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Short Communication

Identities among actin-encoding cDNAs of the Nile tilapia (*Oreochromis niloticus*) and other eukaryote species revealed by nucleotide and amino acid sequence analyses

Andréia B. Poletto[1], Adriane P. Wasko[2], Claudio Oliveira[1], Alexandre Azevedo[1], Robson F. Carvalho[1], Maeli Dal Pai Silva[1], Fausto Foresti[1] and Cesar Martins[1]

[1]*Departamento de Morfologia, Instituto de Biociências, Universidade Estadual Paulista, Campus de Botucatu, Botucatu, SP, Brazil.*
[2]*Departamento de Genética, Instituto de Biociências, Universidade Estadual Paulista, Campus de Botucatu, Botucatu, SP, Brazil.*

Abstract

Actin-encoding cDNAs of Nile tilapia (*Oreochromis niloticus*) were isolated by RT-PCR using total RNA samples of different tissues and further characterized by nucleotide sequencing and *in silico* amino acid (aa) sequence analysis. Comparisons among the actin gene sequences of *O. niloticus* and those of other species evidenced that the isolated genes present a high similarity to other fish and other vertebrate actin genes. The highest nucleotide resemblance was observed between *O. niloticus* and *O. mossambicus* α-actin and β-actin genes. Analysis of the predicted aa sequences revealed two distinct types of cytoplasmic actins, one cardiac muscle actin type and one skeletal muscle actin type that were expressed in different tissues of Nile tilapia. The evolutionary relationships between the Nile tilapia actin genes and diverse other organisms is discussed.

Key words: actin, expression pattern, Nile tilapia, *Oreochromis niloticus.*

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Actin is a ubiquitous protein of eukaryotic cells that has a crucial role in muscle contraction, cell motility, cytoskeletal structure, cell division, intracellular transport, and cell differentiation (Herman, 1993). In yeast and some protozoans, actin is encoded by a single gene only (Hightower and Meagher, 1986; Reece *et al.*, 1997). However, in the nuclei of all animals, plants and in many protozoans examined to date, actin proteins are encoded by a multigene family. In these organisms it seems that actin isoforms are encoded by a set of structurally related genes that resulted from gene duplications followed by functional divergence (Hightower and Meagher, 1986). The number of actin isoforms varies greatly in different lineages. While mammals posses at least six different isoforms (Vandekerckhove and Weber, 1978), teleost fishes contain at least nine (Venkatesh *et al.*, 1996) and echinoderm genomes at least eight (Fang and Brandhorst, 1994) distinct actin isoforms. Similarly, insects have at least six actin genes (Fyrberg *et al.*, 1980). The actin gene family of plants is much larger, comprising 8-44 genes, depending on the taxon (Reece *et al.*, 1992; Drouin and de Sá, 1996).

Send correspondence to Cesar Martins. Departamento de Morfologia, Instituto de Biociências, Universidade Estadual Paulista, Campus de Botucatu, 18618-000 Botucatu, SP, Brazil. E-mail: cmartins@ibb.unesp.br.

The actin gene family can be divided into two broad categories: cytoplasmic (β and γ) and muscle (α) type actins. Invertebrate muscle and cytoplasmic actins seem to be more similar to chordate cytoplasmic actins than to chordate muscle actins (Vandekerckhove and Weber, 1984). It has been suggested that the muscle actins of arthropods differ from the muscle actins of deuterostomes to such an extent that two independent divergence events of muscle actin genes probably occurred, one within the protostome lineage and one within the deuterostome lineage (Mounier *et al.*, 1992).

Although there are data on the evolution of mammalian actin genes, the evolutionary origin, pattern of organization, and the diversity of these genes in other vertebrates, especially fishes, remain to be investigated. To date, an in-depth research on the diversity of actin gene types and tissue expression profiles in a fish species was only performed on *Takifugu rubripes*, revealing nine different actin genes: six muscle-type actin genes that include two α-skeletal actins, three α-cardiac actins, an α-anomalous testis-type actin, and three cytoplasmic actins that include two

β-cytoplasmic actins and one β-cytoplasmic vascular-type actin (Venkatesh *et al.*, 1996). The purpose of the present study was the isolation and characterization of distinct actin cDNAs from different tissues of the Nile tilapia (*Oreochromis niloticus*) - one of the most important food fish species intensively exploited in tropical and subtropical aquaculture (Pullin, 1991) - not only to enhance our knowledge of the species, but also to provide a better understanding of the organization of the actin multigene family in fish genomes.

