7th Workshop on Biological Effects of EMF

The Effects of ELF MFs on Cell Signaling System and Its Interference

Seoul, Korea
October 20, 2003

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Bioelectromagnetics Laboratory, Zhejiang University School of Medicine

Introduction to BEMS Lab at Zhejiang University

History

1970's	involve in RF and MW epidemiological investigation microwave laboratory
1997	Provincial Key Laboratory (Bioelectromagnetics Laboratory)
1997	first key project funded by NSFC
2002	second key project funded by NSFC

Introduction to BEMS Lab at Zhejiang University

Grants

Grants	Number
National	11
Provincial	7
International	2

Note: the grants funded during 1994-2002 were counted in

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Introduction to BEMS Lab at Zhejiang University

Publications

Publication	Number
International Journal	22
Domestic Journal	55
Books	5

Note: the publications during 1994-2002 were counted in

Introduction to BEMS Lab at Zhejiang University

Cell Exposure Systems

ELF MFs

RF EMFs

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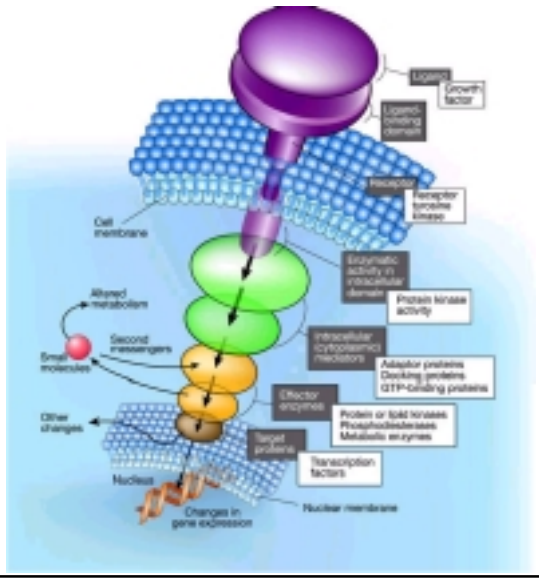
Animal Exposure System

Signal: 900 MHz Mobile Phone Signal

The Possible Interaction Sites of EMF with Cell



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Research Summary of BEMS Laboratory

- The effect of ELF MFs on GJIC and its mechanism of action
- New interaction sites of ELF MFs on cell membrane
- The effects of ELF MFs on signal transduction pathways
- The effects of ELF MFs on cellular transcription factors
- Cloning of MF-response genes
- The role of noise magnetic fields in preventing EMF hazards

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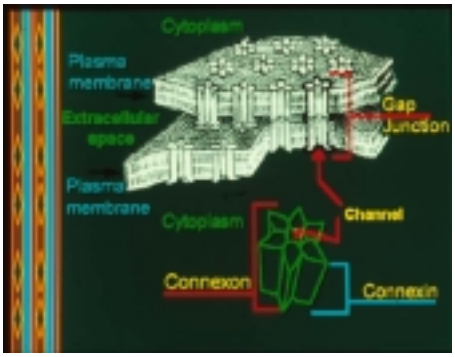
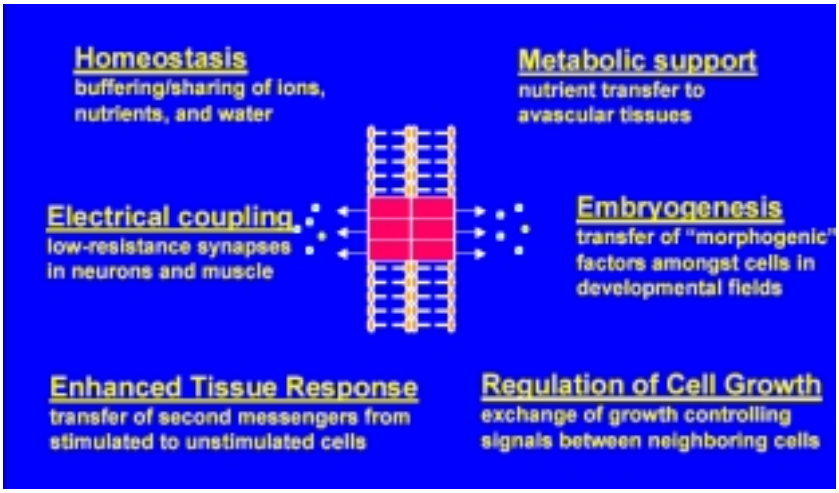
The Effect of ELF MFs on Cellular GJIC

