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Environmental reprogramming of the expression of protein kinase CK2β subunit in fish

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Abstract

The dramatic segregation of the nucleolar components in winter-acclimatized carp is the most striking cellular-phenotypical feature observed during the seasonal adaptation of this fish toward the circannual changes in its habitat. Our studies also show that the carp habitat temperature and photoperiod winter conditions provoke a remarkable reduction of both rRNA transcription and the processing of their precursors. To gain knowledge on the mechanisms involved in the regulation of nucleolar activity during the seasonal adaptation process, we studied the behavior of some genes, specifically snoRNA U3 and protein kinase CK2.

Consistent with the reduction in the synthesis and processing of pre-rRNA observed during the cold season, the level of CK2β expression decreases in winter when compared to that attained in summer. Similarly, in winter, liver and kidney cells contain lower levels of CK2β subunit protein compared to summer. CK2 is associated with or modifies different factors and enzymes involved in the nucleolar activity; therefore, its higher or lower content could be part of the molecular mechanisms underlying the nucleolar seasonal changes that occur during the compensatory acclimatization process. (Mol Cell Biochem **227**: 107–112, 2001)

Key words: protein kinase CK2β subunit, nucleolus, fish acclimatization, carp

Introduction

Acclimatization of a eurythermal fish, i.e. the cyclical adaptation to the seasonal changes of its habitat, involves among other cellular and molecular changes, a clear modulation of gene expression [1–6]. We have studied the mechanisms behind this kind of functional adjustment in *Cyprinus carpio* (carp) and observed that phenotypically, one of the most dramatic cellular modifications occurs during winter when the cytoplasm of the hepatocyte is full of glycogen granules and the nucleolar components appear segregated [7]. Concurrently, the transcription of ribosomal RNA (rRNA) and the processing of their precursor (pre-rRNA) decrease notoriously compared to the summer-adapted fish [8, 9]. During the warm season, however, the hepatocyte cytoarchitecture exhibits all the features typical of a metabolically-active cell [7]. The cytoplasm is therefore empty of glycogen, the nucleolar components are intermingled, and the transcription and processing of rRNA occur at a high rate [7–9].

When carp are treated with insulin, either by oral administration or intra-peritoneal injections, a clear reversion of the winter cell phenotype takes place, rendering all of the ultra-structural features that characterize the summer-adapted hepatocyte [10, 11]. Phosphorylation is needed for rRNA synthesis in the nucleolus of eukaryotic cells [12]. Furthermore, insulin enhancement of rRNA synthesis is correlated with protein kinase CK2 phosphorylation of nucleolin as well as of other protein nucleolar factors [13, 14]. At the cellular level, the nucleolar activity in the carp seems to play a central role in the functional adjustment that the seasonal acclimatization imposes upon the fish, and we therefore deemed it important to examine the possible association of protein kinase CK2 expression events with the remarkable nucleolus reorganization that occurs between summer and winter.

Protein kinase CK2, a tetramer composed of the α catalytic and the β regulatory subunits, is known to be a highly conserved serine/threonine phosphorylating enzyme present in the nucleus and cytoplasm of all eukaryotic cells [15]. To

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study the expression of this protein kinase in a eurythermal fish undergoing seasonal acclimatization, we isolated the cDNA of the carp CK2β subunit and found a significant differential gene expression between winter- and summer-acclimatized carp [16, 17]. We report herein that the CK2β subunit level clearly changes between seasons in carp liver and kidney cells which, in addition to our previous observations [16], provides evidence that the expression of the CK2β subunit is regulated in this fish.

Materials and methods

Animals and tissues

Seasonal-acclimatized male carp were captured and maintained as described previously [2]. The water temperature was 20–22°C in summer and 8–10°C in winter. Tissue sections for immunocytochemistry experiments were prepared and stored as reported [2]. Briefly, the tissue blocks were fixed in Bouin for 24 h at room temperature, then dehydrated in a graded series of ethanol, ending with 1-butanol and then embedded in Paraplast. Sections 5–7 μm thick were placed on glass slides.

