spn-FΔC, wild-type anterior Nod β-GAL staining (arrows in panel C) is abolished (D). In wild-type flies and in 3% of egg chambers from flies expressing Spn-FΔC, kinβ-gal is accumulated at the oocyte posterior (arrow in panel E). kinβ-gal in flies expressing Spn-FΔC either was not detected (F) or was found at the posterior end and in the middle of the oocyte (arrows in panel G) or accumulated only at the center of the oocyte (arrow in panel H).

Expression of C-terminally truncated Spn-F protein affects Ik2 activation. Next, we studied the localization pattern of pIKKε in ovaries expressing C-terminally truncated Spn-F. We found that expression of Spn-F protein lacking its C terminus completely abolished pIKKε in the germ line (Fig. 6B to D) but did not affect Ik2 protein stability (Fig. 6F). The use of the mat-GAL4 promoter, which drives high expression from stage 6 to 7, allows for demonstration of the dramatic effects of Spn-FΔC expression on pIKKε. In stage 5 egg chambers, where GFP:Spn-FΔC expression is low (arrowhead in Fig. 6B), anti-pIKKε immune staining in the oocyte is similar to that seen in the wild type (arrowhead in Fig. 6C). However, in stage 8 egg chambers, where GFP-tagged Spn-FΔC is expressed at high levels (arrow in Fig. 6B), anti-pIKKε antibody staining was abolished (arrowhead in Fig. 6C). Anti-pIKKε immunostaining was also evident in follicle cells (Fig. 6C), since Spn-FΔC was expressed exclusively in the germ line.

Since we found that expression of Ik2 rescues the sterility of females expressing Spn-FΔC, we tested whether Ik2 expression also restores Ik2 activity. We saw that expression of Ik2 is sufficient to also restore Ik2 activity in flies expressing the C-terminally truncated Spn-F protein, as revealed by the restoration of anti-pIKKε immunostaining in the ovaries of these flies (arrows in Fig. 6E).

Spn-FΔC protein can bind Ik2 but not Jvl. To better understand the mechanism by which Spn-FΔC overexpression affects Ik2 activity, we studied the physical interaction between Spn-FΔC and Ik2 or Jvl. Our previous results showed that the C-terminal but not the N-terminal end of the Spn-F protein binds the Ik2 protein (4). The truncated version of Spn-F employed here includes the second coiled-coil domain and the conserved region at the C-terminal region of the protein (spanning from residue 285 to the end of the protein). Since the expression of Spn-FΔC, which includes the second coiled-coil domain but lacks the C-terminal conserved region, affects Ik2 activity, we asked whether Spn-FΔC binds Ik2. Accordingly, we coexpressed myc-Ik2 with either GFP:Spn-F or GFP:Spn-FΔC in S2 cells and then performed immunoprecipitation with anti-GFP antibodies. We found that myc-Ik2...
show that the C-terminal region of Spn-F is able to bind Jvl (Fig. 7D). To verify these results, we considered the ability of Jvl to interact with Spn-F C protein colocalizes with Ik2 and Jvl. Spn-F but not Spn-FΔC protein colocalizes with Ik2 and Jvl. The results showing that different domains within Spn-F bind Jvl and Ik2 suggest that Spn-F, Jvl and Ik2 act as a complex of proteins, where Spn-F acts as a mediator between Ik2 and Jvl. Thus, deleting the Jvl-binding domain in Spn-F will affect the formation of the complex. To test this prediction, we studied the localization pattern of all three proteins in S2 cells. Expression of each of these proteins alone revealed that mCherry-Jvl is localized to a filamentous structure, which resembles the localization of mCherry-Jvl alone (Fig. 8E to G). One the other hand, when GFP-Spn-FΔC was coexpressed with mCherry-Jvl and Myc-tagged Ik2, both Myc-tagged Ik2 and GFP-Spn-FΔC were no longer associated with mCherry-Jvl (Fig. 8H to J).

DISCUSSION

Previously, it was shown that Ik2 is activated locally at the tips of bristles (12). In this study, we have demonstrated that during oogenesis, Ik2 is also locally activated at MT minus-end regions in the oocyte and follicle cells, as well as in the nurse cells, where Ik2 presents a punctate pattern. We found that in the germ line, jvl and spn-F are required for activation of Ik2. To better understand the mechanism by which Spn-F affects Ik2 activation, we performed structure-function analysis of the Spn-F protein. Using this approach, we were able to demonstrate that the C-terminal end of Spn-F is sufficient for protein function. Following the expression of several truncated Spn-F-encoding constructs in the germ line, we noticed that expression of Spn-F lacking 84 amino acids from the C-terminal end produced defects in both the dorsal-ventral and anterior-posterior axes that were similar to those found in ik2 loss-of-function ovaries (2). A high percentage of ventralized eggs and bicaudal embryos are produced by both the ik2 mutant (2) and Spn-FΔC-expressing females. Most importantly, the fact that expression of Ik2 was able to significantly rescue defects in eggshell and embryo development, as detected by Spn-FΔC expression, suggests that the C-terminal end of Spn-F regulates Ik2 protein function.

