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Intercellular mobility and homing of an archaeal rDNA intron confers a selective advantage over intron$^-$ cells of *Sulfolobus acidocaldarius*

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ABSTRACT **Some intron-containing rRNA genes of archaea encode homing-type endonucleases, which facilitate intron insertion at homologous sites in intron$^-$ alleles. These archaeal rRNA genes, in contrast to their eukaryotic counterparts, are present in single copies per cell, which precludes intron homing within one cell. However, given the highly conserved nature of the sequences flanking the intron, homing may occur in intron$^-$ rRNA genes of other archaeal cells. To test whether this occurs, the intron-containing 23S rRNA gene of the archaeal hyperthermophile *Desulfurococcus mobilis*, carried on nonreplicating bacterial vectors, was electroporated into an intron$^-$ culture of *Sulfolobus acidocaldarius*. PCR experiments demonstrated that the intron underwent homing and spread through the culture. By using a double drug-resistant mutant of *S. acidocaldarius*, it was shown that spreading resulted partly from a selective advantage of intron$^+$ cells and partly from intercellular mobility of the intron and homing.**

A special class of introns occur in stable RNA genes of many archaea (1, 2). The RNA introns generate a stable stem–loop "core" structure, and intron excision occurs by endoribonuclease cleavage of a "bulge–helix–bulge" motif that forms at the intron–exon junctions, and at least some of the introns circularize (3–7); the endoribonuclease occurs in a wide range of archaea (4, 5). Some archaeal rRNA introns carry open reading frames containing LAGLI-DADG boxes (8–10) that are common to endonucleases and maturases encoded by mobile group I introns and inteins found in mitochondria, chloroplasts, and nuclei of lower eukaryotes (11, 12). The endonucleases initiate intracellular insertion of an intron at a homologous site in an intron$^-$ allele (homing), probably by the double-strand break and repair mechanism, which results in coconversion of exon sequences (11, 12).

Two archaeal rRNA introns have been shown to encode homing-type endonucleases that can cut, *in vitro*, intron$^-$ rRNA genes of closely related organisms (13, 14) and their DNA recognition sites extend over 15–20 bp (ref. 14; C.A., M. J. Awayez, and R.A.G., unpublished data). The occurrence of these introns in single copy rRNA genes (16), their absence from the corresponding gene of close relatives (2, 9, 17), and the presence of highly conserved sequences bordering the putative homing sites (18) renders it possible that they are mobile between cells. To date, little is known about intercellular mobility of introns. It has been shown that the plasmid-borne group I intron from the nuclear 28S rRNA gene of *Physarum polycephalum* can be transformed into *Saccharomyces cerevisiae* (19). Subsequently, many cells died but resistant colonies were formed, most of which carried mutations in the highly conserved recognition site of the intron-encoded homing enzyme, while a few had undergone homing (19). These experiments suggest, but do not establish, that the intron is mobile between yeast cells.

To test whether the archaeal introns constitute mobile elements, we electroporated the intron-containing 23S rRNA gene from the archaeal hyperthermophile *Desulfurococcus mobilis* on nonreplicating bacterial vectors into an intron$^-$ culture of *Sulfolobus acidocaldarius*. In the presence of I-*Dmo* I, the endonuclease encoded by the *D. mobilis* intron (20), the intron was shown to home in the chromosomal DNA of intron$^-$ cells of *S. acidocaldarius*. Moreover, using a double drug-resistant mutant (21), it was demonstrated that the intron can move from intron$^+$ to intron$^-$ cells of *S. acidocaldarius*.

MATERIALS AND METHODS

Growth and Electroporation of *S. acidocaldarius* Cells. *S. acidocaldarius* cells (DSM no. 639) were grown at 70°C without shaking in 1.3 g of $(NH_4)_2SO_4$, 0.28 g of $MgSO_4 \cdot 7H_2O$, 0.07 g of $CaCl_2 \cdot 2H_2O$, and 1 g of yeast extract (Difco) per liter. The pH was adjusted to 3.0 with H_2SO_4 (22). Under these conditions, the doubling time was 5.5 hr. In some experiments, thiostrepton (Sigma) was added at 25 μg/ml. In electroporation experiments, exponentially growing cells were harvested at a density of A_{600} = 0.3, washed twice with 0.9% NaCl, resuspended in one-half the initial culture volume of ice-cold 0.1 M $MgCl_2$, pelleted, resuspended in 1/20th volume of ice-cold 0.1 M $MgCl_2$, and left on ice for at least 30 min. Aliquots were stored at −80°C in 10% (vol/vol) glycerol after freezing in liquid nitrogen. Before electroporation, cells were washed five times with 10% glycerol. DNA was methylated with *Hae* III methylase (New England Biolabs) to prevent degradation *in vivo*, and the DNA was then deproteinized by treatment with phenol and chloroform and precipitated with ethanol. In some experiments, 1 μg of I-*Dmo* I was added to the DNA and incubated at 70°C for 30 min in 25 mM Tris acetate, pH 8.0/10 mM magnesium acetate/10 mM ammonium acetate/10% glycerol. Electroporation was performed at 0°C in 0.1-cm cuvettes using the following settings: 1.5 kV, 25 mF, and 200 W. The cells were transferred to the growth medium and incubated at 70°C.

