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VASA Mediates Translation through Interaction with a *Drosophila* yIF2 Homolog

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Summary

The *Drosophila* gene *vasa* (*vas*) encodes an RNA-binding protein required for embryonic patterning and germ cell specification. In *vas* mutants, translation of several germline mRNAs is reduced. Here we show that VAS interacts directly with the *Drosophila* homolog of yeast translation initiation factor 2, encoded by a novel gene, *dIF2*. Embryos produced by *vas*/+; *dIF2*/+ females have pattern defects and fewer germline progenitor cells, indicating a functional interaction between endogenous *vas* and *dIF2* activities. Mutations in other translation initiation factors do not enhance the *vas* phenotype, suggesting that dIF2 has a particular role in germ plasm function. We conclude that VAS regulates translation of germline mRNAs by specific interaction with dIF2, an essential factor conserved from bacteria to humans.

Introduction

Translational control is a central genetic regulatory mechanism in germline development (for reviews, see Seydoux, 1996; Saffman and Lasko, 1999). Translational control is often coupled with asymmetric cytoplasmic localization of mRNAs, and in *Drosophila*, several mRNAs, including *oskar* (*osk*) and *nanos* (*nos*), are translationally repressed unless they are localized in the posterior-most cytoplasm of the oocyte, called the pole plasm (Kim-Ha et al., 1995; Dahanukar and Wharton, 1996; Bergsten and Gavis, 1999). Substantial progress has been made in recent years in the identification and analysis of *cis*-regulatory elements in the 3′ untranslated regions (UTRs), and *trans*-acting proteins, which are involved in translational repression of *osk* and *nos* mRNAs (Kim-Ha et al., 1995; Gavis et al., 1996; Smibert et al., 1996; Webster et al., 1997; Saffman et al., 1998; Dahanukar et al., 1999). In contrast, few details are yet clear about factors involved in alleviating repression and activating translation of specific RNAs when they are localized to the pole plasm, although *aubergine* and two unidentified proteins have been implicated in activating translation of *osk* (Wilson et al., 1996; Gunkel et al., 1998).

vasa (*vas*) encodes a DEAD box RNA helicase required for embryonic patterning, germ plasm assembly, and germ cell function (Hay et al., 1988; Lasko and Ashburner, 1988, 1990; Schüpbach and Wieschaus, 1991; Liang et al., 1994). Genetic evidence suggests a role for *vas* protein (VAS) in activating translation of several specific germline mRNAs including *osk*, *nos*, and *gurken* (*grk*) (Dahanukar and Wharton, 1996; Gavis et al., 1996; Styhler et al., 1998; Tomancak et al., 1998; Tinker et al., 1998). However, it is unknown how VAS functions, and more specifically, whether VAS interacts directly with the translational machinery. Here we report the identification of a *Drosophila* initiation factor, dIF2. We show that VAS binds this factor in vitro. *vas* and *dIF2* null mutations genetically interact in germ cell specification and embryonic patterning, showing that the interaction between the two factors also occurs in vivo. The data suggest that dIF2, which carries an essential cellular function, operates in the germ plasm by serving as a link between VAS and the translation machinery.

Results and Discussion

VAS Interacts with a *Drosophila* yIF2 Homolog in the Yeast Two-Hybrid System and In Vitro

To identify proteins that associate with VAS, VAS was used as a bait in yeast two-hybrid assays (Golemis et al., 1997) using a library prepared from ovarian cDNA. From 145 putative interactors that were characterized by sequencing or four-cutter restriction analysis, we obtained eight clones encoding portions of Oskar (OSK; Figure 1A). Recovery of OSK was expected since its interaction with VAS, which represents an essential step in polar granule assembly, had been shown in yeast previously (Breitwieser et al., 1996). Additionally, we obtained six clones producing a novel VAS-interacting protein. To obtain full sequence information on the VAS-interacting protein, we screened various libraries (Brown and Kafatos, 1988; Hovemann, 1991) for cDNA clones and determined the sequence of the longest of 12 clones obtained. This analysis indicated that the VAS-interacting protein is the *Drosophila* homolog of the recently identified yeast initiation factor 2 (yIF2) of *Saccharomyces cerevisiae* (Choi et al., 1998; see below). We therefore termed the *Drosophila* protein dIF2.

The 1144–amino acid dIF2 protein is 53% identical over 592 amino acids to yIF2 (Choi et al., 1998). Still greater similarity (70% identity over 613 amino acids) exists between dIF2 and its human counterpart, hIF2, which can functionally rescue a yeast strain deficient for yIF2 (Lee et al., 1999). dIF2 and yIF2 are more distantly

