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MINIREVIEW

Sp1 Phosphorylation and Its Regulation of Gene Transcription[▽]

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Sp1 is a ubiquitously expressed, prototypic C_2H_2-type zinc finger-containing DNA binding protein that can activate or repress transcription in response to physiologic and pathological stimuli. It was originally found to selectively transactivate the early and late simian virus 40 promoters without influencing numerous other promoters (16) and has since been shown to regulate the expression of thousands of genes implicated in the control of a diverse array of cellular processes, such as cell growth (26, 57), differentiation (48), apoptosis (26), angiogenesis (43), and immune response (25), to name just a few. Sp1 is a 785-amino-acid, 100- to 110-kDa nuclear transcription factor which regulates gene expression via multiple mechanisms. It binds GC-rich motifs (such as 5′-G/T-GGGCGG-G/A-G/A-C/T-3′ or 5′-G/T-G/A-GGCG-G/T-G/A-G/A-C/T-3′) with high affinity (4, 27, 28) and can regulate the expression of TATA-containing and TATA-less genes via protein-protein interactions or interplay with other transcription factors (47), such as Ets-1 (56), c-myc (51), c-Jun (44), Stat1 (6), and Egr-1 (31), and/or components of the basal transcriptional machinery. Sp1 has been linked to chromatin remodeling through interactions with chromatin-modifying factors such as p300 (62) and histone deacetylases (HDACs) (71).

Sp1 was once thought to serve mainly as a constitutive activator of housekeeping genes. However, growing evidence indicates that phosphorylation, acetylation, sumoylation, ubiquitylation, and glycosylation are among the posttranslational modifications that can influence the transcriptional activity and stability of Sp1. Here we will discuss recent developments in our understanding of the role of posttranslational modifications influencing Sp1-dependent transcription, focusing mainly on phosphorylation.

PHOSPHORYLATION OF Sp1

It is now clear that the phosphorylation state of Sp1 influences its transcriptional activity (Table 1). For example, Sp1 phosphorylation is linked with shear stress induction of the tissue factor promoter (39), hepatocyte growth factor-inducible vascular endothelial growth factor (VEGF)/vascular permeability factor gene transcription (46), epidermal growth factor (EGF)-inducible apolipoprotein A-I expression (73), and T-cell receptor-inducible interleukin-21 receptor gene expression (68). Sp1 is phosphorylated by various kinases at different sites within the protein (Table 2), and the influence of these modifications on the function of the transcription factor is only beginning to be understood. Kinases regulating Sp1 binding or transactivity include cyclin-dependent kinase (CDK) (17), atypical protein kinase C-ζ (PKC-ζ) (30, 52, 63), extracellular signal-regulated kinase (ERK) (3, 46), casein kinase II (CKII) (1), and DNA-dependent protein kinase (11). Human Sp1 (NCBI accession number P08047) has 61 putative phosphorylation sites, with 48 of these residues being Ser, 10 Thr, and 3 Tyr (from NetPhos 2.0; data not shown). Sp1 phosphorylation can both positively and negatively influence DNA binding and transcriptional activity. A survey of phosphorylated residues in Sp1 regulating its activity is illustrated in Fig. 1 and discussed below.

The influence of Sp1 phosphorylation on altered gene expression was first observed in the context of human immunodeficiency virus (HIV). HIV type 1 (HIV-1) Tat and Sp1 form a tight protein-protein complex compared with other transcription factors such as Oct and NF-κB (24). This protein-protein interaction was found in a subsequent study by the same group to be mediated via Tat phosphorylation of Sp1 at Ser131 in Sp1 and amino acids 30 to 55 in Tat (11). Phosphorylation of Sp1 is dependent on DNA-dependent protein kinase binding Tat. HIV-1 long terminal repeat expression was limited by Sp1 phosphorylation and not by Sp1 total amount, indicating the importance of Sp1 modification as a trigger of inducible gene expression.

KINASES PHOSPHORYLATING Sp1 INFLUENCE ITS DNA BINDING AND TRANSCRIPTIONAL ACTIVITY

Fojas de Borja et al. showed that, in cyclin A-overexpressing NIH 3T3 cells, Sp1 is phosphorylated by cyclin A-dependent kinase at (murine) Ser61 (corresponding to Ser59 in human Sp1) (17). Moreover, these investigators found that cyclin A forms a complex with CDK, which phosphorylates Sp1 at its N terminus and increases its interaction with the dihydrofolate reductase promoter. Increased DNA binding by Sp1 was independent of changes in total levels of Sp1 (17).

