

Post-Translational Modifications in Activation and Inhibition of Oct-1–DNA Binding Complex in H2B and Other Diverse Gene Regulation: Prediction of Interplay Sites

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ABSTRACT

Octamer DNA binding transcription factors play important roles in housekeeping and specific gene regulations. Octamer DNA binding transcription factor-1 (Oct-1), expressed ubiquitously, is a multifunctional molecule. The binding sites of Oct-1 are the promoters of H2B gene and the genes of snRNA, U2, U6, and 7SK, yet Oct-1 has been described as constitutively expressed transcription factor regulating the expression of housekeeping genes. Diverse tissue-specific genes regulations by Oct-1 include genes for interleukins (IL) 2, 3, 5; the granulocyte-macrophagal colony-stimulating factor, immunoglobulins α , β , Ly9; the endocrine-associated *Pit-1* gene; the genes for gonadoliberin, prolactin, the thyroid transcription factor, and thyrotropin. The most interesting aspect of the gene regulations of Oct-1 includes both activation and inhibition of transcription. These opposite regulations of Oct-1 have been described through presence/absence of a post-translational modification (PTM) in its different domains. We propose a mechanism of interplay of different PTMs or presence/absence of PTMs in the different domains of Oct-1. We also suggest that the absence of phosphorylation and acetylation in G1 and S phases of the cell cycle is associated with interplay of methylation and *O*-GlcNAc modification. This interplay of *O*-GlcNAc modification with the phosphorylation and methylation with acetylation in POU sub-domain of Oct-1 may facilitate the formation of Oct-1–DNA complex, consequently activating H2B gene transcription. Whereas, in G2 and M phases these sites are occupied by phosphate resulting in inhibition of Oct-1–DNA complex formation leading to the suppression of H2B gene transcription. *J. Cell. Biochem.* 114: 266–274, 2013.

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KEY WORDS: HISTONE H2B; INTERPLAY OF PTMS; POST-TRANSLATIONAL MODIFICATIONS; YING YANG SITES

Octamer DNA binding transcription factors belong to a family of structurally related POU (pit, oct, unc) factors containing POU domain. The POU factors are widely found in eukaryotes. The POU domain is a bipartite DNA binding structure consisting of a POU-specific (POUs) domain, which is linked by a flexible linker to a POU homeodomain (POUh) [Herr and Cleary, 1995]. This POU domain is highly conserved among all the members of this family, which is a target for several types of regulatory protein–protein interactions. This is the target region required for sequence-specific

DNA binding [Stern et al., 1989; Ingraham et al., 1990]. Oct family is comprised of different Oct factors including Oct-1, -2, -3, -4, etc. Octamer DNA binding transcription factor-1 (Oct-1) is a DNA binding transcription factor expressed ubiquitously in eukaryotes including mammals [Sturm et al., 1988; Dong and Zhao, 2007]. A consensus octamer sequence 5'-ATGCAAATNA-3' is recognized by Oct-1 regulating the activation of promoters of diverse genes. This consensus sequence is found in promoters and enhancers of diverse vertebrate genes [Verrijzer and Van Der Vliet, 1993]. Though, both

Abbreviations: Oct-1, octamer binding transcription factor-1; *O*-GlcNAc, *N* acetylglucosamine glycosylation; OGT, *O*-GlcNAc transferase; PTMs, post-translational modifications; IL-2, interleukin 2; POU, POU specific domain; POUh, POU homeodomain; Ser, serine; Thr, threonine.

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Oct-1 and Oct-2 recognize the same consensus sequence of DNA, the Oct-1 exhibits the specificity for regulating diverse sets of vertebrate genes [Schaffner, 1989], whereas Oct-2 is specific to bind on promoters and enhancers of immunoglobulin genes in B-cell lineage [Staudt et al., 1988]. The target genes of Oct-1 include promoters of snRNA, mRNA [Tanaka et al., 1992], histone H2B [Fletcher et al., 1987; Labella et al., 1988], interleukins (IL) [Ullman et al., 1991; Wu et al., 1997] and immunoglobulins α , β , and Ly9 [Luo et al., 1992; Strubin et al., 1995].

PTMS SWITCHBOARD IN Oct-1

In the context of Oct-1 binding to promoters of diverse genes, the binding of Oct-1 at one specific promoter will require a specific set of conditions that will be different when binding to the promoter of another gene. In most of the cases post-translational modifications (PTMs), including phosphorylation, *O*-GlcNAc modification, acetylation and methylation, play vital role in regulating the functions of transcription factors and gene expression or repression. Different PTMs can compete on same or neighboring amino acids implicated in regulating different cellular functions. Additionally, presence of one PTM may favor or inhibit the other PTM. A combination of different PTMs on specific amino acids may result in diverse regulations of proteins, hence proteins perform multi-functions. Different amino acids predicted for bearing potential for different PTMs showed that these amino acids are located in different regions of Oct-1, that is, N-terminal, C-terminal, POU domain, POUh domain, and the linker region of POU domain and POUh domain. Switching of one PTM with the other in different transcription factors is the phenomenon that regulates the gene transcription. Moreover, phase-specific PTMs, in cell cycle, seem to be important in regulating the orderly progression of the cell cycle. Phosphorylation of Oct-1 has been documented in regulating the H2B gene expression [Schild-Poulter et al., 2003, 2007]. Involvement of multiple kinases and phosphatases in cell cycle-specific regulation of Oct-1 is evidenced [Roberts et al., 1991]. Sufficient evidences show the involvement of differential phosphorylation of POU domain of different proteins in regulating gene transcription [Tanaka et al., 1992; Caelles et al., 1995; Schild-Poulter et al., 2007]. Additionally, phosphorylation and dephosphorylation is a common mechanism in regulating the functions of different transcription factors [Boulikas, 1995]. This phosphorylation and de-phosphorylation results in regulating the signaling pathways. De-phosphorylation of the Ser/Thr may result in a possibility of an addition of *O*GlcNAc on the -OH function of the same Ser/Thr. Thus, blocking the phosphorylation on those specific sites, consequently resulting in blocking of the function regulated by phosphorylation such as described earlier [Ahmad et al., 2006, 2007].

