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KEY=MICROORGANISMS - TOWNSEND FLORES

Regulated Proteolysis in Microorganisms

Springer Science & Business Media **This book contains an extensive collection of critical reviews, from leading researchers in the field of regulated protein degradation. It covers the role of regulated proteolysis in a range of microorganisms (from Gram positive, Gram negative and pathogenic bacteria to Archaea and the Baker's yeast Saccharomyces cerevisiae).**

Understanding and Harnessing Energy-dependent Proteolysis for Controlled Protein Degradation in Bacteria

Regulated intracellular protein degradation is critical for cellular viability. In many organisms, degradation controls cell-cycle progression, executes responses to stress-inducing environmental changes, and enables the rapid depletion of unwanted or deleterious proteins. In bacteria, most processive protein degradation is carried out by a family of AAA+ compartmentalized proteases. These molecular machines convert the chemical energy of ATP binding and hydrolysis into mechanical work, forcefully unfolding their substrates as a prelude to proteolysis. The AAA+

ClpXP protease, recognizes short peptide tags (degrons) in substrate proteins either directly or with the aid of dedicated specificity factors (adaptors). The prior identification and detailed biochemical characterization of an efficient ClpXP degron (the ssrA tag) and cognate adaptor (SspB) serve as powerful tools and enable the mechanistic studies presented here. In Chapter 2, I describe a collaborative investigation of substrate denaturation and degradation by ClpXP with single-molecule resolution. Detailed kinetic analysis of these experiments revealed homogenous protease activity across the population of enzymes with comparable levels of microscopic and macroscopic ClpXP activity. These experiments required the development of methods to attach ClpXP to surfaces and stabilize the multimeric enzyme at sub-nanomolar concentrations, advances that should be applicable to future single-molecule studies of complex protein machines. Subsequent chapters describe the development of molecular tools that harness our understanding of targeted proteolysis and enable small-molecule control of degradation. By engineering synthetic substrates, adaptors and proteases, I directly test models previously proposed to explain adaptor function and identify the minimal requirements for adaptor-mediated substrate delivery. Many different configurations of protease and adaptor domains lead to efficient, predictable substrate degradation and demonstrate the highly modular nature of this system. These tools allow for facile, small-molecule controlled protein degradation in vivo and should be valuable in basic research and biotechnology. I also describe a family of synthetic insulated promoters that allow predictable, context-independent levels of protein synthesis.

Limited Proteolysis in Microorganisms

Biological Function, Use in Protein Structural and Functional Studies : a Conference

Investigating the Mechanism of

Substrate Delivery by Adaptor Proteins During Regulated Proteolysis in Bacteria

Aspects of Metabolic Regulation in Rumen Bacteria

Regulation of Biomineralization in Magnetotactic Bacteria

Abstract Regulation of Biomineralization by an HtrA Protease in Magnetotactic Bacteria by Patrick Jeffrey Browne Doctor of Philosophy in Microbiology University of California, Berkeley Professor Arash Komeili, Chair Magnetotactic bacteria are a unique group of bacteria characterized by their ability to manufacture magnetic crystals for the purpose of aligning with geo-magnetic fields. They offer a unique opportunity for merging the study of bacterial cellular biology, classic molecular genetics, biochemistry, and evolution, both on the small scale of individual proteins, and a larger system of proteins with a defined complex function. This dissertation will first look at the genetics and evolution of magnetotactic bacteria as a whole before focusing on MamE, an HtrA protease conserved throughout magnetotactic bacteria. HtrA proteases are ubiquitous throughout all domains of life and serves several different functions. This dissertation will show that MamE is a multifunctional protein and will also highlight other magnetosome related genes that either regulate MamE's activity or are targets of proteolysis. The first chapter of this dissertation is a to-be-submitted review article and introduces the genetics of magnetotactic bacteria (MTB), a paraphyletic group of gram-negative bacteria that use around a hundred genes, located in a defined gene island, to create magnetosomes, membrane-bound compartments containing a magnetic iron crystal. By organizing magnetosomes into a chain, cells are able to align with geo-magnetic fields. It will discuss the most well-conserved genes whose general functions are known. It also describes the interesting evolutionary history of the magnetosome gene island (MAI) which despite being an island seemingly capable of horizontal gene transfer, does not contain other hallmarks of recent HGT and closely matches the phylogenetic tree. The second chapter, a published primary research article (Hershey et al., PLOS Biology 2016, and) is focused on

