THE *BAM* H1 RESTRICTION SITE IN THE BACTERIOPHAGE T4 CHROMOSOME IS LOCATED IN OR NEAR GENE 8

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ABSTRACT

Bacteriophage T4 cytosine-containing DNA is cleaved at a single site by the restriction endonuclease, *Bam* H1. The site lies within the late region of the T4 genome, close to, or within, gene 8, one of the structural genes of the phage particle baseplate.

THE chromosome of bacteriophage T4 contains glucosylated hydroxymethylcytosine in place of cytosine (WYATT and COHEN 1953) and, in consequence, is resistant to cleavage by many restriction endonucleases. Multiply mutated derivatives of T4 have been isolated that are unable to carry out this modification and instead incorporate cytosine into their DNA (SNYDER, GOLD and KUTTER 1976). The phage DNA derived from such mutants is sensitive to a wide range of restriction endonucleases and, in general, is cleaved at the frequencies expected on the assumption of random base distribution.

An exception to the rule is the restriction endonuclease, *Bam* H1. The expected number of *Bam* H1 cleavage sites in the T4 chromosome is 16; however, when cytosine-containing T4 DNA is treated with *Bam* H1 and the DNA electrophoresed through agarose gels, no discrete fragment pattern is seen. This observation has led to the suggestion that the T4 chromosome either lacks *Bam* H1 sites altogether or those it possesses remain protected in cytosine-containing DNA (WILSON, TANYASHIN and MURRAY 1977).

We have reexamined T4 DNA treated with *Bam* H1 endonuclease and find evidence for the existence of a single cleavage site. Due to the circularly permuted nature and large size of the T4 chromosome, cleavage at a single site is not readily apparent in single digests with *Bam* H1, but in double digests, using other restriction endonucleases in conjunction with *Bam* H1, the presence of a unique *Bam* H1 cleavage site is demonstrable. TAKAHASHI, et al. (1979) have independently reached the same conclusion. In this report, we confirm that the chromosome of phage T4 is cleaved once by *Bam* H1 and demonstrate that the cleavage site is located close to the junction between T4 genes 7 and 8, probably within gene 8 itself.

MATERIALS AND METHODS

Bacterial strains: Three derivatives of *E. coli* K were used: RR1 (*hsdR*⁻, *hsdM*⁻, *sup*^o; BOLI-VAR *et al.* 1977) as the host for plasmid recombinants, QD Sup3 (*hsdR*⁺, *hsdM*⁺, *sup*3) as the permissive host for T4 amber mutants, and ED 8689 (*hsdR*⁻, *hsdM*⁺, *sup*^o; kindly supplied by NOREEN MURRAY) as the amber-restrictive host in mixed indicator tests and the permissive host for the growth of the cytosine-incorporating T4 mutant, GT7.

Phage strains: The following phage T4 amber mutants, obtained from the Cal Tech collection with the kind assistance of HELEN REVEL, were used: N135 and NG322 (gene 5); NG232, N102 and B250 (gene 6); B16, NG217, NG218, NG437, NG393, NG295 and NG80 (gene 7); N132, NG240 and C80 (gene 8); E343, E17, E570, C215, C226, NG118, and NG577 (gene 9); B255, NG153 and NG430 (gene 10); N128 (gene 11) and N69 (gene 12). The multiply mutated strain T4GT7 (*am* C87, *am* E51, *rNB5060*, *alc*⁻) was used for the preparation of cytosine-containing T4 DNA (WILSON, TANYASHIN and MURRAY 1977).

Bacterial and phage growth: Bacteria were grown at 37° in L-broth, containing per liter: 10 gm Difco Bacto Tryptone, 5 gm Difco Yeast Extract, 5 gm NaCl, 1 gm glucose, pH 7.2, or on L-agar plates containing the same plus 15 gm agar per liter.

Phage were grown at 37° in tryptone broth, containing the same ingredients per liter as L-broth, but without the yeast extract. They were titred on tryptone agar plates consisting of tryptone broth plus 12 gm agar per liter. Indicator cells were poured onto the plates in top agar consisting of tryptone broth plus 6 gm agar per liter.

