Preface

Signal transduction is any process by which a cell converts one kind of signal or stimulus into another. Processes referred to as signal transduction often involve a sequence of biochemical reactions inside the cell, which are carried out by enzymes and linked through second messengers. In many transduction processes, an increasing number of enzymes and other molecules become engaged in the events that proceed from the initial stimulus. Responses of cells to environmental signals, toxins and stressors have profound implications for diverse aspects of human health and disease including development, cystic fibrosis, diabetes, asthma, heart, autoimmune diseases and cancer. The delineation of the signal transduction pathways affected in these and other complex human diseases are likely to present new avenues for therapeutic intervention and understanding of human disease mechanisms.This new book presents the latest research in the field.

Chapter I - Neurotrophins are a family of growth factors implicated in the development and maintenance of different neuronal populations in the nervous system. During development, limited supplies of neurotrophins regulate the balance between survival/death of several neuron classes, whereas in adulthood neurotrophins additionally modulate synaptic strength and plasticity. Neurotrophins bind to two sets of receptors, Trk receptor tyrosine kinases and the $p75^{NTR}$ receptor, to activate multiple signaling pathways that mediate various biological functions. Trk activation leads to receptor tyrosine phosphorylation that serves as docking sites for different enzymes and substrates. Among the pathways triggered by Trk receptors are the well-characterized Ras/Rap-MAPK, PI3K-Akt, and PLCγ-PKC cascades. These events are shared by other receptor tyrosine kinases, but the unique combination of Trk receptor docking sites, recruitment of various adaptors and enzymes, and regulated receptor trafficking elicits specific responses for neurotrophins. Moreover, neurotrophin signal transduction pathways are further modulated by effector modules associated with $p75^{NTR}$, as well as by the ratio of Trk and $p75^{NTR}$ receptors, and by the spatial location and temporal pattern of stimulation. The orchestration of these converging pathways elicited by neurotrophins thus ensures a match between the number of surviving neurons and the appropriate target innervation.

Chapter II - Role of reactive oxygen and nitrogen species in various cellular transduction processes becomes now a leading study of free radicals in biological systems. Just recently major interest of "free radical" scientists has been the study of damaging effects of these

reactive species. Of course it is not surprising because the reactivity of free radicals is well recognized for a long time. It is known that free radicals readily participate in the reactions of hydrogen atom abstraction and addition to the double bonds of organic compounds (Reactions 1 and 2):

$$R^{\cdot} + R'H \Rightarrow RH + R'^{\cdot} \quad (1)$$

$$R^{\cdot} + CHX{=}CHY \Rightarrow RCHXCHY^{\cdot} \quad (2)$$

Therefore, as the participation of free radicals in biological systems was established, their toxic action was expected in many physiological and pathophysiological processes and the ways of their inhibitions with free radical scavengers and antioxidants were suggested.

However at present two novel important phenomena were discovered. First of all it was found that major "physiological" free radicals superoxide $O_2^{\cdot-}$ and nitric oxide NO is relatively inactive species and unable to participate in damaging Reactions 1 and 2. (At the beginning of free radical studies in biology it was thought that the radical anion $O_2^{\cdot-}$ is a reactive oxidant and therefore it was named "superoxide." However brilliant chemical and biological experiments of 1970 – 1980 years demonstrated that $O_2^{\cdot-}$ has no oxidative properties at all and is actually a moderate reductant [1]). NO is also a relatively inactive radical and even its ability to participate in nitrosation reactions proposed in some works seems to be quite questionable. However it was found that both superoxide and nitric oxide are the precursors of very active hydroxyl ($HO^{\cdot}$) and peroxy ($ROO^{\cdot}$) radicals, which can induce a cellular damage under pathophysiological conditions associated with so called free radical pathologies.

Another unexpected discovery was the ability of superoxide and nitric oxide as well as their more stable diamagnetic products hydrogen peroxide H_2O_2 and peroxynitrite $^{-}OONO$ (usually named reactive oxygen species ROS and reactive nitrogen species RNS) to participate in transduction signaling processes including many heterolytic enzymatic reactions. It is imperative to emphasize that the signaling effects of ROS and RNS in cells must not be accompanied by some side toxic effects of these reactive species. It has also been suggested that the concentrations of ROS and RNS is an important factor: smaller concentrations of these species can mostly play a role of signaling molecules while at higher concentrations they might predominantly become damaging agents [2-4].

