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ORIGINAL PAPER

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A single human cell expresses all messenger ribonucleic acids: the arrow of time in a cell

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Abstract Expression of 25 mRNAs in a single human lymphocyte was investigated using the reverse transcription–nested polymerase chain reaction (RT–nested PCR) method. Proteins corresponding to the mRNA investigated were mucin antigen, melanoma antigen, pregnancy-specific β-1 glycoprotein 4, phenylethanolamine-*N*-methyl-transferase, β B3-crystallin, homeobox 4A, interleukin 2, cluster of differentiation 8, progesterone receptor, parathyroid hormone, gastrin, cholecystokinin/pancreozymin, glucagon, insulin, enkephalin, thyroid stimulating hormone, adrenocorticotropic hormone, synapsin I, immunoglobulin (Ig)M, IgD, IgG1, IgG3, IgE, IgA, and T cell receptor α. All mRNAs were detected in single lymphocytes of two individuals, without exception. In addition, transcripts of IgM, IgD, IgG1, IgG3, IgE, IgA, and the T cell receptor α gene were detected in single sperms. The results strongly suggest the possibility that all mRNAs may be expressed in a single human cell, of both somatic and germ lineage. Thus, cells can consume energy in vain to produce functionally meaningless gene transcripts. However, this basal or illegitimate transcription may be essential for the birth of living matter: the arrow of time in a cell. Moreover, the phenomenon implies the potential of using lymphocytes in place of inaccessible tissue for the diagnosis of genetic diseases.

Key words Illegitimate transcription · Single cell · Lymphocyte · Sperm · Arrow of time

Introduction

Recent studies have revealed the regulatory mechanisms of gene transcription in eukaryotes. For cell differentiation in particular, the gene cascade plays an important role in producing specific mRNAs and proteins. A general concept of gene transcription is that only required genes are expressed, in other words, only organ-specific or cell-specific gene expression takes place, while unnecessary genes do not function. However, the so-called house-keeping proteins must be produced constantly in a living cell, which implies a continuous transcription of the corresponding genes.

There are some reports on the ectopic expression of mRNA or the ectopic production of proteins (Humphries et al. 1976; Weintraub and Groudine 1976; Chelly et al. 1988, 1989). Chelly et al. (1988) detected the expression of dystrophin mRNA both in human muscle and in other tissues by PCR and Southern blotting. They concluded in additional experiments that any gene could be transcribed in any cell type and called this phenomenon illegitimate transcription. They calculated this low level of transcription occurred at about one copy per 500–1000 cells.

The reverse transcription–polymerase chain reaction (RT–PCR) method can theoretically detect a single molecule of mRNA. I independently reported a general expression of gene transcripts for 11 proteins in human peripheral blood lymphocytes, gastric mucosal cells, sperm, and 15 malignant tumor cell lines by repeating nested PCR only (Kimoto 1995). This sensitive PCR method detected another six mRNA in a human single lymphocyte (Kimoto 1996) and SRY gene transcripts in a single lymphocyte and male cancer cells (Kimoto 1998b). Most of the proteins are thought to be functionally irrelevant to the function of the cells investigated. In this study, several single lymphocytes as representative somatic cells were analyzed for whether these and other gene transcripts could all exist simultaneously. In addition, the expression of transcripts of immunoglobulin (Ig) genes and of the T cell receptor (TCR) gene, which has been confirmed in non-hematopoietic cancer cells (Kimoto 1998a), was also investigated in single sperms and lymphocytes. The meaning of such basal transcription is discussed.

