**spn-F encodes a novel protein that affects oocyte patterning and bristle morphology in Drosophila**

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The anteroposterior and dorsoventral axes of the Drosophila embryo are established during oogenesis through the activities of Gurken (Grk), a Tgfa-like protein, and the Epidermal growth factor receptor (Egfr). spn-F mutant females produce ventralized eggs similar to the phenotype produced by mutations in the grk-Egfr pathway. We found that the ventralization of the eggshell in spn-F mutants is due to defects in the localization and translation of grk mRNA during mid-oogenesis. Analysis of the microtubule network revealed defects in the organization of the microtubules around the oocyte nucleus. In addition, spn-F mutants have defective bristles. We cloned spn-F and found that it encodes a novel coiled-coil protein that localizes to the minus end of microtubules in the oocyte, and this localization requires the microtubule network and a Dynein heavy chain gene. We also show that Spn-F interacts directly with the Dynein light chain Ddlc-1. Our results show that we have identified a novel protein that affects oocyte axis determination and the organization of microtubules during Drosophila oogenesis.

**KEY WORDS:** Drosophila, Oogenesis, Microtubule, Bristle formation, spn-F

**INTRODUCTION**

The anteroposterior (AP) and dorsoventral (DV) axes of the Drosophila embryo are established during oogenesis by communication between the oocyte and the surrounding follicle cells (Schüpbach, 1987; Ruohola-Baker et al., 1994). This communication is mediated by a single signaling system involving the gurken (grk) and Epidermal growth factor receptor (Egfr) genes (Gonzalez-Reyes et al., 1995; Roth et al., 1995). grk encodes a Tgfa-like protein that is expressed in the germline and is secreted from the oocyte (Neuman-Silberberg and Schüpbach, 1993; Neuman-Silberberg and Schüpbach, 1996). Egfr is expressed throughout the follicular epithelium (Kammermeyer and Wadsworth, 1987; Sapir et al., 1998), where it acts as a receptor for Grk. In earlier stages of oogenesis, localized activation of Egfr at one end of the egg chamber establishes posterior follicle cell fates. The follicle cells signal back to the oocyte and this initiates the formation of the embryonic AP axis (Gonzalez-Reyes et al., 1995; Roth et al., 1995). After the oocyte nucleus has shifted to one side of the egg chamber at stage 8, grk RNA accumulates in a crescent close to the nucleus, and the resulting Gurken protein activates the receptor on one side of the egg chamber to establish dorsal follicle-cell fates. The production and localization of grk RNA and protein within the oocyte is tightly regulated at several levels (for a review, see Nilson and Schüpbach, 1999).

The asymmetric localization of grk mRNA within the developing egg chamber during mid-oogenesis requires a network of polarized microtubules (MTs). Before stage 7, a microtubule-organizing center (MTOC) is located at the posterior of the oocyte, as revealed by the localization of the unconventional kinesin Nod fused to β-galactosidase (Nod:β-gal) (Clark et al., 1997). The first grk signaling event leads to a repolarization of the oocyte MTs and the migration of the oocyte nucleus to the dorsoanterior corner of the oocyte (Gonzalez-Reyes et al., 1995; Roth et al., 1995). During stage 8, the posterior MTOC disassembles and a diffuse MTOC forms at the anterior. The anterior MTOC has been visualized by Nod:β-gal (Clark et al., 1997), Centrosomin (Li and Kaufman, 1996) and the MT-nucleating factors, γ-Tubulin at 37°C (γTub37C) and the γ-Tubulin ring complex protein 75 (Grip75; previously known as Dgrip75) (Schnorrer et al., 2002). Injection of fluorescent grk mRNA into living oocytes revealed that the mRNA is assembled into particles that move in two distinct steps along two different MT arrays, first toward the anterior and then, after an apparent turn, dorsally toward the oocyte nucleus (MacDougall et al., 2003). High-resolution imaging of Tau-GFP (MacDougall et al., 2003; Januschke et al., 2002) and Nod:β-gal distribution in the oocyte suggests that there is a distinct network of MTs that are specifically associated with the oocyte nucleus, and these MTs are responsible for the second step in grk RNA localization. Moreover, recent work suggested that repositioning of the nucleus and its tightly associated centrosome could control MT reorganization in the Drosophila oocyte (Januschke et al., 2006). However, the precise nature and regulatory mechanisms that establish these oocyte nucleus-associated MTs is still unknown.