Although the study of signaling effects of ROS and RNS does not have a long history, there are already several interesting reviews. Finkel [2] has discussed the role of oxygen radicals in cell signaling including the targets of ROS in cells and their activity in pathological states. Irani [5] considered ROS signaling in vascular smooth muscle cells (VSMC) and endothelial cells (EC) in the processes of growth, hypertrophy, survival, and apoptosis. Griendling *et al.* [6] discussed the functions of superoxide and hydrogen peroxide as second messengers to activate and phosphorylate multiple intracellular proteins and enzymes such as mitogen-activated protein kinases Akt/protein kinase B, ERK1/2, and p38, the epidermal growth factor receptor and the others.

In an excellent review Thannickal and Farburg [3] quoted a huge amount of literature data known up to the 2000 year concerning the role of oxygen species in cell signaling including the numerous aspects of ROS chemistry, their cellular sources and regulation, and

the mechanisms of ROS activities. They discussed in detail the mechanism of signal transduction by ROS through the oxidation of the sulfhydryl groups of proteins. In two subsequent reviews Forman *et al.* [4,7] discussed the oxygen radical signaling drawing particular attention to the mechanism of the oxidation of the protein thiol residues by hydrogen peroxide. They considered the possible chemical reactions between ROS and the sulfhydryl groups, which can be responsible for the transduction ways of the enzyme activation and inactivation. (These reactions are regarded below).

Taking into account the previous works, the authors will concentrate their attention on the consideration of earlier and new data from the point of view of intimate mechanisms responsible for the signaling functions of ROS and RNS.

Chapter III - Cell traction forces (CTFs) play a vital role in many biological processes such as angiogenesis, inflammation, and wound healing. Force generation in non-muscle cells is realized by interactions between actin and myosin filaments and is mediated via Rho/Rho-kinase. CTF magnitude in myofibroblasts, which express α-smooth muscle actin (α-SMA), is enhanced by the isometric contraction of stress fibers. CTFs direct a coordinated assembly of stress fibers and an array of focal adhesion (FA) proteins such as vinculin, paxillin, and talin. The transmission of CTF to the extracellular matrix (ECM) is achieved through stress fibers via FA proteins. Modulated by ECM proteins and soluble factors such as transforming growth factor-β (TGF-β), CTF is crucial for many cellular functions including cell migration, ECM organization, and mechanical signal generation. A number of technologies have been developed to measure CTF, among which cell traction force microscopy (CTFM) is one of the most efficient and reliable methods. Future research in the area of CTF should explore the use of CTFM for "cell profiling" applications and will require novel experimental and theoretical methodologies to develop measurement of CTF in 3-D matrices, which may model *in vivo* cell environments more accurately than the current 2-D methods.

Chapter IV – Impedance matching is a very effective way to implement active noise absorbers. Available impedance matching schemes depend on accurate path models such that active controllers are able to cancel outbound waves in noise absorbers. An adaptive controller is presented in this article for model independent impedance matching (MIIM). The system employs the two microphone method to resolve in-/outbound waves from pressure signals measured in a noise absorber. The resolved inbound wave is used as the reference signal for impedance matching. Due to inevitable errors in signal measurement and wave calculation, the resolved inbound wave contains an unknown portion of outbound wave. When this signal is used as the reference signal in a feedforward controller to suppress the outbound wave, a feedback loop exists from the unknown portion of outbound wave hidden in the input signal to the outbound wave to be suppressed by the actuation signal. Closed-loop stability is an important issue to be investigated. It is also very difficult to identify primary and secondary path models accurately in such a system using online measurement signals. The MIIM system uses online estimates of primary and secondary path models, which are not necessarily error-free, for online optimization of an active controller. It is shown analytically that the closed-loop MIIM system is stable in the presence of wave separation errors. Experiment results are presented to demonstrate the reliability and effectiveness of the MIIM system.

