PERSPECTIVE

Metazoan oocyte and early embryo development program: a progression through translation regulatory cascades

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All metazoans that reproduce sexually have the ability to form gametes. The two types of gametes, the egg and the sperm, arise from germ cells, undergo extensive differentiation, and are destined to unite. The outcome of their union, the zygote, maintains and propagates the characteristics of the species. The zygote inherits from the egg not only genetic material but also its cytoplasm, which supports the development of the early embryo through precise expression patterns of maternally inherited messages. The hierarchical organization of these translation regulatory mechanisms is unveiled in the report by Padmanabhan and Richter (2006) in this issue of *Genes & Development*.

Mechanisms for establishing the germline and carrying out oogenesis in evolutionarily distant animals exhibit certain common themes. Gametes develop from primordial germ cells (PGC) that are set aside during early embryogenesis (Matova and Cooley 2001). In most metazoans, PGCs have an extragonadal origin and migrate to reach the somatic gonad, where they proliferate by mitosis to form oocytes in females (Matova and Cooley 2001). Oocytes, in turn, enter meiosis, only to be arrested at the prophase of the first meiotic division (Sagata 1996). This first meiotic arrest may last up to a few years in *Xenopus* or several decades in humans, and is characterized by synthesis and storage of large quantities of dormant mRNA (LaMarca et al. 1973; Rodman and Bachvarova 1976). When later translated, these maternal mRNAs drive the oocyte's re-entry into meiosis (Gebauer et al. 1994; Stebbins-Boaz et al. 1996; Mendez et al. 2000) and control the rate of mitosis during the cleavage divisions after fertilization (Groisman et al. 2000; Oh et al. 2000; Uto and Sagata 2000).

The resumption of meiosis marks the onset of oocyte maturation and is stimulated by progesterone in *Xenopus* (Bayaa et al. 2000; Tian et al. 2000) and by gonadotropins in mouse and human (Faiman and Ryan 1967; Rao et al. 1974). In almost all vertebrates, nuclear and cytoplasmic changes associated with oocyte maturation are completed by the metaphase of the second meiotic division, when oocytes become arrested for a second time and await fertilization (Sagata 1996). A complex network of translational activation and repression of stored maternal mRNAs accompanies oocyte maturation (Gebauer et al. 1994; Stebbins-Boaz et al. 1996; Mendez et al. 2000; Oh et al. 2000), while transcription is limited at best.

The transcriptional silencing that begins with oocyte maturation persists during the initial mitotic divisions of the embryo, which, unlike any other, lack an appreciable G1 or G2 phase. In *Xenopus*, after 12 rapid synchronous cleavages, when the developing embryo is composed of ~4000 cells, the mid-blastula transition occurs and is characterized by lengthening of the cell cycle, inclusion of G1 and G2, and activation of zygotic transcription (Newport and Kirschner 1982a,b). In mouse and human, induction of transcription in the embryo occurs at the two-cell, and four- to eight-cell stages, respectively (Clegg and Piko 1982; Flach et al. 1982; Braude et al. 1988). Despite the earlier occurrence of zygotic transcription, activation of maternally inherited mRNAs in mammals seems to use translation mechanisms similar to those in other vertebrates (Richter 1999; Oh et al. 2000).

On a molecular level, it is known that meiotic reactivation is initiated by translation of specific maternal messages such as those encoding rapid inducer of G2/M progression in oocytes/Speedy (RINGO/Spy), cyclin B1, and cyclin-dependent protein kinase 2 (Cdk2) (Ferby et al. 1999; Mendez and Richter 2001; Eichenlaub-Ritter and Peschke 2002; Dekel 2005). Translation of the RINGO/Spy message is essential since the RINGO/Spy protein, a novel cell cycle regulator with unique kinase-binding and activation domains, is required to activate Cdk2 (Ferby et al. 1999; Lenormand et al. 1999; Terret et al. 2001; Cheng et al. 2005). The subsequent action of these gene products is followed by Aurora A/Eg2 protein kinase activation, which, in turn, promotes polyadenylation of specific transcripts including that of mos serine/threonine kinase (Fig. 1, see orange boxes). Mos is essen-

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tial as it activates a mitogen-activated protein kinase (MAPK) cascade that enables progression through oocyte maturation, maintains activation of a maturation-promoting complex, and is an important component of the cytostatic factor that arrests the matured egg in metaphase II to await fertilization (for review, see Castro et al. 2001; Gandolfi and Gandolfi 2001; Dekel 2005).

