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The Effect of 50 Hz Magnetic Field on GCSmRNA Expression in Lymphoma B Cell by mRNA Differential Display

R. Y. Wu,[1] H. Chiang,[1] G. L. Hu[1]* Q. L. Zeng,[1] and J. L. Bao[2]

[1]Microwave Lab, Medical College, Zhejiang University, Hangzhou 310031, P.R. China
[2]Department of Physiology, Medical College, Zhejiang University, Hangzhou 310031, P.R. China

Abstract Magnetic fields (MFs) of various characteristics can lead to plethora effects in biological system. From a molecular point of view, we hypothesized that there must be a fundamental difference in gene expression between the MF exposed and the unexposed cell. To identify the classes of genes that are regulated, 0.8 mT 50 Hz MF-induced changes in gene expression were examined in a Daudi cell culture using differential display and reverse transcriptase-polymerase chain reaction. A candidate cDNA (signatured as MF-CB) that was observed in the sham-exposed but not in MF-exposed cultures was recovered and reamplified. After verification by Northern blot, the cDNA was cloned and sequenced. It was found that 254-base pair of 5′-end MF-CB cDNA clone was identical to *gcs* in open reading frame (ORF) range. Based on the preliminarily sequence, the prolonged length of 5′-end MF-CB cDNA was obtained by PCR amplification and its sequence analysis showed the same results as its original fragment. In order to further determine whether MF-CB cDNA is from *gcs,* two Northern blots were probed with *gcs* and MF-CB cDNA, respectively, and the data revealed signals of the same size and expression pattern on the two probe filters, which demonstrated that MF-CB is an EST (expression sequence tag) of *gcs*. *gcs* is a gene, identified recently (GenBank accession number D89866), encoding ceramide glucosyltransferase (GCS), which has been implicated as a causal element in human cell growth and differentiation. In an additional experiment, time-dependent changes in the transcription of *gcs* induced by 0.8 mT MF were observed by Northern blot with a sharp and reproducible inhibition effect after 20 min exposure and a reduction after 20–24 h exposure. The study demonstrates for the first time that 50 Hz MF can lead to changes in *gcs* transcription, which provides a new clue to elucidate the mechanism by which MF influence cell growth and differentiation. J. Cell. Biochem. 79:460–470, 2000.

Key words: 50 Hz magnetic field; differential display; GCS mRNA transcription

Numerous epidemiological studies involving both children and adults have strongly implicated job-related, environmental, and residential exposure to extremely low frequency (ELF) magnetic fields (MF) as a possible factor in the development of certain human cancers, especially human lymphoblastic leukemia [Shaikh, 1986; Saritz et al., 1988; Pool, 1990; Floderus et al., 1993, 1994; Taubes, 1993; Feychting and Ahlbom, 1994]. A variety of bioeffects that result from the exposure of tissues and cells to ELF MF are reported in literature. Representative examples include changes in cell surface properties [Phillips et al., 1986a, b; Morron et al., 1988; Liburdy et al., 1993; Paradisi et al., 1993], increased calcium influx [Lindstrom et al., 1993, 1995; Galvanovskis et al., 1999a, b], altered rates of DNA, RNA, and protein synthesis [Marron et al., 1975; Liboff et al., 1984; Goodman et al., 1987; Goodman and Henderson, 1988; Phillips and McChesney, 1991; Goodman et al., 1993; Greene, 1993; Zhao et al., 1999], enhancement of ODC enzyme level [Byos et al., 1987; Mullins et al., 1999], inhibition of nocturnal levels of melatonin [Reiter, 1995; Truong and Yellon, 1997; Brainard et al., 1999], suppression of T-lymphocyte cytotoxicity [Lyle et al., 1988, 1991], and effects on cell growth and proliferation [Cantini et al., 1986; Liburdy et al., 1993; Nakajima et al., 1997; Schimmelpfang et al., 1997; Katsir et al., 1998; Loschinger et al., 1998].

The course of normal development as well as the pathological changes that arise in diseases

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*Correspondence to: Ruiying Wu, Microwave Lab, Zhejiang University, Medical School, Yan An Road 353, Hangzhou, Zhejiang, 310006, PR China

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such as cancer are driven by changes in gene expression. Altered gene expression lies at the heart of the regulatory mechanisms that control cell biology. This raises the possibility that multi-effects of MF must be accompanied by fundamental changes in gene expression. As early as 1983, Goodman and colleagues [1983] reported that two different electromagnetic signals—a single pulse repeated at 72 Hz and pulse train at 15 Hz—each increased gene transcription in dipteran salivary gland cells, compared to unexposed control cells. Furthermore, increased mRNA and rRNA production after MF exposure was reported [Goodman and Henderson, 1986a, b, 1991; Goodman et al., 1989; 1992; Phillips and McChesney, 1991; Greene et al., 1993; Gold et al., 1994; Nagai and Ota, 1994; Heermeier et al., 1998; Miyakoshi et al., 1998].

