

# Wispy, the *Drosophila* Homolog of GLD-2, Is Required During Oogenesis and Egg Activation

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## ABSTRACT

Egg activation is the process that modifies mature, arrested oocytes so that embryo development can proceed. One key aspect of egg activation is the cytoplasmic polyadenylation of certain maternal mRNAs to permit or enhance their translation. *wispy* (*wisp*) maternal-effect mutations in *Drosophila* block development during the egg-to-embryo transition. We show here that the *wisp* gene encodes a member of the GLD-2 family of cytoplasmic poly(A) polymerases (PAPs). The WISP protein is required for poly(A) tail elongation of *bicoid*, *Toll*, and *torso* mRNAs upon egg activation. In *Drosophila*, WISP and Smaug (SMG) have previously been reported to be required to trigger the destabilization of maternal mRNAs during egg activation. SMG is the major regulator of this activity. We report here that SMG is still translated in activated eggs from *wisp* mutant mothers, indicating that WISP does not regulate mRNA stability by controlling the translation of *smg* mRNA. We have also analyzed in detail the very early developmental arrest associated with *wisp* mutations. Pronuclear migration does not occur in activated eggs laid by *wisp* mutant females. Finally, we find that WISP function is also needed during oogenesis to regulate the poly(A) tail length of *dmos* during oocyte maturation and to maintain a high level of active (phospho-) mitogen-activated protein kinases (MAPKs).

MATURE oocytes of most animals are arrested at a species-specific stage of meiosis until certain events occur that convert the oocyte to a state that can undergo embryogenesis. Prior to these events, the mature oocyte is developmentally “poised”: it contains maternal mRNAs and other molecules that are required for embryogenesis. The process that transitions the oocyte to become competent for further development is called egg activation. In vertebrates and marine invertebrates, egg activation is triggered by fertilization. Entry of the sperm causes an increase of free calcium in the egg cytoplasm, which triggers the completion of meiosis (reviewed in DUCIBELLA *et al.* 2006; PARRINGTON *et al.* 2007). In *Drosophila*, egg activation occurs independently of fertilization and appears to be triggered by mechanical stress experienced by the egg when it passes through the female reproductive tract during ovulation (HEIFETZ *et al.* 2001; HORNER and WOLFNER 2008a). A calcium-dependent signaling pathway is involved in activation of *Drosophila* eggs (HORNER *et al.* 2006; TAKEO *et al.* 2006), and many of the events of *Drosophila* egg activation downstream of the initial trigger appear analogous to those in other animals.

Activation events include modifications in egg coverings, resumption and completion of meiosis, degradation of some maternal mRNAs, a decrease in

mitogen-activated protein kinase (MAPK) activity, and changes in the cytoskeleton (reviewed in HORNER and WOLFNER 2008b). An additional crucial aspect of egg activation is the polyadenylation of some maternal transcripts that initiates their translation. In *Drosophila*, transcripts subject to regulation by polyadenylation during egg activation include the *bicoid*, *Toll*, and *torso* mRNAs. These mRNAs encode proteins that are involved in defining embryo polarity. The protein products of these mRNAs are first detected only after egg activation is triggered (DRIEVER and NUSSLEIN-VOLHARD 1988; HASHIMOTO *et al.* 1988; CASANOVA and STRUHL 1989). Initiation of the translation of these mRNAs correlates with an increase in the length of their poly(A) tails (SALLES *et al.* 1994).

Polyadenylation of mRNA takes place initially in the nucleus of eukaryotic cells, but maintenance and regulation of the poly(A) tail occurs in the cytoplasm (SACHS *et al.* 1997). Cytoplasmic polyadenylation is a key regulatory mechanism that controls the function of maternal mRNAs during the early development of *Xenopus* oocytes (reviewed in MENDEZ and RICHTER 2001). A cytoplasmic poly(A) polymerase (PAP) called GLD-2, first identified in *Caenorhabditis elegans*, functions within a protein complex to facilitate cytoplasmic polyadenylation (WANG *et al.* 2002; BARNARD *et al.* 2004). In *Xenopus* oocytes, the cytoplasmic polyadenylation element-binding protein (CPEB) recognizes the cytoplasmic polyadenylation element in the 3'-UTR of the mRNA (HAKE and RICHTER 1994; STEBBINS-BOAZ *et al.* 1996). CPEB asso-

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ciates with the cleavage and polyadenylation specificity factor (CPSF), which recognizes another 3'-UTR element, AAUAAA (DICKSON *et al.* 1999; MENDEZ *et al.* 2000; DICKSON *et al.* 2001). GLD-2 is thus recruited to mRNA through interaction with CPEB and CPSF (BARNARD *et al.* 2004). Several mRNAs required for oocyte maturation in *Xenopus*, such as those encoding cyclin B and Mos, are controlled by this machinery (SHEETS *et al.* 1995; STEBINS-BOAZ *et al.* 1996; BARKOFF *et al.* 2000). Another GLD-2-binding partner in *C. elegans*, GLD-3, is necessary for PAP function, being the subunit that allows the GLD-2-GLD-3 complex to bind to RNA (WANG *et al.* 2002). Cytoplasmic polyadenylation factors are conserved among eukaryotes and some of these factors have also been identified to be important in the *Drosophila* germline. For example, Orb, the *Drosophila* homolog of CPEB, is necessary during oogenesis (LANTZ *et al.* 1994; CASTAGNETTI and EPHRUSSI 2003). However, it has not yet been made clear whether GLD-2 and other GLD-2-associated factors also play a role in *Drosophila* development.

Here we show that the *Drosophila wispy (wisp)* gene encodes a predicted cytoplasmic poly(A) polymerase that is a member of the GLD-2 class of proteins. *wisp* maternal-effect mutations were previously reported to cause defects in *bicoid* mRNA localization and in microtubule-based events of female meiosis, leading to very early developmental arrest (BRENT *et al.* 2000). Early embryos of *wisp* mutant females were also known to fail to destabilize maternal transcripts, a phenotype that suggested a defect in egg activation (TADROS *et al.* 2003). To further dissect *wisp*'s possible role in egg activation, we identified the molecular nature of the *wisp* gene and assessed the ability of *wisp* mutants to undertake various events of egg activation. We found that the WISP protein sequence contains a conserved PAP/25A-associated domain, and our yeast two-hybrid experiments showed that WISP can associate with Bicaudal-C (Bic-C), a *Drosophila* GLD-3 homolog. We also found that *wisp* mutant embryos are defective in the addition of poly(A) to several maternal mRNAs. Embryos from *wisp* mutant mothers also fail to initiate mitosis after fertilization. This problem appears to result in part from abnormalities that occur during the completion of female meiosis in the absence of *wisp* function: the acenriolar microtubule-organizing center between meiosis II spindles is reduced in size, pronuclei fail to appose, and the polar body rosette does not form. WISP function is also required in oocytes for the normal, regulated polyadenylation of *dmos* mRNA and, potentially through this action, for phosphorylation (activation) of MAPKs in mature oocytes prior to egg activation.

## MATERIALS AND METHODS

***Drosophila* stocks and complementation tests:** Oregon-R was used as the wild-type stock. *wisp*<sup>1</sup>/FM6, *wisp*<sup>248</sup>/FM6, and *wisp*<sup>249</sup>/FM6 (TADROS *et al.* 2003) were kindly provided by W.

Tadros and H. Lipshitz (Hospital for Sick Children, University of Toronto). *wisp*<sup>12-3147</sup>/FM0, described by BRENT *et al.* (2000), was obtained from the Bloomington *Drosophila* Stock Center (Bloomington, IN). *sra*<sup>687</sup>/FM6 and *Df(3R)sbd*<sup>45</sup>, *muh*<sup>1</sup> *e*<sup>1</sup>/TM6 were previously described by HORNER *et al.* (2006). *P*-element insertion lines for CG32663, CG1886, CG10353, CG15738, CG15737, CG2467, and CG2471—used for complementation tests as well as *Df(1)RA47*/FM7c used for making *wisp*<sup>1</sup> and *wisp*<sup>248</sup> hemizygotes—were from the Bloomington *Drosophila* Stock Center. We crossed males of *P*-insertion lines to *wisp*<sup>1</sup>/+ or *wisp*<sup>248</sup>/+ virgin females. Fertility of the *wisp*/*P*-insertion daughters of this cross was scored to assess complementation.

