Preface

Molecular chaperones are a ubiquitous class of proteins that play important roles in protein folding and in the protection of cells from several stresses associated with the disruption of three native dimensional structures of proteins. The most important of these proteins are the so-called heat shock proteins (HSPs), also known as stress proteins. This book examines some of the biological aspects of this intriguing family of proteins that are important for consideration of the "proteiomics of HSPs." This book also reviews current research on protein folding in the endoplasmic reticulum (ER) and the functions of ER-resident molecular chaperones in protein folding in the ER. The biochemical, structural and functional information on Redox Enzyme Maturation Proteins (REMPs) are also reviewed in detail. Furthermore, recent progress in molecular biology has provided new insights into the molecular basis of diseases and molecular targets for diagnosis and therapy of human diseases. The role of molecular biology research in molecular imaging is examined, as well as the applications of molecular imaging in diagnostics, gene therapy and drug development. Other chapters in this book explore the role of protists as promising objects for the study of adaptive mechanisms at the biochemical and the molecular level, the different trends in the evolution of molecular adaptations to adverse environmental conditions, and a review of the molecular mechanisms of bicyclol in the protection against liver damage.

Chapter I - An important function of the endoplasmic reticulum (ER) is the folding of newly synthesized polypeptide chains. Several general and specialized chaperones operate in the ER to assist protein folding. The HSP70 class chaperones BiP and GRP170, their DnaJ and GrpE co-chaperones, the HSP90 class chaperone GRP94, and the lectin chaperones calnexin, calmegin, and calreticulin are the major general chaperones of the ER. HSP47 is an example of a specialized chaperone required for collagen folding. Protein foldases such as protein disulfide isomerases and *cis-trans* peptidyl prolyl isomerase catalyze two otherwise rate-limiting steps in protein folding, formation and isomerization of disulfide bonds and the *cis-trans* isomerization of peptidyl-prolyl bonds. Chaperone-assisted folding of the polypeptide chain, disulfide bond formation and posttranslational modification of the polypeptide chain, most notably by addition of large oligosaccharides to exposed asparagine residues by oligosaccharyl transferase interact in the ER in a complex manner to produce properly folded proteins. In addition to functioning in protein folding chaperones participate in other vital functions of the ER, such as import of polypeptide chains by translocation,

quality control of protein folding status and targeting of permanently unfolded proteins for retrograde translocation and proteasomal degradation, and regulation of the activity of ER Ca^{2+} channels. In this chapter I review our current knowledge of protein folding in the ER and of the functions of ER-resident molecular chaperones in protein folding in the ER.

Chapter II - Common to many bacteria is the ability to establish a symbiotic relationship or to evade innate immune responses of an animal, plant, fish or insect host. Most often this capacity is mediated by a type III secretion system (T3SS). The function of these complex molecular machines is likened to a syringe-needle injection device that is dedicated to the translocation of effector proteins directly into target eukaryotic cells. Each translocated effector tends to possess a distinct enzymatic activity that aids in subverting host cell signaling for the benefit of the bacterium. Their translocation requires another class of secreted protein – the translocator – which form pores in the target eukaryotic cell plasma membrane through which the effectors may transit to gain entry into the cell interior. Most often, each secreted substrate requires a dedicated small, non-secreted cytoplasmic chaperone for their efficient secretion. Unlike traditional molecular chaperones, these specialized type III chaperones do not assist in protein folding and are not energized by ATP. Controversy still surrounds their primary role; as bodyguards to prevent premature aggregation or as pilots to direct substrate secretion through the correct T3SS. The later is supported by recent evidence that these chaperones can dock directly to the cytoplasmic face of the T3S machinery, possibly serving as a recognition motif for substrate secretion. Added to this functional complexity is their important contribution to system regulation, which can ultimately confer temporal order to substrate secretion. Moreover, some chaperones display a bewildering propensity to interact with several additional T3S-associated proteins – the relevance of which remains uncertain. Structural data has now appeared for several important type III chaperones, either alone or in complex with their cognate substrate. This is proving a fillip in our attempts to understand the mercurial ways in which these versatile proteins operate in nature. It is hoped that this article will provide information on type III chaperone function, as well as highlighting key recent advances in the field. May it also be a testament to the value of continued intense effort in unravelling the mysteries of type III chaperone biology.

