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The nuclear phenotypic plasticity observed in fish during rRNA regulation entails Cajal bodies dynamics

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Abstract

Cajal bodies (CBs) are small mobile organelles found throughout the nucleoplasm of animal and plant cells. The dynamics of these organelles involves interactions with the nucleolus. The later has been found to play a substantial role in the compensatory response that evolved in eurythermal fish to adapt to the cyclic seasonal habitat changes, i.e., temperature and photoperiod. Contrary to being constitutive, rRNA synthesis is dramatically regulated between summer and winter, thus affecting ribosomal biogenesis which plays a central role in the acclimatization process. To examine whether CBs, up to now, never described in fish, were also sustaining the phenotypic plasticity observed in nuclei of fish undergoing seasonal acclimatization, we identified these organelles both, by transmission electronic microscopy and immunodetection with the marker protein p80-coilin. We found transcripts in all tissues analyzed. Furthermore we assessed that p80-coilin gene expression was always higher in summer-acclimatized fish when compared to that adapted to the cold season, indicating that p80-coilin expression is modulated upon seasonal acclimatization. Concurrently, CBs were more frequently found in summer-acclimatized carp which suggests that the organization of CBs is involved in adaptive processes and contribute to the phenotypic plasticity of fish cell nuclei observed concomitantly with profound reprogramming of nucleolar components and regulation of ribosomal rRNAs.

Keywords: Cajal bodies; Coilin; Seasonal adaptation; Carp

Cajal bodies (CBs) are small organelles found within both plant and animal nuclei. These organelles contain the p80-coilin marker protein, nucleolar proteins fibrillarin and Nopp140 as well as many factors associated with RNA transcription and processing [1–4]. These factors include small nucleolar RNAs that take part in ribosomal biogenesis and small Cajal body-specific RNAs (scaRNA) which accumulate specifically in these organelles and participate in snRNAs modifications [5]. CBs are known to be dynamic and mobile organelles distributing its structure throughout the nucleoplasm, involving interactions with the nucleolus. Accordingly, fibrillarin mobility in the nucleus entrust both CBs and nucleolus suggesting that, as Nopp140, processing and assembly of snoRNPs involves the CBs. In this context, it is interesting to note that the U3 snoRNA precursor is capped by the Tgs1/PIMT methyl transferase enzyme which has been localized within the CBs [6] prior to its interaction with fibrillarin and other proteins constituting the snoRNPs [7]. Therefore, this evidence suggests that both CBs and the nucleolus are involved in ribosomal biogenesis.

We have previously shown that ribosomal RNA synthesis, which is observed in the phenotypic rearrangement of nucleolar components, is reprogrammed as part of the physiological response that fish have evolved to adapt to the environmental cyclic seasonal temperature and photoperiod changes [8,9]. Accordingly, we have found that transcription of 5.8S rRNA is considerably higher in summer- than in winter-acclimatized carp [10]. We have also

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observed that carp nucleolin, the most abundant nucleolar protein, reported to play a key role in repressing rRNA [11], is strongly induced upon cold temperature acclimatization [12] concomitantly with the appearance of a segregated nucleolus [9].

Here, we identify for the first time, CBs in fish. Additionally, we provide evidence that these CBs participate in seasonal acclimatization in carp and that p80-coilin expression is involved in the compensatory response in conjunction with the observed nucleolar dynamics [9] where the mechanism and regulation of ribosomal biogenesis contributes to the remarkable phenotypic plasticity of the nucleus during seasonal acclimatization in fish.

Materials and methods

Animals and tissues preparation. Male carp (*Cyprinus carpio*) of approximately 1000–2000 g were maintained as described in previous studies [13]. Tissues from different organs (liver, brain, pituitary and testis) were obtained, washed with PBS pH 7.4 (137 mM NaCl, 2.7 mM KCl, 10 mM Na_2HPO_4 and 2 mM KH_2PO_4) and immediately processed or stored at −80 °C.

Isolation of the carp p80-coilin cDNA. A 1400 bp fragment corresponding to a partial coding sequence of carp p80-coilin was amplified by nested RT-PCR using carp brain tissue cDNA as a template. Amplification was performed using degenerated primers derived from *Danio rerio* p80-coilin partial sequence (GenBank Accession No.: AF220008). The primers used include: COILRin, 5′-TTYTGGTAGACCAAGTCAAA C-3′; COILRout, 5′-ACTCTCTCTGWGCCATCTGG-3′; COILFin, 5′-TGGTTGCTKGTGGACCTGAAC-3′; COILFout, 5′-ATGTGCTG GTTGCTKGTGGAC-3′.

The 5′- and 3′-terminal regions of carp p80-coilin cDNA were isolated using FirstChoice RLM-RACE kit (Ambion, USA) with adapter and gene-specific primers deduced from the 1400 bp amplified fragment described earlier (P80-5′in: 5′-GACAACTCGGCATTTGTTCAGGT C-3′. P80-5′ou: 5′-TGCTGGAAAGATCTGCGACAACTC-3′. P80-3′in: 5′-GCTGAACCCGGAAAGTTTGACTTG-3′. P80-3′ou: 5′-CTCGGC CTCAAGCTCCAGCTGAAC-3′). The full-length carp p80-coilin cDNA was amplified by RT-PCR using CoilFW-N; 5′-GGAATTCCATATGG CCACCTCCAGTCTCAATAC-3′ and CoilRev-B; 5′-CGCGGATCCA TCAGGGTTCTGATATAC-3′ as primers. The PCR product was cloned in the pGEM-T vector system I (Promega, USA) and named pCoilCM.

