Taking a closer look at stress

Report of the COST 281/FGF/STUK/WHO workshop in Helsinki

by Christoph Bächtle

Under critical physiological conditions, cells activate an emergency program to improve their chances for survival. During this process they release stress proteins also called "heat-shock proteins". At the international workshop „Influences of radio-frequency electromagnetic fields on heat-shock protein expression" on April 28 to 29, 2004, in Helsinki, Finland, experts discussed the question whether even low-frequency electromagnetic fields in the mobile radio frequency range could activate such stress proteins. The conclusion was: "They probably do not ..."

Both the studies of David de Pomerai from the years 2000 and 2002 and the data published by Dariusz Leszczynski in 2002 seem to provide evidence for the assumption that radiofrequency electromagnetic fields could trigger a stress response in different cell lines that would not be due to thermal causes. This concerns above all the small stress proteins biochemically termed Hsp16 and Hsp27. However, this is contradicted by the results of Cleary et al. (1997), Goswami et al. (1999) and Li et al. (1999) who could not find proof of such effects.

Approximately 40 scientists from Europe, Japan and America discussed interesting new aspects of this topic during the workshop jointly organized by COST 281, the FGF, STUK and the WHO. As was done at previous workshops on other topics associated with potential radiofrequency field effects, in addition to the scientists directly active in this field also such colleagues were invited who, although they do not deal with field effects, are acknowledged experts in the area of heat-shock proteins. They opened the meeting by holding survey lectures informing the attendees about the state of the art of research in this special field. In the course of the event, they critically intervened in discussion in their function as experts in the areas of biochemistry and molecular biology.

Heat-shock proteins – a universal system

In his introductory lecture "Heat-shock response and stress signal ways", Matthias Gaestel of the Medical University of Hannover described the fascinating sys-

tem of stress proteins and the mechanisms leading to their expression. Protein expression is defined as the stimulation of the genome, i.e. all the genetic material in a cell, to read the information for a specific protein and to transfer it to a ribosome where the protein is ultimately produced. Consequently, this term itself yet implies an extraordinarily complicated process which, however, was not a direct part of the program of this workshop.

Gaestel demonstrated that this response to physiological stress coming from the outside is a highly conserved mechanism in evolution that occurs at all levels of biological organisation: from bacteria, plants and animals to humans. Thus, science is provided with a large number of model organisms for extensive comparatistic studies. Gaestel not only presented parallels between the systems of stress mechanisms in bacteria and in organisms that have cells containing nuclei, he described the different families of heat-shock proteins and dealt with the structure and activation of genes by which they are expressed, and with the complex intracellular signal paths which are stimulated by stress.

Most heat-shock proteins are so-called chaperones. This term is used to classify these proteins because they help the long chains of amino acids correctly fold into shape as functioning proteins so they do not clump together. They further support the correct refolding of these proteins should their structure be altered by their own functioning or just by external influences. Even if the so-called small heat-shock proteins (= sHsp) cannot directly affect protein folding, they bind misfolded or partially denatured proteins without consumption of energy until other heat-shock proteins which do consume metabolic energy are there to do the refolding.

The transfer of chemical energy from one molecule to the other occurs through phosporylation, i.e. the transfer of a group of phosphates. Mostly, it stems from the adenosine triphosphate (ATP) – the "fuel" of the cell – which is thereby transformed into adenosine diphosphate (ADP). Phosporylation does not only play a part in the repair of disturbed proteins – the heat-shock proteins themselves must be phosporylated first, i.e. be brought into force. This is the task of other proteins, the potein kinases. The suffix "-ase" characterizes them as enzymes that catalytically accelerate these responses. The so-called stress-activated protein kinases (SAPK) play an important role in Hsp phosporylation. However, it is still yet unclear how they are activated themselves.

