**Mag.Dr. Reinhold Hofbauer, Univ.Ass.Prof.**

**Center of Med. Biochemistry, Div.of. Mol. Genetics**

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**Consequences of carnitine deficiency and CSF-1 inhibition**

Born November 14th 1956

Diploma thesis (1980-1981) Mag. rer. nat.

Inst. f. Allgemeine Biochemie, Universität Wien

Ph.D. thesis (1982-1985) Dr. rer. nat.

Inst. f. Molekularbiologie, Universität Wien

Postdoc (1987-1988) at the Cancer Research Lab. with

Prof. Dr. D.T. Denhardt, University of Western

Ontario, London, Canada.

Since Dec 1981 member of the Inst. f. Molekularbiologie

Universität Wien, Universitätsassistent (1983-1996)

Tenure position as Univ.Ass.Prof. since 1997

Since the foundation of the Med. Univ. of Vienna the

Dept. of Med. Biochemistry was created by the fusion

of several Insitutes at the Max F. Perutz Laboratories

including the Inst. f. Molekularbiologie

Group leader at the Div. of Mol. Genetics, Dept. of Med. Biochemistry,

MFPL, Med. Univ. Vienna

**CV**

I have studied biochemistry at the University of Vienna (diploma thesis with Prof. Ruis: establishment of an in vitro protein translation system in yeast, PhD thesis with Prof. Wintersberger: molecular analysis of gene amplification in mouse fibroblasts). Due to Prof. Wintersberger´s influence I got attracted to the G1/S phase specific genes by the molecular analysis of the mouse cytoplasmatic thymidine kinase (Tk1) cDNA and genomic clones (cloning of the cDNA and a mouse genomic pseudogene). My postdoc time was dedicated to the establishment of a differential screening approach to detect low abundant genes induced in G1 and S phase of the mammalian cell cycle. I spent a postdoctoral research time with Dr. David T. Denhardt (Cancer Research Laboratory, University of Western Ontario, London, Canada). This resulted in the generation and partial analysis of cDNA clone collections of growth and cell cycle regulated genes. Since 1982 I am a member of the Dept. of Med. Biochemistry, Div. of Mol. Genetics at the Med. University of Vienna (former Inst. of Molekularbiologie of the University of Vienna) as Ass.Prof. giving lectures and laboratory courses for chemistry, biochemistry, molecular genetics and cell biology being mandatory for students of medicine and natural sciences. In addition in our laboratory permanently students for molecular biology and genetics were attending advanced courses in molecular biology and finally were working on their diploma and/or Ph.D. thesises. In this context I have supervised numerous of students at all levels. Currently four diploma students are members of my group and financially supported by funds originating from my research projects.

Member of different scientific societies:

Österreichische Ges. f. Molekulare Biowissenschaften und Biotechnologie

European Society for the Study of Purine & Pyrimidine

Metabolism in Man (ESSPPMM)

**Research Interests - Current projects**

After my PhD thesis I started my research interests on the characterization of genes induced prior to DNA replication at the G1/S boundary of the growth and/or real cell cycle of mammalian cells. In addition to this project of biomedical relevance a new approach was developed by me to uncover new low abundant G1-S-phase specific genes expressed after growth induction. During my postdoc, I spent with Prof. D.T. Denhardt (now Rutgers University, N.J. USA), I established a very sensitive method for differential screening technique to identify gene functions induced between two states of growth (reverse strand labeling method). I am analyzing novel low abundant genes induced in G1 and at the G1/S boundary. From collections containing hundreds of clones I selected four, which were studied in more detail. The first clone was a real cell cycle regulated "transferase", which turned out to be the murine carnitine acetyl transferase. This gene was the starting point for me to investigate all normal and pathophysiological conditions where low carnitine levels have severe side effects and analyze the exertions on the expression of the genome. The second differentially regulated clone is homologous to the 3´terminal part of the cyclooxygenase 2 (COX2), an early G1-growth inducible RNA, which has the potential to act as "transcription/replication factor". We generated convincing evidence that this mRNA is imported into the mitochondria and directly involved in initiation of the mitochondrial replication machinery. A third differentially regulated cDNA clone (4-2) is a novel butyrate inducible arrestin E homologous gene function known also as Tmem66, which is strictly regulated by growth and butyrate induction. The recombinant expressed protein is very toxic for host cells (bacteria as well as eucaryotic cells) and very likely is involved in signal transduction. This gene function is one the cytotoxic genes which I am currently focusing my research interests. Concomitant to these studies I gathered lots of knowledge about cell biological tasks in mammalian cell culture (elutriation, cell synchronization, cell cycle arrest, mitogenic stimulus, serum induction).

