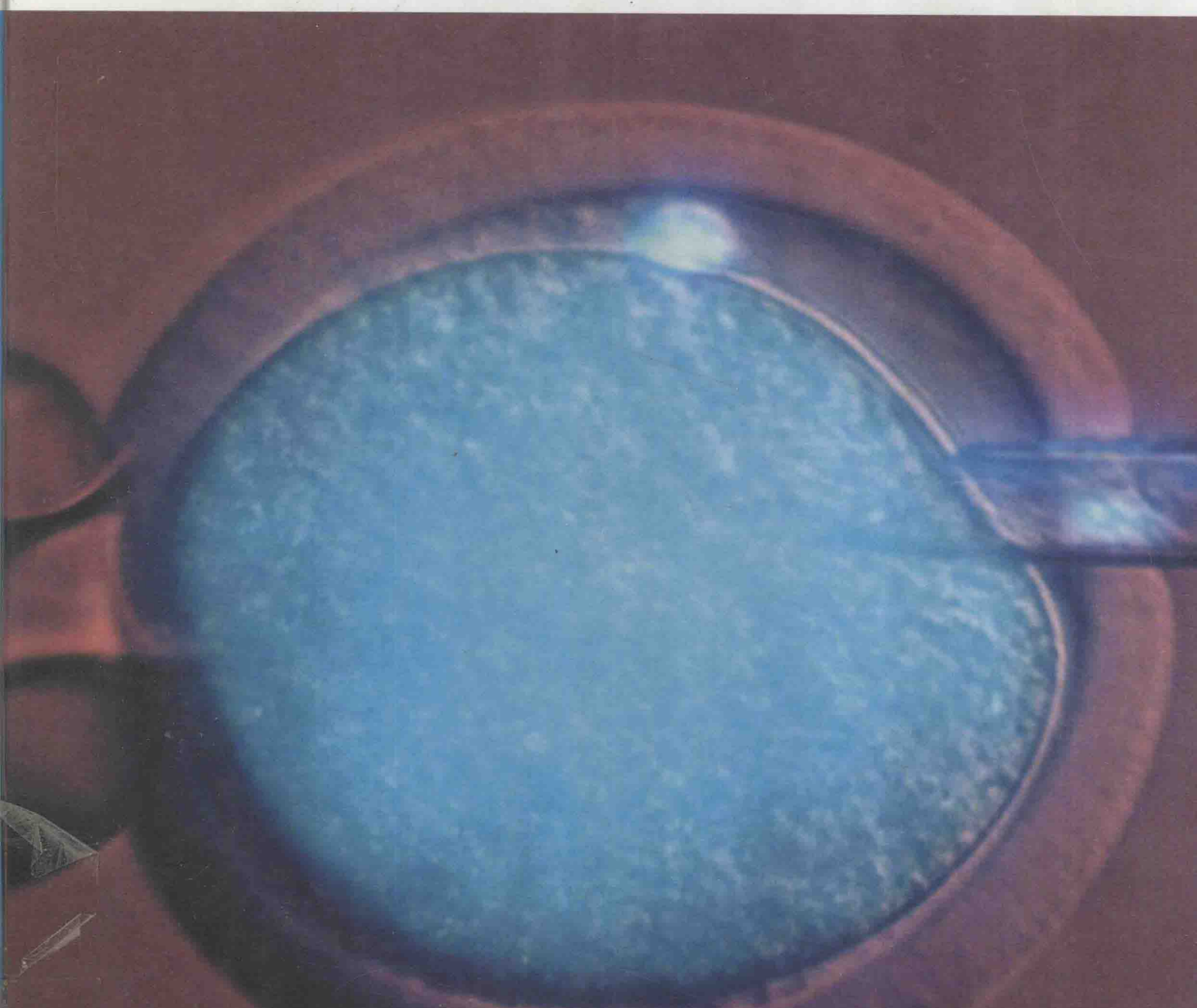


Principles of CLONING



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PRINCIPLES OF CLONING

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We dedicate this book to all the pioneers who contributed, consciously or not, to this new field of science.

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PREFACE

In 1997, Dolly the cloned sheep began a biological revolution. She showed us the way to physiologically dedifferentiate already committed somatic cells, opening the gate to a whole new world of possibilities in the study of basic biological mechanisms that touch the origins of life at its core. It is clear that cloning vertebrates using somatic cells is here to stay. Unfortunately, at this time, we know little about how this process works, and it is difficult to imagine all the applications this technology will bring to fruition. This book tries to address some of these questions.

Part I of *Principles of Cloning* explores the basic known biological processes and lays out critical points to consider when interpreting cloning experiments that are now being conducted. The nucleus itself, chromatin structure, epigenetic changes, and the role of the egg are emphasized. Part II describes the methods utilized during cloning in an attempt to make them available to students and professionals who are interested in nuclear transfer techniques in vertebrates. Parts III–V contain analyses of species that already have been cloned and species that are still being studied, including nonhuman primates. We are delighted to say that the contributors in these sections published the first articles on most of these species. Experts in the area have also contributed to the applications of cloning covered in Part VI; we expect this part of the book to communicate the benefits of cloning to society in the medical and agricultural fields. Part VII addresses the ethical concerns evoked by this technology and is followed by an analysis of what the future may bring.

We hope this book challenges readers, encouraging them to think beyond the cloning procedure itself to the creation of new individuals or pluripotent stem cells. Interesting questions regarding the mechanisms of dedifferentiation must now be addressed; the answers will perhaps allow scientists to recreate the same phenomenon in a more consistent and simpler way.

