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A restriction cleavage map of φX 174 DNA by pulse-chase labelling using E.coli DNA polymerase.

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ABSTRACT

A pulse chase labelling technique using E. coli DNA polymerase I has been used to determine a number of restriction endonuclease cleavage maps of ØX 174 DNA. In addition to verifying the accuracy of the method by confirming some previously published maps, the Hha I and Alu I maps have also been constructed.

INTRODUCTION

Bacteriophage ØX 174 is a small icosahedral virus with a single-stranded circular DNA genome of about 5400 bases. Two forms of double-stranded molecules may be isolated during replication of the DNA: form 1 (RF I) which is a covalently closed superhelical circle, and form 2 (RF II) which contains a single nick in the viral ('plus') strand.

The use of restriction endonucleases to generate small, specific fragments (50-100 base pairs) from ØX 174 RF DNA, and the construction of restriction fragment maps potentially allows the precise location of genetic functions within the genome. Several genes have been located on specific Hind II fragments of ØX 174 RF DNA using a spheroplast assay (1) which also allowed the construction of a partial Hind II map. A similar method led to the characterisation of some of the Hae III digestion products (2), and a complete map of the Hap II fragments(3). RNA polymerase binding sites have been identified in the overlaps of Hind II fragment 2 and Hae III fragment 1, Hind II fragment 6 and Hae III fragment 3, and within Hind II fragment 4 (4). Also, the site for the origin of minus-strand synthesis in the formation of RF DNA and the site for initiation of plus-strand replication are situated in Hind II fragment 3 (5). As further restriction endonucleases become available and more maps are constructed, these and other functions may be further pinpointed. In addition, the use of accurately located restriction fragments as primers for the synthesis of

^{32}P-labelled DNA by E. coli DNA polymerase I (6) allows the nucleotide sequences of specific regions of the DNA to be deduced, possibly leading to a greater understanding of the functioning of the genome.

Current ways of constructing restriction fragment maps are broadly divided into two groups. For the mapping of a few large fragments (e.g. those generated by Eco R1) methods such as genetic deletion mapping (7) or direct visualisation in the electron microscope (8) are applicable, whereas for smaller fragments (e.g. those generated by Hind II) other methods must be resorted to. To date, the most common method of constructing an initial restriction map has been by the analysis of partial restriction enzyme digestion products (9,10,11). Subsequently, maps of different restriction endonuclease fragments may be deduced by digesting fragments from the first restriction map with the second restriction enzyme, and vice versa (reciprocal digestion - 11,12). The method described here approaches the problem differently, using the minus-strand of each restriction fragment from a complete digest as a primer for DNA polymerase, and extending it on a template of whole virus-strand. After a short radioactive 'pulse', the label is diluted out and synthesis allowed to continue, at least past the next restriction site. After subsequent cleavage of the extended DNA by the same restriction endonuclease and separation of the products by gel electrophoresis, the fragment adjacent to the 3'-end of the primer strand is radioactively labelled at high activity, and can be detected by autoradiography. To avoid degradation of the labelled DNA the Klenow modification of E. coli DNA polymerase I lacking the 5'-exonucleolytic activity has been employed.

We have verified the fidelity of the technique by confirming previously published maps of ØX 174 (11) and in addition have constructed the Hha I and Alu I maps. By a slight modification, the method has been applied to mapping double-stranded DNA genomes (22).

MATERIALS AND METHODS

ØX 174 virus DNA and RF double-stranded DNA were gifts of Dr. J. Sedat.

Endonuclease Hind (II and III) was prepared according to Smith and Wilcox (13) (from Haemophilus influenzae strain R_d) and Hae III (from H. aegyptius) by a slight modification of this procedure.

Alu I (Arthobacter luteus) was a gift of Dr. W. Barnes. Hap II (H. aphrophilus) and Hha I (H. haemolyticus) were gifts of Dr. R. Roberts. Hinf I and Hae II were gifts of Dr. N.L. Brown.

E. coli DNA polymerase I (5000 units/mg) and DNA polymerase I (nach Klenow - 5000 units/mg) were purchased from Boehringer.

Unlabelled deoxyribonucleoside triphosphates were purchased from Sigma and α-labelled ^{32}P-deoxyribonucleoside triphosphates (100 Ci/mmole) from New England Nuclear Corp.

A. Gel Electrophoresis

Preparative fractionation of ØX 174 RF DNA restriction fragments and analytical separations were performed on polyacrylamide linear concentration gradient slab gels (either 'long gels': 40 cm x 20 cm x 0.2 cm, or 'short gels': 16 cm x 20 cm x 0.3 cm) prepared as described previously (14). The range of the concentration gradient used for optimum separation of a particular restriction enzyme digest was determined by trial, and is given at the appropriate point in the text.