**Embryo collection, fixation, and staining:** Laid eggs were collected as previously described (HORNER *et al.* 2006). Three- to 4-day-old virgin females were mated either to wild-type Oregon-R males to produce fertilized eggs (embryos) or to spermless males [the sons of *tud*<sup>1</sup> *bw sp* mothers (BOSWELL and MAHOWALD 1985)] to generate unfertilized activated eggs.

Fertilized and unfertilized eggs were collected on grape juice plates for the desired period of time and washed off the plates with egg wash buffer (0.4% NaCl, 0.2% Triton X-100). Hereafter, “0- to 1-hr embryos” will refer to fertilized eggs collected 0- to 1-hr post egg deposition, and other collection lengths will be described in a similar fashion. “Eggs” will refer to eggs laid by females mated to wild-type males, which may be fertilized or unfertilized, and “unfertilized eggs” will refer to eggs laid by females mated to spermless males (described above), unless otherwise indicated. For immunofluorescence analysis, collected eggs were dechorionated in 50% bleach for 2 min, rinsed thoroughly with water, and then permeabilized with heptane and fixed immediately in cold methanol. Fixed eggs were washed in 1× PBST (137 mM NaCl, 2.7 mM KCl, 10.1 mM Na<sub>2</sub>HPO<sub>4</sub>, 1.8 mM KH<sub>2</sub>PO<sub>4</sub>, 0.1% Triton X-100) and incubated at 4° overnight with a 1:200 dilution of a mouse anti- $\alpha$ -tubulin antibody (Sigma, St. Louis) in 1× PBST. RNaseA (Roche Applied Science, Indianapolis) was added to a final concentration of 5  $\mu$ g/ml. Secondary antibody [Alexa Fluor 488-conjugated goat anti-mouse antibody (Invitrogen, Carlsbad, CA) at a dilution of 1:200] was then added for 2 hr at room temperature. Propidium iodide (Invitrogen) was added at a concentration of 10  $\mu$ g/ml to stain DNA. Samples were either mounted in 75% glycerol containing 940 mM *n*-propyl gallate or washed with methanol and mounted in benzyl benzoate: benzyl alcohol (2:1). Staining in fertilized and unfertilized eggs was analyzed using confocal microscopy [Leica TCS SP2 system equipped with an argon-krypton laser and coupled to a Leica DMRBE microscope (Leica Microsystems)]. Leica software was used to collect images and, where appropriate, to project multiple optical sections into a single image and to overlay images.

**RNA extraction and RT-PCR:** Total RNA was extracted from adult flies and 0- to 2-, 2- to 4-, 4- to 6-, or 6- to 24-hr embryos using TRIzol (Invitrogen) and reverse transcribed using SuperScript II reverse transcriptase (Invitrogen). PCR reactions were performed with a High Fidelity kit (Roche Applied Science). Primer sequences were as follows: *wisp* P1 upper, 5'-ACTATCGCAAGTCCGAATCG, and P1 lower, 5'-AGTTGCGCTATGCTCGATGGAC; *wisp* P2 upper, 5'-TACCA GGCGCTAAACACCCAG, and P2 lower, 5'-TTAGCGCAT GCGCTGCAG. The transcript for ribosomal protein RPL32 (FIUMERA *et al.* 2005), amplified using primers 5'-CCGCTT CAAGGGACAGTATC and 5'-GACAATCTCCTTGCGCTTCT, was used as a control for the quality and quantity of the cDNAs used for RT-PCR.

**Western blot analysis:** Samples from ovarian oocytes and 0- to 15-min or 0- to 2-, 2- to 4-, 4- to 6-, 6- to 24-hr embryos were prepared for Western blotting and processed as in SACKTON *et al.* (2007). A polyclonal anti-WISP antibody was generated by

methods described in RAVI RAM *et al.* (2005). Briefly, a glutathione-S-transferase (GST) fusion of the carboxy-terminal 411 amino acids of the predicted WISP protein was purified from *Escherichia coli* cells and used to immunize rabbits (Cocalico Biologicals, Reamstown, PA). Serum was first run through a Sepharose 4B column (Sigma) coupled with GST protein and then affinity purified with the GST-WISP fusion protein. The purified antibody was used at 1:2000 dilution to probe Western blots. Anti-SMG antibody, a kind gift from W. Tadros and H. Lipshitz, was diluted 1:10,000 (TADROS *et al.* 2007). Anti- $\alpha$ -tubulin antibody (Sigma, catalog no. T5168) was diluted 1:10,000. Anti-MAPK antibodies were used as previously described (SACKTON *et al.* 2007).

**Yeast two-hybrid analysis:** We cloned full-length coding sequences from WISP and Bic-C (MAHONE *et al.* 1995) into vectors of the Matchmaker yeast two-hybrid system (CLONTECH, Mountain View, CA). In-frame fusions of each coding region were generated in both the DNA-binding domain vector pGBKT7 and the activation domain vector pGADT7. Yeast cells cotransformed with pGBKT7 and pGADT7 derivatives were grown on  $-Trp -Leu$  synthetic medium and tested for growth on  $-Trp -Leu -His$  and  $-Trp -Leu -His -Ade$  synthetic media.

**Poly(A) test assay:** Oocytes were dissected from 3- to 4-day-old virgin control and virgin *wisp* mutant females. Zero to 1-hr embryos were collected as described above and aged at room temperature for 1 or 2 hr to get 1- to 2- or 2- to 3-hr embryos, respectively. Total RNA was extracted using TRIzol (Invitrogen), and 1  $\mu$ g total RNA from each sample was used for the poly(A) test (PAT) assay. PCR-based PAT assays were performed as described in SALLES and STRICKLAND (1995). Briefly, total RNA from each sample was incubated in the presence of 20 ng 5'-phosphorylated oligonucleotide p(dT)<sub>18</sub> to saturate the poly(A) tails of the mRNAs. The p(dT)<sub>18</sub> was then ligated together in the presence of 10 units (Weiss) of T4 DNA ligase (Fermentas, Glen Burnie, MD) to generate a complementary copy of the poly(A) tail. Two hundred nanograms of oligo(dT)<sub>12</sub> anchor was added and ligated to the 5'-end of the poly(T) strand. The mRNAs were then reverse transcribed (as described above) to synthesize the PAT cDNAs. PCR was performed on the PAT cDNAs using a gene-specific primer and the oligo(dT)<sub>12</sub> anchor to test the length of the poly(A) tail of a specific mRNA. Sequences of primers specific for *bicoid*, *Toll*, and *torso* and the sequence of the oligo(dT)<sub>12</sub> anchor were described by SALLES *et al.* (1994). The sequence of our primer specific for *dmos* is 5'-GCTGAAGCATGAGCTGGAATTC. PCR products from the PAT assays were run on 2% agarose gels or 8% acrylamide gels. Gels were stained with 0.5  $\mu$ g/ml ethidium bromide.

## RESULTS AND DISCUSSION

**The *wisp* gene encodes a member of the GLD-2 poly(A) polymerase family:** To identify the *wisp* gene at the molecular level, we carried out complementation analysis with mutations in 10F1-7, the polytene chromosome region to which *wisp* had been previously mapped (BRENT *et al.* 2000; TADROS *et al.* 2003). We tested for complementation between *wisp* mutant alleles (*wisp*<sup>41</sup> and *wisp*<sup>248</sup>) and the 7 (of 24 total) predicted genes in the 10F1-7 region for which *P*-element insertion lines were available. One line, P{SUP<sub>Or</sub>-P}CG15737<sup>KG05287</sup>, fails to complement both *wisp* alleles. The *P* element in this line is inserted in the coding region of CG15737, 105 bp

downstream of CG15737's start codon according to the latest annotation of the Drosophila genome (<http://www.flybase.org>). Our results suggest that CG15737 corresponds to the *wisp* gene.

