c-Jun Transactivates the Promoter of the Human p21WAF1/Cip1 Gene by Acting as a Superactivator of the Ubiquitous Transcription Factor Sp1*

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The cell cycle inhibitor protein p21WAF1/Cip1 (p21) is a critical downstream effector in p53-dependent mechanisms of growth control and p53-independent pathways of terminal differentiation. We have recently reported that the transforming growth factor-β pathway-specific Smad3 and Smad4 proteins transactivate the human p21 promoter via a short proximal region, which contains multiple binding sites for the ubiquitous transcription factor Sp1. In the present study we show that the Sp1-occupied promoter region mediates transactivation of the p21 promoter by c-Jun and the related proteins JunB, JunD, and ATF-2. By using gel electrophoretic mobility shift assays we show that this region does not contain a binding site for c-Jun. In accordance with the DNA binding data, c-Jun was unable to transactivate the p21 promoter when overexpressed in the Sp1-deficient Drosophila-derived SL2 cells. Coexpression of c-Jun and Sp1 in these cells resulted in a strong synergistic transactivation of this promoter. In addition, a chimeric promoter consisting of six tandem high affinity Sp1-binding sites fused with the CAT gene was transactivated by overexpressed c-Jun in HepG2 cells. The above data propose functional cooperation between c-Jun and Sp1. Physical interactions between the two factors were demonstrated in vitro by using GST-Sp1 hybrid proteins expressed in bacteria and in vitro transcribed-translated c-Jun. The region of c-Jun mediating interaction with Sp1 was mapped within the basic region leucine zipper domain. In vivo, functional interactions between c-Jun and Sp1 were demonstrated using a GAL4-based transactivation assay. Overexpressed c-Jun transactivated a chimeric promoter consisting of five tandem GAL4-binding sites only when coexpressed with GAL4-Sp1-(83–778) fusion proteins in HepG2 cells. By utilizing the same assay, we found that the glutamine-rich segment of the B domain of Sp1 (Be, amino acids 424–542) was sufficient for c-Jun-induced transactivation of the p21 promoter. In conclusion, our data support a mechanism of superactivation of Sp1 by c-Jun, which is based on physical and functional interactions between these two transcription factors on the human p21 and possibly other Sp1-dependent promoters.

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p21WAF1/Cip1 (p21) modulates cyclin-dependent kinase activity resulting in cell growth arrest or progression (1, 2). In addition p21 prevents DNA synthesis and regulates DNA methylation by directly interacting with proliferating cell nuclear antigen, a subunit of DNA polymerase δ (1, 3). Finally, p21 plays important roles in the control of cell senescence, apoptosis, and differentiation (4–6).

p21 gene expression is regulated by a long list of inducers under physiological or pathological conditions. These include the following: (a) tumor suppressors such as p53 (7); (b) factors that control differentiation of diverse cell types such as hematopoietic cells by phorbol esters and steroid superfamily members (8, 9), muscle and skin cells during terminal differentiation (10, 11), hepatocytes during normal liver organogenesis or liver regeneration (12, 13), and nerve cells by nerve growth factor (14, 15); and (c) growth factors, cytokines, hormones, and stress factors such as serum and platelet-derived growth factor (16), tumor necrosis factor α (17), phorbol esters or phosphatase inhibitors (18), interferon γ (19), progesterone (20), and transforming growth factor-β (TGF-β) (21) or activin A (21–23) and their signaling effectors, Smad proteins (24, 25).

Of particular importance has been the elucidation of the transcriptional mechanisms that operate during p21 gene induction by the above listed factors. For many such factors, including p53, retinoic acid, vitamin D3, interferon γ, and others, specific cis-acting DNA motifs have been identified on the p21 promoter in a region that extends between positions −2,300 and −210 relative to the transcriptional initiation site (9, 19, 26). On the other hand, an increasing number of regulatory factors including TGF-β, progesterone, phorbol esters, and phosphatase inhibitors mediate their effects on p21 gene expression via the proximal region of the promoter (−210 to +1 base pairs) (10, 14, 18, 20, 21, 23, 25). The proximal promoter contains characteristic GC-rich motifs that serve as binding sites for members of the Sp1 family of ubiquitous transcription factors (21).

Sp1 belongs to a zinc finger family of transcription factors that recognize GC-rich DNA sequences (27, 28). Sp1 plays an important role in early embryonic development and seems to be required for the maintenance of terminal cell differentiation by regulating the state of DNA CpG island methylation (29). The DNA binding and transactivation activities of Sp1 are regulated by phosphorylation that follows cell cycle-specific patterns (30). Sp1 protein is also stabilized by O-linked glycosylation, which confers resistance to proteasome-dependent
degradation (31). Sp1 has been shown to associate directly with members of the basal transcription machinery such as TFIIID components (32, 33). On the other hand, Sp1 physically interacts and functionally cooperates with several transcriptional activators including NF-kB, GATA, YY1, E2F1, pRb, SREBP-1 (34–37) via complex mechanisms that involve yet unidentified components (38). Thus, although Sp1 has been traditionally considered as a ubiquitous factor closely associated with core promoter activities, it has been recently shown to participate in several cases of regulated gene transcription by multiple signaling pathways and metabolic or differentiation conditions.

Detailed analysis of Sp1-mediated up-regulation of p21 has been provided for the action of TGF-β (21, 23, 25). The cellular effects of TGF-β are elucidated by a signaling pathway that involves membrane serine/threonine kinase receptors and cytoplasmic effectors of which the best understood are the Smad proteins (24, 25, 39). On the other hand, one of the earliest genomic responses of cells to TGF-β is the induction of Jun family members (40, 41). Jun proteins, as constituents of the AP-1 transcriptional complex, also activate several major target genes of TGF-β signaling, including plasminogen activator inhibitor I, pro-α(I)-collagen, retinoic acid receptors, interleukin 11, and c-jun itself among others (Ref. 42 and references therein). The role of Jun transcription factors in TGF-β-regulated gene expression is additionally underscored by the recent identification of specific c-Jun/Smad protein-protein interactions and functional cooperation (43, 44).

We have recently reported that the mechanism of p21 gene induction by TGF-β in hepatoma HepG2 cells is based on functional interactions between Smad3 and -4 proteins and Sp1 (25). We now report that Jun family members can also regulate p21 promoter activity in the same cell system. We demonstrate that c-Jun physically interacts with Sp1 and superactivates p21 promoter activity in a manner that does not require direct binding of c-Jun to the proximal p21 promoter. This newly uncovered mechanism underlying the action of Jun proteins provides a second pathway that could explain TGF-β-mediated p21 up-regulation and could furthermore explain p21 induction by various other stress or pro-inflammatory factors.