Western blot and immunocytochemical detection

Carp liver and kidney protein extracts for the western blot were prepared as described by Figueroa *et al.* [2]. The tissue was homogenized in 50 mM phosphate buffer, pH 7.4, containing 1 mM EDTA and 1 mM EGTA, precipitated at –20°C after the addition of 5 vol. acetone, and the precipitate was recovered by centrifugation. This step was repeated twice and the precipitate was dissolved in 25 mM Tris-HCl, pH 6.8 containing 5% glycerol, 1% SDS, 0.01% bromophenol blue and 1.5% β-mercaptoetanol and it was then fractionated by PAGE, and electrotransferred to nitrocellulose membranes. The filters were then immunostained according to Stenberger *et al*. [18] and Inostroza *et al*. [19]. The antibody was a kind gift from the laboratory of Dr. David Litchfield, and it corresponds to a rabbit polyclonal antiserum against the C-terminal end (198–215) of a consensus sequence of CK2β subunit [20, 21]. The label was quantified using an automated image digitizing system as described earlier [22], in tissue sections from four different carp for each season. The Student's *t*-test was used to assess differences, $p < 0.05$.

Modelization of carp CK2β subunit

A 3-dimensional homology modeling of the carp CK2β subunit was performed with the derived amino acid sequence from carp cDNA, GeneBank accession number AF133088 [16], using the Swiss Model program, version 3.5 [23]. The comparative protein modeling was performed with the ProMod/ProMod II program and the energy minimization with Gromos96. The 3D-structures used as templates were human CK2β (Protein Data Bank accession numbers 1QF8A and 1QF8B, respectively). Figure 1 was prepared with the MOLMOL program [24].

Results and discussion

During our recent studies on the involvement of CK2β in the complex mechanisms that comprise the cellular and molecular compensatory responses that a eurythermal fish requires to survive the cyclical seasonal changes of its habitat, we found that the carp CK2 regulatory subunit cDNA exhibits a high degree of evolutionary conservation [16]. The homology observed in the amino acid derived sequence with other vertebrates [16] suggested a 3-dimensional structure similar to that described by Chantalat *et al.* [25] by crystallographic analyses of a human CK2β dimer lacking 33 residues at the C-terminus. Upon structural protein modeling of the carp CK2β, as shown in Fig. 1, the destruction box, the acidic cluster, and the zinc finger motif can be easily recognized. The carp CK2β cDNA sequence displays a 94 and 87% identity when compared with the zebrafish and human sequences, respectively. The differences primarily map the third codon's nucleotide. In brief, carp CK2β cDNA codes for a 215-amino acid protein completely homologous to the zebrafish and distinctive with respect to the human CK2β primary structure in only three carboxy terminal amino acids.

When we assessed the level of expression of the carp CK2β subunit gene [16] during the seasonal acclimatization of *C. carpio*, hybridizing liver and kidney tissue sections with a specific 20-mer antisense oligonucleotide probe, most of the CK2β mRNA was found in the nuclei. Lower signals were also detected in the cytoplasm [16, 17]. The identification of CK2β transcripts by *in situ* hybridization was visibly more intensive in the liver and kidney sections from summer-acclimatized fish when compared to those from the cold-season adapted carp. Competitive RT-PCR using total RNA from winter- and summer-acclimatized carp liver as a template and the competitor, an RNA template, confirmed the striking CK2β subunit gene transcriptional differences that the *in situ* assays revealed. Quantitatively, the CK2 regulatory subunit gene transcription is 7.2 times higher in the liver of summer-acclimatized fish than that observed in the cold season-acclimatized carp.

To address the question of whether the differential CK2β transcription followed a similar pattern at the translational level, the carp CK2β subunit content was measured by immunocytochemistry analyses. A polyclonal anti-CK2β antise-

