PREFACE

In cell biology, a mitochondrion is a membrane-enclosed organelle found in most eukaryotic cells. Mitochondria are often described as "cellular power plants" because they generate most of the cell's supply of adenosine triphosphate (ATP), used as a source of the chemical energy. In addition to supplying cellular energy, mitochondria are involved in a range of other processes, such as signaling, cellular differentiation, cell death, as well as the control of the cell cycle and cell growth. This book reviews research on the mitochondrial metabolism in age-related neurodegenerative disorders such as Alzheimer's and Parkinson's disease; mitochondria and aging; apoptosis and peritoneal injury; diabetes, mitochondria and brain endothelium dysfunction as a dangerous triad for neurodegeneration.

Chapter I – Around 2 billion years ago, an alliance between the then ancestral cell of all eukaryotes and an ancestor of today's purple bacteria resulted in a proto-eukaryotic cell, with the new endo-symbiotic bacteria becoming the mitochondria. In years that passed by, mitochondria gradually assumed the central respiratory role playing one of the pivotal regulators in maintaining cellular homeostasis. The complex role of mitochondria in mammalian cell apoptosis came into focus when biochemical studies identified several mitochondrial proteins that are able to activate cellular apoptotic programs directly. Apoptosis or targeted cell destruction is critical both in physiological contexts as well as pathological states. Signals converge on mitochondria to induce mitochondrial membrane permeabilization (MMP). A number of agents such as the Bcl-2 family members (both pro- and anti-apoptotic), calcium and reactive oxygen species can all affect mitochondrial membrane depolarization. Anti-apoptotic Bcl-2 family members (e.g. Bcl-2, Bcl-XL) are normally localized on the mitochondrial membranes while the pro-apoptotic factors such as Bax, Bid, Bad, and Bim translocate to mitochondria in response to apoptotic signals. Bid is cleaved by caspase-8, generating an active truncated-Bid that aids the oligomerization and insertion of Bax into the mitochondrial membrane. Bad is sequestered in the cytoplasm by phosphorylation and is associated with 14-3-3 scaffold protein where its dephosphorylation [i.e. by calcineurin (Cn)] promotes translocation to mitochondria. Bim is localized to microtubules via association with the LC8 dynein light chain, and also translocates to mitochondria following apoptotic stimulus. Several other intracellular signals, including DNA damage and endoplasmic reticulum (ER) stress, also converge on mitochondria to induce MMP, which causes the release of pro-apoptotic factors from the intermembrane space (IMS). Among these, cytochrome *c* (Cyt *c*) induces the apoptosis protease-activating factor 1 (APAF-1) and ATP/dATP to assemble the apoptosome, a molecular platform which promotes the proteolytic

maturation of the initiator-caspase-9. Active caspase-9, in turn, cleaves and activates the effector caspases, which finally lead to apoptosis. DNA damage may also signal through the activation of caspase-2, which acts upstream mitochondria to favor MMP. Further, p53, the "guardian of the genome" may favor MMP by direct interactions with Bak, Bax, Bcl-2, and Bcl-X_L at the outer mitochondrial membrane (OM). With the gaining importance of MMP, an increasing number of experimental anticancer drugs, including lonidamine, arsenite, betulinic acid, CD437, and several amphipathic peptides, are thus now being targeted towards it. Moreover in the author lab, work with the anti-neoplastic, immunotherapeutic and apoptotic glycopeptide T11TS also showed MMP during glioma apoptosis. Bid and p53 acted in synergy to activate Bax thereby bringing about T11TS mediated glioma apoptosis via the release of mitochondrial cytochrome c and subsequent caspase activation. Thus the design of mitochondrion-targeted cytotoxic drugs may constitute a novel strategy for overcoming apoptosis resistance where conventional drugs fail to do so.

Chapter II – Apoptosis is a form of programmed cell death (PCD) important for development and homeostasis and its deregulation has implicated as the cause of many diseases, such as cancer or neurodegenerative diseases. Apoptosis may be initiated by a developmental program and by many diverse forms of cell stress. A critical feature of metazoan apoptosis is permeabilization of mitochondrial outer membrane (MOM) that leads to the egress of apoptogenic components from intermembrane space of mitochondria that activates caspases responsible for cell death. The Bcl-2 family of proteins plays a major role in the control of apoptosis as the primary regulators of MOM permeability. The pro-apoptotic Bcl-2 homologues Bax and Bak are activated following the induction of apoptosis and induce MOM permeabilization (MOMP).A second class of Bcl-2 homologues, the BH3-only proteins, is required for activation of Bax and Bak. The activity of these two classes of pro-apoptotic proteins is opposed by anti-apoptotic Bcl-2 family members such as Bcl-2, Bcl-xL, Bcl-W, Bfl-1 (A1), Mcl-1 and Bcl-B. The ratio between pro-survival and pro-apoptotic Bcl-2 family members often dictates the cell fate following apoptotic stimulus. In cancer cells, this balance is often altered thus providing a means for cancer cells to evade apoptosis. Many tumors have been shown to overexpress pro-survival Bcl-2 family members. Strategies that target the pro-survival proteins hold significant therapeutic promise in oncology and several small-molecule inhibitors of antiapoptotic proteins have been identified that induce apoptosis in a variety of tumor cells. However the mechanisms of both of antiapoptotic action of pro-survival proteins and of the inhibition of the antiapoptotic proteins by small-molecule compounds, some of which have advanced into clinical evaluation, are not yet completely understood. In this review, author attempt to summarize the current view of the mechanisms and problems of these two closely related phenomena and outline the areas that are currently under study.

Chapter III – Over the next decades the increase in the elderly population will unavoidably lead to a steadily increasing number of victims of neurodegenerative diseases associated with aging. Due to their chronic and progressive nature and the fact that currently there are no available treatments to slow or stop the disease progression, the disclosure of the causes of idiopathic Alzheimer's and Parkinson's disorders are undeniably needed in order to propose attractive targets for drug therapies and alternative treatments.

