Sp1 nuclear levels have been shown to directly correlate with the proliferative state of the cell. We therefore studied changes in the abundance of Sp1 in a rat pituitary cell line GH4 whose growth rate is regulated by epidermal growth factor (EGF). Nuclear extracts from GH4 cells treated with 10 nM EGF for at least 16 h showed a 50% decrease in Sp1 binding to a GC-rich element present in the gastrin promoter. The decrease in binding correlated with a decrease in cell proliferation, a loss of nuclear Sp1 protein and a 50–60% decrease in Sp1-mediated transactivation through an Sp1 enhancer element in transfection assays. Okadaic acid, a phosphatase inhibitor, was synergistic with the effect of EGF on Sp1 protein levels suggesting that the loss of Sp1 was mediated by phosphorylation events. This result was confirmed by showing a 2-fold increase in orthophosphate-labeled Sp1 with EGF and okadaic acid. Cycloheximide prevented the expected loss of Sp1 mediated by EGF and okadaic acid suggesting that the synthesis of a protease may mediate these events. This hypothesis was tested directly by showing that the cysteine protease inhibitor leupeptin prevented Sp1 degradation. Using the PEST-FIND computer program, the computed PEST score for human and rat Sp1 is 10.4 and 13.7, respectively, indicating that Sp1 has a domain with a high concentration of proline, glutamic acid, serine, and threonine residues as reported for a number of proteins with inducible rates of degradation. Collectively, these results indicate that sustained stimulation of GH4 cells by EGF initiates a cascade of phosphorylation events that promotes Sp1 proteolysis, decreased Sp1 nuclear levels and decreased cellular proliferation.

While Sp1 is generally considered to be a constitutively active housekeeping gene, cellular Sp1 levels have been correlated with tumor cell mitotic rates, cellular proliferation, and cell cycle regulators (1, 2). Sp1 mRNA levels increase in response to SV40 viral infection in CV-1 cells and in response to phorbol ester induction of T-lymphocytes (3). Sp1 protein expression in normal tissue varies markedly during development being highest in the thymus, lung, spleen, and variably expressed in the stomach (2). Post-translational modification of Sp1 by phosphorylation and glycosylation has been correlated with Sp1 transcriptional activation in vitro (4, 5). Thus, Sp1 nuclear levels appear to be closely related to increased cellular proliferation.

Transcription factor Sp1 binds to GC-rich elements in the promoters of both cellular and viral genes and stimulates basal promoter activation through TATA-binding protein-associated factors (6, 7). Overexpression of Sp1 transactivates genes suggesting that regulating the abundance of cellular Sp1 is one mechanism through which Sp1 regulates transcription. Changes in the abundance of Sp1 may occur by regulating Sp1 gene expression, mRNA stability, or post-translational events that result in altered DNA affinity or protein turnover. However, the relationship of these processes to gene activation has not been well characterized.

We have previously studied Sp1 binding to a GC-rich element (GGGGCGGGGTGGGGGG) designated gastrin EGF Response Element (gERE) in the gastrin promoter (8). This element confers both epidermal growth factor (EGF) and phorbol ester responsiveness and consists of two overlapping domains. The 5‘ domain binds Sp1 and the 3‘ domain binds two additional complexes provisionally designated as the gastrin EGF responsive proteins 1 and 2 (gERP 1 and gERP 2) (9). EGF induction of the gastrin promoter occurs within the first 12 h and requires both the 5‘ and 3‘ half-sites. Although EGF induction of the gastrin promoter in GH4 cells appear to require Sp1, there is no significant increase in the binding of this protein with EGF (9). Instead after 16 h of EGF treatment, Sp1 binding decreases. EGF treatment of GH4 cells slows cellular proliferation which correlates inversely with cellular differentiation (10, 11). Collectively, these results suggest that Sp1 nuclear levels correlate directly with cellular proliferation.

