Book Reviews

***Pichia* Protocols**. Methods in Molecular Biology Vol. 103. Edited by D.R. Higgins and J.M. Credd, Humana Press; Totowa, 1998. xi+270 pp. $74.50 (hb). ISBN 0-896-03421-6.

Why work with *Pichia pastoris*? One justification is that unlike *Saccharomyces cerevisiae*, *Pichia* is well suited to the large-scale production of heterologous proteins. Otherwise *Pichia* is quite similar to *Saccharomyces*. For these reasons a group of biotechnology researchers in the 1980s developed genetic and molecular tools for working with *Pichia*. This yeast readily undergoes homologous recombination, and can be used for the constitutive or regulated expression of heterologous genes. A *Pichia* expression system has been marketed by Invitrogen for several years and has now come into widespread use. More recently, *Pichia* has received attention from cell biologists because it has advantages for studying peroxisome biogenesis, autophagy, and the organization of the ER-Golgi system. Hence, *Pichia Protocols* is a timely reference guide for a growing population of researchers.

The editors of this volume are David Higgins and James Cregg, two of the pioneers in the *Pichia* field. They have done a first-rate job. This book is comprehensive but accessible, and it will be valuable both to first-time yeast users and to *Pichia* experts. Most readers will find the first three chapters to be of primary interest. The Introduction provides a historical overview of the *Pichia* expression system and a summary of its properties. The second chapter, by Cregg and coworkers, describes genetic manipulation of *Pichia*. This section provides key information about some of the differences between *Pichia* and *Saccharomyces*. For example, *Pichia* mates only upon nitrogen limitation, yet sporulates much more readily than *Saccharomyces*. In Chapter 3, Cregg and Russell describe methods for the transformation of *Pichia*. All of the protocols listed in these chapters are simple and straightforward. Readers should also pay heed to the accompanying notes, which often contain important information. For example, one note reveals that *ura3* strains of *Pichia* grow slowly even in media supplemented with uracil.

The remainder of the book focuses primarily on heterologous protein production, with some discussion of *Pichia* cell biology. In Chapter 4, Higgins and colleagues describe how a zeocin-resistance cassette has been used as a dominant selectable marker to create convenient expression vectors. The subsequent chapters will be of interest to researchers working to optimize the production of a specific protein. Romanos et al. describe methods for generating *Pichia* strains with multiple copies of a gene of interest; Gleeson et al. explain how protease-deficient strains can be used to maximize the recovery of an overproduced protein; and Stratton et al. discuss strategies for fermentation at high cell densities. These chapters are all well presented, although it is worth noting that similar information can be obtained from online manuals available at the Invitrogen web site. Another important topic is the glycosylation profiling of proteins secreted by *Pichia*. As discussed in a chapter by Cremata et al., the glycosylation state of a secreted protein can influence its activity and especially its antigenicity. Several additional chapters describe strategies and anecdotal results concerning the expression of particular genes in *Pichia*. This information will probably benefit only those workers who are embarking on a detailed optimization of gene expression. A chapter on nucleic acid isolation will also be of limited use, because most investigators now employ commercial kits for this purpose.

Two of the later chapters deal with cell biological topics. Subramani and colleagues are very successfully using *Pichia* to study peroxisome biogenesis, and they provide an overview that includes protocols for subcellular fractionation, immunofluorescence and the generation of temperature-sensitive alleles. In the final chapter, Reiländer and colleagues describe a method for immunoelectron microscopy of *Pichia* cells.

Inevitably, *Pichia Protocols* is already becoming outdated. A search of the recent *Pichia* literature uncovers papers that provide new information about chromosome structure and protein glycosylation, alternative expression vectors, and refined methods for immunofluorescence analysis. For those seeking more comprehensive information, an additional source is patent databases. Because *Pichia* was first studied by industrial researchers, many of the available data have been published only in patent summaries. Two examples are the sequences of the *Pichia URA3* and *PEP4* genes. Unfortunately, *Pichia Protocols* does not include a list of relevant patents. But despite a few minor quibbles, this book is an excellent reference source that will be useful for anyone who works with *Pichia*.

Benjamin S. Glick

Gene Cloning and Analysis. Current Innovations. Edited by B.C. Schaefer, Horizon Scientific Press; Wymondham, 1997. 214 pp. £34.99 (pb). ISBN 1-898486-06-9.

Emerging technologies for gene isolation and characterization are presented in this book in a total of 13 chapters written by 24 authors. Most of these techniques are applicable to any biological system, ranging from bacteria and fungi to plants, yeast, and higher eukaryotes. The methods are categorized into (i) novel technologies, (ii) substantial improvements to recently developed methods which are becoming very popular, but typically plague investigators with artifacts and sub-optimal results, and (iii) elegant and novel refinements of widely used and frequently applied techniques. Considering the multitude of currently used methods in the vast field of molecular cloning and analysis the editor has successfully selected methods of interest to any researcher in the field.

