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Integration of Bacteriophage λ into the Cryptic Lambdoid Prophages of *Escherichia coli*

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Bacteriophage lambda missing its chromosomal attachment site will integrate into *recA*$^+$ *Escherichia coli* K-12 and C at the sites of cryptic prophages. The specific regions in which these recombination events occur were identified in both lambda and the bacterial chromosomes. A *Not*I restriction site on the prophage allowed its physical mapping. This allowed us to identify the locations of Rac, Qin, and Qsr′ cryptic prophages on the *Not*I map of *E. coli* K-12 and, by analogy, to identify the cryptic prophage in *E. coli* C as Qin. No new cryptic prophages were detected in *E. coli* K-12.

The cryptic lambdoid prophages of *Escherichia coli* contain genes related but not identical to those of bacteriophage λ. They were discovered by virtue of their ability to functionally replace defective genes in bacteriophage λ (13, 14, 32). Furthermore, cryptic prophage genes on an *E. coli* chromosome may be expressed and contribute to bacterial phenotype.

Three cryptic prophages have been identified and genetically mapped in *E. coli* K-12: Qsr′ at 12.5 min, Rac at 29.6 to 30.1 min, and Qin, also called Kim, at 34.2 to 34.6 min (1, 10, 12, 13, 19, 22). Partial restriction maps for each cryptic prophage have been constructed, and all three contain sequences that hybridize to sequences from bacteriophage λ (5, 18, 19). The genes in cryptic prophages are usually silent but may be activated by specific mutations or conditions. *cis*-Acting regions may also be activated.

Phage λ can integrate into the cryptic lambdoid prophages. Such integration events are seen when *E. coli* cells are lysogenized with a λ derivative deleted of its attachment site (3, 33). Here, we show that these integration events are dependent on (i) DNA sequence homology found between λ and the cryptic prophages and (ii) the general recombination pathway promoted by the *recA* gene product. We identify those portions of bacteriophage λ that are homologous to the different cryptic prophages in *E. coli* K-12. We determine the physical locations of these cryptic prophages on a recently developed restriction map of the K-12 chromosome (27) and identify the single cryptic lambdoid prophage in *E. coli* C. Our approach can be used to map groups of genetic loci that are homologous to each other such as evolutionarily related genes and active or inactive insertion sequences.

MATERIALS AND METHODS

Phage, bacteria, and plasmids. Phage and bacterial strains and plasmids are listed in Table 1. Strains SY1129 to SY1133 were constructed from SY203, strains SY1135 to SY1139 were constructed from SY924, and strain SY1134 was constructed from SY926 by infection with λ528 as described elsewhere (A. E. Lichens-Park, Ph.D. thesis, Harvard University, Cambridge, Mass., 1988).

Media and chemicals. Media used were as described elsewhere (23). All restriction enzymes and other enzymes used in recombinant DNA work were purchased from New England BioLabs, Inc. Antibiotic concentrations (in milligrams per liter) were as follows: kanamycin, 30; nalidixic acid, 20; chloramphenicol, 10; and tetracycline, 15.

Preparation of DNA samples, restriction enzyme digestion, and electrophoresis. Chromosomal megabase restriction fragments were prepared and fractionated by pulsed-field gel electrophoresis (PFGE) as described previously (25, 29).

Southern blot analysis. In the Southern analysis (30), hybridization was performed in 50% formamide at 42°C. The plasmids from which the probes were prepared are listed in Table 1. Plasmid DNA was purified on CsCl-ethidium bromide gradients. Phage DNA used as probe was prepared from phage purified on CsCl gradients.

Construction of plasmids containing cryptic prophage DNA (pAP1 to pAP6) and pAP7. *Eco*RI fragments from the *E. coli* K-12 genomic library of Kohara et al. (20) were subcloned into the *Eco*RI site of pACYC184 (9). Plasmids pAP1 to pAP3 contain phage 2C3 13.5-, 4.2-, and 14.8-kilobase (kb) *Eco*RI fragments, respectively, which contain Rac DNA. Plasmids pAP4 to pAP6 contain phage 8F11 10.9-, 3.8-, and 0.8-kb *Eco*RI fragments, respectively, which contain Qsr′ DNA. Our goal in constructing these plasmids was to obtain subclones containing all of the cryptic prophage DNA. However, since we cannot be sure where cryptic prophage DNA ends and flanking bacterial DNA begins, we cannot be certain that all of the cryptic prophage DNA is included in our subclones. We believe that a portion of the Qsr′ 6.5-kb *Eco*RI fragment (see below) containing homology to the λ *cos* site and to sequences in the *b*2 region (18) is absent in pAP5. In addition, it is probable that a portion of the Qsr′ 13.8-kb fragment that contains homology to DNA to the right of the λ *cos* site (24) is missing from the 10.9-kb fragment in pAP4. For restriction maps of Qin, Qsr′, and Rac cryptic prophages, including *Eco*RI sites, see references 18, 5, and

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TABLE 1. Bacteria, phage, and plasmids