Constructs of the *D. mobilis* Intron and Bacterial Vectors. Two different recombinants were prepared. In the first, the downstream region of the 23S rRNA gene of *D. mobilis*, containing the intron (positions 300–3004), was inserted into M13mp18 at the *Bam*HI site. The construct lacks archaeal promoters and an archaeal origin of replication (23, 24). In the second construct, the upstream half of the 5S rRNA gene from *D. mobilis* (positions 1–67), and its three putative promoters (23), was fused to the downstream two-thirds of the 23S rRNA gene of *D. mobilis* containing the intron (positions 1046–3004) and inserted into pUC18. This construct contains archaeal promoters but no archaeal origin of replication (23, 24).

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Monitoring Homing of the *D. mobilis* Intron in *S. acidocaldarius*. *S. acidocaldarius* cells were electroporated with each of the constructs described above and grown continuously for 4 weeks. Chromosomal DNA was isolated from aliquots of culture taken at regular intervals (23). A section of the single 23S rRNA gene of *S. acidocaldarius* (18), containing the putative intron homing site (see Fig. 1*A*), was amplified by PCR using primer 5′-GGTTTAACAAAGCCACGAT-3′, which is specific for *S. acidocaldarius* 23S rDNA, and 5′-CCCTCCCACCTACTCTACGC-3′ annealed ≈100 bp upstream and 250 bp downstream, respectively, from the homing site. The products were analyzed by agarose gel electrophoresis. In a control experiment, the same section of the single 23S rRNA genes of the closely related *D. mobilis* (intron$^+$) and *Desulfurococcus mucosus* (intron$^-$) strains (23) were amplified by using primer 5′-GGCACACCCCTGGGACCGC-3′, which is specific for *D. mobilis* 23S rDNA, and 5′-CCCTCCCACCTACTCTACGC-3′. The reaction was performed with 0.2 mM each nucleotide triphosphate in 50 μl of 10 mM Tris·HCl, pH 9.0/2 mM $MgCl_2$/50 mM KCl/0.1% Triton X-100/1 unit of *Taq* polymerase (Promega) in a Biometra (Tampa, FL) Trio-Thermoblock running 30 cycles with the following settings: 40 sec at 58°C, 1.5 min at 72°C, and 1 min at 92°C.

The sensitivity of the PCR assay was established by a competition experiment in which chromosomal DNA from intron$^-$ and intron$^+$ *S. acidocaldarius* cells was mixed in four tubes at the following percentage ratios of intron$^-$/intron$^+$ DNA: 50:50, 90:10, 96:4, and 99:1. Part of each mixture was then amplified by PCR with the *S. acidocaldarius*-specific primers described above. Products were analyzed by agarose gel electrophoresis.

The location of the intron and the sequence of the upstream exon were examined by amplifying a section of chromosomal rDNA extracted from intron$^+$ *S. acidocaldarius* cells using primers 5′-GGTTTAACAAAGCCACGAT-3′ and 5′-CCTCTCTAGCATGTTAGCG-3′ that annealed within the upstream exon (18) and intron (2), respectively. The PCR conditions are described above, except that hybridization was performed at 52°C. The DNA product was cloned into the *Hin*cII site of M13mp18 and sequenced with an ALF sequenator (Pharmacia).

Southern blotting was performed on DNA from the nonreplicating M13 construct and the 4-week transformed culture of *S. acidocaldarius*. An intron-specific probe was generated by PCR from two primers annealed to the intron, 5′-AGGAAACATTAATGCATAATAATGAGA-3′ and 5′-CCTCTCTAGCATGTTAGCG-3′, in the presence of [α-^{32}P]dCTP (Amersham). DNA from the M13 construct and chromosomal DNA were digested with *Pst* I, *Bam*HI, *Pvu* II, *Sac* I, and *Hin*dIII overnight at 37°C in the optimal buffer for each enzyme (Amersham). Hybridization and washing of the filter were performed by standard procedures (25).