Chapters 1 and 2 give detailed overviews, useful improvements and troubleshooting guides for the *yeast two-hybrid system* and the *phage surface display* methodology. *Restriction endonuclease-based gene expression fingerprinting*, a method for the isolation and identification of differentially expressed genes that is based on the differential display technique is presented in chapter 3. Chapter 4 provides basic information about the physical chemistry of PNA (peptide nucleic acids) and its applications. Chapter 5 gives an overview of established techniques for the isolation of unknown genomic DNA regions that flank known DNA sequences, and focuses on a simple and rapid *PCR-based method* that is similar to a technique called RAGE (rapid amplification of genomic DNA ends). Chapters 6 and 7 deal with procedures for the isolation of messenger RNAs and the construction of their full length complementary DNA molecules either by a *CAP retention procedure* called *CAPture* or by *RNA ligase-mediated RACE*. In chapter 8, Chatellier and Vernet describe their method *combinatorial scanning site-directed mutagenesis*, a column splitting technology based on standard DNA chemistry. This technique overcomes limitations of standard oligo-based site-directed mutagenesis protocols posed by the degeneracy of the genetic code and makes it possible to target any residue in a given protein sequence. Chapter 9 gives a comprehensive review of mutagenesis protocols based on PCR strategies. Also, a *rapid method for creating gene mutations and fusions using PCR ligation-PCR mutagenesis* is presented in detail. In chapter

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10, Melton describes the *double replacement gene targeting in embryonic stem cells for the introduction of subtle alterations into endogenous mouse genes*. This technique is useful for situations where the loss of a single codon is required (e.g. to model the common cystic fibrosis mutation ΔF508) instead of a conventional gene knockout system using transgenic mice technology. A simple and fast method to clone any gene into any given vector is provided by the *universal TA cloning* approach in chapter 11. In chapter 12, a PCR-based strategy is presented, which deals with the specific amplification of a large (up to 40 kb) DNA sequence out of a group of highly homologous sequences: *long PCR: rapid restriction mapping and selective suppression by restriction digestion*. The last chapter presents the *high-resolution silver staining-based PCR-single strand conformation polymorphism (SSCP) analysis*, an improvement of the commonly used SSCP method to detect sequence polymorphisms.

Each chapter starts with an abstract and an introduction, followed by a laboratory protocol, many helpful hints and a troubleshooting guide for successful execution. Each chapter is concluded with a comprehensive list of reference articles. The book concludes with a full length index.

Most authors give an objective overview of the methodology they describe, provide references to useful internet pages, indicate commercially available products and give a spectrum of potential applications and future trends. Also, restrictions and limitations of the presented techniques are covered. Good examples are the chapters about the *yeast-two hybrid technology* and the *RNA ligase-mediated RACE*.

In the chapter about PNA, we missed information about the possibility to obtain PNA molecules commercially. In the section 'future trends' we would have welcomed a more detailed discussion of some upcoming issues, such as that PNA may soon play an important role in the construction of high density oligonucleotide arrays (DNA chips) because it may substantially decrease hybridization-dependent problems like sterical hindrance of oligos.

Whereas some chapters are also suitable for 'newcomers', others require substantial prior knowledge. The laboratory protocols presented for all methods are in general detailed and well described with some exceptions. For example, the protocol for the over-expression of a hybrid molecule that is coupled to an IPTG-inducible promoter is given in detail but the step describing the addition of IPTG is missing (chapter 6, page 86).

The book contains many typing errors, although they do not threaten the interpretation of the presented laboratory methods.

These points of criticism, however, do not detract from the usefulness of this book and may be easily eliminated in the next edition.

In summary, the book provides an excellent desk and bench reference and we recommend it to researchers working in the field of molecular biology. ''My hope is that those who purchase *Gene Cloning and Analysis: Current Innovations* will soon find this volume not only useful, but an indispensable laboratory resource''. We are confident that the editor's wish will be fulfilled.

M. Kenzelmann and K. Mühlemann

Immunochemistry in practice. Edited by A. Johnstone and R. Thorpe, Blackwell Science; Oxford, 1996. xv+362 pp. £29.95 (pb). ISBN 0-865-42633-3.

Since all molecules can serve as potential antigens, immunochemistry, in its broadest sense, could encompass all commonly used biochemical and molecular biological procedures. In order to narrow the definition into more manageable proportions, immunochemistry is taken to mean the chemistry of antibodies and their reactions with antigens and it is this definition that the present volume adheres to. *Immunochemistry in Practice* is the third edition of a book that has been useful both as a practical laboratory manual and for teaching classes.

The third edition, appearing 9 years after the second edition, has been revised and restructured with respect to contents and layout. New sections, including methods for conjugating peptides to carrier proteins and for Western blot detection using enhanced chemiluminescence, as well as a new chapter on antibody labelling methods have been added.

The book begins with a chapter on basic techniques, including methods for protein detection, measurement, fractionation, concentration and dialysis. In addition, this chapter describes methods for radioactivity measurement and autoradiography. The whole chapter covers classical methods that have more than stood the test of time. The description is wonderfully clear and all basic chromatographic concepts and principles are admirably well explained. More recent methods like HPLC and FPLC are very briefly addressed (on less than one page) and reference is made to manuals from some of the manufacturers of this type of equipment.