Mitochondrial dysfunction has been shown to be involved in several neurodegenerative diseases. A complex interplay occurs between mitochondria and other cellular machinery that may affect cell survival, since mitochondria play a significant role in electron transport plus

oxidative phosphorylation, and also regulate cell death, marking the point of no return in necrosis and apoptosis. This chapter discusses the mitochondria in all its complexity, starting with mitochondrial metabolism in the brain, mitochondrial dynamics and biogenesis, and finally mitochondrial degradation. In addition author resume some of the findings that provide evidence for the role of mitochondria in neurodegeneration associated with Alzheimer's and Parkinson's diseases, two of the most prevalent neurodegenerative diseases.

Chapter IV - Aside from the well known function of mitochondria in ATP production and cell apoptosis, accumulating evidence implicates mitochondrial functions in a variety of cell fates. In a multicellular organism all types of cells may contain identical nuclear genomes, but some cells will lose functions, some will be eliminated, some will develop into ever-living germ cells, some will remain in a pluripotent state for a prolonged time, and still others may transform into abnormally proliferating cancer cells. In all cases, mitochondria play multifunctional roles in which adenosine triphosphate (ATP) supply presents only one of many different functional components. Physiological regulation or pathological disruption of any anabolic or catabolic pathways in mitochondria, and mutations in mitochondrial DNA (mtDNA) or in nuclear genes that encode mitochondrial proteins can all affect the balance of pro-apoptotic and anti-apoptotic factors localized to mitochondria or other intermediate products of the mitochondria, and then participate in cell fate decisions. Exposing the cell to physical or chemical distresses, mitochondria are typically the most sensitive cellular components that display abnormal morphological and biochemical changes. The responses of mitochondria to distresses are initially intended to rescue the cell from damages. If the distresses exceed mitochondrial capacity in rescuing the cell, mitochondria will direct cellular suicide. In the present review author will summarize the role of mitochondria in germ cell specification and apoptosis during animal gametogenesis, gamete fate and competence, stem cell differentiation, carcinogenesis and other diseases.

Chapter V – Mitochondria are dynamic, semi-autonomous organelles surrounded by a double membrane with their own genome and protein synthesis machinery. They are motile and undergo frequent changes in number and morphology through fusion and fission events. In addition to being a major source of ATP in eukaryotes, they are the site of many important metabolic reactions such as the urea cycle, lipid metabolism, steroid hormone and porphyrin synthesis and inter-conversion of amino acids. Moreover, mitochondria play a central role in complex physiological processes including cellular proliferation, differentiation, reactive oxygen species (ROS) homeostasis and apoptosis. Therefore, mitochondrial dysfunction plays a central role in the pathogenesis of a wide range of diseases including degenerative diseases, cancer and aging that involve disorder in cellular fuel metabolism and survival / death pathways,. The purpose of this review is to present the current state of the author knowledge and understanding of vital roles of mitochondria in relation to cellular survival and death, mitochondrial diseases and therapeutic application.

Chapter VI – Ageing is a fascinating topic that engaged philosophers and scientists for centuries. Nevertheless, it is very difficult to define precisely the term "ageing". There is no simple definition, which would be universally accepted. Ageing is inevitable, a complex biological process which can be characterized as a progressive deterioration of physiological functions and metabolic processes after the reproductive phase of life. In the process of ageing is the gradual accumulation of changes with time, which are connected or responsible for the ever-increasing susceptibility to disease and death with increasing age. One of theory which tries to explain senescence is free radical theory of ageing or oxidative damage theory

of ageing. There is an increase level of reactive oxygen species and sequential accumulation of oxidative damage of biomolecules during ageing. This damage may by the most important contribution to ageing and ageing-related diseases. All organisms live in an environment containing reactive oxygen species and mitochondrial respiratory chain which generate reactive species. Mitochondrial theory of ageing, a hypothesis of free radical theory of ageing, suggests that accumulation of mitochondrial oxidative damage leads to human and animal ageing because mitochondria are the main source and target of reactive oxygen species. There is increasing evidence that bioenergetic function of mitochondria and mitochondrial antioxidant pool decrease with advancing of age in several tissues. Literature is full of controversy results. Oxidative modification of proteins, saccharides, nucleic acid (nuclear and mitochondrial) and lipid peroxidation were observed in different tissues, cells, compartments, including mitochondria with advancing of age. On the other hand also no significant changes in level of oxidative damage products were observed during ageing. Author observed different kind of mitochondrial oxidative changes in the brain and in the heart during ageing. The author understanding of the role played by reactive oxygen species and by mitochondria in disease pathology and ageing is still insufficient. Little is known about mechanisms responsible for the deleterious changes seen during both normal ageing and diseases. Mitochondrial changes, oxidative damages in mitochondria with advancing age and mitochondrial dysfunction in cardiovascular and neurodegenerative diseases will be discussed in this chapter.

Chapter VII – Mitochondria are central to ATP synthesis, regulation of Ca^{2+} homeostasis generation of reactive oxygen species and programmed cell death. They have an essential role in the generation of cell activation signals via released reactive species, as well as small GTPases; mitogen activated kinases located in the outer and inner membranes of mitochondria. While mutations of mitochondrial DNA leading to synthesis of malfunctional proteins are the most understood cause of cellular/organelle pathological changes, the mitochondrial dysfunction induced via oxidative damage to DNA, electron transport chain and structural proteins are also etiologically important events in development and progression in a wide array of diseases including malignancies, diabetes, hypertension, inflammation, hepatitis, neuromuscular and neurodegenerative ones. Although there is a growing amount of data regarding the mechanisms of mitochondrial dysfunction, one of the main challenges in the years to come will be to assess whether functional/structural modulation to mitochondrial proteins and damage to DNA is a cause or a consequence of disease. When mechanistic issues are sorted out, the use of mitochondria as a pharmacological target holds unforeseen promise in the treatment of diseases. Here author provide a short overview on newest discoveries supporting implication of mitochondria in inflammatory and apoptosis-based diseases.

