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Molecular and cytogenetic analysis of the telomeric $(TTAGGG)_n$ repetitive sequences in the Nile tilapia, *Oreochromis niloticus* (Teleostei: Cichlidae)

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Abstract The majority of chromosomes in *Oreochromis niloticus*, as with most fish karyotyped to date, cannot be individually identified owing to their small size. As a first step in establishing a physical map for this important aquaculture species of tilapia we have analyzed the location of the vertebrate telomeric repeat sequence, $(TTAGGG)_n$, in *O. niloticus*. Southern blot hybridization analysis and a Bal31 sensitivity assay confirm that the vertebrate telomeric repeat is indeed present at *O. niloticus* chromosomal ends with repeat tracts extending for 4–10 kb on chromosomal ends in erythrocytes. Fluorescent in situ hybridization revealed that $(TTAGGG)_n$ is found not only at telomeres, but also at two interstitial loci on chromosome 1. These data support the hypothesis that chromosome 1, which is significantly larger than all the other chromosomes in the karyotype, was produced by the fusion of three chromosomes and explain the overall reduction of chromosomal number from the ancestral teleost karyotype of $2n=48$ to $2n=44$ observed in tilapia.

Introduction

Telomeres are the DNA sequences and proteins found at the ends of linear eukaryotic chromosomes (McEachern et al. 2000; Blackburn 2001). The basic structure and function of the telomere has been conserved throughout evolution (Brown 1989; Cross et al. 1989; Brown et al. 1990), reflecting its important role in stabilizing chromosomes and blocking chromosomal end-to-end fusion and degradation (Blackburn and Szostak 1984; McEachern et al. 2000).

Telomeric DNA is composed of tandem arrays of a species-specific 5–8 bp GT-rich repeated sequence, termed the telomeric repeat (Blackburn 2001). The length of these arrays varies from species to species, from as little as 36 bp in *Oxytricha fallax* (Pluta et al. 1982) to 50–150 kb in *Mus musculus* (Kipling and Cooke 1990; Starling et al. 1990; Ziljmans et al. 1997). Telomeric tract lengths are longer in germline tissue than in somatic tissue. For example, in humans, the telomeric repeat arrays in sperm cells are 10–15 kb in length, while in somatic tissue such as peripheral blood lymphocytes these arrays extend for 5–10 kb (Brown 1989; Cross et al. 1989; Brown et al. 1990; de Lange et al. 1990).

Replication at the telomeres is achieved by the addition of telomeric repeats to the 3′ end of the chromosomes by a specialized reverse transcriptase called telomerase (reviewed in Greider 1996). Telomerase is a ribonucleoprotein that has been found in many eukaryotes (Greider 1996; Blackburn 2001). The RNA component of telomerase includes a short sequence that is complementary to the DNA on the G-rich strand of the telomere. This sequence acts as a template for the addition of the telomeric repeat units to the 3′ end of the telomere (Blackburn 1991).

Vertebrates from fish to humans share a common telomeric repeat sequence, $(TTAGGG)_n$. In situ hybridization analysis of two orders of the class Pisces found that the $(TTAGGG)_n$ repeat sequence was present primarily near chromosomal ends and not interstitially (Meyne et al. 1989, 1990). However, Abuín et al. (1996b) found that the nucleolus organizing regions (NORs) in rainbow trout (*Oncorhyncus mykiss*) hybridized strongly to a $(TTAGGG)_n$ probe. We report here on the nature and distribution of telomeric repeats in *Oreochromis niloticus*, the Nile tilapia.

Oreochromis niloticus belongs to the family Cichlidae in the order Perciformes. African cichlids have been

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classified according to morphological characteristics, especially jaw and dental morphology, feeding habits and by their breeding and brooding behavior (Trewavas 1982; Greenwood 1991). More recently, scientists have used molecular biological methods such as allozyme analysis, sequence analyses of mitochondrial DNA, randomly amplified polymorphic DNA analysis and the distribution of microsatellite DNA allele size variants (reviewed by Sültmann and Mayer 1997) to clarify cichlid taxonomy at the genus, species and population levels. The African cichlids can be divided into three major groups: (1) Pelmatochromine cichlids, which inhabit West Africa, (2) Haplochromine cichlids and (3) Tilapiine cichlids (Lowe-McConnell 1991). It is the Haplochromine cichlids of East and Central Africa, especially those of the Rift Valley Lakes, that have undergone a remarkable speciation event that is now considered as a classic example of adaptive radiation (Liem 1991). In Lake Malawi alone, it is believed that there are 500–1000 different cichlid species adapted to specific niches (Stiassny 1991). The Tilapiine tribe, commonly referred to as tilapia, encompasses the genera *Tilapia*, *Sarotherodon* and *Oreochromis*, and a fourth genus, *Danakilia*, which comprises a single species. Cichlids are of scientific interest not only because of their economic importance to tropical aquaculture, but because they have undergone a rapid and extensive speciation event.