Bacterial strain, phage, or plasmid	Genotype or description	Source or reference
Strains		
SY203	X90 Δ(*lac-pro*)*13 ara argE*(Am) *nal rif*	16
SY327	SY203 *recA1*	16
SY924	SY203 *recF143*	This study
SY926	SY203 *recA1 recF143*	This study
X53	F⁻ *gal purE supE* Str^r	J. Beckwith
PLK1165	*trg*::Tn*10*	T. Griffin
C-1a	*E. coli* C, F⁻ prototroph	G. Christy
C4501	C-1a *recA*::chloramphenicol resistance	G. Christy
Phage		
528	λ *b*221 *red*::Kan^r (Δ*bet* Δ*gam* Δ*kil*) *rexB*::IS*50*$_R$ *c*I857 *P*am3	21
2C3	*Sau*3A insert of Rac DNA into λEMBL4 vector	20
8F11	*Sau*3A insert of Qsr′ DNA into λEMBL4 vector	20
2B2	*Sau*3A insert of W3110 chromosomal DNA into λEMBL4 vector	20
Plasmids		
pBS12	331–360 kb[a]	5
pK8	155–163 kb[a] including 0.4 kb of Rac cloned into pBR325	P. L. Kuempel (11)
pK5	390–406 kb[a] 15.2-kb *Eco*RI fragment in pBR325	P. L. Kuempel (18)
pMT521	pUC8::*lsp*	17
pAP1–pAP7	See Materials and Methods	This study
pACYC184	Cm^r Tet^r	R. Kolter (9)

[a] Refers to locations on Bouche's 470-kb physical map of the *E. coli* terminus region (4).

19, respectively. The central *Eco*RI fragment from phage 2B2 was inserted into pACYC184 to create pAP7.

RESULTS

Integration of bacteriophage λ into the cryptic prophages in *E. coli* K-12 is *recA* dependent. Bacteriophage λ deleted for its normal attachment site lysogenizes Rec$^+$ strains of *E. coli* K-12 by integrating into the bacterial chromosome at specific regions (3, 33). These regions appear to correspond to the cryptic lambdoid prophages. Phage λ528 (Fig. 1) was used to map the regions both in the *E. coli* chromosome and in the phage chromosome through which the recombination event occurs. Phage λ528 carries the *b*221 deletion, which removes the *att* site, the Kan^r gene from Tn*903*, which serves as a selectable marker, and an amber mutation in the replication gene *P*. In addition, this phage contains the insertion sequence IS*50*$_R$, which contains a recognition site for the restriction enzyme *Not*I (28). Thus, the location of the phage in the chromosome can be physically mapped by identifying this new restriction site.

TABLE 2. Frequencies of Kan^r transduction by λ528 in *E. coli* strains containing *recA* and *recF* alleles

Strain	Genotype	Multiplicity of infection	Kan^r transductants/ infected cell
SY203	*recA*$^+$ *recF*$^+$	2.7	2×10^{-4}
		0.2	1×10^{-4}
SY327	*recA recF*$^+$	10	$<4 \times 10^{-8}$
		0.6	$<9 \times 10^{-8}$
SY924	*recA*$^+$ *recF*	4.0	1×10^{-4}
		0.3	5×10^{-5}
SY926	*recA recF*	4.7	1×10^{-8}
		0.3	$<5 \times 10^{-8}$
C-1a	*recA*$^+$	0.2	3×10^{-6}
C4501	*recA*	0.2	3×10^{-7}
		0.2	$<2 \times 10^{-7}$

λ528 integrates into the host chromosome either by forming an IS*50*$_R$-mediated cointegrate (21) or by general recombination. This latter point is illustrated by the data in Table 2. The frequency of lysogenization by λ528 is much greater in *recA*$^+$ hosts than in *recA* hosts. This indicates that integration is dependent on the pRecA-promoted general recombination system. To establish this point, we chose five Kan^r transductants of the *recA*$^+$ *recF*$^+$ K-12 host (SY1129 to SY1133), five Kan^r transductants of the *recA*$^+$ *recF* K-12 host (SY1135 to SY1139), and the Kan^r transductant from the *recA recF* K-12 host (SY1134) for further characterization.

λ inserts into K-12 chromosomal regions homologous to λ. The locations of the λ528 prophages were initially mapped by using Southern (30) hybridization techniques. Chromosomal DNA prepared from each of the isolates and from their parental strains was cut with *Eco*RI, and those fragments were probed with wild-type λ DNA (data not shown). Previous hybridization experiments have identified *Eco*RI fragments homologous to λ DNA: a 15.2-kb fragment in Qin (5), 13.6- and 4.6-kb fragments in Rac (19), and 13.8- and 6.5-kb fragments in Qsr′ (18). The lysogens we isolated can be divided into three groups on the basis of missing *Eco*RI fragments. Group I (SY1129, -1130, -1132, and -1137) is missing a 6.5-kb *Eco*RI fragment. This suggests that λ528 has integrated into Qsr′. Group III (SY1131, -1133, -1135, and -1136) is missing the 15.5-kb fragment, suggesting that λ528 has integrated into Qin. In group II (SY1138 and -1139), λ528 appears to be integrated into the 13.6-kb *Eco*RI fragment of Rac.

The location of the λ528 prophage in groups I and II was shown to be near Qsr′ (*purE*) and Rac (*trg*), respectively, by

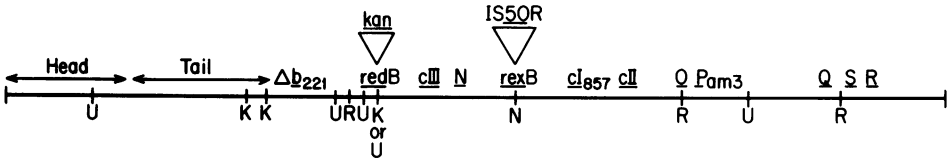

FIG. 1. Map of λ528. Relevant genetic markers are written above the line. Restriction sites are marked below the line: *N*, *Not*I; R, *Eco*RI; K, *Kpn*I; U, *Nru*I. Beginning at the left end of phage λ528, the *Not*I fragments are 27 and 13.3 kb, the *Eco*RI fragments are 21, 10, 5.8, and 3.5 kb, and the *Kpn*I-*Nru*I fragments from left to right are 4.6, 12.5, 1.5, 2.3, 0.7, <1.2 (exact size not known), 11.6, and 6.7 kb. The site marked K or U is either an *Nru*I or a *Kpn*I site that is in the Kan^r gene.