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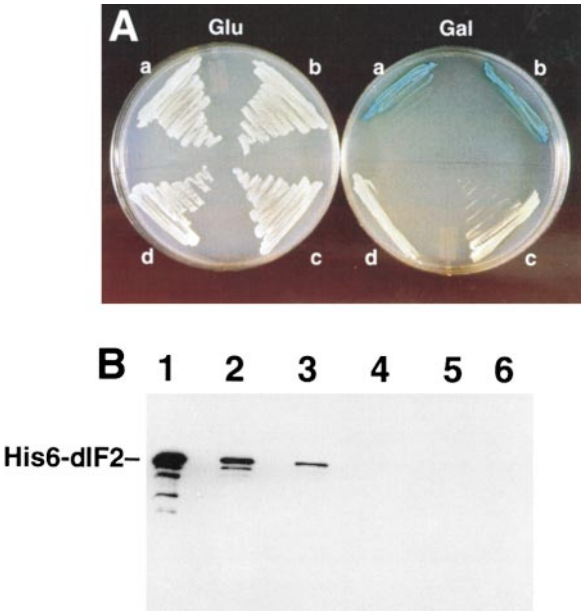

Figure 1. VAS and dIF2 Interact in the Yeast Two-Hybrid Assay and In Vitro

(A) *S. cerevisiae* strain EGY48 was transformed with a "bait" plasmid expressing VAS or a related protein, and with a "prey" plasmid encoding dIF2 or OSK (see Experimental Procedures). Interaction is revealed by activation of β-galactosidase expression on galactose-containing medium (Gal), as opposed to glucose-containing medium (Glu). The yeast strains (a–d) contained plasmids encoding the following proteins: (a) VAS (full-length) and Short OSK (Markussen et al., 1995; aa 5–468), (b) VAS (full-length) and dIF2 (aa 491–1144), (c) eIF4A (Dorn et al., 1993; full-length) and dIF2 (aa 491–1144), and (d) VAS (aa 1–310) and dIF2 (aa 491–1144). Similar results (not shown) were obtained in a growth-based assay for interaction-dependent leucine synthesis.

(B) GST pulldown assay. Purified His_6-tagged dIF2 (aa 491–1144; 15 μg in lane 2, 3 μg in lanes 3–6) was incubated with glutathione-Sepharose beads linked to various proteins as described below. Bound proteins were eluted, separated by SDS-PAGE, and electroblotted. His_6-tagged dIF2 was revealed with a commercial α-RGS-His_6 monoclonal antibody (Qiagen). Lane 1, purified His_6-tagged dIF2 (1.35 μg; aa 491–1144) serving as a marker. Proteins linked to beads were: lane *2*, 15 μg GST-VAS; lane 3, 3 μg GST-VAS; lane 4, 3 μg GST-eIF4A; lane 5, 3 μg GST; and lane 6, beads alone.

related to archaean, mitochondrial, and eubacterial translation initiation factors 2 (IF2; Choi et al., 1998; Wilson et al., 1999). Prokaryotic IF2 and eukaryotic yIF2 were shown to deliver initiator methionine transfer RNA ($Met\text{-}tRNA_i^{Met}$) to the small ribosomal subunit, implying that the mechanism of translation initiation between prokaryotes and metazoans is more similar than had been anticipated (Lee et al., 1999). The VAS-interacting portion of one original *dIF2* clone that was obtained in the two-hybrid screen contained the C-terminal half of dIF2 (amino acids 491–1144; Figure 1A). Furthermore, the interaction between dIF2 and VAS depended on the presence of the domain common to DEAD box proteins in VAS (Linder et al., 1989), since an N-terminal truncated form of VAS containing only amino acids 1–310 failed to interact detectably (Figure 1A).

To test the VAS/dIF2 interaction by an independent assay system, we incubated Sepharose beads linked to glutathione S-transferase–VAS fusion protein (GST–VAS) with the purified His_6-tagged dIF2 partial protein. Figure 1B shows the results of the GST pulldown assay indicating that dIF2 was bound to GST–VAS but not to GST or GST–eIF4A and that purified dIF2 binds strongly to VAS when coincubated in an equimolar ratio. Thus, the results obtained with the yeast two-hybrid system and GST pulldown assays establish that VAS and dIF2 are able to interact directly, both in yeast cells and in vitro.

dIF2 Is Essential for Viability

dIF2 is localized in region 63D2-3 on the left arm of the third chromosome. This region is included in the P1 clone DS01105 for which the DNA sequence is fully established (Berkeley *Drosophila* Genome Project). Comparison with the cDNA sequence allowed us to establish the exon/intron structure of the *dIF2* gene. The 4.1 kb *dIF2* transcript is composed of nine exons (Figure 2B) and appears as a single band on Northern blots (Figure 2C). We used the cDNA clone to probe whole-mount preparations of ovaries and embryos by in situ hybridization and found that *dIF2* is transcribed in germline and somatic tissues throughout development (data not shown), consistent with a general role for dIF2 in translation initiation as observed for yIF2 in yeast. Moreover, the broad distribution of the transcript and the restricted expression pattern of VAS argue against an exclusive and specific function of dIF2 in VAS-dependent translation.

