Edited by Heinz Fraenkel-Conrat and Robert R. Wagner

Comprehensive Virology

7

Reproduction

Bacterial DNA Viruses

Virology

7

Reproduction

Bacterial DNA Viruses

PLENUM PRESS • NEW YORK AND LONDON

Library of Congress Cataloging in Publication Data

Fraenkel-Conrat, Heinz, 1910-
Reproduction, bacterial DNA viruses.

(Their Comprehensive virology; 7)
Includes bibliographies and index.
1. Viruses–Reproduction. 2. Bacteriophage. I. Wagner, Robert R., 1923- Joint author. II. Title. III. Series: Fraenkel-Conrat, Heinz, 1910- Comprehensive virology; 7. [DNLM: 1. DNA replication. 2. Virus replication. QW160 C737 v. 7]
QR357.F72 vol. 7 [QR470] 576'.64'08s [576'.6482]
ISBN 0-306-35147-1 76-40342

© 1977 Plenum Press, New York
A Division of Plenum Publishing Corporation
227 West 17th Street, New York, N.Y. 10011

All rights reserved

No part of this book may be reproduced, stored in a retrieval system, or transmitted, in any form or by any means, electronic, mechanical, photocopying, microfilming, recording, or otherwise, without written permission from the Publisher

Printed in the United States of American

Foreword

The time seems ripe for a critical compendium of that segment of the biological universe we call viruses. Virology, as a science, having passed only recently through its descriptive phase of naming and numbering, has probably reached that stage at which relatively few new—truly new—viruses will be discovered. Triggered by the intellectual probes and techniques of molecular biology, genetics, biochemical cytology, and high-resolution microscopy and spectroscopy, the field has experienced a genuine information explosion.

Few serious attempts have been made to chronicle these events. This comprehensive series, which will comprise some 6000 pages in a total of about 22 volumes, represents a commitment by a large group of active investigators to analyze, digest, and expostulate on the great mass of data relating to viruses, much of which is now amorphous and disjointed, and scattered throughout a wide literature. In this way, we hope to place the entire field in perspective, and to develop an invaluable reference and sourcebook for researchers and students at all levels.

This series is designed as a continuum that can be entered anywhere, but which also provides a logical progression of developing facts and integrated concepts.

Volume 1 contains an alphabetical catalogue of almost all viruses of vertebrates, insects, plants, and protists, describing them in general terms. Volumes 2–4 deal primarily, but not exclusively, with the process of infection and reproduction of the major groups of viruses in their hosts. Volume 2 deals with the simple RNA viruses of bacteria, plants, and animals; the togaviruses (formerly called arboviruses), which share with these only the feature that the virion's RNA is able to act as messenger RNA in the host cell; and the reoviruses of animals and plants, which all share several structurally singular features, the most important being the double-strandedness of their multiple RNA molecules. This grouping, of course, has only slightly more in its favor than others that could have been, or indeed were, considered.

Volume 3 addresses itself to the reproduction of all DNA-containing viruses of vertebrates, a seemingly simple act of classification, even though the field encompasses the smallest and the largest viruses known. The reproduction of the larger and more complex RNA viruses is the subject matter of Volume 4. These viruses share the property of lipid-rich envelopes with the togaviruses included in Volume 2. They share as a group, and with the reoviruses, the presence of enzymes in their virions and the need for their RNA to become transcribed before it can serve messenger functions.

Volumes 5 and 6 represent the first in a series that focuses primarily on the structure and assembly of virus particles. Volume 5 is devoted to general structural principles involving the relationship and specificity of interaction of viral capsid proteins and their nucleic acids, or host nucleic acids. It deals primarily with helical and the simpler isometric viruses, as well as with the relationship of nucleic acid to protein shell in the T-even phages. Volume 6 is concerned with the structure of the picornaviruses, and with the reconstitution of plant and bacterial RNA viruses.

Volumes 7 and 8 deal with the DNA bacteriophages. Volume 7 concludes the series of volumes on the reproduction of viruses (Volumes 2–4 and Volume 7) and deals particularly with the single- and double-stranded virulent bacteriophages.

Volume 8 will be the first of the series on regulation and genetics of viruses, in which the biological properties of the lysogenic and defective phages will be covered, the phage–satellite system P2–P4 described, and the regulatory principles governing the development of selected typical lytic phages discussed in depth.

