DNA Restriction Enzyme from *E. coli*

by
MATTHEW MESELSON
ROBERT YUAN*
The Biological Laboratories,
Harvard University,
Cambridge, Massachusetts

An endonuclease which degrades foreign DNA has been isolated. The enzyme requires S-adenosylmethionine, ATP and Mg^{++}.

MANY strains of *E. coli* can recognize and degrade DNA from foreign *E. coli* strains. Whether a foreign DNA molecule will be rejected can depend on non-heritable characteristics imparted to it by the cell from which it is obtained. Such characteristics are called host-controlled modifications[1-3]. For example, the ability of λ and several other bacteriophages to multiply on *E. coli* strain K depends on the bacterial host in which the phages were last grown. Phages grown in bacteria possessing the modification allele m_K multiply well, but phages grown in bacteria lacking m_K do not. Instead, their DNA is quickly degraded on entering cells of strain K. The ability of strain K to reject or "restrict" DNA from cells lacking m_K is itself under genetic control, the responsible allele being designated r_K (refs. 4 and 5).

More generally, cells with the restriction allele r_i can degrade DNA from cells lacking the corresponding modification allele m_i. Several different modification and restriction alleles are known[6,7]. As well as certain phage DNAs, bacterial DNA transferred between cells by conjugation or transduction is subject to host-controlled modification and restriction, suggesting that these phenomena play a part in regulating the flow of genetic information between bacteria.

There is evidence that the modification character of a DNA molecule is determined by its pattern of methylation[8,9]. The simplest hypothesis for the biochemical basis of restriction is that each restriction allele directs the formation of a nuclease specific for DNA lacking the corresponding modification character. We have detected, isolated and characterized such an enzyme; it is an endonuclease present in strain K that is specifically active against λ DNA from strains lacking m_K.

Restriction by bacterial extracts. Restriction by extracts of r_K bacteria was discovered as an ATP-dependent nucleolytic activity specific for DNA from λ grown on a m_K^- host. Fig. 1 shows the sedimentation profiles of ^3H λ·K DNA and ^{32}P λ·C DNA incubated without extract (*a*), with extract of the r_K strain *E. coli* 1100 (*b*), and with extract of its r_K^- derivative, strain 1100.293 (*c*). The sedimentation of λ·K DNA is seen not to be affected by incubation with either extract. In contrast, the sedimentation rate of λ·C DNA is specifically decreased by incubation with the r_K extract. We conclude that the activity is specific for unmodified DNA and occurs only in the restricting strain.

Purification of the enzyme. Purification of the restriction activity was at first frustrated by its apparent loss on dialysis of bacterial extracts. The difficulty was overcome when we found that both ATP and S-adenosylmethionine (SAM) are needed for restriction to occur. A role for SAM was suggested by the report that methionine, a biosynthetic precursor of SAM, is necessary for optimal restriction *in vivo* by methionineless bacteria (unpublished observations of W. B. Wood, cited in ref. 8).

Following the establishment of suitable assay conditions, the activity was purified as follows: *E. coli* 1100 was grown to 6×10^8 cells/ml. at 37° C in tryptone broth with 1 μg/ml. of thiamine hydrochloride. The cells were sedimented and stored at −20° C. 120 g of cells was suspended in 0·01 M *tris* *p*H 7·4, 2×10^{-4} M $MgCl_2$, 10^{-4} M EDTA, 2×10^{-3} M mercaptoethanol. All subsequent operations were conducted at approximately 4° C. The cells were extracted by blending with glass beads and the extract was centrifuged at low speed and then at 35,000 r.p.m. for two hours in the International *A*-170 rotor. The sediment possessed very little restriction activity and was discarded.

The high speed supernatant (300 ml.) was fractionally precipitated by adding dry $(NH_4)_2SO_4$. The material precipitating between 35 and 55 per cent saturation was dissolved in 100 ml. of 0·02 M potassium phosphate, *p*H 7·0, 10^{-4} M EDTA, 2×10^{-3} M mercaptoethanol (PEM), dialysed against the same buffer, and applied to a column of Whatman *DE* 52 DEAE cellulose 6 cm in diameter and 11 cm long that had been equilibrated with buffer. The column was washed with 600 ml. of buffer and eluted with a 1,000 ml. linear gradient of PEM running from 0·06 to 0·30 M potassium phosphate and collected in 50 ml. fractions. The restriction activity was found in two adjacent fractions with a mean phosphate concentration of 0·15 M.

The active DEAE fractions were combined, dialysed against PEM containing 0·02 M potassium phosphate, and applied to a 1 × 15 cm column of Whatman *P*11 phosphocellulose equilibrated with the same buffer. The column was washed with 40 ml. of buffer and eluted in 9 ml. fractions with a 300 ml. linear gradient running from 0·05 to 0·3 M phosphate. The restriction activity emerged in three adjacent fractions with a mean phosphate concentration of 0·16 M.

