Phosphorylation and Ubiquitination of the Transcription Factor Sterol Regulatory Element-binding Protein-1 in Response to DNA Binding

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Members of the sterol regulatory element-binding protein (SREBP) family of transcription factors control cholesterol and lipid metabolism and play critical roles during adipocyte differentiation. The transcription factor SREBP1 is degraded by the ubiquitin-proteasome system following phosphorylation of Thr426 and Ser430 in its phosphodegron. We now demonstrate that Fbw7-mediated ubiquitination of SREBP1 is dependent on its DNA binding activity. Thus, DNA binding could provide a mechanistic link between the phosphorylation, ubiquitination, and degradation of active transcription factors.

Members of the sterol regulatory element-binding protein (SREBP) family of transcription factors control cholesterol and lipid metabolism and play critical roles during adipocyte differentiation and insulin signaling (1, 2). The SREBP family of transcription factors consists of three different SREBP proteins; SREBP1a, SREBP1c, and SREBP2. The SREBPs are synthesized as large precursor proteins that are inserted into the nuclear and endoplasmic reticulum membranes and are transcriptionally inactive (3). In sterol-depleted cells, SREBPs are processed sequentially by two membrane-associated proteases that release the mature form of the proteins (4, 5). These transcriptionally active fragments of the SREBPs are translocated to the nucleus and bind to the promoters of SREBP target genes (6). It has been demonstrated that the mature forms of SREBPs are modified by phosphorylation (7–9), acetylation (10), sumoylation (11), and ubiquitination (8, 12), and it has been shown that these modifications regulate their stability and/or transcriptional activity.

Many transcription factors, particularly those involved in the control of cell growth, are unstable proteins targeted for degradation by the ubiquitin-proteasome system (13). Numerous observations have pointed to the existence of a close connection between the ubiquitin-proteasome system and transcriptional activation (14, 15). It has been demonstrated that the sequences that specify proteolysis of some activators overlap with their transcriptional activation domains and that components of the proteasome can be recruited to promoters through interactions with transcriptional regulators (16–21). It has also been demonstrated that the activity of certain transcription factors can be enhanced by ubiquitination or through interactions with specific ubiquitin ligases (22–25). However, the mechanistic link between the transcriptional activity of transcription factors and their degradation has been unclear. The transcriptionally active forms of SREBPs are degraded by the proteasome in a ubiquitination-dependent manner (12). It has been suggested that nuclear SREBP molecules are, at least in part, ubiquitinated and degraded as a functional consequence of their transcriptional activity (20). Recently, a phosphodegron in SREBP1a that serves as a recognition motif for the SCF$^{Fbw7}$ ubiquitin ligase was described (8). Fbw7 interacts with nuclear SREBP1a and enhances its ubiquitination and degradation in a manner dependent on the phosphorylation of Thr426 and Ser430 within its phosphodegron by GSK-3β. However, the signals regulating the phosphorylation of the phosphodegron in SREBP1 and its interactions with Fbw7 have been largely unknown. We now demonstrate that the GSK-3-dependent phosphorylation of the phosphodegron in SREBP1 is enhanced in response to specific DNA binding, both in vitro and in vivo. Accordingly, we demonstrate that GSK-3β is recruited to the promoters of SREBP target genes in vivo. Consequently, Fbw7 is recruited to SREBP target promoters and induces the ubiquitination and degradation of SREBP. Thus, DNA binding provides a link between the...
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In Vitro Kinase Assays—Recombinant His$_6$-SREBP1a was incubated in kinase buffer (30 mM HEPES, pH 7.5, 7 mM MgCl$_2$, 3 mM dithiothreitol, and 1 mM ATP) in the absence or presence of HeLa nuclear extract (3.5 μg) and a plasmid DNA template containing the proximal region of the HMG-CoA synthase promoter (SYNSRE-luc, 0.1 μg) or a PCR product containing the same promoter region for 1 h at 30 °C. The reactions were stopped by the addition of Laemmli buffer, and the reaction products were separated on SDS/PAGE. The levels and phosphorylation of SREBP1 were determined by Western blotting.

