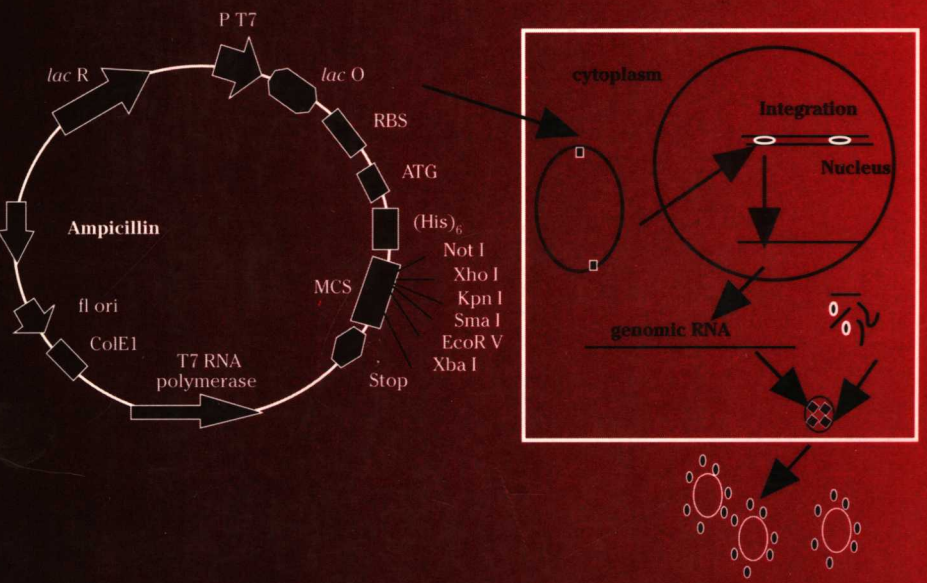
Methods in Gene Biotechnology



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Preface

We are living in an era of a biotechnology revolution. To many molecular and cellular biologists, the flood of information on new methodologies is particularly overwhelming because biotechnology is forging ahead and bringing about rapid changes day after day. Because every single organism depends on molecular actions for survival, molecular biology research has become more and more dominant in multiple disciplines. There appears to be a general tendency for NIH and other funding agencies to award grants with high priority to those research proposals that employ molecular biology approaches. How can one update one's knowledge of biotechnology and use the most recent, proven techniques for novel research? One of the aims of this book is to provide investigators with the tools needed for modern molecular and cellular biology research.

Another aim of this book is to guide graduate students in their thesis research. In our experience, good graduate training mandates independent performance with minimum advice from a mentor. How does one select a novel research project for a thesis? What are the hypotheses, objectives, and experimental designs? How does one grasp technical problems and master current techniques? Where does one begin and what are the predicted results? As a matter of fact, a graduate student needs some help with these questions. This book will provide the clues.

This book covers a wide range of current biotechnology methods that have been developed and are widely used in molecular biology, biochemistry, cell biology, and immunology. The methods and protocols described in the appropriate chapters include the following: approaches to novel research, rapid isolation of specific cDNAs or genes by PCR, construction and screening of cDNA and genomic DNA libraries, preparation of DNA constructs, nonisotopic and isotopic DNA or RNA sequencing, information superhighway and computer databases of nucleic acids and proteins, characterization of DNA, RNA, or proteins by Southern, northern, or western blot hybridization, gene overexpression, gene underexpression and gene knockout in mammalian systems, analysis of cellular DNA or abundance of mRNA by radioactive *in situ* hybridization (RISH), localization of DNA or abundance mRNA by fluorescence *in situ* hybridization (FISH), *in situ* PCR hybridization of low copy genes and *in situ* RT-PCR detection of low abundance mRNAs, new strategies for gene knockout, and large-scale expression and purification of recombinant proteins in cultured cells. Each chapter covers the principle(s), underlying methods, and techniques, in a detailed step-by-step description of each protocol, notes, tips, and troubleshooting guide. We have found that many of the currently available books in molecular biology contain only protocol recipes. Unfortunately, many fail to explain the principles and concepts behind the methods outlined or to inform the reader of possible pitfalls in the methods described. We intend to fill in these gaps.

