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SGI1-K, a Variant of the SGI1 Genomic Island Carrying a Mercury Resistance Region, in *Salmonella enterica* Serovar Kentucky▽

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A multiple-antibiotic-resistant *Salmonella enterica* serovar Kentucky strain was found to contain SGI1-K, a variant form of the *Salmonella* genomic island 1 (SGI1) with an In4-type class 1 integron that contains only one cassette array, *aacCA5-aadA7*, and an adjacent mercury resistance module. Part of the 3′-conserved segment (3′-CS) of the integron, together with the inverted short segment from the right-hand end of the integron transposition module normally found between the 3′-CS and IS*6100* in In4 family integrons, has been removed by an IS*6100*-mediated deletion. IRt, the right-hand inverted repeat found at the outer end of the integron, abuts a mercury resistance region instead of the usual SGI1 backbone segment. The *mer* module is a hybrid of those found in Tn*501* and Tn*21*. This *mer* region and a further uncharacterized segment of at least 10 kb appear to have been incorporated between IRt and the SGI1 backbone. These findings demonstrate that the multidrug resistance region in SGI1 can incorporate new DNA segments in the same way as multiple antibiotic resistance regions in plasmids.

In multiple-antibiotic-resistant strains of *Salmonella enterica* serovar Typhimurium DT104, the antibiotic resistance genes are frequently found clustered within a 43-kb integrated element known as the *Salmonella* genomic island 1 (SGI1) (4, 5). The original SGI1 isolated from *Salmonella* serovar Typhimurium DT104 strains contains five antibiotic resistance genes, all located within the boundaries of a complex class 1 integron (Fig. 1), recently designated In104 (18). Two of these genes, *aadA2* and *blaP1*, are each within a gene cassette, but the cassettes are situated in two different *attI1* sites that are separated by a region that includes the *floR* florfenicol resistance determinant and the *tetRA*(G) tetracycline resistance determinant (2, 4, 6). The fifth gene, *sul1*, is part of the 3′-conserved segment (3′-CS) of the integron. These five genes are responsible for the multiple antibiotic resistance phenotype conferred by SGI1, namely resistance to chloramphenicol and florfenicol (*floR*), ampicillin and carbenicillin (*blaP1*), streptomycin and spectinomycin (*aadA2*), sulfonamides (*sul1*), and tetracycline [*tet*(G) determinant]. SGI1 or variants of it have also been found in several other *S. enterica* serovars, consistent with horizontal gene transfer and mobilization of SGI1 by an IncC plasmid, as recently demonstrated (11). In all cases, SGI1 is found in the bacterial chromosome between the *thdF* and *yidY* genes (3, 9, 12–14, 16, 18, 22). A second integrated element ("retron phage"), located between one end of SGI1 and the *yidY* gene, is found only in *Salmonella* serovar Typhimurium strains (4, 5).

A number of variants of SGI1 containing different sets of resistance genes have also been identified (3, 9, 12–14, 18). These variants, SGI1-A to SGI1-J, appear to have gained, lost, or exchanged resistance genes by gaining and/or losing various segments of DNA, mostly as a result of events that could have occurred by homologous recombination (see references 3, 13, and 18). Some variants have lost the central region of In104 and include only one *attI1* site containing gene cassettes. Others include gene cassettes that differ from those in SGI1, such as the *dfrA1*-orfC or the *aacCA5-aadA7* pairs (13, 14, 18), or have a deletion encompassing an *attI* site (18). Further variants (3, 18) have gained an additional *dfrA10* trimethoprim resistance gene associated with the potential insertion sequence known as CR1, which draws a variety of resistance genes into the complex class 1 integrons that contain it (28). Exchange of the cassette array and acquisition of CR1 (containing orf513) and the adjacent *dfrA10* gene have both been shown experimentally to occur by homologous recombination (25, 27, 28). Recently, further variations that have lost other parts of SGI1 have been reported. One type appears to have been formed by the activity of CR1 and to have lost all of the sequence to the right of CR1 in the CR1-containing SGI1-A variant, with the deletion extending into the adjacent chromosomal genes (12). A variant in which the integrase-encoding end (IRi) of the class 1 integron abuts a different part of the SGI1 backbone has also been described (18). Variation in the structure of SGI1 thus serves as a paradigm for examining the evolution of multiple antibiotic resistance regions occurring within an integron that is found in a defined context and location, namely in the backbone of SGI1, which is integrated within the end of the *thdF* gene in the *S. enterica* chromosome.

