Role of Sp1 in insulin regulation of gene expression

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Abstract
Sp1 is a ubiquitous nuclear factor that plays a key role in maintaining basal transcription of 'house-keeping' genes. However, recent evidence points to a more important function for Sp1 in mediating 'cross-talk' between selected signaling cascades to regulate the target genes that respond to these pathways. The role of Sp1 in mediating the actions of the peptide hormone insulin is of specific interest and serves as a model for detailing effects of intracellular signaling on Sp1 activity. This review summarizes studies suggesting that changes in Sp1 phosphorylation provide one potential mechanism for manipulating activity of this protein. A growing body of evidence reveals that the DNA binding and transcription activity of Sp1 may increase or decrease in response to changes in phosphorylation. This enables 'fine-tuning' of Sp1 activity for regulation of gene transcription. Several mechanisms exist by which Sp1 alters gene activity in response to insulin. These include independent Sp1 activity as well as collaboration or competition with others factors. This review points to an ever-increasing role for Sp1 in regulating the transcription of genes in response to extracellular signals such as insulin.

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Introduction
The peptide hormone, insulin, has a multitude of effects on cellular function. The response to insulin begins with hormone binding to a membrane receptor. This step triggers a cascade of intracellular events leading to a modification of protein activity, changes in gene regulation and cellular function. Many of the physiological effects of insulin arise from its ability to regulate the transcription of specific genes. The insulin responsive gene products are not limited to enzymes that mediate selected metabolic pathways, but also include transcription factors, hormones, oncogenes, and proteins of importance to the cell membrane and architecture. These widespread actions of insulin underlie its effects on cell function and growth (O’Brien & Granner 1996). The scope of this review deals with the role of transcription factors, and specifically Sp1, that mediate the gene regulatory actions of insulin.

There exist many pathways by which insulin increases or represses gene activity. In some cases, well-characterized transcription factors have been shown to mediate insulin action in addition to their other known functions (reviewed by O’Brien & Granner 1996). For example, the insulin responsive sequences within the collagenase and malic enzyme (ME) promoters both contain a consensus AP1 binding sequence (TGACTCA) (Streeper et al. 1998), an element which normally mediates a response to phorbol esters. However, in the context of surrounding gene-specific sequences, both AP1 motifs are able to confer an insulin response on a heterologous promoter, but only the collagenase site responds to phorbol esters (Streeper et al. 1998). Although the c-fos gene also contains an AP1 motif, it is a nearby serum response element (SRE) that is
Role of Sp1 in basal transcription

Sp1 was one of the first eukaryotic transactivators to be isolated. It was purified by fractionation of HeLa cell extracts and shown to activate in vitro transcription in a discriminate manner from the SV40 viral promoter (Dynan & Tjian 1983). These findings prompted the authors to speculate that Sp1 could be involved in the recognition and regulation of other cellular and viral genes (Dynan & Tjian 1983). This is truly an understatement because Sp1 is now recognized as the matriarch of a family of transcription factors that includes Sp2, Sp3, and Sp4 (Kadonaga et al. 1987, reviewed by Suske 1999). The ubiquitous Sp1 and Sp3 factors, and the neural cell-specific Sp4, bind to the ‘GC box’ sequence (GGGCGCGGG), a motif that is common to many viral and eukaryotic promoters. In contrast, Sp2 binds to the GT/CACC sequence.

The Sp isoforms belong to an extended family of transcription regulators, the mammalian Sp/XKLF or ‘Kruppel-like’ factors. The Sp/XKLF proteins share homology with others found in *Drosophila melanogaster*, *Caenorhabditis elegans*, and yeast (reviewed by Phillipson & Suske 1999, Turner & Crossley 1999). Proteins belonging to this extended family bind similar GC-rich motifs because they have homologous DNA binding domains that contain three contiguous carboxyl-terminal Cys2His2 zinc fingers (Fig. 1). However, Sp1 has unique features that include the combination of transcriptional activation domains comprised of two glutamine-rich domains A and B, a weakly basic domain, and a carboxyl-terminal D domain (Fig. 1; Kadonaga et al. 1987, 1988, Courey & Tjian 1988, Pascal & Tjian 1991, Phillipson & Suske 1999). Sp1 may form oligomers through interaction of domains A and B resulting in a tetrameric configuration. These complexes may facilitate DNA looping to juxtapose distant binding sites to the proximal promoter (Mastrangelo et al. 1991, Matsushita et al. 1998). Domain D interactions of Sp1 enable even higher order complexes, such as the stacking of Sp1 tetramers (Matsushita et al. 1998). These interactions appear to facilitate the synergistic transcriptional activation that is observed among Sp1 sites in a single promoter or enhancer.

