Type-Specific Contributions to Chromosome Size Differences in *Escherichia coli*

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Received 10 August 1998/Returned for modification 17 September 1998/Accepted 8 October 1998

The *Escherichia coli* genome varies in size from 4.5 to 5.5 Mb. It is unclear whether this variation may be distributed finely throughout the genome or is concentrated at just a few chromosomal loci or on plasmids. Further, the functional correlates of size variation in different genome copies are largely unexplored. We carried out comparative macrorestriction mapping using rare-restriction-site alleles (made with the Tn10dRCP2 family of elements, containing the *Not*I, *Bln*I, I-*Ceu*I, and ultra-rare-cutting I-*Sce*I sites) among the chromosomes of laboratory *E. coli* K-12, newborn-sepsis-associated *E. coli* RS218, and uropathogenic *E. coli* J96. These comparisons showed just a few large accessory chromosomal segments accounting for nearly all strain-to-strain size differences. Of 10 sepsis-associated and urovirulence genes, previously isolated from the two pathogens by scoring for function, all were colocalized exclusively with one or more of the accessory chromosomal segments. The accessory chromosomal segments detected in the pathogenic strains from physical, macrorestriction comparisons may be a source of new virulence genes, not yet isolated by function.

The gram-negative bacterium *Escherichia coli* occurs commonly as a benign enteric commensal of mammals. Additionally, different types of *E. coli* characteristically cause different diseases (46), including the hemolytic-uremic syndrome (15, 19, 29, 49), urosepsis (1), and newborn sepsis/meningitis (20). Although recent determination of the entire nucleotide sequence from laboratory strain K-12 indicated 4,639 kb (6), estimates for natural isolates range from 4,660 to 5,300 kb (3). This indicates substantial size differences among genome copies of the various *E. coli* strains. How these differences originated and have persisted is unclear.

Genes for some enterobacterial virulence traits, especially those essential to one or another major pathogenic life cycle, may reside on specialized chromosomal elements, i.e., pathogenicity islands (7, 21, 23, 29, 35, 47); in contrast, others, notably antibiotic resistances, typically reside on plasmids (18). Chromosomal virulence traits may be both difficult to isolate by functional means (e.g., if their phenotypes can be scored only in interactions with mammalian hosts) and impossible to isolate by straightforward physical means (i.e., by plasmid preparations, given that genes for them occur integrated on the chromosome). They could be identified by the positional approach to gene discovery (13, 17), however, if the genes conferring them could be distinguished as local alterations to chromosome structure prior to functional analysis. To investigate the applicability of positional gene discovery for finding genes that contribute to E. coli pathogenesis, we mapped the components of chromosomal size differences among laboratory strain K-12 and two pathogenic strains, the uropathogen J96 and the newborn-sepsis-associated strain RS218. Further, we compared the locations of these large, accessory chromosomal segments with the locations of known virulence genes.

MATERIALS AND METHODS

Bacterial genetics techniques. Bacterial strains were grown in LB with aeration or on solid LB or M9-glucose (34). Media were supplemented with kanamycin (50 µg/ml), spectinomycin (100 µg/ml), and/or chloramphenicol (15 µg/ml) as required. Cultures were incubated at 37°C, or at 30°C for P1 infections of RS218 and RS218-chimera cultures (10). Cells were stored long term by being suspended in LB-glycerol (80%/20%, vol/vol) and cooled to -80°C. Bacterio-phage stocks were grown and stored as described by Sternberg and Maurer (45). Double-insertion mutants of strain MG1655 and single- and double-insertion mutants of strains RS218 and J96 were generated by transducing recipient strains with P1 Δ damrev6 lysates of MG1655 insertion mutants (40). Genome structure, assessed by pulsed-field gel electrophoresis (PFGE) in at least six independent isolates from each transduction, was used to confirm P1 transduction fidelity and lack of transduction-associated rearrangements.

Genomic DNA biophysical techniques. Genomic DNAs were purified from 5-ml overnight cultures of wild-type and insertionally mutagenized E. coli in a manner suitable for yielding macrorestriction fragments (50 to 1000 kb), as described previously (40). After digestion of agarose-embedded DNAs with I-SceI (Boehringer Mannheim, Indianapolis, Ind.) for 1 h, NotI (New England Biolabs, Beverly, Mass.) for 4 to 5 h, or BlnI (Panvera, Madison, Wis.) overnight, according to the manufacturers' directions, and after reaction buffer decanting, agarose dots were melted (70°C) and gently pipetted with plastic 200-µl tips into sample wells in 1.2% agarose (PFGE approved; FastLane; FMC, Portland, Maine) gels for electrophoresis in 0.5× TBE buffer (0.045 M Tris borate, 0.045 M boric acid, 0.001 M EDTA) in a PFGE apparatus (Bio-Rad DR-III) according to the manufacturer's instructions. Ramping of PFGE pulse times was determined as described elsewhere (5); ramping from 11 to 21 s over 13 h and from 50 to 56 s over 7 h was used to approximate log-linear separations between 150 and 350 kb. After electrophoresis of samples with Megabase I and/or II DNA standards (Gibco/BRL, Bethesda, Md.), fragments sizes were quantitated as described elsewhere (25).

RESULTS

Structural and functional correlates to genome size variation within species have only recently been attempted (3, 4). The components of genome size were investigated in three strains from different genealogical branches of *E. coli* (43). The strains analyzed were the nonpathogenic laboratory K-12 strain MG1655 (2), the newborn-sepsis strain RS218 (44), and the uropathogenic strain J96 (28). The chromosomes of these strains vary in length from 4,673 kb for strain MG1655 to 5,195 kb for strain RS218 to 5064 kb for strain J96 (Fig. 1). Also, strains RS218 and J96 carry plasmids of 110 and 113 kb, respectively. The additional ~556 kb of chromosomal DNA in

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pathogenic strain RS218 and ~455 kb of chromosomal DNA in pathogenic strain J96 relative to the nonpathogenic strain MG1655 may reside within pathogenicity islands (7, 23, 29, 35), i.e., chromosomal segments on which genes contributing to the virulence of the pathogenic strains reside. Additionally, "black hole" genomic deletions that enhance pathogenicity (32) also need to be considered. Macrorestriction maps of these chromosomes by NotI (22, 23, and 25 fragments in strains MG1655, RS218, and J96, respectively), BlnI (13, 17, and 13 fragments in strains MG1655, RS218, and J96, respectively), and I-CeuI (7 fragments in all strains) digestions are shown in Fig. 1. Further, through macrorestriction analyses we were able to map the positions in the different strains for a set of 20 rare-restrictionsite alleles, made with Tn10dRCP2 insertion elements, which carry the rare-cutting polylinker 2 of rare restriction sites including NotI, BlnI, I-CeuI, and I-SceI.

