Valois, a component of the nuage and pole plasm, is involved in assembly of these structures, and binds to Tudor and the methyltransferase Capsuléen

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Summary

Using the Capsuléen (Csul) methyltransferase as bait in the yeast two-hybrid system, we have identified a novel *Drosophila* protein containing multiple WD repeats and encoded by the *valois* (*vsl*) gene, which acts in pole plasm function. Vls is homologous to human MEP50, which forms a complex with the PRMT5 methyltransferase – the human homologue of Csul. We found that Vls localizes to the nuage in the nurse cells and to the pole plasm in the oocyte. Moreover *vls* is required for the synthesis and/or stability of Oskar and the localization of Tudor (Tud) in both the nuage and at the posterior pole of the oocyte. Furthermore, we show that Vls and a fragment of Tud interact directly in binding assay. As the PMRT5/MEP50 complex is involved in ribonucleoprotein complex assembly, we hypothesize that the Vls complex may play a similar function in assembling the nuage in nurse cells and the polar granules in the oocyte.

Key words: *Drosophila*, Oogenesis, *valois*, *Tudor*, Pole plasm assembly

Introduction

In *Drosophila*, the formation and fate of the germ cells depend on components distinctly located at the posterior pole of the egg – the pole plasm (Geigy, 1931). Functional and structural characteristics of the germ plasm confer specific properties to this region of the egg. First, transplantation of posterior plasm from oocytes or early cleavage embryos induces ectopic formation of pole cells within the recipient embryos (Illmensee and Mahowald, 1974; Okada et al., 1974). Second, the pole plasm is devoid of yolk particles, but exhibits electron-dense, ribonucleoprotein-rich organelles without delimiting membranes, called polar granules (Huettner, 1923; Mahowald, 1962; Illmensee et al., 1976). Third, the respiratory activity of the mitochondria located in the pole plasm is higher than that detected in other regions of preblastoderm embryos (Akiyama and Okada, 1992). Fourth, in addition to its ability to induce formation of ectopic pole cells, the pole plasm also contains an activity required for abdominal development, designated as the posterior determinant (Frohnhöfer et al., 1986; Lehmann and Nüsslein-Volhard, 1986).

Although germline formation and abdominal development represent distinct developmental processes, molecular and genetic analyses of the posterior-grandchild-less group of genes indicate that both processes share a common pathway for producing functional determinants localized at the posterior pole of the embryo. So far four genes are known to be directly required in pole plasm function, including *osk* (Lehmann and Nüsslein-Volhard, 1986), *vasa* (*vas*) (Schüpbach and Wieschaus, 1986), *tudor* (*tud*) (Boswell and Mahowald, 1985) and *valois* (*vls*) (Schüpbach and Wieschaus, 1986). Mutants in all four genes are characterized by the absence or strong reduction of discernible polar granules (Schüpbach and Wieschaus, 1986; Boswell and Mahowald, 1985).

Formation of pole plasm initially depends upon the localization of *osk* mRNA (Ephrussi and Lehmann, 1992), which becomes concentrated at the posterior end of the oocyte in stage 8 egg chambers (Ephrussi et al., 1991; Kim-Ha et al., 1991). Shortly thereafter, *osk* mRNA is translated into two isoforms by use of different initiation codons (Markussen et al., 1995; Rongo et al., 1995), and the short form of Osk induces the assembly of polar granules by recruiting directly the Vas protein, a member of the DEAD-box family of putative RNA helicases (Lasko and Ashburner, 1988; Hay et al., 1988b) at the posterior pole of the oocyte (Breitwieser et al., 1996). Vas may contribute to the synthesis of specific polar granule components, as suggested by its requirement for promoting translation of *nanos* (*nos*) (Gavis et al., 1996) and *gurken* (Styhler et al., 1998; Tomancak et al., 1998). The other factor involved in pole plasm function is Tud (Golumbeski et al., 1991), which accumulates in polar granules but is also detected in the nuage of nurse cells and at the periphery of embryonic nuclei (Bardsley et al., 1993). Although the molecular nature of *vls* remained elusive, this gene appears to be involved in pole plasm function (Schüpbach and Wieschaus, 1986).

Formation of abdomen and pole cells can be mechanistically uncoupled. The *nos* gene contributes to abdominal patterning, albeit not to pole cell formation (Lehmann and Nüsslein-Volhard, 1991). The localization of *nos* mRNA to the posterior pole of the embryo (Wang and Lehmann, 1991) depends upon

genes acting in pole plasm and produces a transient gradient of Nos proteins emanating from the posterior pole (Wang et al., 1994). Unlike the posterior determinant, the determinant involved in pole cell formation is likely to consist of multiple elements. The gene *germ cell-less* (*gcl*) is one of them (Jongens et al., 1992), and ectopic expression of *gcl* mRNA at the anterior pole of the embryo produces nuclei with characteristics of pole cell nuclei with which Gcl protein becomes associated on the inner rim of the nuclear pores (Jongens et al., 1994). Furthermore, mitochondrial ribosomal RNA (mtlrRNA) also contributes to germ-line determination (Iida and Kobayashi, 1998). Transport of the mtlrRNA from mitochondria to the surface of polar granules is mediated by Tud protein through a yet unknown mechanism (Amikura et al., 2001).

During our analysis of the *capsuléen* gene (*csul*; *CG3730*), we found one major protein partner by using the yeast two-hybrid system. In this report, we show that the protein interacting with Csul is encoded by the *vls* gene and describe its function in the process of pole cell formation.

Materials and methods

Molecular biology

Plasmid constructs were generated by amplifying selected fragments by PCR (High Fidelity PCR Master, Roche) and cloned into appropriate vectors. *vas* and *tud* cDNA plasmids were kindly provided by P. Lasko and R. Boswell, respectively. *vls* cDNA (clone GM04727, BDGP-EST project) was purchased from Invitrogen and *gus* cDNA (clone LD34464, BDGP-EST project) was obtained from the *Drosophila* Genomics Resource Center (DGRC; Bloomington, IN). The plasmid pSM 492 (3×HA) was kindly provided by S. Michaelis.

To rescue the *vls* phenotype a 2.04 kb genomic DNA fragment containing the *vls* transcription unit and 535 and 195 nucleotides of the 5′ and 3′ genomic regions, respectively, was cloned into the *P*-element vector *CaSpeR4*. The construct was named *P-[vls]*. Details will be provided upon request. To generate a Vls-HA fusion protein construct, a *Bgl*II site was created at the 3′ end of the *vls*-coding sequence of a *vls* cDNA containing identical 5′ and 3′ untranslated regions as the genomic transgene. A fragment with 3×HA tags was cut with *Bgl*II from plasmid pSM 492 and cloned into the *vsl*-coding sequence. Details will be provided upon request. A clone containing six copies of HA was selected for microinjection.

Sequencing of mutant alleles

The *vls*-coding region was amplified by PCR (High Fidelity PCR Master; Roche) from genomic DNA obtained from single vls^1, vls^2 and vls^3 flies (primers: sense 5′-gttgctcttccttgctggccgattctc-3′, located at position –58 from the translation initiation codon; and antisense, 5′-tgcatttaaactgggctgctgcttcac-3′). The PCR products of 1.46 kb were cloned into pBluescript. Two clones from independent PCR reactions were sequenced for each allele using primers covering the coding region. Nucleotide differences from the wild-type *vls* sequence were distinguished from PCR errors by their appearance in both independent clones.

Yeast two-hybrid screen

Standard yeast two-hybrid methods were employed, using the GAL4 system (*Saccharomyces cerevisiae* strain Y190). The Csul-coding region was cloned into the yeast vector pGBT9 to produce a bait construct. This construct was used to screen the *Drosophila* embryo MATCHMAKER cDNA library (Clontech). About 1.2 millions clones were screened.

GST pull-down assay

Full-length *vls* and *vas* cDNAs were subcloned into pGEX6P2 (Pharmacia), full-length *csul*, *gus* and *tud* cDNAs into pCITE-4 (Novagen). Recombinant proteins were synthesized in vitro using the TNT T7 Coupled Reticulocyte Lysate System (Promega) in the presence of unlabeled amino acids. GST-fusion proteins expressed in *E. coli* were purified with glutathione sepharose (Pharmacia) and washed with the binding buffer [20 mM HEPES (pH 7.8), 10% glycerol, 300 mM NaCl, 0.1% sodium deoxycholate, 0.1% NP40 and 0.1% Triton X-100] plus protease inhibitors (Complete EDTA free from Roche; 1:50 dilution). Recombinant proteins (10% of the reaction volume) were added to this mixture (in 1 ml) and incubated for 3 hours at room temperature. The beads were washed six times (10 minutes each) with binding buffer, boiled in loading buffer, and the proteins were separated by electrophoresis on 8% or 12% SDS-polyacrylamide gels. After transfer to a polyvinylidene difluoride (PVDF) membrane, the bound proteins were detected by Western blotting using an S-protein Alkaline Phosphatase conjugate (Novagen).

Immunoprecipitation

Fly ovaries were homogenized using a plastic pestle in ice-cold IP buffer [145 mM NaCl, 10% glycerol, 1 mM $MgCl_2$, 1.5 mM NaH_2PO_4, 8 mM Na_2HPO_4 (pH 7.4) and 0.5% NP40] containing protease inhibitors. Lysates were clarified by centrifugation at 14,000 ***g*** for 10 minutes at 4°C. The supernatants were removed and mixed with 400 units of RNasin (Promega), 30 μl of anti-HA antibody-coupled protein A beads for 5 hours at 4°C on a rotator. The IP mix was centrifuged at low speed to remove the supernatant. Protein A-beads were then washed four times with IP buffer for 10 minutes each and TAP-Csul was detected on a western blot by using alkaline phosphatase-conjugated IgG (Sigma). TAP-Csul purification was essentially performed as described by Rigaut et al. (Rigaut et al., 1999).