To further understand the role of MTs in grk RNA localization, we molecularly analyzed the spn-F locus. spn-F was first identified as a maternal effect mutation that affects the DV polarity of the eggshell (Tearel and Nüsslein-Volhard, 1987). We found that the ventralization of the eggshell in spn-F mutants is due to defects in the localization and translation of grk mRNA during mid-oogenesis. Moreover, we found that, in addition to the maternal effect, spn-F affects the development of the bristles of the adult fly. We show that transport toward the minus end of the MTs is affected in spn-F mutants, predominantly in the subset of MTs that are required for the transport of grk RNA from the anterior to the dorsal side of the oocyte towards the nucleus. We cloned spn-F and found that it encodes a novel coiled-coil protein that localizes to the minus end of MTs in the oocyte and in a punctate pattern in the nurse cells. Treatment of ovaries with the MT-depolymerization drug colcemid led to abrogation of Spn-F localization at the minus end of the MT and to higher accumulation of the protein in the nurse cells. We show

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that Spn-F interacts directly with the minus end-directed, motor-protein subunit Dynein light chain. Moreover, Spn-F localization to the minus end of the MT requires a Dynein heavy chain (Dhc) gene. Taken together, our results identify a novel protein that is involved in the organization of MTs in the oocyte during mid-oogenesis stages.

MATERIALS AND METHODS

Fly strains

Oregon-R was used as a wild-type control. The following mutant or transgenic flies were used: spn-F234, Df[3R]ll-g, Df[3R]ll-e, Act5C-GAL4 (Drosophila Stock Center, Bloomington), Df[3R]A177der20 and Df[3R]A177der2118, Dhc5-6, Dhc6-12 (Gepner et al., 1996), spn-F2 (Drosophila Stock Center, Szeged; originally designated f(3)si313003). The chromosome designated f(3)si313003 failed to complement the spn-F bristle and ovarian phenotypes. It had been suggested that this line carried two P-element insertions: the first was mapped to 62E by in situ hybridization and the second was uncovered by Df[3R]A177der21 [the second insertion site appears to be a second site mutation where a P-element is no longer associated with the semi-lethality (Deak et al., 1997)]. Indeed, recombinant allowed us to isolate a viable stock containing the spn-F2 mutation. We used the kinesin β-Gal insertion line KZ2503 and the nod-β-Gal insertion line NZ43.2 (Clark et al., 1997), as well as Tau-GFP (Micklem et al., 1997). Germline clones were generated using the ovoD-FLP technique and the FRT82B (3R) (Chou and Perrimon, 1992). CG12114 was expressed in the ovoF using the nanos-Gal4-VP16 expression system (Van Doren et al., 1998). The balancer chromosomes used in this study, as well as the marker mutations, have been described previously (see Lindsley and Zimm, 1992) (see also FlyBase, http://flybase.bio.indiana.edu).

Rescue construct

The entire coding sequence of CG12114 was amplified by PCR from EST (LD01470), using modified primers to create a Kpn1 restriction site at the 5′ end and a Not1 site at the 3′ end. The PCR product was then cloned into pUASp. P-element-mediated germ-line transformation of this construct was carried out according to standard protocols (Spradling and Rubin, 1982).

Sequencing of mutant alleles

Genomic DNA was prepared from flies of the genotype spn-F+/spn-F* according to standard procedures (Sambrook et al., 1989). The coding region was sequenced and the sequences were compared with the wild-type genomic sequence of the parental chromosomes using the MacVector (Kodak/IBI) program.

