Pakistan J. Zool., vol. 39(4), pp. 245-257, 2007.

Histone H1 Sub-types in Mouse: Interplay Between Phosphorylation and *O*-Glycosylation

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Abstract.- Linker histone H1 belongs to the family of proteins that are involved in organizing eukaryotic DNA into a compact structure. Many types of linker histone H1 are found in mammals; and the subtypes are cell specific and their amount in different types of cells varies according to the cell functions. Post-translational modifications occur on different amino acids in each subtype of linker histone H1 that induce conformational changes and consequently allow linker histone H1 to interact with chromatin at different stages during the cell cycle. This results in the regulation of transcription and gene expression. In this study it is proposed that *O*-glycosylation of linker histone H1 promotes condensation of chromatin, while, as it is known, phosphorylation of linker histone H1 activates transcription and gene regulation by decondensation of chromatin. Interplay between phosphorylation and *O*-β-GlcNAc modification on Ser and Thr residues in each subtype of linker histone H1 during the cell cycle may result in diverse functional regulation of proteins. This *in silico* study describes the potential phosphorylation, glycosylation and their possible interplay sites on conserved Ser/Thr residues in various subtypes of linker histone H1 in *Mus musculus*.

Key words: *Mus musculus*, phosphorylation, *O*-glycosylation, post translational modifications, histones.

INTRODUCTION

In eukaryotes, DNA is organized in a compact chromatin structure (Horn and Peterson, 2002), mediated by H1 through its binding with linker DNA (Ausio, 1999) and the DNA which is wrapped on core histones of two adjacent nucleosomes (Duce *et al*., 2006). The binding nature of linker histone H1 regulates chromatin compaction and relaxation during transcription and gene regulation. The super coiled chromatin has strong H1 binding, but the binding of H1 is weak in the case of linear or relaxed circular DNA/chromatin (Freidkin and Katcoff, 2001). Although histones are highly conserved proteins, multicellular organisms contain a variety of subtypes exhibiting significant sequence divergence and these subtypes present distinct patterns of expression during differentiation and development (Saeki *et al*., 2005). Among the histone classes, the H1 linker histones are the most divergent group. Usually nine subtypes of linker histone H1 are present in mammals, including H1.1, H1.2, H1.3, H1.4, H1.5, H10, H1F (H1oo), H1.t (Sarg *et al*., 2005) H1.x (Jedrusik *et al.,* 2002). Linker histone sub-types are classified according to their tightly regulated expression pattern during embronyal development and cell differentiation (Khochbin, 2001). All known sub-types of linker histone H1 contain a short *N*-terminal, a highly conserved central globular domain and a long *C*-terminal domain (Kasinsky *et al*., 2001).

Somatic cells contain almost all sub-types of linker histone H1 (Khochbin, 2001). *In vitro,* H1-containing chromatin shows strong inhibition of transcription (O'Neill and Turner, 1995), whereas transcriptionally active chromatin is depleted in H1 (Santisteban *et al*., 1997). Linker histone H1 binds strongly with super coiled DNA, whereas in the linear or relaxed circular DNA in nucleus H1 depletion results in a dramatic lengthening of chromosomes, which suggests their important role in mitotic chromosome condensation (Woodcock *et al*., 2006). It has been shown that many of the

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mapped modification sites that are considered to be involved in binding to nucleosomal DNA are located within the globular domain region of the different subtypes of the linker histone H1 (Wisniewski *et al*., 2007). The presence of these large numbers of various H1 histone subtypes and their possible post-translational modifications (Talasz *et al*., 1996), make it very clear that H1 histones play numerous structural and functional roles in chromatin. No specific role for various linker histones variants has been established but it is known that the mouse histone H1.2 binds preferentially to a regulatory sequence within a mouse H3.2 replication-dependent histone gene (Kaludov *et al*., 1997).

Post-translational modifications (PTMs) of linker histone H1 play important role in regulation of chromatin structure, transcription, gene activity (Wisniewski *et al*., 2007) and controlling the accessibility of transcription factors to chromatin structure (Parseghian and Luhrs, 2006). Transcriptional activation of genes starts with the dissociation of linker histone H1 from linker DNA (Ashrafi *et al*., 2005). Phosphorylation of linker histone is required for efficient cell cycle progression by the enzyme CDK2 (Contreras *et al*., 2003). This kinase requires a consensus sequence (S/T) PXZ or (S/T) PXK for phosphorylation, where X is any amino acid and Z is a basic amino acid, and this consensus sequence is found in many linker histone H1 variants which become phosphorylated (Paulson *et al*., 1996). It is observed that PKC is also involved in phosphorylation of linker histone variants during regulation of gene expression in the cell cycle (Zhao *et al*., 2004). Phosphorylation of linker histone regulates transcription and gene expression by reducing the electrostatic binding of linker histone to DNA in chromatin (Dou and Gorovsky, 2000). *In vivo* phosphorylation of the linker histone tails influences both the binding to mononucleosomes and the aggregation of polynucleosomes (Wolffe *et al*., 1993). The phosphorylation of linker histones at their *N*- and *C*-terminal tails during the cell cycle influence its functions for enhancing decondensation which in turn regulate transcription and gene expression (Chadee *et al*., 1995). This phosphorylation and dephosphorylation is a common regulatory mechanism for protein functions.

