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Identification of Differentially Expressed Genes in the Mouse Blastocyst: Comparison with Embryonic Stem Cells

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생쥐의 배반포에서 특이적으로 발현되는 유전자 확인: 배아 줄기세포와 비교

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ABSTRACT : Identification of differentially expressed genes at blastocyst stage embryos would provide insights into early development and differentiation. Here, we applied a new differential display reverse transcription polymerase chain reaction(DD RT-PCR) technology, called annealing control primers(ACP) system to identify the genes that are specifically or prominently expressed in mouse blastocysts compared to embryonic stem(ES) cells. Using 100 ACPs, 26 clones were perceived as differentially expressed genes in mouse blastocysts. A BLAST search revealed that cloned genes had significant sequence similarities with known genes in the GenBank/EMBL data base. Among them, 15 genes were selected and conformed by RT-PCR. This analysis suggests that the ACP system is a practical method for the identification of stage-specific genes using small numbers of mouse embryos.

Key words : Embryo, Gene expression, Blastocyst, Embryonic stem cells.

요 약 : 배반포 단계의 난자에서 차이 나게 발현하는 유전자의 발굴을 통해 초기 동물 발생과 분화에 관한 기전을 알 수 있다. 본 연구에서는 새로운 차별발현 역전사효소중합법, 이름하여 에닐링 콘트롤 프라이머(ACP) 방법에 의해 생쥐 배반포에서 차이 나게 발현하는 유전자를 줄기세포와 비교하여 발굴하였다. 총 100개의 ACP를 사용하여 26개의 유전자 단편을 확인하였고, BLAST 탐색에 의해 유전자 정보 은행(GeneBank/EMBL)에 저장된 유전자와 동일하다는 것을 알았다. 이들 유전자 중에 15개의 유전자를 선별하여 역전사효소중합법에 의해 조사한 결과, 배반포에서 차이 나게 발현함을 재확인하였다. 이러한 결과는 ACP 방법이 특정 발달 단계에 있는 소량의 배아 샘플로부터 전사되는 유전자를 확인하는데 실용적으로 사용될 수 있다는 것을 보여주고 있다.

INTRODUCTION

In mammals, blastocyst stage is marked by the segregation of two distinct cell lineages in the blastocyst: the inner cell mass (ICM), which gives rise to the embryo proper and the trophectoderm(TE), which contributes exclusively to the trophoblast portion of the placenta. Thousands of genes seem to be expressed at blastocyst stage, which involved in the cell proliferation, differentiation and implantation. However, to date only a few genes have been characterized with respect to physiological functions(Niemann *et al.*, 2000). Therefore to further understand the molecular basis of early development, it would be of interested to identify differentially expressed genes(DEGs) at specific stage during development and to characterize them in mammalian embryogenesis.

Embryonic stem(ES) cells are derived from the ICM(Evans & Kaufman, 1981; Martin, 1981) and ES cells are pluripotent and can differentiate into all embryonic cell lineages and some extra-embryonic ones. Multiple transcription factors, such as Oct3/4, Stat 3 or Nanog appeared to maintain self renewal and pluripotency in the embryonic stem cells. Regulating gene expression in stem cell line by various transcription factors maintain embryonic

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stem cell lines un differentiation state.

Identification differentially expressed gene in blastocysts comparing with ES cell would provide insights into cell differentiation processes in early mammalian development. However, it is difficult to identify such DEGs in a cell that functions during early embryo development, particularly within preimplantation embryos, because such genes are only expressed at low levels and their transcripts are hard to detect within the cell. To detect DEGs that are transcribed at low levels, highly specific polymerase chain reaction(PCR) amplification is required. Recently, we reported the identification of DEGs in mouse and bovine embryos using a new differential display reverse transcription-polymerase chain reaction(RT PCR) method called the annealing control primer(ACP) system(Hwang *et al.*, 2003; Kim *et al.*, 2004; Hwang *et al.*, 2005; Cui *et al.*, 2005). This is an easy technique without false positives that only allows real products to be amplified.

In the present study we first characterize mouse embryonic stem cells. We used it to analyze blastocyst-specific mRNA expression patterns and to identify differentially expressed transcripts and we also conformed 15 genes by RT-PCR.

