Mobilization of a Tn*402*-Like Class 1 Integron with a Novel Cassette Array via Flanking Miniature Inverted-Repeat Transposable Element-Like Structures[▽]

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A Tn*402*-like class 1 integron was recovered from a prawn-associated bacterium. One of its cassettes included methionine sulfoxide reductase genes, the first example of such genes being captured by an integron. The integron was flanked by direct repeats that resemble miniature inverted-repeat transposable element sequences. Excision of the integron by homologous recombination through these sequences was demonstrated.

Integrons possess a site-specific recombination system that promotes dissemination of mobile gene cassettes (8, 17). About 3% of cells in the general environment contain class 1 integrons, and this integron class is broadly disseminated by lateral gene transfer (1, 6). A subset of class 1 integrons is associated with transposition functions exemplified by transposon Tn*402*, and they are commonly recovered from clinical environments. The Tn*402*-like class 1 integrons have a number of characteristic features, including two conserved segments (CS) that flank the site where mobile gene cassettes are inserted (23) (Fig. 1). The ongoing accumulation of resistance genes in Tn*402*-like integrons makes this integron subtype one of the largest contributors to the spread of multidrug resistance in human pathogens (8, 14).

Tn*402*-like class 1 integrons are also common in commensal bacteria, including those found in humans, and are spreading back into environmental bacteria (6, 12, 21, 24). Such integrons are then exposed to the highly diverse gene cassette metagenome (11), which includes a variety of potential virulence factors (1, 20). Consequently, environmental Tn*402*-like class 1 integrons may be a conduit for additional genes encoding functions other than antibiotic resistance to make their way into human pathogens. As part of a project investigating the role of animal commensal bacteria in the spread of resistance genes, we screened bacteria from the digestive tracts of prawns for the presence of class 1 integrons. Here we describe the complete DNA sequence of a novel Tn*402*-like integron and its surrounds from an *Acinetobacter* sp. strain isolated from a prawn. Digestive tracts from uncooked prawns were used to separately inoculate 5 ml plate count medium (23) and incubated for 24 h at 25°C. Four prawns, harvested from each of 16 distinct locations from Australia, the South Pacific, and Southeast Asia,

were examined. More than 75% of mixed cultures from individual prawns tested positive for class 1 integrons with use of the primers HS915 and HS916 (15). PCR with these primers on pure isolates derived from such mixed cultures revealed that about 3% of 850 colonies were positive for a class 1 integron, a number consistent with findings of other studies (10, 25). We then employed a PCR method that amplified cassette arrays from Tn*402*-like class 1 integrons (13) using the primers HS458 and HS459 (10) (Fig. 1). Sequencing of one of the HS458/459 amplicons revealed an unusual structure. This unusual integron was recovered from an *Acinetobacter* sp. strain (here designated strain NFM2) isolated from a prawn (*Penaeus plebejus*) (19) harvested from ocean waters off the Australian east coast. NFM2 typing was determined by partial 16S rRNA sequencing, with the closest species match being to *Acinetobacter johnsonii*.

A fosmid library of NFM2 was constructed and an *intI1*-positive clone completely sequenced using methods previously described (23). A physical map of the relevant region is shown in Fig. 1. The integron possessed features typical of Tn*402*-derived class 1 integrons, including the presence of a 3′ CS that abutted a truncated *tni* module. However, the integron was flanked by identical copies of a 439-bp direct repeat, sequence comparisons of which are suggestive of them being miniature inverted repeat transposable elements (MITEs), a family of nonautonomous mobile elements (4). MITEs are small, nonautonomous mobile elements broadly dispersed in prokaryotes that have only recently been described (4). These elements are diverse in their sequence and properties but generally include several of the following characteristics: (i) a target site duplication, (ii) short terminal inverted repeats, (iii) high A/T content, (iv) "TA" motif at each of the termini, and (v) binding sites for host integrative factors and methyltransferases. The MITE-like elements in NFM2 include the first three of these (73% A/T). They also include at least three methyltransferase binding sites, although this domain is short (GANTC), so the significance of their presence is unclear. Many MITEs contain open reading frames, and several produce RNA transcripts, the associated secondary structure of which regulates mRNA

