Origin of the AbGRI1 antibiotic resistance island found in the *comM* gene of *Acinetobacter baumannii* GC2 isolates

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Sir,

Multiply, extensively and pan resistant isolates of *Acinetobacter baumannii* are predominantly members of two globally distributed clones, GC1 and GC2. By the mid to late 1970s, these clones had acquired resistance to the antibiotics in use at the time, and the resistance genes were subsequently found to be located in resistance islands in the chromosome, an AbaR-type island in the GC1 clone, and AbGRI1-type and AbGRI2-type islands in GC2 isolates. These islands are present in the earliest GC1 and GC2 isolates still available for study.[1–3] The AbaR and AbGRI1 islands are both located in the *comM* gene, as they are each complex class III transposons with a set of *tniC–tniA–tniB–tniD–tniE* transposition genes that target this location. The resistance genes in AbaR and AbGRI2 islands were recently found to originate from a complex resistance gene cluster in the IncM1 plasmid R1215.[4] Here, we have explored the origin of the AbGRI1 island.

Various forms of the AbGRI1-type island have been found[5–8] and each can be derived from a common ancestor with a structure resembling that shown in Figure 1(a). The wide variety of other forms seen to date can be derived from AbGRI1-0 via addition or loss of additional segments or transposons. For example, a deletion within Tn*6022* gives rise to the commonly detected deletion derivative Tn*6022*D, or the *tet*(B) determinant can be added in via homologous recombination between CR2 in the AbGRI1 and a partial copy of CR2 adjacent to the *tet*(B) determinant. These two events give rise to AbGRI1-Var (Figure 1a). Subsequent introduction of Tn*6022* into the extreme end of the *tetA*(B) gene provides a third *tni* transposon[8] and deletions via homologous recombination between the *tni* genes in this transposon and one of the transposons, Tn*6022* or Tn*6022*D, found at the left end can cause loss of the central segment and the *sul2* gene to give rise to the simpler structure seen in AbGRI1-1 (Tn*6166*) in A320.[5] The Tn*6022* copies can acquire the *oxa23* carbapenem resistance transposon Tn*2006* to form AbaR4 (Tn*6022*::Tn*2006*) and additional antibiotic resistance genes have occasionally been introduced into the region containing the original resistance genes, located near the right end.[5]

We recently found the right-hand defective transposon, Tn*6172*, in a novel type of potentially conjugative plasmid exemplifi by pD4[9] and pA297-3[10] and the adjacent sequence matched most of the central portion of the AbGRI1 ancestor (6055/6205 bp). Our current analysis revealed that a related transposon, here designated Tn*6174*, is also present in the same location in pAB3, a plasmid found recently in ATCC 17978 (GenBank accession number CP012005).[11] Tn*6174* has a transposon backbone (hypothetical transposon Tn*6173*), with a complete set of transposition genes (*tniC–tniA–tniB–tniD–tniE*) and a copy of orf4, that encode proteins 83%–97% identical to the products of the corresponding genes in Tn*6022*, as well as several additional genes (Figures 1 and 2). However, the *tniD* gene is interrupted by a copy of GI*sul2* with ISAba1 upstream of the *sul2* gene. GI*sul2* is a genomic island carrying the *sul2* sulphonamide resistance gene, which was first identifi [12] using the genome of ATCC 17978 available at the time.[13] The presence of both GI*sul2* and several fragments of AbGRI1-2 (then called Tn*6167*) was noted[12] but they were found widely dispersed in the chromosome. This is now known to be due to substantial genome assembly errors, as resequencing has shown that these segments are not in the chromosome but in fact belong in the conjugative plasmid pAB3.[11]

An evolutionary path for the generation of the defective transposon Tn*6172* from Tn*6173* via Tn*6174* can now be proposed as shown in Figure 2(a). An incoming Tn*5393* brought in the *strAB* genes and a recombination event caused deletion of most of GI*sul2* and some of the *tni* genes. However, there is little sequence homology between the sequences giving rise to the deletion (Figure 2b) and the mechanism for this event is unknown.

We noted previously that the segments next to the *tniC* end of Tn*6172* in the plasmid pD4 (GenBank accession number KT779035) and in AbGRI1 are closely related.[9] However, the identity first fell from 99.9% to 95% identity and ended about 150 bp before the orf4 end of Tn*6022* (or Tn*6022*D) at the left end of AbGRI1 (Figure 1b). This dilemma was resolved by the finding that the missing 150 bp are in pAB3 in this location and indeed a copy of Tn*6021* is located in precisely the same position as Tn*6022* in AbGRI1. Tn*6021* is a close relative of Tn*6022* originally found in the *comM* gene of ATCC 17978.[14] Tn*6021* differs from Tn*6022* by a short patch of diverged sequence extending from within *tniC* to within *tniA*, and *tniA* includes a copy of ISAba11.[14] Here, this diverged segment was found to be identical to the corresponding segment in Tn*6172*, Tn*6173* and Tn*6174*.

