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Identification of a Novel Genetic Element in *Escherichia coli* K-12

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Induction of the SOS repair processes of *Escherichia coli* K-12 caused a 14.4-kilobase species of circular deoxyribonucleic acid, called element e14, to be excised from the chromosome. To aid further characterization of this species, an 11.6-kilobase segment of e14 was inserted into the *Hin*dIII site of plasmid pBR313. To map e14 on the *E. coli* K-12 chromosome, the recombinant plasmid, pAG2, was used to transform a *polA* recipient, an event which required integration of pAG2 into the recipient chromosome. This recombinational event was dependent upon the region of homology between the incoming plasmid and the chromosome, as no transformants were scored when either a strain cured of the element was the recipient or pBR313 was the transforming deoxyribonucleic acid. Using these transformants, we have shown that e14 maps between the *purB* and *pyrC* loci near min 25. Several strains of *E. coli* K-12 were found to contain e14; however, one strain, Ymel *trpA36*, did not. In addition, e14 was found to be absent in both *E. coli* B/5 and *E. coli* C. The approach to mapping developed for this work could be used to map other fragments of *E. coli* deoxyribonucleic acid which have no known phenotype.

The genes which have thus far been mapped on the *Escherichia coli* K-12 chromosome are not distributed randomly. Many genetically crowded regions (min 2, 17, 28, 43–45, 72, 84, and 89) and a significant number of unpopulated genetic loci (min 12, 24, 30–35, 65, 77, 86, and 97) exist on the map (2). The reasons for this nonrandom distribution are unclear. The silent regions in particular have been the subject of much speculation as to whether they code for proteins not as yet defined or whether they have some structural significance. Another possibility that has been suggested is that some of the silent regions are the remnants of episomes acquired by some ancestral cell (27).

We have previously reported the study of covalently closed circular (CCC) DNA which can be excised from a tandemly duplicated region of the *E. coli* chromosome (18). These experiments involved UV irradiation of the cultures to stimulate the excision event followed by cell lysis and CsCl-ethidium bromide centrifugation to separate the excised CCC DNA. The predominant species observed was the large CCC DNA characteristic of the tandem duplication mutations. However, smaller CCC DNA was always present in the preparations (R. H. Grafstrom, Ph.D. thesis, Pennsylvania State University, Hershey, 1975), and the characterization of one of these species is described here.

MATERIALS AND METHODS

Bacterial strains and microbiological procedures. The derivations of several of the *E. coli* K-12 mutants used have been described before: PB153 (17); CH428, CH439, and CH440 (19); CH725 and CH734 (9). To prepare CH826, *recA441* (formerly *tif-1*) was introduced into CH734 by Hfr mating, using JM888 (8) as donor and selecting for a Lys^+ temperature-sensitive recombinant. CH902 is an Ilv^+ *metE* derivative of CH725 prepared by P1 transduction (X478 as donor). CH923 is a Met^+ *polA1* derivative of CH902 prepared by P1 transduction (KS219 as donor). CH929 was prepared by mating CH923 with an HfrH derivative, selecting for transfer of the distal $argH^+$ gene, and scoring the recombinants for transfer of the point of origin; CH929 has the genotype *trpA lysA polA1* PO-1. CH931 was a spontaneous Trp^+ revertant of CH929. The derivations or origins of other strains are indicated in the text. Growth media, transductions, Hfr matings (15), and transformations (6) have been described previously.

Isolation of DNA. Isolation of circular DNA from *recA441* strains was accomplished by growing the bacteria in supplemented synthetic medium (32) for two cell doublings at 34°C in the presence of [^{3}H]thymidine (2 Ci/mmol; New England Nuclear Corp.) to an optical density at 590 nm of 0.5. Deoxyadenosine (250 μg/ml) was added 20 min before the [^{3}H]thymidine addition to enhance incorporation of the label. The growing cultures were washed free of the deoxyadenosine and the [^{3}H]thymidine and grown for 150 min at 42°C. The cells were then lysed, and the DNA was extracted by the high-salt–sodium dodecyl sulfate lysis

technique of Guerry et al. (13) and dialyzed against 0.1 × SSC (SSC is 0.015 M NaCl–0.0015 M sodium citrate). For analytical purposes the DNA was fractionated by centrifugation through a 5 to 20% (wt/vol) alkaline sucrose gradient (18). For preparative purposes, the DNA was subjected to CsCl-ethidium bromide density gradient centrifugation followed by centrifugation of the supercoiled DNA through a 5 to 20% (wt/vol) neutral sucrose gradient (18). Fractions representing the peak of interest were pooled, dialyzed, and concentrated by directing a stream of dry, filtered nitrogen across the surface, reducing the volume by 75% or more. The sample was then dialyzed against several changes of 0.1 × SSC.

Total chromosomal DNA was isolated by harvesting exponentially growing cultures of *E. coli* and lysing the cells as previously described (18). The DNA was purified by CsCl-ethidium bromide gradient centrifugation followed by extraction with 1-butanol to remove ethidium bromide. The preparation was then dialyzed against several changes of 0.1 × SSC and concentrated by ethanol precipitation.