For the information of the reader, Chapter 1 was written by William Wu, Peter B. Kaufman, and Michael J. Welsh. Chapters 2, 3, 6, 10, 12 to 14 were written by William Wu.

Chapters 8, 9, 16, and 17 were written by William Wu and Michael J. Welsh. Chapters 5, 7, and 15 were written by William Wu and Peter B. Kaufman. Chapters 4, 11, and 18 were written by William Wu and Helen Zhang. Nonetheless, all four authors have worked as a team.

W. Wu
M.J. Welsh
P.B. Kaufman
H. Zhang

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Dr. Wu is an internationally recognized expert in molecular biology. He has presented and published over 35 research papers at scientific meetings and in national and international journals. As a senior author, Dr. Wu has contributed 34 chapters of molecular and cellular biology methodologies in three books. He has broad knowledge of molecular biology, biotechnology, protein biochemistry, cellular biology, and molecular genetics. He is highly experienced and has extensive hands-on expertise in a variety of current molecular biology techniques. Dr. Wu is a member of the American Association for the Advancement of Science.

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Dr. Welsh is an internationally recognized scientist in molecular and cellular biology. He has published more than 120 research papers and has contributed several chapters to professional books. Dr. Welsh is a leading authority on mammalian heat shock protein (HSP27) and has been awarded several major grants from the U.S. National Institutes of Health to examine the function and mechanisms of HSP27 gene. He is also a Fellow of the American Association for the Advancement of Science.

Peter B. Kaufman, Ph.D., is a Professor of Biology in the Plant Cellular and Molecular Biology Program in the Department of Biology and a member of the faculty of the Bioengineering Program at the University of Michigan, Ann Arbor. Dr. Kaufman received his Ph.D. degree in 1954 in Plant Biology at the University of California, Davis.

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Mrs. Zhang has a strong background in molecular biology, microbiology, biochemistry, and immunology. She is highly experienced in DNA recombination, PCR applications, cell transfection, and gene expression in bacterial and animal systems. Additionally, she has extensive experience in protein/peptide purification, enzymatic assay, and analysis of drug-protein binding.

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Contents

Chapter 1

Approaches to Novel Research

Contents

Introduction

We are living in the era of technological revolution. To many molecular biologists the flood of new information is particularly overwhelming, because biotechnology is forging ahead and bringing about changes day after day. How can one catch up with the new biotechnology, and use the most recent, proven techniques for novel research? As indicated in the preface, one of the aims of this book is to provide investigators with tools for molecular biology research. Perhaps because every single organism depends on molecular actions for survival, molecular biology research becomes more and more dominant in multiple disciplines.[1-21] In fact, there is a general tendency for federal and private funding agencies to increasingly award grants to those research proposals that use molecular biology approaches. Given the fact that

research funding resources are quite limited, that the funding budget is decreasing, and that the number of research proposals rapidly increases year after year, the fundamental question is how can one bring funding to his research to still survive? One strategy is to write an excellent proposal using molecular biology tools. However, what catches the eyes in light of research projects and funding resources? How does one grasp research problems and march towards a new direction? How does one write an important and scientifically sound research proposal with new ideas, comprehensive designs, and methodologies? What is your research plan and where do you start? This chapter can provide some insights and ideas that may help novel research projects to obtain potential funding.

Another aim of this chapter is to guide graduate students in their thesis research. In our experience, good graduate training mandates independent performance with minimum advice from a mentor. How does one select a novel research project for a thesis? What are the hypotheses, objectives, and experimental designs? How does one grasp technical problems and master current techniques? Where does one begin and what are the predicted results? How does one interpret research data and decide on the next experiments? Assuredly, a graduate student needs some help with these questions. This chapter will provide some answers.

The major objective of this book is to help the reader pursue research in molecular biology. The present chapter serves as a guide to the use of current strategies and techniques in the book in order to develop novel research strategies. Several examples of research proposals of 2 to 5 years, with specific aims, strategic designs, and methods, are illustrated below. It should be noted that the following examples are not the actual proposal formats. It is hoped, however, that these examples will be a valuable guide for your novel research, proposal funding, and/or thesis research. The examples can also be adapted and applied to other appropriate novel research projects.

