European Journal of Cell Biology 95 (2016) 563–573

Contents lists available at ScienceDirect

European Journal of Cell Biology

journal homepage: www.elsevier.com/locate/ejcb

Research paper

Formation of *mos* RNA granules in the zebrafish oocyte that differ from *cyclin B1* RNA granules in distribution, density and regulation

Mayu Horie [a], Tomoya Kotani [a,b,*]

[a] *Biosystems Science Course, Graduate School of Life Science, Hokkaido University, Sapporo 060-0810, Japan*
[b] *Department of Biological Sciences, Faculty of Science, Hokkaido University, Sapporo 060-0810, Japan*

ARTICLE INFO

Article history:
Received 30 May 2016
Received in revised form 7 September 2016
Accepted 3 October 2016

Keywords:
Vertebrate
mRNA localization
Oocyte maturation
Translational control

ABSTRACT

Many translationally repressed mRNAs are deposited in the oocyte cytoplasm for progression of the meiotic cell cycle and early development. *mos* and *cyclin B1* mRNAs encode proteins promoting oocyte meiosis, and translational control of these mRNAs is important for normal progression of meiotic cell division. We previously demonstrated that *cyclin B1* mRNA forms RNA granules in the zebrafish and mouse oocyte cytoplasm and that the formation of RNA granules is crucial for regulating the timing of translational activation of the mRNA. However, whether the granule formation is specific to *cyclin B1* mRNA remains unknown. In this study, we found that zebrafish *mos* mRNA forms granules distinct from those of *cyclin B1* mRNA. Fluorescent in situ hybridization analysis showed that *cyclin B1* RNA granules were assembled in dense clusters, while *mos* RNA granules were distributed diffusely in the animal polar cytoplasm. Sucrose density gradient ultracentrifugation analysis showed that the density of *mos* RNA granules was partly lower than that of *cyclin B1* mRNA. Similar to *cyclin B1* RNA granules, *mos* RNA granules were disassembled after initiation of oocyte maturation at the timing at which the poly(A) tail was elongated. However, while almost all of the granules of *cyclin B1* were disassembled simultaneously, a fraction of *mos* RNA granules firstly disappeared and then a large part of them was disassembled. In addition, while *cyclin B1* RNA granules were disassembled in a manner dependent on actin filament depolymerization, certain fractions of *mos* RNA granules were disassembled independently of actin filaments. These results suggest that cytoplasmic regulation of translationally repressed mRNAs by formation of different RNA granules is a key mechanism for translational control of distinct mRNAs in the oocyte.

1. Introduction

Vertebrate oocytes are arrested at prophase I of the meiotic cell cycle and accumulate translationally repressed mRNAs in order to promote oocyte meiosis and development (de Moor et al., 2005; Masui and Clarke, 1979; Mendez and Richter, 2001). These oocytes are unable to be fertilized and are called immature oocytes. Full-grown oocytes in this stage resume meiotic cell division in response to specific signals such as hormones in species-specific breeding seasons (Masui and Clarke, 1979). After resumption of meiosis, oocytes of many vertebrate species are arrested again at meiotic metaphase II. This process is termed oocyte maturation and is necessary for oocytes to acquire fertility. Since transcription becomes quiescent after induction of oocyte maturation, most processes that occur during oocyte maturation and early development are driven by synthesis of proteins from stored mRNAs in oocytes.

Maturation/M-phase-promoting factor (MPF) is one of the important and universal factors for driving the progression of meiotic cell division (Masui and Clarke, 1979; Nurse, 1990). MPF consists of a catalytic subunit of Cdc2 and a regulatory subunit of Cyclin B. In fish and amphibians except *Xenopus*, Cyclin B is absent in immature oocytes (Ihara et al., 1998; Kondo et al., 1997; Kotani and Yamashita, 2002; Tanaka and Yamashita, 1995). Therefore, synthesis of Cyclin B from mRNA that has accumulated in the oocyte cytoplasm is prerequisite for the formation and subsequent activation of MPF, which induces germinal vesicle breakdown (GVBD), chromosome condensation and spindle formation. Although synthesis of Cyclin B is not necessary for GVBD in *Xenopus* and mouse oocytes, newly synthesized Cyclin B is required for meiotic metaphase I to II transition that occurs without DNA replication (Hochegger et al., 2001; Ledan et al., 2001).

