

Transcription and translation

a practical approach

**Edited by
B D Hames**

S J Higgins

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B D Hames

Department of Biochemistry,
University of Leeds, Leeds, England

S J Higgins

Department of Biochemistry,
University of Leeds, Leeds, England



IRL Press Limited
P.O. Box 1,
Eynsham,
Oxford OX8 1JJ,
England

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First published June 1984
First reprinting May 1985

British Library Cataloguing in Publication Data

Transcription and translation. — (The Practical approach series)

I. Biological chemistry — Technique

I. Hames, B.D. II. Higgins, S.J.

III. Series

ISBN 0-904147-52-5

Cover illustration. Electron micrographs of rRNA genes from *Notophthalmus viridescens* in the process of transcription (on the left) and polysomes from *Bombyx mori* showing nascent polypeptides (on the right). The photographs were kindly supplied by Steven McKnight and Oscar L. Miller Jr., The Department of Biology, University of Virginia, USA.

Printed in England by Information Printing, Oxford.

Preface

Our present and future understanding of the mechanism and regulation of gene expression depends upon both direct investigations of gene transcription and the assay of specific messenger RNAs. In addition, the techniques associated with molecular biology and molecular genetics will be required by increasing numbers of researchers in the biological sciences. The aim of this book is to provide detailed practical protocols for these major areas of study. Eukaryotic, prokaryotic and viral genes are all covered, with the transcription of eukaryotic genes being considered mainly with regard to RNA polymerase II. Considerable revisions of some chapters were necessary in order to prevent undue repetition whilst including all the important practical topics and we thank the authors concerned for their understanding during this exercise. While our aim has been to cross-reference between chapters rather than to duplicate practical protocols, where several important approaches to the same technique exist these have been provided in full.

B.D.Hames and S.J.Higgins

Contributors

Michael J. Clemens

Cancer Research Campaign Mammalian Protein Synthesis and Interferon Research Group, Department of Biochemistry, St. George's Hospital Medical School, Cranmer Terrace, London SW17 0RE, UK

Alan Colman

Department of Biological Sciences, University of Warwick, Coventry CV4 7AL, UK

R. Stewart Gilmour

Beatson Institute for Cancer Research, Garscube Estate, Switchback Road, Bearsden, Glasgow G61 1BD, UK

J.B. Gurdon

Cancer Research Campaign Molecular Embryology Group, Department of Zoology, Downing Street, Cambridge CB2 3EJ, UK

I. Barry Holland

Department of Genetics, Adrian Building, University of Leicester, University Road, Leicester LE1 7RH, UK

Ru Chih C. Huang

Department of Biology, Johns Hopkins University, 34 Charles Street, Baltimore, MD 21218, USA

James L. Manley

Department of Biological Sciences, Columbia University, New York, NY 10027, USA

William F. Marzluff

Department of Chemistry, The Florida State University, Tallahassee, FL 32306, USA

Stephen J. Minter

Department of Molecular Biology, University of Manchester Institute of Science and Technology (UMIST), Sackville Street, P.O. Box 88, Manchester M60 1QD, UK

Julie M. Pratt

Department of Genetics, Adrian Building, University of Leicester, University Road, Leicester LE1 7RH, UK

Paul G. Sealey

MRC Mammalian Genome Unit, Department of Zoology, University of Edinburgh, West Mains Road, Edinburgh EH9 3JT, UK

Demetrios A. Spandidos

*Wolfson Laboratory for Molecular Pathology, The Beatson Institute
for Cancer Research, Garscube Estate, Switchback Road, Bearsden,
Glasgow G61 1BD, UK*

Neil G. Stoker

*Imperial Cancer Research Fund Laboratories, P.O. Box 123, Lincoln's
Inn Fields, London WC2A 3PX, UK*

Neil M. Wilkie

*Wolfson Laboratory for Molecular Pathology, The Beatson Institute
for Cancer Research, Garscube Estate, Switchback Road, Bearsden,
Glasgow G61 1BD, UK*