The pooled active phosphocellulose fractions were dialysed against 0·01 M potassium phosphate, *p*H 7·5, 10^{-4} M EDTA, and 5×10^{-4} M dithiothreitol, concentrated to 7·5 ml. by dialysis against dry 'Sephadex *G*-200', and dialysed again. The concentrate was applied in 2·5 ml. portions to 30 ml., 10–25 per cent glycerol gradients made up in the dithiothreitol buffer. After centrifugation at 25,000 r.p.m. for 20 h at 2° C in an International *SB*101 rotor, the activity was found in three adjacent 1 ml. fractions at a position corresponding to a sedimentation coefficient of approximately 12*S*. These were stored at −10° C and have been kept for nearly a year without noticeable inactivation. Table 1 summarizes the purification of the restriction enzyme.

Assays with a limiting amount of pure enzyme were conducted with and without the addition of extract of

* Fellow of the Damon Runyon Memorial Foundation.

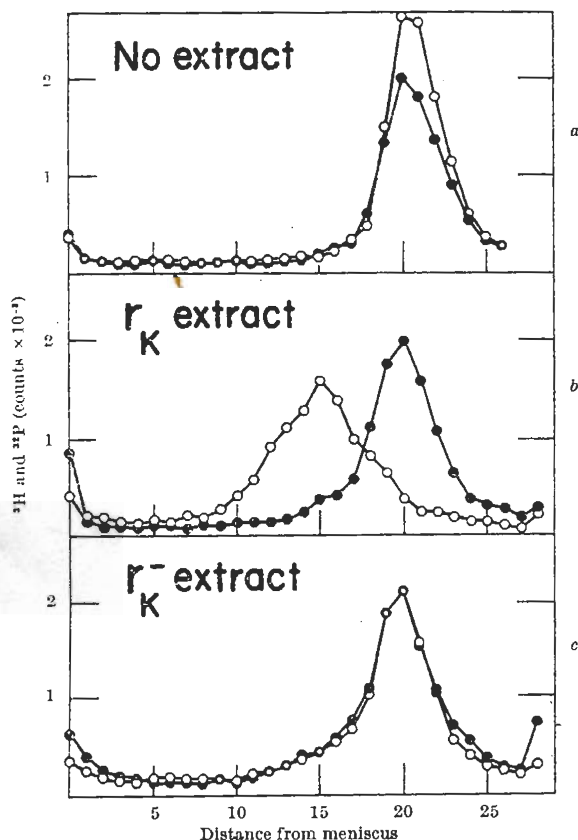


Fig. 1. Sedimentation profiles of a mixture of ^{3}H λ·K DNA and ^{32}P λ·C DNA incubated for 10 min (*a*) without extract, (*b*) with extract of *E. coli* strain 1100, or (*c*) with extract of strain 1100.293. Incubation mixtures (*b*) and (*c*) contained 1 mg/ml. bacterial protein. A 0·25 ml. sample of each mixture was layered on a 3·5 ml. 6–20 per cent sucrose gradient containing 0·01 M *tris* *p*H 8, 0·001 M EDTA, 0·04 per cent sodium dodecyl sulphate and centrifuged for 1·8 h at 55,000 r.p.m. in an International *SB*405 rotor at 20° C. Fractions were collected and counted by standard techniques. ——●——, ^{3}H; ——○——, ^{32}P.

the r_K^- strain 1100.293. The extract had no effect on the extent of the reaction, showing that the activity of r_K extract can be directly compared with that of the purified enzyme in computing the degree of purification achieved. The failure of the r_K^- extract to inhibit the reaction also shows that the r_K^- phenotype results from a reduction in the amount or activity of the restriction enzyme rather than from the presence of an inhibitor.

Requirements of the reaction. Table 2 shows the effects of omitting various components of the reaction mixture. Restriction is measured as the inactivation of infectious λ·C DNA. Enzyme, ATP, Mg^{++} and SAM are all seen to be essential while gelatine, EDTA and mercaptoethanol are not. Fig. 2 shows the effect of lowering the concentration of ATP or SAM. The requirement for SAM cannot be satisfied by l-methionine, 5′-thiomethyladenosine, S-adenosyl-*dl*-homocysteine or S-adenosylethionine. ATP can be replaced by *d*ATP, although at limiting concentrations approximately twice as much must be used for the same degree of inactivation. ATP cannot be replaced by GTP, ADP, AMP, adenosine, pyrophosphate or phosphate. The optimal *p*H for the restriction reaction lies between 7·5 and 8·0.