Chapter III - Chaperones are a large group of unrelated protein families that stabilize unfolded proteins, unfold them for translocation across membranes or degradation, and assist in their correct folding and assembly. They represent one of the most ancient and evolutionarily conserved protective protein families found in nature. A fundamental group of molecular chaperones is the so-called heat shock proteins (HSPs), also known as stress proteins. Originally discovered as inducible molecules capable of maintaining cellular homeostasis against abrupt temperature changes, HSPs were later considered an adaptive physiological response that protects against a variety of different cellular proteotoxic stresses. Early in the study of these proteins, it was evident that these molecules also have physiological roles that facilitate the synthesis, folding, assembly, trafficking, and secretion of specific proteins in various cellular compartments in the absence of significant pathological processes. In summary, these proteins guard the cellular proteome against misfolding and inappropriate aggregation.

From a clinical point of view, modification of the chaperone proteome, mainly the induction of HSPs, has been observed in a wide spectrum of inflammatory and degenerative diseases, including cancer, infectious disease, autoimmune processes, neurodegenerative conditions, and prion disease. The involvement of HSPs in these diverse diseases highlights the importance of the chaperone machinery not only in cell biology, but also in pathophysiology. At the same time, the induction of HSPs in diseases suggests potential clinical applications for molecular chaperones, particularly HSPS, in the diagnosis, prognosis and, above all, therapy of different degenerative and inflammatory human diseases. On this basis, proteomic approaches represent a valuable method to study the roles, structural interrelationships, and intimate molecular mechanisms of the major chaperone families that have been insufficiently characterized, limiting their diagnostic and therapeutic potential.

Chapter IV - A group of bacterial system specific chaperones are involved with the maturation pathway of redox enzymes that utilize the twin-arginine protein translocation (Tat) system. These chaperones are referred collectively as REMPs (Redox Enzyme Maturation Protein). They are proteins involved in the assembly of a complex redox enzyme which itself does not constitute part of the final holoenzyme. These proteins have been implicated in coordinating the folding, cofactor insertion, subunit assembly, protease protection and targeting of these complex enzymes to their sites of physiological function. The substrates of REMPs include respiratory enzymes such as N- and S-oxide oxidoreductases, nitrate reductases, and formate dehydrogenases, which contain at least one of a range of redox-active cofactors including molybdopterin (MoPt), iron sulfur [Fe-S] clusters, and b- and c-type haems. REMPS from *Escherichia coli* include TorD, DmsD, NarJ/W, NapD, FdhD/E, HyaE, HybE and the homologue YcdY. The biochemical, structural and functional information on these REMPs are reviewed in detail here.

Chapter V - Recent progress in molecular biology has provided new insights into the molecular basis of diseases and molecular targets for diagnosis and therapy of human diseases. Molecular imaging is a research discipline aimed at development and testing of novel tools, reagents and methods to image specific molecular pathways *in vivo* that are key targets in disease process and appear much earlier than anatomical and physiological changes. The advancement in the field of imaging and therapy of diseases is mainly due to the vast information available from molecular biology research on new targets with specific ligands and methods to evaluate their application in *in vitro* and *in vivo* systems. Improvement in imaging modalities like single photon emission computed tomography (SPECT), positron emission tomography (PET), magnetic resonance imaging (MRI), computed tomography (CT) and optical imaging (OI) has also contributed in the progress of molecular imaging. First section of this chapter is focused on molecular imaging, different approaches adopted for development of molecular imaging agents and recent imaging modalities and their applications.

Molecular biological techniques used in *in-vitro* diagnostics are being adapted to the special requirements of imaging diagnostics and high affinity imaging is achieved based upon receptor-ligand, antigen-antibody, transporter-substrate and enzyme–substrate interactions. Development of newer approaches based on reporter gene concept are solely dependent on molecular biology research tools. Small animal models of human diseases have become available after completion of human genome project. Noninvasive imaging of molecular,

genetic and cellular processes in animal models complements established *ex vivo* molecular biological assays and imaging provides a new dimension to understanding of various diseases. Role of molecular biology research in molecular imaging is discussed in second section of this chapter. Development of imaging agents based on peptides and role of molecular biology methods in identification of their targets to development of labelled ligands and their evaluation in *in-vitro* and *in-vivo* systems is also discussed.