The book continues with a chapter on antibody production, including brief descriptions on polyclonal and monoclonal antibody production and on the use of peptides as antigens. Use of molecular techniques for antibody production are referred to, but no details are given. Chapter 3 provides an in-depth description of antibody purification and production of antibody fragments. Well established methods for isolating IgG, IgM, IgA, IgE, IgD, IgY and the relevant fragments are clearly and concisely presented. Chapter 4 covers methods used for lymphocyte studies including isolation, manipulation, storage, fractionation and solubilization of lymphocytes. Chapter 5 is concerned with radiolabelling methods for antibodies and antigens. Standard radioiodination methods including the chloramine T, iodogen, lactoperoxidase and the Bolton-Hunter methods are described as are methods for biosynthetic labelling and labelling of glycoproteins and glycolipids. Chapters 6 and 7 cover methods for agar/agarose electrophoresis, immunodiffusion and immunoelectrophoresis, polyacrylamide gel electrophoresis (PAGE) under native and denaturing conditions, isoelectric focussing and two-dimensional electrophoresis. Methods for detecting antigens separated by PAGE are presented in chapter 8, and include crossed immunoelectrophoresis, immunoblotting and filter affinity transfer. A small but useful assortment of recipes for immunoblotting detection includes conventional immunoenzymatic, immuno-autoradiographic and enhanced chemiluminescence techniques. Chapter 9 covers preparative immunoprecipitation and electrophoretic methods while chapter 10 is concerned with affinity chromatography and immunoprecipitation techniques. The brief chapter 11 introduces methods for coupling diverse reporter molecules (fluorochromes, enzymes and biotin) to antibodies and is followed by two chapters describing the use of labelled antibodies or antigens in immunoassay techniques and immunocytochemistry. The final pages of the book contain a list of some manufacturers of chemicals and equipment to whom reference has been made in the text. In addition a seven pages long list of references is provided. The references have been carefully revised and selected in order to provide only key information. Although I find this useful, I do believe that a more extensive reference list covering more references to topics lightly treated in the present volume would have been more helpful.

To whom would this type of book be of use? It is abundantly clearly written and does not assume much previous knowledge. Thus, for instance, the gel chromatography section presents schoolbook formulas for calculating the cross-sectional area and volume of chromatography columns. In addition, the book contains some very clear definitions of chromatographic and electrophoretic principles that probably are better explained here that anywhere else. Thus, this book is highly recommended to students who need to learn the basic facts about immunochemistry. Undoubtedly, this book would also serve as a useful instruction book for practical classes in basic immunochemistry and biochemistry. To more senior scientists it represents an enjoyable trip down memory lane. The lack of attention given to more recent methods and molecular biology does not detract from the general usefulness of this book for practical introductory classes in immunochemistry. I would wish that every one of my own students read it carefully before turning their attention to more fanciful kits and machinery.

Lars-Inge Larsson

Telomeres and telomerase. Edited by D.J. Chadwick and G. Cardew, Wiley; Chichester, 1998. ix+238 pp. £57.50 (hb). ISBN 0-471-97278-9.

Telomeres, repeated DNA sequences $(T_2AG_3)_n$ that serve as guardian of the genome and especially the ends of chromosomes, were first defined by H.J. Muller in 1938. According to Muller's hypothesis, a chromosome must be guarded at both ends by the telomeres and, therefore, he did not believe in the existence of telocentric chromosomes. In 1941, Barbara McClintock, while working on maize chromosomes, provided experimental evidence for the functional aspects of telomeres. Nature does not provide protection to broken chromosome ends. A chromosome with broken ends either has to rejoin at both ends and form a ring or join with other chromosomes to form dicentric and multicentric chromosomes. Chromosomes with such abnormal characteristics follow the breakage-fusion-bridge cycle and eventually perish. With the discovery by C.W. Greider and E.H. Blackburn in 1985 of an enzyme, telomerase, that elongates or stabilizes the length of telomeric DNA in a given chromosome, and the cloning of human telomeric DNA by R.K. Moyzis and associates in 1988, the study of telomere dynamics and aging in the biological sciences in general, and cancer development and apoptosis in particular, has become more challenging. Different functional aspects of telomeric DNA in cell cycle regulation, cellular senescence, DNA repair at chromosomal ends, and cancer development are discussed in detail in this book, *Telomeres and Telomerase*, resulting from the Ciba Foundation Symposium 211, organized by Dean J. Chadarick and Gail Cardew and held on 25–27 February, 1997.

This book is composed of 13 review-type articles, followed by discussions after each presentation, with three general discussions and a succinct summary. The two editors have done a superb job of bringing together excellent subject matter by reputed authors within the field of telomere and telomerase biology. Although some of the functions of telomeres have been known for a long time, the discovery of their special sequences has made the biology of such chromosomal termini a most interesting and enlightening area of current research for apoptologists, oncologists, cell-cyclologists, embryologists, senescensologists, and cell biologists.

The first two chapters by Blackburn and associates and by Cech and Lingner on the biology of telomeres and the discovery of the telomerase enzyme in *Tetrahymena*, by her student Carol Greider in 1985 and 1989, has provided not only an impressive overview of the telomere and telomerase, but also a discussion of different aspects of telomere biology, in normal and mutant conditions, during the anaphase separation of cell division. Nuclear division can be blocked not only by inducing mutations in the telomeric DNA, but also by erosion of these highly repeated telomeric loci. Their research has indicated that the telomere itself is a major controller of telomerase action.

