*Proc. Natl. Acad. Sci. USA*
Vol. 89, pp. 11451–11455, December 1992
Biochemistry

# Activin inhibits binding of transcription factor Pit-1 to the growth hormone promoter

R. SCOTT STRUTHERS*†, DANA GADDY-KURTEN*, AND WYLIE W. VALE*†

*The Clayton Foundation Laboratories for Peptide Biology, The Salk Institute, 10010 North Torrey Pines Road, La Jolla, CA 92037; and †Graduate Program in Biomedical Science, University of California, San Diego, 9500 Gilman Drive, La Jolla, CA 92093-0636

*Contributed by Wylie W. Vale, August 12, 1992*

ABSTRACT **Activin A is a potent growth and differentiation factor related to transforming growth factor β. In somatotrophs, activin suppresses the biosynthesis and secretion of growth hormone (GH) and cellular proliferation. We report here that, in MtTW15 somatotrophic tumor cells, activin decreased GH mRNA levels and inhibited expression of transfected GH promoter–chloramphenicol acetyltransferase fusion genes. Deletion mapping of nucleotide sequences mediating this inhibition led to the identification of a region that has previously been characterized as binding the pituitary-specific transcription factor Pit-1/GHF-1. Characterization of nuclear factor binding to this region demonstrated that binding of Pit-1 to the GH promoter is lost on activin treatment. These results indicate that activin-induced repression of GH biosynthesis is mediated by the loss of tissue-specific transcription factor binding to the GH promoter and suggest a possible general mechanism for other activin responses, whereby activin regulates the function of other POU- or homeodomain-containing transcription factors.**

Activins (1, 2) are members of an extensive family of growth and differentiation factors that includes inhibin, transforming growth factor β, mullerian inhibitory substance, the fly decapentaplegic gene complex, and the product of *Xenopus* Vg-1 mRNA (3). Originally characterized from gonadal extracts based on their ability to stimulate follicle-stimulating hormone secretion by cultures of rat anterior pituitary cells, activins were found to be homodimers of two subunits, $\beta_A$ and $\beta_B$ (1, 2). These polypeptide chains can also combine with a related α subunit to give the αβ heterodimeric inhibins (4–7). Inhibins, for the most part, act in opposition to the activities of activins; therefore, differential subunit association can result in the formation of dimers with opposing biological actions.

Activin/inhibin subunits and their mRNAs are extensively distributed anatomically (8). In addition to their presence in the gonads, they are also found in adrenals, brain, bone marrow, and pituitary [where the α and $\beta_B$ subunits are found within the gonadotrophs (9)]. Activins exert effects on the function and/or proliferation of a variety of non-follicle-stimulating hormone-secreting cells including: gonadal theca interna cells, granulosa cells, Leydig cells, erythroid progenitor cells, hepatocytes, paraventricular oxytocin-producing cells, placental human chorionic gonadotropin- and progesterone-producing cells, pituitary somatotrophs, and corticotrophs (for a review of these actions, see ref. 10). More recently, activin has also been identified as a nerve-cell survival factor (11) and a potent inducer of mesoderm formation during early embryogenesis (12–14), suggesting a key role of this peptide during vertebrate development.

The surprising diversity of sites of both production and action by these "gonadal" peptides suggests a variety of paracrine and autocrine roles in addition to their originally described endocrine functions. Despite this abundance of actions, little is known regarding the intracellular mechanisms of activin's actions. Recently, Mathews and Vale (15) have cloned a family of receptors for activin, and sequence comparisons have suggested these receptors may function as serine/threonine-specific protein kinases. Subsequently, additional activin receptors (16, 17), as well as a related receptor for transforming growth factor β (18), have been cloned. However, pathways downstream from receptor binding have yet to be characterized.

Activin has been identified as a negative regulator of somatotroph function in cultured rat anterior pituitary cells, including growth hormone (GH) biosynthesis and somatotroph proliferation (19, 20). The somatotroph has been the subject of extensive studies to characterize the regulation of GH gene transcription (21–25), as well as the proliferation and development of this highly differentiated cell type (20, 26–30). We therefore focused on the inhibition of GH biosynthesis by activin to characterize pathways that this polypeptide may employ to affect long-term developmental changes. We report here that activin represses GH mRNA levels and expression of transiently transfected GH promoter–chloramphenicol acetyltransferase (CAT) fusion genes. Further, activin treatment results in the loss of binding of the pituitary-specific transcription factor Pit-1/GHF-1 (31, 32) to its cognate recognition sequences in the GH promoter. These results suggest that activin inhibits GH biosynthesis by interfering with the stimulatory actions of the POU/homeodomain transcription factor Pit-1/GHF-1.

## MATERIALS AND METHODS

**Materials.** Recombinant human activin A was a generous gift from J. Mather, A. Mason, L. Bald, and R. Schwall of Genentech.