Immunocytochemistry

Immunostaining was performed with primary antibodies, including anti-Mael from rabbit (Findley et al., 2003), anti-Osk from rabbit (gift of A. Ephrussi), anti-Vas from rabbit and rat (gift of P. Lasko and A. Nakimura, respectively), anti-Tud from rabbit (TUD56; gift of S. Kobayashi), and monoclonal anti-HA (clone 16B12; BAbCO). To reduce background, we incubated first the anti-HA antibodies (1:400) for 3 hours at room temperature with wild-type ovaries and then overnight with experimental ovaries. For immunostaining, the ovaries were fixed for 10 minutes in 4% paraformaldehyde. To visualize components of the nuage, we used the protocol of Findley et al. (Findley et al., 2003). Chromatin was visualized by staining with Oli-Green (Molecular Probes). Cy3-, Cy5- and FITC-conjugated secondary antibodies (Jackson ImmunoResearch Laboratories) were used at 1:200.

Results

Isolation of a major Capsuléen partner

The *capsuléen* gene (*csul*; *CG3730*) is required for pole cell formation in the pathway controlled by *osk* (J.A., R. Ollo, A. Ephrussi and B.M.M., unpublished). To identify Csul-interacting proteins active in pole plasm, we performed a yeast two-hybrid screen. The bait construct consisted of the sequence of the Csul protein (610 amino acid residues) fused to the DNA binding domain (BD) of Gal4. By screening a *Drosophila* cDNA library, we obtained six independent interacting clones of which four of them corresponded to the *CG10728* coding sequence predicted by the *Drosophila* genome project. Conceptual translation of *CG10728* cDNA revealed a protein

of 367 amino acids. Two cDNAs encoded a nearly full-size protein lacking the first 10 amino acid residues, whereas the other two isolates encoded a protein in which 37 residues were missing from the N terminus. Confirmation of the interaction was obtained by performing the reciprocal two-hybrid assay, showing that BD-CG10728 could strongly bind the activation domain of Gal4 fused to Csul (data not shown). Sequence analysis on the Protein Sequence Analysis Server (bmerc-www.bu.edu/psa/) indicated that CG10728 contains four WD repeats (Fig. 1A).

CG10728 codes for *valois*

The *CG10728* gene is located at chromosomal band 38B2. As *vls* was genetically assigned to this region (Schüpbach and Wieshaus, 1986), we investigated whether the *CG10728* sequence was altered in *vls* mutants. To determine the lesions in vls^1, vls^2 and vls^3, we amplified by PCR genomic DNA fragments encompassing the *CG10728*-coding sequence, then cloned and sequenced the amplified fragments from each mutant. For each *vls* mutant we found single nucleotide substitutions that result in premature termination of the coding sequence (Fig. 1A). In vls^1 and vls^2 the nucleotide substitutions changed a TGG Trp codon into TAG and TGA stop codons, producing truncated proteins of 227 and 52 amino acids, respectively. In vls^3, the nucleotide substitution transformed a CGA Arg codon into a TGA stop codon producing a truncated protein of 69 amino acids. To confirm that we cloned *vls*, we constructed two transgenes containing the presumed *vls* sequence, including its 5′ regulatory sequences. The first transgene contained a genomic DNA fragment comprising the *CG10728* transcription unit (Fig. 1B). The second transgene harboured the same sequence to which we fused six copies of the hemaglutinin tag (HA), in frame with the *vls*-coding sequence. Western blots of proteins extracted from ovaries producing HA-Vls probed with anti-HA antibodies displayed a single polypeptide with the expected mass of 50 kDa (Fig.

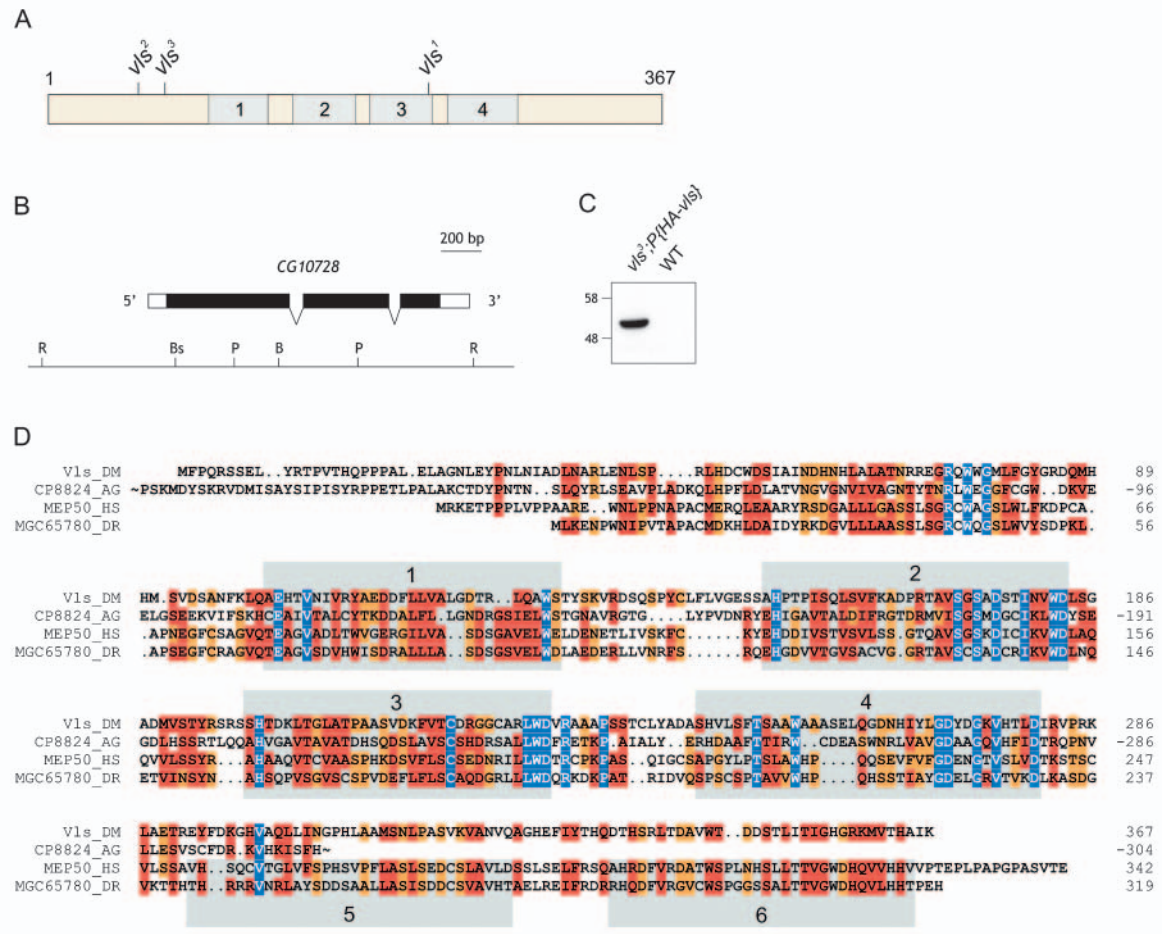


Fig. 1. Molecular analysis of *vls*. (A) Location of three EMS mutations in *vls*. All mutations lead to a premature stop codon. WD repeats are shown in grey. (B) The *vls* transcript and restriction map of the genomic DNA used for generating a *vls* rescue transgene. Exons are drawn as boxes and the putative open reading frame is indicated in black. B, *Bam*HI; Bs, *Bst*EII; P, *Pst*I; R, *Eco*RI. (C) Expression of the *P[HA-Vls]* transgene in ovaries revealed by western blotting using anti-HA antibodies. A band of ~50 kDa is detected in transgenic but not in wild-type ovaries. (D) Alignment of the amino acid sequences of Vls and homologous proteins: CP8824, an incomplete *Anopheles gambiae* homolog; human MEP50; and MGC65780, a zebrafish protein. These proteins display strong conservation of the WD repeats (grey boxes). MEP50 and the zebrafish homolog display six potential WD repeats, whereas *Drosophila* Vls contains only four such repeats, as predicted by the Protein Sequence Analysis server of the BioMolecular Engineering Research Center of Boston University (http://bmerc-www.bu.edu/psa/). Multiple sequence alignment of Vls and related proteins was performed using the Pileup program of the Wisconsin Package (Genetics Computer Group). Gaps in the amino acid sequence, indicated by dots, were introduced for optimal alignment. The GenBank accession numbers of these sequences are the following: CP8824_AG, EAA14269; MEP50_HS, AAL79917; MGC65780_DR, AAH56278.

1C). Both transgenes restored full viability and fertility to embryos produced by *vls*^{1} and *vls*^{3} homozygous females (data not shown). Taken together, our results demonstrate that *CG10728* corresponds to *vls*.