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Materials and methods

Using a cell manipulator under microscopic observation, a single lymphocyte of a healthy adult and a single sperm of a healthy male was collected in 10 µl of H_2O and was allowed to burst osmotically. Total RNA in 10µl of H_2O was reverse transcribed in a mixture containing 1.2 µM random hexamers, 5.6 U RNasin (Promega), and 80 U M-MLV reverse transcriptase (GibcoBRL). Before nested PCR, cDNA was amplified by PCR with random hexamers (8 µM in a total reaction volume of 30 µl) and recombinant *Taq* DNA polymerase (Takara Taq; 1.5 U in 30 µl of reaction mixture) using 15 cycles of 94° C for 40 s, 37° C for 1 min, and 72 ° C for 1 min. All nested or heminested PCRs were carried out using 30 cycles of 94° C for 40 s, 62° C for 1 min, and 72° C for 1 min. Two microliters of the PCR mixture was electrophoresed (constant voltage, 150 V) in an acrylamide gel (8%) that was stained with ethidium bromide. Nested or heminested PCR was repeated with inner primers several times. Sequences of PCR products were confirmed by the method of Sanger et al. (1977). New primers for the detection of mRNA of the human mucin (MUC1) gene (Tsarfty et al. 1990) and the genes coding for melanoma antigens (MAGE1) (van der Bruggen et al. 1991), pregnancy-specific β-1 glycoprotein 4 (PSG4) (Zimmermann et al. 1989), phenylethanolamine-*N*-methyltransferase (PNMT) (Sasaoka et al. 1989), β B3-crystallin (Aarts et al. 1989), homeobox 4A (HOX4A) (Taniguchi et al. 1992), gastrin (Boel et al. 1983), and synapsin I (Suedhof 1990) were designed and applied only to this analysis in order to avoid contamination. In addition, no detection of PCR products in the reagent blank controlled for contamination of mRNA from other cells. Other primers for the detection of mRNA for interleukin 2 (IL-2), cluster of differentiation 8 (CD8), progesterone receptor (PgR), parathyroid hormone (PTH), cholecystokinin/pancreozymin (CCK/PZ), glucagon, insulin, enkephalin, thyroid stimulating hormone (TSH), and adrenocorticotropic hormone (ACTH) were described in a previous report (Kimoto 1995). Primers for TCR (Rabbitts et al. 1985), IgM (Word et al. 1989), IgD (Word et al. 1989), IgE (Flanagan and Rabbitts 1982; Kenten et al. 1982), and IgA (Flanagan et al. 1984) were designed in the heavy-chain constant region, and for IgG1 and IgG3 (Huck et al. 1986), the hinge region was selected. Initially, the primers for TCR and the Igs were applied only for the analysis of sperms in this study. After detection of all Ig and TCR mRNAs in sperms, the primers were then used to investigate single lymphocytes. The map location of the genes, the sizes of the first and the final PCR products, and the numbers of the exons where the primers annealed are described in Table 1.
The following were used:

PCR primers
MUC1. Sense: 5′-GACACCGGGCACCCAGTCTCCTTTC-3′, 5′-CCTGCTGCTGCTCCTCACAGTGC-3′, 5′-GTTGTTACAGGTTCTGGTCATGCAAGC-3′; antisense: 5′-GCCTGAACCGGGGCTGTGGCTGG-3′, 5′-CGCTGCTGGTCATACTCACAGC-3′, 5′-GTAGAGCTGGGCACTGAACTTCTC-3′.
MAGE1. Sense: 5′-GTTTTCAGGGGACAGGCCAACCCAG-3′, 5′-GGATTCCCTGGAGGCCACAGAGGAG-3′, 5′-GGAGAAGATCTGCCTGTGGGTCTTC-3′; antisense: 5′-GTGGGCACCTCCTCCAGGGTGCCC-3′, 5′-CCAGGCCCAGGGCCTCTTGTTGGG-3′, 5′-GGGCTTCCTCAGGCTTGCAGTGCAG-3′.
PSG4. Sense: 5′-CCTTCACCTGTGAACCTAAGAGTG-3′, 5′-GGCTAAATGGTCAGAGCCTCCCTG-3′, 5′-CCTCATTCTACCCAATGTCACGAG-3′; antisense: 5′-GGGGGATAGAGAGCTTTTGTCCTG-3′, 5′-GTCCAAGAATATTGTGCCCGTGGG-3′, 5′-GCAGGACAAGTAGAGGTTTTCTCC-3′.
PNMT. Sense: 5′-GGCGGCGGTGGCTTCGGCCTACC-3′, 5′-CCTCCGCAACAACTACGCGCCCCC-3′, 5′-GCTGCGCTGCTTGGCGCAGACC-3′; antisense: 5′-GGTGCACGTCGATGGGCAGGACCCG-3′, 5′-CCTGCCAGCATTCCCCCTTGCCC-3′, 5′-GGCTGTACATGCTCCAGTTGAAGG-3′.
β B3-crystallin. Sense: 5′-GGGCCTTCCGCGGGGAGCAGTTTG-3′, 5′-GGGATGCCTGGTCCAACAGCCGTG-3′, 5′-GTCCCTCCGGCCTCTGAATATTG-3′; antisense: 5′-CTTCTGGTCACGGATGCGGCGCAC-3′, 5′-GGCGTCCCACTCATTCCAATGGCGG-3′, 5′-CTGGCGCCCACGGTAGCCCGGG-3′.
HOX4A. Sense: 5′-GGAGCTGAACTCAATGGCAGCTGC-3′, 5′-GCAGCCACCACAACCCCCTCCTCC-3′, 5′-GGTGGAGT-