Generation of anti-Spn-F (CG12114) monclonal and polyclonal antibodies

The entire open-reading frame of CG12114 was cloned into pGEX-2T (Pharmacia). The fusion protein was overexpressed in Escherichia coli and the inclusion bodies were purified using a standard protocol (Sambrook et al., 1989). The coding region of mutant alleles

RESULTS

Spn-F affects the pattern of the eggshell and the embryo

Females mutant for spn-F234 produce a centralized eggshell (Tearle and Nüsslein-Volhard, 1987) (Fig. 1A,B, Table 1). A fraction of the eggs are fertilized and the embryos that form inside have a variety of pattern abnormalities (Fig. 1). Apart from centralized embryos that resemble embryos produced by weak gurken mutations, some bicaudal embryos are found inside the eggs, ranging from embryos with the typical reduced head skeleton to rare symmetrical bicauds with several abdominal segments in mirror image symmetry (Fig. 1C,D), and Filzkoerper and telson at both ends. We analyzed the localization of Osk protein, whose posterior concentration depends on endogenous kinesin heavy chain function (Brendza et al., 2002). The localization of oskar RNA in spn-F mutants is similar to that observed in wild type (data not shown); however, we found that the posterior association of Osk protein with the cortex in spn-F stage 10 oocytes is not as tight as in wild type (Fig. 1E,F).
spn-F affects oocyte nuclear morphology, and grk RNA localization and protein accumulation

The ventralization of eggshells in the spn-F mutant is similar to the phenotype produced by mutations in the grk-Egfr pathway. As spn-F is required in the germline (data not shown), we examined the effect of spn-F mutations on the localization and expression of grk RNA. In wild-type ovaries, grk RNA accumulates in the oocyte during earlier stages of oogenesis. In mid-oogenesis, it is localized within the oocyte, first transiently in an anterior-cortical ring (stage 8 and early stage 9), and then to a dorsoanterior domain in the cytoplasm directly overlying the oocyte nucleus (stages 9 and 10, Fig. 1G). In spn-F mutant ovaries, grk RNA is correctly localized to the oocyte in early stages. However, in mid-oogenesis, in the majority of stage 9 and 10 mutant egg chambers, grk RNA is found in a diffuse ring at the anterior margin of the oocyte rather than above the oocyte nucleus (Fig. 1H). In addition to the effect of spn-F on grk RNA localization, the accumulation of Grk protein is also affected. In wild-type egg chambers, Grk is restricted to the oocyte, and in mid-oogenesis it is localized to the dorsoanterior corner of the oocyte, similar to the RNA (Neuman-Silberberg and Schüpbach, 1996) (Fig. 1I). In spn-F mutant ovaries, Grk is also restricted to the oocyte; however, the levels of Grk are variably reduced throughout oogenesis, and in 67% of the stage 9 egg chambers Grk protein was undetectable (Fig. 1J). The DNA within the oocyte nucleus is often fragmented or thread-like in appearance (Fig. 1L), similar to the phenotype produced by spindle class genes (e.g. okra, spn-A, spn-B, spn-D) (Gonzalez-Reyes et al., 1997; Ghabrial et al., 1998; Abdu et al., 2003; Staeva-Vieira et al., 2003).

spn-F does not act through the meiotic checkpoint

Several of the spindle class genes, which are characterized by a variable eggshell ventralization phenotype, were previously found to encode proteins with homology to known DNA repair enzymes, and were shown to be required for the repair of recombination-induced, double-strand DNA breaks during Drosophila oogenesis (Ghabrial et al., 1998; Abdu et al., 2003; Staeva-Vieira et al., 2003). Spindle A, B, C and D patterning defects can be suppressed; for instance, by blocking the formation of double-strand DNA breaks during meiosis using mutations in mei-W68 (Ghabrial and Schüpbach, 1999), or by eliminating the checkpoint by using mutation in the checkpoint genes mei-41 and DmChk2 (lok – FlyBase) (Ghabrial and Schüpbach, 1999; Abdu et al., 2002). To determine whether the ovarian phenotype in spn-F is also due to unrepaird double-strand DNA breaks, flies double mutant for mei-W68 or DmChk2 and spn-F were generated. Our results showed that neither ventralization nor the defects in the appearance of the DNA within the oocyte nuclei of spn-F are suppressed by mei-W68 or DmChk2 (data not shown). These results strongly suggest that spn-F is not involved in the repair of double-strand DNA breaks during meiosis. The DV patterning defects of spn-F mutants therefore appear to arise by a mechanism that operates either downstream or independently of the meiotic checkpoint.