DNA from pBR313 and recombinant plasmids was prepared by the method of Guerry et al. (14) from chloramphenicol-amplified cells, as described by Bolivar et al. (4). The cleared lysate was extracted twice with phenol, digested with 100 μg of RNase A per ml, and extracted once with chloroform-isobutanol (24:1) and once again with phenol. The DNA was ethanol precipitated and dialyzed overnight against several changes of 0.1 × SSC. In some cases, the DNA was further purified by CsCl-ethidium bromide centrifugation (18). P4 and λ DNAs were prepared by phenol extraction of phage particles which had been purified by CsCl centrifugation.

Cloning conditions. One unit of *Hin*dIII restriction endonuclease (Bethesda Research) was added to cleave a maximum of 1 μg of either plasmid pBR313 DNA or e14 DNA in a total volume of 50 μl, containing 20 mM Tris (pH 7.4), 60 mM NaCl, 7 mM $MgCl_2$, and 100 μg of autoclaved gelatin per ml. The digestion was performed at 37°C for 1 h, after which the samples were heated at 65°C for 10 min to destroy the remaining *Hin*dIII enzyme. Bacterial alkaline phosphatase treatment of pBR313 was performed by the method of Ullrich et al. (31). Ligation of these two DNA preparations was carried out by adding 0.7 U of T4 DNA ligase (Bethesda Research) in Tris (50 mM; pH 7.5)–2-mercaptoethanol (10 mM)–bovine serum albumin (0.5 mg/ml) per μg of DNA in a total volume of 60 μl containing 66 mM Tris (pH 7.6), 6.6 mM $MgCl_2$, 10 mM dithiothreitol, and 0.066 mM ATP. Samples were incubated for 19 h at 4°C and were then used to transform the appropriate recipient. The *Hin*dIII fragment carried by pAG2 was purified by preparative agarose gel electrophoresis (25).

Hybridizations. All DNA-DNA hybridizations were performed in the manner described by Sharp et al. (29). A constant amount of ^3H-labeled probe was incubated with unlabeled chromosomal, phage, or plasmid DNA. The amount of hybridized material was determined by resistance to S1 nuclease digestion (30). The data were analyzed by a reciprocal plot described by Wetmur and Davidson (33). DNA concentrations were determined by the diphenylamine test of Burton (5).

RESULTS

UV-induced excision of circular DNA. *E. coli* K-12 strain CH439, the parent of the duplication mutants mentioned above, was irradiated with UV to promote recombination, and the lysozyme-detergent extract was subjected to two successive rounds of CsCl-ethidium bromide centrifugation as described previously (18). No peak of CCC DNA was apparent after the first round of centrifugation, but the region expected to contain CCC DNA was pooled and recentrifuged. After the second round of centrifugation, a small peak of CCC DNA, containing 0.0002 of the total DNA, was observed. Material obtained after both the first and second CsCl gradients was spread for observation with the electron microscope, and spontaneously relaxed circular forms were photographed and measured. Results are shown in Fig. 1. A variety of sizes was seen, but the circular DNA fell into discrete families. By far the most abundant numerically were two small species, sized 12.0 ± 0.3 and 14.6 ± 0.4 kilobases (kb). Some of the larger, less

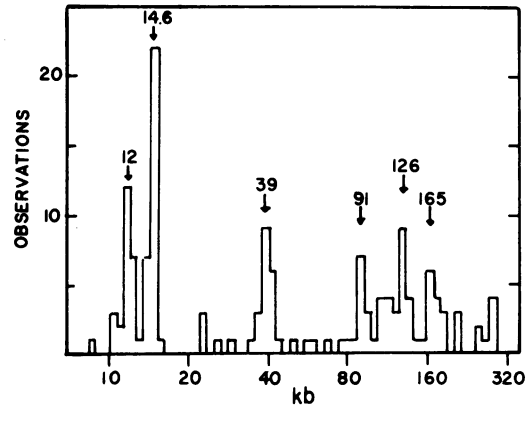

FIG. 1. *UV-induced excision of circular DNA from an E. coli K-12 strain. Strain CH439 was irradiated with UV light, and CCC DNA was purified by one or two successive rounds of CsCl-ethidium bromide centrifugation and spread for electron microscopy, using techniques described previously (18). Two to 14 days elapsed between the CsCl gradient and the spreading of the material for electron microscopy. Phage P4 DNA was included as an internal standard. All open circular species seen were photographed and measured. Most of the largest circles had relaxed by the time of spreading, but only a minority of the smallest had done so. Each bar of the histogram represents an interval of length ±3%. These were generated by expansion of the term* $x(1.03/0.97)^n$, *where x is the length of P4 DNA (10.97 kb) (12) and n is an integer.*

abundant species corresponded in size to the known intervals between the redundant rRNA genes. These will be considered below.

