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*Eco*RI Cleavage Sites in the *argECBH* Region of the *Escherichia coli* Chromosome

EVELYN A. DEVINE, MARY C. MORAN, PETER J. JEDERLINIC, ANTHONY J. MAZAITIS, AND HENRY J. VOGEL*

Department of Pathology, College of Physicians and Surgeons, Columbia University, New York, New York 10032

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The *Eco*RI cleavage of deoxyribonucleic acids (DNAs) from λd*arg* phages, carrying *argECBH*, has been examined. The phages are derived from the heat-inducible, lysis-defective strain λ*y*199, and their bacterial DNA, including *argECBH*, is derived from *Escherichia coli* K-12. Such cleavage of the phage DNAs, in each case, produces the D, E, and F segments of λ. Additionally, these DNAs yield segments, ordered from left to right, of length (in kilobases [kb]) determined by electron microscopy and 0.7% agarose slab gel electrophoresis, as follows: λd*arg13* (*ppc argECBH bfe*), 13.9, 2.8, 1.5, and 5.6; λd*arg14* (*ppc argECBH*), 3.0, 2.0, 17.3, and 6.2; and λd*arg23* (*argECBH*), 18.4 and 6.2. For λd*arg13 sup102* DNA, the segment analogous to the 13.9-kb segment measures 12.2 kb. The direction from left to right corresponds to the clockwise orientation of the *E. coli* genetic map. The *Eco*RI segments define five cleavage sites near the *arg* region of the *E. coli* chromosome. For each of the DNAs, the *arg* genes occur on the largest segment produced. The 17.3-kb segment, being entirely bacterial, represents the *argECBH*-bearing *Eco*RI segment of the *E. coli* chromosome. The location of the *arg* genes was demonstrated electron microscopically in heteroduplex experiments.

To study transcriptional and translational control of enzyme formation in the arginine system of *Escherichia coli*, it became desirable to prepare chromosomal segments containing all or certain portions of the *argECBH* cluster. This gene cluster, with or without deletions, and adjacent bacterial deoxyribonucleic acid (DNA) can be carried by lambda transducing phages. As reported briefly (E. A. Devine, M. C. Moran, P. J. Jederlinic, A. J. Mazaitis, and H. J. Vogel, Genetics 80:s26, 1975), the *Eco*RI restriction endonuclease makes several cuts in the bacterial (as well as in the λ) region of the transducing phage DNA. The present paper describes the location of the *Eco*RI cleavage sites of the phage DNAs examined and also some properties of the segments generated.

MATERIALS AND METHODS

Strains. Transducing phages λd*arg13* (*ppc argECBH bfe*), λd*arg14* (*ppc argECBH*), and λd*arg23* (*argECBH*) were derived from λ*y*199 (*cI857 susS7 susxis*$_6$ *Δb515 Δb519*); λd*arg13 sup102* was constructed from λd*arg13* and carries the *argCB* deletion *sup102*. The λd*arg* strains, isolated by N. Glansdorff and characterized by heteroduplex mapping (6), were carried in *E. coli* MN42 (λ$^-$), doubly lysogenized with a particular λd*arg* phage and with λ*y*199 as helper.

Preparation of phage DNA. The double lysogens were grown with aeration in medium containing 1% tryptone, 0.5% yeast extract, 0.5% NaCl, and 0.1 M tris(hydroxymethyl)aminomethane (Tris)-hydrochloride (pH 7.4). Phage DNA was prepared essentially as described by Zubay (13).

Preparation of *Eco*RI endonuclease and cleavage of phage DNA. The restriction endonuclease *Eco*RI was prepared by the method of Greene et al. (2), as modified by Tanaka and Weisblum (10). The phage DNA was digested with *Eco*RI, essentially as detailed by Greene et al. (2). After incubation for 30 min at 37°C, 5 μl of 25% glycerol plus 0.025% bromophenol blue was added to the digest. The reaction mixture was then heated at 65°C for 10 min and chilled prior to agarose gel electrophoresis.

