Chromosome Research **7**: 363–367, 1999.

Chromosomal localization of 5S rDNA genes in *Leporinus* fish (Anostomidae, Characiformes)

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Received 9 February 1999; received in revised form and accepted for publication by M. Schmid 7 May 1999

Key words: FISH, fish chromosome, *Leporinus*, PRINS, 5S rDNA

Abstract

The large 45S rDNA chromosome sites have often been analyzed in fish. In contrast, little is known about the 5S genes in this animal group. In the genus *Leporinus*, the NOR chromosomal location has been shown to be very diverse. In the present work, chromosome mapping of 5S rDNA in three anostomids, *Leporinus elongatus*, *L. obtusidens* and *L. friderici*, is investigated using fluorescence *in-situ* hybridization (FISH) with PCR-obtained 5S probes and primed *in-situ* labeling (PRINS). Major 5S rDNA chromosomal sites were found to be subterminally located in a small metacentric pair, while minor ones were detected near the centromeric region of a medium-sized submetacentric pair in all studied species. The 5S rDNA genes were not associated with the NORs or sex chromosomes. A highly conserved chromosomal location of these genes appears to characterize the karyotype evolution of this fish group.

Introduction

The 5S rDNA multigene family consists of a highly conserved coding sequence of 120 bp forming arrays of hundreds to thousands of tandem copies, which are kept separated from each other by variable non-transcribed spacers (NTS). These 5S genes are clustered in a single locus or in multiple loci (see Danna *et al*. 1996 for review). In lower eukaryotes, 5S genes can be found interspersed with other multicopy genes, including large 45S pre-rRNA. In higher eukaryotes, the 5S and 45S genes have often been shown to be in separate areas of the genome (Drouin & Moniz de Sá 1995).

The NORs have been detected in fish and other organisms by silver nitrate and GC-specific fluorochrome staining (Galetti *et al*. 1984, 1995, Castro *et al*. 1996, Sola *et al*. 1997, among others). In contrast, little is known about the 5S rDNA location in fish chromosomes (Pendás *et al*. 1994, Martínez *et al*. 1996, Morán *et al*. 1996, Murakami & Fujitani 1998).

In several organisms, the 5S rDNA genes appear strongly constrained to only one chromosome pair, while NORs are often present in multiple chromosomes (Suzuki *et al*. 1996, Mäkinem *et al*. 1997). In amphibians (Schmid *et al*. 1987, Lucchini *et al*. 1993) and some fish species (Murakami & Fujitani 1998), 5S clusters may occur in several chromosomes. Moreover, NOR and 5S rDNA sites may assume a syntenical organization in the same chromosome (Pendás *et al*. 1994, Morán *et al*. 1996) or they can be detected in different chromosomes (Martínez *et al*. 1996, Sajdak *et al*. 1998).

At present no information exists on 5S rDNA chromosome location in neotropical fish species. In contrast, the NORs have been extensively analyzed in this ictiofaune (Galetti 1998 for revision). Particu-

larly within Anostomidae, the NORs have been studied in *Leporinus* and *Schizodon* genera (Galetti *et al*. 1991, 1995, Martins & Galetti 1997, among others). Most *Leporinus* species show NOR sites located in different chromosomes, characterizing important cytotaxonomic markers (Galetti *et al*. 1984, 1991).

In the present work, the 5S rDNA physical chromosome location was analyzed in three *Leporinus* species: *L. elongatus*, *L. obtusidens* and *L. friderici* using fluorescence *in-situ* hybridization (FISH) and primed *in-situ* labeling (PRINS) to investigate the variability of these genes among these fishes. New insights into the evolutionary dynamics of 5S rDNA genes in this animal group are also discussed.

Materials and methods

Individuals of *Leporinus elongatus*, *L. obtusidens* and *L. friderici* were caught in the Mogi-Guaçu river, township of Pirassununga, São Paulo State, Brazil. Mitotic chromosomes were obtained from anterior kidney cells following a conventional air drying technique described elsewhere (Bertollo *et al*. 1978).

For FISH, 5S rDNA probes were obtained from DNA of *Leporinus obtusidens* by PCR (polymerase chain reaction) using primers A (5′-TACGCCCGATCTCGTCCGATC-3′) and B (5′-CAGGCTGGTATGGCCGTAAGC-3′) first designed to amplify 5S RNA genes and their NTS from *Salmo trutta* (Pendás *et al*. 1994). PCR products of about 200 bp were purified using the Sephaglas BandPrep Kit (Pharmacia Biotech) and biotin labeled by nick translation according to the manufacturer's instructions (BioNick labelling System, GibcoBRL). A basic FISH protocol was carried out as described in Martins & Galetti (1998). Sequential silver nitrate staining (Howell & Black 1980) was performed after rinsing the FISH/ slides with tap water followed by dehydration in an alcoholic series.

