Cyanobacterial tRNA$^{\text{Leu}}$(UAA) group I introns have polyphyletic origin

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Abstract

Self-splicing group I introns in tRNA anticodon loops have been found in diverse groups of bacteria (α, β purple bacteria and cyanobacteria). In particular, the cyanobacterial tRNA$^{\text{Leu}}$(UAA) group I introns have attracted considerable attention because of their presumed ancient origin and immobility. In this work, however, we identified tRNA$^{\text{Leu}}$(UAA) group I introns in six out of 16 closely related isolates belonging to the cyanobacterial genus *Microcystis*. Interestingly, these introns are more closely related to the group I introns identified in the α and β purple bacteria (located in tRNA$^{\text{Arg}}$(CCU) and tRNA$^{\text{Ala}}$(CAU), respectively) than to other cyanobacterial introns. Our sequence comparison and phylogetic reconstruction suggest lateral transfer of the intron (possibly through mobility), and a polyphyletic origin of cyanobacterial tRNA$^{\text{Leu}}$(UAA) group I introns.

Keywords: Cyanobacteria; Group I intron; tRNA

1. Introduction

Group I introns share conserved sequence motifs and secondary structure. They occur in a variety of locations, such as in mitochondria, chloroplasts, cyanobacteria and eukaryotic genes, as well as in bacteria and bacteriophages. Many of these introns are mobile, either through mechanisms involving homing endonucleases, or through reverse splicing and transcription [1]. Self-splicing group I introns in tRNA anticodon loops have been found in diverse bacteria, i.e. in the β purple bacterium *Azoarcus* sp. tRNA$^{\text{Gln}}$(CAU), the α purple bacterium *Agrobacterium tumefaciens* tRNA$^{\text{Arg}}$(CCU) [2], and in cyanobacteria tRNA$^{\text{Leu}}$(UAA) [3,4] and tRNA$^{\text{Met}}$(CAU) [5]. In particular, the tRNA$^{\text{Leu}}$(UAA) group I introns have attracted considerable attention because of their presumed immobility and ancient origin [3–5].

In this work we have addressed the mechanisms – mobility or stability – by which group I introns are maintained in the cyanobacterial radiation. A characteristic of mobile introns is a relatively random intron distribution among closely related strains [1]. Thus, as a model system for investigating intron mobility or stability we analyzed the presence of tRNA$^{\text{Leu}}$(UAA) group I introns in 16 closely related cyanobacterial strains belonging to the genus *Microcystis*. These strains have previously been character-
ized by 16S rDNA analysis [6]. Our results show that there has been recent gain or loss of the Microcystis introns, and that unrelated introns are inserted in cyanobacterial tRNA\textsuperscript{Leu}(UAA) genes. Taken together, this evidence suggests lateral transfer of the intron (possibly trough mobility) and a polyphyletic origin of cyanobacterial tRNA\textsuperscript{Leu}(UAA) group I introns.

2. Materials and methods

2.1. Strains, culture conditions and DNA purification

The 16 strains belonging to the genus Microcystis used in this study are listed in Table 1. The majority of the organisms were isolated at the Norwegian Institute for Water Research and cultivated as described by Rudi et al. [6]. DNA for PCR amplification was isolated using a paramagnetic solid phase, while DNA for Southern hybridization analysis was isolated with standard phenol/chloroform methods [7].

2.2. PCR amplification and sequencing

Primers complementary to both the tRNA\textsuperscript{Leu}(UAA) exon and intron sequences were used in the characterizations in this work. Primer pair (CA) 5'-GCGGAATGTTAGACGCTACGGA-3' and (CB) 5'-TGGGGGTGAGGACACTGAAC-3' is complementary to the tRNA\textsuperscript{Leu}(UAA) exons, flanking the (UAA) anticodon loop. The primers (DG) 5'-GTGAGACTAGACGGGCA-3' and (DH) 5'-GTGGTCTGGACTATCCCTTC-3' are complementary to an internal fragment of the Microcystis tRNA\textsuperscript{Leu}(UAA) group I intron.

For primer pair CA-CB the PCR cycling parameters were: 94°C for 30 s, 59°C for 30 s and 72°C for 30s, and for primer pair DG-DH the parameters were: 94°C for 30 s, 48°C for 10 s and 72°C for 30 s. Between 30 and 40 cycles were used in the amplification reactions. All reactions were initiated with 4 min denaturation at 94°C, and ended with 7 min extension at 72°C. The PCR amplification and DNA sequencing sample preparations were done as previously described by Rudi et al. [6].