Standard karotypes have been established for less than 10% of all known fish species (Gold et al. 1990). Standard banding methods (i.e. R-, G-, Q- and T-banding) used for karyotyping and nomenclature for mammalian metaphase chromosomes, like human chromosomes (Craig and Bickmore 1993), are generally unsuccessful in distinguishing fish chromosomes (Blaxhall 1983; Gold et al. 1990; Pendás et al. 1993). Although some individual chromosomes have been identified by silver staining of the NORs of chromosomes (Mayr et al. 1988; Abuín et al. 1996a), the majority of chromosomes cannot be specifically identified for fish. The difficulty stems from problems in obtaining consistent metaphase chromosomal spreads, and the comparatively small size of the majority of fish chromosomes (Blaxhall 1983; Gold et al. 1990). In fish, unlike humans, assignment of chromosomal number is arbitrary owing to the similarity in size of chromosomes (Majumdar and McAndrew 1986; Sánchez et al. 1990). This lack of a defined karyotype has hindered the characterization of the *O. niloticus* genome. Previous cytogenetic studies have shown that the haploid genome of *O. niloticus* consists of 22 chromosomes (Kornfield et al. 1979; Majumdar and McAndrew 1986). Molecular analysis has shown that there are two main satellite DNA sequences in *O. niloticus*: SATA and SATB (Franck et al. 1992; Franck and Wright 1993). SATA is distributed in the centromeric regions of all chromosomes, whereas SATB is restricted to the centromeric region of a single chromosome (Oliveira and Wright 1998).

The limited information available on the structure of fish chromosomes and ongoing studies in genome mapping of *O. niloticus* (Lee and Kocher 1996) have led us to investigate the molecular structure and the distribution of telomeric sequences in the chromosomes of *O. niloticus* as a first step in construction of a physical map for the genome of this species.

Materials and methods

Animals and isolation of genomic DNA

Fifteen adult *O. niloticus* (9 females and 6 males) were obtained from the tilapia culture facility at Dalhousie University (courtesy of Dr. Jim Wright). High molecular weight genomic DNA was prepared from erythrocytes as follows: erythrocytes from 2 ml of blood were harvested (Wright 1989). The cells were resuspended in 0.85% NaCl at a concentration of 3×10^8 cells/ml and mixed with an equal volume of 0.85% NaCl and 1.5% low melting point agarose (Bio-Rad, Low Melt Preparative Grade). The erythrocyte/agarose suspension was allowed to solidify in a plug mold (Bio-Rad) on ice for 10 min before being processed to lyse the erythrocytes and remove the proteins as described by Anand and Southern (1990). Briefly, the agarose plugs were incubated in 1.0% N-lauroyl sarcosine (NDS) [1.0% w/v NDS (Sigma), 0.5 M EDTA, 10 mM TRIS pH 9.5], 1 mg/ml Proteinase K, overnight at 50°C. Subsequently, the plugs were incubated for an additional 24 h at 50°C in fresh 1.0% NDS, Proteinase K solution. After two additional 2 h incubations at 50°C in 1.0% NDS, the agarose plugs were stored at 4°C in 1.0% NDS.

Restriction endonuclease digestion

The DNA suspended in the agarose plugs was digested according to a protocol modified from Anand and Southern (1990). Briefly, a quarter of a plug was washed twice with TE (10 mM TRIS, pH 8.0, 1 mM EDTA, pH 8.0), 0.1 mM PMSF (phenylmethylsulfonylfluoride) for at least 30 min at room temperature to remove residual lysis buffer and denature nucleases, followed by a 30 min wash with TE. The plug quarters were then equilibrated in 500 µl of 1×restriction buffer according to the manufacturer's specifications for 30 min on ice. After equilibration, the buffer was removed, replaced with 60 µl of 1×restriction buffer containing 100 µg/ml of gelatin and 1 mM dithiothreitol and the samples were incubated at room temperature for 10 min. 10 U of restriction enzyme was added to the solution and allowed to diffuse into the plug quarters during a 1 h incubation on ice. The DNAs were digested to completion by incubating for 3 h at 37°C. After the first hour of incubation, another 10 U of restriction enzyme was added. Digestion was stopped by removing the restriction buffer, adding 500 µl of 0.5×TBE (45 mM TRIS-borate, 1 mM EDTA), 10 mM EDTA and incubating on ice for 30 min.