B. Preparation of Restriction Fragments

ØX 174 RF DNA (approx. 0.1 mg/ml) was incubated at 37° with sufficient of the appropriate restriction endonuclease to give complete digestion in 2-4 h, in the following buffer: 10 mM Tris-HCl, pH 7.4; 5 mM $MgCl_2$; 5 mM β-mercaptoethanol; 50 mM NaCl. After incubation the reaction mix was extracted twice with redistilled phenol, saturated with 10 mM Tris-HCl, pH 7.4, 1 mM EDTA, and the residual phenol removed by three ether washes. Finally, the fragmented DNA was precipitated at -20° for 24 h after the addition of sodium chloride to 0.5 M and 2½ volumes of 95% ethanol. The precipitate was collected by centrifugation, washing the pellet with ethanol and finally drying in vacuo.

For electrophoresis, the dried pellet of DNA was dissolved in electrophoresis loading buffer and applied to the gel for separation of the fragments (14). After electrophoresis, the restriction fragments were located by staining and eluted electrophoretically (7).

C. Primed Synthesis Reaction

Primer/template heteroduplexes were prepared as follows: sealed glass capillary tubes containing 50 µl of 20 mM Tris-HCl, pH 7.4, 1-2 µg of ØX 174 DNA and approximately one half molar equivalent of the restriction fragment to be used as primer were heated to 100° for 5 min in a boiling water bath, to separate the strands of the restriction fragment. The tubes were then cooled quickly to 0°, opened, and KCl added to a final concentration of 0.12 M, after which the tubes were resealed and incubated at 65°

for 2 h, to allow hybridisation to occur. The result is a mixture of re-annealed restriction fragments, and heteroduplexes formed between ØX 174 DNA and the minus-strand of the fragment.

For the DNA polymerase reaction, 10 µl of the heteroduplex was mixed with 2 µl of 60 mM $MgCl_2$; 60 mM β-mercaptoethanol and 1 µl (1 unit) of DNA polymerase I (nach Klenow), and the mixture allowed to equilibrate at room temperature for 15 min to allow binding of DNA polymerase to the heteroduplex. The mixture was then cooled to 0° for 15 min and the reaction started (time zero) by addition of 10 µl of a solution containing three of the four deoxyribonucleoside triphosphates at 0.25 mM, and the fourth deoxyribonucleoside triphosphate radioactively labelled in the α-position with ^{32}P (100 Ci/mmole) at 2.5 µM (also cooled to 0°). After 10 min at 0° the ^{32}P-label was diluted out by addition of 2 µl of a 1 mM solution of the corresponding unlabelled deoxyribonucleoside triphosphate, and the reaction tube moved to room temperature (22°) for a further 20 min. At this time a 5 µl aliquot was removed for estimation of the radioactive incorporation by TCA precipitation. Further reaction in the tube was prevented by the addition of 0.2 ml of 50 mM Tris-HCl, pH 7.4, containing 5 mM EDTA and 5 µg/ml of carrier ØX 174 RF DNA.

D. Analysis of ^{32}P-labelled Product

The contents of the reaction tube were freed of residual DNA polymerase activity by extracting once with redistilled phenol, and the phenol removed by washing three times with ether. The α-^{32}P-deoxyribonucleoside triphosphate was then removed from the product in one of two ways:

(a) The solution was made 0.5 M in NaCl and then precipitated for 24 h at -20° by addition of 2½ volumes of 95% ethanol. This treatment left most of the unincorporated ^{32}P-label in solution.

(b) The solution was passed through a 1 ml bed volume column containing Biogel agarose A 0.5 M. The first radioactive peak in the eluant contained the extended heteroduplex which was collected and dried down *in vacuo*. The α-^{32}P-deoxyribonucleoside triphosphate was eluted as a second distinct peak (6).

After purification, the extended DNA was digested to completion with the same restriction endonuclease used to produce the primer fragment, and the resulting products separated by gel electrophoresis. The location of radioactively labelled bands was determined by autoradiography. After autoradiography the fragments produced by digestion of the carrier ØX 174

RF DNA could be located by staining with methylene blue, and by comparison, the radioactive bands identified. In later experiments, a restriction endonuclease digest of ØX 174 RF DNA, labelled by nick-translation (15), was run in parallel to provide markers (see below).

If it was required to compare the radioactivities in gel bands, the band of stained carrier DNA was excised from the gel, and the ^{32}P estimated by detection of emitted Cerenkov radiation in the tritium channel of a liquid scintillation counter.

E. Labelling of ØX 174 RF DNA by Nick-translation

A preparation of DNA polymerase I containing a trace impurity of endonuclease activity was found useful in obtaining uniformly labelled ØX 174 RF DNA. Nicks introduced at random into the DNA by the endonuclease are 'translated' along the DNA strand by the combined polymerase and 5'-exonuclease activities of the DNA polymerase, with consequent labelling of the DNA. 2 μg of ØX 174 RF DNA and 1 μl of the DNA polymerase I preparation (2.5 units) were added to 100 μl of 50 mM Tris-HCl, pH 7.8, 5 mM $MgCl_2$ and 10 mM β-mercaptoethanol, containing three deoxyribonucleoside triphosphates each at 2 μM, and the fourth deoxyribonucleoside triphosphate α-^{32}P-labelled at 1 μM. The mixture was incubated at 22° for 1 h, after which enzyme activity was abolished by heating at 70° for 15 min. 10 μl of this solution, after incubation with the appropriate restriction endonuclease, was electrophoresed in parallel on the gel with the digested primed-synthesis material to produce a set of radioactive fragment markers.