- ELF MFs has been classified as a possible carcinogen
- GJIC disruption is an index to determine if an agent is carcinogic
  most neoplastic cells have decreased gap junction expression and function



Physiological functions of gap junctions



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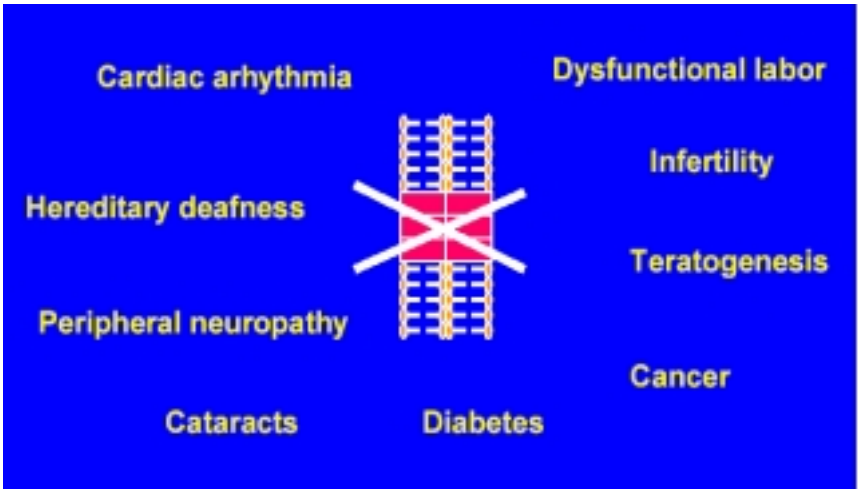
Diseases Associated with Defective Gap Junctions

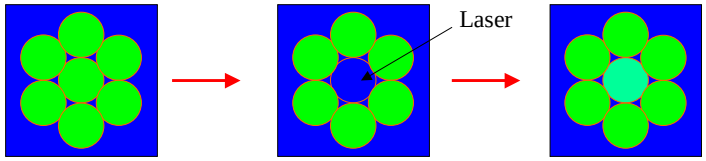


The effect of ELF MFs on Cellular GJIC

Method:

- microinjection: lucifer yellow
  Index: **number of dye-coupled cells (DCC)**

- FRAP (Fluorescence-recovery-after-photobleaching)
  Dye: 5,6-carboxyfluorescein diacetate (5,6-CFDA)
  Index: **percentage of fluorescence recovery**



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ELF MFs inhibit Cellular GJIC

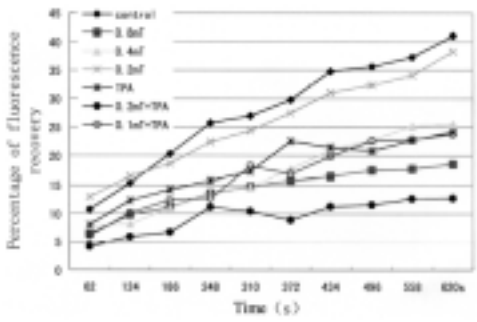


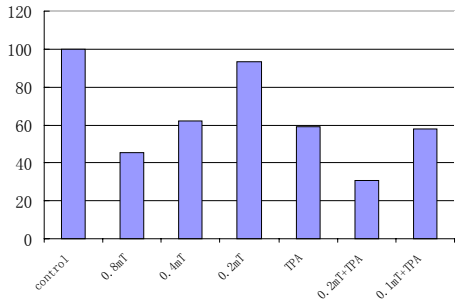
Figure 1. Percentage of fluorescence recovery at each time point.