Similar interplay or interdependence of acetylation and methylation on adjacent Lys residues has also been described in histone H3 [Zhang et al., 2004; Millar and Grunstein, 2006; Taverna et al., 2007] and these acetylated and methylated Lys residues have been documented to function in gene transcription by regulating the interdependent molecular pathways [Lee and Workman, 2007]. Additionally, another interplay of phosphorylation and methylation

on vicinal (Lys 9 and Ser 10; Lys 27 and Ser 28) amino acids has also been evident in histone H3 [Wang et al., 2004] and named as "Methylation/Phosphorylation Switching." This complex interplay of different PTMs regulates diverse protein functions and multi-functions performed by the same protein. Therefore, complex networks of combinatorial PTMs are operative in modulating different functions of proteins. These networks of PTMs can be considered as a "PTMs switchboard" [Everett et al., 2009] regulating protein multi-functionality through various functional switches of different proteins in coordinated manner. Therefore, there is a competition of different enzymes of PTMs to modify same or vicinal amino acids by different modifying groups, for instance, kinases and OGT modify the same or neighboring Ser/Thr either by a phosphate group or by *O*-GlcNAc. These Yin Yang sites predicted to be potential in different domains of Oct-1 may serve as functional switch through differential combination of phosphorylation and *O*-GlcNAc modification at different Ser/Thr in different domains. However, a competition between OGT and different kinases to modify the Oct-1 with a specific combination of phosphorylated and *O*-GlcNAc modified Ser/Thr will depend on the cell cycle stage. Same mechanism of PTMs switching for methylation with acetylation and phosphorylation on adjacent amino acids makes the situation more complex to envisage the functional switches in Oct-1 and other proteins. Unrevealing of the novel mechanisms for these PTMs switchboard will lead to explore new possibilities to maneuver different biological systems and processes from gene expression to signal transduction and cell cycle regulation.

In the current study, utilizing different prediction methods, the modification potential for phosphorylation, *O*-GlcNAc modification, acetylation, and methylation on different amino acid in mouse Oct-1, has been predicted. Additionally, we have also tried to develop an association among different PTMs, including inhibitory and favoring affect of one PTM on the other. The conservation status of these potential sites (amino acids) for different PTMs has also been estimated in diverse vertebrate and invertebrate members to substantiate the modification potential of different amino acid residues predicted by different methods. A mechanism of Oct-1 involvement in H2B gene regulation by interplay sites of different PTMs has been proposed. We suggest that a combination of different PTMs and their interplay with each other may activate or inhibit the Oct-1 function for H2B gene transcription at different stages of the cell cycle.

REGULATION BY PHOSPHORYLATION AND DE-PHOSPHORYLATION

Oct-1 binds directly to the specific DNA motif, but in some cases it requires some co-factors that interact with the DNA binding domain. Binding of these co-factors ultimately result in Oct-1 phosphorylation by different kinases such as protein kinase A (PKA), protein kinase C (PKC), and casein kinase 2 [Grenfell et al., 1996; Inamoto et al., 1997]. Phosphorylation and de-phosphorylation of proteins are important mechanisms to alter the activities and interactions of proteins at specific phases of cell cycle. Phosphorylation of Oct-1 by DNA-protein kinase (DNA-PK) takes place in cell-cycle-dependent manner and this phosphorylation is dependent on physical

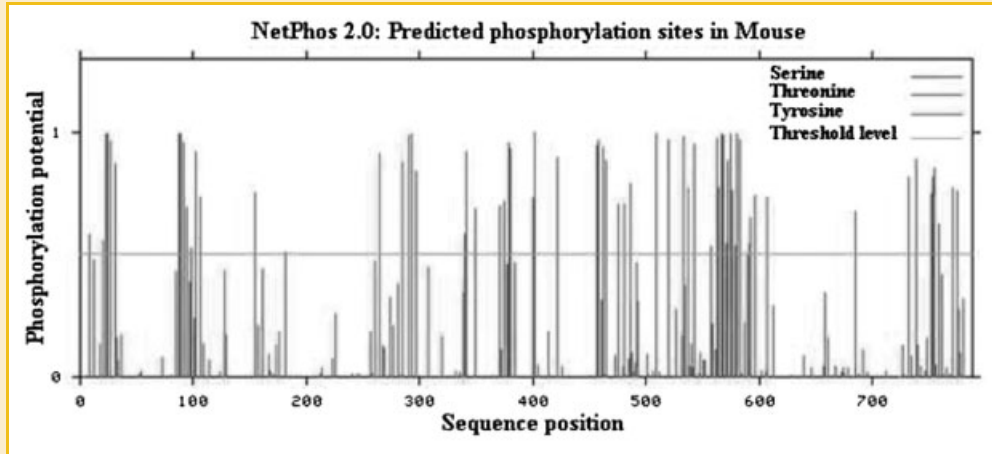


Fig. 1. Graph shows prediction results for phosphorylation by NetPhos 2.0. Vertical lines show phosphorylation potential on Ser (blue color), Thr (green color), and Tyr (red color) in mouse Oct-1 protein. Threshold level is represented by horizontal gray line.

interaction of Oct-1 with Ku antigen [Roberts et al., 1991; Schild-Poulter et al., 2003]. Netphos 2.0 [Blom et al., 1999] has predicted 68 amino acids to have potential for phosphorylation. Amongst the potential predicted 68 sites, the POU sub-domains in mouse Oct-1 include Ser 284, 328, 336, 358, 361, 365, 367, 387, 388, and 409 and Thr 326 and 387 potential phosphorylation sites (Fig. 1, Table I). The conservation status also showed that most of the potential Ser and Thr were conserved in vertebrates (Fig. 3) and invertebrates (Fig. 4). Fifteen specific phosphorylation sites in Oct-1 catalyzed by DNA-PK were already identified [Schild-Poulter et al., 2007]. Out of these, 13 phosphorylation sites lie within the N-terminal domain of Oct-1 protein, a glutamine-rich domain. Moreover, differential phosphorylation in different activation domains of Oct-1 has been described as a mechanism for its activation [Roberts et al., 1991]. A similar control mechanism for Oct-2, a very similar protein to Oct-1 has also been evident [Tanak and Herr, 1990]. It is documented that hyperphosphorylation of Oct-1 during mitosis by p34cdc2 involve in terminating its DNA binding ability [Roberts et al., 1991]. It has been documented that phosphorylation of Oct-1 by PKA also inhibits its DNA binding activity in a sequence-specific manner [Segil et al., 1991]. However, PKA has an interesting feature that it failed to inhibit Oct-1 binding to DNA when there is a simple TAATGARAT motif and an overlapping octamer TAATGRAT motif [Roberts et al., 1991]. Additionally, it has also been reported that regulation of transcription factors mostly takes place by CK-II mediated phosphorylation [Meisner and Czech, 1991]. It has been documented that phosphorylation of Oct-1 results in its failure to bind with DNA during G2 and M-phases [Hwang and Chae, 1989; Schild-Poulter et al., 2003] of the cell cycle.