Vertical slab gel electrophoresis. Digests were analyzed by electrophoresis in a 0.7% agarose gel with the apparatus of Studier (9). The Tris-phosphate buffer (without sodium dodecyl sulfate) of Hayward and Smith (3) was employed. DNA bands were stained (8) by adding an aqueous solution of ethidium bromide (10 mg/ml) to the gel before pouring to a final concentration of 0.5 μg/ml. Digest samples (25 μl) were applied to the gel, and electrophoresis was carried out for 18 h at 30 mA at 25°C. The DNA bands were visualized by subjecting the gel to long-wave ultraviolet irradiation. The gel was then photographed with Polaroid type 107 film through a Kodak no. 23A Wratten gelatin filter.

Isolation of arginine segments by sucrose gradient centrifugation. DNA digests (60 μg, 0.5 ml) were layered on a linear 5 to 20% sucrose gradient

containing 0.02 M Tris-acetate (pH 8.0), 1.0 M NaCl, and 0.001 M ethylenediaminetetraacetate (EDTA), and centrifugation was carried out at 4°C in an SW27.1 rotor at 25,000 rpm for 15 h. Fractions (0.4 ml) were collected and examined by slab gel electrophoresis.

Heteroduplex formation. Samples (0.1 ml) containing equal amounts (1 to 2 μg) of two *arg*-bearing DNA segments isolated by sucrose gradient centrifugation were mixed with an equal volume of 0.2 M NaOH to denature the DNA. After 10 min at 25°C, 0.25 ml of 0.2 M Tris-0.02 M EDTA (pH 8.4) and 0.05 ml of 2.5 M NaCl were added. The mixture was dialyzed for 2 h against the standard renaturation buffer of Sharp et al. (7), followed by dialysis in the cold for 4 h against several changes of 0.01 M Tris-0.001 M EDTA (pH 8.4).

Electron microscopy. The droplet technique of Lang and Mitani (5), as modified by Mazaitis et al. (6), was used. Samples of sucrose gradient fractions were used without prior dialysis. RFII DNA of φX174 (5.25 kilobases [kb]) was the internal standard. Droplets (40 μl) containing 0.05 to 0.1 μg of DNA per ml, 1.3 μg of cytochrome *c* per ml, 0.01 M Tris-0.001 M EDTA (pH 8.4), and 40% formamide were deposited on a sheet of Parafilm in an atmosphere of formaldehyde. After 15 to 20 min at room temperature, samples were taken by touching grids covered with a thin specimen film (1.0% cellulose acetate butyrate-0.8% parlodion) to a droplet surface. The grids were rinsed in water, stained for 30 s in 0.0001 M uranyl acetate in 95% ethanol, rinsed in 95% ethanol, dried, and then decorated with 80% Pt-20% Pd before examination in a Siemens electron microscope. Enlarged images of DNA filaments were traced on paper, and contour lengths were determined with a map measure.

RESULTS

Electrophoresis of *Eco*RI segments of λ*y*199 DNA. The restriction endonuclease *Eco*RI is known to cleave wild-type λ DNA into six segments termed A through F (11). For the transducing phages, the amount of bacterial DNA and its site of insertion in the phage DNA are known from heteroduplex studies (6). Phage λ*y*199, the parent of the transducing phages, has the *b519* and *b515* deletions. In λ*y*199 DNA, the first cleavage site is eliminated by the *b519* deletion, and, instead of the normal A segment, a segment termed A′ is generated that corresponds to the wild-type λ segments A plus B diminished by the *b519* and *b515* deletions. Since the aggregate length of the two deletions very nearly corresponds to the length of the B segment of λ, A′ has approximately the same size as A. The *Eco*RI banding pattern of λ*y*199 DNA produced upon agarose slab gel electrophoresis is shown in Fig. 1a.