To obtain fly strains bearing mutations in *dIF2*, we mobilized a *PlacW* element inserted near the *dIF2* transcriptional start site on the *l(3)L3659* chromosome to produce two partial deletions of *dIF2* (*Δ1* and *Δ2*; Figure 2D). In both mutants, imprecise excision of the P element generated small deletions of genomic DNA, which included the translational start codon and the first 27 and 59 codons, respectively (details in the legend to Figure 2). The lethality of *dIF2* mutants was rescued by P element–mediated introduction of a *dIF2* minigene. In addition, we conducted an extensive ethyl methanesulfonate (EMS) mutagenesis screen for recessive lethal alleles that failed to complement the deletion mutation *Df(3L)HR232* (Wohlwill and Bonner, 1991). A complementation group of four EMS-induced recessive lethal alleles also failed to complement $dIF2^{\Delta 1}$ or $dIF2^{\Delta 2}$; we conclude that all six mutations affect *dIF2*. All *dIF2* mutations are lethal in *trans* to three deficiencies in chromosomal region 63D, including *Df(3L)HR232*, *Df(3L)HR119* (Wohlwill and Bonner, 1991), and *Df(3L)G5*, a small deficiency generated in the EMS screen. Homozygous *dIF2* mutants die as first- or second-instar larvae without expressing a discernible morphological defect in their cuticle pattern. We attribute the normal embryonic development to perdurance of maternal *dIF2* activity (see below).

vas and *dIF2* Interact in Abdominal Patterning and Pole Cell Specification

We used *dIF2* mutants to determine whether *vas* and *dIF2* interact functionally in vivo. $vas^{PH165}/+$ females (Styhler et al., 1998) provide a sensitized background for gene dosage assays when cultured at 29°C, since 10%–30% of the embryos produced by such individuals

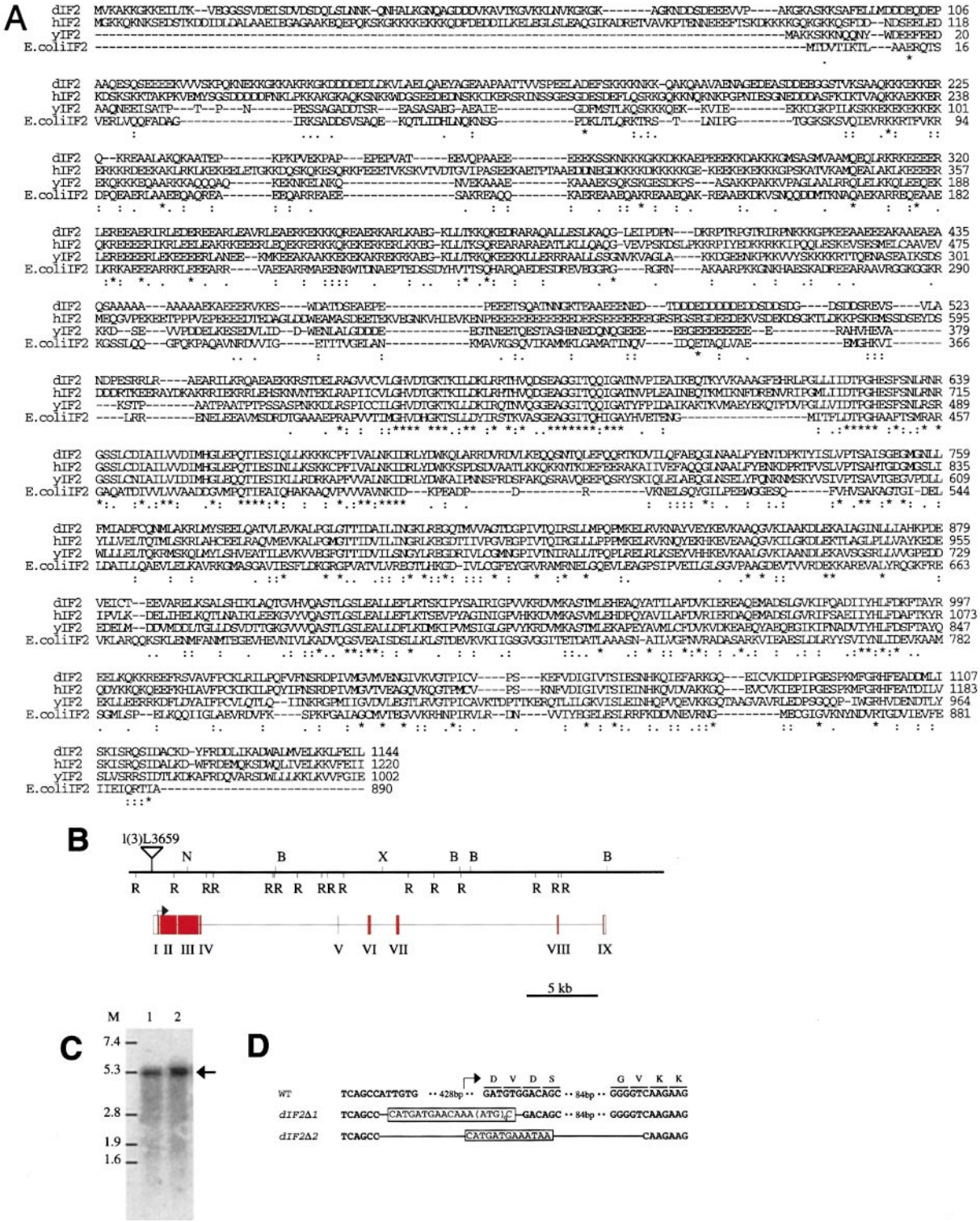

Figure 2. Characterization of the *dIF2* Gene and of *dIF2* Mutations

(A) Alignment of *Drosophila* dIF2 with human and *S. cerevisiae* homologs. Sequence alignment was generated using CLUSTALW (Thompson et al., 1994) version 1.74 at the Pôle Bio-informatique Lyonnais Web Server (http://pbil.ibcp.fr/NPSA/npsa_clustalw.html). Comparison of the *dIF2* cDNA sequence with the genomic sequence of P1 clone DS01105 determined by the BDGP gives 13 nucleotide differences. Of these, 12 are silent, and the remaining one changes Glu-115 to Lys in the predicted translation product.

