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

Perrine BENOIT, Catherine PAPIN, Jae Eun KWAK, Marvin WICKENS and Martine SIMONELIG

Supplementary Information

Supplementary Materials and methods

RNA

Real-time PCR (QRT-PCR) were performed with the Lightcycler System (Roche Molecular Biochemical) with the primers indicated below.

Specific oligos for PAT assays were:

osk	5'AAGCGCTTGTTTGTAGCACA
nos	5'TTTTGTTTACCATTGATCAATTTTTC
bcd	5'CATTTGCGCATTCTTTGACC
cyc B	5'GCTGGCCGAACACATCGGCG
sop	5'GGATTGCTACACCTCGGCCCGT
cort	5'GGCCAAGGACAAGTGCAGCTC
bic	5'GATACGGTTTTCGGAAGAATTG
Arc-p34	5'CTCCGCCAATTTGTTACAGTCATAG
aret	5'TAGTTTCTATTATTTGCTACATCATCC
cdc2	5'GGGTGACTCGGAAATTGACCAG
BicC	5'TAGCTGCGCTCGGGAAAGTGCACAG

cad	5’CTTCAGCCCGTGTAAATAGCCGC
Cdc6	5’CAAACCGCTGGTTAATTGCGCAT
CG3800	5’GCTGGAGAGGATGAGCTAAGCGG
cup	5’GAGTCAAGACCCAAACGGAGCG
CG7033	5’GTCCCTGTCGTGTAATCCTTCTAC
CG7101	5’ATGGATAAATGTAACGTTTTAATAGCG
CG9742	5’CGAAGAACAACATCGGCATGGTGG
CG15092	5’GGAGTGCACAAGGCCGAGGA
CG17018	5’GACTTCCTAAGAATGCAAATAAGAAAC
*His2Av*5’GTTGTAATCATTCTGTGCGCCAGC	
Hrb27C	5’CAAGCATTCTCCTGTACCCCACACTC
RnrS	5’CTGGCCCCGGACATCATTG
Mcm5	5’CGGAGCAGAATATCTTACAGGAC
mod	5’AGAGAATGGTGGTAAATCGTTTG
Pep	5’ATTTGACATTTGCATCCCAATTTGC
phol	5’CACGGATGAAGAGCAGTTCTAAGAG
smt3	5’GTTACTCCTCTTACAACTACACACTT
Set	5’GAGGAAGAGGATGAGGATGACAAG
gnu	5’CCGATTGGCCATGAATATTATCC
cdc2c	5’CGTGTCCAATAGCCCCAGATCTAGC
Df31	5’CAAAACAATTACGGCAAAATAACATATG
dhd	5’GTAAGCGCGAGATGTGGGTAGC
Rca1	5’CAACGTGCCCAGAACCGTGATCCG
mtrm	5’GACCTGGTGTCTTAAAGCTCGTCG
His4	5’GTGCTGCGTGATAACATCCAAGGTA

Hsp83 5' GGAGAAGTACACTGAGGATG

5' CAGGCAGCTCCAGACCCTCC

Oligos for RT-PCR were:

osk 5’GCCATATTGCTGAGCCACGCCC

5'CCAGTAGCGTGGAGGTGCTCG

nos 5'CGATCCTTGAAAATCTTTGCGCAGGT

5’TCGTTGTTATTCTCACAAAAGACGCA

bcd 5’CTGGGTCGACCAATGTCAATGGCG

5’GCTCTTGTCCAGACCCTTCAAAGG

sop 5’CACCCCAATAAAGTTGATAGACCT

5’ATCTCGAACTCTTTGATGGGAAGC

Oligos for QRT-PCR were:

nos 5'CGGAGCTTCCAATTCCAGTAAC

5’AGTTATCTCGCACTGAGTGGCT

rp49 5’CCAAGCACTTCATCCGCCACCAGTC

5’TCCGACCACGTTACAAGAACTCTCA

Oligos to produce a PCR probe for Southern blots of *cort* PAT assays were:

