Applications of Electromagnetic Phenomena in Electrical and Mechanical Systems
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Gene expression profiling of nematode *C. elegans* by exposing the magnetic fields

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Abstract

To investigate the possible mechanism of gene transcription changes induced by ELFMFs, we performed the screening of ELFMFs-responding genes. We identified 56 genes by differential display method and 2 additional genes when nematode *Caenorhabditis elegans* were exposed to magnetic fields up to 0.5 T at 60 Hz for 120 min. By densitometer analysis, thirty-three of 56 genes were up-regulated, and, as for 23 remaining genes were down-regulated by exposure of ELFMFs. Moreover, we demonstrated quantitative RT-PCR to confirm reproducibility of the mRNA expression level of the isolated genes. In cloned candidate genes, *mec-5* and *ncs-2* is primarily expressed in the sensory neurons, the expression level of both mRNAs was higher than the level of control by ELFMF exposure. These results suggest that both genes may be affected by ELFMFs exposure in the nervous system.

1. INTRODUCTION

Although the biological effects of ELFMFs (extremely low frequency magnetic fields) have been well studied in a large number of laboratory experiments, assessments remain contradictory [1-3]. Previously, we studied the effects of ELFMFs on the life cycle of nematode *Caenorhabditis elegans (C. elegans)*. We found that the behavior of *C. elegans* was abnormal in addition to embryonic and post-embryonic development following exposure to ELFMFs [4].

Generally, organisms respond to a difficult environment by producing stress proteins, called heat shock proteins (HSPs) [5, 6]. These proteins have been found in a wide variety of organisms, including bacteria, yeast, plants, nematode, fruit flies, and mammalian cells. We are interested in whether *C. elegans* perceive the ELFMFs as a stress. We focused on *C. elegans* HSPs, and we showed transcriptional levels of HSP16 which is stress-responsible protein were elevated in response to magnetic fields stimulation [7]. Furthermore, to clarify the mechanism of effects of ELFMFs, we have started the screening of ELFMFs responding genes.

2. MATERIALS AND METHODS

2.1 Growth and Maintenance of *C. elegnas*

C. elegans as a model animal is a suitable organism for study of the relationship between ELFMFs and biological stress response by following the reasons.

(1) Simply breeding and observation.
(2) Early lifecycle and expression of inherited character.
(3) Known the completed whole genome sequence.

C. elegans were synchronized, cultured and maintained for 8 hours at 20 ℃ on nematode growth medium (NGM) plates which were seeded with *Escherichia coli* OP50, essentially as described by Brenner [8]. The animals were exposed into a plastic dish 60 mm in diameter to the high magnetic field generator at 20 ℃ (exposed). As a control, worms were also incubated without exposure to ELFMFs at 20 ℃(control).

2.2 High magnetic fields generator

The high magnetic field generator is composed of two E-type cores with their poles placed face to face. The experimental space consists of a 13 mm gap in the centre. By supplying a 60 Hz, 190 A current, an AC magnetic fields with a peak flux density of 0.5 T (up to 1.2T) can be produced.

This machine was cooled by a high performance water circular system, and a quartz thermometer was used to monitor temperatures under the experiment. The fluctuation of temperature in experimental space is less than 0.1 ℃ (Fig. 1 and Table 1).