Chapter concludes with the applications of molecular imaging in diagnostics, gene therapy and drug development. Advancements in biology and medicine is possible due to synergism between various new disciplines, especially molecular biology research has contributed significantly towards progress of molecular imaging.

Chapter VI - Three groups of proteins associated with misfolded protein depending aggregates were identified in *Saccharomyces cerevisiae* cells by using a new approach: comparative analysis of crude lysate pellets of isogenic yeast strains differing by their prion composition or adenine biosynthesis pathway characteristics. 2D electrophoresis followed by MALDI analysis of a recipient [*psi*$^-$] strain and of [*PSI*$^+$] cytoductant permitted identification of 53 proteins whose aggregation state depended on prion content or red pigment accumulation in yeast cells. Further studies allowed identifying an overlapping group of 38 proteins whose aggregation state responded to a shift of prion(s) content and also a rather similar group of more than 40 proteins whose aggregation state depended on accumulation of red pigment. In all these cases nearly one half of the identified proteins belonged to a functional group of chaperones and enzymes involved in glucose metabolism. Notable were proteins involved in oxidative stress response and in translation. The prion dependent group also contained a proteinase. These results are comparable with recent literature data on various misfolded proteins containing aggregates in yeast cells. Being not dependent on cloned heterologous genes, our approach permits a conclusion about universal presence of glucose metabolism enzymes in such aggregates. Most of the identified proteins, although behaving like prions in some experiments (for example, being "transmittable" by cytoduction), seem to be just amyloid-associated and mobilized to pellets in response to presence of prion fibrils. Our model experiments demonstrate that red pigment binds insulin fibrils and blocks their interaction with Thioflavine T. This allows concluding that red pigment impedes mobilization of some prion-associated proteins to prion-containing aggregates and so makes them to appear as pigment depending ones. Also there are some proteins (e.g. Sod1p and Cus1p) that themselves can be "clients" of a hypothetical prion generation pathway dealing with not NQ-rich proteins in yeast.

Chapter VII - The divergent loop cyclophilin, cyclophilin 40 (CyP40), initially discovered in association with the estrogen receptor, is now recognized as an immunophilin cochaperone common to all steroid receptors. This unique peptidylprolyl isomerase, the first tetratricopeptide repeat (TPR) cyclophilin to be identified, contains a C-terminal TPR domain through which it shares structural identity with FKBP52 and other partner cochaperones in steroid receptor-Hsp90 complexes. By dynamically competing for Hsp90 interaction, the cochaperones allow the receptors to establish distinct Hsp90-chaperone complexes, with the potential to exert tissue-specific control over receptor activity. CyP40 regulates Hsp90 ATPase activity during receptor-Hsp90 assembly. Functional deletion of the yeast CyP40 homologue, Cpr7, adversely affected glucocorticoid receptor and estrogen receptor α activity

that could be fully restored, either with wild type Cpr7 or Cpr7 with a cyclophilin domain lacking isomerase activity. We draw parallels with the mechanism already established for FKBP52 and propose that the CyP40 divergent loop, within the N-terminal cyclophilin domain, interfaces with a contact surface on the steroid receptor ligand-binding domain to achieve an optimal orientation for receptor activity. The cyclophilin domain also mediates association with dynein, suggesting a role for CyP40 in nuclear translocation of steroid receptor-Hsp90 complexes from the cytoplasm, as proposed for other immunophilin cochaperones. CyP40 chaperone function has been mapped to a central linker region separating the cyclophilin and TPR domains. Although not essential for viability, CyP40 homologues are important for normal cell growth and mitosis in yeast and in progression of vegetative growth in plants. These may be linked to CyP40 client proteins other than steroid receptors, including diverse kinases and transcription factors. The recent development of CyP40 knockout mouse models provides an attractive opportunity to address fundamental questions regarding the physiological role of CyP40 in mammals.

Chapter VIII - Heat shock proteins (HSP) are closely involved in response of organisms to adverse natural and anthropogenic factors. Within one of the recent directions of HSP studies, attempts are made to uncover mechanisms underlying the organisms' adaptation to various stresses. Promising model objects for this research are protists – lower eukaryotes that are at the same time a cell and a fully fledged organism.

