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Analysis of gene expression in single human oocytes and preimplantation embryos ☆

Steven Shoei-Lung Li [a,*], Yung-Hsien Liu [b], Chao-Neng Tseng [a], Sher Singh [c]

[a] *Department of Biotechnology, Kaohsiung Medical University, Kaohsiung City 807, Taiwan*
[b] *Profertile IVF Center, Kaohsiung County 831, Taiwan*
[c] *Bioinformatics and Biostatistics core, Center for Genomic Medicine, National Taiwan University, Taipei 100, Taiwan*

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Abstract

Little is known about the gene expression in human oocytes and early embryo development because of the rare availability of the materials. The recent advancement of biotechnology has allowed one to analyze the gene expression in single human oocytes and preimplantation embryos. Gene expression of human lactate dehydrogenase isozymes (LDH-A, LDH-B, and LDH-C) and small ubiquitin-like modifier isoforms (SUMO-1, SUMO-2, and SUMO-3) in four oocytes, two 4-cell and three 8-cell embryos was studied using the reverse transcription-polymerase chain reaction. The mRNAs for *SUMO-1*, *SUMO-2*, *SUMO-3*, and *LDH-B* (heart) were detected in all of oocytes, 4- and 8-cell embryos. The mRNA for *LDH-A* (muscle) was detected in two of four oocytes and one of three 8-cell embryos. However, the mRNA for testis-specific *LDH-C* was not detected at all as expected. A cDNA microarray containing 9600 cDNA spots was used to investigate differential expression of human genes in oocyte, 4-cell and 8-cell embryos. The expression of 184, 29, and 65 genes was found to have a value more than twofold above the median value of all genes expressed in oocyte, 4- and 8-cell embryos, respectively, indicating that the expression of some zygotic genes had already occurred at 4-cell embryo.

Keywords: Human; Oocytes; Preimplantation embryos; RT-PCR; cDNA microarray

Mammalian oocyte maturation and early embryo development require the appropriate expression of many genes at different developmental stages. In oocytes, some transcripts are required for oocyte-specific processes and metabolism, while others are presumably stored for use during early embryonic development, prior to the activation of zygotic expression. Fertilization releases the oocyte from cell cycle arrested in second meiotic metaphase and initiates the activation of the embryonic genome. In mice, significant changes in gene expression were found during the early developmental processes, and the stage-specific expressed genes actively promote the advancement of embryos from one stage to the next [1–3]. The gene expression profiling of human oocytes and early embryo development has been difficult to study due to the rare availability of the materials and the associated ethical considerations. However, the recent advancement on linear amplification of very small amounts of RNA samples for successful microarray detection has allowed one to analyze gene expression profiles among single human oocytes and preimplantation embryos obtained through in vitro fertilization (IVF) clinics [4].

Energy metabolism is a key feature of human preimplantation embryo development. Developmental abnormalities may be caused by defective energy metabolism [5], and thus it is important to assess the gene expression of metabolic enzymes in preimplantation embryos. Lactate and pyruvate are the preferred nutrients for the cleavage-stage embryo of several mammalian species, including the human [6]. The interconversion of lactate and pyruvate is

☆ *Abbreviations*: IVF, in vitro fertilization; LDH, lactate dehydrogenase; RT-PCR, reverse transcriptase-polymerase chain reaction; SUMO, small ubiquitin-like modifier.

* Corresponding author.Fax: +886 7 313 5162.
E-mail address: lissl@kmu.edu.tw (S.S.-L. Li).

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catalyzed by lactate dehydrogenases (LDH) with nicotinamide adenine dinucleotide as a coenzyme. Mammalian LDH isozymes are encoded by three different genes: *LDH-A* (muscle), *LDH-B* (heart), and *LDH-C* (testis). Five tetrameric LDH isozymes present in somatic tissues are various combinations of LDH-A and LDH-B subunits, whereas homotetrameric LDH-C isozyme is found only in testis and spermatozoa. LDH-A/B/C isozymes possess distinct catalytic properties [7,8].

Human small ubiquitin-like modifier (SUMO) proteins have recently been identified and they are involved in protein trafficking and targeting through posttranslational modification [9]. SUMO-1, SUMO-2, and SUMO-3 proteins were shown to be located on the nuclear membrane, in the nucleus, and in the cytosol, respectively [10]. The sumoylation participates in a number of cellular processes such as nuclear transport, transcriptional regulation, apoptosis, and so on.

