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Editorial Overview: Biological catalysis at the cross-roads of signaling and metabolism

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Enzymes are the principal catalysts in biology and comprise a large proportion of the proteins encoded by the human genome. Although much has been learned about enzymes over the past century, there are still major gaps in our fundamental knowledge about how enzymes catalyze reactions, what specific substrates are processed by particular enzymes, how enzymes are turned off or on in cellular pathways, and how they influence health and disease. There are increasing numbers of therapeutics that work as enzyme inhibitors and structure-based design is becoming an important part of the arsenal of drug discovery efforts. New technologies involving chemical, biophysical, genetic, and physiologic approaches are changing the landscape of enzyme research and leading to impactful discoveries in enzymology. This issue of *COSTBI* includes a series of articles that summarize recent advances in the enzyme sciences.

A main topic of this issue of *Current Opinion in Structural Biology* are enzymes that post-translationally modify other proteins, with a focus on phosphorylation, methylation, and acetylation. Akt kinases are a textbook example of the Ser/Thr kinase family because of their involvement in the critical steps of cell growth; cell division coupled to nutrient sensing. Cole and co-workers describe recent progress in the field that was made possible by the development of chemical-biology tools that rely on semisynthetic proteins. The key concept is that Akt can integrate multiple cellular signals that modulate its function through post-translational modifications. The Worden and Wolberger article summarizes several recently solved-histone methyltransferase structures including work by the authors on Dot1L. The recent Dot1L structures help settle the longstanding mystery of how ubiquitin attached to histone H2b stimulates Dot1L methylation of histone H3K79 in nucleosomes. The discussion of both the poised and the more reactive state of Dot1L are well-described and beautifully illustrated. The discussion of the DOT1L binding domains and the COMPASS histone H3K4 methyltransferase are also clear and informative. The authors also do a good job of laying out the remaining challenges for understanding COMPASS action and H3K79 methylation function. The manuscript by Tilvawala and Thompson reviews the mechanisms protein arginine deamination as performed by protein arginine deiminases (PADs). The enzymes irreversibly convert, by hydrolysis, Arg residues to citrulline amino

acids in polypeptides. The authors delve into a comparison of methods for detecting citrulline in protein mixtures and cell lysates and describe the proteomic distribution of citrulline and its functions in specific proteins. The article highlights how protein citrullination may contribute to inflammation and various rheumatologic disorders. Despite being one of the first known forms of post-translational modifications, protein acetylation continues to reveal new and unexpected mechanisms. Porter and Christianson contribute an article that discusses the structures and mechanisms of the classical histone deacetylase (HDAC) family members. These metallohydrolase enzymes are responsible for removing acetyl groups from histones and non-histone proteins as well as small molecule polyamines. The authors discuss how different HDAC family members can recognize peptides as well as small molecules and how they utilize an active site centered around a zinc to perform catalysis. These HDAC enzymes are central in the regulation of a myriad of biological processes from gene expression to cell growth and differentiation.

In addition to HDACs, several other metalloenzymes are extensively discussed in this issue. The main theme is that there is continuous flow of discoveries that expand the catalytic versatility of metals. This concept is beautifully illustrated in the review by Hausinger, describing the discovery of a new cofactor in a lactate racemase. In this enzyme, a Nickel-pincer nucleotide functions as the redox cofactor in substrate oxidation. Interestingly, the cofactor resembles the well-known pincer complexes of inorganic chemistry. The Nickel-pincer is only the tip of the iceberg. Many other metal cofactors have been recently discovered and characterized. They include metal-pyrroloquinoline quinones, metal pterins, and metal tetrapyrroles plus various type of metal-sulfur clusters. The chemical complexity of these metal clusters is elegantly discussed in the review by Sugishima and colleagues. They discuss the structures and mechanisms of biliverdin reductase (BVR) and ferredoxin-dependent bilin reductases (FDBR). In BVR, the authors focus on how two biliverdin molecules sit in the BVR active site and how one contributes to the reduction of the other via an interesting proton shuttle. With FDBR, the authors do a nice job describing the complex products that are formed and the role of the iron sulfur cluster. These are fascinating enzymes and the authors effectively summarize the relevant proposed mechanistic features that underlie catalysis. A mononuclear copper metal is the essential cofactor of lytic polysaccharide monooxygenases. These enzymes are in the spotlight for their unique capacity to metabolize the chitin and cellulose polymers. Eijssink and co-workers provide an effective and timely account on the state of the art in the field. Critical issues are the nature of the co-substrate (whether oxygen or hydrogen peroxide), the mechanism of the rupture of the glycosidic, the role in catalysis of the copper, and the structural mechanisms for binding a crystalline substrate. These are hotly debated topics in the literature. In addition to these functional and mechanistic questions, the critical issue is to see how these enzymes can be developed to make them industrially useful in biomass treatment strategies.

