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Distinct roles of two conserved Staufen domains in *oskar* mRNA localization and translation

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Drosophila **Staufen protein is required for the localization of *oskar* mRNA to the posterior of the oocyte, the anterior anchoring of *bicoid* mRNA and the basal localization of *prospero* mRNA in dividing neuroblasts. The only regions of Staufen that have been conserved throughout animal evolution are five double-stranded (ds)RNA-binding domains (dsRBDs) and a short region within an insertion that splits dsRBD2 into two halves. dsRBDs 1, 3 and 4 bind dsRNA *in vitro*, but dsRBDs 2 and 5 do not, although dsRBD2 does bind dsRNA when the insertion is removed. Full-length Staufen protein lacking this insertion is able to associate with *oskar* mRNA and activate its translation, but fails to localize the RNA to the posterior. In contrast, Staufen lacking dsRBD5 localizes *oskar* mRNA normally, but does not activate its translation. Thus, dsRBD2 is required for the microtubule-dependent localization of *osk* mRNA, and dsRBD5 for the derepression of *oskar* mRNA translation, once localized. Since dsRBD5 has been shown to direct the actin-dependent localization of *prospero* mRNA, distinct domains of Staufen mediate microtubule- and actin-based mRNA transport.**

Keywords: *bicoid* mRNA/dsRNA-binding domain/mRNA localization/Staufen/translational control

Introduction

The establishment of cell polarity requires the targeting of specific proteins to the regions of a cell where they are required, and this is often achieved by localizing the mRNAs that encode them (St Johnston, 1995; Bashirullah *et al*., 1998). In many cases, mRNA localization is thought to be an active process that requires the cytoskeleton. For example, mating type switching in *Saccharomyces cerevisiae* is restricted to the mother cell by the myosin-dependent transport of *ash1* mRNA into the emerging daughter cell, and the directed motility of cultured fibroblasts requires the actin-dependent localization of β-actin mRNA (Kislauskis *et al*., 1994; Bertrand *et al*., 1998). Other mRNAs are localized by microtubule-dependent mechanisms, such as Vg1 mRNA, which moves to the vegetal pole of the *Xenopus* oocyte, and *bicoid* (*bcd*) and *oskar* (*osk*) mRNAs, which localize to opposite poles of the *Drosophila* oocyte (Yisraeli *et al*., 1990; Pokrywka and Stephenson, 1991; Clark *et al*., 1994). The importance of microtubule-dependent mRNA localization has been most clearly demonstrated in the case of the latter two transcripts, since their positions define the anterior–posterior axis of the embryo. *bcd* mRNA localizes to the anterior of the egg, and is translated after fertilization to produce a morphogen gradient that patterns the head and thorax of the embryo; the localization of *osk* mRNA to the posterior of the oocyte directs the assembly of the pole plasm, which contains posterior and germline determinants (Ephrussi and Lehmann, 1992; Driever, 1993).

The *cis*-acting signals that direct mRNA localization have been mapped in several transcripts, and in a few cases biochemical approaches have led to the identification of RNA-binding proteins that interact with these signals (Bashirullah *et al*., 1998). However, the best characterized example of an RNA-binding protein required for mRNA localization is *Drosophila* Staufen protein (Stau), which was identified in a genetic screen (Schüpbach and Wieschaus, 1986). Subsequent work has shown that Stau plays an essential role in the localization of three different mRNAs during development. It is required for (i) the localization of *osk* mRNA to the posterior of the oocyte (Ephrussi *et al*., 1991; Kim-Ha *et al*., 1991, 1995); (ii) the anchoring of *bcd* mRNA at the anterior of the egg (St Johnston *et al*., 1989); and (iii) the basal localization of *prospero* mRNA during the asymmetric divisions of embryonic neuroblasts (Li *et al*., 1997; Broadus *et al*., 1998; Matsuzaki *et al*., 1998; Schuldt *et al*., 1998; Shen *et al*., 1998). Although it has not been possible to test whether Stau binds specifically to these mRNAs, it contains five copies of a double-stranded (ds)RNA-binding domain (dsRBD), and the third of these has been shown to bind to dsRNA *in vitro* (St Johnston *et al*., 1992). When mutations that abolish the RNA-binding activity of dsRBD3 are incorporated into a full-length Stau transgene, this construct no longer rescues the localization of either *osk* or *bcd* mRNAs (Ramos *et al*., 2000). Thus, the dsRNA-binding activity of dsRBD3 is required for *bcd* and *osk* mRNA localization, strongly suggesting that Stau binds these RNAs directly.

During stages 7–9 of *Drosophila* oogenesis, *osk* mRNA localizes transiently at the anterior of the oocyte, and then moves to the posterior pole at stage 9 in a microtubule-dependent manner (Ephrussi *et al*., 1991; Kim-Ha *et al*., 1991; Clark *et al*., 1994). In *stau* null mutants, however, *osk* mRNA fails to move to the posterior and remains at the anterior. Several lines of evidence indicate that Stau protein associates with *osk* mRNA to mediate its posterior

localization. (i) Stau protein co-localizes with *osk* mRNA at the anterior of the oocyte and moves with the RNA to the posterior (St Johnston *et al*., 1991). (ii) Stau and *osk* RNA mislocalize to the same ectopic sites in mutants, such as *gurken*, which alter the polarity of the oocyte (González-Reyes *et al*., 1995; Roth *et al*., 1995). (iii) The

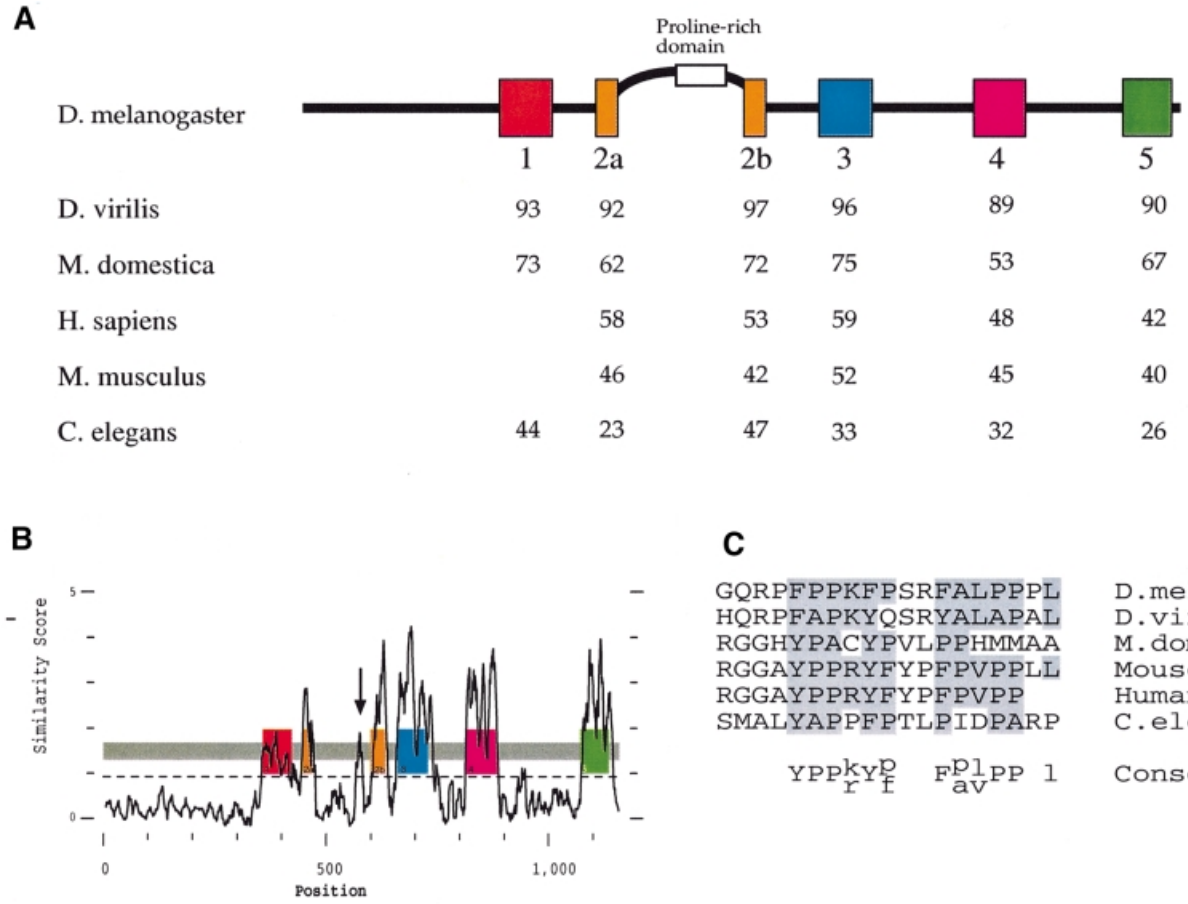

Fig. 1. A comparison of Staufen homologues from different species. (**A**) A diagram showing the positions of the conserved dsRBDs in *D.melanogaster* Staufen protein, and the percentage amino acid identity between these domains and the equivalent domains in other species. (**B**) A PlotSimilarity diagram of a ClustalW alignment of all six Staufen homologues. The positions of the dsRBDs are superimposed on the plot, and show that the similarity between homologues is almost entirely restricted to the dsRBDs. The arrow marks the only other region of similarity, which falls within the insertion in dsRBD2. Note that the degree of similarity shown for dsRBD1 appears lower than that for the other four dsRBDs because this domain is present in only 4/6 homologues. (**C**) Alignment of the conserved region in the dsRBD2 insertion. (**D**) An unrooted tree derived from a ClustalW alignment of dsRBDs from different proteins. Corresponding domains in the different Staufen homologues are more similar to each other than they are to any other dsRBDs, with the exception of CeStau dsRBD5, which is approximately similar to the other Stau dsRBD5s and to the third dsRBD of human TAR RNA-binding protein (Hstrbp) and *Xenopus* Xlrbpa (marked with asterisks). For simplicity, this diagram only includes dsRBDs described up to 1995.

posterior localization of Stau depends on *osk* mRNA (Ferrandon *et al*., 1994). In females carrying extra copies of an *osk* transgene, the increased quantity of *osk* mRNA produced induces a corresponding increase in the amount of Stau that localizes to the posterior pole. Thus, Stau is present in excess, and only the protein that is associated with *osk* RNA localizes to the posterior.