Excision of a small CCC DNA species after thermal shift of a *recA441* mutant. The material observed in Fig. 1 was obtained from UV-irradiated cultures. In experiments in which UV was omitted, we failed to find any CCC DNA. We must emphasize, however, that when the cells were not UV irradiated, the extracted DNA was different in physical character, and this difference could well have interfered with the isolation of minute amounts of CCC DNA. Assuming that the appearance of the various species of CCC DNA observed in Fig. 1 was dependent on the UV irradiation, the UV could have worked by two distinct mechanisms. One would be by the stimulation of homologous recombination, as was observed in previous studies of tandem duplication mutants. The second would be through the induction of the SOS repair system in a fashion more analogous to the excision of the λ prophage after UV irradiation. If the second mechanism were operating, the same CCC DNA should be observed in a *recA441* mutant (formerly called *tif-1*) after a thermal shift (7). The *recA441* mutation causes expression of the SOS repair system at elevated temperature without physical insult to the DNA.

To distinguish between these two alternatives, a *recA441* mutant, CH826, was labeled with [^{3}H]thymidine at the permissive temperature (34°C) and then shifted to the restrictive temperature (42°C) for 150 min. The cells were lysed, and DNA was extracted as described in Materials and Methods. One portion of the lysate was then analyzed by alkaline sucrose gradient centrifugation. A single peak of CCC DNA, relatively small in size, was apparent (Fig. 2). This peak was absent if the thermal shift was omitted or if the culture was *recA*$^+$. The rest of the lysate was purified instead by CsCl-ethidium bromide centrifugation followed by a preparative neutral sucrose gradient (Fig. 3). The peak material was pooled and prepared for electron microscopy. Based on 37 molecules measured with phage P4 DNA as internal standard, the contour length of the CCC element was determined to be 14.4 ± 0.3 kb (Table 1). We therefore concluded that the 14.4-kb element represents a unique DNA species which is excised from the chromosome as a result of derepressing the host SOS repair functions. Hereafter we will refer to this element as e14. Since e14 corresponded closely in size with the 14.6-kb circle seen after UV irradiation of CH439 (Fig. 1), it seemed probable that they were identical.

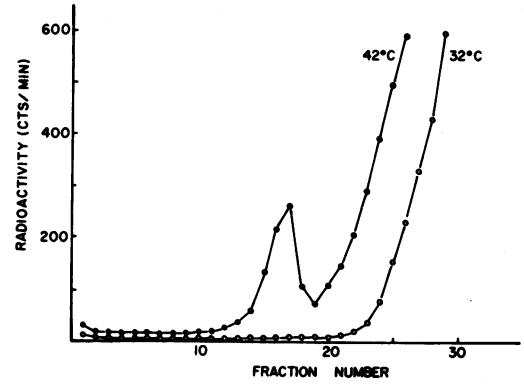

FIG. 2. *Alkaline sucrose density gradient analysis of DNA prepared from CH826 (recA441). Procedures for alkaline sucrose gradient analyses were described previously (18). CH826 was labeled with [^{3}H]thymidine and grown for 150 min at 32°C (○) or 42°C (●). The cells were lysed, and DNA was extracted, dialyzed, and then sedimented through an alkaline sucrose gradient at 40,000 rpm at 20°C for 100 min in a Beckman SW41 rotor. Fractions, 0.4 ml, were collected by bottom puncture, and radioactivity was determined by precipitation with 2 N HCl. A total of 40 fractions were recovered from each gradient.*

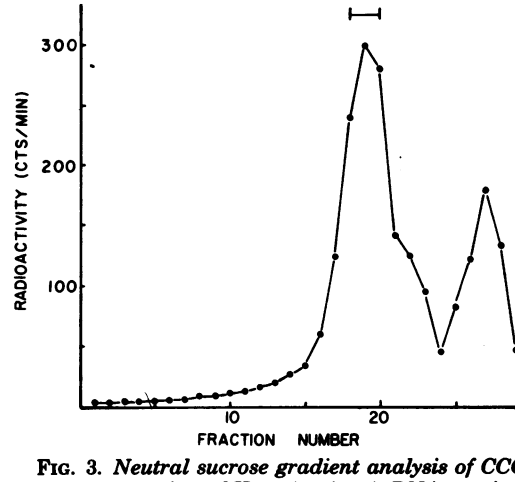

FIG. 3. *Neutral sucrose gradient analysis of CCC DNA prepared from CH826 (recA441). DNA was isolated as described in the text, and the lysate was purified by CsCl-ethidium bromide centrifugation. The peak fractions representing CCC DNA were pooled and sedimented through a neutral sucrose gradient (18) at 40,000 rpm at 20°C for 100 min in a Beckman SW41 rotor. Fractions, 0.4 ml, were collected by bottom puncture, and radioactivity was determined by precipitation of a 5-μl aliquot with 2 N HCl.*

Circle excision in a tandem duplication mutant. A derivative of CH826 was constructed (CH832) which contained a tandem duplication of the *rrnB-rrnE* interval (18). This *recA441* strain was labeled with [^{3}H]thymidine at 34°C

TABLE 1. *Contour lengths of circular DNAs*[a]

Source of DNA	Molecules measured (no.)	Molecular length (kb)	
		Relative to P4	Kilobase pairs (10^3)
e14	37	1.31	14.4 ± 0.3
pBR313	30	0.80	8.8 ± 0.2
pAG2	25	1.86	20.4 ± 0.6