Table 1. PURIFICATION OF THE ENZYME

	Total protein (mg)	Activity
Low speed supernatant	8,000	~1
High speed supernatant	5,700	
$(NH_4)_2SO_4$ precipitate	3,400	
DEAE eluate	260	
Phosphocellulose eluate	2	
Glycerol gradient fraction	≤0·5	$\gtrsim$5,000

Activity is expressed as the reciprocal of the mg/ml. of protein required to break 90 per cent of λ·C duplexes during a 10 min incubation under the assay conditions described in the text.

Table 2. REQUIREMENTS FOR REACTION

	$\log_{10} \frac{\lambda \cdot K}{\lambda \cdot C}$
Complete system	2·0
–EDTA	2·0
–Gelatine	2·0
–Mercaptoethanol	2·0
–$MgCl_2$	0·0
–SAM	0·0
–ATP	0·0

The restriction enzyme is an endonuclease. The action of the enzyme on ^{32}P λ·C DNA liberates little or no non-sedimenting ^{32}P, showing that degradation is endo rather than exonucleolytic. The action of the enzyme on λ twisted circles, DNA molecules without ends, confirms this. Sedimentation analyses of a mixture of ^{3}H λ·K and ^{32}P λ·C twisted circles incubated with and without enzyme (Fig. 3) prove that the enzyme readily degrades λ·C twisted circles. The enzyme, however, has no effect on λ·K twisted circles; neither double nor single chain scissions are produced.

The limit product is duplex DNA. A mixture of ^{3}H λ·K DNA and ^{32}P λ·C DNA was incubated with enzyme, re-isolated by phenol extraction, dialysed against TNE, and again incubated with enzyme. Fig. 4 shows that the second incubation caused little or no additional degradation. A control incubation proved that intact ^{32}P λ·C DNA added to the second incubation mixture was extensively degraded. We conclude that the sedimentation profiles seen in the figure represent the limit product of the restriction enzyme under our reaction conditions. The maxima of these profiles occur close to the position to which λ quarter molecules would sediment. The sedimentation distribution of the limit product in 0·1 M salt was compared with that in 0·01 M salt. No difference was observed. Single DNA chains sediment about three times faster in the stronger salt solution, whereas duplexes sediment at the same rate in both solvents. We conclude that the limit product consists of duplexes containing little or no single stranded DNA.

The limit product is without single-chain breaks. Clearly the enzyme is able to break the λ·C duplex into a number of segments. Conceivably, there are sites within these segments where the enzyme has broken only one of the two polynucleotide chains. Such single-chain breaks would have little effect on the sedimentation behaviour of DNA duplexes. They would, however, become apparent from a comparison of the molecular weight distribution of the reaction product before and after chain separation. A mixture of ^{32}P λ·C DNA and ^{3}H λ·K DNA was extensively incubated with enzyme and then sedimented at 20° C on neutral and on alkali D_2O-H_2O gradients containing 0·9 M NaCl. The alkali solvent dissociates duplexes into their component single chains. Fig. 5 shows the molecular weight distributions calculated from the sedimentation profiles in the two solvents. Molecular weights

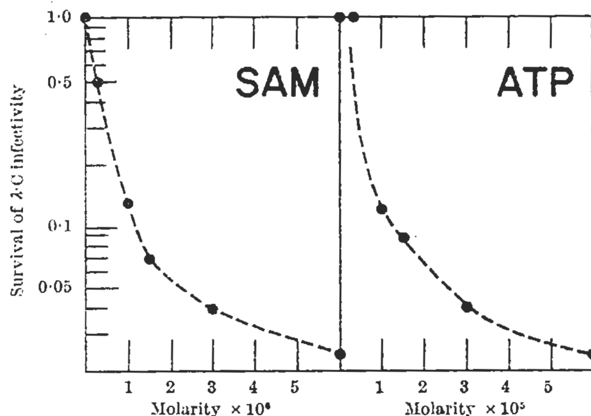


Fig. 2. The dependence of restriction activity on SAM and ATP concentration.