Phage were diluted in 10 mM Tris, pH 7.5, 10 mM MgCl₂ and titred on QD Sup3. Marker rescue tests were performed by spotting serially diluted phage onto lawns seeded with either 100% plasmid-containing RR1 or, when a mixed indicator was preferred, a combination of 20% plasmid-containing RR1 and 80% plasmid-free ED 8689.

DNA purification: Cytosine-containing T4 DNA was isolated from DNase- and RNase-treated lysates of T4 GT7 after concentration and CsCl buoyant banding, followed by phenol extraction (THOMAS and ABELSON 1966). The DNA was subsequently dialyzed against, and stored in, 10 mM Tris, pH 7.5, 1 mM EDTA.

Supercoiled plasmid DNAs were isolated by the CsCl-ethidium bromide buoyant banding method (CLEWELL and HELSINKI 1969). Prior to lysis with lysozyme, EDTA and Triton X-100, cultures were treated with 200 µg per ml chloramphenicol. Ethidium bromide was removed from the plasmids by extraction with n-butanol and subsequently the DNA was dialyzed against, and stored in, 10 mM Tris, pH 7.5, 1 mM EDTA.

Small-scale plasmid preparations were obtained from nonamplified, stationary phase, 5 ml cultures by a similar lysis and clearing procedure, followed by phenol-chloroform extraction, ether extraction, ethanol precipitation and final resuspension in 10 mM Tris, pH 7.5, 1 mM EDTA. Yields were generally in excess of 1 µg, sufficient for restriction analysis and gel electrophoresis.

Restriction endonuclease digestion: Restriction endonucleases *Bam* HI and *Bgl* II were purchased from New England Biolabs. The remaining enzymes, *Eco* R1, *Hind* III, *Pst* I, *Sal* I, *Sst* I, *Xba* I and *Xho* I, were purchased from Bethesda Research Labs. With the exception of *Eco* R1, all digestions were carried out in 10 mM Tris, pH 7.5, 10 mM MgCl₂, 10 mM β-mercaptoethanol, 50 mM NaCl, 0.005% Triton X-100, at 37° for one hr. *Eco* R1 digestions were carried out under identical conditions except that the NaCl concentration was 100 mM. Double digestions were performed simultaneously except in the case of *Bam* HI plus *Eco* R1, which was performed sequentially. Digestions were terminated by heat inactivation of the enzymes at 72° for ten minutes. Enzymes and digested DNA were stored at -20°.

Gel electrophoresis: Electrophoresis was carried out on a 12 cm horizontal slab gel apparatus built to the design of McDONELL, SIMON and STUDIER (1977). 1% agarose (Biorad) gels were routinely used with a tris-acetate running and gel buffer consisting of 40 mM Tris, pH 8.2 20 mM sodium acetate, 1 mM EDTA. DNA samples were mixed with one-third their volume of 10% ficoll 400 (Sigma), 0.025% bromophenol blue prior to loading. Electrophoresis was performed at 60 volts for five to six hr, and gels subsequently stained with 10 µg per ml ethidium bromide

for ten min, washed with several changes of water for 15 min, and photographed under UV light on type 55 Land film with a Polaroid camera system.

DNA ligation and transformation: Following restriction endonuclease digestion and heat inactivation, DNAs intended for ligation and recovery were mixed with one-tenth volume of a ligation cocktail containing, per ml: 600 μ l 1 M Tris, pH 7.5, 25 μ l 0.4 M EDTA, pH 9.0, 100 μ l 1 M $MgCl_2$, 100 μ l 1 M dithiothreitol, 30 μ l 0.1 M ATP. One μ l of T4 DNA ligase (Bethesda Research Labs) was added per 2.5 μ g of DNA in a total reaction volume of 50 μ l, and thereafter the samples were incubated at 17° for six to eight hr, after which they were used in 10 μ l quantities to transform competent bacterial cells.

