Tn5041: a chimeric mercury resistance transposon closely related to the toluene degradative transposon Tn4651

G. Ya. Kholodii,† O. V. Yurieva, Zh. M. Gorlenko, S. Z. Mindlin, I. A. Bass, O. L. Lomovskaya,‡ A. V. Kopteva and V. G. Nikiforov

Author for correspondence: V. G. Nikiforov. Tel: +7 095 196 0015. Fax: +7 095 196 0221.
e-mail: uimg@img.rc.ac.ru

Institute of Molecular
Genetics, Russian Academy
of Sciences, Moscow
123182, Russia

This paper reports the discovery and characterization of Tn5041, a novel-type transposon vehicle for dissemination of mercury resistance in natural bacterial populations. Tn5041 (14876 bp), identified in a *Pseudomonas* strain from a mercury mine, is a Tn3 family mercury resistance transposon far outside the Tn21 subgroup. As in other Tn3 family transposons, Tn5041 duplicates 5 bp of the target sequence following insertion. Tn5041 apparently acquired its *mer* operon as a single-ended relic of a transposon belonging to the classical mercury resistance transposons of the Tn21 subgroup. The putative transposase and the 47 bp terminal inverted repeats of Tn5041 are closely related to those of the toluene degradative transposon Tn4651 and fall into a distinct subgroup on the fringe of the Tn3 family. The amino acid sequence of the putative resolvase of Tn5041 resembles site-specific recombinases of the integrase family. Besides the *mer* operon and putative transposition genes, Tn5041 contains a 4 kb region that accommodates a number of apparently defective genes and mobile elements.

Keywords: Tn5041, *mer* operon, Tn3 family, integrase, transposase

INTRODUCTION

Mercury resistance in bacteria is a widespread phenotype. As a rule, it is determined by several genes organized into a mercury resistance (*mer*) operon. These are often located on plasmids and transposons (Silver & Walderhaug, 1992; Misra, 1992). The first well-characterized mercury resistance transposons, Tn501 and Tn21, were from clinical isolates. Tn501 was isolated from a *Pseudomonas aeruginosa* strain from Australia (Stanisich *et al.*, 1977). Tn21 was found in the plasmid NR1 in *Shigella flexneri* isolated in Japan (Womble & Rownd, 1988; Grinsted *et al.*, 1990). Besides the *mer* operon and transposition genes Tn21 contains an integron carrying the antibiotic resistance genes *sul* and

str (Brown *et al.*, 1996). A plethora of transposons closely related to Tn21 and carrying different sets of antibiotic resistance genes has been detected in clinical isolates (for review see Grinsted *et al.*, 1990). Tn3926, which is homologous to both Tn501 and Tn21, was identified in *Yersinia enterocolitica* isolated from raw milk in France (Lett *et al.*, 1985). Tn21, Tn3926 and Tn501 define a compact group within the Tn3 family of transposons. They are bound by 38 bp inverted repeats (IRs) and contain two genes, *tnpR* and *tnpA*, which are involved in transposition (Grinsted *et al.*, 1990; Osbourn *et al.*, 1995).

Several mercury resistance transposons from environmental bacteria have been described less thoroughly. Tn1861 from *Pseudomonas putida* has a restriction pattern similar to Tn501 (Friello & Chakrabarty, 1980). The apparently identical Tn3402 and Tn3403 (Radford *et al.*, 1981) have a characteristic pattern of *EcoRI* and *HindIII* sites in the transposition genes, similar to that of Tn21 (A. J. Radford, cited by Stanisich *et al.*, 1989). Thus, until recently, all mercury resistance transposons described from Gram-negative bacteria were apparently related to Tn21 and Tn501 with the possible exception

† Present address: The Rockefeller University, New York, NY 10021, USA.

‡ Present address: Microcide Pharmaceuticals, 850 Maude Ave, Mountain View, CA 94043, USA.

Abbreviations: IR, inverted repeat; IS, insertion sequence; PMA, phenylmercuric acetate.

The EMBL accession number for the nucleotide sequence of Tn5041 reported in this paper is X98999.

of the poorly characterized Tn502 (Stanisich *et al.*, 1989; Brown *et al.*, 1996).

The first 'novel-type' *mer* transposon to be characterized was Tn5053 (Kholodii *et al.*, 1993). Tn5053 apparently acquired its *mer* operon, *merRTPFAD*, as a single-ended relic of another transposon which is closely related to the classical mercury resistance transposons Tn21 and Tn501. The transposition module of Tn5053 is quite different from transposition modules of classical transposons of the Tn21 subgroup, but is closely related to a family of transposons carrying integrons (Kholodii *et al.*, 1995; Brown *et al.*, 1996). Tn5053 is bounded by 25 bp terminal inverted repeats and contains four genes involved in transposition, *tniA*, *tniB*, *tniQ* and *tniR*. Transposition of Tn5053 occurs via cointegrate formation mediated by the products of the *tniABQ* genes, followed by site-specific cointegrate resolution. This is catalysed by the product of the *tniR* gene at the *res* region, which is located upstream of *tniR*. Sequence analysis suggests that Tn5053 fell into a recently recognized superfamily of transposons including retroviruses, Tn7, and insertion sequences of the IS3 family (Kholodii *et al.*, 1995).

In this communication we describe the isolation and complete nucleotide sequence of another 'novel-type' mercury resistance transposon, Tn5041, which was identified in a *Pseudomonas* strain from a mercury mine. As is the case with Tn5053, Tn5041 apparently acquired its *mer* operon from a transposon belonging to the Tn21 subgroup. The transposition module of Tn5041 belongs to the Tn3 family but is far outside the Tn21 subgroup.

