Brief Definitive Report

INDUCTION OF IMMUNOGLOBULIN GENE EXPRESSION IN MOUSE FIBROBLASTS BY CYCLOHEXIMIDE TREATMENT

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The DNA rearrangements of immunoglobulin (Ig) genes enable a limited number of variable region (V) gene segments to express diverse specificity of V region–coding DNA sequences by variable-joining region (V-J) or variable-diversity/joining region (V-DJ) joining, and also construct a complete set of a transcriptionally active Ig gene by arranging a specific V gene segment in the vicinity of a transcriptional enhancer element that is located between the J and constant (C) regions (1–3). The transcriptionally active enhancers have been identified in the J-C intron of heavy and κ-light chains of the mouse and human (2–11). The enhancer element associated with the Ig gene functions exclusively in B lymphocyte–derived (myeloma) cells, and not in the cells of other types; i.e., it acts in a cell-specific manner (2–5). Most recently (12), the other types of introns that may regulate Ig gene expression were proposed in the 5′ promoter region. We have cloned an entire gene of a rearranged human γ_1-heavy chain gene. The cloned gene, HIG1, possesses a 5′ promoter region and an enhancer element located 1 kilobasepair (kbp) downstream from the J region exon. A remarkable expression of human γ_1 chain gene was observed by introducing HIG1 gene into mouse myeloma cells, but it was not expressed in mouse fibroblasts (L cells). When the stable transformants of L cells, containing one copy of HIG1 DNA, were pretreated with cycloheximide for a short period, they produced a large amount of mRNA coding for human γ_1 chain, as well as γ_1 Ig in cytoplasm. We discuss a mechanism of the breakdown of cell-specific expression of Ig genes by cycloheximide and propose a crucial role for a repressor molecule in Ig gene expression.

Materials and Methods

Protoplast Fusion. Plasmid pSV2-HIG1 containing the human γ_1-heavy chain gene (HIG1) was constructed by inserting a 21 kbp EcoRI fragment possessing the complete set of a rearranged human γ_1 gene (5′ flanking region, leader sequences, VDJ_6 segment, enhancer element, constant gene segments, membrane exon, and 3′ flanking region) into the EcoRI site of plasmid pSV2*gpt* (13). The transcription orientation of the γ_1 gene is opposite to that of the *gpt* gene. For transfection of the plasmid pSV2-HIG1 to mouse myeloma cells and to mouse L cells, a protoplast fusion technique (14) was used. The transformed cells were selected and cloned in RPMI 1640 medium containing 10% fetal calf serum, xanthine (250 μg/ml), hypoxanthine (15 μg/ml), thymidine (10 μg/ml), and

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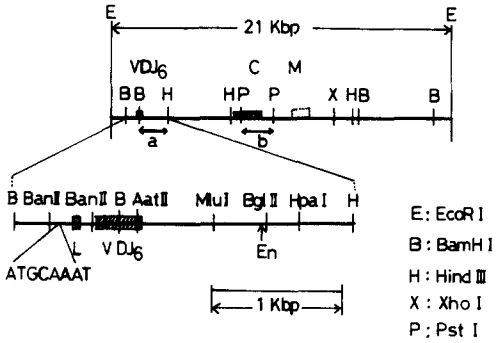

FIGURE 1. Structure and restriction enzyme map of HIG1 gene. A 21 kbp EcoRI fragment contains entire segments of rearranged human γ_1 chain gene. *L*, leader sequence; *V*, variable region; *D*, diversity element; *J*, joining region; *C*, constant region; *M*, membrane exon; *En*, enhancer element; *ATGCAAAT*, conserved sequence found in 5′ ends of mouse and human Ig gene (12). (*a*) 1.8 kb HindIII-BamHI probe; (*b*) 2.0 kb PstI probe.

mycophenolic acid (6 μg/ml). Detection of human γ chain Ig in the cells was performed by using fluorescein isothiocyanate–labeled goat anti-human γ chain–specific antibodies (E. Y. Lab).

