The DAZL and PABP families: RNA-binding proteins with interrelated roles in translational control in oocytes

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Abstract

Gametogenesis is a highly complex process that requires the exquisite temporal, spatial and amplitudinal regulation of gene expression at multiple levels. Translational regulation is important in a wide variety of cell types but may be even more prevalent in germ cells, where periods of transcriptional quiescence necessitate the use of post-transcriptional mechanisms to effect changes in gene expression. Consistent with this, studies in multiple animal models have revealed an essential role for mRNA translation in the establishment and maintenance of reproductive competence. While studies in humans are less advanced, emerging evidence suggests that translational regulation plays a similarly important role in human germ cells and fertility. This review highlights specific mechanisms of translational regulation that play critical roles in oogenesis by activating subsets of mRNAs. These mRNAs are activated in a strictly determined temporal manner via elements located within their 3’UTR, which serve as binding sites for trans-acting factors. While we concentrate on oogenesis, these regulatory events also play important roles during spermatogenesis. In particular, we focus on the deleted in azoospermia-like (DAZL) family of proteins, recently implicated in the translational control of specific mRNAs in germ cells; their relationship with the general translation initiation factor poly(A)-binding protein (PABP) and the process of cytoplasmic mRNA polyadenylation.

Introduction

Impaired fertility affects ~15% of couples worldwide with a significant proportion of cases being due to defective gametogenesis (Matzuk & Lamb 2008). In order to define possible points of therapeutic intervention in infertility, it is necessary to have an in-depth understanding of the normal processes of gametogenesis and, in particular, to delineate the molecular mechanisms by which gametogenesis is regulated. In recent years, a great deal of research has concentrated on the control of gene expression during gametogenesis and, more specifically, has investigated the important role of translational control during oogenesis (Piccioni et al. 2005; see Box 1 and Fig. 1 for an overview of oogenesis). The limited availability of human ovarian tissue and the tractability of organisms such as mice, Xenopus, sheep, cows, Drosophila and Caenorhabditis elegans means that most studies are performed using these models, in which many of the key oogenic and translational control processes are evolutionarily conserved, albeit to varying extents. We primarily discuss studies of 3’UTR-mediated translational activation during oogenesis in small laboratory vertebrates such as Xenopus laevis and mouse.

X. laevis has proved an extremely powerful tool for dissecting the molecular mechanisms and signalling pathways that regulate gene expression during oogenesis. Indeed many key control mechanisms that operate in mammalian oogenesis were first described using X. laevis oocytes. One reason for the popularity of the X. laevis model is that the seasonality of X. laevis ovulation can be uncoupled and hundreds of oocytes of different, clearly identifiable, stages can be recovered from a single ovary. These oocytes can be readily maintained and matured in vitro. Importantly, the large size of these oocytes make them amenable to micro-injection with exogenous DNAs/RNAs/proteins/drugs and subsequent biochemical assays. Most studies have focused on translational regulation during oocyte...
Primordial germ cells (PGCs) originate in the early embryo distinct from the location of the developing gonad. In \textit{Xenopus laevis}, PGCs derive from a cellular subset that inherited oocyte ‘germ-plasm’ during early embryonic asymmetric mitoses (Nieuwkoop & Faber 1994, Sive et al. 1999). Germ-plasm describes a region of mitochondrial-rich ooplasm containing numerous mRNA-rich germinal granules. Human and mouse oocytes have no recognisable germ-plasm and PGCs do not appear to differentiate from a pre-determined cell population. Rather, mouse PGCs differentiate in response to extracellular signals derived from the proximal epiblast or primitive streak of the early embryo. PGCs of each species then proliferate and migrate to the developing gonad. PGC migration is completed prior to ovarian sexual differentiation but the germ cells continue to proliferate. In human and mouse ovaries the oogonia subsequently cease to proliferate and enter meiosis, whereas the \textit{X. laevis} ovary retains a population of mixed primary oogonia (self-renewing) and nests of secondary oogonia (committed to oocyte differentiation; Nieuwkoop & Faber 1994, Sive et al. 1999). The retention of self-renewing primary oogonia in \textit{X. laevis} facilitates seasonal production of large numbers of oocytes, but is fundamentally different from mammalian oogenesis.

The onset of meiosis I in oogonia occurs during embryogenesis in humans and mice and prior to metamorphosis in \textit{X. laevis}. Human oogonia asynchronously begin to enter meiosis I in early second trimester and mice oogonia synchronously enter meiosis I at E13.5. A large proportion of human and mouse oogonia die during meiosis I in a process called atresia, with most cell death occurring during late-pachytene or dichtyate. The dichtyate arrest (prophase I) of surviving human oogonia is complete by late third trimester and persists for up to 45 years. \textit{X. laevis} oogonia begin to enter meiosis I from stage 55 (E32) onwards with a marked increase in entry rate occurring at stage 56 (E38). By stage 62 (E50) most oogonia have entered diplotene of meiosis I, termed stage I oocytes. Several thousand \textit{X. laevis} oocytes then progress through diplotene until many stage VI oocytes are present in the ovary (Nieuwkoop & Faber 1994, Sive et al. 1999).

During diplotene, oocytes synthesise huge amounts of rRNA, tRNAs, mitochondria, proteins and maternal mRNAs to prepare for their post-fertilisation biosynthetic requirements until zygotic transcription resumes. This occurs after 1, 2–3 or 12 post-fertilization mitotic divisions in mouse, human and \textit{X. laevis} respectively (Sive et al. 1999, Matova & Cooley 2001, Bownes & Pathirana 2003). Unlike mammals, \textit{X. laevis} embryos do not receive nutrients placentally and instead oocytes accumulate a nutrient store during diplotene: a process termed vitellogenesis. Liver-secreted vitellogenin accumulates in oocytes until, by stage V, it comprises \(\sim 80\%\) of the ooplasm and accounts for the \(\sim 4000\)-fold greater volume of a \textit{X. laevis} oocyte compared with a mouse oocyte (Nieuwkoop & Faber 1994, Sive et al. 1999). However, although transcription continues during diplotene only \(\sim 2\%\) of the ribosomes in late diplotene \textit{X. laevis} oocytes are engaged in active translation and only \(\sim 20\%\) of the mRNAs are translated; indicating that the majority of the mRNA population is translationally silenced.

Vitellogenesis and ovulation differ between mammals and \textit{X. laevis}, although there are some significant similarities. In humans \(\sim 20\) primordial follicles/day become activated, in a poorly understood process, and then develop in a hormone/steroid-independent manner for \(\sim 65\) days. During this time the granulosa cells (GCs) proliferate, thecal cells derived from the stroma surround the growing follicle and the enclosed granulosa cells begin to differentiate. After this time GC proliferation becomes FSH-dependent, an antral space develops and the oocyte is surrounded by specialized cumulus granulosa cells (termed a Graafian follicle). Ultimately, only a single follicle is sufficiently developed to survive when FSH-levels decline mid-menstrually. It is for this reason that humans are usually mono-ovulatory. Mouse follicles can survive in lower FSH concentrations and, consequently, several follicles progress to ovulation.

In mammals, a surge in circulating LH levels stimulates Graafian follicle oocytes to reinitiate meiosis. In \textit{X. laevis}, the oocyte is surrounded by a single layer of follicle cells which secrete progesterone in response to circulating gonadotrophins and induce the oocyte to resume meiosis. The completion of meiosis I and progression through to metaphase of meiosis II is termed maturation and is highly conserved between mammals and \textit{X. laevis} (see Box 2 and Fig. 2 for a more detailed overview of oocyte maturation; reviewed in (Schmitt & Nebreda 2002, Vasudevan et al. 2006)). Hence, \textit{X. laevis} data have greatly informed studies of oocyte maturation in mammals.
aspects. Their short oestrus and reproductive cycle is also advantageous and the seasonality of reproduction is bred out of most laboratory strains. Additionally, the genetic manipulation of mice has proved extremely valuable to the study of both male and female gametogenesis; a technique that is not as widely applied in the tetraploid *X. laevis*. However, this advantage is countered by the fact that it is difficult to obtain sufficient numbers of mouse oocytes to facilitate molecular and biochemical studies.

In this review, we will focus on the molecular mechanisms of 3’UTR mediated translational activation that play critical roles in oogenesis and highlight the contributions of the *X. laevis* and mouse model systems to the understanding of these regulatory events. In particular, we discuss the emerging importance of the DAZL family of translational activator proteins and their molecular and functional interactions with the PABP family of translation initiation factors.