Translation of unlocalized *osk* mRNA is repressed by the binding of Bruno protein to Bruno-response elements (BRE) in the 3′UTR (Kim-Ha *et al*., 1995; Gunkel *et al*., 1998). An *osk* transgene lacking the BRE (*osk*BRE$^-$) is therefore translated prior to its localization, leading to the production of ectopic Osk, which causes a range of patterning defects in the resulting embryos. Low levels of ectopic Osk result in a loss of head and thoracic segments, while higher levels induce the formation of bicaudal embryos, with abdomens at both ends. Although the translation of *osk*BRE$^-$ mRNA no longer depends on its localization, it still requires Stau protein, since the bicaudal phenotypes are suppressed in a *stau* null mutant background. Thus, Stau plays a role in the translation of *osk*BRE$^-$ mRNA that is independent of its role in posterior localization, suggesting that it may also be involved in the translational regulation of wild-type *osk* mRNA. Finally, Stau has also been implicated in the anchoring of *osk* mRNA at the posterior. When a temperature-sensitive *stau* allele is kept under semi-restrictive conditions, Stau and *osk* mRNA localize to the posterior of the oocyte at stage 10, but are not maintained there in the embryo (St Johnston *et al*., 1991; Rongo *et al*., 1995).

Although Stau is not involved in the initial anterior localization of *bcd* mRNA, it is required to anchor the mRNA during the final stages of oogenesis (St Johnston *et al*., 1989). *bcd* RNA is normally released from the cortex at some time between stage 12 of oogenesis and egg deposition, and remains tightly localized in a spherical region of cytoplasm at the anterior of the egg. In *stau* mutant eggs, however, the RNA forms a shallow anterior–posterior gradient, and the resulting embryos have head defects because there is insufficient Bicoid protein at the very anterior of the embryo. This function of Stau shows several parallels to its role in *osk* mRNA localization (Ferrandon *et al*., 1994). First, Stau protein co-localizes with *bcd* mRNA at the anterior of the egg, and this localization is *bcd* mRNA dependent. Second, when the *bcd* 3′UTR is injected into the egg, it recruits Stau into particles that localize to the poles of the mitotic spindles. Stau, therefore, mediates the microtubule-dependent localization of both *bcd* and *osk* mRNAs, but at two different stages of development. Furthermore, in each case the localization of Stau requires its interaction with the appropriate RNA, suggesting that Stau–RNA complexes are the substrate for localization.

More recently, Stau has been shown to mediate the localization of *prospero* mRNA during the asymmetric divisions of embryonic neuroblasts (Li *et al*., 1997; Broadus *et al*., 1998; Fuerstenberg *et al*., 1998; Matsuzaki *et al*., 1998; Schuldt *et al*., 1998; Shen *et al*., 1998). In contrast to the localization of *bcd* and *osk* mRNAs, the localization of *prospero* mRNA–Stau complexes is disrupted by actin-destabilizing drugs, but not by microtubule-depolymerizing drugs (Broadus and Doe, 1997).

The discovery that Stau can mediate both microtubule- and actin-dependent mRNA localization raises the question of how different Stau–mRNA complexes are coupled to distinct transport pathways. It has previously been shown that Miranda protein binds to the dsRBD5 of Stau to direct the basal localization of *prospero* mRNA (Fuerstenberg *et al*., 1998; Schuldt *et al*., 1998; Shen *et al*., 1998). However, it remains unclear how Stau links *osk* and *bcd* mRNAs to the microtubule-based transport machinery, or how the activation of *osk* mRNA translation at the posterior is achieved. In this paper, we address this question by analysing the domains of Stau to determine which regions of the protein are required for these functions.

Results

Conservation of Stau throughout the animal kingdom

Since the functional domains of a protein can often be identified from their conservation during evolution, we cloned and sequenced homologues of *stau* from two other insect species, *Drosophila virilis* (Dvstau) and *Musca domestica* (Mdstau), which diverged from *Drosophila melanogaster* over 60 and 100 million years ago, respectively. In addition, Wickham *et al.* (1999) and Marión *et al.* (1999) have recently reported Stau homologues in *Caenorhabditis elegans* (Cestau), mouse (Mmstau) and human (Hsstau), and we also identified these on the basis of their homology to the insect genes and sequenced them in their entirety. The predicted amino acid sequences of the invertebrate homologues include five dsRBDs, corresponding to dsRBDs 1–5 of *D.melanogaster* Stau. In contrast, the human and mouse homologues include only four domains, which are most closely related to dsRBDs 2, 3, 4 and 5 of the invertebrate Stau (Figure 1D).

Despite the absence of a dsRBD1 equivalent in the mouse and human sequences, we believe that they represent vertebrate homologues of Stau because of the high degree of similarity to DmStau within the remaining four domains (Figure 1A). A ClustalW analysis of dsRBDs from many proteins reveals that apart from one minor exception for dsRBD5, a given Stau domain is most similar to the equivalent domain in each of the other Stau homologues (Figure 1D). This suggests that evolution is not acting simply to maintain similarity to a dsRBD consensus sequence, but rather that each domain has unique features that have been conserved during evolution. It is particularly notable that in all six homologues, dsRBD2 is split into two parts by up to 118 aa of non-dsRBD sequence. While dsRBDs have been identified in many proteins, the distinctive split in dsRBD2 has only been observed in these six Stau homologues.

Analysis of an alignment of the Stau homologues reveals that the only portions of the protein to have been conserved during evolution are the five dsRBDs (Figure 1B). For example, the *M.domestica* and *D.melanogaster* proteins show an average of 67% amino acid identity within the dsRBDs, but $<15\%$ in the rest of the protein. dsRBD2 and dsRBD5 were originally described as ‘half domains’ showing similarity to the dsRBD consensus only over the C-terminal portion of the domain (St Johnston *et al*., 1992). However, the conservation extends over a region corresponding to the length of a whole domain, and these