In the present study, the expression of human *LDH-A/B/C* and *SUMO-1/2/3*, as well as β-actin, was first analyzed using reverse transcription-polymerase chain reaction (RT-PCR) in order to assess the quality of RNAs isolated from single oocytes, 4- and 8-cell embryos. A cDNA microarray was then used to survey differential expression profiles of human genes in single oocytes, 4- and 8-cell preimplantation embryos, and some zygotic genes were found to be expressed at 4-cell embryo.

Materials and methods

Human oocytes and embryo collection. Four oocytes, two 4- and three 8-cell preimplantation embryos, which were surplus to requirement for infertility treatment, were obtained with the IRB approval from Kaohsiung Medical University and the patients' consent at Profertile IVF Center. Oocytes were treated with hyaluronidase type VIII (80 iμ/ml) to get rid of the surrounding granulosa cells and checked for the first polar body to make sure matured oocytes. Embryos were treated with acid Tyrode's solution for 30 s to free sperm and other cell contamination.

RNA isolation and cDNA synthesis. Single oocytes and embryos were washed with PBS twice and then transferred to Eppendorf in 2 μl PBS, 2 μl lysis buffer [0.5% NP-40 (Vysis, Dowers Grove, IL, USA), 20 mM DTT (Invitrogen, Carlsland, CA, USA)], and 1 μl of 0.5 μg/μl oligo$(dT)_{12–18}$ primer (Invitrogen). Samples were incubated for 5 min at 65 °C to lyse the cells, to release RNAs, and to anneal oligo$(dT)_{12–18}$ primer with RNAs. The cDNA synthesis was carried out by using SURPERSCRIPT One-Step RT-PCR Kit (Invitrogen). For the reverse transcription step, the whole 5 μl of the resuspended RNAs was incubated for 60 min at 42 °C, then 15 min at 72 °C in 50 μl of reaction mixture containing 25 μl of 2×Reaction Mix (Invitrogen) and 1 μl of RT/PLATINUM *Taq* Mix (Invitrogen).

Polymerase-chain reaction. Polymerase-chain reaction (PCR) primers of complementary DNA were designed using Primer 3 web site software (http://www.broad.mit.edu/cgi-bin/primer/primer3_www.cgi). PCR primer sequences for *LDH-A/B/C*, *SUMO-1/2/3*, and β-actin, as well as the sizes of PCR products, are presented in Table 1. PCR amplification of the cDNAs was carried out using 4 μl from the above 50 rmul RT cDNA reaction mixture, added to 25 μl of reaction mixture containing 1×PCR buffer (with 2.0 mM $MgCl_2$), 0.2 mM dNTP, 0.4 μM each primer, and 2.5 U *VioTaq* DNA polymerase (Viogene, Taiwan). The PCR conditions were as follows: initial denaturation for 5 min at 94 °C, followed by 35 cycles of 30 s at 94 °C, 30 s at 56 °C, and 1 min at 72 °C, and final extension for 7 min at 72 °C. The 7.5 μl of the amplified PCR products was electrophoresed on a 3% agarose gel.

Table 1
The PCR primer pairs used to amplify LDH-A/B/C, SUMO-1/2/3, β-actin, and the size of their products

Genes	Primers		Size of products (bp)
LDH-A	F	5′GGCCTGTGCCATCAGTATCT3′	189
	R	5′GCCGTGATAATGACCAGCTT3′	
LDH-B	F	5′AGGATTCATCCCGTGTCAAC3′	169
	R	5′CCCACAGGGTATCTGCACTT3′	
LDH-C	F	5′CCTCTTGGGCTATTGGACTG3′	199
	R	5′GCCTCCTCCTCAGAATTCAA3′	
SUMO-1	F	5′CAGGAGGCAAAACCTTCAAC3′	293
	R	5′TCCATTCCCAGTTCTTTTGG3′	
SUMO-2	F	5′GGATGGTTCTGTGGTGCAGT3′	157
	R	5′TTCCAACTGTGCAGGTGTGT3′	
SUMO-3	F	5′CCAAGGAGGGTGTGAAGACA3′	212
	R	5′TCTCAGCTGTGCTGGAGTGT3′	
β-actin	F	5′GGACTTCGAGCAAGAGATGG3′	234
	R	5′AGCACTGTGTTGGCGTACAG3′	