**Cell Lines.** MtTW15 somatotrophic tumors (33) were passaged in female Wistar–Furth rats (Harlan–Sprague–Dawley) by subcutaneous injection of minced tumor suspended in saline and harvested 3–5 weeks after transplantation. Cells from MtTW15 tumors were routinely dissociated with trypsin/EDTA and established in culture at a density of $5 \times 10^6$ cells per 10-cm dish for 3 days prior to treatment or transfection.

**RNA Blot Analysis.** Total RNA was isolated as described (35). Probes for Northern blot analysis were labeled with [$^{32}$P]dCTP using a random-primer method (36).

**Plasmid Construction.** Plasmids containing GH promoter–CAT fusion genes were constructed by ligation of a *Hin*dIII–*Xba* I fragment from a genomic clone of the rat GH gene (37) and a CAT expression plasmid lacking promoter or enhancer elements, pCAT-Basic (Promega). A *Xho* I–*Xba* I fragment was

The publication costs of this article were defrayed in part by page charge payment. This article must therefore be hereby marked "*advertisement*" in accordance with 18 U.S.C. §1734 solely to indicate this fact.

Abbreviations: GH, growth hormone; CAT, chloramphenicol acetyltransferase; HSV-tk, herpes simplex virus thymidine kinase; TRE, thyroid responsive element.

then excised, followed by repair of the recessed termini and blunt-end ligation to give GH(−1750)CAT, which contained GH promoter sequences from positions −1750 to +8 relative to the native GH transcription start site. GH(−311)CAT was prepared from GH(−1750)CAT by excision of a *Hin*dIII–*Kpn* I fragment and religation of repaired ends. Remaining 5′ deletion constructs (see Fig. 2*B*) were prepared (38) by ligation of PCR-amplified products (with common 3′ termini at position +8) into the *Hin*dIII and *Xba* I sites of pCAT-Basic. The plasmid GH(−183/−48)TKCAT was constructed by ligation of a *Hin*dIII–*Taq* I fragment from GH(−183)CAT into the *Hin*dIII–*Bgl* II sites upstream of the herpes simplex virus thymidine kinase (HSV-tk) promoter in the plasmid pTEN (P. Mellon, University of California at San Diego). All constructions were confirmed by restriction endonuclease mapping or dideoxynucleotide sequencing.

**Transient Transfection Assays.** MtTW15 tumor cells ($5 \times 10^6$ cells per 10-cm plate) were washed thoroughly and cultured in Dulbecco's modified Eagle's medium containing 0.1% fetal calf serum immediately prior to transfection. Cells were transfected with 25 μg of plasmid DNA as a calcium phosphate precipitate followed by glycerol shock (39) and cultured in β-PJ medium (40) containing 1% fetal calf serum. Cells received activin or other treatments immediately after glycerol shock and were allowed to express for 3 days prior to harvest. Transfection efficiencies were normalized using β-galactosidase activity by inclusion of 5 μg of RSV-βGAL plasmid in the calcium phosphate precipitate. CAT activity was analyzed by thin-layer chromatography as described by Gorman *et al.* (41).

**Nuclear Extract Preparation and Mobility-Shift Assays.** Nuclear extracts were prepared from 10-cm dishes of cultured MtTW15 cells as described (42). Electrophoretic-mobility-shift assays were performed on multiple separate extracts from several MtTW15 tumor isolations. Binding reactions were performed at 4°C for 30–45 min in 10 μl of 100–150 mM NaCl/15 mM Tris·HCl, pH 7.5/5 mM dithiothreitol/1 mM EDTA/5 mM $MgCl_2$/12 mM glycerol containing 0.5 or 1 μg of poly(dI-dC) and 0.5 or 1 μg of nuclear extract. A *Hin*dIII–*Xba* I fragment (positions −183 to +8) was labeled by filling in the restriction sites by using the Klenow fragment of DNA polymerase I and purified by acrylamide gel electrophoresis. Double-stranded oligonucleotides were also labeled by filling in the *Hin*dIII cohesive ends and purified by gel filtration thru Sephadex G-25 columns. Each reaction mixture contained 25,000–50,000 cpm of $^{32}$P-labeled DNA probe. Unlabeled competitor DNAs were added with probe DNAs to the reaction buffer prior to the addition of extract. Incubation of extracts with a 1:200 dilution of α132-Pit antiserum or preimmune antisera (53) continued at 4°C for 30 min prior to further incubation for 15 min with labeled DNA probe. Reaction mixtures were immediately loaded on nondenaturing 0.045 M Tris borate/0.001 M EDTA/4% polyacrylamide gels and electrophoresed at 400 V for 2–3 h.

## RESULTS

**Activin Reduces GH mRNA Levels.** Because commonly utilized somatotrophic cell lines (e.g., GC, $GH_3$, and $GH_4$) do not respond to activins (R.S.S. and L. Mathews, unpublished data), we utilized primary cultures of cells dispersed from MtTW15 transplantable somatotrophic tumors. These cells express (R.S.S., L. Mathews, and W.W.V., unpublished data) both type I and II activin receptors and respond to activin by inhibition of basal and GH-releasing factor-stimulated cAMP synthesis.