Evidence is beginning to accumulate indicating that MF-induced changes in gene expression can be involved in the cellular MF response, because MF-induced gene expression is dependent on exposure key parameters (time, frequency, and flux densities) and the cell type. Goodman et al. [1990] reported that when MF were exposed to human HL-60 cells for 20 min, the most pronounced increase in the expression of *c-myc* and Histone H2B transcripts occurred after exposure at 45 Hz and corresponding power strength of 0.7 mT [Wei et al., 1990]. In the experiment reported by Phillips et al. [1992], after CEM-CM3 T-lymphoblastoid cells were exposed to 0.1 mT 60 Hz MF for 15–120 min, time-dependent and cell density-dependent changes in the transcription of *c-fos, c-jun, c-myc,* and protein kinase C (β-form) were observed [Phillips et al., 1992]. However, the number of gene probes used to assess the transcription effects of MF is still very limited. The challenge now is to identify which genes are induced under which key parameters and in which cells, and, ultimately, to determine the function of the induced proteins in the MF responses.

mRNA differential display technique (DD) is a powerful method to select for novel genes by comparing differential expression pattern of genes between two or more cell populations, which can be simultaneously up-regulated as well as down-regulated; genes including known and unknown that are differentially regulated in cells under various conditions [Liang and Pardee, 1992]. In order to obtain more information about genes involved in MF exposure, DD method was used in the present study to screen and clone differentially expressed genes in MF-responsive lymphoma B cells following 50 Hz MF exposure. With a combination of primer, a candidate cDNA fragment (designated as MF-CB) was identified as down-regulated by MF exposure. MF-CB cDNA has been DNA-sequenced and identified as *gcs*. GCS is a recently cloned protein encoding glucosylceramide (GlcCer) transferase (UDP glucose: N-acylsphingosine D-glucosyltransferase, EC 2.4.1.80), which is a key regulatory factor controlling intracellular levels of CerGlc, ceramide, and glycosphingo-lipid (GSL) [Ichikawa et al., 1996]. Recently, GCS and its relative products, including Glcer, GSL, and ceramide, have been implicated not only as causal elements in cell growth and differentiation but also as key components in a signal transduction (ST) pathway for growth and differentiation [Zador et al., 1993; Sando et al., 1996; Hannon, 1997].

MATERIALS AND METHODS

Exposure System

The system was composed of three coils shielded in a mu-metal container within a CO_2 incubator (Model 3164, Forma), a power regulator to regulate the flux densities, and a voltage stabilizer to remove electrical spikes and transients. The coils of upper, middle, and lower are, respectively, 104, 52, and 104 rounds of copper wire in series with 36 cm width and 18 cm apart. The 50 Hz sinusoidal MF was generated from line current with a transformer to provide uniform (0.1%) 0.8 mT MF in the central area ($10 \times 10 \times 10$ cm^3 of three-dimensional space) of the coils. The direction of the applied field in the incubator was horizontal and directed to the north. A similar Forma incubator was used for sham exposure with alternated electrical connections resulting in zero magnetic induction. The stray field in both incubators was < 5–10 μT and the total static MF was 18.5 mT with 14.1 mT horizontal and 12.0 mT vertical components. Model 120-1-25 monitor (Simpson Electrical Co.) with a sensitivity of 0.1 μT was used for checking and adjustment magnetic flux densities before each experiment.

Daudi cells, a human Burkitt lymphoma B line, obtained from Suzhou Medical University, were grown in RPMI-1640 (Gibco-BRL) containing 25 mM Hepes (Sigma) and supple-

mented with 15% fetal bovine serum, penicillin/streptomycin (100 U and 100 μg per ml), and Kanamycin sulfate (100 U per ml). Twelve h prior to field exposure, a bottle of Daudi cells was passaged to a pair of cultures with the final densities of $0.8–1 \times 10^6$ cells/ml in 75 cm^2 Corning flasks. Cell viability at this time was >95% determined by trypan blue exclusion. At the DD experimentation, the cultures were exposed to 50 Hz MF at 0.8 mT or sham-exposed for up to 24 h, respectively.

mRNA Differential Display (DD)

