PREFACE

In this book the authors present further research on the study of cytochrome C as well as cytochrome B. Some of the topics discussed in the book include the regulation of cytochrome C in respiration as well as its role in apoptosis. It also focuses on the structural aspects and touches base on cytochrome B5 as a pleiotropic metabolic modulator.

Chapter 1 - Despite the fact that over 200 phosphorylation sites have been mapped on the mitochondrial oxidative phosphorylation (OxPhos) complexes, very little is known about the relevant cell signaling pathways and the terminal kinases and phosphatases that control these phosphorylations. Within OxPhos, cytochrome *c* (Cyt*c*) plays a special role because it is not only involved in electron transport but is also a key executer of apoptosis when it is released from the mitochondria. It is therefore not surprising that Cyt*c* is regulated by phosphorylation. Four phosphorylation sites have been mapped on mammalian Cyt*c*, two of which have been studied functionally, demonstrating that both respiration and apoptosis are under the control of signaling pathways that have yet to be identified. A review begins here of the regulation and multiple functions of mammalian Cyt*c*, including respiration, reactive oxygen species (ROS) scavenging under healthy conditions, ROS production via $p66^{Shc}$, and cardiolipin oxidation during apoptosis. Then, targeting Cyt*c* by manipulation of signaling cascades as a therapeutic avenue in conditions including neurodegeneration and cancer.

Chapter 2 – Cytochrome b_5 (Cb_5) acts within the cells as a pleiotropic co-factor of multiple enzymes and redox chains that play critical roles for normal function of healthy mammalian organisms. It is maintained in the reduced form largely by the NADH-dependent cytochrome b_5 reductase activity which catalyzes electron transfer to Cb_5 in mammalian cells. The enzyme system

cytochrome b_5 reductase/cytochrome b_5 has been shown to be associated with the endoplasmic reticulum, mitochondria and plasma membrane of mammalian cells, where it is involved in multiple metabolic pathways, such as cholesterol biosynthesis, desaturation and elongation of fatty acids, methemoglobin reduction and steroids and xenobiotics metabolism catalyzed by cytochrome P450s. More recently, this system has been shown to be a component of the 'so-called' redox-chain of the plasma membrane present in mammalian cells, where it clusters within cholesterol-rich and caveolins-rich lipid rafts-associated sub-microdomains of the plasma membrane. The cytochrome b_5 reductase/cytochrome b_5 is also a relevant system in the redox biology of cell death, as it forms a stable complex with cytochrome *c*, a pro-apoptotic signalling molecules, and it is involved in the modulation of superoxide generation in mammalian cells, playing a major role in the NADH-dependent superoxide anion production of the neuronal plasma membrane accounting for most of the superoxide anion overshot that is observed in an early stage of the neuronal apoptosis. In addition, an expanding family of proteins containing a Cb_5-like domain is also present in mammalian cells. Both, the novel cellular functions described for Cb_5 and for novel proteins containing a Cb_5-like domain point out that Cb_5 can be seen as an intracellular signaling molecule in redox biology and as a potential cellular biomarker of health and disease.

Chapter 3 – Cytochrome *c* (cyt *c*) functions as a mobile electron carrier in the respiratory chain anchored to the external side of the inner mitochondrial membrane and shuttling electrons from cyt *c* reductase to cyt *c* oxidase. A further function of cyt *c*, beyond respiration, is realized outside mitochondria in the apoptotic program. Cyt *c* may respond to different environments by changing its fold, thus favouring the exertion of different biological functions in different pathophysiological cell conditions. The binding of lipids (free fatty acids as well as acidic phospholipids) to cyt *c* induces conformational changes and partial unfolding of the protein, strongly influencing cyt *c* oxidase/peroxidase activity. In the early events of apoptosis, the interaction of cyt *c* with a mitochondrion-specific phospholipid, cardiolipin (CL), brings about a conformational transition of the protein and acquirement of peroxidase activity. Transitions among different conformations are regulated by endogenous molecules such as ATP. Apoptosis is strictly connected to the pathogenesis of many human diseases, including neoplastic, neuro-degenerative and cardiovascular diseases, and derangements of cardiolipin biosynthesis and remodeling are crucial in regulation of apoptosis. So as the role of cyt *c* in respiration and apoptosis rely on its interaction with CL, the

present book chapter reviews the structural features of this complex. Then a description of how perturbations in CL amount, acyl composition and CL-driven allosteric modulation of the cyt *c* properties, may contribute to cell fate.

Chapter 4 – For many decades mitochondrial cytochrome *c* was assumed to have a single biological role, namely the transfer of electrons from Q-cytochrome *c* oxido-reductase (cytochrome bc_1) to cytochrome *c* oxidase in the electron transfer chain responsible for the production of the mitochondrial proton motive gradient. Chapter 4 explores the growing range of functions which have been identified for cytochrome *c*. For example, cytochrome *c* has been shown to interact with specific lipid molecules which induce peroxidase enzymic activity in the protein. More recently cytochrome *c* has been shown to have a major role in apoptosis, following its stimulated release from mitochondria into the cytosol, where it provides a 'feed forward' amplification cascade. Evidence is also now emerging that the pro-apoptotic action of cytosolic cytochrome *c*, at least in some cells, can be further modulated by its reactions with other heme proteins.

