Chemistry & Biology *13*, 239–240, March 2006 DOI 10.1016/j.chembiol.2006.02.008

Book Review

Lost and Found in Posttranslation

Posttranslational Modification of Proteins: Expanding Nature's Inventory
Christopher Walsh
Englewood, CO: Roberts and Company Publishers (2005)
576 pp. $99.00 hardcover

The covalent assembly of proteins begins on the ribosome with the formally sequential linear translation of RNA triplet codons, each coding for one of the 20 naturally occurring amino acids, into the nascent polypeptide chain. The rest of a protein's life is decidedly not a linear affair. Beginning with the folding of the polypeptide, proteins can be modified in a number of ways, most notably by interactions with other proteins and by covalent modification of the side chains of selected amino acids or of the peptide groups. A simple interpretation of covalent modifications is that they diversify the amino acid code, thus allowing proteins to do things and behave differently. However, if simple and stoichiometric additions to selected side chains or cleavage of the polypeptide was all that posttranslational modifications entailed, the subject would not be remotely as interesting or likely as important as it is to modern biology. Posttranslational modifications of proteins (PTMs) are highly dynamic, often reversible, and not always stoichiometric changes. In most fields of biochemistry today, discovering and understanding how, where, when, and under what conditions specific PTMs occur is the raison d'être of many researchers. For chemists, the study of PTMs represents an important opportunity, both to contribute to an understanding of these processes—and perhaps to discover new ones—and to overcome the technical challenges of measuring these changes.

The literature on PTMs is vast, but anyone hoping to delve into some aspect of these subjects will find a concise and well-integrated presentation of the key PTMs and their biological functions in Chris Walsh's new book. Walsh starts with basic introductory material on splicing and translation of messenger RNA and then on the chemistry of posttranslational modifications. A series of chapters follow that cover the spectrum of well-known PTMs, as well as some rarer forms and interesting exotica, such as *cis-trans* splicing of polypeptides by inteins and, of course, synthesis of the intrinsically fluorescent proteins ubiquitously utilized as biosensors. As we learn, in all of these reactions, amino acid side chain or peptide groups for the most part serve as nucleophiles in simple, enzymatically catalyzed additions. Each chapter simply and logically presents the basic chemical facts surrounding any particular modification and the consequences and classes of functional consequences. For instance, in a chapter on protein phosphorylation, Walsh provides an overview of the role of phosphorylation in key processes such as signal transduction. Again, sticking strictly to the facts, he explains the enzymology of kinases and phosphatases and provides examples of how these PTMs control specific processes such as modifying the activities of enzymes, creating high-affinity peptide binding motifs for recognition of proteins, or regulating cellular localization.

The organization and content of the book reflect a style we have come to appreciate in previous monographs by the author, in particular the classic 1979 text *Enzymatic Reaction Mechanisms*. The author presents each PTM according to basic reaction mechanisms followed by clear illustrative examples of classes of changes in protein function resulting from individual modifications. The material is simple and should be completely comprehensible to anyone who has taken a single semester course in introductory organic chemistry. Figures are economical in the information they convey. Reaction schemes are straightforward, and cartoon representations of more complex biochemical processes are informative but kept to a minimum. Enzyme structures are also presented, when necessary, by simple MOLSCRIPT-generated cartoons with more detailed ball-and-stick representations of key residues.

A problem and challenge in PTM research is the heterogeneous and nonstoichiometric nature of some of these chemical modifications, which create multiple variants of any individual gene product. This combinatorial explosion of potential products means that for any individual protein there could be multiple modified variants with different activities or functions. Walsh cannot resist making the sort of back-of-the-envelope estimates of how many variants of modified proteins of a particular type may exist, but at the same time he doesn't dwell on the imponderability of the proteomic universe. Instead, a disciplined focus on mechanisms and some choice examples of how the combinatorial complexity of PTMs is used to encode specific biochemical processes are presented.

Two other features of the approach Walsh takes to presenting this subject make for satisfying reading. The first is the effort Walsh makes to provide reasonable explanations or rationales for particular PTMs. For example, I appreciated his summary in chapter 2 of Frank Westheimer's meditation on why nature chose the phosphate group [1]. Such reflection on the chemistry of specific PTM molecules is both instructive and entertaining, and other such arguments are presented for other chemistry throughout the book. On the other hand, some PTMs defy simple chemical arguments and may have occurred as evolutionary accidents. Examples include the PTM of proteins by the 76 amino acid protein ubiquitin, a process that signals directing so-modified proteins to be degraded by an enzyme complex called the proteosome. Obviously, no simple explanation can be given as to why so much energy and material would be given over to such an elaborate process when one could imagine that something simpler would suffice. Another example is glycosylation. The synthesis of complex, branched, and modified oligosaccharides is an expensive process, and yet, to date, we still don't understand its full significance. I had a chuckle when Walsh's otherwise exacting vocabulary turns florid in describing the synthesis and structure of complex carbohydrates; words like "baroque and rococo" reflect well how

most of us feel about these molecules, whether it is wonder at how the synthesis of these molecules is achieved or the potential for complex interactions they could make, or simply frustration that we don't know enough about what these molecules do.