Chapter VIII - Since the discovery of the oxidative phosphorylation (OxPhos) process by Engelhart in 1930 and the establishment of the chemiosmotic theory by Peter Mitchell in 1965, many studies have been performed on the regulation of mitochondrial respiration mostly *in vitro* and bioenergetics was focussed on the studies of mitochondrial inner membrane. However, in recent years mitochondrial outer membrane and mitochondrial surrounding became centres of interest, particularly with development of the methods of studies of mitochondrial respiration in the cells *in situ* and *in vivo*. The aim of the three chapters in this section of the current volume is to review the recent progress in this new and important area in the framework of Molecular Systems Bioenergetics.

Chapter IX – The mitochondrion has evolved as an important organelle in determining cell survival and cell death. This tiny organelle is involved in a plethora of processes in mammalian cells including ATP production, steroid synthesis, and cell division and cell death. Indeed, mitochondrial dysfunction is associated with numerous human maladies including cardiovascular, neurodegenerative and liver diseases as well as diabetes, cancer, and septic shock. Mitochondrial diseases have traditionally been attributed to defects in the electron transport chain (ETC), the major source of mitochondrial reactive oxygen species (ROS), a byproduct of mitochondrial respiration. Superoxide ($O_2^{\bullet-}$) is generated under specific bioenergetics condition at several sites within the ETC and mitochondrial matrix scavengers regulate the level of ROS within a physiological range. Mitochondrial cation channels and exchangers function to maintain matrix homeostasis and are likely involved in modulating mitochondrial function in part by regulating $O_2^{\bullet-}$ generation. The redox state and ROS scavengers largely control the emission (generation – scavenging) of $O_2^{\bullet-}$. Excess ROS (pathological concentrations) can cause a feed-forward effect on further ROS production and lead to increased mitochondrial Ca^{2+} load, both of which are factors that cause damage to mitochondrial DNA and induce apoptosis and necrosis. Therefore, knowledge of the defects in genes regulating the proteins that form the complexes and other mitochondrial proteins has gained increasing importance as mediators of mitochondrial diseases. Mitochondria-related pathologies can occur at any time in life, from early infancy to senescence. Insofar as mitochondria are involved in oxidative damage and cell signaling that leads to apoptosis, antioxidants and other therapeutic strategies that target the organelle appear to be a novel approach to alleviate many diseases. Indeed, recent efforts have been made to design drugs or to engineer proteins with specific sequences or modifications that will enable their uptake into the mitochondrial matrix to protect the organelle against damage. It is worth recognizing that these approaches to target mitochondria to alleviate dysfunction due to genetic alterations in nuclear and/or mitochondrial genes, environmental factors, or metabolic disorders, could lead to selective protection of cells in different tissues and in various pathologic states. This novel approach has gained unprecedented attention recently with a significant potential for future therapeutic purpose. But the effort of targeting mitochondria remains hampered by the accessibility of targeted therapeutic agents through mitochondrial membranes. Moreover, mitochondrial targeting may need to be selective for a target cell or tissue, and a strategy to protect mitochondria in one disease state, e.g. ischemic heart disease, may well hinder mitochondrial function in another disease state, e.g. cancer.

Chapter X – Diabetes is a chronic disease that results from a complex interplay between genetic predisposition and environmental factors and is considered one of the main threats to human health. Neurodegenerative processes such as brain atrophy, white matter and vascular abnormalities, cognitive impairment and dementia are present in a high percentage of diabetic patients. Vascular endothelial dysfunction is accompanied by an imbalance between vasodilator and vasoconstrictor substances produced by the endothelium. Although the way how endothelial function is altered in diabetes is not yet fully understood, the literature suggests that mitochondria play an important role in this process. This review is aimed to discuss the role of brain endothelium in physiologic and diabetes-associated pathologic conditions. The role of mitochondria, oxidative stress and insulin signalling in diabetes-induced endothelium dysfunction is also debated. Finally, some potential therapeutic strategies aimed to counteract diabetes-related endothelium dysfunction will be briefly discussed.

Chapter XI – Brain mitochondria perform several essential functions:
1. they are unique source of ATP for neurones since these are solely dependent on aerobic oxidation of glucose.
2. they are involved in modulation of intracellular calcium transients.
3. they are site of synthesis of some mitochondrial proteins coded by mitochondrial DNA.
4. they are involved in intrinsic pathway of apoptosis as residence and target of pro- and anti-apoptotic proteins.

Previous studies have demonstrated mitochondrial dysfunction and mitochondrial apoptosis after global brain ischemia. However, the mechanism of neuronal cell death due to ischemia-reperfusion injury as well as the relationship between mitochondrial dysfunction and apoptosis remains unknown. The present study was carried out to asses the effect of global brain ischemia on mitochondrial functions and to elucidate possible causal link between mitochondrial dysfunction and initiation of mitochondrial apoptosis. Using standard 4-vesel occlusion model of transient global brain ischemia author have demonstrated that ischemia induces inhibition of both mitochondrial protein synthesis and respiratory complexes I and IV. Inhibition of respiratory complexes was not accompanied with decline in both mitochondrial transmembrane potential and rate of active calcium uptake to mitochondria. Author presume that both mitochondrial transmembrane potential and rate of mitochondrial calcium uptake was not significantly affected by ischemia-reperfusion, apparently due to excess capacity of the complexes I and IV. Finally, ischemia-induced initiation of mitochondrial apoptosis at the level of p53 translocation to mitochondria has been observed. Recent studies have documented the link between inhibition of complex I, oxidative stress and initiation of p53-dependent apoptosis. In order to investigate connection between complex I inhibition, oxidative stress and initiation of p53-dependent apoptosis author have investigated effect of ischemic preconditioning (IPC) on mitochondrial dysfunction and initiation of mitochondrial apoptosis. IPC represents an important adaptation of CNS to sub-lethal ischemia, which results in increased tolerance of CNS to the consequent lethal ischemia. Ischemia-induced inhibition of complex I and stimulation of protein oxidation was not significantly affected by IPC while translocation of p53 to mitochondria was completely abolished by IPC. These results indicate that either ischemia-induced mitochondrial dysfunction is not linked to apoptosis initiation or IPC affects downstream processes connecting mitochondrial dysfunction to apoptosis initiation.