RESULTS AND DISCUSSION

Preliminary experiments (not described here) were performed to obtain conditions that would give a suitable rate of elongation of the primer DNA strand during the labelling stage of the synthesis reaction ('pulse'). Under the conditions detailed in the previous section, the approximate rate of strand elongation during this period was found to be 10 bases/min. At the higher temperature and increased concentration of the fourth deoxyribonucleoside triphosphate used in the second stage of the reaction ('chase'), strand completion should occur within 15-20 min (but see Discussion below).

Hind II

The restriction enzyme Hind II cleaves ØX 174 RF DNA into 13 fragments (Table I) (1). There are no Hind III sites (16) and so Hind II activity was

TABLE I. Estimated sizes of ØX 174 RF DNA fragments produced by restriction endonuclease cleavage

Sizes of the Hind II fragments have been estimated by comparing gel mobilities with markers of known size, and by direct sequencing of fragments 9 and 10 (21). Fragment sizes produced by other restriction endonucleases have been estimated by comparison with the Hind II fragments.

Hind II		Hae III		Alu I		Hha I		Hap II	
Fragment No	Size Base Pairs	Fragment No	Size Base Pairs	Fragment No	Size Base Pairs	Fragment No	Size Base Pairs	Fragment No	Size Base Pairs
1	1000	1	1300	1	1100	1	1630	1	2850
2	760	2	1100	2	840	2	640	2	1650
3	670	3	870	3	690	3	615	3	395
4	510	4	610	4	380	4	540	4	360
5	400	5	320	5	350	5	315	5	230
6a	350	6a	300	6	270	6	310		
6b	345	6b	285	7a	255	7	285		
6c	340	7	230	7b	250	8a & b	200		
7a	300	8	190	7c	240	9a & b	135		
7b	290	9	115	8	200	10	123		
8	205	10	73	9	140	11	100		
9	162			10	115	12	95		
10	79			11	105	13	85		
				12a	86	14	54		
				12b	84				
				12c	81				
				12d	76				
				13	58				
				14	47				
				15a & b	33				
				16	25				
				17	20				
TOTALS	5411		5393		5478		5467		5485

not removed from the Hind II preparation. The fragments are defined here by their relative mobilities when electrophoresed on a 10-20% polyacrylamide concentration gradient gel, fragment 1 being nearest, fragment 10 farthest from the origin. The three bands at position 6 are named 6a, 6b and 6c in that order from the origin; 7a and b correspond to the doublet at position 7.

Figure 1 shows an autoradiograph of the gel defining the order of Hind II restriction fragments in ØX 174 RF DNA. The first column is the result of a control incorporation containing ØX 174 DNA, but no restriction fragment primer. There is a faint background, with some bands running in the positions of known Hind II restriction fragments, and other bands in unknown positions. What causes this background initiation of synthesis is not clear, but in most cases it is too low to interfere with the assignment of neighbours in the primed reactions. The next column shows the product when Hind II fragment 1 is extended: it is clear that the adjacent fragment is one of the three running close together at position 6. Although these bands are not

