# RF-MW non-thermal effect enhanced Beta-galactosidase expression through the induction of Dnak synthesis in *E.coli BL21 DE3.*

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*Abstract:* - Like most of stressful environmental factors RF/MW radiation induce a higher HSP activity in absence of any heating. A low level of RF radiation exposure can also increase other enzyme activity by stimulating their expression in cells in particular the β-galactosidase activity. The aim of our work is to study the rate of accumulation of Dnak (HSP70) and the rate of accumulation of β-galactosidase at the level of RNA messenger. We demonstrate that Dnak and the β-galactosidase synthesis changes at the level of DNAc synthesis, using RT-PCR and detection on agarose gel by UV radiation. The exposure conditions are performed with the nominal radio GSM emitter (Base Transceiver).

*Key words*: - GSM radiation, *E-coli*, low level exposure; Dnak synthesis, *β*-galactosidase activity; *β*-galactosidase synthesis; non-thermal effect.

## 1 Introduction

Based on numerous experimental studies a lot of scientific evidence warrants limiting exposure to high level *RFR* due to their detrimental health effects caused by heating of tissues at specific absorption rate (*SAR*) level that correspond to a few elevation of temperature degrees. *(SAR*) or power density (PD) is a main determinate for the thermal EMF effects. However this does not occur with mobile phones, EMF safety standards were setting so that their radiation absorbed by living tissues does not causes heating. Since establishment of this current safety standards and with the increase in the use of mobile phones, the exposure of general population to this radiation become chronically at varying degrees and level. Furthermore it will continue to increase as the technology advances. In fact biological variables and physicals parameters else temperature like duration of exposure may be important and so far of any heating for so called "*Non thermal*" effect.

A weight of recent studies have addressed some positive finding and indicated that low frequency (EMF) or radio frequencies (RF) some those of the mobile telephony [1, 2] doesn't causes changes in the levels of the protein synthesis, but only on the process of protein synthesis of (HSP) that can repair altered protein, eliminate conformational damaged, regulate folding and assist in maturation of newly synthesized proteins.

This finding is subject of discussing, it is necessary to note that a team, French, does not find any HSP's increase in any cellular lineage studied after exhibition in radiations GSM [4, 5, 6, 7, 8, 14] and other emitted hypothesis that long exposition induce HSP production what can favors cancer provocation [9, 10, 11, 12, 13]. After further study of the nature of interaction between HSP synthesis and enzyme activation, they found that HSP repair and inhibit inactivation of enzymes sequestered in inclusion bodies and that in cells it stabilizes aggregations of denatured proteins. Some experiments studies on *E-Coli β-galactosidase* [15] shows that the *Dnak* (HSP70) protein contributes to *E. coli's* growth not only by protecting some

enzymes from denaturing like the host RNA polymerase enzyme but also by reactive some once they are misfolded or aggregated.

Furthermore several studies have discovered that high of RF radiation can modify the activity level of certain enzymes. For example, ($\beta$-galactosidase expression is increased when cells are exposed to certain frequencies of RF radiation [16, 17, 18]. From here, we can make some hypothesis and investigate on the possibility that *RF-MW* exposure lead not only to the expression of the potential stress marker response *Hsp* as shown as before, but also to increase possibly RNA polymerase activity or simply the *$\beta$-galactosidase* activity at very low SAR and low level of RF exposure.

From a public health stand point the most recent expert report addressing RF/MW radiation examines new data on biological effects, such as enzyme induction, and toxicological effects including genotoxicity, carcinogenicity….That's why we need for further research to clarify the possible associations between RF fields and adverse health outcomes.

That's why the present study was aim to explore the RF/MF non thermal effects on the protein activity, in fact that we have a relationship between $\beta$-galactosidase synthesis increased and Dnak accumulation and that a higher Dnak synthesis responding to the stress factor as shown in our experiment, caused this higher protein activity.

To quantify the RNA expression of ***$\beta$-galactosidase*** and Dnak gene, we purify total RNA and then we proceed by the RT-PCR method. The RT-PCR products obtained by using primers respectively specific to those genes from non-exposed and exposed bacterial cells at *960 MHz, E=6V/m; a* power density (10mW) less than authorized SAR level for 10 days and so far of any heating, to create same conditions of chronicle exposition to BTS used in **Lebanon**, were detected and compared by gel agarose electrophoresis and by dosimeter method.

