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Physical characterization of the genome of the *Myxococcus xanthus* bacteriophage MX-8

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Summary. We have constructed a restriction map for the genome of bacteriophage MX-8 from *Myxococcus xanthus* using the enzymes *Pvu*II, *Mbo*I, and *Eco*RI. The phage genome size, as determined by restriction analysis, is 51.7 ± 0.6 Kb. Double digestions, redigestions of isolated fragments, and crossed-contact hybridization of partial digestion products show that the restriction map is circular. Restriction analysis and Southern hybridization show that the phage DNA molecules are packaged sequentially from a concatemer starting from a specific site which we have mapped. The DNA molecules have an average terminal redundancy of approximately 8% and are circularly permuted over at least 40% of the genome.

Introduction

Myxobacteria are important to microbiologists interested in developmental biology because they are procaryotic cells which display complex social behavior, either as vegetative cells moving within a swarm, or as developmental cells forming multicellular aggregates during sporulation (Kaiser et al. 1979). The behavior of cells suggests that they communicate by intercellular signals (Janssen 1984), and the opportunity for studying cell interactions and cell signals within procaryotes has motivated geneticists to develop genetic tools specific for myxobacteria (Kaiser 1984).

To explore the potential of a bacteriophage as a vector for genes in the myxobacterium *Myxococcus xanthus*, we have studied the bacteriophage MX-8. This phage was isolated and characterized by Martin et al. (1978), who found it to be a generalized transducing phage with a polyhedral head, a short tail, and a chromosome containing approximately 56 kilobase pairs of double-stranded DNA. We have shown that MX-8 can initiate a lysogenic cycle and form a stable prophage by site-specific integration into the host chromosome (Orndorff et al. 1983). In this paper we characterize the MX-8 genome by restriction enzyme analysis and generate a physical map. Although each phage particle contains within it a linear DNA molecule, we observed that the endonuclease cleavage sites for the restriction enzymes *Eco*RI, *Mbo*I, and *Pvu*II map in a circular array. A restriction map for linear DNA molecules could be circular, if the individual DNA molecules within a population differ in DNA sequence by a circular permutation, as observed for the phage P22 (Tye et al. 1974a, b; Jackson et al. 1978a, b).

Our model for MX-8 DNA encapsulation, based on the classical Streisinger model (1967), predicts the intracellular precursor to mature phage DNA is a concatemer. The length of DNA cut from the concatemer equals the amount of DNA which fits into a phage head (one "headful"). If the "headful" unit of DNA were larger than one complete set of genes, each chromosome packaged would be identical in DNA sequence at its ends (terminally redundant). Packaging of more than one headful from a concatemer by a sequential process would generate chromosomes which are both terminally redundant and circularly permuted.

In our restriction analysis of MX-8 DNA, we observed certain DNA fragments that are present in submolar yield, but precise in molecular length. DNA hybridization showed that these are derivatives of full length DNA fragments which can be placed on the circular restriction map. By studying the map position of these precise submolar fragments, we conclude that DNA packaging initiates at a unique sequence (*pac*) within a concatemer. DNA packaging proceeds in sequential fashion along the concatemer and generates DNA monomers with a terminal redundancy of approximately 4.3 kilobase pairs. We detect heterogeneous sized DNA fragments characteristic of a nested set of terminal fragments and show by DNA hybridization that they map to a region of the genome predicted by this model for sequential DNA packaging.

Materials and methods

Bacterial and bacteriophage strains. Myxococcus xanthus MD 2 (Rosenberg et al. 1977) was used for the propagation of phage by the liquid lysate technique described previously (Orndorff et al. 1983). Phage DNA was obtained from a spontaneous clear plaque mutant of MX-8 as described previously (Orndorff et al. 1983).

Restriction endonucleases. Restriction endonucleases were obtained from Bethesda Research Laboratories, Inc. (Rockville, MD, USA) and used as prescribed by the manufacturer. Before loading into the samples wells, the restriction digests were heated to 70° C for 10–15 min, and then 15 µl of 0.8% agarose in electrophoresis buffer (0.09 M Tris base-0.09 M boric acid-0.0025 M Na_2 EDTA, pH 8.3) was added to the digestion mixture.

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Agarose gel electrophoresis. Samples (40 µl) of restriction-enzyme-treated DNA were loaded into wells in horizontal 0.7% agarose slab gels (0.4 × 14 × 25 cm) prepared in electrophoresis buffer and 0.25 µg/ml ethidium bromide. Electrophoresis was carried out at 25–100 V for 6–20 h. Following electrophoresis, the restriction profiles were photographed during illumination with UV light on a transilluminator (Ultra-Violet Products Inc., San Gabriel, CA, USA). Molecular weights of MX-8 DNA restriction fragments were determined by using a *Hin*dIII digest of Lambda DNA as standard.

Isolation of restriction fragments from agarose gels. DNA restriction fragments were excised from agarose gels and placed in dialysis bags (10 mm diameter) containing 0.3 to 1.0 ml of electrophoresis buffer. The restriction fragments contained in the dialysis bags were then eluted from the agarose gels by electrophoresis at 150 V for 1.5–2 h. The electroeluted fragments were dialyzed in 1 l of 50 mM Tris-HCl (pH 7.6), 100 mM NaCl, 5 mM Na_2 EDTA for 6–24 h. The solution containing the electroeluted fragments was removed from the dialysis bags and extracted twice with an equal volume of phenol:chloroform:isoamyl alcohol (50:48:2). The aqueous phase was extracted three times with an equal volume of diethyl ether. The aqueous phase was collected and the residual diethyl ether was removed by heating at 70° C for 15–20 min. The DNA fragments were then precipitated by addition of 2 volumes of ice cold (−20° C) ethanol and stored overnight at −20° C. The precipitate was collected by centrifugation at 15,000 rpm for 30 min in a Sorvall SS-34 rotor, dried, and suspended in sterile distilled water.

Transfer of DNA to nitrocellulose filters and hybridization. Native DNA in agarose gels was denatured in situ and transferred by blotting onto nitrocellulose filters (Schleicher and Schuell) as described by Southern (1975). The filters were rinsed briefly in 2 × SSC (1 × SSC is 0.15 M NaCl, 0.015 M Nacitrate), air-dried, and baked for 3 h at 80° C in vacuo. The prepared nitrocellulose filters were placed in polyethylene bags (Sears, Seal-N-Save) to which was added a prehybridization mixture consisting of 2 × SSC, 1 × Denhardt's solution (0.02% each of Ficoll 400, polyvinylpyrrolidone, and bovine serum albumin (Denhardt 1966), 0.01% sodium dodecyl sulfate, 5 mM Na_2 EDTA, 20 µg of sheared and denatured calf thymus DNA, and 20 mM Tris [pH 7.6]). The bags were sealed and incubated at 70° C overnight. The prehybridization mixture was replaced with a similar hybridization mixture which contained 5×10^6 to 5×10^7 dpm of denatured (100° C for 10 min) radioactive probe in a total volume of 50 µl cm^{-2} of nitrocellulose. The bags were resealed and incubated at 70° C for 18–24 h. Following hybridization, the filters were washed (three times in 2 × SSC, two times in 1 × SSC, and two times in 0.5 × SSC, each wash for 10–15 min) at 70° C, air-dried, and analyzed by autoradiography.

