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Review

The human histone H3 complement anno 2011

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ABSTRACT

Histones are highly basic, relatively small proteins that complex with DNA to form higher order structures that underlie chromosome topology. Of the four core histones H2A, H2B, H3 and H4, it is H3 that is most heavily modified at the post-translational level. The human genome harbours 16 annotated *bona fide* histone H3 genes which code for four H3 protein variants. In 2010, two novel histone H3.3 protein variants were reported, carrying over twenty amino acid substitutions. Nevertheless, they appear to be incorporated into chromatin. Interestingly, these new H3 genes are located on human chromosome 5 in a repetitive region that harbours an additional five H3 pseudogenes, but no other core histone ORFs. In addition, a human-specific novel putative histone H3.3 variant located at 12p11.21 was reported in 2011. These developments raised the question as to how many more human histone H3 ORFs there may be. Using homology searches, we detected 41 histone H3 pseudogenes in the current human genome assembly. The large majority are derived from the H3.3 gene *H3F3A*, and three of those may code for yet more histone H3.3 protein variants. We also identified one extra intact H3.2-type variant ORF in the vicinity of the canonical *HIST2* gene cluster at chromosome 1p21.2. RNA polymerase II occupancy data revealed heterogeneity in H3 gene expression in human cell lines. None of the novel H3 genes were significantly occupied by RNA polymerase II in the data sets at hand, however. We discuss the implications of these recent developments.

1. Histone proteins are the building blocks of chromosomal chromatin

Eukaryotic chromosomes are found in the nucleus as chromatin. The fundamental unit of chromatin is the nucleosome, a protein octamer consisting of two copies of the four core histones H2A, H2B, H3 and H4 that wraps 147 bp of DNA in about 1.6 left-handed superhelical turns [1]. The linker histone H1 can bind nucleosomes, increasing the stretch of bound DNA to about 165 bp [2]. Nucleosomes can form on virtually any DNA sequence, though some are more or less favoured.

Histones are assembled onto chromosomal DNA by histone chaperones [3–6], often in conjunction with ATPases [7–11]. Once assembled, nucleosomes can be moved enzymatically to new locations along the DNA [12–15].

DNA wrapped into nucleosomes is occluded to other DNA binding protein factors [16]. Nucleosomal incorporation endows DNA with particular biophysical properties. For instance, polynucleosome arrays display the same high elasticity as random coil proteins, most likely because the nucleosomes link to one another via histone tails when magnesium is present [17]. Furthermore, and perhaps counter-intuitively, chromatin assembly reduces the apparent persistence length of DNA, both *in vivo* and *in vitro* [18,19].

Biological modulation of the biophysical properties of chromatin occurs via incorporation of histone protein variants as well as via post-translational modification of resident histones. Post-translational histone modifications change histone properties and thus epigenetically mark specific chromatin segments for specific steps in DNA metabolic processes, potentially providing a form of molecular memory that links specific DNA-based processes to specific DNA sequences for as long as the histone modifications persist [20,21].

Of the four core histones H2A, H2B, H3 and H4 it is histone H3 that is most heavily modified, totalling at least 26 potentially modified amino acids in a 135 amino acid protein [22]. It is intriguing to consider that in addition to the variety in possible histone H3 post-translational modifications there also are H3 protein sequence variants that may intrinsically differ in their affinities for histone binding/modifying factors and thus further modulate chromatin properties at loci where they are incorporated.

2. The current human histone H3 complement

2.1. A plethora of annotated human histone H3 proteins

Classically, there are two sources of cellular histone proteins in vertebrate cells. The first class is produced concomitantly with S-phase DNA synthesis [23,24] and the second is produced independently of

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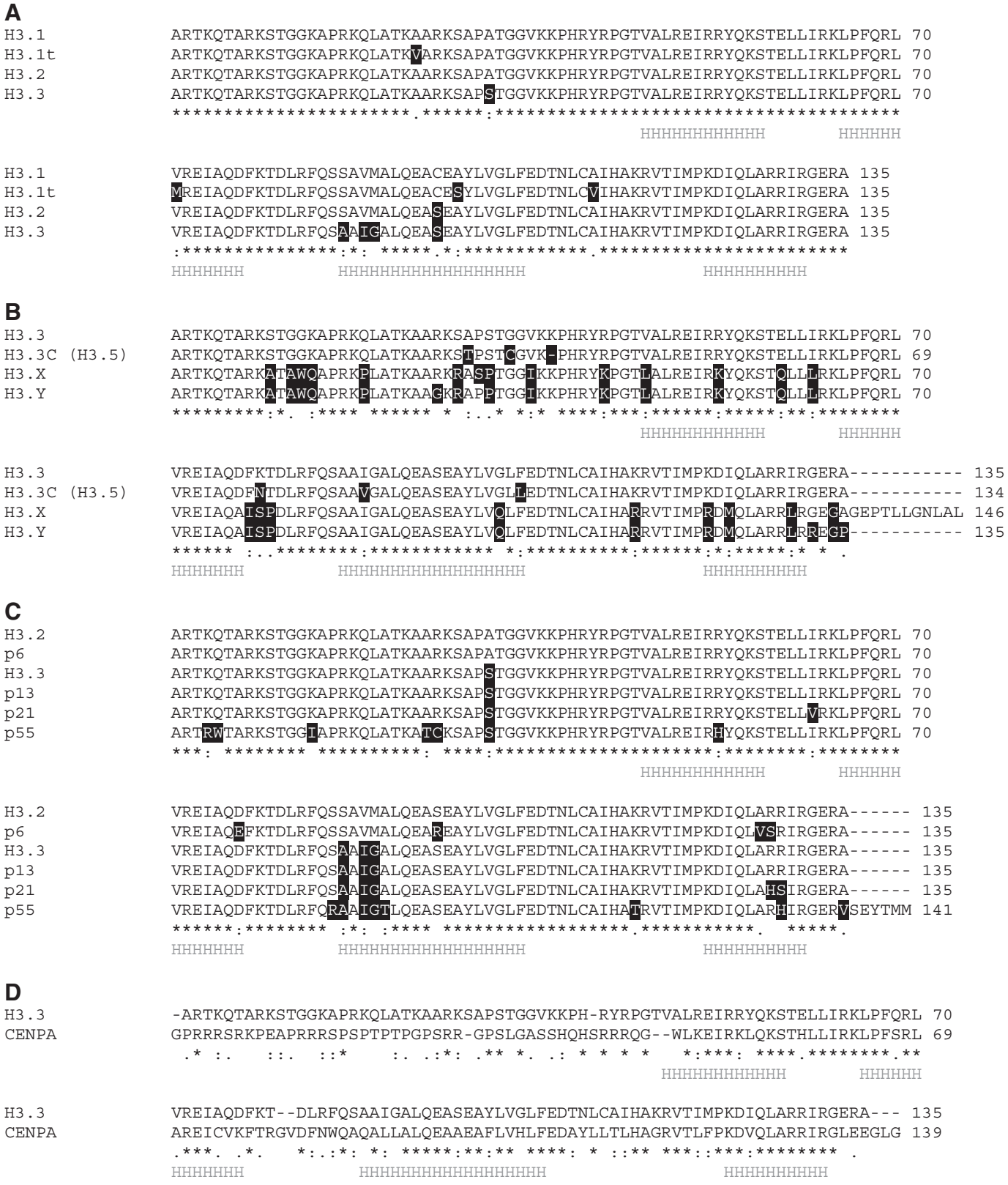

