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Dynamics of 5S rDNA in the tilapia *(Oreochromis niloticus)* genome: repeat units, inverted sequences, pseudogenes and chromosome loci

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Abstract. In higher eukaryotes, the 5S ribosomal DNA (5S rDNA) is organized in tandem arrays with repeat units composed of a coding region and a non-transcribed spacer sequence (NTS). These tandem arrays can be found on either one or more chromosome pairs. 5S rDNA copies from the tilapia fish, *Oreochromis niloticus*, were cloned and the nucleotide sequences of the coding region and of the non-transcribed spacer were determined. Moreover, the genomic organization of the 5S rDNA tandem repeats was investigated by fluorescence *in situ* hybridization (FISH) and Southern blot hybridization. Two 5S rDNA classes, one consisting of 1.4-kb repeats and another one with 0.5-kb repeats were identified and designated 5S rDNA type I and type II, respectively. An inverted 5S rRNA gene and a 5S rRNA putative pseudogene were also identified inside the tandem repeats of 5S rDNA type I. FISH permitted the visualization of the 5S rRNA genes at three chromosome loci, one of them consisting of arrays of the 5S rDNA type I, and the two others corresponding to arrays of the 5S rDNA type II. The two classes of the 5S rDNA, the presence of pseudogenes, and the inverted genes observed in the *O. niloticus* genome might be a consequence of the intense dynamics of the evolution of these tandem repeat elements.

Studies on ribosomal RNA genes have gained prominence in a broad range of animals and plants, especially in relation to species or population characterization and evolutionary relationships. In higher eukaryotes, tandem arrays of ribosomal RNA genes are organized in two distinct multigene families composed of hundreds to thousands of copies (Hadjiolov, 1985). The major class of rDNA comprises the genes that code for the 18S, 5.8S and 28S rRNAs that are related to the nucleolar organizer regions (NORs) (see Long and David, 1980). The rDNA that codes for the 5S rRNA gene represents the second ribosomal family. The general nucleotide sequence and positions of these two multigene families have contributed to understanding the structure, organization and evolution of genomes.

The 5S rDNA array consists of multiple copies of a highly conserved 120 base pair (bp) coding sequence, separated by a variable non-transcribed spacer (NTS) (see Long and David, 1980). Although several papers have considered the usefulness of 5S rDNA sequences as phylogenetic or population markers (Suzuki et al., 1994; Pendás et al., 1995; Baker et al., 2000), special attention must be exercised, mainly in phylogenetic interpretations, as the 5S rDNA family might show a complex organization with the presence of paralogous sequences in the genome (Sajdak et al., 1998; Martins and Galetti, 2001a). Moreover, in some eukaryotes from non-related taxonomic

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Table 1. Description of the primer sets used to amplify distinct 5S rDNA segments of *Oreochromis niloticus*

Primers		Nucleotide sequence (5' - 3')	Original sequence used to design the primers
set 1	5SA	TACGCCCGATCTCGTCCGATC	5S rRNA sequence of rainbow trout (Komiya and Takemura 1979)
	5SB	CAGGCTGGTATGGCCGTAAGC	
set 2	5SC	AGCCCAGACGGACTAGCCAT	internal sequence of the NTS type I of *Oreochromis niloticus* obtained with the primer set 1
	5SD	GGAACTTGTTAACCTTGTAG	
set 3	5SE	CATGAAGCTGCCTCCACTGAG	internal sequence of the NTS type I of *Oreochromis niloticus* obtained with the primer sets 1 and 2
	5SF	AGAGTGCCTCAGATTCGTTTC	
set 4	NTS type IA	GTGGTTAGTGAGTCTTGCG	internal sequence of the NTS type I of *Oreochromis niloticus* obtained with the primer sets 1, 2 and 3
	NTS type IB	TATGGCCGTAAGCGAGAG	
set 5	NTS type IIA	TGCTGCTGTCTGTTTGTG	internal sequence of the NTS type II of *Oreochromis niloticus* obtained with the primer set 1
	NTS type IIB	CAGCGAGGACGACAAACA	

groups, 5S rRNA genes can be found interspersed with other multicopy elements, such as histone genes, 45S rDNA, and repeated trans-spliced leader sequences (Andrews et al., 1987; Drouin, 1999; Drouin and Moniz de Sá, 1995).