Two-dimensional crossed-contact hybridization. The crossed-contact hybridization procedure establishes in a single blotting experiment the overlap relationships which exist between restriction fragments generated by digestion of DNA with two different restriction enzymes. In principal, the technique compares the sequence homology of each individual restriction fragment generated by digestion with one enzyme to that of each individual restriction fragment generated by digestion with a different enzyme.

In this procedure, an unlabeled DNA restriction digest is layered across the top of an agarose slab gel in a single continuous well. Following electrophoresis, the separated restriction fragments appear as continuous bands which extend across the width of the gel. The unlabeled separated restriction fragments then are immobilized on a nitrocellulose sheet by the blotting technique of Southern (1975) as modified by Wahl et al. (1979). DNA fragments generated by digestion with a different restriction enzyme are labeled in vitro with ^{32}P by nick-translation (Rigby et al. 1977). The labeled DNA restriction fragments are layered across the top of an agarose slab gel in a single continuous well and the fragments are separated by electrophoresis. The gel containing the ^{32}P-labeled DNA restriction fragments is placed in contact with the nitrocellulose sheet containing the unlabeled DNA restriction fragments in a manner such that the ^{32}P-labeled bands are rotated 90° with respect to the unlabeled bands. The bands from the labeled and unlabeled DNA therefore criss-cross one another and generate a matrix in which each fragment from the labeled DNA contacts each fragment from the unlabeled DNA. The ^{32}P-labeled DNA is blotted to the nitrocellulose sheet containing the unlabeled DNA under conditions which allow for DNA-DNA hybridization. Consequently, those bands from the two digests which share DNA homology appear as spots in the regions where the two bands intersect. The spot pattern can be used to deduce the overlap relationship which exists between fragments obtained by digestion of a particular DNA genome with two different restriction enzymes. For example, comparison of samples digested with the same restriction enzyme should yield a series of spots positioned on a diagonal line.

The following procedure for mapping DNA restriction fragments is a modification of the procedure for mapping *spo*1 DNA described by Pero et al. (1979).

*(i) Non-radioactive dimension. Eco*RI partial digests of MX-8 DNA (5 µg) were loaded into a singly continuous slot (1.5 × 3 × 120 mm) at the upper end of a 0.5% agarose slab gel (3 × 140 × 240 mm). The fragments were separated by electrophoresis (30 V; 16 h) and transferred onto a nitrocellulose filter by the procedure described by Southern (1975) and modified by Wahl et al. (1979). After transfer, the filters were baked and prehybridized as described above.

(ii) Radioactive dimension. MX-8 DNA (1–2 µg) was digested to completion with *Eco*RI and the *Eco*RI fragments were labeled in vitro by nick-translation using [α-^{32}P]dCTP (Rigby et al. 1977). The ^{32}P-labeled digest ($1–2 \times 10^8$ cpm/µg) was loaded into a single continuous well of a 0.7% agarose slab gel and the fragment separated by electrophoresis (40 V; 14 h).

(iii) Transfer-hybridization. The gel containing the labeled fragments was autoradiographed for 1 to 2 h on Kodak SB-5 X-Ray film to determine the exact number of DNA fragments. The labeled DNA fragments were denatured in situ by incubating the agarose gels for 15 min each in 2 changes of 0.5 N NaOH and 1.5 M NaCl. The gel was neutralized by incubation in 2 changes of 1.0 M Tris-HCl (pH 7.2) and 0.5 M NaCl for 20 min each change. The nitrocellulose filter containing the *Eco*RI partially digested

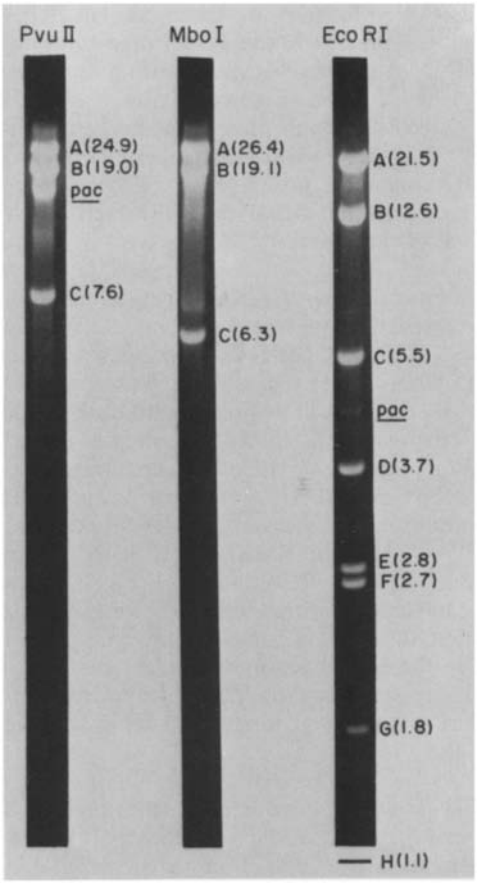

Fig. 1. Restriction digests of the *Myxococcus xanthus* bacteriophage MX-8 DNA with the restriction enzymes *Pvu*II, *Mbo*I, and *Eco*RI

phage DNA was rotated 90° and placed directly on top of the gel containing the ^{32}P-labeled totally digested *Eco*RI fragments.

The transfer-hybridization of the radioactive fragments from the gel to the filter was accomplished according to the procedure of Southern (1975), except: 1) the transfer buffer was 4 × SSC, and 2) the transfer-hybridization was carried out at 70°. The transfer-hybridization was allowed to proceed for 8–10 h after which the nitrocellulose filter was washed as described above. The washed filter was air-dried and autoradiographed.

MX-8 cloning. MX-8 DNA was isolated from purified phage particles by phenol extraction. Plasmid pBR328 (Soberon et al. 1980) and MX-8 DNA were digested with *Eco*RI, mixed and ligated with T4 DNA ligase according to standard procedures. The ligation mixture was used to transform *E. coli* strain LE 392 (Maniatis et al. 1982) for ampicillin and tetracycline resistance. Resistant transformants were transferred to LB agar plates containing 100 μg/ml of chloramphenicol to detect recombinant clones. Chloramphenicol sensitive recombinants were single colony purified and the plasmids in these strains were examined for the presence of MX-8 DNA inserts by *Eco*RI digestion. DNA prepared from *Eco*RI fragments of MX-8 cloned in pBR328 was used for nick-translation and hybridization to mature phage DNA as described in Fig. 4.

Results

Restriction endonuclease sites in MX-8 DNA

We used the DNA restriction enzymes *Eco*RI, *Mbo*I, and *Pvu*II to characterize the genome of the myxobacteriophage MX-8. Other restriction enzymes, such as *Bam*HI, *Bgl*II, *Pst*I, *Xba*I, and *Xho*I fail to cleave MX-8 DNA, while the enzymes *Alu*I, *Ava*I, *Ava*II, *Hae*III, *Hin*cII, *Sal*I, and *Sma*I generate greater than 15 DNA fragments, thereby limiting their use in preliminary mapping analysis.