Combining all of this information, it is now possible to propose that the original AbGRI1 island was derived from a close relative of pAB3 with Tn*6022* in place of Tn*6021* and in which Tn*6174* had already evolved to form Tn*6172* (pHypo in Figure 1c). Using the *tni* genes and the IRl of Tn*6022* together with the IRr of Tn*6172*, this segment has transposed as a single unit into the *comM* gene of the ancestor of the GC2 clone. Subsequently, AbGRI1 has evolved *in situ* into the large variety of forms seen in current GC2 isolates,

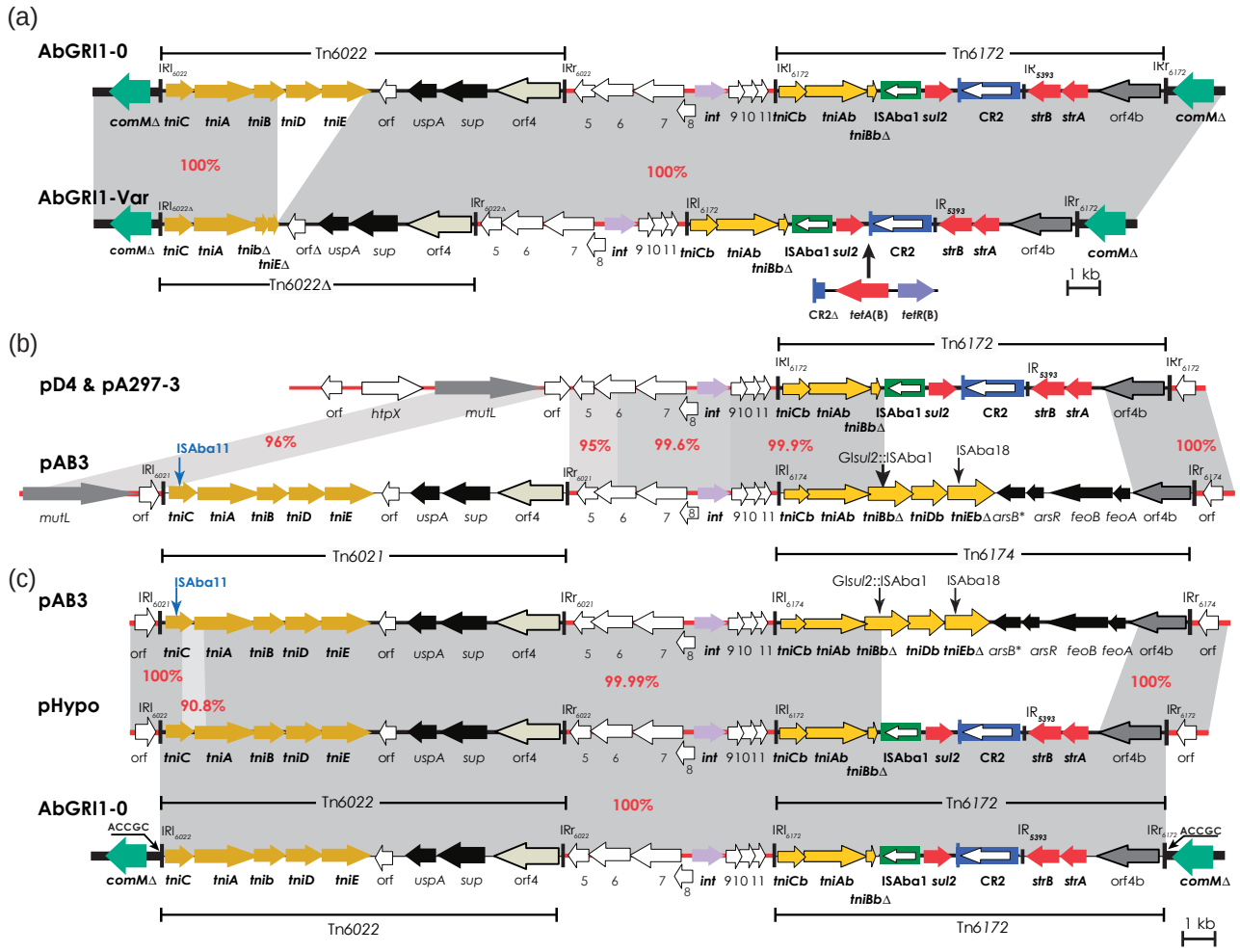

Figure 1. Comparison of AbGRI1 variants and precursors. (a) Comparison of the ancestral form of AbGRI1 islands, AbGRI1-0, and a variant. (b) Comparison of the resistance regions found in pD4, pA297-3 (GenBank accession number KU744946) and pAB3, and (c) evolution of the region in pAB3 that gave rise to AbGRI1-0 in the *comM* gene of GC2 isolates. Red horizontal lines represent plasmid backbones and the chromosome of GC2 strains is represented by a thick horizontal line with the *comM* gene marked on either side of AbGRI1. Arrows represent the extent and orientation of genes or ORFs with their names shown below. Transposition genes of Tn*6021* and Tn*6174* are yellow; resistance genes are red. The extents of Tn*6021*, Tn*6022* and Tn*6174* are shown below and vertical bars at the ends of the elements indicate their IRs. Different shades of grey join regions with significant identities with DNA identities shown by red numbers. ISAba1 and CR2 are shown using a green or blue box with a white arrow indicating the direction of the *tnp* gene. GI*sul2*::ISAba1, ISAba18 and ISAba11 are shown above their location. Figures are to scale and the scale bar is shown. This figure appears in colour in the online version of *JAC* and in black and white in the print version of *JAC*.

some of which have acquired additional antibiotic resistance genes, such as *tet*(B), bla_{PER} or *oxa23* in Tn*2006* or AbaR4.

