Comparison of SXT and R391, two conjugative integrating elements: definition of a genetic backbone for the mobilization of resistance determinants

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Abstract. The SXT element (SXT) is becoming an increasingly prevalent vector for the dissemination of antibiotic resistances in Vibrio cholerae. SXT is a member of a larger family of elements, formerly defined as IncJ plasmids, that are self-transmissible by conjugation and integrate site-specifically into the host chromosome. Comparison of the DNA sequences of SXT and R391, an IncJ element from Providencia rettgeri, indicate that these elements consist of a conserved backbone that mediates the regulation, excision/integration and conjugative transfer of the elements. Both elements have insertions into this backbone that either confer the element-specific properties or are of unknown function. Interestingly, the conserved SXT and R391 backbone apparently contains hotspots for insertion of additional DNA sequences. This backbone represents a scaffold for the mobilization of genetic material between a wide range of Gram-negative bacteria, allowing for rapid adaptation to changing environments.

Key words. SXT; conjugative transposon; R391; gene transfer; Vibrio cholerae; comparative genomics.

Discovery of SXT and related elements in Vibrio cholerae

In late 1992, Vibrio cholerae O139 emerged in India and Bangladesh as the first non-O1 serogroup of V. cholerae to cause epidemic cholera. Microbiological and molecular characterization of V. cholerae O139 revealed that this newly emerged serogroup was closely related to the El Tor biotype of V. cholerae O1 that it initially replaced as the predominant cause of cholera on the Indian subcontinent [1]. Besides the novel serogroup antigen, V. cholerae O139 isolates also differed from El Tor O1 isolates in their antibiotic resistance profiles. Unlike the El Tor strains, O139 strains were resistant to sulfamethoxazole, trimethoprim, chloramphenicol and streptomycin. Initial characterization of the genes encoding these resistances indicated that they resided on a novel mobile genetic element designated SXT (for sulfonamide and trimethoprim) [2]. Although these antibiotic resistance genes were transmissible between V. cholerae strains by conjugation, plasmids were not detected in V. cholerae O139 isolates [2]. Instead, analyses of genomic DNA derived from transconjugant cells by pulsed field gel electrophoresis revealed that these antibiotic resistance determinants were integrated into a single site in the chromosome. Additionally, these transconjugants were able to serve as donors, indicating that SXT was a conjugative, self-transmissible integrating element similar to conjugative transposons [2]. The host range of SXT was not limited to V. cholerae, as the element could be transferred to several Gram-negative bacteria. When El Tor O1 V. cholerae reemerged in India, these strains, unlike their predecessors, were resistant to the same antibiotics as the O139 strains that they replaced [3]. The resistance determinants in these strains were also found to be located on a self-transmissible element closely related but not identical to SXT [2, 4]. Although more recent O139 isolates from India are no longer resistant to sulfamethoxazole or trimethoprim [5], molecular analyses indicate that these isolates still harbor an SXT-related element [6]. In addition, a recent study examining V.
cholerae clinical isolates from Mozambique and South Africa [7] suggests that SXT-like elements are now widespread in Africa as well as Asia. Finally, an SXT-related element has recently been detected in Providencia alcalifaciens clinical isolates from Bangladesh, indicating that the SXT group of conjugative integrating elements is found outside of V. cholerae [6].

SXT is related to IncJ elements

SXT appears to be a member of a larger family of mobile elements [8], once thought to be plasmids of the IncJ group, all mediating resistance to antibiotics and/or metals. Except under unusual laboratory conditions [9, 10], extrachromosomal DNA has not been isolated from any IncJ element [11], suggesting that like SXT, they are conjugative transposons, integrating into their hosts’ chromosomes [12]. These elements have been found in a variety of pathogenic γ proteobacteria. R391, the first described IncJ element, was initially isolated from a Providencia rettgeri clinical isolate in South Africa in 1972 [13] and encodes resistance to mercury and kanamycin. Other reported IncJ elements include pJY1, isolated from Vibrio spp. in the Philippines [14], R997, isolated from Proteus mirabilis in India [15], and pMERPH, isolated from Shewanella putrefaciens in the UK [16]. Although only two of these elements, SXT and R391, have been examined in detail [2, 12], preliminary phenotypic analyses suggest that the other elements are closely related. SXT and the IncJ elements are gaining recognition as widespread mobile elements that can disseminate antibiotic resistances and probably other important properties among bacterial populations.

