

Seasonal environmental changes regulate the expression of the histone variant macroH2A in an eurythermal fish

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Abstract Adaptation to cold and warm conditions requires dramatic change in gene expression. The acclimatization process of the common carp *Cyprinus carpio* L. in its natural habitat has been used to study how organisms respond to natural environmental changes. At the cellular level, adaptation to cold condition is accompanied by a dramatic alteration in nucleolar structure and a down regulation of the expression of ribosomal genes. We show that the enrichment of condensed chromatin in winter adapted cells is not correlated with an increase of the heterochromatin marker trimethyl and monomethyl K20H4. However, the expression of the tri methyl K4 H3 and of the variant histone macroH2A is significantly increased during the winter season together with a hypermethylation of CpG residues. Taking into account the properties of macroH2A toward chromatin structure and dynamics and its role in gene repression our data suggest that the increased expression of macroH2A and the hypermethylation of DNA which occurs upon winter-acclimatization plays a major role for the reorganization of chromatin structure and the regulation of gene expression during the physiological adaptation to a colder environment.

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1. Introduction

Eurythermal fish acclimatization occurring during seasonal changes requires gene expression adjustments to provide the homeostatic condition for survival [1–3]. *Cyprinus carpio* L. lives in a natural environment submitted to a wide range of temperature conditions during winter and summer representing a good experimental system to study adaptation to cold condition on poikilothermic vertebrates [4–6]. Transcriptomes studies of different tissues issue from cold adapted carps indicate that a large number of cDNA (over 3400) were affected by cold [6]. At the cellular level, the most dramatic change that occurs during the acclimatization process is observed in the

nucleolus (Fig. 1). In summer, the nucleoli have a reticulated aspect containing numerous intranucleolar clumps of condensed chromatin. In contrast, in winter, the nucleoli appear as compact masses surrounded by a thick layer of condensed chromatin. These nucleolar rearrangements reveal profound changes in ribosomal biogenesis, like transcription of ribosomal DNA genes that is strongly down regulated, as a consequence of seasonal environmental variations [1]. The molecular mechanisms responsible for the nucleolar rearrangements and the down regulation of ribosomal gene expression are still not known.

Rearrangement of chromatin structure and the regulation of gene expression could be achieved through different mechanisms. Post-translational histone modification and the chromatin remodeling process could be two ways to activate or inactivate specifically gene expression [7–11]. The expression of specific proteins like HMGB1 during the temperature cycle may also regulate global transcription rate [12]. Recently, it was also suggested that the incorporation of histone variant within chromatin could be an other way to regulate transcription [13,14]. For example, the histone variant H2A.Z is implicated in both gene activation [15] and gene silencing [16]. The incorporation of the histone variant H2ABbd into the histone octamer confers lower stability to the H2ABbd nucleosomes and affects nucleosomal DNA organization [17,18]. Since the residues of conventional H2A, which are targets for post-translational modifications, are mutated in H2ABbd, one could expect the function of this histone to be regulated in a distinct way [19]. In vitro transcription experiments have reported that the incorporation of this histone variant in chromatin was associated with an increase in histone acetylation and in transcription [17,20].

MacroH2A is another histone variant whose incorporation into chromatin has been associated with repression of transcription. MacroH2A possesses a C-terminal extremity called the non-histone region (NHR) which is nearly three times the size of H2A [21–23]. Immunofluorescence data suggested that mH2A is enriched in different regions including the inactive X chromosome [21,24–26] and recent proteomic studies indicated that macroH2A was also present in nucleoli [27–29]. In vitro studies have clearly demonstrated that the incorporation of macroH2A within nucleosomes impeded chromatin remodeling by SWI/SNF and ACF [30] and the Gal4-VP16/p300 dependant transcription initiation (Doyen