Fig. 1 shows the DNA restriction fragments produced by *Eco*RI, *Mbo*I, and *Pvu*II digestion of MX-8 DNA obtained from mature phage particles. The profiles of DNA fragments separated by electrophoresis (Fig. 1) reveal that certain individual DNA fragments are present in low yield (e.g., the DNA fragment designated *pac* between *Eco*RI-C and *Eco*RI-D in Fig. 1, lane 3). These DNA fragments that are present in low yield may be precise in size and form

Table 1. Size of phage MX-8 DNA fragments produced by single and double digestions with restriction endonucleases

Fragment	Single digests			Double digests[a]					
	Size (Kb)			*Eco*RI + *Mbo*I		*Eco*RI + *Pvu*II		*Mbo*I + *Pvu*II	
	*Eco*RI	*Mbo*I	*Pvu*II	*Eco*RI Fragment	Kb	*Eco*RI Fragment	Kb	*Mbo*I Fragment	Kb
A	21.5	26.4	24.9	A^{M1}	13.2	A^{P1}	12.6	A^{P1}	17.5
B	12.6	19.1	19.0	B	12.6	A^{P2}	7.6	A^{P2}	9.0
C	5.5	6.3	7.6	A^{M2}	6.3	B^{P1}	7.1	B^{P1}	7.6
D	3.7			C	5.5	C	5.5	B^{P2}	7.5
E	2.8			D	3.7	B^{P2}	5.4	C	6.3
F	2.7			E	2.8	D	3.7	B^{P3}	3.9
G	1.8			A^{M3}	2.0	E	2.8		
H	1.1			G	1.8	F	2.7		
				F^{M1}	1.4	G	1.8		
				F^{M2}	1.3	A^{P3}	1.4		
				H	1.1	H	1.1		

[a] Capital letters followed by superscripts indicate fragments that are present in the double digests but are not present in the single digest described in the column headings of Tables 1–4. For example, A^{M1}, under heading *Eco*RI fragment, indicates that this is the largest double digestion product created by cleavage of *Mbo*I within the *Eco*RI-A fragment

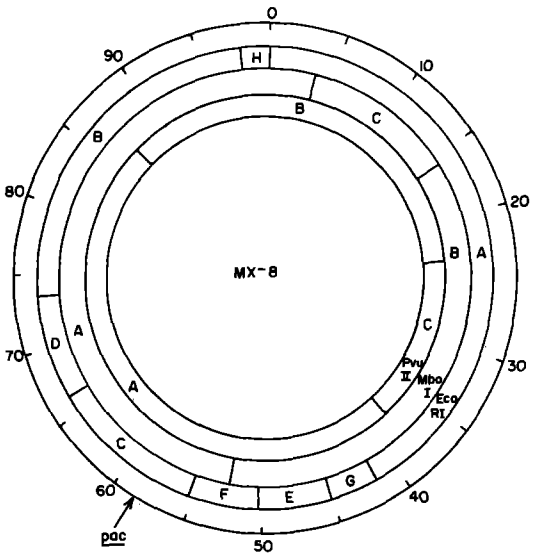

Fig. 2. Circular restriction map of MX-8 DNA for the enzymes *Pvu*II, *Mbo*I, and *Eco*RI

a sharp DNA band or heterogeneous in size and form a wide, diffuse band. The physical mapping data below, based on DNA hybridization, suggest that these submolar fragments are derivatives of major full length restriction fragments and are shorter because they have one terminus determined by the DNA packaging mechanism (see Discussion, MX-8 DNA Packaging). Table 1 presents the size of all full length DNA fragments which have each terminus determined by restriction endonuclease cleavage. These restriction fragments can be placed on a circular restriction map. Fig. 2 shows a DNA restriction map of the MX-8 genome which summarizes our mapping information.

Circular permutation in MX-8 DNA

1. Double digests. To map the restriction sites for the three enzymes used in this study, we first digested phage DNA with pairwise combinations of the restriction enzymes, and then compared the distribution of fragments from double digests with the distribution observed after digestion with each enzyme alone.

Table 1 shows the results of double digests of MX-8 DNA for the combinations *Eco*RI-*Mbo*I, *Eco*RI-*Pvu*II, and *Mbo*I-*Pvu*II. This table compares the sizes of major DNA restriction fragments from single digests with those from double digests. We observed two fragments (*Eco*RI-A and *Eco*RI-F) that are present in the *Eco*RI digest but absent in the *Eco*RI-*Mbo*I double digest, indicating that they contain *Mbo*I cleavage sites. Likewise, two fragments (*Eco*RI-A and *Eco*RI-B) that are present in the *Eco*RI digest are absent in the *Eco*RI-*Pvu*II double digest. The cleavage of two *Eco*RI fragments into five new fragments by *Mbo*I or *Pvu*II indicates that *Mbo*I or *Pvu*II each cleave MX-8 DNA a total of three times.

2. Digestions of isolated fragments. To confirm the relationship between the restriction enzyme fragments as deduced from double digests (Table 1), each of the fragments generated with *Eco*RI, *Mbo*I, *Pvu*II was isolated from preparative agarose gels and digested individually with each of the other enzymes. The results in Table 2 show that *Mbo*I cleaves the *Eco*RI-A fragment twice, yielding fragments *Eco*RI-A^{M1} (13.2 Kb), *Eco*RI-A^{M2} (6.3 Kb), and *Eco*RI-A^{M3} (2.0 Kb). In addition, *Mbo*I cleaves *Eco*RI-F once, generating *Eco*RI-F^{M1} (1.4 Kb) and *Eco*RI-F^{M2} (1.3 Kb). These results verify that a single *Eco*RI fragment (*Eco*RI-A) contains two *Mbo*I cleavage sites, that *Eco*RI-F contains one *Mbo*I site, and that there are a total of only three *Mbo*I restriction sites on the MX-8 genome. Similar results obtained from *Pvu*II digests of isolated *Eco*RI fragments (Table 2) confirm that the MX-8 genome contains only three *Pvu*II restriction sites.

3. The MboI/PvuII circular restriction map. Data presented in Table 3 show that digestion of the isolated *Mbo*I-A fragment with *Pvu*II generates two fragments, *Mbo*I-A^{P1} (17.5 Kb) and *Mbo*I-A^{P2} (9.0 Kb). The digestion of *Mbo*I-B with *Pvu*II generates three fragments, *Mbo*I-B^{P1} (7.6 Kb), *Mbo*I-B^{P2} (7.5 Kb), and *Mbo*I-B^{P3} (3.9 Kb). In the reciprocal digestions (Table 4) two fragments, *Pvu*II-A^{M1} (17.5 Kb) and *Pvu*II-A^{M2} (7.5 Kb) appear after *Mbo*I digestion of the *Pvu*II-A fragment. The results indicate that *Pvu*II-A overlaps *Mbo*I-A and *Mbo*I-B (Fig. 2), since digestion of *Pvu*II-A with *Mbo*I yields two fragments, one each in common to *Pvu*II digestion products of both the *Mbo*I-A and *Mbo*I-B fragments.