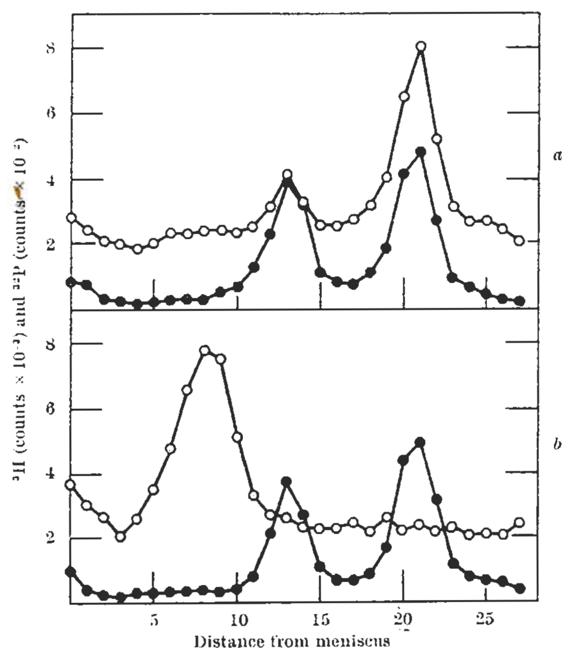


Fig. 3. Sedimentation profiles showing the action of the restriction enzyme on twisted circular λ DNA molecules. (*a*) A mixture of ^{3}H λ·K and ^{32}P λ·C DNA consisting mostly of twisted circular molecules but also containing the less rapidly sedimenting non-twisted circular form. (*b*) The same mixture after 10 min of incubation with enzyme. Samples were centrifuged for 1 h under the conditions given in the legend for Fig. 1. ——○——, ^{3}H; ——●——, ^{32}P.

are plotted as fractions of the weight of the intact duplex or single chain. The near superposability of the two distributions shows that at least most of the limit product consists of duplexes without single chain breaks. Only if located near the ends of the duplexes would any substantial number of single chain breaks have gone undetected by this test.

Single chain scission precedes duplex cleavage. Prolonged action by the restriction enzyme breaks the DNA duplex into several pieces. What is the timing with which individual chains of the duplex are broken? Single and double chain scissions may be sensitively detected and distinguished by their effects on the twisted circular form of λ DNA (ref. 18). Single chain scission converts twisted circles to the more slowly sedimenting non-twisted circular configuration. Double strand scission converts them to even more slowly sedimenting non-circular molecules. Fig. 6 shows the result of incubating a mixture of ^{32}P λ·C twisted circles and ^{3}H λ·K non-circular molecules with enzyme for 30, 60 and 120 s; after an initial lag, untwisted circles are produced before non-circular molecules appear. Thus single strand scission precedes cleavage of the duplex. The same conclusion has been reached by a comparison of neutral and alkali sedimentation analyses performed in parallel on samples taken at various times in the course of the reaction. The occurrence of single chain scission early in the reaction, taken together with the paucity of single chain breaks in the limit product, indicate that the enzyme first breaks only one chain and then, a few seconds later, breaks the complementary chain at a point directly or nearly opposite. Our results do not reveal whether a given enzyme molecule remains bound to catalyse the breaks on both chains or whether the two chains are attacked independently.

The infectivity assay employed to determine the requirements of the reaction is probably insensitive to single strand scissions, for λ DNA can be rather extensively nicked by pancreatic DNase without loss of infectivity[19]. We therefore reinvestigated the requirements for ATP, Mg^{++} and SAM by sedimentation analysis with ^{32}P λ·C twisted circles as substrate. We found that neither double nor single chain scission occurs if any one of the three components is omitted from the reaction mixture.

Duplexes with only one modified chain are not attacked. Separated polynucleotide chains of ^{3}H λ·K DNA and ^{32}P λ·C DNA were mixed in appropriate complementary combinations and annealed to obtain the two possible heteroduplexes (one chain modified, the other unmodified) and both homoduplexes. Incubation with enzyme followed by sedimentation analyses on neutral and alkali gradients showed that unmodified homoduplexes are cleaved but that the enzyme does not act on modified homoduplexes

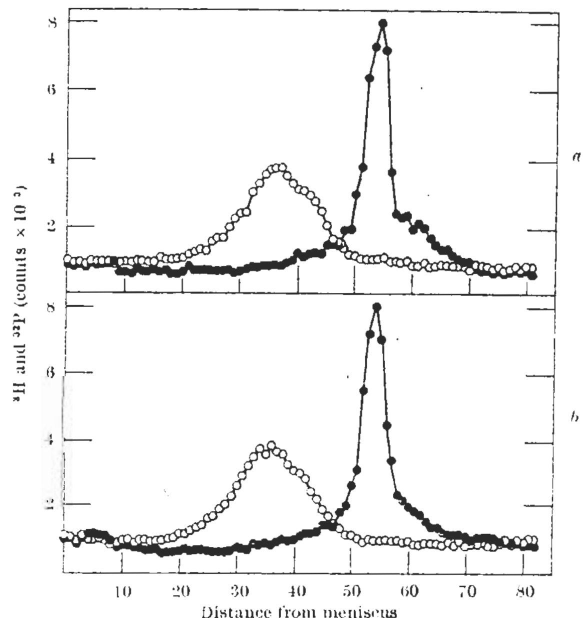


Fig. 4. Sedimentation profiles of a mixture of ^{3}H λ·K DNA and ^{32}P λ·C DNA incubated with enzyme (*a*) once or (*b*) twice. See text for details. Samples of 0·4 ml. were layered on 12 ml. 15–28 per cent sucrose gradients containing 0·01 M *tris* pH 8·0, 0·001 M EDTA, 0·04 per cent sodium dodecyl sulphate and centrifuged for 17 h at 35,000 r.p.m. in an International *SB*283 rotor at 2° C. ——●——, ^{3}H; ——○——, ^{32}P.