DD analysis was carried out as previously described [Liang and Pardee, 1992, 1994; Liang et al., 1994]. Total cellular RNA from the exposed and sham-exposed cells were extracted with TRIZOL (Gibco-BRL) according to the manufacturer's instructions. Contaminating traces of DNA were eliminated from the total RNA sample (50 μg) by digestion at 37°C for 1 h with DNase I (Gibco-BRL) in the presence of RNase inhibitor (Gibco-BRL). After phenol/chloroform extraction and ethanol precipitation, DNA-free total RNA (0.2 μg) from each sample was reverse transcribed in a 20 μl reaction mixture at 37°C for 1 h with Superscript reverse transcriptase (Gibco-BRL) using three anchor primers of T12A, T12C, and T12G, respectively. Polymerase chain reaction (PCR) was then performed in reaction mixture containing 0.1 volume of reverse transcription reaction mixture, 1 × PCR buffer, 20 μM MgCl, 20 μM dNTPs, 10pmol T12M (A,C,G), 10pmol 10-mers arbitrary primers (AP), (α-^{32}P)-dATP-5 μCi/L (DuPont NEN Research Products, Boston, MA) and 1 U Ampli Taq DNA polymerase (Promega) on a Perkin-Elmer 480 thermal cycler. Parameters for PCR reactions were performed at 94°C for 5 min and 40 cycles at 92°C for 1 min, 40°C for 2 min, 70°C for 30 sec and followed by 72°C extension for 10 min. The radioactively labeled PCR fragments from RNAs of the exposed and sham-exposed cells were side-by-side resolved on a 6% DNA-sequencing gel for 5 h. The gel was dried and autoradiographed for 2 days. Any bands evident under one exposure and apparently absent in the other were identified. PCR product bands of interest were recovered from sequencing gels, eluted in buffer as described [Liang et al., 1994], and reamplified in a 40-cycle PCR in the absence of isotope. Reamplified cDNAs ranging > 300 bp were used for clonging into plasmid vectors.

Northern Blot

Samples of total RNAs (30 μg) from the exposed and sham-exposed cells as indicated were fractionated in 2.2 M formaldehyde/1% agarose gels and transferred onto charged nylon membranes (Amersham) by standard capillary blotting techniques. MF-CB band from DD was cut out from the dried gel, amplified by PCR, confirmed in minigels, cloned, and the EcoR I fragment of the plasmid was then purified and used as a probe in Northern analysis to examine the pattern of expression. GCS cDNA clone (sent by Dr. Ichikawa kindly) was digested, and the inserted fragment (1.6 kb) was then purified and used as a probe. The probes were labeled with α-^{32}P using random prime DNA labeling kit (Promega). After hybridization at 42°C and high-stringency washes at 60°C in 0.2 × SSC/0.1 × SDS three times, the blots were exposed to X-ray film overnight at 80°C. After being stripped of previous probes by heating in 0.2 × SSC/0.5 × SDS for 10 min at 100°C, the membranes were reprobed with a glyceraldehyde-3-phosphate dehydrogenase probe (GaPDH) as described above for normalization of mRNA levels. mRNA expression was quantified using a Molecular Imager (Bio-Rad).

cDNA Clone and Sequence Analysis

The candidate PCR-fragments were cloned into pGEM^R-T Easy vector (Promega) with the inserted site as EcoRI and the host bacteria as DH-5α. After the inserted fragments were digested and identified by 3% agarose gel, the cDNA clones were sequenced by the dideoxy method using Taq Track sequence kit (Promega). MF-CB cDNA sequence and the amino acid sequence of the predicted protein product were used to search for similarities in nucleic acid and protein databases through GenBank and EMBL, DDBJ, and PDB databases.

Prolonged Length of MF-CB Clone by PCR

The prolonged MF-CB cDNA was amplified as described by Ohara et al. [1989] with minor modification: the anchored primer was designed complementary to nt 467–447 of MF-CB (5′ CGT AGT TTG GTC CAC CTG ATG A 3′) and the 5′-end AP was corresponding to nt 675–695 of GCS (pM675: 5′-TGC CAG GAT ATG AAG TTG CAAA 3′ (GenBank accession

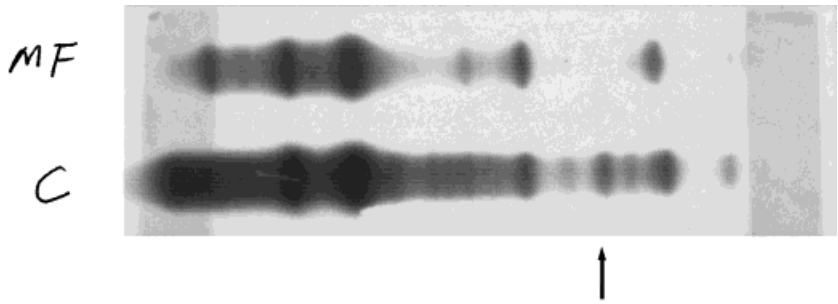


Fig. 1. Identification of differentially expressed genes in Daudi cells following a 24-h exposure to a 50 Hz MF using the differential display technique. A bottle of Daudi cell, was passed into two of 75 flasks, each at a cell density of 0.8×10^6 cells/ml 16 h prior to field exposure. The cultures were then randomly put in closed circles or open circles, respectively, for sham-exposure or exposure to 50 Hz MF at 0.8 mT for up to 24 h. Total RNAs were reverse transcribed using $T_{12}A(G,C)$ anchors, PCR was performed in the presence of α-^{32}P-dATP. One-tenth of the reaction was separated on a 6% sequencing gel as described in Liang and Pardee [1992]. Selected cDNA shown was amplified using the set of T_{12} A -CTTGACGGGG. Both lane C and lane MF represent cDNA transcription obtained by DD from Daudi cells after sham-MF and MF exposure, respectively.