The other 11 articles in the book are grouped according to different scientific philosophies. In the section on the role of the EST genes in yeast telomere replication, Victoria Lundblad and associates describe elegant experiments dealing with the functional aspects of different *Saccharomyces cerevisiae* mutants that mapped to three genes, EST1, EST2 and EST3. Their results have shown that these genes are essential in vivo for telomerase function at the end of the telomere. Following this, the next chapter deals with an unusual mechanism of telomere elongation in *Drosophila* chromosomes by Biessmann and associates. *D. melanogaster* has a telomerase-independent mechanism for the elongation of telomeric repeats. Instead of having short repeats that are synthesized by telomerase, long retrotransposons, HeT-A and TART, transpose to the ends of chromosomes. *Drosophila* telomere length polymorphism is mainly due to terminal retrotransposon arrays that differ between chromosome tips and that change with time. In addition, stable terminal chromosome deletions can be generated that do not contain HeT-A and TART arrays, suggesting that in *Drosophila* the presence of terminal arrays may not be essential for call cycle progression as is required in yeast and humans. David Shore and his group, who present a detailed account of Rap1p in telomere length regulation in the yeast *Saccharomyces cerevisiae*, conclude that regulation of both telomere silencing and length is achieved by separate competing protein complexes that interact with Rap1p. At present no detailed molecular information regarding the mechanism by which Rap1p molecules control telomere length is known. Two proteins, Rif1p and Rif2p, function together as direct mediators of the telomere length regulation function of Rap1p. Their target of action has not yet been identified. The next three chapters deal with aging in human and other mammalian cells in culture. It is now a well-established fact that the limited reproductive life span of normal diploid cells in culture is regulated by the shortening of one or more telomeres that actually serve as 'mitotic clock' for cell division. Many genetic diseases such as Werner's syndrome and progeria indicate that the duration of the life span is also genetically controlled, and is independent of the cessation of cell proliferation. The article by Calvin Harley is a masterpiece on human aging and telomeres. He concludes that sufficient telomere loss on one or more chromosomes in normal somatic cells triggers cell senescence, and the upregulation/reactivation of telomerase is required for cell immortalization. His research supports the following key observations: (a) telomerase is easily detected in mature male and developing female reproductive organs; (b) long telomeres are stably maintained as a function of donor age in sperm; (c) telomerase activity is low or below the level of detection, and telomeres gradually shorten with age in vitro and in vivo in most postnatal somatic cells; (d) telomerase cannot be detected and telomeres continue to shorten in a pre-crisis population of somatic cells transformed with viral oncoproteins; and (e) telomerase is detected and telomeres are stably maintained (at usually a short length) in many post-crisis (immortal) transformed and cancer cell lines.

Since cancer is considered a group of diseases of old age, it is not unreasonable to implicate telomere and telomerase assays in the diagnosis and prognosis of cancer. Jerry Shay and associates have done just that in their article. Almost any clinical specimen can be used to assay telomerase activity, including frozen tissues, needle aspirates, washes and sedimented cells from urine. New strategies to improve the diagnostic value of telomerase determinations include application of in situ hybridization methods for detecting human telomerase RNA expression in paraffin-embedded specimens. This result has shown promise in distinguishing cancer from normal cells and thus may complement the telomerase activity assays. The next chapter by Blasco and associates on a mouse model for the study of telomerase is an interesting departure from the previous one. They have been able to construct a knockout mouse for the mouse telomerase RNA, mTR−/−. These mice and the cell lines derived from them are telomerase deficient. The chapter on genetic control of telomerase and replicative senescence in human and rodent cells provides clues to why rodent cells are easy to transform in vitro and human somatic cells are not. The next two chapters deal with repair and processing events at DNA ends by Lindahl and associates and telomeres in the hemopoietic system by Lansdorp and colleagues, respectively. For the first time, it is shown that all human chromosomes do not have the same amount of telomeric repeats. The newly developed technique, a quantitative fluorescence in situ hybridization (Q-FISH), has the capability to assess the number of telomere repeats at the end of individual chromosomes. This novel tool may provide mechanisms for specific chromosomal alterations (inversions, translocations and deletions) observed in most immortal and cancer cells.

Finally. although this is a collection of several chapters dealing with different aspects of telomeres and telomerase, the book serves as a rich source of useful new information related to the telomere. The part that I like most is the discussion after every presentation. The only thing that is lacking in the book is the role of telomeric DNA in the evolution of different species. I am confident that researchers in the field of oncology, gerontology, apoptosis and evolutionary biology will definitely find this book a great source of frequent consultations. It is, therefore, highly recommended for every research library and teaching institution.

Sen Pathak

Biopharmaceuticals: Biochemistry and Biotechnology. Edited by G. Walsh, Wiley; Chichester, 1998. xvi+431 pp. £29.95 (pb). ISBN 0-471-97789-6.

The biopharmaceutical industry has grown from several hundred small start-up companies in the early 1970s, many of which were based on promise and hype, to a few predominant players which have demonstrated the therapeutic and financial potential of biotechnology. *Biopharmaceuticals: Biochemistry and Biotechnology* provides a balanced overview of the biopharmaceutical industry, starting from a historical perspective of the traditional pharmaceutical industry. As the book progresses, more detail is provided concerning the biopharmaceutical development and manufacturing process. The majority of the book focuses on recently developed biopharmaceuticals, placing major emphasis on polypeptide-based therapeutics. The final chapter discusses the potential of nucleic acid-based drugs.

Chapter 1 begins with an introduction to the history of the pharmaceutical industry. A good overview is provided of the evolution of biotechnology from academic and technical experts who sought to take advantage of developments in the biotechnology area. Most well established large pharmaceutical companies were reluctant to invest in biotech, but did later diversify into this area either through acquisition of smaller start-ups or by formation of strategic alliances (i.e. Genentech and Eli Lilly). Such mergers helped to accelerate the growth of the biotech industry. The last part of chapter 1 is focused on several traditional pharmaceutical substances isolated from biological sources.

Chapter 2 provides a good overview of the drug development process beginning with drug discovery. The reader is introduced to topics including combinatorial chemistry and rational drug design. The critical role of patents in the biotech industry is discussed in some detail. After reviewing the importance of pre-clinical trials, pharmacokinetic and pharmacodynamic studies, toxicity studies and clinical trials, the role of the various regulatory authorities throughout the world is described.