Electrophoresis of *Eco*RI segments of transducing phage DNAs. The λ*darg* phages used are all such that the arm of λ DNA to the right of the attachment site is intact and a portion of the left arm is present (6). Thus, digestion of all the λ*darg* phage DNAs with *Eco*RI results in the production of the λ D, E,

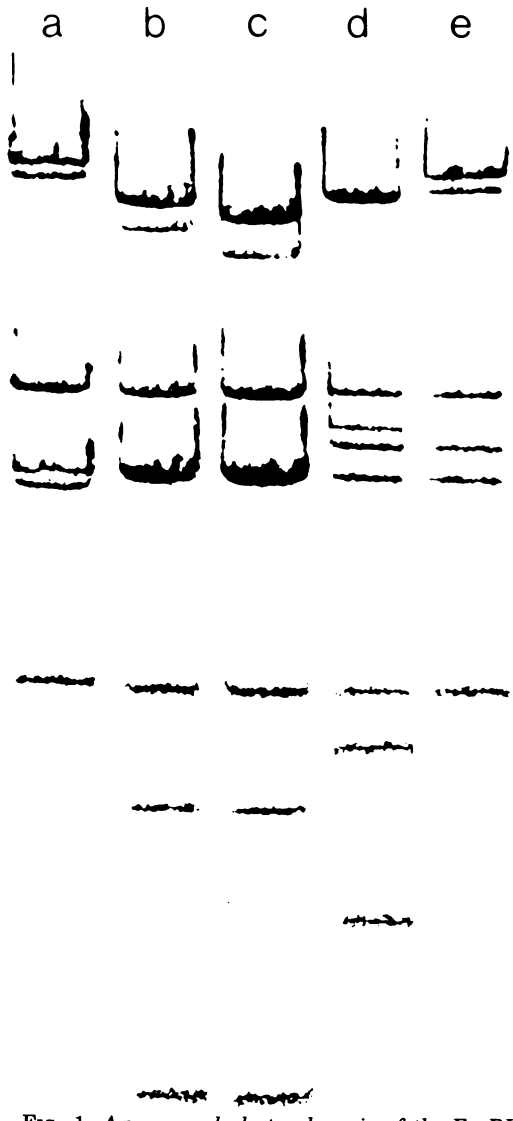

FIG. 1. *Agarose gel electrophoresis of the EcoRI cleavage products of DNAs from (a) λy199, (b) λdarg13, (c) λdarg13 sup102, (d) λdarg14, and (e) λdarg23. The purely λ DNA bands (a) are referred to by letters in the conventional manner; see the text for A′ and A′ + F. The bacterial DNA-containing bands are named according to increasing electrophoretic mobility. From top to bottom, bands are seen as follows: (a) A′ + F, A′, D, E, C, and F; (b) 13-1 + F, 13-1, D, E and 13-2, F, 13-3, and 13-4; (c) 13s-1 + F, 13s-1, D, E and 13s-2, F, 13s-3, and 13s-4; (d) 14-1, D, 14-3 + F, 14-2, E, F, 14-3, and 14-4; and (e) 23-1 + F, 23-1, D, 23-2, E, and F. Segment 13-2 is identical to 13s-2, 13-3 to 13s-3, 13-4 to 13s-4, and 14-2 to 23-2. Segment 13-2 (or 13s-2) comigrates with E.*

and F segments. The banding patterns for *Eco*RI digests of the DNAs from λd*arg13*, λd*arg13 sup102*, λd*arg14*, and λd*arg23* are seen in Fig. 1b through e, respectively. In each case, the segments containing bacterial DNA are numbered according to increasing electrophoretic mobility (Fig. 1). Segments 13-1, 13s-1, 14-3, and 23-1 carry the cohesive left end of λ. Bands corresponding to each of these segments linked to F, known to have the cohesive right end of λ, are present in Fig. 1b to e, respectively.

Measurement of segment length. The electrophoretic mobilities observed (Fig. 1) were used in one method (cf. reference 11) for the measurement of segment length. Figure 2 shows a semilog plot of segment length as a function of segment mobility. The plot was constructed from the values for the A′, C, D, E, and F segments of λ*y*199 DNA. The segments containing bacterial DNA were represented on the plot according to their mobility, and their length was determined from the graph. The values obtained are presented in Table 1. Independently, segment length was measured by electron microscopy (Table 1). The results obtained by the two methods are in good agreement.