The best-studied mechanism regulating the translation of maternally derived mRNAs in the oocyte cytoplasm is polyadenylation. Not only is polyadenylation a nuclear processing event that fashions the 3′-end of almost all pre-mRNAs (Manley 1995; Wahle 1995), but it also takes place in the cytoplasm during oocyte maturation and early embryo development. Several 3′-untranslated region (UTR) motifs have been implicated in the regulation of polyadenylation of maternal mRNAs (Eichenlaub-Ritter and Peschke 2002). These include the cytoplasmic polyadenylation element (CPE), the Pumilio-binding element (PBE), and the embryonic deadenylation element (Gray and Wickens 1998). Additional control mechanisms involving AU-rich elements or microRNAs may also contribute either directly or in concert with the above elements (de Moor et al. 2005; Piccioni et al. 2005).

The article by Padmanabhan and Richter (2006) not only establishes RINGO/Spy as an early inducer of CPE-mediated translation following oocyte activation in *Xenopus*, but also suggests the presence of a regulatory cascade involving multiple mechanisms acting upon distinct 3′-UTRs to control the expression of maternal mRNAs in the oocyte. A predominant theme that emerges is the regulation of the regulators themselves; through an integration of successive translation control mechanisms, with one leading to regulation of the next, temporally precise consequences are generated that correlate with succeeding stages of the developmental program.