Examination of GH mRNA levels in control and activin-treated cells indicates that the inhibition of GH biosynthesis by activin can be attributed, at least in part, to a dramatic reduction in GH mRNA (Fig. 1). Activin repressed both basal and dexamethasone-stimulated GH mRNA levels, effects similar to those observed for GH biosynthesis in cultures of rat anterior pituitary cells (20).

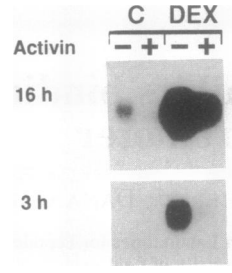


FIG. 1. Activin decreases GH mRNA levels in MtTW15 cells. Cells from transplantable tumors were cultured for 6 days in the presence (+) or absence (−) of recombinant human activin A (0.7 nM) in control (C) or 50 nM dexamethasone (DEX)-supplemented medium. Total RNA (15 μg) was analyzed by Northern blot hybridization (35) to a $^{32}$P-labeled ≈500-bp *Pvu* II fragment of the rat GH cDNA (37). Autoradiographic exposure times are shown.

**Activin Inhibits Rat GH Promoter Activity.** To determine whether activin repression of GH mRNA levels reflected specific changes in the activity of the GH promoter, a series of transient transfection experiments were performed with a CAT reporter gene under the control of 1750 or 320 base pairs (bp) of the rat GH promoter. Results indicate that the activity of the GH promoter is specifically and strongly repressed in response to activin treatment (Fig. 2*A*). Because activin did not repress the somatostatin promoter-containing plasmid [SSΔ(−71)CAT, which consists essentially of a cAMP response element and a "TATA"-box-like element (43)], the effects of activin cannot solely be attributed to moderately decreased intracellular cAMP levels (34).

**An Activin-Responsive Element Is Located Between Bases −133 and −48 in the Rat GH Promoter.** A series of 5′ deletion constructs was tested to localize regions necessary for mediating activin-induced repression of this promoter (Fig. 2*B*). The rat GH promoter has been characterized to contain a thyroid responsive element (TRE, roughly defined from bp −190 to −140) (45–47), two binding sites for the pituitary-specific transcription factor Pit-1/GHF-1 [a distal site from positions −132 to −105 (dPit-1) and a proximal site from positions −94 to −66 (pPit-1)] (31, 32), and a TATA box at bp −30. An additional footprint from positions −144 to −121 was observed in the absence of Pit-1/GHF-1 binding and attributed to an as yet unidentified but ubiquitous nuclear factor, named factor 2 (24). A sequence related to a proposed transforming growth factor β inhibitory element in the transin gene (48) was present at positions −58 to −50 in the GH promoter; however, the potential significance of this sequence, if any, is not clear.

Deletion of 5′ sequences between the *Kpn* I site at positions −311 and −183 did not affect activin repression of the GH promoter. Likewise, further deletion of sequences up to position −133, corresponding to the 5′ border of the distal Pit-1/GHF-1-footprinted region, retained activin repressibility. However, activin repressibility and high basal level expression were both lost with deletion of an additional 9 bp [GH(−124)CAT], despite the presence of an intact proximal Pit-1/GHF-1 site. This dependence on both tissue-specific sites for high basal level transcription has been observed (49) and suggests that cooperativity between the two Pit-1/GHF-1 sites is necessary to achieve high levels of GH gene expression. Deletion of the entire tissue-specific region resulted in a nonrepressible plasmid [GH(−32)CAT] with low basal level expression. The lack of additional repression of these constructs by activin suggests that its inhibitory effects may require disruption of Pit-1/GHF-1-driven high-level expres-