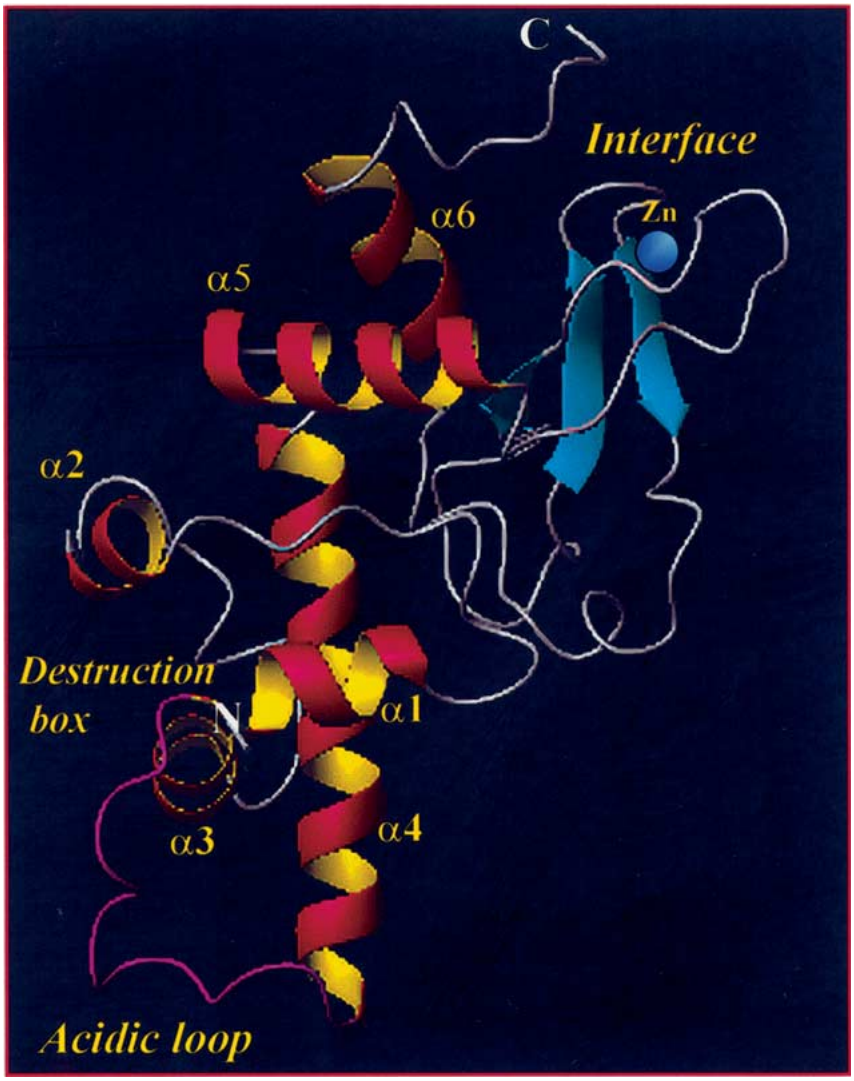

Fig. 1. Ribbon representation of the carp CK2β subunit structure, modeled according to the amino acid sequence derived from carp CK2β cDNA (GeneBank, Accession Number AF133088). A homology model was constructed using the Swiss Model program version 3.5 [23] and the comparative protein modeling was performed with the ProMod/ProMod II program and the energy minimization with Gromos96. The 3D-structures used as templates were human CK2β 1QF8A and 1QF8B (Protein Data Bank). The figure was prepared with the MOLMOL program [24].

rum directed toward the C-terminal synthetic peptide $\beta^{198–215}$ [20, 21], kindly provided by David Litchfield, was probed against the total protein extracted from carp liver and kidney tissue. As depicted in Fig. 2A, the antiserum recognizes only one band in both the carp kidney and liver cells, with a molecular mass between 26 and 27 kDa. Thus the antibody exhibited a clear specificity, identifying a protein of the size expected for the animal CK2 regulatory subunit [15]. In addition to the immunoblotting analyses, we immunostained carp liver sections with the aim of gaining knowledge about the cellular localization of the CK2β subunit protein. Consistent with the reports that map CK2 as a predominantly nuclear enzyme [26–28, Ahmed *et al.*, this issue], we also found that cells were stained mainly at the nuclear and perinuclear levels (Fig. 2B). The results attained with the polyclonal anti-CK2β antiserum allowed the quantification of the carp CK2β subunit *vis à vis* the seasonal adaptive state of the fish. Figure 3 depicts the immunostaining attained when liver (A) and kidney tissue (B) from summer- and winter-acclimatized carp were probed. The stained sections clearly show differences in carp CK2β content between summer and winter. The significant differences in CK2 regulatory subunit content were validated when the digitized signals were quantified. Figure 3 shows that liver cells from summer-acclimatized fish exhibited twice the amount of CK2β subunit when compared to the cold season counterparts. Concurrently, Fig.

