

# Increased protein synthesis by cells exposed to a 1,800-MHz radio-frequency mobile phone electromagnetic field, detected by proteome profiling

Christopher Gerner · Verena Haudek · Ulla Schandl ·  
Editha Bayer · Nina Gundacker · Hans Peter Hutter ·  
Wilhelm Mosgoeller

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## Abstract

**Purpose** To investigate whether or not low intensity radio frequency electromagnetic field exposure (RF-EME) associated with mobile phone use can affect human cells, we used a sensitive proteome analysis method to study changes in protein synthesis in cultured human cells.

**Methods** Four different cell kinds were exposed to 2 W/kg specific absorption rate in medium containing <sup>35</sup>S-methionine/cysteine, and autoradiography of 2D gel spots was used to measure the increased synthesis of individual proteins.

**Results** While short-term RF-EME did not significantly alter the proteome, an 8-h exposure caused a significant increase in protein synthesis in Jurkat T-cells and human fibroblasts, and to a lesser extent in activated primary human mononuclear cells. Quiescent (metabolically inactive) mononuclear cells, did not detectably respond to RF-EME. Since RF exposure induced a temperature increase of less than 0.15°C, we suggest that the observed cellular response is a so called “athermal” effect of RF-EME.

**Conclusion** Our finding of an association between metabolic activity and the observed cellular reaction to low intensity RF-EME may reconcile conflicting results of previous studies. We further postulate that the observed increased protein synthesis reflects an increased rate of protein turnover stemming from protein folding problems caused by the interference of radio-frequency electromagnetic fields with hydrogen bonds. Our observations do not directly imply a health risk. However, vis-a-vis a synopsis of reports on cells stress and DNA breaks, after short and longer exposure, on active and inactive cells, our findings may contribute to the re-evaluation of previous reports.

**Keywords** GSM mobile phone · Protein synthesis · Stress proteins · Toxicology

## Abbreviations

RF-EME Radiofrequency electromagnetic exposure  
SAR Specific absorption rate

## Introduction

Whether or not low intensity radiofrequency electromagnetic field exposure (RF-EME) associated with the use of GSM-1800 mobile phones can have direct effects on cells is a matter of debate. The energy transferred by these fields is certainly too weak to ionize molecules or break chemical bonds (Adair 2003).

So called thermal effects on cells, caused by energy transfer, are directly related to the specific absorption rate (SAR) and are well understood. Investigations of athermal cellular effects caused by low intensity exposure, in contrast, have generated conflicting data (Belyaev 2005). This applies to epidemiologic studies and to laboratory

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C. Gerner · V. Haudek · U. Schandl · E. Bayer · N. Gundacker ·  
W. Mosgoeller (✉)  
Department Med.-1, Institute of Cancer Research,  
Medical University Vienna, Borschkegasse 8a,  
1090 Vienna, Austria  
e-mail: wilhelm.mosgoeller@meduniwien.ac.at

H. P. Hutter  
Center for Public Health, Institute of Environmental Health,  
Medical University Vienna, Kinderspitalgasse 15,  
1095 Vienna, Austria

investigations focusing on cellular effects such as DNA damage or proteome alterations (Blank 2008).

Early epidemiologic studies on mobile phone use did not reveal an associated health risk (Rothman et al. 1996; Valberg 1997). Subsequent studies described some evidence for enhanced cancer risk (Kundi et al. 2004). More recently, an increased brain cancer risk associated with intensive phone use over many years was discussed (Hardell et al. 2008; Hardell et al. 2007; Kan et al. 2008). Sadetzki et al. (2008) reported an exposure-related increase of parotoid gland tumors, which suggests that the possible risk and cellular mechanism is not cell type specific, but may affect various cells.

Repacholi et al. (1997) described an increase in the incidence of lymphoma in sensitized transgenic mice. Their results were, however, not reproduced in follow-up studies (Utteridge et al. 2002). An enhancement of genotoxicity was described (Maes et al. 1996), but again could not be substantiated by the same team (Verschaeve et al. 2006). DNA breaks after RF-EME have been described but could not be reproduced in other laboratories (Diem et al. 2005; Speit et al. 2007).

Several studies have investigated effects of radiation exposure on specific proteins. Thus, Yilmaz et al. (2008), who investigated the effect of RF-EME on the expression level of the anti-apoptotic bcl-2 protein by immunohistochemical staining, reported that exposure to the radiation emitted by a 900-MHz cellular phone for 20 min did not alter the level of bcl-2 in the brain and testes of rats. Sanchez et al. (2008) investigated the mobile phone radiation-induced stress response in human skin cells after exposure for 2 h per day and also found no changes. In contrast, in vitro exposure of EAhy926 cells to 900 MHz GSM microwave radiation induced a transient cellular stress response, judged by an increased phosphorylation of heat shock protein-27 (Leszczynski et al. 2002). Results from Nylund and Leszczynski (2004) support the hypothesis that mobile phone radiation can affect the cytoskeleton and the physiological functions that are regulated by the cytoskeleton. More recently, Karinen et al. (2008) provided evidence that mobile phone radiation can alter protein expression in human skin. Blank (2008) has reviewed examples of direct molecular conformation changes caused by radio frequency radiation exposure.

The observed changes in protein phosphorylation are consistent with the activation of a variety of cellular signal transduction pathways by mobile phone radiation, among them the hsp27/p38MAPK stress response (Leszczynski et al. 2002). Friedman et al. (2007) described the rapid activation of ERK (extracellular-signal-regulated kinase), but not of the stress-related MAPKs (mitogen-activated protein kinase) in response to various frequencies and intensities of RF-EME.

The lack of consensus with regard to the difficulty to reproduce effects of RF-EME may to some extent reflect the large number of experimental variables, such as frequency, amplitude, modulation (Litovitz et al. 1990), exposure time and cell types that must be controlled.

In the present study, we measured the impact of RF-EME on the rate of synthesis of a range of proteins. Because any challenge to cells may eventually trigger an adaptive response, the measurement of protein synthesis offers a highly sensitive screening method to detect subtle effects.

The applied methodology was based on metabolic labeling cells during RF exposure and subsequent resolution of protein extracts by two-dimensional electrophoresis in order to measure de novo protein synthesis and total protein amounts (Gerner et al. 2002). To investigate whether or not cell types respond differently, we exposed different kinds of cells including proliferating Jurkat cells, cultured fibroblasts as well as quiescent and inflammatory stimulated primary human white blood cells.