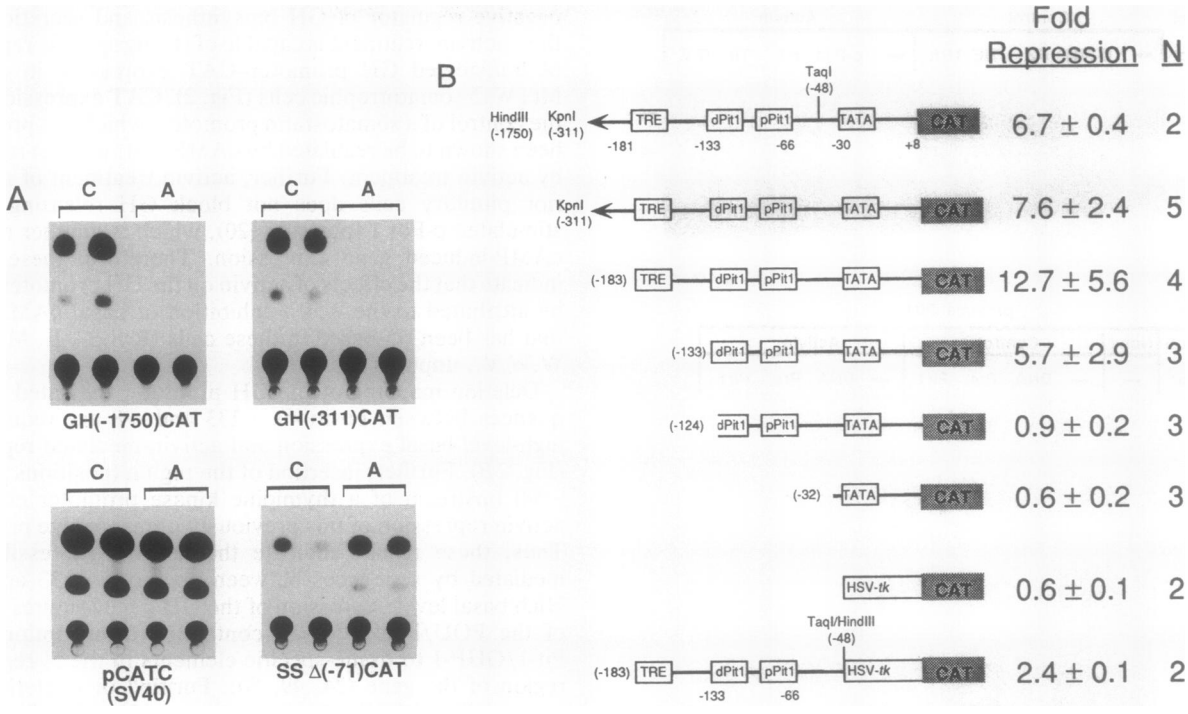


FIG. 2. GH promoter sequences containing Pit-1 recognition sites are required for activin repression of GH–CAT fusion gene expression. (*A*) Repression of the GH promoter by activin. Plasmid DNAs were transiently transfected into MtTW15 cells as calcium phosphate precipitates. Cells were then fed medium alone (lanes C) or medium containing 0.7 nM recombinant human activin A (lanes A) for 3 days prior to harvest and assay of CAT activity. Control plasmids included a simian virus 40 (SV40) promoter and enhancer (pCAT-c) (Promega) or the somatostatin promoter [SSΔ(−71)CAT] (43). β-Galactosidase activity from a cotransfected RSV-βGAL plasmid (5 μg per dish) was measured to normalize transfection efficiencies. A representative experiment is shown. (*B*) Deletion mapping of activin-repressible sequences in the GH promoter. Cultured MtTW15 cells were transfected with plasmids containing the indicated GH–CAT fusion genes and cultured in medium alone or medium containing 0.7 nM activin A. Boxes indicate the locations of previously characterized cis-acting elements: TRE, dPit-1, distal Pit-1; pPit-1, proximal Pit-1; TATA, TATA box/TATA binding protein consensus sequence. Positions are given relative to the native GH transcription initiation site. All plasmids were derived from a common vector backbone (pCAT-Basic, Promega). Activin also repressed GH promoter sequences placed 5′ to a heterologous HSV-tk promoter (44). Values given are the mean ± SEM for the ratio of CAT activity measured in control vs. activin-treated cells from *N* independent experiments, each performed in duplicate.

sion rather than utilizing an independent inducible repressor element.

To determine whether GH promoter sequences could confer activin-induced repression of a heterologous promoter, a plasmid inserting GH sequences upstream of the HSV-tk promoter (44) was constructed and tested by transient transfection (Fig. 2*B*). The minimal HSV-tk promoter was not repressed in response to activin treatment. However, introduction of GH promoter sequences from positions −183 to −48 upstream of the HSV-tk promoter resulted in a chimera that was repressed by activin. Therefore, activin responsiveness can be localized between residues −133 and −48 and does not require the GH promoter TATA element or transcription start site.

**Activin Alters Nuclear Factor Binding to Pit-1/GHF-1 Recognition Sequences.** To determine whether alterations in DNA–protein interactions were contributing, in part, to activin-induced repression of GH gene expression, electrophoretic-mobility-shift experiments were conducted using fragments of the GH promoter and nuclear extracts from control or activin-treated cells. Gel-retardation assays using a $^{32}$P-labeled fragment of the GH promoter from the TRE to just 3′ of the transcription initiation site (positions −183 to +8) resulted in the formation of one major and two minor DNA–protein complexes (Fig. 3*A*). Formation of all complexes was effectively inhibited by addition of excess unlabeled double-stranded oligonucleotide corresponding to the proximal Pit-1/GHF-1 recognition sequence but not by oligonucleotides corresponding to the factor-2 sequence, a possible transforming growth factor inhibitory element-like sequence (TIE), or the TRE. Competition with increasing concentrations of oligonucleotide corresponding to either the proximal or distal Pit-1/GHF-1 site (data not shown) indicated a higher affinity of extract proteins for the proximal site, consistent with what has previously been observed for Pit-1/GHF-1 protein (50).