According to the latest annotation of the Drosophila genome, CG15737 has one predicted open reading frame (ORF) that encodes a protein of 1373 amino acids. BLAST searches revealed that the predicted CG15737 protein has a consensus PAP/25A-associated domain, which is conserved in poly(A) polymerases. As shown in Figure 1A, the predicted CG15737 protein sequence is homologous to GLD-2 proteins from other animals: CeGLD-2 in *C. elegans* (WANG *et al.* 2002), xGLD-2 in *Xenopus laevis* (BARNARD *et al.* 2004), mGLD-2 in mice, and hGLD-2 in humans (KWAK *et al.* 2004; KWAK and WICKENS 2007). CG15737 shares 51–56% similarity in its PAP/25A-associated domain with the previously characterized GLD-2 proteins.

To confirm the assignment of CG15737 as *wisp*, we sequenced CG15737 in four *wisp* mutant alleles: *wisp*<sup>41</sup>, *wisp*<sup>248</sup>, *wisp*<sup>249</sup>, and *wisp*<sup>12-3147</sup>. As shown in Figure 1B, all four alleles have molecular lesions in the predicted ORF of CG15737. *wisp*<sup>41</sup>, *wisp*<sup>248</sup>, and *wisp*<sup>249</sup> were induced in the same genetic background (TADROS *et al.* 2003), so the different molecular lesions found in these alleles do not represent background mutations. *wisp*<sup>41</sup> and *wisp*<sup>249</sup> are single-base-pair changes that cause nonsense mutations resulting in truncated proteins. *wisp*<sup>248</sup> is a point mutation that changes a conserved isoleucine (Ile) to asparagine (Asp) in the predicted PAP/25A domain. *wisp*<sup>12-3147</sup> has two point mutations. One is a change from threonine (Thr) to isoleucine in a residue of the PAP/25A domain adjacent to the one mutated in *wisp*<sup>248</sup>; the other is a methionine- (Met) to-valine (Val) change outside the conserved domain.

We generated an antibody against WISP (see MATERIALS AND METHODS). Our antibody detects a protein of ~140 kDa in ovarian protein extracts of wild-type and *wisp*<sup>248</sup>/*wisp*<sup>248</sup> females, but not in ovarian protein extracts of *wisp*<sup>41</sup>/*wisp*<sup>41</sup> and *wisp*<sup>249</sup>/*wisp*<sup>249</sup> females, consistent with our finding that the *wisp*<sup>41</sup> and *wisp*<sup>249</sup> mutations result in truncated proteins lacking the C-terminal epitopes recognized by this antibody (Figure 1C).

To determine the expression pattern of *wisp*, we performed RT-PCR for *wisp* mRNA using two sets of primers specific for two different regions of the predicted transcript. Transcripts of *wisp* are detected in adult flies and throughout embryogenesis with the primer set P2, which amplifies a region near the 3'-end of the coding sequence (Figure 2A). The primer set P1, located toward the 5'-end of the gene, amplifies a PCR product of the predicted size in cDNAs made from female adult flies and all stages of deposited eggs or embryos, but does not amplify any PCR product in cDNA made from male flies. These results indicate the presence of *wisp* mRNA in females and in eggs/embryos and suggest that males lack the long form of this RNA predicted by bioinformatics.



TABLE 1

Interaction between WISP and Bic-C in yeast two-hybrid assay

	AD	AD-WISP
BD	—	—
BD-Bic-C	—	+

AD, activation domain. BD, DNA-binding domain. +, growth on -Trp -Leu -His and -Trp -Leu -His -Ade media. —, no growth on -Trp -Leu -His or -Trp -Leu -His -Ade media.

levels of WISP protein are also detected in late-stage embryos.

**The WISP protein can interact with Bic- C:**

Sequence comparisons suggest that WISP’s primary amino acid sequence, like those of other GLD-2 proteins (KWAK *et al.* 2004; KWAK and WICKENS 2007), does not contain any predicted RNA recognition motifs. In *C. elegans*, binding of GLD-2 to mRNA is instead mediated by an RNA-binding protein called GLD-3 (WANG *et al.* 2002). In *Xenopus*, xGLD-2 is found in a cytoplasmic polyadenylation complex in which CPEB and CPSF are also present (BARNARD *et al.* 2004). *Drosophila* has homologs of some of these proteins, and *Drosophila*’s CPEB (named Orb) has been shown to interact with Bic-C, the *Drosophila* homolog of GLD-3 (CASTAGNETTI and EPHRUSSI 2003). *Bic-C*, which is present in oocytes and early embryos like *wisp*, is required for anterior-posterior patterning during oogenesis (MAHONE *et al.* 1995; SAFFMAN *et al.* 1998). In the germline, Bic-C has been recently reported to regulate the expression of specific mRNAs by participating in control of their poly(A) tail length (CHICOINE *et al.* 2007).

Using the yeast two-hybrid assay, we found that WISP can interact with Bic-C (Table 1). WISP in an activation domain (AD) fusion can interact with Bic-C in a binding domain (BD) fusion but not with the BD alone (that is, with an empty vector). We observed interaction only between WISP and Bic-C in one orientation because BD-WISP was not detected on Western blots, suggesting that BD-WISP is either not expressed or not stable in yeast (data not shown). The two-hybrid interaction that we observed between WISP and Bic-C suggests that WISP in *Drosophila* might function, analogous to the GLD-2 proteins in other animals, by forming a protein complex with other RNA-binding proteins.

**bicoid poly(A) tail length does not increase in wisp mutant embryos:** Given that the WISP protein is a GLD-2 homolog, we wanted to determine whether it is involved in modulating the length of the poly(A) tail of maternal mRNAs during egg activation. We focused first on *bicoid* (*bcd*), whose product is essential for anterior structure formation in *Drosophila* embryos (DRIEVER and NUSSLEIN-VOLHARD 1988). Maternal *bcd* mRNA remains untranslated until it receives an extended poly(A) tail during egg activation (SALLES *et al.* 1994).

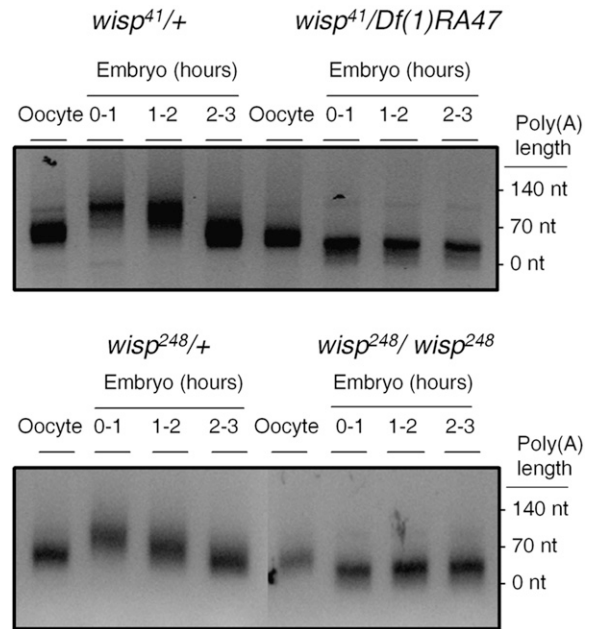


FIGURE 3.—Failure of *bicoid* mRNA polyadenylation in embryos from *wisp* mothers. Total RNA was extracted from ovarian oocytes or different stages of embryos from females of the indicated genotypes. PAT assays were performed to analyze the length of the *bcd* poly(A) tail. Tail lengths were estimated on the basis of molecular size markers. *bcd* poly(A) tail length increased in control embryos after egg activation (left). Polyadenylation of *bcd* mRNA does not occur in *wisp* mutant embryos (right). Rather, there is an ~40-nt decrease in the length of the *bcd* poly(A) tail in embryos from *wisp* females (Figure 3). PAT assays were performed on two independent sets of *wisp*<sup>41</sup> and control samples, and one set of *wisp*<sup>248</sup> and control samples.

