

Books review

Alpha-keto acid dehydrogenase complexes

by M.S. Patel, T.E. Roche and R.A. Harris (eds.) Molecular and cell biology updates (MCBU), Birkhäuser Verlag, Basel, Boston, Berlin; 1996, pp. 321, ISBN 3-7643-5181-0.

Alpha-keto acid (2-oxo acid) dehydrogenases form the ubiquitous family of the largest and most complicated multienzyme complexes which play a regulatory role in crucial steps of metabolism. Essentially, this group of enzymes may be divided into three classes: pyruvate dehydrogenase complex involved in carbohydrate catabolism, α -ketoglutarate (2-oxo glutarate) dehydrogenase located in citric acid cycle and branched-chain-keto acid (branched-chain 2-oxo acid) dehydrogenases responsible for the oxidation of 2-oxo acids derived from the transamination of branched-chain amino acids (valine, leucine and isoleucine). These enzymes have been extensively investigated for 40 years but the rapid development of molecular biology and protein structure techniques which have occurred for last years offers new tools for scientific approach in this field. It has made possible to precisely define the three-dimensional structure of these complexes and the interaction between monomers, groups of the monomers and domains. Channeling of the reactants, molecular mechanism of each catalytic step and the regulation of enzymatic activity create challenging problems in this field. Especially, intrinsic kinase and phosphatase activities tightly bound to the complexes and playing a regulatory function in the response to a wide variety of extracellular stimuli focus the interest of many laboratories. Molecular studies also help to understand similarities and divergences among 2-oxo acid dehydrogenases from various sources. Finally, the knowledge of the abnormalities of the structure of these enzymatic complexes makes possible to understand the molecular basis of some inherited and acquired diseases.

The book contains 21 chapters. Most of them deal with the pyruvate dehydrogenase complex. Only a few concern branched-chain 2-oxo acid or 2-oxo glutarate dehydrogenases. The articles are not actually assembled in thematically separated sections but still one may divide them to a few groups focused on partially different subjects. First eight chapters refer to the structure of individual subunits composing the pyruvate dehydrogenase complex and to interactions between them. The great attention is paid on the structure of active sites of the subunits and on the regulation of enzymatic activity of the whole complex by phosphorylation and dephosphorylation. Interestingly, one of these chapters concerns pyruvate dehydrogenase complexes in plants in which the existence of two completely independent isoenzymes localized in plastides or mitochondria forms a very important regulatory mechanism. Next, three articles are focused on the molecular properties of tightly bound protein kinase and protein phosphatase, their structures and regulatory mechanisms responsible for the modulation of 2-oxo acid dehydrogenase complex activities. The following three chapters refer to the effects of physical exercise and nutritional status of animal on the short- and long-term regulation of activity of different types of 2-oxo acid dehydrogenase complexes. Finally, last four articles are appertained to the medical aspects of 2-oxo acid dehydrogenase deficiency or dysfunction. Additionally, some chapters refer to more detailed aspects concerning 2-oxo acid dehydrogenase complexes, like regulation of pyruvate dehydrogenase activity in parasitic nematode *Ascaris suum*, specificity

of gene coding sperm form of this enzyme and the structure of human 2-oxo glutarate gene.

Each chapter includes the updated reference list containing a substantial number of papers published in last three years. The subject index common for all articles is included at the end of the book.

In conclusion, this book covers a very broad spectrum of aspects concerning the 2-oxo acid dehydrogenase complexes, their structure, regulation of activity, evolutionary interrelationships and molecular aspects of some pathology. So, it would be a very interesting assemblage of the most recent advances in the study on the enzymes catalyzing 2-oxo acid

oxidation. Because of a variety of scientific approaches and of experimental results extending the knowledge in this field, it might be a useful source of information for researchers investigating the 2-oxo acid oxidative systems as well as for lecturers in biochemistry and enzymology and all scientists interested in enzyme complexes, their structures, regulation of activity by phosphorylation and dephosphorylation and also in mitochondrial metabolism and bioenergetics.

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Biotechnology: Proteins to PCR. A Course in Strategies and Lab Techniques

David W. Burden, Donald B. Whitney. Birkhauser. Boston, 1995, pp. 317.
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In recent years biotechnology has become an indispensable tool in the research on living organisms. The most exiting discoveries in biochemistry and physiology in the last 20 years were made using the methods of DNA analysis. In this respect, teaching biotechnology (or, in the narrower sense, molecular biology) is an important part of modern biological education.

The book presented here is a manual of basic biochemical and biotechnology techniques compiled by David W. Burden and Donald B. Whitney from the Biotechnology Training Institute, Lebanon, N. J. It is not intended for advanced researchers, it is rather designed for undergraduate students, teachers, and also for those scientists who wish to use the biotechnology techniques. To give the reader a comprehensive view of the research process the "from protein to gene" approach is used as a strategy for gene cloning. Each chapter contains a short introduction to the topic dealt with, followed by a number of practical protocols for selected procedures. As a practical course, the purification and sequencing of the N-terminus of the α -galactosidase from *Saccharomyces carlsbergensis* (brewer's yeast) is described. The amino acid sequence data are used in the second part of the manual for the designing of an oligonucleotide probe for cloning of the α -galactosi-

dase gene. However, this strategy is limited to those genes for which products are known and can be easily isolated. In many cases, especially with regulatory genes, the protein of interest is unknown or too difficult to purify. So we have to start from DNA sequence and finally the putative protein sequence is determined. The point (and the art of science) is how to find the target sequence among thousands of others within the genome.

The book begins with a chapter introducing the biotechnology laboratory. First, the scope of biotechnology and its applications in agriculture, environment management, medicine and pharmacy are reviewed. Next chapter deals with safety precautions, and finally, the microorganisms commonly used in biotechnology: *E. coli* and yeast are introduced. The first part of the manual (Chapters 2–6) covers the methods of protein analysis. It starts with measurements of enzymatic activity followed by protein purification by various chromatographic techniques (i.e. gel filtration, ion-exchange and affinity chromatography). The last chapter of this part describes checking of protein homogeneity by acrylamide gel electrophoresis and Edman's procedure for protein sequencing.

Chapters 7–14 comprise the information on gene cloning. Chapter 7 is devoted to cloning

strategy based on a probe-DNA derived from protein. Chapter 8 deals with DNA extraction and purification. Protocols for genomic (from yeast) and plasmid DNA isolation are included. The next two chapters deal with construction of a genomic DNA library and transformation of *E. coli* with recombinant plasmids. Various transformation methodologies for both bacterial and higher organisms' cells are presented. I only found surprising that the protocol for transformation of yeast is included separately in another chapter. Chapters 11 and 12 describe screening for recombinant clones using labelled probes and verifying of the selected clones by restriction endonuclease mapping, Southern blotting and sequencing. The last two chapters make clear the advantages of DNA sequence knowledge in both genome and protein analysis, gene cloning and directed mutagenesis. Although PCR is mentioned in the title, it is reviewed only briefly. The applications of this powerful tool increase enormously in all fields of DNA technology and one would expect it is to be described more thoroughly. Also, an additional chapter on gene expression would be a valuable completion of the course.

The book provides a clear account on the gene cloning process. The presentation is given in a systematic way and numerous figures and diagrams provide good explanation to the text. Each chapter is followed by references, study questions and suggestions for further readings. The protocols included help to even an inexperienced reader to perform basic experiments. I think that biotechnologists will profit from having this fine handbook on their lab desks.

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