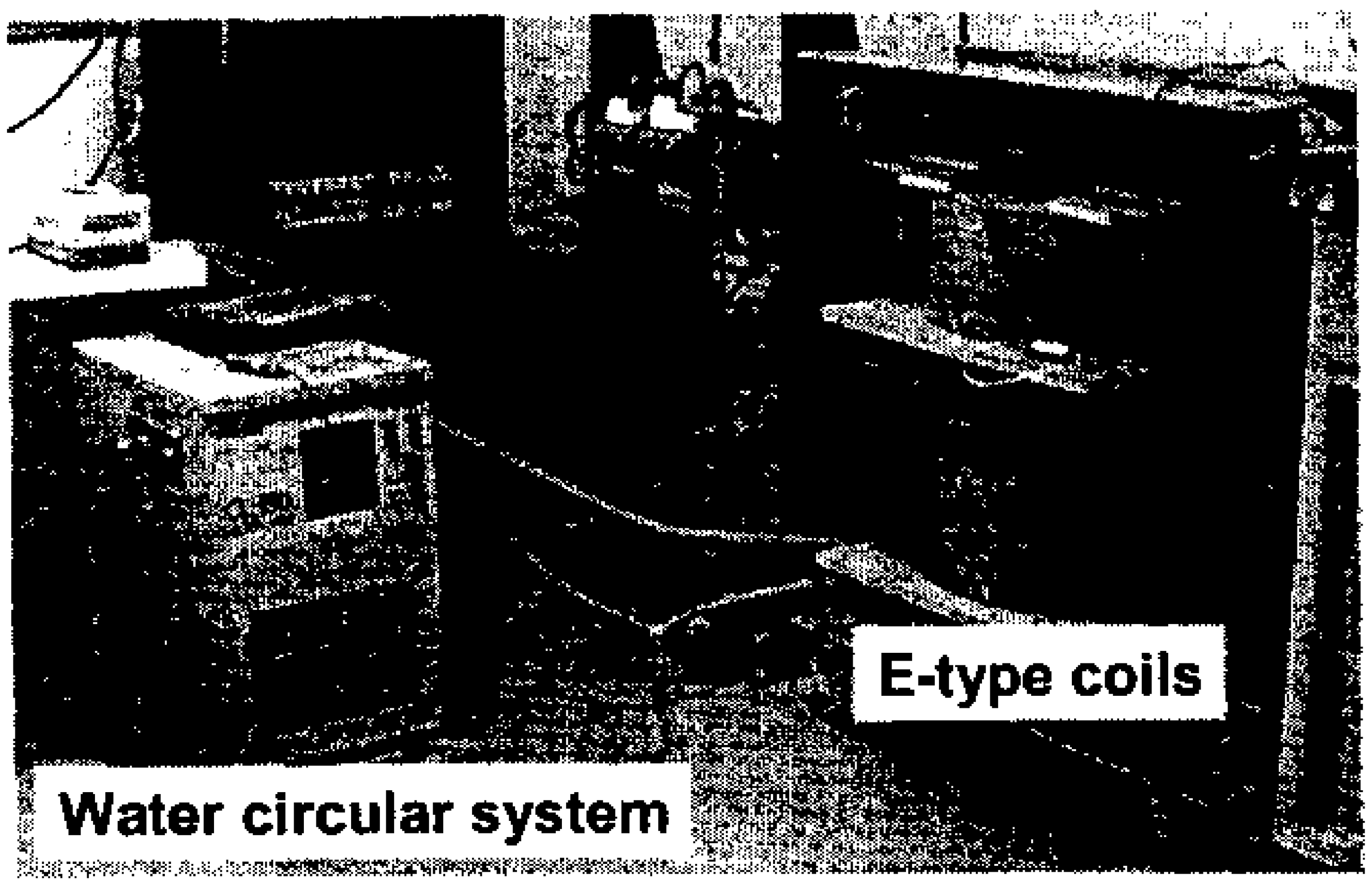


Fig. 1 High magnetic fields generator.

Table 1 Conditions for magnetic exposure.

Experimental area	180 mm×120 mm×13 mm
Frequency f	60 Hz
Magnetic field B	0.5 T
Uniformity in area (magnetic field)	< 2.0 %
Temperature t (℃)	15 <t< 37
Fluctuation in area (temperature)	< 0.1 ℃

2.3 mRNA Differential Display

We carried out the differential display in order to identify genes whose transcription was effected by ELFMFs [9-11]. The strategy of differential display is shown in Fig. 2. Wild type *C. elegnas* were incubated with ELFMFs (exposed) and without ELFMFs (control) at 20 °C for 120 min. After exposing, worms were washed off with distilled water and immediately frozen in liquid N_2. Each total RNA was extracted by homogenizer with ISOGEN (NIPPON GENE), and mRNA were converted to complementary DNA (cDNA) by MMLV reverse transcriptase enzyme including the RNAimage® kit for mRNA differential display systems from GenHunter Corporation. To compare the differences of expression levels between control and exposed case, cDNAs were amplified using both arbitrary primer and one base anchored oligo-dT primer by Taq DNA polymerase from Qiagen. In this reaction, cDNA were labeled by α-[^{33}P]dATP and separated into a 6% polyacrylamide gel by electrophoresis. After electrophoresis, the gel was dried and autoradiographed on Fuji X-ray films at -80 °C. cDNA fragments were excised from the gels. We confirmed unique ELFMF-responsive fragments by measuring its optical density. These gel-purified cDNAs were reamplified and inserted