A considerable amount of information on structural and functional organization of 5S rRNA genes has been described for plants (Hanson et al., 1996; Adacchi et al., 1997; Amarasinghe and Carlson, 1998; among others), mammals (Little and Braaten, 1989; Leah et al., 1990; Suzuki et al., 1994) and some amphibian species (Korn, 1982; Vitelli et al., 1982; del Pino et al., 1992). In most eukaryotes, the 5S rRNA genes are generally detected in distinct areas of the genome, organized as one or more tandemly repeated clusters, and the number of 5S rRNA genes ranges from 100 to 300,000 copies, which is usually higher than the number of the 45S rRNA genes (Hadjiolov, 1985). While in Drosophila, for instance, there is only one cluster of about 160 copies of the 5S rRNA gene, in *Xenopus laevis* the 5S rRNA gene clusters are located at the telomere regions of most, if not all, of its chromosomes (Pardue et al., 1973).

In many vertebrates, the 5S rDNA genes are located on a single chromosome pair, while NORs are often present on multiple chromosomes (Suzuki et al., 1996; Makinem et al., 1997). In amphibian (Schmid et al., 1987; Lucchini et al., 1993) and fish species (Martins and Galetti, 2001b), the 5S rRNA genes can be found on several chromosomes. Moreover, NOR and 5S rDNA loci may assume a syntenic organization in the same chromosome (Pendás et al., 1994; Móran et al., 1996) or can be detected in different chromosome pairs (Martínez et al., 1996; Sajdak et al., 1998; Martins and Galetti, 1999; Wasko et al., 2001). Localization of the 5S rDNA in fishes is important in comprehending the structure and organization of repeated sequences in the chromosomes.

To further our understanding of the dynamics and evolution of 5S rRNA gene arrays in the fish genome, we report here the structure and organization of 5S rDNA in *Oreochromis niloticus*. The detection of two different types of tandem repeats located in distinct chromosome loci and inverted 5S rRNA genes and pseudogenes in this species reflect the intense dynamics of the evolution of tandem repeat elements.

Materials and methods

DNA samples, PCR, cloning and sequencing

Ten adult specimens (five females and five males) of *O. niloticus* were obtained from the tilapia culture facility at Dalhousie University (Halifax, Nova Scotia, Canada) and from Jurema fish farm (Macatuba, São Paulo, Brazil). At least three males and three females were used in each of the analyses performed. DNA was extracted from the liver according to Sambrook and Russell (2001) and PCR amplifications of the 5S rDNA repeats were performed as described in Martins et al. (2000). PCR amplifications were performed using five primer sets (Table 1). The location of the primers employed and expected PCR amplification products are shown in Fig. 1. The PCR-amplified products were cloned in the plasmid pGEM-T (Promega) and used to transform competent cells of the *E. coli* strain DH5α (Gibco-BRL). Clones were sequenced on the OpenGene Automated DNA Sequencing System I (Visible Genetics Inc.) with a Thermo Sequenase Cy 5.5 Terminator Cycle Sequencing kit (Amersham Pharmacia Biotech), and sequence alignments were performed using ClustalW (Thompson et al., 1994). Nucleic acid sequences were subjected to BLAST/N (Altschul et al., 1990) searches at the National Center for Biotechnology Information (NCBI) website (http://www.ncbi.nlm.nih.gov/blast).

Southern blot hybridization

The genomic organization of 5S rDNA was determined by Southern blot hybridization. 10 μg of genomic DNA was partially digested with *Sca*I, *Msp*I, *Hin*dIII, and *Pst*I, and completely digested with *Hin*dIII, *Sac*I, *Msp*I, *Pst*I, and *Pvu*II. The digestion products were subjected to 1% agarose gel electrophoresis and Southern-transferred to a Hybond-N nylon membrane (Southern, 1975). The hybridization of the filter-immobilized DNA was performed using three different probes generated by the primer sets 1, 4, and 5, respectively: (1) 5S rDNA monomer (5S rRNA gene + NTS), (2) NTS type I, and (3) NTS type II. The NTS probes (probes 2 and 3) were obtained with specific primers that do not amplify the 5S rRNA gene sequence. Probes were labelled and detected by the ECL-Direct Nucleic Acid Labelling and Detection System (Amersham Pharmacia Biotech), following the instructions of the manufacturer.

Chromosome hybridization

Mitotic chromosomes were obtained from anterior kidney cells (Bertollo et al., 1978) and submitted to fluorescent *in situ* hybridization (FISH) (Pinkel et al., 1986), following some modifications described in Oliveira and Wright (1998) and Martins and Galetti (2001a). The same three probes (5S rDNA monomer, NTS type I, and NTS type II) employed in the Southern hybridization were also used in the chromosome FISH experiments. Single probes were labelled by nick translation with biotin-14-dATP (Bionick Labelling System, Gibco-BRL) and detected with avidin-FITC conjugate (Sigma) followed by two rounds of signal amplification. Chromosomes were counterstained with propidium iodide (50 μg/ml) and the slides were mounted with antifade (Biorad). Biotin and conjugated FluoroRed dUTP-rhodamine (Amersham Pharmacia Biotech) were used on simultaneous hybridizations (double FISH) to label each of the two NTS probes (NTS type I and NTS type II).

