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**Biological stress responses to radio-frequency
electromagnetic radiation:
Are mobile phones really so (heat)shocking?**

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Short title: Mobile telephone electromagnetic emissions and the induction of altered gene and protein expression in biological systems.

Abstract.

Cells phenotypically adapt to alterations in their intra- and extracellular environment via organised alterations to gene and protein expression. Many chemical and physical stimuli are known to drive such responses, including the induction of oxidative stress and heat shock. Increasing use of mobile telephony in our society, has brought focus on the potential for radio frequency (microwave) electromagnetic radiation to elicit biological stress responses, in association with potentially detrimental effects of this to human health. Here we review evidence suggesting altered gene and protein expression in response to such emissions, with particular focus on heat shock proteins. Non-thermal induction of heat shock proteins has been claimed by a number of investigations in in vitro cellular systems, and appears pleiotropic for many other regulatory events. However, many of these studies are flawed by inconsistencies in exposure models, cell types used and the independent reproducibility of the findings. Further, the paucity of evidence from in vivo experimentation is largely contradictory. Therefore, the validity of these effects in human health risk assessment remain unsubstantiated. Where possible, suggestions for further experimental clarification have been provided.

Keywords: Radio frequency electromagnetic radiation, heat shock proteins, altered gene and protein expression, biological stress response.

Abbreviations : HSP; heat shock protein. RF; radiofrequency. EMF; electromagnetic field. EMR; electromagnetic radiation. ELF; extremely low frequency. GSM; Global Speech Modulation. CW; continuous wave. FDMA; Frequency Division Multiple Access. CDMA; Code Division Multiple Access. TDMA; Time Division Multiple Access. FM; frequency modulation. TEM; transverse electromagnetic. GFP; green fluorescent protein. NOEL; No Observable Effect Level. ICNIRP; International Commission On Non-ionising Radiation Protection. ODC; ornithine decarboxylase. MAPK; mitogen activated protein kinase.

Introduction and scope

The integrity and functionality of all biological material is meticulously maintained by a complex network of biological “sensing” and “correcting” devices, which respond to changes in chemical and physical parameters in the local environment. The basis of most functional adaptation to cellular processes lies either at a) altered gene and protein expression, or b) altered protein activity, resulting from appropriate modulation by signal transduction mechanisms. The overall ability of cells to react in this manner is often collectively termed “stress response”.

The true flexibility of the stress response is well illustrated by the wide variety of chemical and physical stimuli, which have been shown to elicit complex and functionally-co-ordinated alterations to gene and/or protein expression/function in cells. These stimuli classically include exposure to strong oxidants or reductants, resulting in altered intracellular redox states and the induction of oxidative or reductive stress, respectively (1). Chemical insult from reactive electrophiles, such as those routinely generated by the metabolism of xenobiotic agents, may also result in dramatic alterations to cell phenotype (1). In addition to chemical agents, a variety of physical stimuli have been shown to greatly alter cellular phenotype. Perhaps the archetypal example of this is that of altered temperature, resulting in either heat- or cold-shock (2). Mechanical forces have also been shown to alter cellular phenotype, such as that experienced in vascular material under shear stress (3).

The biological logic embodied in the stress response lies in equipping the cell with a more robust phenotype, by securing or enhancing major cellular house keeping functions such as macromolecule (DNA, RNA and protein) synthesis and repair, and chemical energy supply. This adaptive “battle” against loss of function and initiation of cell death has several features that are noteworthy. Firstly, it is a characteristic of all biological systems, and is avidly

preserved throughout evolution, from the simplest prokaryotes, throughout the eukaryotic kingdom. Secondly, although the stress stimulus may vary considerably, the molecular components of the stress response often contain commonalities (1). One example effectively illustrating these points is the case of the heat shock proteins (HSPs), which were initially identified in yeast in response to elevated temperature, but are ubiquitously expressed in eukaryotic cells in response to heat, as well as a variety of other chemical and physical stimuli (2). Here it is also interesting to note that, as well as being involved in the chaperoning of partially-folded and denatured proteins (4), HSPs are also intricately involved in regulating important protein-protein interactions (5, 6), as well as possessing inhibitory effects on the functionality of proteins directly involved in the execution of apoptotic cell demise (7-9).

One source of a stress stimulus which has received less attention, but which is rapidly coming into focus from a biological and human health perspective, is that of electromagnetic radiation. Apart from more traditionally investigated aspects of this field involving exposures to ionising radiation (10) and UV radiation (11), exposure of biological material to radio-frequency (RF) emissions is presently attracting attention, especially due to the use of microwaves in mobile telecommunications systems. Indeed, the scientific community is presently assessing if exposure of the human population to RF emissions and their incumbent magnetic fields is detrimental to health or not. Thus, there is a rapidly accumulating literature describing the potential stress response(s) of biological material to RF energy. This is primarily reflected in studies of altered gene expression and protein synthesis, and of HSPs in particular. However, there are both conflicting aspects within the data and a relative lack of mechanistic explanation for the molecular events observed, particularly in the absence of gross cell/tissue heating effects. Therefore, it is the purpose of this treatise to give an overview of presently available data on RF exposure and the induction of expression of HSPs, both in vitro and in vivo. The review will largely focus on radio emissions in the frequency

range relevant to tele-communication (circa 800-900 MHz (GSM) and circa 1.6 GHz (IRIDIUM), with wavelengths of 33-35 cm and 16-17 cm, respectively). However, this does not negate the fact that there are a number of studies at extremely low frequencies (ELF) radio emissions (50-60Hz), which suggest concerns for human health, as these ranges are employed in electricity transmission lines. This issue has been debated extensively elsewhere, and it should be only mentioned here that many effects indicating stress responses in biological systems have also been noted with such emissions, including effects on HSP expressions (12, 13). At all times efforts will be made to compare and contrast the experiments in terms of a) the exposure, its physical characteristics etc., b) the dose-response characteristics (thresholds etc.), c) biological aspects of the response and d) methodological aspects of the detection of the molecular stress response. The review will also attempt to describe current thoughts on the mechanism(s) underlying the HSP response, as well as speculate how this might or might not be relevant to other gene/protein expression alterations documented in the literature. Although not a central issue for this, essentially biochemical and biological review, a final summation will attempt to provide some suggestions to improve our knowledge in this area, as well as speculating on the potential impact of stress response data on the overall assessment of risks to the human population from exposure to RF emissions from mobile telephony.

Molecular end-points of RF-induced induced-stress

Heat shock proteins

The HSPs are an important group of cell stress response proteins, originally discovered in yeast exposed to elevated temperature (14). The generality of induction of these proteins to other stimuli clearly indicate their collective name to be rather misleading. The HSPs were originally thought to act primarily as molecular chaperones for actively unfolding or partially-folded proteins. Indeed, the mechanism of action of HSP 70, one of the most ubiquitous HSPs, has been closely studied and shown to involve both target protein recognition, binding and catalytic refolding (2). Similarly, the regulation of expression of HSP genes has been closely studied, with the essential characteristics of the activity and response of heat shock factor-1 (HSF-1) well illuminated (15). The activation of transcription results from a disturbance in the balance between HSF-1 and HSP levels, resulting in release of HSF-1 and its trimerisation and transport into the nucleus, where it binds to multiple heat shock elements (consensus NGAAN) in target genes (15). The disruption of the inactive complex seems to have a common stimulus involving damage to cellular proteins and their sequestration by free HSP molecules. The damage may result from both physical (heat) or chemical (oxidation, alkylation etc) stimuli (16). However, more recent work has indicated other specific functions for certain HSPs. For instance, both HSP 27 (7) and HSP 70 (8), have been shown to interfere with post-mitochondrial events in the execution of apoptotic cell deletion. This clearly reveals another point of convergence in the protective function of HSPs against cellular demise stimulated by a variety of obtrusive environmental stimuli.

In discussing effects of RF-electromagnetic radiation (RF-EMR) on HSP it should be borne in mind that most papers refer to gene expression in a manner not wholly related to the assay method used, and/or the biological function of the protein(s). Thus, in most cases either the steady state levels of mRNA or the respective protein are assayed. This may be affected both

by synthesis and/or breakdown rates of the respective macromolecules. Thus, assay of these macromolecules does not necessarily just reflect in induction of transcription rates of the particular gene. This is a weakness we will return to when discussing problems in interpretation.

We will now discuss various experimental results pertaining to the effect of RF emissions in the high MHz frequency range on the heat shock machinery of biological material. At all times we will strive to detail the strengths and weaknesses of the studies, in an effort to determine their relevance to the assessment of what these stress responses may reflect in biological material and potential risks this may present.

Effects of RF-emissions on HSP homeostasis in vitro.

Responses of cell lines and primary cells (Table 1)

The earliest study on the potential effects of high frequency radio emissions on HSP levels in cells was performed by Parker and co-workers (17), where they exposed several rodent cell lines to microwave emission at 2.45 GHz under continuous wave (CW), for up to 20 minutes. These authors were unable to detect increased HSP 70 mRNA levels in L5178Y or CHO cells after delivery of an average specific absorption rate (SAR, accepted measure of biological dose rate) of between 51.75 and 103.5 W/kg, using conventional northern blotting. The study also included controls for elevated temperature and synergy between temperature and RF exposure. Similarly, an early study in HeLa and CHO cell lines exposed at either 27 MHz or 2.45 GHz CW, at an average SAR of 100 W/kg, failed to detect induction of a stress response resembling of heat shock (18). This study, however, was not performed with specific probes, but are reliant on rough protein patterns upon electrophoretic separation.