MamO, a protein that also appears to also be an HtrA protease similar to MamE. However, closer examination shows that is contains no protease activity and is instead a metal-binding protein most likely involved in crystal nucleation. It is also shown to not only be a target of MamE proteolysis but also regulate MamE's activity. Finally, it contains an analysis of MamOs throughout MTB and shows that they are not actually a single protein family. Instead, four separate lineages of MTB have all acquired inactive proteases in a convergent manner. The third chapter, a published primary research article (Hershey et al., Journal of Biological Chemistry 2016) is the first to focus primarily on MamE. Unlike MamO, this chapter shows that MamE is a bonafide protease and identifies several targets of MamE, including MamE itself, MamO, and MamP. It also interrogates the kinetics of MamE's protease activity and shows that MamE processes its targets in a regulated manner. The fourth chapter, to be submitted as a primary research article, also investigates MamE and its targets, this time beginning with a proteomic approach. Cells carrying an inactive form of MamE have deformed smaller crystals. Using a novel method of cell lysis and magnetosome separation, wild-type and mutant magnetosome are compared. This method uncovered a few potential additional targets of MamE proteolysis, one of which was confirmed in vitro. This protein, MamD was also further investigated and determined to possibly function as a biomineralization inhibitor. This chapter also discusses the proteome of the magnetosome as a whole, defining proteins enriched in the magnetosome Finally, the fifth chapter contains unpublished material related to MamE and ends with concluding thoughts and perspectives. It includes a preliminary investigation of how magnetosome proteins reach the magnetosome, a poorly understood topic, but one that MamE also plays a role in. It will also identify one further potential target of MamE proteolysis, MamT, and investigates how the magnetochrome domain, shared by MamE, MamP, and MamT is crucial to the function of all three proteins but not in identical ways. Finally, it will include thoughts on ways to move forward in the field of magnetosome genetics and molecular biology.

Adaptors at Work

Regulation of Bacterial Proteolysis by Adaptor Hierarchies

Regulated protein degradation is essential for all life. Bacteria use energy-dependent proteases to regulate protein degradation. Recognition of a substrate is enabled by the inherent specificity of the protease and by the use of adaptor proteins that widen the spectrum of recognized substrates. In Caulobacter crescentus, the timed destruction of many regulators

including CtrA by the ClpXP protease drives cell cycle progression. Although, in a test tube, ClpXP can degrade CtrA by itself and does not need any helping factors, additional factors such as CpdR, RcdA and PopA are required in vivo. Understanding how these factors modulate protease activity at the mechanistic level is the major focus of this dissertation work. In this work, we show that these factors constitute an adaptor hierarchy where different substrates are destroyed based on the degree of adaptor assembly. The hierarchy builds upon priming of ClpXP by the adaptor CpdR, which promotes degradation of one class of substrates like PdeA and which also recruits the next level of adaptor RcdA to degrade a second class of substrates such as TacA. The third cyclic-di-GMP dependent adaptor PopA binds RcdA to promote destruction of a third class of substrates such as CtrA. Because adaptors must bind their cognate proteases, all adaptors run the risk of themselves being recognized as the substrate and hence degraded by the protease, a process that could limit their effectiveness. Indeed, we find that RcdA is readily degraded by CpdR-activated ClpXP protease when present alone but cargo engagement restrains its degradation. By using chimeric proteins, we find that the ability of a cargo to protect its adaptor is not due to global stabilization but is specific to the native protease recognition elements of that adaptor. We find that this principle extends across several adaptor systems, including the adaptor SspB. Together, this work reveals how hierarchical adaptors orchestrate regulated proteolysis during bacterial cell cycle progression and how, robust adaptor activity can be maintained by cargo engagement. Because of the high degree of conservation of many proteins between species, we speculate that principles found in the Caulobacter system likely generalize to others.

Novel Proteolytic Activities and Regulation of Proteolysis in Lactococcus Lactis

In order to access the main nitrogen source in milk, i.e. caseins, Lactococcus lactis possesses a complex proteolytic system to degrade these milk proteins to peptides and amino acids. The activity and function of a putative member of this system, an aminoacylase from L. lactis MG1363, was analysed in detail. In addition, the underlying regulatory mechanism of the proteolytic pathway, which results in differential expression of its major components in response to medium composition, was shown to be under the control of the transcriptional regulator CodY. Finally, a consensus DNA-binding site for CodY was determined. A putative aminoacylase-encoding gene, designated amd1, was identified on the chromosome of L. lactis MG1363. The deduced protein product of amd1

exhibits similarities to known aminoacylases from bacteria, archaea and plants. Amd1 does not appear to be expressed in L. lactis under laboratory conditions as judged by the lack of aminoacylase activity from wild type crude extracts. The insertion of an IS982 transposon immediately upstream of amd1 is probably the reason why this enzyme is not produced. The observed genetic organization in MG1363 was shown to be retained in seven other dairy strains, indicating, perhaps, an evolutionary retention of this disruption by natural selection in such an environment. The expression of amd1 in L. lactis NZ9800 under the control of the nisA promoter conferred a deacetylating phenotype on the lactococcal host, as judged by the hydrolysis of a number of N-acetylated substrates. Lactococcal aminoacylase was cloned, overexpressed in Escherichia coli and purified. The enzyme was characterized with respect to substrate specificity, pH, temperature and metal dependence. Amd1 exhibited a broad activity range towards N-acetylated-L-amino acids, with pH and temperature optima typical of that normally found inside a growing lactococcal cell (pH 7.0 and 30 oC). Interestingly, Amd1 was also able to hydrolyse several dipeptides. This characteristic also has biological significance since expression of amd1 was shown to complement a growth deficiency in a L. lactis triple peptidase mutant. Hence, two biological functions were proposed for Amd1. The first may have been as a dipeptidase, whose loss due to transposon disruption was compensated by other peptidases. The second function may have involved the turnover of acetylated amino acids and/or peptides, once they had been marked for digestion by the addition of acetyl group.