In this paper, we present some results of our studies on HSP70 level in intact cells of several free-living protists and on the characteristics of its dynamics in the cells in response to the changes in natural environmental factors, salinity and temperature. The protists chosen for the study, the amoebae and the ciliates, possess an essentially different organization of the cell and belong to the most phylogenetically distant groups.

In many cases, a high constitutive level of HSP70 was recorded in intact cells under normal (non-stressful) conditions. It may be considered as a universal pre-adaptation of these protists to possible drastic environmental changes.

The strains of Amoeba proteus and several related species were very similar as to the level of HSP70 and the position of the stained zone on the blots, despite differences in geographic provenance, temperature conditions in natural habitats the strain was isolated from, and the strain age. Species of other genera of freshwater lobose amoebae studied, though close to Amoeba, differed considerably in the HSP70 level.

Out of the seven strains of the facultative parasites Acanthamoeba, only two had a noticeable constitutive level of HSP70. Differences in the constitutive level of HSP70 in the cells of different acanthamoebae strains may reflect their potential pathogenicity.

The ciliates used in the study represented various ecological groups different as to their attitude to environmental salinity. They were shown to employ various strategies of the chaperone system response to increasing and decreasing salinity of the medium. Constitutive level of HSP70 in their cells correlated with the degree of the salinity tolerance.

Chapter IX - The endoplasmic reticulum is an essential cellular compartment with many diverse functions. It is the main Ca^{2+} storage site and is involved in maintaining Ca^{2+} homeostasis. The endoplasmic reticulum is the first compartment of the secretory pathways, making it the entry compartment for approximately one-third of all the proteins synthesized by the eukaryotic cell. The endoplasmic reticulum contains unique enzymes, maintaining an

oxidative environment that allows co- and post-translational modifications such as glycosylation and disulfide bond formation, as well as molecular chaperones that assist in protein folding and quality control of newly synthesized membrane and secretory proteins. Protein folding in endoplasmic reticulum is controlled by endoplasmic reticulum quality control mechanisms. The primary players of endoplasmic reticulum quality control are the molecular chaperones like calnexin and calreticulin that reside in the endoplasmic reticulum. The mechanism of endoplasmic reticulum quality control assures that only correctly folded, functional proteins will exit the endoplasmic reticulum whereas non-native, misfolded proteins will be degraded via endoplasmic reticulum-associated degradation. ERAD pathway is closely connected to unfolded protein response pathway that is involved in its induction and regulation.

Chapter X - HspB8 is one of the recently described members of a large family of human small heat shock proteins. This family is presented by ten members that are characterized by a rather small molecular mass (16–28 kDa) and by the presence of a short (80-100 residues) α-crystallin domain. HspB8 is widely expressed in different human tissues and its content is especially high in muscles and nerves. Expression of HspB8 is dependent on many factors and is induced under certain unfavorable conditions. HspB8 tends to form small homooligomers and seems to interact with other members of the family of small heat shock proteins forming different heterooligomers. HspB8 is phosphorylated by a number of different protein kinases and phosphorylation might affect its structure and chaperone-like activity. HspB8 prevents aggregation of partially denatured proteins both *in vitro* and *in vivo*. The detailed mechanism of chaperone-like activity of HspB8 in the cell remains unclear, however the data of literature indicate that HspB8 is able to directly interact with denatured proteins, activates elimination of denatured proteins by proteasomes, activates autophagy interacting with Bag3 or regulates phosphorylation of α-subunit of the translation initiation factor eIF2. HspB8 interacts with glycolytic enzymes, amyloid proteins, different heat shock proteins, protein kinases, RNA-binding protein SAM68 and biomembranes. The molecular basis underlying interaction of HspB8 with so many diverse substrates remains unknown; however it is supposed that having an intrinsically disordered structure HspB8 can adopt different conformations suitable for interaction with different ligands. Interacting with so many substrates HspB8 seems to be involved in regulation of apoptosis, cell differentiation and proliferation and protects the cell from accumulation of denatured and aggregated proteins. Therefore point mutations of HspB8 correlate with development of certain neurodegenerative diseases and the level of HspB8 expression might affect cardiac hypertrophy and carcinogenesis.