If polymer degradation can be tricky, their synthesis is no less astonishing. Allen and Imperiali showcase the mechanistic and structural diversity that underlie the biosynthesis of glycoconjugates. A challenging aspect of these reactions is that they often take place at the membrane interface dealing with amphiphilic substrates with opposing polarities. As beautifully shown in this review article, enzymes have devised multiple solutions to the

“catalysis-at-the-interface” problem, often adapting protein intricate folds within a single polypeptide that integrate soluble and a membrane-bound portions.

As seen for lytic polysaccharide monooxygenases, a variety of enzymes use oxygen as co-substrate for substrate oxygenations. Often, these reactions are part of secondary metabolism where compounds are made more soluble, and thereby easily secreted, though hydroxylation. A stunning feature of these enzymes is their broad substrate scope, a feature that makes them very attractive for industrial transformations as these reactions are difficult if not impossible to achieve by classical chemical means. As discussed by Fraaije and co-workers, the heme-dependent cytochromes P450 and flavin-dependent monooxygenases are main players in this area of enzymology. The authors have written an unusual and insightful perspective by drawing a comparison between these two classes of monooxygenases. Despite their distinct catalytic mechanisms, some common patterns emerge. Both cytochromes P450 and flavin-dependent monooxygenases feature the outstanding capability to stabilize an intermediate where oxygen is activated and rendered ready for substrate oxygenation. These enzymes often lack a clearly defined site for substrate recognition. Accessibility to the “oxygen-activated” intermediate is the main element that determines the substrate preference.

For monooxygenase and redox enzymes, the incidental generation of reactive oxygen species (ROS) is an unwanted and often damaging reaction. Indeed, a criterion for the evaluation of the effectiveness of these enzymes is their degree of coupling: how much reducing substrate is expended in the unintended generation of ROS byproducts rather than in substrate oxygenation. There are, however, also professional ROS producers, the NADPH oxidases (so-called NOXs). The manuscript by Magnani and Mattevi reviews the structure and biomedical roles these enzymes. The authors highlight how NOX enzymes participate in combating pathogens but also contribute to redox stress, cancer, and inflammation. Recent structural advances from these authors on the NOX enzymes represent breakthroughs in the field, and it is timely to have this structure/function article summarize the state of play and remaining challenges.

The structural and mechanistic complexity of cofactor-dependent enzymes finds a counterpart in sophisticated pathways that lead to their synthesis and generation. This fascinating area of enzymology is addressed in two review articles. Dubois and co-workers guide the reader through the elaborate pathways that generate and disrupt the heme. There is not “a route” for synthesis of heme cofactors. Rather, there are multiple pathways depending on the organism. These biosynthetic enzymes are evolutionarily related, giving the impression of a continuous re-shuffling along the evolution that has allowed organisms to optimize heme biosynthesis depending on metabolic and environmental needs. Likewise, heme-breaking can occur through different enzymatic routes that must also deal with proper usage and/or disposal of the iron liberated by the degraded heme. Once a cofactor is synthesized, it is sometimes taken for granted that it can be easily incorporated into a protein without any external help. This is not always the case. In their review, Iverson and co-workers illustrate the intricacy underlying cofactor incorporation in complex II (the so-called succinate dehydrogenase). Here, a set of protein factors is dedicated to specifically catalyzing the insertion and maturation of the iron-sulfur clusters and the flavin into the

dehydrogenase. The flavin is then covalently ligated to build the fully functional covalent flavoprotein subunit. Failure in this process can be catastrophic as it can cause different types of tumors and the neurodegenerative Leigh's disease.

With their well-defined binding pockets and suitability of binding and inhibition assays, enzymes remain the prime targets for drug development. In this issue, Oliva and co-workers convincingly make the case that the enzymology of parasitic and viral organisms can truly open the way to the discovery of the urgently needed drugs for the treatment of tropical diseases. In addition, "parasite and viral" enzymes often feature unusual and unexpected features that enhance interest in their mechanistic investigation and targeting with small molecules.

Altogether, this collection of articles beautifully document that a thorough understanding of the chemical and structural basis of catalytic transformations remains a cornerstone of the biological sciences.

Biography

Phil Cole graduated from Yale University in 1984 and then spent a year as a Churchill Scholar at the University of Cambridge. Cole went on to obtain M.D. and Ph.D. degrees from Johns Hopkins School of Medicine in 1991. Cole then entered clinical and post-doctoral training at Harvard Medical School prior to joining Rockefeller University in 1996 as a junior lab head. In 1999, Cole returned to Johns Hopkins as professor and director of pharmacology until 2017, when he moved to Harvard Medical School as professor. His research interests are in the area of protein post-translational modifications and chemical biology.

Andrea Mattevi graduated from Pavia University in 1988. He then went on to obtain the Ph.D. degree from the University of Groningen in 1992 under the supervision of Wim Hol. He was then awarded an EMBO fellowship to work in the laboratory of John Walker at the MRC-LMB in Cambridge. In 1994, he returned to the University of Pavia as a research fellow and, since 2001, as a full professor in molecular and structural biology. His research interests are in the area of enzyme redox chemistry and structural biology.