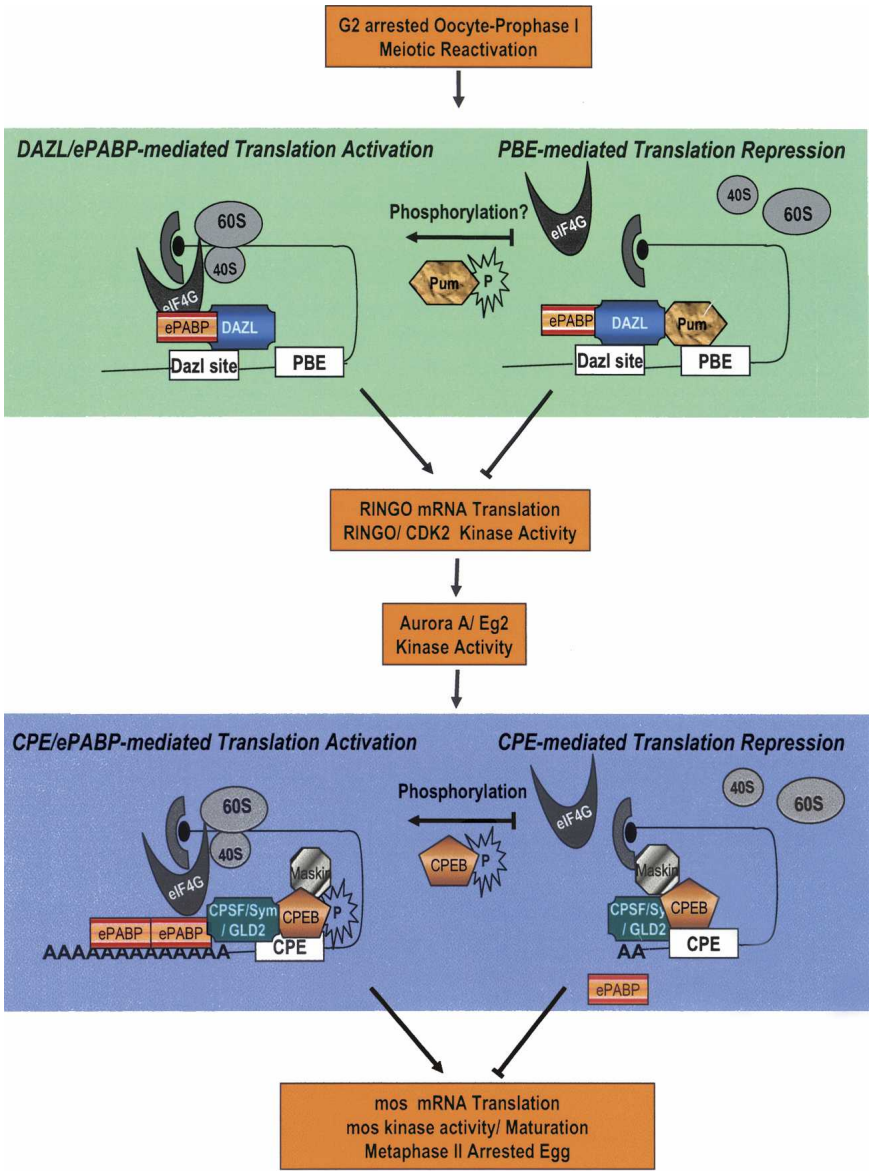

Figure 1. Maternal mRNA translation progresses through a tightly controlled cascade of translation regulatory mechanisms. Maternal mRNAs are regulated by 3′-UTR elements that serve to activate or repress through a hierarchical series of successive translation regulatory mechanisms. In the G2-arrested prophase I oocyte, meiotic reactivation occurs in response to signals such as progesterone. As shown in the green box, this causes loss of Pumilio 2 binding to the PBE and alleviation of Pumilio 2-mediated translation repression of PBE-bearing transcripts. Recruitment of DAZL/ePABP to such a PBE-bearing message, as in the case of the RINGO/Spy mRNA, throws the translational switch through interaction with the eIF4G cap-binding complex. Activity of the RINGO/Spy protein then leads (presumably via activation of the Aurora A/Eg2 protein kinase) to subsequent activation of CPEB, which initiates the next wave of translation regulation as shown in the blue box. CPE-mediated translation repression is imposed by maskin, which binds CPEB and prevents eIF4G association with the cap. CPEB activation by phosphorylation leads to remodeling and activation of the CPEB/CPSF/Symplekin/GLD2 complex, resulting in polyadenylation and recruitment of ePABP, which alleviates CPE-mediated translation repression. Translational activation of messages such as mos protein kinase then permits succeeding steps in maturation, culminating in mos-mediated metaphase II arrest of the mature egg. (The cap and cap-binding eIF4E protein are depicted as a black circle and a semicircle, respectively; 40S and 60S are ribosomal subunits, while Sym depicts Symplekin.)

Cytoplasmic polyadenylation

CPEs (Mendez and Richter 2001), are U-rich sequences in the 3′-UTRs of maternal mRNAs that can either recruit a translation-repressive complex or direct active polyadenylation and resumption of translation (Fig. 1, see blue box). Both activities are dependent on a key RNA-binding protein called CPE-binding protein (CPEB). CPEB engages a repressor called maskin, which blocks cap-dependent translation. Additionally, during meiotic maturation, CPEB phosphorylation leads to activation of a CPEB-associated poly(A) polymerase complex, which contains cleavage and polyadenylation specificity factor (CPSF), Symplekin, and the poly(A) polymerase germline development deficient (GLD2), which then elongates the short poly(A) tail of CPE-containing messages (Barnard et al. 2004). The longer poly(A) tail binds poly(A)-binding protein (PABP), which brings in the eukaryotic initiation factor 4G (eIF4G) to replace maskin in the repressive maskin–cap complex interaction, resulting in increased translation. The switch between the repressed and active states requires the phosphorylation of CPEB, as well as the removal of the repressive factor, maskin.

The CPE pathway is highly networked with the phosphorylation and activation of CPEB coupled to feedback control exerted by the products of CPE-containing messages on several levels. Indeed, the mRNA that encodes the Aurora A/Eg2 kinase (see Fig. 1) required for CPEB activation is itself a CPE-containing message.

The CPE pathway uses additional mechanisms to provide temporally and spatially regulated translation (Wickens 1990; Bouvet et al. 1994; Stebbins-Boaz and Richter 1994). In G2-arrested mouse oocytes, CPE messages undergo CPE-directed deadenylation, reducing their poly(A) tail lengths to 20–40 nucleotides (nt); this limits their translatability (Huarte et al. 1992; Paynton and Bachvarova 1994). The *Drosophila* CPEB (called Orb) is a critical regulator of anterior–posterior patterning and germline differentiation that acts through a similar cytoplasmic polyadenylation mechanism with the added complexity of spatial control (Chang et al. 2001; Castagnetti and Ephrussi 2003). In rat hippocampal neurons, CPEB directs transportation of translationally repressed CPE-bearing messages to dendritic synapses where they are activated (Huang et al. 2003). The human ortholog, hCPEB, has been found to localize in stress granules and direct messages to P bodies, thereby sequestering them from translation (Wilczynska et al. 2005). Therefore, the CPEB/CPE complex may classify messages for translational repression or activation via localization.