We used a PCR-based PAT assay (SALLES and STRICKLAND 1995) to measure the length of the poly(A) tail on *bcd* mRNA in oocytes and laid (that is, activated) embryos. We obtained these oocytes and embryos from mated “*wisp* females” that either are homozygous for a *wisp* allele or carry one *wisp* mutant allele over a deficiency of the *wisp* locus, *Df(1)RA47*. Heterozygous sibling females with one copy of a *wisp* mutant allele and one copy of a balancer chromosome carrying a wild-type *wisp*<sup>+</sup> allele were used as controls. In embryos from *wisp*<sup>41</sup>/<sub>+</sub> and *wisp*<sup>248</sup>/<sub>+</sub> control females, *bcd* poly(A) tail length, which is ~70 nt in oocytes, increases in 0- to 1-hr embryos (after activation), reaching a peak length of ~140 nt (Figure 3). These observations are consistent with previous reports for the magnitude of the change in *bcd* poly(A) tail length during egg activation (SALLES *et al.* 1994). However, in 0- to 1-hr embryos from *wisp*<sup>41</sup>/*Df(1)RA47* and *wisp*<sup>248</sup>/*wisp*<sup>248</sup> females, the poly(A) tail of *bcd* mRNA does not show this increase in length. Rather, there is a small (~40 nt) decrease in the length of the *bcd* poly(A) tail in embryos from *wisp* females (Figure 3). Thus, WISP protein is required for the extension of the *bcd* poly(A) tail upon activation. A short poly(A) tail could affect translation of the BCD protein. Although TADROS *et al.* (2003) reported that BCD is translated in

*wisp* mutants, a shorter poly(A) tail could affect translation efficiency, leading to a reduced level of BCD protein in *wisp* mutants. Unfortunately, we were unable to quantify BCD protein levels because no suitably specific antibody is available. Two other maternal mRNAs, *Toll* and *torso*, that are also polyadenylated upon activation (SALLES *et al.* 1994) similarly fail to acquire extended poly(A) tails in *wisp* mutants (supplemental Figure 1).

Cytoplasmic polyadenylation of maternal mRNAs is a conserved mechanism that regulates translation during both oogenesis and early embryo development. Previous studies of GLD-2 family PAPs revealed that GLD-2 plays an important role in the meiosis/mitosis decision in *C. elegans* (KADYK and KIMBLE 1998) and in oocyte maturation in both *Xenopus* (BARNARD *et al.* 2004) and mice (NAKANISHI *et al.* 2006). However, those studies focused on cytoplasmic polyadenylation events during oogenesis; how cytoplasmic elongation of poly(A) tails is regulated following egg activation remains, in contrast, poorly understood. Mature oocytes contain a remarkable array of maternal mRNAs, representing 20–45% of the genes in the mouse genome and 55% of the genes in *Drosophila* (WANG *et al.* 2004; EVSIKOV *et al.* 2006; TADROS *et al.* 2007). The translation of these maternal mRNAs is a hallmark of egg activation, because two-dimensional gel electrophoresis in mice (XU *et al.* 1994) and sea urchins (ROUX *et al.* 2006) shows a tremendous spike in new protein synthesis within the egg immediately after fertilization. Initiation of the translation of some mRNAs correlates with an increase in the length of their poly(A) tails (SALLES *et al.* 1994). We find that *wisp*, which encodes a *Drosophila* homolog of GLD-2 proteins, is essential for regulating polyadenylation of *bicoid*, *Toll*, and *torso* transcripts upon activation. There might be other maternal mRNAs that are regulated by their poly(A) tail lengths in a *wisp*-dependent manner during *Drosophila* egg activation, and it will be interesting to identify these mRNAs in the future.

It will also be interesting to know how the *wisp*-dependent cytoplasmic polyadenylation machinery is turned on during egg activation. Calcium-signaling pathways are major transducers of the egg activation trigger in many systems (reviewed in DUCIBELLA *et al.* 2006; PARRINGTON *et al.* 2007; HORNER and WOLFNER 2008b), so perhaps cytoplasmic polyadenylation is regulated by a calcium signal. A recent study shows calcium involvement in egg activation in *Drosophila* (HORNER and WOLFNER 2008a). In addition, studies (HORNER *et al.* 2006; TAKEO *et al.* 2006) of the *sarah* (*sra*) gene, which encodes the *Drosophila* calcipressin, indicate that proper regulation of the calcium-dependent phosphatase calcineurin by *Sra* is required for egg activation. HORNER *et al.* (2006) showed that *bcd* mRNA poly(A) tails do not extend in early embryos from *sra* mutant mothers. This latter result suggests that the calcium-signaling pathway might be upstream of polyadenylation

during *Drosophila* egg activation. Consistent with the idea that cytoplasmic polyadenylation could be calcium regulated, a study of hippocampal neurons in mouse showed that CaMKII can phosphorylate a regulatory site on CPEB to activate cytoplasmic polyadenylation (ATKINS *et al.* 2004). In a model where calcium is upstream of cytoplasmic polyadenylation upon egg activation, calcium could act upstream of WISP or upstream of other possible targets such as those GLD-2-associated factors in other organisms whose homologs are also found in *Drosophila*, such as Orb and Bic-C. Since cytoplasmic polyadenylation can be affected by the rate of deadenylation, and deadenylation is also turned on upon activation (TADROS and LIPSHITZ 2005), the calcium-signaling pathway could also potentially act on targets in the deadenylation pathway, such as Smaug (SEMOTOK *et al.* 2005), during activation.

**WISP is not required for SMG translation:** In *Drosophila*, egg activation triggers destabilization of >1600 maternal mRNAs (TADROS *et al.* 2007). This destabilization has been hypothesized to eliminate maternal transcripts so that zygotic transcription can control later embryogenesis. Smaug (SMG) is the major regulator of this mRNA-destabilizing activity. SMG protein is not present in mature oocytes but is present in embryos (SMIBERT *et al.* 1996; DAHANUKAR *et al.* 1999). *smg* mRNA has been reported to be polyadenylated upon egg activation, correlating with, but not absolutely required for, its translation (TADROS *et al.* 2007). Since WISP is also required for maternal transcript destabilization during egg activation (TADROS *et al.* 2003), we tested whether SMG protein is translated in embryos in the absence of *wisp* function. On Western blots, we find that SMG protein is detected in 0- to 2-hr embryos from *wisp*<sup>41</sup> mutant mothers at levels comparable to those in control embryos (Figure 4A). This result indicates that the translation of SMG does not require *wisp* function. In the future it will be interesting to test if *wisp* affects the *smg* transcript's poly(A) tail length.