ELF MFs inhibit Cellular GJIC

Table 1. Effect of ELF and/or TPA on Fluorescence Dye Transfer (at 10 min After Bleaching)

Treatment	n	FRAP ($\bar{x} \pm s$)	% of Control
Control	45	41.04 ± 13.10	100
0.8 mT	60	18.58 ± 7.73*,**	45.27
0.4 mT	47	25.50 ± 9.26*	62.20
0.2 mT	93	38.31 ± 7.23	93.44
TPA	59	24.21 ± 8.74	59.05
0.2 mT + TPA	68	12.69 ± 6.34***	30.95
0.1 mT + TPA	45	23.79 ± 8.78	58.02

n, number of cells evaluated for data shown.
*vs. Control, $P < 0.01$.
**vs. 0.4 mT, $P < 0.01$.
***vs. TPA, $P < 0.01$.



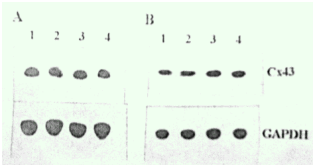
ELF MFs inhibit Cellular GJIC

Summary

- ELF MFs inhibited cellular GJIC
- Dose-dependent response
- Inhibition threshold is 0.4 mT
- 0.2 mT MFs act synergically with carcinogen TPA

Mechanism of GJIC Inhibition Induced by ELF MFs

MFs did not change the transcription of connexin43 mRNA



Cells	n	Control	ELF(0.8mT)	ELF + TPA	TPA
NIH3T3	5	0.804±0.171	0.744±0.218	0.776±0.212	0.956±0.205
CHL	5	0.816±0.155	0.718±0.197	0.847±0.162	0.962±0.208

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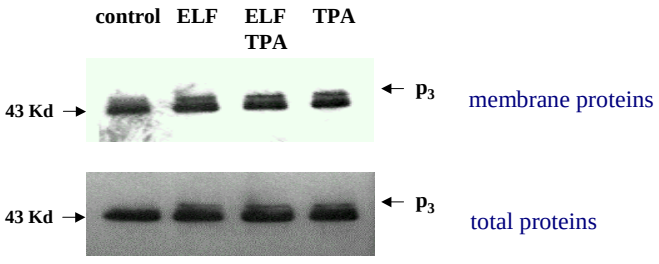
Mechanism of GJIC Inhibition Induced by ELF MFs

Western blot analysis of Connexin 43



Antibody used: rabbit anti-connexin 43 polyclonal antibogy

Mechanism of GJIC Inhibition Induced by ELF MFs

MFs did not change total expression level of connexin43

Treatment	n	total Cx 43 protein
control	4	172.43±23.16
ELF	4	155.85±22.51
ELF+TPA	4	147.64±21.04
TPA	4	144.31±31.26

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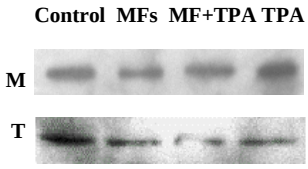
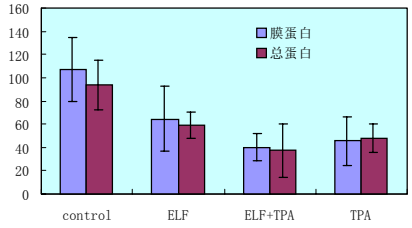
Mechanism of GJIC Inhibition Induced by ELF MFs