METHODS

Bacterial strains and plasmids. The following bacterial strains and plasmids were used: *Pseudomonas* spp. KHP41 and KHP22 (Bogdanova *et al.*, 1988); *Escherichia coli* K-12 strains AB1456 (a recipient in transposition experiments); JM83 (a host strain for pUC19-based recombinant plasmids); HB101 (a host for *mer* operon containing plasmids in mercury resistance testing); RP1 (Pansegrau *et al.*, 1994); RP1::Tn5041 (this paper); R831b (*merB*⁺) (Schottel *et al.*, 1974); pVS982 = pBR322::Tn501 (*merB*) (Grinsted *et al.*, 1982). pWWO containing Tn4651 (Tsuda *et al.*, 1989) was obtained from Dr K. N. Timmis.

Mercury resistance testing. Susceptibility to HgCl₂ and phenylmercuric acetate (PMA) was determined by the disk diffusion method. LB agar (1.8%) was overlaid with 5 ml 0.5% LB agar containing 0.1 ml late-exponential-phase bacterial culture of HB101 with or without a plasmid. Filter paper disks (6 mm diameter) loaded with 5–40 µg HgCl₂ or 2–20 µg PMA were placed on the plates. The growth inhibition zones were measured after incubation at 30 °C for 20 h. All the assays were repeated at least three times. With 10 µg PMA, inhibition zones were about 5 mm for PMA-sensitive strains (HB101 without plasmids or with pVS982, or RP1, or RP1::Tn5041) and about 1.5 mm for the PMA-resistant strain (HB101 bearing R831b). Inhibition zones for HgCl₂ (at 20 µg) were about 6 mm for HB101 without plasmids or with RP1 and about 2 mm for HB101 with any other plasmid mentioned above.

Detection of mercury resistance transposons. This was done by introducing the broad-host-range plasmid RP1 into spontaneous rifampicin-resistant mutants of mercury-resistant *Pseudomonas/Xanthomonas* strains by conjugation from a rifampicin-sensitive *E. coli* donor strain. The matings were performed on solid LB medium. LB agar supplemented with kanamycin (50 µg ml⁻¹) and rifampicin (30 µg ml⁻¹) was used for selecting of RP1-containing transconjugants. After 2–3 serial transfers on selective medium containing kanamycin (50 µg ml⁻¹) and HgCl₂ (4 µg ml⁻¹) the transconjugants were mated with a streptomycin-resistant recipient *E. coli* strain AB1456 (str-r) and RP1-Hg-r recombinant plasmid-containing clones were isolated on LB agar supplemented with HgCl₂ (4–5 µg ml⁻¹) and streptomycin (100 µg ml⁻¹).

Cloning and other DNA manipulations. These were carried out according to Sambrook *et al.* (1989). Tn5041 transposed to RP1 was subcloned in pUC19 and sequenced using the Maxam–Gilbert method (Sambrook *et al.*, 1989). Tn5041-related elements were detected by colony hybridization with ³²P-labelled fragments of transposition genes of Tn5041. Plasmid localization of these elements was checked by Southern hybridization of Tn5041-specific probes with DNA preparations isolated from tested mercury-resistant strains by an alkaline extraction procedure (Birnboim & Doly, 1979). Database search was performed with the BLAST program (Altschul *et al.*, 1990). Sequence alignment of Tn3 family transposases and tree constructions were performed using the program VOSTORG (Zharkikh *et al.*, 1991). In the case of Tn4556 transposase, the published nucleotide sequence (Siemieniak *et al.*, 1990) apparently contained a small number of frame-shifting sequence errors. Our corrections resulted in identification of a well-conserved NH₂-terminal segment (130 residues) of the Tn4556 transposase which was missing in the published amino acid sequence and considerable improvement of the alignment of the following 70 amino acid residues.

RESULTS AND DISCUSSION

Detection of Tn5041 and related elements

For detection of mercury resistance transposons, we used a collection of mercury-resistant *Pseudomonas/Xanthomonas* strains isolated from a mercury mine in Kirgizia, Central Asia (Khesin & Karasyova, 1984; Bogdanova *et al.*, 1988). In most of these strains, *mer* operons were located on 100 MDa narrow-host-range plasmids that were readily transferred by conjugation to their mercury-sensitive plasmid-cured derivatives but not to any *Pseudomonas* or *E. coli* strains tested (Khesin & Karasyova, 1984; Zh. Gorlenko & S. Z. Mindlin, unpublished). We surveyed this collection by introducing the broad-host-range plasmid RP1 and mating out to *E. coli* as described in Methods. As in the case of Gram-negative mercury-resistant strains described by Kelly & Reanney (1984), some strains from our collection (12 out of 23 tested) were refractory to introduction of RP1. For some strains, this may be due to incompatibility or surface exclusion by resident plasmids because plasmids of the same incompatibility group as RP1 can be found in some environmental bacteria (Amuthan & Mahadevan, 1994) and narrow-host-range plasmids showing incompatibility with RP1 are known (Smith & Thomas, 1987). For one strain (KHP22) tested, we demonstrated that the resistance to RP1 was not due

Fig. 1. Genetic organization of Tn5041. The terminal 47 bp IRs are shown by solid arrowheads. The location and polarity of genes and ORFs is shown by open arrows. *T*, *P*, *C* and *D* indicate the corresponding *mer* genes, and 1 and 2 indicate homologues of *urf-1* and *urf-2*, respectively. *P* denotes *orfP* encoding a porin-like protein. *R'* is a truncated copy of *merR* located in the same orientation as the functional copy of *merR*. $\sigma\lambda$ and $\kappa\gamma$ are relics of mobile elements. Solid rectangles represent segments encoding amino acid sequences showing similarity to segments of a rat membrane protein located in the same order (Hallberg *et al.*, 1993). The sequence of a putative *res* site is shown above the 141 bp spacer located between *orfI* and *orfQ* (bases forming a palindrome are underlined, those shared with the *dif* site are boxed). Restriction sites: B, *Bgl*II; E, *Eco*RI; EV, *Eco*RV; H, *Hind*III; N, *Nco*I; Nd, *Nde*I; P, *Pst*I; Pv, *Pvu*II; S, *Sal*I; X, *Xba*I; Xh, *Xho*I.

to the resident plasmid since a mercury-sensitive derivative of KHP22 which was cured of its single 100 MDa plasmid (Khesin & Karasyova, 1984) was still refractory to introduction of RP1.