number D89866). Total RNA was isolated from Daudi cell using RNAzol. First-strand cDNAs were synthesized in a 20 μl-reaction volume as described above, except dT12–18 (5 ng/μl, Promega) was used as a primer. The resulting cDNA was then used as the template of the PCR reaction. The cycling parameter for PCR is as follows: 94°C for 5 min and 35 cycles at 92°C for 1 min, 55°C for 90 s, 72°C for 2 min and followed by 72°C extension for 10 min. The PCR product was cloned and sequenced as described above.

RESULTS

Differentiation Display and Northern Blot Confirmation

To identify transcriptionally regulated genes potentially involved in MF-induced effects, differential mRNA display patterns in Daudi cells after MF exposure and sham exposure were compared. As shown in Figure 1, in the primer combination of the anchor $T_{12}A$ and AP45 (CTTGACGGGGG), MF-CB cDNA was only present in the control culture and did not have a corresponding counterpart in the culture following 0.8 mT 50 Hz MF exposure for 24 h. MF-CB band was recovered from 6% sequence gel and then, prepared as above, used as a probe for Northern analysis. RNA was isolated from Daudi cells following MF and sham MF exposure for 20 min and 24 h, and reduction of MF-CB mRNA transcription was observed by 30% and 50%, respectively, when compared with that in the control (Exposure/control,

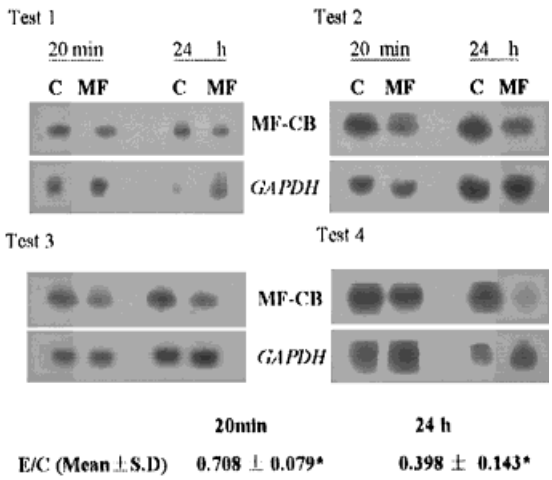
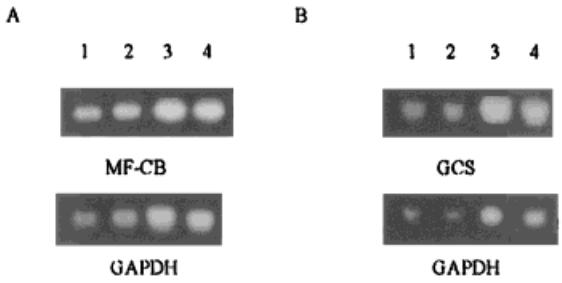


Fig. 2. Inhibition of MF-CB mRNA levels by exposure to 50 Hz MF at 0.8 mT. Northern membrane of RNAs from Daudi cells following 0.8 mT MF exposure for 20 min and 24 h was probed with MF-CB cDNA isolated from DD. The experiments were repeated twice with two plates for each exposure (n = 4). Reduction of MF-CB mRNA transcription was observed for 20 min and 24 h, respectively by 30% and 60%, when compared with that in the control. E/C is defined as the ratio of (MF-CB dpm/GAPDH dpm)$_{exposed}$/(MF-CB dpm/GAPDH dpm)$_{control}$. *$P < 0.01$ showed the statistical significance as compared to that of the sham exposure.

E/C = 0.708 ± 0.079 and 0.398 ± 0.143, respectively). To determine whether the reduced band in irradiated cells could be obtained again and was thus suitable for further analysis, three additional independent Northern blots were performed and revealed the same results as shown in Figure 2. These data indicated that, as suggested by the DD result, MF-CB expression in Daudi cells was inhibited by MF exposure under the test condition.



