Comparative analysis of oocyte transcript profiles reveals a high degree of conservation among species

Maud Vallée, Kazuhiro Aiba¹, Yulan Piao¹, Marie-France Palin², Minoru S H Ko¹ and Marc-André Sirard

Département des Sciences Animales, Centre de Recherche en Biologie de la Reproduction (CRBR), Université Laval, Québec, Québec, G1K 7P4 Canada, ¹Developmental Genomics and Aging Section, Laboratory of Genetics, National Institute on Aging (NIA), National Institutes of Health (NIH), Baltimore, Maryland 21224, USA and ²Dairy and Swine Research and Development Centre, Agriculture and Agri-Food Canada, Lennoxville, Québec, J1M 1Z3 Canada

Correspondence should be addressed to M-A Sirard; Email: marc-andre.sirard@crbr.ulaval.ca

Abstract

Cross-species comparison of gene expression is a powerful approach for discovering genes that have been conserved throughout evolution. Conserved genes are presumably very important in the mechanisms related to the unique molecular functions in oocytes. The objective of this study was to identify genes expressed in the oocyte and conserved across three diverse vertebrate species. We report the global gene expression profiles of *Bos taurus* and *Xenopus laevis* oocytes on an NIA mouse development microarray that consists of 60-mer oligonucleotide probes representing more than 20 000 mouse transcripts derived from stem cell, oocyte, and early embryo cDNA libraries. Analysis based on intensity values revealed that 9853 and 10 046 genes are expressed in bovine and *Xenopus* oocytes respectively. Furthermore, previously published microarray data on preimplantation development in the mouse were used for a comparative analysis of global oocyte gene expression profiles. Interestingly, a substantial proportion of the genes expressed in mouse oocytes is conserved between the three species (74%, 7275 genes). Moreover, functional annotation of these conserved oocyte-expressed genes confirmed that certain functions are conserved among the three species. RNA metabolism and cell cycle were among the over-represented Gene Ontology terms in the biological process category. Finally, a pattern-matching analysis identified 208 conserved maternally expressed genes. Results from these cross-species hybridizations allowed numerous genes expressed in oocytes and conserved between *Mus musculus*, *B. taurus*, and *X. laevis* to be identified. This comparative analysis of oocyte transcript profiles revealed a high degree of conservation among species.

Reproduction (2008) 135 439–448

Introduction

In vertebrates, early embryonic development is supported by maternal mRNAs and proteins synthesized and stored during oogenesis (Rodman & Bachvarova 1976). These maternal factors are critical for development between the fertilization and the maternal–embryonic transition, when transcription of the embryonic genome becomes fully activated. Recent gene-targeting studies have revealed essential developmental roles for oocyte-specific genes: specifically, *Gdf9* (Dong *et al.* 1996), *Bmp15* (Yan *et al.* 2001), *Zp1*, *Zp2*, *Zp3* (Rankin *et al.* 1996, 1999, 2001), *Mater* (Tong *et al.* 2000), *Figla* (Soyal *et al.* 2000), *Npm2* (Burns *et al.* 2003) and *Zar1* (Wu *et al.* 2003), which are all essential for supporting oogenesis, fertilization, or proper early embryonic development prior to embryonic genome activation.

Previous studies have revealed that ~15 700 genes are involved in mouse embryonic development (Stanton *et al.* 2003) and that 196 of them are uniquely expressed

in unfertilized and fertilized eggs (Sharov *et al.* 2003). There is also evidence that genes expressed in oocytes and early embryos are conserved among species, since numerous orthologs have been found in mice and humans by *in silico* analysis (Stanton & Green 2001, 2002). However, our understanding of the oocyte transcriptome and the identity of key oocyte-expressed genes is far from complete. Furthermore, characterization of gene expression in oocytes is necessary and will provide additional insight into the regulation of oocyte maturation, fertility, and preimplantation development.

A variety of approaches have been used to study gene expression in oocytes. RT-PCR is a method of choice for profiling expression of known genes of interest on an individual basis. However, the growing need for genome-wide analysis has motivated the development of high-throughput techniques such as the analysis of expressed sequence tags (EST; Adams *et al.* 1991), differential display RT-PCR (Liang & Pardee 1992),

SAGE analysis (Velculescu *et al.* 1995), and suppressive subtractive hybridization (Diatchenko *et al.* 1996). These techniques have been successfully used during the past few years to identify important genes in oocytes (Ko *et al.* 2000, Neilson *et al.* 2000, Robert *et al.* 2000, Monk *et al.* 2001, Sharov *et al.* 2003, Zeng & Schultz 2003, Pennetier *et al.* 2005, Vallee *et al.* 2005).

Although these approaches have shed some light on the molecular basis of preimplantation development, each has major limitations. Microarray analysis has proved to be the most powerful approach for global gene expression profiling (Schena *et al.* 1995). The development of DNA microarray technology has permitted thousands of genes to be analyzed simultaneously, and the technology has become increasingly popular as a means of identifying differentially expressed genes in complex biological models. Furthermore, a linear amplification method utilizing an *in vitro* transcription reaction is now being widely used for microarray experiments (Eberwine 1996). This approach is particularly interesting since it circumvents many of the problems inherent with the most commonly employed amplification strategies that depend on PCR. Thus, the ability to amplify the small amounts of mRNA present in oocytes, which can only be isolated in limited quantities, makes it feasible to generate enough material for microarray analysis. Numerous studies have been performed to analyze oocytes global gene expression profiling in different species such as mouse, cow, *Xenopus*, rhesus monkey, and human using a variety of microarray platforms (Altmann *et al.* 2001, Bermudez *et al.* 2004, Dobson *et al.* 2004, Hamatani *et al.* 2004, Wang *et al.* 2004, Yao *et al.* 2004, Zeng *et al.* 2004, Arraztoa *et al.* 2005, Misirlioglu *et al.* 2006, Fair *et al.* 2007, Gasca *et al.* 2007, Ghanem *et al.* 2007, Patel *et al.* 2007, Steuerwald *et al.* 2007, Su *et al.* 2007). Another approach to microarray-based research is the use of cross-species hybridizations for comparative analysis to reveal evolutionarily conserved mechanisms and pathways. Several studies have performed different analyses to validate the accuracy and precision of results obtained through microarray cross-species hybridization (Chismar *et al.* 2002, Ji *et al.* 2004, Renn *et al.* 2004, Bar-Or *et al.* 2006). Also, different microarray platforms have been used for cross-species hybridization: custom oligo arrays (Chalmers *et al.* 2005), Affymetrix arrays (Shah *et al.* 2004, Grigoryev *et al.* 2005), cDNA microarray (Adjaye *et al.* 2004, 2007, Donaldson *et al.* 2005), and cDNA macroarray (Robert *et al.* 2002, Dalbies-Tran & Mermillod 2003). Biologically meaningful information has been obtained from all these studies.