Table 1 mRNA investigated (*MUC1* human mucin, *MAGE1* melanoma antigen, *PSG4* pregnancy-specific β-1 glycoprotein 4, *PNMT* phenylethanolamine-*N*-methyltransferase, *HOX4A* homeobox 4A, *IL-2* interleukin 2, *CD*8 cluster of differentiation 8, *PgR* progesterone receptor, *PTH* parathyroid hormone, *CCK*/*PZ* cholecystokinin/pancreozymin, *TSH*β thyroid stimulating hormone, *ACTH* adrenocorticotropic hormone, *Ig* immunoglobulin, *TCR*α T cell receptor α)

Protein	Map location	Size of PCR product (bp)		Primer site (exon)
		First PCR (invisible)	Final PCR (visible)	
MUC1	1q21-23	205	89	1–2
MAGE1	x	281	122	2–3
PSG4	19q13.2	372	181	5–6
PNMT	17	422	212	1–3
βB3-crystallin	22q11.2-12.2	389	205	4–6
HOX4A	2q31-37	508	201	1–2
IL-2	4q26-27	229	127	1–4
CD8	2p12	339	126	2–4
PgR	11q22-23	208	125	5–7
PTH	11p15.4-pter	247	104	2–3
Gastrin	17q	257	114	1–2
CCK/PZ	3pter-21	245	112	2–3
Glucagon	2	343	116	2–4
Insulin	11p15.5	262	104	1–2
Enkephalin	8q23-24	304	109	3–4
TSHβ	1p22	291	106	1–2
ACTH	2p23	286	107	1–3
Synapsin I	xp11.23	299	135	4–7
IgM	14q32.33	364	148	Cµ3-Cµ4
IgD	14q32.33	269	134	Cδ2-Cδ3
IgG1	14q32.33	122	71	Ch1-hinge-Ch2
IgG3	14q32.33	263	194	Ch1-Ch4-C
IgE	14q32.33	506	181	Ch2-Ch4
IgA	14q32.33	508	262	1–3
TCRα	14q11.2	279	153	1–3

GCCTGCCAAGAAGCCC-3′; antisense: 5′-CCTTCTTGTACT TCATGCGCCGG-3′, 5′-CCGTGAGATTCAGCAGGTTGGCC-3′, 5′-CGTGTATGCCGTGCGTACCCGC-3′.
Gastrin. Sense: 5′-GCACTGGCTCTGGCCGCCTTCTCTG-3′, 5′-GGAAGCCCCGCTCCCAGCAGCCAG-3′, 5′-GGTACAGG-GGCCAACAGGGACCTGG-3′; antisense: 5′-GCACTGCGG-CGGCCGAAGTCCATCC-3′, 5′-CTTCTTCCTCCAGCCATGG-TCCCTGC-3′, 5′-CGGGTCTGCCACGAGGTGTGGGGG-3′.
Synapsin I. Sense: 5′-GGGCTGCAGTATGCTGGAATCCCC-3′, 5′-GTCTACAACTTCTGTGACAAGCCC-3′, 5′-GGGTGTTT-GCCCAGATGGTTCGAC-3′; antisense: 5′-GTGGCATACGT-CTTGGTCAGTGCC-3′, 5′-GTCCTGGAAGTCATGCTGG TTGTC-3′, 5′-GCCCCATCTTCACAACCACGGGG-3′.
IgM. Sense: 5′-GGCGAAGCTGTGAAAACCCACACC-3′, 5′-GAGGCCAGCATCTGCGAGGATGAC-3′, 5′-CGGGGAGA GGTTCACGTGCACCG-3′; antisense: 5′-CTCAGGCATTGGG GCGCTGGTCAC-3′, 5′-CCACTGCACGAAGACGTCCGCG GG-3′, 5′-GCCGACTCCCGCAGGTTCAGCTGC-3′.
IgD. Sense: 5′-CACCTGACCTGGGAGGTGGCTGGG-3′, 5′-GCTGCTGGAGCGGCACAGCAACGGC-3′, 5′-GAGCCAGC-ACAGCCGTCTGACCC-3′; antisense: 5′-CAGGAGCCACGA-GGCCGCCTCGGG-3′, 5′-CAGACGAGGCCAGCAGGTT CAGGG-3′, 5′-CGGGTGCCTGCGCAGCGGGTTCTC-3′.
IgG1. Sense: 5′-GCAACACCAAGGTGGACAAGAGAG-3′, 5′-GGTGGACAAGAGAGTTGAGCTC-3′; antisense: 5′-GGGTT-TTGGGGGGAAGAGGAAGAC-3′, 5′-GAGGAAGACTGAC-GGTCCTCCCAG-3′, 5′-GTTCAGGTGCTGGGCACCTGGGC-3′.
IgG3. Sense: 5′-GCAACACCAAGGTGGACAAGAGAG-3′,5′-GGTGGACAAGAGAGTTGAGCTC-3′, 5′-GCTCAAAACCC-CACTTGGTGACAC-3′; antisense: 5′-GGGTTTTGGGGGGA-AGAGGAAGAC-3′, 5′-GAGGAAGACTGACGGTCCTCCCA-G-3′, 5′-GTTCAGGTGCTGGGCACCTTGGGC-3′.
IgE. Sense: 5′-GGACGTGGACTTGTCCACCGCCTC-3′, 5′-GCACTGGCTGTCAGACCGCACCTAC-3′, 5′-GAAGTGTG-CAGATTCCAACCCGAG-3′; antisense: 5′-CCGGGGCAGCA-CGGCGGGCCGCTGG-3′, 5′-CGATCCAGTCTCGGGTGCCC ACCG-3′, 5′-GGTGGAGTGGTTCACAGGCTTCCC-3′.
IgA(α1). Sense: 5′-GGGGACCTGTACACCACGAGCAGC-3′, 5′-GCCGGCAAGTCCGTGACAGTCCAC-3′, 5′-GGATGTGACT-GTGCCCTGCCCAG-3′; antisense: 5′-CCTCGGGCCGGAA-TGTGTTTCCGG-3′, 5′-GGGTGGCGGTTAGCGGGGTCTT GG-3′, 5′-CGGCACAGCCCGGCAGGACACTGG-3′, 5′-GCA-GCCACAGAGGTCACGCTCAGG-3′.
TCR-α. Sense: 5′-GTGCTAGACATGAGGTCTATGGAC-3′, 5′-GCAACAGTGCTGTGGCCTGGAGC-3′, 5′-GCATGTGCAA-ACGCCTTCAACAACAGC-3′; antisense: 5′-GCTGGACCACA-GCCGCAGCGTC-3′, 5′-GCAGATTAAACCCGGCCACTTT-CAGG-3′, 5′-GGATTCGGAAGGGAATCACTGACAGG-3′.

