

## Maternally derived transcripts: identification and characterisation during oocyte maturation and early cleavage

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**Abstract.** The identification and characterisation of differentially regulated genes in oocytes and early embryos are required to understand the mechanisms involved in maturation, fertilisation, early cleavage and even long-term development. Several methods, including reverse transcription–polymerase chain reaction-based suppression subtractive hybridisation, differential display and cDNA microarray, have been applied to identify maternally derived genes in mammalian oocytes. However, conventional gene-knockout experiments to determine specific gene functions are labour intensive and inefficient. Recent developments include the use of RNA interference techniques to establish specific gene functions in mammalian oocytes and early embryos. Regulation of the poly(A) tail length is a major factor in controlling the activities of maternal transcripts in mammals. Further studies are required to clarify the mechanisms by which expression levels of maternally derived transcripts are regulated. In the present review, we focus on the identification and functions of the differentially expressed transcripts during oocyte maturation, fertilisation and early cleavage.

*Extra keywords:* gene expression, maternal genes.

### Introduction

Mammalian oocytes contain maternally derived transcripts necessary for oocyte maturation and early embryogenesis, which occurs in the absence of *de novo* transcription of either parental genome (Nothias *et al.* 1995). During oogenesis, oocytes accumulate transcripts of genes involved in maturation and early embryonic development (Bachvarova 1992). Oocyte maturation is an important process that prepares the egg for fertilisation by a spermatozoon. During oogenesis, the mammalian oocyte enters prophase of the first meiotic division and progresses to the diplotene stage of prophase I, defined as the germinal vesicle (GV) phase. During and following the resumption of first meiosis, chromatin starts to condense, GV breakdown (GVBD) is initiated, the metaphase (M) I spindle is organised and the first polar body is extruded. Immediately thereafter, oocytes enter meiosis II and are re-arrested at the MII stage. Most mRNA transcribed at this time persists in the ooplasm in a stable, but translationally inactive form. Following activation by sperm or parthenogenetic stimuli, oocytes resume meiosis II and complete maturation, emitting the second polar body. The normality of early embryogenesis is directly related to ordered developmentally regulated gene transcription (Van Blerkom 1991). Thus, it is essential to identify and characterise differentially regulated genes in oocytes and early embryos to determine

the mechanisms involved in maturation, fertilisation and early embryonic development.

### Identification of maternal gene transcripts

#### *Reverse transcription–polymerase chain reaction-based methods*

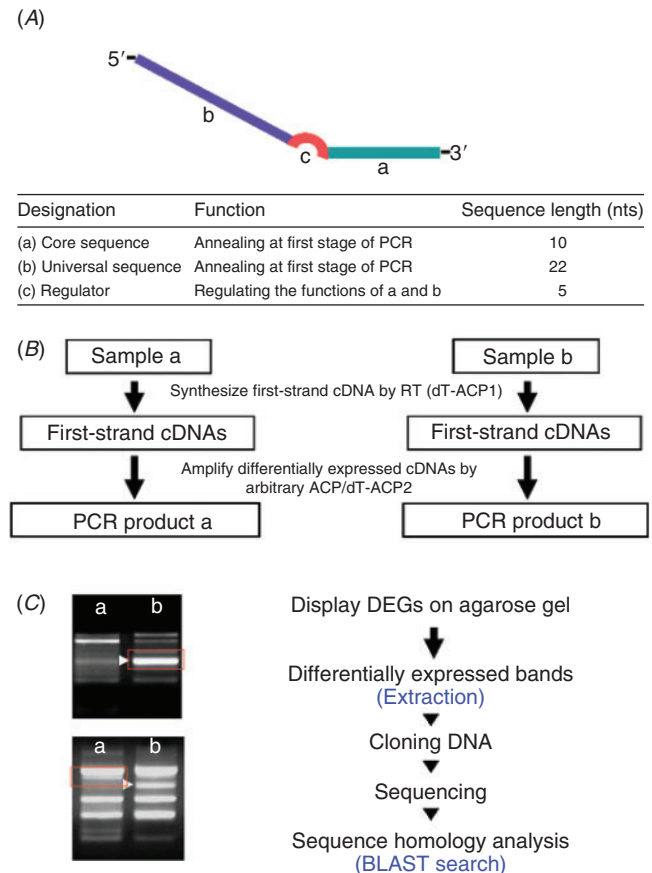
To determine the molecular basis of oocyte maturation and preimplantation development, differentially expressed genes of interest must be identified and analysed in detail. However, the quantity of mRNA in oocytes is limited, which hampers gene identification in oocytes and early preimplantation embryos. To date, several reverse transcription–polymerase chain reaction (RT-PCR)-based techniques have been applied to amplify/identify embryonic transcripts or expressed sequence tags (ESTs) in various species. Serial analysis of gene expression (SAGE) after amplification of RNA by PCR is used to determine gene expression profiles from immature human and mouse oocytes (Neilson *et al.* 2000; Stanton *et al.* 2002). The microSAGE adaptation of SAGE to minute amounts of starting material can be applied to detect genes in mammalian oocytes and embryos (Blomberg and Zuelke 2004). Several investigators have used modified suppression subtractive hybridisation (SSH) to analyse differential gene expression in bovine (Robert *et al.* 2000; Mohan *et al.* 2002; Ponsuksili *et al.* 2002;