Southern Blotting Analysis. DNA from pSV2-HIG1 transformed L cells was digested with EcoRI, electrophoresed on a 0.7% agarose gel, transferred to nitrocellulose paper, and hybridized to nick-translated γ_1 gene (2.0 kbp PstI fragment).

RNA Blotting Analysis. L cells containing one copy of HIG1 DNA or ARH-77 cells were cultured with 10 μg/ml cycloheximide for 8 h. After washing cells with phosphate-buffered saline (PBS), RNA were extracted. Total RNA (~10 μg for each sample) was electrophoresed on a 0.75% agarose–6% formaldehyde gel, transferred to nitrocellulose, and hybridized with nick-translated $C_{\gamma 1}$ probe (2-kbp PstI fragment) or $V_{\gamma 1}$ probe (1.8 kbp HindIII-BamHI fragment).

Results

Human plasma cell leukemia line, ARH-77, produces Ig of secretory as well as membrane-bound IgG (γ_1, κ). A complete set of a human γ_1-heavy chain gene was cloned from a recombinant phage library of ARH-77 DNA. The cloned gene, which is designated HIG1 and 21 kbp EcoRI fragment, contains the 5′ flanking region, leader sequences, rearranged VDJ_6 segment, enhancer element, switch region (S), constant region ($C_{\gamma 1}$), membrane (M) exon, and 3′ flanking region (Fig. 1). The enhancer element is located 1 kbp downstream from the 3′ end of the J_6 exon. The complete nucleotide sequence of the HIG1 gene will be reported elsewhere.[1] HIG1 gene was insertd into the EcoRI site of plasmid pSV2*gpt*, which contains the *Escherichia coli gpt* gene and the SV40 early promoter. The orientation of HIG1 gene was opposite to that of the SV40 promoter. The HIG1 gene has been introduced by the protoplast fusion (14) into mouse myeloma cells (NS-I or J558L cells [15]) and mouse L cells. A large amount of secretory human γ_1 chain was produced in the mouse myeloma cells. On the other hand, the mouse L cells transformed with pSV2-HIG1 did not produce any human γ_1 Ig, indicating that HIG1 gene enhancer(s) act in a cell-specific manner but not in a species-specific manner. About one copy of a complete set of the HIG1 gene was integrated in the host DNA per (stably transformed) mouse L cell (Fig. 2 *a*). The transformed L cells were very stable and retained

[1] Kudo, A., T. Ishihara, Y. Nishimura, and T. Watanabe. *Gene (Amst.)*. In press.