Total RNA samples were obtained from different tissues (gills, heart, ovaries, skeletal muscle, liver, and brain) of two adult individuals of *Oreochromis niloticus* using TRIzol reagent (Gibco-Brl Life Technologies), following the manufacturer's instructions. First-strand cDNA synthesis reactions were performed with the SuperScript First-Strand Synthesis System for RT-PCR (Invitrogen Life Technologies) using random hexamer primers. cDNA amplification was performed using the primer sets αActF (5'-ATGAGACTACCGCCCTTGTG-3') and αActR (5'-AATCCACATCTGCTGGAAGG-3') for α-actin gene amplification, and βActF (5'-TGTTGACAATGGATCCGGTA-3') and βActR (5'-CTGCTGGAAGGTGGAGAGAG-3') for β-actin gene amplification. Both primer sets were designed using the software Primer3 (Rozen and Skaletsky, 2000) based on α-actin and β-actin gene sequences described previously for *T. rubripes* (Venkatesh *et al.*, 1996). RT-PCR products were electrophoresed and visualized on a 1% agarose gel, purified and ligated into pCR2.1 plasmid (TA Cloning Kit, Invitrogen) used to transform DH5α *E. coli* competent cells. Plasmid DNA was purified with the Wizard Plus SV Minipreps DNA Purification System Kit (Promega) and submitted to nucleotide sequencing on an ABI 377 Automated DNA Sequencer (Applied Biosystems).

Nucleic acid and amino acid sequences of *O. niloticus* were analyzed using BLASTn and BLASTx (Altschul *et al.*, 1990). Additionally, sequences from different organisms obtained from GenBank, were aligned with *O. niloticus* sequence data using the software ClustalW (Thompson *et al.*, 1994); alignment was checked by eye and adjusted as necessary. Phylogenetic analyses using the putative aa sequences derived from *O. niloticus* clones and other aa sequences from previously published actin genes in GenBank were performed using MEGA version 3.1 (Kumar *et al.*, 2004). Phylogenetic trees were constructed using the neighbor-joining method (Saitou and Nei, 1987).

RT-PCR of *Oreochromis niloticus* cDNA samples, obtained from total RNA extracted from gills, liver, heart, ovary, skeletal muscle and brain, was performed using the two sets of primers αActF/αActR and βActF/βActR in order to amplify α-actin and β-actin gene sequences, respectively. Irrespective of the tissue or the primer set used, all PCR amplifications resulted in one fragment of approximately 1,100 base pairs (bp) that was cloned and sequenced.

A total of 11 clones were isolated and sequenced in both directions from two individuals of *O. niloticus*. These represented three skeletal muscle type α-actins from gills (*On*1a7, *On*1a8, and *On*1a9); one skeletal muscle type α-actin from heart (*On*3a8); one cardiac muscle type α-actin from heart (*On*3a10c); one β-actin from ovary (*On*4b1); four skeletal muscle type α-actins (*On*5a18, *On*5a10, *On*5a3 and *On*5a5) and one β-actin (*On*5b2) from skeletal muscle tissue (Figure 1). The nucleotide sequences were deposited in the GenBank database under the accession numbers EF206791-EF206801. The obtained sequences of the amplified cDNAs of *O. niloticus* revealed segments ranging in size from 884 to 1,063 bp. The differences in the sequence sizes were due to failures in the sequencing procedure.

Searches in the NCBI database by means of BLASTn indicated that the isolated cDNA nucleotide sequences from *O. niloticus* were very similar to several fish actin gene sequences, especially to α-actins and β-actins of *O. mossambicus* (99% mean nucleotide identity between the two species). The putative amino acid sequences of the isolated cDNAs from *O. niloticus* were also compared to skeletal actin1, skeletal actin2, cardiac1 alpha actin, cytoplasmic actin1 and cytoplasmic actin2 genes of *T. rubripes* (Venkatesh *et al.*, 1996), which resulted in a 98,4% mean identity (Figure 1).