CsuI and Vls interact in vivo

Among the nearest homologues of Vls we identified in protein databases the methylosome protein 50 (MEP50) (Fig. 1D). MEP50, the human homologue of Vls, contains six WD repeats and interacts with PRMT5 (Friesen et al., 2002), the human homologue of CsuI. The finding of an interaction between CsuI and Vls in yeast prompted us to analyze whether such binding occurs in *Drosophila* ovaries. To this end, we constructed transgenic flies bearing both the *HA-vls* transgene and a Tandem Affinity Purification (TAP) (Rigaut et al., 1999) tagged *csul* transgene (*TAP-csul*) shown to restore *csul* development (data not shown). Protein extracts of *P{TAP-csul}; vls*^{3}*; P{HA-vls}* ovaries were immunoprecipitated using rabbit IgG-sepharose beads and the bound CsuI-complexes were released by treatment with recombinant TEV protease. Blots of released proteins probed with anti-HA antibodies showed an immunoreactive band of 50 kDa exhibiting the predicted mass of HA-Vls fusion protein (Fig. 2A). Reciprocally, blots of ovarian proteins immunoprecipitated with anti-HA antibodies and probed with IgG antibodies displayed an immunoreactive band of the mass predicted for TAP-CsuI (90 kDa; Fig. 2B). These results indicate that CsuI and Vls can associate in a protein complex in *Drosophila* ovaries.

Interaction domains in Vls and CsuI

To further characterize the interaction between CsuI and Vls, we mapped the binding domains in each protein by using a GST-pull down assay. For this purpose we fused Vls or fragments of Vls to GST, and CsuI or fragments of CsuI to S•Tag (Kim and Raines, 1993). In vitro translated S•Tag-CsuI polypeptides were incubated with immobilized GST-Vls

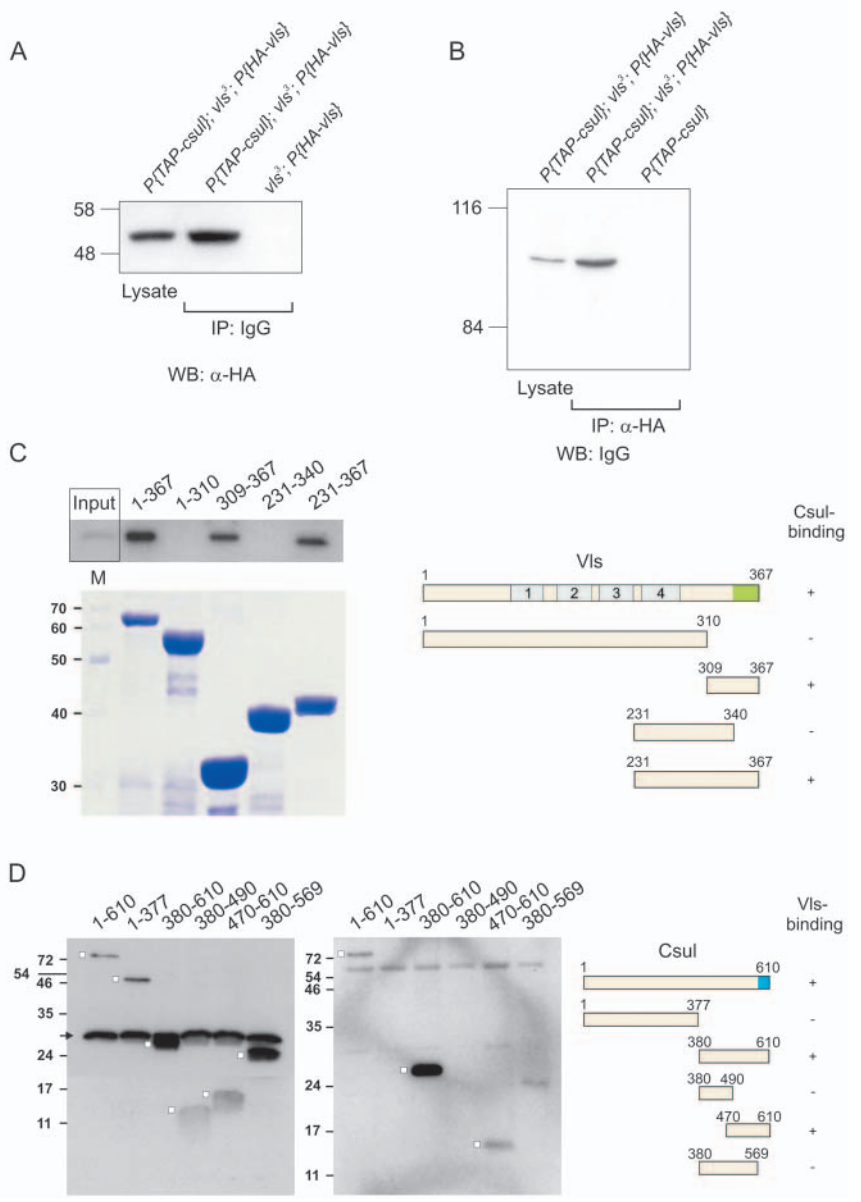
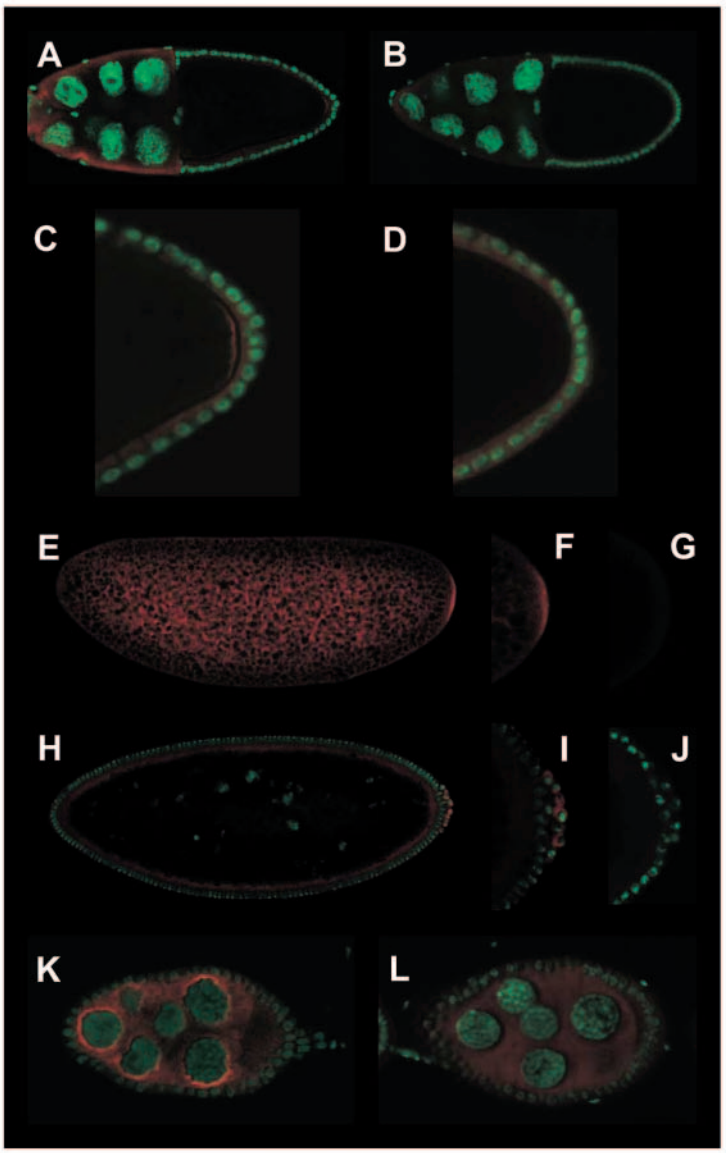


Fig. 2. Vls interacts physically with CsuI. (A) Vls interacts with CsuI in vivo. Ovarian protein extracts of homozygous *vls*^{3} females producing both TAP-CsuI and HA-Vls (lanes 1 and 2), or only HA-Vls (lane 3), were directly loaded on a gel (one tenth of total input, lane1) or immunoprecipitated with Sepharose IgG (lanes 2 and 3). Following separation by SDS-PAGE electrophoresis, bound-HA-Vls was detected by immunoblotting using anti-HA antibodies. (B) Reciprocally, Vls was immunoprecipitated using anti-HA antibodies and bound CsuI was detected by immunoblotting using alkaline phosphatase-conjugated anti-rabbit antibodies. (C) Binding of the C terminus of Vls to CsuI. Full-length GST-Vls or derivatives were purified from bacterial extracts and incubated with S•Tag-CsuI. Bound S•Tag-CsuI (75 kDa) was separated by SDS-PAGE electrophoresis and detected by immunoblotting using alkaline phosphatase-conjugated S proteins. Input: one-tenth of protein extract was loaded on the gel. The amount of used GST-fusion proteins was evaluated by SDS-PAGE followed by Coomassie staining (lower panel). Representation of the GST-Vls constructs used for the mapping and summary of results are indicated on the right. The WD-repeats and the putative CsuI-binding domain of Vls are indicated by grey and green boxes, respectively. (D) The C-terminal region of CsuI interacts with Vls. Full-size S•Tag-CsuI or derivatives were synthesized in vitro and incubated with full-size GST-Vls. Following separation by SDS-PAGE electrophoresis the bound S•Tag-CsuI proteins were detected by immunoblotting using alkaline phosphatase-conjugated S proteins. Left panel: input S•Tag-CsuI proteins. The arrow indicates an endogenous protein synthesized in the reticulocyte system and reactive to alkaline phosphatase-conjugated S proteins. White squares indicate the position of the different S•Tag-CsuI proteins. Middle panel: S•Tag-CsuI proteins bound to GST-Vls are indicated by white squares. Representation of S•Tag-CsuI constructs used for the mapping and summary of the results are indicated in the right panel. The putative Vls-binding domain of CsuI is depicted in blue.

proteins and, after washing, the bound S•Tag-Csul proteins were detected by using S-protein coupled to alkaline phosphatase. As shown in Fig. 2C, Csul bound specifically to immobilized full-length Vls, or to the 30 amino acid C terminal region of Vls, but not to the rest of the protein. GST alone displayed no binding to S•Tag-Csul (data not shown). Conversely, we mapped the region of Csul mediating the interaction with Vls to the C terminal region of Csul (Fig. 2D). Neither the N-terminal region nor the central region of Csul showed strong binding to Vls. Thus, our data indicate that Csul and Vls interact through their C-terminal regions.