Studies by Milanini-Mongiat and colleagues showed that p42/p44 mitogen-activated protein kinase (MAPK) phosphorylates Sp1 at two Thr residues: Thr453 and Thr739 (46). When these sites were mutated to Ala, the MAPK-dependent transcriptional activity of Sp1 in the context of the VEGF promoter, in Sp1-deficient *Drosophila* SL2 cells, decreased by 50%. Both sites are required for Sp1 activity after ERK acti-

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TABLE 1. Agonists, kinases, and Sp1 phosphorylation[a]

Protein	Cell type	Stimulus	Kinase(s)	DNA binding[b]	Transcriptional change[b]	Reference
PDGFR-α	SMCs	FGF-2	ERK	↑	↓	3
PDGF-B	SMCs	NOG	PKC-ζ	↑	↑	52
PDGF-D	SMCs	Ang II	PKC-ζ	↑	↑	63
FasL	SMCs	CAM	PKC-ζ	↑	↑	30
MMP1	Rat endothelial cells	Shear stress	PKC-ζ	↑	↓	33
Nitric oxide synthase	HUVEC	Lysophosphatidylcholine	PP2A	↑	↑	13
VEGF	Fibroblasts	Neu differentiation factor	p42/p44 MAPK	↑	↑	46
Tyrosine kinase	Fibroblasts	Cyclin A	CDK-2	↑	↑	18
DHFR	Fibroblasts	Cyclin A	CDK-2	↑	↑	17
Gastrin	Gastric carcinoma cells	EGF	ERK2	↑	↑	12
VEGF	Keratinocytes	HGF	PI3K, MEK1/2, PKC-ζ		↑	54
Cyclin D3	Megakaryocytes	Thrombopoietin	PP1	↓	↓	67
HIV-1 LTR	T lymphocytes	CD2/CD28	PP2A	↓	↓	35
Aldolase	Hepatoma cells	Glucose	PP1	↓	↓	58
Pyruvate kinase	Hepatoma cells	Glucose	PP1	↓	↓	58
Acetyl-coenzyme A carboxylase	Proadipocytes	Glucose	PP1	↓	↓	14
MMP2	Human lung cancer	NSAID	ERK	↑	↑	50
α-ENaC2	Lung epithelial cells	Thrombopoietin	PP1	↑	↑	9
LHR	Choriocarcinoma and breast cancer cells	TSA	PKC-ζ	↑	↑	69
HO-1	Human embryonic kidney	NGF	PI3K, PKC-ζ, MEK, ERK	↑	↑	55

[a] Abbreviations: CAM, calmodulin; NSAID, nonsteroidal anti-inflammatory drug; NOG, nogalamycin; NGF, nerve growth factor; LTR, long terminal repeat; HUVEC, human umbilical vein endothelial cells; DHFR, dihydrofolate reductase; α-ENaC2, α Na^+ channel 2; MMP1, matrix metalloproteinase 1; HGF, hepatocyte growth factor.
[b] ↑, increase; ↓, decrease.

vation (46). Building on these findings, Legros et al. demonstrated that the inhibitor of Bcr/Abl protein tyrosine kinase imatinib mesylate (STI571) perturbs VEGF transcription in K562 cells by inhibiting ERK and reducing Sp1's and Sp3's affinity for a proximal binding element in the VEGF promoter (38). However, this study did not demonstrate the specific involvement of Thr453 and Thr739 phosphorylation in imatinib's influence on Sp1 binding.

We showed that, in rat pup smooth muscle cells (SMCs), fibroblast growth factor 2 (FGF-2) negatively regulates platelet-derived growth factor (PDGF) receptor alpha (PDGFR-α) expression. Transcription of all known PDGF ligands (15, 31, 32, 40, 45, 53) and at least PDGFR-α (2, 3) is under the control of Sp1 and related zinc finger proteins. Mutation of both Thr453 and Thr739 in Sp1 perturbed FGF-2 repression of PDGFR-α transcription (3). FGF-2 stimulated Sp1 phosphorylation in an ERK- but not p38-dependent manner and increased Sp1 interaction with the PDGFR-α promoter (3). Recent studies by Chuang et al. indicate that Jun N-terminal kinase 1 phosphorylates Sp1 at Thr278 and Thr739 (10), protecting the transcription factor from ubiquitin-dependent degradation and increasing its stability during mitosis in tumor cell lines. Overexpression of green fluorescent protein-Sp1 in HeLa cells increased HeLa cell proliferation, whereas overexpression of green fluorescent protein-Sp1 mutants (T278A and T739A, alone or together) did not. Accumulation of Sp1 in tumors may therefore be a consequence of Jun N-terminal kinase 1-dependent Sp1 phosphorylation (10).

