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Identification of mRNAs that are up-regulated after fertilization in the murine zygote by suppression subtractive hybridization

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Abstract

Transcriptions occur in mouse preimplantation embryos as early as one-cell stage. However, our understanding on gene expression at this stage is lacking. The present study applied suppression subtractive hybridization (SSH) to compared gene expression profiles of mouse zygote and oocyte. Forty-four differentially expressed genes were selected and shown positive signals by reverse dot-blot hybridization. DNA sequences comparison of these putative clones with the GenBank/EMBL databases using BLAST search identified 38 clones with >90% identity to known genes and six novel clones with less than 70% homology to the databases. Eleven out of the 44 differentially expressed clones were either originally isolated from male embryo or testis-specific genes, suggesting that these genes may be derived from paternal genome. Five differentially expressed genes of interest, including bromodomain-containing protein BP75, spindlin, radixin, pituitary tumor-transforming gene (PTTG), and proteoglycan core protein (serglycin) were further studied by semi-quantitative RT-PCR. It is noted that spindlin which involves in cell division is highly expressed in zygote, suggesting that this protein may play an important role in zygotic gene activation (ZGA) and early stage development in 1-cell stage mouse embryos.
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Spermatozoon fertilizes oocyte to form a pronuclear stage zygote that contains a maternal haploid pronucleus from oocyte and a paternal haploid pronucleus from the spermatozoon. Subsequently, the two pronuceli fused together to form a single zygotic genome, which involves a series of complex processes in terms of embryonic nuclear formation, chromatin remodeling, and paternal genome modification [1]. Gradually, the maternal mRNA is degraded and replaced by zygotic transcripts. Therefore, zygotic gene activation (ZGA) is essential for early embryonic development, especially for parental genome uniting [2].

It is well known that zygote transcription is silenced in early stage of embryo development. The function of this silence is to avoid inappropriate epigenetic modification. This period of nonpermissive state for transcripts differs largely between species. It is suggested that global ZGA occurs at the 2-cell stage in mouse embryos [3], but at 4–8-cell stage in human embryos [4]. ZGA of bovine and rabbit has been reported at the 8–16 cell stages [5,6]. However, various studies have found that transcription can occur earlier than previously by conceived. In mouse, transcription was found in late 1-cell embryos, which has been demonstrated by microinjection of reporter genes into the nuclei of the 1-cell mouse embryos [7]. Other lines of evidences on early ZGA in mouse embryo include: (1) endogenous gene expression both in the presence and absence of metabolic inhibitors; (2) expression of integrated reporter genes in oocytes and embryos of transgenic mice; and (3) transplantation of nuclei from one developmental stage to another [reviews on 2, 8]. ZGA also has been detected at the 1-cell stage in other species including the cow [9] and rabbit [10]. Collectively, these data result in a hypothesis that ZGA

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is composed of two waves, a minor phase initiated in the 1-cell embryo which may last over one or several cleavage cycles depending on species and a major wave concurring with the transition from maternal to zygotic control of development in all mammalian species [2].

In the present study, we constructed mouse zygote and oocyte cDNA libraries and contrasted their mRNA expression profiles to isolate genes that are expressed in the zygotes. This comparison provides information of the transcription pattern in the first cell cycle of mouse embryo. It will enhance our understanding of the underlying molecular mechanism in which the roles played by parentally directed control of early embryonic development, especially ZGA and regulation/remodeling of nuclear processes. Characterization of zygotic mRNA expression profile may potentially benefit to optimize the culture condition to sustain the early and later mouse embryonic development.

Materials and methods

Oocyte and zygote collection. MF1 female mice were injected (i.p.) with 5 IU pregnant mare's serum gonadotrophin (PMSG; Sigma, St. Louis, MO) and with 5 IU human chorionic gonadotrophin (hCG; Sigma) 48 h later to induce ovulation as described previously [11]. Zygotes and oocytes were collected from the female mice 24 h post-hCG with and without mating, respectively. The research protocol was approved by the Committee on the Use of Live Animals in Teaching and Research, the University of Hong Kong.

