Linker Histone H1.b is Polymorphic in Grey Partridge (*Perdix perdix*)

Andrzej Kowalski*, Jan Pałyga, and Ewa Górnicka-Michalska

Department of Biochemistry and Genetics, Institute of Biology,
Jan Kochanowski University, Kielce, Poland. E-mail: a.kowalski@ujk.kielce.pl

* Author for correspondence and reprint requests

Z. Naturforsch. **66 c**, 296–304 (2011); received October 12, 2010/March 8, 2011

This study was aimed at characterizing allelic variations of erythrocyte histone H1.b by comparing the electrophoretic patterns of histone H1.b from individuals of grey partridge (*Perdix perdix*) population. As two alloforms, H1.b1 and H1.b2, were discerned in the screening gels, the histone H1.b was regarded to be a polymorphic protein encoded by a gene with two codominant alleles, b^1 and b^2, at a locus. The tested population was found to be at Hardy-Weinberg equilibrium ($\chi^2 = 0.834$, $p = 0.361$), with only a minor heterozygote deficiency (fixation index $F = 0.136$). Since the histone H1.b alloforms were identified in a two-dimensional gel containing sodium dodecyl sulfate, with no significant differences in their migration pattern in an one-dimensional acetic acid polyacrylamide gel, we assumed that the H1.b alloforms possessed a similar net charge and differed in their apparent molecular weights. A comparison of *N*-bromosuccinimide-cleaved and α-chymotrypsin-digested products of histone H1.b alloforms revealed slight differences in the velocity of C-terminal peptides and a similarity in migration of their N-terminal peptides in one-dimensional sodium dodecyl sulfate-polyacrylamide gel. Therefore, it seemed that the histone H1.b alloforms might differ in this amino acid sequence in a protein segment between *N*-bromosuccinimide cleavage site and the very C-terminus.

Key words: Avian Erythrocyte, Histone H1.b, Alloforms, Chemical Cleavage and Limited Proteolysis

Introduction

There are two classes of histone proteins: core nucleosomal histones that form the octamer of nucleosome and H1 histones which are engaged in formation of the higher-order chromatin structure. These small basic proteins, also called linker histones, play an important role in eukaryotic genome organization by binding to the DNA entry/exit points and shifting chromatin to a more condensed state due to stabilization of the nucleosome arrays (Hansen, 2002). Such a compaction may influence gene expression, hence, for many years linker histones have been viewed as global transcriptional repressors (Wolffe, 1992). This assumption was consistent with findings that chromatin regions containing transcriptionally active genes possess a lower level of linker histones relative to those with genes in the repressed states (Kamakaka and Thomas, 1990). A more recent survey of research showed (Zlatanova *et al.*, 2000) that histone H1 might be acting specifically as a gene repressor affecting either initiation or elongation of transcription by hindering the binding of transcriptional factors to DNA (Thomas, 1999), stabilizing the nucleosomes (Vicent *et al.*, 2002) or inhibiting the chromatin-remodeling activity (Hill and Imbalzano, 2000). Moreover, hormone-induced histone H1 phosphorylation increased transcriptional activation in the minichromosomes assembled *in vitro* on the mouse mammary tumour virus promoter due to a lower affinity of phosphorylated H1 for its binding sites in chromatin (Koop *et al.*, 2003).

Because of a highly dynamic but relatively weak binding to chromatin, histone H1-specific gene repression and/or activation seems to be more peculiar as compared with the nucleosomal histones. The direct measurement of histone H1 dynamics in living cells has revealed its mobile nature as shown by alterations in time residence in different chromatin units (Misteli, 2001). A

Abbreviations: 2-ME, 2-mercaptoethanol; PMSF, phenylmethylsulfonyl fluoride; SDS, sodium dodecyl sulfate; NBS, *N*-bromosuccinimide.

transient dissociation of histone H1 from nucleosomes gives other nuclear proteins an opportunity to occupy these sites and induce changes in the chromatin structure that allow specific regulatory factors to bind to their target sites. While linker histones stabilize the higher-order chromatin structure by diminishing the access of transcriptional factors to DNA, the members of the protein family, for example HMGN, can stimulate transcription by reducing the chromatin compactness (Bustin, 2001). Histone H1 may also interact with complexes to modulate their remodeling activity (Ramachandran *et al*., 2003) and is required for efficient homologous recombination (Downs *et al*., 2003). Thus, histone H1 is a DNA-binding protein that ensures a proper organization and function of chromatin. Linker histones are in a constant dynamical competiton with a network of other DNA-binding proteins for nucleosomal targets and modulate the accessibility of nucleosomal DNA (Bustin *et al*., 2005).