MATERIALS AND METHODS

1. Generation of Preimplantation Embryos

To obtain fertilized embryos, 5-week-old C57BL/6 female mice were induced to superovulate by intraperitoneal injections of pregnant mare serum gonadotropin(PMSG, 5 IU) and human chorionic gonadotrophin(hCG, 5 IU) 48h apart. After 3.5 days of hCG injection, blastocysts were harvested from uterus by flushing and washed in Ca^{2+}- and Mg^{2+}-free PBS, snap frozen in liquid nitrogen and stored at −70℃ until use.

2. Establishment of Mouse Embryonic Stem(mES) Cells

Mouse ES cell lines were generated using an adaptation of the protocol(Hogan *et al.*). Early blastocysts(3.5 days postcoitum) were flushed from the uterus of mice(C57BL/6) with Dulbecco's Modified Eagle Medium(DMEM, Gibco BRL) containg 10% fetal bovine serum(FBS, Gibco BRL). The zona pellucida of embryos were digested with 0.5% pronase(Sigma, MO, USA) and transferred onto the feeder cells, which was mitotically inactivated STO(ATCC, USA) cells, in 4-well plates. The culture medium consisted of 15% ES cell qualified FBS(Gibco BRL), 0.1mM MEM nonessential amino acids, 0.1mM β-mercaptoethanol, 1mM glutamine and 1000U/mL leukemia inhibitory factor(LIF, Sigma). After 3~4 days, clumps of cells derived from the inner cell mass were mechanically isolated and replated on the feeder cells. Morphologically ES cell-like colonies were recovered and disaggregated with 0.25% trypsin-EDTA treatment for 5min. The disaggregated cells were washed three times with PBS and reseeded on the feeder cells(passage 1). After 20 passages of subculture, a part of ES cells were frozen with 20% FBS and 10% DMSO, and transferred to liquid nitrogen. In this study, we used the 25 passages of ES cells from thawed and survived ES cells.

3. Establishment of Mouse Embryody Body(EB)

Mouse EB cells were established form mouse ES cells. The maintained mES cells were dissociated with 0.25% trypsin/0.04% EDTA in PBS and plated onto feeder-free culture dish with ES medium for 6~7 days. The cultured and attached mES cells were treated with 0.25% trypsin/0.04% EDTA and plated onto bacterial culture dish in DMEM containing 15% FCS without LIF.

4. Annealing Control Primer Reverse Transcription-Polymerase Chain Reaction(ACP RT-PCR) Analysis

Blastocysts and ES cells colonies were resuspended in 300 μL lysis/binding buffer for 5min and the polyadenylated RNA was prepared by adding 50 μL washed Dynabeads from the Dynabeads mRNA Direct Kit(Dynal Asa, Oslo, Norway). The mRNAs were then subjected to ACP RT-PCR analysis. The mRNA amount of glyceraldehyde-3-phosphate dehydrogenase(GAPDH) was performed to determine the optimal level of blastocyst and ES cell. This experiment revealed that the gel intensities were similar when the mRNA of one blastocyst and diluted ES cell were used. The ACP RT-PCR method is depicted schematically in Fig. 1. The first step is first-strand cDNA synthesis, which is performed using the dT-ACP1 primer(GeneFishing™ DEG kits, Seegene, Korea). The 3'-end core portion of this primer bears a hybridizing sequence that is complementary to the poly A region of mRNA transcripts. Thus, the purified mRNA was incubated with 1 μL of dT-ACP1(10mM) at 80°C for 3min after which the RT reaction was performed in 50mM Tris-HCl(pH 8.3), 75mM KCl, 6mM $MgCl_2$, 2mM DTT, 1mM of each dNTP, 20 U of