# 2 Materials and methods

## 2.1 Bacterial strain and growth conditions.

The bacterial strain, *E-Coli TB1,* was used as hosted cell for transformation of PUC18 plasmids containing lac operon induced with IPTG to further work like, enzymatic Kinetic study of $\beta$-galactosidase.

The genotype of *E. coli* TB1 is: [F *ara*Δ(*lac-pro*AB)*rps* Ψ80*d lacZΔM*15*hsdR17* ($r_k^+$ $m_k^+$)].

The plasmid vector puc18 was used for the $\beta$-galactosidase study contain lac t promoter, an ampicillin (Ap) resistance gene, a lac I repressor and a lac Z fragment to confer blue/white selection. (Sambrook et al, 1989).

The bacterial cells were incubated at 37°C without shaking for overnight after isolation of *E-Coli* colony from culture cells and suspending it in 3ml LB which consisted of 10g bactotryptone (DIFCO,USA), 5g of Yeast extract (DIFCO USA) and 10g NaCl (PROLABO, FRANCE) in 1liter of deionized Water. The PH was adjusted to 7,4 with NaOH. The LB media was supplemented with 75µg/ml of ampicillin.

## 2.2 Preparation of competent cells.

300µL in Erlenmeyer of over night culture was inoculated into 30mL of fresh LB at 37˚C, with shaking at 300 rpm until an OD= 0.4 at 600nm.

The cells were then transferred and cooled on ice for 10 min, harvested by centrifugation at 3000 (rpm) for 5 min at 4˚C. Supernatant was removed and pellet re-suspended in 12mL, volume of ice cold 50mM $CaCl_2$ for 30mL volume of culture and stored on ice for 20 min. Centrifugation was repeated again and the pellet resuspended in 2mL ice cold (50mM) $CaCl_2$ and stored on ice for 2 hours.

## 2.3 Transformation.

We add puc18 plasmid to eppendorff tube containing 300µL of the competent cells then we swirl the tube gently and incubate the reaction on ice for 40 min. After that we heat pulse each transformation reaction in 42˚C water bath for 2 min precisely. The duration of the heat pulse is critical for optimal transformation efficiencies. Then we incubate the reaction on ice for 2 min and add 800µL of LB medium to the transformation reaction and incubate the reaction at 37˚C for 1 hour with shaking at 50 rpm. For the puc18 control transformation, we use a sterile spreader to plate

100µL of the reaction into an LB-ampicillin agar plate + X-Gal (10µL) + IPTG (10µL) and Incubate the plates over night at 37˚C, and then we expected colony numbers for the control transformation.

## 2.4 Microwave exposure.

The microwaves source was generated continuously by BTS (basic station) corresponding to a frequency of 960 MHz for 10 days permanent exposure.
Conditions was as following :
The power output pe=10(mW), Gain BS
Ge=10(dB); Distance = 25cm.
$dP_s$=Pe Ge /(4πD²)=|E|²/η with η= 377ohms.
So at E=6V/m and under controlled temperature conditions (23-25°C).

## 2.5 β-Gal activity study.

### 2.5.1 Bradford's method [19]

Bradford method was used to monitor the concentration of total protein (0.4 mg/ml) and to show the change of total protein concentration of exposed cells compared to non-exposed cells.

### 2.5.2 β-galactosidase activity.

In bacterial culture is usually assayed spectrophotometrically. Because the enzyme cleaves *β*-galactosidic linkage, it will hydrolyze the synthetic chromogenic substrate ONPG, generating O-nitro phenol, which is yellow in aqueous solution.

In our experiment we centrifuge 2ml of medium culture cells induced by IPTG in the logarithmic growth phase, then we dissolve carefully the pellet in TE (TRIS HCL) 10mM, containing 10mM 2-mercaptoethanol, 10mM $MgCL_2$, PH=7,5 and 50µl of lysozyme (50µg/ml), then we centrifuge and keep the supernatant. Finally we prepared 25ml of titration medium: 20 ml of PM2 containing [ 70 mM $Na_2HPO_4$, 3mM $KH_2PO_4$, 2mM Mg EDTA, 1 mM Mg $SO_4$, 0.2mM $MnSO_4$ PH=7 (adjust with HCL)] + 5ml ONPG M/75 (4g/l) + 0,2ml2-mercaptoethanol (12M). We added 1ml of this titration medium to brut extract supernatant 10× diluted with TE. Then we registered the kinetic at 420nm during 1800 sec. Then to established a relation between β-galactosidase activity and the increase of his expression, our study was based on a linear dosimetric function of β-galactosidase concentration (OD=f ([Beta-gal]).