Donnison and Pfeffer 2004; Dode *et al.* 2006), murine (Zeng and Schultz 2003) and rabbit (Pacheco-Trigon *et al.* 2002) preimplantation embryos.

The mRNA differential display RT-PCR (DDRT-PCR) is a procedure to compare mRNA transcript patterns between samples and should facilitate the identification of new and rare transcripts (Liang and Pardee 1992). Several stage-specific genes expressed in oocytes and preimplantation embryos have been identified using this technique (Henrion *et al.* 1997; Brunet-Simon *et al.* 2001; Lee *et al.* 2001; Natale *et al.* 2001; Tesfaye *et al.* 2003). However, despite considerable efforts, conventional differential display methods are labour intensive and lead to a high degree of false positives.

Novel annealing control primer (ACP)-based DDRT-PCR technology regulated by an ACP has been applied to identify differentially expressed genes (DEGs) in embryos using small amounts of mRNA (Hwang *et al.* 2004). Sequence hybridisation is specifically targeted to the template via a polydeoxyinosine (poly(dI)) linker (Hwang *et al.* 2003). The basis of this technology is the unique tripartite structure of the oligonucleotide primer ACP, which consists of: (1) a 3' end region with a target core nucleotide sequence that substantially complements the template nucleic acid for hybridisation; (2) a 5' end region with a non-target universal nucleotide sequence; and (3) a poly(dI) linker bridging the 3' and 5' end sequences (Fig. 1; Hwang *et al.* 2004). The first-strand cDNA from the total RNA was synthesised using dT-ACP1 by reverse transcriptase and second-strand cDNA is amplified by priming the arbitrary ACP and dT-ACP2 at the 3' and 5' ends, respectively (Fig. 1). A poly(dI) linker prevents annealing of the 5' end non-target sequence to the template and facilitates primer hybridisation at the 3' end to the target sequence at specific temperatures, resulting in a marked improvement of annealing specificity.

The ACP-based PCR facilitates the identification of DEGs from samples containing low mRNA levels while reducing false positives (Fig. 1; Hwang *et al.* 2004, 2005a, 2005b; Cui *et al.* 2005a, 2005b; Yoon *et al.* 2005; Lee *et al.* 2006). In our laboratory, Cui *et al.* (2005b) used the ACP-based DDRT-PCR technology to identify genes expressed in MII oocytes. This analysis, which used 60 arbitrary ACPs, disclosed 13 DEGs that are specifically or more prominently expressed in porcine MII oocytes compared with GV oocytes. The expression of four randomly selected genes, namely transcription factor TzP (*Tzp*), annexin A2 (*Anxa2*), hypoxia-inducible protein 2 (*Hig2*) and ATPase subunit 6 (*Atpase6*), during early porcine and murine embryogenesis was characterised by real-time quantitative RT-PCR. The levels of these genes at the different stages of development were normalised relative to histone H2a (H2a) mRNA. Notably, all four genes were markedly up-regulated at the MII stage, after which expression during the early cleavage stages gradually declined (Cui *et al.* 2005b).



