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Interplay of Glycosylation and Phosphorylation in Murine Oct-2 Regulates its POU Domain Binding

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Abstract.- A common mechanism for the regulation of gene expression is through post-translational modification of transcription factors. Oct-1 and Oct-2 exhibit cell-type-specific, differential binding to three octamer binding sequences. Phosphorylation has been shown to modulate differentially the DNA binding to high- and low-affinity binding sites. Similarly, *O*-GlcNAc modification in certain transcription factors has been shown to be dynamic and compatible with phosphorylation, and their interplay at a site regulates vital functions of these proteins. Oct-2 expression is restricted to B-cells and some other neuronal cells rather than displaying ubiquitous expression like that of Oct-1. Phosphorylation of Oct-2 in the POU domain of *Mus musculus* has been described to be involved in regulating its binding. Phosphorylation in POU domain results in decreased binding. It is proposed that *O*-GlcNAc modification blocks phosphorylation at Ser255 in the POU domain and results in its binding and triggering signals for activation of Oct-2 for binding to gene octamer.

Key words: Transcription factors, homeodomain, gene expression.

INTRODUCTION

A 60 amino acid region known as the homeodomain is present in many proteins regulating gene expression, such as Oct-1, Oct-2 and GHF-1/Pit-1, which have a bipartite DNA-binding region known as the POU domain. The POU domain contains the homeodomain and a POU-specific (Pit, Oct, Unc) domain (Johnson and Hirsh, 1990). The 70-75 amino acids long POU domain is located upstream of the homeobox. Proteins with specific motifs bind to specific DNA sequences to cause temporal and spatial regulation of the expression of genes, many of which are involved in the regulation of neuronal development in the central nervous system of mammals (Johnson and Hirsh, 1990). Some other genes are also regulated, including those for immunoglobulin light and heavy chains (Oct-2) (Petryniak *et al*., 1990), and trophic hormone genes, such as those for prolactin and growth hormone (Pit-1). Both elements of the POU-domain are required for high affinity sequence-specific DNA-binding. The domain may also be involved in protein-protein interactions (Mathis *et al*., 1992).

The Oct-2 transcription factor is a founder member of the POU (Pit-Oct-Unc) family of transcription factors (for review see Verrijzer and van der Vliet, 1993; Wegner *et al*., 1993). This factor was initially considered to be expressed only in B lymphocytes, where it is involved in activating the expression of specific genes expressed in these cells (Singh *et al*., 1986; Staudt *et al*., 1988). In agreement with this idea, mice in which the gene encoding Oct-2 has been inactivated show defects in B-cell maturation (Corcoran *et al*., 1993) and fail to express specific genes, such as those encoding CD36 (Konig *et al*., 1995) and Crisp-3 (Pfisterer *et al*., 1996).

The role of post-translational modifications in regulating gene expression of transcription factors is a generally observed mechanism. Phosphorylation and dephosphorylation of transcription factors are the most frequently used mechanisms, which regulate their activity in response to different extra- and intracellular signals and enable convergence of different signaling pathways at the same factor. There is enough evidence to suggest that changes of the phosphorylation status of the POU-domain of proteins modulate their ability to activate

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transcription (Pevzner *et al*., 2000). However, the data about the role of phosphorylation played in the transcriptional potential of the Oct-2 factor is inadequate. It has been repoted that phosphorylated Oct-2 is more competent to activate transcription than non-phosphorylated state (Tanaka and Herr, 1990).

The majority of transcriptional factors appear to be modified by *O*-GlcNAc in presence of the enzyme *O*-glucosaminyltransferase (OGT) in the transcriptional activation domains (Comer and Hart, 1999), and Sp1 is no exception (Jackson and Tjian, 1988), suggesting that OGT plays a critical role in the control of protein-protein interactions involved in transcriptional activation. Using an *in vitro* transcription system, *O*-GlcNAc modification of the SpE peptide markedly inhibited its ability to activate transcription. The first transcription factor shown to bear the *O*-GlcNAc modification was Sp1 (Jackson and Tjian 1988), a ubiquitous transcription factor that plays a vital role in the control of TATA-less housekeeping gene transcription (Pugh and Tjian, 1991).

