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Retrohoming of a Bacterial Group II Intron: Mobility via Complete Reverse Splicing, Independent of Homologous DNA Recombination

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Summary

The mobile group II intron of *Lactococcus lactis*, Ll.LtrB, provides the opportunity to analyze the homing pathway in genetically tractable bacterial systems. Here, we show that Ll.LtrB mobility occurs by an RNA-based retrohoming mechanism in both *Escherichia coli* and *L. lactis*. Surprisingly, retrohoming occurs efficiently in the absence of RecA function, with a relaxed requirement for flanking exon homology and without coconversion of exon markers. These results lead to a model for bacterial retrohoming in which the intron integrates into recipient DNA by complete reverse splicing and serves as the template for cDNA synthesis. The retrohoming reaction is completed in unprecedented fashion by a DNA repair event that is independent of homologous recombination between the alleles. Thus, Ll.LtrB has many features of retrotransposons, with practical and evolutionary implications.

Introduction

Group II introns are catalytic RNAs that are believed to be the progenitors of nuclear spliceosomal introns (Sharp, 1991). Their ability to act as mobile elements is therefore of mechanistic and evolutionary interest. Group II introns in yeast mitochondria are mobile retroelements, capable of homing into cognate intronless alleles. The RNA-mediated mobility event, termed retrohoming, requires the intron-encoded protein, which has three activities: RNA maturase, DNA endonuclease, and reverse transcriptase (RT). The RNA maturase is needed for splicing, which in turn is a prerequisite for mobility, whereas both endonuclease and RT activities are required strictly for homing (reviewed in Curcio and Belfort, 1996). Homing occurs by a target DNA-primed reverse transcription mechanism initiated by the intron endonuclease, which is an RNP complex containing both the intron-encoded protein and the excised intron RNA lariat. First, cleavage of the DNA sense strand (mRNA-like strand) is RNA catalyzed and occurs by a reverse splicing reaction at the intron insertion site. Subsequently, antisense strand cleavage is catalyzed by the intron-encoded protein, after position +10 in the 3′ exon. The 3′ end of the cleaved antisense strand is then used as primer for reverse transcription of either reverse-spliced intron RNA or the intron in pre-mRNA, yielding a cDNA copy that is incorporated into the recipient DNA (Zimmerly et al., 1995a, 1995b; Yang et al., 1996, 1998; Eskes et al., 1997).

Although the work on the yeast group II introns has been extremely illuminating, studies are limited by the paucity of information available on recombination, replication, and repair functions in fungal mitochondria. In contrast, well-characterized bacterial systems are amendable to dissecting the pathways and functional requirements of the complex homing reaction. Indeed, the pathways of group I intron homing have been elaborated by using mutant phage and host functions and found to be strictly DNA-dependent double-strand-break (DSB) repair events that are highly reliant on exon homology (Clyman and Belfort, 1992; Mueller et al., 1996a).

Despite reports of group II introns in bacterial systems (reviewed in Michel and Ferat, 1995), it was not until the discovery of the *Lactococcus lactis* group II intron that both splicing function and mobility were demonstrated in bacteria (Mills et al., 1996, 1997; Shearman et al., 1996). To better understand group II intron function, we developed an expression system for the *L. lactis ltrB* intron (Ll.LtrB) in *Escherichia coli* and showed that it splices efficiently (Matsuura et al., 1997). We further demonstrated that the intron's 70 kDa protein product, LtrA, has maturase, endonuclease, and RT activities. Additionally, DNA endonuclease activity is associated with intron-containing RNP particles. The intron RNA cleaves the sense strand of the DNA at the intron insertion site by reverse splicing, while the intron-encoded protein cleaves the antisense strand at position +9 in the 3′ exon.

Here, we established systems that allow detailed analysis of group II intron mobility. By constructing a "twintron" donor with a group I intron embedded in the group II intron, we demonstrate that Ll.LtrB mobility occurs by an RNA-based retrohoming pathway in both *E. coli* and *L. lactis*. Furthermore, retrohoming has the remarkable properties of occurring in the absence of RecA function,

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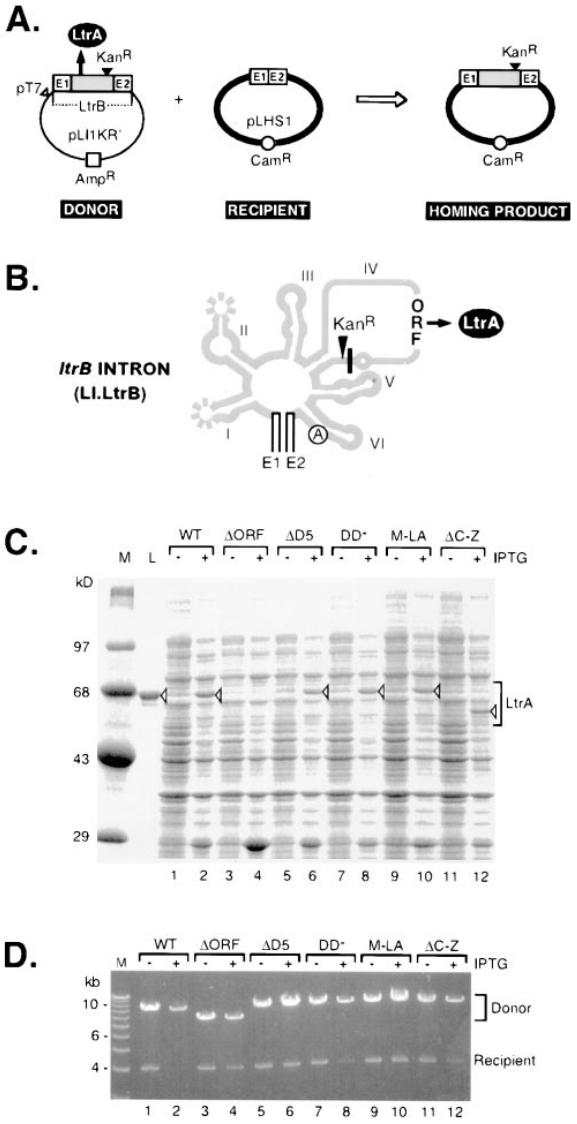

Figure 1. Intron Homing of LI.LtrB in *E. coli*

(A) Genetic assay. Crosses between Amp^RKan^R donors (pLI1KR′ derivatives) and Cam^R recipients (pLHS1) allowed selection for Cam^RKan^R homing products. Intron, shaded; LtrA, product of intron ORF; E1 and E2, exons.

(B) The *ltrB* intron donor. The position of the LtrA-encoding ORF and the location of the *kan*^{R} gene downstream of the stop codon (circle) of the ORF are shown. The position of insertion of the group I intron in the twintron construct (Figure 2) is indicated by a black bar. Dashed lines represent simplifications of the actual structure (Mills et al., 1996).

(C) Expression of LtrA protein. BL21(DE3) cells containing donor and recipient plasmids were harvested 3 hr after incubation in the absence (−) or presence (+) of 2 mM IPTG. Proteins were analyzed in an 8% SDS-polyacrylamide gel. Lanes: M, size markers; L, purified LtrA; 1 and 2, pLI1KR′; 3 and 4, pLIKR′ΔORF; 5 and 6, pLI1KR′ΔD5; 7 and 8, pLI1KR′DD$^-$; 9 and 10, pLI1KR′M-LA; 11 and 12, pLI1KR′Δ-ConZn.

(D) Cleavage of the recipient plasmid. DNA was extracted from aliquots of cultures used in (C), digested with AatII, which linearizes both donor and recipient, and separated in a 1% agarose gel. Labeling of lanes is as in (C).

with, at most, a relaxed requirement for exon homology. Finally, in sharp contrast to retrohoming in yeast mitochondria (Moran et al., 1995; Eskes et al., 1997), retrohoming occurs in bacteria in the absence of flanking marker coconversion. These observations suggest a mechanism involving complete reverse splicing of the intron into the DNA target site followed by full-length cDNA synthesis and a simple DNA repair event. Thus, bacterial retrohoming occurs by retrotransposition between homologous alleles in the absence of standard homologous recombination. These results have both practical and evolutionary significance.