<table>
<thead>
<tr>
<th>Strain*</th>
<th>Intron PCR products$^b$</th>
<th>tRNA PCR products (CA-CB)</th>
<th>tRNA intron primers (DG-DH)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Microcystis botrys N-C 161/1</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Microcystis cf. flos-aquae N-C 144</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Microcystis cf. aeruginosa N-C 118/2</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Microcystis botrys N-C 264</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Microcystis viridis N-C 122/2</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Microcystis cf. isticryobiae N-C 279</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Microcystis viridis N-C 169/7</td>
<td>–</td>
<td>–</td>
<td>+</td>
</tr>
<tr>
<td>Microcystis aeruginosa N-C 123/1</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Microcystis aeruginosa N-C 166</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Microcystis cf. wessenbergii N-C 172/S</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Microcystis aeruginosa N-C 31</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Microcystis aeruginosa N-C 43</td>
<td>–</td>
<td>–</td>
<td>+</td>
</tr>
<tr>
<td>Microcystis aeruginosa N-C 228/1</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Microcystis aeruginosa N-C 143</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Microcystis sp. N-C 324/1</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Microcystis aeruginosa N-C 57</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
</tbody>
</table>

*N-C, strain NIVA-CYA.

$^b$+, amplification of introns. For the exon-specific primer pair (CA-CB) the amplification resulted in a 299 bp PCR product (intron and exon) in addition to a 62 bp product (exon without intron) (see Fig. 3B). For the intron-specific primer pair (DG-DH) the amplification resulted in only a 69 bp product. –, no intron amplification. For the exon-specific primer pair (CA-CB) the amplification resulted in only the 62 bp product. For the intron-specific primer pair (DG-DH) there were no amplification products. The positive signals for the intron- and not exon-specific primers for some of the strains might result from other homologous sequences in the genome.
2.3. Southern hybridization

Approximately 1 µg DNA was digested overnight with 10 U HindIII (Promega, Madison, WI, USA) at 37°C. The restricted DNA was heated to 65°C for 5 min, immediately loaded onto a 1.5% agarose gel and run at 4°C and 45 V for 5 h. The DNA was transferred and crosslinked to GeneScreen\textsuperscript{®} hybridization membrane, according to the manufacturer’s recommendations (NEN, Boston, MA, USA). The membranes were hybridized at 50–55°C, as described by Galau et al. [8]. The \textsuperscript{32}P-labeled probes were generated from PCR amplified DNA using the Random Primer DNA Labeling kit (Boehringer Mannheim GmbH, Mannheim, Germany), as described by Espelund et al. [9].

2.4. Phylogenetic reconstruction

The phylogenetic relationships for the tRNA genes were reconstructed using the parsimony and bootstrap programs from the Phylgeny Inference Package (version 3.5) (J. Felsenstein, Department of Genetics, University of Washington). The intron phylogeny was inferred with the neighbor-joining method, using the Kimura two-parameter model for distance estimation and bootstrap analysis for testing statistical significance of the branching pattern [10].

3. Results and discussion

3.1. Phylogenetic relationships of tRNA group I intron and exon sequences

The tRNA exon sequences from Microcystis were identified as tRNA\textsuperscript{Lys}(UAA) by sequence comparison (the anticodon loop and stem are highly conserved) and phylogenetic reconstruction (Fig. 1A). We

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**Fig. 1.** Sequence comparison and phylogenetic reconstruction of tRNA exon and intron sequences. A: Comparison of the different eu-bacterial tRNA genes containing group I introns. Partial sequences were used in this alignment, the anticodons are highlighted as black boxes, and the phylectic affiliations are indicated to the right. B: Distance tree for group I introns located in the anticodon loops. An alignment containing 162 unambiguously aligned sites was used in the group I intron phylogenetic reconstruction. The sites which could not be properly aligned were not considered in the analysis. The two most divergent sequences for the tRNA\textsuperscript{Lys}(UAA) and tRNA\textsuperscript{Lys}(CAU) introns were used to define these clusters. The following species are displayed: Phormidium Leu, Phormidium sp. tRNA\textsuperscript{Lys}(UAA), group I intron, EMBL accession no. M61163; Anabaena Leu, Anabaena azollae tRNA\textsuperscript{Lys}(UAA), group I intron, EMBL accession no. M38691; Microcystis Leu, Microcystis aeruginosa tRNA\textsuperscript{Lys}(UAA), group I intron, EMBL accession no. Y13474; Synechocystis fMet, Synechocystis sp. tRNA\textsuperscript{Mar}(CAU), group I intron, EMBL accession no. U10482; Dermocarpa fMet, Dermocarpa sp. tRNA\textsuperscript{Mar}(CAU), group I intron, EMBL accession no. U10480; Agrobacterium Arg, Agrobacterium tumefaciens tRNA\textsuperscript{Mar}(CCU), group I intron (from [2]); Azoarcus ile, Azoarcus sp. tRNA\textsuperscript{Mar}(CAU), group I intron (from [2]). For both A and B the numbers at the nodes indicate the percentage of 500 bootstrap trees in which the cluster descending from the node was found.
also identified the conserved anticodon loop of tRNA^{Leu(UAA)} for the amplified genes not containing introns (data not shown). The introns were sequenced from three different *Micocystis* strains (*Micocystis aeruginosa* strains NIVA-CYA 57, 143 and 228/1). All the strains have 226-nt intron sequences with >99.5% identity. The sequence for strain NIVA-CYA 57 has been deposited in the EMBL database, with accession number Y13474. The secondary structure (Fig. 2) clearly resembles the typical structure of group I introns [11]. These sequences are widely divergent (<61.5% identity) from the other known cyanobacterial tRNA^{Leu(UAA)} group I introns (EMBL release no. 50, March 1997). They are, however, more closely related to the introns identified in *Azoarcus* sp. tRNA^{Leu(CAU)} (67.2% identity) and *Agrobacterium tumefaciens* tRNA^{Arg} (CCU) (68.8% identity).