Bal31 nuclease sensitivity assay

Quarters of plugs, containing approximately 4.5 µg of *O. niloticus* DNA, were incubated twice with 1 ml of TE, 0.1 mM PMSF for at least 30 min at room temperature, followed by a 30 min incubation in TE. The samples were then equilibrated in 200 µl of 1×Bal31 buffer (0.65 M NaCl, 10 mM $CaCl_2$, 10 mM $MgCl_2$, 20 mM TRIS-HCl, pH 8.0, 1 mM EDTA) for 30 min on ice. Following a 10 min incubation in 100 µl of 1×Bal31 buffer at room temperature, the plug quarters were incubated for 10 min at 30°C before addition of 6 U of Bal31 exonuclease (Invitrogen). The samples were incubated on ice for 1 h to allow the enzyme to infiltrate the agarose and then the samples were incubated at 30°C. Aliquots were taken at 30 min intervals. After the first 30 min interval, an additional 6 U of enzyme was added to the reactions. The reactions were stopped by removal of the Bal31 buffer and addition of 500 µl of 10 mM EDTA, 50 mM TRIS, pH 8.0, fol-

lowed by incubation for at least 1 h on ice. The plug quarters were then washed overnight in 1 ml of TE at 4°C and the DNA digested to completion with EcoRI as described previously.

Samples of DNA were then subjected to pulsed field gel electrophoresis (PFGE) and were separated on a CHEF-DR II pulsed field electrophoresis system (Bio-Rad). The samples were subjected to electrophoresis at 14°C in 0.5×TBE at 6 V/cm with an initial pulse time of 10 s and ramped to a final pulse time of 40 s for 16 h. Gels were stained for 30 min in 0.5 µg/ml of ethidium bromide, destained for 1 h in 0.5×TBE and photographed using Polaroid 4×5 Land Film, type 55.

Southern blot analysis

The DNA samples separated on the PFGE gels were transferred onto Zetaprobe-GT nylon charged membrane (Bio-Rad) by capillary action under alkaline conditions. Prior to transfer, the gel was subjected to 60,000 µJ of UV radiation in a Stratagene UV Stratalinker 1800. The gel was washed twice for 30 min in 0.4 M NaOH, 1.5 M NaCl before transfer using 0.4 M NaOH.

A $(TTAGGG)_4$ oligonucleotide was end-labeled by incubating 100 ng of DNA with 30 µCi of [γ-^{32}P] ATP and 1 µl of T4 polynucleotide kinase (Invitrogen) at 37°C according to the manufacturer's specifications. The labeling reaction was terminated by incubation at 65°C for 10 min followed by removal of the unincorporated radionucleotides using a Sephadex G-25 (Pharmacia) spin column (Sambrook et al. 1989). The probe was labeled to a specific activity of 1×10^8 cpm/µg.

Before hybridization, nylon membranes were washed at 60°C in 2×SSC (0.3 M NaCl, 30 mM sodium citrate) for 15 min. Membranes were prehybridized in 10 ml of hybridization solution: 6×SSPE (1 M NaCl, 60 mM $NaH_2PO_4{\cdot}H_2O$, 60 mM EDTA), 5×Denhardts [0.1% Ficoll Type 400 (Pharmacia), 0.1% polyvinylpyrrolidone (Sigma), 0.1% BSA (Fraction V, Sigma)], 1% SDS, 100 µg/ml yeast tRNA (Sigma) for 1 h. The $(TTAGGG)_4$ probe was added to a fresh 10 ml of hybridization solution and incubated at 60°C for 2 h. The membranes were washed twice in 2×SSC, 0.1% SDS at 60°C for 30 min. Blots were then exposed to Kodak X-OMAT AR film at –70°C with an intensifying screen.

Chromosome techniques

Mitotic chromosomes were prepared as described by Foresti et al. (1993a). For fluorescent in situ hybridization analysis, a telomeric repeat probe was generated using the polymerase chain reaction with the primers $(TTAGGG)_5$ and $(CCCTAA)_5$ according to IDjo et al. (1991). Probes were labeled with biotin-14-dATP using a nick translation kit (Invitrogen) following the manufacturer's instructions. The size of the nick translation reaction product was controlled by varying the length of time of the reaction. The average size of the nick translation products was determined by gel electrophoresis. In situ hybridization was performed according to Oliveira et al. (1999) except that washes were conducted at 37°C. Hybridization was detected with fluorescein-labeled avidin (Oncor) and the signal was enhanced by incubation with anti-avidin antibody (Oncor) followed by the application of the fluoresceinated avidin. Chromosomes were counterstained with propidium iodide, antifade solution (Oncor).

