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Post-Translational Modifications of Non-Histone High Mobility Group Nucleosomal Proteins

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Abstract.- High mobility group nucleosomal proteins, HMGN-1 and HMGN-2, regulate several DNA-dependent *viz.*, transcription and replication activities of the mammalian nucleus. HMGN-1 decondensates the nucleosome, when it interferes with the linkage of histones to DNA. The HMGN-1 targets histones like H2B, H3 and linker histone H1. The histones-DNA interference is regulated by post-translational modifications at the Ser/Thr/Tyr residues of HMGN-1. Phosphorylation of HMGN-1 inhibits replication/transcription, whereas *O*-GlcNAc modification of HMGN-1 promotes replication/transcription. This *in silico* study proposes that, phosphorylation sites at Ser/Thr residues also exhibit *O*-GlcNAc modification potential. The prediction of *O*-GlcNAc modification sites and possible interplay between phosphorylation and *O*-glycosylation will enhance understanding of gene regulation.

Key words: High mobility group nucleosomal proteins HMGN, phosphorylation, *O*-GlcNAc modification, gene expression.

INTRODUCTION

The high mobility group nucleosomal proteins (HMGN) are evolutionarily conserved non-histone proteins that affect the stability of the higher-order chromatin structure (Bustin *et al*., 1990). These proteins are heterogeneous and are ubiquitously expressed in eukaryotes especially in mammals (Trieschmann *et al*., 1998; Birger *et al*., 2003; Lim *et al*., 2004). The major non-histone nucleosomal proteins are HMGN-1 and HMGN-2, also known as HMG-14 and HMG-17, respectively (Prymakowska-Bosak *et al*., 2001). HMGN proteins bind to the nucleosomal core as homodimers (Postnikov *et al*., 1995), and are the only nucleosomal proteins that specifically bind to the 146/147 bp nucleosome core histone proteins (Bustin and Reeves, 1996; Bustin, 2001). HMGN-1 binds specifically to histone H2B and H3 core proteins and consequently affects the chromatin structure (Trieschmann *et al*., 1998; Lim *et al*., 2004). Due to HMNG proteins involvement in chromatin remodeling, these are also termed as architectural proteins (Muchardt *et al*., 1996; Shirakawa *et al*., 1997).

The HMGN-1 protein binds to the nucleosome core through its nucleosome binding domain (NBD) (Lim *et al*., 2004). Binding of HMGN-1 relaxes the chromatin structure, and promotes the accessibility of transcription factors and co-activators to the transcriptionally induced gene (Birger *et al*., 2003). HMGN-1 competes with the linker histone H1 for bindings sites on the chromatin (Catez *et al*., 2002). H1 stabilizes the higher-order chromatin structure and acts as a general repressor of gene transcription, whereas HMGN proteins destabilizes and decondensates the chromatin structure (Wolffe *et al*., 1997; Cheung *et al*., 2002; Horn *et al*., 2002).

Phosphorylation of HMGN proteins contributes significantly to various nuclear processes (Bustin, 2001). HMGN phosphorylation occurs during the log phase of mitosis (Saffer and Glazer, 1982) suggesting that phosphorylation is a growth associated mechanism. During this phase, HMGN-1 is highly phosphorylated on Ser 20 and 24 in its nucleosome binding domain (NBD) and is translocated into the cytoplasm (Falciola *et al*.,

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1997; Shirakawa *et al*., 1997; Hock *et al*., 1998; Lund and Berg, 1999; Prymakowska-Bosak *et al*., 2001). In late telophase, HMGN proteins relocate into the nucleus with the appearance of the nuclear envelope and co-localize with the chromatin (Hock *et al*., 1998). Furthermore, HMGN-1 modulates post-translational modifications (PTMs) of the core histones. When HMGN-1 is phosphorylated on Ser 20 and 24 during mitosis, it inhibits phosphorylation and acetylation of core histone H3 (Lim *et al*., 2004).