E. coli RR1 was made competent for the uptake of DNA by Ca^{2+} shock treatment (LEDERBERG and COHEN 1974). Cells were grown to log phase at 37° in broth, centrifuged and resuspended first in one-half volume ice-cold 0.1 M $MgCl_2$, then in one-twentieth volume ice-cold 0.1 M $CaCl_2$, and finally kept on ice for one hr before use. Transformation was carried out by mixing 0.25 μ g DNA, 100 μ l of a solution consisting of one part 1X SSC (0.15 M NaCl, 0.015 M sodium citrate) and two parts 0.1 M $CaCl_2$, and 200 μ l of ice-cold competent cells. After 30 min on ice, the cells were heat shocked at 42° for two min, returned to ice for 15 min, then diluted 20-fold into fresh broth and incubated at 30° overnight.

pBRH3B (NEVE, WEST and RODRIGUEZ 1979), a derivative of the ampicillin-resistant, tetracycline-resistant plasmid pBR316 (BOLIVAR *et al.* 1977) was used as a cloning vehicle for T4 DNA fragments. It carries a single restriction target for each of the endonucleases, *Eco* R1 and *Bam* H1. After incubation, transformed cells carrying plasmids were selected by plating in the presence of 20 μ g/ml ampicillin.

RESULTS

Restriction endonuclease digestions: When T4 cytosine containing DNA is treated with *Bam* H1 and the product electrophoresed through agarose gels, it runs as a single, diffuse, high molecular weight band. In contrast, when it is digested with other restriction endonucleases, characteristic sets of discrete bands are generated. We have noticed that the DNA banding patterns of such digests are altered in a consistent manner when they are redigested with *Bam* H1. In most cases examined, *Bam* H1 digestion causes the disappearance of one of the original bands in the electrophoretogram and the concomitant appearance of two new, smaller bands (Figure 1). In those instances where all three fragments can be unambiguously identified, the molecular weights of the two new subfragments add up to that of the lost fragment (Table 1). It is not evident in all of the double-digest combinations that one fragment is lost and two new ones are formed; in these cases, we assume that the pattern is obscured by the superimpositions of other bands. Since we never observe the disappearance of more than one band, nor the formation of more than two new ones, the data argue strongly that there is one, and only one, *Bam* H1 site in the entire chromosome.

Digestion of *Eco* R1-digested T4 DNA with *Bam* H1 results in the disappearance of the 3.7 Kb *Eco* R1 fragment number 11 and the appearance of two new subfragments of 1.1 and 2.6 Kb. The change is rather difficult to see in total T4 digests since all three bands electrophorese close to other T4 fragments, leaving their identification somewhat doubtful. To eliminate the uncertainty and to determine the genetic location of the *Bam* H1 site precisely, we decided to clone the 3.7 Kb *Eco* R1 fragment and analyze it in isolation from the rest of the T4 chromosome. The motivation to do this came from the fact that this particular

FIGURE 1.—Restriction endonuclease digestions of T4 cytosine-containing DNA separated by gel electrophoresis. Eight pairs of digestions are shown: the first member of each pair is the product of single digestion, the second, the product of double digestion in conjunction with *Bam* H1.

Lanes a and b: *Sal* I and *Sal* I + *Bam* H1, respectively;

Lanes c and d: *Xho* I and *Xho* I + *Bam* H1;

Lanes e and f: *Bgl* II and *Bgl* II + *Bam* H1;

Lanes g and h: *Xba* I and *Xba* I + *Bam* H1;

Lanes i and j: *Sst* I and *Sst* I + *Bam* H1;

Lanes k and l: *Pst* I and *Pst* I + *Bam* H1;

Lanes m and n: *Eco* RI and *Eco* RI + *Bam* H1;

Lanes o and p: *Hind* III and *Hind* III + *Bam* H1;

Lane q: Molecular weight markers: mixture of λ CI⁸⁵⁷ DNA digested with *Eco* RI and the same digested with *Hind* III.