INTERPLAY OF PHOSPHORYLATION AND O-GlcNAcylation

De-phosphorylation on -OH function of Ser/Thr provides the chances for other modifications like O-GlcNAc modification. It has been observed that alternation of O-GlcNAc modification with

phosphorylation can occur on the same or neighboring Ser/Thr residues (known as Yin Yang sites) [Comer and Hart, 2000]. This interplay between two modifications on the same or neighboring residues is expected to modulate the specific function(s) of Oct-1 protein either by enhancing or inhibiting its function.

To investigate this competition of glycosylation and phosphorylation, a total of 81 Ser/Thr positive potential O-GlcNAc modification sites including 49 Ser and 32 Thr were predicted in different domains of Oct-1 using YinOYang 1.2 (Table I; Fig. 2). Some of these Ser/Thr residues were found conserved in vertebrates (Fig. 3) while others in invertebrates (Fig. 4). The C-terminal part showed a heavy potential for O-GlcNAc modification on various Ser and Thr residues. Amongst the potential phosphorylation and glycosylation sites in Oct-1 37 Ser/Thr sites were also predicted by YinOYang 1.2 to have potential for both phosphorylation and glycosylation (Yin Yang sites) (Fig. 2). From these 37 probable sites for Yin Yang interplay, five sites including Ser 409, 444, 473, and Thr 252, 271 were screened as highly probable sites to act as possible Yin Yang sites on the bases their complete conservation in vertebrate members and semi conservation in invertebrate members (Table I). Additionally, Ser 361, 365 are also the moderately probable Yin Yang sites in the linker region of POU and POUh sub-domains. Whereas Thr 252 and 271 in N-terminal region and Ser 409 is in POUh domain and other Ser 444 and 473 in the proximal C-terminal domain one of the most probable Yin Yang site. As it has been proposed earlier that differential set of PTMs in DNA binding domain of Oct-2 play vital role in its binding to different DNA octamer motifs, whereas interplay of phosphorylation and O-GlcNAc modification in proximal C-terminal residues can regulate the activation of Oct-2 in mouse and human [Ahmad et al., 2004, 2006]. All the sites described above are fully conserved in mammalian and vertebrate members (Fig. 3), whereas, some of them are also conserved even in invertebrate members (Fig. 4).

Additionally, structural model of mouse POU domain was generated by homology modeling utilizing SwissModel [Kiefer et al., 2009] taking human Oct-1 structure 1cqt as template. The model showed that the amino acids Ser 358, 361, 365, and 409 in

TABLE I. Modification Potential of Different Ser/Thr Residues for Phosphorylation, O-GlcNAc Modification and Yin Yang Sites With Their Conservation Level

Amino acid	Conservation		Modification potential			Amino acid	Conservation		Modification potential		
	Inver.	Vert.	Net phos 2.0	YinOyang 1.2	Yin yang site		Inver.	Vert.	Net Phos 2.0	YinOyang 1.2	Yin yang site
Thr7	NC	SC	+	+	Pr	Thr264	C	SC	-	++	-
Ser10	NC	NC	+	+	-	Ser268	SC	SC	-	+	-
Ser11	NC	SC	+	-	-	Ser270	SC	CS	-	+	-
Ser14	NC	SC	+	-	-	Thr271	SC	C	+	+	HPr
Ser18	NC	NC	+	-	-	Thr277	NC	SC	+	-	-
Ser74	NC	SC	+	+	Pr	Ser279	SC	SC	+	-	-
Ser75	NC	NC	+	-	-	Ser284	SC	C	+	-	-
Ser78	SC	SC	+	-	-	Thr326	C	SC	+	-	-
Ser81	SC	SC	+	+	Pr	Ser328	SC	SC	+	-	-
Ser84	NC	NC	-	+++	-	Ser336	C	CS	+	-	-
Ser85	NC	SC	+	+++	Pr	Ser358	NC	CS	+	++	LPr
Ser89	NC	SC	+	+++	Pr	Ser359	CS	C	-	+	-
Ser93	NC	SC	+	+	Pr	Ser361	NC	SC	+	+	Pr
Ser142	NC	CS	+	+	Pr	Ser365	SC	SC	+	+	Pr
Ser144	NC	SC	-	+	-	Ser367	NC	C	+	-	-
Ser148	NC	SC	-	+	-	Thr387	C	C	+	-	-
Thr153	C	SC	-	+	-	Ser388	C	C	+	-	-
Thr163	CS	C	-	++	-	Ser409	SC	C	+	+	HPr
Ser168	SC	C	+	-	-	Ser444	SC	C	+	+++	HPr
Thr200	NC	SC	-	+	-	Ser445	NC	SC	+	+++	Pr
Ser233	NC	C	-	++	-	Ser449	CS	CS	+	-	-
Ser243	NC	C	-	++	-	Ser451	NC	CS	+	-	-
Ser248	NC	CS	-	+	-	Ser462	NC	SC	+	+	-
Thr251	NC	CS	-	+	-	Thr467	NC	CS	+	+++	LPr
Thr252	SC	C	+	++	HPr	Ser469	NC	C	-	++	-
Thr254	SC	C	-	+	-	Thr472	NC	CS	-	++	-
Ser473	SC	C	+	+	H	Thr574	NC	NC	-	++	-
Thr488	NC	C	-	+	-	Thr576	NC	CS	+	++	L
Ser489	NC	C	-	+	-	Thr577	NC	SC	+	++	P
Ser496	NC	S	+	-	-	Ser578	NC	CS	-	+	-
Ser507	NC	N	+	-	-	Thr579	NC	NC	+	++	-
Thr519	NC	N	-	+	-	Ser583	NC	CS	+	-	-
Thr520	NC	N	+	+	-	Thr593	NC	CS	-	+	-
Thr521	NC	N	-	+	-	Thr594	NC	SC	+	-	-
Thr524	NC	C	+	-	-	Ser626	NC	SC	-	+	-
Thr525	NC	C	-	+	-	Ser671	NC	C	+	-	-
Ser527	NC	N	-	+	-	Ser672	NC	SC	-	++	-
Thr528	NC	N	-	+	-	Thr699	NC	NC	-	+	-
Ser529	NC	N	+	-	-	Ser714	NC	CS	-	+	-
Ser538	NC	C	-	+	-	Ser718	NC	NC	+	+	-
Thr539	NC	N	-	+	-	Ser726	NC	CS	+	++	L
Ser544	NC	C	+	+	P	Thr727	NC	NC	-	++	-
Ser545	NC	C	-	+	-	Thr733	NC	NC	-	++	-
Thr548	NC	C	-	+	-	Ser735	NC	SC	-	+	-
Ser549	NC	C	+	+	P	Ser739	NC	CS	+	-	-
Ser551	NC	C	+	+	P	Ser740	NC	NC	+	-	-
Ser553	NC	C	+	+	L	Thr741	NC	NC	+	++	-
Ser555	NC	C	+	+	P	Ser742	NC	CS	+	++	L
Ser557	NC	N	+	+	-	Ser745	NC	C	+	-	-
Ser559	NC	N	+	+	-	Ser755	NC	NC	-	+	-
Ser561	NC	N	+	-	-	Ser757	NC	CS	+	+	L
Thr562	NC	N	-	+	-	Ser761	NC	SC	+	+	P
Ser563	NC	C	+	+	L	Thr762	NS	CS	-	+	-
Ser566	NC	C	+	-	-	Thr763	NC	NC	-	++	-
Ser567	NC	S	+	-	-	Thr764	NC	C	-	++	-
Ser569	NC	S	+	-	-	Ser767	NC	C	-	++	-