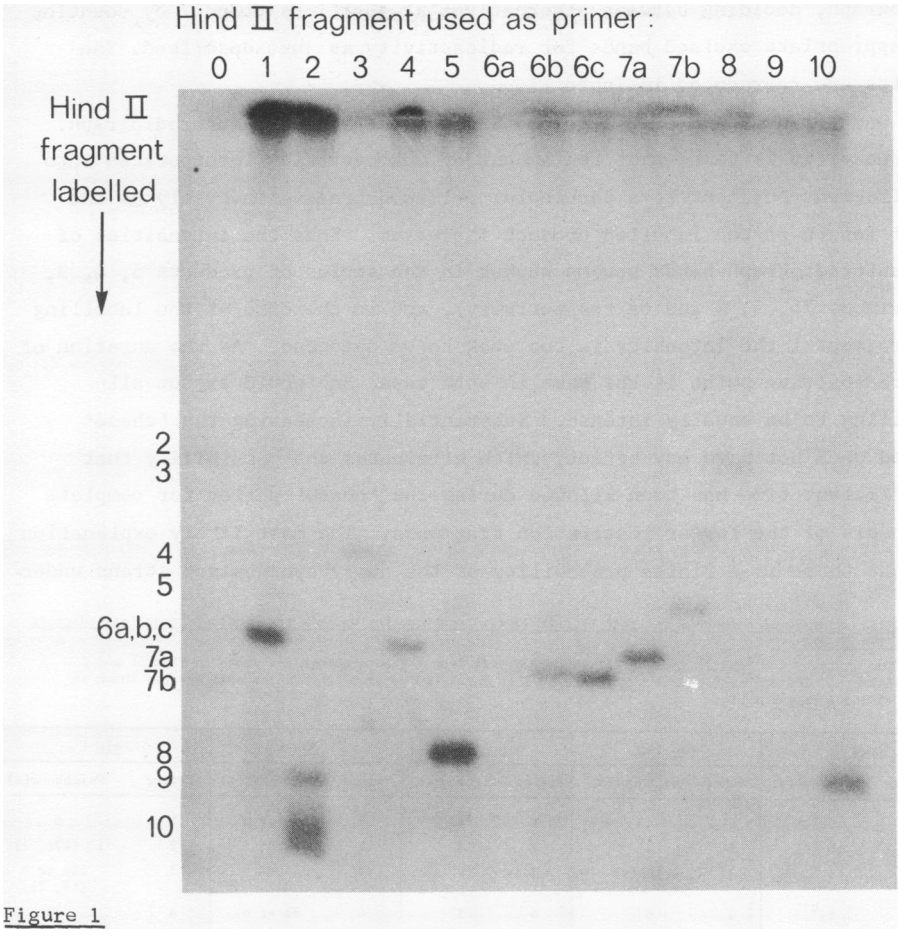

Figure 1

Neighbour analysis of Hind II restriction fragments from ØX 174 RF DNA was performed as described in Materials and Methods. The digested DNA was electrophoresed on a short 10-20% polyacrylamide linear concentration gradient gel, which was then autoradiographed. The column headed '0' is the result of a control experiment using ØX 174 viral DNA, but no restriction fragment primer.

resolved sufficiently on the autoradiograph, they are readily distinguished after staining the bands produced by digestion of the carrier ØX 174 RF DNA. Therefore, after autoradiography, the gel was stained with methylene blue, and the bands at position 6 in the second column were excised and the radioactivity in each measured. The labelled fragment was found to be 6b, and this is therefore adjacent to the primer, fragment 1. The neighbours of each fragment used in turn as primer were similarly assigned from the gel auto-

radiograph, deciding between alternatives at positions 6 and 7 by counting the appropriate excised bands for radioactivity as just described. The results are reproduced in Table II.

One disadvantage of the method is apparent from the autoradiograph. In general it is found that the amount of radioactivity incorporated into the fragment adjacent to a particular primer decreases inversely as the total length of the labelled product increases. Thus the intensities of the autoradiograph bands become weaker in the series of products 5, 4, 3, 2 (primers 7b, 3, 8 and 6a respectively), and in the case of the labelling of fragment 1 the intensity is too weak to be detected. As the duration of the radioactive pulse is the same in each case, one would expect all labelling to be equally intense. Substantially increasing the 'chase' period does not have any effect, which eliminates the possibility that insufficient time has been allowed during the 'chase' period for complete synthesis of the larger restriction fragments. The most likely explanation is that there is a finite probability of the newly synthesised strand under-

TABLE II. Summary of neighbour analyses by pulse-chase technique for various restriction endonuclease digests of ØX 174 RF DNA

The analyses for Hind II and Hae III can be derived from the autoradiographs shown in Figs. 1 and 2 respectively. The information for Hha I and Alu I is partially derived from the autoradiographs shown in Figs. 3 and 4 respectively.

Hind II		Hae III		Hap II		Hha I		Alu I	
Primer	Neighbour(s)	Primer	Neighbour(s)[a]	Primer	Neighbour(s)	Primer	Neighbour(s)	Primer	Neighbour(s)
1	6b[b]	1	4	1	4	1	9a or b	1	6
2	10 (9)	2	-[c]	2	3	2	3	2	13 (12c, 1)
3	4	3	10 (9)	3	-[c]	3	6	3	15a or b (16, 2)
4	6a[b]	4	8	4	5	4	8a or b	4	7a
5	8	5	7	5	-[c]	5	7	5	12a or b
6a	2	6a	6b			6	8a or b	6	5
6b	7a[b]	6b	-[c]			7	10	7a	7b
6c	7b[b]	7	3			8a & 8b	9a or b	7b	14
7a	6c[b]	8	5			9a & 9b	2, 5	7c	12d
7b	5	9	6a			10	12	8	7c
8	3	10	9			11	13	9	3
9	-[c]					12	14	10	11
10	9					13	4	11	4
						14	11	12a & 12b	-[d]
								12c	1
								12d	9
								13	12c
								14	9
								15a & 15b	Not attempted
								16	
								17	

[a] Labelling of primer itself (see text) has not been included in the Table.
[b] Distinguished by excising carrier bands and determining radioactivity.
[c] No band significantly labelled.
[d] Several bands, including both primers, labelled.

going premature termination, which would mean the longer restriction fragments are less likely to be synthesised to completion. Some improvement can be obtained by increasing the 'pulse' time, where all bands are proportionately more intense, but this causes difficulties with the smaller fragments, when more than one may become labelled. We have not yet succeeded in resolving this problem, which remains the major drawback when applying the method to ordering very large restriction products, e.g. those produced by Eco R1 or Hind III. In the results described here the limit seems to be about 1000 bases.