RESULTS

SREBP1 Is Hyperphosphorylated in Response to DNA Binding—In an attempt to identify nuclear factors targeting SREBP1, we performed in vitro kinase assays using recombinant mature SREBP1a and HeLa nuclear extracts. We found that the migration of mature SREBP1a in SDS/PAGE was dramatically shifted to higher molecular species when the kinase assay was supplemented with a promoter-reporter gene containing SREBP-binding sites (Fig. 1A). Similar results were also obtained with mature SREBP2 (Fig. 1B). The shift in apparent molecular weight of SREBP1a in response to DNA binding was reversed by λ-phosphatase treatment (Fig. 1C), indicating that mature SREBP1a is hyperphosphorylated in response to DNA binding. To test this hypothesis, the kinase assay was repeated with a mutant SREBP1 protein unable to bind DNA (DBD$^{-/}$). The DBD$^{-/}$ protein was not phosphorylated following the addition of DNA (Fig. 1D), indicating that DNA binding promotes the phosphorylation of SREBP1a by a kinase present in HeLa nuclear extracts. This idea was supported by our observation that SREBP1a was phosphorylated in response to oligonucleotides containing a wild type SREBP-binding site, but not if the site was mutated so that SREBP1a could no longer bind (Fig. 1E).

The Phosphodegron in SREBP1 Is Phosphorylated in Response to DNA Binding—In an attempt to identify the residues in SREBP1 phosphorylated in response to DNA binding, the kinase assay was performed in the presence of [${}^{32}$P]ATP. Phospho-amino acid analysis of the labeled SREBP1a protein revealed that phosphorylation of serine residues was the dominating modification, with a small amount of radioactivity associated with phospho-threonine (supplemental Fig. S1). Preliminary phospho-peptide mapping of tryptic peptides confirmed that SREBP1a was phosphorylated on multiple residues in response to DNA binding and indicated that a number of the phosphorylation, ubiquitination, and degradation of active SREBP1.

EXPERIMENTAL PROCEDURES

Cell Culture—All of the tissue culture media and antibiotics were obtained from Invitrogen and Sigma. 293T, 293, HeLa, and HepG2 cells were from the American Type Culture Collection.

Reagents and Antibodies—Anti-FLAG antibody (M2 and M5) and standard chemicals were from Sigma. Monoclonal anti-SREBP1 (2A4), anti-tubulin (TU-02), anti-GST (B-14), rabbit anti-SREBP1 (H-160), anti-GSK-3α (H-75), and anti-GSK-3β (H-76) antibodies were from Santa Cruz. The generation of phosphorylation-specific SREBP1 antibodies has been described (8).

Plasmids and DNA Transfections—The mammalian expression vectors for FLAG-tagged SREBP1a and the bacterial expression vectors for His$_6$-SREBP1a and His$_6$-SREBP2 have been described (9). The HMG-CoA synthase (SYNSRE-luc) and low density lipoprotein receptor (LDLR) promoter–reporter constructs and the expression vectors for Fbw7α and cullin1 have been described previously (8, 26–28). Transient transfections were performed using the MBS transfection kit (Stratagene). The control, GSK-3β-galactosidase gene as an internal control for transfection efficiency. Luciferase values were determined in duplicate samples as described previously (8).

In Vitro Ubiquitination Assays—For ubiquitination assays, the in vitro kinase assays were supplemented with E1 (0.1 μg; Biomol), UbcH3 (0.1 μg; Biomol), ubiquitin (10 μg; Sigma), ubiquitin aldehyde (2 μM; Biomol), and MG132 (100 μM; Sigma) and incubated for 1 h at 30 °C in the absence or presence of in vitro translated Fbw7α.

ChIP Assays—The ChIP assays were performed with the indicated antibodies as described previously (9). The PCR amplifications were performed in the presence of 1 μCi of [$^{32}$P]dCTP and analyzed in 6% polyacrylamide gels. The PCR conditions were optimized to remain in the linear range of amplification. The sequences of the primers used for the various promoters are available on request.