In the second part of my research area I focused on the molecular analysis of both eucaryotic thymidine kinases. Both human variants of this enzyme are investigated, whether altered structurally or influenced at expression level in specific tumors (model systems leukemia (APL, AMMOL). Major efforts were invested in the molecular cloning of the mitochondrial thymidine kinase (Tk2). During these studies we have developed a novel single primer based RACE approach to generate and clone structurally blocked 5´termini of cDNA clones. Regarding the TK research my group was part of a European wide cooperation, where we are responsible for the molecular biology of the proposed projects. Finally it was our goal to clarify together with Prof.Dr. Gerd Folkers (Collegium Helveticum, ETH Zürich) the structure and function relationship of wild type and mutant TK isoforms, to develop specific inhibitors for an antitumor therapy. The rationale behind this strategy was comparable to the treatment of viral infections caused by Herpes Simplex Virus by the drug acyclovir. We developed human thymidine kinase 1 mutants with extremely high and low specific activity (super versus feeble TK1) by introducing site-specific mutations. They helped us to discover the dimerization region of the human TK1. This and other TK1 mutants will find their use as key players in gene therapy vector systems.

My group could reveal that L-carnitine has the capacity to act as a nutrigenomical metabolite upon gene expression. The clinical condition of carnitine deficiency itself defines a very critical condition, we therefore use it in a well-defined cell culture model system to investigate the effects of carnitine supplementation in human liver, fibroblast, endothelial, nerve and muscle cells. By chip screen and promoter studies we established a solid basis to study changes on mRNA expression levels and promoter factors. We identified genes being directly involved in the transcriptional regulation of the “L-carnitine effect”, thus being able to approach clinical pathologies of hyperlipidemia, insulin resistance and type 2 diabetes mellitus. We want to reveal “candidate or susceptibility” genes, which are associated with these diseases and have an increased sensitivity to diet. The results of this research will provide better insight in metabolic aspects of pathologies and their regulation as well as mitochondrial function. In addition to the genomic approach we analyzed promoter specific factors directly induced by carnitine by site-specific sequence studies and band shift assays. We were able to reveal several promoter active factors that are influenced by carnitine and are key players in the regulation of fatty acid and glucose metabolism.

In a separate effort we are tracing the effects associated with inhibition of CSF-1. It primarily acts on cells of the mononuclear phagocyte lineage by controlling the differentiation, proliferation and survival of precursor cells as well as the activation of mature macrophages. CSF-1 also has a pivotal role in the pathogenesis of several disorders including cancers, because it regulates the production genes that involved in tissue remodeling and tumor invasion. We did investigate whether inhibition of CSF-1 expression can serve as a valuable tool to fight tumor growth and metastasis. Microarray analyses have revealed very promising candidate genes that are re- or induced during CSF-1 inhibition. Their inhibition should enhance the inhibitory effect of CSF-1 specific antibodies or specific RNAi. Preclinical animal studies with monoclonal antibodies/RNAis are the next experimental aims.

Recently we characterized potentially cytotoxic genes (*e.g.*Tmem66, superactive thymidine kinase 1), that were identified or generated in my group in previous research projects. These genes were cloned into an inducible vector system (pUHD-Hygr) and then transfected into model tumor cells (MCF-7 pretransfected with a puromycin resistance gene carrying a silencer construct). The final aim is to develop a genetic approach to attack tumor cells in mammalian organisms. In parallel we use an adenoviral associated virus (AAV) based expression system that allows us to infect any type of cancer cells. This will be the system of choice for the treatment of xentransplants of human cancer cells in SCID mice.