Gene expression of carp p80-coilin during acclimatization. Total RNA fractions were isolated in testis, brain, pituitary and liver tissues from winter- and summer-acclimatized carp as reported in [8]. Semi-quantitative RT-PCR was performed with StrataScript® One-Tube RT-PCR System (Stratagene, USA), using 0.5 μg of total RNA from each sample as a template and primers CoilFW-N and CoilRev-B. As control, we performed RT-PCR using primers derived from carp mitochondrial 16S ribosomal RNA (16Scarp3′FW: 5′-GGGGTTTACGACCTCGATGT T-3′ and 16Scarp3′REV: 5′-GCTTTAAGTATGGGCCCCCCT-3′) and total RNA obtained from each tissue as template. The RT-PCR products were fractionated in a 1.5% agarose gel and visualized by staining with ethidium bromide.

Construction of a carp p80-coilin expression vector. The coding region containing the first 499 residues of carp p80-coilin protein was amplified by PCR from pCoilCM using primers CoilFW-N and CoilRev-B, which contained NdeI and BamHI restrictions sites, respectively (underlined). This product was digested with NdeI and BamHI and directionally cloned in the pET15b expression vector (Novagen, USA). This clone was named pETCoil.

Expression and purification of recombinant protein. *Escherichia coli* BL21 (DE3) competent cells were transformed with the pETCoil clone. Bacteria containing this clone was grown at 37 °C with agitation in minimal medium plus 50 mg/ml ampicillin and 33 mg/ml chloramphenicol, until log-phase and subsequently induced with 1mM IPTG (isopropyl-1-thio-β-D-galactopyranoside) during 7 h. Recombinant protein was purified by Ni-NTA according to manufacturer instructions (Qiagen, USA), quantified using the Bio-Rad protein assay and analyzed in a 10% SDS–PAGE gel.

Generation of p80-coilin polyclonal antibodies. Specific carp coilin antibodies were prepared by BiosChile S.A. (Chile) by immunizing two rabbits with the purified recombinant p80-coilin protein.

Immunodetection of Cajal bodies. Tissue sections for immunocytochemistry were prepared from pituitary and brain of acclimatized carps according Fernandez et al. [14], and incubated with the carp p80-coilin polyclonal antibody (dilution 1:2000) and FITC-conjugated anti-rabbit IgG (dilution 1:1000, Biosource, USA). The sections were counterstained with 0.5 μg/ml propidium iodide and mounted in 10% DABCO, Sigma (USA) in 90% glycerol. The samples were examined using laser scanning confocal microscopy on an Axiovert 100 M Microscopy (Zeiss).

Transmission electronic microscopy of CBs. Fragments of pituitary from acclimatized carps were fixed for 15–45 min at 4 °C in 1.6% glutaraldehyde in 0.1 M Sörensen's buffer (pH 7.4). The fragments were washed in Sörensen's buffer, incubated for 5 min at 4 °C in Carnoy solution (1:3 acetic acid/ethanol), rehydrated in graded ethanol (5 min each in 100%, 75%, 50%, and 30%), and incubated for 10 min at 60 °C in a freshly prepared solution of 2% gelatin in 1% formic acid and 50% $AgNO_3$ in water. Samples were rinsed in water and immersed for 10 min in a 5% thiosulfate solution and finally rinsed in water before being acetylated as previously described [15]. Ultrathin sections were mounted on collodion-coated cupper grids and stained with uranyl acetate and lead citrate before examination in a Jeol CX100 electron microscope at 60 kV.

Results

We isolated a full length *C. carpio* cDNA sequence (1867 bp, GenBank Accession No. AY585706) encoding 532 amino acids corresponding to carp p80-coilin. The entire sequence of this protein shares 94% identity with its putative ortholog in zebrafish, but only 34% identity with putative mammalian orthologs (human, rat and mouse). However, N- and C-terminal carp p80-coilin domains are more highly conserved (Supplementary Fig. 1). Additionally, the carp p80-coilin contains the conserved RG Box within the C-terminal domain. This RG box is characterized by the presence of several glycine/arginine repeats. Within the central variable region, two characteristic p80-coilin nuclear localization signals (NLSa and NLSb) are located at positions 103–108 and 189–193, respectively (boxed regions in Supplementary Fig. 1).

To assess gene expression of p80-coilin in carp undergoing seasonal acclimatization, RT-PCR assays were performed on different tissues. As shown in Fig. 1, p80-coilin transcript was detected in all tissues analyzed. However, p80-coilin expression was significantly higher in testis, brain and pituitary on summer- compared with winter-acclimatized carp. Liver expression of p80-coilin was weak in both seasons and did not show significant differences between summer- and winter-adapted fish (Fig. 1).

Cajal bodies in carp cells were detected by immunocytochemistry (Fig. 2). We labeled semithin sections of pituitary and brain obtained from winter- and summer-adapted fish with our polyclonal antibody prepared from the purified recombinant carp p80-coilin protein (residues 1–499). The