Volume 8 will be followed by three others dealing with the regulation of gene expression and integration of animal viruses; the genetics of animal viruses; and regulation of plant virus development, covirus systems, satellitism, and viroids. In addition, it is anticipated that there will be two or three other volumes devoted largely to structural aspects and the assembly of bacteriophages and animal viruses, and to special virus groups.

The complete series will endeavor to encompass all aspects of the molecular biology and the behavior of viruses. We hope to keep this series up to date at all times by prompt and rapid publication of all contributions, and by encouraging the authors to update their chapters by additions or corrections whenever a volume is reprinted.

此为试读,需要完整PDF请访问: www.ertongbook.com

Contents

Chapter 3

Reproduction of Large Virulent Bacteriophages

Christopher K. Mathews

CHAPTER 1

The Isometric Single-Stranded DNA Phages

David T. Denhardt
Department of Biochemistry
McGill University
Montreal, Quebec, Canada

"When you are a Bear of Very Little Brain, and you Think of Things, you find sometimes that a Thing which seemed very Thingish inside you is quite different when it gets out into the open and has other people looking at it."

—A. A. Milne, *The House at Pooh Corner*

1. INTRODUCTION

1.1. A Circular, Single-Stranded DNA Molecule within a Tailless Capsid

The subjects of this chapter have an isometric capsid with icosahedral symmetry enclosing a single-stranded DNA genome. The RNA bacteriophages appear to have a similar structure. Some eukaryotic viruses, but no phages that I know of, contain double-stranded DNA enclosed in an isometric capsid, *sans* tail. Isometric structures can be constructed of subunits arranged with cubic symmetry—either tetrahedral, octahedral, or icosahedral. Each of these symmetries requires a set of identical subunits—12, 24, and 60, respectively—arranged on the surface of a sphere. An attribute of icosahedral symmetry is that a subunit of a fixed size can enclose a larger volume than can be enclosed by using either of the other two point group symmetries

(Caspar and Klug, 1962), and it is this kind of symmetry, or derivatives thereof, that seems to be used generally in virus construction, for example in the heads of the tailed phages.

Viruses with single-stranded DNA are among the smallest forms of "life" known. Those replicating in prokaryotic cells contain fewer than ten genes, while the eukaryotic parvoviruses (Rose, 1974) may contain only a single gene. All of the single-stranded DNA viruses have genomes with molecular weights in the range of 1 to 3 $\times$ 10^6. Larger single-stranded DNAs may have been selected against because of the sensitivity of the single-stranded DNA to environmental hazards; if so, those viruses extant now may be the remnants of a once more diverse primitive life form. One reason for interest in these viruses is that because of the small size of their DNA and their limited genetic content they are very dependent on the host cell. Consequently, they provide a probe for various cell functions—particularly those concerned with replicating the genome.

There is no evidence for an evolutionary relationship between the eukaryotic parvoviruses, of which there may be more than one fundamentally different type, and the prokaryotic phages. I have argued elsewhere that the superficially quite distinct filamentous phages, which also contain single-stranded DNA and are reviewed by Ray in Chapter 2 of this volume, are derived from the same primitive ancestor as the isometric phages and that the isometric phages are variations on a common theme (Denhardt, 1975). For the latter, the single-stranded DNA (always of the same polarity) is enclosed in a protein coat about 32 nm in diameter, composed of multiple copies of four different polypeptides. An electron micrograph of ϕX174 is shown in Fig. 1. The isometric phages all seem to have a common organization of the genome and a set of proteins with equivalent functions. The fact that different isolates are serologically unrelated is not necessarily symptomatic of a lack of relationship since this property may be easily affected by mutations (Bone and Dowell, 1973*a*). The base sequence of the genome is capable of changing to a surprising degree without losing its ability to produce a functionally equivalent set of proteins (Godson, 1973). Many of the single-stranded DNA phages, including the filamentous phages, have a high (30–35%) molar ratio of thymine in the viral DNA (Marvin and Hohn, 1969; Sinsheimer, 1968), perhaps because codons containing uracil are favored (Denhardt and Marvin, 1969; Sanger, 1975).

