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Epigenetics and Codon Usage of the Histone Genes in 12 *Drosophila* Species

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Abstract

Histones are proteins that bind to DNA and form nucleosomes. There are several types of histones that differ in chromosome distribution and timing of their expression. In *Drosophila*, each canonical type histone is identical or highly similar in amino acid sequence to its corresponding replacement type histone; however, gene structure and codon usage differ between the two types of histones. Identification of the evolutionary changes responsible for the differences between these two histone types will lead to an understanding of the development of epigenetic regulation. Here, recent findings regarding codon usage for canonical and replacement types of histones are outlined for study of the evolution of these histone genes and their epigenetic regulation in *Drosophila*.

Keywords: Histone gene; Codon usage; *Drosophila;* GC content; Epigenetics

Introduction

The molecular mechanism of epigenetics caused by histone modification and replacement is one of the important problems to be solved in the fields of cell biology and molecular biology. Several types of histones are found in *Drosophila*; one type is expressed dependently with chromosome replication, another type is expressed independently, and other types are histones specific for the centromere region (CEN-P) or are histone-like proteins [1-11]. Most of the early histone studies investigated the replication-dependent type in sea urchin, *Xenopus* and *Drosophila*, and therefore this type is called a 'canonical' histone. The other histone type, which is expressed independently of replication, was called a 'variant' or a 'replacement' because of some amino acid substitutions compared to the corresponding canonical histone [12]. The codon usage for the two histone types is reviewed here; however, many interesting results have also been reported regarding CEN-P and histone-like proteins [3,5,11,13-17]. In addition to the normal 'functional' histone genes, broken genes or pseudogenes have also been found in a genome [18]. The histone genes for canonical histones in *Drosophila* have been reviewed elsewhere [19]. These genetic differences will be helpful for understanding the evolution of these histone genes and their epigenetic systems in *Drosophila*.

Modification and Replacement for Histones

Information other than that on the genetic level has been found to be related to a variety of biological phenomena such as gene expression and cell differentiation [20-28]. Histone modifications and replacements belong to such mechanisms [25,26,29-39]. A chromosome is formed from chromatin, which is modelled from histones and DNA [40,41]. Chromatin remodeling is triggered by subtle changes in nucleosome structure such as modification/unmodification of amino acids or replacement histones [29,32,42-50].

Histone modifications occur at Lys, Arg, Thr, and Ser sites by modifications such as methylation, acetylation, phosphorylation, and ubiquitylation. Effects of histone modifications on chromatin remodeling differ significantly depending on which amino acid is modified or unmodified, which amino acid position is modified, and what kind of modification or unmodification occurs [24,30,51-54]. A replacement of a histone with a different type of histone also causes chromatin remodeling [12,50,55-64].

In *Drosophila melanogaster* five histones, H1, H2A, H2B, H3, and H4, are known as canonical type histones [1,40] and four histones, H2AvD, H3.3A, H3.3B, and H4r are known as replacement type histones (Table 1) [65-68]. A replacement type for H2B has not yet been found in *Drosophila*; however, a pseudogene for H2B was found [18].

Canonical Type	Replacement Type
H1	-
H2A	H2AvD
H2B	-
H3	H3.3A, H3.3B
H4	H4r

Table 1: Genes for canonical type histones and their corresponding replacement type histone in *Drosophila*.

Gene Structure for the Canonical and Replacement Histone Types in *Drosophila*

Large differences in the structural genes of histones in *Drosophila* have been found between the canonical and replacement histone types [65-72]. The structures of the genes for the canonical histone types in *D. melanogaster*, a typical species of *Drosophila*, are shown in Figure 1 and those for the replacement types are shown in Figure 2. The basic

structure of histone genes seems to be the same among different *Drosophila* species [71-78].
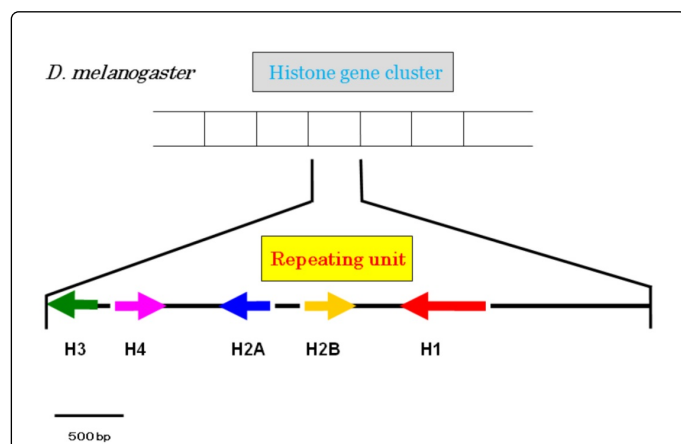