Determination of the Ratio Between *cam^s* and *cam^r* Cells. One milliliter of exponentially growing culture of *S. acidocaldarius* intron$^+$ (*cam^s*) and intron$^-$ (*cam^r*) cells (A_{600} = 0.2) was inoculated into 200 ml of medium and grown continuously at 70°C without shaking. Forty-milliliter samples were taken after 0, 1, 2, and 4 weeks growth, and total RNA was isolated. Primer extension was performed at the mutated site (C2457U), as described earlier (26), except that a nucleotide triphosphate mixture of 2.5 mM dCTP, 2.5 mM dGTP, 2.5 mM dTTP, and 1.0 mM ddATP was used and the extension was performed at 48°C. Band intensities were quantified with an Instant Imager (Packard).

RESULTS

I-*Dmo* I Is Essential for Spreading of the Intron in an *S. acidocaldarius* Culture. Most of the 23S rRNA gene from *D. mobilis*, including the intron (23), was cloned into M13mp18 (Fig. 1*A*). The construct lacked both archaeal promoters (23) and an archaeal origin of replication (24), so that it could neither be transcribed nor replicate in another archaeon. It was electroporated alone, and in the presence of purified I-*Dmo* I (20), into intron$^-$ cultures of *S. acidocaldarius*, which exhibits the same recognition sequence for I-*Dmo* I in its single 23S rRNA gene (13, 18) as occurs in *D. mobilis* (Fig. 1*A*). Cells were grown continuously at 70°C, without shaking, and total DNA was extracted from samples taken after 0, 1, 2, 3, and 4 weeks. A PCR assay was devised in which homing in the host 23S rRNA gene produced a 1000 (1007)-bp (intron$^+$) fragment, while the absence of homing yielded a 400 (387)-bp (intron$^-$) fragment. The results are illustrated in Fig. 1 *B* and *C* and demonstrate that homing occurred only when I-*Dmo* I was added to the M13mp18 construct during electroporation.

There are two alternative explanations for this result. One is that replication and spreading of the vector occurred because of the presence of an unidentified archaeal origin of replication. The other is that intron transposition occurred from another site in the *S. acidocaldarius* genome. Both possibilities were investigated by Southern blot analysis of DNA from the M13 construct and from *S. acidocaldarius* cells 4 weeks after transformation with the construct, using a ^{32}P-labeled probe for the intron. The results are illustrated in Fig. 2. Replication of the M13 construct can be discounted because the fragments deriving from the vector alone exhibit different sizes from those detected in the total cellular DNA isolated from the transformed *S. acidocaldarius* cells (Fig. 2). This result was reinforced by a PCR experiment with primers specific for *D. mobilis*, complementary to positions 2247–2266 and 1881–1899 of the 23S rDNA, which demonstrated that the M13 construct disappeared within 2–4 days of culturing the electroporated *S. acidocaldarius* cells (data not shown). Intron transposition within the *S. acidocaldarius* genome could also be excluded on the basis of the Southern blot experiment (Fig. 2), since the presence of the intron at two or more different sites in the *S. acidocaldarius* genome should have produced more than one fragment band in the lanes with chromosomal DNA.

To confirm that I-*Dmo* I activity is essential for intron spreading in the *S. acidocaldarius* culture, a construct was made in which the upstream half of the 5S rRNA gene of *D. mobilis* and its three putative promoters (23) were fused to the downstream two-thirds of the 23S rRNA gene of *D. mobilis* (Fig. 1*A*) and inserted into the bacterial plasmid vector pUC18, so that transcription, but not DNA replication, could occur in an archaeon. When this was electroporated into the *S. acidocaldarius* cells, spreading of the intron through the culture occurred without addition of I-*Dmo* I. The primary data are not included, but the results correspond to those illustrated in Fig. 1*C* and confirm that addition of I-*Dmo* I during electroporation, or its expression *in vivo*, is essential for intron spreading.