We have previously shown that cross-species hybridizations using cDNA microarrays give very specific results and are very valuable for oocyte gene expression profiling (Vallee *et al.* 2006). We have also shown that many of the genes expressed in oocytes are conserved between

mouse, cow, and *Xenopus* (Vallee *et al.* 2006). In the present study, we have performed cross-species hybridizations of *Bos taurus* and *Xenopus laevis* oocytes on a microarray platform containing 60-mer oligonucleotide probes representing more than 20 000 unique mouse transcripts, assembled primarily from sequences of stem cell, oocyte, and embryo cDNA libraries (Carter *et al.* 2003). We consider this microarray platform to be best suited for comparative analysis of the oocyte transcriptome since it should contain most of the genes expressed in oocytes, including low copy-number genes. Previously published microarray data obtained from a study on mouse preimplantation development, using the same platform (Hamatani *et al.* 2004), were also used in the present analysis. This comparative analysis revealed a high degree of conservation among maternal transcripts expressed in *Mus musculus*, *B. taurus*, and *X. laevis* oocytes. Working simultaneously with three distantly related species should help us better to understand the oocyte transcriptome and the identity of key oocyte-expressed genes with functions in important evolutionarily conserved mechanisms.

Results

Microarray experimental design

The NIA mouse development microarray used in this study consists of more than 20 000 mouse gene features derived from stem cell, oocyte, and early embryo cDNA libraries (Carter *et al.* 2003). We will use the term 'gene' instead of gene feature in descriptions of microarray data. Bovine and *Xenopus* oocyte cRNA were used as probes to perform three replicates of cross-species hybridizations on a mouse development microarray. A universal mouse reference RNA (Stratagene, La Jolla, CA, USA) was used for all hybridizations, so that all developmental stages could be compared (Weil *et al.* 2002). In addition, microarray data from mouse preimplantation development derived from a previous study by Hamatani *et al.* (2004) were used to perform a comparative analysis of global oocyte gene expression profiles and a pattern-matching analysis for identifying conserved maternally expressed genes.

Reproducibility of cross-species hybridizations

For each experiment, reproducibility was verified by comparing the logarithmic signal intensity values of all microarray spots between three replicated experiments. The correlation coefficients for the signal intensities of all microarray spots between these replicates were calculated in a pair-wise manner using the NIA Array Analysis tool (Sharov *et al.* 2005a). The results show that for our cross-species hybridizations the correlation is relatively

Table 1 Correlation coefficients of cross-species hybridization replicate experiments^a.

	Rep1	Rep2	Rep3
Bovine			
Rep1	1	0.91	0.90
Rep2		1	0.95
Rep3			1
<i>Xenopus laevis</i>			
Rep1	1	0.91	0.90
Rep2		1	0.93
Rep3			1

^aPearson correlation for each microarray spots comparing replicate experiments. Correlations were determined using log base(2) fluorescence intensities of the data from every microarray spot.

high between replicates for bovine (0.90–0.95) and *Xenopus* (0.90–0.93; Table 1). These correlation coefficients are comparable with those obtained from same-species hybridizations using mouse oocytes (0.94–0.96; Hamatani *et al.* 2004). Next, an unsupervised hierarchical clustering using all the replicates was performed using the NIA Array Analysis tool (NIA, Baltimore, MD, USA; Sharov *et al.* 2005a). This procedure independently clustered all the replicates by their appropriate species with minimal branch tree distances between species (Fig. 1).

Global analysis of gene expression in bovine and *Xenopus* oocytes

Data from the cross-species microarray hybridizations were uploaded in the NIA Array Analysis tool and a threshold was established to determine which genes were considered as expressed. This threshold was set according to the plot of error function (s.d. (= square root of the error variance) versus expression level (Log intensity)) to ensure minimal s.d. Genes with a mean log signal intensity above the established threshold (log 3.0) were considered to be expressed, whereas genes with signal intensities below the threshold were designated 'absent'. This analysis, based on the intensity values, revealed that the number of genes detected in bovine and *X. laevis* oocytes is fairly similar: 9853 and 10 046 genes are expressed in bovine and *Xenopus*

Figure 1 Hierarchical clustering of replicates. The dendrogram shows that all replicates are clustered by their appropriate species ($n=3$ for bovine and *Xenopus*, $n=4$ for mouse). Mouse microarray data are derived from Hamatani *et al.* (2004). Mm, *Mus musculus*; Bt, *Bos taurus*; Xl, *Xenopus laevis*.

Table 2 Genes detected in oocytes^a.

	Bovine	<i>Xenopus</i>	Common in three species ^b
Genes expressed in oocytes	9853	10 046	7275 (74%)
Average log signal intensity	3.47	3.45	3.55
Oocyte-specific genes ^c	90	92	82 (63%)
Average log intensity	3.42	3.46	3.65

^aGenes with a mean log signal intensity above threshold (log > 3.0).

^bThe proportion of genes common to the three species relative to the number of genes expressed in mouse oocytes is given in (%). ^cGenes recovered only from mouse unfertilized and fertilized egg cDNA libraries and not present in other analyzed tissues (Sharov *et al.* 2003).

oocytes respectively (Table 2). The maximum average log signal intensity observed is 3.47 and 3.45 in bovine and *Xenopus* oocytes respectively (Table 2).