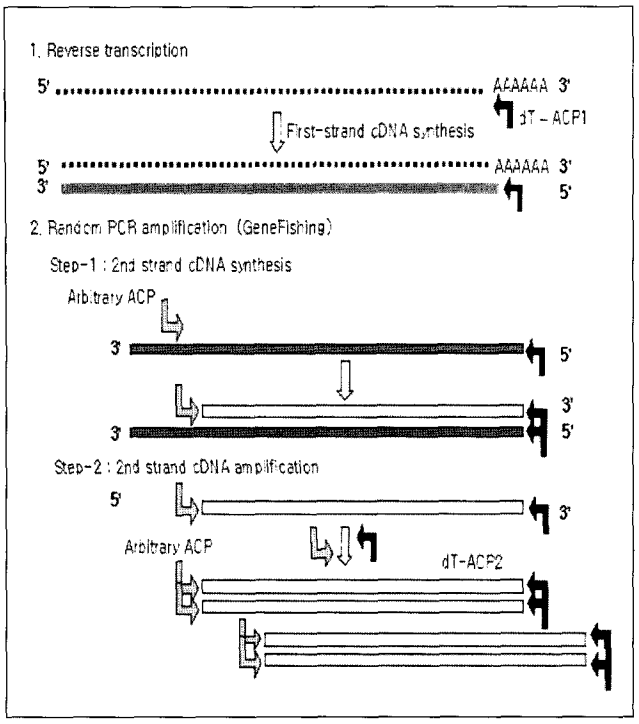

Fig. 1. Schematic illustration of methods using the annealing control primer(ACP) system. Messenger RNA(mRNA) extracted from murine blastocyst(BL) and embryonic stem(ES) cell using oligo(dT) attached magnetic beads(Dynabeads mRNA purification kit) were used for the synthesis of first-strand cDNAs using dT-ACP1. By using a combination of dT-ACP2 (reverse primer) and 100 arbitrary ACPs(forward primer), second-strand cDNAs are then amplified during second-stage PCR, and separated for differentially expressed genes(DEGs) on agarose gels.

RNase inhibitor, and 200 U of Superscript II(Invitrogen Co., Grand Island, NY). The reaction mixture was incubated at 42°C for 90min and then at 94°C for 2min.

The cDNAs were then subjected to second-strand cDNA synthesis by random PCR amplification using dT-ACP2 and one of 100 arbitrary ACPs(DEG101~105, GeneFishing™ DEG kits, Seegene, Korea) as primers. The amplification is conducted so the 3'-end core portion of the dT-ACP2 primer is prevented from annealing to the first-strand cDNAs and only the 3'-end core portion of the arbitrary ACP that bears a hybridizing sequence that is sufficiently complementary to a region on the first-strand cDNA can anneal. Thus, the PCRs were performed in 20mM Tris-HCl(pH 8.4), 50mM KCl, 1.5mM $MgCl_2$, 0.2mM of each dNTP, 10pM of each primer and 2.5 U of Taq DNA polymerase(Promega, Madison, WI) in a final volume of 25 μL. After incubation at 94°C for 3min, 50°C for 3min, and 72°C for 1min, 40 cycles of 94°C for 40sec, 65°C for 40sec, and 72°C for 40sec followed, after which a post-extension was performed at 72°C for 5min. The PCR products were then subjected to electrophoresis on 1.2% agarose gel and stained with 1 μg/mL ethidium bromide.

5. Cloning and Analysis of Subtracted cDNA

After the RT-PCR subtraction procedure described above, the amplified products were cloned into the pCR® 2.1-TOPO vector of the TA cloning kit(Invitrogen Co., Grand Island, NY) and used to transform competent TOP10 *Escherichia coli* cells. The colonies were grown for 16~18h at 37°C on Luria broth agar plates containing ampicillin, X-gal(5-bromo 4-chloro 3-indoyl-b-D-galactopyranoside) and isopropyl-b-D-thiogalactopyranoside for blue/white colony selection. The plasmids were extracted and the inserts were subjected to dideoxy chain termination sequencing (Applied Biosystems, Model 373A Automated Sequencer, USA). The identity of each product was confirmed by sequence homology analysis using the Basic Local Alignment Search Tool (BLAST).

6. Reverse Transcription-Polymerase Chain Reaction RT-PCR

Messenger RNA was prepared from blatocyst stage preimplantation embryos and ES cell using the Dynabeads mRNA Direct Kit(Dynal Asa, Oslo, Norway) according to the manufacturer's instructions. Standard cDNA synthesis by reverse transcription of the RNA was then performed using the $Oligo(dT)_{12\sim18}$ primer and the Superscript reverse transcriptase enzyme(Invitrogen Co., Grand Island, NY). The mRNAs of 15 genes were then detected by RT-PCR with specific primer pairs(Table 1) using reagents supplied with a Taq DNA polymerase kit(Takara Korea Biomedical Inc., Seoul, Korea). The PCR products that were amplified 35 cycles were visualized under ultraviolet light on 1.5% agarose (Invitrogen Co., Grand Island, NY) gels in 1 X TAE buffer containing 1 μg/mL ethidium bromide(Sigma).