**Translational regulation**

Translational regulation is usually defined as a change in the rate of protein synthesis without a corresponding change in mRNA levels. However, this is an oversimplification as translation and mRNA turnover are often tightly coupled and alterations in translation can have downstream effects on mRNA stability (Newbury 2006). Translation can also be spatially regulated to establish protein gradients via localization of mRNAs and/or regulatory factors (Huang & Richter 2004). Translational control therefore facilitates the generation

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**Figure 1** Schematic to illustrate the comparative timing and duration of key events in vertebrate oogenesis. Timings are shown (where known) for the completion of the major primordial germ cell (PGC) pre-meiotic events (orange arrows). Due to the more asynchronous nature of the entry of the PGC/oogonia population into meiosis in human and *X. laevis*, the durations of individual stages of meiosis/folliculogenesis relate to the progress of an individual oocyte/follicle (yellow arrows). See Box 1 (or Matova & Cooley 2001) for a brief comparative review of vertebrate oogenesis. *Xenopus* germ-plasm can be detected throughout early embryogenesis, where it plays an important role in PGC specification, but the PGCs become embedded in the dorsal endoderm by stage 40 prior to migration to the site of genital ridge formation (Nieuwkoop & Faber 1994, Sive et al. 1999). A schematic of particular meiotic stages and pictorial representations of *X. laevis* oogenesis and mammalian folliculogenesis are also shown. GVBD, germinal vesicle breakdown.
Translation can be divided into three basic steps: i) initiation, during which many of the required factors are recruited; ii) elongation, whereupon an mRNA is released. While each of these steps can be regulated, specific regulation affects the translational upregulation of mRNAs encoding key meiotic proteins via modulation of their poly(A) tail length (including c-Mos, cyclin B1 and Wee1). One of the key events in vertebrate oocyte maturation is the translational upregulation of c-Mos, a serine/threonine kinase that promotes the activation of the MAP kinase (MAPK) pathway (Schmitt & Nebreda 2002). This is upstream of the polyadenylation of a large subset of mRNAs. In X. laevis, but not mammals, c-Mos is required for complete activation of maturation promoting factor (MPF), comprising a heterodimer of cyclin B and CDC2, which promotes germinial vesicle membrane breakdown (GVBD) and completion of meiosis I. c-mos null mouse oocytes progress normally through meiosis I but exhibit defects during meiosis II, indicating that c-Mos is required for the second meiotic arrest (Gebauer et al. 1994). Indeed, in both mammals and X. laevis c-Mos is required for cystostatic factor (CF) activity which serves to inhibit the anaphase promoting complex (APC), thereby maintaining MPF activity and causing meiosis II metaphase arrest.

mRNA-specific regulation of translation initiation

Cap-dependent translation provides multiple points at which initiation can be regulated in an mRNA-specific manner (Gray & Wickens 1998, Scheper et al. 2007). Such regulation requires cis-acting elements that are normally located within the 5′- and 3′UTRs, but are occasionally found within the main open reading frame. These elements can confer differential regulation to mRNAs transcribed from the same gene via their inclusion/exclusion due to alternative promoter or polyadenylation site use and/or splicing. To date the majority of examples of mRNA-specific regulation during gametogenesis involve 3′UTR-mediated control (Radford et al. 2008). 3′UTRs act as a repository for elements that regulate mRNA translation, stability or localization; normally by acting as binding sites for a variety of trans-acting factors. These include mRNA translation factors. By contrast, specific regulation affects the translational upregulation of mRNAs normal via sequence elements located within these mRNAs. Global and specific regulation can occur simultaneously to rapidly reprogram protein synthesis (reviewed in Spriggs et al. 2008, Yamasaki & Anderson 2008).

Translational regulation can be divided into two broad categories: global and specific. Global control affects most cellular mRNAs in response to a given signal and is often mediated by changes in the activities of basal translation factors. By contrast, specific regulation affects only a single or subset of mRNAs in a coordinate manner, normally via sequence elements located within these mRNAs. Global and specific regulation can occur simultaneously to rapidly reprogram protein synthesis (reviewed in Spriggs et al. 2008, Yamasaki & Anderson 2008).

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Eukaryotic mRNAs exit the nucleus with a modified 7-methyl guanosine at the 5′end, termed the cap structure, and a poly(A) tail of ~250 nucleotides at the 3′end. These features act as the primary determinants of translational initiation efficiency. Cap-dependent initiation accounts for the majority of cellular translation and although other mechanisms of initiation are also utilized during gametogenesis they are beyond the scope of this review (Spriggs et al. 2008). This pathway is a complex process with several mRNA-dependent steps that are described in detail in Fig. 3. Briefly, initiation proceeds with 1) binding of a protein complex to the 5′ cap, 2) unwinding of mRNA secondary structure, 3) joining of the small (40S) ribosomal subunit, 4) scanning of the 5′UTR by the 40S subunit and 5) recognition of the initiator codon, release of multiple initiation factors and joining of the large (60S) ribosomal subunit.

Intriguingly, although translation initiation occurs at the 5′end of the mRNA, the poly(A) tail at the 3′end of the message is also important. Poly(A) tails are bound by a family of proteins called poly(A)-binding proteins (PABPs) which interact with proteins bound to the 5′end, bringing the 5′- and 3′ends of an mRNA into proximity. This end-to-end configuration is termed the ‘closed-loop’ (illustrated in Fig. 3B), is highly conserved throughout evolution (Gallie 1998, Kalvejian et al. 2001) and forms the basis for understanding regulation by factors bound to the 3′UTR.

Box 2 An overview of the major signalling and gene expression events that regulate vertebrate oocyte maturation (see also Fig. 2).

Maturation is triggered by progesterone in X. laevis, or LH in mammals, causing a rapid inhibition of adenylate cyclase activity (AC) and subsequent decline in cAMP levels, thereby reducing protein kinase A (PKA) activity and initiating a number of intracellular signalling cascades. The earliest regulatory events in maturation are poorly understood but, in X. laevis, rapid inducer of G2/M progression in oocytes (RINGO)/speedy (SPY) mRNA translation is initiated leading to activation of CDK2, aurora A and additional intracellular signalling pathways (the latter is not depicted) (Cheng et al. 2005, Padmanabhan & Richter 2006, Kim & Richter 2007). These events in turn lead to the translational upregulation of mRNAs encoding key meiotic proteins via modulation of their poly(A) tail length (including c-Mos, cyclin B1 and Wee1). One of the key events in vertebrate oocyte maturation is the translational upregulation of c-Mos, a serine/threonine kinase that promotes the activation of the MAP kinase (MAPK) pathway (Schmitt & Nebreda 2002). This is upstream of the polyadenylation of a large subset of mRNAs. In X. laevis, but not mammals, c-Mos is required for complete activation of maturation promoting factor (MPF), comprising a heterodimer of cyclin B and CDC2, which promotes germinial vesicle membrane breakdown (GVBD) and completion of meiosis I. c-mos null mouse oocytes progress normally through meiosis I but exhibit defects during meiosis II, indicating that c-Mos is required for the second meiotic arrest (Gebauer et al. 1994). Indeed, in both mammals and X. laevis c-Mos is required for cystostatic factor (CF) activity which serves to inhibit the anaphase promoting complex (APC), thereby maintaining MPF activity and causing meiosis II metaphase arrest.
binding sites or protein binding sites such as cytoplasmic polyadenylation elements (CPEs). It should be stressed, however, that individual mRNAs often contain multiple cis-acting elements that, rather than acting in a binary manner, confer combinatorial regulatory control over the mRNA (Wilkie et al. 2003). A further level of complexity is added by the fact that many of the trans-acting factors are themselves part of dynamic multiprotein complexes that can confer differential regulation to a bound mRNA. While many mRNAs are translationally regulated, relatively few of the trans-acting factors have been identified and the molecular mechanism of action has only been delineated in a few notable cases. Although these studies indicate that translation initiation can be regulated at almost every step, (Wilkie et al. 2003, Radford et al. 2008), most of the characterized factors are repressors; thus, the mechanism of action of 3'UTR bound activators remains largely enigmatic.

**Translation control during oogenesis: the importance of cytoplasmic polyadenylation**

One important mechanism for the regulation of mRNAs during gametogenesis is via the dynamic modulation of mRNA poly(A) tail length (Fig. 4). In general, an
Increase in poly(A) tail length correlates with translational activation while a decrease correlates with translational silencing. Many X. laevis oocyte mRNAs contain 3' UTR elements that target the mRNA for rapid poly(A) tail shortening following export into the cytoplasm (e.g. cyclin B1 poly(A) length in the cytoplasm of immature oocytes is ~30 nucleotides; Sheets et al. 1994). Such mRNAs remain stable but are stored translationally silent until, under permissive conditions, the poly(A) length is increased again. Critically these regulated, and often dramatic, changes in poly(A) length occur within the cytoplasm by a mechanism that is distinct from nuclear polyadenylation (Radford et al. 2008). Indeed, enucleation of X. laevis oocytes does not block this process (Fox et al. 1989). Pique et al. (2008) recently estimated that ~45% of stored mRNAs undergo cytoplasmic polyadenylation-dependent regulation during X. tropicalis oocyte maturation and that >30% of mouse and human oocyte mRNAs were similarly regulated. Cytoplasmic polyadenylation is critical for gametogenesis in mouse, C. elegans, Drosophila and other model organisms and also occurs in human oocytes (Gebauer et al. 1994, Prasad et al. 2008; reviewed in (Radford et al. 2008)).