Does Spreading Occur via Homing? Intercellular mobility of the intron, followed by homing, would produce an increase in the intron$^+$ fraction of the *S. acidocaldarius* population. However, this increase could also reflect a selective disadvantage for the intron$^-$ cells deriving from different intron-dependent mechanisms (see *Discussion*). Clearly, one or both of these basic mechanisms (mobility homing or negative selection) may be operating. Therefore, we designed an experiment to establish whether one or both of these mechanisms operate. A chloramphenicol-resistant mutant (*cam^r*) of *S. acidocaldarius* was used carrying the transition C2585 → U in the peptidyltransferase loop of 23S rRNA (Fig. 3*A*) (21). Intron$^+$ (*cam^s*) and intron$^-$ (*cam^r*) cells were grown in a mixed culture, and the ratio of *cam^s*/*cam^r* cells was determined after 0, 1, 2, and 4 weeks by a primer-extension assay (26) and compared to the corresponding ratio for a control culture of intron$^-$ (*cam^s*) and intron$^-$ (*cam^r*) cells. The results for the mixed culture of intron$^+$ (*cam^s*) and intron$^-$ (*cam^r*) cells showed a steady

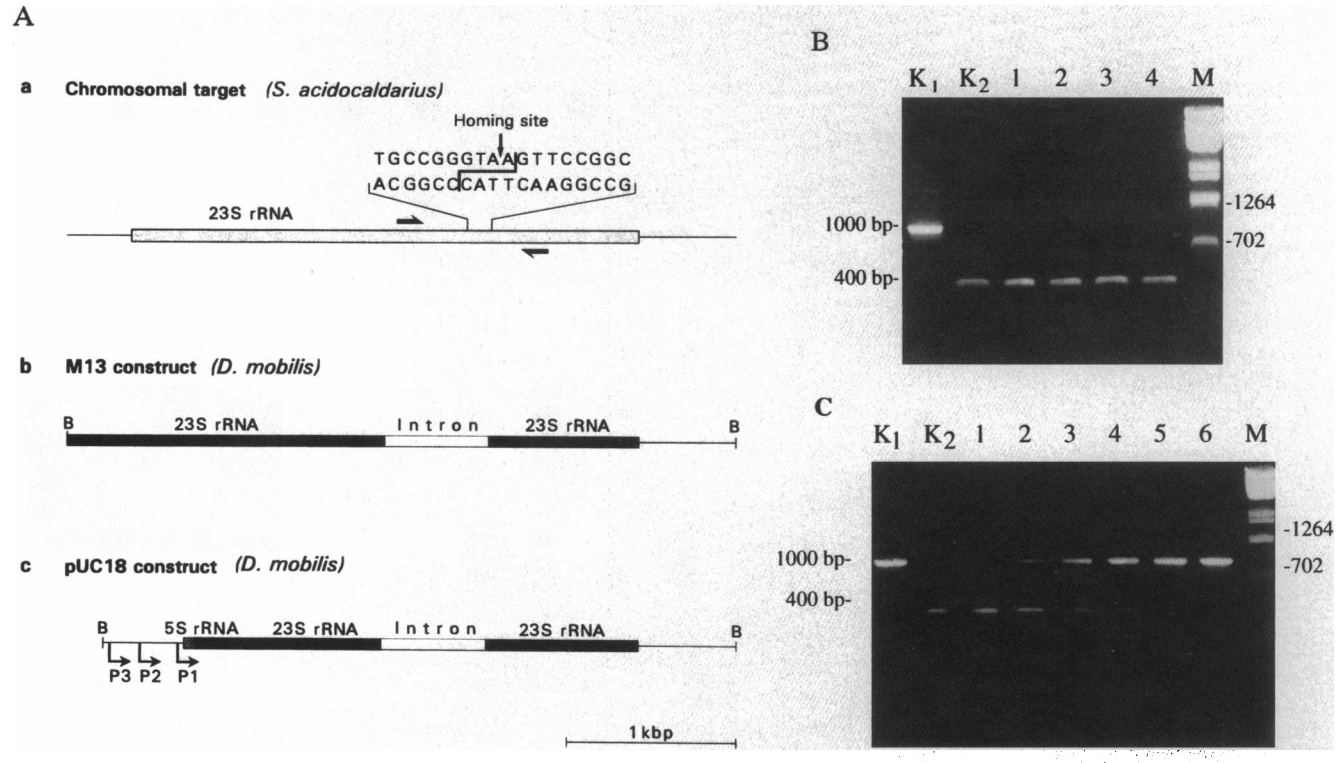

FIG. 1. Spreading of the *D. mobilis* intron through a population of *S. acidocaldarius*. (*A*) The chromosomal homing site of I-*Dmo* I and bacterial vector constructs. (*a*) Recognition sequence of I-*Dmo* I within the single 23S rRNA gene of *S. acidocaldarius*. Cleavage sites are indicated by a thick line and the homing site is marked by an arrow. Half-arrows indicate annealing positions of the two primers used in the PCR experiments. (*b*) Downstream region of the 23S rRNA gene of *D. mobilis*, containing the intron, cloned into M13mp18 (23). (*c*) Upstream half of the 5S rRNA gene from *D. mobilis*, with promoters P1–P3, fused to the downstream two-thirds of the 23S rRNA gene of *D. mobilis* (23) and inserted into pUC18. (*B*) PCR products from *S. acidocaldarius* cells electroporated with the M13mp18 construct, without adding I-*Dmo* I. K_1 and K_2 are control PCR amplifications of the 23S rRNA gene from *D. mobilis* (intron$^+$) and *D. mucosus* (intron$^-$) (2), respectively. Lanes 1–4, 400-bp (intron$^-$) product from the corresponding PCR experiment on chromosomal DNA isolated from *S. acidocaldarius* 0, 1, 2, and 4 weeks after electroporation. Lane M, λ DNA digested with *Bst*EII. (*C*) Same experiment as in *B* except that the M13mp18 construct was incubated with purified I-*Dmo* I (20) before electroporation. Lanes K_1, K_2, and M, control samples as in *B*. Lanes 1–6, 400-bp (intron$^-$) and 1000-bp (intron$^+$) products from chromosomal DNA isolated from *S. acidocaldarius* 0, 5, 10, 15, 20, and 28 days after electroporation using the same primers as in *B*.