**EXPERIMENTAL PROCEDURES**

**Materials**—Restriction and modification enzymes (T4 DNA ligase, T4 polynucleotide kinase, Klrenov fragment of DNA polymerase I, and calf intestinal alkaline phosphatase) were purchased from Minotech, New England Biolabs, or Life Technologies, Inc. Vent DNA polymerase was purchased from New England Biolabs. Sequenase version 2 kit was purchased from Amersham Pharmacia Biotech/U. S. Biochemical Corp. Poly(dIdC), acetyl-CoA, dNTPs, and the GST purification kit were purchased from commercial sources at the purest grade available.

**Plasmid Constructions**—The p21 promoter plasmids —2300/+8 CAT, −210/−8 CAT, −143/+8 CAT, and −2300/+8 (Δ−122−64) p21 luciferase have been described previously (25). The expression vectors pCDNAI/e-Jun and pCDNAI/AIF-T2 were generously provided by Dr. D. Thanos, Department of Biochemistry and Biophysics, Columbia University. Expression vectors encoding the mouse JunB and JunD proteins (pRSV-JunB and pRSV-JunD) were the generous gift of Dr. E. Nikolakaki, Laboratory of Biochemistry, Department of Chemistry, Aristotelian University of Thessaloniki, Greece. The expression vectors encoding the FLAG-tagged human SMAD3 and SMAD4 proteins were the generous gifts of Dr. R. Derynck and Dr. J. Massague, respectively. The expression vectors pmCVc-Jun and pCMVc-Jun (Δ5−122), pCMV-c-Jun A265D In265, and pCMVc-Jun (−1−287) encoding the wild or mutant human c-Jun proteins were the generous gift of Dr. M. Birrer, NICL, National Institutes of Health, Rockville, MD. The p300 expression vector pCMVp300 was a generous gift of Dr. David M. Livingston, Dana Farber Cancer Institute, Boston, MA. The expression constructs pSG424/GAL4-SpLA + B, pSG424/GAL4-Sp1B, pSG424/GAL4-Sp1Bn, pSG424/GAL4-Sp1Bc, the pBGXI plasmid containing the GAL4 DNA binding domain portion only (aa 1–170), as well as the pG2Tl-CAT plasmid containing six tandem Sp1 sites in front of the tk minimal promoter and the CAT gene were the generous gift of Dr. G. Gill, Harvard Medical School, Boston. The pG2Tl-CAT reporter construct containing tandem GAL4 binding sites is from the NIH cell promoter and the CAT reporter gene was the generous gift of Dr. G. Mavrothalassitis, University of Crete Medical School, Greece. The AP1-tk-CAT construct containing a single copy of the collagenase AP1-binding site fused with the minimal tk promoter and the CAT gene was a generous gift of Dr. A. Pintzas, National Institute of Research, Athens, Greece. The bacterial expression vectors pGEX-Spl (−83−778), pGEX-Spl S16C, pGEX-Spl N619, and pGEX-Spl N19 349 were the generous gift of Dr. E. Flavey, Section of Molecular Genetics, Boston University Medical Center, Boston. The original Sp1 mutants were the generous gift of Dr. R. Tjian, University of California, Berkeley. The Drosophila expression vectors pPac-c-Jun, pPac-c-Fos, and pPacO were the generous gift of Dr. J. Noti, Guthrie Research Institute, Sayre, PA (45). The original expression vectors for normalization of transfections in Drosophila SL2 cells was the generous gift of Dr. C. Delidakis, University of Crete, and IMBB, Heraklion.

**Expression of Proteins in Vitro**—Expression of proteins in vitro was performed using the coupled in vitro transcription/translation system (TNT) of Promega according to the manufacturer’s instructions. Labeling of in vitro expressed proteins was done by the inclusion of 20 μCi of [35S]methionine in the TNT reaction mixture.

**Cell Cultures, Transient Transfections, and CAT Assays**—Human hepatoma HepG2 cells, monkey kidney COS-1 cells, human cervical carcinoma HeLa cells, and mouse embryonal carcinoma P19 cells were cultured in Dulbecco’s modified Eagle’s medium supplemented with 10% fetal bovine serum, tr-glutamine, and penicillin/streptomycin at 37 °C, in a 5% CO2 atmosphere. Drosophila Schneider’s SL2 cells were cultured in Schneider’s insect medium supplemented with 10% insect culture-tested fetal bovine serum and penicillin/streptomycin at 25 °C. Transient transfections were performed using the Ca3(PO4)2 coprecipitation method (46). Chloramphenicol acetyltransferase and β-galactosidase assays were performed as described previously (46).

**Gel Electrophoretic Mobility Shift Assay**—Gel electrophoretic mobility shift assays were performed as described previously (47,48). Oligonucleotides corresponding to the p21 promoter regions were synthesized, made double-stranded, labeled with Klenow and [α-32P]dCTP, and incubated with purified proteins. The sequence of the oligonucleotides used in electrophoretic mobility shift assay experiments are as follows: (a) p21 (−122−84), 5′-GAGGGCGGTCCCGGGCGGCGCGGTGGGC- CAGGCGCCGCGCAGGGG-3′; (b) p21 (−122−70), 5′-CCCGGCGTCGCCCCACCCGCCCGAGGGTGCAGGGTCGCGCAGGGCCG-3′; and (c) p21 (−86−70), 5′-GAGGGCGGTCCCGGGCGGCGCGGTGGGC-3′.

**Gelatinolytic Activity Assay**—Gelatinolytic activity was assayed as described previously (47). The assay was performed in Escherichia coli strain DH-10b. Bacteria were grown overnight, diluted 1:25, and after reaching an A600 of 0.7, were stimulated with 250 μM isopropyl-β-D-thiogalactopyranoside for 4 h at 37 °C. Bacteria were then harvested, resuspended in 0.03 volume of phosphate-buffered saline (PBS), sonicated for 1 min in PBS on ice, lysed by the addition of Triton X-100 to final concentration of 1%, and cleared by centrifugation at 10,000 rpm, at 4 °C for 10 min, resulting in a first supernatant enriched in GST fusion protein. The pellets were resuspended in solubilization buffer (1 mM EDTA, 25 mM triethanolamine, 1.5% N-laurylsarcosine) for 30 min, at 4 °C, with gentle agitation. Triton X-100 to final concentration of 2% and CaCl2, to final concentration of 1 mM were added, and the lysates were cleared by centrifugation at 10,000 rpm, at 4 °C, for 10 min, resulting in a second supernatant also enriched in GST fusion proteins. Both supernatants were used for the GST protein-protein interaction experiments. The solubilization of the expressed proteins was monitored by SDS-PAGE and Coomassie Blue staining.