The best known isometric phages are S13 (Burnet, 1927) and ϕX174 (Sertic and Boulgakov, 1935); evidence that they are related was first provided by Zahler (1958). Because of the extensive work done

此为试读,需要完整PDF请访问: www.ertongbook.com

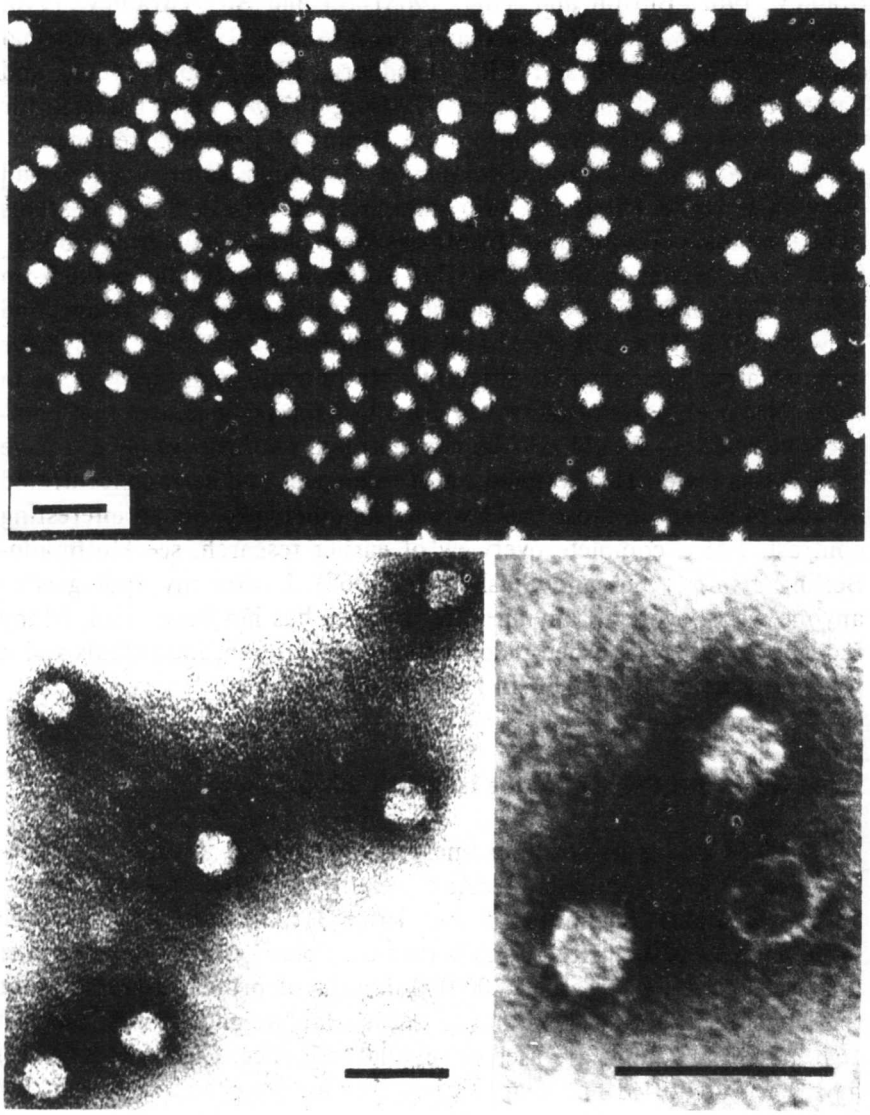

Fig. 1. Electron micrograph of ϕX174 negatively stained with phosphotungstic acid. The bar represents 0.05 μm. I thank L. Guluzian and C. Hours for the electron microscopy.

with ϕX174 (Sinsheimer, 1968), it can be used as the archetype with which the others can be contrasted. These others include ϕR (Kay and Bradley, 1962; Burton and Yagi, 1968), α3 and St-1 (Bradley, 1970; Bowes and Dowell, 1974), ϕA and ϕK (Taketo, 1974, and personal communication), 6SR and BR2 (Lindberg, 1973), U3 (Watson and Paigen, 1971), and the G phages (Godson, 1974*b*). Host bacteria for these various isolates are appropriate strains of Enterobacteriaceae—*Escherichia, Shigella,* and *Salmonella.* Although some isolates will grow on *E. coli* K (St-1, U3, ϕK) and others on B (α3), *E. coli* C seems to be the most common host. Insofar as these phages have been studied, there is no reason to think that they differ in any fundamental from ϕX174. Mutants of ϕX174 capable of growing on K12 (Bone and Dowell, 1973*a*,*b*) and B (Denhardt unpublished, see Razin, 1973) have been isolated. The fact that different isolates made at different times in widely separate parts of the world are all so similar indicates that comparative research should now be concentrated on those we have. I have limited this review to a selected set of references and have concentrated on ϕX174 except in those cases where the others provide an interesting contrast. For a complete overview of earlier research, see Hoffmann-Berling *et al.* (1966) and Sinsheimer (1968). I offer my apologies to anyone whose work in any specific instance has not been cited. Many facts have been established independently by different individuals and it was not practical to include all possible references.

