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METHODOLOGY

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MS_HistoneDB, a manually curated resource for proteomic analysis of human and mouse histones

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Abstract

Background: Histones and histone variants are essential components of the nuclear chromatin. While mass spectrometry has opened a large window to their characterization and functional studies, their identification from proteomic data remains challenging. Indeed, the current interpretation of mass spectrometry data relies on public databases which are either not exhaustive (Swiss-Prot) or contain many redundant entries (UniProtKB or NCBI). Currently, no protein database is ideally suited for the analysis of histones and the complex array of mammalian histone variants.

Results: We propose two proteomics-oriented manually curated databases for mouse and human histone variants. We manually curated >1700 gene, transcript and protein entries to produce a non-redundant list of 83 mouse and 85 human histones. These entries were annotated in accordance with the current nomenclature and unified with the "HistoneDB2.0 with Variants" database. This resource is provided in a format that can be directly read by programs used for mass spectrometry data interpretation. In addition, it was used to interpret mass spectrometry data acquired on histones extracted from mouse testis. Several histone variants, which had so far only been inferred by homology or detected at the RNA level, were detected by mass spectrometry, confirming the existence of their protein form.

Conclusions: Mouse and human histone entries were collected from different databases and subsequently curated to produce a non-redundant protein-centric resource, MS_HistoneDB. It is dedicated to the proteomic study of histones in mouse and human and will hopefully facilitate the identification and functional study of histone variants.

Keywords: Histone, Histone variants, Chromatin, Mass spectrometry, Proteomics

Background

In eukaryotic cells, the nucleosome is the basic unit of chromatin organization. Nucleosomes are composed of an octamer of four core histones, H2A, H2B, H3 and H4, wrapped by DNA [1]. An additional linker histone, H1, can be deposited near the DNA entry–exit points [2, 3]. The dynamic organization of chromatin impacts many cellular events, including the regulation of gene transcription, DNA replication and the maintenance of genome integrity through DNA repair mechanisms [4, 5]. These pathways signal to chromatin by different mechanisms including DNA methylation, non-coding regulatory RNAs, recruitment of remodelling factors, incorporation of histone variants and covalent modifications of histones [6–12]. Histones are decorated by many post-translational modifications, the most common of which are acetylation, methylation, phosphorylation and ubiquitination [13, 14]. Some of these modifications favour transcription activation, while others are associated with repression of transcription [15]. In addition to transcription, histone modifications are involved in numerous regulatory circuits, such as chromosome dynamics [16], DNA repair [17] or the establishment and maintenance of heterochromatin [18]. Furthermore, dedicated molecular machineries can load and mobilize nucleosomes along the DNA (for review, see [4]). These

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chromatin remodellers play an important role in the regulation of transcription by organizing the nucleosomal positions at critical regulatory regions [19, 20]. Finally, non-allelic variants of canonical histones, named histone variants, are important elements in chromatin signalling pathways [21, 22]. Some variants are general players—expressed ubiquitously, contributing to various aspects of transcription and epigenetic regulations—while others are only expressed in certain cell types, such as germ cells [23]. Some of these variants are specifically expressed during sperm differentiation and are annotated TS for testis-specific [24–26]. Altogether, histone variants have been described for H3, H2A, H2B and H1; H4 is the only histone for which no variant has been identified in mammals, but some organisms, such as the urochordate *Oikopleura dioica*, ciliates and trypanosomes, have evolved H4 variants [10, 27–29].

Histone variants were initially discovered using classical biochemical approaches. Recently, the development of mass spectrometry (MS) techniques, with constant increases in sensitivity and speed of analysis, has facilitated their identification and functional characterization [14, 30–34]. In order to utilize these technologies, histones are first biochemically enriched taking advantage of their highly basic nature. Then, they are proteolyzed with proteases to form short peptides, which are then analysed by MS/MS. The acquired MS/MS spectra are interpreted and converted into amino acid sequences, from which the identity of the original histone protein and the possible presence of post-translational modifications on specific residues can be determined [35]. However, these analyses still remain restricted for a number of reasons. One of these is that the interpretation of MS/MS spectra relies on matching experimental data to theoretical peptide sequences obtained by an *in silico* proteolysis of a list of proteins. Therefore, the content of the theoretical protein sequence database conditions the interpretation of the experimental spectra and the subsequent identification of histones. Classical databases such as Swiss-Prot, trEMBL and NCBI are usually used with success. However, histones have not been precisely annotated in these resources. Manually curated databases such as Swiss-Prot lack several histones, while others, such as trEMBL or NCBI protein database, are more extensively populated with non-reviewed data. The latter contain more histone entries, but the degree of redundancy and the precision of the descriptions can make protein identification results difficult to interpret. Finally, naming of histones has been recently revisited with a new unified nomenclature [36]. The recent release of HistoneDB 2.0 consolidated the sequence information of a large variety of histones and their sequence variants in many organisms [37]. However, it has not yet been integrated in the above databases and the same variant can go by different names. For instance, the coding gene *H2afb1* refers to proteins H2A.L.2 or H2A.Lap3 in the literature and to H2A-Bbd type 1 in the NCBI RefSeq and UniProtKB databases [38, 39]. In addition, a different protein coded by *Gm14920* is also named H2A.Bbd.1 in other publications [40]. Here, a unified name is presented to identify uniquely each ambiguous entry and is also associated with its other names to facilitate its relationship with previously published work.

We have collected redundant histone entries from a number of public databases, gathering >700 entries for mouse and >1000 entries for human histones. We manually curated these lists to obtain a final count of 83 and 85 histone entries for mouse or human, respectively. Their annotations have been revisited to match the current histone nomenclature in accordance with the new resource "HistoneDB 2.0—with variants" [37]. About 30% of these entries have a fuzzy UniProtKB protein annotation, such as "predicted" or "inferred by homology", and we performed MS analysis to clearly identify several of these imprecisely characterized entries (some of which had formerly been described to be detected by western blot).

Results

MS_HistoneDB, a resource containing unique and non-redundant histones

Our initial aim with this work was to generate an exhaustive and non-redundant resource that would facilitate histone analysis by MS. We identified and collected all the information available on human and mouse histones from the public databases of NCBI, Ensembl and UniProtKB (Table 1). This work was aided by the recent release of an updated version of the Histone Database, named "HistoneDB 2.0—with variants" [37]. This database contains 38,664 entries from 1624 species, with 761 and 1039 entries for mouse and human, respectively. In addition, several histones were also considered based on published articles [41–45].

The dataflow is presented in Fig. 1a. This curating process is exemplified with human H2A.Z on Fig. 1b. A total of 23 entries were collected from Swiss-Prot, Uniprot-trEMBL and NCBI databases. Fourteen were duplicated and removed to obtain nine unique entries, which were annotated as H2A.Z.1 and eight spliced isoforms of H2A.Z.2 using the release R86 of the Ensembl database (Fig. 1c, d). In summary, the following rules were applied. First, each entry is protein-centric and therefore defined by the final product, a unique mature protein. Second, it must be associated with gene, transcript and protein accession numbers in NCBI and/or Ensembl, unless published data document its existence. Third, histone names are not always consistent within the

Table 1 Histone entries in various publicly accessible databases

	Mouse			Human		
	NCBI HistoneDB 2.0	UniProtKB	This study	NCBI HistoneDB 2.0	UniProtKB	This study
H1	170	26	16	126	14	12
H2A	238	44	37	313	44	35
H2B	121	19	16	239	32	21
H3	151	17	13	189	22	16
H4	81	4	1	172	4	1
Total	761	110	83	1039	1126	85