(B) Restriction map of the *dIF2* locus and structure of the cDNA. Exons (open boxes) are numbered with Roman numerals (I–IX); closed portion, coding region. The translation initiation start site (arrow) is located in exon I. The triangle shows the P element *l(3)L3659* insertion site that is immediately after position 35,615 in the BDGP P1 clone DS01105 (GenBank accession number AC004356) and is located 360 bp upstream of the initiation codon. B, BamHI; N, NotI; R, EcoRI; X, XbaI.

(C) Northern blot of 10 μg of poly(A)$^+$ RNA from 0–24 hr wild-type embryos (lane 1) and adult flies (lane 2), probed with a 1.8 kb fragment from the coding region of *dIF2* cDNA. RNA size standards are indicated.

(D) The *dIF2*$^{\Delta 1}$ and *dIF2*$^{\Delta 2}$ alleles result from an imprecise excision of *l(3)L3659*, in which 440 bp and 535 bp, respectively, of genomic DNA are replaced with 41 bp and 13 bp from the P element (sequences in boxes), respectively. Both deletions remove the translational start codon. *dIF2*$^{\Delta 1}$ removes the first 27 codons, and *dIF2*$^{\Delta 2}$ the first 59 codons. The translation start site is indicated by the arrow.

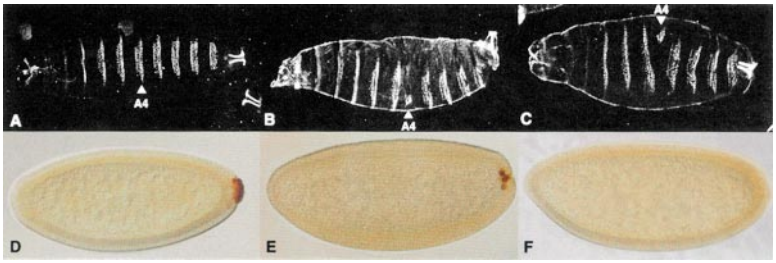

Figure 3. *vas* and *dIF2* Interact Genetically

Embryos were collected from (A and D) wild-type or (B, C, E, and F) $vas^{PH165}/+$; $dIF2^{\Delta1}/+$ females. (A–C) Dark-field photographs of cuticle preparations (Wieschaus and Nüsslein-Volhard, 1986). The fourth abdominal segment (A4) is identified in all three embryos. (D–F) Cellular blastoderm stage embryos stained with α-VAS antiserum and detected by DAB staining to visualize pole cells (Lasko and Ashburner, 1990). Note that many pole cells are covered by VAS-negative cells (embryo in [E]) and that pole cells are occasionally completely absent (embryo in [F]).

fail to hatch and exhibit variable and minor patterning defects affecting both the anteroposterior and dorso–ventral axes. When one copy of *dIF2* was removed from such females by introducing either the $dIF2^{\Delta1}$ or the *Df(3L)G5* chromosome, the frequency of unhatched embryos increased from 10%–30% to as high as 89%. Many (30%–47%) of the unhatched embryos from $vas^{PH165}/+$; *dIF2*/+ mothers exhibited severe segmentation defects, and an additional 26% showed a *vas*-like phenotype in which the fourth abdominal segment (A4) is completely or partially deleted (Figures 3A–3C). Segments A4 and A5 have been shown to be most sensitive to the activity of posterior group genes such as *vas* in previous studies (Schüpbach and Wieschaus, 1986). Partial or total deletion of A4 was never observed among 62 embryos examined from control $vas^{PH165}/+$; *TM6B*/+ females, nor among those from $dIF2^{\Delta1}/+$ females. In addition, and even more strikingly, we found evidence for the genetic interaction between *vas* and *dIF2* by monitoring the number and position of germline precursor cells, termed pole cells. We observed a marked reduction in the number of pole cells, formed in progeny of $vas^{PH165}/+$; $dIF2^{\Delta1}/+$ *trans*-heterozygotes, when compared to wild-type embryos or the progeny of females heterozygous for only vas^{PH165} or $dIF2^{\Delta1}$ (Figures 3D–3F). In one set of experiments, almost all (90%) embryos produced by $vas^{PH165}/+$; $dIF2^{\Delta1}/+$ *trans*-heterozygotes had fewer than 15 pole cells, as opposed to an average of 35–40 in wild-type embryos at the cellular blastoderm stage, although some experimental variability in the absolute, but not the relative, numbers was observed (see below and Table 1). Occasionally pole cells are completely absent in progeny of $vas^{PH165}/+$; $dIF2^{\Delta1}/+$ *trans*-heterozygotes (Figure 3F). Also, unlike in wild-type embryos, pole cells in progeny of $vas^{PH165}/+$; $dIF2^{\Delta1}/+$ *trans*-heterozygotes are often interspersed with or positioned beneath somatic cells.