Chapter 3, the longest chapter in the book, is entitled 'The drug-manufacturing process'. A significant overview is provided of the facilities needed to make a biopharmaceutical product, the different recombinant expression systems available, upstream and downstream processing and product characterization. More discussion of Chinese hamster ovary (CHO) cells as an expression system could have been provided since this is becoming the vector of choice in the industry due to its ability to glycosylate proteins more like the naturally occurring human form of the molecule. The importance of glycosylation to the clearance of proteins could also have been included. The chapter provides a thorough description of product formulation and stability.

Chapters 4 through 10 focus on recently developed products including cytokines and interferons, interleukins, tumor necrosis factor, hemopoietic growth factors, growth factors, hormones, blood products, antibodies, vaccines and adjuvants. Each of these chapters provides a thorough scientific review which leaves the reader with a sound understanding of these types of molecules. The final chapter provides an introduction to nucleic-based therapeutics.

In summary, *Biopharmaceuticals: Biochemistry and Biotechnology* is well written and provides an excellent overview of the biotechnology industry. The author has suggested that the major target audience is that of advanced undergraduates or post-graduate students pursuing courses in relevant aspects of the biological sciences, biotechnology, biochemistry, the pharmaceutical sciences and medicine in addition to those already employed in the biopharmaceutical sector who wish to gain a better overview of their industry. The book achieves its purpose. It is unique in that it integrates an overview of the development and manufacturing processes together with a review of recently developed biotechnology products. The book is well referenced and provides numerous suggestions for further reading at the end of each chapter. As with any book which attempts to provide a review of a complex and rapidly evolving industry, there are some areas which could have been covered in more detail such as an expanded discussion on CHO cell fermentation processes or the role of glycosylation in biotech products. The book is still an excellent and balanced source of information about the biotechnology industry.

Wayne Gombotz

Adenovirus Methods and Protocols. Methods in Molecular Medicine. Edited by W.S.M. Wold, Humana Press; Totowa, 1998. xiii+352 pp. $99.50 (hb). ISBN 0-896-03551-4.

This book is one in a rather substantial series that is devoted to methods in molecular medicine. A stated primary purpose of this text is to focus on adenoviruses as a relevant model system for studying cell biology. As a means of assisting new researchers in conducting studies on adenoviruses, and helping established researchers gain access to new techniques, the multiple authors have adhered to a consistent format. Each chapter contains two principal elements, a general introductory background for the topic and a collection of step-by-step methods that are relevant to the subject. Included in the first seven chapters are methods for growing and titering adenoviruses and for creating mutants; in the next five chapters are methods for measuring apoptosis induced by cytokines, primed immune cells and intrinsic effector cells. Several chapters are devoted to in vitro methods for studying transcription and splicing, and the latter chapters address various specialized topics.

The chapter sections dealing with the methodology are rather comprehensive and provide much useful information, particularly for the reader who has not had much formal training in virology. Basic issues such as virus quantitation, large-scale preparation, and gradient purification are addressed in the first chapter while the next several chapters describe various methods for the construction of mutations, with a focus on early region (E) genes. Given the widespread interest in adenoviruses as gene therapy vectors, the construction of deletion mutants is a necessary first step in creating recombinant vectors that contain specific transgenes. Essential elements of these discussions on characterization of adenoviral recombinants are: (1) the description of viral particle assembly; (2) the enumeration of important *cis*-acting packaging sequences, and (3) the assays necessary to measure the packaging of mutant virus DNA into intact virus particles.

The chapters on immune-mediated apoptosis are particularly relevant to current research as it has become readily apparent that the immune response to adenovirus vectors can impose serious limitations on the longevity of transgene expression. Since the immune system cannot distinguish between wild-type adenovirus and recombinant adenovirus vectors, it is increasingly clear that some type of host immune modulation may be necessary to improve the functions of this virus as a gene delivery vehicle.

Two chapters are devoted to a discussion of regulatory proteins and although this might be described as standard fare, it is absolutely essential to a comprehensive text on adenoviruses. Particular attention is given to the E1A transcription unit that encodes two major proteins, one of which acts as a transcriptional activator of adenovirus early genes, while the other can induce cell cycle progression, immortalize cells, and transform cells in cooperation with other oncogenes.

Some attention is given to morphometric techniques as one chapter describes the use of fluorescently labeled wild-type adenovirus 2 to track the transport from cell cytoplasm to the nucleus. Presumably this technique would be equally applicable to recombinant adenoviruses and thus would offer an important strategy for analyzing the effectiveness of gene transfer in a variety of target cells. A second chapter details the techniques for performing fluorescence in situ hybridization (FISH) and immunostaining for the simultaneous detection of RNA and proteins in adenovirus-infected cells.

Studies of the structure and function of the adenovirus fiber protein remain of fundamental interest since this protein mediates the initial attachment of the virus capsid to a cell-surface receptor. Appropriately, a chapter is devoted to enumerating the methods for purification of the fiber from virus infected cells, including both DEAE-Se-

pharose-hydroxylapatite and mono Q and mono S chromatography. Analogous chapters address the purification of adenovirus hexon and protease.

In summary, this book constitutes an excellent reference text if one is interested in methods necessary for studying adenovirus biology. It takes cognizance of the fact that adenoviruses have been of intense interest to researchers in the field of gene transfer. Although considerable attention is given to creating virus deletion mutants, it would have been worthwhile to have an additional chapter that covers fully deleted or 'gutted' adenovirus recombinants; these constructs are particularly appealing because they may abrogate some of the host immune responses and they possess a large transgene capacity (approximately 28 kb). Nonetheless this book is replete with useful information for both the virologist and the non-virologist who is interested in working with adenoviruses.