Messenger RNA isolation. Polyadenylated RNA from mouse oocytes and zygotes were isolated using Dynabeads mRNA DIRECT kit (Dynal AS, Oslo, Norway) as described previously [12]. In brief, zona pellucida of mouse oocytes and zygotes were removed by acid Tyrode treatment. A known number of oocytes and zygotes (120 for suppression subtractive hybridization and 50–60 for semi-quantitative RT-PCR) was lysed and mixed with Dynabeads oligo(dT_{25}). Then, repetitive washing and elution of poly$(A)^+$ RNA was followed. Messenger RNA was eluted with 10–50 μl of diethyl pyrocarbonate (DEPC)-treated water.

Oocyte and zygote cDNA synthesis. SMART cDNA synthesis kit (Clontech Laboratories, Palo Alto, CA) was used to reverse transcribe mRNA extracted from the oocytes and zygotes according to manufacturer's instructions. Briefly, 3 μl mRNA sample was mixed with 1 μl of 10 μM SMART oligonucleotide (5′-TACGGCTGCGAGAAGA CGACAGAAGGG-3′) and 1 μl CDS/3′ PCR primer. The mixture was incubated at 72 °C for 2 min and cooled on ice for 2 min. Then, 2 μl 5× first-strand buffer, 1 μl 20mM DTT, 1 μl 10 mM dNTP mix, and 1 μl MMLV reverse transcriptase (200 U/μl) were added and incubated at 42 °C for 1 h for first-strand cDNA synthesis. Two microliters first-strand cDNA was combined with 10 μl 10× KlenTaq PCR buffer, 2 μl dNTP mix, 2 μl 5′PCR primer (5′-TACGGCTGCGAGAAGACGAC AGAA-3′), 2 μl CDS/3′ PCR primer, 2 μl 50× Advantage KlenTaq Polymerase Mix, and 80 μl deionized water. The sample was amplified by the following PCR program: 1 cycle at 95 °C for 1 min, then 26 cycles at 95 °C for 15 s and 68 °C for 5 min. The PCR products were used for the following suppression subtractive hybridization experiment.

Suppression subtractive hybridization. Suppression subtractive hybridization (SSH) was performed with the PCR-Select cDNA Subtraction Kit (Clontech Laboratories, Palo Alto, CA) as described previously [13,14] with some modifications. In brief, 43.5 μl of SMART-amplified zygote DNA (tester) and 43.5 μl of SMART-amplified oocyte DNA (driver) were used. Primary PCR condition was 94 °C for 30 s, 66 °C for 30 s, and 72 °C for 90 s for 30 cycles in a reaction volume of 50 μl. One microliter of one-tenth diluted primary PCR product was added into a new PCR tube for a second round of PCR. The secondary PCR condition was 94 °C for 30 s, 68 °C for 30 s, and 72 °C for 90 s for 12 cycles. The subtracted PCR products generated by SSH were cloned into the pGEM-T Easy vector (Promega) for screening. Two hundred clones were screened from the forward subtracted library.

Evaluation of the ligation and subtraction efficiency. To evaluate the efficiency of the ligation reaction, the relative amount of β-actin cDNA present in adaptor-ligated cDNA populations were compared by PCR using β-actin forward (5′-GTGCCCATCTACGAGGGCTATGCT-3′) and reverse (5′-TACCCAAGAAGGAAGGCTGGAAAA-3′) primers, or β-actin forward and PCR primer 1 (5′-CTAATACGACTCACTA TAGGGC-3′, which locates on the adaptor sequences). PCRs were carried out for 26 cycles at 68 °C annealing temperature. To evaluate the efficiency of the subtraction, the relative amount of β-actin cDNA present in the subtracted and unsubtracted cDNA populations after SSH was examined by PCR amplification using the β-actin forward and reverse primers.