Vls is present in nuage and pole plasm

To determine the distribution of Vls during oogenesis, we analyzed the localization of HA-Vls protein using anti-HA antibodies. Immunostaining of vls^3; *P{HA-vls}* egg chambers revealed that Vls accumulates at the posterior pole of the oocyte in stage 10 egg chambers (Fig. 3A,C) and in the pole plasm of early syncytial embryos (Fig. 3E,F). We also found Vls in pole cells (Fig. 3H,I) and in migrating primordial germ cells (data not shown). During oogenesis, Vls decorates a region surrounding the nurse cell nuclei (Fig. 3K), which bears strong similarity to the nuage (Findley et al., 2003). By contrast, immunostaining of wild type ovaries using anti-HA antibodies displayed only an overall residual staining (Fig. 3B,D,G,J,L). These data show that Vls is a component of both the nuage and the pole plasm.



Distribution of pole plasm and nuage components in *vls* egg chambers

Embryos produced by *vls* females fail to form pole cells at the posterior pole (Schüpbach and Wieschaus, 1986) (Fig. 4B). To check whether pole cell formation strictly depends on *vls* activity, we introduced an *osk-bcd* 3′UTR transgene into vls^1 and vls^3 backgrounds, and found no evidence for ectopic formation of pole cells at the anterior pole (Fig. 4D), although we could detect residual Vas staining in this region of the egg (data not shown). This result concurs with the suppressor effect of vls^1 on ectopic pole cell formation observed after overexpression of *osk* (Smith et al., 1992), but contrasts the data using an *osk-bcd* 3′UTR transgene expressed in vls^2/*Df(2R)TW2* flies by Ephrussi and Lehmann (Ephrussi and Lehmann, 1992). From our data, we conclude that *vls* function is essential for pole cell formation.

To determine *vls* hierarchical position among known components of the pole plasm, we investigated the distribution of Osk, Vas and Tud in vls^1 and vls^3 egg chambers. Use of specific antibodies against Osk revealed that Osk was normally localized at the posterior pole from stage 9 onwards egg chambers in *vls* (Fig. 4F). However, we often noticed that the amount of Osk protein was reduced in *vls* in comparison with wild type. To determine whether *vls* regulates Osk protein accumulation, we examined by immunoblotting the amount of Osk protein produced in wild-type and *vls* ovaries. We found that the level of Osk short form was markedly reduced in *vls* in comparison with that in wild type (Fig. 4G) (Rongo et al., 1995) and even more reduced in *aubergine* (*aub*) (Wilson et al., 1996). However, the phosphorylation of this isoform seems unaffected in *vls*. In addition, we noticed that the slower migrating form of the short Osk protein was particularly reduced in abundance in *aub* protein extract, suggesting a defective phosphorylation in *aub* ovaries. This result extends the previous observation of Wilson et al. (Wilson et al., 1996), showing a strongly reduced level of short Osk in *aub* ovaries and may explain the *aub* requirement for *osk* activity induced anteriorly by the *OB1 osk-bcd* 3′UTR transgene. Examination of Vas distribution showed a normal localization at the posterior pole in stage 10 *vls* egg chambers (Fig. 4I), albeit at a reduced level compared with wild-type egg chambers (Fig. 4H).

Fig. 3. Vls is a component of nuage and pole plasm. Fixed, whole-mount egg chambers stained with Oli-Green for DNA and anti-HA antibodies in homozygous vls^3 flies expressing *P{HA-Vls}* (A,C,E,F,H,I,K) and in wild-type flies as controls (B,D,G,J,L). HA-Vls is detected at the posterior pole of the oocyte in transgenic vls^3*; P{HA-Vls}* stage 10 egg chambers (A,C). (C,D) Enlarged views of similar stage 10 oocytes, as shown in A and B, respectively. HA-Vls is observed at the posterior pole of early vls^3*; P{HA-Vls}* embryos and in pole cells (E,F,H,I). (F,G,I,J) Enlarged views of similar syncytial and blastoderm embryos, as shown in E and H, respectively. HA-Vls is detected in the nuage surrounding the nurse cell nuclei (K).

Checking the distribution of Tud during oogenesis revealed that Tud was absent from the posterior pole of *vls* mutant oocytes in stage 10 egg chambers (Fig. 4K), indicating that Vls may play a role in pole plasm accumulation of Tud. As Tud is also a component of the nuage encircling nurse cell nuclei (Bardsley et al., 1993), we looked at this structure by examining the distribution of three components of the nuage including Tud, Vas (Hay et al., 1988a) and Maelstrom (Mael) (Findley et al., 2003). The use of a transgene expressing a GFP-Vas fusion protein (Sano et al., 2002) associated with the *vls* chromosome revealed that GFP-Vas was normally localized in the nuage of *vls* nurse cells, albeit at a lesser degree compared with wild type (Fig. 5B) (Hay et al., 1990; Lasko and Ashburner, 1990). Examination of Mael showed that this protein is also present in the nuage, although its distribution diverges from the wild-type pattern. We found that Mael is concentrated in brighter spots in *vls* than in wild type at the periphery of the nurse cell nuclei (Fig. 5D). By contrast, we were unable to detect Tud in the nuage of *vls* nurse cells (Fig. 5F), although Tud accumulates in *vls* oocytes (Fig. 5F) and transiently localizes at their anterior margin (data not shown). These findings indicate that: (1) *vls* acts downstream of Vas for the recruitment of Tud in the nuage; and (2) events occurring in the nuage are dispensable for the transport of Tud from the nurse cells to the oocyte but are required for Tud recruitment in the pole plasm.

Examination of *vls* egg chambers revealed also a modification in the structure of the oocyte nucleus in which the karyosome is fragmented into two fuzzy spots (Fig. 5H) instead of forming a single compact dot in wild-type nuclei (Fig. 5G). A similar phenotype was described for *spindle* (González-Reyes et al., 1997) and *vas* mutants (Tomancak et al., 1998). As *vls* oocytes undergo normal meiosis and support normal embryonic development, this finding suggests that Vls may exert a dispensable function on the karyosome organization.

Vls physically interacts with Tud in vitro

The presence in Vls of four WD repeats able to interact with other proteins (reviewed by Smith et al., 1999) prompted us to examine whether Vls could physically interact with proteins localized in the nuage. For this purpose, we performed pull-down assays using tagged Tud, Vas and Gustavus (Gus) (Styhler et al., 2002) polypeptides.