Comparative analyses of genes expressed in mouse, bovine, and *Xenopus* oocytes

A substantial proportion of genes expressed in mouse oocytes are common to the three species (74%, 7275 genes; Table 2, Fig. 2). Furthermore, genes expressed in oocytes show a wide range of signal intensities from faint to high expression for the three species (Fig. 2). Interestingly, the signal intensities in the three species were also similar (Fig. 2). Those results suggest that genes conserved in oocytes of all three species also have similar expression levels. Genes with low expression levels in the mouse oocytes also have low expression levels in bovine and *Xenopus* oocytes, and the same applies to highly expressed genes. The species distribution of oocyte-expressed genes is presented as a Venn diagram according to their detection in oocytes of one, two, or three species (Fig. 3). Over 1000 genes appear to be expressed only in mouse oocytes, suggesting either a lack of homology between the species for these specific oligonucleotides and/or genes or possibly an actual interspecific difference in oocyte gene expression. Detection of genes expressed only in *B. taurus*, *X. laevis*, and *M. musculus* could reflect real differences in oocyte physiology between species.

To characterize further the subpopulation of genes conserved in all three species, we used the large preimplantation EST collections in which 196 genes recovered only from unfertilized and fertilized mouse egg cDNA libraries, and not present in other tissues analyzed, were identified (Sharov *et al.* 2003). Of these 196 genes, 154 were on the NIA Mouse 22K Microarray and 134 showed statistically significant expression changes during preimplantation stages (Hamatani *et al.* 2004). Among those genes, 82 were found to be expressed in oocytes of all three species (Table 2). As previously mentioned, these oocyte-specific genes also showed a wide range of signal intensities from faintly expressed to highly expressed (Fig. 2).

Figure 2 Scatter plot of mean log signal intensities of oocyte-specific genes conserved among species. Mean log signal intensity for (A) bovine and (B) *Xenopus laevis* oocytes plotted against mean log signal intensity for mouse oocytes. Gray spots represent all the genes on the microarray and blue spots represent oocyte-specific genes (82/154). The red dotted line corresponds to the threshold value ($\log 3.0$). Mm, *Mus musculus*; Bt, *Bos taurus*; Xl, *Xenopus laevis*.

GO terms associated with oocyte-expressed genes conserved in all three species

To find the Gene Ontology (GO) terms associated with the 7275 conserved genes expressed in oocytes, we used the tool in the NIA Mouse Gene Index 8.0 (Sharov *et al.* 2005b). The NIA Mouse Gene Index 8.0 contains all transcripts found in the microarray platform used in this study. This tool associates the proper GO terms from the Gene Ontology Consortium annotation categories (biological processes, cellular components, and molecular functions) for each gene and generates a list of over-represented GO terms in our population (Ashburner *et al.* 2000). From this analysis, several over-represented categories were identified from our candidate genes. RNA metabolism, ribonucleoprotein complex, and RNA binding were the most over-represented GO terms in conserved oocyte-expressed genes for the biological process, cellular component, and molecular function categories respectively (Fig. 4). Example of genes in these over-represented categories that are conserved in oocytes are: *Cpeb1*, *Eif4e2*, *Gemin6*, *Pabpn1*, *Paip1*, *Pum1*, *Rnpc2*, *Slbp*, and *Snrpd2*. Cell cycle and cellular division were also among the biological processes over-represented in the genes conserved in oocytes. *Bub1* and *Mphosph6*, known for their association with the cell cycle and/or cell division and previously identified as oocyte specific, were found in the present study to be associated with this category.

Conserved maternally expressed genes in development

Finally, to examine further the possible roles of genes according to their expression patterns during embryonic development, the conserved oocyte-expressed gene list was used for pattern-matching analysis. The spindlin (*Spin*) gene was used as template to identify conserved maternal genes. The results of this pattern-matching analysis for conserved oocyte-expressed genes revealed that 208 genes display the characteristics of conserved maternally expressed genes in the mouse (Fig. 5A). The list of known genes is presented in Fig. 5B. Among those

Figure 3 Venn diagram representing clones present in oocytes of one, two, or all three species. Transcripts are detected when their mean log signal intensities are above the threshold ($> \log 3.0$).

Figure 4 Selected GO categories over-represented in conserved oocyte-expressed genes. GO categories associated with conserved genes expressed in oocytes were identified using the NIA Mouse Gene Index 8.0 tool. Graphic representation of the GO term frequencies associated with (A) biological process, (B) cellular component, and (C) molecular function categories.

identified, some were previously known as maternal genes, such as *Bcl2l10*, *Gdf9*, *Tcl1* and *Zar1*. More interestingly, this analysis also identified uncharacterized transcripts such as *D6Ertd474e*, *BC066140*, *2610019P18Rik*, and *C230081A13Rik*. These maternally expressed genes are particularly interesting, mainly because of their expression patterns during embryonic development but also because they are conserved between the three species.

Discussion

The main objective of this study was to identify conserved oocyte-expressed genes. We believe that a comparative analysis performed on three distantly related species is a powerful tool for identifying key

Figure 5 Identification of conserved maternally expressed genes through pattern matching. The analysis was performed using the NIA microarray analysis tool with *Spindlin* gene (Z00008605-1) as template (tenfold change threshold and 0.90 correlation threshold). (A) Expression profiles of 208 genes identified through pattern matching. The y-axis indicates the relative expression levels of each gene and the x-axis indicates each developmental stage. The bold black line shows the pattern of the template gene (*Spindlin*), gray lines are centered gene intensities, and the red line is the average intensity of the genes found. Oo, oocyte; F, fertilized egg; 2c, 2-cell; 4c, 4-cell; 8c, 8-cell; M, morula; B, blastocyst. (B) List of known genes identified through this pattern-matching analysis.

oocyte-expressed genes with important functions in evolutionarily conserved mechanisms and should provide a better understanding of the oocyte transcriptome.

An essential criterion for the application of cross-species experiments is data reproducibility. The comparison of correlation coefficients between same-species and cross-species hybridizations revealed that the latter were sufficiently high to confirm acceptable reproducibility between experimental replicates. However, the cluster dendrogram revealed a cross-species effect. The sequence mismatches between bovine or *Xenopus* and mouse sequences probably cause bovine and *Xenopus* to be clustered together. In the absence of this cross-species effect, if microarray hybridization were all performed on a same-species

array, we would expect bovine and mouse to group closer together and *Xenopus* further apart. However, the fact that replicates cluster together by their appropriate species gives confidence in the reliability of the cross-species hybridizations.

