High level, anterior pituitary-specific expression of the rat growth hormone (rGH) promoter requires cooperative actions of several different transcription factors. Previously, we described a series of multisubunit, tissue-general, transcription factor complexes that bound to the GHF3 activation site and strongly regulated rGH promoter activity. A 43-kDa DNA-binding subunit common to each of the different GHF3 complexes is identified here as the transcription factor, C/EBPα. In human monocyte U937 cells, which do not express the endogenous or transfected GH genes, co-expression of C/EBPα and Pit-1 synergistically activated the transfected rGH promoter. Full-length C/EBPα was present in the GH-secreting GC, and prolactin-secreting 235-1, pituitary cell lines, but not in GHFT1-5 cells, which are transformed at a stage in development immediately prior to GH expression. Transient expression of C/EBPα in GHFT1-5 cells strongly activated the co-transfected rGH promoter through the GHF3 binding site; a second activation site mapped to evolutionary conserved GH promoter sequences between −106 and −33. C/EBPα activation was synergistic with phorbol 12-myristate 13-acetate and forskolin, activators of protein kinases C and A, respectively. Thus, C/EBPα is an important regulator of rGH promoter activity that appears to function in synergy with Pit-1, activators of A and C protein kinases and possibly other factors.

The rat growth hormone (rGH) gene is expressed selectively in a subpopulation of anterior pituitary cells. Transient promoter sequences between −237 and +8 (where +1 is the transcription start site) drive the maximal expression of a linked reporter gene in cultured pituitary cells (reviewed in Refs. 1–3). Pituitary mutagenesis and transient transfection demonstrated that several different sequence elements are critical for rGH promoter activity. The nonadditive effects of those promoter mutants suggested that cooperation between those factors was crucial to the high level and pituitary-specific regulation of the rGH promoter (3). The importance of those cooperative activities was underscored by studies showing that the rGH promoter could be strongly activated in nonpituitary cells (4–6) only by the co-expression of the pituitary-specific factor (7–13) Pit-1, and either the thyroid hormone receptor (T3R) or ZN15. The transcriptional consequences of repositioning binding sites within the rGH promoter (7, 14, 15) further suggested that the integration of binding factor activities is crucial to promoter function. Thus, a detailed knowledge of the factors that bind to the rGH promoter and their cooperative and/or mutually disruptive activities will be required to understand the physiologic and ontologic regulation of GH transcription.

Previously, we identified a factor termed GHF3 that binds to rGH promoter sequences between −239 and −219 and is one of the strongest determinants of rGH promoter activity; promoter mutants that disrupted the GHF3 binding site reduced the activity of the rGH promoter transfected into GH-secreting rat pituitary GC cells by 75% (16). Gel mobility shift experiments showed that complexes of differing electrophoretic mobility bound to the GHF3 binding site and that the identical site was footprinted in each of those complexes (16). Two-dimensional gel mobility shift assays showed that each complex shared a DNA-binding subunit of similar electrophoretic mobility (16). Consistent with the presence of a common DNA-binding subunit, UV cross-linking of the different complexes to the GHF3 binding site demonstrated that each complex contained a DNA cross-linked factor of identical size, approximately 46 kDa (16). This agreed well with the predominant 43-kDa GHF3 DNA-binding subunit detected by a Southwestern blot using a radiolabeled GHF3 DNA binding site to probe crude GC cell extracts separated electrophoretically according to size (16). Thyrotroph embryonic factor (TEF), which binds to multiple sites within the promoter for the gene encoding the β-subunit of the thyroid-stimulating hormone, also bound to the rGH promoter over the GHF3 site (17). However, the 29.3-kDa size of TEF (17) does not agree well with the −43-kDa size of the predominant GHF3 DNA-binding subunit present in GC cells or even with other minor 35-, 58-, 72-, and 140-kDa GHF3-binding proteins detected by Southwestern blot (16).