Bristle defects in spn-F mutants

In contrast to the other spindle class genes, spn-F also affects the development of the bristles (Fig. 2). When compared with wild type, spn-F mutants have considerably shorter and thicker bristles.

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**Table 1. Relative percentage of the different ventralized eggshell phenotypes found in spn-F mutants**

<table>
<thead>
<tr>
<th>Genotype</th>
<th>Class 1 – similar to wild type</th>
<th>Class 2 – appendages fused at base</th>
<th>Class 3 – single appendage</th>
<th>Class 4 – no appendage</th>
</tr>
</thead>
<tbody>
<tr>
<td>spnF1</td>
<td>4%</td>
<td>7%</td>
<td>9%</td>
<td>80%</td>
</tr>
<tr>
<td>spnF/Df</td>
<td>6%</td>
<td>5%</td>
<td>10%</td>
<td>79%</td>
</tr>
<tr>
<td>spnF2</td>
<td>3%</td>
<td>8%</td>
<td>5%</td>
<td>84%</td>
</tr>
<tr>
<td>spnF/Df</td>
<td>7%</td>
<td>1%</td>
<td>4%</td>
<td>88%</td>
</tr>
</tbody>
</table>

The classification of eggshell ventralization is based on that of Ghabrial et al. (Ghabrial et al., 1998). The deficiency Df(3R)tl-1/e (Df) was used in these experiments.
Scanning electron micrographs of *spn-F* bristles showed that, in addition to the aberrant length and width, the morphology of *spn-F* bristles was altered; the *spn-F* bristles do not have finely tapered ends (Fig. 2B), and, in some extreme cases, the direction of bristle growth at some points along the bristle shaft is altered (Fig. 2C,D).

**Actin and microtubule network in *spn-F* mutant ovaries**

Because *spn-F* mutants affect the formation of the bristles and also the localization of grk RNA, we investigated whether the actin and/or the MT network are altered during oogenesis. To analyze the organization of the MT network in *spn-F* mutant egg chambers, we used two different markers, α-tubulin staining and localization of Tau-GFP, which labels MTs (Micklem et al., 1997) (Fig. 3). In wild-type egg chambers at early stages, we found that MTs are equally distributed in the oocyte cytoplasm (Fig. 3A). However, in *spn-F* early stages, the α-tubulin accumulated abnormally around the oocyte nuclear membrane (Fig. 3B). In wild-type stage 9 egg chambers, α-tubulin is enriched at the anterior region of the oocyte with a slightly higher accumulation around the oocyte nucleus (Fig. 3C). In *spn-F* stage 9 egg chambers, α-tubulin is abnormally associated with the oocyte nuclear periphery (Fig. 3D). The oocyte nucleus does, however, move normally to the dorsoanterior corner in the mutant egg chambers. Using Tau-GFP in stages 6-9 egg chambers (Fig. 3E-H), we observed that, in early stages in wild type and mutants, MTs were uniformly seen in the oocyte cytoplasm (Fig. 3A). In *spn-F* mutants, we found abnormal accumulation of Tau-GFP is visible, associated with the mutant oocyte nucleus. Tau-GFP forms a gradient from anterior to posterior at stage 9 in both wild type and mutant; however, an abnormal accumulation of Tau-GFP occurs at the oocyte nuclear membrane in the mutant. Tau-GFP, green; DNA, blue. Abnormal clumps of actin can be seen in the mutant oocyte chamber (Fig. 3J), compared with wild type (Fig. 3I).

To examine the actin network, ovaries were stained with Rhodamine-conjugated phalloidin (Fig. 3I,J). In wild-type stage 8-9 egg chambers, most actin was uniformly and evenly distributed at the cortical surface (Fig. 3I); however, in 60% of *spn-F* mutant egg chambers, small irregularly shaped actin aggregates and actin clumps along the anterior ring below the actin-rich cortical layer were observed (Fig. 3J).