1.2. Genes and Proteins of the Isometric Phages

ϕX174 has as its chromosome one molecule of a circular, single-stranded DNA with a molecular weight of about 1.7×10^6, or 5370 nucleotides (Section 1.4.5 and Table 3). The viral DNA has the same polarity as the mRNA and is thus the "plus" strand. A genome of this size can code for about 200,000 daltons of protein assuming that the "average" amino acid has a molecular weight of 120 and all 1670 potential codons are used once, and only once. Eight genes (A–H) have been identified and mapped and the protein products of most of them identified unambiguously. The genetic map (see Fig. 2) is circular (Baker and Tessman, 1967). A list of the genes, their earlier designations, and the molecular weights and functions of the proteins insofar as they are known is presented in Table 1. The direction of transcription and translation is clockwise (Vanderbilt *et al.*, 1971).

Structural proteins found in the virion include the products of genes F, G, and H and have molecular weights of about 50,000, 20,000,

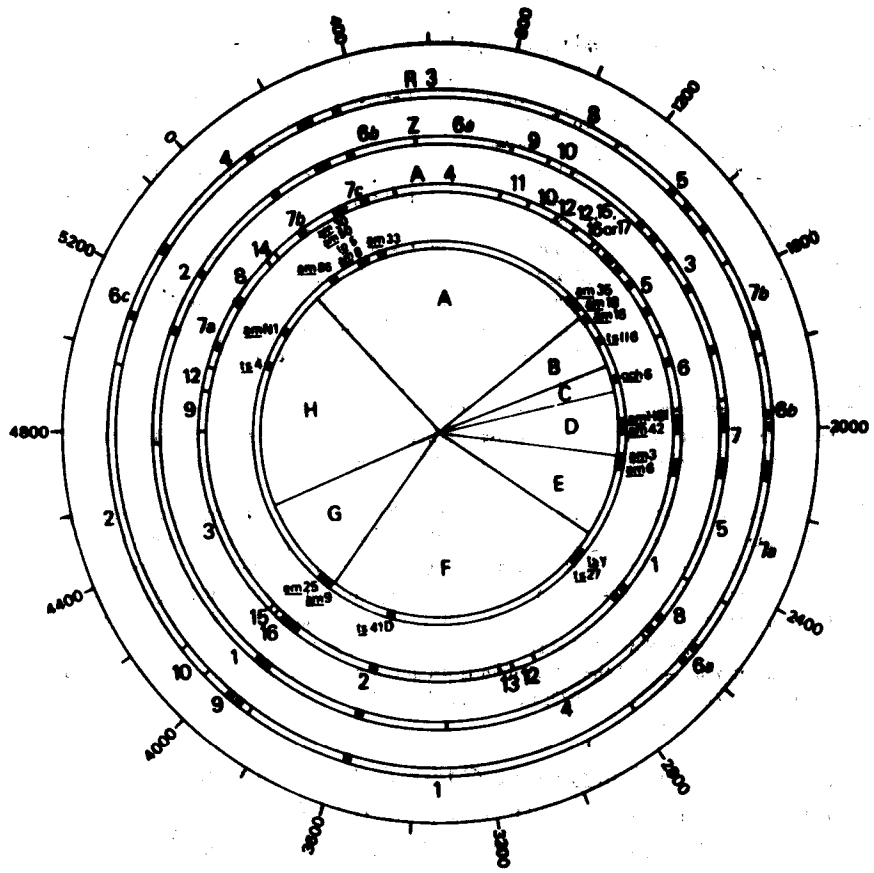

Fig. 2. Genetic and restriction enzyme maps of the ϕX174 genome. The outer circle is arbitrarily divided into 5500 base pairs; 5370 is probably more accurate. The inner circle is divided into pie-shaped portions each of which is allocated to a gene on the basis of either the known size of the protein or the size estimated from the physical mapping data. The three concentric circles labeled R, Z, and A give the location of the various cleavage products produced by the restriction enzymes *Hin*dII, *Hae*III, and *Alu*I, respectively. The sites of various nonsense and temperature-sensitive mutants used to correlate the genetic and physical maps are indicated; *to*5 and *em*25 are tourmaline and emerald mutations, respectively, classified according to their ability to grow on *Shigella sonnei* V64 (Borrias *et al.*, 1969). Reproduced from Weisbeek *et al.* (1976), with permission.

