APPLICATIONS OF CHIMERIC GENES AND HYBRID PROTEINS

Volume 327

Jeremy Thorner

Methods in Enzymology

Volume 327

APPLICATIONS OF CHIMERIC GENES AND HYBRID PROTEINS

Part B

Cell Biology and Physiology

METHODS IN ENZYMOLOGY

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Applications of Chimeric Genes and Hybrid Proteins

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Cell Biology and Physiology

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Table of Contents

Section III. Tools for Analysis of Membrane Proteins

Section IV. Signals for Addressing Proteins to Specific Subcellular Compartments

Section V. Application of Chimeras in Monitoring and Manipulating Cell Physiology

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Preface

The modern biologist takes almost for granted the rich repertoire of tools currently available for manipulating virtually any gene or protein of interest. Paramount among these operations is the construction of fusions. The tactic of generating gene fusions to facilitate analysis of gene expression has its origins in the work of Jacob and Monod more than 35 years ago. The fact that gene fusions can create functional chimeric proteins was demonstrated shortly thereafter. Since that time, the number of tricks for splicing or inserting into a gene product various markers, tags, antigenic epitopes, structural probes, and other elements has increased explosively. Hence, when we undertook assembling a volume on the applications of chimeric genes and hybrid proteins in modern biological research, we considered the job a daunting task.

To assist us with producing a coherent work, we first enlisted the aid of an Advisory Committee, consisting of Joe Falke, Stan Fields, Brian Seed, Tom Silhavy, and Roger Tsien. We benefited enormously from their ideas, suggestions, and breadth of knowledge. We are grateful to them all for their willingness to participate at the planning stage and for contributing excellent and highly pertinent articles.

A large measure of the success of this project is due to the enthusiastic responses we received from nearly all of the prospective authors we approached. Many contributors made additional suggestions, and quite a number contributed more than one article. Hence, it became clear early on that given the huge number of applications of gene fusion and hybrid protein technology—for studies of the regulation of gene expression, for lineage tracing, for protein purification and detection, for analysis of protein localization and dynamic movement, and a plethora of other uses—it would not be possible for us to cover this subject comprehensively in a single volume, but in the resulting three volumes, 326, 327, and 328.

Volume 326 is devoted to methods useful for monitoring gene expression, for facilitating protein purification, and for generating novel antigens and antibodies. Also in this volume is an introductory article describing the genesis of the concept of gene fusions and the early foundations of this whole approach. We would like to express our special appreciation to Jon Beckwith for preparing this historical overview. Jon's description is particularly illuminating because he was among the first to exploit gene and protein fusions. Moreover, over the years, he and his colleagues have

continued to develop the methodology that has propelled the use of fusion-based techniques from bacteria to eukaryotic organisms. Volume 327 is focused on procedures for tagging proteins for immunodetection, for using chimeric proteins for cytological purposes, especially the analysis of membrane proteins and intracellular protein trafficking, and for monitoring and manipulating various aspects of cell signaling and cell physiology. Included in this volume is a rather extensive section on the green fluorescent protein (GFP) that deals with applications not covered in Volume 302. Volume 328 describes protocols for using hybrid genes and proteins to identify and analyze protein–protein and protein–nucleic interactions, for mapping molecular recognition domains, for directed molecular evolution, and for functional genomics.

We want to take this opportunity to thank again all the authors who generously contributed and whose conscientious efforts to maintain the high standards of the *Methods in Enzymology* series will make these volumes of practical use to a broad spectrum of investigators for many years to come. We have to admit, however, that, despite our best efforts, we could not include each and every method that involves the use of a gene fusion or a hybrid protein. In part, our task was a bit like trying to bottle smoke because brilliant new methods that exploit the fundamental strategy of using a chimeric gene or protein are being devised and published daily. We hope, however, that we have been able to capture many of the most salient and generally applicable procedures. Nonetheless, we take full responsibility for any oversights or omissions, and apologize to any researcher whose method was overlooked.

Finally, we would especially like to acknowledge the expert assistance of Joyce Kato at Caltech, whose administrative skills were essential in organizing these books.

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VOLUME VI. Preparation and Assay of Enzymes (*Continued*)
Preparation and Assay of Substrates
Special Techniques
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Edited by RAY WU

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VOLUME 70. Immunochemical Techniques (Part A)
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