RESULTS

SREBP1 Is Hyperphosphorylated in Response to DNA Binding—In an attempt to identify nuclear factors targeting SREBP1, we performed in vitro kinase assays using recombinant mature SREBP1a and HeLa nuclear extracts. We found that the migration of mature SREBP1a in SDS/PAGE was dramatically shifted to higher molecular species when the kinase assay was supplemented with a promoter-reporter gene containing SREBP-binding sites (Fig. 1A). Similar results were also obtained with mature SREBP2 (Fig. 1B). The shift in apparent molecular weight of SREBP1a in response to DNA binding was reversed by λ-phosphatase treatment (Fig. 1C), indicating that mature SREBP1a is hyperphosphorylated in response to DNA binding. To test this hypothesis, the kinase assay was repeated with a mutant SREBP1 protein unable to bind DNA (DBD$^{-/}$). The DBD$^{-/}$ protein was not phosphorylated following the addition of DNA (Fig. 1D), indicating that DNA binding promotes the phosphorylation of SREBP1a by a kinase present in HeLa nuclear extracts. This idea was supported by our observation that SREBP1a was phosphorylated in response to oligonucleotides containing a wild type SREBP-binding site, but not if the site was mutated so that SREBP1a could no longer bind (Fig. 1E).

The Phosphodegron in SREBP1 Is Phosphorylated in Response to DNA Binding—In an attempt to identify the residues in SREBP1 phosphorylated in response to DNA binding, the kinase assay was performed in the presence of [$^{32}$P]ATP. Phospho-amino acid analysis of the labeled SREBP1a protein revealed that phosphorylation of serine residues was the dominating modification, with a small amount of radioactivity associated with phospho-threonine (supplemental Fig. S1). Preliminary phospho-peptide mapping of tryptic peptides confirmed that SREBP1a was phosphorylated on multiple residues in response to DNA binding and indicated that a number of the
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To understand how DNA binding promotes the phosphorylation of SREBP1, we needed to establish the timing of phosphorylation relative to DNA binding. To address this issue, recombinant SREBP1a was incubated in the presence of DNA for various times, and the phosphorylation of the Fbw7-dependent phosphodegron was detected with phosphorylation-specific antibodies directed against phospho-Thr\(^{426}\) (pT426) and phospho-Ser\(^{430}\) (pS430). In parallel experiments, the total phosphorylation of SREBP1a in response to DNA binding was monitored in the presence of \(^{[32P]}\)ATP. The DNA binding activity of recombinant SREBP1a over time was monitored in electromobility shift assays. Only the shifted SREBP1a-DNA complex is shown (bottom panel). C, His\(_{5}\)-SREBP1a, either wild type or the DBD\(^{-}\) mutant, was incubated in the absence or presence of DNA (SYNSRE-luc) in the presence of HeLa nuclear extract. The reaction products were subjected to SDS/PAGE and Western blot analysis for the detection of SREBP1. D, 293T cells were transfected with mature SREBP1a, either wild type (0.3 \(\mu\)g) or the DBD\(^{-}\) mutant (0.075 \(\mu\)g). Total cell lysates were subjected to SDS/PAGE, and the levels and phosphorylation of SREBP1 were detected by Western blot analysis (top three panels). In a parallel experiment, the total phosphorylation of SREBP1a, either wild type (WT) or the DBD\(^{-}\) mutant, was incubated in the absence or presence of DNA (SYNSRE-luc) in the presence of HeLa nuclear extract. The reaction products were subjected to SDS/PAGE and Western blot analysis for the detection of SREBP1a, either wild type or the DBD\(^{-}\) mutant, was incubated in the absence or presence of an increasing amount of a double-stranded oligonucleotide corresponding to the SREBP-binding site in the LDL receptor promoter, either wild type (5'-ATACCCAC-3') or mutated (5'-ATAAAACCCAC-3'), in the presence of HeLa nuclear extract. The reaction products were subjected to SDS/PAGE and Western blot analysis for the detection of SREBP1.