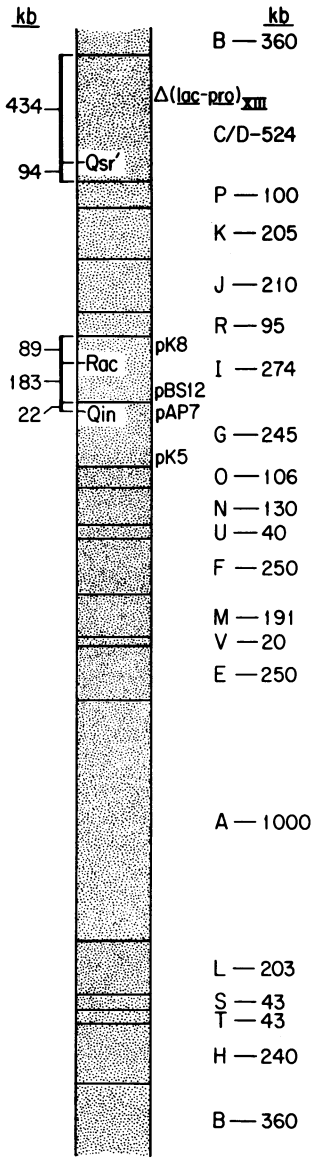

FIG. 2. *Not*I restriction map of SY203 containing the locations of cryptic lambdoid prophages Rac, Qsr', and Qin. Whereas the precise location of probe DNA within the *Not*I restriction map of EMG2 (27) is known only for linking clones, the positions of the cryptic prophages in the SY203 *Not*I fragments are quite accurately mapped because they were marked with *Not*I sites (see text). Only the *Not*I fragments that contain cryptic prophages were identified in SY203 by using cloned probes. The other *Not*I fragment sizes in SY203 are listed only by analogy with those in EMG2. For the orientation of λ528 inserted into each cryptic prophage, see text. The scale of the map is in kilobases.

P1 transduction (data not shown). The *kan* gene in the group I lysogens was 6% linked to *purE*, and the *kan* gene in group II lysogens was 3 to 10% linked to *trg*.

Physical mapping of the cryptic lambdoid prophages of *E. coli* K-12. The restriction enzyme *Not*I cuts the *E. coli* K-12 genome into 22 fragments, which can be separated by PFGE (29). These fragments were ordered to construct a complete *Not*I restriction map of the *E. coli* K-12 genome (Fig. 2; 27). Any chromosomal locus that has been marked with a *Not*I site can be easily mapped by comparing the pattern of *Not*I fragments in the parental, unmarked chromosome with that of the marked chromosome (26, 28). Genetic loci that have homology to even a small cloned fragment can be mapped by integrating a *Not*I site into a nearby gene on the chromosome by RecA-dependent general recombination. The parental *Not*I fragment will be converted to two fragments in a marked chromosome. This will locate the marked locus to one of two map positions. A true position can be identified by determining the polarity of the new fragments by hybridization experiments using nearby genetic markers as probes. We demonstrate this technique by mapping the cryptic lambdoid prophages, using the *Not*I site in λ528 as our marker.

The *Not*I restriction map was originally constructed for the *E. coli* K-12 wild-type strain EMG2 (27), whereas in this study *E. coli* K-12 strain SY203 was used as the parent. Two major differences are apparent when *Not*I fragments of SY203 and EMG2 are compared (compare lanes 2 in Fig. 3A and 4A with Fig. 1 and Table 1 of reference 27). SY203 contains a fragment of about 524 kb that replaces *Not*I fragments C (306 kb) and D (275 kb) in EMG2 because of the Δ(*lac-pro*)*13* deletion in SY203 that removes over 100 kb of DNA. The second major difference is that fragment I is 230 kb in EMG2, whereas in SY203 it is 274 kb. The larger size of fragment I in SY203 is accounted for by the fact that this fragment includes the terminus of replication, which is highly polymorphic in size among different laboratory strains of *E. coli* K-12 (28).

Determination of physical map position of Qsr'. Figure 3A is an ethidium bromide-stained PFGE fractionation of *Not*I-digested DNA from SY203 and each of the 11 derivative λ528 Kan^r transductants. The second-largest fragment (the C-D fusion fragment) was missing in the group I strains (lanes 3 to 6). A new (461-kb) fragment was apparent in this group. A 107-kb fragment was also seen in a different PFGE experiment designed to fractionate smaller DNA (Fig. 4A, lanes 3 to 6). The amount of λ528 DNA in each fragment was determined as described below.

The orientation of the new fragments in group I was determined by hybridization to a plasmid containing the *lps* gene, pMT521 (Fig. 3B). This gene contains a *Not*I site linking *Not*I fragments B and D (Fig. 2). Since the 461-kb fragment hybridized to pMT521, it must be next to fragment B and the new *Not*I site must be 461 kb away from the proximal end of the C-D fusion fragment in group I strains.

Determination of the physical map position of Rac. PFGE resolution of smaller *Not*I fragments by using shorter pulse times (Fig. 4A) revealed that the group II strains were missing a 274-kb fragment and had gained fragments of 196 and 116 kb. The 274-kb fragment was from the region of the *E. coli* chromosome that includes Rac.