This rough survey alone shows the complexity of the mechanism that may be triggered by stress in the cell. Moreover, other reactions that can achieve the same purpose with different speed constants occur in parallel. Some stress responses are triggered within a few minutes after the stress occurred, others reach their whole potential only hours later. Not all damaging effects on the cell can be repaired by stress proteins. In some cases, apoptosis or necrosis mark the end of the cell. In contrast to necrosis – the uncontrolled dissolution of cellular structures –, apoptosis is an organized "dissection" of structures where highly organized components can be reapplied at other sites. These two types of cell death can be clearly distinguished microscopically.

Molecular mechanisms of cellular temperature probes

In his lecture titled "Feeling the heat shock: Structural and functional investigation of GrpE, the nucleotide exchange factor of the DnaK chaperone sys-

tem“, John Grimshaw of the Biochemical Institute at the University of Zurich presented a reaction cycle of bacterial heat-shock response. This DnaK system in non-nucleic bacteria, the so-called prokaryotes, corresponds with the Hsp70 proteins in nucleic cells of higher organisms (eukaryotes). It is again the phosphorylation of the protein, i.e. the interaction with an energy-laden ATP, that leads to an activation here. This process in turn is catalyzed by a protein kinase called „GrpE“.
This working group now for the first time succeeded in clarifying the molecular structure of this GrpE molecule. It is a dimer, i.e. a double molecule, each containing a long spiralling alpha helix. This helix obviously is the thermometer of the cell. In a narrow temperature range above 40° C, this spiral reversibly reshapes, thereby steering the functionality of the whole molecule. Through this temperature sensitive adaptation of the DnaK cycle, the proteins repaired within the cycle are restrained until the surrounding temperature again has reached protein compatible values.

Thermolabile luciferase as a test system

“Proteins and membranes as sensitive thermometers: The role of heat-shock proteins in sensing and repairing stress damages in bacteria and plants“ – this was the title of the lecture of Pierre Goloubinoff from the University of Lausanne. It was a more methodologically oriented contribution on the topic of the workshop. The enzyme luciferase – known from the bacteria causing the glow of fireflies as symbionts – is temperature sensitive itself and served as an indicator of heat responses in the experiments of this group. These reactions were triggered by strong direct current pulses in moss. Thermally induced heat-shock proteins could protect luciferase to a certain extent from being damaged. According to Goloubinoff, different temperature dependent changes in the cell membrane are especially involved in heat detection.

Detailed comparison of literature

Martin L. Meltz from the Department of Radiation Oncology at the University of Texas, San Antonio, presented a differentiated inventory of already finished and published studies of heat-shock proteins, apoptosis, the activation of DNA repair mechanisms, or specific protein kinases as a consequence of influences from radiofrequency electromagnetic fields.
As the workshop was meant specifically to deal with stress proteins, Meltz separated studies dealing with the activation of heat-shock proteins from those investigating other stress-induced response paths. Five studies, including three in vivo and two in vitro studies, examined influences from radiofrequency electromagnetic fields on stress proteins: Cleary et al. (1997), Fritze et al. (1997), Daniells et al. (1998), De Pomerai et al. (2000), and Leszczynski et al. (2002). Only the investigations of De Pomerai and Leszczynski showed a clear activation of stress proteins at small field strengths. Meltz raised critical questions and objections regarding the experiments and result interpretations in these two studies.
Other stress-induced signal paths were examined by: Li et al. (1999), Walters et al. (1995), Natarajan et al. (2002), Goswami et al. (1999), and Nayak et al. (2004 a, b). The studies of Li and Walters found no field influence on the examined parameters. The conclusion of Goswami was that, though there was a specific response of cells to the applied electromagnetic signals, there was no general stress response. The results of Nayak from the first cited study showed no effects; the second study indicated influences on gene regulation.
In view of the heterogeneous results, Martin Meltz pointed out that a biological reaction, even if it reveals characteristics of a stress response, must not necessarily be one: “This is possible if a stress response occurs in a non-specific way, and is only one of several changes that occur.“

Narrow basis: Lack of dosimetry makes result assessment difficult

Sianette Kwee from the University of Aarhus, Denmark, presented results according to which heat-shock proteins might be activated by radiofrequency electromagnetic fields. Moreover, she pointed to own research done in 1999 where she found an induction of heat-shock proteins also due to extremely low 50 Hertz magnetic fields. In her 2001 studies, she exposed amnion cells to a 960 MHz field of the GSM standard (20 minutes, 2.1 mW/kg). Heat-shock proteins developed, and the cell cycle was affected. Asynchronously growing cultures being synchronized in turn led to increased proliferation.