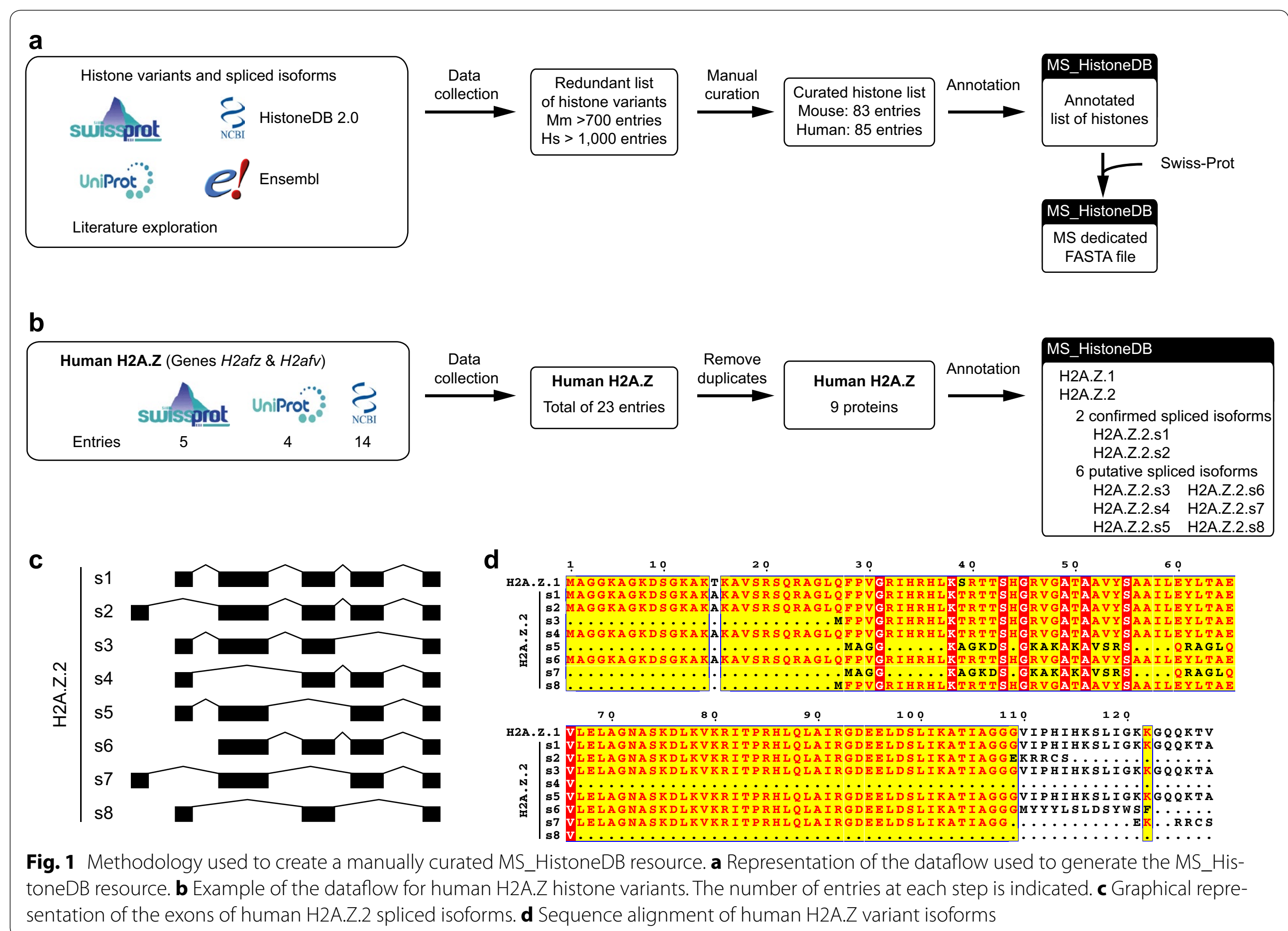

Fig. 1 Methodology used to create a manually curated MS_HistoneDB resource. **a** Representation of the dataflow used to generate the MS_HistoneDB resource. **b** Example of the dataflow for human H2A.Z histone variants. The number of entries at each step is indicated. **c** Graphical representation of the exons of human H2A.Z.2 spliced isoforms. **d** Sequence alignment of human H2A.Z variant isoforms

existing public databases. Some were renamed following the Talbert et al. nomenclature and in agreement with the HistoneDB 2.0 resource as detailed in the following sections [36, 37]. The final list of histone entries is presented as a phylogenetic tree in Fig. 2 and in Tables 2 and 3 for mouse and human species, respectively. We did not provide here an extensive review of the functional roles of each histone variant, which are already available elsewhere [4, 21, 23, 36, 46].

Canonical histones

Canonical histones constitute the bulk of the proteins that organize DNA into chromatin. They are synthesized and incorporated into chromatin during replication [41]. Their expression is carefully regulated to provide enough proteins to be loaded onto newly synthesized DNA while preventing the accumulation of free histones [47, 48]. For this reason, they are denominated "replication-dependent" and their mRNA adopts a unique organization (for

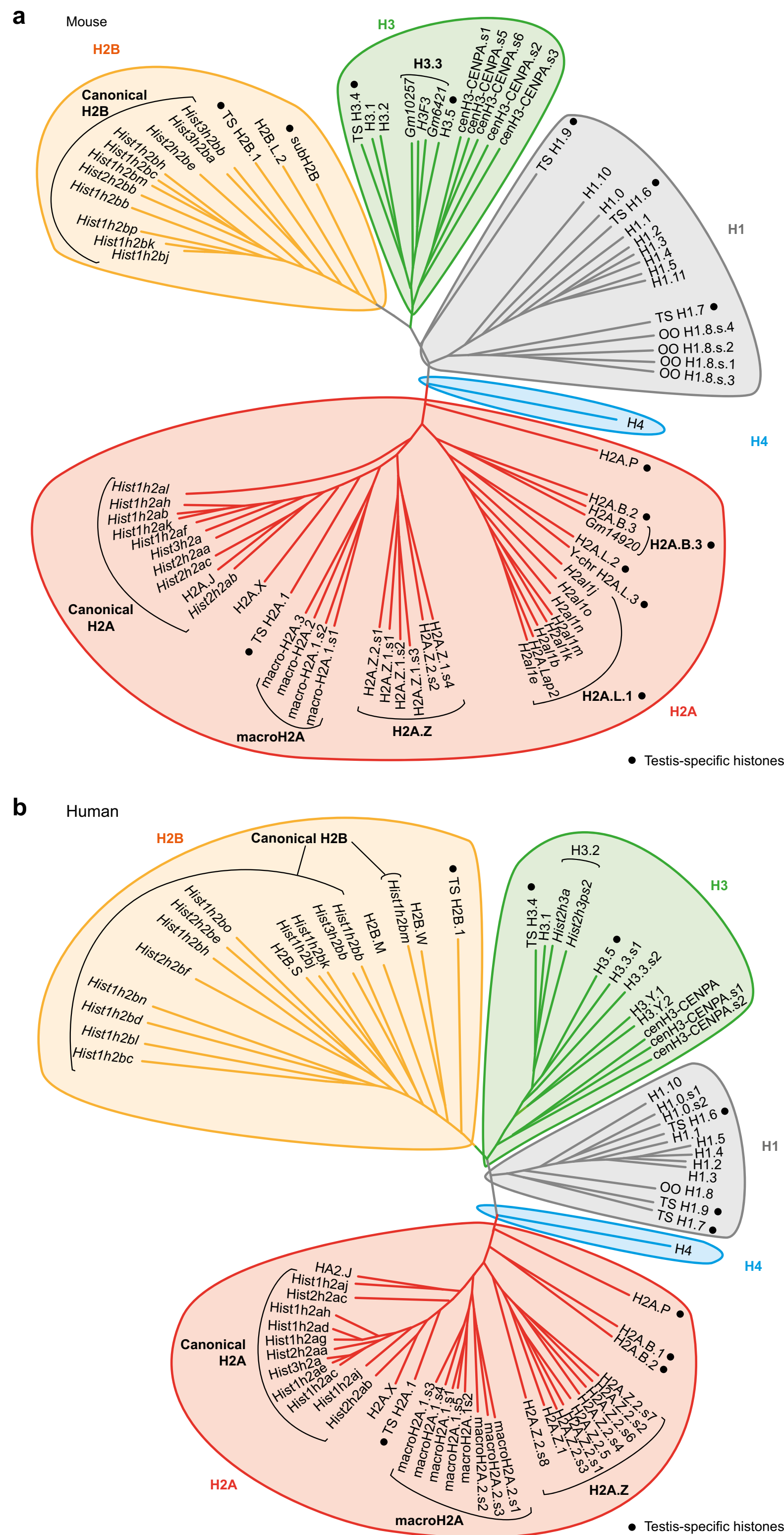

Fig. 2 Phylogenetic trees for mouse and human histone entries in MS_HistoneDB. Please note that for clarity, some putative spliced isoforms of canonical histones were not included, as well as other very short spliced isoforms for some histone variants. The full lists are presented in Tables 2 and 3. Gene names are indicated in *italic* to identify histone isoforms grouped under a generic term (see Tables 2 and 3; Additional files 4, 5). *Black dots* highlight testis-specific variants