The *O*-GlcNAc modification was found to be as dynamic and regulatory as phosphorylation (Comer and Hart 1999; Wells *et al*., 2001; Kamemura *et al*., 2002). An interplay between GlcNAc modification and phosphorylation has been observed in many nucleoplasmic and cytoplasmic proteins (Comer and Hart 1999; Wells *et al*., 2001; Kamemura *et al*., 2002). *O*-GlcNAc modification in transcription factors was shown to be involved in modulating the function of these proteins (Yang *et al*., 2002). We propose the involvement of *O*-GlcNAc modification along with its interplay with phosphorylation in modulating the function of Oct-2 binding in its POU domain.

MATERIALS AND METHODS

Sequence data

The sequence of Oct-2 was described (Wirth *et al*., 1991; Matsuo *et al*., 1992) in the early nineties. The sequence data used for predicting phosphorylation and glycosylation sites for oct-2 of *Mus musculus* was retrieved from SWISSPROT sequence database with entry name PO22_MOUSE and primary accession number Q00196. The sequence of human oct-2 was also retrieved from Entrez (NCBI sequence viewer) that was found to be identical to SWISSPROT entry. Indeed, no difference was found with respect to number and sequence position of amino acids between the sequences retrieved from the two sources.

Prediction methods

Prediction methods used to predict potential glycosylation sites involved four methods: three for potential *O*-linked glycosylation sites and one for predicting *N*-linked glycosylation sites. The methods used for predicting potential *O*-linked glycosylation sites include *NetOGlyc* 3.1 (http://www.cbs.dtu.dk/services/NetOGlyc/), that predicts *O*-glycosylation sites in mucin type proteins (*i.e.,* for *O*-GalNAc sites), *DictyOGlyc* 1.1 (http://www.cbs.dtu.dk/services/DictyOGlyc/) predicts *O*-GlcNAc sites in proteins, and YinOYang 1.2 (http://www.cbs.dtu.dk/services/YinOYang/), that predicts *O*-GlcNAc sites compatable with phosphorylation sites in proteins. The *NetNGlyc* 1.0 (http://www.cbs.dtu.dk/services/NetNGlyc/) was used for predicting *N*-glycosylation sites. The above-mentioned four methods for predicting the glycosylation sites are neural network based. For prediction of phosphorylation sites in Oct-2 *NetPhos* 2.0 (http://www.cbs.dtu.dk/services/NetPhos/) was used. The *NetPhos* 2.0 is also a neural network based program designed by training the neural networks through protein phosphorylation data from phosphobase 2.0.

RESULTS

Prediction results of Oct-2 for different *O*-linked and *N*-linked glycosylation sites show that the protein has the potential for both *N*-linked and *O*-linked glycosylation. Among *O*-linked glycosylation sites *O*-GalNAc and *O*-GlcNAc are the most frequent ones. The prediction results for *O*-GlcNAc modification show that there are a total of 22 potential sites highly predicted to be modified by *O*-GlcNAc at 16 Ser residues, at positions 52, 68, 133, 157, 255, 267, 342, 355, 369, 373, 376, 387, 414, 428, 432, and 436, and at 6 Thr residues at positions 150, 384, 419, 425, 426, 429 (Fig. 1). Apart from these, there are many other Ser and Thr