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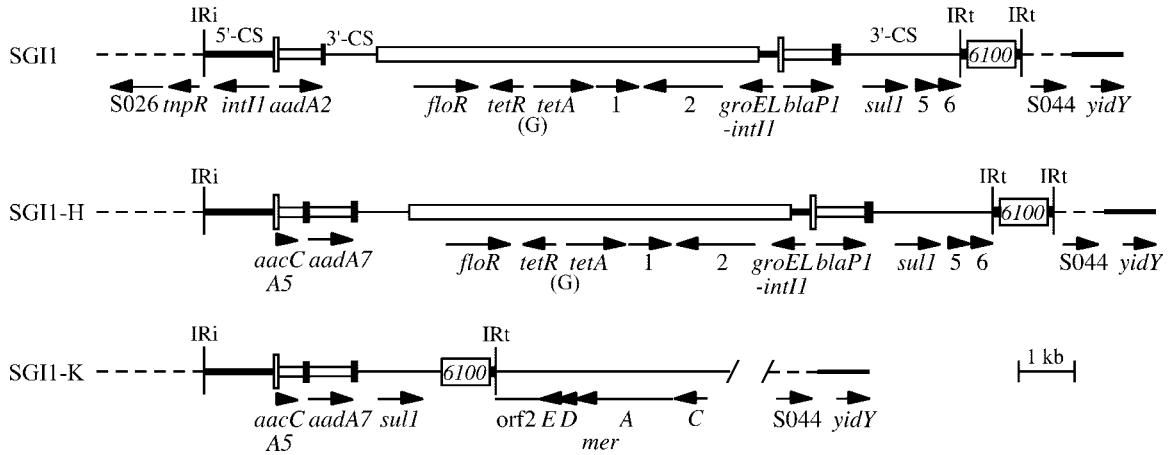

FIG. 1. Integrons in SGI1 and variants. The map of In104, the integron in the original SGI1, was generated from the sequence in GenBank accession no. AF261825. For SGI1-H, the cassettes from AY458224 were substituted on the left. The complete sequence of the integron in SGI1-K and its immediate surroundings is from this study (AY463797.3). Dashed lines represent the surrounding SGI1 backbone, and the medium-width line on the right represents the adjacent chromosome. Narrow vertical bars represent the inverted repeats (IRi and IRt) of class 1 integrons. The 5′-CS, 3′-CS, and *tni* regions of class 1 integrons are indicated by lines of different thicknesses, with *attI1* as a tall open box and gene cassettes as open boxes with a black bar at one end, indicating the 59-base element (59-be). IS*6100* and the central, non-integron-derived region are open boxes. Arrows indicate the position and orientation of genes and open reading frames, which are named or numbered.

We recently reported the sequence of an *aacCA5-aadA7* gene cassette array in a class 1 integron from a multiple-antibiotic-resistant *Salmonella enterica* serovar Kentucky strain that was isolated in 2001 from spice imported into Australia from India (19). Here, this integron was localized to a variant SGI1 and both the complete structure of the integron and its context were investigated.

MATERIALS AND METHODS

Bacterial strains. *Salmonella* serovar Kentucky strain SRC73 was isolated in 2001 from spice imported into Australia from India. It is resistant to ampicillin, gentamicin, streptomycin, spectinomycin, sulfathiazole, tetracycline, and nalidixic acid (19) and known to contain the cassette array *aacCA5-aadA7* conferring resistance to gentamicin (*aacCA5*) and streptomycin and spectinomycin (*aadA7*), as well as the resistance genes bla_{TEM} (ampicillin resistance), *sul1* (sulfonamide resistance), *strAB* (streptomycin resistance), and *tet*(A) (tetracycline resistance), but not the *aadA2* gene or *tet*(B), *tet*(G) determinants (19). The *blaP1* (or *blaP2*) and *floR* genes were also absent (R. S. Levings, unpublished observations).

PCR mapping. SRC73 cultures were grown overnight at 37°C on MacConkey agar (Becton Dickinson and Company). Whole-cell DNA was isolated using standard methods (31) and used as the template for PCR amplification reactions using various combinations of the primers listed in Table 1. Amplification was carried out in PCR buffer (Roche Molecular Biochemicals, Mannheim, Germany) containing each deoxynucleoside triphosphate (dNTP) at 160 μM, 20 pmol of each primer, approximately 10 to 50 ng of template, and 1 U of *Taq* DNA polymerase (Roche). Reaction conditions, described in detail elsewhere (18), were generally 94 to 96°C for 3 to 5 min, followed by 30 to 40 cycles of denaturation (94 to 96°C for 30 s), annealing (52 to 62°C for 30 to 60 s), and extension (72°C for 30 s for 2 min), and a final incubation at 72°C for 10 to 15 min. Products were separated on agarose gels, and sizes were estimated using 100-bp Plus (Fermentas, Vilnius, Lithuania) or Hyperladder I (Bioline, London, United Kingdom) and known amplification products from SGI1 as standards.