The precise location of the λ528 prophage in group II was determined by hybridization with pBS12 (Fig. 4B). This clone maps to 33.1 to 33.7 min on the genetic map (Table 1). It hybridizes to the 274-kb fragment from *E. coli* SY203 and from its group I and III derivatives and to the new 196-kb *Not*I fragment of group II. The new 116-kb fragment hybridizes to plasmid pK8, a region of the *E. coli* chromosome flanking Rac and including a small portion of Rac. Thus, the new *Not*I site in group II is 116 kb from the proximal end of fragment I.

Determination of the physical map position of Qin. There was no obvious difference in the *Not*I fragment pattern between the SY203 parent and its group III derivatives (Fig. 3A and 4A). This could indicate that λ528 inserted very close to a *Not*I site. Plasmid pK5, which contains DNA from Qin (18), was used to probe *Not*I-digested DNA from SY203 and

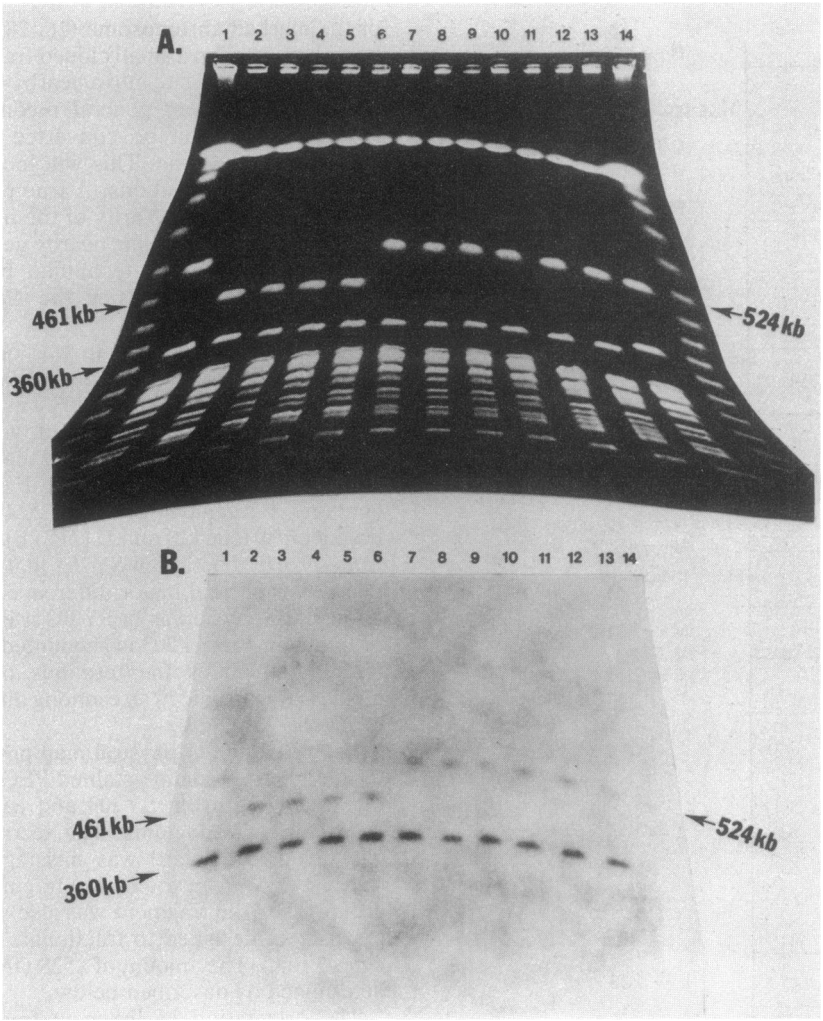

FIG. 3. Precise location of Qsr' on the *Not*I restriction map. (A) Ethidium bromide-stained PFGE experiment of *Not*I-digested DNA from Kan^r λ528 transductants: SY203 (lane 2), SY1137 (lane 3), SY1129 (lane 4), SY1130 (lane 5), SY1132 (lane 6), SY1131 (lane 7), SY1133 (lane 8), SY1135 (lane 9), SY1136 (lane 10), SY1138 (lane 11), SY1139 (lane 12), SY1134 (lane 13). Lanes 1 and 14 contain linear concatemers of λ *c*I857 DNA starting from 48.5 kb. Fractionation was for 40 h at 330 V, using a 60-s pulse time. (B) Hybridization of the gel shown in panel A with plasmid pMT521.

the group III strains. In each of these strains, pK5 exposed a band in a cluster of bands between 240 and 250 kb (data not shown). The genetic map position of Qin predicts that λ528 should integrate near the proximal end of *Not*I fragment G (245 kb). This prediction was tested by hybridizing *Not*I-digested DNA with plasmid pAP7. This clone includes DNA from 1,630 to 1,645 kb on the physical map of Kohara et al. (20) of the *E. coli* chromosome, which corresponds to DNA at about 34.4 min on the genetic map. The probe hybridizes to a 245-kb fragment in DNA from SY203 but to a fragment of 35 kb in the DNA from group III strains SY1131 and SY1133 (data not shown). This finding shows that the new *Not*I site in the group III strains is 35 kb from the proximal end of fragment G. The missing 35 kb from fragment G was not detected in group III because of the insertion of 27 kb from λ528 (see below).