Results

The *ltrB* Intron Can Home into a Cognate Intronless Allele in *E. coli*

Because of the facility of performing mechanistic studies in *E. coli*, it was first desirable to determine whether LI.LtrB homing could be observed in that host. A two-plasmid genetic assay was developed, based on a kanamycin resistance (Kan^R) marker engineered into the intron immediately downstream of the intron open reading frame (ORF), which encodes the LtrA protein (Figures 1A and 1B). The donor plasmid expresses the Kan^R-marked *ltrB* intron flanked by exon sequences and also confers ampicillin resistance (Amp^R). Donors were derivatives of pLI1KR′, with LI.LtrB under control of the phage T7 promoter (Matsuura et al., 1997). Recipient plasmids contain contiguous exons without the intron and confer chloramphenicol resistance (Cam^R) (Figure 1A). Recipient pLHS1 contains the LI.LtrB homing site within 800–900 bp of homology to the donor on each side of the intron insertion site. Donor and recipient plasmids were cotransformed into BL21(DE3). Inheritance of the Kan^R-marked intron by the recipient was monitored after induction of the resident T7 polymerase gene with IPTG to drive transcription of LI.LtrB. Cam^RKan^R homing products were distinguished from the Amp^RKan^R donor and the Cam^R recipient on selective media (see Experimental Procedures). Homing was thereby estimated at 1.3×10^{-3} per recipient, representing an ~25-fold increase of Kan^R recombinants over uninduced levels (Table 1A, cross 1).

The process was examined for its anticipated dependence on LtrA protein and on a splicing-competent intron by using mutant intron donors. The production of LtrA with different donors was monitored on polyacrylamide gels (Figure 1C). A 70 kDa band, corresponding to LtrA, was induced from the wild-type donor pLI1KR′ (lane 2), from splicing-defective intron mutant ΔD5 (lane 6), RT mutant DD$^-$ (lane 8), and maturase mutant M-LA (lane 10), but not from the ΔORF donor with a deletion in the *ltrA* gene (lane 4). Furthermore, the ΔConZn donor with a deletion of the endonuclease domain of LtrA yielded a smaller protein product, consistent with the size of the deletion (lane 12).

Cleavage of the recipient plasmid was checked on agarose gels (Figure 1D; Table 1A). Efficient cleavage induced by the wild-type donor pLI1KR′ on both strands is reflected by a sharp decrease of the recipient band (Figure 1D, cf. lanes 1 and 2). As in group I intron homing,

Table 1. Intron Homing in *E. coli* and *L. lactis*

A. Homing in *E. coli*

#	Donor	Characteristic	Clvg[b]	Homing Frequency[a]	
				-IPTG	+IPTG
1	pLI1KR'	Wild-type kan^R	+	$4.8 \pm 0.8 \times 10^{-5}$	$1.3 \pm 0.2 \times 10^{-3}$
2	pLI1KR'ΔORF	LtrA deletion	-	$1.4 \pm 0.9 \times 10^{-5}$	$1.3 \pm 0.2 \times 10^{-5}$
3	pLI1KR'ΔD5	Splicing-defective	-	$3.8 \pm 0.9 \times 10^{-6}$	$5.7 \pm 1.8 \times 10^{-6}$
4	pLI1KR'DD$^-$	RT-defective	+/-	$2.1 \pm 1.0 \times 10^{-5}$	$4.0 \pm 2.7 \times 10^{-5}$
5	pLI1KR'M-LA	Maturase-defective	-	$2.5 \pm 0.1 \times 10^{-5}$	$1.8 \pm 0.7 \times 10^{-5}$
6	pLI1KR'ΔConZn	Endo-defective	+/-	$1.5 \pm 0.3 \times 10^{-5}$	$2.2 \pm 0.6 \times 10^{-5}$

B. Retrohoming in *E. coli*

#	Twintron Donor	Splicing[c]		Homing Frequency[a]		% GI Intron Loss[d]	
		GII	GI	-IPTG	+IPTG	-IPTG	+IPTG
1	pLI1*td*$^+$KR'	+	+	$6.5 \pm 0.4 \times 10^{-5}$	$1.2 \pm 0.2 \times 10^{-3}$	49 ± 6%	77 ± 6%
2	pLI1*td*$^-$KR'	+	-	$6.9 \pm 0.2 \times 10^{-5}$	$9.2 \pm 0.3 \times 10^{-4}$	<0.5%	<0.5%
3	pLI1ΔORF*td*$^-$KR'	-	+	$2.3 \pm 0.9 \times 10^{-5}$	$2.0 \pm 0.5 \times 10^{-5}$	<0.5%	<0.5%

C. Retrohoming in *recA*$^+$/*recA*$^-$ hosts in *E. coli*

#	*recA*$^+$ Host			#	*recA*$^-$ Host			*recA*$^-$/*recA*$^+$	
	Strain	Homing Frequency[a]	% GI Loss[d]		Strain	Homing Frequency[a]	% GI Loss[d]	Homing	GI Loss
1	BL21(DE3)	$1.0 \pm 0.2 \times 10^{-3}$	82 ± 2	4	BLR(DE3)	$1.4 \pm 0.4 \times 10^{-3}$	95 ± 5	1.4	1.2
2	KL16(DE3)	$2.3 \pm 0.3 \times 10^{-4}$	77 ± 16	5	KL16*recA*(DE3)	$9.5 \pm 2.6 \times 10^{-4}$	88 ± 12	4.1	1.1
3	AB1157(DE3)	$2.0 \pm 0.9 \times 10^{-4}$	87 ± 13	6	AB1157*recA*(DE3)	$2.6 \pm 0.4 \times 10^{-4}$	75 ± 4	1.3	0.9

D. Homing in *recA*$^+$/*recA*$^-$ hosts in *L. lactis*

Donor[e]	#	*recA*$^+$ Host			#	*recA*$^-$ Host			*recA*$^-$/*recA*$^+$	
		Strain[f]	Homing Frequency[g]	% GI Loss[h]		Strain[f]	Homing Frequency[g]	% GI Loss[h]	Homing	GI Loss
pLE12	1	MG1363	5.6×10^{-2}	_	5	MG1363*rec*$^-$	1.1×10^{-1}	_	2.0	_
pLE12	2	LM0230	4.4×10^{-2}	_	6	MMS372	2.0×10^{-1}	_	4.5	_
pLE12*td*$^+$	3	MG1363	7.6×10^{-2}	90%	7	MG1363*rec*$^-$	1.3×10^{-1}	85%	1.7	0.9
pLE12*td*$^+$	4	LM0230	3.4×10^{-2}	94%	8	MMS372	1.1×10^{-1}	92%	3.2	1.0

[a] Crosses were performed as in Figure 1A. Kan^R homing products per recipient are given with standard error for at least three independent experiments.
[b] Cleavage of the recipient was monitored on agarose gels as in Figure 1D (+, cleavage; +/−, partial cleavage; −, no cleavage).
[c] Splicing proficiency of group II (GII) or group I (GI) intron.
[d] Group I intron loss determined by probing Cam^{R}Kan^R homing products with group I splice junction probe (Figure 3, P3).
[e] Donors contain wild-type LI.LtrB without (pLE12) or with (pLE12*td*$^+$) the splicing-proficient group I *td* intron.
[f] Crosses 1 and 5, 2 and 6, 3 and 7, and 4 and 8 were done in *recA*$^+$ and *recA*$^-$ isogenic hosts, respectively.
[g] Homing efficiency is represented by the number of group II intron homing products per recipient in crosses performed as in Figure 4.
[h] Group I intron loss determined by probing Spc^R homing products with group I splice junction probe (Figure 4, P3).

the cleaved recipient is degraded by host nucleases and therefore does not appear as a discrete band (Quirk et al., 1989). The RT mutant also induces cleavage of the recipient, although less than that of the wild type (Figure 1D, lanes 7 and 8). In contrast, the recipient band persists when LtrA is absent (lanes 3 and 4) or when

splicing is blocked by mutation of either the intron RNA (lanes 5 and 6) or the maturase domain of LtrA (lanes 9 and 10), indicating that both the protein and RNA are required to make the DSB. Interestingly, the endonuclease-defective ΔConZn donor induced partial disappearance of the recipient band (lanes 11 and 12). Since reverse splicing into the sense strand occurs in this mutant, but antisense-strand cleavage is blocked in vitro (Matsuura et al., 1997), this result is likely to reflect creation of a DSB through antisense-strand cleavage by cellular nucleases.