Since the initial discovery of Sp1, it has generally been defined as a ‘basal’ transcription factor. It is
believed to have a key role in maintaining expression of 'house-keeping' genes that lack a TATA-box. The TATA-box binds a protein complex called TFIID. This complex contains the TATA-box binding protein, TBP, and associated factors called TAFs. In the absence of a TATA-box, Sp1 facilitates binding of TFIID to the promoter, which, in turn, recruits RNA polymerase II (Pol II) transcription machinery (Blake et al. 1990, Smale et al. 1990, Javahery et al. 1994, Kaufmann & Smale 1994). Sp1 physically interacts with TFIID through TAFs, specifically human TAF\textsubscript{II}130, TAF\textsubscript{II}55 and Drosophila TAF\textsubscript{II}110 (Hoey et al. 1993, Gill et al. 1994, Chiang & Roeder 1995, Tanese et al. 1996). An additional 9 sub-unit co-factor, CRSP, separate from TAFs, also appears to be essential for Sp1 activation of Pol II transcription (Ryu et al. 1999).

Sp1 may also play a role in maintenance of housekeeping genes by preventing gene silencing by DNA methylation. Multiple GC boxes have been found in the CpG-rich regulatory regions of a number of constitutively expressed genes. These motifs are likely the binding sites of a nuclear factor in vivo, presumably Sp1 (Gardiner-Garden & Frommer 1987, Pfeifer et al. 1990, Macleod et al. 1994). The removal of the Sp1 motifs exposes these regulatory regions to DNA methylation (Macleod et al. 1994). Further support that Sp1 protects the DNA from being methylated comes from the insertion of ectopic Sp1 sites upstream of methylated β-globin regulatory sequences.
This manipulation results in demethylation of the surrounding sequences (Brandeis et al. 1994). However, Sp1 gene knockouts in mice have shown that the methylation-free status of the APRT, DHFR, and ApoA1 genes is maintained without Sp1, although overlapping contributions of other Sp family members may provide an explanation for this observation (Marin et al. 1997).

In addition to the participation of Sp1 in basal transcription, there is evidence now emerging that shows the interplay among Sp proteins in modulating cell-type-specific gene expression. Both Sp1 and its relative, Sp3, are ubiquitous proteins that bind the same motif. Therefore, it is reasonable to expect they also compete for binding to promoters containing GC-boxes. In fact, these two factors have been shown to display differential activity, including parallel or opposing transcription effects depending on the promoter (Conn et al. 1996, Kennett et al. 1997, Hata et al. 1998, Hoppe & Francone 1998, Nielsen et al. 1998, Rajakumar et al. 1998, Yajima et al. 1998, Merchant et al. 1999, Vines & Weigent 2000). Cell-type-specific levels of Sp1 and Sp3 variants have the potential to be an important mode of regulating the contribution of Sp1 to promoter activity. Also, numerous Sp1/Sp3 experiments have been performed in the Sp-null background of Drosophila cells, so that cell-type-specific differences in the abundance of Sp-family proteins cannot be responsible for their differential activity. It is more likely that inherent features of the promoter will prove to be important (Rajakumar et al. 1998).

Similar to the interplay between Sp1 and Sp3, the Sp proteins functionally interact with the early growth response factor, Egr-1, leading to either opposing or parallel actions. Egr-1 is an immediate-early gene product that also binds to a GC-rich consensus GCGC/(G/T)GGCG (Cao et al. 1993). Phorbol esters or serum stimulation of cells increases the synthesis and DNA binding activity of Egr-1. Increased abundance of Egr-1 displaces Sp1 bound to an overlapping sequence (Cao et al. 1993). Similar opposing Sp1/Egr-1 interactions are also found in the human tumor necrosis factor alpha (TNF-α), human interleukin 2, platelet derived growth factor (PDGF) genes and several other promoters (Kramer et al. 1994, Khachigian et al. 1995, Skerka et al. 1995). With other promoters, Egr-1 and Sp1 may cooperate rather than oppose each other’s action. For example, such interactions are found for regulation of the human interleukin-2 receptor β-chain promoter (Lin & Leonard 1997) and the 5-lipoxygenase promoter (Silverman et al. 1997). Others (Huang et al. 1997) have defined the promoter architecture that enables Sp1 and Egr-1 to work in a cooperative or opposing fashion. These investigators noted that if the Sp1 binding site overlaps an Egr-1 site, then increasing abundance of Egr-1 displaces Sp1 from the DNA leading to a repression of Sp1-activated transcription. In contrast, juxtaposition of the sites without overlap yields a promoter where Egr-1 cooperates with Sp1.