Four out of eleven RP1-accepting strains gave mercury-resistant transconjugants with frequencies about 10^{-5} per RP1-containing transconjugant. One of these strains contained a novel mercury resistance transposon, Tn5053, which was described previously by Kholodii *et al.* (1993, 1995). Restriction analysis of plasmid DNA of mercury-resistant transconjugants obtained from *Pseudomonas* sp. strain KHP41 revealed insertions of about 15 kb in a segment of RP1 located between *oriV* and Tn1. Sequencing of the junction between RP1 and the inserted element in three independent transconjugants revealed insertions of the same sequence after positions (numbering according to Pansegrau *et al.*, 1994) 11019 of RP1 (the beginning of the *kIcB* gene), 12025 and 12212 (a non-coding region at the border of *oriV*). Insertions of the element, Tn5041, resulted in duplications of 5 bp of the target sequence (GTATT, CTTTG and GGCAC, respectively).

Most mercury resistance determinants with a few possible exceptions (Witte *et al.*, 1986; Mahler *et al.*, 1986; Wang *et al.*, 1987; Shiratori *et al.*, 1989; Kholodii *et al.*, 1993) were found on plasmids. A 4.2 kb plasmid, which was too small to accommodate Tn5041, and no large plasmids were found in strain KHP41 using both an alkaline extraction procedure (Birnbom & Doly, 1979) and agarose gel electrophoresis of crude extracts by the method described by Eckhardt (1978). Chromosomal DNA preparations from KHP41 showed hybridization with Tn5041-specific DNA probes while plasmid DNA preparations did not (data not shown).

Thus, Tn5041 was apparently localized chromosomally. As in other reported cases of apparent chromosomal location of mercury resistance determinants, the possibility was not excluded that Tn5041 was actually located on a megaplasmid which often go undetected by common methods (Barton *et al.*, 1995). DNA elements showing hybridization with Tn5041 transposition genes were found in 100 MDa plasmids readily detected in three other *Pseudomonas* strains isolated from the same mine as KHP41 (see Methods). We failed to check transposability of these elements since their host strains were refractory to introduction of RP1. We demonstrated that about 10% of environmental *Pseudomonas* mercury-resistant strains collected in other parts of the world contained DNA elements showing hybridization with Tn5041 transposition genes. One of these strains contained a functional transposon similar to Tn5041 while other were either resistant to RP1 or transposition deficient. We found no elements related to Tn5041 in our collection of *Acinetobacter* and enterobacterial strains.

Tn5041 belongs to the Tn3 family

We determined the complete nucleotide sequence of Tn5041 on both strands as described in Methods. Results of sequence analysis are summarized in Fig. 1. Tn5041 is bound by 47 bp imperfect inverted repeats which are closely related to IRs of the toluene degradative transposon Tn4651 (Tsuda *et al.*, 1989). The 38 outermost base pairs of these IRs show similarity to IRs of the Tn3 family transposons (Fig. 2a).

At the right arm of Tn5041, a large ORF is situated encoding a protein (995 amino acid residues) showing 96.1% identity with a transposase of a Tn3 family

Fig. 2. Comparison of terminal IRs of (a) Tn5041 and (b) of an $\kappa\gamma$ element located within Tn5041 with selected IRs of the Tn3 family transposons. Letters following names of transposons are showing locations of IRs in those cases when the IRs are not the same ('t' indicates the *tnpA* end and 'o' the other end). Dots represent bases identical with those in the upper sequence. Accession nos (or references): Tn5041, X98999; Tn4651 (Tsuda *et al.*, 1989); *avrB*, M21965; Tn5396, U04360 and U04362; *mkfA*, X16098; 'res501' (located between *resIII* and *tnpR* in Tn501), X03406; Tn1721, X61367. Other accession nos are listed in the legend to Fig. 3.

toluene degradative transposon Tn4651 (EMBL accession no. X83686; Dr R. Horak). Accordingly, we suggest that this ORF which we designated *tnpA* (Fig. 1) encodes the Tn5041 transposase.

To find the exact position of Tn5041 in the Tn3 family we aligned its putative transposase with 20 Tn3 family transposases which we found in the databases. Analysis of the alignment demonstrated that most of the transposases clustered around three previously recognized subgroups. The first cluster united a compact Tn21 subgroup (including Tn21, Tn3926, Tn501 and Tn1721) (Grinsted *et al.*, 1990) with the more distantly related Tn2501 (Turner & Grinsted, 1987). Tn5393 (Chiou & Jones, 1993) and Tn163 (Ulrich & Puehler, 1994) can be also affiliated to this cluster on the basis of analysis of the more conserved COOH-terminal part of the transposase protein (Fig. 3). The second cluster contained transposons from Gram-positive bacteria only: Tn917 (An & Clewell, 1991), Tn5422 (Lebrun *et al.*, 1994), Tn1546 (Arthur *et al.*, 1993), Tn4430 (Mahillon & Lereclus, 1988), and a putative transposon from *Bacillus firmus* (Ivey *et al.*, 1992). The third cluster included Tn3 and Tn1000 (Grinsted *et al.*, 1990), Tn5396 (Elhai *et al.*, 1994), Tn1412 (accession no. L36547), Tn5401 (Baum,

Fig. 3. Evolutionary tree of Tn3 family transposons based on comparison of the COOH-terminal-third of their transposases. Accession nos: Tn21, X04891; Tn3926, X14236; Tn501, X03406; Tn2501, Y00502; Tn5393, M96392; Tn5403, X75779; Tn163, L14931; Tn917, M11180; Tn5422, L28104; Tn1546, M97297; Tn4430, X07651; Tn3, V00613; Tn1000, X60200; Tn1412, L36547; Tn5401, U03554; Tn4556, M29297; IS1071, M65135; Tn4651, X83686; Tn5041, X98999.