Reverse dot-blot analysis. The DNA inserts from the selected clones were amplified by PCR. In brief, a bacterial colony of 2 mm in size was picked and re-suspended in 50 μl water. Five microliters of bacterial suspension was mixed with 20 μl 1× PCR buffer (10 mM Tris–HCl; pH 9.0, 50 mM KCl, 1.5 mM $MgCl_2$, and 0.1% Triton X-100), 200 nM primers (NP1: 5′-TCGAGCGGCCGCCCGGGCAGGT-3′; NP2R: 5′-AGCGTGGTCGCGGCCGAGGT-3′), 200 μM dNTPs, and 5 U Taq DNA polymerase (Roche Molec. Biochem., Indianapolis, IN). PCR was carried out at 94 °C for 10 min, then 30 cycles of 94 °C for 30 s, 60 °C for 30 s, and 72 °C for 60 s. The purified PCR products (50 ng) were dotted onto Hybond N^+ membranes (Amersham Pharmacia Biotech.) as described previously [13,14]. Non-subtracted cDNA probes from testers and drivers were ^{32}P-labeled using PCR-select Subtraction Hybridization Screening Kit (Clontech) in the presence of [α-^{32}P]dCTP (Dupont NEN, Boston, MA). The labeled probes were purified by spin column. The membranes were pre-hybridized with Rapid Hyb-buffer (Amersham Pharmacia Biotech.) for 2 h at 65 °C. Hybridizations were performed at 65 °C for 18 h in the Rapid Hyb-buffer containing radioactive probes (1×10^6 cpm). Membranes were washed once in 2× SSC, 0.1% SDS and once in 1× SSC, 0.1% SDS at 45 °C for 20 min each, then subjected to autoradiography (BioMax autoradiographic film, Eastman Kodak, Rochester, NY) for 12–48 h. The hybridization signals were analyzed using LabWorks analysis software (Ultra-Violet Products, Cambridge, UK).

DNA sequence analysis. DNA Sequencing was performed using an automated ABI 310 Genetic Analyzer (Perkin–Elmer Applied Biosys., Foster City, CA). Sequencing reactions were carried out with the dRhodamine Terminator Cycle Sequencing Kit (Perkin–Elmer) and T7 and SP6 primers. The sequences obtained were compared against GenBank/EMBL database using the online computer BLAST program [15].

Semi-quantitative reverse transcriptase-polymerase chain reaction. The expression of five clones of interest and mouse β-actin was evaluated and compared in zygotes and oocytes by using semi-quantitative RT-PCR as described [11]. The gene description, accession number, and primer used in RT-PCR of these clones are listed in Table 1. In brief, mRNA of mouse zygotes and oocytes were isolated using Dynabeads mRNA DIRECT Kit (Dynal) as described above. The mRNAs were subjected to semi-quantitative RT-PCR using the one-tube Access RT-PCR system (Promega). Two micoliters of mRNA was reverse transcibed and PCR amplified in 50 μl of reaction volume containing 1× avian myeloblastosis virus (AMV)/thermus flavus (Tfl) buffer, 5 U AMV reverse transcriptase, 5 U Tfl DNA polymerase, 1 mM $MgSO_4$, 200 μM dNTP, and 1 μM gene-specific primer (Table 1). The reverse transcription was carried out at 48 °C for 45 min and followed by 2 min at 94 °C to inactivate the AMV reverse transcriptase.

Table 1
Oligonucleotide primers used for reverse transcription-polymerase chain reaction

Clone	Identity	Accession No.	Primer sequence (forward and reverse)	Nucleotide	Size (bp)
ZD3	Bromodomain-containing protein BP75	AF084259	5′-GAGGAAGAAAGGCGAGAA-3′	405–422	137
			5′-TAGGGACATGACACTGGAGA-3′	541–522	
ZD26	Spindlin	U48972	5′-ACCCATTAGAAGGAAGATTG-3′	3687–3706	140
			5′-CACAGACATTTCCACCACAA-3′	3826–3807	
ZD45	Radixin	NM_009041	5′-TACAGAAGATGCAGAAGCCT-3′	3372– 3391	240
			5′-TTGTCCTACAGAGTCCAAAA-3′	3611–3592	
ZD85	Pituitary tumor-transforming 1	NM_013917	5′-CGAGTCGGCAAAGTGTTCAA-3′	438–457	266
			5′-TGCTCCCCAGGCAGGTCAAA-3′	703–684	
ZD146	Proteoglycan core protein	M33497	5′-GCTTGTGCCTGACTCTGTGC-3′	826–845	271
			5′-CTACCCATGATGAAACTCCA-3′	1096–1078	
ATC	β-Actin	X03672	5′-GTGCCCATCTACGAGGGCTATGCT-3′	567–590	319
			5′-TACCCAAGAAGGAAGGCTGGAAAA-3′	885–862	

The PCR conditions were 22–36 cycles at 94 °C for 30 s, 60 °C for 1 min, and 68 °C for 2 min. The PCR products were separated on 2.0% NuSieve 3:1 agarose (FMC Bioproducts, Rockland, MN). The images were quantified by densitometric scanning followed by analysis with ImageQuant version 3.3 (Molecular Dynamics, Sunnyvate, CA). Intensities of bands were normalized and expressed relative to the intensity of the band of β-actin. The means ± SD of three independent experiments were compared and analyzed in each clone.

