Biochem. J. (1977) **163**, 503–509
Printed in Great Britain

A Map of the Cleavage Sites for Endonuclease *Aval* in the Chromosome of Bacteriophage Lambda

By STEPHEN G. HUGHES
Department of Molecular Biology, University of Edinburgh, Edinburgh EH9 3JR, Scotland, U.K.

(*Received* 28 *January* 1977)

The linear order of nine fragments generated by the action of endonuclease *Aval* on the DNA of bacteriophage λ was determined from the altered fragmentation patterns of bacteriophages containing known deletions and of hybrids of bacteriophages λ and $\phi 80$. Digestion of 5′-terminally ^{32}P-labelled bacteriophage-λ DNA was used to identify the terminal fragments. Measurement of relative fragment lengths permitted rough mapping of the endonuclease-*Aval* cleavage sites relative to the ends of the bacteriophage-λ chromosome. The fragment order was confirmed and the map refined by analysis of the fragmentation of derivative phages containing single cleavage sites for endonuclease *Eco*RI.

The chromosome of bacteriophage λ is a single DNA molecule of 30.8×10^6 daltons (Davidson & Szybalski, 1971). Bacteriophage λ has been the object of great attention by both geneticists and biochemists studying the molecular basis of complex regulatory systems, and its chromosome contains several regions, defined both genetically and physically, which are of special interest. The ability to isolate defined fragments of the bacteriophage λ chromosome promises to be useful in extending the study of such regions, and has been exploited in the elucidation of the nucleotide sequence of the operator and promoter regions (Maniatis *et al.*, 1975; Pirrotta, 1975). Sequence-specific endonucleases, including type-II restriction endonucleases (for review see Nathans & Smith, 1975), cut the DNA of bacteriophage λ to give discrete fragments, and the positions of the cuts have been determined for a number of the enzymes. Endonuclease *Eco*RI cuts bacteriophage λ DNA at five sites, which have been mapped accurately (Thomas & Davis, 1975), and endonuclease *Hin*dIII cuts at six sites, which also have been mapped (Murray & Murray, 1975). These restriction enzymes have also been used with specially constructed derivative chromosomes to construct bacteriophages carrying additional DNA from other prokaryotic and eukaryotic sources (Murray *et al.*, 1975). Information on the position of cleavage sites for additional site-specific endonucleases is of use not only for the isolation of defined fragments by using endonucleases, singly or in concert, but also in the analysis of newly constructed hybrid bacteriophage chromosomes and deletion mutants. In constructing a cleavage-site map of a viral chromosome or plasmid DNA molecule the deduction of a linear fragment order and a rough assignment of cleavage-site positions deduced from fragment sizes is now relatively easy. The assignment of accurate map positions is more complex, especially since different methods of establishing fragment sizes usually yield conflicting results (Murray & Murray, 1975; Helling *et al.*, 1974). Accurate mapping of cleavage sites based on fragment-length measurement has been achieved by using sophisticated electron-microscopic methods (Thomas & Davis, 1975). Such maps provide a reference point for those without access to such sophisticated techniques from which to produce a rough map that can then be refined by consideration of the positions of the individual sites with respect to neighbouring accurately located sites on the chromosome. The latter approach is adopted in this paper in constructing a map of the eight sites in bacteriophage-λ DNA cut by endonuclease *Aval* (see Murray *et al.*, 1976).

Materials and Methods

Conventions and nomenclature

The map of the wild-type bacteriophage-λ chromosome is presented in the vegetative linear form as drawn by Davidson & Szybalski (1971). Throughout the text 'left' and 'right' refer to this orientation. Map positions are expressed as percentage of the total map distance from left to right. Endonuclease-cleavage sites are numbered consecutively from the left (Arber & Linn, 1969) and given the prefix *savaI*, *srI* or *shindIII*. DNA fragments from digests of wild-type bacteriophage-λ DNA are lettered alphabetically in the upper case according to their size as reflected by their mobility in agarose gels, starting from the longest (i.e. slowest-moving). Lower-case letters are used for the labelling of gel bands before the determination of the identity of the fragments that they contain.

In the nomenclature of restriction enzymes the

convention of Smith & Nathans (1973) has been followed.

Strains

(*a*) *Bacteriophages.* Bacteriophage λ, quoted as wild-type, carried the heat-inducible mutation cI857 (Goldberg & Howe, 1969). Bacteriophages IV, VII, VIII, IX, X, XI and XII have been fully described by Murray & Murray (1974). Bacteriophage 29 contains the deletion *b*508 (Parkinson, 1971) and was provided by Dr. N. E. Murray. Bacteriophage 500 was described by Murray & Murray (1975) and the *trp*-transducing phage BG2 by Brammar *et al.* (1974). Bacteriophage λsk^0, a hybrid of bacteriophages λ and φ80, was described by Murray *et al.* (1973). Bacteriophages 501, 507 and 509 came from the collection of Dr. N. E. Murray, and had been constructed by normal genetic crosses: they contain the substitutions and deletions shown in Table 1.

(*b*) *Bacteria. Escherichia coli* W3350 was used for the propagation of bacteriophage and *E. coli* 803 SuIII (Wood, 1966) for the assay of bacteriophage stocks.

Preparation of bacteriophage DNA

Where possible, bacteriophages were propagated by growth and induction at 42°C of an *E. coli* lysogen.