The linker histones, composed of several nonallelic subtypes, are the most heterogeneous group of all histone classes and include an array of somatic subtypes as well as differentiation-associated ($H1^0$ and H5) variants and both testis- (H1t and others) and oocyte-specific (H1oo) components (Khochbin, 2001; Happel and Doenecke, 2009). The existence of multiple forms of histone H1 is likely associated with subtype-related functions as exemplified among others by specific gene repression during development (*Xenopus* H1A), recombination during spermatogenesis (mouse H1t), stabilization of chromatin structure during differentiation (mouse $H1^0$), and activation of gene repression (mouse H1c) (Brown, 2001). Moreover, it has been postulated that additional H1 subtype heterogeneity, such as polymorphic variations (Sarg *et al*., 2005; Górnicka-Michalska *et al*., 2006) and/or posttranslational modifications (Wiśniewski *et al.*, 2007), may have diversified potential subtype activity and hence influence H1-dependent chromatin processes. Out of the six main histone H1 variants in bird erythrocytes, the histones H1.a, H1.b, and H1.z are polymorphic (Pałyga, 1998a; Pałyga *et al*., 2000; Kowalski *et al*., 1998, 2004; Górnicka-Michalska *et al*., 2006) and depending on a species and/or breed; they may be represented by two or three alloforms (Pałyga *et al*., 2000; Kowalski *et al*., 2004).

In the present study we have found polymorphisms of erythrocyte histone H1.b in grey partridge which, unlike typical breeding flocks so far tested, is mostly a wild-living species that is quite rarely kept in captivity in larger groups and therefore is unique for research. It appeared that histone H1.b phenotypes were at the Hardy-Weinberg equilibrium, however, a lower number of heterozygotes might indicate some effect of inbreeding in this grey partridge population. As we have found that the C-terminal peptides generated by *N*-bromosuccinimide (NBS) cleavage or α-chymotrypsin digestion from each of the histone H1.b alloforms differed in their migration in the gel, we assumed that they might possess a differential amino acid region located between the NBS cleavage site in the globular domain and the very C-terminus of the molecule.

Materials and Methods

Animals

The study was conducted on 59 grey partridge (*Perdix perdix*) individuals (Table I) bred at the Department of Poultry Breeding of the University of Technology and Life Sciences, Bydgoszcz, Poland.

Isolation and electrophoretic separation of perchloric acid-soluble proteins

Histone H1 proteins were isolated from whole blood erythrocytes by double extraction of saponin nuclei with perchloric acid (1 M and 0.5 M) (Neelin *et al*., 1995). The proteins precipitated with 20% trichloroacetic acid were first dried on cool air, then washed once with acetone acidified with concentrated HCl (500:1, v/v), and finally twice with acetone alone. One-mg protein samples were dissolved in 200 μL solution containing 8 M urea, 0.9 M acetic acid, and 10% 2-mercaptoethanol (2-ME), and aliquots were separated in both 15% acrylamide, 0.9 M acetic acid, 8 M urea gel and 13.5% acrylamide, 0.1% sodium dodecyl sulfate (SDS) gel. Electrophoresis was done in 240 cm x 240 cm x 0.8 cm slab gels according to Pałyga (1991a).

In order to obtain concentrated and purified proteins for subsequent structural analyses, about ten Coomassie Blue R-250-stained gel strips with histone H1.b alloforms resolved in the acetic acid polyacrylamide gel were excised, soaked (2 x 15 min) in an adaptation buffer containing 2.1% SDS, 2% 2-ME, 10% glycerol, and 0.124 M

Tris-HCl, pH 6.8, and applied in stacks separately for each alloform on top of the one-dimensional SDS-polyacrylamide gel. Histone H1.b bands were cut out from the preparative SDS gel, and protein was in-gel cleaved with NBS or digested by α-chymotrypsin, and/or *Staphylococcus aureus* protease V8.

Cleavage of histone H1.b with NBS and digestion with α-chymotrypsin

For NBS cleavage, the gel pieces containing the alloforms of histone H1.b were incubated in 1 mL of 0.1% NBS in 50% acetic acid. The reaction was conducted in the dark for 2 x 30 min with freshly prepared NBS solution, and following incubation in the adaptation buffer (2 x 15 min, see above), the gel stripes containing specific isoforms of histone H1.b were separately electrophoresed in one-dimensional 13.5% SDS-polyacrylamide gel.

Limited degradation by α-chymotrypsin or *Staphylococcus aureus* protease V8 was performed in a stacking SDS-polyacrylamide gel. At first, the fragments of the gel with alloforms of histone H1.b were incubated twice in the adaptation buffer (0.1% SDS, 10% glycerol, 0.125 M Tris-HCl, pH 6.8, 1 mM EDTA) for 15 min, and then were applied on top of the gel and overlayered with 2 μL solution containing either α-chymotrypsin or *Staphylococcus aureus* protease V8 dissolved in the adaptation buffer (0.25 μg enzyme/2 μL buffer).