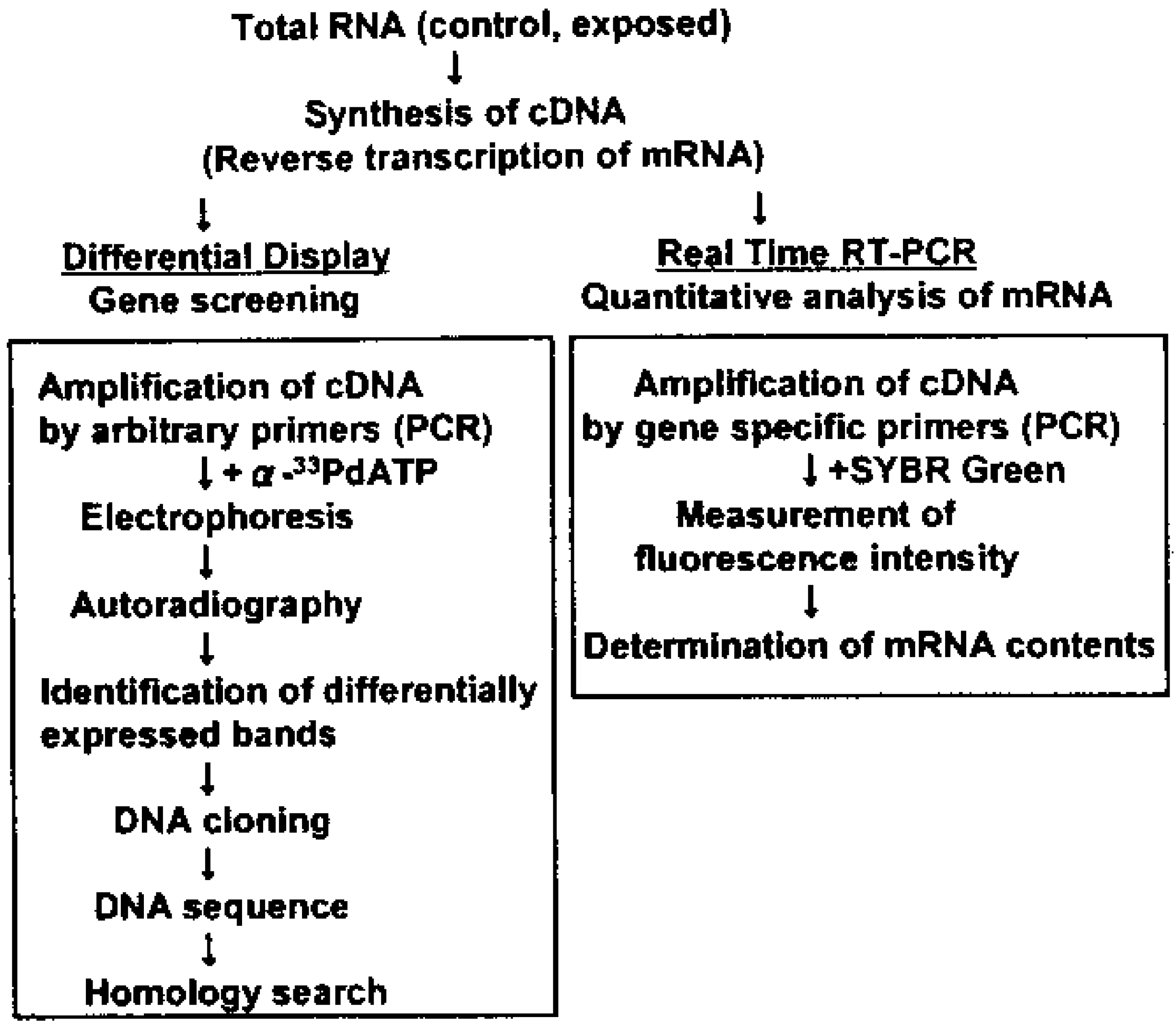


Fig. 2 Analysis of the mRNA expression.

into pGEM plasmid vector from Promega. The cloned cDNAs were subsequently sequenced. The nucleotide sequence of the differentially expressed DNA fragments were compared against the *C. elegans* genome databases. In addition, all clones were identical to *C. elegans* cosmid sequence.

2.4 Quantitative RT-PCR

To determine quantity of the expression level of isolated genes, mRNA was quantified with SYBR Green assay by using the quantitative RT-PCR machine, ABI Prism 7700 Sequence Detection System.

RT-PCR (Reverse Transcriptase-Polymerase Chain Reaction) can perform more quantitative expression of mRNA and identification of their reproducibility. Similarity to differential display, mRNA were converted to cDNA by MMLV reverse transcriptase, and amplified by PCR with the gene specific primers. When extension reaction in the processing PCR cycle steps amplifies PCR products, SYBR Green as a fluorescence dye intercalates the double stranded DNA and detects fluorescence intensities and plots the amplification curve in real-time. Finally, this system detects mRNA contents as fluorescence intensities and it is possible to determine the quantity of mRNA expressions. In order to correct of the mRNA concentrations, we employed *act-1* gene as an endogenous control at the same reaction. As expression of *act-1* theoretically dose not change by activation or proliferation of cells, it is possible to normalize quantitation of each mRNA target for differences in the amount of mRNA added to each reaction. A sham exposed control is standardized as 100 arbitrary units and compared to exposed case.