**GST Protein Interaction Assay**—Glutathione-Sepharose 4B beads were equilibrated in PBS and mixed with 1 volume of bacterially expressed GST fusion proteins on a rotary shaker for 60 min at 4 °C. The beads were washed three times with 10 volumes of PBS and equilibrated in washing buffer (20 mM Hepes (pH 7.0), 100 mM KCl, 5 mM MgCl2, 0.2% Nonidet P-40, 2 mM phenylmethylsulfonyl fluoride, 10
Transactivation of Human p21 Promoter by Jun

RESULTS

Transactivation of the Human p21WAF1 Promoter by Jun

Proteins Is Mediated by the Proximal −122 to −64 Region—The proximal region of the human p21 promoter extending between positions −122 and −64 is GC-rich and contains five sequences that resemble or match exactly the recognition sequence of the ubiquitous transcription factor Sp1 (5'-GGGGCGG-3', Fig. 1A, double underline). One of these Sp1 sites was shown previously to be required for the stimulation of the p21 promoter by TGF-β in HaCaT keratinocytes (designated TβRE in Fig. 1A) (21), whereas at least one of these Sp1 sites was shown to mediate stimulation of the same promoter by phorbol esters (designated phorbol 12-myristate 13-acetate in Fig. 1A) during U937 cell differentiation (18). Both TGF-β and phorbol ester signal transduction pathways are mediated, at least in part, by AP1 proteins (Jun and ATF-2), we investigated the role of different AP1 family members in human p21 gene regulation. For this purpose, reporter constructs containing different p21 promoter fragments (−2300/+8, −210/+8, and −143/+8) fused with the bacterial CAT reporter gene were transiently cotransfected into the human hepatoma HepG2 cells along with an expression vector for the protooncogene c-jun or the empty vector as a control. As shown in Fig. 1B, overexpression of rat c-jun in HepG2 cells resulted in a 1.4–7-fold transactivation depending on the p21 promoter fragment tested. The greatest induction of transactivation by c-jun was achieved with a short proximal promoter that extends 143 bases 5' and 8 bases 3' to the transcription initiation site of the p21 gene (−143/+8 p21 CAT). Transactivation of the −143/+8 p21 promoter by c-jun was dose-dependent and was also observed in non-hepatic cell lines such as the human cervical carcinoma HeLa cells and the mouse embryonic carcinoma P19 cells to levels comparable to the ones achieved in HepG2 cells (data not shown). These findings suggest that the transactivation of the p21 promoter by c-jun does not depend on hepatocyte-specific auxiliary factors.

To investigate further the contribution of the proximal GC-rich −122/−64 p21 promoter region to the c-jun-mediated transactivation, HepG2 cells were transiently cotransfected with the −2300/+8 p21 promoter-luciferase construct bearing an internal deletion between nucleotides −122 and −64 (−2300/+8 (Δ−122/−64) p21 luciferase) along with the expression vector for rat c-jun or the empty vector as a control. As shown in Fig. 1B, this mutated promoter was only 5% as active as the wt promoter and was not responsive to c-jun overexpression in HepG2 cells. This finding confirms the importance of this proximal 60-base pair region for the c-jun-mediated transactivation of the p21 promoter in hepatocytes.

To investigate the ability of other members of the AP1 family of transcription factors (JunB, JunD, and ATF-2) to transactivate the p21 promoter, HepG2 cells were transiently cotransfected with the −143/+8 p21 CAT reporter construct along with expression vectors for the mouse JunB and JunD and the human ATF-2 proteins. As shown in Fig. 1C, overexpression of JunB, JunD, or ATF-2 caused a 5.8, 6.2, and 1.6-fold transactivation of the −143/+8 p21 promoter, respectively. No statistically significant change in transactivation was observed when the above three proteins were individually coexpressed along with c-jun (Fig. 1C and data not shown).

Overall, the findings of Fig. 1 indicate that transcription

μg/ml aprotinin). Fifty microliters of a 1:1 bead slurry in washing buffer was combined with 5 μl of a 35S-labeled reticulocyte lysate in a final volume of 500 μl of washing buffer, 10% glycerol (Interaction buffer) on a rotary shaker for 90 min at 4 °C. The beads were then washed 5 times with 20 volumes of washing buffer, and the bound proteins were eluted by boiling in Laemmli SDS-PAGE loading buffer and subjected to SDS-PAGE. Bound proteins were visualized by autoradiography.
Transactivation of Human p21 Promoter by Jun

Transactivation of Human p21 Promoter via the bZip Domain

The observation that all members of the AP1 family tested (c-Jun, JunB, JunD, and ATF-2) were able to transactivate the p21 promoter prompted us to identify domain(s) of these proteins required for this transactivation function. For this purpose, a preliminary structure-function analysis of human c-JUN protein was performed based on three mutant forms of c-Jun (Fig. 2A). The first mutant, c-Jun-(ΔS–122), lacks the N-terminal amino acids 3–122 that include the two serine residues (Ser^63 and Ser^73) that are phosphorylated by the c-Jun N-terminal kinase/stress-activated protein kinase (47). The second mutant, c-Jun A265D In265, contains a substitution of alanine to aspartic acid at position 265 as well as an insertion of three negatively charged amino acids after residue 265. This residue is localized within the positively charged basic region of c-Jun that serves as the DNA binding domain. This mutant c-Jun protein has lost its ability to bind to AP1 sites as shown previously (48). The third mutant, c-Jun-(1–287), lacks the C-terminal amino acids 288–331, which include the dimerization interface between AP1 proteins, the so-called leucine zipper domain. This mutant is unable to form dimers with other Jun proteins (48). The wt or mutant c-Jun proteins were transiently cotransfected into HepG2 cells along with the −143/+8 p21 CAT reporter, and the activity of the p21 promoter in the absence or presence of c-Jun was determined. This analysis showed the following. (a) Overexpression of human wt c-JUN protein (residues 1–331) resulted in a 12-fold transactivation of the p21 promoter, almost twice as high as its rat homologue (lanes 1 and 2 of Fig. 2A). (b) Deletion of amino acids 3–122 caused a 17-fold transactivation of the p21 promoter that represents a 40% increase in transactivation relative to the transactivation achieved by wild type c-Jun. (c) Mutagenesis of the basic region of c-Jun by the insertion of four negative charges did not cause a statistically significant change in the transactivation potential on the p21 promoter relative to wt c-Jun (lane 4). This mutation eliminated transactivation of a synthetic collagenase AP1-tk-CAT reporter construct in HepG2 cells (data not shown). (d) Deletion of the leucine zipper domain of c-Jun decreased its transactivation potential on the p21 promoter by 60% (5-versus 12-fold, lanes 2 and 6).