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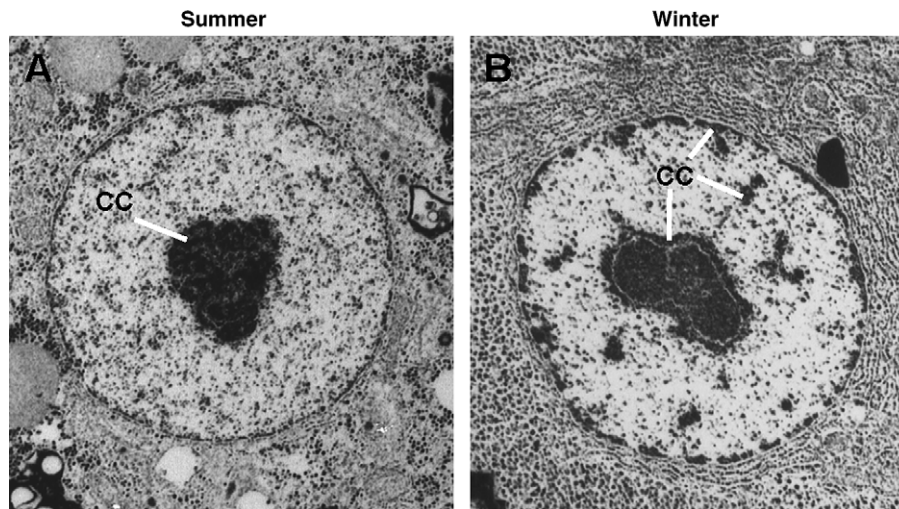


Fig. 1. The nucleolar structure is drastically modified upon acclimatization. Ultrastructural analysis of liver cells from summer (A) and winter (B) adapted carps. During the cold season the nucleolar compartment undergoes a profound rearrangement with the formation of perinucleolar condensed chromatin (CC).

et al., submitted). Interestingly, this repression of transcription is solely determined by the NHR of macroH2A (Doyen et al., submitted). The presence of macroH2A within the nucleoli suggest that macroH2A could be involved in the regulation of expression of ribosomal DNA genes, however, macroH2A has not been yet found associated with rDNA.

In this report, we describe that under the physiological condition of the acclimatization process, the expression of macroH2A and the methylation state of DNA are drastically affected. These data suggest that macroH2A could be an important factor for the global reorganization of chromatin domain and the regulation of gene expression during the acclimatization process.

2. Material and methods

2.1. Cell fractionation, protein purification and analysis

Male carp (*C. carpio*) were captured and maintained at the seasonal summer-(20–22 °C) and winter-(8–10 °C) environmental conditions [1]. Nuclear extracts were performed essentially as described previously [29]. Tissue lysates were prepared in HB buffer (250 mM Sucrose, 3 mM CaCl₂, 1 mM PMSF in 20 mM Tris pH 7.4) and centrifugated at 1500 rpm per 10 min at 4 °C in a Sorvall rotor HB6. The pellet was collect and washed with PBS at least for three times. Then, the nuclear fraction was applied on a cushion of 0.8 M Sucrose and centrifugated for 10 min at 2400 rpm on the HB6 rotor. Following quantification of the nuclear proteins, they were electrophoretically separated by SDS-PAGE and subsequently transferred to nitrocellulose. Immunoblots were probed using antibodies against either macroH₂A (1/3000), H₂A (1/250), trimethyl K20H₄ (1/1000, Abcam), Monomethyl K20H₄ (1/1000, Abcam), MeK36H₃ (1/1000, Abcam), MeK34H₃ (1/1000, Abcam) and developed using the ECL system (Amersham Pharmacia Biotech).

2.2. RT-PCR and macroH2A cloning

A 900 pb fragment from the macroH2A2 gene was amplified using primers oligonucleotides (CmacroR) (ATCTTGGCCATCTCCTGCAGGTA) and CmacroF (GTCTACATGGCAGCTGTCATTGA) derived from a macroH2A sequence obtained from *Danio rerio* [31], using 4 µg of total RNA extracted from liver of winter-acclimatized carp. RT-PCR product was cloned into vector pGEM-T (Promega) and sequenced. Gene Bank Accession No. DQ173494.

2.3. Immunofluorescence

Tissues were fixed in 4% paraformaldehyde (15 min at room temperature) and permeabilized for 15 min with 0.1% Triton-X 100 in PBS. After three washes with PBS/Tween 20 (0.1%), slides were pre-incubated in TNB blocking buffer (0.1 M Tris-HCl, pH 7.5, 0.15 M NaCl, 0.5% Blocking Reagent (w/v)) for 30 min at room temperature, then incubated overnight with a 1/100 dilution of macroH₂A antibody in TNB buffer. Slides were washed three times in TNT buffer (0.1 M Tris-HCl, pH 7.5, 0.15 M NaCl, 0.05% Tween 20) and then incubated for 2 h at room temperature with FITC conjugated anti rabbit antibody (Biosource). Slides were washed three times again with TNT and incubated 5 min with DAPI (KPL). After DAPI incubation, slides were washed three times for 10 min on PBS and mounted on glycerol (Merck). Images were obtained using an Olympus BX41 microscope with fluorescence source coupled to an Olympus digital camera.