Bacteriophages lacking the cI857 mutation or which were unable to lysogenize were propagated by infection of an exponentially growing culture as described by Murray & Murray (1975).

Bacteriophage lysates were purified and concentrated by pelleting at 45000*g*, followed by equilibrium centrifugation in 41.5% (w/w) CsCl (Kaiser & Hogness, 1960).

Uniformly ^{32}P-labelled bacteriophage-λ DNA was obtained from bacteriophage propagated in low-phosphate medium (Grossman, 1967) containing 0.05mCi of [^{32}P] orthophosphate/ml. DNA was isolated from bacteriophage as described previously (Murray *et al.*, 1976).

Enzymes and digestion

Endonuclease *Eco*RI was isolated by the method of Yoshimori (1971) and endonuclease *Hin*dIII as described by Old *et al.* (1975). Endonuclease *Ava*I was purified from *Anabaena variabilis* as described by Murray *et al.* (1976). Endonuclease digests were carried out in the presence of 5mM-$MgCl_2$ and 50mM-Tris/HCl, pH7.5, at 37°C for 40–60min, in a volume of 20–30μl (dependent on the concentration of the DNA preparation). The amount of each endonuclease required for complete digestion under these conditions of 1μg of bacteriophage-λ DNA was determined by a series of trial digestions with increasing amounts of enzyme. These were analysed by zone electrophoresis in agarose gels (see below). Analytical digests were done as a routine with 1–2μg of DNA unless stated otherwise, as in the legends to Figures. Polynucleotide kinase was prepared as described by Richardson (1965), as adapted in our laboratory (Murray, 1973; Hughes & Brown, 1973), and was used for labelling 5′-termini of DNA molecules with ^{32}P as described by Murray (1973).

Table 1. *Analysis of bacteriophage-λ chromosomes with deletions and substitutions*

DNA (2μg) of each of the bacteriophages below was digested with endonuclease *Ava*I and the digests were analysed in parallel on a 1% agarose gel. The extent and location of each deletion quoted and the structure of the chromosomes of the hybrid bacteriophages λsk^0 and λ*trp*BG2 are shown on Fig. 1. The isolated right arm of bacteriophage λ was recovered from an endonuclease *Eco*RI digest of bacteriophage 430 (Skalka, 1971) as described in detail by Murray & Murray (1974).

Bacteriophage	Deletion	Substitution	Endonuclease *Ava*I fragments with altered mobility
VII	*b*527		C
VIII	*b*519		C, H
IV	*b*538		C, E
500	*b*519*nin*R5		B, C, H
XII	*srI* 1–*srI* 2		C
501	*b*538	*imm*^{434}	C, D, E, G
507	*b*2	*imm*^{21}	C, D, H
λsk^0		Left arm of bacteriophage φ80	A, C, H
λ*trp*BG2		Left arm of bacteriophage φ80+*trp* operon of *E. coli*	A, C, E, F, H
509	*b*538	Small segment of bacteriophage φ80 chromosome in extreme right arm	C, E
Isolated right arm of bacteriophage λ (from bacteriophage 430)			Only fragments B, D and G present

Electrophoretic analysis of DNA digests

Digests of bacteriophage-λ DNA with various endonucleases and combinations of endonucleases were analysed by zone electrophoresis in gels of 1% agarose containing ethidium bromide (Sharp *et al.*, 1973), as described in detail by Thompson *et al.* (1974).

Gels were photographed on Ilford FP4 film by using a 4× red filter with the gel illuminated by short-wavelength u.v. light. For radioautography, gels were dried on glass plates in a hot-air oven at 70°C.

Results and Discussion

Identification of terminal fragments

An endonuclease *Ava*I digest of bacteriophage-λ DNA was divided and the two samples were analysed on adjacent tracks on a gel after one had been heated to separate the naturally cohesive 5′-ends of bacteriophage-λ DNA (Plate 1). Micro-densitometer traces of the gel photograph show that, after heating, band a* is lost and bands b and d have increased in intensity relative to the other bands and therefore contain the two terminal fragments. Moreover, band d now gives a broader peak than any of the other bands, suggesting that it contains more than one component. This analysis was confirmed by the finding that in digests of 5′-terminally ^{32}P-labelled DNA the label was located only in bands a*, b and d. At this point a provisional assignment of fragments to gel bands was made. Fragments D and D′ were assigned to band d, otherwise fragments A–H were assigned to bands a–h. Fragment D′ was assigned to the terminal fragment in the doublet band d. Fragment B therefore contains the other terminus.

Preliminary alignment of fragments

The linear order of the endonuclease *Ava*I fragments on the bacteriophage-λ chromosome was determined by considering the altered fragmentation patterns of the chromosomes of bacteriophages with known deletions, or hybrid bacteriophages carrying fractions of the bacteriophage-λ chromosome. This type of analysis is open to the objection that in the interpretation of band patterns on agarose gels it is possible to be misled into concluding that a particular fragment is unaltered by the presence of a deletion or substitution in a bacteriophage genome, whereas an unaltered band on the gel could equally well represent a new fragment resulting from the deletion. For this reason, information was collected from a number of overlapping deletions where available to give the preliminary alignment of fragments.