That's why we added to each tubes of the 11 prepared tubes except the buffer one, a variation of 10x diluted brut extract volume, at concentration of (0,4 mg/ml); 10µl for the first tube to 100µl for the last tube. And we adjust to 1 ml with (PM2 + 0.1 ml 2-mercaptoethanol). Then we incubated them at 28˚C and run the colorimetric reaction by addition of ONPG (M/75) 4g/l.

Finally we stopped the reactions each 5 min with adding 0.5ml of $Na_2CO_3$ (1ml) to each tubes.

RT-PCR was employed for the determinant of DNAc Dnak and DNAc *β*-galactosidase level.

## 2.6 RNA extraction

We homogenize 2mL of bacteria with 1mL of TRIR; the homogenate separates into 2 phases after extraction with 0.2mL of chloroform, mixed vigorously, incubated in ice for 5 min, and centrifuged at 12000g for 15 min (4°C).The total RNA was precipitated from aqueous phase by addition with 0.8mL of isopropanol, and then we centrifuged at 12000g, 15 min, 4°c after 2 hours of incubation at -20°C.

Pellet of RNA was washing with 1mL of ethanol 75% and then solubilised, after drying at 75°C for 3min, with DEPC-water. Cell densities of culture used for RT-PCR were $10^7$ cells/ml.

## 2.7 RT-PCR

We mixed the following components in a 0.2mL Eppendorf tube: 10µL RNA template (less than 1µg); 5µL of one step buffer 10× (15mM of $MgCl_2$); 1µL of actins primer sense (10µM); 1µL actins primer antisense (10µM); 4µL dNTPs (2.5mM); 1µL RTase (stork is 5 U/µL diluted 1 in 4) 1µL (1.25U) Taq polymerase (stork is 5 U/µL diluted 1 in 4) and the volume was adjust to 50µL with sterile water samples which were placed in a thermal cycler and cycle as follows: first strand synthesis (47°C, 30min); RTase blend inactivation and initial denaturation (94°C, 2min/cycle); denaturation (94°C, 20sec); annealing (56°C, 30-40cycles); extension (72°C, 1min/Kb); final extension (72°C, 10min/cycle).

The PCR and RT-PCR primers used for the amplification of 103 bp (Dnak) [20] were as follow:
Primer (sense):

**5'CGC-ACC-ACG-CCT-TCT-ATC-ATT3'**

Primer(antisense):
**5'CCA-ATC-AGG-CGT-TTA-ATC-GCA-3'**
And for the amplification of β-galactosidase (21) 119 bp:

Primer(sense):
**5'GTA-AAA-CGA-CGG-CCA-GTG-CCA3'**
Primer(antisense):
**5'CAG-GAA-ACA-GCT-ATG-ACC-ATG3'**

A semi-quantitative analysis of RNA expression by RT-PCR, was used to compare bands intensity corresponding to same deposed volume (20µL) of β-galactosidase or Dnak DNAc exposed and non-exposed. DNA and RNA concentration were determined by densitometry. Ulltraviolet light was used to visualize the Ethidium bromide staining of the Dnak and β-galactosidase bands.

Quantification of the bands was performed using the supplied software and loading was normalized by the intensity of the ethidium bromide staining of the DNA ladder bands

# 3 Results and discussion

## 3.1 Kinetic study

The result of MF exposure on the β-galactosidase activity in *E-coli* culture demonstrate that the enzymatic Kinetic increases linearly as function of time during 1800 sec (**Fig.1**). This indicates the possibility to quantify and analyse β-galactosidase enzyme activity to 10 days exposed cells at 960 MHz compared to control cells induced by IPTG.

The figure obtained in this study suggests that β-galactosidase activity for exposed bacteria is 1.7 fold higher than β-galactosidase activity corresponding to non-exposed Bacteria.

From here we can suggest the hypothesis that a higher rate of β-galactosidase activity is due to a conformation change or to an increase in β-galactosidase protein level.

## 3.2 Effect of β-galactosidase expression on it's activity:

In order to confirm that β-galactosidase activity under MF exposure is due to his higher protein synthesis, a bradford [19] method was used to monitor the protein concentration and to show that total protein concentration of exposed bacteria are higher than non exposed bacteria.

Difference was indeed found in the level of intracellular protein of exposed culture compared to non-exposed cells. The level of protein concentration in exposed cells increased by 1.69 times over than that from control cells, respectively.

This result can indicate that we had possibly a higher expression of β-galactosidase for exposed cells.