MFs decreased the quantity of unphosphorylated Connexin 43



Antibody: mouse anti-Cx 43 monoclonal antibody

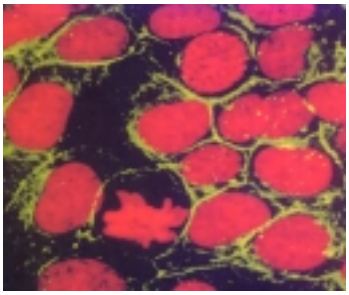
Treatment	Membrane (n=5)	Total protein (n=4)
Control	107.33±27.42	93.73±21.76
ELF(0.8mT)	64.69±27.88 *	58.89±11.46 *
ELF+TPA	39.96±11.93 **	37.49±22.96 *
TPA	45.54±20.54 **	47.86±12.44 *



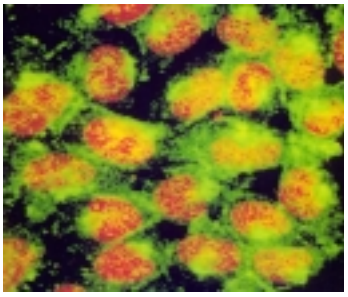
* P<0.05 *vs.* control
** P<0.01 *vs.* control

Mechanism of GJIC Inhibition Induced by ELF MFs

MFs induced Internalization of Connexin 43



Control



Exposured cells

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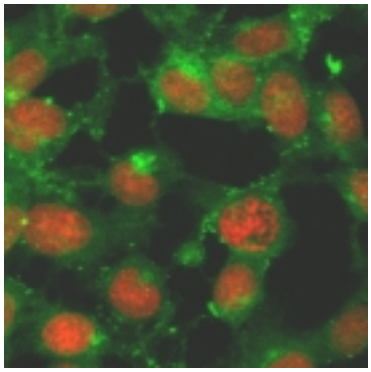
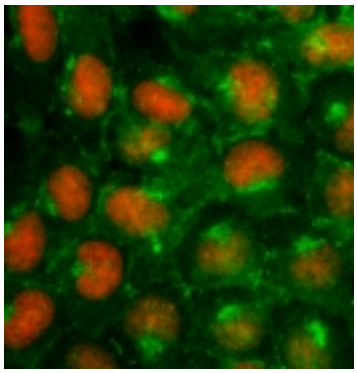
Mechanism of GJIC Inhibition Induced by ELF MFs

MFs induced Internalization of Connexin 43



TPA



ELF MFs + TPA

Mechanism of GJIC Inhibition Induced by ELF MFs

Western Blot Analysis of Connexin 43 in Cytoplasm

Control MFs TPA

Data Suggest: MFs did increase the amount of Cx 43 in cytoplasm

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Mechanism of GJIC Inhibition Induced by ELF MFs

PKC was involved in the GJIC suppression

Group	DCC (means±SD)
control	10.01 ±2.01
MF (0.8 mT)	4.79 ±1.55 *
MF (0.8 mT) +STS	
0	5.00 ±1.09
5	6.93 ±0.73 **
10	8.71 ±1.07 **
20	8.13 ±1.85 **

* P<0.01 *vs.* control.

** P<0.05 *vs.* DMSO solvent without STS (staurosporine, a PKC inhibitor)

ELF MFs inhibit Cellular GJIC

Conclusion

- ELF MFs inhibited cellular GJIC
- The mechanism of GJIC suppression induced by MFs is:
  - Hyperphosphorylation of Cx43 (through PKC)
  - Internalization of Cx43

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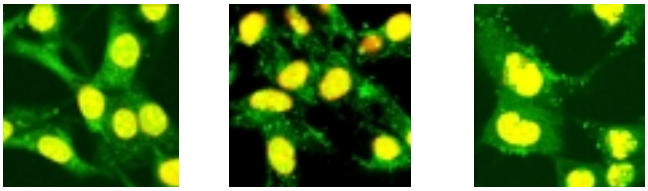
ELF MFs Induced Receptor Clustering