**Fig. 1.** Schematic depiction of the annealing control primer (ACP) reverse transcription–polymerase chain reaction GeneFishing procedure. (A) The ACP primer contains three regions: a target region with a complementary sequence to template DNA (a), a non-target universal sequence region (b), and a regulator composed of a polydeoxyinosine linker that bridges these two regions (c). (B) The mRNA sequences isolated from samples a and b were used for the synthesis of first-strand cDNA using the dTACP1 primer. Second-strand cDNAs were amplified during second-stage PCR using a combination of dT-ACP2 (reverse primer) and one of 120 arbitrary ACP primers (forward primer). Products were separated on an agarose gel to identify the differentially expressed genes that are highly expressed in sample a or b.

#### *Global analysis using cDNA microarray or in silico identification*

Microarray analysis is currently the most powerful approach for global gene expression profiling (Schena *et al.* 1995). Global gene expression profiles of mouse and human preimplantation embryos from the GV oocyte to blastocyst stages have been established via microarray analyses using *in vitro*-transcribed antisense RNA as amplified target material (Hamatani *et al.* 2004; Tanaka and Ko 2004; Wang *et al.* 2004; Zeng *et al.* 2004). Recently, global gene expression profiles have been compared in porcine (Whitworth *et al.* 2004, 2005) and bovine embryos (Dalbies-Tran and Mermillod 2003; Mohan *et al.* 2004; Vallee *et al.* 2005).

A comparison of transcripts from the GV stage to MII oocytes using microarray analysis disclosed changes in a large number of genes in both directions (a more than three-fold increase in 737 genes and a more than threefold decrease in 1082 genes; Wang *et al.* 2004). Although degradation and/or deadenylation of some maternal mRNA occur during meiotic maturation, new transcription essentially ceases after re-entry into meiosis (Bachvarova 1985; Paynton *et al.* 1988). Because the current RNA amplification procedure involves annealing between the poly(A) tail and an oligo-(dT) primer, an elongated poly(A) tail may increase annealing efficiency and, therefore, transcript detection. Thus, the transcript increase in specific stage oocytes possibly reflects poly(A) length-mediated gene regulation (Gosden 2002; Wang *et al.* 2004).

Global gene expression using microarray analysis confirmed considerable maternal mRNA degradation (Hamatani *et al.* 2004). Maternal transcripts in the early preimplantation embryo are subdivided into two classes during preimplantation development according to expression levels. The first class of maternal mRNA, common to the oocyte and early embryo, is replenished after zygotic genome activation. The second class is oocyte specific and not subsequently expressed from zygotic genes. This mRNA may not only be unnecessary, but also detrimental to early development after fertilisation (Wang *et al.* 2004).

Using the *in silico* subtraction approach, Dade *et al.* (2003) recently identified six genes of the oogenesis family present on chromosome 4 in a cluster of almost 1 Mb composed of 12 oogenesis paralogous genes. The group further identified six genes displaying similarities with Nacht, leucine-rich repeat and pyrin domain-containing 5 (Nalp5; old symbol, Mater), which are specifically expressed in the mouse oocyte. Three of these are clustered on chromosome 7 (Dade *et al.* 2004). The finding that these two groups of genes are localised in two clusters suggests that they originate from the duplication of an ancestral gene (Paillisson *et al.* 2005).

#### *Real-time reverse transcription–polymerase chain reaction*

Quantification of specific genes of interest at the total mRNA level is informative for evaluating changes in cell physiology resulting from differentiation or in response to various parameters. Classical RNA analysis techniques, such as Northern blotting (Thomas 1980) and dot- or slot-blotting (White and Bancroft 1982), are unsuitable for mRNA identification in mammalian oocytes or preimplantation embryos because they are not sufficiently sensitive to distinguish low transcript levels. Reverse transcription–polymerase chain reaction allows the amplification of low-abundance mRNA and permits expression analysis of rare transcripts (Rappolee *et al.* 1988; Brenner *et al.* 1989). However, owing to differential reaction efficiencies and kinetics in RT-PCR, the amount of final product obtained after amplification does not