Table 2 Manually curated list of mouse histones

Histone	Protein name	Entry name for MS analysis	Gene name	UniProtKB	References
H1	H1.1	H1.1	*Hist1h1a*	P43275	[41, 91]
	H1.2	H1.2	*Hist1h1c*	P15864	[41, 92]
	H1.3	H1.3	*Hist1h1d*	P43277	[41]
	H1.4	H1.4	*Hist1h1e*	P43274	[41]
	H1.5	H1.5	*Hist1h1b*	P43276	[41]
	H1.0 (H1°)	H1.0 (H1°)	*H1f0*	P10922	[93]
	TS H1.6 (H1T)	TS H1.6 (H1T)	*Hist1h1t*	Q07133	[54]
	TS H1.7 (H1T2, HANP1)	TS H1.7 (H1T2, HANP1)	*H1fnt*	Q8CJI4	[55, 56]
	OO H1.8 (H1oo)	OO H1.8.s1 (H1oo)	*H1foo*	Q8VIK3	[59]
		OO H1.8.s2	*H1foo*	Q8VIK3-2	[60]
		OO H1.8.s3 (putative spliced isoform)	*H1foo*	E0CZ52	*
		OO H1.8.s4 (putative spliced isoform)	*H1foo*	E0CYL2	Short**
		OO H1.8.s5 (putative spliced isoform)	*H1foo*	A0A0N4SV54	Short*
	TS H1.9 (HILS1)	TS H1.9 (HILS1)	*Hils1*	Q9QYL0	[57, 58]
	H1.10	H1.10	*H1fx*	Q80ZM5	*
	H1.11	H1.11 gene: Gm6970	*Gm6970*	F7DCP6	*
H2A	Canonical H2A	Canonical H2A genes: Hist1h2ab, Hist1h2ac, Hist1h2ad, Hist1h2ae, Hist1h2ag, Hist1h2ai, Hist1h2an, Hist1h2ao, Hist1h2ap	*Hist1h2ab*	P22752	[41]
			Hist1h2ac	P22752	[41]
			Hist1h2ad	P22752	[41]
			Hist1h2ae	P22752	[41]
			Hist1h2ag	P22752	[41]
			Hist1h2ai	P22752	[41]
			Hist1h2an	P22752	[41]
			Hist1h2ao	P22752	[41]
			Hist1h2ap	P22752	[41]
		Canonical H2A gene: Hist1h2af	*Hist1h2af*	Q8CGP5	[41]
		Canonical H2A gene: Hist1h2ah	*Hist1h2ah*	Q8CGP6	[41]
		Canonical H2A gene: Hist1h2ak	*Hist1h2ak*	Q8CGP7	[41]
		Canonical H2A gene: Hist1h2al	*Hist1h2al*	F8WIX8	*
		Canonical H2A genes: Hist2h2aa1, Hist2h2aa2	*Hist2h2aa1*	Q6GSS7	[41]
			Hist2h2aa2	Q6GSS7	[41]
		Canonical H2A gene: Hist2h2ab	*Hist2h2ab*	Q64522	[41]
		Canonical H2A gene: Hist2h2ac	*Hist2h2ac*	Q64523	[41]
		Canonical H2A gene: Hist3h2a	*Hist3h2a*	Q8BFU2	[41]
	H2A.J (putative variant)	H2A.J.s1 (putative variant)	*H2afj*	Q8R1M2	*
		H2A.J.s2 (putative variant, putative spliced isoform)	*H2afj*	A0A0N4SV66	*
	H2A.X	H2A.X	*H2afx*	P27661	[61, 62, 94]
	H2A.Z.1	H2A.Z.1.s1	*H2afz*	P0C0S6	[43]
		H2A.Z.1.s2 (putative spliced isoform)	*H2afz*	Q3UA95	*
		H2A.Z.1.s3 (putative spliced isoform)	*H2afz*	G3UWL7	Short*
		H2A.Z.1.s4 (putative spliced isoform)	*H2afz*	G3UX40	Short**
	H2A.Z.2	H2A.Z.2.s1	*H2afv*	Q3THW5	[43]
		H2A.Z.2.s2 (putative spliced isoform)	*H2afv*	Q8R029	Short*
	Macro-H2A.1	Macro-H2A.1.s1	*H2afy*	Q9QZQ8	[95]
		Macro-H2A.1.s2	*H2afy*	Q9QZQ8-2	[45]
	Macro-H2A.2	Macro-H2A.2	*H2afy2*	Q8CCK0	[96, 97]
	Macro-H2A.3	Macro-H2A.3 (pseudogene) gene: H2afy3	*H2afy3*	Q9D3V6	***
	TS H2A.1	TS H2A.1 (TH2A)	*Hist1h2aa*	Q8CGP4	[41]
	H2A.L.1 (H2A.Lap2)	H2A.L.1 (H2A.Lap2) genes: H2al1a, GH2al1c,H2al1d, H2al1f,H2al1g, H2al1h,H2al1i	*H2al1a*	Q5M8Q2	[38, 39]
			H2al1c	Q5M8Q2	[38, 39]
			H2al1d	Q5M8Q2	[38, 39]

Table 2 continued

Histone	Protein name	Entry name for MS analysis	Gene name	UniProtKB	References
			H2al1f	Q5M8Q2	[38, 39]
			H2al1g	Q5M8Q2	[38, 39]
			H2al1h	Q5M8Q2	[38, 39]
			H2al1i	Q5M8Q2	[38, 39]
		H2A.L.1 gene: H2al1b	*H2al1b*	A0A087WP11	*
		H2A.L.1 gene: H2al1e	*H2al1e*	Q810S6	*
		H2A.L.1 gene: H2al1j	*H2al1j*	A2BFR3	*
		H2A.L.1 gene: H2al1k	*H2al1k*	J3QP08	*
		H2A.L.1 gene: H2al1m	*H2al1m*	Q9DAD9	*
		H2A.L.1 gene: H2al1n	*H2al1n*	Q497L1	*
		H2A.L.1 gene: H2al1o	*H2al1o*	L7MU04	*
	H2A.L.2 (H2A.Lap3, H2A.B.1)	H2A.L.2 (H2A.Lap3, H2A.B.1) gene: H2afb1	*H2afb1*	Q9CQ70	[38, 39]
	Y-chr H2A.L.3	Y-chr H2A.L.3 genes: H2al2b, H2al2c	*H2al2b*	A9Z055	[98]
			H2al2c	A9Z055	[98]
	H2A.P (H2A.L3, H2A.Lap4)	H2A.P (H2A.L3, H2A.Lap4) gene: Hypm	*Hypm*	Q9CR04	[38, 39]
	H2A.B.2	H2A.B.2 gene: H2afb2	*H2afb2*	S4R1M3	[40, 99]
	H2A.B.3	H2A.B.3 gene: H2afb3	*H2afb3*	S4R1G7	[40, 99]
		H2A.B.3 (H2A.Lap1) gene: Gm14920	*Gm14920*	S4R1E0	[39, 40, 99]
H2B	Canonical H2B	Canonical H2B gene: Hist1h2bb	*Hist1h2bb*	Q64475	[41]
		Canonical H2B genes: Hist1h2bc, Hist1h2be, Hist1h2bg	*Hist1h2bc*	Q6ZWY9	[41]
			Hist1h2be	Q6ZWY9	[41]
			Hist1h2bg	Q6ZWY9	[41]
		Canonical H2B genes: Hist1h2bf, Hist1h2bj, Hist1h2bl, Hist1h2bn, Hist1h2bq, Hist1h2br	*Hist1h2bf*	P10853	[41]
			Hist1h2bj	P10853	[41]
			Hist1h2bl	P10853	[41]
			Hist1h2bn	P10853	[41]
			Hist1h2bq	P10853	[41]
			Hist1h2br	P10853	[41]
		Canonical H2B genes: Hist1h2bq, Hist1h2br (putative spliced isoform)	*Hist1h2bq*	Q8CBB6	*
			Hist1h2br	Q8CBB6	*
		Canonical H2B gene: Hist1h2bh	*Hist1h2bh*	Q64478	[41]
		Canonical H2B gene: Hist1h2bk	*Hist1h2bk*	Q8CGP1	[41]
		Canonical H2B gene: Hist1h2bm	*Hist1h2bm*	P10854	[41]
		Canonical H2B gene: Hist1h2bp Spliced isoform 1 (main)	*Hist1h2bp*	Q8CGP2	[41]
		Canonical H2B gene: hist1h2bp (putative spliced isoform)	*Hist1h2bp*	Q8CGP2-2	[41]
		Canonical H2B gene: Hist2h2bb	*Hist2h2bb*	Q64525	[41]
		Canonical H2B gene: Hist2h2be	*Hist2h2be*	Q64524	[41]
		Canonical H2B gene: Hist3h2ba	*Hist3h2ba*	Q9D2U9	[41]
		Canonical H2B gene: Hist3h2bb	*Hist3h2bb*	Q8CGP0	*
	TS H2B.1 (TH2B)	TS H2B.1 (TH2B)	*Hist1h2ba*	P70696	[41, 79]
	subH2B (H2BL.1)	subH2B (H2BL.1)	*1700024p04rik*	Q9D9Z7	[38, 100]
	H2B.L.2	H2B.L.2	*H2bfm*	Q9DAB5	[38]
H3	Canonical H3.1	Canonical H3.1	*Hist1h3a*	P68433	[41]
			Hist1h3g	P68433	[41]
			Hist1h3h	P68433	[41]
			Hist1h3i	P68433	[41]
	Canonical H3.2	Canonical H3.2	*Hist1h3b*	P84228	[41]
			Hist1h3c	P84228	[41]
			Hist1h3d	P84228	[41]
			Hist1h3e	P84228	[41]
			Hist1h3f	P84228	[41]
			Hist2h3b	P84228	[41]