SwrA Transcriptional Activity is Controlled by Adaptor-mediated Proteolysis

Regulated Proteolysis of DnaA Coordinates Cell Growth with Stress Signals in Caulobacter Crescentus

DNA replication is an essential process in all domains of life. Replication must be precisely regulated, especially at the step of initiation. In bacteria, the replication initiator DnaA is regulated by multiple post-translational regulations to ensure timely replication. Caulobacter crescentus has the most strict replication regulation that DNA only replicates once per cell cycle, and proteolysis of DnaA identified in this species is the only irreversible way to inhibit DnaA, suggesting it might be pivotal to

restricting DNA replication. However, the responsible protease(s) and mechanism for its degradation remain unclear since its first discovery in 2005. In this thesis, I describe the efforts to characterize the proteolysis regulation on C. crescentus DnaA. I identified and characterized DnaA degradation by two different proteases, Lon and ClpAP. Lon is the dominant protease for DnaA degradation, and my work on this degradation revealed a novel allosteric regulation mechanism by which Lon links unfolded substrate concentration with DnaA proteolysis, and provides a way for Lon to rapidly eliminate DnaA and arrest replication during proteotoxic stress. Mechanistic studies of Lon-dependent degradation shows that a complicated mechanism governs the recognition and degradation of DnaA, including the existence of multiple degradation determinants and the dependency of DnaA activity state. In contrast, ClpAP plays an auxiliary role on DnaA degradation, but this degradation is enhanced during nutrient starvation stress. Interestingly, Lon degrades DnaA more rapidly when it is in a complex with DnaA loaded on the replication origin DNA, but a specific structure of DNA, G-quadruplex, strongly inhibits general substrate degradation by Lon. Taken together, the studies in this thesis revealed the complex mechanisms on DnaA degradation in Caulobacter crescentus, and provided insights on how cells interrogate proliferation status in changing environments by modulating the levels of a replication factor.

Bacterial Physiology and Metabolism

Cambridge University Press **Recent determination of genome sequences for a wide range of bacteria has made in-depth knowledge of prokaryotic metabolic function essential in order to give biochemical, physiological, and ecological meaning to the genomic information. Clearly describing the important metabolic processes that occur in prokaryotes under different conditions and in different environments, this advanced text provides an overview of the key cellular processes that determine bacterial roles in the environment, biotechnology, and human health. Prokaryotic structure is described as well as the means by which nutrients are transported into cells across membranes. Glucose metabolism through glycolysis and the TCA cycle are discussed, as well as other trophic variations found in prokaryotes, including the use of organic compounds, anaerobic fermentation, anaerobic respiratory processes, and photosynthesis. The regulation of metabolism through control of gene expression and control of the activity of enzymes is also covered, as well as survival mechanisms used under starvation conditions.**

A Novel Adapter Mechanism Regulates the Caulobacter Cell Cycle by Promoting the Degradation of the Transcriptional Regulator CtrA.

Caulobacter crescentus is a powerful model organism for understanding cellular differentiation, cell polarity and cell cycle regulation in bacteria. An elaborate network of two-component signaling proteins works to orchestrate the developmental program that characterizes the Caulobacter cell cycle. The essential DNA-binding response regulator CtrA is at the center of this regulatory scheme and acts to control the transcription of>100 genes that are required for cell cycle progression, motility, DNA methylation, morphology and other processes. Because CtrA also inhibits chromosome replication at specific stages of the Caulobacter cell cycle, its activity must be temporarily eliminated in order for DNA replication to occur. Inactivation of CtrA is achieved though dephosphorylation and regulated degradation by the broadly conserved energy-dependent protease ClpXP. In this dissertation, I analyze the roles of three proteins that are required for CtrA degradation in living cells. These are a single domain response regulator CpdR, a protein with no predicted function, RcdA, and a cyclic diguanylate (cdG)-binding protein, PopA. Structure-directed mutagenesis of RcdA was used to probe RcdA function. Results from these studies undermine the prevailing model for RcdA function, which suggest that RcdA does not participate directly in delivering CtrA to ClpXP, but instead acts simply as a localization factor increasing the concentration of CtrA at the cell pole where the protease is located. Additionally, I reconstituted the regulated proteolytic reaction in vitro and probed the role of all three accessory proteins and the small molecule cdG in promoting CtrA degradation. Although ClpXP alone is known to degrade CtrA in vitro, I observed a dramatic acceleration of proteolysis in the presence of the accessory proteins and cdG. This accelerated proteolysis was characterized by a nearly 10-fold reduction in the KM of the reaction, which is consistent with predictions for an adaptor mediated mechanism. I began to characterize protein-protein interactions within the proteolytic complex using in vivo and in vitro techniques. These experiments demonstrate that CtrA interacts directly with PopA in a cdG-dependent fashion. CtrA also interacts directly with RcdA and with ClpX. The CtrA-PopA(cdG) and CtrA-RcdA interactions are weakened or abolished by

mutations in the receiver domain of CtrA that slow its proteolysis in vivo. We propose a mechanism in which CtrA forms a ternary complex with PopA and RcdA in response to rising cdG concentrations in the cell. In this complex, PopA and RcdA act as a multi-protein adaptor complex to enhance the delivery of CtrA to the catalytic pore of ClpX. CpdR is required for accelerated CtrA proteolysis, but its precise role is still unknown. The accessory proteins were able to stimulate CtrA degradation even in the presence of a DNA fragment containing a CtrA binding site, which is known to inhibit CtrA proteolysis. Future work will determine if the accessory factors prevent the formation of inhibitory CtrA-DNA complexes or actively disassemble them. This dissertation alters the concept of proteolytic adaptors to include multi-protein complexes and expands the range of mechanisms by which proteolytic adaptors are controlled to include direct regulation by the small molecule cdG.