The results of the current study demonstrated that in terms of mRNA localization, the expression of Spn-FΔC protein produced, similarly to the case for ik2 mutants, high percentages of bicaudal...
embryos due to defects in osk mRNA localization. Previously, we showed that females mutant for spn-F produce a low percentage of bicaudal embryos, ranging from embryos with the typical reduced head skeleton to rare symmetrical bicaudals (1). Thus, we believe that Ikk2 function in oocyte anterior-posterior patterning has two components, one that depends on Spn-F and one that does not.

One can thus ask how Ikk2 activity affects osk mRNA localization. The role of the cytoskeleton in transporting osk mRNA to its final destination required cooperation between MTs and between MTs and actin motor proteins. Initially, osk mRNAs are transcribed in nurse cells and transported into the MT minus end at the anterior of the oocyte by dynein, along with the accessory factors Bic-D and Egalitarian (35,36). Within the oocyte, it was shown that the localization of osk to the posterior end required MTs, Khc, and myosin V (37–39). Several models explaining how osk transcripts are transported toward the posterior of the oocyte have been proposed, including active transport to the posterior (38), diffusion and trapping (40), or exclusion from the anterior and lateral cortex (41). One recent model suggested that osk mRNA is actively transported along microtubules in all directions, with a slight bias toward the posterior (42). As to the role of Khc in osk mRNA transport, it was suggested that Khc is required either directly (38,41) or indirectly (43–45). In the present study, we have demonstrated that whereas Ikk2 physically interacts with Spn-F C and the Ik2 and Jvl proteins. Previously, we had shown that Ikk2 is able to bind Ikk2 and Jvl. In this study, we demonstrated that whereas Ikk2 physically interacts with Spn-F C, Jvl was not able to bind Spn-F C. We also found that Ikk2 binds to the second coiled-coil domain of Spn-F (Hayashi, unpublished data), while Jvl interacts with the conserved C-terminal region of this protein. Moreover, we were able to show that Spn-F but not Spn-FΔC protein forms a complex with Ikk2 and Jvl. We believe that expression of Spn-FΔC, which is able to bind Ikk2 but not Jvl, interfered with the interaction between Ikk2 and Jvl. These results suggest that specific interference with the interaction between Ikk2

We have demonstrated that the defects in the dorsoventral axis in flies expressing Spn-FΔC are due to the Grk protein but not mRNA mislocalization. Moreover, we showed that Grk protein, a secreted protein, as well as spectrin, a microfilament-related protein, is localized to ectopic actin clumps in the oocyte. The localization of Grk to ectopic actin clumps was reported for several mutants, including Bic-C (26,27), trailer hitch (trail) (26,27), spn-F (1), and ik2 (2) mutants. In all of these mutants, grk mRNA was mislocalized but in a different pattern than was Grk protein, suggesting that defects in grk mRNA localization cannot account for defects in Grk protein localization. It was suggested that Bic-C and trail are part of the same pathway that regulates efficient Grk secretion (26,27). Accumulation of ectopic Grk protein in the oocyte was also found in Khc and Dhc mutants, and it was suggested that both genes are also required for Grk protein exocytosis (44). Thus, we suggest that the aberrant localization of Grk protein in ik2 mutants and in flies expressing Spn-FΔC reveals a role of these proteins in regulating Grk protein secretion.

To better understand the mechanism by which overexpression of Spn-FΔC affects Ikk2 activity, we studied interactions between Spn-FΔC and the Ikk2 and Jvl proteins. Previously, we had shown that Spn-F is able to directly bind Ikk2 and Jvl. In this study, we demonstrated that whereas Ikk2 physically interacts with Spn-FΔC, Jvl was not able to bind Spn-FΔC. We also found that Ikk2 binds to the second coiled-coil domain of Spn-F (Hayashi, unpublished data), while Jvl interacts with the conserved C-terminal region of this protein. Moreover, we were able to show that Spn-F but not Spn-FΔC protein forms a complex with Ikk2 and Jvl. We believe that expression of Spn-FΔC, which is able to bind Ikk2 but not Jvl, interfered with the interaction between Ikk2 and Jvl. These results suggest that specific interference with the interaction between Ikk2
and Jvl, as revealed upon Spn-FΔC expression, is critical for Ik2 core functions during oogenesis. The fact that Spn-F mediates Ik2 interaction with Jvl, an MT-associated protein, and the finding that Ik2 is activated at the MT minus end, together with the specific effects of ik2 on oocyte MT organization (2), suggest that Ik2 plays a crucial role in MT organization and/or function during oogenesis.

ACKNOWLEDGMENTS

We thank Anna Ephrussi and the Bloomington Stock Center for generously providing fly strains and reagents.

This research was supported by Israel Science Foundation grant 968/10 (to U.A.).

REFERENCES

Asmalem et al.