cort 5’GGCCAAGGACAAGTGCAGCTC

5’GGAGAGGCCCATCTTCTTGTGTTC

Western blots and immunostaining

Western blots and immunostaining were performed as reported (Benoit et al., 2005; Benoit et al., 1999). Antibody dilutions for western blots were: anti-Wisp 1:3000, rabbit anti-Bic-C (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 1:10000 (Whitfield et al., 1990), anti-Cort 1:2000 (Pesin and Orr-Weaver, 2007), anti-α-tubulin (Sigma T5168) 1:10000. Dilutions for immunostaining were: anti-Wisp 1:2000, anti-Osk (Kim-Ha et al., 1995) 1:500, anti-Nos (gift from A. Nakamura) 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, methanol fixation was performed as reported (Brent et al., 2000) and dilution of anti-α-tubulin (Sigma T9026) was 1:200. Meiotic spindles in stage 14 oocytes were visualized as previously described (Endow and Komma, 1997) using FITC-conjugated anti-α-tubulin (Sigma F2168).

Immunoprecipitations

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

References

Anderson, L.K., Royer, S.M., Page, S.L., McKim, K.S., Lai, A., Lilly, M.A. and Hawley, R.S. (2005) Juxtaposition of C(2)M and the transverse filament protein C(3)G within the central region of Drosophila synaptonemal complex. *Proc Natl Acad Sci U S A*, **102**, 4482-4487.

Benoit, B., Mitou, G., Chartier, A., Temme, C., Zaessinger, S., Wahle, E., Busseau, I. and Simonelig, M. (2005) An essential cytoplasmic function for the nuclear poly(A) binding protein, PABP2, in poly(A) tail length control and early development in Drosophila. *Dev Cell*, **9**, 511-522.

Benoit, B., Nemeth, A., Aulner, N., Kühn, U., Simonelig, M., Wahle, E. and Bourbon, H.M. (1999) The *Drosophila* poly(A)-binding protein II is ubiquitous throughout *Drosophila* development and has the same function in mRNA polyadenylation as its bovine homolog *in vitro*. *Nucleic Acids Res.*, **27**, 3771-3778.

Brent, A.E., MacQueen, A. and Hazelrigg, T. (2000) The Drosophila wispy gene is required for RNA localization and other microtubule-based events of meiosis and early embryogenesis. *Genetics*, **154**, 1649-1662.

Endow, S.A. and Komma, D.J. (1997) Spindle dynamics during meiosis in Drosophila oocytes. *J Cell Biol*, **137**, 1321-1336.

Juge, F., Zaessinger, S., Temme, C., Wahle, E. and Simonelig, M. (2002) Control of poly(A) polymerase level is essential to cytoplasmic polyadenylation and early development in Drosophila. *EMBO J.*, **21**, 6603-6613.

Kim-Ha, J., Kerr, K. and Macdonald, P.M. (1995) Translational regulation of oskar mRNA by bruno, an ovarian RNA-binding protein, is essential. *Cell*, **81**, 403-412.

Kosman, D., Small, S. and Reinitz, J. (1998) Rapid preparation of a panel of polyclonal antibodies to *Drosophila* segmentation proteins. *Dev. Genes Evol.*, **208**, 290-294.

Pesin, J.A. and Orr-Weaver, T.L. (2007) Developmental Role and Regulation of cortex, a Meiosis-Specific Anaphase-Promoting Complex/Cyclosome Activator. *PLoS Genet*, **3**, e202.

Saffman, E.E., Styhler, S., Rother, K., Li, W., Richard, S. and Lasko, P. (1998) Premature translation of oskar in oocytes lacking the RNA-binding protein bicaudal-C. *Mol Cell Biol*, **18**, 4855-4862.