Results

Suppression subtractive hybridization

The mouse oocyte and zygote (fertilized oocyte) adaptor-ligated cDNA libraries were constructed. We adopted the SMART amplification protocol to amplify picogram amount of mRNA presence in the pooled oocyte and zygote populations. With the use of the adaptor sequences, the present amplification protocol allows us to amplify the cDNA in an unbiased manner irrespective of their abundances. In the forward subtraction experiment, transcripts preferentially expressed in the zygote relative to the oocyte were selected. The subtraction efficiency was evaluated using housekeeping gene β-actin. The amount of β-actin transcripts was reduced 40-fold after subtraction (Fig. 1). After ensuring that the housekeeping gene had been extensively removed in the subtraction reactions, the subtracted cDNA was cloned into a pGEM-T easy vector for screening.

Two hundred clones of oocyte or zygote subtracted samples were selected at random and inserts were re-amplified by PCR. The cDNAs were dot-blotted on Hybond N^+ membrane and probed with reverse transcribed ^{32}P-labeled cDNA from the unsubtracted oocyte and zygote samples (Fig. 2). Positive signals were detected using zygote cDNA in 44 out of 200 re-amplified clones (22%). All the positive clones from reverse dot-blot analysis were sequenced and summarized in Table 2. DNA sequence comparisons with the GenBank/EMBL database using BLAST search identified 38

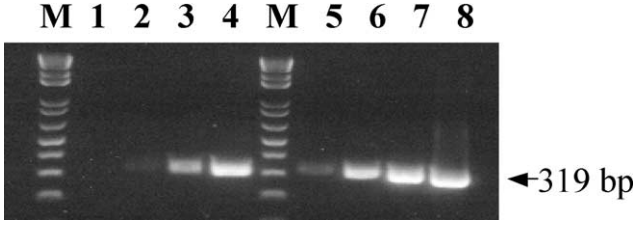

Fig. 1. Evaluation of efficiency of suppression subtractive hybridization. The forward subtracted library was subjected to PCR amplification using β-actin primer. Relative amount of β-actin products were shown (marked with an arrow) in subtracted (lanes 1–4 for 18, 23, 28, and 33 cycles, respectively) and un-subtracted (lanes 5–8 for 18, 23, 28, 33 cycles, respectively). M: 1-kb plus DNA marker (Amersham Bio-Sciences).

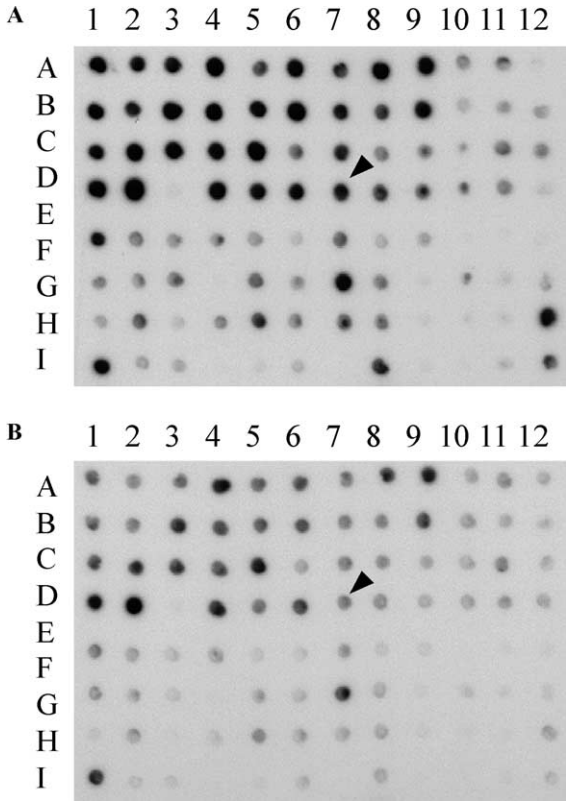

Fig. 2. Typical results of reverse dot blot analysis using oocyte and zygote cDNA. Two identical membranes were hybridized with radioactive labeled probes prepared from (A) zygote cDNA and (B) oocyte cDNA. One of the differentially expressed clones was marked with arrows.