Phylogenetic reconstruction also strongly suggests clustering of the *M. aeruginosa*, *Azoarcus* sp. and *A. tumefaciens* introns (Fig. 1B), supporting a polyphyletic tRNA^{Leu(UAA)} intron origin. Only the introns have been transferred laterally, since they are located in different tRNA genes. Divergent branch lengths of more than 30% between the introns in the phylogenetic tree suggest, however, that these event(s) of lateral transfer are relatively ancient in the eubacterial radiation.

### 3.2. Distribution of tRNA^{Leu(UAA)} group I introns in the *Micocystis* genus

We identified, by Southern hybridization, *Micocystis* strains both with tRNA genes interrupted by introns and genes without introns (Fig. 3A). The exon probe gave a clear single band, while the intron
Fig. 3. Southern hybridization and PCR amplification for selected *Microcystis* strains. A: Southern hybridization of tRNA-1eu(UAA) exon probe and intron probe. Genomic DNA digested with *Hind*III was separated, blotted and hybridized as described in Section 2. The exon probe was generated using CA-CB amplification products from the cyanobacterium *Anabaena lemmermannii* strain NIVA-CYA 266/1, confirmed not to contain introns (unpublished results). The intron probe was obtained by nested PCR amplification with the primers (DG-DH), using (CA-CB) amplification products from strains NIVA-CYA 57 as a template. B: Amplification products for the exon-specific primer pair (CA-CB). The samples were electrophoresed in an ethidium bromide-stained 1.5% agarose gel for 30 min at 100 V. Twenty percent of the amplification products was loaded in each lane. The 299 nt bands are amplified fragments with introns, while the 62 nt bands are fragments without introns. The NIVA-CYA clone numbers are given for each lane in both A and B.

The strains belonging to the *Microcystis* genus are tightly clustered (≥99% identity) based on 16S rDNA analysis. These sequences are given in EMBL accession numbers Y12604 to Y12614, and in [6]. Since the strains are genetically closely related the gain or loss of introns in the *Microcystis* lineage must be relatively recent events. The presence of gene copies both with and without introns might indicate two or more copies of tRNA1eu(UAA) genes in the genome, or that the introns are so highly mobile that the cultures are heterogeneous populations of clones both with and without introns. Further investigations are required to determine the exact nature of these observations, and whether or not they reflect intron mobility.

3.3. Evolution of eubacterial tRNA group I introns

Preliminary results obtained by comparing 16S rDNA and tRNA1eu(UAA) intron phylogeny within the cluster defined by the species *Phormidium* sp. and *Anabaena azollae* (Fig. 1B) also suggest events of lateral transfer of tRNA1eu(UAA) introns (K. Rudi and K.S. Jakobsen, unpublished results). Thus, lateral transfer of introns might be a universal mechanism to maintain introns in the eubacterial radiation, as opposed to the presumed immobility of tRNA1eu(UAA) group I introns [3–5].

Eubacterial and chloroplast (of eubacterial origin) group I introns located in tRNA anticodon loops, however, form a monophyletic lineage within the evolutionary tree of all known group I introns (data not shown), suggesting that these introns are of ancient origin in the eubacterial radiation, rather than being recent invaders. Further evolutionary and biochemical studies are needed to elucidate the frequencies, and by which mechanisms these introns are transferred laterally.

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References