A second aspect I appreciated is Walsh's attention to thematic completeness. By this I mean tying together common themes and chemistries of PTMs such as the symmetry of addition/removal that distinguishes PTM from simple stoichiometric modifications and the differences and similarities among PTM chemistries. For example, in chapter 3, on protein tyrosine sulfotransferases, he discusses the analogy between transfer of sulfate and phosphate groups. Unlike phosphate PTMs, there is no evidence for tyrosine sulfatases in mammals, so instead, Walsh chooses to discuss another class of enzymes, the aryl sulfatases, which cleave sulfate groups from N-SO_3 and O-SO_3 linkages in glycosaminoglycans. Why bother describing enzymes that don't even act on proteins? First, that cleavage of sulfate linkages can occur shows that it can, and how it happens, and just because we haven't discovered a mechanism for reversal of a particular modification doesn't mean it doesn't exist. This is well illustrated for methylation of lysine side chains on the nucleosome histone proteins as discussed in chapter 5. Until three years ago, it was assumed that this PTM was not reversible, until a novel class of enzymes that do just that was discovered. This discovery changed our view of the dynamics of this important modification and its role in gene regulation. Thus the lesson is that if a covalent modification or removal can occur somewhere in biology, there is a reasonable possibility that it could function elsewhere, and one should search for these activities rather than accepting contemporary dogma.

Walsh doesn't spend much time dwelling on the techniques used to study PTMs. There is wisdom in this approach because there is a dearth of really good techniques, and detailed discussions of existing techniques would detract from the interesting mechanistic and functional details of PTMs presented in this book. Instead, overviews of key techniques, their limitations, and challenges to improve them are presented. For instance, in chapter 2 Walsh provides an overview of the use of mass spectrometric techniques to identify phosphorylation sites on proteins. Mass spectrometry is and will likely remain the dominant method for identifying specific PTMs on proteins. In spite of challenges due to the nonstoichiometric nature of PTMs, their biochemical lability, and variation with cellular states—issues Walsh presents concisely—the endless ability of spectroscopists to adapt novel sample preparations and improvements to detection techniques assure that present day challenges will be likely met quickly. At the same time, Walsh alludes to the fact that we have no way of knowing if we have discovered all of the PTMs that nature uses and that if there are more, likely they will turn up sometime in a mass spectrum.

Among the most interesting sections of the book are those in which Walsh brings everything together, providing lucid accounts of how a series of consecutive or parallel PTMs on proteins perform what one might call "biocomputing"—temporal, spatial, as well as regiospecific integration of posttranslational modifications to drive specific processes. In these sections, Walsh builds on or anticipates PTM mechanisms described in other, detailed chapters so that the reader is shown what he or she may already suspect: that PTMs are part of dynamic, integrated processes. We are introduced to this idea in the example of the "histone code" (Figure 6.3), a sequence of different, sometimes competing PTMs that occur on the histone core complex of nucleosomes. The nucleosomes are the basic substructures of chromatin fibers, the "string of pearls" arrangement of double-stranded DNA wound around the histone complex. The number and types of PTMs of the histone complex proteins determine whether the DNA with which the histones are associated can be replicated or transcribed or remains silent. How, when, and on what regions of the chromosomes the combinations of PTMs that code for a specific event occur are the subject of intense efforts, both to understand the fundamental processes of gene transcription and DNA replication, but also to understand genetic and allelic instability such as occurs in cancers.

So what's not to like? Not much. As I mentioned earlier, PTM research presents many opportunities for fundamental discovery, but the field is in great need of new techniques to study PTMs, particularly in intact cells or in vivo. Besides advances in sample preparation and mass spectrometric techniques, a number of novel experimental strategies have been developed to address aspects of PTM dynamics. Some discussion of the more elegant among these could be both instructive and inspiring. For example, a strategy to selectively inhibit kinases is an excellent case of how a combination of thoughtful structural and chemical ideas can be put together to study the specific biochemical and phenotypic consequences of phosphorylation [2]. At the same time, PTM reactions have inspired a whole host of ideas for probing biochemical processes. An entire chapter describes the fluorescent proteins, their engineering into useful probes of biochemical processes, and the strategies for modifying proteins via intein reactions. Perhaps a more encompassing chapter on the topic of measuring and exploiting PTMs and outstanding problems in the detection of PTMs would be useful.

The message should be clear: readers at any level who find themselves lost in the bewildering literature of posttranslational modification will find a clear guide in this text, and any young or youngish investigator looking to make important contributions to modern biology would be well advised to read this book cover to cover to get an idea of what needs to be done. There is gold in them there hills.

Stephen W. Michnick
Département de Biochimie and Robert Cedergren
Centre for Genomics and Bioinformatics
Université de Montréal
C.P. 6128, Succ. Centre-ville
Montréal, Québec H3C 3J7
Canada

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