Determination of λ528 orientation when integrated into cryptic prophages. The orientation of λ528 in each of the cryptic prophages was determined by hybridizing IS*50* DNA that lies to the left of the *Not*I site (Fig. 1) to PFGE-fractionated *Not*I fragments from group I, II, and III strains. The 461-, 116-, and 250-kb fragments were exposed by this probe in groups I, II, and III, respectively. Thus, if we define the orientation of the λ prophage integrated at the λ *att* site as clockwise (i.e., with head and tail genes below the rest of the phage in Fig. 2), the group I and II prophage would be clockwise but the group III prophage would be counterclockwise. This information was also used to more precisely map the location of the group I, II, and III prophage integration sites (see below).

Identification of the cryptic lambdoid prophage in *E. coli* C. Once we had determined the physical map location of the cryptic prophages in *E. coli* K-12, it was relatively simple to identify the cryptic prophage in *E. coli* C, which can also be infected with λ528. Anilionis and Riley (2) determined that there was only one *Eco*RI fragment and only one *Hin*dIII fragment in *E. coli* C that was homologous to λ DNA, implying that strain C had only one cryptic lambdoid prophage. Integration of λ528 was *recA* dependent in *E. coli* C as well as in *E. coli* K-12 (Table 2).

Chromosomal DNA from *E. coli* C-1a and five Kan^r transductants cut with *Not*I and fractionated by PFGE was

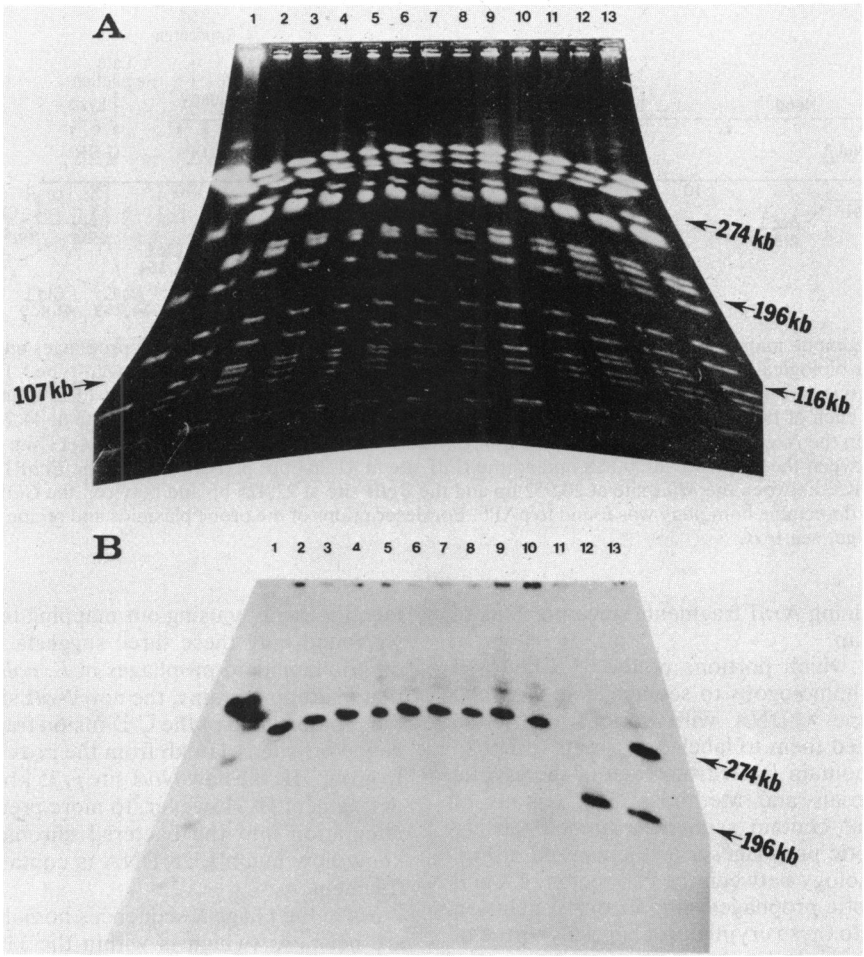

FIG. 4. Precise location of Rac on the *Not*I restriction map. (A) Ethidium bromide-stained PFGE experiment of *Not*I-digested DNA from Kan^r λ528 transductants: SY203 (lane 2), SY1137 (lane 3), SY1129 (lane 4), SY1130 (lane 5), SY1132 (lane 6), SY1131 (lane 7), SY1133 (lane 8), SY1135 (lane 9), SY1136 (lane 10), SY1138 (lane 11), SY1139 (lane 12), SY1134 (lane 13). Lane 1 contains linear concatemers of λ *c*I857 DNA starting from 97 kb. Fractionation was for 40 h at 330 V, using an 18-s pulse time. (B) Hybridization of the gel shown in panel A with plasmid pBS12.

hybridized with IS*50* DNA (data not shown). The *Not*I fragment pattern of *E. coli* C was similar to that of *E. coli* K-12. Fragments of 250 and 35 kb in size were detected in all of the transductants. This result is similar to that obtained with the group III *E. coli* K-12 strains, which contain λ528 inserted into Qin prophage. Furthermore, plasmid pAP7, used to map Qin in K-12, hybridized to the 35-kb fragment in four of the five Kan^r transductants. Plasmid pK5, which contains DNA from Qin, hybridized to a 245-kb fragment in the parent and to 250-kb fragments in DNA from each of the Kan^r transductants. Comparing these results with those obtained in K-12, we conclude that the cryptic prophage in *E. coli* C is most likely Qin.