A comparison of bacteriophages VIII and 500 (Table 1 and Fig. 1) showed that the *nin*R5 deletion lies within endonuclease *Ava*I fragment B. Fragment B is therefore from the right-hand terminus, because deletion *nin*R5 lies near to the right-hand end of the bacteriophage-λ chromosome (Fig. 1), and fragment D′ is from the left-hand terminus.

Analyses of bacteriophages with deletions in the central region (Fig. 1 and Table 1) show that fragments C, E and H lie in this region. Since fragment H is absent only from deletion *b*519 (which is the one extending furthest left; see Fig. 1) and fragment C is altered by all the deletions in the central region, fragment C must lie to the right of fragment H. Similarly fragment E is altered only by deletions *b*538 and *b*508, which places it to the right of fragment C, giving the order HCE. The analysis of chromosomes of two hybrid bacteriophages carrying segments from only the right arm of bacteriophage λ assigns fragments to the right or to the left arm. Since bacteriophage λ*trp*BG2 has only fragments B, G and D or D′ in common with bacteriophage λ, and fragment D′ has already been assigned to the left-hand terminus, fragments B, G and D must come from the right arm. Fragment B contains the right-hand terminus; therefore the order of these three fragments is (G,D)-B. The hybrid bacteriophage λsk^0 contains a larger segment from the right arm of bacteriophage λ than does bacteriophage λ*trp*BG2 (Fig. 1; Brammar *et al.*, 1974), and in addition to fragments B, G and D it has fragments E and F in common with bacteriophage λ (Table 1). Fragments E and F therefore lie to the left of fragments G and D. Further, since fragment E is adjacent to and to the right of fragment C, it must lie to the left of fragment F, which must then have fragment G or D on its right. Only fragment A remains unaccounted for, and it cannot lie between them. The only remaining possibility requires the fragment order D′-A-H-C-E-F(D,G)B. Orientation of fragments D and G was shown to be D-G, as described below.

Location of cleavage sites

Relative lengths of fragments were estimated as fractions of the total length of the bacteriophage-λ chromosome by comparing the relative yield of ^{32}P in fragments isolated from a digest of uniformly ^{32}P-labelled bacteriophage-λ DNA (Table 2) with the sum of the yields of all the fragments. Fragment sizes were also estimated by comparing the electrophoretic mobility of individual endonuclease *Ava*I fragments on agarose gels with those of the six endonuclease *Eco*RI fragments whose fractional lengths are known (Thomas & Davis, 1975). These results are in Table 3. Both methods are open to error. In the first, random breakage of bacteriophage-λ DNA due to shear or ^{32}P decay is likely to produce less than stoicheiometric yields of the larger fragments, resulting in a cumulative error. In the second method, base composition rather than size alone may also affect mobility of DNA fragments in agarose gels. However, the two sets of results show reasonable correspondence for the

