Proc. Natl. Acad. Sci. USA
Vol. 75, No. 10, pp. 5099–5103, October 1978
Genetics

Bacteriophage λ carrying the *Escherichia coli* chromosomal region of the replication origin

(transducing phages/*dnaA-ilv* region/minichromosome)

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Communicated by Arthur B. Pardee, July 10, 1978

ABSTRACT **A transducing phage λ*asn* was isolated. The late gene region of its genome was found to have been substituted by an *Escherichia coli* chromosomal segment containing the genes *bglR, bglC, glmS, uncA,* and *asn.* Restriction endonuclease cleavage mapping and electron microscopic analysis of the λ*asn* DNA revealed that the size of the bacterial segment is approximately 1.75×10^7 daltons, corresponding to about 26.4 kilobases. The circular DNA of λ*asn* was digested with restriction endonuclease *Eco*RI, diluted, and sealed with DNA ligase. When the reaction mixture was used to transform a recipient *E. coli* strain, a small plasmid of about 1×10^7 daltons (named pMCR115) was obtained. Restriction endonuclease cleavage mapping of pMCR115 and other evidence suggested that it contained the replication origin (*oriC*) of the *E. coli* chromosome.**

The chromosome of *Escherichia coli* is a single molecule of circular DNA of approximately 2.5×10^9 daltons (1). A cycle of replication is initiated from a unique origin (1–3), which has been proposed to reside somewhere near the *dnaA* and *ilv* genes on the chromosome (4–7). The origin was named *oriC* by Hiraga (7). Now we have undertaken to determine its exact location. One of the approaches we have chosen for this purpose was to insert the genome of bacteriophage λ within the *dnaA-ilv* region, and to isolate a series of λ strains with the capability of transducing various markers in the region. If any one of the transducing λ strains contained *oriC* in the phage genome, digestion of the genome with a restriction endonuclease would release an *E. coli* DNA fragment which would form, upon ligation and subsequent transformation into a suitable cell, a small plasmid capable of autonomous replication. In this way, we expected to clone *oriC* using the λ genome as a vehicle. This *in vivo* cloning should ensure identification of *oriC* in its native integrity.

Thus we obtained a series of λ strains with the capability of transducing consecutively a set of markers in the *dnaA-ilv* region of the *E. coli* chromosome. Among them, one phage strain called λ*asn* was found to contain a bacterial DNA segment from which we were able to construct a small autonomously replicating plasmid named pMCR115. This paper describes the isolation and characterization of these phage strains and the plasmid. Especially, data will be presented to demonstrate that the plasmid pMCR115 carries the *asn* gene of *E. coli,* but lacks the replication origin of phage λ originally present in λ*asn* DNA. Based on these data and other observations, we conclude that a nucleotide sequence which may be interpreted as *oriC* is located very close to the *asn* gene. This nucleotide sequence was found to reside, according to cleavage mapping and electron microscopic analysis, in the region some 24,000 base pairs away from the *bglB* gene toward the *ilv* gene.

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MATERIALS AND METHODS

Bacterial and Phage Strains, Media, and Transduction. Bacterial strains used were derivatives of *E. coli* K-12 as listed in Table 1. A *tna*-transducing phage strain λ*tna imm*^{21} (11) was a generous gift from W. J. Brammar. Other phage strains were our laboratory stocks. L-broth (12) was used for transduction with P1*vir* (13), and also to grow cells lysogenic for λ*c*I857*S*7. Medium E (14), with 2 μg of thiamine per ml, was used as a synthetic minimal medium, which was supplemented as required; e.g., with 0.4% glucose for selecting asparagine prototroph (Asn$^+$) clones, or with 1% glycerol, 25 μg of indole per ml, and 100 μg of 5-methyltryptophan per ml for selecting clones with active tryptophanase (Tna$^+$) (11). In both cases the medium was further supplemented with 0.2% Casamino acids (Difco), and in the following cases with 0.02% Casamino acids. For selecting clones with active membrane-bound Mg^{2+},Ca^{2+}-ATPase (Unc$^+$), the medium was supplemented with 0.5% sodium succinate (15). For selecting clones that used salicin (Sal$^+$) or arbutin (Arb$^+$), it was supplemented with 0.5% salicin or with 0.5% arbutin, respectively (16).