Proposal 1. Identification of a New Drug-Targeting Protein(s) and Isolation of a Novel Gene(s)

Drug discovery is one of the most interesting research goals to public health and is certainly invaluable to pharmaceutical companies. Once a new drug is produced, it will open up broad areas for new research. The fundamental question concerns its cure mechanism. Many kinds of research can be conducted using the drug. In our view, the most promising research proposal offers identification of the drug-targeting protein(s) and isolation of a novel gene(s). Because molecular interaction is the basis for the cure of a disease with a new drug, it is reasonable to hypothesize that there may be one or more proteins or genes that are potentially targeted by the drug. Because the discovery of a new drug is of great concern to the public, this plan would most likely get funded by NIH, pharmaceutical companies, or other private sector groups. Figure 1.1 illustrates the strategies, research design, and molecular biology methodologies that one can use, along with references to the appropriate chapters.

Proposal 2. Exploration of the Functions or Roles for the Expression of a Gene Targeted by a New Drug

Once a novel protein or gene targeted by a new drug or an important chemical has been identified, further research needs to be done. One logical and promising funding project is determining the function of the targeted protein or gene. The information from the research

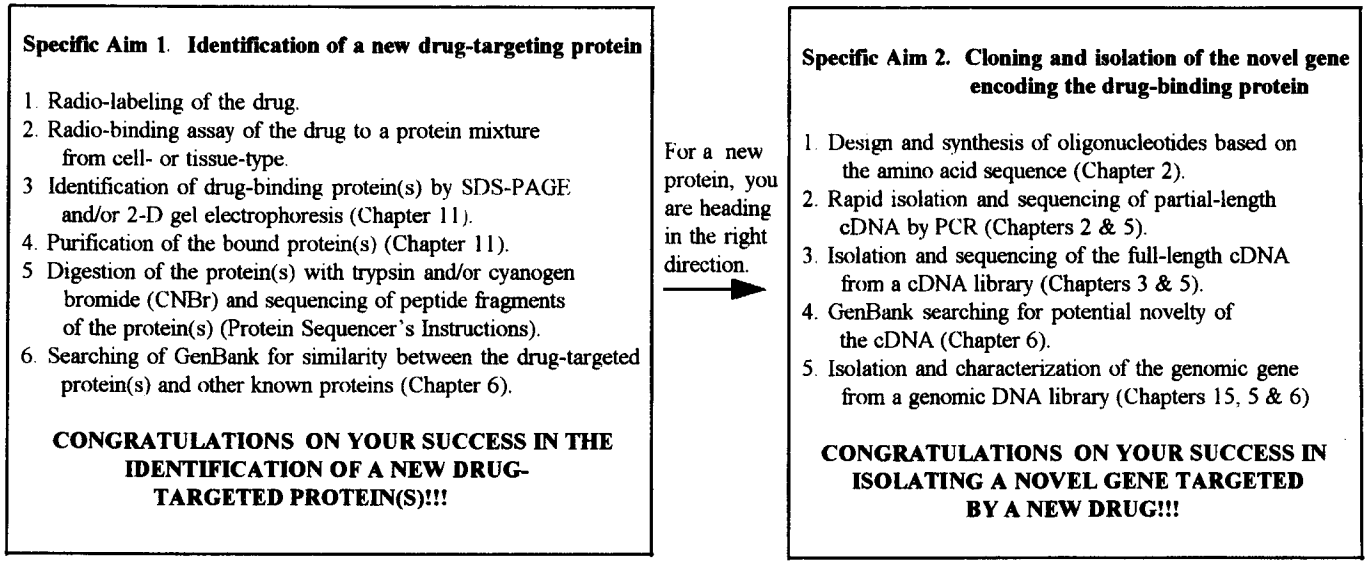

FIGURE 1.1
Research design and methodologies for the identification of a novel gene(s) targeted by a new drug.

will provide crucial evidence for the cure mechanism of a disease by the drug. This involves sophisticated skills, including generation and use of transgenic mice as an animal model (Figure 1.2).