O-Glycosylation is also an important PTM of proteins. During *O*-glycosylation a molecule of *N*-acetylglucosamine (*O*-GlcNAc) is introduced on Ser or Thr residue by *O*-GlcNAc transferases (OGT). Addition of *O*-GlcNAc can inhibit phosphorylation on Ser or Thr residue (Wells *et al.*, 2004). Interplay between *O*-GlcNAc modification and phosphorylation on the same amino acid residues has been observed in several nuclear and cytoplasmic proteins (Kamemura *et al*., 2002). These PTMs are dynamic and result in temporary conformational changes and regulate many functions of the proteins. The interplay of these modifications on the same or neighboring residues may modulate the specific function of the proteins either by introducing or eliminating stereo-electronic effects. Residues where *O*-GlcNAc and phosphorylation compete for each other are known as Yin Yang sites (Zachara and Hart, 2002). These Yin Yang sites can be predicted and analyzed using various computer-assisted neural network-based programs, which can help to determine the regulatory functions of proteins by assessing their modification potentials. The present study describes potential phosphorylation, *O*-glycosylation and their possible interplay that influence condensation, decondensation as well as transcriptional and gene regulation during the cell cycle in various subtypes of linker histone H1.

MATERIALS AND METHODS

The sequences of different types of linker histone H1 of mammals have been described (Wisniewski *et al*., 2006). The sequence data used for predicting phosphorylation and glycosylation sites for different subtypes of linker histone H1 of *Mus musculus* was retrieved from the SWISS-PROT sequence databases (Boeckmann *et al*., 2003). The primary accession numbers for each subtype of linker histone in *Mus musculus* are P43275 (H1.1), P15864 (H1.2), P43277 (H1.3), P43274 (H1.4), P43276 (H1.5), P10922 (H1.0), Q07133 (H1.T), QSIVK3 (H1.F) and GI 38348566 (H1.X). BLAST search was made using the NCBI database of non-redundant sequences (Altschul *et al*., 1997). The search was made for all organisms' sequences with

expect value set to 10 using blosum 62 matrix and low complexity filter selecting nr database. Hits with highest bits score and zero expect value were selected to find out conserved residues in *Mus musculus* linker histone H1. All selected sequences were multiply aligned using CLUSTALW (Thompson *et al*., 1994).

Post-translational modifications prediction methods

Phosphorylation sites on Ser, Thr and Tyr residues were determined by using NetPhos 2.0 (http://www.cbs.dtu.dk/services/NetPhos/) server (Blom *et al*., 1999). NetPhos 2.0 is a neural network-based method for the prediction of potential phosphorylation sites.

NetPhosK 1.0 (http://cbs.dtu.dk/services/NetPhosK) server (Blom *et al*., 2004) was used to predict kinase specific phosphorylation sites in mouse histone H1 subtypes. The NetPhosK 1.0 predicts the kinase specific acceptor substrates sites in proteins.

Phospho.E.L.M database (http://phospho.elm.eu.org/) was used for verification of the experimentally known phosphorylation sites (Diella *et al*., 2004) present in various linker histone H1 subtypes in different species. The Phospho.ELM database contains a collection of experimentally verified serine, threonine and tyrosine sites in eukaryotic proteins.

The *O-β*-GlcNAc modification sites were predicted using Yin*O*Yang 1.2 (http://www.cbs.dtu.dk/services/YingOYang/). This method was also used to predict the potential phosphorylation sites and hence predicting the Yin Yang sites.

Neural networks-based prediction methods

Artificial neural networks based methods have been extensively used in biological sequence analysis and predicting the potentials for modifications (Baldi and Brunak, 2002). The methods developed using machine learning approach includes memorizing the neural networks with the sequence environment windows of phosphorylated/ glycosylated and non-phosphorylated/non-glycosylated sites. During this learning process the input data of sequence window containing phosphorylated/glycosylated and non-phosphorylated/non-glycosylated sites is presented to the neural networks in the form of binary codes of 21 digits for each amino acid. A threshold value in form of bits is set for positive hit and zero for negative hits. The learning process and performance is checked with the data reserved for cross validation using statistical equations. During learning, the error is computed and weights given to each neuron are set to get the maximum correct predictions. This reduces the error and hence decreases the false positive and false negative predictions.

RESULTS

Prediction of phosphorylation sites

Potential sites predicted by NetPhos 2.0 for phosphorylation of possible serine and threonine residues among all known subtypes of linker histone H1 has been shown in Figure 1. A detail of all positively predicted serine and threonine residues for phosphorylation are given in Table I.

Prediction of kinases involved in phosphorylation

Different kinases are found to be involved in phosphorylation of serine and threonine residues of linker histone H1 subtypes. Almost each kinase predicted to be potential for phosphorylation of H1 subtypes shows potential for more than two Ser/Thr residues. The predicted kinases involved in phosphorylation of different H1 subtypes by NetPhos K 1.0 are shown in Table II.