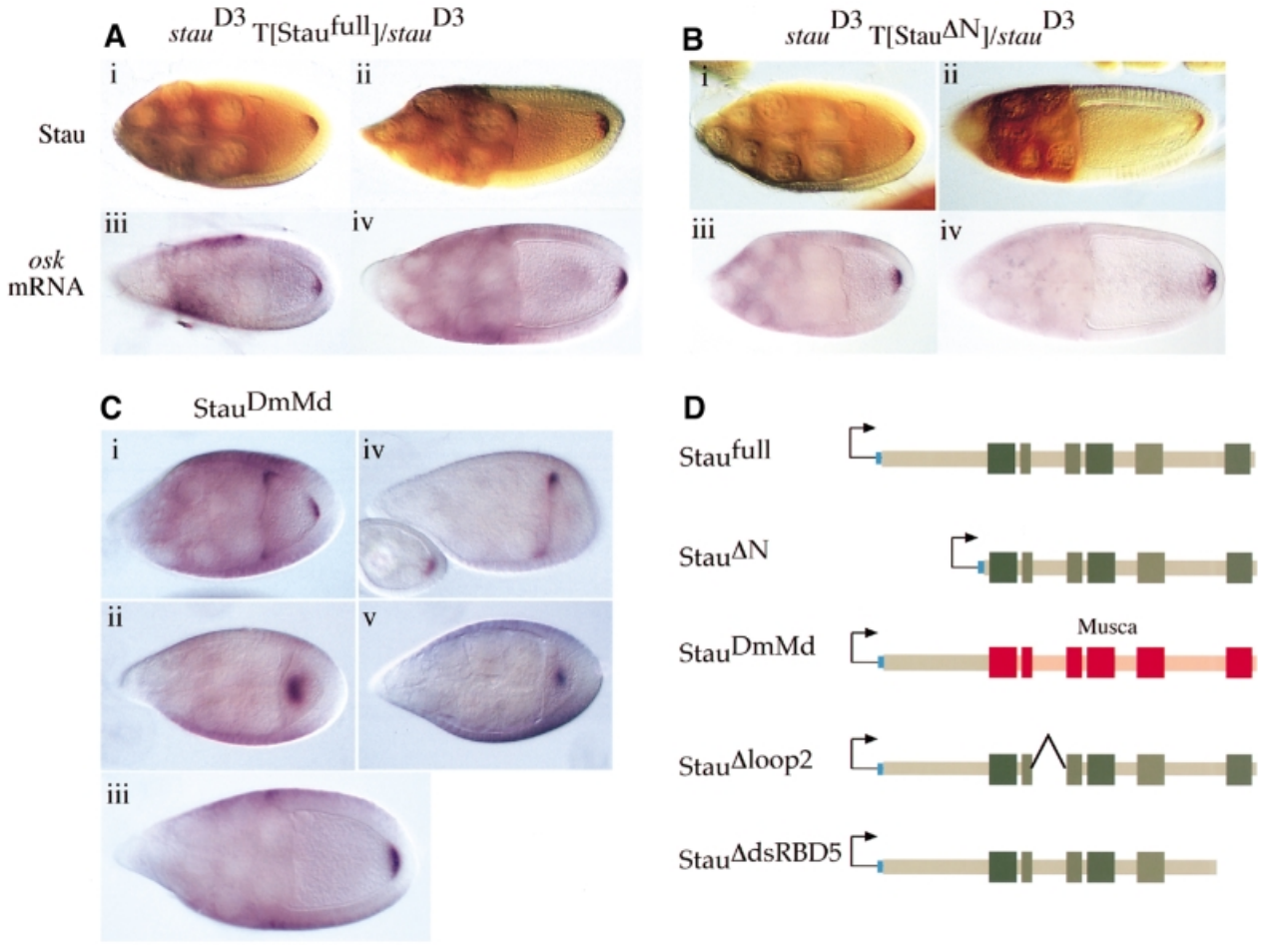

Fig. 2. The dsRBDs are the only conserved regions of Stau required for *osk* mRNA localization. (**A**) The localization of Staufen protein (i and ii) and *osk* mRNA (iii and iv) in stage 9 and 10 oocytes from *stau*^{D3} T[Stau^{full}]/*stau*^{D3} females. Full-length Staufen protein expressed from the transgene localizes normally to the posterior of the oocyte, and rescues the *osk* mRNA posterior localization defect of a *staufen* null mutation. (**B**) The localization of Stau protein (i and ii) and *osk* mRNA (iii and iv) in stage 9 and 10 oocytes from *stau*^{D3} T[Stau$^{\Delta N}$]/*stau*^{D3} females. Stau protein lacking the non-conserved N-terminal 282 aa also localizes normally and rescues *osk* mRNA localization and anchoring. (**C**) (i) In wild-type egg chambers, *osk* mRNA shows a transient localization to the anterior of the oocyte during stage 9. (ii) In *stau*^{D3} T[Stau^{DmMd}]/*stau*^{D3} egg chambers, *osk* mRNA fails to accumulate at the anterior margin at stage 9, and localizes instead to the centre of the oocyte. (iii) However, the mRNA shows a normal localization at the posterior pole by stage 10. (iv) In *stau*^{D3} egg chambers, all *osk* mRNA remains anchored at the anterior of the oocyte. (v) *osk* mRNA shows a transient localization to a point in the centre of the oocyte, when Stau^{DmMd} is expressed in the presence of wild-type *Drosophila* Stau protein. (**D**) A diagram showing the structure of the Stau proteins encoded by the Stau^{full}, Stau$^{\Delta N}$, Stau^{DmMd}, Stau$^{\Delta loop2}$ and Stau$^{\Delta dsRBD5}$ transgenes. The boxes indicate the positions of the dsRBDs. The short leader peptide containing the myc epitope tag is labelled in blue, *Drosophila* sequences in green and *M.domestica* sequences in red or pink.

domains should therefore be considered as complete, albeit divergent, dsRBDs, in agreement with the results of Gibson and Thompson (1994). The only other obvious homology between these proteins is a short region within the insertion in the middle of dsRBD2 that is rich in proline and aromatic amino acids (Figure 1B and C). Since the regions of the protein essential for its activity are expected to be conserved during evolution, the dsRBDs and this proline-rich region are likely to mediate all of the functions of Stau, including its ability to bind both mRNA and the factors that localize Stau–mRNA complexes.

To determine whether the dsRBDs are indeed the only part of Stau necessary for its function, we generated a transgene in which the large non-conserved N-terminal region of DmStau was deleted, and crossed this construct into a *stau* null mutant background (*stau*^{D3}). Like the full-length protein expressed from the same vector (Stau^{full}), Stau$^{\Delta N}$ localizes normally to the posterior of the oocyte, and completely rescues the posterior localization and anchoring of *osk* mRNA (Figure 2A and B). Furthermore, the eggs laid by Stau$^{\Delta N}$ females show a wild-type localization of *bcd* mRNA at the anterior pole (data not shown). This rescue of the maternal function of *stau* is also reflected in the phenotype of the embryos produced by Stau$^{\Delta N}$ females. Whereas the embryos laid by *stau*^{D3} mutant females die with head defects and no abdomen, almost all of the progeny of Stau$^{\Delta N}$ females hatch into larvae, and have normal heads and almost wild-type abdominal segmentation (Table I). Furthermore, a similar proportion of the adult offspring of Stau$^{\Delta N}$ females had gametic ovaries (85%) compared with those of females carrying Stau^{full} (88%), indicating that this construct leads to the production of the high levels of Osk activity that are necessary to specify the germline (Table I). Thus, the portion of Stau that includes the dsRBDs is able to mediate all of Stau's functions during oogenesis, including localization of both *osk* and *bcd* mRNAs, activation of

Table I. Rescue of the *stau* null phenotype by *stau* transgenes

Genotype	Average No. of abdominal denticle belts	% adults with gametic ovaries	% normal heads
wild type	8 ± 0	100	100
$stau^{D3}$	0.04 ± 0.002	n/a	0
$stau^{D3}$ T[$Stau^{full}$]	7.1 ± 0.21	88	100
$stau^{D3}$ T[$Stau^{\Delta N}$]	6.9 ± 0.18	85	100
$stau^{D3}$ T[$Stau^{DmMd}$]	7.4 ± 0.16	100	86
$stau^{D3}$ T[$Stau^{\Delta loop2}$]	0.14 ± 0.06	n/a	74
$stau^{D3}$ T[$Stau^{\Delta dsRBD5}$]	0.06 ± 0.03	n/a	68

osk translation and maintenance of pole plasm at the posterior.

As a more stringent test of whether the functional domains of Stau have been conserved during evolution, we generated transgenic lines in which the dsRBD-containing region of DmStau is replaced with the corresponding region from *M.domestica* ($Stau^{DmMd}$), and found that this transgene rescues all the phenotypes of a *stau* null mutation at least as well as the full-length Stau construct (Figure 2C; Table I). Since the only regions that are conserved between DmStau and MdStau are the five dsRBDs and the short sequence in the insertion in dsRBD2, it is likely that the most important functional domains of the protein reside in these regions.

Although the localization of *osk* mRNA to the posterior of the oocyte during stage 9 appears normal in $stau^{D3}$ females carrying $Stau^{DmMd}$, *osk* mRNA localizes through an atypical intermediate stage. In wild-type flies, *osk* mRNA shows a transient association with the anterior pole of the oocyte before moving to the posterior, whereas all of the mRNA remains at the anterior in $stau^{D3}$ homozygous females (Figure 2C, i and iv). In $Stau^{DmMd}$ ovaries, however, *osk* mRNA does not accumulate at the anterior of the oocyte, and instead forms a 'blob' in the middle of the oocyte, which disperses as localization proceeds (Figure 2C, ii and iii). Furthermore, this effect of $Stau^{DmMd}$ is dominant: in the absence of endogenous *D.melanogaster* Stau this 'blob' is diffuse, but in a wild-type background it forms a much sharper, well defined spot (Figure 2C, v). The nature of the cytoplasmic blob is unclear, but it is intriguing that *D.virilis osk* mRNA is localized through a similar intermediate when introduced into *D.melanogaster* (Webster *et al*., 1994).

dsRBD2 and dsRBD5 do not bind dsRNA

The discovery that the dsRBDs of Stau are the only conserved regions of the protein that are required for its function raises the question of whether all of these domains bind to dsRNA, and we therefore examined the ability of the five dsRBDs to bind to dsRNA on Northwestern blots (Figure 3A). As previously reported, dsRBD3 binds strongly to dsRNA, whereas a control domain in which 5 aa that contact the RNA have been mutated does not (Ramos *et al*., 2000). dsRBDs 1 and 4 also bind dsRNA, irrespective of its sequence, although this binding is weaker than that observed with dsRBD3. In contrast, dsRBD2 and dsRBD5 do not bind to any of the dsRNAs tested in this assay.

NMR and mutational analysis of Stau dsRBD3–dsRNA complexes have revealed that the dsRBD binds RNA through conserved amino acids that cluster on one face of the domain (Figure 3C) (Ramos *et al*., 2000). The corresponding positions in dsRBDs 1, 4 and 5 can be identified both by sequence alignment and by 'threading' their sequences onto the structure of dsRBD3. While dsRBDs 1 and 4 contain identical or similar conserved amino acids to dsRBD3, the amino acids on this face of dsRBD5 are much less well conserved, and are of a different type from those found in the other domains (Figure 3C). The lack of conservation of the dsRNA-contacting amino acids, coupled with the observed inability of dsRBD5 to bind dsRNA *in vitro* strongly suggest that this domain does not function as a dsRBD *in vivo*. However, the structural amino acids that comprise the hydrophobic core of the domain are highly conserved when compared with other dsRBDs, and amino acids on the other faces of dsRBD5 are also conserved across the different species. It is therefore likely that domain 5 folds into a typical dsRBD structure, but performs a distinct conserved function unrelated to dsRNA binding.