C, conserved; NC, non-conserved; CS, conserved substitution; SC, semi-conserved; Pr, probable as Yin Yang site; HPr, highly probable as Yin Yang site; LPr, lower probable; +, positive prediction; ++, highly positive prediction; +++, very highly positive prediction; -, negative.

POU domain of mouse Oct-1 are in the structural positions, which can affect their binding with DNA octamer motif (Fig. 5) differently with different PTMs. Amongst these four sites the three sites including Ser 358, 361, and 365 are located in the linker region of two POUh and POU sub-domains, since the linker region of these sub-domains is involved in producing flexibility resulting in differential binding patterns of the two sub-domains leading to a

capability of Oct-1 to bind on promoters and enhancers of different genes. A phosphate group being small and bearing negative charge is expected to produce different structural and/or conformational changes compared to GlcNAc, a bulky and multiple -OH group containing molecule. A differential combination of phosphorylation and O-GlcNAc modification of these three sites (Ser 358, 361, and 365) in developing flexibility by different is quite probable (Fig. 5).

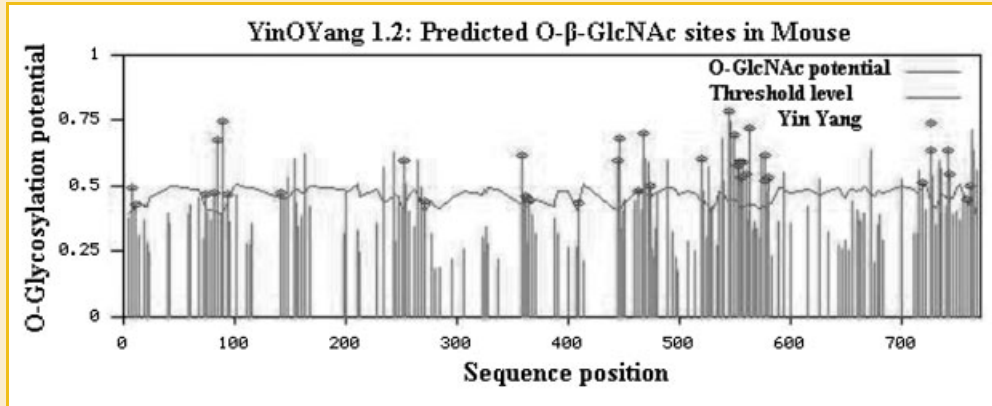


Fig. 2. Graphic presentation of potential for *O*-GlcNAc modification on Ser and Thr (vertical green lines) predicted by YinOYang 1.2 in Mouse Oct-1 protein. The Yin Yang sites (Ser/Thr bearing potential both for phosphorylation and *O*-GlcNAc modification) are represented by an asterisk on the top of vertical lines. The threshold level is represented by horizontal variable blue line.

Similarly, phosphorylated/*O*-GlcNAc modified Ser 409 located in distal part of POUh sub-domain can also produce the structural/conformational changes that can favor/disfavor Oct-1 binding with DNA. Consequently, these possible Yin Yang sites provide the basis for experimental investigations for their role in Oct-1 binding and activation. On the basis of the findings that phosphorylation and dephosphorylation is a common mechanism in regulating the functions of different transcription factors [Boulikas, 1995], and phosphorylated Oct-1 results in repression of binding of Oct-1 with DNA [Roberts et al., 1991], it can be logical to propose that dephosphorylation of Oct-1 facilitates its binding to DNA and ultimate gene expression (Fig. 6). We propose the occupation of the dephosphorylated sites with *O*-GlcNAc resulting in facilitation of binding of Oct-1 with DNA. Moreover, phosphorylation of Oct-1 results in its failure to bind with DNA during G2 and M-phases [Hwang and Chae, 1989; Schild-Poulter et al., 2003] of the cell cycle (Fig. 6). Therefore, we propose a mechanism for H2B gene transcription that during G1 and S-phase the *O*GlcNAc modification on dephosphorylated amino acids may result in activation of the transcription machinery (Fig. 6). We also suggest that during G1 and S phases the Oct-1 may be modified by GlcNAc on one or more dephosphorylated Ser/Thr, in DNA binding domain, acting as Yin Yang sites. Thus, three potential Yin Yang sites, including Ser 361, 365 and 409, in DNA binding domain, two Yin Yang sites, Thr 252 and 271, in N-terminal region and three Yin Yang sites, Ser 444, 445, and 473, in proximal C-terminal domain can serve as the interplay sites for phosphorylation and *O*-GlcNAc modification for H2B gene regulation. When the transcription process is terminated, an enzyme *O*GlcNAcase (OGN) removes the *O*-GlcNAc and these Ser/Thr residues in DNA binding domain, are available for kinase action to phosphorylate them (Fig. 6). Phosphorylation of Oct-1 in its DNA binding domain results in its removal from DNA [Hwang and Chae, 1989; Schild-Poulter et al., 2003] and the cell enters in G2 phase spanning the M-phase (Fig. 5), consequently, inhibition of transcription process of H2B gene during G2 and M phases of the cell cycle may result (Fig. 5).

