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RNA-Binding Proteins in Early Development

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ABSTRACT RNA-binding proteins play a major part in the control of gene expression during early development. At this stage, the majority of regulation occurs at the levels of translation and RNA localization. These processes are, in general, mediated by RNA-binding proteins interacting with specific sequence motifs in the 3′-untranslated regions of their target RNAs. Although initial work concentrated on the analysis of these sequences and their *trans*-acting factors, we are now beginning to gain an understanding of the mechanisms by which some of these proteins function. In this review, we will describe a number of different families of RNA-binding proteins, grouping them together on the basis of common regulatory strategies, and emphasizing the recurrent themes that occur, both across different species and as a response to different biological problems.

KEYWORDS *C. elegans, Drosophila*, oocyte, RNA localization, translational control, *Xenopus*

INTRODUCTION

The formation of a complex organism from a single cell requires a series of highly regulated changes in gene expression. Although cell specification later on in development often relies on the hierarchical expression of specific transcription factors, earlier events are usually completely dependent on regulation at the level of RNA, rather than DNA. This is primarily due to the fact that the zygotic nucleus is not transcribed during the first stages of development. As has been well characterized in model organisms such as *Drosophila* and *Xenopus*, the production of new proteins relies on the utilization of mRNAs which have been maternally transcribed and then stored in an inactive form until the appropriate time.

The key post-transcriptional processes that are regulated during development are RNA localization and translation. This allows gene expression to be controlled in both a spatial and a temporal fashion. The targeting of an mRNA to a specific region of an egg or developing embryo, in conjunction with the translational repression of the mRNA until it is correctly localized, results in localization of the protein product and a generation of polarity, a vital requirement for establishing an asymmetric organism from a single egg. In addition, the masking of an mRNA from the translational machinery until a particular time during development allows an ordered series of events to occur.

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The concerted use of both translational control and RNA localization has been best characterized in *Drosophila*. In flies, for example, generation of the anteroposterior axis relies on the localization of *oskar* mRNA (Ephrussi *et al.*, 1991; Kim-Ha *et al.*, 1991), and subsequently *nanos* mRNA, to the posterior pole of the oocyte (Gavis & Lehmann, 1992; Wang & Lehmann, 1991), and the localization of *bicoid* mRNA to the anterior pole (Driever & Nusslein-Volhard, 1989). All these RNAs are translationally repressed before localization (Gavis & Lehmann, 1994; Kim-Ha *et al.*, 1995). In *Xenopus*, Vg1 mRNA provides the paradigm of a vertebrate message regulated at the level of localization and translation, during the lengthy period of oogenesis.

For the study of the temporal, rather than spatial, control of protein synthesis, *Xenopus* has provided an important model system. When mature oocytes are stimulated with progesterone to complete meiosis I and enter meiosis II (a period known as meiotic maturation, marked by germinal or nuclear envelope breakdown (GVBD), and after fertilization of the egg, previously quiescent mRNAs are translationally stimulated by cytoplasmic polyadenylation to provide the dividing cells with the necessary polypeptides, such as *c-mos* and cyclins, whereas housekeeping mRNAs are deadenylated and translationally repressed.

These modes of control are revisited later in development, for example, in situations where regulated protein synthesis needs to occur at a site distant from the nucleus, such as in neurons where the synthesis of specific proteins occurs at the nerve synapses far from the cell body (reviewed in Glanzer & Eberwine, 2004; Huang & Richter, 2004; Steward & Schuman, 2003), or when transcription is again impossible, such as during spermatogenesis in mammals, when the spermatid nucleus is inactivated by chromatin condensation (reviewed in Kleene, 2003).

Analysis of many different examples of the regulation of RNA localization or translation has shown that the control is often effected by RNA-binding proteins interacting with specific sequence motifs usually located in the untranslated regions (UTRs) of the regulated RNAs, most commonly in the 3′-untranslated regions (reviewed in Gebauer & Hentze, 2004; Kuersten & Goodwin, 2003; Wilkie *et al.*, 2003). The RNA-binding proteins known to be involved in development are many and varied and cannot be exhaustively covered in this review. We will focus on some of the key strategies employed by the cell to regulate gene expression, describing some of the specific RNA-binding proteins involved and the extent of their conservation in different organisms.

THE MECHANISM OF TRANSLATION INITIATION AND ITS CONTROL

Translation Initiation

In order to understand how translation can be regulated during development, it is useful to first review the basic process of protein synthesis. Translation can be divided into three main stages: initiation, elongation, and termination. Whereas translational control could theoretically happen at any of these points, most cases are known to act at the level of initiation and, therefore, we will concentrate on this stage (reviewed in Hershey & Merrick, 2000; Preiss & Hentze, 2003).

Translation initiation begins with the formation of the 43S pre-initiation complex, comprising the 40S ribosomal subunit, the initiator methionyl tRNA, and the translation initiation factor eIF2. The 43S complex must then be recruited to the 5′-end of the mRNA from where it scans along the mRNA until it reaches the AUG initiation codon. Upon recognition of the start codon, eIF5 triggers GTP hydrolysis by eIF2, resulting in the release of all the translation initiation factors that have associated with the 40S ribosomal subunit prior to scanning. The 60S ribosomal subunit can then join to form the 80S ribosome, and the elongation phase commences.

Recruitment of the 43S complex to the mRNA is a key step and requires the action of the eIF4F complex. eIF4F contains the eukaryotic initiation factor eIF4G, the cap-binding protein eIF4E, and the DEAD-box RNA helicase eIF4A. eIF4G acts as a scaffolding protein, providing a framework to bring together many of the factors associated with translation initiation. In addition to binding eIF4E and eIF4A, it also possesses binding sites for the poly(A) binding protein (PABP), the multi-subunit initiation factor eIF3, and the eIF4E kinase Mnk1. eIF4A is thought to unwind secondary structure in the 5′ UTR of the mRNA. Its helicase activity is stimulated by the initiation factor eIF4B. eIF4E binds tightly to the cap structure at the 5′-end of the mRNA, localizing the eIF4F complex to the 5′-tip. eIF4G interacts with eIF3, which in turn binds the 43S subunit, to recruit the preinitiation complex to the mRNA. Most cellular mRNAs are translated in a cap-dependent manner.

Influence of the 3′-End of the mRNA on Initiation at the 5′-End

While translation initiation occurs at the 5′-end of the mRNA, the 3′-end can also influence the efficiency of initiation. The majority of mRNAs possess a poly(A) tail at their 3′-ends, which is bound by the poly(A) binding protein PABP. This protein has been shown to have key roles in both translation and RNA stability. It comprises four RNA recognition motifs (RRMs), one of the best-characterized RNA-binding domains, together with a C-terminal domain approximately 70 amino acids in length, PABC, a peptide-binding domain that serves to recruit the termination factor eRF3, and the translational regulators Paip1 and 2 (reviewed in Kühn & Wahle, 2004). RRM1+2, which bind poly(A) with the same affinity as the full-length protein (Kühn & Pieler, 1996), bind 12 adenosine residues, but the binding site is extended by RRM3+4 to protect 25 adenosines in total (Sachs *et al.*, 1987). Therefore, when a poly(A) tail is degraded to less than 25 adenosines, PABP will no longer bind.

The poly(A) tail and PABP have been shown to influence translation. Although the poly(A) tail is not essential for the process (Proweller & Butler, 1994; Searfoss & Wickner, 2000), it does promote the translation of adenylated mRNAs over non-adenylated mRNAs in a competitive environment (Preiss & Hentze, 1998). Most strikingly, transfection experiments in yeast showed that the poly(A) tail acts synergistically with the cap to stimulate translation so that RNAs that are both capped and polyadenylated are translated to a much greater extent than RNAs that have been modified at just one end (Gallie, 1991). This finding was confirmed in cell-free systems from yeast (Iizuka *et al.*, 1994; Tarun Jr. & Sachs, 1995), *Drosophila* embryos (Gebauer *et al.*, 1999), and mammalian cells (Michel *et al.*, 2000). An explanation of this observation became apparent when it was found that yeast PABP could interact directly with eIF4G (Tarun & Sachs, 1996), resulting in circularization of the RNA, as shown directly by atomic force microscopy (Wells *et al.*, 1998). Such an interaction has also been observed between plant (Le *et al.*, 1997), human (Imataka *et al.*, 1998; Piron *et al.*, 1998), and *Xenopus* PABP and eIF4G (Wakiyama *et al.*, 2000). It occurs between RRM2 of PABP (Kessler & Sachs, 1998) and an N-terminal PABP binding domain in eIF4G (Tarun & Sachs, 1996). The synergism in translational stimulation could therefore be explained by a cooperativity between the components of the tertiary eIF4E-eIF4G-PABP complex stabilizing the association of eIF4F with the mRNA and resulting in more effective recruitment of the 43S complex. This is termed the 'closed-loop' model of translation initiation (Jacobson, 1996; Kahvejian *et al.*, 2001) (Figure 1).

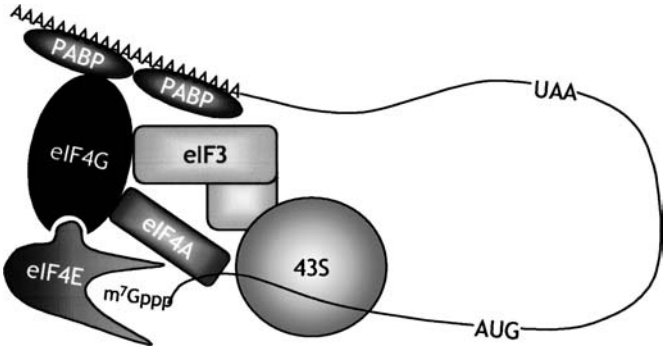


FIGURE 1 **The closed loop model of translation initiation. The translation initiation factor eIF4G provides a scaffold for the recruitment of multiple other initiation factors. The interaction of eIF4G with both the cap-binding protein eIF4E and the poly(A)-binding protein PABP results in circularization of the mRNA through the interaction of these proteins with the cap structure at the 5′-end and the poly(A) tail at the 3′-end respectively. eIF4G recruits the 43S preinitiation complex to the mRNA through its interaction with eIF3, while the presence of the helicase eIF4A allows unwinding of the 5′ UTR with the aid of eIF4B (not shown).**

Strategies for Regulating Initiation

This gives us two obvious mechanisms by which translation initiation can be down-regulated–by disrupting the interaction between eIF4G and eIF4E and by disrupting the interaction between eIF4G and PABP. One of the first details to be established when examining a new mechanism of translational control is thus whether it is poly(A)- or cap-dependent. As the study of the control mechanism usually involves the attachment of the regulatory sequences to a reporter gene and then expression of *in vitro* transcribed reporter mRNAs in a system of choice, the mRNA can be manipulated in various ways. mRNAs lacking a poly(A) tail are often still translated to a reasonable extent in cell-free extracts or after injection into the organism of interest, making it relatively straightforward to establish whether or not the mechanism is poly(A)-dependent. Uncapped mRNAs are often very poorly translated, however, and it would thus be difficult to determine whether the translational control is still occurring. (Moreover, uncapped RNA is usually subject to rapid degradation in extracts or in cells.) In this case, internal ribosome entry sites (IRES's) derived from a variety of viruses are often used. These

sites allow the virus to disable the usual mechanism of cap-dependent translation initiation by which cellular mRNAs are translated, but still allow translation of the viral RNAs by recruiting the ribosome internally to the RNA (Jackson, 2000). By introducing an IRES into reporter mRNAs, it is possible to circumvent the need for a cap in order to translate the mRNA and thus solely examine its requirement for translational control. As different IRES require different canonical translation initiation factors (Hellen & Sarnow, 2001), it is often possible to dissect further the mechanism of regulation. Using these approaches, the translational control of a number of developmentally regulated mRNAs has been shown to function in either a cap-dependent or poly(A)-dependent fashion, and the means by which RNA-binding proteins enable this to occur will be discussed in detail later.

Disruption of the eIF4E-eIF4G interaction has been well characterized as a means of enabling the cell to respond to environmental changes. The treatment of cells with certain growth factors results in an up-regulation of cap-dependent translation, achieved by modifying the activity of a family of proteins known as the 4E-binding proteins or 4E-BPs (Lin *et al.*, 1994; Pause *et al.*, 1994). These proteins interact with eIF4E in a manner that prevents it from interacting with eIF4G, and thus they repress translation initiation (Haghighat *et al.*, 1995) (Figure 2A). Analysis of the sequences of eIF4G and the 4E-BPs revealed that they contain a consensus sequence motif, YXXXXLΦ, where X is variable and Φ is any hydrophobic residue (Mader *et al.*, 1995). Crystal structures of eIF4E bound to cap analog and complexed with the peptides representing the conserved motifs from either 4E-BP1 or eIF4G revealed that both peptides form similar α-helical structures that lie along the convex region of eIF4E, on the opposite surface to the cap (Marcotrigiano *et al.*, 1999; Matsuo *et al.*, 1997). After cells are treated with the appropriate growth factors, the 4E-BPs become hyperphosphorylated, which destroys their ability to bind eIF4E, thus allowing eIF4E to bind eIF4G (Lin *et al.*, 1994; Pause *et al.*, 1994). The interaction between eIF4E and eIF4G appears to produce significant conformational changes in both proteins, resulting in an enhanced affinity of eIF4E for the cap, and the promotion of translation initiation (Gross *et al.*, 2003).

Recently, it has been shown that a similar approach has been adopted in some cases of developmental regulation. For example, RNA-binding proteins, such as the

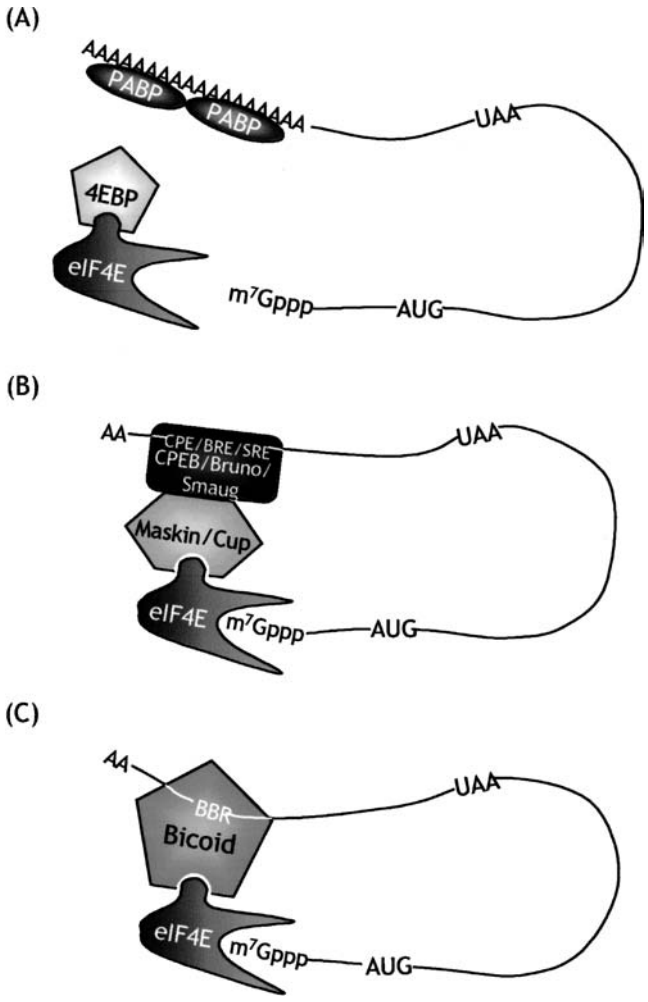


FIGURE 2 **(*A*) Translational repression by the 4E-BPs. The 4E-BPs repress translation in the absence of specific growth factors (Lin *et al.*, 1994; Pause *et al.*, 1994). As they interact with eIF4E at the same site as eIF4G, using a similar binding motif, the binding of the two proteins is mutually exclusive (Haghighat *et al.*, 1995). Thus, the 4E-BPs prevent formation of the closed loop. eIF4E bound to 4E-BP has a lower affinity for the cap compared to eIF4E bound to eIF4G (Gross *et al.*, 2003). (*B*) Maskin and Cup repress translation in a manner analogous to the 4E-BPs. RNAs containing CPE, BRE or SRE elements are repressed by their interaction with CPEB, Bruno and Smaug proteins, respectively. CPEs are found in multiple mRNAs that are translationally down-regulated during *Xenopus* oogenesis. In *Drosophila*, *oskar* mRNA is controlled by Bruno and *nanos* mRNA by Smaug. Each of these proteins interacts directly with an eIF4E-binding factor containing a peptide with homology to the eIF4E-binding peptide identified in the 4E-BPs. In the case of CPEB, this factor is maskin (Stebbins-Boaz *et al.*, 1999), while Bruno and Smaug both interact with Cup (Nakamura *et al.*, 2004), Nelson *et al.*, 2004). Cup contains a high affinity canonical eIF4E-binding site (Nakamura *et al.*, 2004; Nelson *et al.*, 2004), Wilhelm *et al.*, 2003; Zappavigna *et al.*, 2004). Interaction of the proteins with eIF4E prevents the association of eIF4G, thus repressing translation. (*C*) Bicoid binds eIF4E directly through a consensus eIF4E-binding site. In *Drosophila* embryos, Bicoid binds to the Bicoid binding region (BBR) in the 3′ UTR of *caudal* mRNA (Dubnau & Struhl, 1996; Rivera-Pomar *et al.*, 1996). Translational repression requires the eIF4E-binding motif of Bicoid, and the interaction of Bicoid with eIF4E is dependent on binding of the protein to the *caudal* mRNA (Niessing *et al.*, 2002). Again, the complex that is formed prevents recruitment of eIF4G.**

cytoplasmic polyadenylation binding protein (CPEB) in *Xenopus* and the translational repressor Bruno in *Drosophila*, which bind to specific motifs in their target 3′ UTRs, in turn interact with accessory proteins that contain the consensus eIF4E binding motif and prevent the cap binding protein interacting with eIF4G (Nakamura *et al.*, 2004; Nelson *et al.*, 2004; Stebbins-Boaz *et al.*, 1999; Wilhelm *et al.*, 2003). A slightly modified approach would be to directly prevent eIF4E from binding to the cap and this is believed to be utilized by the *Drosophila* RNA-binding protein Bicoid (Niessing *et al.*, 2002).

Examples of the disruption of the interaction between PABP and eIF4G have also been described. The rotaviral RNAs are capped (Imai *et al.*, 1983) but not polyadenylated (McCrae & McCorquodale, 1983). They terminate in a short sequence motif, which is bound by the viral protein NSP3 (Poncet *et al.*, 1993). NSP3 acts synergistically with the cap to promote translation in a similar manner to PABP (Vende *et al.*, 2000). The virus also promotes translation of its own RNAs by inhibiting the translation of cellular RNAs (Michel *et al.*, 2000). It interacts with the PABP-binding domain of eIF4G, with a higher affinity than that of PABP itself, to evict PABP from eIF4G (Piron *et al.*, 1998). Although there are no similarities in the primary or tertiary structures of NSP3 and PABP, site-directed mutagenesis and isothermal titration calorimetry experiments showed that NSP3 and PABP use analogous strategies to recognize eIF4G (Groft & Burley, 2002).

While the interaction between PABP and eIF4G is, at present, the best-characterized means of translational stimulation by PABP, it is likely that PABP participates in additional interactions to stimulate translation. For example, yeast PABP can stimulate the translation of capped, non-adenylated RNAs in *in vitro* translation extracts when added in excess. Mutagenesis experiments show that this activity does not require the PABP-eIF4G interaction but may involve additional factors interacting with RRM2 or RRM4 of PABP (Otero *et al.*, 1999). Experiments in *Xenopus* oocytes in which PABP was tethered to a reporter RNA independently of a poly(A) tail, showed that while RRMs 1+2 can stimulate translation through an interaction with eIF4G, RRMs 3+4, which do not interact with eIF4G, will also stimulate translation (Gray *et al.*, 2000). These interactions, which have not yet been characterized, could also be the targets of regulatory mechanisms.

In most cases, the amount of PABP bound to an mRNA depends on the length of the poly(A) tail. Translational control can, therefore, also be achieved by varying poly(A) tail length to either stimulate or repress translation (Richter, 2000). This mechanism presumably operates by affecting the amount of PABP bound and the degree of stimulation of translation accomplished by the PABP-eIF4G, and other putative, interactions. Cytoplasmic polyadenylation is a highly regulated and conserved mechanism, first observed in *Spisula* oocytes, that dramatically increases translation during meitotic maturation and after fertilization (reviewed in Richter, 2000). This phenomenon has subsequently been observed in a variety of other organisms including *Urechis* (Rosenthal & Wilt, 1986), starfish (Standart *et al.*, 1987), flies (Sallés *et al.*, 1994), *Xenopus* (Paris *et al.*, 1988; Paris & Philippe, 1990) and mice (Sallés *et al.*, 1992). Cytoplasmic polyadenylation is promoted by sequences in 3′ UTRs, which include one or more copies of a U-rich cytoplasmic polyadenylation element (CPE), with the consensus sequence $U_{4\text{-}6}A_{1\text{-}3}U$, located in fairly close proximity to the ubiquitous nuclear polyadenylation signal, AAUAAA, as shown by microinjection into maturing eggs or in cell-free lysates. Both elements are required to support cytoplasmic poly(A) extension and to stimulate translation during oocyte maturation (Fox *et al.*, 1989; McGrew *et al.*, 1989; Richter, 2000; Sheets *et al.*, 1994). Many mRNAs in a wide range of organisms have now been shown to be translationally regulated by CPEs. These include biologically important mRNAs such as those encoding *Xenopus* cyclin B1 (Barkoff *et al.*, 2000; de Moor & Richter, 1999), wee-1 (Charlesworth *et al.*, 2000), mouse tissue-type plasminogen activator (tPA) and cyclin B1 (Stutz *et al.*, 1998; Tay *et al.*, 2000), clam ribonucleotide reductase and cyclin A (Minshall *et al.*, 1999), and *Drosophila bicoid* (Sallés *et al.*, 1994). A particularly striking example is provided by *c-mos* mRNA, whose polyadenylation and consequent translation is a pivotal regulatory step in the meiotic maturation of *Xenopus* and mouse oocytes (de Moor & Richter, 1997; Gebauer *et al.*, 1994; Mendez *et al.*, 2000a; Sheets *et al.*, 1995). In *Xenopus*, mRNAs lacking a CPE-motif in their 3′ UTR, such as ribosomal protein and actin mRNAs, lose their poly(A) tail by default at meiotic maturation, and are concomitantly released from polysomes, providing further evidence of the tight connection between poly(A) tail length and translational efficiency (Fox & Wickens, 1990; Sheets *et al.*, 1994; Varnum & Wormington, 1990).

Examples of developmentally-regulated mRNAs have been identified that still undergo control in the absence of both a cap and a poly(A) tail. For example, in *Drosophila*, the sex-lethal protein (SXL) represses the translation of the male-specific lethal protein 2 (MSL-2) to prevent over-transcription of the two X-chromosomes in female flies. SXL binds to both the 5′- and 3′ UTRs of the *msl-2* mRNA (Gebauer *et al.*, 1999) and prevents association of the 43S ribosomal subunit with the message (Gebauer *et al.*, 2003). As a cap is not required, the repression must occur at a stage subsequent to 43S subunit recruitment by eIF4F, and it is proposed that SXL destabilizes the scanning complex, dissociating it from the mRNA (Gebauer *et al.*, 2003). Repression of the rabbit 15-lipoxygenase (r15-LOX) mRNA during erythropoiesis also occurs at a later step in translation initiation, even later than control by SXL. The repression is mediated by an element in the r15-LOX 3′ UTR called the differentiation control element (DICE) and its specific binding factors, hnRNPs K and E1/E2 (Ostareck-Lederer *et al.*, 1994; reviewed by Ostareck-Lederer & Ostareck, 2004). Repression by DICE has been shown to require no canonical initiation factors using the Cricket Paralysis Virus IRES which implies that the element interferes with 60S subunit joining, the last stage of translation initiation (Ostareck *et al.*, 2001).

Thus it can be seen that a variety of different approaches to regulation are possible. We will now discuss the role of specific RNA-binding proteins in translational control in more detail.

DISRUPTION OF THE INTERACTION BETWEEN eIF4E AND eIF4G

The Cytoplasmic Polyadenylation Element Binding (CPEB) Proteins: A Conserved Family of RNA-Binding Proteins

CPEB is a critical regulator of gene expression in early development. It was first cloned and characterized in *Xenopus* oocytes as a 62 kDa protein that bound specifically to the CPEs that mediate cytoplasmic polyadenylation (Hake & Richter, 1994; Stebbins-Boaz *et al.*, 1996). A decade later, the *Xenopus* protein has become the founding member of a large and important family of RNA-binding proteins, including clam (*Spisula solidissima*) p82 (Walker *et al.*, 1999), *Drosophila* Orb (Christerson & McKearin, 1994; Lantz *et al.*, 1992), *C. elegans* CPB-1-4 (Luitjens *et al.*, 2000), sea hare (*Aplysia californica*) CPEB (Liu & Schwartz, 2003; Si *et al.*, 2003a), as well as the more closely related mouse, zebrafish, and human homologs (Bally-Cuif *et al.*, 1998; Gebauer & Richter, 1996; Kurihara *et al.*, 2003; Theis *et al.*, 2003; Welk *et al.*, 2001). The functions of these proteins now extend well beyond oogenesis and egg maturation.

While *Xenopus* has only one characterized CPEB, several homologs have been identified in worms and mammals. For example, the nematode *C. elegans* possesses four CPEB homologs in total. CPB-1 is required for progression through meiosis in spermatocytes while FOG-1 controls germ cell fates; none of the homologs are required for oogenesis, however (Jin *et al.*, 2001; Luitjens *et al.*, 2000). Four mammalian CPEB proteins have also been identified. Mouse CPEB-1 (mCPEB-1), the homolog of *Xenopus* CPEB, regulates cyclin B1 mRNA expression in oocytes (Gebauer & Richter, 1996; Tay *et al.*, 2000) and is also expressed in brain (Wu *et al.*, 1998); specifically, in the dendritic layers of the hippocampus, at synapses in cultured neurons, and in postsynaptic densities of adult rat brain (Theis *et al.*, 2003; Wu *et al.*, 1998). Evidence suggests that CPEB-1 may regulate synaptic plasticity by controlling the translation and localization of specific mRNAs in dendrites (Huang *et al.*, 2003; Huang *et al.*, 2002; Wu *et al.*, 1998). Visual experience induces rapid polyadenylation and translational activation of Ca^{2+} calmodulin-dependent protein kinase II mRNA (α-CaMKII) in the visual cortices of dark-raised rats (Wu *et al.*, 1998). α-CaMKII mRNA contains two CPE sequences in its 3′ UTR and encodes a key component in long-term potentiation and synaptic differentiation. Both female and male CPEB-1−/− mice display severe fertility effects (Tay & Richter, 2001), but long term potentiation is only modestly affected, hinting at protein redundancy, with the function in neurons being compensated by one of the other mCPEB homologs (Alarcon *et al.*, 2004; Wells *et al.*, 1998). Indeed, the highly related mCPEB-2 homologue is abundantly expressed in testis and brain (Kurihara *et al.*, 2003; Theis *et al.*, 2003); its postmeiotic expression during spermatogenesis suggests a possible role in translational regulation of stored mRNAs in transcriptionally inactive haploid spermatids (Kurihara *et al.*, 2003). mCPEB-3 mRNA is strongly expressed in heart and brain and mCPEB-4 mRNA in embryos and adult brain, as well as kidney, lung, and heart (Theis *et al.*, 2003). The mouse mCPEB-3 and -4 isoforms are

most similar to mCPEB-2 and show less homology to mCPEB-1. mCPEB2-4 are subject to alternative splicing, which generate tissue-specific isoforms that may undergo differential phosphorylation (Theis *et al.*, 2003). Human homologues of all four mouse proteins have also been identified (Kurihara *et al.*, 2003; Welk *et al.*, 2001).

All CPEB proteins (Table 1) share two C-terminal RNA recognition motifs (RRMs) and two zinc-finger RNA-binding domains downstream from the RRMs (Hake & Richter, 1994; Walker *et al.*, 1999). The RRM is the most widespread and well-characterized RNA-binding domain, consisting of two short consensus sequences, RNP1 (octamer) and RNP2 (hexamer), located in a structurally conserved domain of about 80 amino acids, with a $\beta\alpha\beta\beta\alpha\beta$ topology. RNA recognition occurs on the four-stranded anti-parallel β-sheet surface, where the RNP1 and RNP2 motifs are juxtaposed on the two middle strands (reviewed in Varani & Nagai, 1998). Zinc finger proteins vary widely in structure, as well as in function, which ranges from DNA or RNA binding to protein–protein interactions and membrane association. The classical Cys_2His_2 zinc finger is the founding member of a rapidly expanding family of zinc-binding modules (reviewed in Laity *et al.*, 2001). A detailed analysis of deletions and point mutations in the *Xenopus* CPEB protein showed that the two RRM domains and the highly conserved set of cysteine and histidine residues forming two unusual zinc finger regions (C_4, C_2H_2) are all required to bind CPE-containing RNA *in vitro* (Hake *et al.*, 1998). The importance of these domains for CPEB function was recently confirmed in a broad mutational analysis that identified the molecular lesions associated with 33 independent *fog-1* mutations in *C. elegans*. All of the single missense mutations altered one of the RRM or Zn finger RNA-binding domains (Jin *et al.*, 2001).

The N-termini of CPEB proteins are far more varied in sequence, although some members share certain features whose functional importance will be discussed in detail in later sections. Vertebrate CPEB-1 isoforms and clam p82 CPEB contain a short motif, S(D/E)S(D/E)TSGFSS, part of a PEST sequence, which, in *Xenopus*, mediates CPEB degradation, by ubiquitination, during meiotic maturation, and in response to cdc2 phosphorylation (Mendez *et al.*, 2002; Reverte *et al.*, 2001; Thom *et al.*, 2003). *Xenopus* and mouse CPEB-1 proteins also contain consensus Aurora A (Eg2) kinase phosphorylation sites (LDS/TR). These are phosphorylated early during meiotic maturation (Hodgman *et al.*, 2001; Mendez *et al.*, 2000a, 2000b; Tay *et al.*, 2003) as progesterone stimulation of maturation induces the activation of Aurora A (Andresson & Ruderman, 1998), although there is some disagreement regarding the exact timing of its activation (Castro *et al.*, 2003; Frank-Vaillant *et al.*, 2000; Maton *et al.*, 2003). mCPEB-2-4 and all invertebrate isoforms lack Aurora A phosphorylation sites (Theis *et al.*, 2003; Thom *et al.*, 2003). Some of the alternatively-spliced mouse products instead contain potential recognition sites for protein kinase A, α-CAMKII and p70 (S6 kinase) (Liu & Schwartz, 2003; Theis *et al.*, 2003), suggesting control of mouse CPEB family members by distinct signalling pathways.

The final noteworthy feature of these proteins, which is not shared by all the homologs, is the glutamine-rich domain, located N-terminal to the RRMs and originally noted in clam and fly CPEB (Christerson & McKearin, 1994; Lantz *et al.*, 1992; Walker *et al.*, 1999). It was recently shown to be also present in the N-terminus of mCPEB-3 (Theis *et al.*, 2003) and neuronal *Aplysia* CPEB (Liu & Schwartz, 2003; Si *et al.*, 2003a). Strikingly, this Q-rich region of *Aplysia* CPEB has been reported to confer prion-like properties upon the protein (Si *et al.*, 2003b).

Translational Repression by CPEB During Oogenesis

In addition to its positive role in promoting translational activation of target RNAs during meiotic maturation by cytoplasmic polyadenylation (reviewed in more detail below), CPEB represses translation by interacting with the U-rich 3′ UTR CPE elements in clam, frog, and mouse oocytes (de Moor & Richter, 1999; Minshall *et al.*, 1999; Tay *et al.*, 2000).

The pioneering work of Vassalli and colleagues (Stutz *et al.*, 1998; Stutz *et al.*, 1997) on mouse tissue plasminogen activator mRNA lays the framework for the model that a single type of 3′ UTR element may perform dual roles in regulating maternal mRNA translation. In this study, the UA-rich adenylation control element (ACE), which supports deadenylation in growing oocytes as well as the subsequent meiotic readenylation, was also shown to mediate translational repression in primary oocytes, by interaction with a titratable repressor protein. However, despite the apparent homology of function and sequence between CPEs and

TABLE 1 **Schematic representation of the domain architecture of the RNA-binding proteins reviewed in this article, alongside their binding sites where known, and comments on protein family classification, and roles in gene expression control. For further details and references, see text. The order of the proteins in the figure mirrors their appearance in the text. Note that, for clarity, we present all proteins as of one size, but the exact length of a representative protein is indicated in terms of the number of amino acids. The lengths and positions of sub-domains are not to exact scale. (*Continued*)**

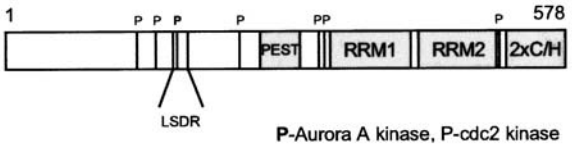
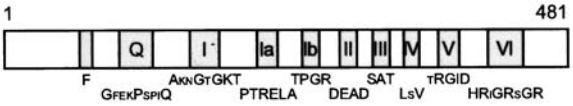
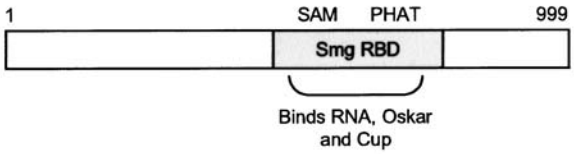
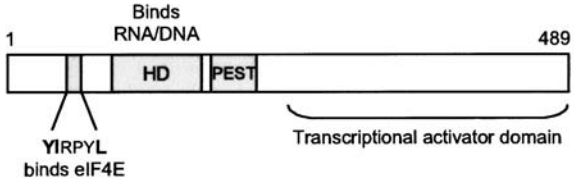
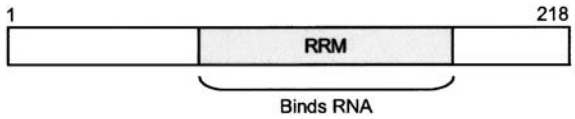
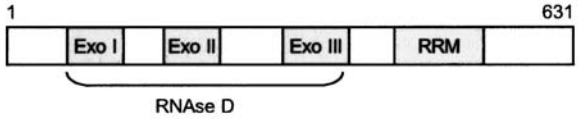
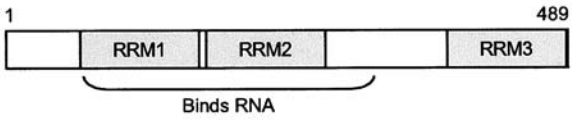
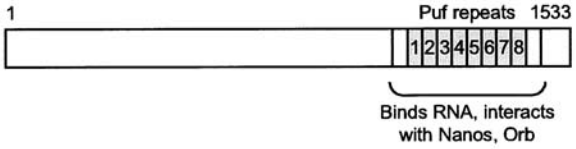
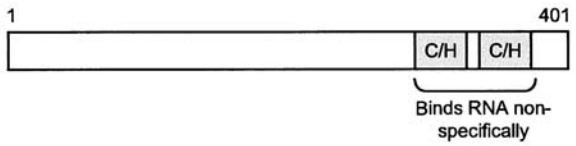
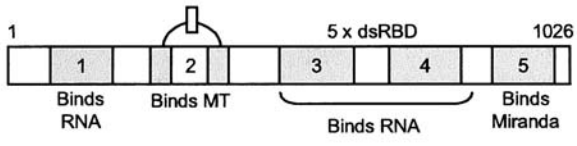
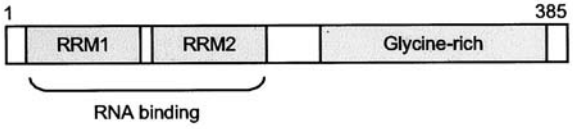
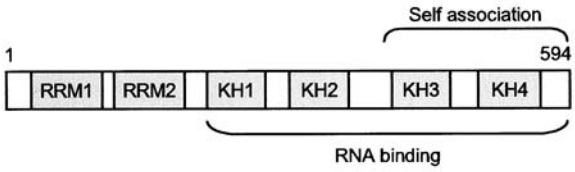
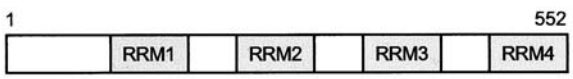
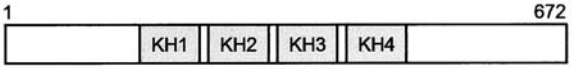
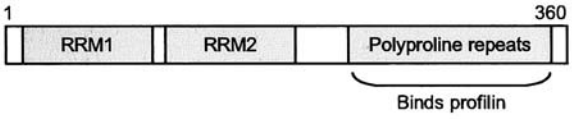
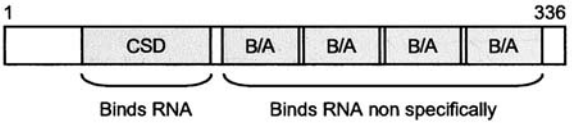
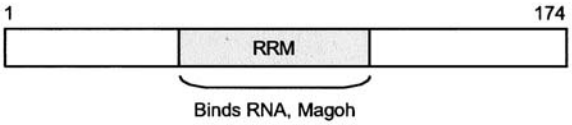
Name	Domain structure	RNA Binding site	Comments
X. l. CPEB		$U_{4-6}A_{1-2}U$ Consensus CPE (Cytoplasmic polyadenylation element)	Roles in translational repression and cytoplasmic polyadenylation/translational activation
X. l. Xp54		Unknown	Member of the rck family of DEAD-box RNA helicases Role in translational repression
D. m. Bruno		**AAUGUAUGU UAAUUGUAU GUAUU**A BRE (Bruno response element)	Member of the *elav* family Role in translational repression
D. m. Smaug		 *nanos* 3' UTR stemloop II SRE	Role in translational repression
D. m. Bicoid		*Caudal* 3' UTR	Homeodomain protein Role in translational repression
X. l. ePABP2		Poly A	Homologous to nuclear PABP2. Xenopus protein is cytoplasmic. Possible role in control of poly(A) tail length
X. l. PARN		Poly A	Member of the DEDD family of deadenylases Role in default deadenylation

TABLE 1 *(Continued)*

Name	Domain structure	RNA Binding site	Comments
X. l. ePABP		Poly A	Homologous to PABP1, expressed in oocytes and early embryos. Role in regulating deadenylation
X. l. EDEN-BP		**UAUAUGUAUG UGUUGUUUUU AUGUGUGUGU GUGUG**CU *c-mos* EDEN (embryonic deadenylation element)	Member of the *elav* family Role in deadenylation
C. e. GLD-1		UACU(C/A)A SBE (STAR binding element)	Member of the STAR/GSG family Role in translational repression
D. m. Pumilio		UGUAN$_{2-4}$UA Consensus Pumilio binding site (*D. m.*, *Xenopus*, mouse and man)	Member of the Puf family Role in translational repression/ deadenylation
D. m. Nanos		AUUAUUUU**GU UGU**CGAAA**AU UGUA**CAUAAG CC *D. m.* NRE (Nanos response element)	Role in translational repression
X. l. SLBP1		 Histone 3′ UTR SLBP binding site	Role in translational activation
D. m. Staufen		dsRNA, non-sequence specific *in vitro*	Role in RNA localization and translational activation

(Continued on next page)

TABLE 1 *(Continued)*

Name	Domain structure	RNA Binding site	Comments
D. m. Hrp48		Binds oskar 5' UTR between 2 alternative AUG codons. Also binds 3' UTR at the BRE (see Bruno for sequence)	Member of the hnRNP A/B family Role in translational control and mRNA localization
X. l. Vg1RBP		UUCAC E2 motif in the Vg1, VegT localization element	Member of the ZBP/VICKZ family Role in RNA localization
X. l. hnRNPI/ PTB		UUUCUA VM1 motif in the Vg1, VegT localization element	Role in RNA localization
X. l. Vg1RBP 71		**GUAUAU**AA**CC** **U**GAU**GA**C**UUU** **UCUAUUU** In the Vg1 localization element	Member of the FBP2/KSRP family Possible role in localization
X. l. Prrp		Vg1 localization element	Similarity in the RNA binding domain to the hnRNP A/B family Possible role in mRNA localization
X. l. FRGY2		AACAUC Selex CSD	Role in RNA packaging and repression
H. s. Y14		Binds RNA weakly and non-specifically	Nucleocyto-plasmic shuttling protein, deposited in EJCs. Role in RNA localization

the ACE, the ACE-binding protein, ~80 kDa, does not correspond in size to mouse oocyte CPEB, and remains to be characterized (Stutz *et al.*, 1998). The identification of the clam masking element-binding protein p82 (Standart *et al.*, 1990; Walker *et al.*, 1996) as a CPEB homologue (Walker *et al.*, 1999) provided one of the first hints that CPEB itself may have dual roles in regulating translation in early development. This was shown by the different effects that anti-p82 antibodies display in oocyte and egg lysates. In oocyte lysates, anti-p82 antibodies specifically activate translation of masked mRNAs. In egg lysates, the clam p82 binding sites in the ribonucleotide reductase 3′ UTR, which resemble the U-rich CPEs, are required for polyadenylation, and here anti-p82 antibodies prevent polyadenylation. These data suggest that p82/CPEB acts first as a repressor of translation in immature oocytes and subsequently participates in the activation of translation by cytoplasmic polyadenylation (Minshall *et al.*, 1999).