Enzymes. Restriction endonucleases *Eco*RI, *Hin*dIII, and *Bam* I and T4 DNA ligase were purchased from the Miles Laboratory. T Okazaki and M. Takanami generously supplied us *Bam* I and *Hin*dIII, respectively. The method described by Robinson and Landy (17) was used for endonuclease digestions and ligase reactions.

Preparation of DNA and Transformation. The method of Meyers *et al.* (18) was used for isolation of plasmid DNA. Phage DNA was prepared from particles that were purified by CsCl equilibrium density gradient centrifugation. The phage suspension was treated with 1/100 vol of 0.25 M Na_2EDTA (pH 7.9), 1/50 vol of 1 M sodium dodecyl sulfate, and 1 vol of phenol; after low-speed centrifugation the aqueous phase was recovered. It was dialyzed overnight against 10 mM Tris·HCl, pH 7.9/1 mM Na_2EDTA/10 mM NaCl. DNA transformation was carried out as described by Cohen *et al.* (19).

Agarose Gel Electrophoresis. A slab-gel preparation contained 0.7% or 1.2% agarose II (Wakenyaku Co.) in a buffer solution composed of 10.8 g of Tris base, 0.93 g of Na_2EDTA, and 5.5 g of boric acid per liter. Electrophoresis was carried out at 40 V for 12 hr; the specimen was stained with 7 μg of ethidium bromide per ml in Tris borate buffer, and was illuminated by a UV lamp (Maruzen Electric Co., FL20 × 1).

Electron Microscopy. The method for heteroduplex analysis of λ DNA by electron microscopy was a modified version (20) of the formamide technique (21). A Parlodion flake (Mallinkrodt Co.) was generously given by T. Ando and T. Arai.

Abbreviation: MDal, megadalton.

Table 1. *E. coli* K-12 strains used

Strain	Relevant markers	Source (ref.)
"W3110"*	*trpB9578, tna-2, str*	C. Yanofsky
KY7243	*trpB9578, tna-2, str* (λ*tna imm*^{21}::*tna*)	This work
KH693	*dnaA46, bglB, bglR, tna*	T. Horiuchi†
KY7302	*trpB9578, tna-2, str, bglR* (λ*tna imm*^{21}::*tna*)	This work
KH712	*bglR,* Δ*gal-att*λ*-bio*	T. Horiuchi†
KY7126	*bglR,* Δ*gal-att*λ*-bio* (λ*c*I857*S*7::*bglB*)	This work
SH97	*dna-167, trpE, tyr*	This work
KY7200	*trpE, tyr* (λ*c*I857*S*7::*bglB*)	This work
KL16-99	Hfr/*recA1*	B. J. Bachmann (8)
KY2350	*glmS, gal, lac*	T. Sato†
KY2377	*uncA401, gal, lac, ton*	T. Sato†
ER	F$^+$/*asn-31, thi*	B. J. Bachmann (9)
KH716	F$^+$/*asn-31, thi, rif*	T. Horiuchi†
KY7512	F$^-$/*asn, thi, rif, recA*	This work

For gene symbols, see Bachmann *et al.* (10). The mark :: indicates that the phage genome is inserted in the bacterial gene or the vicinity. Δ designates deletion.
* A derivative of W3110 carrying markers as indicated.
† Unpublished results.