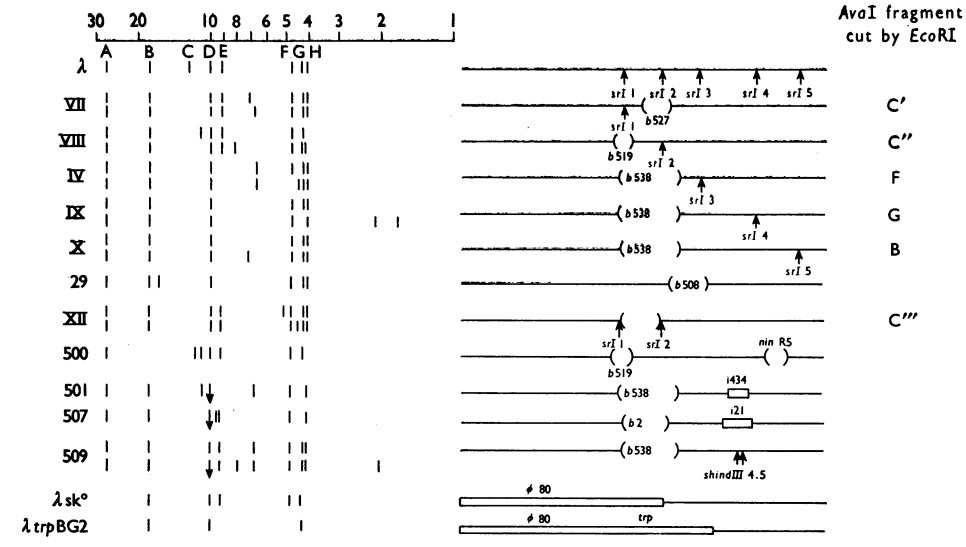
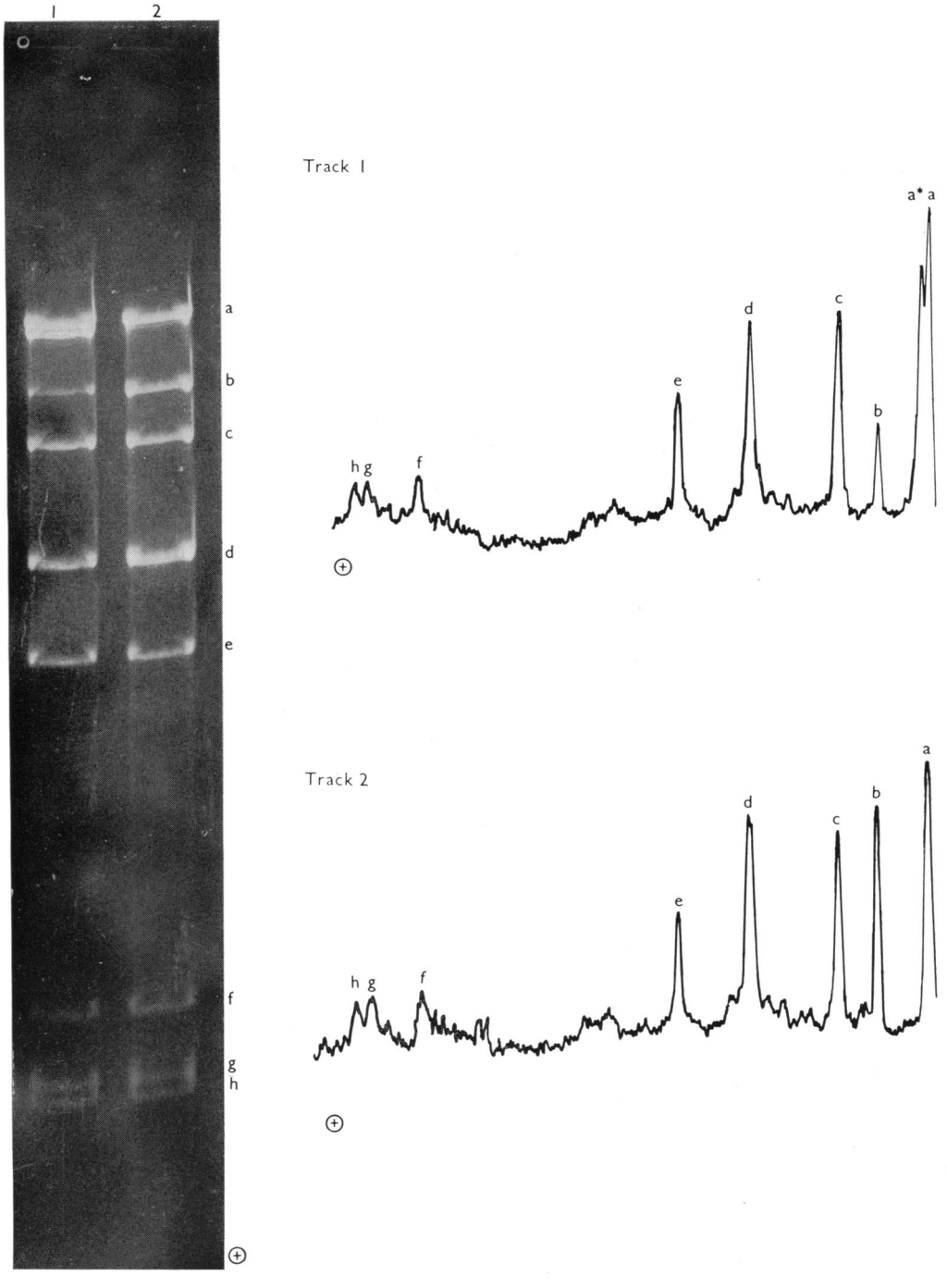


Fig. 1. *Schematic representation of the bacteriophages analysed in Table* 1 *and elsewhere in the text, and their fragmentation patterns*