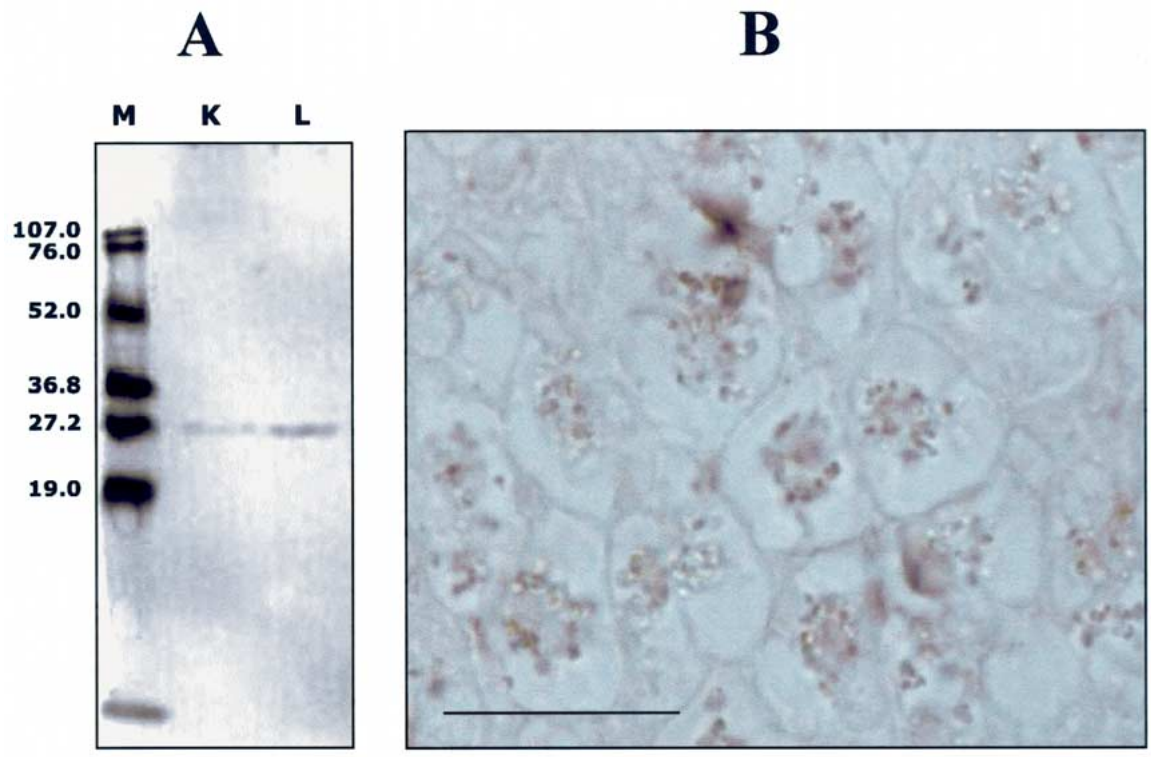

Fig. 2. Carp CK2β immunodetection with a rabbit polyclonal anti-CK2β antiserum directed against the C-terminal synthetic peptide $\beta^{198-215}$ kindly provided by David Litchfield. (A) Immunoblotting of fractionated protein extracts of liver and kidney (1:100). Lane M: pre-stained molecular weight standard, lane K: 60 μg carp kidney total protein, lane L: 60 μg carp liver total protein. (B) Immunocytochemical staining of liver section with anti-CK2β antiserum, dilution 1:100. (Bar: 20 μm).

3 depicts the significant variation attained upon quantification of the CK2β level in kidney cells where those from the warm-season-adapted carp display 3 times more than the winter-acclimatized carp immunostained cells. Thus the seasonal modulation of the CK2β subunit gene transcription exhibits a consistent correlation with the pattern of appearance of the CK2 regulatory subunit within the framework of the complex adaptive process that implies a physiologically coordinated response of the fish toward the stress that the changes in the physical parameters of their habitat imposes.

Several reports have shown shifts of CK2 expression primarily affecting a variety of tumor and leukemic cells [14, 29, Seldin *et al.*, this issue]. Thus protein kinase CK2 does not behave as a classical constitutive enzyme [Pyerin *et al.*, this issue]. More recently, Gerber *et al.* [27] have approached the study of the cellular dynamics of protein kinase CK2 subunits using specific stressors. Interestingly, they found that CK2α and CK2β are differentially targeted inside the nucleolus, whereas the CK2α subunit is preferentially accumulated.