*Pvu*II-B digestion with *Mbo*I generates three fragments, a 9.0 Kb fragment common to *Mbo*I-A, the entire 6.3 Kb *Mbo*I-C fragment, and a 3.9 Kb fragment common to *Mbo*I-B. The results clearly demonstrate that *Mbo*I-C lies between *Mbo*I-A and *Mbo*I-B, and that each *Mbo*I fragment is flanked on either side by the other *Mbo*I fragments, thereby confirming the circular nature of the *Mbo*I restric-

Table 2. Digestion of isolated *Eco*RI fragments from MX-8 DNA

*Eco*RI Fragment (Kb)	Second digestion	
	MboI (Kb)	PvuII (Kb)
A (21.5)	*Eco*RI-A^{M1}(13.2), *Eco*RI-A^{M2}(6.3), *Eco*RI-A^{M3}(2.0)	*Eco*RI-A^{P1}(12.6), *Eco*RI-A^{P2}(7.6), *Eco*RI-A^{P3}(1.4)
B (12.6)	–	*Eco*RI-B^{P1}(7.1), *Eco*RI-B^{P2}(5.4)
C (5.5)	–	–
D (3.7)	–	–
E (2.8)	–	–
F (2.7)	*Eco*RI-F^{M1}(1.4), *Eco*RI-F^{M2}(1.3)	–
G (1.8)	–	–
H (1.1)	–	–

Table 3. Digestion of isolated *Mbo*I fragments from MX-8 DNA

*Mbo*I Fragment (Kb)	Second digestion	
	*Eco*RI (Kb)	*Pvu*II (Kb)
A (26.4)	*Mbo*I-A^{E1}(12.6), *Mbo*I-A^{E2}(5.5), *Mbo*I-A^{E3}(3.7) *Mbo*I-A^{E4}(2.0), *Mbo*I-A^{E5}(1.3), *Mbo*I-A^{E6}(1.1)	*Mbo*I-A^{P1}(17.5), *Mbo*I-A^{P2}(9.0)
B (19.1)	*Mbo*I-B^{E1}(13.2), *Mbo*I-B^{E2}(2.8), *Mbo*I-B^{E3}(1.8) *Mbo*I-B^{E4}(1.4)	*Mbo*I-B^{P1}(7.6), *Mbo*I-B^{P2}(7.5), *Mbo*I-B^{P3}(3.9)
C (6.3)	–	–

Table 4. Digestion of isolated *Pvu*II fragments from MX-8 DNA

*Pvu*II Fragment (Kb)	Second digestion	
	*Eco*RI (Kb)	*Mbo*I (Kb)
A (24.9)	*Pvu*II-A^{E1}(7.1), *Pvu*II-A^{E2}(5.5), *Pvu*II-A^{E3}(3.7), *Pvu*II-A^{E4}(2.8), *Pvu*II-A^{E5}(2.7), *Pvu*II-A^{E6}(1.8), *Pvu*II-A^{E7}(1.4)	*Pvu*II-A^{M1}(17.5), *Pvu*II-A^{M2}(7.5)
B (19.0)	*Pvu*II-B^{E1}(12.6), *Pvu*II-B^{E2}(5.4), *Pvu*II-B^{E3}(1.1)	*Pvu*II-B^{M1}(9.0), *Pvu*II-B^{M2}(6.3), *Pvu*II-B^{M3}(3.9)
C (7.6)	–	–

Table 5. Digestion of isolated *Eco*RI partial digestion products

Partial digestion fragments (Kb)		Limit digestion products of partial digestion fragments							
		A(21.5 Kb)	B(12.6 Kb)	C(5.5 Kb)	D(3.7 Kb)	E(2.8 Kb)	F(2.7 Kb)	G(1.8 Kb)	H(1.1 Kb)
A	16.3		+		+				
B	13.7		+						+
C	12.6		+						
D	9.2			+	+				
E	8.2			+			+		
F	7.3					+	+	+	
G	5.5			(+)		+	+		
H	4.6					+		+	
I	3.7				+				
J	2.8					+			
K	2.7						+		
L	1.8							+	
M	1.1								+

tion map (Fig. 2). Similar results for the *Pvu*II fragments (Table 4) indicate the *Pvu*II restriction map is circular.

We deduced the absolute map positions of the *Pvu*II restriction sites relative to the *Mbo*I restriction sites on the MX-8 genome from reciprocal double-digestion data. The *Pvu*II restriction sites which define *Pvu*II-A are located 17.5 Kb and 7.5 Kb from the *Mbo*I A/B border, respectively. *Pvu*II-B extends from the end of the 17.5 Kb *Pvu*II-A fragment in *Mbo*I-A to the end of *Mbo*I-A, a distance of 9.0 Kb, continues through the entire *Mbo*I-C fragment (6.3 Kb), and terminates 3.9 Kb into the *Mbo*I-B fragment. The *Pvu*II-C fragment (7.6 Kb) occupies a position between *Pvu*II-A and B, located 3.9 Kb from the *Mbo*I-B/C border and 7.5 Kb from the *Mbo*I-A/B border.

4. The EcoRI circular restriction map. We established the order of the *Eco*RI fragments by partial digestion, reciprocal double digestion, and crossed-contact hybridization.

Digestion of the isolated *Pvu*II-B restriction fragment with *Eco*RI generates a 12.6 Kb fragment which overlaps an end present in *Eco*RI-A, the entire *Eco*RI-H (1.1 Kb) fragment, and a 5.4 Kb fragment common to *Eco*RI-B (Table 4). This result assigns *Eco*RI-H a position between *Eco*RI-A and *Eco*RI-B.

In additional experiments, *Eco*RI digestion of the *Mbo*I-B fragment yields a 13.3 Kb fragment which overlaps *Eco*RI-A, the entire *Eco*RI-E and G fragments, and a 1.35 Kb derivative of *Eco*RI-F (Table 3). These data place *Eco*RI-F distal to *Eco*RI-A with respect to *Eco*RI-E and G. The relative order of the fragments is therefore F-(EG)-A-H-B.

We determined the exact order of *Eco*RI-E and G relative to *Eco*RI-F by digestion of the isolated partial products to completion with *Eco*RI. The results (Table 5) demonstrate that a 4.6 Kb partial digestion product contains the *Eco*RI-E (2.8 Kb) fragment and the *Eco*RI-G (1.8 Kb) frag-

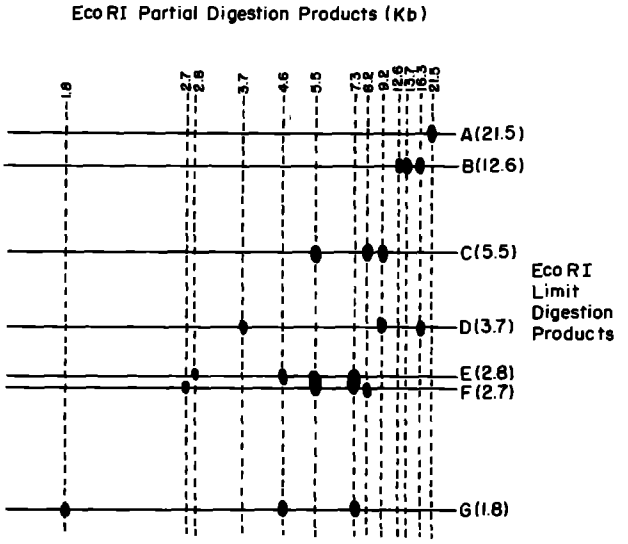

Fig. 3. Crossed-contact hybridization of *Eco*RI partial digestion products of MX-8 DNA with ^{32}P-labeled *Eco*RI limit digestion products of MX-8 DNA (see Materials and Methods). A spot indicates homology between the partial digestion product and the limit digestion product

ment. The complete digestion of a partial digestion product which comigrates with *Eco*RI-C (5.5 Kb) produces *Eco*RI-C, as expected, but also produces *Eco*RI-E (2.8 Kb) and *Eco*RI-F (2.7 Kb) fragments. Taken together, these data locate *Eco*RI-E between *Eco*RI-F and *Eco*RI-G. Since *Eco*RI-F is the most distal of *Eco*RI-E, F, or G from *Eco*RI-A, the order of the fragments must be F-E-G-A-H-B.

