An Excision-Competent and Exogenous Mosaic Transposon Harbors the *tetM* Gene in Multiple *Mycoplasma hominis* Lineages

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The presence of the tetracycline resistance determinant *tetM* has been reported for *Mycoplasma hominis* (1, 2) and association with Tn916 genes established (3). To identify the mobile genetic element harboring this gene, genome analysis of *M. hominis* Sprott (isolated from a nongonococcal urethritis patient before 1978 [4]) and targeted PCR/amplicon sequencing of *M. hominis* 1630 (1984, arthritis isolate [5]) were undertaken. These analyses revealed that in both isolates, truncated Tn916 resides within an uncharacterized transposon, closely related to several from streptococci that site-specifically integrate into *rumA* (6, 7). Although Tn916 truncation precludes mobility of that conjugative transposon (CTn), the larger mosaic element retains competency for excision and circularization in *M. hominis*. While this letter was in preparation, this element was also noted in the *M. hominis* placental strain PL5 (isolated between 1991 and 1996 [8]).

Comparison of the Sprott genome sequence (GenBank accession number CP011538 [9]) to those from *M. hominis* LBD-4 (10) and PG21 (11) disclosed a 25,257-nucleotide (nt) insertion, which included a truncated *tetM*-harboring Tn916 unit (13.3 kb). The

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FIG 1 Structure of the *M. hominis* *tetM* mosaic transposon and detection of an extrachromosomal form. (A) Stylized representation of mosaic transposons in the GBS GB0555 and *M. hominis* Sprott. Arrows represent selected genes in the elements or flanking regions. The ~75-kb Tn from GB0555 corresponds to nt 3648 to 78587 in GenBank accession number [ALTN01000021](#). The ~25-kb element in Sprott corresponds to nt 573817 to 599077 in GenBank accession number [CP011538](#). The “empty site” of strain LBD-4 is shown at the bottom. Bars and arrows shown in green correspond to regions of Tn916; blue or red arrows correspond to regions of a conjugative transposon (in GB0555) which is present with a deletion in Sprott. The deletion occurs between a membrane protein-encoding gene (*mp*) and an antirestriction protein ORF of Tn916 (*ardA*), both of which are truncated following the deletion (designated *mp'* and *ardA'* in Sprott). The boundaries of the mosaic transposon span from the C-terminal *RumA*-encoding region to the C-terminal region of a serine recombinase (SR) gene. Insertion of the element extends the *RumA* C terminus by 102 aa (to generate *RumA'*), and the SR gene product acquires a C-terminal 8-aa extension (*) that is derived from *rumA*. (B) A related transposon with identical Tn916 and deletion boundaries is present in *M. hominis* 1630. PCR products corresponding to the junctions of the element and deletions are shown. Lanes a to c correspond to the amplicon regions depicted by solid black bars. M is a 1-kb DNA ladder (Promega). (C) Detection of an empty site and extrachromosomal forms of the *M. hominis* Sprott element. An empty site was detected by PCR (lane 1) using the left primer for the PCR product (panel B, lane a) and the right primer for that of amplicon c. An extrachromosomal form is shown in lane 2 using outwards-facing oligonucleotides (black triangles in the *M. hominis* Sprott diagram). In this structure, the 3' region of SR fuses in frame with the 102-aa-C-terminus-encoding region of *rumA* (red triangles). All PCR products were sequenced by Sanger sequencing.

insertion was 36.2% G+C, in contrast to the core *M. hominis* genome (27.1%). The 25.3-kb insertion appears to derive from a larger, uncharacterized CTn based on 98% identity to a 75-kb *Streptococcus agalactiae* GB0555 CTn that contains a complete 18-kb Tn916 copy. The *M. hominis* derivative evidently underwent deletion of ~50 kb that eliminated 5 kb from Tn916 and 45 kb (encompassing conjugation genes) from the GB0555-related transposon (Fig. 1). In *M. hominis*, the transposon contains an 8.8-kb region with 100% nucleotide identity to a gene cluster encoding putative efflux proteins from GB0555 and that is also present, at 97% nucleotide sequence identity, in ICE*Sp*2905 (12).

An increasing number of mobile genetic elements (MGE) that target the *rumA* 3' region have been reported (6, 7). The GB0555 and Sprott elements insert at the same nucleotide, resulting in a translational fusion where the *RumA* C terminus acquires a 102-residue extension that derives from the transposon. Thus, a burgeoning number of *RumA* proteins in GenBank have almost identical C termini (derived from related transposons) despite having quite divergent sequences otherwise. For example, those from *M. hominis* and GB0555 are 29% identical (amino acids [aa] 1 to 440) but 100% identical in their 102-residue C termini. Furthermore, following integration, the serine recombinase (SR) open reading frame (ORF) product that is predicted to catalyze integration acquires a C-terminal tag (<20 aa) derived from the host *RumA* ORF.

An extrachromosomal form of the *M. hominis* Sprott element could be detected by inverse PCR, sequence analysis of which verified the *rumA* excision site. Furthermore, the 102-residue *RumA* C-terminal extension is fused in frame to the SR C terminus, suggesting that the variant that mediates integration may require this domain for activity.

Although *tetM* and Tn916 sequences have been reported in *M. hominis*, the data from three *M. hominis* lineages (different patients, collected in different decades) suggest that the MGE responsible for tetracycline resistance is actually the composite unit. This is in contrast to the situation in *Ureaplasma urealyticum* serovar 9 (strain ATCC 33175), in which a full-length Tn916 unit is present (13) but not the composite element described herein. One of several possible scenarios for interspecies transfer involves conjugation of a composite CTn (analogous to that in GB0555) from a group B streptococcus (GBS) (*S. agalactiae*) to *M. hominis* in the urogenital tract, with subsequent deletion. The recent report of MGE-mediated exchange of mycoplasmal chromosomal loci (14) suggests a plausible mechanism for further intraspecies dissemination.

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