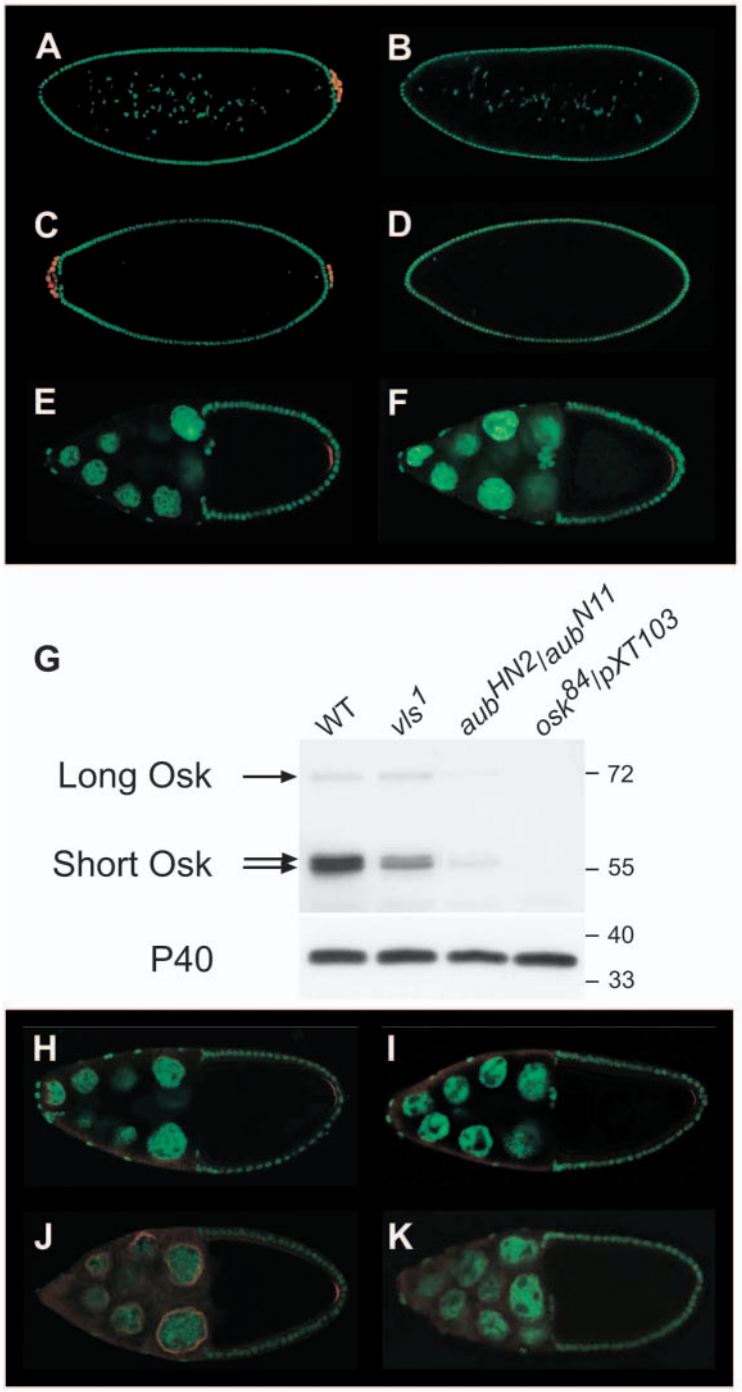


Fig. 4. Defective pole plasm assembly in *vls*. (A,B) Absence of pole cell formation in vls^3. Staining of Vas with rat anti-Vas antibodies (red) and DNA with Oli-Green (green) in wild-type (A) and vls^3 (B) blastoderm embryos. (C,D) *vls* suppresses ectopic anterior pole cell formation induced by *osk-bcd 3′UTR*. Staining of Vas protein (red) and DNA (green) in *osk-bcd 3′UTR* (C) and vls^3; *osk-bcd 3′UTR* (D) blastoderm embryos. No pole cells form at the anterior pole of vls^3; *osk-bcd 3′UTR* embryos. (E,F) Osk is localized at the posterior pole of the oocyte in vls^3 stage 10 egg chambers, albeit in reduced amount. (E) Wild-type and (F) vls^3 egg chambers. (G) The level of Osk protein is reduced in *vls*. Ovarian protein extracts of wild-type (WT), vls^1, aub^{N11}/aub^{HN2} and $osk^{84}/Df(3R)pXT103$ females were separated by SDS-PAGE electrophoresis, blotted and detected using anti-Osk antibodies. The abundance of short Osk is reduced in *vls* mutant ovaries, whereas the long form is maintained at the wild-type level. Both Osk forms are strongly reduced in *aub* ovaries, with a more substantial effect on short Osk. In contrast to *aub*, the phosphorylation of short Osk is not affected in *vls*. The same blot was successively probed for ribosomal P40, as loading control (Török et al., 1999). (H,I) Vas is localized at the posterior pole of the oocyte in wild type (H) and vls^3 (I) stage 10 egg chambers. There is less localized Vas in vls^3 compared with wild type. Ovaries were stained with rabbit anti-Vas antibodies (red) and Oli-Green for DNA (green). (J,K) Tud is detected in nuage and at the oocyte posterior pole in wild-type stage 10 egg chambers (J) but is not detected at these locations in vls^3 stage 10 egg chambers (K). Ovaries were stained with anti-Tud antibodies (red) and Oli-Green for DNA (green).

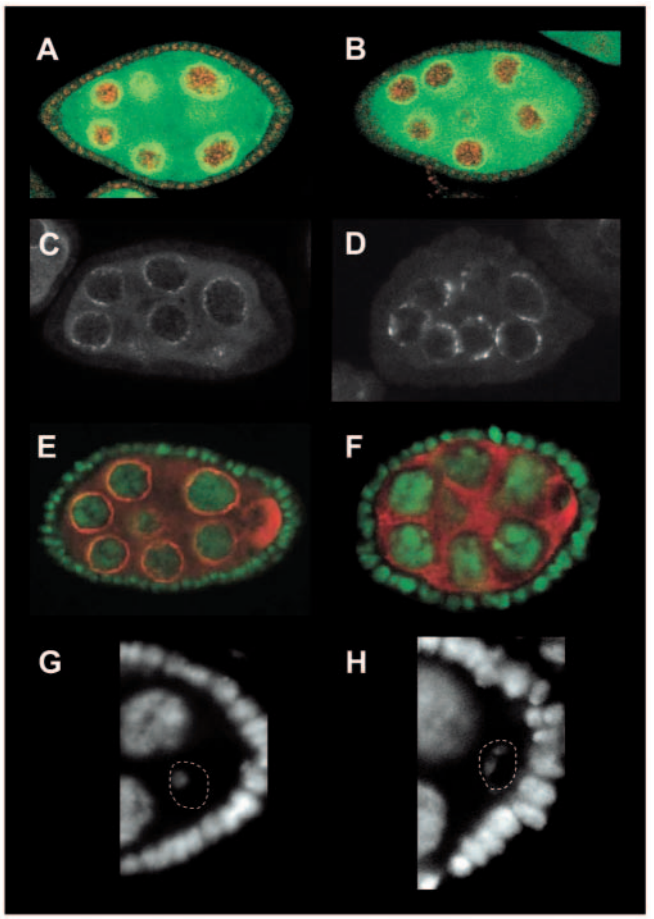


Fig. 5. Displacement of nuage components during early *vls* oogenesis. (A,B) GFP-Vas and (C,D) Mael accumulate in nuage in stage 7 egg chambers in wild type (A,C) and vls^3 (B,D). GFP-Vas (green) was detected in fixed egg chambers stained with Hoechst 33258 for DNA (red). (E,F) Tud accumulates in nuage in wild type stage 7 nurse cells (E), whereas it is homogenously dispersed in the nurse cell cytoplasm in vls^3 stage 7 egg chambers (F). Ovaries were stained using anti-Tud antibodies (red) and Oli-Green for DNA (green). Tud is found at high levels in wild type and vls^3 oocytes. (G,H) Staining of DNA with Oli-Green reveals that the karyosome, which appears as a single compact dot in wild-type oocytes (G), is frequently fragmented in vls^3 oocytes (H). The oocyte nuclear membrane is indicated by a broken line.

Five segments of Tud fused to the S•Tag, including JOZ (amino acid residues 3-273), 9A1 (residues 198-1199), 3ZS+L-N (residues 1198-1981) and 3ZS+L-C (residues 1941-2515) (Golumbeski et al., 1991), together comprising the complete Tud protein (Fig. 6A), were in vitro translated and the synthesized polypeptides were incubated with immobilized full-length and truncated GST-Vls proteins. Of the five Tud fragments, we found that only the 9A1 fragment could interact with Vls, whereas JOZ and the two subfragments of 3ZS+L showed only weak binding (Fig. 6C). The 9A1 fragment was further divided into two fragments. The 9A1-N and 9A1-C fragments (residues 198-770 and 751-1199, respectively) displayed a strong binding to Vls (Fig. 6C).

In the reciprocal experiment, we found that the Tud 9A1-N fragment can bind to the region encompassing residues 90-190 of Vls. When this segment was further divided into two A and B fragments, containing residues 90-139 and 139-192, respectively, we discovered that only the fragment A was able to bind to Tud 9A1-N. These results indicate that the first WD repeat of Vls can directly interact with Tud (Fig. 7).

No specific Vls binding with Vas and Gus polypeptides was detected in GST pull-down assays using GST, GST-Vls and GST-Vls (residues 90-190). Similarly, no interaction could be uncovered between Vls and Vas in the yeast two-hybrid assay (data not shown). These data indicate that the binding between Vls and Tud represents a specific interaction.

Discussion

By analyzing potential interactors of the putative Csul methyltransferase, we isolated the *vls* gene. The confirmation that we cloned *vls* was obtained by determining the molecular lesions in three EMS-induced *vls* mutations and by showing that development and fertility of *vls* mutant flies is restored by transgenes containing the *vls* genomic sequence.

Attempts to isolate additional partners of Vls by using the yeast-two hybrid system were unsuccessful because of the occurrence of a too large number of clones growing on selective medium (data not shown), presumably reflecting the occurrence of WD repeats that are known to mediate interactions with numerous proteins in eukaryotic cells (Smith et al., 1999). By using direct binding assays with proteins involved in pole plasm function, we found that Vls interacts with Tud.

Vls is a member of the WD-repeat protein superfamily

The feature characterizing the superfamily of WD-repeat proteins is the WD motif, a ~40 amino acid stretch typically containing a GH dipeptide 11-24 residues from its N-terminus and a WD dipeptide at its C terminus, albeit exhibiting only limited amino acid sequence conservation (Yu et al., 2000). When present in a protein, the WD motif typically occurs in multiple tandem repeated units. Based on structural analysis, the conformation of the WD repeat is defined as a series of four-stranded anti-parallel β sheets, which fold into a higher-order structure termed a β-propeller. At least four repeats are required to constitute a β-propeller (Chothia et al., 1997). We found that Vls contains four conserved WD repeats and can potentially form a β-propeller structure. Interestingly, the nearest mammalian homologue of Vls, the human MEP50 protein, displays six WD repeats. Although the sequence of the two additional repeats is conserved overall in *Drosophila* Vls, no characteristic GH and WD di-peptides could be found in these repeats (Fig. 1D).

WD-repeat proteins act as scaffolding/anchoring proteins for a number of binding partners (Smith et al., 1999). WD-repeat motifs within one protein can simultaneously bind several proteins and foster transient interactions with other proteins. Moreover, WD-repeat proteins occur in relatively stable protein complexes in which they play a structural role. A similar function can be assigned to Vls in promoting either permanent or transient interaction with other proteins.

Evolutionary conserved interaction between Vls and Csul

The human homologue of Vls, the MEP50 protein, was

identified through its association with PRMT5 (Friesen et al., 2002), the human homologue of *Drosophila* Csul methyltransferase. We show that the domain of Vls required for binding to Csul is small and confined to its C-terminal end. By contrast, the domain of MEP50 specifically involved in the interaction with PRMT5 remains unknown.