Co- and Post-Translational Modifications of Therapeutic Antibodies and Proteins

John Wiley & Sons **A Comprehensive Guide to Crucial Attributes of Therapeutic Proteins in Biological Pharmaceuticals With this book, Dr. Raju offers a valuable resource for professionals involved in research and development of biopharmaceutical and biosimilar drugs. This is a highly relevant work, as medical practitioners have increasingly turned to biopharmaceutical medicines in their search for safe and reliable treatments for complex diseases, while pharmaceutical researchers seek to expand the availability of biopharmaceuticals and create more affordable biosimilar alternatives. Readers receive a thorough overview of the major co-translational modifications (CTMs) and post-translational modifications (PTMs) of therapeutic proteins relevant to the development of biotherapeutics. The majority of chapters detail individual CTMs and PTMs that may affect the physicochemical, biochemical, biological, pharmacokinetic, immunological, toxicological etc. properties of proteins. In addition, readers are guided on the methodology necessary to analyze and characterize these modifications. Thus, readers gain not only an understanding of CTMs/PTMs, but also the ability to design and assess their own structure-function studies for experimental molecules. Specific features and topics include: Discussion of the research behind and expansion of biopharmaceuticals Twenty chapters detailing relevant CTMs and PTMs of proteins, such as glycosylation, oxidation, phosphorylation, methylation, proteolysis, etc. Each chapter offers an introduction and guide to the mechanisms and biological significance of an individual CTM or PTM, including practical guidance for experiment design and analysis An**

appendix of biologic pharmaceuticals currently on the market, along with an assessment of their PTMs and overall safety and efficacy This volume will prove a key reference on the shelves of industry and academic researchers involved in the study and development of biochemistry, molecular biology, biopharmaceuticals and proteins in medicine, particularly as biopharmaceuticals and biosimilars become ever more prominent tools in the field of healthcare.

Cell Cycle Regulation and Development in Alphaproteobacteria

Springer Nature

Microbial Metabolism and Disease

Academic Press **Microbiome Metabolic Pathways and Disease provides insight into the interaction of microbial metabolic pathways in the human body and the impact these can have on a variety of diseases. By analyzing these pathways the book seeks to investigate how these metabolic processes can be targeted and manipulated in order to treat various disorders and diseases. Topics covered in the book include microbial shikimate pathways, protein biosynthesis, tryptophan metabolites, microbiome metabolic engineering, fecal microbiota transplantation, and virulence factors. Additionally, a variety of conditions are covered, such as disorders associated with metabolic syndromes, serotonin syndromes, Alzheimer's disease, and Covid-19, providing a detailed overview of how metabolic pathways of microbiome can impact health and disease in the human body. Explores microbial metabolic pathways in the human body and implications for disease Investigates specific steps involved in metabolic reactions in the human microbiome, including shikimate pathways and tryptophan pathways Considers a variety of diseases and disorders, such as Alzheimer's disease, metabolic syndromes, Crohn's disease and Covid-19 Includes analysis of various amino acids and enzymes in microbial and human cells and how these can impact health**

Regulation of Bacteriophage^ Development

The Lytic-lysogenic Decision

Prokaryotic Metabolism and Physiology

Cambridge University Press **Extensive and up-to-date review of key metabolic processes in bacteria and archaea and how metabolism is regulated under various conditions.**

Microbial Enzymes in Aquatic Environments

Springer Science & Business Media **Organic matter in aquatic environments consists mostly of large compounds which cannot be taken up and utilized directly by microbial cells. Prior to incorporation, polymeric materials undergo degradation by cell-bound and extracellular enzymes produced by these microbes; in fact, such enzymatic mobilization and transformation is the key process which regulates the turnover of organic as well as inorganic compounds in aquatic environments. This volume brings together studies on enzymatic degradation processes from disciplines as diverse as water and sediment research, bacterial and algal aquatic ecophysiology, eutrophication, and nutrient cycling and biogeochemistry, in both freshwater and marine ecosystems. Its scope extends from fundamental research exploring the contribution of microbial enzymatic processes to whole ecosystem functioning to practical applications in water biotechnology. The first comprehensive publication providing an overview of this emerging field of enzymology, Microbial Enzymes in Aquatic Environments will be of great interest to ecologists and microbiologists alike.**

Regulation of Bacterial Chromosome Replication Origins Through Binding of 'response Regulator' Transcription Factors

and Regulated Proteolysis

The Regulation of DnaA in "Caulobacter Crescentus"