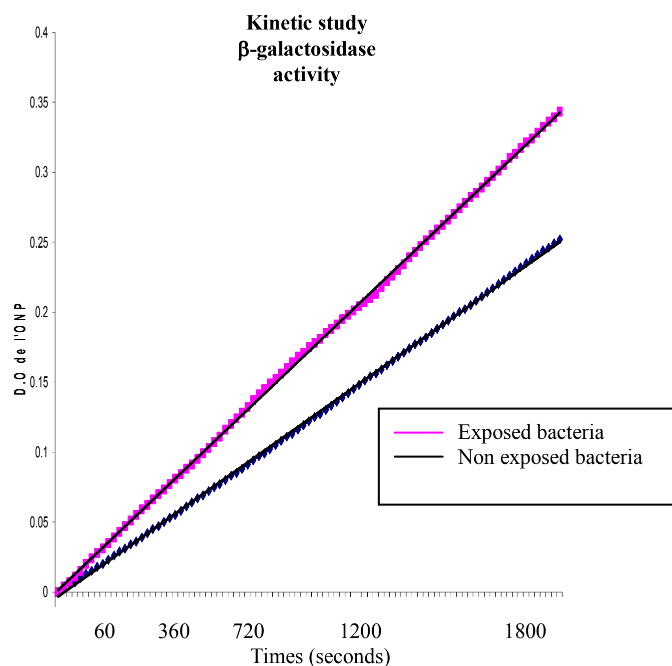



**Fig.1** ***Effect of magnetic field exposure (960 MHz) on the beta-galactosidase activity in E-coli.***

Then we examined the change caused by the electric field on β-galactosidase expression using the semi quantitative analysis method, the RT-PCR. The result of electric exposure on the rate of RNAm β-galactosidase expression is illustrated in **Fig.2**.

We obtained in this study a higher expression for the RT-PCR product corresponding to exposed β-galactosidase gene obtained by using primers specific compared to the RT-PCR product corresponding to non-exposed β-galactosidase gene.

We compared and examined the two product by spectrophotometric method; [ OD=f(λ260nm)].After quantifying RT-PCR product and the bands using the supplied software, we found that *E-coli* exposed to electric field showed a significantly change in level of intracellular β-galactosidase RNAm compared to cells incubated without field exposure.

For the *E.coli* culture that had been exposed, it gave a higher Beta-gal concentration (960 ng/µl for (a) and 994 ng/µl for (b)) that than non-exposed culture (565 ng/µl for (c) and 585 for (d)).

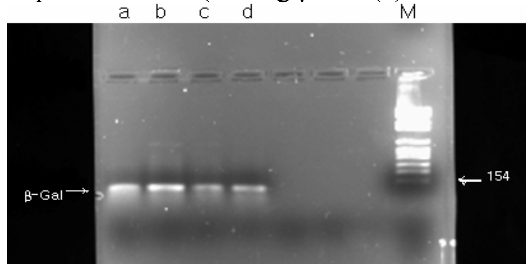



**Fig.2** ***Semi-quantitative analysis of RNA expression by RT-PCR. The RT-PCR product obtained by using primers specific to B-galactosidase gene. Lane M, 100bp DNA ladder, lane a, b, B-gal in EF exposed cells; lane c ,d, B-gal in control cells***

The change of β-galactosidase RT-PCR product exposed 10 days to MF clearly shows that *E-coli* exposed to MF increased the transcription level of gene encoding β-galactosidase. Then using RT-PCR we reported that β-galactosidase expression directed by RNAm were elevated following exposure to 960 MHz.

Then an enzyme activity study was done **Fig.3** to prove that β-galactosidase activity increased due to his higher expression under microwave exposure.

We quantified the activity taken at various total protein concentration following the start of MF exposure and non exposure.

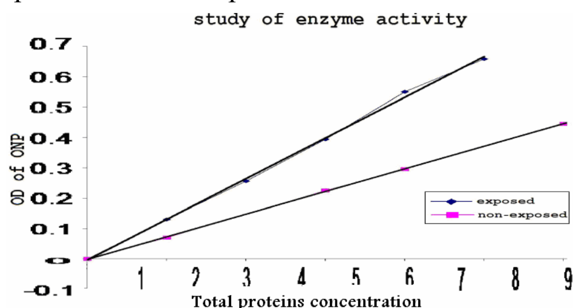



**Fig.3** ***Effect of B-gal synthesis on it's activity***

A significant change of β-galactosidase activity was shown under exposure for 10 days to the electric field. The fig.3 does show that the increase of both B-galactosidase activity and total proteins expression are significantly proportional.