MEP50 acts as an adaptor binding to a subset of the Sm proteins and contributing to their methylation by the PRMT5

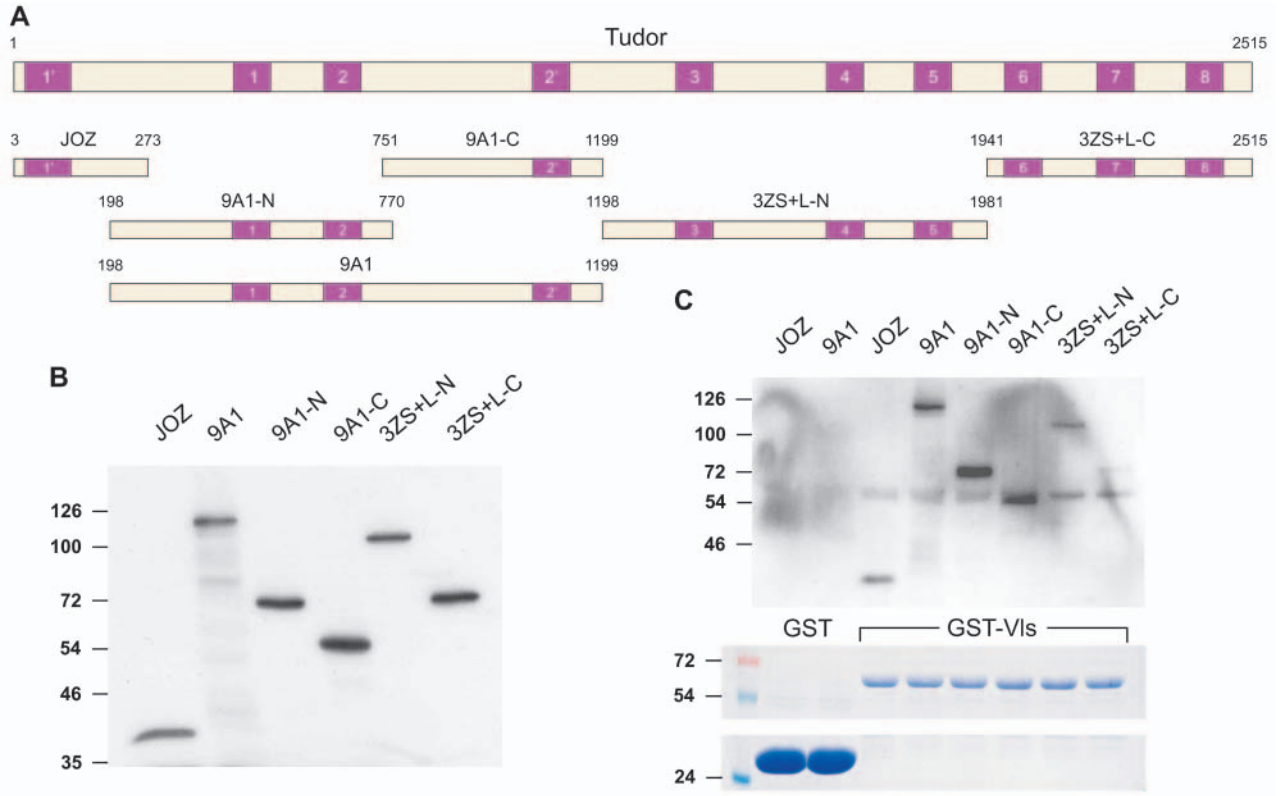


Fig. 6. Vls physically interacts with Tud. (A) The eight Tudor (1-8) and two Tudor-like domains (1′-2′) are depicted as purple boxes. Fragments of Tud (Golumbeski et al., 1991) used in pull-down assays are indicated below the map. N- and C-terminal amino acid residues of each fragment are indicated. (B) Western blot analysis of in vitro produced S•Tag-Tud fragments detected by using alkaline phosphatase-conjugated S proteins. (C) After incubation with GST or GST-Vsl, the bound Tud fragments were detected by immunoblotting using alkaline phosphatase-conjugated S proteins: the Tud-9A1-N and Tud-9A1-C fragments display a strong binding to GST-Vls, whereas Tud-JOZ and Tud-3ZS+L bind only weakly to it. GST alone (negative control) exhibits no binding to Tud-JOZ and Tud-9A1. Lower panels: input GST-Vls and GST proteins separated on a SDS-PAGE gel and stained with Coomassie.

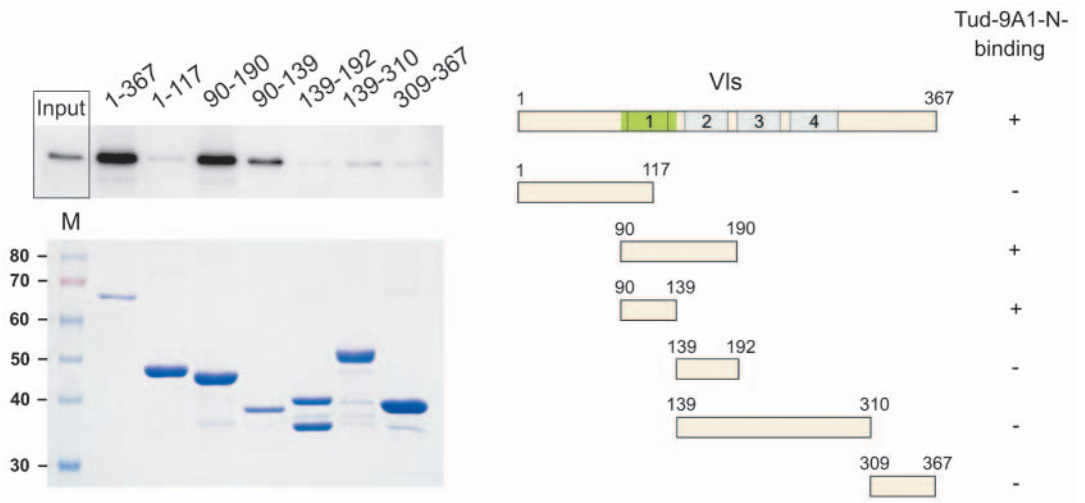


Fig. 7. Tud-binding domain in Vls. As performed in Fig. 2C, full-length GST-Vls or derivatives were incubated with S•Tag-Tud9A1-N. Bound S•Tag-Tud9A1-N was detected as described in Fig. 6B. Input: one-tenth of the S•Tag-Tud9A1-N extract. The smallest fragment of Vls showing binding to Tud9A1-N encompasses the first WD domain shown in green. The amount of GST-Vls proteins was visualized by Coomassie staining (lower panel). Representation of the GST-Vls fragments used for mapping and summary of the results are indicated on the right panel.

methyltransferase (Friesen et al., 2002). Biochemical assays indicate that MEP50 is necessary for the methyltransferase activity of the methylosome, as anti-MEP50 antibodies significantly reduce the methylation of Sm proteins (Friesen et al., 2002). However, the precise role of MEP50 remains elusive. Its possible functions include a regulation of the enzymatic activity of PMRT5 and the control of the positioning of the substrate for methylation.

The human methylosome complex is involved in the assembly of spliceosomal U-rich small nuclear ribonucleoproteins (snRNPs) mediated by the survival motoneuron (SMN) protein, a gene product that is affected in spinal muscular atrophy (Lefebvre et al., 1995; Meister et al., 2002). SMN is produced ubiquitously and contains a single Tudor domain that associates with SmB/B′, SmD1-D3 and SmE proteins of snRNPs (Liu et al., 1997; Buhler et al., 1999; Selenko et al., 2001). The assembly of snRNPs mediated by SMN occurs in the cytoplasm and is stimulated by the PMRT5-methylosome complex that converts specific arginine residues in the Sm proteins into dimethylarginines (Meister at al., 2001; Friesen et al., 2001a; Branscombe et al., 2001), facilitating the binding of the Sm proteins to SMN and their association with snRNA molecules (Friesen et al., 2001b; Brahms et al., 2001). Ultimately, the assembled snRNPs are released and targeted to the nucleus, whereas the SMN-PRMT5 complex may dissociate before its components associate again for a new round of assembly.

In *Drosophila*, the Tud protein differs from SMN by containing eight Tudor domains and two Tudor-like domains (Callebaut and Mornon, 1997), whereas SMN contains a single Tudor domain (Pontig, 1997). It is thus possible to envisage that *Drosophila* Tud may bind different categories of cytoplasmic RNPs through its multiple Tudor domains. However, in contrast to the PMRT5/MEP50 complex that apparently binds SMN through other protein(s) present in the complex, we found that Vls can directly bind to Tud through its first WD repeat. As the C-terminal tail of Vls binds to Csul, it is possible that the *Drosophila* Csul/Vls methylosome associates with Tud through Vls. Thus, we propose that the association between the methylosome and Tud promotes binding and assembly of specific RNPs on Tud. Further experiments are needed to unravel the relationship between these proteins, the targets of Csul methyltransferase activity and the nature of the RNPs associated to Tud.

vls functions in nuage and pole plasm

vls mutations causing a grandchild-less phenotype are characterized by agametic larvae exhibiting defects in abdominal patterning (Schüpbach and Wieschaus, 1986). Eggs laid by homozygous *vls* females are devoid of polar granules and consequently the embryos produce no pole cells (Schüpbach and Wieschaus, 1986). In these embryos, Tud is absent from the posterior pole (Bardsley et al., 1993), and Vas rapidly disappears from this location during the period of nuclear cleavage (Hay et al., 1990; Lasko and Ashburner, 1990). Our analysis reveals that localization of Tud is already absent from the nuage surrounding the *vls* nurse cell nuclei. However, the occurrence of Vas and Mael in the nuage of *vls* nurse cells indicates that aspects of this structure can be made independently of Tud.