N-acetylglucosamine glycosylation (*O*-GlcNAc) is an enzyme dependent protein modification that commonly occurs on nucleosomal proteins (Comer and Hart, 2000). *O*-GlcNAc modification and phosphorylation can be reciprocal and occur on the same or adjacent Ser/Thr residues (Haltiwanger *et al*., 1997; Hart, 1997). However, the interplay between phosphorylation and *O*-GlcNAc modification depends on the specific function of the protein required in the particular environment (Comer and Hart, 2000). The alternation of these two modifications on the same or neighboring residue may modulate the specific function of the proteins either by enhancing or inhibiting the functional capacity. We propose that, amino acid residues where phosphorylation occurs can also accept *O*-GlcNAc modification suggesting possible interplay between *O*-GlcNAc glycosylation and phosphorylation in HMGN-1. This possible interplay between different post-translational modifications (PTMs) on HMGN-1 may control gene expression.

MATERIALS AND METHODS

Selection of model organisms

The HMGN-1 protein was selected for the determination of potential phosphorylation and *O*-GlcNAc modification sites in *Mus musculus*. The sequence data was retrieved from the Swiss-Prot sequence database with accession no. P18608 (Boeckmann *et al*., 2003).

Phosphorylation/O-GlcNAc modification prediction sites

Phosphorylation sites on Ser, Thr and Tyr 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. YinOYang 1.2 database (http://www.cbs.dtu.dk/services/YinOYang/) was used for the prediction of *O*-GlcNAc modification sites on Ser and Thr residues. Predicted phosphorylation sites were analyzed at 0.5 threshold value (in between zero and one). Zero threshold value was used for negative and one threshold value for positive possibility of phosphorylation prediction sites. However, the threshold value in the Yin Yang database prediction varies depending upon the surface accessibility.

BLAST searching and sequence alignment

BLAST searching was done by using NCBI database (http://www.ebi.ac.uk/blastall/) (Altschul *et al*., 1997) of non-redundant sequences. The sequences were aligned by using ClustalW database (Thompson *et al*., 1994) (http://www.ebi.ac.uk/clustalw/). Four mammals [*Homo sapiens* (Human with RefSeq, P05114), *Pan troglodytes* (Chimpanzee with RefSeq, XP_514899), *Macaca mulatta* (Rhesus monkey with RefSeq, XP_001098642) and *Bos taurus* (Cattle with RefSeq, NP_001029944 XP_871660)] were selected for sequence alignment.

Abbreviations

HMGN - High mobility group nucleosomal proteins, NBD - Nucleosome binding domain, PTMs - Post-translational modifications, OGT - *O*-GlcNAc transferase, OGT- *O*-GlcNAc transferase.

RESULTS AND DISCUSSION

The HMGN proteins have the ability to facilitate replication and/or transcription by unfolding the higher-order chromatin structure (Trieschmann *et al*., 1995; Ding *et al*., 1997). These proteins perform this function specifically by binding to the nucleosome core. The compaction of the chromatin is due to the presence of the linker histone H1, which specifically binds to the *N*-terminal of the core histones through its *C*-terminal tail (Postnikov *et al*., 1997). Histone H1 and dephosphorylated HMGN-1 compete with each

other for the nucleosomal interaction. The target site of HMGN-1 in the nucleosome core is close to that