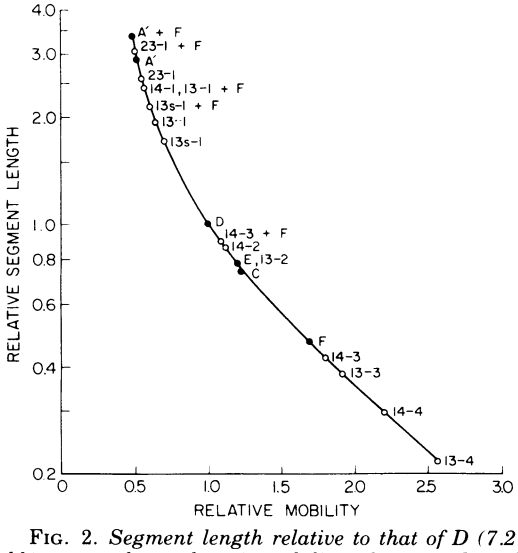

FIG. 2. *Segment length relative to that of D (7.2 kb) versus electrophoretic mobility relative to that of D (average absolute mobility, 48.8 mm), based on the banding patterns in Fig. 1. The plot was constructed from the values for the segments of λy199 DNA* (●), *and points corresponding to the bacterial DNA-containing segments* (○) *were entered on the curve according to their respective mobilities. The lengths of the purely λ DNA segments are (in kilobases): A′ + F, 24.0; A′, 20.7; D, 7.2; E, 5.6; C, 5.3; and F, 3.3.*

TABLE 1. *Length of EcoRI-generated λdarg segments containing bacterial DNA*[a]

Segment	Length of segment (kb) determined by:	
	Electrophoretic mobility	Electron microscopy
13-1 + F	17.3	17.2
13-1	13.9	13.9
13-2	5.6	5.6
13-3	2.7	2.8
13-4	1.5	1.5
14-1	17.3	17.3
14-3 + F	6.4	6.3
14-2	6.2	6.2
14-3	3.0	3.0
14-4	2.1	2.0
23-1 + F	21.8	21.7
23-1	18.4	18.4
23-2	6.2	6.2

[a] The electrophoretic mobility values were computed from the plot in Fig. 2; values below 3.3 kb were obtained by extrapolation. Each electron microscope determination represents the average of at least 50 measurements; in electron micrographs of λd*arg13* segments, the 5.6-kb segment appears with approximately twice the frequency as that of the other segments, showing the identical lengths (within experimental error) of 13-2 and E. For the deletion-bearing DNA from λd*arg13 sup102*, the segment corresponding to 13-1 is shortened by the length of the deletion, i.e., by 1.7 kb, as determined by either method. Segment 23-2 is identical to segment 14-2.

Mapping of cleavage sites. The ordering of the various *Eco*RI segments produced, and hence the establishment of a cleavage site map, could be achieved on the basis of segment size, taken together with the known structures of the intact transducing phage DNAs (6). In the case of λd*arg23* DNA, there are only two segments containing *E. coli* DNA, measuring 18.4 and 6.2 kb. Since the left end of λd*arg23* DNA is known to contain 9.5 kb of purely λ DNA (not having an *Eco*RI cleavage site), the 6.2-kb segment cannot be at the left end, and the sequence of segments (from left to right) must be 23-1, 23-2, D, E, and F (see Fig. 3, diagram 4 from top, and cf. top diagram).