As a next step, we investigated the function and polarity of the MTs in *spn-F* mutant ovaries. First, the transport to the posterior pole of the oocyte was analyzed using the MT plus-end marker kin:β-gal (Clark et al., 1997). We found that kin:β-gal localization in *spn-F* mutants is similar to that observed in wild-type stage 9 egg chambers (Fig. 4A), in that the fusion protein is concentrated at the posterior end of the oocyte (Fig. 4B).

To analyze transport to the anterior pole, we used the MT minus-end marker Nod:β-gal (Clark et al., 1997). In wild-type stage 6 egg chambers, Nod:β-gal is concentrated at the posterior cortex of the oocyte (Fig. 4C). By stage 8, the protein is no longer detectable at the posterior of the oocyte but instead concentrates along the anterior cortex of the oocyte with highest accumulation flanking the oocyte nucleus (Fig. 4E). In *spn-F* mutant ovaries, neither the
early posterior localization (Fig. 4D) nor the anterior ring localization in mid-oogenesis (Fig. 4F) are detected. These results indicate that transport to the minus end of the MTs or some aspect of anchoring cargoes to the MT network is not functioning properly in spn-F mutant egg chambers. To assay transport to the minus end of the MTs in the anterior ring during mid-oogenesis versus transport to the dorsoanterior corner of oocyte, we analyzed the localization of bicoid, encore (Fig. 5) and js(1)K10 RNA (data not shown), which all accumulate in an anterior ring, but are not further localized to the dorsal corner. We found that the localization of these RNAs in spn-F mutants is similar to that observed in wild type. We also found that Centrosomin, a MTOC component that marks MT minus ends (Li and Kaufman, 1996), is localized to the anterior ring of the oocyte in spn-F mutants, as in wild type (data not shown).

Molecular characterization of the spn-F locus

spn-F234 had been mapped to position 3-100 on chromosome 3R. We used chromosomal deficiencies to show that spn-F234 is uncovered by Df(3R)Ill-e and Df(3R)A177der21, but not by Df(3R)Ill-g and Df(3R)A177der20, indicating that spn-F is located between 100B1-100B9. To identify additional spn-F alleles, we crossed a set of P-element lines (Deak et al., 1997) that were initially mapped to this region to spn-F234. One of the lines, l(3)s113003 failed to complement the spn-F bristle and ovarian phenotypes. After we recombined this insertion away from a second insertion on the same chromosome (see Materials and methods), we were able to obtain a homozygous viable line that contained a second spn-F allele (spn-F2). Both hemizygous and homozygous spn-F individuals are female sterile and show bristle defects similar to those observed in spn-F234. We also found that there was no difference in the severity of DV defects of the eggshell between the hemizygous spn-F234 and spn-F2, suggesting that both alleles are genetically null (Table 1).

Databank searches of region 100B1-100B9 revealed two likely candidate genes for spn-F. We sequenced both the bottleneck gene and CG12114 in spn-F234 homozygotes, and found a nonsense mutation in CG12114 at base pair 178 of its mRNA that results in a truncation of the predicted protein after amino acid 59. In spn-F2, there is a 67-nucleotide deletion starting from nucleotide 726.

To demonstrate that these mutations in the CG12114 gene are responsible for the defects observed in spn-F mutants, we expressed the entire CG12114 open reading frame in the germline under UAS control, using a nanos-Gal4 driver line in the spn-F234 and spn-F2 mutant backgrounds, and found that this transgene fully rescues the female sterility (data not shown). Also, expressing CG12114 using an actin-Gal4 driver rescued both the female sterility and the bristle defects. These results verify that spn-F indeed corresponds to the CG12114 gene.

Blast searches of protein databases identify proteins in Drosophila pseudoobscura (GA11408; 70% identity and 78% similarity) and in Anopheles gambiae (GenBank number: XP_311294; 34% identity and 49% similarity) as the proteins most closely related to CG12114. From sequence analysis, the spn-F gene is predicted to encode a 40 kDa protein with coiled-coil domains between amino acids 32-114 and 210-243. No homologous protein has been found in the databases outside the Dipteran species mentioned above.