Integrated macrorestriction mapping with transposons that carry rare restriction sites can distinguish accessory chromosomal segments from conserved chromosomal segments in the physical maps of different chromosomal copies (40); this requires determination of reference loci and of the physical distances separating those loci. Reference loci were determined, and gene order conservation was assessed in these three strains by introduction of Tn10dRCP2 insertions carrying the I-SceI restriction site (9). I-SceI is an ultra-rare-cutting megaendonuclease which recognizes an 18-bp nucleotide, TAGGGATA A \downarrow CAGGGTAAT, generating 3' cohesive ends (36). Statistically, the I-SceI restriction site occurs once in $\sim 6.9 \times 10^{10}$ bp. Therefore, it is not surprising that this sequence does not occur in *E. coli* sequences of ~ 5 Mb. The Tn10dRCP2 family of insertion elements occurs in three antibiotic resistance varieties. Previously, MG1655::Tn10dKanRCP2, MG1655::Tn10d SpcRCP2, and MG1655::Tn10dCamRCP2 insertion mutants were isolated (9). From this strain collection, eight MG1655:: Tn10dKanRCP2, nine MG1655::Tn10dSpcRCP2, and three MG1655::Tn10dCamRCP2 strains (Table 1) were chosen to facilitate comparisons among the chromosomes of E. coli MG1655, RS218, and J96 and to localize chromosomal additions/deletions. These MG1655::Tn10dRCP2 mutants were chosen to give 20 I-SceI insertions separated from one another by approximately ~250 kb (i.e., ~5,000 kb of E. coli genome/ 20). By this attention to spacing, the ability to resolve chromosomal segment size in the three strain backgrounds between neighboring pairs of I-SceI fragments was optimized. This was because all of the I-SceI fragments generated by adjacent pairs of these evenly spaced insertions could be determined to equivalent accuracy with a single set of PFGE parameters designed to afford log-linear separations between 150 and 350 kb (5). The 20 Tn10dRCP2, I-SceI cleavage site landmarks were introduced around the chromosome within either the RS218 or the J96 strain background by P1 transduction (Table 1). The locations of the I-SceI insertions within each strain were mapped relative to that strain's macrorestriction map based on the artificial NotI, BlnI, and/or I-CeuI site introduced on the Tn10dRCP2 element. Locations of the Tn10dRCP2 inserts in all three strain backgrounds are shown on a linearized schematic of the E. coli chromosome opened at the thrA gene at 0 min (Fig. 1). The clockwise order of the 20 insertions was maintained in all three backgrounds, indicating a lack of detectable inversions or translocations. This was despite the potential for inversion between even the different laboratory derivatives of strain K-12 (26, 38) but was indeed expected from general conservation of the E. coli genetic map throughout the species and the family Enterobacteriaceae (39).

The crossing of pairs of rare-restriction-site alleles between different *E. coli* strain backgrounds allows physical distance