During the past few years, a number of studies have successfully used cross-species hybridizations while others have tried to assess their specificity and reliability in reflecting biological processes. Kane *et al.* (2000) showed that probe specificity requires target genes to be at least 75% similar over the target region when 50-mer oligonucleotide microarrays are used. In addition, if the target region is marginally similar (50–75%), a stretch of complementary sequence of more than 15 contiguous bases will allow hybridization. Ji *et al.* (2004) reached fairly similar conclusions when they created a simple mathematical model for cross-species hybridization and concluded that a contiguous-matched oligonucleotide of 16 bp was sufficient to generate a specific hybridization signal. In our previous study, nucleotide sequence similarity for genes expressed in the oocyte was evaluated between mouse, bovine, and *X. laevis*. We found that, on average, bovine sequences showed an 86% identity to mouse sequences while 80% identity was observed between *Xenopus* and mouse sequences (Vallee *et al.* 2006). Another group quantified the agreement in gene expression profiles across different species. Although the agreement was most robust when the target RNA was derived from closely related species (<10 million years divergence), consistent profiles for more distantly related species (~65 million years divergence) and, to a lesser extent, even very distantly related species (>200 million years divergence) were also obtained (Renn *et al.* 2004). Finally, a recent study demonstrated that once the data are filtered by restricting proper cut-offs of probe homology, cross-species hybridization can closely reflect the biological process analyzed by same-species hybridization (Bar-Or *et al.* 2006). Although cross-species hybridizations have proven to be relatively specific, the identification of specific paralogues might prove to be difficult and need to be taken into consideration when results are analyzed. Occasional low sequence homology between common genes could preclude identification of the totality of transcripts conserved across species but, will not affect the validity of the genes identified as conserved in oocytes in the present study.

Moreover, the degree of homology between probes and targets when cross-species hybridizations are performed can be extremely variable. In the presence of sequence mismatches, relative hybridization intensities will reflect both differences in transcript abundance and differences in hybridization kinetics. In addition, intensities can be variable when two different species are used for cross-species hybridization, especially when the species studied are not equally divergent. In view of these limitations, the goal of this study was not to assess

gene expression levels but rather to identify transcripts present in the oocytes of all three species. The microarray analysis revealed that a substantial number of genes are detected in bovine and *Xenopus* oocytes; 9853 and 10 046 genes respectively. Previously, these labeling and hybridization protocols on this microarray platform and for all genes tested were extensively validated by quantitative real-time PCR; a log signal intensity >2.0 confirmed their expression (Carter *et al.* 2003). Thus, adjusting our threshold to above log 3.0 in the present study should be adequate. Moreover, a recently published study using the same microarray dataset also used a log-intensity signal >3.0 as the criterion for a positive signal (Mager *et al.* 2006). Finally, we previously showed through validation by RT-PCR and gene-specific microarray hybridization that cross-species microarray hybridizations with oocytes from these three species were highly specific for the candidate genes tested (Vallee *et al.* 2006).

When we performed comparative analyses of genes expressed in mouse, bovine, and *Xenopus*, the results showed that 74% of genes expressed in mouse oocytes are common to the three species, providing evidence that most genes expressed in oocytes are conserved among species. This conforms with our previous study in which cross-species hybridizations on a cDNA multi-species microarray showed that 81% of the genes expressed in mouse oocytes were also expressed in bovine and *Xenopus* oocytes (Vallee *et al.* 2006). Another study compared the ESTs generated from a mouse oocyte cDNA library to genes expressed in *X. laevis* and *Ciona intestinalis* oocytes (Evsikov *et al.* 2006). This analysis revealed that for 80% of the genes expressed in the mouse oocytes, homologs transcribed in eggs of either *X. laevis* or *C. intestinalis* were found (Evsikov *et al.* 2006). Interestingly, a recent study on yeast has demonstrated that proteins with essential functions evolve more slowly than less essential proteins (Zhang & He 2005). It concluded that this is likely to apply to mammalian genes as well. The authors suggest that when a large dataset of mouse knockout studies becomes available, it will be possible to demonstrate the same phenomena in mammals.

GO terms identified as being over-represented in oocyte-expressed genes conserved in all three species reflect the principal functions of the oocyte, such as RNA metabolism, ribonucleoprotein complex, RNA binding, cell cycle, and cellular division. It is well known that oocytes accumulate large quantities of maternal mRNAs during their growth phase (Rodman & Bachvarova 1976) and that these maternal mRNAs are very stable with a half-life of 8–12 days (Brower *et al.* 1981). Thus, their timely recruitment for translation and/or degradation during early embryonic stages is critical for successful development. Genes expressed in the oocyte associated with cell cycle are critical for early embryonic development since maternal transcripts have to support

cell cycles prior to embryonic genome activation, which occurs at different stages in different species (Telford *et al.* 1990). All these maternal factors, which are degraded following maturation (Piko & Clegg 1982), are critical for the interval between fertilization and the maternal–embryonic transition when transcriptional activity of the embryonic genome becomes fully functional. Pattern matching can be used to find genes with expression patterns similar to that of a selected template gene. In order to identify maternal genes, we used the spindlin (*Spin*) gene as template, a maternal transcript (Oh *et al.* 1997) that is known to be conserved in the three species (Vallee *et al.* 2006). *Spin* is known to be an abundant maternal transcript in the unfertilized egg and also in the 2-cell but not the 8-cell mouse embryo (Oh *et al.* 1997). SPIN protein is a substrate in the MOS/MAP kinase pathway that associates with the meiotic spindle and is suggested to play a role in cell-cycle regulation during the transition from gamete to embryo (Oh *et al.* 1997, 1998). Previously published microarray data on mouse preimplantation development (Hamatani *et al.* 2004) were used in this pattern-matching analysis to identify conserved maternally expressed genes. Expression profiles from these microarray data had previously been analyzed by a *k*-means nonhierarchical clustering method to identify expression trends in mouse embryonic development (Hamatani *et al.* 2004). The results of these studies showed that three different expression patterns corresponded to maternally expressed genes, and *Spin* was found in one of those clusters (Hamatani *et al.* 2004). Different studies have used microarray experiments to identify maternal transcripts in mouse oocytes (Hamatani *et al.* 2004, Wang *et al.* 2004, Zeng *et al.* 2004, Cui *et al.* 2007, Su *et al.* 2007), bovine oocytes (Misirlioglu *et al.* 2006, Adjaye *et al.* 2007, Fair *et al.* 2007), and in *Xenopus* (Evsikov *et al.* 2006, Graindorge *et al.* 2006), but to our knowledge, it has never been done for identifying conserved maternally expressed transcripts during development. The present pattern-matching analysis allowed us to identify which of our conserved oocyte-expressed genes are expressed in mouse oocytes and degraded throughout embryonic development. We believe that genes identified in this analysis are inclined to be maternally expressed in bovine and *X. laevis*. This suggests that they have important functions in oogenesis, oocyte maturation, fertilization and/or early embryonic development and should therefore be further characterized.