Since the λd*arg14* and λd*arg23* DNAs are known to have identical DNA sequences extending from the attachment site to the left for a distance of approximately 11 kb, it follows that λd*arg14* DNA must give rise to a segment corresponding to 23-2. Segment 14-2 has the required size, namely, 6.2 kb, of which 3.8 kb must be λ DNA sequences (reflecting the known distance between the attachment site and the left end of the D segment of λ DNA). The left end of 14-2, therefore, is located at 2.4

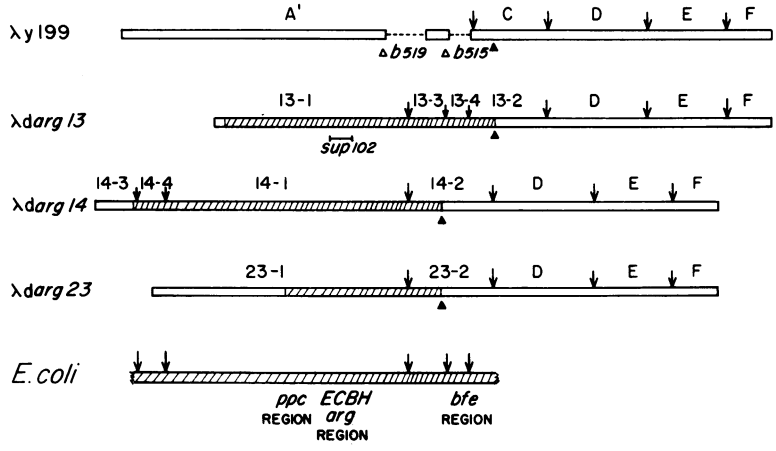

FIG. 3. *EcoRI cleavage map of DNAs from λy199, λdarg13, λdarg13 sup102, λdarg14, and λdarg23 and of the argECBH region of the E. coli chromosome. Symbols: ↓, cleavage sites; ▲, λ attachment site; ▨, E. coli DNA; and ▭, λ DNA. Alignment is made on the basis of the E. coli chromosomal DNA.*

kb to the left of the attachment site, leaving to its left more than 8 kb of bacterial DNA without a cleavage site. Thus, segments 14-3 and 14-4, measuring 3.0 and 2.0 kb, respectively, cannot be to the immediate left of 14-2; rather, 14-1 must be in this position. Insofar as intact λd*arg14* DNA carries 2.6 kb of λ sequences at its left terminus, 14-4, which is only 2.0 kb long, cannot be at the left end; consequently, the terminus must be carried by 14-3, and 14-4 must lie between 14-3 and 14-1. The sequence of the λd*arg14* segments from left to right, therefore, is: 14-3, 14-4, 14-1, 14-2, D, E, and F (Fig. 3, diagram 3 from top).

Because the secondary attachment site for λd*arg13* is located 3.8 kb to the right of that for λd*arg14* and λd*arg23*, the bacterial portion of λd*arg13* DNA extends correspondingly further to the right and includes the *bfe* region. For all three transducing phage DNAs, however, the λ DNA sequences to the right of the attachment site are identical, and the distance between the attachment site and the nearest cleavage site in the λ DNA sequences measures 3.8 kb. Since two of the λd*arg13* segments are only 1.5 and 2.8 kb long, neither of them can be adjacent to the left end of D. Segment 13-1 (13.9 kb) likewise cannot be adjacent to D, insofar as there must be a cleavage site approximately 10 kb (i.e., 6.2 kb, representing the length of 14.2, plus 3.8 kb, representing the distance between the attachment sites) to the left of D. Therefore, 13-2 must abut D. Similarly, 13-1 cannot be next to 13-2; rather, a stretch of approximately 4.4 kb (i.e., approximately 10 kb minus 5.6 kb) has to be accounted for. In other words, 13-3 and 13-4, with an aggregate length of 4.3 kb, must lie between 13-1 and 13-2. Segment 13-4 (1.5 kb) cannot abut 13-1, since to the immediate right of 13-1 there are 2.4 kb of bacterial DNA without a cleavage site (cf. the data for λd*arg14* DNA). Consequently, the sequence of segments for λd*arg13* DNA, from left to right, is: 13-1, 13-3, 13-4, 13-2, D, E, and F (Fig. 3, diagram 2 from top). For λd*arg13 sup102* DNA, the sequence is analogous, with the place of 13-1 being taken by 13s-1, which is shortened by the length of the *sup102* deletion (1.7 kb).