REGULATION BY METHYLATION AND ACETYLATION

Similarly, the competition of methylation with acetylation on same or adjacent Lys can be as promising as that for phosphorylation and *O*-GlcNAc modification. Prediction of acetylation by PAIL [Li et al., 2006] have shown 15 residues with a higher potential for acetylation including the Lys 9, 273, 297, 302, 316, 338, 342, 344, 384, 385, 399, 436, 438, 454, and 768. While prediction of methylation on Lys residues by MeMo [Chen et al., 2006] resulted in only two potential sites including Lys 73 and 385. Only Lys 385 located in POU domain, also conserved completely in all vertebrates (Fig. 3) and invertebrates (Fig. 4), has been predicted to be potential switch site for methylation and acetylation. Acetylation of basal transcription factors on Lys has been documented to regulate the activity of transcription factors [Imhof et al., 1997]. Additionally, YY1, a sequence-specific DNA binding transcription factor, is also differentially regulated by reversible phosphorylation and acetylation in its different domains involving both activation and repression of different genes during development and differentiation processes [Yao et al., 2001]. Therefore, acetylation of murine Oct-1 Lys 385 located in POUh subdomain can result in providing further strength for repressing the binding of Oct-1 with DNA octamer motif with a mechanism similar to phosphorylation as both bearing negative charges on them (Fig. 6). Similar to phosphorylation, reversible acetylation and deacetylation also act as a mechanism of protein regulations. Methylation of deacetylated Lys 385 can result in inhibition of acetylation mediated binding repression of Oct-1 to DNA octamer motif (Fig. 6).

REGULATION BY METHYLATION AND PHOSPHORYLATION

Moreover, Lys methylation and Ser/Thr phosphorylation switch on vicinal amino acids can also result in some diverse regulations of

