Development 135, 1969-1979 (2008) doi:10.1242/dev.021444

PAP- and GLD-2-type poly(A) polymerases are required sequentially in cytoplasmic polyadenylation and oogenesis in *Drosophila*

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Cytoplasmic polyadenylation has an essential role in activating maternal mRNA translation during early development. In vertebrates, the reaction requires CPEB, an RNA-binding protein and the poly(A) polymerase GLD-2. GLD-2-type poly(A) polymerases form a family clearly distinguishable from canonical poly(A) polymerases (PAPs). In *Drosophila*, canonical PAP is involved in cytoplasmic polyadenylation with Orb, the *Drosophila* CPEB, during mid-oogenesis. We show that the female germline GLD-2 is encoded by *wispy*. Wispy acts as a poly(A) polymerase in a tethering assay and in vivo for cytoplasmic polyadenylation of specific mRNA targets during late oogenesis and early embryogenesis. *wispy* function is required at the final stage of oogenesis for metaphase of meiosis I arrest and for progression beyond this stage. By contrast, canonical PAP acts with Orb for the earliest steps of oogenesis. Both Wispy and PAP interact with Orb genetically and physically in an ovarian complex. We conclude that two distinct poly(A) polymerases have a role in cytoplasmic polyadenylation in the female germline, each of them being specifically required for different steps of oogenesis.

KEY WORDS: Cytoplasmic polyadenylation, *Drosophila*, GLD-2, Meiosis, Metaphase I, Translational control

INTRODUCTION

In many species, the oocyte and early embryo develop in the absence of transcription. Therefore, the first steps of development depend on maternal mRNAs and on their regulation at the level of translation, stability and localization. Regulation of mRNA poly(A) tail length is a common mechanism of translational control. Deadenylation or poly(A) tail shortening results in mRNA decay or translational repression. Conversely, poly(A) tail elongation by cytoplasmic polyadenylation results in translational activation (Richter, 2000; Wickens et al., 2000). How the poly(A) tail length of a particular mRNA and, consequently, its level of translation are determined has been a matter of investigation for many years. It is becoming clear that poly(A) tail length results from a balance between concomitant deadenylation and polyadenylation (Kim and Richter, 2006).

The molecular mechanisms of cytoplasmic polyadenylation have been investigated in *Xenopus* oocytes. The specific RNA-binding protein in the reaction is CPEB (Cytoplasmic polyadenylation element binding protein), which binds the CPE in the 3′-UTR of regulated mRNAs. Two other factors, CPSF (Cleavage and polyadenylation specificity factor) and Symplekin, are required in addition to a poly(A) polymerase (Barnard et al., 2004; Richter, 2007). Before meiotic maturation, the polyadenylation complex also contains PARN, a deadenylase whose activity counteracts poly(A) tail elongation (Kim and Richter, 2006). At meiotic maturation, CPEB phosphorylation results in the release of PARN from the complex, thus leading to polyadenylation and translational activation.

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Accepted 8 April 2008

CPSF and Symplekin are also required for nuclear polyadenylation, a cotranscriptional reaction that leads to the synthesis of a poly(A) tail at the 3′ end of all mRNAs (Edmonds, 2002). A canonical poly(A) polymerase (PAP) is responsible for poly(A) tail synthesis during nuclear polyadenylation. Particular isoforms of PAP were first thought to be required for cytoplasmic polyadenylation (Ballantyne et al., 1995). Moreover, TPAP (Papolb – Mouse Genome Informatics), a testis-specific PAP in mouse, is cytoplasmic in spermatogenic cells and has been shown, using a *Tpap* knockout, to be required for cytoplasmic polyadenylation of specific mRNAs and for spermiogenesis (Kashiwabara et al., 2002; Zhuang et al., 2004). More recently, a new family of atypical poly(A) polymerases, the GLD-2 family, has been characterized, with a first member identified in *C. elegans* (Wang et al., 2002). GLD-2-type proteins exist in all eukaryotes, where they have different functions (Buhler et al., 2007; Kwak and Wickens, 2007; Rissland et al., 2007).

In *C. elegans*, GLD-2 is required for entry into meiosis from the mitotic cycle in the gonad, and for meiosis I progression (Kadyk and Kimble, 1998). *C. elegans* GLD-2 has a poly(A) polymerase activity in vitro (Wang et al., 2002) and in vivo (Suh et al., 2006). In *Xenopus* oocytes, GLD-2 is found in the cytoplasmic polyadenylation complex, within which it directly interacts with CPEB and CPSF, and it has a poly(A) polymerase activity in vitro in the presence of the other factors of the complex (Barnard et al., 2004). GLD-2 is in complexes with mRNAs, such as *cycB1* and *mos*, that are regulated by cytoplasmic polyadenylation (Rouhana et al., 2005). It is thus very likely that GLD-2 plays a role in cytoplasmic polyadenylation during *Xenopus* meiotic maturation. However, although cytoplasmic polyadenylation of *mos* and *cycB1* mRNAs is required for meiotic maturation (Sheets et al., 1995; Stebbins-Boaz et al., 1996), the functional role of *Xenopus* GLD-2 in meiotic maturation has not been addressed. Unexpectedly, although mouse GLD-2 (Papd4 – Mouse Genome Informatics) is found in oocytes at metaphases I and II, a recent study shows that oocyte maturation in GLD-2 knockout mice is not altered,

demonstrating that if mouse GLD-2 acts as a poly(A) polymerase at this stage, another protein acts redundantly (Nakanishi et al., 2006; Nakanishi et al., 2007).

In *Drosophila*, poly(A) tail regulation by deadenylation and cytoplasmic polyadenylation is essential for controlling mRNAs involved in axis patterning and other aspects in early development (Benoit et al., 2005; Castagnetti and Ephrussi, 2003; Kadyrova et al., 2007; Morris et al., 2005; Semotok et al., 2005; Vardy and Orr-Weaver, 2007; Zaessinger et al., 2006). In ovaries, cytoplasmic polyadenylation regulates the translation of *oskar* (*osk*), the posterior determinant, and of *CycB* mRNAs, and this polyadenylation depends on Orb, the *Drosophila* homolog of CPEB (Benoit et al., 2005; Castagnetti and Ephrussi, 2003; Chang et al., 1999; Juge et al., 2002). Orb is required at the earliest steps of oogenesis for the regulation of the synchronous divisions of a cystoblast that lead to the production of sixteen germ cells per cyst, and for the restriction of meiosis to one oocyte (Huynh and St Johnston, 2000). A single gene, *hiiragi* (*hrg*), which encodes one isoform of canonical PAP, exists in the *Drosophila* genome (Juge et al., 2002; Murata et al., 2001). Genetic interactions have implicated *orb* and *hrg* in the cytoplasmic polyadenylation of *osk* mRNA and accumulation of Osk protein at the posterior pole of the oocyte during mid-oogenesis. This led to the conclusion that canonical PAP has a role in cytoplasmic polyadenylation at this stage (Juge et al., 2002).

Cytoplasmic poly(A) tail elongation is also crucial in early embryos to activate the translation of mRNAs, including that of *bicoid* (*bcd*), which encodes the anterior morphogen (Salles et al., 1994). Polyadenylation and translation occur upon egg activation, a process that also induces the resumption of meiosis from the metaphase I arrest in mature oocytes, and which is triggered by egg laying, the passage of the egg through the oviduct (Heifetz et al., 2001). A link has been established between cytoplasmic polyadenylation and meiotic progression at egg activation because mutants defective for meiotic progression are also defective for poly(A) tail elongation (Horner et al., 2006; Lieberfarb et al., 1996; Page and Orr-Weaver, 1996).

Here, we analyze the function of *Drosophila* GLD-2 in the female germline. We show that this protein is encoded by *wispy* (*wisp*), a gene previously identified genetically (Brent et al., 2000), and we therefore refer to this protein as Wisp. We find that Wisp has a poly(A) polymerase activity in vitro and in vivo, and that it is required for poly(A) tail elongation of maternal mRNAs during late oogenesis and early embryogenesis. Wisp is required for meiotic progression in mature oocytes. A key target of Wisp during this process is *cortex* (*cort*) mRNA, which encodes a meiosis-specific activator of the anaphase-promoting complex (APC). This demonstrates the role of polyadenylation and translational activation in meiotic progression. In addition, we investigate the respective roles of conventional PAP and of Wisp in oogenesis and show that PAP and Orb are involved earlier than Wisp and Orb. Our results establish the requirement of two poly(A) polymerases for cytoplasmic polyadenylation at different steps of oogenesis.