* Corresponding author at : Department of Biological Sciences, Faculty of Science, Hokkaido University, North 10 West 8, Sapporo, Hokkaido 060-0810, Japan.
E-mail address: tkotani@sci.hokudai.ac.jp (T. Kotani).

http://dx.doi.org/10.1016/j.ejcb.2016.10.001
0171-9335/

Mos protein plays crucial roles during the progression of meiotic cell division and in metaphase II arrest as a component of the cytostatic factor (CSF). Mos is synthesized after induction of oocyte maturation and immediately activates the mitogen-activated protein kinase (MAPK) pathway. Mos-deficient oocytes and oocytes treated with MAPK inhibitors undergo GVBD but fail to form normal spindles in fish, frogs and mice (Araki et al., 1996; Choi et al., 1996; Dupre et al., 2002; Fisher et al., 1999; Gross et al., 2000; Kajiura-Kobayashi et al., 2000; Kotani and Yamashita, 2002; Verlhac et al., 1996). These oocytes form nuclei in meiotic metaphase I to II transition and fail to arrest at metaphase II due to a decrease in the MPF activity after entry into the second meiotic division.

In addition to the synthesis of Cyclin B and Mos from stored mRNAs, temporally ordered translation of these mRNAs after induction of oocyte maturation is also important for normal progression of meiotic cell division. For instance, it was shown that precocious activation of MPF caused defects in spindle formation and chromosome segregation in frog and mouse oocytes (Davydenko et al., 2013; Kotani and Yamashita, 2002). In addition, the timing of activation of the MAPK pathway prior to the activation of MPF is important for chromosome condensation and microtubule organization to form a normal spindle apparatus in frog oocytes (Kotani and Yamashita, 2002).

One of the mechanisms promoting translational activation of the dormant mRNAs with a short poly(A) tail is cytoplasmic polyadenylation of the mRNAs (McGrew et al., 1989; Sheets et al., 1994; Vassalli et al., 1989). The cytoplasmic polyadenylation element (CPE) located on the 3′ untranslated region (3′UTR) of certain numbers of mRNAs including *mos* and *cyclin B1* and CPE-binding protein CPEB function in both repression and direction of the cytoplasmic polyadenylation (Barkoff et al., 2000; de Moor and Richter, 1999; Gebauer et al., 1994; Tay et al., 2000). Pumilio-binding element (PBE) located on the 3′UTR of *cyclin B1* mRNA and its binding protein Pumilio are involved in translational activation at the timing that is specific to *cyclin B1* (Nakahata et al., 2001, 2003; Ota et al., 2011; Pique et al., 2008). Musashi-binding element (MBE) located on the 3′UTR of *Xenopus mos* mRNA and its binding protein Musashi direct translational activation in an early period after induction of oocyte maturation (Charlesworth et al., 2002, 2006). These studies demonstrated the importance of particular *cis*-acting elements and *trans*-acting factors that regulate the state of poly(A) length. However, the precise cytoplasmic regulation of dormant mRNAs in oocytes remains to be investigated.

We previously reported the identification of *cyclin B1* RNA granules in the zebrafish and mouse oocyte cytoplasm (Kotani et al., 2013). These RNA granules disassembled shortly after induction of maturation at the timing at which poly(A) tails are elongated. The disruption and stabilization of RNA granules induced acceleration and prevention of translational activation of the mRNA, respectively. Therefore, the assembly and dissociation of *cyclin B1* RNA granules appear to direct translational repression and activation of the mRNA. However, whether this regulation is specific to *cyclin B1* mRNA remains unknown.

In this study, we found that the zebrafish *mos* mRNA forms RNA granules within the animal polar cytoplasm of immature oocytes as in the case of the *cyclin B1* mRNA. The *mos* RNA granules were, however, different from those of *cyclin B1* in distribution and density. In addition, the *mos* RNA granules gradually disassembled at the timing of their polyadenylation after induction of oocyte maturation, in contrast to rapid disassembly of *cyclin B1* RNA granules. Our results indicate the presence of distinct cytoplasmic regulation of dormant mRNAs as formation of different granules and suggest the importance of formation and disassembly of RNA granules in temporal and spatial control of mRNA translation in oocytes.