DNA sequencing and sequence analysis. PCR products were purified using the QIAquick PCR purification kit (QIAGEN, Inc., Valencia, CA) following protocols supplied by the manufacturer. Automated sequencing was carried out as described previously (18, 28). The sequence of overlapping PCR fragments was determined on both strands for all regions except for parts of the 5′-CS and 3′-CS, where only one strand was sequenced. Assembly and analysis of sequences were carried out as described previously (28).

Southern hybridization. Whole-cell DNA was digested with various restriction enzymes, and the fragments were resolved by electrophoresis through 0.7% agarose. The DNA was transferred to N^+ nylon membrane (Amersham, Buckinghamshire, United Kingdom) by capillary action and was cross-linked to the membrane by baking at 80°C for 2 h. The membrane was exposed to denatured digoxigenin-labeled probes overnight at 42°C and then washed twice with 2× SSC (1× SSC is 0.15 M NaCl plus 0.015 M sodium citrate)–0.1% sodium dodecyl sulfate for 15 min at room temperature and twice more stringently with 2× SSC–0.1% sodium dodecyl sulfate for 15 min at 68°C. Digoxigenin-labeled molecular mass markers (molecular marker set II; Roche Diagnostics Corporation) were included on all membranes, and bands were visualized fluorescently using standard protocols supplied by the manufacturer (Roche Diagnostics Corporation). Probes for *intI1*, *merA*, S024, and S044 were generated by PCR using primers listed in Table 1. The S024 probe (primers RL-D3 and S024-RV) is equivalent to p1-9 (4, 5) and was used to confirm that the XbaI restriction fragments carrying S012 to S026 were present.

Nucleotide sequence accession number. The 4.36 kb of nucleotide sequence from the SGI1-K integron and flanking regions reported in this paper has been added to GenBank accession no. AY463797.1 to give AY463797.3. The sequences of the left- and right-hand boundaries of SGI1 (the *thdF-int* and S044-*yidY* junctions) are also included in GenBank accession no. AY463797.3.

RESULTS

Serovar Kentucky SRC73 contains a variant of SGI1. Published primers (listed in Table 1) were used to detect the presence of SGI1 and the integron within it in the serovar Kentucky SRC73 strain. Products of the expected size (500 bp) were obtained with primers U7-L12 (in *thdF*) with LJ-R1 (in SGI1) and 104-RJ (in SGI1 and S044) with 104-D (in *yidY*), indicating that the standard junctions between an SGI1-like region and the *thdF* and *yidY* genes on the chromosome are present. The "retron phage" insertion found in SGI1-containing *Salmonella* serovar Typhimurium strains (4) was not present. To confirm this, the PCR products were sequenced. The sequences flanking SGI1 were, with the exception of a few base changes, identical to a continuous region extending from within the *thdF* gene to within the *yidY* gene in genome sequences of serovars Typhi, Paratyphi A, and Choleraesuis (GenBank accession no. AL627280, AE014613, CP000026, and AE017220). However, a 100-bp segment found adjacent to the right-hand end of the SGI is not present in the DT104 se-