## Materials and methods

### Exposure apparatus

We used the sXc1800 exposure unit (IT'IS, Zürich, Switzerland) to test radio frequency electromagnetic field exposures from mobile communication devices (Schuderer et al. 2004). The unit was installed in a conventional cell incubator with 5% CO<sub>2</sub> and saturated humidity. The exposure unit has two wave guides, which serve as chambers for cell growth and RF exposure. In every experiment, it allows for (and requires the) comparison of control cells and those exposed to modulated GSM 1,800 MHz fields. ELF magnetic fields may actively contribute cellular effects (Mild et al. 2009). However, in our experiments, the background fields were identical between sham and real exposure and therefore cannot be held responsible for the observed differences.

### Double-blind experimental design

Approximately  $10 \times 10^6$  cells were used for each experiment. Cells were either exposed or mock-exposed to RF-EM under blinded conditions, followed by protein extraction and analyses. RF exposure was controlled by a computer program, which switched on the exposure in one waveguide while the other served as exposure control. The exposure settings were recorded in a coded file, and after the biochemical analysis of exposed and control cells, decoding was carried out by a coauthor (HPH) who was not involved in the exposure and biochemical analysis. In this

manner, we excluded any direct and indirect investigator bias of the results.

### Exposure conditions

In this study, we used modulations closely reflecting the technical specifications of GSM-1800. A GSM signal is modulated, i.e. it has different superordinated structures according to the transmission mode (“GSM-basic” for speech uplink or GSM-DTX for listening). A GSM basic signal is a multi-frame signal consisting of 26 frames, of which every 26<sup>th</sup> frame is blanked, which creates a low frequency (8 Hz) component. The GSM-DTX signal consists of periodical single bursts, with some multi-frames interspersed. For details see “[www.itis.ethz.ch](http://www.itis.ethz.ch)”. A typical phone conversation is a mixture of listening (GSM-DTX) and talking (GSM basic). In the current study, we used a modulation mixture that consisted of about 66% GSM basic (talking) and 34% GSM-DTX (listening). The exposure time was 8 h. The intermittence pattern was 5 min. RF exposure “on” and 10 min “off” (no exposure), to compare the very same conditions, which revealed DNA breaks (Diem et al. 2005; Schwarz et al. 2008). The exposure was 2 W/kg during the “on” phase. With the chosen parameters, the controlled temperature difference between the two chambers (control and real exposure) was below 0.15°C, which, according to our experience, excludes a thermally induced effect in our system (Gerner et al. 2002).

### Cell preparation

Human Jurkat T-cells were cultured in RPMI supplemented with 10% FCS under standard cell culture conditions. Primary human diploid fibroblasts (ES1 cells) were a kind gift of the workgroup Rüdiger in Vienna. It allowed us to investigate the proteomes of the very same cell line and culture conditions, which upon radiation revealed DNA breaks (Diem et al. 2005; Schwarz et al. 2008). These cells were cultured in Dulbecco’s modified Eagle’s Medium (DMEM, Gibco), 10,000 IU/ml penicillin/streptomycin, 200 mM L-glutamine, 40 µg/ml neomycin and 10% FCS. Peripheral blood mononuclear cells (white blood cells—WBC) were isolated from heparinized whole blood obtained from healthy donors (mixed with 2 vol. HBSS) by standard density gradient centrifugation with Ficoll-Paque (Pharmacia Biotech). The interface cells were washed and resuspended in autologous (donor) plasma. Inflammatory activation of the cells was accomplished by the addition of 5 µg/mL phytohaemagglutinin (PHA-P; Sigma) and 10 ng/mL LPS (Sigma).

Cells were metabolically labeled with 0.2 mCi/mL <sup>35</sup>S protein labeling mix containing <sup>35</sup>S-methionine and <sup>35</sup>S-cysteine (Trans35label, Biomedica, MP Biomedicals)

during control exposure and real RF-EME at 37°C in a humidified atmosphere containing 5% CO<sub>2</sub>. The incubation and labeling times were 2 and 4 h in exploratory experiments and 8 h in the final series with three independent repetitions per exposure condition.

### Subcellular fractionation

After incubation and labeling of cells, cytoplasmic proteins were isolated as follows. Cells were lysed in 0.25 M sucrose, 3 mM MgCl<sub>2</sub>, 0.5% Triton X-100 in lysis buffer (10 mM HEPES/NaOH, pH 7.4, 10 mM NaCl, 3 mM MgCl<sub>2</sub>). The cytoplasmic fraction was separated from the nuclei by centrifugation through a 30% sucrose gradient at 3,500 rpm for 5 min at 4°C. After ethanol precipitation, the pelleted cytoplasmic protein fraction was directly solubilized in sample buffer. All buffers used were supplemented with the protease inhibitors PMSF (1 mM), aprotinin, leupeptin and pepstatin A (all at 1 µg/mL).

### 2D Page

High-resolution 2D gel electrophoresis was carried out as described previously (Gerner et al. 2002), using the Protean II xi electrophoresis system (Bio-Rad, Hercules, CA). The protein samples were dissolved in sample buffer (7.5 M urea, 1.5 M Thiourea, 4% CHAPS, 0.05% SDS, 100 mM DTT). To optimize the solubilization of proteins, we saturated the protein solution with solid urea. Protein concentrations were determined using a standard Bradford assay.

Solubilized protein (300 µg per gel) was diluted to 280 µl with sample buffer freshly adjusted to 0.2% Bio-Lyte® 3/10 Ampholytes (Bio-Rad) and 0.002% bromophenol blue. After 12 h rehydration of pH 5–8, 17-cm IPG strips (Bio-Rad, Hercules, CA) at room temperature, IEF of protein samples was performed in a stepwise fashion (1 h 0–500 V linear; 5 h 500 V; 5 h 500–3,500 V linear; 12 h 3,500 V). After IEF, the strips were equilibrated with 100 mM DTT and 2.5% iodacetamide according to the manufacturer’s instructions (Bio-Rad Hercules, CA). For SDS–PAGE, focused and equilibrated IPG strips were placed on top of 1.5 mm 12% polyacrylamide slab gels and overlaid with 0.5% low melting agarose. The gels were run at 15°C at 150 mA for about 4–5 h and then stained with 400 nM solution of Ruthenium II tris (bathophenanthroline disulfonate; RuBPS) as described by Rabilloud et al. (2001). Fluorescence scanning was performed with a Fluor-Imager 595 (Amersham Biosciences, Amersham, UK) at a resolution of 100 µm. After scanning, gels were placed on Whatman 3MM chromatography paper, covered with cling film, and dried at 60°C using a slab gel dryer SE110 (Hoefer, San Francisco, CA, USA). Exposure to phosphor storage screens (Molecular Dynamics) was carried out at

room temperature for 24 h. Screens were subsequently scanned with a Phosphorimager SI (Molecular Dynamics) at a resolution of 100  $\mu\text{m}$ .