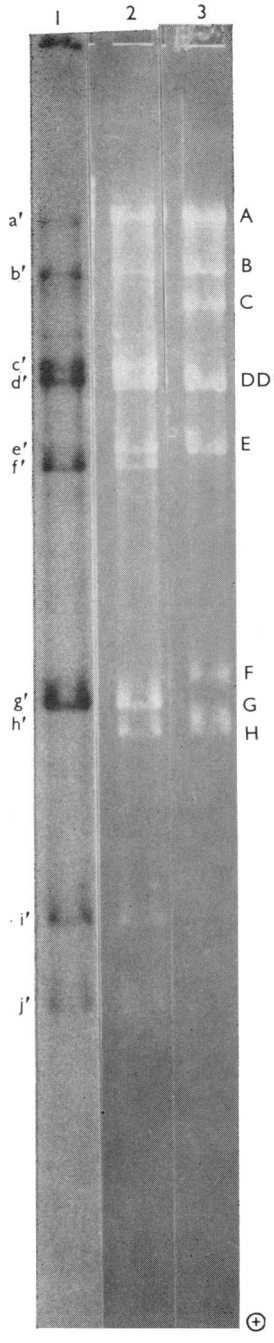
Where two such patterns are shown for a particular bacteriophage, the lower one represents an endonuclease *Eco*RI/endonuclease *Ava*I double digest or, for bacteriophage 509, an endonuclease *Hin*dIII/endonuclease *Ava*I double digest. For bacteriophages λsk^0 and λ*trp*BG2 only fragments common to bacteriophage λ and these bacteriophages are shown. Where fragments D, D′ are shown as a solid arrow this indicates that fragment D is absent. In bacteriophages 501, 507 and 509 it is possible to see that fragment D is altered, despite the problem of band d containing two fragments, since the residual fragment D′ is in low yield and gives only a faint band, as it occurs also in band 1a annealed to fragment B. The size of endonuclease *Eco*RI cleavage products of endonuclease *Ava*I fragments was estimated from a plot of electrophoretic mobility versus fragment size, constructed by using the fragment sizes from Table 2. The location of end points of deletions were taken from Davis & Parkinson (1971). Fragments C′, C″ and C‴ are fragments which replace fragment C and adjacent fragments (H or E) in bacteriophages with deletions. The prefix *b* denotes deletions in the central region of the bacteriophage-λ chromosome. In bacteriophage XII, which lacks the region between sites *srI* 1 and *srI* 2, endonuclease *Ava*I fragment C is decreased to 5% of the size of bacteriophage-λ DNA. When cut with endonuclease *Eco*RI a fragment of 4.3% is obtained identical in mobility with a new fragment in the double digest of bacteriophage VIII. These bacteriophages have in common the region between sites *srI* 3 and *savaI* 4, which must be the interval from which the 4.3% fragment is derived. This places site *savaI* 4 at 58.4%. By subtraction, the fragment between sites *savaI* 3 and *srI* 1 must therefore be 5−4.3% = 0.7%, which places site *savaI* 3 at 43.8%. The deletion *b*519 shortens and combines fragments C and H to give a fragment containing site *srI* 2, which is cut by endonuclease *Eco*RI to give segments of 8.2% and 4.3%. The segment of 4.3% comes from between sites *srI* 2 and *savaI* 2. The distance of site *savaI* 2 from *srI* 2 is therefore 8.2% plus the length of deletion *b*519, which places site *savaI* 2 at 40%. Endonuclease *Ava*I fragment F is cut by endonuclease *Eco*RI at site *srI* 3 in bacteriophage VIII to yield a fragment of 3.8% and a fragment too small to be resolved, which by subtraction is 0.25%. An isolated endonuclease *Eco*RI fragment from bacteriophage XI extending from *srI* right towards the end of the chromosome when digested with endonuclease *Ava*I gave a fragment of identical electrophoretic mobility with the above 3.8% fragment, which shows that this fragment comes from between *srI* 3 and *savaI* 6. This places *savaI* 6 at 69.4% and *savaI* 5 at 65.2%. Endonuclease *Ava*I fragment G is cut by endonuclease *Eco*RI acting at *srI* 4 to give fragments of 2.1 and 1.7%. The orientation of the two fragments has not been determined. Therefore *savaI* 8 and 7 can only be refined between limits defined by the lengths of the smaller and larger fragments in the two possible orientations, which places site *savaI* 7 between 78.9 and 79.3% and site *savaI* 8 between 82.7 and 83.1%.

longer fragments. If the size of fragment A is estimated by subtraction of the sum of the relative lengths of other fragments, determined by electrophoretic mobility, from 100%, a value of 32% is obtained, which is greatly at variance with the value of 27.2% estimated by ^{32}P content. An independent measurement of the length of fragment A was therefore made by electron microscopy (Table 4), giving a result of 26.0±1%, which agrees well with the value obtained from relative ^{32}P content. Table 2 shows that gel band d contains either one fragment of 20.4% or two fragments of 10.2%. Table 3 gives a size of 9.3% for this fragment, which confirms that band d contains two fragments.



EXPLANATION OF PLATE 1

Analysis of an endonuclease AvaI digest of wild-type bacteriophage-λ DNA by electrophoresis on agarose gel
Wild-type bacteriophage-λ DNA was digested with endonuclease *Ava*I and the digest divided into two. The sample in track 2 was heated at 75°C for 10min before being applied to the gel, but that on track 1 received no additional treatment. The gel was photographed and negatively scanned with a Joyce–Loebl micro-densitometer.



EXPLANATION OF PLATE 2

Analysis of an endonuclease AvaI*/endonuclease* Eco*RI co-digest of wild-type bacteriophage-λ DNA*

Bacteriophage-λ DNA was digested with endonuclease *Eco*RI and the 5′-ends of the resulting fragments were labelled with ^{32}P. A 2μg sample of this DNA containing 1000c.p.m. was digested with endonuclease *Ava*I and was analysed by gel electrophoresis. The gel was photographed (track 2), then dried and radioautographed (track 1). Track 3 shows a parallel digest of wild-type bacteriophage-λ DNA with endonuclease *Ava*I. Only fragments produced by cutting endonuclease *Ava*I fragments at endonuclease-*Eco*RI cleavage sites contain label, therefore intact endonuclease *Ava*I fragments contain no label. Band a′ corresponds to endonuclease *Ava*I fragment A and contains no label. Band b′ contains endonuclease *Ava*I fragment D′ annealed to the right-hand fragment obtained by cutting endonuclease *Ava*I fragment B at site *srI* 5 and contains label. Band c′ contains the left-hand fragment obtained by cutting endonuclease *Ava*I fragment B at site *srI* 5 and therefore contains no label. Band d′ corresponds to endonuclease *Ava*I fragments D and D′ and endonuclease *Eco*RI fragment C and therefore contains several labelled components. Band e′ corresponds to endonuclease *Ava*I fragment E which is not cut by endonuclease *Eco*RI and therefore contains no radioactivity. Band *f*′ contains *Eco*RI fragment F, which is not cut by endonuclease *Ava*I and contains label. Band g′ contains the right-hand fragment from cutting endonuclease *Ava*I fragment C at site *srI* 2 and the right-hand fragment from cutting *Ava*I fragment F at site *srI* 3 and contains label. Band h′ corresponds to endonuclease *Ava*I fragment H and therefore contains no label. Bands i′ and j′ were generated by cutting *Ava*I fragment G at *srI* 4 and both therefore contain label.