Results

Pulsed field gel electrophoretic analysis of *O. niloticus* $(TTAGGG)_4$-hybridizing DNA

Southern blot analysis of *O. niloticus* DNA indicated that the telomeric repeat array in *O. niloticus* is indeed $(TTAGGG)_n$. However, the restriction fragments containing these terminal repeat arrays were too large to be resolved using conventional agarose gel electrophoresis (Chew 1997). In order further to characterize these large terminal $(TTAGGG)_n$-containing restriction fragments, high molecular weight DNA was digested to completion with restriction enzymes that cut frequently in the genome: EcoRI, BamHI, HindIII, HaeIII, or Sau3AI. The resulting fragments were resolved by PFGE (Fig. 1a). All restriction digests produced a heterogeneous smear of fragments, the majority of which had a mobility of less than 145 kb for the 6 bp site recognition enzymes and less than 23 kb for the 4 bp site recognition enzymes. Several discrete high molecular weight restriction fragments were also evident for each digest. Southern blot hybridization analysis using a radiolabeled $(TTAGGG)_4$ probe revealed that the majority of the restriction fragments detected by this probe ranged from 50–100 kb, with a smear extending down to 5 kb (Fig. 1b) for DNA digested with EcoRI, BamHI or HindIII. The probe also hybridized to 4–10 kb Sau3AI or HaeIII restriction fragments. The heterogeneity in size of the fragments is consistent with these being derived from different chromosomal ends. Furthermore, owing to the natural variability among cells, individual chromosomal ends may also have variable numbers of the terminal telomeric repeats for any single chromosomal end

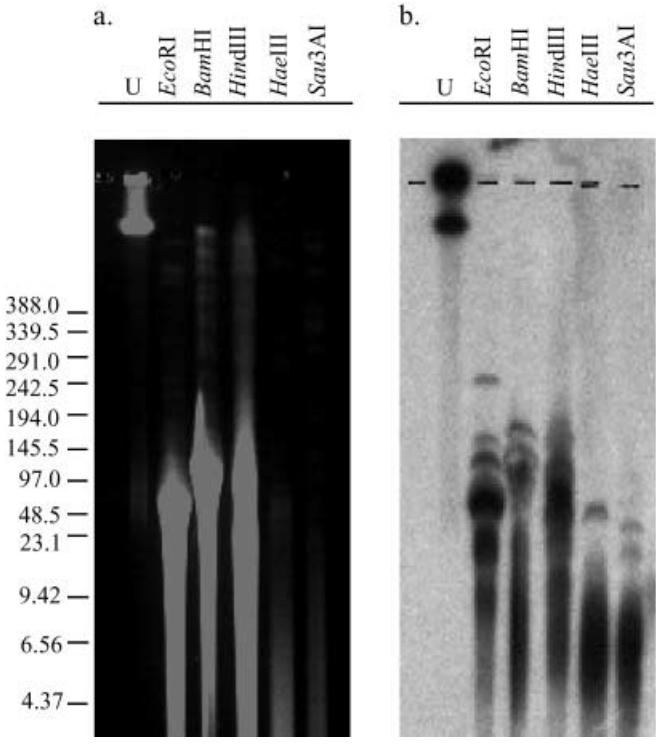

Fig. 1a, b *Oreochromis niloticus* TTAGGG-hybridizing restriction fragments. **a** An ethidium bromide-stained 1.0% pulsed field gel on which *O. niloticus* DNA embedded in agarose plugs was size-fractionated either undigested (*U*) or after digestion to completion with the indicated restriction enzymes. The size in kilobases and mobility of molecular weight markers are shown at the left. **b** Autoradiogram of the Southern blot of the gel shown in **a** after hybridization with radiolabeled $(TTAGGG)_4$ probe

(Brown et al. 1990; de Lange et al. 1990). In the EcoRI-digested sample, there appeared to be three major clusters of fragments averaging 9, 50 and 100 kb in size. It is unlikely that the discrete high molecular weight $(TTAGGG)_4$-hybridizing bands were due to partial digestion of the *O. niloticus* DNA since other high molecular weight bands were seen in the PFGE analysis (Fig. 1a) and these did not hybridize to the $(TTAGGG)_4$ probe (Fig. 1).

