Ann. Anim. Sci., Vol. 17, No. 2 (2017) 385–398 *DOI: 10.1515/aoas-2016-0063*

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DISTRIBUTION OF NON-ALLELIC HISTONE H1 SUBTYPES IN FIVE AVIAN SPECIES*

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Abstract

The arrays of histone H1 subtypes from five avian species (chicken, grey partridge, pheasant, quail and duck) were compared to evaluate their intra- and inter-species variability. The electrophoretic patterns of linker histone preparations revealed the presence of subtypes that occur in all species (H1.a, H1.b, H1.c, H1.c′, H1.d and H5) and those which are confined to some species only (H1.a′, H1.b′, H1.z). In the densitometric profiles of histone H1 bands resolved in one-dimension acetic acid-urea polyacrylamide gel, the quantitative differences were observed both within a species (the ratio of H1.b to H1.d = 8.13 in quail) and between species (the ratio of H1.d in grey partridge and quail = 8.37). The comparable levels of abundant histone H5 that constitute from 53.62% (quail) to 60.86% (duck) of whole linker histone complement were detected in all species. Likewise, the quantification of H1 protein spots separated in a two-dimension SDS-polyacrylamide gel indicated that their intensity ratios could vary up to about 17-fold within a species (the ratio of H1.d to H1.a′ in grey partridge) and up to 10-fold between species (the ratio of pheasant H1.d to quail H1.d). Differences ($P<0.05$) in the histone H1 subtype levels were found both within and between avian species. A low to moderate range for the coefficients of H1 spot variation (from 0.13 to 0.72) was obtained for several independent histone H1 preparations.

Key words: avian, histone H1, non-allelic subtypes, quantification, variability

Histone H1 is a basic protein essential for proper chromatin organization and function. This protein stabilizes nucleosome structure by binding both to DNA segment associated with histone octamer and to DNA linker between consecutive nucleosomes. Consequently, H1 promotes a higher-order chromatin structure (Hansen, 2002; Routh et al., 2008). Due to dynamic mobility (Catez et al., 2006) and propensity for intrinsic disorder (Peng et al., 2012; Kowalski, 2015), the histone H1 influences gene expression by acting either as a global (Hashimoto et al., 2010) or local regulator (Fan et al., 2005). Additionally, the histone H1 is engaged in epigenetic events correlated with the modulation of histone H3 and DNA methylation marks

*The study was financed from statutory activity, project no. 612433.

(Zhang et al., 2012; Yang et al., 2013) and large-scale reprogramming of chromatin (She et al., 2013). The histone H1 function is also coupled with the control of chromatin activity by participating in interactions with partner proteins (Kalashnikova et al., 2016). For example, while the interaction with BAF factor (Montes de Oca et al., 2005) may consolidate active chromatin regions, and that with protein HP1 (Daujat et al., 2005) is implicated in the formation of inactive chromatin, the interactions with ribosomal protein L22 (Ni et al., 2006) and other nucleolar proteins (Kalashnikova et al., 2016) as well as genetic interactions with a remodeling enzyme CHD1 (Kavi et al., 2015) are engaged in the coordinated transcriptional regulation. Thus, the H1 histone may act as an interface for coordinating a plethora of chromatin interactions that may eventually lead to changes in the cell function. The multifaceted action of histone H1 (Hergeth and Schneider, 2015) is a result of its heterogeneity caused by the occurrence of several non-allelic subtypes (Shannon and Wells, 1987; Parseghian, 2015). Although these subtypes are considered to share a similar role in the function and organization of chromatin (Fan et al., 2001), they may also act individually by influencing various cell activities, including transcription and cell cycle progression (Happel et al., 2009), DNA damage response and repair (Soria et al., 2012), and apoptosis (Garg et al., 2014).

Table 1. Histone H1 subtype nomenclature and histone H1 identifiers in the UniProt database. A standard somatic mammalian nomenclature (h – human; m – mouse) for histone H1 subtype genes and corresponding proteins was unified by Talbert et al. (2012). Chicken (ch) histone H1 subtype nomenclature was adopted from Shannon and Wells (1987) and Pałyga (1991). The histone H1 subtypes of remaining avian species (grey partridge, pheasant, duck and quail) whose sequences are not annotated in the UniProt database are named according to Pałyga (1991). Duck (*Anas platyrhynchos*) histone H5 gene and protein name is designated with the prefix du

	Gene name	Protein name	UniProt (human/mouse)
Mammalian	h*HIST1H1A/* m*Hist1h1a*	h/mH1.1	Q02539/P43275
	h*HIST1H1C*/m*Hist1h1c*	h/mH1.2	P16403/P15864
	h*HIST1H1D*/m*Hist1h1d*	h/mH1.3	P16402/P43277
	h*HIST1H1E*/m*Hist1h1e*	h/mH1.4	P10412/P43274
	h*HIST1H1B*/m*Hist1h1b*	h/mH1.5	P16401/P43276
	h*H1F0*/m*H1f0*	h/mH1.0	P07305/P10922
	h*H1FX*/m*H1fx*	h/mH1.10	Q92522/Q80ZM5
Avian	ch*H1.11L*	chH1-a	P08287
	ch*H1.11R*	chH1-a’	P08288
	ch*H1.03*	chH1-b	P08285
	ch*H1.10*	chH1-c	P08286
	ch*H1.01*	chH1-c’	P08284
	ch*H1.02*	chH1-d	P09987
	ch*H1F0* (H5)	chH5	P02259
	du*H1F0* (H5)	duH5	R0LE74