There are studies, too numerous to discuss here, that report the functional and physical co-operation of Sp1 with other transcription factors. Examples include cell-type-specific regulators, such as octamer transcription factor Oct-1 (Janson & Pettersson 1990), oncogenes, such as c-Myc (Kyo et al. 2000), and nuclear receptors for estrogens or androgens (Krishnan et al. 1994, Lu et al. 2000, Salvatori et al. 2000). The interpretation of such studies could be that a basal level of Sp1 activity co-operates with induced factors to contribute to activation of gene expression. However, current evidence suggests that the activity of Sp1 is neither basal nor static. Sp1 does not wait in anticipation to aid the actions of newly induced activators. Instead, Sp1 is believed to mediate gene-specific responses to a variety of signals in the cellular environment independent of co-operative interactions with ‘inducible’ transcription factors. The key question to be answered is how the actions of this ubiquitous transcription factor are regulated, thus allowing it to mediate specific cell-type and temporal patterns of gene expression. Part of the answer lies in the unfolding story of Sp1 post-translational modifications which give it the ability to respond to signal transduction cascades independent of other transcription factors.

**Sp1 as a target of signal transduction cascades**

A topic of rising interest is the potential participation of Sp1 as an effector of signal transduction cascades. Soon after Sp1 was isolated and purified, it was shown to be modified by phosphorylation of serine and threonine residues
et al. (MAPK) (Kreegipuu (CK) 1 and 2, protein kinases (PK) A and C, and ing calmodulin kinases (CamKs), casein kinases phosphorylation sites for numerous kinases includ-
primary sequence of Sp1 contains consensus of signal transduction pathways (Fig. 1). The response to extracellular stimuli through a variety not a constitutive modification and is altered in proteosome degradation (Han & Kudlow 1997).

Recent studies show that Sp1 phosphorylation is not a constitutive modification and is altered in response to extracellular stimuli through a variety of signal transduction pathways (Fig. 1). The primary sequence of Sp1 contains consensus phosphorylation sites for numerous kinases including calmodulin kinases (CamKs), casein kinases (CK) 1 and 2, protein kinases (PK) A and C, and mitogen activated protein kinases Erk 1 and 2 (MAPK) (Kreegipuu et al. 1999, Merchant et al. 1999, Zheng et al. 2001). At this juncture, the volume of literature on Sp1 regulation by phosphorylation continues to grow. Unfortunately, so does the complexity surrounding the possible signaling pathways involved, and the effects of phosphorylation on Sp1 binding and transcription activity. It is not difficult to imagine that different kinases and phosphatases could target selected motifs and thus alter Sp1 phosphorylation in a cell-type-specific manner. This could lead to differential effects that may be positive or negative on transcriptional activity, DNA binding, as well as interactions with other activators and/or the transcription machinery. Table 1 summarizes much of the current literature on Sp1 as a target of cellular kinases and phosphatases, and effects on DNA binding. This table also notes effects on transcription activity, but the evidence is indirect and gathered using inhibitors or co-expressed kinases and phosphatases, rather than in vitro transcription systems. Figure 1 provides a schematic summary of the pathways that have been shown to converge on Sp1.

Evidence supporting the role of PKC in Sp1 regulation comes from the positive effect of phorbol esters, and presumed PKC activation, on Sp1. Although studies suggest that the effect of phorbol esters may be at the level of increased synthesis (D’Angelo et al. 1996, Ries et al. 1998, Langmann et al. 1999, Tanaka et al. 2000, Noe et al. 2001), our laboratory showed that Sp1 binding and transcription activity in human HepG2 cells is increased by phorbol esters without requiring Sp1 synthesis (Zheng et al. 2000, 2001). Similarly, Kang-Park et al. (2001) have shown Sp1 phosphorylation and activation by PKCa co-transfection in HepG2 cells. However, only atypical PKCζ has been shown to physically interact with and phosphorylate Sp1 in vitro, with no apparent modification by other PKC isoforms (Pal et al. 1998, 2001, Garcia et al. 2000, Rafit & Khachigian 2001).

Sp1 may also be directly modified in vitro by DNA-PK (Jackson et al. 1990), PKA (Rohllf et al. 1997), and Erk 2 (Merchant et al. 1999) with a resultant increase in DNA binding activity demonstrated for the latter two kinases. PKA also appears to have a positive effect on Sp1 activity in transfection (Ahlgren et al. 1999, Wang et al. 1999a, Zheng et al. 2000). Casein kinase 2 (CK2) is also known to phosphorylate Sp1 in vitro, but this modification has a negative effect on DNA binding due to modification of threonine-579, a residue located in the second zinc finger of Sp1 (Armstrong et al. 1997, Zhang & Kim 1997, Wang et al. 1999b).