Jose Cibelli

FOREWORD

Every living mammal originates from the transplantation of a sperm nucleus into an egg. Biparental inheritance and genetic diversity have been the rewards of this natural form of nuclear transplantation. Modification of this process for laboratory use was first accomplished in the middle of the past century. The 27 chapters in *Principles of Cloning* not only provide a fitting 50th anniversary tribute to this pioneering achievement, but also serve as a record of the notable scientific advances that have emanated from cloning experiments.

Nuclear transplantation has had a much more profound impact on our understanding of the processes of development and differentiation than is often appreciated. It has demonstrated not only that cell differentiation takes place without the loss or permanent inactivation of genes, but has also provided the basis for the new field of genomic imprinting. In addition, elegant studies based on the transfer of nuclei between somatic cells have yielded important insights into the mechanisms of differentiation and have contributed to gene mapping and the identification of tumor suppressor genes. If the past achievements of nuclear transplantation have been to extend significantly our knowledge of nuclear plasticity and differentiation, what problems and progress can be expected from the cloning experiments of the future?

Many investigators have reported that fully differentiated cells in amphibia and mammals revert to a totipotent state when exposed to the cytoplasm of oocytes and eggs. Although widely acclaimed, this important advance is still beset by major difficulties and uncertainties arising from the exceedingly small percentage of differentiated nuclei that develop into viable young after transplantation. The current emphasis on cloning an ever-increasing range of mammals has demonstrated the universality of the problem; irrespective of the species studied, only 1 to 2% of cloned embryos survive to birth. In my view, the repetitious and disappointing nature of these results argues strongly for a redirection of effort. Would it not be more rewarding to move away from the cloning of ever more animals and focus heavily instead on the molecular and cellular events associated with nuclear reprogramming? Many fundamental issues associated with cloning deserve investigation. For example, does the high rate of failure indicate that the capacity to undergo full reprogramming resides in a small and specialized subset of adult nuclei only? Alternatively, are all differentiated nuclei equally capable of undergoing reprogramming but prevented from doing so due to imperfections in the cloning procedures? Likewise, what is the nature of the epigenetic and higher order events that are associated with nuclear reprogramming? Priority given to these studies will not only be of importance to cloning but will also contribute to a more comprehensive understanding of developmental processes in both embryos and stem cells.

A sharper focus on the mechanisms of reprogramming will be significant both in restoring cloning to its original role of answering important biological questions and also in redressing the balance between the academic and the commercial aspects of cloning.

Although the identification of nuclear changes underpinning reprogramming is essential, this advance may well be eclipsed by studies on cytoplasmic proteins involved in the initiation and regulation of nuclear reprogramming. The identification of reprogramming

proteins will be important for both scientific and medical reasons. In addition to a likely role in the preconditioning of somatic nuclei before transplantation, cytoplasmic reprogramming proteins may well have an even more vital future role in the preparation of somatic cells for direct use in tissue repair procedures. These considerations, together with the intellectual challenge of discovering how the reprogramming proteins act at the level of the nucleus, provide adequate incentives for a major focus on this emerging area of research. However, it would be unhelpful to minimize the problems that face investigators working on either the cytoplasmic or the nuclear aspects of reprogramming of cloned embryos. These difficulties include working with very limited amounts of material and having an inadequate set of markers with which to delineate the progress of nuclear reprogramming. Although these difficulties are substantial, the present advances in molecular technology are sufficiently great to ensure the problems will be overcome.

Although I believe fundamental studies offer the best long-term solutions to the problems of cloning, it is nevertheless apparent that the major interest at present is focused firmly on the potential of cloning for medical and biotechnological use. To be of value, the debate on the commercialization of cloning must be realistic, flexible, broadly based, and comparative in nature. In addition, it is imperative that the deep concerns felt by a substantial proportion of the public about some aspects of cloning are addressed.

Before looking toward future commercial developments, it is instructive to recall some of the existing biomedical successes achieved using nuclear transplantation techniques. Perhaps the least controversial but most important of its biotechnological successes has been in the production of monoclonal antibodies for therapeutic and experimental purposes. A comparable success has been made in the transplantation of nuclei for the treatment of male infertility; the so-called intracytoplasmic sperm injection procedure. These two examples demonstrate that nuclear transplantation can have a significant but acceptable impact on widely disparate areas of human well-being. Controversy emerged a few years ago when sheep were cloned by somatic nuclear transplantation amidst a fanfare of publicity. There followed a period of largely unfavorable press comment and unrealistic commercial expectations. Now a new and balanced approach is emerging in which technical problems, ethical considerations, and alternative technologies are being carefully weighed. The debate about therapeutic cloning exemplifies this more measured approach and has led to a consensus in which embryonic stem cells are adjudged to offer the best short-term prospects for tissue repair, while adult stem cells seem destined to predominate in the longer term. The need for flexibility is, however, paramount because rapid developments in adult stem cell biology are likely to challenge the prevailing consensus and may well point to the advantages of making more immediate use of the patient's own cells for tissue repair. A development of this nature would have major implications for the role of therapeutic cloning.

In conclusion, cloning is in transition from a distinguished past to a new future. If used in an imaginative manner, this move will ensure that nuclear transplantation continues to serve as a beacon of scientific excellence and as an important contributor to human health.

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