increase in the cam^s/cam^r ratio with time; the cam^r cells decreased from 44% to 10% of the population within 1 week and to 4% after 4 weeks (Fig. 3*B*). In the control culture, the cam^s/cam^r ratio (both intron$^-$) remained constant (Fig. 3*B*). Thus, cam^s cells (initially intron$^+$) outgrew cam^r cells (initially intron$^-$). These results clearly indicate that the intron$^-$ (cam^r) cells were at a selective disadvantage. Moreover, this effect is attributable, in some way, to the intron: (*i*) because of the result of the control experiment, and (*ii*) because a PCR analysis of the 4-week culture (as in Fig. 1*C*) revealed a 1000-bp (intron$^+$) fragment but no 400-bp (intron$^-$) fragment.

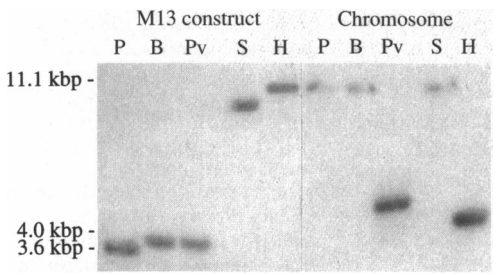

FIG. 2. Southern blot analysis of the M13 construct DNA and total DNA isolated from *S. acidocaldarius* cultures grown for 4 weeks after transformation with the M13 construct (Chromosome). The probe used was specific for the intron DNA. DNA was digested with the following enzymes: P, *Pst* I; B, *Bam*HI, Pv, *Pvu* II; S, *Sac* I; H, *Hin*dIII. Approximate sizes of the fragments from the M13 construct are 3.6, 4.0, 4.1, 10.3, and 11.1 kbp, respectively.

The question remains, does the intron mobility-homing mechanism operate? If it does, then the residual cam^r cells (4% of mixed culture after 4 weeks; Fig. 3*B*) should be, at least partly, intron$^+$. Failure to detect the 400-bp (intron$^-$) fragment in the 4-week culture (see above) suggested strongly that the cam^r cells had become intron$^+$, given that the PCR method should have detected a component that was ≥1% of the total DNA (see *Materials and Methods*). To provide positive support for this result, the pool of cam^r cells was amplified and reanalyzed. This was possible because the cam^r strain of *S. acidocaldarius* carried an additional ribosomal mutation conferring thiostrepton resistance (ths^r). Given the different sites of action of the two drugs on 23S rRNA (27), growth in the presence of thiostrepton would not induce spontaneous chloramphenicol revertants in intron$^-$ cells. The 4-week-old culture, containing 96% intron$^+$ (cam^s, ths^s) and 4% (cam^r, ths^r) cells, was incubated for a further 2 weeks in the presence of thiostrepton (25 μg/ml), during which time the cam^r, ths^r fraction increased to 26% (Fig. 3*C*). This value did not increase further with time, probably because the intron$^+$ (cam^s, ths^s) cells gradually became thiostrepton resistant. A PCR analysis of DNA from this cell culture again produced the 1000-bp band, but no 400-bp fragment (Fig. 3*D*), which unequivocally demonstrates that intron mobility and homing had occurred in the 23S rRNA gene of the cam^r, ths^r cells.