There is also evidence that CK2 has an indirect role in regulating Sp1 phosphorylation, through known interactions of CK2 with protein phosphatase 2A (PP2A) (Cieslik et al. 1998, 1999). PP2A forms stable complexes with a long list of protein kinases in vitro (Millward et al. 1999) including the catalytic alpha subunit of CK2 (reviewed by Allende & Allende 1998). Cieslik et al. (1998, 1999, 2000) have teased out the pathway by which lysophosphatidylcholine (LPC) induces endothelial nitric-oxide synthase (eNOS) gene transcription. The positive effect of LPC is dependent on Sp1, and is blocked by okadaic acid treatment of cells in culture or nuclear extracts in vitro, implicating PP2A (Cieslik et al. 1998, 1999). CK2 phosphorylates PP2A, resulting in down-regulation of PP2A activity (Cieslik et al. 1999). This decrease in PP2A activity is an indirect path to augmenting Sp1 phosphorylation, decreasing its activity (Cieslik et al. 1998, 1999). These authors provide further data to support a model by which LPC treatment of cells results in activation of a cascade of phosphatidylinositol 3-kinase (PI-3Kγ), Janus kinase (Jak2), mitogen activated protein kinase kinase (MEK-1/ MAPKK), and finally Erk-1 and Erk-2 (Fig. 1; Cieslik et al. 1999). Erk-1/2, in turn, phosphorylate and inhibit CK2 so that it cannot inactivate PP2A,
<table>
<thead>
<tr>
<th>Modifier</th>
<th>Cell Type</th>
<th>Promoter</th>
<th>Inducer</th>
<th>Modification</th>
<th>Sp1 binding</th>
<th>Transfection Activity</th>
<th>Reference</th>
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<tr>
<td>DNA-PK</td>
<td>SV40 infected CV1-L/HeLa cells</td>
<td>SV40 intergenic control region</td>
<td>HIV type 1 TAT protein</td>
<td>Phosphorylation (Ser/Thr; N-terminus Ser-131)</td>
<td>No effect</td>
<td>Increased</td>
<td>Jackson et al. (1990) Chun et al. (1998) Rohlff et al. (1997)</td>
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<td>PKA</td>
<td>HL-60/HL-60 AR leukemia cells</td>
<td>SV40 promoter</td>
<td>Inhibited by 8-Cl-cAMP &amp; RpcAMP[S] Forskolin/8-CPT-cAMP ACTH</td>
<td>Phosphorylation (Thr-366)</td>
<td>Increased by PKA in vitro</td>
<td>Increased by co-transfected PKA</td>
<td>Ahlgren et al. (1999)</td>
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<td></td>
<td>Human H295R/mouse Y1 adrenocortical cells</td>
<td>P450 CYP11A</td>
<td>None</td>
<td>N.D.</td>
<td>No effect</td>
<td>Increased</td>
<td>Zheng et al. (2000)</td>
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<td>PKCα</td>
<td>Human hepatoblastoma HepG2 cells</td>
<td>Human H358 estrogen receptor negative lung cancer cells</td>
<td>Apolipoprotein A1</td>
<td>Forskolin phorbol dibutyrate</td>
<td>N.D.</td>
<td>Increased</td>
<td>Increased</td>
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<td></td>
<td>Human hepatoblastoma HepG2 cells</td>
<td>p21Cip1/Waf1 cyclin kinase</td>
<td>None</td>
<td>Phosphorylation by cotransfected PKCα and Erk 2</td>
<td>Increased</td>
<td>Increased by PKCζ mutant</td>
<td>Decreased by dominant negative PKCζ mutant</td>
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<tr>
<td>Atypical PKCζ</td>
<td>Human renal cell carcinoma 768-0</td>
<td>IGF-II promoter 4</td>
<td>Hepatitis B virus X protein</td>
<td>Phosphorylation in zinc finger region</td>
<td>Increased</td>
<td>Decreased by dominant negative PKCζ mutant</td>
<td>Decreased by dominant negative PKCζ mutant</td>
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<td>PP2A</td>
<td>Rat pup aortic smooth muscle cells WKY12-22</td>
<td>PDGF B-chain</td>
<td>Nogalamycin</td>
<td>Phosphorylation (Thr 410)</td>
<td>Increased</td>
<td>Decreased by okadaic acid</td>
<td>Increased in transfection</td>
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<td>PP1</td>
<td>Human ECV-304 umbilical vein endothelial cells</td>
<td>Endothelial nitric oxide synthase</td>
<td>Lysophosphatidylcholine</td>
<td>Dephosphorylation</td>
<td>Decreased by okadaic acid</td>
<td>Increased in transfection</td>
<td>Daniel et al. (1996)</td>
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<td></td>
<td>Y10/L8057 megakaryocyte cells</td>
<td>Cyclin D3</td>
<td>Mpl ligand/MGDF</td>
<td>Dephosphorylation in vitro by PP1</td>
<td>Increased by in vitro PP1</td>
<td>Decreased by okadaic acid</td>
<td>Increased in transfection</td>
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<td></td>
<td>Mouse 30A5 pre-adipocytes</td>
<td>Acetyl-CoA carboxylase promoter II</td>
<td>Glucose</td>
<td>Dephosphorylation in vitro by PP1</td>
<td>Decreased by okadaic acid</td>
<td>N.D.</td>
<td>Daniel et al. (1996)</td>
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<td>Cell Type</td>
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<td>Modification</td>
<td>Sp1 binding</td>
<td>Transfection Activity</td>
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<td>CK2</td>
<td>Rat liver &amp; K562 cells</td>
<td>RCE of the DBP promoter</td>
<td>Terminal differentiation</td>
<td>Decreased by in vitro CK2</td>
<td>N.D.</td>
<td>Armstrong et al. (1997)</td>
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<td>Phosphorylation (C-terminal D domain Ser/Thr-579 in second zinc finger)</td>
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<td>Leggett et al. (1995)</td>
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<td>Increased by in vitro Erk2</td>
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<td>Kang-Park et al. (2001)</td>
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<td>Increased by co-transfected Erk2</td>
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<td>Increased by Ras or Erk2 co-expression</td>
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<td>Decreased by dominant negative Ras Increased by CamK II/IV co-transfection</td>
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<td>p44 MAPK (Erk2)</td>
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<td>Gastrin EGF responsive element</td>
<td>EGF</td>
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<td>HepG2 cells</td>
<td>IGF-II promoter 4</td>
<td>Hepatitis B virus X protein</td>
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<td></td>
<td>HepG2 cells</td>
<td>Apolipoprotein A1</td>
<td>Insulin/EGF</td>
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<td>Phosphorylation (Thr-266 in second glutamine rich domain)</td>
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<td>Ca²⁺/CamK II and IV</td>
<td>Mouse AtT-20/16-16 pituitary tumor cells</td>
<td>RCE of c-fos Depolarization with K⁺</td>
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<td>60 kDa kinase</td>
<td>P-19 teratocarcinoma cells</td>
<td>Acetylcholine receptor ε</td>
<td>Neuregulin (Neu differentiation factor)</td>
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<td>‘Sp1 kinase’</td>
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<td>subunit DHFR</td>
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<td>‘MEK 1 dependent’</td>
<td>Mouse Balbc &amp; 3T3 cells</td>
<td>Serum</td>
<td>Phosphorylation in vitro</td>
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<td>‘Sp1 kinase’</td>
<td>Human AGS gastric adenocarcinoma cells</td>
<td>Gastrin EGF responsive element</td>
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ACTH, adrenocorticotropic; CK2, casein kinase 2; CamK, calmodulin kinase; DBP, D-site binding protein; DHFR, dihydrololate reductase; DNA-PK, dsDNA dependent protein kinase; EGF, epidermal growth factor; IGF-II, insulin-like growth factor II; MEK, MAP/ERK kinase; MGDF, PEG-rHuMGDF (truncated Mpl ligand thrombopoietin); PKA, protein-kinase A; PP2A, protein-phosphatase 2A; PP1, protein phosphatase 1; PDGF, platelet-derived growth factor; VEGF, vascular permeability/endothelial growth factor; RCE, retinoblastoma control element; N.D., not determined.
thus freeing PP2A to activate Sp1 (Fig. 1; Cieslik et al. 1999).

