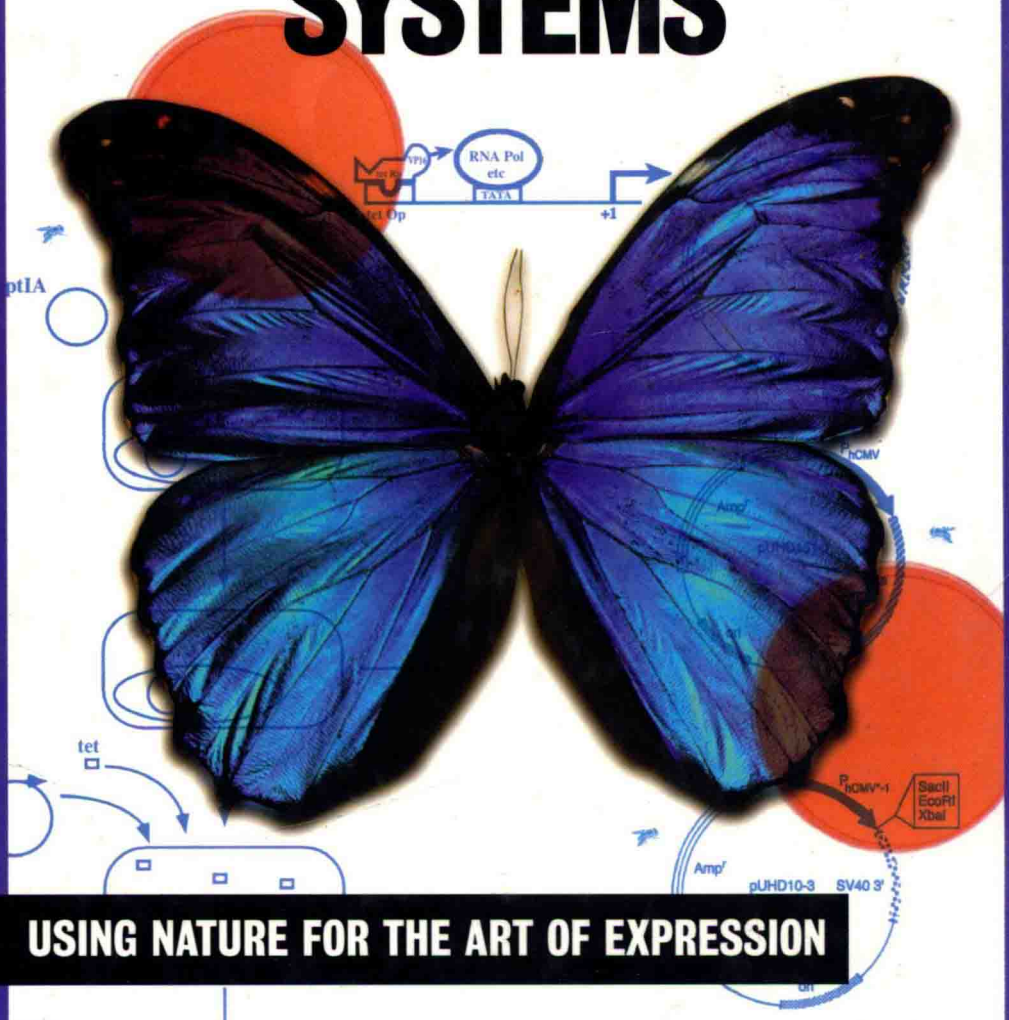
GENE EXPRESSION SYSTEMS



USING NATURE FOR THE ART OF EXPRESSION

EDITED BY

JOSEPH M. FERNANDEZ

JAMES P. HOEFFLER

Gene Expression Systems

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Joseph M. Fernandez and James P. Hoeffler

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CONTRIBUTORS

Numbers in brackets indicate the pages on which the authors' contributions begin.

Rula Abbud [367], Department of Pharmacology, Case Western Reserve University, Cleveland, Ohio 44106

Roger N. Beachy [429], Division of Plant Biology, The Scripps Research Institute, La Jolla, California 92037

Robert P. Bennett [259], Invitrogen Corporation, Carlsbad, California 92008

Marc Better [95], XOMA Corporation, Santa Monica, California 90404

Jürgen Brosius [45], Institute for Experimental Pathology, Center for Molecular Biology of Inflammation (ZMBE), University of Münster, D-48149 Münster, Germany

E. S. Cole [399], Genzyme Corporation, Framingham, Massachusetts 01701

James M. Cregg [157], Department of Biochemistry and Molecular Biology, Oregon Graduate Institute of Science and Technology, Portland, Oregon 97291

John M. Curling [399], John Curling Consulting AB, S-75329 Uppsala, Sweden

Russell Durbin [9], Department of Hematology and Oncology, Wexner Institute for Pediatric Research, Children's Hospital, Columbus, Ohio 43205