The fragment sizes from Table 2 were used with the preliminary fragment alignment to produce a rough map (map 1, Fig. 2) of the cleavage sites and fragment locations for endonuclease *Ava*I. Similarly map 2 was drawn from the data from Table 3. The two maps show reasonable correspondence at the ends, with increasing discrepancy towards the centre of the chromosome. This is as expected, since the two maps are aligned by the ends of the bacteriophage-λ chromosome, and emphasizes the need for refinement of the map by consideration of the positions of individual sites relative to internal markers rather than the ends of the molecule. A study was made of the fragmentation by endonuclease *Ava*I and endonuclease *Ava*I and endonuclease *Eco*RI together of bacteriophages carrying single targets for endonuclease *Eco*RI (Fig. 1). This showed that the endonuclease-*Eco*RI cleavage sites fall in the *Ava*I fragments predicted by map 1 (Fig. 2). Also, it was possible to determine the position of sites *savaI* 2, 3, 4, 5, 6, 7 and 8 relative to adjacent endonuclease-*Eco*RI cleavage sites (Fig. 1) and produce a refined map (Fig. 2). The position of site *savaI* 7 resolves the ambiguity in the order of fragments G and B, showing that the longer fragment (B) in the G+B interval lies to the left of the smaller (G). This order is confirmed by bacteriophages IX and 509. Bacteriophage 509 contains only two cleavage sites for *Hin*dIII (*shindIII* 4 and 5) which lie within fragment *D* (Fig. 1) and to the left of site *srI* 4, which lies within fragment G (Plate 2). Unfortunately sites *shindIII* 4 and 5 are not unambiguously mapped (Murray & Murray, 1975; Allet & Solem, 1974; Blattner *et al.*, 1974), so they could not be used to refine further the positions of sites *savaI* 6 and 7. Additional limits to the positions of sites *savaI* 7 and *savaI* 8 were set from a study of other deletion and substitution mutants of bacteriophage λ (Table 1). The deletion *nin*R5 (bacteriophage 500) alters fragment B but not fragment G, and therefore site *savaI* 8 must lie to the left of deletion *nin*R5. The substitution of imm^{434} or imm^{21} for *immλ* (bacteriophages 501 and 507) alters both fragment D and fragment G; therefore site *savaI* 7 must lie to the left of the rightmost limit of the imm^{434} substitution. It is likely therefore that the larger (2.1%) fragment derived by cutting

Table 2. *Determination of the relative sizes of fragments from their radioisotope content*

Uniformly ^{32}P-labelled bacteriophage-λ wild-type DNA was digested with endonuclease *Ava*I, heated at 75°C for 10 min and then analysed by electrophoresis on a 1% agarose gel. After electrophoresis the gel was covered with a film of cellophan and radioautographed by direct contact with X-ray film. The developed film was used to locate bands on the gel containing radioactivity, which were cut out and counted for radioactivity (Clausen, 1967) in a Beckman liquid-scintillation spectrometer to a counting error of less than 5%. '% of total' was calculated by dividing the ^{32}P content of individual fragments by the sum of the ^{32}P contents of all of the fragments.

Endonuclease *Ava*I fragment	^{32}P content (c.p.m.)	^{32}P content (% of total)
A	1840	27.3
B	1220	18.0
C	830	12.3
DD′	1380	2×10.2
E	620	9.2
F	320	4.7
G+H	550	2×4.1

Table 3. *Determination of the relative sizes of fragments from their electrophoretic mobility on agarose gels*

The relative sizes of fragments were obtained by comparing their mobility with fragments of bacteriophage-λ DNA generated by endonuclease *Eco*RI which are of known relative size (Allet *et al.*, 1973; Thomas & Davis, 1975). The mobilities of endonuclease *Ava*I fragments and endonuclease *Eco*RI fragments were measured on the same agarose gel. A plot was made of gel mobility versus log [relative length (% of whole bacteriophage-λ DNA)] for the endonuclease *Eco*RI fragments and the curves were used to estimate the lengths of the endonuclease *Ava*I fragments relative to the whole bacteriophage-λ DNA.

Endonuclease *Ava*I fragment	Mobility (mm)	Length (% of bacteriophage-λ DNA)	Endonuclease *Eco*RI fragment	Mobility (mm)	Length (% of bacteriophage-λ DNA)
A					
B	60	18	A	40	44.5
C	78	12.5	B	68	15.4
DD′	96	9.3	C	80	12.0
E	112	8.4	D	84	11.3
F	168	3.3	E	94	9.8
G	180	2.75	F	116	7.0
H	184	2.6	—	—	—