RESULTS

Isolation of λ Strains with *dnaA-glmS* Region. A Tna$^-$ bacterial strain W3110 *trpB9578 tna-2 str* was infected with a transducing phage λ*tna imm*^{21}, and Tna$^+$ transductants with the phage immunity (*imm*^{21}) were selected. One such Tna$^+$ (*imm*^{21}) strain, KY7243, was found to have integrated the genome of λ*tna imm*^{21} within or in the vicinity of the bacterial *tna* gene by general recombination (Fig. 1). When a phage lysate prepared by UV irradiation of KY7243 was used to infect KH693 (*dnaA46*), which does not grow at 42° (Ts), a number of transductants able to grow at 42° (Ts$^+$) were obtained. From one such Ts$^+$ transductant, two *dnaA*-transducing phage strains

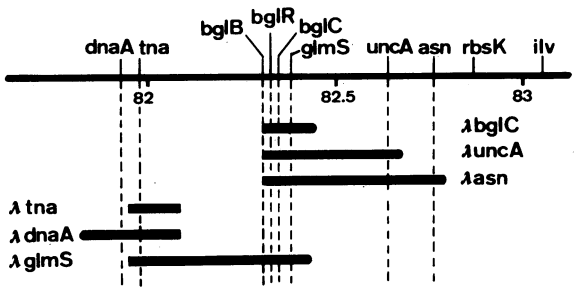

FIG. 1. Genetic structure of transducing λ strains isolated. Scale shows the map distance in minutes of the *dnaA-ilv* region of the *E. coli* chromosome (10). Positions of *dnaA, tna, bglB, bglR, asn,* and *ilv* were determined in this experiment by phage P1-mediated transductional mapping (13, 22). These agree well with the published standard map (10), with two exceptions. First, *tna* was found to be much closer to *dnaA* than previously thought, as already pointed out by Saitoh and Hiraga (23). Second, the position of *asn* proved to be drastically different from that shown on the standard map (10). Although our data of mapping *asn* by P1-mediated transduction were not clear enough to determine its exact position, they permitted us to conclude that *asn* resided between *bgl* and *ilv,* in agreement with Cedar and Schwartz (9). This conclusion is also consistent with the results of our merodiploid analyses. Recently, von Meyenburg *et al.* (24) obtained the same result. The positions of *bglC, rbsK,* and *glmS* are from the standard map of Bachmann *et al.* (10), and the position of *uncA* is from Saitoh and Hiraga (23). In order to isolate transducing λ carrying a part of the region consecutively in each λ genome, we chose two markers, *tna* and *bglB,* into which the λ genome was to be inserted. Bars below the scale schematically illustrate the region that was deduced to be integrated in the genome of each transducing phage thus isolated. The transducing activity was determined by examining complementation of the chromosomal marker in the lysogen of each phage.

λ*dnaA imm*^{21} were isolated (Fig. 1). They are plaque forming, as are the parental λ*tna imm*^{21}, but have lost the ability to be integrated into the normal *att*λ site on the bacterial chromosome. The phage acquired transducing ability for *dna-167* and *dnaA5* besides *dnaA46,* and retained the original ability for *tna-2.*

From a lysate of KY7302, which is a *bglR* mutant derived from KY7243, a *glmS*-transducing phage strain λ*glmS imm*^{21} was also isolated (Fig. 1). This phage was defective, and acquired the ability to transduce the *bglB, bglR,* and *bglC* genes besides *glmS.*

Isolation of λ Strains with *bglB-asn* Region. The method of Shimada *et al.* (25) was used to isolate a lysogen whose *bglB* gene was inactivated by insertion of λ*c*I857*S*7 DNA within the gene. A bacterial strain KH712 (*bglR* Δ*att*λ) was infected with λ*c*I857*S*7 at a multiplicity of infection of 10. KH712 can use salicin as a carbon source, as well as arbutin. This is possible because KH712 has a mutated *bglR* gene which allows expression of the *bglB* gene as well as the *bglC* gene (16). When *bglB* is inactivated, but not *bglC,* the cell becomes Sal$^-$Arb$^+$ (unable to use salicin and able to use arbutin). This distinguishes the desired clones from the unwanted Sal$^-$Arb$^-$ clones, which frequently occur due to spontaneous reversion of BglR$^-$ to BglR$^+$.