When the donor contained the ΔORF *ltrA* deletion, the homing frequency dropped 100-fold from 1.3×10^{-3} to 1.3×10^{-5} per recipient (Table 1A, crosses 1 and 2). Additionally, splicing-defective mutant ΔD5 and LtrA mutants defective in maturase (M-LA), RT (DD^-), and endonuclease (ΔConZn) activities showed 30- to 230-fold reductions in homing frequency (Table 1A, crosses 3–6). Homing frequencies were at least 10-fold lower in *E. coli* than in *L. lactis* (see below), likely owing to the relative instability of *ltrB* intron RNA in *E. coli* (Matsuura et al., 1997). Nevertheless, homing was dependent on the intron RNA and protein, and the system was therefore considered suitable for developing an assay to probe the role of an RNA intermediate in homing.

An Artificial Twintron Construct as a Test for Retrohoming

To verify that the observed mobility events with LI.LtrB represent RNA-based retrohoming, a twintron donor was constructed containing the phage T4 group I *td* intron (Figure 2A). The *td* intron lacking its own mobility apparatus was cloned with its requisite exon sequences between the LtrA ORF and the *kan*^{R} gene of the *ltrB* intron (Figures 1B, 2A, and 2B, schematic). In a DNA-based mobility event, the group I intron should be maintained in all group II intron (Cam^RKan^R) homing products (I^+II^+), whereas in an RNA-based mobility event, the group I intron should be spliced out of some fraction of the twintron RNAs, resulting in group I intron loss in that fraction of products (I^-II^+) (Figure 2A).

Splicing of both the group I and group II introns was first verified in the twintron constructs. The phage T4 group I *td*Δ1–3 intron, a splicing-proficient variant with a large deletion of the intron ORF (Belfort et al., 1987), was used to make twintron construct pLI1*td*$^+$ and its *kan*^{R}-containing counterpart pLI1*td*$^+$KR′. Equivalent constructs pLI1*td*$^-$ and pLI1*td*$^-$KR′, containing a splicing-defective *td* intron (*td*ΔSG1), were also made. Splicing of the *td* intron, measured in a primer-extension-termination assay using 3′ exon primers (Zhang et al., 1995), yielded precursor:product ratios for both *td*$^+$ twintron constructs of ~1:1, implying that about 50% of the group I intron had spliced in vivo (data not shown). Predictably, no splicing was observed for the *td*$^-$ constructs.

To examine the effect of the *kan*^{R} gene and the group I intron on group II intron splicing, a critical event in retrohoming, primer extension assays were performed (Figure 2B). The use of a group II intron-specific primer (P1) allowed detection of the precursor (cDNAs of 292 and 324 nt, corresponding to two different 5′ ends) and the excised intron lariat where the RT stops at the

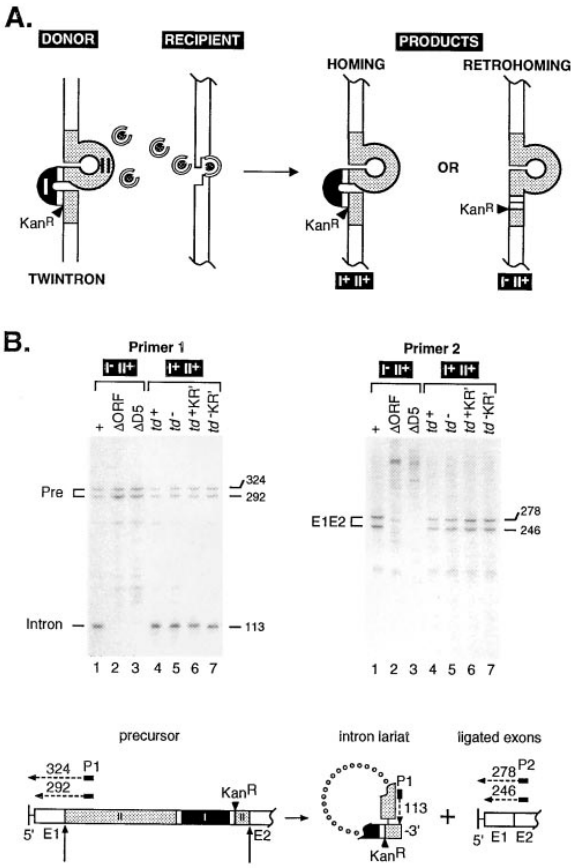

Figure 2. Test for Retrohoming

(A) The twintron as a test for retrohoming. The twintron (left) serves as donor in a mobility cross. Group II intron, shaded; group I intron, black; exons, unshaded; LtrA-intron RNP, encircled PacMan symbol. DNA-based homing will yield strictly I^+II^+ products; RNA-based retrohoming will yield some I^-II^+ products.

(B) Efficiency of group II intron splicing in twintron constructs. Primer extension analysis was performed with RNAs extracted from BL21(DE3) 1 hr after induction of intron donor plasmids derived from pLI1 (Matsuura et al., 1997). The cDNA bands, of lengths indicated in nucleotides to the right of each panel, correspond to precursors (Pre) and intron lariat for intron-specific primer 1, and ligated exons (E1E2) for exon 2–specific primer 2, as indicated in the schematic below. Designations above the lanes correspond to the pLI1 variants or twintron constructs. Schematic: cDNA extension products, dashed lines; primers, black bars; group II intron splice sites, upward-directed arrows.

branched nucleotide (cDNA of 113 nt). An exon 2–specific primer (P2) produced cDNAs of the ligated exons (cDNAs of 246 and 278 nt, again corresponding to the two 5′ ends) (Matsuura et al., 1997). Splicing ratios, represented by intron/(precursor + intron), were similar, regardless of whether or not the group II intron contained a group I intron and/or a *kan*^{R} gene (Figure 2B, cf. lanes 4–7 with lane 1). These ratios, determined with primer P1, were 62% for wild-type LI.LtrB (Figure 2B, lane 1) and 56%–76% for all the twintron constructs, whether or not the *td* intron was splicing proficient (lanes 4 and 5) or contained the *kan*^{R} gene (lanes 6 and 7). In contrast, *ltrA* deletion (ΔORF) and intron core deletion (ΔD5) constructs failed to splice (ratios <3%), confirming the reliability of the assay (lanes 2 and 3). These results indicate

that group I intron splicing is not required for splicing of the group II intron, and that group II intron splicing proceeds as efficiently in the presence of the *kan*^{R} gene and the group I intron as in their absence. The twintron construct therefore provides a viable group II intron donor for tests of retrohoming.

Retrohoming in *E. coli*

With Kan^R twintron constructs pLI1*td*$^+$KR′ and pLI1*td*$^-$KR′ as the group II intron donors, the frequency of homing was again established at $\sim 10^{-3}$ per recipient, indicating that the presence of the group I intron, regardless of its splicing status, is consistent with homing of the group II intron (Figure 3A; Table 1B, crosses 1 and 2). When the twintron donor was deleted for the *ltrA* ORF, homing frequencies dropped ~60-fold (Table 1B, cross 3), confirming that the events observed were dependent on LtrA.