Independent evidence for the identity of the left terminal segments. The assignment of 13-1, 14-3, and 23-1 to their positions as left terminal segments of their respective DNAs was confirmed by electron microscopy. DNA segments linked through the λ cohesive termini are known to be recognizable electron microscopically through the occasional formation of "pulled-apart molecules" (11). In this manner, associations between 13-1 and F (Fig. 4A), 14-3 and F (Fig. 4B), and 23-1 and F were demonstrated.

Heteroduplex experiments. A heteroduplex formed between segments 13s-1 and 23-1 is shown in Fig. 4C, providing direct evidence that these two segments carry the λ left terminus and thus are the left terminal segments of their respective DNAs (cf. reference 6). Additionally, this heteroduplex visualizes the location of the *argCB* deletion *sup102*. Therefore, the location of *argECBH* is indicated for 13-1 as well as for 23-1, and hence for 14-1.

DISCUSSION

For the purpose of in vitro studies of repression, at the level of translation or transcription

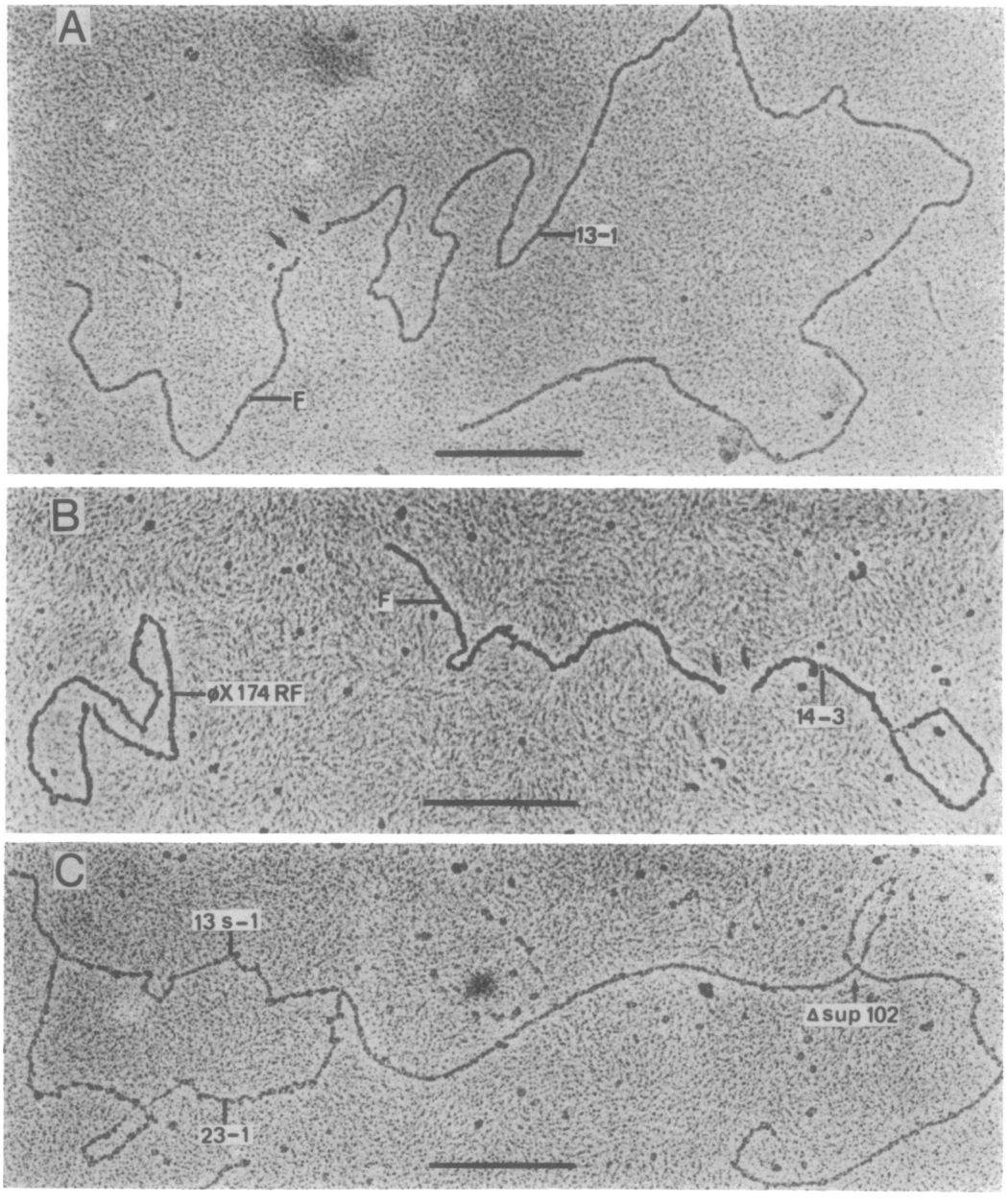

FIG. 4. *Electron micrographs of "pulled-apart molecules" and a heteroduplex of λdarg DNA segments produced by EcoRI. (A) Terminal segments of λdarg13 DNA with pulled-apart cohesive ends (arrows); (B) pulled-apart terminal segments of λdarg14 DNA; (C) heteroduplex between segments 13s-1 and 23-1, showing the sup102 deletion loop and the nonhomologous region. The sup102 deletion marks the position of the argECBH cluster. The bar represents 1 kilobase (1,000 base pairs).*

it is desirable to have available, on a preparative scale, *arg* DNA as free as possible from extraneous DNA. For translational investigations (12), the preparative availability of *arg* genes would make possible the isolation of individual *arg* messages obtained by in vitro transcription. Thus, the preparation of substantially enriched *arg* DNA was undertaken.

The divergently transcribed four-gene, two-operon *argECBH* cluster (1, 4) seemed espe-