1994) and Tn4556 (Siemieniak *et al.*, 1990). Only three transposases were definitely outside the aggregate of the above clusters even when only the more conserved COOH-terminal part of the transposase protein were taken into account. These are a solitary transposase of IS1071 (Nakatsu *et al.*, 1991) and a pair of closely related transposases of Tn5041 and Tn4651 (Fig. 3). Thus, sequence analysis of Tn5041 and Tn4651 transposases puts them on the fringe of the Tn3 family. Yet, Tn5041 and Tn4651 are genuine Tn3 family transposons. Their transposases contain most of the invariant amino acid residues found in other transposases of the Tn3 family including a triad of acidic residues, D-D-E, which are candidates for catalytic residues (Yurieva & Nikiforov, 1996).

Tn5041 encodes a protein showing homology to site-specific recombinases of the integrase family

Typically, transposons of the Tn3 family encode enzymes of the resolvase family which are used for resolution of cointegrates formed during transposition. The exceptions include IS1071 which lacks resolvase (Nakatsu *et al.*, 1991) and Tn4430 and Tn5401, both

	Domain 1	Domain 2
	*	* *
OrfI Tn5041	GAFFRSELVTLEMQY	HSLRRGLATSAHRAGADFLEIKRQGGWRHDGT VH-GYIE
Cre P1	TLL.IA.IARIRVKD	.A.V.A.RDMA...VSIP.MQA...TNVNI.M-N..R
XerD <i>E. coli</i>	TGL.V...G.T.SD	.V..HAF..HLLNH...LRVVQMLL.HSDLS.TQ-I.TH
XerC <i>E. coli</i>	AGL.L...G.DIKH	.K..HSF..HMLESSG.LRGVQELL.HANLS.TQ-I.TH
Int integron	TGM.I..GLQ.RVKD	.T..HSF..ALL.S.Y.IRTVQDLL.HSDVS.TM-I.TH
TnpA Tn554	.GL.IG.VLS.RLED	.M..HTH..QLI.E.W.VAFVQKRL.HA.VQ.TLNT.VH
TnpI Tn4430	TGV.I..ALSIK.ND	.Q..HFFC.N.IEK.FSIH.VAN.A.HSNIH.TL-L.TN

Fig. 4. Integrase-like domains (Argos *et al.*, 1986; Abremski & Hoess, 1992) of a putative resolvase of Tn5041 encoded by *orfI*. OrfI is aligned with its nearest neighbours from the integrase family in the order of closest similarity over the sequences shown. Dots represent bases identical with those in the upper sequence. Dashes represent bases absent in other sequences. Invariant amino acid residues which are believed to be involved in catalysis are marked by asterisks. Accession nos: Cre P1, X03453; XerD *E. coli*, M54884; XerC *E. coli*, P22885; Int of integrons, J02967; TnpA Tn554, X03216; TnpI Tn4430, X07651.

from *Bacillus thuringiensis*, which use integrase-like proteins as resolvases (Mahillon & Lereclus, 1988; Baum, 1994, 1995). Database search revealed no Tn5041 genes encoding resolvase/invertase family proteins but detected that *orfI*, one of the two divergent ORFs located at the left arm of Tn5041, encoded a protein showing homology to phage P1 Cre and *E. coli* XerD proteins. These proteins are well known representatives of a large family of integrases involved in various site-specific recombination processes (Nash, 1996). Although the members of the integrase family exhibit a large diversity in their sequences, they share four invariant amino acid residues, the RHRY tetrad, in two characteristic regions, named domain 1 and domain 2 (Argos *et al.*, 1986; Abremski & Hoess, 1992). The tetrad is believed to be involved in catalysis (Chen *et al.*, 1992). The predicted OrfI protein of Tn5041 shows 28–34% similarity to its nearest neighbours (including phage, chromosomal, integron and transposon integrases) in the two conserved domains (Fig. 4); similarity to more distant members of the family is about 20%. Tn5041 OrfI contains all of the RHRY tetrad residues in the proper positions (Fig. 4). On this basis we suggest that *orfI* encodes a functional protein involved in recombination, most probably in the resolution of cointegrates formed during transposition of Tn5041. Identification of an integrase-like OrfI protein encoded by Tn5041 suggests that integrase-like resolvases are not unique to transposons of *B. thuringiensis*. Direct comparison of resolution systems of Tn5041 and its relative, Tn4651, is not possible since Tn4651 resolution genes mapped by Tsuda *et al.* (1989) are not yet sequenced. Our Southern hybridization experiments revealed no homology of Tn5041 *orfI* or divergently transcribed *orfQ* of unknown function with a toluene degradative plasmid pWWO which contains Tn4651 (data not shown). This demonstrates that the resolution systems of Tn5041 and Tn4651 are not as closely related as their IRs and transposases.