Figure 3 Overview of mRNA translation initiation. (A) The mRNA-dependent steps of cap-dependent translation initiation. (1) The eIF4F complex (eIF4A, eIF4E and eIF4G) binds the 5' end of the mRNA via the cap-binding protein eIF4E. eIF4G coordinates the binding of eIF4E to the cap and the ATP-dependent RNA helicase activity of eIF4A, which is stimulated by eIF4B. (2) eIF4A helicase activity removes secondary structure within the 5'UTR. (3) Removal of secondary structure facilitates small ribosomal subunit (40S) binding at or near the cap. The 40S also carries with it a number of initiation factors; including the initiator tRNA complex (eIF2-GTP-tRNAi), eIF1, eIF1A, eIF3 and eIF3. Multiple interactions, including that between eIF4G and eIF3, may facilitate this step. (4) 40S and its associated factors then 'scan' the 5'UTR, in a poorly defined process, to locate an appropriate initiator codon (most frequently the first AUG encountered). (5) Initiator codon recognition results in GTP hydrolysis of eIF2-GTP, release of multiple initiation factors and large ribosomal subunit (60S) joining to form the translationally competent 80S ribosome. A number of additional eIFs drive this process. Initiation ends with the Met-tRNAi based paired to the AUG in the P-site of the 80S ribosome, with the A-site being available for delivery of tRNA. For clarity, the function of some initiation factors that are not pertinent to this review have been omitted (for a more detailed review see (Gray & Wickens 1998, Scheper et al. 2007)), and the spatial arrangement of factors does not depict all in vivo interactions.

(B) The closed-loop model of translation initiation. PABP interacts with eIF4G, functionally linking the ends of the mRNA via the interaction of eIF4G with the cap-binding protein eIF4E. This interaction increases both cap (panel A, step 1) and poly(A)-binding by the end-to-end complex. PABP also interacts with eIF4B and PAIP1, both of which interact with eIF4A; thus PABP may influence unwinding of secondary structure within the 5'UTR via eIF4A (panel A, step 2). PAIP1 may also influence eIF3 function, enhancing or stabilizing interaction of the 40S with the mRNA (panel A, step 3). While PABP-eIF4G interactions are the most studied, each of these interactions may play an incremental role in initiation complex stabilization, thereby enhancing various steps of translation initiation. A single molecule of PABP is shown for simplicity. Numbered factors, eIFs. Filled black circle, 5' cap. Dotted lines denote lack of interaction between overlapping factors.
but has been most intensively studied during *X. laevis* oocyte maturation.

In order for stage VI *X. laevis* oocytes to mature they need to exit prophase I-arrest and recommence meiosis, a process that can be induced by progesterone (Box 2 and Fig. 2). This requires the translational activation of many mRNAs (e.g. c-Mos and cyclin B1) in a highly orchestrated temporal sequence. This is achieved via specific cis-acting elements within their 3'UTRs, which promote cytoplasmic polyadenylation (Bello et al. 2008, Radford et al. 2008).

The critical requirement for the cytoplasmic polyadenylation of individual mRNAs for oocyte maturation was demonstrated in seminal studies in *X. laevis* and mouse. Ablation of endogenous c-Mos mRNA polyadenylation in *X. laevis* oocytes via selective removal of its 3'end, resulted in inhibition of c-Mos translation and progesterone-induced maturation; both of which were rescued by annealing a prosthetic, polyadenylation-dependent 3'end or a prosthetic poly(A) tail to the mRNA (Sheets et al. 1995, Barkoff et al. 1998). Similarly, Gebauer et al. (1994) demonstrated the importance of c-Mos polyadenylation for mouse oocyte maturation.

Mouse knock-out/down models have revealed that other stages of mammalian gametogenesis are also directed by this process. By deleting a factor required for the adenylation of many mRNAs (CPE-binding protein (CPEB)), a lack of cytoplasmic polyadenylation was shown to result in the arrest of both male and female germ cells at the pachytene stage of meiotic prophase I (Tay & Richter 2001). This may be due, at least in part, to the loss of synaptonemal complex protein (Sycp) 1 and Sycp3 translation, which correlates with a failure of homologous chromosome pairing and resultant meiotic failure. When CPEB expression was reduced after completion of the pachytene stage of prophase I, via expression of an siRNA under the control of the zona pellucida 3 (ZP3) promoter, the loss of polyadenylation of a number of key mRNAs was demonstrated (Racki & Richter 2006). These include growth differentiation factor 9 (GDF9), a critical folliculogenic growth factor expressed by the oocyte; SMAD5, which functions in the transforming growth factor (TGF) β signalling pathway; and H1Foo, an oocyte-specific histone 1 variant which is essential for oocyte maturation. A progressive decrease in oocyte and follicle number was observed in these CPEB-knockdown mice. At the dictyate stage, a number of oocyte phenotypes were observed including apoptosis, abnormal polar bodies and parthenogenetic cell division. Additionally, oocyte-granulosa cell interactions are disrupted leading to oocyte detachment from the cumulus granulosa cells and impaired folliculogenesis and these mice often developed ovarian cysts with age. Several of the phenotypes associated with these CPEB-knockdown mice have led to the proposal that they may model aspects of human premature ovarian failure syndrome (POF; Racki & Richter 2006). Together, these findings indicate that cytoplasmatic polyadenylation is required at multiple steps during the female meiotic program (recently reviewed in (Bello et al. 2008)). However, CPEB also functions as a translational repressor and loss of mRNA repression may contribute to these phenotypes, but this remains to be addressed. Interestingly, CPEB expression (and cytoplasmic polyadenylation) is not restricted to germ cells but its functions in somatic cells are beyond the scope of this review and are detailed elsewhere (Richter 2007).

**Sequences required for cytoplasmic polyadenylation**

All cytoplasmically polyadenylated mRNAs contain varying combinations of *cis*-acting sequences that determine the precise timing and extent of their polyadenylation (reviewed in detail in (Bello et al. 2008)). CPEs are the best characterized and have been predominantly studied in *X. laevis* oocytes (McGrew et al. 1989). CPEs function in collaboration with the hexanucleotide (hex) signal AAUAAA (see (Mandel et al. 2008) for review of nuclear polyadenylation), and both elements are binding sites for multiprotein complexes that can interact and are reviewed in detail elsewhere (Radford et al. 2008). Briefly, CPEB bound to the CPE(s) interacts with cytoplasmic polyadenylation and specificity factor (CP5F) bound to the hex. CPEB then recruits a cytoplasmic poly(A) polymerase GLD-2 and the poly(A) RNase (PARN) which acts antagonistically. During many oogenic stages, PARN activity exceeds that of GLD-2, resulting in deadenylation and translational silencing of the mRNA. Thus, CPEs also play a role in rapid deadenylation and translational silencing of regulated mRNAs as they exit the nucleus. Under permissive conditions (e.g. during oocyte maturation) CPEB is phosphorylated and PARN dissociates from the complex, facilitating GLD-2-mediated mRNA translation.
polyadenylation. Recent data suggest that PABPs may interact with polyadenylation complex components, raising the possibility that this may facilitate rapid PABP binding to newly synthesized poly(A) tails to promote the mRNA closed-loop conformation (Fig. 3B) thereby stimulating translation and protecting against PARN-mediated deadenylation (Richter 2007, Radford et al. 2008).

CPE-containing mRNAs exhibit varying patterns of translational activation during oogenesis; with the sequence, number and position of CPEs with respect to the hex being important. Recent studies further defined rules which determine at what point during X. laevis oocyte maturation, and with how many adenosines, a given CPE-containing mRNA will be polyadenylated (reviewed in (Bellloc et al. 2008)). Regulation conferred by CPEs/CPEB can be further modulated by the presence of additional cis-acting elements in the mRNA 3′UTR, such as the pumilio 2 (PUM2)-binding element (PBE). PUM2 is a member of the PUM/Fem-3 mRNA-binding factor (FBF) (PUF) family of RNA-binding proteins which have well-defined roles in translational repression in the germ-line of many species (Wickens et al. 2002, Kimble & Crittenden 2007). However, both CPEB-mediated polyadenylation, as well as repression, appears to be augmented by PUM2 in X. laevis oocytes.