For identification of 2D gel spots, protein samples of unlabeled cells were separated by 2D-PAGE followed by silver staining as described (Gerner et al. 2002).

Gels were warped to a reference gel with the TT900 S2S software (version 2006.0.2389, Nonlinear dynamics, Carlsbad, CA) and evaluated with the Progenesis software PG200 (version 2006, Nonlinear, Newcastle upon Tyne, UK) using the “same spot” algorithm. Spot assignment, background correction, normalization and statistical calculations (analysis of variance, ANOVA) were performed using this software package. Factors indicating up-regulation of proteins in 2D gels were obtained using normalized integrated spot intensities. For most accurate quantification, we only considered spots with an integrated intensity at least three-fold higher than the corresponding spot background value. Integrated intensities from fluorescence detection and autoradiography were normalized against the sum of all matched spots.

### Tryptic digestion

Protein spots were excised, de-stained with 15 mM  $\text{K}_3\text{Fe}(\text{CN})_6/50\text{ mM Na}_2\text{S}_2\text{O}_3$  and extensively washed with a methanol (50%)/acetic acid (10%) mixture. The pH was then adjusted with 50 mM  $\text{NH}_4\text{HCO}_3$ , the proteins were reduced with 10 mM DTT/50 mM  $\text{NH}_4\text{HCO}_3$  for 30 min at 56°C and finally alkylated with 50 mM iodacetamide/50 mM  $\text{NH}_4\text{HCO}_3$  for 20 min in the dark. Afterward the gel pieces were dried with acetonitrile and rapidly dried in a vacuum centrifuge (Heto, Denmark). Between each step, the tubes were shaken for 5–10 min (Eppendorf thermomixer Comfort).

The dried gel spots were treated with trypsin (0.0125 g/ml; trypsin sequencing grade, Roche Diagnostics, Germany)/50 mM  $\text{NH}_4\text{HCO}_3$ , for 20 min on ice, covered with 50 mM  $\text{NH}_4\text{HCO}_3$  and subsequently incubated overnight at 37°C.

The digested peptides were eluted from the gel spots by addition of 50 mM  $\text{NH}_4\text{HCO}_3$  and sonication for 10 min. The supernatants were then transferred to siliconized tubes, and the extraction procedure repeated a further two times with 5% formic acid/50% acetonitrile. After this, the extracted peptide solutions were concentrated to a volume appropriate for further analysis.

### Mass spectrometry analysis

Proteins were identified by mass spectrometric analysis. Peptides were loaded on a Zorbax 300SB-C8 (5  $\mu\text{m}$ ,

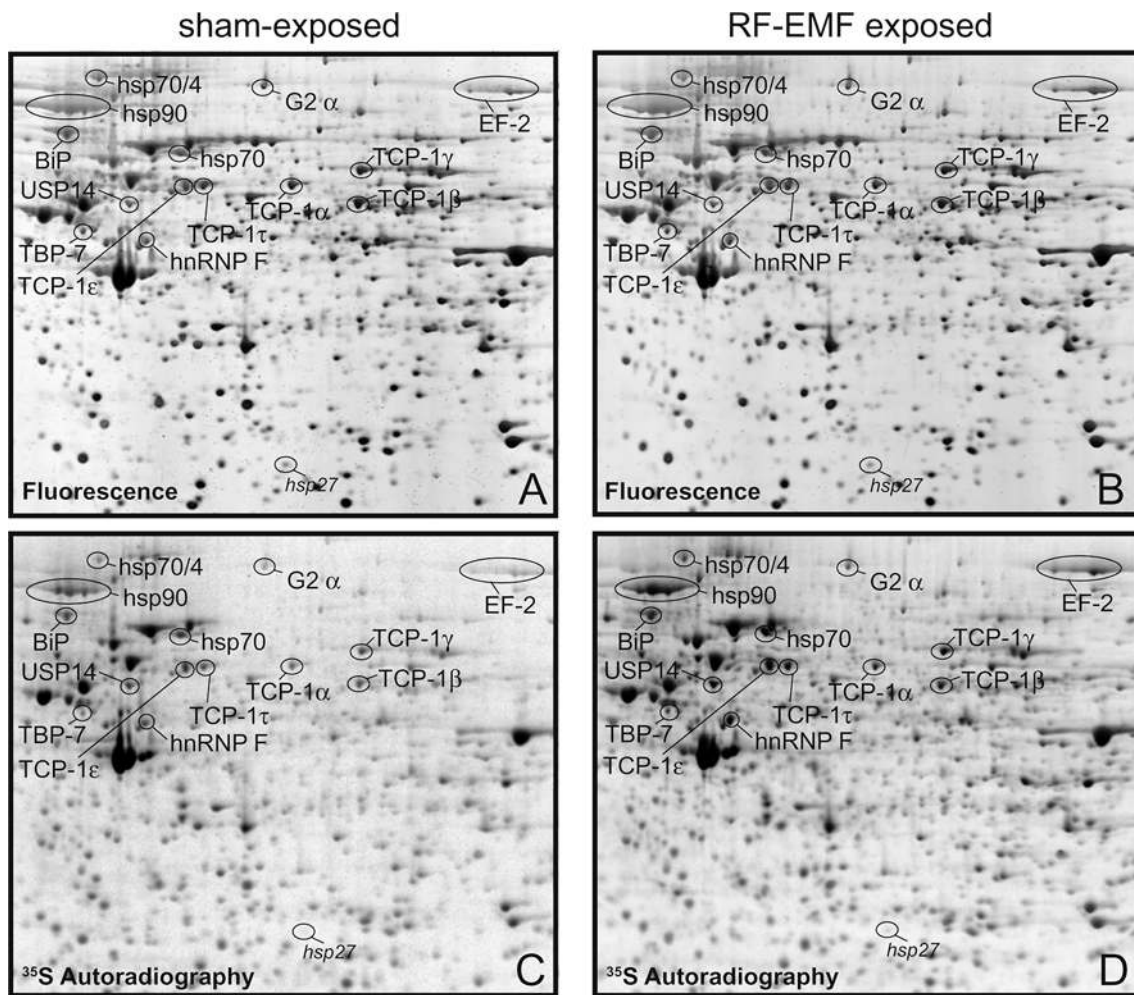
0.3 mm  $\times$  5 mm) column and separated by nanoflow liquid chromatography (1100 Series LC system, Agilent, Palo Alto, CA) using a Zorbax 300SB-C18 (5  $\mu\text{m}$ , 75  $\mu\text{m} \times$  150 mm) column at a flow-rate of 250 nl/min and using a gradient from 0.2% formic acid and 3% acetonitrile to 0.2% formic acid and 45% acetonitrile over 12 min. Peptide identification was accomplished by MS/MS fragmentation analysis with an ion trap mass spectrometer (XCT-Ultra, Agilent) equipped with an orthogonal nanospray ion source. The MS/MS data were interpreted by the Spectrum Mill MS Proteomics Workbench software (Version A.03.03, Agilent) and searched against the SwissProt Database version 20061207 allowing the initial search algorithm a precursor mass deviation of 1.5 Da, a product mass tolerance of 0.7 Da and a minimum matched peak intensity (%SPI) of 70%. Due to previous chemical modification, carbamidomethylation of cysteines was set as fixed modification. No other modifications were considered here. Peptide scores were essentially calculated from sequence tag lengths, but also considered mass deviations. To assess the reliability of the peptide scores, we performed searches against the corresponding reverse database. 6.0% positive hits were found with peptides scoring  $>9.0$ , while no positive hits were found with peptides scoring  $>13.0$ . All spots were identified with at least two different peptides including one scoring at least higher than 13.0. The details of protein identifications, including peptide sequences, peptide scores and sequence coverage are provided in the electronic supplementary data.