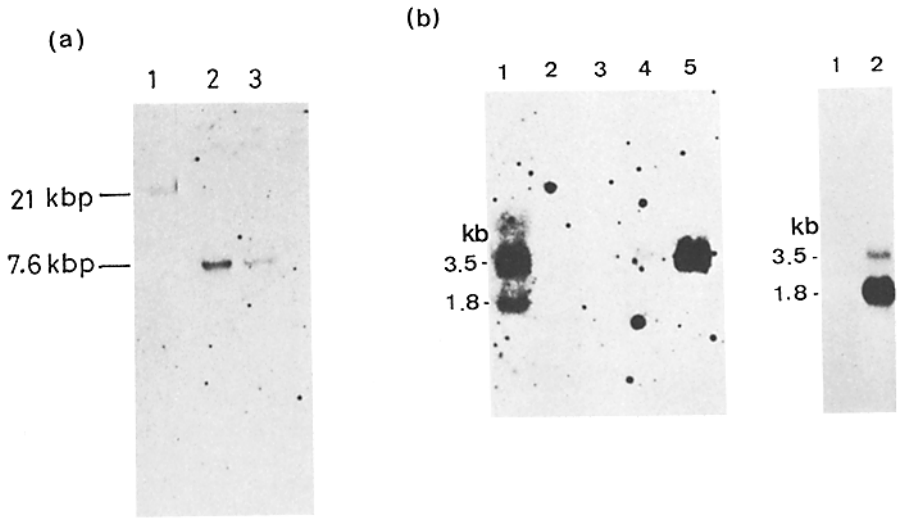

FIGURE 2. DNA and RNA blotting analysis of pSV2-HIG1-transformed L cells. (*a*) Southern blotting analyses. (Lane *1*) DNA from pSV2-HIG1-transformed L cells. The probe is 2.0 kbp PstI fragment of $C\gamma_1$ gene; (lane *2*) one copy of DNA of 7.8 kbp HindIII fragment of $C\gamma_1$; (lane *3*) three copies of $C_{\gamma 1}$ HindIII fragment. (*b*) RNA blotting analysis. Total RNA (10 μg per lane) was electrophoresed on a 0.75% agarose–6% formaldehyde gel, transferred to nitrocellulose, and hybridized with nick-translated $V_{\gamma 1}$ probe (1.8 kbp HindIII-BamHI fragment). 3.5 kb mRNA is membrane bound mRNA, and 1.8 kb mRNA is secretory mRNA. (*Left*) lane *1*, ARH-77; lane *2*, normal L cells; lane *3*, CX-treated L cells; lane *4*, pSV2-HIG1-transformed L cells; lane *5*, CX-treated, pSV2-HIG1-transformed L cells. (*Right*) lane *1*, NS-I cells; lane *2*, pSV2-HIG1-transformed NS-I cells.

the HIG1 gene in their DNA for >6 mo so far. They did not produce any human γ_1 chain molecules in the cells. The transformed L cells were then treated with 10 μg/ml of cycloheximide (CX) for 8 h in vitro. The RNA was extracted and analyzed by Northern blotting using the $V_{\gamma 1}$ gene fragment as a probe (Fig. 2 *b*). The CX-treated pSV2-HIG1-transformed L cells produced at least 100-fold more human γ_1 mRNA compared with untreated L cells, which produced trace amount of the γ_1 mRNA. Such treatment elevated the level of γ_1 mRNA in ARH-77 cells by only two- to threefold (data not shown). The level of the γ_1 mRNA in CX-treated L cell transformants was comparable to that in NS-I myeloma cells containing the HIG1 gene. The size of γ_1 mRNA produced in CX-treated L cell transformants (i.e., membrane-bound γ_1 heavy chain [γ_m] mRNA) was exclusively 3.5 kb. The cells were treated with CX for 4 h, pulsed with actinomycin D for 1 h, washed, and cultured for another 24 h. As shown in Fig. 3, human γ_1 chain was detected in the cytoplasm of the CX-treated transformed L cells. Surface Ig was negative. These data indicated that the human heavy chain gene is silent in pSV2-HIG1-transformed mouse L cells, possibly due to regulation by the cell-specific enhancer(s), but it becomes actively transcribed and translated by a short-term treatment with a protein synthesis inhibitor, such as CX.

Discussion

It is well known that hybrids between myeloma and B lymphoid cells continue to produce Ig and that the Ig genes derived from both parental cells are