Proposal 3. Verification of Potential Function of a Specific Gene by the Gene Knockout Approach

To verify the potential role of a novel important gene, the best strategy is to knock out the expression of the gene. For example, if it is reasonable to believe that a gene plays a key role in heart development, a smart approach is to target the gene *in vivo* by knockout. If the gene becomes null, diseases such as failure of heart development would be predicted to occur based on the hypothesis. This is certainly a very sound proposal with promising funding for 3 to 4 years, assuming that no previous grants have been awarded for this type of proposal to other laboratories. The general approaches and methods are diagrammed in Figure 1.3.

Proposal 4. Identification of the Functional Domain of a Protein by Site Specific Mutagenesis

Very often, the functions of a novel protein have been demonstrated but no one knows which specific fragment of the protein is the active domain or binding site. If the protein or enzyme is very important, there is a good reason to write a 2-year proposal on the identification of the functional domain of the novel protein or enzyme. The strategies for doing this are outlined in Figure 1.4.

Proposal 5. Identification of Toxicant-Binding Protein(s) and Isolation of a Novel Gene that Is Related to the Toxicant and Heart Hypertrophy Using an Animal Model

The heart is the first organ formed in animals, and diseases such as heart hypertrophy, heart attack, or failure are of great concern to the public. Additionally, toxicity mediated by toxicants becomes more and more of an environmental concern. If there is good reason to hypothesize that a toxicant (e.g., chemicals, proteins, drugs, carbohydrates) may be an inducer of heart hypertrophy, a 4- to 5-year proposal would be profound and promising for funding. This is a long-term research project that may need collaboration between laboratories. Specific aims, strategies, designs, expected results, and methodologies, as found in the appropriate chapters, are outlined in Figure 1.5.

Proposal 6. Rescue of an Immune-Deficient System Via Gene Therapy

It is well known that an immune deficiency disease, such as HIV, is of great concern to the public at the present time. Many important proteins, such as the CD families, have been discovered to play a significant role in the deficient immune system. In order to increase the