Prediction of O-linked glycosylation sites

Prediction results for *O*-linked glycosylation sites showed that all subtypes of linker histone H1 have very high potential for *O*-GlcNAc modification. There are many predicted Yin Yang sites in each subtype of linker histone which are shown in Figure 2 by an asterisk.

Identification of false-negative sites

Conserved serine and threonine residues showing a potential very close to threshold value for *O*-GlcNAc modification and with a very high potential for phosphorylation were identified as false-negative sites (FN-sites) as proposed earlier (Ahmad *et al*., 2006). All the serine and threonine residues which were predicted false-negatively with

high conservation status and phosphorylation potential among different subtypes of linker histone H1 are given in Table III.

Table I.- Phosphorylation and *O*-GlcNAc site map of *Mus Musculus.*

Substrate		Phosphorylation site	Yin yang sites	Conserved	Conserved sub
H1.1	Ser	40, 50, 51, 52, 56, 105, 114, 122, 158, 162, 164, 179	50, 51, 52, 122, 158, 162	41, 43, 51, 53, 60, 106, 182	1, 48, 52, 104,
	Thr	14, 23, 118, 119, 148, 161	14, 23, 161	94	11, 101, 137, 145, 151, 164, 190, 203
H1.2	Ser	50, 54, 103, 112, 172, 186	50	1, 40, 58, 77, 102, 104, 172, 188	35, 85, 88, 112
	Thr	145, 153, 166	166	44, 91, 95, 98	3, 153
H1.3	Ser	36, 51, 55, 104, 113, 171, 173, 188, 200, 205	36, 51, 171, 188	36, 41, 51, 58, 79, 89, 102, 104, 188	1, 86
	Thr	17, 92, 146, 150, 154		3, 45, 92, 96, 99	154
H1.4	Ser	35, 50, 54, 103, 112, 149, 171, 186, 203	35, 50, 186, 203	1, 35, 40, 50, 54, 57, 78, 85, 88, 101, 103, 112, 150, 171, 186	172, 188
	Thr	17, 34, 91, 145, 153, 192, 202	17, 34, 145, 192, 202	3, 17, 91, 95, 98, 145	141, 154, 202
H1.5	Ser	17, 40, 48, 50, 103, 112, 169, 185, 219	17, 40, 219	1, 43, 60, 80, 104, 106, 115	17, 53, 88, 91, 172
	Thr	23, 24, 35, 91, 134, 147, 151, 206	23, 24, 35, 206	38	3, 8, 47, 98, 101, 154
H1.0	Ser	6, 18, 21, 44, 48, 65, 70, 103, 130, 134	6, 21, 44, 130, 134, 190	4, 6, 21, 28, 44, 45, 55, 65, 70, 89, 91, 103, 130, 170, 184, 185	18, 115
	Thr	109,118,140, 152,177,	177	1, 5, 22, 76, 77, 83, 109, 134, 140, 152	
H1.T	Ser	20, 21, 40, 50, 52, 105, 109, 114, 124, 126, 138, 140, 143, 144, 160, 162, 177, 188	20, 21, 50, 52, 138, 140, 143, 160, 177, 188	1,40, 42, 50, 52,59, 79, 103,105,138,140,162, 177	8, 37, 124, 126, 135, 143, 184, 185,
	Thr	155, 166		3, 21, 97, 100, 146, 155	10, 48, 131, 145,
H1.F	Ser	8, 10, 11, 12, 13, 16, 20, 22, 36, 110, 147, 219, 221, 236, 246	8, 10, 11, 12, 16, 20, 110	5, 8, 12, 13, 20, 67, 110, 118, 221, 236,	7, 122, 219, 231, 241, 249
	Thr	19, 66, 84, 127, 270, 274, 298	19, 66, 127	66, 81, 97, 116, 231	19, 209
H1.X	Ser	13, 91, 112, 130, 133, 134, 160, 168	13, 130, 133, 134, 160, 168	48, 64, 65, 91, 111	13, 27, 133
	Thr	17, 21, 54	21	100	12, 31, 54, 86

Proposed Yin Yang sites within different subtypes of linker histone H1

The proposed Yin Yang sites for the interplay of phosphorylation and *O*-GlcNAc modification are given in Table III. These Yin Yang sites are proposed on the basis of conservation status and modification potential of Ser/Thr residues in each subtype of linker histone H1.