Poly(A)-dependent translation control

The role of the polyadenylation process and resulting poly(A) tail in maternal mRNA gene expression is crucial, dictating either deadenylation or translation (Jackson and Standart 1990; Wormington et al. 1996; Richter 1999). Interestingly, there is no decay of *Xenopus* messages following deadenylation through oocyte maturation or in the early stages of embryo development (until the mid-blastula transition), suggesting a reversible regulatory process that can shift mRNAs between repressed and translationally active states (Audic et al. 1997; Voeltz and Steitz 1998). Tethering of poly(A) polymerase leads to premature activation of translation of such messages (Dickson et al. 2001; Rouhana et al. 2005). Thus, either the act of polyadenylation and/or the poly(A) tail itself is critical for meiotic activation and subsequent maturation-dependent translation of CPE-containing messages.

In clear contrast, during early oogenesis prior to meiotic arrest, many non-CPE messages remain fully polyadenylated and are translated. However, following meiotic reactivation, these messages are specifically deadenylated by a maturation-activated deadenylase that is released from the nucleus upon germinal vesicle (nucleus) breakdown, thereby repressing their translation and promoting the translation of CPE-bearing transcripts (Wickens 1990; Wormington 1993).

There are at least three possible polyadenylation-linked mechanisms that could individually or cooperatively function to activate translation. First, since the process of polyadenylation itself appears to impact post-transcriptional processes—reminiscent of the role of nuclear history in dictating downstream events in the life of an mRNA (for review, see Moore 2005)—the polyadenylation machinery may modify or load a factor conducive for translation. Richter and colleagues have suggested that 2′-O-ribose methylation of the 5′-cap of the mRNA may be such a modification, but how the cytoplasmic poly(A) polymerase complex would orchestrate cap methylation remains mysterious (Mendez and Richter 2001). Second, a translationally negative complex formed on a partially deadenylated message may simply be overridden by extension of a short poly(A) tail. Earlier, Richter's group demonstrated that maskin is tethered by such a 3′-end in a way that prevents the cap-binding protein eIF4E from forming a complex with eIF4G, a normal requirement for ribosome recruitment and translation (Cao and Richter 2002; Groisman et al. 2002). Third, extension of the poly(A) tail may relocate the message from repressed bodies to the translation apparatus through recruitment of PABP (Brengues et al. 2005; Kedersha et al. 2005).

Polyadenylation-independent translational control

It has become increasingly evident that mechanisms distinct from cytoplasmic polyadenylation are required in parallel with CPE regulation to control *Xenopus* gene expression in a transcript-specific and temporal manner (de Moor et al. 2005; Piccioni et al. 2005). As revealed in the article by Padmanabhan and Richter (2006), such processes can also occur prior to and be required for activation of the subsequent CPE translation control mechanism (see Fig. 1).

Padmanabhan and Richter (2006) have found a second important 3′-UTR element, the PBE, in the *Xenopus* RINGO/Spy mRNA. The PBE is a defined binding site for certain members of the Pumilio family of proteins (White et al. 2001; Fox et al. 2005). Previously, in *Dro-*