TABLE 2. Residues phosphorylated in Sp1

Region in Sp1	Residue	Reference(s)
N-terminal activation domain	Ser59	17, 65
N-terminal activation domain	Ser101	22, 47a
N-terminal activation domain	Ser131	11
N-terminal activation domain	Thr278	10
N-terminal activation domain	Thr355	73
N-terminal activation domain	Thr453	3, 46
High-charge-density region/zinc finger 1	Ser641	69
Zinc finger 2	Thr668	1, 63
Zinc finger 2	Ser670	63
Zinc finger 2 (just outside)	Thr681	63, 65
C-terminal D domain	Thr739	3, 10, 46

Sp1 phosphorylation at Thr355 (Thr266 in the originally reported shorter form of Sp1) activates the ApoA1 promoter in HepG2 cells exposed to EGF and insulin (72). This modification of Sp1 by EGF and insulin was mediated via the MAPK/ERK pathway. However with insulin, PKC also influenced Sp1 phosphorylation. Deletion and mutational analysis revealed that the insulin-responsive core element within the ApoA1 promoter was responsible for the actions of EGF. This motif bound Sp1 specifically but not Sp2 or Sp3 (72). When Thr355 in Sp1 was mutated to Ala in cells stimulated with EGF, ApoA1 was no longer induced compared with the wild type, indicating the functional importance of this residue (72).

Studies performed a decade ago with rat hepatoma cells revealed that Sp1 is phosphorylated by CKII at a consensus site located within the second zinc finger, Thr579 (corresponding to Thr668 in human Sp1) (1). Phosphorylation of Sp1 at this site reduced Sp1's capacity to bind DNA without affecting total levels of Sp1. Mutation of Thr579 in the consensus CKII site did not eliminate CKII phosphorylation of Sp1, though it did perturb CKII's ability to inhibit Sp1 binding to DNA in vitro. Treatment of K562 cells with okadaic acid to inhibit endoge-

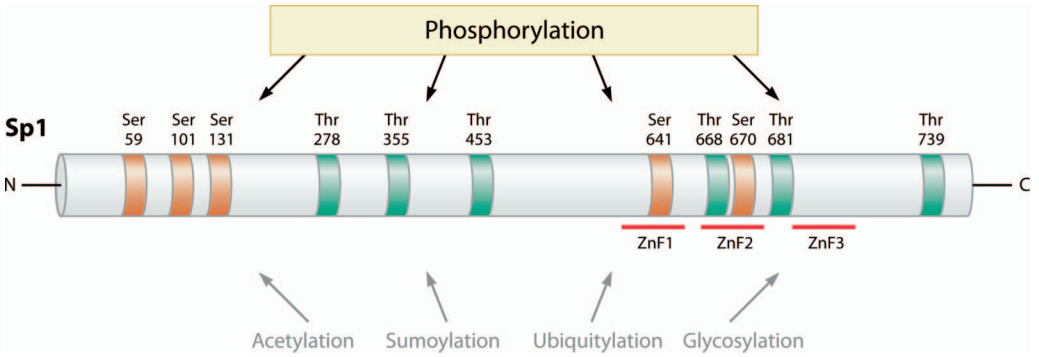

FIG. 1. Published Ser and Thr phosphorylation sites in Sp1. Ser and Thr residues are indicated in red and blue, respectively. ZnF, zinc finger domain. Acetylation, sumoylation, ubiquitylation, and glycosylation are among other posttranslational modifications that influence the transcriptional activity and stability of Sp1. See text for details.

nous phosphatase increased Sp1 phosphorylation and reduced DNA binding activity (1).