Similarly, the glucose regulation of the acetyl-CoA carboxylase PII promoter also appears to be modulated by interaction between CK2 and cellular protein phosphatases (Daniel & Kim 1996, Daniel et al. 1996, Zhang & Kim 1997). In pancreatic β-cells, glucose induction of Sp1 is abrogated by expression of the CK2 catalytic subunit or by treatment with okadaic acid or immunodepletion to decrease PP1 activity (Zhang & Kim 1997).

Other groups also have demonstrated the importance of cellular protein phosphatases in the regulation of Sp1 activity. However, there is difficulty in reconciling some contradictory observations. In vitro, treatment of Sp1 with calf intestinal phosphatase (Leggett et al. 1995, Alroy et al. 1999, Chupreta et al. 2000, Merchant et al. 1999, Ray et al. 1999, Noc et al. 2001), potato acid phosphatase (Kumar & Butler 1998), or PP2A (Rolff et al. 1997) decreases DNA binding activity. Others have shown that alkaline phosphatase (Boreilini et al. 1990) or PP1 (Daniel et al. 1996, Armstrong et al. 1997, Wang et al. 1999b) increases binding. Obviously, some of these differences could be explained by differing experimental approaches, such as omission of phosphatase inhibitors during nuclear extract preparation (Edmead et al. 1999).