Figure 1: Structure of the genes for the canonical type histones in *Drosophila melanogaster*. A repeating unit encodes the five histone genes and is repeated about 110 times. Arrows show the direction of transcription.
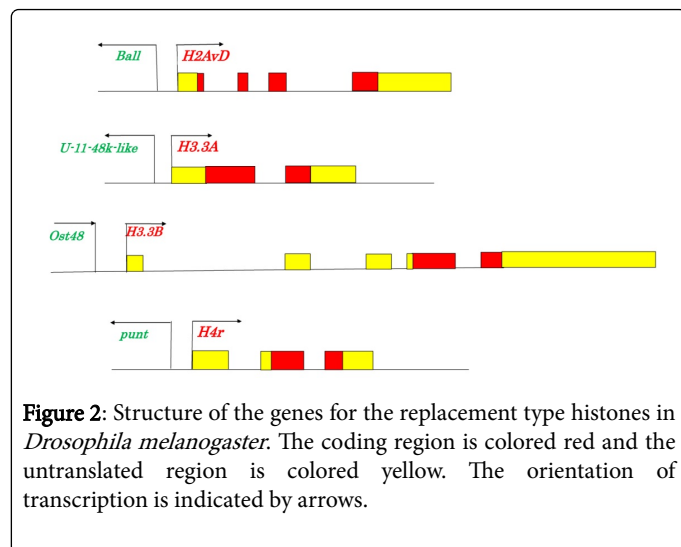

Figure 2: Structure of the genes for the replacement type histones in *Drosophila melanogaster*. The coding region is colored red and the untranslated region is colored yellow. The orientation of transcription is indicated by arrows.

Genes for the canonical histone type are clustered by tandem duplication, and a repetitive unit is 5 kb (L) or 4.8 kb (S) in *D. melanogaster*. The difference between the L and S units is due to the presence or absence of a tRNA-derived element [69]. The histone gene cluster is located at the D-E region of chromosome II and the unit is repeated more than 100 times [79,80]. An ordinary repeating unit codes for 5 histone genes, termed a 'quintet' (H1, H2A, H2B, H3, and H4), and an exceptional unit codes for 4 histone genes, termed a 'quartet' (H2A, H2B, H3, and H4). In *Drosophila virilis* and *Drosophila americana*, quintets and quartets coexist in the genome [27,75,81,82]. A similar quartet cluster was also found in *Mytilus edulis* [83]. The repeating units of the cluster in a species are highly similar in terms of DNA sequence and have evolved in a concerted fashion [70,74,77]. No intron has been found for any gene for the canonical histone type. A hair-pin loop structure is present in the downstream region of each histone gene and transcription stops at that point [69,84]. A poly(A) tail is not usually added in the transcripts of

canonical histones [85] although some exceptions have been reported [86]. The presence of a signal for polyadenylation has been indicated [69,86].

On the other hand, the genes for the replacement type histones are either single copy (H2AvD and H4r) or a few copies (H3.3A and H3.3B) [65-68], and have evolved independently of those for the canonical type [87-89]. These histone genes are split by 1-4 introns [65-68] (Figure 2) and a poly(A) tail is added to the transcripts [65,67].

The GC Content at the 3rd Codon Position of the Histone Genes in *Drosophila*

The GC content at the 3rd codon position of the genes for the canonical and replacement histone types was analysed and compared for 12 *Drosophila* species [87-89] for which the genome project has already been completed. Analyses for the H1 and H2B genes have not been presented before because of the absence of corresponding replacement histones. Results for these genes are shown together with those for the other histone genes in Figure 3. The GC content at the 3rd codon position is clearly affected by at least two factors; one factor is 'species' and the other factor is 'genes' [76,90,91]. In the species comparison, the GC content for any histone gene in *Drosophila willistoni* was considerably lower than that for other species (Figure 3). The same tendency for a drop in GC value in *D. willistoni* has been reported for other genes [92,93]. Table 2 shows the GC content at the 3rd codon position for each histone gene averaged over the 12 *Drosophila* species. For any comparison of canonical and replacement type histones, the GC content of the replacement type is always higher than that of the canonical type. Comparison of the average GC content of the two types shows that the average GC content is 8.9% higher in the replacement type (62.8%) than that in the canonical type (53.9%) (Figure 3 and Table 2). The highest GC content among the canonical types was observed for H2B (61.7%). The reason for this finding is not known. One possibility is the absence of a corresponding replacement type in spite of the fact that H2B is a core histone. The lowest GC content among the canonical types was observed for H1 (48.0%). This finding is probably related to the fact that the expression level of H1, which functions as a linker, is half that of the core histones. As described below, the GC content at the 3rd codon position is relevant to the usage of codons.