Properties of SXT and R391

SXT was found to be integrated near the 5’ end of the prfC gene in V. cholerae [4]. The integrated element appears to be very stable, as loss of SXT has never been observed, even after growth without selection for many generations [4]. Following transfer to a new host, SXT integrates into the same site in the V. cholerae and Escherichia coli chromosomes [2, 4]. SXT site-specific integration into and its excision from the chromosome require an element-encoded integrase, Int, that bears similarity to phage-encoded tyrosine recombinases. SXT chromosomal integration and excision are similar to the chromosomal integration and excision described for lambdoid phages. These similarities include (i) formation of a circular extrachromosomal intermediate through recombination of sequences at the left and right ends (attL and attR, respectively) of the integrated element, (ii) recombination between relatively short element (attP) and chromosomal (attB) sequences in a recA-independent fashion and (iii) the requirement of a tyrosine recombinase to mediate this recombination [4]. SXT integration disrupts the 5’ end of prfC, a nonessential gene encoding RF3, a protein involved in the termination of translation. The 3’ end of SXT encodes a novel 5’ coding sequence for prfC and a promoter that leads to expression of functional RF3. SXT excision from the chromosome restores the wild-type copy of prfC [4]. This disruption/restoration phenomenon has also been observed in integration of the Gifsy-1 phage into a transfer RNA (tRNA) gene [18]. R391 was found to be integrated between 98.0 and 99.5 minutes on the E. coli chromosome [19]. Subsequently, Hochhut et al. localized the insertion site to prfC and identified an R391-encoded integrase nearly identical to that of SXT, indicating that the mechanisms for R391 integration and excision are virtually identical to those of SXT [8].

Although an extrachromosomal circular form of SXT has been observed and is thought to be the transfer intermediate, an autonomously replicating circular form of SXT has not been identified [2, 4]. Consistent with this observation, analysis of the SXT DNA sequences did not reveal any genes related to known replication factors [20]. Also, transconjugant formation requires int expression in recipient cells, suggesting that the circular extrachromosomal form of the element cannot be stably maintained without its integration [4]. Instead, SXT maintenance apparently requires its integration into the chromosome. Under certain experimental conditions an extrachromosomal, circular form of R391 and R997 has successfully been isolated [9, 10]. When the investigators transferred R391 into a recA-deficient strain containing R997, both elements were able to coexist, and an extrachromosomal element corresponding to the approximate size of R391 was isolated. The same was true for R997 in the converse experiment [10]. It remains to be determined whether these observations reflect detection of an autonomous replicative form of these elements or a shift in equilibrium between the excised and integrated forms of R391 and R997 in cells containing both elements.

DNA sequence analysis indicates that the conjugative apparatus utilized by SXT and R391 is related to that of the F plasmid [20, 21]. Following transfer of R391 and R997 to ‘bald’ strains of E. coli, long, flexible pili were observed [22]. The transfer frequency of both R391 and SXT are relatively low (10^4–10^5 exconjugants per donor in E. coli, and even lower in V. cholerae) [2, 12, 13], while R997 transfers at a higher frequency (10^3 per recipient) [22]. While both SXT and R391 are able to transfer on solid media, only R391 is able to transfer at a relatively high frequency in broth [13]. Also, the elements differ in their requirement for recA in conjugative transfer. The frequency of SXT transfer drops dramatically in the absence