### Statistical analysis

In each experiment, we compared proteins from cells kept under identical culture conditions. The only difference was that they were exposed under sham or real conditions. The gel from sham exposed cells (reference) was compared to the gel from the cells with real exposure, using the TT900 S2S software (version 2006.0.2389, Nonlinear dynamics, Carlsbad, CA) and then evaluated with the Progenesis software PG200 (version 2006, Nonlinear) using the “same spot” algorithm. Spot assignment, background correction, normalization and statistical calculations (one way analysis of variance, ANOVA, calculated from three independent experimental replicates per group) were performed using this software package. If the “*P*-value” for a protein was  $\geq 0.05$ , this was considered “not significant”.

For each data set (cell type), in each table, we summarized three independent experiments (pairs of sham and real exposed cells), where the differences between sham and real exposure are expressed as average “increase factor” (x-fold higher) in real exposed cells.





**Fig. 1** RF-EME exposure of Jurkat T-cells generally increased <sup>35</sup>S incorporation rates, indicating induction of protein synthesis. The cells (exposed and controls) were metabolically labeled for 8 h during exposure. **a, b** Fluorescence detection of protein amounts, separated by 2D-

PAGE. **c, d** De novo synthesis (<sup>35</sup>S autoradiographs) of proteins depicted in **a** and **b**, respectively. While protein amounts did not show significant alterations, protein synthesis was generally increased due to RF-EME. Annotated proteins are further detailed in Table 1

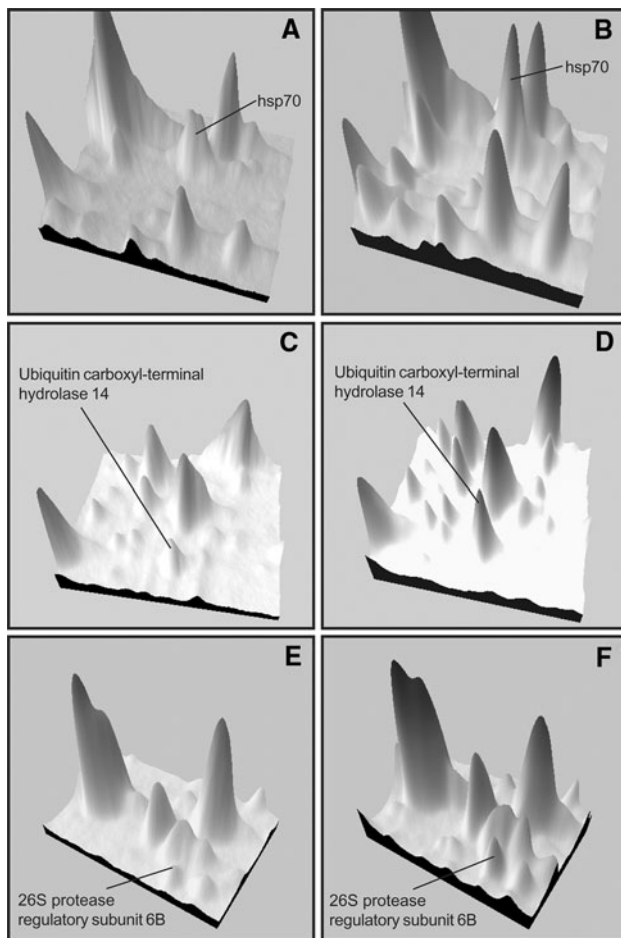
## Results

### Jurkat cells

Figure 1 shows corresponding 2D gels derived from exposed and control cells. The separated proteins were quantified for protein amounts (fluorescence detection) and protein synthesis (<sup>35</sup>S-autoradiography). The spot pattern obtained was very highly reproducible: 855 spots were consistently detected in six gels from three independent experiments, each with exposed and corresponding control cells. The average standard deviation of fluorescence spot intensities (18.8%) was determined from the three independent control cell gels. Fluorescence spot intensities for some individual proteins appeared to reveal an increased level in response to RF-EME. Application of strict spot quantification criteria, however, indicated that there were no significant differences between RF-EME-exposed and control

cells. Autoradiographs of the same gels, however, revealed significant differences in the rate of de novo synthesis of several proteins (greater than 2 fold) between RF-EME and control cells. Figure 1c, d shows the higher general autoradiograph intensity observed for radiation exposed cells. On average, the total <sup>35</sup>S autoradiograph intensity was almost doubled [the measured increase was  $93 \pm 28\%$  ( $n = 3$ )]. Actually all detectable protein spots displayed an increased <sup>35</sup>S incorporation rate.