Phosphorylated residues resided in the C-terminal domain of mature SREBP1, including Thr\(^{426}\) and Ser\(^{430}\) in the Fbw7-dependent phosphodegron (not shown). To confirm that the C-terminal domain in SREBP1a was phosphorylated in response to DNA binding, wild type SREBP1a and mutant proteins in which either the N- or C-terminal domains were deleted were used for in vitro kinase assays. As illustrated in Fig. 2A, deletion of the N terminus (ΔN; deletion of amino acid residues 1–90) did not affect the shift in molecular weight in response to DNA binding. However, deletion of the C-terminal domain (ΔC; deletion of amino acid residues 418–490) blocked the shift almost completely, indicating that the major phosphorylation sites reside in the C-terminal domain.
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When expressed in cells, the DBD-/- mutant accumulates to high levels, presumably as a result of inefficient ubiquitin-dependent degradation (20). When similar levels of wild type and DBD-/- SREBP1a protein were expressed in 293T cells, the DBD-/- mutant migrated slightly faster than the wild type protein (Fig. 2D), indicating a difference in phosphorylation. Indeed, the phosphorylation of both Thr426 and Ser430 was significantly reduced in the DBD-/- mutant when compared with the wild type protein (Fig. 2D), suggesting that DNA binding contributes to the phosphorylation of the phosphodegron in SREBP1 in vivo. The minor phosphorylation signals seen with the DBD-/- protein indicate that DNA binding may not be the only requirement for phosphorylation in vivo. Taken together, our results indicate that SREBP1 is phosphorylated on multiple C-terminal amino acid residues in response to DNA binding. Two of these residues, Thr426 and Ser430, reside in the phosphodegron of SREBP1 that are phosphorylated under these conditions will be a major future challenge. In the current study, we will focus on the phosphorylation of Thr426 and Ser430 because of the critical importance of these residues in the phosphorylation-dependent degradation of SREBP1 (8).

GSK-3 Is Involved in the DNA Binding-dependent Phosphorylation of SREBP1—It has been shown that Thr426 and Ser430 can be phosphorylated by GSK-3β, both in vitro and in vivo (8). Thus, we wanted to determine whether GSK-3β was involved in the DNA binding-dependent phosphorylation of these residues. The phosphorylation of both Thr426 and Ser430 in response to DNA binding was attenuated in the presence of lithium, a pharmacologic GSK-3 inhibitor (Fig. 3A). The phosphorylation of Thr426 and Ser430 was also attenuated in the presence of SB-415286, a specific GSK-3 inhibitor (supplemental Fig. S2). The effect was specific, because none of the other kinase inhibitors tested had any significant effect on the phosphorylation of these residues in response to DNA binding (supplemental Fig. S2).

To further test the role of GSK-3 in the DNA-dependent phosphorylation of Thr426 and Ser430, nuclear extracts were prepared from cells transfected with either control siRNA or
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FIGURE 4. Rapid accumulation of phosphorylated SREBP1 on target promoters following proteasome inhibition. A, HeLa cells were treated with MG132 (25 μM) or MeSO and processed for ChIP analysis of the HMGS, LDLr, FPPS, and GAPDH promoters, using anti-SREBP1, anti-pT426, and anti-pS430 antibodies for immunoprecipitation. B, HeLa cells were treated with MeSO or increasing concentrations of MG132 for 4 h. Total RNA was used to determine the expression of the HMGS, LDLr, and GAPDH genes by semiquantitative RT-PCR. C, HepG2 cells were transfected with the SREBP-responsive SYN5SRE-luc and LDL-luc reporter genes. Twenty-four hours after transfection, the cells were treated with MG132 (25 μM) or MeSO for 4 h, and the luciferase activity was measured. The expression of each reporter in the absence of MG132 was set to 1. The data represent the averages ± S.D. of three independent experiments. D, HeLa cells were left untreated or treated with MG132 (25 μM) for the indicated times and processed for ChIP analysis of the LDLr (left) and HMGS (right) promoters, using anti-SREBP1, anti-pT426, and anti-hemagglutinin (HA, negative control) antibodies for immunoprecipitation. E, HeLa cells were treated with MG132 as in D, and the levels and phosphorylation (pT426) of SREBP1 in whole cell lysates (WCL) were determined by Western blot (WB) analysis. Ab, antibody.

siRNA directed against GSK-3α and GSK-3β. As illustrated in Fig. 3B, the phosphorylation of both residues was decreased in response to siRNA-mediated inactivation of GSK-3. Knockdown of GSK-3β alone was as effective in attenuating the phosphorylation as the combined knock-down of both isoforms, indicating that GSK-3β could be the more important isoform in the phosphorylation of Thr426 and Ser430. The depletion of GSK-3α and β in these experiments was not complete, because the complete removal of GSK-3 affected cell viability, making it difficult to interpret the results. Taken together, our results suggest that GSK-3β is involved in the phosphorylation of both Thr426 and Ser430 in response to DNA binding. In support of this hypothesis, we found that endogenous GSK-3β in HeLa cells was recruited to SREBP-binding sites in DNAP assays (Fig. 3C). Importantly, less GSK-3β was recruited to DNA when cells had been treated with excess sterols to lower the levels of mature SREBP1. To determine whether the recruitment of GSK-3β to SREBP-regulated promoters is dependent on a functional SREBP-binding site in the promoter, we performed DNAP assays with HeLa nuclear extracts and the proximal promoter from the LDLr gene, either wild type or a mutant in which the SREBP-binding site has been deleted. As illustrated in Fig. 3D, endogenous GSK-3β was recruited to the wild type promoter and not to the promoter lacking a functional SREBP-binding site. Taken together with our previous results, these results establish that GSK-3β is recruited to SREBP1-regulated promoters by direct interactions with SREBP1.