Coconversion of the Upstream Exon Can Occur on Intron Homing. To establish that the homing process was accurate, and to investigate whether exon sequences had coconverted, 10 clones of 23S rDNA were isolated from the 4-week culture

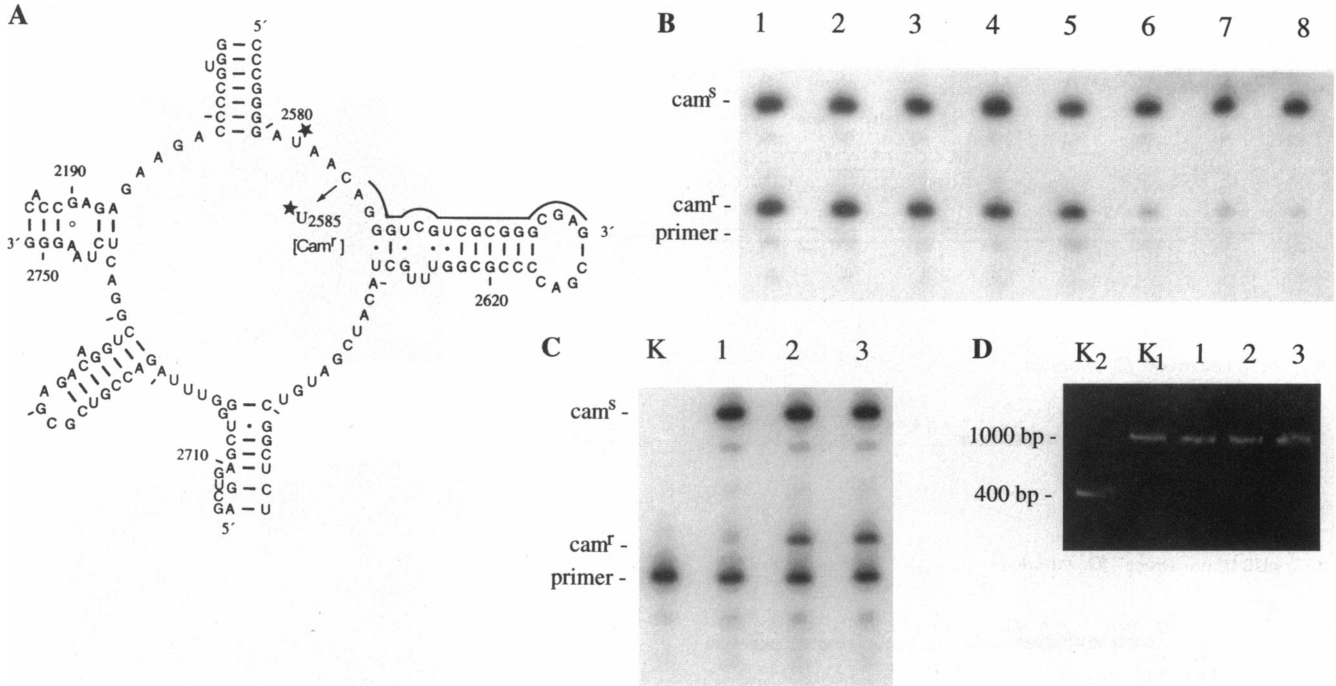

FIG. 3. Determination of the ratio between *cam^s* (intron⁺) and *cam^r* (intron⁻) cells in a continuously growing culture of *S. acidocaldarius.* (*A*) Section of the peptidyltransferase loop in the 23S rRNA of *S. acidocaldarius* where the *cam^r* mutation C2585 → U (21) is indicated. Primer binding site (positions 2586–2613) is overlined and the two uridine residues that produce reverse transcriptase stops are indicated by stars. (*B*) Autoradiogram showing primer extension from the site overlined in *A* on total RNA isolated from mixed cultures of intron⁺ (*cam^s*) and intron⁻ (*cam^r*) *S. acidocaldarius.* Lanes 1–4, control culture of *cam^s* and *cam^r* cells (both intron⁻); lanes 5–8, culture of intron⁺ (*cam^s*) and intron⁻ (*cam^r*) cells. Samples were taken after 0 weeks (lanes 1 and 5), 1 week (lanes 2 and 6), 2 weeks (lanes 3 and 7), and 4 weeks (lanes 4 and 8). (*C*) Autoradiogram showing results for the same primer-extension procedure after selecting for the *S. acidocaldarius* mutant (*cam^r, ths^r*) in the presence of thiostrepton (25 μg/ml). Lane K, primer only. Lane 1, 4-week culture of intron⁺ (*cam^s*) and intron⁻ (*cam^r*) cells (cf. lane 8 in *B*); lanes 2 and 3, the 4-week culture grown for 1 and 2 weeks more, respectively, in the presence of thiostrepton (25 μg/ml). (*D*) PCR products from *S. acidocaldarius* cultures grown in the presence of thiostrepton. Lanes K_1 and K_2, size markers as in Fig. 1*B*; lanes 1–3, cell samples taken 0, 1, and 4 weeks, respectively, after adding thiostrepton (25 μg/ml) to the growth medium.