Table 2 Candidate for ELFMFs –responding genes.

primers	cosmid	DD	RT-PCR	gene	function/ product/ homology	references
related protein						
T11G/AP3	Y105C5A	8. 8↓		Y105C5A.8	putative protein family member	
T11G/AP4	Y39G10A	1. 2↑	4. 7↑	Y39G10AR.2	putative cytoplasmic protein of bilaterial origin	
T11G/AP5	R57	100↑	3. 8↓	R57.1	PA domain containing protein family member	
T11G/AP5	T20G5	100↓		T20G5.4	putative membrane protein	
T11G/AP6	Y67D8A	1. 8↑	1. 2↑	Y67D8A.1	putative prenylated protein family member of ancient origin	
T11C/AP3	R107	5. 7↑		R107.2	uncharacterized protein family UPF0031	
T11C/AP8	C42D8	1. 8↑	1. 5↓	*vit-2*	yolk protein VIT ellogenin structural gene VIT-2	[12-14]
T11A/AP3	T19C9	5. 6↑		T19C9.8	putative protein family member	
T11G/AP7	C07A12	2. 2↑	1. 2↑	*pdi-2*	essential protein disulfide isomerase	[15]
T11C/AP8	ZK455	5. 9↓	1. 7↓	*gei-22*	GEX interacting protein GEI-22	[16]
Transcription						
T11A/AP5	H14A12	4. 2↑	1. 4↑	*fum-1*	putative nuclear protein	
T11C/AP3	F43D2	16. 8↑	2. 0↑	F43D2.2	putative nuclear protein family member	
T11A/AP6	Y14H12B	3. 8↑	ND	Y14H12B.2	putative nuclear protein family member	
T11G/AP7	Y49E10	2. 6↑	ND	*pie-1*	transcriptional repressor family member	[17-23]
T11G/AP7	W01B11	1. 9↑		W01B11.3	essential SAR DNA-bindig like	
T11G/AP7	C54G10	3. 3↑	1. 6↑	*rfc-1*	DNA replication factor C family RFC-1	
T11C/AP5	Y47G6A	35. 7↓	3. 9↓	*cm-1*	similarity to Pfam domains PF01367	[24]
T11G/AP3	F08G2	1. 9↓	1. 9↓	F08G2.4	putative nuclear protein 20123	
T11G/AP7	C05G5	12. 5↓	2. 6↓	C05G5.3	putative nuclear protein, with a coiled coil-4 domain, of bilaterial origin	
T11G/AP7	F47A4	43. 4↓	2. 5↓	*dpy-22*	nucleic acid binding inferred from electronic annotation	[25]
Cuticle or Constrictor						
T11A/AP2	K10B2	16. 6↑		K10B2.3	lectin C-type domain	
T11A/AP3	C24D10	44. 8↑		C24D10.6	similarity to Mus musculus Hypothetical protein	
T11A/AP3	M199	2. 5↑		M199.5	collagen-like	
T11C/AP5	E03G2	2. 3↑	1. 7↑	*mec-5*	collagen required for mechanotransduction, MEC-5 precursor	[26-29]
T11G/AP7	C36E6	100↑	1. 7↑	*mlc-2*	myosin light chain 2	[30-32]
T11G/AP3	Y22D7AF	2. 2↑		Y22D7AR.10	putative secreted or extracellular protein precursor	
T11A/AP2	B0280	1. 4↑		B0280.5	chitin binding peritrophin-A domain and aggrecan core protein repeat precursor family member	
T11C/AP4	T04C12	41. 8↑		*act-2*	similarity to Pfam domain	[33]
T11G/AP5	W02A2	11. 2↓	3. 4↓	*fat-2*	delta 12 fatty acid desaturase FAT-2	[34]
T11C/AP4	F31D5	6. 6↓		F31D5.2	similarity to homo sapiens unc-93 homolog B1	
Apoptosis						
T11C/AP4	F57B10	10. 8↓	2. 4↓	*bag-1*	BAG family (BLC-2 binding athanogene) molecular chaperone regulator	[35]
T11C/AP8	Y73B6BL	4. 1↓	2. 1↓	*csp-2*	CaSPase CSP-2 alternative variant b caspase-related protein	[36, 37]
Redox						
T11A/AP6	MTCE	3. 1↑		MTCE.4, 26	cytochrome oxidase subunit I (C01), NADH dehydrogenase subunit 4 (ND4)	
Translation, Amino Acid Import and Metabolism						
T11G/AP3	T11G6	6. 3↓		*hrs-1*	histidyl tRNA synthetase HRS-1	
T11A/AP8	F42D1	1. 8↓		F42D1.2	tyrosine aminotransferase	
T11G/AP2	F22B3	7. 3↑		F22B3.4	similarity to human glucosamin-fructose-6-phosphate aminotransferase	
T11A/AP8	M02D8	2. 0↓		M02D8.4	asparagine synthetase, alternative variant b	
T11G/AP7	D1007	2. 9↑	1. 2↑	*rps-10*	essential ribosomal protein, small subunit RPS-10	
T11C/AP5	K10B3	34. 4↑		K10B3.1	bacilius subtilis hypothetical lipoprotein ybbD precursor	
T11A/AP8	Y50D4C	1. 2↑		Y50D4C.5	similarity to equine herpesvirus type 1 glycoprotein X precursor	
T11C/AP1	K01G5	4. 4↑	1. 8↑	*ran-1*	essential RAN (nuclear import/ export) related RAN-1	
T11G/AP4	Y71F9AM	1. 2↓	1. 2↓	*nxt-1*	essential nuclear transport factor 2-related, nuclear protein export NXT-1, binds ran-GTP	
T11C/AP8	T22C1	100↓	4. 8↓	T22C1.5	hypothetical protein	
T11A/AP3	K02E7	4. 8↑		K02E7.9	BTB POZ domain family member	
T11A/AP4	C14F11	1. 8↓		C14F11.6	dTDP-4-dehydrorhamnose3 5-epimerase	
T11A/AP6	F37C12	4. 9↑		F37C12.7	similarity to Pfam domain PF00501 (AMP-binding enzymes)	
T11A/AP6	F44E7	5. 1↑		F44E7.2	haloacid dehalogenase-like hydrolase family	
T11A/AP6	M88	2. 4↑		M88.5	similar in places to hnRNP K	
T11A/AP8	M02F4	2. 2↓		M02F4.7	regenerating islet-derived 1 like family member	
T11G/AP2	F23B2	8. 6↑		*pcp-2*	member of the prolyl carboxy peptidase like gene class	
T11G/AP3	Y87G2A	7. 6↓		Y87G2A.8	glucose-6-phosphate isomerase	
T11G/AP5	W09C2	2. 7↓		*mca-1*	member of the membrane calcium ATPase gene class	[38]
T11C/AP8	R02C2	43. 4↓		R02C2.4	nuclear hormone receptor family member	
T11A/AP4	Y56A3A	1. 9↓		*mif-1*	macrophage migration inhibitory factor related MIF-1	[39]
T11G/AP5	ZK470	2. 9↓		ZK470.4	similarity to rattus norvegicus non-muscle caldesmon	
Neuron Specific						
	C44C1	—	1. 5↓	*ncs-1*	neuronal calcium binding protein, neuronal calcium sensor 1	[40-42]
T11A/AP8	F10G8	1.2↑	2. 5↑	*ncs-2*	neuronal calcium binding protein, neuronal calcium sensor 2	[41, 42]
	K03E6	—	3. 4↓	*ncs-3*	neuronal calcium binding protein, neuronal calcium sensor 3	[41, 42]