MG1655 K-L2 prototype J96 Nethorn manipality prototype J96 MG1055:Tn1/0dKanRCP2, Km² M2008 xM4000 xb1/20, (316), [312], [319] xM2009 xM6020 xb1/20, (316), [312], [319] xM2009 xM6030 xM4020 xb1/20, (318, [319], [320], [321] xM2008 xM6080 xM4080 xb1/20, (318, [312], [347], [347], [347], [340], [342] xM2009 xM3080 xM4080 xb1/20, (313, [350], [351], [354], [354], [354], [352], [352], [354], [356], [352], [354], [356], [352], [356], [35	Strain	RS218 transductant	J96 transductant	Description ^b
NS218 Newborn menupts prototype Pj6 Pjelouephritis prototype M2006 \$\phi4008 \$\phi4008 \phi2020 \phi3031 \$\phi4008 \$\phi-120, (613), [53], [MG1655			K-12 prototype
J96 Predenegrating prototype xM2008 xM5008 xM4008 sch.106, (316), (312), (319) xM2012 xM5012 xM4012 sch.106, (316), (312), (319) xM2014 xM5013 xM4013 sch.106, (316), (312), (319) xM2015 xM5064 xM4064 puF-164, (2419), (255), (2474) xM2009 xM5080 xM4080 cp.106, (257), [153], (2522), (2474) xM2019 xM5095 xM4095 sch.205, (536), [371), (3640) xM2107 xM5107 xM4107 sch.205, (536), [371), (3640) xM2102 xM5002 xM4004 sch.106, (535), [571, [373) xM2014 xM5014 xM4014 sch.110, (1013), (1013), (1013) xM2016 xM5061 xM4016 sch.166, (270), [292], (1803) xM2016 xM5061 xM4016 sch.166, (270), [291, [473], [922], (1803) xM2016 xM5061 xM4016 sch.166, (101, [204], [2723], [301] xM2016 xM5061 xM4016 sch.166, (101, [304], [312],	RS218			Newborn meningitis prototype
MG1005: (M4008 zml-108, G110, G121, G19) xM2020 xM5020 xM4020 zml-128, G110, G	190			Pyelonephritis prototype
				MG1655::Tn10dKanRCP2; Km ⁴
$\begin{array}{cccccccccccccccccccccccccccccccccccc$	χM2008	χ M 3008	χ M4008	<i>zah-108</i> , (316), [312], {319}
xM2031 xM2064 xM2064 xM4061 zeh-131 (267), [1439], (1285) xM2060 xM2080 xM4080 xM4080 cgs-1809 (2675), [3158], [2922] xM2095 xM4095 xM4095 zh-19, [3570], [3640], xM2017 xM5107 xM4107 zid-207, [3670], [4209], [4448] xM2012 xM5127 xM5127 zm4117 zid-227, (4504), [4494], [4895] xM2012 xM5002 xM5002 (xM4002 car-102, [351, [35], [35] xM2014 xM5014 xM4014 zbd-114, (575), [597], (573) xM2016 xM5066 xM4046 zdh-146, (575), [597], (573) xM2016 xM5066 xM4046 zdh-146, (575), [130], (1031) xM2061 xM5066 xM4046 zdh-146, (575), [130], (1031) xM2061 xM5066 xM4046 zdh-146, (1773), [1221], [1303] xM2061 xM5066 xM4046 zdh-146, (226), [2400], [2281] xM2070 xM5070 xM4070 zfh-770, (2671), [2414], (2723) xM2088 xM5088 xM4088 zdb-188, (3149), [1452], (4596) xM2088 xM5088 xM4088 zdb-188, (1499), [1668], (1564] xM2090 xM5090 xM4090 zhi-099, [1668], (1564] xM207 xM5033 xM4053 zed-133, (1085), [161], (2012) xM215 xM5115 xM4115 zi-221, (4151), [1642], (4357) xM208 xM5208 xM4208 zdb-138, (1499), [1668], [1564] xM209 xM5209 xM4209 zc-126 (57) zh-120 (Km7) xM2209 xM5209 xM4209 zc-126 (57) zh-130 (Km7) xM2209 xM5209 xM4209 zc-126 (57) zh-131 (Km7) xM2210 xM5211 xM4211 zi-221, (4151), [161], [2012] xM211 xM5211 xM4211 zi-221, [4151, [4000], [4438], [4357] xM2209 xM5209 xM4209 zc-126 (57) zh-131 (Km7) xM2209 xM5209 xM4209 zc-126 (57) zh-131 (Km7) xM2210 xM5213 xM4213 zh-131 (Km7) xM2210 xM5214 xM4211 zi-221, [416], [457] xM2214 xM5214 xM4211 zi-221, [417] xM2215 xM3125 xM4213 zh-130 (Km7) zc-126 (57) xM2214 xM5214 xM4214 zi-221 (57) ji xM2215 xM4215 zh-180 (Km7) zc-126 (57) xM2216 xM5216 xM4216 zh-131 (Km7) zc-126 (57) xM2217 xM5217 xM4217 cg-188 (57) fi-107 (57) xM2218 xM4218 zh-121 (Km7) zi-180 (Km7) xM2219 xM5214 xM4216 zh-120 (Km7) xM2210 xM5215 xM4215 zh-180 (Km7) zc-126 (57) xM2211 xM5217 xM4217 cg-188 (57) fi-107 (57) xM2212 xM5225 xM4225 zh-131 (Km7) zh-131 (Km7) xM2214 xM5216 xM4216 zh-120 (Km7) xM2226 xM5225 xM4225 zh-131 (Km7) zh-135 (Cm7) xM2226 xM5225 xM4225 zh-163 (Cm7) zh-255 (Cm7) xM2226 x	χM2020	χM3020	χM4020	<i>zbh-120</i> , (813), [836], {812}
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XM2053 XM3053 XM4053 zed-153, (1985), [2161], [2012] XM2115 XM3115 XM4115 zii-215, (4080), [4443], [4357] MG1655 (Tn1/0dSpcRCP2) (Tn1/0dKanRCP2) XM2207 XM3207 xM4207 car-102 (Sp') zah-108 (Km') XM2208 XM3208 XM4209 zcc-126 (Sp') zzh-131 (Km') XM2210 XM3210 XM4210 zef-158 (Sp') zzh-131 (Km') XM2211 XM3211 XM4211 zef-158 (Sp') zzh-131 (Km') XM2212 XM3213 XM4213 zef-158 (Sp') zzh-131 (Km') XM2213 XM3213 XM4213 zef-168 (Sp') zzh-20 (Km') XM2214 XM3213 XM4213 zef-168 (Sp') zij-227 (Km') XM2215 XM3215 XM4214 zig-221 (Sp') zij-227 (Km') XM2214 XM3214 XM4214 zig-221 (Sp') zij-227 (Km') XM2215 XM3215 XM4216 zah-108 (Km') zgf-188 (Sp') XM2216 XM3216 XM4217 cys-180 (Km') zgf-188 (Sp') XM2217 XM3217 XM4218 purb-164 (Km') zdf-188 (Sp') XM2216 XM3216 XM4218 purb-170 (Sp') XM2219	vM2038	vM3038	vM4038	zdb-138. (1499). [1668]. {1564}
² M2115	xM2053	xM3053	xM4053	zed-153, (1985), [2161], {2012}