Clustering of EGF receptor



Control EGF MFs

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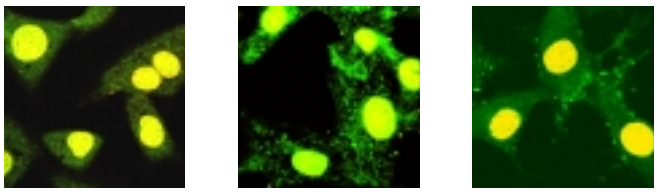
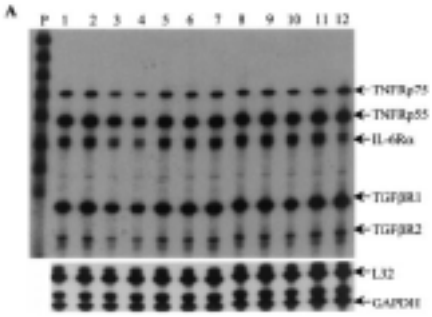
ELF MFs Induced Receptor Clustering

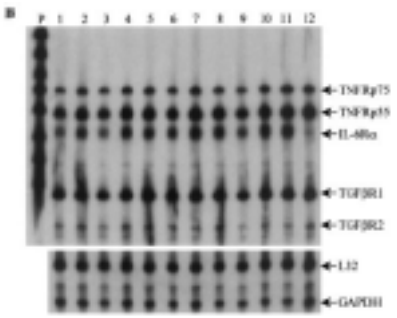
Clustering of TNF receptor



Control TNF MFs

RPA Autoradiographs of Cytokine Receptor mRNA Expression in HL60 Cells

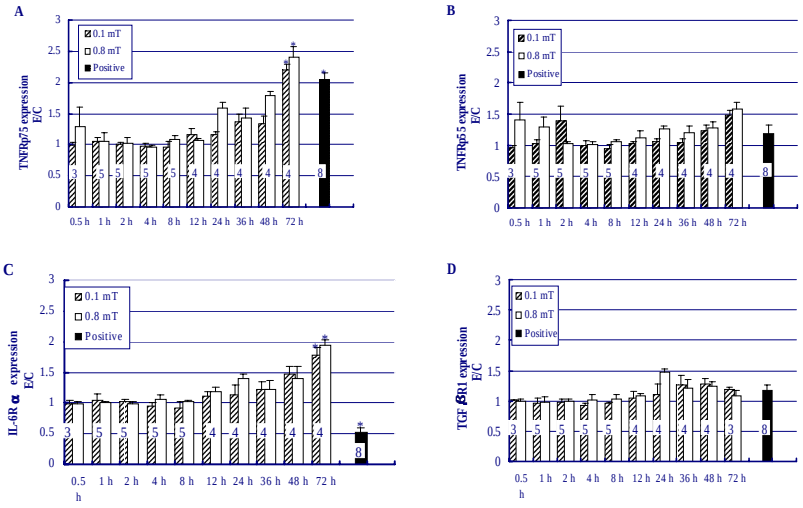




1. Sham 2. 0.1 mT 30 min 3. 0.8 mT 30 min 4. 0.1 mT 1 h
5. 0.8 mT 1 h 6. 0.1 mT 2 h 7. 0.8 mT 2 h 8. 0.1 mT 4 h
9. 0.8 mT 4 h 10. 0.1 mT 8 h 11. 0.8 mT 8 h
12. PMA 10 nM 8 h

1. Sham 2. 0.1 mT 12 h 3. 0.8 mT 12 h 4. 0.1 mT 24 h
5. 0.8 mT 24 h 6. 0.1 mT 36 h 7. 0.8 mT 36 h 8. 0.1 mT 48 h
9. 0.8 mT 48 h 10. 0.1 mT 72 h 11. 0.8 mT 72 h
12. PMA10 nM 8 h

The Effects of MFs on Cytokine Receptor mRNA Expression in HL60 Cells



The Effects of MFs on Cytokine Receptor mRNA Expression in HL60 Cells

Conclusion

- MFs exposure for 72 h increased TNFR p75 and IL-6Rα transcription
- MFs didn't change gene expression levels of TNFR p55 and TGFβR1