Treatment of cells with recombinant activin A (0.7 nM) for 3 days prior to preparation of nuclear extracts resulted in decreased formation of the major complex (II) (Fig. 3*A*) but did not appear to affect the two minor species. Similar results were obtained for at least 18 extracts prepared from cells isolated from at least five tumors (data not shown). Similar reduction of complex formation upon activin treatment was also observed for additional GH promoter fragments (−133/+8 and −133/−48) containing various amounts of sequence external to the Pit-1/GHF-1 sites (data not shown).

Gel-retardation assays using labeled oligonucleotides corresponding to the proximal Pit-1/GHF-1-footprinted region revealed several retarded species that could be specifically competed with the corresponding oligonucleotide (Fig. 3*B*). One major complex (II) and a minor complex (I) were formed using a 32-bp Pit-1-specific oligonucleotide as probe (Fig. 3*B*). Activin treatment of cells prior to extract preparation prevented formation of complex II and partially inhibited formation of complex I. A slower migrating minor band was observed in some experiments but was not diminished by activin treatment. In addition, complexes I and II were specifically retarded upon addition of Pit-1-specific antisera, indicating that Pit-1 protein is a component of these complexes. Similar results were obtained with a distal Pit-1 site oligonucleotide probe. Parallel mobility-shift experiments with labeled oligonucleotides corresponding to transcription

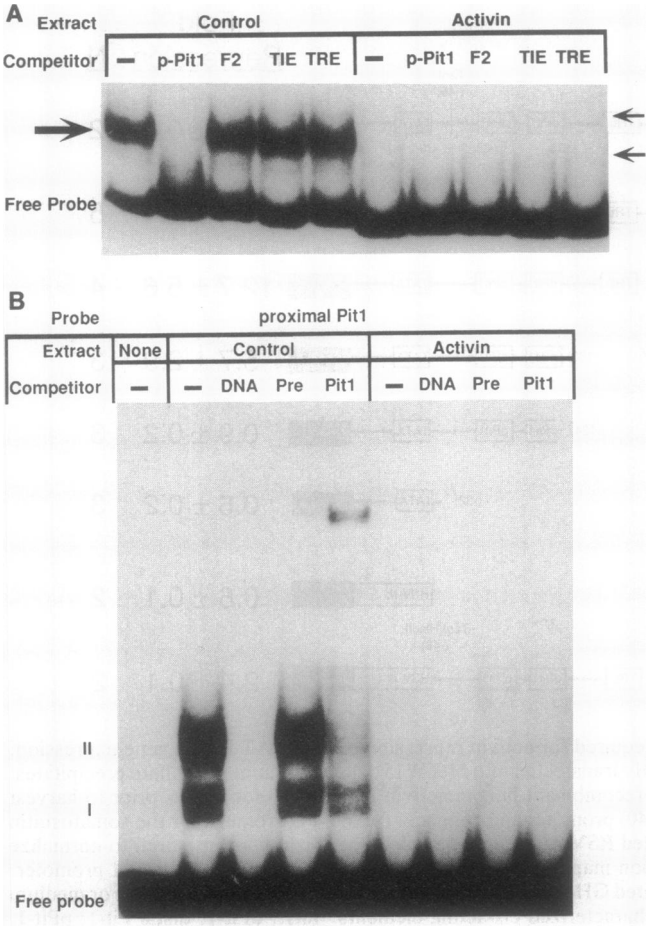


FIG. 3. Activin represses Pit-1 binding to the proximal Pit-1 site of the GH promoter. (*A*) Activin treatment altered binding of MtTW15 extracts to a Pit-1 site of the GH promoter in electrophoretic-mobility-shift assays. A $^{32}$P-labeled GH promoter fragment (positions −183 to +8; *Hin*dIII–*Xba* I) (1.6 ng) was incubated with nuclear extracts (42) from control or 3-day activin-treated cells in the absence (−) or presence of excess competing oligonucleotides: pPit-1, proximal Pit-1 site (positions −97 to −66); F2, site of GH factor 2 footprint (positions −159/−130) (24); TIE (positions −63/−46) (48); TRE, positions −183/−152 (45). Major (large arrow) and minor (small arrows) complexes are indicated. (*B*) Activin inhibited Pit-1 protein binding to the pPit-1 site. Mobility-shift assay using the pPit-1 oligonucleotide probe (positions −97 to −66) (0.9 ng) and control extracts demonstrated a DNA–protein complex pattern similar to that seen with intact GH promoter probe. Complex formation was inhibited by addition of a 50-fold molar excess of the pPit-1 oligonucleotide (DNA). Activin treatment resulted in the loss of complexes I and II. Incubation of Pit-1-specific antisera, but not preimmune sera (Pre), with control extract specifically decreased mobility of both complex I and complex II but had little effect on DNA–protein complexes from activin-treated extracts.

factor CREB or AP1 recognition sequences showed no changes upon activin treatment (data not shown). This result excludes the possibility that activin treatment results in a general decrease in transcription factor binding to DNA. These data and previous reports showing that the Pit-1 protein alone can account for the footprint activity of somatotroph nuclear extracts to these sites (31) indicate that activin repression of GH transcription occurs via decreased binding of immunoreactive Pit-1 to its cognate binding sites on the GH promoter.

