BIOTECHNOLOGY OF MAMMALIAN CELLS

Development for the Production of Biologicals

Edited by Makoto Umeda, Hideki Koyama, Jun Minowada, and Michio Oishi

PREFACE

This is unquestionably the age of high technology. We human beings encourage its development because it is expected to contribute greatly towards human welfare. Advanced technology is also considered something which we should hand down to the next generation who will live on into the 21st century.

In general, the introduction of new technology to human society requires establishment of a new consensus with a change in our evaluative sense. The impact of high technology, however, is so great and its development is so rapid that some people are finding it difficult to accept. Its influence is felt in such diverse fields as politics, economics, human culture, and our daily lives. The field of biotechnology, in particular, will surely aid us in the resolution of challenges in areas such as medical care, food, and energy, even though its development will require changes in our custom, ethics, and way of thinking.

This publication focused on advances made and problems encountered in biotechnology using mammalian cultured cells for the production of valuable biological products. While such technology enables us to produce some of these products using bacterial cells or

yeasts, it is now recognized that the production of more intact and useful substances such as those glycosylated or with complicated tertial structure is impossible. Therefore, biotechnological manufacturing utilizing mammalian cells is looked to for the production of these substances. The main subjects covered in this volume are the raising and exploitation of useful mammalian cell lines and vectors, a procedure for the large-scale production of substances by the cells, and the understanding of the safety factors involved with the products.

From the viewpoint of scientists, the obtaining of adequate information is essential for making a proper judgement. Placing faith and trust in the knowledge and judgement of specialists in this field, those engaged in politics, economics, and the improvement of social conditions will conduct impartial assessments and arrive at agreements about future directions for our world. We hope that the information on these pages will benefit these individuals as well as readers in other disciplines by allowing a greater understanding of the challenges facing biotechnology, and thereby fulfilling the ultimate goal of contributing to the betterment of human welfare.

This publication has specially been edited from the presentations and discussions which took place at the 2nd Yokohama Forum for the 21st Century which was held in Yokohama, Japan, in 1986.

INTRODUCTION

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Tremendous progress in molecular biology and bacterial genetics during the last three decades gave birth to an evolutional technology termed recombinant DNA technology or genetic engineering. This new technology was immediately applied to the industrial manufacturing of biologically active substances. As one outcome of its rapid and steady development, genetically engineered bacterial cells, especially *Escherichia coli*, into which cloned animal genes are introduced, have permitted production of a number of biologically active substances such as human interferon β, insulin, growth hormone, *etc.*, that otherwise have been extremely difficult to obtain. From these earlier successes, *E. coli* was first thought to be a proper substrate on which to produce such biologicals. The bacterium grows rapidly to a mass, making it easier to manufacture biologicals industrially, and it has been believed safe for mankind since much information has been known of its biology and physiology.

However, it became evident that this was not the case. It is now well recognized that there are severe limitations in producing more complex proteins of animal cell origin by genetically engineered bac-

teria. In *E. coli*, for example, some proteins are not translated properly, others are not modified by glycosylation or amidation after translation, or the conformation of some proteins is not properly formed. It is also sometimes very hard to purify a final product, since the product is accumulated within the bacterial cell.

It is with mammalian cells that we are now able to produce such complex proteins. Cultured mammalian cells, especially human cells, are suitable for this purpose, because some synthesize useful biologicals and others, even if they do not, may become cell substrates into which cloned recombinant genes are introduced and expressed. Cultured cells also seem feasible for the process of industrial manufacturing on the analogy of bacteria or yeasts living as single cells. Nevertheless, it has only been relatively recently that the importance of the use of such mammalian cells was realized and the development of appropriate technology began, so that a number of problems remain to be solved. We therefore believe it to be the right time to publish up-to-date information about these issues.

The focus of this format is on biotechnology utilizing mammalian cells in culture for the production of valuable biologicals. The contents are divided into four sections: 1) raising of useful mammalian cell lines, 2) exploitation of mammalian cell vectors, 3) technologies for the large-scale production of such biologicals by the cell lines, and 4) the safety features involved in use of those cell lines, in other words, the safety of the final products.

With respect to the first section, it is important to expand those stocks of established mammalian cell lines which may have potential to synthesize certain useful materials, or which may become better substrates suitable for the application of recombinant DNA technology. Generally, two methods are employed to establish cell lines from mammalian tissues. The first, which is conventional, is to serially cultivate primary cells until immortalized variant cells occur spontaneously. The second method, which has been developed recently is to obtain immortalized cells by transforming primary cells with so-called immortalizing genes. Among the trials described to establish cell lines, two papers concerning lines from human hematopoietic tumors established by the former method are presented by Namba

et al., and Minowada. They summarize the characteristics and utility of myeloma, and leukemia-lymphoma cell lines.

The next two papers are concerned with the second method of cell establishment. Onodera *et al.* summarize the function of the E1A gene, which is part of the adenovirus 12 genome and possesses immortalizing activity. They describe changes in cellular property after introducing their E1A-containing plasmids into a variety of mammalian cell lines. Griffin describes the usefulness of cloned DNA fragments from Epstein-Barr virus DNA in order to immortalize epithelial cells of green monkey, human, or marmoset that are very refractory to the establishment.

Mammalian cell vectors have inevitably been taken advantage of for the introduction of foreign recombinant genes into mammalian cells and the manufacture of biologicals. Okayama presents a new vector system comprising techniques for high efficiency cloning of full length cDNAs and two vectors, an SV40-based cDNA expression vector and a transducing vector for lambda phage-based cDNA library. Oishi *et al.* present a full description of a new and promising plasmid they recently discovered in mouse L cells, construction of composite vectors using the plasmid, and transfection of mammalian cells with these vectors.