To study the effect of EGF on Sp1 in greater detail, changes in Sp1 binding and abundance were studied by EMSAs, immunoblots, and metabolically labeling endogenous Sp1. We found that Sp1 protein levels decreased in response to prolonged treatment with EGF. Since EGF receptor activation results in a cascade of phosphorylation events and Sp1 is known to be a phosphoprotein (4, 12, 13), we reasoned that EGF stimulation of Sp1 proteolysis may be related to its phosphorylation state. We therefore examined the effect of combining EGF with a phosphatase inhibitor, okadaic acid to sustain Sp1 phosphorylation. In addition, since the decrease in cellular Sp1 levels indicated that Sp1 may undergo proteolysis, we investigated whether this process required protein synthesis and was inhibitory.
Inducible Sp1 Proteolysis

Cell Culture—GH3 cells (derived from a rat pituitary adenoma) (14) were cultured in Dulbecco's modified Eagle's medium (DMEM, Life Technologies, Inc.) at 37 °C in a humidified atmosphere of 5% CO2 and 95% air. Complete medium contained 8% horse serum, 6% newborn calf serum, penicillin at 100 μg/ml, and streptomycin at 100 μg/ml. Where indicated, the cells were stimulated with 10 nM EGF with or without 50 nM okad acid in the above medium for 16 h prior to the preparation of nuclear extracts by detergent extraction using a final concentration of 1% Nonidet P-40 (15). All buffers contained phosphatase inhibitors, 10 mM sodium fluoride, 10 mM sodium vanadate, 10 mM sodium pyrophosphate, 5 mM sodium phosphate and protease inhibitors, 1 mM dithiothreitol, 5 μM/ml aprotinin, 1 μg/ml leupeptin, 1 μg/ml pepstatin, 0.1 mM phenylmethylsulfonyl fluoride, 0.1 mM sodium metabisulfite, 5 μg/ml antipain, 5 μg/ml chymostatin. Leupeptin, a tripeptide aldehyde with an amino-terminal acetyl group (Ac-Leu-Leu-Arginal), was purchased from Sigma and okadaic acid was purchased from LC Laboratories, Woburn, MA. MG-132 was a gift from Proscript, Cambridge, MA. MG-132 is a tripeptide aldehyde benzoyloxycarbonyl (Z)-Leu-Leu-Leucinal at 1 μM.

Electrophoretic Mobility Shift Assays (EMSAs)—Oligonucleotide probes were Klenow end-labeled with [α-32P]dATP after hybridizing complementary strands. All gel shift reactions were carried out in a final volume of 20 μl that contained 2.0 μg of nuclear extract, radiolabeled oligonucleotide probe at a final concentration of (~30,000 cpm/μg) 10 μM Tris-Cl, pH 7.5, 100 mM KCl, 1 mM MgCl2, 3 mM EDTA, 1 mM dithiothreitol, 10% glycerol, 300 ng of poly(dI-dC), and 1 mM ZnCl2. After binding for 20 min at 25 °C, DNA-protein complexes were resolved on a 4% nondenaturing polyacrylamide gel containing 45 μM Tris base, 45 μM boric acid, 1 mM EDTA and quantified on a PhosphorImager (Molecular Dynamics). The sense strands of the double stranded oligonucleotide probes were WT-gERE (GGGGCGGGGTGGGGGG) and a consensus Oct-1 sequence (TGGTCAATGTCACCATTACACTAGA).

Orthophosphate Labeling of Sp1—GH3 cells (107 cells) were incubated in 3 ml of phosphate-deficient DMEM with 10% dialyzed fetal calf serum (Life Technologies, Inc.) for 1 h prior to the addition of labeling medium. The cells were incubated for 4 h with 100 μCi/ml [32P]-orthophosphate (2500–3500 Ci/mmol) in phosphate-deficient medium that also contained 0.1% bovine serum albumin and the supernatants combined. The incubations were terminated by removing the labeling medium, rinsing the cells with PBS and preparing nuclear extracts by detergent lysis according to the method of Schreiber (15). Twenty-five micrograms of nuclear protein was then used to immunoprecipitate Sp1. Nonspecific protein was precleared using magnetized Dynabeads (Dynal Inc., Oslo, Norway) coated with rabbit IgG. The beads were washed twice in PBS containing 0.1% bovine serum albumin and the supernatants combined. The protein eluted by heating the sample to 65 °C for 2 min was resolved on a 7.5% SDS-polyacrylamide gel. The gel.