3. RESULTS

3.1 Identification of ELFMF-responding genes

As results of the analysis by differential display, the differentially expressed cDNA fragments were derived from 56 independent clones. Of the 56 cDNA fragments isolated, 33 of the clones were up-regulated, 23 of the clones were down-regulated by exposure of ELFMFs and 22 cDNA clones in 56 have been known genes.

In particular, it is worthy of notice that there are 22 ELFMFs-responding genes that related factors of transcription, cuticle, constrictor and neuronal function. The resultant of ELFMFs exposure showed that expression of *mec-5* was 2.3-times up-regulated (Fig. 3a), and *fat-2* was 11.2-times down-regulated (Fig. 3b) as compared with the sham exposed control. Though, *mec-5* is coded a collagen gene, which has reported that required for mechanosensation in sensory neuron [26-29]. *fat-2* functions as a delta fatty acid desaturase that is important for normal *C. elegans* cuticle function [34]. All genes cloned by differential display are listed, and each function is shown in Table 2.

3.2 Quantitative analysis of mRNAs

Fig. 4 shows a relative quantity of *mec-5* and *fat-2* mRNA to compare between control and exposed case. As similar to results of differential display, expression of *mec-5* was increased (Fig. 4a), and expression of *fat-2* was decreased (Fig. 4b) by ELFMFs exposure.

ncs-2, which is neuronal calcium sensor (NCS) gene, have reported primarily expressed in the neuronal cells [41, 42], though an expressed cell are different with *mec-5* gene. We also confirmed the reproducibility of *ncs-2* mRNA expressions by the same way. The expression of *ncs-2* was 2.5-times up-regulated under 0.5 T at 20 °C for 120 min (Fig. 5a). *ncs* gene has three homologues, *ncs-1*, *ncs-2* and *ncs-3*, in the nematode *C. elegans* [40-42]. However, it could not be identified *ncs-1* and *ncs-3* by using differential display. When we investigated *ncs-1* and *ncs-3* gene, expression of *ncs-1* and-*3* mRNA were differ from *ncs-2* one and decreased by ELFMFs exposure (Fig. 5b and 5c). To determine the expression level of *ncs* genes' in time-dependent manner under an ELFMFs at 20 °C, we extracted each total RNA for 15, 30, 60 and 120 min, and performed real-time RT-PCR. No significant changes of each *ncs* mRNA were observed under sham exposed control. When exposed ELFMFs, *ncs-2* was significantly up-regulated in time-dependent manner, and reached at the maximum level to 2.5 times for 120 min. *ncs-1* and *ncs-3* were significantly down-regulated at maximum 1.7 times and 4.0 times each.