We recently demonstrated that Thr668, along with Ser670 (and Thr681), is a target of phosphorylation by PKC-ζ using a combination of approaches including in vitro peptide and protein phosphorylation analysis with Ala mutant counterparts, mass spectrometry phosphopeptide analysis, and coimmunoprecipitation analysis (63). This follows investigations by Pal et al. demonstrating that PKC-ζ binds to and phosphorylates the zinc finger region of Sp1 (49). Phospho-specific antibodies targeting pThr668/pSer670 or pThr681 were used in a variety of applications to demonstrate that angiotensin II (Ang II) stimulates PKC-ζ (pThr410) and Sp1 phosphorylation via the AT1 receptor in SMCs without influencing total levels of Sp1. Extending our previous findings that Ang II activates the PDGF-D promoter and that Sp1 binds the PDGF-D promoter (40), we found that Ang II-inducible PDGF-D expression involves PKC-ζ-dependent Sp1 phosphorylation at Thr668, Ser670, and Thr681. Triple mutation, but not single and double mutations, of these amino acids blocked Ang II activation of the PDGF promoter despite the fact that the triple mutation did not perturb Sp1's ability to bind DNA.

We found phosphorylated Sp1 (p681) in SMCs of human atherosclerotic plaques, as well as in SMCs of the mechanically injured rat carotid artery wall. This is the first demonstration of phosphorylated Sp1 in diseased animal and human tissue (63). We previously showed that activated PKC-ζ (pThr410) is also expressed in atherosclerotic plaques in the context of the Fas ligand (29). Numerous other studies have linked aberrant phosphorylation of Tyr, Ser, or Thr residues to cancer (36), diabetes (41), hypertension (21), and cardiac hypertrophy (20). Earlier studies by Vicart et al. found that Thr681 and Ser59 are targets for protein phosphatase 2A (PP2A) (65). Interestingly these investigators found that Ser59 and Thr681 can function independently or together for PP2A regulation of Sp1 activity during interphase (65).

CHROMATIN RECRUITMENT AND DNA DAMAGE

More recently, Vicart and colleagues found that the phosphorylation state of Ser59 influences Sp1's association with chromatin and Sp1 global O glycosylation (65). Dephosphorylated Sp1 is preferentially recruited to chromatin, and Ser59 is a target of PP2A. Although a detailed mechanism is yet to be established, the increased association of Sp1 with chromatin via PP2A's effects on Ser59 may serve as a permissive step in transcription. They further showed that Thr681, which resides in the second zinc finger linker, is another target of PP2A.

Sp1 is hyperphosphorylated during DNA damage. Iwahori et al. recently demonstrated that Ser101 is phosphorylated in response to ionizing radiation, UV irradiation, or hydroxyurea-induced replicative stress (22). Ser101 is phosphorylated by ataxia telangiectasia mutated (ATM) kinase (22, 47a). The proportion of chromatin-bound Sp1 phosphorylated at Ser101 increases rapidly after ionizing radiation (22), and Sp1 colocalizes in the nuclei with ATM, which is itself phosphorylated at Ser1981, at sites of double-strand breaks.

Sp1 phosphorylation plays a role in the derecruitment of repressor proteins from the promoter. Zhang and colleagues used coimmunoprecipitation studies to demonstrate that treatment with the HDAC inhibitor trichostatin A (TSA) stimulated Sp1 and PKC-ζ interaction in a TSA dose-dependent manner (69). TSA activated PKC-ζ (Thr410 phosphorylation [8]) and Sp1 phosphorylation at Ser, which was blocked by PKC-ζ small interfering RNA or a dominant negative, whereas Sp3 was not phosphorylated in response to TSA. Mutational analysis revealed that PKC-ζ phosphorylation of Sp1 at Ser641 is required for a critical role in TSA-inducible luteinizing hormone receptor (LHR) gene activation (69). Further studies using wortmannin or LY294002 demonstrated that phosphatidylinositol 3-kinase (PI3K) acts upstream of PKC-ζ and that the activity of this kinase is needed for PKC-ζ-mediated phosphorylation of Sp1. Sp1 phosphorylation (at Ser641) is required for the release of the pRB homologue p107 inhibitor protein from the LHR gene promoter, where it acts as a repressor (70). Interestingly, cell-type-specific differences were apparent despite the involvement of Ser641. TSA stimulated LHR gene promoter-localized histone hyperacetylation in choriocarcinoma (JAR) cells without influencing DNA methylation status, whereas in mammary carcinoma cells (MCF-7) cells, TSA induced a 160-fold derepression of LHR gene expression through histone hyperacetylation and DNA demethylation at its promoter (69).