Also, it is possible that the amount and location of phosphorylated residues could differ among cell types and the methods of extract preparation. For example, hyperphosphorylated Sp1 could be dephosphorylated to a more active state, while in other experiments, the baseline phosphorylation may be lower such that further phosphorylation decreases activity. What appears to be most consistent is the observation that in vivo treatment with okadaic acid, a cellular phosphatase inhibitor, increases Sp1 activity in the majority of reports (Daniel et al. 1996, Armstrong et al. 1997, Cieslak et al. 1998, 1999, Alroy et al. 1999, Wang et al. 1999b). Exceptions are the HIV long terminal repeats (LTR) and the serum amyloid A gene where okadaic acid treatment is reported to increase Sp1 DNA binding and transcription activity (Vlach et al. 1995, Ray et al. 1999).

It is clear from the preceding discussion that the regulation of Sp1 phosphorylation and activity is complex. Sp1 is regulated by a number of different signal transduction pathways (Fig. 1). Beyond inducible transcription, evidence for the importance of cellular protein phosphatases in the regulation of Sp1 activity is mounting (reviewed by Black et al. 2001). Sp1 phosphorylation is known to be dynamic throughout the cell cycle (Leggett et al. 1995, Black et al. 1999) and Sp1 can mediate a response to mitogenic signals, such as serum, through Ras-pathway activation (Miltenberger et al. 1995, Kumar & Butler 1998, Black et al. 1999, Spencer & Misra 1999). Furthermore, it is involved in the regulation of cell cycle proteins, such as multiple cyclin-dependent kinase inhibitors (Kivinen et al. 1999, Pagliuca et al. 2000, Wang et al. 2000), cyclin-dependent kinase-2 (Tikoo et al. 2000), and cyclin D3 (Wang et al. 1999b), and is itself regulated by cyclin activity (Shao & Robbins 1995). Recent experiments also implicate Sp1 as an essential player in activation of apoptotic events (Kavurma et al. 2001). The intimate role of Sp1 in mediating growth and environmental signals makes it an attractive candidate as a final target for the peptide hormone, insulin.

**Sp1: regulation by insulin**

Sp1 binding sites are found in the insulin-responsive regions of numerous genes, as summarized in Table 2. This finding points to the participation of Sp1 in the insulin regulation of gene activity. The role of Sp1 in insulin regulation is not uniform and is highly dependent on the inherent characteristics of each promoter. A survey of the insulin-responsive promoters listed in Table 2 reveals three different mechanisms by which Sp1 mediates insulin action. First, Sp1 acts alone in mediating the effects of insulin. Secondly, Sp1 binding sites may be closely juxtaposed to those of other insulin-responsive transcription factors, thus suggesting a co-operative interaction is required for insulin induction. Thirdly, Sp1 binding to an insulin-responsive promoter may lead to basal activity, but dissociation of Sp1 from this site permits the actions of another factor or factors to modulate gene activity in response to insulin.