Independent LI.LtrB Cam^{R}Kan^R homing products from a cross with the *td*$^+$ twintron were analyzed by restriction digest and by PCR to probe the presence of the *td* intron (Figure 3B). Depending on the presence or absence of the *td* intron, cleavage with KpnI generates fragments of 4.8 or 4.4 kb, whereas PCR using primers on each side of the *td* intron insertion site in LI.LtrB generates fragments of 1.8 or 1.4 kb, respectively (Figures 3A and 3B). The two assays were corroborative, indicating that the majority of the homing products had lost the *td*$^+$ intron during homing (I^-II^+) (Figure 3B). To screen many recombinants, we utilized a colony or patch hybridization assay for group I splicing, in which a probe to the *td* splice junction (P3) gives a positive signal only upon group I intron loss (Belfort et al., 1990) (Figures 3A and 3C). With the splicing-proficient *td*$^+$ intron, ~80% of group II intron homing products had lost the group I intron and gave a hybridization signal. In contrast, none of the homing products with the splicing-defective *td*$^-$ intron gave a signal, indicating that all retained the group I intron (Figure 3C; Table 1B, crosses 1 and 2). These data confirm that group I intron loss with the *td*$^+$ intron results from the use of an RNA template. Some group I intron loss occurred in the absence of IPTG (Table 1B, cross 1), suggesting leaky LI.LtrB expression in uninduced cells. Nevertheless, together the results argue strongly that homing occurs in *E. coli* by an RNA-based mechanism. Furthermore, considering the efficiency of group I intron splicing (~50%) and group I intron loss from homing products (>75%), it seems likely that the retrohoming pathway generated virtually all of the recombinant products, a conclusion verified below.

Retrohoming in *L. lactis*

Retrohoming was also studied in *L. lactis* using the twintron strategy, with shuttle plasmid pLE12*td*$^+$, which again contains the *td*$^+$ intron in LI.LtrB (Figures 1B, 2, and 4A). In analogy to experiments in *E. coli*, primer extension assays confirmed that the *td* intron splices accurately and efficiently and that its presence does not perturb LI.LtrB splicing in *L. lactis* (data not shown). The homing efficiency was assayed after crossing Cam^R group II intron donors pLE12 or pLE12*td*$^+$ with a spectinomycin-resistant (Spc^R) recipient, pMN1343, by cotransformation. Spc^R recipient plasmids from the cross were selected by retransformation into *E. coli* DH5α, and the homing frequency was assessed by probing colonies for acquisition of LI.LtrB using intron probe P4 (Figure

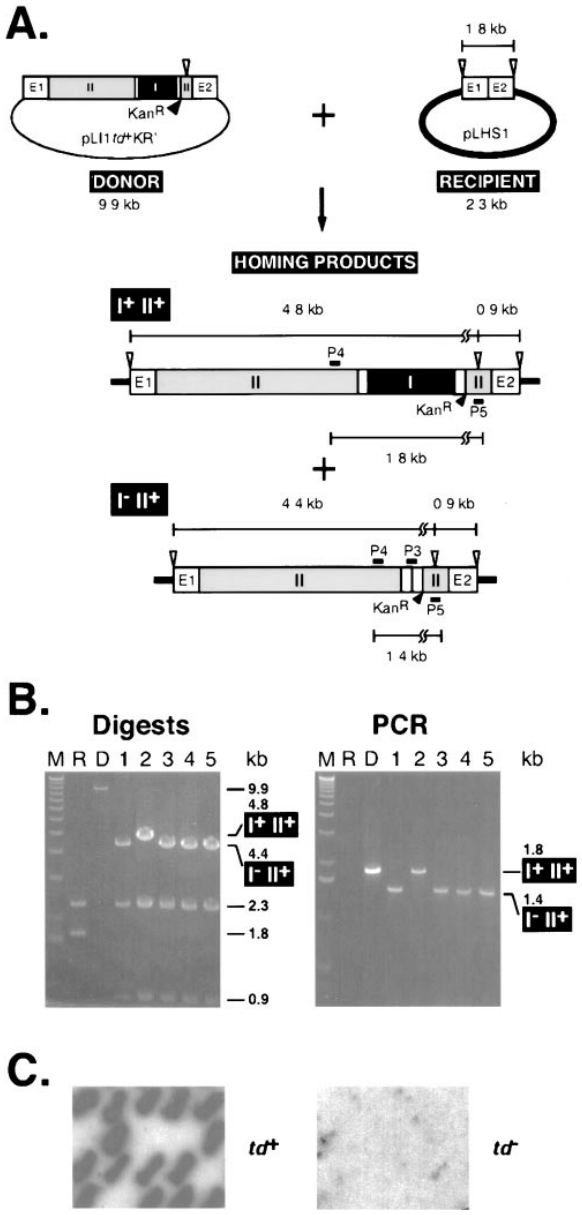

Figure 3. Retrohoming in *E. coli*

(A) Mobility cross. The Kan^R-marked twintron donor pLI1*td*$^+$KR′ and recipient pLHS1 are shown above, and the I^+II^+ and I^-II^+ homing products are shown below, with sizes of KpnI fragments and PCR products. KpnI sites are marked with open arrowheads. Intron and exon designations are as in Figure 2B. P3 is a probe to the *td* splice junction for detecting group I intron loss, whereas P4 and P5 correspond to PCR primers that flank the group I intron insertion site.

(B) Representative homing products. Plasmid DNA from independent Cam^{R}Kan^R colonies was digested with KpnI (left) or subjected to PCR analysis with P4 and P5 (right). Lanes: M, 1 kb DNA ladder; R, recipient; D, donor; 1–5, homing products.

(C) Hybridization analysis of retrohoming products. P3 was used to detect the *td* splice junction (Belfort et al., 1990) in I^-II^+ clones among homing products generated with *td*$^+$ (left) and *td*$^-$ (right) twintron donors.

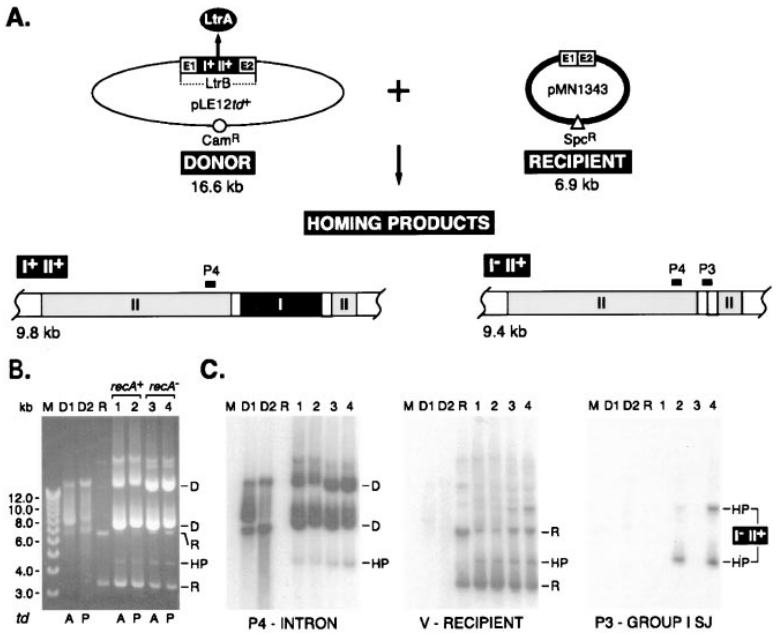

Figure 4. Retrohoming in *L. lactis*

(A) Schematic of assay. Intron donors were pLE12 or pLE12*td*$^+$ (Cam^R) (twintron depicted), and the recipient was pMN1343 (Spc^R). Conventions are as in Figure 2B. Probes P4 (group II intron) and P3 (*td* splice junction) were used to calculate, respectively, intron homing efficiencies and the percentage of group I intron loss.

(B) Agarose gel analysis of DNA from crosses. Undigested plasmids from the homing experiments were separated on 0.5% agarose gels. Lane M, 1 kb ladder; lanes D1 and D2, donor plasmids pLE12 and pLE12*td*$^+$, respectively; lane R, recipient plasmid pMN1343; lanes 1–4, crosses performed with pLE12 (lanes 1 and 3) or pLE12*td*$^+$ (lanes 2 and 4) in a *recA*$^+$ (lanes 1 and 2) or *recA*$^-$ (lanes 3 and 4) background. The absence (A) or presence (P) of the *td* intron are indicated below the lanes. D, donor; R, recipient; HP, homing product. Lanes 1, 2, 3, and 4 correspond to crosses 1, 3, 5, and 7 in Table 1D.