Table 2 continued

Histone	Protein name	Entry name for MS analysis	Gene name	UniProtKB	References
			Hist2h3c1	P84228	[41]
			Hist2h3c2	P84228	[41]
	H3.3	H3.3 genes: H3f3a, H3f3b	*H3f3a*	P84244	[101, 102]
			H3f3b	P84244	[101, 102]
		H3.3 gene: Gm6421	*Gm6421*	EDL18362.1	[103]
		H3.3 gene: Gm10257	*Gm10257*	XP_003084990.1	[103]
	cenH3-CENPA	cenH3-CENPA.s1	*Cenpa*	O35216	[104]
		cenH3-CENPA.s2 (putative spliced isoform)	*Cenpa*	D6RCV6	Short**
		cenH3-CENPA.s3 (putative spliced isoform)	*Cenpa*	D6RJ71	**
		cenH3-CENPA.s4 (putative spliced isoform)	*Cenpa*	A0A0G2JEV0	*
		cenH3-CENPA.s5 (putative spliced isoform)	*Cenpa*	A0A0G2JGI2	*
		cenH3-CENPA.s6 (putative spliced isoform)	*Cenpa*	A0A0G2JEV2	**
	H3.5	H3.5	*H3f3c*	P02301	***
	TS H3.4 (H3T)	TS H3.4 (H3T)	*Gm12260*	NP_001304932.1	[74]
H4	H4	H4	*Hist1h4a*	P62806	[41, 105]
			Hist1h4b	P62806	[41, 105]
			Hist1h4c	P62806	[41, 105]
			Hist1h4d	P62806	[41, 105]
			Hist1h4f	P62806	[41, 105]
			Hist1h4h	P62806	[41, 105]
			Hist1h4i	P62806	[41, 105]
			Hist1h4j	P62806	[41, 105]
			Hist1h4k	P62806	[41, 105]
			Hist1h4m	P62806	[41, 105]
			Hist1h4n	P62806	[41, 105]
			Hist2h4	P62806	[41, 105]
			Hist4h4	P62806	[41, 105]

Their protein names have been adapted to improve their identification and analysis by mass spectrometry. Indeed, the column "Entry name for MS analysis" represents the information present in the FASTA file (Additional file 1) used as a database to identify peptides and proteins after an MS analysis. The last column indicates studies on histones that described evidence of transcript and/or protein existence. For the sake of completeness, histone entries lacking a related publication were retained and the classification currently proposed by the Ensembl database was specified, as follows

* "Protein coding", genes and/or transcript that contains an open reading frame (ORF)

** "Nonsense mediated decay", transcript is thought to undergo nonsense mediated decay

*** "Pseudogene", genes containing frameshift and/or stop codon(s) that disrupt the ORF

The term "Short" indicates that the putative protein is significantly smaller than conventional histones; its incorporation into chromatin and its biological function is then doubtful. Additional file 4 presents links to gene, transcripts and protein entries to Ensembl and UniProtKB databases

review, see [49]). They are the only RNA polymerase II transcripts which are not polyadenylated but instead possess a 3′ stem-loop, formed during the maturation of their mRNA and which is essential for their regulation [49]. However, polyadenylation events of replication-dependent histone mRNA have recently been identified in terminally differentiated cells and suggested to provide a replacement pool of canonical histones [50].

H2A and H2B canonical histones have minor sequence variations, and it is not clear yet whether these have a functional significance [51]. MS analysis can differentiate between these isoforms and their denomination had to be adapted for proteomic analysis. Here, we propose that canonical H2A and H2B isoforms can be regrouped under the generic term "canonical H2A" or "canonical H2B", complemented by the gene name of each isoform (Tables 2, 3).

Histone variants are mostly replication-independent

In contrast to canonical histones, almost all histone variants are synthesized independently of the cell cycle and named "replication-independent" [49]. Their mRNA is polyadenylated and these histones are incorporated into chromatin at any time of the cell cycle. Two exceptions are the testis-specific (TS) histone variants TS H2A and TS H2B, which possess a 3′ stem-loop in their mRNA. For this reason, they have been classified as replication-dependent [49] even if expressed in differentiating