In Fig. 1, although Hind II fragment 1 is not found as a labelled product of any of the primed reactions, by a process of elimination it must be adjacent to fragment 9 which, when used as a primer does not lead to any band being significantly labelled above background. Thus the entire sequence of Hind II restriction fragments can be deduced, and is shown in Fig. 5. In Fig. 1 both bands 9 and 10 are labelled when fragment 2 is used as primer. The reason for this is clear from the final order of fragments, where 9 follows 10, and thus represents continuation of synthesis through the junction between 9 and 10 before the label is diluted out. As fragment 10 is 79 bases long (Table I) this confirms the approximate rate of synthesis by DNA polymerase I under the conditions used as approximately 100 bases/10 min 'pulse' period.

The order of fragments deduced here is the same as that obtained by Lee and Sinsheimer (11) from analysis of partial digestion products, with the additional placement of fragment 10. However, it should be noted that the fragments we have called 6a, 6b and 6c correspond to Lee and Sinsheimer's fragments 6.1, 6.3 and 6.2 respectively. The differences in relative mobilities of these fragments when run on different gel electrophoresis systems is not unprecedented (7). Molecular weight is clearly not the only factor determining mobility of double-stranded DNA on polyacrylamide gels.

Hae III

Digestion of ØX 174 RF DNA by restriction endonuclease Hae III leads to eleven products (Table I) (2), which can be separated by electrophoresis on a 3.5-7.5% polyacrylamide concentration gradient gel. Again, they are defined here by their mobility on the gel, the double band at position 6 being sub-classified as 6a and 6b. Figure 2 shows the pattern of labelled bands obtained when Hae III restriction fragments are used as primers, and

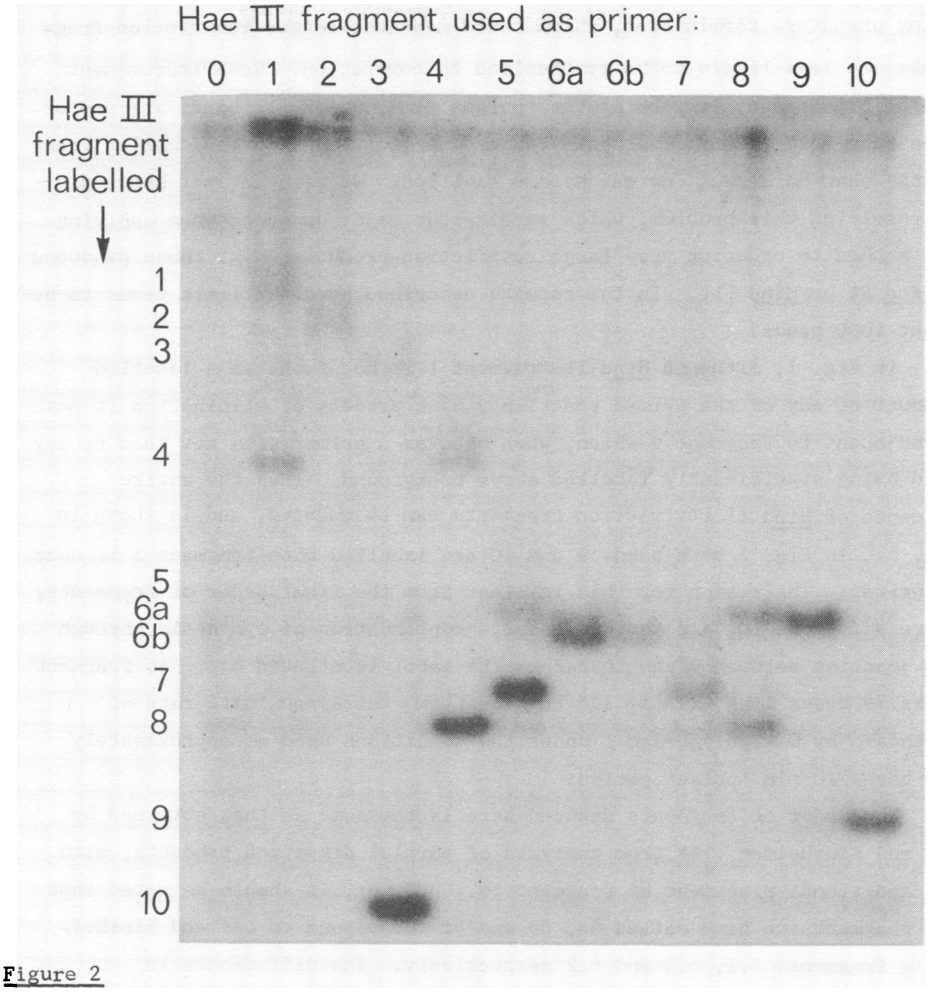

Figure 2

Neighbour analysis of Hae III restriction fragments from ØX 174 RF DNA. The digested DNA was electrophoresed on a short 3.5-7.5% polyacrylamide linear concentration gradient gel, which was then autoradiographed. Column '0' is a control experiment containing no added primer.

