Edited by
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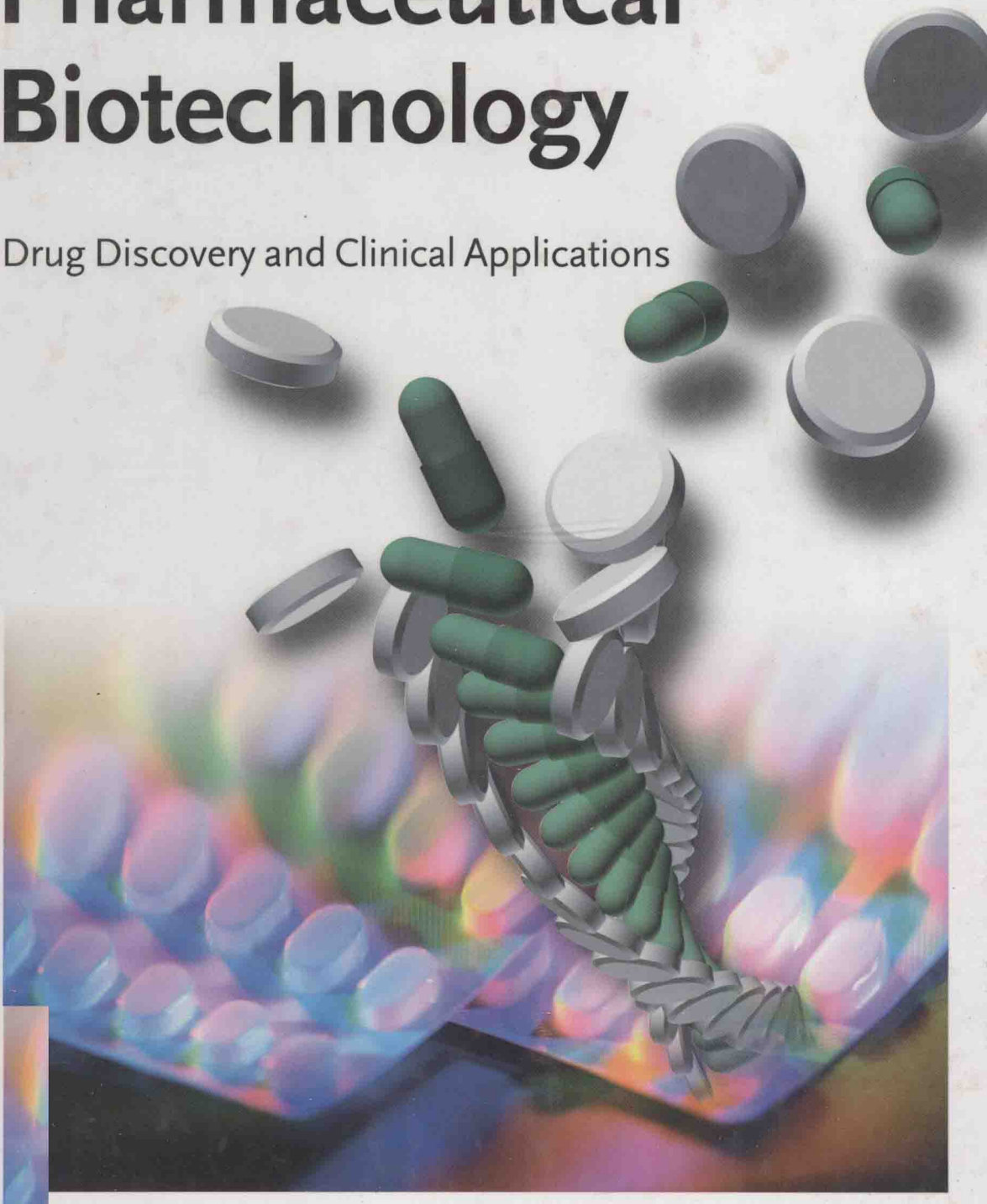
Pharmaceutical Biotechnology