(C) Southern blot analysis. Blots of the gel from (B) were probed with intron probe P4 (left), recipient vector probe (middle), and *td* splice-junction probe P3 (right) (Sambrook et al., 1989; Belfort et al., 1990). Double bands (D/D and R/R in [B]; D/D, R/R, and HP/HP in [C]) result from plasmid multimerization.

4A). Homing frequencies were 3.4–7.6 $\times$ 10^{-2} recombinants per recipient (Table 1D, crosses 1–4). Using pLE12 and pLE12*td*$^+$, it was verified that the presence of the group I *td* intron within the group II intron had no detectable effect on the homing frequency of LI.LtrB in *L. lactis* (cf. crosses 1 and 3, 2 and 4, 5 and 7, 6 and 8). The frequency of group I intron loss, determined by probing the homing products with the *td* splice junction probe (P3), was 85%–94% in two different strains of *L. lactis* (MG1363 and LM0230), suggesting that, as in *E. coli*, the retrohoming pathway is used in a very high fraction of events.

Homing and retrohoming were also examined on agarose gels with DNA extracted from *L. lactis* cultures in which crosses were performed as above. Homing products in addition to intact donor and recipient plasmids were evident upon staining with ethidium bromide (Figure 4B). Southern blots of the same gel confirmed homing products with the group II intron probe (P4) and the recipient vector (V) probe (Figure 4C). Furthermore, with the *td* splice junction probe (P3), it is clear that the twintron donor plasmid generated homing products that had lost the group I intron, indicative of retrohoming (Figure 4C, P3, lanes 2 and 4).

RecA Is Not Required for Retrohoming

It was important to investigate the recombinase requirement for retrohoming in bacteria, particularly since group II homing events in yeast implicate homologous recombination pathways (Moran et al., 1995; Eskes et al., 1997), whereas those in *L. lactis* do not (Mills et al., 1997). In *E. coli*, in three different *recA*$^+$/*recA*$^-$ isogenic strain pairs, retrohoming was somewhat more efficient in the absence of RecA than in its presence (Table 1C, *recA*$^-$/*recA*$^+$ ratios of 1.3 to 4.1). Similarly, in *L. lactis*, the retrohoming efficiency was 1.7- to 4.5-fold elevated in the *recA*$^-$ background in two different isogenic strain pairs (Table 1D, cf. crosses 5–8 with crosses 1–4). These results are corroborated in the Southern blot with the group I splice junction probe (Figure 4C), where I$^-$II$^+$ homing products in the *recA*$^-$ host are 2- to 2.5-fold more intense than in the *recA*$^+$ host (Figure 4C, P3, cf. lanes 2 and 4).

Elevated retrohoming in *recA*$^-$ cells does not result from channeling of substrates from RecA-mediated DSB repair into retrohoming pathways, as the proportion of retrohoming relative to total mobility events is not increased in *recA*$^-$ hosts (Table 1C and 1D, *recA*$^+$/*recA*$^-$ ratios of 0.9 to 1.2). Further, elevated homing in *recA*$^-$ hosts does not result from RecA's role in the SOS response (Walker, 1984), as we found no difference in retrohoming levels in a *lexA* mutant in which the SOS response cannot be induced (data not shown). Rather, considering RecA's role in degrading linearized DNA (Kuzminov and Stahl, 1997), it is likely that cleaved substrates are stabilized in *recA*$^-$ hosts, thereby boosting retrohoming levels. Regardless, bacterial retrohoming is independent of the major RecA-dependent recombination pathway.

Homology Requirements

In light of the RecA independence of retrohoming on one hand and group II intron movement to homologous alleles on the other, the homology dependence of the process was determined. To this end, a set of recipient alleles was constructed with varying lengths of homology to the donor in each exon (Figure 5A, constructs 1–12). Given the likelihood that short homing sites are not adequate cleavage substrates, each homing site

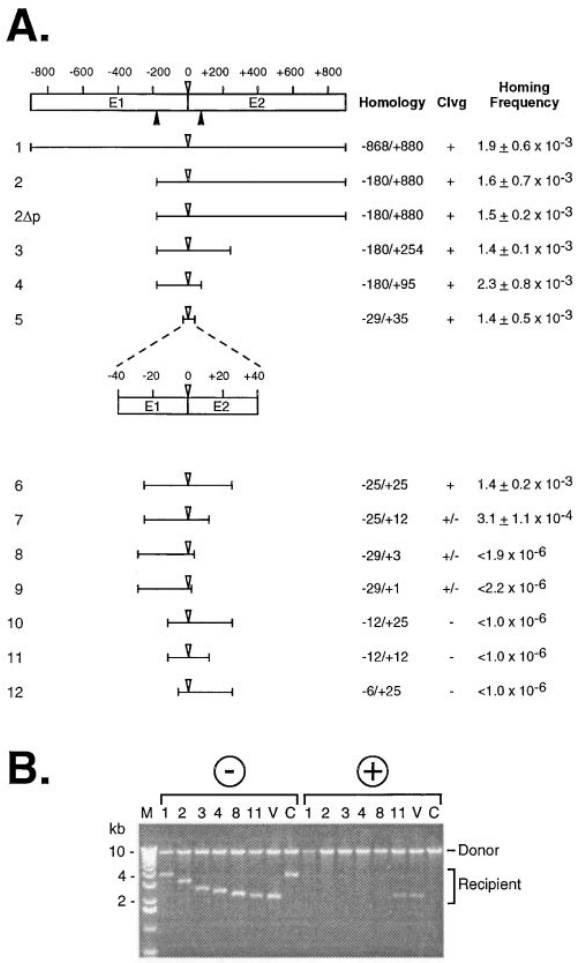

Figure 5. Homology Dependence of Retrohoming

(A) Homing frequency as a function of exon homology. Top, map of homing site of recipient (construct 1) with maximum homology to donor, pLI1*td*$^{+}$KR′. White arrowhead, intron insertion site; black arrowheads, boundary between *ltrB* and vector sequences. Constructs 1–12 corresponding to pLHS1-pLHS12 represent homing sites of varying lengths, with constructs 6–12 shown on an expanded scale. Construct 2Δp is a promoter deletion with equivalent homology to construct 2. The extent of homology with the donor, cleavage ([B]: +, cleavage; −, no cleavage; +/−, degrees of partial cleavage), and homing frequency with standard error for at least three independent experiments are listed on the right of each construct.

(B) Cleavage of homing sites. Digestion of representative plasmids from a mobility cross with SapI and SacI reveals linear donor and recipient bands, respectively (−, no IPTG). Disappearance of the recipient after induction with 2 mM IPTG for 3 hr (+) reflects cleavage of the homing site. M, size markers. Numbered lanes correspond to constructs in (A); lane V, vector without homing site; lane C, coconversion recipient pLHS-CR (see Figure 6).

was tested for cleavability by the intron RNP in vivo (Figures 5A and 5B).

Retrohoming occurred at wild-type levels of 1 to 2 × 10^{-3} per recipient with homologies between 868 and 25 bp in exon 1 and between 880 and 25 bp in exon 2 (constructs 1–6). These results are in striking contrast to group I intron mobility, where a homing site with 56 bp of exon homology (21 bp in exon 1 and 35 bp in exon 2) resulted in a 200-fold drop in homing levels (Parker et al., 1996). LI.LtrB retrohoming levels were similar regardless of whether or not the homing site was preceded by a promoter (cf. constructs 2 and 2Δp). Furthermore, a construct with only 25 bp of homology in exon 1 and 12 bp of homology in exon 2 supported homing to ~20% of wild-type levels (construct 7). In contrast, with homing sites having 12 bp or less of exon 1 (constructs 10–12), or 3 bp or less of exon 2 (constructs 8 and 9), homing was reduced 10^3-fold. Interestingly, while there was little cleavage of homing sites 10 to 12 compared to the vector without the homing site (V), cutting occurred, albeit at reduced levels, with homing sites 8 and 9 (Figures 5A and 5B, data shown for constructs 8, 11, and V). The latter homing sites retain sufficient 5′ exon sequences to support reverse splicing but lack 3′ exon sequences required for antisense-strand cleavage (G. Mohr and A. M. L., unpublished data). Thus, as with the endonuclease-defective LtrA mutant (Figure 1D, lanes 11 and 12), cleavage may reflect the ability of the intron lariat to reverse splice into the sense strand of these targets, which could then undergo antisense-strand cleavage by a cellular nuclease to form dead-end homing intermediates. Similarly, experiments in *L. lactis* with the −25/+25 homing site (Figure 5A, construct 6) in vector pDL278 resulted in equivalent retrohoming levels to those with longer exons (ca. 6.0 × 10^{-2} per recipient). These results indicate that while short stretches of homology are required for the RNP-homing site interaction, retrohoming can occur efficiently in the absence of extensive exon homology.