Pulse-Chase Analysis—GH3 cells were cultured to a density of 70% confluency on 75-cm2 flasks (107 cells) in control DMEM with a total of 14% serum and 10 nM EGF, 50 nM okad acid, 2 μM leupeptin, or combinations of these agents as indicated for 10 h. After culturing, the media was removed and the cells were rinsed once in DMEM minus methionine and cysteine with 10% dialyzed fetal calf serum. The cells were incubated for 1 h in the deficient medium then replaced with labeling medium. The cells were labeled in 10 ml of cytochrome and methionine-deficient DMEM with 10% dialyzed fetal calf serum that contained 90 μCi/ml [35S]methionine (~1000 Ci/mmol) (Amersham). After 1 h, the cells were switched to complete medium containing unlabeled methionine and 10 nM EGF, 50 nM okad acid, 10 nM EGF plus 50 nM okad acid, 2 μM leupeptin or leupeptin plus EGF and okad acid. After time intervals up to 8 h in the complete medium, the cells were washed three times in PBS alone then lysed in ice-cold Nonidet P-40 lysis buffer containing 1% Nonidet P-40, 50 mM Tris, pH 8.0, 150 mM NaCl, 10 mM EDTA, 1 mM dithiothreitol, protease inhibi-
tors, and phosphatase inhibitors as described above. The whole cell lysates were immediately frozen in liquid N2 and stored at −80 °C until use.

Thawed lysates were clarified by centrifuging at 13,000 rpm for 30 min. Protein was determined on the supernatant using the method of Bradford (18).

Cell Culture—GH3 cells were treated with 10 nM EGF for 2, 4, or 16 h (Fig. 1A). In the presence of 1 mM ZnCl2, there was an increase in Sp1 and gERP 2 complex binding as described previously (9). No change in

RESULTS

EGF Stimulates a Decrease in Sp1 Binding and Protein—To assess whether EGF induces changes in Sp1 binding, GH3 cells were treated with 10 nM EGF for 2, 4, or 16 h (Fig. 1A).
Sp1 or gERP binding was observed within 4 h. However, after at least 16 h of EGF treatment, Sp1 binding decreased without a significant change in the gERP 1 and 2 complexes. To assess whether the decrease in Sp1 binding was due to a change in Sp1 binding activity or a change in the level of nuclear Sp1, immunoblot analysis was performed using Sp1 antibody. Nuclear extracts prepared from GH4 cells were treated for 16 h with EGF and resolved by SDS-PAGE. The results shown in Fig. 1B indicate that there was a decrease in the nuclear levels of Sp1. In addition, no Sp1 was detected by immunoblot analysis of cytosolic extracts after 16 h of EGF treatment indicating that the decrease in Sp1 was not due to retention of Sp1 in the cytoplasm (data not shown).

A Decrease in Sp1 Protein Correlates with a Decrease in Sp1-mediated Transcriptional Activation—To demonstrate that the lower levels of Sp1 generated by chronic EGF treatment also correlate with a decrease in Sp1 transactivation, several gastrin-reporter constructs were tested in GH4 cells. GH4 cells were pretreated with 10 nM EGF 24 h prior to transfection with gastrin-reporter constructs and compared with cells not pretreated with EGF prior to transfection. Three reporter constructs containing elements ligated upstream of a minimal gastrin promoter were transfected: the WT gERE element, a mutation of the gERE element that only binds Sp1 (M6) and an Sp1 consensus element from the human metallothionein promoter (Sp1). The binding specificity and activity of these constructs have been reported previously (9). The results shown in Table I demonstrate that pretreatment with EGF had little effect on the basal activity of the gastrin promoter in the presence of the WT element. However, those elements capable of binding only Sp1 were significantly affected by EGF pretreatment. In particular, both the mutated gERE element (M6) and the Sp1 element which normally confer higher basal activity than the WT element showed a 50–60% decrease in basal promoter activity. Since these elements have a higher affinity for Sp1 compared with the gERP complexes (9), the observed decrease in basal activation was consistent with a decrease in the abundance of Sp1.