Gel image processing and statistical analysis

Two-dimensional polyacrylamide gel pattern analyses were performed by capturing the gel images with the Doc-Print II gel documentation system (Vilber Lourmat, Eberhardzell, Germany). Then, the images were processed by means of ImageJ 1.42q software (www. rsbweb.nih.gov/ij). The volumes of histone H1.b spots in the total histone H1 gel patterns from three independent individuals with the appropriate phenotype were assessed based on the grey intensity of the spots, and expressed as percentage of the H1.b spot relative to the total grey intensity of all histone H1 spots for each individual. The coefficient of variation (CV), *i.e.* the standard deviation (SD) divided by the mean, was calculated for each histone H1.b phenotype to estimate the H1.b variability between independent protein preparations.

Results

Polymorphic variation of erythrocyte histone H1.b in grey partridge

Histone H1 preparations obtained by the extraction of saponin-lysed erythrocyte nuclei with a dilute perchloric acid solution were resolved in an acetic acid polyacrylamide gel into six bands (a, b, b′, c, c′, and d) with a chicken-like array of histone H1 variants (Pałyga, 1991b) (Fig. 1A). While each of the histone H1 bands resolved in the acetic acid gel migrated with similar electrophoretic

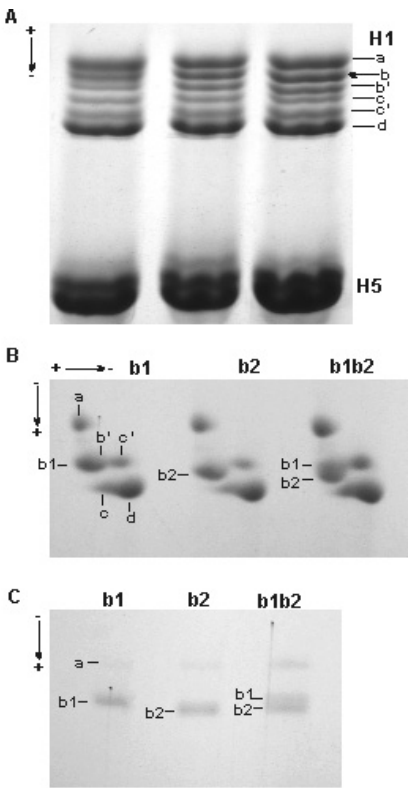

Fig. 1. Total histone H1 extracted from grey partridge erythrocyte nuclei with a dilute perchloric acid solution was resolved into six non-allelic subtypes (a, b, b', c, c', and d) in one-dimensional acetic acid-urea polyacrylamide gel (A). Two alloforms (H1.b1 and H1.b2) of grey partridge erythrocyte histone H1.b which could form three phenotypes (b1, b2, and b1b2) were detected in the two-dimensional (B) and one-dimensional SDS-polyacrylamide gel (C). The two-dimensional polyacrylamide gel patterns of histone H1 were obtained by separating first the perchloric acid-soluble protein preparations in the acetic acid-urea gel (from left to right) and then in the second dimension in the SDS gel (from top to bottom).

Table I. Distribution of histone H1.b phenotypes and alleles in grey partridge population.

Phenotype	Number of individuals observed/expected	Frequency of phenotype observed/expected	Allele	Frequency of allele
b1	28/26.40	0.475/0.447	b^1	0.670
b2	8/6.46	0.135/0.109		
b1b2	23/26.13	0.389/0.442	b^2	0.330
$\chi^2 = 0.834$; $p = 0.361$; $F = 0.136$				

mobility in all individuals screened (Fig. 1A), an altered migration of the histone H1.b spots was, however, observed in the two-dimensional SDS-polyacrylamide gel (Fig. 1B). We have identified two alloforms of histone H1.b: a slower moving H1.b1 and a faster migrating H1.b2 spot (Fig. 1B). A shift in the electrophoretic migration of H1.b1 and H1.b2 proteins was also apparent after separation of the individual H1.b bands, excised from the acetic acid gel, in one-dimensional SDS-polyacrylamide gels (Fig. 1C). The same rate of migration of the histone H1.b in the acetic acid polyacrylamide gel and a differential phenotype-dependent mobility in the two-dimensional polyacrylamide gel indicated that the histone H1.b alloforms possessed a similar net charge and differed in their apparent molecular weights.

Three phenotypes of histone H1.b (b1, b2, and b1b2) were differently distributed in the tested grey partridge population. The homozygous individuals b1 occurred at frequency 0.475 while the remaining phenotypes were less abundant (Table I). Although the grey partridge population was at Hardy-Weinberg equilibrium, as estimated by the chi-square goodness-of-fit (χ^2) test, it, however, exhibited a deficit of heterozygous individuals (inbreeding coefficient $F = 0.136$) (Table I). Moreover, allele b^1 was more common (frequency 0.670) than allele b^2 (frequency 0.330) in the grey partridge population (Table I).

The coefficients of variation (CV) calculated for the histone H1.b protein spots in all grey partridge phenotypes were below a threshold value of 0.25 (Table II), indicating a low variability in a relative spot density between separate protein preparations.