SMG triggers transcript degradation by recruiting the CCR4/POP2/NOT deadenylase complex to specific maternal transcripts to destabilize these mRNAs (SEMOTOK *et al.* 2005; TADROS *et al.* 2007). Our finding that SMG is translated in embryos from *wisp* mutant mothers in conjunction with our previous observation of a small (~40 nt) decrease in the length of the *bcd* poly(A) tail in *wisp* mutant embryos (Figure 3) suggests that the shortening of the *bcd* poly(A) tail in *wisp*-deficient embryos might be due to a SMG-dependent increase in deadenylase activity upon activation. Upon egg activation, SMG-dependent deadenylation of the *bcd* transcript would normally be counteracted by polyadenylation, but this deadenylation could become detectable when polyadenylation is disrupted, as in the *wisp* mutant. Given that polyadenylation of the *bcd* transcript is affected in embryos from *sra* mutants (HORNER *et al.* 2006), we also considered the role of SMG-dependent

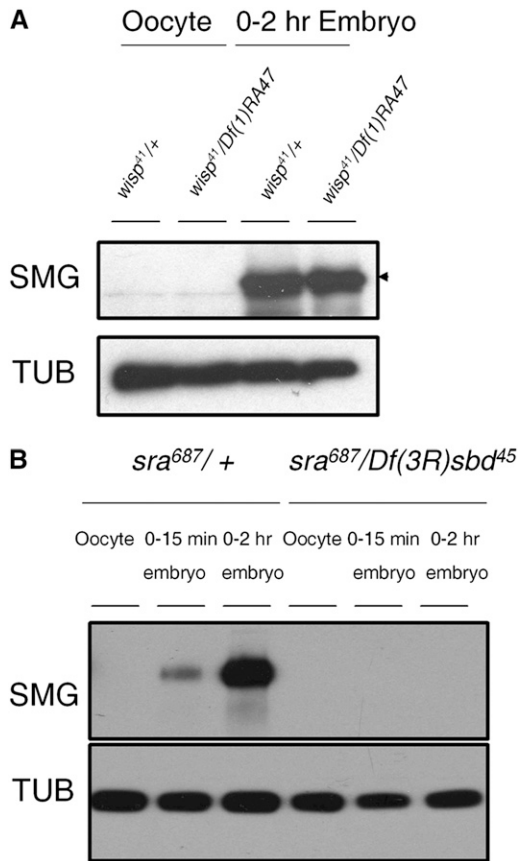


FIGURE 4.—SMG protein is translated in *wispy* mutant embryos, but not in *sra* embryos. (A) Total protein extracts of ovarian oocytes (left) and laid 0- to 2-hr embryos (right) from *wisp<sup>+/Df(1)RA47</sup>* or *wisp<sup>+/+</sup>* females were separated by SDS-PAGE, blotted, and probed for Smaug (SMG) and  $\alpha$ -tubulin (TUB; loading control). SMG protein is translated normally in laid embryos from *wispy* mutant females; there is no statistically significant difference between control and mutant embryos in the amount of SMG signal intensity, normalized to TUB signal intensity, in five independent replicates (matched pairs *t*-test: *t*-ratio = -0.205, 4 d.f., *P* = 0.848). (B) Total protein extracts of oocytes, 0- to 15-min embryos and 0- to 2-hr embryos from *sra<sup>687/+</sup>* or *sra<sup>687/Df(3R)sbd<sup>45</sup></sup>* females were isolated, blotted, and probed for SMG and  $\alpha$ -tubulin (TUB; loading control). SMG protein is not detected in embryos from *sra* mutant females.

deadenylation in *sra* mutants. HORNER *et al.* (2006) showed that *bcd* mRNA's poly(A) tail is not extended in embryos laid by *sra* mutant mothers, but in contrast to our observation for *wispy* mutants, these investigators saw no decrease in the length of the *bcd* mRNA poly(A) tail in the absence of *sra*. If the deadenylation of *bcd* mRNA in *wispy* mutants is caused by the activation of SMG protein, then the lack of *bcd* transcript poly(A) decrease in *sra* embryos might suggest that *sra* eggs and embryos do not activate SMG translation. Consistent with this prediction, we found that embryos from *sra* mutant females lack detectable SMG (Figure 4B).

Both WISP and SMG are required for maternal transcript destabilization during egg activation (TADROS *et al.*

2003). The function of WISP in regulating maternal transcript destabilization cannot be mediated through regulation of SMG translation, because SMG is still translated in *wispy*-deficient early embryos. Translation of SMG protein upon egg activation is instead known to be activated by the PAN GU (PNG) kinase complex (TADROS *et al.* 2007), and PNG also plays a role in promoting the polyadenylation of *smg* mRNA (TADROS *et al.* 2007) and *cyclin B* mRNA (VARDY and ORR-WEAVER 2007). Future studies are needed to determine whether WISP functions in mRNA destabilization downstream of PNG or instead functions in parallel to the PNG-SMG pathway.

**Embryos from *wispy* mutant females arrest subsequent to the completion of female meiosis but prior to the apposition of female and male pronuclei:** Because *wispy* mutants fail to carry out two aspects of egg activation [polyadenylation of maternal mRNAs (this study) and maternal mRNA destabilization (TADROS *et al.* 2003)], we asked whether other aspects of egg activation are affected in *wispy* mutants. Modifications to the egg coverings are an essential part of egg activation in many species. The vitelline membrane, one of *Drosophila*'s egg coverings, is permeable to small molecules before activation, but after activation vitelline membrane proteins become crosslinked to render the activated egg impermeable to small molecules (LEMOSEY and HASHIMOTO 2000; HEIFETZ *et al.* 2001). Activated eggs are consequently impermeable to bleach and remain resistant to lysis after a 2-min incubation in 50% bleach, while ~100% of eggs that have not yet been activated are lysed by this treatment (MAHOWALD *et al.* 1983). Zero- to 1-hr eggs laid by either *wisp<sup>+/+</sup>* (control) or *wisp<sup>+/wisp</sup>* females were collected and assayed for bleach resistance. In one representative experiment, 90.5% of *wisp<sup>+/+</sup>* (*N* = 412) eggs and 92.2% of *wisp<sup>248/+</sup>* (*N* = 516) eggs were resistant to 50% bleach after a 2-min incubation, while 60.7% of the eggs (*N* = 384) laid by *wisp<sup>+/wisp<sup>41</sup></sup>* (a presumptive null mutation) females and 68.1% of the eggs (*N* = 426) laid by *wisp<sup>248/wisp<sup>248</sup></sup>* females were resistant to bleach, suggesting that vitelline membrane crosslinking occurs in *wispy* mutants, but might be incomplete or delayed relative to wild type (*P* < 0.0001). While the exact percentage values vary among other repetitions of this experiment, the difference between controls and *wispy* mutants remains in the same direction and always statistically significant.

Another aspect of egg activation is the resumption and completion of female meiosis. BRENT *et al.* (2000) reported that embryos laid by *wisp<sup>12-3147</sup>*, *wisp<sup>11-600</sup>*, and *wisp<sup>14-1299</sup>* females arrest after an abnormal meiosis. Since *wisp<sup>12-3147</sup>* is a missense (or double missense) mutation and the molecular lesions in the other two alleles tested by BRENT *et al.* (2000) are unknown, we were uncertain if they reflect the null phenotype. We therefore chose to analyze two other alleles, *wisp<sup>41</sup>* and *wisp<sup>248</sup>*, because the *wisp<sup>41</sup>* allele has a nonsense mutation that

TABLE 2

## Distribution of developmental stages of 0- to 2-hr-old embryos

Genotypes	Meiosis	Mitosis	Arrested
<i>wisp<sup>41</sup>/FM6</i>	2/39	37/39	0/39
<i>wisp<sup>41</sup>/wisp<sup>41</sup></i>	0/20	0/20	20/20
<i>wisp<sup>41</sup>/Df(1)RA47</i>	1/24	0/24	23/24
<i>wisp<sup>248</sup>/FM6</i>	3/33	30/33	0/33
<i>wisp<sup>248</sup>/wisp<sup>248</sup></i>	0/31	0/31	31/31
<i>wisp<sup>248</sup>/Df(1)RA47</i>	0/27	0/27	27/27

Embryos collected from females of the indicated genotype were fixed and stained with propidium iodide and anti- $\alpha$ -tubulin antibody. Stages were determined on the basis of spindle and chromosome configurations.

removes the majority of the protein, including the entire conserved PAP domain, and thus is likely to be the strongest allele available. We further analyzed the progression of cell cycle phenotype due to *wisp<sup>41</sup>* and *wisp<sup>248</sup>* and determined that both meet the genetic definition of null alleles (Table 2). Both *wisp<sup>41</sup>* and *wisp<sup>248</sup>* females are viable and capable of laying eggs, but these eggs never hatch. We examined the phenotypes of these arrested eggs by using immunofluorescence microscopy. Control and *wisp* females were mated to wild-type males, and the resultant eggs were collected. They were then fixed and stained with an antibody against  $\alpha$ -tubulin to visualize the spindle and with propidium iodide to reveal the chromosomes. Eggs from hemizygous [*wisp<sup>41</sup>/Df(1)RA47* or *wisp<sup>248</sup>/Df(1)RA47*] females show the same phenotype as those from homozygous (*wisp<sup>41</sup>/wisp<sup>41</sup>* or *wisp<sup>248</sup>/wisp<sup>248</sup>*) females. Since the *wisp<sup>41</sup>* allele has a nonsense mutation that removes the majority of the protein, including the entire conserved PAP domain, and meets the genetic definition of a null, we focused further phenotypic analysis on this allele.