Chapter XI - Small stress or heat shock proteins (sHSP) are involved in protective activity against some of the most important pathologies affecting human health including cancer and neurodegeneration. The fundamental molecular mechanism for the function(s) of small stress proteins (sHSPs or small heat shock proteins) are just beginning to be understood. The archetype for small stress proteins is human alphaB crystallin where multiple interactive sequences were identified using protein pin arrays and confirmed using site directed mutagenesis. A dynamic equilibrium between subunits of alphaB crystallin and with large polydisperse complexes has been described. The interactive domains were mapped to a homology model of the surface of the small stress proteins. The surface exposure of the

interactive domains varies with unfolding and self assembling proteins. Peptides were synthesized on the basis of the interactive sequences and were found to be active in assays for protection against proteins associated with protein unfolding diseases. The equilibrium between sHSP subunits and assembled complexes may be a dynamic mechanism for regulation of stress protein function. Characterization of the functional mechanism of small stress protein action is expected to lead to novel therapies for diseases of aging.

Chapter XII - The chaperonin containing TCP-1 (CCT) is found in the cytosol of all eukaryotic cells. It is an oligomer formed from two back to back rings, each containing eight subunits that surround a central cavity. Each CCT subunit contains three domains: an equatorial domain containing an ATP binding site, a substrate binding domain which displays the least sequence similarity between other CCT subunits and a linker domain. The folding cycle of CCT is ATP driven and the subunits of each chaperonin ring hydrolyse ATP in a sequential manner. For actin and tubulin it has been demonstrated that these proteins initially bind to CCT in an open conformation. Following the nucleotide cycle of CCT they become compact whilst remaining bound to one or more chaperonin subunits. This is in contrast to the mechanism of the prokaryotic chaperonins for which it is thought that folding substrates are encapsulated within the chaperonin cavity without direct interactions with chaperonin subunits.

The sequence diversity of the CCT substrate binding domains provides a complex binding interface for potential substrates. The major folding substrates of CCT are the abundant cytoskeletal proteins actin and tubulin, whilst other less abundant proteins such as the cell cycle regulating proteins Cdc20 and Cdh1 are known to require interactions with CCT to reach their native state. The numbers of CCT substrate proteins and the way in which CCT binds to its substrates is a matter for debate. At present it is estimated that in yeast up to 300 proteins may bind to CCT but it is not yet known how many proteins are obligate substrates. There are two major theories regarding how CCT recognises its folding substrates. The first proposes that CCT is a relatively general chaperone, recognising hydrophobic binding determinants, whilst the second, at least in the case of actin, proposes that binding to CCT is sequence specific.

The mechanisms of CCT action and the diversity of potential CCT substrates will be discussed in relation to the activity of CCT having far reaching implications on the many cellular functions that depend on the activity of its folding substrates.

Chapter XIII - Plant growth is greatly affected by abiotic stresses, such as drought, high salinity and cold. Therefore, plants need to have defending systems against many stresses for survival. Molecular chaperone interacts with unfolded or partially folded protein subunits, e.g. nascent chains emerging from the ribosome, leading to stabilizing native proteins, preventing aggregation of denatured proteins and degradation of defective or improperly folded proteins. Many molecular chaperones are stress proteins and most of them, but not all, have been identified as heat shock proteins (HSPs). HSPs/molecular chaperones and the heat stress elements in their promoters are conserved in all the eukaryotes, suggesting that they essentially have same function in response to high temperature stress. Participation of HSPs/molecular chaperones in other stresses has been also reported in plants. Furthermore, other types of proteins with chaperone functions, such as protein disulfide isomerase (PDI) and calnexin/calreticulin are up-

regulated by the stress. They play a role for preventing aggregation by assisting refolding of nonnative proteins. It has been shown that expression of HSPs/molecular chaperones increases for assisting the deposition/assembly of high abundant secretory storage proteins in endoplasmic reticulum (ER) lumen during seed development. HSPs/molecular chaperones in plants are not well understood, however, they are elucidated little by little.

In this review, we first describe the characteristics of HSPs/molecular chaperones in plants. Then we describe the roles of HSPs/molecular chaperones in abiotic stress (heat and cold etc.) and ER stress during seed development, including enhanced chaperones accompanying by deposition of recombinant products in transgenic plants.