Fig. 1. Histone H3 protein alignments. Multiple alignments were performed on the amino acid sequences of histone H3 variants with ClustalW2 (http://www.ebi.ac.uk/) using standard protein settings. A. Alignment of the four classical histone H3 variants H3.1, H3.1t, H3.2 and H3.3. B. Alignment of the recently reported histone variants H3.3C (H3.5) [32] and H3.X and H3.Y [31] with the evolutionarily ancestral histone H3 variant H3.3. C. Four putative novel histone H3 protein variants derived from translating the *p6*, *p13*, *p21* and *p55* H3 pseudogenes are aligned to the classic histone H3 variants H3.2 and H3.3. D. Alignment of H3.3 and the centromeric CENPA H3 variant. A middle case (–) symbol in the sequence represents a deletion (H3.3C K36/37). A star symbol (*), double point (:), single point (·) or an open space below the sequence alignment represents respectively invariance, highly conservative, weakly conservative and non-conservative amino acid substitutions. The position of alpha-helical secondary protein structures of histone H3 proteins are indicated with 'H' under the alignments.

cellular DNA synthesis activity. Coordinating DNA and histone synthesis makes sense since during S-phase, a human cell must assemble ~$3 \cdot 10^7$ new nucleosomes in a span of 8 h, so as to match chromosomal DNA replication. The human genes coding for replication-coupled histone H3 are termed canonical histone H3 genes. They can code for the H3 protein variants H3.1 or H3.2 (Fig. 1A) which respectively harbour a cysteine or a serine at position 96 [25].

Post-mitotic cells and cells residing outside the S-phase may also need newly synthesised histones. Intriguingly, for histone H3 this involves production of the H3.3 variant. H3.3 was first reported in

