Ian Gibson (Ed.)

Antisense and Ribozyme Methodology

Laboratory Companion

With 10 Figures, some in Color

CHAPMAN & HALL
an International Thomson Publishing company I(T)P®

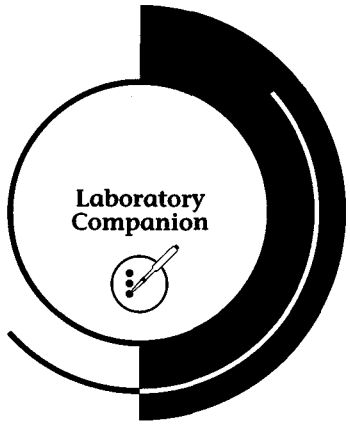
London · Glasgow · Weinheim · New York · Tokyo · Melbourne · Madras

This Page Intentionally Left Blank

Ian Gibson (Ed.)

Antisense and Ribozyme Methodology

Laboratory Companion



The *Laboratory Companion* series by Chapman & Hall focuses on state of the art methodology in modern bio-medical sciences. The titles of this series are more than just another collection of proven and easy to use protocols – they provide the experimenter with the valuable background information that other laboratory manuals often omit:

- fundamental discussion of the potential for application of methodology,
- competent advice for decisions between alternative approaches,
- strategies for optimisation of methodology,
- comprehensive troubleshooting guides,
- extensive discussion of data acquisition and critical evaluation of results,
- listings of reagents, equipments and suppliers,
- biohazard warnings and safety recommendations.

ISBN 3-8261-0079-4

Ian Gibson (Ed.)

Antisense and Ribozyme Methodology

Laboratory Companion

With 10 Figures, some in Color

CHAPMAN & HALL
an International Thomson Publishing company I(T)P®

London · Glasgow · Weinheim · New York · Tokyo · Melbourne · Madras

Dr. Ian Gibson
School of Biological Sciences
University of East Anglia
UK-Norwich NR4 7TJ

Library of Congress and British Library Cataloging-in-Publication Data applied for

Die Deutsche Bibliothek – CIP-Einheitsaufnahme

Antisense and ribozyme methodology / Ian Gibson (ed.). – London ; Glasgow ; Weinheim ; New York ; Tokyo ; Melbourne ; Madras : Chapman and Hall, 1997 (Laboratory companion)
ISBN 3-8261-0079-4

Book Production: PRO EDIT GmbH, 69126 Heidelberg, Germany
Typesetting: Mitterweger Werksatz GmbH, 68723 Plankstadt, Germany
Printing: Appl OHG, 86650 Wemding, Germany
Cover Design: punto design, 68167 Mannheim, Germany
Printed in the Federal Republic of Germany

Preface

The discovery of new drugs is a difficult, expensive, and long term activity, and often ends in tears after clinical failures. Approximately 80 % of applied research programs in this area fail at various technical hurdles. A rational drug discovery program involves hypothesis, identifying molecular targets involved in the physiology of the disease, and developing a *selective* agent to interact with this target. Eventually, despite success *in vitro*, clinical trials must ensue. At this point considerations of selectivity, safety, and pharmacokinetics are important. Further development requires animal cell models, and here relevance to the human condition is of the essence. All of these considerations are prevalent now in the field of antisense and ribozyme technology. The inhibition of restenosis in rats and pigs, and of leukemia cell proliferation and tumor growth in mice have been recorded. Further tests against the human immunodeficiency virus-1, the cytomegalovirus (CMV), and the human papilloma virus are now undergoing clinical trials. As the molecular and cellular biology of these biological systems are investigated, new therapeutic targets emerge. This monograph attempts to underline the approach to the targeting of nucleic acid informational systems via antisense DNA molecules and ribozymes (RNA). Key stages in disease processes may then be inhibited. Specific nucleic acid sequences may be targeted, and this should enable the normal sequence to escape the effect of the antisense DNA or ribozyme.

The work in this field has, however, thrown up discrepancies in that non-antisense mechanisms gave biological effects on, for example, cellular proliferation. This has led on the one hand to a wide skepticism of the positive claims with antisense or ribozymes, especially when effects could not be repeated, or controls gave similar effects. A careful analysis of conditions of the experiments, the use of a wide range of controls and, most importantly, attempts to show specific effects on target m-RNA and protein levels have been highlighted as essential stages in any analysis. The latter may be particularly difficult due to long turnover times of the key protein. As secondary structures for RNA become more predictable from current crystallization studies, then the discrepancies may yet find an explanation.

The field is certainly evolving at a fast rate, and groups are vying with each other to increase the specificity of targeting, the stability of the molecules, and maximum uptake into cells. This monograph

appears at an early stage in the testing of such molecules, and is written with the aim of giving the research worker a focal point from where they can start to carry out experiments. It does not pretend to have the 'answer' to the problems, but with the help of active practitioners in the field takes a view on 'the best practice'.

Chapter 2 is the work of Dr David Tidd of the Department of Biochemistry, University of Liverpool who is a leading expert on chimeric antisense molecules. Chapter 3 is the work of Dr. George Sczakiel who is an expert on ribozyme construction and development. Chapter 4 is the work of a PhD student at the University of East Anglia in Norwich, Ciara Twomey, and myself, who are both involved in the development of delivery systems of ribozymes into cells.

An active research field such as this is almost certain to render some of these developments redundant in a year or so. However, if this monograph serves to excite and enthuse others to enter the field, then it will have worked.

Norwich, 1997 Ian Gibson

A note on the layout of this book:

In order to facilitate the use of this book as a methodological source for your bench work, a wide page format has been chosen.
Due to the type of durable binding used, the book has the advantage of lying flat on your bench top for convenient use. In addition, a wide margin leaves room for your own notes and provides some key notes and pictograms to assist you in finding the relevant information:

a pipette symbol marks the start of a step by step protocol section, a grey bar runs down the margin of the whole protocol section

this symbol draws your attention to potential hazards and safety suggestions

comments on the key steps in methodology are highlighted by a key symbol

a "good idea" symbol marks useful hints for optimization of methodology

this pictogram indicates discussions of alternative approaches

the tool indicates troubleshooting guides that should help you in finding out what could or did go wrong and in solving and avoiding problems

suggestions for monitoring quality and reliability of the experimental procedure are highlighted by the magnifying glass