A number of X. laevis mRNAs are activated by polyadenylation very early in oocyte maturation, in an apparently CPE-independent manner. To date, two further cis-acting cytoplasmic polyadenylation elements are described; Musashi/polyadenylation response elements (PREs) that are bound by Musashi (Charlesworth et al. 2000, 2004) and the translational control sequence (TCS) whose trans-acting factor(s) is unknown (Wang et al. 2008). It is proposed that these elements function during early oocyte maturation prior to CPE-dependent polyadenylation. The temporal regulation of cytoplasmic polyadenylation is a contentious issue since, in most of the cases described to date, PREs and TCSs are present in mRNAs that also contain CPEs. However, this may reflect a role of multiple cis-acting elements in the intricate regulation of translation via combinatorial control. mRNAs that do not contain CPEs are normally silenced following maturation-induced germinal vesicle breakdown, via a default pathway in which their poly(A) tails are removed, further enhancing the reprogramming of translation (Fox & Wickens 1990, Varnum & Wormington 1990).

**PABPs**

The primary function of PABPs is to transduce the signal provided by the poly(A) tail and convey it to the translational machinery (see Fig. 3B). Indeed, tethering PABPs to the 3′UTR of non-adenylated reporter mRNAs can functionally replace poly(A) tails in X. laevis oocytes (Gray et al. 2000, Wilkie et al. 2005). Thus, cytoplasmic polyadenylation is thought to exert its effects on translation largely by recruiting PABPs to the newly extended poly(A) tails. Consistent with a role of PABP in poly(A) tail mediated changes during oogenesis, over-expression of PABP1 in X. laevis oocytes blocks the maturation-induced deadenylation and translational inactivation of mRNAs that lack CPEs, as well as inhibiting the recruitment of some CPE-containing mRNAs onto polysomes (Wormington et al. 1996). A role for PABP in oogenesis is also indicated by early meiotic defects in PAB-1-deficient C. elegans (Maciejewski et al. 2005).

Cytoplasmic PABPs are evolutionarily conserved from yeast to humans and have multiple roles in translation and mRNA stability (Table 3; reviewed in (Gorgoni & Gray 2004)). Drosophila only encode a single cytoplasmic PABP which, like PABP in budding yeast, is required for viability, whereas C. elegans encodes two cytoplasmic PABPs and vertebrate genomes encode multiple cytoplasmic PABP genes (Fig. 5A; Gorgoni & Gray 2004). No vertebrate PABP-deficient phenotypes have yet been described and only a handful of non-synonymous single nucleotide polymorphisms are documented within human PABP genes, possibly reflecting an incompatibility of PABP mutation with viability.

In vertebrates, only the molecular functions of the prototypical PABP1 (also known as PABPC1), and to a lesser extent ePABP, have been studied in any detail (Voeltz et al. 2001, Gorgoni & Gray 2004, Wilkie et al. 2005). As few as 12 adenosines are required to bind a single PABP molecule, but each PABP has a footprint of ~26 adenosines, binding cooperatively and contiguously such that long poly(A) tails may be associated with multiple PABPs (reviewed in Gorgoni & Gray 2004). PABP1 comprises four highly conserved RNA recognition motifs (RRMs) that exhibit differing specificities for RNA sequences and interacting proteins, such as the translation initiation factor, elF4G (Figs 3 and 5B); a less well-conserved proline-rich region that functions in cooperative PABP binding to poly(A) (Melo et al. 2003) and a C-terminal domain (termed the PABC domain) which exhibits homology to HECT domains of E3-ubiquitin ligases (Kozlov et al. 2001; Fig. 5B). Neither the proline-rich nor the PABC domain binds the RNA but both interact with proteins; the latter binding proteins which contain a PABC-binding domain called PAM2, such as the PABP-interacting protein 1 (PAIP1; Figs 3 and 5B; Kozlov et al. 2001, Albrecht & Lengauer 2004, Kozlov et al. 2004, Lim et al. 2006).

PABP4, testis-specific PABP (tPABP -PABP2 in mouse and PABP3 in human) and ePABP exhibit a similar domain structure to PABP1 (Yang et al. 1995, Feral et al. 2001, Guezeloglu-Kayisli et al. 2008), whereas PABP5 has neither a proline-rich linker region nor a PABC domain (Blanco et al. 2001). Vertebrates also encode an additional protein, ePABP2, which, although apparently
The expression patterns of vertebrate PABPs are documented to varying degrees. PABP1 is expressed in a wide variety of tissues in humans, mice and X. laevis and available evidence suggests that PABP4 mRNA may also be widely expressed in these species (Kleene et al. 1994, Gu et al. 1995, Yang et al. 1995, Cosson et al. 2002; GS Wilkie & NK Gray, unpublished observations). Both PABP1 and PABP4 mRNAs are detected in the testis and ovary of humans, mice and X. laevis. Other PABPs appear to be more restricted in their expression. RT-PCR analyses of human PABPS suggest low-level expression in a variety of tissues, with higher levels being detected in the brain and gonads (Blanco et al. 2001). Mouse tPABP mRNA and protein and human tPABP mRNA are only expressed in pachytenic spermatocytes and round spermatids (Kleene et al. 1994, 1998, Kimura et al. 2009). ePABP mRNA is detected in both mouse and human oocytes and is downregulated during early embryogenesis (Seli et al. 2005, Guzeloglu-Kayisli et al. 2008, Sakugawa et al. 2008). ePABP mRNA is also expressed at low levels in mouse testis (Wilkie et al. 2005) but appears to be more widespread in humans. ePABP protein expression has been studied in X. laevis oogenesis where a developmental switch between PABP1 and ePABP appears to occur (Cosson et al. 2002). Thus, ePABP is the predominant PABP present during most of oogenesis, and during oocyte maturation and early embryogenesis when regulated poly(A) tail changes drive the translational selection of mRNAs (Voeltz et al. 2001). The physiological basis for this switch to ePABP remains to be determined, although ePABP stimulates translation analogously to PABP1 (Wilkie et al. 2005).

PABP1 regulates translation at multiple steps and by multiple mechanisms (Gray et al. 2000, Kalvejian et al. 2005). This is in keeping with the fact that PABP1 contains several domains that can stimulate translation and can interact with numerous translation factors (Figs. 3B and 5B). The best-characterized interaction is between PABP1 and elf4G, a component of the cap-associated complex elf4F (Figs 3 and 5B), and this interaction is conserved from yeast to man (Derry et al. 2006). Interaction of PABP1 with elf4G is proposed to increase both the poly(A)-binding affinity of PABP1 and the cap-binding affinity of the elf4F complex (Fig. 3A, step 1), establishing the ‘closed-loop’ mRNP conformation (Fig. 3B). This conformation is believed to be optimal for translational initiation and ribosomal recycling. Consistent with this, mutations that disrupt the PABP1–elf4G interaction result in compromised translational activity (Taran et al. 1997). PABP1 also binds to elf4B (Bushell et al. 2001) and PAIP1 predominantly cytoplasmic, is more closely related to the nuclear PABPN1 (Cosson et al. 2004). ePABP2 may, like its nuclear counterpart PABPN1, regulate poly(A) tail length rather than translation (Good et al. 2004) and is beyond the scope of this review.
(Craig et al. 1998; Figs 3 and 5B), with both interactions proposed to promote elf4A RNA helicase activity which aids unwinding of secondary structure within the 3’UTR (Fig. 3A, step 2). PAIP1 may also influence elf3 function (Martineau et al. 2008) which is thought to facilitate the 40S subunit joining step (Fig. 3A, step 3). PABP1 can also enhance later steps in translation including 60S subunit joining (Sachs & Davis 1989, Kahvejian et al. 2005) and translation termination. PABP1 regulates termination via an interaction with the eukaryotic translation termination factor 3 (eRF3; Hoshino et al. 1999) and recent work in yeast described a potential role for the PAB-1–eRF3 interaction in 40S recruitment; but existence of this mechanism has not been described in vertebrates (Amrani et al. 2008). The basis for the PABP1-mediated regulation of 60S joining remains enigmatic (Kahvejian et al. 2005). By necessity most PABP1 interactions have been studied in isolation and it is unclear to what extent either a single PABP1 molecule or a cooperatively bound PABP1 oligomer can support multiple interactions with the same or multiple protein partners. Therefore, while significant progress has been made, many important questions remain pertaining to the mechanisms of action of PABP1 and the functions of other PABPs (reviewed in greater detail in (Gorgoni & Gray 2004, Derry et al. 2006)).