MATERIALS AND METHODS

Drosophila stocks and genetics

The w^{1118} stock was used as control. The $wisp^{KG5287}$ (previously called $CG15737^{KG5287}$) mutant was from the Berkeley *Drosophila* Genome Project. The *P*-element (*SUPor-P*) was mobilized in the presence of *P* transposase and internal deletions of the *P*-element were recovered. Several female fertile revertants were also recovered among which two were sequenced and found to have retained a small portion of the *P*-element ends (33 bp and 39 bp, respectively). As the insertion point is downstream of the start codon and the remaining short insertions are coding and in frame with Wisp in both of the revertants, we believe these revertants produce slightly longer functional Wisp proteins. A 1.7 kb genomic region overlapping the conserved domains in $wisp^{12\text{-}3147}$, was PCR amplified and sequenced. Two point mutations, T1151I and I1292V, were found. The stock y^1 $fs(1)K10^4$ cv^1 v^1 $f^1/FM0$ that was generated in the same mutagenesis as $wisp^{12\text{-}3147}$ (Mohler, 1977) was used as a source of parental chromosome, and the same region of *wisp* was sequenced in this stock. The conservative I1292V mutation was present in this control stock, whereas T1151I was not.

Analysis of RNA

Analysis of poly(A) tail length by PCR (the PAT assay) and RT-PCRs were performed as reported previously (Benoit et al., 2005; Zaessinger et al., 2006). RNA preparations were from 20 embryos, 10 ovaries, and from dissected germarium-to-stage 8, stage 9-10, and stage 14 egg chambers from 10 ovaries. RT-PCRs were performed on the same RNA preparations used for the PAT assays, using serial dilutions of the cDNAs. Dilutions 1:10 are shown. Oligos for PAT assays were (5′ to 3′): *osk*, AAGCGCT-TGTTTGTAGCACA; *CycB*, GCTGGCCGAACACATCGGCG; *nos*, TTTTGTTTACCATTGATCAATTTTTC; *bcd*, CATTTGCGCATTCT-TTGACC; *cort*, GGCCAAGGACAAGTGCAGCTC; and *sop*, GGA-TTGCTACACCTCGGCCCGT. Oligos for RT-PCR were (5′ to 3′): *osk*, GCCATATTGCTGAGCCACGCCC and CCAGTAGCGTGGAGGT-GCTCG; *nos*, CGATCCTTGAAAATCTTTGCGCAGGT and TCG-TTGTTATTCTCACAAAAGACGCA; *bcd*, CTGGGTCGACCAATGT-CAATGGCG and GCTCTTGTCCAGACCCTTCAAAGG; and *sop*, CACCCCAATAAAGTTGATAGACCT and ATCTCGAACTCTTT-GATGGGAAGC. Whole-mount RNA in situ hybridizations were performed by standard methods. The RNA antisense probes were made from pKSbcdwt (*bcd*), pN5 (*nos*) and *osk* cDNA clones.

Poly(A) polymerase assays in *Xenopus* oocytes

To express MS2 fusion proteins in *Xenopus* oocytes, the C-terminal half of Wisp (residues 702-1373) and full-length *Homo sapiens* GLD-2 (*Hs*GLD-2) were cloned into the pCSMS2 vector using *Nhe*I and *Xho*I (Rouhana et al., 2005). Wisp D1031A and *Hs*GLD-2 D215A mutations were created by site-directed mutagenesis. For in vitro transcription, Wisp and *Hs*GLD-2 clones were linearized with *Xba*I and *Not*I, respectively, and transcribed using the SP6 Megascript Kit (Ambion). *Xenopus* oocyte manipulation, injection, luciferase assays and labeled RNA analysis were performed as described (Dickson et al., 1999; Kwak et al., 2004). To test MS2 fusion protein expression, oocytes were harvested 6 hours after mRNA injection and analyzed by western blotting using α-HA_{11} antibody (1:2000; Covance). Two oocytes were loaded per lane.

GST pull-down assays

GST recombinant proteins were produced by cloning the C-terminal half of Wisp (residues 702-1373) into the pBAH vector using *Eco*RI and *Xho*I, and the N-terminal region of Wisp (residues 11-547) digested with *Bgl*II into pGEX-5X-2 digested with *Bam*HI. In vitro interactions were performed as described (Benoit et al., 2002), in the presence of 0.2 μg/μl RNase A. Orb was in vitro translated from the *orb* D5 cDNA cloned into pBluescript using *Eco*RI and *Hin*dIII.

Antibodies, western blots and immunostaining

Antibodies against Wisp were obtained by cloning a portion of *wisp* cDNA LD18468 encoding residues 702 to 1373 into the pMAL vector. The Wisp-MBP fusion protein was expressed in *Escherichia coli* and purified using amylose beads (NEB). The purified fusion protein was injected into guinea pigs. Western blots and immunostaining were performed as described (Benoit et al., 2005; Benoit et al., 1999). Antibody dilutions for western blots were: anti-Wisp 1:3000, rabbit anti-BicC (Saffman et al., 1998) 1:1000, rat anti-PAP (Juge et al., 2002) 1:500, anti-Orb 6H4 (Developmental Studies Hybridoma Bank) 1:20, rabbit anti-Cyclin A (Whitfield et al., 1990) 1:10,000, anti-Cort (Pesin and Orr-Weaver, 2007) 1:2000, anti-α-tubulin (Sigma T5168) 1:10,000. Dilutions for immunostaining were: anti-Wisp 1:2000, anti-Osk (Kim-Ha et al., 1995) 1:500, anti-Nos (gift from A. Nakamura, RIKEN Center for Developmental Biology, Kobe, Japan) 1:1000, anti-Bcd (Kosman et al., 1998) 1:200, mouse anti-C(3)G (Anderson et al., 2005) 1:500. To visualize meiotic or mitotic spindles in embryos,

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methanol fixation was performed as described (Brent et al., 2000) and dilution of anti-α-tubulin (Sigma T9026) was 1:200. Meiotic spindles in stage 14 oocytes were visualized as described (Endow and Komma, 1997) using FITC-conjugated anti-α-tubulin (Sigma F2168).

Immunoprecipitation

Immunoprecipitations (IPs) were performed as described (Zaessinger et al., 2006). Each IP was with 60 ovaries from well-fed 3- to 4-day-old females and 5 μl of serum (immune or pre-immune) or 10 ml of hybridoma supernatant (Orb 6H4 or irrelevant 12CA5).

RESULTS

The female germline GLD-2 poly(A) polymerase in *Drosophila*

Two genes, GC5732 and CG15737, encoding GLD-2 homologs are present in the *Drosophila* genome. The corresponding proteins share the characteristics of GLD-2 family members in other species. They have a catalytic DNA polymerase β-like nucleotidyltransferase domain containing three conserved aspartic acid residues that is included in a larger conserved central domain, a PAP/25A-associated domain, and they lack an RNA-binding domain (Fig. 1A). The region that is N-terminal to the central domain is variable in size and non-conserved in the *Drosophila* GLD-2. Several CG5732 cDNAs described in FlyBase are from adult testis, indicating that CG5732 is expressed in this tissue. We verified by RT-PCR that CG5732 is not expressed in ovaries (data not shown). We focused on CG15737 (Fig. 1B), which was expressed in ovaries.

An antibody against the C-terminal half of the protein (residues 702-1373) was developed. In western blots, this antibody revealed a band of 180 kDa that was present in adult females and ovaries and absent from adult males (Fig. 1C). This band corresponds to the protein encoded by CG15737 as it was absent when the gene was mutated (see below). In embryos, expression of the protein peaked