Although our studies were performed assessing the transcription and content of the protein kinase CK2β subunit, the results obtained determined that the enzyme is regulated under physiological conditions, particularly in our model system that demands a completely different cellular strategy than homeotherms to attain the homeostasis needed for survival. We are studying the carp pituitary gland to assess its possible role in the complex process that transduces the periodic external physical milieu changes into the needed signaling molecules that build the compensatory response of the fish [30]. Nucleolar activity appears to be of the utmost importance for the proper cell-functional adjustment in the seasonal adaptive process of the fish. Assessing small nucleolar RNA (snoRNA) expression, we determined a remarkable decrease in winter compared to summer, a drop off that could be linked to the reduction of the processing of pre-rRNA observed in winter [8]. We have also documented a consistent behavior of genes related to the nucleolar activity such as 5.8S rRNA and U3 snoRNA [8, 9].

The localization of CK2 in the nucleolus, along with its kinase activity, appears to play a key role in the regulation of rDNA transcription [28]. We are currently involved in the study of carp nucleolin, a major substrate of CK2. Nucleolin is implicated in the control of ribosome biogenesis through the regulation of rRNA transcription [31]. Additionally, this nucleolar protein plays a role in the processing of pre-rRNA and the packaging of ribosome particles.

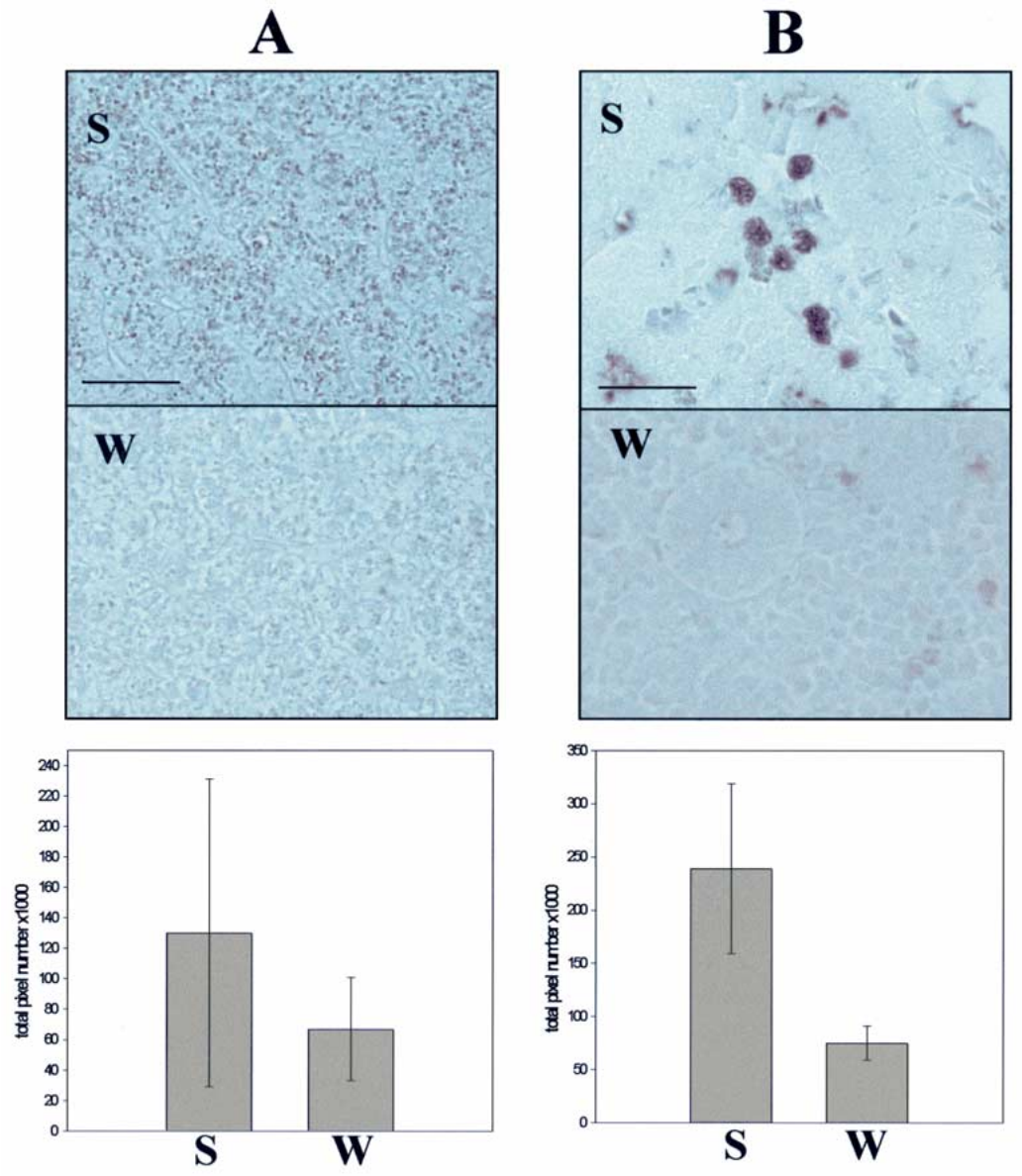

Fig. 3. Immunocytochemical staining and semi-quantitative analyses of CK2β subunit content in liver (A) and kidney (B) sections from summer- (S) and winter-acclimatized (W) carp. The detection was performed with a rabbit polyclonal anti-CK2β antiserum [19, 20], dilution 1:100. (Bar: 100 μm). Semi-quantitative analyses of the immunolabeling are represented graphically. The histograms depict the mean total pixel values (± S.D.) in four different individuals corresponding to each season. In both tissues, the analyses rendered significant differences at Student *t*-test $p < 0.05$.

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References

1. Krauskopf M, Amthauer R, Araya A, Concha M, León G, Ríos L, Vera MI, Villanueva J: Temperature acclimatization of the carp. Cellular and molecular aspects of the compensatory response. Arch Biol Med Exp 21: 151–157, 1988
2. Figueroa J, Molina A, Alvarez M, Villanueva J, Reyes A, León G, Krauskopf M: Prolactin gene expression and changes of prolactin pituitary level during the seasonal acclimatization of the carp. Comp Biochem Physiol 108B: 551–560, 1994