However, result discussion ended when the referee was unable to provide exact details on her exposure system and the field properties of these experiments, as well as on the methods of dosimetry.

Field sensitive DNA component said to trigger gene activation

Reba Goodman – in cooperation with Martin Blank from the Columbia University, New York (Weisbrot et al. 2003) – described her results from experiments with radiofrequency electromagnetic fields performed in the fruitfly *drosophila melanogaster* (sponsored by the “Goodman Foundation“). The field was generated by a mobile phone with GSM signal kept in a petri dish that played music during exposure. According to Goodman, the maximum SAR was 1.4 W/kg, the surrounding temperature 25° C. 100 flies were exposed to the field over a period of ten days, two times per day; 80 flies served as a control. Measured were: the number of offspring, Hsp70 concentration inside the salivary cells, as well as two transcription factors that are relevant for the growth and the development of the animals. All four examined parameters showed signficantly ($p=0.01$) changed values. Goodman demanded that such stress responses be considered in the safety guidelines. Further, she addressed own previous studies, partially using low-frequency fields, where she was convinced to have found a specific field sensitive gene (Goodman and Blank 2002). If mutations were inserted into the field sensitive segment of the DNA, the sensitivity to electromagnetic fields was lost.

Exposure conditions selected by this group were several times criticized during discussion. Since the output power of the mobile phone is strongly influenced by the base station and the base station in turn adapts to alternating transmission and reception conditions, these experiments do not allow to draw conclusions on field distribution, SAR and other important dosimetric data. This negligent handling of dosimetry prompted one of the attendees to ask whether perhaps the music played could have triggered the described effects.

The Leszczynski scenarios – what are the consequences of protein phosporylations?

Dariusz Leszczynski of the Finnish Radiation Protection Authority (STUK) presented his to date published resp. prepared extensive results. He investigated the effects of radiofrequency electromagnetic fields on the protein pattern of endothelial cells, i.e. inner lining cells. These results have already been presented during the BEMS meeting 2003 in Maui and at the EBEA conference 2004 in Budapest. Therefore, the elaborate experimental strategies will not be described here. In cooperation with other institutes, the working group applied modern methods of protein analysis (proteomics, genomics) to quickly gain an overall picture of potential changes in several thousand genes and proteins.

According to Leszczynski’s present results, exposure changes the pattern of phosphorylation of several hundred proteins, also including Hsp27. The endothelial cells EA.hy926 applied by his working group were exposed to a GSM 900 or a GSM 1800 field.

The average SAR was 2.4 W/kg, the increase in temperature during exposure was 0.1 to 0.3° C. Experimental conditions and field distributions were explained in detail, as well as the approach used for protein analysis. According to the results of the working group, radiofrequency field exposure causes an increase in the concentration of phosporylated Hsp27, averaged across all field experiments, by 28 %.
Further analyses demonstrated that a whole series of proteins are affected, especially those determining the mechanical properties of the cell (cytoskeleton). Some of the examined genes were more strongly expressed after exposure, others less strongly, and the expression of a third group remained unchanged.
From his observations, Leszczynski developed far-reaching hypotheses on the consequences of these effects at the cellular level. He developed cause-effect scenarios speculating on possible consequences. However, in contrast to previous lectures, he did not derive health risks for organs or the whole organism from these hypotheses.