2.4. Methylation at the promoter of rDNA

1 µg of DNA extracted from liver tissues from each season was digested with 20 U of *Hpa*II enzyme before PCR amplification using four different primers in three combinations, in order to evaluate the CCGG site at positions –30 and +45. For quantification, PCR was performed with LightCycler (Roche). The relative resistance to *Hpa*II digestion was calculated by normalizing the amount of DNA amplified with a pair of primers (–28/–10; +24/+45) located between the two studied CpG residues.

3. Results and discussion

3.1. Reorganization of nucleolar structure upon acclimatization

As a natural process, acclimatization involves a seasonal reprogramming of molecular and cellular functions [5,6,12] for the adaptation of the cells to opposed seasonal conditions. A striking cellular change can be visualized by electron microscopy of hepatocyte cells of winter (W) and summer (S) carps (Fig. 1). The nucleolar organization is clearly different in these two physiological conditions. In winter-adapted carp, the fibrillar component appears as a unique mass surrounded by several granular caps whereas in summer-adapted carp the fibrillar component reach the surrounding granular masses by forming few granular cordons. In the hepatocyte nucleolus of summer-adapted carp, the condensed chromatin appears as structures formed by filaments that are found inside the

nucleolar body. In contrast, in winter-adapted carp the condensed chromatin associated with the nucleolus appears as densely-contrasted masses that are mainly located around the nucleolus, within the nucleoplasm and attached to the nuclear membrane. A recent analysis using acetylation method with the TdT-immunogold labelling procedure has clearly detected an enrichment of condensed chromatin around the nucleolus in winter-adapted cells (Thiry et al., submitted). These changes in nucleolar ultrastructure reflect variations in the expression of rRNA gene which is higher in summer-adapted carp than in winter-adapted carp [1].

3.2. Detection of heterochromatin markers and of macroH2A histone variant

The dramatic change of chromatin organization during acclimatization, and the accumulation of condensed chromatin in winter adapted carp must be accompanied by modification of chromatin composition. It has been shown that heterochromatin formation is intimately linked to histone methylation, in particular of Lys4, Lys9, Lys27, Lys36, Lys79 on H3 and Lys20 on H4 [32]. To determine if the accumulation of condensed chromatin in winter carp was correlated with an increased of these histone modifications we used different antibodies against histone H4 and H3 modifications (Fig. 2). Antibodies against mono and tri methyl K20 histone H4 have been used to determine if the expression of these heterochromatin markers was modified during acclimatization. Hepatocytes nuclear extracts were prepared from winter (W) and summer (S) adapted carps and used for western blotting. Interestingly, none of the heterochromatin mono and tri methyl

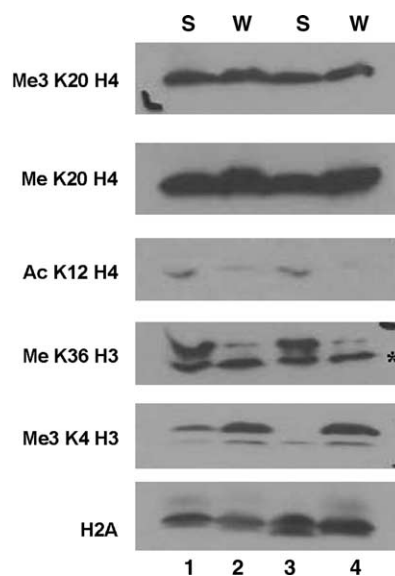


Fig. 2. Histone H4 and H3 modification during acclimatization. Liver nuclear protein extracts were prepared from summer (lanes 1 and 3) or winter (lanes 2 and 4) adapted carps and used in western blotting experiments. Antibodies against trimethyl and monomethyl K20 H4, and triMeK4H3 were used as markers of heterochromatin. Acetyl K12 H4 and MeK36H3 were used as markers of euchromatin. The asterisk in the MeK36H3 panel indicates a non-specific band detected by the antibody as described by Abcam (Ab9048). The same extracts were also probed with an antibody against H2A histone. Each lane corresponds to a different fish. Analysis of other individual fish showed similar pattern (data not shown).