Table 3 Manually curated list of human histones

Histone	Protein name	Entry name for MS analysis	Gene name	UniProtKB Accession	References
H1	H1.1	H1.1	*Hist1h1a*	Q02539	[41, 106, 107]
	H1.2	H1.2	*Hist1h1c*	P16403	[41, 106, 107]
	H1.3	H1.3	*Hist1h1d*	P16402	[41, 106, 107]
	H1.4	H1.4	*Hist1h1e*	P10412	[41]
	H1.5	H1.5	*Hist1h1b*	P16401	[41, 108]
	H1.0 (H1°)	H1.0 (H1°)	*H1f0*	P07305	[109]
	TS H1.6 (H1t)	TS H1.6 (H1t)	*Hist1h1t*	P22492	[41]
	TS H1.7 (H1T2, HANP1)	TS H1.7 (H1T2, HANP1)	*H1fnt*	Q75WM6	[110]
	OO H1.8 (H1oo)	OO H1.8.s1 (H1oo)	*H1foo*	Q8IZA3-1	[111, 112]
		OO H1.8.s2 (putative spliced isoform)	*H1foo*	Q8IZA3-2	*
	TS H1.9 (Hils)	TS H1.9 (Hils)	*Hils1*	P60008	[57]
	H1.10	H1.10	*H1fx*	Q92522	[113]
H2A	Canonical H2A	Canonical H2A genes: Hist1h2ag, Hist1h2ai, Hist1h2ak, Hist1h2al, Hist1h2am	*Hist1h2ag*	P0C0S8	[41]
			Hist1h2ai	P0C0S8	[41]
			Hist1h2ak	P0C0S8	[41]
			Hist1h2al	P0C0S8	[41]
			Hist1h2am	P0C0S8	[41]
		Canonical H2A gene: Hist1h2ac	*Hist1h2ac*	Q93077	[41]
		Canonical H2A gene: Hist1h2ad	*Hist1h2ad*	P20671	[41]
		Canonical H2A gene: Hist1h2ae	*Hist1h2ae*	P04908	[41]
		Canonical H2A gene: Hist1h2ah	*Hist1h2ah*	Q96KK5	[41]
		Canonical H2A gene: Hist1h2aj	*Hist1h2aj*	Q99878	[41]
		Canonical H2A gene:Hist2h2aa4	*Hist1h2aa4*	Q6FI13	[41]
		Canonical H2A gene: Hist2h2ab	*Hist2h2ab*	Q8IUE6	[41]
		Canonical H2A gene: Hist2h2ac	*Hist2h2ac*	Q16777	[41]
		Canonical H2A gene: Hist3h2a	*Hist3h2a*	Q7L7L0	[41]
		Canonical H2A (pseudogene)	*Hist1h2Aps4*	Q92646	***
	H2A.J (putative variant)	H2A.J.s1	*H2afj*	Q9BTM1-1	**
		H2A.J.s2 (putative spliced isoform)	*H2afj*	Q9BTM1-2	**
		H2A.J.s3 (putative spliced isoform)	*H2afj*	H0YFX9	Short**
	H2A.X	H2A.X	*H2afx*	P16104	[61, 114]
	H2A.Z.1	H2A.Z.1	*H2afz*	P0C0S5	[115, 116]
	H2A.Z.2	H2A.Z.2.s1	*H2afv*	Q71UI9-1	[116]
		H2A.Z.2.s2	*H2afv*	Q71UI9-2	[65]
		H2A.Z.2.s3 (putative spliced isoform)	*H2afv*	Q71UI9-4	[65]
		H2A.Z.2.s4 (putative spliced isoform)	*H2afv*	Q71UI9-5	[65]
		H2A.Z.2.s5 (putative spliced isoform)	*H2afv*	Q71UI9-3	[65]
		H2A.Z.2.s6 (putative spliced isoform)	*H2afv*	C9J0D1	*
		H2A.Z.2.s7 (putative spliced isoform)	*H2afv*	C9J386	Short*
		H2A.Z.2.s8 (putative spliced isoform)	*H2afv*	E5RJU1	Short*
	macroH2A.1	macroH2A.1.s1	*H2afy*	O75367	[117]
		macroH2A.1.s2	*H2afy*	O75367-2	[66]
		macroH2A.1.s3 (putative spliced isoform)	*H2afy*	B4DJC3	*
		macroH2A.1.s4 (putative spliced isoform)	*H2afy*	D6RCF2	***
		macroH2A.1.s5 (putative spliced isoform)	*H2afy*	O75367-3	*
	macroH2A.2	macroH2A.2.s1	*H2afy2*	Q9P0M6	[96, 97]
		macroH2A.2.s2 (putative spliced isoform)	*H2afy2*	Q5SQT3	*
	TS H2A.1 (TH2A)	TS H2A.1 (TH2A)	*Hist1h2aa*	Q96QV6	[71]
	H2A.B.1	H2A.B.1	*H2afb1*	P0C5Y9	[118, 119]

Table 3 continued

Histone	Protein name	Entry name for MS analysis	Gene name	UniProtKB Accession	References
	H2A.B.2	H2A.B.2	*H2afb2*	P0C5Z0	*
			H2afb3		
	H2A.P	H2A.P	*Hypm*	O75409	*
H2B	Canonical H2B	Canonical H2B gene: Hist1h2bb	*Hist1h2bb*	P33778	[41]
		Canonical H2B genes: Hist1h2bc, Hist1h2be, Hist1h2bf, Hist1h2bg, Hist1h2bi	*Hist1h2bc*	P62807	[41]
			Hist1h2be	P62807	[41]
			Hist1h2bf	P62807	[41]
			Hist1h2bg	P62807	[41]
			Hist1h2bi	P62807	[41]
		Canonical H2B gene: Hist1h2bd	*Hist1h2bd*	P58876	[41]
		Canonical H2B gene: Hist1h2bh	*Hist1h2bh*	Q93079	[41]
		Canonical H2B gene: Hist1h2bj	*Hist1h2bj*	P06899	[41]
		Canonical H2B gene: Hist1h2bj (putative spliced isoform)	*Hist1h2bj*	U3KPT8	*
		Canonical H2B gene: Hist1h2bk	*Hist1h2bk*	O60814	[41]
		Canonical H2B gene: Hist1h2bl	*Hist1h2bl*	Q99880	[41]
		Canonical H2B gene: Hist1h2bm	*Hist1h2bm*	Q99879	[41]
		Canonical H2B gene: Hist1h2bn	*Hist1h2bn*	Q99877	[41]
		Canonical H2B gene: Hist1h2bn (putative spliced isoform)	*Hist1h2bn*	U3KQK0	[41]
		Canonical H2B gene: Hist1h2bo	*Hist1h2bo*	P23527	[41]
		Canonical H2B gene: Hist2h2be	*Hist2h2be*	Q16778	[41]
		Canonical H2B gene: Hist2h2bf (putative spliced isoform)	*Hist2h2bf*	Q5QNW6	*
		Canonical H2B gene: Hist2h2bf (putative spliced isoform)	*Hist2h2bf*	Q5QNW6-2	*
		Canonical H2B gene: Hist3h2bb	*Hist3h2bb*	Q8N257	[41]
	H2B.S (putative variant)	H2B.S (putative variant)	*H2bfs*	P57053	*
	H2B.M (putative variant)	H2B.M.s1 (putative variant)	*H2bfm*	P0C1H6	*
		H2B.M.s2 (putative variant, putative spliced isoform)	*H2bfm*	A9UJN3	Short*
	H2B.W	H2B.W	*H2bfwt*	Q7Z2G1	[72, 120, 121]
	TS H2B.1 (TH2B)	TS H2B.1 (TH2B)	*Hist1h2ba*	Q96A08	[41, 71]
H3	Canonical H3.1	Canonical H3.1 genes: Hist1h3a, Hist1h3b, Hist1h3c, Hist1h3d, Hist1h3e, Hist1h3f, Hist1h3g, Hist1h3h, Hist1h3i, Hist1h3j	*Hist1h3a*	P68431	[41]
			Hist1h3b	P68431	[41]
			Hist1h3c	P68431	[41]
			Hist1h3d	P68431	[41]
			Hist1h3e	P68431	[41]
			Hist1h3f	P68431	[41]
			Hist1h3g	P68431	[41]
			Hist1h3h	P68431	[41]
			Hist1h3i	P68431	[41]
			Hist1h3j	P68431	[41]
	Canonical H3.2	Canonical H3.2 genes: Hist2h3a, Hist2h3c, Hist2h3d	*Hist2h3a*	Q71DI3	[41]
			Hist2h3c	Q71DI3	[41]
			Hist2h3d	Q71DI3	[41]
		Canonical H3.2 (pseudogene)	*Hist2h3ps2*	Q5TEC6	*

Table 3 continued

Histone	Protein name	Entry name for MS analysis	Gene name	UniProtKB Accession	References
	H3.3	H3.3.s1	*H3f3a*	P84243	[122, 123]
			H3f3b	P84243	[122, 123]
		H3.3.s2 (putative spliced isoform)	*H3f3a*	B4DEB1	*
			H3f3b	B4DEB1	*
		H3.3.s3 (putative spliced isoform)	*H3f3b*	K7EK07	*
		H3.3.s4 (putative spliced isoform)	*H3f3b*	K7EMV3	*
		H3.3.s5 (putative spliced isoform)	*H3f3b*	K7EP01	*
		H3.3.s6 (putative spliced isoform)	*H3f3b*	K7ES00	*
	H3.Y.1	H3.Y.1	*H3.Y*	Translated from NG_012784.2	[44]
	H3.Y.2 (H3.X)	H3.Y.2 (H3.X)	*H3.X*	Translated from NG_023411.2	[44]
	H3.5	H3.5	*H3f3c*	Q6NXT2	[75]
	cenH3-CENPA	cenH3 - CENPA	*Cenpa*	P49450-1	[124]
		cenH3.s1 (putative spliced isoform)	*Cenpa*	P49450-2	*
		cenH3.s2 (putative spliced isoform)	*Cenpa*	F8WD88	Short**
	TS H3.4 (H3t)	TS H3.4 (H3t)	*Hist3h3*	Q16695	[41]
H4	H4	H4	*Hist1h4a*	P62805	[41]
			Hist1h4b	P62805	[41]
			Hist1h4c	P62805	[41]
			Hist1h4d	P62805	[41]
			Hist1h4e	P62805	[41]
			Hist1h4f	P62805	[41]
			Hist1h4h	P62805	[41]
			Hist1h4i	P62805	[41]
			Hist1h4j	P62805	[41]
			Hist1h4k	P62805	[41]
			Hist1h4l	P62805	[41]
			Hist2h4a	P62805	[41]
			Hist2h4b	P62805	[41]
			Hist4h4	P62805	[41]

Please refer to the Table 2 for legend. The corresponding FASTA file is presented as Additional file 2. Additional file 5 presents links to gene, transcripts and protein entries to Ensembl and UniProtKB databases

germ cells which replicate their DNA only once before meiosis.

