Iranian Biomedical Journal 8 (4): 179-183 (October 2004)

Time Course of Degradation and Deadenylation of Maternal c-mos and Cyclin A2 mRNA during Early Development of One-Cell Embryo in Mouse

Zohreh Alizadeh[*1], Parichehr Pasbakhsh[1], Aligholi Sobhani[1], Mohammad Barbarestani[1], Marefat Ghafari[2], Faride Etesam[1]

[1]*Dept. of Anatomy, Faculty of medicine, Tehran University of medical sciences;* [2]*Dept. of Endocrinology, Avesina Research Center, Shahid Beheshti University, University of medical sciences, Tehran, Iran*

Received 14 October 2003; revised 17 April 2004; accepted 20 June 2004

ABSTRACT

Early in the development of many animals, before transcription begins, any change in the pattern of protein synthesis is attributed to a change in the translational activity or stability of mRNA in the egg and early embryo. As a result, translational control is critical for a variety of developmental decisions, including oocyte maturation and initiation of preimplantation development. In this study, using real-time RT-PCR method, we defined the time course of degradation and deadenylation of an oocyte specific gene (c-mos) more precisely and a gene that is re-synthesized after ZGA (cyclin A2). Our data indicate that oocyte-specific transcript, c-mos, degrades rapidly while cyclin A2 mRNA does not and the deadenylation of c-mos mRNA precedes the process of degradation. Our findings suggest that time-dependent elimination of different maternal mRNA is a way for regulation of translation in early development of mouse embryos. *Iran. Biomed. J. 8 (4): 179-183, 2004*

Keywords: Maternal mRNA, Mouse embryo, mRNA degradation, c-mos, Cyclin A2

INTRODUCTION

In many species, transcription is effectively silent during the meiotic maturation and the early stage of embryo development. Maternally synthesized RNA and proteins that are loaded into the cytoplasm of developing oocyte program the completion of meiosis and developmental progression in the early embryo [1]. Therefore, the regulation of gene expression at post-transcriptional level (localization, translation and stability) probably plays a critical role in the control of early development [2]. Furthermore, changes in the stability of specific maternal mRNA are a way of removing transcripts that encode proteins harmful for embryonic development, as suggested for *Cdc25* gene product in Drosophila [3].

Maternal mRNA both supports and directs early development at least up to the time when the embryonic genome is activated. Zygotic genome activation (ZGA) is the major developmental transition that occurs following fertilization and corresponds to the stage of development when zygotic transcription becomes necessary [4]. The times when this transition takes place largely differ among different organisms. In mouse embryo, detection of new transcripts that are capable of being translated (i.e. functional transcripts) is clearly evident by the two-cell stage [5].

The resumption of transcription is accompanied by a massive degradation of the maternal derived transcripts [6]. In mouse, most of the poly-A containing mRNA is degraded, by mid two-cell stage [7]. For example, total RNA decreases by 20% during meiotic maturation from 430 pg/oocyte to 350 pg/egg, and by the mid two-cell stage, more than 90% of many species of maternally derived mRNA are destroyed [8, 9].

The control of mRNA translation and/or stability, as a means of regulating gene expression in eukaryotic cells, is now recognized as a mechanism of widespread importance. In many cases, this

*Corresponding Author; Tel. (+98-21) 611 2954; E-mail: alizade_397@yahoo.com

[DOR: 20.1001.1.1028852.2004.8.4.3.4]

control is exerted via the 3´-terminal poly-A tail [10]. In general, mRNA with a poly-A tail is much more actively translated and stable than mRNA with a short or no poly-A tail. Therefore, cytoplasmic activities that alter the length of the poly-A tail are potent regulators of gene expression [11].