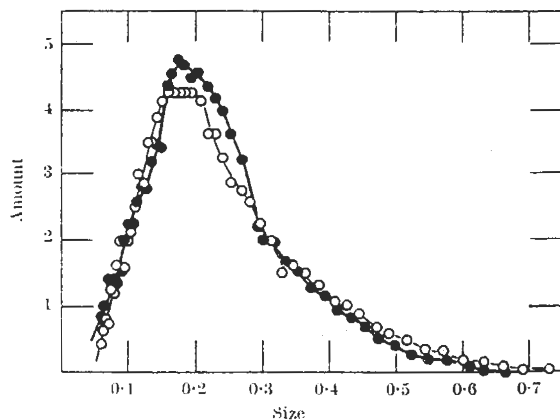
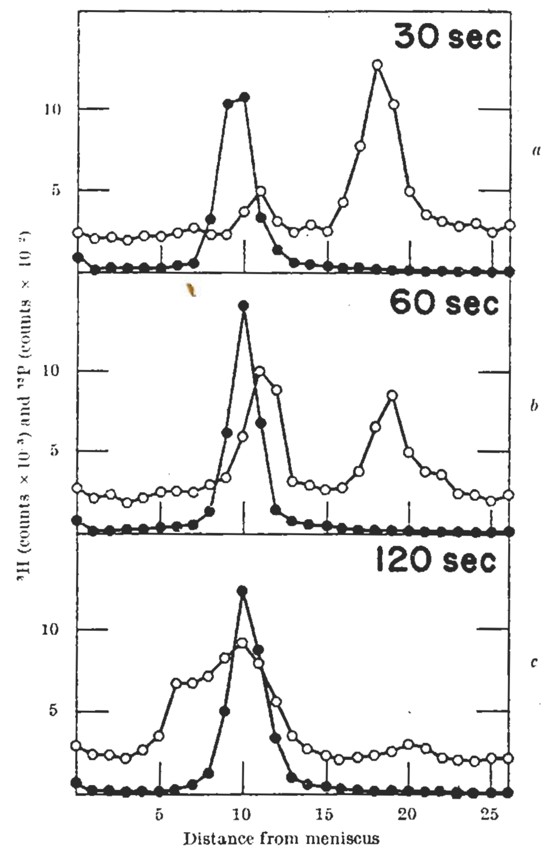


Fig. 5. Molecular size distributions of the reaction product before (——○——) and after (——●——) strand separation. Each point represents a fraction collected from the corresponding gradient. A mixture of ^{3}H λ·K DNA and ^{32}P λ·C DNA was extensively incubated with enzyme and then analysed on 11·2 ml. D_2O–H_2O gradients containing 0·9 M NaCl, 0·001 M EDTA and either 0·04 per cent sarcosyl *NL*97, 0·01 M *tris* pH 8·0 or 0·1 M NaOH. The gradients were centrifuged in the *SB*283 rotor at 41,000 r.p.m. for 3·3 h at 20° C, which moves the intact ^{3}H reference DNA about 85 per cent of the way down the gradient. Sedimentation coefficients were computed for each fraction, taking into account the variation of acceleration, solution density, and viscosity along the gradient. Molecular size relative to intact ^{3}H λ·K duplexes or single chains taken as unity was obtained from the sedimentation coefficients[17]. The ordinate for each point is the ^{32}P counting rate corrected for background and normalized to give the amount of DNA per unit molecular size. No correction has been made for the effect of finite band width. Parallel centrifugations of the DNA mixture incubated without enzyme showed the ^{32}P DNA to sediment in both neutral and alkali solution as a band with a half-width of three fractions at half its maximum concentration. In the neutral gradient, the trailing edge was sharp while in alkali it displayed a tail indicating that approximately 1/4 of the polynucleotide chains were broken. In neutral solution 10 per cent of the material sedimented slightly ahead of the leading edge while in alkali the leading edge was sharp.



ig. 6. Sedimentation distributions of a mixture of ³H λ·K non-circular NA and ³²P λ·C twisted circular DNA after incubation with enzyme r (a) 30, (b) 60 and (c) 120 s. The distributions of the untreated DNA ixture and of the mixture incubated with enzyme in a reaction mixture cking ATP and SAM were essentially the same as that seen in (a). amples were centrifuged for 1·1 h under the conditions given in the legend for Fig. 1. ——●——, ³H; ——○——, ³²P.

on either of the two heteroduplexes (Fig. 7). Thus eroduplexes are resistant even to single chain scissions.