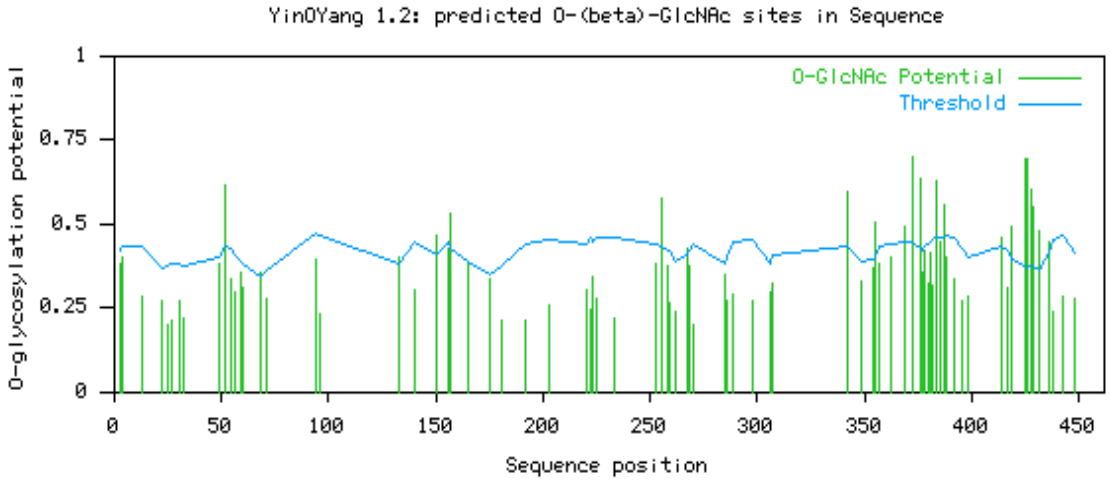

Fig. 1. Graph showing the potential of serine and threonine residues for modification by O-GlcNAc in Oct-2 of *Mus musculus*. (This graph shows potential for serine and threonine: it is representing only one molecule: serine or threonine?). Blue vertical lines are for both Ser and Thr, means each vertical green line shows either a serine or a threonine.

residues which were predicted to be non-glycosylated, but they are very close to the threshold and are likely to be modified by *O*-GlcNAc (Fig. 1).

Similarly, the prediction results by Netphos 2.0 for possible phosphorylation sites show that Oct-2 (*Oct-4 ?? or Oct-2 ?)* possesses a high potential for phosphate modification. A total of 31 residues are predicted to be phosphorylated (Fig. 2). These 31 residues include 24 Ser (at 13, 25, 27, 52, 54, 59, 68, 71, 175, 225, 233, 255, 258, 262, 268, 286, 307, 355, 373, 378, 380, 432, 436, and 438); 7 Thr (at 32, 223, 285, 354, 392, 419 and 425), and 0 Tyr. The elevated number of Ser and very low number of Tyr residues for predicted phosphate modification suggest a high possibility of *O*-GlcNAc modification in Oct-2.

There are a number of Yin Yang sites according to the prediction results, especially in the C-terminal region of the protein. However, the important ones that we are proposing for *O*-GlcNAc and phosphorylation interplay include Ser175 and 255 and Thr 150 in Oct 2.

DISCUSSION

Gene expression can be modulated through post-translational modification of transcription factors. Phosphorylation/dephosphorylation of transcription factors is a common mechanism that regulates the binding of these proteins to specific DNA sequences and the activation of gene transcription (reviewed in Hunter and Karin, 1992; Karin, 1994). Phosphorylation of Oct-1 by protein kinase A (PKA) inhibits its DNA-binding activity (Segil *et al*., 1991), and the calcium/calmodulin-activated phosphatase, calcineurin, has been shown to augment Oct-1-dependent transcription (Clipstone and Crabtree, 1992).

There is sufficient evidence to suggest the involvement of phosphorylation of octamer factors in presenting a mechanism of differential regulation of octamer factor binding to high- and low-affinity binding sites (Kapiloff *et al*., 1991; Grenfellet *et al*., 1996). Hyperphosphorylation of Oct-2 induced by okadaic acid on binding of this factor to three octamer sequences has been analyzed by Pevzner *et al*. (2000). One is a classical high-affinity binding octamer site present in the promoters of immuno-globulin genes or in the H2B gene promoter (Herr and Cleary, 1995); the other two are the non-canonical sequences identified in the promoters of the human and murine *BLR1* genes (Wolf *et al*., 1998). The latter two differ from each other by a single nucleotide substitution at position 3 and both