[a] Covalently closed circular DNA was isolated as described in the text. Before spreading, the DNA was dialyzed against 0.1 × SSC and heated at 75°C for 90 min to induce thermal nicking (11). The DNA was then spread according to the protocols of Davis et al. (10). Phage P4 DNA of length 10,970 base pairs (12) was included as an internal standard.

and then treated in one of two ways. One-half of the culture was UV irradiated and grown at 34°C for 150 min. The other half was not irradiated, but was immediately diluted and grown for 150 min at 42°C. The cells were lysed, their DNA was extracted, and the respective lysates were run on alkaline sucrose gradients. In the profile of the UV-irradiated cultures (Fig. 4A), a peak of CCC DNA (fractions 19 to 20), corresponding in size to the duplicated *rrnB-rrnE* interval (39 kb), was present. However, under conditions of thermal induction, no peak of this size was evident (Fig. 4B). This was consistent with the hypothesis that the excision of a tandem duplication results from a *recA*-dependent recombinational event (17), an event which is enhanced by UV irradiation but not by induction of the SOS repair processes (34).

As expected, the excision of the 14.4-kb circle did occur when CH832 was thermally induced (Fig. 4B, fraction 28). Any 14.4-kb material present in the extract of the UV-irradiated culture (Fig. 4A) would not have been resolved from the fragments of chromosomal DNA produced by the UV irradiation.

These experiments demonstrated that the mechanism for excision of e14 was substantially different from the excision of a circle by simple homologous recombination between tandemly duplicated segments. Excision of e14 clearly depended on the induction of the SOS repair processes.

Cloning of a portion of e14. To further investigate the nature of this novel cryptic element, it was useful to clone at least a major segment of its genome. The vector chosen was pBR313, a plasmid which carries the genes for ampicillin and tetracycline resistance and which has one site for *Hin*dIII endonuclease cleavage occurring within the tetracycline resistance gene (4). Both pBR313 and e14 DNAs were cleaved with *Hin*dIII enzyme, mixed, and ligated, and

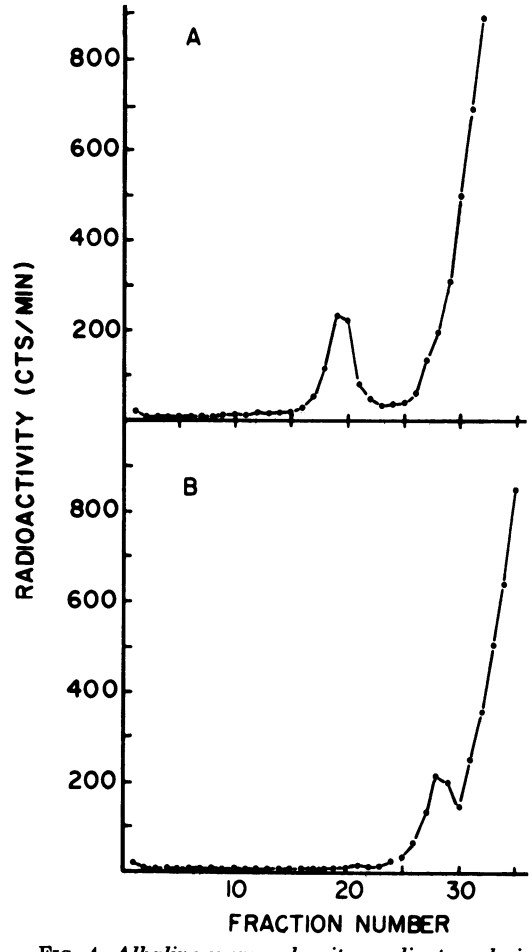

FIG. 4. *Alkaline sucrose density gradient analysis of DNA prepared from CH832 (a recA441 mutant bearing a 39-kb tandem duplication). Centrifugation was performed at 40,000 rpm at 20°C for 50 min in an SW41 rotor. (A) DNA prepared after 40 s of UV irradiation and 150 min of growth at 32°C before lysis. (B) DNA prepared after thermal shift to 42°C for 150 min before lysis. Forty fractions were recovered from each gradient. Other conditions were as described in the legend to Fig. 2.*

the mixture was used to transform CH428. Amp^r transformants were selected, and each was scored for Tet sensitivity, indicating a possible insertion into the tetracycline resistance gene. One colony was found to be $Amp^r Tet^s$; it proved to contain a recombinant plasmid, pAG2. When purified pAG2 was cleaved with *Hin*dIII, two bands were apparent upon agarose gel electrophoresis, one equivalent in size to pBR313 (8.9 kb) and the other approximately 12 kb in length. pAG2 was then measured by electron microscopy against phage P4 and pBR313 DNA stand-

ards (Table 1). Since it had a total contour length of 20.4 ± 0.6 kb, we concluded that a segment 11.6 ± 0.6 kb in size was inserted into pBR313.

pAG2 was then tested by DNA-DNA hybridization with purified e14 DNA (isolated as in the preceding section) to be certain that the inserted fragment did indeed come from the 14.4-kb element. The results presented in Fig. 5 show that pAG2 contained a segment of e14 DNA.

