N⁶-Methyladenosine Sequencing Highlights the Involvement of mRNA Methylation in Oocyte Meiotic Maturation and Embryo Development by Regulating Translation in Xenopus laevis*‡

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During the oogenesis of Xenopus laevis, oocytes accumulate maternal materials for early embryo development. As the transcription activity of the oocyte is silenced at the fully grown stage and the global genome is reactivated only by the mid-blastula embryo stage, the translation of maternal mRNAs accumulated during oocyte growth should be accurately regulated. Previous evidence has illustrated that the poly(A) tail length and RNA binding elements mediate RNA translation regulation in the oocyte. Recently, RNA methylation has been found to exist in various systems. In this study, we sequenced the N⁶-methyladenosine (m⁶A) modified mRNAs in fully grown germinal vesicle-stage and metaphase II-stage oocytes. As a result, we identified 4207 mRNAs with m⁶A peaks in germinal vesicle-stage or metaphase II-stage oocytes. When we integrated the mRNA methylation data with transcriptome and proteome data, we found that the highly methylated mRNAs showed significantly lower protein levels than those of the hypomethylated mRNAs, although the RNA levels showed no significant difference. We also found that the hypomethylated mRNAs were mainly enriched in the cell cycle and translation pathways, whereas the highly methylated mRNAs were mainly associated with protein phosphorylation. Our results suggest that oocyte mRNA methylation can regulate cellular translation and cell division during oocyte meiotic maturation and early embryo development.

During oogenesis in animals like Xenopus laevis, mouse, and human, germinal vesicle (GV)-stage oocytes gradually achieve maximum size, and genomic transcription activity is silenced (1, 2). After that, the fully grown GV oocytes resume meiosis and develop to metaphase of the second meiosis (MII) stage. Then the oocyte is fertilized by sperm to form a zygote. The zygote starts mitosis, and embryo development is initiated. As embryo genomic transcription is not reactivated until embryo development to the mid-blastula stage for X. laevis (3) or the 2-cell to 16-cell stages for mammals (4–7), oocytes or embryos need the maternal RNAs accumulated during oocyte growth to support new protein synthesis.

As the synthesis of new proteins such as cyclins is of importance for the meiotic and mitotic events, the translation of maternal mRNAs during oocyte meiotic maturation and early embryo development should be precisely controlled. Previous data have shown that there are mainly two methods for cells to control the maternal mRNA translation in oocyte or early embryo protein binding to the cytoplasmic polyadenylation elements (CPEs) in maternal mRNAs and controlling the polyadenylation of the mRNA poly(A) tails (8). In most cases, shortened poly(A) tails of mRNAs repress their translation, whereas elongated poly(A) tails activate translation (9). The poly(A) tail length of oocyte mRNA is mainly associated with cytoplasmic polyadenylation, which is controlled by RNA binding proteins and associated proteins. The maternal mRNAs, whose 3′ UTR contains a cis-element CPE, could be bonded by CPE binding protein (CPEB) (10). When CPEB is phosphorylated under stimulation of progesterone, it recruits and binds to the cleavage and polyadenylation-specific factor. The cleavage and polyadenylation-specific factor then recruits poly(A) polymerase to the mRNA end and mediates poly(A) tail elongation and promotes mRNA translation (11).

In addition to the poly(A) tail and CPE-mediated mRNA translation regulation, recent evidence revealed a correlation between RNA translation and an RNA-specific modification called N⁶-methyladenosine (m⁶A) (12, 13). It has been shown that m⁶A modifications have different effects on mRNA translation when the m⁶A modifications occur at different regions of the mRNAs (14–16). RNA methylation at the 5′ UTR could promote mRNA translation, whereas RNA methylation at the last exons participated in the 3′ UTR regulation of mRNAs (15, 16). By knocking out the m⁶A writer Mettl3, mRNA methylation was also found to play important roles in...
other cellular events such as cell differentiation and RNA degradation (17).