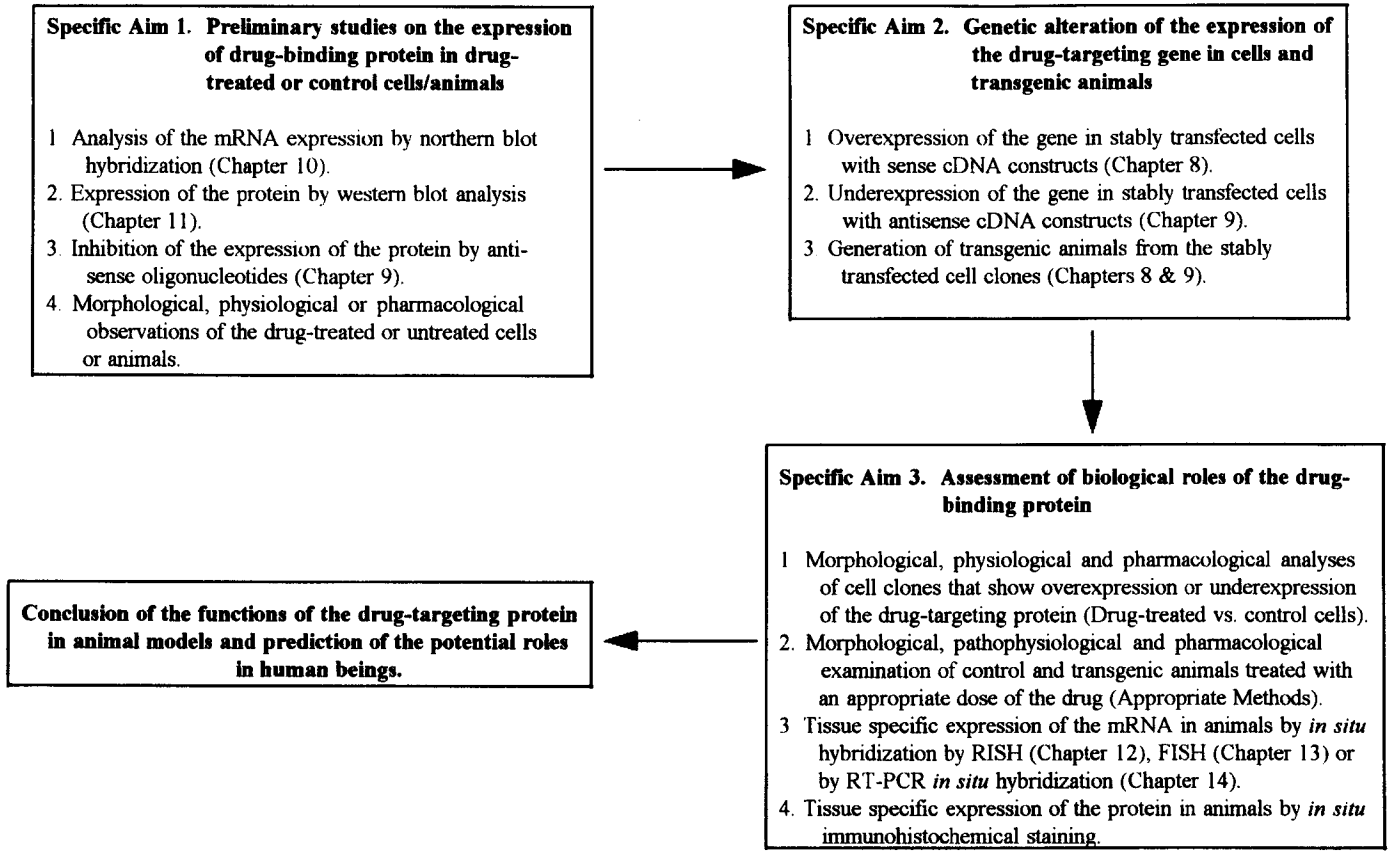

FIGURE 1.2
Research approaches and methods for exploration of the potential functions of a new drug-targeting protein using cultured cells and animal models.