Although the amplitude of translational activation during oogenesis is related to the length of the poly(A) tail added/number PABPs bound, this relationship is complex. Upon injection into X. laevis oocytes, reporter mRNAs with the same length poly(A) tail but different 5′UTRs are translated to differing extents (Gallie et al. 2000) and some endogenous oocyte mRNAs are activated without changes in poly(A) tail length (e.g. Wang et al. 1999) (Fig. 4). Moreover, poly(A) tails of sufficient length to support multiple PABP binding do not always confer translational activation. In immature X. laevis oocytes, for instance, c-Mos mRNA has an average poly(A) tail length of 50 nucleotides but is not translated, although reporter mRNAs containing the same length poly(A) tail are very efficiently translated (Sheets et al. 1994). This complexity is likely to be mediated, at least in part, via a poorly understood interplay between trans-acting repressors/activators bound to 3′UTR cis-acting elements and the poly(A) tail/PABP (Fig. 4). For example, repressors may prevent PABP binding to poly(A) tails or may modulate PABP activity. In this regard, Kim & Richter (2007) recently showed that ePABP associates with CPEB in a repressive complex in X. laevis oocytes prior to maturation. The ePABP–CPEB interaction is subsequently lost in maturing oocytes and ePABP binds the de novo synthesized poly(A) tails. However, our understanding has been hampered by the relatively few trans-acting factors that have been identified to date. Recently, we and others have gained insights into the links between poly(A) tail length, PABP function and the DAZL proteins, a protein family with critical roles in gametogenesis.

**DAZL family of proteins**

DAZ, DAZL and BOULE comprise a family of proteins (Fig. 6A) that have important roles in gametogenesis. Deleted in azoospermia (DAZ) is located within the azoospermia factor locus (AZFc) on the human Y-chromosome and was first identified as a candidate factor underlying diverse azoospermia and oligosper-mia defects in 5–10% of infertile men (Reijo et al. 1995, Reynolds & Cooke 2005). Human DAZL (hDAZL) and BOULE (hBOULE) are both autosomal and their roles in human fertility are less clear. hDAZL SNPs have been linked to Sertoli cell-only syndrome, oligospermia and POF (Reynolds & Cooke 2005, Fassnacht et al. 2006, Tung et al. 2006a, 2006b). An association between a lack of hBOULE protein and adult male germ cell meiotic arrest was observed (Luetjens et al. 2004); although this does not provide any evidence that hBOULE deficiency is causative. Not all family members are conserved across species with DAZ being found in humans and old world monkeys, while invertebrates only encode boule (Fig. 6A).

Germ cells are the main site of DAZL family protein expression. However, the mammalian germ cell expression patterns of the DAZL family members are complex, although only DAZL has been detected in oocytes (detailed in Table 1; reviewed in detail in (Reynolds & Cooke 2005)). In humans, mice and X. laevis DAZL expression is detectable in primordial germ cells (PGCs) and all oogenic stages examined so far (Table 1). DAZL has also been reported to be expressed in human and mouse granulosa cells (Ruggiu et al. 1999, Dorfman et al. 1999), human theca interna cells (Nishi et al. 1999) and in the granulosa-luteal cells of human corpus lutea (Pan et al. 2002, 2008), but this remains controversial. In invertebrates, DAZL family protein expression is not restricted to the germ cells/gonads with Boule being expressed in Drosophila larval neurons and adult brain (Joiner & Wu 2004, Hooper et al. 2008).

Knock-down of X. laevis DAZL (xDAZL) leads to a defect in PGC migration and proliferation and thus a loss of both female and male gametes (Houston & King 2000). Deletion of mouse Dazl (mDazl) results in a complete loss of female germ cells before birth and a failure to advance past meiotic prophase I in spermatogenesis (Ruggiu et al. 1997, Lin & Page 2005). However, it should be noted that the timing of male germ cell loss appears to vary depending on the genetic background; with the loss of male germ cells occurring earlier on an inbred background (reviewed in Reynolds & Cooke 2005). Consistent with this, DAZL was recently demonstrated to be an intrinsic factor required for germ cell entry into meiosis (Lin et al. 2008). Only oogenesis is affected in worms; loss-of-function mutations in the
**DAZL family proteins are translational regulators**

DAZL family proteins are identified by the presence of two functional domains; RRMs and DAZ domains, the latter of which appears to be unique to this family. RRMs are found in many RNA-binding proteins (such as PABPs) which function in processes as diverse as mRNA splicing, localization, translation and stability. Indeed, several studies have demonstrated that DAZL family proteins are bona fide RNA-binding proteins (Reynolds & Cooke 2005), consistent with a role in nuclear and/or cytoplasmic post-transcriptional gene regulation. The subcellular localization of DAZL family proteins changes during gametogenesis and, while DAZL family proteins may have undefined nuclear functions, it is of note that the timing of germ cell loss in outbred male mDAZL null mice corresponds to the meiotic stages during which mDAZL becomes localized to the cytoplasm (Reynolds & Cooke 2005, Anderson et al. 2007). The importance of a cytoplasmic, rather than nuclear, function was elegantly demonstrated in *Drosophila*, wherein nuclear exclusion of Boule did not disrupt germ-cell entry into meiosis (Cheng et al. 1999). In humans and mice, DAZL is located in the cytoplasm throughout oogenesis.

The first link to a role in translation came from genetic experiments in *Drosophila*, where Twine/Cdc25A mRNA translation was found to be defective in *boule* null flies. However, it remains unclear whether Boule mediates its effect directly on the translation of *Twine/Cdc25A* mRNA or indirectly via alterations in the expression of unknown mRNAs upstream of *Twine/Cdc25A* (Maines & Wasserman 1999). In vertebrates, a translational or mRNA stability role for DAZL family proteins was intimated by the detection of mDAZL, and subsequently exogenously
Table 1 Immunohistochemical analysis of DAZL family proteins in mammalian germ cells.

<table>
<thead>
<tr>
<th>Germ cell stage</th>
<th>BOULE</th>
<th>DAZL</th>
<th>DAZ</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Hs</td>
<td>Mm</td>
<td>Hs</td>
</tr>
<tr>
<td>PGC</td>
<td>No</td>
<td>Xu et al. (2001)</td>
<td>c/N Reijo et al. (2000), Ruggiu et al. (2000) and Anderson et al. (2007)</td>
</tr>
</tbody>
</table>


C Reijo et al. (2000)
<table>
<thead>
<tr>
<th>Stage</th>
<th>Expression</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>Round spermatids</td>
<td>No</td>
<td>Xu et al. (2001), Luetjens et al. (2004), Tung et al. (2006a) and Kostova et al. (2007)</td>
</tr>
<tr>
<td>Elongating spermatids</td>
<td>No</td>
<td>Xu et al. (2001), Luetjens et al. (2004), Tung et al. (2006a) and Kostova et al. (2007)</td>
</tr>
<tr>
<td>Mature sperm</td>
<td>No</td>
<td>Xu et al. (2001), Moore et al. (2003) and Tung et al. (2006a)</td>
</tr>
<tr>
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<td>n.d</td>
<td>Moore et al. (2003)</td>
</tr>
<tr>
<td>Primordial follicle oocytes</td>
<td>n.d</td>
<td>Moore et al. (2003)</td>
</tr>
<tr>
<td>Pre-antral follicle oocytes</td>
<td>n.d</td>
<td>Moore et al. (2003)</td>
</tr>
<tr>
<td>Antral follicle oocytes</td>
<td>n.d</td>
<td>Moore et al. (2003)</td>
</tr>
</tbody>
</table>

Being Y-chromosomal hDAZ is not expressed in ovary. n.d., not determined; No, not observed in immunohistochemistry; C, exclusively cytoplasmic localization; N, exclusively nuclear localization; C/N, detected in both compartments; Cn, predominantly cytoplasmic localization; C/N, predominantly nuclear localization. Where conflicting reports exist these are presented on individual lines; Hs, Homo sapiens; Mm, Mus musculus.
expressed zebrafish DAZL (zDAZL), on actively translating polyribosomes (polysomes; Tsui et al. 2000a, 2000b, Maegawa et al. 2002).

Direct evidence supporting a translational role for DAZL family proteins came from two sets of observations. First, zDAZL upregulated reporter protein synthesis in an in vitro translation assay (Maegawa et al. 2002). Second, tethering of DAZL family members to the 3'UTR of reporter mRNAs microinjected into X. laevis oocytes provided evidence that zDAZL, mDAZL, hDAZL, hDAZ and hBOULE exhibit a conserved and robust ability to stimulate the translation of mRNAs to which they were bound. This suggested a role in mRNA-specific, rather than global, regulation of translation (Collier et al. 2005). It is of note that this functional conservation occurs despite the relatively low sequence homology between some of these proteins outside the RRM and DAZ domains. Finally, the 3'UTRs from two potential mDAZL target mRNAs conferred DAZL-dependent translational regulation to reporter mRNAs, in microinjected X. laevis oocytes, and the levels of the protein products of these endogenous target mRNAs were reduced in the remaining germ cells in tests of mDazl null mice (see below; Reynolds et al. 2005, 2007).