PRINS was carried out according to Kock *et al*. (1989) and Coullin *et al*. (1997) with some modifications, employing the same primer set used in FISH. Chromosomes were denatured in formamide 70% 2 × SSC at 70°C for 2 min, followed by dehydration in an ice-cold ethanol series (75%, 85% and 100%) for 5 min in each solution and subsequent air drying. A reaction mixture containing 100 pmol of both oligonucleotides (primers A and B) 10 nmol each of dATP, dGTP and dCTP, 5 nmol each of dTTP and Bio-16-dUTP (Boehringer Mannheim), 10% glycerol, 10% *Taq* buffer and 3 U of *Taq* DNA polymerase (Pharmacia Biotech) was prepared yielding a final volume of 75 μl/slide. The mixture was transferred to slides and covered with a coverslip. The slides were then transferred to an aluminium plate, in which the polymerization reaction was performed for 20–30 min at annealing temperature (63°C). The reaction was stopped in 500 mmol/L NaCl/50 mmol/L EDTA, pH 8, solution at 72°C for 1 min. The detection reaction used two layers of avidin–FITC. Propidium iodide (0.2%) was used for chromosome counterstaining and antifade (25 μl/slide) was added to the slides under the coverslip.

Results and discussion

Giemsa karyotype, C-banding, Ag-NOR and GC-fluorochrome staining of *Leporinus elongatus*, *L. obtusidens* and *L. friderici* have been reported previously (Galetti *et al*. 1984, 1991, 1995, Koehler *et al*. 1997) showing $2n = 54$ in both sexes. All presented a ZZ/ZW sex chromosome system, except *L. friderici*. Both FISH and PRINS revealed a major 5S cluster located subterminally in a small metacentric chromosome pair (17th pair) of all studied species (Figure 1). Additionally, a minor 5S cluster was detected near the centromeric region on the long arm of a medium-sized submetacentric pair (10th pair). Size and fluorescence intensity polymorphisms of this minor signal were frequently observed between the homologous chromosomes. 5S sites were not associated with NOR genes or sex chromosomes in *Leporinus* species. Sequential Ag-staining revealed NOR sites in a large submetacentric pair (2nd pair) of *L. elongatus* and a large metacentric (2nd pair) of *L. obtusidens* and *L. friderici* (Figure 1), as previously reported (Galetti *et al*. 1984, Molina *et al*. 1998).

Similarly, independent chromosomal distributon of NOR and 5S rDNA sites has already been reported in *Anguilla anguilla* (Martínez *et al*. 1996), *Salmo truta* (Morán *et al*. 1996), *Coregonus artedi* and *C. zenithicus* (Sajdak *et al*. 1998), which so far is the configuration most frequently observed in vertebrates (Lucchini *et al*. 1993, Suzuki *et al*. 1996). Thus, in all these cases, the 5S and 45S clusters are located in