No gene activation by GSM fields found in vitro or in vivo

The results of these investigations performed by Leszczynski could not be confirmed by the studies of Florence Poulletier de Gannes of the University of Bordeaux, France. For their experiments, the researchers selected cells from tissues that are more strongly exposed to electromagnetic fields during mobile phoning: cells from the brain and the skin. Besides single cells, they also applied cell layers similar to artificially restored skin (epidermis) as a model of cell binding, as well as hairless rats as an animal model. Moreover, they replicated the studies of Dariusz Leszczynski with the same endothelial cell line – EA.hy926 – he had used.
The test cells were exposed with a SAR of 2 W/kg to a 900 MHz GSM field. During sham exposure, the antenna was switched off. The temperature difference between exposed cells and sham-exposed controls was maximally 0.1° C. Brain cells were exposed for either one hour or 24 hours. Skin cells were exposed to the electromagnetic field for 48 hours. For the positive control, the stress proteins were induced in the brain cells by a 20-min heat shock at 43° or 45° C, skin cells activated by UVB light. The qualitative and quantitative proof of the occurrence of the stress proteins Hsp27, Hsp70 and Hsc70 was provided by immuno-histochemical methods (ELISA).
Heat-shock proteins could not be induced in any of the tested brain cell lines by the electromagnetic field. The GSM field had no influence on the heat-shock proteins Hp27 and Hsp70 in different skin types, either. The protein Hsc70 was less strongly expressed in human binding-tissue cells (fibroblasts) at exposure than in controls. There were no effects triggered by the electromagnetic field on the examined proteins in the restored skin model.
The hairless rats were exposed both acutely and chronically; one side of the body was exposed, the other was used for controls (GSM 900, GSM 1800, 4 W/kg, 2 hours/day, 5 days/week, 12 weeks). These in vivo tests do not confirm the decreasing tendency in activation of Hsc70 proteins as was found in the in vitro tests. The experiments are continuing.
The replication of the Leszczynski study with the endothelial cell line EA.hy926 could not measure any effects. Neither the cells from Leszczynski's laboratory nor those from the laboratory of the University of North Carolina showed a significant activation of Hsp27. Only a non-significant increase in Hsp27 by 26 % was recorded after 1-hour exposure. Additional experiments with other positive controls will follow.

Investigation of growth, expression and phosphorylation

Two remarkably well documentated studies were presented by the Japanese scientist Junji Miyakoshi from the Hirosaki University (see Tian et al. 2002 and

Miyakoshi et al. 2000). Miyakoshi first reported on double-blinded tests on the expression of Hsp27 and Hsp70, as well as on growth of human brain tumor cultures (glioma cells) (MO54) at exposure to 1950 MHz fields (10 to 120 minutes, 1, 2 or 10 W/kg). Even at maximum exposure, the tests showed neither changes in growth rates nor in the distribution of Hsp27 or Hsp70. At field exposure of at least 1 hour, and at 10 W/kg however, the constitutive phosphorylation of Hsp27 on serine 78 decreased. Nonetheless, according to Miyakoshi, adverse effects on the cell culture are highly improbable; ultimately, no morphological changes of the cells could be found. Cells subjected to heat shock treatment of 43° C instead sprang into a distinctly rounder shape; in some of the cells, the shape of cell nuclei additionally changed. Miyakoshi's results quite strongly contradict the studies of Leszczynski emphasizing the results presented by Florence Poulletier.

The second study of Miyakoshi concerns the effect of strong 2.45 GHz fields on the expression of Hsp70 proteins in the same cells. In these tests, petri dishes with three concentric compartments were applied. Thus, SAR and temperature profiles could be determined for each of the three ring-shaped compartments. There was an average SAR between 1.3 and 100 W/kg. The temperature increased maximally by 5° C. The study measured cell death rate and the change in Hsp70 concentration.