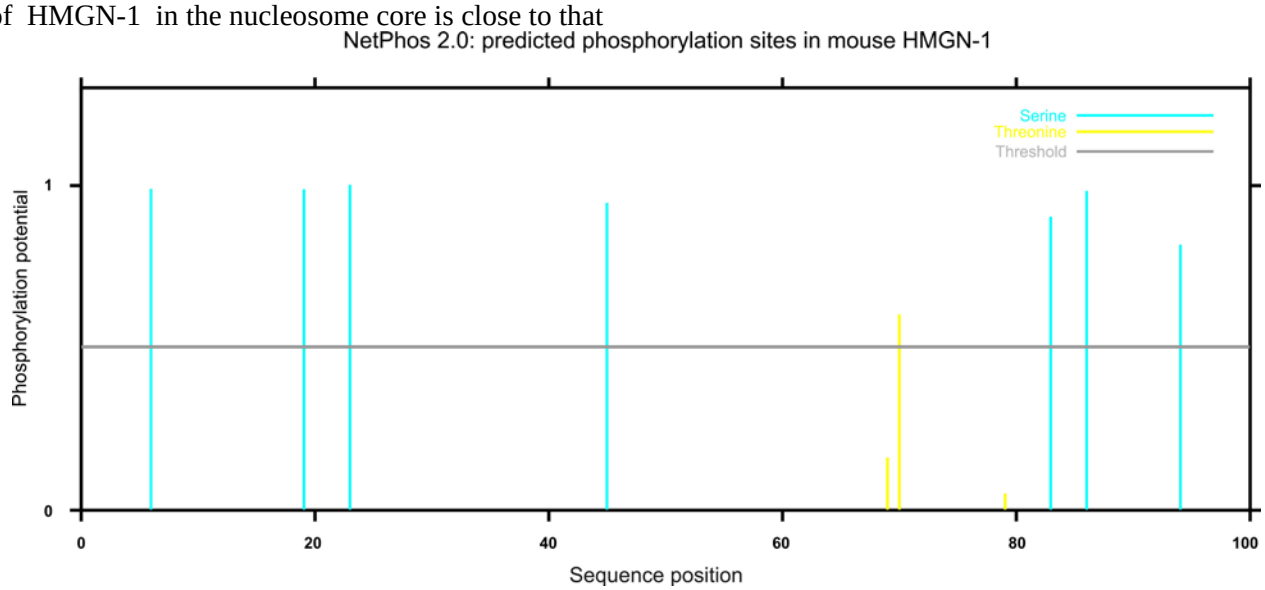

Fig. 1. Simple line graph represents the phosphorylation potential of Ser and Thr residues of mammalian HMGN-1 (Light green vertical line shows the potential for Thr and blue vertical line shows the potential for Ser. Horizontal light grey colored line showing the threshold value at 0.5).

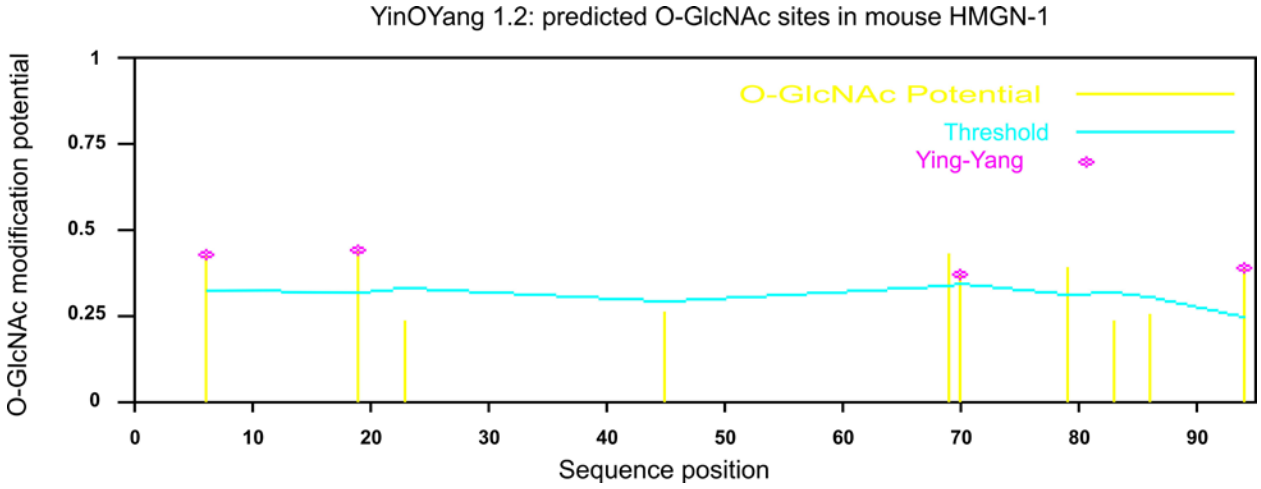

Fig. 2. Simple line graph represents the *O*-GlcNAc potential of Ser and Thr residues of mammalian HMGN-1 (Light green vertical pointed lines show the potential for Ser and Thr. Non-pointed light green lines show false negative potential for *O*-GlcNAc modification. Horizontal blue colored line shows the threshold value)

of H1 (Alfonso *et al*., 1994), thus it competes with and hinder almost all the H1 mediated activities (Ding *et al*., 1997). In the nucleosome binding process, the *C*-terminal of HMGN-1 is involved (Trieschmann *et al*., 1998), and binds to the *N*-terminal of H3 in the nucleosome core (Arents and Moudrianakis, 1993; Luger *et al*., 1997). Thus, the *C*-terminal of HMGN-1 is necessary for replication and transcription processes. But, the functional significance of the *N*-terminal region is not clear (Trieschmann *et al*., 1998). *N*-termini of core histones are involved in various processes leading to the rearrangement in the structure of chromatin and stimulation of DNA-dependent activities (Wolffe,

1995; Hansen, 1997). When HMGN-1 is attached to the *N*-terminal of H3, H3 binding to H1 is weakened (Trieschmann *et al*., 1998). As a result, the interaction of H1 and H3 is destabilized, which

Table I.- Predicted true and false phosphorylation sites for possible *O*-GlcNAc modification.

