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Review

Evolutionary persistence of tripartite integrative and conjugative elements

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ABSTRACT

Integrative and conjugative elements (ICEs) are generally regarded as regions of contiguous DNA integrated within a bacterial genome that are capable of excision and horizontal transfer via conjugation. We recently characterized a unique group of ICEs present in *Mesorhizobium* spp., which exist as three entirely separate but inextricably linked chromosomal regions termed α, β and γ. These regions occupy three different recombinase attachment (*att*) sites; however, they do not excise independently. Rather, they recombine the host chromosome to form a single contiguous region prior to excision and conjugative transfer. Like the single-part ICE carried by *M. loti* R7A (ICE*Ml*Sym^{R7A}), these "tripartite" ICEs (ICE^{3}s) are widespread throughout the *Mesorhizobium* genus and enable strains to form nitrogen-fixing symbioses with a variety of legumes. ICE^{3}s have likely evolved following recombination between three separate ancestral integrative elements, however, the persistence of ICE^{3} structure in diverse mesorhizobia is perplexing due to its seemingly unnecessary complexity. In this study, examination of ICE^{3}s revealed that most symbiosis genes are carried on the large α fragment. Some ICE^{3}-β and γ regions also carry genes that potentially contribute to the symbiosis, or to persistence in the soil environment, but these regions have been frequently subjected to recombination events including deletions, insertions and recombination with genes located on other integrative elements. Examination of a new ICE^{3} in *M. ciceri* Ca181 revealed it has jettisoned the genetic cargo from its β region and recruited a serine recombinase gene within its γ region, resulting in replacement of one of the three ICE^{3} integration sites. Overall the recombination loci appear to be the only conserved features of the β and γ regions, suggesting that the tripartite structure itself provides a selective benefit to the element. We propose the ICE^{3} structure provides enhanced host range, host stability and resistance to destabilization by tandem insertion of competing integrative elements. Furthermore, we suspect the ICE^{3} tripartite structure increases the likelihood of gene capture from integrative elements sharing the same attachment sites.

1. Introduction

The prokaryotic mobile gene pool, also termed the mobilome, accelerates bacterial adaptation by facilitating the single-step horizontal acquisition of novel genetic traits. The bacterial mobilome largely consists of conjugative or mobilizable plasmids (Smillie et al., 2010) and chromosomal mobile genetic elements (MGEs) including prophage, transposons, integrons, integrative and conjugative elements (ICEs) and integrative and mobilizable elements (IMEs) (Frost et al., 2005; Johnson and Grossman, 2015; Wozniak and Waldor, 2010). An analysis of approximately 1100 bacterial genomes has revealed that ICEs are probably the most abundant conjugative elements in prokaryotes (Guglielmini et al., 2011). ICEs are DNA regions flanked by attachment sites (*att*), which recombine through activity of an ICE-encoded site-specific recombinase, facilitating excision of the intervening DNA prior to horizontal transfer via conjugation (Johnson and Grossman, 2015; Wozniak and Waldor, 2010). As well as encoding genes required for excision and conjugation, ICEs often carry genes associated with host fitness benefits. ICEs can be categorized based on the function of the "genetic cargo", such as metabolism, antimicrobial resistance, pathogenicity or symbiosis (Hochhut and Waldor, 1999; Juhas et al., 2009; Rice, 1998; Schmidt and Hensel, 2004; Sullivan and Ronson, 1998; Sullivan et al., 1995). Symbiosis ICEs such as ICE*Ml*Sym^{R7A} of *Mesorhizobium loti* carry genes enabling soil bacteria to engage in a

Abbreviations: ICE, integrative and conjugative element; ICE^{3}, tripartite integrative and conjugative element; MGE, mobile genetic element; IME, integrative and mobilizable element; *att*, ICE core attachment sites; RDF, recombination directionality factor; bv., biovariant; TA, toxin-antitoxin; DR, direct repeat

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nitrogen-fixing symbiotic relationship with plants of the *Fabaceae* (*Leguminoseae*) (Sullivan and Ronson, 1998; Sullivan et al., 1995; Sullivan et al., 2002). Symbiotic nitrogen fixation is one of the most ecologically and agriculturally important biochemical processes on the planet (Herridge and Peoples, 2008; Jones et al., 2007).