In summary, the results obtained from this cross-species hybridization allowed numerous conserved oocyte-expressed genes to be identified in mouse, bovine, and *Xenopus*, and also allowed conserved maternally expressed genes to be identified. Globally, this comparative analysis of oocyte transcript profiles revealed a high degree of conservation among species and clearly establishes the feasibility of working with a well-characterized microarray platform from another

species. Since our understanding of the oocyte transcriptome and the identity of key oocyte-expressed genes are far from complete, the strategic use of cross-species microarray hybridizations – an original and non-standard use of microarray – allowed the extraction of a smaller number of candidate genes that have important roles from a large cohort of maternal transcripts detected by microarrays. Finally, this study adds a substantive amount of new information that is extremely valuable to the scientific community working on fertility and early embryonic development. It will contribute to the elucidation of crucial molecular mechanisms and pathways regulating oogenesis and embryogenesis in different species including in humans, which may lead to the solutions for infertility problems or improvements in assisted reproductive technologies.

Materials and Methods

Tissue collection

Bovine ovaries were collected from a slaughterhouse. Cumulus–oocyte complexes (COCs) from 3 to 6 mm follicles were collected by aspiration; germinal vesicle (GV) oocytes were mechanically denuded, washed several times in PBS to prevent cumulus cell contamination. Three groups of 200 GV oocytes were then frozen in a minimal volume of PBS in liquid nitrogen. *X. laevis* oocytes were obtained from an adult female anesthetized in 0.1% methanesulfonate salt of 3-aminobenzoic acid ethyl ester (MS222, Sigma–Aldrich) for 20 min. A piece of ovary was isolated and oocytes were defolliculated for 1 h at 18 °C in OR2 saline containing 0.15% collagenase (Sigma). Stage IV–V oocytes (~1 mm diameter) were collected in OR2 medium at 18 °C. Three groups of 20 oocytes were washed in PBS and frozen in liquid nitrogen until RNA extraction. Animals were cared for according to the respective recommended codes of practice and killed by an acceptable method approved by the local Animal Care Committee following the guidelines of the Canadian Council on Animal Care (1993).

RNA extraction

Bovine and *Xenopus* total RNA were extracted using the Absolutely RNA Microprep Kit (Stratagene) according to the manufacturer's instructions. An additional phenol/chloroform purification step was performed on *Xenopus* oocytes prior to total RNA extraction to remove excess lipids. The extracted RNA was dissolved in water and the integrity and concentration were evaluated using a 2100-Bioanalyzer (Agilent Technologies, Palo Alto, CA, USA) with the RNA PicoLab Chip (Agilent Technologies).

Labeling and hybridization on the NIA 22 K 60-mer oligo microarray

Three RNA aliquots (100 ng) for each species were labeled with Cy3-dye by two-round linear amplification labeling to make

each cRNA target using a Fluorescent Linear Amplification Kit (Agilent Technologies) as previously described (Carter *et al.* 2003). In brief, mRNA was used to synthesize double-stranded cDNA with Moloney Murine Leukemia Virus (MMLV) reverse transcriptase in a reaction scaled down to a total volume of 4 µl, with half the standard T7-oligo-dT primer concentration and 125 ng/µl T4gp32 single-stranded DNA-binding protein (United States Biochemical, Cleveland, OH, USA). Linear amplification was performed in a total volume of 16 µl, with half the standard NTP concentration and no labeled CTP. For the second round of amplification, the product of the first reaction was labeled using the manufacturer's standard protocol, with the addition of T4gp32 in the cDNA synthesis reaction. The quality and size distribution of targets were determined by RNA 6000 Nano Lab-on-chip Assay (Agilent Technologies) and quantified using a NanoDrop microscale spectrophotometer (NanoDrop, Wilmington, DE, USA). Similarly, universal mouse reference RNA (Stratagene), labeled with Cy5-dye by one round of linear amplification, was used as control in all hybridizations (Carter *et al.* 2003). cRNA targets (750 ng) from oocytes and universal reference were assembled into a hybridization reaction on the NIA Mouse 22K Microarray v1.0 (manufactured by Agilent Technologies; Carter *et al.* 2003). Three replicate cross-species hybridizations were performed for bovine and *Xenopus* for a total of six hybridizations and 121 680 gene expression measurements. This microarray platform contains 60-mer oligonucleotide probes representing 20 281 mouse transcripts, assembled primarily from sequences of stem cell, oocyte, and early embryo cDNA libraries. A complete list of the annotated gene content of the microarray can be found on the NIA mouse cDNA project website (<http://lgsun.grc.nia.nih.gov/cDNA/>).

Microarray data analysis

For our cross-species hybridizations, spot intensity was extracted from scanned microarray images using Feature Extraction 5.1.1 software (Agilent Technologies), which performs background subtractions and dye normalization as described previously (Carter *et al.* 2003). The microarray experiments presented in this study adhere to the standards proposed by the Microarray Gene Expression Data Society Microarray (www.mged.org). Data for the microarray experiments reported herein are stored in the public repositories ArrayExpress (E-MEXP-1339) (www.ebi.ac.uk/arrayexpress). Comparative analyses were performed using mouse preimplantation development microarray data from a study by Hamatani *et al.* (2004). Their results were obtained from the hybridization of mouse oocytes and embryos (unfertilized oocyte, fertilized oocyte, 2-cell, 4-cell, 8-cell, morula, and blastocyst) in four replicates on the NIA Mouse 22K Microarray v1.0. Microarray data from all three species were uploaded in the NIA Array Analysis tool (Sharov *et al.* 2005a) where background threshold was determined according to the plot of error function (S.D. (=square root of the error variance) versus expression level (Log intensity)). For each gene on the array, a mean log signal intensity was calculated on the basis of the signal intensities obtained from the three replicate hybridizations. Genes with a mean log signal intensity above the