Drug Discovery and Clinical Applications



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Preface

Pharmaceutical biotechnology has a long tradition and is rooted in the last century, first exemplified by penicillin and streptomycin as low molecular weight biosynthetic compounds. Today, pharmaceutical biotechnology still has its fundamentals in fermentation and bioprocessing, but the paradigmatic change affected by biotechnology and pharmaceutical sciences has led to an updated definition. Upon a suggestion by the European Association of Pharma Biotechnology (EAPB), pharmaceutical biotechnology is defined as a science covering all technologies required for the production, manufacturing, and registration of biotechnological drugs.

The biopharmaceutical industry has changed dramatically since the first recombinant protein (Humulin®) was approved for marketing in 1982. The range of resources required for the pharmaceutical industry has expanded from its traditional fields. Advances in the field of recombinant genetics allows scientists to routinely clone genes and create genetically modified organisms that can be used in industrial production processes. Also, specific therapeutic proteins can be synthesized in nonbiological ways, and recombinant proteins can be isolated from complex mixtures in commercially viable processes. In contrast to academic research, industrial development and manufacturing is guided by cost and time effectiveness, patent protection, exclusivity periods, and regulatory compliance. There are many critical industry issues that companies have to face; hence there is a need for new pharmaceutical biotechnology textbooks focussing on industrial needs.

Therapeutic proteins and the recently approved antisense oligonucleotide Fomivirsen® represent new and innovative biotech drugs that are different from classical drugs in the development and production process. In this area, pharmaceutical companies are confronted with new challenges to develop new products and to apply new technologies. Industrial needs are particularly different and are either not discussed or are only marginally discussed in existing textbooks, which is why we feel that there is a need for a new pharmaceutical biotechnology textbook.

We asked experts from the pharmaceutical biotech area to present their integrated view to answer questions focussing on industrial needs in the discovery and manufacture of recombinant drugs and new therapies. We are glad that a majority of contributors, active in the pharmaceutical industry, have participated and shared their views on new developments in protein production, production organisms, DNA vaccines, bioinformatics, and legal aspects. Distinct problems related to recombinant proteins that

have arisen in recent years, such as drug stability, pharmacokinetics, and metabolization, are discussed in detail. It should be mentioned that for the first time the topic of generic recombinant drugs is presented in this textbook.

Biotechnology is a fast-moving area and crucial topics for future technologies can be recognized today. We wanted to give an insight into these future enterprise technologies and had asked for contributions to highlight new developments in gene therapy, tissue engineering, personalized medicine, and xenotransplantation having a realistic chance of being used in industrial applications.

In this textbook, you will find updated facts and figures about the biotech industry, product approvals, and discussions of how biotechnology is applied in human and animal health care, and in industrial and environmental processes. We address how biotech is being employed in national security efforts as well as the ethical issues that are frequently debated when people discuss the use of biotechnology in health sciences.

We would like to thank all contributors for their contributions, because we know that time was short and most of the papers were written alongside their regular duties. Special thanks to Dr. Andrea Pillmann, Wiley VCH, for her support in the layout, proofreading, and production of this textbook.

We are convinced that this textbook is filling a niche and covering industrial needs and interests in the pharmaceutical biotech area. Our point of view is that this textbook will cater to scientists and decision makers in pharmaceutical and biotechnological companies, venture capitals/finance, and politics.

O. Kayser
R.H. Müllers

Berlin, December 2003

Foreword

Pharmaceutical Biotechnology is a multidisciplinary scientific field undergoing an explosive development. Advances in the understanding of molecular principles and the existence of many regulatory proteins have established biotechnological or therapeutic proteins as promising drugs in medicine and pharmacy. More recent developments in biomedical research highlight the potential of nucleic acids in gene therapy and antisense RNAi technology that may become a medical reality in the future.