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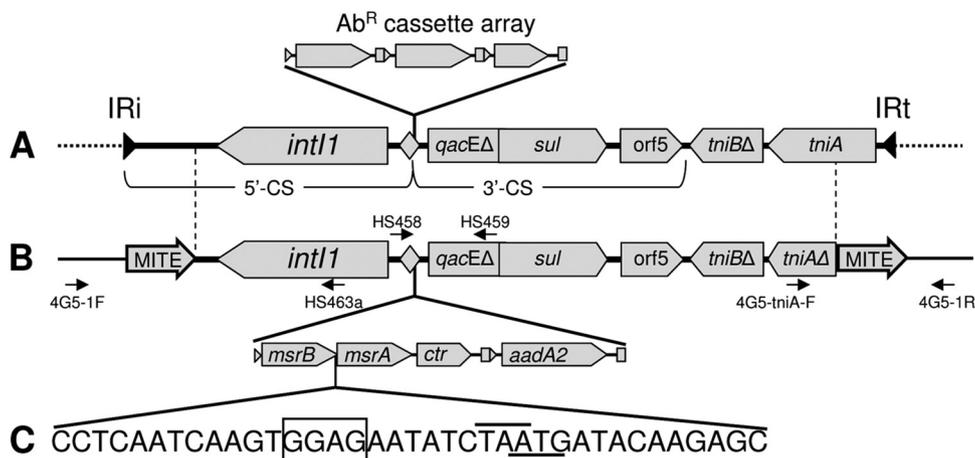
FIG. 1. Features of Tn*402*-like class 1 integrons. (A) Generalized structure of a Tn*402*-like class 1 integron as commonly recovered from multidrug-resistant pathogens. Black filled horizontal arrows indicate the inverted repeats IRi and IRt, which define the ends of Tn*402*-like transposons. The gray-shaded diamond represents the *attI1* site, at which mobile cassettes are inserted. Ab[R], antibiotic resistance. A three-cassette array is depicted here, but the type and number of cassettes are variable. The extents of the 5′ CS) and 3′ CS are indicated. (B) The vertical dashed lines define the extent of the sequence of the NFM2 integron common to that of a typical Tn*402*-like class 1 integron. The open diamond represents the *attI1* site with the structure of the two-cassette array in the NFM2 integron indicated immediately below. The small horizontal arrows indicate the relative positions of primers HS458 and HS459 and primers used to assess excision of the integron by homologous recombination between the MITE elements. (C) The sequence of the region at the end of *msrB* and the start of *msrA*. Overlined bases are the stop codon for *msrB* and the underlined bases the start codon of *msrA*. The boxed sequence is a putative ribosome binding site for *msrA*. Abbreviations: *intI1*, class 1 integron integrase gene; *qacEΔ1*, remnant of quaternary ammonium compound resistance gene; *sul*, sulfonamide resistance gene; orf5, open reading frame encoding unknown function; *tniA* and *tniB*, Tn*402*-associated transposition genes ("Δ" symbol indicates partial gene deletion). See the text for other abbreviations. The figure is not necessarily drawn to scale.

(3). The MITEs found in NFM2 do not possess obvious open reading frames. NFM2 MITE transcripts, if produced, would be likely to possess extensive secondary structures; however, we have no information as to the role, if any, in mRNA regulation.

The outer ends of the two MITE direct repeats were in turn flanked by a 5-bp direct repeat (GTTGC). We hypothesize that the MITE sequences captured the class 1 integron in a structure analogous to a composite transposon, and this has subsequently transposed to its present location. The points of insertion of the MITE-like elements are inside the normal boundary of a Tn*402*-like integron, in that the first 76 bases of the 5′ CS, including IRi, are missing at one end, and IRt and most of *tniA* are missing at the other end (Fig. 1). In silico analysis by us revealed that portions of the MITE-like sequences in NFM2 are also found in sequences derived from several *Acinetobacter* sp. clinical isolates derived from soft tissue and bloodstream infections (see reference 16 and accession numbers therein). These MITE-like examples also flank a class 1 integron at the 5′-CS segment end with the same insertion point as seen here. In addition, it has recently been found that a class 1 integron from *Enterobacter cloacae* is also flanked by MITEs, although these are not related in sequence to those found here (18). In addition, the locations of each of the MITEs at both ends of the respective integrons are different, further suggesting independent capture events.