A highly conserved feature of all six Stau homologues is the presence of the large loop interrupting dsRBD2, and this most probably accounts for the inability of this domain to bind dsRNA *in vitro*. The NMR structure of the dsRBD3–dsRNA complex reveals that the dsRNA-binding regions of the domain span one turn of a dsRNA helix, and that their relative positions within the whole domain are crucial for RNA binding (Ramos *et al*., 2000). The insertion in loop 2 separates the two halves of dsRBD2, and the RNA-binding amino acids are therefore unlikely to have the correct spacing to contact RNA. Although the presence of the insertion in loop 2 makes it impossible to predict the structure of dsRBD2, the sequence of the domain suggests that it could bind dsRNA if it adopted a conformation in which these two halves were juxtaposed. To test this hypothesis, we constructed a version of dsRBD2 in which the extended loop 2 is replaced by the corresponding 8 aa loop of dsRBD3. When examined in the Northwestern assay, this dsRBD2Δloop2 binds dsRNA almost as efficiently as dsRBD3 (Figure 3). Thus, dsRBD2 can bind dsRNA when the removal of the large insertion allows the correct folding of the domain, suggesting that this domain binds dsRNA *in vivo* in the context of full-length protein.

The insertion in domain 2 is required for Stau–osk mRNA localization

To determine what role, if any, the extended loop in dsRBD2 plays in Stau function, we constructed a transgene ($Stau^{\Delta loop2}$) in which the normal domain 2 is replaced by the truncated dsRBD2Δloop2 described above, and crossed this into a $stau^{D3}$ mutant background. Although this transgene expresses high levels of a protein of the appropriate molecular weight (Figure 6B), it gives little or no rescue of the *stau* posterior phenotype; almost all *osk* mRNA and Stau protein fail to be transported to the posterior of the oocyte and remain trapped instead at the anterior margin (Figure 4A–E). However, a small amount of mRNA is occasionally seen at the posterior at stage 9. As a result of this defect in *osk* mRNA localization, none of the progeny of these flies hatch into larvae, and almost all develop less than one abdominal segment (Table I).

A trivial explanation for the inability of $Stau^{\Delta loop2}$ to

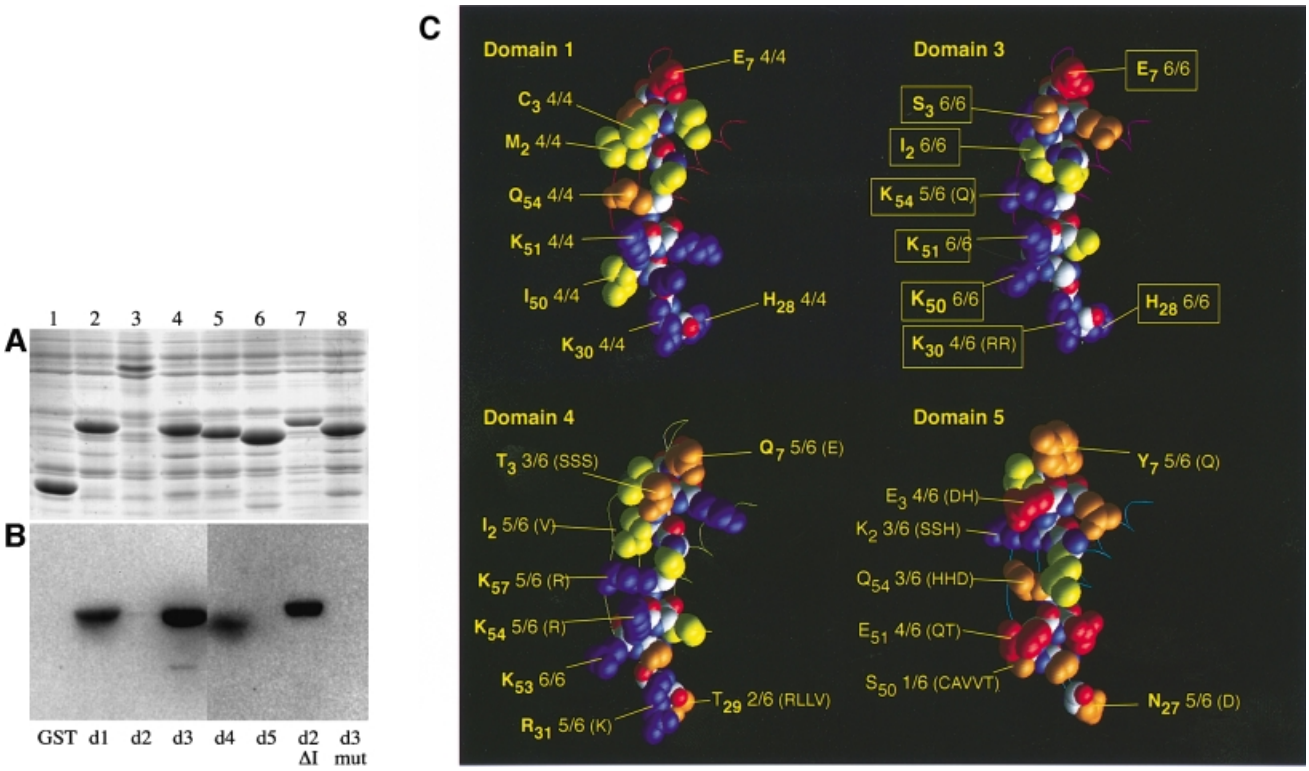

Fig. 3. dsRBDs 2 and 5 do not bind to dsRNA *in vitro*. (**A**) A Coomassie-stained SDS–PAGE gel showing the expression of the Staufen dsRBDs fused to glutathione *S*-transferase (GST). Lane 1, GST alone; lane 2, GST–dsRBD1; lane 3, GST–full-length dsRBD2; lane 4, GST–dsRBD3; lane 5, GST–dsRBD4; lane 6, GST–dsRBD5; lane 7, GST–dsRBD2 in which the large insertion has been replaced by the short loop 2 from dsRBD3; lane 8, GST–dsRBD3 containing five amino acid substitutions in residues that contact dsRNA (Ramos *et al*., 2000). (**B**) A Northwestern blot of the same samples as in (A) probed with [^{32}P]dsRNA. The right hand side of this blot has been exposed approximately four times longer than the left to reveal the weak dsRNA-binding activity of dsRBD4. (**C**) A comparison of the RNA-binding faces of dsRBDs 1, 3, 4 and 5, showing the amino acids in domain 3 that are required for dsRNA binding (yellow boxes), and the identity and conservation of the amino acids in equivalent positions in the other domains (yellow). The structures of dsRBDs 1, 4 and 5 have been modelled by 'threading' them onto the known structure of dsRBD3 (Bycroft *et al*., 1995). Blue, basic residues; red, acidic; yellow, non-polar; orange, polar and uncharged. The amino acids are numbered from the first conserved residue of the domain (Ramos *et al*., 2000).

localize *osk* mRNA is that the removal of the extended loop in dsRBD2 disrupts the folding of the protein and prevents it from binding to the RNA. While it is not possible to test the binding of full-length Stau to *osk* mRNA *in vitro*, three lines of evidence suggest that this is not the case. First, dsRBD2Δloop2 binds to RNA *in vitro*, whereas the wild-type domain does not, indicating that this deletion facilitates the folding of the domain into the RNA-binding configuration. Secondly, Stau$^{\Delta loop2}$ co-localizes with *osk* mRNA to the anterior of the oocyte (Figure 4F). The localization of Stau to the oocyte requires its association with *osk* mRNA, since the protein remains in the nurse cells in an *osk* mRNA null mutant (M.Weston and D.St Johnston, unpublished results). The normal co-localization of the mutant protein to the anterior of the oocyte with *osk* mRNA therefore indicates that it still binds to the RNA.

The third argument to suggest that Stau$^{\Delta loop2}$ interacts with *osk* mRNA makes use of the *osk*BRE^{-} transgene to uncouple *osk* mRNA translation from localization. In wild-type ovaries, the ectopic Osk protein produced from *osk*BRE^{-} mRNA suppresses head development in about half of the embryos, and causes a duplication of abdominal segments at the anterior (bicaudal phenotype) in over a third (Table I). Even though the translation of this mRNA does not require its localization to the posterior, it is not efficiently translated in the absence of Stau protein: none of the embryos laid by *stau*^{D3};*osk*BRE^{-} mothers develop the bicaudal phenotype, and almost all form only one abdominal segment at the posterior (Figure 4G). In contrast, Stau$^{\Delta loop2}$ activates translation of *osk*BRE^{-} mRNA almost as effectively as wild-type Stau: 36% of the embryos laid by Stau$^{\Delta loop2\ stauD3}$;*osk*BRE^{-} females develop a bicaudal phenotype, and another third show a suppression of head development and partial or complete rescue of the abdomen (Figure 4H and I). Thus, while Stau$^{\Delta loop2}$ is unable to localize *osk* mRNA, it is able to activate its translation, providing strong evidence that the protein is bound to the mRNA. This mutant protein therefore retains some of the functions of wild-type Stau, but has lost the ability to mediate the transport of Stau–*osk* mRNA complexes from the anterior to the posterior of the oocyte.