As transcription is silenced in fully grown oocytes and early embryos, mRNA translation regulation is essential for biological events during this period. To investigate whether m^6^A modification takes part in RNA translation regulation in the *X. laevis* oocyte and its potential roles in oocyte maturation and embryo development, we sequenced m^6^A-modified mRNAs in fully grown GV-stage and MII-stage *X. laevis* oocytes and compared the m^6^A-seq data with the transcriptome and proteome data.

**Results**

m^6^A-Seq of GV- and MII-stage Oocytes in *X. laevis*—m^6^A-modified mRNAs of GV- and MII-stage oocytes from *X. laevis* (supplemental Fig. S1) were isolated and analyzed according to the methods described by Dominissini et al. (18). After mapping the methylated RNA fragments to the transcriptome of *X. laevis* (19), we found that 4207 mRNAs (4128 in GV oocytes and 3820 in MII oocytes) were methylated in GV- or MII-stage oocytes (supplemental Dataset S1).

According to the height of the m^6^A peaks, we divided these mRNAs into three classes: m^6^A high mRNAs, m^6^A medium mRNAs, and m^6^A low mRNAs. From these results we found that the m^6^A modification was maintained in 1674 mRNAs during oocyte maturation from GV to MII stage, but in 2400 mRNAs the m^6^A levels were decreased, and in 133 mRNAs the m^6^A levels were increased (Fig. 1A).

Using the m^6^A peak-corresponding mRNA sequences, we predicted the conservative m^6^A motifs in *X. laevis* oocytes. As described for human and mouse cells, mRNA methylation in *X. laevis* oocytes also occurred at the GGACU motifs (Fig. 1B). By analyzing the m^6^A peak positions along the mRNAs, we found that the m^6^A peaks were mainly distributed downstream of the coding DNA sequence (CDS) start sites and around the CDS end sites (Fig. 1C).

To analyze the association between m^6^A peak height and mRNA level in oocytes, we integrated the m^6^A-seq data with the transcriptome data published by Charlier et al. (20) (supplemental Figs. S1 and S2). From the transcriptome data, we found 1030 genes with detected m^6^A modifications. The m^6^A levels of these genes decreased from GV stage to MII stage (p < 0.01, supplemental Fig. S2, A and B). In addition, we also found that there was no obvious relativity between the m^6^A peak heights and the mRNA levels (supplemental Fig. S2, C and D).

Next we compared the ratios of m^6^A peak height/mRNA level in GV and MII oocytes and found that most ratios of m^6^A peak height/mRNA level were decreased from GV stage to MII stage (supplemental Fig. S2, E–H).

**Gene Set Enrichment Analysis of the Methylated mRNA-associated Pathways in *X. laevis* Oocytes**—To know whether RNA methylation participates in oocyte maturation and embryo development, we analyzed the m^6^A-modified, mRNA-enriched Kyoto Encyclopedia of Genes and Genomes (KEGG) pathways through the DAVID tools (21). From the results (supplemental Dataset S2), we could see that the highly or moderately methylated mRNAs mainly enriched in pathways like ErbB signaling, progesterone-mediated oocyte maturation, and cell cycle. However, the hypomethylated mRNAs mainly enriched in pathways like RNA degradation, DNA replication, ribosome, spliceosome, pentose phosphate pathway, and glycolysis/glucoseogenesis.

To further assess the biological functions of these m^6^A-modified mRNAs in *Xenopus* oocytes, we annotated the *Xenopus* mRNAs using BLAST on the Biocloud platform. From the gene ontology annotation results, we could find that the oocyte m^6^A-modified mRNAs were mainly associated with biological processes like transcription, protein phosphorylation, and cell division. Interestingly, in the m^6^A-modified mRNAs, there were 443 whose proteins had ATP binding functions, and 354 had zinc ion binding functions (supplemental Dataset S3).