accurately reflect the initial mRNA concentration. Competitive RT-PCR procedures have been developed to overcome the problems associated with end-point analysis (Becker-Andre and Hahlbrock 1989; Wang *et al.* 1989; Stieger *et al.* 1991). The levels of the gene product of interest could be compared with the standard and then quantified as a relative expression with respect to the endogenous gene. However, these methods are time-consuming, because they require the design of standardised cDNA templates. Real time RT-PCR with fluorescence monitoring has been introduced to determine the concentrations of low-abundance mRNAs of interest (Higuchi *et al.* 1993). A unique feature of this system is the use of fluorescent reporters, such as TaqMan probes and SYBR Green dye (Morrison *et al.* 1998; Bustin 2000). Reliable quantitative data can be obtained in the exponential phase of amplification by analysis of fluorescence generated during the course of the reaction. The analysis programme determines a threshold cycle ( $C_t$ ) whereby the fluorescent signal of the sample exceeding the threshold level rises significantly above the mean of baseline fluorescence.

Successful use of this system has been reported for gene expression analysis in single oocytes and preimplantation embryos of mammals (Steuerwald *et al.* 1999, 2000; Hartshorn *et al.* 2002; Hayashi *et al.* 2003; Lindeberg *et al.* 2004; Cui *et al.* 2005a, 2005b, 2006). To improve efficiency, quantitative real-time RT-PCR should be combined with reliable determination of RNA as internal standards, as well as accurate normalisation methods. In most cells and tissues, glyceraldehyde-3-phosphate dehydrogenase (*Gapdh*), cytoplasmic  $\beta$ -actin (*Actb*) and rRNA (*Rnr*) are commonly used as control housekeeping genes (Suzuki *et al.* 2000). Semiquantitative or real-time RT-PCR is the most frequently used technique for studies on gene expression and regulation during preimplantation development. The process involves normalisation of RNA by adding a known amount of external control RNA or using endogenous transcripts of housekeeping genes as an internal control.

A prerequisite for efficiency in normalisation is that the expression levels of housekeeping gene do not vary markedly through preimplantation development or in response to different experimental conditions. The mRNA level in preimplantation embryos is variable for most genes, including housekeeping genes, owing to the dynamics of gene expression in early development (Bilodeau-Goeseels and Schultz 1997). This variability introduces a considerable bias when target gene values are normalised to those of an inconsistent housekeeping gene in bovine preimplantation embryos (Robert *et al.* 2002). The H2A histone family member Z (*H2a.z*) gene is currently the best internal standard because transcript levels remain relatively similar throughout the preimplantation period (Robert *et al.* 2002; Cui *et al.* 2005a, 2005b, 2006; Jeong *et al.* 2005). However, H2a.z expression levels vary during oocyte and embryo development. Consequently, a more suitable endogenous control should be

found and used in combination with an exogenous mRNA standard.

### Translation of maternal mRNA

To elucidate embryonic cellular function, detailed characterisation is necessary at the protein level. Conventional immunocytochemistry with conventional or confocal laser scanning is widely used to determine protein synthesis and localisation in oocytes and early embryos (Kim *et al.* 1996a, 1996b, 1996c; Fan *et al.* 2003; Fan and Sun 2004; Villa-Diaz and Miyano 2004; Koo *et al.* 2005; Sutovsky *et al.* 2005; Cui *et al.* 2006). For example, oogenesis is expressed during oogenesis and early embryogenesis in mouse (Minami *et al.* 2003). The protein is synthesised from the oocyte to four-cell embryo stages, suggesting a possible role during oocyte maturation and/or embryonic genome activation (Minami *et al.* 2003).

The proteomics approach was recently applied to analyse mouse and porcine oocytes during *in vitro* maturation (Calvert *et al.* 2003; Coonrod *et al.* 2004). In mouse, two-dimensional (2D) electrophoresis, avidin blotting and tandem mass spectrometry techniques were used to identify and characterise abundant molecules from mouse egg proteome (Calvert *et al.* 2003). These novel developments in mass spectrometry, using expression profiling and peptide sequencing to identify proteins involved in biological processes, are revolutionary (Hale *et al.* 2003; Shankar *et al.* 2005). However, the large quantity of starting material required remains a stumbling block for the introduction of this technology in the field of oocyte and early embryo physiology.