Table 1. Effects of *vas, dIF2, eIF4A,* and *eIF4E* Mutations on Pole Cell Number in Progeny Embryos

Maternal Genotype	Number of Embryos Examined	Pole Cell Number
Oregon-R	61	33.0 ± 6.6
$vas^{PH165}/+$; *TM6B*/+	60	24.2 ± 6.0
+/+; $dIF2^{\Delta1}/+$	60	29.4 ± 8.1
$vas^{PH165}/+$; $dIF2^{\Delta1}/+$	66	16.1 ± 5.9
$vas^{PH165}/eIF4A^{02439}$	65	26.4 ± 5.4
$vas^{PH165}/eIF4A^{1013}$	60	29.5 ± 4.2
$vas^{PH165}/+$; $eIF4E^{589/11}/+$	65	23.4 ± 4.5

Females were mated to Oregon-R wild-type males and maintained at 29°C. The counts are reported as mean ± standard deviation.

VAS Levels Are Not Specifically Reduced in *dIF2*/+ Heterozygotes

We considered the possibility that the genetic interaction we observed in $vas^{PH165}/+$; $dIF2^{\Delta1}/+$ *trans*-heterozygotes might result from lower VAS levels brought about as a general reduction in translation resulting from the $dIF2^{\Delta1}$ mutation. To test this idea, we compared VAS levels in ovaries from $vas^{PH165}/+$; $dIF2^{\Delta1}/+$ females, and females *trans*-heterozygous for vas^{PH165} and one of three other mutations affecting translation initiation factors, eIF4A or eIF4E ($eIF4A^{02439}$, $eIF4A^{1013}$, and $eIF4E^{589/11}$; Galloni and Edgar, 1999; P. Lachance and P. Lasko, unpublished results). We also counted pole cells in embryos produced by females of these different genotypes. As shown in Figure 4, the level of VAS is reduced approximately 3-fold in $vas^{PH165}/+$; $eIF4E^{589/11}/+$ ovaries, as compared to $vas^{PH165}/+$; $dIF2^{\Delta1}/+$ ovaries. $vas^{PH165}/eIF4A$ ovaries contain VAS at a level similar to $vas^{PH165}/+$; $dIF2^{\Delta1}/+$. However, examination of embryos revealed that, unlike for $vas^{PH165}/+$; $dIF2^{\Delta1}/+$, no reduction in pole cell number was observed in progeny from either $vas^{PH165}/+$; $eIF4E^{589/11}/+$ *or* $vas^{PH165}/eIF4A$ females (Table 1). Thus, the genetic interaction between *vas* and *dIF2* mutations cannot be explained simply on the basis of a nonspecific reduction of VAS translation, as the much stronger effect of the $eIF4E^{589/11}$ mutation on the VAS level does not lead to a reduction in pole cell number.

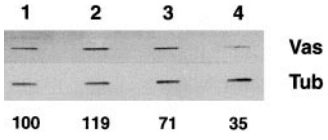

Figure 4. The Effects of *dIF2*, *eIF4A*, and *eIF4E* Mutations on the Ovarian VAS Level

Protein extracts from (lane 1) $vas^{PH165}/+$; $dIF2^{\Delta1}/+$, (lane 2) $vas^{PH165}/+$; $eIF4A^{02439}/+$, (lane 3) $vas^{PH165}/+$; $eIF4A^{1013}/+$, and (lane 4) $vas^{PH165}/+$; $eIF4E^{589/11}/+$ ovaries were electroblotted onto a nitrocellulose membrane. VAS was detected using a rabbit anti-VAS antiserum (1:2000, Styhler et al., 1998) and compared to α-tubulin levels, detected using a mouse anti-α-Tub antiserum (1:2000, Sigma). Bands were quantitated using the NIH Image 1.60 software. The numbers below the images report the ratio of VAS/Tub protein in the various extracts, as normalized to $vas^{PH165}/+$; $dIF2^{\Delta1}/+$, which was arbitrarily set to 100.

The pole cell number is approximately proportional to the *osk* gene dosage (Ephrussi and Lehmann, 1992). In the strongest *vas* allelic combination that permits oogenesis to proceed to completion (*vas*^{PD}/*vas*^{D1}), levels of the active Short OSK protein isoform are somewhat reduced, and phosphorylation of Short OSK is undetectable (Markussen et al., 1995, 1997). These results suggest that *osk* may be a target RNA for VAS, and that the effects we observed on pole cell number in progeny of *vas*^{PH165}/+; *dIF2*$^{\Delta 1}$/+ females might be explained on the basis of a specific reduction in *osk* translation brought about by the *dIF2* mutation. To test this, we assayed OSK levels in protein extracts prepared from ovaries of the following genotypes: wild-type, *vas*^{PH165}/+; *dIF2*$^{\Delta 1}$/+, *vas*^{PH165}/*eIF4A*^{1013}, and *vas*^{PH165}/+; *eIF4E*$^{589/11}$/+. With two different α-OSK antisera, we did not detect any specific alteration in the Short OSK level or electrophoretic mobility in *vas*^{PH165}/+; *dIF2*$^{\Delta 1}$/+ extracts (data not shown). As developmentally significant alterations in the level or distribution of maternal patterning proteins often escape direct detection in immunological assays (Dahanukar et al., 1999, for example), our negative results do not exclude the possibility that dIF2 may have a specific function in regulating *osk* translation.