In parallel studies, the repressive role of vertebrate CPEB was extensively documented. *Xenopus* cyclin B1 and wee-1, and mouse cyclin B1, 3′ UTRs repress reporter mRNA translation in oocytes, as long as low levels of reporter mRNA are injected. Higher levels of mRNA are not repressed, implicating the action of a saturable masking factor. Deletion and mutational analyses pointed to the CPEs as the primary repression elements (Barkoff *et al.*, 2000; Charlesworth *et al.*, 2000; de Moor & Richter, 1999; Tay *et al.*, 2000). Because these were known to bind CPEB during oogenesis, it was concluded that CPEs and CPEB cause translational repression in oocytes, in additional to their role in polyadenylation in eggs. In an important extension of these observations, de Moor & Richter (1999) demonstrated that CPE-mediated repression was 5′-cap-dependent, as insertion of an EMCV-derived internal ribosome entry site (IRES) upstream of a reporter coding region, circumventing the requirement for a 5′-cap in translation, prevented its repression by cyclin B1 CPE elements in the 3′ UTR.

Maskin: A CPEB and eIF4E-Interacting Protein

Several partners of CPEB have been proposed to mediate its role in translational repression including maskin (Cao & Richter, 2002; Stebbins-Boaz *et al.*, 1999), the DEAD-box RNA helicase Xp54 (Minshall & Standart, 2004; Minshall *et al.*, 2001) and Pumilio (Nakahata *et al.*, 2001; Nakahata *et al.*, 2003). Both maskin and Xp54 have been proposed to interfere with the access of eIF4F to the 5′-cap structure of CPE-containing mRNAs, whereas the role of Pumilio in repression, most likely mediated at the level of poly(A) tail shortening, will be discussed later.

Maskin was identified as a *Xenopus* CPEB-interacting protein in co-immunoprecipitation, pull-down and yeast two hybrid assays (Stebbins-Boaz *et al.*, 1999). The 150 kDa maskin protein is a member of the transforming acidic coiled-coil-containing (TACC) family (reviewed in Gergely, 2002), which has important roles in cell division and cellular organization in both embryonic and somatic systems in a wide variety of organisms. These closely related proteins have been implicated in microtubule stabilization, acentrosomal spindle assembly, haematopoietic development, and cancer progression (Gergely, 2002). *Xenopus* maskin contains a peptide (TEADFLL) resembling the eIF4E-binding site found in eIF4G and the regulatory 4E-BPs, and binds eIF4E via this peptide (Stebbins-Boaz *et al.*, 1999). Indeed, the wild-type, but not a mutant (AEADAAA), peptide disrupts eIF4G binding to eIF4E bound to an immobilized cap column, and the wild-type, but not mutant, peptide inhibits all translation in oocytes (Stebbins-Boaz *et al.*, 1999), presumably by inactivating all the available eIF4E. Consistent with its proposed role as a repressor, the injection of antibodies against maskin activates cyclin B1, *c-mos* and wee-1 translation in oocytes (Cao & Richter, 2002). Importantly, maskin-eIF4E, but not CPEB-maskin, interactions are disrupted during oocyte maturation to allow translational initiation. eIF4E partially dissociates from maskin, perhaps as a result of maskin phosphorylation (Stebbins-Boaz *et al.*, 1999), but principally due to cytoplasmic polyadenylation and PABP binding to the newly elongated poly(A) tail (Cao & Richter, 2002). If PABP is prevented from binding eIF4G, using a peptide whose sequence is derived from the portion of eIF4G that interacts with PABP (Wakiyama *et al.*, 2000), maskin remains complexed with eIF4E in eggs (Cao & Richter, 2002). These findings led Richter and colleagues to propose the attractive and widely known maskin model (Figures 2B and 3), based on the closed-loop model of eukaryotic mRNA translation initiation (Gallie, 1998; Jacobson, 1996; Kahvejian *et al.*, 2001; Sachs, 2000). In oocytes, *Xenopus* CPEB, through its bridging partner maskin, represses translation of CPE-containing mRNAs by preventing eIF4E-eIF4G interactions required for translational initiation

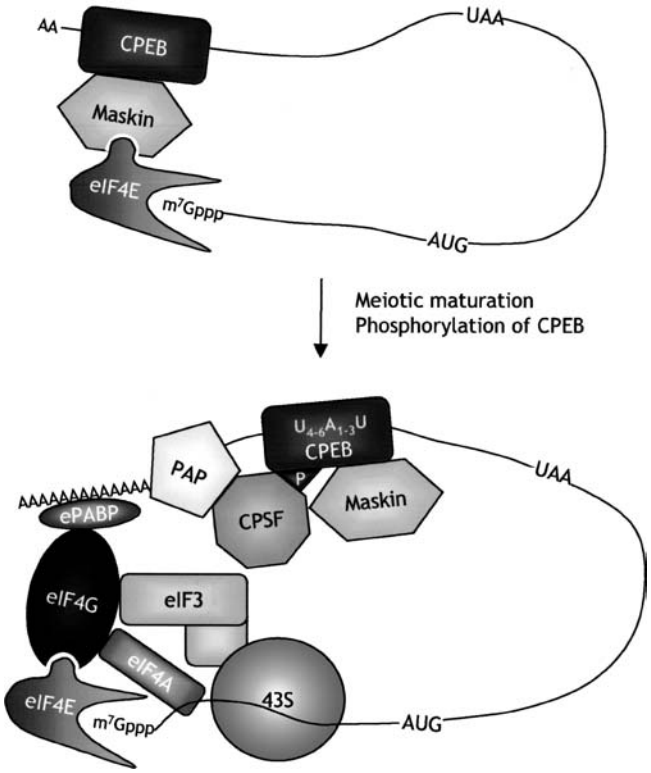


FIGURE 3 **Model showing the translational activation of CPE-containing mRNAs. As shown in Figure 2, during *Xenopus* oogenesis, mRNAs containing CPEs are repressed by the action of maskin, which is recruited via its interaction with CPEB. Upon meiotic maturation, CPEB is phosphorylated by Aurora A kinase (Mendez *et al*., 2000b), increasing the affinity of CPEB for the cleavage and polyadenylation specificity factor (CPSF) (Mendez *et al*., 2000b). Recruitment of CPSF results in cytoplasmic polyadenylation of the mRNA, which in turn recruits ePAB, the *Xenopus* form of PABP present during oogenesis and early embryogenesis. Polyadenylation and the presence of PABP disrupts the maskin-eIF4E interaction, resulting in translational activation of the mRNA (Cao & Richter, 2002). The maskin-CPEB interaction is maintained during maturation (Stebbins-Boaz *et al*., 1999).**

at the 5′-end (Stebbins-Boaz *et al*., 1999). Thus, maskin acts as a type of mRNA-specific 4E-BP. During meiotic maturation, CPEB is phosphorylated by Aurora A kinase and activated as a cytoplasmic polyadenylation factor, leading to polyadenylation of the mRNA (Mendez *et al*., 2000b), This results in the dissociation of the inhibitory maskin-eIF4E link, presumably due to the fact that PABP increases the affinity of eIF4G for eIF4E (Cao & Richter, 2002; Stebbins-Boaz *et al*., 1999).

The maskin model provides the paradigm for explaining how RNA-binding proteins, bound to mRNA-specific 3′ UTR sequences, prevent translational initiation, and is echoed in subsequent studies, as discussed below, of the *Drosophila* proteins Cup and Bicoid. Nevertheless, some characteristics of maskin indicate that the translational repressor role of this protein may not be universal. Maskin levels are regulated during oogenesis, with significant amounts only detectable during late stage V/VI (Groisman *et al*., 2000), suggesting that repression earlier in oogenesis relies on other mechanisms. Secondly, the maskin eIF4E-binding site appears to be absent from other TACC homologs (Gergely, 2002), although this does not exclude non-canonical maskin-eIF4E interactions.

Association of Xp54, a Conserved DEAD-Box RNA Helicase, with CPEB

Co-immunoprecipitation experiments have shown an interaction between CPEB and the DEAD-box RNA helicase, p47, in *Spisula* oocytes (Minshall *et al*., 2001), and with the *Xenopus* homolog of p47, Xp54, in *Xenopus* oocytes (Minshall & Standart, 2004). Clam p47 and *Xenopus* Xp54 are members of the highly conserved rck/DDX6 DEAD box RNA helicase family (Minshall *et al*., 2001). RNA helicases are involved in a variety of cellular processes, including splicing, ribosome biogenesis, RNA transport, degradation, and translation, although their precise contribution to most of these processes is not known. They are characterized by nine conserved domains, including the eponymous DEAD motif and the recently-identified Q motif (Cordin *et al*., 2004; Tanner *et al*., 2003), with varying roles in substrate binding and catalysis. Helicases use ATP hydrolysis to unwind short RNA duplexes and may also influence rearrangements of large RNA structures or protein-RNA interactions. Their ATPase activity is stimulated by (or dependent on) RNA binding, but it is unclear to what extent helicases recognize specific RNA sequences (reviewed in Fuller-Pace, 1994; Linder, 2003; Lüking *et al*., 1998; Rocak & Linder, 2004). The rck family, named after the human protein (Akao *et al*., 1995), includes *Drosophila* Me31B (Nakamura *et al*., 2001), *C. elegans* Cgh-1(Navarro *et al*., 2001), *S. cerevisisae* Dhh1 (Coller *et al*., 2001; Fischer & Weis, 2002), and *S. pombe* Ste13 (Maekawa *et al*., 1994).

As the *Xenopus* and human proteins can complement Dhh1Δ yeast cells, at least some of the function(s) of rck helicases appear to be conserved (Tseng-Rogenski *et al*., 2003; Westmoreland *et al*., 2003). Consideration of the known features of the family members can provide clues regarding their functions. The *Drosophila* homolog Me31B (Maternal expression at 31B) (de Valoir *et al*., 1991) was identified as a cytoplasmic particle component in germline cells during oogenesis in an

impressive screen of a GFP-cDNA expression library, looking for proteins that distribute in a granular pattern (Nakamura *et al.*, 2001). Me31B is essential for oogenesis, and co-localizes with several mRNAs localized in oocytes during their transport from nurse cells to the oocyte, as well as with Exuperantia (Exu) and Ypsilon Schachtel (Yps; FRGY2 homologue) (Nakamura *et al.*, 2001), components of the *oskar* RNP (Wilhelm *et al.*, 2000b). Interestingly, the loss of Me31B causes the premature translation of *oskar* and *Bicaudal D* mRNAs, during their transport to the oocyte, suggesting that Me31B is a translational repressor (Nakamura *et al.*, 2001).

Dhh1 stimulates mRNA decapping in yeast. This process requires deadenylation followed by dissociation of eIF4E from the cap structure, allowing the decapping enzyme Dcp1 to remove the 5′-cap and leaving an end susceptible to 5′-3′ exonucleolytic degradation (reviewed in Coller & Parker, 2004). In Dhh1Δ mutants, mRNAs accumulate as deadenylated, capped species. The role of Dhh1 in decapping appears to be direct, as Dhh1 physically interacts with several proteins involved in mRNA decapping including Dcp1, as well as Lsm1 and Pat1p/Mrt1, which function to enhance the decapping rate (Coller *et al.*, 2001; Fischer & Weis, 2002), and Pop2, a subunit of the mRNA deadenylase (Coller *et al.*, 2001; Hata *et al.*, 1998).

Rck helicases are present in distinct cytoplasmic RNP particles in many cell types. In oocytes, stored mRNAs are components of large granules, variously named polar granules, P granules and the Balbiani body depending on the species. P granules and the Balbiani body become segregated to germline cells, and their RNA and protein constituents direct germline development (Houston and King, 2000). Cgh-1 is localized to P granules and other mRNP in worms (Navarro *et al.*, 2001), while Me31B is concentrated in the sponge bodies in germline cells during *Drosophila* oogenesis (Nakamura *et al.*, 2001) and Xp54 is present in the Balbiani body in early *Xenopus* oocytes (Smillie & Sommerville, 2002). In yeast, and in human cells, Dhh1/rck is a component of discrete cytoplasmic processing bodies, which function in decapping and 5′-3′ degradation (Cougot *et al.*, 2004; Sheth & Parker, 2003; Tseng-Rogenski *et al.*, 2003). Collectively, these studies highlight the conserved and critical role of this RNA helicase in down-regulating mRNA expression, and hint at a possible link between decapping and translational masking.

Xenopus p54, present at constant levels throughout oogenesis, has RNA duplex-unwinding activity and has been implicated in the nuclear assembly of stored mRNP particles in early oocytes, where it shuttles between the nucleus and cytoplasm (Ladomery *et al.*, 1997; Smillie & Sommerville, 2002; Thom *et al.*, 2003). In view of its interaction with CPEB, its potential involvement in translation was examined using the tethered function assay (Coller & Wickens, 2002). MS2-Xp54 was tethered via MS2-binding sites to the 3′ UTR of a reporter firefly luciferase mRNA (Minshall *et al.*, 2001). MS2-Xp54 repressed translation three- to fivefold, relative to MS2 alone, and somewhat surprisingly, mutations in the DEAD (ATP hydrolysis) and HRIGR (ATP binding) helicase motifs of the helicase activated translation three- to fourfold. These effects were at the level of translation, rather than RNA stability, required MS2 binding sites, and were observed in both oocytes and in progesterone-matured eggs (Minshall & Standart, 2004; Minshall *et al.*, 2001). Altogether, the data implied that the helicase activity of Xp54 is required for repression (Minshall *et al.*, 2001). Moreover, we also showed that RNA-dependent Xp54 oligomerization correlates with its ability to repress translation, although the role of self-association in repression is not known (Minshall & Standart, 2004). Interestingly, Xp54 interacts, not only with CPEB, but also with eIF4E, in an RNA-independent manner in oocytes. In eggs, however, CPEB, due to its large-scale proteolysis during meiotic maturation (Reverte *et al.*, 2001; Thom *et al.*, 2003), is not detectable in the immunoprecipitates, and the interaction between Xp54 and eIF4E now becomes RNA-dependent (Minshall & Standart, 2004). On the basis of these dynamic changes, we proposed that tethered Xp54 (localized either via MS2 or CPE/CPEB), sequesters eIF4E from the translational machinery in oocytes, thus repressing translation, whereas in progesterone-matured eggs, Xp54, presumably no longer anchored to the CPE motifs owing to reduced levels of CPEB, interacts with RNA in an altered manner, and eIF4E is released to allow translation (Minshall & Standart, 2004).

Cup—The *Drosophila* Equivalent of Maskin?

Drosophila Cup is a germline-specific protein required for oogenesis which is expressed until the blastoderm stage of embryogenesis (Keyes & Spradling, 1997; Verrotti & Wharton, 2000). Cup came to prominence at the turn of 2004 when three complementary studies

attested to its critical role in 3′ UTR-based translational control in *Drosophila* oocytes and embryos.

These studies showed that, although Cup itself does not bind RNA directly, it is recruited by a number of different RNA-binding proteins to specific RNAs. Cup, a large protein of about 150 kDa, binds directly to eIF4E. Immunoprecipitation, pull-down, and yeast two hybrid assays led to the identification in Cup of a high affinity and canonical eIF4E binding site, YTRSRLM (Nakamura *et al.*, 2004; Nelson *et al.*, 2004; Wilhelm *et al.*, 2003; Zappavigna *et al.*, 2004), and a nearby second, unconventional and lower affinity eIF4E binding site, ELEGRLR (Nelson *et al.*, 2004).

Furthermore, *cup* and *eIF4E* interact genetically, since lowering eIF4E levels affects the development and growth of ovaries bearing homozygous *cup* mutant alleles (Zappavigna *et al.*, 2004). Surprisingly, eIF4E in developing oocytes is not distributed homogeneously throughout the cell, as one might predict for a general translation initiation factor, but rather localizes to the posterior pole along with Cup, in a *cup*-dependent manner (Wilhelm *et al.*, 2003; Zappavigna *et al.*, 2004).

Cup and eIF4E were both identified as proteins that co-immunoprecipitate with Me31B, in an RNA-dependent manner, and also co-localize with the helicase during oogenesis. As in the case of loss of Me31B (Nakamura *et al.*, 2001), *oskar* mRNA, which is normally repressed until correctly localized to the posterior pole, is prematurely translated in *cup* mutant ovaries, although its localization is unaffected (Nakamura *et al.*, 2004). Interestingly, Cup interacts with Bruno, a well known translational repressor of *oskar* mRNA, *in vivo* as well as in yeast cells (Nakamura *et al.*, 2004).

As mentioned previously, *oskar* is required for defining the posterior of the embryo, and also for the formation of germ cells (Lehmann & Nusslein-Volhard, 1986). Both *oskar* mRNA and the encoded protein must be properly localized to the posterior pole of the oocyte for correct development (Ephrussi & Lehmann, 1992; Kim-Ha *et al.*, 1995). Translational repression of *oskar* mRNA during its localization is mediated in part by the RNA-binding protein Bruno, which binds specific repeated sequences in the 3′ UTR of *oskar* RNA, called Bruno response elements (BREs; A, B and C regions) (Kim-Ha *et al.*, 1995). Deletion of these BREs results in inappropriate translation of Oskar protein at the anterior end of the oocyte leading to embryos with two posterior poles. Bruno is an RRM-containing protein, related to the *elav* (embryonic lethality and abnormal visual system) family of RNA-binding proteins (Webster *et al.*, 1997). Proteins within this family have the same domain structure: an N-terminal domain, two consecutive RRMs that are the principal RNA-binding domains and then a linker region of 50–70 amino acids followed by a C-terminal third RRM (reviewed in Good, 1997; Good *et al.*, 2000; Soller & White, 2004). Direct evidence for a role for Bruno in the repression of *oskar* mRNA translation came from studies using a *Drosophila in vitro* translation system (Castagnetti *et al.*, 2000; Lie & Macdonald, 1999). Although ovary extracts, which contain Bruno, repress BRE-containing mRNA, embryonic lysates lacking Bruno freely translate firefly luciferase-BRE reporter mRNA. Addition of Bruno to the latter represses translation (Castagnetti *et al.*, 2000). The interaction between Cup and Bruno, mediated by the Q-rich C-terminus of Cup and the linker domain of Bruno, in addition to the ability of Cup to bind eIF4E, led Nakamura *et al.* (2004) to propose the model, based on the maskin model, whereby Bruno, bound to the 3′ UTR of *oskar* mRNA, prevents its translation by sequestering the cap-binding protein from productive association with eIF4G (Figure 2C).

Early studies suggested that *oskar* translation is poly(A)-independent (Lie & Macdonald, 1999; Sallés *et al.*, 1994). However, recent re-evaluations document that *oskar* mRNA is subject to cytoplasmic polyadenylation *in vivo* (Castagnetti & Ephrussi, 2003; Chang *et al.*, 1999). Nevertheless, while a long poly(A) tail is required for efficient *oskar* translation, both *in vivo* and *in vitro*, it is not sufficient to overcome BRE-mediated repression (Castagnetti & Ephrussi, 2003). Rather, activation of *oskar* mRNA translation at the posterior pole of the oocyte, which requires a specific *cis*-element in the 5′ UTR of the RNA (Gunkel *et al.*, 1998), may involve the as-yet-unidentified factor, p68, which binds this element, interfering with eIF4E-Cup-Bruno interactions to promote translation. An additional protein p50, which has recently been identifed as the hnRNP Hrp48, also binds to the 5′-*cis*-acting element, in addition to the BREs in the 3′ UTR, and acts as a co-repressor of *oskar* translation (Gunkel *et al.*, 1998). Hrp48 will be discussed in more detail later. Derepression does not appear to involve repressor degradation or modification or cytoplasmic polyadenylation, but rather an interaction between proteins linking the 5′- and 3′-ends of the mRNA (Gunkel *et al.*, 1998).

In parallel studies, Cup, isolated as a component of the *oskar* RNP by virtue of its interaction with GFP-Exu

(Wilhelm *et al.*, 2000b), was shown to be required for both translational repression of *oskar* mRNA, and for *oskar* mRNA localization (Wilhelm *et al.*, 2003). The *cup* mutants used in this study differed to those used by (Nakamura *et al.*, 2004), and disrupted *oskar* mRNA localization by failing to recruit the plus end-directed microtubule transport factor Barentsz, thus linking the control of localization and translation. The data suggest that Cup is required early in the assembly of the *oskar* RNP when it recruits Barentsz, and subsequently kinesin, to localize *oskar* mRNA. Once anchored, the RNP would rearrange to allow translation (Wilhelm *et al.*, 2003). The authors noted that Cup bears partial homology to a large human protein called 4E-T(ransporter), an eIF4E-binding protein which mediates the nuclear import of eIF4E in HeLa cells (Dostie *et al.*, 2000). During mitosis, 4E-T is phosphorylated, and its association with eIF4E much reduced (Pyronnet *et al.*, 2001), leading to the proposal that *oskar* translational activation is regulated by modification of Cup at the posterior pole (Wilhelm *et al.*, 2003).

Bruno also regulates the translation of *gurken* mRNA during *Drosophila* oogenesis, and binds the *gurken* 3′ UTR in a BRE-dependent manner (Filardo & Ephrussi, 2003). Yet, despite these similarities between *oskar* and *gurken* mRNA control, *gurken* translation was not affected in *cup* mutants (Nakamura *et al.*, 2004; Wilhelm *et al.*, 2003), suggesting that Bruno-Cup-eIF4E interactions are dispensible for the regulation of *gurken* translation.

Nelson *et al.* (2004) showed that Cup also functions in translational repression in embryos. *nanos* mRNA, which encodes the localized component of the *Drosophila* posterior body patterning determinant, is normally translated only at the posterior pole of the embryo; unlocalized *nanos* mRNA is translationally repressed. A discrete translational control element (TCE) within the *nanos* 3′ UTR acts independently of the localization signal to mediate translational repression of unlocalized *nanos* RNA (Dahanukar & Wharton, 1996; Gavis *et al.*, 1996; Smibert *et al.*, 1996). The TCE contains a pair of redundant hairpins each bearing the loop sequence CUGGC, which bind the RNA-binding protein Smaug and are known as Smaug recognition elements (SREs). Smaug is distributed throughout the embryo and functions as a translational repressor of *nanos* mRNA (Dahanukar *et al.*, 1999; Smibert *et al.*, 1999; Smibert *et al.*, 1996). Smaug interacts with the RNA through an unusual RNA-binding domain (RBD) composed of a sterile-alpha motif (SAM) domain neighbouring a pseudo-HEAT repeat analogous topology (PHAT) domain (Table 1). Surprisingly, it is largely the SAM domain that interacts specifically with the *nano*s mRNA TCE regulatory sequence. Thus, in addition to their previously characterized roles in protein-protein interactions, SAM domains can also play crucial roles in RNA binding. (Aviv *et al.*, 2003; Green *et al.*, 2003).

Pull-down experiments have shown that Smaug interacts specifically with Cup, through the RNA-binding domain of the former (Nelson *et al.*, 2004). Moreover, Smaug-mediated translational repression of luciferase reporter mRNA containing 3 SREs in its 3′ UTR requires Cup (Nelson *et al.*, 2004). Therefore, Smaug may also repress translation through recruiting Cup to disrupt the interaction between eIF4E and eIF4G. According to this scenario, Smaug represses at the level of translation initiation. However, other investigators have found that repressed *nanos* mRNA associates with polysomes, implying repression is imposed after the initiation step (Clark *et al.*, 2000). The TCE contains another stem-loop motif, in addition to the SREs, which is responsible for repression in oocytes in the absence of Smaug (Forrest *et al.*, 2004), and which binds as-yet-unidentified proteins. One of these could act as a repressor at another level, or Smaug itself may use multiple mechanisms to control *nanos* mRNA. How is the repression of *nanos* by Cup alleviated? A plausible model utilizes the observations that both Smaug and Cup (Keyes & Spradling, 1997) are distributed throughout the embryo whereas Oskar protein, which is required for *nanos* mRNA translation, is localized to the posterior (Ephrussi *et al.*, 1991; Gavis & Lehmann, 1994; Kim-Ha *et al.*, 1991). As Oskar interacts with Smaug via the RBD region of the latter (Dahanukar *et al.*, 1999), as does Cup (Nelson *et al.*, 2004), localized Oskar protein may displace Cup from Smaug, and thus sever the inhibitory Smaug-eIF4E link. Alternatively Cup modification may release eIF4E.

Drosophila Cup and *Xenopus* maskin are unrelated proteins, but they share the ability to bind both sequence-specific 3′ UTR RNA binding proteins, Bruno and Smaug and CPEB respectively, and eIF4E, thereby limiting translation initiation (Figure 2B). It will be of interest to determine whether the vertebrate Cup homologue, 4E-T, also acts as a specific translational repressor. Over-expression of wild-type 4E-T in HeLa cells, but not of a mutant form that cannot bind eIF4E, inhibits the translation of reporter RNA (Pyronnet *et al.*,

2001); similar experiments in oocytes, in the context of high levels of RNA-binding proteins, may reveal a more specific role.

Direct Binding of eIF4E by Bicoid, a Specific 3′ UTR-Binding Translational Repressor

Formation of the anterior-posterior axis during early *Drosophila* embryogenesis depends on the formation of opposing concentration gradients of the Hunchback and Caudal transcription factors. Whereas establishment of the Hunchback gradient relies on the regulation of *oskar* and *nanos* RNAs, described above, the gradient of Caudal depends on the prior formation of an anterior to posterior gradient of Bicoid, created by the localization of *bicoid* mRNA to the anterior pole. In embryos lacking Bicoid activity as a result of mutation, the Caudal gradient fails to form and Caudal becomes evenly distributed throughout the embryo (Driever & Nüsslein-Volhard, 1988) (reviewed in Ephrussi & St. Johnston, 2004; Wickens *et al.*, 2000).

Bicoid was shown to act as a specific translational repressor of *caudal* mRNA in the anterior region of the *Drosophila* embryo via a distinct Bicoid-binding region (BBR) in the 3′ UTR (Dubnau & Struhl, 1996; Rivera-Pomar *et al.*, 1996). Bicoid also acts as a transcription factor and contains a homeodomain within its N-terminal half and an activation domain in the C-terminal region (Table 1). Translational repression requires the homeodomain, and a downstream PEST sequence (Dubnau & Struhl, 1996; Niessing *et al.*, 1999; Rivera-Pomar *et al.*, 1996), although translational repression by Bicoid is unlikely to involve ubiquitin-dependent protein degradation (Niessing *et al.*, 1999). The Bicoid homeodomain contains an arginine-rich motif (ARM) in its helix III, similar to the RNA-binding domain of the HIV-1 protein REV, needed for both RNA and DNA recognition. Replacement of arginine 54, within this motif, alters the RNA- but not the DNA-binding properties of the homeodomain. Corresponding Bicoid mutants fail to repress *caudal* mRNA translation, whereas the transcriptional target genes are still activated (Niessing *et al.*, 2000).

Bicoid represses translation in a 5′-cap-dependent manner in cell culture, shown using a bicistronic transcript with an IRES derived from the *Antennapedia* leader (Niessing *et al.*, 1999), hinting that it interferes with the initial recruitment of the 43S complex. Consistent with this, Bicoid was found to bind eIF4E via an eIF4E binding site (YIRPYLP) upstream of the homeodomain. This binding is competed by the YDRKFLM peptide of human 4E-BP1, which competes efficiently for binding at the eIF4G-binding site of eIF4E (Marcotrigiano *et al.*, 1999; Ptushkina *et al.*, 1999), but not by a peptide in which the conserved Y and L residues, which are critical for interaction with eIF4E, are mutated to A and R, respectively (Niessing *et al.*, 2002). Translational repression requires the eIF4E-binding motif of Bicoid, as shown in *bcd* mutant embryos, in which wild-type Bicoid, but not an AIRPYLR version, rescued transgene-dependent translational repression (Niessing *et al.*, 2002). The Bicoid-eIF4E interaction requires the *caudal* 3′ UTR (Niessing *et al.*, 2002). Thus, in a modified maskin/Cup model of translational inhibition, Bicoid, when bound to its target RNA, sequesters eIF4E itself, rather than acting through an intermediary protein (Figure 2C).

CONTROL OF TRANSLATION BY THE REGULATION OF POLY(A) TAIL LENGTH

Cytoplasmic Polyadenylation

Conserved Importance of Cytoplasmic Polyadenylation in Eukaryotes and Identification of the Cytoplasmic Polyadenylation Machinery

Until recently, cytoplasmic polyadenylation was generally considered to be a peculiarity confined to oocytes, eggs, and early embryos. However, investigations in the last two years have left no doubt that polyadenylation in the cytoplasm is a widespread method of controlling gene expression–for example, in regulating genes involved in DNA metabolism and genome maintenance in yeast (Read *et al.*, 2002; Saitoh *et al.*, 2002), during spermatogenesis in mice (Kashiwabara *et al.*, 2002), and in as-yet-uncharacterized roles in humans (Kwak *et al.*, 2004).

Polyadenylation in the cytoplasm is performed by a set of factors, similar to those that add a poly(A) tail cotranscriptionally to pre-mRNA in the nucleus, namely poly(A) polymerase and cleavage and polyadenylation specificity factor (CPSF) (reviewed in Edmonds, 2002; Hall, 2002; Proudfoot *et al.*, 2002; Wahle & Ruegsegger, 1999). In *Drosophila*, the single poly(A) polymerase (PAP) gene, *hiiragi*, is involved in both

nuclear and cytoplasmic polyadenylation *in vivo*, and regulation of the level of Hiiragi protein is critical for early development; over-expression leads to a dramatic elongation of poly(A) tails and a loss of specificity during cytoplasmic polyadenylation, resulting in embryonic lethality (Juge *et al.*, 2002). Fission yeast contain related enzymes, the cid1 and cid13-like genes, that elongate poly(A) tails in the cytoplasm, and appear to enhance, in this case, mRNA stability (Read *et al.*, 2002; Saitoh *et al.*, 2002). Indeed, the fission yeast Cid13/Cid1 and budding yeast Trf4/5 proteins are members of a newly identified polβ-nucleotidyl transferase superfamily conserved from yeast to man, which includes eukaryotic nuclear poly(A) polymerases, CCA-adding enzymes, and DNA polymerases (Kwak *et al.*, 2004; Read *et al.*, 2002; Saitoh *et al.*, 2002). The *C. elegans* gene GLD-2, a divergent family member, is a regulator of the mitosis/meiosis decision and other germline events and encodes the catalytic moiety of a cytoplasmic poly(A) polymerase that is associated with P granules in early embryos. Although GLD-2 lacks the RNA-binding motifs required for nuclear PAP activity, and has little PAP activity on its own, it is stimulated *in vitro* by GLD-3 (Wang *et al.*, 2002a), or by tethering to the 3′ UTR of a reporter RNA (Kwak *et al.*, 2004). GLD-3 is also a developmental regulator involved in sex-determination, and belongs to the Bicaudal-C family of RNA-binding proteins (Kwak *et al.*, 2004; Wang *et al.*, 2002a). Thus, the GLD-2-GLD-3 heterodimer provides both enzyme and RNA-binding activities. As some mammalian GLD-2 homologs are also cytoplasmic poly(A) polymerases, as shown in tethered function assays (Kwak *et al.*, 2004), the control of RNA translation and/or stability by extending poly(A) tails is likely to be a universal mechanism of gene regulation, and identification of mammalian GLD-2 ligands that serve the role of the GLD-3 partner in cytoplasmic polyadenylation could point to gene-specific control. In *Xenopus* oocytes, catalytically active and cytoplasmic forms of poly(A) polymerase have been identified, similar in size and sequence to mammalian somatic nuclear poly(A) polymerase, but lacking the C-terminal nuclear localization signal (Ballantyne *et al.*, 1995; Gebauer & Richter, 1995). Whether *Xenopus* oocytes also contain GLD-2 type cytoplasmic polymerases is not yet known.

CPSF was first characterized in nuclear cleavage and polyadenylation. In the nucleus, both the cleavage and subsequent poly(A) addition reactions require the sequence AAUAAA, typically located 10 to 30 nucleotides 5′ of the polyadenylation site. CPSF binds directly to this sequence and is required for both activities. Mammalian CPSF consists of four subunits of 160, 100, 73 and 30 kDa. The 160 kDa subunit interacts with the AAUAAA sequence, recruiting poly(A) polymerase, which has little or no intrinsic specificity for RNA, to the substrate mRNA. The 30 kDa subunit may also bind AAUAAA and, moreover, binds preferentially to U-rich sequences (reviewed in Edmonds, 2002; Hall, 2002; Proudfoot *et al.*, 2002; Wahle & Ruegsegger, 1999). Cytoplasmic forms of the 160, 100, and 30 kDa subunits of CPSF have been characterized in *Xenopus* oocytes (Dickson *et al.*, 1999; Mendez *et al.*, 2000b). In addition to location, cytoplasmic polyadenylation differs to nuclear polyadenylation in that, in addition to AAUAAA, it requires U-rich CPE sequences, which are normally located upstream from the hexanucleotide, and hence only targets a subset of RNAs.

The Role of CPEB in Cytoplasmic Polyadenylation

The role of CPEB in translational repression has already been described. This protein, however, also plays a part in cytoplasmic polyadenylation and translational stimulation. Cytoplasmic polyadenylation is readily assayed *in vitro* in egg lysates, by monitoring RNAseH/oligo(dT)-sensitive elongation of exogenous labelled RNA. Mutations in CPEs abolish both CPEB binding and cytoplasmic polyadenylation (Minshall *et al.*, 1999; Stebbins-Boaz *et al.*, 1996). In *Xenopus* lysates immunodepleted of CPEB, the polyadenylation of several CPE-containing RNAs, including B4 mRNA (Hake & Richter, 1994) and G10, *c-mos*, cdk2 and cyclins A1, B1, and B2 mRNAs (Stebbins-Boaz *et al.*, 1996) is abolished, and the addition of *in vitro* synthesized CPEB restores polyadenylation (Hake & Richter, 1994), demonstrating the requirement for CPEB in the polyadenylation of CPE-containing mRNAs. Similarly, anti p82/CPEB antibodies inhibited polyadenylation of the ribonucleotide reductase 3′ UTR in *Spisula* egg lysates (Minshall *et al.*, 1999). The critical role of CPEB in cytoplasmic polyadenylation was further demonstrated in *Xenopus* oocytes microinjected with CPEB antibodies. When progesterone was added, these oocytes not only failed to polyadenylate RNA, but also did not undergo meiotic maturation, due to a lack of *c-mos* mRNA translational activation (Stebbins-Boaz *et al.*, 1996).