Overall, the findings of Fig. 2 indicate that the transactivation of the p21 promoter by c-Jun is mediated by the C-terminal 122–331 aa region which includes the bZip region. Furthermore, the dimerization property of c-Jun seems to be essential for transactivation, whereas an intact DNA binding domain is not required.

c-Jun Can Act as a Superactivator of the Ubiquitous Transcription Factor Sp1—Inspection of the nucleotide sequence of the proximal p21 promoter −143 to +8 did not reveal any identity or homology with the consensus AP1-binding site for c-Jun (5′-TGA G TCA-3′). The lack of consensus AP1 sites in this region in conjunction with the results of Fig. 2 led us to the hypothesis that c-Jun transactivates the p21 promoter not by binding directly to the DNA but rather by acting via other DNA-bound transcription factors through protein-protein interactions. This “superactivation” hypothesis was thoroughly tested by direct DNA binding, transactivation experiments, and in vitro protein-protein interaction assays. First, direct DNA binding of c-Jun to the −122/−70 p21 promoter region was tested by gel electrophoretic mobility shift analysis using the −86/−70 and −122/−84 regions as probes and purified c-Jun or Sp1 proteins (Fig. 3A). This analysis showed that c-Jun could not bind to the −86/−70 or −122/−84 p21 probes (lanes 1 and 4), whereas it bound efficiently to its cognate site on a collagenase AP1 probe used a control (compare lanes 1, 4, and 7). As expected, Sp1 bound strongly to both oligonucleotides (lanes 2 and 5). Interestingly, coinubcation of c-Jun and Sp1 led to the formation of a stronger Sp1-DNA complex (compare lanes 2 with 3 and 5 with 6), whereas no visible shift in the electrophoretic mobility of this Sp1-DNA complex was observed in the presence of c-Jun (lanes 3 and 6).

To study further the role of Sp1 in the c-Jun-mediated transactivation of the p21 promoter, we utilized the Sp1-deficient, Drosophila-derived, Schneider’s SL2 cells (49). Background levels of the −143/+8 p21 promoter were observed in these cells. Overexpression of Sp1 resulted in a 33-fold increase in transactivation of the human −143/+8 p21 promoter (lane 2) in accordance with previous findings (18, 21). In agreement with the DNA binding data of Fig. 3A, overexpression of c-Jun in SL2 cells did not increase the −143/+8 human p21 promoter activity. In contrast, a strong transactivation of a collagenase AP1-tk-CAT reporter construct was observed by c-Jun overexpression in the same cells (Fig. 3B, lanes 1, 3, 7, and 8). Coexpression of c-Jun and c-Fos also did not transactivate the p21 promoter, whereas c-Fos decreased by 40% the c-Jun-
mediated transactivation of the collagenase AP1-tk promoter in SL2 cells (lanes 5 and 9). Most importantly, coexpression of Sp1 with c-Jun or c-Jun/c-Fos caused a potent synergistic transactivation of the −143/+8 p21 promoter (120- and 155-fold versus 33-fold, lanes 4 and 6 of Fig. 3B). These findings suggest that transactivation of the p21 promoter by c-Jun is strictly Sp1-dependent.

Additional experiments were performed to establish functional interactions between c-Jun and Sp1. Transient cotransfection experiments in HepG2 cells showed that c-Jun transactivated a synthetic promoter consisting of six tandem high affinity Sp1-binding sites in front of the tk minimal promoter (6×Sp1 CAT) by 3.2-fold (compare lanes 1 and 2 in Fig. 3C). In addition, overexpression of c-Jun protein in HepG2 cells transactivated a synthetic promoter consisting of five tandem high affinity binding sites for the yeast protein GAL4 in front of the E1B minimal promoter (5×GAL4 CAT) only when it was coexpressed with a GAL4(DBD)-Sp1 chimeric protein consisting of the DNA binding domain of GAL4 (aa 1–170) fused with Sp1 (aa 83–778) (Fig. 3D). Overexpression of c-Jun in HepG2 cells enhanced the transactivation potential of GAL4-Sp1-(83–778) on the 5×GAL4 promoter by 7.3-fold (compare lanes 4 and 5). Control experiments showed that the GAL4 protein alone or in the presence of c-Jun had no effect on the activity of the 5×GAL4 promoter (lanes 2 and 3). The combined data of Fig. 3 strongly suggest that cooperative interactions between c-Jun and Sp1 can transactivate promoters containing multiple Sp1-binding sites.

Physical Interactions between c-Jun and Sp1—The ability of c-Jun to superactivate transcription in a strict Sp1-dependent manner strongly suggested that c-Jun and Sp1 are able to interact physically. To obtain direct evidence for such interactions, the GST interaction assay was employed. A fusion protein consisting of wt human Sp1 (aa 83–778) or mutated forms fused at the C terminus of glutathione S-transferase (GST-Sp1-(83–778)) or the GST portion alone (Fig. 4A) were used in these analyses along with 35S-labeled in vitro transcribed-translated wild type c-Jun protein. As shown in Fig. 4C, c-Jun could not bind to the GST beads (lane 2), whereas it bound efficiently to the GST-Sp1-(83–778) beads (lane 3), suggesting that physical interactions between the two factors are specific. To obtain additional information concerning the region(s) of Sp1 that mediate physical interactions with c-Jun, the following Sp1 mutant proteins were employed: Sp1 516C that lacks the N-terminal amino acids 1–261 (region A); Sp1 Δint 349 containing an internal deletion of amino acids 263–609 (regions B and C); and Sp1 N619 which lacks the C-terminal amino acids 703–778 (region D). This analysis showed that none of the Sp1 deletions was able to eliminate binding of c-Jun to Sp1 (Fig. 4C, lanes 6–8). The only difference was observed in the c-Jun/GST-Sp1 N-619 interaction that was weaker by approximately 50% relative to c-Jun/GST-Sp1-(83–778) interaction (lane 8). Again, no specific interaction was observed between c-Jun and the GST control (lane 5). The data of Fig. 4 suggest that c-Jun/Sp1 interactions require at least one of the two homologous Gln/ Ser/Thr-rich regions A and B and/or the DNA binding domain of Sp1 (located between regions C and D, Fig. 4A, labeled zinc fingers).

