

*Methods in Enzymology*

*Volume 273*

*RNA Polymerase and  
Associated Factors*

*Part A*

EDITED BY

*Sankar Adhya*

NATIONAL CANCER INSTITUTE  
NATIONAL INSTITUTES OF HEALTH  
BETHESDA, MARYLAND



ACADEMIC PRESS

San Diego London Boston New York Sydney Tokyo Toronto

# Table of Contents

CONTRIBUTORS TO VOLUME 273. . . . .	ix
PREFACE. . . . .	xiii
VOLUMES IN SERIES . . . . .	xv

## Section I. Promoter Elements and RNA Polymerase Components

1. Promoters and Basal Transcription Machinery in Eubacteria and Eukaryotes: Concepts, Definitions, and Analogies	JAN S. FASSLER AND GARY N. GUSSIN	3
2. <i>Escherichia coli</i> Promoter Sequences: Analysis and Prediction	GERALD Z. HERTZ AND GARY D. STORMO	30

## Section II. Biochemical Assays of Transcription Initiation

3. Kinetic Analysis of RNA Polymerase-Promoter Interactions	GARY N. GUSSIN	45
4. Quantitative Parameters for Promoter Clearance	LILIAN M. HSU	59
5. <i>In Vitro</i> Assay for Reiterative Transcription during Transcriptional Initiation by <i>Escherichia coli</i> RNA Polymerase	FENGXIA QI, CHONGGUANG LIU, LUCIE S. HEATH, AND CHARLES L. TURNBOUGH, JR.	71
6. Rigorous and Quantitative Assay of Transcription <i>in Vitro</i>	HAILAN ZHANG, NANCY ILER, AND CORY ABATE-SHEN	86
7. Global Steps during Initiation by RNA Polymerase II	JAY D. GRALLA	99
8. Purification and Analysis of Functional Preinitiation Complexes	STEFAN G. E. ROBERTS AND MICHAEL R. GREEN	110

### Section III. RNA Polymerase and Its Subunits in Prokaryotes

9. Reconstitution of RNA Polymerase	NOBUYUKI FUJITA AND AKIRA ISHIHAMA	121
10. <i>Escherichia coli</i> RNA Polymerase Holoenzyme: Rapid Reconstitution from Recombinant $\alpha$ , $\beta$ , $\beta'$ , and $\sigma$ Subunits	HONG TANG, YOUNGGYU KIM, KONSTANTINE SEVERINOV, ALEX GOLDFARB, AND RICHARD H. EBRIGHT	130
11. $\sigma$ Factors: Purification and DNA Binding	ALICIA J. DOMBROSKI	134
12. Purification of Overproduced <i>Escherichia coli</i> RNA Polymerase $\sigma$ Factors by Solubilizing Inclusion Bodies and Refolding from Sarkosyl	RICHARD R. BURGESS	145
13. RNA Polymerase $\sigma$ Factors of <i>Bacillus subtilis</i> : Purification and Characterization	KATHLEEN M. TATTI AND CHARLES P. MORAN, JR.	149

### Section IV. RNA Polymerase and Associated Factors from Eukaryotes

14. Nuclear RNA Polymerases: Role of General Initiation Factors and Cofactors in Eukaryotic Transcription	ROBERT G. ROEDER	165
15. Yeast RNA Polymerase II Holoenzyme	YANG LI, STEFAN BJORKLUND, YOUNG-JOON KIM, AND ROGER D. KORNBERG	172
16. Purification of Yeast RNA Polymerase II Holoenzymes	ANTHONY J. KOLESKE, DAVID M. CHAO, AND RICHARD A. YOUNG	176
17. Phosphorylation of Mammalian RNA Polymerase II	MICHAEL E. DAHMUS	185
18. Purification of RNA Polymerase II General Transcription Factors from Rat Liver	RONALD C. CONAWAY, DANIEL REINES, KARLA PFEIL GARRETT, WADE POWELL, AND JOAN WELIKY CONAWAY	194
19. Reconstitution of TATA-Binding Protein-Associated Factor/TATA-Binding Protein Complexes for <i>in Vitro</i> Transcription	JIN-LONG CHEN AND ROBERT TJIAN	208
20. Purification, Characterization, and Role of CCAAT-Binding Factor in Transcription	SANKAR N. MAITY AND BENOIT DE CROMBRUGGHE	217
21. Purification, Assay, and Properties of RNA Polymerase I and Class I-Specific Transcription Factors in Mouse	ANDREAS SCHNAPP AND INGRID GRUMMT	233

22. RNA Polymerase III and Class III Transcription Factors from <i>Saccharomyces cerevisiae</i>	JANINE HUET, NATHALIE MANAUD, GIORGIO DIECI, GÉRALD PEYROCHE, CHRISTINE CONESA, OLIVIER LEFEBVRE, ANNY RUET, MICHEL RIVA, AND ANDRÉ SENTENAC	249
23. Basal and Activated <i>in Vitro</i> Transcription in Plants by RNA Polymerase II and III	HAO FAN AND MASAHIRO SUGIURA	268

### Section V. Genetic Analysis of Transcription and Its Regulation

24. Analysis of Two-Component Signal Transduction Systems Involved in Transcriptional Regulation	REGINE HAKENBECK AND JEFFRY B. STOCK	281
25. Mutational Analysis of Structure-Function Relationship of RNA Polymerase in <i>Escherichia coli</i>	DING JUN JIN AND YAN NING ZHOU	300
26. Vectors with Bidirectional Reporter Genes for Studying Divergent Promoters	VINAY K. JAIN	319
27. Gene Identification Using the Yeast Two-Hybrid System	CHANG BAI AND STEPHEN J. ELLEDGE	331
AUTHOR INDEX . . . . .		349
SUBJECT INDEX . . . . .		369