Meiotic maturation in oocyte and mitotic cell cycle progression in one-cell embryo are accompanied by recruitment of many maternal mRNA [12]. Two such mRNA are: c-mos and cyclin A2 mRNA. The c-mos mRNA is recruited during oocyte maturation and its mobilization results in the activation of a protein whose activity is required to maintain arrest at metaphase II in mature oocyte [13]. The cyclin A2 mRNA is recruited after fertilization and inhibiting this mRNA recruitment inhibits entry into S phase of the one-cell embryo [14]. For this reason, we chose to study the stability of these two maternal mRNA that are recruited at two different stages: meiotic maturation and mitotic cell division.

In spite of timely dependence of maternal transcripts, degradation in the early embryo is necessary for developmental progression and for the passage of developmental control to zygotic genome, however, there is little information about mouse embryo. In this study, we have more precisely defined the time course of degradation and its relationship between deadenylation of maternal cyclin A2 and moloney sarcoma oncogene (c-mos) mRNA.

Our data indicate that each maternal mRNA displays a unique pattern of behavior and deadenylation is followed by degradation.

MATERIALS AND METHODS

Collection and culture of eggs and embryos. Female BDF1 mice (3 weeks old) and mature male ICR mice were purchased from SLC (Shizuoka, Japan). Female mice were superovulated by injection with 5 IU of pregnant mare's serum gonadotropin (PMSG) followed 48 h later by 5 IU of human chorionic gonadotropin (hCG) intra-peritoneally.

The ovulated metaphase II arrested eggs were collected from oviducts 15-16 hours after hCG injection. Cumulus cells were removed by short incubation in Whitten medium [15] containing hyaluronidase. The eggs were washed several times in a fresh medium.

To obtain embryos, ovulated metaphase II arrested eggs were inseminated with capacitated sperm from ICR male mouse. The sperms had been incubated at 37°C atmosphere of 5% CO_2 and 95% air. Two hours after insemination, the embryos were washed with glucose-free CZB medium [16] and then cultured in CZB medium containing 20 µg/ml alpha-amanitin at 37°C atmosphere of 5% CO_2 and 95% air. The embryos were collected 3, 6, 9 and 12 hours after insemination.

Semi-quantification of mRNA by real-time RT-PCR. Relative changes in Cyclic A2 (Accession number NM 009828) and c-mos (Accession number J00372) expression were determined by real-time RT-PCR assay using Smart Cycler System (Cephid, Sunnyvale, USA. and TaKaRa, Shiga, Japan). The total RNA was isolated from 30 eggs and 30 embryos at various developmental stages using ISOGEN (Nippon Gene, Tokyo, Japan) and was subjected to reverse transcription by adding oligo-dT primer (0.5 µg/µl) and dNTP mixture (10 mM) (Invitrogen, Carlsbad). To determine the degradation pattern, antisense specific primer was used in this stage. The mixture was incubated at 70°C for 4 min. Reverse transcription was followed by adding reaction buffer (provided with the enzyme), DTT (0.1 M), reverse transcriptase Reverse-Script II (200 units) and RNase inhibitor (Wako, Japan). Samples were incubated by the following program: 42°C for 50 min, 51°C for 30 min and 70°C for 15 min. Rabbit globin mRNA (500 pg) (Sigma Chemical Co., St. Louis, USA) was added before the isolation of total RNA. This served as an external control to evaluate the efficiency of RNA extraction and reverse transcription. Following reverse transcription, two oocytes or embryo equivalents were used as a template for each PCR reaction. PCR was performed using a set of following primers:

Cyclin A2 (14)**:** 5'-GAGGTGGGAGAAGAATA TAA-3' (sense) and 5'-ACTAGGTGCTCCATTCT CAG-3' (antisense)

Rabbit globin (14)**:** 5'-GCAGCCACGGTGGCG AGTAT-3' (sense) and 5'-GTGGGACAGGAGCTT GAAAT-3' (antisense)

c-mos**:** 5'-CTCCGGAGATCCTGAAAGGA-3' (sense) and 5'-CAGTGTCTTTCCAGTCAGGG-3' (antisense)

[DOR: 20.1001.1.1028852.2004.8.4.3.4]

For c-mos amplification, a pair of primers were designed based on the cDNA sequence (Accession number J00372) using Genetix software.