The sites of action of site-specific recombinases are often located upstream of their genes and comprise a functional core composed of a segment, called the overlap region, flanked by inverted repeats. For different members of the integrase family, the overlap region is

6–8 bp long while the repeats are 10–12 bp long. The invertases recognize the inverted half-sites and cleave and exchange DNA strands via the transient formation of a covalent linkage of the conserved Tyr residue with the 3' ends flanking the overlap region (for review see Nash, 1996). Inspection of the sequence upstream of *orfI* revealed a 34 bp non-perfect palindromic sequence as a candidate functional core of the Tn5041 resolution site (Fig. 1). This putative site has a striking similarity to the *E. coli* chromosomal *dif* site. This suggests that the OrfI-mediated strand cleavage/exchange occur 3' to the ATAA dyad portions (shared with *dif* and boxed in Fig. 1) separated by 6 bp central region. It should be noted that *dif* is operated by two proteins, XerD and XerC, showing 37% overall sequence identity (Blakely *et al.*, 1993), but no such closely related partner of OrfI is encoded by Tn5041. Besides the functional core, the recombination locus may include extra binding sites for the recombinase and/or binding sites for accessory proteins. This is believed to provide additional asymmetry to recombinational loci to ensure directionality of the recombination process (Sherratt *et al.*, 1995). For example, the Tn5041 locus involved in cointegrate resolution contains two extra binding sites for a transposon-encoded integrase-like TnpI protein. These extra sites are represented by direct repeats of single copies of the half-sites of the functional core (Baum, 1995). No extra core half-site copies or obvious binding sites for accessory proteins such as those found in ColE1 *cer* (Stirling *et al.*, 1988) were found around the putative resolution site of Tn5041. Further characterization of the putative resolution locus of Tn5041 requires a biochemical approach.

Tn5041 contains a functional *mer* operon showing 70% sequence similarity to the *mer* operons of Tn21 and Tn501

The central part of Tn5041 is occupied by a well-recognized *mer* operon (Fig. 1) showing 70% similarity to *mer* operons of classical mercury resistance transposons Tn21 and Tn501. The *mer* operon of Tn5041 is preceded by a 24 bp element showing 75% sequence similarity to the outermost part of IRs typical for Tn21-like transposons. As in Tn21-like transposons, the

	10	20	30	40	50	60	70	80	90	
	<-- end of merR									
Tn21	GGGGGCACCTCAGAAAACGGAAAATAAAGCACGCTAAGGCATA-----GCTGACCTTGCCAGGCGCTGCTT-CGCCCTGTAGTG									
pKLH2	...T.GT...TT...G..GTCACCCCGTGACTCCCCCGC..C..TGCA..G..CTTC.T.C...T.T..C...									
Tn5041	...-.G..T.CGC...G.....T..GTTAA.G...A.....T.GT.C..A..C..C..									
pDU1358	..A..G.GTGACC.TCT.ATT.....G..ACT...TCG...A.A...TC.G.ACCA.GC...TCTT.CT.C..T..C..C..									
Tn5053	..A.T.G.....G..GTTC..T..G...A.A...TCCG.AC...GC..TTCTT.CTTC..T..C...									
Tn501	...T.GT.....C..A.C...A..T...C..A.....									
	<----- 38-bp IR -----									
	101	111	121	131	141	151	161	171	181	191
Tn21	ACGCGATCAACGGGCAGGAACATTCCCTTTTCGTGTCATGGCAGGCGCACACGAGTTTCAGACAGCAGCGGTTTCCATGCGCGCCAAGTCGGCCATCTTCTC									
pKLH2	...A...G.....G.....T..C..C.....TC.....CC.....T.....									
Tn5041	..AA.....G...A.....G...G.GG.TC...CAA...T..TGA..C...GCTC..AA..T.AC..G..C..TCG..GA..C..G.G...GC..									
pDU1358	..A..A...G.....A.....G.....T.GC..C.....A...A...G..T.....G.....T...T..T..									
Tn5053	...A...G.....G.....C..C.....C.AA.....CC.....G...A.....C....									
Tn501	G.....G.....C...C.....C.AC.....CC.....G...A...T....									

Fig. 5. Sequence alignment of the beginning of the *mer* operon of Tn5041 with the *mer* operons from various Gram-negative bacteria. The inverted repeats (IRs) are underlined. Note that the IRs of Tn501 and Tn5053 shown are located within the corresponding transposons (Grinsted & Brown, 1984; Kholodii *et al.*, 1993). The stop codon position and direction of translation of *merR* gene of Tn21 is shown by arrow. Dots represent bases identical with those in the upper sequence. Dashes represent bases absent in other sequences. Accession nos: Tn21, J01730; pKLH2, L04303; pDU1358, M24940; Tn5053, L40585; Tn501, X03406.

truncated IR is fused to a regulatory gene *merR* (Fig. 5). The *merR* gene is followed by the structural genes *merT*, *P* and *C* which control transport of mercury ions and *merA* which encodes mercury reductase. Then follows an ORF, *orfY*. In this position, some *mer* operons contain a *merB* gene which encodes organomercurial lyase that confers resistance to organomercurials (Misra, 1992). However *orfY* shows no homology to known *merB* (Reniero *et al.*, 1995) or any other genes. Moreover, our experiments have demonstrated that the *mer* operon of Tn5041 does not confer organomercurial resistance (for details see Methods). Thus the function of *orfY* remains obscure. This is then followed by *merD* (which is thought to be involved in the fine regulation of *mer* operons) and two ORFs, *urf-1* and *urf-2*, of unknown function. The latter contains a large deletion compared with Tn501.

Tn5041 contains a 4 kb region with relics of mobile elements and shortened genes

The 4 kb region between *orfQ* and the *mer* operon of Tn5041 contains traces of multiple rearrangements resulting in formation of apparently non functional genes and mobile elements. One such element ($\kappa\gamma$ in Fig. 1) is bound by 38 bp non-perfect IRs which neatly fall into the family of IRs of the Tn3 family (Fig. 2b). We noticed that IRs of $\kappa\gamma$ are closely related to the 38 bp sequence (*res501* in Fig. 2b) which is located between the end of the *resIII* site and the start codon of *tnpR* in Tn501 and Tn1721 but is absent in Tn21 and Tn3926. By searching nucleotide sequence databases we have identified previously unnoticed solitary 38 bp elements closely related to IRs of $\kappa\gamma$ in the vicinity of the *avrB* gene of *Pseudomonas syringae* (Tamaki *et al.*, 1988) and *mkaD* (*mkfA*) gene found in virulence plasmids of numerous *Salmonella* strains (Taira & Rhen, 1990; Matsui *et al.*, 1990). Another defective element ($\sigma\lambda$ in Fig. 1) is a truncated copy of an IS element showing 60.6% nucleotide sequence similarity to IS2 and 59.4%