The procedures of signal detection and amplification were carried out only for the biotin-labelled probe, since these steps are not necessary for Fluoro-Red. Chromosomes were counterstained with DAPI (0.3 μg/ml).

Results

PCR amplification of the 5S rDNA of *O. niloticus* using the primer set 1 generated one band of approximately 500 bp, according to the results described by Martins et al. (2000). Primer sets 2 and 3 revealed PCR products of approximately 1.4 kb and 450 bp, respectively. DNA sequences were determined for 37 positive clones of all the obtained PCR products. After the sequence alignment, the DNA sequences were distributed in two classes: one with monomers of 1,405 bp (denominated 5S rDNA type I) and another one with monomers of 475 bp (denominated 5S rDNA type II) (Fig. 1). The 5S rDNA sequences were subjected to BLAST/N searches (Altschul et al., 1990) at NCBI, which enabled us to identify a region that corresponds to the coding sequence of a putative 5S rRNA bona fide gene (position 1–120 of the 5S rDNA type I and type II). Moreover, an inverted 5S rRNA putative pseudogene (position 511–577) and an inverted putative 5S rRNA bona fide gene (position 960–1078) were also seen in the 5S rDNA type I sequence (Fig. 1). Sequencing of the 5S rDNA of *O. niloticus* also permitted the identification of two conserved sequences designated C1 and C2 (Fig. 1). The C1 sequence is downstream of both 5S rRNA genes of the 5S rDNA type I. The C2 sequence is located downstream the 5S rRNA gene of type II and also downstream the first 5S rRNA gene copy of type I. A TATA-like element was observed upstream of the 5S rRNA genes of both 5S rDNA types including the inverted 5S rRNA gene (Fig. 1).

Based on the sequences of the 5S rDNA type I and type II of *O. niloticus*, two sets of oligonucleotides (primer sets 4 and 5) were designed in order to amplify specific NTS segments of each type of 5S rDNA. The isolated NTSs were termed NTS