We also investigated whether ovaries with a *dIF2* null germline would resemble *vas* null ovaries. We used the FLP-DFS mitotic recombination system (Chou and Perrimon, 1996) to produce *dIF2*$^{\Delta 1}$ homozygous germline clones. However, the ovaries of heat-shocked individuals were extremely rudimentary in their development (data not shown), indicating that large clones of *dIF2*$^{\Delta 1}$ cells cannot be recovered. Thus, unlike the case in yeast where a disruption mutant that lacks yIF2 retains partial viability (Choi et al., 1998), in *Drosophila* the corresponding gene is essential for organismal and possibly also for cellular viability.

The Association between VAS and dIF2 Links VAS to the Translational Machinery

Our functional identification of a yIF2 homolog in *Drosophila* and its similarity to products predicted from mammalian genes indicate that the yIF2 translation initiation factor is highly conserved among metazoans. The physical interaction between VAS and dIF2 in vitro, the interaction between transfected *vas* and *dIF2* in yeast cells, and that between the two endogenous genes in *Drosophila* all indicate that VAS may regulate translation of specific mRNAs through a direct link with the translation initiation machinery via dIF2. Our failure to recover any other genes encoding known translation initiation factors from the interaction screen and the lack of a genetic interaction between *vas* and *eIF4A* or *eIF4E* mutations support this idea. Other DEAD box helicases related to VAS may function similarly; *S. cerevisiae ded1* mutants exhibit defects in translation initiation (Chuang et al., 1997; de la Cruz et al., 1997). However, the precise step at which VAS exerts its function in the translation of a specific set of mRNAs is unclear. Since VAS is an RNA-binding protein (Liang et al., 1994) that presumably interacts with mRNA, it appears likely that VAS acts at the level of mRNA recruitment to the ribosome. However, yIF2 has been implicated in promoting Met-tRNA$_i^{Met}$ binding to ribosomes, which takes place prior to the recruitment of mRNA (Merrick and Hershey, 1996; Lee et al., 1999). Further analysis of VAS and dIF2 will provide additional insight into the specific functions of both of these molecules, which are conserved in mammals (Fujiwara et al., 1994; Lee et al., 1999; Wilson et al., 1999), and how they cooperate to initiate translation.

Experimental Procedures

Yeast Two-Hybrid Screen

The entire VAS-coding region was subcloned in the yeast vector pEG202 to produce a bait construct. This was used to screen an ovarian cDNA library cloned in the vector pJG4-5 (complexity 1.5 × 10^6; kindly provided by J. Grosshans and C. Nüsslein-Volhard). Over 300 Leu$^+$ colonies were recovered, restreaked, and tested for β-galactosidase expression. The inserts from 145 confirmed positive clones were amplified by PCR and digested with HaeIII; the resulting restriction fragment patterns allowed the classification of the clones into several groups. For sequence determination, pJG4-5 plasmids were recovered in *E. coli* using standard techniques. Plasmid DNA was prepared and sequenced manually using standard dideoxy techniques, using a primer complementary to vector sequence adjacent to the 5′ end of the inserts.

Pulldown Assays

His$_6$-tagged dIF2 (aa 491–1144) was expressed in *E. coli* cells using the vector pQE32 (Qiagen) and purified by affinity chromatography to Ni-NTA agarose (Qiagen). Glutathione S-transferase (GST), GST–eIF4A, and GST–VAS fusion proteins were produced in *E. coli* cells using the pGEX vector series (Pharmacia) and purified by affinity chromatography to glutathione-Sepharose beads (75% suspension, Pharmacia). For the pulldown assay, 25 μl of glutathione-Sepharose bead suspension was washed three times in 1 ml of binding buffer (50 mM HEPES-NaOH [pH 7.4], 100 mM NaCl, 1 mM EDTA, 0.5% Triton X-100). To link the GST fusion proteins, 10 or 50 μg of GST–VAS, GST–eIF4A, or GST in 500 μl of BBPI (binding buffer supplemented with 1 mM PMSF, 1× protease inhibitor cocktail [Boehringer-Mannheim]) plus 0.5 mg/ml bovine serum albumin (BSA) was added to the beads, and the suspension was incubated on a rotator for 3 hr at 4°C. The beads were washed three times in 1 ml of BBPI, then incubated for 4 hr on a rotator at 4°C with 500 μl of BBPI + 0.5% BSA, to which was added 10 μg of purified His$_6$-tagged dIF2 (aa 491–1144). To remove proteins that bound nonspecifically, the beads were washed four times in 1 ml of BBPI. Bound proteins were then removed by adding 20 μl of SDS sample buffer and boiling for 5 min. Proteins were separated on a 10% SDS-PAGE gel and transferred by electroblotting to a nitrocellulose membrane (Hybond C Extra, Amersham). To reveal the presence of His$_6$-tagged dIF2, the membrane was incubated with a commercial α-RGS-His$_6$ monoclonal antibody (Qiagen), followed by α-mouse IgG conjugated with horseradish peroxidase (Amersham). Detection was by chemiluminescence using the ECL Plus kit (Amersham).