A pivotal role in the organization of the nuage was assigned to Vas on the basis of its involvement in localizing specific components such as Aub (Wilson et al., 1996) and Mael (Clegg et al., 1997) in this structure (Harris and Macdonald, 2001; Findley et al., 2003). To confirm a Vas-dependence of Tud localization in the nuage, we examined Tud distribution in *vas* egg chambers and found that the localization of Tud around nurse cell nuclei is fully abolished (data not shown). Similarly the absence of Tud in the nuage of *vls* nurse cells shows that Tud localization in the nuage is *vls* dependent. However, we detected significant amounts of Tud in *vls* oocytes and at their anterior border, indicating that Tud accumulation in the nuage is not required for Tud transport into the oocyte and its anterior margin. Furthermore, as Vls-HA does not accumulate in early oocytes nor localize at their anterior margin (data not shown), we conclude that the interaction between Vls and Tud should be spatiotemporally regulated.

We show that Vls is a component of the nuage and pole plasm. Only a limited number of proteins display a similar pattern of distribution, including Vas (Hay et al., 1988a), Aub (Harris and Macdonald, 2001) and Tud (Bardsley et al., 1993). The dual location of these proteins indicates that they either perform distinct functions at each location or exert a function in the nuage required for their accumulation in the pole plasm. The finding that Vas absence in the pole plasm correlates with its absence in the nuage supports the latter possibility (Liang et al., 1994).

Inactivation of *vls* function exerts a further effect on the production of the short form of Osk protein. As *osk* mRNA localization and amount seems normal in *vls* embryos (Ephrussi et al., 1991), we assume that the lower amount of this form detected by immunoblotting in *vls* ovaries corresponds to either a defect in Osk synthesis or stability. We notice, however, that the level of Osk abundance varies considerably between individual *vls* oocytes with an apparently normal level in a small number of oocytes and a markedly reduced level in the majority of oocytes. The lower amount of Vas detected at the posterior pole of stage 10 *vls* or vls^2*/Df(2L)TW2* oocytes (Hay et al., 1990), can be interpreted as a consequence of the reduced amount of Osk protein, as Vas is absent from the posterior pole of *osk* oocytes (Hay et al., 1990; Lasko and Ashburner, 1990).

The mechanism by which Vls regulates Osk synthesis and/or stability remains unknown. However, on the basis of Vls localization during oogenesis, we envisage that *vls* could regulate the production of the short form of Osk by two distinct mechanisms. One the one hand, *vls* can regulate Osk synthesis by recruiting specific enhancing factors in the pole plasm. On the other hand, Osk synthesis may be dependent on events mediated by *vls* occurring in the nuage. Similarly, efficient *osk* mRNA translation in the pole plasm could also be mediated by Aub in the nuage (Harris and Macdonald, 2001). Furthermore, recent data point out that the nuage may function in assembling or reorganizing ribonucleoprotein complexes, particularly those involving localized or translationally regulated mRNAs (Snee and Macdonald, 2003).

The formation of polar granules fully depends upon *vls* activity (Schüpbach and Wieshaus, 1986), but only partially upon *tud* function as polar granules in reduced number and altered morphology are observed in amorphic *tud* pre-blastoderm embryos (Thomson and Lasko, 2004). This raises the question of what the targets of *vls* function are in addition

to *tud* and *osk*. Further experiments will reveal the components required for *vls*-dependent formation of polar granules.

As this work was being completed, another group reported the cloning of *vls* by positional mapping (Cavey et al., 2005). Although Cavey and colleagues identified only two WD repeats in Vls, they clearly recognized that its nearest homologue is MEP50. As the human methylosome is formed by MEP50 and PMRT5 homologous to *Drosophila* Vls and Csul, respectively, our finding that Vls can specifically bind to Csul indicates that it is the orthologue of MEP50 and not a 'divergent WD protein'. In contrast to Cavey and colleagues, we found a restricted and dynamic Vls distribution during oogenesis, first in the nuage and then at the posterior pole of the growing oocyte. Finally, we observed that Vls is preferentially incorporated in the forming pole cells. These findings show that *vls* may crucially act in the nuage, germ plasm and pole cells, and are consistent with the *vls* mutant phenotype. Similar to Cavey and colleagues, we detected a lower amount of Osk at the posterior pole of growing *vls* oocytes, but, in a discrepancy that we cannot explain, we found that Osk levels were already lower in stage 9 egg chambers, whereas Cavey et al. only observed a decrement of Osk later during oogenesis in stage 11 egg chambers.

In conclusion, we demonstrate that Vls can interact physically with at least two proteins, Csul and Tud, which are specifically involved in germ-line determination. Vls, in association with Csul, constitutes the first example of a partner of a dimethylarginine protein methyltransferase whose function has been characterized in vivo. These findings reinforce their cardinal function in a pathway first elucidated through genetic investigations. Our work sets the basis for further investigations on the role of Vls, its dependence upon Csul and its involvement in specific localization of cytoplasmic proteins during the formation of a functional pole plasm.

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References

Akiyama, T. and Okada, M. (1992). Spatial and developmental changes in the respiratory activity of mitochondria in early *Drosophila* embryos. *Development* **115**, 1175-1182.

Amikura, R., Hanyu, K., Kashikawa, M. and Kobayashi, S. (2001). Tudor protein is essential for the localization of mitochondrial RNAs in polar granules of *Drosophila* embryos. *Mech. Dev.* **107**, 97-104.

Bardsley, A., McDonald, K. and Boswell, R. E. (1993). Distribution of Tudor protein in the *Drosophila* embryo suggests separation of functions based on site of localization. *Development* **119**, 207-219.

Boswell, R. E. and Mahowald, A. P. (1985). *tudor*, a gene required for assembly of the germ plasm in *Drosophila melanogaster*. *Cell* **43**, 97-104.

Breitwieser, W., Markussen, F.-H., Horstmann, H. and Ephrussi, A. (1996). Oskar protein interaction with Vasa represents an essential step in polar granule assembly. *Genes Dev.* **10**, 2179-2188.

Brahms, H., Meheus, L., de Brabandere, V., Fischer, U. and Lührmann, R. (2001). Symmetrical dimethylation of arginine residues in spliceosomal Sm protein B/B′ and the Sm-like protein LSm4, and their interaction with the SMN protein. *RNA* **7**, 1531-1542.

Branscombe, T. L., Frankel, A., Lee, J.-H., Cook, J. R., Yang, Z.-h., Pestka, S. and Clarke, S. (2001). PRMT5 (Janus kinase-binding protein 1) catalyzes the formation of symmetric dimethylarginine residues in proteins. *J. Biol. Chem.* **276**, 32971-32976.

Bühler, D., Raker, V., Lührmann, R. and Fischer, U. (1999). Essential role fort he tudor domain of SMN in spliceosomal U snRNP assembly: implications for spinal muscular atrophy. *Hum. Mol. Genet.* **8**, 2351-2357.

Callebaut, I. and Mornon, J.-P. (1997). The human EBNA-2 coactivator p100: multidomain organization and relationship to the staphylococcal nuclease fold and to the tudor protein involved in *Drosophila melanogaster* development. *Biochem. J.* **321**, 125-132.

Cavey, M., Hijal, S., Zhang, X. and Suter, B. (2005). *Drosophila valois* encodes a divergent WD protein that is required for Vasa localization and Oskar protein accumulation. *Development* **132**, 459-468.

Chothia, C., Hubbard, T., Brenner, S., Barns, H. and Murzin, A. (1997). Protein folds in the all-beta and all-alpha classes. *Annu. Rev. Biophys. Biomol. Struct.* **26**, 597-627.

Clegg, N. J., Frost, D. M., Larkin, M. K., Subrahmanyan, L., Bryant, Z. and Ruohola-Baker, H. (1997). *maelstrom* is required for an early step in the establishment of *Drosophila* oocyte polarity: posterior localization of *grk* mRNA. *Development* **124**, 4661-4671.

Ephrussi, A. and Lehmann, R. (1992). Induction of germ cell formation by *oskar*. *Nature* **358**, 387-392.

Ephrussi, A., Dickenson, L. K. and Lehmann, R. (1991). *oskar* organizes the germ plasm and directs localization of the posterior determinant *nanos*. *Cell* **66**, 37-50.

Findley, S. D., Tamanaha, M., Clegg, N. J. and Ruohola-Baker, H. (2003). *Maelstrom*, a *Drosophila spindle*-class gene, encodes a protein that colocalizes with Vasa and RDE1/AGO1 homolog, Aubergine, in nuage. *Development* **130**, 859-871.

Friesen, W. J., Massenet, S., Paushkin, S., Wyce, A. and Dreyfuss, G. (2001a). SMN, the product of the spinal muscular atrophy gene, binds preferentially to dimethylarginine-containing protein targets. *Mol. Cell* **7**, 1111-1117.

Friesen, W. J., Paushkin, S., Wyce, A., Massenet, S., Pesiridis, G. S., van Duyne, G., Rappsilber, J., Mann, M. and Dreyfuss, G. (2001b). The methylosome, a 20S complex containing JBP1 and pICln, produces dimethylarginine-modified Sm proteins. *Mol. Cell. Biol.* **21**, 8289-8300.

Friesen, W. J., Wyce, A., Paushkin, S., Abel, L., Rappsilber, J., Mann, M. and Dreyfuss, G. (2002). A novel WD repeat protein component of the methylosome binds Sm proteins. *J. Biol. Chem.* **277**, 8243-8247.

Frohnhöfer, H. G., Lehmann, R. and Nüsslein-Volhard, C. (1986). Manipulating the anteroposterior pattern of the *Drosophila* embryo. *J. Embryol. Exp. Morph. Suppl* **97**, 169-179.

Gavis, E. R., Lunsford, L., Bergsten, S. E. and Lehmann, R. (1996). A conserved 90 nucleotide element mediates translational repression of *nanos* RNA. *Development* **122**, 2791-2800.