Spn-F protein is localized to the minus end of the microtubule and interacts with the Dynel light chain Ddlc-1 (Cut up)

To analyze the intracellular localization of Spn-F, we generated monoclonal antibodies against the CG12114-encoded protein (Fig. 6). In wild-type ovaries, during early stages of oogenesis, Spn-F protein is localized to the posterior pole of the oocyte (Fig. 6A); in later stages Spn-F becomes localized to the anterior margin, with higher accumulation around the oocyte nucleus (Fig. 6B). These results strongly suggest that Spn-F is localized to theminus end of the MTs in the oocyte. We also found that, in the nurse cells, Spn-F is found in a punctate pattern (Fig. 6B). The antibody is specific for Spn-F, as such staining was not observed in spn-F mutant ovaries.

We next investigated the role of the MT network in Spn-F localization during oogenesis. Treatment of Drosophila females with colcemid, a MT depolymerizing drug, simultaneously affects all MTs in the oocyte (Pokrywka and Stephenson, 1995). We found that treatment of ovaries with colcemid abrogates Spn-F localization at the minus end of the MTs both during early (data not shown) and mid-oogenesis (Fig. 6C). Moreover, we found that in colcemid-
treated ovaries, a more punctate distribution of Spn-F protein accumulated in the nurse cells when compared with wild-type ovaries (Fig. 6C). These results suggest that Spn-F is transported from the nurse cells to the oocyte via the MT network, and requires MTs for its localization to the posterior in early oocytes, and to the anterior in older oocytes.

In a two-hybrid-based, protein-interaction map of the fly proteome, several proteins were found to interact with Spn-F, among these, although with somewhat low confidence, is cut up, which encodes a Dynein light chain (Ddlc) (Giot et al., 2003). Ddlc is a highly conserved light-chain subunit of cytoplasmic Dynein, a MT minus-end motor protein that is thought to play a fundamental role in both the assembly of the motor complex and the recruitment of cargo. To investigate the direct association of Spn-F with Ddlc-1, a GST pull-down assay was conducted. First, recombinant GST alone (Fig. 6E, lane 1), GST-Ddlc (Fig. 6E, lane 2) and His tagged-Spn-F (Fig. 6E, lane 3) proteins were purified and analyzed by gel electrophoresis. Next, GST-Ddlc-1 bound to GSH-agarose (Fig. 6E, lane 4) or GST bound to GSH-agarose (Fig. 6E, lane 5) was incubated with purified Spn-F protein and a pull-down assay was performed. We found that Spn-F binds efficiently to GST-Ddlc-1 (Fig. 6E, lane 4), but not to GST alone (Fig. 6E, lane 5) or to any of the other GST-fusion proteins tested (data not shown).

We also investigated the in vitro binding of recombinant Ddlc-1 to Spn-F using pull-down assays (Fig. 6F). We incubated a fusion protein containing glutathione-S-transferase (GST) and Ddlc-1 with extracts from wild-type ovaries and precipitated the fusion protein with GSH-agarose. Co-precipitated Spn-F protein was then detected by western blot analysis. Spn-F could be co-precipitated with GST-Ddlc-1 (Fig. 6F, lane 3), but not with GST protein alone (Fig. 6F, lane 1), or with any of the other GST fusion proteins tested (data not shown). These results indicate that the Spn-F interacts specifically with Ddlc-1.

To test whether Spn-F localization to the minus end of the anterior of the oocyte is dependent on Dynein, we studied the localization of Spn-F in Dynein heavy chain (Dhc64) mutants. We tested the localization of Spn-F in hypomorphic semi-viable dhc allelic combinations (Gepner et al., 1996) and found that, in 70% of the egg chambers, Spn-F protein was not localized to the anterior minus end of the oocyte (Fig. 6D), and a more punctate distribution of Spn-F was found in the nurse cells. These results, together with the direct interaction of Spn-F with Dynein light chains, suggest that Spn-F transport from the nurse cells to the oocyte anterior is dependent on the minus end-directed motor Dynein.