Table 2
Summary of the subtracted clones from the zygote cDNA library

	Number
Putative positive after SSH	200
Confirmed by reverse dot-blot analysis	44
Number of clones sequenced	44
>90% homology	38
Novel	6

sequences with >90% identity to known genes and six putative novel sequences with less than 70% nucleotide sequence homology to GenBank/EMBL database (Table 2). Overall, 11 of 44 high-quality sequences (25%, Table 3) were homologous to five different genes described previously. Four of them were found in duplicates on reverse dot-blot analysis. They were DNA J protein (BC003420), *Mus musculus* 10, 11 days embryo cDNA (AK012939), spindlin (U48972), and *M. musculus* adult male testis cDNA (AK015399). Moreover, pituitary tumor-transforming gene (NM013917) was repeated thrice in the screening.

Confirmation of SSH by semi-quantitative RT-PCR

To confirm the results of SSH, five different clones that gave strong positive signals on reverse dot-blot analysis were selected for semi-quantitative RT-PCR. The primer sequences used in RT-PCR were summarized in Table 1. Fig. 3 shows results of RT-PCR. The signals

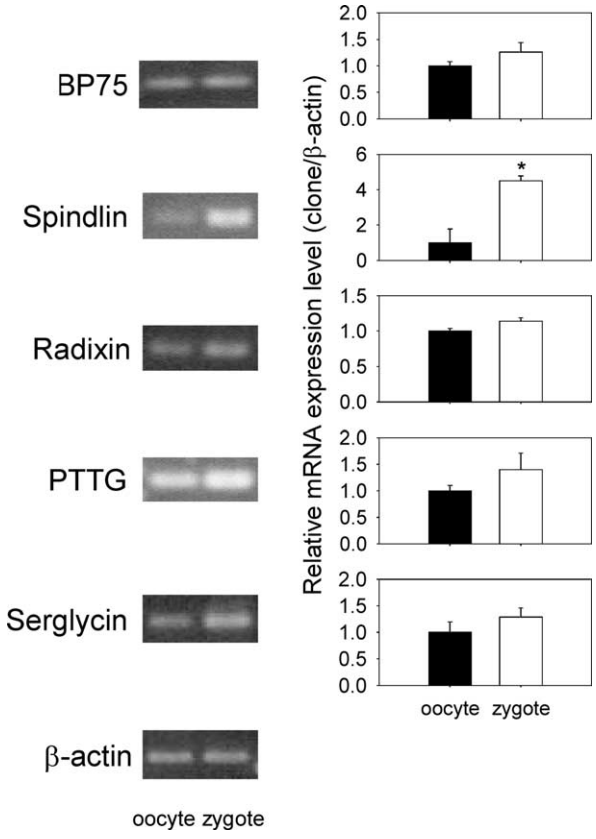

Fig. 3. Comparison of seven differentially expressed seven genes between oocyte (O) and zygote (Z) using semi-quantitative PCR. Relative mRNA expression level was presented by dividing the intensity of gene with β-actin. Values are means ± SD of three independent experiments. $^{*}p < 0.05$ in comparison with oocyte.

Table 3
Identities of the subtracted zygote clones

Clone	Repetition	Accession No.	Identities	Homology	Size (bp)
ZD85	3	NM_013917	Pituitary tumor-transforming 1	4.00E – 88	550
ZD26	2	U48972	Spindlin	1.00E – 47	400
ZD173	2	AK015399	Adult male testis cDNA	1.00E – 95	650
ZD25	2	AK012939	10, 11 days embryo cDNA	2.00E – 75	500
ZD8	2	BC003420	DNA J protein	2.00E – 47	500
Others	1				

were normalized with housekeeping gene β-actin for comparisons (Fig. 3). It was found that the mRNA levels of bromodomain-containing protein BP75 (BP75), radixin, pituitary tumor-transforming gene (PTTG), and proteoglycan core protein (serglycin) in zygote were 26%, 14%, 39%, and 29% higher than in oocyte, respectively. Interestingly, the expression of spindlin in zygote was 4.5-fold higher ($p < 0.05$) than that in the oocyte.