A	Lane 1	Lane 2	Lane 3	Lane 4
MF-CB dpm/GAPDH dpm	1.13	1.20	1.16	1.13
E/C		1.06	1.03	1.0
B				
GCS dpm/GAPDH dpm	2.19	2.24	2.36	2.09
E/C		1.02	1.07	0.95

Fig. 3. Northern blot verification of the relationship between MF-CB and GCS mRNA. RNA was isolated from the Daudi culture as the indicated time. Two identical filters were prepared and hybridized with MF-CB cDNA and GCS cDNA (1.1 kb), respectively. mRNA expression was normalized by GAPDH expression. The result revealed the same signals in size (about 3.8 kb) and in expression pattern on the two probe filters (E/C of the lanes 2, 3, and 4 from MF-CB and GCS mRNA is almost the same). **A:** MF-CB transcription; **B:** GCS mRNA transcription. Lanes: (1) control; (2) 0.8 mT, 5 min; (3) 0.8 mT, 2 h; (4) 0.8 mT, 4 h. The ratio E/C is defined as the ratio of (MF-CB dpm/GAPDH dpm)$_{exposed}$/(MF-CB dpm/GAPDH)$_{control}$.

Clong and Sequence Analysis of MF-CB

The nucleotide sequence and the predicted amino acid sequence of the cDNA were compared with nucleic acid and protein databases using the BLAST Server network service. The analysis revealed that MF-CB cDNA was 496 bp in length and over 254 bp at 5′ end of the cDNA was 95% identical to GCS mRNA in open reading frame (ORF) (Fig. 3A). The preliminary sequence was contrary to usual results in which the 3′ primer recognizes the 3′ end of the mRNA. In this instance, the 3′ primer hybridized with an internal sequence within the coding region of *gcs*. As a result, the PCR fragment of about 254 bases was entirely within the coding regions and thus permitted unambiguous identification of the gene by comparison with the published sequence. However, as to MF-CB cDNA here, 254 bp of 496 bp identical to GCS mRNA, it is still needed to be verified whether MF-CB is an EST of *gcs*. The prolonged length of 5′ end MF-CB cDNA was first obtained by anchor PCR with the anchored primer complementary to nt 467–447 of 5′end MF-CB, and 5′end AP corresponding to nt 675–695 of *gcs*. After cloned and sequenced, the inserted PCR product was shown 456 bp in length with 99% homologue to *gcs* in ORF (Fig. 3B), which further supported that MF-CB is an EST of *gcs*.

Northern Blot Verification of the Relationship Between MF-CB and *gcs* cDNA

The sequencing result of MF-CB cDNA as above has shown it very high homologue to *gcs* in ORF. Therefore, it is hypothesized that blot signals of MF-CB probe can be observed as the same as that of *gcs* probe if MF-CB cDNA is the EST of *gcs*. Subsequent Northern blots were performed. RNAs from Daudi cells after different MF exposure were prepared into two identical filters, which were then hybridized with MF-CB cDNA and GCS cDNA, respectively. Both the signals from MF-CB and GCS filters were shown 3.8 kb in size. As to the signal densities, each filter showed higher levels of messenger RNA in lane 3 (MF for 2 h) and lane 4 (MF for 4 h) when compared to lane 1 (control) and lane 2 (MF for 5 min). However, there was no difference among the lanes when normalized by GAPDH. As shown in Figure 3, the ratio of MF-CB dpm/GAPDH dpm from MF-CB cDNA filter was 1.13, 1.20, 1.16, and 1.13 in lanes 1, 2, 3, and 4 ($P > 0.05$), and that from GCS cDNA filter was 2.19, 2.24, 2.36, and 2.09 in lanes 1, 2, 3, and 4 ($P > 0.05$). From the ratio of E/C, no differences were found among the lanes in each filter and between the corresponding lanes in the two filters (E/C of MF for 5 min, 2 h, and 4 h were, respectively, 1.06,

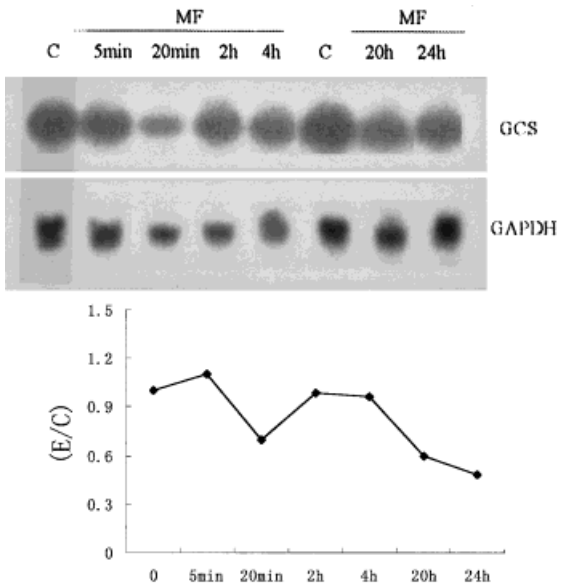


Fig. 4. Time course of GCS expression induced by MF. Daudi cultures were exposed to MF for 5 min, 20 min, 2 h, 4 h, 20 h, and 24 h. RNAs were isolated after MF-indicated exposure and probed with GCS cDNA. No changes in *gcs* expression were found as early as 5 min, while an decreased of 30% was detected after 20 min exposure when compared to the control, it recovered to a normal level within 2 h and maintained the control level until undergoing 20–24 h MF exposure, when a second reduction of *gcs* transcript levels by 60% was measured. The ratio E/C is defined as the ratio of (MF-CB dpm/GAPDH dpm)$_{exposed}$/(MF-CB dpm/GAPDH dpm)$_{control}$.