Domain 5 is required to activate the translation of osk mRNA at the posterior pole

To determine which functions of Stau are mediated by dsRBD5, we constructed a transgene in which the C-terminus of the protein is deleted (Stau$^{\Delta dsRBD5}$). When introduced into flies lacking wild-type Stau, Stau$^{\Delta dsRBD5}$ completely rescues the posterior localization of *osk* mRNA, and co-localizes with the RNA to the posterior (Figure 5A–D). Thus, the mutant protein retains the ability both to

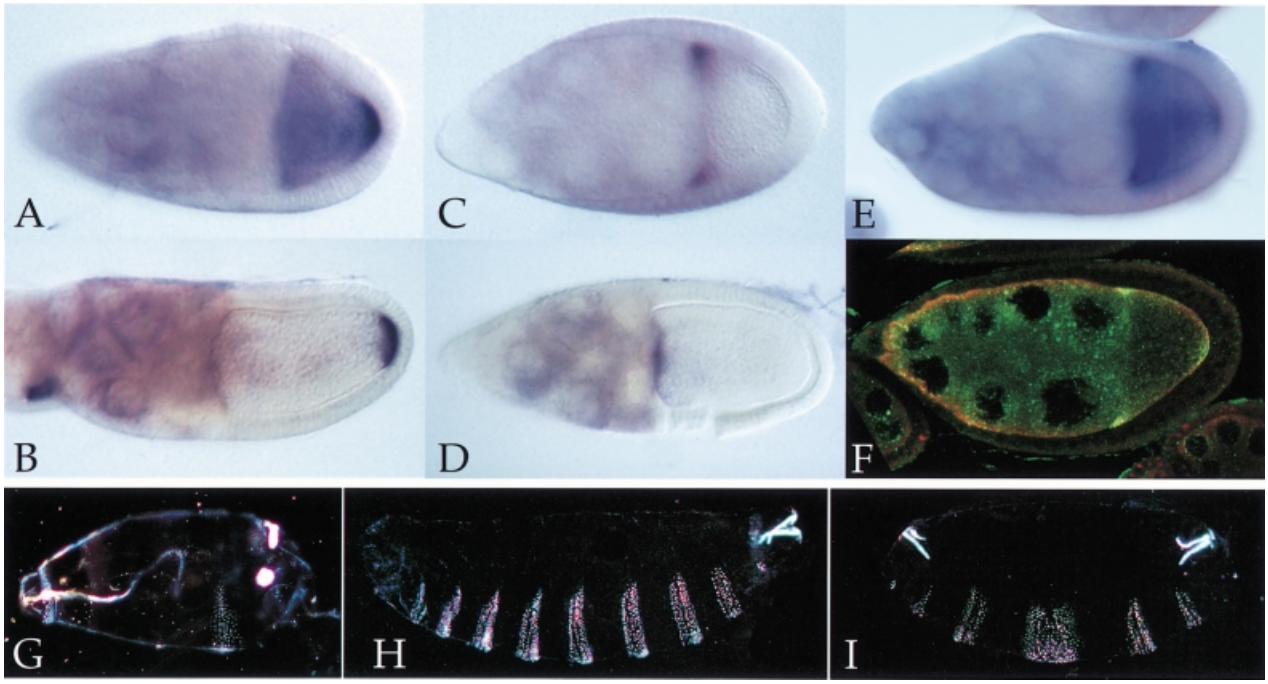

Fig. 4. The insertion in dsRBD2 is required for the posterior localization of *osk* mRNA. (**A** and **B**) Wild-type stage 9 and 10A egg chambers showing the normal localization of *osk* mRNA to the posterior of the oocyte. (**C** and **D**) *stau*^{D3} T[Stau$^{\Delta loop2}$]/*stau*^{D3} egg chambers, in which all *osk* mRNA remains anchored at the anterior of the oocyte. (**E**) A *stau*^{D3} T[Stau$^{\Delta loop2}$]/*stau*^{D3} stage 9 egg chamber, showing a small amount of *osk* mRNA at the posterior. (**F**) Stau$^{\Delta loop2}$ protein (yellow) co-localizes with *osk* mRNA to the anterior of the oocyte. (**G**) A cuticle preparation of a typical embryo from a *stau*^{D3};*osk*BRE^{-} female, with a normal head and only one abdominal segment. (**H** and **I**) Typical embryos from Stau$^{\Delta loop2\ stauD3}$;*osk*BRE^{-} females, showing the loss of head structures and rescue of the abdomen (H), and the stronger symmetric bicaudal phenotype (I).

bind *osk* mRNA and interact with the factors required for transport to the posterior. However, this construct shows almost no rescue of the *stau*^{D3} abdominal phenotype, and neither *osk* mRNA nor Stau$^{\Delta dsRBD5}$ protein are localized at the posterior by the time the egg is laid (Table I; data not shown). These observations show that dsRBD5 is required for a function of Stau that occurs after *osk* mRNA localization, suggesting that it may play a role in the translation of Osk protein. In wild-type ovaries, localized *osk* mRNA is translated to produce a tight posterior crescent of Osk protein at stage 10a (Figure 5E). In contrast, no detectable Osk is produced at the posterior of Stau$^{\Delta dsRBD5}$ oocytes, even though *osk* mRNA is correctly localized at this stage (Figure 5F). This requirement of Stau dsRBD5 for *osk* mRNA translation explains the failure of this transgene to rescue the development of the abdomen, and also accounts for the loss of *osk* mRNA and Stau from the posterior at later stages of oogenesis, since Osk protein has been shown to be necessary for the anchoring of Stau–*osk* mRNA complexes (Ephrussi *et al*., 1991; Kim-Ha *et al*., 1991).

To examine the role of dsRBD5 in the regulation of *osk* mRNA translation further, we crossed the Stau$^{\Delta dsRBD5}$ transgene into the *stau*^{D3};*osk*BRE^{-} background. Like Stau$^{\Delta loop2}$, Stau$^{\Delta dsRBD5}$ activates the translation of de-repressed *osk* mRNA. The resulting embryos develop an average of 5.2 abdominal denticle belts, compared with 1.3 for *stau*^{D3};*osk*BRE^{-} alone, and the majority show head defects caused by anterior Osk activity (Table II). This construct differs from Stau$^{\Delta loop2}$, however, in that it causes a lower frequency of bicaudal embryos, and this may be because *osk* mRNA is localized to the posterior rather than the anterior of the oocyte. Thus, Stau$^{\Delta dsRBD5}$ can activate the localization-independent translation of *osk*BRE^{-} mRNA, but not the translation of wild-type *osk* mRNA at the posterior pole, suggesting that dsRBD5 is specifically required for the activation of translation once the mRNA has been localized.

bcd mRNA localization requires dsRBD2 and dsRBD5

Since Stau is also required for the anterior anchoring of *bcd* mRNA, we examined whether the Stau$^{\Delta dsRBD5}$ and Stau$^{\Delta loop2}$ transgenes could rescue the *bcd* mRNA localization defect of a *stau* null mutation. Although both mutant proteins are expressed at similar levels to endogenous Stau, neither anchors *bcd* mRNA at the anterior (Figure 6A and B). Surprisingly, both constructs almost completely rescue the *stau* head phenotype, even though they do not restore the wild-type localization of the *bcd* mRNA. Whereas 100% of the embryos laid by *stau*^{D3} homozygous females at 18°C lack all or part of the head skeleton, over two-thirds of the embryos laid by the transgenic *stau*^{D3} females have wild-type heads, and the rest have much milder head defects than in *stau*^{D3} alone (Table I). This suggests that Stau plays a second role in the regulation of *bcd* mRNA expression that is independent of its function in localization. In contrast to its role in anchoring, this activity does not require dsRBD5 or the insertion in dsRBD2.

Discussion

The only regions of Stau to have been maintained through evolution are the dsRBDs and a short region within the