These early studies both possess two major weaknesses with respect to extrapolation to the potential effects of emissions from mobile telephones. Firstly, they were performed with

emissions not representative of the normal frequency ranges used for modern GSM telephony, i.e. both use emissions in the unmodulated form. Secondly, they were performed with extremely high SARs, well above levels emitted by telephony devices. Thus, in a more recent study by Kwee and co-workers, transformed human amnion cells were exposed at 960 MHz fields modulated at 217 Hz in a transverse electromagnetic (TEM) cell at an average SAR of 2.1 mW/kg, for 20 minutes. The levels of HSPs 27 and 70 were detected by immunocytochemistry and positive cells scored by eye (19). The authors claim that these emissions cause a large induction of HSP 70 protein levels in the cells, in the absence of thermal heating. However, it is difficult to rationalise these results in terms of the kinetics of the responses reported, thus detracting from their use in extrapolations. Maximal HSP 70 immuno-reactivity, on a cell basis, was noted at the end of the 20-minute exposure period. This is far faster than the kinetics of induction of protein levels in response to heat and other treatments. The data also lack any quantitative evaluation of the levels of HSP 70 using western blotting, in the culture as a whole.

In an interesting recent addition to the field, Leszczynski and co-workers (20) have provided perhaps some of the most relevant in vitro data thus-far reported, by studying the effects of 900 MHz emissions pulsed at GSM signal modulation schemes. The emission was also adjusted to give an average SAR of 2 W/kg over a one-hour exposure period, which is the currently accepted safety limit standard defined by the International Commission On Non-Ionising Radiation Protection (ICNIRP). The cell system chosen was that of a human endothelial cell line (EA.hy926), which has direct relevance to possible vascular effects of RF emissions in close proximity to the ear. The data indicate, very interestingly, a non-thermally-related induction of the levels of HSP 27 protein in the cells in response to RF exposure, as well as a hyper-phosphorylation of the protein. Unlike the previous in vitro studies, this induction was coherently revealed by a variety of independent protein analytical techniques.

The data also revealed that activation of p38 mitogen activated kinase (MAPK) was partially responsible for the phosphorylation of the HSP. This well performed study, including heat shock controls, also revealed other biomarkers of the treatment. Thus, the consequences of activation of the p38MAPK-HSP 27 pathway are discussed at length below.

It should be mentioned here, however, that unpublished results presented by Poulltier de Gannes at a recent workshop on “The influence of RF fields on the expression of stress proteins” in Helsinki, Finland (April, 2004), failed to reproduce the above results using endothelial cells exposed to 2 W/kg 900 MHz GSM modulated RF for 1 hour.

Convincing evidence for the induction of HSP 70 by non-thermal effects of RF emissions in vitro also comes from the work of Tian et al., using human glioma cells (21), also a cellular target of relevance to the issue of RF emissions and human health. The emission was set at 2.45 GHz (CW), therefore not strictly applicable to RF fields emitted from GSM phones. The authors did, however, make great efforts both to study the effects of varying intensity of emission, and in calculating the variation in SAR over the flat culture dishes used for the exposures. The data revealed induction of HSP 70 protein by immuno-cytochemistry, which was clearly related to the variation in SAR over the plate surface. The induction was detected at an SAR of 20 W/kg, roughly 10-fold the safety standard, which should be taken into account before the data is used in the assessment of risks.

In a recently published paper by Guisasola et al. (22), designed to check the biological effects of the components of magnetic resonance imaging, L-132 cells were exposed to a 1.5-T magnetic field and various frequencies of RF applied. However, unlike the other in vitro studies detailed above, this work confined the emissions to a frequency range between 3 and 300 MHz, which are more relevant to emissions from magnetic resonance imaging apparatus. The work failed to detect changes in either HSP 70 or HSP27 protein or their respective mRNA levels at steady state, however the work provides no details on the exposure

conditions, no attempts to calculate the SAR experienced by the cells during the exposure and no attempts to study dose-response relationships. The paper does report, however, small, but significant increases in intracellular calcium content in response to RF emissions. Although a fuller treatise of this particular stress response is out of the scope of the present review, these data do illustrate that other stress responses, particularly within the function of important signal transduction mechanisms, may provide alternative, sensitive biomarkers of exposure to HSP expression levels.

The tissues of the head and neck are clearly those which are primarily exposed to microwave emissions from mobile telephones. Despite this, apart from the glioma cell study detailed above, there have been few reports of *in vitro* experiments utilising human cells of direct relevance to the potential exposure of neuronal tissue in the CNS, in particular the auditory apparatus. There are a few ongoing studies reported in the International EMF Project database, published by the WHO (23). French (24) has issued a preliminary report on the exposure of human primary astrocytes to 900 MHz, at an SAR of 0.2 W/kg for either 1 hour only, or 1 hour on 4 consecutive days. The authors report no significant alterations to HSP gene expression on average, and point to large inter-experimental variations in the response. This study has not been fully reported in the literature, and therefore lacks acceptable scrutiny. A similar ongoing study of the effects of GSM (900 and 1800 MHz) on primary astrocyte lines and microglial cell lines is reported by Poullétier de Gaines and Veyret (25) within the database. Again no data is available for scrutiny from this study, which also focuses on HSP expression in rat brain and skin, using immunohistochemical approaches. Despite the lack of unequivocal data from these studies, the experimental design of these astrocyte studies brings an important issue into focus with regards the choice of model systems for study. Variance in response of primary cell cultures derived from experimental animals must also translate into at least equivalent variation in cells derived from human donors. However, due to the lack of

availability of relevant human primary culture cellular models, this important issue remains unexplored with respect to HSP responses in vitro.

Finally, as yet unpublished results presented by Miyakoshi at the recent Conference on “The influence of RF fields on the expression of stress proteins” (Helsinki, April 2004) demonstrated that human glioma cells exposed to 1950 MHz (CW) for 1-2 hours at SARs between 1-10 W/kg, failed to induce HSP 27 or HSP 70 immuno-reactivity, and actually suppressed HSP 27 phosphorylation at the highest exposure.

Responses in simple transgenic organisms (Table 1)

Modern genetic methods have facilitated the generation of transgenic organisms, with simplified cellular structures. These offer the possibility of producing experimental biological systems of intermediate complexity between isolated and cultured cells and entire organisms. Such organisms are beginning to be explored for their usefulness in studying biological responses to RF emissions. The pioneering work in this area was performed by Daniells and co-workers (26), who inserted a β -Gal reporter gene construct, driven by the endogenous HSP-16 promoter, into the nematode *C-elegans*. Using a standard TEM cell set-up, the worms were exposed to frequencies between 300 MHz and 750 MHz CW, at varying power settings and for extended periods of up to 16 hours. The transgenic worms were exposed at 25 °C in multi-well plates, and some effort was made to match the degree of biological effect to the average SAR experienced in the individual cells. This did not, however, extend to the level of individual worms in the cell, which were all treated as an average with respect to the exposure. The data clearly reveal induction of the reporter construct in response to the microwaves, with some degree of correlation to the closeness to source, all in the absence of gross heating effects in the medium. Interestingly, a bell-shaped response curve with respect to the energy of emission was observed, with energies at 21 dBm producing a greater effect

than 27 dBm. This might suggest a “power-window” in which such stress responses might be activated, and argues against a general relationship to energy transfer and heating, be it generalised in the organisms, or localised around cellular proteins.

This work was later confirmed using a refined transgenic worm model in a similar TEM chamber design. This study also included animals where a green fluorescent protein (GFP) construct driven by the endogenous HSP-16 promoter had been introduced into *C.elegans* (27). Induction of HSP16 in response to long-term (18 hour) exposure to 750 MHz CW was confirmed with both reporter constructs, and the effect was shown to synergise with increasing temperature of incubation. Interestingly, the GFP transgene allowed visualisation of the induction of expression in over half of the worm’s individual cells, although the authors did not report on any systematic analysis of the cells involved. The authors calculated an SAR at the centre of the worm exposure chambers of 1 mW/kg, which is lower than that for mobile telephones (0.02 to 1 W/kg). If the calculations of SAR are correct in this study, then this would considerably lower the no observable effect level (NOEL) for this effect in biological test systems.

Continuing in this model, in one of the rare attempts to define the biological consequences of induction of HSPs in model systems, de Pomerai and co-workers (28) used the transgenic worms, exposed at 1 GHz CW and an average SAR of 1 mW/kg, and demonstrated small, but significant increases both in the growth rate of larvae and in the number of egg-bearing adults. Irrespective of whether one considers these events as related to the induction of HSPs in the animals or to other adaptive responses, these data again point to significant biological effects occurring in integrated cellular systems in response to low levels of exposure to GSM-like microwaves.

Finally, a double transgenic *C.elegans* model has been very recently described (29). Using similar conditions to those reported above, co-induction of galactosidase activity and GFP in

response to microwave exposures were demonstrated. Again, the authors do not present any visual data on the distribution of expression of the reporter gene-GFP, referring to the response as generalised throughout the animal.