K20H4 marker shows significant difference between winter and summer conditions, indicating that these histones modifications are probably not associated with the drastic reorganization of chromatin and the regulation of gene expression during this process. We next looked at the expression of the tri methyl K4H3 modification which is generally associated with active gene [33], however, recent reports indicate that it could also be associated with heterochromatin region [34] or that these modification could be found flanking the heterochromatin region [35]. Interestingly, we found that the tri methyl K4H3 is significantly more expressed in winter conditions (Fig. 2) which could then be in agreement with the enrichment of heterochromatin in winter. The acetylation level of K12H4 and the mono methylation of K36H3 which are modifications usually associated with gene activation, seems more important in the summer condition (Fig. 2), which could be also in agreement with a higher transcriptional activity during that period.

Incorporation of histone variant could be another way to regulate chromatin structure and function [13,14]. One of these variants, macroH2A has a structural and particular localization suggesting that its incorporation within specific chromatin domains could be involved in gene silencing. Immunofluorescence studies indicate that macroH2A is preferentially located on the inactive X chromosome [21,24–26] and macroH2A could be involved in generation or maintenance of the hypo-acetylated state of the histones (Doyen et al., submitted) suggesting that macroH2A could be an important factor for the establishment or maintenance of repressed chromatin together with other epigenetic chromatin markers [36–38]. Furthermore, recent proteomic studies of the nucleolar compartment have identified the variant histone macroH2A within the nucleolar compartment [27–29]. However it has not been shown yet that macroH2A is associated with rDNA sequences or that it is involved in the silencing of rDNA. In order to determine if the expression of macroH2A could play a role in the reorganization of chromatin, we undertook to characterize the expression of macroH2A in these two extreme physiological conditions. We first cloned and sequenced carp macroH2A2 (Gene Bank accession number DQ173494; data not shown). Sequence analysis showed that the H2A-like and NHR domains of carp macroH2A2 are 87% and 67% identical, respectively, to the human and mouse protein.

To analyze the level of macroH2A2 mRNA in liver cells from winter and summer adapted carps we performed RT-PCR using total RNA (Fig. 3A). This experiment revealed that the expression of macroH2A2 mRNA was drastically different under these two conditions. MacroH2A2 mRNA was undetectable in the summer condition, whereas it was well expressed in cells isolated from winter-adapted animals. This data suggest that the down regulation of the expression of many genes (including ribosomal DNA genes) and the formation of condensed chromatin during acclimatization to the winter conditions are correlated with an increased expression of the macroH2A2 variant mRNA. To determine if this increased level of macroH2A2 mRNA was associated with an increased protein level a western blot analysis was performed on nuclear protein extracts from both conditions (Fig. 3B). Although the global pattern of nuclear protein is very similar in summer and winter-adapted liver cells (Fig. 3B, compare lane 1 and 2), the macroH2A protein level shows a significant increase (7–8-fold) during the winter acclimatization (compare

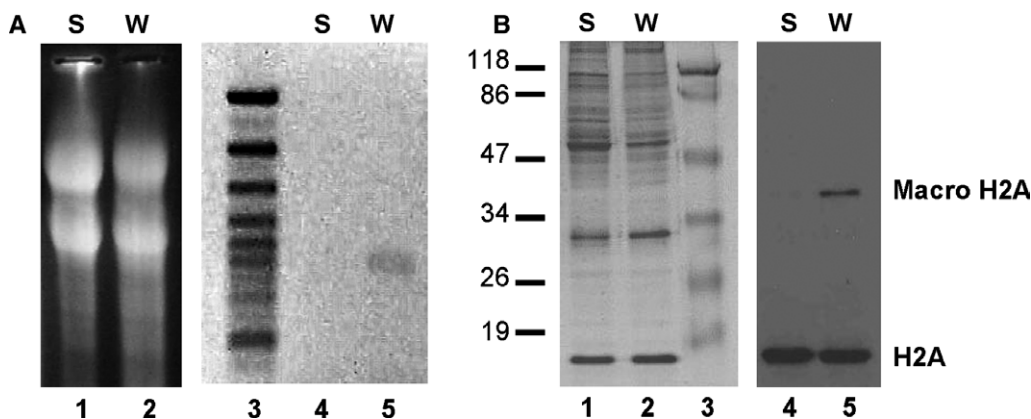


Fig. 3. macroH2A expression in summer- and winter-acclimatized liver cells. (A) RT-PCR analysis of macroH2A mRNA expression. Total RNA extracted from liver of summer- (S, lane 1) and from winter-adapted (W, lane 2) carp were used for the RT-PCR. Lanes 1 and 2 show the ethidium bromide staining of the RNA. After the RT-PCR reaction as described in the material and methods, the PCR product was analyzed on a 1% agarose gel and revealed by ethidium bromide staining (lanes 3–5). (B) Western blot analysis of the expression of macroH2A. Nuclear proteins from liver of winter (W)- and summer (S)-adapted carps were stained with Coomassie (lanes 1 and 2) and then probed with antibodies against macroH2A (lanes 4 and 5). The same blot was then probed with antibodies against H2A. Molecular weight is shown in lane 3.