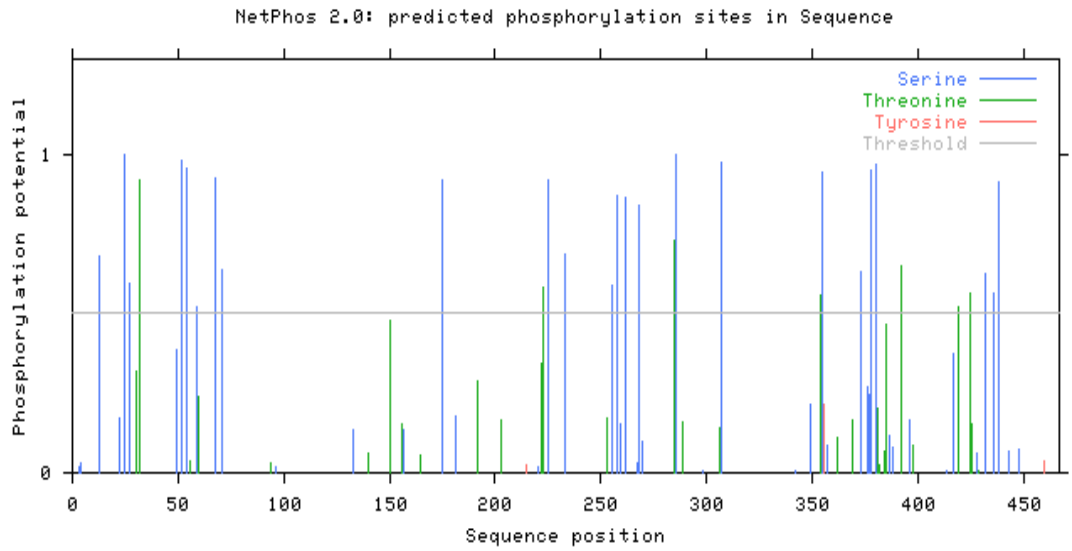

Fig. 2. Graphic representation of potential of all Ser, Thr, and Tyr residues for modification by phosphate in Oct-2 of *Mus musculus*.

have a substitution at position 2 unlike the classical motif 5´-ATGCAAAT-3´. Pevzner *et al.* (2000) showed that Oct-2 bound to the octamer site of the human BLR1 promoter with higher affinity than to its murine counterpart and with much lower affinity to both these sites as compared to the classical octamer motif. The data obtained reveal that phosphorylation of Oct-2 causes a dramatic reduction of its ability to bind to the murine BLR1 non-canonical octamer sequence. At the same time, binding to the octamer sequence of the human BLR1 promoter or to the classical octamer site remained unaffected under the same conditions**.** The latter observation contradicts the previously described data showing inhibition of Oct-2 binding to the octamer site of the H2B promoter (Grenfell *et al.*, 1996). However, the opposite effects of the okadaic acid treatment of intact cells and inhibition of phosphatases in nuclear extracts may reflect the differential activation of kinases *in vivo* and *in vitro*.

A possible explanation for the observed effect of Oct-2 phosphorylation on binding-site recognition may be a direct electrostatic interference of the phosphates coupled to the protein domain responsible for DNA contact, therefore preventing effective binding.

The dynamic *O*-GlcNAc modification may block phosphorylation at the same site. For example, c-myc is *O*-GlcNAc-modified at Thr 58, a known site of phosphorylation and a mutational hot spot in lymphomas (Chou *et al.*, 1995). Recent work suggests that glycosylation of c-myc plays a role in protein stability and subcellular localization of the transcription factor (Kamemura *et al.*, 2002).The Sp-1 transcription factor is one of the best studied *O*-GlcNAc-modified proteins. Work has shown that *O*-GlcNAc modification of Sp-1 appears to modulate its transactivation capability (Roos *et al.*, 1997). Interestingly, OGT activity was shown to be required for maximal transcriptional repression. Furthermore, increased *O*-GlcNAc modification of p53 and NF-UL is associated with increased transcriptional activity (Vosseller *et al.*, 2002). Thus, there is convincing data showing that *O*-GlcNAc modification of the transcriptional machinery is modulating the activity of the protein. It remains to be elucidated how *O*-GlcNAc is modulating the activity at the molecular level and how the *O*-GlcNAc modification of the transcription machinery is itself being regulated.

On the basis of prediction results and available data, we propose that phosphorylation/ dephosphorylation at Ser and Thr residues in the POU domain and C-terminal region are

accompanied by GlcNAc modification. Dephosphorylation of Ser or Thr residues actually involves blocking of that residue by *O*-GlcNAc modification. This suggests that Ying Yang or interplay sites are important when these are involved in regulating Oct-2 functions. In the N-terminal region, which lies in the Oct-2 binding inhibition domain, Thr 150, potential for *O*-GlcNAc modification (Fig. 1), also shows higher potential for phosphorylation, narrowly escaping to touch the threshold line (Fig. 2). This can act as an interplay site in binding inhibition of Oct-2 and in the same fasion Ser 175 may also act as a possible interplay site in regulating binding inhibition of Oct-2 (Figs.1-2). Similarly, Ser255 in the POU domain of murine Oct-2, when phosphorylated, reduces its binding ability to the gene octamer motif to activate the transcription process, whereas dephosphorylation of Ser255 results in increased binding at the time when Ser255 is blocked by *O*-GlcNAc that favours some Oct-2 activation signals for binding to the gene octamer.

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