sophila embryos, Pumilio had been shown to bind a 3′-UTR element dubbed the Nanos regulatory element (NRE) and interact with another repressor, Nanos, in *Drosophila* embryos to prevent the translation of *hunchback* mRNA (Zamore et al. 1997; Wharton et al. 1998). Pumilio 2 binding to the PBE likewise effects translation repression of the RINGO/Spy mRNA through the participation of two other RNA-binding proteins, deleted in azoospermia (DAZ)-like protein (DAZL) and embryonic PABP (ePABP). Whether the process involves active deadenylation, as is known for some Pumilio orthologs (Wickens et al. 2002), or instead builds a repressed mRNP complex independent of the poly(A) tail, the case for other Pumilio proteins (Chagnovich and Lehmann 2001), is not yet clear. In fact, the poly(A) status of the RINGO/Spy message at this stage of development has not been established. Since artificial tethering of DAZL and ePABP was previously shown to lead to significant translation activation independent of any signaling or without a poly(A) tail (Collier et al. 2005), it seems more likely that Pumilio 2 recruits a repressive complex to the RINGO/Spy mRNA or alters the mRNP to prevent translation. Indeed, overexpression of the N terminus of Pumilio 2 titrates away the inhibition, suggesting the existence of a cofactor for repression (Padmanabhan and Richter 2006). As is the case for CPEB (see above), data on the yeast Pumilio homolog Puf6 suggest that repression may be localization mediated (Gu et al. 2004); bound transcripts become sequestered in translationally silenced complexes reminiscent of stress granules of mammalian cells (Brengues et al. 2005; Kedersha et al. 2005).

Temporal precision by a synergistic mechanism

The above pathways collaborate to provide temporal precision as well as substrate specificity to the sequential translation of different maternal transcripts in the *Xenopus* oocyte (see Fig. 1). The PBE-containing RINGO/Spy message is repressed by Pumilio 2 in conjunction with DAZL and ePABP, as demonstrated in the Padmanabhan and Richter (2006) article. Upon meiotic reactivation, Pumilio 2 loses its interactions with both the PBE and the DAZL and ePABP proteins, permitting the DAZL/ePABP complex to activate translation either independently or through additional unidentified cofactors (Fig. 1, green box). RINGO/Spy is then expressed, leading to activation of CPEB by phosphorylation, which, in turn, elicits polyadenylation and translation activation of the mRNA for a critical oocyte maturation factor, the mos kinase (Fig. 1, blue box).

A defining feature of this regulatory network is the presence of a DAZL-binding site on the same transcript as the PBE. The interplay between these two translational control elements orchestrates precise translation of the RINGO/Spy mRNA (Padmanabhan and Richter 2006). DAZL binds to a consensus sequence with a GUUC/U-rich core (Jiao et al. 2002; Maegawa et al. 2002; Fox et al. 2005), and apparently recruits ePABP/PABP to the mRNA (Collier et al. 2005). Yet, this translation-activating component is subject to the overriding dominance of PBE-bound Pumilio 2, which enforces translational repression. Upon meiotic reactivation, Pumilio 2 loses its affinity for the PBE and for DAZL, but DAZL and ePABP remain bound to the mRNA (Fig. 1, green box). Therefore, the specificity of maternal transcript translation is imparted by which regulators recognize the message and when each regulator is active as a consequence of a preceding translation regulation event.

Meanwhile, other CPE-bearing transcripts, such as cyclin B1 mRNA, are additionally repressed through an xPumilio site (xPB) that recruits a different Pumilio homolog (Nakahata et al. 2003). In this case, selective temporal control is imposed on cyclin B1 mRNA expression through a two-pronged effect on translation. First, cyclin B1 mRNA is repressed predominantly by the CPEB mechanism discussed earlier (Cao and Richter 2002). Second, xPumilio can interact with CPEB through its PUF RNA-binding motif and may thereby impose an additional constraint on CPEB-mediated translation regulation (Nakahata et al. 2003). This limitation may involve a mechanism similar to the one discovered by Padmanabhan and Richter (2006), where xPumilio bound to the xPB site interacts in *cis* with additional 3′-UTR element regulatory complexes such as CPEB or DAZL/ePABP (Moore et al. 2003; Nakahata et al. 2003). Repression of cyclin B1 mRNA is released upon maturation through activation of CPEB and loss of xPumilio binding to the mRNA and to CPEB; as a consequence, translation proceeds through a CPE-mediated polyadenylation mechanism where loss of repression requires ePABP recruitment (Cao and Richter 2002; Nakahata et al. 2003). This regulation does not affect non-CPE messages or the CPE-only messages. Rather, the presence of both a CPE and an xPB site in certain transcripts or of a DAZL site and a PBE site on others could impart a highly precise temporal order of expression in a transcript-specific manner. Consequently, translation activation of messages with one element such as that of the CPE-bearing mos would precede those with additional elements, as in cyclin B1. This scenario suggests that the RNA-binding protein players—Pumilio, DAZL, and CPEB, which can individually function as translation regulators—reassociate with each other to form multiple transcript-specified combinations. They thereby achieve a more refined translational control, although using a common ePABP/PABP complex.