Clearly the *C.elegans* transgene models offer great possibilities to study integrated responses of a simple organism to “whole body exposure” to microwaves, at levels relevant to the power emissions of mobile telephones. Further experiments might focus around the functional significance of endogenous HSP induction in the wild type organism, for instance with respect to thermotolerance and inhibition of cellular apoptosis, which is a very organised and precise process, and is indeed well studied in this organism (30). It should be noted, however, that these observations have been largely made by one research group, and await corroboration from other groups and in other simplified transgenic organisms. Therefore, as a point of caution, it is notable that more recent, unpublished results presented by de Pomerai and co-workers at the recent conference on “Influence of RF fields on the expression of stress proteins” (Helsinki, April 2004) demonstrate that their original observations in the transgenic worm system may be related to small, but significant temperature elevations in the exposure system.

Effects of RF-emissions on HSP homeostasis in vivo (Table 1).

A number of studies have attempted to determine if exposure to microwaves under conditions similar to GSM emissions can induce a heat shock response in vivo. In one of the first studies, Fritze and co-workers (31) exposed immobilized rats at 900 MHz emissions, at SARs ranging from 0.3 W/kg (modulated mode) to 7.5 W/kg CW, for 4 hours. In situ hybridisation revealed slight, but non-significant effects on HSP70 steady state mRNA levels in sections of brain in response to the highest exposure, in the absence of immuno-reactive HSP70 protein, probed by in situ immunocytochemistry. Western-blot analyses of protein extracts from specific areas

of the brain were not reported. On the other hand, when Walters and co-workers studied the effects of emissions in the high-powered microwave (HPM) region, the reported data clearly demonstrate that HSP70 can be induced in vivo in various brain regions in young and aged rats, under conditions of restricted calorific intake (32). However the relevance of this study to GSM microwave emissions must be questioned as the 2.06 GHz, 2.2 W/cm² emission clearly resulted in localised heating effects in the brain (32).

Several studies similar to those performed in rodents have also been performed in chick embryos. In two closely related studies from the same laboratory, Shallom and co-workers (33) and Di Carlo et al. (34) report somewhat conflicting data on the effects of 915 MHz (nominal frequency) on chick embryos exposed in a TEM cell. In some experiments a modulation frequency (ELF-EM noise at 30-90 Hz) was additionally applied and the SAR was estimated to be between 1.75 and 2.5 W/kg. In the former study increases in HSP70 protein immuno-reactivity were demonstrated in whole embryo extracts by western blotting, but the data were not internally standardised against “house-keeping” proteins. Neither were attempts made to perform immuno-cytochemical analysis of sectioned embryos. Interestingly, however, the authors demonstrated HSP70 induction to be related to an increased survival of chick embryos to hypoxic stress. Further the authors of this study also allude to the possibility of a specific window of emission which might be most effective in this respect, with higher exposures resulting in diminished cross-protection. The data also suggest that ELF-EM noise may disturb or even abrogate the biological response of the embryos to CW emission (33). On the other hand, extension of the exposure time to a period of days has been shown to have inhibitory effects on the expression of HSP70 in chick embryos (34). This resulted in a deprotection of the animals from hypoxic stress, and was interpreted by the authors to suggest that chronic exposures to microwave emissions, similar to those emitting from mobile telephones, might represent a health risk based on diminished function of HSP70. The validity

of these conclusions can be questioned in a number of ways, however. Firstly, the western blot data on HSP70 data was confined to the effects of chronic ELF-EMF emissions, and no data presenting effects of long-term exposure to microwaves were presented. Similarly, most of the western blot data presented in these studies is non-internally standardised for loading variation and concerns the entire embryo, and no in situ immuno-cytochemical analyses were performed.

In addition to the above in vivo experiments, a number of workers have debated potential pathophysiological consequences of the induction or suppression of HSPs in response to microwaves, particularly in terms of chronic exposures in vivo. Thus, French and co-workers (35) have argued that induction of HSP responses could lead to an increased risk for cancer, based on the fact that HSPs can interfere with normal cell cycle progression by inhibiting the action of p53-dependent check-pointing. This would potentially interfere with DNA repair in response to damage. Additionally, evidence of elevated HSP expression in tumour metastases was incited to substantiate these arguments. That HSPs are anti-apoptotic is today a more defined concept, as work from our laboratory (7) and other (8, 9) clearly demonstrate the proteins to interfere with the normal function of mitochondrially-stimulated, apoptosome-dependent apoptosis in mammalian cells. Several major draw-backs in this hypothesis concerning the risks for cancer from induction of HSPs exist, however. Firstly, the concept is based on correlative suppositions. Thus, no functional correlations, perhaps by the use of transgenic animals to perform carcinogenicity studies, have been established. Additionally, it should be borne in mind that exposure to RF-EMF has been shown to induce altered expression of a variety of other genes involved in important cell signalling pathways (see below), whose collective functionality will determine the out-come of the exposure. The complexity of this response will become more apparent as gene and protein profiling techniques are applied to appropriate biological material from well-controlled exposure

studies in cell and animal models. This issue will be returned to later in this treatise. Finally, the data obtained in chick embryos, and detailed above, clearly questions this hypothesis, in the light of claims of suppressed expression of HSP70 in response to chronic exposures.

Due to the close proximity to the microwave source, the tissues of the head, neck and ear are thought to be at most risk from the potentially detrimental effects of microwave emissions from mobile phones. However, there has been little specific work done on the induction of HSP responses in the tissues of these areas *in vivo*, and the literature is generally concerned with illuminating the risks for the generation of cancer within these regions in epidemiological studies (36, 37).

Other genes and proteins whose expression may be affected by exposure to RF-emissions

Although this review is primarily focussed on the effects of RF-EMF emissions on HSP gene and protein expression, it is extremely relevant at present to reinforce the concept of multiplicity of genetic and biochemical adaptations to stressful stimuli. Thus, HSP responses are highly unlikely to occur in isolation of other adaptive events, and definition of other events within the overall pattern of the stress response may not only be able to more clearly explain the mechanism(s) by which microwaves elicit their responses in biological systems, but also add to our overall appreciation of the risks to human health from such exposures.

Using conventional gene/protein expression methods, coupled to logical suppositions concerning potential biochemical targets, several genes and proteins involved in regulating the mammalian cell cycle have been hitherto studied with respect to RF-EMF emissions.

Ornithine decarboxylase (ODC)(Table 2)

Ornithine decarboxylase (ODC) is a key enzyme involved in the regulation of polyamine synthesis during the progression of the cell cycle, particularly during S-phase. Litovitz and Penafiel (38) showed that the exposure of mouse L929 fibroblasts to 835 MHz CW, at an SAR of 2.5 W/kg did not induce the expression of ODC protein. However, Time Division Multiple Access (TDMA) modulation of the signal clearly induced ODC protein and activity, but this effect was abrogated by introduction of random speech patterns into the modulation, suggesting that coherent modulation is responsible for the up-regulation of ODC in the presence of microwaves. In extension of this work, the same authors reported on the effects of exposure of L929 cells to microwaves at 835 MHz CW, at 2.5 W/kg, with a variety of superimposed modulations. Continuous wave exposures gave a small but significant rise in ODC activity, however modulation at various frequencies between 16.5 Hz and 60 Hz gave significant increases in ODC activity, whilst the use of frequencies above and below this gave no response. Modulation using a 50 Hz pulsed signal was also shown to increase ODC activity (39).

Mitogen activated protein (MAP) kinases (Table 2)

Mitogen activated protein kinases (MAPKs) are a large family of cell signalling proteins of critical importance in the regulation of the eukaryotic cell cycle (40). Certain members of the family act as critical plexus points through which differing physical and chemical stimuli are encoded into signals and “funnelled” towards the genome to elicit adaptive alterations to gene expression. Thus, it is of interest to determine if RF-EMF can affect these critical processes. In a recent seminal contribution, Leszczynski and co-workers (20) studied the effect of exposure of EA.hy 926 endothelial cells to 900MHz emissions modulated to match GSM. The

authors used several methods to carefully calculate and regulate the exposure of the cells to be matched to the ICNIRP SAR limit of 2 W/kg. By employing rather advanced phospho-protein proteomic techniques, exposure to the RF-EMF was shown to stimulate the phosphorylation of over 324 cellular proteins and the de-phosphorylation of 67 specific phosphoproteins. Interestingly, the authors detected an increased phosphorylation of HSP27, in association with increase in number of HSP27-expressing cells. This was confirmed by immuno-precipitation of the HSP27 protein from control and exposed cells, although phosphatase-treated controls were omitted. Intriguingly, pre-incubation of the cells with SB203580, a specific inhibitor of the p38MAPK protein, led to a diminished degree of the phosphorylation of HSP27. The authors also demonstrated that the expression of p38MAPK protein itself was induced by the RF-EMF exposure. Despite these observations, the authors did not speculate as to the origin of the cell signalling resulting in the activation of the p38MAPK pathway, but did suggest the hypothesis that increased expression of HSP27 and its hyper-phosphorylation may present risks to biological material due to its established anti-apoptotic properties at the level of the mitochondrion (7) and the apoptosome (8, 9). Inhibition of apoptosis resulting from such MAPK-dependent cell signalling was postulated to increase the risks for fixation of spontaneous and other mutations in affected cells, thus increasing the risks for development of cancer. However, it must be argued that these suggestions require definitive study, perhaps involving experiments designed to see if exposure to RF-EMF can indeed increase mutation frequencies in cells exposed to genotoxic stimuli. This should also include the potential role of release of caspase-2 from the nuclei following DNA damage (41), and the involvement of p53-dependent mechanisms in response to primary DNA damage. Similarly, it should be remembered that the cells utilized in these experiments were transformed in their nature and constitutively expressed HSP27 at high levels. This has been noted in other transformed cells, such as human lung type II epithelial carcinoma cells, which are also intrinsically resistant to

apoptotic stimuli (42, 43). Similar experiments in primary cultures of endothelial cells might thus be indicated.