Accession no	Scientific name	Common name	Predicted sites for phosphorylation		Predicted sites for *O*-GlcNAc modification	
			Ser residues	Thr residues	Ser residues	Thr residues
P18608	*Mus musculus*	Mouse	6, 19, 23, 45, 83, 86, 94	70	6, 19, 94	70
NP_001029944 XP_871660	*Bos taurus*	Cattle	7, 21, 25, 47, 87, 90, 100	–	7, 21, 100	–
P05114	*Homo sapiens*	Human	6, 20, 24, 45, 85, 88, 98	–	6, 20, 98	–
XP_001098642	*Macaca mulatta*	Rhesus monkey	7, 25, 46, 85, 88, 98	–	7, 98	–
XP_514899	*Pan troglodytes*	Chimpanzee	7, 21, 25, 46, 86, 89, 99	–	7, 21, 99	–

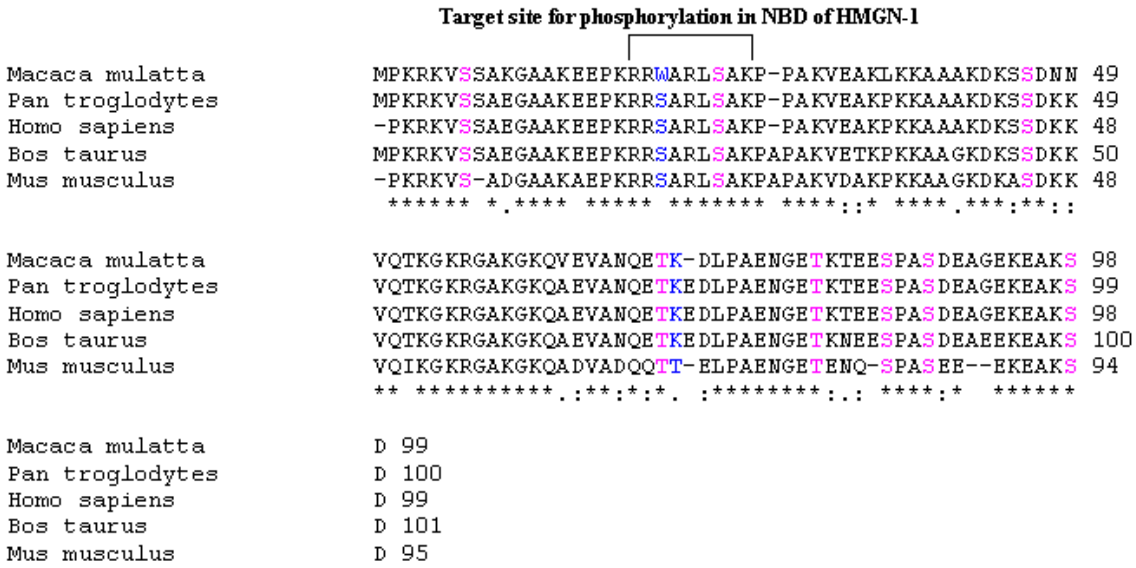

Fig. 3. HMGN-1 sequence alignment of different mammals showing the conserved (pink in color) and non-conserved (blue in color) regions of Ser and Thr residues.

leads to decompaction of the nucleosomal core (Trieschmann *et al*., 1998).

We have predicted phosphorylation sites for the HMGN-1 by using the NetPhos database (Blom *et al*., 1999). These results suggest that, Ser 6 and Ser 19 are the potential sites for phosphate modification in mammalian HMGN-1 (Fig. 1). These results are in agreement with the previous work, which showed that Ser 6 and 19 residues are phosphorylation sites in HMGN-1 in *Mus musculus* (Walton *et al*., 1984; Spaulding *et al*., 1991; Barratt *et al*., 1994). Furthermore Walton and Gill (1983) and Walton *et al*. (1985) confirmed possible phosphorylation sites in human HMGN-1 at Ser 88 and 98, which also are in agreement with our results (Table I).