Nucleic Acid Hybridizations and Clone Isolation

Northern hybridizations and cDNA library screening by Southern hybridization were performed using standard techniques. cDNAs were isolated from a plasmid library prepared from imaginal disk tissue (Brown and Kafatos, 1988) and a phage library prepared from 0–18 hr embryos (Hovemann, 1991). All cDNA clones correspond to the same transcript. The sequence of the longest cDNA clone (4052 bp) has been submitted to GenBank under accession number AF143207.

Generation of *dIF2*-Null Alleles

l(3)L3659 is a viable *PlacW* insertion at 63D1,2, but the chromosome carries a separate lethal mutation. Excision lines of *l(3)L3659* were generated by crossing in a third chromosome carrying *Δ2-3* transposase. One hundred white-eyed (*w*$^-$) males from independent excision events were isolated and scored for lethality in *trans* to a deficiency uncovering the 63D1,2 region. The two chromosomes carrying lethal excision events were established as balanced stocks. These alleles were checked by Southern blots and PCR to determine whether

they carried a deletion in *dIF2*. The *dIF2* genomic region close to the *l(3)L3659* insertion site was amplified by PCR, and two independent PCR products of each excision line were sequenced.

Genetic Interaction Studies

dIF2$^{\Delta 1}$/*TM6B* or *Df(3L)G5*/*TM6B* females were mated with heterozygous males carrying a null mutation in *vas* (*vas*^{PH165}/*CyO*; Styhler et al., 1998). Mothers that were heterozygous for both *vas*^{PH165} and *dIF2*$^{\Delta 1}$ (*Df(3L)G5*) were selected and cultured with wild-type males for 2 or 3 days at 29°C. Embryos were then collected from these females and aged for 2 days, all at 29°C. The number of hatching larvae was determined as a fraction of the total number of fertilized eggs. As controls, heterozygous flies for *vas*^{PH165} and *TM6B*, or for *vas*^{PH165} only were treated and analyzed in parallel. For pole cell counts, females of various genotypes were cultured with wild-type males at 29°C. After a minimum of 3 days, embryos were collected, fixed, and stained with anti-VAS antibody (Lasko and Ashburner, 1990). Pole cells were counted in stage 10–12 embryos, when they are migrating individually and are well dispersed.

Generation of Germline Clones

Homozygous *dIF2*$^{\Delta 1}$ clones were produced by the FLP-DFS method using the following breeding scheme: *y w HSFlp; FRT2A ovo*^{D1}/*TM3 Sb* males were mated with *dIF2*$^{\Delta 1FRT2A}$ *w*$^{+}$/*TM3Ser* virgin females. Flies were transferred to fresh food every 24 hr, and F1 larvae were heat shocked at 37°C for 2 hr during the second and third instar larval stages to induce mitotic recombination in proliferating germ cells. Ovaries from non-Sb, non-Ser females were examined.

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References

Bergsten, S.E., and Gavis, E.R. (1999). Role for mRNA localization in translational activation but not spatial restriction of *nanos* RNA. Development *126*, 659–669.

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.

Brown, N.H., and Kafatos, F.C. (1988). Functional cDNA libraries from *Drosophila* embryos. J. Mol. Biol. *203*, 423–437.

Choi, S.K., Lee, J.H., Zoll, W.L., Merrick, W.C., and Dever, T.E. (1998). Promotion of met-tRNA$_i^{Met}$ binding to ribosomes by yIF2, a bacterial IF2 homolog in yeast. Science *280*, 1757–1760.

Chou, T.B., and Perrimon, N. (1996). The autosomal FLP-DFS technique for generating germline mosaics in *Drosophila melanogaster*. Genetics *144*, 1673–1679.

Chuang, R.Y., Weaver, P.L., Liu, Z., and Chang, T.H. (1997). Requirement of the DEAD-box protein ded1p for messenger RNA translation. Science *275*, 1468–1471.

Dahanukar, A., and Wharton, R.P. (1996). The Nanos gradient in *Drosophila* embryos is generated by translational regulation. Genes Dev. *10*, 2610–2620.

Dahanukar, A., Walker, J.A., and Wharton, R.P. (1999). Smaug, a novel RNA-binding protein that operates a translational switch in *Drosophila*. Mol. Cell *4*, 209–218.

de la Cruz, J., Iost, I., Kressler, D., and Linder, P. (1997). The p20 and Ded1 proteins have antagonistic roles in eIF4E-dependent translation in *Saccharomyces cerevisiae*. Proc. Natl. Acad. Sci. USA *94*, 5201–5206.