We categorized a protein as specifically up-regulated if the normalized integrated <sup>35</sup>S autoradiograph spot intensity was at least two-fold greater than the corresponding control cell spot with an ANOVA *P*-value of less than 0.05. Using this criterion, fourteen proteins were found to be specifically up-regulated and were subsequently identified by mass spectrometry as described in Materials and Methods (Table 1 and supplementary data). Figure 2 provides three examples of proteins specifically up-regulated by RF-EME:



**Fig. 2** 3D-landscapes of selected gel areas. Relative spot intensities from controls (**a, c, e**) and RF-EME exposed cells (**b, d, f**) are depicted as spot heights to demonstrate the specific induction of some proteins relative to the local spot environment. The indicated proteins are also listed in Table 1

heat shock protein 70, ubiquitin carboxyl-terminal hydrolase 14 and 26S protease regulatory subunit 6B. Figure 3 shows peptide fragmentation mass spectra of peptides derived from ubiquitin carboxyl-terminal hydrolase 14 and 26S protease regulatory subunit 6 in order to demonstrate the high degree of reliability of the protein identification procedure used. The RF-EME induced increase in protein synthesis (Fig. 4a–f) was highly reproducible.

#### Fibroblasts

Cultured human fibroblasts showed the highest level of responsiveness to RF-EME (Fig. 4e, f; Table 2) with an average protein synthesis increase of  $128 \pm 22\%$  (three independent experiments). Thirteen of the fourteen proteins whose rate of de novo synthesis was increased in Jurkat cells were also synthesized at a higher rate in fibroblasts. As well as these, the rates of synthesis of annexin A1 and A5 were found to be significantly increased (Table 2). This

finding suggests that the proteome alterations in responsive cells induced by RF-EME exposure are characteristic for this kind of cell stress.

#### White blood cells

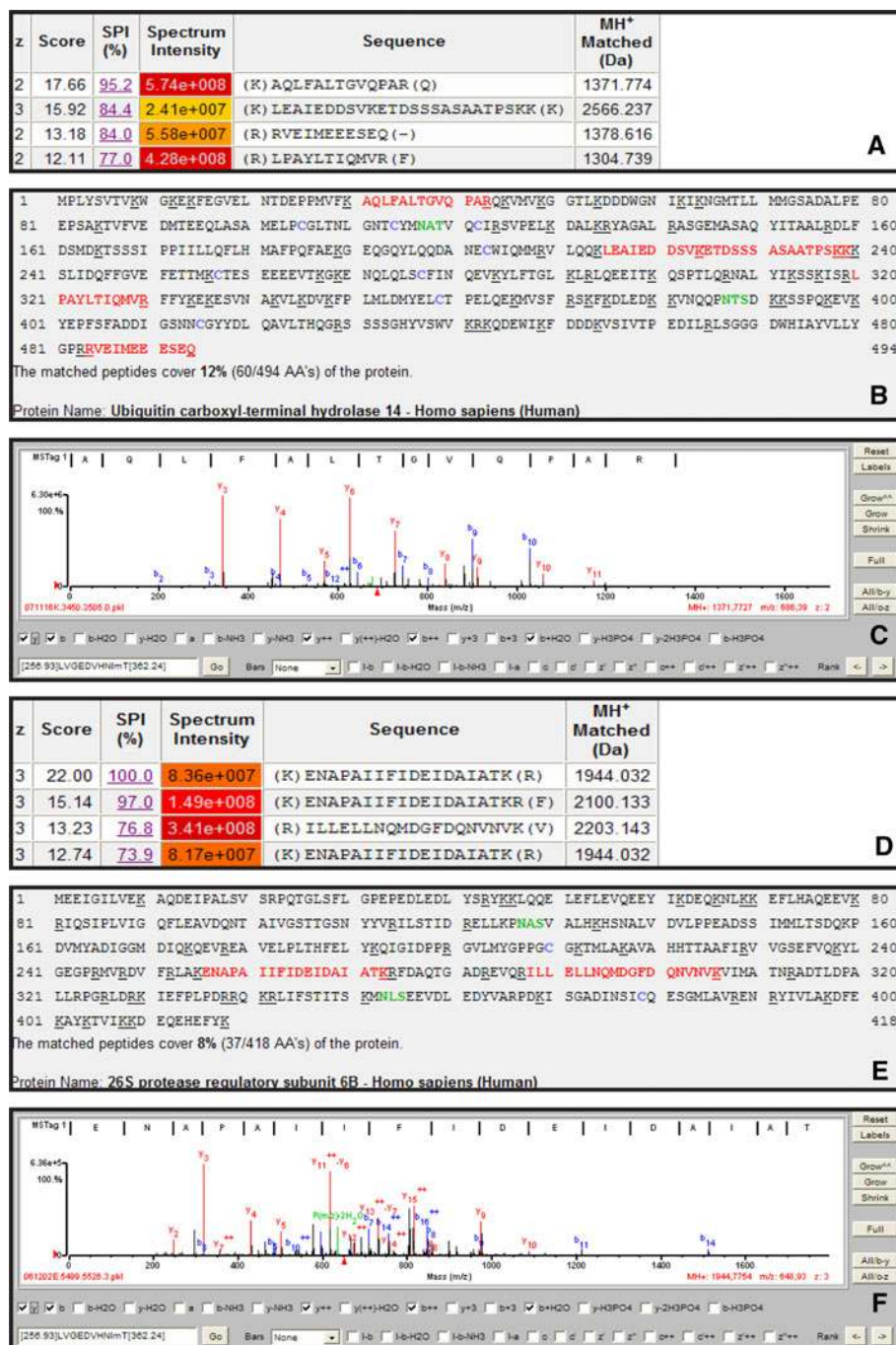
Primary mononuclear cells isolated from peripheral blood (white blood cells, WBC) responded only marginally to RF-EME (Fig. 4g, h; Table 3). The apparent increase in  $^{35}\text{S}$  incorporation was less than 10%, which is within the margin of error of the applied methodology. Inflammatory stimulation of WBCs by treatment with lipopolysaccharide and phytohaemagglutinin increased the level of protein synthesis by these cells (compare Fig. 4g–i), which is consistent with the induction of cell proliferation as previously described in more detail (Traxler et al. 2004). Notably, RF-EME further increased the level of  $^{35}\text{S}$  incorporation by the stimulated cells (compare Fig. 4i–j) by an average of  $43 \pm 13\%$  (three independent experiments with three different donors). The proteome alterations were, however, less compared to those observed in Jurkat cells and fibroblasts. Only one protein, hsp60, was induced more than two-fold (Table 4).

#### Discussion

We used a highly sensitive method of measuring protein synthesis rates and protein amounts to investigate the potential effects of low-intensity mobile phone radiation exposure on cells. Our results show that the rate of protein synthesis in proliferating cells is increased by long-term (8 h) RF-EME, while no effect was detectable in quiescent white blood cells treated in the same manner. Although the observed changes reached no statistical significance at short exposure times, we observed some trends consistent with but also extend observations made by Nylund and Leszczynski (2004), who used the same exposure system, but only measured protein amounts (and not de novo synthesis).