Treatment of digested T4 cyt-DNA with *Bam* H1 causes the fragment pattern to change; with the enzymes *Xba* I, *Pst* I and *Hind* III, it is clear that one band disappears and two new ones form. Though less easy to distinguish, the same change can be seen with the enzymes *Xho* I, *Sst* I and *Eco* RI; with *Sal* I and *Bgl* II, the change is almost completely obscured. A molecular size scale, calibrated in kilobase pairs, is shown on the left side of the figure.

TABLE 1

T4 restriction fragments cleaved by *Bam* H1

Restriction Endonuclease	Fragment Cleaved by <i>Bam</i> H1	Molecular Size (Kb)	
		Subfragment Products	
<i>Xho</i> I	18	10	+ 8
<i>Xba</i> I	12	6.5	+ 5.5
<i>Sst</i> I	22	12	+ 10
<i>Pst</i> I	3.8	1.9	+ 1.9
<i>Eco</i> RI	3.7	2.6	+ 1.1
<i>Hind</i> III	11	6.0	+ 5.0

The approximate molecular sizes (in kilobase pairs) of the restriction fragments of T4 DNA cleaved by *Bam* H1 and the resulting subfragment products.

T4 fragment is one of the few whose genetic composition has been determined; it has been shown to contain part of the late T4 genes, 7 and 8 (WILSON, TANYASHIN and MURRAY 1977). We anticipated that cloning the fragment and successively deleting the sequences on either side of the *Bam* H1 site would enable us to determine with precision its location relative to genes 7 and 8.

Plasmid recombinants: From a "shotgun" molecular cloning experiment, 144 plasmid recombinants carrying inserted fragments of *Eco* RI-cleaved T4 cytosine containing DNA were isolated and screened electrophoretically to identify those carrying the 3.7 Kb fragment. Several such recombinants were found; of these, two, which carried the fragment in opposite orientations (see below), were chosen for further analysis.

The two recombinants are referred to as p11R and p11S. Both were found to carry only the 3.7 Kb *Eco* RI fragment, which, as anticipated, contained a single *Bam* H1 site. On double digestion with *Eco* RI and *Bam* H1, the 3.7 Kb *Eco* RI fragment was cleaved into the two subfragments of 2.6 and 1.1 Kb that were noted earlier in total T4 DNA digestions (Figure 2). The plasmid vehicle also carries a single *Bam* H1 cleavage site, within the tetracycline-resistance gene, 0.4 Kb away from the *Eco* RI insertion site. Digestion of p11R and p11S with *Bam* H1 alone gave complementary electrophoretic patterns consistent with their carrying the T4 fragment in opposite orientations; digestion of p11R with *Bam* H1 generated a 1.5 Kb fragment, evidently comprising the smaller T4 subfragment plus the beginning of the tetracycline resistance gene, while digestion of p11S generated the alternative 3.0 Kb composite fragment (Figures 2 and 3).

Following cleavage of the two recombinants with *Bam* H1 and subsequent ligation, deleted derivatives that lacked the composite *Bam* H1 fragment were isolated. Five transformant colonies from each ligation were chosen at random and screened, and in all ten instances the plasmids that they carried were found to have lost the appropriate *Bam* H1 fragment. One recombinant from each set was retained; p11R Δ , derived from p11R, carries only the 2.6 Kb subfragment of T4 DNA, while p11S Δ , derived from p11S, carries the alternative 1.1 Kb T4

FIGURE 2.—Restriction fragment analysis of the plasmid recombinants:

Lanes a, b and c: *Eco* R1 digestions of p11S, p11R and T4 cyt-DNA respectively;

Lanes d, e and f: *Eco* R1 + *Bam* H1 double digestions of T4 cyt-DNA, p11S and p11R respectively;

Lanes g, h, i, j and k: *Bam* H1 digestions of p11S, p11R, T4 cyt-DNA, p11S Δ and p11R Δ respectively;

Lanes l, m and n: *Eco* R1 + *Bam* 1 double digestions of p11S Δ , p11R Δ and T4 cyt-DNA respectively;

Lanes o, p and q: *Eco* R1 digestions of T4 cyt-DNA, p11S Δ and p11R Δ respectively.