Mapping of e14. pBR313 is a multicopy plasmid derived from the naturally occurring plasmid ColE1. It has been shown previously that replication of ColE1 and pBR313 is dependent upon host DNA polymerase I (22). If the *polA1* allele (which codes for a defective DNA polymerase I) is transduced into a plasmid-containing strain, replication of such plasmids will cease and it will be lost as the host continues to divide and replicate. In addition, it has been shown that ColE1, and thus pBR313, cannot be transferred into a *polA* recipient (22), since the incoming plasmid cannot replicate and is unable to establish itself as an autonomous replicon. However, we believed that if an incoming plasmid, such as pAG2, contained an area of homology with the host chromosome, it could integrate by homologous recombination at that site. If so, pAG2 transformants of a *polA* recipient could be used to locate the chromosomal locus which contains the area of homology.

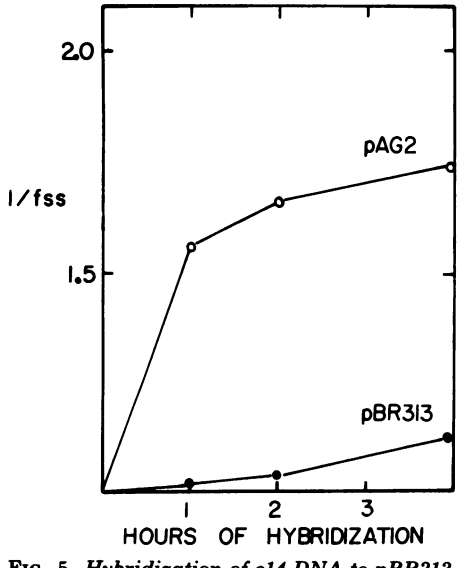

FIG. 5. *Hybridization of e14 DNA to pBR313 and pAG2. A 5-ng portion of e14 DNA (8 × 10⁴ cts/μg) was incubated at 68°C with 60 ng of plasmid DNA for the times indicated. The amount of material hybridized was determined by resistance to S1 nuclease (30). Radioactivity was measured by precipitation with 12% trichloroacetic acid. Each point represents the average of two results. fss, Fraction of the DNA remaining single stranded.*

To test this hypothesis, a *polA1* derivative of CH902 was constructed (CH923). Both pBR313 and pAG2 were able to transform CH902 (*polA*⁺) at relatively high frequencies (Table 2). pBR313, which shares no homology with the host chromosome, was unable to transform either of the *polA1* mutants, CH923 or CH985 (Table 2). However, pAG2, which carried 11.6 kb of DNA derived from e14, was able to transform both *polA1* mutants, although at a greatly reduced frequency (Table 2). Examples of the transformants were shown to lack autonomous plasmid DNA by alkaline sucrose gradient analysis of their lysates. The ampicillin resistance gene carried by pAG2 in these transformed colonies was presumably integrated into the resident e14 locus. Proof that the entire pAG2 plasmid was retained in CH923 was obtained by transducing the *polA*⁺ allele back into one such transformant. We reasoned that, in this situation, the integrated pAG2 would provide an active origin of replication and would be detrimental. Variants wherein the pAG2 had been looped out by homologous recombination between it and the resident e14 would not be handicapped by the multiple initiation of replication within the bacterial chromosome. In fact, when two independent *polA*⁺ derivatives were analyzed by alkaline sucrose gradient analysis, both were found to contain plasmid DNA. The gel electrophoretic patterns of both of these plasmids were indistinguishable from that of pAG2.

To map the position of the *E. coli* chromo-

TABLE 2. *Transformations with circular DNA*[a]

Transforming DNA	Recipient	Transformants per μg of DNA
pBR313	CH902 (*polA*⁺)	2×10^6
pAG2	CH902 (*polA*⁺)	1.4×10^6
pBR313	CH923 (*polA*)	<1
pAG2	CH923 (*polA*)	23
pBR313	CH985 (*polA recA441*)	<1
pAG2	CH985 (*polA recA441*)	15
pBR313	CH1035 (cured)	1.2×10^6
pAG2	CH1035 (cured)	1.0×10^6
pBR313	CH1073 (*polA*, cured)	<1
pAG2	CH1073 (*polA*, cured)	<1

[a] Circular DNA was prepared as described in the text. The procedure for transformation was described by Capage and Hill (6). Selection for Amp^r transformants was done on L-plates (5 g of NaCl, 10 g of tryptone, and 5 g of yeast extract per liter) containing 25 μg of ampicillin per ml.

some where e14 resides, a *polA* HfrH strain was constructed (CH931); it was then transformed by pAG2. The resultant strain, CH931/pAG2, was used as a donor in interrupted mating experiments to determine the time of entry of the ampicillin resistance gene, and thus, e14. The results suggested that pAG2 mapped somewhere near the *pyrC-purB* region. To accurately determine the map position, P1 lysates were prepared from four independent transformants of CH931 and from CH985/pAG2, an F⁻ *polA recA441* transformant derived in an independent experiment. We found that ampicillin resistance was 18 to 44% cotransducible with *purB*, depending on which strain the P1 lysate was grown (Table 3). Ampicillin resistance and *purB* were not cotransducible when a *polA*⁺ pAG2 transformant served as donor (Table 3). It was also determined that ampicillin resistance was 0.5 to 1.5% cotransducible with *pyrC* (Table 3), whereas *purB* and *pyrC* were not cotransducible in any of these strains (Table 3). Therefore, we concluded that the ampicillin resistance gene, and thus, e14, mapped between the *pyrC* and *purB* loci, more closely linked to the *purB* gene. This placed the integrated element near min 25 on the standard genetic map of *E. coli* K-12 (2).