Given the importance of the GHF3 site, it was necessary to identify the factor that binds to it to understand the control of rGH expression. This factor was of additional interest because it is the DNA-binding subunit common to the array of alternate multisubunit GHF3 complexes. We demonstrate here, by antibody interference with gel mobility shift complexes and by Western blots of GHF3 factors purified by DNA affinity chromatography, that the GHF3 DNA-binding subunit contains C/EBPα. A role for C/EBPα in the pituitary-specific regulation of the GH gene was further supported by a number of observations: 1) C/EBPα and Pit-1 synergistically activated the rGH promoter in human monocye U937 cells; 2) full-length C/EBPα was detected in extracts of GH-secreting GC cells and prolactin-secreting 235-1 cells but not in Pit-1-containing, GHFT1-5 pituitary progenitor cells transformed at a developmental stage prior to the expression of any mature pituitary hormones (18);
and 3) transient expression of C/EBPα in GHFT1-5 cells strongly activated a co-transfected rGH promoter, implying that C/EBPα may be limiting for rGH expression in these pituitary progenitor cells. 60% of the C/EBPα activation in GHFT1-5 cells required the GHF3 binding site, whereas the remaining activation required rGH promoter sequences between -106 and -33. rGH promoter activation by C/EBPα was synergistic with cultivating the GHFT1-5 cells with PMA and forskolin, activators of protein kinases C and A, respectively (19, 20) further demonstrating the importance of integrative functions in the regulation of high level rGH promoter activity. Thus, C/EBPα is an important factor regulating high level transcription of rGH promoter activity and appears to function in synergy with Pit-1, activators of protein kinases A and C, and possibly other factors through the GHF3 and secondary activation sites.

EXPERIMENTAL PROCEDURES

Western Blots—Affinity-purified Pit-1 and 20 μg of nuclear extracts, prepared as described previously (21), were separated by discontinuous SDS-polyacrylamide gel electrophoresis and electroblotted to nitrocellulose. GHF3 purified 600-fold by one round of DNA binding site affinity chromatography (16) was subjected to a second round of affinity purification and simultaneously blotted. Blots were blocked with 5% nonfat dry milk powder and probed with the indicated primary antibodies, C/EBPα, C/EBPβ, and C/EBPβ7. Polyclonal antibodies were a gift from W.-C. Yeh and S. L. McKnight (Tularik, South San Francisco). Anti-TEF antibodies were obtained from D. Drolet and M. G. Rosenfeld (University of California, San Diego), and anti-DBP antibodies were purchased from Berkeley Antibody Company (BAbCo). The blot was then probed with anti-rabbit horseradish peroxidase-linked secondary antibodies (Amersham Corp., respectively) and developed using the ECL chemiluminescence solution (Amersham). Gel Mobility Shift Assays—Gel mobility shift assays were conducted essentially as described previously (21) except that affinity-purified GHF3 (16) was first incubated for 15 min at room temperature with the indicated antibodies or nonimmune serum. Following preincubation, a radiolabeled 87-base pair fragment containing rGH promoter sequences between -285 and -198 that span the GHF3 site (239 to -219), was added with or without 50 ng of a cold competitor oligonucleotide (240 to -216) (16). pGHF1 and GHF2 control competitor oligonucleotides were previously described (11). Because of nonspecific DNA binding activities in the nonimmune and C/EBPα-primer sera, 4 μg of poly(dI- dC) (Pharmacia Biotech Inc.) had to be added to each incubation. Poly(dI-dC) was added at the time of probe and oligonucleotide addition.

Promoter Constructs: Requirement for a Modified pUC Vector—The -237/-18 promoter and -230/-226 mutation thereof (see Fig. 6, mut/237) (16), the -106/-8 (8), the -33/-8, and the -33/+8 promoter to which six copies of the GHF3 binding site were appended (16) were previously described. +1 is the transcription start site. All rGH promoters were cloned upstream of the coding sequences of the bacterial chimaeraphenolic acetyltransferase (CAT) gene carried in a pUC vector deleted of sequences between AatI and EcoRI (4, 22). Use of the modified pUC vector was critical, since the -33/+8 rGH promoter carried in the standard pUC vector was strongly activated by C/EBPα expression (data not shown), whereas parallel transfected -33/+8 promoters carried in the modified pUC vector were not (see Figs. 6 and 7). Thus, there appears to be a C/EBPα activation site present in the standard pUC vector, but this site was eliminated in the experiments described here.