RESULTS

1. DEGs between Mouse Blastocysts and ES Cells

Table 1. Primers used in RT-PCR

Genes	GenBank Aceesion No.	Primer sequence	Annealing temperature	Base pairs
GAPDH	BC083065	F: 5'- aac gac ccc ttc att gac -3' R: 5'- ttc acg aca tac tca gca c -3'	50℃	191
Atp6ap2	NM_027439	F: 5'- tca aga aaa ctc cct tct ca-3' R: 5'- tag aag cat ccc tga act gt-3'	55℃	236
Dbc2	AF420002	F: 5'- acc ctg aac aac acc acc at -3' R: 5'- cgt aag atc ccg tca ctg gt -3'	63℃	151
Kdelr3	BC024420	F: 5'- tac agt tac acg ccg atc ga -3' R: 5'- cca gtt ggc cag ata gag ga -3'	63℃	168
Ddb1	NM_015735	F: 5'- gtc aaa gag gtg gga atg ta-3' R: 5'- atc ctg gta gac aaa gca aa-3'	59℃	399
Cdc42	U76960	F: 5'- tac ttt taa cca aca tgg gg -3' R: 5'-ggg gaa gga gat tac cta aa-3'	55℃	205
Gm2a	NM_010299	F: 5'- tgg tcc ctg gag atg tag tc-3' R: 5'-agg gta gag aac cgt agg ag-3'	59℃	522
Dpp3	BC004600	F: 5'- tga aga aga gta cca ggc at-3' R: 5'- gca ctg agg ttc tga gag tc-3'	59℃	330
Whsc2	AK088384	F: 5'- aac aag aat gcc ctg acc ac -3' R: 5'- ctt cgg aat gcc ttt gag ag -3'	63℃	225
Uqcr	AK003881	F: 5'- gcg atc tac gag cac atc aa -3' R: 5'- cat agc ttg ccc aca cac tg -3'	60℃	197
Arpc51	AK008255	F: 5'- ctt tgc gga act cac caa tc -3' R: 5'- cag cta agg cct ttt cat gc -3'	56℃	231
Sap18	NM_009119	F: 5'- ggg tct tca cca cca aca ac-3' R: 5'- agt cat cag tgc cct tcc tg-3'	60℃	264
Set	BC018255	F: 5'- cac gaa gag cca gag agc tt -3' R: 5'- tct tcg tcg tcg tca tca tc -3'	56℃	176
Dppa1	BC052149	F: 5'- aca ctc acc ttc ttg gac ac-3' R: 5'- aga acc act tct tgc tgg ta-3'	60℃	385
Rent1	BC052149	F: 5'- aca ctc acc ttc ttg gac ac-3' R: 5'- aga acc act tct tgc tgg ta-3'	60℃	385
Esd	NM_016903	F: 5'- gtg gtc att gcc cct gat ac -3' R: 5'- agc tcc tcg gtg acg taaga -3'	56℃	155

After 40 PCR cycles using the cDNA synthesis GeneFishing DEG kits, agarose gel electrophoresis revealed cDNA bands ranging in size from 120 bp to 1.4kb for both blastocyst and embryonic stem cell. Using a combination of 100 arbitrary(DEG 101~105 kit, Seegene) primers and two anchored oligo(dT) primers(dT-ACP 1 and dT-ACP2), a total of 26 clones was selected after ACP-based DD-RT-PCR, and we obtained partial sequence information following sequencing. Example of the identification of three blastocyst DEGs(ATPase, H+ transporting, lysosomal accessory protein 2 [Atp6ap2], Cell division cycle 42 [Cdc 42] and ganglioside activator protein [Gm2a]) by this analysis are shown in Fig. 2. The partial sequences obtained from all clones were compared with known sequences in the GenBank(National Center for Biotechnology Information, Bethesda, MD) database.

Among the 26 DEGs, alpha-tropomyosin and E46 protein shared similarity(>20%) with sequences from other species(rat or human) whereas others showed significant similarity(>93%) with the coding regions of known mouse genes. Their categories, putative identity, nucleotide homologies with other known sequences, and insert size are shown in Table 2.