calculated threshold ($> \log 3.0$) were considered as expressed. Correlation coefficients of signal intensities of all microarray spots between replicated experiments ($n=3$ for bovine and *Xenopus*, $n=4$ for mouse) were calculated in a pair-wise manner using the NIA Array Analysis tool (Sharov *et al.* 2005a). Furthermore, data processing including hierarchical clustering and pattern matching was also performed through the NIA microarray analysis tool. The pattern-matching algorithm can identify a group of genes, the expression patterns of which match to a selected template gene. The analysis was performed using microarray data from bovine and *Xenopus* oocyte and mouse preimplantation development microarray data from Hamatani *et al.* (2004) using the spindlin gene (Z00008605-1) as template, a tenfold change threshold and a 0.90 correlation threshold. The NIA Mouse Gene Index 8.0 (Sharov *et al.* 2005b) was used to find the GO terms over-represented among the conserved genes expressed in oocytes. This tool associates the proper GO terms from the Gene Ontology Consortium annotation categories for each gene and generates a list of over-represented GO terms in our population (Ashburner *et al.* 2000). The GO Consortium has created a defined vocabulary of terms describing the biological processes, cellular components, and molecular functions of all genes. It assigns genes to GO terms providing annotation and biological context for individual genes.

Acknowledgements

The authors acknowledge Drs Alexei A Sharov and Mark G Carter for their help in planning the experiment and analysis and Drs Susan Novak and Julie Fradette for critical reading of the manuscript and for providing valuable comments. MV is supported by NSERC fellowship. This research was supported in part by the Intramural Research Program of the NIH, the National Institute on Aging, the Canada Research Chair and Natural Science and Engineering Research Council of Canada. Lennoxville Dairy and Swine R & D Center Contribution No. 936. The authors declare that there is no conflict of interest that would prejudice the impartiality of this scientific work.

References

- Adams MD, Kelley JM, Gocayne JD, Dubnick M, Polymeropoulos MH, Xiao H, Merril CR, Wu A, Olde B, Moreno RF *et al.* 1991 Complementary DNA sequencing: expressed sequence tags and human genome project. *Science* **252** 1651–1656.
- Adjaye J, Herwig R, Herrmann D, Wruck W, Benkahl A, Brink TC, Nowak M, Carnwath JW, Hultschig C, Niemann H *et al.* 2004 Cross-species hybridisation of human and bovine orthologous genes on high density cDNA microarrays. *BMC Genomics* **5** 83.
- Adjaye J, Herwig R, Brink TC, Herrmann D, Greber B, Sudheer S, Groth D, Carnwath JW, Lehrach H & Niemann H 2007 Conserved molecular portraits of bovine and human blastocysts as a consequence of the transition from maternal to embryonic control of gene expression. *Physiological Genomics* **31** 315–327.
- Altmann CR, Bell E, Sczyrba A, Pun J, Bekiranov S, Gaasterland T & Brivanlou AH 2001 Microarray-based analysis of early development in *Xenopus laevis*. *Developmental Biology* **236** 64–75.
- Arraztoa JA, Zhou J, Marcu D, Cheng C, Bonner R, Chen M, Xiang C, Brownstein M, Maisey K, Imarai M *et al.* 2005 Identification of genes expressed in primate primordial oocytes. *Human Reproduction* **20** 476–483.

