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A novel mRNA 3′ untranslated region translational control sequence regulates *Xenopus* Wee1 mRNA translation

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Abstract

Cell cycle progression during oocyte maturation requires the strict temporal regulation of maternal mRNA translation. The intrinsic basis of this temporal control has not been fully elucidated but appears to involve distinct mRNA 3′ UTR regulatory elements. In this study, we identify a novel translational control sequence (TCS) that exerts repression of target mRNAs in immature oocytes of the frog, *Xenopus laevis*, and can direct early cytoplasmic polyadenylation and translational activation during oocyte maturation. The TCS is functionally distinct from the previously characterized Musashi/polyadenylation response element (PRE) and the cytoplasmic polyadenylation element (CPE). We report that TCS elements exert translational repression in both the Wee1 mRNA 3′ UTR and the pericentriolar material-1 (Pcm-1) mRNA 3′ UTR in immature oocytes. During oocyte maturation, TCS function directs the early translational activation of the Pcm-1 mRNA. By contrast, we demonstrate that CPE sequences flanking the TCS elements in the Wee1 3′ UTR suppress the ability of the TCS to direct early translational activation. Our results indicate that a functional hierarchy exists between these distinct 3′ UTR regulatory elements to control the timing of maternal mRNA translational activation during oocyte maturation.

Introduction

Fully grown (immature) vertebrate oocytes are typically arrested in prophase I of the first meiotic division. In response to the appropriate external stimulus, immature oocytes re-enter the cell cycle and resume meiotic progression before arresting again at metaphase of Meiosis II. This progression through meiosis is termed oocyte maturation and results in oocytes that can be fertilized by sperm. During oocyte maturation and the developmental period immediately following fertilization, gene transcription is actively repressed and altered patterns of protein expression are determined by translation of pre-existing mRNAs (maternal mRNAs) present in the oocyte (Heikinheimo and Gibbons, 1998; Newport and Kirschner, 1982). The immature oocyte contains a pool of nontranslated, maternal derived mRNAs that are stored as ribonucleoprotein complexes. At various stages in development, specific mRNAs are recruited for translation (Davidson, 1986). This regulated translation of maternal mRNAs is critical for early developmental processes (Colegrove-Otero et al., 2005; de Moor et al., 2005; Kuersten and Goodwin, 2003).

Regulation of maternal mRNA translation has been best characterized in oocytes of the frog, *Xenopus laevis*. Maternally derived mRNAs may be classed as “early” or “late” based on the order of their translational activation during oocyte maturation. Late class mRNAs are activated coincident with or after breakdown of the oocyte nucleus (termed the germinal vesicle)

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and completion of Meiosis I. Early class mRNAs are translationally activated prior to germinal vesicle breakdown (GVBD). Activation of the late class mRNAs requires early class mRNA translation (Ballantyne et al., 1997; Charlesworth et al., 2006; de Moor and Richter, 1997). The strict temporal order of early and late class mRNA translation is essential to ensure hormone-dependent progression through oocyte meiotic maturation (Ferby et al., 1999; Freeman et al., 1991; Howard et al., 1999; Murakami and Vande Woude, 1998; Nakajo et al., 2000; Roy et al., 1991; Sheets et al., 1995). The mechanisms that specifically recognize early and late class mRNA species and induce translation differentially have not been fully elucidated.

Recent work has underscored the role of regulatory elements in the 3′ untranslated region (3′ UTR) of targeted mRNAs for orchestrated developmental changes in proteomic profiles (reviewed in (de Moor et al., 2005; Kuersten and Goodwin, 2003)). Two distinct 3′ UTR regulatory elements have been described which have unique temporal induction properties during *Xenopus* oocyte maturation. Cytoplasmic polyadenylation elements (CPEs) can enforce late mRNA translational activation (Charlesworth et al., 2000; Tung et al., 2007) while Musashi/polyadenylation response elements (PREs) direct early mRNA translational activation (Charlesworth et al., 2002). However, some early class mRNAs do not contain consensus Musashi/PRE or CPE sequences suggesting that other, as yet undefined, 3′ UTR regulatory elements may contribute to early mRNA translational activation.

In this study, we have characterized a novel regulatory element that confers early mRNA polyadenylation and translational activation. This element, designated a translational control sequence (TCS), mediates both mRNA translational repression in immature *Xenopus* oocytes and directs early mRNA translational activation in response to progesterone stimulation. We show that TCS elements contribute to the repression in immature oocytes of both the late class Wee1 mRNA (which encodes a protein involved in regulation of CDK1 activity; McGowan and Russell, 1993; Mueller et al., 1995; Parker and Piwnica-Worms, 1992) and the Pcm-1 mRNA (which encodes a protein involved in the assembly of centrosomes and microtubule networks; Balczon et al., 1994; Dammermann and Merdes, 2002). We demonstrate that TCS function directs early class mRNA translational activation exerted by the 3′ UTR of the Pcm-1 mRNA. Interestingly, CPE sequences flanking the TCS elements in the Wee1 3′ UTR suppress the ability of the TCS to exert early translational activation. These findings indicate that multiple mechanisms exist to direct both repression and early translational activation of mRNAs and a functional hierarchy between distinct 3′ UTR regulatory elements may refine the temporal patterns of maternal mRNA translational activation during oocyte maturation.