Transposition of MITEs requires that additional functions be provided in *trans* (4). The presence of a direct repeat implies that the class 1 integron in NFM2 moved into its identified location by transposition. Indeed, transposition of the *Enterobacter cloacae* integron mobilization unit has been demonstrated (18). However, attempts to transpose the class 1

integron from NFM2 into a conjugative plasmid in a mating-out assay with an *Escherichia coli* recipient were unsuccessful (data not shown), suggesting that the required additional transposon functions are not present in this strain. However, the two copies of the MITE-like sequences are identical, and given they are oriented as direct repeats, it seemed highly likely that the integron could be readily excised from the genome by homologous recombination. To test this, PCR primers were designed to detect excision via recombination. The position of these primers is indicated in Fig. 1. Primers 4G5-1F (5′ CCC ACACAATAAACGCCG) and 4G5-1R (5′ TGGCGATGGC TCAATGTC) should generate a product of 819 bp, comprising a single MITE-like sequence remaining in the genome if the integron is deleted. Primers HS463a (11) and 4G5-tniA-F (5′ TGCGACAAGGTACGGTAGG) should recover a 1,231-bp sequence that includes the junction of the circularized, excised product. A PCR with each of these primer pairs on NFM2 genomic DNA generated PCR products of the predicted lengths (data not shown). DNA sequencing of each PCR product revealed boundaries consistent with excision via homologous recombination from a point within the MITE-like sequences.

The NFM2 integron carried two cassettes, the second of which was an *aadA2* gene cassette commonly seen in Tn*402*-like class 1 integrons. This was consistent with the fact that NFM2 was resistant to streptomycin (25 μg/ml). The first cassette was unusual for a number of reasons. It was 1,874 bases in length and included three genes. These are probably contained in a single transcript and in order are *msrB* (best match; 78% protein product identity to *Xanthobacter autotrophicus* Py2 [accession no. CP000781]), *msrA* (83% protein product identity to *Rhodopirellula baltica* SH 1 [accession no. BX294154]),

and a gene encoding a hypothetical protein (*orf255*). *msrA* and *msrB* encode methionine sulfoxide reductases, involved in the repair of proteins damaged by oxidative stress (7). The A and B forms reduce alternative isomers of methionine sulfoxide (5). The stop codon of *msrB* overlaps the start codon of *msrA* by one base (Fig. 1). The presence of a ribosome binding site immediately upstream of *msrA* suggests that this gene is translated by the process of translational coupling (9). This is the first report of *msr*-related genes being located in a mobile gene cassette. The *attC* site associated with this unusual cassette is 71 bases in length and conforms to all the criteria associated with this family of sites, including an overall imperfect repeat structure and two simple sites with the conserved domains 1R, 2R, 2L, and 1L (22). The predicted circular form of the *msr* cassette would have complementary 1R and 1L sites. We could not identify an obvious promoter in this *attC* site, so it is likely that the *aadA2* gene is expressed from the integron promoter, P_c.

Most Tn*402*-like class 1 integrons are defective transposons (2), although mobilization by transposition can still occur if the required proteins are provided in *trans*. The requirement for exogenous components limits mobilization in comparison to autonomous elements. The element found here and that seen in an *Enterobacter cloacae* isolate (18) represent two independent examples whereby class 1 integrons may be mobilized by families of elements unrelated to Tn*402*. The fact that one of these is from a clinical isolate and the other from a marine invertebrate further underscores the potential for mobilization of gene cassettes between human pathogens and bacteria found in the general environment. Finally, the NFM2 integron has an unusual cassette in that it includes three genes that may be implicated in a phenotype unrelated to the neutralization of antibiotics. This suggests that Tn*402*-like class 1 integrons are beginning to recruit new types of functions in some bacteria.

Nucleotide sequence accession numbers. The integron sequence described here is contained within the fosmid clone 4G5 and is deposited in GenBank under accession number FJ711439. Accession number GQ377756 contains the 16S rRNA sequence from NFM2.

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