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The stem-loop binding protein regulates translation of histone mRNA during mammalian oogenesis

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Abstract

Although messenger RNAs encoding the histone proteins are among the most abundant in mammalian oocytes, the mechanism regulating their translation has not been identified. The stem-loop binding protein (SLBP) binds to a highly conserved sequence in the 3′-untranslated region (utr) of the non-polyadenylated histone mRNAs in somatic cells and mediates their stabilization and translation. We previously showed that SLBP, which is expressed only during S-phase of proliferating cells, is expressed in growing oocytes at G2 of the cell cycle and accumulates substantially during meiotic maturation. We report here that elevating the amount of SLBP in immature (G2) oocytes is sufficient to increase translation of a reporter mRNA bearing the histone 3′-utr and endogenous histone synthesis and that this effect is not mediated through increased stability of the encoding mRNAs. We further report that translation of the reporter mRNA increases dramatically during meiotic maturation coincident with the accumulation of SLBP. Conversely, when SLBP accumulation during maturation is prevented using RNA interference, both translation of the reporter mRNA and synthesis of endogenous histones are significantly reduced. This effect is not mediated by a loss of the encoding mRNAs. Moreover, following fertilization, SLBP-depleted oocytes also show a significant decrease in pronuclear size and in the amount of acetylated histone detectable on the chromatin. These results demonstrate that histone synthesis in immature and maturing oocytes is governed by a translational control mechanism that is directly regulated by changes in the amount of SLBP.

Keywords: Oocyte; Histones; Translational control; SLBP; Meiotic maturation

Introduction

During growth, the mouse oocyte accumulates maternal products that are utilized during the late stages of oogenesis or after fertilization. For example, certain mRNAs synthesized by the growing oocyte are not translated immediately but instead are stored in a silent form until they are recruited for translation during meiotic maturation of the oocyte (Huarte et al., 1987; Vassalli et al., 1989; Stutz et al., 1998; Oh et al., 2000). Conversely, other mRNAs are actively translated during oocyte growth but undergo silencing during maturation (Bachvarova et al., 1989; Paynton and Bachvarova, 1994). The molecular mechanisms underlying silencing and activation of oocyte mRNAs are best understood in the context of the cytoplasmic polyadenylation element (CPE) and the CPE-binding protein (CPEB). The CPE is a U-rich sequence found in the 3′-untranslated region (utr) of certain mRNAs and is typically located within 100 nt of the polyadenylation signal (Oh et al., 2000). In *Xenopus* oocytes, CPEB binds to the CPE and also to a protein termed maskin. Maskin binds to eIF4E, and this prevents initiation of translation (Paris and Richter, 1990; Hake and Richter, 1994; Stebbins-Boaz et al., 1999). During maturation, phosphorylation of CPEB allows recruitment of the cleavage and polyadenylation specificity factor (CPSF), which contributes to the elongation of the polyA tail. This indirectly leads to the displacement of maskin from eIF4E, thus

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allowing translational activation of the mRNA (Mendez et al., 2000; Hodgman et al., 2001; Cao and Richter, 2002). Numerous maternally provided mRNAs contain CPEs and are differentially polyadenylated and translated during mouse oocyte maturation and early embryogenesis (Oh et al., 2000).

Among the most abundant mRNAs in the oocyte are those encoding the histones (Giebelhaus et al., 1983; Graves et al., 1985). A single oocyte contains as much histone mRNA as a blastocyst, despite the enormous difference in the number of nuclei (Graves et al., 1985), and synthesis of specific subtypes has been reported (Wiekowski et al., 1997; Fu et al., 2003). Some of the histone mRNA species in oocytes are likely polyadenylated; for example, the oocyte-specific linker histone, H1foo, contains a potential CPE (Tanaka et al., 2001). However, the bulk of the histone mRNAs in oocytes appears to be of the so-called replication-dependent class (Graves et al., 1985). These transcripts are not polyadenylated but instead carry a highly conserved 3′-utr that ends in a stem-loop structure (Birchmeier et al., 1982; Dominski and Marzluff, 1999). Thus, their translation in oocytes cannot be regulated by a CPE-based process but must be controlled through a different mechanism.