Although the nucleotide sequences of the isolated cDNAs of *O. niloticus* and the actin genes of *T. rubripes* differ by several nucleotides, their inferred aa sequences present a high degree of similarity, approximately 98%, since most nucleotide variation corresponds to synonymous substitutions and, thus, their aa residues are mostly identical (Figure 1). Diagnostic aa positions that distinguish α-muscle actin from β-cytoplasmic actin of Nile tilapia could be observed (Figure 1). These aa positions were also identified in the isolated cDNAs of *T. rubripes* (Venkatesh *et al.*, 1996). Most amino acids that distinguish fish α-striated muscle actins from β-cytoplasmic actins correspond to those that also distinguish mammalian α- and β-actins (Mounier and Sparrow, 1997). Diagnostic amino acids 281, 321 and 325 that distinguish *T. rubripes* β-cytoplasmic actin2 from β-cytoplasmic actin1 were identified in the isolated cDNA sequence *On*4b1 of *O. niloticus*, which validates the occurrence of a second type of cytoplasmic actin gene expressed in fish species. This is a novel finding, since the expression of β-cytoplasmic actin2 has not yet been reported, only its genomic sequence (Venkatesh *et al.*, 1996).

A molecular phylogenetic analysis of some actin aa sequences was performed to examine the evolutionary relationship of the actin isoforms isolated from Nile tilapia and several other eukaryote species (Table 1, Figure 2). The