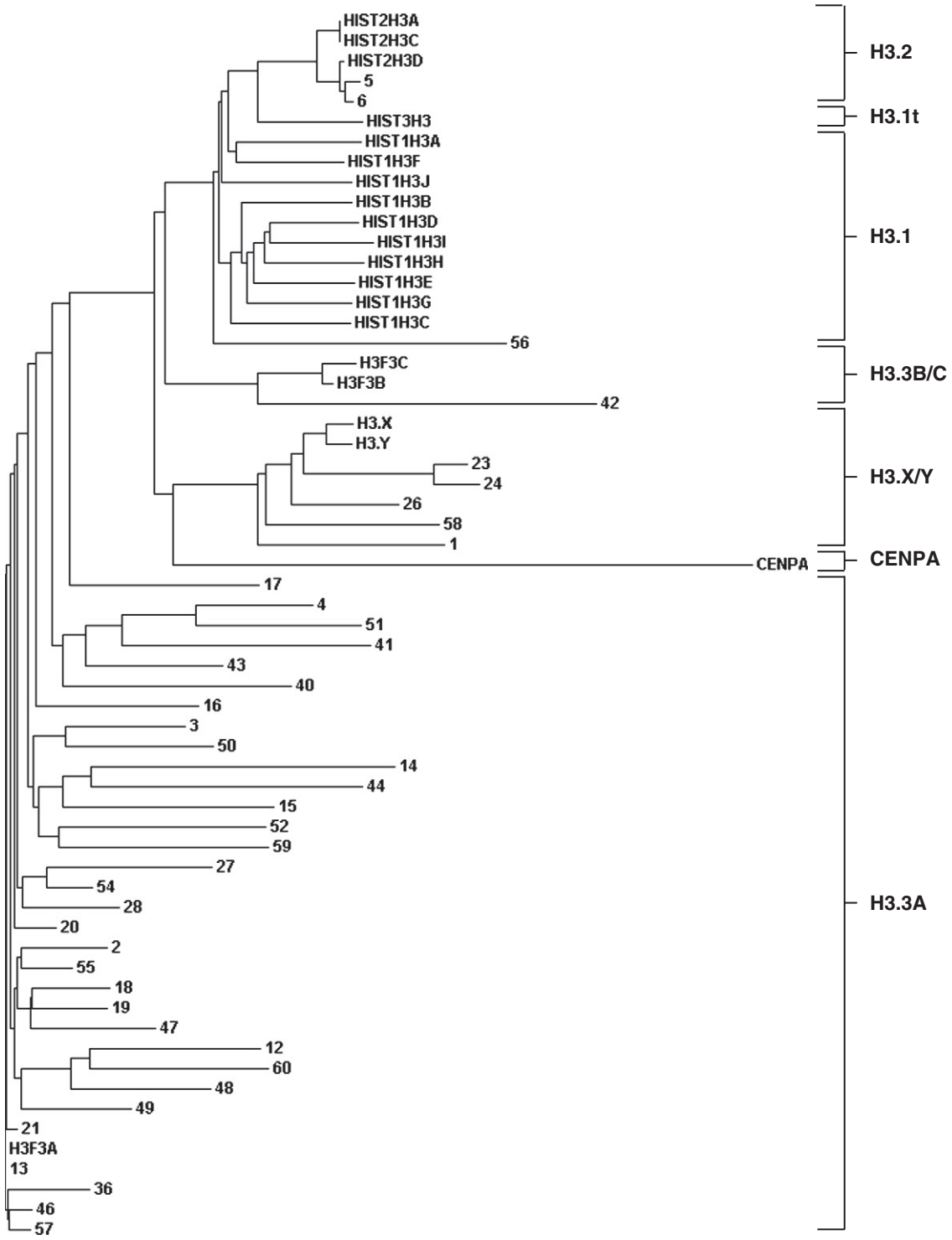

Fig. 2. Human histone H3 gene and pseudogene homology tree. DNA homology amongst the ORFs of the 19 currently annotated human H3 genes and of 41 H3 pseudogenes is displayed as a phylogram that is based on aligning the H3 ORF-derived sequences of each locus. The multiple alignment was performed with ClustalW2 (http://www.ebi.ac.uk/) using standard DNA settings except that the gap opening penalty was raised from 10 to 25. The DNA sequences cluster into five broad classes indicated on the right; H3.1, H3.2/H3.1t, H3.3B/C, H3.X/Y and H3.3A. Note that 32 pseudogenes fall into the H3.3A variant class. The pseudogenes *p5* and *p6* cluster with H3.2 genes and *p56* with the H3.1 genes (see also Fig. 4). Note also the cluster of five pseudogenes (*p1*, *p23*, *p24*, *p26* and *p58*) which show homology to the recently discovered H3.X/Y variants and which, like H3.X/Y, are located at 5p15.1 (see Fig. 3). Four pseudogenes, *p6*, *p13*, *p21* and *p55*, were found to contain a full length ORF (see Fig. 1.C), making them candidates for novel human histone H3 proteins.

1977 [25] and was defined 4 years later as a histone H3 protein that is expressed in a DNA-synthesis independent fashion [26]. Like H3.2, H3.3 has a serine at position 96. However, it further differs from the canonical histones at five positions, four of which are clustered in alpha helix 2 (Fig. 1A) and overlap a putative histone H3 chaperone specificity site [3,6,27].

A more recently discovered histone H3 protein variant is commonly called H3.1t, although it is also known as H3t and H3.4 [28]. H3.1t bears the H3.1 variant hallmark cysteine 96 but includes three extra amino acid substitutions (Fig. 1A) that reduce the stability of nucleosomes containing it [29]. This variant was found to be expressed in primary human spermatocytes at the pachytene stage, similar to the human testis-specific histone H1t variant [30], where it appears therefore to take part in the process of spermatogenesis.

In 2010, two related human histone H3 variants were reported, namely H3.X and H3.Y [31]. These proteins differ profoundly from H3.3 as they harbour at least 22 amino acid substitutions (Fig. 1B). Nevertheless, convincing fluorescence recovery after photobleaching (FRAP) experimental evidence demonstrated their capacity to be incorporated into chromosomal nucleosomes in cultured cell lines [31].

In 2011, a second proposed testis-specific transcript coding for a histone H3 variant called H3.3C was reported [32]. This human-specific H3.3 variant is encoded by a retrotransposed copy of the H3.3 coding gene *H3F3B* (see Fig. 2) that is called *H3F3C*. Relative to the basal H3.3 histone protein, H3.3C harbours five amino acid substitutions, including deletion of one of the two lysines at positions 36 and 37 (Fig. 1B). Conspicuously, transcripts could only be detected in testis tissue [32]. Although nuclear localisation of recombinant H3.3C protein product was demonstrated [32], no FRAP experiments [31] were performed. The biochemical evidence towards H3.3C as a *bona fide* human histone H3 is therefore less extensive as for H3.X. If it were to be functional, the H3.3C processed pseudogene product would be a human-specific ORFs since it is not conserved at the amino acid level in the chimpanzee, gorilla or orang-utan genomes [32].

Last but not least, CENPA is a very special histone H3 variant that is evolutionarily conserved in eukaryotes. CENPA has a very divergent N-terminus (Fig. 1D) [33] and it is incorporated at centromeres where it epigenetically marks these vital chromosome regions [34].

Altogether, anno 2011 there are eight human histone H3 protein variants reported in the literature; CENPA, H3.1, H3.2, H3.3, H3.1t, H3.X and H3.Y, and H3.3C (also known as H3.5). In Section 3.1 we identify four un-annotated human ORFs that code for an additional four putative human histone H3 protein variants shown in Fig. 1C.

2.2. Canonical human histone genes

Canonical human histones are found in clusters featuring local duplications and inversions that also harbour H2A, H2B, H4 and H1 histone genes (see Fig. 5B and C for examples). The ten H3.1 variant coding genes are all located in the histone cluster locus called *HIST1* on chromosome 6, while the three H3.2 coding genes are found on chromosome 1 in the histone cluster locus called *HIST2* (Table 1). Remarkably, the mouse genome displays a very similar arrangement of orthologous canonical histone genes [35].

One major peculiarity of the canonical histone genes is that they produce transcripts that have no introns and that lack a poly-A tail. The 3′ processing pathway of canonical histone mRNAs relies on the U7 snRNA [36,37] and takes place in Cajal body-related histone locus bodies (HLBs) that assemble on the histone clusters in normal cells [38–40]. The fact that canonical histone mRNAs have a unique 3′ processing mechanism probably reflects the imperative need to translate large quantities of histones in a controlled fashion and a defined time window so as to keep up with chromosome synthesis during S-phase.

Table 1
The human H3 protein complement. Overview of the 19 human histone H3 genes currently described in the literature plus four new H3 ORFs reported here (Fig. 1C). The canonical replication-dependent H3.1 and H3.2 coding genes are ordered according to their chromosomal position, which sorts them by subtype. Other subtypes are scattered around the genome.