Chapter XII - The main focus of this chapter is VDAC regulation by dimeric tubulin, one of the most abundant cytoskeleton proteins. Recently, author have found that nanomolar concentrations of mammalian tubulin induce reversible blockage of VDAC channel reconstituted into planar phospholipid membranes. Tubulin-VDAC interaction requires the presence of negatively charged C-terminal tails of tubulin. Tubulin lacking the C-terminus did not induce VDAC closure. Author suggested a model of tubulin-VDAC interaction in which the tubulin C-terminus penetrates into the channel lumen, interacting with VDAC with high specificity and partially blocking channel conductance.

Chapter XIII – For long time mitochondria have only been considered for their role in the aerobic energy production in eukaryotic cells, being the sites of oxidative phosphorylation, which couples the electron transfer from respiratory substrates to oxygen with ATP synthesis. Subsequently, the discovery that electron transfer along the inner mitochondrial membrane carriers is associated with formation of radicals and other reactive

oxygen species, commonly indicated as ROS, able to damage cellular components, suggested the mitochondrial involvement in degenerative processes linked to several diseases and aging. More recently, a new role for mitochondria, as a system able to supply protection against cellular oxidative damage, is emerging. Experimental evidence suggests that within mitochondria mechanisms are operative which can scavenge ROS, such as superoxide radical and hydrogen peroxide, produced by other cellular ROS sources. This action is particularly relevant in several physio-pathological conditions leading to increased cellular ROS production.

Moreover, mitochondrial population appears to be able to regulate its own rate of generation of reactive oxygen species. This regulation is principally linked to the capacity of mitochondria to commit suicide by a process called mitoptosis. Such a process constitutes a negative feedback loop, since it is induced by enhanced ROS generation and leads to the purification of the intracellular population of mitochondria from the ROS-overproducing ones. Moreover, it has to be regarded as the last line of defense against the processes, induced by severe cellular oxidative damage, which lead to cell death.

Chapter XIV – Mitochondrial diseases (MD) are disorders caused by impairment of the mitochondrial respiratory chain, or electron transport chain (ETC). The genetic error can affect mitochondrial (mtDNA) or nuclear DNA (nDNA). Clinical phenotypes are polymorphic and range from pure myopathies to multisystemic disorders. The estimated prevalence of MD is 1-2 in 10000, which makes them among the commonest inherited neuromuscular disorders. At present, the diagnosis of MD requires a complex approach, including clinical and family data, measurement of serum lactate, exercise testing, electromyography, muscle histology and enzymology, and genetic analysis. Ragged red fibers, ragged blue fibers and cytochrome c oxidase (COX)-negative fibers are pathological hallmarks. However, reliable biomarkers for these disorders are still needed. Because mtDNA genes encode subunits of the mitochondrial ETC, oxidative base modifications to mtDNA can lead to bioenergetic dysfunctions, thus generating a vicious cycle of mitochondrial dysfunction, increased oxidative stress, and cellular death.

The treatment of MD is still inadequate. Therapies that have been attempted include ETC cofactors, other metabolites secondarily decreased in MD, antioxidants and agents acting on lactic acidosis. However, their role in the treatment of the majority of MD remains unclear. This chapter reviews the diagnostic and therapeutic approaches to mtDNA-related disorders.

Chapter XV – The cellular oxygen sensing system of the body ensures appropriate adaptation of cellular functions towards hypoxia by regulating ion channel activity and gene expression with an impact on ventilation, blood circulation, vascularisation, metabolism and O_2 transport capacity. Deconvolution of hypoxia induced light absorption changes in oxygen sensing tissues - like rat carotid body in vitro, human tumor cells or mouse embryonic stem cells both grown in three dimensional tissue culture - is an ideal tool to study and prove the function of non mitochondrial and mitochondrial heme proteins within oxygen sensing pathways. According to these photometric studies and confirmed by patch clamp studies or molecular biology techniques the oxygen-sensing signal cascades are suggested to include the following: an isoform of the neutrophil NADPH oxidase, different electron carrier units of the mitochondrial electron transport chain (METC) like cytochrome a592 of the terminal cytochrome c oxidase (COX) or COX isoform-IV2 as well as mitochondrial flavine adenine dinucleotide (FAD)-binding sulfhydryl oxidase (Erv1), heme oxygenase-2 (HO-2), the trimeric AMP activated protein kinase and a subfamily of 2-oxoglutarate dependent

dioxygenases termed HIF (hypoxia inducible factor) prolyl-hydroxylase (PHDs) and HIF asparagyl hydroxylase called factor-inhibiting HIF (FIH-1). A complex system of specific oxygen-sensing cascades involving gaso-transmitters like oxygen (O_2), reactive oxygen species (ROS), nitric oxide (NO), carbon monoxide (CO) or hydrogen sulphide (H_2S) as well as AMP as second messengers or modulators may help to tailor various adaptive responses according to differences in tissue oxygen availability by means of their different oxygen sensitivities as well as cell-specific and subcellular localizations. Herein, author propose an integrated model for these various oxygen-sensing mechanisms to provide a deeper understanding of the hypoxic response that might be useful for new therapeutic strategies in ischemic disease.

Chapter XVI - The nuclear and mitochondrial genomes are both subject to various types of DNA damage caused by exogenous sources, such as *u*ltraviolet (UV) radiation and genotoxic drugs, and to endogenous damage like continuously produced *r*eactive *o*xygen *s*pecies (ROS). Persistent DNA damage will be encountered by the replication and transcription machineries and has been shown to result in a decay of DNA and RNA synthesis. Failed repair and replication can lead to mutation or loss of both nuclear and *mit*ochondrial DNA (mtDNA).