- Ashburner M, Ball CA, Blake JA, Botstein D, Butler H, Cherry JM, Davis AP, Dolinski K, Dwight SS, Eppig JT *et al.* 2000 Gene ontology: tool for the unification of biology. The Gene Ontology Consortium. *Nature Genetics* **25** 25–29.
- Bar-Or C, Bar-Eyal M, Gal T, Kapulnik Y, Czosnek H & Koltai H 2006 Derivation of species-specific hybridization-like knowledge out of cross-species hybridization results. *BMC Genomics* **7** 110.
- Bermudez MG, Wells D, Malter H, Munne S, Cohen J & Steuerwald NM 2004 Expression profiles of individual human oocytes using microarray technology. *Reproductive Biomedicine Online* **8** 325–337.
- Brower PT, Gizang E, Boreen SM & Schultz RM 1981 Biochemical studies of mammalian oogenesis: synthesis and stability of various classes of RNA during growth of the mouse oocyte *in vitro*. *Developmental Biology* **86** 373–383.
- Burns KH, Viveiros MM, Ren Y, Wang P, DeMayo FJ, Frail DE, Eppig JJ & Matzuk MM 2003 Roles of NPM2 in chromatin and nucleolar organization in oocytes and embryos. *Science* **300** 633–636.
- Canadian Council on Animal Care 1993 *Guide to the Care and Use of Experimental Animals*, Ottawa: Canadian Council on Animal Care.
- Carter MG, Hamatani T, Sharov AA, Carmack CE, Qian Y, Aiba K, Ko NT, Dudekula DB, Brzoska PM, Hwang SS *et al.* 2003 *In situ*-synthesized novel microarray optimized for mouse stem cell and early developmental expression profiling. *Genome Research* **13** 1011–1021.
- Chalmers AD, Goldstone K, Smith JC, Gilchrist M, Amaya E & Papalopulu N 2005 A *Xenopus tropicalis* oligonucleotide microarray works across species using RNA from *Xenopus laevis*. *Mechanisms of Development* **122** 355–363.
- Chismar JD, Mondala T, Fox HS, Roberts E, Langford D, Masliah E, Salomon DR & Head SR 2002 Analysis of result variability from high-density oligonucleotide arrays comparing same-species and cross-species hybridizations. *BioTechniques* **33** 516–518 (520, 522 *passim*).
- Cui XS, Li XY, Yin XJ, Kong IK, Kang JJ & Kim NH 2007 Maternal gene transcription in mouse oocytes: genes implicated in oocyte maturation and fertilization. *Journal of Reproduction and Development* **53** 405–418.
- Dalbies-Tran R & Mermillod P 2003 Use of heterologous complementary DNA array screening to analyze bovine oocyte transcriptome and its evolution during *in vitro* maturation. *Biology of Reproduction* **68** 252–261.
- Diatchenko L, Lau YF, Campbell AP, Chenchik A, Moqadam F, Huang B, Lukyanov S, Lukyanov K, Gurskaya N, Sverdlov ED *et al.* 1996 Suppression subtractive hybridization: a method for generating differentially regulated or tissue-specific cDNA probes and libraries. *PNAS* **93** 6025–6030.
- Dobson AT, Raja R, Abeyta MJ, Taylor T, Shen S, Haqq C & Pera RA 2004 The unique transcriptome through day 3 of human preimplantation development. *Human Molecular Genetics* **13** 1461–1470.
- Donaldson L, Vuocolo T, Gray C, Strandberg Y, Reverter A, McWilliam S, Wang Y, Byrne K & Tellam R 2005 Construction and validation of a bovine innate immune microarray. *BMC Genomics* **6** 135.
- Dong J, Albertini DF, Nishimori K, Kumar TR, Lu N & Matzuk MM 1996 Growth differentiation factor-9 is required during early ovarian folliculogenesis. *Nature* **383** 531–535.
- Eberwine J 1996 Amplification of mRNA populations using aRNA generated from immobilized oligo(dT)-T7 primed cDNA. *BioTechniques* **20** 584–591.
- Eviskov AV, Graber JH, Brockman JM, Hampl A, Holbrook AE, Singh P, Eppig JJ, Solter D & Knowles BB 2006 Cracking the egg: molecular dynamics and evolutionary aspects of the transition from the fully grown oocyte to embryo. *Genes and Development* **20** 2713–2727.
- Fair T, Carter F, Park S, Evans AC & Lonergan P 2007 Global gene expression analysis during bovine oocyte *in vitro* maturation. *Theriogenology* **68** S91–S97.
- Gasca S, Pellestor F, Assou S, Loup V, Anahory T, Dechaud H, De Vos J & Hamamah S 2007 Identifying new human oocyte marker genes: a microarray approach. *Reproductive Biomedicine Online* **14** 175–183.
- Ghanem N, Holker M, Rings F, Jennen D, Tholen E, Sirard MA, Torner H, Kanitz W, Schellander K & Tesfaye D 2007 Alterations in transcript abundance of bovine oocytes recovered at growth and dominance phases of the first follicular wave. *BMC Developmental Biology* **7** 90.
- Graindorge A, Thuret R, Pollet N, Osborne HB & Audic Y 2006 Identification of post-transcriptionally regulated *Xenopus tropicalis* maternal mRNAs by microarray. *Nucleic Acids Research* **34** 986–995.
- Grigoryev DN, Ma SF, Simon BA, Irizarry RA, Ye SQ & Garcia JG 2005 *In vitro* identification and *in silico* utilization of interspecies sequence similarities using GeneChip technology. *BMC Genomics* **6** 62.
- Hamatani T, Carter MG, Sharov AA & Ko MS 2004 Dynamics of global gene expression changes during mouse preimplantation development. *Developmental Cell* **6** 117–131.
- Ji W, Zhou W, Gregg K, Yu N & Davis S 2004 A method for cross-species gene expression analysis with high-density oligonucleotide arrays. *Nucleic Acids Research* **32** e93.
- Kane MD, Jatkoe TA, Stumpf CR, Lu J, Thomas JD & Madore SJ 2000 Assessment of the sensitivity and specificity of oligonucleotide (50mer) microarrays. *Nucleic Acids Research* **28** 4552–4557.
- Ko MS, Kitchen JR, Wang X, Threat TA, Hasegawa A, Sun T, Grahovac MJ, Kargul GJ, Lim MK, Cui Y, *et al.* 2000 Large-scale cDNA analysis reveals phased gene expression patterns during preimplantation mouse development. *Development* **127** 1737–1749.
- Liang P & Pardee AB 1992 Differential display of eukaryotic messenger RNA by means of the polymerase chain reaction. *Science* **257** 967–971.
- Mager J, Schultz RM, Brunk BP & Bartolomei MS 2006 Identification of candidate maternal-effect genes through comparison of multiple microarray data sets. *Mammalian Genome* **17** 941–949.
- Misirlioglu M, Page GP, Sagirkaya H, Kaya A, Parrish JJ, First NL & Memili E 2006 Dynamics of global transcriptome in bovine matured oocytes and preimplantation embryos. *PNAS* **103** 18905–18910.
- Monk M, Holding C & Goto T 2001 Isolation of novel developmental genes from human germ cell, oocyte and embryo cDNA by differential display. *Reproduction, Fertility, and Development* **13** 51–57.
- Neilson L, Andalibi A, Kang D, Coutifaris C, Strauss JF III, Stanton JA & Green DP 2000 Molecular phenotype of the human oocyte by PCR-SAGE. *Genomics* **63** 13–24.
- Oh B, Hwang SY, Solter D & Knowles BB 1997 Spindlin, a major maternal transcript expressed in the mouse during the transition from oocyte to embryo. *Development* **124** 493–503.
- Oh B, Hampl A, Eppig JJ, Solter D & Knowles BB 1998 SPIN, a substrate in the MAP kinase pathway in mouse oocytes. *Molecular Reproduction and Development* **50** 240–249.
- Patel OV, Bettgeowda A, Ireland JJ, Coussens PM, Lonergan P & Smith GW 2007 Functional genomics studies of oocyte competence: evidence that reduced transcript abundance for follistatin is associated with poor developmental competence of bovine oocytes. *Reproduction* **133** 95–106.
- Pennetier S, Uzbekova S, Guyader-Joly C, Humblot P, Mermillod P & Dalbies-Tran R 2005 Genes preferentially expressed in bovine oocytes revealed by subtractive and suppressive hybridization. *Biology of Reproduction* **73** 713–720.
- Piko I & Clegg KB 1982 Quantitative changes in total RNA, total poly(A), and ribosomes in early mouse embryos. *Developmental Biology* **89** 362–378.
- Rankin T, Familari M, Lee E, Ginsberg A, Dwyer N, Blanchette-Mackie J, Drago J, Westphal H & Dean J 1996 Mice homozygous for an insertional mutation in the Zp3 gene lack a zona pellucida and are infertile. *Development* **122** 2903–2910.
- Rankin T, Talbot P, Lee E & Dean J 1999 Abnormal zonae pellucidae in mice lacking ZP1 result in early embryonic loss. *Development* **126** 3847–3855.
- Rankin TL, O'Brien M, Lee E, Wigglesworth K, Eppig J & Dean J 2001 Defective zonae pellucidae in Zp2-null mice disrupt folliculogenesis, fertility and development. *Development* **128** 1119–1126.
- Renn SC, Aubin-Horth N & Hofmann HA 2004 Biologically meaningful expression profiling across species using heterologous hybridization to a cDNA microarray. *BMC Genomics* **5** 42.
- Robert C, Barnes FL, Hue I & Sirard MA 2000 Subtractive hybridization used to identify mRNA associated with the maturation of bovine oocytes. *Molecular Reproduction and Development* **57** 167–175.
- Robert C, Hue I, McGraw S, Gagne D & Sirard MA 2002 Quantification of cyclin B1 and p34(cdc2) in bovine cumulus-oocyte complexes and expression mapping of genes involved in the cell cycle by complementary DNA macroarrays. *Biology of Reproduction* **67** 1456–1464.
- Rodman TC & Bachvarova R 1976 RNA synthesis in preovulatory mouse oocytes. *Journal of Cell Biology* **70** 251–257.
- Schena M, Shalon D, Davis RW & Brown PO 1995 Quantitative monitoring of gene expression patterns with a complementary DNA microarray. *Science* **270** 467–470.