A molecular size scale (in kilobase pairs) is shown to the left of the figure.

Digestion of p11S and p11R with *Eco* R1 demonstrate that both recombinants carry the 3.7 Kb T4 *Eco* R1 fragment number 11 (lanes a, b and c). When digested with *Eco* R1 plus *Bam* H1, the fragment is cleaved into 1.1 Kb and 2.6 Kb subfragments (lanes d, e and f), and the asymmetric location of the *Bam* H1 site within the T4 fragment reveals that p11S and p11R carry it in opposite orientations (lanes g and h). The deleted plasmid derivatives, p11S Δ and p11R Δ , carry separate *Eco* R1 + *Bam* H1 subfragments (lanes j, k, l, m, p, and q).

FIGURE 3.—The structures of the plasmid recombinants p11R and p11S and their deleted derivatives p11RΔ and p11SΔ. Only part of the plasmid vehicle, represented by a single line, is shown in each case. The arc-shaped blocks represent the T4 sequences carried by the recombinants. The sections shown stippled are present in the plasmids, while those indicated by dashed lines have been removed by *Bam* H1 cleavage. Open arrow heads indicate *Eco* R1 cleavage sites, filled arrow heads, *Bam* H1 cleavage sites. The entire T4 *Eco* R1 fragment is 3.7 Kb in length; the two *Eco* R1-*Bam* H1 subfragments are 2.6 Kb and 1.1 Kb long. The *Bam* H1 site in the plasmid vehicle is approximately 0.4 Kb from the *Eco* R1 insertion site.

subfragment (Figures 2 and 3). The parental plasmids and their deleted derivatives were subsequently tested *in vivo* with a number of T4 amber mutants to establish their genetic composition.

Marker rescue: Each recombinant plasmid was tested for its ability to support the growth of T4 amber mutants in genes 5 through 12, covering the region of the T4 genome from which the 3.7 Kb *Eco* R1 fragment is known to originate. The tests were performed by spotting dilutions of the amber phage onto plates seeded with the *E. coli* strain RR1 (BOLIVAR *et al.* 1977) harboring the plasmids. This host was chosen because it was nonpermissive for the growth of many of the amber phages used; in those instances in which it proved either "leaky" or permissive, it was used in conjunction with a less permissive plasmid-free host as a mixed plating indicator. Whether pure or mixed indicator lawns were used, vigorous growth of the phage on the bacterial lawn was dependent upon rescue of the wild-type allele from the T4 fragment resident on the plasmid. Provided that sufficient mutant phage ($>10^4$) were spotted, the ability of the plasmid to support marker rescue could be determined unambiguously.

The parental plasmids p11R and p11S behaved identically in the tests, rescuing all the gene 8 amber mutants with which they were tested, and some in the flanking genes 7 and 9. Neither of the plasmids rescued ambers in genes 5, 6, 10, 11 or 12 (Table 2). Since the direction of transcription in this region is from gene 5 toward gene 12 (STAHL *et al.* 1970), these results indicate that the 3.7 Kb *Eco* R1 fragment contains the C-terminal-encoding end of gene 7, the whole

TABLE 2

Marker rescue of plasmid-borne alleles by T4 amber mutants

T4 genes:	5, 6	7	8	9	10, 11, 12
	N135	B16	NG218	N132	E343 E17 B255
	NG322	NG217	NG437	NG240	E570 NG153
ambers:	NG232		NG393	C80	C215 NG430
	N102		NG295		C226 N128
	B250		NG80		NG118 N69
					NG577
p11R	—	—	+	+	+
p11S	—	—	+	+	+
p11RΔ	—	—	+	—	—
p11SΔ	—	—	—	+	—
Restriction targets:					
		↑	↑		↑
		(EcoR1)	Bam H1		(EcoR1)

The plating behavior of T4 amber mutants in genes 5 through 12 on bacterial hosts harboring the recombinant plasmids. A plus sign indicates the occurrence of marker rescue, a minus sign, its absence. The deleted plasmid derivatives rescue either exclusively gene 7 amber mutants or exclusively gene 8 amber mutants, demonstrating that the *Bam* H1 cleavage site is located close to the junction between the two genes.

of gene 8, and the N-terminal-encoding portion of gene 9, in agreement with previous findings (WILSON, TANYASHIN and MURRAY 1977).