To detect mRNA for PTH and ACTH in a single lymphocyte, two further antisense primers were added to the primers described in Kimoto (1995): 5′-CTTACGCAGCCATTCTACTCTCTCC-3′ and 5′-GCCACGCACTTCCATGGAGGCCTG-3′, respectively.

Results

The genes examined in this study have plural exons and the primers designed for their detection were selected to anneal to different exons, so that the PCR product sizes matched only those of the mRNAs directly or cDNA transcripts, not those from genomic DNA. All PCR product sequences, confirmed by the method of Sanger et al. (1977), were intronless. In these experiments, no lens, brain, gastrointestinal or endocrine cells were probed; several single lymphocytes and sperms were the only cells examined.

MUC1, MAGE1, PSG4, PNMT, β B3-crystallin, and HOX4A gene transcripts were detected in five single lymphocytes of a healthy male (Fig. 1). Gastrin gene transcripts existed in five single lymphocytes of another individual simultaneously with gene transcripts of IL-2, CD8, PgR, PTH, CCK/PZ, glucagon, insulin, enkephalin, and TSH (Fig. 2). The heavy-chain constant region of Ig gene transcripts and the TCR-α gene transcript were detected in six single sperms (Fig. 3) and nine single lymphocytes (Fig. 4) of the male. The same lym-