DISCUSSION

Human linker histones have eight sub-types, all consisting of a highly conserved globular domain

and less conserved *N*- and *C*-terminal tails. It is interesting to note that the sequences of the terminal tails of different subtypes of linker histone H1 within a species are much less conserved (Fig. 3),

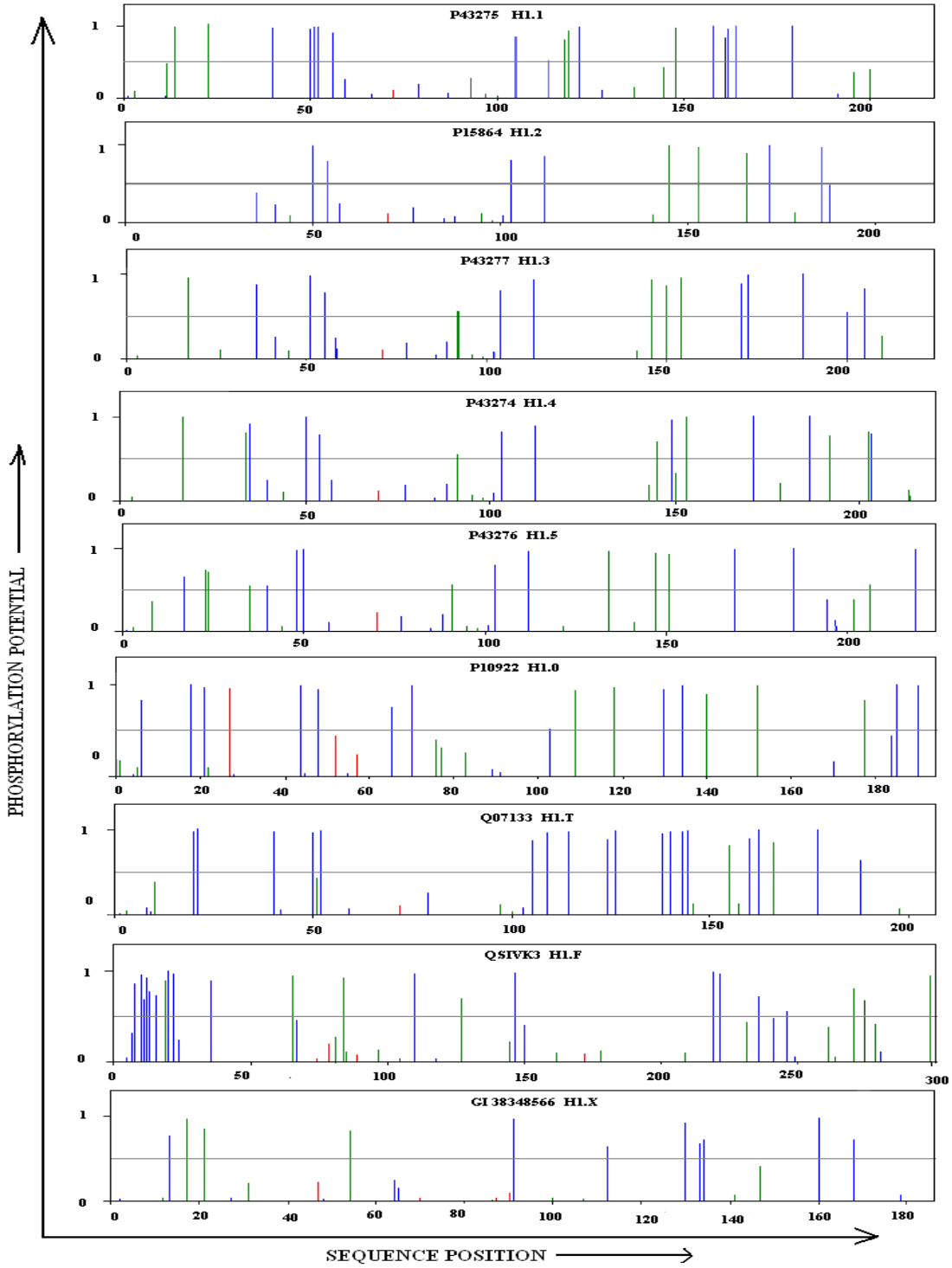

Fig. 1. Graphical representation of potential for phosphate modification at serine, threonine and tyrosine residues

in different subtypes of linker histone H1 in *Mus musculus*. Here blue vertical lines show the phosphorylation potential of Ser, green vertical lines show the phosphorylation potential of Thr residues, red lines show phosphorylation potential of Tyr residues, and gray horizontal lines show threshold for modification potential in each subtype of linker histone H1.