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The effects of ELF MFs on Cellular Signal Transduction

1. MFs induced protein tyrosine phosphorylation in CHL cells

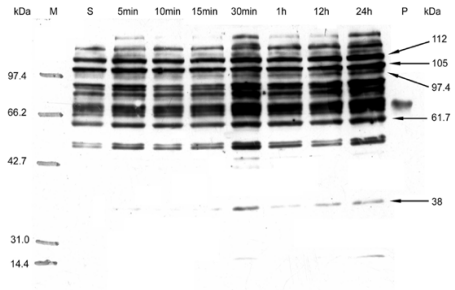

FIGURE 1a. The effects of 0.4mT MF on protein tyrosine phosphorylation
M: marker S: sham exposure P: positive control

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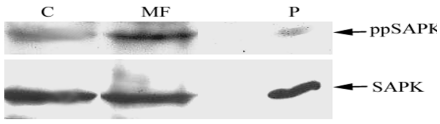
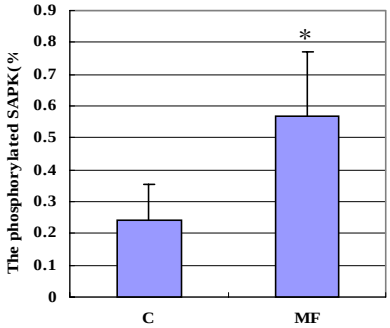
The effects of ELF MFs on Cellular Signal Transduction

2. MFs activated SAPK/JNK in CHL cells

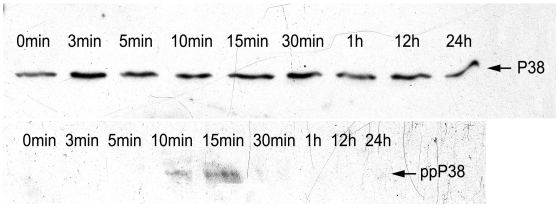


0.4 mT MFs, 15 min exposure



The effects of ELF MFs on Cellular Signal Transduction

3. MFs activated p38 MAPK in CHL cells



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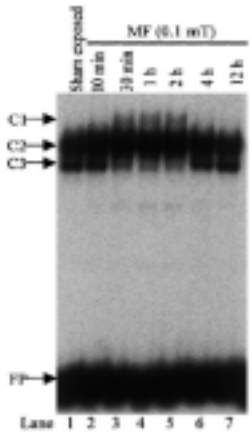
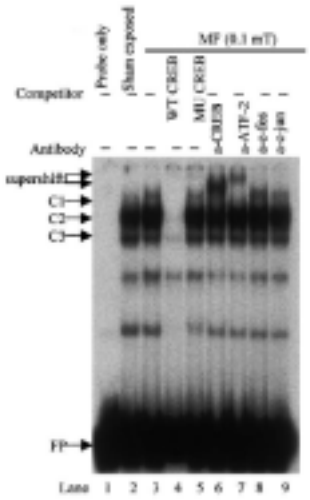
The Effects of ELF MFs on DNA Binding Capability of CREB

MFs Induced a Time-dependent Activation of CREB



- Method: EMSA
- Probe: a 21-mer cAMP responsive element (CRE) consensus oligo
- Results: three protein-DNA complexes (C1, C2, and C3)

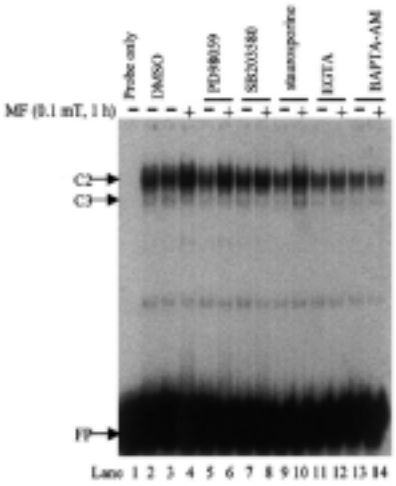
MFs Increased CREB Binding activity



Methods: competition assay
supershift assay

- CRE bound with proteins specifically
- Complex C1 was a homodimer of ATF-2
- CREB was a component of complex C2