Usefully, our results appear to reconcile a number of conflicting previous findings. First, we found both RF-EME responsive and RF-EME-insensitive cells (compare Tables 1, 2 with Table 3). The RF-EME insensitive quiescent WBCs (Table 3) were rendered sensitive to RF-EME by inflammatory activation (Fig. 4). Inflammatory activation of WBC induces T-cell proliferation and consequently an increased rate of protein synthesis (Traxler et al. 2004). Thus, our data suggest that proliferating cells with high protein synthesis rates are more sensitive to RF-EME than cells with lower protein production. Many studies have been performed with quiescent white blood cells, which were also insensitive under our experimental conditions.

**Fig. 3** Identification details of isolated 2D gel spots. After tryptic digestion and peptide separation by nano-flow liquid chromatography, isolated peptides were fragmented in an ion trap mass spectrometer. **a–c** Peptides identified in the spot identified as ubiquitin carboxyl-terminal hydrolase 14 (z, peptide charge; Score, Spectrum Mill peptide score; SPI, scored peak intensity). **b** Assignment of identified peptides to protein sequence. **c** MS2 spectrum of the peptide AQLFALTGVQPAR. **d–f** Peptides identified in the spot identified as 26S protease regulatory subunit 6B, **e** assignment of identified peptides to protein sequence, **f** MS2 spectrum of the peptide ENAPAIIFIDEIDAIATK



Second, the exposure time seems to be a critical factor. In our preliminary experiments, we did not observe significant effects with 2 and 4 h exposure times (data not shown). An 8-h exposure was required to obtain reproducible and significant effects, a time much longer than the longest exposure time used in most other studies. Third, the determination of protein amounts by spot integration is not very precise. Silver staining in particular, does not produce reliable quantitative data (White et al. 2004). Standard deviations obtained with the much more accurate fluores-

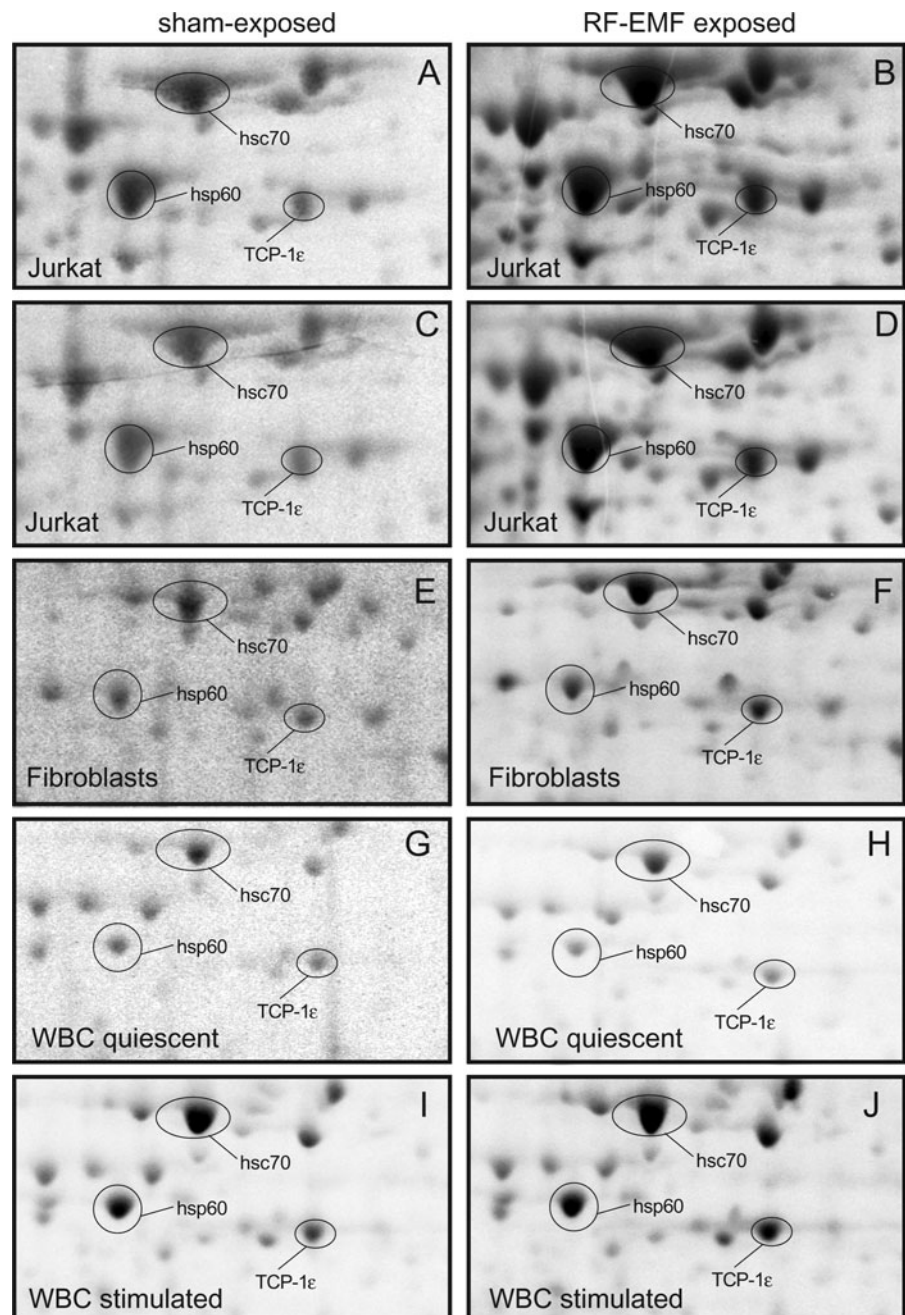
cence detection methods are usually of the order of 25%. Consequently, subtle alterations may easily be missed due to limited sensitivity.