A repertory of non-allelic histone H1 subtypes may range from a single form in protists and other lower eukaryotes to several regular and specialized variants in higher eukaryotes (Kowalski and Pałyga, 2012 a). The set of mammalian histone H1 non-allelic subtypes consists of ubiquitous somatic histones (H1.1, H1.2, H1.3,

H1.4, H1.5, H1.0 and H1.10), of which the histone H1.0 is essentially restricted to differentiated cells. Germ-cell linker histones include sperm-specific subtype H1t, H1T2 and HILS1 as well as oocyte-specific histone H1oo (Happel and Doenecke, 2009). The family of avian linker histones contains several common as well as species-specific H1 variants (Kowalski and Pałyga, 2012 a), complemented by abundant subtype H5 restricted to differentiated erythrocytes only (Kowalski and Pałyga, 2011). Avian somatic H1 histones are represented by common subtypes H1.a, H1.b, H1.c, H1.c′ and H1.d, and species-specific subtypes H1.a′, H1.b′ and H1.z confined to certain species like chicken, quail, turkey, duck and goose (Kowalski and Pałyga, 2012 a). A coherent nomenclature of mammalian and avian histone H1 subtypes is presented in Table 1.

The purpose of this work was to estimate a range of both within- and between-species variation in the avian histone H1 complements.

Material and methods

Animals

Approximately 1-year-old birds used in this study were obtained from the Department of Poultry Breeding of the University of Technology and Life Sciences in Bydgoszcz (Poland) – chicken (*Gallus gallus*, Silkie breed), grey partridge (*Perdix perdix*) and pheasant (*Phasianus colchicus*); the Waterfowl Breeding Station in Dworzyska of the National Research Institute of Animal Production (Poland) – duck (*Anas plathyrhynchos*, Khaki Campbell breed); and the Chair for Biological Bases of Animal Production of the University of Life Sciences in Lublin (Poland) – quail (*Coturnix coturnix japonica*, Pharaoh breed).

Extraction and electrophoresis of H1 histones

Blood from individual birds was collected from wing vein into propylene tubes containing SSC solution (0.15 M NaCl-0.015 M sodium citrate). Erythrocyte nuclei were prepared by lysis with 3% saponin in 0.1 M sodium phosphate buffer (pH 7.0) followed by several washes with 0.1 M sodium phosphate buffer (pH 7.0).

H1 histones were extracted from freshly isolated erythrocyte nuclei using 0.5 M and 1 M perchloric acid solutions according to the method of Neelin et al. (1995). Histone H1 samples were prepared by dissolving 1 mg of protein preparation in 200 mL solution containing 8 M urea, 0.9 M acetic acid and 10% 2-mercaptoethanol. The 5-μL aliquots of histone H1 (5 μg of protein) were loaded into separate wells of an acetic acid-urea slab gel, containing 15% acrylamide, 0.5% methylenebisacrylamide and 8 M urea, and electrophoresed. The resolved histone H1 protein bands were cut out from the gel, equilibrated in a buffer containing 100 mM Tris-base, 10% glycerol, 2.1% SDS and 2% 2-mercaptoethanol and transferred for the separation in an SDS slab gel containing 13.5% polyacrylamide and 0.1% SDS. One dimension acetic acid-urea and two-dimension SDS long polyacrylamide gels (24 cm) were electrophoresed either at 150 V for about 48 h or at 30 mA for about 24 h, respectively. The gels were stained sequentially with the solutions of Coomassie Blue R-250 dye

(0.05% and 0.0035%) and then repeatedly destained with 10% acetic acid (Kowalski and Pałyga, 2012 b).

Gel image processing and histone H1 quantification

The electrophoretic patterns of separated histone H1 subtypes were registered by using Doc-Print II gel documentation system (Vilber Lourmat), transferred to a computer and processed with the ImageJ 1.42q software (www.rsbweb.nih.gov/ij). The abundance of histone H1 subtypes was estimated based on densitometric profiles of the protein bands resolved in one-dimension polyacrylamide gel and expressed as a peak area of an H1 band in relation to a total volume of all histone H1 bands in a given species. The two-dimension polyacrylamide gels with separated histone H1 spots were scanned, and then the intensities of the protein spots were recorded as integrated densities which represent the sum of the pixels in the area of a selected protein spot. For the correct calculation of the integrated densities, the gel images were reversed to reduce background as much as possible to count the pixels which fall within a protein spot. The quantification of each histone H1 subtype was repeated for 10 individuals of the given species.

Statistical analysis

The statistical significance of differences in the histone H1 subtype abundance within and between species was checked using Student's t-test. The coefficients of variation (CV) for protein spots were computed as a ratio of standard deviation (SD) and the mean (M). The CV values below 0.25 and those between 0.25 and 0.75 were regarded as indicating low and moderate relative variability of the protein spot, respectively.

Results

Differences in electrophoretic patterns of non-allelic histone H1 subtypes among avian species

One-dimension acetic acid-urea polyacrylamide gel patterns of perchloric acid-soluble erythrocyte proteins revealed an abundant histone H5 band with a comparable electrophoretic migration in all species (Figure 1) and a partially resolved suite of histone H1 subtypes. The chicken and duck H1.c′ and H1.d subtypes (Figure 2) co-migrated as a single band because of their similar sizes and/or net charges. Moreover, the chicken and grey partridge subtype H1.a′ migrated together with adjacent subtypes (H1.a and/or H1.b) forming more intensely stained bands in the gel. A complete set of histone H1 subtype spots was detected in two-dimension SDS-polyacrylamide gel patterns after resolving first the protein preparations in the acetic acid-urea polyacrylamide gel. Based on the differences in their migration patterns, eight histone H1 subtypes (H1.a, H1.a′, H1.b, H1.b′, H1.c, H1.c′, H1.d and H1.z) were distinguished, among which histones H1.a, H1.b, H1.c, H1.c′ and H1.d occurred in all tested species (Figure 2). The remaining subtypes were characteristic for certain species only. For example, the histone H1.a′ was present in chicken and grey

partridge, the H1.b′ in pheasant and quail, and the H1.z in quail and duck. Both one-dimension gel patterns of the protein bands and their densitometric tracings demonstrated that electrophoretically similar H1 subtypes from different species exhibited differential migration and intensities. As compared to the relatively constant electrophoretic migration of subtypes H1.a, H1.c′ and H1.d, the variable in-gel location of histones H1.b and H1.c was revealed.