The Signal Led to CREB Binding Activation

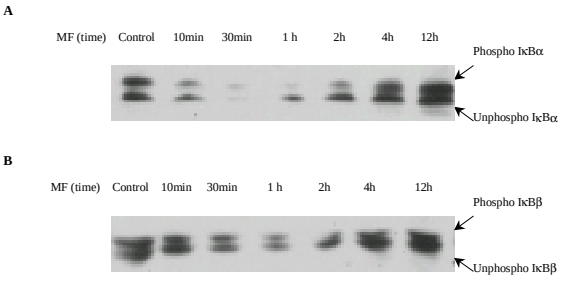


PD98059: ERK inhibitor
SB203580: p38 MAPK inhibitor
Staurosporine: PKA/PKC inhibitor
EGTA: extracellular Ca^{2+} chelator
BAPTA-AM: intracellular Ca 2+ chelator

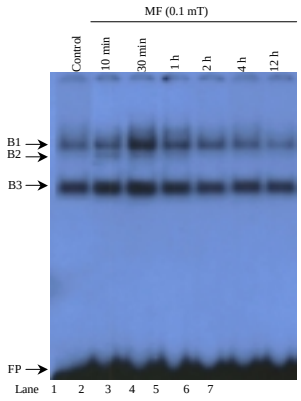
Data suggest:
- Both extracellular and intracellular Ca 2+ play a ciritcal role
- The activation was independent of PKA, PKC, ERK, and p38 MAPK

The Effects of ELF MFs on DNA Binding Capability of NF-κB

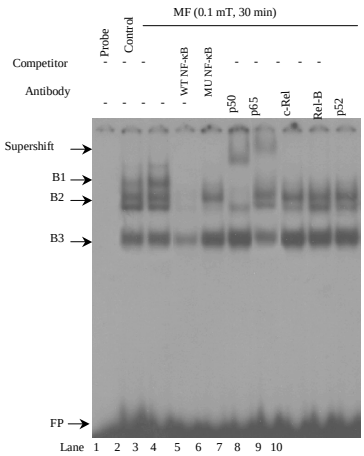
MFs Induced Degradation of NF-κB Inhibitory Proteins IκBα and β



MFs Increased the DNA Binding Capability of NF-κB



MFs Increased the DNA Binding Capability of NF-κB



Data suggest:

- ➤NF-κB bound to DNA specifically
- ➤B3 band was a non-specific binding band
- ➤Complex B1 was a p50/p65 heterodimer
- ➤Complex B2 was a p50/p50 homodimer

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Cloning of MF-response Genes

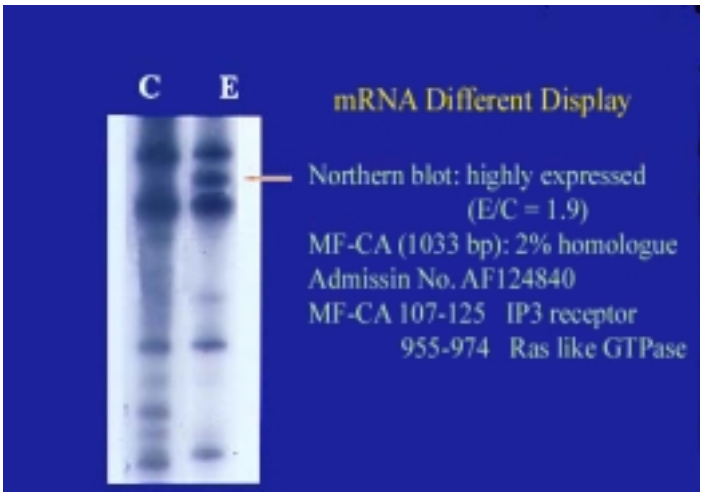
Cells: Daudi Cells

Method: Differential Display of Gene Expression

ELF MFs intensity: 0.8 mT

Results:
11 differential displayed fragments
7 fragments confirmed
3 genes cloned: novel gene; GCS; CO1