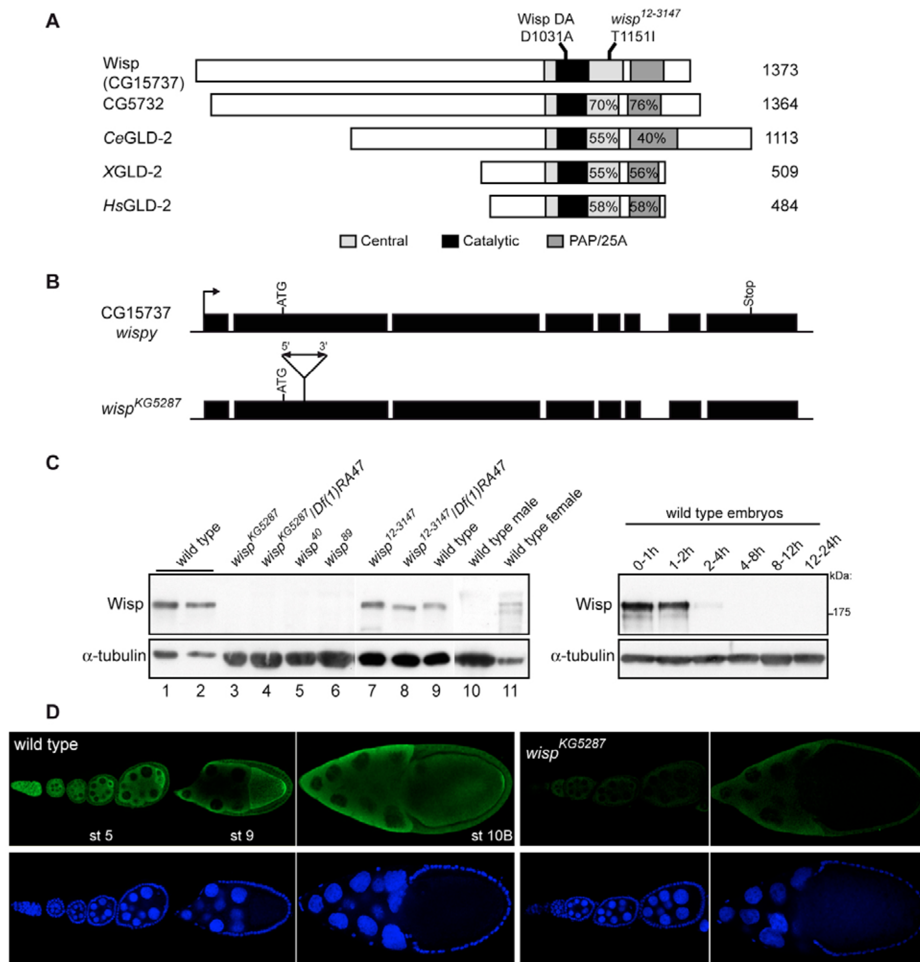


Fig. 1. GLD-2 genes and proteins in *Drosophila*. (**A**) Schematic of GLD-2 proteins from different species. Accession numbers are NP_572766 for Wisp, NP_651012 for CG5732-encoded protein, NP_491842 for *C. elegans*, AAT98005 for *Xenopus* and NP_776158 for human. The regions showing homology are in gray and black; percentage similarity with Wisp in the central (including catalytic) domain and in the PAP/25A domain are indicated. The mutation D1031A in the catalytic domain that precludes poly(A) polymerase activity, and the point mutation in *wisp*$^{12\text{-}3147}$, are shown. (**B**) Schematic of *wisp* (CG15737) locus and mutant. Black boxes are exons. The arrow indicates the transcription start site. The three cDNAs LD18468, RE03648 and RE14825 were sequenced. RE03648 and RE14825 are full-length and start and end at identical nucleotides, whereas LD18468 is incomplete at both ends. The *P*-element (not drawn to scale) in *wisp*^{KG5287} is shown. The insertion site was verified by sequencing. (**C**) Western blots with anti-Wisp, showing Wisp expression in females and ovaries and the lack of protein in *wisp*^{KG5287}, *wisp*^{40} and *wisp*^{89} mutant ovaries. Protein extracts were from 0.4 (lane 1) or 0.2 (lanes 2, 9) wild-type ovaries, from one (lanes 3-6) or 0.2 (lanes 7, 8) mutant ovaries, from 0.3 male (lane 10) or 0.1 female (lane 11), and from 20 wild-type embryos (right panel). α-tubulin was used as a loading control. (**D**) Immunostaining of ovaries with anti-Wisp, showing cytoplasmic expression (green) throughout oogenesis and the lack of expression in *wisp*^{KG5287} mutant ovaries. Nuclei are visualized with DAPI (blue). Anterior is oriented towards the left.

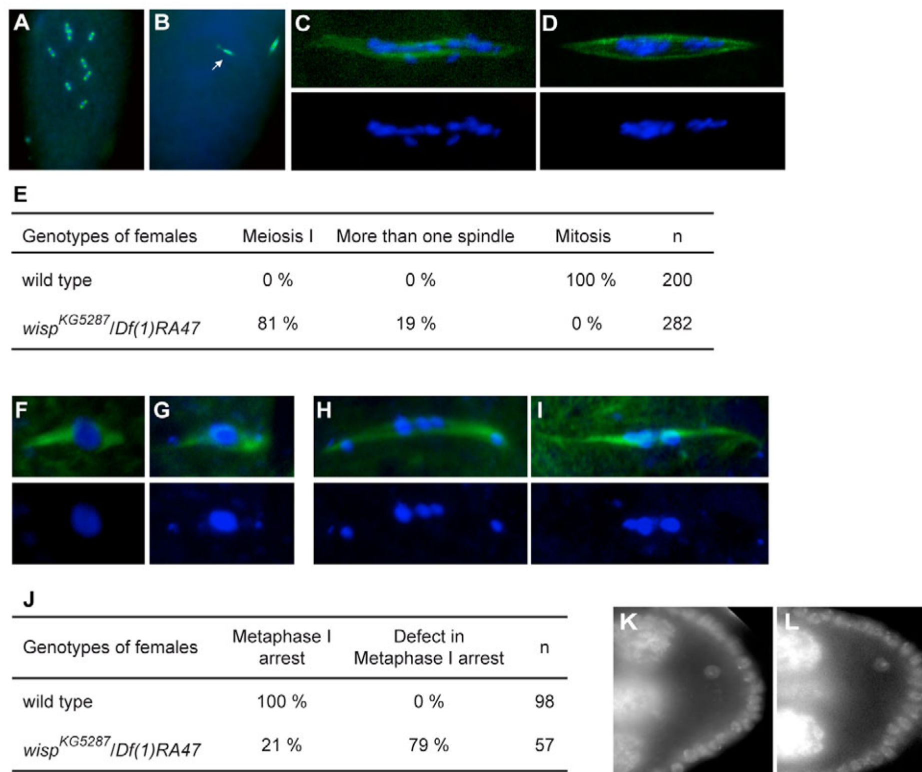
at 0-2 hours of development, decreased at 2-4 hours and was undetectable in later stages, consistent with a maternal expression. Immunostaining of ovaries showed that the protein is expressed throughout oogenesis (Fig. 1D). It was cytoplasmic, present both in nurse cells and the oocyte and accumulated in the oocyte from stage 5 onwards. In stages 9 and 10, protein accumulation was visible at the posterior pole of the oocyte. Staining of ovaries mutant for CG15737 was reduced to background levels, indicating that the antibody specifically recognized the protein encoded by this gene.

The female germline GLD-2 poly(A) polymerase is encoded by *wispy* and is required for metaphase of meiosis I

A *P*-element insertion in CG15737 (*CG15737^{KG5287}*) was available at the Berkeley *Drosophila* Genome Project (BDGP), in which the insertion occurred 36 residues downstream of the initiation codon (Fig. 1B). Homozygous *CG15737^{KG5287}* males were viable and fertile, whereas homozygous *CG15737^{KG5287}* females were viable and sterile. Oogenesis did not appear affected and these females laid a normal number of eggs, but none of the embryos from mutant females crossed with wild-type males showed any development (Fig. 2A,B). Because the protein encoded by CG15737 was lacking in ovaries from *CG15737^{KG5287}* homozygous females (Fig. 1C,D) and the phenotypes of *CG15737^{KG5287}* homozygotes were identical to those of *CG15737^{KG5287}/Df(1)RA47* (a deficiency overlapping the region) females, we believe that *CG15737^{KG5287}* is a null allele. Mobilization of the *P*-element in *CG15737^{KG5287}* generated either new mutant alleles (*CG15737^{40}* and *CG15737^{89}*) corresponding to internal deletions of the *P*-element, or nearly complete deletions of the *P*-element that restored female fertility. A close examination of *CG15737^{KG5287}* phenotypes showed that the gene is required at metaphase of meiosis I.

These phenotypes were similar to those of *wisp* mutants, a gene previously identified genetically and which is located in the same chromosome region as CG15737 (Brent et al., 2000; Mohler, 1977). Complementation tests between *CG15737^{KG5287}* and *wisp$^{12-3147}$* showed that CG15737 is *wisp*. Sequencing of the conserved domains in *wisp$^{12-3147}$* identified a point mutation, T1151I, in the central domain of the protein, changing a residue that is conserved in the other species (Fig. 1A). This mutation did not prevent the production of Wisp in ovaries (Fig. 1C).