Spliced and putative isoforms

More than 40 spliced isoforms for all mouse and human histones are present in the Ensembl database. However, this information, mainly based on transcriptional data, remains questionable; notably whether the corresponding proteins are expressed and incorporated into chromatin is uncertain. Some spliced isoforms correspond to very short isoforms that lack the globular domain and are probably, if expressed, non-functional (mouse: cenH3-CENPA.s2, cenH3-CENPA.s3, cenH3-CENPA.s4, cenH3-CENPA.s5, OO H1.8.s.4, OO H1.8.s.5, H2A.Z.1.s3, H2A.Z.1.s4, H2A.Z.2.s2; human: H2A.J.s3, H2A.Z.2.s7, H2A.Z.2.s8, canonical H2B.s2, cenH3-CENPA.s2). Even though their expression remains highly uncertain, they have been included in MS_HistoneDB for their identification by MS to be possible. Observing the presence of a shorter non-functional sequence at the expense of the full-length histone would indeed constitute interesting information. Following the same rationale, several putative isoforms or pseudogenes have been included in this resource (Tables 2, 3). Their detection by MS will constitute an indispensable step to confirm the expression of their protein form.

H1 histones

H1 histones (or linker histones) are different from core histones with respect to their structure, function and evolution. Therefore, it is not possible to single out one of its isoforms as canonical. H1 variants are known to

encompass isoforms named H1.0–H1.10. H1.1–H1.5 from histone gene cluster 1 and orphan genes H1.0 (H1°) and H1.10 are usually referred to as somatic variants [36]. The linker variant H1.0 has been described to be involved in cell differentiation (for review [2, 52]). H1.10 has been identified in human and plays an essential role for mitotic progression [53]. H1 variants also include the TS proteins TS H1.6 [54], TS H1.7 [55, 56], TS H1.9 [57, 58] and the oocyte-specific OO H1.8 variant [59, 60]. Finally, a new mouse entry, H1.11, was identified here while performing an *in silico* search using sequence alignments.

H2A variants

H2A variants comprise H2A.X, H2A.Z, macro-H2A and a number of TS variants, TS H2A, H2A.L/H2A.P and H2A.B.

Only one H2A.X protein has been described; this variant is involved in double-strand break repair, genome stability and chromatin remodelling and silencing in male meiosis [61–64]. H2A.Z is involved in transcription regulation and is encoded by two different genes, *H2afz* and *H2afv* [43]. In mouse, four putative spliced isoforms may be expected in addition to the two original sequences, while in human eight H2A.Z.2 isoforms have been suggested, of which two have been demonstrated to be stable at the protein level [65]. The specific functional roles of these isoforms are not well understood yet, but in some specific tissues, such as in the brain, some H2A.Z spliced isoforms could provide context-specific signalling information [65].

Macro-H2A is the largest histone variant with a long C-terminal domain [66]. This histone variant is associated with transcription repression, although recent evidence suggests that in some conditions it may also promote transcription (reviewed in [51]). Macro-H2A is known to be encoded by two or three different genes, for human and mouse, respectively, some of which are differentially spliced. These variable forms allow differential binding of NAD [67].

Finally, many H2A variants are specifically expressed in the testis. First, TS H2A.1 was originally identified in 1982 in the testis, where it plays an important role and was later detected in the ovary [41, 68–71].

Fourteen other mouse TS H2A variants have been grouped into three main classes, H2A.L, H2A.B and H2A.P (Fig. 3). This class also regroups human variants, with two H2A.B and one H2A.P proteins (Fig. 3). They are involved in transcription regulation and the final chromatin reorganization during post-meiotic differentiation of sperm cells [26, 38, 39]. The mouse variants have been described by different research groups [38, 39], and a denomination used here follows previous publications [36, 37]. When potential protein products of different genes have only minor sequence variations

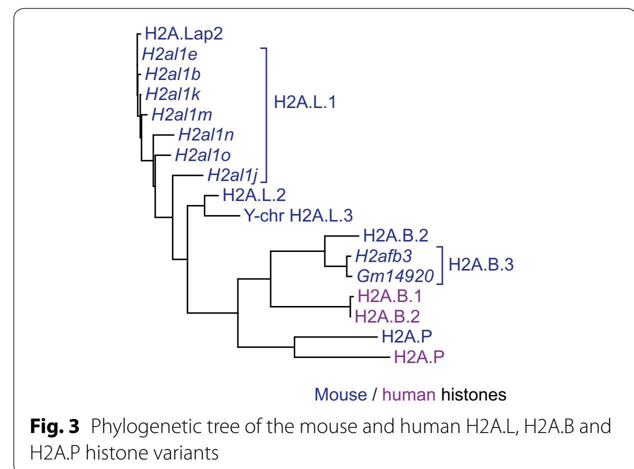

Fig. 3 Phylogenetic tree of the mouse and human H2A.L, H2A.B and H2A.P histone variants

and no functional difference has been characterized, we grouped them under the name with the same number suffix (e.g. H2A.L.1); however, the gene name is provided in the name of the entry as the second qualifier. Future studies might warrant splitting of such groups of proteins if functional differences between the members are detected. Currently, H2A.B.2 and H2A.B.3 are proposed to be numbered following their gene name, i.e. *H2AFB2* and *H2AFB3*, respectively. In 2007, new TS H2A variants were identified and named H2AL1 and H2AL2. [38]. A few years later, these histones were independently identified and named H2A.Lap2 and H2A.Lap3, respectively [39]. This latter work also reported the identification and functional characterization of a third member baptized H2A.Lap1 which falls into H2A.B group and is proposed to be regrouped with the highly similar protein H2A.B.3. H2A.L.3 was originally identified by S. Khochbin's group [38] and is the same as H2A.Lap4, also identified by D. Tremethick's group [39]. However, it forms a separate phylogenetic clade in placental mammals and is named H2A.P here according to [36].

H2B variants

Variants TS H2B.1, H2B.L and H2B.W were first identified as TS. In the testes, these proteins are involved in the chromatin-to-protamine transition [38, 69, 72, 73]. Then, TS H2B.1 and TS H2A.1 were also identified in human oocytes, where they favour the generation of induced pluripotent cells [70, 71]. In human, some genes (e.g. *H2BFM*, *H2BFS*) still await characterization and have been denoted as putative variants in this work (Table 3).

H3 variants

H3 has several isoforms: H3.1 and H3.2 are replication-dependent; H3.3 is considered to be a replication-independent histone variant, while TS H3.4 and H3.5 are TS

[74, 75]. Several new isoforms of H3.3 were included in the database developed here along with two other human H3 histone variants, H3.X and H3.Y [44].

CenH3/CENPA is a well-known centromeric H3 variant with many spliced isoforms. Its name has been the subject of heated discussion, which is out of the scope of our work [36, 76, 77]. We therefore propose to use both names, cenH3-CENPA, until a consensus has been reached by the community.

Generation of MS-based databases

De novo MS data interpretation methods are naive and do not rely on pre-existing databases. However, MS data acquired on histones are generally matched to a database containing all the protein sequences that could theoretically be found in the sample. Using this approach, a given histone protein cannot be identified if its sequence is not present in the database explored by the MS/MS data interpretation software. We used MS_HistoneDB to create a new search space dedicated to the analysis of histones. Basically, mouse or human non-redundant and well-annotated Swiss-Prot FASTA files were cleared of their histone sequences and then repopulated using MS_HistoneDB. This resource is included as Additional files 1 and 2, providing resources to study histones in mouse and human samples, respectively.