**mRNA targets of DAZL family protein-mediated regulation of translation**

The phenotypes associated with the loss of DAZL family function suggest that the targets of DAZL-mediated translational regulation are likely to be mRNAs that encode proteins with key functions in oogenesis and/or spermatogenesis. Initial studies indicated that DAZL exhibited a general affinity for poly(U) RNA and suggested that the affinity may be increased for poly(U) interspersed by G or C residues (Venables et al. 2001). These studies defined a loose consensus sequence, UUU[G/C]UUU, which was later refined to GUUC and U_{2-10}[G/C]U_{2-10} for zDAZL (Maegawa et al. 2002) and mDAZL (Reynolds et al. 2005) respectively. Tethered-function and reporter mRNA studies in X. laevis oocytes (Collier et al. 2005) and zDAZL-transfected cells respectively (Maegawa et al. 2002) suggest that functional DAZL-binding sites were likely to be located in 3'UTRs of target mRNAs. Furthermore, the tethering of multiple molecules of DAZL stimulates translation to a greater extent than tethering of a single DAZL molecule; suggesting that multiple DAZL-binding sites may be required for efficient translational activation of an mRNA (Collier et al. 2005). Consistent with this, several DAZL target mRNAs contain multiple DAZL-binding motifs (Reynolds et al. 2005, 2007) and identification of such 3'UTR motif clusters may prove important in identifying bona fide target mRNAs. While the DAZL-binding consensus occurs relatively frequently throughout vertebrate genomes; mRNAs with a 3'UTR that contains multiple binding sites occur much less frequently.

Efforts to identify in vivo mRNA targets of mammalian DAZL family proteins utilized varied approaches but have been mainly restricted to mDAZL (reviewed in (Reynolds & Cooke 2005)) and have identified multiple putative targets (Jiao et al. 2002, Reynolds et al. 2005, 2007). These studies focused on testis-expressed mRNAs but it is likely that at least a subset of the identified mRNAs are regulated during oogenesis.

Mouse vasa homolog (Mvh) and Syce3 mRNAs were isolated by co-immunoprecipitation with mDAZL from UV-crosslinked mouse testis extracts (Reynolds et al. 2005, 2007). The male phenotypes of Mvh null and Syce3 null mice correspond to distinct blocks in meiotic prophase I, reminiscent of the final block in the mDazl null mice (Tanaka et al. 2000, Yuan et al. 2000). Combinations of in vitro RNA-binding assays and translation reporter assays in injected X. laevis oocytes indicated that mDAZL could regulate the translation of reporters containing the 3'UTR from these mRNAs. At least for Syce3, stimulation was dependent on the presence of intact mDAZL-binding sites. Importantly, MVH and SYCP3 protein levels were significantly reduced in the surviving germ cells of mDazl null testis (5 or 7 days post-partum respectively), implicating these mRNAs as the first physiological targets of a vertebrate DAZL protein (Reynolds et al. 2005, 2007).

However, MVH expression is dispensable for normal mouse oogenesis (Tanaka et al. 2000). Thus, while Mvh mRNA may be regulated by mDAZL in oocytes, the loss of its translation does not underlie the defective oogenesis in mDazl null mice. In Syce3 null female mice, oogenesis and folliculogenesis progress to completion but the mature oocytes exhibit a high degree of aneuploidy and a resultant failure of embryogenesis (Yuan et al. 2002). The lack of a correlation between the female phenotypes of the mDazl null and Syce3 null mice indicates that defective Syce3 translation is not the primary lesion leading to the loss of germ cells in mDazl null females, but does not exclude DAZL-mediated Syce3 translation contributing to the production of viable female gametes. It would be desirable to generate a conditional mDazl null mouse, to induce mDAZL deficiency during later stages of oogenesis and thereby facilitate the study of the roles of putative mDAZL target mRNAs; for instance during the diplotene arrest and maturation of oocytes.

Studies aimed at identifying human mRNA targets of DAZL family proteins have proposed CDC25 and severe depolymerization of actin 1 (SDAD1) as putative targets. Cdc25 was first suggested as a Boule target mRNA in Drosophila (Maines & Wasserman 1999), subsequently as a mDAZL target mRNA in mouse (Venables et al. 2001, Jiao et al. 2002) and CDC25 protein was absent in testicular biopsies from...
men with reduced hBOULE expression (Luetjens et al. 2004). However, studies have disagreed regarding which CDC25 isoform is the putative target mRNA and regarding the location of the binding sequence for DAZL family members within the mRNA, although this disparity may be, in part, the result of species differences (Maines & Wasserman 1999, Venables et al. 2001, Jiao et al. 2002, Maegawa et al. 2002). Nonetheless, the potential for DAZL-mediated regulation of CDC25 translation warrants further study since Cdc25b null female mice are sterile (Lincoln et al. 2002). SDAD1 was identified in a yeast-based screen that recovered mRNAs associated with both human PUM2 (hPUM2) and hDAZL (Fox et al. 2005). hDAZL binds to the SDAD1 mRNA 3′UTR in vitro but, to date, it is not known whether hDAZL regulates SDAD1 mRNA translation in vitro or in vivo.

To date, no vertebrate DAZL mRNA targets have been definitively identified in female germ cells and a concerted effort is required to redress this lack of knowledge, in order to better understand the role of hDAZL in human fertility. However, a bona fide DAZL family target mRNA with a role in oogenesis has been identified in the hermaphroditic worm C. elegans. Translation of FBF mRNAs, which encode proteins required for germline stem cell maintenance and the sperm-oocyte switch, is enhanced by DAZ-1 binding during the switch to oogenesis (Otori et al. 2006).

In vertebrates, a potential oocyte target mRNA was suggested in X. laevis oocytes. RINGO/SPY activates CDK2, initiating a phosphorylation cascade that is critical for oocyte maturation (Box 2 and Fig. 2). In prophase I-arrested oocytes RINGO/SPY mRNA is translationally repressed by a complex containing PUM2 (xPUM2), xDAZL and ePABP. RINGO/SPY translation is required prior to the onset of CPE-mediated cytoplasmic polyadenylation and its mRNA does not contain CPEs. When maturation is triggered xPUM2 exits the complex and RINGO/SPY translation is activated, presumably in an ePABP and/or xDAZL-dependent manner (Padmanabhan & Richter 2006). A demonstration that RINGO/SPY translational activation is mediated by xDAZL in X. laevis would raise the possibility that it is similarly regulated during mammalian oogenesis.

**Mechanism of DAZL-mediated translational stimulation**

The observation that multiple DAZL family members from a variety of species can stimulate the translation of specific mRNAs suggests that this conserved function is critical to their roles in oogenesis (Collier et al. 2005, Reynolds et al. 2007). Therefore, it is important to understand the mechanism(s) by which they stimulate translation. Since the function of mRNA-specific translational activators is poorly understood, few paradigms exist for the function of DAZL family proteins. Sucrose gradient analysis of translation intermediates from microinjected X. laevis oocytes revealed that mDAZL regulates translation initiation (Collier et al. 2005), similar to characterized mRNA-specific repressors; thereby raising the possibility that DAZL influences initiation factor function.

A conserved interaction between DAZL family members and both PABP1 and ePABP was detected in vivo (Collier et al. 2005; Fig. 6B). Importantly, several lines of evidence demonstrated protein-protein interactions between DAZL family members and PABP1 or ePABP (Table 2), rather than simply detecting DAZL and PABP associated with the same mRNA, thereby implicating PABP1 and ePABP as attractive candidates for DAZL-regulated initiation factors.

Two further observations support the hypothesis that a PABP-DAZL interaction is critical for DAZL family proteins to stimulate translation. First, deletion of the PABP interaction site, but not other regions, within mDAZL completely abrogated its ability to stimulate translation (Collier et al. 2005). This loss of activity was independent of mDAZL RNA-binding activity since the protein was artificially tethered to the reporter mRNA. Second, the relative ability of mDAZL to activate reporter mRNA translation is reduced when the mRNA is polyadenylated; indicating that the amplitude of mDAZL-mediated translational activation is altered when the mRNA can recruit multiple PABPs via the poly(A) tail (Collier et al. 2005). However, the determination of the extent to which PABPs are required for DAZL function in vertebrate oogenesis will require the genetic confirmation of a role for the DAZL-PABP interaction. Interestingly, the C. elegans DAZ-1 loss-of-function phenotype is highly reminiscent of the C. elegans PAB-1-deficient phenotype, both exhibiting early meiotic blocks in the female germline (Maciejowski et al. 2005, Maruyama et al. 2005).