Further analysis of *Eco*RI partial digestion products establishes linkage between *Eco*RI-C and F, *Eco*RI-C and D, and *Eco*RI-B and D (Table 5). These results show the order for *Eco*RI fragments in the MX-8 genome is A-G-E-F-C-D-B-H.

A second method, termed crossed-contact hybridization (see Methods) was used to confirm a portion of the *Eco*RI mapping data. Figure 3 presents the results of crossed-contact hybridization between a labeled *Eco*RI partial digest of MX-8 DNA and a ^{32}P-labeled *Eco*RI limit digest. Analysis of the spot pattern from crossed-contact hybridization (Table 5) reveals that the 9.2 Kb partial digestion product contains sequences homologous to the *Eco*RI-C and D fragments. Furthermore, the 8.2 Kb partial digestion product is homologous to *Eco*RI-C and F, while the 7.4 Kb partial digestion product hybridizes to *Eco*RI-E, F, and G. These results show homology between specific partial digestion products and complete digestion products, which directly proves the overlaps predicted from data in Table 5.

Homology relationships between EcoRI, MboI, and PvuII restriction fragments

We verified by DNA hybridization experiments the homology relationships between *Eco*RI, *Mbo*I, and *Pvu*II DNA restriction fragments predicted by a region of the restriction map in Fig. 2. *Eco*RI-A, B, C, and D restriction fragments from MX-8 DNA first were cloned into the *E. coli* plasmid vector pBR328 and then labeled in vitro by nick-translation. Each of these labeled fragments was used individually to probe *Eco*RI, *Mbo*I, and *Pvu*II DNA fragments produced in single digests of mature MX-8 phage DNA (see Materials and methods). The DNA bands depicting DNA hybridization appear in Fig. 4.

The dark bands in Fig. 4 represent in most cases DNA hybridization between the *Eco*RI probe and homologous full length DNA restriction fragments present in relatively high yield in the different restriction digests. The *Eco*RI-A DNA fragment overlaps on the DNA restriction map (Fig. 2) the *Mbo*I-A, B, and C restriction fragments, and Fig. 4 shows the *Eco*RI-A DNA sequences hybridize to each full length *Mbo*I DNA restriction fragment to an extent reflecting the homology predicted by the map (Fig. 4, column 1, lane 2). Similarly, the *Eco*RI-A probe overlaps mapwise *Pvu*II-A, B, and C restriction fragments (Fig. 2), and this probe hybridizes to each *Pvu*II fragment to an extent approximately proportional to this overlap (Fig. 4,

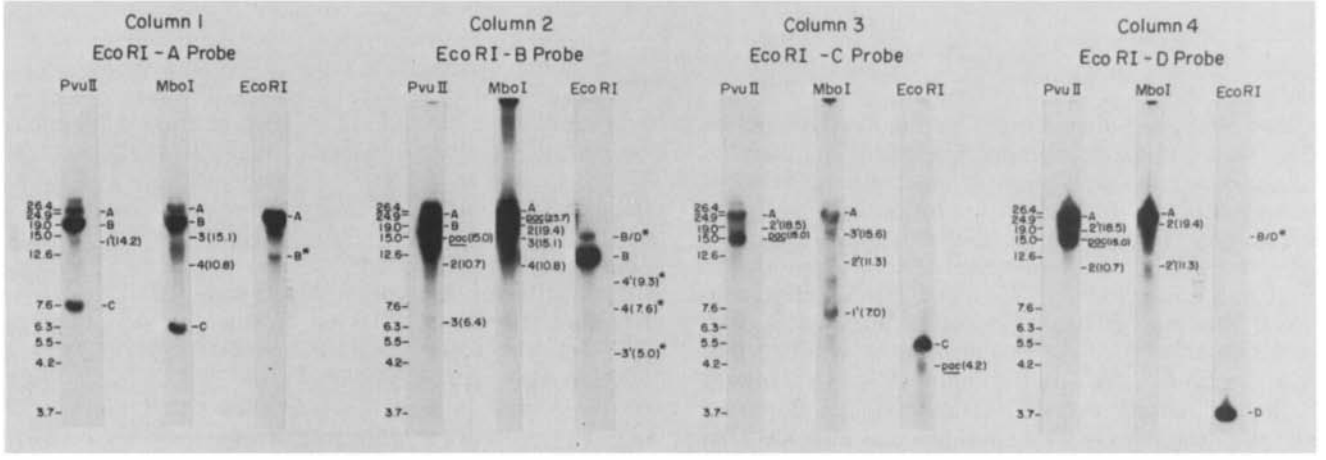

Fig. 4. Autoradiograph of *Mbo*I, *Pvu*II, and *Eco*RI restriction digests of MX-8 DNA probed with ^{32}P-labeled *Eco*RI-A, B, C and D fragments. Letters indicate full length restriction fragments. Numbers indicate "heterogeneous-sized fragments". B* indicates DNA band corresponding to non-specific hybridization of the *Eco*RI-A probe to the *Eco*RI-B restriction fragment (12.6 Kb). B/D* indicates DNA band corresponding to hybridization of the probe to a partial restriction fragment containing *Eco*RI-B and *Eco*RI-B and *Eco*RI-D. Asterisk for *Eco*RI termini denoted 3′, 4, and 4′ (column 2) indicates that DNA bands are more clearly visible in an autoradiograph made with longer exposure

column 1, lane 1). As expected, the *Eco*RI-B probe hybridizes to *Eco*RI-B in the control and to *Mbo*I-A (Fig. 4, column 2, lane 2) with which it overlaps mapwise. The *Eco*RI-B probe also hybridizes to the *Pvu*II-A and B fragments (Fig. 4, column 2, lane 1), with which it overlaps mapwise. The *Eco*RI-C probe hybridizes to *Eco*RI-C in the control, to *Mbo*I-A (Fig. 4, column 3, lane 2) with which it overlaps mapwise and to *Pvu*II-A, (Fig. 4, column 3, lane 1) with which it overlaps mapwise. The *Eco*RI-D probe hybridizes to *Eco*RI-D, to *Mbo*I-A, and to *Pvu*II-A (Fig. 4, column 4) as predicted by the restriction map in Fig. 2.

