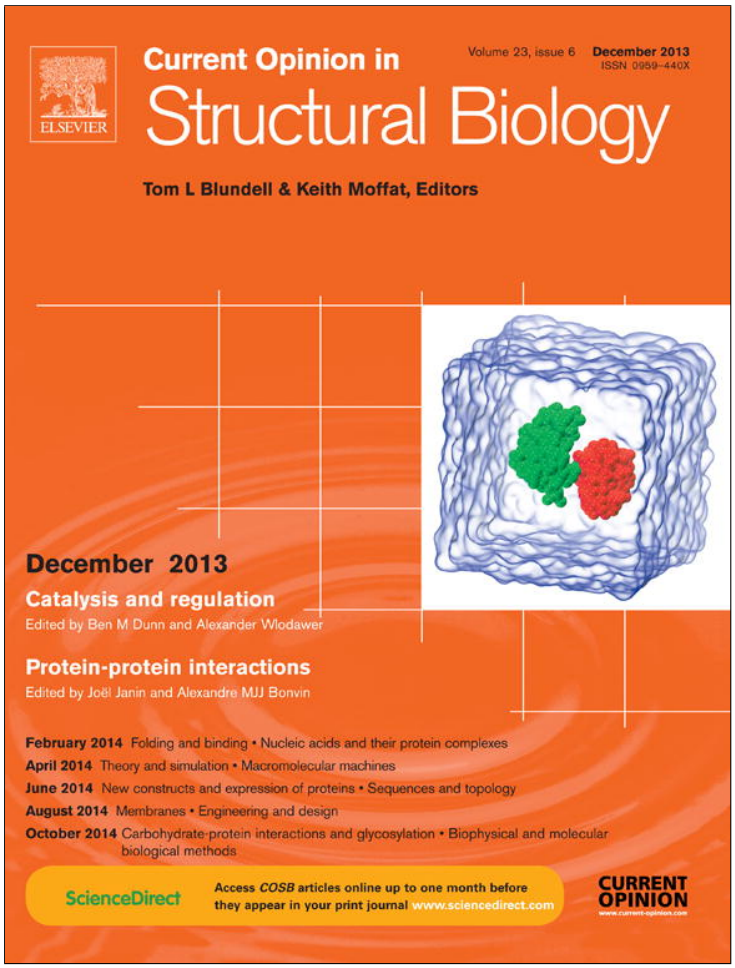

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The regulation of proteolysis around the World

Editorial overview

Ben M Dunn and Alexander Wlodawer

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Ben M Dunn
Department of Biochemistry and Molecular Biology, University of Florida, 1600 SW Archer Road, Gainesville, FL 32610-0245, USA
e-mail: bdunn@ufl.edu

Ben M Dunn is Distinguished Professor of Biochemistry & Molecular Biology in the College of Medicine at the University of Florida. His laboratory has developed methods for the quantitative study of the catalytic properties of proteins, especially proteolytic enzymes. These methods also permit the study of potential inhibitors of the enzymes and this has led to many collaborations with Alex Wlodawer to solve the structures of enzyme–inhibitor complexes. Professor Dunn's laboratory studies drug-resistant variants of HIV-1 protease from several subtypes of the retrovirus. They also study potential drug targets from the malaria parasite and the tuberculosis bacteria.

Alexander Wlodawer
Macromolecular Crystallography Laboratory, Center for Cancer Research, National Cancer Institute, Building 536, Room 5, P.O. Box B, Frederick, MD 21702-1201, USA
e-mail: wlodawer@nih.gov

Alexander Wlodawer is Chief of the Macromolecular Crystallography Laboratory, National Cancer Institute. His laboratory is involved in investigation of the relationship between protein structure and function, mainly by the technique of high-resolution X-ray diffraction. For over 25 years he has been studying proteases and protease inhibitors (often in collaboration with Ben Dunn), succeeding in the determination of a number of structures of enzymes from retroviruses, including HIV. He has also been involved in extensive investigations of ribonucleases, cytokines and their receptors, and a number of other proteins, as well as in development of crystallographic methodology.

Interest in proteolytic enzymes has spanned the modern era of biochemistry starting in the '60s–'70s when newly developed methods of column chromatography permitted the isolation of large amounts of naturally abundant enzymes from a variety of organisms. The availability of pure enzymes, mainly of the single-domain type, made possible detailed studies of enzymatic mechanisms and brought the advent of the age of structure determination, initially by crystallography, and later also by NMR. The availability of structural data allowed many laboratories to develop structure-based drug design which accelerated therapeutic development. However, the observation that the use in humans of compounds that were thought to be selective for an enzyme from a particular pathogenic organism frequently led to off-target effects was one element in the current era of interest in the regulation of proteolytic activity in cells and organisms. As frequently occurs in science, it has been the development of advanced methods of structure determination that has driven the field forward.