The deleted plasmid derivatives each rescued an exclusive subset of the amber mutants rescued by the parents. p11RΔ rescued all of the gene 7 amber mutants for which the parents were permissive, but none of the gene 8 or 9 ambers. Conversely, p11SΔ rescued the gene 8 and 9 amber mutants, but none of those in gene 7 (Table 2). Clearly the larger *Eco* R1-*Bam* H1 subfragment derives mainly or entirely from gene 7 and the smaller subfragment mainly or entirely from genes 8 and 9. The *Bam* H1 site thus separates all the amber sites examined in gene 7 from all those in gene 8 and must therefore be close to the junction between the two genes—in either the C-terminal region of gene 7, the N-terminal region of gene 8 or within the intergenic spacer region. Data presented below suggest the second alternative is correct.

Efficiency of plating: The bacterial host used for the marker rescue tests, RR1, is nonpermissive for the growth of two C-terminal gene 7 amber mutants, all three gene 8 ambers and the solitary gene 9 amber mutant rescued by the plasmids. Serial dilutions of these phages were spotted onto homogeneous lawns of RR1 harboring each of the plasmids, and the resulting plaque titres were compared with those obtained on a fully permissive, plasmid-free host in order to determine the plating efficiency (eop) of each mutant (Table 3).

The parental plasmids p11R and p11S carry only part of genes 7 and 9, and were found to plate gene 7 and 9 amber mutants with correspondingly low efficiency, around 10^{-2} . The presence of the deletion in p11RΔ did not substantially affect the eop of the gene 7 amber mutants, nor the deletion in p11SΔ, the eop of the gene 9 amber mutant. In contrast, the gene 8 amber mutants grew with

TABLE 3

Eop of T4 amber mutants

gene: amber:	7 NG295	NG80	N132	8 NG240	C80	9 E343
p11R :	3×10^{-2}	5×10^{-2}	1	1	1	5×10^{-3}
p11S :	3×10^{-2}	5×10^{-2}	1	1	1	5×10^{-3}
p11RA:	3×10^{-2}	5×10^{-2}	$< 10^{-6}$	$< 10^{-5}$	$< 10^{-4}$	$< 10^{-6}$
p11SA:	$< 10^{-6}$	$< 10^{-6}$	2×10^{-3}	1×10^{-3}	1×10^{-2}	5×10^{-3}

The efficiency of plating of various T4 amber mutants on nonpermissive, plasmid-carrying *E. coli* RR1, relative to plasmid-free, fully permissive, QD *Sup3*. The 100- to 1000-fold reduction in *eop* of the gene 8 amber mutants that occurs following introduction of the *Bam* H1 generated deletion in plasmid 11S argues that the cleavage site is located within the promoter or coding region of gene 8.

an *eop* of one on strains containing the parental plasmids, known to carry a complete gene 8, but with a much lower efficiency—between 10^{-3} and 10^{-2} —on the deleted derivative p11SA. We would like to argue that an *eop* of unity reflects marker rescue augmented by complementation, while a low *eop* reflects marker rescue alone. If this is the case, then since the parental plasmids complement gene 8 ambers, while the *Bam* H1-deleted derivative p11SA does not, the *Bam* H1 site must lie within the functional domain of gene 8—either in its promoter or in the structural gene (Figure 4).

FIGURE 4.—A simplified map of the chromosome of bacteriophage T4, taken from WOOD and REVEL 1976. The location of the *Bam* H1 site is shown together with the map locations of several prominent T4 genes. The two open arrowheads within the circle indicate the positions of the *Eco* R1 cleavage sites on either side of the 3.7 Kb *Eco* R1 fragment enclosing the *Bam* H1 site.