## DISCUSSION

The results presented here show that activin A is a negative regulator of GH mRNA levels (Fig. 1), consistent with the observations of Vale and coworkers (19, 20) that activin is a negative regulator of GH biosynthesis and secretion. Further, activin treatment is capable of strong specific repression of transfected GH promoter–CAT expression in cultured MtTW15 somatotrophic cells (Fig. 2). CAT expression under the control of a somatostatin promoter, which has previously been shown to be regulated by cAMP (43), was not repressed by activin treatment. Further, activin treatment of rat anterior pituitary cells does not block GH releasing factor-stimulated c-Fos expression (20), which is another target of cAMP-induced gene expression. Therefore, these results indicate that the effects of activin on the GH promoter cannot be attributed to the ≈50% inhibition of basal cAMP levels that has been observed in these cells (R.S.S., L. Mathews, W.W.V., unpublished data).

Deletion mapping of the GH promoter indicated that sequences between positions −133 and +8 are required for high-level basal expression and activin-mediated repression (Fig. 2*B*). Further, insertion of the region (positions −183 to −48) upstream of a thymidine kinase promoter conferred activin repression of this previously unresponsive promoter. Thus, these results indicate that activin repressibility is mediated by sequences between positions −133 and −48. High basal level expression of the GH gene requires binding of the POU/homeodomain-containing transcription factor Pit-1/GHF-1 to tissue-specific elements in the 5′ regulatory region of the gene (24, 49, 50). Purified or bacterially expressed Pit-1/GHF-1 footprints were identified in two regions in the rat GH promoter, a distal site from positions −132 to −105 and a proximal site from positions −94 to −66 (31, 32). Additional nuclear factor binding sites have not been demonstrated within this region other than the TATA binding protein recognition sequence (TATA box) at position −30. Therefore, our results suggest that the same regions that confer tissue-specific gene expression are also required for activin-mediated repression. On the other hand, contributions from sequences flanking the Pit-1/GHF-1 footprints cannot be definitively excluded by these deletion mapping experiments.

Deletions extending into the Pit-1/GHF-1-footprinted regions resulted in low-level expression that was not further repressed by activin treatment. This result is consistent with a model in which activin acts through interference with Pit-1/GHF-1 function rather than through an independent repressor element. This hypothesis is strongly supported by the observation that activin-treated extracts were no longer capable of forming the predominant Pit-1–DNA complex with the proximal Pit-1/GHF-1 recognition sequence in gel-shift experiments (Fig. 3). This major complex was formed in control extracts using several different length promoter fragments and was always lost upon activin treatment. These results and experiments showing that the Pit-1/GHF-1 protein alone can account for the footprint activity of somatotroph nuclear extracts to these sites (31) suggest that activin inhibition of GH gene expression is the result of decreased binding of this POU/homeodomain transcription factor to its cognate sequences in the GH promoter.

The pathways by which activin causes the loss of protein binding to the tissue-specific region of the GH promoter remain to be characterized. Several potential mechanisms have been reported by which Pit-1/GHF-1 or other POU-domain proteins can be negatively regulated. At the level of post-translational regulation, Pit-1/GHF-1 can be phosphorylated *in vivo*, and phosphorylation *in vitro* of residue Tyr-220 in its POU homeodomain has been correlated with decreased binding to the proximal site in the rat GH promoter, but not the distal site (51). POU-domain proteins have also been shown to form various homo- and heterodimers. Pit-1 exists in solution as a monomer but can associate as a homodimer on its cognate DNA response element (52). Pit-1 can also form a heterodimer with another POU-domain protein, Oct-1

(53). These heterodimers bind preferentially to some Pit-1 response elements (including the proximal site in the rat GH promoter) while other Pit-1 response elements are only capable of binding the homodimers (such as the distal site in the rat GH promoter) (53). Heterodimer formation between Cf1-a and I-POU in *Drosophila* neurons gives rise to a complex that can no longer bind DNA (54).

It is also interesting to note that two cis-acting Pit-1/GHF-1 response elements have been characterized in the Pit-1/GHF-1 gene itself and are proposed to mediate autoregulation of Pit-1/GHF-1 gene expression (34, 55). This model would predict that reduction of the activity of the Pit-1/GHF-1 protein may have the long-term consequence of decreasing its own synthesis, as well as the synthesis of GH, thereby interrupting a positive feedback loop proposed to maintain the differentiated state of somatotrophs and lactotrophs. Because Pit-1/GHF-1 has also been shown to be necessary for proliferation of somatotrophic cell lines *in vitro* (30), as well as for the development of somatotrophic, lactotrophic, and thyrotrophic cell lineages in mice (28), the long-term antiproliferative effects of activin on the somatotroph (20) may be mediated also by loss of Pit-1/GHF-1 function.