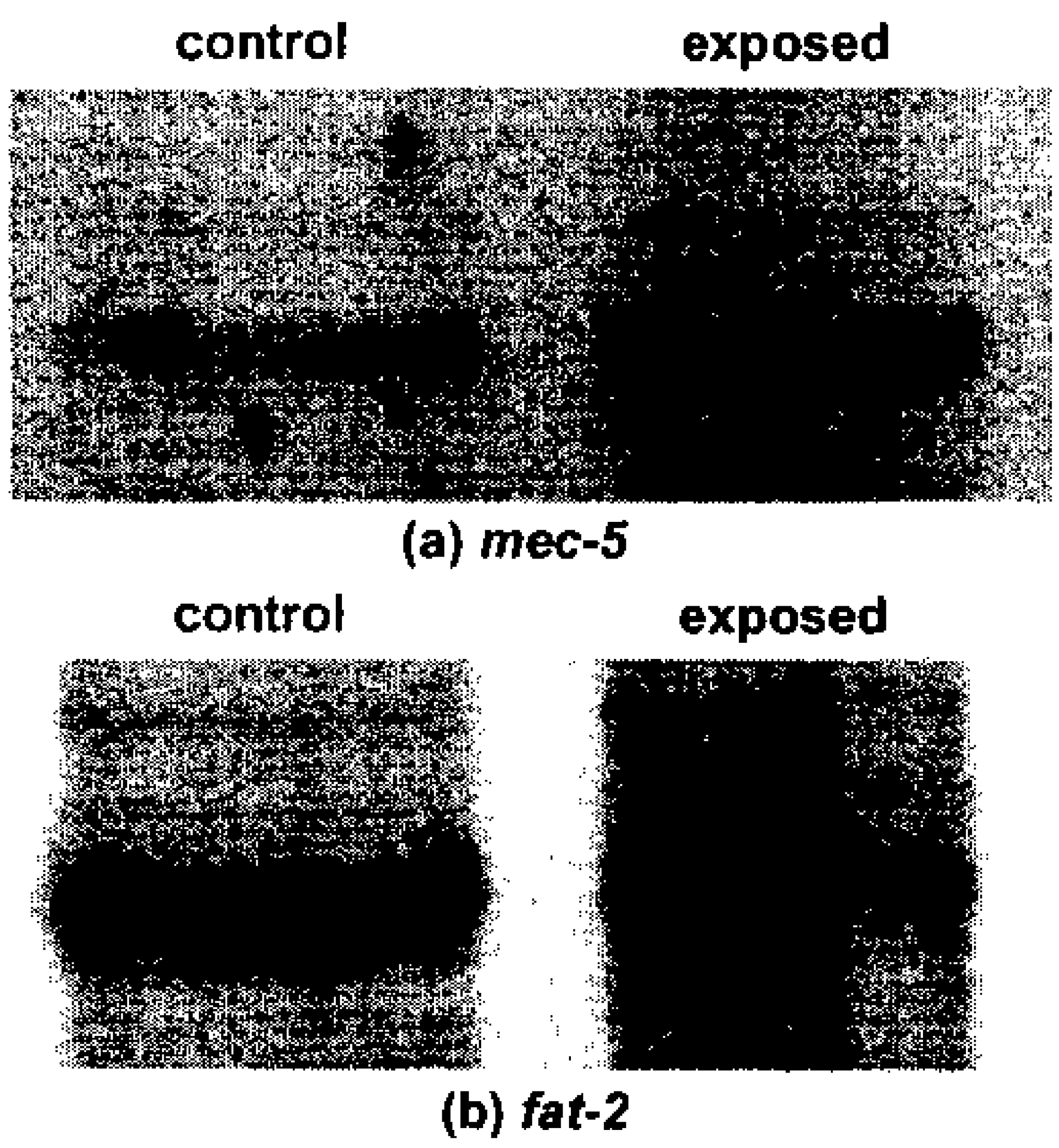


Fig. 3 Autoradiogram of *mec-5* and *fat-2*.