Finally, data presented by Swicord and RotiRoti at this meeting, where HELA cells were exposed to 836 MHz TDMA at 5 W/kg for 2 and 24 hours, also failed to demonstrate hyperphosphorylation of HSP 27. However, these experiments were not performed under conditions similar to those used by Leszczynski et al.

In another study using a nylon membrane cDNA microarray assay, Pacini demonstrated that the expression of MAPKK3 and G2/mitotic-specific cyclin G1 increased in skin fibroblasts in response to 900 MHz GSM for 1 hour, at an average SAR of 0.6 W/kg (44), in association with increased DNA synthesis. The author suggested that these effects might present significant biological risks for abnormal skin fibroblast proliferation in exposed human skin. Again, no attempt was made by the authors to relate their findings to primary biochemical events in the cells in response to the EMF stimulus.

Transcriptional regulator NF κ B and AP-1 (Table 2)

The transcription factor NF κ B and AP-1 have been shown to be pivotal in coordinating gene expressional changes in cells exposed to a wide variety of physical and chemical stress stimuli, including oxidative stress and various kinds of irradiation. Natarajan and Meltz (45) reported that exposure of the MonoMac-6 monocytic cell line to either 2.45 GHz (CW), or 8.2 GHz (pulsed wave), at an SAR close to 10 W/kg, increased the binding of NF κ B to its consensus DNA sequence, using conventional electromobility gel shifting. However, no stimulated down-stream gene expressions could be detected, indicating the lack of pro-inflammatory response of the cells. Although tantalising in view of the potential relationship to inflammatory processes, these data are clearly in need of verification in primary cultures of

human cells, at more realistic SARs, more relevant exposure times and at frequencies more relevant to conventional GSM telephone emissions.

Considerably more studies on the expression of AP-1 have been performed with a focus on short- and long-term exposure of cells to RF-EMF. In an early study, PC12 rat pheochromocytoma cells exposed to 836 MHz, (0.41-41 mW/kg), showed increased expression of cJun, one of the components of AP-1, but only at the highest SAR and after 20 minutes of exposure (46). Similarly, Goswami and Roti Roti have reported on the exposure of C3H 10T1/2 fibroblasts to either 835 MHz (FM) or 847 MHz (Code Division Multiple Access, CDMA) at an SAR of 0.6 W/kg, for up to 6 days. The results clearly show induction of the steady state level of mRNA for c-Jun, but no increased binding of AP-1 to its consensus binding sequence using gel shifting. The authors also demonstrated that the binding of other transcription factors, such as the TATA-binding protein and NF κ B were unaffected by the exposures, but that AP-2-dependent DNA binding was clearly increased. The biological significance of these small changes remain uncertain from these studies (47).

When considering potential effects of RF emissions on transcription factor binding to consensus DNA sequences, and resultant down-stream signalling events, it should be borne in mind that small (in the range of 50-100%) increases in binding obtained in in vitro systems may not represent events of considerable physiological importance in vivo. Thus, The expression of these important transcription factors has also been monitored in a number of in vivo studies. Fritze and Hossmann (48) clearly showed a lack of effect of exposure to 900 MHz (GSM) for 4 hours, at either 0.3W/kg or 1.5 W/kg (GSM), or 7.5 W/kg (CW) on the accumulation of c-Fos and c-Jun proteins in rat brain. Similarly, Morrissey et al. demonstrated a lack of induction of levels of c-Fos protein in the brain after exposure of mice to 1.6 MHz (IRIDIUM or CW) for 1 hour at SARs between 0.23 and 1.19 W/kg, whilst some induction was apparent at levels above this. The author concluded, however, that the induction at these

upper levels were probably due to heat shock, based on comparisons with animals subjected to conventional heat shock treatment (49). These data are supported by the work of Stagg *et al* (50), who failed to demonstrate induction of steady state levels of c-Jun and c-Fos mRNA in the brains of rats exposure to 1.6 GHz (IRIDIUM) for 2 hours at an average SARs between 0.16 and 5 W/kg.

Several studies centred around these transcription factors and the expression of early response genes are cited as ongoing in the International EMF project data base from the WHO (51).

Diverse other genes involved in cellular metabolism and regulation (Table 2).

In addition to the HSPs and the cell-cycle related genes detailed above, the expression of several other genes has been shown to be affected by RF-EMF. To this end, two strategies have been apparently employed. The first involves the targeting of particular genes for study. The second involves the use of emerging technologies within genomic and proteomic analysis to screen for changes in gene/protein expression.

In a study where pregnant mice were exposed under the entire period of gestation to 2.45 GHz (CW or modulated at 50 Hz) for 100 minutes a day, and at a whole-body average SAR of 4.23 W/kg, maternal and foetal brain and liver aminoacyl-tRNA activities were examined and shown to be significantly decreased in brain (CW only) and increased in liver (both modulation modes) (52). The significance of these findings for the overall protein synthesis capacity of the organs involved was not further investigated. It should be mentioned, however, that this is one of the only published studies where the potential teratogenic effects of RF-EMF on animals has been studied. It is therefore interesting to note that there were no effects of the exposure on post-natal weight-gain, either of the organs examined or of the new-born pups (52). These data await independent confirmation and it should be bourn in

mind that the exposure level is above the adverse effect threshold that the ICNIRP standards are based upon.

In addition to the above published data the International EMF data base reports on several other unpublished studies focussing on the expression of EGF receptor and other growth factors receptors (53), ATP synthase in E.Coli (54), or NOS II activity in C6 glioma cells, U937 monocytes and primary murine macrophages (55), and nicotinic receptors in several neuronal cell lines (56). However, it should be emphasised that none of the primary data are available for further scrutiny.

In the second strategy, recent advances in technology have facilitated the ability screen for patterns of mRNA and protein expression using DNA microarray and proteomic technologies, respectively. In an initial study utilizing membrane-based cDNA microarray, Harvey and French studied the effects 864.3 MHz (CW) on HMC-1 human monocytes. The exposure was carefully controlled and averaged at an SAR of 7 W/kg, almost double the established adverse effect level. The cells were exposed for 20 minutes every 4 hours, over a period of 7 days. cDNA Microarray revealed consistent alterations in steady state mRNA levels of 3 of the 558 genes represented on the membranes. These probes were for the stem cell factor receptor cKit (increased), the negative regulator of apoptosis DAD1 (decreased) and nucleoside diphosphate kinase (NDPK), a potential tumour suppressor gene (decreased) (57). However, there was considerable variability between the two experiments reported and the changes to steady state mRNA levels were small (<50%), and their significance must be questioned. The authors do not report on the use of other more quantitative estimates of these events as confirmation, which is generally accepted as necessary when determining the significance of such small changes in probed mRNA levels on DNA microarrays.

Interestingly, in the same study the authors report on an activation of protein kinase C (PKC) in RF-EMF exposed cells (57). This was demonstrated as a shift towards more membrane-

bound kinase protein after the exposure. The authors discuss the possibility of involvement of altered calcium homeostasis in this effect, without further investigating this possibility more specifically. Neither did they explore down-stream events to which PKC activation is coupled, such as the phosphorylation of specific protein substrates. Additionally, from the design of the experiment, it is uncertain if de novo induction of expression of PKC occurs as a result of the exposure.

At the recent annual meeting of the Bioelectromagnetics Society, Kuokka and Leszczynski reported on an extension of their phosphor-proteomic study discussed previously. After exposure of EA.hy926 cells to 900 MHz (modulated), at an SAR of 2.4 W/kg for one hour, proteomic analysis revealed altered levels of a few protein spots, but no identification has yet been reported (58). This group also presented a collaboration designed to study the whole genome expression profile of human cells utilizing nylon membrane arrays spotted with over 75 000 human cDNA clones (59). A variety of cell lines (ES-1 fibroblasts, HL-60 promyelocytes, NB69 neuroblastoma cells and E.hy926 endothelial cells) were exposed to 1.8 GHz (CW) or 900 MHz (modulated), at SARs of between 1 and 2.5 W/kg, intermittently for up to 24 hours. The authors report on several hundreds of genes whose steady state mRNA levels were reproducibly altered by the exposures, many involved in the regulation of cell cycle, differentiation and DNA husbandry. A full description of this data is awaited, and the results will surely expand our knowledge of the depth and breadth of the stress response of mammalian cells to RF-EMF. As a matter of caution, however, at the same meeting Whitehead and co-workers reported on the exposure of C2H 10T1/2 cells to CDMA or Frequency Division Multiple Access (FDMA) RF at 5W/kg for 24 hours, and concluded that no consistent alterations of steady state mRNA levels could be detected when probed by a 12488 feature Affymetrics chip device (60). However, the same cells were used by Park and Kim, along with WI 38 human fibroblasts and the DO11.10 murine T cell line, to study the

effects of 836.5 MHz (CW) and 1.76 GHz (CDMA), at SARs up to 38 W/kg for up to 72 hours, using DNA microarrays probing up to 10 000 mRNAs. Many alterations were reported upon, with both suppressions of steady state mRNA levels for some metabolism genes and induction of some mRNAs for pro-differentiation and stress response genes detected (61). Again, these results await further scrutiny by peer review.