Thus, the surviving λ lysogens of KH712 after the phage infection were subjected to twice-repeated penicillin screening to concentrate Sal$^-$ derivatives. Undesirable nonlysogens that survived due to inability of adsorbing phage were killed by infecting λ*cIh*^{80} and λ*cIb2.* From the Sal$^-$ derivatives, KY7126 was finally selected as Arb$^+$, and immune to λ at 30°. At 42° the majority of KY7126 cells were killed owing to the temperature-sensitive repressor of the prophage λ*c*I857*S*7, but among the survivors were those which were cured of the prophage, and they invariably reverted to Sal$^+$.

Strain KY7126 was therefore judged to be a lysogen in which prophage λ*c*I857*S*7 was inserted within the *bglB* gene. This was further supported by the fact that phage P1-mediated transductional linkage between *ilv* and *tna* (which ordinarily has a cotransduction frequency of 10%) in KY7126 was entirely lost (data not shown). Since KY7126 was suspected of being a multiple lysogen, carrying at least two more prophages at sites other than *bglB,* the *dnaA-bglB*(λ)-*bglC* region of KY7126 was transferred by phage P1-mediated transduction into another strain SH97, and a single lysogen KY7200 was obtained.

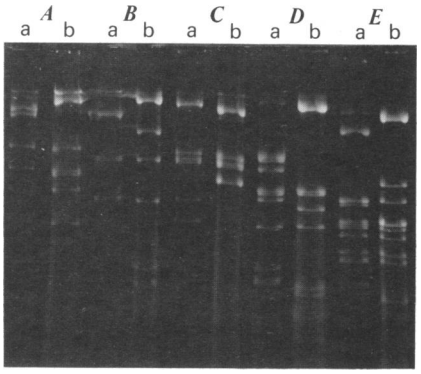

FIG. 2. Example of agarose gel electrophoresis patterns of DNAs from (a) λ*asn*-5 and (b) λc1857*S*7, which were digested with restriction endonucleases (*A*) *Eco*RI, (*B*) *Hin*dIII, and (*C*) *Bam* I. The DNA samples were also codigested with (*D*) *Eco*RI and *Hin*dIII, or with (*E*) *Eco*RI and *Bam* I.

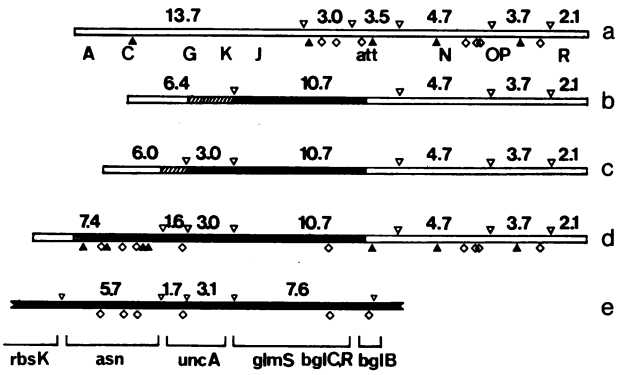

FIG. 3. Cleavage map of DNAs from (a) λc1857*S*7, (b) λ*bglC*-2, (c) λ*uncA*-1, and (d) λ*asn*-5. DNA samples were digested with *Eco*RI (▽), *Hin*dIII (◇), and/or *Bam* I (▲), and were analyzed by agarose gel electrophoresis (see Fig. 2). Open bar represents λ DNA; closed segment represents *E. coli* DNA (transducing segment). The shadowed portion is an uncertain region of either λ or *E. coli* DNA. Numerals represent the size of each *Eco*RI-digested segment in megadaltons (MDal). Data of the wild-type λ DNA (a) are from refs. 17, 26, and 27; these served as the reference for identification of fragments produced by digestion of transducing λ DNAs shown in b, c, and d. The consistent appearance of the 10.7-MDal fragment in b, c, and d, but not in a, and of the 3.0-MDal fragment in c and d, but not in b, permitted us to construct an initial map of the transducing segment. Furthermore, the DNA from λ*asn* contained additional segments of 1.6 and 7.4 MDal. The order of these cannot primarily be determined, but comparing our data (see Fig. 2) with those of Marsh and Worcel (28), in e, the transducing segment of λ*asn* DNA was mapped as shown in d. Combining the genetic evidence (see Fig. 1) with the cleavage map, the transducing markers were assigned to each segment as shown at the bottom.