Table 2. Identity, size, and percentage homology of mouse specific cDNA clones compared to known sequences in GenBank

Categories[a]	Identity	GenBank	Base pairs	Homology[b]
Metabolism				
Nucleoside, nucleotide and nucleic acid	SET translocation(Set)	XM-135011	1200	98%(821/837)
	Regulator of nonsense transcripts 1(Rentl)	BC052149	250	100%(179/179)
	Metallothionein 2(Mt2)	NM-008630	500	100%(379/379)
	Damage specific DNA binding protein 1(Ddb1)	NM_015735	1100	97%(840/861)
	Sin3-associated polypeptide 18(Sap18)	AK085992	650	99%(547/548)
Protein	Dipeptidylpeptidase 3(Dpp3)	BC004600	120	100%(82/82)
	Ribosomal protein L40	XM-126432	500	99%(409/411)
	Eukaryotic translation initiation factor 2B, subunit 1(Eif2b1)	BC003426	1200	99%(610/612)
	Ribosomal protein S3a	BC039659	1000	97%(744/760)
	Cell division cycle 42(CDC42)	U76960	500	100%(188/188)
Lipid, fatty acid and steroid	GM2 ganglioside activator protein(Gm2a)	NM_010299	850	95%(650/679)
Nitrogen	HESB-like protein homolog(Hbld2)	AK041946	1000	99%(658/662)
Carbohydrate	Alpha-tropomyosin 5a/ b	M34134	450	Rat.97%(344/352); Hum.92%(273/295)
Signal transduction	DBC2 protein(Dbc2)	AF420002	1200	99%(533/534)
	Developmental pluripotency associated 1(Dppa1)	NM-178247	300	99%(245/246)
Protein traffic	KDEL (Lys-Asp-Glu-Leu) endoplasmic reticulum protein retention receptor 3(Kdelr3)	BC024420	1300	98%(764/778)
Electron transport	Ubiquinol-cytochrome-c reductase(Uqcr)	AK003881	440	99%(384/385)
Structure and motility	Actin-related protein 2/3 complex subunit 5(Arpc5l)	AK008255	200	100%(144/144)
Hydrolase	Esterase D/formylglutathione hydrolase(Esd)	NM_016903	1200	97%(824/841)
Unclassification	Wolf-Hirschhorn syndrome candidate 2 protein homolog(Whsc2)	AK088384	1300	99%(789/793)
	ATPase, H+ transporting, lysosomal accessory protein 2(Atp6ap2)	NM-027439	1400	97%(372/383)
	GK003	AK011852	1200	98%(713/725)
	Unknown EST	AK041989	900	99%(378/380)
	Hypothetical RNI-like structure containing protein(D5Ertd577e)	AK087741	1300	97%(715/732)
	PP238	AK008304	1300	96%(509/527)
	E46 protein	AF119662	1200	Hum.82%(578/699)

[a] Categories are based on PANTHER(https://panther.appliedbiosystems.com) classification system.

[b] Percentage are based on BLAST searches of the GenBank database. The numbers in parentheses show the number of bases(query/subject) that were compared.

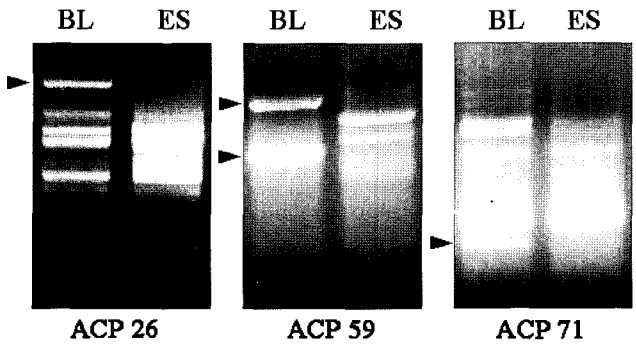

Fig. 2. Representative band patterns of murine blastocyst(BL, the left side of each gel) and embryonic stem(ES, the right side of each gel) cell following ACP-PCR. The arrows indicate the DEGs that show specifically expressed in the BL embryos, The left blot shows the identification of Atp6ap2 using the ACP 26 primer, while the middle blot shows the identification of Dbc2(up) and Gm2a(down) using the ACP 59 primer and the right bolt shows Dppa1 by ACP 71 primer.