The apparent requirement for an interaction with PABP, a known translation initiation factor, provides a model for DAZL function. In this model, DAZL bound to a 3′UTR recruits PABP which, in turn, interacts with 5′ end-associated factors to enhance the assembly of the closed-loop mRNP conformation (Fig. 7, see also Fig. 3B). The interaction of DAZL with the C-terminal region of PABP should permit PABP to simultaneously scaffold interactions with the key factors required to promote translation initiation, such as eIF4G (Fig. 5B). However, it remains to be examined whether PABP maintains all its interactions with translation factors when complexed with DAZL. Nor is it known which step(s) of initiation DAZL regulates as PABP has pleiotropic effects on initiation. Moreover, this model does not rule out the possibility that other DAZL-interacting factors may modulate or co-operate with PABP to promote DAZL-mediated stimulation.
Table 2 Available evidence for best-characterized DAZL-interacting proteins.

<table>
<thead>
<tr>
<th>Protein</th>
<th>Interacts with</th>
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<th>Methods used</th>
<th>RNase used?</th>
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<td></td>
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<td></td>
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<td>No</td>
<td>Y2H</td>
<td></td>
<td></td>
<td>Urano et al. (2005)</td>
</tr>
<tr>
<td>XI DAZL</td>
<td>XI PABP1</td>
<td>No</td>
<td>Y2H, oCoP, eCoIP</td>
<td>RNase 1</td>
<td>Collier et al. (2005)</td>
</tr>
<tr>
<td></td>
<td>XI ePABP</td>
<td>No</td>
<td>eCoIP, Y2H</td>
<td>RNase 1</td>
<td>Collier et al. (2005)</td>
</tr>
<tr>
<td></td>
<td>XI PUM2</td>
<td>No</td>
<td>oCoIP</td>
<td>RNase A</td>
<td>Padmanabhan &amp; Richter (2006)</td>
</tr>
<tr>
<td>Ce DAZ-1</td>
<td>Ce CPB-3</td>
<td>No</td>
<td>Y2H, oCoIP, IHC</td>
<td></td>
<td>Hasegawa et al. (2006)</td>
</tr>
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</table>

Yeast two-hybrid (Y2H) analysis is frequently provided as evidence of a direct protein interaction but interactions can, in some cases, be bridged by endogenous yeast proteins or RNA. * indicates yeast-based screen with multiple components. GST pull-downs (GST) utilizing recombinant proteins purified to homogeneity indicate direct protein–protein interactions. When recombinant proteins are used to pull-down proteins from cell lysates or endogenous yeast proteins or RNA. * indicates yeast-based screen with multiple components. GST pull-downs (GST) utilizing recombinant proteins.

DAZL interacting proteins and other roles of DAZL in post-transcriptional regulation in the cytoplasm

The recently defined role of DAZL family proteins as translational activators does not preclude them from having additional translational/non-translational functions within the cytoplasm and/or nucleus. Indeed, many mRNA-binding proteins interact with different partners in the same cell, as well as in different cell types or developmental stages, to perform different functions (Abaza & Gebauer 2008). Thus, the composition of DAZL-containing complexes may change throughout gametogenesis in order to enable reprogramming of protein synthesis.

A number of DAZL family-interacting proteins have been identified (Fig. 6B and Table 2) including PUM2, dynein light chain (DLC) and DAZL-associated protein 1 (DAZAP-1). The previously characterized molecular functions of these proteins (Table 3) may provide insight into their potential roles in DAZL-mediated regulation. However, little is actually known regarding their in vivo functions with DAZL. Additional partners are also described for which no obvious functional role in DAZL-mediated regulation can be ascribed: DAZAP-2 (Tsui et al. 2000a, 2000b) and DZIP1, 2 and 3 (Moore et al. 2004). Table 2 summarises the evidence for the best-characterized interactions with DAZL proteins.

The PUF family protein PUM2 is expressed in human, mouse and X. laevis oocytes and is also highly expressed in human and mouse PGCs, gonocytes and spermatogonia and at lower levels in primary and secondary spermatocytes (Moore et al. 2003, Xu et al. 2007). xPUM2 acts as a translational repressor via binding to PBEs (see sequences required for cytoplasmic polyadenylation), and also contributes to regulated cytoplasmic...
polyadenylation (Table 3) (Nakahata et al. 2001, 2003). In immature X. laevis oocytes xPUM2 regulates RINGO/SPY translation (see mRNA targets), and interacts with xDAZL that is also bound to this mRNA (Padmanabhan & Richter 2006). The association of xPUM2, xDAZL and ePABP with translationally repressed RINGO/SPY mRNA in X. laevis does not contain putative DAZL binding sites in its 3′UTR (Urano et al. 2005). Conversely, it has been suggested that the mRNA targets of PUM2 may be altered as a function of the interaction with specific DAZL family proteins (Urano et al. 2005).

While data supports a role for xPUM2 in X. laevis oogenesis (Padmanabhan & Richter 2006), it remains unclear whether any of its functions are mediated via xDAZL. Genetic evidence for a physiologically important role in mammalian oogenesis is absent since Pum2 null mice are fertile. However, male mice do display a significant reduction in testis size (Xu et al. 2007) and the lack of ovarian phenotype could be due to a level of redundancy with mouse PUM1. Thus, further work is required to clarify the functional and physiological consequences of DAZL–PUM2 interactions.

mDAZL also interacts in vitro with dynein light chain, a component of the microtubule-associated dynein–dynactin motor complex which has an important role in mRNA localization (Lee et al. 2006). Indeed, when mDAZL was ectopically expressed in cultured somatic cells, it was able to regulate the cytoplasmic distribution of reporter mRNAs containing either the CDC25C (but interfering with the poly(A) tail, rather than xDAZL, as described for Drosophila pumilio (Wreden et al. 1997). It also remains possible that xDAZL is an active component of the repression complex. Thus, it is unclear whether xPUM2 is repressing the stimulatory action of xDAZL, whether xDAZL is an active component of the repressive complex or even whether xDAZL is involved in the subsequent activation of this mRNA.

It has also been suggested that PUM2/DAZL interactions may affect RNA-binding. Human PUM2 (hPUM2) interacts with hBOULE and hDAZL (Moore et al. 2003, Urano et al. 2005) and it has been suggested that hPUM2 may aid the recruitment of these proteins to mRNAs. Consistent with this, RINGO/SPY mRNA in X. laevis does not contain putative DAZL binding sites in its 3′UTR (Urano et al. 2005). Conversely, it has been suggested that the mRNA targets of PUM2 may be altered as a function of the interaction with specific DAZL family proteins (Urano et al. 2005).

The described molecular functions are from a variety of species, some of which have multiple family members. These functions are independent of an interaction with DAZL family members. Experimental evidence for DAZL-mediated function refers to experiments aimed at directly testing the function of these proteins when participating in complexes with DAZL.

Table 3 Molecular functions of DAZL interacting proteins.

<table>
<thead>
<tr>
<th>DAZL-interacting protein</th>
<th>Molecular function(s)</th>
<th>Experimental evidence for DAZL-dependent function</th>
</tr>
</thead>
</table>
| PABPs                    | i) Translation initiation factor  
                           | ii) mRNA stability and deadenylation  
                           | iii) Translational repressor  
                           | iv) Nonsense-mediated mRNA decay | i) Tethered function assays; Collier et al. (2005) |
| PUM2                     | i) Translational repressor of CPE-dependent translation  
                           | ii) Translational activator of CPE-dependent translation  
                           | iii) Direct translational repressor | – |
| Dynemin light chain      | i) Transport of mRNAs and proteins along microtubules | i) Intracellular mRNA transport studies; Lee et al. (2006) |
| DAZAP-1                  | ?                     | – |
| DAZAP-2, DZIP1-3         | ?                     | – |

The described molecular functions are from a variety of species, some of which have multiple family members. These functions are independent of an interaction with DAZL family members. Experimental evidence for DAZL-mediated function refers to experiments aimed at directly testing the function of these proteins when participating in complexes with DAZL.
not CDC25A), TPX1 or MVH 3′UTRs (see DAZL targets). While mRNA localization is heavily linked to translational control in organisms whose oocytes contain germ-plasm, the role of this process in mammalian oocytes is less well explored. It would be interesting to determine the extent of mDAZL involvement in the localization of mRNAs that it subsequently activates in vivo. Moreover, localized mRNAs are normally repressed prior to their translation, which could involve partners such as PUM2 or even DAZAP-1.

The mRNA-binding protein DAZAP-1 (also known as proline-rich protein (PRRP)) is widely expressed in human and mouse, with high-level expression in testis (Tsui et al. 2000a, 2000b, Dai et al. 2001, Kurihara et al. 2004, Hori et al. 2005, Pan et al. 2005). Where investigated, DAZAP-1 appears to interact with DAZ motifs, a region important for the polysomal localization of DAZL, suggesting that it could contribute to DAZL-activated translation or modulate this function by preventing the binding of other proteins to this region. Interestingly, in this regard, human DAZAP-1-associated DAZ does not interact with PABP in vitro (Morton et al. 2006), although the minimal binding site for PABP does not include the DAZ motif.