The reaction mixture consisted of each cDNA, dNTP mixture (10 mM), Mg+ solution (250 mM), Ex Taq R-PCR (5 U/µl) (TaKaRa, Shiga, Japan), reaction buffer (provided with the enzyme), SYBER®Green I (BioWhittaker Molecular Applications, Rockland, USA) and a pair of specific primers (10 pmol/ml). The PCR conditions were as follows: initial denaturation at 95°C for 30 s was followed by 40 cycles for both cyclin A2 and c-mos and 35 cycles for globin of denaturation at 95°C for 15 s, annealing at either 58°C for c-mos and globin or 56°C for cyclin A2, for 30 s, and extension at 72°C for 30 s. The expected length of PCR products was 172 bp for c-mos, 236 bp for cyclin A2 and 257 bp for globin. The fluorescence intensity was measured during every cycle. The relative amount of each sample was determined using a standard curve obtained by amplifying a 10-fold dilution series of cDNA that was prepared by PCR.

RESULTS AND DISCUSSION

In order to determine the dynamics of maternal transcript degradation in early one-cell stage of mouse embryo, we focused on an oocyte-specific maternal transcript, c-mos [17] and one maternally encoded mRNA, cyclin A2 that were shown to be present in the two-cell stage [14].

In order to characterize more precisely the relative stability of c-mos and cyclin A2 maternal messengers during early hours of the one-cell embryo, we conducted semi-quantitative RT-PCR in unfertilized eggs and embryos spanning 3 h interval during the first 12 h after fertilization. The embryos were cultured in a medium containing alpha-amanitin, an inhibitor of RNA polymerase II, to block initiation of transcription. In mammals, it has been shown that alpha-amanitin treatment of one-cell stage rat embryo extends the synthesis of several maternal encoded proteins probably by stabilizing the effect of some maternal mRNA, but there is no such evidence in the mouse [18]. The expression pattern of c-mos mRNA shows a dramatic decrease in the amount of this maternal transcript by 6 h after fertilization and is near to the limit of detection in 9 h, more rapidly than cyclin A2 mRNA (Fig. 1). Paynton *et al.* [8] studied the degradation pattern of different maternal mRNA in late one-cell and two-cell stages. They also concluded that each mRNA has its own pattern of changes in poly-A length and degradation.

To distinguish between deadenylation and degradation of these mRNA, we used two different kinds of primers, oligo-dT and antisense specific primers, for reverse transcription stage of RT-PCR in an unfertilized egg and 6 h embryo.

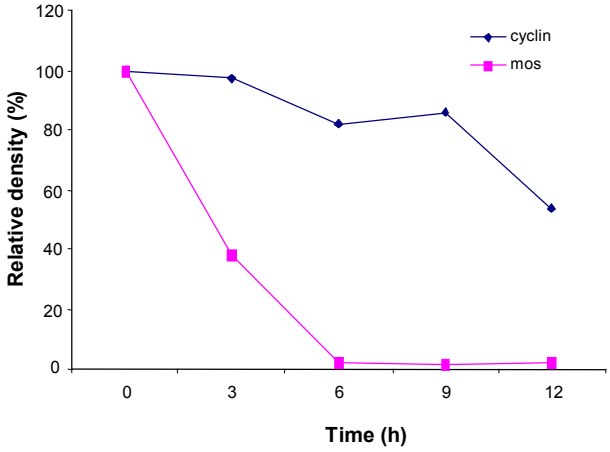
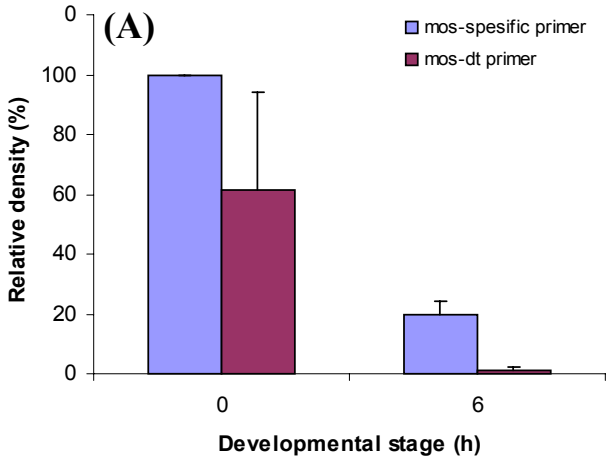