EGF Inhibits GH4 Cell Proliferation—Tashjian and co-workers (10) showed previously that EGF inhibits GH4 proliferation and DNA content within 72 h. Thus the decrease in Sp1 binding and abundance parallels a decrease in cellular proliferation. To confirm that EGF has a negative effect on GH4 proliferation, the proliferative rate of GH4 cells over 72 h was studied. Five thousand cells were plated per microtiter well with or without 10 nM EGF. Cellular proliferation was quantified at the times indicated and clearly showed that EGF negatively affected cellular proliferation (Fig. 2).

Okadaic Acid Augments the Effect of EGF on Sp1 Protein Levels—To determine whether the effect of EGF was related to the level of cellular phosphorylation, immunoblot analysis was performed on nuclear extracts prepared from cells treated with the phosphatase inhibitor okadaic acid or with both EGF and okadaic acid. The results shown in Fig. 3 demonstrate that treatment of the cells with okadaic acid alone resulted in a similar or greater decrease in Sp1 nuclear levels compared with EGF alone. Treatment of the cells with both EGF and okadaic acid had a synergistic effect, further depressing nuclear levels of Sp1. These results are consistent with studies showing that okadaic acid treatment of GH4 cells inhibits proliferation in response to decreased phosphatase activity (19). Since okadaic acid alone and in concert enhanced the effect of EGF on the abundance of Sp1, these results suggested that the decrease in Sp1 is related to its phosphorylation state.

EGF and Okadaic Acid Stimulate Sp1 Phosphorylation—Since okadaic acid alone stimulated an increase in Sp1 degradation and okadaic acid and EGF had a synergistic effect on the decrease in Sp1 abundance, we investigated whether these treatments affected Sp1 phosphorylation. GH4 cells were labeled with [32P]orthophosphate, then stimulated with EGF or okadaic acid alone or EGF and okadaic acid together. After 4 h of stimulation, the cells were lysed, nuclear extracts were prepared and Sp1 was precipitated with Sp1 antibody. The results shown in Fig. 4 indicate that all three treatments increase the phosphorylation state of endogenous Sp1. EGF, okadaic acid, and okadaic acid plus EGF resulted in 1.3- to 2.7- and 2.2-fold increase in phosphorylation over control levels, respectively. Thus, okadaic acid and EGF plus okadaic acid had the greatest effect on phosphorylation levels due to the inhibition of endogenous phosphatases 2A and 1 (20). Moreover, this result was

![Image](image-url)

**Fig. 1.** EGF decreases Sp1 binding activity in GH4 cells. A, EMSA of GH4 nuclear extracts (2 μg) binding to a radiolabeled WT probe in the absence (lanes 1, 2, 5, 6, 9, and 10) or presence (lanes 3, 4, 7, 8, 11, and 12) of 1 mM Zn2+; GH4 cells were treated for 2 h (lanes 1–4), 4 h (lanes 5–8), or 16 h (lanes 9–12) with control medium (lanes 1, 3, 5, 7, 9, and 11) or medium containing 10 nM EGF (lanes 2, 4, 6, 8, 10, and 12). The three gERE binding complexes are identified as Sp1 (arrowhead), gERP 1 and gERP 2 (arrows). B, immunoblot analysis of nuclear extracts (50 μg) from GH4 cells treated for 16 h with 10 nM EGF. Lane 1, untreated; lane 2, EGF-treated. Sp1 protein is indicated (arrowhead).

**Table I**

Luciferase specific activity of gastrin reporter constructs before and after pretreatment with EGF

| EGF pretreatment | WTALuc | M6ALuc | Sp1ALuc |
|------------------|--------|--------|---------|
|                   | −      | +      | −       | +      |
| Experiment 1      | 1,914  | 1,495  | 13,540  | 4,114  | 11,392 |
|       | 1,784  | 2,037  | ND*    | ND     | 9,965  |
| Experiment 2      | 1,784  | 2,037  | ND*    | ND     | 9,965  |

* ND, not determined.
consistent with prior reports that okadaic acid prevents Sp1 dephosphorylation (21).