Surface-enhanced laser desorption–ionisation time of flight mass spectrometry (SELDI-TOF MS), an alternative proteomic technology based on capturing proteins and peptides by chemically modified surfaces, is highly sensitive for the analysis of complex biological samples (Siebert *et al.* 2004; Xiao *et al.* 2005). Different classes of proteins (hydrophobic, hydrophilic, acidic or basic) on the chip

surface are captured for analysis. Laser activation liberates gaseous ions and TOF MS is used to accurately determine the mass/charge ratio ( $m/z$ ) of once-bound proteins (Merchant and Weinberger 2000). Recently, Katz-Jaffe *et al.* (2006) developed a SELDI-TOF MS system capable of analysing the proteome of an individual human blastocyst. Identification of differentially expressed proteins of mammalian oocytes and early embryos may lead to an improved understanding of preimplantation embryonic development and the critical events occurring immediately before implantation.

### Functional studies of maternally derived transcripts

#### *Conventional gene-knockout studies*

Maternal effect genes produce mRNA or proteins that accumulate in the egg during oogenesis and are involved in embryonic development (Tong *et al.* 2000). Several maternal effect genes have been characterised using knockout mouse models (Table 1; Christians *et al.* 1999; Tong *et al.* 2000; Howell *et al.* 2001; Gurtu *et al.* 2002). During folliculogenesis, factor in germline  $\alpha$  (Fig $\alpha$ ), a basic helix–loop–helix (bHLH) transcription factor, is initially detected in oocytes at embryonic day (E) 13.5 and persists in adults. Homozygous null Fig $\alpha$  female mice are unable to form primordial follicles, resulting in massive depletion of oocytes and sterility (Soyal *et al.* 2000). In addition, Fig $\alpha$  is required for the expression of genes encoding for zona pellucida proteins Zp1, Zp2 and Zp3 (Liang *et al.* 1997), implying that the protein plays a key regulatory role in the expression of multiple oocyte-specific genes, including those initiating folliculogenesis and encoding the zona pellucida.

Fibroblast growth factor (Fgf) 8 is present in maturing oocytes (Valve *et al.* 1997). The embryonic lethality of Fgf8-null mutants has precluded genetic analysis of its role in follicular growth (Sun *et al.* 1999). Growth differentiation factor (Gdf) 9, a member of the transforming growth factor (Tgf)- $\beta$  family, is obligatory for proper folliculogenesis

**Table 1. Important maternal effect genes and their proposed roles**

Gene name	Gene symbol	Proposed roles	Reference
Factor in germline $\alpha$	<i>Fig<math>\alpha</math></i>	Folliculogenesis, encoding the zona pellucida	Soyal <i>et al.</i> (2000)
Follicular growth factor 8	<i>Fgf8</i>	Follicular growth	Sun <i>et al.</i> (1999)
Transforming growth factor- $\beta$	<i>Tgf<math>\beta</math></i>	Folliculogenesis, fertility	Carabatsos <i>et al.</i> (1998), Matzuk and Lamb (2002)
Heat shock factor 1	<i>Hsf1</i>	Cleavage	Christians <i>et al.</i> (2000)
Nucleoplasmin 2	<i>Npm2</i>	Fertility, nucleolar biogenesis	Burns <i>et al.</i> (2003)
NACHT, leucine-rich repeat and PYD9-containing 5	<i>Nalp5</i> (old symbol <i>Mater</i> )	Cleavage	Tong <i>et al.</i> (2000)
Zygote arrest 1	<i>Zar1</i>	Cleavage	Wu <i>et al.</i> (2003)
Stem cell enriched protein	<i>Stella</i>	Embryo development	Payer <i>et al.</i> (2003)
Zinc finger protein 36 like 2	<i>Zfp3612</i>	Cleavage	Ramos <i>et al.</i> (2004)
Basonuclin	<i>Bnc</i>	Cleavage	Ma <i>et al.</i> (2006)

beyond the primary stage and fertility in female mice (Carabatsos *et al.* 1998; Matzuk and Lamb 2002). In addition, heat-shock factor (Hsf) 1 is a maternal effect gene. Most embryos lacking Hsf1 fail to develop beyond the one-cell stage and those that cleave show ultrastructural anomalies in nuclei (Christians *et al.* 2000). Nucleoplasmin 2 (Npm2) has been identified using subtractive hybridisation (Burns *et al.* 2003). The Npm2-knockout females display fertility defects as a result of reduced cleavage to the two-cell stage. In Npm2-null oocytes and zygotes, the absence of coalesced nucleolar structures and the loss of heterochromatin and deacetylated histone H3 are observed, suggesting that Npm2 is critical for nuclear and nucleolar organisation and embryonic development (Burns *et al.* 2003).