Identification of new histones in mouse

About 30% of the proteins in MS_HistoneDB have imprecise protein annotations in UniProtKB and are presented in Tables 4 and 5. These tables regroup histones that are annotated in the UniProtKB and NCBI databases as "inferred from homology" or "predicted". Even though a certain number of these histones have already been

Table 4 Mouse histone variants with poor annotation status in the UniProtKB database

Names	Accession number	Protein status	Method of detection			References
			This study (number of identified peptides)	Other MS-based studies	Not MS-based studies	
H1.0 (H1°)	P10922	Transcript	Yes (5)	Yes	RT-PCR; WB	[128]
TS H1.7 (H1T2, HANP1)	Q8CJI4	Transcript	Yes (6)		NB; WB	[55]
OO H1.8 (H1oo)	Q8VIK3	Transcript			WB	[59, 60, 129]
H1.11	F7DCP6	Inferred				
Macro-H2A.3	Q9D3V6	Transcript				
H2A.L.1 gene: *H2al1b*	A0A087WP11	Inferred	Yes (1)			
H2A.L.1 gene: *H2al1j*	A2BFR3	Inferred				
H2A.L.1 gene: *H2al1 k*	J3QP08	Inferred				
H2A.L.1 gene: *H2al1 m*	Q9DAD9	Transcript				
H2A.L.1 gene: *H2al1n*	Q497L1	Transcript	Yes (2)			
H2A.L.1 gene: *H2al1o*	L7MU04	Inferred				
H2A.L.2 (H2A.B1,H2A.Lap3)	Q9CQ70	Transcript	Yes (3)	Yes	RT-PCR; NB; WB	[38, 39, 125]
Y-chr H2A.L.3	A9Z055	Transcript			RT-PCR	[98]
H2A.P (H2A.L3,H2A.Lap4,)	Q9CR04	Inferred			RT-PCR	[38, 39]
H2A.B.2	S4R1M3	Inferred			RT-PCR; WB	[40, 99]
H2A.B.3 gene: *H2afb3*	S4R1G7	Inferred			RT-PCR; WB	[40, 99]
H2A.B.3 (H2A.Lap1) gene: *GM14920*	S4R1E0	Inferred			RT-PCR; WB	[39, 40, 99]
H2B.L.2	Q9DAB5	Transcript	Yes (4)	Yes	RT-PCR; WB	[38]
H3.3 gene: *GM6421*	EDL18362.1	Predicted	Yes (1)	Yes	RT-PCR	[103]
H3.3 gene: *GM10257*	XP_003084990.1	Record removed	Yes (1)		RT-PCR	[103]
CENPA-cenH3	O35216	Transcript		Yes	qPCR; WB	[126, 127]
H3.5	P02301	Inferred			RT-PCR	
TS H3.4 (H3t)	NP_001304932.1	Predicted		Yes	RT-PCR	[103]

The "protein status" was retrieved from UniProtKB: "Evidence at transcript level" (noted "Transcript") or "Inferred from homology" (noted "Inferred", update of July 2016). Three variants are predicted in NCBI database and are absent in UniProtKB. Information about the detection of some variants at the mRNA level (e.g. by RT-PCR) or at the protein level (e.g. by WB or MS) was further completed with publications and compared to the MS identification results obtained in the present study

NB northern blot, *WB* western blot, *MS* mass spectrometry

Table 5 Human histone variants with poor annotation status in the UniProtKB database

Name (other names)	Protein status	Accession number	Method of detection	References
TS H1.6 (H1t)	Transcript	P22492	WB	[41]
TS H1.7 (H1T2, HANP1)	Transcript	Q75WM6	NB; WB	[110]
OO H1.8 (H1oo)	Transcript	Q8IZA3-1	RT-PCR	[111, 112]
H2A.1.ps	Inferred	Q92646		
H2A.B.1 (H2A.Bbd)	Transcript	P0C5Y9	WB	[118, 119, 130]
H2A.B.2 (H2A.Bbd)	Transcript	P0C5Z0	WB	[118, 119, 130]

The "protein status" was retrieved from UniProtKB: "Evidence at transcript level" (noted "Transcript") or "Inferred from homology" (noted "Inferred", update of July 2016). Information about the detection of some variants at the mRNA level (e.g. by RT-PCR) or at the protein level (e.g. by WB or MS) was further completed with publications

NB northern blot, *WB* western blot, *MS* mass spectrometry

described in publications, which provide clear evidence of their existence at mRNA and protein levels, they may not have been identified by MS yet. This could explain their poor annotation status in UniProtKB.

At the RNA level, almost all histone variants have been detected in the testis [38–40, 55, 57, 68, 78–81]. Moreover, the expression profile of the mouse H2A.L.1 isoforms which are described in this study has been explored. RNA-seq data from nine mouse tissues have been obtained from a recently published dataset [82]. Expression data were available for 7 out of the 8 H2A.L.1 mouse histone entries and confirm that all of them are mainly expressed in the testis, similarly to H2A.L.2 and H2A.P (Fig. 4a) [38, 39]. Gene expression profiles during spermatogenesis have been obtained from Ref. [83]. It also confirms that H2A.L.1 isoforms are expressed in the post-meiotic stage in spermatids, similarly to H2A.L.2 and H2A.P (Figs. 3c, 4b) [38, 39].

We next decided to test whether MS_HistoneDB would allow the identification by MS of histone entries with imprecise protein annotation using mouse testis. Histones were purified from whole testis or from elongating spermatids (Fig. 4c). Mass spectrometry analysis combined with MS_HistoneDB allowed identification of nine of these poorly annotated proteins (Table 4; Additional file 3). Each newly MS-identified variant was detected by 1–10 specific peptide sequences. The current guidelines for the identification of previously undetected human proteins ("missing proteins") require the identification of two different peptide sequences of at least nine amino acids in length [84]. To stringently apply the same rules to validate new histone variants would be demanding, given the very high level of sequence homology between some variants. However, out of the nine histone variants detected here for the first time at the protein level by MS-based approaches, six were identified with at least two non-overlapping peptides of length ≥9 amino acids. Almost all the newly identified variants are TS. This analysis thus confirmed the existence of H2A.L.1 encoded by *H2al1b* in mouse testes (Fig. 4d). In addition, this analysis confirmed the existence of the histone variant H3.3 encoded by the gene *Gm10257*, for which a specific peptide has been identified, even if its corresponding NCBI protein record has been recently removed (XP_003084990.1).

Other variants may not be detectable by MS in our analysis. For example, a trypsin digestion does not generate any peptides distinguishing mouse *H2al1k* protein from highly homologous H2A.L.1 variants. Its specific detection would require a more extensive analytical work, e.g. using an alternative protease for protein sample processing, which is beyond the scope of the current work.

Conclusions

MS is a powerful technique to identify histones, their variants and their post-translational modifications but relies on databases with contradictory naming and excessive redundancy. Here we exhaustively collected histone sequences for mouse and human and used manual curation to establish a protein-centric list, MS_HistoneDB, dedicated to the proteomic study of mouse and human histones. Histone variants whose protein status is uncertain in UniProtKB and NCBI but whose protein existence has been established by experimental evidence described in the literature have been included. This work confirmed the expression of isoforms of previously identified TS histone variants and allowed the detection of one H3.3 isoform whose status was so uncertain that its record had been deleted from the NCBI protein database. We hope that this resource will facilitate the study of histone variants, especially by MS, and their functional roles in physiological and pathological contexts.

Methods

Phylogenetic tree representation

Multiple sequence alignments of mouse and human histones were performed using Clustal Omega [85]. Tree data were downloaded in aln format and displayed with iTOLv3 [86].