**The Relationship between mRNA Methylation and mRNA Translation**—To investigate whether mRNA methylation is related to mRNA translation, we integrated our mRNA methylation data and the transcriptome and proteome data from Smits et al. (22). In the transcriptome and proteome data, Smits et al. (22) revealed the RNA and protein levels in the egg and embryo (supplemental Fig. S1) of *X. laevis*. Through BLAST, the information of 723 m^6^A-modified mRNAs were identified in the RNA/protein profile data. From the profile data, we found that the RNA levels (log10 RNA RPKM values) of high m^6^A peak mRNAs, detected in GV or MII stage oocytes, were higher than the levels of the total RNAs (in eggs or embryos); however, the protein levels (log10 protein amount/femtomole) were lower than the levels of total proteins (Fig. 2). Compared with the highly methylated mRNAs, the hypomethylated RNAs showed significantly higher protein levels (Fig. 3; p values shown in supplemental Dataset S4). These results suggest that m^6^A modification may be associated with mRNA translation in oocytes or even in early embryos.

To analyze whether the mRNA positions (5’ UTR, CDS, and 3’ UTR) of RNA methylation are associated with translation, we extracted mRNAs whose m^6^A modification only occurred at the 5’ UTR, 5’-terminal CDS, 3’-terminal CDS, or 3’ UTR from the highly methylated RNAs in GV oocytes (Fig. 4 and supplemental Fig. S3). In Fig. 4 we can see that the mRNAs whose 5’-terminal CDSs or 3’-terminal CDSs were methylated showed lower protein levels compared with the other sets. In addition, mRNAs whose m^6^A modification occurred at the 3’ UTR, in contrast, showed higher median protein levels (p values are shown in supplemental Dataset S4). These results suggest that CDS region RNA methylation may suppress mRNA translation.

In addition, we also integrated our data with the *X. laevis* embryo (supplemental Fig. S1) proteome data published by Peshkin et al. (23). As a result, we also found that the hypermethylated mRNAs in eggs have a lower protein concentration distribution. As for the hypermethylated RNAs, these RNAs, whose m^6^A modification mainly occurred in CDS regions, showed a lower protein level distribution (supplemental Fig. S4). From the protein profile data calculated by Peshkin et al. (23), we found that hypermethylated RNAs showed a lower protein synthesis rate (supplemental Fig. S5).
When *X. laevis* oocytes develop to the fully grown GV stage, their transcription activity is silenced. After maturation (about 6 h), MII-stage oocytes are fertilized by spermatozoa, and the oocytes are transformed into fertilized eggs. The fertilized eggs then develop to mid-blastula stage embryos (50–90 min), at which stage the embryonic genome is reactivated, and global transcription is initiated. From the fully grown GV oocytes to the mid-blastula embryos, oocyte maturation and embryo development mainly rely on the maternal factors accumulated in oocytes (24). For oocyte mRNAs, their translation is controlled strictly to satisfy the development requirements. Previous evidence showed that oocyte mRNA translation is controlled by two mechanisms: poly(A) tail length-correlated translation efficiency and 3′ UTR element-mediated mRNA translation activation. Recently, evidence suggested a possible association of translation with RNA methylation (12, 16, 25), indicating that the 5′ UTR m6A modification could initiate the translation of non-capped mRNAs. Through integrating the m6A-seq data and transcriptome/proteome data in *X. laevis*, we investigated m6A modification and possible roles of oocyte mRNAs. We found that mRNAs in which the CDS region was...
m^6^A-modified showed low protein levels, but mRNA with an m^6^A-modified 3' UTR showed high protein levels. Our results suggest that RNA methylation is involved in RNA translation regulation in _X. laevis_ oocyte maturation and further embryo development. Thus, we reveal a mechanism regulating maternal mRNA dormancy or translation in early development. Our data also suggest that m^6^A may play different roles according to their positions. The correlation of CDS region m^6^A modification and low protein level may suggest that m^6^A modification represses the translation elongation of mRNAs.