DISCUSSION

The data presented here indicate that the chromosome of phage T4 is cleaved once by the restriction endonuclease *Bam* H1, at a site within or near the beginning of gene 8. Since T4 has a circularly permuted chromosome, cleavage at this unique site will generate not two fixed length pieces, but a spectrum of equally frequent fragments of all possible sizes up to the genome length, around 170 Kb (KIM and DAVIDSON 1974). If linearly separated according to size, a *Bam* H1 digestion of T4 DNA would distribute itself as a uniform smear. Gel electrophoresis separates fragments on a logarithmic rather than linear basis and fails to separate effectively above a certain size limit; hence, we observe *Bam* H1-digested T4 DNA electrophoresing as a discrete band with a faint preceding smear.

If the T4 chromosome were not randomly permuted, but displayed a preference for particular end points as some permuted phages are known to do (TYE, HUBERMAN and BOTSTEIN 1974; BÄCHI and ARBER 1977; GILL and MACHATTIE 1976), a banding pattern would probably be evident in the electrophoretogram of *Bam* H1-digested DNA. The absence of discrete bands points to the absence of preferred end points, at least within 15 to 20 Kb of the *Bam* H1 site.

If the nucleotides in the T4 chromosome were distributed randomly, we would expect about 16 *Bam* H1 cleavage sites, rather than one. Similar significant departures from the expected number of cleavage sites can be seen for other restriction endonucleases as well. For example, both *Bgl* II (PIRROTTA 1977) and *Xba* I (ZAIN and ROBERTS 1977) are expected to cleave at about 60 sites each, but in fact cleave at only 12 and 20 sites, respectively. Clearly, the distribution of bases in the T4 chromosome is not random.

The presence of a single *Bam* H1 site in the T4 chromosome has practical implications. It allows the formation of at least one fixed end point in studies concerning the replication and transcription of the chromosome, and will be useful for aligning different restriction maps of T4 with respect to one another and to the genetic map. The occurrence of a single restriction site in a viral or plasmid replicon often signals its use as a cloning vector. Two obstacles confront the use of T4 as a cloning vehicle. First, the *Bam* H1 site is probably within the essential gene 8, whose function would be disturbed by the insertion of foreign DNA; and second, the chromosome is circularly permuted so that many of the products of *in vitro* ligation would be genetically incomplete. Neither problem is insurmountable, however; gene 8 function might be bypassed by extragenic suppression or complementation, and occasionally incomplete recombinants would only reduce the efficiency of cloning rather than preventing it altogether.

One of the interesting aspects of the work described is the possibility of complementation between T4 DNA in the parental plasmids, p11R and p11S, and the T4 gene 8 amber mutants. Bacteria carrying these plasmids are permissive for T4 gene 8 amber mutants in the sense that they permit their growth with an eop of one. This result is provocative because complementation is unexpected; since the T4 fragment carried by the plasmids contains cytosine rather than glu-

cosylated hydroxymethylcytosine, its integrity should be disrupted soon after infection by the phage-encoded nucleases WIBERG 1966; WARNER *et al.* 1970; HERCULES *et al.*, 1971) and its transcription inhibited by the product of the phage *alc* gene (SNYDER, GOLD and KUTTER 1976; SIROTKIN, WEI and SNYDER 1977). MATTSON *et al.* (1977) have shown, moreover, that complementation does not occur between T4 amber mutants and plasmid recombinants carrying another of the T4 late genes, gene 22. These objections would not arise if it were supposed that the plasmids express gene 8 constitutively prior to infection and complement the phage mutants by furnishing a pool of pre-existing gene 8 product. *A priori* this is unlikely, however, since gene 8 is one of the late T4 genes, whose expression is normally dependent upon the presence of several T4-encoded RNA polymerase subunits (STEVENS 1972; RATNER 1974; RABUSSAY and GUIDUSCHEK 1977) that would be absent from the uninfected cell. The alternative explanation, that gene 8 is transcribed by read-through from a resident plasmid promoter, remains feasible, but unlikely, since its expression is independent of the orientation of the fragment within the plasmid. Experiments to clarify this aspect of the work are in progress.

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