"All growing cells must ensure that their genetic material is faithfully replicated and divided equally to the newly formed daughter cells. Once chromosome replication has begun the cell must assure that it does not start replication until the next cell cycle. In nearly all bacteria chromosome replication is initiated by the highly conserved DnaA protein. In Escherichia coli the activity of the initiator protein DnaA is down regulated by the regulatory inactivation of DnaA (RIDA) system. Shortly after the initiation of replication, the intrinsic ATPase activity of DnaA is stimulated by Hda, a protein with homology to the ATPase domain of DnaA. Therefore, Hda converts DnaA from its active ATP bound form to its inactive ADP bound form. The current models of chromosome replication have been developed using the model bacteria E. coli. However, DnaA proteins form other bacteria differ from DnaA in E. coli in their structures and biochemical properties. A regulatory model based solely on E. coli does not take into account the diversity found among bacteria. Another model is provided by the free-living Gram-negative bacterium Caulobacter crescentus which is found in nutrient poor aqueous environments such as fresh water lakes. Genomic analysis predicts C. crescentus encodes a protein (HdaA) with homology to Hda of E. coli. C. crescentus also makes use of novel regulatory mechanisms not found in E. coli to regulate DnaA. Unlike DnaA of E. coli the DnaA protein of C. crescentus is unstable in growing cells, rapidly degraded during stationary phase and upon nutrient starvation. I have created a strain of C. crescentus with hdaA expressed under the control of a xylose dependent promoter. I demonstrated that C. crescentus depends upon a RIDA mechanism to prevent multiple initiations of chromosome replication in the same cell cycle, as blocking hdaA expression causes an increased frequency of chromosome replication as well as a blockage of cell division. I have also uncovered an unexpected role for HdaA in the stability control of DnaA. Removing HdaA from C. crescentus stabilizes the DnaA protein in growing cells and prevents complete DnaA protein removal from stationary phase cells. My experiments have identified a new role for HdaA in DnaA proteolysis that presumably works in exponentially growing cells to aid the RIDA mechanism that restricts chromosome replication to once per cell cycle. I also demonstrate that HdaA participates in the programmed transition from exponentially growing cells to the stationary phase. C. crescentus also contains a second gene (hdaB) predicted to encode a protein with homology to the DNA binding domain of DnaA. The hdaB gene is not essential for the normal

growth of C. crescentus. The fact that homologs of HdaB are found in the genomes of other alphaproteobacteria suggests that, hdaB has an evolutionary conserved role in these bacteria. Form these studies I propose that HdaA not only alters DnaA activity but also DnaA protein stability. This hypothesis further suggests that C. crescentus employs both nucleotide binding/hydrolysis, as seen in E. coli and a novel proteolytic mechanism to regulate DnaA. However, no previous studies have directly addressed the nucleotide binding and ATP hydrolysis of C. crescentus DnaA. To clarify the link between ATPase activity and protein stability I created four independent single amino acid mutations in two conserved positions of C. crescentus DnaA, DnaAR300 and DnaAR357. I showed that mutations in either position reduce DnaA ATPase activity in vitro. Further in vivo studies showed that mutations C. crescentus DnaAR357 increased chromosome replication and increased DnaA stability. These results indicate that the stability of DnaA protein is linked with its ATPase activity state. Combined with the established E. coli RIDA mechanism, my results imply a similar feedback mechanism that also ties DnaA proteolysis with C. crescentus cell cycle progression. " --

Divergent Proteolysis of Anti-sigma Proteins Controls Cell-surface Signalling in Pseudomonas Aeruginosa

A Thesis Submitted for the Degree of Doctor of Philosophy at the University of Otago, Dunedin, New Zealand

Cumulated Index Medicus

Molecular Biology of the Cell

The Role of the N Domain in Substrate Binding, Oligomerization, and Allosteric Regulation of the AAA+ Lon Protease

For cells and organisms to survive, they must maintain protein homeostasis in varied and often harsh environments. Cells utilize proteases and chaperones to maintain their proteomes. In bacteria, most cytosolic proteolysis is performed by self-compartmentalized AAA+ proteases, which convert the chemical energy of ATP binding and hydrolysis into mechanical work to unfold and translocate substrates into an internal degradation chamber. Substrates are targeted to AAA+ proteases by degradation tags (degrons). In E. coli, the Lon protease is responsible for the degradation of numerous regulatory proteins, including the cell-division inhibitor SulA, but also recognizes and degrades the majority of misfolded proteins. How Lon recognizes and prioritizes such a vast array of substrates is poorly understood. Active Lon is a homohexamer in which each subunit contains an N domain, a AAA+ module that mediates ATP binding and hydrolysis, and a peptidase domain. Degron binding allosterically regulates Lon activity and can shift Lon into conformations with higher or lower protease activity, but the mechanistic basis of this regulation is unknown. The low-protease conformation of Lon may serve as a chaperone. In Chapter 2, I describe the development and characterization of fluorescent model substrates that Lon degrades in vitro and in vivo. In Chapter 3, I describe collaborative experiments that show that Lon equilibrates between a hexamer and a dodecamer. Based on biochemical analysis and a low-resolution EM dodecamer structure, Lon appears to shift its substrate profile by changing oligomeric states and contacts between N domains appear to stabilize the dodecamer. In Chapters 4 and 5, 1 identify a binding site for the sul20 degron (isolated from SulA) in the Lon N domain and demonstrate that substrate binding to this site allosterically regulates protease and ATPase activity. I also show that the E240K mutation in the N domain alters Lon activity and stabilizes dodecamers. Finally, I provide evidence that E. coli Lon can act as a chaperone in vivo. These experiments demonstrate that the N domain integrates substrate binding, oligomerization, and regulation of the catalytic activities of Lon.