Homology of e14 with other DNA. We wished to test for the presence of e14 in various *E. coli* strains and to determine whether it is related to other episomes such as bacteriophage λ and P4. This was accomplished by DNA-DNA hybridization as follows. The 11.6-kb fragment from ^{3}H-labeled pAG2 DNA, isolated by preparative agarose gel electrophoresis, served as the hybridization probe. The ^{3}H-labeled probe was hybridized to unlabeled DNA from a number of sources. The amount of unlabeled DNA was adjusted to provide four equivalents, taking into account the genetic complexity. The time of hybridization was plotted against the inverse of the fraction of labeled DNA which remained single stranded and, thus, susceptible to S1 nuclease digestion as described by Wetmur and Davidson (33). We found that e14 was present in the parent strain, CH439 (Fig. 6A), and in distantly related strains of *E. coli* K-12, W3102 and PB153, a Cavalli-Hfr derivative (Fig. 6B). However, e14 was not present in *E. coli* B/5 (Fig. 6A), *E. coli* C (Fig. 6B), or a K-12 derivative, Ymel *trpA36* (Fig. 6B). When four equivalents of pure pAG2 were mixed with the *E. coli* B/5 DNA, the rate of reannealing (Fig. 6A) was very similar to the rate observed for the four equivalents of CH439, W3102, or the Cavalli-Hfr derivative. This suggested that there was one equivalent of e14 per chromosome of these *E. coli* K-12 derivatives. Finally, we found that the 11.6-kb *Hin*dIII fragment of e14 shared no detectable homology with bacteriophage λ (Fig. 6D) or P4 (Fig. 6C) and plasmid pBR313 (Fig. 6D).

Loss of element e14. We wished to determine whether e14 carried essential gene sequences or whether a "cured" derivative could be obtained. To test this, CH826 (*recA441*) was inoculated into minimal medium and incubated for 8 h at 42°C. The culture was then streaked onto a minimal agar plate for single-colony isolation and incubated for 24 h at 42°C. Eight individual clones were examined to see whether or not they gave rise to the 14.4-kb circle upon

TABLE 3. *Cotransduction frequencies of purB, pyrC, and ampicillin resistance*[a]

Donor	Recipient[b] (relevant markers)	Marker selected	Marker scored	Cotransduction (%)[c]
CH985/pAG2	*pyrC* Amp^s	Pyr$^+$	Amp^r	1.4
CH985/pAG2	*purB pyrC* Amp^s	Pyr$^+$	Amp^r	0.5
CH931/pAG2, no. 1[d]	*purB pyrC* Amp^s	Pyr$^+$	Amp^r	1.5
CH931/pAG2, no. 1	*purB pyrC* Amp^s	Pyr$^+$	Pur$^+$	<0.2
CH985/pAG2	*purB pyrC* Amp^s	Pyr$^+$	Pur$^+$	<0.2
CH439	*purB pyrC*	Pyr$^+$	Pur$^+$	<0.1
CH931/pAG2, no. 1	*purB* Amp^s	Pur$^+$	Amp^r	40.8
CH931/pAG2, no. 2	*purB* Amp^s	Pur$^+$	Amp^r	17.7
CH931/pAG2, no. 3	*purB* Amp^s	Pur$^+$	Amp^r	43.9
CH931/pAG2, no. 4	*purB* Amp^s	Pur$^+$	Amp^r	31.3
CH985/pAG2	*purB* Amp^s	Pur$^+$	Amp^r	28.0
CH428/pAG2	*purB* Amp^s	Pur$^+$	Amp^r	<0.1

[a] Procedures for P1 transductions have been described previously (15).

[b] The *pyrC* Amp^s recipient was strain MA1008 (3). The *purB pyrC* Amp^s recipient was strain X7014a (28). The *purB* Amp^s recipient was strain PC0254 (Phabagen Collection, Utrecht, The Netherlands).

[c] Percent cotransduction was derived by replica plating or spot printing the selected transductants for the unselected marker.

[d] CH931/pAG2 no. 1 through 4 were four independent Amp^r transformants of the *polA1* mutant CH931.