To address the question of how DNA binding promotes GSK-3β-dependent phosphorylation of SREBP1, we performed co-immunoprecipitation assays with recombinant SREBP1a, either wild type or a mutant lacking the C-terminal domain (ΔC), and in vitro translated GSK-3β in the absence or presence of DNA. Wild type SREBP1a and the ΔC mutant interacted equally well with GSK-3β in the absence of DNA (Fig. 3E, first and third lanes). Interestingly, the addition of DNA dramatically enhanced the interaction between wild type SREBP1a and GSK-3β (Fig. 3E, second lane). Although the ΔC protein bound DNA with the same affinity as the wild type protein (supplemental Fig. S3 and Ref. 9), no enhanced interaction between the ΔC protein and GSK-3β was observed in response to DNA binding (Fig. 3E, fourth lane). Thus, our results demonstrate that DNA binding enhances the interaction between SREBP1 and GSK-3β in a manner dependent on the C-terminal domain in SREBP1.

Using GSK-3β-specific antibodies in chromatin immunoprecipitation (ChIP) assays, we found that endogenous GSK-3β was associated with the promoters of the HMG-CoA synthase (HMGS), LDLr, and FPP synthase (FPPS) genes, which are all positively regulated by SREBP (Fig. 3F). Taken together, our results indicate that SREBP1 is hyperphosphorylated when associated with DNA and that the recruitment of GSK-3β to DNA-bound SREBP1 contributes to the phosphorylation of Thr426 and Ser430 in its phosphodegron. However, the overall phosphorylation of SREBP1 in response to DNA binding was unaffected by the inhibition of GSK-3 (Fig. 3, A and B, and supplemental Fig. S2), suggesting that SREBP1 is also targeted by other kinases in response to DNA binding. The identification of these kinases and the residues they modify will be important in the future.

Rapid Accumulation of Phosphorylated SREBP1 on Target Promoters in Response to Proteasome Inhibition—Treating cells with the proteasome inhibitor MG132 stabilizes the mature form of endogenous SREBP1 by inhibiting ubiquitin-dependent degradation of SREBP1 molecules phosphorylated on Thr426 and Ser430 (8). Thus, we speculated that endogenous SREBP1 phosphorylated on Thr426 and Ser430 should accumulate on the promoters of SREBP target genes in response to proteasome inhibition. To test this hypothesis, we performed ChIP assays on HeLa cells treated in the absence or presence of MG132. As expected, endogenous SREBP1 accumulated on target promoters in response to proteasome inhibition (Fig. 4A). When the ChIPs were performed with phospho-specific anti-SREBP1 antibodies, we could demonstrate that SREBP1 molecules phosphorylated on Thr426 and Ser430 are associated with target promoters in vivo. Inactivation of the proteasome resulted in a dramatic increase in the amount of phosphorylated SREBP1 associated with promoters (Fig. 4A). Quantification of the amount of [32P]CTP incorporated in the PCR products demonstrated that the relative accumulation of phos-
The phosphorylated form of the protein, accumulates very rapidly (within 1 h) on target promoters following inhibition of the proteasome. The results demonstrate that SREBP1, and especially the phosphorylated (pT426) SREBP1 was monitored in total cell lysates. In parallel experiments, the accumulation of total and phosphorylated SREBP1 on target promoters in response to MG132 was greater than the accumulation of total SREBP1, both total and phosphorylated, did not reach their peak until 3 or 4 h after the addition of MG132 (Fig. 4E). These results strongly suggest that phosphorylated SREBP1 molecules associated with target promoters is the primary target for the proteolytic activity of the proteasome and support our hypothesis that DNA binding-dependent phosphorylation of the phosphodegron in SREBP1 is linked to the rapid degradation of the transcription factor.