To determine whether the decrease in Sp1 protein was related to a decrease in Sp1 gene expression, Northern blot analysis was performed. The results shown in Fig. 5 indicate that treatment of cells with EGF and okadaic acid, which significantly decrease Sp1 protein levels, did not decrease Sp1 gene expression. Instead, okadaic acid alone and the combination of EGF and okadaic acid treatment stimulated Sp1 gene expression possibly due to a feedback regulatory loop that mediates activation of the Sp1 gene as a result of a significant decrease in Sp1 protein in the cell.

The effect of EGF and okadaic acid on Sp1 levels was reversible upon removal of these agents from the culture medium (Fig. 6). In complete medium, Sp1 binding activity increased with time (Fig. 6A). In contrast, the addition of EGF and okadaic acid for 16 h resulted in a >60% decrease in Sp1 binding activity that was detectable by 24 h. There were minimal changes in binding of the gERP factor complex and the ubiquitous transcription factor Oct-1 (Fig. 6B). The decreased ratio of Sp1 to Oct-1 binding was lowest at 24 h and returned to control levels after the removal of EGF and okadaic acid (Fig. 6C).

Cycloheximide Treatment Blocks Sp1 Degradation—To assess whether the decrease in Sp1 protein levels required protein synthesis, GH4 cells were incubated with cycloheximide for 1 h prior to treatment with EGF and okadaic acid. Cycloheximide showed a dose-dependent inhibition of the expected decrease in cellular Sp1 levels (Fig. 7A). In addition to an inhibition of Sp1 degradation, cycloheximide treatment also decreased the appearance of several lower molecular weight species detected by Sp1 antibody (see also Fig. 1B) or EGF and okadaic acid treatment and decreased with the inhibition of protein synthesis.

A Protease Inhibitor Prevents Sp1 Degradation—The dependence of Sp1 degradation upon protein synthesis suggested that Sp1 turnover may be related to increased protease activity. Therefore, to determine whether the decrease in Sp1 levels was reversed by specific protease inhibitors, GH4 cells were pretreated with two membrane-permeable protease inhibitors MG-132 and leupeptin. MG-132 is a proteosome-restricted protease inhibitor; whereas leupeptin is a cysteine protease that inhibits lysosomal and calcium-activated proteases. In addi-
agonists and 2 mM unlabeled methionine in DMEM. The half-time of Sp1 turnover may not be related to the proteasome.

EGF and okadaic acid decrease the abundance of Sp1 in the cell. Moreover, EGF and okadaic acid together stimulate an increase of total nuclear Sp1 observed on immunoblots and the time lag required for Sp1 degradation. These results correlated with the loss of total nuclear Sp1 observed on immunoblots and the time lag required for Sp1 degradation.

**Inducible Sp1 Proteolysis**

**PEST Sequence Identification**—Several targets of inducible proteolysis contain a region with an unusually high concentration of the amino acids proline (P), glutamic (E), or aspartic acid (D), serine (S), and threonine (T); whereas fewer that 5% of a random survey of the GENEPRO data bank contain such PEST sequences (23, 24). PEST-FIND, a program ranking the amino acid domains within proteins produced significant scores (>5.0) for a number of proteins with rapid rates of degradation: cdc25 (15.6), IkBα (5.1), and c-Fos (10.1). In contrast, proteins with low degradation rates produced low scores, e.g. adenylyl kinase (~11.8). We evaluated the amino acid sequence of Sp1 to determine if it also contained a PEST domain. Human Sp1 produced a score of 10.4 and rat Sp1...
produced a score of 13.7, consistent with an internal PEST sequence between amino acids 437 and 458, (Fig. 10). Targeted degradation of proteins containing PEST sequences is a mechanism for rapidly controlling transcription factor activity (23). Thus proteolysis of Sp1 correlated with the presence of a PEST domain.