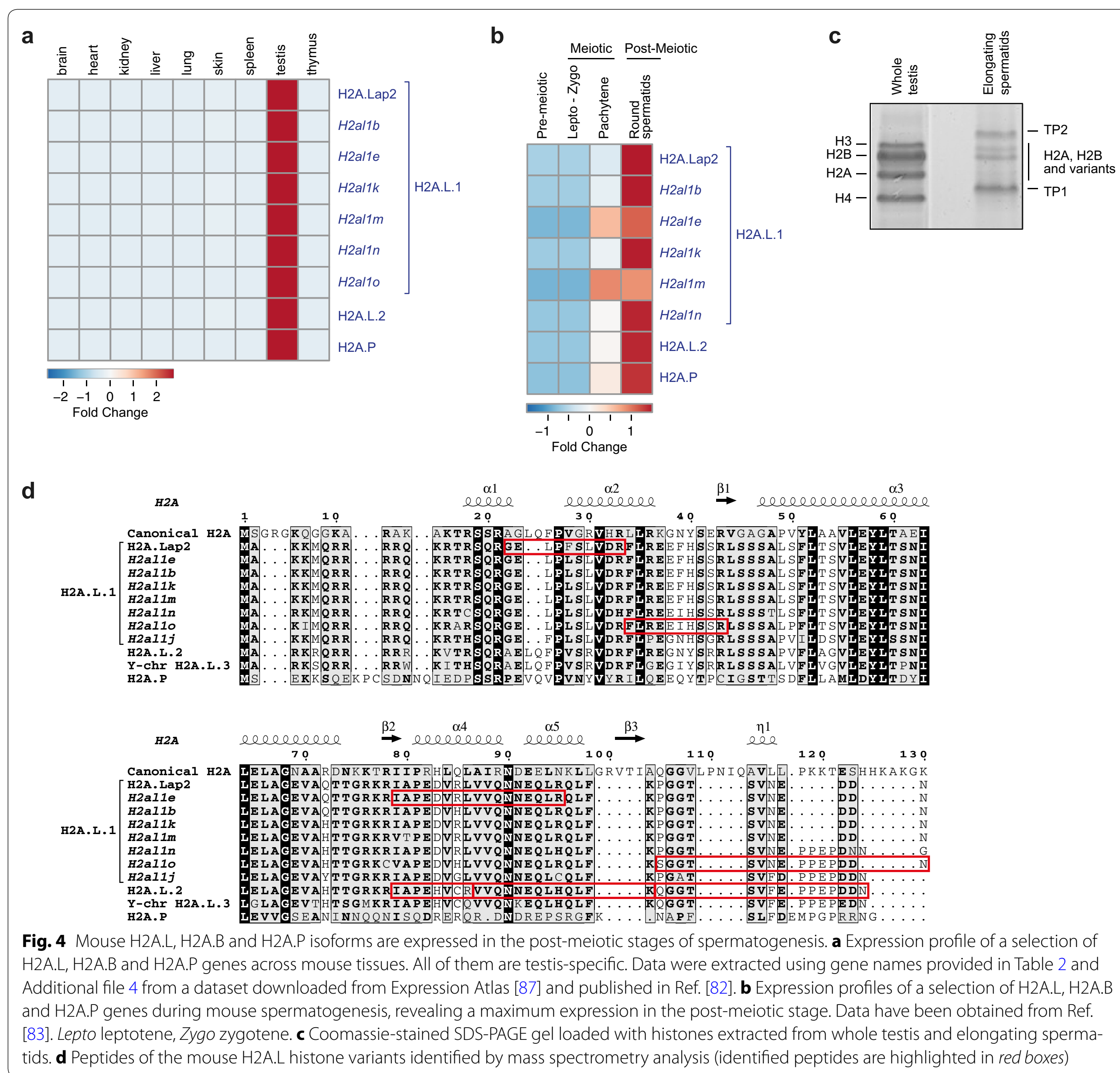

Fig. 4 Mouse H2A.L, H2A.B and H2A.P isoforms are expressed in the post-meiotic stages of spermatogenesis. **a** Expression profile of a selection of H2A.L, H2A.B and H2A.P genes across mouse tissues. All of them are testis-specific. Data were extracted using gene names provided in Table 2 and Additional file 4 from a dataset downloaded from Expression Atlas [87] and published in Ref. [82]. **b** Expression profiles of a selection of H2A.L, H2A.B and H2A.P genes during mouse spermatogenesis, revealing a maximum expression in the post-meiotic stage. Data have been obtained from Ref. [83]. *Lepto* leptotene, *Zygo* zygotene. **c** Coomassie-stained SDS-PAGE gel loaded with histones extracted from whole testis and elongating spermatids. **d** Peptides of the mouse H2A.L histone variants identified by mass spectrometry analysis (identified peptides are highlighted in *red boxes*)

Analysis of RNA-seq data

Tissue-specific expression data were obtained from Huntley et al. [82] through the Expression Atlas Repository [87]. RNA-seq data at different stages of spermatogenesis were obtained from da Cruz et al. [83]. Data were imported and treated in R using the pheatmap library (https://CRAN.R-project.org/package=pheatmap).

Purification of histones from mouse testis

Histones were extracted from two types of biological samples, namely whole testis and elongated spermatids, to maximize the number of histone variants identified at specific maturation stages of male germ cells. Pure fractions of spermatid nuclei were obtained by sonicating mouse testes, as previously described [38]. Histones were isolated from testis cells and spermatids using sulfuric acid [38] or saline extraction [88]. They were then separated by SDS-PAGE, and proteins were visualized by Coomassie staining.

Sample preparation and analysis by MS

Histones were reduced and alkylated as described previously [89]. Histones were either derivatized with propionic anhydride before and after in-gel trypsin digestion

[90], or only submitted to trypsin digestion [89]. The dried extracted peptides were resuspended in 2.5% acetonitrile and 0.05% trifluoroacetic acid and analysed via online nano-LC–MS/MS using an Ultimate 3000 LC system coupled to an LTQ-Orbitrap instrument (CID fragmentation mode) or a Q Exactive Plus instrument (HCD fragmentation mode) (Thermo Fisher Scientific).

Protein sequence database search and manual verification

MS RAW files produced by LC–MS/MS analysis of proteolyzed histones were processed as follows. All MS/MS spectra were submitted to the Mascot program (version 2.5.1) for searching against the MS_HistoneDB protein sequence database. The parse rules for MS_HistoneDB Fasta files in Mascot are using the accession rule >\([^]*\) and the description rule \(.*\). In addition, the taxonomy and sequence report sources are indicated as "Swiss-Prot FASTA" and "FASTA file", respectively. No taxonomy was specified when using MS_HistoneDB with Mascot Daemon.

Classical histone modifications were included in the variable modifications: N-terminal protein acetylation; Lys acetylation; and Lys and Arg mono- or di-methylation. For all Mascot searches, the tolerance on mass measurement was set to 5 ppm for peptides and to 0.6 or 0.025 Da for fragment ions when considering LTQ-Orbitrap or Q Exactive acquisitions, respectively. Up to four tryptic missed cleavages were allowed for samples that were not propionylated in vitro, as trypsin does not cleave acetylated lysine, a frequent modification. The enzyme ArgC and up to two missed cleavages were specified for the interpretation of data acquired on propionylated samples. All MS/MS spectra leading to the identification of tryptic peptides specific to newly described variants were carefully manually examined: all major intensity fragment peaks had to be interpreted in terms of y/b ions; a continuous sequence of at least five amino acids had to be read in all cases for validation. Proteomics data are available from ProteomeXchange (PXD005489).

Additional files

Additional file 1. FASTA file containing MS_HistoneDB mouse entries in a mouse Swiss-Prot FASTA file.

Additional file 2. FASTA file containing MS_HistoneDB human entries in a human Swiss-Prot FASTA file.

Additional file 3. MS/MS spectra assigned to peptides of H2A and H2B variants with an uncertain protein annotation state in UniProtKB or NCBI.

Additional file 4. MS_HistoneDB mouse entries with links to gene, transcript and protein identifiers.

Additional file 5. MS_HistoneDB human entries with links to gene, transcript and protein identifiers.

Abbreviations

CID: collision-induced dissociation; FASTA: text-based format for representing protein sequences; HCD: higher-energy collisional dissociation; MS: mass spectrometry; MS/MS: tandem mass spectrometry, used to fragment ions; LC–MS/MS: coupling between liquid chromatography and tandem mass spectrometry; NB: northern blot; PAGE: polyacrylamide gel electrophoresis; TS: testis-specific; WB: western blot.

Authors' contributions

SEK, JG and DP designed the project. SEK collected the data relative to histone entries, which was subsequently curated by SEK, AKS, SK, ARP, DL, DP and JG. Mass spectrometry experiments were performed by SEK and AA, and the MS acquired data analysed by SEK, AA, DP and CB. The first version of the manuscript was written by SEK, DP and JG and then critically revised by AKS, ARP, SK and DL. All authors read and approved the final manuscript.

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Competing interests

The authors declare that they have no competing interests.

Availability of data and materials

Proteomics data are available from ProteomeXchange under the identifier PXD005489 [131]. RNA-seq data were downloaded from the supplementary information of their original publications [83] or through the Expression Atlas/Array Express repositories [82, 87].

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