2. Materials and methods

2.1. Preparation of ovaries

All animal experiments in this study were approved by the Committee on Animal Experimentation, Hokkaido University. Zebrafish ovaries were dissected from adult females in zebrafish Ringer's solution (116 mM NaCl, 2.9 mM KCl, 1.8 mM $CaCl_2$, and 5 mM HEPES; pH 7.2). For *in situ* hybridization analysis, zebrafish ovaries were fixed with 4% paraformaldehyde in PBS (4% PFA/PBS) overnight at 4 °C. For sucrose density gradient ultracentrifugation analysis, zebrafish ovaries were homogenized with an equal volume of ice-cold extraction buffer (EB: 100 mM β-glycerophosphate, 20 mM HEPES, 15 mM $MgCl_2$, 5 mM EGTA, 1 mM dithiothreitol, 100 μM (*p*-amidinophenyl)methanesulfonyl fluoride, 3 μg/ml leupeptin; pH 7.5) containing 100 units/ml RNasin Plus RNase Inhibitor (Promega). After centrifugation at 5000 rpm for 5 min at 4 °C, the supernatant was collected and used for ultracentrifugation.

2.2. Section in situ hybridization

Section *in situ* hybridization was performed according to the procedure reported previously (Kondo et al., 2001). Fluorescent *in situ* hybridization (FISH) with the tyramide signal amplification (TSA) Plus DNP system (PerkinElmer) was performed according to the procedure reported previously (Kotani et al., 2013). Briefly, fixed ovaries and oocytes were dehydrated, embedded in paraffin, and cut into 10-μm-thick sections. A digoxigenin (DIG)-labeled antisense RNA probe for the full length, 5′ half (1-609 bases) or 3′ half (613-1168 bases) of *mos* was used for detection of the *mos* gene transcript. No signal was detected with sense probes. After hybridization and washing, samples were incubated with anti-DIG-horseradish peroxidase (HRP) antibody (Roche) (1:500 dilution) for 30 min. The reaction with tyramide-dinitrophenyl (DNP) was performed according to the manufacturer's instructions. The samples were then incubated overnight with anti-DNP-Alexa 488 antibody (Molecular Probes) (1:500 dilution). To detect nuclei, the samples were incubated with 10 μg/ml Hoechst 33258 for 10 min. After being mounted with Prolong Antifade Kit (Molecular probes), the samples were observed under an LSM5LIVE confocal microscope (Carl Zeiss). The number of *mos* and *cyclin B1* RNA granules was quantified using ImageJ software, which enables detection of granules according to size (larger than 0.2 μm) and intensity at the center of granules.

Double *in situ* hybridization of *mos* and *cyclin B1* transcripts was performed as follows. A fluorescein-labeled antisense RNA probe for *cyclin B1* was used for detection of the *cyclin B1* gene transcript. Ten-μm-thick sections of zebrafish ovaries were hybridized with a mixture of *mos* and *cyclin B1* antisense RNA probes. After detection of the DIG-labeled antisense *mos* RNA probe, the samples were incubated with 1% H_2O_2 in PBS for 1 h for inactivating HRP. After washing with PBS, the samples were incubated with anti-Fluorescein-HRP antibody (Roche) (1:200 dilution) for 30 min. The reaction with tyramide-Cy3 was performed according to the manufacturer's instructions. A fluorescein-labeled antisense RNA probe for *mos* and a DIG-labeled antisense RNA probe for *cyclin B1* were also used for double *in situ* hybridization. Detection of the DIG- and fluorescein-labeled RNA probes was performed as described above. After staining with Hoechst 33258, the samples were mounted and observed under the LSM5LIVE confocal microscope.

2.3. Sucrose density gradient ultracentrifugation

Sucrose density gradient ultracentrifugation was performed according to the procedure reported previously (Takahashi et al., 2014) with modifications. Briefly, 500 μl of ovary extracts was