3. Goldspink G: Adaptation of fish to different environmental temperature by qualitative and quantitative changes in gene expression. J Therm Biol 20: 167–174, 1995
4. Tiku PE, Gracey AY, Macartney AI, Beynon RJ, Cossins AR: Cold-induced expression of Δ^9-desaturase in carp by transcriptional and posttranscriptional mechanisms. Science 271: 815–818, 1996
5. Kausel G, Vera MI, San Martín R, Figueroa J, Molina A, Muller M, Martial J, Krauskopf M: Transcription factor Pit-1 expression is modulated upon seasonal acclimatization of eurythermal ectotherms: Identification of two Pit-1 genes in the carp. J Cell Biochem 75: 598–609, 1999
6. Sarmiento J, Leal S, Quezada C, Kausel G, Figueroa J, Vera MI, Krauskopf M: Environmental acclimatization of the carp modulates the transcription of β-actin. J Cell Biochem 80: 223–228, 2000
7. Sáez L, Zuvic T, Amthauer R, Rodríguez E, Krauskopf M: Fish liver protein synthesis during cold acclimatization. Seasonal changes of the ultrastructure of the carp hepatocyte. J Exp Zool 230: 175–186, 1984
8. Vera MI, Norambuena L, Alvarez M, Figueroa J, Molina A, León G, Krauskopf M: Reprogramming of nucleolar gene expression during the acclimatization of the carp. Cell Mol Biol Res 39: 665–674, 1993
9. Vera MI, Ríos HM, de la Fuente E, Figueroa J, Krauskopf M: Seasonal acclimatization of the carp involves differential expression of 5.8S ribosomal RNA in pituitary cells. Comp Biochem Physiol 118B: 777–781, 1997
10. Sáez L, Amthauer R, Rodríguez E, Krauskopf M: Effects of insulin on the fine structure of hepatocytes from winter-acclimatized carp: Studies on protein synthesis. J Exp Zool 230: 187–197, 1984
11. Vera MI, Romero F, Figueroa J, Amthauer R, León G, Villanueva J, Krauskopf M: Oral administration of insulin in winter-acclimatized carp (*Cyprinus carpio*) induces hepatic ultrastructural changes. Comp Biochem Physiol 106A: 677–682, 1993
12. Hannan R, Taylor L, Cavanaugh A, Hannan K, Rothblum LI: UBF and the regulation of ribosomal DNA transcription. In: M.R. Paule (ed). Transcription of Ribosomal RNA Genes by Eukaryotic RNA Polymerase I. Springer-Verlag, Berlin, Heidelberg, 1998, pp 221–228
13. Csermely P, Schnaider T, Chatman B, Olson MOJ, Kahn CR: Insulin induces the phosphorylation of nucleolin. J Biol Chem 268: 9747–9752, 1993
14. Guerra B, Issinger O-G: Protein kinase CK2 and its role in cellular proliferation, development and pathology. Electrophoresis 20: 391–408, 1999
15. Allende JE, Allende CC: Protein kinase CK2: An enzyme with multiple substrates and puzzling regulation. FASEB J 9: 313–323, 1995
16. Vera MI, Kausel G, Barrera R, Leal S, Figueroa J, Quezada C: Seasonal adaptation modulates the expression of the protein kinase CK2β subunit gene in the carp. Biochem Biophys Res Com 271: 735–740, 2000