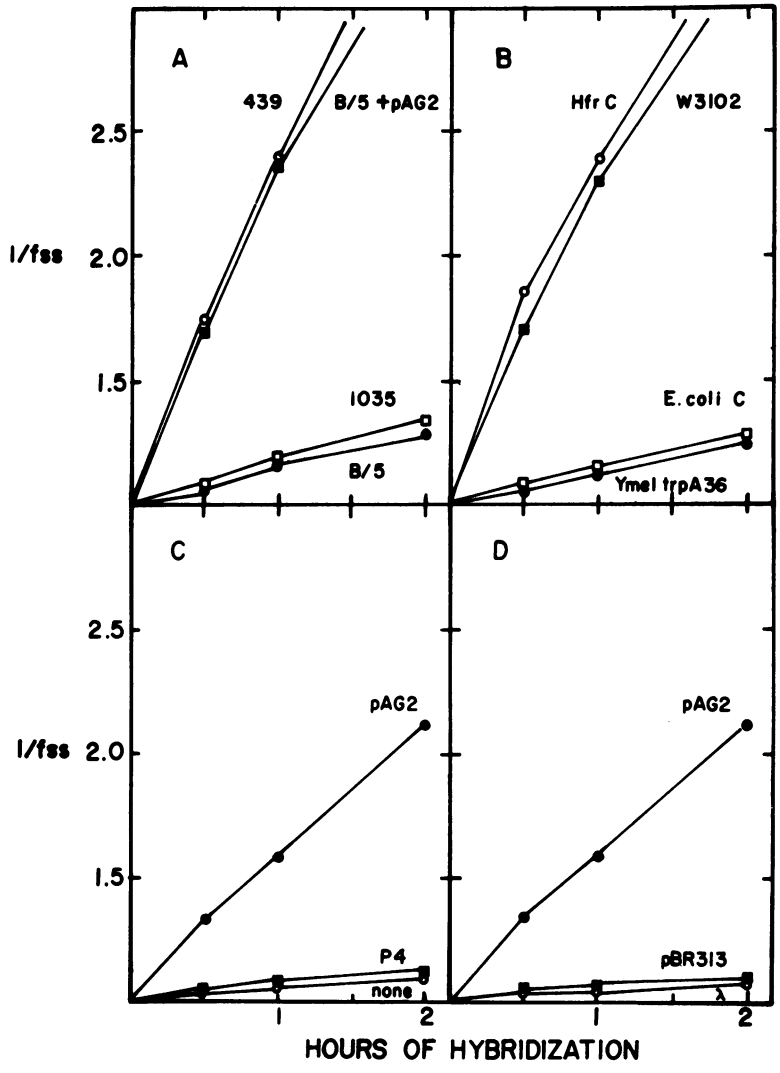

FIG. 6. *Hybridization of the 11.6-kb fragment of e14 to DNA from various sources. In (A) and (B), 7.1 ng of the labeled fragment (2.4 × 10^4 cts/μg) was incubated at 68°C with 11.4 μg of chromosomal DNA (four equivalents) isolated from various E. coli strains. The Cavalli-Hfr derivative used was PB153. In (C) and (D), 7.1 ng of the probe was incubated at 68°C with four equivalents of phage or plasmid DNA (number of equivalents based on size of unlabeled DNA added compared with the 11.6-kb fragment). The amount of material hybridized was determined by resistance to S1 nuclease. Radioactivity was measured by precipitation with 12% trichloroacetic acid. Each point represents the average of two results.*

thermal induction. Of the eight which were screened, five no longer showed a CCC DNA peak corresponding to e14 on alkaline sucrose gradients. These cured strains showed no new growth factor requirements and no measurable change in doubling time. One of these (CH1035) was further tested by DNA-DNA hybridization to the cloned 11.6-kb fragment to ascertain whether the element was indeed lost or whether a mutation had occurred which interfered with its ability to give rise to CCC molecules. The hybridization experiments (Fig. 6A) showed that the cryptic element had been lost from CH1035.

Further evidence that CH1035 no longer carried the e14 sequence was obtained by transformation of a *polA* derivative of CH1035 (CH1073) by pAG2. Although pAG2 could transform a *polA* derivative of an e14-containing strain, it was unable to transform CH1073 (Table 2). This suggested that the homologous DNA necessary for pAG2 to integrate was not present in the cured strain.

DISCUSSION

We have characterized a 14.4-kb element which is excised from the *E. coli* K-12 chromosome under SOS-inducing conditions. This element, designated e14, has been shown to reside in the genetically unpopulated area near min 25 by the following criteria.

(i) Transformation of a *polA* strain by pAG2 (which contained an 11.6-kb fragment of the e14 DNA) occurred at a low but measurable frequency. Transformation by pBR313 did not. This implied that the incoming plasmid contained a region of homology with the chromosome to serve as a site for recombination between the two.

(ii) Transformation of a cured *polA* strain by pAG2 did not occur, indicating that pAG2 could not recombine with a chromosome which no longer contained any homology to the plasmid.

(iii) The ampicillin resistance gene of pAG2 and the *purB*$^+$ allele were cotransducible by phage P1 prepared from five independently transformed *polA* colonies. In addition, those transformants tested also showed cotransducibility of *pyrC* and ampicillin resistance, whereas we determined that *pyrC* and *purB* were less than 0.1% cotransducible.