TABLE 1. Characteristics of the primers used in this study

Primer	Sequence (5′→3′)	Location	Nucleotide positions	Accession no.	Source or reference[a]
U7-L12	ACACCTTGAGCAGGGCAAAG	*thdF*	1–20	AF261825.2	5
LJ-R1	AGTTCTAAAGGTTCGTAGTCG	*int*	500–480	AF261825.2	5
RL-D3	ATTGGTATGAGCCATGATGG	S024	20531–20550	AF261825.2	This study
S024-RV	GGTACGGTATCGCCTAAGTG	S024	21930–21911	AF261825.2	9
S026-FW	TCGGGTAATCTCAGCAGAGC	S026	25021–25040	AF261825.2	9
int-RV	GGGCATGGTGGCTGAAGGACC	*intI1*	27266–27246	AF261825.2	9
L2	GACGATGCGTGGAGACC	*intI1*	27612–27628	AF261825.2	30
L3	CTTGCTGCTTGGATGCC	5′-CS	27908–27892	AF261825.2	21
QS-1	ATGAAAGGCTGGCTTTTTCTTG	*qacEΔ1*	28953–28974, 38413–38424	AF261825.2	4
QS-2	TGAGTGCATAACCACCAGCC	*sul1*	29674–29655, 31934–31915	AF261825.2	4
sul1-F	GTGACGGTGTTCGGCATTCT	*sul1*	29297–29316, 35757–35776	AF261825.2	17
sul1-R	TTTACAGGAAGGCCAACGGT	*sul1*	39424–39405	AF261825.2	17
orf5-F	AGGTTGTGCGGCTGATGC	orf5	39776–39793	AF261825.2	18
orf5-R2	CGAGTTCTAGGCGTTCTGC	orf5	40213–40195	AF261825.2	18
orf6-R	ACTATCTTCGGCCTTCACACG	orf6	40508–40488	AF261825.2	18
DB-T1	TGCCACGCTCAATACCGAC	IS*6100*	41120–41138	AF261825.2	3
IS6100-Rv2	AATGGTGGTTGAGCATGCC	IS*6100*	41475–41457	AF261825.2	18
MDR-B[b]	GAATCCGACAGCCAACGTTCC	S044	41905–41884	AF261825.2	3
104-RJ	TGACGAGCTGAAGCGAATTG	S044	42373–42392	AF261825.2	5
104-D	ACCAGGGCAAAACTACACAG	*yidY*	47130–47111	AF261825.2	5
RL-D1	TTGTCCCTGATGAGACTGC	S044	41994–42012	AF261825.2	This study
RL-D2	TCGGGATGATTGTGGCTCC	S044	42225–42207	AF261825.2	This study
73QS-R1	GTGGCAGCAACATCCTTTGG	*aadA7*	2843–2824	AY463797.2	This study
RH365	CGATATGCACGCTCACC	Tn*501mer*	3090–3074	Z00027	This study
RH383	CTTCGTGAAATCAGCCCAG	Tn*501mer*	384–366	Z00027	This study
RH389	GTGCCGTCCAAGATCATG	Tn*501mer*	1753–1770	Z00027	This study
RH444	GACTCGCCGATGTTTCCACG	Tn*501mer*	4303–4284	Z00027	This study
RH445	GAACGCCTTGCCGTAATCG	Tn*501mer*	2140–2158	Z00027	This study
RH446	CTTACTGCGGTCAATCGTAGG	Tn*501mer*	1860–1840	Z00027	This study
RH447	CACACCAACTCAGACAGCACG	Tn*501mer*	205–225	Z00027	This study
RH448	CGGTGTTGGAACCCTATCG	Tn*501mer*	753–771	Z00027	This study
CV-D1	CAGCCGCAGTTCGTCTATG	Tn*501mer*	2548–2566	Z00027	This study[c]
CV-D2	TCGTCAGGTAGGGGAACAAC	Tn*501mer*	2941–2922	Z00027	This study[c]

[a] Reference numbers are for the primer.
[b] The sequence from AF261825.2 is GAATCCGACAGCCAACG**C**TTCC (the C in bold is not present in the primer).
[c] Primer designed by C. Venturini.

quence or in the *S. enterica* serovar Typhimurium LT2 genome sequence (GenBank accession no. AF261825 and AE008879).

Using primers that detect the boundaries of the integron with the SGI1 backbone, namely S026-FW (in S026) with int-RV (in *intI1*) and DB-T1 (in IS*6100*) with MDR-B (in S044), only the S026-FW with int-RV product was obtained, indicating that the left end of an integron is at the normal position within the SGI1 backbone but that the right-hand end is not. The sequence of the S026-FW with int-RV product confirmed that the IRi end of the integron is in precisely the same position as in SGI1 (see GenBank accession no. AF261825).

The fact that a single 1.6-kb PCR product containing the *aacCA5-aadA7* cassette array was obtained previously with primers L1 and R1 in the integron 5′-CS and 3′-CS (19) indicates that only one *attI1* site is present in SRC73. Consistent with this conclusion, the *tet*(G) or *floR* genes that are found between the two *attI1* sites in SGI1 were not detected using PCR primers within each gene and neither of the cassettes found in SGI1, *aadA2* and *blaP1*, was present (see Table 1 in reference 18 for primer details). Hence, SRC73 contains a variant form of SGI1. Linkage of the *aacCA5-aadA7* cassette array to the SGI1 backbone was demonstrated by PCR, using a primer in *aadA7* (73QS-R1) with one in the SGI1 backbone (S026-FW). The variant therefore differs from its closest relative, SGI1-H (GenBank accession no. AY458224), found in serovar Newport (14), which contains two *attI1* sites with the *aacCA5-aadA7* cassette array replacing the *aadA2* cassette of SGI1 (Fig. 1). Using the next available letter, the SGI1 variant in *Salmonella* serovar Kentucky was named SGI1-K.