The facultative anaerobe baker's yeast, *Saccharomyces cerevisiae* (*S. cerevisiae*), is able to survive without a functional respiratory chain and as such is a useful model for studying genomic instability in mitochondria. However, some mitochondrial functions are essential for cell viability, such as flavin mononucleotide synthesis or the biogenesis of *i*ron-*s*ulphur *c*lusters (ISCs). Interestingly, impaired biogenesis of ISCs, or incorporation of mtDNA sequences into nuclear DNA, are sources of nuclear genome instability. Damaged mtDNA also affects nuclear gene expression and is proposed to activate a checkpoint-like response in order to repair mtDNA and to restore mitochondrial function. In the coming years, the exploration of mitochondria-specific checkpoint responses will be a challenging and new area of research.

In addition to the aging process, many human diseases have been associated with mtDNA mutation or copy number dysregulation, including muscular dystrophies and cancer. Therefore, there is an increased need to understand how cells maintain a functional mitochondrial genome. In this chapter author will discuss known mtDNA repair pathways and explore the cellular consequences of mitochondrial dysfunction observed in yeast, including *petite* formation, and changes in mitochondrial morphology and copy number. Author will also review the current research on how mitochondrial dysfunction can lead to nuclear genome instability.

Chapter XVII – A modern eukaryotic cell is the result of α-proteobacteria and primitive eukaryotic progenitor mutualism, so cytoplasm is the sort of ambient environment for mitochondria. The driving force of endosymbiosis had become a number of metabolic advantages, which had been taken both organisms from coexistence. The mutual adaptation of host and endosymbiotic organisms has taken place in evolution progress. On the one hand, this adaptation was directed to provide host cell with essential compounds (H^+ and ATP, for example), on the other hand, to supply endosymbiotic pre-mitochondrial cell with carbon source and environment buffering. As a result of horizontal gene transfer from endosymbiotic to host genome, mitochondrial physiological state and replicative process are entirely controlled by nucleus in accordance with the environmental changes. The mechanisms of

mutual correction were stabilized genetically, which is now marked by anterograde and retrograde gene expression regulation.

A focus point of this chapter is a phosphate (Pi) metabolism contribution to the mitochondrial biogenesis and mtDNA stability control. Intracellular phosphate concentration decrease triggers digestion of another phosphate sources: ATP, phosphoenolpyruvates, sugar-phosphates, polyphosphates etc., but these take place as a last resort only. If phosphate concentration is low, cell corrects cytoplasmic phosphate concentration by activating transcription of phosphatases genes *PHO5*, *PHO11*, *PHO12*, *PHO8*, *HOR2*; phosphate transporter genes *PHO84*, *PHO89*, *PHO86*; CDK-inhibitor genes *PHO81*, *SPL2*; ATP-phosphoribosyltransferase gene *HIS1*, polyphosphate synthesis genes *PHM1, 2, 3, 4* (*PHO*-regulated genes). With yeast transition from aerobic to anaerobic metabolism cell makes maximal reserve of phosphate. This indicates that phosphate plays an important role in glycolysis. Phosphate acts as a substrate and effector of a great number of enzymes (phosphofructokinase, for example), influences intracellular pH level. Deletion of CDK gene *PHO85* leads to a number of phenotypic effects, among them are fast accumulation of respiratory deficient cells, constitutive expression of *PHO*-regulated genes, which leads to phosphate transport increase even under intracellular phosphate abundance.

Thus in evolutions course some mitochondrial activity regulation pathways have been formed. Regulatory proteins of different metabolism pathways are able not only to correct mitochondrial functions, but influence mtDNA quantity and even the presence.

Chapter XVIII – In smooth muscle cells (SMC), Ca^{2+} regulates several key functions like cell division, growth and cell death, as well as smooth muscle contractility. Mitochondria are intracellular compartments capable of Ca^{2+} uptake and release mainly via the mitochondrial uniporter and the mitochondrial Ca^{2+}-Na^{+} exchanger, respectively. Thus mitochondria can considerably participate to Ca^{2+} homoesostasis and Ca^{2+} signalling in SMC. In addition to their rôle as Ca^{2+} store, mitochondria contribute to the control of SMC function and in particular to smooth muscle tone via reactive oxygen species (ROS) production. Mitochondrial ROS production can modulate both ion channels that influence the pattern of the calcium signal, and the sensitivity of the contractile apparatus to the calcium signal. This review gives an overview of Ca^{2+} signaling and the contractile apparatus of smooth muscle cells, witrh particular focus on airway and vascular smooth muscle, and summarizes (i) the rôle of mitochondria as active Ca^{2+} stores, including mitochondria-sarcoplasmic reticulum coupling and Ca^{2+} microdomains, and their consequence on SMC Ca^{2+} homeodynamics and signalling (ii) the rôle of mitochondria in ROS production and its subsequent modulation of smooth muscle contraction. Examples of mitochondrial involvement in smooth muscle dysfunction are also presented. Moreover, an overview is given of theoretical models that have been developed for the understanding of mitochondrial impact on the spatiotemporal pattern of Ca^{2+} signalling of the SMC.

Chapter XIX – The interaction of tubulin and mitochondria will be the subject of the sections that follow. This interaction occurs both at the level of MT and mitochondria as well as at the level of the unpolymerized tubulin dimer and mitochondria. This latter interaction is the one featured in the previous two chapters by Saks, et al., and by Rostovtseva, and is the focus here as well. In the following sections, relevant features of MT and tubulin structure and evolution will be discussed, followed by mitochondrial structure and variation. Finally the coevolution of tubulin and mitochondria will be considered in light of the binding of

tubulin's tail peptides to the mitochondrial outer membrane (MOM) through the voltage dependent anion channel (VDAC), also known as mitochondrial porin.