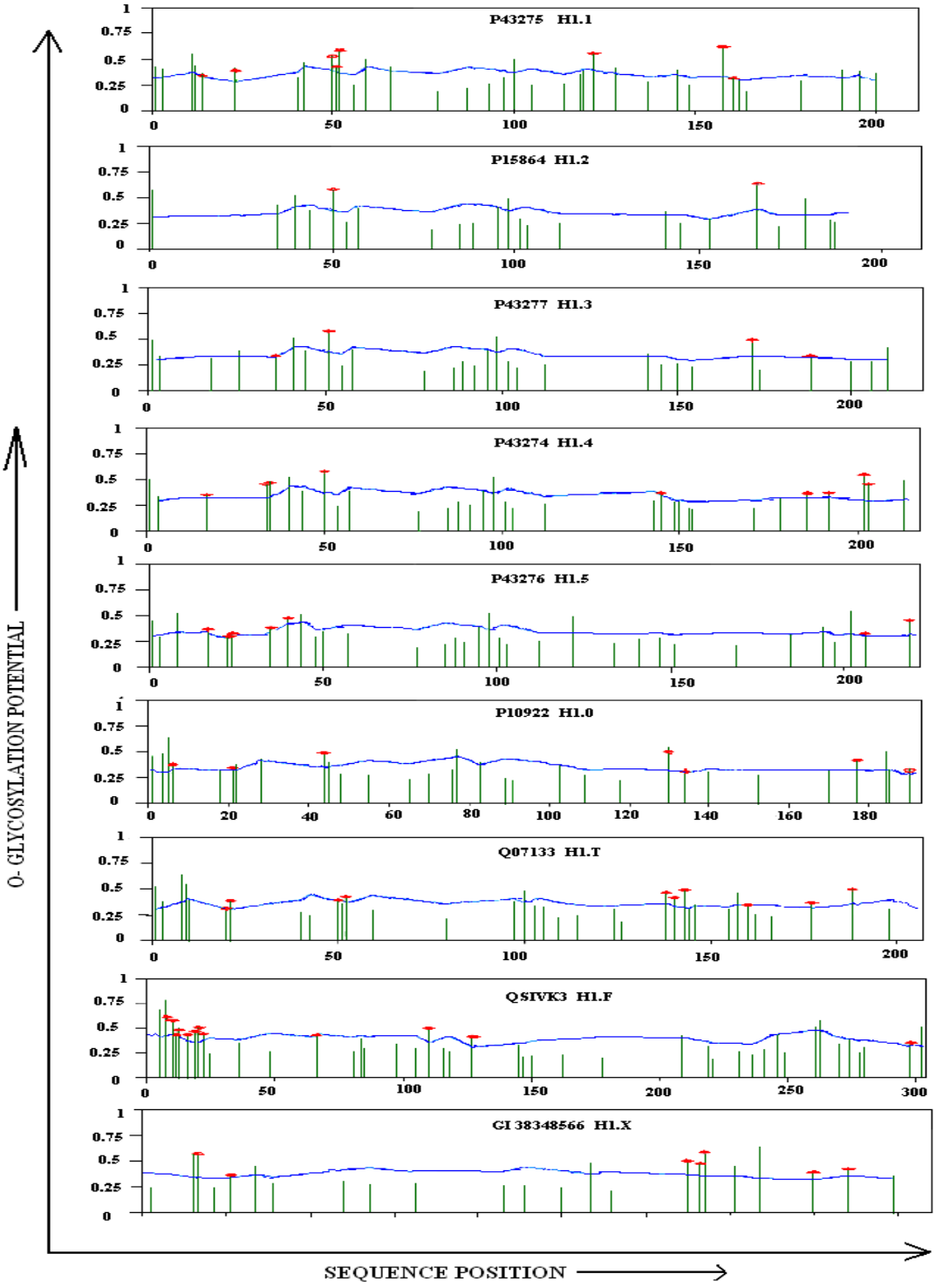

Fig. 2. Graphical representation of potential for *O*-GlcNAc modification in serine and threonine residues in the different subtypes of linker histone H1 in *Mus musculus*. Green vertical lines show the potential of Ser/Thr residues for *O*-GlcNAc modification and light blue horizontal wavy lines show threshold for modification potential.

Table II.- Protein kinases involved in phosphorylation of different subtypes of linker histone H1 in *Mus musculus*

Substrate		Protein kinases*							
		PKC	PKA	CDC2	CDK5	GSK3	P38 MAPK	RSK	PKG
H1.1	Ser	51, 59, 105, 114, 122, 128, 158, 162, 164, 191,	40, 66, 87	52,					
	Thr	12, 23, 93, 100, 118, 119, 145, 148, 161, 196		3,	148, 200	200	148, 200		23, 137
H1.2	Ser	50, 57, 85, 101, 103, 112, 172, 188	35	50					35, 186
	Thr	98, 141, 153, 166, 179			145				153, 179
H1.3	Ser	51, 58, 86, 102, 104, 113, 171, 173, 188, 100, 205	26,	51	188			188	36, 171, 200
	Thr	26, 92, 99, 142, 150, 154, 210			17,				26, 150,
H1.4	Ser	50, 57, 85, 101, 103, 112, 149, 171, 203	35,	50,	186	186	186	35, 171	35, 149, 203
	Thr	91, 98, 143, 145, 150, 153, 178, 192, 202, 213	34,		153		17		178, 213
H1.5	Ser	85, 101, 103, 112, 169, 185, 194, 197, 219		50	17, 169, 185	169, 185	185		194
	Thr	23, 24, 35, 91, 98, 122, 134, 141, 147, 151, 202, 206	35		134, 151		134		23, 35, 122, 141, 202
H1.O	Ser	18, 44, 45, 55, 70, 91, 103, 130, 134, 170, 184, 185, 190	18, 28, 44, 89, 190	4, 6, 21,					18, 185,
	Thr	22, 76, 109, 152, 177,			152,		118, 140		22, 109
H1.F	Ser	20, 21, 59, 103, 105, 109, 126, 140, 143, 144, 146, 160, 162, 188	8, 40, 79	1, 9, 42, 52. 109	140, 177	140	140, 177	40, 177	
	Thr	100, 146, 157, 166					155		
H1.T	Ser	5, 8, 13, 36, 67, 118, 147, 150, 241, 249,	219	5, 12, 13,	20,	20,		20,	219, 221,
	Thr	84, 97, 105, 127, 145, 261, 270		19					
H1.X	Ser	27, 112, 133, 160, 168, 179	48, 64,130	2,64, 133	134			130,	179
	Thr	17, 21, 31, 54, 86,			147		147		21