Structure of the SGI1-K integron. The complete sequence of the In104-K integron backbone (from IRi to IRt) was determined using overlapping PCR products as substrates (Fig. 1). The 5′-CS of In104-K contains the strong version of the P_c promoter [TTGACA(17)TAAACT], which drives transcription of the cassette genes (10), whereas both 5′-CS regions in the original SGI1 (GenBank accession no. AF261825) have P_c weak [TGGACA(17)TAAGCT], and a G residue replaces the more usual C at the 16th position of the 17-bp P_c spacer. The left-hand 5′-CS region in SGI1-H (AY458224), which has the same cassette array as SGI1-K, also has the strong version of P_c. This is consistent with a model in which, during formation of SGI1-H and SGI1-K, the new cassette array came into the integron within SGI1 by homologous recombination, bringing with it the P_c region and simultaneously removing the *aadA2* cassette and associated P_c. However, SGI1-K has also lost the central region of the integron containing the *floR* and *tet*(G) resistance determinants together with the right-hand *attI1* site and *blaP1* cassette found in SGI1 and SGI1-H (Fig. 1).

The primer 73L2-F1, situated in the *aacCA5* gene, or sul1-F

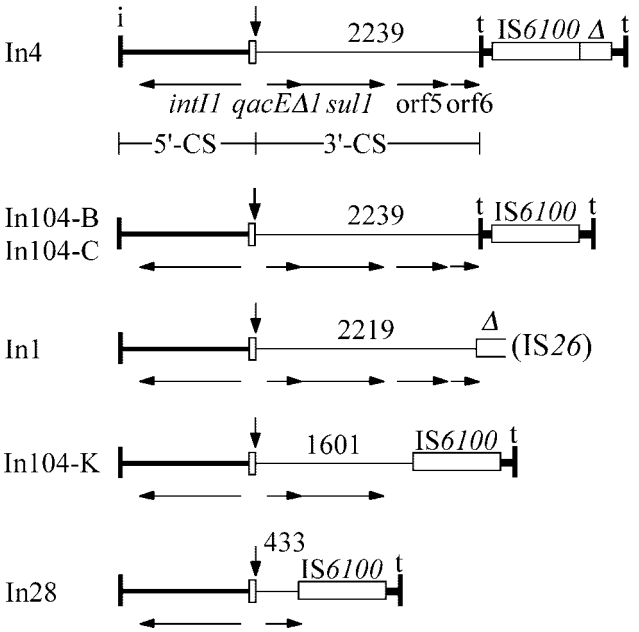

FIG. 2. Backbone structures of In4-type class 1 integrons and derivatives with IS*6100*-mediated deletions. Symbols are as in Fig. 1, and the vertical arrow marks the position at which the cassettes (not shown) are inserted. The extents of the 3′-CS are indicated above by the position of the last base present. Partial copies of IS*6100* are shown as open boxes labeled Δ. The extents of genes are shown by horizontal arrows and are named under In4 only. The sources for the sequences used are as follows: In4, GenBank accession no. U12338; In104-B and In104-C, which were deduced from SGI1, AF261825; In1, AY046276; and In28, AF313472. In104-K is from this study (AY463797.3).

or orf5-F, in the 3′-CS, together with primer IS1600-Rv2 in IS*6100* yielded PCR products, indicating that IS*6100* is adjacent to the 3′-CS. However, the estimated sizes of the products (3.5 kb, 2.0 kb, and 1.0 kb, respectively) were all about 0.7 kb smaller than predicted for the standard In4-type integron configuration (26), which is present at the right-hand end of In104 in SGI1 (Fig. 1) and SGI1-A to SGI1-J. The sequence of the orf5-F/IS6100-Rv2 product revealed that only nucleotides 1 to 1601 of the standard 3′-CS, which includes the complete *sul1* gene, immediately precede IS*6100* (Fig. 2). Hence, 638 bp of the 3′-CS, which is 2,239 bp in In4 and In104, and the 123 bp of *tni* sequence (including IRt) found in inverse orientation between IS*6100* and the 3′-CS of In4 and In104 have been lost. This indicates that an IS*6100*-mediated deletion event has occurred (Fig. 2), and similar deletions that remove the inverted *tni* end fragment as well as different lengths of the 3′-CS are found in other IS*6100*-containing class 1 integrons (29). There is also a single base difference (T218C) in the IS*6100* of SGI1-K in SRC73 that is not seen in In104 from SGI1 or in any other example of IS*6100* currently in GenBank.