Location of an altered protein segment between the grey partridge erythrocyte histone H1.b alloforms

Single histone H1.b1 and H1.b2 bands were cut out from the acetic acid gel containing repeatedly resolved histone H1.b preparations isolated from appropriate homozygous individuals. After incubation in an adaptation buffer (see Materials and Methods), approximately ten gel pieces with the bands of the relevant alloform of histone H1.b were applied into the well of the SDS preparative gel (Fig. 2). After electrophoresis, a sufficient amount of pure histone H1.b alloforms separated from the adjacent histone H1.a was obtained for subsequent analyses of the protein structure.

NBS cleavage and α-chymotrypsin digestion were used in order to split specifically the peptide bonds at the carboxyl site of a single tyrosine and phenylalanine residue, respectively, in both histone H1.b alloforms. As the tyrosine residue is located at the position 74 in the molecule of histone H1.b subtype in chicken (Shannon and Wells, 1987), we inferred – on the basis of high evolutionary conservation of globular domain sequences in vertebrate histone H1 (Coles *et al.*, 1987) – that the NBS cleavage of grey partridge

Table II. Average coefficient of variation (CV = SD/mean) of the volumes of histone H1.b spots for particular H1.b phenotypes in grey partridge. The volume of H1.b protein spots, measured as a level of grey (in pixels) in the images of two-dimensional gel from three independent individuals, was expressed as a percentage of H1.b spot(s) relative to all histone H1 spots in the preparation.

Phenotype	Ratio of the grey level of H1.b/total H1 (%)	Mean (%)	SD	CV[a]
b1	69.11/404.2 (17.1) 76.7/405.8 (18.8) 75.9/400.5 (18.9)	18.26	1.0	0.055
b2	72.22/440.4 (16.4) 78.6/413.6 (19.0) 86.3/452.2 (19.0)	18.13	1.5	0.082
b1b2	73.9/424.8 (17.4) 75.5/382.2 (19.4) 101.7/474.8 (21.4)	19.4	2.0	0.1

[a] CV values below 0.25 indicate a low relative spot variability.

H1.b should produce two peptides: an N-peptide from the N-terminal amino acid to a single Tyr residue (conserved among vertebrate H1 somatic H1 subtypes) and a C-peptide from the next residue onwards. The short N-terminal peptides cleaved by NBS were invisible after staining with Coomassie Blue, therefore, the longer C-terminal peptides (NBS-Cb1 from histone H1.b1 and NBS-Cb2 from histone H1.b2) that migrated with different electrophoretic mobilities in the SDS-polyacrylamide gel were only detected (Fig. 3). A dissimilar electrophoretic mobility of the C-terminal peptides from the histone H1.b alloforms indicated that differences in their primary structures were likely to be located between the NBS-cleaved site and the very C-terminus of their molecules. To limit a region which comprises a presumed sequence segment that may differ between the histone H1.b alloforms, we employed α-chymotrypsin digestion at a single Phe residue (Phe108 in the chicken H1.b; conserved in vertebrate linker histone superfamily) that provided both N- and C-terminal peptides. As shown in Fig. 4, the products of α-chymotrypsin digestion of histones H1.b1 and H1.b2 from both species encompassed the faster migrating N-terminal peptides, N-b1 and N-b2, with a similar electrophoretic mobility and slower moving C-terminal peptides, C-b1 and C-b2, which differed in their migration in the SDS-polyacrylamide gel. Since the peptide C-b2 derived from the histone H1.b2 migrated slightly faster than the C-b1 peptide from histone H1.b1, it is plausible that a variable region of histone H1.b alloforms may be located between the consecutive amino acid residue, Arg, also conserved in the majority of chicken histone H1 subtypes (Coles *et al.*, 1987), and the C-terminal end.

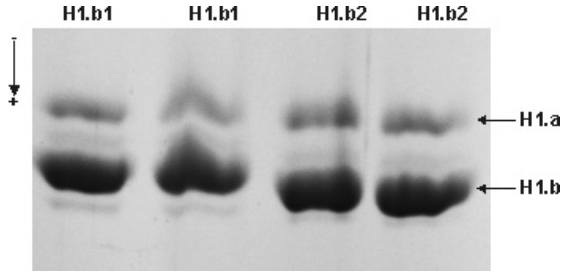

Fig. 2. Electrophoretic purification of grey partridge erythrocyte histone H1.b alloforms. About 10 gel pieces containing either histone H1.b1 or H1.b2 bands were cut out from the acetic acid polyacrylamide gel and separately electrophoresed in the SDS preparative gel to obtain concentrated protein bands free from neighbouring histone H1.a.