Chapter V - Recent literature data indicate that oxidative stress is a common feature of either clinical and experimental conditions of chronic liver disease of different aetiology. Reactive oxygen species (ROS), aldehydic end-products of lipid peroxidation such as 4-hydroxy2,3-nonenal and malonyldialdehyde, nitric oxide and other oxidative stress – related reactive intermediates are now suggested to play a role in the pathogenesis of fibrotic progression of chronic liver diseases (i.e., liver fibrosis) towards the end-stage of liver cirrhosis. This chapter includes a brief survey of basic knowledge concerning oxidative stress (basic concepts, antioxidant defenses, intra- and extracellular sources of oxidative stress, major modulating factors) and is dedicated to review the proposed role of oxidative stress and its reactive intermediates in the modulation of cell death and liver injury as well as of signaling processes, gene expression, proliferation and, more generally, the response of potential target liver cells. Major emphasis is dedicated to the relationships between oxidative stress, inflammatory response and the role of activated pro-fibrogenic cells (hepatic stellate cells/myofibroblast – like cells). A section is dedicated to the potential role of antioxidant therapy based on recent literature data.

Chapter VI - The mitogen-activated protein kinase (MAPK) is a family of serine/threonine protein kinase that plays a critical role in governing the growth, proliferation, differentiation and survival of many cell types. There are three major subfamilies of MAPKs: the extracellular-signal regulated kinases-MAPK (ERK-MAPK); the c-jun N-terminal kinase or stress-activated protein kinases (JNK or SAPK)-MAPK; and p38 MAPK. The ERK-MAPK (Ras/Raf/MEK/ERK) pathway is one of the most important and intensively studied signaling pathways, disregulated in various diseases, ranging from cancer to immunological disease and inflammatory, and thus represents an important drug target. The gastrointestinal (GI) tract is the major organ for digesting and absorbing nutrient substance, dietary components may be a significant environmental factor that can exacerbate or interfere with carcinogenesis through MAPKs signaling pathways. GI cancers are the most common, and are the major cause of life quality and cancer death worldwide, GI carcinogenesis is a complex multistep process involving progressive disruption of GI epithelial cell proliferation, apoptosis, differentiation, and survival mechanisms, the constitutively active Ras/MAPK can induce transformation of intestinal cell and MAPK activities are elevated in tumors. The BRAF mutation is frequently seen in sessile serrated adenoma and in sporadic high level of microsatellite instability colorectal cancers, both of which are associated with DNA methylation. Stimulation of the MAPK signal transduction pathway can active mitogen- and stress-activated protein kinases (MSK) 1 and 2, which are histone H3 kinases, that MSK1/2 activity and H3 phosphorylation have roles in chromatin remodeling and transcription of the immediate-early genes. Many dietary components such as short-chain fatty acid (SCFA), the phenolic compounds and the isothiocyanates have been shown to be associated with the biological consequences and activity modulation of MAPK signaling pathway. Over recent years, there is a wide variety of models of the MAPK pathway which have led to some novel insights and predictions as to how this system functions, small-molecule inhibitors designed to target various steps of this pathway have entered clinical trails. In the present review, we introduce the features and functions of the MAPK signaling pathway, comparing the available human cell lines and animal models. Focus on the MAPK pathway in the GI carcinogenesis and metastasis, and its potential as an

approach to cancer treatment, also introduce the efficiency of dietetic supplements that affect MAPK pathway in cancer prevention and treatment. Future challenges for the assessment of these targeted agents in the clinic are also presented.

Chapter VII - Angiogenesis plays pivotal roles in pathophysiological conditions, such as solid tumor growth, ischemic retinopathy, and collateral formation after the occlusion of arteries. Inhibition of angiogenesis is anticipated to control human advanced malignant tumors. In animal studies, various anti-angiogenic strategies significantly reduce the size of preexisting, transplanted tumors, or retard the growth of tumors, leading to the extension of survival of tumor-bearing animals. However, in humans, almost no anti-angiogenic agent has solely been reported to cause regression of advanced tumors and only a few clinical studies showed enhanced survival of patients. In this regard, we may have to re-evaluate the anti-angiogenic strategies for the remedy of patients with advanced malignant tumors. Assessment of anti-angiogenic agents against transplanted tumors in rodent may yield different results from those in humans. Furthermore, a positive effect for anti-angiogenic therapy in animal studies does not necessarily guarantee inhibition of tumor angiogenesis in humans. It is also possible that the dose of anti-angiogenic agents used in human may not be sufficient to cause regression of tumors. In this chapter, the author discusses the impact of attenuation of the anti-angiogenic actions by particular agents and the possible studies that could solve these problems.