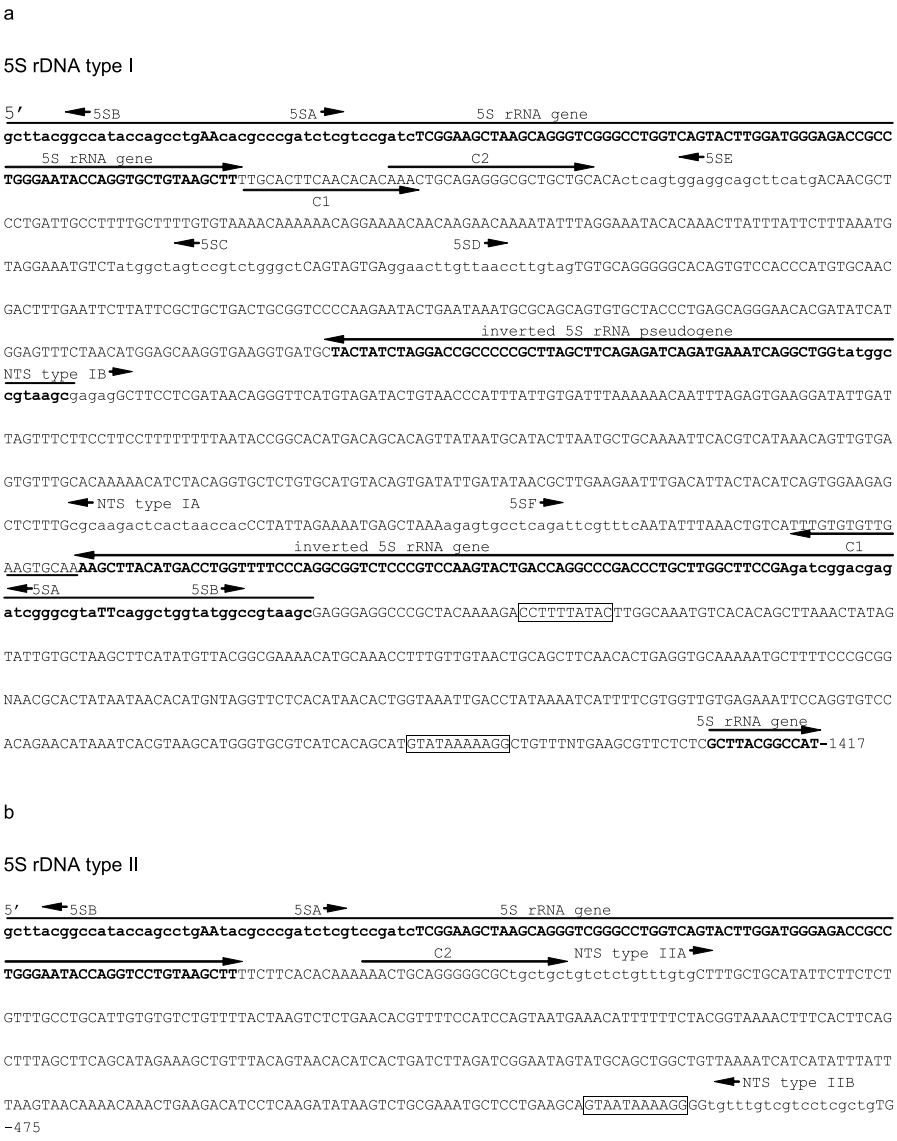

Fig. 1. DNA sequences of the two 5S rDNA unit classes identified in *Oreochromis niloticus:* (a) 5S rDNA type I; (b) 5S rDNA type II. The coding sequences of both 5S rDNA classes are in boldface type and arrows indicate the direction of transcription of the 5S rRNA genes. Also indicated are the two conserved sequences (C1 and C2) in the NTSs. TATA-like elements are evidenced in boxes. Primers used to obtain the sequences are in lower case and an arrowhead indicates the direction of the amplification. The sequences are deposited in GenBank under the accession numbers AF478461 and AF478462.

type I, related to the 5S rDNA type I, and NTS type II, related to the 5S rDNA type II.

Southern blot hybridization analysis was conducted using several restriction endonucleases, selected for their recognition sites within the 5S rDNA sequences of *O. niloticus*. Hybridization using probe 1 (5S rDNA monomer) showed a complex organization of the 5S rDNA in the genome of this species (Fig. 2a). Clearly hybridization signals indicated the presence of 5S rDNA monomers of 1.4 kb, identified by the enzymes *Sac*I and *Msp*I, and bands of 0.5 kb, evidenced by the enzymes *Hin*dIII, *Pst*I and *Pvu*II. Moreover, the enzymes *Hin*dIII and *Pst*I also detected bands of 0.8 kb and 1.2 kb, respectively.

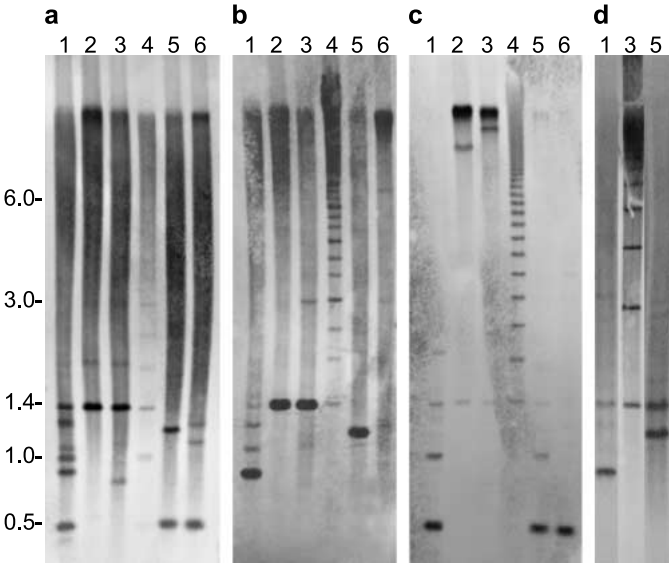

Fig. 2. Southern blot-hybridization of genomic DNA from *Oreochromis niloticus* using three 5S rDNA probes: 5S rDNA monomer (a), NTS type I (b and d), and NTS type II (c). (1) *Hin*dIII, (2) *Sac*I, (3) *Msp*I, (4) *Sca*I, (5) *Pst*I, (6) *Pvu*II. A partially digested pattern was obtained with *Sca*I (a, b, and c). Partially digested DNA samples were also visualized with *Hin*dIII, *Msp*I, and *Pst*I (d). Molecular weight markers are indicated on the left of the panels.