A phage lysate prepared by heat induction of KY7126 was used to infect KL16-99 (*bglR*$^+$) and KY2377 (*uncA*), and Arb$^+$ and Unc$^+$ transductants, respectively, were selected. From them we obtained transducing phage strains λ*bglC* and λ*uncA* (Fig. 1). They are defective, and λ*bglC* transduces the *bglR*, *bglC*, and *glmS* genes; λ*uncA* transduces the same set of genes besides *uncA*. A phage lysate of KY7200 was then used to infect an asparagine auxotroph KH716 (Asn$^-$), and asparagine prototroph (Asn$^+$) transductants were selected. From one of them, an *asn*-transducing phage strain λ*asn*-5 was isolated. It is able to transduce, besides *asn*, the *bglR*, *bglC*, *glmS*, and *uncA* genes (Fig. 1).

Cleavage Mapping of Phage DNA. The physical structure of genomes of the transducing phage strains isolated was examined by cleaving the genome with restriction endonucleases *Eco*RI, *Hin*dIII, and *Bam* I. An example of typical gel patterns of the digestion fragments is shown in Fig. 2. Some bands of the reference λc1857*S*7 DNA are missing, and new bands appear in DNA of the transducing phage, indicating that a part of the λ genome was replaced by an *E. coli* DNA segment. A series of such experiments and systematic comparison of the band patterns enabled us to reconstitute physical maps of DNA from λ*bglC*, λ*uncA*, and λ*asn* (Fig. 3). The data are consistent with the genetic structure (Fig. 1) deduced from the transducing activity of each phage. Especially noteworthy is the finding that λ*asn* contains a large part of the *E. coli* DNA segment [the *Eco*RI fragment of 5.7 megadaltons (MDal)] which was identified by Marsh and Worcel (28) to incorporate [^{3}H] thymidine earliest in a DNA replication cycle (compare Fig. 3 d and e); this finding prompted us to study λ*asn* further.

Electron Microscopic Analysis of λ*asn* DNA. Examination of heteroduplex molecules formed between DNAs from λ*asn* and λ*imm*^{21} (Fig. 4) revealed the following. The λ*asn* genome was partially substituted by a bacterial DNA segment in the region starting at the 7.3% point from the left end and terminating at the 57.2% point. The latter is known to be the *att* site (32), at which the λ genome is integrated into the bacterial chromosome through site-specific recombination. Thus, λ*asn* can be thought to have been produced by an illegitimate excision of a prophage that resided in the middle of the bacterial *bglB* gene using the *att* site (25). The excision occurred in such a way as to substitute a part of the λ genome (containing the late genes) with the bacterial DNA segment starting at the middle of the *bglB* gene and ending somewhere beyond the *asn* gene (Fig. 1). The length of this bacterial DNA segment was estimated to be 56.7% (SD 2.18) λ unit (Fig. 4), corresponding to 26,366 (SD 1,014) base pairs long, when 1% λ unit was taken to be 465 base pairs (30). This corresponds to approximately 1.75 × 10^7 daltons, and is consistent with the result obtained by cleavage mapping (Fig. 3).