*CDC2, cell division control 2; CDK5, cyclin-dependent kinase-5; GSK3, glycogen synthase kinase-3; p38MAPK, p38 mitogen-activated protein kinase; PKA, protein kinase A; PKC, protein kinase C; PKG, cyclin GMP-dependent protein kinase G; RSK, ribosomal S6 kinase.

whereas the sequences of terminal tails of a specific subtype are well conserved among different species (Goytisolo *et al*., 1996). In addition to heterogeneity of their primary structures, the histone tails are also post-translationally modified under various biological conditions (Lennox, 1984). The proportion of linker histone H1 subtypes varies in a tissue- and species-specific manner (Khochbin and Wolffe, 1994), and the expression of each subtype varies throughout development and differentiation (Helliger *et al*., 1992). Studies of the structure of different subtypes of linker histone H1 and their interaction with the nucleosome and their roles in controlling gene activity indicate that linker histones have both an essential architectural function and an important task in regulating transcription (Alami *et al*., 2003). The functions of linker histones arising due to modifications are not yet fully understood, but it is known that different linker histone variants are preferentially localized in particular chromosomal

Table III.- Proposed Ser/Thr residues for interplay of phosphorylation and *O*-GlcNAc modification in different subtypes of linker histone H1 in *Mus musculus*.

Substrate		Proposed yin yang sites	Proposed fn-yin yang sites	Yin yang sites by similarity
H1.1	Ser		51, 52	
	Thr			
H1.2	Ser	50	112, 172	172 (Human)
	Thr			
H1.3	Ser	36, 51, 188	104	188 (Human)
	Thr		92, 154	
H1.4	Ser	35, 50, 186	54, 103, 112	186 (Human)
	Thr	202		17, 45 (Human)
H1.5	Ser	17, 172		17 (Human)
	Thr			137 (Human)
H1.0	Ser	6, 21, 44, 130, 134	65, 70, 103	
	Thr		109, 140, 152	
H1.T	Ser	50, 52, 138, 140, 177		
	Thr		155	
H1.F	Ser	8, 12, 20, 110	131, 221, 236	
	Thr	19, 66		
H1.X	Ser		13, 133	
	Thr		54	