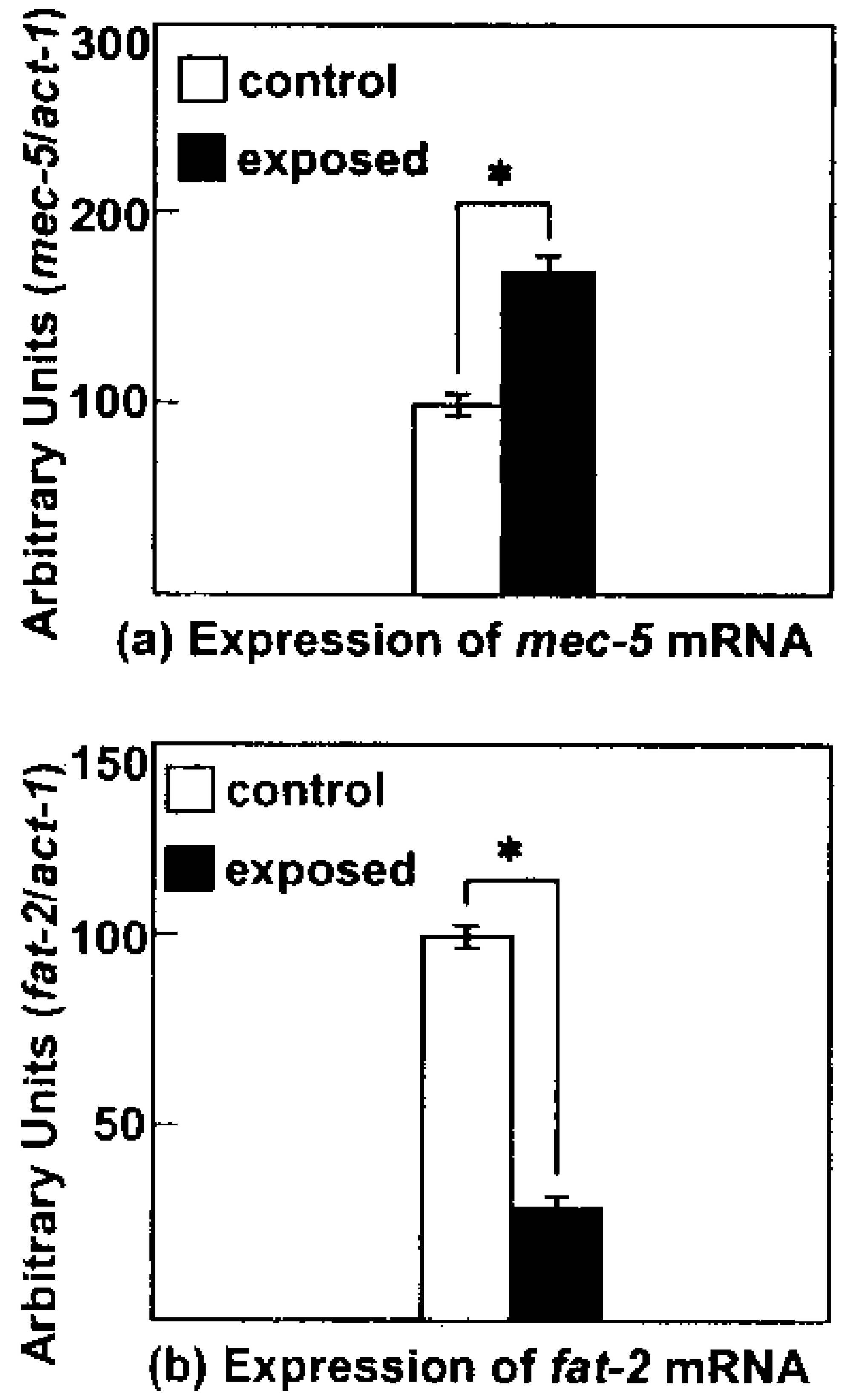
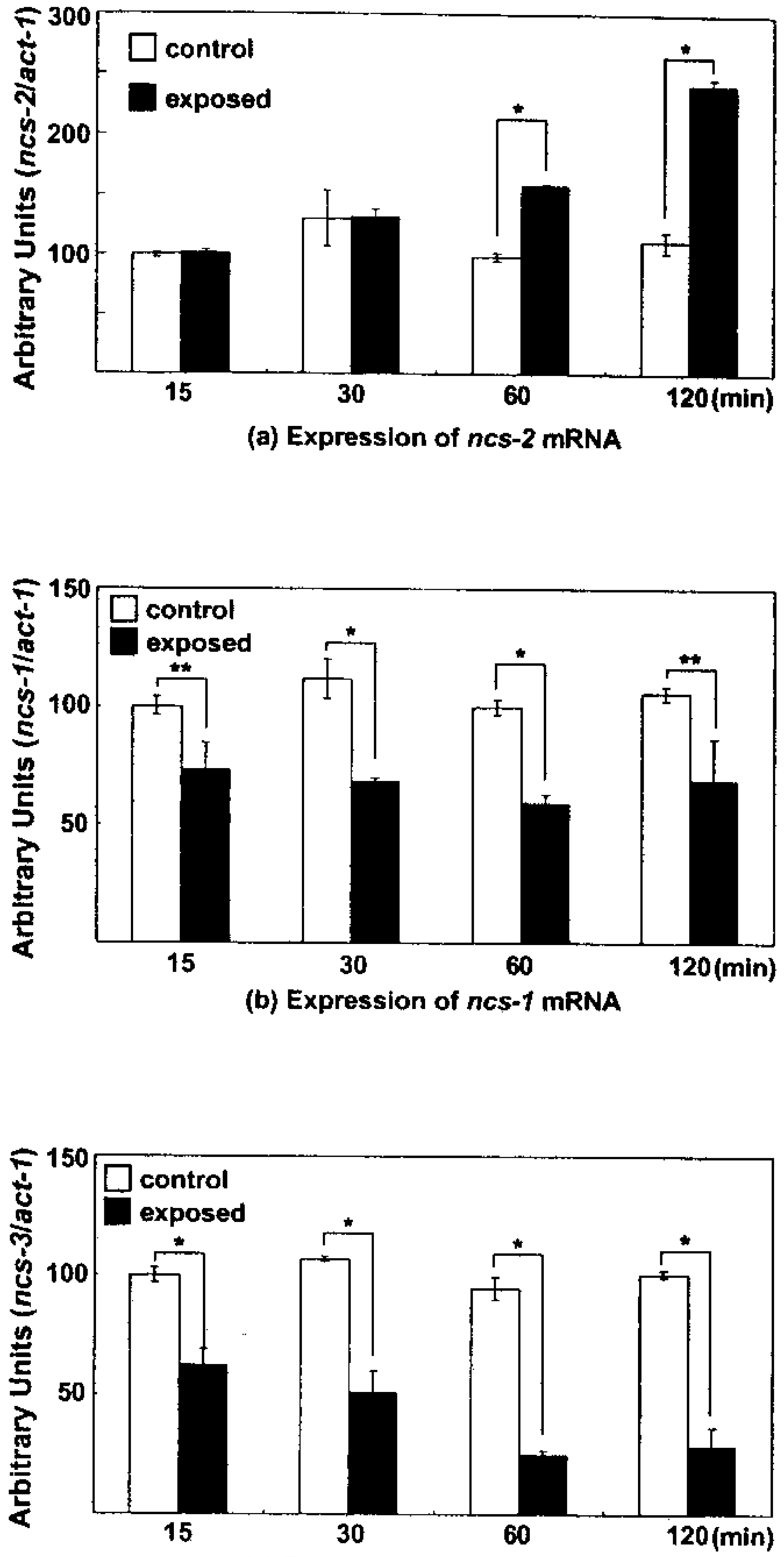