The $(TTAGGG)_n$ repeats in *O. niloticus* are Bal31 exonuclease sensitive

In order to determine the length and the sequence of the telomeric repeat array in *O. niloticus*, a Bal31 sensitivity assay was conducted. Any sequences at the very ends of chromosomes will gradually be lost as the Bal31 double-stranded DNA exonuclease progressively degrades inward from the chromosomal ends. A decrease in the size and signal obtained in a Southern blot analysis is expected if the sequences recognized by the probe reside near the ends of the chromosomes. High molecular weight DNA was subjected for increasing lengths of time to Bal31 exonuclease digestion. Subsequently, the Bal31-treated DNAs were incubated with EcoRI and analyzed by gel electrophoresis (Fig. 2a) and Southern blot hybridization with the $(TTAGGG)_4$ oligonucleotide probe (Fig. 2b). Prior to digestion with Bal31, the $(TTAGGG)_4$ probe recognized discrete EcoRI fragments of 160, 195 and 300 kb and a smear of EcoRI fragments clustered in three size groups ranging from 8–11, 15–48 and 84–138 kb. After 30 min of Bal31 digestion, when approximately 2.5 kb of DNA on average had been removed from chromosomal ends, there was a decrease in the intensity of hybridization signal associated with the smear of EcoRI fragments. The 300 kb EcoRI fragment was no longer detected and the smallest pool of fragments decreased to 5–9 kb in size. After 1 h, the discrete high molecular weight EcoRI fragments had disappeared but the three main pools of EcoRI fragments were still visible, with sizes of 5–7, 11–32 and 74–118 kb. After 120 min incubation with Bal31, the cluster of approximately 100 kb-sized fragments, although diminished in intensity, remained unchanged in size (Fig. 2b). Ethidium bromide staining of the gel (Fig. 2a) showed a homogenous smear of equal intensity in each lane, indicating that equal amounts of DNA had been loaded and that the mobility of most fragments was unaffected by incubation with the exonuclease. Furthermore, reprobing of the Southern blot with a radiolabeled probe that recognizes several internal chromosomal loci in the *O. niloticus* genome, produced a diagnostic pattern of bands of equal intensity across all the lanes (data not shown). These results indicate that the DNA was cut to completion with EcoRI and not nonspecifically degraded by the Bal31 treatment. Thus, the Bal31 sensitivity of the $(TTAGGG)_n$ sequences demonstrates that $(TTAGGG)_n$ is indeed found at the termini of *O. niloticus* chromosomes, consistent with it being the telomeric repeat. A subset of the $(TTAGGG)_n$-hybridizing EcoRI fragments of approximately 100 kb in size were resistant to enzymatic degradation by the Bal31 enzyme, suggesting that they were located at an internal chromosomal site at least 5–20 kb away from chromosomal ends.

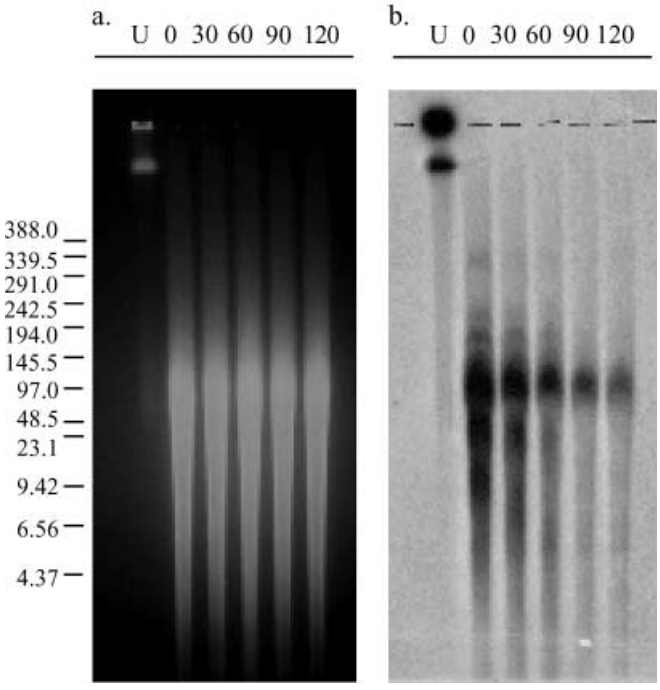

Fig. 2a, b Bal31 sensitivity of *Oreochromis niloticus* TTAGGG-hybridizing sequences. **a** An ethidium bromide-stained 1.0% pulsed field gel on which *O. niloticus* DNA embedded in agarose plugs has been size-fractionated after incubation with Bal31 for the indicated time (in minutes), followed by digestion to completion with EcoRI. The size in kilobases and mobility of molecular weight markers are shown at the left. **a** *Lane U* contains DNA untreated with Bal31 or EcoRI. **b** Autoradiogram of the Southern blot of the gel shown in **a** after hybridization with radiolabeled $(TTAGGG)_4$ probe