**Sp1 alone mediates insulin action**

The plasminogen activator inhibitor type 1 (PAI-1) gene is up-regulated by insulin, with over twofold induction in Hep G2 cell transfection experiments (Banfi et al. 2001). Using signaling pathway...
<table>
<thead>
<tr>
<th>Gene promoter</th>
<th>No. of Sp1 sites</th>
<th>Sequence</th>
<th>Other nearby factors</th>
<th>Cells</th>
<th>Inducer</th>
<th>Fold induction</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>GADPH, glyceraldehyde 3-phosphate dehydrogenase</td>
<td>1</td>
<td>-471-ACCTTTCCGCCCTCTCAGCTTTGAAAG−436</td>
<td>IRE-ABP</td>
<td>3T3 adipocytes</td>
<td>1000 mU insulin 16 h</td>
<td>4</td>
<td>Nasrin et al. (1990)</td>
</tr>
<tr>
<td>Apolipoprotein A1</td>
<td>1</td>
<td>-419-ACCTTTGAGGCCGGGATGAGT−388</td>
<td>None known</td>
<td>H35 hepatoma</td>
<td>100 mU/l insulin 24 h</td>
<td>2</td>
<td>Murao et al. (1988), Zheng et al. (2001)</td>
</tr>
<tr>
<td>Fatty acid synthase</td>
<td>2</td>
<td>-57-GTGGCGGCCGGCGGGATGGCCGCG−35</td>
<td>Ebox (USF) CATGTG</td>
<td>Rat hepatocyte</td>
<td>20 mM glucose/0.1 µM insulin 48 h</td>
<td>1.5</td>
<td>Fukada et al. (1997b), Fukada &amp; Iritani (1999)</td>
</tr>
<tr>
<td>Rat leptin</td>
<td>1</td>
<td>-101-GGGCCGGAGTTGGCGCTC−83</td>
<td>None known</td>
<td>Rat hepatocytes</td>
<td>20 mM glucose/0.1 µM insulin 48 h</td>
<td>2</td>
<td>Fukada &amp; Iritani (1999)</td>
</tr>
<tr>
<td>Insulin-like growth factor I region V</td>
<td>1</td>
<td>+140-GCCTCATTATTCTGCCCACCAATT</td>
<td>IRBP binding ATTATT</td>
<td>H4IIE hepatoma</td>
<td>10 nM insulin 48 h</td>
<td>3</td>
<td>Kaytor et al. (2001a, 2001b)</td>
</tr>
<tr>
<td>Calmodulin</td>
<td>3</td>
<td>−393 to +1</td>
<td>Upstream H4IIE hepatoma</td>
<td>10 000 µU/ml</td>
<td>1.5</td>
<td>Solomon et al. (1997), Pan et al. (2001)</td>
<td></td>
</tr>
<tr>
<td>Rat ATP citrate-lyase</td>
<td>1</td>
<td>−64-TGATGGGGCGGGAGGAGGAGCCCG−41</td>
<td>None known</td>
<td>Rat hepatocytes</td>
<td>20 mM glucose/0-1 µM insulin 48 h</td>
<td>2</td>
<td>Fukada et al. (1996, 1997a)</td>
</tr>
<tr>
<td>Human insulin receptor gene</td>
<td>4</td>
<td>−618-GGGCGGGGGCGGGGCGGGGACCGB GCCCG−93</td>
<td>None known</td>
<td>Rat hepatocytes</td>
<td>20 mM glucose/0-1 µM insulin 48 h</td>
<td>2</td>
<td>Fukada et al. (2001)</td>
</tr>
<tr>
<td>Plasminogen activator inhibitor type 1</td>
<td>1</td>
<td>−93-CCCAGAGCCAGTGAGTGGGTGGGTGGCTGGAAC−62</td>
<td>None known</td>
<td>Human Hep G2 hepatoblastoma</td>
<td>100 nM insulin 16 h</td>
<td>3.5</td>
<td>Banfi et al. (2001)</td>
</tr>
<tr>
<td>Rat malic enzyme</td>
<td>1</td>
<td>−175-CCCGCCCGCGCTCCTCGCA−156</td>
<td>Egr-1 (IRF)</td>
<td>H35 hepatoma</td>
<td>N.A.</td>
<td>N.A.</td>
<td>Garcia-Jimenez et al. (1994), Barroso &amp; Santisteban (1999)</td>
</tr>
</tbody>
</table>

GADPH, glyceraldehyde 3-phosphate dehydrogenase; USF, upstream stimulating factor; IRE-ABP, insulin responsive element-A binding protein; Egr-1, early growth response factor-1; IRBP, insulin receptor binding protein; IRF, insulin response factor; N.A., not applicable.
Inhibitors, it appears that insulin induction is Ras independent, but is mediated instead through a cascade involving phosphatidylinositol (PI) 3-kinase, PKC, and MAP/ERK (MEK) kinase. Insulin induces nuclear protein binding to three different regions of the PAI-1 promoter, all of which contain Sp1 binding sites. However, only the most proximal sequence (−93/−62) binds Sp1 specifically, and this binding is induced by insulin. A tetramer of this region confers a significant insulin response on a minimal promoter suggesting that Sp1 may mediate the response to insulin in the context of immediately surrounding PAI-1 promoter sequences.

Insulin regulation of rat apolipoprotein (Apo) A1 gene expression provides another example of Sp1 independently mediating the actions of insulin. Studies from our laboratory have shown that insulin induces, but glucose inhibits, the activity of the apoA1 promoter in transfected Hep G2 cells (Murao et al. 1998). A mutant promoter lacking an intact insulin responsive core element, IRCE (−411 to −404), cannot respond to insulin. Importantly, Sp1 specifically binds the IRCE motif in gel mobility assays (Zheng et al. 2000). Insulin action is mediated by pathways involving the participation of PI-3K, PKC and MAP/ERK kinases, similar to the PAI-1 promoter presented above (Zheng et al. 2001). Co-transfection of Sp1 along with the ApoA1 IRCE reporter construct augments the actions of insulin. Further, the use of antisense RNA to lower the amount of Sp1 protein in the cell attenuates the insulin response.