Our results also showed a decrease in RNA methylation during oocyte development from the GV stage to MII stage. As there was no transcription activity in these oocytes, we inferred that there were two events that may induce this RNA methylation decrease: active RNA demethylation and RNA degradation in the MII stage. The RNA methylation pattern changes during oocyte meiosis may also be a mechanism for the oocyte to control mRNA translation. Recent evidence has shown that, in addition to Mettl3, there were other enzymes, like Mettl14 (26), Wilms tumor 1-associated protein (Wtap) (27), and KIAA1429 (28), also involved in the RNA methylation process. On the other hand, two m^6^A erasers, fat mass and obesity-associated (Fto) (29) and alkB homolog 5 RNA demethylase (Alkbh5) (30), have been found to be involved in m^6^A modification in mammalian cells (31). These findings suggest that the regulation of RNA methylation in eukaryotic cells is finely regulated through these m^6^A modification-associated factors.

Microarray data about mouse oocyte development (32) have shown that both the m^6^A writers Mettl3 and Mettl14 and the m^6^A eraser Alkbh5 were expressed highly in growing oocytes, indicating that mRNA m^6^A modifications may exist in a dynamic pattern during oocyte growth. In any case, the functions of these m^6^A-associated enzymes and cofactors should be further analyzed to reveal the regulation of RNA methylation in oocytes and their biological functions.

In our study, we found numerous oocyte meiosis- and early embryo development-associated genes whose mRNAs are methylated. For example, cyclin B2 (_ccnb2_) is required for _X. laevis_ oocyte bipolar spindle formation, and evidence showed that its protein levels increased in MII-stage oocytes (33). _mos_ is also essential for _X. laevis_ oocyte meiosis resumption (34) and MII stage maintenance (35, 36). Our data showed that the m^6^A modification in _ccnb2_ and _mos_ mRNAs decreased in the 3' terminal of the CDS region but showed no obvious change at the 5' UTR (supplemental Fig. S6). _cdt1_, whose m^6^A modification decreased at the CDS region, has been proven to play roles in DNA replication in embryos (37–39). The m^6^A modification changes of these key factors indicate that RNA methylation is involved in meiosis regulation and early embryo development.

**Experimental Procedures**

_X. laevis Oocyte Collection_—Feeding and handling of _X. laevis_ were conducted in accordance with the Animal Research Committee policies of the Institute of Zoology, Chinese Acad-
emy of Sciences. Sexually mature *X. laevis* females were injected with 50 IU of pregnant mare serum gonadotropin 5 days before collection of GV-stage oocytes. Before the surgery, the *X. laevis* was buried under ice for about 30 min until it reached complete anesthesia. After making a small incision off the middle line of the belly, a part of the ovary was removed with forceps and scissors and placed in M199-HEPES medium. We then sutured the incision and let the *X. laevis* recover under a moist tissue until fully awake, and then individuals were submerged in water supplemented with penicillin. The excised ovary was cut into small pieces and transferred to a 50-ml tube containing 40 ml of M199-HEPES with 0.2% collagenase type I. These oocytes were washed with fresh M199-HEPES and aliquoted in 1.5-ml tubes. After snap-freezing in liquid nitrogen, these oocytes were stored at −80 °C until use. For the ovulation of MII eggs, the *X. laevis* was injected with 500 IU of human chorionic gonadotropin. After ovulation, we collected the healthy MII oocytes and aliquoted the oocytes in 1.5-ml tubes. After snap-freezing in liquid nitrogen, these oocytes were stored at −80 °C until use.

**RNA Preparation**—Total RNAs of GV- or MII-stage oocytes were extracted using TRIzol reagent (Life Technologies). To avoid DNA contamination, all samples were treated with Turbo DNase (Life Technologies). RNA samples were chemically fragmented into about 100-nt fragments by incubating in fragmentation buffer (10 mM ZnCl₂ and 10 mM Tris-HCl (pH 7)) for 15 min at 94 °C. The fragmentation reaction was stopped by adding 0.05 M EDTA, and the RNAs were collected by standard ethanol precipitation.