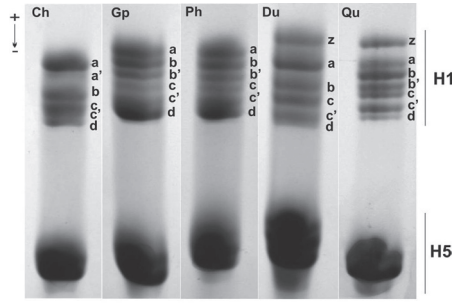

Figure 1. Acetic acid-urea polyacrylamide gel patterns of total erythrocyte histone H1 complement (H1 and H5) from five avian species (Ch, chicken; Gp, grey partridge; Ph, pheasant; Du, duck; Qu, quail)

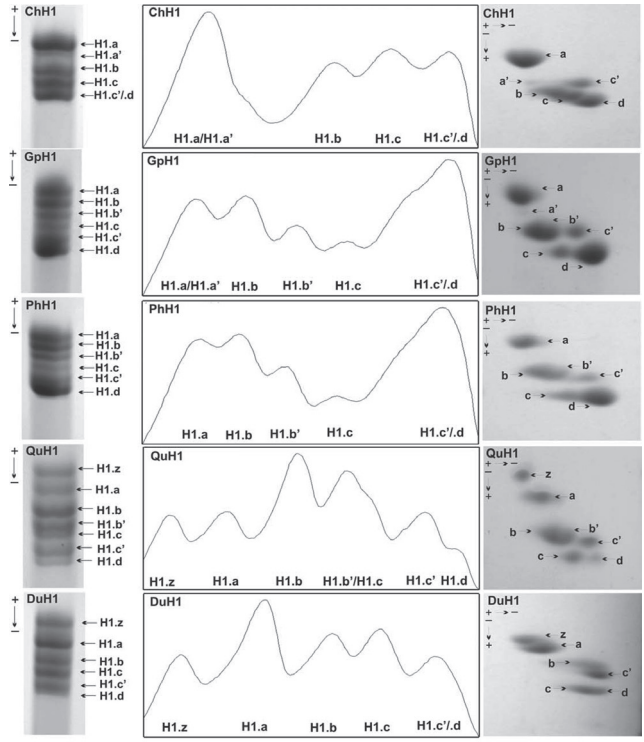

Figure 2. Electrophoretic patterns of erythrocyte histone H1 variants (H1.a, H1.a', H1.b, H1.b', H1.c, H1.c', H1.d and H1.z) from chicken (ChH1), grey partridge (GpH1), pheasant (PhH1), quail (QuH1) and duck (DuH1). Histone H1 preparations resolved in one-dimension acetic acid-urea polyacrylamide gel are placed leftmost and their densitometric profiles are in the middle, while the corresponding second-dimension SDS-polyacrylamide gel patterns are on the right

The differential intensities of histone H1 bands and spots in one- and two-dimension polyacrylamide gels, respectively, have been reflected in quantitative differences in the peak and spot area sizes. These differences were particularly pronounced in the two-dimension gel patterns (Figure 2), in which the chicken and grey partridge histone H1.a spots were much more deeply stained than in other species. Similarly, the levels of histone H1.b were low in chicken, pheasant, quail and duck and high in grey partridge. A considerable variation in the intensity of histone H1.d spots was observed because this protein, almost undetectable in quail and duck, was expressed at higher levels in chicken and pheasant and reached the highest levels in grey partridge (Figure 2).

Quantification of within- and between-species variation in histone H1 subtypes

The amount of a histone H1 subtype was expressed as a ratio of its densitometric peak area to the whole area of total histone H1 in a particular species, determined from multiple measurements (n = 10) of the histone H1 band areas in one-dimension polyacrylamide gel profiles (Figure 1 and Figure 2). Apart from subtypes H1.a+H1.a′ (chicken) and H1.c′+H1.d (chicken and duck) that were recorded as single peaks, representing 37.85% (chicken H1.a+H1.a′), 16.78% (chicken H1.c′+H1.d) or 14.77% (duck H1.c′+H1.d) of total histone H1, the levels of remaining well separated H1 subtypes ranged from 3.39% (quail H1.d) to 30.35% (duck H1.a) (Table 2). The moderate levels of H1.c′ (13.67%–15.42%) were present in grey partridge, pheasant, quail and duck. The levels of histone H1.c were low in grey partridge, pheasant and quail and almost twice as high in duck (19.50%) and chicken (24.47%). Quantitative differences were also apparent in the histone H1.d (Table 2) that ranged from high levels in pheasant (28.38%) and grey partridge (27.92%) to low amount in quail (3.39%). The abundance of histone H1.z was similar in quail and duck while that of H1.a was twice as high in duck (30.35%) than in quail (15.85%). The content of histone H5, constituting more than a half of overall linker histone complement (mean value 52.17%), was similar in all species (Table 3).