The broad spectrum of activin's developmental and antiproliferative effects indicate that the scope of its intracellular actions extends well beyond regulation of Pit-1/GHF-1. Because of the key role activin plays in early vertebrate differentiation and development, it is tempting to speculate that other activin responses may also involve modulation of the activity of POU/homeodomain-containing transcription factors. This could provide a general mechanism by which activin exerts tissue-specific-transcriptional, antiproliferative, and developmental effects, depending on the complement of POU/homeodomain proteins expressed in the target cell.

The contributions of R.S.S. and D.G.-K. to this manuscript were equivalent and both should be considered as primary authors. We thank Marc Montminy, James Leonard, and Richard Kurten for many helpful discussions, Lawrence Mathews for critical reading of the manuscript, and J. Voss and M. G. Rosenfeld for providing antisera. This work was supported by National Institutes of Health Grants DK26741 and HD13527, Sanofi, The Harry and Grace Steele Foundation and was conducted by the Foundation for Medical Research, Inc. W.W.V. is a Foundation for Medical Research Senior Investigator. R.S.S. was supported by the Paul Stock Foundation. D.G.-K. was supported by the Kleberg Foundation and is a National Research Service Award recipient.

1. Vale, W., Rivier, J., Vaughan, J., McClintock, R., Corrigan, A., Woo, W., Karr, D. & Spiess, J. (1986) *Nature (London)* **321**, 776–779.
2. Ling, N., Ying, S. Y., Ueno, N., Shimasaki, S., Esch, F., Hotta, M. & Guillemin, R. (1986) *Nature (London)* **321**, 779–782.
3. Massague, J. (1987) *Cell* **49**, 437–438.
4. Rivier, J., Spiess, J., McClintock, R., Vaughan, J. & Vale, W. (1985) *Biochem. Biophys. Res. Commun.* **133**, 120–127.
5. Robertson, D., Foulds, L., Leversha, L., Morgan, F., Hearn, M., Burger, H., Wettenhall, R. & Kretser, D. (1985) *Biochem. Biophys. Res. Commun.* **126**, 220–226.
6. Miyamato, K., Hasegawa, Y., Fukuda, M., Nomura, M., Igarashi, M., Kangawa, K. & Matsuo, H. (1985) *Biochem. Biophys. Res. Commun.* **129**, 396–403.
7. Ling, N., Ying, S., Ueno, N., Esch, F., Denoroy, L. & Guillemin, R. (1985) *Proc. Natl. Acad. Sci. USA* **82**, 7217–7221.
8. Meunier, H., Rivier, C., Evans, R. M. & Vale, W. (1988) *Proc. Natl. Acad. Sci. USA* **85**, 247–251.
9. Roberts, V., Meunier, H., Vaughan, J., Rivier, J., Rivier, C., Vale, W. & Sawchenko, P. (1989) *Endocrinology* **124**, 552–554.
10. De Paolo, L. (1991) *Proc. Soc. Exp. Biol. Med.* **198**, 500–512.
11. Schubert, D., Kimura, H., LaCorbiere, M., Vaughan, J., Karr, D. & Fisher, W. (1990) *Nature (London)* **344**, 868–870.
12. Thomsen, G., Woolf, T., Whitman, M., Sokol, S., Vaughan, J., Vale, W. & Melton, D. (1990) *Cell* **63**, 485–493.
13. Smith, J., Price, B., Nimmen, K. & Huylebroeck, D. (1990) *Nature (London)* **345**, 729–731.
14. van den Eijnden-Van Raaij, A. J. M., van Zoelent, E. J. J., van Nimmen, K., Koster, C. H., Snoek, G. T., Durston, A. J. & Huylebroeck, D. (1990) *Nature (London)* **345**, 732–734.
15. Mathews, L. S. & Vale, W. W. (1991) *Cell* **65**, 973–982.
16. Attisano, L., Wrana, J., Cheifetz, S. & Massague, J. (1992) *Cell* **68**, 97–108.
17. Mathews, L. S., Vale, W. W. & Kintner, C. R. (1992) *Science* **255**, 1702–1705.
18. Lin, H., Wang, X., Ng-Eaton, E., Weinberg, R. & Lodish, H. (1992) *Cell* **68**, 775–785.
19. Bilezikjian, L. M., Corrigan, A. & Vale, W. W. (1990) *Endocrinology* **126**, 2369–2365.
20. Billestrup, N., Gonzalez-Manchon, C., Potter, E. & Vale, W. (1990) *Mol. Endocrinol.* **4**, 356–362.
21. Evans, R. M., Birnberg, N. C. & Rosenfeld, M. G. (1982) *Proc. Natl. Acad. Sci. USA* **79**, 7659–7663.