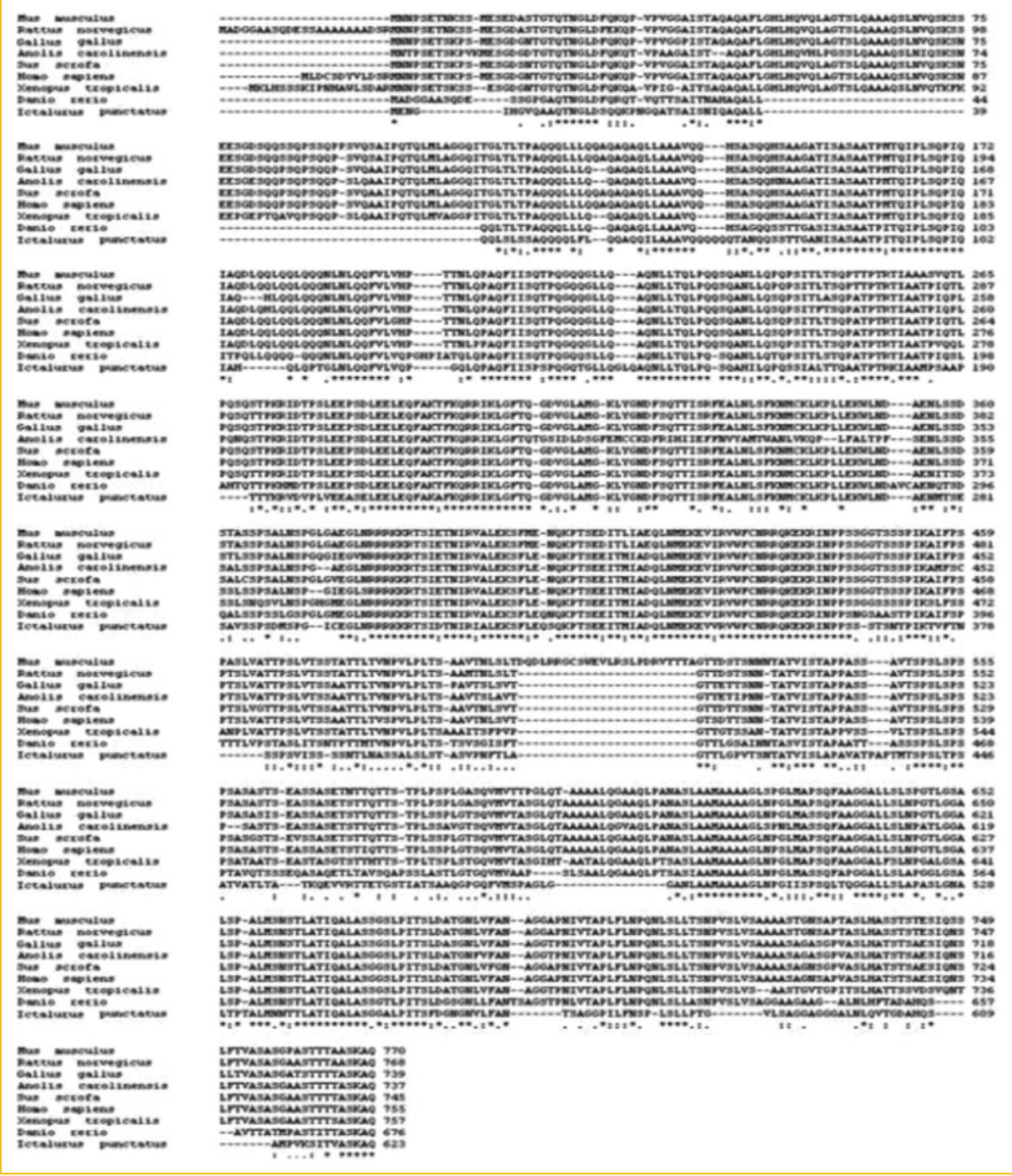


Fig. 3. ClustalW [Thompson et al., 1994] results show the alignment of Oct-1 protein of *Mus musculus* (P25425) retrieved from the Swiss-Prot database [Boeckmann et al., 2003] with eight orthologues from different vertebrates including *Homo sapiens* (GenBank AAM7792.1), *Sus scrofa* (Swiss-Prot Q29076.1), *Rattus norvegicus* (Swiss-Prot P31503) *Gallus gallus* (Ref Seq NM_205472.1), *Xenopus tropicalis* (EMBL CR94271.2), *Danio rerio* (Ref Seq NP_001082798), and *Ictalurus punctatus* (EMBL CAA03984.1) selected by BLAST search [Altschul et al., 1997]. Conservation status of amino acids in different vertebrates is shown by asterisk for conservation in all members, double dot represent conserved substitution, whereas single dot represents semi conserved amino acid residue.

Oct-1. The possible methylation and phosphorylation switch sites in Oct-1 can be Lys 73 with Ser 74 and Lys 385 with Ser 387 and 388. Methylation of these Lys 74 and 385 can result in reduced or complete absence of phosphorylation of the vicinal Ser residues (including Ser 74 and Ser 387 and 388).

CONCLUSIONS

In essence, we suggest that different PTMs and their interplay can regulate gene expression by controlling the transcriptional activation and repression in different phases of the cell cycle.



Fig. 4. A multiple alignment of mouse Oct-1 with two invertebrate orthologs selected by BLAST search utilizing ClustalW. Conservation level of amino acids in the two invertebrates [*Drosophilla melanogaster* (P31368) and *Caenorhabditis elegans* (Ensembl K02B12.1)] is shown by asterisk for conservation in all members, double dot represent conserved substitution, whereas single dot represents semi-conserved amino acid residue.

Switching of one PTM with the other, in general, and interplay of phosphorylation and *O*-GlcNAc modification on -OH function of Ser/Thr, in particular, is quite probable in different domains of murine Oct-1 and can be investigated experimentally as a possible mechanism for modulation of H2B and other genes regulation. Here, we suggest an involvement of acetylation for

producing similar affects as those of phosphorylation and those of methylation similar to those of *O*-GlcNAc modification (Fig. 6). The PTMs switching with each other can act as a functional switch of proteins resulting to produce an ability in proteins to perform multifunctions. This can serve as a general mechanism to regulate gene transcription initiation and inhibition in

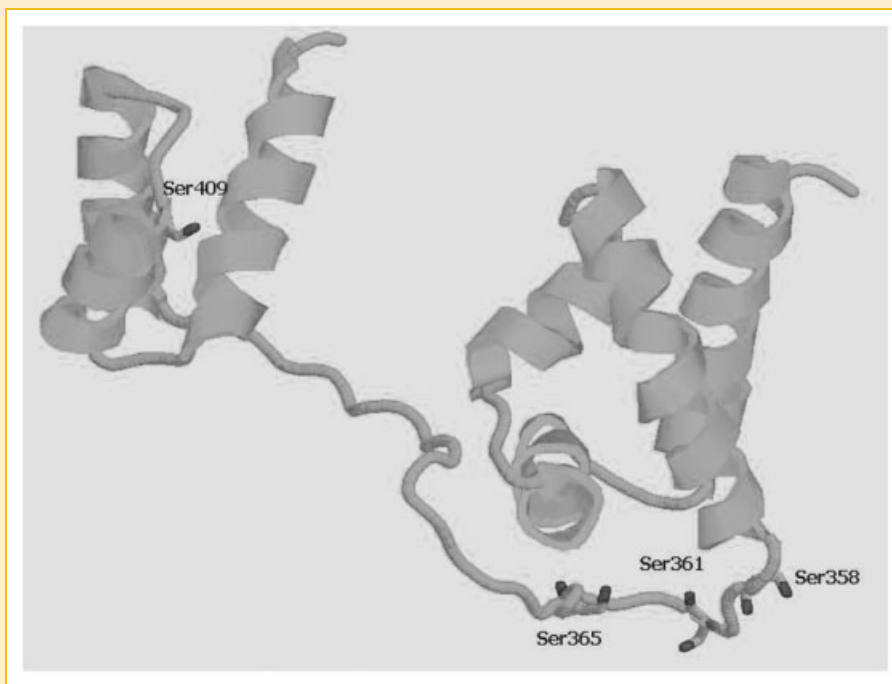


Fig. 5. Homology model of POU domain of mouse Oct-1 showing the four sites (Ser358, 361, 365, and 409) in stick style with CPK color scheme. Three of them (Ser 358, 361, and 365), in the linker region of POUAs and, POUH are in a position that can affect the flexibility of linker region by differential combinations of different PTMs resulting in diverse gene regulations by Oct-1. The fourth amino acid, Ser 409, is located in distal part of POUH sub-domain and different PTMs can also result in structural/conformational changes leading to diverse gene regulations.