In addition to these in vitro studies, one study on the application of DNA microarray analysis has been reported in vivo. Rats were exposed to 900 MHz (modulated), at SARs between .04 to 0.4 W/kg, for 2 hours. Using an Affymetrics chip analysis small but significant changes in the steady state levels of a dozen mRNAs were detected in brain tissue samples, some of which were purported to be related to blood-brain barrier function (62). However, it should be born in mind that these kinds of analyses are restricted by their relative insensitivity, and that small changes in mRNA steady state levels may not necessarily reflect in a discernable functional change at the level of respective proteins.

Despite the lack of critical review of the above studies, it is clear that the application of emerging genomic and proteomic techniques to the study of the response of biological material to RF-EMF may help to unravel the mechanism(s) underlying the interaction. The studies may also reveal novel biomarker candidates which might be used to study the effects of exposure of humans to microwave emissions from mobile phones, for example by determining potential gene or protein alterations in circulating lymphocytic populations or skin biopsies. However, future studies should focus more stringently on the time of exposure (1 hour may not be sufficient to allow for altered gene/protein expression) and the tissue/cell type under study, with reference to the need to provide biomarkers for human exposures and risk assessments. The relatively qualitative data must also find confirmation in specific assays, followed out to the level of the protein, and, importantly find independent validation. The studies must also address currently emerging concerns, voiced at the recent conference on

“Influence of RF fields on the expression of stress proteins (Helsinki, April 2004), that effects on stress proteins in the absence of apparent heat shock, may still be elicited by thermal mechanisms in the test systems employed.

Mechanistic considerations

One of the key issues to be dealt with in the context of rationalising the potential effects of RF-emissions on biological systems is the explanation of the physical basis of underlying the observed effects, with particular reference to 1) the HSP response, 2) its apparent dependence on discrete frequency windows and 3) the potential influence of secondary modulation of CW emissions on this. At the same time it should be born in mind that the literature is in a state of uncertainty as to the validity of these concepts.

In terms of the HSP response, which has been detailed above, there is a consensus of opinion that the effects generated in the in vitro systems, for instance, are not coupled to gross heating effects in the cell culture medium surrounding the cells. This has tended to label the effects as “HSP-inducing, by a non-thermal heating effect”. However, the term “heating” must be clearly defined in physical terms in order to clarify the possibilities. Heating is a physical process whereby the energy would be converted from, in this case, the vibrational energy in the RF emission, into vibrational energy in bio-molecules. Laurence and co-workers (63) clearly point out that we generally measure temperature at an equilibrium situation over a number of seconds, using conventional thermometers and thermocouples. Changes in vibrational states of molecules do or coarse, theoretically, occur much faster than this. Therefore, we label them as non-thermal, although they are defined by the basic laws of thermodynamics. Therefore, in their recent hypothesis paper, Laurence et al (63) have elegantly discussed various aspects of the HSP response detected in biological systems, in terms of theoretical physical events in the biological setting. The authors consider various

aspects of the exposure to RF emissions, such as the pulse times of exposure, characteristics of “cooling” of molecules such as proteins in the aqueous environment of the cell, absorption of microwave energy into the vibrational mode of a particular protein molecule and characteristic times for protein folding/unfolding events. In a more recent paper, the same authors present new theoretical calculations, based on their original hypothesis, that show that, even at maximum exposure levels allowed by ICNIRP, the thermal diffusivity of biological systems negate steady state fluctuations in temperature over macromolecular or cellular distance scales (64).

Although a deeper consideration of these issues is out of the scope of the present treatise, these authors clearly show that direct “heating” of individual proteins within the intracellular milieu, is not only theoretically possible, but is likely as a response to RF emissions of the kinds used in modern mobile telephony. In simple terms, this results from the induction of molecular temperature transients, which result in significant protein-specific effects on protein conformation. Expanding on this, they propose that this possibility might explain the apparent non-linear relationship between RF power in the exposure and the degree of biological HSP response, where loss of function without induction of HSP response at low power emissions transits into an HSP stress response as the extent of “semi-molton” proteins increases in the cell, over to overt toxicity at high exposures, where extensive protein aggregation might occur. Similarly, it is clearly theoretically possible that secondary modulation of the primary frequency can enhance the delivery of vibrational energy to protein molecules (63).

In a more recent treatise by Balzano and Sheppard (65), the importance of low frequency modulation of CW emissions was discussed in terms of a two compartment model, where the water phase of biological systems acts linearly with the emission according to equilibrium thermodynamics, and the remaining components of the biological system interact according to non-linear thermodynamic principles.

Much of the theoretical consideration of the mechanism of RF-induced alteration to protein structure and function is focussed on the characteristics of the RF emission and its interaction with aqueous-based systems. However, it is important to remember that cellular proteins are all different in their structure and that they will behave individually when exposed to RF. Each has a unique structure with a unique “vibrational signature”. Additionally, many proteins are in intimate electrostatic contact with others within the gel-like consistency of the cytoplasm. Therefore, predicting which proteins in a cell might be affected by RF emissions must also carefully consider this situation. This is not only important when considering the HSP effect, but also important as we move into an era where many other biological processes are beginning to emerge as potential “targets”. Here it is instructive to consider the variability of protein structure, versus the structure of the major classes of nucleic acids in the cell.

In order to further substantiate the risks from RF emissions in biological systems, there is therefore an immediate need to improve our understanding of the nature of the interaction within living systems. Indeed, there is still contention in the literature concerning non-thermal effects elicited by RF emissions. Recently Adair has argued that the intrinsic molecular “noise” within biological systems would essentially abrogate any specific effect RF emissions might have on particular molecules (66).

A key issue therefore seems to be to demonstrate that RF emissions can directly affect the higher order organisation of individual proteins. To this end, a number of model studies, using purified enzymes or proteins exposed to RF in aqueous solutions, have shown clear effects on the physical and biochemical properties of the tested proteins. These proteins include β -lactoglobulin, where both denaturation and renaturation kinetics were shown to be affected (67), and a variety of thermophilic enzymes, whose stability and activities were generally affected by the exposures (68). On the other hand, the polymerisation-depolymerisation behaviour of isolated microtubule protein was not affected by similar emissions (69). Very recently de

Pomerai and colleagues exposed serum albumin or insulin to 1 GHz emissions at an SAR of 0.5 W/kg and demonstrated that the exposure increased the aggregation behaviour of both proteins, with the formation of organised amyloid fibrils in the latter case. Interestingly, they also showed that recombinant HSP16 was able to prevent the aggregation of BSA in this simple model (70). Clearly, there is a need to pursue this direction of investigation with other major classes of cellular proteins and with increasingly complex mixtures of proteins where aggregation behaviour is implicit in their biological mode of action. There is also a need to conduct exposures which more closely relate to RF emissions from mobile telephones, particularly regarding the issues of non-linear responses to CW emissions, and potential amplificatory effects from secondary frequency modulation.

Summary and thoughts for future investigations.

Mobile telephony is becoming increasingly integrated into our everyday life. Millions of individuals are exposed to RF emissions on a daily basis and concerns are being raised as to potential risks to human health from the energy contained in such emissions. Due to the microwave character of mobile telephone emissions, it was suggested that such emissions might affect biological systems in a manner similar to other microwave emissions, by inducing a heat shock response (Figure 1). During the last 10 years a number of studies in in vitro systems have consistently shown that CW RF emissions, in the range between 750 MHz and 2.4 GHz, are able to induce the expression of HSPs in a large variety of eukaryotic cell systems. Similarly, it has been shown that secondary frequency modulation of the emission has considerable effect on the response of the biological system to the primary frequency. Although each study has been performed utilizing differing exposure conditions, including the use of different cell systems, differing RF sources, varying CW emissions, varying secondary frequency modulations, varying power of the emission resulting in varying SAR and different

methods to assess the induction of an HSP response, there is now accumulating evidence in the literature to substantiate the effect in vitro. However, many of the findings await independent corroboration, and there is particular concern when labelling the effects as being non-thermally derived. Irrespective of the mechanism of induction of the stress response in vitro, it is more difficult to surmise if this effect occurs within exposed tissues (epidermis, peripheral neuronal tissue and the CNS) in the limited number of in vivo experiments that have been conducted.

Thus, issues concerning the risks to human tissues from RF emissions in vivo are still clouded by a number of inconsistencies and controversies in the literature with respect to the HSP response, which must be clarified by novel research.

The cellular models: There is a need to concentrate efforts on choosing human cellular systems relevant to the in vivo exposure situation, for further in vitro studies. Experimentation should include primary cultures of normal human skin keratinocytes and dermally-derived, organotypic cell models. Primary cultures of human neuronal tissues are also strongly *indicated for study*.