C/EBPα Co-purifies with GHF3 Binding Activity—Although TEF transcripts were detected in GH-secreting GC cells (17) and TEF protein was capable of binding to the GHF3 site in the rGH promoter (17), the size of TEF is different from the size of the major GHF3-binding protein found in GC cells (16). We also did not detect TEF in Western blots (data not shown) of GHF3 DNA-binding subunit purified from GC cell extracts by GHF3 binding site affinity chromatography (16), although the lack of a Western signal with extracts from a variety of pituitary and nonpituitary cells limited the conclusion that no anti-TEF cross-reacting material is present in affinity-purified GHF3. TEF is a member of a large family of transcription factors, the bZIP proteins, that bind to similar DNA sequences and share homology in their DNA binding and dimerization domains (23, 24). Since only the portions of TEF that were conserved with other bZIP proteins were necessary for binding to the GHF3 site (17), it was possible that the authentic GHF3 DNA-binding subunit was another bZIP factor. Western blots using polyclonal antibodies directed against a 42-kDa bZIP protein, C/EBPα (25, 26), detected an appropriately sized factor present in the GHF3-binding fractions enriched from GC cell extracts by DNA affinity chromatography (Fig. 1A, purified GHF3). 34- and 30-kDa proteins that cross-react with the C/EBPα antibody were also detected in the affinity-purified material. In contrast, C/EBPβ antibodies did not cross-react with Pit-1 prepared from the same extracts by DNA affinity chromatography (purified Pit-1), although Pit-1 antibodies were cross-reactive with affinity-purified Pit-1 (Fig. 1B).
GHFT1-5 cells are an excellent cell line for studying the regulation of rGH gene expression, since they represent an embryonic pituitary cell type transformed during the developmental window in which Pit-1 is expressed prior to its commitment to the GH+, TSH-, or PRL-secreting cell lineages (18) (Fig. 1B). Therefore, factors other than Pit-1 expression must limit rGH expression in these cells. The 43-kDa C/EBPα species was not detectable in Western blots of GHFT1-5 extracts, although C/EBPα was observed in GC cells, prolactin-secreting 235-1 cells, and human cervical carcinoma HeLa cells (Fig. 1A). A 34-kDa factor that cross-reacted with the anti-C/EBPα antibody was detected in extracts from all three pituitary cell types (Fig. 1A), and a 30-kDa cross-reacting factor was detected in the GC and 235-1 cell extracts. Neither the 30- nor the 34-kDa species were detected in HeLa cell extracts (Fig. 1A).

The wild type -237/-8 rGH promoter was weakly active when transfected into GHFT1-5 cells (Fig. 4). Co-expression of C/EBPα resulted in a 4.5-fold, on average (n = 9), enhancement of CAT activity expressed from the rGH promoter. In contrast, Pit-1 co-expression did not activate the rGH promoter and did not further enhance the C/EBPα-activated rGH promoter (Fig. 4). The Pit-1 expression vector is active in GHFT1-5 cells under these conditions, since its transfection strongly activates the prolactin promoter,3 minimal promoters to which rGH Pit-1 binding sites are multimerized and appended (6), and even the -237/-8 rGH promoter, but only if TR is co-expressed and the transfected GHFT1-5 cells are incubated with PMA and forskolin (6).