Temperatures of more than 39° C applied over a period of 16 hours had no influence on the death rate of sham-exposed cells. In contrast, the survival rate in the cells exposed at 100 W/kg decreased by 30%. The longer the exposure, the stronger the expression of Hsp70. This effect only failed to show at a SAR of 5 W/kg. But also the cells cultivated at 39° C as a temperature control developed more Hsp70 with increasing time. However, cells exposed at a SAR of 50 W/kg expressed more Hsp70 than the temperature controls at 39° C. According to Hiyakoshi's results, MO54 cells increasingly express Hsp70 from a SAR of 20 W/kg onwards only due to field exposure, even if the influence of the temperature increase is considered. Such high SARs are not relevant in mobile phoning, though.

De Pomerai revises results

David de Pomerai has published results from tests performed with the worm *caenorhabditis elegans*, according to which radiofrequency electromagnetic fields elicit an activation of small heat-shock proteins (De Pomerai et al. 2000, 2003). This much examined threadworm expresses heat-shock proteins if it is exposed to temperatures above 25° C. Lower temperatures have no effect. In his publications, de Pomerai had shown that specific Hsp16 genes inducing heat-shock protein expression can be activated by continuous radiofrequency fields of 750 to 1000 MHz, at a mean SAR of 5 to 40 mW/kg. Exposure periods ranged from two to 24 hours. At that time, he suggested a non-thermal effect for gene activation. But the subsequent validation of the applied TEM cell showed that there was a power loss within the cell that can be responsible for local temperature rises. In the meantime, the TEM unit has been improved, and the temperature rise now has been diminished to below 0.1° C. De Pomerai repeated his tests and found out that his results were not reproducible. These experiments illustrate the need for exact calibration of field exposure systems; even slight temperature changes must always be considered.

The methods of genomics – fast and comprehensive genetic analysis

Christian Maercker of the German Resource Center for Genome Research, Heidelberg (Deutsches Ressourcenzentrum für Genomforschung), introduced an approach that is based on the use of extremely small plates for analysis, so-called microarrays. Automated methods allow to validate changes in the activity of

several thousand genes in a very short time. This biosystemic approach is similar to the method of proteomics used by Lesczcynski. However, the microarrays applied by Maercker do not analyze poteins but DNA segments, i.e. ultimately genes.
First, the RNA is isolated from the examined cells and translated into so-called complementary DNA (cDNA) by an appropriate enzyme. Fluorescent dyes are inserted into these cDNA – later on they will allow to find specific cDNA molecules on the analysis plate. 75,000 cDNA molecules with defined sequences are fixed to the microarray tray; they represent a large part of the known genes. The cDNA segments from sample and control can bind to these fixed molecules, as far as they have the matching complementary sequence. The fluorescent signal can be used to determine which of the cDNA molecules fixed to the tray have found their match. Since the cDNA molecules from the sample and the control are marked with different fluorescent dyes, it is rather easy to distinguish whether a fluorescent signal stems from a cDNA molecule from the sample, from the control or from both. There is either a pure colored or a blended color signal. This way, one can determine whether the expression of a gene from the sample is more or less pronounced, or whether it is unchanged. Maercker exposed HL-60 cells to a continuous 1800 MHz signal (1.3 W/kg, 24 hours). An influence on single genes and slightly increased ribosome synthesis could be found. Heat-shock gene expression remained unchanged by field exposure. However, Maercker indicated that the time period between field exposure and analysis was the same in all experiments. Thus, conclusions on potential delayed effects cannot be drawn.

No influence on Hsp27 phosphorylation

Mays Swicord of Motorola, USA, presented information on test series performed by Joseph L.R. Roti at Washington University. Roti wanted, at least in part, to reproduce the results of Dariusz Leszczynski. Instead of entholial cells, he applied HeLa-S3 cell cultures (breast cancer cells) and selected an electromagnetic field of the North American TDMA standard (800 MHz) with a SAR of 5 W/kg. Exposure was applied over two or 24 hours. The experimental goal, however, was identical: It was examined whether Hsp27 phosh porylation changes at exposure. As a positive control, cells were exposed at 41° C to a slight, or at 45° C to an intense heat shock in order to compare field efects with known stressors.
Roti's results did not confirm the studies of Leszczynski. The electromagnetic field did not change the phosphorylation of Hsp27. The heat-shock experiments showed that the degree of phosporylation changes proportionally with the incubation temperature of the heat shock. At 45° C, repeatedly phosporylated Hsp27 shapes could be detected; at 41° C, the concentration of phosporylated Hsp27 was distinctly higher than in controls and in the exposed samples.