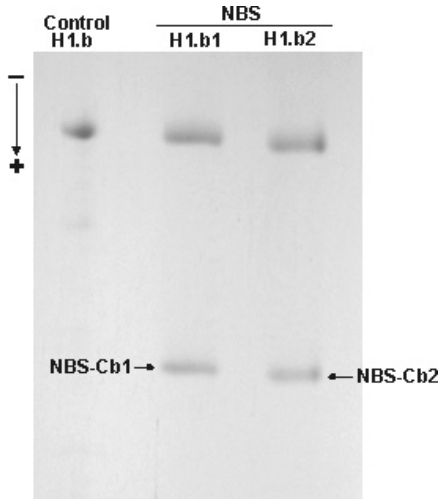

Fig. 3. *N*-Bromosuccinimide (NBS) cleavage of grey partridge ethrocyte histone H1.b alloforms from grey partridge. A differential electrophoretic mobility of C-peptide bands, NBS-Cb1 and NBS-Cb2, from slow histone H1.b1 and fast histone H1.b2, respectively, was detected in the SDS-polyacrylamide gel. Control H1.b, undigested grey partridge histone H1.b.

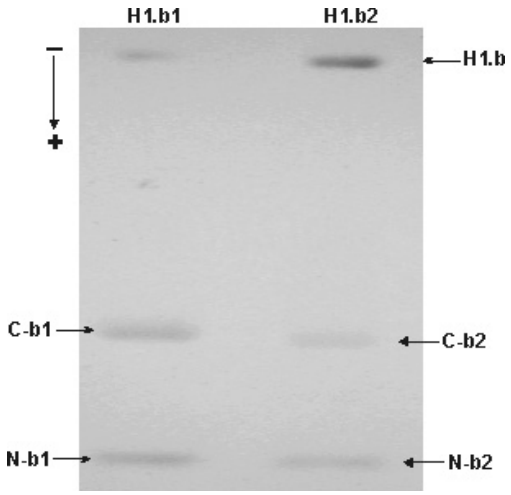

Fig. 4. α-Chymotrypsin digestion of grey partridge erythrocyte histone H1.b alloforms. The C-terminal peptides from histone H1.b1 (slower moving peptide C-b1) and histone H1.b2 (faster migrating C-b2) and N-terminal peptides (N-b1 and N-b2) migrating with a similar velocity were detected in the SDS-polyacrylamide gel.

It seems that the grey partridge histone H1.b alloforms may differ in the same C-terminal sequence segment due to amino acid variation.

Discussion

A specific allelic variation of erythrocyte histone H1.b was previously detected in duck and quail populations (Pałyga, 1991c; Pałyga *et al.*, 2000). While histone H1.b in Pekin-type duck lines is composed of three alloforms (H1.b1, H1.b2, and H1.b3) (Pałyga *et al.*, 2000), only two alloforms of H1.b (H1.b1 and H1.b2) were detected in Japanese quail (Pałyga, 1991c). Usually, polymorphic variants of histone H1 detected by electrophoresis may differ in the net charge (in the acetic acid polyacrylamide gel) and/or molecular weight (in the SDS-polyacrylamide gel). For example, quail and duck histones H1.b1 and H1.b2 differ in their apparent molecular masses. Here, a similar pattern of grey partridge histone H1.b alloform variation was observed in two-dimensional gel (Fig. 1B) as well as in the tracings of one-dimensional SDS-polyacrylamide gels (not shown). As depicted in Figs. 1B and 1C, the histone H1.b was composed of high- (H1.b1) and low (H1.b2)-molecular weight alloforms, which might combine to form the phenotypes b1, b2, and b1b2. Thus, histone H1.b is encoded by a gene with two alleles (b^1 and b^2) at a locus. In spite of some heterozygote deficiency, no deviation from the Hardy-Weinberg equilibrium in grey partridge population was found. In our previous work we reported (Pałyga, 1998b; Pałyga *et al.*, 2000) that the allele frequency of polymorphic histone H1 subtypes tend to fluctuate within genetic lines and/or strains in duck and quail, even leading to the elimination of a particular allele in successive generations under some selective conditions. The alloforms of certain polymorphic variants of histone H1 are likely to be associated with some traits judging from the selection-induced changes in histone H1.b allele frequencies in quail (Pałyga, 1998b) and the occurrence of allele b^2 only in coloured Pekin-type duck strains (Pałyga *et al.*, 2000). However, it is not clear from a limited data how histone H1 alloforms may modulate the chromatin structure to govern gene activity, but the reports on the various characteristics of histone H1 non-allelic subtypes (Alami *et al.*, 2003; Konishi *et al.*, 2003; Lin *et al.*, 2004) seem to suggest that individual alleles might have been also involved in mediating specific functional alterations in chromatin.