2. RT-PCR Confirmation of DEGs

Selected 15 genes identified by ACP-PCR were used for RT-PCR to validate their differential expression. To confirm the presence of the blastocysts specific genes, PCR was performed using the blastocyst subtracted and embryonic stem cell subtracted cDNA with 15 genes [ATPase, H+ transporting, lysosomal accessory protein 2(Atp6ap2), DBC2 protein(Dbc2), KDEL (Lys-Asp-Glu-Leu) endoplasmic reticulum protein retention receptor 3(Kdelr3), damage specific DNA binding protein 1(Ddb1), cell division cycle 42(Cdc42), GM2 ganglioside activator protein (Gm2a), dipeptidylpeptidase 3(Dpp3), Wolf-Hirschhorn syndrome candidate 2 protein homolog(Whsc2), Ubiquinol-cytochrome-c reductase 7k protein(Uqcr), Actin-related protein 2/3 complex subunit 5(Arpc5l), Sin3-associated polypeptide 18(Sap18), SET translocation(Set), developmental pluripotency associated 1(Dppa1), regulator of nonsense transcripts 1(Rent1) and Esterase D(Esd)] specific primers(Table 1). PCR(35cycles) was performed as follows: 94℃ for 30sec, 55℃/60℃ for 30sec, and 72℃ for 30sec. The RT-PCR assays revealed that in agreement with the ACP differential display. All transcripts had similar expression patterns when DEGs from blastocyst were compared with ES cell(Fig. 3).

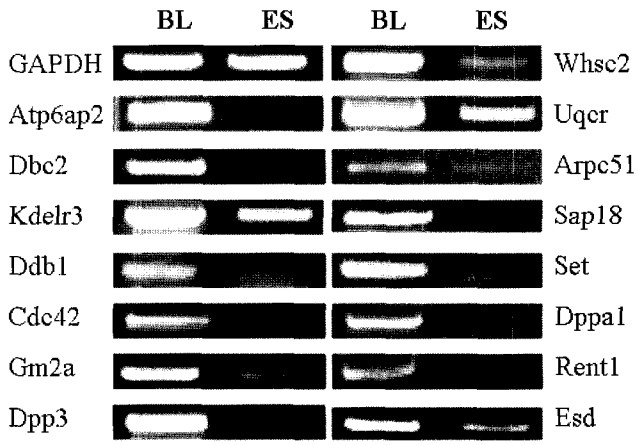

Fig. 3. Confirmation of the mRNA expression patterns of 15 genes that were identified by ACP- RT-PCR as being representative DEGs in blastocyst(BL) and embryonic stem(ES) cell by RT-PCR. Amplified DNA products were separated on a 1.5% standard agarose gel, and stained with ethidium bromide. The mouse GAPDH was used as a control to confirm the integrity of the mRNA expression.

DISCUSSION

One key developmental differentiation during preimplantation development is blastulation, which occurs after compaction and trophoblasts and inner cell mass are accompanied by changes in cell structure and changes in developmental fate. The ES cells has pluripotency and maintains undifferentiated state. The information on DEGs between blastocysts and ES cells might be important to understand mechanism of early differentiation of embryos.

We applied the ACP methods to identify the DEGs. It was efficacious and 26 DEGs were identified by cloning and sequencing. BLAST search revealed that cloned genes or ESTs had significant sequence similarities with known genes of other species in the GenBank/EMBL data base. These genes are categorized according their possible functions in the previous research results into metabolism, signal transduction, protein traffic, electron transport, structure and motility, hydrolase and unclassification(Table 2).

Protein synthesis is critical, proteolysis, on the other hand, is equally vital to the upkeep of appropriate levels of short-lived and regulatory proteins that are mostly involved in basic cellular processes such as regulation of cell cycle and cell division, cell differentiation, metabolism, stress response, modulation of cell-surface receptors and ion channels, transcription, and signaling factors. Recently we observed in mouse(Cui *et al.*, 2005) and porcine(Hwang *et al.*, 2005) blastocyst, many protein synthesis related gene expression were observed in the blastocyst stage

comparing 2-cell stage(Hwang *et al.*, 2005). We found that of the DEGs in blastocysts comparing ES cells express ribosomal proteins. Similarly, in bovine blastocysts, several different types of ribosomal RNA have been observed(Mohan *et al.*, 2002). It is known that the transcription of the rRNA genes is first detectable late in the third cell cycle in pigs and cattle(Viuff *et al.*, 1998; Viuff *et al.*, 2002). Although it is not clear what role ribosomal RNA plays in specific protein translation, the expression of various types of rRNA early in preimplantation development suggests that they may play critical roles in specific protein synthesis for early-development.