Fig. 1. Time course of c-mos and cyclin A2 mRNA expression. The relative amounts of c-mos and cyclin A2 mRNA were determined by real-time RT-PCR, using oligo-dT primer for RT stage, in unfertilized egg (0), 3 h after insemination (3), 6 h after insemination (6), 9 h after insemination (9), and 12 h after insemination (12). The amount presented in unfertilized egg, which had the highest relative amount of transcripts, was set as 100% and the values obtained for other developmental stages were expressed relative to this value. The results of three separate experiments were averaged.

Using antisense specific primers of c-mos and cyclin A2, we showed deadenylation and degradation of these mRNA. Then, the results were compared with the data obtained from dT primer. Using oligo-dT primer for reverse transcription stage, we showed polyadenylation state of mRNA. For c-mos, it was shown that the mRNA was deadenylated and degraded in 6 h after fertilization (Fig. 2A), but cyclin A2 mRNA was partially degraded and did not show deadenylation during the same period (Fig. 2B). This result is consistent with adenylation state in cyclin A2 mRNA in unfertilized egg and one-cell stage reported by others [14]. They used Northern-blot analysis and showed that cyclin A2 was polyadenylated in one-cell embryo.

To determine the time course of c-mos and cyclin A2 mRNA degradation during one-cell stage, the unfertilized eggs and embryos spanning 3 h interval were collected and relative amounts of mRNA were examined using specific primers for RT stage of RT-PCR. The results were compared using oligo-dT

[DOR: 20.1001.1.1028852.2004.8.4.3.4]



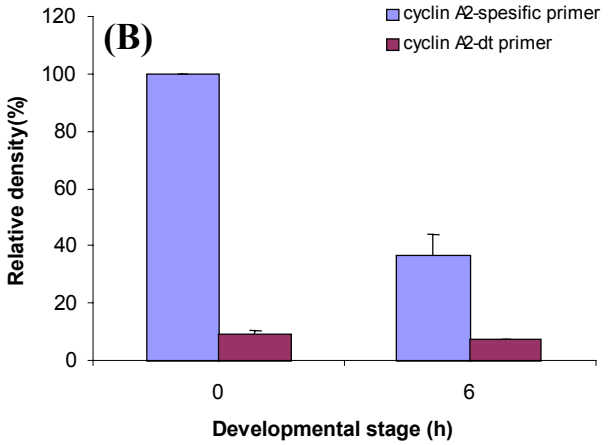


Fig. 2. Temporal pattern of c-mos **(A)** and cyclin A2 **(B)** mRNA stability in unfertilized egg (0) and 6 h after insemination (6), using specific and dT primer in RT stages. Using specific primer, the value obtained in unfertilized egg was set as 100% and the values obtained for the other were expressed relative to this value. The results of three separate experiments were averaged.

primer (Fig. 3 A and B). The data show that the c-mos (Fig. 3A) and cyclin A2 (Fig. 3B) mRNA show different patterns of degradation and that c-mos mRNA degradation is followed by deadenylation.