#### Possible mechanisms

During electromagnetic exposure, we applied 5 min of “exposure on” and 10 min of “off” on the same cell types and/or conditions, which revealed DNA breaks (Diem et al. 2005; Franzellitti et al. 2010; Schwarz et al. 2008). Interestingly,



**Fig. 4** The RF-EME induced increase of  $^{35}\text{S}$  incorporation rates was reproducibly observed in different cell types. **a b** and **c, d** show two independent experiments with Jurkat cells. **e, f** is a representative example for cultured human fibroblasts showing the highest induction of  $^{35}\text{S}$  incorporation rates by RF-EME, **g, h** shows a representative example of quiescent (metabolically inactive) primary human white blood cells (WBC). Here, RF-EME hardly induced detectable increases in  $^{35}\text{S}$  incorporation rates; compared to untreated controls (**g**), activated WBC (**i**) displayed higher  $^{35}\text{S}$  incorporation rates, RF-EME induced a further increase in  $^{35}\text{S}$  incorporation rates (**j**), which indicates that activity renders cells sensitive to RF-EME



we found the same cells reactive (e.g. fibroblasts, Table 2) or nonreactive (e.g. naïve lymphocytes, Table 3), when investigating protein synthesis. This may suggest a common underlying mechanism between DNA breaks and increased protein synthesis in reactive cells.

With this exposure regime, the temperature difference between exposed cells and control cells was less than  $0.15^{\circ}\text{C}$ , we exclude a heat-related response. Heat-induced proteome alterations detectable with our proteome profiling methodology would require temperature differences greater than  $1^{\circ}\text{C}$ . Furthermore, a temperature increase of even  $1^{\circ}\text{C}$  does not affect e.g. TCP-1 family members (Gerner et al.

2002). We conclude that the warming of the cell cultures caused by RF exposure was too low to account for the present observations.

Most of the proteins found to be induced by RF-EME are chaperones, which are mediators of protein folding. Since the applied electromagnetic fields were very weak, the direct and active denaturation of existing proteins by RF-EME exposure appears unlikely to underlie the observed increased level of protein synthesis. Resonance phenomena may concentrate radiation exposure-mediated physical energy on hot spots and have already been suggested to cause biological effects (Belyaev 2005). Indeed, exposure to



**Table 1** Jurkat cells: proteins displaying a specific up-regulation of  $^{35}\text{S}$  incorporation by real exposure

Acc-no	Protein name	Abbreviations	Increase factor	ANOVA ( <i>P</i> )
P43686	26S protease regulatory subunit 6B	TBP-7	2.6	<0.001
P11021	78-kDa glucose-regulated protein	BiP	2.5	0.005
P13639	Elongation factor 2	EF-2	4.4	0.017
<i>P10809</i>	<i>60-kDa heat-shock protein, mitochondrial</i>	<i>hsp60</i>	<i>1.4</i>	<i>&gt;0.05</i>
P08107	Heat-shock 70-kDa protein 1	hsp70	2.4	0.004
P43932	Heat-shock 70-kDa protein 4	hsp70/4	4.0	<0.001
P08238	Heat-shock protein 90	hsp90	2.4	<0.001
P52597	Heterogeneous nuclear ribonucleoprotein F	hnRNP F	3.5	0.004
Q14697	Neutral alpha-glucosidase AB	G2 $\alpha$	3.5	<0.001
P17987	T-complex protein 1, alpha subunit	TCP-1 $\alpha$	2.8	0.001
P78371	T-complex protein 1, beta subunit	TCP-1 $\beta$	2.3	0.026
P48643	T-complex protein 1, epsilon subunit	TCP-1 $\epsilon$	2.6	0.002
P49368	T-complex protein 1, gamma subunit	TCP-1 $\gamma$	2.4	0.033
P50990	T-complex protein 1, theta subunit	TCP-1 $\tau$	2.9	0.001
P54578	Ubiquitin carboxyl-terminal hydrolase 14	USP14	3.5	<0.001
P04083	Annexin A1	A-I	1.5	0.031
<i>P08758</i>	<i>Annexin A5</i>	<i>A-V</i>	<i>1.2</i>	<i>&gt;0.05</i>

Proteins are depicted in Fig. 1 and annotated with the listed abbreviations. The increase factor and the ANOVA *P*-values are derived from three independent experimental replicates to compare spot intensities from RF-EMF exposed cells and controls. Proteins printed in italics did not show relevant alterations. They are listed to be complete in comparison with the other cell types analyzed (Tables 2–4). Accession numbers and protein names are according to the SwissProt database. Details of mass analysis results are provided electronically in the supplementary data

**Table 2** Protein alterations detected in fibroblasts, legend as in Table 1

Acc-no	Protein name	Abbreviations	Increase factor	ANOVA ( <i>P</i> )
P43686	26S protease regulatory subunit 6B	TBP-7	2.5	<0.001
P11021	78-kDa glucose-regulated protein	BiP	3.5	<0.001
P13639	Elongation factor 2	EF-2	2.2	0.033
<i>P10809</i>	<i>60-kDa heat-shock protein, mitochondrial</i>	<i>hsp60</i>	<i>2.3</i>	<i>&gt;0.05</i>
P08107	Heat-shock 70-kDa protein 1	hsp70	4.7	<0.001
P43932	Heat-shock 70-kDa protein 4	hsp70/4	4.7	<0.001
P08238	Heat-shock protein 90	hsp90	2.6	0.023
P52597	Heterogeneous nuclear ribonucleoprotein F	hnRNP F	2.5	0.02
Q14697	Neutral alpha-glucosidase AB	G2 $\alpha$	3.1	0.011
<i>P17987</i>	<i>T-complex protein 1, alpha subunit</i>	<i>TCP-1<math>\alpha</math></i>	<i>1.8</i>	<i>0.043</i>
P78371	T-complex protein 1, beta subunit	TCP-1 $\beta$	2.3	0.007
P48643	T-complex protein 1, epsilon subunit	TCP-1 $\epsilon$	4.7	<0.001
P49368	T-complex protein 1, gamma subunit	TCP-1 $\gamma$	2.5	0.042
P50990	T-complex protein 1, theta subunit	TCP-1 $\tau$	2.6	0.011
P54578	Ubiquitin carboxyl-terminal hydrolase 14	USP14	2.5	<0.001
P04083	Annexin A1	A-I	2.4	<0.001
P08758	Annexin A5	A-V	2.7	<0.001

low frequency electromagnetic fields caused effects, which were reduced by noise signals (Litovitz et al. 1997), providing further support for the concept of resonance as an underlying condition. Hydrogen bonds are known to resonate with microwaves. Folding of newly synthesized proteins in the endoplasmic reticulum occurs via a process involving

the formation of intermediate folded structures (Arai and Kuwajima 2000) mediated by several chaperones (Deuring and Bukau 2004). This process could potentially respond in a very sensitive fashion to radiation-induced excitation of hydrogen bonds as this could cause a temporary disturbance of spatial orientation. An increased rate of