The exposure systems. There is need to refine and co-ordinate the manner of exposure to RF. Concerted effort should be applied to defining “effect windows” in particular exposure models, by consequently varying the frequency of CW emission, in combination with consequential variation of relevant secondary frequency modulation and SARs. In the case of the later issue, most in vitro models employ exposures within a Petri-dish format. Here recent work by Schuderer and Kuster (70) clearly indicate that theoretical calculations of the exposure and SAR of in vitro systems must be re-evaluated and refined. Technical possibilities also exist to conduct cellular exposures in other types of exposure supports, such as carrier beads.

The analysis methods: Due to the constraints of varying exposures in conventional in vitro systems, more attention must be applied to methods able to define adaptive gene and protein expressional alterations within individual cells. This might include the use of laser-capture methods to “fence-in” cells where exposure characteristics can be more exactly calculated. Further, there is an urgent need to probe the depth of the adaptive stress response, using appropriate proteomic and genomic techniques, in appropriate cellular models, exposed with the above considerations in mind. Here it is interesting to note that it is now technically feasible to perform mass spectral analysis of the proteome of individual cells (71).

The mechanism of the response: The physical basis of the induction of an HSP response within biological material is still a matter of theoretical debate, where considerations of “athermal heating” have been invoked at the level of individual proteins in the aqueous environment of the cell. There is thus an urgent need to conduct more work with purified proteins and protein complexes, which probe effects of RF on the comparative sensitivity of individual proteins to “molecular melting” and loss of function. Similarly, proof of concept must be sought within the intracellular milieu. Therefore, it can be envisaged that the state of folding of target proteins could be probed by studying changes to fluorescence characteristics of transfected proteins, such as green fluorescent protein, or fluorescent chimeric proteins.

The extent of the response: There are emerging indications of a much wider stress response in biological systems in response to RF than the HSP response. These emerging data must be allowed to mature and be subjected to rigorous statistical analyses and comparisons, as well as explorative pathway analyses. Cross-validation of the functional significance of other gene expression alterations than those which are HSP-related must be also performed, with focus on particular signal transduction pathways.

The consequences of the phenotypic adaptations: A major obstacle to the application of these basic cell biological findings to risk assessment is the controversy concerning the

functional significance of the HSP response. Clearly the interpretation of the effect of this adaptation depends on the cell type under study, the duration of the response and other factors controlling and integrating the cell cycle in complex cellular matrices. Many of these are, of course, minimalised or absent in the simplified in vitro systems utilized. Thus, the induction of an HSP response may be construed to be either positive in terms of chaperoning unfolding cellular proteins, or viewed negatively if the structural integrity of otherwise damaged proteins is maintained abnormally. Similarly, the anti-apoptotic function of HSPs can be viewed either positively or negatively, depending on whether one considers this to confer additional robustness to the cell with respect to other potentially damaging stimuli, such as oxidative stress (1) or prevent normal apoptotic deletion of damaged cells. This quandary will not be simplified as we discover other components of the stress response to RF emissions. This author is convinced that solutions to the interpretations of the relevance of the stress response to human risk assessment will require integration of all of the above issues, in cooperation with other more fundamental discoveries in cell biology.

In vivo effects. Translation of the concepts and mechanisms defined in vitro systems into the in vivo setting is an essential component of assessing how they might realistically contribute to a human health risk from RF emissions from mobile telephones. There is, therefore, an immediate need to consolidate data from relevant in vivo exposures, modelled to mimic human exposures. Here, in terms of more chronic exposures, it is interesting to note that a very recent addition to the literature, describing the exposure of mice to 848.5 MHz or 1.76 GHz RF at between 2.4 and 12.2 W/kg, twice daily for 45 minutes, 5 days per week and for 10 weeks failed to demonstrate altered expression of HSPs 70, 90 or 25, or the phosphorylation of ERK1/2, JNK1/2 or p38 MAPK, which have been strongly indicted from in vitro studies. This correlated to an absence of effects of exposures on cell gross tissue morphology within the brain (71).

So are mobile telephones really so (heat)shocking? The answer to this question is certainly that this is a probability if biological material is adequately exposed, but that we are absolutely not in a position at present to interpret these effects, obtained in largely model in vitro systems, in terms of risks to human health (Figure 1).

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References

- 1) Cotgreave IA, Morgenstern R, Jernström B and Orrenius S (2000). Current Molecular and Cellular Concepts in Toxicology. General and Applied Toxicology. Volume 1, Ballantyne et al Eds., pp155-175, Macmillan.
- 2) Morimoto RI, Kline MP, Bimston DN, Cotto JJ (1997). The heat-shock response: regulation and function of heat-shock proteins and molecular chaperones. *Essays Biochem.* 32:17-29.
- 3) Ishida T, Takahashi M, Corson MA, Berk BC (1997). Fluid shear stress-mediated signal transduction: how do endothelial cells transduce mechanical force into biological responses? *Ann N. Y. Acad. Sci.*, 15;811:12-23.
- 4) Buchner J (1996). Supervising the fold: functional principles of molecular chaperones. *FASEB J.* 1996 Jan;10(1):10-9.
- 5) Craig TA, Lutz WH, Kumar R (1999). Association of prokaryotic and eukaryotic chaperone proteins with the human 1alpha,25-dihydroxyvitamin D(3) receptor. *Biochem.Biophys.Res.Commun.*, 260:446-42.
- 6) Bidmon B, Endemann M, Muller T, Arbeiter K, Herkner K, Aufricht C (2002). HSP-25 and HSP-90 stabilize Na,K-ATPase in cytoskeletal fractions of ischemic rat renal cortex. *Kidney Int.*, 5:1620-1627.
- 7) Concannon CG, Orrenius S and Samali A (2001). HSP27 inhibits cytochrome c-mediated caspase activation by sequestering both pro-caspase 3 and cytochrome c. *Gene expression.* 9; 195-201
- 8) Zhang L, Pelech S, Uitto VJ (2004). Bacterial GroEL-like heat shock protein 60 protects epithelial cells from stress-induced death through activation of ERK and inhibition of caspase 3. *Exp. Cell Res.*, 292: 231-240.

- 9) Garrido C, Schmitt E, Cande C, Vahsen N, Parcellier A, Kroemer G (2003). HSP27 and HSP70: potentially oncogenic apoptosis inhibitors. *Cell Cycle*. 2: 579-584.
- 10) Dent P, Yacoub A, Contessa J, Caron R, Amorino G, Valerie K, Hagan MP, Grant S, Schmidt-Ullrich R. (2003). Stress and radiation-induced activation of multiple intracellular signaling pathways. *Radiat. Res.*, 159: 283-300.
- 11) Ichihashi M, Ueda M, Budiyo A, Bito T, Oka M, Fukunaga M, Tsuru K, Horikawa T. (2003). UV-induced skin damage. *Toxicology*. 189: 21-39.
- 12) Kavet R, Stuchly MA, Bailey WH, Bracken TD. (2001). Evaluation of biological effects, dosimetric models, and exposure assessment related to ELF electric- and magnetic-field guidelines. *Appl. Occup. Environ. Hyg.*, 16: 1118-1138
- 13) Tokalov SV, Gutzeit HO. (2004). Weak electromagnetic fields (50 Hz) elicit a stress response in human cells. *Environ. Res.*, 94: 145-51.
- 14) McAlister L, Finkelstein DB (1980). Heat shock proteins and thermal resistance in yeast. *Biochem. Biophys. Res. Commun.*, 93: 819-824.
- 15) Sorger PK, Lewis M and Pelham HR (1987). Heat shock factor is regulated differently in yeast and HeLa cells. *Nature*. 329 :81-84.
- 16) Sorger PK and Pelham HR (1988). Yeast heat shock factor is an essential DNA-binding protein that exhibits temperature-dependent phosphorylation. *Cell*. 54 :855-864.
- 17) Parker JE, Kiel JL, and Winters WD (1988). Effect of radiofrequency radiation on mRNA expression in cultured rodent cells. *Physiol. Chem. And Phys. Med. NMR.*, 20: 129-134.
- 18) Cleary SF, Cao G, Liu LM, Egle PM, Shelton KR. Stress proteins are not induced in mammalian cells exposed to radiofrequency or microwave radiation. *Bioelectromagnetics*. 18:499-505.

- 19) Kwee S, Raskmark P and Velizarov S (2001). Changes in cellular proteins due to environmental non-ionizable radiation. I. Heat shock proteins. *Electro- and Magnetobiology* 20; 141-152.
- 20) Leszczynski D, Joenväärä S, Reivinen J and Kuokka R. (2002). Non-thermal activation of the HSP27/p38MAPK stress pathway by mobile phone radiation in human endothelial cells: Molecular mechanism for cancer and blood-brain barrier-related effects. *Differentiation*, 70;120-129.
- 21) Tian F, Nakahara T, Wake K, Taki M and Miyakoshi J (2002). Exposure to 2.45 HHZ electromagnetic fields induces hsp70 at a high SAR of more than 20W/Kg but not at 5 W/Kg in human glioma MO54 cells. *Int. J. Radiat. Biol.*, 78: 433-440.
- 22) Guisasola C, Desco M, Millan O, Villanueva FJ and Garcia-Barreno P (2002). Biological Dosimetry of Magnetic Resonance Imaging. *J. Magnetic Resonance Imaging*, 15: 584-590.
- 23) <http://www10.who.int/peh-emf/emfstudies>
- 24) <http://www10.who.int/peh-emf/emfstudies/viewstudy.cfm?ID=859>
- 25) <http://www10.who.int/peh-emf/emfstudies/viewstudy.cfm?ID=982>
- 26) Daniells, C, Thomas D, Sewell P, Tattersall J and de Pomerai D (1998). Transgenic nematodes as biomonitors of microwave-induced stress. *Mut. Res.*, 399, 55-54.
- 27) de Pomerai D, Daniells C, David H, Allan J, Duce I, Mutwakil M, Thomas D, Sewel D, Tattersall J, Jones D and Candido P (2001). Non thermal Heat shock response to Microwaves, *Nature* 405, 417-418.
- 28) de Pomerai D, Dawe A, Djerbib L, Allan J, Brunt G and Daniells C (2002). Growth and maturation of the nematode *Caenorhabditis elegans* following exposure to weak microwave fields. *Enzymes and Microbial Technology*, 30, 73-79.