Chapter XX - When cereal grains and animal feed are colonised by moulds there is a significant risk of contamination with the secondary metabolites of these fungi. A number of these fungal compounds are endowed with toxic effects towards animals and human beings called mycotoxins. They induce diverse and powerful biological effects in test systems: some are carcinogenic, mutagenic, teratogenic, estrogenic, hemorrhagic, immunotoxic, nephrotoxic, hepatotoxic, dermotoxic and neurotoxic. Zearalenone (ZEN), T-2 toxin and ochratoxin A (OTA) are among the mycotoxins fitting into this major group. The diseases (mycotoxicoses) caused by these mycotoxins are quite varied and involve a wide range of susceptible animal species including humans. Most of these diseases occur after consumption of mycotoxin contaminated food and feed. Apoptotic cell death pathways have been implicated in the toxic events induced by these mycotoxins leading to diseases installation.
This cell death, in both its physiological and pathological forms, is closely linked to mitochondrial structure and (dys)function. The mitochondrial membrane permeabilization (MMP) is frequently the decisive event that delimits the frontier between survival and death. Pathological cell death induced by mycotoxins relies on MMP as a critical event. The discovery that many proteins which have essential functions within mitochondria may also serve as caspase-dependent and -independent cell death proteins placed mitochondria in a center stage as potential orchestrators of cell death.
In this review, author are trying to expose the major findings which point to a central role of mitochondria in the toxic processes induced by ZEN, T-2 toxin and OTA and to provide new insights into the molecular mechanisms by which these mycotoxins might promote diseases in order to identify interesting targets to overcome mycotoxins toxicity.

Chapter XXI – Mitochondria protect neurons from the deleterious effects of abnormal Ca^{2+} influx, which leads to the activation of hydrolytic and degradation pathways associated with apoptosis and necrosis. In this work different characteristics of the MPT in mouse non synaptic brain cortex mitochondria were studied. Methodological approaches for MPT characterization will be described. Mitochondrial swelling and membrane potential were evaluated after a single bolus of 100 μM Ca^{2+} addition. Respiratory function and production of reactive oxygen derived molecules, were analysed before and after calcium induction of MPT. Energized organelles after MPT induction with 100 μM Ca^{2+}, showed mitochondrial depolarization and partial swelling that was maximal after addition of alamethicin. Mitochondrial swelling induced by 100 μM Ca^{2+} was associated with an impaired respiratory function. Mitochondrial hydrogen peroxide (H_2O_2) production, dependent of a high proton force was increased after 100 μM Ca^{2+} or alamethicin. Mitochondrial CsA pretreatment was able to restore respiratory function transmembrane potential and H_2O_2 levels to those observed in untreated mitochondria. As expected, alamethicin alone or with 100 μM Ca^{2+} induced swelling accompanied by a clear mitochondrial depolarization, a 113% increase in state 4 respiratory rate and a 66% decrease in state 3 respiratory rate. The effect of NO on MPT and apoptotic cell death will be discussed, as well as the role of MPT induction in brain pathology.

Chapter XXII – The maximum rates (Vmax) of some mitochondrial enzymatic activities related to energy transduction such as citrate synthase, succinate dehydrogenase, malate dehydrogenase for Krebs' cycle; NADH-cytochrome c reductase (both total and

rotenone sensitive), succinate CoQ reductase, succinate cytochrome c reductase, cytochrome oxidase, for the electron transfer chain (ETC); glutamate dehydrogenase, glutamate-pyruvate-transaminase, glutamate-oxaloacetate-transaminase for glutamate and aminoacids metabolism were evaluated in non-synaptic and intra-synaptic mitochondria from cerebral cortex of *Macaca fascicularis* (*Cynomolgus* monkey). These three types of mitochondria were isolated from dopaminergic terminals of monkey's cerebral cortex after a Parkinson's Disease like syndrome induced by *e.v.* injections of MPTP neurotoxin.

In control (vehicle-treated) animals, the biochemical machinery is differently expressed in non-synaptic mitochondria with respect to "light" and "heavy" intra-synaptic ones and also between intra-synaptic themselves. The activities of citrate synthase, succinate dehydrogenase, malate dehydrogenase, NADH-cytochrome c reductase, succinate cytoch-rome c reductase, glutamate dehydrogenase and glutamate-pyruvate- and glutamate-oxaloacetate-transaminases are lower, while cytochrome oxidase is higher in intra-synaptic "light" and "heavy" mitochondria than those in non-synaptic ones, being succinate cytochrome c reductase activity the same in all mitochondria types. Some enzyme activities are lower in "heavy" than in "light" intra-synaptic mitochondria, confirming that in various types of brain mitochondria also from primates, a different metabolic machinery exists, due to their different location *in vivo*, in soma or synapses.

In the MPTP-treated animals (Parkinson's like syndrome), the systemic treatment with the neurotoxin (*i.v.*, 0.3 mg/kg/day for 5 days) increased the activity of succinate dehydrogenase and succinate CoQ reductase only in "light" intra-synaptic mitochondria (decreasing the glutamate dehydrogenase activity) and the neurotoxin decreased also cytochrome oxidase activities in all mitochondria types and that of glutamate-pyruvate-transaminase of non-synaptic mitochondria.

Thus, MPTP stimulated the succinate metabolism, increasing the activity of Complex II, but decreasing the activity of Complex IV, the most sensitive Complex to neurotoxin, not affecting the activities of Complex I, Complex I-III, Complex II-III; also glutamate metabolism is affected by MPTP, decreasing the activity of glutamate dehydrogenase ("light" intra-synaptic mitochondria) and particularly that of glutamate-pyruvate-transaminase on non-synaptic mitochondria.

The data on *functional proteomics* of these different types of mitochondria suggest that the treatment with MPTP, as a model of Parkinson's Disease, affected the activities linked mainly to succinate metabolism of "light" intra-synaptic mitochondria, instead of Complex I, as previously hypothesized, indicating that the link between the Krebs' cycle and ETC seems to be of primary pathogenetic significance, indicating *a specific subcellular trigger site* of action. These results presented in this chapter also suggest *a specific molecular trigger mode* of action on energy metabolism of cerebral dopaminergic terminals, being the neurotoxin effective also on enzyme activities of non-synaptic mitochondria for transamination of pyruvate, while Complex IV activity was already decreased by MPTP, thus impairing the ATP synthesis in any case.