Thus, while no role in translation has been established for DAZAP-1, these studies raise the possibility that DAZAP-1 may repress, activate or modulate DAZL family-mediated translation.

DAZAP-1 may also have other roles. Human DAZAP-1 is a nucleocytoplasmic shuttling protein (Lin & Yen 2006) and its X. laevis ortholog, PRRP, associates with mRNA localization elements in Vg1 and VegT mRNAs (Zhao et al. 2001). However, it is unclear whether DAZAP-1 plays an active role in mRNA localization and its role on these mRNAs appears to be independent ofxDAZL. The wide expression pattern of DAZAP-1 also suggests that it frequently functions independently of DAZL proteins. DAZAP-1 is a substrate of the ERK2 kinase, raising the possibility that its interaction with protein partners may be subject to regulation by phosphorylation. Consistent with this notion, phosphomimetic forms of human DAZAP-1 exhibit reduced DAZ binding activity in vitro (Morton et al. 2006).

Recently, Hsu et al. (2008) showed that most Dazap-1 null (or hypomorphic) mice die perinatally due to growth defects, indicating roles for DAZAP-1 in normal growth and development. The few surviving DAZAP-1 hypomorphic male progeny have no post-pachytene spermatocytes indicating a role for DAZAP-1 in spermatogenesis. However, Dazap-1 null mice exhibit apparently normal oogenesis, indicating that an mDAZL–DAZAP-1 interaction is not an absolute requirement for mouse oogenesis, although DAZAP-1 is normally expressed in the ovary.

Finally, a role for DAZL family proteins in regulating mRNA stability may be indicated by the decrease in abundance of a subset of mRNAs in mDazl null mouse testis (Maratou et al. 2004). However, these changes in mRNA abundance may be a direct consequence of germ cell loss or an indirect consequence of mDAZL deficiency. Nonetheless, changes in mRNA stability are often linked to changes in mRNA translation. Thus, DAZL-mediated changes in the translation of target mRNAs may alter their stability, but no evidence currently supports a distinct role in mRNA stability.

A model for DAZL as a translational activator of mRNAs during oogenesis

Observations in C. elegans and mouse suggest that DAZL may function at various defined times during gametogenesis (Collier et al. 2005, Reynolds & Cooke 2005, Reynolds et al. 2007). DAZL function may change during the complex, multistage oogenesis program in order to regulate different aspects of mRNA metabolism; changes which may depend on protein partner interactions. However, to date, only a role in activating mRNA translation has been demonstrated. Cytoplasmic polyadenylation is an important mechanism for regulating the translation of a large number of oligoadenylated mRNAs, but not all mRNAs appear to require polyadenylation for translational activation (Radford et al. 2008). While loss of repression could contribute to the activation of these mRNAs, it appears likely that activating proteins may be required to compensate for their short poly(A) tail status (Wang et al. 1999). Since DAZL family proteins appear to be mRNA-specific translational regulators that stimulate efficient translation of mRNAs with short poly(A) tails, it was suggested that the binding of DAZL proteins (and their associated PABPs) may be one mechanism by which such mRNAs can be activated (Collier et al. 2005, Reynolds et al. 2005, 2007; Figs 4 and 7). This would allow the activation of small subsets of mRNAs, for instance at times that do not coincide with waves of cytoplasmic polyadenylation. This hypothesis does not preclude the DAZL-mediated translational activation of mRNAs with longer poly(A) tails but, rather, predicts that the magnitude of their stimulation would be reduced.

It seems unlikely that there is no link between DAZL-mediated translational activation and cytoplasmic polyadenylation. Indeed, the cytoplasmic polyadenylation of some DAZL family target mRNAs may be required for their maximal translational activation (Fig. 4). For instance, translation of Sycp3 is attenuated in both mDazl null and Cpeb null mouse germ cells. Sycp3 mRNA contains both mDAZL binding sites and a CPE and has a short poly(A) tail (~20 As) in Cpeb null oocytes, indicating that CPEB-dependent cytoplasmic polyadenylation is required for full translational activation of Sycp3 expression. The mDAZL binding site(s) in the Sycp3 3′UTR overlaps with the CPE and, although it has not been formally excluded that both sites can be occupied simultaneously, the inability to co-immuno-precipitate CPEB and mDAZL makes this unlikely.
(Reynolds et al. 2007). The translation of Symp3 may be initially activated by mDAZL which is then either replaced or further activated by CPEB to maintain and/or promote Symp3 translation. In contrast, in C. elegans, an interaction between the BOULE ortholog DAZ-1 and the CPEB ortholog CPB-3 has been detected (Hasegawa et al. 2006) raising the possibility that DAZL family proteins can activate translation both directly via DAZL/PABP interactions and indirectly by facilitating cytoplasmic polyadenylation. However, no DAZL family members have yet been shown to promote polyadenylation and, given the dual role of CPEB in repression as well as polyadenylation, the functional consequences of this interaction remain unclear. Greater insight into the molecular mechanisms of DAZL action and its relationship with other protein complexes will be required to understand how regulatory elements and associated factors, both individually and in combination, function to achieve the changes in gene expression required to complete oogenesis.

**Perspectives**

While other putative functions remain to be elucidated, it appears that DAZL proteins fulfil their roles in oogenesis, at least in part, by activating the translation of target mRNAs. However, it is appealing to speculate that DAZL may also participate in complexes that localise and repress target mRNAs prior to their activation at defined times during oogenesis. The extent to which other family members may share these functional properties also remains to be determined, but while DAZ and BOULE mRNA targets may overlap with those of DAZL in spermatogenesis this appears unlikely in vertebrate oocytes.

A greater understanding of the roles of DAZL proteins during vertebrate oogenesis is hampered by the current dearth of identified female DAZL target mRNAs, although further study of RINGO/SPY in X. laevis appears warranted. This mRNA is activated early in maturation, lacks CPEs, is bound by complexes containing xDAZL and is critical for oocyte maturation. The DAZL binding consensus sequence needs to be further defined prior to its use in bioinformatic searches for putative target mRNAs. Moreover, these searches may require a clearer understanding of how these elements function together with, or independently of, other control sequences such as CPEs and PBEs. Furthermore, it will be important to determine whether DAZL-interacting proteins alter the RNA-binding specificity of DAZL proteins. Ultimately, the functional definition and in vivo validation of DAZL target mRNAs will require a combination of molecular and genetic studies in models such as Xenopus and mouse.

In oogenesis, DAZL-mediated translation may predominantly play a role in the activation of mRNAs with short poly(A) tails. Consistent with this, Symp3 has a short poly(A) tail in CPEB-deficient mice. While molecular studies support an important role of PABP in DAZL-mediated activation, the physiological role of this interaction remains to be clarified. The targeted mutation of the PABP-binding region of DAZL by genetically disrupting this interaction in a model organism, such as the mouse, would determine the extent to which DAZL utilises PABP for translational activation. Such a model would also provide insight into some of the roles of PABPs during gametogenesis. Ultimately, further definition of the molecular mechanism of DAZL-mediated stimulation may require a detailed understanding of the mechanisms by which PABP activates the initiation pathway and which of these activities is modulated by DAZL.

However, PABPs are also likely to have other essential roles in oogenesis, including the translational activation of cytoplasmically polyadenylated mRNAs and both default and adenosine/uridine-rich element (ARE)-mediated deadenylation of mRNAs. Expression of multiple PABPs within certain cell types, including X. laevis oocytes, raises the possibility that PABPs have both overlapping and distinct roles during oogenesis. It is therefore tempting to speculate that PABPs may share some of their poly(A)-mediated functions but differ in their ability to control specific mRNAs, for instance via proteins such as DAZL. Generation of PABP-deficient vertebrate models will be crucial to increase our understanding of the molecular and physiological roles of this multi-functional protein family in gametogenesis. Moreover, it is clear that the study of PABPs will be relevant to other areas of reproductive and non-reproductive biology.

The study of DAZL function may also have unforeseen implications because DAZL-mediated recruitment of PABP in a poly(A)-independent manner raises the question of how many other 3’UTR-binding proteins function analogously. The idea that such a mechanism may be more widespread is supported by the recent observation that proteins such as BRCA1 similarly utilise PABP (Dizin et al. 2006); thus, studies of DAZL may provide a paradigm for understanding the function of other translational activators in oocytes as well as other cell types.

In conclusion, defining the roles, mechanisms and RNA targets of these interrelated DAZL and PABP families in gametogenesis may ultimately identify potential sites of therapeutic intervention in infertility. However, it will be important to ascertain to what extent these animal studies accurately model human reproductive disorders.

**Declaration of interest**

The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research reported.
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DAZL and PABP mediated translation in oocytes

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