Fig. 4 Expressions of *mec-5* and *fat-2* mRNA.
(＊: P<0.001, N=3)



(a) Expression of *ncs-2* mRNA

(b) Expression of *ncs-1* mRNA

(c) Expression of *ncs-3* mRNA

Fig. 5 Expressions of NCS family mRNA.
(＊: $P<0.01$, ＊＊: $P<0.1$, $N=4$)

4. CONCLUSION

As results of screening of ELFMFs-responding genes, we identified 56 differentially expressed clones independently. By measuring the optical density of differentially expressed bands, we found that 33 of the genes were up-regulated, and 23 of the genes were down-regulated by ELFMFs exposure.

We are interested in the effects of ELFMFs in neuron. Particularly we discovered two candidate genes that are associated with neural function of the worm; *mec-5* is coded a collagen like gene and *ncs-2* is known to as a neural calcium sensor gene in the *C. elegans* sensory neurons. We found that *mec-5* mRNA was 1.7 times up-regulated and *ncs-2* mRNA was 2.5 times up-regulated under ELFMFs exposure by using RT-PCR. Whereas *ncs-2* mRNA was significantly up-regulated in time-dependent manner under an ELFMFs, *ncs-1* and *ncs-3* mRNAs were decreased under same condition. This shows that *ncs* genes function at different mechanisms respectively.

As electrical activity in neuronal cells plays an important role in development of neurons and neurotransmission, it is likely that ELFMFs effect on these functions. We assume that as the result of which *C. elegans* perceive ELFMFs as a stress, the expression level of mRNAs is changed, and affected on behaviors of the nematode *C. elegans* [4, Ikeda and Harada. unpublished data].

5. ACKNOWLEDGMENTS

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