22. Barinaga, M., Yamonoto, G., Rivier, C., Vale, W., Evans, R. & Rosenfeld, M. G. (1983) *Nature (London)* **306**, 84–85.
23. Nelson, C., Crenshaw, E., Franco, R., Lira, S., Albert, V., Evans, R. & Rosenfeld, M. (1986) *Nature (London)* **322**, 557–562.
24. Lefevre, C., Imagawa, M., Dana, S., Grindlay, J., Bodner, M. & Karin, M. (1987) *EMBO J.* **6**, 971–981.
25. Lira, S. A., Crenshaw, III, E. B., Glass, C. K., Swanson, L. W. & Rosenfeld, M. G. (1988) *Proc. Natl. Acad. Sci. USA* **85**, 4755–4759.
26. Mayo, K. E., Hammer, R. E., Swanson, L. W., Brinster, R. L., Rosenfeld, M. G. & Evans, R. M. (1988) *Mol. Endocrinol.* **2**, 606–612.
27. Billestrup, N., Swanson, L. W. & Vale, W. (1986) *Proc. Natl. Acad. Sci. USA* **83**, 6854–6857.
28. Li, S., Crenshaw, E. B., Rawson, E. J., Simmons, D. M., Swanson, L. W. & Rosenfeld, M. G. (1990) *Nature (London)* **347**, 528–533.
29. Struthers, R. S., Vale, W. W., Arias, C., Sawchenko, P. E. & Montminy, M. R. (1991) *Nature (London)* **350**, 622–624.
30. Castrillo, J., Theill, L. & Karin, M. (1991) *Science* **253**, 197–199.
31. Ingraham, H. A., Chen, R., Mangalam, H. J., Elsholtz, H. P., Flynn, S. E., Lin, C. R., Simmons, D. M., Swanson, L. & Rosenfeld, M. G. (1988) *Cell* **55**, 519–529.
32. Bodner, M., Castrillo, J. L., Theill, L. E., Deerinck, T., Ellisman, M. & Karin, M. (1988) *Cell* **55**, 505–518.
33. Furth, J., Clifton, K. H., Gadsden, E. L. & Buffett, R. F. (1956) *Cancer Res.* **16**, 608–616.
34. McCormick, A., Brady, H., Theill, L. E. & Karin, M. (1990) *Nature (London)* **345**, 829–832.
35. Chomczynski, P. & Sacchi, N. (1986) *Anal. Biochem.* **162**, 156–159.
36. Feinberg, A. & Vogelstein, B. (1983) *Anal. Biochem.* **132**, 6–13.
37. Barta, A., Richards, R., Baxter, J. & Shine, J. (1981) *Proc. Natl. Acad. Sci. USA* **78**, 4867–4871.
38. Saiki, R. K., Gelfand, D. H., Stoffel, S., Scharf, S. J., Higuchi, R., Horn, G. T., Mullis, K. B. & Erlich, H. (1988) *Science* **239**, 487.
39. Graham, F. & van der Eb, A. (1973) *Virology* **52**, 546.
40. Vale, W., Vaughan, J., Yamamoto, G., Bruhn, T., Douglas, C., Dalton, D., Rivier, C. & Rivier, J. (1983) *Methods Enzymol.* **103**, 565–577.
41. Gorman, C., Moffat, L. & Howard, B. (1982) *Mol. Cell. Biol.* **2**, 1044–1051.
42. Schreiber, E., Matthias, P., Muller, M. & Schaffner, W. (1989) *Nucleic Acids Res.* **17**, 6419.
43. Montminy, M. R., Sevarino, K. A., Wagner, J. A., Mandel, G. & Goodman, R. H. (1986) *Proc. Natl. Acad. Sci. USA* **83**, 6682–6686.
44. McKnight, S., Gavis, E., Kingsbury, R. & Axel, R. (1981) *Cell* **25**, 385–398.
45. Koenig, R., Brent, G., Warne, R., Larsen, P. & Moore, D. (1987) *Proc. Natl. Acad. Sci. USA* **84**, 5670–5674.
46. Flug, F., Copp, R., Casanova, J., Horowitz, Z., Janocko, L., Plotnick, M. & Samuels, H. (1987) *J. Biol. Chem.* **262**, 6373–6382.
47. Norman, M., Lavin, T., Baxter, J. & West, B. (1989) *J. Biol. Chem.* **264**, 12063–12073.
48. Kerr, L., Miller, D. & Matrisian, L. (1990) *Cell* **61**, 267–278.
49. Bodner, M. & Karin, M. (1987) *Cell* **50**, 267–275.
50. Nelson, C., Albert, V., Elsholtz, H., Lu, L. & Rosenfeld, M. (1988) *Nature (London)* **239**, 1400–1405.
51. Kapiloff, M., Farkash, Y., Wegner, M. & Rosenfeld, M. (1991) *Science* **253**, 786–789.
52. Ingraham, H., Flynn, S., Voss, J., Albert, V., Kapiloff, M., Wilson, L. & Rosenfeld, M. (1990) *Cell* **61**, 1021–1033.
53. Voss, J., Wilson, L. & Rosenfeld, M. (1991) *Genes Dev.* **5**, 1309–1320.
54. Treacy, M., He, X. & Rosenfeld, M. (1991) *Nature (London)* **350**, 577–584.
55. Chen, R., Ingraham, H. A., Treacy, M. N., Albert, V. R., Wilson, L. & Rosenfeld, M. G. (1990) *Nature (London)* **346**, 583–586.