lane 4 and 5). This increased macroH2A protein level is not the result of a general increased of histone proteins since the same blot revealed with an anti-H2A antibody show that the expression of H2A protein is not affected during the winter-acclimatization process. Immunofluorescence analysis was then performed to further characterize the expression of macroH2A in winter and summer acclimatized cells (Fig. 4). In summer cells no significant signals could be detected in nuclei confirming the data of the western blot (Fig. 3B) showing that the amount of macroH2A protein is very low in these cells. In contrast, in winter cells, a strong signal is found throughout the nucleus. Altogether these data show that during the winter acclimation, the dramatic change in gene expression and chromatin organization is accompanied by a modification of histone composition and not by an increase of classical

heterochromatin markers histone H4 mono and trimethyl modifications.

3.3. Ribosomal DNA genes are hypermethylated in winter-adapted hepatocytes

Many studies have pointed out the importance of cytosine base methylation of CpG dinucleotides for the establishment of constitutive heterochromatin and in some case of facultative heterochromatin. It has been shown that silent ribosomal genes are methylated at CpG residues [39]. Since the dramatic change in nucleolar structure during the winter-acclimatization process is accompanied by the down regulation of ribosomal gene expression, we wanted to determine if this down regulation of ribosomal gene expression was correlated to a modification of CpG methylation within the ribosomal promoter sequence. DNA was extracted from cells exposed to both conditions, submitted to *HpaII* digestion (inhibited by methylation) then analysed by PCR using different pairs of primers (Fig. 5). We analyzed two different CCGG sites at -30 and $+45$. If these CpG residues are methylated, DNA will be resistant to *HpaII* cleavage, and a PCR product will be detected. As a control we used a pair of primers ($-28/-10$; $+24/+45$) located between the 2 studied CpG residues, which will give a PCR product independently of the methylation state of DNA. As shown in Fig. 5, DNA isolated from winter-adapted animals is significantly more resistant to *HpaII* cleavage than DNA isolated from summer adapted animals. Quantitative PCR indicated a 3.5-fold (-30) and 3.2-fold ($+45$) resistance to cleavage in winter compared to summer conditions, indicating that in cold condition, ribosomal DNA promoter sequences are hypermethylated.

Our study shows that the expression of the histone variant macroH2A is drastically modified upon acclimatization. MacroH2A interferes with transcription factor binding, SWI/SNF nucleosome remodeling [30] and polymerase II transcription (Doyen et al. submitted) potentially through enhancing the interaction of the histone octamer with the nucleosomal DNA [40]. Furthermore, the C-terminal non-histone domain of macroH2A may be involved in ADP-ribosylation of chromatin with potential implications for transcriptional silencing

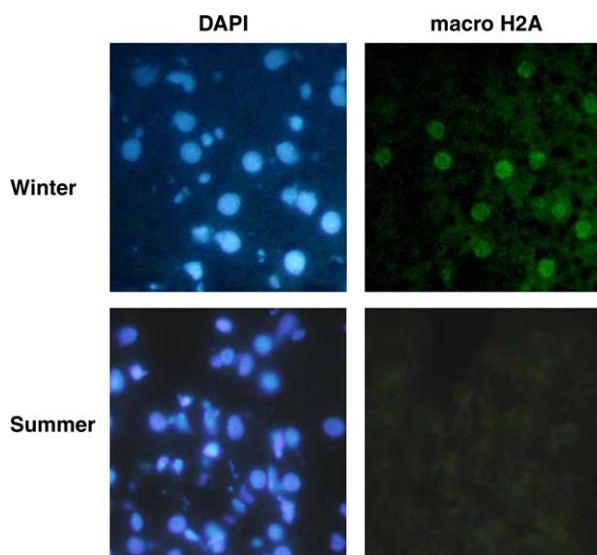


Fig. 4. Immunofluorescence analysis of the expression of macroH2A. Liver fragments from winter- and summer-acclimatized animals were frozen then treated for staining with macroH2A antibodies. Nuclei were stained with DAPI.