Four sites sere predicted to act as Yin Yang sites, Ser 6, 19, 94 and Thr 70 (Fig. 2). Results

obtained after sequence alignment of different mammals (human, Rhesus monkey, cattle and chimpanzee) against mouse showed that the Ser and Thr residues in HMGN-1 at Ser 6, 19, 23, 45, 83, 86 and 94 are highly conserved with the exception of Thr 70. Thr 70 was replaced by Lysine (K) residue

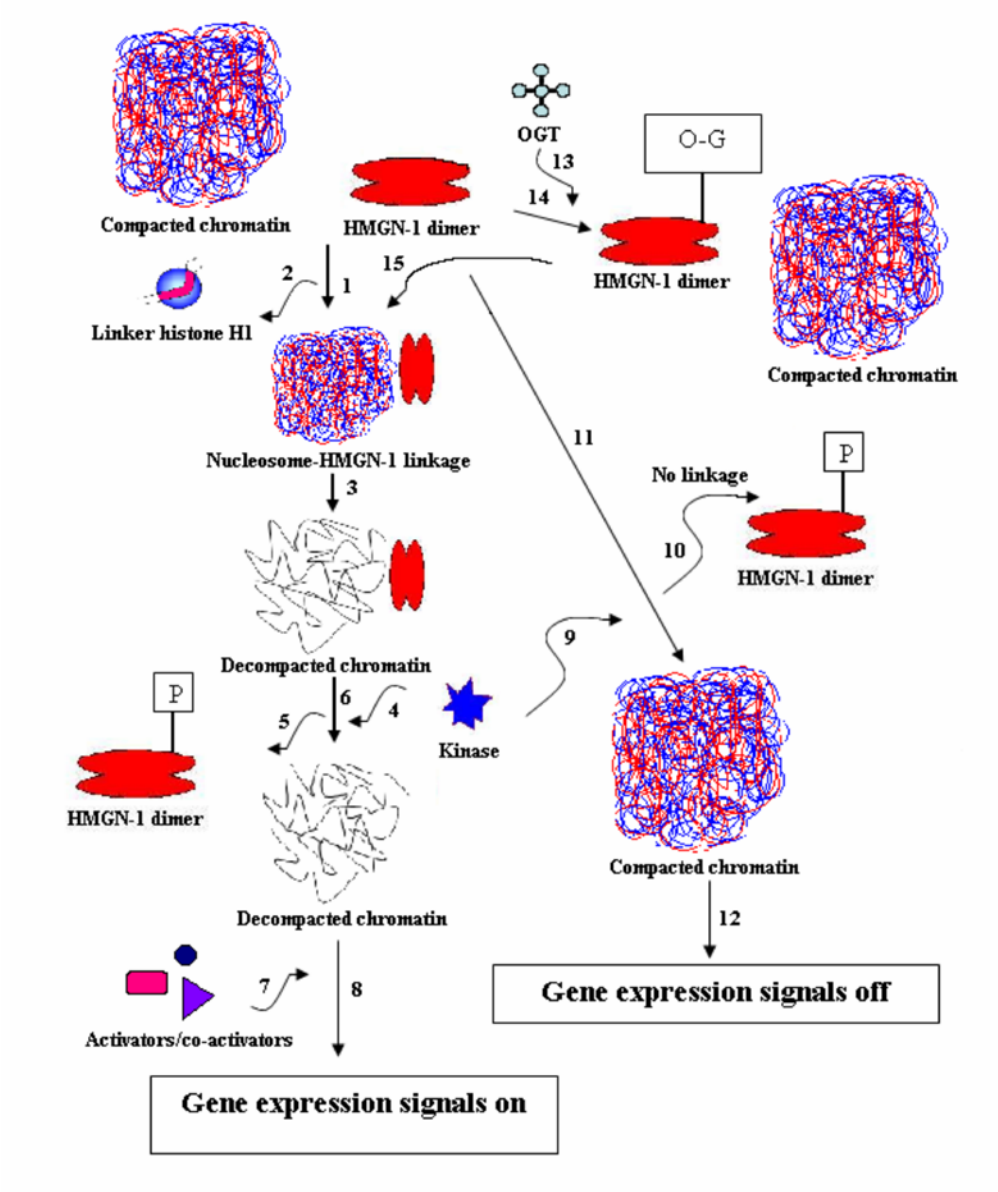

Fig. 4. Interplay between phosphorylation and *O*-GlcNAc modification of HMGN-1 in the regulation of gene expression signals in mammals. (1) HMGN-1 and H1 compete with each other to bind with the nucleosome core. (2) When the HMGN-1 gets bind with nucleosome core, H1 detach from nucleosome core. (3) HMGN-1 binds to the compacted chromatin, and relaxes the structure. (4) Kinase enzyme like protein kinase C phosphorylates HMGN-1. (5) Phosphorylated HMGN-1 leaves the chromatin and is transported into the cytoplasm. (6) Decompacted chromatin is now ready to access the replication/transcription activators/co-activators. (7, 8) Activators/co-activators get attached to the chromatin and enhance the replication/transcription rate (gene expression). (9) The kinase phosphorylates HMGN-1 prior to binding with nucleosome core. (10) Phosphorylated HMGN-1 is unable to bind with the nucleosome core. (11) Chromatin remains in compacted form. (12) Compacted chromatin is not accessed for the

replication/transcription activators/co-activators and gene expression signals remain off. (13) OGT transfer *O*-GlcNAc moiety to HMGN-1. (14) HMGN-1 becomes *O*-GlcNAc modified. (15) *O*-GlcNAc modified HMGN-1 easily binds with nucleosome core and promotes gene expression. P = phosphorylated HMGN-1, O-G = *O*-GlcNAc modified HMGN-1.

in all the mammals (Fig. 3) and Ser 19 was replaced by Tryptophan (W) only in Rhesus monkey. When mouse HMGN-1 sequence was aligned with that of human, it was found that Ser 19 of mouse corresponds to Ser 21 and Ser 23 to Ser 24 in human HMGN-1.

