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Single-Stranded DNA Transposition Is Coupled to Host Replication

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SUMMARY

DNA transposition has contributed significantly to evolution of eukaryotes and prokaryotes. Insertion sequences (ISs) are the simplest prokaryotic transposons and are divided into families on the basis of their organization and transposition mechanism. Here, we describe a link between transposition of IS*608* and IS*Dra2,* both members of the IS*200*/IS*605* family, which uses obligatory single-stranded DNA intermediates, and the host replication fork. Replication direction through the IS plays a crucial role in excision: activity is maximal when the "top" IS strand is located on the lagging-strand template. Excision is stimulated upon transient inactivation of replicative helicase function or inhibition of Okazaki fragment synthesis. IS*608* insertions also exhibit an orientation preference for the lagging-strand template and insertion can be specifically directed to stalled replication forks. An in silico genomic approach provides evidence that dissemination of other IS*200*/IS*605* family members is also linked to host replication.

INTRODUCTION

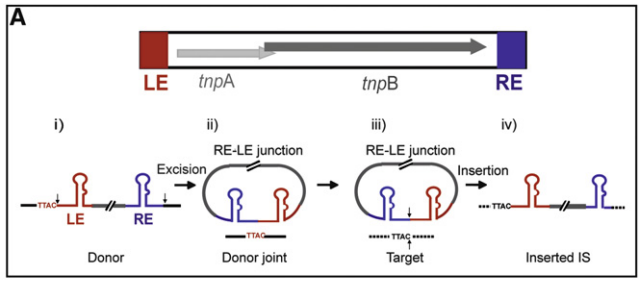
DNA transposition involves movement of discrete DNA segments (transposons) from one genomic location to another. It occurs in all kingdoms of life and has contributed significantly to evolution of eukaryotes and prokaryotes. Transposable elements can represent a significant proportion of their host genomes (Biémont and Vieira, 2006). They have been particularly well studied in bacteria where they are major motors of broad genome remodeling, play an important role in horizontal gene transfer, and can sequester and transmit a variety of genes involved in accessory cell functions, such as resistance to antimicrobial agents, catabolism of unusual compounds, and pathogenicity, virulence, or symbiosis. They are also important as genetic tools in identifying specific gene regulatory regions by insertion and are being developed as delivery systems for gene therapy applications.

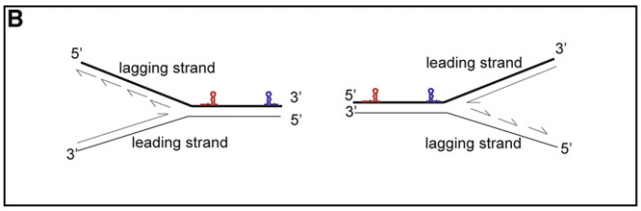
A variety of structurally and mechanistically distinct enzymes (transposases) have evolved to carry out transposition by several different pathways (Turlan and Chandler, 2000; Curcio and Derbyshire, 2003). They all possess an endonuclease activity allowing them to cleave, excise, and insert transposon DNA into a new location. Depending on the system (Curcio and Derbyshire, 2003), different types of nucleophile can be used by transposases to attack a phosphorus atom of a backbone phosphodiester bond and cleave DNA. These include water (generally activated by enzyme-bound metal ions), a hydroxyl group at the 5′ or 3′ end of a DNA strand, or a hydroxyl group of an amino acid of the transposase itself, such as serine or tyrosine.

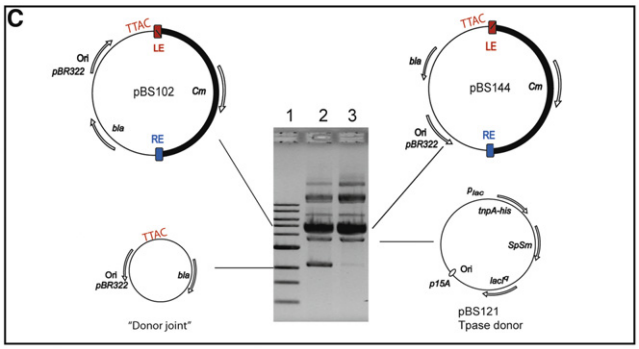
Many mobile DNA elements move using a "cut-and-paste" mechanism by excision of a double-stranded copy from one genomic location and insertion at another. Recently, a family of bacterial insertion sequences (ISs), the IS*200*/IS*605* family, has been found that uses a completely different pathway and an unusual transposase with a catalytic tyrosine (a Y1 transposase).

Studies of one member, IS*608* (Figure 1A), provided a detailed picture of their transposition (Ton-Hoang et al., 2005; Ronning et al., 2005; Guynet et al., 2008; Barabas et al., 2008). In vitro, this requires single-stranded DNA (ssDNA) substrates and is strand specific: only the "top" strand is recognized by the element-encoded transposase, TnpA, and is cleaved and transferred, whereas the "bottom" strand does not transpose. Excision of the top strand as a transposon circle with joined left and right ends is accompanied by rejoining of the DNA flanks. The circle junction then undergoes TnpA-catalyzed integration into an ssDNA target in a sequence-specific reaction. Insertion involves transfer of both the 5′ and 3′ ends of the single-strand circle junction into the ssDNA target. The left (5′) IS*608* end always inserts specifically just 3′ of the tetranucleotide, 5′-TTAC-3′ (Kersulyte et al., 2002), which is also essential for subsequent transposition (Ton-Hoang et al., 2005).