ePABP: a common translation effector

ePABP is a distinctive member of an evolutionarily conserved family of PABPs. PABPs are present from yeast to humans and play vital roles in mRNA metabolism, through biogenesis, localization, translation, and turnover of mRNAs (Mangus et al. 2003; Kuhn and Wahle 2004). These RNA-binding proteins have very high affinity for homopolymeric poly(A) tracts and bear one or four RNA recognition motifs (RRMs), as well as a conserved C-terminal region with a PABP signature motif (see Fig. 2).

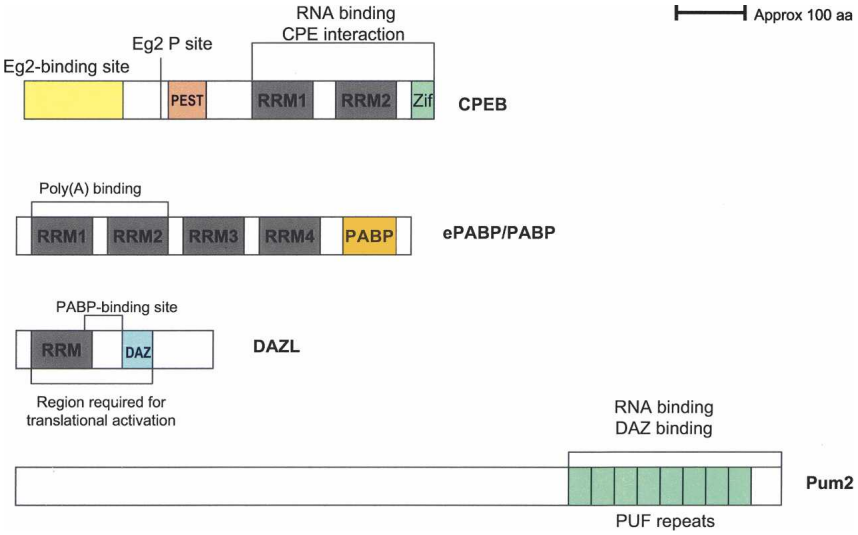

Figure 2. Schematic representation of four key RNA-binding proteins involved in the regulation of maternal mRNA translation in oocytes. Domain structures and known interacting regions for CPEB (Mendez and Richter 2001), ePABP/PABP1 (Seli et al. 2005), DAZL (Moore et al. 2003; Collier et al. 2005), and Pumilio (Wharton et al. 1998) are shown. The structure for ePABP/PABP1 is shared by all cytoplasmic PABPs, whereas nuclear PABPs contain one RRM (Mangus et al. 2003). Pumilio-2 belongs to the PUF family (Pum [Pumilio] and FBF [*fem-3* mRNA-binding factor]), characterized by a highly conserved C-terminal RNA-binding domain, composed of eight tandem repeats (Spassov and Jurecic 2003a).

ePABP has been characterized in *Xenopus* and mouse (Voeltz et al. 2001; Seli et al. 2005) and is ~70% identical at the amino acid level to classical PABP, with the most marked differences near the C terminus. Since this region is essential for interaction with PABP-specific regulators that can modulate function, truncation as well as variations in the C-terminal region of ePABP relative to PABP may define novel roles. Yet, PABP and ePABP have been demonstrated to be interchangeable for many PABP functions, including rescue of a *pab1*Δ lethality in *Saccharomyces cerevisiae*, interactions with the cap-binding eIF4G complex, and with termination factor eRF3, poly(A), and AU-rich element binding, as well as preventing deadenylation of the mRNA (Voeltz et al. 2001; Cao and Richter 2002; Cosson et al. 2002). A feature that distinguishes ePABP from the normal cytoplasmic PABP is its expression pattern: ePABP has so far been found to be expressed exclusively in an oocyte- and embryo-specific manner (Voeltz et al. 2001; Seli et al. 2005). The realization that ePABP is the predominant PABP present during oocyte maturation and early embryogenesis in both *Xenopus* and mouse begs the question of how it may function differently from cytoplasmic PABP, which replaces it later in development.