In the P1 transduction experiments described above, the fate of the DNA entering the recipient is unclear. Since pAG2 contained a viable origin of replication, the incoming DNA (if it contained that origin) might simply have recircularized and replicated autonomously in the *polA*$^+$ recipients rather than have been recombined into the chromosome. Nevertheless, the cotransduction frequencies clearly measured the likelihood of two markers being brought in on the same P1 fragment rather than their fate once inside the recipient cell. Therefore, the reported frequencies depended entirely on the linkages in the *polA1* donor.

To be certain that pAG2 integrated at a unique location rather than at numerous sites, a recombinant plasmid, consisting of a segment of *E. coli* DNA from the *xyl-mtl* region (min 80) cloned in pBR313, was used to transform CH923 (R.-J. Lin and C. W. Hill, unpublished data). P1 lysates grown on this transformant showed cotransduction frequencies of 28.4 and 13.6% between the ampicillin resistance gene carried by the recombinant plasmid and the bacterial *mtl* and *xyl* loci, respectively. In addition, ampicillin resistance was less than 0.1% cotransducible with *purB*. From this we concluded that transformation of a *polA* recipient by a plasmid carrying a portion of the *E. coli* chromosome occurred by integration into the region of homology only.

This novel genetic element shares some properties with known episomes. Like the lambdoid prophages, e14 is excised from the host chromosome upon SOS induction. We have no evidence that it undergoes replication after excision. Therefore, it may turn out that it is more accurately classified as a defective episome, since some form of autonomous replication is essential for an element to be considered a true episome (20).

We have shown that prolonged growth of a *recA441* strain under conditions which derepress the host SOS repair functions caused e14 to excise and give rise to daughter cells cured of the element. Since no differences in growth between the e14-containing strain and the cured strain were apparent, we infer that the element encodes nonessential gene sequences. Also of interest is that not every clone tested after these prolonged SOS-inducing conditions had been cured of the element (only five of eight were cured). This suggests that the excision process is not entirely efficient.

Extraction of CCC DNA from UV-irradiated CH439 resulted in the observation of a variety of circular DNA species (Fig. 1). Based on the coincidence of size, the 14.6 ± 0.4-kb species was almost certainly e14. Larger circles tended to fall into size categories corresponding to known intervals between the directly repeated rRNA genes (*rrnB-rrnE*, 39 kb; *rrnC-rrnA*, 91 kb; *rrnA-rrnB*, 126 kb; *rrnA-rrnE*, 165 kb). A likely explanation for these circles, therefore, is that they were excised by homologous intrachromosomal recombination between *rrn* genes. The observation of these large circles lends support to a model for the generation of tandem duplications wherein such circles could reintegrate by homologous recombination into the same region of a sister chromatid (15).

A discrete 12.0 ± 0.3-kb species was also observed in the extracts of UV-irradiated CH439. No indication of 12-kb material was obtained after thermal induction of the *recA441* strain CH826, even though 12-kb circles would not have been resolved from the 14.4-kb circles by the preparative steps (Fig. 3). The measurements of the 37 circles observed in the material derived in Fig. 3 ranged from 13.8 to 15.2 kb. Since 75% of the circles were relaxed, we are confident that 12-kb circles either are not produced by the thermal shift or are produced at a much lower frequency than the e14 circles. Although closely related, CH826 and CH439 differ at several loci, the consequence of several different matings, so it is also possible that CH826 does not even harbor the 12-kb element.

The existence of several biases in the measurements tabulated in Fig. 1 should be emphasized. The largest CCC molecules are undoubt-

edly preferentially lost during the prolonged preparative steps due to nicking and shear. On the other hand, since no effort was made to nick the preparations before spreading, the smallest molecules tended to remain as supercoils and, therefore, were unmeasurable.

It has been postulated that a contributing factor in the evolution of the enteric bacterial chromosome involves the addition (and loss) of episomes (27). Original isolates of *E. coli* K-12 contained both the F^+ sex factor and bacteriophage λ in the lysogenic state (1). In addition, it has recently been shown that a cryptic prophage called *rac* is a normal substituent of the *E. coli* K-12 chromosome and resides in the large silent region surrounding the terminus of replication (21, 23). The *rac* prophage is thought to be nonessential as "nonlysogenic" strains have been isolated. Evidence has been presented by a number of sources (14, 21) which suggested that there exist other cryptic elements which are normal constituents of the *E. coli* chromosome and which map at locations distinct from the *rac* prophage.

Element e14 was shown to be present in W3102 and Cavalli-Hfr, two sublines of *E. coli* K-12 which diverged early in the development of laboratory strains (1). Therefore, e14 may well have been present in the original K-12 stock. One K-12 stock examined, Ymel *trpA36*, did not contain DNA homologous to e14. Ymel *trpA36* was a UV-induced mutant of Ymel (24) and it is possible that this mutant was cured either at that time, or at some earlier stage in the development of Ymel. It is clear that e14 is not universal among *E. coli* strains since it was not found in either *E. coli* B/5 or *E. coli* C. The presence or absence of e14 has not been observed to affect the phenotype of the host cell in any way.

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