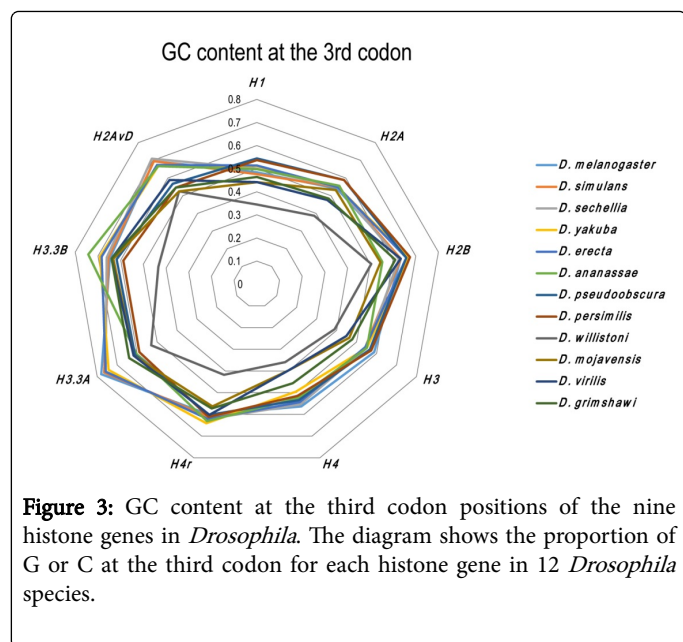| Histone gene	Canonical type	Replacement type	
H1	0.480	-	-
H2A	0.529	-	0.617
H2B	0.617	-	-
H3	0.521	A	0.667
		B	0.636
H4	0.491	-	0.591
Mean type	0.527	-	0.628
Mean type (Without H1)	0.539	-	-

Figure 3: GC content at the third codon positions of the nine histone genes in *Drosophila*. The diagram shows the proportion of G or C at the third codon for each histone gene in 12 *Drosophila* species.