Histone H3 gene name	Locus (HG-19)	Entrez gene ID	Histone H3 Prot. variant	Stemloop (+/−)	Length (AA)
HIST1H3A	6p22.1	8350	H3.1	+	135
HIST1H3B	6p22.1	8358	H3.1	+	135
HIST1H3C	6p22.1	8352	H3.1	+	135
HIST1H3D	6p22.1	8351	H3.1	+	135
HIST1H3E	6p22.1	8353	H3.1	+	135
HIST1H3F	6p22.1	8968	H3.1	+	135
HIST1H3G	6p22.1	8355	H3.1	+	135
HIST1H3H	6p22.1	8357	H3.1	+	135
HIST1H3I	6p22.1	8354	H3.1	+	135
HIST1H3J	6p22.1	8356	H3.1	+	135
HIST2H3D	1q21.2	653604	H3.2	+	135
HIST2H3A	1q21.2	333932	H3.2	+	135
HIST2H3C	1q21.2	126961	H3.2	+	135
H3F3A	1q42.12	3020	H3.3	−	135
HIST3H3	1q42.13	8290	H3.1t (H3.4)	+	135
H3F3B	17q25.1	3021	H3.3	−	135
H3F3C	12p11.21	440,093	H3.3C (H3.5)[a]	−	134
H3.X	5p15.1	340,096	H3.X[b]	−	146
H3.Y	5p15.1	391,769	H3.Y[b]	−	135
p06[c]	1q21.2	N/A	H3.2-like	+	135
p13[c]	2q31.1	N/A	H3.3-like	−	135
p21[c]	4q31.1	N/A	H3.3-like	−	135
p55[c]	Xp11.22	N/A	H3.3-like	−	141

[a] See [32].
[b] See [31].
[c] This work (see Figs. 1C, 2).

Notably, the fact that canonical histone gene mRNAs are usually processed in HLBs by the U7 snRNP, does not fully preclude production of polyadenylated messages from these loci [26,41–44].

At the DNA and RNA levels it is quite straightforward to identify canonical histone genes because they invariably harbour a 3′ UTR sequence that can form a stem loop (Fig. 4), which serves as a binding site for SLBP, the stem loop binding protein which also assembles the U7 snRNP onto the histone downstream element.

In summary, there are ten genes for the H3.1 variant, all of which are located in the *HIST1* canonical histone cluster on 6p22.1, while the three H3.2 genes are located in the *HIST2* canonical histone cluster at 1q21.2. The gene for the spermatocyte restricted H3.1t variant is located at 1q42.13, at the edge of the *HIST3* canonical histone cluster (Table 1).

2.3. The replication independent human H3 genes

There are two basal histone H3 genes that code for identical H3.3 histone proteins; *H3F3A* and *H3F3B*. *H3F3A* lies about 2 Mb proximal to the *HIST3* cluster, while *H3F3B* is located at 17q25.1 (Table 1). Both genes harbour introns and produce poly-adenylated mRNA, and both lack a 3′ stem loop structure. These two H3.3 genes are expressed in most tissues although relative levels differ [45]. For instance, *H3F3A* but not *H3F3B* is highly expressed in human germ cells [46].

2.4. The genes for the new variants H3.X, H3.Y and H3.3C

Wiedemann et al. [31] reported the existence of two highly similar histone H3 variant ORFs that strongly diverge from all other H3 histones. These were called H3.X and H3.Y. Endogenous H3.X expression was documented. The genes for H3.X and H3.Y are located at 5p15.1 in a region displaying extreme patterns of local duplications (Fig. 3). Neither H3.X nor H3.Y harbour introns characteristic for basal histones and neither the 3′ stem loop characteristic for canonical histones. At the DNA sequence level, the 5p15.1 encoded H3 genes