cDNA microarray. 28.5 μl of the cDNAs present in above 50 μl RT reaction mixture purified by Microarray Target Purification Kit was used as templates to amplify cDNA by Microarray Target Amplification Kit (Roche Applied Science, Indianapolis, ID, USA). The complementary RNAs (cRNAs) were synthesized from the purified 200 ng cDNA by Microarray RNA Target Synthesis Kit (Roche Applied Science). The purified 5 μg cRNAs from single oocyte, 4- and 8-cell embryos were analyzed by the cDNA Microarray Core Facility for Genomic Medicine at National Taiwan University. The cDNA microarray contains 9600 PCR-amplified cDNA fragments from Integrated Molecular Analysis of Genomes and their Expression [IMAGE] human cDNA clones, with each spot representing a putative gene cluster with an assigned gene name in the Unigene clustering. The details of cDNA microarray experiments, including probe preparation, hybridization, and color development, were described previously [11,12]. After hybridization and color development of cDNA microarrays, the spots of good quality having a signal over background at least 1.5-fold were included in the further analysis, and the raw data were normalized per gene to median value.

Results

RT-PCR analysis of LDH-A/B/C and SUMO-1/2/3 gene expression in single human oocytes and preimplantation embryos

The gene expression of lactate dehydrogenase isozymes (LDH-A, LDH-B, and LDH-C) and small ubiquitin-like modifier isoforms (SUMO-1, SUMO-2, and SUMO-3), as well as β-actin, in four oocytes, two 4-cell and three 8-cell preimplantation embryos was analyzed using RT-PCR (Fig. 1). The mRNAs for *SUMO-1*, *SUMO-2*, *SUMO-3*, and *LDH-B* (heart) were detected in all of oocytes, 4- and 8-cell embryos. The mRNA for *LDH-A* (muscle) was detected only in two of four oocytes and one of three 8-cell embryos. The mRNA for testis-specific *LDH-C* was not detected at all as expected. The β-actin was used as positive control.

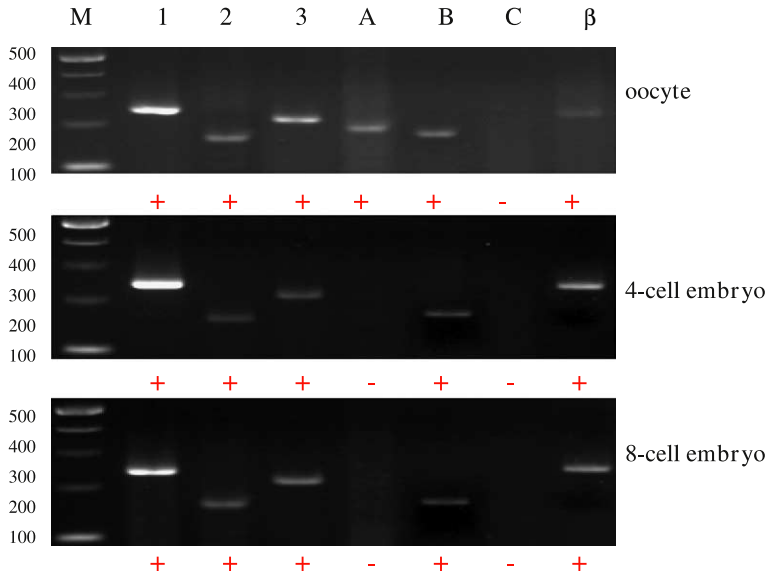

Fig. 1. RT-PCR detection of SUMO-1/2/3 and LDH-A/B/C gene expression in single human oocyte, 4- and 8-cell embryos. The lanes are M, DNA size (bp) maker; 1, SUMO-1; 2, SUMO-2; 3, SUMO-3; (A) LDH-A; (B) LDH-B; (C) LDH-C; and β, β-actin as positive control. + and − indicate the presence and absence of mRNAs, respectively.

cDNA microarray analysis of gene expression in single human oocyte and preimplantation embryos