Y. Echelard [399], Genzyme Transgenics Corporation, Framingham, Massachusetts 01701

Joseph M. Fernandez [1], Invitrogen Corporation, Carlsbad, California 92008

Eugenio Ferrari [65], Genencor International, Inc., Palo Alto, California 94304

Michael Galleno [331], Invitrogen Corporation, Carlsbad, California 92008

S. Groet [399], Genzyme Transgenics Corporation, Framingham, Massachusetts 01701
M. Harvey [399], Genzyme Transgenics Corporation, Framingham, Massachusetts 01701
James P. Hoeffler [1], Invitrogen Corporation, Carlsbad, California 92008
Robert B. Kirkpatrick [289], Department of Gene Expression Sciences, SmithKline Beecham Pharmaceuticals, King of Prussia, Pennsylvania 19406
H. M. Meade [399], Genzyme Transgenics Corporation, Framingham, Massachusetts 01701
Brian Miller [65], Genencor International, Inc., Palo Alto, California 94304
Glen R. Nemerow [111], Department of Immunology, The Scripps Research Institute, La Jolla, California 92037
John H. Nilson [367], Department of Pharmacology, Case Western Reserve University, Cleveland, Ohio 44106
Christopher K. Raymond [193], Department of Protein Expression Technology, ZymoGenetics, Inc., Seattle, Washington 98102
Marijane Russell [235], Invitrogen Corporation, Carlsbad, California 92008
Allan Shatzman [289], Gene Expression Sciences Department, SmithKline Beecham Pharmaceuticals, King of Prussia, Pennsylvania 19406
August J. Sick [331], Invitrogen Corporation, Carlsbad, California 92008
T. E. Smith [399], Genzyme Transgenics Corporation, Framingham, Massachusetts 01701
Mark F. Stinski [211], Department of Microbiology, University of Iowa, Iowa City, Iowa 52242
Andreas Voloudakis [429], Department of Cell Biology, The Scripps Research Institute, La Jolla, California 92037
Daniel J. Von Seggern [111], Department of Immunology, The Scripps Research Institute, La Jolla, California 92037
Yanhai Yin [429], Department of Medicine and School of Medicine, University of California, San Diego, La Jolla, California 92037
M. W. Young [399], Genzyme Transgenics Corporation, Framingham, Massachusetts 01701
C. A. Ziomek [399], Genzyme Transgenics Corporation, Framingham, Massachusetts 01701

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Introduction

SO MANY POSSIBILITIES: HOW TO CHOOSE A SYSTEM TO ACHIEVE YOUR SPECIFIC GOAL

Joseph M. Fernandez and James P. Hoeffler
Invitrogen Corporation, Carlsbad, California 92008

The Art of Expression

Our understanding of gene expression has increased greatly since the mid-1980s, resulting in new developments in protein expression system technology. A key element in creating efficient and economic expression systems has been the construction of vectors that include, along with the gene of interest, the appropriate promoter and other regulatory sequences. Recombinant DNA techniques have enabled unique pairings of promoters and structural genes in a wide variety of vectors for expression of desired recombinant proteins. These new gene combinations are currently being utilized in numerous prokaryotic and eukaryotic organisms to produce recombinant products of both academic and industrial importance. Notably, all the elements that constitute an expression system—structural genes, control sequences, markers, and induc-

Gene Expression Systems: Using Nature for the Art of Expression

ers—are present in nature; scientists have only had to mix and match them from a variety of organisms to create diversity and flexibility in the regulation of gene expression, much as a painter mixes colors on a palette to obtain the desired shades. The result is a wide range of effective expression systems available to researchers to achieve their specific objectives: from high-level expression of recombinant products for large-scale production, to subtle expression for studying protein function in the cell, from prokaryotes to transgenic animals. Selecting the most suitable system depends on a series of parameters, such as time, resources, and intended use. Often, after weighing advantages and disadvantages, there is not always a clear winner, but there may be some areas of overlap among alternatives. The choice is indeed so wide and complex that selecting the right system has become an art in itself, further emphasizing the fact that Nature is a palette for the art of expression.

Purpose of This Book

The aim of this book is to provide the latest information on state-of-the-art protein expression systems and to help the reader select one that will best suit individual goals and resources. Leading scientists in the field review the most popular prokaryotic and eukaryotic expression systems: from bacteria (Section I) to yeast (Section II) and to insects (Section III), mammalian cell cultures (Section II), and transgenic animals and plants (Section IV). Advantages and disadvantages of each system are surveyed and summarized for easy reference. Each chapter illustrates how a system works and what proteins are most likely to work well, addressing potential problems and suggesting solutions. Although some information is offered on methods for generating recombinants, detailed protocols are beyond the scope of this book and, for these, most authors provide references. In light of the increasingly important role of protein expression, most chapters also contribute insight into future developments. Finally, in an effort to provide further guidance to the reader, prokaryotic and eukaryotic systems are compared for commonly desirable characteristics in a convenient, easy-to-read chart.