Table 4. *Electron-microscopic measurement of endonuclease AvaI fragment A from bacteriophage-λ DNA*
Endonuclease *Ava*I fragment A was located on an agarose gel, and the gel band was cut out, minced and centrifuged at 140000*g* for 30min in a 3ml tube (MSE Super-Speed 65 Mk.II centrifuge, 3×3ml swing-out rotor). Then 0.1ml of the supernatant was diluted into ammonium acetate and mounted for electron microscopy by the method of Lang & Mitani (1970) on carbon grids. Bacteriophage-fd DNA was mounted similarly. Grids were shadowed with platinum from an angle of 7°, and screened in a Siemens Emiscop instrument at magnification ×18000. Suitably spread molecules were photographed, ensuring that each field photographed contained at least one linear fragment and one bacteriophage-fd circular molecule. Each photographic negative was projected on a piece of paper by using a commercial episcope and the DNA molecules were traced. The tracings were then measured in mm by using a simple map measurer. A size of 30.8×10^6 daltons was taken for whole bacteriophage-λ DNA. The size of bacteriophage fd (3.4×10^6 daltons) was taken from the data of Marvin & Schaller (1966) and Sinsheimer (1959), and the size of fragment A in daltons was determined by multiplying this value by the ratio of the lengths of fragment A and bacteriophage-fd DNA (Davis *et al.*, 1971). The size of fragment A as a percentage of whole bacteriophage-λ DNA was calculated assuming a size of 30.8×10^6 daltons for whole bacteriophage-λ DNA. S.D. was similarly calculated in terms of daltons and percentage of whole bacteriophage-λ DNA.

DNA molecule measured	Number of molecules measured	Mean length (mm)	S.D. (mm)	Size (daltons)	Size (% of bacteriophage-λ DNA)	S.D. (% of whole bacteriophage-λ DNA)
*Ava*I fragment A from bacteriophage-λ DNA	29	845.7	34.4	8.01×10^6	26.0	±1
Bacteriophage fd-DNA (replicative form)	38	359.2	39.8	3.4×10^6	—	—

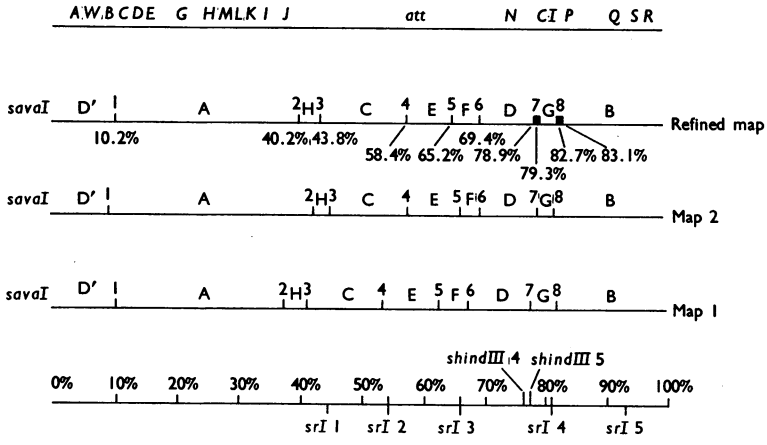


Fig. 2. *Preliminary and refined maps of the cleavage sites for AvaI in the bacteriophage chromosome*
The distribution of genes (represented by capital italics) shown on the top line is taken from Davidson & Szybalski (1971). Below map 1 a linear scale marked in percentages is presented. The positions of relevant cleavage sites for endonuclease *Eco*RI (*srI*) and endonuclease *Hin*dIII (*shindIII*) are shown on this scale. Map 1 was drawn from the data in Table 2 and map 2 from the data in Table 3. The refined map was produced from the data in Fig. 1. A–H on maps 1 and 2 and the refined map represent endonuclease *Ava*I fragments and are allocated as described in the text.

fragment G at site *srI* 4 comes from the left of this fragment (see Fig. 1). This would place site *savaI* 7 at 78.9% and *savaI* 8 at 82.7%. For lack of confirmatory evidence this information has not been included in the refined map.

It is possible that, during the construction of the mutant bacteriophage genomes carrying single cleavage sites for endonuclease *Eco*RI, the distribution of cleavage sites for endonuclease *Ava*I may have been altered more extensively than by the deletions which these genomes contain. An endonuclease *Eco*RI/endonuclease *Ava*I co-digest of wild-type bacteriophage-λ DNA was analysed. To simplify the analysis, the endonuclease *Eco*RI digestion products were 5′-terminally labelled before endonuclease *Ava*I digestion (Plate 2). This showed that the distribution of fragments in the double digest of wild-type bacteriophage-λ DNA was consistent with

the distribution of cleavage sites determined from the single-site mutants.

Owing to the difficulty of obtaining accurate measurements of fragment sizes, restriction site mapping, as suggested by Roberts (1976), must be regarded as an evolutionary process, and small adjustments to maps must be made in the light of inconsistencies which arise in fitting together maps obtained independently for different restriction enzymes. In this regard endonuclease *Ava*I/endonuclease *Hin*dIII and endonuclease *Ava*I/endonuclease *Bam*I double digests have shown that the refined map of endonuclease *Ava*I sites presented here is consistent with the published maps of sites of cleavage for endonuclease *Hin*dIII and endonuclease *Bam*I (Perricaudet & Tiollais, 1975; Haggerty & Schleif, 1976). Furthermore double digests with endonuclease *Ava*I and endonuclease *Sma*I have shown that the three cleavage sites for the endonucleases *Sma*I and *Xma*I in the bacteriophage-λ chromosome are coincident with or close to endonuclease-*Ava*I cleavage sites. The cleavage sites for endonucleases *Sma*I and *Xma*I have been mapped at 40.6, 65.6 and 82.5% (McParland *et al.*, 1976), which is consistent with them being coincident with sites *sava*I 2, *sava*I 5 and *sava*I 7. This interpretation is supported by studies of the fragmentation of other DNA species and by data from nucleotide-sequence analysis at each of the eight sites in bacteriophage-λ DNA cut by endonuclease *Ava*I (S. G. Hughes & K. Murray, unpublished work).