(Fig. 1*C*) and sequenced through the upstream exon–intron junction. All yielded a normal exon–intron sequence at the junction (Fig. 4) compatible with accurate homing. Moreover, the results revealed four chimeric 23S rRNA genes containing sequences from both *S. acidocaldarius* and *D. mobilis* (Fig. 4). The lengths of the coconverting exon sequences from *D. mobilis* 23S rDNA correspond to one between 143 and 174 bp and three identical ones between 33 and 43 bp. For the remaining six clones, no coconversion was detected above the lower limit of detection (31 bp).

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         200               180              160              140
   ****  *** **  ***   *    |* *  *   *       |                |  *
D.m: GGGCACACCCTGGGACCGCAGGCCGCAGTGCCTAGGGGGGCCTGACTGTTTAATAAAAACATAGGTCCCCGCAAGCCCGAAAG
a  : GGTTTAACAAAGCCACGATAGGTCGCAGTGACCAGAGGGAcctgactgtttaataaaaacataggtccccgcAAGCCCGAAAG
b  : GGTTTAACAAAGCCACGATAGGTCGCAGTGACCAGAGGGACCTGACTGTTTAATAAAAACATAGGTCCCCGCTAGCCCGAAAG
c  : GGTTTAACAAAGCCACGATAGGTCGCAGTGACCAGAGGGACCTGACTGTTTAATAAAAACATAGGTCCCCGCTAGCCCGAAAG
S.a: GGTTTAACAAAGCCACGATAGGTCGCAGTGACCAGAGGGACCTGACTGTTTAATAAAAACATAGGTCCCCGCTAGCCCGAAAG

         120               100              80               60
      *   |      *          |  *   **  *   * *   *    *      *  * *        *
D.m: GGTTTGTACGGGGGCTGAATCCTGGCCACTGGCGGTCCGTGAAACCGGGGTCCAACCCGGCGAAGCGCCGCTGAAGGCCGGGA
a  : GGTTTGTACGGGGGCTGAATCCTGGCCACTGGCGGTCCGTGAAACCGGGGTCCAACCCGGCGAAGCGCCGCTGAAGGCCGGGA
b  : GGTGTGTACGGGGGCTAAATCCTGGCCACTGGTGGTTGGTTAAATCCGGGTTCAACCGGGCGAAGCCCCACCGAAGGCCGGGG
c  : GGTGTGTACGGGGGCTAAATCCTGGCCACTGGTGGTTGGTTAAATCCGGGTTCAACCGGGCGAAGCCCCACCGAAGGCCGGGG
S.a: GGTGTGTACGGGGGCTAAATCCTGGCCACTGGTGGTTGGTTAAATCCGGGTTCAACCGGGCGAAGCCCCACCGAAGGCCGGGG