The bZip Region of c-Jun Is Essential for Both Physical and Functional Interactions between c-Jun and Sp1—To gain insight into the specific domain(s) of c-Jun required for physical interaction with Sp1, wt or mutant c-Jun proteins were utilized in GST interaction assays. The following c-Jun mutants were utilized for this analysis (Fig. 5A): c-Jun-(3–122), c-Jun A265D, c-Jun A265D In265 and c-Jun-(1–287) (described in Fig. 2), c-Jun-(1–175), and c-Jun-(226–331). As shown in Fig. 5B, none of the c-Jun proteins could bind to the GST beads. Deletion of amino acids 1–226 or 3–122 did not affect the ability of c-Jun to bind to Sp1 (c-Jun-(226–331) and c-Jun-(3–122)). In contrast, mutagenesis of the basic region or deletion of the leucine zipper domain of c-Jun decreased drastically but did not eliminate physical interactions between c-Jun and Sp1 (c-Jun A265D In265 and c-Jun-(1–287)). Finally, binding of c-Jun to Sp1 was totally abolished by deletion of the c-Jun region between amino acids 176 and 331 which includes the entire bZip domain.

In conclusion, the combined data of Figs. 4 and 5 suggest that physical interactions between c-Jun and Sp1 are mediated via
the bZip region of c-Jun and at least one of the two homologous regions A and B and/or the DNA binding domain of Sp1.

The Gln-rich Domain of Sp1 Is Sufficient to Mediate Functional Interactions between Sp1 and c-Jun—We next focused on the ability of the homologous A and B (Gln-Ser/Thr)-rich domains of Sp1 to interact functionally with c-Jun. For this purpose, various GAL4-Sp1 fusion proteins were utilized in the GAL4-based transactivation assay. The Sp1 mutants employed are shown in Fig. 6A, GAL4-Sp1-A + B, which contains only the A and B domains of Sp1 (amino acids 1–542); GAL4-Sp1-B, which contains only domain B (amino acids 263–542); GAL4-Sp1-Bn, which contains only the Ser/Thr-rich segment of domain B of Sp1 (amino acids 263–424); and GAL4-Sp1-Bc, which contains the Gln-rich part of domain B (amino acids 424–542). The ability of c-Jun protein to superactivate the various GAL4-Sp1 mutants was assessed by transient transfection experiments in HepG2 cells. As shown in Fig. 6B, overexpression of GAL4-Sp1-A + B caused a 10-fold transactivation of the 5×GAL4 promoter (compare lanes 1 and 3). Coexpression of c-Jun with GAL4-Sp1 A + B increased the transactivation of the 5×GAL4 promoter from 10- to 43-fold (compare lanes 3 and 4). Deletion of the domain A of Sp1 had no effect on the transactivation of the 5×GAL4 promoter (compare lanes 3 and 5), whereas cotransfection of c-Jun with the GAL4 Sp1 B fusion protein increases the transactivation of the 5×GAL4 promoter by 7.8-fold (compare lanes 5 and 6). The GAL4-Sp1 fusion protein retaining only the C-terminal part of domain B (GAL4-Sp1-Bc) transactivated the 5×GAL4 promoter to the same extent as the GAL4-Sp1-A + B form (compare lanes 3 and 7). c-Jun superactivated the GAL4-Sp1-Bc protein by 7.5-fold (compare lanes 7 and 8). Finally, the GAL4-Sp1-Bn mutant, which contains only the N-terminal part of domain B (Bn), was totally inactive either in the absence (lane 9) or in the presence of coexpressed c-Jun (lane 10).

These findings indicate that the N-terminal domains A and B of Sp1 are by themselves sufficient to mediate superactivation of the Sp1 protein by c-Jun. The mechanism of superactivation seems to involve functional interactions between c-Jun and at least one of the two Gln-rich domains of Sp1, although functional interactions between c-Jun and other parts of the Sp1 molecule such as the DNA binding domain cannot be excluded.

The p300 Cointegrator Enhances the Transactivation Potential of c-Jun and Smads on the p21 Promoter—We have recently shown that the −210/+8 and −143/+8 p21 promoter regions can be transactivated by Smad3 and Smad4 proteins via functional interactions with Sp1 (25). It has also been reported recently that c-Jun and Smad3 interact physically...
with one another and activate synergistically transcription from target promoters containing AP1 and/or Smad-binding elements (43, 44, 50, 51). To investigate putative synergistic interactions between Smads and c-Jun on the p21 promoter, HepG2 cells were cotransfected transiently with a p21 reporter construct (2210/118 p21 CAT) along with expression vectors for c-Jun, Smad3, and Smad4, or both. As shown in Fig. 7A, coexpression of c-Jun and Smads had a synergistic effect on the p21 promoter activity (6-fold by c-Jun, lane 2; 80-fold by Smad3/4, lane 3; and 115-fold by both c-Jun and Smad3/4, lane 4) indicating that Smads and c-Jun can transactivate the p21 promoter in a cooperative fashion.

It has been established previously by many independent investigations that the p300 cointegrator interacts physically with Smad3 and potentiates Smad3-mediated transactivation of TGF-β-responsive promoters (52, 53). It is also known that p300 contains an interaction interface for c-Jun (54). To investigate the putative role of p300 cointegrator in the c-Jun- and Smad-mediated transactivation of the p21 promoter, HepG2 cells were transiently cotransfected with the 2210/118 human p21 promoter-CAT construct (3 μg) in the absence (−) or presence (1 μg each) of expression vectors for c-Jun and/or Smad3 and Smad4. As shown in Fig. 7B, p300 caused a 2-fold enhancement in the c-Jun-mediated transactivation of the p21 promoter (7-fold versus 3.5-fold, lanes 3 and 4). Overexpression of p300 had a similar effect on the Smad3/4-mediated transactivation of the p21 promoter (14.8-fold versus 8.8-fold, lanes 5 and 6). These findings indicate that c-Jun and Smads regulate the transcription potential of both c-Jun and Smad3 and Smad4 on the p21 promoter. C, proposed model for the transactivation of the human p21 promoter by Smads, Jun, and the p300 cointegrator.