Nelson A. Wivel

Tissue Engineering Methods and Protocols. Methods in Molecular Medicine. Edited by J.R. Morgan and M.L. Yarmush, Humana Press; Totowa, 1999. xvi+629 pp. $99.50 (hb). ISBN 0-896-03516-6.

Scientists, clinicians, and engineers have recognized that by pooling their experiences and backgrounds from their respective fields, intractable problems related to perennially complex tissue regeneration problems can be solvable. The field of tissue engineering has been formed specifically to provide a multidisciplinary platform through which significant medical problems can be addressed. A vexing issue not only for tissue engineering neophytes but also for experienced investigators has been the lack of a comprehensive set of standard protocols and methods for use in their studies.

This book, well-edited by Morgan and Yarmush, contains a nearly exhaustive series of protocols related to biomaterials, cells, composites of cells and biomaterials, and measurement techniques. The editors are known and respected in the field and, as such, were able to attract some of the stalwart tissue engineering investigators. Each chapter begins with a brief, in numerous cases inadequate introduction to the problem at hand. Of course, one needs to be reminded that by design this book is not intended to provide an exhaustive introduction to the myriad tissue engineering problems, but rather to walk one through the actual experimental steps of implementing a procedure. Thus, some familiarization with each chapter's main focus is assumed. The introduction is followed by a step-by-step description of each method, not unlike what one may encounter in one's lab notebook or in a company's compendium of standard operating procedures.

The biomaterials section contains 11 chapters on preparation of constructs of natural elements, such as collagen, as well as synthetic polymers, such as polylactides. There are excellent sections, containing 25 chapters, on methods for isolating, culturing, and using cells alone or in conjunction with biomaterials. The last seven chapters describe quantitative techniques to evaluate cell function. Useful diagrams and photographs are included in many of the chapters, along with substantial reference list.

As expected, a book which purports to cover the entire field of tissue engineering is bound to exhibit certain deficiencies. The sequence of the main sections does not appear to be in a cogent order. For example, the section entitled clinical applications (which actually contains chapters on cells) precedes the section on measurement techniques. One would expect that the last section of this book would be clinical or experimental applications in animal models. The book could be helped significantly if it contained a section on assessment techniques of tissue engineered products and not just cells. For example, when it comes to musculoskeletal tissues, biomechanical assessment of the tissue engineered product is of paramount importance in determining functionality. As in many books which are essentially collections of disparate chapters, uniformity is absent in style, depth of coverage, and chapter length.

These minor criticisms could not and should not take anything away from the usefulness of *Tissue Engineering Methods and Protocols*. This book is intended to become an indispensable aid to students and seasoned investigators in tissue engineering.

Kyriacos Athanasiou

Molecular and Cellular Basis of Inflammation. Edited by C.N. Serhan and P.A. Ward, Humana Press; Totowa, 1999. xii+338 pp. $125.00 (hb). ISBN 0-896-03595-6.

Much progress has been made in recent years in our understanding of the molecular and cellular basis of acute and chronic inflammatory processes. *Molecular and Cellular Basis of Inflammation* focusses on recent research in this field. The editors have enlisted 39 authors for 15 chapters, each of which deals with important aspects of inflammation. The first chapters provide an actual overview of the role of complement factors, arachidonic acid and lipoxygenases in inflammatory processes. These chapters are very well written and precisely describe the current knowledge in this field. Further chapters are dedicated to the role of angiogenesis, isoprostanes, endothelial cells, adenosine, granulocytes and gamma/delta T cells in inflammation. Finally, two chapters address new molecular data on the pathogenesis of rheumatoid arthritis and lupus erythematodes. Helpful diagrams and illustrations add to the value of the book as an educational volume, but it is perhaps too specialized for students and more appropriate for experts in the field. As regards the clinical part of the book, other relevant inflammatory diseases in humans (such as hepatitis, inflammatory bowel diseases) should have been discussed. Furthermore, it would have been helpful if the editors had imposed greater uniformity of presentation between the chapters. Some chapters appear to cover at least partially redundant information (e.g. the two chapters on isoprostanes).

Taken together, this book provides a comprehensive description of our current knowledge in inflammation. It contains some useful figures and tables that provide the reader with a very good overview of the knowledge gained until 1998. The book will be an important read for scientists and clinicians who are interested in inflammation. The less knowledgeable individual will need to go to other sources for more extensive description and references. Nevertheless, the book fills a useful niche and I recommend it to any person who is interested in molecular and cellular aspects of inflammation.

Markus F. Neurath

Mycoplasmal Protocols. Methods in Molecular Biology, Vol. 104; Edited by R. Miles and R. Nicholas, Humana Press; Totowa, 1998. xiii+330 pp. $79.50 (hb). ISBN 0-896-03525-5.

By and large this book fulfills its promise of providing up-to-date, easy-to-read protocols for mycoplasmologists working within the fields of biochemistry, immunology and molecular biology. Each chapter is lucidly presented and referenced. After a short introduction the necessary materials are listed followed by a recipe-like method section. Helpful tips are included in the subsequent notes. The protocols, especially those dealing with growth, media and identification of mycoplasmas, are clear and should present the newcomer to mycoplasmology with good basic laboratory protocols for getting started. For those established in mycoplasmology the different chapters provide insight into species perhaps different from one's own specialities. Each chapter is written so that it can be read in isolation and therefore there is a deal of repetition; however, as the book is probably not intended to be read in its entirety this is not necessarily a disadvantage.