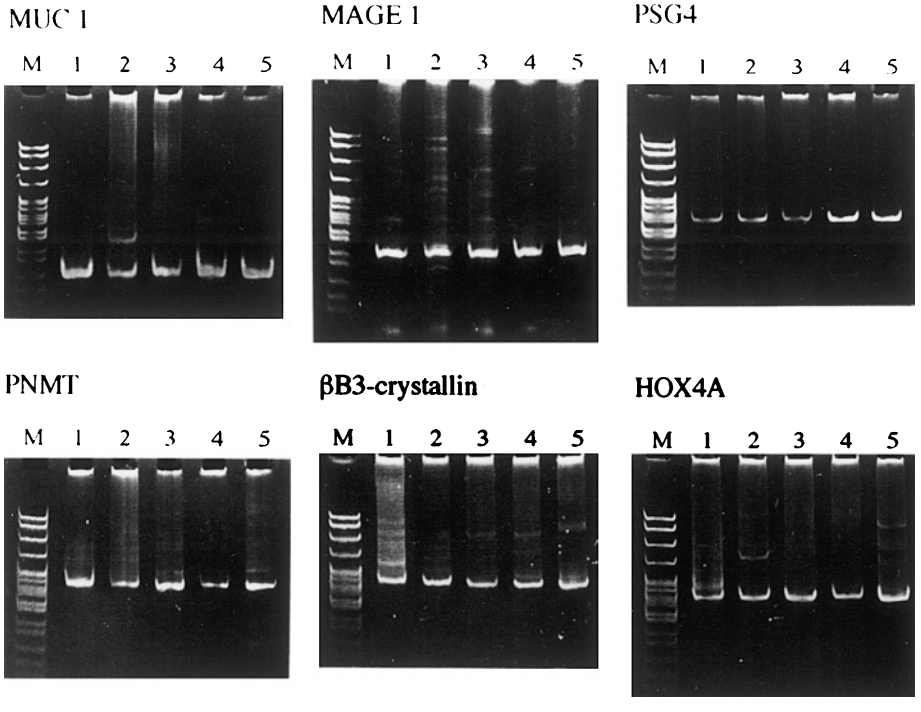

Fig. 1 PCR products amplified from mRNAs for MUC1, MAGE1, PSG4, PNMT, β B3-crystallin and HOX4A in single human male lymphocytes. *M* pBR322/*MspI* digest. 1–5 Five single lymphocytes; *identical number* means the same lymphocyte

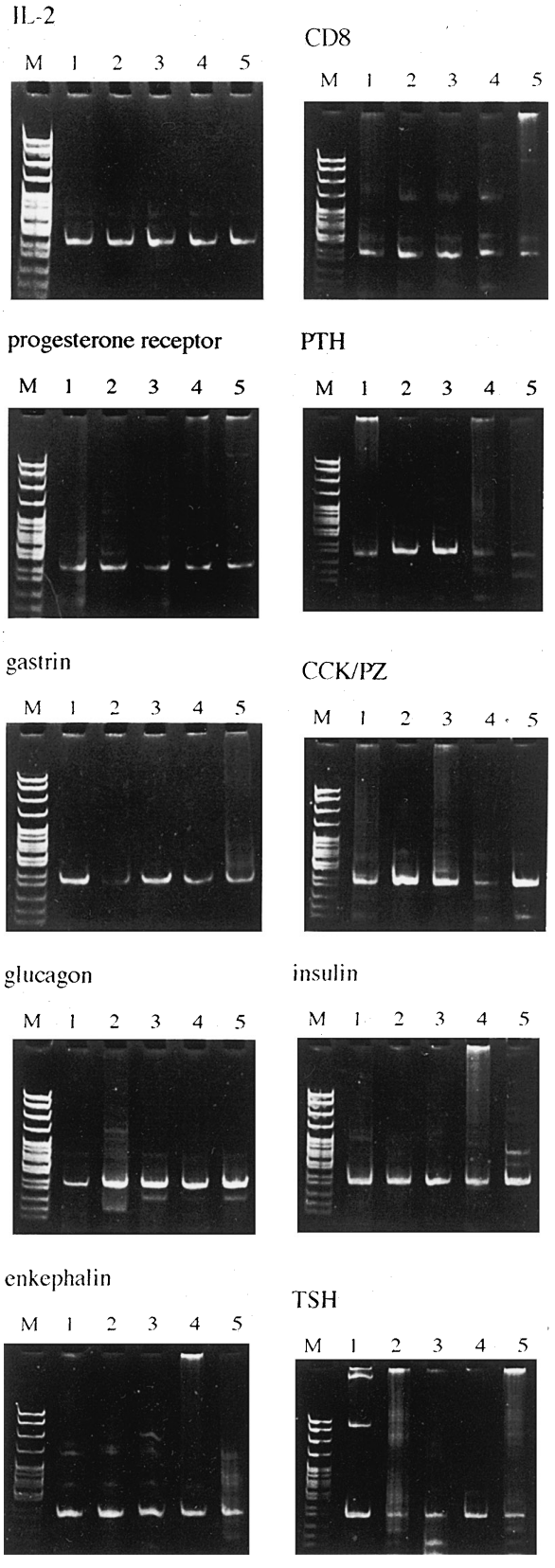

Fig. 2 PCR products amplified from mRNAs for IL-2, CD8, PgR, PTH, gastrin, CCK/PZ, glucagon, insulin, enkephalin, and TSH in single human female lymphocytes. *M* pBR322/*MspI* digest. 1–5 Five single lymphocytes; *identical number* means the same lymphocyte

phocytes also expressed the mRNA for ACTH (Fig. 4). Although synapsin I is believed to be neuron specific, transcripts of synapsin I were detected in single lymphocytes and a sperm (Fig. 5). In addition, as a negative control, pure water and 1 μg of mRNA obtained from chicken splenocytes, a small fish (*Medaka*) and chickweed leaf were used for the detection of human MUCl, MAGEl, and IgD mRNAs, using the primers described here. These three mRNAs could most easily be detected in human single lymphocytes in only one experiment. Aimed PCR products were never detected in the other species, while simultaneous experiments revealed the PCR products in human lymphocytes (Fig. 6).