MG1655 (Tn1/dSpcRCP2) (Tn1/dKanRCP2) xM2207 xM3207 xM4207 car-102 (Sp) zah-108 (Km') xM2208 xM3208 xM4209 zdc-114 (Sp') zah-108 (Km') xM2209 xM3209 xM4209 zcc-126 (Sp') zah-104 (Km') xM2210 xM3210 xM4210 zah-161 (Sp') zah-170 (Km') xM2211 xM3211 xM4211 zfh-170 (Sp') zah-170 (Km') xM2212 xM3212 xM4211 zfh-170 (Sp') zah-170 (Km') xM2212 xM3212 xM4211 zfh-170 (Sp') zah-270 (Km') xM2213 xM3214 xM4213 zahi-199 (Sp') zah-270 (Km') xM2216 xM3215 xM4216 zah-108 (Km') zah-114 (Sp') xM2217 xM3216 xM4217 cyb-180 (Km) zah-188 (Sp') xM2218 xM3218 xM4217 cyb-180 (Km') zah-188 (Sp') xM2219 xM3219 <td< td=""><td>χM2115</td><td>xM3115</td><td>χM4115</td><td>zii-215, (4080), [4443], {4357}</td></td<>	χM2115	xM3115	χM4115	zii-215, (4080), [4443], {4357}
$\begin{array}{cccccccccccccccccccccccccccccccccccc$				MG1655 (Tn10dSpcRCP2) (Tn10dKanRCP2)
XM2208 XM3208 XM4208 Zbd-114 (Sp') zbh-120 (Km') XM2209 XM3209 XM4209 zcc-126 (Sp') zch-131 (Km') XM2210 XM3210 XM4210 zch-161 (Sp') purF-164 (Km') XM2211 XM3211 XM4211 zfh-170 (Sp') cys-180 (Km') XM2212 XM3212 XM4212 zgf-188 (Sp') zh-195 (Km') XM2213 XM3213 XM4214 zji-207 (Km') XM2214 XM3215 XM4215 zah-109 (Sp') zji-227 (Km') XM2216 XM3216 XM4217 cys-180 (Km') zgf-188 (Sp') XM2217 XM3217 XM4216 zbh-120 (Km') zcr-126 (Sp') XM2216 XM3217 XM4217 cys-180 (Km') zgf-188 (Sp') XM2217 XM3217 XM4218 puF-164 (Km') zfh-170 (Sp') XM2219 XM3219 XM4219 zji-227 (Km') car-102 (Sp') XM2219 XM3218 XM4219 zji-227 (Km') zfh-164 (Sp') XM2220 XM3220 XM4220 zhc-195 (Km') zfh-170 (Sp') XM2221 XM3223 XM4220 zhc-195 (Km') zfh-164 (Sp') XM2222 XM3223 XM4223 zed-133 (Cm') zh-161 (Sp')	xM2207	xM3207	xM4207	car-102 (Sp ^r) $zah-108$ (Km ^r)
$\begin{array}{ccccc} \chi M2209 & \chi M3209 & \chi M4209 & zcc-126 \ (Spr) zch-131 \ (Kmr) \\ \chi M2210 & \chi M3210 & \chi M4210 & zch-161 \ (Spr) purf-164 \ (Kmr) \\ \chi M2211 & \chi M3211 & \chi M4211 & zfh-170 \ (Spr) cys-180 \ (Kmr) \\ \chi M2212 & \chi M3212 & \chi M4212 & zgf-188 \ (Spr) zhc-195 \ (Kmr) \\ \chi M2213 & \chi M3213 & \chi M4213 & zhi-199 \ (Spr) zid-207 \ (Kmr) \\ \chi M2214 & \chi M3214 & \chi M4214 & zje-221 \ (Spr) zji-227 \ (Kmr) \\ \chi M2216 & \chi M3216 & \chi M4216 & zhh-120 \ (Kmr) zcc-126 \ (Spr) \\ \chi M2217 & \chi M3217 & \chi M4217 & cys-180 \ (Kmr) zgf-188 \ (Spr) \\ \chi M2216 & \chi M3216 & \chi M4216 & zhh-120 \ (Kmr) zgf-188 \ (Spr) \\ \chi M2216 & \chi M3217 & \chi M4217 & cys-180 \ (Kmr) zgf-188 \ (Spr) \\ \chi M2217 & \chi M3217 & \chi M4217 & cys-180 \ (Kmr) zgf-188 \ (Spr) \\ \chi M2218 & \chi M3218 & \chi M4218 & purf-164 \ (Kmr) zgf-188 \ (Spr) \\ \chi M2219 & \chi M3219 & \chi M4219 & zji-227 \ (Kmr) \ curl cys-180 \ (Kmr) zgf-188 \ (Spr) \\ \chi M2219 & \chi M3219 & \chi M4219 & zji-227 \ (Kmr) \ curl cys-190 \ (Spr) \\ \chi M2220 & \chi M3220 & \chi M4220 & zhc-195 \ (Kmr) \ zhi-199 \ (Spr) \\ \chi M2221 & \chi M3222 & \chi M4223 & zch-191 \ (Spr) \\ \chi M2222 & \chi M3223 & \chi M4223 & zch-191 \ (Spr) \\ \chi M2224 & \chi M3224 & \chi M4224 & zdh-146 \ (Spr) \ zdh-161 \ (Spr) \\ \chi M2224 & \chi M3224 & \chi M4224 & zdh-146 \ (Spr) \ zdh-161 \ (Spr) \\ \chi M2225 & \chi M3225 & \chi M4225 & zch-131 \ (Kmr) \ zhi-138 \ (Cmr) \\ \chi M2226 & \chi M3226 & \chi M4226 & zid-207 \ (Kmr) \ zhi-138 \ (Cmr) \\ \chi M2226 & \chi M3226 & \chi M4224 & zdh-146 \ (Spr) \ zdh-161 \ (Spr) \\ \chi M2224 & \chi M3224 & \chi M4224 & zdh-146 \ (Spr) \ zdh-161 \ (Spr) \\ \chi M2224 & \chi M3224 & \chi M4224 & zdh-146 \ (Spr) \ zdh-161 \ (Spr) \\ \chi M2225 & \chi M3225 & \chi M4225 & zch-131 \ (Kmr) \ zhi-138 \ (Cmr) \\ \chi M2226 & \chi M3226 & \chi M4226 & zid-207 \ (Kmr) \ zhi-138 \ (Cmr) \\ \chi M2226 & \chi M3225 & \chi M4225 & zch-131 \ (Kmr) \ zhi-138 \ (Cmr) \\ \chi M2226 & \chi M3226 & \chi M4226 & zid-207 \ (Kmr) \ zhi-138 \ (Cmr) \\ \chi M2226 & \chi M3226 & \chi M4226 & zid-207 \ (Kmr) \ zhi-138 \ (Cmr) \\ \chi M2226 & \chi M3226 & \chi M4226 & zid-207 \ (Kmr) \ zhi-138 \ (Cmr) \\ \chi M2226 & \chi M3226 & \chi M4226 & zid-207 \ (Kmr) \ zhi-137 \ (Kmr) \ zhi-138 \ (Cmr) \ zhi$	xM2208	xM3208	xM4208	zbd-114 (Sp ^r) $zbh-120$ (Km ^r)
² M2210 ² M3210 ² M4210 ² m4211 ² m4211 ² m4211 ² m4212 ² m4212 ² m4212 ² m4212 ² m4212 ² m4213 ² m4213 ² m4213 ² m4213 ² m4213 ² m4213 ² m4214 ² m4215 ² m4114 ² m4215 ² m4114 ² m4215 ² m4216 ² m4217 ² m421 ² m4219 ² m4220 ² m4222 ² m422	xM2209	xM3209	xM4209	zcc-126 (Sp ^r) $zch-131$ (Km ^r)
xM2211 xM3211 xM4211 zfh-170 (Sp ⁺) cys-180 (Km ⁺) xM2212 xM3212 xM4212 zgf-188 (Sp ⁺) zhc-195 (Km ⁺) xM2213 xM3213 xM4213 zhhi-199 (Sp ⁺) zid-207 (Km ⁺) xM2214 xM3214 xM4214 zje-221 (Sp ⁺) zji-227 (Km ⁺) xM2215 xM3215 xM4215 zah-108 (Km ⁺) zbd-114 (Sp ⁺) xM2216 xM3216 xM4216 zbh-120 (Km ⁺) zcc-126 (Sp ⁺) xM2217 xM3217 xM4216 zbh-120 (Km ⁺) zgf-188 (Sp ⁺) xM2218 xM3216 xM4216 zbh-120 (Km ⁺) zgf-188 (Sp ⁺) xM2217 xM3217 xM4217 cys-180 (Km ⁺) zgf-188 (Sp ⁺) xM2218 xM3218 xM4218 purf-164 (Km ⁺) zdf-170 (Sp ⁺) xM2219 xM3219 xM4219 zji-227 (Km ⁺) car-102 (Sp ⁺) xM2220 xM3220 xM4210 zih-196 (Sm ⁺) zih-170 (Sp ⁺) xM2221 xM3222 xM4221 zdb-138 (Cm ⁺) zih-170 (Sp ⁺) xM2222 xM3223 xM4220 zih-196 (Sp ⁺) xM2224 xM3223 xde1422 zih-136 (Cm ⁺) zih-221 (Sp ⁺) xM2222 xM3223 xd42	χM2210	xM3210	χ M4210	zeh-161 (Sp ^r) $purF-164$ (Km ^r)