Hybridization of phage λ DNA to *Not*I-cut, PFGE-fractionated DNA from *E. coli* K-12, C, and B revealed three λ-homologous *Not*I fragments in *E. coli* B of sizes similar to those found in *E. coli* K-12 (data not shown). Though one of these fragments was slightly larger than the 306-kb fragment C in K-12 and one was slightly smaller than the 230-kb fragment I in K-12, these results suggest that *E. coli* B also contains Qsr′, Rac, and Qin. As expected, only one λ-homologous *Not*I fragment was present in *E. coli* C, and it was slightly larger than the 245-kb fragment G in K-12. These results provide evidence that Qin is on fragments of the same or similar size in all three strains. These results further support the apparent physical conservation of genome organization that has been suggested by similar experiments (C. L. Smith, unpublished observations).

Regions in λ affected by integration into the cryptic prophages. The regions within λ528 through which integration occurred were determined by hybridizing labeled λ DNA to genomic DNA from the lysogens and the SY203 parent cut with *Kpn*I and *Nru*I and with *Eco*RI (data not shown). Group I and II lysogens contain *Kpn*I-*Nru*I bands of 16 and 14.5 kb and of 18.5 and 7.5 kb, respectively, that are not present in parental DNA or in the circular λ528 (Fig. 1). The group I and II lysogens do not contain the 11.3-kb *Nru*I fragment in λ528 that contains the λ *cos* site. The loss of this 11.3-kb *cos*-containing fragment indicates that the integration region used by λ528 maps to this *Nru*I fragment. In group III, no obvious *Kpn*I-*Nru*I fragment size changes were detected, although the largest band in SY203 may be somewhat larger in these strains. The Southern blot of the *Eco*RI fragments from the λ528 lysogens indicates that integration into the cryptic prophages occurs through the 24.5-kb *Eco*RI fragment of λ528; this result is consistent with integration into

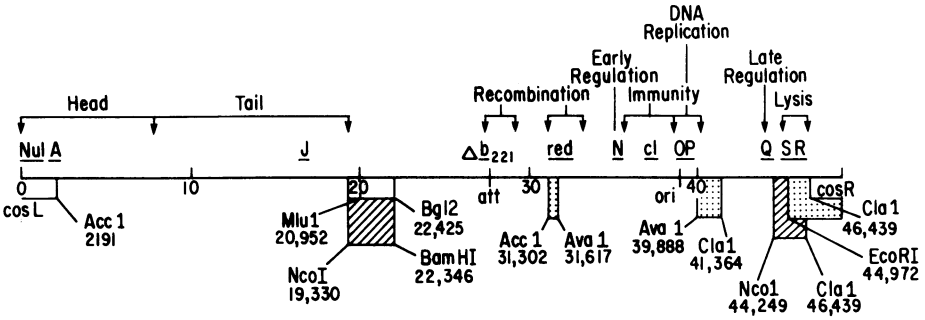

FIG. 5. Physical and genetic map of bacteriophage λ, including regions of homology to the cryptic prophages and clusters of genes whose products serve particular biological functions. Boxed areas below the map are restriction fragments in wild-type λ DNA that are partially but not completely homologous to one of the cryptic lambdoid prophages. Homologies to Qin (□), Qsr' (▒), and Rac (▨) are shown. Regions of homology to each of the probe plasmids are as follows: pAP1 and pAP3, between the *Nco*I site at 44,249 bp and the *Cla*I site at 46,439 bp; pAP2, between the *Nco*I site at 19,330 bp and the *Bam*HI site at 22,346 bp; pAP4, between the *Acc*I site at 31,302 bp and the *Ava*I site at 31,617 bp and between the *Ava*I site at 39,888 bp and the *Cla*I site at 41,364 bp; pAP5, between the *Eco*RI site at 44,972 bp and the right end at 48,514 bp; pK5, between the *Mlu*I site at 20,952 bp and the *Bgl*II site at 22,425 bp and between the *Cla*I site at 46,439 bp and the *Acc*I site at 2,191 bp. No detectable homology was found to pAP6. For descriptions of the probe plasmids and regions of λ528 used to integrate into each cryptic prophage, see text.

the 11.3-kb *cos*-containing *Nru*I fragment, since portions of these fragments overlap.

To further identify which portions of the 11.3-kb *Nru*I fragment of λ528 are homologous to sequences in Qsr' and Rac, we cut wild-type λ DNA with various restriction enzymes and hybridized them to labeled plasmids (pAP1 to pAP6 and pK5) that contain DNA from each of the cryptic prophages (see Materials and Methods). The regions of wild-type λ DNA that contain sequences homologous to sequences of the cryptic prophages are summarized in Fig. 5. The regions of homology between the *cos* region of λ and the Qsr' and Rac cryptic prophages support our conclusion that λ528 integrates into these cryptic prophages by virtue of the DNA homology through the *cos* region.

DISCUSSION

Bacteriophage λ missing its attachment site will integrate into cryptic prophage sequences in *recA*$^+$ *E. coli* K-12 and C. We have physically mapped the regions in both the bacterial chromosome and bacteriophage λ through which the integration reaction occurs. Phage λ528 contains a *Not*I restriction site. Thus, the location of integrated phage λ528 could be localized on the *Not*I restriction map of the *E. coli* chromosome. This method can be generalized either to directly map the physical location of any cloned DNA sequence on a low-resolution genomic map or to begin the ordering of large restriction fragments for such a map. The requirements in either case would be (i) a cloned gene or, in the case of essential genes, the appropriate recognition sequence for a restriction enzyme cutting infrequently which could be inserted into the region under study and (ii) a selectable marker. Obviously, the chromosome would need to contain a sequence of at least 20 to 30 base pairs (bp) of homology to the region under study that can be integrated by general recombination. A more precise position of homologous sites can be determined if clones are available which can be used to orient the new *Not*I fragments in the transformants and transductants.