Discussion

MUCl glycoprotein is a mucin isolated as a human breast cancer-associated tumor antigen, gene transcripts of which were reported to be expressed in epithelial cells (Wreschener et al. 1990) and tumor cells. This antigen is recognized by cytotoxic T lymphocytes (CTL). MAGEl codes for a melanoma antigen which combines with the major histocompatibility molecule to form antigen MZ2-E, recognized also by CTL (Traversari et al. 1992). The result in this study that lymphocytes can express both MUCl and MAGEl gene transcripts, suggests that patients' autologous lymphocytes, which could recognize MUCl or MAGEl (MZ2-E) molecules, also contain both gene transcripts. In lymphocytes, therefore, translation of these mRNAs to their corresponding proteins, processing, and antigen presentation could occur. If so, these antigens are not tumor specific, and both lymphocytes and tumor cells share the same gene transcripts. Under these conditions, autologous lymphocytes could recognize these antigens; MUCl glycoprotein may even be secreted by lymphocytes. The function of the MAGEl protein is still unknown. A small number of molecules of both proteins are probably expressed on human lymphocytes and also on malignant tumor cells. The proteins expressed on tumor cells might be biochemically modified or damaged, which could be recognized as non-self by autologous CTL. Another explanation is that there exists an autoimmune system even in the normal body and recognition of self proteins depends on the balance of the amounts of recognized and recognizing molecules.

PSG4 is secreted from trophoblasts during pregnancy, but has also been identified in several organs and tumor cells. This implies that PSG4 is not a pregnancy-specific protein. This gene belongs to the Ig gene superfamily. In this study, the PCR products from the constant region of PSG4 mRNA were detected in a male lymphocyte. Detection of PNMT gene transcripts suggests that even a lymphocyte can produce adrenaline. β B3-crystallin is a lens-specific protein. The HOX4A gene belongs to the family of highly conserved homeobox-containing genes. HOX proteins regulate embryonic morphogenesis. Both gene transcripts were also expres-

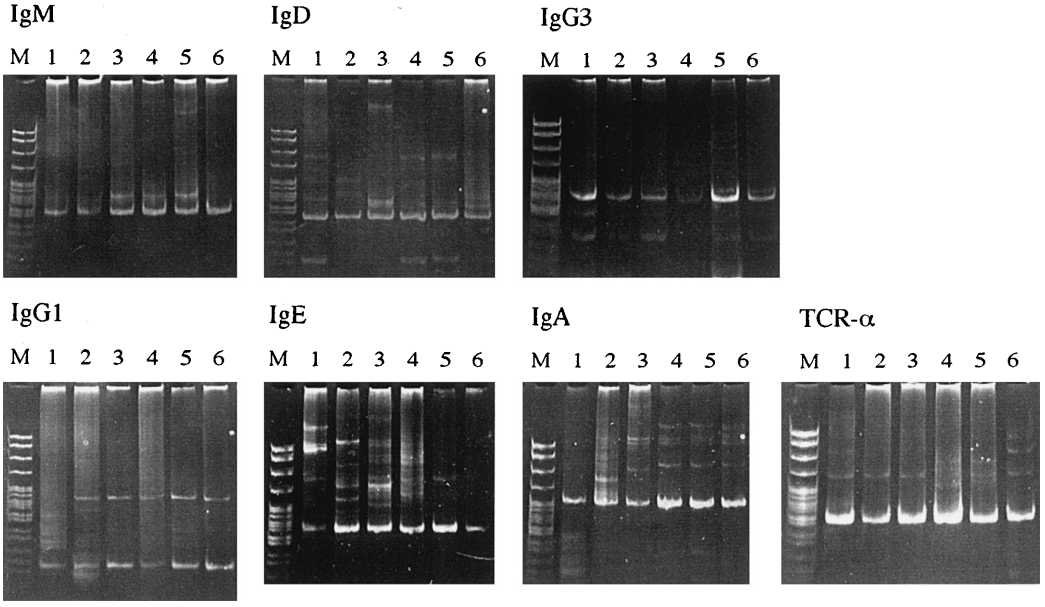

Fig. 3 PCR products amplified from mRNAs for IgM, IgD, IgG1, IgG3, IgE, IgA, and TCR-α in single human sperms. *M* pBR322/*MspI* digest. 1–6 Six single sperms; identical number means the same sperm. IgG3 hinge region is encoded by three repeating sequences of 45 bp, Ch2, Ch3, and Ch4, which are sometimes reflected in three different sizes of PCR products, 194 bp, 149 bp, and 104 bp, respectively. The sequences of these three PCR products were also confirmed by the method of Sanger et al. (1977)