**Phosphorylation-dependent Recruitment of Fbw7 to SREBP1 in Response to DNA Binding**—The interaction between Fbw7 and SREBP1 is dependent on the phosphorylation of Thr426 and Ser430. Our data suggest that the phosphorylation of Thr426 and Ser430 is enhanced in response to DNA binding, indicating that the interaction between SREBP1 and Fbw7 could be enhanced in response to DNA binding. To test this hypothesis, recombinant SREBP1a, either wild type or the DBD mutant, was incubated in the absence or presence of DNA and nuclear extracts, and the interaction between SREBP1 and Fbw7a was tested following immunoprecipitation of SREBP1. As illustrated in Fig. 5A, DNA-dependent phosphorylation of wild type SREBP1a enhanced its interaction with Fbw7a, whereas the interaction between Fbw7a and the DBD mutant was unaffected.

We have previously demonstrated that phosphorylation of Thr426 and Ser430 in SREBP1 is critical for Fbw7-mediated ubiquitination and degradation of the transcription factor. The data presented in the current study demonstrate that the phosphorylation of both these residues is enhanced in response to DNA binding, indicating that Fbw7 could be recruited to SREBP-regulated promoters and induce the ubiquitination and degradation of SREBP. To address this issue, we performed DNAP assays, using extracts from 293 cells expressing mature SREBP1a, either wild type or the DBD mutant, was incubated in the absence or presence of DNA and nuclear extracts, and the interaction between SREBP1 and Fbw7a was investigated following immunoprecipitation of SREBP1. As illustrated in Fig. 5A, DNA-dependent phosphorylation of wild type SREBP1a enhanced its interaction with Fbw7a, whereas the interaction between Fbw7a and the DBD mutant was unaffected.
in the presence of dominant-negative cullin1 to attenuate Fbw7-dependent degradation of SREBP (8). When anti-FLAG antibodies were used in the ChIP assays, we found that transfected Fbw7α was associated with the HMGS, LDLr, and FPPS promoters (Fig. 5C), indicating that Fbw7 can associate with SREBP1 on target promoters in vivo. Interestingly, the amount of Fbw7 associated with SREBP target promoters was enhanced in response to MG132 treatment. Taken together, our results suggest that phosphorylation of SREBP1 in response to DNA binding leads to the recruitment of the ubiquitin ligase Fbw7 to SREBP target genes in vivo.

**DISCUSSION**

Numerous reports have demonstrated a close connection between the activity and ubiquitination of transcription factors. Our current findings provide a mechanistic link between the expression of SREBP1 in response to DNA binding. The Fbw7-dependent ubiquitination of wild type SREBP1a in response to DNA binding was attenuated in the presence of LiCl (Fig. 6C), suggesting that GSK-3-dependent phosphorylation of SREBP1 is critical for its ubiquitination in response to DNA binding. When the membrane was reprobed with antibodies directed against phosphorylated SREBP1 (pT426), it became clear that SREBP1 molecules phosphorylated in response to DNA binding were highly ubiquitinated (Fig. 6C, lower panel).