1.03, and 1.0 in MF-CB filter and were 1.02, 1.07, and 0.95 in GCS filter (shown in Fig. 4). These results, the same signals in size and in expression pattern from both MF-CB and GCS probes, strongly demonstrate that MF-CB is an EST of *gcs*.

Time Course of GCS Expression Induced by MF

In order to determine the time dependency of MF effect on *gcs* gene transcription, Northern blot analysis was performed using a DNA probe prepared from *gcs* cDNA clone. Daudi cultures were exposed to MF for 5 min, 20 min, 2 h, 4 h, 20 h, and 24 h, and RNAs were isolated after MF-indicated exposure. No change in GCS *mRNA* expression was found as early as 5-min after exposure, while a decrease of 30% was detected after a 20 min exposure, it recovered to a normal level within 2 h and maintained the control level until undergoing 20–24 h MF exposure, when a second reduction of *gcs* transcript levels by 50%–60% was measured (Fig. 5). These data indicated that, under the present condition, *gcs* expression could be reduced by MF exposure in Daudi cells as suggested by the DD results, and such MF effect has shown time dependent.

DISCUSSION

Numerous recent studies involving genes responsive to MF have exhibited a time-dependent effect. For human HL-60, changes in the expression of *c-myc* and histone 2B transcription lead to a peak after being exposed to MF for 20 min [Wei et al., 1990]; in CEM-CM3 T-lymphoblastoid cell, *c-fos* transcription increased after 20-min exposure and led to a mean maximal after 30 min; *c-myc* and protein kinase C (β-form) transcription increased to a peak after 20 min, three of the gene expressions recovered to the control level after 1 h [Phillips et al., 1992]. In the yeast model, the expression of *URA3* and *SSA1* heat-shock gene transcription can be affected by exposure to 60 Hz MF for 15–20 min, the most dramatic effect for *URA3* is after a 15-min exposure and for *SSA1* after a 20-min exposure [Weisbrot et al., 1993]. Using the bacterium *Escherichia coli,* the intracellular level of sigma 32 mRNA was enhanced following a 15-min exposure to a 1.1 mT 60 Hz MF [Cairo et al., 1998]. Even in the transcription/translation system a similar time-dependent pattern was observed for 72 Hz sinusoidal MF at 1.1 mT exposure [Goodman et al., 1993]. Evaluating from the reports as above, if there was transcription activation after the MF exposure point in a cellular system, it generally led to a peak at 15–30 min and recovered to a normal level in 1 h. In the present study, after MF exposure of early stage for 5 min, 20 min, 2 h, and 4 h, only at the 20 min point *gcs* gene transcription was observed inhibited, and it recovered to a normal level after 2 h. Daudi cells showing such a response to MF in gene levels is consistent with the data available.

However, there were many reports involving cellular effects after relatively long-term ELF MF exposure. For example, enhanced DNA synthesis was observed in human fibroblast cells exposed for 16–20 h to sinusoidal varying magnetic fields (SVMF) [Liboff et al., 1984]. Cell proliferation was detected after exposure to 0.7 mT MF in chick embryo fibroblasts (CEF) cell for 24 h [Katsir and Parola, 1998; Katsir et al., 1998] and in human peripheral bloods for 72 h [Scarfi et al., 1999]. In addition, the change in plasma membrane (a decrease in membrane fluidity and reorganization of cy-

A:

CTTGACGGGGCAAAAGAAGAAAACGGAGCTAGGCAATTTCATACTGATCAAAGCTCGAATTTTATTGTTGCGACACCAGTT
ATGAAGGAAGGGGGAGGGGACCCGATTCCCGCCGAATAATCTCTGGTCCAGTAGAAAGGTGCACGGGTGTGGCTCCGCAGT
TCCAGCAGTGGGCGTGGCAGAACGAATGAGCAGGAAGCTCCACCCCGAGCAAGCAGGTTTCAGGCTGGGGGAGGGGAGA
CTACACCTGTCTGTAAATTATTGTAGCAGGAAGCATGTTAATTCTCAATTTGGTCCACCTGATTCTCAATTTGGTCCACCTGAT
CATTCTGGATTGAAACTGAGAAATTGAGTACGAACCAGAGTTTTGGCATTTGGCATTGCAACTTGAGTAGACATTGAAAACC
TCCAACCTCGGTCGGCTATTGCTTTGGCCATAAAGTAATCTTCAGCAATGTACTGAGCAAAGGCTATGAGCCCTCCTGCCTGA
TCTAGCACATCCTTCCTCATCAAACAAGACATCCCCGTCAAG.