Staining of embryos from *wisp^{KG5287}* or *wisp^{KG5287}/Df(1)RA47* females (thereafter called *wisp* embryos) with anti-α-tubulin and DAPI to visualize meiotic figures showed that these embryos did not complete meiosis (Fig. 2A-E). In most embryos (74%), a single meiotic spindle was visible, which could be thin or distorted and on



E

Genotypes of females	Meiosis I	More than one spindle	Mitosis	n
wild type	0 %	0 %	100 %	200
wisp^{KG5287}/Df(1)RA47	81 %	19 %	0 %	282

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Genotypes of females	Metaphase I arrest	Defect in Metaphase I arrest	n
wild type	100 %	0 %	98
wisp^{KG5287}/Df(1)RA47	21 %	79 %	57

Fig. 2. *wisp* function in the *Drosophila* germline. (**A-E**) Meiotic arrest as visualized in embryos. Immunostaining of 0- to 20-minute wild-type embryos and 0- to 2-hour *wisp^{KG5287}/Df(1)RA47* embryos with anti-α-tubulin (green) and DAPI (blue). (A) Wild-type embryo showing mitoses. (B) *wisp^{KG5287}/Df(1)RA47* embryo showing one metaphase I anastral female spindle and one mitotic-like spindle with a centrosome, associated with the male pronucleus (arrow). (C,D) Examples of meiosis I spindle in *wisp^{KG5287}/Df(1)RA47* embryos showing scattered chromosomes along the spindle (C), and asymmetric pools of chromosomes (D). Bottom panels are stained with DAPI alone. (E) Scoring of meiotic arrest as visualized with anti-α-tubulin and DAPI staining. (**F-J**) Meiotic defects in stage 14 oocytes visualized with anti-α-tubulin (green) and DAPI (blue). (F) Prometaphase I in wild-type stage 14 oocyte. (G) Wild-type-like metaphase I in *wisp^{KG5287}/Df(1)RA47* embryo; the fourth chromosomes are separated from the main chromosome pool (metaphase I arrest in J). (H,I) Abnormal metaphase I spindles in *wisp^{KG5287}/Df(1)RA47* oocytes. Bottom panels are stained with DAPI alone. (J) Scoring of meiotic figures as visualized with anti-α-tubulin and DAPI. (**K**,**L**) DAPI staining of stage 8 oocytes, showing that the karyosome is not affected in the *wisp* mutant. (K) Wild type; (L) *wisp^{KG5287}/Df(1)RA47*.

which the chromosomes were scattered or separated in asymmetric pools (Fig. 2C,D). A second small spindle was sometimes present, which was nucleated by one or several lost chromosomes (8% of embryos). These figures (81%, Fig. 2E) correspond to a block in meiosis I. Among the remaining embryos, 15% were identified as blocked in meiosis II by visualization of two meiotic spindles, which in most cases were abnormally arranged. This phenotype is stronger than that of $wisp^{12\text{-}3147}$/*Df(1)RA47* embryos, most of which were arrested at or after metaphase II (Brent et al., 2000). Meiotic figures in *wisp* mutant embryos correspond to either abnormal metaphase I or anaphase I. In the wild type, a meiotic arrest occurs at metaphase I in mature oocytes (Fig. 2F,G). Meiosis I resumes at egg activation, driven by egg laying, and meiosis II is rapidly completed without further arrest.

Meiotic figures were analyzed in $wisp^{KG5287}$/*Df(1)RA47* mature (stage 14) oocytes and we found that in most oocytes (79%), metaphase I arrest was not maintained properly (Fig. 2H-J). The chromosomes separated along the spindle and did so asymmetrically, the fourth chromosomes often migrating out of the spindle. The spindle appeared thin or irregular (Fig. 2H,I). Therefore, *wisp* mutants showed a defect in metaphase I arrest, and meiosis is then blocked at this stage as abnormal meiotic figures are similar before and after egg activation in *wisp* mutant embryos. Because a defect in metaphase I arrest could result from a defect earlier in meiosis, we analyzed earlier aspects of meiosis. We found that the karyosome in $wisp^{KG5287}$/*Df(1)RA47* stage 8 oocytes appeared as in the wild type (Fig. 2K,L). Entry into meiosis, as well as meiosis restriction to one oocyte in the germarium, as visualized by the formation of the synaptonemal complex using anti-C(3)G antibody (Anderson et al., 2005), were also as in the wild type (data not shown).

We conclude that the GLD-2-type poly(A) polymerase in the *Drosophila* female germline is encoded by *wisp* and is required for metaphase I arrest and for progression of meiosis after this stage.

Wisp is a poly(A) polymerase and is involved in poly(A) tail elongation during late oogenesis

A tethering assay was previously developed in *Xenopus* oocytes to analyze the poly(A) polymerase activity of candidate proteins (Kwak et al., 2004). The tested protein is tethered to mRNAs through MS2, an exogenous RNA-binding protein, and poly(A) addition as well as translational activation of the targeted mRNA are assayed. A chimeric mRNA encoding an MS2-Wisp fusion protein was injected into *Xenopus* oocytes. Two reporter mRNAs, luciferase with MS2 binding sites and β-galactosidase lacking MS2 binding sites, were then co-injected. Translational activation was calculated by comparing luciferase activity to β-galactosidase activity (Fig. 3A). *Hs*GLD-2 was used as a positive control and induced translational activation that was abolished when the catalytic site was destroyed by mutation of an essential aspartic acid residue (*Hs*GLD-2 DA). Wisp protein strongly stimulated translation of the reporter RNA and the stimulatory effect was abolished by a point mutation in the catalytic domain (Wisp DA) (Fig. 1A). Poly(A) tail addition to a short RNA containing MS2 binding sites was measured and a robust polyadenylation, which depended on the catalytic domain integrity, was observed in the presence of either MS2-*Hs*GLD-2 or MS2-Wisp (Fig. 3B). These results demonstrate that Wisp is a poly(A) polymerase.

We confirmed the role of Wisp in poly(A) tail elongation in vivo using PAT assays, an RT-PCR-based technique that allows measurements of poly(A) tail length. Cytoplasmic polyadenylation of *osk* and *CycB* mRNAs during oogenesis has been reported and this depends on Orb (Benoit et al., 2005; Castagnetti and Ephrussi, 2003). We determined whether it also depended on the Wisp poly(A) polymerase. Cytoplasmic polyadenylation of *nanos* (*nos*) and *bcd* mRNAs was also analyzed. Egg chambers of progressive stages were dissected and poly(A) tails were measured (Fig. 3C). For all tested mRNAs, poly(A) tails lengthened during oogenesis in the wild type, with a moderate lengthening between early stages (germarium to stage 8) and stages 9-10, and a pronounced elongation between stages 9-10 and stage 14. For *bcd* mRNA, this lengthening is not sufficient for translational activation, which occurs after egg activation (Salles et al., 1994). In $wisp^{KG5287}$ egg chambers, the lengthening at stage 9-10 was slightly affected and the strong elongation at stage 14 was completely abolished.

To determine whether Wisp has a general role in regulating mRNAs, we analyzed the poly(A) status of 30 mRNAs that we have identified to be regulated by cytoplasmic polyadenylation and the poly(A) tails of which undergo a robust lengthening in mature oocytes (I. Busseau and M.S., unpublished). For all tested mRNAs, the poly(A) tail lengthening in $wisp^{KG5287}$ mature oocytes was impaired (see Fig. S1 in the supplementary material).

Together, these data show that Wisp is the poly(A) polymerase responsible for cytoplasmic polyadenylation of mRNA targets during late oogenesis.

cort mRNA is a meiotic target of Wisp

To determine whether the meiotic defect observed in *wisp* mutants resulted from defects in mRNA polyadenylation and translational activation, we identified a Wisp target required for meiotic progression. *cort* encodes a female meiosis-specific activator of the APC that is necessary for female meiosis (Chu et al., 2001). Eggs from *cort* mutant females show aberrant chromosome segregation in meiosis I and eventually arrest in metaphase II (Lieberfarb et al., 1996; Page and Orr-Weaver, 1996). Cort is required for sequential degradation of CycA, B and B3 during meiosis, with CycA being degraded earlier, prior to metaphase I arrest (Pesin and Orr-Weaver, 2007; Swan and Schupbach, 2007). Cort protein expression peaks during oocyte maturation (stages 13 and 14) and correlates with *cort* mRNA poly(A) tail lengthening (Pesin and Orr-Weaver, 2007). We found that *cort* poly(A) tail elongation was abolished in $wisp^{KG5287}$ stage 14 oocytes (Fig. 4A). This led to a defect in Cort protein accumulation at this stage (Fig. 4B). The earliest target of Cort is CycA, the degradation of which fails in *cort* mutants by metaphase I arrest (Pesin and Orr-Weaver, 2007). We verified that the lack of Cort accumulation in $wisp^{KG5287}$ mature oocytes resulted in a defect of CycA destruction: CycA levels were elevated in $wisp^{KG5287}$ mature oocytes, consistent with impaired destruction (Fig. 4C).

These results identify *cort*, an mRNA required for meiotic progression, as a Wisp target, the poly(A) tail elongation and translation of *cort* being dependent on Wisp. They strongly suggest that the poly(A) polymerase function of Wisp is required during the progression of meiosis.