Hae III is used to cleave the synthesised product. Column 1 is the control, with no added primer. Column 2 was primed by Hae III fragment 1. Two bands are labelled above background, namely 1 and 4. In this particular experiment, in each column there are two labelled products - the fragment used to prime the synthesis faintly labelled in addition to the main product. This is presumably because of exonuclease contamination in the Hae III used to prepare the primer fragments, causing slight degradation at their 3'-ends.

Thus on priming, some radioactivity is incorporated into the newly synthesised product before the first restriction site, leading to the priming fragment itself becoming labelled, as observed. However, this does not obscure the primer-neighbour relationship and, for example, the fragment adjacent to 1 is clearly 4 (second column, Fig. 2). The other neighbours are similarly defined, and are listed in Table II. As observed in the case of Hind II, the intensity of labelling again decreases as the size of the products increases, and here neither fragment 1 nor fragment 2 appear as labelled products, and fragment 3 is only just labelled above background when primed by fragment 7. Nevertheless, because of the circular nature of ØX 174 DNA, there is sufficient information to define the complete fragment order (Fig. 5). To agree with the previously published map (11) the relative electrophoretic mobilities of the doublet at position 6 are again apparently reversed by the gel system described here.

Hap II

Restriction endonuclease Hap II cleaves ØX 174 RF DNA at five sites (Table I) (3), the resultant five fragments being resolved on a 3.5-7.5% polyacrylamide concentration gradient gel. The three smaller fragments were labelled by the 'pulse-chase' method (Table II) but fragments 1 and 2 were found to be too large to show a significant incorporation. However, there is sufficient information to order the fragments (Fig. 5) and this order was verified by reciprocal digestion, against Hind II and Hae III (data not included).

Hpa II and Hap II recognise the same restriction site (C-C-G-G) (17, 18) and the Hpa I cleavage site (G-T-T-A-A-C) (17) is a subset of the Hind II recognition sites (G-T-Py-Pu-A-C) (19). Thus by comparing the combined Hpa (I and II) map (11) and the Hind II map, the Hap II map may also be deduced, and when this is done the result agrees with the direct determination described in this paper. A map with fragments 4 and 5 inverted has also been published (3).

Hha I

Digestion of ØX 174 RF DNA with Hha I generates 16 fragments (Table I) which may be fractionated on a 4-12% polyacrylamide concentration gradient gel (see Fig. 3). Apart from the two poorly resolved doublets at positions 8 and 9, each fragment may have its neighbour unambiguously assigned by the 'pulse-chase' technique. The results of priming with fragments 8-14 are

shown in Fig. 3. The unresolved doublet at position 8 when used as primer gives only one major product, at position 9, and some faint bands in positions not corresponding to any of the complete digestion products, and presumed to be products of incomplete digestion. The absent major product

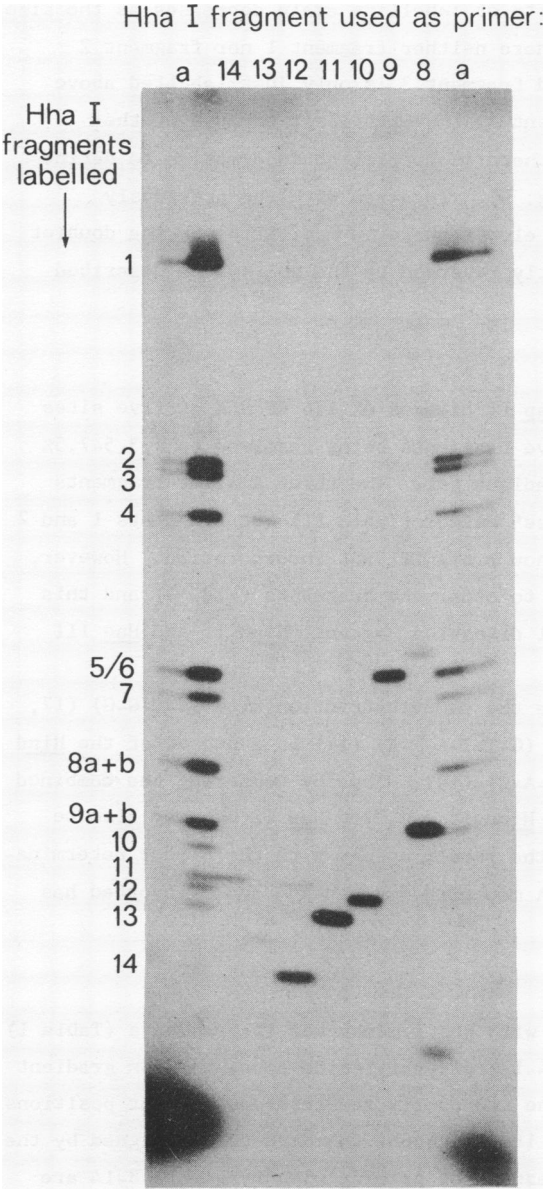

Figure 3

Neighbour analysis of Hha I restriction fragments 8-14 from ØX 174 RF DNA on a long 4-12% polyacrylamide linear gradient gel. Columns headed (a) contain restriction fragments produced by Hha I digestion of 'nick-translated' ØX 174 RF DNA.