and 37,000, respectively (Burgess, 1969*a*; Mayol and Sinsheimer, 1970; Godson, 1971*a*). Three proteins involved in DNA replication, A, C, and D, have molecular weights of about 60,000, 10,000, and 14,000, respectively (Burgess and Denhardt, 1969; Godson, 1971*a*; Borras *et al.*, 1971; van der Mei *et al.*, 1972*b*). A* results from a translational restart within

TABLE 1

Genes and Gene Functions of the Isometric Phages

Gene designation	Molecular weight of the protein product	Mutant phenotype or function(s) of the protein
A (VI, C, IV)[a]	60,000[b]	RF replication and SS DNA synthesis
B (IV, B, II)	20,000	SS DNA synthesis and virion morphogenesis
C (VIII, H, VI)	12,000	SS DNA synthesis
D (V, D, VII)	14,000	SS DNA synthesis, probably virion morphogenesis
E (I, G, V)	(10,000?)	Lysis of the cell
F (VII, E, I)	50,000	Capsid protein, SS DNA synthesis
G (III, F, IIIa)	20,000	Spike protein, SS DNA synthesis
H (II, A, IIIb)	37,000	Spike protein, pilot protein (?)
A*	37,000	? (the *C*-terminal portion of A)[c]

[a] The designations in parentheses are those originally given to these genes by Sinsheimer (Benbow *et al.*, 1971), Hayashi (Hayashi and Hayashi, 1971), and Tessman (Tessman *et al.*, 1971), respectively.

[b] The molecular weights given are approximate. Different laboratories have reported slightly different values, and the molecular weights of the corresponding proteins coded for by different isolates of the isometric phages vary somewhat. The relative mobilities of proteins A*/H and B/G can vary with the gel system. (Data compiled from the results of Burgess and Denhardt, 1969; Godson, 1971*a*, 1973; Borras *et al.*, 1971; van der Mei *et al.*, 1972*b*; Linney *et al.*, 1972; Benbow *et al.*, 1972*b*; Siden and Hayashi, 1974.)

[c] A* is part of gene A, and the A* protein is produced as the result of a translational start signal within gene A (Linney *et al.*, 1972; Linney and Hayashi, 1973).

gene A and corresponds to the *C*-terminal portion of the gene A protein (Linney and Hayashi, 1974). The gene B protein is required for virion formation and appears to have a molecular weight of about 20,000 (Benbow *et al.*, 1972*b*; Siden and Hayashi, 1974). The gene E protein has not been clearly identified, but if we assume that the *del*E deletion (see Fig. 10 and Section 7.1) is congruent with that gene then it would have a molecular weight of about 14,000. A protein of about this size was uncovered in SDS gels of virus-coded proteins when the similarly sized gene D protein was eliminated by an amber mutation. The molecular weights of these proteins were determined by electrophoresis in polyacrylamide gels in the presence of sodium dodecylsulfate and mercaptoethanol (see Fig. 11) and there is thus uncertainty in their precise molecular weights. Various substituents on the protein can alter its mobility, and the relative mobilities of certain proteins (H vs. A*, B vs. G) vary with the cross-linker (Linney and Hayashi, 1974; Siden and Hayashi, 1974). These proteins total about 200,000 daltons, and it appears that all of the coding capacity of the genome is accounted for. Two additional genes (gene I, Hayashi and Hayashi, 1971, and gene J, Benbow *et al.*, 1972*b*) have been reported, but current evidence does not

confirm their existence (M. Hayashi, personal communication; Weisbeek *et al.*, 1976).

1.3. Enzymatic and Chemical Studies

1.3.1. Restriction Enzyme Maps

A number of researchers have developed restriction enzyme maps of the viral replicative form DNA, and in some cases have assigned particular genetic loci to particular cleavage products. The fairly detailed correlation reported by Weisbeek *et al.* (1976) is illustrated in Fig. 2. Mutations can be assigned to DNA fragments by formation of partial heteroduplexes between intact mutant viral strands and a complementary strand fragment derived from the restriction enzyme cleavage product derived from wild-type RF (Hutchison and Edgell, 1971; Edgell *et al.*, 1972). Wild-type progeny will be produced in spheroplasts infected with these heteroduplexes only when the restriction enzyme fragment carries the relevant portion of the wild-type allele.