In somatic cells, several factors associate with the 3′-utr of replication-dependent histone mRNAs (hereafter termed stem-loop histone mRNAs): notably, the stem-loop binding protein (SLBP), which contains a unique RNA-binding domain and interacts with the stem-loop (Wang et al., 1996), and the U7 snRNP, whose RNA component associates with a purine-rich element, termed the histone downstream element (HDE), that is located 3′ of the stem-loop sequence on the histone pre-mRNA. In conjunction with SLBP and a zinc-finger protein termed hZPF100, the U7 snRNP directs cleavage within the nucleus of newly synthesized histone transcripts between the stem-loop and the HDE (Cotten et al., 1988; Dominski et al., 2002). This processing reaction protects the transcripts from rapid degradation. As well, an exonuclease, termed 3′hExo, also interacts with the stem-loop and is thought to function in histone mRNA degradation during G2 of the cell cycle (Dominski et al., 2003).

In addition to its nuclear role, SLBP is also associated with stem-loop histone mRNAs in the cytoplasm. Indeed, in myeloma cells, much of the SLBP is cytoplasmic and is associated with polysomal histone mRNAs (Hanson et al., 1996; Whitfield et al., 2004). Furthermore, SLBP can activate the translation of a reporter mRNA carrying the histone stem-loop, both in vitro and in *Xenopus* oocytes (Sanchez and Marzluff, 2002). Moreover, SLBP co-purifies with translation initiation factors and physically interacts with eIF4G (Ling et al., 2002). These results suggest that, likely through interaction with factors bound to the 5′-end of the mRNA, SLBP stimulates translation of stem-loop histone mRNAs.

In somatic cells, SLBP is detectable only during S-phase of the cell cycle (Whitfield et al., 2000). To investigate whether SLBP might also play a role in regulating histone mRNA metabolism in mammalian oocytes, we previously characterized its expression in these cells (Allard et al., 2002). We found that SLBP is present in immature oocytes, which are at late G2 of the cell cycle, where it is enriched in the nucleus (germinal vesicle or GV). Upon initiation of meiotic maturation, SLBP begins to accumulate. This increase in SLBP begins shortly after germinal vesicle breakdown and continues throughout maturation, such that a mature metaphase II oocyte contains 10- to 15-fold more SLBP than an immature prophase-I-arrested oocyte (Allard et al., 2002). Thus, in contrast to its S-phase-restricted expression in somatic cells, SLBP is present in oocytes at G2 and M-phase of the cell cycle. These observations raised the possibility that SLBP might play a central role in regulating translation of the stem-loop histone mRNAs in oocytes. We tested this by monitoring endogenous histone synthesis, injecting reporter mRNAs bearing the 3′-utr of histone stem-loop mRNAs and using RNA interference (RNAi) and mRNA injection to manipulate SLBP levels within the oocyte.

Materials and methods

Oocyte collection and culture

Fully grown meiotically immature oocytes were collected from 21-day-old CD-1 female mice (Charles River Canada) by puncture of the ovarian antral follicles as previously described (Clarke et al., 1992). The oocytes were cultured in bicarbonate-buffered minimal essential medium (MEM) supplemented with sodium pyruvate, antibiotics, 3 mg/ml bovine serum albumin (BSA) and 0.1 mg/ml dibutyryl cyclic AMP (dbcAMP) at 37°C, in 5% CO_2 in air. Resumption of meiosis was initiated by transferring the oocytes into medium without dbcAMP.

SLBP overexpression

SLBP cDNA sequence corresponding to −2 to +884, encompassing the entire coding sequence, was excised by *Nco*I/*Stu*I digest, blunt-ended and cloned into the Cs2+ vector (gift from Dr. Mark Featherstone) that had been digested with *Bam*HI and blunt-ended. After linearization, SLBP cDNA was transcribed using the SP6 phage promoter and Ambion mMessage mMachine kit. The mRNA was purified by lithium chloride precipitation followed by three washes in 70% ethanol. The pellet was resuspended in Rnase-free water and stored at −80°C. SLBP mRNA was injected into immature oocytes at 1 μg/μl in Rnase-free water. The oocytes were incubated in culture medium for 16 h and collected for immunoblotting or radiolabeling.

Double-stranded RNA preparation and oocyte microinjection

Double-stranded RNA (dsRNA) was prepared from a pGEM cloning vector containing a 1.2 kb *Nco*I fragment of