Though PCR products that were expected to include the IRt end of the integron (using primer DB-T1 with either MDR-B or 104-RJ) were not obtained, the sequence of the adjacent region was obtained fortuitously. In initial PCR mapping, the orf5-F primer paired with orf5-R2 gave a product, which was unexpected granted that the 3′-CS region in SGI1-K does not include the orf5-R2 primer site. However, the size of this product (1.6 kb) was larger than the expected product. Furthermore, a product of the same size was generated when the orf5-F primer was used with MDR-B. The sequence of the product obtained with orf5-F/MDR-B indicated that it was in fact generated by priming of orf5-F (AGGTTGTGCGGCTG ATGC) at the correct position in the 3′-CS and again fortuitously in a sequence beyond the IRt end of the integron (see below). The 152-bp segment from the IRt end of class 1 integrons that is normally located to the right of IS*6100* in In4 and In4-type integrons (26, 29) was present (Fig. 1 and 2), but it was not located adjacent to S044 as it is in SGI1 (Fig. 1) and most of the known SGI1 variants.

Context of the IRt end of the integron. The sequence beyond IRt matched part of the Tn*501* mercury resistance (*mer*) module, and the fortuitous priming site for orf5-F was identified as AGGgcGTGCtGgCTGATGC (mismatched bases in lowercase), which lies within the open reading frame orf2 in the *mer* module of Tn*501* (7) and Tn*5051* (23). IRt of the SRC73 integron abuts base 3925 of Tn*501* (counting from the IR_{tnp} end; 3925 corresponds to position 4430 in GenBank accession no. Z00027) (Fig. 3). This point is 191 bp from the resolution crossover position in the *resI* subsite of the *res* site. Three class 1 integrons for which sequence adjacent to the IRi end has recently been reported, AF355189 (15), AJ515707 (34), and AJ550807 and AJ628135 (24), appear to have been inserted at the same position in the Tn*501 mer* module. Though only the context of the IRi end of these integrons is available, IRi abuts position 3930 (from the IR_{tnp} end) of Tn*501* (or 196 bp from between A and T in the *resI* subsite), as expected when the 5-bp duplication formed during transposition is taken into account (Fig. 3A). However, the transposon within which they reside is more closely related to Tn*5051* (GenBank accession no. Y177190) (23) which includes the same *mer* module as Tn*501* with a different, but related, transposition (*tnp*) module.

Extent of the *mer* region. To examine if the complete Tn*501 mer* module is present in SGI1-K, further PCR mapping using pairs of primers either specific for Tn*501 mer* or that will detect Tn*501 mer* and other related *mer* modules (Table 1 and Fig. 3B) was carried out. The plasmid pVS1, which is the original source of Tn*501* (33), was used as a positive control. With whole-cell DNA from SRC73 as a substrate, PCR products of the predicted size were obtained with RH444/RH445 and with RH365/RH389 (Fig. 3B), indicating that the *mer* region in SGI1-K extends to at least partway through the *merA* gene. However, a primer within this amplified region (RH446) paired with a primer in the *merR* gene (RH447) or a primer in the *merT* gene (RH448) yielded products 0.5 kb larger than the predicted size for Tn*501 mer*, suggesting that there is an insertion of approximately 0.5 kb within this segment. The sizes of these PCR fragments are consistent with the presence of a *mer* region containing the *merC* gene between *merA* and *merP* (20). The sequence of the *merC* region was identical to that of Tn*21*, and the *mer* module is a hybrid with the *merD* and *-E* genes and most of the *merA* gene derived from Tn*501* and the *merC*, *-T*, and *-R* genes derived from Tn*21* (GenBank accession no. AF071413). Consistent with the presence of a complete *mer* module, SRC73 was found to be resistant to mercuric chloride (20 μg/ml).

The right-hand end of SGI1-K. One possible explanation for the configuration found in SGI1-K is that a plasmid (or nonreplicating circular molecule) containing a class 1 integron in