In wild-type embryos, female meiosis completes upon egg activation and four haploid meiotic products form (FOE *et al.* 1993). If the egg is fertilized, the sperm nucleus remodels (in part by exchanging protamines for histones) and becomes the male pronucleus. One of the four female meiotic products migrates toward, and apposes with, the male pronucleus, and then the two apposed pronuclei together enter the first mitotic division. The other three female meiotic products stay at the cortex of the embryo and form a polar body rosette. We examined whether or not *wisp* eggs can be fertilized, and whether meiosis and pronuclear behaviors are normal. Asters can be observed using  $\alpha$ -tubulin antibody and the sperm tail can be observed using an antibody against the sperm tail in embryos from *wisp<sup>41</sup>* mutant females (supplemental Figure 2) as was also stated for *wisp<sup>12-3147</sup>* mutants (BRENT *et al.* 2000). Thus, the lack of development of *wisp* embryos is not due to lack of fertilization.

As expected, the majority of 0- to 1-hr fertilized eggs (embryos) laid by *wisp<sup>41</sup>/+* heterozygous females un-

dergo embryonic mitotic divisions (Figure 5A). In contrast, no embryos from *wisp* homozygous mutant females enter a normal zygotic mitosis. Instead, they are arrested at an aberrant metaphase-like stage with very few (usually five or fewer) spindles (Figure 5B). The chromosomes associated with the spindle structures are condensed and appear to have entered a division cycle, since their chromatin can be stained by an antibody against the mitosis marker phospho-H3 (data not shown). The spindles in these arrested embryos are clearly different from mitotic spindles, since in general they are not associated with microtubule-organizing centers (MTOC). However, one spindle deep inside the embryo, with two aster structures, can be observed in some of these fertilized eggs (Figure 5B inset). Centrosomes in *Drosophila* embryos are paternally derived (FOE *et al.* 1993), suggesting that the centrosome-containing spindle in *wisp* embryos is associated with the male pronucleus while the other spindles are associated with the four female meiotic products. The arrest phenotype that we see for *wisp<sup>41</sup>* is like that described for *wisp<sup>12-3147</sup>* (BRENT *et al.* 2000), suggesting that the point mutations that we mapped in the latter allele completely disrupt *wisp* function.

Given that *wisp* embryos arrest before embryonic mitosis, we examined whether or not WISP function is essential for fertilized eggs (embryos) to complete female meiosis. We stained 5- to 15-min embryos from either *wisp<sup>41</sup>/+* heterozygous control females or *wisp<sup>41</sup>/Df(1)RA47* mutant females and checked the progression of female meiosis after these females were mated to wild-type males. All stages of female meiosis are observed in *wisp* mutants (Table 3), although one aspect of meiosis appears abnormal. In controls, the two meiosis II spindles are tandemly linked, and bright  $\alpha$ -tubulin staining can be seen in an acentriolar MTOC between the two spindles in metaphase II (Figure 5C) and telophase II (Figure 5E). However in *wisp* mutants, the staining of the acentriolar MTOC between the two meiosis II spindles is greatly diminished (Figure 5 D and F).

Although meiosis can complete in *wisp* mutants, we see abnormalities in pronuclear behavior thereafter. In embryos from *wisp* mutant females, the paternal chromosomes decondense to form a male pronucleus (insets of Figure 5, D, F, and H), as in control embryos (insets of Figure 5, C, E, and G). The aster duplicates in the embryos from *wisp* mutant females (inset Figure 5F). However, none of the female meiotic products migrates toward the male pronucleus, and there is thus no pronuclear apposition (Figure 5H). Several considerations suggest that the effects of *wisp* mutants on pronuclear migration might reflect *wisp*-mediated control of the synthesis or levels of a microtubule-associated protein or a regulator of this association. In embryos from *wisp* mutant females, the two centrosome-associated proteins  $\gamma$ -tubulin and CP190 are reduced in the



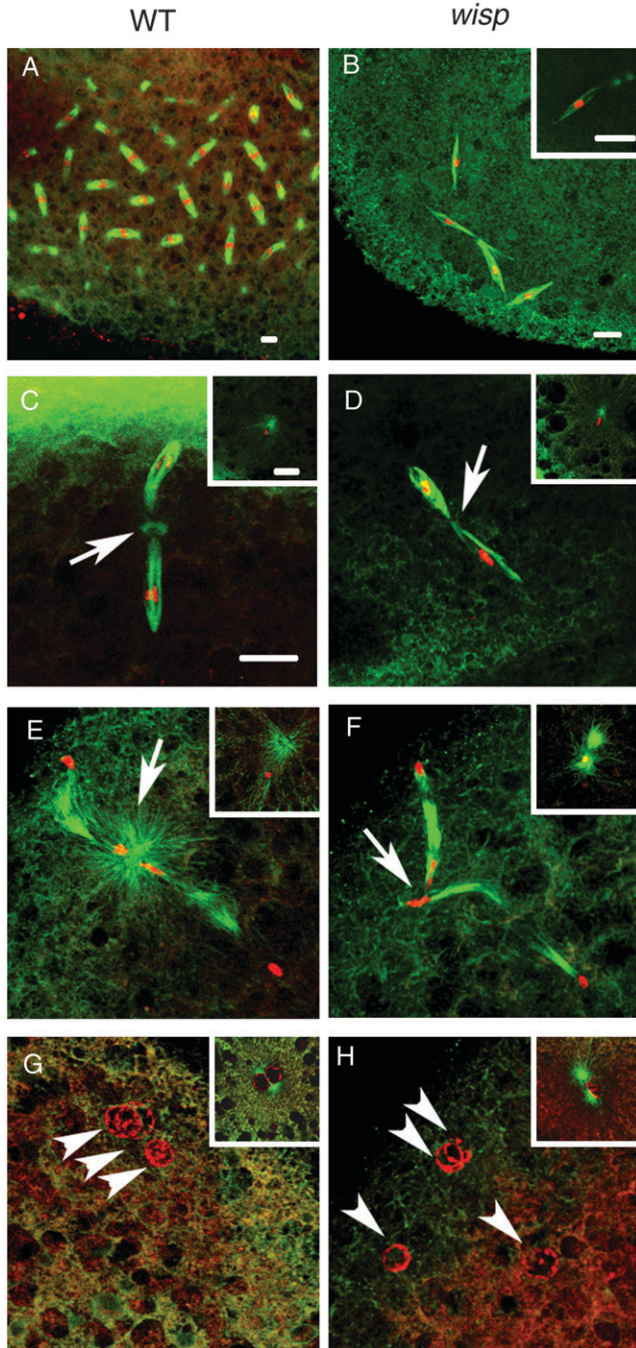


FIGURE 5.—Fertilized eggs from *wisp* mutant females arrest subsequent to the completion of female meiosis. Embryos derived from *wisp<sup>41</sup>/Df(1)RA47* or *wisp<sup>41</sup>/+* control females were fixed and stained with propidium iodide to visualize DNA (red) and with anti- $\alpha$ -tubulin antibody to visualize microtubules (green). Insets show the male nucleus in each embryo. A control 0- to 1-hr-embryo undergoes mitotic divisions (A) while a 0- to 1-hr embryo from a *wisp<sup>41</sup>* mutant female is arrested with multiple spindle structures (B). Five- to 15-min embryos were used to observe female meiosis (C–H). Compared to control embryos' metaphase II (C) and telophase II (E), metaphase II (D) and telophase II (F) in *wisp<sup>41</sup>* mutant embryos have reduced green staining of the acentriolar MTOC between the two linked meiotic spindles (arrows). Female meiosis in control embryos results in four products, three near the surface of the embryo (G, arrowheads) and