Eukaryotic translation initiaton factor 2B is a heteromeric guanine nucleotide exchange factor that plays an important role in regulating mRNA translation(Wang *et al.*, 2001). eIF2B converts eIF2□GDP to eIF2□GTP, which induces the formation of ternary complex of eIF2□GTP□Met-tRNAi(Hellen & Sarnow, 2001; Hennebusch, 2000; Hershey & Merrick, 2000; Pestova *et al.*, 2001). This in turn promotes binding of Met-tRNAi to 40S ribosomal subunits. This process is an essential step for initiation and it controls total protein synthesis(Inamura *et al.*, 2003).

Cdc 42, a member of Rho family of small guanosine triphosphatase(GTPase) proteins, is highly conserved, and regulates multiple cell functions including motility, proliferation, apoptosis and cell morphology. In order to fulfill these diverse roles, the timing and location of CDC42 activation must be controlled by effector proteins. Cdc42 is important for maintaining cell polarity and spindle orientation in C. elegances. Many Cdc42 dependant processes are mediated through down stream effector containing a conserved CRIP(Cdc42/rac interactive binding) proteins. The Wiskoff-Aldrich syndrome protein(WASP) is a CRIB motif-containing Cdc42 effector and critical component pathways that link extracellular signals to the actin cytoskeleton. The actin-related protein(Arp) 2/3 complex has been implicated in the control of actin polymerization in cells(Welch *et al.*, 1997). Taken together identification of both CDC42 and actin-related protein(Arp) 2/3 at the blastocyst stage comparing to the ES cells suggest that the complex promotes seems to be important to maintain cell structure and polarity at the time of blastocyst formation.

Hamaguchi *et al.*(2002) found that RHOBTB2, which they called DBC2, lies at the epicenter of deletions in chromosome 8p21 associated with breast cancer. It was homozygously deleted in 3.5% of breast tumors. Expression of DBC2 in breast cancer cells lacking DBC2 transcripts caused growth inhibition. By yeast 2-hybrid analysis and coimmunoprecipitation of endogenous proteins from HeLa and HEK293 cells, Wilkins *et al.*(2004) found that RHOBTB2 bound to the ubiquitin ligase scaffold protein CUL3. RHOBTB2 did not bind to any other cullin family members, and the interaction required the first BTB domain of RHOBTB2. RHOBTB2 carrying a tyr284-to-asp (Y284D) mutation in the first BTB domain, which was found in a lung cancer cell line, was unable to bind CUL3 and avoided degradation by the ubiquitin/proteasome system, resulting in increased RHOBTB2 protein levels *in vivo*.

Carlson *et al.*(1998) studied the role of SET in the regulation of renal cell proliferation and tumorigenesis. The mRNA encoding SET was expressed at much higher levels in transformed human and rodent cell lines than in cultured renal epithelial and primary endothelial cells. Previous findings during renal development were extended by demonstrating that SET protein expression is also much greater in developing rat and human kidney than in fully differentiated, mature kidney. Finally, high levels of SET mRNA and SET protein expression were found in Wilms tumor but not in renal cell carcinoma, adult polycystic kidney disease, or in transitional cell carcinoma. The SET domain(Tschiersch *et al.*, 1994) is found in proteins that are involved in embryonic development(Tripoulas *et al.*, 1996). Collectively, our observations in the blastosyst stage, suggest that this gene is related with early differential provesses in the embryos.

Hopkinson *et al.*(1973) described a red cell esterase that they called esterase D. Although studied in red cell hemolysates, esterase D was found in many different tissues including cultured fibroblasts and lymphocytoid cells. Genetic polymorphism was discovered in European, black, and Indian populations. Cowell *et al.*(1986) showed that there is a consistently lower level of esterase D enzyme activity in persons with the 2-2 phenotype when activity is measured in certain ways. Sparkes *et al.*(1980) found that quantitative and qualitative expression of esterase D in 5 persons with partial deletions or duplications of chromosome 13 supported localization of the gene to 13q14. Mohandas *et al.*(1982) found that mouse-human cell hybrid clones retaining a human X/13 translocation did not express esterase D. They suggested that this may reflect spreading of inactivation

into the autosomal part of the translocation chromosome.

In conclusion, the results of this study suggest that the ACP-based strategy is a reliable technique for identifying DEGs in mouse embryos. We showed that several genes that are differentially expressed in blastocyst stage comparing to ES cells were identified using ACP-Technology. Although the detailed function of the genes and their products remains to be determined, it is likely that the mechanisms of the cell-to-cell compaction and formation of blastocyst *in vitro* can be elucidated genetically by the analysis of differentially expressed genes in the future.

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