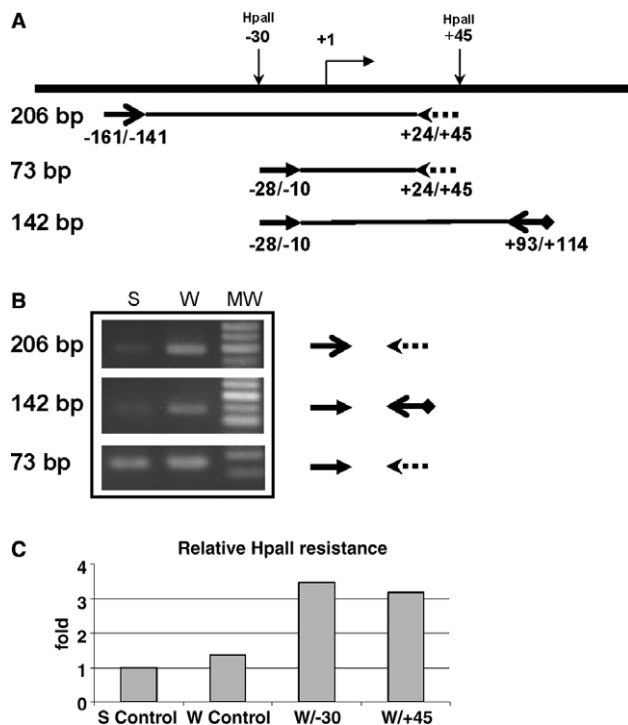


Fig. 5. Ribosomal genes are hypermethylated in winter-acclimatized animals. DNA was extracted from hepatocytes cells isolated from summer (S) and winter (W) acclimatized carps and methylation at CpG site was analyzed by QPCR after *HpaII* digestion. (A) Map of the carp ribosomal DNA gene with the position of the -30 and +45 studied CpG sites. Primers used for the PCR reactions are indicated together with the length of the expected PCR product. (B) PCR analysis with the different primers as indicated on the left on the figure. On the right of the panel are indicated the length of the PCR fragment. Amplification with internal primers (lower panel) is used as internal control since amplification is independent on the presence of the methylated state of DNA. (C) Quantification of the PCR assays.

[14,40]. Recent reports indicate that macroH2A may accumulate in constitutive heterochromatin and could contribute to maintaining its repressed state [37] and that it could contribute to transcriptional silencing acting in synergy with other repressive markers such as DNA methylation, histone deacetylation and methylation [36]. Expression of macroH2A seems to vary greatly according to the cell type [41], and in this study, we show that its expression is drastically up regulated during the cold acclimatization process. The regulated expression of histones variants, and in particular of macroH2A as shown in this study, could be key regulatory factors for the global reorganization of chromatin structure and the regulation of gene expression.

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References

[1] Vera, M.I., Norambuena, L., Alvarez, M., Figueroa, J., Molina, A., Leon, G. and Krauskopf, M. (1993) Reprogramming of nucleolar gene expression during the acclimatization of the carp. *Cell. Mol. Biol. Res.* 39, 665–674.

[2] Vera, M.I., Kausel, G., Barrera, R., Leal, S., Figueroa, J. and Quezada, C. (2000) Seasonal adaptation modulates the expression of the protein kinase CK2 beta subunit gene in the carp. *Biochem. Biophys. Res. Commun.* 271, 735–740.

[3] Buckley, B.A., Owen, M.E. and Hofmann, G.E. (2001) Adjusting the thermostat: the threshold induction temperature for the heat-shock response in intertidal mussels (genus *Mytilus*) changes as a function of thermal history. *J. Exp. Biol.* 204, 3571–3579.

[4] Tiku, P.E., Gracey, A.Y., Macartney, A.I., Beynon, R.J. and Cossins, A.R. (1996) Cold-induced expression of delta 9-desaturase in carp by transcriptional and posttranslational mechanisms. *Science* 271, 815–818.

[5] Gracey, A.Y. and Cossins, A.R. (2003) Application of microarray technology in environmental and comparative physiology. *Annu. Rev. Physiol.* 65, 231–259.