OTHER POSTTRANSLATIONAL MODIFICATIONS INFLUENCING Sp1 ACTIVITY

Acetylation, sumoylation, ubiquitylation, and glycosylation are among other posttranslational modifications that influence the transcriptional activity and stability of Sp1. Glycosylation occurs at *O*-GlcNAc linkages at Ser and Thr residues in Sp1 (23) and is reversible by the action of *O*-GlcNAc-selective *N*-acetyl-D-glucosaminidase (34). Sp1 glycosylation can either stimulate or repress DNA binding and transcription (23). Studies with rat hepatoma cells showed that Sp1 glycosylation is critical for nuclear localization; however, once in the nucleus, Sp1 may be phosphorylated, thereby activating calmodulin gene expression (42). When Sp1 is hypoglycosylated under conditions of nutrition insufficiency, it is quickly proteolytically degraded (19), indicating that *O*-GlcNAc modification of Sp1 may play a role as a nutritional checkpoint (19).

Acetylation of Sp1 occurs in the DNA binding domain (62), and numerous studies investigating this modification have used HDAC inhibitors such as TSA. Recent studies by Chen et al. suggested that TSA treatment inhibits EGF induction of 12(*S*)-lipoxygenase by increasing Sp1 acetylation, which reduced Sp1 binding to the 12(*S*)-lipoxygenase promoter (7). Acetylation of Sp1 in this region along with the recruitment of c-Jun transcription factor and p300 increases the activity of the 12(*S*)-lipoxygenase gene promoter (7). The acetylation of Sp1 appears to play a protective role in neuronal cells undergoing oxidative stress via elevated cyclooxygenase 2 (COX-2) expression (37). COX-2 also plays a protective role in the context of vascular injury. COX-2-deficient mice undergo increased ischemic injury in the heart (5).

More recently it has been discovered that Sp1 is sumoylated at the N terminus under basal conditions, which negatively regulates Sp1 transcriptional activity (59). Sumoylation controls transcription by initiating chromatin structure changes that render DNA inaccessible to the transcriptional machinery. Sp3 sumoylation can suppress transcription by compacting repressive chromatin and provoking local heterochromatic gene silencing (61). SUMO-dependent transcriptional repression appears to be independent of HDACs (64). Horowitz and colleagues found that Sp3 isoforms (Sp3, M1, and M2) are sumoylated at Lys551 (60). Recent studies indicate that levels of sumoylated Sp1 are attenuated during tumorigenesis (66). Sumoylated Sp1 is found in the cytosol interacting with rpt6, and this interaction leads to increased Sp1 proteolysis and degradation (66). Decreased levels of cytosolic Sp1 as a consequence of sumoylation reduce the pool of Sp1 that would otherwise migrate to the nucleus (60). Modifications of Sp1 can occur simultaneously and synergistically. For example, glycosylation of Sp1 can lead to its translocation from the cytoplasm to the nucleus, where it is modified via phosphorylation to activate calmodulin gene expression (42). Sp1 phosphorylation and desumoylation can also occur simultaneously (59).

The influence of phosphorylation on the transcriptional activity of Sp1, particularly how it changes Sp1's affinity for DNA and/or other proteins, its roles in pathobiology, and the impact of other posttranslational modifications, is only beginning to become apparent, and further work is needed. For example, although we understand that certain kinases and residues in Sp1 mediate Sp1-dependent transcription, as in the case of FasL (30), PDGF B-chain (52), PDGF-D (63), PDGFR-α (3), and VEGF (49), it is unclear whether or how Sp1 phosphorylation influences chromatin remodeling, conformational changes, the recruitment of coactivators or repressors, and other posttranslational modifications. The availability of phospho-specific antibodies, such as those targeting pSer59 (65), pThr453 (46), p668/670 (63), p681 (63), and pThr739 (46), will serve as important tools in future investigations to more precisely define the roles of phosphorylation in the functions of this important transcription factor in health and disease.

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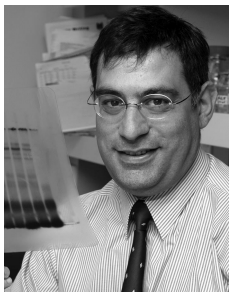

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