Pathophysiological Aspects of

Proteases

Springer **This book provides a comprehensive overview of the multifaceted field of protease in the cellular environment and focuses on the recently elucidated functions of complex proteolytic systems in physiology and pathophysiology. Given the breadth and depth of information covered in the respective contributions, the book will be immensely useful for researchers working to identify targets for drug development. Multidisciplinary in scope, the book bridges the gap between fundamental and translational research, with applications in the biomedical and pharmaceutical industry, making it a thought-provoking read for basic and applied scientists engaged in biomedical research. Proteases represent one of the largest and most diverse families of enzymes known, and we now know that they are involved in every aspect of a given organism's life functions. Under physiological conditions, proteases are regulated by their endogenous inhibitors. However, when the activity of proteases is not correctly regulated, disease processes such as tumour progression, vascular remodelling, atherosclerotic plaque progression, ulcer, rheumatoid arthritis, Alzheimer's disease and inflammation can result. Many infective microorganisms require proteases for replication or use them as virulence factors, which has facilitated the development of protease-targeted therapies for a variety of parasitic diseases.**

Analysis of an Uncharacterized Gene in Caulobacter Crescentus and Its Novel Connections with Cell Cycle Regulatory Machinery

Cell division and differentiation are complex biological phenomena that occur in all kingdoms of life. Understanding the molecular mechanisms that underlie these complex processes often requires the study of experimentally tractable model organisms. As a Gram-negative bacteria with less than four thousand genes, Caulobacter crescentus exhibits cell differentiation, highly regulated chromosome replication and segregation, and asymmetric cell division with every turn of the cell cycle. To achieve these behaviors, Caulobacter utilizes spatial control mechanisms such as sub-cellular compartmentalization and protein localization. The cell poles are particularly enriched for cell cycle regulatory proteins. Many of these proteins are localized by the hub protein PopZ, which forms a three-dimensional scaffold that also aids in chromosome segregation. The PopZ scaffold also includes proteolysis activity, which regulates cell cycle

progression in a manner that is analogous to well-known eukaryotic systems. In this dissertation, I characterized an evolutionarily conserved protein of unknown function, which is now named SpbR (Swarmer pole blocking factoR). SpbR is a pole-localized protein that has co-evolved with PopZ and other polar proteins. Strikingly, SpbR over-production exhibited a severe chromosome segregation phenotype, in which the newly replicated centromere failed to travel across the cell to its normal destination at the opposite pole. SpbR overproduction results in its accumulation at the old pole, where it physically interacts with PopZ. This prevents the relocation of PopZ to the new pole, thereby eliminating a positional cue for centromere translocation. Consistent with this, the centromere translocation phenotype of SpbR overproducing cells is further enhanced in genetic backgrounds that accumulate higher SpbR or reduce chromosome segregation activity. We find that pole-localized SpbR is normally cleared by proteolysis before the time of chromosome segregation, indicating that SpbR turnover is part of the cell cycle-dependent program of polar development.

Current Topics in Cellular Regulation

Elsevier **Current Topics in Cellular Regulation, Volume 9 presents the fundamental mechanisms involved in the regulation of diverse cellular activities, including cellular differentiation, intermediary metabolism, and the transfer of genetic information. This book provides information pertinent to the various aspects of cellular regulation. Organized into six chapters, this volume begins with an overview of the properties of the various hemoglobins present in trout blood. This text then introduces an interesting system for the study of differentiation and morphogenesis, the unicellular prokaryote Caulobacter crescentus. Other chapters consider the possible function of intracellular proteases in the regulation of enzyme activity in microorganisms and in their adaptation to changes in environmental conditions. This book discusses as well the physiological role and regulation of malic enzymes. The final chapter provides a comprehensive kinetic analysis of the phenomenon of enzyme induction in both eukaryotic and prokaryotic cells. This book is a valuable resource for biochemists, biologists, and research workers.**