While studies in *Xenopus* provide the most direct evidence for the role of CPEB as a cytoplasmic polyadenylation factor, investigations of the *Drosophila* homolog of CPEB, Orb, strengthen this conclusion. Orb was originally characterised as a protein required for anteroposterior and dorsoventral patterning during *Drosophila* oogenesis. Strong *orb* mutants arrest oogenesis early, before the formation of the 16-cell cyst that normally differentiates into a single oocyte and surrounding nurse cells. Using a weaker allele, orb^{mel}, Orb was shown to be required for anchoring of *oskar* mRNA at the posterior pole of the oocyte (Christerson & McKearin, 1994; Lantz *et al.*, 1992; Lantz *et al.*, 1994). As Oskar protein is needed to anchor its own mRNA, the effect of orb^{mel} could be due to low *oskar* mRNA translation. Indeed, subsequent studies showed that Oskar protein expression in oocytes depends on Orb, which interacts with *oskar* mRNA *in vivo*, and which can be cross-linked to the *oskar* CPE-containing 3′ UTR *in vitro* (Chang *et al.*, 1999). The reduced level of Oskar was associated with a shortened *oskar* poly(A) tail in *orb* mutant ovaries (Chang *et al.*, 1999). In line with the proposed role of Orb in cytoplasmic polyadenylation, Simonelig, Wahle and colleagues demonstrated that *hiiragi* interacts genetically with *orb* to cooperate specifically in polyadenylation of *oskar* mRNA (Juge *et al.*, 2002). Extending these observations, Castagnetti and Ephrussi showed that Orb functions in posterior patterning by enhancing *oskar* mRNA translation through the addition or maintenance of a long poly(A) tail (Castagnetti & Ephrussi, 2003).

Returning to the more biochemical environment of the *Xenopus* system, we can ask how CPEB promotes polyadenylation, and whether all CPE-containing mRNAs become polyadenylated to the same extent. Early in meiotic maturation, *Xenopus* (and mouse) CPEBs are phosphorylated by Aurora A/Eg2 kinase on Ser174 (Hodgman *et al.*, 2001; Mendez *et al.*, 2000a), which enhances CPSF binding to CPE-containing mRNAs, in turn, presumably recruiting poly(A) polymerase and thus promoting cytoplasmic polyadenylation (Dickson *et al.*, 2001; Mendez *et al.*, 2000b). Immunoprecipitation assays show that, although CPEB phosphorylation during maturation increases binding to the 160 kDa CPSF subunit about fourfold (Mendez *et al.*, 2000b), it does not appreciably increase binding to the 100 kDa subunit (Dickson *et al.*, 2001). Whether this reflects a differential timing of CPEB-CPSF subunit interactions or simply differences in reagents and experimental protocols is unclear. Nevertheless, both studies detected a pre-existing interaction between CPEB and CPSF in the oocyte, suggesting that CPSF binding to the mRNA does not entirely depend on CPEB phosphorylation. Assessment of a variety of synthetic polyadenylation substrate RNAs, with or without a potential CPE close to the AAUAAA, showed that, somewhat surprisingly, the sequence specificity of polyadenylation in egg lysates is comparable to that observed with highly purified mammalian CPSF and recombinant poly(A) polymerase. However, when the same RNAs were examined by injection into oocytes, polyadenylation was considerably more efficient and differed in sequence requirements. While CPEB may not be strictly necessary for CPE-dependent polyadenylation *in vitro*, *in vivo* it clearly enhances the reaction, with different RNAs showing different degrees of enhancement (Dickson *et al.*, 2001). In sum, CPEB is a factor that mediates cytoplasmic polyadenylation by association with CPSF, but the extent of its involvement seems to vary, sometimes subtly, between different CPE-containing mRNAs.

Differential Control Mechanisms of CPE-Containing RNAs

In regulated *Xenopus* maternal mRNAs, the consensus CPE sequence, $U_{4\text{-}6}A_{1\text{-}3}U$, is generally found in close proximity to (usually up to 25 nt from), and upstream of, the AAUAAA hexanucleotide, although they can be found in non-canonical positions (see Charlesworth *et al.*, 2004, for an updated list). mRNAs that are regulated during meiotic maturation may contain more than one CPE and the number of CPEs present appears to affect the mechanism of control. *Xenopus* cyclin B1 is probably the best-characterised mRNA with four CPE sequences. These cooperate to function in both repression in the oocyte and in cytoplasmic polyadenylation in eggs (Barkoff *et al.*, 2000; de Moor & Richter, 1999; Nakahata *et al.*, 2001; Stebbins-Boaz *et al.*, 1996). In contrast, 3′ UTR sequences containing CPEs derived from *Xenopus* cyclin A1, B2 and *c-mos* mRNA, which do promote cytoplasmic polyadenylation (Fox *et al.*, 1989; McGrew *et al.*, 1989; Sheets *et al.*, 1994), do not support translational repression of a reporter mRNA (Barkoff *et al.*, 2000). These latter mRNAs contain only one CPE in close proximity to the AAUAAA hexanucleotide. As one CPE is sufficient for polyadenylation, while repeated copies appear to be necessary for repression, this could imply that the tighter binding of multiple CPEBs is required

to prevent translation. These studies also highlight another interesting contrast–some mRNAs, such as cyclin B1 mRNA, strictly require polyadenylation in maturing oocytes for derepression (unmasking) (Barkoff *et al.*, 2000; de Moor & Richter, 1999; Tay *et al.*, 2000) while others, such as *Xenopus* wee-1 and mouse tPA, can be unmasked without extensive polyadenylation (Charlesworth *et al.*, 2000; Stutz *et al.*, 1998). Similarly, polyadenylation and unmasking are uncoupled in the clam lysate (Standart *et al.*, 1990). It seems that individual mRNAs regulated by CPEs are differentially subject to control by repression and polyadenylation–some mRNAs require a secondary level of activation (polyadenylation) over and above a simple relief of repression (unmasking); others do not. Whether this reflects differences in the interaction of regulatory factors, or the temporal context of activation is not yet clear (for a fuller discussion, see Wickens *et al.*, 2000).

Early and Late Cytoplasmic Polyadenylation During Meiotic Maturation

In addition to differences in the involvement of repression and polyadenylation in their regulation, CPE-containing mRNAs in *Xenopus* can also undergo temporally different patterns of polyadenylation and translational activation upon meiotic maturation (Figure 4). Several laboratories have investigated the control of these mRNAs with the aim of identifying the underlying causes of their differences. Recent results, in particular, emphasise that cytoplasmic polyadenylation is not simply controlled by CPEB binding to CPEs.

In the early studies, the control of several maternal mRNAs was classified as being independent (class I) of *c-mos* and CDK1 kinase activities, whereas polyadenylation of the second class requires both of these activities. Class I polyadenylation, through its effects on *c-mos* mRNA, is required for class II polyadenylation (Ballantyne *et al.*, 1997; de Moor & Richter, 1997). Interestingly, subtle changes in the CPE sequence can, at least partially, switch an RNA from Class I to Class II (Ballantyne *et al.*, 1997). Recently, the timing of polyadenylation of an extensive set of mRNAs was simultaneously determined in a sensitive RNA-ligation RT-PCR assay, and scored as occurring 'early', prior to GVBD, or 'late,' at or following GVBD. The early class of mRNAs include histone-like B4, D7, Eg2, FGFR1, G10 and *c-mos* mRNAs and the late class of mRNAs include cyclin B1, cyclin B4, wee1 and cyclin A1 (Charlesworth *et al.*, 2004). In support of this classification, the expression of cyclin B1 and B4 was previously noted to be strongly activated after meiosis I (Hochegger *et al.*, 2001). Neither the sequence of CPEs, their number, nor their location

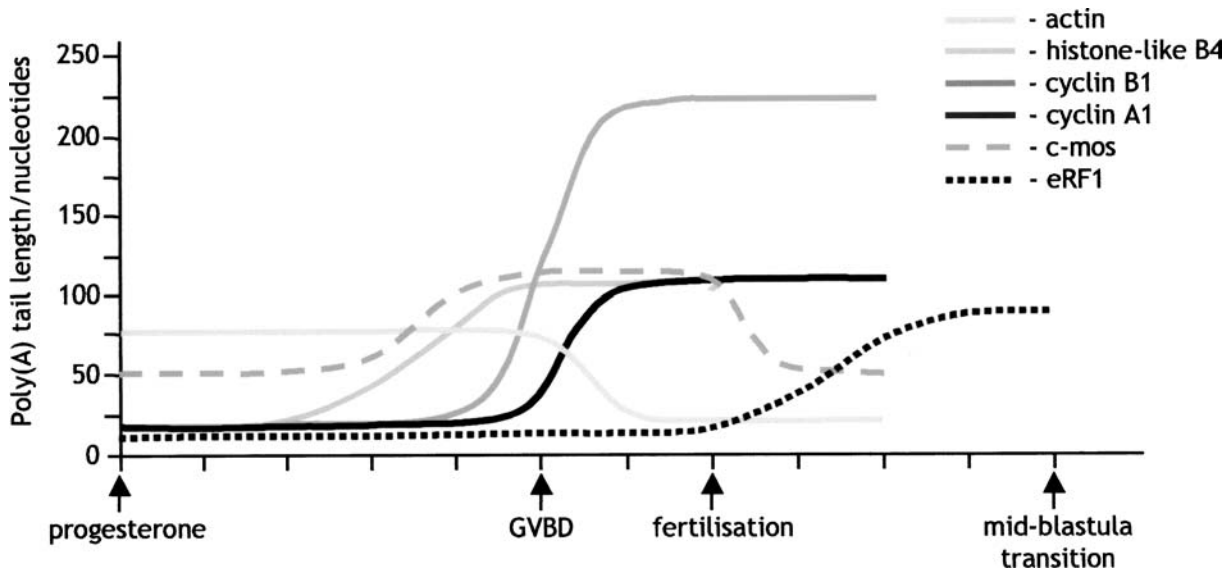


FIGURE 4 **Patterns of polyadenylation and deadenylation during meiotic maturation and early embryogenesis in *Xenopus*. The graph shows the characteristic changes observed in poly(A) tail length for each described class of mRNA. Timings are not shown to scale. Histone-like B4 (Charlesworth *et al.*, 2004) and *c-mos* mRNAs both belong to the early class of polyadenylated mRNAs, but *c-mos* becomes deadenylated again on fertilization (Fox *et al.*, 1989; McGrew *et al.*, 1989; Sheets *et al.*, 1994). The late class of polyadenylated mRNAs shows more heterogeneity with respect to timing—adenylation of cyclin B1 mRNA begins approximately one hour before germinal vesicle breakdown (GVBD) while cyclin A1 is not adenylated until after GVBD (Charlesworth *et al.*, 2004). Actin mRNA, which lacks CPEs, is active during oogenesis but becomes deadenylated at GVBD (Sheets *et al.*, 1994). The Cl1/eRF1 mRNA is not adenylated until after fertilization has occurred (Simon & Richter, 1994). For the majority of the mRNAs shown, poly(A) tail length has not been characterized in the embryo.**

distinguish between the two classes, at least at first sight. However, all late mRNAs contain a CPE that overlaps with the AAUAAA hexanucleotide, as in cyclin B1 mRNA (UUUUAAUAAA) (Charlesworth *et al.*, 2004), possibly indicating a steric occlusion of the $CPSF^{160}$ binding site by CPEB early in maturation, before CPEB is proteolysed (Mendez *et al.*, 2002). More interestingly still, Charlesworth *et al.* (2002, 2004), overturning current dogma, report that early maternal mRNA polyadenylation and translational activation during *Xenopus* oocyte maturation occurs in a CPE- and CPEB-independent manner. They define a new polyadenylation response element (PRE), present in the 3′ UTRs of early class mRNAs, which directs temporally-early cytoplasmic polyadenylation and translational activation. By contrast, the cytoplasmic polyadenylation of CPE- and CPEB-dependent mRNAs is a temporally late event. The two classes can be distinguished by the use of a dominant negative form of CPEB (CPEB-AA; (Mendez *et al.*, 2000a) that has alanine residue substitutions in the two tandem Aurora A/Eg2 consensus phosphorylation sites. This mutant significantly delays progesterone-mediated GVBD, and affects the cytoplasmic polyadenylation and translational activation of the 'late class' of mRNAs, but not that of the 'early class' of CPE mRNAs (Charlesworth *et al.*, 2004; Mendez *et al.*, 2000a). Charlesworth *et al.* (2004) propose a model whereby PRE-mediated polyadenylation results in a short poly(A) tail and the initiation of protein synthesis, and subsequent CPE-mediated polyadenylation extends the tail and continues protein synthesis, implying that early class mRNAs are subject to sequential regulation. Detailed studies of the role of the PRE, a relatively loosely conserved A and U-rich sequence (Charlesworth *et al.*, 2004), await the identification and characterisation of the PRE-binding factor.

Cytoplasmic Polyadenylation—Does Length Matter?

We are beginning to glean some of the mechanistic details underlying differential temporal control of cytoplasmic polyadenylation during meiotic maturation (Charlesworth *et al.*, 2004). But–dare we ask–does length matter? As was clearly illustrated in the seminal study of Sheets *et al.* (1994), which determined the poly(A) tail length of several CPE-containing mRNAs in oocytes, maturing eggs, and embryos, and was corroborated in subsequent investigations (Ballantyne *et al.*, 1997; Charlesworth *et al.*, 2004; Stebbins-Boaz *et al.*, 1996), different RNAs receive poly(A) tails of different lengths at different times in early development (Figure 4). Early studies noted an approximate correlation between the extent of polyadenylation and translational efficiency. In meiotically-maturing *Xenopus* eggs, cyclin B1 mRNA receives an additional 220 adenosines, cyclin A1 around 100 residues, and *c-mos* 70 residues, and they are translationally stimulated by about 75×, 5× and 10× respectively (Sheets *et al.*, 1994). In *in vitro* translation extracts derived from *Drosophila* embryos it has also been noted that translational efficiency responds sensitively to reporter mRNA poly(A) tail length. No significant enhancement, relative to A_0, was observed with tails of 31 or fewer residues, mRNAs with 50 to 150 adenosines were increasingly better templates, and the translation of an mRNA with >200 A residues was dramatically stimulated, with no changes in RNA stability (Castagnetti & Ephrussi, 2003; Gebauer *et al.*, 1999). The simplest interpretation of these observations is that the more PABP molecules that bind a poly(A) tail, the more frequently translation is initiated on the mRNA. Interactions between PABP and eIF4G are critical for efficient translation in *Xenopus* oocytes (Wakiyama *et al.*, 2000), as they are in mammalian cells (Imataka *et al.*, 1998) and yeast (Tarun & Sachs, 1996), and may increase the functional affinity of eIF4E for the 5′-cap (Borman *et al.*, 2000; von der Haar *et al.*, 2000). A higher density of PABP may stabilize interactions with eIF4G more than would a lower density, although it is not clear how the concentration effect would operate.

Very little is known about how the extent of polyadenylation of each individual mRNA is controlled to give characteristic poly(A) tail lengths. *Xenopus* oocytes were recently found to contain a cytoplasmic form of PABP2 (Cosson *et al.*, 2004; Good *et al.*, 2004), a homolog of the single RRM class of poly(A)-binding proteins that function in nuclear poly(A) length control (reviewed in Kühn & Wahle, 2004). The *Xenopus* form is predominantly expressed in embryogenesis and in adult ovary tissues (Cosson *et al.*, 2004; Good *et al.*, 2004), suggesting that it could be involved in regulating cytoplasmic poly(A) tail length in early development.

Cytoplasmic Polyadenylation in the Embryo

Polyadenylation during early development is not simply confined to the period of meiotic maturation but also operates in early embryogenesis (Paris &

Philippe, 1990). The mRNAs that are polyadenylated in the cytoplasm and recruited to polysomes after fertilization encode such proteins as the polypeptide chain release factor eRF1 (Cl1, homologue of *S. cerevisiae* SUP45), Cl2 (of unknown function), and the activin receptor (Simon & Richter, 1994; Simon *et al.*, 1992; Simon *et al.*, 1996; Tassan *et al.*, 1993). Polyadenylation of these mRNAs in embryos requires an embryonic CPE, $U_{12–27}$, in addition to the AAUAAA hexanucleotide. An eCPE can function at a long distance from the 3′-end of the mRNA, up to almost 300 nt in the case of Cl1, compared with 54 nt in Cl2 (Simon & Richter, 1994). Mutation of the hexanucleotide prevents both polyadenylation and translational activation in embryos (Simon *et al.*, 1992; Simon *et al.*, 1996). The translation of preadenylated injected mRNA, with a poly(A) tail similar in length to that of endogenous embryonic RNA, is, however, not enhanced, suggesting that ongoing polyadenylation, rather than a certain number of adenosines, mediates activation (Simon *et al.*, 1992). Noting that Cl1 mRNA is fully polyadenylated hours later in embryogenesis than Cl2 mRNA, Simon and colleagues investigated the effect of shortening the spacing between the eCPE and the hexanucleotide in Cl RNAs injected into fertilized eggs. Interestingly, bringing the elements closer affects both the temporal and length control aspects, the mutated mRNAs are polyadenylated earlier and receive longer tails than normal (Simon & Richter, 1994; Simon *et al.*, 1992), implying that proximity of their binding factors may enhance polyadenylation efficiency. Precocious polyadenylation in maturing oocytes is prevented by long 'masking' elements in the 3′ UTR, which include the eCPE. If these upstream masking elements are removed, the eCPE will support robust polyadenylation in eggs, as well as in embryos, indicating that the embryonic polyadenylation machinery is present in both stages (Simon & Richter, 1994; Simon *et al.*, 1992). A 36 kDa protein was found to bind to the eCPE (Simon & Richter, 1994; Simon *et al.*, 1996) and was identified as ElrA, a member of the *elav* family of RNA-binding proteins, but an extensive series of experiments failed to show a role in cytoplasmic polyadenylation (Wu *et al.*, 1997). However, expression of a truncated ElrA, containing the principal RNA-binding RRM1 and RRM2 domains in injected embryos results in abnormal development beginning at the gastrula stage, suggesting that it plays an important, albeit unspecified, role in early development (Wu *et al.*, 1997). Recently, it has been reported that maturation-type CPEs, and CPEB, also promote polyadenylation and translational activation in the *Xenopus* embryo, in a cell cycle-regulated manner in the case of cyclin B1 mRNA (Groisman *et al.*, 2002).

Cytoplasmic Deadenylation

Deadenylation During Meiotic Maturation: The Role of the Poly(A)-Specific Ribonuclease PARN

While cytoplasmic polyadenylation is used to activate translation, deadenylation can be used to produce the opposite effect. Poly(A) removal not only initiates mRNA turnover in yeast and in somatic metazoan cells (reviewed in Parker & Song, 2004), but is also used to silence maternal mRNAs translationally during oocyte maturation and embryogenesis in diverse species (Richter, 2000). In maturing *Xenopus* eggs, polyadenylated mRNAs that lack a CPE in their 3′ UTR, such as actin and ribosomal protein mRNAs, are deadenylated at GVBD; inclusion of a CPE within such mRNAs prevents deadenylation. Importantly, loss of the poly(A) tail releases the mRNAs from polysomes. Deadenylation is thus a default reaction by which mRNAs that lack specific sequence information are translationally silenced during meiotic maturation (Fox & Wickens, 1990; Varnum & Wormington, 1990). To date, with the exception of histone mRNAs (Sanchez & Marzluff, 2004), deadenylation in maturing *Xenopus* eggs has been coupled with translational repression. Repression is caused, in part at least, by the loss of PABP-binding sites in the 3′ UTR, since over-expressing PABP in *Xenopus* oocytes inhibits both maturation-specific deadenylation and translational inactivation (Wormington *et al.*, 1996). Unlike yeast and somatic metazoan cells (Parker & Song, 2004), deadenylation in *Xenopus* eggs does not induce decapping (Gillian-Daniel *et al.*, 1998) and subsequent RNA degradation. Decapping activity is absent from oocytes and eggs and is, in fact, not detected till later, in embryogenesis, at the mid-blastula transition (MBT) (Zhang *et al.*, 1999b). However, deadenylation is required for degradation later in development (Audic *et al.*, 1997; Voeltz & Steitz, 1998).

The deadenylase activity is initially nuclear in mature oocytes as poly(A) removal is a late maturation event that cannot be detected prior to nuclear envelope breakdown and is prevented if oocytes are enucleated prior to maturation (Varnum *et al.*, 1992). Indeed, poly(A)-specific ribonuclease (PARN), the default deadenylase,

is found in the nucleus of stage VI oocytes; its release at GVBD deadenylates all non-CPE-containing target mRNAs at the same time (Copeland & Wormington, 2001).

PARN was purified from oocytes by several chromatographic procedures, culminating in a poly(A) column, and its peptide sequences used to clone the *Xenopus* cDNA (Copeland & Wormington, 2001; Korner *et al.*, 1998). The 74 kDa *Xenopus* PARN is highly homologous to human PARN, cloned independently (Korner *et al.*, 1998; Martinez *et al.*, 2000). Mutagenesis of the active site residues of PARN confirmed that it resembles the active site of the 3′-exonuclease domain of *E. coli* DNA polymerase I functionally and structurally and identifies the deadenylase as a member of the conserved family of DEDD nucleases, containing tripartite RNAse D exonuclease domains (Korner *et al.*, 1998; Ren *et al.*, 2002; Thore *et al.*, 2003). A putative RRM is located downstream of the exonuclease domains and is presumably responsible for binding poly(A) (Copeland & Wormington, 2001) (Table 1). PARN, the only mammalian poly(A)-specific 3′-exoribonuclease identified and characterised thus far, is an oligomeric and highly processive 3′-exonuclease. A reaction pathway for degradation by PARN has been proposed, the key characteristics of which are the release of 5′-AMP as the mononucleotide product and the requirement for a 3′-located adenosine residue with a free 3′-hydroxyl group (Copeland & Wormington, 2001; Korner *et al.*, 1998; Martinez *et al.*, 2000; Ren *et al.*, 2002).

Deadenylation by amphibian and mammalian PARN is stimulated by the presence of an m^7-guanosine cap on substrate RNAs. PARN exhibits intrinsic cap-binding activity that is stimulated by a poly(A) tail and competed by eIF4E, implying that interactions between the 5′-cap and the 3′-poly(A) tail integrate translational efficiency with mRNA stability (Dehlin *et al.*, 2000; Gao *et al.*, 2000; Martinez *et al.*, 2001) (Figure 5). PARN is the major deadenylase activity in mammalian lysates (Gao *et al.*, 2000), is essential for embryogenesis in higher plants (Reverdatto *et al.*, 2004) and, although not present in the *S. cerevisiae* nor *D. melanogaster* genomes, is the closest homolog of the yeast DEDD poly(A) nuclease Pop2/Caf1 (Thore *et al.*, 2003), one of two deadenylase activities present in the large Ccr4-Not complex and responsible for mRNA deadenylation. The relative importance of Pop2/Caf1, and the second activity, Ccr4, in deadenylation in yeast is not yet clear (Temme *et al.*, 2004; Thore *et al.*, 2003; Tucker *et al.*, 2002). Nevertheless, it is interesting to note that nocturnin, the *Xenopus* Ccr4 homologue, is rhythmically expressed in the cytoplasm of retinal photoreceptor cells, while PARN is constitutively present in most retinal cells, including the photoreceptors. The distinct spatial and temporal expression of nocturnin and PARN suggests that there may be specific mRNA targets of each deadenylase (Baggs & Green, 2003). Whether nocturnin/Ccr4 is also expressed in oocytes is not known.

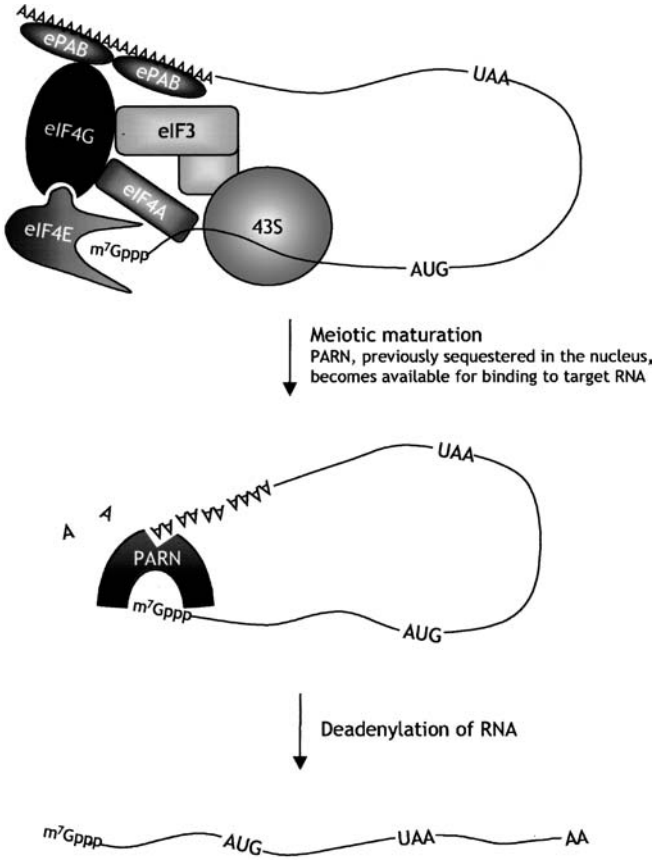


FIGURE 5 **Model showing the translational inactivation of mRNAs by PARN. During oogenesis, mRNAs that do not contain CPE sequences are polyadenylated and actively translated. Upon meiotic maturation and GVBD, the poly(A)-specific ribonuclease (PARN), previously sequestered in the nucleus, becomes available to interact with polyadenylated mRNAs (Copeland & Wormington, 2001; Korner *et al.*, 1998). PARN interacts directly with the cap structure, as shown by its ability to photo-crosslink to the cap (Dehlin *et al.*, 2000; Gao *et al.*, 2000; Martinez *et al.*, 2001). Presence of the cap stimulates the activity of PARN and capped substrates are efficiently deadenylated (Dehlin*et al.*, 2000; Gao*et al.*, 2000; Martinez*et al.*, 2001). This results in their release from ribosomes and translational repression.**

Post-Fertilization Deadenylation: The Embryonic Deadenylation Element (EDEN) and EDEN-BP

The maternal *Xenopus* mRNAs encoding Eg1 (cdk2), Eg2 (Aurora A kinase), Eg5 (kinesin-related) and *c-mos*

display a characteristic polyadenylation/deadenylation behavior (Figure 4). These mRNAs, which contain a functional CPE, are polyadenylated and translationally activated during oocyte maturation, and then deadenylated and translationally repressed after fertilization (Paris *et al.*, 1988; Paris & Philippe, 1990). Silencing the translation of previously activated mRNAs is an important mechanism to regulate cell cycle progression. For example, the arrest of Mos synthesis upon fertilization of *Xenopus* eggs is a prerequisite for the initiation of embryonic divisions. The rapid deadenylation of Eg mRNA is only observed in embryos and in egg extracts that have been activated with the calcium ionophore A23817 to simulate fertilization (Legagneux *et al.*, 1995; Sheets *et al.*, 1994; Stebbins-Boaz & Richter, 1994). Post-fertilization deadenylation in *Xenopus* embryos is sufficient to cause the degradation of these RNAs at the blastula stage (Audic *et al.*, 1997; Voeltz & Steitz, 1998), the delay, as discussed earlier, arising from the lack of decapping activity prior to MBT (Gillian-Daniel *et al.*, 1998; Zhang *et al.*, 1999b).

Sequences that promote deadenylation of Eg RNAs in embryos have been identified as embryo deadenylation elements (EDEN) (reviewed in Paillard & Osborne, 2003). EDENs are composed of U/purine dinucleotide repeats, the number of which varies between individual mRNAs. The *c-mos* mRNA 3′ UTR contains a 36 nt EDEN, which is sufficient to confer rapid deadenylation upon a polyadenylated reporter mRNA in activated egg lysates. This EDEN is composed of two blocks, of six and eight UR repeats, and is located several hundred nucleotides away from the CPE at the 3′-end (Paillard *et al.*, 1998). Similar UR repeats are required for deadenylation in Eg2 and Eg5 RNAs (Paillard & Osborne, 2003). Using synthetic 3′ UTR EDEN sequences, it was shown that the number of repeats seems to correlates with activity–nine repeats of UGUA confer rapid deadenylation, whereas $(UGUA)_6$ RNA is less efficiently deadenylated (Audic *et al.*, 1998). Deadenylation, and translational silencing, mediated by EDEN elements may be enhanced by $(AUU)_3$, located just 5′ of the poly(A) tail (Audic *et al.*, 1998; Stebbins-Boaz & Richter, 1994) and by AUUUA elements (Ueno & Sagata, 2002).

EDEN elements promote deadenylation by interacting specifically with a 55 kDa protein, first identified in UV-crosslinking studies. The importance of this factor was shown in experiments in which injection of excess EDEN RNA into eggs restored the translation of *c-mos* RNA (Ueno & Sagata, 2002). Mutations in EDEN that reduced or abolished deadenylation did not bind the 55 kDa protein, which was subsequently purified on EDEN RNA columns and named EDEN-BP (Paillard *et al.*, 1998). Importantly, immunodepleting EDEN-BP from an egg extract abolished the EDEN-mediated deadenylation activity, but did not affect default deadenylation by PARN (Paillard *et al.*, 1998). Like the *Drosophila* translational repressor Bruno, EDEN-BP (Table 1) is a member of the *elav* family of RNA-binding proteins with three RRMs, the second and third of which are separated by a linker region (reviewed in Good, 1997; Soller & White, 2004). Specific recognition of EDEN RNA is mediated by RRM1+2 and a portion of the linker region (Bonnet-Corven *et al.*, 2002). EDEN-BP self-associates in a yeast two hybrid assay, and an analysis of its binding to short and long repeat sequences suggests that the functional form of the protein is a dimer (Bonnet-Corven *et al.*, 2002). EDEN-dependent translational repression of maternal mRNA is functionally conserved between *Xenopus* and *Drosophila*, as shown in transgenic flies (Ezzeddine *et al.*, 2002). An EDEN inserted into the 3′ UTR of a maternally-expressed lacZ reporter gene represses its translation in *Drosophila* oocytes, without affecting its localization or stability. Interestingly, *Drosophila* oocytes do not appear to deadenylate the inhibited reporter mRNA (Ezzeddine *et al.*, 2002), pointing to differences in the mechanism of EDEN-mediated translational repression between species. The closest *Drosophila* EDEN-BP homologue (with 50% similarity) is Bruno. Despite this sequence conservation, and the similarity in target RNA sequences (Table 1), but in line with the lacZ-EDEN reporter data (Ezzeddine *et al.*, 2002), Bruno represses the translation of *oskar* mRNA in a poly(A)-independent manner (Castagnetti & Ephrussi, 2003) and there is no evidence that it functions in deadenylation. However, human CUG-BP (better known as a factor regulating alternative splicing, (Gromak *et al.*, 2003)) and *Xenopus* EDEN-BP, which are 88% identical and also have very similar RNA-binding specificities, are functional homologues in deadenylation assays (Paillard *et al.*, 2002; Paillard *et al.*, 2003).

Post-Fertilization Deadenylation: AU-Rich Elements (AREs) and ePABP

In mammalian cells, certain mRNAs encoding cytokines or proto-oncogenes are especially unstable, due to the presence of sequence elements in their 3′ UTRs

named A/U-rich elements (AREs). AREs cause RNA instability by promoting the rapid shortening of the poly(A) tail of the mRNA (Chen *et al.*, 1995; Shyu *et al.*, 1991). They have been separated into three different classes (Chen & Shyu, 1995). Classes I and II both contain the pentamer AUUUA; a class I element consists of one to three copies of the motif with a nearby U-rich region, while in class II there are at least two overlapping copies of the nonamer UUAUUUA(U/A)(U/A) located in a U-rich region. The class III RNAs are less well characterized, decay seems to depend on the presence of a U-rich domain. The deadenylation of mRNAs mediated by class I/II AREs is conserved in *Xenopus* embryos. The AREs of *Xenopus* c-myc II and the human granulocyte-macrophage colony-stimulating factor (GMCSF) mRNAs both direct deadenylation of chimeric mRNAs in an AUUUA-dependent manner. ARE-mediated mRNA deadenylation can be uncoupled from ARE-mediated mRNA decay, and AUUUAs directly signal deadenylation, but not degradation, during *Xenopus* early development. AUUUA-specific deadenylation appears to be developmentally regulated: low deadenylation activity is observed in the oocyte, whereas rapid deadenylation occurs following egg activation or fertilization (Voeltz & Steitz, 1998). Earlier experiments in *Xenopus* oocytes showed that AREs derived from several cytokine mRNAs, including that encoding GMCSF, conferred translational inhibition upon a reporter mRNA (Kruys *et al.*, 1989). In the fertilized egg, AREs could also repress translation, but somewhat surprisingly in the light of the later studies, independently of the state of polyadenylation of the injected RNA (Marinx *et al.*, 1994).

An *in vitro* system that recapitulates the *in vivo* effect of AREs on mRNA deadenylation was developed from *Xenopus* A23817-activated egg extracts. This allowed the purification of a novel high affinity ARE-binding protein called ePAPB (for embryonic poly(A)-binding protein) by ARE RNA affinity selection (Voeltz *et al.*, 2001) (see Table 1). ePABP exhibits 72% identity to mammalian and *Xenopus* PABP1, contains the four highly-conserved RRMs as well as the PABC domain, and is the predominant poly(A)-binding protein expressed in the stage VI oocyte and during *Xenopus* early development. In contrast, the levels of *Xenopus* PABP1 are extremely low in oocytes, and the protein is only readily detected in embryos obtained one day after fertilization or later (Cosson *et al.*, 2002; Voeltz *et al.*, 2001). Immunodepletion of ePABP increases the rate of both ARE-mediated and default deadenylation *in vitro*, whereas the addition of even a small excess of ePABP inhibits deadenylation (Voeltz *et al.*, 2001). Similarly, over-expression of *Xenopus* PABP1 in oocytes prevents maturation-specific deadenylation and translational inactivation (Wormington *et al.*, 1996). These data argue that ePABP is the principal poly(A)-binding protein that controls poly(A) tail length in oocytes, and is thus a potential regulator of mRNA deadenylation and translation during early development. ePABP resembles the zygotic isoform, not only in its ability to bind poly(A) (Cosson *et al.*, 2002; Voeltz *et al.*, 2001), but also in its ability to interact with eIF4G (Cao & Richter, 2002) and eRF3, and the ePABP gene can complement a pab1Δ mutation in *S. cerevisiae* (Cosson *et al.*, 2002). It remains to be seen whether the sequence differences, largely confined to the C-terminal region between RRM4 and PABC, implicated in mediating PABP-PABP interactions (Melo *et al.*, 2003), affect other functions. Recently, human PABP was shown to bind AU-rich RNA via RNA-binding domains 3 and 4 (Sladic *et al.*, 2004), and it is therefore likely that ePABP binds AREs via RRM3+4, in contrast to the high-affinity poly(A) binding by RRM1+2 of PABP1 (reviewed by Kühn & Wahle, 2004).

Lastly, the c-Jun ARE, a representative of class III (non-AUUUA) AREs, also provokes the deadenylation of a reporter RNA in *Xenopus* embryos. The rapid deadenylation of RNAs that contain the c-Jun ARE, but not an AUUUA ARE, requires EDEN-BP, as shown in immunodepletion and immunoneutralization experiments (Paillard *et al.*, 2002).

Thus, three main deadenylation pathways have been characterized in early *Xenopus* development, none of which signals immediate RNA degradation, but rather are used to silence translation. During meiotic maturation, PARN deadenylates non-CPE-containing mRNAs, such as actin mRNA. After fertilization, CPE-containing RNAs with additional EDEN (e.g., *c-mos*) or ARE elements (e.g., c-*myc*), are deadenylated in a manner requiring EDEN-BP or are regulated by ePABP, respectively. The poly(A) nucleases that mediate EDEN and AUUUA-dependent deadenylation in embryos have not yet been identified, but appear to be different to each other, and to PARN.

Translational Repression and Potential Deadenylation by GLD-1

Translational repression is equally vital for the early development of *C. elegans*, and the nematode also

exploits the process of deadenylation. *C. elegans* has two sexes: hermaphrodites and males. Hermaphrodites are essentially female animals that make sperm and then oocytes. The sex-determining gene *tra-2* is required for female development and is predicted to encode a large transmembrane protein, TRA-2A, that is involved in the inhibition of downstream male determinants. Proper male development requires the repression of *tra-2* activity. Dominant gain-of-function (*gf*) mutations have been identified that result in high *tra-2* activity leading to inappropriate female development. The *gf* mutations all map to a direct repeat located in the 3′ UTR of the *tra-2* mRNA, which functions by repressing its translation (Goodwin *et al.*, 1993; Jan *et al.*, 1999). The two 28 nt long *tra-2* and *GLI* elements (TGEs) are separated by a 4 nt spacer (Jan *et al.*, 1999; Jan *et al.*, 1997).