2. Symbiosis ICEs

ICE*Ml*Sym^{R7A} is a 502-kb symbiosis ICE initially discovered within the chromosome of *Mesorhizobium loti* R7A (Kelly et al., 2014b; Sullivan and Ronson, 1998). ICE*Ml*Sym^{R7A} was identified through its ability to transfer to indigenous non-symbiotic mesorhizobia present in New Zealand soil, converting them in a single evolutionary step into symbiotic strains that could nodulate *Lotus corniculatus*. Transfer was also demonstrated under laboratory conditions (Sullivan and Ronson, 1998; Sullivan et al., 1995). Further work confirmed that ICE*Ml*Sym^{R7A} encodes both the majority of genes required for entering a root nodule symbiosis and a suite of genes that facilitate excision and horizontal dissemination through conjugation (Sullivan et al., 2002). ICEs resembling ICE*Ml*Sym^{R7A} have subsequently been identified in many other *Mesorhizobium* spp. (Haskett et al., 2016a; Kaneko et al., 2000; Ling et al., 2016; Reeve et al., 2014; Shimoda et al., 2016).

3. Regulation of monopartite ICE excision and transfer

ICE*Ml*Sym^{R7A} is integrated within the 3′ end of the sole *phe*-tRNA gene in the single chromosome of *M. loti* R7A. ICE*Ml*Sym^{R7A} excises from the chromosome through site-specific recombination between direct repeats (DRs) termed *attL* and *attR* located within recombinase attachment sites flanking each end of the integrated ICE*Ml*Sym^{R7A} (Ramsay et al., 2006; Sullivan and Ronson, 1998) (Fig. 1A). Recombination between $attL_S$ (subscripts are used to distinguish attachment sites for distinct recombinases described in Section 4) and $attR_S$ produces the new attachment sites $attP_S$ on circularized ICE*Ml*Sym^{R7A} and restores the $attB_S$ site within the *M. loti* R7A *phe*-tRNA gene. The tyrosine recombinase, IntS, catalyses both the excisive (i.e. $attL_S + attR_S \rightarrow attP_S + attB_S$) and integrative (i.e. $attP_S + attB_S \rightarrow attL_S + attR_S$) reactions. Excision additionally requires the recombination directionality factor (RDF; also known as excisionase) RdfS (Ramsay et al., 2006). Like other RDFs, RdfS likely stimulates IntS-mediated excision by binding to *att* sites. Excision and transfer are positively regulated by quorum-sensing. However, a combination of transcriptional activation, anti-activation and low-frequency ribosomal frameshifting robustly suppress *rdfS* expression and hence ICE*Ml*Sym^{R7A} excision in most cells (Ramsay et al., 2013; Ramsay et al., 2009; Ramsay et al., 2015b). Overexpression of *rdfS* cures R7A of ICE*Ml*Sym^{R7A}, producing the non-symbiotic derivative R7ANS (Ramsay et al., 2006).

4. Recombination of tripartite ICEs (ICE^3)

We recently characterized a structurally distinct symbiosis ICE (ICE*Mc*Sym^{1271}) existing in the chromosome of *M. ciceri* bv. biserrulae WSM1271, a Sardinian symbiont of the pasture legume *Biserrula pelecinus*. Transfer of ICE*Mc*Sym^{1271} to native non-symbiotic mesorhizobia in Australian soils confers on them the ability to nodulate *B. pelecinus* (Haskett et al., 2016c; Nandesena et al., 2006; Nandesena et al., 2007). In contrast to ICE*Ml*Sym^{R7A}, which exists as a single contiguous chromosomal segment, ICE*Mc*Sym^{1271} is composed of three separate chromosomal regions, named α, β, and γ, which assemble into a single region and then excise as a circular element through site-specific recombination prior to horizontal transfer. Thus, ICE*Mc*Sym^{1271} is termed a "tripartite" ICE (Here termed ICE^3). In addition to ICE*Mc*Sym^{1271}, transfer of a further three different ICE^{3}s located within the genomes of three *Mesorhizobium loti* strains, NZP2037, NZP2042 and SU343, has been demonstrated in the laboratory. A further 11 ICE^{3}s have been identified on the basis of sequence analysis in the genomes of a diverse range of *Mesorhizobium* spp. (Haskett et al., 2016c).