DISCUSSION

The present study shows that stimulation of GH4 cells with physiologic concentrations of EGF results in a decrease in the cellular content of Sp1 and that okadaic acid augments this effect. These results reflect a decrease in both immunoreactive Sp1, metabolically-labeled Sp1, as well as Sp1 DNA binding and transcriptional activity. Okadaic acid specifically binds to and inhibits the catalytic subunits of serine/threonine phosphatases 1 and 2A with different potencies (13). It therefore has been used to study the effect of unopposed kinase activity on transcription and DNA-protein interactions (25).

SV40 viral infection of CV1-L cells stimulates Sp1 phosphorylation by a DNA-dependent protein kinase (4). This increase in Sp1 phosphorylation may also be related to viral inhibition of an opposing phosphatase since the small T antigen of SV40 interacts with protein phosphatase 2A (26), the same phosphatase inhibited by okadaic acid. In GH4 cells, 10 nM okadaic acid inhibits GH4 cell proliferation and interferes with the formation of the mitotic spindle, thereby slowing cellular progression through mitosis (19). Similarly, EGF inhibits cellular proliferation of GH4 cells (10) suggesting that both EGF and okadaic acid may function through overlapping pathways. One pathway of overlap appears to be the ability of both agents to affect the abundance of Sp1. The loss of Sp1 was observed with EGF and augmented by okadaic acid, suggesting that activation of the EGF receptor phosphorylates Sp1 through increased intracellular serine/threonine protein kinase activity. No decrease in Sp1 mRNA was observed with stimulation indicating that the loss of Sp1 was post-transcriptional. Since phosphorylation of Sp1 can be detected within 4 h well before significant degradation is detected, the results suggest that sustained Sp1 phosphorylation targets the protein for turnover. The loss of Sp1 results from increased proteolytic activity since a cysteine protease inhibitor, leupeptin, blocked the degradation expected. In addition, degradation was also inhibited in a dose-dependent fashion by cycloheximide at concentrations that have been shown to inhibit protein synthesis. The cycloheximide result is consistent with induction of Sp1 degradation and the time required to synthesize an Sp1-specific protease. The effect of EGF and okadaic acid on Sp1 was specific since a constitutive transcription factor Oct-1 remained unchanged, as did the gERP complexes.

While the effect of EGF stimulation on a general transcription factor in GH4 cells appears paradoxical there is clear precedence. Phorbol esters, EGF, and okadaic acid were previously shown to be antiproliferative in GH4 cells resulting in phenotypic changes consistent with differentiation (10, 11, 19). Van Doulah et al. (19) relates this antiproliferative effect to the slowed progression through mitosis and sustained phosphorylation of the retinoblastoma gene product Rb. Subsequent studies in GH4 cells have shown that the ability to dissociate the differentiating effects of EGF from its mitogenic response is dependent upon the concentration of other serum factors (27). Okadaic acid mimics EGF or phorbol ester activation of c-fos gene expression, AP-1 binding, and NFκB transactivation (20, 28, 29). Thus increasing protein phosphorylation or decreasing phosphoprotein phosphatase activity similarly affect transcriptional regulation and have been implicated as common pathways for cell transformation and tumor promotion. In GH4 cells, these extracellular regulators inhibit proliferation and

![Figure 9. EGF and okadaic acid stimulate the degradation of radiolabeled Sp1.](image-url)
promote differentiation.

Sp1 abundance and activity are clearly related to proliferation perhaps through its ability to cooperate with the retinoblastoma gene product Rb and the ability to regulate Rb gene expression. Rb stimulates the transcription of several growth-related genes (e.g. insulin growth factor-2, c-fos, transforming growth factor-β1) through complexes that contain Sp1 (30). Moreover, the promoter elements regulated by Rb are also binding sites for Sp1 (31). One potential mechanism by which Rb cooperates with Sp1 is that Rb releases a negative coregulator from Sp1 (32). Sp1 may also regulate Rb gene expression since the Rb promoter contains an important Sp1 site that when mutated to abolish binding contributes to low-penetrance hereditary retinoblastoma (33).