**DISCUSSION**

Jun Proteins Transactivate the Human p21WAF1 Promoter via the Proximal −122 to −64 Region—In the present study we investigated the potential involvement of Jun proteins in human p21 gene regulation. By using transient transfection experiments in HepG2 cells as well as gel electrophoresis mobility shift assays and GST interaction analyses, we established that c-Jun is a positive regulator of the human p21 promoter activity (Figs. 1 and 2). The highest degree of transactivation by c-Jun was achieved when a short proximal promoter segment extending 143 bases upstream and 8 bases downstream to the putative mRNA start site (TATA box) was used. As shown in Fig. 7C, p300 enhances the transcriptional activity of c-Jun on the p21 promoter by recruiting the p300 cointegrator.
transcription initiation site (−143/+8 p21 CAT) was used (Fig. 1B). Moreover, a p21 promoter construct bearing an internal deletion between nucleotides −122 and −64 (−2300/+8 Δ−122−/−64 p21 luciferase) was not transactivated by c-Jun. This GC-rich region contains the five sequences that resemble or match exactly the recognition sequence of the ubiquitous transcription factor Sp1 (Fig. 1A, double underline).

In addition to c-Jun, three other members of the AP1 family of transcription factors were found capable of transactivating the −143/+8 p21 promoter: JunB, JunD, and ATF-2 (activating transcription factor-2) (Fig. 1C). JunB and JunD proteins share extensive amino acid sequence similarity with c-Jun in two regions as follows: the C-terminal basic/leucine zipper domain involved in DNA binding and dimerization and the N-terminal acidic transactivation domain (Fig. 2B) (55). Jun proteins form homodimers and heterodimers with each other via the leucine zipper domain. In addition, c-Jun forms heterodimers with other AP1 members such as c-Fos and ATF-2 resulting in the recognition of the AP1 and the cyclic AMP-responsive elements, respectively (56, 57). The junB gene, similar to c-jun, is a target for TGF-β signaling (41). Activating transcription factor 2 (ATF-2) was shown recently to be a key effector in TGF-β signaling by acting as a common nuclear target of Smad and the mitogen-activated protein kinase kinase kinase TAK1 (58).

All these findings indicate that functional complexes between Jun, ATF-2, and Smad proteins could mediate the functional cross-talk between signal transduction cascades that result in the modulation of gene expression of target genes during stress or other growth conditions depending on the extracellular stimulus.

p21 Promoter Regulation by c-Jun Is Independent from Its DNA-binding Properties and Requires Sp1—Inspection of the nucleotide sequence in the proximal −143/+8 p21 promoter region did not reveal any homology with the AP1 or cyclic AMP-responsive element recognition motifs suggesting that c-Jun does not function as a typical AP1 transactivator in p21 gene regulation. We thus hypothesized that c-Jun acts as superactivator of other nuclear factor binding to the proximal p21 promoter.

We investigated more thoroughly the putative superactivating function of c-Jun by various analyses. First, purified c-Jun protein could not bind to the −122−/−64 p21 promoter region in a gel electrophoretic mobility shift assay (Fig. 3A). Second, a DNA-binding deficient c-Jun mutant (c-Jun A265D In 265) transactivated the p21 promoter as effectively as wt c-Jun in transient transfection experiments (Fig. 2A). Third, c-Jun could not transactivate the p21 promoter in the Drosophila SL2 cells, which lack proteins highly related to mammalian Sp1 (Fig. 3B). In contrast, coexpression of c-Jun with Sp1 resulted in a strong synergistic transactivation of the p21 promoter (Fig. 3B). Fourth, c-Jun transactivated a synthetic promoter consisting of six high affinity binding sites for Sp1 in front of the thymidine kinase (tk) minimal promoter (32) in transient transfection experiments in HepG2 cells (Fig. 3C). Fifth, c-Jun activated transcription via Sp1 in a GAL4-based transactivation system (Fig. 3D). A similar result had been obtained previously in a different cell system (59). Thus, the combined data of Figs. 2 and 3 suggest that protein-protein interactions between c-Jun and Sp1 are involved in the transactivation of natural (p21) or artificial Sp1-dependent promoters by c-Jun.

Sp1 Physically Interacts with c-Jun—The functional interactions observed between c-Jun and Sp1 prompted us to investigate potential physical interactions between these two factors. Our analysis showed that c-Jun/Sp1 interactions require at least one of the two homologous regions A and B and/or the DNA binding domain of Sp1, whereas domains C and D are not required (Fig. 4). The involvement of the zinc finger DNA binding domain of Sp1 in physical interactions with other proteins is not unprecedented. For example, Sp1 was shown to physically interact via its DNA binding domain with transcriptional activators such as the p65/RelA subunit of NF-κB (60), the erythroid factor GATA-1 (61), the Inr binding protein YY1 (34), and the cell cycle regulator E2F (36, 62). In all of the above cases, physical interactions were associated with functional cooperation among the partners on target promoters that contain binding sequences, usually closely spaced, for the two proteins. In the case of the p21 promoter, the synergistic mechanism of transactivation by c-Jun and Sp1 seems to be different from the above examples. First, c-Jun cannot bind to the p21 promoter, thus functional cooperation could not be the result of a correct juxtaposition of the two proteins on the DNA. Second, the B domain of Sp1 is sufficient for the superactivation of Sp1 by c-Jun, whereas this domain is dispensable for the interaction of Sp1 with all of the above listed factors. Third, the basic leucine zipper (bZip) domain of c-Jun was shown to be required for physical interaction and functional synergism with Sp1 (Figs. 2 and 5). The bZip domain of c-Jun does not display any homology with the domains of the above factors that are essential for interaction with Sp1 (zinc finger domains in YY1 and GATA-1, the Rel homology domain of p65, and the cyclin A binding domain of E2F). Notably, E2F also contains a leucine zipper domain, but in contrast with c-Jun, this domain was not required for physical interaction with Sp1.