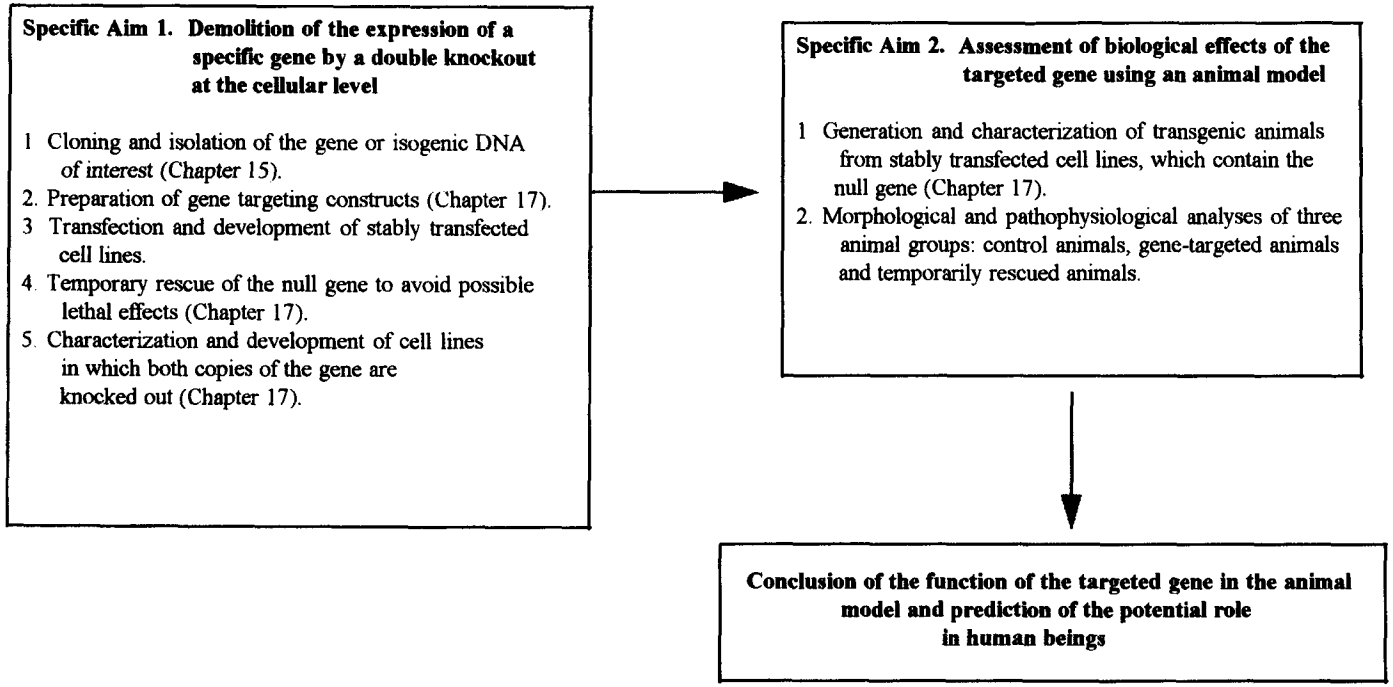

FIGURE 1.3
Research approaches and methods for exploration of the potential functions of a new drug-targeting protein using cultured cells and animal models.

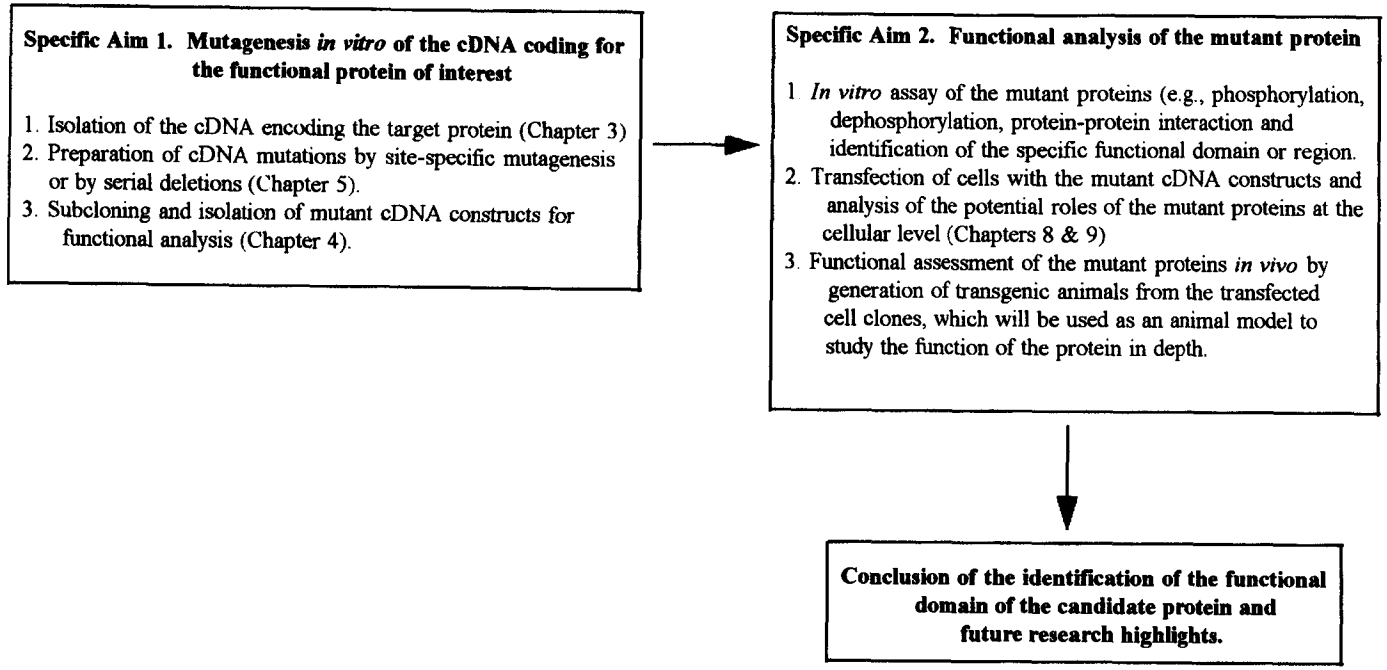

FIGURE 1.4
Design and methods for identification of a functional domain(s) for a given protein by mutagenesis.