was thought to be fragment 1, which would not be detected because of its size (Table I) and this was indeed verified by the subsequent data. Priming with the two unresolved fragments from position 9 gave rise to two labelled products, fragments 2 and 5. The relative radioactive intensities of the two products are consistent with their sizes, and the general size effect described earlier.

When using fragment 12 as primer, its neighbour, fragment 14, is completely synthesised during the 'pulse' and synthesis continues into fragment 11 before the label is diluted out, thus leading to labelling of both fragments.

The neighbours of fragments 1-7 were determined in an identical manner, and the combined results are shown in Table II. Because of the unresolved doublets at positions 8 and 9, it is not possible to distinguish between two possible physical orders for the fragments from this data alone. The extra information required to resolve the ambiguity was obtained by digesting some purified ^{32}P-labelled Hha I fragments with Hind II and Hae III. The results obtained, listed in Table III, allow the Hha I fragments to be aligned with respect to the Hind II and Hae III maps, thereby completing the ordering of the Hha I fragments. Although the doublets at positions 8 and 9 are incompletely resolved, it is possible to divide both doublets into the band fronts, being enriched in the faster migrating components (fragments 8b and 9b) and the band tails, likewise enriched in the more slowly migrating components (fragments 8a and 9a). When this was done, and the extracted DNA digested by Hind II, it was clear that fragments 8b and 9b were cleaved, and 8a and 9a were unaffected. The Hha I map is therefore completely defined, and shown in Fig. 5. Closer alignment of the Hha I and Hind II maps is possible because Hha fragment 10 is part of a sequenced region of the ØX 174 genome, contained in the Hind II fragment 6b (20).

Alu I

Twenty-three fragments are produced by Alu I cleavage of ØX 174 RF DNA (Table I and ref. 12). When separated on a polyacrylamide concentration gradient gel a complex band pattern is obtained (see Fig. 4) including a triplet at position 7, a quadruplet at position 12 and a doublet at position 15. All except bands 15a and 15b can be resolved on the gel.

Fragments 1-14 were used as primers for the 'pulse-chase' synthesis reaction, and Fig. 4 shows an autoradiograph of the gel on which were run the Alu I cleaved products of priming with fragments 1-7. A longer 'pulse'

TABLE III. Redigestion of the Hha I fragments by Hind II and Hae III

Nick-translated ØX 174 RF DNA was digested with Hha and the fragments separated on a 5% acrylamide gel, eluted and recleaved with Hind II or Hae III.

Hha fragment	Size	Products with Hind II	Product size	Products with Hae III	Product size
1	1630	R 3	670	Z 6.1	290
		R 8	205	Z 6.2	285
		ΔR 4	470	Z 9	115
		ΔR 5	290	Z10	73
			1635	ΔZ 2	500
				ΔZ 3	400
					1663
2	640	ΔR 2	390	ΔZ 2	520
		ΔR 6a	250	ΔZ 1	140
			640		660
3	615	R10	79	Uncleaved	
		ΔR 2	390		
		ΔR 9	150		
			619		
4	540	R 6c	340	Z 7	230
		ΔR 7b	165	ΔZ 3	280
			505		510
5	315	-[a]	-	Uncleaved	
6	310	ΔR 1	300	-	
7	285	ΔR 6b	135	ΔZ 4	260
		ΔR 1	120		
			255		
8a	200	Uncleaved		-	
8b	200	ΔR 7b	125	-	
		ΔR 5	70		
			195		
9a	135	Uncleaved		ΔZ 1	90
				ΔZ 4	50
					140
9b	135	ΔR 6a	80	Uncleaved	
		ΔR 4	60		
			140		
10	123	-		-	
11	100	-		-	
12	95	ΔR 6b	75	ΔZ 5	50
				ΔZ 8	40
					90
13	85	-		-	
14	54	-		-	

[a] These fragments were not tested.