The book attempts to provide a balanced view of the biotechnological industry, and the number of experts from the industry sharing their knowledge and experience with the readers gives the book an outstanding value. All contributors provide with each chapter an up-to-date review on key topics in pharmaceutical biotechnology. Section 1 serves as an introduction to basics in protein production and manufacturing. Particular emphasis not only on production organisms like microorganisms and plants but also on industrial bioprocessing will be appreciated by the reader.

The advent and development of recombinant proteins and vaccines is described in detail in Part 2. Biotech drugs have created a number of unique problems because of their mostly protein nature. The production, downstream processing, and characterization is in many aspects different from conventional low molecular weight drugs and is highlighted by selected experts still in touch with the lab bench. Bringing the therapeutic protein to the patient is a major challenge. Protein formulation, biopharmaceutical aspects, and drug regulation are fields that are fast developing and well recognized by their new and innovative techniques. Drug regulation has a major impact on the whole drug manufacturing process, which is why special chapters on the drug approval process in Europe and the United States, and biogenerics are of high interest. Finally, in Part 4, experts provide an outlook on potential drugs and therapeutic strategies like xenotransplantation that are under investigation. Hopefully, some of these concepts will find clinical application in the following years.

I believe that there is a distinct need for a pharmaceutical biotech book focusing on the industrial needs of recombinant drugs and providing detailed insight into industrial processes and clinical use. Therefore, this work is not only a valuable tool for the industrial expert but also for all pharmacists and scientists from related areas who wish to work with biotech drugs. In life-learning courses and the professional environment, this compact book is the basis for a solid understanding for those who wish to gain a better overview of the industry they are working in.

Robert Langer
MIT Boston, November 2003

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Color Plates

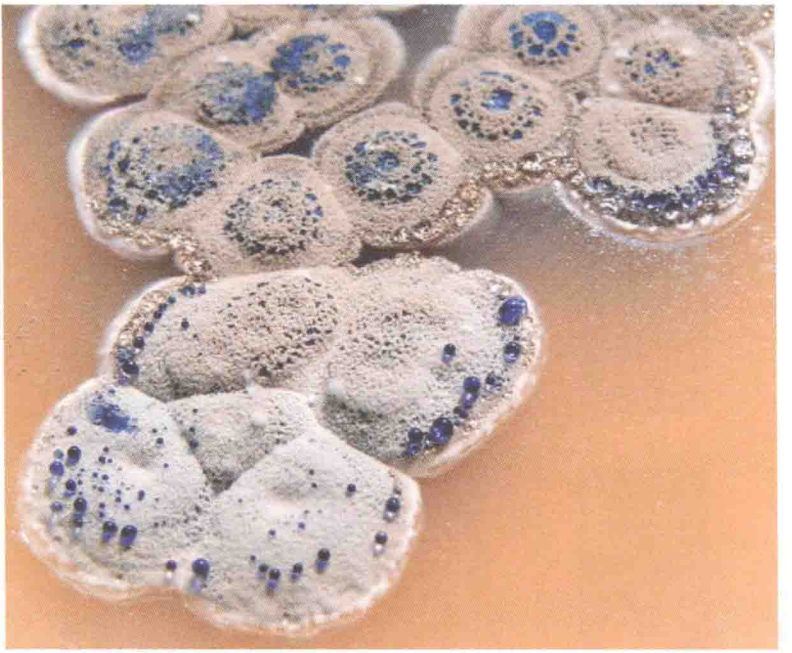

Fig. 2.1 Photography of a sporulated *Streptomyces* strain growing on solid medium. The blue drops indicate the production of an antibiotic (aromatic polyketide).