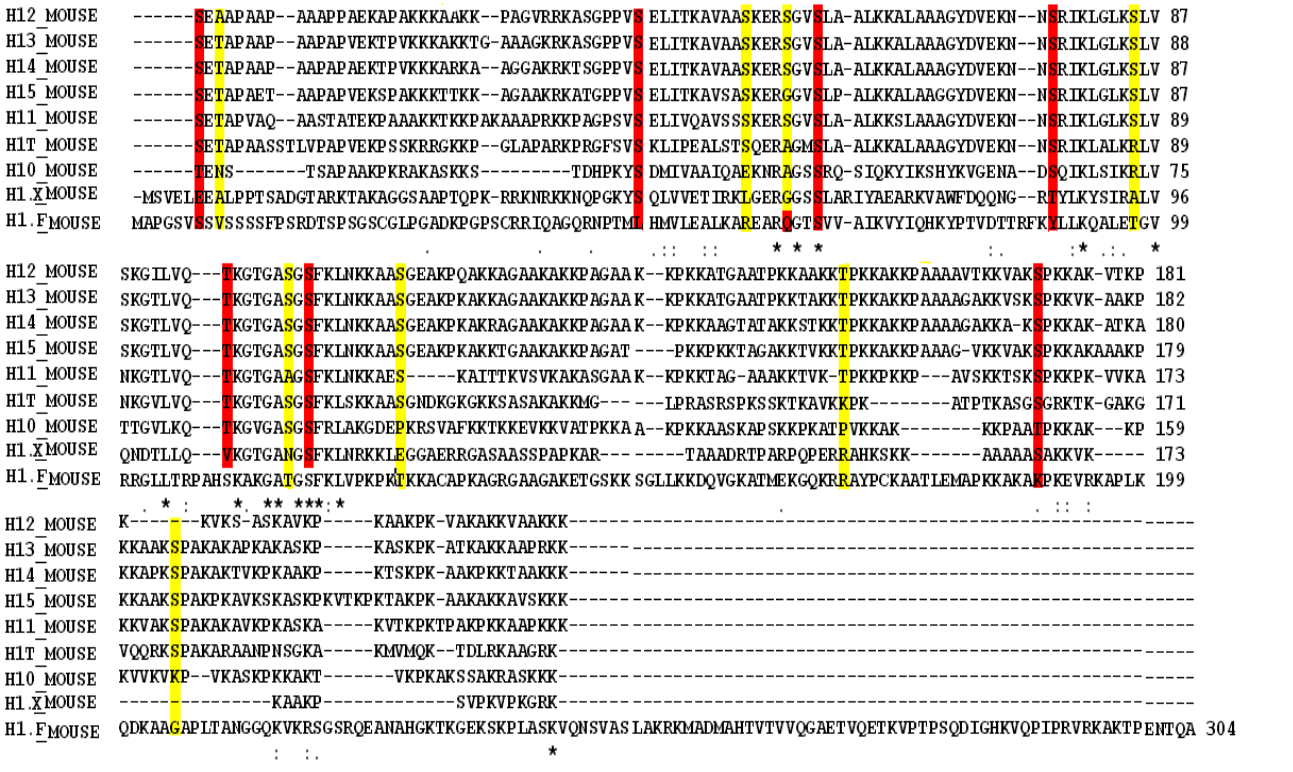

Fig. 3. Sequence alignment of different subtypes of linker histone H1 present in *Mus musculus*. The residues highlighted in red show conserved substitution regions in Ser and Thr residues, while the regions highlighted in yellow show Ser and Thr residues which are conserved in majority of subtypes but not present in all of the subtypes in linker histone H1.

domains after modifications (Sarg *et al*., 2005). The sequences within the globular domain of linker histone H1 are thought to be responsible for differential effect of overproduction of different linker histone variants on gene expression (Brown *et al.*, 1997), while the *N*- and *C*-terminal domains of linker histone H1 are responsible for condensation of chromatin (Bharath *et al*., 2002). The *N*-terminal region of linker histone H1 binds with linker DNA (Vila *et al*., 2001) and the *C*-terminal of linker histone H1 has binding affinity for core histones (Goytisolo *et al*., 1996). Different linker histone H1 subtypes have different chromatin condensing abilities (De Lucia *et al*., 1994). All linker histone H1 subtypes differ not only in primary sequence but also in turnover rate, timing of synthesis during development and extent of phosphorylation (Lennox, 1984), and they also have the potential to add a great deal of flexibility to chromatin structure and transcriptional activation (Khadake and Rao, 1997).

Linker histone H1 is required for longitudinal compaction of replicated chromosome. Enrichment of linker histone H1 onto chromatin requires passage through interphase, when DNA replication takes place. Thus, linker histone H1 contributes to chromosome condensation in vertebrates (Maresca and Heald, 2006). In mouse, depletion of linker histone H1 caused chromatin structure changes which include decreased global nucleosome spacing, reduced chromatin compaction and decrease in certain histone modifications like methylation (Fan *et al*., 2005). *In vitro* experiments showed that linker histone H1 represses transcriptional promoters and factors by condensing the chromatin material (Zlatanova and van Holde, 1992) but *in vivo* studies showed that linker histone H1 does not function as a global transcriptional repressor, but instead participates in complexes that either activate or repress specific genes (Wolffe *et al.*, 1997). Differences between linker histone H1 subtypes for both binding and the capacity to aggregate polynucleosome into condensed structure implies functional differences among different linker histone H1 subtypes during cell cycle and development of organism (Talasz *et al*., 1998). Sub-fractions of H1 histones differ in their effectiveness in condensing DNA fibers into ordered aggregates (Liao *et al*., 1981). Furthermore, each of linker histone H1 variants shows different binding capacity with DNA (Hill *et al*., 1991).

Hale *et al*. (2006) showed that phosphorylation of linker histone H1 provides a signal for the disassembly of higher order chromatin structure during cell cycle. Linker histone H1 is phosphorylated in a cell-cycle dependent manner. For instance, in G_1 phase levels of H1 phosphorylation are usually lowest and then rises continuously during S and G_2 phase (Lennox *et al*., 1982). The M-phase, where chromatin is highly condensed, shows the highest number of phosphorylated sites (Roth and Allis, 1992). Phosphorylation of linker histone H1 subtypes occurs on specific serine and threonine residues during the cell cycle in the presence of different protein kinases (Ewen, 2000). In the interphase, phosphorylation occurs mainly on serine residues while during mitosis, phosphorylation takes place on threonine (Moreno and Nurse, 1990). Linker histone H1.5 is phosphorylated in both the *C*- and *N*-terminal regions, while linker histones H1.2, H1.3 and H1.4 are exclusively phosphorylated in the *C*-terminal regions (Sarg *et al*., 2006).