Chapter XIV - Intrinsically disordered proteins (IDP) are unfolded/unstructured under native conditions. They play important roles in living organisms, mostly in signal transduction and regulation of biochemical pathways. Biochemical studies showed that some IDPs also have protective effect on partner molecules, or enhance folding of a specific protein, i.e. they have chaperone activity. In our studies we have analyzed the chaperone activity of six IDPs and found that all of them are active in a variety of assays employed. The studies were carried out with two plant stress proteins from the dehydrin family (ERD10 and ERD14, *A. thaliana*), and four *E. coli* ribosomal proteins (L15, L16, L18, L19). We have tested these proteins in both the prevention of substrate aggregation and deactivation, and in the refolding/reactivation of a denatured partner molecule. We found that ERD10 and ERD14 hinder aggregation and also deactivation, however they do not assist refolding. The ribosomal proteins significantly enhance the refolding and reactivation of a denaturated protein and also prevent the deactivation of substrate molecules under denaturing conditions. A combination of their chaperone activities and molecular properties (tolerance against mutation, resistance against aggregation, etc.) elevate these IDPs to a unique level of functionality and stability, due to which they play an essential role in the survival of strong environmental stress conditions and probably also in sudden changes in protein stability caused by a mutation for example.

Chapter XV - Fluorescence spectroscopy of tryptophan residues and the 8-anilino-1-naphthalenesulfonate probe and light scattering were used to study some properties of bovine α-crystallin, in particular, its thermo- and photo-induced aggregation. The effective diameter of the native α-crystallin globule, calculated from the polarization and life-time of 8-anilino-1-naphthalenesulfonate (ANS) using the Levshin–Perrin equation, amounts to 90 Å and increases during aggregation to at least 140 Å. The decrease in the tryptophan fluorescence intensity in the course of α-crystallin thermo- and photo-denaturation and aggregation is caused by local conformational alterations in the environment of the tryptophan residues and by light scattering. Tryptophan residues in the aggregates are hidden in the interior. Thermal aggregation of the protein takes place not only at high temperatures. Extrapolation of the experimental time dependence of slow spontaneous aggregation to the long time range allows one to find the "denaturation time" t_e. The t_e value for α-crystallin (at a concentration of 0.8 mg/ml in phosphate buffer at pH 8.4) is about 100 h. Using steady-state, polarized, and phase-modulation fluorometry, the DTT-induced denaturation of insulin and formation of its complex with α-crystallin in solution were studied. Prevention of the aggregation of insulin by α-crystallin is due to formation of a chaperone complex, i.e. tight interaction of chains of the denatured insulin with α-crystallin. The conformational changes in α-crystallin that occur

during complex formation are rather small. It is unlikely that N-termini are directly involved in the complex formation. It has been shown that ANS is not sensitive to the complex formation. ANS emits mainly from α-crystallin monomers, dimers, and tetramers, but not from oligomers or aggregates. The possibility of highly sensitive detection of aggregates by light scattering using a spectrofluorometer with crossed monochromators is demonstrated.

Chapter XVI - Bicyclol is a new potent anti-hepatitis drug and has been approved to treat viral hepatitis in China since 2004. Pharmacologically, bicyclol protects against drug and chemical-induced liver injury, and inhibits hepatitis virus replication in duck viral hepatitis and in a HepG2.2.15 cell line. Bicyclol exerts most of its effects by eliminating free radicals, maintenance of mitochondrial glutathione redox status, anti-inflammation and anti-apoptosis. However, further elucidation of the molecular mechanism of bicyclol in protection against liver damage is still needed.

Heat shock proteins (HSPs) are a family of constitutive and inducible expressed gene products that collectively function to maintain cellular protein conformation during stressful conditions. HSPs can be induced during acute or chronic stress as a result of protein misfolding, aggregation or disruption of regulatory complexes. Prior induction of HSPs protects cells from subsequent lethal insults. The up-regulation of HSPs expression constitutes an important cellular defense mechanism. Therefore, it would be of great therapeutic benefit to discover compounds that are clinically safe and able to enhance the expression of HSPs.

Since bicyclol has cytoprotective action against liver injury, a question arises whether the effect of bicyclol on liver injury is mediated through induction of hepatic HSPs. Our study demonstrated that bicyclol significantly induces the hepatic HSP27 and HSP70, which play a key role in protection against liver injury.