Major technology	Mammalian (CHO) cells	Transgenic mammal milk	Transgenic chicken eggs	Transgenic plants
Companies	Amgen (Thousand Oaks, CA) Genentech (S. San Francisco, CA) other current biologics manufacturers; Crucell (Leiden, Netherlands) uses human cells	GTC Biotherapeutics (Framingham, MA) PPL Therapeutics (Edinburgh, UK) BioProtein (Paris, France)	Avigenics (Athens, GA) Origen Therapeutics (Burlingame, CA) TranXenoGen (Shrewsbury, MA) Viragen (Plantation, FL) GeneWorks (Ann Arbor, MI) Vivalis (Nantes, France)	Croptech (Blacksburg, VA) Epicyte (San Diego, CA) Large Scale Biology (Owensboro, KY) Meristem Therapeutics (Clermont-Ferrand, France) Prodigene (College Station, TX)
Estimated cost (cost/g raw material)*	$150	$1–$2	$1–$2	$0.05

*Company estimates

Fig. 3.2 Companies and technologies in biomanufacturing. A comparison of different expression systems shows the big differences in terms of costs, ranging from US$150 per gram for CHO cells to US$0.05 per gram for transgenic plants [11].

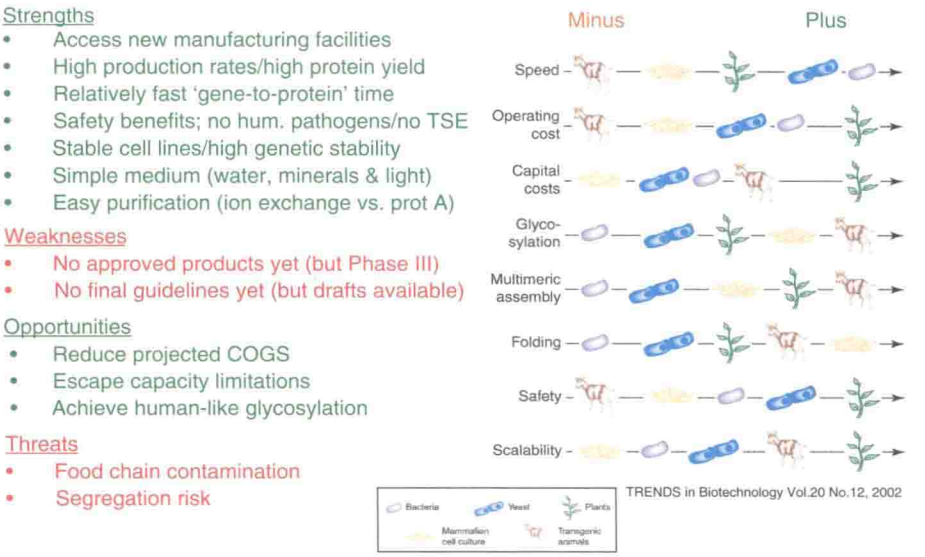

Fig. 3.3 SWOT analysis of plant expression systems. Plant expression systems have a lot of advantages (plus) over other systems and are therefore mostly shown on the right-hand side of the picture (Raskin I et al., Plants and human health in the twenty-first century. *Trends in Biotechnol.* **2002** *20*, 522–531.). Herein different systems (transgenic animals, mammalian cell culture, plants, yeast, and bacteria) are compared in terms of speed (how quickly they can be developed), operating and capital costs and so on, and plants are obviously advantageous. Even for glycosylation, assembly and folding, where plants are not shown on the right-hand side (meaning other systems are advantageous), some plant expression systems are moving in that direction (as will be shown exemplarily in the section for moss). Also, the weaknesses and threats can be dealt with, using the appropriate plant expression system [20].