Selecting a Suitable Expression System: Considerations

When selecting a protein expression system, a number of considerations must be made, including the intended use, time frame, availability of resources, and the characteristics of the recombinant product. These considerations will affect the choice of expression system and type of promoter to be used. As for the intended use, this may be the isolation of the recombinant protein or the study of protein function in a cell or organism. If the objective is large-scale production of the gene product, then a yeast such as *Pichia pastoris* (discussed in Chapter 6) or a transgenic animal system (see Chapter 14) may be suitable options. Both systems allow for high-level production of exogenous eukaryotic proteins, but differ in a number of characteristics related to additional considerations. If time and cost are of concern, the yeast offers the advantages of rapid growth in inexpensive medium and easy handling of microbes, which translates into economical high-level production of the gene product. A transgenic system, conversely, involves higher costs and time-consuming activities associated with transgenic animal development, dairying, and testing; this system, however, is able to produce correctly processed protein and would therefore be a better choice when the expressed protein requires posttranslational modifications not possible in the yeast.

When the user's objective is to study the function of a recombinant protein in a cell or organism, the ability to regulate expression may be important, leading to the choice of a system that allows for maximum optimization. There are numerous stable and transient expression systems, for regulated or constitutive production, that can be used for functional studies. The choice will depend on factors such as the need for posttranslational processing to obtain a biologically active product, toxicity of the recombinant product on the host, the amount of protein needed, and the time frame of the study. Viruses, for instance, make powerful tools for the expression of heterologous gene products in higher eukaryotes because of their high transfection efficiencies and the high level of recombinant protein expression. The Sindbis virus (reviewed in Chapter 10) is an example of an effective vector system particularly suitable for transient expression, as infection with this virus results in eventual death of the host cells. In contrast, retroviruses integrate into the host genome

and therefore represent an attractive system if stable protein expression is desired, allowing for optimization of a cell line.

One important consideration is the effect of the recombinant protein on the host. If the protein is toxic to or if it inhibits growth of the host cells, a transient system or a system that uses an inducible promoter may be desirable. In Chapter 4, the reader will find information on the arabinose expression system, which allows for the tightly regulated expression of recombinant prokaryotic and eukaryotic proteins in bacteria, and Chapter 9 discusses inducible mammalian expression systems that enable regulated inducible expression, including prokaryotic control elements in eukaryotic cells, as well as the novel insect system based on regulatory elements from *Drosophila*.

Finally, to help the reader in the identification of a suitable choice, we have provided a quick comparison chart (Table 1) in

Table 1
Comparison of Expression Systems

	Expression system			
Desired characteristics	Bacteria	Yeast	Insect	Mammalian cell culture
Cell growth	Rapid	Rapid	Slow	Slow
Complexity of growth medium	Minimum	Minimum	Complex	Complex
Cost of growth medium	Low	Low	High	High
Expression level	High	Low to high	Low to high	Low to moderate
Extracellular expression	Secretion to periplasm	Secretion to medium	Secretion to medium	Secretion to medium
Posttranslational modifications				
Protein folding	Refolding usually required	Refolding may be required	Proper folding	Proper folding
N-linked glycosylation	None	High mannose	Simple, no sialic acid	Complex
O-linked glycosylation	No	Yes	Yes	Yes
Phosphorylation	No	Yes	Yes	Yes
Acetylation	No	Yes	Yes	Yes
Acylation	No	Yes	Yes	Yes
γ-Carboxylation	No	No	No	Yes

which the expression systems featured in this book are compared for commonly desired characteristics. Each system category (bacteria, yeast, insect, mammalian cell cultures) is reviewed, respectively, in Section I, II, III and IV of this book. Whatever the challenge, selecting the right system is an important key to success.

Genomics and the Future of Protein Expression Systems

Developments in DNA array technology have made it possible to define gene expression profiles as never before, enabling the simultaneous analysis of thousands of genes, large-scale gene discovery, and mapping of genomic DNA clones. DNA arrays are used to measure the expression levels of prokaryotic and eukaryotic genes and can quickly elucidate the correlation between gene expression and biochemical pathways. In addition, they have provided information on the expression patterns of many previously unknown genes. DNA chip technology is advancing rapidly, with applications in diagnostics (mutation detection), gene discovery, gene expression, and mapping, as well as pharmaceutical development. Because of the abundance of data generated by this technology, protein expression systems will be increasingly more important, as researchers study the structure and function of gene products identified by DNA arrays. However, with the plethora of expression systems available, one thing to bear in mind is that there is always an element of unpredictability in the behavior of any given protein in a system. No matter how advanced the state of technology and how carefully all parameters are evaluated when selecting a system, no system is totally predictable and ultimately; the only sure way to find out if a system will work is to try and experiment with it. As Mark Ratner pointed out in Bio/Technology 1989, "Expression systems are protein specific. You must be able to play around with each one, insert your gene of choice, tweak it, and then see what you've got."