Transcription and translation

a practical approach

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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.

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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

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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 x 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	*Herpes simplex* 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 proteolyic 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 those whose components are mainly defined, have proved especially valuable in the initial recognition as well as the subsequent purification of transcriptional and translational factors. On the other hand, living cells can be used for such an analysis only under exceptional circumstances, for example, when a type of cell is available which is known to lack a factor which can be extracted from another cell type. The disadvantage of cell-free systems is that the range of steps in gene expression which takes place is limited and that the efficiency (or rate) of each step may be $10^2 - 10^5$ times less than in an injected cell. The significance of this greater efficiency is that the control of a particular reaction in gene expression can be studied more validly when that reaction is proceeding more closely to normal than when it is taking place at less than 1% of the rate *in vivo*. In conclusion, it is important to know the rate of gene expression in any experimental system used for analysis of the control of gene expression but this is not necessary for analysis of the mechanism of gene expression.

During recent years, experimental systems have greatly improved both in the range and efficiency of the gene expression steps which they carry out. Furthermore, there has been a great proliferation in the types and sources of systems which can be usefully applied to a particular problem. I therefore believe that the present volume will be very widely welcomed. The chapters have been contributed by those who have extensive experience of the procedures involved, and who, in many cases, have been directly involved in their development.

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