Discussion

Fertilization occurs when a spermatozoon penetrates into an oocyte. The fertilized oocyte, zygote (one-cell embryo) starts a series of complex morphological changes, including several early cleavage divisions, the activation of zygotic transcription, blastomere compact, formation of blastocyst cavity, differentiation of inner cell mass, and trophectoderm, before implantation onto the uterus [1]. In mouse embryos, the first cell cycle is about 18–20 h long [16]. The S phase of the first cycle lasts 4–8 h and G2 + M phases from 3 to 8 h, while G1 phase takes 1–2 h [1]. Various studies found that the zygote is transcriptionally active [2,8] and its gene activation is initiated at S phase of the first cell cycles. The first event of ZGA is a transition of zygotic cytoplasm from a transcriptionally nonpermissive state to a permissive state [17]. Zygotic chromatin undertakes remodeling to allow active transcription of genes essential for post-fertilization embryonic development [2,8]. In the present study, we demonstrated that some genes were highly expressed in mouse zygote when compared to oocyte, suggesting that (1) spermatozoa may carry transcripts essential for first embryonic division to the oocyte during fertilization and (2) paternal genome was actively transcribed after fertilization and before the first cell division in the mouse embryo.

SSH is one of the most common methods used to contrast and isolate genes that are differentially expressed in two samples populations [13,14]. Applying this method, we constructed a subtracted zygote cDNA library by the oocyte driver cDNA. Forty-four differentially expressed genes were confirmed by reverse dot blot analysis and sequenced. It is believed that the majority of maternal mRNA was degraded in 2-cell stage embryo [2]. Therefore, the cDNA subtraction library of zygote by oocyte should reflect the transcripts produced by the zygote after fertilization. These results provide further evidence that zygote in first cell cycle is transcriptional activity.

There were a number of testis-specific genes isolated from the subtracted zygote cDNAs, suggesting that these genes were from paternal origin. These paternal derived genes account for about 25% (11/44) of our total differentially expressed genes by SSH. They include mouse DNA J protein (BC003420), pituitary tumor-transforming gene (NM013917), and adult male testis cDNA (AK015399). A number of studies showed that early transcription is preferentially triggered by paternal pronucleus. Ferreira and Carmo-Fonseca [18] found that paternal and maternal pronuclei replicated asynchronously. The dynamics of this embryonic genome replication correlate with gross chromatin structure transition in mouse early embryonic development. It has been reported that the incorporation of Br-UTP by the male pronucleus was four to five times greater than that of the female pronucleus [19]. Besides, addition of histone deacetylase inhibitor, sodium butyrate, stimulates promoter activities in maternal pronuclei, but not in paternal pronuclei [20]. Various paternal transcripts for the Y-linked genes were detected in 1-cell stage embryos, including SRY and ZFY [21,22]. Therefore, it is hypothesized that the paternal pronucleus is in a transcriptionally permissive state, while the maternal pronucleus is in a nonpermissive state in 1-cell stage embryo. Cloning of paternal derived genes in this study may provide further evidences that transcription starts in the paternal pronucleus.