Flanking Marker Coconversion

To distinguish between different pathways of intron homing, a coconversion analysis of flanking markers was performed in *E. coli* (Figure 6). Whereas DNA-dependent homing predicts extensive bilateral coconversion in each exon (Moran et al., 1995; Mueller et al., 1996b), retrohoming via either partial or complete reverse splicing pathways predicts more limited transfer of flanking markers (Lazowska et al., 1994; Moran et al., 1995; Eskes et al., 1997) (Figures 7A and 7B). To examine the possibilities, a coconversion recipient, pLHS-CR, was constructed that contains six strategically positioned polymorphic sites, five of which are new restriction sites (Figures 6A and 6B). These sites extend from −7 to −400 bp upstream of the intron insertion site and from +7 to +387 bp downstream, including sites close to the intron, with one between the intron insertion site and antisense-strand cleavage site at +9. Not only is pLHS-CR cleaved well by the LI.LtrB RNP, but the homing frequency is comparable to pLHS1, the wild-type recipient (Figure 5B, lanes C and data not shown). Forty independent mobility crosses were performed with this polymorphic substrate and the twintron donor pLI1*td*$^{+}$KR′. One retrohoming product ($I^{-}II^{+}$) was isolated from each cross and examined by restriction digests for marker coconversion. The mutation at +7 did not create a restriction site and was analyzed by DNA sequencing. Remarkably, there was no detectable coconversion of any of the markers in any of the retrohoming products (Figures 6A and 6C).

Group I intron-containing homing products ($I^{+}II^{+}$) were

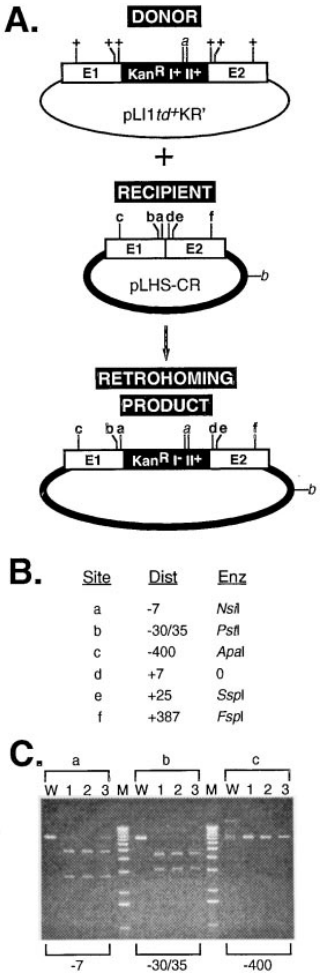

Figure 6. Coconversion Analysis

(A) Mobility cross. The Kan^R twintron pLI1*td*$^+$KR′ with wild-type exons and the recipient pLHS-CR with multiply marked exons (a–c in exon 1, d–f in exon 2) are shown. Bold letters (a–f) correspond to newly created sites in the exons; italics (a and b) correspond to existing sites elsewhere in the plasmids. The lack of coconversion is reflected by the persistence of polymorphic sites in the retrohoming products (I^-II^+).

(B) Polymorphic sites. The distance of each site from the intron insertion site is listed.

(C) Representative data. Results are shown for three retrohoming products (lanes 1–3) in the polymorphic recipient pLHS-CR and one in the wild-type recipient (lane W). Sites a, b, and c examined at −7 (NsiI), −30/−35 (PstI, two mutations to create site), and −400 (ApaI) are shown. Retention of sites a and b results in formation of two bands (one band with the linearized wild-type recipient), whereas retention of site c results in formation of one linearized band (an uncleaved plasmid with the wild-type recipient). M, DNA size markers.

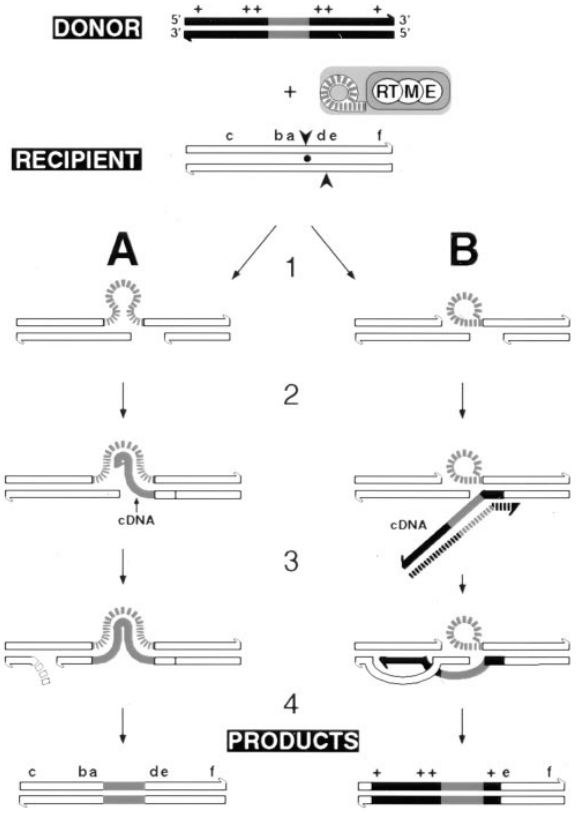

Figure 7. Retrohoming Pathways

The donor DNA (exons, black; intron, shaded; 3′ ends, half arrowheads; 5′ ends, blunt) and recipient DNA (exons, white; cleavage site, arrowheads; intron insertion site, dot) are shown with the LtrA/intron ribonucleoprotein (lariat plus trifunctional protein on gray background). RNA molecules, dashed lines; three activities of LtrA, encircled letters: RT, reverse transcriptase; M, maturase; E, endonuclease. Two possible pathways of retrohoming occur by recombinase-independent (A) and recombinase-dependent (B) mechanisms. In (A), the intron acts as template for cDNA synthesis, whereas in (B) the pre-mRNA acts as template. Steps: 1, reverse splicing and antisense strand cleavage; 2, cDNA synthesis; 3, repair synthesis without (A) or with synapsis (B); 4, completion of repair process. The predictions for coconversion of flanking markers are depicted in the retrohoming products.

also subjected to coconversion analysis, with 23 of 24 events showing no coconversion, indicative of retrohoming. The one exception displayed extensive bidirectional coconversion (>400 bp on each side) and reflects either standard homologous recombination or a DNA-based DSB-repair event. Thus, 63 of 64 independent events represent retrohoming, indicating that this pathway accounts for >98% of homing products. Likewise, in *L. lactis*, with the pLE12*td*$^+$ donor and polymorphic recipient, none of 20 independent retrohoming products (I^-II^+) showed coconversion. These data indicate that retrohoming in bacteria occurs by a complete reverse-splicing pathway that is independent of homologous recombination (Figure 7A).

Discussion

Group II intron homing in bacteria presents unique opportunities for studying mobility mechanisms. A group I–group II twintron construct was instrumental in showing that mobility of the *L. lactis ltrB* intron in *E. coli* and *L. lactis* occurs by an RNA-mediated retrohoming pathway, analogous to the demonstration that Ty1 elements transpose through an RNA intermediate (Boeke et al., 1985). Bacterial retrohoming is unprecedented in that it does not require recombinase function, is not critically dependent upon exon homology, and occurs without marker coconversion in flanking exons.