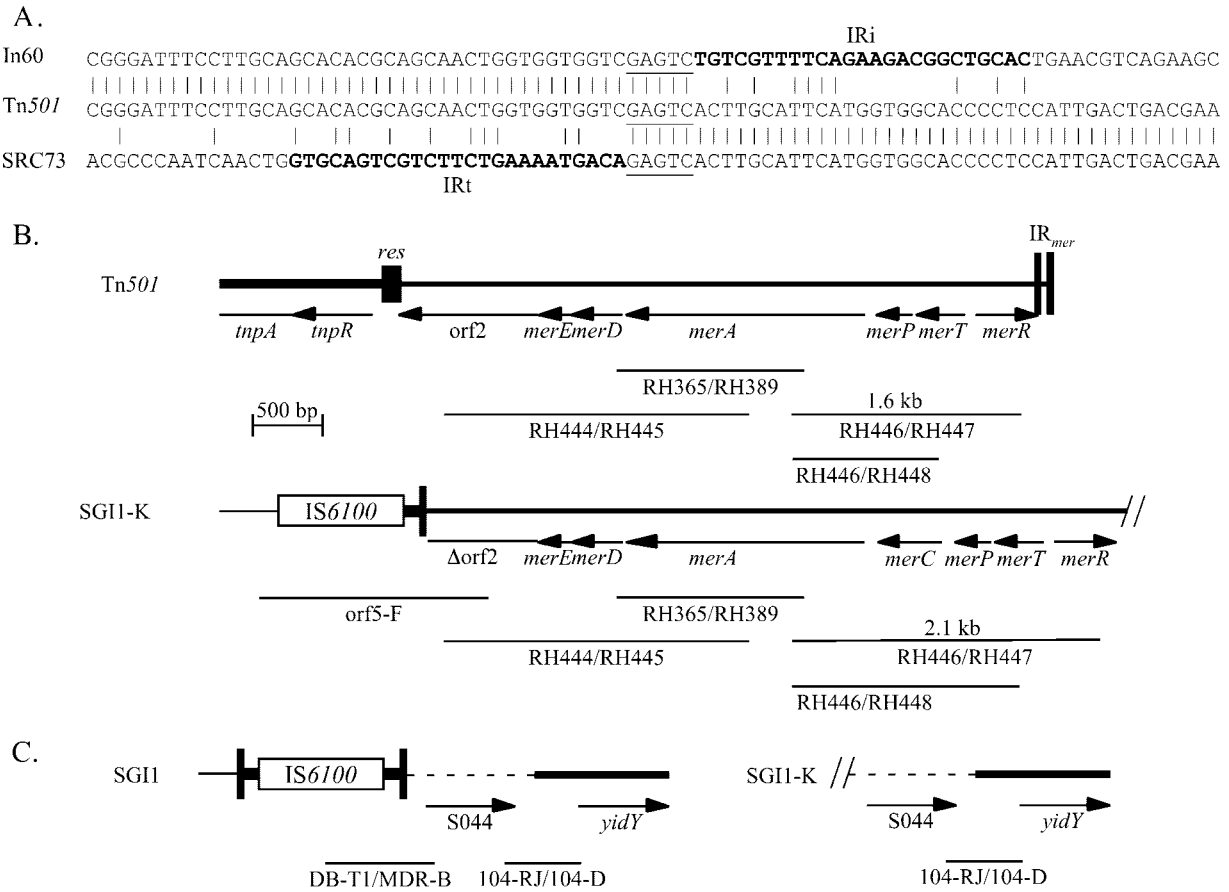

FIG. 3. Location of In104-K. (A) The sequence of the IRt boundary in SGI1-K is aligned with the sequences of Tn*501* from GenBank accession no. Z00027 and the IRi boundary of integrons in AF355189 (In60), AJ515707, and AJ550807. The IRi and IRt regions are in boldface type, and the 5-bp duplication is underlined. (B) Structure of the adjacent *mer* segment. The map of the *mer* region of Tn*501* (and Tn*5051*) is derived from Z00027, with the positions of specific PCR fragments indicated below. The SGI1-K map is derived from AY463797.3, the Tn*501 mer* sequence, and the Tn*21 mer* sequence. Features are as described in the legend to Fig. 1.

the Tn*501* or Tn*5051* context has become incorporated into the SGI via homologous recombination between regions of identity (e.g., 5′-CS, 3′-CS, or IS*6100*) in the two integrons. However, if this were the case, a second integron should be present adjacent to S044. In SGI1 and most of its variants, the region between IRt of the integron and the right-hand end of SGI1 is 880 to 898 bp long and includes only a single gene, S044 (see Fig. 1). At least part of this region is present in SGI1-K, as the priming site for 104-RJ, which is located within S044, is present (Fig. 3C). However, a copy of IS*6100* is not found near this end of SGI1 as the primer DB-T1 (in IS*6100*) paired either with MDR-B (in S044) or with 104-D (in *yidY*) did not yield products (Fig. 3C). This indicates that the final configuration of SGI1-K did not arise simply as the result of a single recombination event between identical regions (e.g., 5′-CS) in the integron in SGI and a plasmid containing a class 1 integron in the Tn*5051* context, which would leave the IRt-S044 boundary intact. Hence, if the additional *mer*-containing segment entered in this way, further events must have occurred. Attempts to establish linkage between the *mer* module and S044 using long-range PCR were unsuccessful. Southern blots of SRC73 genomic DNA, digested with a variety of restriction enzymes, revealed an XbaI fragment of over 23 kb that hybridized to probes for *intI1*, *merA*, and S044. Assuming that the ends of this fragment are the XbaI sites in *tnpR* and S044 (see Fig. 1 for positions of these genes), a minimum size of 13 kb can be calculated for the region between the right-hand end of the *mer* region and S044.