In order to examine the correlation between the 5S rDNA type I and the 5S rDNA type II sequences of *O. niloticus* and the 5S rRNA gene hybridization pattern obtained with probe 1, Southern hybridization was also performed using probe 2 (NTS type I) and probe 3 (NTS type II) (Fig. 2b, c, d). The NTS type I hybridized to repeat units of 1.4 kb digested by *Sac*I and *Msp*I and to smaller units of 1.2 kb (*Pst*I) and 0.8 kb (*Hin*dIII) (Fig. 2b). Partial digestions with *Hin*dIII and *Pst*I showed that the smaller units are included within the large *Msp*I 1.4-kb monomer that is tandemly arrayed (Fig. 2d). As the enzyme *Pvu*II does not have restriction sites in the 5S rDNA type I, an intense signal of high molecular weight was observed in the hybridization of probe 2 to the *Pvu*II-digested DNA of *O. niloticus* (Fig. 2b). Southern hybridizations with probe 3 revealed that the NTS type II hybridized to 0.5-kb monomers of *Hin*dIII, *Pst*I, and *Pvu*II-digested DNA of *O. niloticus* and to multimeric units generated by *Sca*I partially-digested DNA (Fig. 2c). An intense band of high molecular weight was evident in the DNA samples digested by *Sac*I and *Msp*I, enzymes that do not have a recognition site in the 5S rDNA type II sequence (Fig. 2c). The NTS type I and NTS type II hybridization results correspond to the band pattern observed in the Southern hybridization with the 5S rRNA gene (probe 1) (Fig. 2a).

The chromosomal localization of the 5S rRNA genes and both NTSs in *O. niloticus* was achieved by FISH and double-FISH using probes 1 (5S rDNA monomer), 2 (NTS type I) and 3 (NTS type II). The 5S rDNA was detected in an interstitial position in the long arm of two pairs of subtelo-acrocentric chromosomes and terminally in the short arm of one subtelo-acrocentric chromosome pair (Fig. 3a). FISH using probes of each NTS separately and double-FISH with both NTSs showed

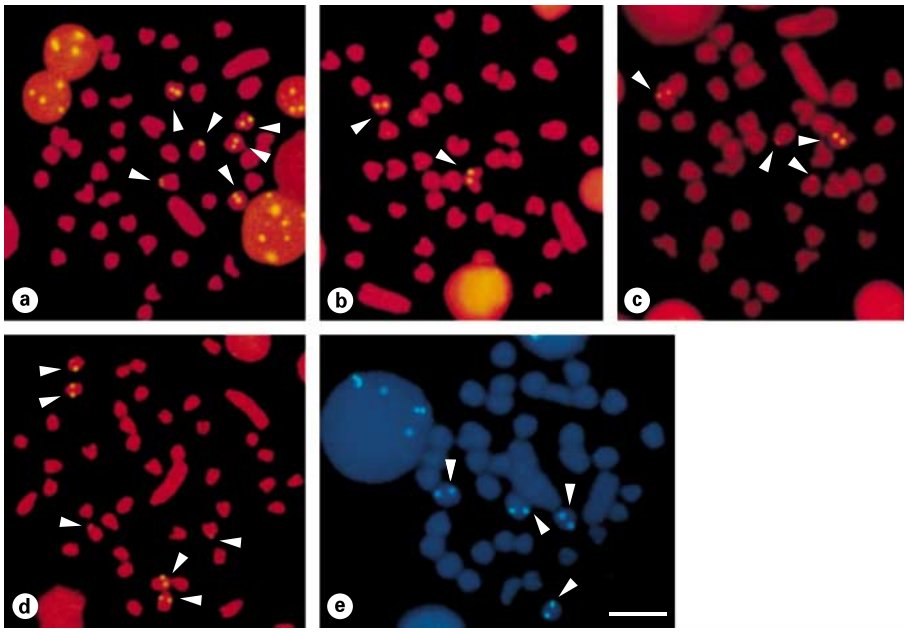

Fig. 3. Chromosome metaphase spreads of *Oreochromis niloticus*. FISH probed with 5S rDNA monomer (a), NTS type I (b), NTS type II (c), and NTSs type I + NTS type II (d). Double-FISH probed with NTS type I (red) and NTS type II (green) (e). Arrows indicate the detected loci. Bar represents 5 μm.