An internal inconsistency which is of concern is that the sizes of fragments D and D′, shown to be the same by electrophoretic mobility, differ by 0.8–1.2% on the refined map. It is possible that a difference in base composition gives fragments D and D′ identical mobility even though they are different in size.

Note Added in Proof (Received 6 April 1977)

Recent nucleotide sequence and 'mapping' studies have shown that site *sava*I 6 is coincident with the single site in bacteriophage-λ DNA cut by endonuclease XhoI.

I thank Dr. K. Murray for help and advice, particularly in the preparation of the manuscript, Dr. N. E. Murray for generous gifts of bacteriophage stocks and Mrs. Sandra Bruce for the preparation of many samples of bacteriophage-λ DNA.

References

Allet, B. & Solem, R. (1974) *J. Mol. Biol.* **85**, 476–484
Allet, B., Jeppeson, P. G. N., Katagiri, D. J. & Delius, H. (1973) *Nature (London)* **241**, 120–122
Arber, W. & Linn, S. (1969) *Annu. Rev. Biochem.* **38**, 467–500
Blattner, F. R., Fiendt, M., Hass, K. K., Those, P. A. & Szybalski, W. (1974) *Virology* **62**, 458–471
Brammar, W. J., Murray, N. E. & Winton, S. (1974) *J. Mol. Biol.* **90**, 635–647
Clausen, T. (1967) *Ann. Biochem.* **22**, 70–73
Davidson, N. & Szybalski, W. (1971) in *The Bacteriophage λ* (Hershey, D., ed.), pp. 46–82, Cold Spring Harbor Laboratory, Cold Spring Harbor
Davis, R. W. & Parkinson, J. S. (1971) *J. Mol. Biol.* **56**, 403–423
Davis, R. W., Simon, M. & Davidson, N. (1971) *Methods Enzymol.* **21**, 413–428
Goldberg, A. R. & Howe, M. (1969) *Virology* **38**, 200–202
Grossman, L. (1967) *Methods Enzymol.* **12**, 700–702
Haggerty, D. & Schleif, R. (1976) *J. Virol.* **18**, 659–663
Helling, R. W., Goodman, H. M. & Boyer, H. W. (1974) *J. Virol.* **14**, 1235–1244
Hughes, S. G. & Brown, P. R. (1973) *Biochem. J.* **131**, 583
Kaiser, A. D. & Hogness, D. S. (1960) *J. Mol. Biol.* **2**, 312–415
Lang, D. & Mitani, M. (1970) *Biopolymers* **9**, 373–379
Maniatis, T., Jeffrey, A. & Kleid, D. G. (1975) *Proc. Natl. Acad. Sci. U.S.A.* **72**, 1184–1188
Marvin, D. A. & Schaller, H. (1966) *J. Mol. Biol.* **15**, 1–7
McParland, R. H., Brown, L. R. & Pearson, G. D. (1976) *J. Virol.* **19**, 1006–1011
Murray, K. (1973) *Biochem. J.* **131**, 569–583
Murray, K. & Murray, N. E. (1974) *Nature (London)* **251**, 476–481
Murray, K. & Murray, N. E. (1975) *J. Mol. Biol.* **98**, 551–564
Murray, K., Murray, N. E. & Brammar, W. J. (1975) *FEBS Symp.* **38**, 193–207
Murray, K., Hughes, S. G., Brown, J. & Bruce, S. A. (1976) *Biochem. J.* **159**, 317–322
Murray, N. E., Batten, P. L. & Murray, K. (1973) *J. Mol. Biol.* **81**, 395–407
Nathans, D. & Smith, H. O. (1975) *Annu. Rev. Biochem.* **44**, 273–293
Old, R. W., Murray, K. & Roizes, G. (1975) *J. Mol. Biol.* **92**, 331–339
Parkinson, J. S. (1971) *J. Mol. Biol.* **56**, 385–401
Perricaudet, M. & Tiollais, P. (1975) *FEBS Lett.* **56**, 7–11
Pirrotta, V. (1975) *Nature (London)* **254**, 114–117
Richardson, C. C. (1965) *Proc. Natl. Acad. Sci. U.S.A.* **54**, 158–165
Roberts, R. (1976) *CRC Crit. Rev. Biochem.* **4**, 122–164
Sharp, P. A., Sugden, J. & Sambrook, J. (1973) *Biochemistry* **12**, 3055–3063
Sinsheimer, R. L. (1959) *J. Mol. Biol.* **1**, 43–53
Skalka, A. (1971) *Methods Enzymol.* **21**, 341–350
Smith, H. O. & Nathans, D. (1973) *J. Mol. Biol.* **81**, 419–423
Thomas, M. & Davis, R. W. (1975) *J. Mol. Biol.* **91**, 315–328
Thompson, R., Hughes, S. G. & Broda, P. (1974) *Mol. Gen. Genet.* **133**, 141–149
Wood, W. B. (1966) *J. Mol. Biol.* **16**, 118–133
Yoshimori, R. N. (1971) Ph.D. Thesis, University of California