The results obtained in this β-galactosidase study suggest that indeed, β-galactosidase activity increased due to a higher β-galactosidase expression.

Numerous teams showed that low frequency (ELF) exposure provoke an increase of the synthesis of HSP and their activation [13]. So we examined the hypothesis that the cells exposed to electric field affect in a manner similar to other environmental stress factor, such as heat shock exposition.

The expression levels of Hsp70 were investigated using RT-PCR. A dosimetric method was used to analyze Dnak concentration after RT-PCR. A difference was found in the levels of Dnak of exposed cells compared to control cells.

The culture that had been exposed for 10 days at 960 MHz, gave a higher Dnak concentration (775 ng/µl for (a) and 516.6 ng/µl for (c)) that than non-exposed culture (415 ng/µl for (b) and 276.4 ng/µl for (d), c and d are 1.5 diluted samples respectively to a and b).

Using RT-PCR and quantification of the bands using supplied software we found in agarose gel (**Fig.4**) evidence increase of 1.87 in Dnak level following exposure to continuous wave or GSM-modulated RFR at 960 MHZ for a period of 10 days which indicates that indeed electric field (960 MHz Volt/m) out of any heating or thermal effect ,were an environmental stress factor.

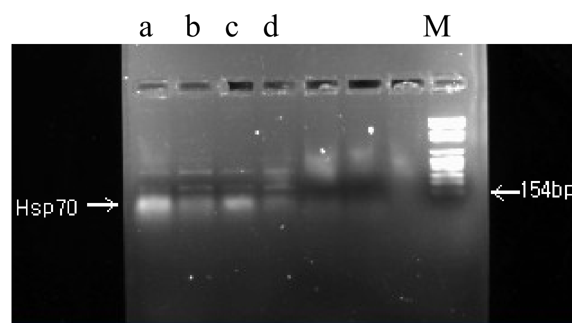



**Fig4**. ***Semi-quantitative analysis of RNA expression by RT-PCR.. The RT-PCR product obtained by using primers specific to Dnak gene. Lane M, 100bp DNA ladder, lane a,c, Dnak (HSP70) in EF exposed cells; lane b,d, Dnak in control cells.***

### 3.3 Dnak accumulation and β-galactosidase expression.

The **Fig.5** shows that the accumulation of both Dnak and β-galactosidase in *Escherichia coli* is responsive to the electric field exposure. Conceivably as Dnak and β-galactosidase are responsive to the stressing factor, the reason of this higher enzyme activity seems to be related to the accumulation of the major heat shock protein of *E.coli* Dnak.

That's why a complementary study have been done, consisting to identify if heat shock protein is involved in the higher β-galactosidase activity.

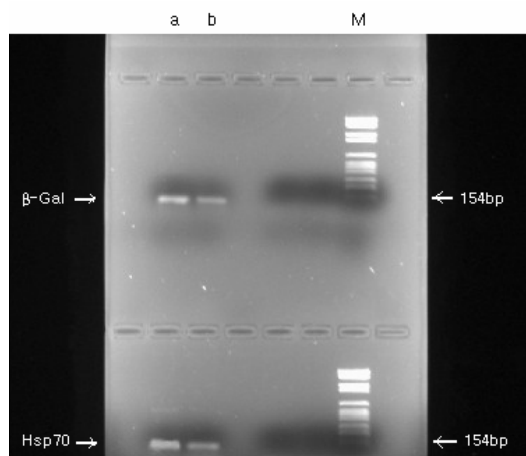
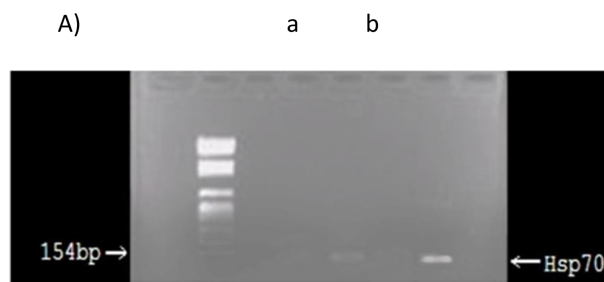



**Fig.5** ***Comparison of B-gaactosidase and Dnak RT-PCR product exposed (a) and non-exposed (b). . Lane M, 100bp DNA ladder.***

We transformed the *E.coli TB1* with plasmid that carry RT-PCR product Dnak obtained using primers specific and control cells with a plasmid that does not carry any gene encoding Dnak as the negative control. The transformed culture was induced with IPTG. The β-galactosidase, activity as shown as in **Fig.6**, shown that cells, that carried recombined PUC18 gave a higher level of ONP, corresponding to β-galactosidase activity compared to control cells.