Chapter XXIII – The review considers modern data concerning the role of inorganic polyphosphates (polyP) in mitochondria. PolyP are linear polymers containing a few to several hundred residues of orthophosphate (P_i) linked by energy-rich phosphoanhydride bonds. PolyP was found in the mitochondria of yeast, mammalian and insect cells. They are located in the membranes and inter-membrane space of mitochondria. Mitochondrial membranes possesses polyP/Ca^{2+}/polyhydroxybutyrate complexes. Biopolymers with a short

chain length (~15 phosphate residues) were found in the mitochondria of *Saccharomyces cerevisiae* at first by ^{31}P-NMR. Later, polyP was extracted from the pure mitochondrial fraction of *S. cerevisiae* and assayed by chemical and electrophoretic methods. They comprised ~7-10 % of the total polyP content of the yeast cell. Its accumulation is typical of promitochondria but not of functional active mitochondria. Yeast mitochondria possess two exopolyphosphatases splitting P_i from the end of the polyP chain. One of them, encoded by the *PPX1* gene, is located in the matrix; the other one, encoded by the *PPN1* gene, is membrane-bound. Formation of well-developed mitochondria in the cells of *S. cerevisiae* after glucose depletion is accompanied by decrease of the polyP level and chain length. In *PPN1* mutants, polyP chain length increased under glucose consumption, and the formation of well-developed mitochondria was blocked. These mutants were defective in respiration functions and consumption of oxidable carbon sources such as lactate and ethanol. This fact suggests that the *PPN1* gene is essential for mitochondrial functioning in *S. cerevisiae*.
Since polyP is a compound with high-energy bonds, its metabolism vitally depends on the cell bioenergetics. The maximal level of short chain acid soluble polyP was observed in *S. cerevisiae* under consumption of glucose, while the long-chain polyP prevailed under ethanol consumption. It is assumed that accumulation of separate polyP fractions with specific localization and chain length differently correlates with the energy-providing pathways.
As regards insects, polyP in the mitochondria changed drastically under ontogenetic development. The content and chain length of mitochondrial polyP were observed to decline between the stages of *Rhipicephalus microplus* embryo cellularization and segmentation, indicating involvement of the polymers in regulation of mitochondrial metabolism under ontogenesis. In mammalian cells, specific reduction of mitochondrial polyP under expression of yeast exopolyphosphatase PPX1 significantly modulates mitochondrial bioenergetics and transport: the potential of the inner membrane was reduced, the NADH level decreased, and the calcium accumulation capacity increased.
These results demonstrate the crucial role of polyP in mitochondrial functions and dysfunctions in yeast and animal cells.

Chapter XXIV – The history of plant mitochondrial potassium channel/s began about ten years ago, when the first channel was described on a functional basis in durum wheat mitochondria. This channel was named Plant Mitochondrial Potassium Channel ATP sensitive (PmitoK$_{ATP}$) in analogy with the animal counterpart (mitoK$_{ATP}$). The PmitoK$_{ATP}$ shows interesting features, being able to deeply affect mitochondrial bioenergetics and to control mitochondrial reactive oxygen species (ROS) production, thus playing a role as a mechanism acting against oxidative/environmental stresses in mitochondria/cell/plant. To date, mitochondrial potassium channels have been also described in about ten plant species. However, these channels display at the same time analogies and differences with respect to the original PmitoK$_{ATP}$. The intrinsic activity may vary significantly among channels, the pattern of modulators is different and even some of these channels are ATP-insensitive. Finally, different physiological roles have been proposed. Awaiting for the identification of the molecular nature of each channel, here author point out the question whether the observed differences may be attributed to the same channel differently modulated in mitochondria from different sources or it may be more appropriate to consider different channels. At the moment, it appears more appropriate to refer to different channels, thus suggesting that a fit nomenclature should be consistently used.

Chapter XXV - Mitochondria contribute to cellular function in ways that extend far beyond their well-described role in energetic metabolism. Their capacity in ATP generation is only one of the many ways in which they exhibit control over cellular processes. In particular, their role in cardiac physiology and pathology has been an area of increasingly exciting research. Mitochondria have been shown to be a critical component of the pathways governing hypertrophy, ischemia-reperfusion injury, and cell death. Additionally, it has been shown that mitochondria are the downstream mediators of cardioprotective compounds that activate plasma membrane receptors. Their role in cardioprotective signal transduction is of particular interest here. Author have recently proposed that these cardioprotective signaling events are coordinated by plasma membrane microdomains called caveolae. These domains pack the cardioprotective receptors as well as the crucial signaling enzymes of a particular pathway into signaling platforms (signalosomes), which are then delivered to mitochondria. The signalosome hypothesis, as author have described it, proposes that diverse signaling responses can be mediated by redundant kinases because specificity is provided by the specific signalosome. Interaction of the signalosome with mitochondria instigates opening of the mitochondrial ATP-sensitive K^+ channel ($mitoK_{ATP}$) and triggers intramitochondrial signaling events that result in reduced cardiac damage by inhibiting the onset of the mitochondrial permeability transition (MPT). Herein, author describe the processes instigated by the signalosome-mitochondria interaction and its implications for further research in this paradigm.

Chapter XXVI – During apoptosis, the Apoptosis Inducing Factor (AIF) is known to be released from the mitochondrial intermembrane space to the cytosol, and moves to the nucleus. Author reviewed, here, the basic morphological and molecular features of apoptosis and the role played by AIF in the intrinsic apoptotic pathway. In particular, based on multicolor immunocytochemistry at conventional and confocal fluorescence microscopy, author discussed the dynamic relocation of AIF throughout the apoptotic process in mammalian cells driven to apoptosis by different agents (etoposide, actinomycin D, cisplatinum, carboxamide) or photosensitized with the fluorogenic substrate Rosa Bengal acetate. Author observed changes in the structure and function of mitochondria, and found that in early apoptosis, independently of the stimulus used, the progressive chromatin condensation and early DNA degradation into High Molecular Weight fragments entails translocation of cytochrome c and AIF from mitochondria to the nucleus. Remarkably, when the apoptotic program proceeds through the activation of the caspase cascade that provokes DNA degradation into oligonucleosome-sized fragments, AIF does not locate in the nucleus anymore but is visible in the cytoplasm, where it seems to be mainly confined to mitochondria. This is consistent with the hypothesis that AIF is the caspase-independent executioner, responsible for the onset of type 1 chromatin condensation (stage I) but not for internucleosomal DNA fragmentation (stage II); in this respect, its release from mitochondria should be considered as an early hallmark of apoptosis.