Finally, our findings also establish that the association of c-Jun with Sp1 can occur in the absence of DNA. However, in the presence of DNA, the interaction of the two proteins results in enhanced DNA binding of Sp1 to its cognate site (Fig. 3A). This phenomenon has also been observed previously in the cases of Rb/Sp1 and SREBP-1/Sp1 interactions (63, 64). Thus, we are tempted to speculate that the association of c-Jun with Sp1 results in a conformational change in the Sp1 molecule, the new conformation displaying enhanced DNA-binding properties. We could also hypothesize that specificity in Sp1 function could result from the different configurations that Sp1 could adopt as a result of its interaction with different transcription factors.

The Gln-rich Domain of Sp1 Is Sufficient to Mediate Functional Interactions between Sp1 and c-Jun—By using the GAL4-based transactivation system, we showed that the Gln-rich C-terminal part of Sp1 domain B (Be) is sufficient to mediate functional interactions with c-Jun. Previous work had established that this glutamine-rich hydrophobic patch present in Sp1 contacts directly TAF111,110 and TAF130,130, components of the TFIIID complex in eukaryotes, thus linking Sp1 to the initiation complex and mediating transcriptional activation (32, 65). Based on these observations and the data presented in the current study, we hypothesize that the Sp1/c-Jun interactions result in enhanced transactivation of Sp1-dependent promoters by stabilizing the interactions between Sp1 and the basal transcriptional machinery factors TAF111,110, TAF130,130, and/or other yet unidentified components.

Finally, it is of interest that the Gln-rich region of the B domain of Sp1 has also been shown to be required for the transcriptional induction of Sp1-dependent promoters by transforming-growth factor-β (66). This observation along with our recent findings that overexpression of Smad proteins in HepG2 cells superactivate Sp1 via the Gln-rich domain Be2 strongly suggest that c-Jun and Smad proteins regulate Sp1-dependent transcription via a similar mechanism.
Integration of Different Signal Transduction Cascades Could Be Mediated by Functional Jun-Smad-Sp1 Complexes and the p300 Coactivator—We have recently reported that the −122/−64-proximal p21 promoter region mediates the transcriptional activation of this promoter by TGF-β and Smads (25). Utilizing GAL4-Sp1 fusion proteins we had showed that the mechanism of activation by Smad proteins involves their interaction with the glutamine- and serine-threonine-rich N-terminal domains of Sp1. The similarity between the mechanisms of Sp1-dependent superactivation of the p21 promoter by Smads and c-Jun prompted us to investigate whether c-Jun and Smads act synergistically on the p21 promoter. Our results shown in Fig. 7A of this study showed that coexpression of c-Jun and Smad3 and −4 had a synergistic effect on the −143/+8 p21 promoter suggesting that Smads and c-Jun can transactivate the p21 promoter in a cooperative fashion. The latter hypothesis implies that c-Jun and Smad3 and/or Smad4 could form a higher order transcription complex with either free or DNA-bound Sp1. Thus one could in principle obtain transcription factor-specific, synergistic, or additive and sustainable gene expression by signaling modules that can activate Jun, Smads, and or Sp1. For example, TGF-β could activate the p21 promoter by two different pathways as follows: via the c-Jun N-terminal kinase/c-Jun and via the Smad cascade. Both Jun and Smads can also induce jun gene expression thus forming an autoregulatory loop. Thus one can have (i) direct and immediate early effects caused by rapid transcriptional induction of target genes via Smad-Sp1 or Jun-Sp1 interactions; (ii) indirect and long lasting effects that form autoregulatory mechanisms of transcriptional induction of the c-jun genes.

The c-Jun-Smad-Sp1 complexes could also be stabilized by coactivator/auxiliary proteins. For example, it has been well established that both c-Jun and Smad3 can bind to CBP/p300 (52–54). In contrast, direct association between Sp1 and p300 coactivator/auxiliary proteins. For example, it has been well established that both c-Jun and Smad3 can bind to CBP/p300 (52–54). In contrast, direct association between Sp1 and p300 could be modulated by the transcription coactivator CBP/p300 and/or possibly other yet unidentified auxiliary factors.

Generality of Sp1-Jun Interactions in Eukaryotic Gene Regulation—Since a large number of eukaryotic promoters contain proximal Sp1-binding sites, the question of whether all Sp1-dependent promoters can be transactivated by c-Jun is of particular importance. Jun could in principle transactivate promoters that contain multiple tandem proximal Sp1-binding sites in the absence of other regulatory regions. However, in more complex promoters in which the Sp1-binding sites are present as single copies and are dispersed among other binding regions, the function of c-Jun could be predicted to depend on the identity and the role of the other factors that modulate the expression of the target promoter. In addition, eukaryotic promoters with GC boxes could possibly involve binding of other Sp family members such as Sp3 or Sp4, ZIF268, TIEG1 and TIEG2, and more (68–70). Thus, future analysis of the specificity of Jun interactions with any of these factors and their involvement in the physiological regulation of target genes such as p21 will clarify the spectrum of regulatory networks in which Jun can participate.