[6] Gracey, A.Y., Fraser, E.J., Li, W., Fang, Y., Taylor, R.R., Rogers, J., Brass, A. and Cossins, A.R. (2004) Coping with cold: an integrative, multitissue analysis of the transcriptome of a poikilothermic vertebrate. *Proc. Natl. Acad. Sci. USA* 101, 16970–16975.

[7] Kornberg, R.D. and Lorch, Y. (1999) Chromatin-modifying and -remodeling complexes. *Curr. Opin. Genet. Dev.* 9, 148–151.

[8] Travers, A. (1999) An engine for nucleosome remodeling. *Cell* 96, 311–314.

[9] Peterson, C.L. and Workman, J.L. (2000) Promoter targeting and chromatin remodeling by the SWI/SNF complex. *Curr. Opin. Genet. Dev.* 10, 187–192.

[10] Langst, G. and Becker, P.B. (2001) Nucleosome mobilization and positioning by ISWI-containing chromatin-remodeling factors. *J. Cell Sci.* 114, 2561–2568.

[11] Kornberg, R.D. and Lorch, Y. (1999) Twenty-five years of the nucleosome, fundamental particle of the eukaryote chromosome. *Cell* 98, 285–294.

[12] Podrabsky, J.E. and Somero, G.N. (2004) Changes in gene expression associated with acclimation to constant temperatures and fluctuating daily temperatures in an annual killifish *Austrofundulus limnaeus*. *J. Exp. Biol.* 207, 2237–2254.

[13] Kamakaka, R.T. and Biggins, S. (2005) Histone variants: deviants?. *Genes Dev.* 19, 295–310.

[14] Ladurner, A.G. (2003) Inactivating chromosomes: a macro domain that minimizes transcription. *Mol. Cell* 12, 1–3.

[15] Santisteban, M.S., Kalashnikova, T. and Smith, M.M. (2000) Histone H2A.Z regulates transcription and is partially redundant with nucleosome remodeling complexes. *Cell* 103, 411–422.

[16] Dhillon, N. and Kamakaka, R.T. (2000) A histone variant, Htz1p, and a Sir1p-like protein, Esc2p, mediate silencing at HMR. *Mol. Cell* 6, 769–780.

[17] Bao, Y., Konesky, K., Park, Y.J., Rosu, S., Dyer, P.N., Rangasamy, D., Tremethick, D.J., Laybourn, P.J. and Luger, K. (2004) Nucleosomes containing the histone variant H2A.Bbd organize only 118 base pairs of DNA. *EMBO J.* 23, 3314–3324.

[18] Gautier, T., Abbott, D.W., Molla, A., Verdel, A., Ausio, J. and Dimitrov, S. (2004) Histone variant H2A.Bbd confers lower stability to the nucleosome. *EMBO Rep.* 5, 715–720.

[19] Chadwick, B.P. and Willard, H.F. (2001) A novel chromatin protein, distantly related to histone H2A, is largely excluded from the inactive X chromosome. *J. Cell Biol.* 152, 375–384.

[20] Angelov, D., Verdel, A., An, W., Bondarenko, V., Hans, F., Doyen, C.M., Studitsky, V.M., Hamiche, A., Roeder, R.G., Bouvet, P. and Dimitrov, S. (2004) SWI/SNF remodeling and p300-dependent transcription of histone variant H2A.Bbd nucleosomal arrays. *EMBO J.* 23, 3815–3824.

[21] Chadwick, B.P., Valley, C.M. and Willard, H.F. (2001) Histone variant macroH2A contains two distinct macrochromatin domains capable of directing macroH2A to the inactive X chromosome. *Nucleic Acids Res.* 29, 2699–2705.

[22] Pehrson, J.R. and Fujii, R.N. (1998) Evolutionary conservation of histone macroH2A subtypes and domains. *Nucleic Acids Res.* 26, 2837–2842.

[23] Pehrson, J.R. and Fried, V.A. (1992) MacroH2A, a core histone containing a large nonhistone region. *Science* 257, 1398–1400.

[24] Costanzi, C. and Pehrson, J.R. (1998) Histone macroH2A1 is concentrated in the inactive X chromosome of female mammals. *Nature* 393, 599–601.