Southern blot analysis of *O. niloticus* genomic DNA treated with restriction enzymes that recognize a 4 bp site and hybridized with the $(TTAGGG)_4$ probe indicated that the length of the terminal repeat array itself is at least 4–10 kb. The range in length of the telomeric repeat array in *O. niloticus* erythrocytes is similar to the 5–10 kb telomeric repeat array observed in human peripheral lymphocytes (Cross et al. 1989; DeLange et al. 1990). The data also indicate that the length of the terminal EcoRI fragments in *O. niloticus* erythrocytes ranges from 4 to 300 kb. This heterogeneity in terminal EcoRI fragments is not unexpected as these fragments represent all possible terminal EcoRI fragments from all the chromosomal ends in *O. niloticus*. Individual chromosomal ends may also have variable numbers of the terminal telomeric repeats and thus a range of sizes of terminal fragments would not be unusual. Large terminal restriction fragments have previously been observed for

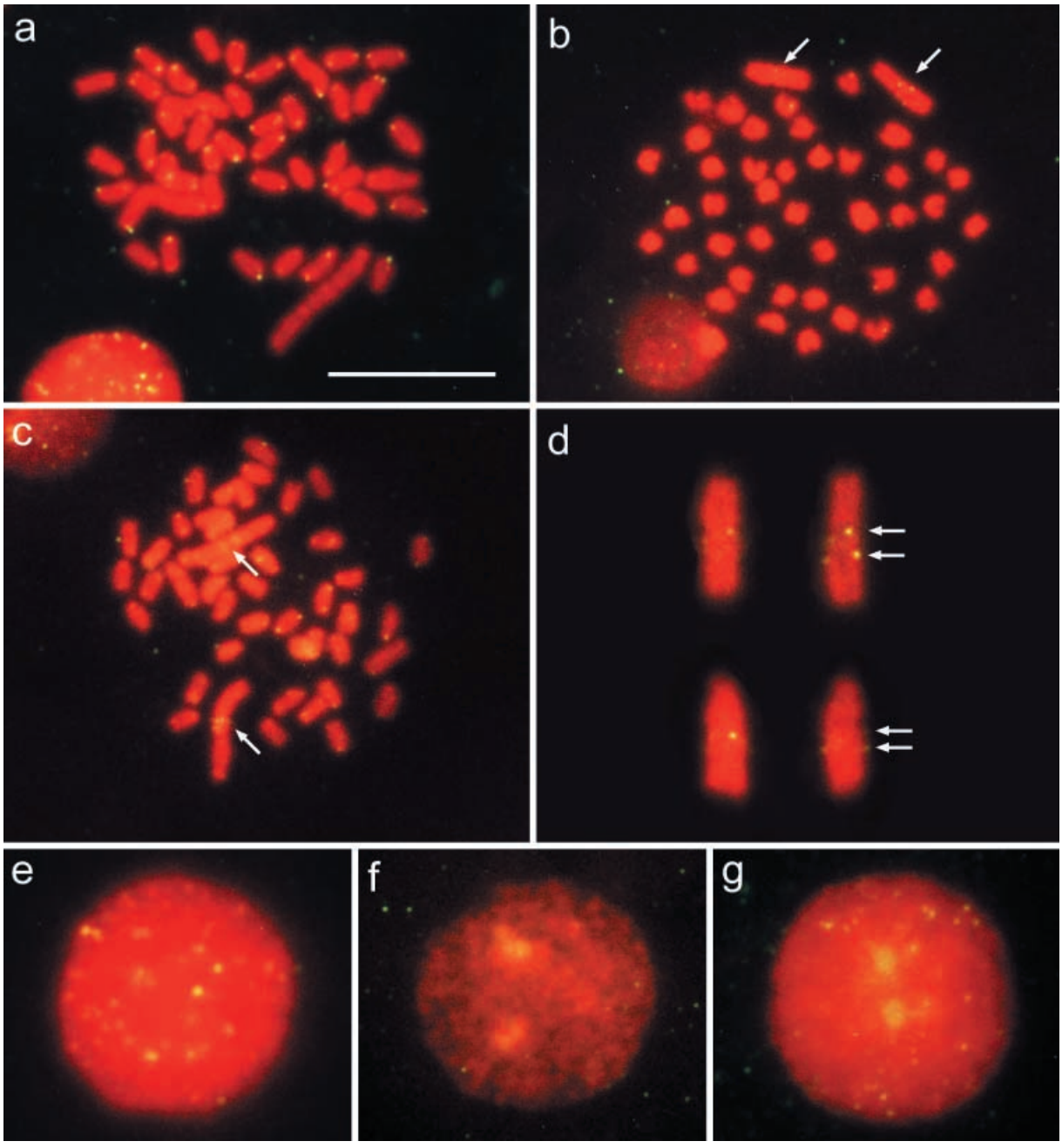

Fig. 3a–g Fluorescent in situ hybridization using biotinylated $(TTAGGG)_n$ probe to chromosomes of *Oreochromis niloticus*. Metaphase chromosomal spreads showing the presence of $(TTAGGG)_n$ sequences at **a** the ends of all chromosomes (*bar* represents 10 μm), **b** in the interstitial region of chromosome 1 (*arrows*), and **c** at the termini of all chromosomes and in the interstitial region of chromosome 1 (*arrows*). **d** Enlarged chromosome 1 pairs showing hybridization signal in two interstitial positions (*arrows*). **e, f, g** Interphase nuclei showing the presence of small and large clusters of telomeric repeats

chromosomes from *Xenopus laevis* (Bassham et al. 1998) and either reflect the heterogeneity found at chromosomal ends or the presence of repetitive sequences adjacent to the telomeric tract that lack recognition sites for common restriction enzymes.

Chromosomes of *O. niloticus* contain interstitial tracts of $(TTAGGG)_n$

Fluorescent in situ hybridization of biotinylated $(TTAGGG)_n$ probes to denatured metaphase spreads and interphase nuclei showed that the signals produced were dependent on the probe size. Probes with an average size of about 150 bp mainly hybridized to all chromosomal termini (Fig. 3a), while probes with an average size of about 400 bp primarily recognized two interstitial regions in chromosome 1 (Fig. 3b, d). A combination of the small and large probes resulted in the simultaneous labeling of all chromosomal ends and two interstitial regions of chromosome 1 (Fig. 3c). The analysis of interphase nuclei after hybridization with the small probes