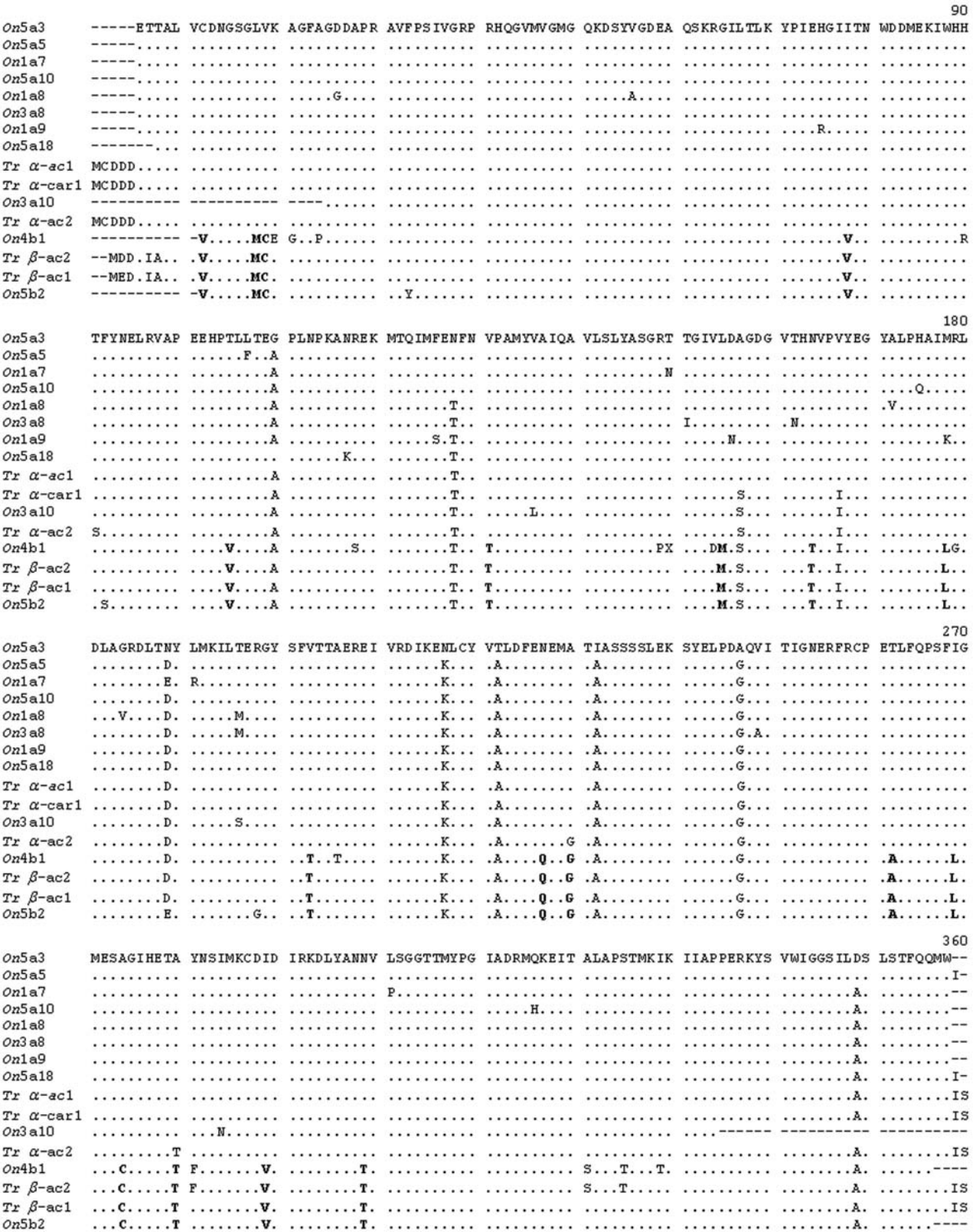

Figure 1 - Alignment of putative amino acid sequences of actin cDNAs isolated from *Oreochromis niloticus* (*On*) and cDNAs of *Takifugu rubripes* (*Tr*) obtained from NCBI database. Dashes indicate gaps introduced in the sequences to optimize the alignment. Diagnostic aa positions that distinguish α-muscle actin from β-cytoplasmic actins are in bold face type. Sources and accession numbers for the actin sequences are described in Materials and Methods.

Table 1 - Species and accession numbers of actin cDNA sequences obtained from GenBank.

Species	Actin type and GenBank entries
Ambystoma mexicanum (salamander)	α-actin: AF276076
Arabidopsis thaliana (arabidopsis)	β-actin: NM179953
Bos taurus (cattle)	α-actin: NM174225; β-actin: AY141970
Ciona intestinalis (ascidian)	α-actin: AK115759
Danio rerio (zebrafish)	α-actin: BC065435; β-actin: NM131031
Dictyostelium discoideum (myxamoeba)	β-actin: XM632417
Dipsosaurus dorsalis (iguana)	α-actin: AF503591
Drosophila melanogaster (fruit fly)	α-actin: NM079643; β-actin: NM079076
Gallus gallus (chicken)	α-actin: X02212; β-actin: NM205518
Homo sapiens (human)	α-actin: BC012597; β-actin: NM001101
Mus musculus (mouse)	α-actin: M12866; β-actin: NM007393
Oryza sativa (rice)	β-actin: AB047313
Rattus norvegicus (rat)	α-actin: NM019212; β-actin: NM031144
Spodoptera exigua (moth)	α-actin: AY507963
Strongylocentrotus purpuratus (sea urchin)	α-actin: J01202; β-actin: NM214529
Sus scrofa (pig)	β-actin: AY550069
Takifugu rubripes (fugu)	α-actins: U38850, U38958, U38959, U38960, U38961; β-actins: U37499, U38848, U38849
Xenopus laevis (African clawed frog)	α-actin: BC041197
Xenopus tropicalis (pipid frog)	β-actin: BC064155