Next, several production systems with mammalian cells are outlined, and technological aspects and related matters discussed. Obijeski presents cases in which such biologicals as hepatitis B antigen are successfully produced by only recombinant mammalian cells and are as active as those obtained from natural sources. For production of human-human hybridomas, Murakami *et al.* report the establishment of fusion partners from a lymphoblastoid line, the development of fusion methods for establishing human-human hybridomas, and the design of serum-free media for the growth of the hybridomas. The mass production of interferon α by human lymphoblastoid cells using large culture tanks is presented by Finter and his colleagues. Sano *et al.* cite the production of human interferon γ and interleukin 2 in recombinant mammalian cells that are prepared by introducing cloned respective human genes into mouse or hamster cells in culture.

Finally, we come to the question of whether or not the continu-

ous growing of mammalian cells in culture is safe for the production of biologicals, in other words, whether or not the biologicals produced by the cells are safe for humans. On the basis of his thorough purity testing of products derived from such mammalian cells, Obijeski clarifies what the safety issues are and recommends how these issues should be dealt with and resolved. He strongly advocates analysis of these issues by industry, by the medical community, and by control authorities to develop an appropriate consensus about the safe use of the final products. Petricciani reviews the safety issues involved in the use of continuous cell lines for the production of biologicals. He summarizes various discussions on these issues which have taken place at national and international levels during recent years and cites the relevant activity of the World Health Organization. These discussions will greatly help the reader to understand and reflect on the current status of the safety factors involved with these new biologicals.

We believe that the effectiveness and profit of using mammalian cells to obtain biologically active products at an industrial level have obviously been established. Anticipating the future development of this biotechnology, however, there still remain many problems to be solved or settled: efforts should be continued to raise established mammalian cell lines with the ability to yield valuable materials or having favorable properties as substrates for the establishment of recombinant mammalian cells. For this, the use of immortalizing genes could be promising to facilitate the establishment of cell lines.

New mammalian cell vectors should be sought which exist as plasmids within host cells without deteriorating the cells, are able to express inserted genes only when stimulated, and assure a stable and abundant yield of gene products.

Regarding the manufacturing of biologicals using mammalian cell cultures, technologies for mass culture should be developed. New devices of mass culture, new methods of sterilization, and development of serum-free medium will greatly facilitate the production. In particular, since monoclonal antibodies produced by human-human hybridomas are applicable to diagnosis and treatment of cancers, proper fusion partners of human myeloma-type cells should be raised. In this case, availability of serum-free medium to support the stable

proliferation of hybridomas is extremely important to obtain the most purified antibody.

Finally, we are of the opinion that the safe use of continuous mammalian cell lines or, ultimately, the safety of their final products has been considerably evaluated by scientific data in various disciplines. Prompt development of an appropriate consensus allowing public acceptance of such products, for example as medicines is hoped for.

It is our great desire that this volume will help the reader to understand more clearly the various aspects of biotechnology in the utilization of mammalian cells.

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CULTIVATION OF HUMAN MYELOMA CELLS

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Human myeloma cell lines are useful for various fields of biological sciences, such as for production of human monoclonal antibodies, production by gene transfer of certain biologically active substances other than antibodies, and in studies on the cell biology of human myelomas: anticancer drug sensitivity, cytogenetics, the behavior of myeloma cell growth, B cell differentiation, and etiology of diseases. In addition, myeloma cells themselves may produce some substances like lymphokines or growth factors.

To date, as shown in Table I, 15 myeloma cell lines have been reported. The case of Togawa *et al.* in the table is our first established

TABLE I
Human Myeloma Cell Lines Reported as of March 1986

References		Cell line
Burk *et al.* (1978)	*Cancer Res.*, **38**, 2508	ARH-77
Diehl *et al.* (1978)	*Blut*, **36**, 331	L363
Durie *et al.* (1985)	*Blood*, **66**, 548; *Blood*, **66**, 229	LB-831, LB-832, LB-833, LB-834
Jobin *et al.* (1974)	*J. Exp. Med.*, **140**, 494	LA49
Karpas *et al.* (1982)	*Science*, **216**, 997	Karpas 707
Katagiri *et al.* (1985)	*Int. J. Cancer*, **36**, 241	OPM-1, OPM-2
Matsuoka *et al.* (1967)	*Proc. Soc. Exp. Biol. Med.*, **125**, 1246	RPMI 8226
Miller *et al.* (1982)	*Cancer*, **49**, 2091	Fr
Nilsson *et al.* (1970)	*Clin. Exp. Immunol.*, **7**, 477	266 Bl (U266), 268 Bm
Togawa *et al.* (1982)	*Int. J. Cancer*, **29**, 495	KMM-1

cell line whose characteristics will be described later in detail. At present, however, these established human myeloma cell lines seem to be inappropriate as partners for human-human hybridomas. The myeloma cells used in preparation of hybridomas should have such properties as high hybridization rate, high cloning efficiency, lack of immunoglobulin production, and stable production of immunoglobulins after hybridization.

Since 1980 we have been successful in establishing four cell lines from myeloma patients. We will present here their characteristics and discuss problems to be solved for further establishment of new human myeloma cell lines.

I. CHARACTERISTICS OF OUR ESTABLISHED CELL LINES

Although we have been trying since 1980 to establish human myeloma cell lines, we have to date been able to establish only 4 cell lines from patients with multiple myeloma (Table II). We started the first culture 6 years ago. While we failed to culture myeloma cells from the bone marrow of the patient, we succeeded in the cultivation of myeloma cells from a subcutaneous tumor. This KMM-1 cell line has continued to grow and it is still producing lambda chains in the culture medium,