Oral administration of bicyclol alone significantly induced hepatic HSP27 and HSP70 expression both in protein and mRNA levels in a time- and dose-dependent manner through activation of heat shock factor-1 (HSF1) in mice, but no inducing effect on HSP27 and HSP70 in mouse spleen and kidney was observed. In *in vitro* study of HepG2 cells, bicyclol can enhance HSP27 and HSP70 promoter activities, indicating that bicyclol induces transcription of the HSPs genes.

The hepatoprotective effect of HSP27 and HSP70 induced by bicyclol was confirmed in acetaminophen and concanavalin A (ConA) induced liver injury in mice. HSP27 and HSP70 suppressed the elevation of serum alanine aminotransferase (ALT) and aspartate aminotransferase (AST), liver tissue necrosis, the release of cytochrome c and apoptosis inducing factor (AIF) from mitochondria as well as hepatic DNA fragmentation caused by acetaminophen and ConA. Moreover, overexpression of HSP27 and HSP70 by bicyclol attenuated NF-κB activation induced by ConA either by inhibiting IκB degradation or by directly supressing the NF-κB transcriptional activity, and also inhibited D-galactosamine-induced activation of JNK signal transduction pathway. These results suggest that the protective action of bicyclol against liver injury is mediated by its induction of HSP27 and HSP70, which provide new evidence for elucidating the mechanism of cytoprotective effect of bicyclol against liver injury both in animals and patients.

Chapter XVII - Similarly to all other organisms plants are constantly exposed to different environmental abiotic and biotic factors. Some of these agents can exert strongly negative

aggregated proteins, maintenance of the cell cycle, interaction with steroid hormone receptors and signal transduction pathway for steroid hormone).

Over the last few years we have been focusing on various stress stimuli in homeothermic livestock, namely, environmental, nutritional and psychological. Our findings led us to hypothesize that upon stress, the Hsp response may be bi-directional regarding the type of stimulus and the tissue tested. While in some cases Hsp levels increase, in other instances it goes the other way round. In those situations (nutritional stress) where Hsp levels decrease, a concomitant induction of other genes, related to the function of the specific tissue has occurred. This is in marked difference from the classical Hsp response, in which, in parallel with the induction of Hsp the expression of other proteins is silenced.

Chapter XX - The chaperonin containing T-complex polypeptide (CCT) is the major cytosolic chaperonin in eukaryotes and has been estimated to interact with up to 15% of all cellular proteins. The CCT holoenzyme is a hexadecameric molecule comprised of two copies each of eight discrete subunits, each of which shares partial homology to the others, as they are thought to have evolved from a single common ancestor gene. CCT has principally been implicated in the folding of cytoskeletal proteins such as tubulin and actin, and has been noted as an important factor in a variety of cellular processes, including cell proliferation, embryogenesis, ciliary biogenesis, etc.

The majority of studies on CCT function have thus far focused on the 16-mer holoenzyme; however, an increasing body of evidence suggests that the individual subunits of CCT may have independent function and significance. This evidence derives from several different types of observations: the effects of naturally occurring (or experimentally-induced) mutations in individual CCT subunits, the differential patterns of expression and localization displayed by various CCT subunits, and direct experimental demonstration of individual CCT subunit physiology in a capacity without the CCT aggregate enzyme.

Multiple spontaneous and induced mutations in CCT subunits have been identified that lead to discrete phenotypes: in CCT-epsilon (causing mutilating sensory neuropathy with spastic paraplegia in humans), in CCT-delta (causing the mutilated foot phenotype in rats), and in CCT-gamma (leading to the *no tectal neuron* phenotype in zebrafish, interfering with eye development). Investigations on expression of CCT subunits have demonstrated that not all subunits are coordinately expressed, and that some subunits localize within cells as monomers or as microcomplexes much smaller than the 16-mer CCT enzyme. In some instances a specific biological activity has been imputed to a single CCT subunit. The CCT-eta has been found to be a biological partner for the soluble guanylyl cyclase, affording it a role within nitric oxide signaling, and CCT-alpha has been shown to preferentially inhibit the polyglutamine-mediated toxicity of the huntingtin protein This seemingly disparate collection of observations together suggest that individual CCT subunits may have particular physiological roles apart from the CCT holoenzyme.