Structural and Biochemical Elucidation of the Mechanism of C-

di-GMP Mediated Inside-out Signaling Controlling Periplasmic Proteolysis

Bacteria have developed multiple strategies to adapt to diverse ecological niches and hostile environments. One such strategy involves formation and maintenance of multicellular communities known as biofilms. In these microbial aggregates, sessile bacterial cells are encased in an extracellular matrix. It has now been established that c-di-GMP, a ubiquitous bacterial second messenger, is a central regulator of this developmental process in bacteria. It exerts its effects on transcriptional, translational and post-translational levels. While diguanylate cyclases and phosphodiesterases with conserved GGDEF and EAL (and HD-GYP) domains are responsible for the production and degradation of the dinucleotide, respectively, the receptors form a more diverse group with degenerate, catalytically inactive GGDEFEAL domain-containing proteins representing a major subfamily. One such protein, LapD from Pseudomonas fluorescens, uses an inside-out signaling mechanism to relay intracellular c-di-GMP concentration to control the localization of an outer-membrane anchored large adhesin protein LapA at the cell surface, by sequestering a periplasmic cysteine protease, LapG. When free, LapG cleaves the N-terminus of LapA, releasing it from the cell surface and ultimately leading to biofilm dispersal. Based on our structure-function analysis, here we propose a mechanism for the c-di-GMP-mediated, regulation of periplasmic proteolysis by LapD. We first elucidate the molecular basis of signal recognition and relay by P. fluorescens LapD and identify orthologous systems in multiple other bacteria including many pathogens such as Legionella pneumophila. This is followed by our work on L. pneumophila LapG, which provides us with the first atomic models of a bacterial protease of the DUF920 family and we are able to identify a highly conserved Ca2+-binding motif integral to its function. We then characterize the LapD-ortholog CdgS9 from L. pneumophila which confirms a common molecular mechanism. The crystal structure of the periplasmic output domain module reveals novel conformations and sheds new light on the mode of activation of the receptor. We finally report the structure of a complex between CdgS9output and P. fluorescens LapG which led to the discovery of the pharmacologically relevant binding interface between the output domain and LapG.

Microbiology of Fruits and Vegetables

CRC Press **Fresh and fresh-cut fruits and vegetables have an excellent safety record. However, surveillance data from the U.S. Centers for Disease Control and Prevention and recent foodborne illness outbreaks have demonstrated that the incidence of foodborne illnesses linked to the consumption of contaminated fresh fruit and vegetable products may in fact be**

Microbial Proteomics: Development in Technologies and Applications

Bentham Science Publishers **This volume brings current knowledge of proteomics technologies and related developments with special reference to diseases caused by microbes. The editor has compiled chapters written by expert academicians which distill the information about useful methods in microbial proteomics for the benefit of readers. Chapters cover several methods used to investigate the microbial proteome and special topics such as antimicrobial drug resistance mechanisms, biomarker developments, post translational modifications. Key Features: -overview of several biochemical methods in proteomics -full-color, high quality images of the most frequent technologies and applications -concise, well organized, and didactic format -updates in basic applied information -bibliographic references -information on proteomics for tuberculosis treatment This reference work is intended for researchers seeking information on laboratory techniques applied in proteomics research and microbiology.**

Genetics and Regulation of Nitrogen Fixation in Free-Living Bacteria

Springer Science & Business Media **Genetics and Regulation of Nitrogen-Fixing Bacteria This book is the second volume of a seven-volume series, which covers all fields of research related to nitrogen fixation - from basic studies through applied aspects to environmental impacts. Volume II provides a comprehensive and detailed source of information concerning the genetics and regulation of biological nitrogen fixation in free-living prokaryotes. This preface attempts to provide the reader with some insight into how**

this volume originated, how it was planned, and then how it developed over the several years of its production. Once the editorial team was established, the first job was to decide which of the many free-living diazotrophs that have been subjected to genetic analysis should be included in this volume. Would we need to develop specific criteria for selection or would the organisms, in effect, select themselves? Of course, Klebsiella pneumoniae and Azotobacter vinelandii, which have served (and still serve) as the main model organisms for the genetic analysis of diazotrophy, plus some of the other bacteria described in this volume, did indeed select themselves. However, there was considerable discussion surrounding well-characterized fixing species, like Azorhizobium caulinodans and Herbaspirillum seropedicae, both of which are able to fix atmospheric N under free-living conditions.

Microbial Contamination and Food Degradation

Academic Press **Microbial Contamination and Food Degradation, Volume 10 in the Handbook of Food Bioengineering series, provides an understanding of the most common microbial agents involved in food contamination and spoilage, and highlights the main detection techniques to help pinpoint the cause of contamination. Microorganisms may cause health-threatening conditions directly by being ingested together with contaminated food, or indirectly by producing harmful toxins and factors that can cause food borne illness. This resource discusses the potential sources of contamination, the latest advances in contamination research and strategies to prevent contamination using key methods of analysis and evaluation. Presents modern alternatives for avoiding microbial spoilage and food degradation using preventative and intervention technologies Provides key methods for addressing microbial contamination and preventing food borne illness through research and risk assessment analysis Includes detailed information on bacterial contamination problems in different environmental environments and the methodologies to help solve those problems**