Abbreviations

APH	aminoglycoside phosphotransferase
bp	base pairs
BPV	bovine papilloma virus
BSA	bovine serum albumin
CAT	chloramphenicol acetyltransferase
cDNA	complementary DNA
Ci	Curie (3.7×10^{10} Bq)
c.p.m.	counts per minute
DEAE	diethylaminoethyl
DEPC	diethylpyrocarbonate
DHFR	dihydrofolate reductase
DMSO	dimethyl sulphoxide
d.p.m.	disintegrations per minute
DTT	dithiothreitol
EDTA	ethylenediamine tetraacetic acid
EGTA	ethyleneglycobis(β -aminoethyl)ether tetraacetic acid
EMC	encephalomyocarditis virus
HAT medium	hypoxanthine-aminopterin-thymidine medium
Hepes	N-2-hydroxyethylpiperazine-N'-2-ethanesulphonic acid
Hg-RNA	mercury-substituted RNA
Hg-UTP	5'-mercurated UTP
HGPRT	hypoxanthine-guanine phosphoribosyltransferase
HMBA	hexamethylene bisacetamide
HnRNA	heterogeneous nuclear RNA
HSV-1	<i>Herpes simplex</i> virus type 1
kb	kilobases
LTR	long terminal repeat
MMTV	mouse mammary tumour virus
MoMuSV	Moloney murine sarcoma virus
Mops	3-(N-morpholino)propanesulphonic acid
mRNA	messenger RNA
NHP	non-histone protein
NP-40	Nonidet P-40
PAGE	polyacrylamide gel electrophoresis
PBP	penicillin-binding protein
PBS	phosphate-buffered saline
p.f.u.	plaque forming units
Pipes	piperazine-N,N'-bis-2-ethanesulphonic acid
PMSF	phenylmethylsulphonyl fluoride
p.s.i.	pounds per square inch (lb/in ²)
RNP	ribonucleoprotein
rRNA	ribosomal RNA
SDS	sodium dodecyl sulphate
SDS-PAGE	polyacrylamide gel electrophoresis in the presence of SDS

α -S-RNA	RNA synthesised with α -thionucleotides
γ -S-RNA	RNA synthesised with γ -thionucleotides
SV40	simian virus 40
TCA	trichloroacetic acid
TEMED	N,N,N',N'-tetramethylethylenediamine
TET	tetracycline-resistance protein
TK	thymidine kinase
t.l.c.	thin-layer chromatography
TMV	tobacco mosaic virus
Tricine	N-[2-hydroxy-1,1-bis(hydroxymethyl)ethyl] glycine
tRNA	transfer RNA
XGPRT	xanthine-guanine phosphoribosyltransferase

Introduction

J.B. GURDON

The value of experimental systems for the analysis of gene expression will be obvious to all who work in the areas of cell biology and molecular biology, but it may be helpful to distinguish two different objectives of work in this area. One is to determine the *mechanism* of gene expression, and the other to analyse the *control* of this process. The former is concerned with identifying molecules required to obtain the expression of a gene. The type of information sought is which of several DNA clones codes for a certain gene product, and which of many fractions of RNA contain the mRNA required. These answers can be readily provided by the use of appropriate cell-free systems. With cell-free systems containing purified components it is also possible to identify factors required for the accurate transcription of DNA and translation of mRNA. The second, much more difficult, objective is to understand the control of gene expression. This requires a knowledge of the rate at which each step in gene expression proceeds, and identification of the components which are limiting in these steps. The reason why a meaningful analysis of gene control is so hard to achieve is that any component involved in a reaction can become limiting under particular experimental conditions even though most of these conditions may never normally exist *in vivo*. There is no simple way of determining whether a component which is limiting *in vitro* is also limiting *in vivo*. The same problem does not apply to an analysis of the *mechanism* of gene expression since even if the components in a cell-free system are present at concentrations different from normal, the coding capacity and requirement for essential factors should not be altered.

The ideal towards which everyone strives is a cell-free system which reflects normal gene expression and which consists entirely of known components. Very few such systems exist. Nearly all commonly-used cell-free systems involve the use of crude extracts to which purified components, such as cloned DNA or mRNA, are added. The great majority of systems described in this volume fall into this class. However, another type of system which has proved more successful than might have been predicted initially consists of a living cell into which purified components are injected. When a cell is disrupted, the lysate usually contains large amounts of DNase, RNase and proteolytic activities, so that these activities must be removed or reduced in the initial steps in the preparation of cell-free systems. However, when a living cell is injected with a solution of DNA or mRNA comprising as much as 10% of its volume, little degradation of the injected molecules takes place. Not surprisingly, therefore, microinjection of DNA and mRNA into living cells is an important and useful technique in the analysis of gene expression. Various methods and systems for microinjection are described in this volume.

Finally, it is important to be aware of the relative merits of cell-free systems and injected living cells for studying gene expression. Cell-free systems, and especially

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