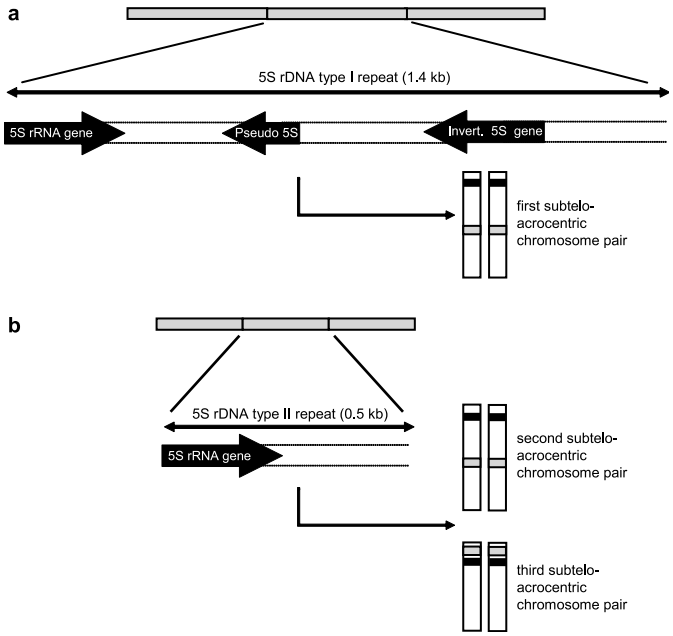

Fig. 4. Scheme of the organization of the 5S rDNA type I (a) and 5S rDNA type II (b) in the *O. niloticus* genome. The chromosome loci are represented below and on the right of the DNA sequence scheme. Dark bands represent the chromosome centromeres. Light bands indicate the 5S rDNA chromosome localization. The 5S rDNA is located in three chromosome pairs: the first pair corresponds to the position of the NTS type I (first subtelocentric-acrocentric pair); the second (second subtelocentric-acrocentric pair) and the third (third subtelocentric-acrocentric pair) correspond to the sites of the NTS type II.

that the NTS type I was present in one of the subtelo-acrocentric chromosome pair bearing the interstitial 5S rRNA gene cluster, while the NTS type II showed strong hybridization to the other interstitially located 5S rRNA gene cluster and faint hybridization to the terminal region of the third chromosome pair bearing the 5S rRNA genes (Fig. 3b, c, d). Double-FISH experiments were also performed to determine if both NTS classes were coincident/adjacent and interstitially located in the same subtelo-acrocentric chromosome pair. The results showed that each NTS type was localized at a distinct subtelo-acrocentric chromosome pair (Fig. 3e). The terminal 5S rDNA cluster was not detected by double-FISH using both NTSs as probes (Fig. 3e). A schematic summarizing of the genomic organization of the 5S rDNA in *O. niloticus* is presented in Fig. 4.

Discussion

5S rDNA tandem repeats

While the 5S rRNA gene is conserved even among non-related taxa, the non-transcribed spacer shows extensive length and sequence variation, which can give an accentuated dynamism to the 5S rRNA genes (Williams and Strobeck, 1985). Studies of 5S rDNA sequences among fish species have identified variant types of the 5S rDNA tandem repeats characterized by remarkable differences in the NTSs. The presence of two types of tandem repeats of this ribosomal DNA has been observed for several fish species of Characiformes (Martins and Galetti, 2001a; Wasko et al., 2001), Perciformes (Martins et al., 2000) and Salmoniformes (Pendás et al., 1994; Morán et al., 1996; Sajdak et al., 1998). Based on this information, Martins and Galetti (2001b) have suggested that two classes of 5S rDNA tandem repeats could represent a general feature for 5S rDNA in fishes.

In the tilapiine cichlid fish *O. niloticus,* two distinct 5S rDNA units (designated 5S rDNA type I and 5S rDNA type II) were also identified. Although previous analysis also detected the presence of two 5S rDNA types in *O. niloticus* (Martins et al. 2000), the obtained sequences were incomplete and no information about their organization in the genome was described. For the present paper, new results on the sequence and genome organization of the two tandem repeat classes of 5S rDNA were obtained. The exact size of the repeats, the presence of pseudogenes and inverted sequences in one of them, and their organization in the genome were determined. Each 5S rDNA class is characterized by a distinct NTS that varied in nucleotide sequence and length between the loci. Moreover, an inverted putative pseudogene and a 5S rRNA bona fide gene sequence were detected within the 5S rDNA type I. 5S rDNA variants related to NTS variations and pseudogenes have also been reported for mammals and amphibians (Jacq et al., 1977; Fedoroff and Brown, 1978; Little and Braaten, 1989; Leah et al., 1990; Suzuki et al., 1994; Frederiksen et al., 1997). A dual 5S rRNA gene system, differently regulated in somatic and oocyte cells, has been described for a number of vertebrate species, including fish and amphibians (Komiya et al., 1986). In these organisms both 5S rDNAs were highly conserved in their coding sequence, but their NTSs were very distinct. In *X. laevis*, for example, the oocyte unit is about 750 bp and it includes the 120-bp coding region, an NTS and a pseudogene (Jacq et al., 1977; Fedoroff and Brown, 1978), while the somatic unit has approximately 880 bp and does not contain pseudogenes (Peterson et al., 1980). The variant classes of the 5S rDNA observed in different fish species, including *O. niloticus*, could represent the oocyte and somatic 5S rRNA gene types well documented for *Xenopus*.