Several important bands in Fig. 4 show DNA hybridization between the *Eco*RI probe and DNA products in the restriction digest which are not unique full length DNA restriction fragments portrayed by the restriction map in Fig. 2. These DNA products may be precise in size, such as the *pac* fragment (Fig. 1, lanes 1 and 3) assumed to arise from the left terminus of the first chromosome packaged from the concatemer during DNA maturation (see Discussion); other DNA products may be heterogeneous in size and form a wide, diffuse band in the profile (Fig. 1).

The *Eco*RI-C probe (Fig. 4, column 3, lane 3) hybridizes to a DNA fragment 4.2 Kb in size which is present in the *Eco*RI restriction digest as a faint, sharp DNA band (designated *pac* in Fig. 1, lane 3). Whereas the *Eco*RI 4.2 *pac* fragment (Fig. 1) shows homology to the *Eco*RI-C restriction fragment (5.5 Kb), it lacks homology to the *Eco*RI-A, B, and D fragments (Fig. 4, columns 1, 2 and 4, lane 3). The 4.2 Kb *Eco*RI fragment therefore appears to be a short derivative of the full length *Eco*RI-C DNA restriction fragment.

The *Eco*RI-B, C and D probes hybridize to a DNA fragment 15 Kb in size in the *Pvu*II restriction digest (Fig. 4, columns 2, 3 and 4, lane 3). This 15 Kb *Pvu*II fragment appears as a sharp DNA band in *Pvu*II single digests (Fig. 1, lane 1). Since this 15 Kb *Pvu*II DNA fragment is homologous to DNA sequences within *Eco*RI-B, C, and D, which mapwise overlap *Pvu*II-A (Fig. 2), this 15 Kb *Pvu*II fragment (*pac*) appears to be a short derivative of *Pvu*II-A.

Discussion

The MX-8 genome size

Digestion of MX-8 DNA with *Eco*RI to a limit product yields eight full length restriction fragments. These are labelled A-H, according to their molecular weight, A being the largest. Digestion to a limit product with either *Mbo*I or *Pvu*II produces three full length restriction fragments, labelled A–C. Addition of the molecular weights of the eight unique full length *Eco*RI fragments (A–H), the three full length *Mbo*I fragments (A–C), or the three *Pvu*II fragments (A–C), produces the sum of 51.7 Kb, 51.8 Kb, or 51.5 Kb, respectively. The average sum for the three digests (51.7 ± 0.6 Kb) represents the molecular weight of the DNA corresponding to unique genetic information within the MX-8 DNA molecule.

An electron microscope study which measured the length of 104 MX-8 DNA molecules showed the average length to be approximately 56 Kb, relative to ϕX174 (Martin et al. 1978). These MX-8 DNA molecules ranged in size from approximately 50 Kb to approximately 62 Kb. The difference between the average value determined by electron microscopy (56 Kb), which measures the physical chromosome, and restriction analysis (51.7 Kb), which reflects the unique genetic information, can be resolved by proposing that MX-8 DNA molecules are terminally redundant. The terminal redundancy is an average of approximately 4.3 Kb or 8% of the genome. DNA molecules as large as 62 Kb in length would have a redundancy of approximately 10 Kb, or 20% of the genome.

The MX-8 circular restriction map

The individual restriction fragments for each enzyme (*Eco*RI, *Mbo*I and *Pvu*II) are placed on a map by first determining which fragments are adjacent. The neighbor relationships we deduce imply that each individual fragment must be flanked on each side, in some DNA molecules, by other full length restriction fragments. Conversely, no individual *Mbo*I, *Pvu*II or *Eco*RI restriction fragment contains DNA sequences uniquely present at the terminus of all MX-8 DNA molecules in the population. We conclude the restriction sites for *Mbo*I, *Pvu*II and *Eco*RI map in a circular array, and we draw the restriction map as a circle (Fig. 2).

Isolation of the *Eco*RI DNA restriction fragments from a limit digest and subsequent digestion by either *Mbo*I or *Pvu*II proves unequivocally that the MX-8 genome contains three *Mbo*I restriction sites and three *Pvu*II restriction sites. Since DNA molecules from mature phage particles are linear as viewed in the electron microscope, the three *Mbo*I or *Pvu*II restriction sites might be expected to generate four unique *Mbo*I or *Pvu*II restriction fragments in single digests. This expectation would arise if the population of MX-8 DNA molecules were homogeneous with all molecules identical in DNA sequence.

The digestion of MX-8 DNA with *Mbo*I, however, yields only three full length unique fragments in single digests. The digestion of MX-8 DNA by *Pvu*II also generates only three full length DNA restriction fragments; a fourth DNA fragment, approximately 15 Kb in size, appears in the restriction profile (Fig. 1, lane 1), but this fragment is not unique, since it is shown by DNA hybridization to be a derivative of the *Pvu*II-A fragment. Redigestion of each of the three major *Mbo*I or *Pvu*II fragments by a second enzyme satisfies the rule that the sum of the molecular weights for the subfragments derived from a particular major fragment always equals the molecular weight of this major fragment. This suggests no hypothetical fourth fragment is comigrating with another major fragment. Moreover, the size of the genome (51.7 ± 0.6 Kb) agrees for each of the three digests (*Eco*RI, *Mbo*I and *Pvu*II), suggesting that in the restriction profile for *Mbo*I and *Pvu*II the total number of full length restriction fragments observed (three) is the correct number.

A linear restriction map for a population of identical linear MX-8 DNA molecules would contradict this number of fragments. If the three restriction sites for *Mbo*I or *Pvu*II were placed upon a circular restriction map (Fig. 2), endonuclease cutting could generate the three unique fragments observed for each enzyme. This argument suggests the mature linear DNA molecules from a population of MX-8 phage particles are heterogeneous and differ in DNA sequence by circular permutations.

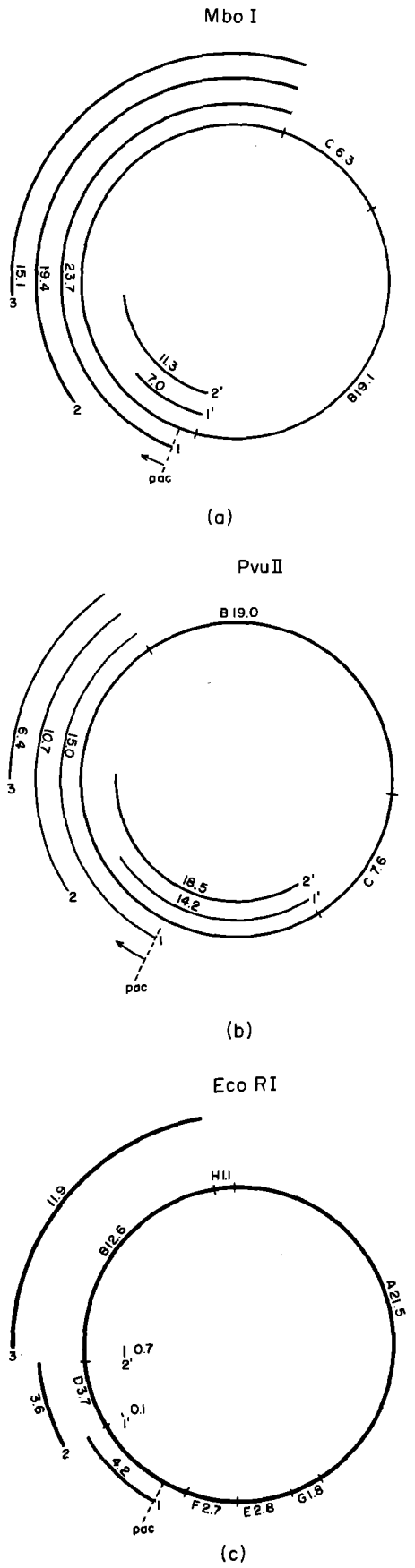

Fig. 5. *Mbo*I, *Pvu*II, and *Eco*RI packaging models for MX-8 DNA. These models show the *pac* fragments and heterogeneous sized fragments that would be expected for each restriction digest if sequential packaging starts at the *pac* site