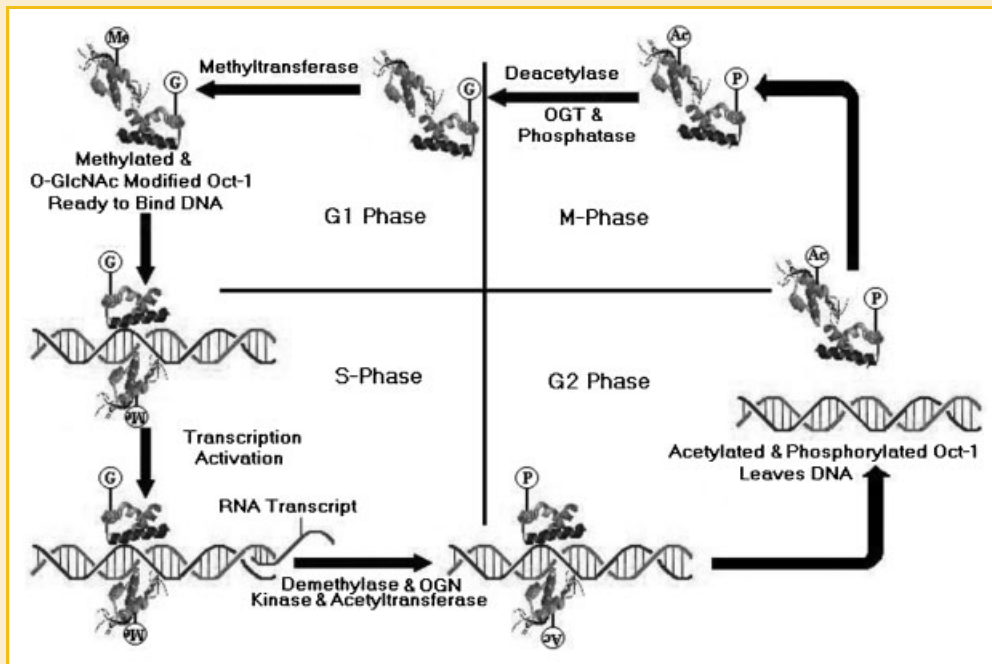


Fig. 6. A scheme of the proposed mechanism for activation and inhibition of DNA-Oct-1 complex formation regulated by alternative and cell cycle phase specific phosphorylation, acetylation with *O*-GlcNAc modification and methylation, respectively. During G2 and M phase phosphorylation and acetylation of Oct-1 in its DNA binding domain result in inhibition of DNA-Oct-1 complex formation, whereas in G1 and S phases of the cell cycle, the *O*-GlcNAc modification and methylation result in activation of DNA-Oct-1 complex formation.

different phases of the cell cycle for different transcription factors including Oct-1.

Specifically, interplay of phosphorylation and *O*-GlcNAc modification on Ser 358, 361 and 365 in linker region of POU_s and POU_h sub-domains can result in changing its flexibility resulting in binding of Oct-1 to different genes promoters and enhancers. Similarly, Ser 409 in POU_h domain can also play a same role through interplay of phosphorylation and *O*-GlcNAc modification by inducing structural/conformational changes resulting in differential binding of POU_h sub-domain to different gene promoters and/or enhancers.

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REFERENCES

- Ahmad I, Walker-Nasir E, Shakoori AR, Saleem M, Rafik MS, Hussain SA, Nasir-ud-Din. 2004. Interplay of glycosylation and phosphorylation in Murine Oct-2 regulates its POU domain binding. *Pak J Zool* 36:333-338.
- Ahmad I, Hoessli DC, Walker-Nasir E, Rafik SM, Shakoori AR, Nasir-ud-Din. 2006. Oct-2 DNA binding transcription factor: Functional consequences of phosphorylation and glycosylation. *Nucleic Acids Res* 34:175-184.
- Ahmad I, Hoessli DC, Gupta R, Walker-Nasir E, Rafik SM, Choudhary MI, Shakoori AR, Nasir-ud-Din. 2007. *In silico* determination of intracellular glycosylation and phosphorylation sites in human selectins: Implication for biological function. *J Cell Biochem* 100:1558-1572.
- Altschul SF, Madden TL, Schaffer AA, Zhang J, Zhang Z, Miller W, Lipman DJ. 1997. Gapped BLAST and PSIBLAST: A new generation of protein database search programs. *Nucleic Acids Res* 25:3389-3402.
- Blom N, Gammeltoft S, Brunak S. 1999. Sequence- and structure-based prediction of eukaryotic protein phosphorylation sites. *J Mol Biol* 294:1351-1362.
- Boeckmann B, Bairoch A, Apweiler R, Blatter MC, Estreicher A, Gasteiger E, Martin MJ, Michoud K, O'donovan C, Phan I, Pilboud S, Schneider M. 2003. The SWISS-PROT protein knowledgebase and its supplement TrEMBL in 2003. *Nucleic Acids Res* 31:365-370.
- Boulikas T. 1995. Phosphorylation of transcription factors and control of the cell cycle. *Crit Rev Eukaryot Gene Expr* 5:1-77.
- Caelles C, Hennemann H, Karin M. 1995. M-phase-specific phosphorylation of the POU transcription factor GHF-1 by a cell cycle-regulated protein kinase inhibits DNA binding. *Mol Cell Biol* 15:6694-6701.
- Chen H, Xue Y, Huang N, Yao X, Sun Z. 2006. MeMo: A web tool for prediction of protein methylation modifications. *Nucleic Acids Res* 34:W249-W253.
- Comer FI, Hart GW. 2000. *O*-Glycosylation of nuclear and cytosolic proteins—Dynamic interplay between *O*-GlcNAc and *O*-phosphate. *J Biol Chem* 275:29179-29182.
- Dong B, Zhao FQ. 2007. Involvement of the ubiquitous Oct-1 transcription factor in hormonal induction of β -casein gene expression. *Biochem J* 401:57-64.
- Everett L, Vo A, Hannehalli S. 2009. PTM-Switchboard—A database of posttranslational modifications of transcription factors, the mediating enzymes and target genes. *Nucleic Acids Res* 37:D66-D71.
- Fletcher C, Heintz N, Roeder RG. 1987. Purification and characterization of OTF-1, a transcription factor regulating cell cycle expression of a human histone *H2b* gene. *Cell* 51:773-781.