Distinct features of particular histone H1 variants seem to be a consequence of differences in the sequence and structure of two positively charged terminal domains (N- and C-tails) which, in contrast to a central highly conserved globular domain, are known to be more diverged throughout the evolution and varied in both the length and sequence (Kasinsky *et al.*, 2001). Structural properties of histone H1 subtypes may specify their residence time and binding affinities to chromatin (Lu and Hansen, 2004; Th'ng *et al.*, 2005) as well as site-specific modifications influencing their interactions with chromatin (Wiśniewski *et al.*, 2007). The variable regions in avian erythrocyte histone H1 alloforms are usually restricted to the C-terminal tail, as evidenced by a chemical cleavage and/or limited enzymatic digestion of duck H1.z (Pałyga *et al.*, 1993) and chicken H1.a (Górnicka-Michalska *et al.*, 2006) subtypes. The results obtained in the present work support these observations by demonstrating that the C-terminal peptides from grey partridge histone H1.b alloforms obtained after NBS cleavage or α-chymotrypsin and protease V8 digestion differently migrated in the SDS-polyacrylamide gel. Previously, we revealed (Górnicka-Michalska *et al.*, 2006) that a variable region in chicken histone H1.a spans a very distal part of globular domain and the whole C-terminal one. As is schematically depicted in Fig. 5, it is quite likely that an expected sequence difference between grey partridge histone H1.b alloforms, as well as between guinea fowl H1.b alloforms (Kowalski *et al.*, 2011), might be also located in their C-terminal tails.

The C-terminal domain of histone H1 was shown to be important for chromatin folding and condensing into higher-order structures (Khadake and Rao, 1995), especially by conserved S/TPKK repeat motifs that determined, the correct binding of H1 to chromatin (Bharath *et al.*, 2003). The experiments with *in vitro* generated C-terminal H1 mutants demonstrated a 45% decrease in the DNA condensation when all S/TPKK motifs were deleted and a nearly 90% decrease in the DNA condensation when a 34 amino acid stretch, from residue 145 to residue 178, was removed (Bharath *et al.*, 2002). The similar changes in the binding capacity of the histone H1, up to almost complete elimination of its binding, were observed *in vivo* when all of the 70 C-terminal amino acids were

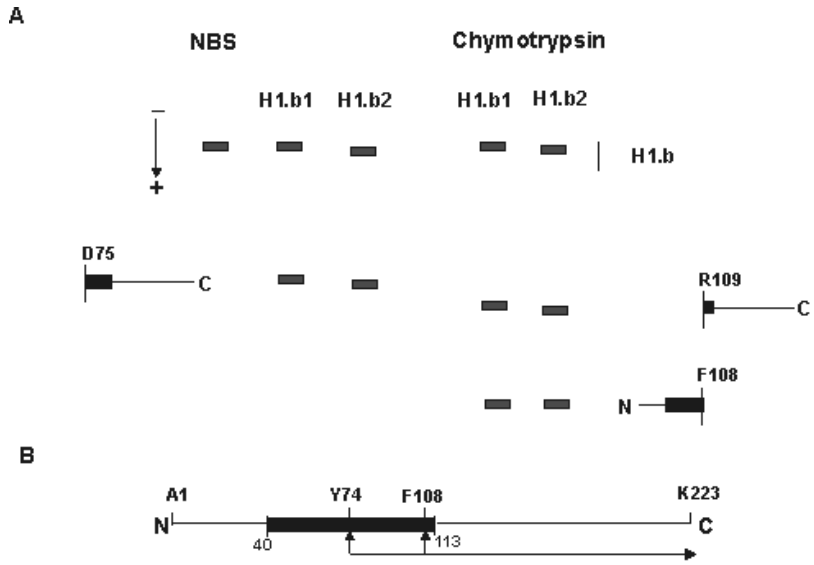

Fig. 5. (A) Schematic pattern of electrophoretic mobility of the main peptides obtained by *N*-bromosuccinimide (NBS) cleavage and α-chymotrypsin digestion of histone H1.b alloforms and (B) location of a region with presumed amino acid sequence differences in the histone H1.b molecule. As the C-peptides generated from the histone H1.b alloforms by cleavage with NBS at Tyr74 (D75–K223) and α-chymotrypsin at Phe108 (R109–K223) differed in their electrophoretic migration, and α-chymotryptic N-peptides (A1–F108) possessed the same electrophoretic mobility in the SDS-polyacrylamide gel, the putative difference in the amino acid sequence between histone H1.b alloforms seems to be located in the region indicated by a horizontal arrow, spanning from a putative Asp75 residue to the very C-terminus. This region includes a part of globular domain (from residue 75 to 113) and a C-terminal domain (from residue 114 to 223). The amino acid positions as well as globular domain borders shown in the figure refer to those in the chicken histone H1.b sequence (Shannon and Wells, 1987).

deleted, following surprisingly, the substitution of a single Thr152 with glutamic acid (Hendzel *et al.*, 2004). Therefore, it appeared that even slight structural alterations in the histone H1 C-termini might substantially contribute to changes in the chromatin properties in living cells. Such changes in the positively charged C-terminal domain might differentially influence the strength of histone H1 binding and alter the rate of protein exchange between chromatin binding sites (Catez *et al.*, 2006). The important functional property of the C-terminal histone H1 tail is its capability of transition from an almost unstructured state in a dilute solution to a stable secondary structure in a DNA-bound state (Roque *et al.*, 2005). Thus, it seems that both histone H1 binding to chromatin and structural transition within this molecule might be influenced by amino acid substitutions in the C-terminal domain.