Chapter XXI - Hsp26 from *Saccharomyces cerevisiae* is represented by a 24-mer oligomer with the overall organization resembling that of a hollow globular sphere, which possesses co-chaperone activity. It functions in the recovery of misfolded proteins and prevent aggregation, but its *in vivo* role in protein homeostasis remains unclear. sHsps from some organisms are known to be posttranslational regulated by phosphorylation, where those covalent modifications regulate function and quaternary structure. Global analysis studies of

influence upon crop growth. Heat shock protein synthesis appears to be one of the major anti-stress responses conserved among all known organisms on our planet -from bacteria, plants and animals to human, which has unique nuance in plants. Studying and understanding of this defensive mechanism, despite the difficulties in finding appropriate model systems in plants, have great importance for the detection of markers against different kind of stresses and their practical application in genetically transferred crops.

This review summarizes and comments the main part of the researches for different heat shock protein families that can act as molecular chaperones in maintaining the cellular homeostasis. This paper demonstrates the significant role of these proteins in normal and stress conditions in plants, which are discussed in parallel. Classification of heat shock protein families and subfamilies and regulation of heat shock protein expression are presented, and there is information about different kinds of stress inductors in plants. This review explains the understanding for the interaction between different heat shock proteins in one general molecular chaperone network. Additionally, the article discusses thermotolerance and the main participants in this acclimation process.

Chapter XVIII - Heat shock proteins (Hsps) provide cellular and whole body adaptation of animals to various adverse environmental conditions due to their diverse chaperone properties. Hsp70 is apparently the major player underlying biological adaptation in all organisms studied so far.

Therefore, in our experiments to analyze the patterns of heat shock system firing in different Diptera families we predominantly concentrated on the *hsp70* gene family expression at the transcription and translation levels. We have investigated several species in three families: Drosophilidae, Stratiomyidae and Chironomidae belonging to three suborders (Cyclorrhapha, Orthorrhapha and Nematocera).

All *Drosophila* species studied so far did not reveal any level of Hsps under normal physiological conditions but responded to heat shock (HS) by rapid synthesis of all Hsps. On the contrary, four Stratiomyidae species studied are characterized by extraordinary high concentration of Hsp70 in cells (phenomenon especially pronounced at the larval stage) under normal physiological conditions and hardly detectable in control but inducible synthesis of correspondent RNAs. Such a pattern, which implies high stability of Hsps in species belonging to this family, is probably responsible for wide range of Stratiomyidae species in various habitats, including highly aggressive ones. Preliminary studies of Chironomidae inhabiting cold running waters (larvae of two species in the subfamily Diamesinae) indicate that representatives of this subfamily exhibit high constitutive level of *hsp70* RNA in cells under normal conditions and seem to lose the ability to respond to heat shock treatment.

The data accumulated suggest different trends in the evolution of molecular adaptations to adverse environmental conditions occurring in the same insect order.

Chapter XIX - Heat shock proteins (Hsp) are considered cellular protective agents against a wide range of stressors; inducible expression of Hsp prevents misfolded proteins from aggregation, interfere with the cell death program and help cells recover and survive.

In normal resting cells Hsp are involved in various "house keeping" missions in the cell, form complexes with client proteins and act as molecular chaperones. This includes protein folding, assembly and translocation between compartments, degradation of misfolded or

yeast phosphoproteome identified phosphorylated peptide sequences in Hsp26. To study the *in vivo* phosphorylation of yeast Hsp26, the gene enconding Hsp26 was overexpressed from a multicopy plasmid using its own promoter. Hsp26 was purified from stationary phase cells to homogeneity by a procedure already described in our lab. The purification method used consisting of three steps: ethanol precipitation, sucrose gradient ultracentrifugation, and heat inactivation of residual contaminants produced native Hsp26 protein. The purified Hsp26 was shown to be phosphorylated in its serine-peptides. Hsp26 was resolved in four isoforms, displaying the same molecular masses but different isolelectric points. A MALDI/MS analysis of the isoforms led to the identification of a phosphopeptide 37-QLANT**(p)**PAK-44 at the N-terminus of Hsp26. If the isoforms represent multiple phosphorylated forms of Hsp26 is an open question.