**m⁶A-Seq**—m⁶A sequencing was performed as described previously Dominissini et al. (18) with little modification. Briefly, 200 μl of protein A bead slurry (Thermo Fisher Scientific) was blocked by incubating at room temperature for 1 h with 1 ml of immunoprecipitation buffer (150 mM NaCl, 0.1% Igepal CA-630, and 10 mM Tris-HCl (pH 7.4)) supplemented with 50 mg/ml BSA and 200 units of RNasin (Promega). Then the fragmented RNAs (3 mg) were incubated at 4 °C for 1 h with 50 μl of blocked protein A beads to reduce nonspecific binding. After centrifugation at 4 °C, 2500 × g for 3 min, we collected the RNA supernatant and incubated it at 4 °C for 2 h with 12.5 μg of purified rabbit anti-m⁶A polyclonal antibody (Synaptic Systems). The RNA/antibody mixture was incubated with 50 μl of blocked protein A beads for an additional 2 h at 4 °C. After extensive washing, the bound RNAs were eluted by competition with 0.5 mg/ml N⁶-methyladenosine (Sigma-Aldrich). The eluted RNAs were precipitated with ethanol and resuspended.
in nuclease-free water. Finally, the RNA library was generated with an mRNA sequencing kit (Illumina) and sequenced on an Illumina HiSeq2000 platform. The raw data of the sequencing reads were uploaded to the sequence read archive (accession no. SRR3667693).

**m6A-Seq Data Analysis**—The *X. laevis* oocyte m6A-seq data were analyzed as described in the protocol by Dominissini et al. (18) with some modifications. Briefly, the RNA-seq reads were mapped to the mRNAs of *X. laevis* collected by Xenbase (19) using Bowtie software (40). The Bowtie-generated SAM files were treated using MACS software (41) to identify the m6A peak positions in the RNAs. The 100-nt RNA fragments including methylation sites were extracted and analyzed with MEME software (42) to construct the conservative m6A motif in X. laevis. To analyze the m6A sites distribution, we extracted the UTR and CDS information of *X. laevis* mRNAs from NCBI GenBank. To visualize the m6A peaks in RNAs, we wrote Python scripts and displayed the m6A peaks in SVG format. For the RNAs with m6A peaks, according to the height of the peak, we divided the m6A-modified RNAs into three classes: high (read depth > 60), medium (read depth between 30 and 60) and low (read depth < 30) methylated RNAs. To annotate the *X. laevis* mRNAs, we blasted the mRNA sequences to the GO dataset using Anno-function software on the Biocloud website.

**Integration of m6A-Seq Data with Transcriptome and Proteome Data**—The *X. laevis* oocyte and embryo omic data published by Smits et al. (22) and Peshkin et al. (23) were used for integration analysis of oocyte m6A data. As different reference genome or gene IDs were used in different research groups, we searched the corresponding gene ID using BLAST. All gene expression data we used were calculated by the authors themselves.

**Author Contributions**—S. T. Q., J. Y. M., and Q. Y. S. designed the experiments. S. T. Q., Z. B. W., L. G., and Y. H. performed the experiments. J. Y. M. analyzed the data, and J. Y. M. and Q. Y. S. wrote the paper.

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**References**

1. Bellier, S., Dubois, M. F., Nishida, E., Almouzni, G., and Bensaude, O. (1997) Phosphorylation of the RNA polymerase II largest subunit during *Xenopus laevis* oocyte maturation. *Mol. Cell. Biol.* 17, 1434–1440

2. Miyara, F., Migne, C., Dumont-Hassan, M., Le Mure, A., Cohen-Bacrie, P., Aubriot, F. X., Glissant, A., Nathan, C., Douard, S., Stanovici, A., and Dehe, P. (2003) Chromatin configuration and transcriptional control in human and mouse oocytes. *Mol. Reprod. Dev.* 64, 458–470
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3. Lee, M. T., Bonneau, A. R., and Giraldez, A. J. (2014) Zygotic genome activation during the maternal-to-zygotic transition. Annu. Rev. Cell Dev. Biol. 30, 581–613

4. Prather, R. S. (2010) Whatever happened to the “cell-block” during mammalian embryogenesis? Mol. Reprod. Dev. 77, NA

5. Graf, A., Krebs, S., Heininen-Brown, M., Zakhartchenko, V., Blum, H., and Wolf, E. (2014) Genome activation in bovine embryos: review of the literature and new insights from RNA sequencing experiments. Animal Reprod. Sci. 149, 46–58