similarity to ISRm1 (Watson & Wheatcroft, 1991). A truncated copy of *merR* (*R'* in Fig. 1) showing 94.3% nucleotide sequence similarity to the functional copy *merR* of Tn5041 is located between $\sigma\lambda$ and $\kappa\gamma$ (Fig. 1). Traces of two membrane protein genes can be pinpointed in the 4 kb region. One of these is represented by an ORF, *orfP*, with a reasonable Shine-Dalgarno sequence (Fig. 1). The predicted translation product shows about 30% sequence similarity to a central segment of *P. aeruginosa* porins D2 and E1. No specific functions have been ascribed to this segment in the porin molecule (Yamano *et al.*, 1993). Relics of another putative membrane protein gene are represented by four short reading frames. Segments of proteins translated from these frames show similarity to different segments of a rat integral membrane protein described by Hallberg *et al.* (1993) (Fig. 1). These speculations can be confirmed only by finding more closely related functional bacterial porins.

Tn5041 is a chimera composed of several modules that have different origins

In conclusion, our sequence analysis demonstrates that both the *mer* operon module and the transposition module of Tn5041 show relationships to known mobile elements. The *mer* operon module of Tn5041 displays 70% nucleotide sequence similarity to the *mer* operons of the well-known Tn21-like transposons and carries 3' to the stop codon of its *merR* a truncated copy of IR typical of these transposons. The transposase gene and IRs of Tn5041 are very similar to those of a toluene degradative transposon, Tn4651, but are very distantly related to those of Tn21-like transposons. Accordingly, we consider Tn5041 as a chimera which obtained its *mer* operon as a result of insertion of a transposon belonging to Tn21 subgroup into a transposon related to Tn4651 followed by deletion of primary (Tn21-type) trans-

position genes. Similar insertion-deletion events occurred during the genesis of quite different mercury resistance transposons, Tn5053 (Kholodii *et al.*, 1993, 1995) and Tn501 (Grinsted & Brown, 1984). It seems that relays of transposition modules from the primary Tn21-like transposons to various other types is a common feature of the lifestyle of *mer* operons. Besides the *mer* operon and putative transposition genes, Tn5041 contains a 4 kb region of unknown origin and significance which accommodates a number of apparently defective mobile elements. This suggests that Tn5041-like transposition modules were used as vehicles by numerous passenger genes other than mercury resistance or toluene degradative operons.

ACKNOWLEDGEMENTS

This work was supported in part by the Russian State Program Frontiers in Genetics, and National Institutes of Health FIRCA Grant TW00396-02. We thank Dr R. Novick for support, Dr N. Brown and two anonymous referees for comments and helpful suggestions, and Drs K. N. Timmis and J. Grinsted for gifts of plasmids.

