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The wide reach of enzymology: from bioorganic chemistry to chemical biology and beyond Mechanisms

Editorial Overview

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Dan Herschlag's interests focus on elucidating fundamental mechanisms in biology, including those of ribozyme and protein enzyme catalysis and RNA folding, and on applying this basic understanding to uncovering the roles of RNA processing in the control and regulation of gene expression.

We remember well the endless conversations as graduate students before the era of site-directed mutagenesis, plentiful structures and genome sequences: ''*What's the future of enzymology?*''; ''*Is enzymology dead?*''; ''*How can enzymology die when we still don't fully understand a single enzyme?*'' As Mark Twain famously said after reading of his own death in the morning papers, ''*The rumors of my death have been greatly exaggerated.*'' Likewise, enzymology, although much eulogized, has, on the one hand, stayed the course and, on the other hand, adapted to and adopted new perspectives, opportunities and approaches. How fitting that enzymology, the vital component of bioorganic chemistry, a field born nearly a half century ago, has re-emerged as a central element of the new era of 'chemical biology'.

Of course, much has been learned about enzymes over the decades. And there are new tools, new applications, and new twists. We start this section with a catalytic twist uncovered 25 years ago — RNA enzymes. In recent years, our understanding of how these non-standard enzymes or 'ribozymes' work has progressed enormously. **Bevilacqua and Yajima** focus on one of the more recent realizations in ribozymology: the ability of nucleic acid bases to participate directly in catalytic events such as proton transfers. Here, the mechanistic challenges come closer and closer to those faced in uncovering the precise mechanisms of action of protein enzymes, yet the dissection of RNA catalysts provides a revealing perspective into fundamental aspects of biological catalysis and a window into the behavior of nucleic acids.

A basic problem faced by enzymes is that certain species, such as ammonia and indole, that are generated in metabolic processes can be harmful to or lost from the cell if released from active sites. Many years ago, Yanofsky showed that indole was not released in the process of synthesizing tryptophan. Decades later, the structure of tryptophan synthase solved by Miles and Davies [1] led to one of those beautiful and rare moments in biology when seeing a structure solves a mystery: a tunnel inside the enzyme allows indole to be released at one active site and migrate to the enzyme's second active site 30 Å away without ever leaving the enzyme! **Week, Lund, and Raushel** review this exciting and elegant physical solution to a biological problem, describing lessons from many enzymes now known to use this strategy.

Much of the focus of chemical biology has been on tool development, with an emphasis on approaches that can directly probe the concentrations,

activities, complexes and localization of cellular components. But a second, less-heralded opportunity for chemical biology also comes from its intersection with modern genomic biology. The emergent properties of biological systems arise from the interaction of cellular components. The powerful tools such as mass spectrometry, two-hybrid methods, and other genomic approaches to determine which constituents interact is just a starting point for understanding these emergent properties. Thus, a fundamental biological need intersects with one of the frontiers of enzymology, understanding multi-step, multi-component reactions and processes carried out by enzyme 'machines' and stable and transient complexes.

Two case studies of the enzymology of complex processes are reviewed, one that is rather well-worked out at a basic level and one where understanding is just now emerging. It is thought that a high percentage of all proteins are membrane associated, and much of biology occurs at the membrane interface. The classic work on phospholipases reviewed by **Jain and Berg** provides a paradigm for investigating and understanding the distinct behaviors and properties of systems that operate at the membrane interface. Protein synthesis is of course critical to biology, and an important control point in gene expression. Although there has been an overall model for steps in translation initiation for decades and many interactions between initiation components have been identified, investigations of the inner workings of this complex orchestration of dozens of protein components along with the 60S and 40S ribosomal subunits are just beginning to emerge. **Algire and Lorsch** focus on the critical step of start-codon recognition and describe recent advances from biochemical, structural and genetic studies that lead to new models and clarify the many remaining mechanistic and structural questions.

A critical tool for dissecting translation initiation (Algire and Lorsch) was the incorporation of fluorescent probes via the construction of semi-synthetic proteins. **Pellois and Muir** provide recent elegant examples of mechanistic insights obtained using semi-synthetic proteins with unnatural amino acids and other substitutions. The ability to integrate chemical diversity to more incisively probe mechanistic models represents an exciting and compelling alliance of chemical biology and mechanistic enzymology.

Genome sequencing and the explosion of protein structures have fueled the ascension of a new area of enzymological investigation that we refer to as 'evolutionary enzymology'. Understanding the relationships between enzymes that have structural similarities but catalyze different reactions is exciting detective work with implications for understanding the evolution of protein function, for the design, engineering and selection of proteins with new activities, and for accurate gene annotation. **Glasner, Gerlt and Babbitt**} discuss the extensive emerging understanding of the relationships between enzyme superfamilies, and ***Khersonsky, Roodveldt and Tawfik*** focus on a particularly fascinating aspect of evolutionary relationships, properties of 'catalytic promiscuity' — the low level of activity of an enzyme for a reaction other than its current biological function. Such activities are thought to be both evolutionary vestiges and raw material for future evolution. Thus, a better understanding of catalytic promiscuity can help reveal how nature has evolved new activities, how features in and around the active site 'tune' enzyme mechanisms, and how we might mimic or modify nature to engineer or select enzymes with desirable catalytic functions.

The review by **Hancok, Vaughan and Withers** describes uses of the impressive mechanistic knowledge of glycosidase and glycosyltransferase enzymes and mechanisms to engineer existing enzymes to better synthesize many types of glycosides. Screening, rational mutagenesis, directed evolution and even whole-cell metabolic engineering successes are described. It will be fascinating to monitor the impact of such engineering in the coming years.

Last but not least is a review by **Hedstrom and Gan** on the enzyme IMP dehydrogenase that encompasses many of exciting and important areas of enzymology. IMP dehydrogenase has a complex reaction pathway that utilizes a series of conformational transitions. Beyond its fascinating mechanistic properties, IMP dehydrogenase appears to control guanine nucleotide pools and is an important drug target for immunosuppressive, antiviral and cancer chemotherapies. But this enzyme does even more, exhibiting a 'moonlighting' activity in binding to RNA and a reversible ring-like aggregated state of unknown function. Work on this enzyme underscores a critical but rarely discussed aspect of drug 'design' — the need for detailed and holistic understanding of protein function that goes well beyond annotation of a 'primary' activity and development of potent inhibitors. Our ability to be truly effective in combating disease and controlling metabolic function will require a mechanistic understanding that incorporates catalysis within the context of additional interactions and overall cellular logic. We anticipate that the tools of enzymology and Chemical Biology will ultimately be foundational for the deep understanding of biology that is both desired from an intellectual perspective and required from a practical perspective.

We hope that the reader of this special section will enjoy these articles and the synergy of chemical biology and enzymology for years to come.

Reference

1. Hyde CC, Ahmed SA, Padlan EA, Miles EW, Davies DR: **Three-dimensional structure of the tryptophan synthase $\alpha_2\beta_2$ multienzyme complex from *Salmonella typhimurium***. *J Biol Chem* 1988, **263**:17857-17871.