Recombinase and Homology Independence of Retrohoming

The independence of RecA function sets retrohoming apart from group I intron homing, which is strictly recombinase dependent, requiring either *E. coli* RecA or phage T4 UvsX (Clyman and Belfort, 1992; Mueller et al., 1996a). Rather, homology-independent, RT-dependent movement to target DNAs are properties in common between group II introns and non-long terminal repeat (non-LTR) retrotransposons. Indeed, target-primed reverse transcription at a break on the DNA target was first described for the R2Bm non-LTR retrotransposon of *Bombyx mori* (Luan et al., 1993). Interestingly, it has also been reported that gene-conversion events in yeast involving a cDNA donor are independent of RecA homologs RAD51, RAD55, and RAD57 (Derr, 1998).

Remarkably, the exon homology requirement for retrohoming is limited to the RNP recognition sequence of the homing site (Figure 5). LI.LtrB achieves target-site recognition by both intron RNA–DNA and by protein–DNA interactions, as do the yeast aI1 and aI2 introns (Guo et al., 1997; Matsuura et al., 1997; Yang et al., 1998; G. Mohr and A. M. L., unpublished data). Intron RNA base pairs with positions −13 to +1 of the DNA homing site (Mills et al., 1996; Matsuura et al., 1997), whereas flanking regions −25 to −13 of exon 1 and +2 to +10 of exon 2 are recognized by the LtrA protein (G. Mohr and A. M. L., unpublished data). This RNP recognition span from -25 to +10 corresponds to that needed for retrohoming (Figure 5), with no apparent requirement for other homologous sequences to complete the repair process.

Pathway for Retrohoming in Bacteria

In principle, retrohoming may occur via partial reverse splicing (PRS) or complete reverse splicing (CRS), using integrated intron or pre-mRNA as template for cDNA synthesis, with or without homologous recombination (Zimmerly et al., 1995a; Eskes et al., 1997) (Figure 7). Together, our results suggest that retrohoming of LI.LtrB occurs by the mechanism proposed in Figure 7A. The pathway initiates with the LtrA-assisted CRS of the intron into the sense strand of the recipient DNA. The antisense strand is then cleaved by the DNA endonuclease activity of the LtrA protein at position +9 of the 3′ exon (Figure 7A, step 1). The 3′ end of the cleaved antisense strand is used as a primer for reverse transcription of the reverse-spliced intron RNA (Figure 7A, step 2). DNA synthesis into the 5′ exon of the recipient may then either displace the antisense strand of the exon through the action of a helicase or replace this strand following degradation by cellular nucleases (Figure 7A, step 3). After digestion of the RNA template, presumably by cellular RNase H, second strand synthesis could be primed by the exposed 3′ end of 5′ exon DNA. Ligation of both strands would complete the retrohoming event (Figure 7A, step 4). This pathway is fully consistent with the RecA and homology independence of bacterial retrohoming and with the finding that >98% of events occur without coconversion of donor exons. Furthermore, the synaptase-independent mechanism, where the nascent DNA strand simply assimilates with upstream exon sequences, is precedented by RecA-independent recombination in phage λ–infected *E. coli* (Stahl et al., 1997).

Whereas the absence of flanking marker coconversion indicates that retrohoming in bacteria occurs via CRS, most of the RNA-DNA intermediates found in vitro result from PRS (Matsuura et al., 1997). It remains unclear whether this difference results from inefficient CRS in vitro or from an inability of bacterial cells to repair PRS intermediates, much as they cannot efficiently repair DSBs (Thaler et al., 1987; Clyman and Belfort, 1992). Notably, the lack of coconversion of flanking exon sequences is also consistent with CRS of the intron into an RNA substrate, followed by reverse transcription and integration of the cDNA into the recipient DNA. However, the RecA independence and relaxed homology requirements argue against this scenario because the cDNA would have to recombine with the recipient DNA via exon homology by RecA-dependent recombination. Furthermore, the observation that a promoter-minus recipient supported homing to the full extent of its promoter-plus counterpart (Figure 5A) argues against reverse splicing into an RNA substrate.

The predominant retrohoming pathways in bacteria and yeast mitochondria differ dramatically in their reliance on homologous recombination. Retrohoming of the yeast aI2 intron is proposed to initiate via PRS (Zimmerly et al., 1995b) (Figure 7B, step 1). After antisense strand cleavage, cDNA synthesis from the 3′-OH of the antisense strand could use pre-mRNA as template to allow repair in the upstream exon (Figure 7B, step 2). Synapsis in the 5′ exon (Figure 7B, step 3) results in limited 3′- and extensive 5′-exon marker coconversion after ligation and resolution (Figure 7B, step 4). In contrast, retrohoming of the yeast aI1 intron occurs via CRS, with the majority of events involving coconversion of upstream but not downstream exon markers (Eskes et al., 1997). These results are most readily explained by a process that initiates similarly to bacterial retrohoming (Figure 7A, steps 1 and 2), after which the cDNA copy of the intron invades an intron-containing allele to initiate DSB repair, with upstream marker coconversion. For both the yeast aI1 and aI2 introns, in addition to retrohoming, a significant fraction of the homing events have the characteristics of DNA-mediated DSB repair pathways. This is in sharp contrast to bacterial group II homing, where only one of 84 events has been observed with bidirectional coconversion, characteristic of DSB repair. Again, this result is consistent with wild-type *E. coli* lacking the machinery to efficiently repair DSBs (Thaler et al., 1987; Clyman and Belfort, 1992). It remains to be determined whether the mechanistic flexibility of group II introns, and their ability to move by homology-dependent or -independent CRS, PRS, or DSB-repair pathways, is an intrinsic property of the intron or attributable to its cellular environment.

Utility and Evolutionary Implications of Recombination-Independent Retrohoming

Besides the relative ease of manipulating bacterial systems, the *ltrB* intron is advantageous for gene targeting for several reasons. First, targeting to ectopic sites would be facilitated in the absence of a strict homology requirement. Second, the absence of coconversion would minimize undesirable changes to the target sequence. Third, the bacterial group II introns are shown

here to readily tolerate lengthy foreign sequences without compromising their catalytic activity or mobility. By altering the exon binding sites to change the specificity of the reverse splicing reaction (Eskes et al., 1997; Guo et al, 1997; Matsuura et al., 1997) foreign sequences might be directed to ectopic sites at will. Alternatively, LI.LtrB might be developed as a retrotransposon that integrates at nonallelic sites by randomizing the intron sequences that base-pair with the DNA target. Since the Gram-positive *ltrB* intron is functional in the Gram-negative *E. coli*, indicating a broad host range, LI.LtrB may provide a means for developing a retrotransposon for genome manipulation of bacteria (e.g., pathogens) or archaea with poorly developed genetic systems. Moreover, the finding that mobility does not require homologous recombination strengthens the idea that group II intron RNPs might be adapted for insertion of foreign sequences into a variety of eukaryotes that lack efficient homologous recombination systems (e.g., insects, plants, and mammals).

Precisely those features of bacterial retrohoming that are conducive to manipulating genomes and mobilizing sequences favor intron dissemination within and across species lines. Not only is the search for homology obviated because targeting is attributable entirely to intron–exon pairings and the recognition properties of the RNP, but also the intron landing site is preserved through the absence of coconversion. Since homologous recombination plays no role in the homing reaction, transposition to ectopic sites, again guided by intron–exon pairings, would be permitted, as demonstrated for group II introns in fungal mitochondria (Mueller et al., 1993; Sellem et al., 1993). Additionally, the bacterial retrohoming pathway favors intron spread because interspecies movement is not limited by a host's natural ability to support demanding repair processes. *E. coli*, for example, lacks the machinery to mobilize group I introns unless its recombination apparatus is supplemented by that of resident or infecting phage (Clyman and Belfort, 1992). A recombination-independent retrohoming pathway therefore expands the range of biological niches, likely contributing to the diverse phylogenetic distribution of group II introns. This freedom of movement could account for similar group II introns in bacteria, fungal, and plant organelles and might also have led to their dispersal in eukaryotic nuclear genomes, prior to their hypothesized degeneration into present-day spliceosomal introns (Sharp, 1991).