The protein GLD-1, a member of the STAR/GSG (signal transduction and activation of RNA/ GRP33, Sam68, GLD-1) family of RNA-binding proteins (reviewed in Lukong & Richard, 2003), was isolated in a yeast three hybrid screen (Bernstein *et al.*, 2002) as a TGE-specific RNA-binding protein (Jan *et al.*, 1999). The hallmark of this family is a single KH (hnRNP K homology) domain flanked by two conserved domains called QUA1 and QUA2, comprising approximately 200 amino acids in total (Table 1). The KH motif, which has a $\beta\alpha\alpha\beta\beta\alpha$ topology, is approximately 70 amino acids long and has a characteristic pattern of aliphatic residues, with the most conserved IGxxGxxI core located roughly in the middle of the domain (Adinolfi *et al.*, 1999; Grishin, 2001; Ostareck-Lederer *et al.*, 1998). The recent crystal structure of the Nova-2 KH domain bound to a 20-mer RNA hairpin confirmed that the KH consensus sequence is in direct contact with nucleic acid (Lewis *et al.*, 2000). KH domains may be present in single or (more usually) multiple copies and are present in a wide variety of proteins, from bacteria to man, that function in close association with RNA (reviewed by Adinolfi *et al.*, 1999). The QUA1 domain allows STAR proteins to form functional homodimers (Chen *et al.*, 1997; Chen & Richard, 1998), whereas QUA2, in association with the KH domain, serves to extend the RNA interaction surface (Liu *et al.*, 2001).

GLD-1 has multiple roles in germline development; it is essential for oogenesis, and has non-essential roles in germline proliferation and sex determination. In sex determination, GLD-1 is necessary for hermaphrodite spermatogenesis, resulting from the repression of *tra-2* translation. GLD-1 can repress the translation of reporter RNAs containing TGEs both *in vitro* and *in vivo*, and is needed to maintain low levels of TRA-2A protein in the germline (Jan *et al.*, 1999). Translational control by TGE motifs was shown to be conserved in the related nematode *C. briggsae* and in mammalian cells (Jan *et al.*, 1997), raising the possibility that GLD-1 homologs utilise a similar mechanism to control gene activity. The STAR/GSG protein family includes the *quaking* proteins (QKI-5, QKI-6 and QKI-7), which are essential for embryogenesis and myelination in mouse (Lukong & Richard, 2003). Indeed, QKI-6 can specifically bind TGEs from *C. elegans*, *C. briggsae* and human mRNAs and repress the translation of TGE-containing RNAs both *in vitro* in yeast and *in vivo* in *C. elegans*, implying a conservation of function amongst some STAR family members (Saccomanno *et al.*, 1999). However, other members of this family, such as the splicing factor SF1, control other post-transcriptional events, so STAR protein functions extend beyond the control of protein synthesis (Lukong & Richard, 2003).

Recently, the RNA target specificity of GLD-1 was determined (Ryder *et al.*, 2004). The minimal consensus STAR-binding element (SBE) is a single, high affinity, 6 nt element, UACU(C/A)A. Re-examination of the two TGE regions of *tra-2* mRNA reveals three consensus binding sites, one in each of the TGE repeats and the third comprising the last two nucleotides of the first repeat together with the 4 nucleotide spacer. Recombinant GLD-1 binds RNA as a preformed dimer, and dimerization, as the earlier studies reported (Chen *et al.*, 1997; Chen & Richard, 1998), requires the QUA1 domain (Ryder *et al.*, 2004). Additional targets of GLD-1 had previously been identified, including *mes-3* (Xu *et al.*, 2001) and *cej-1*, *exo-3*, *lin-45*, *puf-5*, *puf-6*, *puf-7* and *rme-2* (Lee & Schedl, 2001). These RNAs all contain an SBE in their 3′ UTRs (Ryder *et al.*, 2004), attesting to the importance of this motif for high-affinity binding by GLD-1. The results from database searches for further genes containing the conserved nucleotides suggested that GLD-1 may have additional functions in X-chromosome silencing and early embryogenesis (Ryder *et al.*, 2004). Moreover, the SBE is present in mRNA targets of mouse Qk1 and *Drosophila* How, which are translationally repressed by these proteins, suggesting that STAR protein RNA-binding specificity is highly conserved (Xu *et al.*, 2001). However, it is important to note that STAR/GSG proteins whose RNA-binding regions diverge more extensively from that of GLD-1

have a different high affinity binding site; for example, human Sam68 binds AUUAAAA (Lin *et al.*, 1997).

The details of the mechanism of translational repression by GLD-1 involving *tra-2* TGEs are not yet clear, but a role in deadenylation, or maintenance of short poly(A) tails has been suggested. While wild-type *tra-2* RNA in *C. elegans* was noted to have a poly(A) tail of 50 to 70 adenosines, an RNA which lacked the TGEs had a longer poly(A) tail of 100 to 150 A residues (Jan *et al.*, 1997). Additional evidence came from analyzing the effects of TGEs placed downstream of a reporter mRNA, microinjected into fertilised *Xenopus* eggs. The TGEs promote translational repression, in a poly(A)-dependent manner, suggesting they may disrupt the interaction between PABP and the 5′-end of the RNA. Moreover, TGEs result in rapid poly(A) shortening in *Xenopus* embryos, giving an accelerated appearance of fully deadenylated mRNAs (Thompson *et al.*, 2000). However, the *trans*-acting factors bound to the TGEs in *Xenopus* have not been identified and, therefore, it is not possible to predict whether GLD-1 is directly involved in deadenylation.

The Puf Family Proteins and Poly(A) Tail Shortening

The Puf Family: Homologues, Domains and Structure

The involvement of deadenylation in translational repression has been further highlighted by the study of the Puf family of RNA-binding proteins (Table 1). The founder members of this family are *Drosophila* Pumilio and *C. elegans* FBF (*fem-3* mRNA binding factor) (reviewed by Spassov & Jurecic, 2003; Wickens *et al.*, 2002). Puf proteins are readily identified by the presence of eight tandem copies of an imperfectly repeated, 36 amino acid sequence motif, the PUM repeat (Zamore *et al.*, 1997; Zhang *et al.*, 1997), which, together with conserved flanking sequences, form a sequence-specific RNA binding domain, the Pumilio homology domain (PUM-HD) (Zamore *et al.*, 1997) or Puf domain (Zhang *et al.*, 1997). Some Puf homologs contain additional RNA-binding domains, such as putative RRMs and zinc fingers (e.g., Puf1/2 and Puf3/4 respectively in yeast (Gerber *et al.*, 2004), and these may confer additional specificity in RNA binding.

Whereas insects only have one Puf protein, there are eleven proteins in *C. elegans* with the signature RNA-binding domain, six in *S. cerevisae* and two in vertebrates, including man, mouse, zebrafish, and *Xenopus* (Spassov & Jurecic, 2003; Wickens *et al.*, 2002). The Puf family of proteins is conserved throughout evolution with homologs also identified in plants, parasites (malaria, *Leishmania* and *Trypanosomes*) and prokaryotes, (Cui *et al.*, 2002; Spassov & Jurecic, 2003; Wickens *et al.*, 2002).

The high degree of conservation of the PUM-HD regions–for example, ~80% identity between murine and human PUM2 and *Drosophila* Pumilio (Moore *et al.*, 2003; White *et al.*, 2001)–suggests that they recognise similar RNA sequences. Indeed, a variety of approaches, including Selex experiments using the mouse and *Xenopus* PUM2-HD domains and a comparison of the 3′ UTR sequences in mRNAs associated with yeast Puf3-5 homologs, have revealed a highlyconserved 8 nt-long binding site, UGUANAUA, whose identity was supported by the structure determination of the human PUM1-HD-RNA complex (Gerber *et al.*, 2004; Nakahata *et al.*, 2001; Wang *et al.*, 2002b; White *et al.*, 2001). *Drosophila* Pumilio and *C. elegans* FBF recognize similar sequences in their target RNAs; the Nanos response elements (NREs) in the 3′ UTRs of the *hunchback* and *bicoid* RNAs (Murata & Wharton, 1995) and the regulatory region of *fem-3* RNA respectively (Zhang *et al.*, 1997).

Crystal structures of the human PUM1 and the *Drosophila* Pumilio RNA-binding domains reveal that the eight PUM repeats correspond to eight copies of a single, repeated structural motif, which pack together to form a right-handed superhelix. This yields an extended, rainbow-shaped molecule. The distribution of side chains on the inner and outer faces of this arc suggests that the inner face of the PUM-HD binds RNA while the outer face could interact with protein ligands (Edwards *et al.*, 2001; Wang *et al.*, 2001; reviewed in Gavis, 2001). Subsequent determinations of the structures of the RNA binding domain of hPUM1 bound to the 8 nt high-affinity RNA ligand, and of the complex of *Drosophila* Pumilio bound to its interacting partner Brain Tumor (Brat) protein bore out these predictions (Edwards *et al.*, 2003; Wang *et al.*, 2002b). The RNA binds the concave surface of the molecule, where each of the eight repeats of the domain makes contacts with a different RNA base via three conserved amino acid side chains. Thus, the high affinity and specificity of the PUM-HD for RNA is achieved using multiple copies of a simple repeated motif (Wang *et al.*, 2002b). Brat is a member of the conserved NHL family of proteins

(reviewed in Slack and Ruvkun, 1998), and a component of the Pumilio quaternary complex involved in translational control (Sonoda & Wharton, 2001). A model of the Pumilio-Brat complex derived from *in silico* docking experiments, and supported by mutational analysis of the protein-protein interface, reveals the recognition of the outer, convex surface of the Pumilio Puf domain by Brat (Edwards *et al.*, 2003).

The Role of Puf Proteins in Deadenylation

Members of the Puf family have been implicated in deadenylation in a number of organisms, including *Drosophila, C. elegans, Xenopus* and yeast. In *Drosophila* embryos, Pumilio plays a key role in posterior patterning by binding to maternal *hunchback* mRNA (Murata & Wharton, 1995). This maternally-supplied mRNA is distributed throughout the egg and, before fertilization, has a short poly(A) tail and is translationally repressed (Wreden *et al.*, 1997). Upon fertilization, *hunchback* mRNA is activated by polyadenylation. However, in the posterior of the embryo, *hunchback* translation is repressed by the concerted activities of Pumilio, Nanos and Brat (Parisi & Lin, 2000; Sonoda & Wharton, 2001). Early in *Drosophila* embryogenesis, Pumilio and Brat proteins are uniformly distributed (Macdonald, 1992; Sonoda & Wharton, 2001), whereas Nanos is initially detected at the posterior end of the embryo and then in the pole cells (Wang & Lehmann, 1991). Pumilio binds to the NREs and recruits first Nanos, and then Brat. Formation of the quaternary complex is essential for translational control of *hunchback* mRNA (Sonoda & Wharton, 2001). Pumilio and Nanos were shown to promote the deadenylation of *hunchback* mRNA at the posterior pole, thereby inhibiting its translation (Wreden *et al.*, 1997). However, this may be an oversimplification, as *hunchback* mRNA is regulated both by poly(A) removal (Chagnovitch and Lehmann, 2001; Wreden *et al.*, 1997) and by a poly(A)-independent pathway (Chagnovitch and Lehmann, 2001).

The Pumilio-Nanos partnership (reviewed in Jaruzelska *et al.*, 2003; Parisi & Lin, 2000) in *Drosophila* is not only critical for posterior patterning, but has also been implicated in germline development in embryos (Asaoka-Taguchi *et al.*, 1999), in eye development (Wharton *et al.*, 1998), optic nerve development (Schmucker *et al.*, 1997), neuronal excitability (Schweers *et al.*, 2002), and dendrite morphogenesis in peripheral neurons (Ye *et al.*, 2004), extending the possible involvement of this means of translational control to eye and neuronal development as well as early embryogenesis.

In the hermaphrodite *C. elegans*, the switch from spermatogenesis to oogenesis relies on repression of the sex-determining *fem-3* mRNA. Gain-of-function *fem-3* mutants only make sperm; such mutations map to the *fem-3* 3′ UTR, and concentrate in the six nucleotide point mutation element (PME), UCuUGu (capital letters denote nucleotides affected by the *gf* mutations). Over-expression of the wild-type *fem-3* 3′ UTR masculinises the nematode germ line, consistent with the titration of a repressor (Ahringer & Kimble, 1991; Ahringer *et al.*, 1992). FBF (*fem-3* mRNA binding factor) was isolated in a yeast three-hybrid screen with the PME RNA as bait (Zhang *et al.*, 1997). FBF, consisting of FBF-1 and FBF-2, two nearly identical proteins that contain the Puf domain, is required for the sperm/oocyte switch, as RNAi-mediated depletion results in a dramatic masculinization of the germ line. It also has roles in spermatogenesis (Luitjens *et al.*, 2000) and the control of germline stem cells (Crittenden *et al.*, 2002).

NANOS-3, one of three Nanos homologues in the nematode, participates in the sperm/oocyte switch by its physical interaction with FBF (Kraemer *et al.*, 1999). NOS-3 itself binds RNA non-specifically, and is recruited to the *fem-3* RNA by an interaction with the Puf RNA-binding domain of FBF (Kraemer *et al.*, 1999). The role of this conserved interaction (see below) in *fem-3* mRNA control is not yet clear. However, the recent demonstration that FBF is inhibited by GLD-3, the Bicaudal-C homologue, raises some interesting speculations regarding its mode of action as a repressor. GLD-3 inhibits the action of FBF, by interfering with FBF binding to the *fem-3* PME (Eckmann *et al.*, 2002). GLD-3 does not itself appear to recognize the PME, but rather, by directly binding FBF, prevents its interaction with the PME (Eckmann *et al.*, 2002). GLD-3 also binds and enhances the activity of GLD-2, the cytoplasmic poly(A) polymerase (Wang *et al.*, 2002a). Therefore, GLD3 may antagonise FBF by promoting polyadenylation of its target mRNA (Eckmann *et al.*, 2002).

Xenopus Pumilio 2 (XPUM2), a 137 kDa protein, is expressed in oocytes and binds to an UGUA motif in the 3′ UTR of cyclin B1 mRNA, just upstream of the CPEs (Nakahata *et al.*, 2001). Mutation of the XPUM2 binding site leads to a slight alleviation of the repression exerted by the 3′ UTR when fused to a reporter mRNA (Nakahata *et al.*, 2003). Consistent with a repressor role, injection of anti-XPUM2 antibody

accelerated GVBD, by enhancing the translation of cyclin B1 mRNA, while over-expression of the C-terminal half of XPUM2 (XPUM2-RBD) delayed cyclin B1 synthesis and GVBD (Nakahata *et al.*, 2003). Repression was specific to cyclin B1 mRNA, as translation of another CPE-containing mRNA, encoding *c-mos*, was unaffected. XPUM2 co-immunoprecipitates via its Puf domain with CPEB, in an RNA-independent manner in oocytes, but not with phosphorylated CPEB in eggs (Nakahata *et al.*, 2001; Nakahata *et al.*, 2003). It is proposed that XPUM2 cooperates with CPEB to repress cyclin B1 mRNA in oocytes, and dissociation of XPUM2 from phosphorylated CPEB in maturing eggs may help to destabilize repression by the CPEB-maskin-eIF4E complex, and/or enhance cytoplasmic polyadenylation, resulting in the translational activation of cyclin B1 mRNA. XPUM2-mediated repression, in oocytes over-expressing the XPUM2-RBD, correlates with short poly(A) tails on cyclin B1 mRNA, in line with a role for *Xenopus* PUM2 in deadenylation or prevention of polyadenylation (Nakahata *et al.*, 2003).

The functions of Pumilio in flies, nematodes and frogs all point to a role in the deadenylation or maintenance of short poly(A) tails, resulting in translational repression. In *Drosophila* and *C. elegans,* this occurs through the formation of a complex with Nanos. In this regard, it is of interest that XPUM2 can interact with Xcat-2, the *Xenopus* Nanos homologue, *in vitro* (Nakahata *et al.*, 2001). While this precise pairing may not occur in oocytes, as Xcat-2 mRNA, a germ plasm mRNA, is translationally repressed until the blastula stages (MacArthur *et al.*, 1999), such an interaction may occur either later in development or with an alternative, as-yet-unknown, *Xenopus* Nanos homologue. In humans, NOS1, a close relative of Xcat-2, also interacts with hPUM2 (Jaruzelska *et al.*, 2003).

There are six proteins (referred to as Puf1/Jsn1, Puf2, Puf3, Puf4, Puf5/Mpt5 and Puf6) in the *S.cerevisiae* genome that contain six to eight copies of the characteristic Puf repeat sequence (Gerber *et al.*, 2004). Puf5 negatively regulates expression of the HO gene, at a post-transcriptional level, by binding a UUGU element in the HO mRNA 3′ UTR (Tadauchi *et al.*, 2001), while Puf3 enhances the rate of deadenylation, and subsequent degradation, of COX17 mRNA, and binds the COX17 mRNA 3′ UTR *in vitro* (Olivas and Parker, 2000). Puf3 binds nearly exclusively to cytoplasmic mRNAs that encode mitochondrial proteins, such as COX17, with a consensus UGUAAAUA motif in their 3′ UTRs (Gerber *et al.*, 2004). The rapid deadenylation of the COX17 mRNA, enhanced by Puf3, is mediated by the Ccr4p/Pop2p/Not deadenylase complex (Tucker *et al.*, 2002). Importantly, in contrast to the situation in *Drosophila*, *C. elegans* and *Xenopus*, yeast Puf3 does not affect the translation of COX17 mRNA, but rather its stability (Olivas & Parker, 2000). This is presumably due to the difference in fate of deadenylated RNAs in yeast and early embryos. While deadenylated RNAs are rapidly degraded in yeast, this pathway is inactive during early embryogenesis. These results imply that Pumilio-mediated deadenylation in the multicellular eukaryotes is not the consequence of translation initiation inhibition, but rather, its cause.

Histone mRNA Activation by SLBP and by Deadenylation—The Exception to the Rule

While the majority of metazoan mRNAs terminate in a poly(A) tail, replication-dependent histone mRNAs end in a conserved stem-loop structure. The NMR structure of the stem-loop shows that the hairpin adopts a novel UUUC tetraloop, which is closed by a conserved U-A base pair that terminates the canonical A form stem (DeJong *et al.*, 2002; Zanier *et al.*, 2002). The stem-loop binds a class of 45 kDa proteins known as the stem-loop binding proteins (SLBP) or hairpin binding proteins (HBP) which mediate histone RNA 3′-end formation, in concert with U7 snRNP, in the nucleus, and also regulate histone mRNA stability and translation in the cytoplasm (reviewed in Marzluff & Duronio, 2002).

In *Xenopus*, there are two SLBPs:xSLBP1 (Table 1), the homologue of the mammalian SLBP, which is required for processing of histone pre-mRNA and is located both in the nucleus and in the cytoplasm, and xSLBP2, which is expressed only during oogenesis, is confined to the cytoplasm, and is bound to the stored histone mRNA in *Xenopus* oocytes (Wang *et al.*, 1999). The centrally located RNA binding domain (RRM) is highly conserved between xSLBP1 and xSLBP2, but otherwise the two frog SLBPs are not similar in protein sequence, suggesting that they have distinct activities in histone mRNA metabolism (Wang *et al.*, 1999).

In one of its cytoplasmic functions, the stem-loop is required for efficient translation of histone mRNAs and substitutes for the poly(A) tail in promoting translation (reviewed in Marzluff & Duronio, 2002). When capped luciferase mRNAs ending in the stem-loop were injected into *Xenopus* oocytes after over-expression of one or other SLBP protein, only xSLBP1 stimulated

translation, while xSLBP2 reduced translation of the reporter RNA. A small region in the N-terminal portion of xSLBP1 was found to be responsible for the translational stimulation both *in vivo* and *in vitro*. As MS2-tethered SLBP1 fusion protein can activate translation of a reporter mRNA ending in an MS2 binding site, xSLBP1 only needs to be recruited to the 3′-end of the mRNA and does not need to be directly bound to the histone stem-loop to activate translation (Sanchez & Marzluff, 2002). In *Xenopus* oocytes, the translation of histone mRNA is activated during meiotic maturation, to provide the embryo with sufficient histone proteins for incorporation into chromatin during the rapid cleavage stages. During the later stages of oogenesis, the histone mRNAs are bound by the SLBP2 protein and are translationally repressed. At oocyte maturation, xSLBP2 is degraded, thus apparently allowing xSLBP1 to associate with the histone mRNA (Wang *et al.*, 1999), and this switch in the isoform bound to the stem-loop is proposed to lead to translational activation (Figure 6).

The translation of mRNAs ending in the histone stem-loop is also stimulated in *S. cerevisiae* cells expressing mammalian SLBP (Ling *et al.*, 2002). In yeast cells, the translational function of SLBP genetically requires eIF4E, eIF4G,and eIF3, and expressed SLBP co-isolates with initiation factor complexes that bind the 5′-cap in a manner dependent on eIF4G and eIF3. Furthermore, eIF4G co-immunoprecipitates with endogenous SLBP in mammalian cell extracts and recombinant SLBP and eIF4G co-isolate, indicating that SLBP stimulates the translation of histone mRNAs through a functional interaction with both the mRNA stem-loop and the 5′-cap, which is mediated by eIF4G and eIF3 (Ling *et al.*, 2002). The interaction between SLBP and eIF4G, leading to the specific stimulation of translation of histone mRNA, is reminiscent of the approach used by rotaviruses to promote the translation of their mRNAs. As mentioned previously, rotaviruses use the viral protein NSP3 both to stimulate translation of their own RNAs by binding to the 3′-end and interacting with eIF4G (Piron *et al.*, 1998) and also to shut off host cell translation by preventing PABP interacting with eIF4G through binding to the PABP-binding domain of eIF4G (Imataka *et al.*, 1998; Piron *et al.*, 1998). SLBP-mediated stimulation of translation does not interfere with the translation of other cellular mRNAs, however, and so it seems unlikely that SLBP binds eIF4G via the PABP-binding domain.

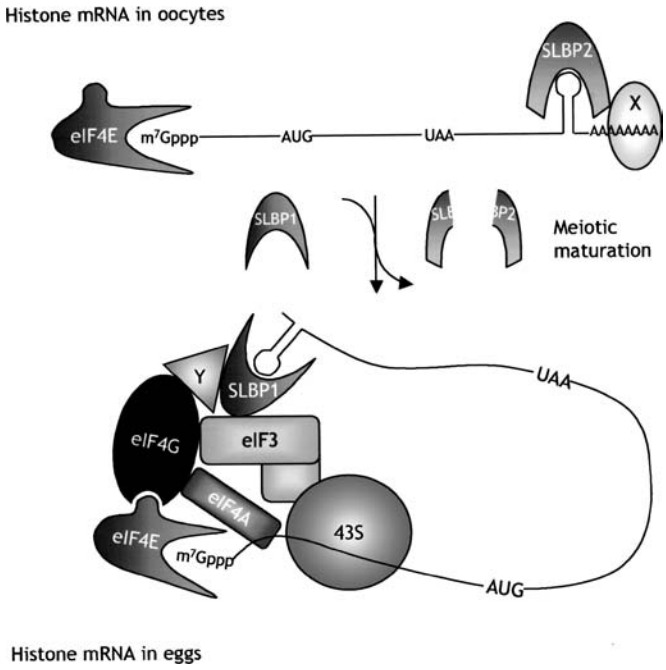


FIGURE 6 **Model showing the translational activation of histone mRNA during meiotic maturation. In *Xenopus*, histone mRNAs are repressed during oogenesis and then translationally activated upon meiotic maturation. The mRNAs are not polyadenylated but possess a conserved stem-loop structure in their 3′ UTRs. Two proteins are found in *Xenopus* that are capable of interacting with the stem-loop, stem-loop binding proteins 1 and 2 (xSLBP1 and xSLBP2). xSLBP1 can stimulate translation while xSLBP2 does not have this effect (Sanchez & Marzluff, 2002). In oocytes, the stem-loop is bound by xSLBP2, preventing the stimulatory xSLBP1 from binding and thus repressing translation (Wang *et al.*, 1999). On meiotic maturation, xSLBP2 is degraded and replaced by SLBP1, activating translation of the histone mRNAs (Wang *et al.*, 1999). SLBP1 interacts with eIF4G and eIF3 (Ling *et al.*, 2002), directly or indirectly (protein Y). The histone mRNAs also possess a short oligo(A) tail in oocytes that has an additional inhibitory effect on translation. The tail is too short to bind PABP but is suggested to interact with as-yet-unknown oocyte-specific factors (X) to prevent xSLBP1 interacting with the histone mRNA (Sanchez & Marzluff, 2002; Sanchez & Marzluff, 2004). The oligo(A) tail is also degraded upon meiotic maturation (Sanchez & Marzluff, 2002; Sanchez & Marzluff, 2004).**

In addition to exchange of the xSLBPs, the histone mRNAs themselves undergo structural changes during development, specifically, the addition of a short poly(A) tail early in oogenesis and its subsequent removal at oocyte maturation. In an unexpected twist to the preconception that deadenylation silences translation, a recent report indicates that, on the contrary, the oligo$(A)_8$ tail (which is too short to bind PABP (Sachs *et al.*, 1987) actively represses histone mRNA translation in oocytes, and that its removal during maturation activates translation (Sanchez & Marzluff, 2004). It is proposed that the oligoadenylated histone mRNA binds oocyte-specific factors that are involved

in preventing xSLBP1 interacting with the stem-loop. Altogether, translational activation requires both degradation of xSLBP2 and removal of the oligo(A) tail (Sanchez & Marzluff, 2002; Sanchez & Marzluff, 2004).

RNA Localization

The second post-transcriptional process that is heavily exploited during development, often in conjunction with translational regulation, is RNA localization. It is possible to envisage various mechanisms by which RNAs could become targeted to a specific region of a cell (reviewed in de Heredia & Jansen, 2004; Johnstone & Lasko, 2001; Palacios & St. Johnston, 2001; Tekotte & Davis, 2002; Zhou and King, 2004). For example, an RNA may form a tight association with a previously localized cell component. Any RNA that diffuses into the vicinity of the localized binding partner would essentially be trapped within this domain. By a progressive process of RNA diffusion and entrapment, RNAs would become concentrated within the desired region. Alternatively, RNAs could be confined to a particular area by ensuring the regulated degradation of non-localized RNAs and concomitant stabilization of localized RNAs. The most direct method of RNA localization would be the active transport of an mRNA to a specific location within the cell by, for example, the movement of motor proteins such as kinesins and dyneins along the microtubule network. Obviously, individual RNAs would need to be recruited to the motor proteins and, again, the interaction of RNA-binding proteins with specific elements in these mRNAs has a part to play. Effective localization may require a combination of these approaches.

RNA localization has been most extensively studied in *Drosophila*. The *bicoid*, *oskar* and *nanos* mRNAs, responsible for the establishment of the anterior-posterior axis, are all synthesized within the nurse cells which surround the oocyte, transported into the oocyte and then localized by different mechanisms (reviewed in Cooperstock & Lipshitz, 2001; Johnstone & Lasko, 2001) (Figure 7). At a specific stage of oogenesis, the

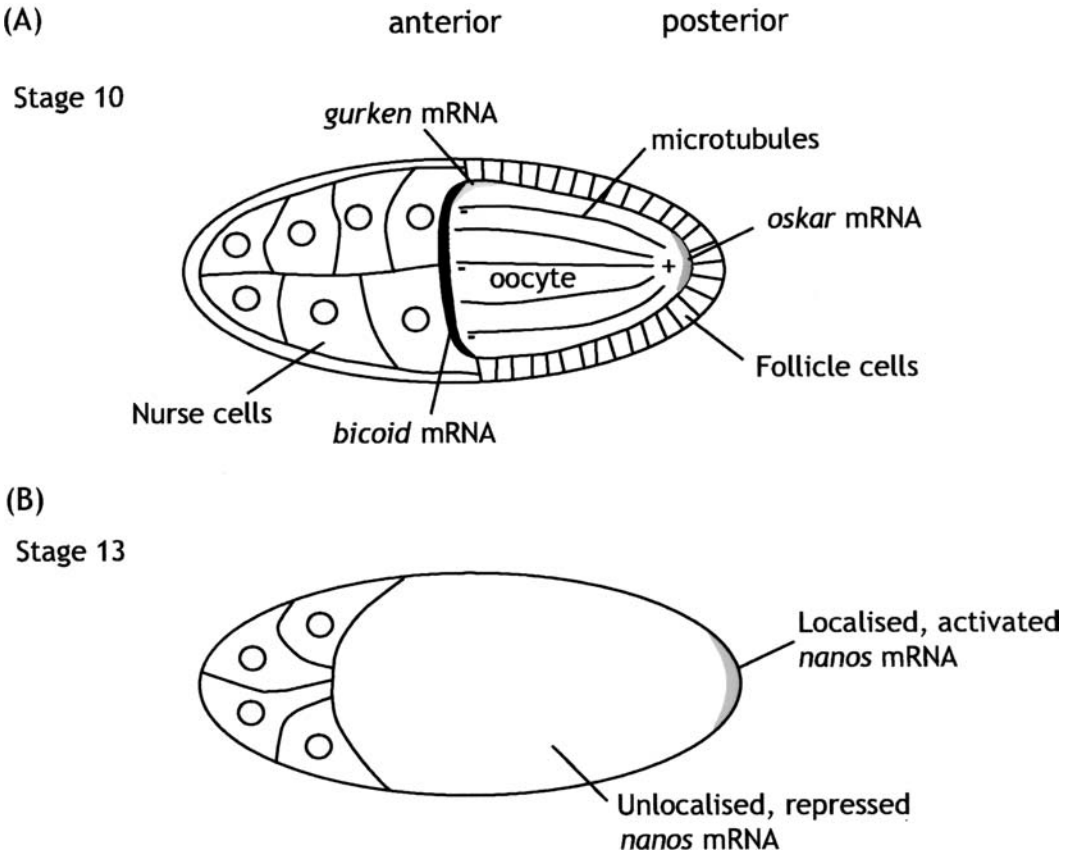


FIGURE 7 **Localization of *Drosophila* mRNAs during oogenesis. (A) In stage 10 oocytes, *oskar* mRNA is localized to the posterior pole, *bicoid* to the anterior pole and *gurken* to the anterior-dorsal corner. Microtubules are oriented with their plus ends toward the posterior, allowing directional transport to occur (Theurkauf *et al.*, 1992). (B) In stage 13 oocytes, a proportion of *nanos* mRNA has become localized to the posterior pole, in addition to the previously localized mRNA. Localised *nanos* mRNA is translationally activated while the majority of *nanos* mRNA, which is not localized, remains repressed (Bergsten and Gavis, 1999).**

microtubules become organised with the plus ends pointing towards the posterior pole (Theurkauf *et al.*, 1992). *bicoid* is localized through an attachment to a dynein protein, which is maintained at the minus ends of the microtubules at the anterior pole (Schnorrer *et al.*, 2000), while the *oskar* mRNP, on the other hand, is bound by Kinesin I and actively transported to the posterior pole (Brendza *et al.*, 2000). The double-stranded RNA binding protein Staufen is involved in the localization of both *bicoid* and *oskar* as will be described shortly. *nanos* becomes localized by virtue of its interaction with Oskar protein, which is only present at the posterior (Ephrussi *et al.*, 1991). As the majority of *nanos* mRNA remains unlocalized (Bergsten and Gavis, 1999), translational repression is particularly pertinent in this case.

RNA localization has also been well studied in *Xenopus laevis*, where a number of RNAs are localized to either the animal or vegetal pole of the oocyte during oogenesis. There are two different pathways that have been described for vegetally localized RNAs. The early, or messenger transport organiser (METRO), pathway localizes germ cell determinants, such as Xcat2 and Xpat RNAs, in stage I oocytes (oogenesis is divided into six stages) to the mitochondrial cloud, a structure which is composed of mitochondria, lipids and other electron-dense materials and which seems to correspond to the polar granule material in *Drosophila* and *C. elegans*. These RNAs are then transported to the vegetal cortex during stage II through their association with the mitochondrial cloud. RNAs that are localized by the late pathway are initially uniformly distributed in stage I oocytes but become confined to a wedge-shaped zone, which also contains endoplasmic reticulum (ER), within the vegetal hemisphere during stage II and then concentrated into a tight sub-cortical layer during stages III and IV (reviewed in King *et al.*, 1999; Palacios & St. Johnston, 2001; Zhou and King, 2004).

Fewer RNAs have been assigned to the late localization pathway. They include VegT (Stennard *et al.*, 1996), Vg1 (Melton, 1987; Weeks & Melton, 1987), the *Xenopus* Bicaudal-C homologue (Wessely and De Robertis, 2000) and Xvelo1 (Claussen & Pieler, 2004). Vg1 is the best studied (Figure 9A). It encodes a member of the transforming growth factor β (TGF-β) superfamily involved in mesoderm induction and the establishment of left/right asymmetry in the developing embryo (Hyatt & Yost, 1998; Weeks & Melton, 1987). Vg1 mRNA translocation to, and anchoring at, the vegetal cortex involves microtubules and microfilaments, respectively (Yisraeli *et al.*, 1990), the actin meshwork serving as a framework for the network of cytokeratin filaments (Alarcon & Elinson, 2001). The *cis*-acting element for localization of Vg1 has been mapped within the 3′ UTR. It comprises a 340 nt element, the Vg1 localization element (VLE) (Mowry & Melton, 1992), which contains short repeated motifs required for localization (Bubunenko *et al.*, 2002; Deshler *et al.*, 1998; Havin *et al.*, 1998; Kwon *et al.*, 2002). Localization of VegT mRNA, which encodes a T-box transcription factor and, like Vg1 mRNA, is implicated in establishing the primary germ layers (Zhang *et al.*, 1998), is mediated by similar motifs within its localization element (Bubunenko *et al.*, 2002; Kwon *et al.*, 2002).

The study of Vg1 mRNA suggests a link between localization and translational control in *Xenopus*, as in *Drosophila*. Vg1 protein cannot be detected until stage IV of oogenesis, after RNA localization has been completed (Dale *et al.*, 1989; Tannahill & Melton, 1989). Indeed, we and others have shown that the Vg1 3′ UTR contains a 250 nt long UA-rich Vg1 translational element (VTE), downstream of the VLE, which mediates the translational repression of unlocalized RNA *in vivo* and *in vitro*, in a cap-dependent but poly(A)-independent manner (Otero *et al.*, 2001; Wilhelm *et al.*, 2000a). The major protein shown to interact with the VTE by UV-crosslinking is ElrB, a member of the *elav* family. Members of this family were initially characterized as being involved in the stabilization of RNAs that possess a destabilizing AU-rich element in their 3′ UTRs. They have more recently been implicated in translational control (Antic *et al.*, 1999; Jain *et al.*, 1997; Kullman *et al.*, 2002). Mutation of a motif in the VTE that matches the consensus binding site described for the human ElrB homologue, HelN-I (Gao *et al.*, 1994) abolishes the ability of the VTE to repress translation and dramatically reduces ElrB binding. As multiple copies of the ElrB binding motif will both repress translation and compete for VTE-dependent repression, ElrB is strongly implicated in the translational control of Vg1 mRNA (Colegrove-Otero *et al.*, 2004).

We will now discuss in more detail some of the proteins involved in RNA localization in *Drosophila* and *Xenopus*.

The Double-Stranded RNA-Binding Protein Staufen

The Role of Staufen in the Localization and Translational Activation of oskar

The localization of both *bicoid* and *oskar* RNAs within the *Drosphila* oocyte requires the action of the double-stranded RNA-binding protein Staufen. During oogenesis,Staufen is required to anchor *bicoid* transcripts to the anterior pole of the oocytes and to localize *oskar* mRNA to the posterior pole (reviewed in Palacios & St. Johnston, 2001). Staufen co-localizes with these mRNAs, and co-localization requires the RNA-binding activity of the protein (Ferrandon *et al.*, 1994; Ramos *et al.*, 2000; St Johnston *et al.*, 1992). Thus, it seems probable that Staufen binds directly to mRNA, and perhaps couples this complex to the translocation machinery. This machinery involves microtubules and the plus end-directed motor protein Kinesin I, to mediate transport of *oskar* mRNA to the posterior pole. Interestingly, both the minus end-directed motor Dynein and Kinesin I cooperate in the transport of *bicoid* mRNA to the anterior pole; both motors are components of the same complex and appear to cooperate in recycling each other to the opposite microtubule pole (Brendza *et al.*, 2000; Duncan & Warrior, 2002; Januschke *et al.*, 2002; Palacios & St. Johnston, 2002). An additional specific component of the *oskar* mRNA localization complex is the hydrophilic protein Barentsz, which is required for the Kinesin-mediated transport step and is recruited to the complex by Cup (van Eeden *et al.*, 2001; Wilhelm *et al.*, 2003).

Staufen has roles in addition to the part it plays in the localization of *oskar* and *bicoid* during oogenesis. As discussed earlier, it is very important that Oskar protein is only produced at the posterior of the oocyte, and the translation of unlocalized *oskar* mRNA is, therefore, repressed through the binding of factors, including Bruno, Cup and Hrp48 (Kim-Ha *et al.*, 1995; Micklem *et al.*, 2000; Nakamura *et al.*, 2004; Yano *et al.*, 2004). This repression must be relieved once *oskar* mRNA has been localized, and Staufen, which remains associated with the RNA at the posterior pole, performs a second function in the activation of *oskar* translation (Micklem *et al.*, 2000). Later, during neurogenesis, Staufen asymmetrically localizes *prospero* mRNA, in an actin-dependent manner, to the apical crescent of dividing neuroblasts (Broadus *et al.*, 1998; Li *et al.*, 1997). Thus, *Drosophila* Staufen is a common component of RNA transport in oocytes and neurons (Roegiers & Jan, 2000), and is also involved in translational regulation. Recent large-scale screens to identify genes involved in the consolidation of long-term memory in *Drosophila* unambiguously identified a requirement for the machinery of mRNA localization and translational regulation, including *staufen*, *pumilio* and *orb/CPEB*. In particular, Staufen is up-regulated in adult flies in memory formation, and *staufen* mutants have defects in long-term memory (Dubnau *et al.*, 2003).