Wisp and PAP poly(A) polymerases are both in a cytoplasmic polyadenylation complex with Orb

Cytoplasmic polyadenylation of *osk* and *CycB* mRNAs during oogenesis is Orb-dependent, and GLD-2 is in a complex with CPEB in *Xenopus* oocytes (Barnard et al., 2004; Rouhana et al., 2005). We therefore analyzed whether Wisp and Orb were present in a complex, by co-immunoprecipitations in ovary extracts. Orb was able to co-precipitate with Wisp and this interaction was RNA-independent (Fig. 5A). Conversely, Wisp co-precipitated with Orb, and the interaction again was RNA-independent (Fig. 5B). A direct

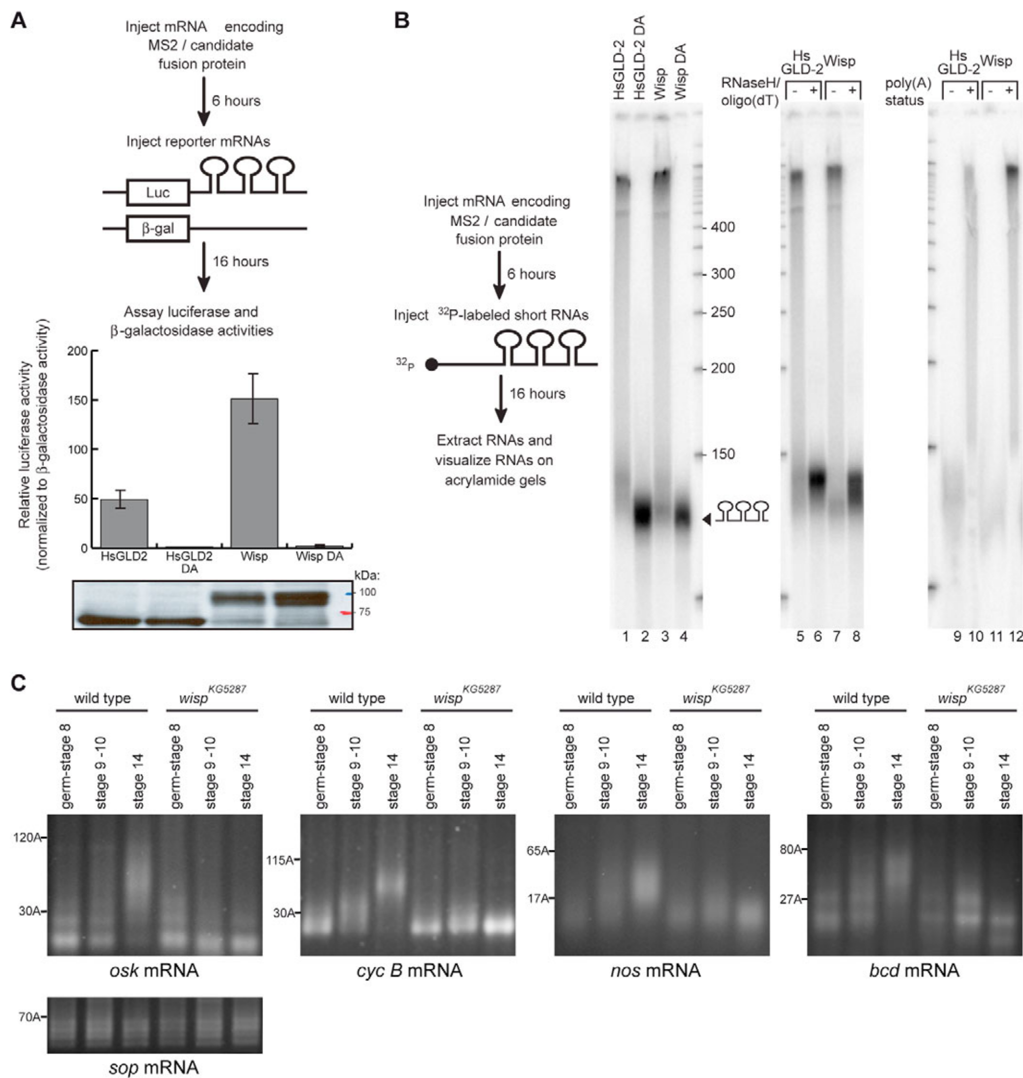

Fig. 3. Poly(A) polymerase activity of Wisp in a tethering assay and during oogenesis. (**A**,**B**) Tethering assay in *Xenopus* oocytes. (A) Wisp and a control protein, *Hs*GLD-2, were tethered to luciferase reporter mRNA using MS2. β-galactosidase-encoding mRNA lacking MS2 binding sites was used as an internal control. Translational stimulation was assayed by measuring luciferase activity. Wild-type and point-mutant (DA) proteins were expressed at similar levels, as determined by western blotting with anti-HA_{11} (bottom). (B) 130 nt, ^{32}P-labeled RNA was injected and then purified from oocytes. (Lanes 1-4) Tethered *Hs*GLD-2 and Wisp added long poly(A) tails onto labeled RNA. Active site mutations disrupted elongation. (Lanes 5-8) Tails added by wild-type Wisp were removed by RNase H/oligo(dT) treatment, confirming that they were poly(A). –, RNase H only; +, RNase H plus oligo(dT). (Lanes 9-12) RNAs elongated by Wisp were bound to an oligo(dT) column. –, RNAs that did not bind; +, RNAs that bound. (**C**) PAT assays measuring *osk*, *CycB*, *nos* and *bcd* poly(A) tail lengths in *Drosophila* egg chambers of different stages (germarium to stage 8, stages 9 and 10, and stage 14) from wild-type and *wisp*^{KG5287} ovaries. *sop* mRNA, which encodes a ribosomal protein and is not regulated by cytoplasmic polyadenylation, was used as a control.

interaction between Wisp and Orb was confirmed by in vitro binding assays, in which in vitro translated Orb bound to recombinant GST-Wisp(702-1373), but not to GST-Wisp(11-547) or to GST alone (Fig. 5E).

In *C. elegans*, GLD-2 directly interacts with the RNA-binding protein GLD-3 (Wang et al., 2002), a homolog of *Drosophila* Bicaudal C (BicC). In a co-immunoprecipitation between Wisp and BicC, Wisp co-precipitated with BicC. This interaction depended on the presence of RNA, suggesting that BicC and Wisp can be present in common RNP complexes, but do not interact directly (Fig. 5C).

We reported previously that poly(A) tail elongation of *osk* mRNA in ovaries, and Osk protein accumulation at the posterior pole of oocytes, depend on Orb and the canonical PAP (Juge et al., 2002). We now find that cytoplasmic polyadenylation of *osk* mRNA requires Wisp, another poly(A) polymerase. To understand the role of two different poly(A) polymerases in poly(A) tail lengthening of the same targets, we investigated the presence of PAP in the cytoplasmic polyadenylation complex. PAP co-precipitated with Orb in ovary extracts, independently of the presence of RNA (Fig. 5B). More strikingly, Wisp co-precipitated with PAP and the interaction was maintained in the absence of RNA (Fig. 5D).

These data show that Wisp is recruited to mRNAs through a direct interaction with Orb, and are consistent with the role of both PAP and Wisp in cytoplasmic polyadenylation with Orb.

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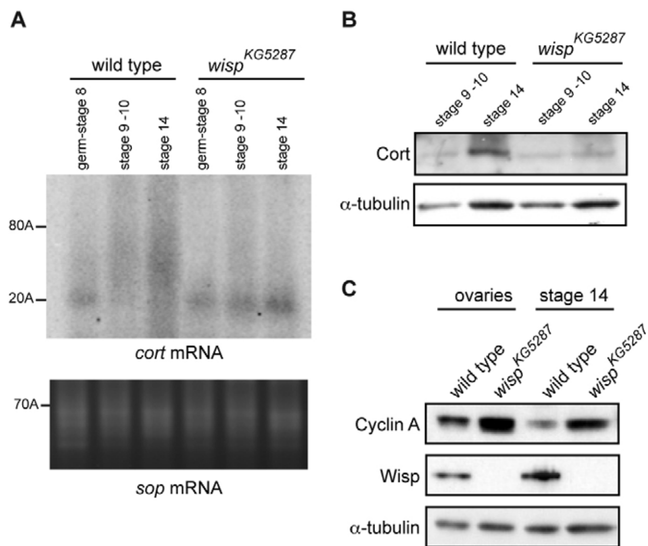


Fig. 4. *cort* mRNA is a meiotic target of Wisp. (**A**) PAT assays measuring *cort* poly(A) tail lengths in *Drosophila* egg chambers of progressive stages from wild-type and $wisp^{KG5287}$ ovaries. *sop* mRNA was used as a control. (**B**) Western blots showing that Cort protein levels are reduced in $wisp^{KG5287}$ stage 14 oocytes as compared with wild type. Protein extracts were from 30 egg chambers at stage 9-10 and from 15 oocytes at stage 14. (**C**) Western blots showing that CycA levels are higher in the ovaries and stage 14 oocytes of $wisp^{KG5287}$ than of wild-type. Protein extracts were from 0.5 ovary and 20 oocytes at stage 14. α-tubulin was used as a loading control in B and C.

Functions of PAP and Wisp during oogenesis

To investigate the respective roles of PAP and Wisp in oogenesis, we analyzed genetic interactions between *orb* and *wisp* and between *orb* and the PAP-encoding gene *hrg*. Strong *hrg* mutants are lethal and do not produce late egg chambers in germline clones (Juge et al., 2002). Therefore, the role of PAP and Orb in cytoplasmic polyadenylation had been inferred from strong genetic interactions between heterozygous *hrg* alleles and the homozygous weak *orb* allele, orb^{mel}. The combination of both mutants strongly reduces female fertility and oogenesis stops around stage 8 in a number of ovarioles (Juge et al., 2002).