xM2212 xM3212 xM4212 zgf-188 (Sp ⁷) zhc-195 (Km ⁷) xM2213 xM3213 xM4213 zhi-199 (Sp ⁷) zid-207 (Km ⁷) xM2214 xM3214 xM4214 zje-221 (Sp ⁷) zid-207 (Km ⁷) xM2215 xM3215 xM4215 zdh-104 (Sp ⁷) zid-207 (Km ⁷) xM2216 xM3215 xM4215 zdh-108 (Km ⁷) zbd-114 (Sp ⁷) xM2216 xM3217 xM4216 zbh-120 (Km ⁷) zcr-126 (Sp ⁷) xM2217 xM3217 xM4217 cys-180 (Km ⁷) zf-170 (Sp ⁷) xM2218 xM3218 xM4217 cys-180 (Km ⁷) zf-170 (Sp ⁷) xM2219 xM3219 xM4219 zji-227 (Km ⁷) car-102 (Sp ⁷) xM2220 xM3212 xM4219 zji-227 (Km ⁷) car-102 (Sp ⁷) xM2212 xM3212 xM4219 zji-227 (Km ⁷) car-102 (Sp ⁷) xM2220 xM3220 xM4200 zhc-195 (Km ⁷) zdh-146 (Sp ⁷) xM2221 xM3222 xM4223 zde-153 (Cm ⁷) zdh-146 (Sp ⁷) xM2222 xM3223 xM4223 zde-153 (Cm ⁷) zdh-161 (Sp ⁷) xM2224 xM3223 xM4224 zdh-146 (Sp ⁷) zd-153 (Cm ⁷) xM2224 xM3224	χM2211	χM3211	χM4211	zfh-170 (Sp ^r) $cys-180$ (Km ^r)
χM2213 χM3213 χM4213 zhi-199 (Sp') zid-207 (Km') χM2214 χM3214 χM4214 zje-221 (Sp') zji-227 (Km') xM2215 χM3215 χM4215 zah-108 (Km') zbd-114 (Sp') χM2216 χM3216 χM4216 zbh-120 (Km') zc-126 (Sp') χM2216 χM3216 χM4217 cys-180 (Km') zgf-188 (Sp') χM2217 χM3217 χM4216 zbh-120 (Km') zgf-188 (Sp') χM2217 χM3217 χM4216 zbh-120 (Km') zgf-188 (Sp') χM2218 χM3217 χM4218 purF-164 (Km') zfh-170 (Sp') χM2219 χM3219 χM4219 zji-227 (Km') car-102 (Sp') χM2220 χM3220 χM4220 zhc-195 (Km') zhi-199 (Sp') χM2221 χM3221 χM4220 zhc-195 (Km') zhi-199 (Sp') χM2222 χM3223 χM4220 zhc-195 (Km') zhi-199 (Sp') χM2222 χM3223 χM4223 zii-215 (Cm') zii-221 (Sp') χM2222 χM3223 χM4223 zii-215 (Cm') zii-221 (Sp') χM2223 χM3223 χM4223 zii-215 (Cm') zii-217 (Sp') χM2224 χM3224 χM4224 zdh-146 (Sp') zel-153 (Cm')<	χM2212	χM3212	χM4212	<i>zgf-188</i> (Sp ^r) <i>zhc-195</i> (Km ^r)
χM2214 χM3214 χM4214 zje-221 (Sp ^r) zji-227 (Km ^r) χM2215 χM3215 χM4215 zah-108 (Km ^r) zbd-114 (Sp ^r) χM2216 χM3216 χM4216 zbh-120 (Km ^r) zc-126 (Sp ^r) χM2217 χM3217 χM4217 cys-180 (Km ^r) zc-126 (Sp ^r) χM2216 χM3217 χM4217 cys-180 (Km ^r) zc-126 (Sp ^r) χM2217 χM3217 χM4217 cys-180 (Km ^r) zc-126 (Sp ^r) χM2217 χM3217 χM4217 cys-180 (Km ^r) zc-126 (Sp ^r) χM2217 χM3217 χM4217 cys-180 (Km ^r) zgf-188 (Sp ^r) χM2218 χM3218 χM4218 purF-164 (Km ^r) zfh-170 (Sp ^r) χM2219 χM3219 χM4219 zji-227 (Km ^r) car-102 (Sp ^r) χM2220 χM3220 χM4220 zhc-195 (Km ^r) zhi-199 (Sp ^r) χM2221 χM3222 χM4220 zhc-195 (Km ^r) zhi-199 (Sp ^r) χM2222 χM3223 χM4223 zde-133 (Cm ^r) zhi-146 (Sp ^r) χM2223 χM3223 χM4223 zde-153 (Cm ^r) zhi-161 (Sp ^r) χM2224 χM3224 χM4224 zdh-146 (Sp ^r) zdb-138 (Cm ^r) χM2225 χM3225	χM2213	χM3213	χM4213	<i>zhi-199</i> (Sp ^r) <i>zid-207</i> (Km ^r)
$\begin{array}{c c c c c c c c c c c c c c c c c c c $	χM2214	χM3214	χM4214	<i>zje-221</i> (Sp ^r) <i>zji-227</i> (Km ^r)
xM2215 xM3215 xM4215 zah-108 (Km ¹) zbh-114 (Sp ¹) xM2216 xM3216 xM4216 zbh-120 (Km ¹) zcc-126 (Sp ¹) xM2217 xM3217 xM4217 cys-180 (Km ¹) zcf-188 (Sp ¹) xM2216 xM3216 xM4216 zbh-120 (Km ¹) zcc-126 (Sp ¹) xM2216 xM3216 xM4216 zbh-120 (Km ¹) zcc-126 (Sp ¹) xM2217 xM3217 xM4216 zbh-120 (Km ¹) zcf-188 (Sp ¹) xM2218 xM3218 xM4218 purF-164 (Km ¹) zfh-170 (Sp ¹) xM220 xM3219 xM4219 zji-227 (Km ¹) car-102 (Sp ¹) xM2210 xM3220 xM4220 zhc-195 (Km ¹) zhi-199 (Sp ¹) xM2221 xM3220 xM4220 zhc-195 (Km ¹) zhi-199 (Sp ¹) xM2222 xM3222 xM4221 zdb-138 (Cm ¹) zh-146 (Sp ¹) xM2222 xM3223 xM4223 zdb-138 (Cm ¹) zie-161 (Sp ¹) xM2224 xM3223 xM4223 zdb-133 (Cm ¹) xM224 xM4224 zdh-146 (Sp ¹) zed-153 (Cm ²) xM224 xM4224 zdh-146 (Sp ¹) zed-153 (Cm ²) xM225 xM3225 xM4225 zch-131 (Km ¹) zdb-138 (Cm ²) </td <td></td> <td></td> <td></td> <td>MG1655 (Tn10dKanRCP2) (Tn10dSpcRCP2)</td>				MG1655 (Tn10dKanRCP2) (Tn10dSpcRCP2)
χM2216 χM3216 χM4216 zbh-120 (Km²) zcc-126 (Sp²) χM2217 χM3217 χM4217 cys-180 (Km²) zgf-188 (Sp²) χM2216 χM3216 χM4216 zbh-120 (Km²) zcc-126 (Sp²) χM2217 χM3217 χM4216 zbh-120 (Km²) zcc-126 (Sp²) χM2217 χM3217 χM4216 zbh-120 (Km²) zgf-188 (Sp²) χM2218 χM3217 χM4218 purF-164 (Km²) zgh-170 (Sp²) χM2219 χM3219 χM4219 zji-227 (Km²) car-102 (Sp²) χM2220 χM3220 χM4220 zhc-195 (Km²) zh-146 (Sp²) χM2221 χM3222 χM4221 zdb-138 (Cm²) zh-146 (Sp²) χM2222 χM3222 χM4223 zed-153 (Cm²) zel-161 (Sp²) χM2223 χM3223 χM4223 zel-153 (Cm²) zel-161 (Sp²) χM2224 χM3223 χM4223 zel-153 (Cm²) zel-161 (Sp²) χM2224 χM3224 χM4224 zdh-146 (Sp²) zel-153 (Cm²) χM2225 χM3225 χM4225 zch-131 (Km²) zdb-138 (Cm²) χM2226 χM3226 χM4226 zid-207 (Km²) zii-215 (Cm²)	χM2215	χM3215	χM4215	zah-108 (Km ^r) zbd-114 (Sp ^r)
χM2217 χM3217 χM4217 cys-180 (Km ¹) zgf-188 (Sp ¹) χM2216 χM3216 χM4216 zbh-120 (Km ¹) zcc-126 (Sp ¹) χM2217 χM3217 χM4217 cys-180 (Km ¹) zgf-188 (Sp ¹) χM2217 χM3217 χM4217 cys-180 (Km ¹) zgf-188 (Sp ¹) χM2218 χM3218 χM4218 purF-164 (Km ¹) zfh-170 (Sp ¹) χM2219 χM3219 χM4219 zji-227 (Km ¹) car-102 (Sp ¹) χM2220 χM3220 χM4220 zhc-195 (Km ¹) zhi-199 (Sp ¹) χM2221 χM3222 χM3220 zM4220 zhc-195 (Km ¹) zhi-199 (Sp ¹) χM2222 χM3223 χM4220 zhc-195 (Km ¹) zhi-199 (Sp ¹) zmi-199 (Sp ¹) χM2222 χM3223 χM4220 zii-215 (Cm ¹) zie-221 (Sp ¹) zmi-196 (Sp ¹) χM2223 χM3223 χM4223 zed-153 (Cm ¹) zie-221 (Sp ¹) zmi-161 (Sp ¹) χM2224 χM3224 χM4224 zdh-146 (Sp ¹) zed-161 (Sp ¹) zmi-161 (Sp ¹) χM2225 χM3225 χM4225 zch-131 (Km ¹) zdb-138 (Cm ¹) zmi-130 (Cm ¹) χM225 χM3226 χM4226 zid-207 (Km ¹) zii-215 (Cm ¹)	χM2216	χM3216	χM4216	<i>zbh-120</i> (Km ^r) <i>zcc-126</i> (Sp ^r)