The MX-8 DNA packaging process

Fig. 5 presents a model for the origin of circular permutation within a population of MX-8 DNA molecules. We assume that the precursor to mature DNA within phage particles is a concatemer. The model predicts that DNA encapsulation initiates at a unique site on the concatemer, *pac*, located 58 map units from the origin on the circular restriction map. (The origin of the map is arbitrarily defined as the junction between *Eco*RI-A and *Eco*RI-H.). The model also predicts that DNA packaging proceeds sequentially along the concatemer in a clockwise direction relative to the map.

Fig. 5 depicts the termini for individual MX-8 chromosomes predicted from the model. The left terminus of each headful of DNA sequentially packaged (1, 2 or 3) is designated by a solid curved line (1, 2 or 3) drawn outside the circle. The right terminus is designated by the curved line (1′, 2′) drawn inside the circle. The length of the curved offset line reflects the approximate size of the restriction fragment derivative corresponding to the terminus of the MX-8 DNA molecule after DNA restriction. The numeral 1 refers to the fragment originating at the *pac* site, which determines the left terminus of the DNA in the first headful packaged. The numeral 1′ represents the DNA fragment from the right terminus of the first chromosome packaged. The right terminus occurs at a site located in the model approximately 51.7 plus 4.3 Kb clockwise around the map. This site also defines the initiation site for packaging the second headful unit of DNA; the second DNA unit packaged therefore has termini displaced on the map from the first headful unit by the length of the terminal redundancy (on average 4.3 Kb). This model makes specific predictions about the map position for the termini of molecules packaged sequentially within the first three headfuls.

DNA packaging initiating at a specific site (*pac*) at 58 map units will create a terminus for one precisely sized DNA restriction fragment in the DNA restriction profile. For *Eco*RI, there is one precisely sized DNA fragment [4.2 Kb] present in low yield which migrates between *Eco*RI-C [5.5 Kb] and *Eco*RI-D [3.7 Kb] (Fig. 1, lane 3). This fragment hybridizes to an *Eco*RI-C probe, but not to an *Eco*RI-A, B or D probe, indicating it is a short derivative of the full length *Eco*RI-C restriction fragment. For *Pvu*II, there is one precisely sized DNA fragment migrating at the 15 Kb position (Fig. 1, lane 1). This fragment is not a unique full length *Pvu*II restriction fragment, because it is homologous to DNA sequences in the *Pvu*II-A fragment. It hybridizes to the *Eco*RI-B, C, and D probes, but not to the *Eco*RI-A probe. Since *Eco*RI-B, C, and D define a region on the *Pvu*II-A restriction fragment distal from the region in which *Eco*RI-A and *Pvu*II-A overlap (Fig. 2), the 15 Kb *Pvu*II fragment terminus corresponds to a site 15 Kb from the *Pvu*II-A/B border (Fig. 5b). Oriented relative to the *Eco*RI map, the terminus for the 15 Kb *Pvu*II fragment is within the *Eco*RI-C region 4.2 Kb from the *Eco*RI-C/D border. The 15 Kb *Pvu*II fragment and the 4.2 Kb *Eco*RI fragment therefore support the model in Fig. 5b and Fig. 5c, respectively, that the left end of the first DNA molecule packaged from the concatemer maps to a position 58 map units from the origin. For *Mbo*I, the fragment defining *pac* should originate in *Mbo*I-A and extend 23.7 kB to the *Mbo*I-A/C border (Fig. 5a). Unfortu-

nately, this band is obscured by its proximity to the *Mbo*I-A fragment 26.4 Kb in size (Fig. 1, lane 2).

If the "headful" measuring process in DNA packaging were to be imprecise, the termini of MX-8 DNA molecules would produce heterogeneous sized DNA fragments upon restriction. These terminal fragments would form a nested set and appear characteristically in a restriction profile as a faint diffuse DNA band. A model for DNA packaging makes specific predictions about the map position for "heterogeneous" sized DNA fragments.

In the *Mbo*I restriction digest, the right terminus of the first chromosome packaged should lead to heterogeneous sized fragments approximately 7.0 Kb in size (that is, 2.7 Kb+4.3 Kb; Fig. 5a). Figure 1 shows that for *Mbo*I there is a class of heterogeneous sized DNA fragments at the 7.0 Kb position. Figure 4 (lane 2) shows these DNA fragments hybridize to the *Eco*RI-C probe, but not to the *Eco*RI-A, B or D probes. This maps the heterogeneous sized fragments approximately 7.0 Kb in size to a region within *Mbo*I-A, proximal to the *Mbo*I-A/B border and distal to the *Mbo*I-A/C border, as predicted by the model.

Packaging of the second headful should produce chromosomes with termini which produce heterogeneously sized fragments approximately 19.4 Kb and 11.3 Kb in size after *Mbo*I digestion (designated "2" and "2′", respectively, Fig. 5a). Although the specific size of such bands cannot be discerned in the *Mbo*I restriction profile (Fig. 1, lane 2), they do appear to be present. Fig. 4 shows clearly that heterogeneously sized DNA fragments about 11–12 Kb in size do exist. These DNA fragments hybridize to both *Eco*RI-C and D probes, which confirms that they contain DNA sequences extending from the *Mbo*I-A/B border to a position 11.3 Kb into the *Mbo*I-A fragment. Figure 4 also shows that the heterogeneously sized 19.4 Kb *Mbo*I fragments are present and hybridize strongly to *Eco*RI-D but very weakly to *Eco*RI-C. The hybridization shows that these fragments extend from the *Mbo*I-A/C border to a position in *Mbo*I defined by the *Eco*RI-C/D border, a distance of approximately 19.4 Kb.

For the *Pvu*II restriction digest, the model for DNA packaging (Fig. 5b) predicts a 14.2 Kb DNA band for heterogeneous sized DNA fragments corresponding to the right terminus of the first DNA headful packaged. The DNA hybridization probes *Eco*RI-A and *Eco*RI-C clearly detects heterogeneous sized DNA fragments this size (Fig. 4, column 1, lane 1).