Because the phenotype of the orb^{mel} mutant tended to become stronger with time, we backcrossed orb^{mel} in a new background (Canton S) to produce a weaker phenotype. In this background, orb^{mel} females produced a low level of maternal-effect embryonic lethality (15%), which was increased to 31% and 45% in the $hrg^{-}/+;\ orb^{mel}$ combinations, consistent with the previously described interactions (Fig. 6A). Embryonic lethality from $wisp^{-}/+;\ orb^{mel}$ females also increased to similar levels of 35-50%. These genetic interactions corroborate the physical interactions between Wisp and Orb (Fig. 5) and provide additional support for the proposed combined role of Wisp and Orb in cytoplasmic polyadenylation. A number of embryos from orb^{mel} females are ventralized owing to defects in *gurken* (*grk*) mRNA localization and translation during mid-oogenesis (Chang et al., 2001). Whereas the percentage of ventralized embryos did not increase in interactions between *wisp* and *orb*, they were enhanced for embryos from $hrg^{-}/+;\ orb^{mel}$ females (26-31%) (Fig. 6A). These results suggest that *hrg* and *orb* interact during early or mid-oogenesis (before or during stages 9-10), whereas *wisp* and *orb* interact later (after stage 10).

We then compared the phenotypes of genetic interactions between *orb* and *hrg* and between *orb* and *wisp* during oogenesis. Oogenesis stopped at stage 8 in a number of ovarioles from $hrg^{PAP21}/+;\ orb^{mel}$ females (34%) (Fig. 6B). Staining with anti-C(3)G to visualize the oocyte was impaired in arrested $hrg^{PAP21}/+;\ orb^{mel}$ egg chambers, indicating either degeneration or the lack of oocyte determination (Fig. 6C). Lack of oocyte determination was confirmed by the presence of sixteen nurse cells (10% of ovarioles). Consistent with the possible function of PAP with Orb during early oogenesis, PAP and Orb expression

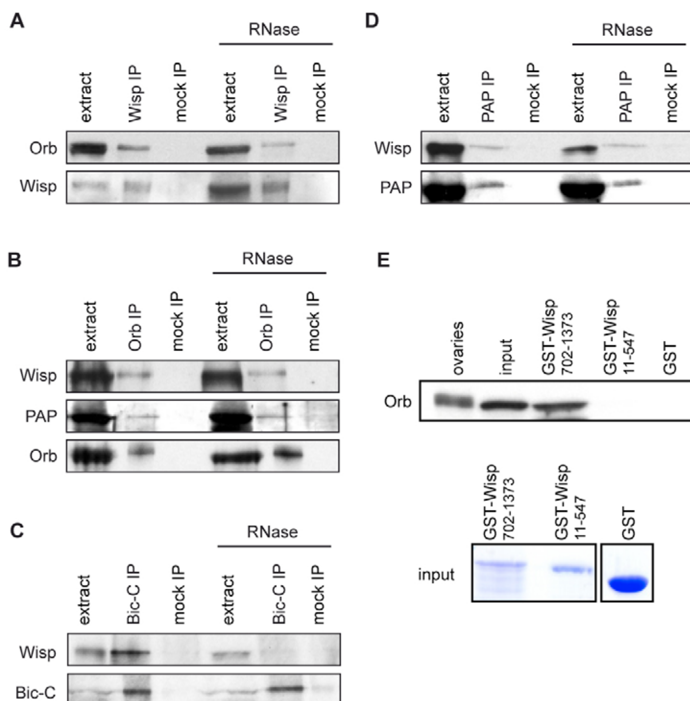
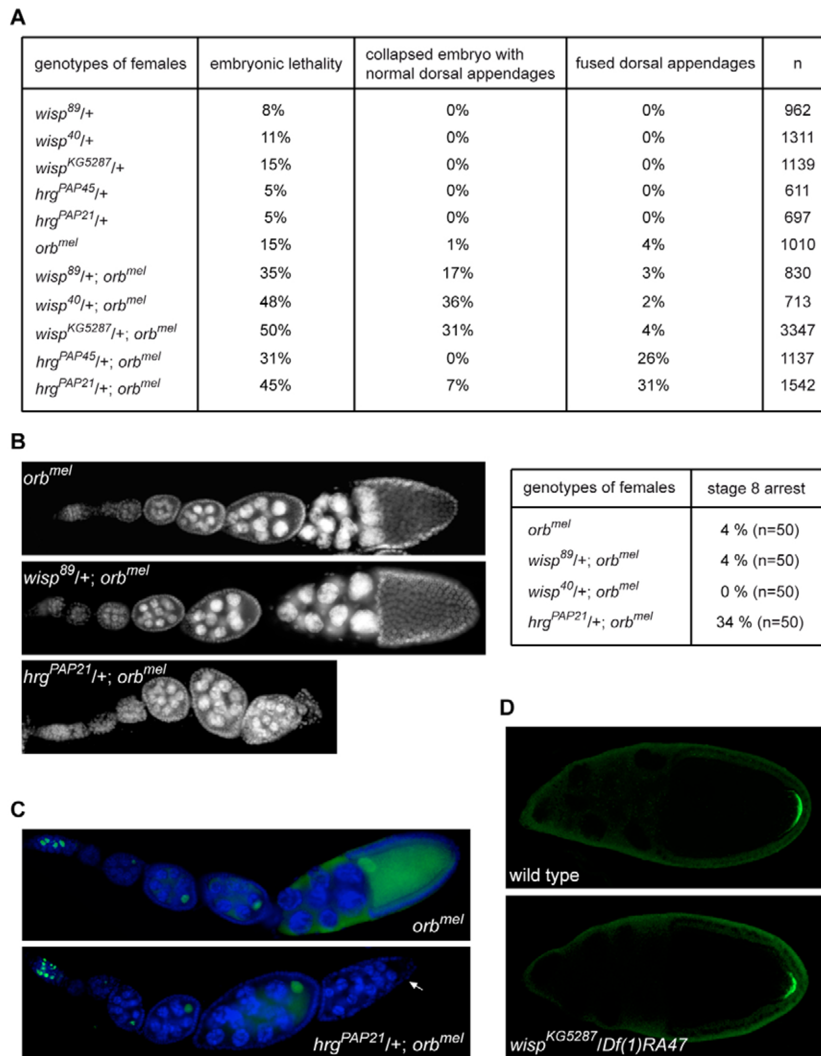


Fig. 5. Cytoplasmic polyadenylation complexes in *Drosophila* ovaries. (**A-D**) Immunoprecipitations (IPs) were performed in ovary extracts either in the presence of RNase inhibitor or in the presence of RNase A (RNase). Co-immunoprecipitated proteins were identified by western blot. Mock IPs were with preimmune serum for Wisp and PAP IPs, with rabbit serum for BicC IP and with an irrelevant monoclonal antibody for Orb IP. Extract (1/20) prior to IP was loaded. (**E**) In vitro interaction assays showing that Orb directly interacts with recombinant GST-Wisp(702-1373), but not with GST-Wisp(11-547) or GST alone. Orb was revealed by western blot. 1/10 of in vitro synthesized Orb before the interaction assay was loaded (input). Protein extract from 0.5 ovary was also loaded (upper panel). Recombinant and GST proteins are shown (input, bottom panel).



genotypes of females	embryonic lethality	collapsed embryo with normal dorsal appendages	fused dorsal appendages	n
$wisp^{89}$/+	8%	0%	0%	962
$wisp^{40}$/+	11%	0%	0%	1311
$wisp^{KG5287}$/+	15%	0%	0%	1139
hrg^{PAP45}/+	5%	0%	0%	611
hrg^{PAP21}/+	5%	0%	0%	697
orb^{mel}	15%	1%	4%	1010
$wisp^{89}$/+; orb^{mel}	35%	17%	3%	830
$wisp^{40}$/+; orb^{mel}	48%	36%	2%	713
$wisp^{KG5287}$/+; orb^{mel}	50%	31%	4%	3347
hrg^{PAP45}/+; orb^{mel}	31%	0%	26%	1137
hrg^{PAP21}/+; orb^{mel}	45%	7%	31%	1542

genotypes of females	stage 8 arrest
orb^{mel}	4 % (n=50)
$wisp^{89}$/+; orb^{mel}	4 % (n=50)
$wisp^{40}$/+; orb^{mel}	0 % (n=50)
hrg^{PAP21}/+; orb^{mel}	34 % (n=50)