6. Yu, C., Ji, S. Y., Dang, Y., Zhou, J. J., Wang, Z. W., Hu, B., Sun, Q. Y., Sun, S. C., Tang, F., and Fan, H. Y. (2016) BTG4 is a meiotic cell cycle-coupled maternal-zygotic-transition licensing factor in oocytes. Nat. Struct. Mol. Biol. 23, 387–394

7. Radford, H. E., Meijer, H. A., and de Moor, C. H. (2008) Translational control by cytoplasmic polyadenylation in Xenopus oocytes. Biochem. Biophys. Acta 1779, 217–229

8. Stebbings-Boaz, B., Hale, E. L., and Richter, J. D. (1996) CPEB controls the cytoplasmic polyadenylation of cyclin, CDk2 and c-mos mRNAs and is necessary for oocyte maturation in Xenopus. EMBO J. 15, 2582–2592

9. Hale, E. L., and Richter, J. D. (1994) CPEB is a specificity factor that mediates cytoplasmic polyadenylation during Xenopus oocyte maturation. Cell 79, 617–627

10. Mendez, R., Murthy, K. G., Ryan, K., Manley, J. L., and Richter, J. D. (2000) Phosphorylation of CPEB by Eg2 mediates the recruitment of CPSF into an active cytoplasmic polyadenylation complex. Mol. Cell 6, 1253–1259

11. Zhang, Y., Wang, Z., Wang, W., Song, H. P., Li, Y., Zhang, Y., Zhao, X., Li, A., Yang, Y., Dahal, U., and Lou, X. M., et al. (2014) Mammalian WTAP is a regulatory subunit of the RNA N6-methyladenosine methyltransferase. Cell Res. 24, 177–189

12. Cheng, Z., Huang, C. M., Li, C. J., Vågbø, D. F., and Bobe, J. (2012) Oocyte-somatic cells interactions, lessons from evolution. BMC Genomics 13, 560

13. Huang da, W., Sherman, B. T., and Lempicki, R. A. (2009) Systematic and integrative analysis of large gene lists using DAVID bioinformatics resources. Nat. Protoc. 4, 44–57

14. Smits, A. H., Lindeboom, R. G., Perino, M., van Heeringen, S. I., Veenstra, G. J., and Vermeulen, M. (2014) Global absolute quantification reveals tight regulation of protein expression in single Xenopus eggs. Nucleic Acids Res. 42, 8980–8981

15. Peshkin, L., Wühr, M., Pearl, E., Haas, W., Freeman, R. M., Jr., Gerhart, J. C., Kleinf, A. M., Horb, M., Gygi, S. P., and Kirschner, M. W. (2015) On the relationship of protein and mRNA dynamics in vertebrate embryonic development. Dev. Cell 35, 383–394

16. Heesman, J. (2006) Patterning the early Xenopus embryo. Development 133, 1205–1217

17. Lin, S., Choe, J., Du, P., Triboulet, R., and Gregory, R. I. (2016) The m(6)A methyltransferase METTL3 promotes translation in human cancer cells. Mol. Cell 62, 335–345

18. Liu, J., Yue, Y., Han, D., Wang, X., Fu, Y., Zhang, L., Jia, G., Yu, M., Lu, Z., Deng, X., Dai, Q., Chen, W., and He, C. (2014) A METTL3-METTL14 complex mediates mammalian nuclear RNA N6-adenosine methylation. Nat. Chem. Biol. 10, 93–95

19. Ping, X. L., Sun, B. F., Wang, L., Xiao, W., Yang, W., Wang, J. W., Adhikari, S., Shi, Y., Lv, Y., Chen, Y. S., Zhao, X., Li, A., Yang, Y., Dahal, U., and Lou, X. M., et al. (2014) Mammalian WTAP is a regulatory subunit of the RNA N6-methyladenosine methyltransferase. Cell Res. 24, 177–189