Excision of ICE*Mc*Sym^{1271} is a three-step process requiring sequential action of three recombinases IntS, IntG and IntM (Fig. 1B). Each α, β, and γ ICE^3 region is flanked by an *attL* site for one recombinase and an *attR* site for a different recombinase but taking the three regions together, *attL* and *attR* sites for each recombinase are present (Haskett et al., 2016c). Sequential recombination between pairs of matching *attL* and *attR* sites on α, β, and γ promotes a series of chromosomal inversions that result in the stepwise assembly of the three regions into a single contiguous region comprising α, β, and γ. The final recombination step excises the assembled ICE^3 to form a circular element carrying three distinct *attP* sites ($attP_S$, $attP_G$ and $attP_M$). ICE^3 assembly and excision restores the chromosome to a "naïve" state in which the arrangement of the *phe*-tRNA, *guaA* and *met*-tRNA genes that contain the *attB* sites is identical to that in mesorhizobial chromosomes lacking an ICE^3. Although our model of ICE^3 assembly and excision suggests that the recombination reactions catalyzed by IntS, IntG and IntM may occur in any sequence to form the circular ICE^3 element, some pathways may inviably segregate the chromosome. The disassembly process of the circular ICE^3 into α, β, and γ has been reconstructed in vivo using a synthetic minimal ICE^3 and ectopic expression of the three ICE*Mc*Sym^{1271} recombinase genes *intS*, *intG*, and *intM* (Haskett et al., 2016c). Once transferred to a recipient *Mesorhizobium*, the ICE^3 may integrate at any one of the three chromosomal *attB* sites within the 3′-end of the *phe*-tRNA, *guaA* or *met*-tRNA

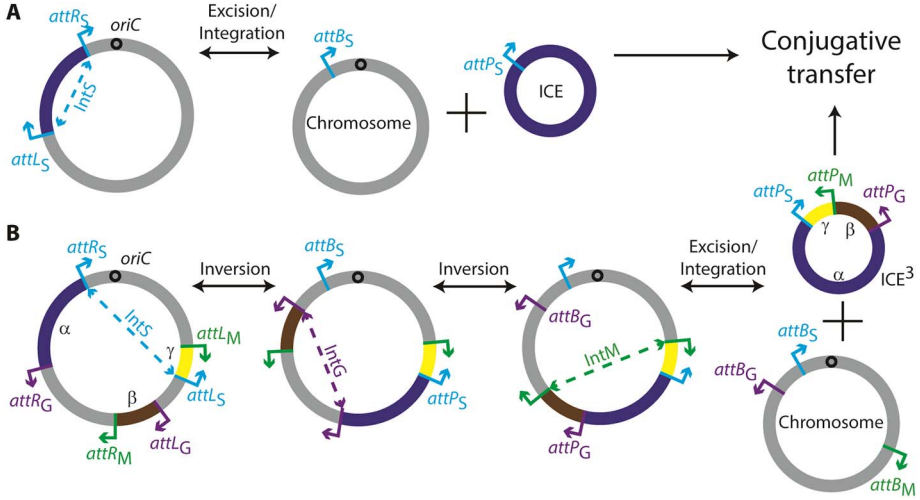

Fig. 1. Recombination and circularization of single-part and tripartite ICEs. (A) A single reversible recombination reaction is driven by IntS on the $attL_S$ and $attR_S$ sites of the integrated ICE*Ml*Sym^{R7A} to produce the excised ICE and the $attP_S$ and $attB_S$ sites. (B) Three sequential reactions between three pairs of attachment sites through the action of three recombinases IntS, IntG and IntM are necessary for excision of ICE*Mc*Sym^{1271}. The α, β, and γ regions are coloured blue, brown and yellow respectively. The attachment sites are shown as arrows pointing in the direction of the attachment-site core sequence and are coloured cyan, green and magenta for IntS, IntM and IntG, respectively. Note that the three recombination reactions may proceed in any order, however, our QPCR and mini-tripartite-ICE experiments are consistent with IntM being the last or lowest-rate recombination reaction (Haskett et al., 2016c). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)