B

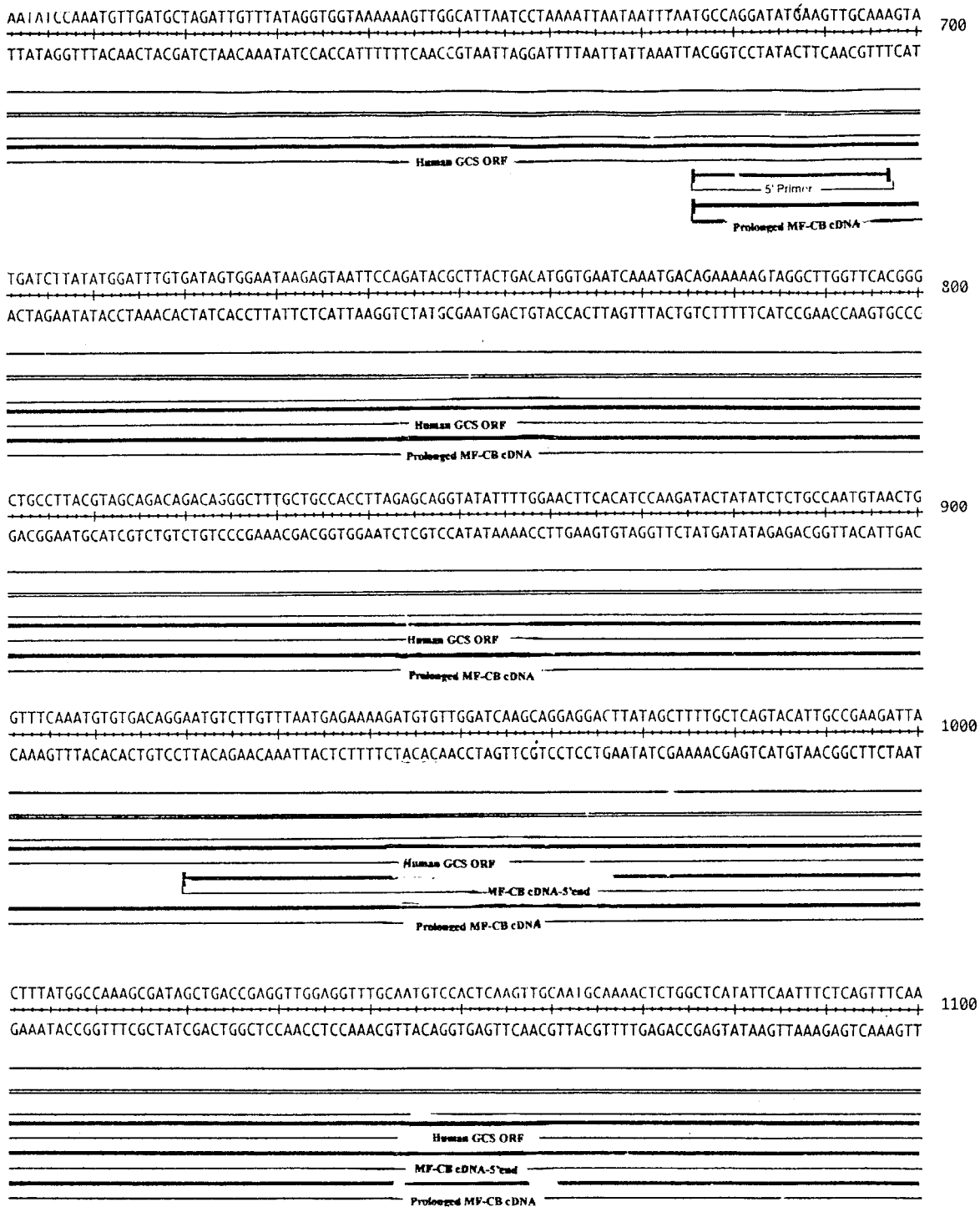


Figure 5.