REFERENCES

- Abremski, K. E. & Hoess, R. H. (1992). Evidence for a second conserved arginine residue in the integrase family of recombination proteins. *Protein Eng* 5, 87–91.
- Altschul, S. F., Gish, W., Miller, W., Myers, E. W. & Lipman, D. J. (1990). Basic local alignment search tool. *J Mol Biol* 215, 403–410.
- Amuthan, G. & Mahadevan, A. (1994). Replicon typing of plasmids of phytopathogenic xanthomonads. *Plasmid* 32, 328–332.
- An, F. Y. & Clewell, D. B. (1991). Tn917 transposase: sequence correction reveals a single open reading frame corresponding to the *tnpA* determinant of Tn3-family elements. *Plasmid* 25, 121–124.
- Argos, P., Landy, P., Abremski, K., Egan, J. B., Haggard-Ljungquist, E., Hoess, R. H., Kahn, M. L., Kalionis, B., Narayana, S. V. L., Pierson, L. S., III, Sternberg, N. & Leong, J. M. (1986). The integrase family of site-specific recombinases: regional similarities and global diversity. *EMBO J* 5, 433–440.
- Arthur, M., Molinas, C., Depardieu, F. & Courvalin, P. (1993). Characterization of Tn1546, a Tn3-related transposon conferring glycopeptide resistance by synthesis of depsipeptide peptidoglycan precursors in *Enterococcus faecium* BM4147. *J Bacteriol* 175, 117–127.
- Barton, B. M., Hardling, G. P. & Zuccarelli, A. J. (1995). A general method for detecting and sizing large plasmids. *Anal Biochem* 226, 235–240.
- Baum, J. A. (1994). Tn5401, a new class II transposable element from *Bacillus thuringiensis*. *J Bacteriol* 176, 2835–2845.
- Baum, J. A. (1995). TnpI recombinase: identification of sites within Tn5401 required for TnpI binding and site-specific recombination. *J Bacteriol* 177, 4036–4042.
- Birnboim, H. & Doly, J. (1979). A rapid alkaline extraction procedure for screening recombinant plasmid DNA. *Nucleic Acids Res* 7, 1513–1523.
- Blakely, G., May, G., McCulloch, R., Arciszewska, L. K., Burke, M., Lovett, S. T. & Sherratt, D. J. (1993). Two related recombinases are required for site-specific recombination at *dif* and *cer* in *E. coli* K12. *Cell* 75, 351–361.
- Bogdanova, E. S., Mindlin, S. Z., Kalyaeva, E. S. & Nikiforov, V. G. (1988). The diversity of mercury reductases among mercury-resistant bacteria. *FEBS Lett* 234, 280–282.
- Brown, H. J., Stokes, H. W. & Hall, R. M. (1996). The integrons In0, In2, and In5 are defective transposon derivatives. *J Bacteriol* 178, 4429–4437.
- Chen, J.-W., Lee, J. & Jayaram, M. (1992). DNA cleavage in trans by the active site tyrosine during Flp recombination: switching protein partners before exchanging strands. *Cell* 69, 647–658.
- Chiou, C.-S. & Jones, A. L. (1993). Nucleotide sequence analysis of a transposon (Tn5393) carrying streptomycin resistance genes in *Erwinia amylovora* and other gram-negative bacteria. *J Bacteriol* 175, 732–740.
- Eckhardt, T. (1978). A rapid method for identification of plasmid desoxyribonucleic acid in bacteria. *Plasmid* 1, 584–588.
- Elhai, J., Cai, Y. & Wolk, P. C. (1994). Conduction of pEC22, a plasmid coding for MR. *EcoT221*, mediated by a resident Tn3-like transposon, Tn5396. *J Bacteriol* 176, 5059–5067.
- Friello, D. A. & Chakrabarty, A. M. (1980). Transposable mercury resistance in *Pseudomonas putida*. In *Plasmids and Transposons: Environmental Effects and Maintenance Mechanisms*, pp. 249–259. Edited by C. Stuard & K. R. Roze. New York: Academic Press.
- Grinsted, J. & Brown, N. L. (1984). A Tn21 terminal sequence within Tn501: complementation of *tnpA* gene function and transposon evolution. *Mol Gen Genet* 197, 497–502.
- Grinsted, J., de la Cruz, F., Altenbuchner, J. & Schmitt, R. (1982). Complementation of transposition of *tnpA* mutants of Tn3, Tn21, Tn501, and Tn1721. *Plasmid* 8, 276–286.
- Grinsted, J., de la Cruz, F. & Schmitt, R. (1990). The Tn21 subgroup of bacterial transposable elements. *Plasmid* 24, 163–189.
- Hallberg, E., Wozniak, R. W. & Blobel, G. (1993). An integral membrane protein of the pore membrane domain of the nuclear envelope contains a nucleoporin-like region. *J Cell Biol* 122, 513–521.
- Ivey, D. M., Guffanti, A. A., Shen, Z., Kudyan, N. & Krulwich, T. A. (1992). The *cadC* gene product of alkaliphilic *Bacillus firmus* OF4 partially restores Na⁺ resistance to an *Escherichia coli* strain lacking an Na⁺/H⁺ antiporter (NhaA). *J Bacteriol* 174, 4878–4884.
- Kelly, W. J. & Reanney, D. C. (1984). Mercury resistance among soil bacteria: ecology and transferability of genes encoding resistance. *Soil Biol Biochem* 16, 1–8.
- Khesin, R. B. & Karasyova, E. V. (1984). Mercury-resistant plasmids in bacteria from a mercury and antimony deposit area. *Mol Gen Genet* 197, 280–285.
- Kholodii, G., Yurieva, O., Lomovskaya, O., Gorlenko, Zh., Mindlin, S. & Nikiforov, V. (1993). Tn5053, a mercury resistance transposon with integron's ends. *J Mol Biol* 230, 1103–1107.
- Kholodii, G. Ya., Mindlin, S. Z., Bass, I. A., Yurieva, O. V., Minakhina, S. V. & Nikiforov, V. G. (1995). Four genes, two ends, and a *res* region are involved in transposition of Tn5053: a paradigm for a novel family of transposons carrying either a *mer* operon or an integron. *Mol Microbiol* 17, 1189–1200.
- Lebrun, M., Audurier, A. & Cossart, P. (1994). Plasmid-borne cadmium resistance genes in *Listeria monocytogenes* are present on Tn5422, a novel transposon closely related to Tn917. *J Bacteriol* 176, 3049–3061.
- Lett, M.-C., Bennett, P. M. & Vidon, D. J.-M. (1985). Characterization of Tn3926, a new mercury-resistance transposon from *Yersinia enterocolitica*. *Gene* 40, 79–91.