Codon Usage of the Histone Genes in *Drosophila*

Codon usage for nine histone genes, five canonical (H1, H2A, H2B, H3, and H4) and four replacement (H2AvD, H3.3A, H3.3B, and H4r) genes is shown in Figure 4. A group of two synonymous codons is shown on the left-hand side. Groups of three, four, and six synonymous codons are shown on the right-hand side. For convenience, a 3rd codon with A or U is indicated at the lower end of each bar, and a 3rd codon with a G or C is indicated at the upper end of each bar. In this way, the GC content at the 3rd codon can be imaged by summing the G and C blocks at the upper end of the bars. Codon usage cannot be compared for Cys, because of no usage or a small number. When codon usage of the two histone types is compared, generally speaking G or C is used more frequently at the 3rd codon position for the replacement type than for the canonical type. In some exceptional cases, although the number of cases is small, the usage of a synonymous codon was almost the same for the two histone types (for example, in a comparison of codons for Glu between H2A and H2AvD, or of codons for Tyr between H3 and H3.3B) or showed an inverse tendency (in comparisons of codons for Asp or Ile between H3, H3.3A, and H3.3B). Therefore, the GC content difference of the 3rd codon between the two types seems to reflect a general tendency rather than reflect several unique usages for a specific amino acid.
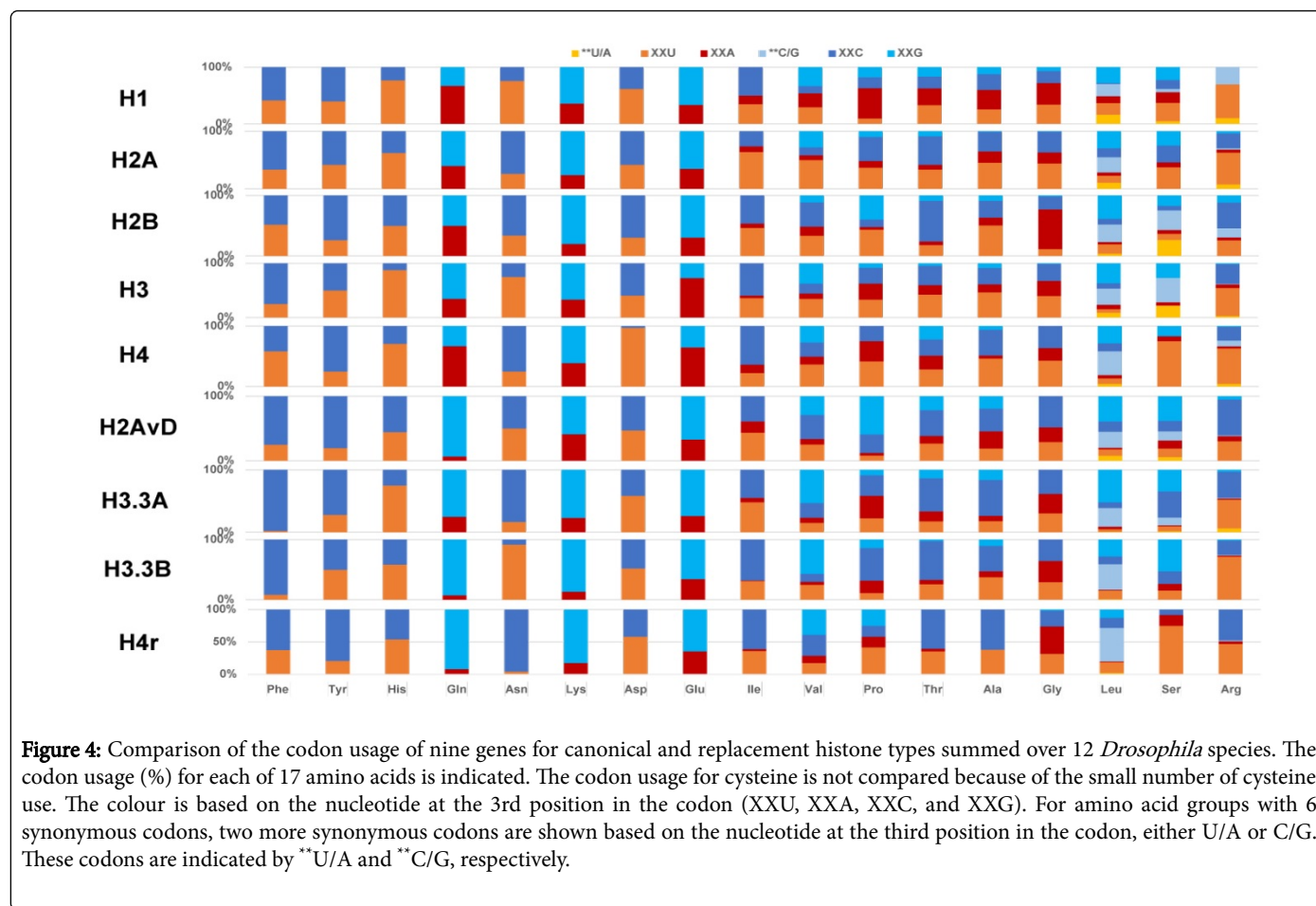

Figure 4: Comparison of the codon usage of nine genes for canonical and replacement histone types summed over 12 *Drosophila* species. The codon usage (%) for each of 17 amino acids is indicated. The codon usage for cysteine is not compared because of the small number of cysteine use. The colour is based on the nucleotide at the 3rd position in the codon (XXU, XXA, XXC, and XXG). For amino acid groups with 6 synonymous codons, two more synonymous codons are shown based on the nucleotide at the third position in the codon, either U/A or C/G. These codons are indicated by **U/A and **C/G, respectively.

Codon Usage at the Histone Modification Sites

The relationship between codon usage and histone modification (at Lys, Arg, Thr, or Ser) was analysed for four histones in *Drosophila*

[87-89]. The modification pattern of Lys was complicated because of many sites of modification; several kinds of modifications such as methylation, acetylation, phosphorylation, and ubiquitylation; and multiple modifications at a single site such as 1-3 methylations,

methylation and acetylation [20,45,46,51,52,94]. Although the relationship was not clear-cut, when the codon AAA was frequently used, this Lys site tended to be modified.