Domain Structure and Function of Staufen Homologs

Drosophila Staufen contains five double-stranded RNA-binding domains (dsRBDs; Table 1). The dsRBDs do not recognize particular nucleotide sequences *in vitro*, allowing non-specific binding to dsRNAs and to ssRNAs with extensive secondary structure, suggesting that target specificity may be governed by interactions with other proteins (Saunders & Barber, 2003; St Johnston *et al.*, 1992). *In vivo*, Staufen protein is specifically recruited to the site of injection of the 3′ UTR of *bicoid* mRNA, but not a control RNA, to form particles that move in a microtubule-dependent manner (Ferrandon *et al.*, 1994). The regions recognized by Staufen in this *in vivo* assay are predicted to form three stem-loop structures involving extensive double-stranded stretches (Ferrandon *et al.*, 1997; Wagner *et al.*, 2001). *Drosophila* dsRBDs 1, 3 and 4 bind dsRNA *in vitro*, but dsRBDs 2 and 5 do not (Micklem *et al.*, 2000; St Johnston *et al.*, 1992). An insert present in *Drosophila* dsRBD2, which separates the $\beta 1$ and $\beta 2$ strands based on the $\alpha\beta\beta\beta\alpha$ dsRBD3 NMR structure (Ramos *et al.*, 2000), destroys its ability to bind dsRNA *in vitro* (Micklem *et al.*, 2000). Indeed, all known Staufen proteins contain an insert at the same point in dsRBD2, albeit of somewhat varying sequence and length, leading to lack of RNA-binding by this domain (Micklem *et al.*, 2000). Full-length Staufen protein lacking the dsRBD2 insertion binds *oskar* mRNA and activates its translation, but fails to localize the RNA to the posterior pole. In contrast, Staufen that is lacking dsRBD5 localizes *oskar* mRNA normally, but does not activate its translation. Thus, dsRBD2 is required for the microtubule-dependent localization of *oskar* 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 (Micklem *et al.*, 2000).

Mammals–including man, mouse, and rat–have two genes for Staufen, both of which undergo alternative splicing to generate a number of isoforms, differing at their N- and C-termini (Duchaine *et al.*, 2002; Marion *et al.*, 1999; Wickham *et al.*, 1999). Staufens 1 and 2 are highly related at the sequence level and contain dsRBD2, 3 and 4, of which dsRBD3 and 4 are the principal RNA-binding domains, and the most highly conserved regions of the protein. Staufens 1 (Stau1) lack dsRBD1, while Staufens 2 (Stau2) appear to have a truncated dsRBD5. Lying between dsRBD4 and dsRBD5 is the so-called tubulin-binding domain (TBD), unique to vertebrate Staufens, and absent in *Drosophila*. The TBD bears partial homology to the tubulin-binding domain of MAP1B, and binds tubulin *in vitro* (Wickham *et al.*, 1999; Zauner *et al.*, 1992).

Stau1 and Stau2 transcripts appear to be fairly ubiquitously expressed, with evidence of enhanced expression of Stau1 in ovary, and Stau2 in brain, in mouse, rat and man (Duchaine *et al.*, 2002; Marion *et al.*, 1999; Saunders *et al.*, 2000; Wickham *et al.*, 1999). In cultured cells, human Stau1 (hStau1) co-localizes with markers of the rough endoplasmic reticulum, but not with endosomes, cytoskeleton, or Golgi apparatus (Marion *et al.*, 1999; Wickham *et al.*, 1999). Furthermore, sedimentation analyses indicate that hStau1 associates with ribosomes, via protein-protein interactions mediated by the dsRBD4 and TBD domains, in conjunction with the RNA-binding activity of dsRBD3 (Luo *et al.*, 2002), consistent with a possible role in translation.

In neurons, Stau1 is found in the soma and dendrites, and is absent from axons. Mammalian Stau1 localizes to the somatodendritic domain of cultured hippocampal neurons, where it concentrates in the vicinity of the ER and microtubules (Kiebler *et al.*, 1999; Kohrmann *et al.*, 1999; Krichevsky & Kosik, 2001; Monshausen *et al.*, 2001). Indeed, hStau1-GFP granules move bidirectionally along the dendritic shaft in a microtubule-dependent manner (Kohrmann *et al.*, 1999). Stau2 also localizes to the somatodendritic domain of neurons, but, importantly, Stau1 and 2 particles do not co-localize in distal dendrites (Duchaine *et al.*, 2002). A dominant negative form of Stau2 reduced, while overexpression of the wild-type protein increased, dendritic mRNA content in cultured hippocampal neurons, suggesting that Stau2 plays an important role in the transport of RNA to dendrites (Tang *et al.*, 2001). RNAs are transported in motile granules, which are highly enriched in Staufen and contain ribosomes (Krichevsky & Kosik, 2001). Biochemical characterization of Staufen-containing complexes from rat and mouse brain documented their association with ribosomes and ER, and also identified Kinesin as a component (Mallardo *et al.*, 2003; Ohashi *et al.*, 2002). Barentsz interacts with Stau1 in an RNA-dependent manner; possible candidates being the dendritically localized brain cytoplasmic 1 (BC1) transcripts (Macchi *et al.*, 2003; Mallardo *et al.*, 2003). Moreover, Barentsz and Stau1 colocalise within particles in the cell body and, to a more variable extent, in dendrites of mature hippocampal neurons, suggesting that the mRNA transport machinery is conserved during evolution (Macchi *et al.*, 2003).

Little was known until recently of the role of Staufen proteins in early vertebrate development, when regulation at the level of RNA localization and translation is also critical. Two reports indicate that the *Xenopus laevis* genome encodes homologs to the mammalian Staufen genes, XStau1 and XStau2 (Allison *et al.*, 2004; Yoon & Mowry, 2004). *Xenopus* Staufen 1 and 2 proteins are highly homologous to *Drosophila* Staufen, and to their mouse, human, and rat counterparts (Duchaine *et al.*, 2002; Kiebler *et al.*, 1999; Monshausen *et al.*, 2001). Oocytes contain one major form of XStau1, of 79 kDa, most similar to the mammalian Stau1^{63} alternatively spliced isoform, and three, less abundant, maternal XStau2 proteins of around 80 kDa (Allison *et al.*, 2004; Yoon & Mowry, 2004). XStau1 is the principal Staufen protein in oocytes, eggs and embryos, (Allison *et al.*, 2004). Consistent with a role in RNA transport, the two *Xenopus* Staufen proteins are found in the vegetal cytoplasm, a region to which important mRNAs including Vg1 and VegT mRNAs become localized, and XStau1 interacts with Vg1 and Veg T mRNAs, but not with non-vegetally-localized mRNAs (Allison *et al.*, 2004; Yoon & Mowry, 2004). XStau1 was found to be concentrated in the vegetal cortical region from stage II onwards. It shows partial co-distribution with endoplasmic reticulum (ER), raising the possibility that Staufen may anchor mRNAs to specific ER-rich domains. The imprecise co-localization of XStau1 and ER suggests that their association is indirect, and may depend on the presence of other structural components. XStau2 in oocytes appears to be distributed more uniformly than XStau1, suggesting that XStau2 complexes are essentially cytoplasmic (Allison *et al.*, 2004; Yoon & Mowry, 2004).

Significantly, XStau was shown to interact with Kinesin I, and to function in vegetal RNA localization, as shown by over-expression of a dominant negative form containing dsRBD2-4 (Yoon & Mowry, 2004). Previously, Kinesin II was implicated in Vg1 RNA transport (Betley *et al.*, 2004). Moreover, XStau proteins are phosphorylated by the MAPK pathway early during meiotic maturation (Allison *et al.*, 2004), a period during which RNAs such as Vg1 RNA are released from their tight localization at the vegetal cortex (Forristall *et al.*, 1995; Klymkowsky *et al.*, 1991). Together these findings support the previously unrecognized potential for Staufen to be involved in targeting and/or anchoring of maternal determinants to the vegetal cortex of the oocyte in *Xenopus* (Allison *et al.*, 2004; Yoon & Mowry, 2004), though the precise roles of the two Staufens remain to be determined.

Role of the hnRNP Hrp48 in Localization and Translational Control

Staufen is just one of several proteins which have been implicated as playing a role in the localization of *oskar* mRNA. Recent studies have provided a direct link between the localization of this mRNA and its translational repression. As described earlier, the involvement of the translational repressor Bruno in the control of *oskar* mRNA has been well characterized and is now known to act through Cup. It had been previously shown, however, that an unidentified protein p50 also crosslinks to both the translational repressor region in the 3′ UTR of *oskar* and a derepression region in the 5′ UTR (Gunkel *et al.*, 1998). Mutations that disrupted the binding of p50, but not Bruno, resulted in a loss of translational repression, implying that this protein was also involved in the translational control.

Recently, p50 was identified as Hrp48, a member of the hnRNP A/B family of RNA-binding proteins (Huynh *et al.*, 2004; Yano *et al.*, 2004). Members of this family are characterized by two N-terminal RRMs followed by a C-terminal Glycine-rich domain (GRD) (Matunis *et al.*, 1992, Table 1). Yano *et al.* found, in three *hrp48* mutants in which the levels of Hrp48 protein were markedly reduced, that *oskar* mRNA was not localized to the posterior pole (Yano *et al.*, 2004). Staufen protein was also mislocalized. In addition, unlocalized *oskar* mRNA was no longer translationally repressed. The work of Huynh *et al.* confirmed the role of Hrp48 in *oskar* localization, utilising mutants that apparently uncoupled the two activities (Huynh *et al.*, 2004). The positioning of the latter point mutations within Hrp48 may yield some clues as to the involvement of the protein in localization. Two of the mutations are located in the C-terminal GRD, which is thought to function as an oligomerization domain (Cartegni *et al.*, 1996). Huynh *et al.* suggest that these mutations may prevent localization by disrupting the formation of higher-order *oskar* RNP complexes required for transport. Oligomerization of Hrp48 may also play a part in the translational control since deletion of the Hrp48 binding site in the 5′ UTR of *oskar* mRNA results in a relief of translational repression. Binding of Hrp48 to both 5′ and 3′ sequences (Gunkel *et al.*, 1998), followed by self-association, may promote circularization of the RNA (Yano *et al.*, 2004), in turn facilitating repression by Cup, recruited to the 3′ UTR by Bruno (Nakamura *et al.*, 2004) (Figure 8).

Goodrich *et al.* have further extended our knowledge of the involvement of Hrp48 in localization and translational control by showing that it also regulates *gurken* mRNA (Goodrich *et al.*, 2004). The localization of *gurken* RNA to the dorsal-anterior region of the oocyte (Figure 7A) is required for the establishment of the dorso-ventral axis (Neuman-Silberberg & Schupbach, 1993), and, as might be expected, unlocalized RNA is translationally repressed. The hnRNP Squid had previously been shown to play a part in both processes (Norvell *et al.*, 1999). A two-hybrid assay to identify Squid-interacting proteins showed an interaction between Squid and Hrp48 (Goodrich *et al.*, 2004). Examination of *hrp48* mutants showed defects in *gurken* mRNA localization that corresponded to dorsalized phenotypes. Antibody staining of these mutants revealed that mislocalized *gurken* mRNA is translated in the absence of Hrp48. In the case of *gurken*, Squid and Hrp48 appear to cooperate to promote localization and repress translation.

Therefore, the studies of these proteins show that the two processes of RNA localization and translational control, which often functionally complement each other, can undergo coordinate control by a single protein.

The Xenopus Protein Vg1RBP/Vera

Homologues, Features and Expression

Interest in RNA-binding proteins that mediate localization in *Xenopus* oocytes was initiated about a decade ago, with the analysis by band shift assay of complexes formed between the 340 nt long Vg1 localization

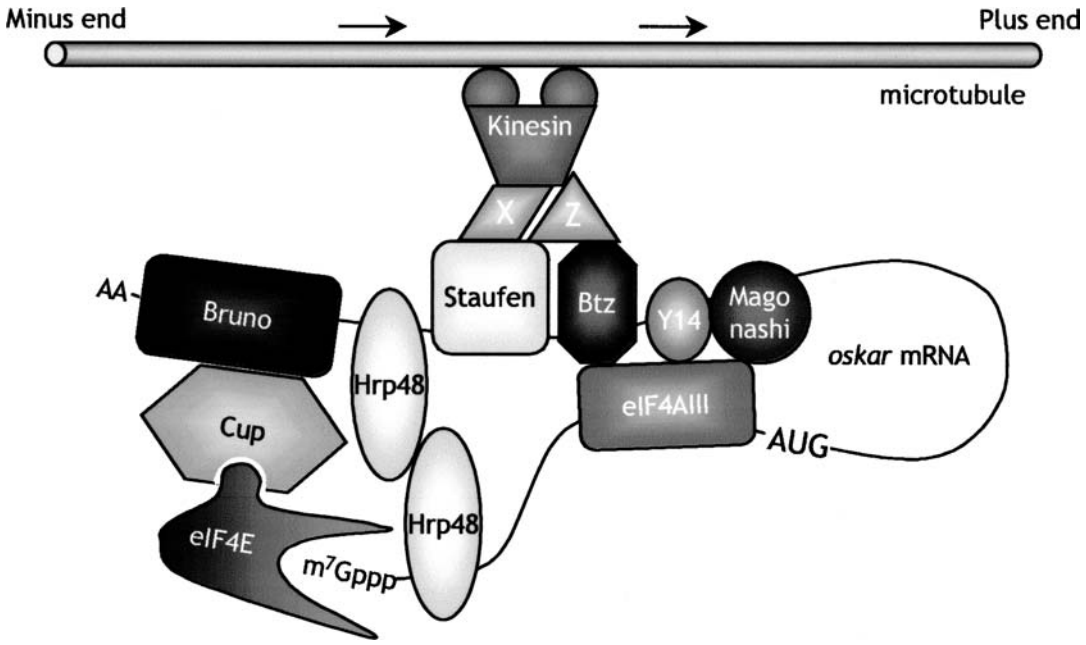


FIGURE 8 **Model showing components involved in the translational repression and localization of *Drosophila oskar* mRNA during oogenesis. As shown in Figure 2, the translational repressor Cup interacts directly with Bruno, bound to the *oskar* 3′ UTR, and with eIF4E (Nakamura *et al*., 2004; Wilhelm *et al*., 2003). Hrp48, which has specific binding sites in both the 5′ and 3′ UTRs, has been implicated in both repression and localization (Gunkel *et al*., 1998). It is suggested that oligomerization of Hrp48, together with its binding to both ends of the RNA, may assist circularization of the mRNA, facilitating repression by Cup (Huynh *et al*., 2004; Yano *et al*., 2004). Oligomerization may also play a part in the correct formation of higher-order complexes required for transport. Hrp48 has not yet been shown to interact with any of the other proteins involved in the complex. Barentsz is recruited to the complex by Cup and is required for the kinesin-mediated transport step (van Eeden *et al*., 2001; Wilhelm *et al*., 2003). However, no direct interaction has been shown between Barentsz and Cup, the kinesin heavy chain or Staufen, although Barenstz co-immunoprecipitates with Staufen in an RNAse-sensitive manner (Macchi *et al*., 2003). Barentsz does interact with directly with eIF4AIII, which in turn interacts with the Mago-Y14 dimer (Palacios *et al*., 2004). The double-stranded RNA binding protein Staufen moves with *oskar* mRNA to the posterior pole (St Johnston *et al*., 1992) and is suggested to couple the complex to the translocation machinery. However, although Kinesin I has been shown to be required for *oskar* localization (Brendza *et al*., 2000; Palacios & St. Johnston, 2002), no direct interaction has been observed between Kinesin I and any of the proteins known to be present in the complex. Therefore, there may still be additional factors required which have not yet been identified.**

element (VLE) and proteins present in oocyte lysates and the characterization of *trans*-acting factors by UV-crosslinking. Six proteins (called p78, p69, p60, p40, p36, and p33, after their size in kDa) crosslinked to VLE RNA, and not to control sequences (Mowry, 1996; Schwartz *et al.*, 1992). p69, subsequently identified as Vg1RBP/Vera (Deshler *et al.*, 1998; Havin *et al.*, 1998), and p60, hnRNP I/PTB (Cote *et al.*, 1999), have been the most investigated proteins, with documented roles in RNA localization. p78 can most probably be ascribed to VgRBP71, a homologue of ZBP-2 (Kroll *et al.*, 2002), and p40 to Prrp, a proline-rich hnRNP protein that interacts with vegetally localized RNAs and with profilin, an actin-associated protein that appears to regulate microfilament assembly (Zhao *et al.*, 2001).

Early biochemical studies pointed to interesting features of Vg1RBP/Vera, supporting its involvement in RNA localization. Vg1RBP/Vera could mediate the association of Vg1 RNA with microtubules *in vitro* and co-fractionated with endoplasmic reticulum membranes in concentrated lysates, hinting at a role in associating RNAs with a distinctive subcompartment of the ER in the wedge-shaped region in mid-oogenesis, and their transport to the cortex along microtubules (Deshler *et al.*, 1997; Elisha *et al.*, 1995).

p69, independently cloned as Vg1RBP (Havin *et al.*, 1998) and Vera (VLE and ER association) (Deshler *et al.*, 1998), turned out to be a member of a highly-conserved family of RNA-binding proteins whose founder member is the chicken zipcode-binding protein (ZBP1), implicated in localising β-actin mRNA via its short 3′ UTR zipcode to the leading edge of fibroblasts (Ross *et al.*, 1997). The proposed participation of these proteins in targeting different mRNAs in oocytes, fibroblasts, and subsequently neurons (Zhang *et al.*, 2001) suggested that Vg1RBP is a common component of diverse localization pathways. Other members of the ZBP1 (or VICKZ, reviewed in (Yaniv & Yisraeli, 2002)) family include the mouse c-*myc* coding region determinant-binding protein (CRD-BP) implicated in regulating RNA stability

(Doyle *et al.*, 1998), and three human insulin-like growth factor II mRNA-binding proteins (IMP1-3) whose binding to the 5′-leader of IGF-II mRNA represses translation in late development (Nielsen *et al.*, 1999). IMP1 (CRD-BP) is essential for normal growth and development; *Imp1*(−/−) mice are significantly smaller than average, show impaired gut development and high perinatal mortality (Hansen *et al.*, 2004). The pattern of ZBP1 family expression in flies, frogs, zebrafish, and mice is biphasic and includes a maternal component followed by zygotic expression in the early developing nervous system (Hansen *et al.*, 2004; Müeller-Pillasch *et al.*, 1999; Nielsen *et al.*, 2000; Zhang *et al.*, 1999a). So far, significant levels of ZBP1 proteins have not been detected in adult tissues, but are frequently found in transformed cells, and hence they are known as oncofetal proteins (reviewed in Yaniv & Yisraeli, 2002). Indeed, targeted expression of CRD-BP in mammary epithelial cells in transgenic mice results in them developing mammary adenocarcinomas that are capable of metastasizing (Tessier *et al.*, 2004).

Vertebrate ZBP1 proteins (Table 1) contain the novel combination of two tandem RRM domains followed by four KH domains, whereas the *Drosophila* homologue lacks the two N-terminal RRMs (Git & Standart, 2002; Nielsen *et al.*, 2000). The KH domains mediate RNA-binding (Farina *et al.*, 2003; Git & Standart, 2002; Nielsen *et al.*, 2002), and self-association (Git & Standart, 2002; Nielsen *et al.*, 2004); the function of the RRMs is not yet clear (Figure 9B). The four KH domains are organized as two pairs of didomains, with short and conserved linkers separating KH1 from KH2 and KH3 from KH4, while longer and more variable loops separate the RRMs from KH1, and KH2 from KH3. The two pairs of didomains are not equivalent. KH3+4 mediate protein self-association that results in sequential, cooperative binding on RNA (Git & Standart, 2002; Nielsen *et al.*, 2004). Different combinations of these didomains are used to bind RNA in different homologues, perhaps reflecting their varied target RNAs. For example, KH3+4 in chicken ZBP1 exhibit a preference for RCACCC (where R is a purine) in Selex experiments and in the physiological ligand, β-actin mRNA (Farina *et al.*, 2003), while KH1+2 bind this zipcode with ~100-fold less affinity. In contrast, all four KH domains are required to promote high affinity binding of H19 RNA by IMP1 (Nielsen *et al.*, 2002), and of Vg1 VLE by Vg1RBP (Git & Standart, 2002). Several repeated UUCAC motifs, called E2 elements, which are interspersed in the long localization elements of Vg1 and the similarly-regulated VegT mRNA, bind Vg1RBP (Bubunenko *et al.*, 2002; Deshler *et al.*, 1998; Kwon *et al.*, 2002; Lewis *et al.*, 2004), possibly explaining the involvement of all four KH domains. The four KH domains in IMP1, and the *Drosophila* homolog, have also been shown to mediate interactions with microtubule-associated protein (MAP) preparations and MAP-enriched tubulin (Nielsen *et al.*, 2002).

The Role of Vg1RBP/Vera in RNA Localization

Vg1RBP is distributed throughout the oocyte in early stages, and a low but significant amount can even be detected in the germinal vesicle nucleus (Zhang *et al.*, 1999a). The presence of Vg1RBP in the nucleus, initially puzzling, is now clearer. Isolation of the B3 transcription factor of the *Xenopus* TFIIIA gene, by DNA affinity chromatography, revealed B3 to be identical to Vg1RBP (Griffin *et al.*, 2003); thus extending the functions of this protein to DNA-, as well as RNA-, binding. Moreover, IMP1 can translocate into the nuclei of fibroblasts, although there is no simple import signal, and be exported into the cytoplasm, via conserved NES signals present in the KH2 and KH4 domains, indicating that this class of proteins is capable of shuttling (Nielsen *et al.*, 2003). As oogenesis progresses, nuclear staining disappears and Vg1RBP accumulates first in a wedge-shaped region and, later, along the vegetal cortex, mirroring the pattern of Vg1 mRNA localization (Chang *et al.*, 2004; Zhang *et al.*, 1999a). Analysis of sequences within the VLE that are required to support RNA localization on the one hand, and Vg1RBP binding on the other, showed a very good correlation, implying the involvement of Vg1RBP in targeting RNA to the vegetal cortex (Deshler *et al.*, 1998; Havin *et al.*, 1998). However these early studies, somewhat curiously, pointed to two different sets of repeat elements: three interspersed UUUCUA elements (also called VM1) (Gautreau *et al.*, 1997; Havin *et al.*, 1998) and five interspersed UUCAC elements (called E2 (Deshler *et al.*, 1998; Deshler *et al.*, 1997; Kwon *et al.*, 2002)). The importance of CAC-rich elements, such as the E2 motifs, in vegetal RNA localization, not only in *Xenopus* oocytes but in chordates in general, was independently verified in a computational screen (Betley *et al.*, 2002). More recent re-evaluation of the roles of the E2 and VM1 repeats proposes that it is the clustering of several copies of each element that is critical for RNA localization (Bubunenko *et al.*, 2002; Lewis

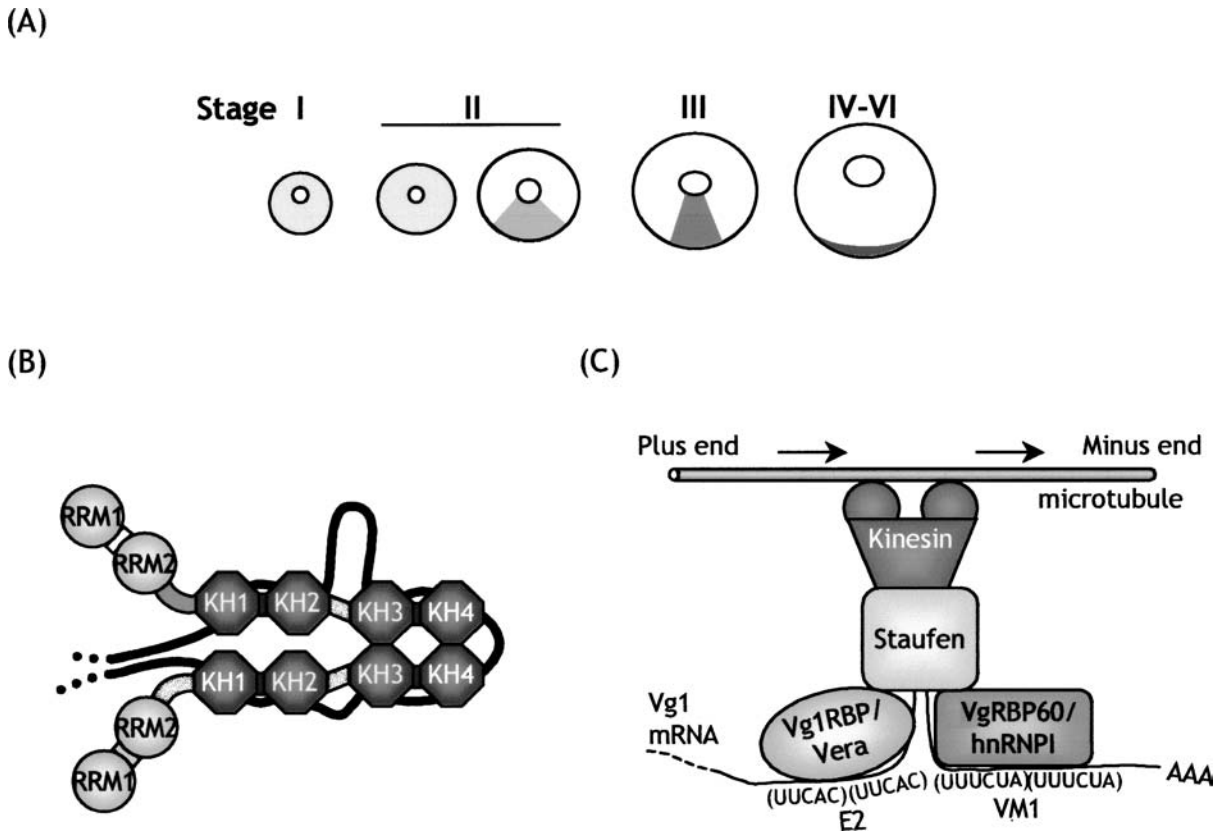


FIGURE 9 **(*A*) Late pathway of RNA localization in *Xenopus* oocytes. Vg1, VegT and Xvelo1 are among are RNAs localized by this pathway to the vegetal cortex of *Xenopus* oocytes. The RNAs are initially distributed throughout the cytoplasm, they become confined to a wedge-shaped region of the vegetal hemisphere during stage II of oogenesis and then become further restricted to the vegetal cortex during stage III and early stage IV. (*B*) Model of the RNA binding and self-association of Vg1RBP/Vera. Vg1RBP/Vera possesses two N-terminal RRM domains and four KH domains arranged as two didomains. All four KH domains are involved in RNA binding while KH3+4 allow self-association (Git & Standart, 2002). This dimerization results in sequential cooperative binding on the RNA (Git & Standart, 2002; Nielsen *et al.*, 2004). A function has not yet been shown for the RRMs. (*C*) Model showing some of the components involved in Vg1 mRNA localization. The localization process requires microtubules (Yisraeli *et al.*, 1990). Analysis of the Vg1 localization element (VLE) has shown that the clustering of short repeat elements is required for localization (Lewis *et al.*, 2004). The E2 repeats, with the sequence UUCAC, bind Vg1RBP/Vera while the VM1 repeats (UUUCUA) interact with VgRBP60/hnRNPI (Bubunenko *et al.*, 2002; Lewis *et al.*, 2004). Clusters of only one type of element cannot support localization suggesting that both proteins are required for localization (Lewis *et al.*, 2004). Vg1RBP/Vera and VgRBP60/hnRNPI are present in a complex with the double-stranded binding protein Staufen (Kress *et al.*, 2004) and Staufen co-immunoprecipitates with the motor protein kinesin I (Yoon & Mowry, 2004). However, it is not clear as yet whether these proteins are interacting directly or are simply present on the same mRNA molecule. The situation is also complicated by the observation that the majority of microtubules in oocytes are orientated with their minus ends toward the cortex (Pfeiffer and Gard, 1999). Kinesins are normally plus end-directed but examples of minus-end directed kinesins do exist (Woehlke & Schliwa, 2000).**

et al., 2004). In line with their proposed dual role, both E2 and VM1 elements, and their relative positions, are conserved between Vg1 mRNA from *X. laevis* and the pseudotetraploid *X. borealis* (Lewis *et al.*, 2004), as well as in the more distantly related diploid *X. tropicalis* (unpublished). UUCAC elements provide the binding sites for Vg1RBP, while UUUCUA elements interact with VgRBP60 (Bubunenko *et al.*, 2002; Lewis *et al.*, 2004). Clusters of only one type of element are not sufficient to promote localization (Lewis *et al.*, 2004), suggesting the requirement for both Vg1RBP and VgRBP60 in the localization process. VgRBP60 was identified as hnRNP I (Table 1), or polypyrimidine-tract binding protein (PTB) (Cote *et al.*, 1999), a protein previously associated with the control of alternative splicing (reviewed in Valcarcel and Gebauer, 1997), and picornaviral internal initiation of translation (Kaminski & Jackson, 1998). In stage III *Xenopus* oocytes, although most of VgRBP60/hnRNP I is nuclear, the cytoplasmic fraction co-localizes with Vg1 mRNA in the vegetal cortex (Cote *et al.*, 1999). VM1 and E2 elements, and binding by Vg1RBP and hnRNP I, are required not only to localize Vg1 mRNA, but also VegT mRNA (Bubunenko *et al.*, 2002; Kwon *et al.*, 2002). The 300 nt long localization element of VegT mRNA contains a cluster of redundant elements, although their precise number and relative arrangement

differs to that found in the Vg1 VLE (Bubunenko *et al.*, 2002). It seems, therefore, that rather than sharing a common primary sequence or secondary structure, the localization signals of these mRNAs provide a critical grouping of neighboring elements that bind a common set of *trans*-acting factors. Importantly, injection of antibodies raised against Vg1RBP into oocytes reduces the ability of Vg1 and VegT RNA to localize (Kwon *et al.*, 2002); thus confirming and extending the mutagenesis and RNA-binding experiments, and demonstrating an involvement for Vg1RBP in this process. Vg1RBP and Vg1RBP60/hnRNP I may function by forming bridging links between the 3′ UTR E2/VM1 elements of localized mRNAs, Staufen and Kinesin motor proteins (Betley *et al.*, 2004; Yoon & Mowry, 2004) (Figure 9C). Co-immunoprecipitation experiments reveal contacts between Vg1RBP and Vg1RBP60/hnRNP I and Staufen, and between Staufen and Kinesin I, although it is not yet known whether these are direct (Kress *et al.*, 2004; Yoon & Mowry, 2004). Additional c*is*-elements may also promote vegetal RNA transport (Claussen & Pieler, 2004).

In addition to its role in RNA localization in oocytes, Vg1RBP is required for cell migration during early neural development (Yaniv *et al.*, 2003). Reduction of Vg1RBP levels, achieved using antisense morpholino oligonucleotides, severely diminished neural crest migration. The effect was shown to be specific, as expression of an mRNA encoding Vg1RBP lacking the regions of complementarity with the morpholino oligonucleotide rescued this defect. The cells forming the roof plate of the neural tube were properly determined, but immobilized at their site of origin (Yaniv *et al.*, 2003). In migrating neural crest cells, Vg1RBP is found enriched in the extended processes, suggesting that it may sort particular mRNAs to the leading edge of cells (Yaniv *et al.*, 2003). This is a particularly attractive model in view of the properties of ZBP1, in mediating β-actin mRNA localization in fibroblasts (Farina *et al.*, 2003; Ross *et al.*, 1997), growth cones (Zhang *et al.*, 2001), and dendrites and spines of hippocampal neurons (Eom *et al.*, 2003; Tiruchinapalli *et al.*, 2003). Chicken embryo fibroblasts localize β-actin mRNA to their lamellae, resulting in high local concentrations of actin monomers, which are hypothesized to regulate actin polymerisation dynamics at the leading edge of the cell. Antisense oligonucleotides targeted to the 3′ UTR 54 nt long zipcode or over-expression of the four KH domains of ZBP-1 delocalise β-actin mRNA and impair cell polarity and motility (Farina *et al.*, 2003; Kislauskis *et al.*, 1997). The RNA-binding KH domains form granules that associate with the actin cytoskeleton, while the RRMs are required to localize these granules to the periphery of the fibroblasts (Farina *et al.*, 2003). Formation of an RNP complex between the zipcode sequence and ZBP1 was similarly required for β-actin mRNA localization to, and motility of, growth cones (Zhang *et al.*, 2001), as well as for localization to dendrites of cultured neurons, thereby facilitating filopodial growth (Eom *et al.*, 2003). In NIH-3T3 fibroblasts, IMP1, fused to GFP, is also found in large granules, some of which accumulate in lamellipodia near the leading edge and around the nucleus (Nielsen *et al.*, 2002).

A detailed understanding of the role of ZBP proteins in RNA transport will require knowledge of a more complete set of RNA targets, leading to better understanding of their binding sites as well as their protein ligands, and may need to take into account the possibility of additional functions in translation and RNA stability. It would also, no doubt, be very informative to exploit the power of a genetic approach in examining the role of the *Drosophila* homolog (Nielsen *et al.*, 2000).

Involvement of the VLE-Binding Protein VgRBP71 (ZBP2, MARTA1) in RNA Localization

The *Xenopus* Vg1 VLE also binds VgRBP71 (Table 1), a homologue of a human transcription factor FBP2 (FUSE-binding protein), which was also independently isolated as the splicing regulatory factor KSRP (Kroll *et al.*, 2002). Consistent with these functions, the *Xenopus* protein localizes to both the germinal vesicle and the cytoplasm. VgRBP71 also contains 4 KH domains, but does not otherwise resemble Vg1RBP/Vera. Its precise role in localization is not yet clear. Since it is found associated with both RNAs localized to the vegetal pole and to the animal pole, and the cytoplasmic form is found enriched in the cortical region, it may perform a common anchoring function, possibly in association with Prrp, with which it interacts (Kroll *et al.*, 2002). It has also been proposed to have a function in translational activation of Vg1 mRNA in mid-oogenesis, by stimulating a nuclease that cleaves the 3′ UTR between the VLE and VTE regions to provide a new polyadenylation site (Kolev & Huber, 2003), and to remove the translational repressor element (Kolev & Huber, 2003; Otero *et al.*, 2001; Wilhelm *et al.*, 2000a). Whether such a scission of cortical Vg1 mRNA occurs *in vivo* remains to

be determined; hitherto, Northern blot analysis of total Vg1 mRNA isolated throughout oogenesis has not revealed any significant levels of such a truncated mRNA (for example, (Melton, 1987).

Interestingly, the β-actin mRNA zipcode interacts with the homologues of VgRBP71, called ZBP2, in chicken and rat (Gu *et al.*, 2002; Snee *et al.*, 2002). This protein also shuttles between the nucleus and the cytoplasm (Gu *et al.*, 2002). Expression of a truncated ZBP2 inhibits the localization of β-actin mRNA in both fibroblasts and neurons, suggesting that ZBP2, although predominantly a nuclear protein, has a role in cytoplasmic RNA localization (Gu *et al.*, 2002). The same protein has also been implicated in dendritic sorting of microtubule-associated protein 2 (MAP2) mRNA, which is mediated by the 640 nt 3′ UTR dendritic targeting element (DTE). Of the two major DTE *trans*-acting factors, MARTA1 and MARTA2, MARTA1 has been identified as a ZBP2 homologue. Again, MARTA1 is primarily intranuclear in rat brain neurones and cultured neurones derived from superior cervical ganglia, but it is also present in the somatodendritic cytoplasm and, thus, MARTA1 may play a role in nucleocytoplasmic mRNA targeting (Rehbein *et al.*, 2002).

NUCLEAR INFLUENCES ON CYTOPLASMIC CONTROL

A wealth of data, discussed earlier, illustrates that maternal mRNAs that are synthesised *in vitro* and microinjected into (usually *Xenopus* but also *Drosophila*) oocyte cytoplasm are subject to the appropriate stage-specific regulation, at the level of translation and localization, when the oocytes are subsequently matured and the eggs fertilized. Moreover, these properties of stage-specific regulation can be transferred to reporter mRNAs through appending the regulatory (almost always 3′ UTR) element for translation assays, or simply examined by analysing the localization of the 3′-UTR RNA. It has also become increasingly clear that the translation and localization of mRNAs is influenced by nuclear events, including transcription, splicing, and association with shuttling proteins (reviewed in Dreyfuss *et al.*, 2002; Farina and Singer, 2002; Le Hir *et al.*, 2003); leading to the conclusion that the study of RNA that has not had the nuclear experience may not entirely reflect its entire control in the cell.

Influences on Translational Control

In pioneering studies in the *Xenopus* system, transcription in the nucleus, or injection of *in vitro* transcribed RNA into the nucleus, resulted in inefficiently translated RNA compared to RNA injected directly into the cytoplasm, in the absence of defects in RNA export or changes in RNA levels. Packaging of newly synthesized transcripts by the relatively non-sequence-specific RNA-binding protein FRGY2 was also found to be important for the repressed or masked state (Bouvet *et al.*, 1994; Braddock *et al.*, 1994). Injection of anti-FRGY2 antibodies into the nucleus relieved repression, while over-expression of FRGY2 or injection of FRGY2-precoated mRNA enhanced the repressed state (Bouvet *et al.*, 1994; Braddock *et al.*, 1994).

FRGY2, a doublet of so-called mRNP3 and 4 proteins of about 55 kDa, is a member of the Y-box family. Eukaryotic Y-box proteins, so named due to their ability to bind to Y-box promoters carrying an inverted CCAAT box, interact with both DNA and RNA to control transcription and translation. All contain a cold-shock domain (CSD), also found in prokaryotic cold-shock proteins that function as RNA chaperones to destabilize RNA secondary structure and control translation at low temperatures. Vertebrate homologs also contain a variable N-terminal domain rich in alanine and proline, which may serve as a *trans*-activation domain, and a C-terminal tail domain. The latter consists of alternate regions of predominantly basic or acidic amino acids, each of which are about 30 amino acids in length, called a B/A repeat, which has a strong affinity for single-stranded nucleic acids, and may also function as a charged zipper and facilitate multimerization (reviewed by (Kohno *et al.*, 2003; Matsumoto & Wolffe, 1998; Sommerville, 1999) (Table 1). The CSD, one of the most evolutionarily conserved nucleic acid-binding domains yet identified, comprises a five-stranded β-barrel that includes the two RNA-binding sequence motifs RNP1 and RNP2 also found in RRMs (Manival *et al.*, 2001). RNA-binding by FRGY2 is mediated by both the CSD and the B/A repeat domains, with evidence, from Selex experiments, of a sequence preference by the CSD for AACAUC (Bouvet *et al.*, 1995).