Our results appear to suggest that grey partridge erythrocyte histone H1.b alloforms may differ in a variable region in the C-terminal domain, as it was found in other avian species (Pałyga *et al.*, 2000). If mutation- or even post-translational modification-induced changes in the C-terminal domain of histone H1 were able to alter vital functional properties of chromatin, then similar effects might also arise following allele-specific deposition of the polymorphic histone H1 subtype. Depending on the C-terminal sequence features, the histone H1 alloforms could have differentially affected folding and/or unfolding of the chromatin regions leading to their structural and functional alterations.

Acknowledgements

We thank Professor Zenon Bernacki and Professor Marek Adamski, Department of Poultry Breeding, University of Technology and Agriculture, Bydgoszcz, Poland, for allowing us to use their bird stocks.

Alami R., Fan Y., Pack S., Sonbuchner T. M., Besse A., Lin Q., Greally J. M., Skoultchi A. I., and Bouhassira E. E. (2003), Mammalian linker-histone subtypes differentially affect gene expression *in vivo*. Proc. Natl. Acad. Sci. USA **100**, 5920–5925.

Bharath M. M., Ramesh S., Chandra N. R., and Rao M. R. (2002), Identification of a 34 amino acid stretch within the C-terminus of histone H1 as the DNA-condensing domain by site-directed mutagenesis. Biochemistry **41**, 7617–7627.

Bharath M. M., Chandra N. R., and Rao M. R. (2003), Molecular modeling of the chromatosome particle. Nucleic Acids Res. **31**, 4264–4274.

Brown D. T. (2001), Histone variants: are they functionally heterogeneous? Genome Biol. **2**, rev0006.1-0006.6.

Bustin M. (2001), Chromatin unfolding and activation by HMGN* chromosomal proteins. Trends Biochem. Sci. **26**, 431–437.

Bustin M., Catez F., and Lim J. H. (2005), The dynamics of histone H1 function in chromatin. Mol. Cell **17**, 617–620.

Catez F., Ueda T., and Bustin M. (2006), Determinants of histone H1 mobility and chromatin binding in living cells. Nat. Struct. Mol. Biol. **13**, 305–310.

Coles L. S., Robins A. J., Madley L. K., and Wells J. R. E. (1987), Characterization of the chicken histone H1 gene complement. J. Biol. Chem. **262**, 9656–9663.

Downs J. A., Kosmidou E., Morgan A., and Jackson S. P. (2003), Suppression of homologous recombination by the *Saccharomyces cerevisiae* linker histone. Mol. Cell **11**, 1685–1692.

Górnicka-Michalska E., Pałyga J., Kowalski A., and Cywa-Benko K. (2006), Sequence variants of chicken linker histone H1.a. FEBS J. **273**, 1240–1250.

Hansen J. C. (2002), Conformational dynamics of the chromatin fiber in solution: determinants, mechanisms and functions. Ann. Rev. Biophys. Biomol. Struct. **31**, 361–392.

Happel N. and Doenecke D. (2009), Histone H1 and its isoforms: contribution to chromatin structure and function. Gene **431**, 1–12.

Hendzel M. J., Lever M. A., Crawford E., and Th'ng J. P. (2004), The C-terminal domain is the primary determinant of histone H1 binding to chromatin *in vivo*. J. Biol. Chem. **279**, 20028–20034.

Hill D. A. and Imbalzano A. N. (2000), Human SWI/SNF nucleosome remodeling activity is partially inhibited by linker histone H1. Biochemistry **39**, 11649–11656.

Kamakaka R. T. and Thomas J. O. (1990), Chromatin structure of transcriptionally competent and repressed genes. EMBO J. **9**, 3997–4006.

Kasinsky H. E., Lewis J. D., Dacks J. B., and Ausio J. (2001), Origin of H1 linker histones. FASEB J. **15**, 34–42.

Khadake J. R. and Rao M. R. (1995), DNA- and chromatin-condensing properties of rat testes H1a and H1t compared to those of rat liver H1bdec; H1t is a poor condenser of chromatin. Biochemistry **34**, 15792–15801.

Khochbin S. (2001), Histone H1 diversity: bridging regulatory signals to linker histone function. Gene **271**, 1–12.

Konishi A., Shimizu S., Hirota J., Takao T., Fan Y., Matsuoka Y., Zhang L., Yoneda Y., Fujii Y., Skoultchi A. I., and Tsujimoto Y. (2003), Involvement of histone H1.2 in apoptosis induced by DNA double-strand breaks. Cell **114**, 673–688.