sed in the same single lymphocyte of an adult man. $CD8^+$ T lymphocytes occupy about 20–30% of whole peripheral blood lymphocytes by analysis using anti-CD8 monoclonal antibody. The probability that all five single lymphocytes investigated were $CD8^+$ was very low; $(0.2)^5$–$(0.3)^5$. Thus, $CD8^-$ lymphocytes could express CD8 mRNA. The mRNA of IL-2, a major cytokine secreted by lymphocytes, was detected in a single lymphocyte. Gene transcripts for PTH, digestive, and cerebral hormones were also expressed in the same lymphocytes.

Igs are secreted by B lymphocytes when stimulation by antigens induces the class switch phenomenon. TCR is believed to be expressed only in T lymphocytes. In a sperm, a single paternal gene coding for IgM through IgA and a gene for TCR exist. Even though IgG2 and IgG4 were not investigated, detection of mRNA for the heavy-chain constant region of Igs and TCR in a single sperm suggests that spontaneous transcription occurs in a cell. There is no evidence that rearrangement or class switch takes place in a sperm.

Each single lymphocyte possesses mRNAs for constant regions of all Igs and TCR, whatever the phenotype of the lymphocyte. In lymphocytes, the process of Ig production includes allelic exclusion of one gene. Therefore, the mRNAs detected in this study, except one which corresponds to phenotypic expression, were considered to be transcribed from either an excluded allele or are sterile transcripts which had been initiated from promoters just upstream of the constant region genes.

Taken together with previous reports, these results strongly suggest that, in the human, all gene transcripts are expressed in all cells, both of somatic and germ lineage, at basic levels. Not only the genes of cell skeleton proteins and enzymatic proteins, the so-called housekeeping proteins, but also the genes for other proteins, which are probably useless for differentiated cells and germ cells, are considered to be transcribed in a cell. If it is only actively transcribing genes that are unmethylated, then expression of any mRNA is not cell specific, but cell dominant.

The mechanism of gene transcription, in particular the positive and negative regulatory systems have been studied at the molecular level. Some transcription factors, DNA motifs or elements, and their complicated feature have been revealed. Conversely, the most fundamental problem of cell differentiation is still unsolved. Consensus sequences of silencer and enhancer elements have been reported, and the existence of nuclear or cytoplasmic factors which bind to the DNA motif has been revealed. However, each cell must carry the same gene in an individual. In addition, the same DNA sequences encoding the DNA-binding factors are also preserved in every cell. It is possible that both silencer and enhancer factors function almost simultaneously. Thus, the net balance of negative and positive regulatory factors may determine further transcription and cell differentiation.

Recently, a general transcription factor (TF), TFIIH, has been reported to consist of RNA polymerase II, CDK-activating kinase (cdk7 and cyclin H), DNA helicase, and other subunits (Zawel and Reinberg 1995). This means cell division, transcription, and repair cooperate with each other. Thus, a minimal amount of mRNA, without functional meaning, could be expressed purely by a chemical reaction and be consumed or vanish as time passes; cells consume energy in vain to produce functionally meaningless gene transcripts. Chelly et al. (1988, 1989) reported the occurrence of