**DISCUSSION**

Mutations in *spn-F* cause specific patterning defects during oogenesis, resulting in eggs and embryos that show variable alterations along the DV and AP axes. We have demonstrated that grk mRNA is mislocalized to the anterior circumference of the oocyte in late-stage egg chambers, instead of accumulating in the dorsoanterior region above the oocyte nucleus. The mislocalized mRNA is not translated efficiently, resulting in reduced levels of Grk in the oocyte. The decrease in Grk protein explains the ventralized eggshell and embryo phenotypes produced by *spn-F* mutations. Previous studies have indicated that grk is translationally repressed when not localized and is specifically derepressed at its site of localization in the dorsoanterior corner of the oocyte (Hawkins et al., 1997; Saunders and Cohen, 1999; Norvell et al., 1999).

In mutations of *squid*, which encodes an hnRNP, the mislocalized grk mRNA along the anterior ring is translated, suggesting that Sqd protein is involved in grk mRNA localization and translation regulation (Norvell et al., 1999). Although our results indicate that Spn-F is also involved in grk mRNA localization, in *spn-F*, the mislocalized grk mRNA is not relieved from its translational repression.

We also observed a small fraction of *bicaudal* embryos, indicating that there was a problem with anterior posterior pattern formation; however, we did not observe ectopic Oskar protein at the anterior end of *spn-F* mutant oocytes, as might be expected, given the *bicaudal* phenotype. We did observe a minor mislocalization of Oskar protein at the posterior, which might have arisen as a result of the abnormalities in the MT or actin cytoskeleton, but this minor mislocalization cannot be the cause of the *bicaudal* phenotype. Given that only a small percentage of the embryos show the AP...
positioning abnormalities, it is likely that there is a small amount of ectopic Oskar protein present at the anterior in a few egg chambers that was not detected in our experiments.

What is the mechanism by which spn-F affects grk mRNA localization? Various lines of evidence have suggested that there are two separable steps in the localization of grk RNA. Mutations in several genes, such as squid and fs(1)K10, allow the accumulation of grk RNA at the anterior, but interfere with the localization to the dorsal corner (Serano and Cohen, 1995; Neuman-Silberberg and Schüpbach, 1993; Norvell et al., 1999). Furthermore, after injection of labeled grk RNA into live oocytes, it was observed that grk mRNA particles move in two distinct steps, which are likely to involve two distinct arrays of MTs. Initially the particles move towards the anterior, subsequently they turn and move dorsally towards the oocyte nucleus (MacDougall et al., 2003). Both steps of grk mRNA localization require intact MTs and cytoplasmic Dynein (MacDougall et al., 2003). We have analyzed the organization and polarity of MTs in spn-F mutant oocytes through the imaging of Tau-GFP α-tubulin and Nod:β-gal. We detected an abnormal accumulation of GFP-Tau protein and tubulin in the periphery of the oocyte nucleus. We also tested the accumulation of Nod:β-gal, which is localized to the MT minus end during early and mid-oogenesis in wild-type oocytes. We observed that this accumulation does not occur in spn-F egg-chambers. However, a number of probes that normally associate with the anterior cortex of the oocyte in a MT-dependent manner, such as bicaudal RNA and Centrosomin, appeared unaffected in spn-F mutants. It is possible that molecules whose anchoring at the anterior cortex is stabilized by interaction with other molecules (Pokrywka and Stephenson, 1995; Schnorrer et al., 2000) may accumulate seemingly normally at the anterior, whereas molecules that depend solely on the MT network (Nod:β-gal) are more strongly affected in the spn-F mutant. It may be significant that grk RNA itself, while localizing to the anterior, only forms a broad and fuzzy ring at the anterior of spn-F mutants (which is different from its accumulation in mutations such as squid), as grk RNA has been shown to require continuous transport for its anchoring (MacDougall et al., 2003). In any case, the effect of spn-F on the organization of the minus ends that are positioned along the anterior cortex appears to be more subtle, whereas the effect on MTs around the oocyte nucleus is much more severe, resulting in a visible disorganization and clumping of the network. The effect of spn-F on the organization of the MT minus end in the oocyte seems to be relatively specific, as no defects in the localization of kin:β-gal to the MT plus end were observed. We also found that Spn-F protein is localized to MT minus end in the oocyte, and in a punctate form in the nurse cells. We showed that the localization to the MT minus ends requires an intact MT network and the subunit of the motor complex Dhc. We also observed higher Spn-F accumulation in the nurse cells after colcemid treatment.