- Shah G, Azizian M, Bruch D, Mehta R & Kittur D 2004 Cross-species comparison of gene expression between human and porcine tissue, using single microarray platform—preliminary results. *Clinical Transplantation* **18** 76–80.
- Sharov AA, Piao Y, Matoba R, Dudekula DB, Qian Y, VanBuren V, Falco G, Martin PR, Stagg CA, Bassey UC *et al.* 2003 Transcriptome analysis of mouse stem cells and early embryos. *PLoS Biology* **1** E74.
- Sharov AA, Dudekula DB & Ko MS 2005a A web-based tool for principal component and significance analysis of microarray data. *Bioinformatics* **21** 2548–2549.
- Sharov AA, Dudekula DB & Ko MS 2005b Genome-wide assembly and analysis of alternative transcripts in mouse. *Genome Research* **15** 748–754.
- Soyal SM, Amleh A & Dean J 2000 FIGa, a germ cell-specific transcription factor required for ovarian follicle formation. *Development* **127** 4645–4654.
- Stanton JL & Green DP 2001 A set of 840 mouse oocyte genes with well-matched human homologues. *Molecular Human Reproduction* **7** 521–543.
- Stanton JL & Green DP 2002 A set of 1542 mouse blastocyst and pre-blastocyst genes with well-matched human homologues. *Molecular Human Reproduction* **8** 149–166.
- Stanton JL, Macgregor AB & Green DP 2003 Gene expression in the mouse preimplantation embryo. *Reproduction* **125** 457–468.
- Steuerwald NM, Bermudez MG, Wells D, Munne S & Cohen J 2007 Maternal age-related differential global expression profiles observed in human oocytes. *Reproductive Biomedicine Online* **14** 700–708.
- Su YQ, Sugiura K, Woo Y, Wigglesworth K, Kamdar S, Affourtit J & Eppig JJ 2007 Selective degradation of transcripts during meiotic maturation of mouse oocytes. *Developmental Biology* **302** 104–117.
- Telford NA, Watson AJ & Schultz GA 1990 Transition from maternal to embryonic control in early mammalian development: a comparison of several species. *Molecular Reproduction and Development* **26** 90–100.
- Tong ZB, Gold L, Pfeifer KE, Dorward H, Lee E, Bondy CA, Dean J & Nelson LM 2000 Mater, a maternal effect gene required for early embryonic development in mice. *Nature Genetics* **26** 267–268.
- Vallee M, Gravel C, Palin MF, Reghenas H, Stothard P, Wishart DS & Sirard MA 2005 Identification of novel and known oocyte-specific genes using complementary DNA subtraction and microarray analysis in three different species. *Biology of Reproduction* **73** 63–71.
- Vallee M, Robert C, Methot S, Palin MF & Sirard MA 2006 Cross-species hybridizations on a multi-species cDNA microarray to identify evolutionarily conserved genes expressed in oocytes. *BMC Genomics* **7** 113.
- Velculescu VE, Zhang L, Vogelstein B & Kinzler KW 1995 Serial analysis of gene expression. *Science* **270** 484–487.
- Wang QT, Piotrowska K, Ciemerych MA, Milenkovic L, Scott MP, Davis RW & Zernicka-Goetz M 2004 A genome-wide study of gene activity reveals developmental signaling pathways in the preimplantation mouse embryo. *Developmental Cell* **6** 133–144.
- Weil MR, Macatee T & Garner HR 2002 Toward a universal standard: comparing two methods for standardizing spotted microarray data. *BioTechniques* **32** 1310–1314.
- Wu X, Viveiros MM, Eppig JJ, Bai Y, Fitzpatrick SL & Matzuk MM 2003 Zygote arrest 1 (Zar1) is a novel maternal-effect gene critical for the oocyte-to-embryo transition. *Nature Genetics* **33** 187–191.
- Yan C, Wang P, DeMayo J, DeMayo FJ, Elvin JA, Carino C, Prasad SV, Skinner SS, Dunbar BS, Dube JL *et al.* 2001 Synergistic roles of bone morphogenetic protein 15 and growth differentiation factor 9 in ovarian function. *Molecular Endocrinology* **15** 854–866.
- Yao J, Ren X, Ireland JJ, Coussens PM, Smith TP & Smith GW 2004 Generation of a bovine oocyte cDNA library and microarray: resources for identification of genes important for follicular development and early embryogenesis. *Physiological Genomics* **19** 84–92.
- Zeng F & Schultz RM 2003 Gene expression in mouse oocytes and preimplantation embryos: use of suppression subtractive hybridization to identify oocyte- and embryo-specific genes. *Biology of Reproduction* **68** 31–39.
- Zeng F, Baldwin DA & Schultz RM 2004 Transcript profiling during preimplantation mouse development. *Developmental Biology* **272** 483–496.
- Zhang J & He X 2005 Significant impact of protein dispensability on the instantaneous rate of protein evolution. *Molecular Biology and Evolution* **22** 1147–1155.

Received 30 July 2007

First decision 24 August 2007

Revised manuscript received 4 December 2007

Accepted 7 January 2008