Packaging of the second headful of DNA should lead to DNA fragments in the *Pvu*II digest approximately 10.7 Kb in size for the left terminus and 18.5 Kb for the right terminus. Heterogeneous sized DNA fragments do appear at the position for 10.7 Kb DNA fragments and hybridize to the *Eco*RI-B and *Eco*RI-D probes, but not the *Eco*RI-A or *Eco*RI-C probe, which supports the model (Fig. 4, lane 1). Moreover, heterogeneous sized DNA fragments 18.5 Kb in size appear as predicted and hybridize to the *Eco*RI-C and *Eco*RI-D probes.

In the *Eco*RI digest, the terminal fragments from the left end for the first three DNA headfuls (designated 1, 2, and 3, Fig. 5c) should be 4.2 Kb, 3.6 Kb, and 11.9 Kb in size. The 4.2 Kb DNA fragments appear as a sharp DNA band, as expected for *pac* fragments. Unfortunately, the 3.6 Kb and 11.9 Kb classes of heterogeneous fragments are almost equal in size to the major full length restriction fragment hybridizing to the probe (*Eco*RI-D and *Eco*RI-B, respectively), and are therefore obscured in the restriction profile. Moreover, the right termini (designated 1′ and 2′, Fig. 5c) are predicted to be 0.1 Kb and 0.7 Kb, which are too small to detect by the hybridization probes (Fig. 4). Verification of the packaging model for the *Eco*RI digest emerges, however, for the third and fourth DNA headfuls packaged. The model predicts that fragments corresponding to the 3′, 4, and 4′ chromosomal ends should produce heterogeneous sized fragments approximately 5.0 Kb, 7.6 Kb, and 9.3 Kb in size. DNA hybridization (Fig. 4, column 2, lane 3) verifies that these DNA fragments exist. They hybridize specifically to the *Eco*RI-B probe, and not to the *Eco*RI-A, C or D probes, as predicted.

MX-8 DNA integration

During lysogenization MX-8 phage DNA integrates into the host chromosome by recombination between a specific DNA site on the host chromosome (called *att* B) and a specific DNA site on the phage chromosome (called *att* P). The phage integration site (*att* P) resides within the *Eco*RI-B restriction fragment leading to cleavage of this region of the MX-8 genome during prophage formation (Orndorff et al. 1983). Figure 2 shows the relative position of the *Eco*RI-B fragment in the map for mature phage DNA. This restriction map indicates the DNA site for integration within *Eco*RI-B is physically distant from the *pac* site for DNA packaging. Therefore, cleavage of the MX-8 genome during integration, and cleavage during DNA packaging, probably occur by independent processes.

The sequential packaging process initiating at *pac* produces linear MX-8 DNA molecules that are circularly permuted and terminally redundant within phage particles. Upon injection into the cell, a terminally redundant linear DNA molecule could form a circle through recombination between its ends, as shown classically for phage P22 (Botstein and Martz 1970). Circle formation by this mechanism predicts each full length *Eco*RI restriction fragment will be present in prophage DNA (except *Eco*RI-B, which is split into two fragments during integration). DNA hybridization probes to characterize the structure of prophage DNA verified this prediction (Orndorff et al. 1983).

The map position for *att* P within *Eco*RI-B suggests this DNA fragment might encode the genetic information sufficient for integration. To test this possibility, we introduced into *M. xanthus* the *Eco*RI-B fragment isolated and cloned into the plasmid vector pBR328. This element of the MX-8 genome was transferred from *E. coli* into *M. xanthus* cells using a suicide vector (*E. coli* phage P1), which fortuitously adsorbs to *M. xanthus* and injects foreign DNA (Kaiser and Dworkin 1975). We observed the cloned *Eco*RI-B fragment transferred to *M. xanthus* integrates into the *att* B site on the host chromosome by the normal MX-8 integration process. This raises the possibility of using the integration genes residing in the *Eco*RI-B DNA fragment to integrate cloning vectors into *M. xanthus* at the unique *att* B site on the chromosome. This could lead to the production of partially diploid strains valuable for genetic complementation.

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References

Botstein D, Martz MJ (1970) A recombination function essential to the growth of bacteriophage P22. J Mol Biol 54:417–440

Denhardt DT (1966) A membrane filter technique for the detection of complementary DNA. Biochem Biophys Res Commun 23:641–646

Jackson E, Jackson D, Deans R (1978a) *Eco*RI analysis of bacteriophage P22 DNA packaging. J Mol Biol 118:365–388

Jackson E, Miller H, Adams M (1978b) *Eco*RI restriction endonucleast cleavage site map of bacteriophage P22 DNA. J Mol Biol 118:347–363

Janssen G (1984) Ph D Thesis, Cell-cell interactions during development of lysis of *Myxococcus xanthus*. University of Minnesota

Kaiser D (1984) Genetics of myxobacteria. In: Rosenberg E (ed) Myxobacteria: Development and cell interactions. Springer, Berlin Heidelberg New York Tokyo

Kaiser D, Dworkin M (1975) Gene transfer to a myxobacterium by *Escherichia coli* phage P1. Science 187:653–654

Kaiser D, Manoil C, Dworkin M (1979) Myxobacteria: Cell interactions, genetics and development. Ann Rev Microbiol 33:595–639

Maniatis T, Fritsch EF, Sambrook J (1982) Molecular cloning: A laboratory manual. Cold Spring Harbor, New York, p 504

Martin S, Sodergren E, Masuda T, Kaiser D (1978) Systematic isolation of transducing phages of *Myxococcux xanthus*. Virology 88:44–53

Orndorff P, Stellwag E, Starich T, Dworkin M, Zissler J (1983) Genetic and physical characterization of lysogeny by bacteriophage MX8 in *Myxococcus xanthus*. J Bacteriol 154:772–779

Pero J, Hannett N, Talkington C (1979) Restriction cleavage map of SPO1 DNA: General location of early, middle and late genes. J Virol 31:156–171

Rigby PWJ, Dieckman M, Rhodes C, Berg P (1977) Labeling deoxyribonucleic acid to high specific activity in vitro by nick translation with DNA polymerase I. J Mol Biol 113:237–251

Rosenberg E, Keller K, Dworkin M (1977) Cell density-dependent growth of *Myxococcus xanthus* on casein. J Bacteriol 129:770–777

Soberon X, Covarrubias C, Bolivar F (1980) Construction and characterization of new cloning vehicles. IV. Deletion derivatives of pBR322 and pBR325. Gene 9:287–305

Southern EM (1975) Detection of specific sequences among DNA fragments separated by gel electrophoresis. J Mol Biol 98:503–517

Streisinger G, Emrich J, Stahl M (1967) Chromosome structure in phage T 4. III. Terminal redundancy and length determination. Proc Natl Acad Sci USA 57:292–295

Tye B-K, Chan R, Botstein D (1974b) Packaging of an oversize transducing genomes by *Salmonella* phage P22. J Mol Biol 85:485–500

Tye B-K, Huberman J, Botstein D (1974a) Nonrandom circular permutations of phage P22 DNA. J Mol Biol 85:501–532

Wahl G, Stern M, Stark G (1979) Efficient transfer of large DNA fragments from agarose gels to diazobenzylexymethyl-paper and rapid hybridization by using dextran sulfate. Proc Natl Acad Sci USA 76:3683–3687

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