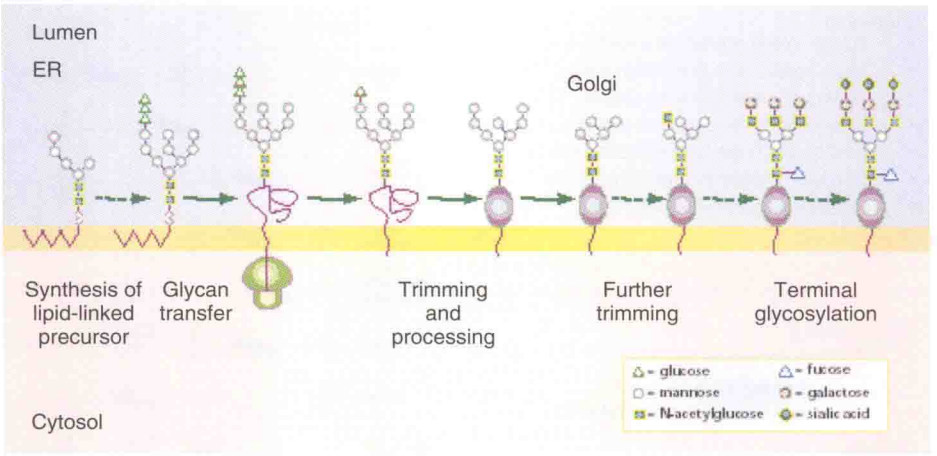

Fig. 3.4 The glycosylation pathway via ER and Golgi apparatus. In the cytosol carbohydrates are attached to a lipid precursor, which is then transported into the lumen of the ER to finish core glycosylation. This glycan is now attached to the nascent, folding polypeptide chain (which is synthesized by ribosomes attached to the cytosolic side of the ER from where it translocates into the lumen) and subsequently trimmed and processed before it is folded and moved to the Golgi apparatus. Capping of the oligosaccharide branches with sialic acid and fucose is the final step on the way to a mature glycoprotein [23].

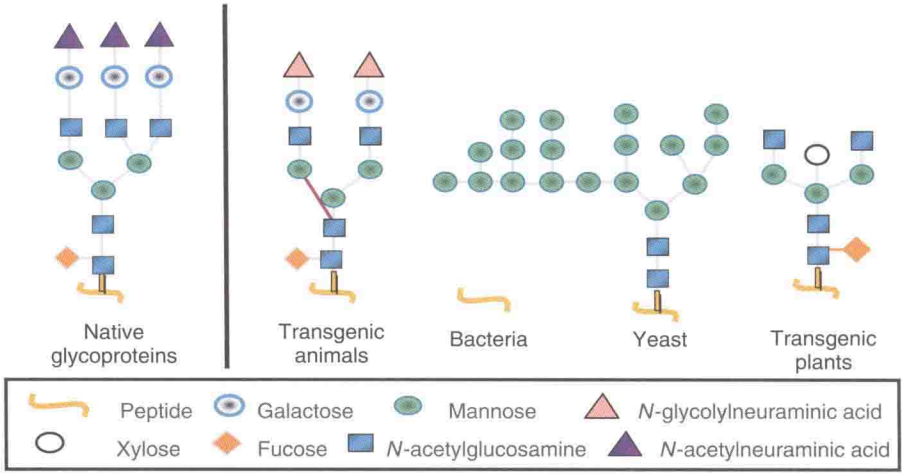

Fig. 3.5 Engineering plants to humanlike glycosylation. The first step to achieve humanlike glycosylation in plants is to eliminate the plant glycosylation pattern, that is, the attachment of β-1-2-linked xylosyl and α-3-linked fucosyl sugars to the protein. Because these two residues have allergenic potential, the corresponding enzymes xylosyl and fucosyl transferase are knocked out. In case galactose is relevant for the final product, galactosyl transferase is inserted into the host genome. Galactose is available in the organism so that this single-gene insertion is sufficient to ensure galactosylation [24].

Fig. 3.6 Secretion of the biopharmaceuticals via tobacco roots. The tobacco plants are genetically modified in such a way, that the protein is secreted via the roots into the medium ("rhizosecretion"). In this example, the tobacco plant takes up nutrients and water from the medium and releases GFP (green fluorescent protein). Examination of root-cultivation medium by its exposure to near-ultraviolet illumination reveals the bright green-blue fluorescence characteristics of GFP in the hydroponic medium (left flask in panel lower left edge). The picture also shows a schematic drawing of the hydroponic tank, as well as tobacco plants at different growth stages, for example, callus,–fully grown and greenhouse plantation [24].