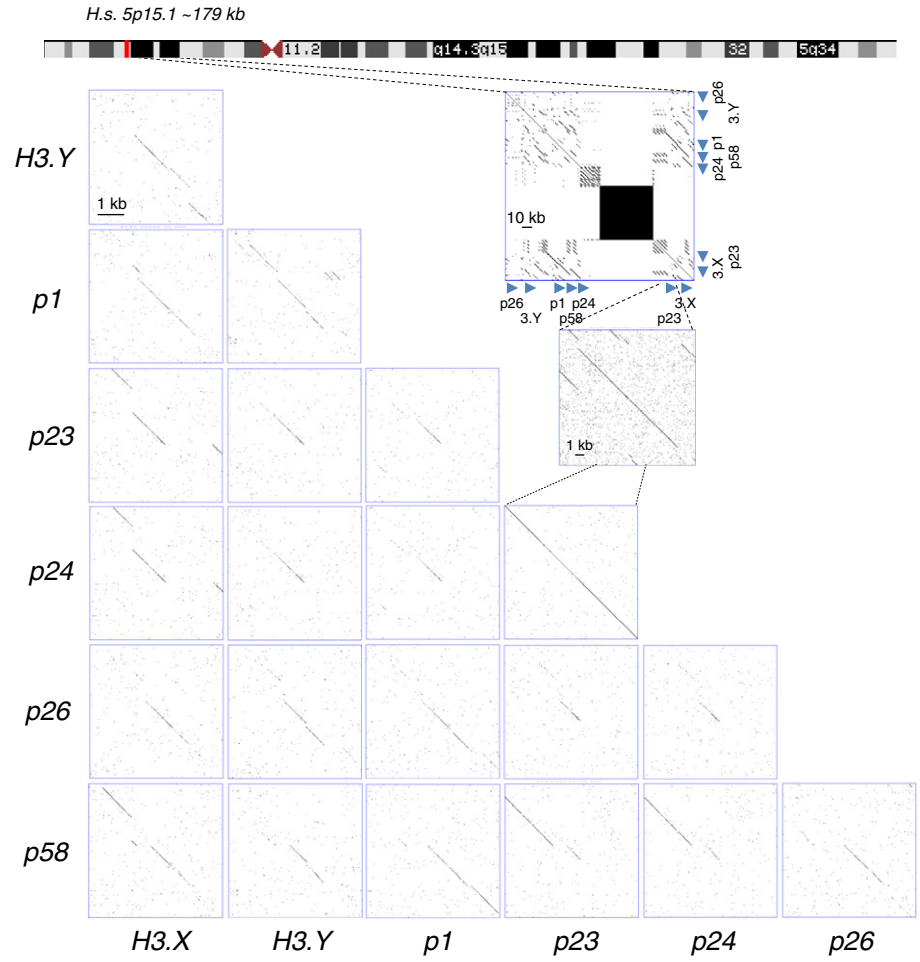

Fig. 3. The human 5p15.1 locus encompasses seven histone H3 pseudogenes over a locus length of 179 kb, including the H3.X and H3.Y coding variants [31] and is devoid of other core histone genes. Twenty-one pair-wise comparisons are shown for the seven 5p15.1 H3 sequences using nucleic acid dot plot matrices (http://www.vivo.colostate.edu/; window size 11, mismatch<2) for 5 kb regions centred on the start codons. Note the prevalence of fragmented duplications. The comparison of *p23* and *p24* generated a dot plot matrix which indicates identity over the entire 5 kb region. A dot plot (window size 11, mismatch<2) on a 14 kb fragment centred on their start codons shows that the *p23/p24* duplication involves ~11 kb. When zoomed out to the complete 5p15.1 pseudogene cluster of 179 kb (window size 31, mismatch<3), one can appreciate the large number of duplicated segments. Here, the 5p15.1 H3 pseudogenes are illustrated by blue triangles indicating ORF orientation. The big black-box in the dot plot matrix is a stretch of 50,000 uncalled nucleotides.

are closely related (Fig. 2). In fact, the 5p15.1 region harbours an additional 5 histone H3 sequences that are definitely pseudogenes since they carry frame shifts and/or nonsense mutations (Table 2). Phylogenetically, the H3.X and H3.Y variants appear to be Catarrhine-specific, an order containing old world monkeys, the apes and humans.

Like H3.X and H3.Y, the gene for the histone H3.3C variant [32] located at chromosome 12 lacks introns and a 3′ stem loop (Table 1). Sequence comparisons suggest that H3.3C represents a processed pseudogene of the intron-bearing H3.3 coding *H3F3B* gene. We prefer the H3.3C designation over H3.5 [32] for this human histone H3 ORF because the former name more clearly reflects its evolutionary origin (Figs. 1B, 2).

3. How many more human histone H3 ORFs are there?

The human genome has approximately 25,000 protein coding genes and some 20,000 pseudogenes [47]. In 1987, Wells et al. [48] reported the existence of 20 to 30 H3.3 processed pseudogenes using Southern blotting with *H3F3A* exon probes.

Considering the recent reports of novel human H3 histone variants, we wondered how many histone H3 ORFs there actually are in the human genome. Hence we queried the most recent build of the human genome for additional potential histone H3 protein coding loci. To this end we employed the on-line UCSC BLAT program (http://genome.ucsc.edu/) and the 135 amino acid sequence of histone H3 as query. This approach yielded 60 loci. CENPA was not recovered by this approach. However, all 19 currently annotated human histone H3 genes discussed in Sections 2.2, 2.3 and 2.4 were included. Of the 41 un-annotated loci, only four had an ORF that was not interrupted (Fig. 1C). None of the 37 remaining sequences could code for a full length histone H3 (Table 2). In order to deduce similitude amongst the 60 sequences we aligned all their H3 ORF-derived DNA sequences using ClustalW2 [49] and obtained a phylogram that approximates their relatedness (Fig. 2).

Three of the un-annotated H3 pseudogenes clustered with the canonical replication dependent H3 genes. The *p56* pseudogene is located on chromosome X and it is most related to the canonical H3.1-coding genes located in the *HIST1* histone cluster on chromosome 6 (Figs. 1C, 2, Table 2). On the other hand, the *p5* and *p6* are pseudogenes that are most closely related to H3.2 canonical *HIST2H3D* (Fig. 2) and they are both located on chromosome 1, about 29 Mb and 0.38 Mb proximal to the *HIST2* cluster, respectively. The *p5*, *p6* and *p56* pseudogenes display 3′ stem loop sequences, however only the stem loop of *p6* is still intact (Fig. 4). Curiously, *p6* is one of the four pseudogenes that has an uninterrupted ORF, displaying only four amino acid substitutions relative to canonical histone H3.2 (Fig. 1C).

Table 2
This table lists the 19 reported histone H3 genes and the complete set of 41 pseudogenes identified in this review. The histone H3 protein variant type is indicated where appropriate. The presence of a stem loop motif, an intact start codon and premature stop codons are indicated. Coordinates are listed for each gene (human genome build hg19, UCSC at http://genome.ucsc.edu/), as are coding strand orientation and intron presence. Putative pseudogene parents are indicated, as derived from DNA homology analysis (Fig. 2).