Cloning of MF-response Genes



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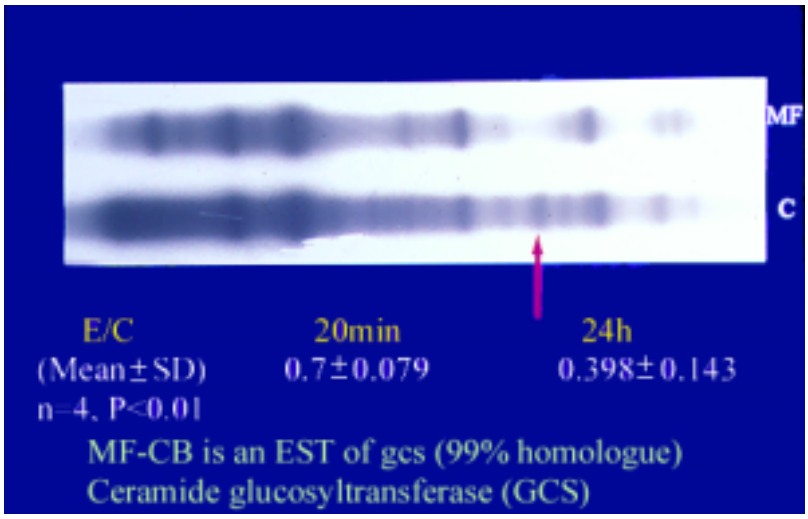
Cloning of MF-response Genes



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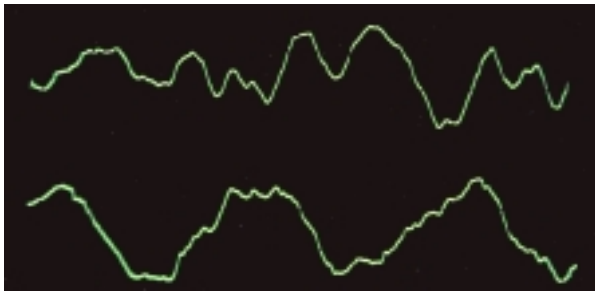
The Magnetic Fields Used in the Experiment

50Hz sinusoidal MF signal



Noise MF signal

The combined MF signal

Noise Magnetic Fields Abolished Bioeffects Induced By MFs

- noise MF abolished MF-induced GJIC suppression
- noise MF blocked MF-induced SAPK activation
- noise MF interfered EGF and TNF receptor clustering induced by MFs

Demonstrate:

noise magnetic field is one of the flexible approaches to interfere the biological effects of ELF-MF

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Research Summary of BEMS Laboratory

- 0.4 mT ELF MFs inhibited GJIC
  0.2 mT MF enhanced GJIC suppression induced by TPA
- 0.1 mT ELF MFs interacted with proteins on cell membrane
- 0.4 mT ELF MFs interfered multiple cellular signal transduction pathways.
  There are cross-talk between different pathways
- 0.1 mT ELF MFs activated key cellular transcription factors
- Cloned three genes responded to 0.8 mT ELF MFs
- Demonstrated the role of noise magnetic fields in preventing EMF hazards

Data implied:

The exposure limit for ELF MFs should be lower than 0.1 mT in order to prevent its possible health hazards.

What we are doing now

- Reveal the bioeffects of ELF MFs at the whole genome level
  - genomic approach
  - proteomic approach
- Determine the bioeffects of RF

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