These largely cytoplasmic and highly abundant RNA-binding proteins are the major components of translationally inactive mRNP in *Xenopus* oocytes. Consistent with a role in translational repression, the abundance of FRGY2 decreases during embryogenesis and

it has disappeared completely by the time maternal mRNA stores are depleted (Matsumoto *et al.*, 1998; Sommerville, 1999).

The role of FRGY2 in translational repression can be extended to its homologues. MSY4, a mouse testis homologue of FRGY2, represses translation of several mRNAs in spermatids, thereby inhibiting their differentiation (Giorgini *et al.*, 2002). In somatic cells, the major protein of mRNP is the highly related protein p50 or YB-1 (Evdokimova *et al.*, 1995). Recent investigations indicate that, coincident with translational repression at the level of initiation, p50 stabilizes mRNA, in a non-sequence-specific, but 5′-cap-dependent, manner (Evdokimova *et al.*, 2001; Nekrasov *et al.*, 2003). Inhibitors of translation, such as cap analogs or 4E-BP1, that interfere with the assembly or function of the eIF4E cap-binding complex, promote p50 interactions with the 5′-cap, and prevent RNA degradation. Thus, p50 can stabilize capped RNA in situations where the association of eIF4E with the cap is impaired. The Y-box proteins are the prototypes of RNA-binding proteins that associate with nascent mRNA in the nucleus in a non-sequence-specific manner, and are transported into the cytoplasm in the form of untranslated mRNP. Subsets of these mRNP would be translationally awakened at appropriate times by the action of sequence-specific binding regulators.

The translational fate of mRNA is also responsive to its prior splicing history. In the *Xenopus* injection experiments, the addition of a spliceable intron into the plasmid construct relieved repression (Braddock *et al.*, 1994; Matsumoto *et al.*, 1998). Moreover, the effect of splicing appears to be dependent on intron identity and location. While an intron inserted at the 5′-end of the transcript relieved repression, and an intron at the 3′-end further repressed translation in the study of Matsumoto *et al.* (1998), a different 3′-intron activated translation in the Braddock *et al.* (1994) investigation. It remains to be determined how intron identity and location influences translation.

In mammalian cells, as well as in *Xenopus* oocytes, it has been reported that splicing enhances translation as a result of the deposition of exon junction complexes (EJC) (Nott *et al.*, 2004; Nott *et al.*, 2003). Splicing in mammalian cells deposits the multiprotein EJC approximately 20 nucleotides upstream of exon-exon junctions in spliced RNA, irrrespective of RNA sequence. The EJC functions in nonsense-mediated decay (NMD), a surveillance mechanism that degrades RNA with premature termination codons (PTCs). NMD requires splicing and translation and is believed to occur during a pioneer round of translation, when newly synthesised RNAs are checked for PTCs and the EJC complex is removed (reviewed (Maquat, 2004; Tange *et al.*, 2004). Splicing-mediated translational stimulation can be replicated by tethering the individual EJC proteins Y14 and Magoh or the yeast Upf NMD factors to an intronless reporter mRNA, within the reporter ORF. Importantly, increased translational yield does not reflect enhanced RNA stability or nucleocytoplasmic transport. Rather, the EJC promotes polysomal association of the mRNA, in addition to its previously characterized role in NMD (Nott *et al.*, 2004).

Factors that are normally associated with the control of constitutive and alternative splicing, the Ser-Arg-rich (SR) proteins, such as SF2/ASF, which are known to shuttle continuously between the nucleus and the cytoplasm, also have an enhancing role in translation, as shown by tethering and over-expression studies in *Xenopus* oocytes and in HeLa cells (Sanford *et al.*, 2004). It is interesting to note that, while splicing enhances translational yields three- to fourfold (Nott *et al.*, 2004; Sanford *et al.*, 2004), cytoplasmic polyadenylation can result in up to an approximately 100-fold increase in protein levels (Sheets *et al.*, 1994), although this difference may be partly attributable to the cellular context–the former assays are performed in *Xenopus* oocytes, and the latter in eggs. Finally, it is noteworthy that at least some of the well-known mRNAs that are translationally inactive in the *Xenopus* oocyte, including *c-mos* and core histones, lack introns, whereas translationally-efficient messages, such as TFIIIA, are spliced (Matsumoto *et al.*, 1998). With the completion of the *Xenopus tropicalis* genome, it will be of interest to extend this analysis to other regulated mRNAs, to test the generality of this rule.

Influences on RNA Localization

Splicing of the first intron of *oskar* RNA was shown to be essential for its localization at the posterior pole (Hachet and Ephrussi, 2004). Since mRNA synthesized from an intronless *oskar* gene fails to be properly localized, how were previous conclusions of the sufficiency of the *oskar* 3′ UTR for localization reached? In the earlier experiments, the intronless reporter *lacZ* mRNA was appended to the *oskar* 3′ UTR (for example in the study of (Gunkel *et al.*, 1998). The Ephrussi lab showed that localization of the chimeric reporter requires

endogenous *oskar* mRNA, suggesting that *lacZ-oskar* 3′ UTR RNA hitchhikes on the endogenous *oskar* RNP, whose assembly requires splicing (Hachet and Ephrussi, 2004). So, localization of *oskar* mRNA at the posterior pole requires not only the *oskar* 3′ UTR and its associated factors, such as Staufen, but also the process of splicing. Consistent with the splicing-dependent assembly of the *oskar* RNP, several EJC proteins, originally characterized in NMD in mammalian cells, are present in the *oskar* RNP, and are required for its posterior localization. These include the core heterodimer of Mago nashi and Y14 (Hachet and Ephrussi, 2001; Micklem *et al.*, 1997; Mohr *et al.*, 2001), in addition to eIF4AIII (Palacios *et al.*, 2004), which functions in NMD (Chan *et al.*, 2004; Ferraiuolo *et al.*, 2004; Palacios *et al.*, 2004; Shibuya *et al.*, 2004). eIF4AIII bridges the interactions between Mago nashi/Y14 and Barentsz (Palacios *et al.*, 2004), the plus end-directed microtubule transport factor recruited to the *oskar* RNP by Cup (Wilhelm *et al.*, 2003) (Figure 8). Barentsz is also essential for NMD in HeLa cells (Palacios *et al.*, 2004); hence, somewhat unexpectedly, a conserved complex functions in NMD and in RNA localization (Palacios *et al.*, 2004). eIF4AIII, a nuclear shuttling protein (Chan *et al.*, 2004; Ferraiuolo *et al.*, 2004; Shibuya *et al.*, 2004), ~65 % identical to the eIF4AI/II initiation factors, is also an ATPase-dependent helicase, and interacts with eIF4G and its co-factor eIF4B. However, it is inhibitory to translation *in vitro*, possibly because it only binds the central eIF4A binding site of eIF4G, rather than both the central and C-terminal binding sites, which its active eIF4AI/II counterparts do (Li *et al.*, 1999). Because of its close similarity to initiation factors and loading onto RNA in the nucleus, it has been proposed that eIF4AIII substitutes for eIF4AI/II (Ferraiuolo *et al.*, 2004) during the pioneer round of translation in NMD (Ishigaki *et al.*, 2001). eIF4AIII is thus a key structural and functional link between splicing in the nucleus and localization, RNA degradation and translation in the cytoplasm.

Consistent with the recent elegant demonstration of the tight coupling between splicing and localization of *oskar* mRNA (Hachet and Ephrussi, 2004), it had previously been proposed that proteins that associate with pre-mRNA in the nucleus accompany and regulate the localization of the cytoplasmic message (reviewed in Farina and Singer, 2002). For example, hnRNP A2, a shuttling RNA-binding protein, participates in the localization of myelin basic protein mRNA in oligodendrocytes (Kwon *et al.*, 1999). The ZBP1 family members are found in the human spliceosome (IMP3) (Zhou *et al.*, 2002), and associate with β-actin pre-mRNA transcripts in nuclei, shown using high speed imaging, prior to targeting the RNA to the lamellae in the cytoplasm (ZBP1) (Oleynikov and Singer, 2003). In *Xenopus* oocytes, Vg1RBP and hnRNP I bind the vegetally localized Vg1 and VegT mRNAs in the nucleus and the cytoplasm. The two proteins interact in both compartments, as well as *in vitro*, but the cytoplasmic, unlike the nuclear, interaction is RNA-dependent. In line with the possibility of mRNP remodelling during export, other components of the Vg1 RNP complex, Prrp and Staufen, only co-purify with the cytoplasmic heterodimer (Kress *et al.*, 2004).

REGULATING THE REGULATOR

Last, we briefly revisit the areas of translation and localization to review the types of mechanisms that are used to regulate the activities of RNA-binding proteins. One way to alter gene expression in development is through the differential expression of protein isoforms with conserved RNA-binding domains but different functions, exemplified by the histone stem loop binding proteins SLBP1/2 (reviewed in Marzluff & Duronio, 2002). We also note that vertebrates, as well as invertebrates, contain several isoforms of CPEB, PABP, Pumilio and Staufen proteins, among others, that are differentially expressed, and potentially perform different regulatory functions.

Modification of RNA-binding proteins is also used to regulate their activities, in response to the activation of critical signalling pathways. Notably, phosphorylation can influence protein localization. For example, hnRNP I phosphorylated by protein kinase A (PKA) is restricted to the *Xenopus* cytoplasm in oocytes (Xie *et al.*, 2003). As PKA is down-regulated during meiotic maturation, hnRNP I may then relocate to the nucleus. Phosphorylation also impacts on protein stability, as illustrated by cdc2 modification of CPEB and its subsequent PEST-mediated proteolysis (Mendez *et al.*, 2002; Reverte *et al.*, 2001; Thom *et al.*, 2003), and ligand binding, also exemplified by CPEB phosphorylation by Aurora A and cdc2 kinases, which enhance CPSF interactions (Dickson *et al.*, 2001; Mendez *et al.*, 2000b), and reduce Pumilio binding (Nakahata *et al.*, 2003), respectively, during oocyte maturation. The increase in EDEN-dependent deadenylation following egg activation is achieved, at least partially, via dephosphorylation

and/or phosphorylation of regulatory proteins, including dephosphorylation of EDEN-BP, in a manner independent from MPF inactivation (Detivaud *et al.*, 2003). While, to our knowledge, phosphorylation has not been documented to influence binding to RNA in the developmental examples discussed hitherto, *c-src* kinase-mediated phosphorylation of hnRNP K drives translational activation of specifically silenced mRNAs by reducing RNA-binding (Ostareck-Lederer *et al.*, 2002). On the other hand, acetylation correlates with enhanced RNA-binding in the case of the STAR/GSG protein Sam68, which is modified in tumor cell lines (Babic *et al.*, 2004).

CLOSING REMARKS

Thus, it can be seen that a number of recurring themes arise in the regulation of translation and RNA localization by RNA-binding proteins. These proteins often use common RNA-binding motifs such as the RRM or KH domain. While these domains provide basic RNA-binding functions, small variations in the amino acid sequence of individual domains, and the use of several types of domains in combination, can result in the recognition of very different RNA sequence motifs. Such motifs most often lie in the 3′ UTRs of the RNAs, even those that regulate the initiation of translation at the 5′-end. It will be important to derive more well defined consensus binding sites for these proteins than exist at present, to mine the expanding genome and EST databases and predict novel target RNAs, as it seems unlikely that our current lists are complete. In terms of the control of translation initiation, the most commonly utilized strategies appear to be to regulate ribosomal recruitment by disrupting interactions within the eIF4F complex or to modulate poly(A) tail length, either by adenylation or deadenylation, to affect the degree of translational stimulation by PABP. As regards RNA localization, the interaction of RNA-binding proteins with their target RNAs most often allows the recruitment of additional factors that direct the RNA to the cytoskeleton for active transport. It will be interesting to observe whether future work brings to light novel strategies adopted by other RNA-binding proteins or whether additional, as-yet-uncharacterized, mechanistic pathways will also begin to converge. The study of conserved regulatory proteins in diverse model systems–some with genetics, some with biochemistry, others with cytology–will be beneficial to derive a complete and detailed view of the roles of RNA-binding proteins in early development. Finally, it is exciting to note that insights gained from such studies of translation and localization control mechanisms bear directly on our understanding of these processes in neuronal cells, as many critical components are shared (reviewed in Bassell & Kelic, 2004; Glanzer & Eberwine, 2004; Huang & Richter, 2004; Jiang & Schuman, 2002; Kiebler & DesGroseillers, 2000; Steward & Schuman, 2003).

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REFERENCES

Adinolfi, S., Bagni, C., Castiglione Morelli, M.A., Fraternali, F., Musco, G., and Pastore, A. 1999. Novel RNA-binding motif: The KH module. *Biopolymers* 51:153–164.

Ahringer, J. and Kimble, J. 1991. Control of the sperm-oocyte switch in *Caenorhabditis elegans* hermaphrodites by the *fem-3* 3′ untranslated region. *Nature* 349:346–348.

Ahringer, J., Rosenquist, T.A., Lawson, D.N., and Kimble, J. 1992. The *Caenorhabditis elegans* sex determining gene *fem-3* is regulated post-transcriptionally. *EMBO J* 11:2303–2310.

Akao, Y., Marukawa, O., Morikawa, H., Nakao, K., Kamei, M., Hachiya, T., and Tsujimoto, Y. 1995. The rck/p54 candidate proto-oncogene product is a 54-kilodalton D-E-A-D box protein differentially expressed in human and mouse tissues. *Cancer Res* 55:3444–3449.

Alarcon, J.M., Hodgman, R., Theis, M., Huang, Y.S., Kandel, E.R., and Richter, J.D. 2004. Selective Modulation of Some Forms of Schaffer Collateral-CA1 Synaptic Plasticity in Mice With a Disruption of the CPEB-1 Gene. *Learn Mem* 11:318–327.

Alarcon, V.B. and Elinson, R.P. 2001. RNA anchoring in the vegetal cortex of the *Xenopus* oocyte. *J Cell Sci* 114:1731–1741.

Allison, R., Czaplinski, K., Git, A., Adegbenro, E., Stennard, F., Houliston, E., and Standart, N. 2004. Two distinct Staufen isoforms in *Xenopus* are vegetally localized during oogenesis. *RNA* 10:1751–1763.

Andresson, T. and Ruderman, J.V. 1998. The kinase Eg2 is a component of the *Xenopus* oocyte progesterone-activated signaling pathway. *EMBO J* 17:5627–5637.

Antic, D., Lu, N., and Keene, J.D. 1999. ELAV tumor antigen, Hel-N1, increases translation of neurofilament M mRNA and induces formation of neurites in human teratocarcinoma cells. *Genes Dev* 13:449–461.

Asaoka-Taguchi, M., Yamada, M., Nakamura, A., Hanyu, K, and Kobayashi, S. 1999. Maternal Pumilio acts together with Nanos in germline development in *Drosophila* embryos. *Nat Cell Biol* 1:431–437.

Audic, Y., F., O. and Osborne, H.B. 1997. Postfertilisation deadenylation of mRNAs in *Xenopus laevis* embryos is sufficient to cause their degradation at the blastula stage. *Mol Cell Biol* 17:209–218.

Audic, Y., Omilli, F., and Osborne, H.B. 1998. Embryo deadenylation element-dependent deadenylation is enhanced by a *cis* element containing AUU repeats. *Mol Cell Biol* 18:6879–6884.

Aviv, T., Lin, Z., S., L., Rendl, L.M., Sicheri, F., and Smibert, C.A. 2003. The RNA-binding SAM domain of Smaug defines a new family of post-transcriptional regulators. *Nat Struct Biol* 10:614–621.

Babic, I., Jakymiw, A., and Fujita, D.J. 2004. The RNA binding protein Sam68 is acetylated in tumor cell lines, and its acetylation correlates with enhanced RNA binding activity. *Oncogene* 23:3781–3789.

Baggs, J.E. and Green, C.B. 2003. Nocturnin, a deadenylase in Xenopus laevis retina: a mechanism for posttranscriptional control of circadian-related mRNA. *Curr Biol* 13:189–198.

Ballantyne, S., Bilger, A., Astrom, J., Virtanen, A., and Wickens, M. 1995. Poly (A) polymerases in the nucleus and cytoplasm of frog oocytes: Dynamic changes during oocyte maturation and early development. *RNA* 1:64–78.

Ballantyne, S., Daniel, J., D.L., and Wickens, M. 1997. A dependent pathway of cytoplasmic polyadenylation reactions linked to cell cycle control by *c-mos* and CDK1 activation. *Mol Biol Cell* 8:1633–1648.

Bally-Cuif, L., Schatz, W.J., and Ho, R.K. 1998. Characterisation of the zebrafish Orb/CPEB-related RNA-binding protein and localization of maternal components in the zebrafish oocyte. *Mech Dev* 77:31–47.

Barkoff, A.F., Dickson, K.S., Gray, N.K., and Wickens, M. 2000. Translational control of cyclin B1 mRNA during meiotic maturation: coordinated repression and cytoplasmic polyadenylation. *Dev Biol* 220:97–109.

Bassell, G.J. and Kelic, S. 2004. Binding proteins for mRNA localization and local translation, and their dysfunction in genetic neurological disease. *Curr Opin Neurobiol* 14:574–581.

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

Bernstein, D., Buter, N., Stumpf, C., and Wickens, M. 2002. Analyzing mRNA-protein complexes using a yeast three-hybrid system. *Methods* 26:123–141.

Betley, J.N., Frith, M.C., Graber, J.H., Choo, S., and Deshler, J.O. 2002. A ubiquitous and conserved signal for RNA localization in chordates. *Curr Biol* 12:1756–1761.

Betley, J.N., Heinrich, B., Vernos, I., Sardet, C., Prodon, F., and Deshler, J.O. 2004. Kinesin II Mediates Vg1 mRNA Transport in *Xenopus* Oocytes. *Curr Biol* 14:219–224.

Bonnet-Corven, S., Audic, Y., Omilli, F., and Osborne, H.B. 2002. An analysis of the sequence requirements of EDEN-BP for specific RNA binding. *Nucleic Acids Res* 30:4667–4674.

Borman, A.M., Michel, Y.M., and Kean, K.M. 2000. Biochemical characterisation of cap-poly(A) synergy in rabbit reticulocyte lysates: the eIF4G-PABP interaction increases the functional affinity of eIF4E for the capped mRNA 5′-end. *Nucl Acids Res* 28:4068–4075.

Bouvet, P., Matsumoto, K., and Wolffe, A.P. 1995. Sequence-specific RNA recognition by the *Xenopus* Y-box proteins. An essential role for the cold shock domain. *J Biol Chem* 270:28297–28303.

Bouvet, P., Omilli, F., Arlot-Bonnemains, Y., Legagneux, V., Roghi, C., Bassez, T., and Osborne, H.B. 1994. The deadenylation conferred by the 3′ untranslated region of a developmentally controlled mRNA in *Xenopus* embyos is switched to polyadenylation by deletion of a short sequence element. *Mol Cell Biol* 14:1893–1900.

Braddock, M., Muckenthaler, M., White, M.R.H., Thorburn, A.M., Sommerville, J., Kingsman, A.J., and Kingsman, S.M. 1994. Intronless RNA injected into the nucleus of *Xenopus* oocytes accesses a regulated translation control pathway. *Nucl Acids Res* 22:5255–5264.

Brendza, R.P., Serbus, L.R., Duffy, J.B., and Saxton, W.M. 2000. A function for kinesin I in the posterior transport of *oskar* mRNA and Staufen protein. *Science* 289:2120–2122.

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.

Bubunenko, M., Kress, T.L., Vempati, U.D., Mowry, K.L., and King, M.L. 2002. A consensus RNA signal that directs germ layer determinants to the vegetal cortex of *Xenopus* oocytes. *Dev Biol* 248:82–92.

Cao, Q. and Richter, J.D. 2002. Dissolution of the maskin-eIF4E complex by cytoplasmic polyadenylation and poly(A)-binding protein controls cyclin B1 mRNA translation and oocyte maturation. *EMBO J* 21: 3852–3862.

Cartegni, L., Maconi, M., Morandi, E., Cobianchi, F., Riva, S., and Biamonti, G. 1996. hnRNP A1 selectively interacts through its Gly-rich domain with different RNA-binding proteins. *J Mol Biol* 259:337–348.

Castagnetti, S. and Ephrussi, A. 2003. Orb and a long poly(A) tail are required for efficient *oskar* translation at the posterior pole of the *Drosophila* oocyte. *Development* 130:835–843.

Castagnetti, S., Hentze, M.W., Ephrussi, A., and Gebauer, F. 2000. Control of oskar mRNA translation by Bruno in a novel cell-free system from *Drosophila* ovaries. *Development* 127:1063–1068.

Castro, A., Mandart, E., Lorca, T., and Galas, S. 2003. Involvement of Aurora A kinase during meiosis I-II transition in *Xenopus* oocytes. *J Biol Chem* 278:2236–2241.

Chagnovitch, D. and Lehmann, R. 2001. Poly(A)-independent regulation of maternal *hunchback* translation in the *Drosophila* embryo. *Proc Natl Acad Sci USA* 98:11359–11364.

Chan, C.C., Dostie, J., Diem, M.D., Feng, W., Mann, M., Rappsilber, J., and Dreyfuss, G. 2004. eIF4A3 is a novel component of the exon junction complex. *RNA* 10:200–209.

Chang, J., Tan, L., and Schedl, P. 1999. The *Drosophila* CPEB homolog, orb, is required for Oskar protein expression in oocytes. *Dev Biol* 215:91–106.

Chang, P., Torres, J., Lewis, R., Mowry, K., Houliston, E., and King, M.L. 2004. Localization of RNAs to the mitochondrial cloud in *Xenopus* oocytes by entrapment and association with endoplasmic reticulum. *Mol Biol Cell* 15:4669–4681.

Charlesworth, A., Cox, L.L., and MacNicol, A.M. 2004. CPE- and CPEB-independent mechanisms regulate early class maternal mRNA translational activation in *Xenopus* oocytes. *J Biol Chem* 279:17650–17659.

Charlesworth, A., Welk, J., and MacNicol, A.M. 2000. The temporal control of wee1 mRNA translation during *Xenopus* oocyte maturation is regulated by cytoplasmic polyadenylation elements within the 3′-untranslated region. *Dev Biol* 227:706–719.

Chen, C.-Y., and Shyu, A.-B. 1995. AU-rich elements: characterisation and importance in mRNA degradation. *Trends Biochem Sci* 20:465–470.

Chen, C.-Y., Xu, N., and Shyu, A.-B. 1995. mRNA decay mediated by two distinct AU-rich elements from c-fos and granulocyte-macrophage colony-stimulating factor transcripts: different deadenylation kinetics and uncoupling from translation. *Mol Cell Biol* 15:5777–5788.

Chen, T., Damaj, B.B., Herrera, C., Lasko, P., and Richard, S. 1997. Self-association of the single-KH-domain family members Sam68, GRP33, GLD-1, and Qk1: role of the KH domain. *Mol Cell Biol* 17:5707–5718.

Chen, T. and Richard, S. 1998. Structure function analysis of Qk1: a lethal point mutation in mouse *quaking* prevents homodimerisation. *Mol Cell Biol* 18:4863–4871.

Christerson, L. and McKearin, D. 1994. *orb* is required for anteroposterior and dorsoventral patterning during *Drosophila* oogenesis. *Genes Dev* 8:614–628.

Clark, I.E., Wyckoff, D., and Gavis, E.R. 2000. Synthesis of the posterior determinant Nanos is spatially restricted by a novel cotranslational regulatory mechanism. *Curr Biol* 10:1311–1314.

Claussen, M. and Pieler, T. 2004. Xvelo1 uses a novel 75-nucleotide signal sequence that drives vegetal localization along the late pathway in *Xenopus* oocytes. *Dev Biol* 266:270–284.

Colegrove-Otero, L.J., Devaux, A., and Standart, N. 2005. A Novel Role for *Xenopus* ELAV Proteins in Translational Repression during Oogenesis. (Submitted).

Coller, J. and Parker, R. 2004. Eukaryotic mRNA decapping. *Ann Rev Biochem* 73:861–890.

Coller, J. and Wickens, M. 2002. Tethered function assays using 3′ untranslated regions. *Methods*. 26:142–150.

Coller, J.M., Tucker, M., Sheth, U., and Parker, R. 2001. The DEAD box helicase, Dhh1p, functions in mRNA decapping and interacts with both the decapping and deadenylase complexes. *RNA* 7:1717–1727.

Cooperstock, R.L. and Lipshitz, H.D. 2001. RNA localization and translational regulation during axis specification in the *Drosophila* oocyte. *Int Rev Cytol* 2001:541–566.

Copeland, P.R. and Wormington, M. 2001. The mechanism and regulation of deadenylation: identification and characterisation of *Xenopus* PARN. *RNA* 7:875–886.

Cordin, O., Tanner, N.K., Doere, M., Linder, P., and Banroques, J. 2004. The newly discovered Q motif of DEAD-box RNA helicases regulates RNA-binding and helicase activity. *EMBO J* 23:2478–2487.

Cosson, B., Braun, F., Paillard, L., Blackshear, P., and Osborne, H.B. 2004. Identification of a novel *Xenopus* laevis poly (A) binding protein. *Biol Cell* 96:519–527.

Cosson, B., Couturier, A., Le Guellec, R., Moreau, J., Chabelskaya, S., G., Z., and Philippe, M. 2002. Characterization of the poly(A) binding proteins expressed during oogenesis and early development of *Xenopus laevis*. *Biol Cell* 94:217–231.

Cote, C.A., Gautreau, D., Denegre, J.M., Kress, T.L., Terry, N.A., and Mowry, K.L. 1999. A *Xenopus* protein related to hnRNP I has a role in cytoplasmic RNA localization. *Mol Cell* 4:431–437.

Cougot, N., Babajko, S., and Seraphin, B. 2004. Cytoplasmic foci are sites of mRNA decay in human cells. *J Cell Biol* 165:31–40.

Crittenden, S.L., Bernstein, D.S., Bachorik, J.L., Thompson, B.E., Gallegos, M., Petcherski, A.G., Moulder, G., Barstead, R., Wickens, M., and Kimble, J. 2002. A conserved RNA-binding protein controls germline stem cells in *Caenorhabditis elegans*. *Nature* 417:660–663.

Cui, L., Fan, Q., and Li, J. 2002. The malaria parasite *Plasmodium falciparum* encodes members of the Puf RNA-binding protein family with conserved RNA binding activity. *Nucl Acids Res* 30:4607–4617.

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.

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

Dale, L., Matthews, G., Tabe, L., and Colman, A. 1989. Developmental expression of the protein product of Vg1, a localised maternal mRNA in the frog *Xenopus laevis. EMBO J* 8:1057–1065.

de Heredia, M.L. and Jansen, R.-P. 2004. mRNA localization and the cytoskeleton. *Cur Opin Cell Biol* 16:80–85.

de Moor, C. and Richter, J.D. 1999. Cytoplasmic polyadenylation elements mediate masking and unmasking of cyclin B1 mRNA. *EMBO J* 18:2294–2303.

de Moor, C.H. and Richter, J.,D. 1997. The mos pathway regulates cytoplasmic polyadenylation in *Xenopus* oocytes. *Mol Cell Biol* 17:6419–6426.

de Valoir, T., Tucker, M.A., Belikoff, E.J., Camp, L.A., Bolduc, C., and Beckingham, K. 1991. A second maternally expressed *Drosophila* gene encodes a putative RNA helicase of the "DEAD box" family. *Proc Natl Acad Sci USA* 88:2113–2117.

Dehlin, E., Wormington, M., Korner, C.G., and Wahle, E. 2000. Cap-dependent deadenylation of mRNA. *EMBO J* 19:1079–1086.

DeJong, E.S., Marzluff, W.F., and Nikonowicz, E.P. 2002. NMR structure and dynamics of the RNA-binding site for the histone mRNA stem-loop binding protein. *RNA* 8:83–96.

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.

Deshler, J.O., Highett, M.I., and Schnapp, B.J. 1997. Localization of *Xenopus* Vg1 mRNA by Vera protein and the endoplasmic reticulum. *Science* 276:1128–1131.

Detivaud, L., Pascreau, G., Karaiskou, A., Osborne, H.B., and Kubiak, J.Z. 2003. Regulation of EDEN-dependent deadenylation of Aurora A/Eg2-derived mRNA via phosphorylation and dephosphorylation in *Xenopus laevis* egg extracts. *J Cell Sci* 116:2697–2705.

Dickson, K.S., Bilger, A., Ballantyne, S., and Wickens, M.P. 1999. The cleavage and polyadenylation specificity factor in *Xenopus laevis* oocytes is a cytoplasmic factor involved in regulated polyadenylation. *Mol Cell Biol* 19:5707–5717.

Dickson, K.S., Thompson, S.R., Gray, N.K., and Wickens, M. 2001. Poly(A) polymerase and the regulation of cytoplasmic polyadenylation. *J Biol Chem* 276:41810–41816.

Dostie, J., Ferraiuolo, M., Pause, A., Adam, S.A., and Sonenberg, N. 2000. A novel shuttling protein, 4E-T, mediates the nuclear import of the mRNA 5′ cap-binding protein, eIF4E. *EMBO J* 19:3142–3156.

Doyle, G.A., Betz, N.A., Leeds, P.F., Fleisig, A.J., Prokipcak, R.D., and Ross, J. 1998. The c-myc coding region determinant-binding protein: a member of a family of KH domain RNA-binding proteins. *Nucl Acids Res* 26:5063–5044.

Dreyfuss, G., Kim, V.N., and Kataoka, N. 2002. Messenger-RNA-binding proteins and the messages they carry. *Nat Rev Mol Cell Biol* 3:195–205.

Driever, W. and Nusslein-Volhard, C. 1989. The Bicoid protein is a positive regulator of hunchback transcription in the early *Drosophila* embryo. *Nature* 337:138–143.

Driever, W. and Nüsslein-Volhard, C. 1988. A gradient of Bicoid protein in *Drosophila* embryos. *Cell* 54:83–93.

Dubnau, J., Chiang, A.-S., Grady, L., Barditch, J., Gossweiler, S., McNeil, J., Smith, P., Buldoc, F., Scott, R., Certa, U., Broger, C., and Tully, T. 2003. The *staufen/pumilio* pathway is involved in *Drosophila* Long-term memory. *Curr Biol* 13:286–296.

Dubnau, J. and Struhl, G. 1996. RNA recognition and translational regulation by a homeodomain protein. *Nature* 379:694–699.

Duchaine, T.F., Hemraj, I., Furic, L., Deitinghoff, A., Kiebeler, M.A., and DesGroseillers, L. 2002. Staufen2 isoforms localise to the somatodendritic domain of neurons and interact with different organelles. *J Cell Sci* 115:3285–3295.

Duncan, J.E. and Warrior, R. 2002. The cytoplasmic dynein and kinesin motors have interdependent roles in patterning the *Drosophila* oocyte. *Curr Biol* 12:1982–1991.

Eckmann, C.R., Kraemer, B., Wickens, M., and Kimble, J. 2002. GLD-3, a bicaudal-C homolog that inhibits FBF to control germline sex determination in *C. elegans*. *Dev Cell* 3:697–710.

Edmonds, M. 2002. A history of poly A sequences: from formation to factors to function. *Prog Nucleic Acid Res Mol Biol* 71:285–389.

Edwards, T.A., Pyle, S.E., Wharton, R.P., and Aggarwal, A.K. 2001. Structure of Pumilio reveals similarity between RNA and peptide binding motifs. *Cell* 105:281–289.

Edwards, T.A., Wilkinson, B.D., Wharton, R.P., and Aggarwal, A.K. 2003. Model of the brain tumor-Pumilio translation repressor complex. *Genes Dev* 15:2508–2513.

Elisha, Z., Havin, L., Ringel, I., and Yisraeli, J. 1995. Vg1 RNA binding protein mediates the association of Vg1 RNA with microtubules in *Xenopus* oocytes. *EMBO J* 14:5109–5114.

Eom, T., Antar, L.N., Singer, R.H., and Bassell, G.J. 2003. Localization of a beta-actin messenger ribonucleoprotein complex with zipcode-binding protein modulates the density of dendritic filopodia and filopodial synapses. *J Neurosci* 23:10433–10444.

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.

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

Ephrussi, A. and St Johnston, D. 2004. Seeing is believing: the bicoid morphogen gradient matures. *Cell* 116:143–152.

Evdokimova, V., Ruzanov, P., Imataka, H., Raught, B., Svitkin, Y., Ovchinnikov, L.P., and Sonenberg, N. 2001. The major mRNA-associated protein YB-1 is a potent 5′ cap-dependent mRNA stabilizer. *EMBO J* 20:5491–5502.

Evdokimova, V.M., Wei, C.-L., Sitikov, A., Simonenko, P., Lazarev, O., Vasilenko, K., Ustinov, V., Hershey, J., and Ovchinnikov, L. 1995. The major protein of messenger ribonucleoprotein particles in

somatic cells is a member of the Y-box binding transcription factor family. *J Biol Chem* 270:3186–3192.
Ezzeddine, N., Paillard, L., Capri, M., Maniey, D., Bassez, T., Ait-Ahmed, O., and Osborne, H.B. 2002. EDEN-dependent translational repression of maternal mRNAs is conserved between *Xenopus* and *Drosophila*. *Proc Natl Acad Sci USA* 99:257–262.
Farina, K.L., Huttelmaier, S., Musunuru, K., Darnell, R., and Singer, R.H. 2003. Two ZBP1 KH domains facilitate β-actin mRNA localization, granule formation and cytoskeletal attachment. *J Cell Biol* 160:77–87.
Farina, K.L. and Singer, R.H. 2002. The nuclear connection in RNA transport and localization. *Trends Cell Biol* 12:466–471.
Ferraiuolo, M.A., Lee, C.S., Ler, L.W., Hsu, J.L., Costa-Mattioli, M., Luo, M.J., Reed, R., and Sonenberg, N. 2004. A nuclear translation-like factor eIF4AIII is recruited to the mRNA during splicing and functions in nonsense-mediated decay. *Proc Natl Acad Sci USA* 101:4118–4123.
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 that move in a microtubule-dependent manner. *Cell* 79:1221–1232.
Ferrandon, D., Koch, I., Westhof, E., and Nüsslein-Volhard, C. 1997. RNA-RNA interaction is required for the formation of specific *bicoid* mRNA 3′ UTR-Staufen ribonucleoprotein particles. *EMBO J* 16:1751–1758.
Filardo, P. and Ephrussi, A. 2003. Bruno regulates *gurken* during *Drosophila* oogenesis. *Mech Dev* 120:289–297.
Fischer, N. and Weis, K. 2002. The DEAD box protein Dhh1 stimulates the decapping enzyme Dcp1. *EMBO J* 21:2788–2797.
Forrest, K.M., Clark, I.E., Jain, R.A., and Gavis, E.R. 2004. Temporal complexity within a translational control element in the *nanos* mRNA. *Development* 131:5849–5857.
Forristall, C., Pondel, M., Chen, L., and King, M. 1995. Patterns of localization and cytoskeletal association of two vegetally localised RNAs, *Vg1* and, *Xcat-2*. *Development* 121:201–208.
Fox, C.A., Sheets, M.D., and Wickens, M.P. 1989. Poly(A) addition during maturation of frog oocytes: distinct nuclear and cytoplasmic activities and regulation by the sequence UUUUUAU. *Genes Dev* 3:2151–2162.
Fox, C.A. and Wickens, M. 1990. Poly(A) removal during oocyte maturation: a default reaction selectively prevented by specific sequences in the 3′-UTR of certain maternal mRNAs. *Genes Dev* 4:2287–2298.
Frank-Vaillant, M., Haccard, O., Thibier, C., Ozon, R., Arlot-Bonnemains, Y., Prigent, C., and Jessus, C. 2000. Progesterone regulates the accumulation and the activation of Eg2 kinase in *Xenopus* oocytes. *J Cell Sci* 113:1127–1138.
Fuller-Pace, F.V. 1994. RNA helicases:modulators of RNA structure. *Trends Cell Biol* 4:271–274.
Gallie, D.R. 1991. The cap and poly(A) tail function synergistically to regulate mRNA translational efficiency. *Genes Dev* 5:2108–2116.
Gallie, D.R. 1998. A tale of two termini: A functional interaction between the termini of an mRNA is a prerequisite for efficient translation initiation. *Gene* 216:1–11.
Gao, F.-B., Carson, C., Levine, T., and Keene, J. 1994. Selection of a subset of mRNAs from combinatorial 3′ untranslated region libraries using neuronal RNA-binding protein Hel-N1. *Proc Natl Acad Sci USA* 91:1207–11211.
Gao, M., Fritz, D.T., Ford, L.P., and Wilusz, J. 2000. Interaction between a poly(A)-specific ribonuclease and the 5′ cap influences mRNA deadenylation rates *in vitro*. *Mol Cell* 5:479–488.
Gautreau, D., Cote, C.A., and Mowry, K.L. 1997. Two copies of a subelement from Vg1 RNA localization sequence are sufficient to direct vegetal localization in *Xenopus* oocytes. *Development* 124:5013–5020.
Gavis, E.R. 2001. Over the rainbow to translational control. *Nat Struct Biol* 8:387–389.
Gavis, E.R. and Lehmann, R. 1992. Localization of *nanos* RNA controls embryonic polarity. *Cell* 71:301–313.
Gavis, E.R. and Lehmann, R.L. 1994. Translational regulation of *nanos* by RNA localization. *Nature* 369:315–318.
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.
Gebauer, F., Corona, D.F.V., Preiss, T., Becker, P., and Hentze, M.W. 1999. Translational control of dosage compensation in *Drosophila* by Sex-lethal: cooperative silencing via the 5′ and 3′ UTRs of *msl-2* mRNA is independent of the poly(A) tail. *EMBO J* 18:6146–6154.
Gebauer, F., Grskovic, M., and Hentze, M. 2003. *Drosophila* sex-lethal inhibits the stable association of the 40S ribosomal subunit with *msl-2* mRNA. *Mol Cell* 11:1397–1404.
Gebauer, F. and Hentze, M.W. 2004. Molecular mechanisms of translational control. *Nat Rev Mol Cell Biol* 5:827–835.
Gebauer, F. and Richter, J. 1996. Mouse cytoplasmic polyadenylylation element binding protein: An evolutionary conserved protein that interacts with the cytoplasmic polyadenylylation elements of *c-mos* mRNA. *Proc Natl Acad Sci USA* 93:14602–14607.
Gebauer, F. and Richter, J.D. 1995. Cloning and characterisation of a *Xenopus* poly(A) polymerase. *Mol Cell Biol* 15:1422–1430.
Gebauer, F., Xu, W., Cooper, G., and Richter, J. 1994. Translational control by cytoplasmic polyadenylation of *c-mos* mRNA is necessary for oocyte maturation in the mouse. *EMBO J* 13:5712–5720.
Gerber, A.P., Herschlag, D., and Brown, P.O. 2004. Extensive Association of Functionally and Cytotopically Related mRNAs with Puf Family RNA-Binding Proteins in Yeast. *PLoS Biol* 2:E79.
Gergely, F. 2002. Centrosomal TACCtics. *BioEssays* 24:915–925.
Gillian-Daniel, D.L., Gray, N.K., Astrom, J., Barkoff, A., and Wickens, M. 1998. Modifications of the 5′ cap of mRNAs during *Xenopus* oocyte maturation: independence from changes in poly(A) length and impact on translation. *Mol Cell Biol* 18:6152–6153.
Giorgini, F., Davies, H.G., and Braun, R.E. 2002. Translational repression by MSY4 inhibits spermatid differentiation in mice. *Development* 129:3669–3679.
Git, A. and Standart, N. 2002. The KH domains of *Xenopus* Vg1RBP mediate RNA binding and self-association. *RNA* 8:1319–1333.
Glanzer, J.G. and Eberwine, J.H. 2004. Mechanisms of translational control in dendrites. *Neurobiol Aging* 24:1105–1111.
Good, P.J. 1997. The role of elav-like genes, a conserved family encoding RNA-binding proteins, in growth and development. *Sem Cell Dev Biol* 8:577–584.
Good, P.J., Abler, L., Herring, D., and Sheets, M.D. 2004. *Xenopus* embryonic poly(A) binding protein 2 (ePABP2) defines a new family of cytoplasmic poly(A) binding proteins expressed during the early stages of vertebrate development. *Genesis* 38:166–175.
Good, P.J., Chen, Q., Warner, S.J., and Herring, D.C. 2000. A family of human RNA-binding proteins related to the *Drosophila* Bruno translational regulator. *J Biol Chem* 275:28583–28592.
Goodrich, J.S., Clouse, K.N., and Schupbach, T. 2004. Hrb27C, Sqd and Otu cooperatively regulate *gurken* RNA localization and mediate nurse cell chromosome dispersion in *Drosophila* oogenesis. *Development* 131:1949–1958.
Goodwin, E.B., Okkema, P.G., Evans, T.C., and Kimble, J. 1993. Translational regulation of *tra-2* by its 3′ untranslated region controls sexual identity in *C. elegans*. *Cell* 75:329–339.
Gray, N., Coller, J., Dickson, K., and Wickens, M. 2000. Multiple portions of poly(A)-binding protein stimulate translation *in vivo*. *EMBO J* 19:4723–4733.
Green, J.B., Gardner, C.D., Wharton, R.P., and Aggarwal, A.K. 2003. RNA recognition via the SAM domain of Smaug. *Mol Cell* 11:1537–1548.
Griffin, D., Penberthy, W.T., Lum, H., R.W., S., and Taylor, W.L. 2003. Isolation of the B3 transcription factor of the *Xenopus* TFIIIA gene. *Gene* 313:179–188.
Grishin, N.V. 2001. KH domain:one motif, two folds. *Nucleic Acids Res* 29:638–643.
Groft, C.M. and Burley, S.K. 2002. Recognition of eIF4G by rotavirus NSP3 reveals a basis for mRNA circularization. *Mol Cell* 9:1273–1283.