Fig. 6. PAP has an earlier role in *Drosophila* oogenesis than Wisp. (**A**) Genetic interactions between *orb* and *wisp* and between *orb* and *hrg*. Females of the indicated genotype were crossed with wild-type males and the embryonic lethality of their progeny was scored. The percentage of embryonic lethality from orb^{mel} mothers is increased in the presence of both heterozygous *wisp* or *hrg* mutants. *hrg* mutations only are able to dominantly increase the ventralization phenotype of orb^{mel}. Note that collapsed embryos with normal dorsal appendages is a common phenotype of *wisp* mutant embryos. This phenotype suggests a defect in vitelline membrane cross-linking, an early event at egg activation. (**B**) Ovaries of orb^{mel} single mutant females, or in the presence of $wisp^{-}$/+ or hrg^{-}/+ mutants, visualized with DAPI. Oogenesis progresses normally in orb^{mel}, $wisp^{89}$/+; orb^{mel} and $wisp^{40}$/+; orb^{mel} females, but is blocked at stage 8 in hrg^{PAP21}/+; orb^{mel} ovaries. Fifty ovarioles of the indicated genotype were scored and the percentage of abnormal egg chambers arrested at stage 8 is indicated. For stage 8 arrests that were scored in orb^{mel} or $wisp^{-}$/+; orb^{mel} ovaries, the oocyte was present. (**C**) Characterization of stage 8 arrest in hrg^{PAP21}/+; orb^{mel} ovaries. Immunostaining of ovaries with anti-C(3)G antibody (green) and DAPI (blue), showing the presence of the oocyte in orb^{mel} and its absence in arrested hrg^{PAP21}/+; orb^{mel} egg chambers (arrow). (**D**) Osk protein accumulation is not affected in *wisp* mutant oocytes during mid-oogenesis. Immunostaining of wild-type and $wisp^{KG5287}$/*Df(1)RA47* mutant ovaries with anti-Osk antibody, showing that Osk accumulation at the posterior pole is similar in wild-type and *wisp* mutant stage 10 oocytes.

overlapped at these stages (see Fig. S2 in the supplementary material). Note that these data do not preclude a role for PAP later in oogenesis.

By contrast, early defects did not appear in $wisp^{-}$/+; orb^{mel} ovaries (Fig. 6B). Moreover, translation of *osk* mRNA has been quantified previously in the orb^{mel} mutant and found to be strongly affected in stage 9-10 oocytes (Castagnetti and Ephrussi, 2003). We found that Osk protein accumulation was not affected in $wisp^{KG5287}$/*Df(1)RA47* oocytes at these stages (Fig. 6D) (*n*=128).

We conclude that PAP is required with Orb during early oogenesis, whereas Wisp has an essential function with Orb after stage 10 of oogenesis.

Wisp is required for cytoplasmic polyadenylation in early embryos

Upon egg activation, cytoplasmic polyadenylation leads to a robust poly(A) tail elongation and translation of a number of maternal mRNAs, including *bcd*. In *wisp* mutant embryos, poly(A) tail elongation of *bcd* mRNA was abolished (Fig. 7A). This short poly(A) tail was stable during the first 3 hours of embryogenesis and, consistently, *bcd* mRNA was not completely destabilized. *bcd* mRNA was also detected in embryos by in situ hybridization; however, the transcript was delocalized in the anterior region and to a lesser extent in the rest of the embryo (Fig. 7B). Maternal mRNA delocalization in *wisp* mutant embryos has been reported previously, but was weaker, probably owing to the utilization of weaker alleles (Brent et al., 2000). Consistent with previous data establishing that poly(A) tail elongation of *bcd* mRNA is necessary and sufficient for its translation in embryos (Salles et al., 1994), the lack of poly(A) lengthening in *wisp* mutant embryos prevented Bcd protein accumulation (Fig. 7C, see Fig. S3 in the supplementary material).

We measured poly(A) tail lengths of *osk* and *nos* mRNAs in embryos. In wild-type embryos, pools of *osk* and *nos* mRNAs are localized at the posterior pole, but another fraction of these mRNAs is widespread in the bulk cytoplasm (Bergsten and Gavis, 1999) and then degraded. In agreement with this, both *osk* and *nos* mRNAs analyzed by PAT assays and RT-PCR were deadenylated and destabilized during the first 3 hours in wild-type embryos (Fig. 7A). We have shown previously that *nos* mRNA destabilization in the bulk cytoplasm of the embryo depends on deadenylation by CCR4 (*twin* – FlyBase) (Zaessinger et al., 2006). In *wisp* mutant embryos, *osk* and *nos* mRNAs were destabilized prematurely in the first hour of embryogenesis and pools of these mRNAs were not stabilized at the posterior pole (Fig. 7A,B, see Fig. S3B,C in the supplementary material). Accordingly, Osk and Nos proteins were completely lacking in *wisp* mutant embryos (Fig. 7C). We propose that premature destabilization of *osk* and *nos* mRNAs in *wisp* mutant embryos results from increased shortening of poly(A) tails in the absence of

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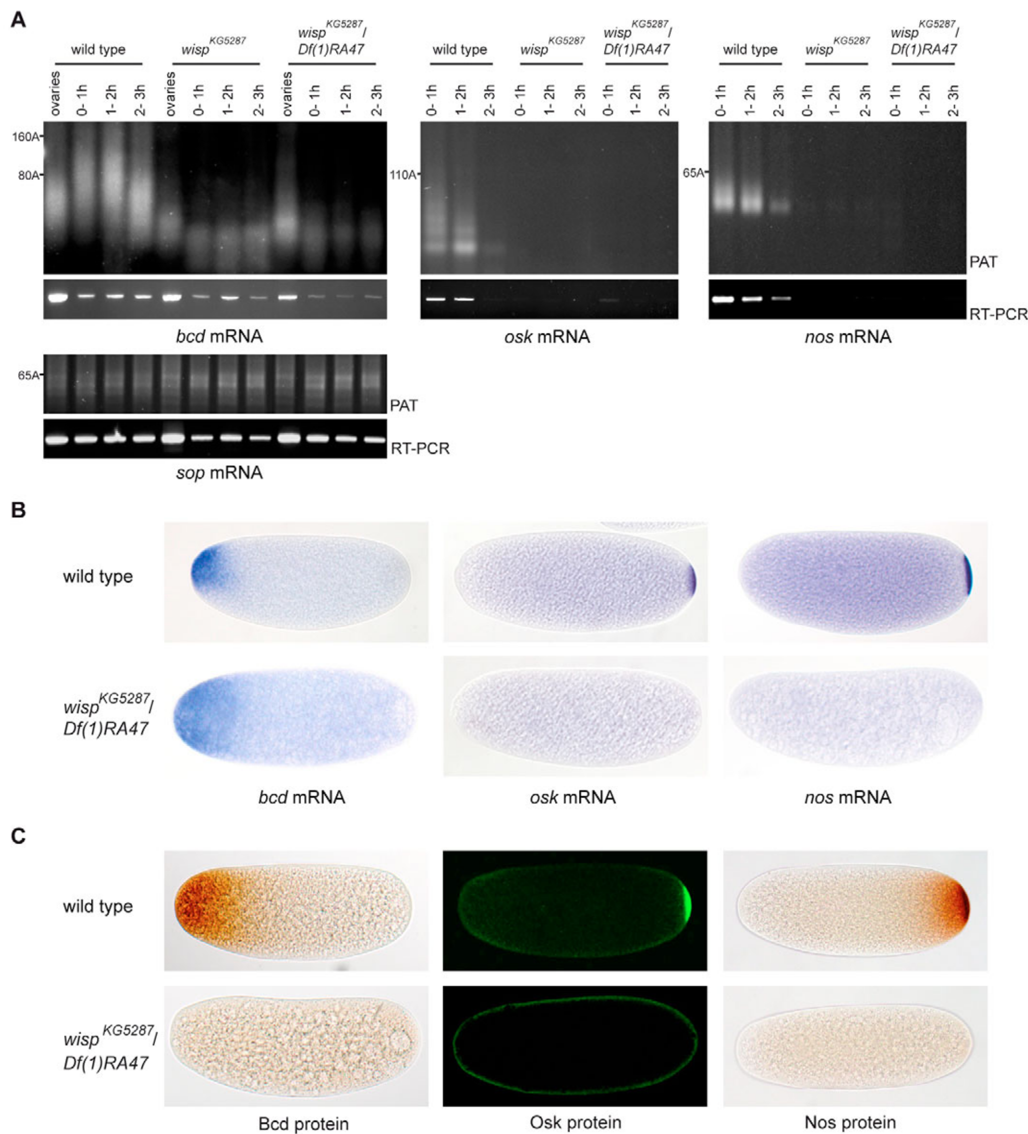

Fig. 7. Role of Wisp in cytoplasmic polyadenylation in early *Drosophila* embryos. (**A**) PAT assays and RT-PCR of *bcd*, *osk* and *nos* mRNAs in embryos from 0-1, 1-2 and 2-3 hours of development, from wild-type, $wisp^{KG5287}$ or $wisp^{KG5287}$/*Df(1)RA47* females. In the absence of Wisp, *osk* and *nos* mRNAs are destabilized and the poly(A) tails of *bcd* mRNA are not elongated in embryos. *sop* mRNA was used as a control. The *sop* RT-PCR is the loading control for *bcd*, *osk* and *nos* RT-PCR. For *bcd* mRNA, PAT assays from ovaries were loaded to show the poly(A) tail elongation between ovaries and early embryos in the wild type. (**B**) In situ hybridizations of 0- to 1-hour wild-type and $wisp^{KG5287}$/*Df(1)RA47* embryos showing *bcd*, *osk* and *nos* mRNA. (**C**) Immunostaining of 0- to 1-hour embryos with anti-Bcd, anti-Nos and anti-Osk antibodies, showing that the lack of poly(A) tail elongation in $wisp^{KG5287}$/*Df(1)RA47* mutant embryos, leading or not to mRNA decay, results in the lack of corresponding proteins. Note that the lack of *nos* mRNA/protein at the posterior pole could also result from the lack of Osk protein, as Osk is required for *nos* mRNA stabilization.

poly(A) tail elongation by Wisp. As Smaug protein is required for *nos* mRNA destabilization, through the recruitment of the deadenylation complex (Zaessinger et al., 2006), we verified that Smaug levels were unaffected in *wisp* mutant embryos (data not shown).