Table 2. Abundance of histone H1 subtypes (H1) expressed as the ratio of respective densitometric peak area relative to the whole tracing of H1 bands resolved in one-dimension acetic acid-urea polyacrylamide gels. The values represent mean±standard deviation of measurements conducted for 10 individuals for each species

H1	Chicken	Grey partridge	Pheasant	Quail	Duck
H1.a	0.3785±0.049[a)]	0.1917±0.021	0.2020±0.023	0.1585±0.020	0.3035±0.026
H1.b	0.2088±0.014	0.1865±0.030	0.1808±0.023	0.2759±0.015	0.2106±0.014
H1.b′		0.1182±0.013	0.1129±0.019	0.1907±0.010	
H1.c	0.2447±0.027	0.0828±0.015	0.0706±0.014	0.0851±0.009	0.1950±0.020
H1.c′	0.1678±0.00[9b)]	0.1367±0.010	0.1542±0.018	0.1370±0.014	0.1477±0.017[b)]
H1.d		0.2838±0.024	0.2792±0.014	0.0339±0.009	
H1.z				0.1185±0.018	0.1430±0.027

[a)]Combined value for subtypes H1.a+H1.a′. [b)]Combined value for subtypes H1.c′+H1.d.

Table 3. Abundance of histone H1 subtypes (H1) and histone H5 (H5) in the complement of total linker histones in several avian species. The values (mean±standard deviation) represent band areas for H1 and H5 relative to the whole area for total linker histones protein bands resolved in one-dimension acetic acid-urea polyacrylamide gels. The measurements were conducted for 10 individuals in each species

Species	H1	H5[a)]
Chicken	0.4366±0.021	0.5634±0.010
Grey partridge	0.4408±0.041	0.5592±0.021
Pheasant	0.4543±0.018	0.5456±0.021
Quail	0.4638±0.016	0.5362±0.036
Duck	0.3913±0.026	0.6086±0.027

[a)]No difference (P=1.0000) in the levels of histone H5 between avian species was found.

Table 4. Abundance (mean±standard deviation) of histone H1 subtypes (H1) estimated as integrated intensity (the measurement of mean grey value in pixels) for particular protein spots (n=10) resolved in two-dimension SDS-polyacrylamide gels

H1	Chicken	Grey partridge	Pheasant	Quail	Duck
H1.a	0.2257±0.019	0.1585±0.036	0.2377±0.019	0.1763±0.034	0.2864±0.021
H1.a’	0.0225±0.026	0.0177±0.031			
H1.b	0.2657±0.015	0.2024±0.039	0.1604±0.037	0.2465±0.019	0.1704±0.033
H1.b’		0.1110±0.057	0.1122±0.033	0.2146±0.034	
H1.c	0.1287±0.022	0.1118±0.040	0.0929±0.017	0.0933±0.028	0.1816±0.072
H1.c’	0.1037±0.023	0.0954±0.044	0.0674±0.028	0.1113±0.041	0.1432±0.044
H1.d	0.2237±0.038	0.3022±0.023	0.4024±0.013	0.0392±0.041	0.0835±0.052
H1.z				0.1175±0.032	0.1342±0.023

Table 5. The values for the coefficient of variation (CV) for the H1 spots resolved in two-dimension SDS-polyacrylamide gels, calculated as the quotient of standard deviation (SD) and a mean (M). The CV values below 0.25 and those from 0.25 to 0.75 were regarded as low and moderate spot variability, respectively

H1s	Chicken	Grey partridge	Pheasant	Quail	Duck
H1.a	0.19	0.36	0.19	0.34	0.21
H1.a’	0.27	0.32			
H1.b	0.15	0.37	0.38	0.20	0.33
H1.b’		0.69	0.34	0.29	
H1.c	0.22	0.41	0.17	0.28	0.72
H1.c’	0.21	0.44	0.28	0.41	0.44
H1.d	0.38	0.24	0.13	0.53	0.53
H1.z				0.32	0.23

Histone H1 subtypes were also evaluated quantitatively by multiple measurements (n = 10) of the protein spot areas in the images of two-dimension SDS polyacrylamide gels and expressed as spot integrated densities. The mean values for the protein spot intensities are presented in Table 4, confirming the variable levels of histone H1 subtypes both within and among species. On average, the amount of

subtypes H1.a and H1.b was about 9- to 11-fold higher compared to a low-abundant H1.a′ subtype in chicken and grey partridge. Histone H1.d in quail and duck was found to be at least 5 times less abundant than in chicken, grey partridge and pheasant. Although the subtype H1.c exhibited comparable levels, the content of pheasant H1.c' was, on average, lower by at least half compared to chicken, quail and duck. The ratio between the most and least abundant histone H1 spot within a species was high in grey partridge (the ratio of 17.0 between H1.d and H1.a′) and chicken (the ratio of 10.0 between H1.a and H1.a′), lower in quail (the ratio of 6.3 between H1.b and H1.d) and pheasant (the ratio of 5.9 between H1.d and H1.c′), and the lowest in duck (the ratio of 3.4 between H1.a and H1.d). Since the values for coefficients of variation ranged from 0.13 to 0.72, the variability of histone H1 protein spots was regarded to be low to moderate (Table 5).