χM2216 χM3216 χM4216 zbh-120 (Km ¹) zcc-126 (Sp ¹) χM2217 χM3217 χM4217 cys-180 (Km ¹) zgf-188 (Sp ¹) χM2218 χM3218 χM4218 purF-164 (Km ¹) zfh-170 (Sp ¹) χM2219 χM3219 χM4219 zji-227 (Km ¹) car-102 (Sp ¹) χM2220 χM3220 χM4220 zhc-195 (Km ¹) zh-179 (Sp ¹) χM2221 χM3220 χM4220 zhc-195 (Km ¹) zh-199 (Sp ¹) χM2221 χM3221 χM4220 zdb-138 (Cm ¹) zh-146 (Sp ¹) χM2222 χM3222 χM4223 zdb-138 (Cm ¹) zh-161 (Sp ¹) χM2223 χM3223 χM4223 zed-153 (Cm ¹) zh-161 (Sp ¹) χM224 χM3224 χM4223 zed-153 (Cm ¹) zeh-161 (Sp ¹) χM225 χM3224 χM4224 zdh-146 (Sp ¹) zed-153 (Cm ¹) χM225 χM3225 χM4225 zch-131 (Km ¹) zdb-138 (Cm ¹) χM225 χM3226 χM4226 zid-207 (Km ¹) zii-215 (Cm ¹)	χM2217	χM3217	χM4217	<i>cys-180</i> (Km ^r) <i>zgf-188</i> (Sp ^r)
χM2217 χM3217 χM4217 cys-180 (Km²) zgf-188 (Sp²) χM2218 χM3218 χM4218 purF-164 (Km²) zfh-170 (Sp²) χM2219 χM3219 χM4219 zji-227 (Km²) car-102 (Sp²) χM2220 χM3220 χM4220 zhc-195 (Km²) zh-199 (Sp²) χM2221 χM3221 χM4220 zdb-138 (Cm²) zh-146 (Sp²) χM2222 χM3222 χM4222 zii-215 (Cm²) zje-221 (Sp²) χM2223 χM3223 χM4223 zed-153 (Cm²) zie-221 (Sp²) χM224 χM3223 χM4223 zed-153 (Cm²) zie-161 (Sp²) χM224 χM3224 χM4224 zdh-146 (Sp²) zed-153 (Cm²) χM225 χM3225 χM4225 zch-131 (Km²) zdb-138 (Cm²) χM225 χM3226 χM4226 zid-207 (Km²) zie-215 (Cm²)	χM2216	χM3216	χM4216	zbh-120 (Km ^r) $zcc-126$ (Sp ^r)
χM2218 χM3218 χM4218 purf-164 (Km²) zfn-170 (Sp²) χM2219 χM3219 χM4219 zfi-227 (Km²) car-102 (Sp²) χM2220 χM3220 χM4220 zhc-195 (Km²) zhi-199 (Sp²) χM221 χM3221 χM4220 zdb-138 (Cm²) zhi-199 (Sp²) χM2221 χM3221 χM4221 zdb-138 (Cm²) zhi-164 (Sp²) χM2221 χM3221 χM4221 zdb-138 (Cm²) zhi-164 (Sp²) χM2222 χM3222 χM4222 zii-215 (Cm²) zhi-164 (Sp²) χM2223 χM3223 χM4223 zdi-133 (Cm²) zhi-161 (Sp²) χM2224 χM3223 χM4223 zdi-153 (Cm²) zhi-161 (Sp²) χM2224 χM3224 χM4224 zdh-146 (Sp²) zei-153 (Cm²) χM2225 χM3225 χM4225 zch-131 (Km²) zib-138 (Cm²) χM2226 χM3226 χM4226 zid-207 (Km²) zii-215 (Cm²)	χM2217	χM3217	χM4217	cys-180 (Km ¹) $zgf-188$ (Sp ¹)
χM2219 χM3219 χM4219 2μ-22/ (Km²) car-102 (Sp²) χM2220 χM3220 χM4220 zhc-195 (Km²) zhi-199 (Sp²) χM221 χM3221 χM4221 zdb-138 (Cm²) zhi-146 (Sp²) χM2222 χM3222 χM4222 zii-215 (Cm²) zhi-161 (Sp²) χM2223 χM3223 χM4223 zed-153 (Cm²) zhi-161 (Sp²) χM2224 χM3223 χM4223 zed-153 (Cm²) zhi-161 (Sp²) χM2224 χM3224 χM4224 zdh-146 (Sp²) zed-153 (Cm²) χM2225 χM3225 χM4225 zch-131 (Km²) zdb-138 (Cm²) χM2225 χM3225 χM4226 zid-207 (Km²) zie-215 (Cm²)	χM2218	χM3218	χM4218	purF-164 (Km ¹) $zfh-170$ (Sp ¹)
χM2220 χM3220 χM4220 zhc-195 (Km²) zh-199 (Sp²) χM2221 χM3221 χM4221 zdb-138 (Cm²) zdh-146 (Sp²) χM2222 χM3222 χM4222 zii-215 (Cm²) zje-221 (Sp²) χM2223 χM3223 χM4223 zed-153 (Cm²) zeh-161 (Sp²) χM2224 χM3224 χM4224 zdh-146 (Sp²) zeh-161 (Sp²) χM2225 χM3224 χM4224 zdh-146 (Sp²) zeh-153 (Cm²) χM2225 χM3225 χM4225 zch-131 (Km²) zdb-138 (Cm²) χM2226 χM3226 χM4226 zid-207 (Km²) zii-215 (Cm²)	χM2219	χM3219	χM4219	$z_{JI}-227$ (Km ²) $car-102$ (Sp ²)
χM2221 χM3221 χM4221 zdb-138 (Cm ^r) zdh-146 (Sp ^r) χM2222 χM3222 χM4222 zii-215 (Cm ^r) zdh-146 (Sp ^r) χM2223 χM3223 χM4223 zed-153 (Cm ^r) zdh-161 (Sp ^r) χM2224 χM3224 χM4224 zdh-146 (Sp ^r) zdh-161 (Sp ^r) χM2225 χM3224 χM4224 zdh-146 (Sp ^r) zdh-153 (Cm ^r) χM2225 χM3225 χM4225 zdh-146 (Sp ^r) zdh-153 (Cm ^r) χM2225 χM3225 χM4225 zch-131 (Km ^r) zdh-138 (Cm ^r) χM2226 χM3226 χM4226 zid-207 (Km ^r) zii-215 (Cm ^r)	χM2220	χM3220	χM4220	zhc-195 (Km ²) $zhi-199$ (Sp ²)
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χM2222 χM3222 χM4222 zui-215 (Cm²) zje-221 (Sp²) χM2223 χM3223 χM4223 zed-153 (Cm²) zeh-161 (Sp²) χM2224 χM3224 χM4224 zdh-146 (Sp²) zed-153 (Cm²) χM2225 χM3225 χM4225 zdh-146 (Sp²) zed-153 (Cm²) χM2225 χM3225 χM4225 zch-131 (Km²) zdb-138 (Cm²) χM2226 χM3226 χM4226 zid-207 (Km²) zii-215 (Cm²)	χM2221	χM3221	χM4221	zdb-138 (Cm ⁴) $zdh-146$ (Sp ⁴)
χM2225 χM3225 χM4225 zea-155 (Cm ⁺) zeh-161 (Sp ⁺) γM2224 χM3224 χM4224 zdh-146 (Sp ⁺) zed-153 (Cm ⁺) γM2225 χM3225 χM4225 zch-131 (Km ⁺) zdb-138 (Cm ⁺) γM2226 χM3226 χM4226 zid-207 (Km ⁺) zii-215 (Cm ⁺)	χM2222	χM3222	χM4222	zu-215 (Cm ²) $zje-221$ (Sp ²)
χM2224	χΜ2223	χιν13223	χ14223	<i>zea-155</i> (Cm) <i>zen-101</i> (Sp)
χM2224 χM3224 χM4224 zdh-146 (Sp ^r) zed-153 (Cm ^r) χM2225 χM3225 χM4225 xM4225 zch-131 (Km ^r) zdb-138 (Cm ^r) χM2226 χM3226 χM4226 zid-207 (Km ^r) zii-215 (Cm ^r)				MG1655 (Tn10dSpcRCP2) (Tn10dCamRCP2)
χM2225 χM3225 χM4225 χM4225 zch-131 (Km ^r) zdb-138 (Cm ^r) χM226 χM3226 χM4226 zid-207 (Km ^r) zii-215 (Cm ^r)	χM2224	χM3224	χM4224	<i>zdh-146</i> (Sp ^r) <i>zed-153</i> (Cm ^r)
χM2225 χM3225 χM4225 zch-131 (Km ^r) zdb-138 (Cm ^r) χM2226 χM3226 χM4226 zid-207 (Km ^r) zii-215 (Cm ^r)				MG1655 (Tn10dKanRCP2) (Tn10dCamRCP2)
χ M2226 χ M3226 χ M4226 $zid-207$ (Km ^r) $zii-215$ (Cm ^r)	χM2225	xM3225	χM4225	<i>zch-131</i> (Km ^r) <i>zdb-138</i> (Cm ^r)
	χM2226	χM3226	χM4226	<i>zid-207</i> (Km ^r) <i>zii-215</i> (Cm ^r)