The introductory chapters 1–3, dealing with the medical and veterinary significance of mycoplasmas, are short, concise resumés and interestingly written. It is a little surprising that the HIV-associated mycoplasma species (*M. fermentas* strain incognitus/*M. penetrans*) are not mentioned at all.

The following chapters 4–7 cover the recovery of mycoplasmas (including ureaplasmas) from human and animal specimens and their cultivation and provide much useful information in easy-to-read sections; however, the important pig pathogen *M. hyorhinis* is not described at all in chapter 5 and is only dealt with as a cell culture contaminant in chapter 23.

Chapter 8 goes into the importance of quality control testing for mycoplasma media, a subject which has not been dealt with before as a detailed protocol, and we found this chapter a useful guide for practical lab work.

Three chapters follow describing the biochemistry of mycoplasmas. Chapter 9 covers the biochemical characteristics of mycoplasmas used in species identification, in itself a difficult field, but does so well and thoroughly, while chapter 10 is a comprehensive and elegantly written section focusing on function assays for three enzymes common to all mycoplasmas. The necessary preparation of mycoplasmas for such sensitive and reliable assaying is concisely described. Subsequently protocols on the measurement of substrate metabolism by monitoring oxygen use, pH change and nitroblue tetrazolium reduction are covered in chapter 11. These three chapters should offer much useful advice for those working in the field of mycoplasma biochemistry.

Seven chapters deal with the detection and identification of mycoplasmas by the use of immunological methods. Chapter 12 contains protocols for the serological testing of mycoplasmas in the growth and metabolism inhibition assays. These are standard protocols published previously in books such as *Methods in Mycoplasmology* by Razin and Tully (1983). Nevertheless any book covering mycoplasmas would be incomplete without them. Chapter 13, covering dot immunobinding, mentions the pitfalls of variable mycoplasma surface antigens and supposedly specific antigens as an identification method. This chapter could have perhaps been added to that of the serological testing of mycoplasmas as these are essentially similar subjects. Immunofluorescence identification of mycoplasmas both in tissue and on agar plates is clearly written and filled with adequate detail in chapter 14 and further immunological methods are described for the detection of mycoplasmas in cell cultures (chapter 24) and can also be found in chapter 33, where gold labelled antibodies against surface antigens are used in electron microscopy. Again, this is no doubt covered in other non-mycoplasma texts, but there are some details which appear to be mycoplasmas-specific and this chapter should help those wishing to venture into this area.

Chapter 15 describes an interesting sandwich ELISA which combines mycoplasma enrichment by growth in test wells over 3 days with a standard ELISA subsequent to this. In this way the antigen amount is increased and in addition one has the cultured organism at hand. Last but not least the immunohistochemical staining protocol in chapter 16 is not really mycoplasma-specific but informative.

The section from chapters 17 to 29 (excluding chapters 23 and 24) is devoted to molecular biological methodologies and starts in chapter 17 with the extraction of genomic DNA from mycoplasmas by two commonly used methods: firstly, a phenol/chloroform extraction with RNase and proteinase K treatment and secondly, a guanidinium thiocyanate method. Both methods are clearly presented, and complemented by slightly different preparation methods included in chapters 19, 20 and 22.

The broad usage of PCR in mycoplasmology is shown in chapters 18–20. Chapter 18 elaborates the stepwise, PCR-based development of a 16S-related phylogenetic tree, which for interested readers contains a wealth of details. A few programs are quoted, which are partly available through the world wide web, making the choice of such programs easier. This presents to the patient reader engaged in phylogeny a guide to the detection of the relatedness of different species.

M. mycoides ssp. *mycoides* was used as a source of DNA to describe, in chapter 19, the usage of a commercially available PCR kit from AMRAD. As the methodology is applicable to all bacteria, the specificity to mycoplasma is obvious only in the specimen collection and possibly in the sample preparation.

The characterization of mycoplasmas by random amplification of polymorphic DNA (RAPD) or arbitrarily primed PCR (AP-PCR) is presented in detail in chapter 20. In general these methods enable the differentiation of mycoplasma species and moreover can also help to detect strain-specific heterogeneity.

Chapter 21 shows that also in mycoplasma research the old and tested method of DNA hybridization analysis is of value.

An overview of insertion sequences (IS) in mycoplasmas is presented in chapter 22 with a strategy for the identification of useful IS elements in the characterization of new mycoplasma species. It was refreshing to read the section on the critical interpretation of the ensuing results.

In the chapter on the transformation of mycoplasmas (chapter 25) the authors have succeeded in converting four methods for transforming bacteria to the specific problems one may encounter with mycoplasmas. A further method for inserting foreign DNA into mycoplasma by use of a conjugal donor *E. faecalis* follows in chapter 26. It describes the rapid examination of numerous mycoplasma strains as putative recipients of the transposon Tn*916* for transposon mutagenesis analysis, but gives only a few examples for its concrete applicability.

The methods to demonstrate extrachromosomal elements in mycoplasmas (chapter 27) focus on the isolation of bacteriophages and those for heterologous expression of mycoplasmal genes in *A. laidlawii* (chapter 28) and in *E. coli* (chapter 29) are well described. Chapter 28 seems to give a complete overview of the genes that have been cloned and expressed in mollicutes to date and protocols for construction and screening of an *A. laidlawii* DNA library followed by detection of expression. Chapter 29 is mainly restricted to methods belonging to the screening of genomic libraries in λ-phage or individual recombinant phages for heterologous expression. These methods include the use of tryptophan suppressors to circumvent a premature translational stop at the TGA codons which encode tryptophan in mycoplasmas but function as a stop codon in *E. coli*. The expression of mycoplasma genes cloned in plasmids is treated briefly and no overview is given concerning suitable expression plasmids and strategies for point mutagenesis of TGA codons.