Chapter XXVII – In recent years, the role of mitochondria in apoptotic cell death has received considerable attention. One of the key events in apoptosis is the increase in mitochondrial membrane permeability, which leads to the release of apoptogenic factors from mitochondria into the cytoplasm. Cytochrome *c* released from mitochondria activates the cascade of caspases and downstream targets of apoptotic cell death. Bcl-2 family is one of the best characterized protein groups that directly regulates mitochondrial functions. A major role of Bcl-2 family proteins is to alter mitochondrial membrane permeability, thus controlling

cytochrome *c* release. Recent reports describe novel apoptogenic regulators other than Bcl-2 family in regulation of mitochondrial function. Tumor necrosis factor receptor-associated protein 1 (TRAP1) is a member of the heat-shock family of mitochondrial proteins, and is homologous to the members of the 90-kDa family of heat-shock proteins (HSP90). TRAP1 appears to have specific functions different from those of other members of the HSP90 family. Reduced expression of TRAP1 enhances cytochrome *c* release from mitochondria. Moreover, reactive oxygen species (ROS) are involved in the regulation of TRAP1 expression, indicating that TRAP1 can act as a sensor of ROS-mediated regulation of apoptosis. Here, author describe the mechanism by which TRAP1 controls mitochondrial functions during apoptosis.

Chapter XXVIII – Protomitochondria (PrM) are intracellular mitochondrial germ organelles, precursors of mitochondria in specialized cells of animals. PrM have been isolated from rat liver by centrifugation and filtration through Millipore filters with pore diameters of 0.1 to 0.45 micron and characterized by fluorescence-based assay. PrM, having the volume smaller than that of light mitochondria (LM), did not differ much from the latter in protein, lipid and DNA composition, membrane charge, and some other parameters. At the same time, the activity of some enzymes of the respiratory chain (NADH dehydrogenase, succinate dehydrogenase) in PrM was even higher than that in mature mitochondria. The results obtained are important for understanding the processes of mitochondrial biogenesis in specialized cells of mammals.

Chapter XXIX – The physiological role of exogenous nitrate as protective agent under extreme conditions of anaerobic stress was examined by investigation of mitochondrial membrane ultrastructure of 4 - day - old pea (*Pisum sativum*) and rice (*Oryza sativa)* seedling root tips incubated under anoxia in the presence or absence of exogenous KNO_3 (10mM). Destruction and subsequentl degradation of mitochondrial membrane ultrastructure was observed already after six to nine (pea) and six to eight (rice) hours anaerobic incubation in KNO_3 - free medium.

In the presence of KNO_3 no mitochondrial membrane destruction was observed after 6 and even 9 hours anoxic treatment of pea roots although some modifications in the mitochondrial ultrastructure took place: in some cells mitochondrial cristae were arranged in parallel rows. In case of rice roots, signs of destruction of mitochondrial membrane was observed only after 8 hours of anaerobic treatment in the presence of KNO_3. Postanaerobic growth capacity of roots was observed only after incubation in the presence of KNO_3 The data obtained indicate a protective role of exogenous nitrate in oxygen-free medium and suggest the advisability of use of nitrate as nitrogen fertilizer on hypoxic and anoxic soils.

The results of the present investigations are discussed and compared with reports of either markedly damaging or favourable effects of exogenous nitrate on the growth, metabolism and energetic of rice, pea and other plant roots under hypoxic and anoxic conditions.

Chapter XXX - Apoptosis is an active mode of cell death of possible therapeutic relevance since it is regulated by the activation of intracellular lethal molecules. Apoptosis and other forms of cell death are often controlled at one or more crucial steps involving the mitochondria. Numerous pro-apoptotic signal-transducing molecules and pathological stimuli converge on mitochondria to induce mitochondrial outer membrane permeabilization (MOMP). Pro- and anti-apoptotic members of the Bcl-2 family control the permeability of the outer mitochondrial membrane. MOMP is lethal because it results in the release of caspase-

activating molecules and caspase-independent death effectors, metabolic failure in the mitochondria, or both.

Chapter XXXI – Proteomics based studies have gained international recognition during the last decade holding significant promise in several fields of science from the discovery of biomarkers of disease to applications in agriculture, animal and marine sciences. Most of the proteomics studies above mentioned use 2DE (two-dimensional electrophoresis) coupled to mass spectrometry in order to observe changes in the patterns of expression of individual proteins as a result of a particular experimental condition. Standard 2DE separates proteins according to their isoelectric point (pI) and molecular mass. In 2DE proteins are previously denatured by customarily used extraction buffers. The denaturing step of protein extracts, characteristic of 1DE and 2DE PAGE renders the study of protein interactions and their complex dynamics impossible. In order to study the complexome (proteome of protein complexes) of the organelles, particularly mitochondria, an approach using protein in their native tridimensional structure is mandatory. This technique was designated as BN-Blue Native and was established in the early nineties. It combines a first step where protein complexes are separated by molecular mass under non-denaturing conditions, and a second dimension where complexes are separated into their constituent proteins through the use of denaturing conditions. This method allows the study of protein complexes composition and differences of expression of individual proteins in the organelle membrane.

Chapter XXXII – This article attempts to show a short overview on a new role of mitochondria in Ca^{2+}-signal pathway involved in autocrine/paracrine release of ATP from cells.

At first, the possible mechanisms underlying ATP release postulated in recent studies are listed. The next, from the publications during a few decade, the Ca^{2+}-signal transducing system from the endoplasmic reticulum (ER) and other organella to mitochondria is briefly summarized.