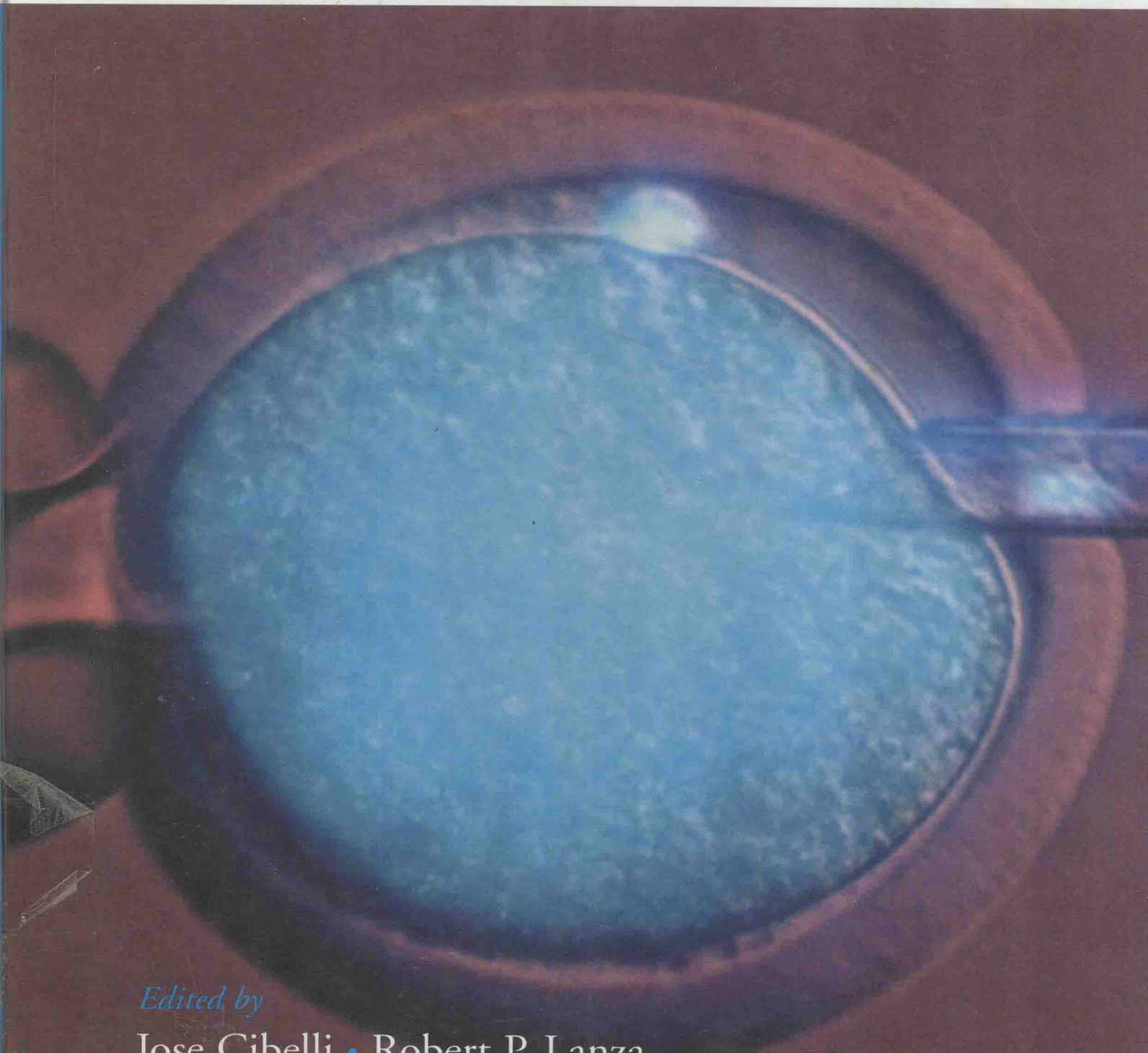
Principles of CLONING



Edited by

Jose Cibelli • Robert P. Lanza

Keith H.S. Campbell • Michael D. West

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PRINCIPLES OF CLONING

Edited by

JOSE CIBELLI
Advanced Cell Technology
Worcester, Massachusetts

ROBERT P. LANZA
Advanced Cell Technology
Worcester, Massachusetts

KEITH H. S. CAMPBELL
Department of Animal Physiology
University of Nottingham
Loughborough
Leicestershire, United Kingdom

MICHAEL D. WEST
Advanced Cell Technology
Worcester, Massachusetts

ACADEMIC PRESS

An imprint of Elsevier Science

AMSTERDAM BOSTON LONDON NEW YORK OXFORD PARIS SAN DIEGO SAN FRANCISCO SINGAPORE SYDNEY TOKYO

This book is printed on acid-free paper. ♾

Academic Press
An Elsevier Science Imprint
525 B Street, Suite 1900, San Diego, California 92101-4495, USA
http://www.academicpress.com

Academic Press
84 Theobalds Road, London WC1X 8RR, UK
http://www.academicpress.com

Library of Congress Catalog Card Number: 2002101649

International Standard Book Number: 0-12-174597-X

PRINTED IN THE UNITED STATES OF AMERICA
02 03 04 05 06 07 MM 9 8 7 6 5 4 3 2 1

PRINCIPLES OF CLONING

EDITORIAL BOARD

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

In particular, we want to mention those no longer with us: Alan Wolffe, Jacques Loeb, Hans Spemann, Robert Briggs, Thomas King, and Stephen Jay Gould.