                                    Exon ↔ Intron
      40               20              1
       |     *          |              |             -320-
D.m: GTAACTCTGACTCTCTTAAGGTAGCCAAATGCCTTGCCGGGTA-AGGGCAGGGGG.........CGCTAACATGCTAGAGAGG
a  : GTAACTCTGACTCTCTTAAGGTAGCCAAATGCCTTGCCGGGTA-AGGGCAGGGGG.........CGCTAACATGCTAGAGAGG
b  : gtaactctgacTCTCTTAAGGTAGCCAAATGCCTTGCCGGGTA-AGGGCAGGGGG.........CGCTAACATGCTAGAGAGG
c  : GTAACTCTGACCctcttaaggtagccaaatgccttgccgggta-AGGGCAGGGGG.........CGCTAACATGCTAGAGAGG
S.a: GTAACTCTGACCCTCTTAAGGTAGCCAAATGCCTTGCCGGGTA
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FIG. 4. Summary of the aligned sequences of the upstream exon and exon–intron junction of the 23S rDNA from 10 clones of intron⁺ *S. acidocaldarius.* Top and bottom sequences are from *D. mobilis* (D.m) (23) and *S. acidocaldarius* (S.a) 23S rDNA (18), respectively, where differences are marked by asterisks. *D. mobilis* sequences are underlined and both sequences are numbered in an upstream direction from the homing site. Rows a, b, and c, three different sequences observed for 1, 3, and 6 clones, respectively, that exhibit decreasing lengths of coconverted exon sequence (see text for details). Lowercase letters begin at the upstream limits of coconverted sequences. Exon–intron junction is marked and priming sites used for PCR are denoted by dotted lines.

DISCUSSION

The results demonstrate that an archaeal intron can home *in vivo* (Fig. 1*C*). The initial process requires addition of I-*Dmo* I to the electroporation mixture with *S. acidocaldarius* cells (Fig. 1*B*) or its expression *in vivo*. This finding, together with the observed coconversion of *D. mobilis* exon sequences in at least 4 of the 10 clones studied (Fig. 4), implies that intron homing occurs at the DNA level, by the double-strand break and repair mechanism, as proposed for homing of group I introns and inteins (11, 12). Failure to detect coconversion above the lower limit (31 bp) in 6 of the clones suggests that very short sequences are adequate at least for crossover (Fig. 4). Moreover, although there may be strong pressure on the mismatches in the coconverted *D. mobilis* sequence to revert to the *S. acidocaldarius* sequence, in order to restore optimal activity of an important ribosomal site (28), no reversion was detected after ≈120 generations in the 4 clones exhibiting altered sequences (Fig. 4).

The results also show that the *D. mobilis* intron can spread rapidly through a culture of *S. acidocaldarius* (Fig. 1*C*) by at least two mechanisms; one involves the intron conferring a selective advantage on intron$^+$ cells and the other (possibly related) involves intercellular movement and homing in intron$^-$ cells. The selective advantage was strong enough to overcome the disadvantage of sequence mutations incurred in the functionally important domain IV of *S. acidocaldarius* 23S rRNA (ref. 28; Fig. 4). It could have resulted from inefficient or defective homing, observed earlier for *S. cerevisiae* transformed with a nuclear group I intron, Pp LSU3, under the control of an inducible promoter (19) when most surviving colonies exhibited mutations in the highly conserved homing sequence. However, we could conclude that this phenomenon was not prevalent in *S. acidocaldarius* because no 400-bp (intron$^-$) fragment was detected in the transformed cells after 4 weeks (Fig. 1*C*). Possibly, in the archaeon, the selective advantage results primarily from infection of intron$^-$ cells with I-*Dmo* I (29), or the stable RNA intron (7), either of which could lead to DNA cleavage, but not insertion, which in turn would require DNA repair, leading to slower cell growth and possibly death. This difference between the archaeon and lower eukaryote may reflect that although mutation of highly conserved nucleotides around their neighboring homing sites impairs protein biosynthesis (28), these effects are relatively more detrimental for growth of the archaeon, with its single rRNA gene copies (16).

The observed mobility of the intron from intron$^+$ to intron$^-$ *S. acidocaldarius* cells, where it homes, establishes that genetic transfer can occur directly between chromosomes of different cells. The most likely mechanism by which the intron invades intron$^-$ cells is via mating and it has been demonstrated, at least for the euryarchaeotal branch of the archaea, and in contrast to bacteria, that mating involves bidirectional transfer of DNA (30), probably via cell fusion (15). Assuming that this is also true for the crenarchaeotal branch, then introns will tend to be maintained among closely related organisms, which is consistent with the archaeal rRNA introns occurring in clusters of related organisms (17). Thus, despite the highly conserved recognition sequences of archaeal (and other) homing enzymes, intron homing in more distantly related organisms will be a rare event and limited to the archaeal domain by the archaeal-specific nature of the RNA splicing enzymes (4, 5). However, more distantly related archaea, and other organisms, may still be vulnerable to invasion by a homing enzyme, or the stable RNA intron in which it is encoded, within natural environments.

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