Table 6. A significance of differences (Student's t-test) in the levels of histone H1 subtypes within avian species: chicken (Ch), grey partridge (Gp), pheasant (Ph), quail (Qu) and duck (Du)

Ch	H1.a	H1.a′	H1.b	H1.c	H1.c′	H1.d	Gp	H1.a	H1.a′	H1.b	H1.b′	H1.c	H1.c′	H1.d
H1.a		***	**	**	***	—	H1.a		—	—	—	***	—	***
H1.a′	***		—	*	—	—	H1.a′	—		—	*	*	—	***
H1.b	**	—		**	***	—	H1.b	—	—		*	*	**	**
H1.c	**	*	**		—	*	H1.b′	—	*	*		—	—	***
H1.c′	***	—	***	—		**	H1.c	***	*	*	—		—	***
H1.d	—	—	—	*	**		H1.c′	—	—	**	—	—		***
							H1.d	***	***	**	***	***	***	
Ph	H1.a	H1.b	H1.b′	H1.c	H1.c′	H1.d	Qu	H1.a	H1.b	H1.b′	H1.c	H1.c′	H1.d	H1.z
H1.a		**	***	***	***	***	H1.a		**	—	**	*	***	**
H1.b	**		—	**	**	***	H1.b	**		*	***	***	***	***
H1.b′	***	—		**	**	***	H1.b′	*	—		***	***	***	***
H1.c	***	**	**		***	***	H1.c	**	***	***		**	**	**
H1.c′	***	**	**	***		***	H1.c′	*	***	***	**		**	**
H1.d	***	***	***	***	***		H1.d	***	***	***	**	**		**
							H1.z	**	***	***	**	**	**	
Du	H1.a	H1.b	H1.c	H1.c′	H1.d	H1.z								
H1.a		**	**	*	***	***								
H1.b	**		*	***	—	***								
H1.c	**	*		***	—	**								
H1.c'	*	***	***		—	—								
H1.d	***	—	—	—		—								
H1.z	***	***	**	—	—									

— P>0.05; * P<0.05; ** P<0.01; *** P<0.001.

In general, whereas no difference (P>0.05) in the content of histone H5 was detected between the species (Table 3), the amount of histone H1 subtypes differed (P<0.05) both within (Table 6) and between (Table 7) the species.

Table 7. A significance of differences (Student's t-test) in the levels of histone H1 subtypes between bird species: chicken (Ch), grey partridge (Gp), pheasant (Ph), quail (Qu) and duck (Du)

H1.a	Ch	Gp	Ph	Qu	Du	H1.b	Ch	Gp	Ph	Qu	Du	H1.c	Ch	Gp	Ph	Qu	Du	H1c′	Ch	Gp	Ph	Qu	Du
Ch		*	**	***	***	Ch		***	**	**	—	Ch		***	**	**	**	Ch		—	—	—	
Gp	*		—	*	**	Gp	***		***	*	*	Gp	***		*	—	**	Gp	—		**	—	—
Ph	**	—		***	***	Ph	**	***		*	*	Ph	**	*		**	**	Ph	—	**		*	—
Qu	***	*	***		***	Qu	**	*	*		—	Qu	**	—	**		*	Qu	—	—	*		—
Du	***	**	***	***		Du	—	*	*	—		Du	**	**	**	*		Du	—	—	—	—	
H1.a′	Ch					H1.b′	Gp	Ph	Qu			H1.d	Ch	Gp	Ph	Qu	Du	H1.z	Qu				
Gp	—					Gp		**	*			Ch		***	***	***	***	Du	*				
						Ph	**		—			Gp	***		—	***	***						
						Qu	—	*				Ph	***	—		***	***						
												Qu	***	***	***		**						
												Du	***	***	***	**							

— P>0.05; * P<0.05; ** P<0.01; *** P<0.001.

Discussion

A differential distribution of histone H1 subtypes was reported in both mammalian cell lines (Ajiro et al., 1981) and tissues (Lennox and Cohen, 1984). For example, the relative proportions of histone H1 subtypes in mouse liver varied from 1.4% to 48.4% for histone H1.1 and H1.3/H1.4, respectively, with the intermediary levels for remaining subtypes: H1.5 (7.6%), H1.2 (15.4%) and H1.0 (27.2%) (Medrzycki et al., 2012). Likewise, human fetal fibroblasts exhibited variable contents of histone H1 subtypes in active chromatin with predominance of histone H1.3, compared to the subtypes H1.1, H1.2 and H1.4 (Parseghian et al., 2000). Moreover, qualitative differences were also detected among human histone H1 complement, with subtypes H1.1, H1.3 and H1.5 occurring in some cell lines only (Meergans et al., 1997). An uneven contribution of each subtype was also found within the set of the avian histone H1. Shannon and Wells (1987) showed that chicken erythrocyte subtypes H1.a+H1.a′ were most abundant (36–38%) as compared to underrepresented subtypes H1.c+H1.c′, constituting collectively 23%, and H1.b (20%) or H1.d (18–19%). Similar proportions of chicken H1 subtypes were also detected in this work, with histones H1.a+H1.a′ and H1.b representing 37.85% and 20.88%, respectively, of total H1 proteins. Likewise, the content of remaining subtypes, H1.c, H1.c′ and H1.d, amounted to 41.25%. The histone H1 subtype distribution in the chicken differed from that in other species. Histone H1.a constituted only 15.85% of total histone H1 in quail while histone H1.c in grey partridge, pheasant and quail was approximately three times less abundant than in the chicken. In addition to the differential distribution of histone H1 subtypes within a species, the disproportions in their contents were also apparent between the species. In contrast to quantitative variations in the histone H1 subtypes, the levels of linker histone H5 were roughly similar in all avian species. The heterogeneous histone H1 family represents less than a half of the total amount of erythrocyte linker histone set. The remaining part is represented by the abundant linker histone H5 which heavily influences avian chromatin by forming highly compacted and, thus, repressed regions (Kowalski and Pałyga, 2011). Our findings are in agreement with the data provided by Koutzamani et al. (2002) who found that depending on the method of extraction, the histone H5 may represent from 51 ± 5% to 59 ± 8% of the whole histone H1 complement in the chicken erythrocytes.