It seems possible that tandemly arrayed multigene families evolve according to homogenization processes governed by molecular drive (Dover, 1986) and concerted evolution (Elder and Turner, 1995), resulting in a sequence similarity of the repeating units that is greater within than between species. However, even within the same genome, different classes of tandem repeats can be detected, as observed in the 5S rDNA, which suggests a complex pattern of organization of this ribosomal gene class. The organization of *O. niloticus* 5S rDNA indicates that the sequence similarity of the repeating units is greater within the same array, but can differ extensively between loci.

The 5S rRNA gene is transcribed by the RNA polymerase III and it contains an internal control region (ICR), which functions as a promoter for the gene (Hallenberg et al., 1994). Until recently it was believed that NTSs had no function. However, it seems likely that a TATA sequence located in the NTS plays an important role in regulation of 5S rRNA gene expression in sev-

eral mammals (Nederby-Nielsen et al., 1993; Suzuki et al., 1996). TATA-like sequences have been observed upstream the 5S rRNA gene in several fish species such as *Salmo salar* (Pendás et al., 1994), *Carassius auratus* (Murakami and Fugitani, 1998), *Coregonus* (Sajdak et al., 1998), *Gasterosteus aculeatus* (Rocco et al., 1999), *Acheilognathus tabira, Cyprinus carpio* (Inafuku et al., 2000), *Brycon* (Wasko et al., 2001) and *Leporinus* (Martins and Galetti, 2001a), suggesting that this element may influence the transcription control of the 5S rRNA genes. TATA-like elements (GTATAAAAG) were also observed upstream the 5S rRNA genes of both 5S rDNA classes detected in *O. niloticus*, suggesting their transcriptional activity.

Pseudogenes and inverted sequences

Inverted 5S rRNA genes have been reported only within the 45S rDNA array of some non-related eukaryotes (Drouin and Muniz de Sá, 1995). The inverted putative 5S rRNA bona fide gene detected in *O. niloticus* corresponds to the first description of such sequence inside a 5S rDNA array. The inverted gene and the 5S rRNA gene observed in the 5S rDNA type I of *O. niloticus* were very similar, presenting 120 bp and just two nucleotide substitutions. Moreover, a conserved short-sequence, C1, was identified downstream from both 5S rRNA genes of 5S rDNA type I. Whether the first or the second 5S rRNA gene of the type I sequence is inverted was not clearly elucidated. However, the presence of a pseudogene in the same orientation of the second 5S rRNA gene suggests that the first 5S rRNA gene may have originated the second copy and the pseudogene through the occurrence of an inversion/duplication. The presence of TATA-like elements downstream from the 5S rRNA inverted gene of *O. niloticus* also suggests that this inverted segment could be transcriptionally active, since similar TATA-like nucleotide regions (GTATAAAAGG) were also observed upstream the 5S rRNA gene of both 5S rDNA classes of the species and these sequences seem to play an important role in regulation of 5S rRNA gene expression (Nederby-Nielsen et al., 1993; Suzuki et al., 1996).

BLAST/N searches (Altschul et al., 1990) of the NTS sequences of the 5S rDNA type I from *O. niloticus* showed that the nucleotide sequence between positions 577 and 510 (3′→5′ direction) was similar to the sequence between positions 1–76 of the 5S rRNA gene of several vertebrates. The isolated putative 5S rRNA pseudogene of *O. niloticus* is very similar to the 5S rRNA gene of the species, differing by base substitutions and deletion of part of the gene and it also shows similarity to mouse and human 5S rDNA pseudogenes (Leah et al., 1990; Hallenberg et al., 1994). Pseudogenes are a consequence of gene duplication that can occur in two fundamentally different ways: by transposition or via genomic DNA duplication (Mighell et al., 2000). 5S rDNA variants and pseudogenes seem to be common in mammals (Emerson and Roeder, 1984; Doran et al., 1987; Leah et al., 1990; Hallenberg et al., 1994) and have already been isolated from rat, mouse and human cells (Nederby-Nielsen et al., 1993). Among fish, 5S rRNA pseudogenes have only been described for one other species, *Carassius auratus langsdorfi* (Murakami and Fujitani, 1998). Once the *O. niloticus* 5S rRNA pseudogene is in the same direction of the inverted 5S rRNA gene, this element might have arisen as a consequence of the mechanisms involved in the inversion of the 5S rRNA gene. Whether the inverted 5S rRNA gene and the pseudogene are transcribed or not remains to be elucidated.