Histone H3 gene name	Histone H3 protein variant	Stemloop (+/−)	Start-codon (+/−)	Premature stop-codon (+/−)	Putative pseudogene parent	Chr.	Genome loci coordinates in human genome (HG-19)		Strand (+/−)	Introns (+/−)
p02		−	+	+	H3F3A	1	24275505	24275892	−	−
p03		−	+	+	H3F3A	1	53409206	53409603	−	−
p04		−	+	+	H3F3A	1	93214735	93214848	−	−
p05		−	+	+	HIST2H3D	1	120904675	120905090	+	−
p06[a]	H3.2	+	+	−	HIST2H3D	1	149400132	149400542	−	−
HIST2H3D	H3.2	+	+	−		1	149784826	149785236	−	−
HIST2H3A	H3.2	+	+	−		1	149812319	149812729	−	−
HIST2H3C	H3.2	+	+	−		1	149824217	149824627	+	−
H3F3A	H3.3	−	+	−		1	226252053	226259180	+	+
HIST3H3	H3.1t (H3.4)	+	+	−		1	228612616	228613026	−	−
p12		−	−	+	H3F3A	2	30432896	30433176	+	−
p13[a]	H3.3	−	+	−	H3F3A	2	175584636	175585046	+	−
p60		−	−	+	H3F3A	2	178209171	178209566	+	−
p14		−	−	+	H3F3A	2	203369717	203370064	−	−
p15		−	−	+	H3F3A	2	209330990	209331283	−	−
p16		−	−	+	H3F3A	3	25482624	25483027	−	−
p17		−	−	+	H3F3A	3	31310509	31310889	+	−
p18		−	+	+	H3F3A	3	109128525	109128931	−	−
p19		−	−	+	H3F3A	3	179367250	179367607	−	−
p20[b]		−	+	+	H3F3A	4	113486144	113486554	+	−
p21[a]	H3.3	−	+	−	H3F3A	4	140619298	140619708	−	−
H3.X	H3.X	−	+	−		5	17491877	17491434	−	−
p23		−	−	+	H3.X/Y	5	17502149	17502481	−	−
p24		−	−	+	H3.X/Y	5	17614992	17615221	−	−
p58		−	+	+	H3.X/Y	5	17620640	17621048	−	−
p01		−	+	−	H3.X/Y	5	17625659	17626068	−	−
H3.Y	H3.Y	−	+	−		5	17655647	17655237	−	−
p26		−	−	+	H3.X/Y	5	17670277	17670692	−	−
p27		−	−	+	H3F3A	5	87898586	87898966	+	−
p28[b]		−	+	+	H3F3A	5	115106283	115106690	+	−
HIST1H3A	H3.1	+	+	−		6	26020718	26021128	+	−
HIST1H3B	H3.1	+	+	−		6	26031878	26032288	−	−
HIST1H3C	H3.1	+	+	−		6	26045639	26046049	+	−
HIST1H3D	H3.1	+	+	−		6	26197068	26197478	−	−
HIST1H3E	H3.1	+	+	−		6	26225383	26225793	+	−
HIST1H3F	H3.1	+	+	−		6	26250423	26250833	−	−
HIST1H3G	H3.1	+	+	−		6	26271202	26271612	−	−
p36		−	−	+	H3F3A	6	26322104	26322520	−	−
HIST1H3H	H3.1	+	+	−		6	27777852	27778262	+	−
HIST1H3I	H3.1	+	+	−		6	27839683	27840093	−	−
HIST1H3J	H3.1	+	+	−		6	27858160	27858570	−	−
p59		−	−	+	H3F3A	6	76295836	76296240	+	−
p40		−	−	+	H3F3A	6	156983186	156983581	+	−
p41		−	−	+	H3F3A	9	1011085	1011437	+	−
p42		−	+	+	H3F3B	9	21638284	21638712	+	−
p43		−	−	+	H3F3A	9	26880171	26880547	−	−
p44		−	−	+	H3F3A	11	11878019	11878424	−	−
H3F3C	H3.3C (H3.5)	−	+	−		12	31944693	31945100	−	−
p46		−	−	+	H3F3A	13	72249196	72249606	+	−
p47		−	−	+	H3F3A	14	23764363	23764700	+	−
p48		−	−	+	H3F3A	15	40243569	40243925	−	−
p49		−	−	+	H3F3A	15	45507743	45508144	+	−
p50		−	−	+	H3F3A	15	94711576	94711989	+	−
p51		−	+	+	H3F3A	17	26743929	26744191	+	−
p52		−	−	+	H3F3A	17	58393193	58393579	+	−
H3F3B	H3.3	−	+	−		17	73774676	73775255	−	+
p54[b]		−	+	+	H3F3A	X	28677179	28677586	−	−
p55[a]	H3.3	−	+	−	H3F3A	X	50648438	50648866	+	−
p56		+	−	+	HIST1H3	X	101870400	101870744	−	−
p57		−	−	+	H3F3A	X	122648321	122648728	+	−

[a] Pseudogenes that were found to harbour full length ORFs (see Fig. 1C).
[b] Pseudogenes that were found to contain only one premature stop codon in their predicted ORFs.

Five pseudogenes, *p1*, *p23*, *p24*, *p26* and *p58*, mapped to chromosome 5, within a 179 kb chromosome segment that also harbours H3.X and H3.Y (Fig. 3, Table 2). The 5p15.1 locus is one of the unfinished regions of the human genome, harbouring an 'insert' of 50 kb of uncalled bases (UCSC hg19) and it displays a complex pattern of segmental duplications (Fig. 3). The *p23* and *p24* pseudogenes for instance, are clearly derived from a segmental duplication event as they lie within an exact 11 kb duplication while they share reciprocal interrupted sequence homology stretches with the other H3 pseudogenes at this locus (Fig. 3). It has been noted previously that 5p15.33 harbours conserved non-coding gene-poor segments [50] but this is more than 13 Mb removed from 5p15.1. Comparison with the great

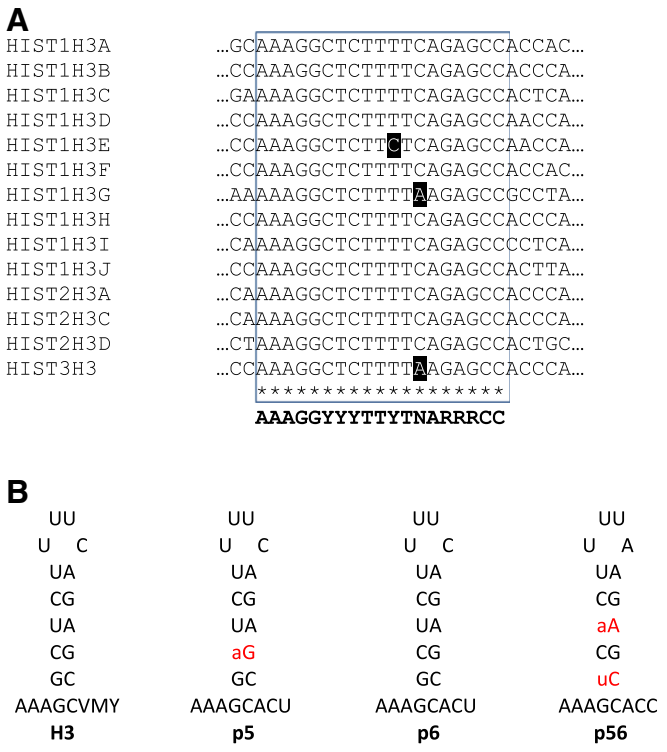

Fig. 4. Histone H3 stem loops. A conserved stem loop motif is present in the 3′ end UTR of the mRNAs of all the replication dependent, canonical H3 mRNAs. A. Multiple DNA alignment of 3′ ends of the 14 known canonical human histone H3 mRNAs produced from the *HIST1/2/3* histone clusters. The human H3 consensus region is delineated by a grey box. B. The H3 stem loop hairpin structure is illustrated. Furthermore, the hairpins of the only human H3 pseudogenes which were found to contain a stem loop motif (*p5*, *p6* and *p56*) are shown. The lower case red nucleotides mark mutations that compromise the hairpin structure. 'Y' refers to pyrimidines, 'R' to purines, 'M' represents 'A' or 'C', 'V' all but 'U', 'N' any base.