cially attractive for in vitro experiments. Lambda transducing phages carrying this cluster have been obtained and characterized (6). As shown in Fig. 3, the bacterial portions of the transducing phage DNAs, in each case, contain the *argECBH* region. In addition, the DNAs from λd*arg13* and its deletion-bearing derivative λd*arg13 sup102* carry the *E. coli ppc* and *bfe* regions; λd*arg14* DNA has *ppc*, but not *bfe*; and λd*arg23* DNA has part of *ppc*, but not *bfe*. The direction from left to right corresponds to the clockwise orientation of the *E. coli* chromosome. An objective of this investigation was to use the DNA from these phages as starting material for the preparation of homogeneous stretches of DNA carrying the desired *arg* genes. This was accomplished with the aid of the *Eco*RI endonuclease.

The position of the *Eco*RI cleavage sites in the bacterial portion of the DNAs was determined by ordering the segments obtained. The order of the segments was deduced from the measurement of segment length and the identification of segments bearing the cohesive termini of λ, taken together with the known structure of the intact phage DNAs and the known cleavage sites in the λ sequences of these DNAs. The bacterial DNA-containing segments of λd*arg23* were readily ordered on the basis of their size, which permitted the ordering of the λd*arg14* DNA segments, which in turn permitted the ordering of the λd*arg13* and λd*arg13 sup102* DNA segments, again based on segment size. The inferred position of the left terminal segment was verified in each case by the direct demonstration that one λ cohesive end was present on a segment of the appropriate size. For all of the λd*arg* phage DNAs examined, the *argECBH* cluster was concluded to be present on the largest segment formed. The position of the *arg* genes on the segments was located in heteroduplex experiments.

Each of the *arg*-bearing segments (13-1, 13s-1, 14-1, and 23-1) is readily obtained on a preparative scale by *Eco*RI digestion of the intact DNAs followed by sedimentation in a 5 to 20% sucrose gradient. The 13-1 segment may be the most advantageous since it is the smallest of the *arg*-containing segments and easily obtainable. This segment carries a relatively small stretch (0.7 kb) of λ DNA. Segment 14-1 is composed entirely of bacterial DNA and thus represents the *argECBH*-bearing *Eco*RI segment of the *E. coli* chromosome.

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