Chapter 5 – Novel redox-active metallotherapeutic agents were used to develop effective antimicrobial remedies differing from standard antibiotics in their mechanism of action with participation of free-radical intermediates. For this purpose redox-active compounds carrying sterically hindered 1,2-dihydroxybenzene moieties along with metal ions in a single scaffold have been synthesized and characterized by means of chemical and physic chemical methods as well as screened for their antimicrobial activity against Gram-negative bacteria (*Escherichia coli, Pseudomonas aeruginosa, Serratia marcescens*), Gram-positive bacteria (*Bacillus subtilis, Sarcina lutea, Staphylococcus aureus, Mycobacterium smegmatis*), yeasts (*Cryptococcus laurentii, Lipomyces lipofer, Candida albicans, Candida boidinii, Candida utilis, Saccharomyces cerevisiae*) and fungi (*Aspergillus niger, Alternaria alternata, Mucor spp., Botrytis cinerea, Monilia spp, Fusarium spp., Penicillium lividum, Sclerotinia sclerotiorum*). This screening revealed silver(I) complexes that may be considered as potential chemotherapeutic agents with activity higher than or comparable to that of some standard drugs such as tetracycline, streptomycin, chloramphenicol, isoniazid, amphotericin B, fluconazole, terbinafine. Using the method of cyclic voltammetry, it shows some phenolic ligands as well as their silver (I) complexes to be also of a pronounced reducing ability. Bacterial cytochrome *c*-like enzymes being among the first targets for redox-active antimicrobials on their way into the cell, spectrophotometric investigation was carried out in order to estimate the rate of bovine heart cytochrome *c* reduction with the compounds synthesized.

Oxidation of 1, 2-dihydroxybenzene derivatives and their silver (I) complexes *in vitro* under anaerobic conditions can include two successive one-electron steps of oxidation of their ionic forms to yield *o*-benzoquinones on interaction with cytochrome *c* *via* intermediate *o*-benzosemiquinone formation. The results obtained are discussed in view of presumed correlation between the capability of the compounds synthesized for reducing cytochrome *c*, antimicrobial activity, and physico-chemical characteristics (redox properties determined electrochemically, lipophilicity, ionization constants of the ligands and stability constants of the metal complexes).

Chapter 6 – Cytochrome *c* (cyt *c*) is a mitochondrial membrane hemoprotein of high physiological importance. First, cyt *c* is one of the key elements of respiration chain transferring electrons from cyt *c* reductase (*bc*1 complex) to cyt *c* oxidase. Second, release of cyt c from the intermembrane space of mitochondria into the cytosol triggers the apoptotic pathway. The idea that specific interactions between cyt *c* and cardiolipin (CL), the main lipid component of mitochondrial membrane, are crucial to the protein biological activities, constantly receives further corroboration from both theoretical and experimental studies. Despite considerable progress achieved in the field of cyt *c* – CL biophysics, the detailed structural description of protein-lipid complexation is still lacking. The present study applies Förster resonance energy transfer (RET) technique to give comprehensive characterization of cyt *c* binding to the model lipid membranes composed of the mixtures of zwitterionic lipid phosphatidylcholine (PC) with anionic lipids phosphatidylglycerol (PG), phosphatidylserine (PS) or cardiolipin (CL) in different molar ratios. The donor-acceptor pairs were represented by either anthrylvinyl-labeled PC (AV-PC) or anthrylvinyl-labeled CL (AV-CL) incorporated in trace amounts in lipid vesicles, and heme moiety of cyt *c*. Association of the protein with the lipid bilayers led to the decrease in donor fluorescence reflecting energy transfer from AV fluorophore to heme. The most effective RET was found for CL-containing membranes. This observation has been interpreted in terms of higher affinity of cyt *c* to CL as compared to other anionic lipids. In order to get understanding of protein specificity to CL, RET was measured as a function of CL content and ionic strength. Monte Carlo analysis of multiple datasets revealed a complex interplay between several processes, namely i) lipid demixing; ii) CL transition into extended conformation; iii) formation of hexagonal phase. The switch between these states was found to be controlled by CL content and salt concentration. These characteristics of cyt *c* – CL interaction are of great interest not only in the

context of regulating cyt *c* electron transfer and apoptotic propensities, but also from the viewpoint of the protein biogenesis.

Chapter 7 – The initial stage of a number of neurodegenerative diseases, including Alzheimer's disease (AD), is characterized by oxido-reductive imbalance leading towards free radicals production as well as towards the impaired respiration in mitochondria. Both the decreased activity of cytochrome *c* oxidase (COX), i.e. the Complex IV of the respiratory chain, and the altered cholinergic transmission are found in AD patients brain.

Aluminium is a metal whose role in the etiology and/or pathogenesis of AD could not be rejected. It has multiple effects, such as cellular respiration damage and oxidative stress induction. In the experimental model the study, uses aluminium chloride was applied intrahippocampally (i.h.) to the adult male Wistar rats. That was followed by the decreased activity of three enzymes in the brain regions that are most affected in AD, such as the forebrain cortex, hippocampus and basal forebrain. The activity of glucose-6-phosphate dehydrogenase (G6PDH) was more than halved, while the activity of COX and acetylcholinesterase (AChE) was almost exhausted. All this was accompanied by deteriorated learning and memory established by a two-way active avoidance test.

The intrahippocampal application of G6PDH, the enzyme of the pentose monophosphate pathway, just before aluminium, reverted the activity of COX to the control values. The rats were also i.h. pretreateated with fresh prepared green tea leaf extract, which primarily has strong antioxidant effects. Such pretreatment resulted in statistically significant improvement of the activity of COX and AChE in comparison with the aluminium treated rats, although these values did not achieve the levels of controls. Besides that, green tea leaf extract reverted the decreased learning and memory to control values, as well.

Conclusion: Neurotoxicity of aluminium was demonstrated through the decreased activity of G6PDH, COX and AChE, that was clinically expressed as impaired learning and memory. G6PDH and green tea leaf extract showed protective effects against this toxicity.