Thus, our results indicate that SREBP molecules that are unable to bind DNA, such as the DBD−/− mutant, would be poor substrates for Fbw7-mediated degradation in vivo. To test this hypothesis, 293T cells were transfected with mature SREBP1a, either wild type or the DBD−/− mutant, in the absence or presence of increasing amounts of Fbw7α. In support of our hypothesis, the DBD−/− protein was less sensitive to Fbw7α-mediated degradation compared with the wild type protein (Fig. 6D). This result is in agreement with the fact that the DBD−/− protein accumulates to very high levels when expressed in cells (20).
DNA binding activity of SREBPs and their phosphorylation-dependent ubiquitination and degradation (Fig. 7). We have previously demonstrated that transcriptionally active SREBP molecules are rapidly degraded, whereas inactive proteins, including the DBD–/– mutant, are stable (20). We now demonstrate that SREBP1a is phosphorylated on multiple residues in response to DNA binding, and we have identified two of the phosphorylated residues. These residues, Thr^{426} and Ser^{430}, reside in the Fbw7-dependent phosphodegron in SREBP1a, and our data indicate that GSK-3β could be involved in the DNA binding-dependent phosphorylation of both residues. DNA binding promoted the interaction between the C-terminal domain of SREBP1 and GSK-3β. One possibility is that DNA binding induces a conformational change within the C-terminal domain of SREBP1 that enhances its interaction with GSK-3β. The latter hypothesis is in agreement with our observation that there is a delay between DNA binding and the phosphorylation of Thr^{426} and Ser^{430}. However, it is also possible that phosphorylation of other residues in the C-terminal domain of SREBP1 in response to DNA binding could contribute to the recruitment of GSK-3β and thereby affect the phosphorylation of Thr^{426} and Ser^{430}. This hypothesis is supported by the fact that SREBP1a is hyperphosphorylated in response to DNA binding and by our observation that the overall phosphorylation of SREBP1a precedes the phosphorylation of Thr^{426} and Ser^{430}. Alternatively, phosphorylation of other residues than target promoters should be better substrates for Fbw7-mediated degradation. This hypothesis was supported by our observation that the phosphorylation of both Thr^{426} and Ser^{430} was reduced in the DBD–/– mutant. As a result, the DBD–/– mutant was less sensitive to Fbw7-mediated degradation in vivo compared with the wild type protein. The hypothesis that promoter-associated SREBP1c molecules are rapidly degraded was further strengthened by our observation that SREBP1c phosphorylated on Thr^{426} rapidly accumulated on target promoters following MG132-mediated inhibition of the proteasome. The accumulation of SREBP1 on target promoters in response to proteasome inhibition preceded the overall cellular accumulation of mature SREBP1 in response to MG132 treatment, suggesting that phosphorylated SREBP1c molecules associated with promoters are the primary targets of the proteasome.

The other two SREBP isoforms, SREBP1c and SREBP2, are also targeted by Fbw7-mediated degradation in manner dependent on amino acid residues corresponding to Thr^{426} and Ser^{430} in SREBP1a. In addition, both SREBP1c and SREBP2 are hyperphosphorylated in response to DNA binding. Thus, it is possible that the proteolytic pathway described in the current study applies to the entire SREBP family of transcription factors. Such a mechanism to regulate the stability of transcription factors ensures that the activation of target genes is linked to the absolute levels of the transcription factor and that the intracellular levels of these proteins are maintained at a low level.
thereby allowing cells to respond rapidly to environmental signals. Thus, sterol-regulated cleavage and activation of the precursor forms of SREBPs (5) and DNA binding-dependent phosphorylation and degradation of the active transcription factor enable cells to respond rapidly to changes in cholesterol levels and control the duration of SREBP-dependent gene expression (Fig. 7). Consequently, sustained activation of SREBP target genes would require a continuous supply of mature SREBP molecules through sterol-regulated processing of precursor molecules. Taken together with our previous work (8), the current study suggests that the phosphorylation and Fbw7-dependent degradation of active SREBP molecules plays an important role in SREBP-regulated transcription. The activity of GSK-3 is controlled by extracellular signals, including insulinsignaling. Thus, the DNA binding-dependent phosphorylation and degradation of active SREBP molecules could be regulated by growth factor signaling.

It has been suggested that the activities of certain transcription factors, including c-Myc and Gcn4, are enhanced by ubiquitination and/or proteasome-mediated degradation (23–25). However, the expression of SREBP target genes is enhanced in cells lacking Fbw7 (8) and in response to inactivation of the proteasome (Fig. 4, B and C). Thus, it appears as if Fbw7-mediated ubiquitination and proteasome-dependent degradation are not required for the transcriptional activity of SREBPs. Rather, ubiquitin-dependent degradation of SREBPs appears to be required to remove promoter-bound transcription factors to allow binding of new molecules in a process that could be termed “destruction by activation” (15). However, more detailed studies of the potential role of mono- and polyubiquitination of SREBPs for their activity are warranted. SREBP molecules cannot be degraded as soon as they associate with target promoters. Their degradation must be delayed until transcription has been initiated. It is possible that these processes are controlled by the phosphorylation or dephosphorylation of other residues in SREBP1. It will therefore be important to identify all the residues in SREBP1 that are phosphorylated in response to DNA binding. The identification of other post-translational modifications associated with promoter-bound SREBP1, as well as detailed structural studies of SREBP1 bound to DNA, will also be helpful to determine how DNA binding enhances the phosphorylation of SREBP1.

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