The presence of the conserved sequence C2 at the 3′-end of both types of 5S rRNA gene copies suggests a common origin for the 5S rDNA type I and type II of *O. niloticus*. Searches of C2 sequence at NCBI did not detect homology of C2 with any 5S rDNA sequence of other fish species. Moreover, the occurrence of this conserved sequence in both classes of 5S rDNA and the occurrence of an inverted 5S rRNA gene in the same orientation of the pseudogene in the 5S rDNA type I suggest that events of insertion/deletion and inversion might have played a role in the evolution of the 5S rDNA type I of *O. niloticus*. The organization of the 5S rDNA in tandem repeats probably facilitates the origin of such elements by duplication of genomic segments. As the 5S rRNA pseudogene of *O. niloticus* is maintained in the tandem repeat copies of the 5S rDNA type I, it suggests that this stretch of DNA might have arisen at the same time as the differentiation of both 5S rDNA types.

The evolution of repetitive sequences is thought to be subjected to the action of several molecular mechanisms such as gene conversion, unequal crossing-over, transposition, slippage-replication and RNA-mediated exchanges, that cause non-Mendelian segregation ratios leading to a particular pattern of evolution (Dover, 1986). Such mechanisms can lead to the occurrence of accentuated variations in the repeat units of a tandemly arrayed multigene family.

Chromosome location

The chromosomal locations of the 5S rRNA genes have been described for 48 fish species representing distinct groups, such as Acipenseriformes, Anguilliformes, Cypriniformes, Characiformes, Salmoniformes, Perciformes, and Tetraodontiformes. For cichlid fishes, the chromosomal location of the 5S rDNA has only been determined in *O. niloticus* (Martins et al., 2000; present data). In almost all the fish species studied, the location of the 5S rDNA occurs at an interstitial position in the chromosomes. This same 5S rDNA chromosomal location has been observed in mammals (Mellink et al., 1996; Frederiksen et al., 1997; Mäkinem et al., 1997; among others) and amphibians (Vitelli et al., 1982; Schmid et al., 1987; Lucchini et al., 1993), suggesting that such organization may not be casual. An interstitial distribution for the 5S rRNA gene loci could represent some advantage related to the organization of these genes in the vertebrate genome (Martins and Galetti, 2001b).

Although previous FISH studies have identified two chromosome pairs bearing the 5S rDNA loci in *O. niloticus* (Martins et al., 2000), the present study revealed a third 5S rDNA locus. While the 5S rDNA type I was detected in an interstitial position in the long arm of a subtelo-acrocentric chromosome pair, the 5S rDNA type II was identified interstitially in the long arm of a different subtelo-acrocentric pair and at the terminal region in the short arm of another subtelo-acrocentric chromosome pair. The assignment of different classes of 5S rDNA to distinct chromosome loci was previously described for the fish *Leporinus* (Martins and Galetti, 2001a). The results described for the 5S rDNA organization in *O. niloticus* are in agreement with the data observed for *Leporinus*, reinforcing

the idea that distinct 5S rDNA classes occupy different chromosome loci. The distinct 5S rDNA classes and their different chromosome location reflect the absence of non-homologous chromosome exchange between the chromosome pairs bearing 5S rDNA clusters. This scenario is in agreement with the idea that individual chromosomes occupy specific territories in the nucleus (Lamond and Earnshaw, 1998) and the chromosomes bearing 5S rDNA clusters seem to be evolving independently in individual nuclear environments.

The presence of the conserved C2 sequence downstream of the 5S rRNA coding region of both 5S rDNA types in *O. niloticus* suggests that the 5S rDNA type I and the 5S rDNA type II have derived from the same ancestral array. The identification of variants such as a pseudogene and an inverted gene in the 5S rDNA type I and the detection of the 5S rDNA type II in two chromosome loci and the 5S rDNA type I in just one chromosome locus suggests that the 5S rDNA type I is derived from the 5S rDNA type II.

Conclusion

Although the exact number of different 5S rDNA tandem repeats remains to be clearly elucidated, the existence of different 5S rDNA classes seems to be a rule for fishes. However, we could not eliminate the possibility of the existence of other 5S rDNA sequences organized in tandem repeats or dispersed copies in the genome. As demonstrated for *O. niloticus*, as a consequence of the intense dynamics of the tandem repeat units, the different 5S rDNA classes might present not only very distinct NTS, but also other variants such as pseudogenes and inverted gene sequences. Further investigation of the functional role of the pseudogenes and inverted genes present in the 5S rDNA types of *O. niloticus* may clarify the role, if any, of such sequences in the genome. The investigation of the 5S rDNA organization in other species of *Oreochromis*, as well as in other species of Tilapiine, will contribute to the elucidation of the level of complexity of 5S rDNA organization and evolution in this fish group. Moreover, further studies on several members of different fish orders are needed to improve on 5S rDNA data in this vertebrate group.

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