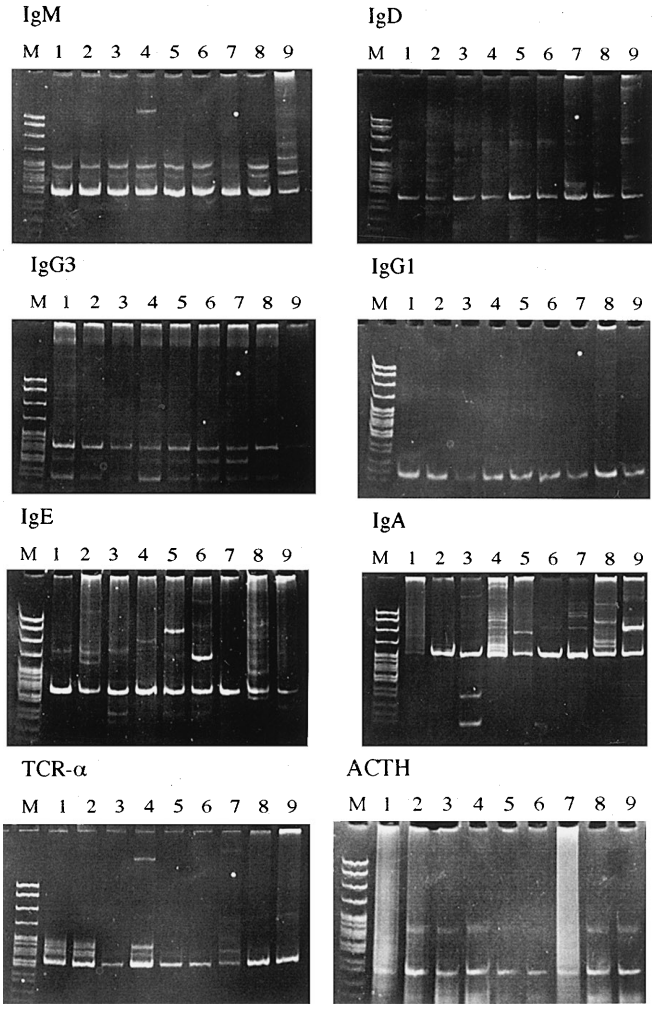

Fig. 4 PCR products amplified from mRNAs for IgM, IgD, IgG1, IgG3, IgE, IgA, TCR-α, and ACTH mRNA in single human lymphocytes. *M* pBR322/*MspI* digest. 1–9 Nine single lymphocytes; *identical number* means the same lymphocyte

illegitimate transcription in any cell type and anticipated that all promoters could be minimally active when ubiquitous transcriptional factors reach their cognate DNA element, leading to very low, but probably not null, gene transcription. The present study confirmed their prediction and the phenomenon occurs in each cell.

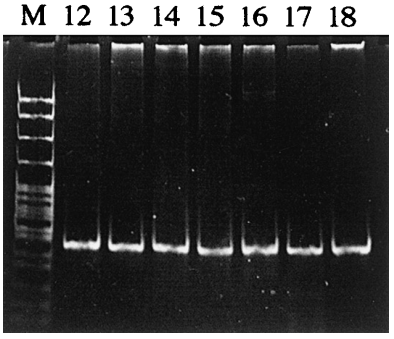

Fig. 5 Expression of mRNA for synapsin I in single lymphocytes and a sperm. *M* pBR322/*MspI* digest. 12–17 Single lymphocyte, 18 single sperm

Eventually, the diagnostic implication for genetic screening will be realised. For example, the availability of any mRNA of cell dominantly expressed genes in inaccessible tissues such as neurons or deep organs could be studied on the cDNA level from lymphocytes prepared from any patient with ease (Cooper et al. 1994).

All genes may be transcribed constantly in the DNA → RNA basal flow direction. This minimal amount of mRNA should be preserved in any single cell of human beings or eukaryotes, and could be ignored for life, although mRNAs and their translated proteins can function biologically only at greater amounts. The constant transcription or preservation of this minimal amount of mRNA might simply represent the result of a pure chemical reaction, which never stops unless the temperature becomes 0 K. Even though these chemical reactions need energy derived from adenosine triphosphate, the total response must be regulated by the second law of thermodynamics. Overall transcription and translation, so-called differentiation and the construction of life and body, may be considered simply as a series of chemical reactions and be directed by the balance of these chemical reactions.

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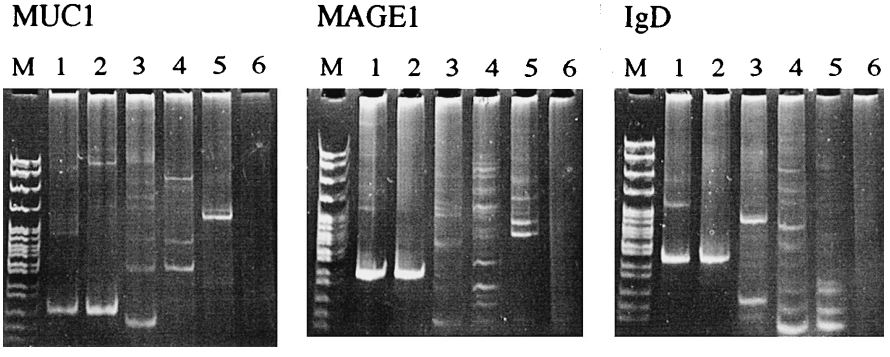

Fig. 6 Human MUC1, MAGE1, and IgD mRNAs are expressed in human lymphocytes but not in chicken, fish, and chickweed leaf. *1* Human lymphocytes, *2* human IL-2-activated lymphocytes, *3* chicken splenocytes, *4* fish, *Medaka*, *5* chickweed leaf, *6* H_2O

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