These results demonstrate that Wisp is responsible for cytoplasmic polyadenylation in early embryos and that poly(A) tail elongation is required for the production of the major determinants in embryo anteroposterior patterning.

DISCUSSION

We have characterized Wisp, one of the two GLD-2-type poly(A) polymerases in *Drosophila*, which has a function in the female germline. We show that Wisp is a bona fide poly(A) polymerase: it has poly(A) polymerase activity in a tethering assay that depends on a conserved residue in the catalytic domain. Wisp is required for poly(A) tail lengthening of a pool of mRNAs in late stages of oogenesis. GLD-2 poly(A) polymerases do not have an RNA-binding domain; instead, they interact with RNA through their association with RNA-binding proteins. In *Xenopus* oocytes, GLD-2 interacts with CPEB in a complex that is active in cytoplasmic polyadenylation (Barnard et al., 2004; Rouhana et al., 2005). We find that Wisp interacts directly with Orb. Consistent with a role of Wisp and Orb together in an ovarian cytoplasmic polyadenylation complex, *wisp* mutants are dominant enhancers of a weak *orb* allele. In *C. elegans*, GLD-2 has been reported to interact with the KH-domain RNA-binding protein GLD-3, which has homology with *Drosophila* BicC. Although we find Wisp and BicC together in an ovarian RNP complex, their association is mediated by RNA, suggesting that the proteins do not interact directly. We recently reported that BicC functions in deadenylation: BicC recruits the CCR4-NOT deadenylase complex to mRNAs (Chicoine et al., 2007). However, we also found a role of BicC in poly(A) tail elongation during oogenesis (Chicoine et al., 2007).

In addition to its function in oogenesis, Wisp-dependent cytoplasmic polyadenylation is required for the translation of essential determinants of the anteroposterior patterning of the embryo. *bcd* mRNA poly(A) tail elongation was known to be required for the deployment of the Bcd gradient from the anterior pole of the embryo (Salles et al., 1994). We now show that Osk and Nos accumulation at the posterior pole also depends on Wisp. This highlights the general role of poly(A) tail length regulation in *Drosophila* early development.

Meiotic progression and translational control

In *Drosophila*, meiosis starts in the germarium, where several cells per germline cyst enter meiotic prophase. Meiosis is then restricted to a single oocyte that remains in prophase I during most of oogenesis. Progression to metaphase I (oocyte maturation) occurs in stage 13, with maintenance of metaphase I arrest in mature stage 14 oocytes (King, 1970). Arrested oocytes are then activated by egg laying, which induces the resumption of meiosis (Heifetz et al., 2001).

The earliest phenotypes in *wisp*-null mutant are defects in metaphase I arrest and in the progression beyond this stage. This suggests that Wisp-dependent cytoplasmic polyadenylation and translational activation are essential for meiosis during and after metaphase I (but not for oocyte maturation). Consistent with this, massive translation appears to be dispensable for the completion of meiosis (Page and Orr-Weaver, 1997), but translational activation of specific mRNAs, at least of *cort*, is required (Pesin and Orr-Weaver, 2007). We identify *cort* as a Wisp target: *cort* poly(A) tail elongation and Cort accumulation in mature oocytes require Wisp. Moreover, defects in Cort accumulation in *wisp* mutant oocytes result in impaired CycA destruction, an event thought to be critical for meiotic progression (Pesin and Orr-Weaver, 2007; Swan and Schupbach, 2007). We find that Wisp regulates many mRNAs at oocyte maturation, several of which might be involved at various steps of meiosis. Identification of these specific targets will be necessary to fully unravel the role of Wisp during meiosis.

Cytoplasmic polyadenylation has been linked to meiotic progression at egg activation given that some maternal mRNAs undergo poly(A) tail elongation at egg activation (Benoit et al., 2005; Salles et al., 1994; Vardy and Orr-Weaver, 2007). Moreover, *bcd* polyadenylation is affected in mutants that are defective in meiosis, such as *cort* mutants (Lieberfarb et al., 1996). It has been proposed that the link between cytoplasmic polyadenylation and egg activation results from the inactivation of canonical PAP activity by phosphorylation via the MPF (Mitotic promoting factor: Cdc2/CycB) (Chu et al., 2001). CycB degradation by APC-Cort would both induce meiotic progression and release PAP inactivation, leading to polyadenylation.

This model can be adapted with results presented here and in the recent literature (Pesin and Orr-Weaver, 2007; Vardy and Orr-Weaver, 2007). Two waves of cytoplasmic polyadenylation occur successively, one during oocyte maturation and one at egg activation. They both depend on Wisp poly(A) polymerase. The first wave is Orb-dependent and the pathway that triggers its activation is unknown. This polyadenylation induces the synthesis of Cort (and probably other proteins), which in turn is required for the second wave of cytoplasmic polyadenylation at egg activation. Cort could act in this process through the destruction of cyclins or of other proteins more specifically involved in the regulation of the polyadenylation machinery.

Two poly(A) polymerases function in translational control during oogenesis

A striking result in this paper is the requirement of two poly(A) polymerases for cytoplasmic polyadenylation during oogenesis. Since the discovery of GLD-2 poly(A) polymerases, it has been assumed that these proteins were responsible for cytoplasmic polyadenylation. Our data reveal a higher level of complexity to this regulation. The phenotypes of *wisp* mutants indicate a function of Wisp late in oogenesis. We find that entry into meiosis and restriction of meiosis to one oocyte, as well as DNA condensation in the karyosome, are unaffected in *wisp* mutants. By contrast, *orb*-null mutants arrest oogenesis in the germarium, with defects in the synchronous mitoses of cystoblasts and in the restriction of meiosis to one oocyte (Huynh and St Johnston, 2000) (I. Busseau and M.S., unpublished). We find that *orb* phenotypes corresponding to early defects in oogenesis, including oocyte determination and dorsoventral patterning, are dominantly enhanced by *hrg* mutants, strongly suggesting that canonical PAP and Orb act together in cytoplasmic polyadenylation during the first steps of oogenesis. Because Orb forms complexes with both PAP and Wisp, the same pools of mRNAs can be regulated by the two different complexes, at different steps of oogenesis. The inclusion of one or other poly(A) polymerase could allow for different types of regulation. In addition, it is possible that the presence of both poly(A) polymerases together in the complex could be required for some step of oogenesis.

In *Xenopus*, GLD-2 catalyzes polyadenylation during oocyte maturation (Barnard et al., 2004; Rouhana et al., 2005), but the enzymes involved after fertilization have not been identified. Moreover, polyadenylation at earlier stages of oogenesis remains unexplored.

CPEB function has been addressed genetically in mouse and the defect in the female germline of *Cpeb*-knockout mice was found to be during prophase I (Tay and Richter, 2001). By contrast, GLD-2 expression in the oocytes appears to start at metaphase I (Nakanishi et al., 2006). Moreover, no female germline defective phenotype was observed in GLD-2 knockout mice (Nakanishi et al., 2007). This demonstrates some level of redundancy in poly(A) polymerase function in mouse female meiosis, and indicates that the involvement of different types of poly(A) polymerase for translational activation in oogenesis and meiotic progression is common to other species.

We thank A. Nakamura, P. Macdonald, P. Lasko, J. Reinitz, S. Hawley, D. Glover, J. Pesin and T. Orr-Weaver for gifts of antibodies; the Bloomington Stock Center for *Drosophila* stocks; M. Wolfner for sharing unpublished data; and I. Busseau for comments on the manuscript. This work was supported by the CNRS UPR1142, 'ANR Blanche' (ANR-06-BLAN-0343) and FRM (Equipe FRM 2007) to M.S., and by NIH (GM31892 and 50942) grants to M.W. P.B. held awards from the Ministère de la Recherche and from the Association pour la Recherche sur le Cancer. J.E.K. held a Mary Shine Peterson Fellowship.

Supplementary material

Supplementary material for this article is available at http://dev.biologists.org/cgi/content/full/135/11/1969/DC1

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