Synergistic Activation by C/EBPα, PMA, and Forskolin—Since Pit-1 and TR activate the rGH promoter only in PMA- and forskolin-induced GHFT1-5 cells, the effect of PMA and forskolin on the activation of the rGH promoter by C/EBPα and/or Pit-1 was investigated. Incubation of C/EBPα-transfected GHFT1-5 cells with PMA and forskolin caused a 15.4-fold, on average (n = 9), enhancement of the rGH promoter, much greater than the sum of the 1.8- and 4.5-fold effects for the respective single treatments (15.4/1.8 > (1.8 + 4.5)/2).

Cross-linking and Western blot analysis (16). The rGH promoter is inactive in U937 cells. CAT activity expressed from the -237/-8 rGH promoter transiently transfected into U937 cells with (+) or without (0) vectors expressing the Pit-1 or C/EBPα cDNA is shown. Cells were treated with 10^-8 M PMA and 10^-5 M forskolin 24 h before collection. wt, wild-type Pit-1. 72/125, mutation of Pit-1 that selectively inhibits synergistic activation with TR (6). The data represent the mean ± S.D. of three independent experiments normalized to the CAT activity of the Pit-1- and C/EBPα-activated promoter (100%).
caused by independent PMA/forskolin incubation and C/EBP\(\alpha\) activation, respectively (Fig. 4). Pit-1 did not enhance C/EBP\(\alpha\) activation of the rGH promoter even when the GHFT1-5 cells were incubated with PMA and forskolin.

Synergistic activation by C/EBP\(\alpha\) and PMA/forskolin incubation was maximal when both protein kinase inducers were present (Fig. 5). C/EBP\(\alpha\) activation after incubation with both PMA and forskolin was 1.8-fold higher than the sum of the increases by independent PMA and forskolin incubation over C/EBP\(\alpha\) activation in the absence of PMA and forskolin. PMA was independently able to synergize with C/EBP\(\alpha\), albeit more poorly than when both PMA and forskolin were present.

Two rGH Promoter Sites for C/EBP\(\alpha\) Activation—Point mutations disrupting the GHF3 binding site (16) in the −237/+8 rGH promoter showed that 60% of the C/EBP\(\alpha\) activation in GHFT1-5 cells, in either the presence or absence of Pit-1 or PMA/forskolin, required the GHF3 binding site (Fig. 6, compare wild type (wt/237) promoter with mutant (mut/237) promoter). Whereas the rGH promoter truncated to −106 was activated by C/EBP\(\alpha\) expression to the same extent as the −237/+8 promoter mutated in the GHF3 binding site, the rGH promoter truncated to −33 was not activated by C/EBP\(\alpha\) expression, even at high amounts of transfected C/EBP\(\alpha\) expression vector (Fig. 7A). The −33/+8 promoter could be made strongly responsive (43.2-fold activation on average when co-transfected with 10 \(\mu\)g of C/EBP\(\alpha\) expression vector) to C/EBP\(\alpha\) by inserting six copies of the GHF3 binding site immediately upstream of it (Fig. 7B), demonstrating that the −33/+8 promoter was competent for C/EBP\(\alpha\) activation.

**DISCUSSION**

The GHF3 site is a major locus controlling the transcription of the rGH promoter and is bound by a series of different multisubunit complexes via a single, common DNA-binding subunit (16). As a first step toward elucidating the structures and activities of the different GHF3 complexes, we have determined in the following ways that the common DNA-binding subunit contains C/EBP\(\alpha\). C/EBP\(\alpha\) co-fractionated with factors enriched greater than 600-fold in the minimal DNA-binding subunit of GHF3 (Fig. 1A); antibodies directed against C/EBP\(\alpha\) disrupted GHF3 gel mobility shift complexes (Fig. 2) formed with affinity-purified GHF3; Southwestern blots previously identified the predominant GHF3-binding factor as having a molecular mass of 43 kDa (16), similar to the 42-kDa size of C/EBP\(\alpha\) (25, 26) (Fig. 1A); and the GHF3 binding site contains strong similarity (Fig. 8) to an optimal, palindromic C/EBP\(\alpha\) motif (27). C/EBP\(\alpha\) is a member of the bZIP family of transcription factors, and the known ability of C/EBP\(\alpha\) to heterodimerize with independent PMA/forskolin incubation and C/EBP\(\alpha\) activation, respectively (Fig. 4). Pit-1 did not enhance C/EBP\(\alpha\) activation of the rGH promoter even when the GHFT1-5 were incubated with PMA and forskolin. Synergistic activation by C/EBP\(\alpha\) and PMA/forskolin incubation was maximal when both protein kinase inducers were present (Fig. 5). C/EBP\(\alpha\) activation after incubation with both PMA and forskolin was 1.8-fold higher than the sum of the increases by independent PMA and forskolin incubation over C/EBP\(\alpha\) activation in the absence of PMA and forskolin. PMA was independently able to synergize with C/EBP\(\alpha\), albeit more poorly than when both PMA and forskolin were present.