Materials and methods

Plasmid constructions and RNA synthesis

Standard PCR mutagenesis was used to generate the 3′ UTR reporter constructs utilized in this study. A detailed methodology of plasmid construction is provided as Supplementary data.

The sequence integrity of all 3′ UTR constructs was confirmed by DNA sequencing. For *in vitro* transcription, all plasmids were linearized with Pst1 and 5′ capped RNA synthesized *in vitro* with SP6 RNA polymerase as previously described (Melton et al., 1984). The resulting RNAs lacked poly[A] tails but became oligoadenylated following injection into immature oocytes (typically receiving 5–10 adenylate residues), consistent with prior studies (Charlesworth et al., 2000; de Moor and Richter, 1997; Gillian-Daniel et al., 1998; Sheets et al., 1994).

Oocyte culture and injection

Oocytes were defolliculated by collagenase digestion as previously described (Charlesworth et al., 2000). Dumont stage VI immature oocytes (≥1200 μm in diameter) were isolated and injected with 1 ng of reporter RNA. Where indicated, real-time PCR was employed to verify the levels of reporter mRNAs present in the injected oocyte samples. The levels of GST reporter mRNA were normalized to the levels of the endogenous cyclin B1 mRNA (see Supplementary data for real-time PCR primer sequence and reaction parameters). To reveal progesterone-inducible polyadenylation, oocytes were stimulated with 2 μg/ml progesterone and the rate of germinal vesicle breakdown (GVBD) was monitored morphologically by the appearance of a white spot on the animal hemisphere. Pools of 10 oocytes were harvested at each time point, along with time-matched immature oocyte controls. Because oocytes from different frogs mature at different rates in response to progesterone, the culture times were standardized between experiments to the time taken for 50% of oocytes to undergo germinal vesicle breakdown ($GVBD_{50}$).

Western blot analyses

Oocytes were lysed in 10 μl of ice cold Nonidet P-40 (NP-40) lysis buffer per oocyte (MacNicol et al., 1993), and insoluble material and lipid were separated by centrifugation at 13,000×*g* for 10 min at 4 °C. RNA and protein lysate were prepared from the same oocyte samples, as previously described (Charlesworth et al., 2000). The lysates were normalized for the amount of total protein, separated on sodium dodecyl sulfate (SDS)-12% polyacrylamide gels, and transferred to a 0.2-μm-pore-size nitrocellulose filter. The filter was blocked with 5% nonfat dried milk in TBS. Filters were incubated with antibody and visualized with an appropriate horseradish peroxidase-linked secondary antibody by enhanced chemiluminescence (ECL). Antiserum to GST was obtained from Santa Cruz Biotechnology, antiserum to tubulin was obtained from Sigma, and antiserum to Pcm-1 was a gift from Dr. Andreas Merdes (Dammermann and Merdes, 2002). GST accumulation was visualized and quantitated by ECL western blotting as previously described (Charlesworth et al., 2000, 2002, 2004) using ChemiGlow West, a ChemiImager 5500 and AlphaEaseFC software (AlphaInnotech Corp.). One-way analysis of variance and post hoc Newman–Keuls multiple comparison test were performed to analyze differences between the means and differences in *P*-value less than 0.05 were considered statistically significant.

Polyadenylation assays

Reporter mRNA polyadenylation was assessed by northern blotting or RNA ligation-coupled PCR as previously described (Charlesworth et al., 2000, 2002; Rassa et al., 2000). For RNA ligation PCR, a forward primer to the β-globin 3′ UTR was used (GCG GAA TTC ACA CTT ACA AAA TGT TGT) to analyze polyadenylation of reporter constructs, except in the case of the 5′ boundary mutants where a primer to the GST coding region was used instead (Prasad et al., 2008). For analysis of endogenous mRNA polyadenylation, the following gene-specific forward primers were employed: Pcm-1, AAG CCT GTC TTT TTC CTC TC; Cyclin A1, CAT TGA ACT GCT TCA TTT TCC CAG; and Cyclin B1, GTG GCA TTC CAA TTG TGT ATT GTT. All PCR products were resolved on a 3% agarose gel and visualized using ethidium bromide staining. An increase in PCR product size is indicative of poly[A] tail extension (Charlesworth et al., 2002) and where noted polyadenylation was verified by direct sequencing of the PCR products. Where indicated, the mode of the PCR product population poly[A] tail length extension was determined using AlphaEaseFC Software (Alpha Innotech) snap-to-peak analyses.