Experimental Procedures

Bacterial Strains

The standard *E. coli* host was lysogen BL21(DE3), which expresses T7 RNA polymerase from a λ prophage upon induction with IPTG (Studier et al., 1990). BLR(DE3) is its *recA* (Δ*srL-recA*)306::Tn10 counterpart (Novagen). λDE3 lysogens were prepared from strains KL16 (*E. coli* Genetic Stock Center, #4245), KL16*recA* (Δ*srL-recA*) 306::Tn10, AB1157 (R. Cunningham), AB1157*recA* (ΔrecA-srL304) (R. Kolodner), and AB1157*lex*A3 (*lexA*3*zja*::Tn10) (prepared by P1 transduction from N0145). The standard *L. lactis* strains were MG1363 (P. Duwat) and LM0230 (L. McKay), and their isogenic *recA* derivatives were MG1363recA (P. Duwat) and MMS372 (L. McKay).

Plasmid Constructs

Donor plasmids in the pLI1KR′ series (Figure 1A) for use in *E. coli* are derivatives of pLI1 (Matsuura et al., 1997). The *kan*^{R} gene from pUC4K (Pharmacia, Piscataway, NJ) was cloned in the same orientation as LtrA on a 1.1 kb PCR fragment into a SalI site engineered into loop IV of LI.LtrB by inserting the oligonucleotide 5′-TGAATGT CGACAG-3′ immediately downstream of the LtrA stop codon in pLI1 (C. Beall, unpublished data). LI.LtrB mutants are as follows: ΔORF, a >500 amino-acid deletion of LtrA; ΔD5, a splicing-defective intron mutant; DD$^-$, an RT mutant; M-LA, a maturase mutant; ΔConZn, an endonuclease mutant (Matsuura et al., 1997; H. Ma, unpublished data). The twintron constructs were generated from pLI1KR′ by cloning the group I *td* intron immediately upstream of the *kan*^{R} gene in the SalI site of pLI1KR′. The splicing-proficient (*td*$^+$) or splicing-defective (*td*$^-$) intron was cloned into this site from *td*Δ1-3 and *td*ΔSG1 (Belfort et al., 1987), respectively, on 417 and 385 bp PCR fragments, which include 12 bp of *td* exon sequence on each side.

The primary intron recipient for use in *E. coli* was Cam^R pLHS1, which was generated by first cloning the 271 bp HindIII fragment containing the LI.LtrB homing site (Mills et al., 1997) into the XbaI site of pET11a (Studier et al., 1990) by blunt-end ligation to give pLHS0. A PCR fragment was generated from pLHS0 using vector primers with KpnI ends on either side of the homing site to extend homology between donor and recipient (868 bp on the exon 1 side, 880 bp on the exon 2 side). This fragment was cloned into the KpnI site of pSU18 (Bartolome et al., 1991) to generate pLHS1. The pLHS2-4 series, which contains decreasing extents of homology surrounding the homing site (Figure 5A), was generated in the same way as pLHS1, with appropriate primers. Constructs pLHS5, pLHS8, and pLHS9, which have even less homology (Figure 5A), were generated by annealing complementary kinased oligonucleotides LL0 and LL0B (pLHS5), LL0 and CB62 (pLHS8), and LL0 and CBΔ3 (pLHS9), filling in with Klenow polymerase (BRL), and cloning into the SmaI site of pBSKS$^-$ (Stratagene). Plasmid pLHS5 was generated by cloning the SacI and HindIII fragments from the pBSKS$^-$ intermediate into pSU18, while pLHS8 and pLHS9 were generated by cloning the KpnI and XbaI fragments from the pBSKS$^-$ intermediate into pSU18. Plasmids pLHS6, pLHS7, and the pLHS10-12 series (Figure 5A) were generated by annealing kinased complementary oligonucleotides, filling in with Klenow, and cloning directly into the SmaI site of pSU18. All homing sites are in the same orientation as the replication origin. Plasmid pLHS-CR, the coconversion recipient, is a derivative of pLHS1 with mutations indicated in Figure 6, generated with the Gene-Editor Kit (Promega) using mutagenic oligonucleotides.

L. lactis donor construct pLE12 is a Gram$^-$/Gram$^+$ Cam^R shuttle plasmid containing the *ltrB* gene on a 7.5 kb PstI fragment (Mills et al., 1996). pLE12*td*$^+$ was constructed by replacement cloning from pLI1*td*$^+$ after partial digestion of pLE12 with KpnI. The linear band was redigested with BsrGI, and the BsrGI-KpnI fragment from pLI1*td*$^+$ was cloned into pLE12 (BsrGI-KpnI) to generate the twintron pLE12*td*$^+$. The recipient plasmid, pMN1343, used in *L. lactis* contains the 271 bp homing site on a HindIII fragment in the Spc^R shuttle vector pDL278 (Mills et al., 1997).

Oligonucleotides

Primers and probes are as follows: P1 (W598) 5′-CCGTGCTCTGTTC CCGTATCAGC-3′ and P2 (W596) 5′-CTTTAGGAATGACTTTCC AGT-3′ were for primer extension assays (Figure 2). P3 (W932) 5′-ATTAAACGGTAGACCCAAGAAAAC-3′ is the probe used to distinguish retrohoming products, while P4 (W645) 5′-GTATGGCTATG CCCGGAATAC-3′ and P5 (W682) 5′-ACTGGTTTGCACCACCCTC TTC-3′ are LI.LtrB-specific PCR primers used to characterize homing products (Figure 3). Oligonucleotides LL0 (5′-CCCACGTCGATC GTGAACACATCCATA-3′), LL0B (5′-CGGAATTCCCAGTATAAAGAT TCGTAGAATTAAAAATGATATGGTTATGGATGTG-3′), CB62 (5′-CGC AAAAAGGCCATTGAGCAGTCGCATCCCGGATGGCGATGGTTA TGGATGTGTC-3′), and CBΔ3 (5′-CGGAATTCCCAGAAAAAAGAA AGGATCTTAATTTTTACTATAGGTTATGGATGTG-3′) were used to generate clones pLHS5, pLHS8, and pLHS9.

Genetic Assay for Homing in *E. coli*

Fresh transformants of λDE3 lysogens containing Amp^R Kan^R donors (pLI1KR′ derivatives) and Cam^R recipients (pLHS1 derivatives) were diluted 1/100 from overnight cultures in TBYE (1% Bacto tryptone, 0.5% NaCl, 0.1% Bacto yeast extract) with appropriate antibiotics, grown to OD_{600} of 0.2, and induced with 2 mM IPTG for 3 hr. Plasmid

DNA extracted from 2 ml aliquots was digested with SapI, PpuMI, and AvaII, enzymes that cut only the donor, to enrich for homing products. The digests were electrotransformed into DH5α and plated onto three different media selecting for the following: Cam^R transformants (recipients, homing products, and cotransformants of donor and recipient plasmids), Cam^RKan^R transformants (homing products and cotransformants), and $Cam^RKan^RAmp^R$ cotransformants (to subtract from the number of Cam^RKan^R colonies and quantitate true homing products). Cam^RKan^R colonies were also patched onto plates with ampicillin to ensure that they were homing products (Cam^RKan^R) and not cotransformants ($Cam^RKan^RAmp^R$). The homing frequency was determined by calculating the number of Cam^RKan^R relative to Cam^R colonies.

Homing Assay in *L. lactis*

L. lactis cells containing recipient pMN1343 were transformed with donors pLE12 or pLE12*td*$^+$. Culture conditions and DNA manipulations were as described by Mills et al. (1997). Cotransformant colonies were grown overnight in GM17 medium (M17 from Difco laboratories containing 0.5% glucose) with chloramphenicol (10 μg/ml) and spectinomycin (300 μg/ml) to late exponential phase at 30°C. Plasmids extracted from harvested cells were analyzed for group II intron homing by agarose gel analysis and hybridization. Spc^R plasmid retransformants in DH5α were also probed for homing (P4) and retrohoming (P3).

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