Advances in Microbial Physiology

Academic Press **Advances in Microbial Physiology**

Distinct Roles for Two Trypsin-like

Proteases in Magnetosome Biomineralization

The ability of living organisms to transform inorganic elements into insoluble crystalline structures is an underexplored theme in biology. A group of aquatic bacteria, called magnetotactic bacteria, produces chains of nanometer-sized magnetic crystals within their cells, allowing them to align in the earth's geomagnetic fields. Understanding the mechanism for this process has become increasingly important as the demand for customized nanoparticles in medical and industrial applications has grown. In particular, the protein factors required to transform soluble iron into magnetite (Fe3O4) are likely to contain novel mechanisms for manipulating insoluble inorganic compounds. This thesis describes the biochemical and genetic features of two such factors, MamO and MamE. A historical account is provided describing the discovery of magnetotactic bacteria, the development of Magnetospirillum magneticum AMB-1 as a model system and the identification of specific genes required to produce magnetite. This previous body of work led to the identification of MamO and MamE as predicted trypsin-like proteases that are required for biomineralization in AMB-1. Genetic, biochemical and structural studies reported here showed that the MamO protease domain is catalytically inactive and incapable of serine protease activity. Instead it has a novel di-histidine motif that participates in direct binding to transition metals. This motif is required for biomineralization in vivo, confirming that the MamO protein is a repurposed trypsin-like scaffold that promotes magnetite nucleation by binding directly to iron precursor atoms. Genomic and phylogenetic analysis of related serine proteases in other magnetotactic bacteria showed that the repurposing of trypsin-like proteases has occurred numerous times independently during the evolution of magnetosome formation. Also described is the observation that three biomineralization factors, MamE, MamO and MamP are proteolytically processed in AMB-1. MamE and MamO are both required for these proteolytic events, as are the predicted catalytic residues from MamE. However, consistent with its newly assigned pseudo-protease classification, the predicted MamO active site is dispensable. This suggested that MamE directly processes these targets in a manner that requires MamO. The proteolytic activity of MamE was reconstituted in vitro with a recombinant form of the protein. MamE cleaved a custom peptide substrate based on an in vivo cleavage site in MamO with positive cooperativity, and its auto-proteolytic activity could be stimulated by both substrates and peptides that bind to its regulatory domains. These enzymatic properties suggested that a switch-like regulatory mechanism modulated MamE-dependent proteolysis during biomineralization. This regulatory paradigm was confirmed by showing that both catalytically inactive and constitutively active alleles of mamE caused

severe biomineralization defects in vivo. Although the genes required for biomineralization were known previously, the molecular mechanisms by which each protein promotes magnetite synthesis had not been explored. The results of these studies define biochemical functions for two of the four factors required for magnetite nucleation in AMB-1. Furthermore, describing the evolutionary repurposing of a trypsin scaffold along with the phylogenetic description of its evolutionary history add broad biological interest to magnetosome research.

Prokaryotic Communications: From Macromolecular Interdomain to Intercellular Talks (Recognition) and Beyond

Frontiers Media SA

Enzymes of Psychrotrophs in Raw Food

CRC Press **This book draws together theoretical and applied aspects of extracellular hydrolytic enzymes in spoilage, and thus provides information and analysis of interest to microbiologists and biochemists, as well as up-to-date methods and recommendations of value to food scientists and processors. The first section deals with psychrotroph proteinases, lipases, and phospholipases in milk and dairy products, and covers such aspects as producer microorganisms, biochemical classification of enzymes, physical and biochemical properties, thermal stability, regulation and control of synthesis and assay methods. Particular emphasis is placed on commercially important areas such as physical and biochemical effects in food components and influence on shelf life and product quality. The problems of standardization and control of enzymes in dairy products, as well as areas for future research, are critically examined. The poorly understood role of psychrotroph extracellular enzymes in meat, fish, and poultry is also discussed in a separate section under such headings as physical and biochemical effects on tissue and contribution to growth and penetration of the producer organism.**

Research Awards Index

Bioactive Peptides Produced by Limited Proteolysis

Morgan & Claypool Publishers **Proteins are considered supremely important for the organization, survival, and functioning of living organisms. They were considered stable and static molecules until the early 1940s, when RudolphSchoenheimer demonstrated that proteins exist in a constant dynamic process of synthesis and degradation (proteostasis), absolutely essential for life. Since then, general and limited protein degradation became some of the most fascinating aspects of biological sciences. This book is focused on a particular aspect of protein degradation, namely, limited proteolysis, which gives rise to bioactive peptides as a result of the enzymatic action of proteinases and peptidases, which are enzymes that hydrolyze specific peptide bonds of proteins and peptides, respectively. In a broad sense, bioactive peptides are any fragment of endogenous or exogenous proteins able to elicit either physiological or pathological activities. Here, we aim at presenting to the readers that bioactive peptides are not merely produced through random processes during protein degradation, but rather through a well-organized enzymatic process that is deeply integrated in the homeostatic processes of living organisms. Table of Contents: Overview and Historical Background / Bioactive Peptides Produced by Extracellular Proteolysis / Bioactive Peptides Generated by Intracellular Proteolysis / Proteolytic Enzymes / Concluding Remarks / References / Author Biographies**

Biochemistry of Nickel

Springer Science & Business Media **In this timely monograph, the author summarizes the rapidly growing body of knowledge regarding nickel by providing a balanced discussion of its harmful and beneficial effects. Coverage includes a history of nickel; the chemistry of nickel, descriptions of the four known enzymes which contain nickel; and nickel metabolism in microbes, plants, and animals. Taken as a whole, Dr. Hausinger's work will highlight key features of this important element and help define future research.**

Regulation of Gene Expression in

Eukaryotic Cells

A Symposium