The last four chapters deal with the separation (chapter 30) and characterization of proteins, especially those in the membrane (chapters 31–33). Whereas in chapter 30 the main methods of general protein research are repeated with one- and two-dimensional gel electrophoresis, wet blotting and immunostaining (Western blotting), the methods summarized in chapter 31 enable the identification and immunological and biochemical characterization of mycoplasma membrane proteins. The descriptions are interspersed by figures depicting the different preparations. An important hint is given that prokaryotic consensus sequences, such as lipoprotein signal sequences, do not always match those of mycoplasmal proteins.

In chapter 32 two methods for the detection and analysis of mycoplasmal adhesins are presented. The first comprises a Western blot adherence assay with radioactively labelled host cells as the binding partner, first presented at the 10th IOM in 1994. The second method describes an adherence assay carried out in tissue culture plates. A useful critique of both methods is included. All in all this book represents a collection of well-written protocols in the field of mycoplasmology by 38 different authors but lacks cohesion in both style and contents. This is evident in the large degree of repetition and the lack

of focus on real mycoplasma-specific problems. For example no word is wasted on the impact of genetic variation within strains and between mycoplasma species. This is, in our opinion, extremely important and relevant for molecular biology research in mycoplasmology.

The study of the organism *Mycoplasma* and its interaction with its environment can be pursued using the instruments provided by this book, but by and large it does not supply the reader with an insight into new and innovative mycoplasma-specific techniques but rather mirrors the methods currently in use by mycoplasma researchers. Thus, the book achieves its goal in providing laboratory protocols to researchers in the field of mycoplasmology but along the same road all other researchers have trod.

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Booklist No. 151

1. Gersen, S.L. and Keagle, M.B. (eds.) The Principles of Clinical Cytogenetics. Humana Press; Totowa, 1999. ix+558 pp. $79.50 (hb).
2. Kjems, J. et al. (eds.) Analysis of RNA-protein complexes in vitro. Laboratory Techniques in Biochemistry and Molecular Biology. Elsevier; Amsterdam, 1998. x+237 pp. NLG 125.00 (pb).
3. Galat, A. and Rivière, S. (eds.) Peptidyl-Prolyl *Cis/Trans* Isomerases. Protein Profile. Oxford University Press; Oxford, 1998. xii+117 pp. £25.00 (pb).
4. Greulich, K.O. (ed.) Micromanipulation by Light in Biology and Medicine. The Laser Microbeam and Optical Tweezers. Birkhäuser; Basel, 1999. xii+300 pp. CHF 168.00 (hb).
5. Jenkins, N. (ed.) Animal Cell Biotechnology. Methods and Protocols. Methods in Biotechnology. Humana Press; Totowa, 1999. xiv+302 pp. $99.50 (hb).
6. Bjornsti, M.-A. and Osheroff, N. (eds.) DNA Topoisomerase Protocols. DNA Topology and Enzymes. Methods in Molecular Biology, Vol. 64. Humana Press; Totowa, 1999. xv+327 pp. $79.50 (hb).
7. Hajibagheri, M.A.N. (ed.) Electron Microscopy Methods and Protocols. Methods in Molecular Biology, Vol. 117. Humana Press; Totowa, 1999. xi+283 pp. $89.50 (pb).
8. Wu-Pong, S. and Rojanasakul, Y. (eds.) Biopharmaceutical Drug Design and Development. Humana Press; Totowa, 1999. xii+435 pp. $69.50 (hb).
9. Dunford, H.B. (ed.) Heme Peroxidases. Wiley; Chichester, 1999. xiii+507 pp. £126.00 (hb).
10. Hall, B.K. (Chair) Homology. Novartis Foundation Symposium 222. Wiley; Chichester, 1999. viii+256 pp. £75.00 (hb).
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12. Conn, P.M. (ed.) Methods in Enzymology, Vol. 302. Green Fluorescent Protein. Academic Press; San Diego, 1999. xxxii+490 pp. $99.95 (hb).
13. Kammer, G.M. and Tsokos, G.C. (eds.) Lupus. Molecular and Cellular Pathogenesis. Humana Press; Totowa, 1999. xx+708 pp. $195.00 (hb).
14. Rollins, B.J.R. (ed.) Chemokines and Cancer. Contemporary Cancer Research. Human Press; Totowa, 1999. xii+320 pp. $125.00 (hb).
15. Margesin, R. and Schinner, F. (eds.) Biotechnological Applications of Cold-Adapted Organisms. Springer; Heidelberg, 1999. viii+338 pp. DM 298.00 (hb).
16. Neidle, S. (ed.) Oxford Handbook of Nucleic Acid Structure. Oxford University Press; Oxford, 1999. xi+662 pp. £75.00 (hb).
17. Drenth, J. (ed.) Principles of Protein X-Ray Crystallography. Springer; Heidelberg, 1999. xv+341 pp. DM 129.00 (hb).
18. Cotterill, S. (ed.) Eukaryotic DNA Replication. A Practical Approach. Oxford University Press; Oxford, 1999. xxii+281 pp. £29.95 (pb).
19. Cass, T. and Ligler, F.S. (eds.) Immobilized Biomolecules in Analysis. A Practical Approach. Oxford University Press; Oxford, 1998. xviii+216 pp. £29.95 (pb).
20. Li, M. (ed.) NMDA Receptor Protocols. Methods in Molecular Biology. Humana Press; Totowa, 1999. x+196 pp. $69.50 (hb).