apes suggests that the 5p15.1 region is unstable since it does not align well across species (data not shown), although, at this point we cannot exclude that such inter-species differences merely reflects incomplete assembly of this chromosomal region in human and apes. Importantly, the 5p15.1 histone H3 pseudogene cluster does not harbour any sequences with homology to histone H1 or other core histone genes, indicating that 5p15.1 cannot be equated to the three canonical histone clusters discussed in Section 2.2 above, as could be expected from the absence of 3′ stem loops in the H3.X/Y H3 genes.

Only one of the 41 pseudogenes, *p42*, clusters with the H3.3 basal *H3F3B* gene in our phylogram and, like the processed pseudogene *H3F3C* on chromosome 12, *p42* on chromosome 9 was therefore derived via retrotransposition.

Strikingly, 32 pseudogenes show homology to the basal H3.3 histone *H3F3A* gene (Fig. 2). This is in accord with published genomic Southern blot data [48]. It therefore appears that 76% of the human histone H3 pseudogenes are derived from one histone H3 gene. This may relate to the fact that *H3F3A*, but not *H3F3B*, has been detected by microarray analysis in human germ cells [46]. The processed *H3F3A* pseudogenes *p13*, *p21* and *p55* have an ORF that could code for new histone H3 variants (Fig. 1C). Further experimental data will be needed to determine whether these and the *p6* pseudogene actually code for functional histone proteins.

4. RNA polymerase ChIP seq data

Our department has reported genome-wide RNA polymerase II chromatin immunoprecipitation (ChIP-seq) experiments on the NB4 promyelocytic leukaemia and MCF-7 mammary gland cancer cell lines [51,52]. We undertook it to mine these data sets to characterise the human H3 gene complement as a function of RNA polymerase II occupancy (Fig. 5). The results are similar to what was reported using a limited number of canonical human H3 genes, and indicate that not every replication-dependent H3 gene is transcribed in every immortalised cell line [53]. Furthermore, heterogeneity in RNA polymerase II occupancy patterns extended to other canonical core histone genes, as shown for the histone H4 gene *HIST1H4L* (Fig. 5, panel C, data not shown). The basal histone H3.3 replacement gene *H3F3B* tends to be much more occupied by RNA polymerase II than *H3F3A* in these cell lines (Fig. 5A).

Finally, the three H3.3 histone pseudogenes, *H3.X*, *H3.Y* and *H3F3C*, as well as the spermatocyte-specific H3.1t/H3.4 variant gene were not significantly occupied by RNA polymerase II in the RNA polymerase ChIP-seq data sets we analysed. The same was true for the H3 *p6*, *p13*, *p21* and *p55* pseudogenes which harbour full length histone H3 ORFs, suggesting that they do not contribute significantly to the histone H3 proteome of these cell lines.

5. Conclusion

Deep phylogenetic analyses indicate that the ancestral form of eukaryotic histone H3 is the variant known as H3.3 in vertebrates [27,54]. There are two human genes, *H3F3A* and *H3F3B*, that code for this variant. They harbour introns and produce polyadenylated transcripts [28] but differ in promoter structure [45]. H3.3 is produced throughout the cell cycle, accumulates at transcribed regions [55] and is a major histone type in post-mitotic cells [56,57]. Notably, histone H3.3 has a chaperone repertoire that is distinct from that of the replication dependent canonical H3.1 and H3.2 histones [3] and this is at least in part due to the primary amino acid sequence of the H3.3 variant [6].

The H3.1t, H3.1 and H3.2 variants are encoded by clustered, intronless genes that are recruited into Cajal-like histone locus bodies where canonical histone mRNA 3′ end processing takes place [37,40]. Transcriptional control of canonical histone H3 genes has been studied but much remain to be discovered yet [58–63]. As we performed our *in silico* search for additional H3 ORFs, we noticed that histone H3 pseudogenes tend to be marked as predicted CpG islands in the UCSC genome browser, reflecting codon usage in human histone H3 genes. Indeed, presumed *cis*-acting sites have been proposed in the ORFs of core histones, including H3 [59,60]. It may therefore be that some H3 pseudogenes affect gene expression around their integration sites.

Recently, three new human histone H3 protein variants were reported [31,32]. All appear to be processed H3.3 pseudogenes. Such novel H3 histone protein variants might play specialised molecular roles by virtue of their primary sequence divergence that could endow them with unique properties with regards to their repertoire of possible chaperones, post-translational modifications or interactions with other histones. Since the H3.X/Y variants are ape-specific and H3.3C is human-specific these novel H3 variants might underlie species-specific traits.

We were curious as to how many more histone H3 variants are potentially encoded by our genome. Using BLAT on the human genome web server at UCSC, we could identify 41 H3 pseudogenes. None harboured introns. Three were most related to canonical H3 genes and one of those, *p6*, has a full length ORF and an intact stem loop (Fig. 1C). In accord with a 1987 report [48], three quarters of the H3 pseudogenes we identified appear to be derived from the *H3F3A* H3.3 variant gene (Fig. 2). We presume that this strong bias reflects germ line expression of *H3F3A* but not *H3F3B*[46]. Three *H3F3A*-derived pseudogenes have intact ORFs (Fig. 1C) and could therefore warrant further investigation. However, we did not detect significant RNA polymerase II occupancy for any of the novel H3 variant genes, including *H3.X/Y* and *H3F3C* (Fig. 5, data not shown).