Geigy, R. (1931). Action de l'ultraviolet sur le pôle germinal dans l'oeuf de *Drosophila melanogaster*: castration et mutabilité. *Rev. Suisse Zool.* **38**, 187-288.

Golumbeski, G. S., Bardsley, A., Tax, F. and Boswell, R. E. (1991). *tudor*, a posterior-group gene of *Drosophila melanogaster*, encodes a novel protein and an mRNA localized during mid-oogenesis. *Genes Dev.* **5**, 2060-2070.

González-Reyes, A., Elliott, H. and St Johnston, D. (1997). Oocyte determination and the origin of polarity in *Drosophila*: the role of the *spindle* genes. *Development* **124**, 4927-4937.

Harris, A. N. and Macdonald, P. M. (2001). Aubergine encodes a Drosophila polar granule component required for pole cell formation and related to eIF2C. *Development* **128**, 2823-2832.

Hay, B., Ackerman, L., Barbel, S., Jan, L. Y. and Jan, Y. N. (1988a). Identification of a component of *Drosophila* polar granules. *Development* **103**, 625-640.

Hay, B., Jan, L. Y. and Jan, Y. N. (1988b). A protein component of the *Drosophila* polar granules is encoded by vasa and has extensive sequence similarity to ATP-dependent helicases. *Cell* **55**, 577-587.

Hay, B., Jan, L. Y. and Jan, Y. N. (1990). Localization of vasa, a component of *Drosophila* polar granules, in maternal-effect mutants that alter embryonic anteroposterior polarity. *Development* **109**, 425-433.

Huettner, A. F. (1923). The origin of the germ cells in *Drosophila melanogaster*. *J. Morphol.* **37**, 385-423.

Iida, T. and Kobayashi, S. (1998). Essential role of mitochondrially encoded large rRNA for germ-line formation in *Drosophila* embryos. *Proc. Natl. Acad. Sci. USA* **95**, 11274-11278.

Illmensee, K. and Mahowald, A. P. (1974). Transplantation of posterior polar

plasm in *Drosophila*. Induction of germ cells at the anterior pole of the egg. *Proc. Natl. Acad. Sci. USA* **71**, 1016-1020.

Illmensee, K., Mahowald, A. P. and Loomis, M. R. (1976). The ontogeny of germ plasm during oogenesis in *Drosophila*. *Dev. Biol.* **49**, 40-65.

Jongens, T. A., Hay, B., Jan, L. Y. and Jan, Y. N. (1992). The *germ cell-less* gene product: a posteriorly localized component necessary for germ cell development in *Drosophila*. *Cell* **70**, 569-584.

Jongens, T. A., Ackerman, L. D., Swedlow, J. R., Jan, L. Y. and Jan, Y. N. (1994). Germ cell-less encodes a cell type-specific nuclear pore-associated protein and functions early in the germ-cell specification pathway of *Drosophila*. *Genes Dev.* **8**, 2123-2136.

Kim, J.-S. and Raines, R. T. (1993). Ribonuclease S-peptide as a carrier in fusion proteins. *Protein Sci.* **2**, 348-356.

Kim-Ha, J., Smith, J. L. and Macdonald, P. M. (1991). *oskar* mRNA is localized to the posterior pole of the *Drosophila* oocyte. *Cell* **66**, 23-35.

Lasko, P. and Ashburner, M. (1988). The product of the *Drosophila* gene *vasa* is very similar to eukaryotic initiation factor-4A. *Nature* **335**, 611-617.

Lasko, P. and Ashburner, M. (1990). Posterior localization of *vasa* protein correlates with, but is not sufficient for, pole cell development. *Genes Dev.* **4**, 905-921.

Lefebvre, S., Bürglen, L., Reboullet, S., Clermont, O., Burlet, P., Viollet, L., Benichou, B., Cruaud, C., Millasseau, P., Zeviani, M. et al. (1995). Identification and characterization of a spinal muscular atrophy-determining gene. *Cell* **80**, 155-165.

Lehmann, R. and Nüsslein-Volhard, C. (1986). Abdominal segmentation, pole cell formation, and embryonic polarity require the localized activity of *oskar*, a maternal gene of *Drosophila*. *Cell* **47**, 141-152.

Lehmann, R. and Nüsslein-Volhard, C. (1991). The maternal gene *nanos* has a central role in posterior pattern formation in the *Drosophila* embryo. *Development* **112**, 679-691.

Liang, L., Diehl-Jones, W. and Lasko, P. (1994). Localization of vasa protein to the *Drosophila* pole plasm is independent of its RNA-binding and helicase activities. *Development* **120**, 1201-1211.

Liu, Q., Fischer, U., Wang, F. and Dreyfuss, G. (1997). The spinal muscular atrophy disease gene product, SMN, and ist associated protein SIP1 are in a complex with spliceosomal snRNP proteins. *Cell* **90**, 1013-1021.

Mahowald, A. P. (1962). Fine structure of pole cells and polar granules in *Drosophila melanogaster*. *J. Exp. Zool.* **151**, 201-215.

Markussen, F.-H., Michon, A.-M., Breitwieser, W. and Ephrussi, A. (1995). Translational control of *oskar* generates Short OSK, the isoform that induces pole plasm assembly. *Development* **121**, 3723-3732.

Meister, G., Eggert, C., Bühler, D., Brahms, H., Kambach, C. and Fischer, U. (2001). Methylation of Sm proteins by a complex containing PRMT5 and the putative U snRNP assembly factor pICln. *Curr. Biol.* **11**, 1990-1994.

Meister, G., Eggert, C. and Fischer, U. (2002). SMN-mediated assembly of RNPs: a complex story. *Trends Cell Biol.* **12**, 472-478.

Okada, M., Kleinman, I. A. and Schneiderman, H. A. (1974). Restoration of fertility in sterilized *Drosophila* eggs by transplantation of polar cytoplasm. *Dev. Biol.* **37**, 43-54.

Pontig, C. P. (1997). Tudor domains in poteins that interact with RNA. *Trends Biochem. Sci.* **22**, 51-52.

Rigaut, G, Shevchenko, A., Rutz, B., Wilm, M., Mann, M. and Séraphin, B. (1999). A generic protein purification method for protein complex characterization and proteome exploration. *Nat. Biotechnol.* **17**, 1030-1032.

Rongo, C., Gavis, E. R. and Lehmann, R. (1995). Localization of *oskar* RNA Regulates *oskar* translation and requires Oskar protein. *Development* **121**, 2737-2746.

Sano, H., Nakamura, A. and Kobayashi, S. (2002). Identification of a transcriptional regulatory region for germline-specific expression of *vasa* gene in *Drosophila melanogaster*. *Mech. Dev.* **112**, 129-139.

Schüpbach, T. and Wieschaus, E. (1986). Maternal-effect mutations altering the anterior-posterior pattern of the *Drosophila* embryo. *Roux's Arch. Dev. Biol.* **195**, 302-317.

Selenko, P., Sprangers, R., Stier, G., Bühler, D., Fischer, U. and Sattler, M. (2001). SMN Tudor domain structure and its interaction with the Sm proteins. *Nat. Struct. Biol.* **8**, 27-31.

Smith, J. L., Wilson, J. E. and Macdonald, P. M. (1992). Overexpression of *oskar* directs ectopic activation of *nanos* and presumptive pole cell formation in *Drosophila* embryos. *Cell* **70**, 849-859.

Smith, T. F., Gaitatzes, C., Saxena, K. and Neer, E. J. (1999). The WD repeat: a common architecture for diverse functions. *Trends Biochem. Sci.* **24**, 181-185.

Snee, M. J. and Macdonald, P. M. (2003). Live imaging of nuage and polar granules: evidence against a precursor-product relationship and a novel role of Oskar in stabilization of polar granule components. *J. Cell Sci.* **117**, 2109-2120.

Styhler, S., Nakamura, A., Swan, A., Suter, B. and Lasko, P. (1998). *vasa* is required for Gurken accumulation in the oocyte, and is involved in oocyte differentiation and germline cyst development. *Development* **125**, 1569-1578.

Styhler, S., Nakamura, A. and Lasko, P. (2002). VASA localization requires the SPRY-domain and SOCS-box containing protein, GUSTAVUS. *Dev. Cell* **3**, 865-876.

Thomson, T. and Lasko, P. (2004). Drosophila *tudor* is essential for polar granule assembly and pole cell specification, but not for posterior patterning. *Genesis* **40**, 164-170.

Tomancak, P., Guichet, A., Zavorszky, P. and Ephrussi, A. (1998). Oocyte polarity depends on regulation of *gurken* by Vasa. *Development* **125**, 1723-1732.

Török, I., Herrmann-Horle, D., Kiss, I., Tick, G., Speer, G., Schmitt, R. and Mechler, B. M. (1999). Down-regulation of RpS21, a putative translation initiation factor interacting with P40, produces viable minute imagos and larval lethality with overgrown hematopoietic organs and imaginal discs. *Mol. Cell. Biol.* **19**, 2308-2321.

Wang, C. and Lehmann, R. (1991). *nanos* is the localized posterior determinant in *Drosophila*. *Cell* **66**, 637-647.

Wang, C., Dickinson, L. K. and Lehmann, R. (1994). Genetics of *nanos* localization in *Drosophila*. *Dev. Dyn.* **199**, 103-115.

Wilson, J. E., Connell, J. E. and Macdonald, P. M. (1996). *aubergine* enhances *oskar* translation in the *Drosophila* ovary. *Development* **122**, 1631-1639.

Yu, L., Gaitatzes, C., Neer, E. and Smith, T. F. (2000). Thirty-plus functional families from a single motif. *Protein Sci.* **9**, 2470-2476.