Tadros, W., Houston, S.A., Bashirullah, A., Cooperstock, R.L., Semotok, J.L., Reed, B.H. and Lipshitz, H.D. (2003) Regulation of maternal transcript destabilization during egg activation in Drosophila. *Genetics*, **164**, 989-1001.

Whitfield, W.G.F., Gonzalez, C., Maldonado-Colina, G. and Glover, D.M. (1990) The A- and B-type cyclins of *Drosophila* are accumulated and destroyed in temporally distinct events that define separable phases of the G2-M transition. *EMBO journal*, **9**, 2563-2572.

Zaessinger, S., Busseau, I. and Simonelig, M. (2006) Oskar allows nanos mRNA translation in Drosophila embryos by preventing its deadenylation by Smaug/CCR4. *Development*, **133**, 4573-4583.

Supplementary Figure Legends

Supplementary Figure 1: mRNA targets of Wisp during late oogenesis

PAT assays of 30 mRNAs showing that Wisp has many targets. The poly(A) tail lengths of the 30 mRNAs were reduced in *wisp*^{KG5287} stage 14 oocytes compared to wild type. The top of each poly(A) tail is indicated. *His4* has two poly(A) sites, both mRNAs are regulated by Wisp. *sop* mRNA was used as a control.

Supplementary Figure 2: PAP and Orb expressions overlap during early oogenesis

(A-C) Immunostaining of germarium and stage 3 egg chamber with anti-PAP (A) (1:500) and anti-Orb (B) (1:5000, acite produced from 6H4). (C) merge. In addition to PAP nuclear

expression, substantial amounts of PAP are present in the cytoplasm of germline cells. This cytoplasmic expression overlaps with Orb expression. Single confocal sections are shown.

Supplementary Figure 3: Lack of Bcd accumulation and premature mRNA destabilization in *wisp* mutant embryos

(A) Immunostaining of 0-1 h embryos with anti-Bcd showing that Bcd protein does not accumulate in $wisp^{12\text{-}3147}$/*Df(1)RA47* embryos. Bcd protein gradient has been shown earlier to be unaffected in $wisp^{181}$ mutant embryos (Tadros et al., 2003). We could not reproduce this result using two different *wisp* mutant combinations, $wisp^{KG5287}$/*Df(1)RA47* (Figure 7C) and $wisp^{12\text{-}3147}$/*Df(1)RA47*. In these two *wisp* mutant combinations, one of which corresponds to the null, Bcd protein does not accumulate in embryos (in a small number of embryos, faint amounts of unlocalized Bcd protein can be seen).

(B) Quantification of *nos* mRNA levels in 0-1h and 2-3h wild-type and $wisp^{KG5287}$ embryos by QRT-PCR. *nos* levels were normalized with *rp49*. The ratio of *nos* mRNA/*rp49* mRNA was set to 100 in 0-1h wild-type embryos. In the wild type, *nos* mRNA is destabilized in 2-3h embryos. In *wisp* mutant embryos, *nos* mRNA levels are low both in 0-1 h and in 2-3 h embryos showing that destabilization occurs prematurely in 0-1 h embryos. Two experiments with independent RNA preparations are shown.

(C) RT-PCR of *Hsp83* mRNA in 0-1 h, 1-2 h and 2-3 h wild-type and $wisp^{KG5287}$/*Df(1)RA47* embryos showing that *Hsp83* mRNA is destabilized earlier in *wisp* mutant embryos than in wild type. *sop* mRNA was used as a loading control. Maternal mRNA destabilization was reported previously to be prevented in *wisp* mutant embryos, using *Hsp83* as a test mRNA (Tadros et al., 2003). Therefore, we analysed *Hsp83* mRNA stability in $wisp^{KG5287}$/*Df(1)RA47* embryos. We could not reproduce the lack of *Hsp83* mRNA destabilization. In contrast,

Hsp83 mRNA destabilization was premature in *wisp*^{KG5287}*/Df(1)RA47* embryos (C), as it is the case for destabilization of *nos* and *osk* mRNAs.