The dangers of SAR analysis

In their observations, Kari Jokela and Jürgen Schuderer dealt with exposure systems. They showed how relevant parameters can be determined and evaluated. At the same time, they demonstrated how the selection of exposure setups alone may affect the result, and emphasized the relevance of exact dosimetry for the quality of measurement results. If, for example, SAR and temperature are not accurately determined, or if numerical analyses of the SARs prior to experiments are left out, this may considerably affect the overall conclusiveness of later measurements. If conclusive results are to be achieved and meaningless data to be avoided, diligent dosimetry is indispensable.
Kari Jokela dealt with the development of an exposure chamber for in vitro tests at exposure to 900 MHz fields. Jokela, who works with the Finnish Radiation Protection Authority STUK, searches for ways to

detect SARs and temperatures during exposure as near to the cells as possible. He proposed minimal standards that are to be met by modern exposure units.

Moreover, Jokela introduced horizontal and vertical exposure setups developed at the STUK that may be applied for exposure of petri dishes. His data and diagrams from the tests with horizontal exposure impressively illustrated e.g. the strong deviations of the SAR across a petri dish over a radial distance of only 25 mm. At one specific point inside the petri dish, the SARs are considerably larger: on the surface of the medium, exactly at the border between medium and vessel wall, i.e. at the so-called meniscus. When the SAR in the middle of the culture dish was 1 W/kg, at the above described point at the meniscus measurements showed a value of 73 W/kg. At vertical exposure, this meniscus effect is no problem.

Kari Jokela summarizes three conclusions from his test results:

- it is very difficult to exactly determine the SAR in in vitro tests
- the SAR has to be determined by means of other methods
- the problem of heating must not be neglected in in vitro tests with a SAR larger than 1 W/kg.

Purposeful selection of exposure setup improves quality of results

In his lecture, Jürgen Schuderer from the Foundation for the Research on Information Technologies, Zurich, Switzerland (Stiftung für die Erforschung von Informationstechnologien), spoke about the technical aspects of in vitro exposure of cells to 900 and 1800 MHz fields. Not every exposure system will be appropriate to examine all kinds of cell cultures, as cell monolayers e.g. have other properties than cell suspensions, and these differences considerably affect SAR distribution and temperature rises. The aim of his study was to describe and evaluate exposure setups that would expose cells to electromagnetic fields according to standardized and exactly defined conditions. He focused on technological requirements that have to be met by exposure systems, depicted the conditions for a reasonable dosimetry, and compared the performance of different systems. Based on his data, Schuderer developed recommendations for the exposure of monolayer cultures and cell suspensions. Schuderer underlined the minimum requirements suggested by Kari Jokela as part of the experimental framework conditions, but additionally demanded to maintain a maximum temperature rise of 0.1° C at a SAR of 2 W/kg. Moreover, signal transmitters would have to provide a variety of possibilities. Besides continuous fields with freely selectable frequencies, modulation should be possible, as well as signal patterns according to GSM, TDMA and other standards, like e.g. GPRS and DECT. All signal components should simulate the normal situation and should be exactly detectable and evaluable.

For a reliable control of the experimental exposure to be feasable, the environmental parameters of the control unit have to absolutely identical with those of the test setup. These potential influential factors, as well as other biological and technical parameters, have to be measurable as exactly as possible. External field influences have to be excluded, and the setup has to allow a double-blinded aproach.

Schuderer proposed very strict requirements also for dosimetry. SARs have to be determined and validated both numerically and metrologically. SAR deviations, SAR distribution and temperature changes during exposure have to be documentated.