Pituitary tumor-transforming gene (PTTG) is one of the paternal derived genes that were found thrice in this study. PTTG and four other genes (Table 1) that were highly expressed in zygote by reverse dot-blot analysis were selected for confirmation using semi-quantitative RT-PCR. PTTG is a recently cloned oncogene and is detected in a variety of tumor cell lines. It is highly expressed in testis but low in thymus and placenta [23–25]. Various studies suggested that PTTG is an important transcription activator [24,26,27]. PTTG transcription activity could be stimulated by the epidermal growth factor induced activation of the mitogen-activated protein (MAP) kinase cascade [28]. Our present study detected a 39% increase in PTTG transcript in zygote than in oocyte, suggesting that PTTG may play a role in ZGA. Interestingly, two Sp1 binding sites were found between −462 and −745 bp of the PTTG promoter using DNase I footprinting assay [29]. Sp1 is a ubiquitously expressed transactivator that binds to GC box sequence and regulates basal transcription of many housekeeping genes [30,31]. Sp1 protein was detected in the 1-cell stage mouse embryos and its concentration was greater in the male pronucleus than that in the female pronucleus [32]. In addition, it was reported that PTTG could interact with DNA J protein, a member of heat shock protein family [33]. DNA J protein was suggested to promote translation initiation and correct protein folding [33]. In our present study, two DNA J protein genes were also cloned in the subtracted library. The presence of mRNA of both PTTG and DNA J protein in the zygote implicates that PTTG may play some role in protein synthesis via DNA J protein.

Apart from paternal gene transcription, various maternally transcribed genes were detected in 1-cell stage

mouse embryo [34]. Spindlin is one of the most abounding transcripts recently isolated from mouse oocyte and preimplantation embryo cDNA library [34,35]. Spindlin is maternally transcribed with a stage-specific expression pattern and detected in the unfertilized oocyte, 1-cell and 2-cell stage mouse embryo, but not 8-cell stage embryo [34,35]. It exhibits high homology to a multicopy gene, Y-linked spermiogenesis-specific transcript (Ssty). Oh and coworkers [35] found that spindlin was associated with the meiotic spindle and was phosphorylated in a cell-cycle-dependent fashion. These studies suggested that spindlin might play a role in cell-cycle regulation during the transition from gamete to embryo. In the present study, spindlin was detected both in oocyte and zygote with higher expression in zygote than in oocyte. In line with previous studies [34,35], our findings suggest that transcription of spindlin occurs after fertilization and during early stage of embryonic development.

Bromodomain is originally described as highly conserved 88 amino acid domain in human, Drosophila, and yeast proteins [36,37]. In the present study, bromodomain-containing protein BP75 was highly expressed in zygote. In fact, bromodomain-containing protein is expressed in many tissues including lung, stomach, thymus, kidney, and testis [38]. Using fluorescent double-labeling technique, Cuppen [38] demonstrated that BP75 localizes predominantly in nucleus. It has been suggested that bromodomains mediate protein–protein interactions in the nucleus, influencing the assembly and activity of multiprotein complexes at specific chromosomal sites. Thus, BP75 may have a function that regulates chromatin remodeling and transcriptional activation [38]. It is known that both chromatin remodeling and gene activation are important events during transition from oocyte to embryo. Therefore, it is strongly suggested that BP75 may play an important role in the process of ZGA.

In yeast two-hybrid screening, BP75 was found to interact with mouse protein tyrosine phosphatase-BAS-like (PTP-BL), while PTP-BL harbors an ezrin/radixin/moesin (ERM) domain [39]. ERM is a protein family that consists of three closely related proteins, ezrin, radixin, and moesin [40]. Various studies demonstrated that ERM proteins are involved in the formation and/or maintenance of cortical actin filaments and plasma membranes [41]. It is interesting that a clone coding for radixin was also isolated from the zygote subtracted library. After fertilization, sperm nuclear envelope break down and a new pronucleus membrane forms. Possible role of the co-existence and interaction of BP75 with ERM proteins in zygote warrant further investigation.

Serglycin is a Ser–gly-repeat-containing protein belonging to a family of proteoglycan core proteins [42]. The function of serglycin is still unknown, but it is likely that it may involve in packaging of proteins into secretory granules and/or directing the secretion of such molecules as cytokines or chymases [43]. Ho and coworkers detected serglycin mRNA in mouse E11.5–E14.5 embryos using in situ hybridization [44]. They suggested that serglycin has an important role in post-implantation development. In the present study, we detected mRNA of proteoglycan core protein gene encoding serglycin both in oocyte and zygote, with zygote having higher expression level. However, the role of serglycin in early embryo development remains unknown.

Our study represents the first use of the SSH technique to contrast the transcripts in the first cycle of preimplantation mouse embryos development. The finding of paternal derived genes coding for transcription factors associates with transcription activity and cell cycle regulation in one-cell embryos strongly suggests that paternal transcripts may play important roles in ZGA in early stage embryo.

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