A restriction enzyme from P1 lysogenized cells. We have tly purified a restriction activity from cells lysogenic phage P1 which specifically breaks DNA duplexes of ge λ grown on strains not lysogenic for P1. Like the triction enzyme from strain K, it requires Mg^{++}, ATP l SAM. The product of its action also consists mainly entirely of relatively large fragments of the λ duplex. propose that these enzymes be designated *E. coli* lonuclease III·K and III·P, respectively.

Discussion. The degree of purification achieved and diversity of the fractionation procedures employed ke it most unlikely that the restriction activity we e isolated corresponds to more than a single molecular cies, although it may well be a stable aggregate of two more sub-units. Indeed, the enzyme's high sedimenta- coefficient, the complexity of its action and co- tor requirements suggest an aggregate.

he ATP and SAM requirements of endonucleases III·K l III·P are unprecedented and we do not yet know ether either compound is consumed in the reaction or s only catalytically, possibly as an allosteric effector. requirement for SAM is especially interesting because the likelihood that modification corresponds to the cific methylation of DNA[8,9], presumably using SAM a methyl donor. It appears that the enzyme interacts ctly with ATP, for it is rapidly inactivated when they incubated together if SAM is absent.

akano, Watanabe and Fukasawa[20] have described a cific inactivation of unmodified infectious λ DNA by racts of bacteria carrying certain restrictive episomes. ATP was added to their reaction mixtures. In our experiments and in an earlier investigation (unpublished results of S. Lederberg and M. Meselson) using the λ infectivity assay system, neither K nor P restriction activity was detected in bacterial extracts without added ATP; the addition of SAM was unnecessary in our crude extracts. It remains to be seen whether the enzymes associated with the restrictive episomes studied by Takano *et al.* differ in their co-factor requirements from the K and P enzymes or whether there was adequate ATP (and SAM) naturally present in their extracts.

The size distribution of the DNA fragments in the limit digest of the restriction enzyme poses several problems. The very limited action of the enzyme *in vitro* contrasts sharply with the nearly complete and very rapid breakdown of unmodified DNA to acid soluble products seen *in vivo*[21,22]. Perhaps the production of an end, or a certain type of end, quite generally exposes a DNA duplex to such breakdown within the cell, even though it is not clear that the known nucleases of *E. coli* would be sufficient for this purpose[23]. Although it seems very likely that the restriction enzyme attacks DNA at fixed sites, no clear indication of discrete species was seen in the sedimentation profiles of the limit product. Depending on