The RNA samples of good quality one each from single oocyte, 4-cell and 8-cell embryos were used for cDNA microarray analysis. A total of 2123 cDNA spots had signals over background at least 1.5-fold in three microarrays of oocyte, 4- and 8-cell embryos, and 631 of them were found to have more than twofold values either above or below the expression median value in at least one array (Table 2 and Supplementary Table S1). In oocyte, 184 genes were found to express more than twofold above the median value, but only two genes were at least twofold below the median value. In 4-cell embryo, 29 genes expressed more than twofold above the median value, and 98 genes expressed at least twofold below the median value. In 8-cell embryo, 65 genes expressed more than twofold above the median value, and 287 genes expressed at least twofold below the median value. These 631 genes were further analyzed and organized by hierarchical clustering (Fig. 2). Table 3 lists the 45, 14, and 12 genes expressed more than threefold above the median value in oocytes, 4- and 8-cell embryos, respectively.

Table 2
The number of genes expressed more than twofold above or below the median value of all genes analyzed in single oocyte, 4- and 8-cell preimplantation embryos

Oocyte/embryos	Twofold above median	Twofold below median	Sum
Oocyte	184	2	186
4-Cell embryo	29	98	127
8-Cell embryo	65	287	352
Total	278	387	665[a]

[a] A total of 631 genes expressed more than twofold values above or below the median value, and 34 genes exhibited twofold above/below medium common in two stages.

Discussion

In this investigation, human *LDH-B* (heart) mRNAs were found to be present in all of four oocytes, two 4-cell and three 8-cell embryos (Fig. 1). Mouse *LDH-B* mRNAs were previously found to accumulate to very high levels in fully grown oocytes [13]. These findings suggest the importance of LDH-B isozyme in the interconversion of lactate and pyruvate during the oocyte maturation and preimplan-

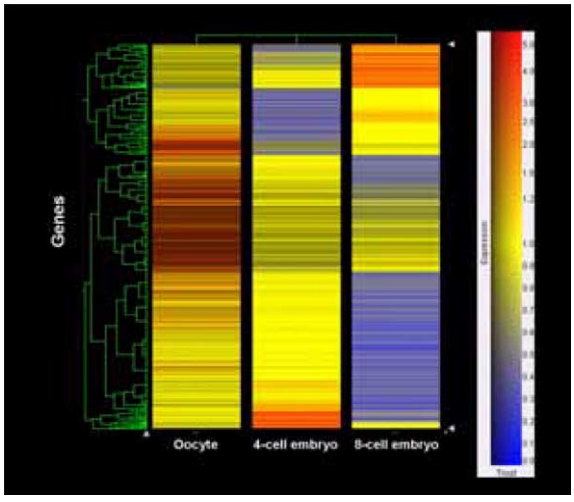

Fig. 2. The hierarchical cluster analysis of 631 genes expressed more than twofold above or below the median value in single oocyte, 4- and 8-cell embryos. The colors indicate the relative expression levels of each gene, with red indicating the highest expression above the median value and blue indicating the lowest expression below the median value.