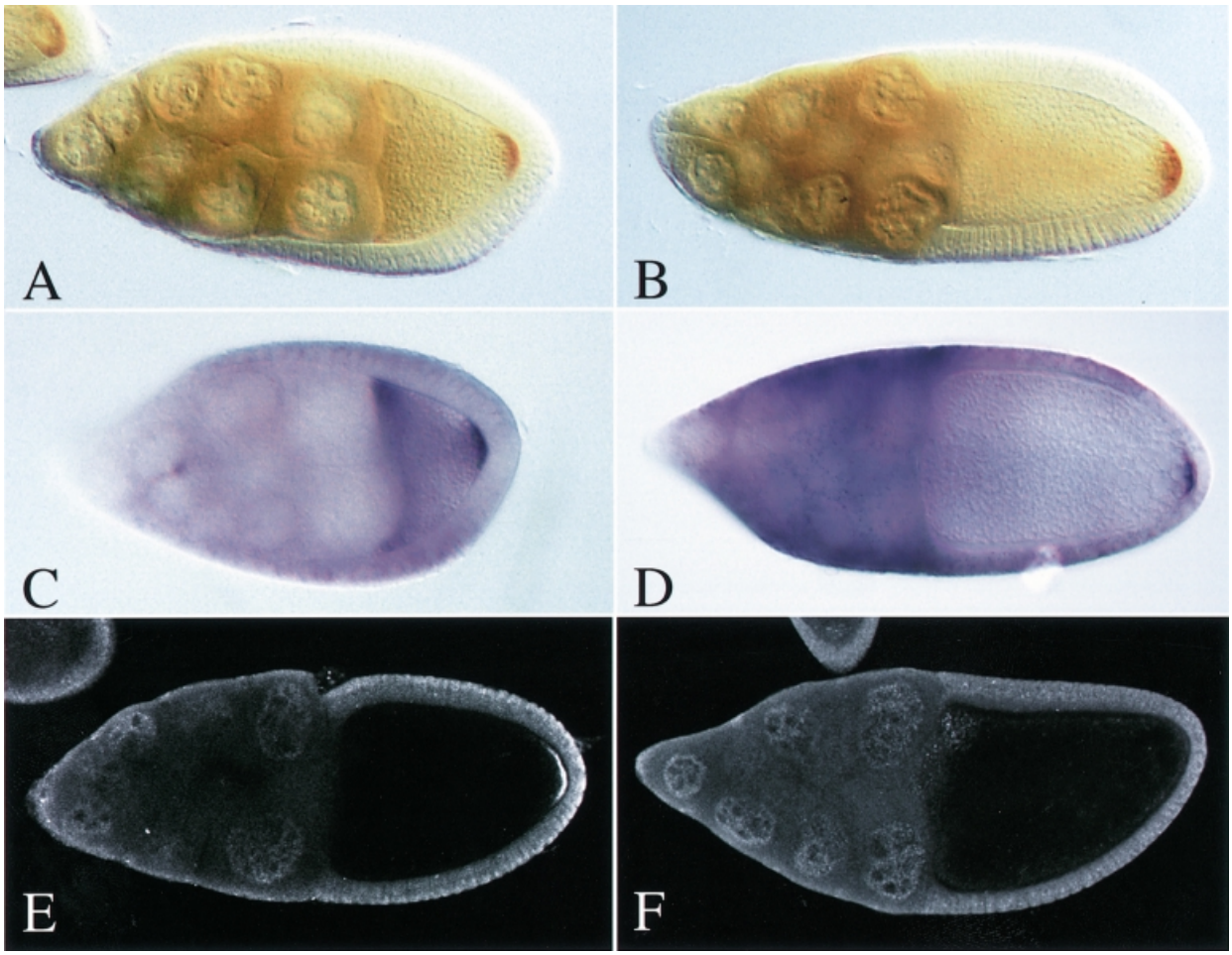

Fig. 5. dsRBD5 is required for *osk* mRNA translation. (A–D) Both Stau$^{\Delta dsRBD5}$ protein (**A** and **B**) and *osk* mRNA (**C** and **D**) localize normally to the posterior of stage 9 (A and C) and 10 oocytes (B and D). (E and F) Osk antibody stainings of wild-type (**E**) and *stau*^{D3} T[Stau$^{\Delta dsRBD5}$]/*stau*^{D3} oocytes (**F**).

Table II. Stau$^{\Delta loop2}$ and Stau$^{\Delta dsRBD5}$ activate the translation of *osk BRE*$^{-}$

Genotype	% wild type	% anterior defects with full or partial abdomen	% bicaudal	% *stau*^{D3}-like	Average No. of abdominal denticle belts
+;*osk*BRE^{-}	11	53	36	0	6.5
stau^{D3};*osk*BRE^{-}	1	5[a]	0	93	1.3
stau^{D3} T[Stau$^{\Delta loop2}$];*osk*BRE^{-}	7	33[a]	36	24	5.0
stau^{D3} T[Stau$^{\Delta dsRBD5}$];*osk*BRE^{-}	5	52[a]	9	34	5.2

[a]Embryos were only assigned to this class if their head defects were stronger than those seen in *stau*^{D3} alone, to distinguish between the phenotype caused by the *stau* defect in *bcd* mRNA localization, and that caused by the ectopic anterior expression of Oskar protein.

insertion in dsRBD2. Furthermore, the dsRBD-containing region from *M.domestica* retains all the essential functions of Stau during oogenesis. Thus, these conserved domains are likely to mediate all of the activities of Stau, such as binding to *bcd* and *osk* mRNAs, and its interactions with the factors that mediate mRNA transport and translational control. The alignment of the dsRBDs from all six species shows that natural selection is acting to maintain the unique characteristics of each. Consistent with this, only domains 1, 3 and 4 bind to dsRNA *in vitro*, while domains 2 and 5 are required for other activities of Stau.

The presence of a loop splitting dsRBD2 is the most pronounced conserved feature of all the Stau homologues. Replacement of this loop with the corresponding residues from dsRBD3 disrupts the posterior localization of almost all Stau and *osk* mRNA complexes, although a small amount is sometimes seen at the posterior of a few oocytes. A very similar variable localization of trace amounts of *osk* mRNA is also seen in all other mutants that specifically disrupt *osk* mRNA localization, such as *mago nashi* and *Tropomyosin II*, suggesting that this RNA may reach the posterior by a parallel translation-dependent pathway (Newmark and Boswell, 1994; Erdélyi *et al*., 1995). Wild-type *osk* mRNA is only translated once it is localized, and the resulting Osk protein anchors its own mRNA and Stau at the posterior pole. Thus, any RNA that diffuses

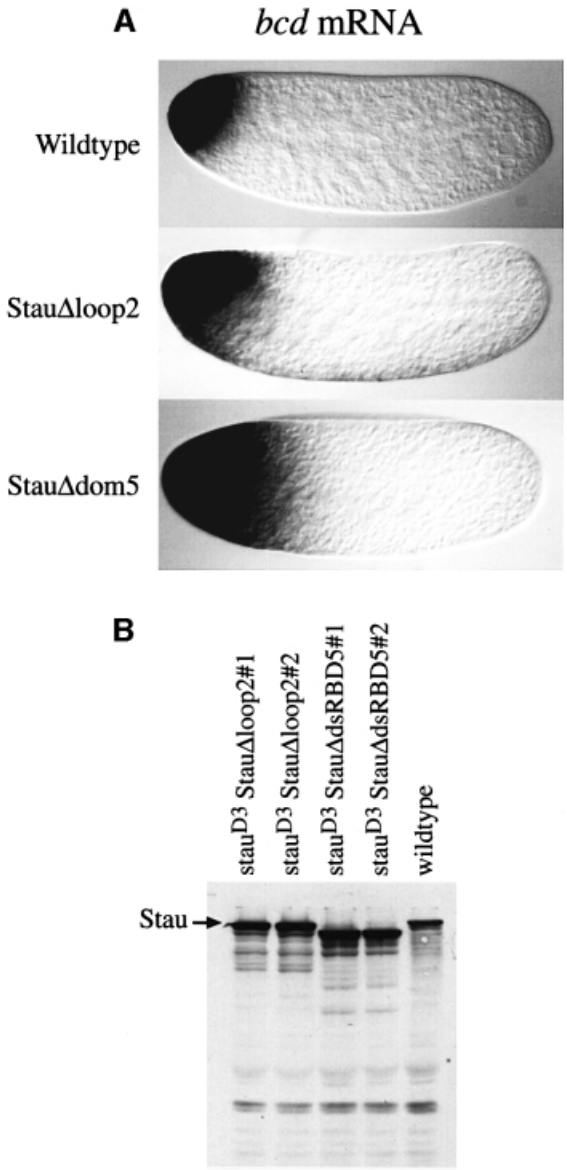

Fig. 6. dsRBD5 and the insertion in dsRBD2 are required for the anchoring of *bcd* mRNA. (**A**) *bcd* mRNA localization in freshly laid eggs from wild-type, *stau*^{D3} T[Stau$^{\Delta loop2}$]/*stau*^{D3} and *stau*^{D3} T[Stau$^{\Delta dsRBD5}$]/*stau*^{D3} females. (**B**) Western blot analysis of Stau expression in *stau* null mutant ovaries carrying the Stau$^{\Delta loop2}$ and Stau$^{\Delta dsRBD5}$ transgenes. Stau is expressed at higher levels than in the wild type in two independent lines of each transgene. Note that the Stau$^{\Delta dsRBD5}$ protein migrates slightly faster than the wild-type and Stau$^{\Delta loop2}$ proteins because it contains a larger deletion.

to the posterior should be trapped by localized translation and anchoring, and this could localize a tiny fraction of the mRNA in the absence of the normal transport pathway.

Despite its inability to localize *osk* mRNA to the posterior, Stau$^{\Delta loop2}$ still associates with the RNA at the anterior of the oocyte, and activates the translation of *osk*BRE^{-} RNA. These observations strongly suggest that Stau$^{\Delta loop2}$ interacts with *osk* mRNA, but that the removal of the insertion in dsRBD2 prevents the resulting complexes from associating with the transport machinery. This insertion probably disrupts the binding of the domain to dsRNA *in vitro* by changing the relative positions of the RNA-binding amino acids on either side. The domain binds dsRNA when the insertion is removed, however, and the domain contains several of the amino acids that contact RNA in other dsRBDs. Since these residues have been maintained by natural selection, it seems very likely that the domain interacts with RNA *in vivo* in the context of the full-length protein. Indeed, it is easy to envisage that the interaction of dsRBDs 1, 3 and 4 with RNA presents a very high local concentration of dsRNA to dsRBD2, which induces the domain to adopt the RNA-binding configuration.

Stau localization in both the oocyte and early embryo requires its association with the appropriate mRNA, suggesting that the protein undergoes a conformational change on binding RNA that allows it to associate with the factors that mediate RNA transport. Since the insertion in dsRBD2 is required for localization, it is attractive to propose that this conformational change occurs in dsRBD2. The two halves of dsRBD2 must come together for the domain to bind RNA, and this should loop out the insertion, which could then interact with the factors that transport Stau–RNA complexes. Thus, dsRBD2 could act as a conformational switch that senses the presence of bound RNA, and couples these complexes to the localization machinery. Alignment of the loops in dsRBD2 from different species reveals that they are highly divergent in both sequence and length, but there is a short block of amino acids that might represent a conserved motif with which these transport factors could interact (Figure 1B and C).