Dorn, R., Morawietz, H., Reuter, G., and Saumweber, H. (1993). Identification of an essential *Drosophila* gene that is homologous to the translation initiation factor eIF-4A of yeast and mouse. Mol. Gen. Genet. *237*, 233–240.

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

Fujiwara, Y., Komiya, T., Kawabata, H., Sato, M., Fujimoto, H., Furusawa, M., and Noce, T. (1994). Isolation of a DEAD-family protein gene that encodes a murine homolog of *Drosophila* vasa and its specific expression in germ cell lineage. Proc. Natl. Acad. Sci. USA *91*, 12258–12262.

Galloni, M., and Edgar, B.A. (1999). Cell-autonomous and non-autonomous growth-defective mutants of *Drosophila melanogaster*. Development *126*, 2365–2375.

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.

Golemis, E.A., Serebriiskii, I., Gyuris, J., and Brent, R. (1997). Interaction trap/two-hybrid system to identify interacting proteins. In Current Protocols in Molecular Biology, F.M. Ausubel et al., eds. (New York: Wiley), unit 20.1, p. 1–35.

Gunkel, N., Yano, T., Markussen, F.-H., Olsen, L.C., and Ephrussi, A. (1998). Localization-dependent translation requires a functional interaction between the 5′ and 3′ ends of *oskar* mRNA. Genes Dev. *12*, 1652–1664.

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

Hovemann, B. (1991). Construction of a random primed embryonic *Drosophila* cDNA expression library cloned into phage lambda-gt11. Dros. Inf. Serv. *70*, 250–251.

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.

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

Lee, J.H., Choi, S.K., Roll-Mecak, A., Burley, S.K., and Dever, T.E. (1999). Universal conservation in translation initiation revealed by human and archaeal homologs of bacterial translation initiation factor IF2. Proc. Natl. Acad. Sci. USA *96*, 4342–4347.

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

Linder, P., Lasko, P.F., Ashburner, M., Leroy, P., Nielsen, P.J., Nishi, K., Schnier, J., and Slonimski, P.P. (1989). Birth of the D-E-A-D box. Nature *337*, 121–122.

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.

Markussen, F.-H., Breitwieser, W., and Ephrussi, A. (1997). Efficient translation and phosphorylation of Oskar require Oskar protein and the RNA helicase Vasa. Cold Spring Harb. Symp. Quant. Biol. *62*, 13–17.

Merrick, W.C., and Hershey, J.W.B. (1996). The pathway and mechanism of eukaryotic protein synthesis. In Translational Control, J.W.B. Hershey, M.B. Mathews, and N. Sonenberg, eds. (Cold Spring Harbor, NY: Cold Spring Harbor Laboratory Press), pp. 31–69.

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.

Saffman, E.E., and Lasko, P. (1999). Germline development in vertebrates and invertebrates. Cell. Mol. Life Sci. *55*, 1141–1163.

Schüpbach, T., and Wieschaus, E. (1986). Maternal-effect mutations

altering the antero-posterior pattern of the *Drosophila* embryo. Roux's Arch. Dev. Biol. *195*, 302–317.

Schüpbach, T., and Wieschaus, E. (1991). Female-sterile mutations on the second chromosome of *Drosophila melanogaster*. II. Mutations blocking oogenesis or altering egg morphology. Genetics *129*, 1119–1136.

Seydoux, G. (1996). Mechanisms of translational control in early development. Curr. Opin. Genet. Dev. *6*, 555–561.

Smibert, C.A., Wilson, J.E., Kerr, K., and Macdonald, P.M. (1996). smaug protein represses translation of unlocalized *nanos* mRNA in the *Drosophila* embryo. Genes Dev. *10*, 2600–2609.

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.

Thompson, J.D., Higgins, D.G., and Gibson, T.J. (1994). CLUSTAL W: improving the sensitivity of progressive multiple sequence alignment through sequence weighting, position-specific gap penalties and weight matrix choice. Nucleic Acids Res. *22*, 4673–4680.

Tinker, R., Silver, D., and Montell, D.J. (1998). Requirement for the vasa RNA helicase in *gurken* mRNA localization. Dev. Biol. *199*, 1–10.

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

Webster, P.J., Liang, L., Berg, C.A., Lasko, P., and Macdonald, P.M. (1997). Translational repressor bruno plays multiple roles in development and is widely conserved. Genes Dev. *11*, 2510–2521.

Wieschaus, E., and Nüsslein-Volhard, C. (1986). Looking at embryos. In *Drosophila*: a Practical Approach, D.B. Roberts, ed. (Washington, DC: IRL Press), pp. 199–227.

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

Wilson, S.A., Sieiro-Vazquez, C., Edwards, N.J., Iourin, O., Byles, E.D., Kotsopoulou, E., Adamson, C.S., Kingsman, S.M., Kingsman, A.J., and Martin-Rendon, E. (1999). Cloning and characterization of hIF2, a human homologue of bacterial translation initiation factor 2, and its interaction with HIV-1 matrix. Biochem J. *342*, 97–103.

Wohlwill, A., and Bonner, J.J. (1991). Genetic analysis of chromosome region 63 of *Drosophila melanogaster*. Genetics *128*, 763–775.

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