Given these results, one possible interpretation would be that spn-F mutants affect transport toward the minus end of MTs, predominantly in the subset of MTs that are required for the transport of grk RNA from the anterior to the dorsal side of the oocyte towards the nucleus. The observed interaction with the Dynein light chain would support such a mechanism. Another possibility is that Spn-F has a role in the organization of the minus end of MTs, with particularly strong effects on the MT network that surrounds the oocyte nucleus. The association of Spn-F with the Dynein light chain might be only transient under this model. Finally, given the collapsed aspect of the MT network in the vicinity of the oocyte nucleus, seen in the mutant egg chambers, it is also possible that Spn-F functions to provide a stabilizing connection between the minus ends of MTs and the actin cytoskeleton. However, to gain more insight into the direct role of Spn-F in MT organization, further experiments are needed. Analyzing the role of MT polymerization, MT polarity, and identifying further interaction partners of Spn-F will provide further insight into the function of the Spn-F protein.

In addition to the effects on the oocyte, we have found that spn-F affects the development of the bristles. The highly elongated bristles of Drosophila have proven to be a valuable model system for studying cellular morphogenesis. Developing bristles in Drosophila pupae contain 7-11 bundles of crosslinked actin filaments and a large population of MTs. During bristle growth, the rate of cell elongation increases with bristle length. It has been suggested that actin filaments and MTs play different roles during bristle elongation in Drosophila (Tilney et al., 2000). Whereas actin assembly is crucial for bristle cell elongation, MTs must provide other functions, such as providing bulk to the bristle cytoplasm, as well as playing a role in vesicle transport. It has also been shown that MT antagonists such as vinblastine and colchicine resulted in a decreased axial length and a compensatory increase in width, so that the volume of the bristle was not significantly changed (Fei et al., 2002). These data suggest that the MT cytoskeleton is of central importance for growth to be polarized in the axial direction (Fei et al., 2002). Mutations in the Dynein heavy chain resulted in shorter and thicker bristles (Gepner et al., 1996); mutations in kinesin also resulted in shorter and thicker bristles, and the tips of bristles were often contorted, exhibiting flattened, flared or twisted tips (Brendza et al., 2000). Although the spn-F bristle phenotype is comparable to the kinesin mutant bristles, the spn-F bristle phenotype is nevertheless unique, suggesting a specific role in the process that is slightly different from that of either Dynein or Kinesin.

In a global two-hybrid screen, Spn-F (CG12114) was found to interact with the Ik2 (CG2615) protein (Giot et al., 2003). Mutations in this protein have been isolated and characterized by Shapiro and Anderson (Shapiro and Anderson, 2006). Interestingly, ik2 mutants share many phenotypes with spn-F, including a very similar bristle phenotype and specific effects on MT organization in oogenesis. However, ik2 mutants are lethal, whereas spn-F homozygotes survive. In addition, whereas spn-F mutations have only mild effects on Oskar protein localization and a low frequency of biclaudal phenotypes, such effects are more pronounced in the ik2 mutants. Nevertheless, the striking similarities strongly suggest that the two genes function in a common pathway that affects certain types of MT more strongly than others. In normal mitotic cells, the minus ends of MTs are usually focused by the centrosomes in the interior of the cell, and plus ends contact the cortex. However, in specialized cells, such as the Drosophila oocyte, there are minus ends that make contact with the cortex (Cha et al., 2002). It is therefore possible that Spn-F and Ik2 are required for providing a stable connection between such cortical MT minus ends and cortical actin for subsets of MTs involved in specialized transport processes. Future experiments will address the interactions of Spn-F and Ik2 directly, and will determine whether, for instance, Spn-F might be a target of Ik2.

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