Reverse splicing of a mobile twin-ribozyme group I intron into the natural small subunit rRNA insertion site

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Abstract
A mobile group I intron containing two ribozyme domains and a homing endonuclease gene (twin-ribozyme intron organization) can integrate by reverse splicing into the small subunit rRNA of bacteria and yeast. The integration is sequence-specific and corresponds to the natural insertion site (homing site) of the intron. The reverse splicing is independent of the homing endonuclease gene, but is dependent on the group I splicing ribozyme domain. The observed distribution of group I introns in nature can be explained by horizontal transfer between natural homing sites by reverse splicing and subsequent spread in populations by endonuclease-dependent homing.

Natural distribution of nuclear group I introns
A number of eukaryotic microorganisms contain autocatalytic group I intron insertions in nuclear rRNA genes [1,2]. More than 1600 nuclear group I intron sequences are available in DNA sequence databases [3] at highly conserved sites within the SSU (small-subunit; ~50 sites) or the large-subunit (~50 sites) rRNA genes. The ribozyme domains of group I introns are conserved at the secondary and tertiary structure levels, and comparison of the intron sequences on the basis of these conserved structural features gives valuable information on the relationships between introns. Numerous analyses show almost exclusively that introns present at homologous sites (same rDNA insertion site), which in distantly related host species are very similar [4,5]. Thus group I intron phylogeny supports widespread and site-specific horizontal transfer between homologous sites (intron homing) in nature.

Mechanisms of group I intron transfer
There are two different mechanisms of group I intron homing that may explain the observed distribution pattern in nature (Figure 1). Intron homing at the DNA level involves a homology-dependent gene conversion event promoted by an intron-encoded homing endonuclease [6]. This mechanism, dependent on sexual mating, results in a unidirectional and highly efficient spread of complex group I introns at the population level. Experimental evidence of DNA level homing from mating experiments has been reported for the Physarum intron and Tetrahymena intron (Tth.L1925). Here, both introns were successfully integrated into all the approx. 150 genomic copies of rDNA locus in Saccharomyces [9–11]. Insertion of group I intron sequences into the natural intron insertion sites at the DNA level represents a different homing approach. Here, intron insertion depends on the reversal of the forward self-splicing reaction (reverse splicing), a subsequent reverse transcription of the recombined RNA and, finally, genomic integration of the resulting cDNA. Experimental evidence for the complete homing pathway is still lacking, but intron integration of the Tetrahymena intron by reverse splicing has been reported in Escherichia coli [12–14]. These studies show that the Tetrahymena intron is able to integrate both into its natural site (homing site) as well as various heterologous rRNA sites.

Reverse splicing experiments in bacteria and yeast
We have investigated reverse splicing of the complex Didymium intron Dir.S956-1 into SSU rRNA of bacteria (E. coli) and yeast (S. cerevisiae). The Didymium intron, in contrast with the previously studied Tetrahymena intron, represents a functionally and biologically adequate group I intron. The Didymium intron has a twin-ribozyme organization (Figure 2A) and is the most complex group I intron known [15,16]. It contains three distinct structural and functional domains: (i) a novel processing ribozyme (DiGIR1) [17,18], (ii) a regular group IE splicing ribozyme (DiGIR2) [15,19–21] and (iii) an HEG (homing endonuclease gene; I-DirI HEG) expressing an endonuclease protein that is involved in intron homing at the DNA level [8,22,23]. The intron was targeted to its natural site (site 956) in the SSU rDNA [7,8]. Furthermore, artificial horizontal transfers at the DNA level into yeast have been demonstrated for the Physarum intron and Tetrahymena intron (Tth.L1925). Here, both introns were successfully integrated into all the approx. 150 genomic copies of rDNA locus in Saccharomyces [9–11]. Insertion of group I intron sequences into the natural intron insertion sites at the RNA level represents a different homing approach. Here, intron insertion depends on the reversal of the forward self-splicing reaction (reverse splicing), a subsequent reverse transcription of the recombined RNA and, finally, genomic integration of the resulting cDNA. Experimental evidence for the complete homing pathway is still lacking, but intron integration of the Tetrahymena intron by reverse splicing has been reported in Escherichia coli [12–14]. These studies show that the Tetrahymena intron is able to integrate both into its natural site (homing site) as well as various heterologous rRNA sites.

Key words: group I intron, horizontal gene transfer, reverse splicing, ribozyme, small subunit rRNA, twin-ribozyme.

Abbreviations used: HEG, homing endonuclease gene; SSU, small subunit.

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Endonuclease-mediated homing at the DNA level involves the following steps: step 1, sexual fusion of intron-containing (+) and intron-deficient (−) haploid (1n) Didymium amoebae; step 2, I-DirI endonuclease expression in the diploid (2n) zygote leads to endonuclease-mediated spread of Dir.S956-1 to the intron-deficient allele; step 3, development of the zygote into a plasmodium by synchronous nuclear divisions without cytokinesis; step 4, the plasmodium develops into sporangia and the production of haploid spores by meiosis results in haploid intron-containing amoebae by germination. The RNA-level homing is presented in two steps: step 1, a haploid intron-deficient amoeba acquires the Dir.S956-1 intron as a free intron RNA or as an intron full-length circle (FLC) RNA; step 2, inside the nucleolus, the intron reverse-splices into its natural site in the uninterrupted SSU rRNA. Reverse transcription and subsequent genomic integration of the resulting recombinant cDNA ensures stable genome integration of the intron, i.e. the amoeba becomes I+. 

Concluding remarks
In summary, the observed distribution of group I intron in nature is explained by horizontal transfers between the natural sites. We suggest that reverse splicing, in concert with the endonuclease-mediated homing, accounts for group I intron spread among the natural sites of different strains and species. In future work, we aim to generate stable genome integration of the Didymium intron based on reverse transcription of the recombined RNA and subsequent genomic integration of the resulting cDNA.
References


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