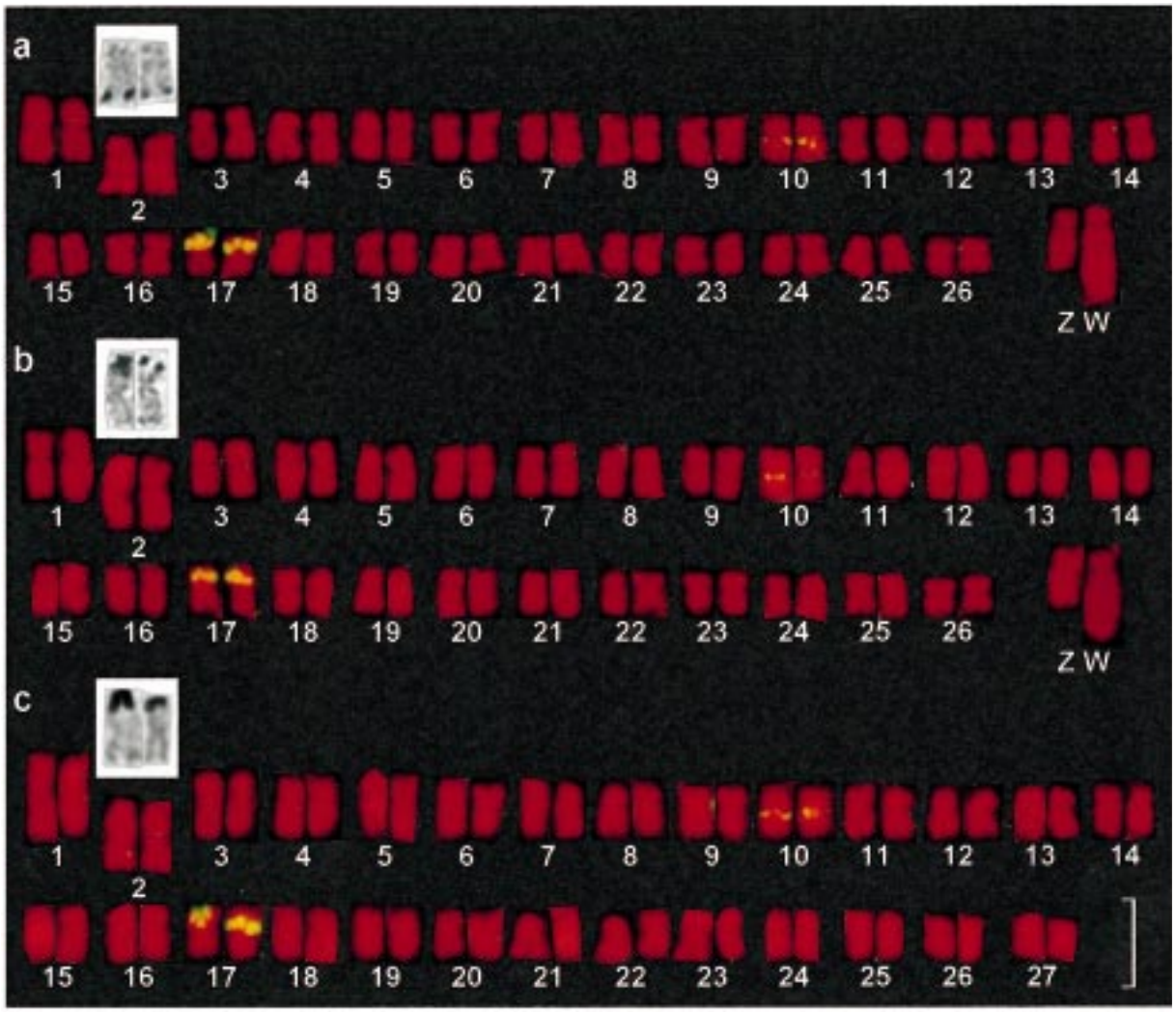

Figure 1. Karyotypes of *Leporinus elongatus* female (a), *L. obtusidens* female (b) and *L. friderici* male (c) after *in-situ* hybridization (FISH) showing 5S rDNA sites (pairs 10 and 17). The Ag-NOR-bearing chromosome pair is shown in the white box. Scale bar = 5 μm.

different chromosomal environments, following unrelated evolutionary pathways.

In contrast, in *Salmo salar* and *Oncorhynchus mykiss* (Pendás *et al*. 1994, Morán *et al*. 1996) the 5S and 45S sites may be linked in the same chromosome and probably evolve together. Gene conversion and unequal crossing-over are important in the maintenance of a conserved and multiple array of these multigene families (Dover 1986). It appears that both mechanisms can be more efficient if 5S and 45S clusters remain separated instead of linked in the same chromosome area. In this latter case, disruptive interference, such as undesired translocation of 5S sequences inside 45S arrays might occur. This hypothesis might explain why most vertebrates have these clusters located in distinct chromosomes.

The number of 5S clusters is also quite diverse in the diploid genome of different living plants and animals (Vitelli *et al*. 1982, Lomholt *et al*. 1995, Prado *et al*. 1996, among others). The 5S rDNA genes are often clustered in a single chromosome pair in fish (Pendás *et al*. 1994, Martínez *et al*. 1996), probably representing a more ancient condition among this animal group. In *Leporinus* and some other fish (Murakami & Fujitani 1998), the occurrence of major and minor rDNA sites may represent derived conditions in their evolutionary dynamic. Major and minor 5S rDNA sites have also been

described in humans (Lomholt *et al*. 1995), monkeys (Lomholt *et al*. 1996) and other mammals (Suzuki *et al*. 1996, Christensen *et al*. 1998). In several vertebrates, including fish and amphibians, a dual oocyte and somatic cell 5S rDNA gene system has been reported, and two classes of these genes are differently regulated in each cellular type (Komiya *et al*. 1986). These two 5S rDNA gene types may be nested in distinct chromosome loci and could represent both major and minor 5S rDNA loci in *Leporinus*.

No polymorphism related to the fluorescent signal intensity of the major 5S rDNA locus were detected within or between individuals and species. In contrast, the fluorescent signals over the minor 5S rDNA locus were heteromorphic both in FISH and PRINS, although in very low frequency. The small size of this locus *per se* could impede its detection. Alternatively, such heteromorphisms might be related to variations in the number of copies between both homologous 5S rDNA array tandem repeats. The enhanced signals used in FISH (three layers of avidin–FITC) were fundamental in visualizing these small clusters. In PRINS, the use of only two layers of avidin–FITC suggests greater efficiency in detecting the minor 5S rDNA locus in these fish.

A highly conserved chromosomal location of these 5S rDNA clusters, such as that observed in the three species here studied, may suggest a common feature in the genus *Leporinus*. Further studies using molecular approaches should help to improve understanding of the 5S rDNA organization in the genome of this fish.

Acknowledgements

The authors thank Fundação de Amparo à Pesquisa do Estado de São Paulo (FAPESP) and Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq) for financial support and Dr. L. Sola for help with and suggestions concerning PRINS experiments.

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