Construction of a Plasmid *In Vitro* from λ*asn* DNA. The cohesive ends of DNA extracted from λ*asn* particles were annealed by keeping the DNA in a buffer solution at 4° for 4 days. The DNA molecules were then completely digested with an excess amount of *Eco*RI. In order that the digested fragments form circles by themselves, the DNA solution was diluted 1:10, and was treated with T4 DNA ligase. After the treatment was completed, the DNA sample was used to transform KY7512 (*asn recA*) cells, and 15 Asn$^+$ clones were selected. They were all not immune to λ. It was found that 4 out of the 15 Asn$^+$ clones segregated Asn$^-$ subclones at relatively high frequencies during growth in a nonselective medium. Furthermore, agarose gel electrophoresis of DNA extracted from the 15 Asn$^+$ clones revealed that the 4 unstable clones without exception produced a new DNA band of approximately 10 MDal, while the remaining 11 stable clones yielded no such DNA that can be detected (data not shown). The DNA fraction of 10 MDal from one of the four unstable Asn$^+$ clones was purified and used to transform KY7512; again a number of Asn$^+$ transformants appeared. These experiments were repeated to show reproducibility; thus, the 10-MDal DNA was confirmed to be an extrachromosomal plasmid capable of autonomous replication in *E. coli* cells. The plasmid series was named pMCR.

In order to identify which of the *Eco*RI-digested fragments of λ*asn* DNA formed the plasmid pMCR115, it was purified from the second-step Asn$^+$ transformant, codigested with *Eco*RI and *Bam* I, and analyzed by agarose gel electrophoresis. As shown in Fig. 5*A*, except for the largest fragment with a size of 5.0 MDal, all corresponded to the *Bam* I-digested fragments that constituted the left-most segment of the λ*asn* DNA created by *Eco*RI digestion (Fig. 3). The 5.0-MDal fragment corresponded to a conglomerate between the right-most *Eco*RI fragment and the left-most *Bam* I fragment joined at the co-