^{*a*} This study was the source for all strains listed except strain MG1655 (2), strain RS218 (44), strain J96 (28), and χM strains 2002, 2008, 2014, 2020, 2026, 2031, 2038, 2046, 2053, 2061, 2064, 2070, 2080, 2088, 2095, 2099, 2107, 2115, 2121, and 2127 (9).

^b Locus designations were relative to established transposon insertions, determined as described previously (40), or were determined by auxanography (25). Physical map coordinates are clockwise from the position 25 kb counterclockwise to the (conserved) native *Not* site nearest the car locus (25). The insertions' coordinates refer to kilobases in the MG1655 (parentheses), RS218 (brackets), and J96 (braces) strain backgrounds; these were determined from the distances between insertions by I-SceI digestions from double mutants (Fig. 3B).

comparisons between pairs of corresponding points in different copies of the *E. coli* genome (9). Biophysical comparison of corresponding genome segments between strains MG1655 and pathogenic strains RS218 and J96 was carried out following

introduction of pairs of Tn10dRCP2 insertion alleles. This was done by sequential P1 transductions; the distinct antibiotic resistances carried by the neighboring Tn10dRCP2 inserts in the set enabled construction of double mutants in the second



FIG. 2. Determination of macrorestriction fragment length polymorphism on the *E. coli* chromosome. The macrorestriction digestion patterns of genomic DNAs from different double mutants, all bearing the same insertions (*zcc-12d* and *zch-131*) in the MG1655 background (lanes 1 and 2), the RS218 background (lanes 3 and 4), and the J96 background (lanes 5 and 6), are shown. Missing native fragments (white bars) and novel subfragments (black bars) generated by *Not*I restriction (lanes 1, 3, and 5) indicated the positions of the insertions in the different strain backgrounds relative to native sites (Fig. 1). (Open bars indicate the electrophoretic positions of 5-kb novel *Not*I subfragments generated by *I-SceI* restriction (lanes 2, 4, and 6) indicate by contrast, in readily comparable units allowed by the absence of native sites, the distances between the insertions in the different strain backgrounds (Fig. 3).

step of this process (Table 1). In this way, the chromosome was divided into 20 contiguous and nonoverlapping intervals in each of the three strain backgrounds. A representative macrorestriction-PFGE analysis of corresponding double-insertion mutants, with strains χ M2211, χ M3211, and χ M4211, is shown in Fig. 2. A genomic NotI digestion of strain xM2211 (MG1655 zcc-126::Tn10dSpcRCP2, zch-131::Tn10dKanRCP2) is shown (Fig. 2, lane 1). The NotI pattern serves to verify the Tn10dRCP2 insertions into native fragments J_N and I_N . This strain carrying a pair of Tn10dRCP2 inserts was detected by the loss of the J_N and I_N bands and the generation of four new subfragments (Fig. 2, lane 1). This same strain was also digested with I-SceI, resulting in a single band of 229 kb (Fig. 2, lane 2). The corresponding double mutants χ M3211 (RS218 zcc-126::Tn10dSpcRCP2 zch-131::Tn10dKanRCP2) and χM4211 (J96 zcc-126::Tn10dSpcRCP2 zch-131::Tn10dKanRCP2) were also digested with NotI and I-SceI. For verification of the double insertion into the RS218 background, loss of $C_{\rm N}$ and M_N was sought (Fig. 2, lane 3); for verification in the J96 background, loss of D_N and M_N was sought (Fig. 2, lane 5). The different sizes of the genome interval, measured in unit fragments following I-SceI digestion, were determined to be 309 kb within χ M3211 for RS218 (Fig. 2, lane 4) and 354 kb

within χ M4211 for J96 (Fig. 2, lane 6). These data indicate that both pathogenic *E. coli* strains RS218 and J96 contain added chromosomal segments within this interval, potentially containing virulence factors (see below).