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Transparency declarations

None to declare.

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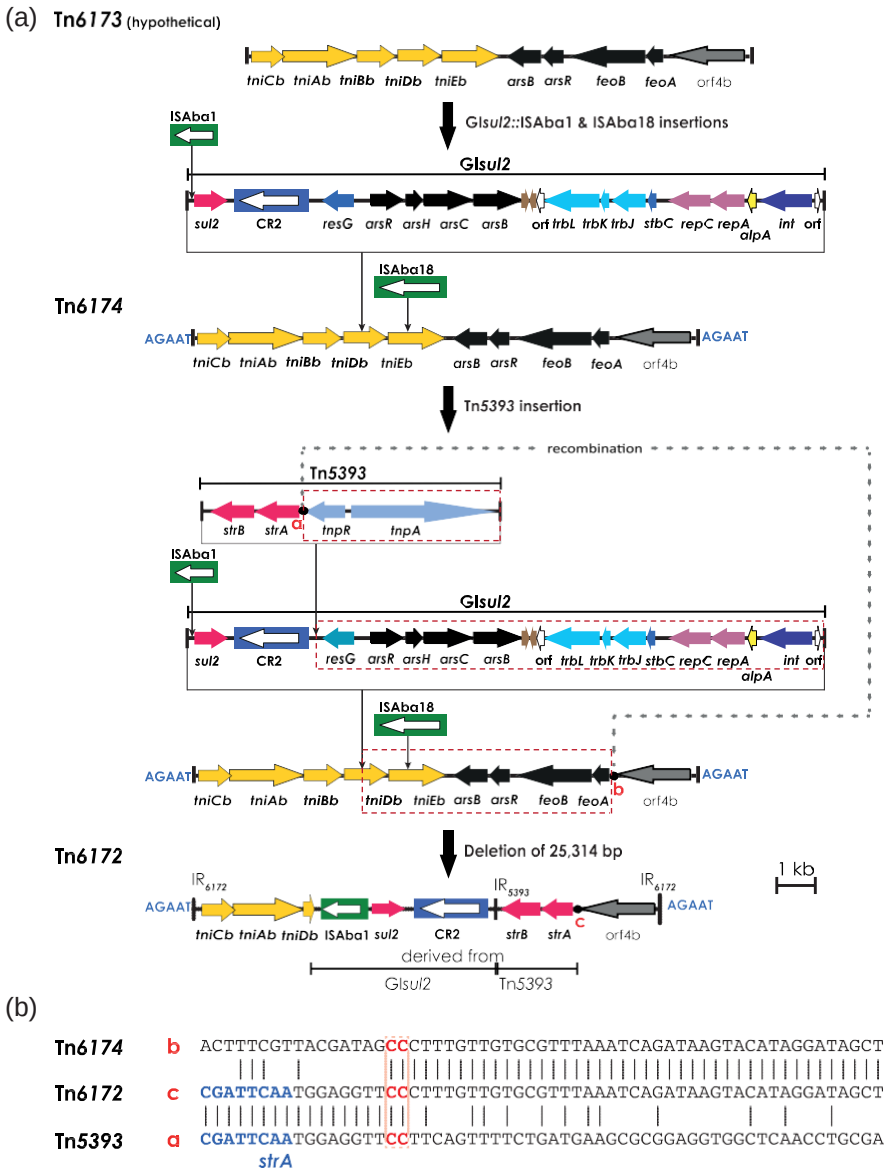

Figure 2. Schematic of the evolution of Tn*6172* from Tn*6173*. (a) Events generating Tn*6172*. Black central lines indicate the backbone of transposons with short vertical lines at each end representing the terminal IRs. Arrows indicate the extent and the direction of genes or ORFs. ISs are shown as filled green boxes with a white arrow indicating the orientation of the transposase gene and the IS name above. Thin vertical arrows pointing to various parts of Tn*6174* and GI*sul2* indicate the insertion point of the element shown above the arrow. The grey line made of small arrows indicates the deletion endpoints and the segments deleted are enclosed by red dotted line boxes. (b) Sequence of the regions involved in the deletion removing large parts of the GI*sul2*, Tn*5393* and Tn*6173*. Sequence lines marked a, b and c correspond to the positions a, b and c indicated by black dots in (a). This figure appears in colour in the online version of *JAC* and in black and white in the print version of *JAC*.

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