17. Leal S, Kausel G, Figueroa J, Escobar J, Pinto R, San Martín R, Vera MI: Quantification of the expression of CK2β subunit during the acclimatization of the fish *C. carpio*. Biol Res: R-90, 2000
18. Stenberger LA, Hardy PH, Cuculis JJ, Meyer HG: The unlabeled antibody enzyme method of immunochemistry. J Histochem Cytochem 18: 315–333, 1970
19. Inostroza J, Vera MI, Goicoechea O, Amthauer R, Krauskopf M: Apolipoprotein A-I synthesis during the acclimatization of the carp (*Cyprinus carpio*). J Exp Zool 256: 8–15, 1990
20. Litchfield DW, Lozeman FJ, Cicirelli MF, Harrylock M, Ericsson LH, Piening CJ, Krebs EG: Phosphorylation of the beta subunit of casein kinase II in human A431 cells. Identification of the autophosphorylation site and a site phosphorylated by $p34^{cdc2}$. J Biol Chem 266: 20380–20389, 1991
21. Litchfield DW, Luscher B, Lozeman FJ, Eisenman RN, Krebs EG: Phosphorylation of casein kinase II by p34cdc2 *in vitro* and at mitosis. J Biol Chem 267: 13943–13951, 1992
22. Kausel G, Vera M-I, Figueroa J, Hernández J, San Martin R, Molina A, Chavez VM, Muller M, Martial J, Krauskopf M: Effect of seasonal acclimatization on the expression of the carp transcription factor Pit-1. Biochem Mol Biol Int 45: 813–821, 1998
23. Peitsch MC: ProMod and Swiss-Model: Internet-based tools for automated comparative protein modeling. Biochem Soc Trans 24: 274–279, 1996
24. Koradi R, Billeter M, Wüthrich K: MOLMOL: A program for display and analysis of macromolecular structures. J Mol Graphics 14: 51–55, 1996
25. Chantalat L, Leroy D, Filhol O, Nueda A, Benitez MJ, Chambaz EM, Cochet C, Dideberg O: Crystal structure of the human protein kinase CK2 regulatory subunit reveals its zinc finger-mediated dimerization. EMBO J 18: 2930–2940, 1999
26. Krek W, Maridor G, Nigg EA: Casein kinase II is a predominantly nuclear enzyme. J Cell Biol 116: 43–55, 1992
27. Gerber D, Souquere-Besse S, Puvio F, Dubois M-F, Bensaude O, Cochet C: Heat-induced relocalization of protein kinase CK2. Implication of CK2 in the context of cellular stress. J Biol Chem 275: 23919–23926, 2000
28. Faust M, Monternah M: Subcellular localization of protein kinase CK2. A key to its function? Cell Tissue Res 301: 329–340, 2000
29. Faust RA, Gapany M, Tristani P, Davis A, Adams GL, Ahmed K: Elevated protein kinase CK2 activity in chromatin of head and neck tumors: Association with malignant transformation. Cancer Lett 101: 31–35, 1996
30. Figueroa J, Reyes A, Ríos M, Vera, MI, Krauskopf M: Effect of temperature and photoperiod on prolactin transcription in *Cyprinus carpio.* Zool Sci 14: 353–357, 1997
31. Ginisty H, Sicard H, Roger B, Bouvet P: Structure and functions of nucleolin. J Cell Sci 112: 761–772, 1999