CONTRIBUTORS

Jennifer D. Ambroggio
IVF Clinic
University of Southern California
Los Angeles, California 90033
Chapter 19

Michael Bader
Department of Pediatrics
Northwestern University Medical School
Children's Memorial Institute for Education and Research
Chicago, Illinois 60614
Chapter 21

Esmail Behboodi
Genzyme Transgenics Corporation
Framingham, Massachusetts 01701
Chapter 25

Zeki Beyhan
Department of Animal Sciences
University of Wisconsin—Madison
Madison, Wisconsin 53706
Chapter 19

Michele Boiani
Germline Development Group at the Center for Animal Transgenesis and Germ Cell Research
University of Pennsylvania
Kennett Square, Pennsylvania 19348
Chapter 6

J. A. Byrne
Wellcome CRUK Institute
and Department of Zoology
University of Cambridge
Cambridge CB2 1QR, United Kingdom
Chapter 14

Keith H. S. Campbell
School of Biosciences
University of Nottingham
Sutton Bonington, Loughborough
Leicestershire LE12 5RD, United Kingdom
Chapter 20

Pascale Chavatte-Palmer
Biologie du Développement et Biotechnologies
Unité Mixte de Recherche Institut National de la Recherche Agronomique/Ecole Nationale Vétérinaire d'Alfort
78352 Jouy en Josas, France
Chapter 12

LiHow Chen
Genzyme Transgenics Corporation
Framingham, Massachusetts 01701
Chapter 25

Philippe Collas
Institute of Medical Biochemistry
University of Oslo
Oslo 317, Norway
Chapter 2

Margaret M. Destrempes
Genzyme Transgenics Corporation
Framingham, Massachusetts 01701
Chapter 25

András Dinnyés*
Department of Animal Science/Biotechnology Center
University of Connecticut
Storrs, Connecticut 06269
Chapter 17

Tanja Dominko
Advanced Cell Technology
Worcester, Massachusetts 01605
Chapter 22

Patrick W. Dunne
Department of Veterinary Anatomy and Public Health
Center for Animal Biotechnology and Genomics
Texas A&M University
College Station, Texas 77843
Chapter 11

Yann Echelard
Genzyme Transgenics Corporation
Framingham, Massachusetts 01701
Chapter 25

Kevin Eggan
Whitehead Institute for Biomedical Research
and Department of Biology
Massachusetts Institute of Technology
Cambridge, Massachusetts 02142
Chapter 4

Neal L. First
Department of Animal Sciences
University of Wisconsin—Madison
Madison, Wisconsin 53706
Chapter 19

*Current affiliations: Roslin Institute, Department of Gene Expression and Development, Roslin, United Kingdom; and Research Group for Animal Breeding, Hungarian Academy of Sciences and Szent, Istvan University, Godollo, Hungary.

Rafael A. Fissore
Paige Laboratories
Department of Veterinary and Animal Sciences
University of Massachusetts
Amherst, Massachusetts 01003
Chapter 2

Robert H. Foote
Department of Animal Science
Cornell University
Ithaca, New York 14853
Chapter 1

Vasiliy Galat
Max Delbrück Center for Molecular Medicine
D-13092 Berlin, Germany
Chapter 21

David K. Gardner
Colorado Center for Reproductive Medicine
Englewood, Colorado 80110
Chapter 9

R. L. Gardner
Department of Zoology
University of Oxford
Oxford OX1 3PS, United Kingdom
Chapter 27

Ronald M. Green
Ethics Institute
Dartmouth College
Hanover, New Hampshire 03755
Chapter 26

J. B. Gurdon
Wellcome CRUK Institute
and Department of Zoology
University of Cambridge
Cambridge CB2 1QR, United Kingdom
Chapter 14

Jonathan R. Hill
College of Veterinary Medicine
Cornell University
Ithaca, New York 14853
Chapter 12

Philip Iannaccone
Department of Pediatrics
Northwestern University Medical School
Children's Memorial Institute for Education and Research
Chicago, Illinois 60614
Chapter 21

Kimiko Inoue
RIKEN Bioresource Center
Ibaraki 305-0074, Japan
Chapter 8

Rudolf Jaenisch
Whitehead Institute for Biomedical Research
and Department of Biology
Massachusetts Institute of Technology
Cambridge, Massachusetts 02142
Chapter 4