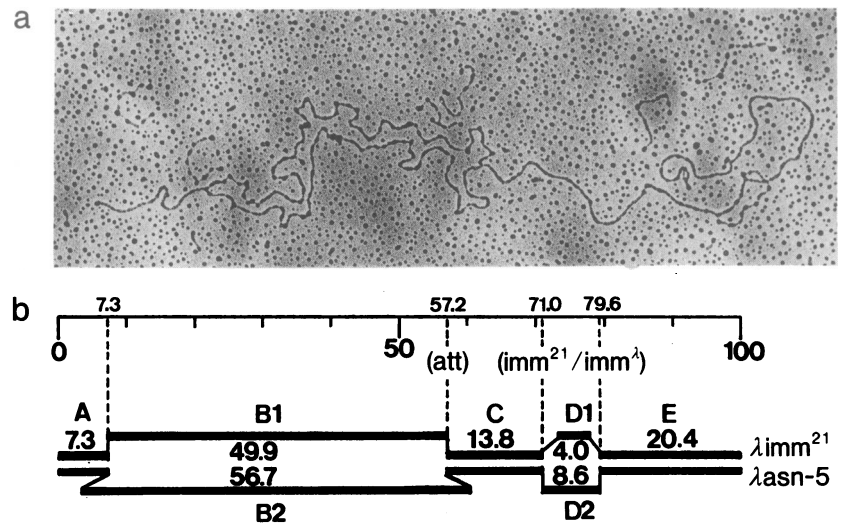

FIG. 4. Electron microscopic analysis of heteroduplex DNA from λ*asn* (*imm*$^{\lambda}$) and λ*imm*^{21}. (*a*) Obtained by spreading annealed DNA on a copper grid covered with a Parlodion film; the specimen was stained with uranyl acetate, shadowed with Pt/Pd, and examined in a JEM-100B. (×5400.) (*b*) Schematic representation of the heteroduplex DNA. Altogether 28 molecules were analyzed by enlarging the image on each negative film 10-fold with a Nikon 6c-2 projection comparator; the image was traced and the length of each segment was measured with a curvimeter. Since the right end of the *imm*^{21}/*imm*$^{\lambda}$ substitution loop is 79.6% from the left end of the λ genome (29), the length of segment E is 20.4% λ unit, and this served as an internal reference to estimate the length of the double-stranded segments (A and C) of each heteroduplex. Thus, the lengths A = 7.3% (SD 0.38) and C = 13.8% (SD 0.48) were obtained. We knew that bouyant densities of λ*asn* and λcI857S7 particles were approximately 1.498 and 1.492 g·cm^{-3}, respectively, giving the DNA molecular weight difference (30) of about 6%. We judged therefore that the longer segment (B_2) of the single-stranded substitution loop belonged to λ*asn*, and the shorter one (B_1) to λ*imm*^{21}. Since the left end of the *imm*^{21}/*imm*$^{\lambda}$ loop is 71.0% (29), the length of segment B_1 was calculated to be 49.9% (= 71.0 − A + C) λ unit. This again served as an internal reference to estimate the length of segment B_2, and we obtained the value of 56.7% (SD 2.18). Segment D_2 (*imm*$^{\lambda}$) can also serve as an internal reference to estimate single-stranded segments (29). Thus, when D_2 = 8.6% λ unit was given, B_1 = 49.3% (SD 3.85) and B_2 = 56.0% (SD 3.98) were obtained, confirming the other estimations. Segment D_1 (*imm*^{21}) was likewise estimated to be 4.0% (SD 0.65), not far different from the published value of 3.8% (31). Summing segments A, B_2, C, D_2, and E, we obtain a total length of λ*asn* DNA of 108.6% λ unit, confirming the bouyant density result.

hesive ends (Fig. 3). The cleavage map of pMCR115 thus constructed is schematically shown in Fig. 5*B*. We conclude that pMCR115 DNA replicates autonomously and carries the *asn* gene. Since the DNA segment that contains the replication origin of λ (the *Eco*RI fragment of 4.7 MDal; ref. 33) was excluded from pMCR115, the replication origin of this plasmid must have been derived from the *E. coli* chromosomal segment of the λ*asn* DNA. Otherwise one would have to imagine that a new replication origin was created by the construction of pMCR115.

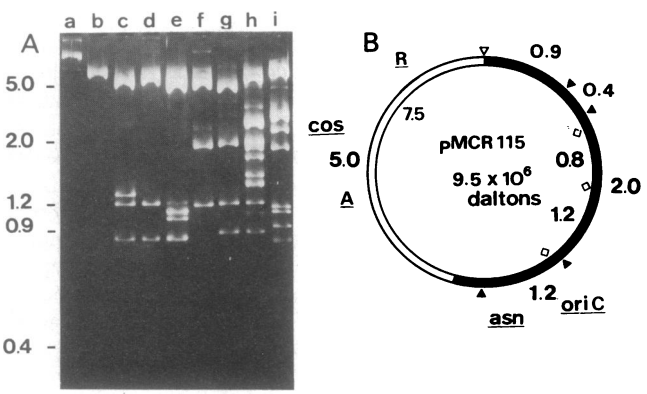

FIG. 5. Cleavage mapping of pMCR115 DNA. (*A*) Agarose gel electrophoresis of pMCR115 DNA (a–g), λ*asn*-5 DNA (h), and λ DNA (i) as a molecular size reference. DNA was digested with no enzyme (a), *Eco*RI (b), *Hin*dIII (d), *Bam* I (f), *Eco*RI plus *Hin*dIII (c and i), *Hin*dIII plus *Bam* I (e), or *Eco*RI plus *Bam* I (g and h). (*B*) Cleavage map of pMCR115. Filled region represents DNA derived from the *E. coli* chromosome; open region, DNA from the λ genome. Genes: *cos*, cohesive end site; *A* and *R*, λ genes; *asn*, *E. coli* gene; *oriC*, replication origin of the *E. coli* chromosome. They are placed at approximate locations. Sites cleaved by (▵) *Eco*RI, (◊) *Hin*dIII, and (▲) *Bam* I. Numerals denote the size of each segment in megadaltons.