Color graphics are presented at the end of the chapter.

Chapter VIII - Microglia are central nervous system (CNS) resident phagocytic cells, which function as brain macrophages. They migrate to area of injured nervous tissue, and they engulf and destroy microbes and cellular debris, as do macrophages in periphery. They also play a pivotal role in brain inflammation by producing a variety of inflammatory mediators. Activation of microglia may be intended to protect neurons at first. However, activation of microglia and inflammatory products derived from them has been also implicated in the neuronal destruction commonly observed in various neurodegenerative diseases. Therefore, the auto-regulatory mechanisms that control the microglial activation may exist *in vivo*, and the failure of these auto-regulatory mechanisms may be in part responsible for the deleterious effects of microglial activation associated with CNS pathologies. It has been shown that microglia undergo apoptosis upon inflammatory activation in a manner similar to activation-induced cell death of lymphocytes. Inflammatory stimuli such as lipopolysaccharide and interferon-γ played a multiple role in the microglial apoptosis: they not only induced cytotoxic nitric oxide (NO) production through interferon regulatory factor-1 (IRF-1), but also initiated NO-independent apoptotic signaling pathways via induction of caspase-11 or antiproliferative BTG1 gene. The auto-regulatory apoptosis of activated microglia was mediated through Toll-like receptor-4. Further elucidation of molecular mechanisms underlying the auto-regulation of microglial activation may enhance our understanding of pathogenesis of neurodegenerative diseases.

Chapter IX – Recently a large-scale expression-profiling approach to identify genes differently regulated during the symbiotic interaction has been successfully used in search for genes involved in signal transduction pathway (Mitra et al., 2004). Comparative analysis of *M. truncatula* wild type and mutant plants revealed at least 6 symbiotic genes necessary for triggering symbiosis-specific genes in *Medicago*: *NFP*, *DMI1*, *DMI2*, *DMI3* and *NSP1*,

NSP2. All these genes could be involved in subsequent steps of NF signal transduction (Catoira et al., 2000). The *DMI1* gene encodes a putative membrane-spanning cation channel related to NF signal transduction (Ane et al., 2004). It has been suggested that the *Sym8* gene of pea and probably *POLLUX* of *L. japonicus* are orthologous to *DMI1* (Ane et al., 2004; Imaizumi-Anraku et al., 2004). The *DMI2* encode the protein from the family of leucine-rich repeat (LRR) receptor kinases (Endre et al., 2002). The *Sym19* gene from pea is orthologous to *DMI2 M. truncatula*, *NORK* from *M. sativa* and *SYMRK* gene from *L. japonicus* (Schneider et al., 1999; Stracke et al., 2002). Protein encoded by the *DMI3* gene belongs to the family of Ca^{2+}/calmodulin-dependent protein kinases and may act as calcium-sensitive effector that operates downstream of calcium spiking (Levy et al., 2004). The *Sym9/30* gene from pea is orthologous to *DMI3*. Both *NSP1* and *NSP2* encode transcription factors of GRAS family and may be involved in DNA binding with the gene-targets (Smit et al., 2005; Kalo et al., 2005). The *Sym7* gene from pea was shown to be orthologous to *NSP2* from *M. truncatula* (Kalo et al., 2005). Thus, in pea, six loci (*Sym 8, Sym 10, Sym 19, Sym9/30* and *Sym7*) seem to control early steps of symbiosis development between legume plants and rhizobia. As expression of most of the symbiosis-specific proteins in *Medicago* depends on *NFP, DMI1, DMI2, DMI3* and *NSP1, NSP2*, we suggested that orthologous components of NF signal transduction pathway in pea (*Sym 10, Sym 8, Sym 19, Sym9/30* and *Sym7*) should be involved in symbiosis-specific gene activation.

To test our supposition, we analysed the expression of nodulin genes in pea mutants blocked at different stages of symbiosis development. Information about nodulin expression in pea mutants is very limited. Previously only pea plants mutated in the symbiotic gene *sym8* were assessed and showed no expression of some nodulin genes (Albrecht et al., 1998). Here we present a dissection of the NF signalling in pea using specific gene markers that are consequently triggered in the signalling and infection phases of NF-induced signal transduction pathway.