Linker histones not only regulate gene expression and transcription but also have roles in ageing, DNA repair and apoptosis, which suggest their importance in maintaining chromatin and genomic integrity (Workman and Kingston, 1998). These regulations occur in response to changes in the ionic environment by electrostatic interactions between DNA, histone proteins, and free ions (Bednar *et al*., 1998). Decondensation of chromatin mediated through phosphorylation of linker histone weakens the electrostatic interactions between the negatively charged DNA and positively charged *C*-terminal tails of linker histone subtypes and vice versa (Dou and Gorovsky, 2002). During mitosis, linker histone H1.1 is phosphorylated on two residues, *i.e.* Thr-152 and Ser-182 (Hendzel *et al*., 2004), histone H1.2 is phosphorylated on Ser-172, histone H1.3 on Ser-188, histone H1.4 on three residues including the two serine residues 171 and 186, and one threonine residue 145, while linker histone H1.5 is phosphorylated on four residues, two serine residues 17 and 172, and two threonine residues 137 and 154 (Sarg *et al*., 2006). Linker

histone H1.T phosphorylates on three residues Ser-177, Thr-158 and -159, while H1.X also phosphorylates three residues, Ser-2, -31 and -33 (Olsen *et al*., 2006). There is no experimental data available about the phosphorylated sites of the other two remaining linker histone subtypes H1.F and H1.0 in mammals. It was shown that during interphase, phosphorylation occurs on serine residues, while during mitosis the threonine residues are phosphorylated. This indicates the dual effect of linker histones phosphorylation during the cell cycle; first during interphase, the phosphorylation of serine residues of all subtypes of linker histone H1 promotes DNA replication, transcription and gene regulation; then during mitosis, phosphorylation of threonine residues of linker histone H1.4, H1.5 and H1.T may be required for recruiting proteins that are involved in condensation by an unknown mechanism (Strunikov *et al*., 1995).

Our results of NetPhos K 1.0 for the prediction of phosphorylation potential of all serine and threonine residues show that these residues are phosphorylated by different kinases during the cell cycle (Table II). These experimentally verified residues are conserved in all subtypes of linker histones in mammals and we conclude that these phosphorylated sites may be present on linker histones of other mammals "by similarity" where these phosphorylation sites are not yet experimentally known. *O*-GlcNAc modification can occur on these serine and threonine residues where kinases are involved in phosphorylation, as it is well known that kinases and OGT compete for same site modification (Haltiwanger *et al*., 1997). This suggests a possibility for interplay between phosphorylation and *O*-GlcNAc modification on these residues. Yin*O*Yang 1.2 prediction results had indicated that all subtypes of linker histone H1 of mouse have high potential for *O*-linked glycosylation, as shown in Figure 2. The proteins modified by *O*-GlcNAc are more concentrated on condensed chromatin as compared with transcriptionally active regions (Slawason *et al*., 2005); thus the *O*-GlcNAc modification acts in a reciprocal manner to phosphorylation. Chromatin and several transcription factors are also found to be modified by *O*-GlcNAc (Majumdar *et al*., 2003; Love and Hanover, 2005).

The serine and threonine residues of linker histone H1 that are known to be experimentally phosphorylated showed positive potential for *O*-GlcNAc modification at Ser-188 of H1.3, Ser-186 and Thr-145 of H1.4, Ser-17 of H1.5 and Ser-177 of linker histone H1.T. These predicted sites can also be phosphorylated by different kinases (Table II) and may act as possible switch for *O*-GlcNAc modification. The remaining serine and threonine residues of linker histone subtypes which are conserved in different species and either known or predicted to be phosphorylated, showed negative potential for *O*-GlcNAc modification but are very close to the threshold value and consequently identified as FN-Yin Yang sites. These conserved sites can be accessed by different kinases and therefore it is likely that these sites have also potential for OGT modification. Therefore, these sites may also enable interplay for phosphorylation and *O*-GlcNAc. The binding of DNA with nucleosome can be increased with the mutation of serine and threonine phosphorylation sites to alanine residues at different subtypes of linker histone H1 (Contreras *et al*., 2003). This phenomenon showed that these serine and threonine residues are involved in transcription and gene regulation during the cell cycle through interplay of phosphorylation and *O*-GlcNAc modification.

The above results reveal that all the conserved phosphorylated residues which show positive potential for *O*-GlcNAc modification (Yin Yang sites) or predicted as FN-Yin Yang sites, as shown in Table III, may be involved in modulating the functions through interplay between phosphorylation and *O*-GlcNAc modification among different subtypes of linker histone H1. Primarily these linker histone H1 subtypes are phosphorylated on specific serine residues at the *N*-terminal region and enhance the process of DNA replication, transcription and gene regulation by decondensation of chromatin material during interphase. This decondensation process can be blocked by *O*-GlcNAc modification on these specific serine residues, which may then result in chromatin condensation and repress transcription of DNA. The interplay between phosphorylation and *O*-GlcNAc modification on threonine residues during mitosis may also activate proteins that are

involved in the condensation mechanism. Thus we conclude that phosphorylation in different subtypes of linker histone H1 on proposed Ser/Thr residues (Table III) is involved in decondensation of chromatin structure which leads to transcription regulation and gene expression, while the *O*-GlcNAc modification occurring on the same Ser/Thr residues is involved in condensation of chromatin.

ACKNOWLEDGEMENT

Nasir-ud-Din acknowledges support from Pakistan Academy of Sciences for this research effort and thanks Tehmina Saleem Khan for useful discussion.

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(Received 20 December 2006, revised 1 February 2007)