Table 3
Genes abundantly expressed in oocyte, 4- and 8-cell embryos

Symbol	Gene description	UniGene	Folds
A. Oocyte			
GTF2IRD1	GTF21 repeat domain containing 1	Hs.156302	12.46
TPM3	Tropomyosin 3	Hs.146070	8.74
NUP88	Nucleoporin 88 kDa	Hs.554767	8.39
NEK9	NIMA (never in mitosis gene a)-related kinase 9	Hs.7200	7.46
ALPL	Alkaline phosphatase, liver/bone/kidney	Hs.250769	6.62
ACTA1	Actin, α1, skeletal muscle	Hs.1288	5.92
PHLDB2	Pleckstrin homology-like domain, family B, member 2	Hs.477114	5.78
NUBP2	Nucleotide binding protein 2	Hs.256549	5.53
FOXH1	Human homolog of *Xenopus* forkhead activin signal transducer-1	Hs.449410	5.43
LOC374395	Collagen, type V, α1	Hs.381134	5.29
MTX1	Glucosidase, β; acid (includes glucosylceramidase)	Hs.247551	5.12
SEC6L1	SEC6-like 1 (*Sccharomyces cerevisiae*)	Hs.481464	5.08
POLR2G	RNA polymerase II subunit hsRPB7 mRNA, complete cds	Hs.14839	4.94
KIAA0905	mRNA for KIAA0905 protein, complete cds	Hs.436549	4.89
AQP7	Aquaporin 7	Hs.368607	4.58
LOC139202	XIST, coding sequence "a" mRNA (locus DXS399E)	Hs.444102	4.30
NRD1	*H. sapiens* mRNA for NRD1 convertase	Hs.4099	4.19
HERC1	Guanine nucleotide exchange factor p532 mRNA, complete	Hs.133411	4.15
PSMA3	Proteasome (prosome, macropain) subunit, α type, 3	Hs.246240	4.15
SULT2B1	Hydroxysteroid sulfotransferase SULT2B1b (HSST2)	Hs.406998	4.13
C6	Complement component 6	Hs.1282	4.12
CBX3	Chromobox homolog 3	Hs.381189	4.01
UMOD	Uromodulin (uromucoid, Tamm–Horsfall glycoprotein)	Hs.130005	3.99
IGFBP5	Insulin-like growth factor binding protein 5 (IGFBP5) mRNA	Hs.380833	3.78
HSPE1	Heat shock 10 kDa protein 1 (chaperonin 10)	Hs.1197	3.76
LOC285941	Hypothetical protein LOC285941	Hs.413394	3.63
STS	Arylsulfatase C, isozyme S	Hs.79876	3.59
TAC1	Tachykinin 2 (substance K, neurokinin A, neurokinin 2, neuromedin L, neurokinin α, neuropeptide K, neuropeptide gamm)	Hs.2563	3.55
CSPP 1	Centrosome spindle pole associated protein 1	Hs.370147	3.54
AHDC1	AT hook, DNA binding motif containing 1	Hs.469280	3.43
AASS	mRNA for lysine-ketoglutarate reductase/saccharopine dehydrogenase	Hs.433075	3.40
BET1	Golgi vesicular membrane trafficking protein p18	Hs.23103	3.38
IL18RAP	Interleukin 18 receptor accessory protein	Hs.158315	3.29
BCL2L2	BCL2-like 2	Hs.410026	3.24
CHASM	Ortholog of mouse calponin homology-associated smooth muscle	Hs.68756	3.19
NIFIE14	mRNA for putative seven transmembrane domain protein	Hs.9234	3.17
ACD	Adrenocortical dysplasia homolog (mouse)	Hs.78019	3.11
IGF2	Putative insulin-like growth factor II associated protien	Hs.523414	3.10
CHRNB1	Cholinergic receptor, nicotinic, β-polypeptide 1 (muscle)	Hs.330386	3.06
KCNAB1	Potassium voltage-gated channel, shaker-related subfamily, β	Hs.157818	3.06
TRNT1	TRNA nucleotidyl transferase, CAA-adding 1	Hs.506382	3.04
COL4A5	Collagen, type IV, α5 (Alport syndrome)	Hs.169825	3.03
POLR2F	Gene for RNA polymerase II subunit 14.4 kDa	Hs.46405	3.03
SELE	Selectin E (endothelial adhesion molecule 1)	Hs.89546	3.00
ACTL6A	Actin-like 6A, BAF53A	Hs.435326	3.00
B. 4-Cell embryo			
MAG	Myelin associated glycoprotein	Hs.515354	6.36
IER2	Transcription factor ETR101 mRNA, complete cds	Hs.501629	6.09
FLT1	fms-Related tyrosine kinase 1 (vascular endothelial growth factor/vascular permeability factor receptor)	Hs.507621	5.25
RCOR1	Clone 24709 mRNA sequence	Hs.510521	5.04
MGC34648	Gene from PAC 426I6	Hs.96607	4.33
LOC284208	Clone 23614 mRNA sequence	Hs.435541	3.99
HSF1	Heat shock transcription factor 1	Hs.132625	3.88
PCTK3	PCTAIRE protein kinase 3	Hs.445402	3.88
RBMS1	RNA binding motif, single stranded interacting protein 1	Hs.470412	3.75
KRT1B	Keratin 1B	Hs.334989	3.47
DYM	Dymeclin	Hs.162996	3.46
—	EST from ovary and testis	Hs.131415	3.44
DNASE1L2	Deoxyribonuclease I-like 2	Hs.103503	3.34
PTPN21	mRNA for protein–tyrosine phosphatase D1	Hs.437040	3.22