- 29) David HE, Dawe AS, de Pomerai DI, Jones D, Candido PM and Daniells C (2003). Construction and evaluation of a transgenic hsp-16-GFP-lacZ *Caenorhabditis elegans* strain for environmental monitoring. *Env. Toxicol. And Chemistry*, 22, 111-118.
- 30) Brenner, Schulston and Horwitz, Nobel Lectures 2002. <http://www.nobel.se/medicine/laureates/2002/index.html>
- 31) Fritze K, Wiessner C, Kuster N, Sommer C, Gass p, Hermann DM, Kiessling M and Hossman KA. 1997. Effect of Mobile Communication Microwave Exposure on the genomic response of rat brain. *Neuroscience* 81, 627-639.
- 32) Walters TJ, Ryan KL and Mason PA. Regional distribution of HSP70 in the CNS of young and old food-restricted rats following hyperthermia. (2001) *Brain Res. Bull.*, 55, 367-374.
- 33) Shallom JM, Di Carlo AL, Ko D, Penafiel ML, Nakai A and Litovitz TA. Microwave exposure induces HSP70 and confers protection against hypoxia in chick embryos. *J. Cell Biochem.*, 85, 490-466.
- 34) Di Carlo A, White N, Guo F, Garrett P and Litovitz T. Chronic electromagnetic field exposure decreases HSP70 levels and lowers cytoprotection. (2002) *J. Cell Biochem.*, 84, 447-454.
- 35) French PW, Penny R, Laurence JA and McKenzie DR (2000). Mobile phones, heat shock proteins and cancer. *Differentiation*, 67, 93-97.
- 36) Hardell L, Nasman A, Pahlson A and Hallquist A. (2002). Case-control study on radiology work, medical x-ray investigations, and use of cellular telephones as risk factors for brain tumors. *Medscape General Medicine*. 2: E2.
- 37) Hardell L, Mild KH, Pahlson A and Hallquist A (2001). Ionizing radiation, cellular telephones and the risk for brain tumours. *European Journal of Cancer Prevention*. 10: 523-529.

- 38) Litovitz, TA and Penafiel L (1993). The role of coherence time in the effect of microwaves on ornithine decarboxylase activity in vitro. *Bioelectromagnetics* 14, 395-403.
- 39) Litovitz TA and Penafiel L. (1997) Role of modulation in the effect of microwaves on ornithine decarboxylase activity in L929 cells. *Bioelectromagnetics*. 18, 132-141.
- 40) Garrington T and Johnson G (1999). Organization and regulation of mitogen-activated protein kinase signaling pathways. *Current Opinion in Cell Biology*. 11: 211-218.
- 41) Robertson JD, Enoksson M, Suomela M, Zhivotovsky B and Orrenius S (2002). Caspase-2 acts upstream of mitochondria to promote cytochrome c release during etoposide-induced apoptosis. *Journal of Biological Chemistry*. 277: 29803-2980.
- 42) Dandrea T, Bajak E, Wärngård L, Cotgreave IA. (2002). Protein S-glutathionylation correlates to selective stress gene expression and cytoprotection. *Archives of Biochemistry & Biophysics*. 406: 241-252
- 43) Dandrea T, Hellmold H, Jonsson C, Zhivotovsky B, Hofer T, Wärngård L and Cotgreave IA (2004). The transcriptional response of human A549 lung cells to a hydrogen peroxide-generating system. Relationship to DNA damage, cell cycle arrest and caspase activation. *Free Rad. Biol. Med.*, 36: 881-896.
- 44) Pacini S, Ruggiero M, Sardi I, Aterini S, Gulisano F, Gulisano M. (2002). Exposure to global system for mobile communication (GSM) cellular phone radiofrequency alters gene expression, proliferation, and morphology of human skin fibroblasts. *Oncology Research*. 13: :19-24.
- 45) Natarajan M and Meltz ML (2002). Signalling pathways down-stream of NF κ B activation after 2450 MHz exposure of human monocytes in vitro. *Bioelectromagnetics* 23, 271-277.

- 46) Ivaschuk OI, Jones RA, Ishida-Jones T, Haggren W, Adey WR and Phillips JL (1997). Exposure of nerve growth factor-treated PC12 rat pheochromocytoma cells to a modulated radiofrequency field at 836.55 MHz: effects on c-jun and c-fos expression. *Bioelectromagnetics*. 18:223-229.
- 47) Goswami PC, Albee LD, Parsian AJ, Baty JD, Moros EG, Pickard WF, Roti Roti JL and Hunt CR (1999). Proto-oncogene mRNA levels and activities of multiple transcription factors in C3H 10T 1/2 murine embryonic fibroblasts exposed to 835.62 and 847.74 MHz cellular phone communication frequency radiation. *Radiation Research*. 151:300-309.
- 48) Fritze K and Hoffman KA (1997). Effect of global mobile communication microwave exposure on the genomic response of the rat brain in vivo. *Neuroscience* 81; 627-639.
- 49) Morrisey JJ (1999). IRIDIUM Exposure increases cFos expression in mouse brain only at levels which likely result in tissue heating. *Neuroscience* 92; 1539-1546.
- 50) Stagg R, Adey WR and Byus C (2001). Effect of immobilization and concurrent exposure to pulse-modulated microwave field on core body temperature, plasma ACTH and corticosteroid, and brain ornithine decarboxylase, Fos and Jun mRNA. *Radiation Research* 155, 584-592.
- 51) <http://www10.who.int/peh-emf/emfstudies/viewstudy.cfm?ID=346> or **ID=272** or **ID=233**.
- 52) Kubinyi G, Somosy Z, Thuroczy G (1996). Biological effects of exposure to RF at 900 and 1800 MHz with GSM-like modulated waveforms. *Bioelectromagnetics* 17; 497-503.
- 53) <http://www10.who.int/peh-emf/emfstudies/viewstudy.cfm?ID=722>
- 54) <http://www10.who.int/peh-emf/emfstudies/viewstudy.cfm?ID=781>
- 55) [Http://www10.who.int/peh-emf/emfstudies/viewstudy.cfm?ID=820](http://www10.who.int/peh-emf/emfstudies/viewstudy.cfm?ID=820)

56) <http://www10.who.int/peh-emf/emfstudies/viewstudy.cfm?ID=347>

57) Harvey C and French PW (1999). Effects on protein kinase C and gene expression in human mast cell line HMC-1, following microwave exposure. *Cell Biol. International*, 23; 739-748.

58) Kuokka R and Leszczynski D (2003). Applicability of proteomics in studying effects of mobile phone radiation using two variants of human endothelial cell line. *Proceedings of the 25th Annual Meeting of the Bioelectromagnetics Society*. Poster 20.

59) Maercker c, Kuokka R, Reivinen S, Ivancsits S, Ruediger J, Schuderer j, Kuster N, Fornasari D, Clementi F, Schlatterer K, Tauber R, Fitzner R, Adlkofer F and Leszczynski D (2003). Whole genome gene expression profiling: A big challenge to find out the molecular answer to EMF exposure. . *Proceedings of the 25th Annual Meeting of the Bioelectromagnetics Society*. Poster 200.

60) Whitehead TD, Hook GJ, Moros EG, Cha BA ad Roti Roti JL. (2003). Exposure of c3H 10T1/2 cells to CMA or FDMA radiofrequencies does not significantly modify gene expression. *Proceedings of the 25th Annual Meeting of the Bioelectromagnetics Society*. Poster 345.