While the microtubule-dependent localization of *osk* mRNA requires the insertion in dsRBD2, but not dsRBD5, the converse is true for the actin-dependent localization of *prospero* mRNA in embryonic neuroblasts. In this case, localization is mediated by the binding of Miranda protein to dsRBD5 (Fuerstenberg *et al*., 1998; Matsuzaki *et al*., 1998; Schuldt *et al*., 1998; Shen *et al*., 1998). Thus, distinct domains of Stau mediate microtubule- and actin-dependent mRNA localization, presumably by recruiting different *trans*-acting factors. Although Stau is the first example of an RNA-binding protein that can direct localization along both actin and microtubules, it is likely that other proteins will also have this capacity. Chicken ZBP-1 protein, which binds to part of the β-actin mRNA localization sequence, is the homologue of VERA/Vg1RBP, which binds to the localization element that directs the microtubule-dependent localization of *Xenopus* Vg1 mRNA (Ross *et al*., 1997; Deshler *et al*., 1998; Havin *et al*., 1998). Thus, this protein is implicated in both microtubule- and actin-based localization, albeit in different organisms, but it remains to be seen whether distinct domains of the protein are required for each process.

While the localization of *osk* and *prospero* mRNAs requires either dsRBD5 or the insertion in dsRBD2, both domains are necessary for the Stau-dependent anchoring of *bcd* mRNA at the anterior of the egg. Very little is known about the steps in *bcd* mRNA localization that occur at the end of oogenesis, because it has been impossible to visualize the distribution of the RNA once the vitelline membrane is deposited around the egg. However, these results raise the possibility that Stau needs to interact with both the microtubule and actin cytoskeletons to anchor *bcd* mRNA.

Role of Stau in translational control

It has previously been difficult to investigate the role of Stau in *osk* mRNA translation for two reasons. First, *stau*

null mutations disrupt the localization of *osk* mRNA, and it is not translated unless it is localized to the posterior pole (Markussen *et al*., 1995; Rongo *et al*., 1995). Second, it is difficult to distinguish between the effects of weak *stau* alleles on translation and anchoring, because Osk protein is required to anchor its own RNA, but the mRNA needs to be anchored at the posterior to be translated (Ephrussi *et al*., 1991; Kim-Ha *et al*., 1991). However, Stau$^{\Delta dsRBD5}$ seems to have a specific defect in *osk* mRNA translation, as *osk* mRNA is localized normally to the posterior at stage 10 in these ovaries, but no detectable Osk protein is produced. Furthermore, *osk*BRE^{-} RNA produces significant amounts of Osk activity in these ovaries, indicating that Stau$^{\Delta dsRBD5}$ can function in the translation of derepressed *osk* mRNA. Taken together, these results strongly suggest that dsRBD5 is required to relieve Bruno repression once the mRNA has reached the posterior. This requirement cannot be absolute, however, since some Osk protein must be present early in oogenesis to anchor Stau–*osk* mRNA complexes.

Since dsRBD5 does not bind RNA, it presumably mediates its function in Osk translation through protein–protein interactions. Although Miranda binds to this domain, this interaction is unlikely to play any role during oogenesis, since *miranda* null germline clones have no phenotype (Matsuzaki *et al*., 1998). Thus, dsRBD5 presumably interacts with other proteins to regulate *osk* translation. A very similar translation defect is observed in *osk* transgenes that lack binding sites for 68 and 50 kDa proteins in the 5′UTR, whereas Stau is thought to associate with the localization signal in the 3′UTR (Gunkel *et al*., 1998). Thus, derepression is likely to involve cooperation between proteins bound to both ends of the RNA.

In addition to its role in derepressing *osk* translation at the posterior, Stau is required for the efficient expression of derepressed *osk*BRE^{-} RNA. Since neither the insert in dsRBD2 nor dsRBD5 are necessary for this activity, it presumably depends on the dsRBDs that bind RNA. It is possible that these dsRBDs also interact with other proteins, since only one face of the domain contacts RNA, and several amino acids on the other faces of these domains have been conserved during evolution. Alternatively, the binding of Stau may enhance *osk* mRNA translation indirectly, for example, by altering the folding of the RNA so that other factors can bind more efficiently.

Both Stau$^{\Delta loop2}$ and Stau$^{\Delta dsRBD5}$ partially rescue the *stau* head phenotype, even though they do not restore the wild-type localization of *bcd* mRNA. Thus, more Bcd activity must be produced from the mislocalized mRNA in the presence of these mutant proteins than in *stau*^{D3} alone, indicating that they provide a function of Stau that is independent of its role in anchoring. A comparison of the phenotypes produced by *vasa exu* and *stau exu* double mutants also indicates that Stau has a second function in the regulation of *bcd* mRNA. *exu* mutants block the localization of *bcd* mRNA early in oogenesis, and result in a uniform distribution of the RNA along the anterior–posterior axis of the embryo, while both *vasa* and *stau* mutants prevent the formation of the pole plasm, and therefore lack Nanos activity, which represses *bcd* mRNA translation (St Johnston *et al*., 1989; Wang and Lehmann, 1991; Wharton and Struhl, 1991; Wang *et al*., 1994). Despite the identical distributions of *bcd* RNA in these genotypes, *vasa exu* embryos develop anterior head structures everywhere, indicating that they contain high levels of Bcd activity, whereas *stau exu* form only thoracic structures (Schüpbach and Wieschaus, 1986). Thus, the removal of Stau reduces the level of Bcd expression, in the absence of any effect on mRNA localization. We can envisage two explanations for this localization-independent function of Stau. Stau binding could protect *bcd* RNA from degradation, and therefore increase the total amount of RNA. Alternatively, Stau could enhance the efficiency of *bcd* translation, in much the same way as it does for *osk* mRNA.

Since Stau has been conserved throughout animal evolution, it seems likely that the homologues will fulfil similar functions in mRNA localization and translational control in other organisms. In support of this view, recent evidence indicates that mammalian Stau mediates mRNA transport along microtubules in neurons (Köhrmann *et al*., 1999). The mouse and human Stau genes share an extra region of homology not found in the insect homologues, which resembles the microtubule-binding domain of MAP1B, and this region of HsStau binds to microtubules *in vitro* (Marión *et al*., 1999; Wickham *et al*., 1999). It will therefore be interesting to see whether this domain or the insertion in dsRBD2 is required for the microtubule-dependent movement of Stau in neurons.

Materials and methods

Cloning Stau homologues

Drosophila virilis and *M.domestica* homologues were obtained by low stringency screens of genomic libraries (Thummel, 1993; Curtis *et al*., 1995). These sequences have been submitted to the DDBJ/EMBL/GenBank under accession Nos AF225924 and AF225925. The human homologue (DDBJ/EMBL/GenBank accession No. T06248) was identified in the EST database (Adams *et al*., 1993). The corresponding clone (HFBDQ83) was obtained from the American Type Culture Collection and sequenced. The mouse homologue was obtained by low stringency screening of a 7.5 d.p.c. mouse embryonic cDNA library (L.-L.Li, unpublished) with HFBDQ83. *Cestau* was identified in cosmid F55A4 by BLAST searching, and corresponds to the predicted gene F55A4.5. ESTs directly confirm 6/10 of the predicted splice junctions. All but one of the remaining splice sites were confirmed by direct examination of the sequencing traces.

Drosophila mutants and transgenic lines

The *osk*BRE^{-} stock was a kind gift from Paul MacDonald. *stau*^{D3} is a protein null (St Johnston *et al*., 1991).

Transgenes were expressed from a P-element transformation vector, pCaTubMycSTOP, which drives maternal germline expression from the α4 tubulin promoter (Micklem *et al*., 1997). The Stau^{full}, Stau$^{\Delta dsRBD5}$ and Stau$^{\Delta loop2}$ constructs are identical to D288, Stau$^{\Delta dRBD5}$ and Stau$^{\Delta dRBD2}$ described previously (Schuldt *et al*., 1998). Stau$^{\Delta N}$ was generated in a similar manner, but lacks the first 281 aa of Stau, upstream of the *Cla*I site at position 1140. Stau^{DmMd} is identical to Stau^{full} from the start codon to 6 aa before the start of dsRBD1 (up to and including the sequence TSSSGRG). This portion is fused to the dsRBDs of MdStau, starting at an *Xma*I site 4 aa before the start of dsRBD1 (i.e. from REKTPMCLV). A single Ala residue replaces the Gly of DmStau (or the Ser of MdStau) 5 aa before the start of dsRBD1. Further details of these constructs are available on request.

Northwestern blots

Northwestern blots were performed using the procedure described in St Johnston *et al.* (1992), using a blocking buffer of 2.5% (v/v) Tween-20, 1% (w/v) milk, 25 mM NaCl, 10 mM $MgCl_2$, 10 mM HEPES pH 8.0, 100 μM EDTA, 1 mM dithiothreitol (DTT), and a binding buffer of 2.5% Tween-20, 1% milk, 50 mM NaCl, 10 mM $MgCl_2$, 10 mM HEPES pH 8.0, 100 μM EDTA and 1 mM DTT containing 500 000 c.p.m./ml of probe.

Phenotypic analysis

Antibody stainings and *in situ* hybridizations were performed as described in St Johnston *et al.* (1991). Antibodies were used at the following dilutions: rabbit α-Stau, 1/1000; rabbit α-Osk, 1/200.

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References

Adams,M.D., Kerlavage,A.R., Fields,C. and Venter,J.C. (1993) 3,400 new expressed sequence tags identify diversity of transcripts in human brain. *Nature Genet.*, **4**, 256–267.