(*continued on next page*)

Table 3 (*continued*)

Symbol	Gene description	UniGene	Folds
C. 8-Cell embryo			
HBE1	Hemoglobin epsilon chain	Hs.117848	5.95
GAL	Galanin	Hs.278959	4.44
GPS2	G protein pathway suppressor 2	Hs.438219	4.00
GOSR1	Golgi SNAP receptor complex member 1	Hs.462680	3.43
LOC389362	Hypothetical LOC389362	Hs.207069	3.41
NARS	Asparaginyl-tRNA synthetase	Hs.465224	3.33
SYK	Spleen tyrosine kinase	Hs.371720	3.32
CDS2	CDP-diacylglycerol synthase (phosphatidate cytidylyltransferase) 2	Hs.472027	3.22
LDB2	LIM domain binding 2	Hs.23748	3.17
PAX3	Paired box gene 3 (Waardenburg syndrome 1)	Hs.42146	3.07
SNX3	Sorting nexin 3	Hs.12102	3.03
CD1A	CD1A antigen, a polypeptide	Hs.1309	3.01

tation embryo development. Human *LDH-A* (muscle) mRNAs were found only in two of four oocytes and one of three 8-cell embryos, and the failure to detect *LDH-A* mRNAs in some others might be due to the various quality of samples. Human testis-specific *LDH-C* mRNAs were not detected in any oocyte, 4- and 8-cell embryos, and this is consistent with the fact that LDH-C was previously found only in testis and spermatozoa [7,8,14]. On the other hand, human *SUMO-1/2/3* mRNAs were found to be present in all oocytes, 4- and 8-cell embryos, indicating that these three SUMO-1/2/3 proteins may play important roles in oocyte maturation and preimplantation embryo development.

Only 2123 cDNA spots were fully analyzed in these cDNA microarray experiments, and a total of 631 human genes was shown to exhibit differential expression in oocyte, 4- and 8-cell preimplantation embryos. The 184 highly expressed genes in oocyte were replaced by 29 different genes in 4-cell embryo. These 29 highly expressed genes in 4-cell embryo were replaced by another 65 different genes in 8-cell embryo (Table 2, Fig. 2). These results indicate that the linear amplification procedure described in this report can indeed be used for the gene expression profiling among single human oocytes and preimplantation embryos, and that some zygotic mRNA expression had already occurred at 4-cell embryo. The timing of transition from maternal to zygotic expression was reported to be at the 2-cell stage in mice, the 4–8-cell stage in humans and cows, and 8–16-cell stage in sheep and rabbits [15,16]. The specific roles of these abundantly expressed human genes (Table 3) during oocytes maturation and early embryo development remain to be fully determined.

Human early embryogenesis is currently the focus of intense interest because of its potential for therapeutic cloning. Embryo quality is also a major concern in human assisted reproduction. The full transcriptomes of immature and mature oocytes, fertilized eggs, 2-, 4-, 8-, and 16-cell embryos, and blastocysts (including inner cell mass and trophectoderm) will have to be analyzed using DNA microarrays containing a much more complete set of human UniGenes in order to profile fully the differential expression during oocyte maturation and preimplantation embryo development [4]. The existence of a list of UniGenes expressed in human preimplantation embryo development makes it possible to build DNA arrays targeted at human preimplantation embryos and embryonic stem cells. The targeted DNA microarrarys promise the possibility of diagnostics for assessing human embryo quality in human assisted reproduction. These DNA microarrarys will also allow assessment of gene expression in human embryonic stem cells, leading to advances in the manipulation of these cells and their use in tissue transplantation therapy. The immortality, pluripotency, and motility of early embryonic cells suggest that the human embryonic genes identified may be candidate genes for a role in tumorigenesis. A panel of cDNAs prepared from cancer cell lines and tumors can be screened using this targeted DNA microarray for their expression in order to identify embryonic/ cancer genes [17]. Then these identified genes/cDNAs could be used in cancer therapy and the preparation of DNA vaccines.

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Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bbrc.2005.11.149.

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