The obligatorily single-stranded nature of IS*200*/*IS605* transposition in vitro raises the possibility that it is limited in vivo by the availability of its ssDNA substrates. A number of cellular







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Substrate	Transposase	Transposition frequency	fold
1) pBS102	NO (pAPT110)	8.1×10^{-8} (1.4)	-
2) pBS102	YES (pBS121)	5.6×10^{-5} (0.8)	27
3) pBS144	YES (pBS121)	2.1×10^{-6} (0.3)	
4) pBS102ter1	YES (pBS121)	4.2×10^{-5} (0.6)	21
5) pBS144ter1	YES (pBS121)	2.0×10^{-6} (0.4)	
6) pBS102ter2	YES (pBS121)	7.0×10^{-5} (0.4)	23
7) pBS144ter2	YES (pBS121)	3.1×10^{-6} (0.2)	

Figure 1. IS*608* Excision Depends on the Direction of Replication

(A) IS*608* organization and a simplified transposition model. Gray arrows, *tnpA* and *tnpB* orfs; red and blue boxes, left (LE) and right (RE) ends (color code retained throughout).

(Ai) Schematized single-stranded IS*608* showing secondary structures of LE and RE, the flanking TTAC, and cleavage positions at the ends (vertical black arrows).

(Aii) Excision and ssDNA circle formation with an RE-LE junction and a sealed donor joint (black line) retaining TTAC.

(Aiii) TnpA brings together the transposon junction with a new TTAC-carrying target (dotted black line). Vertical black arrows, points of cleavage and strand transfer.

(Aiv) IS*608* insertion into the target.

(B) Orientation of the IS*608* derivative with respect to replication direction. The disposition of the IS*608* active (top) strand with respect to replication direction is shown when the fork approaches from one direction (left) when it is part of the lagging-strand template or the other (right) when it is part of the leading strand. This is described in more detail in Figure S1 and its legend.

(C) Excision measured directly in vivo by the appearance of "donor joint" plasmids, deleted for the IS*608* derivative. Left: pBS102 with the active IS*608* strand as part of the lagging-strand template. Right: pBS144 with the active IS*608* strand as part of the leading-strand template. Ori, pBR322 origin of replication; Cm, *bla*, SpSm, chloramphenicol, β-lactamase and, streptomycin/spectinomycin resistance genes; P_{lac}, *lac* promoter; *tnpA-his*, C-terminal his6-tagged *tnpA* gene. Directions of DNA replication and transcription are indicated. Agarose gel (0.8%) showing separation of plasmid DNA from overnight cultures of strains carrying pBS102 + pBS21 (lane 2) and pBS144 + pBS121 (lane 3). Lane 1, 1 kb standard.

(D) Mating-out assays. Left-hand column, transposon donor plasmid; middle, presence or absence of TnpA (relevant plasmids in parentheses); right, measured transposition frequencies (standard error in parentheses; n > 3). Plasmids pBS102ter1 and pBS144ter1 carry a single set of origin proximal terminators; pBS102ter2 and pBS144ter2 carry two sets of terminators flanking both transposon ends (Extended Experimental Procedures).

See also Figure S1A.

processes generate or occur using ssDNA, including DNA repair, natural transformation, conjugative plasmid transfer, single-stranded phage infection, and replication (where the DNA serving as the template for Okazaki fragment synthesis on the lagging strand of the replication fork is single stranded).

Here, we investigate the link between IS*608* transposition and the availability of ssDNA during replication in vivo. Our results demonstrate that transposition of the IS*200*/IS*605* family is closely integrated into the host cell cycle and takes advantage of the presence of ssDNA on the lagging-strand template at the replication fork for dissemination. We also show that IS*608* transposition is affected by perturbing the fork: transitory inactivation of crucial replication proteins increased excision from the lagging-strand template, and stalling of the fork resulted in insertions directed to the lagging strand of the blocked fork.

Our results also suggest that insertion and excision of the related element, IS*Dra2*, also depends on the lagging-strand template in its host, the radiation-resistant bacterium *Deinococcus radiodurans*, and that this dependency can be abolished with irradiation. We have extended our analysis to a number of related IS*200*/IS*605* elements in a variety of sequenced bacterial genomes. The results of this in silico analysis are also consistent with a strong bias of insertion into the lagging-strand template in these organisms. Together, the results demonstrate the importance of the lagging-strand template for IS*608* and IS*Dra2* activity and suggest that all IS*200*/IS*605* family members have evolved a mode of transposition that exploits ssDNA at the replication fork.

RESULTS

IS*608* Excision Depends on the Direction of Replication

To investigate whether replication direction affects IS*608* transposition, we used a plasmid assay in *E. coli* to monitor the excision step of transposition (Ton-Hoang et al., 2005). In this assay, the IS-carrying plasmids included an IS*608* derivative in which the *tnpA* and *tnpB* genes (Figure 1A) were replaced by a chloramphenicol resistance (Cm^R) cassette (Figure 1C). In one case, the active (top) IS*608* strand was located in the lagging-strand template (pBS102), and in the second, the replication origin was inverted (Figures 1B and 1C), placing the transposionally active top strand on the leading-strand template (pBS144). A second compatible plasmid supplied TnpA in *trans* under