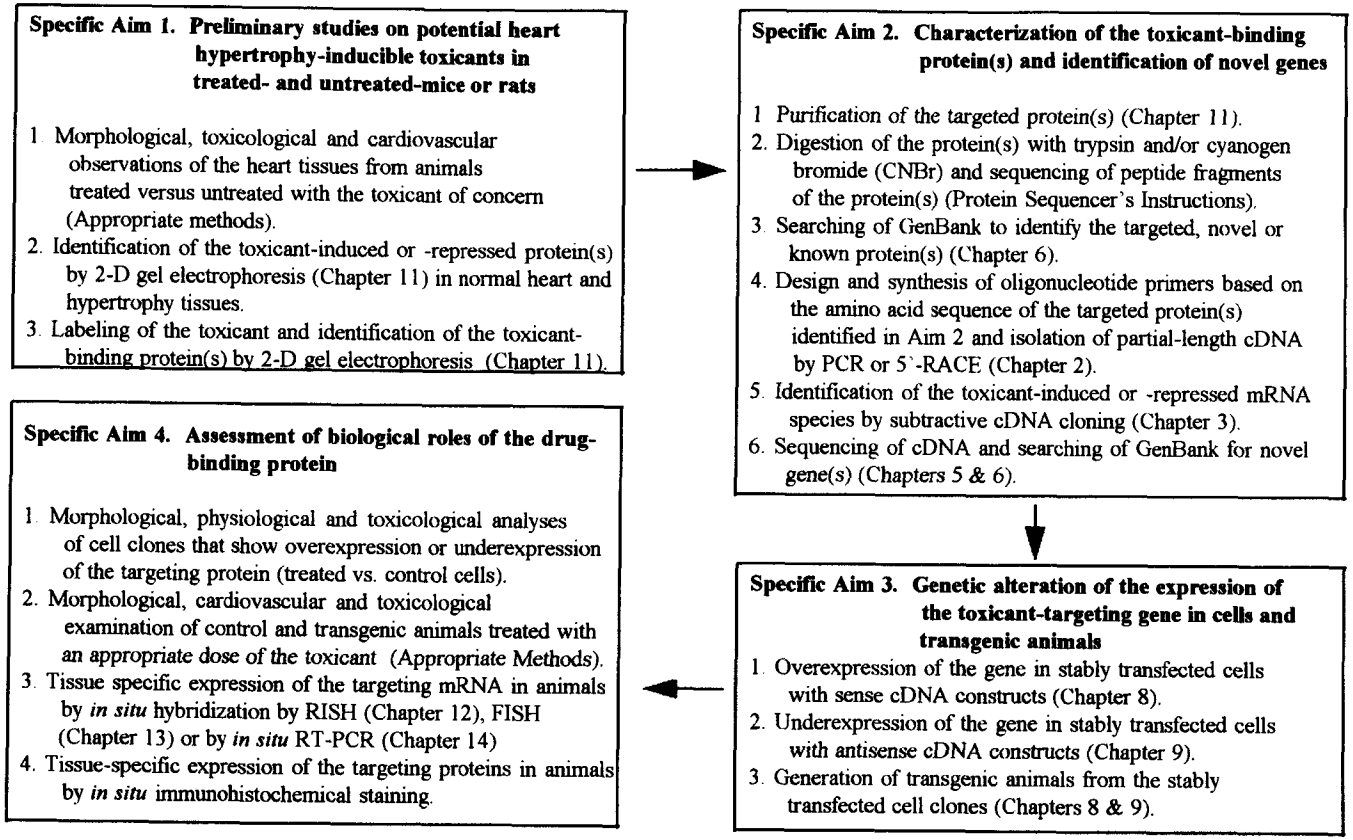

FIGURE 1.5
Comprehensive research design and methods for identification of a novel protein(s) and gene(s) in heart hypertophy induced by a toxicant using an animal model.