Another maternal effect gene, namely *Mater*, is a single-copy gene transcribed in growing oocytes (Tong *et al.* 2000). Although its transcripts are degraded during meiotic maturation, the MATER protein persists up to the blastocyst stage. Female mice lacking this 125-kDa cytoplasmic protein produce no offspring owing to an embryonic block at the early cleavage stage (Tong *et al.* 2000). *Mater* is localised in both mitochondria and nuclei. These findings suggest that *Mater* participates in both cytoplasmic and nuclear events for early development (Tong *et al.* 2004). Zygote arrest 1 (*Zar1*) is the maternal effect gene identified in mouse. The mRNA of *Zar1* is present in oocytes and one-cell embryos, but decreases significantly at the two-cell stage and is not detected at further stages of development or in tissues other than the ovary. Compared with *Mater*-null embryos, *Zar1*-null embryos are blocked predominantly at the one-cell stage and genome activation is almost completely suppressed (Wu *et al.* 2003).

Stem cell enriched protein (*Stella*) is a known mammalian maternal effect gene and the phenotypic effect on embryonic development is mainly a consequence of the maternal *Stella* mutant genotype. *Stella* is a novel gene specifically expressed in primordial germ cells, oocytes, preimplantation embryos and pluripotent cells. *Stella*-deficient females exhibit severely reduced fertility owing to the lack of maternally inherited *Stella* protein in their oocytes and embryos without *Stella* display compromised preimplantation development and rarely reach the blastocyst stage (Payer *et al.* 2003). Zinc Finger Protein 36 Like 2 (*Zfp3612*) belongs to an unusual family of zinc finger proteins containing tandem zinc-binding motifs (Varnum *et al.* 1991). The *Zfp3612*-null females apparently cycle and ovulate normally and their ova can be fertilised. However, the embryos do not progress beyond the two-cell stage of development (Ramos *et al.* 2004).

### RNA interference

Targeted mRNA degradation is mediated by double-stranded RNA (dsRNA), which is a useful tool in terms of RNA

interference (RNAi), which has been adapted to allow the transient or stable knockdown of gene expression in several systems. The process of RNAi is defined as the ability to block the activity of a cellular gene by introducing homologous RNA into cells (Fire 1999). A novel innovative method, RNAi is currently a powerful biological tool for gene function analysis in plants, invertebrates and vertebrates (Sharp 2001). A few RNAi studies reported in mouse oocytes or preimplantation embryos involve microinjection of dsRNA (Svoboda *et al.* 2000; Wianny and Zernicka-Goetz 2000), short interfering RNA (siRNA; Kim *et al.* 2002; Shin *et al.* 2005; Cui *et al.* 2006) or electroporation of dsRNA (Grabarek *et al.* 2002). In these experiments, expression of endogenous genes with known functions, such as *c-mos* (*c-Mos*), E-cadherin (*Cdh1*), plasminogen activator, tissue (*Plat*) Oct-3/4 (*Pou5f1*), cytochrome *c* oxidase (*Cox*) and cell division cycle 42 (*Cdc42*), was successfully inhibited. Results were comparable to those from knockout animals, confirming the effects of RNAi in mammalian oocytes and embryos. Svoboda *et al.* (2000) demonstrated specific and significant reduction of transcript levels in mouse oocytes following direct injection of dsRNA into GV stage oocytes.