61) <http://www10.who.int/peh-emf/emfstudies/viewstudy.cfm?ID=838>

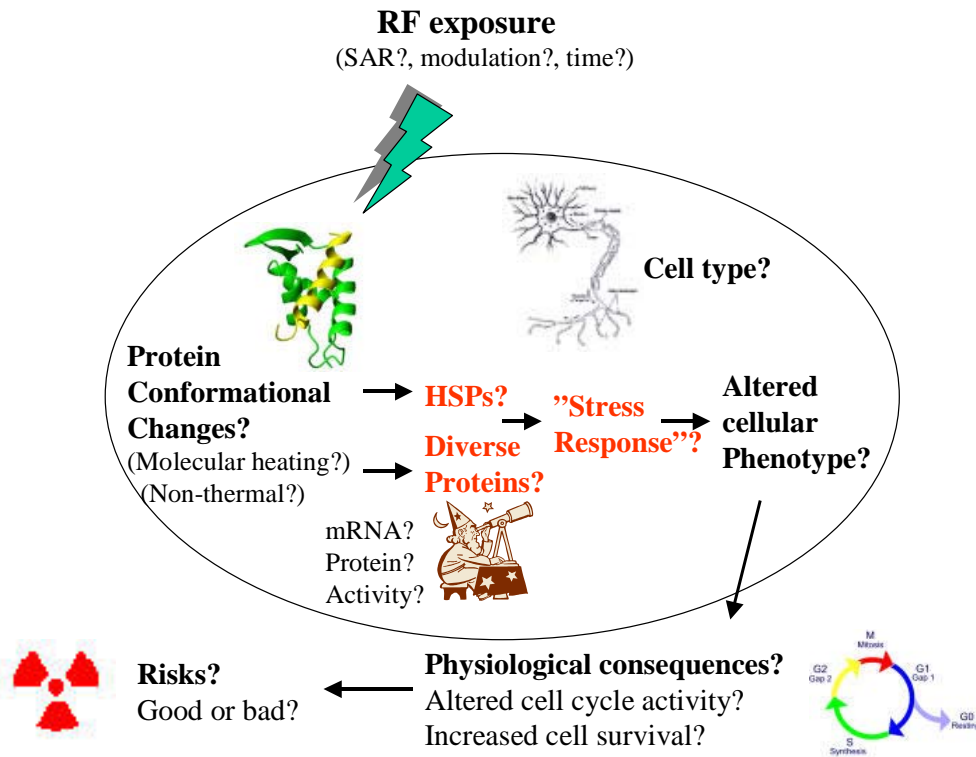
62) <http://www10.who.int/peh-emf/emfstudies/viewstudy.cfm?ID=1062>

63) Laurence JA, French PW, Linder RA and McKenzie DR (2000). Biological effects of electromagnetic fields. Mechanisms for effects of pulsed wave radiation on protein conformation. *J. Theor. Biol.*, 206; 291-298.

64) Lawrence JA, McKenzie DR and Foster KR (2003). Application of the heat equation to the calculation of temperature rises from pulsed microwave emissions. *J. Theoretical Biol.*, 222; 403-405.

- 65) Balzano Q and Sheppard A (2003). RF nonlinear interactions in living cells-I: Nonequilibrium thermodynamic theory. *Bioelectromagnetics* 24: 473-482.
- 66) Adair RK (2003). Biophysical limits on athermal effects of RF and microwave radiation. *Bioelectromagnetics*. 24: 39-48.
- 67) Porcelli M, Cacciapuoti G, Fusco S, Massa R, d'Ambrosio G (1997). Non-thermal effects of microwaves on proteins: Thermophilic enzymes as model systems. *FEBS Lett.*, 402, 102-106.
- 68) Ortner MJ, McRee Diand Galvin MJ (1983). The effect of 2450-MHz microwave radiation during microtubule polymerisation in vitro. *Radiation Res.*, 93: 353-363.
- 69) De Pomerai, DI, Smith B, Dawe A, North K, Smith T, Archer DB, Duce IR, Jones D and Candido PM (2003). Microwave radiation can alter protein conformation without bulk heating. *FEBS Lett.*, 543: 93-97.
- 70) Schuderer J and Kuster N (2003). Effect of meniscus at the solid/liquid interface on the SAR distribution and flasks. *Bioelectromagnetics* 24; 103-108.
- 71) Schubert W (2003). Topological proteomics, toponomics, MELK-technology. *Advances in Biochemical Engineering-Biotechnology*. 83: 189-209.
- 72) <http://www10.who.int/peh-emf/emfstudies/viewstudy.cfm?ID=994> Lee JS and Seo JS (2004). *Bioelectromagnetics* in press.

Figure 1. Schematic representation of the interaction of radio-frequency emissions with the cell.



Legend:

The figure attempts to summarise current thinking on the molecular mechanisms of interaction of RF emissions with biological material. This review has centred around gathering information on the possible components of the stress response of the cell (in red), as well as presenting theoretical appraisals of possible physiological consequences and risks. However, it should be noted that all components of the Figure are associated with question marks, and today there is a lack of concordance if RF emissions indeed are able to induce stress responses, particularly by non-thermal mechanisms.

Table 1 Summary of the published studies related to altered HSP gene/protein expression and function in response to radio frequency emission exposures in vitro and in vivo

HSP under study	Biological model	RF source	Exposure	General comments	Ref.
HSP70 (m) ¹	L5178Y cells, CHO cells	2.45 GHz (CW)	51.75-103.5 W/kg,	No induction	17
HSP70 (p)	HeLa cells, CHO cells	27 MHz, 2.45 GHz (CW)	100 W/kg	No induction	18
HSP 27 (p) HSP 70 (p)	Human amnion cells	960 MHz (217 Hz mod)	2.1 mW/kg,	Induction	19
HSP27 (p)	EA.hy926 cells	900 MHz (GSM)	2 W/kg,	Induction and hyper-phosphorylation	20
HSP 70 (p)	Human glioma cells	2.45 GHz (CW)	20 W/kg	Induction	21
HSP 70 (m, p) HSP27 (m, p)	L-132 cells	3-300 MHz (CW)		No induction	22
HSP 70 (m) HSP 27 (m)	Human astrocytes	900 MHz	0.2 W/kg	No induction	24
HSP 16-bGAL ² (p, a)	C.elegans	300, 750 MHz, (CW)	Various	Induction of promoter	26
HSP 16-GFP ² (p, a)	C.elegans	750 MHz (CW)	1 mW/Kg	Induction of promoter	27
HSP70 (m, p)	Rat (Brain)	900 MHz (modulated and CW)	0.3-7.5 W/Kg	Induction (m) No induction (p)	31
HSP 70 (p)	Rat (Brain)	2.06 GHz	2.2 W/cm ²	Induction due to localised heating	32
HSP 70 (p, a)	Chick embryo	915 MHz (modulated and CW)	1.57-2.5 W/Kg	Induction Resistance to hypoxia	33
HSP 70 (p, a)	Chick embryo	915 MHz (modulated and CW)	1.57-2.5 W/Kg	Suppression after extended exposure periods. Loss of protection from hypoxia	34

¹ m=mRNA, p=protein, a=activity. ² Promoter construct

GAL=galactosidase, GFP= Green Fluorescent Protein, CW= Continuous Wave, GSM=Global Signal Modulation

Table 2 A summary of the published studies related to the effects of radio frequency emissions on the expression of genes or proteins other than HSPs.

Gene/protein under study	Biological model	RF source	Exposure	General comments	Ref.
ODC (p)	L929 cells	835 MHz (CW, TDMA)	2.5 W/Kg	No induction (CW) Induction (TDMA)	38
ODC (p)	L929 cells	835MHz (CW, various modulations)	2.5 W/Kg	Induction (CW) Enhanced induction (modulation between 16 and 60 Hz)	39
p38MAPK (p, a)	EA.hy926 cells	900 MHz (GSM)	2 W/Kg	Induced (p, a) 324 and 67 other cellular proteins hyper/hypo-phosphorylated, respectively.	20
MAPKK3 (m)	Skin fibroblasts	900 MHz (GSM)	0.6 W/Kg	Induction. Microarray study	44
Cyclin G1 (m)	Skin fibroblasts	900 MHz (GSM)	0.6 W/Kg	Induction Microarray study	44
NF _κ B (a)	MonoMac-6	2.45 GHz (CW) 8.2 GHz (pulsed wave)	10 W/kg	Increased binding to DNA	45
AP-1 (cJun, p)	PC12 cells	836 MHz (CW)	0.41-41 mW/Kg	Induction at highest exposures only	46
AP-1 (cjun, m, a)	C3H 10T1/2	835 MHz (FM), 847 MHz (CDMA)	0.6 W/Kg	Induction (m) No induction (a)	47
AP-2	C3H 10T1/2	835 MHz (FM), 847 MHz (CDMA)	0.6 W/Kg	Induction	47
TATA-binding protein (m)	C3H 10T1/2	835 MHz (FM), 847 MHz (CDMA)	0.6 W/Kg	No induction	47
Aminoacyl t-RNA (a)	Rat (Foetal liver and brain)	2.45 GHz (CW, FM)	4.23 W/Kg	Suppression (Brain, CW) Induction (Liver, FM)	51
cKIT (m)	HMC-1 cells	864 MHz	7 W/Kg	Suppression, microarray study	52
DAD-1 (m)	HMC-1	864 MHz	7 W/Kg	Induction, microarray study	52
NDPK (m)	HMC-1	864 MHz	7 W/Kg	Suppression, microarray study	52
Various unidentified proteins	EA.hy926 cells	900 MHz (GSM)	2 W/Kg	Induction, proteomic study	20
AP-1 (cJun, cFos, p)	Rat (Brain)	900 MHz (GSM)	0.3, 1.5 and 7.5 W/Kg	No induction	48
AP-1 (cFos, p)	Mouse (Brain)	1.6 GHz (CW or IRIDIUM)	0.23-1.19 W/Kg	No induction	49
AP-1 (cFos, cJun, m)	Rat (Brain)	1.6 GHz (IRIDIUM)	0.16-5 W/Kg	No induction	50

¹ m=mRNA, p=protein, a=activity

CW= Continuous Wave, GSM=Global Signal Modulation, TDMA= Time Division Multiple Access, FM=Frequency Modulation, CDMA=Code Division Multiple Access.