Groisman, I., Huang, Y.-S., Mendez, R., Cao, Q., Therkauf, W., and Richter, J. 2000. CPEB, maskin, and cyclin B1 mRNA at the mitotic apparatus: Implications for local translational control of cell division. *Cell* 103:435–447.

Groisman, I., Jung, M.Y., Sarkissian, M., Cao, Q., and Richter, J.D. 2002. Translational control of the embryonic cell cycle. *Cell* 109: 473–483.

Gromak, N., Matlin, A.J., Cooper, T.A., and Smith, C.W. 2003. Antagonistic regulation of alpha-actinin alternative splicing by CELF proteins and polypyrimidine tract binding protein. *RNA* 9:443–456.

Gross, J.D., Moerke, N.J., von der Haar, T., Lugovskoy, A.A., Sachs, A.B., McCarthy, J.E., and Wagner, G. 2003. Ribosome loading onto the mRNA cap is driven by conformational coupling between eIF4G and eIF4E. *Cell* 115:739–750.

Gu, W., Pan, F., Zhang, H., Bassell, G.J., and Singer, R.H. 2002. A predominantly nuclear protein affecting cytoplasmic localization of β-actin mRNA in fibroblasts and neurons. *J Cell Biol* 156:41–51.

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.

Hachet, O. and Ephrussi, A. 2001. *Drosophila* Y14 shuttles to the posterior of the oocyte and is required for *oskar* mRNA transport. *Curr Biol* 11:1666–1674.

Hachet, O. and Ephrussi, A. 2004. Splicing of *oskar* RNA in the nucleus is coupled to its cytoplasmic localization. *Nature* 428:959–963.

Haghighat, A., Mader, S., Pause, A., and Sonenberg, N. 1995. Repression of cap-dependent translation by 4E-binding protein 1: competition with p220 for binding to eukaryotic initiation factor-4E. *EMBO J* 14:5701–5709.

Hake, L.E., Mendez, R., and Richter, J.D. 1998. Specificity of RNA binding by CPEB: Requirement for RNA recognition motifs and a novel zinc finger. *Mol Cell Biol* 18:685–693.

Hake, L.E. and Richter, J.D. 1994. CPEB is a specificity factor that mediates cytoplasmic polyadenylation during *Xenopus* oocyte maturation. *Cell* 79:617–627.

Hall, T.M. 2002. Poly(A) tail synthesis and regulation: recent structural insights. *Curr Opin Struct Biol* 12:82–88.

Hansen, T.V., Hammer, N.A., Nielsen, J., Madsen, M., Dalbaeck, C., Wewer, U.M., Christiansen, J., and Nielsen, F.C. 2004. Dwarfism and Impaired Gut Development in Insulin-Like Growth Factor II mRNA-Binding Protein 1-Deficient Mice. *Mol Cell Biol* 24:4448–4464.

Hata, H., Mitsui, H., Liu, H., Bai, Y., Denis, C.L., Shimizu, Y., and Sakai, A. 1998. Dhh1p, a putative RNA helicase, associates with the general transcription factors Pop2p and Ccr4p from *Saccharomyces cerevisiae*. *Genetics* 148:571–579.

Havin, L., Git, A., Elisha, Z., Oberman, F., 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.

Hellen, C.U.T. and Sarnow, P. 2001. Internal ribosome entry sites in eukaryotic mRNA molecules. *Genes Dev* 15:1593–1612.

Hershey, J.W.B. and Merrick, W.C. 2000. Pathway and mechanism of initiation of protein synthesis. In: *Translational Control of Gene Expression*, Sonenberg, N., Hershey, J., and Mathews, M. (eds.) Cold Spring Harbor Laboratory Press, pp. 33-88. Cold Spring Harbor, New York.

Hochegger, H., Klotzbucher, A., Kirk, J., Howell, M., le Guellec, K., Fletcher, K., Duncan, T., Sohail, M., and Hunt, T. 2001. New B-type cyclin synthesis is required between meiosis I and II during *Xenopus* oocyte maturation. *Development*. 128:3795–3807.

Hodgman, R., Tay, J., Mendez, R., and Richter, J.D. 2001. CPEB phosphorylation and cytoplasmic polyadenylation are catalyzed by the kinase IAK1/Eg2 in maturing mouse oocytes. *Development* 128:2815–2822.

Houston, D.W. and King, M.L. 2000. Germ plasm and molecular determinants of germ cell fate. *Curr Top Dev Biol* 50:155–181.

Huang, Y.-S. and Richter, J.D. 2004. Regulation of local mRNA translation. *Cur Opin Cell Biol* 16:308–313.

Huang, Y.S., Carson, J.H., Barbarese, E., and Richter, J.D. 2003. Facilitation of dendritic mRNA transport by CPEB. *Genes Dev* 17:638–653.

Huang, Y.S., Jung, M.Y., Sarkissian, M., and Richter, J.D. 2002. N-methyl-D-aspartate receptor signaling results in Aurora kinase-catalyzed CPEB phosphorylation and alpha CaMKII mRNA polyadenylation at synapses. *EMBO J* 21:2139–2148.

Huynh, J.R., Munro, T.P., Smith-Litiere, K., Lepesant, J.A., and Johnston, D.S. 2004. The *Drosophila* hnRNPA/B Homolog, Hrp48, Is Specifically Required for a Distinct Step in *osk* mRNA Localization. *Dev Cell* 6:625–635.

Hyatt, B.A. and Yost, H.J. 1998. The left-right coordinator:The role of Vg1 in organising left-right axis formation. *Cell* 93:37–46.

Iizuka, N., Najita, L., Franzusoff, A., and Sarnow, P. 1994. Cap-dependent and cap-independent translation by internal initiation of mRNAs in cell extracts prepared from *Saccharomyces cerevisiae*. *Mol Cell Biol* 14:7322–7330.

Imai, M., Akatani, K., Ikegami, N., and Furuichi, Y. 1983. Capped and conserved terminal structures in human rotavirus genome double-stranded RNA segments. *J Virol* 47:125–136.

Imataka, H., Gradi, A., and Sonenberg, N., 1998. A newly identified N-terminal amino acid sequence of human eIF4G binds poly(A)-binding protein and functions in poly(A)-dependent translation. *EMBO J* 17:7480–7489.

Ishigaki, Y., Li, X., Serin, G., and Maquat, L.E. 2001. Evidence for a pioneer round of mRNA translation:mRNAs subject to nonsense-mediated decay in mammalian cells are bound by CBP80 and CBP20. *Cell* 106:607–617.

Jackson, R. 2000. Comparative view of initiation site selection mechanisms. In: *Translational Control of Gene Expression*, pp. 127–183. Sonenberg, N., Hershey, J., and Mathews, M. (eds.), Cold Spring Harbor Laboratory Press, Cold Spring Harbor, New York.

Jacobson, A. 1996. Poly(A) metabolism and translation:the closed-loop model. In: *Translational Control*, pp. 451–480. Hershey, J.W.B., Mathews, M.B., and Sonenberg, N. (eds.), Cold Spring Harbor Laboratory Press, New York.

Jain, R., Andrews, L., McGowan, K., Pekala, P., and Keene, J. 1997. Ectopic expression of Hel-N1, an RNA-binding protein, increases glucose transporter (GLUT1) expression in 3T3-L1 adipocytes. *Mol Cell Biol* 17:954–962.

Jan, E., Motzny, C., Graves, L., and Goodwin, E. 1999. The STAR protein, GLD-1, is a translational regulator of sexual identity in *Caenorhabditis elegans*. *EMBO J* 18:258–269.

Jan, E., Yoon, J.W., Walterhouse, D., Iannaccone, P., and Goodwin, E.B. 1997. Conservation of the *C. elegans tra-2* 3′UTR translational control. *EMBO J* 16:6301–6313.

Januschke, J., Gervais, L., Dass, S., Kaltschmidt, J.A., Lopez-Schier, H., St Johnston, D., Brand, A.H., Roth, S., and Guichet, A. 2002. Polar transport in the *Drosophila* oocyte requires Dynein and Kinesin I cooperation. *Curr Biol* 12:1971–1981.

Jaruzelska, J., Kotecki, M., Kusz, K., Spik, A., Firpo, M., and Reijo Pera, R.A. 2003. Conservation of a Pumilio-Nanos complex from Drosophila germ plasm to human germ cells. *Dev Genes Evol* 213:120–126.

Jiang, C. and Schuman, E.M. 2002. Regulation and function of local protein synthesis in neuronal dendrites. *Trends Biochem Sci* 27:506–513.

Jin, S.-W., Arno, N., Cohen, A., Shah, A., Xu, Q., Chen, N., and Ellis, R.E. 2001. In *Caenorhabditis elegans*, the RNA-binding domains of the cytoplasmic polyadenylation element binding protein FOG-1 are needed to regulate germ cell fates. *Genetics* 159:1617–1630.

Johnstone, O. and Lasko, P. 2001. Translational regulation and RNA localization in *Drosophila* oocytes and embryos. *Annu Rev Genet* 35:365–406.

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

Kahvejian, A., Roy, G., and Sonenberg, N. 2001. The mRNA closed-loop model: the function of PABP and PABP-interacting proteins in mRNA translation. *Cold Spring Harb Symp Quant Biol* 66:293–300.

Kaminski, A. and Jackson, R.J. 1998. The polypyrimidine tract binding protein (PTB) requirement for internal initiation of translation of cardiovirus RNAs is conditional rather than absolute. *RNA* 4:626–638.

Kashiwabara, S., Noguchi, J., Zhuang, T., Ohmura, K., Honda, A., Sugiura, S., Miyamoto, K., Takahashi, S., Inoue, K., Ogura, A., and Baba, T. 2002. Regulation of spermatogenesis by testis-specific, cytoplasmic poly(A) polymerase TPAP. *Science* 298:1999–2002.

Kessler, S.H. and Sachs, A.B. 1998. RNA recognition motif 2 of yeast Pab1p is required for its functional interaction with eukaryotic translation initiation factor 4G. *Mol Cell Biol* 18:51–57.

Keyes, L.N. and Spradling, A.C. 1997. The *Drosophila* gene fs(2)cup interacts with otu to define a cytoplasmic pathway required for the structure and function of germ-line chromosomes. *Development* 124:1419–1431.

Kiebler, M.A. and DesGroseillers, L. 2000. Molecular insights into mRNA transport and local translation in the mammalian nervous system. *Neuron* 25:19–28.

Kiebler, M.A., Hemraj, I., Verkade, P., Kohrmann, M., Fortes, P., Marion, R.M., Ortin, J., and Dotti, C.G. 1999. The mammalian staufen protein localises to the somatodendritic domain of cultured hippocampal neurons:Implications for its involvement in mRNA transport. *J Neurosci* 19:288–297.

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

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.

King, M.L., Zhou, Y., and Bubunenko, M. 1999. Polarizing genetic information in the egg: RNA localization in the frog oocyte. *BioEssays* 21:546–557.

Kislauskis, E.H., Zhu, X.-C., and Singer, R.H. 1997. β-actin messenger RNA localization, and protein synthesis augment cell motility. *J Cell Biol* 136:1263–1270.

Kleene, K.C. 2003. Patterns, mechanisms, and functions of translation regulation in mammalian spermatogenic cells. *Cytogenet Genome Res* 103:217–224.

Klymkowsky, M.W., Maynell, L.A., and Nislow, C. 1991. Cytokeratin phosphorylation, cytokeratin filament severing, and the solubilization of the maternal mRNA Vg1. *J Cell Biol* 114:787–797.

Kohno, K., Izumi, H., Uchiumi, T., Ashizuka, M., and Kuwano, M. 2003. The pleiotropic functions of the Y-box-binding protein, YB-1. *BioEssays* 25:691–698.

Kohrmann, M., Luo, M., Kaether, C., DesGroseillers, L., Dotti, C.G., and Kiebeler, 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 Biol Cell* 10:2945–2953.

Kolev, N. and Huber, P. 2003. VgRB71 stimulates cleavage at a polyadenylation signal in Vg1 mRNA, resulting in the removal of a cis-acting element that represses translation. *Mol Cell* 11:745–755.

Korner, C.G., Wormington, M., Muckenthaler, M., Scneider, S., Dehlin, E., and Wahle, E. 1998. The deadenylating nuclease (DAN) is involved in poly(A) tail removal during the meiotic maturation of *Xenopus* oocytes., *EMBO J* 17:5427–5437.

Kraemer, B., Crittenden, S., Gallegos, M., Moulder, G., Barstead, R., Kimble, J., and Wickens, M. 1999. NANOS-3 and FBF proteins physically interact to control the sperm-oocyte switch in *Caenorhabditis elegans*. *Curr Biol* 9:1009–1018.

Kress, T.L., Yoon, Y.J., and Mowry, K.L. 2004. Nuclear RNP complex assembly initiates cytoplasmic RNA localization. *J Cell Biol* 165:203–211.

Krichevsky, A.M. and Kosik, K.S. 2001. Neuronal RNA granules:a link between RNA localization and stimulation-dependent translation. *Neuron* 32:683–696.

Kroll, T.T., Zhao, W., Jiang, C., and Huber, P.W. 2002. A homolog of FBP2/KSRP binds to localized mRNAs in *Xenopus* oocytes. *Development* 129:5609–5619.

Kruys, V., Marinx, O., Shaw, G., Deschamps, J., and Huez, G. 1989 Translational blockade imposed by cytokine-derived UA-rich sequences. *Science* 245:852–855.

Kuersten, S. and Goodwin, E.B. 2003. The power of the 3′ UTR:Translational control and development. *Nature Rev Gen* 4:626–637.

Kühn, U. and Pieler, T. 1996. *Xenopus* poly(A) binding protein: Functional domains in RNA binding and protein-protein interaction. *J Mol Biol* 256:20–30.

Kühn, U. and Wahle, E. 2004. Structure and function of poly(A) binding proteins. *BBA* 1678:67–84.

Kullman, M., Gopfert, U., Siewe, B., and Hengst, L. 2002. ELAV/Hu proteins inhibit p27 translation via an IRES element in the p27 5′UTR. *Genes Dev* 16:3087–3099.

Kurihara, Y., Tokuriki, M., Myojin, R., Hori, T., Kuroiwa, A., Matsuda, Y., Sakurai, T., Kimura, M., Hecht, N.B., and Uesugi, S. 2003. CPEB2, a novel putative translational regulator in mouse haploid germ cells. *Biol Reprod* 69:261–268.

Kwak, J.E., Wang, L., Ballantyne, S., Kimble, J., and Wickens, M. 2004. Mammalian GLD-2 homologs are poly(A) polymerases. *Proc Natl Acad Sci USA* 101:4407–4412.

Kwon, S., Abramson, T., Munro, T.P., John, C.M., Kohrmann, M., and Schnapp, B.J. 2002. UUCAC and Vera dependent localization of VegT RNA in *Xenopus* oocytes. *Curr Biol* 12:558–564.

Kwon, S., Barbarese, E., and Carson, J.H. 1999. The cis-acting RNA trafficking signal from myelin basic protein mRNA and its cognate transacting ligand hnRNP A2 enhance cap-dependent translation. *J Cell Biol* 147:247–256.

Ladomery, M., Wade, E., and Sommerville, J. 1997. Xp54, the *Xenopus* homologue of human RNA helicase p54, is an integral component of stored mRNP particles in oocytes. *Nucl Acids Res* 25:965–973.

Laity, J.H., Lee, B.M., and Wright, P.E. 2001. Zinc finger proteins: new insights into structural and functional diversity. *Curr Opin Struct Biol* 11:39–46.

Lantz, V., Ambrosio, L., and Schedl, P. 1992 The *Drosophila orb* gene is predicted to encode sex-specific germline RNA-binding proteins and has localised transcripts in ovaries and early emryos. *Development* 115:75–88.

Lantz, V., Chang, J., Horabin, J., Bopp, D., and Schedl, P. 1994. The *Drosophila orb* RNA-binding protein is required for the formation of the egg chamber and establishment of polarity. *Genes Dev* 8:598–613.

Le, H., Tanguay, R.L., Balasta, M.L., Wei, C.-C., Browning, K.S., Metz, A.M., Goss, D.J., and Gallie, D.R. 1997. Translation initiation factors eIF-iso4G and eIF4B interact with the poly(A)-binding protein and increase its RNA binding activity. *J Biol Chem* 272:16247–16255.

Le Hir, H., Nott, A., and Moore, M.J. 2003. How introns influence and enhance eukaryotic gene expression. *Trends Biochem Sci* 28:215–220.

Lee, M.H. and Schedl, T. 2001. Identification of in vivo targets of GLD-1, a maxi KH-motif containing proten required for *C.elegans* germ cell development. *Genes Dev* 15:2408–2420.

Legagneux, V., Omilli, F., and Osborne, H.B. 1995. Substrate-specific regulation of RNA deadenylation in *Xenopus* embryo and activated egg extracts. *RNA* 1:1001–1008.

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

Lewis, H.A., Musunuru, K., Jansen, K.B., Edo, C., Chen, H., Darnell, R.B., and Burley, S.K. 2000. Sequence-specific RNA binding by a Nova KH domain: implications for paraneoplastic disease and the fragile X syndrome. *Cell* 100:323–332.

Lewis, R.A., Kress, T.L., Cote, C.A., Gautreau, D., Rokop, M.E., and Mowry, K.L. 2004. Conserved and clustered RNA recognition sequences are

a critical feature of signals directing RNA localization in *Xenopus* oocytes. *Mech Dev* 121:101–109.
Li, P., Yang, X., Wasser, M.Y.C., and Chia, W. 1997. Inscuteable and Staufen mediate asymmetric localization and segregation of prospero RNA during *Drosophila* neuroblast cell divisions. *Cell* 90:437–447.
Li, Q., Imataka, H., Morino, S., Rogers Jr., G.W., Richter-Cook, N.J., Merrick, W.C., and Sonenberg, N. 1999. Eukaryotic translation initiation factor 4AIII (eIF4AIII) is functionally distinct from eIF4AI and eIF4AII. *Mol Cell Biol* 19:7336–7346.
Lie, S.Y. and Macdonald, P.M. 1999. Translational regulation of *oskar* mRNA occurs independent of the cap and poly(A) tail in *Drosophila* ovarian extracts. *Development* 126:4989–4996.
Lin, Q., Taylor, S.J., and Shalloway, D. 1997. Specificity and determinants of Sam68 RNA binding. Implications for the biological function of K homology domains. *J Biol Chem* 272:27274–27280.
Lin, T.-A., Kong, X., Haystead, T., Pause, A., Belsham, G., Sonenberg, N., and Lawrence Jr, J. 1994. PHAS-1 as a link between mitogen-activated protein kinase and translation initiation. *Science* 266:653–656.
Linder, P. 2003. Yeast RNA helicases of the DEAD-box family involved in translation initiation. *Biol Cell* 95:157–167.
Ling, J., Morley, S.J., Pain, V.M., Marzluff, W.F., and Gallie, D.R. 2002. The histone 3′-terminal stem-loop-binding protein enhances translation through a functional and physical interaction with eukaryotic initiation factor 4G (eIF4G) and eIF3. *Mol Cell Biol* 22:7853–7867.
Liu, J. and Schwartz, J.H. 2003. The cytoplasmic polyadenylation element binding protein and polyadenylation of messenger RNA in *Aplysia* neurons. *Brain Res* 959:68–76.
Liu, Z., Luyten, I., Bottomley, M.J., Messias, A.C., Houngninou-Molango, S., Sprangers, R., Zanier, K., Kramer, A., and Sattler, M. 2001. Structural basis for recognition of the intron branch site RNA by splicing factor 1. *Science* 294:1098–1102.
Luitjens, C., Gallegos, M., Kraemer, B., Kimble, J., and Wickens, M. 2000. CPEB proteins control two key steps in spermatogenesis in *C elegans*. *Genes Dev* 14:2596–2609.
Lüking, A., Stahl, U., and Schimdt, U. 1998. The protein family of RNA helicases. *Crit Rev Biochem Mol Biol* 33:259–296.
Lukong, K.E. and Richard, S. 2003. Sam68, the KH domain-containing superSTAR. *Biochim Biophys Acta* 1653:73–86.
Luo, M., Duchaine, T.F., and DesGroseillers, L. 2002. Molecular mapping of the determinants involved in human staufen-ribosome association. *Biochem J* 365:817–824.
MacArthur, H., Bubunenko, M., Houston, D.W., and King, M. 1999. Xcat2 RNA is a translationally sequestered germ plasm component in *Xenopus*. *Mech Dev* 84:75–88.
Macchi, P., Kroening, S., Palacios, I.M., Baldassa, S., Grunewald, B., Ambrosino, C., Goetze, B., Lupas, A., St. Johnston, D., and Kiebler, M. 2003. Barentsz, a new component of the Staufen-containing ribonucleoprotein particles in mammalian cells, interacts with Staufen in an RNA-dependent manner. *J Neurosci* 23:5778–5788.
Macdonald, P.M. 1992. The Drosophila *pumilio* gene: an unusually long transcription unit and an unusual protein. *Development* 114:221–232.
Mader, S., Lee, H., Pause, A., and Sonenberg, N. 1995. The translation initiation factor eIF-4E binds to a common motif shared by the translation factor eIF-4G and the translational repressors 4E-binding proteins. *Mol Cell Biol* 15:4990–4997.
Maekawa, H., Nakagawa, T., Uno, Y., Kitamura, K., and Shimoda, C. 1994. The *ste13*$^{+}$ gene encoding a putative RNA helicase is essential for nitrogen starvation-induced G1 arrest and initiation of sexual development in the fission yeast *Schizosaccharomyces pombe*. *Mol Gen Genet* 244:456–464.
Mallardo, M., Deitinghoff, A., Muller, J., Goetze, B., Macchi, P., Peters, C., and Kiebler, M.A. 2003. Isolation and characterisation of Staufen-containing ribonucleoprotein particles from rat brain. *Proc Natl Acad Sci USA* 100:2100–2105.
Manival, X., Ghisolfi-Nieto, L., Joseph, G., Bouvet, P., and Erard, M. 2001. RNA-binding strategies common to cold-shock domain- and RNA recognition motif-containing proteins. *Nucleic Acids Res* 29:2223–2233.
Maquat, L.E. 2004. Nonsense-mediated mRNA decay: splicing, translation and mRNP dynamics. *Nat Rev Mol Cell Biol* 5:89–99.
Marcotrigiano, J., Gingras, A.C., Sonenberg, N., and Burley, S.K. 1999. Cap-dependent translation initiation in eukaryotes is regulated by a molecular mimic of eIF4G. *Mol Cell* 3:707–716.
Marinx, O., Bertrand, S., E., K., Huez, G., and Kruys, V. 1994. Fertilisation of *Xenopus* eggs imposes a complete translational arrest of mRNAs containing 3′ UUAUUUAU elements. *FEBS Let* 345:107–112.
Marion, R.M., Fortes, P., A., B., Dotti, C., and Ortin, 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.
Martinez, J., Ren, Y.G., Nilsson, P., Ehrenberg, M. and Virtanen, A. 2001. The mRNA cap structure stimulates rate of poly(A) removal and amplifies processivity of degradation. *J Biol Chem* 276:27923–27829.
Martinez, J., Ren, Y.G., Thuresson, A.C., Hellman, U., Astrom, J., and Virtanen, A. 2000. A 54-kDa fragment of the Poly(A)-specific ribonuclease is an oligomeric, processive, and cap-interacting Poly(A)-specific 3′ exonuclease. *J Biol Chem* 275:24222–24230.
Marzluff, W.F. and Duronio, R.J. 2002. Histone mRNA expression:multiple levels of cell cycle regulation and important developmental consequences. *Curr Opin Cell Biol* 14:692–699.
Maton, G., Thibier, C., Castro, A., Lorca, T., Prigent, C., and Jessus, C. 2003. Cdc2-cyclin B triggers H3 kinase activation of Aurora-A in *Xenopus* oocytes. *J Biol Chem* 278:21439–21449.
Matsumoto, K., Wassarman, K.M., and Wolffe, A.P. 1998. Nuclear history of a pre-mRNA determines the translational activity of cytoplasmic mRNA. *EMBO J* 17:2107–2121.
Matsumoto, K. and Wolffe, A.P. 1998. Gene regulation by Y-box proteins: coupling control of transcription and translation. *Trends Cell Biol* 8:318–323.
Matsuo, H., Li, H., McGuire, A.M., Fletcher, C.M., Gingras, A.C., Sonenberg, N., and Wagner, G. 1997. Structure of translation factor eIF4E bound to m7GDP and interaction with 4E-binding protein. *Nat Struct Biol* 4:717–724.
Matunis, E.L., Matunis, M.J., and Dreyfuss, G. 1992. Characterization of the major hnRNP proteins from *Drosophila melanogaster*. *J Cell Biol* 116:257–269.
McCrae, M.A. and McCorquodale, J.G. 1983. Molecular biology of rotaviruses. V. Terminal structure of viral RNA species. *Virology* 126:204–212.
McGrew, L.L., Dworkin-Rastl, E., Dworkin, M.B., and Richter, J.D. 1989. Poly(A) elongation during *Xenopus* oocyte maturation is required for translational recruitment and is mediated by a short sequence element. *Genes Dev* 3:803–815.
Melo, E.O., Dhalia, R., Martins de Sa, C., Standart, N., and de Melo Neto, O.P. 2003. Identification of a C-terminal PABP-PABP interaction domain: Role in cooperative binding to poly(A) and efficient cap distal translational repression. *J Biol Chem* 278:46357–46368.
Melton, D. 1987. Translocation of a localised maternal mRNA to the vegetal pole of *Xenopus* oocytes. *Nature* 328:80–82.
Mendez, R., Barnard, D., and Richter, J.D. 2002. Differential mRNA translation and meiotic progression require cdc2-mediated CPEB destruction. *EMBO J* 21:1833–1844.
Mendez, R., Hake, L.E., Andresson, T., Littlepage, L.E., Ruderman, J.V., and Richter, J.D. 2000a. Phosphorylation of CPE binding factor by Eg2 regulates translation of *c-mos* mRNA. *Nature* 404:302–307.
Mendez, R., Murthy, K.G.K., Ryan, K., Manley, J.L., and Richter, J.D. 2000b. Phosphorylation of CPEB by Eg2 mediates the recruitment of CPSF into an active cytoplasmic polyadenylation complex. *Mol Cell* 6:1253–1259.
Michel, Y.M., Poncet, D., Piron, M., Kean, K.M., and Borman, A.M. 2000. Cap-Poly(A) synergy in mammalian cell-free extracts. Investigation

of the requirements for poly(A)-mediated stimulation of translation initiation. *J Biol Chem* 275:32268–32276.
Micklem, D.R., Adams, J., Grunert, S., and St. Johnston, D. 2000. Distinct roles of two conserved Staufen domains in *oskar* mRNA localization and translation. *EMBO J* 19:1366–1377.
Micklem, D.R., Dasgupta, R., Elliott, H., Gergely, F., Davidson, C., Brand, A., Gonzalez-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.
Minshall, N. and Standart, N. 2004. The active form of Xp54 RNA helicase in translational repression is an RNA-mediated oligomer. *Nucleic Acids Res* 32:1325–1334.
Minshall, N., Thom, G., and Standart, N. 2001. A conserved role of a DEAD box helicase in mRNA masking. *RNA* 7:1728–1742.
Minshall, N., Walker, J., Dale, M., and Standart, N. 1999. Dual roles of p82, the clam CPEB homolog, in cytoplasmic polyadenylation and translational masking. *RNA* 5:27–38.
Mohr, S.E., Dillon, S.T., and Boswell, R.E. 2001. The RNA-binding protein Tsunagi interacts with Mago Nashi to establish polarity and localize *oskar* mRNA during *Drosophila* oogenesis. *Genes Dev* 15:2886–2899.
Monshausen, M., Putz, U., Rehbein, M., Scweizer, M., DesGroseillers, L., Kuhl, D., Richter, D., and Kindler, S. 2001. Two rat brain staufen isoforms differentially bind RNA. *J Neurochem* 76:155–165.
Moore, F.L., Jaruzelska, J., Fox, M.S., Urano, J., Firpo, M.T., Turek, P.J., Dorfman, D.M., and Pera, R.A.R. 2003. Human pumilio-2 is expressed in embryonic stem cells and germ cells and interacts with DAZ (Deleted in AZoospermia) and DAZ-like proteins. *Proc Natl Acad Sci USA* 100:538–543.
Mowry, K. 1996. Complex formation between stage-specific oocyte factors and a *Xenopus* mRNA localization element. *Proc Natl Acad Sci USA* 93:14608–14613.
Mowry, K.L. and Melton, D.A. 1992 Vegetal messenger RNA localization directed by a 340-nt RNA sequence element in *Xenopus* oocytes. *Science* 255:991–994.
Müeller-Pillasch, F., Pohl, B., Wilda, M., Lacher, U., Beil, M., Wallrapp, C., Hameister, H., Knochel, W., Adler, G., and Gress, T.M. 1999. Expression of the highly conserved RNA binding protein KOC in embryogenesis. *Mech Dev* 88:95–99.
Murata, Y. and Wharton, R. 1995. Binding of pumilio to maternal *hunchback* mRNA is required for posterior patterning in *Drosophila* embryos. *Cell* 80:747–756.
Nakahata, S., Katsu, Y., Mita, K., Inoue, K., Y., N., and Yamashita, M. 2001. Biochemical identification of *Xenopus* Pumilio as a sequence-specific cyclin B1 mRNA-binding protein that physically interacts with a Nanos homolog (Xcat-2) and a cytoplasmic polyadenylation element-binding protein (CPEB). *J Biol Chem* 276:20945–20953.
Nakahata, S., Kotani, T., Mita, K., Kawasaki, T., Katsu, Y., Nagahama, Y., and Yamashita, M. 2003. Involvement of *Xenopus* Pumilio in the translational regulation that is specific to cyclin B1 mRNA during oocyte maturation. *Mech Dev* 120:865–880.
Nakamura, A., Amikura, R., Hanyu, K., and Kobayashi, S. 2001. Me31B silences translation of oocyte-localising RNAs through the formation of cytoplasmic RNP complex during *Drosophila* oogenesis. *Development* 128:3233–3242.
Nakamura, A., Sato, K., and Hanyu-Nakamura, K. 2004. *Drosophila* Cup Is an eIF4E Binding Protein that Associates with Bruno and Regulates *oskar* mRNA Translation in Oogenesis. *Dev Cell* 6:69–78.
Navarro, R.E., Shim, E.Y., Kohara, Y., Singson, A., and Blackwell, T.K. 2001. *cgh-1* a conserved predicted helicase required for gametogenesis and protection from physiological germline apoptosis in *C. elegans Dvelopment* 128:3221–3232.
Nekrasov, M.P., Ivshina, M.P., Chernov, K.G., Kovrigina, E.A., Evdokimova, V.M., Thomas, A.A., Hershey, J.W., and Ovchinnikov, L.P. 2003. The mRNA-binding protein YB-1 (p50) prevents association of the eukaryotic initiation factor eIF4G with mRNA and inhibits protein synthesis at the initiation stage. *J Biol Chem* 278:13936–13943.
Nelson, M.R., Leidal, A.M., and Smibert, C.A. 2004. *Drosophila* Cup is an eIF4E-binding protein that functions in Smaug-mediated translational repression. *EMBO J* 23:150–159.
Neuman-Silberberg, F.S. and Schupbach, T. 1993. The *Drosophila* dorsoventral patterning gene gurken produces a dorsally localized RNA and encodes a TGF alpha-like protein. *Cell* 75:165–174.
Nielsen, F.C., Nielsen, J., Kristensen, M.A., Koch, G., and Christiansen, J. 2002. Cytoplasmic trafficking of IGF-II mRNA-binding proteins by conserved KH domains. *J Cell Sci* 115:2087–2297.
Nielsen, J., Adolph, S.K., Rajert-De Meyts, E., Lykke-Andersen, J., Koch, G., Christiansen, J., and Nielsen, F.C. 2003. Nuclear transit of the human zipcode-binding protein IMP1. *Biochem J* 376:383–391.
Nielsen, J., Christiansen, J., Lykke-Andersen, J., Johnsen, A.H., Wewer, U.M., and Nielsen, F.C. 1999. A family of insulin-like growth factor II mRNA-binding proteins represses translation in late development. *Mol Cell Biol* 9:1262–1270.
Nielsen, J., Kristensen, M.A., Willemoes, M., Nielsen, F.C., and Christiansen, J. 2004. Sequential dimerization of human zipcode-binding protein IMP1 on RNA: a cooperative mechanism providing RNP stability. *Nucl Acids Res* 32:4368–4376.
Nielsen, J., Nielsen, F.C., Jakobsen, R.K., and Christiansen, J. 2000. The biphasic expression of IMP/Vg1-RBP is conserved between vertebrates and *Drosophila*. *Mech Dev* 96:129–132.
Niessing, D., Blanke, S., and Jackle, H. 2002. Bicoid associates with the 5′-cap-bound complex of *caudal* mRNA and represses translation. *Genes Dev* 16:2576–2582.
Niessing, D., Dostatni, N., Jackle, H., and Rivera-Pomar, R. 1999. Sequence interval within the PEST motif of Bicoid is important for translational repression of *caudal* mRNA in the anterior region of the *Drosophila* embryo. *EMBO J* 18:1966–1973.
Niessing, D., W., D., Sprenger, F., Taubert, H., Jackle, H, and Rivera-Pomar, R. 2000. Homeodomain position 54 specifies transcriptional versus translational control by Bicoid. *Mol Cell* 5:395–401.
Norvell, A., Kelley, R.L., Wher, K., and Schupbach, T. 1999. Specific isoforms of Squid, a *Drosophila* hnRNP, perform distinct roles in *gurken* localization during oogenesis. *Genes and Dev* 13:864–876.
Nott, A., Le Hir, H., and Moore, M.J. 2004. Splicing enhances translation in mammalian cells: an additional function of the exon junction complex. *Genes Dev* 18:210–222.
Nott, A., Meislin, S.H., and Moore, M.J. 2003. A quantitative analysis of intron effects on mammalian gene expression. *RNA* 607–617.
Ohashi, S., Koike, K., Omori, A., Ichinose, S., Ohara, S., Kobayashi, S., Sato, T.-A., and Anzai, K. 2002. Identification of mRNA/protein (mRNP) complexes containing Purα, mStaufen, Fragile X protein, and Myosin Va and their association with rough endoplasmic reticulum equipped with a kinesin motor. *J Biol Chem* 277:37804–37810.
Oleynikov, Y. and Singer, R.H. 2003. Real-time visualization of ZBP1 association with β-actin mRNA during transcription and localization. *Curr Biol* 13:199–207.
Olivas, W. and Parker, R. 2000. The Puf3 protein is a transcript-specific regulator of mRNA degradation in yeast. *EMBO J* 19:6602–6611.
Ostareck, D.H., Ostareck-Lederer, A., Shatsky, I.N., and Hentze, M.W. 2001. Lipoxygenase mRNA silencing in erythroid differentiation:The 3′ UTR regulatory complex controls 60S ribosomal subunit joining. *Cell* 104:281–290.
Ostareck-Lederer, A., D.H., O., Cans, C., Neubauer, G., Bomsztyk, K., Superti-Furga, G., and Hentze, M. 2002. c-Src-mediated phosphorylation of hnRNP K drives translational activation of specifically silenced mRNAs. *Mol Cell Biol* 22:4535–4543.
Ostareck-Lederer, A. and Ostareck, D.H. 2004. Control of mRNA translation and stability in haematopoietic cells:the function of hnRNPs K and E1/E2. *Biol Cell* 96:407–411.
Ostareck-Lederer, A., Ostareck, D.H., and Hentze, M.W. 1998. Cytoplasmic regulatory functions of the KH-domain proteins hnRNP K and E1/E2. *Trends Biochem Sci* 23:409–411.
Ostareck-Lederer, A., Ostareck, D.H, Standart, N., and Thiele, B.J. 1994. Translation of 15-lipoxygenase mRNA is controlled by a protein that

binds to a repeated sequence in the 3′ untranslated region. *EMBO J* 13:1476–1481.