In both yeast and mammalian cells, poly-A intermediates are not normally observed, which implies that the rate at which the deadenylated RNA are produced does not exceed the capacity of degradation machinery in these cells [19]. In *Xenopus laevis* embryos, the situation is somewhat different as there is a transient accumulation of poly-A mRNA, presumably due to a lag between deadenylation and degradation [20]. In this species, the maternal Eg mRNA, in addition to their postfertilization deadenylation, also undergoe characteristic changes in their stability during early development. These mRNA appear to accumulate as poly-A transcripts before being degraded [21]. The decay of these mRNA becomes apparent during the midblastula transition [21].

In summary, the results indicate that each of the maternal mRNA displays a unique pattern of behavior with respect to the time course of degradation in early embryo and deadenylation is followed by degradation. Our findings suggest that the time dependent elimination of different maternal mRNA is a way for regulation of gene expression in early mouse embryo.

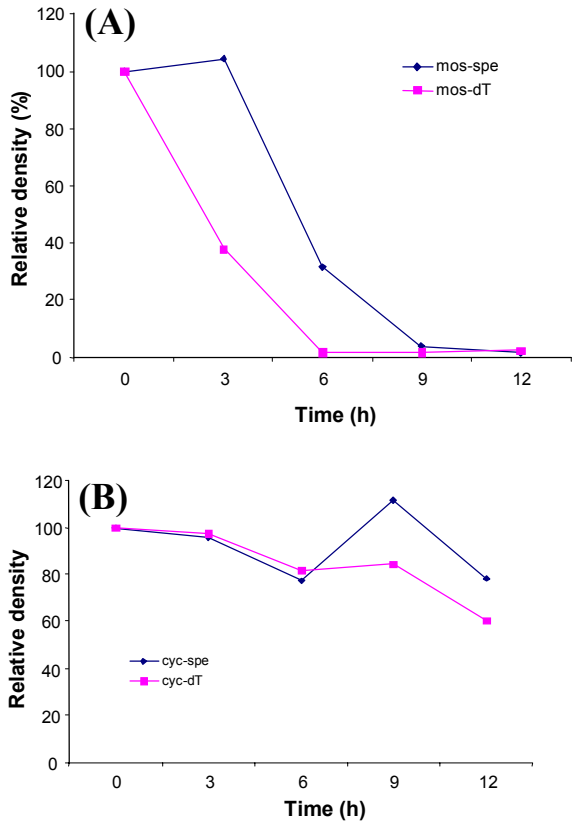


Fig. 3. Time course of c-mos **(A)** and cyclin A2 **(B)** mRNA deadenylation and degradation in unfertilized egg (0), 3 h after insemination (3), 6 h after insemination (6), 9 h after insemination (9), and 12 h after insemination (12). The amount presented in the unfertilized egg is arbitrarily set at 100 and the values obtained for other developmental stages were expressed relative to this value. The results of three separate experiments were averaged.

ACKNOWLEDGMENTS

The authors wish to thank the Bioresorce regulation Lab of Tokyo University for providing technical support.

[DOR: 20.1001.1.1028852.2004.8.4.3.4]