Restriction endonucleases that have been used to characterize isometric phage DNA are listed in Table 2 together with a summary of the number of fragments produced from the different genomes. In the literature, the sizes of the individual fragments have usually been calculated assuming that the size of the ϕX174 genome was close to 5500 nucleotides. Since the correct nucleotide content is probably closer to 5370 (Section 1.4.5), the sizes of the individual fragments that have been reported may be some 2% too high. Enzymes which put only one cleavage into viral RF molecules include *Eco*R1, *Mbo*I, *Pst*I, *Ava*I, *Kpn*I, and *Bgl*I. Restriction of ϕX174 replication *in vivo* by F factors (Groman, 1969) and by *Eco*B (Schnegg and Hofschneider, 1969) has been reported.

There is disagreement between Weisbeek *et al.* (1976) (see Fig. 2) and Lee and Sinsheimer (1974*a*) on the relative positions of the *Hae*III 6a and 6b fragments. Grosveld *et al.* (1976) also mapped these fragments, and the *Hin*dII 6a, 6b, 6c, 7a, and 7b fragments, but named them according to their location in the genome. The fragments in each group have similar mobilities in gels and are difficult to separate cleanly. A terminology based on the purported size deduced from relative migration rates in acrylamide and/or agarose may be awkward because differences in base composition or sequence may reverse the relative migration rates of certain fragments under different condi-

TABLE 2

Number of Cleavages of Isometric Phage RF by Restriction Enzymes[a]

Enzyme (cell)	Sequence	φX174	S13	G4	St-1
*Hin*dII (*Haemophilus influenzae* serotype d)	GTPy/PuAC	13 (a,d,j)	13 (j)	6 (i)	6
*Hae*III (*Haemophilus aegyptius*)	GG/CC	11 (b,d,h)	10 (j)	12	12
*Hae*II (*Haemophilus aegyptius*)	PuGCGC/Py	7	5	3	—
*Hpa*II (*Haemophilus parainfluenzae*)	C/CGG	5 (f,i) 8 (c,d)	7	7 (i)	14
*Hpa*I (*Haemophilus parainfluenzae*)	GTT/AAC	3	3	—	—
*Hap*II (*Haemophilus aphirophilus*)	C/CGG	5 (e)	7 (e)	—	—
*Hin*H (*Haemophilus influenzae H-1*)	PuGCGCPy	8 (e)	3 (e)	—	—
*Hin*f (*Haemophilus influenzae* serotype f)	G/ANTC	16	17	13	15
*Hha*I (*Haemophilus haemolyticus*)	GCG/C	14 (h)	19	12	16
*Hph*I (*Haemophilus parahaemolyticus*)	GGTGA[b]	6	7	10	11
*Hga*I (*Haemophilus gallinarum*)	—	14	15	14	11
*Alu*I (*Arthrobacter luteus*)	AG/CT	23 (g), 24	25	10	20
*Mbo*I (*Moraxella bovis*)	/GATC	0	1	2	4
*Mbo*II (*Moraxella bovis*)	GAAGA[b]	11	17	18	18
*Eco*RI (*Escherichia coli* resistance transfer factor 1)	G/AATTC	0 (i)	0 (i)	1 (i)	4
*Pst*I (*Providencia stuartii* 164)	CTGCA/G	1	1	1	0
*Ava*I (*Anabaena variabilis*)	CGPu/PyCG	1	1	0	0
*Bgl*I (*Bacillus globiggi*)	—	0	0	2	1
*Kpn*I (*Klebsiella pneumoniae* OK 8)	—	0	0	1	1

[a] References: (a) Edgell *et al.* (1972), (b) Middleton *et al.* (1972), (c) Johnson *et al.* (1973), (d) Lee and Sinsheimer (1974*a*), (e) Hayashi and Hayashi (1974), (f) Godson and Boyer (1974), (g) Vereijken *et al.* (1975), (h) Blakesley and Wells (1975), (i) Godson (1975*a*), (j) Grosveld *et al.* (1976). If no reference is given, the number of cleavages is taken from Godson and Roberts (1976). These workers screened 26 restriction enzymes. (In a few cases, the number of cleavages may be underestimated.) In some of the *Hin*dII preparations, *Hin*dIII was also present but there appear to be no sites in φX174 sensitive to this enzyme. Johnson *et al.* (1973) and Lee and Sinsheimer (1974*a*) used an enzyme preparation from *H. parainfluenzae* that probably contained both *Hpa*I and *Hpa*II activities.

[b] Cleavage is some 8 nucleotides away. See Roberts (1976) for other references and additional details.

此为试读,需要完整PDF请访问: www.ertongbook.com