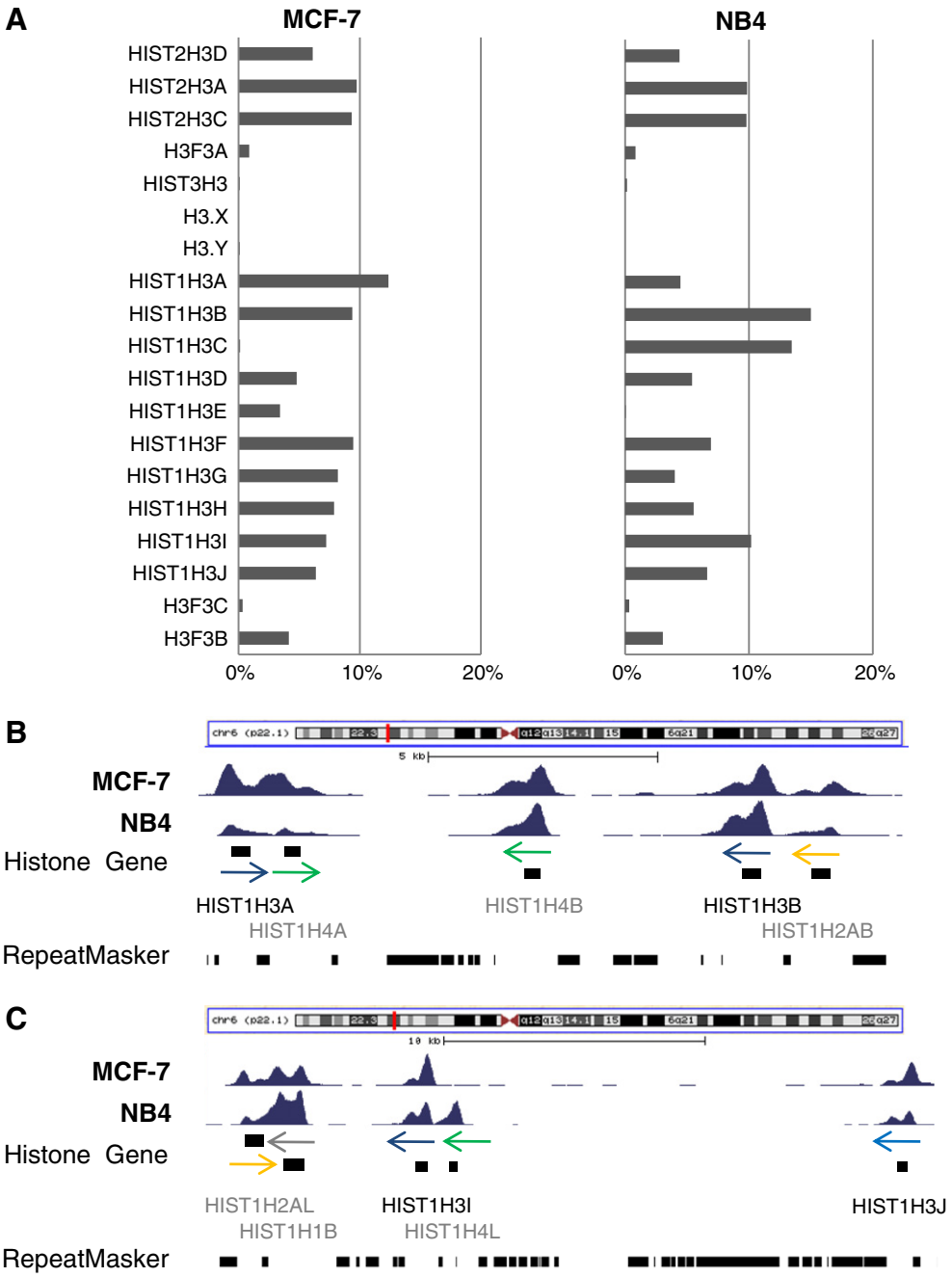

Fig. 5. RNA polymerase II profiling. Chromatin immunoprecipitation (ChIP) experiments were performed with the 8wg16 antibody (Abcam, Diagenode), targeting RNA polymerase II in NB4 [52] and MCF-7 cells [51]. A. ChIP-seq tags were counted for the 19 annotated histone H3 genes. The percent tags over the 19 histone H3 genes is displayed. B. Shown in this illustration is an optimised screenshot from the UCSC browser (http://genome.ucsc.edu/) for some of the data shown in A. The region covers a stretch of 16 kb at chromosome 6p22.1 containing five histone genes including the *HIST1H3A* and *HIST1H3B* H3 genes. C. Similar to B, but here a region of 28 kb is shown (6p22.2) with variable RNA polymerase II occupancy. Again, five histone genes are shown including the two *HIST1H3I* and *HIST1H3J* histone H3 genes. Arrows above or under the gene-bars indicate gene transcription direction. Arrow colours stand for; grey: H1, yellow: H2A, blue: H3 and green: H4. RepeatMasker software implemented in UCSC was used to show repetitive regions such as LINE, SINE, LTR, and DNA transposons.

The highly divergent human H3.X and H3.Y variants are encoded by a fascinating locus (5p15.1) that harbours seven H3 pseudogenes within ~179 kb. This region is devoid of other core histone ORFs, indicating that it is structurally different from the canonical *HIST* histone gene clusters. The relatedness of all the 5p15.1 H3 pseudogenes at the DNA level suggests that they were derived from one single H3.3 processed pseudogene via segmental duplications. Despite their high primary sequence divergence, epitope-tagged H3.X and H3.Y could be incorporated into nucleosomes and endogenous expression was documented; at the RNA level via reverse transcription-coupled PCR in some tumour samples and in the brain, as well as at the protein level in one cell line under starvation and overgrowth conditions [31]. Data indicating a physiological function for the 3.3C variant is limited to reverse transcription-coupled PCR assays on testis tissue and nuclear localisation of recombinant H3.3C in transient transfection experiments [32].

The available evidence supporting physiological roles for the recently reported ape- and human-specific histone H3 variants is therefore still rather scant at this point in time.

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