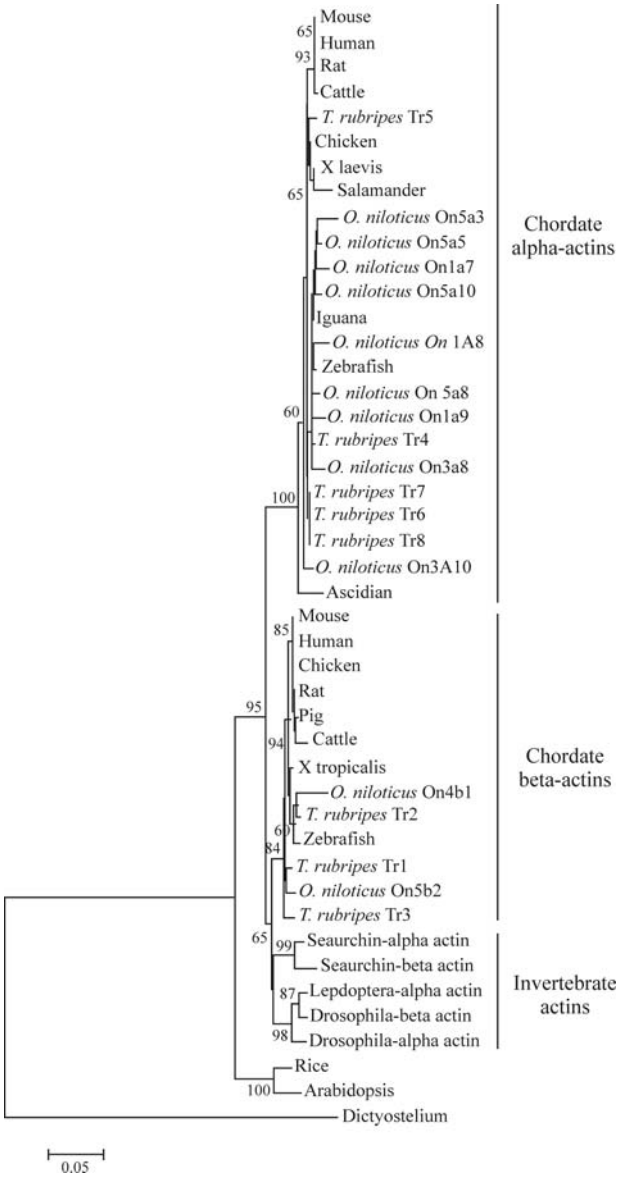

Figure 2 - Molecular phylogenetic tree inferred by the neighbor-joining method from predicted amino acid (aa) sequences of actin genes. The myxamoeba species *Dictyostelium discoideum* was used as an outgroup. Branch lengths are proportional to evolutionary distances. Scale bar indicates an evolutionary distance of 0.05 aa substituition per position in the sequences. The numbers at each node indicate the percentage recovery (> 60%) of the particular node (500 bootstrap replicates) in which the same internal branch was recovered. Sources and accession numbers for the actin sequences are described in Material and Methods.

myxamoeba species *Dictyostelium discoideum* was used as an outgroup. The animal actins were clearly discriminated from plant actins in 95% of the recovered trees. Not surprisingly, a close relationship was observed between Nile tilapia and other vertebrate α-actins. The clustering of the isolated Nile tilapia α-actins with other vertebrate and ascidian muscle actins in the same clade was strongly supported in 100% of bootstrap replicates. This strongly supported relationship between vertebrate and ascidian muscle actins has been already demonstrated by the comparison of diagnostic aa and phylogenetic analyses, suggesting that the chordate muscle-type actins probably diverged from a nonmuscle-like actin before the divergence of urochordates and vertebrates, but presumably after the divergence of echinoderms and chordates (Kusakabe *et al.*, 1997). The actin genes allowed the discrimination of higher taxa, but were not informative for lower taxonomic levels because of the high conservation at DNA sequence level.

The clade composed of the isolated Nile tilapia cytoplasmic actins and vertebrate cytoplasmic actins is supported in 84% of the bootstrap replicates. An interesting point was the presence of invertebrate muscle and cytoplasmic actins in the clade of vertebrate cytoplasmic actins (tree node percentage recovery of 65%). This relationship is in accordance with previous analyses that suggest that non-

chordate muscle actin genes are more closely related to vertebrate cytoplasmic actins than to vertebrate muscle actins (Kusakabe *et al.*, 1997). The actins expressed in muscle cells of non-chordates have traditionally been considered to be cytoplasmic-like (Vandekerckhove and Weber, 1984), and non-muscle actins are likely to represent ancestral actin forms.

As evidenced for other organisms, different fish actin types also seem to be under different evolutionary selection pressures, leading to the conjecture that these isoforms seem to have somewhat different roles. Further analyses comparing the organization of distinct actin isoforms from several species will be useful for understanding the molecular evolution and function of these genes in fishes.

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Internet Resources

BLAST: The Basic Local Alignment Search Tool (BLAST) is a WWW service of National Center for Biotechnology Information (NCBI). Available from http://ncbi.nml.nih.gov/blast/ (May 20, 2006).

ClustalW: WWW Service at the European Bioinformatics Institute. Available from http://www.ebi.ac.uk/clustalw (May 20, 2006).

GenBank: GenBank® is the National Institutes of Health (NIH) genetic sequence database, an annotated collection of all publicly available DNA sequences. Available from http://www.ncbi.nlm.nih.gov/GenBank/ (January 03, 2007).

Primer3: Primer3 is a widely used program for designing PCR primers. Available from http://fokker.wi.mit.edu/primer3/ (May 20, 2006).

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