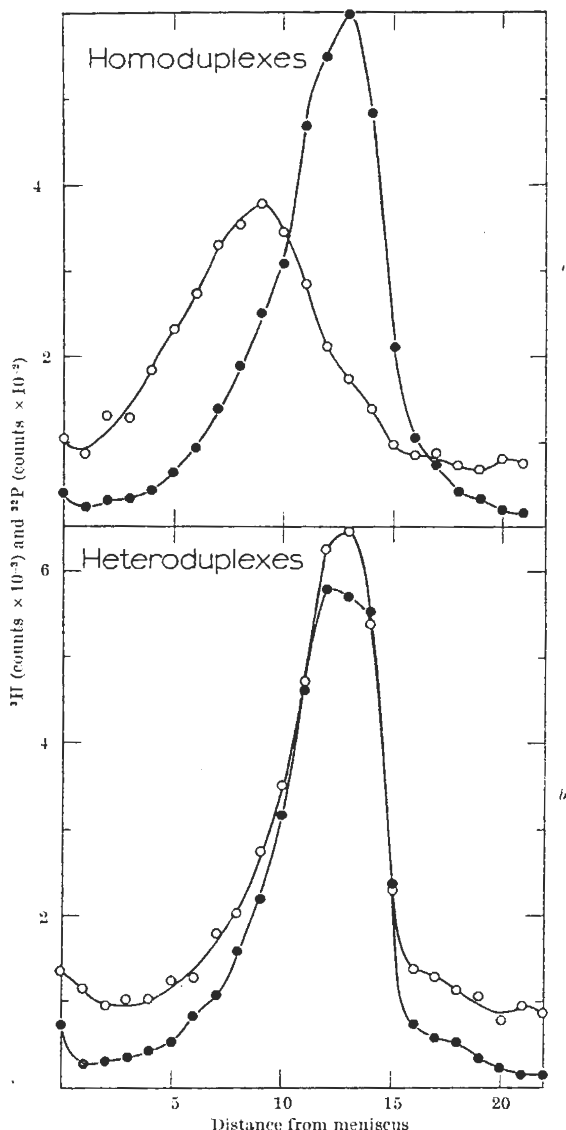


Fig. 7. Alkali sedimentation profiles of (a) reconstituted ³H λ·K and ³²P λ·C homoduplexes and (b) the two possible heteroduplexes after incubation with enzyme. Samples of 0·2 ml. were layered onto 3·5 ml. 6–20 per cent sucrose gradients containing 0·005 M EDTA and 0·3 M NaOH and centrifuged 1·8 h at 55,000 r.p.m. in the *SB*405 rotor at 20° C. ——●——, ³H; ——○——, ³²P.

the distribution of susceptible sites, however, it is possible that different classes of fragments would not have been resolved under our conditions of analysis.

The molecular size distribution of the limit product indicates that not all λ chromosomes are broken to the same degree. For example, although there are segments of approximately 40 per cent length, they comprise much less than 40 per cent of the digest, as though an appreciable number of chromosomes did not react to give such segments. If Fig. 5 accurately portrays the molecular size distribution of the limit product, it may be that the initial population of DNA molecules was heterogeneous; some may already have been modified at one or a few sites. Alternatively, the restriction enzyme itself may occasionally render some sites resistant to cleavage. Conceivably the restriction enzyme is also the modifying enzyme but with its modifying activity only weakly manifested in our assay system. The presence of SAM in the incubation mixture, the close genetic linkage between r^- and m^- mutations, and the fact that a large proportion of r^- mutants are also m^- (refs. 4, 5 and 24), are consistent with this interpretation, although other explanations can be given.

It is definitely known that one, and somewhat less well established that both, of the two possible λ chromosomes with only one modified polynucleotide chain are able to infect and multiply normally in a restricting host[3]. Our finding that both types of λ heteroduplex are resistant to the restriction enzyme confirms these conclusions. This shows that both polynucleotide chains of λ·K DNA are modified at every site of potential attack. The fact that heteroduplexes are resistant even to single chain scission indicates that the enzyme somehow takes account of the structure of both chains before cutting either. The resistance of heteroduplexes may serve to protect newly replicated bacterial DNA from attack by the cells' own restriction enzyme, allowing time for modification of the newly synthesized chain. Indeed, if restriction and modification are accomplished by the same enzyme, the choice between the two reactions may normally be governed by whether the substrate is an unmodified homoduplex or a heteroduplex, respectively. The extreme destructive potential of the restriction system may have enforced selection for additional mechanisms to ensure that cellular DNA newly synthesized by replication or repair is modified before it can be restricted. Various protective control mechanisms can obviously be devised involving the cofactor requirements for restriction and the sensitivity of the restriction enzyme to ATP when SAM is absent.

If DNA synthesis in restricting bacteria could be continued beyond one generation after methylation has been blocked, the resulting unmodified homoduplexes should be vulnerable to cleavage and degradation by the cells' own restriction system. Having made this prediction, we were struck by the report of Lark[25] that DNA synthesis in methionineless *E. coli* 15T⁻ halts one generation after methionine is replaced with ethionine or norleucine and that much of the cellular DNA may then become acid soluble. Although the level of SAM may be very low during methionine deprivation, even one cleavage of the bacterial chromosome by restriction enzyme might stop replication and trigger extensive degradation. According to this picture, DNA synthesis should continue beyond one generation and without degradation if Lark's experiments were repeated with a strain deficient in restriction.

Finally, endonucleases III·K and III·P may provide a model for other systems that cleave duplexes or cut single chains at specific locations, not only in connexion with restriction phenomena, but possibly also in replication, recombination or transcription.

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Materials and Methods. *Bacteria and bacteriophages.* Strain C600 (re 10), its derivative CR34 *thy* (ref. 11) and the DNA endonuclease I deficier strain 1100 (ref. 12) have the restriction and modification properties $r_K m$ Strain 1100.293 is an $r_{K_1}^- m_K$ mutant of strain 1100. Strains C600.4 and C600 are $r_K^- m_K^-$. Bacteriophage λ is the wild type of Kaiser[14] and λc is a clear plaqu forming mutant. Phages grown on m_K^- strains are designated λ·C, followin the custom based on the fact that *E. coli* strain C is m_K^-. Taking the platin efficiency on strain C600.4 as unity, that on all other sensitive r_K^- strair employed was 1·0 and on r_K^- strains was 1·0 for λ·K and 10^{-4} for λ·C.