The Padmanabhan and Richter (2006) article describes a new role for ePABP in controlling translation of an upstream regulator of CPEB upon meiotic activation and subsequent maturation. Since the modulation of translation of the RINGO/Spy mRNA through the PBE operates in the absence of CPEB, ePABP appears to be involved in a novel cytoplasmic polyadenylation-independent process. Previous data with a tethering system indicated that DAZL or ePABP itself is sufficient to promote translation of a reporter in *Xenopus* oocytes (Collier et al. 2005; Wilkie et al. 2005). Here it may be significant that ePABP was originally identified not as a PABP but through its direct interaction with an AU-rich upstream sequence in the 3′-UTR (Voeltz et al. 2001). Since tethering a mutant DAZL lacking its ePABP-binding domain was unable to stimulate translation (Collier et al. 2005), a key function of DAZL may be to bind and coordinate ePABP with negative translation regulators bound to the 3′-UTR of the message. One such negative regulator, the Pumilio/PBE complex, functions to repress translation, presumably through its interactions with the DAZL/ePABP complex (Fig. 1, green box; Moore et al. 2003; Padmanabhan and Richter 2006). Conversely, in the absence of negative elements such as the PBE, as in Figure 3 of the article by Padmanabhan and Richter (2006), translation was activated without a need for meiotic activation or subsequent maturation signals. Therefore, the Padmanabhan and Richter data suggest a model in which an adaptive ePABP complex is the key switch that allows oscillation between repressed and translationally active states.

In all these processes, whether polyadenylation dependent or independent, ePABP/PABP plays a critical role by promoting protection of the transcript from deadenylation and in enhancing translation (Voeltz et al. 2001; Wilkie et al. 2005). Tethering ePABP and also cytoplasmic PABP to a reporter stimulated translation in immature oocytes by eightfold (Wilkie et al. 2005), suggesting that both proteins can up-regulate translation. It is also possible that an additional factor contributes to ePABP-mediated translation or that ePABP undergoes modification upon meiotic activation and subsequent maturation. In *Spisula* embryogenesis, PABP is somehow masked in maturing oocytes and unable to bind polyadenylated RNA, revealing the existence of regulatory mechanisms operating on this class of proteins (de Melo Neto et al. 2000). Accordingly, PABP overexpression prevented maturation-dependent deadenylation and translation inactivation of maternal transcripts but did not interfere with CPE-mediated polyadenylation (Wormington et al. 1996). Since ePABP is replaced by increasing levels of PABP in later stages of development after zygotic gene activation (Voeltz et al. 2001; Seli et al. 2005), this programmed substitution of the universal translation effector is likely pivotal for guiding further specifi-

cation toward a distinct maturation-controlled gene expression program.

DAZL, lessons from different species

The other RNA-binding protein that the article by Padmanabhan and Richter (2006) sheds new light on is DAZL. Unlike Pumilio, CBEB, and ePABP, which were characterized in studies of model developmental organisms, our knowledge of DAZL originated in human disease.

DAZL was first identified by its homology to DAZ, a gene on the long arm of the Y chromosome that is frequently deleted in infertile men with nonobstructive azoospermia. DAZL and BOULE, two autosomal homologs of DAZ, exist in numerous species. BOULE is considered the ancestor of the DAZ family, with orthologs in *Caenorhabditis elegans*, *Drosophila*, mice, and humans (Eberhart et al. 1996; Karashima et al. 2000; Xu et al. 2001). DAZL orthologs are found in vertebrates only, while DAZ genes are restricted to old world monkeys and humans, suggesting that DAZL gave rise to DAZ during primate evolution.

The DAZ/DAZL/BOULE family of proteins is characterized by its nearly exclusive expression in germ cells and by a highly conserved RRM and unique DAZ repeat of 24 amino acids. Both BOULE and DAZL are single-copy genes that contain only one DAZ repeat (see Fig. 2), whereas most men possess four DAZ genes with one to three RRMs and seven to 24 DAZ repeats.