Otero, L.J., Ashe, M.P., and Sachs, A.B. 1999. The yeast poly(A)-binding protein Pab1p stimulates *in vitro* poly (A)-dependent and cap-dependent translation by distinct mechanisms. *EMBO J* 18:3153–3163.

Otero, L.J., Devaux, A., and Standart, N. 2001. A 250-nucleotide UA-rich element in the 3′ untranslated region of *Xenopus laevis* Vg1 mRNA represses translation both in vivo and in vitro. *RNA* 7:1–15.

Paillard, L., Legagneux, V., Maniey, D., and Osborne, H.B. 2002. c-Jun ARE targets mRNA deadenylation by an EDEN-BP (embryo deadenylation element-binding protein)-dependent pathway. *J Biol Chem* 277:3232–3235.

Paillard, L., Legagneux, V., and Osborne, H.B. 2003. A functional deadenylation assay identifies human CUG-BP as a deadenylation factor. *Biol Cell* 95:107–113.

Paillard, L., Omilli, F., Legagneux, V., Bassez, T., Maniey, D., and Osborne, H.B. 1998. EDEN and EDEN-BP, a cis element and an associated factor that mediate sequence-specific mRNA deadenylation in Xenopus embryos. *EMBO J* 17:278–287.

Paillard, L. and Osborne, H.B. 2003. East of EDEN was a poly(A) tail. *Biol Cell* 95:211–219.

Palacios, I.M., Gatfield, D., St. Johnston, D., and Izaurralde, E. 2004. An eIF4AIII-containing complex required for mRNA localization and nonsense-mediated mRNA decay. *Nature* 427:753–757.

Palacios, I.M. and St. Johnston, D. 2001. Getting the message across: the intracellular localization of mRNAs in higher eukaryotes. *Annu Rev Cell Dev Biol* 17:569–614.

Palacios, I.M. and St. Johnston, D. 2002. *Kinesin light chain*-independent function of the *Kinesin heavy chain* in cytoplasmic streaming and posterior localization in the *Drosophila* oocyte. *Development* 129:5473–5485.

Paris, J., Osborne, H.B., Couturier, A., Le Guellec, R., and Philippe, M. 1988. Changes in the polyadenylation of specific stable RNA during the early development of *Xenopus laevis*. *Gene* 72:169–176.

Paris, J. and Philippe, M. 1990. Poly(A) metabolism and polysomal recruitment of mRNAs during early *Xenopus* development. *Dev Biol* 140:221–224.

Parisi, M. and Lin, H. 2000. Translational repression: a duet of Nanos and Pumilio. *Curr Biol* 10:R81–R83.

Parker, R. and Song, H. 2004. The enzymes and control of eukaryotic mRNA turnover. *Nat Struct Mol Biol* 11:121–127.

Pause, A., Belsham, G., Gingras, A.-C., Donzé, O., Lin, T.-A., Lawrence Jr, J., and Sonenberg, N. 1994. Insulin-dependent stimulation of protein synthesis by phosphorylation of a regulator of 5′-cap function. *Nature* 371:762–767.

Pfeiffer, D.C. and Gard, D.L. 1999. Microtubules in *Xenopus* oocytes are oriented with their minus-ends toward the cortex. *Cell Motility Cytoskeleton* 44:34–43.

Piron, M., Vende, P., Cohen, J., and Poncet, D. 1998. Rotavirus RNA-binding protein NSP3 interacts with eIF4GI and evicts the poly(A) binding protein from eIF4F. *EMBO J* 17:5811–5821.

Poncet, D., Aponte, C., and Cohen, J. 1993. Rotavirus protein NSP3 (NS34) is bound to the 3′ end consensus sequence of viral mRNAs in infected cells. *J Virol* 67:3159–3165.

Preiss, T. and Hentze, M.W. 1998. Dual function of the messenger RNA cap structure in poly(A)-tail-promoted translation in yeast. *Nature* 392:516–520.

Preiss, T. and Hentze, M.W. 2003. Starting the protein synthesis machine: eukaryotic translation initiation. *BioEssays* 25:1201–1211.

Proudfoot, N.J., Furger, A., and Dye, M.J. 2002. Integrating mRNA processing with transcription. *Cell* 108:501–512.

Proweller, A. and Butler, S. 1994. Efficient translation of poly(A)-deficient mRNAs in *Saccharomyces cerevisiae. Genes Dev* 8:2629–2640.

Ptushkina, M., von der Haar, T., Karim, M.M., Hughes, J.M., and McCarthy, J.E. 1999. Repressor binding to a dorsal regulatory site traps human eIF4E in a high cap-affinity state. *EMBO J* 18:4068–4075.

Pyronnet, S., Dostie, J., and Sonenberg, N. 2001. Suppression of cap-dependent translation in mitosis. *Genes Dev* 15:2083–2093.

Ramos, A., Grunert, 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.

Read, R.L., Martinho, R.G., Wang, S.W., Carr, A.M., and Norbury, C.J. 2002. Cytoplasmic poly(A) polymerases mediate cellular responses to S phase arrest. *Proc Natl Acad Sci USA* 99:12079–12084.

Rehbein, M., Wege, K., Buck, F., Schweizer, M., Richter, D., and Kindler, S. 2002. Molecular characterization of MARTA1, a protein interacting with the dendritic targeting element of MAP2 mRNAs. *J Neurochem* 82:1039–1046.

Ren, Y.G., Martinez, J., and Virtanen, A. 2002. Identification of the active site of poly(A)-specific ribonuclease by site-directed mutagenesis and Fe(2+)-mediated cleavage. *J Biol Chem* 277:5982–5987.

Reverdatto, S.V., Dutko, J.A., Chekanova, J.A., Hamilton, D.A., and Belostotsky, D.A. 2004. mRNA deadenylation by PARN is essential for embryogenesis in higher plants. *RNA* 10:1200–1214.

Reverte, C.G., Ahearn, M.D., and Hake, L.E. 2001. CPEB degradation during *Xenopus* oocyte maturation requires a PEST domain and the 26S proteasome. *Dev Biol* 231:447–458.

Richter, J.D. 2000. Influence of polyadenylation-induced translation on metazoan development and neuronal synaptic function. In: *Translational control of gene expression*. Sonenberg, N., Hershey, J., and Mathews, M. (eds.), pp. 785–806. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, New York.

Rivera-Pomar, R., Niessing, D., Schmidt-Ott, U., Gehring, W.J., and Jackle, H. 1996. RNA binding and translational suppression by *bicoid. Nature* 379:746–749.

Rocak, S. and Linder, P. 2004. DEAD-box proteins: the driving forces behind RNA metabolism. *Nat Rev Mol Cell Biol* 5:232–241.

Roegiers, F. and Jan, Y.N. 2000. Staufen:a common component of mRNA transport in oocytes and neurons? *Trends Cell Biol* 10:220–224.

Rosenthal, E.T. and Wilt, F.H. 1986. Patterns of maternal messenger RNA accumulation and adenylation during oogenesis in *Urechis caupo*. *Dev Biol* 117:55–63.

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

Ryder, S.P., Frater, L.A., Abramovitz, D.L., Goodwin, E.B., and Williamson, J.R. 2004. RNA target specificity of the STAR/GSG domain post-transcriptional regulatory protein GLD-1. *Nat Struct Mol Biol* 11:20–28.

Saccomanno, L., Loushin, C., Jan, E., Punkay, E., Artzt, K., and Goodwin, E.B. 1999. The STAR protein QKI-6 is a translational repressor. *Proc Natl Acad Sci USA* 96:12605–12610.

Sachs, A. 2000. Physical and functional interactions between the mRNA cap structure and the poly(A) tail. In: *Translational control of gene expression*. Sonenberg, N., Hershey, J., and Mathews, M. (eds.), Cold Spring Harbor Laboratory Press, Cold Spring Harbor, New York.

Sachs, A.B., Davis, R.W., and Kornberg, R.D. 1987. A single domain of yeast poly(A)-binding protein is necessary and sufficient for RNA binding and cell viability. *Mol Cell Biol* 7:3268–3276.

Saitoh, S., Chabes, A., McDonald, W.H., Thelander, L., Yates, J.R., and Russell, P. 2002. Cid13 is a cytoplasmic poly(A) polymerase that regulates ribonucleotide reductase mRNA. *Cell* 109:563–573.

Sallés, F.J., Darrow, A.L., O'Connell, M.L., and Strickland, S. 1992. Isolation of novel murine maternal mRNAs regulated by cytoplasmic polyadenylation. *Genes Dev* 6:1202–1212.

Sallés, F.J., Lieberfarb, M.E., Wreden, C., Gergen, J.P., and Strickland, S. 1994. Coordinate initiation of *Drosophila* development by regulated polyadenylation of maternal messenger RNAs. *Science* 266:1996–1999.

Sanchez, R. and Marzluff, W.F. 2002. The stem-loop binding protein is required for efficient translation of histone mRNA *in vivo* and *in vitro. Mol Cell Biol* 22:7093–7104.

Sanchez, R. and Marzluff, W.F. 2004. The oligo(A) tail on histone mRNA plays an active role in translational silencing of histone mRNA during *Xenopus* oogenesis. *Mol Cell Biol* 24:2513–2525.
Sanford, J.R., Gray, N.K., Beckmann, K., and Caceres, J.F. 2004. A novel role for shuttling SR proteins in mRNA translation. *Genes Dev* 18: 755–768.
Saunders, L.R. and Barber, G.N. 2003. The dsRNA binding protein family: critical roles, diverse cellular functions. *FASEB J* 17:961–983.
Saunders, P.T., Pathirana, S., Maguire, S.M., Doyle, M., Wood, T., and Bownes, M. 2000. Mouse staufen genes are expressed in germ cells during oogenesis and spermatogenesis. *Moll Hum Reprod* 6:983–991.
Schmucker, D., Jackle, H., and Gaul, U. 1997. Genetic analysis of the larval optic nerve projection in *Drosophila*. *Development* 124: 937–948.
Schnorrer, F., Bohmann, K., and Nusslein-Volhard, C. 2000. The molecular motor dynein is involved in targeting *swallow* and *bicoid* RNA to the anterior pole of *Drosophila* oocytes. *Nat Cell Biol* 2:185–190.
Schwartz, S.P., Aisenthal, L., Elisha, Z., Oberman, F., and Yisraeli, J.K. 1992. A 69-kDa RNA-binding protein from *Xenopus* oocytes recognises a common motif in two vegetally localised maternal mRNAs. *Proc Natl Acad Sci USA* 89:11895–11899.
Schweers, B.A., Walters, K.J., and Stern, M. 2002. The *Drosophila melanogaster* translational repressor Pumilio regulates neuronal excitability. *Genetics* 161:1177–1185.
Searfoss, A. and Wickner, R. 2000. 3′poly(A) is dispensable for translation. *Proc Natl Acad Sci USA* 97:9133–9137.
Sheets, M., Fox, C., Hunt, T., Vande Woude, G., and Wickens, M. 1994. The 3′-untranslated regions of *c-mos* and cyclin mRNAs stimulate translation by regulating cytoplasmic polyadenylation. *Genes Dev* 8:926–938.
Sheets, M.D., Wu, M., and Wickens, M. 1995. Polyadenylation of *c-mos* mRNA as a control point in *Xenopus* meiotic maturation. *Nature* 374:511–516.
Sheth, U. and Parker, R. 2003. Decapping and decay of messenger RNA occur in cytoplasmic processing bodies. *Science* 300:805–808.
Shibuya, T., Tange, T.O., Sonenberg, N., and Moore, M.J. 2004. eIF4AIII binds spliced mRNA in the exon junction complex and is essential for nonsense-mediated decay. *Nat Struct Mol Biol* 11:346–351.
Shyu, A.-B., Belasco, J.G., and Greenberg, M.E. 1991. Two distinct destabilizing elements in the c-fos message trigger deadenylation as a first step in rapid mRNA decay. *Genes and Dev* 5:221–231.
Si, K., Giustetto, M., Etkin, A., Hsu, R., Janisiewicz, A.M., Miniaci, M.C., Kim, J.H., Zhu, H., and Kandel, E.R. 2003a. A neuronal isoform of CPEB regulates local protein synthesis and stabilizes synapse-specific long-term facilitation in Aplysia. *Cell* 115:893–904.
Si, K., Lindquist, S., and Kandel, E.R. 2003b. A neuronal isoform of the aplysia CPEB has prion-like properties. *Cell* 115:879–891.
Simon, R. and Richter, J. 1994. Further analysis of cytoplasmic polyadenylation in *Xenopus* embryos and identification of embryonic cytoplasmic polyadenylation element-binding proteins. *Mol Cell Biol* 14:7867–7875.
Simon, R., Tassan, J.-P., and Richter, J.D. 1992. Translational control by poly(A) elongation during *Xenopus* development: differential repression and enhancement by a novel cytoplasmic polyadenylation element. *Genes Dev* 6:2580–2591.
Simon, R., Wu, L., and Richter, J.D. 1996. Cytoplasmic polyadenylation of activin receptor mRNA and the control of pattern formation in *Xenopus* development. *Dev Biol* 179:239–250.
Slack, F.J. and Ruvkun, G. 1998. A novel repeat domain that is often associated with RING finger and B-box motifs. *Trends Biochem Sci* 23:474–475.
Sladic, R.T., Lagnado, C.A., Bagley, C.J., and Goodall, G.J. 2004. Human PABP binds AU-rich RNA via RNA-binding domains 3 and 4. *Eur J Biochem* 271:450–4457.
Smibert, C., Lie, Y., Shillinglaw, W., Henzel, W., and Macdonald, P. 1999. Smaug, a novel and conserved protein, contributes to repression of *nanos* mRNA translation *in vitro*. *RNA* 5:1535–1547.
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.
Smillie, D.A. and Sommerville, J. 2002. RNA helicase p54 (DDX6) is a shuttling protein involved in nuclear assembly of stored mRNP particles. *J Cell Sci* 115:395–407.
Snee, M., Kidd, G.J., Munro, T.P., and Smith, R. 2002. RNA trafficking and stabilization elements associate with multiple brain proteins. *J Cell Sci* 115:4661–4669.
Soller, M. and White, K. 2004. ELAV. *Curr Biol* 14:R53.
Sommerville, J. 1999. Activities of cold-shock domain proteins in translation control. *BioEssays* 21:319–325.
Sonoda, J. and Wharton, R.P. 2001. *Drosophila* Brain Tumor is a translational repressor. *Genes and Dev* 15:762–773.
Spassov, D.S. and Jurecic, R. 2003. The PUF family of RNA-binding proteins: does evolutionarily conserved structure equal conserved function? *IUBMB Life* 55:359–366.
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.
Standart, N., Dale, M., Stewart, E., and Hunt, T. 1990. Maternal mRNA from clam oocytes can be specifically unmasked in vitro by antisense RNA complementary to the 3′-untranslated region. *Genes Dev* 4:2157–2168.
Standart, N., Minshull, J., Pines, J., and Hunt, T. 1987. Cyclin synthesis, modification and destruction during meiotic maturation of the starfish oocyte. *Dev Biol* 124:248–258.
Stebbins-Boaz, B., Cao, Q., de Moor, C.H., Mendez, R., and Richter, J.D. 1999. Maskin is a CPEB-associated factor that transiently interacts with eIF-4E. *Mol Cell* 4:1017–1027.
Stebbins-Boaz, B., Hake, L.E., and Richter, J.D. 1996. CPEB controls the cytoplasmic polyadenylation of cyclin, Cdk2 and *c-mos* mRNAs and is necessary for oocyte maturation in *Xenopus EMBO J* 15:2582–2592.
Stebbins-Boaz, B. and Richter, J.D. 1994. Multiple sequence elements and a maternal mRNA product control cdk2 RNA polyadenylation and translation during early *Xenopus* development. *Mol Cell Biol* 14:5670–5880.
Stennard, F., Carnac, G., and Gurdon, J.B. 1996. The *Xenopus* T-box gene, *Antipodean*, encodes a vegetally localised maternal mRNA and can trigger mesoderm formation. *Development* 122:4179–4188.
Steward, O. and Schuman, E. 2003. Compartmentalized synthesis and degradation of proteins in neurons. *Neuron* 40:347–359.
Stutz, A., Conne, B., Huarte, J., Gubler, P., Völkel, V., Flandin, P., and Vassalli, J.D. 1998. Masking, unmasking, and regulated polyadenylation cooperate in the translational control of a dormant mRNA in mouse oocytes. *Genes Dev* 12:2535–2548.
Stutz, A., Huarte, J., Gubler, P., Conne, B., Belin, D., and Vassalli, J.-D. 1997. In vivo antisense oligodeoxynucleotide mapping reveals masked regulatory elements in an mRNA dormant in mouse oocytes. *Mol Cell Biol* 17:1759–1767.
Tadauchi, T., Matsumoto, K., Herskowitz, I., and Irie, K. 2001. Post-transcriptional regulation through the HO 3′UTR by Mpt5, a yeast homolog of Pumilio and FBF. *EMBO J* 20:552-561.
Tang, S.J., Meulemans, D., Vazquez, L., Colaco, N., and Schuman, E. 2001. A role for a rat homologue of staufen in the transport of RNA to neuronal dendrites. *Neuron* 32:463–475.
Tange, T.O., Nott, A., and Moore, M.J. 2004. The ever-increasing complexities of the exon junction complex. *Curr Opin Cell Biol* 16:279–284.
Tannahill, D. and Melton, D.A. 1989. Localized synthesis of the Vg1 protein during early *Xenopus* development. *Development* 106:775–785.
Tanner, N.K., Cordin, O., Banroques, J., Doere, M., and Linder, P. 2003. The Q motif: A newly identified motif in DEAD box helicases may regulate ATP binding and hydrolysis. *Mol Cell* 11:127–138.
Tarun, Jr., S. and Sachs, A. 1995. A common function for mRNA 5′ and 3′ ends in translation initiation in yeast. *Genes Dev* 9: 2997–3007.

Tarun, S.Z., Jr. and Sachs, A.B. 1996. Association of the yeast poly(A) tail binding protein with translation initiation factor eIF4G. *EMBO J* 15:7168–7177.

Tassan, J.P., Le Guellec, K., Kress, M., Faure, M., Camonis, J., Jacquet, M., and Philippe, M. 1993. In *Xenopus laevis*, the product of a developmentally regulated mRNA is structurally and functionally homologous to a *Saccharomyces cerevisiae* protein involved in translation fidelity. *Mol Cell Biol* 13:2815–2821.

Tay, J., Hodgman, R., and Richter, J. 2000. The control of cyclin B1 mRNA translation during mouse oocyte maturation. *Dev Biol* 221: 1–9.

Tay, J., Hodgman, R., Sarkissian, M., and Richter, J.D. 2003. Regulated CPEB phosphorylation during meiotic progression suggests a mechanism for temporal control of maternal mRNA translation. *Genes and Dev* 17:1457–1462.

Tay, J. and Richter, J.D. 2001. Germ cell differentiation and synaptonemal complex formation are disrupted in CPEB knockout mice. *Dev Cell* 1:201–213.

Tekotte, H. and Davis, I. 2002. Intracellular mRNA localization: motors move messages. *Trends Genet* 18:636–642.

Temme, C., Zaessinger, S., Meyer, S., Simonelig, M., and Wahle, E. 2004. A complex containing the CCR4 and CAF1 proteins is involved in mRNA deadenylation in Drosophila. *EMBO J* 23:2862–2871.

Tessier, C.R., Doyle, G.A., Clark, B.A., Pitot, H.C., and Ross, J. 2004. Mammary tumor induction in transgenic mice expressing an RNA-binding protein. *Cancer Res* 64:209–214.

Theis, M., Si, K., and Kandel, E.R. 2003. Two previously undescribed members of the mouse CPEB family of genes and their inducible expression in the principal cell layers of the hippocampus. *Proc Natl Acad Sci USA* 100:9602–9607.

Theurkauf, W.E., Smiley, S., Wong, M.L., and Alberts, B.M. 1992. Reorganization of the cytoskeleton during *Drosophila* oogenesis: implications for axis specification and intercellular transport. *Development* 115:923–936.

Thom, G., Minshall, N., Git, A., Argasinska, J., and Standart, N. 2003. Role of cdc2 kinase phosphorylation and conserved N-terminal proteolysis motifs in cytoplasmic polyadenylation-element-binding protein (CPEB) complex dissociation and degradation. *Biochem J* 370:91–100.

Thompson, S., Goodwin, E., and Wickens, M. 2000. Rapid deadenylation and poly(A)-dependent translational repression mediated by the *Caenorhabditis elegans* tra-2 3′ untranslated region in the *Xenopus* embryos. *Mol Cell Biol* 20:2129–2137.

Thore, S., Mauxion, F., Seraphin, B., and Suck, D. 2003. X-ray structure and activity of the yeast Pop2 protein: a nuclease subunit of the mRNA deadenylase complex. *EMBO Rep* 4:1150–1155.

Tiruchinapalli, D.M., Oleynikov, Y., Kelic, S., Shenoy, S.M., Hartley, A., Stanton, P.K., Singer, R.H., and Bassell, G.J. 2003. Activity-dependent trafficking and dynamic localization of zipcode binding protein 1 and beta-actin mRNA in dendrites and spines of hippocampal neurons. *J Neurosci* 23:3251–3261.

Tseng-Rogenski, S.S.-I., Chong, J.-L., Thomas, C.B., Enomoto, S., Berman, J., and Chang, T.-H. 2003. Functional conservation of Dhh1p, a cytoplasmic DExD/H-box protein present in large complexes. *Nucleic Acids Res* 31:4995–5002.

Tucker, M., Staples, R.R., Valencia-Sanchez, M.A., Muhlrad, D., and Parker, R. 2002. Ccr4p is the catalytic subunit of a Ccr4p/Pop2p/Notp mRNA deadenylase complex in *Saccharomyces cerevisiae. EMBO J* 21:1427–1436.

Ueno, S. and Sagata, N. 2002. Requirement for both EDEN and AUUUA motifs in translational arrest of mos mRNA upon fertilization of *Xenopus* eggs. *Dev Biol* 250:156–167.

Valcarcel, J. and Gebauer, F. 1997. Post-transcriptional regulation: the dawn of PTB. *Curr Biol* 7:R705–708.

van Eeden, F.J., Palacios, I.M., Petronczki, M., Weston, M.J., and St. Johnston, D. 2001. Barentsz is essential for the posterior localization of *oskar* mRNA and colocalizes with it to the posterior pole. *J Cell Biol* 154:511–523.

Varani, G. and Nagai, K. 1998. RNA recognition by RNP proteins during RNA processing. *Annu Rev Biophys Biomol Struct* 27:407–445.

Varnum, S.M., Hurney, C.A., and Wormington, W.M. 1992. Maturation-specific deadenylation in *Xenopus* oocytes requires nuclear and cytoplasmic factors. *Dev Biol* 153:283–290.

Varnum, S.M. and Wormington, W.M. 1990. Deadenylation of maternal mRNAs during *Xenopus* oocyte maturation does not require specific cis-sequences: a default mechanism for translational control. *Genes Dev* 4:2278–2286.

Vende, P., Piron, M., Castagne, N., and Poncet, D. 2000. Efficient translation of rotavirus mRNA requires simultaneous interaction of NSP3 with the eukaryotic translation initiation factor eIF4G and the mRNA 3′end. *J Virol* 74:7064–7071.

Verrotti, A.C. and Wharton, R.P. 2000. Nanos interacts with Cup in the female germline of Drosophila. *Development* 127:5225–5232.

Voeltz, G.K., Ongkasuwan, J., Standart, N., and Steitz, J.A. 2001. A novel embryonic poly(A) binding protein, ePAB, regulates mRNA deadenylation in *Xenopus* egg extracts. *Genes Dev* 15:774–788.

Voeltz, G.K. and Steitz, J.A. 1998. AUUUA sequences direct mRNA deadenylation uncoupled from decay during *Xenopus* early development. *Mol Cell Biol* 18:7537–7545.

von der Haar, T., Ball, P.D., and McCarthy, J.E. 2000. Stabilization of eukaryotic initiation factor 4E binding to the mRNA 5′-Cap by domains of eIF4G. *J Biol Chem* 275:30551–30555.

Wagner, C., Palacios, I., Jaeger, L., St. Johnston, D., Ehresmann, B., Ehresmann, C., and Brunel, C. 2001. Dimerisation of the 3′UTR of *bicoid* mRNA involves a two-step mechanism. *J Mol Biol* 313:511–524.

Wahle, E. and Ruegsegger, U. 1999. 3′-End processing of pre-mRNA in eukaryotes. *FEMS Microbiol Rev* 23:277–295.

Wakiyama, M., Imataka, H., and Sonenberg, N. 2000. Interaction of eIF4G with poly(A)-binding protein stimulates translation and is critical for *Xenopus* oocyte maturation. *Curr Biol* 10:1147–1150.

Walker, J., Dale, M., and Standart, N. 1996. Unmasking mRNA in clam oocytes: Role of phosphorylation of a 3′ UTR masking element-binding protein at fertilization. *Dev Biol* 173:292–305.

Walker, J., Minshall, C., Hake, L., Richter, J., and Standart, N. 1999. The clam 3′UTR masking element-binding protein p82 is a member of the CPEB family. *RNA* 5:14–26.

Wang, C. and Lehmann, R. 1991. Nanos is the localized posterior determinant in Drosophila. *Cell* 66:637–647.

Wang, L., Eckmann, C.R., Kadyk, L.C., Wickens, M., and Kimble, J. 2002a. A regulatory cytoplasmic poly(A) polymerase in *Caenorhabditis elegans. Nature* 419:312–316.

Wang, X., McLachlan, J., Zamore, P.D., and Hall, T.M. 2002b. Modular recognition of RNA by a human pumilio-homology domain. *Cell* 110:501–512.

Wang, X., Zamore, P.D., and Hall, T.M. 2001. Crystal structure of a Pumilio homology domain. *Mol Cell* 7:855–865.

Wang, Z.F., Ingledue, T.C., Dominski, Z., Sanchez, R., and Marzluff, W.F. 1999. Two *Xenopus* proteins that bind the 3′ end of histone mRNA: implications for translational control of histone synthesis during oogenesis. *Mol Cell Biol* 19:835–845.

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.

Weeks, D.L. and Melton, D.A. 1987. A maternal mRNA localized to the vegetal hemisphere in *Xenopus* eggs codes for a growth factor related to TGF-β. *Cell* 51:861–867.

Welk, J.F., Charlesworth, A., Smith, G.D., and MacNicol, A.M. 2001. Identification and characterisation of the gene encoding human cytoplasmic polyadenylation element binding protein. *Gene* 263:113–120.

Wells, S.E., Hillner, P.E., Vale, R.D., and Sachs, A.B. 1998. Circularization of mRNA by eukaryotic translation initiation factors. *Mol Cell* 2:135–140.

Wessely, O. and De Robertis, E.M. 2000. The Xenopus homologue of Bicaudal-C is a localized maternal mRNA that can induce endoderm formation. *Development* 127:2053–2062.

Westmoreland, T.J., Olson, J.A., Saito, B.S., Huper, G., Marks, J.R., and Bennett, C.B. 2003. Dhh1 regulates the G1/S-checkpoint following DNA damage or BRCA1 expression in yeast. *J Surg Res* 113:62–73.

Wharton, R.P., J., S., Lee, T., Patterson, M., and Murata, Y. 1998. The pumilio RNA-binding domain is also a translational repressor. *Mol Cell* 1:863–872.

White, E.K., Moore-Jarrett, T., and Ruley, H.E. 2001. PUM2, a novel murine puf protein, and its consensus RNA-binding site. *RNA* 7:1855–1866.

Wickens, M., Bernstein, D.S., Kimble, J., and Parker, R. 2002. A PUF family portrait:3′UTR regulation as a way of life. *Trends Genet* 18:150–158.

Wickens, M., Goodwin, E., Kimble, J., Strickland, S., and Hentze, M. 2000. Translational control of developmental decisions. In: *Translational Control of Gene Expression*, pp. 295–370. Sonenberg, N., Hershey, J., and Mathews, M. (eds.). Cold Spring Harbor Laboratory Press, Cold Spring Harbor, New York.

Wickham, L., Duchaine, T., Luo, M., Nabi, I.R., and DesGroseillers, L. 1999. Mammalian Staufen is a double-stranded RNA-and tubulin-binding protein which localises to the rough endoplasmic reticulum. *Mol Cel Biol* 19:2220–2230.

Wilhelm, J., Vale, R., and Hegde, R. 2000a. Coordinate control of translation and localization of Vg1 mRNA in *Xenopus* oocytes. *Proc Natl Acad Sci USA* 97:13132–13137.

Wilhelm, J.E., Hilton, M., Amos, Q., and Henzel, W. 2003. Cup is an eIF4E binding protein required for both the translational repression of *oskar* and the recruitment of Barentsz. *J Cell Biol* 163:1197–1204.

Wilhelm, J.E., Mansfield, J., Hom-Booher, N., Wang, S., Turck, C.W., Hazelrigg, T., and R.D., V. 2000b. Isolation of a ribonucleoprotein complex involved in mRNA localization in *Drosophila* oocytes. *J Cell Biol* 148:427–440.

Wilkie, G.S., Dickson, K.S., and Gray, N.K. 2003. Regulation of mRNA translation by 5′- and 3′-UTR-binding factors. *Trends Biochem Sci* 28:182–188.

Woehlke, G. and Schliwa, M. 2000. Walking on two heads: the many talents of kinesin. *Nat Rev Mol Cell Biol* 1:50–58.

Wormington, M., Searfoss, A., and Hurney, C. 1996. Overexpression of poly(A) binding protein prevents maturation-specific deadenylation and translational inactivation in *Xenopus* oocytes. *EMBO J* 15:900–909.

Wreden, C., Verrotti, A.C., Schisa, J.A., Lieberfarb, M.E., and Strickland, S. 1997. *Nanos* and *pumilio* establish embryonic polarity in *Drosophila* by promoting posterior deadenylation of *hunchback* mRNA. *Development* 124:3015–3023.

Wu, L., Good, P., and Richter, J. 1997. The 36-kilodalton embryonic-type cytoplasmic polyadenylation element-binding protein in *Xenopus laevis* is ElrA, a member of the ELAV family of RNA-binding proteins. *Mol Cell Biol* 17:6402–6409.

Wu, L., Wells, D., Tay, J., Mendis, D., Abbott, M.-A., Barnitt, A., Quinlan, E., Heynen, A., Fallon, J.R., and Richter, J.D. 1998. CPEB-mediated cytoplasmic polyadenylation and the regulation of experience-dependent translation of α-CaMKII mRNA at synapses. *Neuron* 21:1129–1139.

Xie, J., Lee, J.A., Kress, T.L., Mowry, K.L., and Black, D.L. 2003. Protein kinase A phosphorylation modulates transport of the polypyrimidine tract-binding protein. *Proc Natl Acad Sci USA* 100:8776–8781.

Xu, L., Paulsen, J., Yoo, Y., Goodwin, E.B., and Strome, S. 2001. Caenorhabditis elegans MES-3 is a target of GLD-1 and functions epigenetically in germline development. *Genetics* 159:1007–1017.

Yaniv, K., Fainsod, A., Kalcheim, C., and Yisraeli, J.K. 2003. The RNA-binding protein Vg1 RBP is required for cell migration during early neural development. *Development* 130:5649–5661.

Yaniv, K. and Yisraeli, J.K. 2002. The involvement of a conserved family of RNA binding proteins in embryonic development and carcinogenesis. *Gene* 287:49–54.

Yano, T., De Quinto, S.L., Matsui, Y., Shevchenko, A., and Ephrussi, A. 2004. Hrp48, a *Drosophila* hnRNPA/B Homolog, Binds and Regulates Translation of *oskar* mRNA. *Dev Cell* 6:637–648.

Ye, B., Petritsch, C., Clark, I.E., Gavis, E.R., Jan, L.Y., and Jan, Y. 2004. Nanos and Pumilio are essential for dendrite morphogenesis in *Drosophila* peripheral neurons. *Curr Biol* 14:314–321.

Yisraeli, J.K., Sokol, S., and Melton, D.A. 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.

Yoon, Y.J. and Mowry, K.L. 2004. *Xenopus* Staufen is a component of a ribonucleoprotein complex containing Vg1 RNA and kinesin. *Development* 131:3035–3045.

Zamore, P.D., Williamson, J.R., and Lehmann, R. 1997. The Pumilio protein binds RNA through a conserved domain that defines a new class of RNA-binding proteins. *RNA* 3:1421–1433.

Zanier, K., Luyten, I., Crombie, C., Muller, B., Schumperli, D., Linge, J.P., Nilges, M., and Sattler, M. 2002. Structure of the histone mRNA hairpin required for cell cycle regulation of histone gene expression. *RNA* 8:29–46.

Zappavigna, V., Piccioni, F., Villaescusa, J.C., and Verrotti, A.C. 2004. Cup is a nucleocytoplasmic shuttling protein that interacts with the eukaryotic translation initiation factor 4E to modulate *Drosophila* ovary development. *Proc Natl Acad Sci USA* 101:14800–14805.

Zauner, W., Kratz, J., Staunton, J., Feick, P., and Wiche, G. 1992. Identification of two distinct microtubule binding domains on recombinant rat MAP1B. *Eur J Cell Biol* 57:66–74.

Zhang, B., Gallegos, M., Puoti, A., Durkin, E., Fields, S., Kimble, J., and Wickens, M.P. 1997. A conserved RNA-binding protein that regulates sexual fates in the *C. elegans* hermaphrodite germ line. *Nature* 390:477–484.

Zhang, H.L., Eom, T., Oleynikov, Y., Shenoy, S.M., Liebelt, D.A., Dictenberg, J.B., Singer, R.H., and Bassell, G.J. 2001. Neurotrophin-induced transport of a beta-actin mRNP complex increases β-actin levels and stimulates growth cone motility. *Neuron* 31:261–275.

Zhang, J., Houston, D.W., King, M.L., Payne, C., Wylie, C., and Heasman, J. 1998. The role of maternal VegT in establishing the primary germ layers in *Xenopus* embryos. *Cell* 94:515–524.

Zhang, Q., Yaniv, K., Oberman, F., Wolke, u., Git, A., Fromer, M., Taylor, W.L., Meyer, D., Standart, N., Raz, E., and Yisraeli, J.K. 1999a. Vg1 RBP intracellular distribution and evolutionarily conserved expression at multiple stages during development. *Mech Dev* 88:101–106.

Zhang, S., Williams, C.J., Wormington, M., Stevens, A., and Peltz, S.W. 1999b. Monitoring mRNA decapping activity. *METHODS:A Companion to Methods in Enzymology* 17:46–51.

Zhao, W.M., Jiang, C., Kroll, T.T., and Huber, P. 2001. A proline-rich protein binds to the localization element of *Xenopus* Vg1 mRNA and to ligands involved in actin polymerization. *EMBO J* 20:2315–2325.

Zhou, Y. and King, M.L. 2004. Sending RNAs into the future:RNA localization and germ cell fate. *IUBMB Life* 56:19–27.

Zhou, Z., Licklider, L.J., Gygi, S.P., and Reed, R. 2002. Comprehensive proteomic analysis of the human spliceosome. *Nature* 419:182–185.