DISCUSSION

SRC73, an *S. enterica* serovar Kentucky strain isolated in Australia in 2001 from a spice imported from India (19), represents the 13th *Salmonella* serovar that has been found to include an SGI1-type genomic island carrying multiple antibiotic resistance determinants (see reference 18). Though others have reported sequencing the boundaries between SGI1 and the chromosome for serovars Agona, Paratyphi B, and Albany (4, 11, 13), the sequences have not been released, nor were any differences between serovar Typhimurium and other serovars in the intergenic region between *thdF* and *yidY* mentioned. The sequence reported here for the chromosomal segments adjacent to the site of integration of SGI1-K matches those of serovars Typhi, Paratyphi A, and Choleraesuis in this region and SGI1-K is inserted within the end of the *thdF* gene. The first 100 bp adjacent to the right-hand end of SGI1-K is missing

from the sequence of the *S. enterica* serovar Typhimurium genome (strain LT2) and from the region flanking SGI1 in the DT104 strain. It is replaced by the so-called "retron phage," which lies adjacent to the end of *thdF* in LT2 or at the right-hand end of SGI1 in DT104 (5).

SGI1-K, the SGI1 variant found in SRC73, has undergone a number of changes relative to SGI1. It is related to the SGI1-H variant found in *S. enterica* serovar Newport (14) because both include the *aacCA5-aadA7* cassette array together with a P_c promoter different from that found in most SGI1 variants. In both cases, the cassette array is likely to be derived from another DNA molecule—e.g., a plasmid, integrating element or other nonreplicating circle present in the same cell—with these cassettes having been gained by the SGI1 variants via homologous recombination within both CS (i.e., 5′-CS and 3′-CS) flanking cassettes in class 1 integrons (25, 27). An alternative is that for SGI1-K, a single crossover in the 5′-CS incorporated the complete extra molecule, which included a class 1 integron in the Tn*501*/Tn*5051* context with subsequent events yielding the final configuration. The *Salmonella* serovar Newport strain containing the *aacCA5-aadA7* cassette array in SGI1-H was isolated in France in 2001 from a patient recently returned from Egypt (14). The same cassette array has also been found in *Vibrio fluvialis* strains isolated in 2002 from patients with cholera in Calcutta, India (GenBank accession no. AB114632 and AY605484) (1, 32). It is also present in a *Salmonella* serovar Haifa strain isolated in Spain from a patient with traveler's diarrhea who had recently returned from Egypt (AY563051) (8) and serovar Kentucky isolates from Slovakia (AM039633). Thus, the *aacCA5-aadA7* cassette array, which was only identified recently, has already been found in several countries and multiple species, demonstrating how readily cassette arrays containing new resistance genes can spread.

At least five further changes have clearly occurred in the course of the creation of SGI1-K from SGI1. First, the central region of the complex integron found in SGI1 including the right-hand *attI1* site has been lost. This event is likely to have occurred by homologous recombination between the right- and left-hand copies of either the 5′-CS or the 3′-CS, but if SGI1-K is a derivative of SGI1-H, the recombination must have occurred in the 3′-CS. Second, an IS*6100*-mediated deletion has removed part of the 3′-CS. This event could have occurred either within the SGI or previously in the incoming Tn*501* (Tn*5051*)-associated integron. Third, a *mer* module has become incorporated into the SGI. This change could have occurred simultaneously with the cassette exchange if only a single crossover were involved in that step. Fourth, the *mer* module, which is a hybrid between Tn*501*/Tn*5051 mer* and the *merC*-containing *mer* module from Tn*21*, has probably arisen by homologous recombination (23). The sequence switches from identity with Tn*501* to identity with Tn*21* in a short stretch of sequence identity near the beginning of the *merA* gene. Finally, the boundary between the IRt end of the integron and the SGI1 backbone has been disrupted. To explain the presence of only one integron and the absence of the standard boundary seen at the right-hand end of the integron in SGI1 and other SGI1 variants, it is necessary to invoke at least one further step, probably a deletion, that occurred after the postulated homologous recombination event incorporated a plasmid or nonreplication circular molecule into the SGI. However, further analysis of the large region (over 13 kb) between the *mer* module and S044 will be needed to resolve this. The locations of the remaining resistance genes known to be present in SRC73, namely bla_{TEM}, *strAB*, and *tet*(A), also remain to be established, as plasmids were not detected using standard extraction procedures (19).

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