DNA preparations. Stocks of λ·K and λc·C were made on strains C60 and C600.4, respectively, by the agar layer method. Stocks of ^{3}H-thymidir λ·K and ^{32}P λ·C were produced by ultraviolet light induction of CR34 (and C600.4 (λ) in radioactive media. Phages were purified by two cycl of differential centrifugation followed by equilibrium sedimentation in Cs solution. DNA was extracted with water-saturated phenol, extensive dialysed against 0·01 M *tris*, 0·01 M NaCl, 2×10^{-4} M EDTA, *p*H 7·4 (TN and stored at 0° C. Before use in assays, DNA solutions were kept at 55° for 5 min in order to disassociate end-to-end aggregates. Twisted circul λ DNA was prepared by infection of *E. coli* C600.4 (λ) or C600 (λ) wi 10 ^{32}P λ·C or ^{3}H λ·K per cell, respectively. After incubation for 30 min 37° C in tryptone broth, the infected cells were sedimented, washed wi 0·03 M *tris* *p*H 8·0, 0·01 M NaCl, and resuspended in the same medium 5×10^9 cells/ml. The suspension was made 0·02 per cent in lysozyme, i cubated for 1 min at 37° C, made 0·01 M in EDTA, incubated for an addition minute, and lysed by the addition of 10 mg/ml. sodium dodecyl sulphat The lysate was sheared by vigorous passage through a No. 26 hypoderm needle, extracted with water-saturated phenol, dialysed against TNE, ar sedimented through a neutral sucrose gradient containing 10^{-3} M EDT After shear treatment, host cell DNA sediments much more slowly tha λ twisted circles, which are relatively insensitive to shearing. The fracti of the gradient containing twisted circles was dialysed against TNE ar stored at 0° C.

Polynucleotide chain separation and annealing. Separated polynucleoti chains were prepared according to Hradecna and Szybalski[15], using po UG rather than poly IG. Broken chains and poly UG were removed b sedimentation through an alkali D_2O–H_2O gradient. Complementary mi tures containing approximately 10 μg/ml. of purified single chains we annealed by overnight dialysis at 37° C against 0·001 M EDTA, 0·1 M Na NaOH *p*H 10·5 followed by dialysis against TNE (private communicati from W. Doerfler and D. Hogness). More than 80 per cent of the anneal material sedimented at the same rate as native DNA duplexes in neutr 0·01 M salt. Sedimentation analysis in alkali showed that most of the anneal duplexes were free of single chain breaks.

Enzyme assays. Restriction activity was assayed in a mixture containin per ml., 50 μmoles *tris*, 5 μmoles $MgCl_2$, 0·2 μmoles EDTA, 5 μmoles mercapt ethanol, 100 μg gelatine, 0·2 μmoles ATP, 0·02 μmoles S-adenosylmethioni (SAM), approximately 10^{10} phage units of ^{32}P λ·C DNA and ^{3}H λ·K DN and the enzyme sample to be assayed. SAM was purified from the co mercial product by elution from 'Biorex 70' with acetic acid. The *p*H the reaction mixture was 7·7 at 20° C. After incubation for the desired ti at 37° C, 1/10 volume of 0·4 M EDTA *p*H 9·5 was added and the mixture w subjected to sedimentation analysis.

In some assays, radioactive DNA was replaced with approximately 1 phage units of a mixture of λc·C DNA and λ·K DNA. After incubation, t reaction mixture was diluted 1 : 100 in TNE and assayed for infectivi Restriction was measured as a decrease in the ratio of clear plaques to turb plaques, relative to the ratio for the untreated DNA mixture. λ·K DNA not inactivated by the purified enzyme. The infectivity assay was that Kaiser and Hogness[16] except for the following: strain C600.5 was used recipient and C600.4 (λi^{434}) as indicator. Recipient cells were prepared 4·5 h growth at 37° C from a 1 : 3 dilution of an overnight culture. Glyce rather than glucose was used as carbon source. The bacteria were sediment resuspended at 2×10^9/ml. in 0·01 M $MgSO_4$, infected with 5 λi^{434} phages p cell, incubated for 7 min at 37° C, diluted 1 : 1 with iced 0·01 M *tris* *p*H 8 0·01 M $CaCl_2$, 0·01 M $MgSO_4$, sedimented, and resuspended at 2×10^9/ml. the same solution. The cells were kept at 0° C for 2–12 h before use. T efficiency of the assay was approximately 0·01 plaques per phage unit λ DNA. Infectivity assays were useful for detecting restriction activity o after the phosphocellulose chromatography step in the purification of t enzyme. Less purified enzyme preparations caused severe non-speci inactivation of infectivity.

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