Additional support for the inverse correlation of Sp1 nuclear levels with differentiation has been observed in embryonal carcinoma cells that differentiate into parietal endoderm (22). In this system, Sp1 nuclear levels decreased with retinoic acid induction of differentiation. The decrease was due to a cysteine protease localized in the nucleus since leupeptin and antipain prevented the loss of Sp1 protein. Thus, as reported here, anti-proliferative effects or differentiation increases nuclear protease activity and decreases the level of specific transcription factors including Sp1. Moreover, both the current study and the experiments by Scholtz et al. (22) indicate that a cysteine protease mediates Sp1 degradation. There are no studies reporting Sp1 ubiquitination; thus, ubiquitin-mediated degradation of Sp1 cannot be excluded. Even so, it is not clear that all ubiquitinated proteins are degraded by the proteasome (34, 35).

Regulation of transcription from promoters containing Sp1-binding sites is thought to involve several poorly characterized mechanisms including; modulation of Sp1 glycosylation (5), inducible Sp1 phosphorylation (4), control of Sp1 abundance (36), cooperative interactions with tissue-specific transcription factors (31, 37), or control of Sp1 affinity for DNA and transcription factors within the TFIID complex (38). Previous reports support the role of tissue specific changes in Sp1 levels during development (2), but this was in a heterogeneous cell population and may represent relative increases in only some cell types. A recent study reported that decreased Sp1 binding activity in terminally differentiated liver tissue correlates with protein phosphorylation (39). However, this study did not assess total immunoreactive Sp1 levels. The present study examines both Sp1 DNA binding and protein levels in a homogenous tissue culture system. The results indicate that Sp1 degradation represents another means of regulating its transcriptional activity.

Inducible proteolysis as a means of regulating transcription factor activity has been established in other systems. Two models in eukaryotes are: 1) protease induced relocation of a protein with gain of DNA-binding function, and 2) protease degradation of a DNA-binding protein with loss of function. NFκB is sequestered in the cytoplasm until cytokine induced phosphorylation of the inhibitory IκB subunit results in ubiquitinylation, proteosomal degradation, and subsequent translocation of the Rel homology domain to the nucleus (16). A similar mechanism regulates the sterol regulatory element 1 domain within the 5′-flanking region of the low density lipoprotein-receptor gene. SREBP-1, a nuclear envelope/endoplasmic reticulum-bound transcription factor, is released by proteolysis in sterol-depleted cells, translocates to the nucleus, and activates transcription via cooperation with a weak adjacent Sp1-binding site (40, 41). An example of the second model is seen in the inactivation of the activity of the yeast transcription factor Gcn4, a regulator of amino acid and purine biosynthesis. Turnover of Gcn4 is regulated by extracellular amino acid concentration, with high amino acid levels resulting in ubiquitin-dependent degradation of Gcn4 (42). Likewise, characterization of the turnover of c-Jun showed that the δ-domain is necessary but not sufficient to mediate ubiquitin-dependent degradation of the transcription factor (43). The δ-domain is absent from the unregulated retroviral homologue v-Jun suggesting that the inability to degrade the viral homologue may represent the means for viral protein escape from cellular control.

In summary, EGF stimulation of GH3 cells results in the proteolysis of Sp1. Sp1 binding to gERE has been shown to be required for hormone induction of the gastrin promoter in this cell line (9). The time course determined for Sp1 degradation suggests that this may represent one mechanism for the desensitization of EGF hormone stimulation. Alternatively, it may represent a part of the process affecting an antiproliferative and differentiated phenotype for these cells in response to EGF. The dysregulation of cellular proteases has been identified in a number of disease states and proteolysis is important in the control of differentiation (22, 44, 45). Phenotypic changes may result from inducible degradation of transcription factors resulting in the alteration of cell fate (16, 43, 46). In GH3 cells, inducible expression of the gastrin gene coincides with the differentiated state of these cells since prolatin levels are also

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**Fig. 10. Location of Sp1 PEST domain.** The program PEST-FIND was used to predict the location of all significant PEST domains within the primary sequence of human Sp1. Only a single domain with a PEST score greater than 5.0 was identified. This sequence is shown relative to the location of zinc finger, acidic, and basic domains.
induced with EGF (10). Thus, the results reported here link these well documented observations to a series of specific molecular events that will likely be applicable to other biologic systems.

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