However, the use of RNAi by injecting dsRNA or siRNA targeting maternal transcripts may be limited owing to dependence on translation over the whole of oogenesis (i.e. well before injection) and on the stability of the corresponding protein. The RNAi strategy may be significantly improved by transgenesis of growing oocytes using the oocyte-specific Zp3 promoter (Yu *et al.* 2004). With this transgenic RNAi approach, basenuclin (*Bnc*) deficiency in mouse oocytes perturbed both RNA polymerase I- and II-mediated transcription and oocyte morphology was affected. Fertilised *Bnc*-deficient eggs failed to develop beyond the two-cell stage, suggesting that the protein is a novel member of the mammalian maternal effect gene family (Ma *et al.* 2006).

### Control of expression of maternally derived transcripts

Unlike the period of mouse oocyte growth, during meiotic maturation, marked qualitative changes in protein synthesis are observed and more than half the mRNA is either deadenylated or even degraded (Bachvarova 1985; Paynton and Bachvarova 1994). Selective degradation of maternal transcripts during oocyte maturation is a developmentally regulated event preceding the transition of gene expression from maternal to zygotic control. Although the synthesis of new transcripts essentially ceases after nuclear envelope breakdown, poly(A) tails of some classes of existing transcripts are elongated, leading to increased translation and protein levels (Bachvarova 1992). Thus, regulation of the poly(A) tail length is a major mechanism for controlling maternal transcript activity. During oogenesis in many species, cytoplasmic polyadenylation of a set of maternal

mRNAs regulates their translation. Cytoplasmic polyadenylation of the poly(A) tail of maternal mRNAs is precisely controlled at the translational level when oocyte maturation commences (Huarte *et al.* 1987; Strickland *et al.* 1988; Bachvarova 1992). In bovine oocytes, the polyadenylation level of several selected genes decreases during *in vitro* maturation. Moreover, the poly(A) tails of these transcripts are shorter in oocytes with lower developmental competence (Brevini-Gandolfi *et al.* 1999; Lequarre *et al.* 2004). Cytoplasmic polyadenylation of cyclin B1 is linked with the translation/appearance of the protein before *in vitro* maturation in bovine (Tremblay *et al.* 2005).

In *Xenopus* and mouse oocytes, two *cis* elements, the nuclear poly(A) addition signal (e.g. AAUAAA) and cytoplasmic polyadenylation element (CPE; e.g. UUUUUAU) at the 3'-untranslated region (UTR), are essential for cytoplasmic polyadenylation (Vassalli *et al.* 1989; Gebauer and Richter 1996). The cytoplasmic polyadenylation element binding protein (CPEB) is a sequence-specific RNA-interacting factor necessary to achieve adequate poly(A) addition within the cytoplasm. A long poly(A) tail probably allows mRNA to acquire a circular structure before the initiation of translation (Piccioni *et al.* 2005). The cytoplasmic somatic cell poly(A)-binding protein (PABP1) is not expressed until later on in embryogenesis. Seli *et al.* (2005) identified an embryonic poly(A)-binding protein (ePAB) in *Xenopus* and mouse. Expression of mouse ePAB before zygotic gene activation is implicated in the translational activation of maternally derived mRNAs during mammalian oocyte and early preimplantation embryo development. Both CPE and CPEB are implicated in the temporal activation of specific mRNA during vertebrate oocyte maturation (Piccioni *et al.* 2005). However, Charlesworth *et al.* (2004) reported that in progesterone-stimulated *Xenopus* oocytes, early cytoplasmic polyadenylation and translational activation of multiple maternal mRNAs occur in a Cpe- and Cpeb-independent manner. Thus, further studies are required to clarify the mechanisms by which expression levels of maternally derived transcripts are regulated.

## Conclusions

Full exploitation of maternal effect genes will take considerable time, but should provide insights into the molecular processes during oocyte maturation, cleavage and even long-term development. Improved RT-PCR-based differential display, real-time RT-PCR, cDNA microarray and *in silico* mining have been applied to identify maternally derived genes in mammalian oocytes. Moreover, conventional gene knockout and RNAi techniques have been used to characterise the specific functions of maternally derived transcripts. The regulatory mechanisms of the activities of maternally derived genes in mammals are currently under investigation. These findings may subsequently be applied to animal

biotechnology procedures, such as improvement of culture systems for *in vitro* maturation, *in vitro* embryo production, cloning by nuclear transfer and *in vitro* fertilisation in the clinic.

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