DISCUSSION

The present results suggest that λ*asn*, one of phage λ strains transducing markers in the *dnaA-ilv* region of the *E. coli* chromosome, may contain a replication origin of that chromosome. This conclusion is based on the following observations. A small plasmid (pMCR115) was constructed by *Eco*RI digestion of the λ*asn* DNA, ligation, and subsequent transformation into a bacterial strain. This plasmid contains the left-most *Eco*RI fragment of λ*asn* DNA (Fig. 3), including the *asn* gene, but excluding the replication origin of λ (Fig. 3). Thus, the replication origin of pMCR115 can most easily be attributed to a nucleotide sequence present somewhere in the left-most region of the *E. coli* chromosome that substituted the late gene region of the λ genome in λ*asn* (Fig. 4).

Direct evidence is not available, however, to state that the replication origin of pMCR115 is the same as the nucleotide sequence that functions as the genuine origin of the *E. coli* chromosome replication. We cannot exclude the possibility, for instance, that a new replication origin was created during construction of the plasmid pMCR115.

Nonetheless, the following facts support our conclusion. First, the *E. coli* chromosomal segment that was identified by Marsh and Worcel (28) to be the earliest to replicate during a cycle of synchronous replication almost exactly coincided with the left-most part of the *Eco*RI fragment of λ*asn* DNA (Fig. 3). Second, the replication origin of plasmid pSY211, constructed *in vitro* by Yasuda and Hirota (34), seems to have been derived from the *E. coli* chromosomal region hemmed in between the *uncA* and *rbsK* genes. This genetic location, and their results of cleavage mapping with restriction endonucleases of the plasmid, precisely agree with our data. Third, von Meyenburg and coworkers (24) recently reported that a site which may be interpreted as the replication origin can be mapped somewhere between *bglB* and *asn*; this again is consistent with our results.

The earlier observation of Hiraga (7) led him to propose that *oriC* might reside in the *dnaA-bglB* region (*bglB* previously designated *bglA*), but more recent data amend it to the locality consistent with the present result (35). Finally, we noted in the present work that pMCR115 is rather unstable and easily lost from the host cell during growth. The instability of pMCR115 may also be interpreted as characteristic of a replicon with the same specificity as the *E. coli* chromosome (2, 7). In any event, it is at least certain that there is, very close to the *asn* gene, a nucleotide sequence that can function as a replication origin. Electron microscopic analysis and cleavage mapping of λ*asn* DNA indicate that this nucleotide sequence lies in the region that is some 24,000 base pairs apart from the *bglB* gene toward the *ilv* gene (Figs. 3 and 4).

If the λ*asn* DNA indeed contained *oriC*, in addition to the replication origin of λ, this phage would provide an interesting experimental system for studying regulatory mechanisms of DNA replication. Further investigations on λ*asn* and pMCR115 should contribute to deciphering initiation events of DNA replication.

Note Added in Proof. We have now constructed a composite plasmid pMCF1 consisting of a part of the *E. coli* chromosomal region present on pMCR115 and the major part of pSC138, a mini-F plasmid carrying the ampicillin resistance gene (36). This composite plasmid can replicate on *E. coli* Hfr strains, and this character (Poh$^+$) is retained even when the DNA fragment carrying the replication origin of F (*oriV*) was deleted from pMCF1 *in vitro*, supporting the notion (7) that the Poh$^+$ phenotype is attributed to *oriC*. All the deletion mutants of pMCF1 contained a *Bam* I-generated 1.2-MDal fragment and two small *Bam* I fragments (approximately 0.06 and 0.05 MDal, as detected by polyacrylamide gel electrophoresis), suggesting that these fragments may constitute the functional *oriC*.

We are deeply indebted to Drs. T. Okazaki and M. Takanami for enzymes; to Drs. C. Yanofsky, B. J. Bachmann, W. J. Brammar, T. Horiuchi, and T. Sato for bacterial and phage strains; to Drs. T. Ando and T. Arai for a Parlodion flake; to Drs. H. Yamagishi and A. Matsumoto who taught us electron microscopy; and to Dr. K. von Meyenburg who kindly informed us of his work prior to publication. This work was supported in part by grants from the Ministry of Education of Japan.

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