- Grenfell SJ, Latchman DS, Thomas NS. 1996. Oct-1 and Oct-2 DNA-binding site specificity is regulated *in vitro* by different kinases. *Biochem J* 315:889–893.
- Herr W, Cleary MA. 1995. The POU domain: Versatility in transcriptional regulation by a flexible two-in-one DNA-binding domain. *Genes Dev* 9:1679–1693.
- Hwang I, Chae CB. 1989. S-phase-specific transcription regulatory elements are present in a replication-independent testis-specific H2B histone gene. *Mol Cell Biol* 9:1005–1013.
- Imhof A, Yang XJ, Ogryzko VV, Nakatani Y, Wolffe AP, Ge H. 1997. Acetylation of general transcription factors by histone acetyltransferases. *Curr Biol* 7:689–692.
- Inamoto S, Segil N, Pan ZQ, Kimura M, Roeder RG. 1997. The cyclin dependent kinaseactivating kinase (CAK) assembly factor, MAT1, targets and enhances CAK activity on the POU domains of octamer transcription factors. *J Biol Chem* 272:29852–29858.
- Ingraham HA, Flynn SE, Voss JW, Albert VR, Kapiloff MS, Wilson L, Rosenfeld MG. 1990. The POU specific domain of pit-1 is essential for sequence-specific, high affinity DNA binding and DNA-dependent pit-1 interactions. *Cell* 61:1021–1033.
- Kiefer F, Arnold K, Künzli M, Bordoli L, Schwede T. 2009. The SWISS-MODEL Repository and associated resources. *Nucleic Acids Res* 37:387–392.
- Labella F, Sive HL, Roeder RG, Heintz N. 1988. Cell-cycle regulation of a human histone *H2b* gene is mediated by the H2B subtype-specific consensus element. *Genes Dev* 2:32–39.
- Lee KK, Workman JL. 2007. Histone acetyltransferase complexes: One size doesn't fit all. *Nat Rev Mol Cell Biol* 8:284–295.
- Li A, Xue Y, Jin C, Wang M, Yao X. 2006. Prediction of Nepsilon-acetylation on internal lysines implemented in Bayesian Discriminant Method. *Biochem Biophys Res Commun* 350:818–824.
- Luo Y, Fujii H, Gerster T, Roeder RG. 1992. A novel B cell-derived coactivator potentiates the activation of immunoglobulin promoters by octamer-binding transcription factors. *Cell* 71:231–241.
- Meisner H, Czech MP. 1991. Phosphorylation of transcriptional factors and cell-cycledependent proteins by casein kinase II. *Curr Opin Cell Biol* 3:474–483.
- Millar CB, Grunstein M. 2006. Genome-wide patterns of histone modifications in yeast. *Nat Rev Mol Cell Biol* 7:657–666.
- Roberts SB, Segil N, Heintz N. 1991. Differential phosphorylation of the transcription factor Oct1 during the cell cycle. *Science* 253:1022–1026.
- Schaffner W. 1989. How do different transcription factors binding the same DNA sequence sort out their jobs? *Trends Genet* 5:37–38.
- Schild-Poulter C, Matheos D, Novac O, Cui B, Giffin W, Ruiz MT, Price GB, Zannis-Hadjopoulos M, Haché RJ. 2003. Differential DNA binding of Ku antigen determines its involvement in DNA replication. *DNA Cell Biol* 22:65–78.
- Schild-Poulter C, Shih A, Tantin D, Yarmowich NC, Soubeyrand S, Sharp PA, Haché RJ. 2007. DNA-PK phosphorylation sites on Oct-1 promote cell survival following DNA damage. *Oncogene* 26:3980–3988.
- Segil N, Roberts SB, Heintz N. 1991. Mitotic phosphorylation of the Oct-1 homeodomain and regulation of Oct-1 DNA binding activity. *Science* 254:1814–1816.
- Staudt L, RG M, Clerc H, Singh JH, Lebowitz PA, Baltimore SD. 1988. Cloning of a lymphoid-specific cDNA encoding a protein binding the regulatory octamer DNA motif. *Science* 241:577–580.
- Stern S, Tanaka M, Herr W. 1989. The Oct-1 homeodomain directs formation of a multiprotein-DNA complex with the HSV transactivator VP16. *Nature* 341:624–628.
- Strubin M, Newell JW, Matthias P. 1995. OBF-1, a novel B cell-specific coactivator that stimulates immunoglobulin promoter activity through association with octamer-binding proteins. *Cell* 80:497–506.
- Sturm RA, Das G, Herr W. 1988. The ubiquitous octamer-binding protein Oct-1 contains a POU domain with a homeobox subdomain. *Genes Dev* 2:1582–1599.
- Tanak M, Herr W. 1990. Differential transcriptional activation by Oct-1 and Oct-2: Interdependent activation domains induce Oct-2 phosphorylation. *Cell* 60:375–386.
- Tanaka M, Lai JS, Herr W. 1992. Promoter-selective activation domains in Oct-1 and Oct-2 direct differential activation of an snRNA and mRNA promoter. *Cell* 68:755–767.
- Taverna SD, Ueberheide BM, Liu Y, Tackett AJ, Diaz RL, Shabanowitz J, Chait BT, Hunt DF, Allis CD. 2007. Long-distance combinatorial linkage between methylation and acetylation on histone H3N termini. *Proc Natl Acad Sci USA* 13(104):2086–2091.
- Thompson JD, Higgins DG, Gibson TJ. 1994. CLUSTALW: Improving the sensitivity of progressive multiple sequence alignment through sequence weighting, position-specific gap penalties and weight matrix choice. *Nucleic Acids Res* 22:4673–4680.
- Ullman KS, Flanagan WM, Edvards CA, Crabtree GR. 1991. Activation of early gene expression in T lymphocytes by Oct-1 and an inducible protein, OAP. *Science* 254:558–562.
- Verrijzer CP, Van Der Vliet PC. 1993. POU domain transcription factors. *Biochim Biophys Acta* 1173:1–21.
- Wang Y, Fischle W, Cheung W, Jacobs S, Khorasanizadeh S, Allis CD. 2004. Beyond the double helix: Writing and reading the histone code. *Novartis Found Symp* 259:3–17.
- Wu CD, Lai EJ, Huang N, Wen X. 1997. Oct-1 and GGAAT/enhancer-binding protein (C/EBP) bind to overlapping elements within the interleukin-8 promoter. *J Biol Chem* 24:2396–2403.
- Yao Y-L, Yang W-M, Seto E. 2001. Regulation of transcription factor YY1 by acetylation and deacetylation. *Mol Cell Biol* 21:5979–5991.
- Zhang K, Siino JS, Jones PR, Yau PM, Bradbury EM. 2004. A mass spectrometric “Western blot” to evaluate the correlations between histone methylation and histone acetylation. *Proteomics* 4:3765–3775.