showed the presence of many signals dispersed over all the nuclei (Fig. 3e) while hybridization of the large probes to interphase nuclei primarily generated two intense signals (Fig. 3f). Hybridization with both the small and large probes resulted in the labeling of many small discrete signals and two larger signals in all interphase nuclei examined (Fig. 3g).

Discussion

It is believed that the ancestral karyotype of teleosts was 2*n*=48 chromosomes (reviewed in Denton 1973 and references therein). Primitive fish have acrocentric chromosomes, while, for more advanced fish, a greater proportion of their chromosomes are metacentric. The correlation between a change in chromosomal number and a change in chromosomal morphology has been used to support the idea that karyotypic evolution and the creation of new species are mostly due to chromosomal rearrangements (Denton 1973).

Changes in chromosomal morphology can be caused by: (1) deletions due to chromosomal breakages; (2) duplications; (3) inversions – pericentric inversions of the centric regions in acrocentric chromosomes give rise to metacentric chromosomes while an inversion of a metacentric chromosome gives rise to a large acrocentric chromosome; and (4) translocations, fusions or exchanges between nonhomologous chromosomes. The fusion of two acrocentric or two telocentric nonhomologous chromosomes to create a single metacentric chromosome is termed a Robertsonian (Rb) fusion or translocation. The centric portion of one of the fused chromosomes is either deleted or inactivated (reviewed in Slijepcevic 1998). The metacentric chromosome can then undergo a pericentric inversion, resulting in a large acrocentric chromosome. Robertsonian fusions are the most common type of translocation in fish and have played an important role in the evolution of the salmonid karyotype (Denton 1973).

Cytogenetic studies conducted in 115 species of the family Cichlidae show that 51% of the species studied have a karyotype with 2*n*=48 chromosomes (Klinkhardt et al. 1995). Moreover, a large number of Neotropical cichlids, i.e. *Cichlosoma*, *Geophagus*, which are the primitive sister group of African cichlids, have 2*n*=48 acrocentric chromosomes (Thompson 1979). In the Tilapiine tribe, most species have 2*n*=44, and the presence of 2*n*=48 chromosomes in *Oreochromis alcalicus* is seen to be plesiomorphic and possibly predates the overall reduction to 2*n*=44 in all other tilapiine species (Majumdar and McAndrew 1986). We hypothesize that three chromosomes may have been involved in a combination of Rb fusion and pericentric inversion events to generate the large acrocentric chromosome 1. The observation of two interstitial $(TTAGGG)_n$ signals within chromosome 1 reported here supports this hypothesis for the origin of the observed chromosomal number reduction in tilapia.