Thirteen arginine sites are known to be methylated in a total of four histones. The amino acid at position 73 of H2B in *Drosophila* is substituted with a different amino acid from arginine and therefore this site is not methylated. At three arginine positions in canonical type histones, position 76 in H2A, 128 in H3, and 92 in H4, the arginine codon AGA was used the most. All three of these positions were modified (Figure 5). Thus the codon AGA was used with a frequency of more than 50%, only for modified sites. There may thus be a relationship between AGA codon usage and arginine methylation.
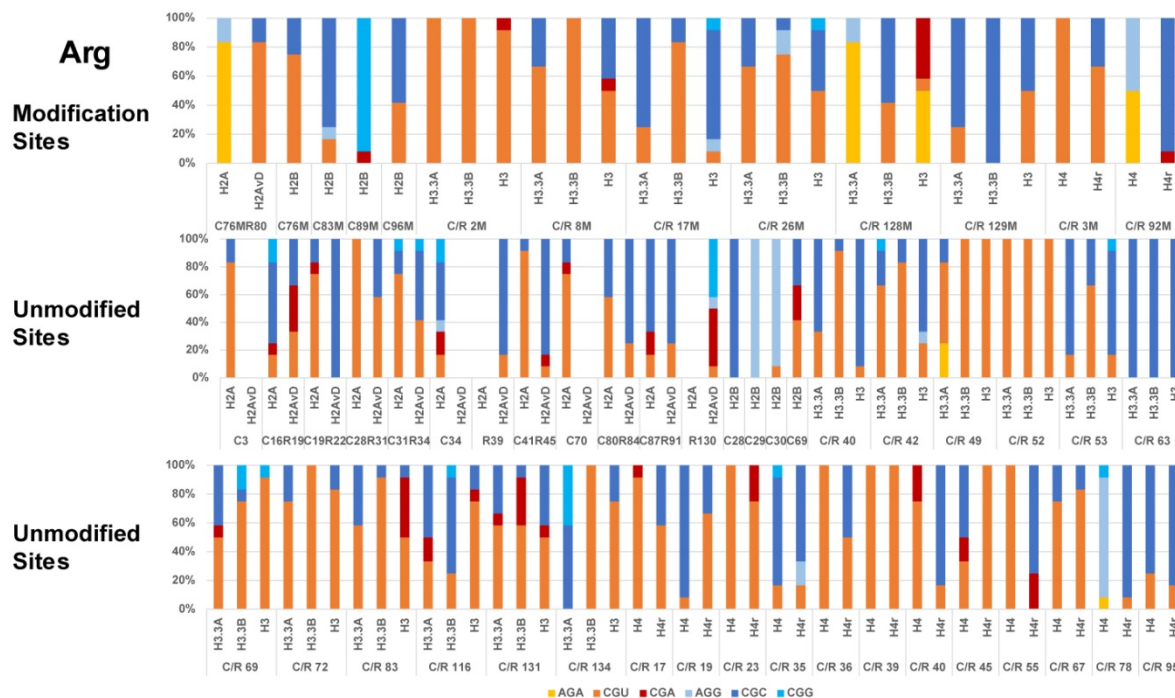

Figure 5: Codon usage for the modified or unmodified arginine sites in histones summed over 12 *Drosophila* species. The position numbers for arginines in the canonical (C) or replacement (R) histone types, and the codon usage for these arginines are indicated. The codon usages (%) are labeled with the same colours as in Figure 4. The protein length of H2A in *Drosophila* differs from that of human/mouse H2A by six amino acids. Indels in human/mouse and *Drosophila* may cause gaps in the amino acid numbers defined from the N-terminal end for each species; therefore, caution is needed regarding the amino acid position numbers of the modification sites. The "M" on the X axis refers to a site for methylation.

Five serine sites are phosphorylated in total (position 1 in H2A, 33 in H2B, 10 and 28 in H3, and 1 in H4). There is an amino acid substitution for the non-modified amino acid at position 14 of H2B. For the canonical type, the codons UCU and AGU are used more than the other codons for serine at 7 sites, and 3 of these sites (position 1 in H2A, 28 in H3, and 1 in H4) correspond to sites of serine modification (Figure 6). There is thus possibly a connection between the phosphorylation of serine and the usage of UCU and AGU codons.

Four threonine sites are phosphorylated in total (position 119 in H2A, and 3, 11, and 118 in H3). There was no obvious tendency for specific codon usage for threonine. In *Drosophila* H4, the amino acid at position 47 is substituted with a non-modified amino acid and therefore this site would not be phosphorylated.