In other promoters, Sp1 may oppose the effects of insulin. In rat hepatocytes, the fatty acid synthase (FAS) promoter is induced by insulin together with glucose, while it is inhibited by polyunsaturated fatty acids (PUFA) (Fukada et al. 1997b). The FAS response is mediated by a motif within a −57/−35 fragment with 70% homology to the GC box (Fukada et al. 1997b). A second group has also identified this region (−68/60) as essential for insulin/glucose regulation in adipocytes (Moustaid et al. 1993, 1994). Sp1 binds this sequence in a specific manner as demonstrated by competition studies and supershifts with Sp1 antibodies. Interestingly, over-expression of Sp1 in rat hepatocytes leads to a decrease in FAS-promoter activity, suggesting that it plays a negative transcriptional role at this site (Fukada et al. 1997b).

In a similar fashion, this group has studied other insulin-regulated genes such as leptin, ATP citrate-lyase (ACL), and the insulin receptor (IR), to extend their model of Sp1 action (Table 1). Insulin and glucose induction of the leptin and ACL promoters only require a single proximal Sp1 site (Fukada et al. 1997a, Fukada & Iritani 1999), as with the FAS promoter, while the response of the IR promoter is dependent on four closely apposed Sp1 binding sites (Fukada et al. 2001). Sp1 and Sp3 bind specifically to the ACL, leptin and IR sites as demonstrated in antibody supershift experiments (Fukada et al. 1997a, 2001, Fukada & Iritani 1999). As with the FAS promoter, Sp1 co-expression inhibits transfection activity.

It is possible that Sp1 truly mediates a negative effect on the insulin response of the ACL, IR, leptin, and FAS promoters. Sp1 binding to the ACL promoter site is decreased in liver extracts from rats fed a high carbohydrate diet compared with fasting (Fukada et al. 1997a). The investigators hypothesize that this result represents an inhibition of Sp1 activity due to the higher insulin levels in the carbohydrate-fed rats. Further, there is evidence that induction of the FAS promoter by insulin involves an E-box motif at −65, which overlaps the Sp1 site (Wang & Sul 1998). However, the ACL, leptin, and IR promoters do not contain an E-box in their insulin responsive regions. An alternative explanation is that the observed inhibition of transfected promoter activity by co-expressed Sp1 is due to supra-physiological levels of Sp1 in the cell, such that other factors required for the transcription machinery are titrated out and unavailable for transcription. This would be similar to the phenomenon of ‘squelching’ which was first described for in vitro transcription systems (Ptashne & Gann 1990).

**Sp1 acts co-operatively with other insulin-regulated factors**

In contrast to the apparently independent actions of Sp1 in the preceding section, other insulin-responsive regions appear to require Sp1 activity in concert with additional insulin-responsive factors. Kaytor et al. (2001a,b) have investigated the sequences that mediate an insulin response of the insulin-like growth factor (IGF)-I promoter. Insulin induction of IGF-I from the third transcription start site, IS3, is dependent on ‘region V’. This site
mediates differential liver-specific expression in diabetic and control rats. Region V is a 24 bp motif comprised of both AT- and GC-rich domains at opposite ends. Mobility shift assays show that the 3′ GC-rich end of region V binds Sp1 or Sp3. In contrast, the AT-rich end binds a second factor, insulin responsive binding protein (IRBP). IRBP binding is activated two- to sixfold by insulin treatment in a dose-responsive manner. IRBP binding to region V is possible following mutations treatment in a dose-responsive manner. IRBP binding is activated two- to sixfold by insulin insulin responsive binding protein (IRBP). IRBP contrast, the AT-rich end binds a second factor, GC-rich end of region V binds Sp1 or Sp3. In

Displacement of Sp1 mediates insulin action

ME is an important lipogenic enzyme that is controlled by insulin. The ME promoter is GC-rich and contains numerous Sp1 motifs (Moroika et al. 1988, Garcia-Jimenez et al. 1994). Transcription factor AP-1 has previously been shown to play a role in mediating insulin regulation of the ME promoter (Streeper et al. 1998). However, Barroso & Santisteban (1999) have studied one of the GC-rich sites resembling the IRE-A motif of the GAPDH promoter, called IRE-II (−175 to −156). IRE-II binds Sp1 and Sp3 constitutively, while Egr-1 binds to an overlapping recognition site, increasing in response to insulin. In transfection experiments, Egr-1 expression inhibited the response to insulin by the ME promoter. Further, increasing amounts of Egr-1 displaced Sp1 from its binding site. Overall, these experiments suggest that the ME promoter initially is inhibited in response to insulin through displacement of Sp1 or Sp3 by Egr-1 at the IRE-II site.

Summary

It is clear that Sp1 is much more than a basal transcription factor. Sp1 activity is modified by its phosphorylation state in response to a multitude...
of signals, implicating Sp1 as a key mediator of ‘cross-talk’ between signaling pathways and gene transcription. The finding that Sp1 binding sites are common to many insulin responsive promoter regions suggests that it is a major factor in mediating the effects of insulin. However, its role appears to be particularly diverse, with positive or negative effects depending on the promoter.

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