Interstitial telomeric sequences have been found in pericentric regions in a number of vertebrate species (Meyne et al. 1990), suggesting that Rb fusions can occur without a resulting loss of telomeric sequences. Recently evolved species are thought to have nontelomeric sites of these repeats. These interstitial sites of the telomeric sequence have been interpreted as "footprints" of previous chromosomal fusions (Phillips and Reed 1996). The presence of these sites, especially in the pericentric region, may promote the fission/fusion of chromosomes during karyotypic evolution (Meyne et al. 1990). Robertsonian fusions have also played a major role in mammalian karyotypic evolution. An end-to-end fusion of two tracts of telomeric repeats at a subtelomeric location in human chromosome 2 has been identified as a relic of an ancient telomere-telomere fusion. Two ancestral ape chromosomes must have fused to give rise to human chromosome 2 in what must have been a relatively recent event after the separation of the lines that led to great apes (orangutan, chimpanzee and gorilla) and humans (IDjo et al. 1991), respectively.

Fluorescent in situ hybridization studies of *O. niloticus* chromosomes show that the short arms of some chromosomes and all centromeric regions have large amounts of a satellite DNA sequence called SATA (Oliveira and Wright 1998). The absence of SATA sequences in interstitial regions of chromosome 1 suggests that, if chromosomal fusion has occurred, the satellite DNA sequences, and possibly the inactive centromeres, must have been eliminated by deletion or mutation. The distribution of $(TTAGGG)_n$ sequences among 13 fish species showed that these sequences were primarily localized in the telomeres (Meyne et al. 1990; Phillips and Reed 1996; Gornung et al. 1998) but some nontelomeric sites have been observed (Reed and Phillips 1995). Pericentric blocks of $(TTAGGG)_n$ have been reported in other species such as Chinese hamster (Meyne et al. 1990) and a recent study by Abuín et al. (1996b) found that the NORs in rainbow trout (*O. mykiss*) hybridized strongly to a $(TTAGGG)_n$ probe. Interestingly, in three fish species, the $(TTAGGG)_n$ sequence hybridized with the entire NOR (Salvadori et al. 1995; Abuín et al. 1996a), and in *Salvelinus namaycush*, three of the NOR sites were flanked by the telomeric repeat (Reed and Phillips 1995). In tilapia, NORs are found in a terminal position on three chromosomal pairs (Foresti et al. 1993a, b). We did not observe more intense hybridization of the $(TTAGGG)_4$ probe to any particular subset of chromosomal ends, suggesting that extensive tracts of telomeric repeats do not flank the NORs.

Telomere-associated sequences (TAS) are middle repetitive DNA sequences found adjacent to the telomeric repeat tract. These TAS repeats are often organized in arrays and often held in common between subsets of nonhomologous chromosomal ends. The regions containing these TAS repeats vary in length from chromosome to chromosome and can be up to several hundred kilobases in length (reviewed in Zakian 1989; Beissmann and Mason 1992). The function of these sequences

is unknown, but there has been some speculation that they might be involved in chromosomal movement, telomere-telomere interactions and interactions between telomeres and the nuclear envelope (Blackburn and Szostak 1984; Murphy and Karpen 1995). The TASs show a polymorphic distribution and have proven useful as markers in genome mapping and identifying specific sets of chromosomes in humans (Moyzis et al. 1987; Brown 1989; Brown et al. 1990; de Lange et al. 1990). The majority of terminal EcoRI fragments observed for *O. niloticus* chromosomes fall in one of three size classes (8–11, 15–48 and 84–138 kb), suggesting that these could represent different classes of chromosomal ends. The results of the Southern blot and Bal31 analyses suggest that the discrete EcoRI fragments of 160, 195 and 300 kb that hybridized to the $(TTAGGG)_4$ probe likely represent individual chromosomal ends at which the telomere-associated DNA lacks a recognition site for EcoRI. Possible candidates for these ends could be chromosomes bearing satellite DNA sequences or the NORs at terminal positions. These large EcoRI fragments would be good candidates for use in generating probes that may be used to distinguish these three chromosomal ends. Cloning of the other terminal EcoRI fragments would allow us to determine whether the telomere-associated DNA contains TASs that could be used as probes to identify subsets of chromosomal ends.

In conclusion, we have determined that the telomeric repeat array in *O. niloticus* is in the size range of 4–10 kb. The 100 kb Bal31-insensitive EcoRI fragments are probably present on chromosome 1, which has two interstitial $(TTAGGG)_n$ sites. These interstitial sites likely represent remnants of the fusion of three chromosomes to generate the large chromosome 1. Further analysis of the chromosomal distribution of telomeric sequences in New World cichlids like *Crenicara filamentosa* and *Apistogramma* (2*n*=46) and Old World cichlids like *Pelvicachromis pulcher* (2*n*=48) and *Etroplus maculatus* (2*n*=46) (Klinkhardt et al. 1995) would help to decipher chromosomal evolution in this fascinating group of fishes.

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