These developments in the areas of the regulation of proteolysis parallel those in other fields, where the basic understanding of the reactions of, for example, replication of DNA and production of new proteins has been followed by interest in the regulation of transcription and translational control. Studies of the regulation of biological processes have been driven by ever-increasing understanding of the structural basis of many of the catalytic processes that are critical for control.

It is now becoming clear that regulation of the activity of a growing number of proteolytic systems depends on the multi-domain or multi-subunit character of the enzymes. This now demands improved methodology for determination of the three-dimensional structures of the highly regulated systems. In the papers that follow, the authors describe systems where either new structures have revealed details of regulatory events or where tantalizing glimpses into organization of elements of the systems promise new discoveries to come.

Due to the considerable practical interest in the field of regulation of proteolysis and its potential applications in biotechnology and medicine, it is not surprising that much of the research is done in pharmaceutical companies. Three papers in this volume have been contributed by the scientists from Genentech, a company with extensive experience in that field. Weiru Wang, Yichin Liu, and Robert Lazarus summarized the current understanding of the allosteric inhibition of β-secretase (BACE1) by an exosite-binding antibody. Whereas many small-molecule inhibitors of this enzyme which is implicated in the development of Alzheimer's disease have

been described, not much is known about its inhibition by proteins and the authors have shown that an antibody may regulate its function through an allosteric mechanism. The allosteric site was identified through binding of peptides monitored by phage display, and an antibody developed against this region was shown to act as a noncompetitive manner with only partial inhibition (max. 77%). Oscar Huang and Andrea Cochran described the modes of regulation of the proteolytic activity of deubiquitinase, an enzyme which functions to reverse the process of the conjugation of ubiquitin to a specific substrate. Such regulation is accomplished by a variety of post-translational modifications and/or by the choice of protein binding partners, and is dependent on the presence of reactive oxygen species. Other Genentech authors, Jeremy Murray and Adam Renslo, discussed allosteric modulation of the activity of caspases, aspartate-specific cysteine proteases that mediate apoptosis and inflammation. Despite the importance of this family of enzymes as potential drug targets for diseases such as cancer, inflammation, or neurodegeneration, no small molecules directed against their active sites have successfully passed human clinical trials. For that reason, their allosteric regulation offers another path to inhibitor development and the authors discuss new modes of modulation of caspase activity by targeting areas beyond the active site.

Several groups located at the Monash University in Melbourne, Australia have been very active in the area of the regulation of proteolytic activity and three of them summarized their results in this issue. Robert Pike and Lakshmi Wijeyewickrema described the recent developments in the understanding of the mechanisms that control the interactions between complement proteases of the classical and lectin pathways and their substrates. Understanding of these pathways is of considerable importance since the complement system is involved in host defenses against pathogens and is also implicated in inflammatory diseases. The subject of Sheena McGowan's review is metalloaminopeptidases from *Plasmodium falciparum*, a parasite transmitting malaria. These enzymes are potential targets for the development of novel drugs against this disease that affects a large proportion of humans. In particular, the review discusses a variety of mechanisms whereby loops can interact with active sites to block entry of substrates. In addition, the formation of a hexameric structure has been found to restrict access to the active site in a manner that is subject to spatial and temporal control. Ruby Law, Diana Abu-Ssaydeh, and James Whisstock reviewed the current understanding of the process of plasminogen activation that leads to production of plasmin, an enzyme responsible for dissolution of fibrin clots and cleavage of other proteins involved in immunity and tissue repair. The activation of this multi-domain protein is a complicated process that is still not fully understood in structural terms, despite quite extensive studies.

The plants utilize proteolytic enzymes for defense against pathogens, with the latter producing macromolecular inhibitors that can be very specific. Anja Hörger and Renier van der Hoorn (Max Planck Institute for Plant Breeding Research, Cologne) have discussed recent findings regarding the selectivity of plant proteases from the model plant tomato and evolutionary adaptation processes involving the enzymes and their inhibitors in a continual war of attack and defense.

Whereas most proteolytic enzymes that have been studied to date are soluble, some proteases exist within cell membranes. Such proteases include rhomboids that are the subject of a review by Kutti Vinothkumar (Cambridge) and Matthew Freeman (Oxford). These serine proteases control a variety of biological processes by cleaving other membrane proteins present in the bilayer. They are often regulated by segregating the enzyme from the substrate, or by competition with the inactive forms of these enzymes called iRhoms that interact with the substrates and prevent their cleavage.

The contributions have been arranged in a geographical progression beginning in California, moving to Australia, and then on to Europe and the UK. These reviews capture many exciting developments in our understanding of regulatory events at the molecular level and indicate that there is world-wide interest in these topics. However, it must be understood that similar progress has been achieved in many laboratories in a variety of other locations and the ones featured here are only representative of the broader progress now made possible by achievements in structural resolution of more complex assemblies.