Two rGH Promoter Sites for C/EBP\(\alpha\) Activation—Point mutations disrupting the GHF3 binding site (16) in the −237/+8 rGH promoter showed that 60% of the C/EBP\(\alpha\) activation in GHFT1-5 cells, in either the presence or absence of Pit-1 or PMA/forskolin, required the GHF3 binding site (Fig. 6, compare wild type (wt/237) promoter with mutant (mut/237) promoter). Whereas the rGH promoter truncated to −106 was activated by C/EBP\(\alpha\) expression to the same extent as the −237/+8 promoter mutated in the GHF3 binding site, the rGH promoter truncated to −33 was not activated by C/EBP\(\alpha\) expression, even at high amounts of transfected C/EBP\(\alpha\) expression vector (Fig. 7A). The −33/+8 promoter could be made strongly responsive (43.2-fold activation on average when co-transfected with 10 \(\mu\)g of C/EBP\(\alpha\) expression vector) to C/EBP\(\alpha\) by inserting six copies of the GHF3 binding site immediately upstream of it (Fig. 7B), demonstrating that the −33/+8 promoter was competent for C/EBP\(\alpha\) activation.

**DISCUSSION**

The GHF3 site is a major locus controlling the transcription of the rGH promoter and is bound by a series of different multisubunit complexes via a single, common DNA-binding subunit (16). As a first step toward elucidating the structures and activities of the different GHF3 complexes, we have determined in the following ways that the common DNA-binding subunit contains C/EBP\(\alpha\). C/EBP\(\alpha\) co-fractionated with factors enriched greater than 600-fold in the minimal DNA-binding subunit of GHF3 (Fig. 1A); antibodies directed against C/EBP\(\alpha\) disrupted GHF3 gel mobility shift complexes (Fig. 2) formed with affinity-purified GHF3; Southwestern blots previously identified the predominant GHF3-binding factor as having a molecular mass of 43 kDa (16), similar to the 42-kDa size of C/EBP\(\alpha\) (25, 26) (Fig. 1A); and the GHF3 binding site contains strong similarity (Fig. 8) to an optimal, palindromic C/EBP\(\alpha\) binding site (31, 32). C/EBP\(\alpha\) is a member of the bZIP family of transcription factors, and the known ability of C/EBP\(\alpha\) to heterodimerize...
C/EBPα Activation of the rGH Promoter

The relative lack of full-length (42-kDa) C/EBPα in pituitary progenitor GHFT1-5 cells compared with adult-derived GC or 235-1 pituitary tumor cells (Fig. 1), combined with the strong activation of the rGH promoter in GHFT1-5 cells by C/EBPα expression (Fig. 4), provided additional evidence for an important role of C/EBPα in the pituitary-specific expression of the rGH promoter. Previously reported distributions of C/EBPα RNA or protein in rats (28), mice (28, 29), or humans (30) did not specifically include the pituitary gland. C/EBPα expression is associated with the terminally differentiated state in other tissues and has antiproliferative actions in some cells (39–43), making it possible that C/EBPα expression promotes both rGH expression (Fig. 4) and concomitant differentiation into a nonproliferative cell type. Pituitary-specific activation of the rGH promoter by C/EBPα is likely to be dependent upon Pit-1 (Fig. 3), although the presence of both Pit-1 and C/EBPα in lactotroph 235-1 cells (Fig. 1) that do not express GH indicates a role for other factors.