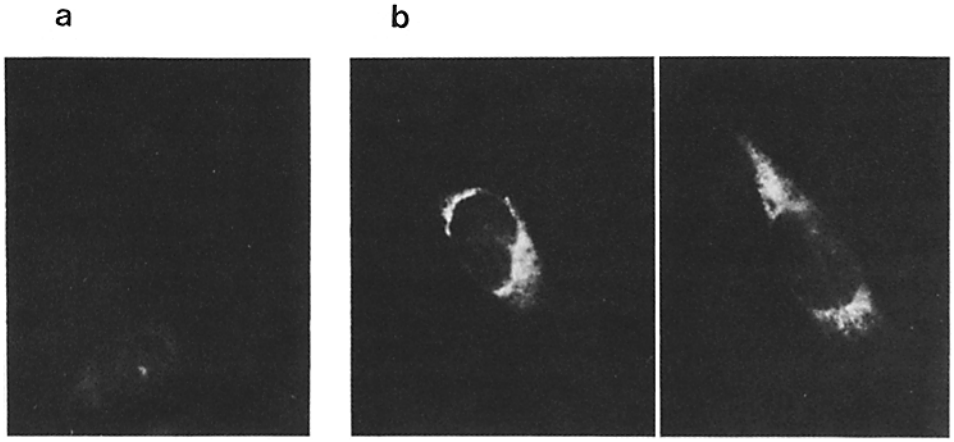

FIGURE 3. Human γ_1 chain was produced in the cytoplasm of L cells containing the HIG1 gene, after treatment with CX. The transformed L cells containing one copy of HIG1 gene were cultured with 10 μg/ml CX for 4 h, pulsed with 10 μg/ml actinomycin D for 1 h, washed, and recultured without CX and actinomycin D for another 24 h. The fixed cells were stained with fluorescein isothiocyanate–labeled $F(ab')_2$ of goat anti-human γ chain–specific antibodies (E. Y. Lab). (*a*) Nontreated L cells containing the HIG1 gene, (*b*) CX- and actinomycin D–treated transformed L cells.

codominantly expressed (16, 17). On the other hand, the hybrids between two cells that show various differentiated characteristics generally lose the ability to show some of these characteristics (18, 19). When Ig-secreting myeloma cells are fused with fibroblasts, Ig synthesis completely ceases (19). One of the ideas proposed (20) to interpret this suppression is that the cells, such as fibroblasts, may contain a diffusable repressor molecule, which is *trans*-acting and causes the cessation of Ig gene expression. Induction and superinduction by CX have been reported concerning various genes, such as interferon (21, 25), c-myc (22), interleukin 1 (IL-1) (23), and IL-2 (24). One explanation proposed for induction by CX is that CX might inhibit the synthesis of the labile repressor protein (21, 22, 25). Treatment of the transformed cells with CX for a short time might inhibit synthesis of the labile repressor protein, which in turn would increase the expression of human γ_1 chain gene in L cells. We suppose that mouse L cells possess transcriptional apparatus or factors similar to those present in B lymphoid cells, which may function through enhancers, and that a rearranged Ig gene will be transcribed in L cells unless a repressor is functioning. A repressor molecule that acts on the Ig gene may be continuously produced in L cells but not in myeloma cells. Some active mechanisms acquired during B cell development may suppress the formation of the repressor molecule in antibody-forming cells.

To identify the repressor molecule(s), we have extracted nuclear protein from L cell nuclei and transferred it into myeloma cells by a microinjection technique (26). Human γ_1 chain mRNA synthesis in mouse myeloma cells containing HIG1 gene appears to be remarkably affected by microinjection of L cell nuclear protein (manuscript in preparation). Such factor(s) may be related to the DNA-binding nuclear proteins that regulate gene expression in mammalian cells.

The transcript produced in CX-treated transformed L cells was exclusively of a membrane-bound cell surface receptor molecule (γ_m). It has been reported

(27) that a form of developmentally regulated RNA processing is responsible for the level of membrane-bound (γ_m) and secretory-type (γ_s) mRNA. Apparently, L cells do not have regulatory molecules that are responsible for cleavage of nascent transcripts or that induce polyadenylation at the first site (3′ end of the $C_{\gamma 3}$) to produce γ_s mRNA; polyadenylation occurred only at the second site (3′ side of the membrane exon).

Summary

A complete set of a rearranged human γ_1-heavy chain gene, HIG1, was cloned from human plasma cell leukemia line, ARH-77, and transferred into mouse cells. It was strongly expressed in mouse myeloma cells but not in mouse L cells, indicating that immunoglobulin gene expression is not species-specific but cell-specific. However, a remarkable production of human γ_1 chain was induced in mouse L cells containing HIG1 gene when the cells were treated with cycloheximide for a short period. The role of a labile repressor molecule in the expression of the immunoglobulin gene is proposed.

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