In *E. coli* K-12, phage λ528 integrates into three chromosomal loci, which allowed their precise physical mapping (Fig. 2). These loci were Qsr' (group I), Rac (group II), and Qin (group III). If *E. coli* K-12 contained more than these three cryptic prophages, we should have been able to identify them by using our mapping technique. The fact that we found only these three suggests that they are the only cryptic lambdoid prophages in *E. coli* K-12. Our data show that in group I strains, the new *Not*I site is 461 kb away from the proximal end of the C-D fusion fragment. In group II, the new *Not*I site is 116 kb from the proximal end of fragment I. In group III, the new *Not*I site is 35 kb from the proximal end of fragment G. However, to more precisely locate the site of integration into the bacterial chromosome, we needed to know how much λ528 DNA is contained in each new *Not*I fragment.

Since the phage λ sequences homologous to Rac and Qsr' are near *cos* (which is within the 11.3-kb *Nru*I fragment), some of those sequences were probably used to integrate λ528 into Qsr' and Rac. Phage λ528 contains only a subset of the sequences in wild-type λ which we know to be homologous to Qin (Fig. 5). Thus, phage λ528 probably has available only those sequences between the *Cla*I site at 46,439 bp and the *Acc*I site at 2,191 bp to integrate into Qin. One of the new *Not*I fragments in the *E. coli* K-12 group I, II, and III strains contains 27 kb of λ528 DNA, and the other contains 13 kb (Fig. 1). Since the orientation of each new *Not*I fragment was determined in these strains, we showed that λ was integrated in Qsr' 434 kb away from the proximal end of the C-D fusion fragment in SY203, in Rac 89 kb from the proximal end of *Not*I fragment I, and in Qin 22 kb from the proximal end of fragment G (Fig. 2).

In *E. coli* C, there is a single cryptic prophage that corresponds in map position to Qin from *E. coli* K-12. We have not completely eliminated the possibility that λ528 has integrated into a cryptic prophage in *E. coli* C that is not Qin but is at the same physical location as that of Qin in K-12. However, we not only mapped the λ528 integration site to the same *Not*I restriction fragment of C that contains Qin in K12 but also determined its precise location within that fragment. We believe that it would be highly coincidental that the location of a different cryptic lambdoid prophage in C would be indistinguishable from the location of Qin in K-12.

The genetic and physical maps are congruent to each other. Our mapping data indicate that Qin is 205 kb away from Rac. Bouche et al. (5) also located Qin about 200 kb away from Rac. Rac and Qsr' are about 840 kb away from

each other on the map of Kohara et al. (20). The PFGE mapping experiments show that Rac and Qsr′ are separated by 793 kb in our strain. It was not possible to align restriction map information for Qin onto the map of Kohara et al. (20). Qin appears to be absent from the laboratory *E. coli* K-12 strain W3110 that was used to construct their map.

There are multiple regions in bacteriophage λ that serve as attachment points for RecA-dependent integration. Assuming that integration occurs via DNA homology-dependent recombination, multiple sites simply reflect crossover events at different points in regions of homology. The ability of members of the lambdoid family of phages to recombine with each other is relevant to the modular theory of virus evolution, originally proposed by Hershey (15) and subsequently modified (7, 31). This theory states that the lambdoid phages evolved as a family whose members may exchange functional modules of DNA. The DNA in each module serves a separate biological function (e.g., DNA replication, DNA recombination, and host cell lysis; Fig. 5); because members of the lambdoid family have a common organization of functional units (7), this allows modules from one family member to work properly in the genome of another member. This theory raised the possibility that modules contained special recombination sites that facilitated modular rearrangements. However, this does not seem to be the case. As shown by Campbell and co-workers (6, 8), functional modules are exactly contiguous without apparent intervening recombination sites. Our results are consistent with the absence of specialized recombination sites, since recombination appears to occur by virtue of the DNA homology of the functional modules. The importance of modular rearrangements for lambdoid phage evolution is reduced if general recombination is the only means by which different lambdoid phage can rearrange their modules. The requirement for contiguous regions of homology will effectively prevent modular rearrangements between distantly related phages.