Further, he dealt with coupling mechanisms. Based on several parameters affecting field absorption, he clarified that biological coupling processes are possible in cell cultures which are different from those occurring in the whole organism. By example of the fluid meniscus that develops at the wall of the petri dish due to adhesion, he showed how strongly SARs

deviate, dependent on each other, and dependent on the fact whether the meniscus is included in the calculations or not. If the meniscus is not considered, the calculated SAR is too small.
Schuderer examined the following three exposure designs comparing their adequacy for the exposure of cell monolayers and cell suspensions: the waveguide systems sXc900 and sXc1800, a TEM unit where the petri dishes were H-polarized, and a wire patch system. According to his results, the waveguide systems are best for the exposure of monolayers. The petri dishes, however, must be arrayed in the maximum radiofrequency field; only then, the heterogeneity of the SAR distribution is below 30 % and it meets the requirements. The temperature rise in these systems with 0.03° C is the smallest, and the SAR is the most efficient with 50 W/kg input power.

None of the examined exposure designs met the minimum requirements regarding SAR distribution for cell suspensions. The best result was a value of 46 % achieved in a TEM cell. The temperature rise was 0.05° C per W/kg. The TEM cell provided reliable results at the exposure of cell suspensions when petri dishes were arrayed at the level of K-polarization. SAR gradients occurring during cell suspension exposure do not lead to local temperature peaks, since the heat is sufficiently dispersed by the medium.

Conclusion: Dosimetry is the key

The workshop emphasized existing doubts concerning the assumption that weak radiofrequency electromagnetic fields may lead to the development of heat-shock proteins by non-thermal influences. Well-documentated test series, like those of Miyakoshi, provide no evidence of interactions between mobile radio electromagnetic fields and heat-shock proteins or their genes. Studies where such interactions were observed, could not yet be reproduced or showed considerable flaws regarding dosimetry or exposure that were duely criticized by the attendees. An exact dosimetry is indispensable. Analyzing heat-hock proteins is a balancing act. As experimental target molecules are temperature sensitive, thermal effects have to be excluded with certainty in tests.
In the studies of Kwee and Goodman, besides other experimental flaws, temperature influences that are due to flawed exposure setups and insufficient dosimetry, cannot be excluded. The results of Lesczcynski could not be reproduced by independent researchers, either in the same cell line or in other in vitro models. So there is still much to do regarding verification.
The presented tests on heat-shock proteins basically go in two directions: on one side, gene activation is examined, on the other, the focus is on phosporylation of existing proteins. Phosphorylations are signals that can initiate subsequent processes and have their own dynamics. This was shown by the presentations of Dariusz Leszczynski and Matthias Gaestel. Which cascade is activated and how, the side paths that are concerned, and the consequences for cells or organisms – all this is difficult to determine at the moment. The time constants of these processes can be very different.
The technically orientated presentations made clear that parameters like temperature and SAR are difficult to control in the exposed samples within an exposure systems. This makes working with temperature sensitive, physiologically effective molecules like stress proteins complicated. David de Pomerai e.g. could not prove his concept of a non-thermal effect in the threadworm *caenorhabditis* caused by the influence of weak fields, because temperature deviated more strongly in his exposure system than he expected. So especially dosimetry has to be carefully performed and documentated, for there are many sources of errors. The studies of Kari Jokela and Jürgen Schuderer provide important suggestions how to improve quality.

Presentations and subsequent discussions led to the developing of recommendations. There was agreement on the fact that further studies, both in vitro and in vivo, are necessary; results of new studies should be validated as early as possible. In particular, the replication of the tests of Kwee, Goodman and Leszczynski were demanded. The modern systembiologic genomic and proteomics techniques already applied by Maercker and Leszczynski in their research should be used more often in future. However, the biological and methodological significance of results must be reliably assessable. For the investigation and evaluation of microthermal effects, new ways have to be found. These difficult to determine influences can seriously disturb in vitro and in vivo experiments alike and therefore provoke misinterpretations.

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