- Mahillon, J. & Lereclus, D. (1988).** Structural and functional analysis of Tn4430: identification of an integrase-like protein involved in the cointegrate-resolution process. *EMBO J* 7, 1515–1526.
- Mahler, I., Levinson, H. S., Wang, Y. & Halvorson, H. O. (1986).** Cadmium- and mercury-resistant *Bacillus* strains from a salt marsh and from Boston Harbor. *Appl Environ Microbiol* 52, 1293–1298.
- Matsui, H., Kawahara, K., Terakado, N. & Danbara, H. (1990).** Nucleotide sequence of a gene encoding a 29 kDa polypeptide in mba region of the virulence plasmid, pKDSC50, of *Salmonella choleraesuis*. *Nucleic Acids Res* 18, 1055.
- Misra, T. K. (1992).** Bacterial resistances to inorganic mercury salts and organomercurials. *Plasmid* 27, 4–16.
- Nakatsu, C., Ng, J., Singh, R., Straus, N. & Wyndham, C. (1991).** Chlorobenzoate catabolic transposon Tn5271 is a composite class I element with flanking class II insertion sequences. *Proc Natl Acad Sci USA* 88, 8312–8316.
- Nash, H. A. (1996).** Site-specific recombination: integration, excision, resolution, and inversion of defined DNA segments. In *Escherichia coli and Salmonella typhimurium: Cellular and Molecular Biology*, 2nd edn, vol. 2, pp. 2363–2376. Edited by F. C. Neidhardt and others. Washington, DC: American Society for Microbiology.
- Osourn, S. E. V., Turner, A. K. & Grinsted, J. (1995).** Nucleotide sequence within Tn3926 confirms this as Tn21-like transposable element and provides evidence for the origin of the *mer* operon carried by plasmid pKLH2. *Plasmid* 33, 65–69.
- Pansegrau, W., Lanka, E., Barth, P. T., Figurski, D. H., Guiney, D. G., Haas, D., Helinski, D. R., Schwab, H., Stanisich, V. A. & Thomas, C. M. (1994).** Complete nucleotide sequence of Birmingham IncP plasmids. Compilation and comparative analysis. *J Mol Biol* 239, 623–663.
- Radford, A. J., Oliver, J., Kelly, W. J. & Reaney, D. C. (1981).** Translocatable resistance to mercuric and phenylmercuric ions in soil bacteria. *J Bacteriol* 147, 1110–1112.
- Reniero, D., Galli, E. & Barbieri, P. (1995).** Cloning and comparison of mercury- and organomercurial resistance determinants from a *Pseudomonas stutzeri* plasmid. *Gene* 166, 77–82.
- Sambrook, J., Fritsch, E. F. & Maniatis, T. (1989).** *Molecular Cloning: a Laboratory Manual*, 2nd edn. Cold Spring Harbor, NY: Cold Spring Harbor Laboratory.
- Schottel, J., Mandal, A., Clark, D., Silver, S. & Hedges, R. W. (1974).** Volatilization of mercury and organomercurials determined by inducible R-factor systems in enteric bacteria. *Nature* 251, 335–337.
- Sherratt, D. J., Arciszewska, L. K., Blakely, G., Colloms, S., Grant, K., Leslie, N. & McCulloch, R. (1995).** Site-specific recombination and circular chromosome segregation. *Phil Trans R Soc Lond B* 347, 37–42.
- Shiratori, T., Inoue, C., Sugawara, K., Kusano, T. & Kitagawa, Y. (1989).** Cloning and expression of *Thiobacillus ferrooxidans* mercury ion resistance genes in *Escherichia coli*. *J Bacteriol* 171, 3458–3464.
- Siemieniak, D. R., Slightom, J. L. & Chung, S.-T. (1990).** Nucleotide sequence of *Streptomyces fradiae* transposable element Tn4556: a class-II transposon related to Tn3. *Gene* 86, 1–9.
- Silver, S. & Walderhaug, M. (1992).** Gene regulation of plasmid- and chromosome-determined inorganic ion transport in bacteria. *Microbiol Rev* 56, 195–228.
- Smith, C. A. & Thomas, C. M. (1987).** Comparison of the organization of the genomes of phenotypically diverse plasmids of incompatibility group P: members of the IncP β subgroup are closely related. *Mol Gen Genet* 206, 419–427.
- Stanisich, V. A., Bennett, P. M. & Richmond, M. H. (1977).** Characterization of a translocation unit encoding resistance to mercuric ions that occurs on a nonconjugative plasmid in *Pseudomonas aeruginosa*. *J Bacteriol* 129, 1227–1233.
- Stanisich, V. A., Arwas, R., Bennett, P. M. & de la Cruz, F. (1989).** Characterization of *Pseudomonas* mercury-resistance transposon Tn502, which has a preferred insertion site in RP1. *J Gen Microbiol* 135, 2909–2915.
- Stirling, C. J., Sztmari, G., Stewart, G., Smith, M. C. M. & Sherratt, D. J. (1988).** The arginine repressor is essential for plasmid-stabilizing site-specific recombination at the ColE1 *cer* locus. *EMBO J* 7, 4389–4395.
- Taira, S. & Rhen, M. (1990).** Nucleotide sequence of *mkaD*, a virulence-associated gene of *Salmonella typhimurium* containing variable and constant regions. *Gene* 93, 147–150.
- Tamaki, S., Dahlbeck, D., Staskawicz, B. & Keen, N. T. (1988).** Characterization and expression of two avirulence genes cloned from *Pseudomonas syringae* pv. *glycinea*. *J Bacteriol* 170, 4846–4854.
- Tsuda, M., Minegishi, K.-I. & Iino, T. (1989).** Toluene transposons Tn4651 and Tn4653 are class II transposons. *J Bacteriol* 171, 1386–1393.
- Turner, A. K. & Grinsted, J. (1987).** DNA sequence of the transposase gene of the new category of class II transposon, Tn2501. *Nucleic Acids Res* 15, 10049.
- Ulrich, A. & Puehler, A. (1994).** The new class II transposon Tn163 is plasmid-borne in two unrelated *Rhizobium leguminosarum* biovar *viciae* strains. *Mol Gen Genet* 242, 505–516.
- Wang, Y., Mahler, I., Levinson, H. S. & Halvorson, H. O. (1987).** Cloning and expression in *Escherichia coli* of chromosomal mercury resistance genes from a *Bacillus* sp. *J Bacteriol* 169, 4848–4851.
- Watson, R. & Wheatcroft, R. (1991).** Nucleotide sequence of *Rhizobium meliloti* insertion sequence ISRm1: homology to IS2 from *Escherichia coli* and IS426 from *Agrobacterium tumefaciens*. *DNA Seq* 2, 163–172.
- Witte, W., Green, L., Misra, T. K. & Silver, S. (1986).** Resistance to mercury and to cadmium in chromosomally resistant *Staphylococcus aureus*. *Antimicrob Agents Chemother* 29, 663–669.
- Womble, D. D. & Rownd, R. H. (1988).** Genetic and physical map of plasmid NR1: comparison with other IncFII antibiotic resistance plasmids. *Microbiol Rev* 52, 433–451.
- Yamano, Y., Nishikawa, T. & Komatsu, Y. (1993).** Cloning and nucleotide sequence of anaerobically induced porin protein E1 (OprE) of *Pseudomonas aeruginosa* PAO1. *Mol Microbiol* 8, 993–1004.
- Yurieva, O. & Nikiforov, V. (1996).** Catalytic center quest: comparison of transposases belonging to the Tn3 family reveals an invariant triad of acidic amino acid residues. *Biochem Mol Biol Int* 38, 15–20.
- Zharkikh, A. A., Rzhetsky, A. Yu., Morosov, P. S., Sitnikova, T. L. & Krushkal, J. S. (1991).** VOSTORG: a package of microcomputer programs for sequence analysis and construction of phylogenetic trees. *Gene* 101, 251–254.

Received 20 November 1996; revised 10 March 1997; accepted 15 April 1997.