When HMGN-1 is bound to the chromatin, it inhibits the ability of kinases to phosphorylate histone H3 at Ser 10 (Lim *et al*., 2004). Once, gene expression begins, HMGN-1 gets phosphorylated at Ser 19 and 23 (corresponds to 20 and 24 in human). Phosphorylation of HMGN-1 disrupts the binding between HMGN-1 and chromatin (Louie *et al*., 2000; Prymakowska-Bosak *et al*., 2001; Lim *et al*., 2004) as these Ser residues are located in the NBD, and relocates the protein into the cytoplasm (Bustin and Reeves, 1996; Louie *et al*., 2000) until the completion of cell cycle. In consistency with phosphorylation, disruption of chromatin interaction and translocation of HMGN-1 into the cytoplasm, peptide or major sequence, RRSARLSAK is involved (Palvimo and Maenpaa, 1988; Lund and Berg, 1991; Bustin and Reeves, 1996). This peptide/ sequence represent the NBD of the HMGN-1 and are conserved in the mammals (with the exception of Rhesus monkey) (Fig. 3).

Our predicted Yin Yang sites, Ser 19 and 23, are located in HMGN-1's NBD, and *O*-GlcNAc transferase (OGT) may play an equally important role in HMGN-1's ability to bind to the chromatin structure. *O*-GlcNAc modification is a highly dynamic process in the regulation of a protein-dependent pathway in the cell (Kearse and Hart, 1991). Chromatin is known to be modified by OGT (Love and Hanover, 2005), and several transcription factors like CREB (Lamarre-Vincent and Hsieh-Wilson, 2003) and Sp1 (Majumdar *et al*., 2003) are also found to be *O*-GlcNAc modified. The HMGN-1 protein is known to be phosphorylated by protein kinase C (Prymakowska-Bosak *et al*., 2001), and an inversible relationship between protein kinase C and OGT has been documented (Wells *et al*., 2001). The *O*-GlcNAc modification, which acts in a reciprocal manner to phosphorylation, prevents HMGN-1 from being phosphorylated. This suggests that *O*-GlcNAc modification of HMGN-1 promotes gene expression in mammals. If these Ser residues are *O*-GlcNAc modified by the *O*-GlcNAc transferase (OGT), the chance of PKC mediated phosphorylation of HMGN-1 minimizes (Fig. 4). These *O*-GlcNAc modification potential sites regulates the gene expression in mammals as these *O*-GlcNAc modified HMGN-1 gets easily attached to the nucleosome, so gene expression is unaffected.

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