Similar comparative analyses were repeated for each of the 20 ~250-kb genomic intervals. The results are summarized schematically in Fig. 3A relative to the MG1655 background. The sizes of the I-SceI intervals for the double-Tn10dRCP2 strains are given in Fig. 3B. An interval size difference of >7 kb between corresponding I-SceI fragments was taken to indicate substantial addition or deletion. Detected differences of ≤ 7 kb were considered to have resulted from small rearrangements including transposon and insertion sequence migrations. It is conceivable, however, and a general shortcoming of comparative mapping with rare-restriction-site insertions, that the differences of ≤ 7 kb could have reflected larger additions or deletions canceling out each other's contributions to size within a given interval. The RS218 chromosome contained 10 unique segments relative to the MG1655 chromosome: zah to zbd (26 kb), zbh to zcc (69 kb), zcc to zch (80 kb), zdh to zed (27 kb), zeh to purF (66 kb), zfh to cys (40 kb); cys to zgf (50 kb), zid to zii (24 kb), zje to zji (70 kb), and zji to thrA (85 kb). The RS218 chromosome had one deletion of 20 kb relative to the MG1655 chromosome between zdb and zdh. The J96 chromosome had two deletions relative to strain MG1655: one of 35 kb within the same *zdb*-to-*zdh* interval (as in RS218), and a second of 53 kb between zch and zdb. The J96 chromosome had four unique segments relative to the MG1655 chromosome: zcc to zch (125 kb), zed to zeh (28 kb), cys to zgf (230 kb), and zje to zii (110 kb). Three of these J96 unique segments mapped to the same intervals as different unique segments in strain RS218: those at zcc to zch (22 to 27 min), cys to zgf (cys to 65 min), and zje to zii (94 to 98 min). These locations contain the virulence factors sfa (22 to 27 min in strains RS218 and J96) kpsA (64 min in strain RS218), hlyB/D and pap (64 min in strain J96), and *hlyB/D*, prs, and cnf (94 to 98 min in strain J96). Interestingly, some of the same virulence factors (hlyB/D and prs) were originally mapped to yet other chromosomal loci in uropathogenic strain 536 (12).

DISCUSSION

Previously we have shown that insertions containing rare restriction sites can facilitate integrated genome mapping (40). In subsequent work we have shown that pairs of insertions containing the unique I-SceI restriction site allow purification of the genomic intervals that they flank (9). In this study, we combined integrated genome mapping with the isolation of genomic intervals between I-SceI insertions to identify chromosomal segments that distinguish pathogenic from nonpathogenic E. coli strains. Through comparisons of corresponding chromosomal segments, we anticipated finding evidence of insertions and/or deletions that contributed to genome size variation and perhaps to pathogenic traits. We identified 11 such chromosomal differences between strains RS218 and MG1655 (10 additions and 1 deletion) and 6 between strains J96 and MG1655 (4 additions and 2 deletions) of genomic segments of 15 kb or larger. These relatively few, large additions and deletions accounted for nearly all genome size differences.

The *E. coli* strains that we examined, MG1655, RS218, and J96, exhibit distinct modes of interaction with mammalian hosts. Strain MG1655 is a nonpathogenic derivative of *E. coli* K-12. Strains RS218 and J96 are pathogenic, especially in targeted subpopulations of the host. These strains also exhibit extensive (up to \sim 500 kb) variation in chromosome size. De-

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٩	اًاً :	MG1655 RS218 J96	FIG. 3. Three-cop copies encoded genea the allele numbers of by P1 transduction. Ir (gaps). The additions including insertion-sec <i>prs</i> , and <i>cnf</i> [47]) and <i>i</i> intervals in the gridw

234 RODE ET AL. spite their extensive structural and functional divergence, overall chromosomal gene order is conserved among these three strains; i.e., the 20 I-SceI insertions are in the same order along all three chromosomes. In addition, the data indicated five different classes of genomic intervals: (i) seven intervals carrying genomic segments of the same length in all three strains, (ii) eight intervals carrying additional genomic segments in one or the other of the two pathogenic strains, (iii) three intervals carrying additional genomic segments in both pathogenic strains, (iv) one interval carrying a genomic deletion relative to strain MG1655 in one of the two pathogenic strains, and (v) one interval carrying different genomic deletions relative to strain MG1655 in both pathogenic strains (Fig. 3). The conservation of overall gene order and of many physical distances among the chromosomes of the three strains indicates a previously unimagined degree of structural identity-by-descent among them. They represent various instances of the E. coli chromosome in which different combinations of accessory components and/or deletions have been acquired.

Associated with a majority of the strain-specific chromosomal segments from the two pathogens were genes contributing to some of the key virulence traits distinguishing them. For instance, newborn-sepsis-associated strain RS218 carries genes putatively for penetration of epithelial basement membranes (sfa) (24), for immune evasion by molecular mimicry of a fetal brain antigen (kpsA) (22), and for penetration of the blood-brain barrier (*ibe-10*) (27). The coordinates for these virulence genes within the E. coli chromosome are as follows: sfa, 24 min (41); kpsA, 64 min (8, 16, 48); ibe-10, 87 min (7); and *ibeB*, 98 min (7). Herein we demonstrate the association of these virulence factors with the RS218-specific segments at zcc to zch (\sim 24 min), zeh to purF (\sim 47 min), cys to zgf (\sim 64 min), zid to zii (~ 87 min), and zii to thrA (~ 98 min), respectively. The uropathogenic strain J96 carries genes putatively for penetration of epithelial basement membrane (sfa) (37, 42), for ascendance of the host's ureters (pap) (30), for disruption of eukaryotic cells by α -hemolysin (*hly*) and by cytotoxic necrotizing factor 1 (cnf) (11), and for adhesion to host tissues (prs) (31). The coordinates for these virulence genes within the E. coli chromosome are as follows: sfa at 24 min (41); hlyB/D and pap at 64 min (47); and hlyB/D, prs, and cnf at 94 min (47). Again, these J96 virulence factors are associated with J96specific segments at zcc to zch ($\sim 24 \text{ min}$), cys to zgf ($\sim 64 \text{ min}$), and zje to zji (~94 min), respectively. The acquisition of different strain-specific pathogenicity islands within the same genomic regions indicates that these loci are potential hot spots for evolution of pathogenic traits. Insertions of many known pathogenicity islands into the E. coli chromosome are at tRNA genes: at the phenylalanine gene pheV for the pap gene of J96 (47) and the kpsA gene of strain RS218 (16); and at the selenocysteine gene *selC* for the locus of enterocyte effacement element (33), and at the phenylalanine gene pheR for the prs and hly genes, of strain J96 (11). Further, strains RS218 and J96 both have large genomic deletions relative to strain MG1655 at zdb to zdh (~31 min). Strain J96 has a second deletion occurring at *zch* to *zdb* (\sim 27 min). These deletions may constitute virulence black holes (loss of genes enhancing a strain's virulence), like that recently reported for the evolution of Shigella spp. and enteroinvasive E. coli (32). This inverse complement to pathogenicity islands may also contribute to the evolution of these pathogens by enhancing the pathogen's survival through the loss of chromosomal sequences.

Our findings of large unique components to the *E. coli* chromosome in pathogenic strains are consistent with the work of others showing that virulence factors tend to be clustered both on pathogenicity islands (23) and within particular

branches of the *E. coli* tree (14). Further, the segments uniquely absent from the chromosomes of pathogenic strains are consistent with Maurelli et al.'s black-hole concept that loss of chromosomal components may be important in the evolution of pathogenesis (32). In the two pathogenic strains, the unique regions identified by physical chromosomal alignments relative to strain MG1655 were colocalized with known virulence genes, and it is possible that the unaccounted-for coding capacity of these regions may contain new unidentified virulence factors. Also, the unique chromosomal segments in the three strains accounted for most of the genome size differences among them, which may suggest an explanation for the correlation between genome size variation and conventional genetic distance in *E. coli*.

ACKNOWLEDGMENTS

This work was supported by grants R29-AI31419 and R01-AI40074 from the National Institutes of Health to C.A.B.

We thank S. Hanash for kind and enthusiastic support of this work, J. Adams for critically reviewing the manuscript, and Erin McDaid-Kelly and Janice Hatch for technical assistance in preparation of the manuscript.

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