In the future, it is anticipated that new histone modifications and more biological meanings for histone modification will be found. As for the timing of these modifications, it was recently reported that a certain modification (H3K9) occurred during translation, but not post translation [95]. The possible connection between modification and codon use such as between methylation of Arg and the use of AGA,

and between phosphorylation of Ser and UCU or AGU usage suggested that histone modification might be associated with a specific tRNA, leading to one of the modification mechanisms at a specific site in the protein. It is a possibility that some amino acids within histones are modified during translation.

Histone Genes and Epigenetics Evolution

Gene structure of the two histone types is very different. Large amounts of the canonical histones need to be produced within a short period during early development in *Drosophila*. This can be accomplished by multiple gene copies, tandem gene clusters, and no splicing. On the other hand, large amounts of the replacement histones are not required; however, they should be expressed at the proper time. Therefore, for the replacement histones, a single or a few gene copies should be sufficient or better than multiple gene copies.

Exon-intron structure is also remarkably different between the genes of the two histone types; no intron has been found for the canonical type, but 1-4 introns have been found for the replacement type. Therefore, control of histone expression by splicing is only

possible for the replacement type. Although the detailed mechanisms regarding the control of histone expression by splicing remains unknown, several conserved sequences at splicing sites have been found for the replacement type [87-89].
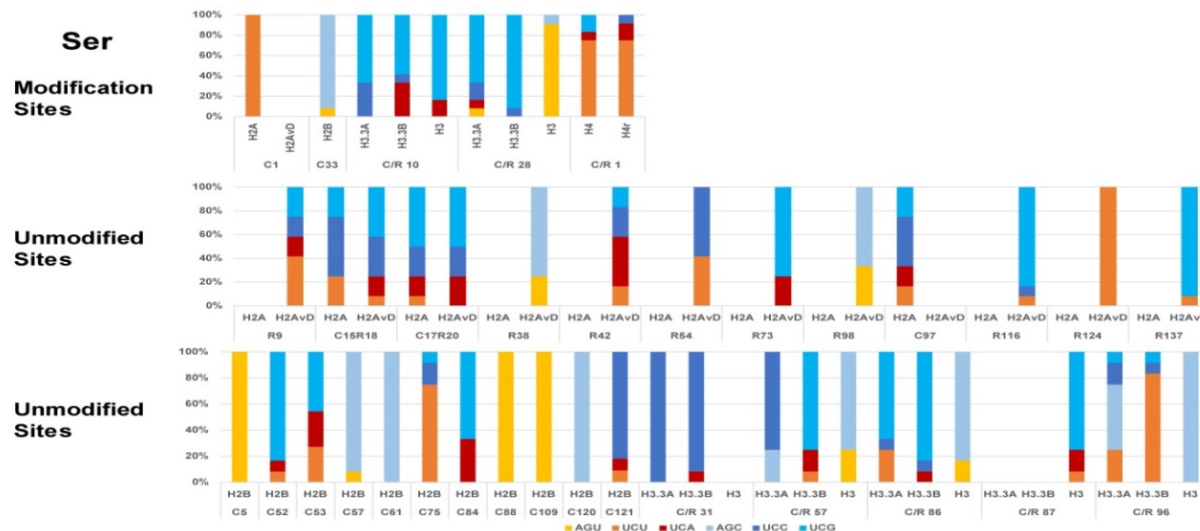

Figure 6: Codon usage for the modified or unmodified serine sites in histones summed over 12 *Drosophila* species. The position numbers for serines in the canonical (C) or replacement (R) histone types, and the codon usage for these serines are indicated. The codon usages (%) are labeled with the same colours as in Figure 4.

Transcriptional control plays an important role in controlling the expression of the canonical type [19,69]. However, a transcriptional control region was not found in the upstream region of the genes for the replacement type histones [87,88] except for the H2AvD gene [89]. Thus, for the replacement type histones, transcriptional control is only possible for the H2AvD gene since this is the only replacement type histone gene to have a conserved transcriptional control sequence upstream of the H2AvD gene [89].

Another difference between canonical and replacement type histones is their codon usage. The replacement type used G or C at the 3rd codon position more often than the canonical type. Codon use may affect the translation efficiency in conjunction with the composition of tRNA pools in the cell. Furthermore, the extraordinary biased codon usage at the sites of histone modification suggested a functional difference in codon usage.

Acknowledgements

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