Koop R., Croce L. D., and Beato M. (2003), Histone H1 enhances synergistic activation of the MMTV promoter in chromatin. EMBO J. **22**, 588–599.

Kowalski A., Pałyga J., Górnicka-Michalska E., and Krajewska W. M. (1998), Allelic polymorphism of histone H1.a in duck erythrocytes. Biochem. Genet. **36**, 183–191.

Kowalski A., Pałyga J., and Górnicka-Michalska E. (2004), Identification of histone H1.z components in a Muscovy duck (*Cairina moschata* L.) population. Comp. Biochem. Physiol. Past B: Biochem. Mol. Biol. **137**, 151–157.

Kowalski A., Pałyga J., and Górnicka-Michalska E. (2011), Two polymorphic linker histone loci in Guinea fowl erythrocytes. C. R. Biol. **334**, 6–12.

Lin Q., Inselman A., Han X., Xu H., Zhang W., Handel M. A., and Skoultchi A. I. (2004), Reductions in linker histone levels are tolerated in developing spermatocytes but cause changes in specific gene expression. J. Biol. Chem. **279**, 23525–23535.

Lu X. and Hansen J.C. (2004), Identification of specific functional subdomain within the linker histone $H1^0$ C-terminal domain. J. Biol. Chem. **279**, 8701–8707.

Misteli T. (2001), Protein dynamics: implications for nuclear architecture and gene expression. Science **291**, 843–847.

Neelin J. M., Neelin E. M., Lindsay D. W., Pałyga J., Nichols C. R., and Cheng K. M. (1995), The occurrence of a mutant dimerizable histone H5 in Japanese quail erythrocytes. Genome **38**, 982–990.

Pałyga J. (1991a), Genetic polymorphism of histone H1b in duck erythrocytes. Hereditas **114**, 85–89.

Pałyga J. (1991b), A comparison of the histone H1 complements of avian erythrocytes. Int. J. Biochem. **23**, 845–849.

Pałyga J. (1991c), Genetic polymorphism of erythrocyte histone H1 in Japanese quail. Biochem. Genet. **29**, 431–445.

Pałyga J. (1998a), Genes for polymorphic H1 histones are linked in the Japanese quail genome. Biochem. Genet. **36**, 93–103.

Pałyga J. (1998b), Distribution of allelic forms of erythrocyte H1 histones in Japanese quail populations divergently selected for amount of weight loss after transient starvation. Biochem. Genet. **36**, 79–92.

Pałyga J., Górnicka-Michalska E., and Kowalski A. (1993), Genetic polymorphism of histone H1.z in duck erythrocytes. Biochem. J. **294**, 859–863.

Pałyga J., Górnicka-Michalska E., Kowalski A., and Książkiewicz J. (2000), Natural allelic variation of duck erythrocyte histone H1.b. Int. J. Biochem. Cell. Biol. **32**, 665–675.

Ramachandran A., Omar M., Cheslock P., and Schnitzler G. R. (2003), Linker histone H1 modulates nucleosome remodeling by human SWI/SNF. J. Biol. Chem. **278**, 48590–48601.

Roque A., Iloro I., Ponte I., Arrondo J. L., and Suau P. (2005), DNA-induced secondary structure of the carboxyl-terminal domain of histone H1. J. Biol. Chem. **280**, 32141–32147.

Sarg B., Green A., Soderkvist P., Helliger W., Rundquist I., and Lindner H. H. (2005), Characterization of sequence variations in human histone H1.2 and H1.4 subtypes. FEBS J. **272**, 3673–3683.

Shannon M. F. and Wells J. R. E. (1987), Characterization of the six chicken histone H1 proteins and alignment with their respective genes. J. Biol. Chem. **262**, 9664–9668.

Th'ng J. P., Sung R., Ye M., and Hendzel J. M. (2005), H1 family histones in the nucleus: control of binding and localization by the C-terminal domain. J. Biol. Chem. **280**, 27809–27814.

Thomas J. O. (1999), Histone H1: location and role. Curr. Opin. Cell Biol. **11**, 312–317.

Vicent G., Mella M. J., and Beato M. (2002), Asymmetric binding of histone H1 stabilizes MMTV nucleosomes and the interaction of progesterone receptor with the exposed HRE. J. Mol. Biol. **324**, 501–517.

Wiśniewski J. R., Zougman A., Krüger S., and Mann M. (2007), Mass spectrometric mapping of linker histone H1 variants reveals multiple acetylations, methylations and phosphorylation as well as differences between cell culture and tissue. Mol. Cell. Proteomics **6**, 72–87.

Wolffe A. (1992), Chromatin: Structure and Function. Academic Press, London.

Zlatanova J., Caiafa P., and van Holde K. (2000), Linker histone binding and displacement: versatile mechanism for transcriptional regulation. FASEB J. **14**, 1697–1704.