Despite a lack of detailed data on the functional features of avian H1 histones, it seems that the subtypes may act either individually or in combination to produce unique chromatin effects (Kowalski and Pałyga, 2016). A partial redundancy attributable to the histone H1 functioning (Milan-Ariño et al., 2016), has been confirmed by Lu et al. (2009). They found that mouse somatic H1 histones (H1.0, H1.1, H1.2, H1.3 and H1.4) have similar relative DNA binding affinities and equally stabilize chromatin structure. However, there is a number of studies demonstrating that the histone H1 subtype-specific activities are conducted through their differential abilities to bind DNA and condense chromatin (for recent review see Hergeth and Schneider, 2015; Parseghian, 2015; Kowalski and Pałyga, 2016). In mammals, H1 histones are thought to play a unique role in the organization of chromatin structure and may

individually regulate its action (Izzo et al., 2008; Over and Michaels, 2014). It has been shown (Clausell et al., 2009) that H1 subtypes have weak (H1.1 and H1.2), intermediate (H1.3) and strong (H1.0, H1.4, H1.5 and H1x) chromatin condensing capabilities. While the genes that control replication and recombination remain under the influence of histone H1.2, the genes regulating amino acid metabolism and protein synthesis are targeted by histone H1.0 (Bhan et al., 2008). In addition, the histones H1.2 and H1.0 fulfill a common function by affecting the genes related to a wide range of cell activity such as growth, signaling and death (Bhan et al., 2008). A specialization of histone H1 subtypes is also reflected by a characteristic distribution in the chromatin regions. Apart from the H1.0 histone that is mainly associated with euchromatin but may be also located in the heterochromatin domains, the remaining histone H1 subtypes were found to be distributed either in euchromatin (subtypes H1.1–H1.3) or in heterochromatin (subtype H1.4 and H1.5) regions (Th'ng et al., 2005). Moreover, the histone subtypes H1.2–H1.5 were preferentially located in the inactive chromatin regions but the histone H1.1 was associated with active chromatin domains (Izzo et al., 2013). Therefore, the histone H1 subtypes may function either as specific or general modulators of chromatin organization and function.

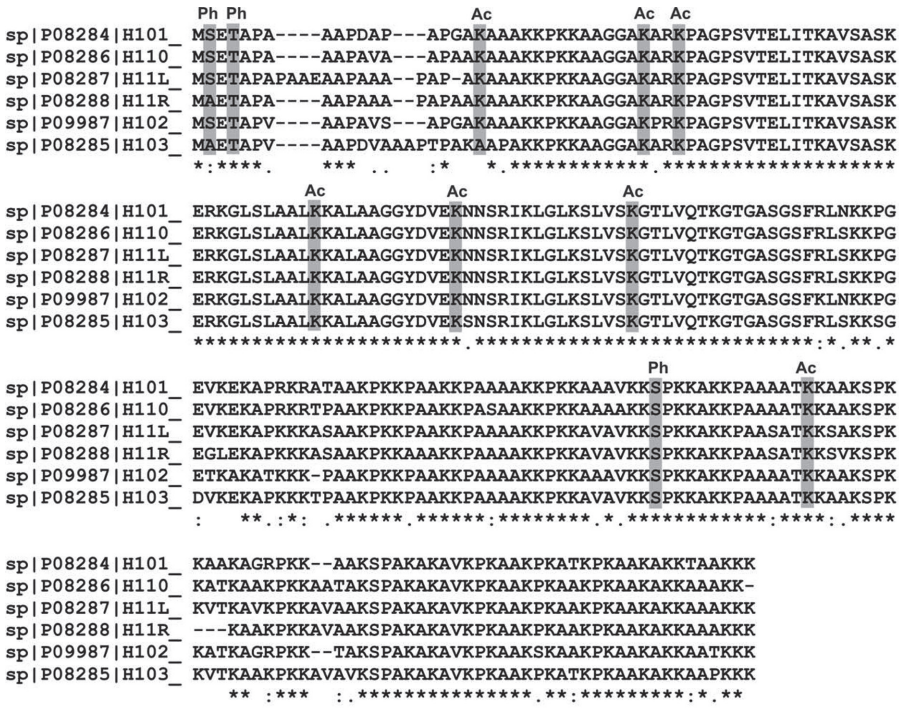

Figure 3. Sequence alignment of chicken histone H1 subtypes (H101 – H1.c'; H110 – H1.c; H11L – H1.a; H11R – H1.a'; H1_CH – H1.d and H103 – H1.b) in the CLUSTAL format obtained by MAFFT sequence alignment software v7.273. A degree of conservation in the consensus sequence is indicated as '*' (identical residues), ':' (highly conserved residues) and '.' (weakly conserved residues). Shaded boxes indicate acetylated (Ac) and phosphorylated (Ph) residues in all sequences (Sarg et al., 2015)

The specialized functions of histone H1 subtypes might also be inferred from a degree of their sequence conservation. The high similarity (more than 90%) of the chicken H1 subtype sequences (Figure 3) may suggest that they have a limited functional individualization with respect to more divergent mammalian H1 variants with sequence identity of 73% (Sarg et al., 2014) and, thus, able to perform more divergent functions (Parseghian, 2015). A range of covalent posttranslational modifications strongly influences the histone H1 activity (Izzo and Schneider, 2016). The modifications at the same sites in different H1 subtypes may evoke similar function, while the H1 modifications specific for a particular subtype might promote more diversified function. The shared function of avian histone H1 subtypes might be ascribed to the conserved sites of acetylation and phosphorylation (Figure 3) which were also present in the mammalian histone H1 subtypes (Sarg et al., 2015). The alignment of avian and mammalian H1 histone sequences revealed at least 59% sequence identity with ten sites of conservative modifications (four sites of phosphorylation and six sites of lysine methylation). As some positions in chicken erythrocyte histone H1 sequences are differently modified than those in the mammalian cells, they may be associated with cell-specific effects of linker histones (Sarg et al., 2015).