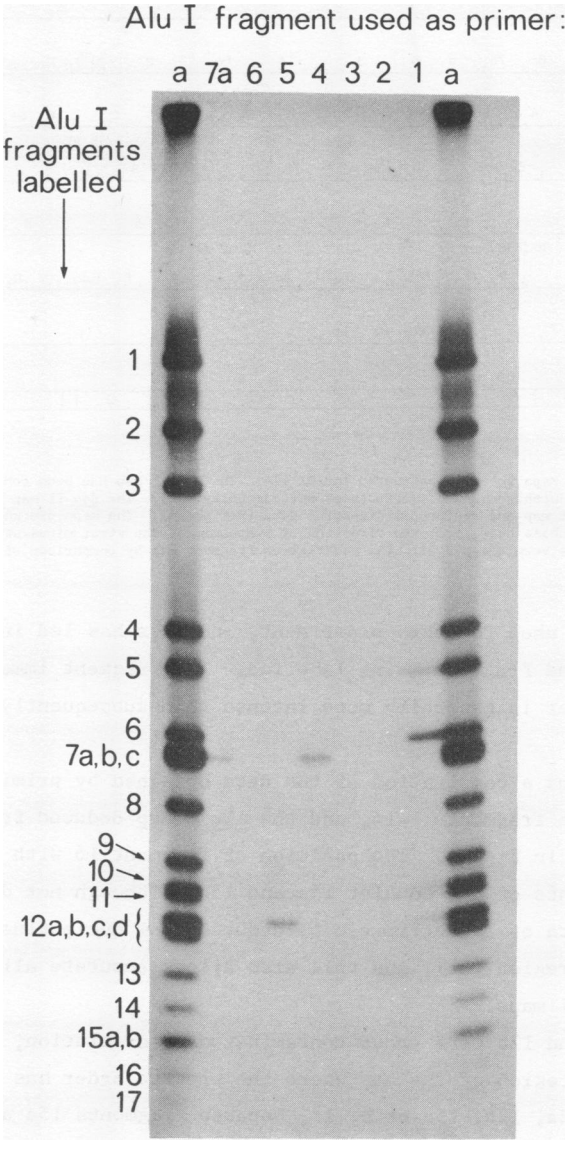

Figure 4

Neighbour analysis of Alu I restriction fragments 1-7a from ØX 174 RF DNA on a long 4-12% polyacrylamide linear gradient gel. Columns headed (a) contain marker fragments generated by digestion of 'nick-translated' ØX 174 RF DNA with Alu I.

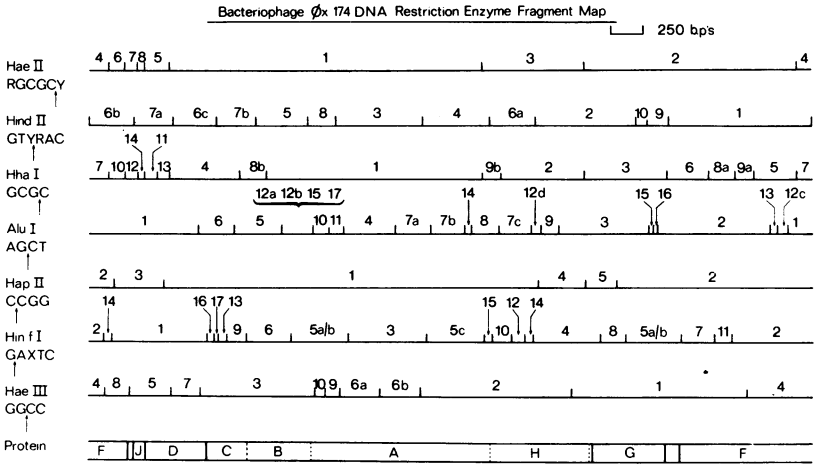

Figure 5

Combined restriction cleavage maps so far constructed for ØX 174. The Hinf I map has been constructed by B.G. Barrell, G.M. Air, C.A. Hutchison and D. Jaffe (personal communication). The Hae II map was constructed from comparison with the Hha I map and reciprocal digestion data (not shown). The maps are shown in the figure in the polarity of the pulse-chase data, i.e. the direction of synthesis of the viral minus-strand is from left to right. The genetic map has been aligned with the restriction fragment map by comparison of known amino acid and DNA sequences (21).

period of 20 min was used for this experiment, and this has led in several cases to more than one fragment being labelled. The fragment immediately adjacent to the primer is generally more intense than subsequently labelled products.

Table II contains a compilation of the data obtained by priming in turn with each of the fragments 1-14, and the Alu I map deduced from this information is shown in Fig. 5. The position of fragment 16 with respect to one of the fragments of the doublet 15a and 15b, although not defined by the 'pulse-chase' data of Table II, can be deduced from nucleotide sequence information in this region (21), and this also allows accurate alignment of the Alu I and Hind II maps.

Fragments 12a and 12b were cross-contaminated after elution, and this has resulted in one region of the map where the precise order has not been determined, namely 12a, 12b, 15a or b, 17, because fragments 15a and 15b and 17 have not been used as primers. This uncertainty is as yet unresolved.

By confirming restriction cleavage maps of ØX 174 DNA previously determined by other means, we have verified the accuracy of 'pulse-chase' labelling using E. coli DNA polymerase for the construction of further maps, such as Alu I and Hha I. The procedure is ideally suited to small and

medium sized fragments (up to approximately 1000 bases) and the final fragment order can often be deduced from a single set of experiments.

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