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REFERENCES
1. Sherr, C. J., and Roberts, J. M. (1995) Genes Dev. 9, 1149–1163
2. Cheng, M., Olivier, P., Diehl, J. A., Fero, M., Roussel, M. F., Roberts, J. M., and Sherr, C. J. (1999) EMBO J. 18, 1571–1583
3. Cheng, L. S., Jan, H. L., Ng, H. H., Xu, G., and Li, B. F. (1997) Science 277, 1996–2000
4. Brown, J. P., Wei, W., and Sedivy, J. M. (1997) Science 277, 831–834
5. Podlak, K., Waldman, T., Ho, T. C., Kinzler, K. W., and Vogelstein, B. (1996) Genes Dev. 10, 1450–1952
6. Jacks, T., and Weinberg, R. A. (1998) Science 290, 1035–1036
7. El-Deiry, W. S., Tokino, T., Velculescu, V. E., Levy, D. B., Parsons, R., Trent, J. M., Lin, D., Mercer, W. E., Kinzler, K. W., and Vogelstein, B. (1994) Cell 75, 817–825
8. Jiang, H., Lin, J., Su, Z. Z., Collart, F. R., Huberman, E., and Fisher, P. B. (1994) Oncogene 9, 3397–3406
9. Liu, M., Iavarone, A., and Freedman, L. P. (1996) J. Biol. Chem. 271, 31725–31728
10. Prowse, D. M., Bolgan, L., Molnar, A., and Dotto, G. P. (1997) J. Biol. Chem. 272, 1308–1314
11. Zhang, P., Wang, C., Liu, D., Finegold, M., Harper, J. W., and Elledge, S. J. (1999) Genes Dev. 13, 213–224
12. Wu, H., Wade, M., Kral, R., Grimson, J., Xiong, Y., and Van Dyke, T. (1996) Genes Dev. 10, 245–260
13. Albrecht, J. H., Meyer, A. H., and Hu, M. Y. (1997) Hepatology 25, 557–563
14. Yan, G.-Z., and Ziff, E. B. (1997) J. Neurosci. 17, 6122–6132
15. Erhardt, J. A., and Pittman, R. N. (1998) J. Biol. Chem. 273, 23517–23523
16. Michieli, P., Chedid, M., Lin, D., Pierce, J. H., Mercer, W. E., and Givel, D. (1994) Cancer Res. 54, 3391–3395
17. Akashi, M., Hachiyi, M., Sato, Y., Spiloti, K., Suzuki, G., and Koeffler, H. P. (1995) J. Biol. Chem. 270, 19181–19187
18. Biggs, J. R., Kudlow, J. E., and Kraft, A. S. (1996) J. Biol. Chem. 271, 901–906
19. Chen, Y. E., Kitagawa, M., Sie, C. S., You, Z.-H., Iwamoto, Y., and Fu, X.-Y. (1996) Science 272, 719–722
20. Owen, G. I., Richer, J. K., Tung, L., Takimoto, G., and Horwitz, K. B. (1998) J. Biol. Chem. 273, 10696–10701
21. Dan, M. B., Yu, Y., and Wang, X.-F. (1995) J. Biol. Chem. 270, 28623–28628
22. Zauberman, A., Oren, M., and Zipori, D. (1997) Oncogene 15, 1705–1711
23. Li, J. M., Datto, M. B., Shen, X., Hu, P. P., Yu, Y., and Wang, X.-F. (1998) Nucleic Acids Res. 26, 2207–2212
24. Hunt, K. K., Fleming, J. B., Abramian, A., Zhang, L., Evans, D. B., and Chiao, P. J. (1998) Cancer Res. 58, 5565–5566
25. Murtakasa, A., and Kardassi, D. (1998) Proc. Natl. Acad. Sci. U. S. A. 95, 6733–6738
26. El-Deiry, W. S., Tokino, T., Waldman, T., Oliner, J. D., Velculescu, V. E., Burrell, M., Hill, D. E., Healy, E., Rees, J. L., Hamilton, S. R., Kinzler, K. W., and Vogelstein, B. (1995) Cancer Res. 55, 2910–2919
27. Berg, J. M. (1992) Proc. Natl. Acad. Sci. U. S. A. 89, 11109–11110
28. Lania, L., Majello, B., and De Luca, P. (1997) Int. J. Biochem. Cell Biol. 29, 1313–1323
29. Marin, M., Caris, V., Visser, P., Grosveld, F., and Philipsen, S. (1997) Cell 89, 619–628
30. Han, I., and Kudlow, J. E. (1997) Mol. Cell. Biol. 17, 2550–2558
31. Gill, G., Pascale, E., Taeng, Z. H., and Tjian, R. (1994) Proc. Natl. Acad. Sci. U. S. A. 91, 192–196
32. Saluja, D., Vassallo, M. F., and Tanese, N. (1998) Mol. Cell. Biol. 18, 5734–5743
33. Lee, J. S., Galvin, K. M., and Shi, Y. (1993) Proc. Natl. Acad. Sci. U. S. A. 90, 6145–6149
34. Udasin, A. J., Templeton, D. J., and Horvitz, J. M. (1995) Proc. Natl. Acad. Sci. U. S. A. 92, 3953–3957
35. Lin, S. Y., Black, A. R., Kostiec, D., Pajovic, S., Hoover, C. N., and Azizkhan, J. C. (1996) Mol. Cell. Biol. 16, 1668–1675
36. Hirano, F., Tanaka, H., Hirano, Y., Hirohata, M., Handa, H., Makino, I., and Scheidereit, C. (1996) Mol. Cell. Biol. 16, 1266–1274
38. Naair, A. M., Beaurang, P. A., Robinson, K. M., Oliner, J. D., Avizionsis, D., Scheek, S., Zwicker, J., Kadonaga, J. T., and Tjian, R. (1998) *Genes Dev.* 12, 3020–3031
39. Pertoavaara, L., Sistonen, L., Bos, T. J., Vogt, P. K., Keski-Oja, J., and Alitalo, K. (1989) *Mol. Cell. Biol.* 9, 1255–1262
40. Li, L., Hu, J.-S., and Olson, E. N. (1990) *J. Biol. Chem.* 265, 1556–1562
41. Kardassis, D., Zannis, V. I., Cladaras, C. (1992) *J. Biol. Chem.* 267, 2622–2632; Correction (1992) *J. Biol. Chem.* 267, 7956
42. van Dam, H., Duyndam, M., Rottier, R., Bosch, A., de Vries-Smits, L., Herrlich, P., Zantema, A., Angel, P., and van der Eb, A. J. (1993) *EMBO J.* 12, 479–487
43. Halazonetis, T. D., Georgopoulos, K., Greenberg, M. E., and Leder, P. (1988) *Cell* 55, 887–898
44. Courey, A. J., and Tjian, R. (1988) *Cell* 55, 887–898
45. Yieh, L., Sanchez, H. B., and Osborne, T. F. (1995) *Proc. Natl. Acad. Sci. U. S. A.* 92, 6102–6106
46. Li, J. M., Datto, M. B., Shen, X., Hu, P. P., Yu, Y., and Wang, X. F. (1998) *Nucleic Acids Res.* 26, 2449–2456
47. Gerritsen, M. E., Williams, A. J., Neish, A. S., Shi, Y., and Collins, T. (1997) *Proc. Natl. Acad. Sci. U. S. A.* 94, 2927–2932
48. Xiao, H., Hasegawa, T., and Isobe, K. (1999) *J. Biol. Chem.* 274, 2927–2932
49. Kim, Y., Ratziu, V., Choi, S.-G., Lalazzaro, A., Theiss, G., Dang, Q., Kim, S.-J., and Friedman, S. L. (1998) *J. Biol. Chem.* 273, 33750–33758
50. Cook, T., Gebelein, B., Mesa, K., Mladek, A., and Urrutia, R. (1998) *J. Biol. Chem.* 273, 25929–25936