Despite the similarities in structure and expression patterns among DAZ/DAZL/BOULE family members, the impact of their absence on germ cell maturation varies between species. In *Drosophila*, Boule expression is limited to males and its loss results in azoospermia because of a defect in the G2/M transition (Eberhart et al. 1996). Conversely, in *C. elegans*, loss of the single DAZ homolog Daz-1 results in a block at the pachytene stage of meiosis I in oocytes but does not affect spermatogenesis (Karashima et al. 2000). In *Xenopus*, the DAZ-like gene (Xdazl) is expressed in adult *Xenopus* ovary and testis but not in any of the somatic tissues (Houston and King 2000); it appears to play a critical role in the development of PGCs (Houston and King 2000). In the mouse (a species that does not have a DAZ gene on the Y chromosome), Dazl expression is limited to germ cells in gonads (Cooke et al. 1996), and targeted disruption of Dazl results in infertility in both males and females (Ruggiu et al. 1997). In the Dazl knockout mouse, both male and female germ cells are lost before the first meiotic arrest (Saunders et al. 2003) despite the fact that these mice contain a functional Boule gene. This is probably because Boule expression in mouse testes does not start until after germ cell development has already been impaired in the Dazl knockout mouse. Interestingly, the expression pattern of DAZL in humans is somewhat different and is not limited to germ cells. DAZL can be detected in somatic cells of the gonad as well as in later stages of the human preimplantation embryo, long after activation of zygotic gene expression. This suggests that, in contrast to other species, human DAZL may play roles in embryogenesis beyond germ cell development (Cauffman et al. 2005).

Until recently, the molecular basis of DAZL function had not been identified. Using *Xenopus laevis* oocytes as a model system, Collier et al. (2005) showed that Xdazl, mouse Dazl, human DAZL, human DAZ, and human BOULE all possess the ability to stimulate translation. They also demonstrated that these proteins interact with PABP1 and ePABP. The article by Padmanabhan and Richter (2006) now describes an additional role for DAZL coupled with ePABP in effecting repression or translational activation of the RINGO/Spy mRNA. While the loss of germ cells observed in the mouse DAZL knockout remains to be explained, these exciting findings hint that the DAZ/DAZL/BOULE family of proteins plays additional roles earlier in gametogenesis, likely in a species-specific fashion.

Parallels and prospects

The evidence discussed above for the roles of specific RNA-binding proteins in regulating waves of translation of maternal mRNAs derives in large part from studies in *Xenopus* oocytes and embryos. Can we project findings in *Xenopus* to explain the biology of the gamete in evolutionarily distant species, especially mammals? While we believe the answer is yes, it is important to be vigilant of differences between species (e.g., those discussed in the above section on DAZL).

Indeed, the analysis of mammalian orthologs of genes important for oocyte and early embryo development in model organisms (e.g., *Xenopus*, *Drosophila*, *C. elegans*), even following confirmation of their specific expression in germ cells, can have surprising outcomes. Orthologs of the proteins studied by Padmanabhan and Richter (2006) have been identified in mouse (Cooke et al. 1996; Gebauer and Richter 1996; Spassov and Jurecic 2003b; Cheng et al. 2005; Seli et al. 2005). Studies of CPEB (Tay and Richter 2001) and DAZL (Ruggiu et al. 1997) knockout mice both revealed loss of oocytes prior to meiotic reactivation. This hints at additional functions for these RNA-binding proteins prior to their presumed roles in regulating maternal mRNA translation in mouse oocytes as in *Xenopus*. Conditional knockout vectors with the potential to manipulate expression at particular stages of oogenesis and embryogenesis may be more useful for delineating the roles of specific proteins in the translational regulation of maternal mRNA expression. Alternatively, knockdown approaches in mouse oocytes using RNAi are also conceivable (Stein et al. 2003).

It is clear that the work of Padmanabhan and Richter (2006), and of others discussed above, sheds new light on our understanding of gamete development, and accumulating evidence suggests that similar proteins and mechanisms are present in developmentally distant species. As indicated by the CPEB and Dazl knockout mice, additional steps that involve the same RNA-binding proteins may occur earlier in the process. It would not be surprising to find that these additional steps are likewise

organized into translation regulatory cascades. The *Xenopus* model system, used by Padmanabhan and Richter, will continue to be a powerful tool for studying mechanistic questions that arise from gene deletion approaches in other species.

Acknowledgments

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