20. Schwartz, S., Mumbach, M. R., Jovanovic, M., Wang, T., Maciag, K., Bushkin, G. K., Mertins, P., Ter-Ovanesyan, D., Habib, N., Cacchiarelli, D., Sanjana, N. E., Freinkman, E., Pacold, M. E., Satija, R., Mikkelsen, T. S., et al. (2014) Perturbation of m(6)A writers reveals two distinct classes of mRNA methylation at internal and 5’ sites. Cell Rep. 8, 284–296

21. Jia, G., Fu, Y., Zhao, X., Dai, Q., Zheng, G., Yang, Y., Yi, C., Lindahl, T., Pan, T., Yang, Y. G., and He, C. (2011) N6-methyladenosine in nuclear RNA is a major substrate of the obesity-associated FTO. Nat. Chem. Biol. 7, 885–887

22. Zheng, G., Dahl, J. A., Niu, Y., Fedorcsak, P., Huang, C. M., Li, C. I., Vågbø, C. B., Shi, Y., Wang, W. L., Song, S. H., Lu, Z., Bosmans, R. P., Dai, Q., Hao, Y. I., Yang, X., et al. (2013) ALKBHS is a mammalian RNA demethylase that impacts RNA metabolism and mouse fertility. Mol. Cell 49, 18–29

23. Cao, G. L., H. B., Yin, Z., and Flavell, R. A. (2016) Recent advances in dynamic m(6)A RNA modification. Open Biol. 6, 160003

24. Pan, H., O’Brien, M. J., Wigglesworth, K., Eppig, J. J., and Schultz, R. M. (2005) Transcript profiling during mouse oocyte development and the effect of gonadotropin priming and development in vitro. Dev. Biol. 286, 493–506

25. Yoshimoto, S., Furuno, N., Prigent, C., and Hashimoto, E. (2012) The subcellular localization of cyclin B2 is required for bipolar spindle formation during Xenopus oocyte maturation. Biochem. Biophys. Res. Commun. 422, 770–775

26. Peter, M., Labbé, J. C., Doree, M., and Mandart, E. (2002) A new role for Mos in Xenopus oocyte maturation: targeting Myt1 independently of MAPK. Development 129, 2129–2139

27. Colledge, W. H., Carlton, M. B., Udy, G. B., and Evans, M. J. (1994) Disruption of c-mos causes parthenogenetic development of unfertilized mouse eggs. Nature 370, 65–68

28. Araki, K., Naito, K., Haraguchi, S., Suzuki, R., Yokoyama, M., Inoue, M., Aizawa, S., and Sato, E. (1996) Meiotic abnormalities of c-mos knockout mouse oocytes: activation after first meiosis or entrance into third meiotic metaphase. Biol. Reprod. 55, 1315–1324

29. Narasimhachar, Y., and Coué, M. (2009) Geminin stabilizes Cdt1 during meiosis in Xenopus oocytes. J. Biol. Chem. 284, 27235–27242

30. Arentson, E., Faloon, P., Seo, J., Moon, E., Studits, J. M., Fremont, D. H., and Choi, K. (2002) Oncogenic potential of the DNA replication licensing protein Cdt1. Oncogene 21, 1150–1158

31. Arias, E. E., and Walter, J. C. (2005) Replication-dependent destruction of c-mos causes parthenogenetic development of unfertilized mouse eggs. Nature 370, 65–68

32. Charlier, C., Montfort, J., Chabrol, O., Brisard, D., Nguyen, T., Le Cam, A., Richard-Parpaillon, L., Moreeves, F., Pontarotti, P., Uzbekova, S., Chesnel, F., and Bobe, J. (2012) Oocyte-somatic cells interactions, lessons from evolution. BMC Genomics 13, 560

33. Huang da, W., Sherman, B. T., and Lempicki, R. A. (2009) Systematic and integrative analysis of large gene lists using DAVID bioinformatics resources. Nat. Protoc. 4, 44–57

34. Smits, A. H., Lindeboom, R. G., Perino, M., van Heeringen, S. I., Veenstra, G. J., and Vermeulen, M. (2014) Global absolute quantification reveals