- [25] Costanzi, C. and Pehrson, J.R. (2001) MACROH2A2, a new member of the macroH2A core histone family. *J. Biol. Chem.* 276, 21776–21784.
- [26] Mermoud, J.E., Costanzi, C., Pehrson, J.R. and Brockdorff, N. (1999) Histone macroH2A1.2 relocates to the inactive X chromosome after initiation and propagation of X-inactivation. *J. Cell Biol.* 147, 1399–1408.
- [27] Andersen, J.S., Lam, Y.W., Leung, A.K., Ong, S.E., Lyon, C.E., Lamond, A.I. and Mann, M. (2005) Nucleolar proteome dynamics. *Nature* 433, 77–83.
- [28] Andersen, J.S., Lyon, C.E., Fox, A.H., Leung, A.K., Lam, Y.W., Steen, H., Mann, M. and Lamond, A.I. (2002) Directed proteomic analysis of the human nucleolus. *Curr. Biol.* 12, 1–11.
- [29] Scherl, A., Couste, Y., Deon, C., Calle, A., Kindbeiter, K., Sanchez, J.C., Greco, A., Hochstrasser, D. and Diaz, J.J. (2002) Functional proteomic analysis of human nucleolus. *Mol. Biol. Cell* 13, 4100–4109.
- [30] Angelov, D., Molla, A., Perche, P.Y., Hans, F., Cote, J., Khochbin, S., Bouvet, P. and Dimitrov, S. (2003) The histone variant macroH2A interferes with transcription factor binding and SWI/SNF nucleosome remodeling. *Mol. Cell* 11, 1033–1041.
- [31] Sprague, J., Doerry, E., Douglas, S. and Westerfield, M. (2001) The Zebrafish Information Network (ZFIN): a resource for genetic, genomic and developmental research. *Nucleic Acids Res.* 29, 87–90.
- [32] Sims III, R.J., Nishioka, K. and Reinberg, D. (2003) Histone lysine methylation: a signature for chromatin function. *Trends Genet.* 19, 629–639.
- [33] Santos-Rosa, H., Schneider, R., Bannister, A.J., Sherriff, J., Bernstein, B.E., Emre, N.C., Schreiber, S.L., Mellor, J. and Kouzarides, T. (2002) Active genes are tri-methylated at K4 of histone H3. *Nature* 419, 407–411.
- [34] Spada, F., Vincent, M. and Thompson, E.M. (2005) Plasticity of histone modifications across the invertebrate to vertebrate transition: histone H3 lysine 4 trimethylation in heterochromatin. *Chromosome Res.* 13, 57–72.
- [35] Sullivan, B.A. and Karpen, G.H. (2004) Centromeric chromatin exhibits a histone modification pattern that is distinct from both euchromatin and heterochromatin. *Nat. Struct. Mol. Biol.* 11, 1076–1083.
- [36] Hernandez-Munoz, I., Lund, A.H., van der Stoop, P., Boutsma, E., Muijters, I., Verhoeven, E., Nusinow, D.A., Panning, B., Marahrens, Y. and vanLohuizen, M. (2005) Stable X chromosome inactivation involves the PRC1 Polycomb complex and requires histone macroH2A1 and the CULLIN3/SPOP ubiquitin E3 ligase. *Proc. Natl. Acad. Sci. USA* 102, 7635–7640.
- [37] Grigoryev, S.A., Nikitina, T., Pehrson, J.R., Singh, P.B. and Woodcock, C.L. (2004) Dynamic relocation of epigenetic chromatin markers reveals an active role of constitutive heterochromatin in the transition from proliferation to quiescence. *J. Cell Sci.* 117, 6153–6162.
- [38] Ma, Y., Jacobs, S.B., Jackson-Grusby, L., Mastrangelo, M.A., Torres-Betancourt, J.A., Jaenisch, R. and Rasmussen, T.P. (2005) DNA CpG hypomethylation induces heterochromatin reorganization involving the histone variant macroH2A. *J. Cell Sci.* 118, 1607–1616.
- [39] Santoro, R. and Grummt, I. (2001) Molecular mechanisms mediating methylation-dependent silencing of ribosomal gene transcription. *Mol. Cell* 8, 719–725.
- [40] Abbott, D.W., Chadwick, B.P., Thambirajah, A.A. and Ausio, J. (2005) Beyond the Xi: macroH2A chromatin distribution and post-translational modification in an avian system. *J. Biol. Chem.* 280, 16437–16445.
- [41] Abbott, D.W., Laszczak, M., Lewis, J.D., Su, H., Moore, S.C., Hills, M., Dimitrov, S. and Ausio, J. (2004) Structural characterization of macroH2A containing chromatin. *Biochemistry* 43, 1352–1359.