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LITERATURE CITED

1. **Anilionis, A., P. Ostapchuk, and M. Riley.** 1980. Identification of a second cryptic lambdoid prophage locus in the *E. coli* K12 chromosome. Mol. Gen. Genet. **180:**479–481.
2. **Anilionis, A., and M. Riley.** 1980. Conservation and variation of nucleotide sequences within related bacterial genomes: *Escherichia coli* strains. J. Bacteriol. **143:**355–365.
3. **Bender, J., and N. Kleckner.** 1986. Genetic evidence that Tn*10* transposes by a nonreplicative mechanism. Cell **45:**801–815.
4. **Bouche, J. P.** 1982. Physical map of a 470 × 10^3 base-pair region flanking the terminus of DNA replication in the *Escherichia coli* K12 genome. J. Mol. Biol. **154:**1–20.
5. **Bouche, J. P., J. P. Gelugne, J. Louarn, J. M. Louarn, and K. Kaiser.** 1982. Relationships between the physical and genetic maps of a 470 × 10^3 base-pair region around the terminus of *Escherichia coli* K12 DNA replication. J. Mol. Biol. **154:**21–32.
6. **Campbell, A.** 1988. Phage evolution and speciation, p. 1–14. *In* R. Calendar (ed.), The bacteriophages. Plenum Publishing Corp., New York.
7. **Campbell, A., and D. Botstein.** 1983. Evolution of lambdoid phages, p. 365–380. *In* R. W. Hendrix, J. W. Roberts, F. W. Stahl, and R. A. Weisberg (ed.), Lambda II. Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.
8. **Campbell, A., D. P. Ma, M. Benedik, and R. Limberger.** 1986. Reproductive isolation in prokaryotes and their accessory DNA elements. Banbury report 24: antibiotic resistance genes: ecology, transfer and expression. Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.
9. **Chang, A. C. Y., and S. N. Cohen.** 1978. Construction and characterization of amplifiable multicopy DNA cloning vehicles derived from the P15A cryptic miniplasmid. J. Bacteriol. **134:**1141.
10. **Diaz, R., P. Barnsley, and R. H. Pritchard.** 1979. Location and characterization of a new replication origin in the *E. coli* K12 chromosome. Mol. Gen. Genet. **175:**151–157.
11. **Diaz, R., and K. Kaiser.** 1981. Rac$^-$ *E. coli* K12 strains carry a preferential attachment site for λ rev. Mol. Gen. Genet. **183:**484–489.
12. **Espion, D., K. Kaiser, and C. Dambly-Chaudiere.** 1983. A third defective lambdoid prophage of *Escherichia coli* K12 defined by the λ derivative, λ qin III. J. Mol. Biol. **170:**611–633.
13. **Gottesman, M. M., M. E. Gottesman, S. Gottesman, and M. Gellert.** 1974. Characterization of bacteriophage λ reverse as an *Escherichia coli* phage carrying a unique set of host-derived recombination functions. J. Mol. Biol. **88:**471–487.
14. **Henderson, D., and J. Weil.** 1975. The nature and origin of a class of essential gene substitutions in bacteriophage λ. Virology **67:**124–135.
15. **Hershey, A.** 1971. Comparative molecular structure among related phage DNA's. Carnegie Inst. Washington Yearb. **1970:**3–7.
16. **Hopkins, J. D., M. B. Clements, T.-Y. Liang, R. R. Isberg, and M. Syvanen.** 1980. Recombination genes on the *E. coli* sex factor specific for transposable elements. Proc. Natl. Acad. Sci. USA **77:**2814–2818.
17. **Innis, M. A., M. Tokunaga, M. E. Williams, J. M. Loranger, S. Y. Chang, S. Chang, and H. C. Wu.** 1984. Nucleotide sequence of the *Escherichia coli* prolipoprotein signal peptide (*lsp*) gene. Proc. Natl. Acad. Sci. USA **81:**3708–3712.
18. **Kaiser, K.** 1980. The origin of Q-independent derivatives of phage λ. Mol. Gen. Genet. **179:**547–554.
19. **Kaiser, K., and N. Murray.** 1979. Physical characterization of the "Rac prophage" in *Escherichia coli* K12. Mol. Gen. Genet. **175:**159–174.
20. **Kohara, Y., K. Akiyama, and K. Isono.** 1987. The physical map of the whole *Escherichia coli* chromosome: application of a new strategy for rapid analysis and sorting of a large genomic library. Cell **50:**495–508.
21. **Lichens-Park, A., and M. Syvanen.** 1988. Cointegrate formation by IS*50* requires multiple donor molecules. Mol. Gen. Genet. **211:**244–251.
22. **Low, B.** 1973. Restoration by the *rac* locus of recombinant forming ability in *recB*$^-$ and *recC*$^-$ merozygotes of *Escherichia coli* K12. Mol. Gen. Genet. **122:**119–130.
23. **Miller, J. H.** 1972. Experiments in molecular genetics. Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.
24. **Redfield, R. J., and A. M. Campbell.** 1987. Structure of cryptic λ prophages. J. Mol. Biol. **198:**393–404.
25. **Smith, C. L., and C. R. Cantor.** 1987. Preparation and manipulation of large DNA molecules: advances and applications. Trends Biochem. Sci. **12:**284–287.
26. **Smith, C. L., G. Condemine, S.-Y. Chang, E. McGary, and S. Chang.** 1989. Insertion of rare cutting sites nearby genes allows their rapid physical mapping: localization of the *E. coli* map locus. Nucleic Acids Res. **17:**817.
27. **Smith, C. L., J. G. Econome, A. Schutt, S. Klco, and C. R. Cantor.** 1987. A physical map of the *Escherichia coli* K12 genome. Science **236:**1448–1453.
28. **Smith, C. L., and R. D. Kolodner.** 1988. Mapping of the *E. coli* chromosomal Tn*5* and F insertions by pulsed field gel electrophoresis. Genetics **119:**227–236.
29. **Smith, C. L., P. E. Warburton, A. Gaal, and C. R. Cantor.** 1986. Analysis of genome organization and rearrangements by pulsed field gradient gel electrophoresis, p. 45–70. *In* J. Setlow and A.

Hollaender (ed.), Genetic engineering, vol. 8. Plenum Publishing Corp., New York.
30. **Southern, E. M.** 1975. Detection of specific sequences among DNA fragments separated by gel electrophoresis. J. Mol. Biol. **98**:503–517.
31. **Susskind M., and D. Botstein.** 1978. Molecular genetics of bacteriophage P22. Microbiol. Rev. **42**:385–413.
32. **Szybalski, W., and E. H. Szybalski.** 1974. Visualization of the evolution of viral genomes, p. 563–582. *In* E. Kurstak and K. Maramorosch (ed.), Viruses, evolution and cancer. Academic Press, Inc., New York.
33. **Young, R., D. S. Grillo, R. Isberg, J. Way, and M. Syvanen.** 1980. Transposition of the kanamycin-resistance transposon Tn*903*. Mol. Gen. Genet. **178**:681–689.