Y. Kato
Laboratory of Animal Reproduction
College of Agriculture
Kinki University
Nara 631-8505, Japan
Chapter 13

Manabu Kurokawa
Paige Laboratories
Department of Veterinary and Animal Sciences
University of Massachusetts
Amherst, Massachusetts 01003
Chapter 2

Michelle Lane
Colorado Center for Reproductive Medicine
Englewood, Colorado 80110
Chapter 9

Crista Martinovich
Departments of Obstetrics-Gynecology and Reproductive Sciences and Pittsburgh Development Center of Magee—Women's Research Institute
University of Pittsburgh School of Medicine
Pittsburgh, Pennsylvania 15213
Chapter 22

Harry M. Meade
Genzyme Transgenics Corporation
Framingham, Massachusetts 01701
Chapter 25

Narumi Ogonuki
RIKEN Bioresource Center
Ibaraki 305-0074, Japan
Chapter 8

Atsuo Ogura
RIKEN Bioresource Center
Ibaraki 305-0074, Japan
Chapter 8

Kenjiro Ozato
Bioscience Center
Nagoya University
Nagoya 464-8601, Japan
Chapter 15

Raymond L. Page
Advanced Cell Technology
Worcester, Massachusetts 01605
Chapter 7

Anthony C. F. Perry[†]
Advanced Cell Technology
Worcester, Massachusetts 01605
Chapter 16

Jorge A. Piedrahita
Center for Animal Biotechnology and Genomics
College of Veterinary Medicine
Texas A&M University
College Station, Texas 77843
Chapter 11

[†]Current affiliation: Laboratory of Mammalian Molecular Embryology, RIKEN Center for Developmental Biology, Chuou-ku, Kobe 650-0047, Japan.

Randall S. Prather
Department of Animal Sciences
University of Missouri—Columbia
Columbia, Missouri 65211
Chapter 18

Gerald Schatten
Departments of Obstetrics-Gynecology and Reproductive Sciences and Pittsburgh Development Center of Magee—Women's Research Institute
University of Pittsburgh School of Medicine
Pittsburgh, Pennsylvania 15213
Chapter 22

Hans R. Schöler
Germline Development Group at the Center for Animal Transgenesis and Germ Cell Research
University of Pennsylvania
Kennett Square, Pennsylvania 19348
Chapter 6

George E. Seidel, Jr.
Animal Reproduction and Biotechnology Laboratory
Department of Physiology
Colorado State University
Fort Collins, Colorado 80523
Chapter 10

Calvin Simerly
Departments of Obstetrics-Gynecology and Reproductive Sciences and Pittsburgh Development Center of Magee—Women's Research Institute
University of Pittsburgh School of Medicine
Pittsburgh, Pennsylvania 15213
Chapter 22

Jeremy Smyth
Paige Laboratories
Department of Veterinary and Animal Sciences
University of Massachusetts
Amherst, Massachusetts 01003
Chapter 2

Steven L. Stice
Department of Animal and Dairy Science
University of Georgia
Athens, Georgia 30602
Chapter 24

M. Azim Surani
Wellcome CRUK Institute
Physiological Laboratory
University of Cambridge
Cambridge CB2 1QR, United Kingdom
Chapter 5

Masako Tada
Department of Development and Differentiation
Institute for Frontier Medical Sciences
Kyoto University
Kyoto 606-8507, Japan
Chapter 5

Takashi Tada
Department of Development and Differentiation
Institute for Frontier Medical Sciences
Kyoto University
Kyoto 606-8507, Japan
Chapter 5

X. Cindy Tian
Department of Animal Science/Biotechnology Center
University of Connecticut
Storrs, Connecticut 06269
Chapter 17

Alan Trounson
Institute of Reproduction and Development
Monash University
Clayton, Victoria 3168, Australia
Chapter 23

Y. Tsunoda
Laboratory of Animal Reproduction
College of Agriculture
Kinki University
Nara 631-8505, Japan
Chapter 13

Fyodor D. Urnov
Sangamo BioSciences, Inc.
Pt. Richmond Tech Centre
Richmond, California 94804
Chapter 3

Yuko Wakamatsu
Bioscience Center
Nagoya University
Nagoya 464-8601, Japan
Chapter 15

Teruhiko Wakayama[‡]
Advanced Cell Technology
Worcester, Massachusetts 01605
Chapter 16

Alan P. Wolffe[§]
Sangamo BioSciences, Inc.
Pt. Richmond Tech Centre
Richmond, California 94804
Chapter 3

Xiangzhong Yang
Department of Animal Science/Biotechnology Center
University of Connecticut
Storrs, Connecticut 06269
Chapter 17

[‡]Current affiliation: RIKEN Center for Developmental Biology, Chuou-ku, Kobe 650-0047, Japan.
[§]Deceased.

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.

Robert M. Moor
The Babraham Institute
Cambridge CB2 4AT, United Kingdom

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