centrosomes compared to wild type, while CP60 is not detected (BRENT *et al.* 2000). Failure of pronuclear migration as observed in *wisp*-deficient embryos also occurs in embryos from *asp* or *KLP3A* mutant females, which lack certain microtubule-associated proteins (WILLIAMS *et al.* 1997; RIPARBELLI *et al.* 2002). Moreover, defective acentriolar MTOC of meiosis II, reduced sperm aster growth, and failure of pronuclear migration, all seen in *wisp*-deficient embryos, have also been reported in mutant embryos lacking POLO, which regulates the organization of microtubule-associated structures (RIPARBELLI *et al.* 2000).

Wild-type *Drosophila* eggs can activate without fertilization. Such unfertilized eggs can complete female meiosis but do not develop further (DOANE 1960; FOE *et al.* 1993). The four haploid nuclei derived from female meiosis retreat to the egg surface and fuse to form a polar body rosette structure; the chromosomes in the rosette are condensed in a mitotic-like arrest (PAGE and ORR-WEAVER 1997). We examined the four maternally derived nuclei in unfertilized eggs from *wisp<sup>41</sup>/Df(1)RA47* females. In control eggs, a polar body rosette structure forms normally (Figure 6, A and C). However, the four maternally derived nuclei in unfertilized *wisp*-deficient eggs become associated with spindles that resemble meiotic spindles (Figure 6B). In some cases, the four haploid nuclei fuse to associate with an irregular-sized spindle, but still no rosette-like structure can be observed (Figure 6D). These findings suggest that the arrest of these *wisp* mutant eggs is different from the arrest of the polar body nuclei in wild-type unfertilized eggs.

**Levels of some active (phospho-) MAPKs are decreased in *wisp* mutant oocytes:** Because we observed WISP protein both in ovaries and in embryos after egg activation, and because *wisp* mutant oocytes have a defect during the meiosis I arrest prior to egg activation (BRENT *et al.* 2000), we considered the possibility that WISP might have functions in mature oocytes immediately before egg activation. In *Drosophila* oocytes, levels of active (that is, phosphorylated) MAPKs are high, but upon egg activation (independent of fertilization), the amounts of the active forms of the MAPKs decrease although the total amounts of the MAPK proteins do not change (SACKTON *et al.* 2007). Three classes of MAPK [extracellular signal-related kinase (ERK), Jun N-terminal kinase (JNK), and p38] are present in *Drosophila*, as in other organisms. The change in MAPK activity between mature oocytes and activated eggs suggests that MAPK-signaling cascades have a necessary

one opposing the male nucleus (G inset). All four female meiotic products are found near the surface of the *wisp<sup>41</sup>* mutant embryo (H, arrowheads); none apposed to the male pronucleus (H inset). Bar, 10  $\mu$ m. D–H are at the same magnification as C.

**TABLE 3**  
**Meiotic progression in 5- to 15-min-old embryos**

Genotype	Embryos scored	Metaphase I (%)	Anaphase I (%)	Metaphase II (%)	Anaphase II (%)	Telophase II (%)	Pronuclei (%)	Mitosis (%)	Arrested (%)
<i>wisp<sup>41</sup>/+</i>	128	0 (0)	5 (3.9)	16 (12.5)	9 (7.0)	26 (20.3)	23 (10.2)	49 (38.3)	0 (0)
<i>wisp<sup>41</sup>/Df(1)RA47</i>	103	1 (1.0)	5 (4.9)	11 (12.1)	4 (3.9)	16 (15.5)	16 (15.5)	0 (0)	50 (48.5)

Embryos were collected 10 min after deposition by females of the indicated genotype, fixed for 5 min, and stained with propidium iodide and anti- $\alpha$ -tubulin antibody to score meiotic and mitotic figures. Stages were determined on the basis of spindle and chromosome configurations.

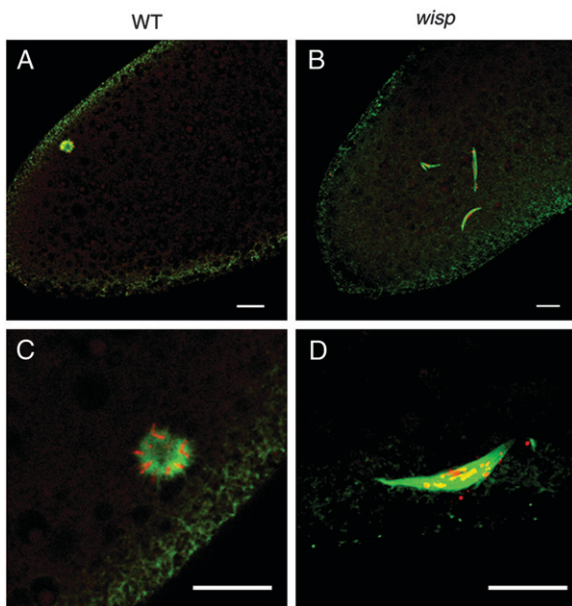
function in oocytes and lack of MAPK signaling could be important upon egg activation.

To determine if *wisp* affects MAPK activity at or before egg activation, we examined the amount of phospho-MAPKs in *wisp* mutant oocytes and embryos. In *wisp<sup>41</sup>/+* controls (Figure 7A, left), the level of phospho-ERK is high in mature (stage 14) oocytes and decreases upon egg activation as seen previously in wild type (SACKTON *et al.* 2007). In contrast, the level of phospho-ERK in *wisp* mutant mature oocytes is much lower than in control mature oocytes (Figure 7A, right). Similarly, stage 14 *wisp* oocytes also contain an unusually low level of phospho-JNK relative to controls (Figure 7B), although their levels of phospho-p38 are normal (data not

shown). Upon egg activation, the levels of phospho-ERK and phospho-JNK in *wisp* embryos are very low or undetectable, as in wild-type embryos (Figures 7, A and B). These results suggest that *wisp* plays a role in regulating MAP kinase activity during oogenesis.

Because total ERK protein levels are normal in *wisp* mature oocytes, WISP regulates the activation of this kinase, not its translation. WISP could regulate translation of an upstream activating kinase or kinases in oocytes, so that these kinase(s) in turn can regulate the phosphorylation and activity of ERK and JNK. Studies in *Xenopus* have shown that CPEB-mediated polyadenylation regulates the translation of *mos* mRNA during oocyte maturation (SHEETS *et al.* 1995; STEBBINS-BOAZ *et al.* 1996) and that MOS phosphorylates MAPK/ERK kinase (MEK), a kinase that then phosphorylates MAPKs in mature oocytes (MALLER *et al.* 2002). Decreased levels of phospho-ERK and phospho-MEK were observed in oocytes from *dmos* germline clones in *Drosophila* (IVANOVSKA *et al.* 2004). MOS has also been shown to activate JNK in *Xenopus* eggs and embryos (BAGOWSKI *et al.* 2001; MOOD *et al.* 2004).