In conclusion, common erythrocyte histone H1 subtypes (H1.a, H1.b, H1.c, H1.c′ and H1.d) are always present in all tested bird species, in contrast to the minor subtypes (H1.a′, H1.b′ and H1.z) occurring in some species only. It seems plausible that both abundant and faintly expressed avian histone H1 subtypes may operate individually and/or in a combinatorial way by affecting the chromatin structure and function.

References

Ajiro K., Borun T.W., Solter D. (1981). Quantitative changes in the expression of histone H1 and H2B subtypes and their relationship to the differentiation of mouse embryonal carcinoma cells. Dev. Biol., 86: 206–211.

Bhan S., May W., Warren S.L., Sittman D.B. (2008). Global gene expression analysis reveals specific and redundant roles for H1 variants, H1c and $H1^0$, in gene expression regulation. Gene, 414: 10–18.

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

Clausell J., Happel N., Hale T.K., Doenecke D., Beato M. (2009). Histone H1 subtypes differently modulate chromatin condensation without preventing ATP-dependent remodeling by SWI/SNF or NURF. PLoS One, 4: e0007243. doi: 10.1371/journal.pone.0007243.

Daujat S., Zeissler U., Waldmann T., Happel N., Schneider R. (2005). HP1 binds specifically to Lys^{26}-methylated histone H1.4, whereas simultaneous Ser^{27} phosphorylation blocks HP1 binding. J. Biol. Chem., 280: 38090–38095.

Fan Y., Sirotkin A.M., Russel R.G., Ayala J., Skoultchi A.I. (2001). Individual somatic H1 subtypes are dispensable for mouse development even in mice lacking the H1(0) replacement subtype. Mol. Cell. Biol., 21: 7933–7943.

Fan Y., Nikitina T., Zhao J., Fleury T.J., Bhattacharyya R., Bouhassira E.E., Stein A., Woodcock C.L., Skoultchi A.I. (2005). Histone H1 depletion in mammals alter global chromatin structure but causes specific changes in gene regulation. Cell, 123: 1199–1212.

Garg M., Perumalsamy L.R., Shivashankar G.V., Sarin A. (2014). The linker histone H1.2 is an intermediate in the aptoptotic response to cytokine deprivation in T-effectors. Int. J. Cell Biol., 2014: 674753. doi: 10.1155/2014/674753.

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., Doenecke D. (2009). Histone H1 and its isoforms: contribution to chromatin structure and function. Gene, 431: 1–12.
Happel N., Warneboldt J., Hänecke K., Haller F., Doenecke D. (2009). H1 subtype expression during cell proliferation and growth arrest. Cell Cycle, 8: 2226–2232.
Hashimoto H., Takami Y., Sonoda E., Iwasaki T., Iwano H., Tachibana M., Takeda S., Nakayama T., Kimura H., Shinkai Y. (2010). Histone H1 null vertebrate cells exhibit altered nucleosome architecture. Nucleic Acids Res., 38: 3533–3545.
Hergeth S.P., Schneider R. (2015). The H1 linker histones: multifunctional proteins beyond the nucleosomal core particle. EMBO Rep., 16: 1439–1453.
Izzo A., Schneider R. (2016). The role of linker histone H1 modifications in the regulation of gene expression and chromatin dynamics. Biochim. Biophys. Acta, 1859: 486–495.
Izzo A., Kamieniarz K., Schneider R. (2008). The histone H1 family: specific members, specific functions? Biol. Chem., 389: 333–343.
Izzo A., Kamieniarz-Gdula K., Ramirez F., Noureen N., Kind J., Manke T., van Steensel B., Schneider R. (2013). The genomic landscape of the somatic linker histone subtypes H1.1 to H1.5 in human cells. Cell Rep., 3: 2142–2154.
Kalashnikova A.A., Rogge R.A., Hansen J.C. (2016). Linker histone H1 and protein-protein interactions. Biochim. Biophys. Acta, 1859: 455–461.
Kavi H., Lu X., Xu N., Bartholdy B.A., Vershilova E., Skoultchi A.I., Fyodorov D.V. (2015). A genetic screen and transcript profiling revealed a shared regulatory program for *Drosophila* linker histone H1 and chromatin remodeler CHD1. G3, 5: 677–687.
Kowalski A. (2015). Abundance of intrinsic structural disorder in the histone H1 subtypes. Comput. Biol. Chem., 59: 16–27.
Kowalski A., Pałyga J. (2011). Chromatin compaction in terminally differentiated avian blood cells: the role of linker histone H5 and non-histone protein MENT. Chromosome Res., 19: 579–590.
Kowalski A., Pałyga J. (2012 a). Linker histone subtypes and their allelic variants. Cell Biol. Int., 36: 981–996.
Kowalski A., Pałyga J. (2012 b). High-resolution two-dimensional polyacrylamide gel electrophoresis: A tool for identification of polymorphic and modified linker histone components. In: Gel Electrophoresis – Principles and Basics, Magdeldin S. (ed.). In Tech (Croatia), pp. 117–136.
Kowalski A., Pałyga J. (2016). Modulation of chromatin function through linker histone H1 variants. Biol. Cell, 108: 1–18.
Koutzamani E., Loborg H., Sarg B., Lindner H.H., Rundquist I. (2002). Linker histone subtype composition and affinity for chromatin *in situ* in nucleated mature erythrocytes. J. Biol. Chem., 227: 44688–44694.
Lennox R.W., Cohen L.H. (1984). The alterations in histone H1 complement during mouse spermatogenesis and their significance for H1 subtype function. Dev. Biol., 103: 80–84.
Lu H., Hamkalo B., Parseghian M.H., Hansen J.C. (2009). Chromatin condensing functions of the linker histone C-terminal domain are mediated by specific amino acid composition and intrinsic protein disorder. Biochemistry, 48: 164–172.
Medrzycki M., Zhang Y., Cao K., Fan Y. (2012). Expression analysis of mammalian linker-histone subtypes. J. Vis. Exp., (61). doi: 10.3791/3577.
Meergans T., Albig W., Doenecke D. (1997). Varied expression patterns of human histone H1 genes in different cell lines. DNA Cell Biol., 16: 1041–1049.
Millãn-Ariño L., Izquierdo-Bouldstridge A., Jordan A. (2016). Specificities and genomic distribution of somatic mammalian histone H1 subtypes. Biochim. Biophys. Acta, 1859: 510–519.
Montes de Oca R., Lee K.K., Wilson K.L. (2005). Binding of barrier to autointegration factor (BAF) to histone H3 and selected linker histones including H1.1. J. Biol. Chem., 280: 42252–42262.
Neelin J.M., Neelin E.M., Lindsay D.W., Pałyga J., Nichols C.R., Cheng K.M. (1995). The occurrence of a mutant dimerizable histone H5 in Japanese quail erythrocytes. Genome, 38: 982–990.
Ni J.Q., Liu L.P., Hess D., Rietdorf J., Sun F.L. (2006). *Drosophila* ribosomal proteins are associated with linker histones H1 and suppress gene transcription. Gene. Dev., 20: 1959–1973.