**Table 3** WBC quiescent: for legend see Table 1

Acc-no	Protein name	Abbreviations	Increase factor	ANOVA ( <i>P</i> )
P43686	26S protease regulatory subunit 6B	TBP-7	1.2	>0.05
P11021	78-kDa glucose-regulated protein	BiP	1.1	>0.05
P13639	Elongation factor 2	EF-2	1.3	>0.05
P10809	60-kDa heat shock protein, mitochondrial	hsp60	1.1	>0.05
P08107	Heat-shock 70-kDa protein 1	hsp70	1.0	0.040
P43932	Heat-shock 70-kDa protein 4	hsp70/4	1.1	>0.05
P08238	Heat-shock protein 90	hsp90	0.8	>0.05
P52597	Heterogeneous nuclear ribonucleoprotein F	hnRNP F	1.0	>0.05
Q14697	Neutral alpha-glucosidase AB	G2 $\alpha$	nd	nd
P17987	T-complex protein 1, alpha subunit	TCP-1 $\alpha$	1.0	0.037
P78371	T-complex protein 1, beta subunit	TCP-1 $\beta$	1.0	0.023
P48643	T-complex protein 1, epsilon subunit	TCP-1 $\epsilon$	1.2	<0.001
P49368	T-complex protein 1, gamma subunit	TCP-1 $\gamma$	1.0	>0.05
P50990	T-complex protein 1, theta subunit	TCP-1 $\tau$	1.1	>0.05
P54578	Ubiquitin carboxyl-terminal hydrolase 14	USP14	1.2	>0.05
P04083	Annexin A1	A-I	0.9	>0.05
P08758	Annexin A5	A-V	0.8	>0.05

**Table 4** WBC stimulated: for legend see Table 1

Acc-no	Protein name	Abbreviations	Increase factor	ANOVA ( <i>Pf</i> )
P43686	26S protease regulatory subunit 6B	TBP-7	1.2	>0.05
P11021	78-kDa glucose-regulated protein	BiP	1.1	>0.05
P13639	Elongation factor 2	EF-2	1.0	>0.05
P10809	60-kDa heat-shock protein, mitochondrial	hsp60	2.7	<0.001
P08107	Heat-shock 70-kDa protein 1	hsp70	1.5	0.031
P43932	Heat-shock 70-kDa protein 4	hsp70/4	0.9	>0.05
P08238	Heat-shock protein 90	hsp90	0.9	>0.05
P52597	Heterogeneous nuclear ribonucleoprotein F	hnRNP F	1.2	>0.05
Q14697	Neutral alpha-glucosidase AB	G2 $\alpha$	nd	nd
P17987	T-complex protein 1, alpha subunit	TCP-1 $\alpha$	1.3	0.037
P78371	T-complex protein 1, beta subunit	TCP-1 $\beta$	1.3	0.023
P48643	T-complex protein 1, epsilon subunit	TCP-1 $\epsilon$	1.5	<0.001
P49368	T-complex protein 1, gamma subunit	TCP-1 $\gamma$	1.0	>0.05
P50990	T-complex protein 1, theta subunit	TCP-1 $\tau$	1.0	>0.05
P54578	Ubiquitin carboxyl-terminal hydrolase 14	USP14	1.0	>0.05
P04083	Annexin A1	A-I	1.1	>0.05
P08758	Annexin A5	A-V	1.2	>0.05

inappropriate folding of newly synthesized proteins would not affect existing proteins and thus render cell function intact for some time (unless key labile proteins are affected). Furthermore, such a mechanism would not necessarily have a significant impact on total protein amounts. However, later on it would increase the protein synthesis rate in response to an increased rate of turnover of the newly folded proteins. This interpretation plausibly explains the reported increased level of protein synthesis.

Essentially all detectable proteins displayed an increased synthesis rate, which indicates a general compensatory response, e.g. to a hampered supply of functional proteins. Proteins with the highest response (Tables 1, 2) are involved in the chaperoning of newly synthesized proteins and protein turnover. Chaperones such as 78-kDa glucose-regulated protein, heat-shock proteins and T-complex protein 1 family members are directly involved in protein folding and assist folding of newly synthesized proteins

(Deuerling and Bukau 2004). Neutral alpha-glucosidase AB is an important endoplasmic reticulum protein responsible for quality control and glycoprotein processing (Ellgaard and Helenius 2003). Ubiquitin carboxyl-terminal hydrolase 14, also termed deubiquitinating enzyme 14, is required for proteasomal processing of ubiquitinated substrates (Koulich et al. 2008). The 26S protease regulatory subunit 6B is also involved in ATP-dependent degradation of ubiquitinated proteins and in transcriptional regulation (Choi et al. 1996). Elongation factor 2 is actually indispensable for protein synthesis (Perentesis et al. 1992).

### Exposure time matters

Our data complement those of Lee et al. (2006) who did not find changes in the expression levels of HSP90, HSP70, and HSP27, or MAPK phosphorylation in Jurkat cells exposed to RF-EM for 30 min and 1 h. In our experiments, increased protein synthesis was only observed after an 8-h exposure time and was in fact fully reversible within 2 h (data not shown). This is also in agreement with Sanchez et al. (2008) and Yilmaz et al. (2008) who found no changes associated with exposure times of 2 h and 20 min, respectively, i.e. changes in the rate of protein synthesis are induced by long exposures to low intensity RF-EM.

### Conclusions

Our data describe cell responses to RF-EME exposure specifically observed in actively proliferating cells. When investigating protein synthesis, we found the same cell types nonreactive or reactive, compared to those to reveal DNA breaks (Diem et al. 2005; Schwarz et al. 2008). Active cells continuously repair DNA-damage as it occurs as side product of DNA transcription or respiratory chain products (Alberts et al. 2001; Branden and Tooze 1999). Therefore, a subtle inhibition of any part of the antioxidant protection or the DNA repair system would accumulate damaged DNA. Consequently, interference with protein expression may explain the DNA changes found by others (Belyaev et al. 2005; Diem et al. 2002; Schwarz et al. 2008) as indicator for a risk associated with long-term exposure.

The observed proteome alterations support a novel mechanistic model for the understanding of RF-EME induced bioeffects: this model is based on radiation-induced disturbances of hydrogen bonds, which may be essential during the protein folding process. Our results do not directly indicate a health risk. However, the finding that metabolically active and/or proliferating cells are more responsive to RF-EME implies a higher sensitivity of growing organisms.

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**Conflict of interest statement** None.

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