REFERENCES

1. Curtis, D., Lehmann, R. and Zamore, PD. (1995) Translational regulation in development. *Cell 81: 171-178*.
2. Bashirullah, A., Halsell, S.R., Cooperstock, R.L., Kloc, M., Karaiskakis, A., Fisher, W.W., Fu, W., Hamilton, J.K., Etkin, L.D. and Lipshitz, H.D. (1999) Joint action of two RNA degradation pathways controls the timing of maternal transcript elimination at the midblastula transition in Drosophila melanogaster. *EMBO J. 18 (9): 2610-2620*.
3. Edgar, B. and Datar, SA. (1996) Zygotic degradation of two maternal Cdc25 mRNAs terminates Drosophila early cell cycle program. *Genes Dev. 10: 1966-1977*.
4. Schultz, RM. (1999) The regulation and reprogramming of gene expression in the preimplantation embryo. *Adv. Dev. Biochem. 5: 127-162*.
5. Telford, N.A., Watson, A. and J. Schultz, G.A. (1990) Transition from maternal to embryonic control in early mammalian development: a comparison of several species. *Mol. Reprod. Dev. 26: 90-100*.
6. Gandolfi, T. and Gandolfi, F. (2001) The maternal legacy to the embryo: Cytoplasmic compartments and their effect on early development. *Theriogenology 55: 1255-1276*.
7. Schultz, R.M. (1993) Regulation of zygote gene activation in the mouse. *Bioassay 15 (8): 531-538*.
8. Paynton, B.V., Rample, R. and Bachvarova, R. (1988) Changes in state of adenylation and time course of degradation of maternal mRNAs during oocyte maturation and early embryonic development in the mouse. *Dev. Biol. 129: 304-314*.
9. Bachvarova, R., Deleon, V., Johnson, A., Kaplon, G. and Paynton, B.V. (1985) Changes in total RNA, polyadenylated RNA, and actin mRNA during meiotic maturation of mouse oocytes. *Dev. Biol. 108: 325-331*.
10. Richter, J.D. (1999) Cytoplasmic polyadenylation in development and beyond. *Microbiol. Mol. Biol. Rev. 63: 446-456*.
11. Paillard, L., Legagneux, V., Maniey, D. and Osborne, H.B. (2002) c-Jun ARE targets mRNA deadenylation by an EDEN-BP (embryo deadenylation element-binding protein)-dependent pathway. *J. Biol. Chem. 277: 3232-3235*.
12. Schultz, R.M., Letourneau, G.E. and Wassarman, P.M. (1979) Program of early development in the mammal: changes in the patterns and absolute rates of tubulin and total protein synthesis during oocyte growth in the mouse. *Dev. Biol. 73 (1): 120-133*.
13. Kigami, D., Minami, N., Takayama, H. and Imai, H. (2003) MuERV-L is one of the earliest transcribed genes in mouse one-cell embryos. *Biol. Reprod. 68 (2): 651-654*.
14. Fuchimoto, D.I., Mizukoshi, A., Schultz, R.M., Sakai, S. and Aoki, F. (2001) Posttranscriptional regulation of Cyclin A1 and Cyclin A2 during mouse oocyte meiotic maturation and preimplantation development. *Biol. Reprod. 65: 986-993.*
15. Whitten, W.K. (1971) Nutrient requirement for the culture of preimplantation embryos. *Adv. Biosci. 6: 129-139*.
16. Chatot, C.L., Ziomek, C.A., Bavister, B.D., Lewis, J.L. and Torres, I. (1989) An improved culture medium supports development of random-bred one-cell embryos *in vitro*. *J. Reprod. Fertil. 86: 679-682*.
17. Rajkovic, A. and Matzuk, M.M. (2002) Functional analysis of oocyte-expressed genes using transgenic models. *Mol. Cell Endocrinol. 187: 5-9*.
18. Richoux, V., Renard, J.P. and Bahinet, C. (1991) Synthesis and developmental regulation of an egg specific protein translated from maternal mRNA. *Mol. Reprod. Dev. 28: 218-229*.
19. Chen, C.Y. and Shyu, A.B. (1995) AU-rich elements: Characterization and importance in mRNA degradation. *Trends Biochemisci. 20: 465-470*.
20. Audic, Y., Omilli, F. and Osborne, B. (1997) Postfertilization deadenylation of mRNA in *Xenopus laevis* embryos is sufficient to cause their degradation at the balstula stage. *Mol. Cell Biol. 17 (1): 209-218*.
21. Obsorne, P.J.H., Coutorier, A., LeGuellec, K. and Philippe, M. (1998) Changes in the polyadenylation of specific stable RNAs during the early development of *Xenopus laevis*. *Gene 72: 169-176*.

[DOR: 20.1001.1.1028852.2004.8.4.3.4]