Over R.S., Michaels S.D. (2014). Open and closed: the roles of linker histones in plants and animals. Mol. Plant, 7: 481–491.

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

Parseghian M.H. (2015). What is the role of histone H1 heterogeneity? AIMS Biophys., 2: 724–772.

Parseghian M.H., Newcomb R.L., Winokur S.T., Hamkalo B.A. (2000). The distribution of somatic H1 subtypes is non-random on active vs. inactive chromatin: distribution in human fetal fibroblasts. Chromosome Res., 8: 405–424.

Peng Z., Mizianty M.J., Xue B., Kurgan L., Uversky V.N. (2012). More than just tails: intrinsic disorder in histone proteins. Mol. Biosyst., 8: 1886–1901.

Routh A., Sandin S., Rhodes D. (2008). Nucleosome repeat length and linker histone stoichiometry determine chromatin fiber structure. Proc. Natl. Acad. Sci. USA, 105: 8872–8877.

Sarg B., Lopez B., Lindner H., Ponte I., Suau P., Roque A. (2014). Sequence conservation of linker histones between chicken and mammalian species. Data Brief, 1: 60–64.

Sarg B., Lopez R., Lindner H., Ponte I., Suau P., Roque A. (2015). Identification of novel post-translational modifications in linker histones from chicken erythrocytes. J. Proteomics, 113: 162–177.

Shannon M.F., 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.

She W., Grimanelli D., Rutowicz K., Whitehead M.W.J., Puzio M., Kotliński M., Jerzmanowski A., Baroux C. (2013). Chromatin reprogramming during the somatic-to-reproductive cell fate transition in plants. Development, 140: 4008–4019.

Soria G., Polo S.E., Almouzni G. (2012). Prime, repair, restore: the active role of chromatin in the DNA damage response. Mol. Cell, 46: 722–734.

Talbert P.B., Ahmad K., Almouzni G., Ausio J., Berger F., Bhalla P.L., Bonner W.M., Cande W.Z., Chadwick B., Chan S.W.L., Cross G.A.M,, Cui L., Dimitrov S.I., Doenceke D., Eirin-Lopez J.M., Gorovsky M.A., Hake S.B., Hamkalo B.A., Holec S., Jacobsen S.E., Kamieniarz K., Kchohbin S., Ladurner A.G., Landsman D., Latham J.A., Loppin B., Malik H.S., Marzluff W.F., Pehrson J.R., Postberg J., Schneider R., Singh M.B., Smith M.M., Thompson E., Torres-Padilla M-E., Tremethick D.J., Turner B.M., Waterborg J.H., Wollmann H., Yelagandula R., Zhu B., Henikoff S. (2012). A unified phylogeny-based nomenclature for histone variants. Epigenet. Chromatin, 5: 7. doi: 10.1186/1756-8935-5-7.

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

Yang S-M., Kim B.J., Norwood Toro L., Skoultchi A.I. (2013). H1 linker histone promotes epigenetic silencing by regulating both DNA methylation and histone H3 methylation. Proc. Natl. Acad. Sci. USA, 110: 1708–1713.

Zhang Y., Liu Z., Medrzycki M., Cao K., Fan Y. (2012). Reduction of *Hox* gene expression by histone H1 depletion. PLoS One, 7:e38829. doi: 10.1371/journal.pone.0038829.

Received: 28 I 2016
Accepted: 11 X 2016