The level of β-galactosidase activity corresponding to cells transformed by recombined plasmid, increased by approximately 1.63 Fold times over that than from control cells. The results obtained in this study suggest that the higher β-galactosidase activity was due to Dnak accumulation.

A)




B)

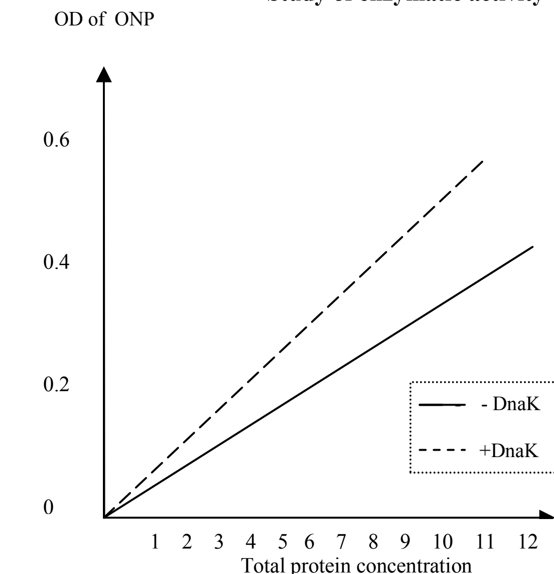



**Fig.6.** *A) Detection Dnak induction in E.coli cells carrying recombined PUC18 (a) and cells carrying not recombined PUC18 (b). The Dnak accumulation was detected by RT-PCR, using primers respectively specific to Dnak gene in culture as induced by IPTG with a concentration of (30µM)*

*B) Effect of Dnak accumulation on Beta-gal activity in E.coli.*
*a-refer to negatives cells (that not carry gene encoding Dnak).*
*b-refer to the cells that carry gene encoding Dnak(Hsp70)*

Dnak is known to interact directly in RNA transcription. However experiment shown that *E.coli* Hsp70 contributes to *E.coli*'s growth not only by protecting and disaggregate some enzymes, like RNA polymerase from denaturation , but also by reactivating them [14].

It's also known that when cells are exposed to certain frequencies radiation, the β-galactosidase expression [16, 17, and 18] and Hsp70 level [12, 13] are increased.

With these two lines of references, it prompts us to propose that the stimulatory effect of EF at 960 MHz likely to be used by mobile telephony lead not

only to increase the accumulation of the transcription factor (23) of stress promoter in *E.coli* of Dnak but also to increase RNA polymerase activity or simply the β-galactosidase activity trough Dnak induction. In which we suggest that a higher β-galactosidase activity is due to Dnak accumulation.

# 4 Conclusion

Our present discovery that EF exposure of mobile telephony can enhanced the βgalactosidase protein synthesis under the control of the major HSP of *E.coli* (Dnak) responding to the stress factor (EF), has raised two interesting issue.

First, the chronicle exposition of mobiles phones used in Lebanon are potential carcinogen problem as certain cancers showing Hsp's surexpression [22] and as the stimulatory effect of electric field on Dnak surexpression are linked as shown in our experiment.

We have to indicate at this point that the SAR corresponding to the electrictic field that we used was lower that than the normal authorized.

Second, the discovery that, Dnak under the control of EF, can be a positive finding for the gangliosidosis GM1, an autosomal inherited disease known to be responsible of a neurological disorder caused by deficiency of β-galactosidase activity and expression, which leads to an accumulation of GM1 ganglioside, and sphingolipids in neurons and others organs and that it can enhance any specific insert encoding for a gene recombined, which could be used for medical endings (insulin production for diabetics uses, etc…).

Our study has many interesting issues and can confer an other dimension of evolutionary potential to RF effect on culture cells.

However, what we should have to verify, it's if the accumulation of Hsp that protect some enzymes from denaturation and aggregation can always protect protein from denaturation and aggregation at a higher level of protein expression in response to a higher level of RF than that used in our experiment.

We could envisaged at a high level of RF the appearance of a biological mechanism which could lead the decrease of the protein synthesis.

Our conclusion: that the non thermal effect increases the protein synthesis at the RNAm level, and stimulate protein activation at low level of RF.

We can also agree that HSP may have a role in the alteration of the DNA [24]. However other experiments should be done.

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