The decreased levels of phospho-ERK and phospho-JNK in *wisp*-deficient *Drosophila* mature oocytes could be attributable to upstream regulation by DMOS. We explored the possibility that *dmos* transcripts' poly(A) tails could be extended during oocyte maturation, as is the case for *Xenopus mos*, and for *Drosophila cortex* and *cyclin B* transcripts (BENOIT *et al.* 2005; PESIN and ORR-WEAVER 2007), and that *wisp* could mediate this, by analogy to the requirement for xGLD-2 for CPEB-mediated polyadenylation during *Xenopus* oogenesis (BARNARD *et al.* 2004). We used PAT assays to examine *dmos* mRNA's poly(A) tail length in immature (stages 1–12) and mature (stage 14) oocytes from control females or *wisp* mutant females. In the controls (Figure 7C, two lanes on left), *dmos* transcripts have shorter poly(A) tails in stage 1–12 (immature) oocytes; their poly(A) tails are longer by ~40 nt in stage 14 (mature) oocytes. Thus, *dmos* mRNA poly(A) tails lengthen during oocyte maturation in *Drosophila*, as in *Xenopus*. In contrast to the situation in controls, the length of the poly(A) tails of *dmos* mRNA in mature oocytes of *wisp<sup>41</sup>* mutant females is not significantly different from that in immature oocytes of these females (Figure 7C, two



**FIGURE 6.**—Female meiotic products in unfertilized *wisp* eggs. Laid unfertilized eggs from *wisp<sup>41</sup>/+* control (A and C) or *wisp<sup>41</sup>/Df(1)RA47* (B and D) mothers were fixed and stained with propidium iodide to visualize DNA (red) and with anti- $\alpha$ -tubulin antibody to visualize microtubules (green). Ninety-three percent ( $n = 56$ ) of control eggs formed the polar body rosette. In 100% ( $n = 57$ ) of the eggs from mutant mothers, the female meiotic products associate with anastral spindles (B). In ~45% of these eggs, apposition of multiple female meiotic products can be observed, but no polar rosette is formed (D). Bar, 10  $\mu$ m.

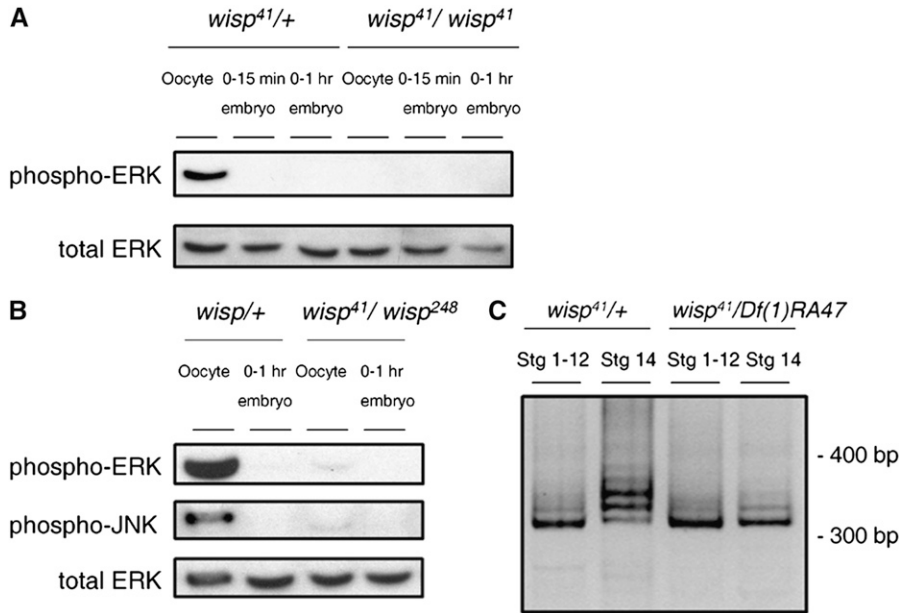


FIGURE 7.—Levels of phospho-ERK and phospho-JNK and lengths of *dmso* poly(A) tails are decreased in *wisp* mutant oocytes. (A) Western blot analysis was performed on total protein extracts of mature (stage 14) ovarian oocytes, 0- to 15-min or 0- to 1-hr embryos from *wisp<sup>41/wisp<sup>41</sup></sup>* or *wisp<sup>41/+</sup>* females. Anti-ERK was used to detect total ERK protein while anti-phospho-ERK was used to detect the active form of ERK. (B) Anti-phospho-JNK was used to detect the active form of JNK in total protein extracts of mature oocytes or 0- to 1-hr embryos from *wisp<sup>41/wisp<sup>248</sup></sup>* or heterozygous control females. Anti-ERK was used here as a loading control. (C) Stages 1–12 and stage 14 oocytes were hand dissected from *wisp<sup>41/+</sup>* control and *wisp<sup>41/Df(1)RA47</sup>* mutant females. PAT assays were performed to examine the poly(A) tail length of *dmso* mRNA. PAT assay products were separated by DNA PAGE. Molecular size markers

are shown on the right. In the controls (two lanes on left), the length of the *dmso* mRNA poly(A) tail is ~40 nt longer in stage 14 oocytes than in stages 1–12 (two lanes on left), indicating extension of the *dmso* mRNA poly(A) tails during oocyte maturation. In contrast, this increase is not seen in *wisp* mutant oocytes (two lanes on right).

lanes on right). Therefore, *wisp* function is needed for poly(A) tail extension on *dmso* mRNA during oocyte maturation.

The shorter poly(A) tail in mature *wisp* oocytes could lead to a failure or inefficiency of *dmso* translation that would in turn explain the decreased levels of phospho-ERK and phospho-JNK. We unfortunately are unable to examine DMOS protein levels in *Drosophila* because there is no suitably specific antibody available. However, we find that our preliminary data indicate that phospho-MEK levels are low in *wisp* mutant mature oocytes (data not shown), supporting the hypothesis that MAPK phosphorylation is regulated through the upstream kinases MEK and DMOS during *Drosophila* oocyte maturation and that *wisp* controls this by controlling the polyadenylation of *dmso* mRNA (at least) during egg maturation.

Although levels of phospho-ERK and phospho-JNK are low or undetectable in *wisp* mutant mature oocytes, meiosis can still complete upon activation of those eggs. This result is consistent with a previous report that oocytes from *dmso* mutant germline clones have decreased levels of phospho-ERK yet they can complete meiosis (IVANOVSKA *et al.* 2004). Although MAPK activity downstream of DMOS is not necessary for completion of meiosis in *Drosophila*, the regulation of MAPKs or other genes by WISP may be important for other aspects of late oocyte development or for making the oocyte competent to activate.

**Conclusions:** We have shown that the *wisp* gene encodes a *Drosophila* member of the GLD-2 cytoplasmic PAP family and that the product of this gene is necessary for several aspects of egg activation and

oogenesis. WISP is required for the polyadenylation of *bicoid*, *Toll*, and *torso* mRNAs upon egg activation. However, translation of SMG, which has been previously reported to correlate with but not depend on polyadenylation of *smg* mRNA upon activation (TADROS *et al.* 2007), is WISP independent. *wisp* maternal-effect mutations lead to very early developmental arrest prior to embryonic mitosis. Eggs lacking maternal WISP function are capable of completing female meiosis to form decondensed female meiotic products. Although the sperm nucleus appears capable of remodeling and forming a male pronucleus in the absence of WISP function, the sperm asters fail to grow and pronuclear migration does not occur. All the nuclei condense their chromosomes and become associated with a spindle structure, but then arrest at this point. These data are consistent with *wisp*'s being essential for some aspect of the tubulin cytoskeleton in oocytes and embryos. WISP also has functions during oogenesis because levels of active (phospho-) MAPKs are severely reduced in *wisp* oocytes and *dmso* mRNA poly(A) tails fail to extend in *wisp* oocytes during oocyte maturation.

Given its molecular identity, it is likely that WISP affects these aspects of egg activation and oogenesis by a single mechanism: determination of the poly(A) tail length of certain maternal mRNAs. It is likely that there are more mRNA targets of WISP in addition to the ones identified in this study. Future identification of these mRNA targets whose poly(A) tail increase during oogenesis or upon egg activation is *wisp* dependent will help to elucidate whether and how products of these genes play roles in oogenesis and egg activation.

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