Bashirullah,A., Cooperstock,R.L. and Lipshitz,H.D. (1998) RNA localization in development. *Annu. Rev. Biochem.*, **67**, 335–394.

Bertrand,E., Chartrand,P., Schaefer,M., Shenoy,S.M., Singer,R.H. and Long,R.M. (1998) Localization of ASH1 mRNA particles in living yeast. *Mol. Cell*, **2**, 437–445.

Broadus,J. and Doe,C. (1997) Extrinsic cues, intrinsic cues and microfilaments regulate asymmetric protein localization in *Drosophila* neuroblasts. *Curr. Biol.*, **7**, 827–835.

Broadus,J., Fuerstenberg,S. and Doe,C.Q. (1998) Staufen-dependent localization of *prospero* mRNA contributes to neuroblast daughter cell fate. *Nature*, **391**, 792–795.

Bycroft,M., Grünert,S., Murzin,A.G., Proctor,M. and St Johnston,D. (1995) NMR solution structure of a dsRNA binding domain from *Drosophila* Staufen protein reveals homology to the N-terminal domain of ribosomal protein S5. *EMBO J.*, **14**, 3563–3571.

Clark,I., Giniger,E., Ruohola-Baker,H., Jan,L. and Jan,Y. (1994) Transient posterior localisation of a kinesin fusion protein reflects anteroposterior polarity of the *Drosophila* oocyte. *Curr. Biol.*, **4**, 289–300.

Curtis,D., Apfeld,J. and Lehmann,R. (1995) *nanos* is an evolutionarily conserved organizer of anterior–posterior polarity. *Development*, **121**, 1899–1910.

Deshler,J.O., Highett,M.I., Abramson,T. and Schnapp,B.J. (1998) A highly conserved RNA-binding protein for cytoplasmic mRNA localization in vertebrates. *Curr. Biol.*, **8**, 489–496.

Driever,W. (1993) In Bate,M. and Martínez-Arias,A. (eds), *Maternal Control of Anterior Development in the Drosophila Embryo*. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY, Vol. 1, pp. 301–324.

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

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

Erdélyi,M., Michon,A., Guichet,A., Bogucka Glotzer,J. and Ephrussi,A. (1995) A requirement for *Drosophila* cytoplasmic tropomyosin in *oskar* mRNA localization. *Nature*, **377**, 524–527.

Ferrandon,D., Elphick,L., Nüsslein-Volhard,C. and St Johnston,D. (1994) Staufen protein associates with the 3′UTR of *bicoid* mRNA to form particles which move in a microtubule-dependent manner. *Cell*, **79**, 1221–1232.

Fuerstenberg,S., Peng,C.-Y., Alvarez-Ortiz,P., Hor,T. and Doe,C.Q. (1998) Identification of Miranda protein domains regulating asymmetrical cortical localization, cargo binding and cortical release. *Mol. Cell. Neurosci.*, **12**, 325–339.

Gibson,T.J. and Thompson,J. (1994) Detection of dsRNA-binding domains in RNA helicase A and *Drosophila* maleless: implications for monomeric RNA helicases. *Nucleic Acids Res.*, **22**, 2552–2556.

González-Reyes,A., Elliott,H. and St Johnston,D. (1995) Polarization of both major body axes in *Drosophila* by *gurken-torpedo* signalling. *Nature*, **375**, 654–658.

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.

Havin,L., Git,A., Elisha,Z., Oberman,F., Yaniv,K., Schwartz,S.P., Standart,N. and Yisraeli,J.K. (1998) RNA-binding protein conserved in both microtubule- and microfilament-based RNA localization. *Genes Dev.*, **12**, 1593–1598.

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.

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

Kislauskis,E.H., Zhu,X. and Singer,R.H. (1994) Sequences responsible for intracellular localization of β-actin messenger RNA also affect cell phenotype. *J. Cell Biol.*, **127**, 441–451.

Köhrmann,M., Luo,M., Kaether,C., DesGroseillers,L., Dotti,C.G. and Kiebler,M.A. (1999) Microtubule-dependent recruitment of Staufen–green fluorescent protein into large RNA-containing granules and subsequent dendritic transport in living hippocampal neurons. *Mol. Cell. Biol.*, **10**, 2945–2953.

Li,P., Yang,X., Wasser,M., Cai,Y. and Chia,W. (1997) Inscuteable and Staufen mediate asymmetric localization and segregation of *prospero* RNA during *Drosophila* neuroblast cell divisions. *Cell*, **90**, 437–447.

Marión,R.M., Fortes,P., Belosco,A., Dotti,C. and Ortín,J. (1999) A human sequence homologue of Staufen is an RNA-binding protein that is associated with polysomes and localizes to the rough endoplasmic reticulum. *Mol. Cell. Biol.*, **19**, 2212–2219.

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.

Matsuzaki,F., Ohshiro,T., Ikeshima-Kataoka,H. and Izumi,H. (1998) *miranda* localizes *staufen* and *prospero* asymmetrically in mitotic neuroblasts and epithelial cells in early *Drosophila* embryogenesis. *Development*, **125**, 4089–4098.

Micklem,D.R., Dasgupta,R., Elliott,H., Gergely,F., Davidson,C., Brand,A., González-Reyes,A. and St Johnston,D. (1997) The *mago nashi* gene is required for the polarisation of the oocyte and the formation of perpendicular axes in *Drosophila*. *Curr. Biol.*, **7**, 468–478.

Newmark,P.A. and Boswell,R.E. (1994) The *mago nashi* locus encodes an essential product required for germ plasm assembly in *Drosophila*. *Development*, **120**, 1303–1313.

Pokrywka,N.J. and Stephenson,E.C. (1991) Microtubules mediate the localization of *bicoid* RNA during *Drosophila* oogenesis. *Development*, **113**, 55–66.

Ramos,A., Grünert,S., Adams,J., Micklem,D.R., Proctor,M.R., Freund,S., Bycroft,M., St Johnston,D. and Varani,G. (2000) RNA recognition by a Staufen double-stranded RNA binding domain. *EMBO J.*, **19**, 997–1009.

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

Ross,A.F., Oleynikov,Y., Kislauskis,E.H., Taneja,K.L. and Singer,R.H. (1997) Characterization of a β-actin mRNA zipcode-binding protein. *Mol. Cell. Biol.*, **17**, 2158–2165.

Roth,S., Neuman-Silberberg,F.S., Barcelo,G. and Schüpbach,T. (1995) *cornichon* and the EGF receptor signaling process are necessary for both anterior–posterior and dorsal–ventral pattern formation in *Drosophila*. *Cell*, **81**, 967–978.

Schuldt,A.J., Adams,J.H.J., Davidson,C.M., Micklem,D.R., St Johnston,D. and Brand,A. (1998) Miranda mediates the asymmetric protein and RNA localisation in the developing nervous system. *Genes Dev.*, **12**, 1847–1857.

Schüpbach,T. and Wieschaus,E. (1986) Maternal-effect mutations altering the anterior–posterior pattern of the *Drosophila* embryo. *Roux Arch. Dev. Biol.*, **195**, 302–317.

Shen,C.P., Knoblich,J.A., Chan,Y.M., Jiang,M.M., Jan,L.Y. and Jan,Y.N. (1998) Miranda as a multidomain adapter linking apically localized Inscuteable and basally localized Staufen and Prospero during asymmetric cell division in *Drosophila*. *Genes Dev.*, **12**, 1837–1846.

St Johnston,D. (1995) The intracellular localization of messenger RNAs. *Cell*, **81**, 161–170.

St Johnston,D., Driever,W., Berleth,T., Richstein,S. and Nüsslein-Volhard,C. (1989) Multiple steps in the localization of *bicoid* RNA to the anterior pole of the *Drosophila* oocyte. *Development*, (*Suppl.*), **107**, 13–19.

St Johnston,D., Beuchle,D. and Nüsslein-Volhard,C. (1991) *staufen*, a gene required to localize maternal RNAs in the *Drosophila* egg. *Cell*, **66**, 51–63.

St Johnston,D., Brown,N.H., Gall,J.G. and Jantsch,M. (1992) A conserved double-stranded RNA-binding domain. *Proc. Natl Acad. Sci. USA*, **89**, 10979–10983.

Thummel,C. (1993) Compilation of *Drosophila* cDNA and genomic libraries. *Drosoph. Inf. Serv.*, **72**, 180–183.

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* localisation in *Drosophila. Dev. Dyn.*, **199**, 103–115.
Webster,P., Suen,J. and Macdonald,P.M. (1994) *Drosophila virilis oskar* transgenes direct body patterning but not pole cell formation or maintenance of RNA localization in *D.melanogaster. Development*, **120**, 2027–2037.
Wharton,R.P. and Struhl,G. (1991) RNA regulatory elements mediate control of *Drosophila* body pattern by the posterior morphogen nanos. *Cell*, **67**, 955–967.
Wickham,L., Duchaîne,T., Luo,M., Nabi,I.R. and DesGroseillers,L. (1999) Mammalian Staufen is a double-stranded-RNA- and tubulin-binding protein which localizes to the rough endoplasmic reticulum. *Mol. Cell. Biol.*, **19**, 2220–2230.
Yisraeli,J., Sokol,S. and Melton,D. (1990) A two-step model for the localization of maternal mRNA in *Xenopus* oocytes: involvement of microtubules and microfilaments in the translocation and anchoring of Vg1 mRNA. *Development*, **108**, 289–298.

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