Glucosylceramide Synthase mRNA Transcription and Magnetic Field

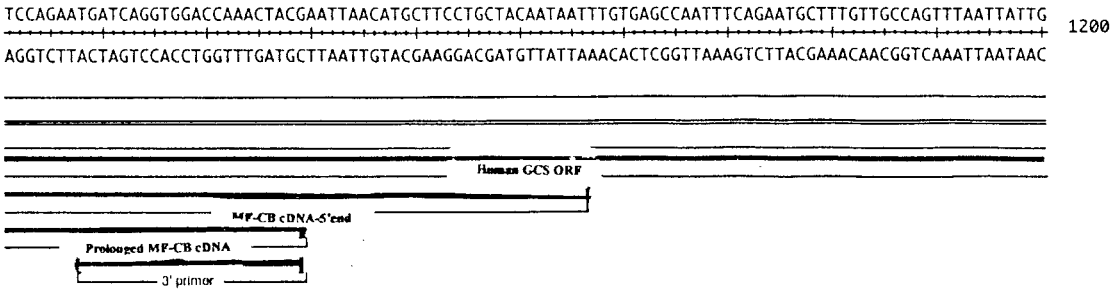


Fig. 5. MF-CB cDNA clone sequencing and its sequencing homology. The nucleotide sequence of the cDNA was compared with GenBank using the BLAST server network service. The analysis revealed that original MF-CB cDNA was 496 bp in length and over 254 bp at 5′ end of the cDNA was 95% identical to *gcs* in (ORF). The prolonged length of 5′ end MF-CB cDNA obtained was shown 456 bp in length with 99% homologue to *gcs* in ORF. **A:** MF-CB cDNA sequence. **B:** MF-CB cDNA and the prolonged length of 5′ end MF-CB clone homology to *gcs* (GenBank D89866) in ORF.

toskeletal components) in human B lymphoid cells (Raji) was found following 2 mT SVMF exposure for 72 h [Santore et al., 1997]. Changes in cell-surface structure and physiology in K562 cells were detected following 2.5 mT MF exposure for 96 h [Paradisi et al., 1993]. It is widely accepted that, on a functional level, diverse cellular process is mediated by the modulation of secondary response gene expression. Based on data as above, we speculated that MF exposure induces biological responses most likely by activating signaling pathways that lead to the induction of early response genes, which subsequently activate the transcription of secondary response genes. In the present study, using DD method, *gcs* gene transcription was newly identified being reduced after 50 Hz MF exposure. The changes in the transcription level showed time dependent, besides peaking at MF exposure for 20 min; MF exposure for 20 h and 24 h was another sensitively responsive point. The results provide direct evidence that MF can affect both primary responsive genes as well as secondary responsive genes. The changes in GCS mRNA transcription suggested that multieffects induced by MF may be mediated via *gcs* gene-inducing actions.

GCS, also termed glucosylceramide synthase, catalyzes the reaction of UDPGlc and ceramide to form UDP and GlcCer, which, in most cell types, serves mainly as a precursor for complex glycolipids or as a plasma membrane component [Ichikawa et al., 1996]. Effects of GCS inhibitors on specific cellular processes underscored the crucial roles of GCS in ganglioside metabolism, cell surface recognition, and adhesion properties, as well as regulation of cell growth [Zador et al., 1993; Ichikawa et al., 1994; Rani et al., 1995; Sando et al., 1996]. In a mouse B16 melanoma cell line with deficient GCS activity and undetectable glycolipids, altered morphology and a reduced growth rate was reported as compared to the parental line [Ichikawa et al., 1994]. Rani and colleagues [1995] also reported that the efficient GCS inhibitor PDMP (threo-1-phenyl-2-decanoylamine-3-morpholino-1-propanol) could lead to the cell arrest at the G1/S and G2/M transitions, subsequently reducing cell growth rate.

Data on epidemiologic studies have suggested that ELF magnetic field may contribute to the risk of childhood acute lymphoblastic leukemia and it is thought that MF may participate in leukemogenesis by influencing their

proliferation, suvival, and/or differentiation programs (Shaikh, 1986; Sartiz et al., 1988; Floderus et al., 1993, 1994; Zhang et al., 1995; Linet et al., 1997). As to acute lymphoblastic leukemia, leukemia cell growth rate is usually suppressed when compared to the other cancer cells. The pathogenesis of leukemia is characterized by retardation of cell development and differentiation, accumulation of large numbers of unmatured cells, and the inhibition of normal lymphoblastic cell proliferation [Inokuchi et al., 1989].

The present study has demonstrated that *gcs* transcription in lymphoma B cell can be inhibited by MF exposure, at least at early and later stages. From the view of *gcs* gene transcription and its corresponding function, the inhibition of *gcs* transcription may lead to down-regulated GCS enzyme activity, which can, consequently, retard leukemia cell development and differentiation [Rani et al., 1995]. Therefore, the present study provides new important evidence supporting MF effects on leukemogenesis.

In addition, there is nothing known about the mechanisms of regulation of *gcs* expression (e.g., promoter regions and relevant transcription factors), although the cDNA for human *gcs* expression has been cloned, it will be interesting to use *gcs* gene as a new end point to assess MF effects, which might provide new directions to explore its possible mechanism mediating via GCS route and the mechanism of MF interacting with biological systems.

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