C/EBPα antibodies also cross-reacted with 30- and 34-kDa proteins present both in affinity-purified GHF3 and in extracts from the GC and 235-1 cells; only the 34-kDa species seemed to be present in GHFT1-5 cells (Fig. 1A). A 30-kDa C/EBPα variant originating from an internal translation initiation site within the C/EBPα mRNA has been described previously in mice, rats, and chickens (43–45). This amino-terminal truncated, 30-kDa C/EBPα lacks the transcriptional activation functions (45) and antiproliferative activity (43) of C/EBPα but retains its DNA binding and dimerization domains, consistent with its purification by DNA affinity chromatography (Fig. 1A). The previously reported extinction of GH gene expression in fusion of GH-secreting GC cells and L-cells may involve factors binding to the GHF3 site (46), and it is possible that an altered balance of activating 42-kDa C/EBPα and inactive 30-kDa C/EBPα could contribute to this suppression. The origins and activity of the 34-kDa form have not been reported, and it may represent a pituitary-specific isofrom of C/EBPα. Basal rGH promoter activity in GHFT1-5 cells was inhibited by a point mutation (Fig. 6, mut/237) that inhibits GHF3 factor binding, suggesting that GHFT1-5 cells contain an activating factor, possibly the 34-kDa C/EBPα variant, that binds to the GHF3 site.

The same promoter mutation reduced, but did not eliminate, activation of the rGH promoter by ectopic C/EBPα expression in GHFT1-5 cells (Fig. 6). Because the promoter containing the GHF3 binding site mutation was still activated by C/EBPα, it was impossible to differentiate whether C/EBPα activation through the GHF3 binding site was a result of direct binding of C/EBPα to the GHF3 site or whether C/EBPα was binding to a separate site and merely synergizing with other GHF3-binding factors present in GHFT1-5 cells. C/EBPα activation of the −33/+8 rGH promoter truncated to contain little more than the TATA box was wholly GHF3 binding site-dependent (Fig. 7B), suggesting that C/EBPα activation can occur directly through the GHF3 binding site. Thus, it is likely that C/EBPα directly binds to the GHF3 site in vivo (Fig. 7) as well as in vitro (Figs. 1 and 2) to cause the activation of the rGH promoter.

The current data also suggest that C/EBPα participates, directly or indirectly, in the cAMP- and PMA-dependent effects on the rGH promoter. rGH promoter activation by C/EBPα was synergistic with incubating GHFT1-5 cells with PMA and forskolin, activators of protein kinases C and A, respectively (Figs. 4–6). GH expression is tightly regulated by the hypothalamic factor, growth hormone-releasing factor, through a cAMP in-

FIG. 7. GHF3 binding sites confer C/EBPα activation to the nonresponsive −33/+8 rGH promoter. A, CAT activity expressed from the wild type −237/+8 (wt/237 rGH), −230−226 mutant (mut/237 rGH), or −33/+8 (33 rGH) rGH promoters in response to increasing amounts of co-transfected C/EBPα expression vector. B, the same data plotted in Fig. 7A only including the C/EBPα activation profile of the −33/+8 rGH promoter to which six copies of the GHF3 binding site had been appended. Note the difference in scale. All points were collected from the collection of extracts. The data represent the mean ± S.D. from three independent experiments.

GAGATCTTGCTAACCATTGC
GATTGCACATC
GHF3 Footprint
C/EBP Consensus

with some other bZIP factors (26, 33–37) might implicate other bZIP proteins in the GHF3 complexes. Current evidence suggests that C/EBPα is likely to be the only DNA-binding factor in the minimal DNA-binding subunit, since protein-DNA uv cross-linking of the minimal GHF3 DNA-binding subunit detected exclusively a 46-kDa cross-linked species (16). Similarly, Western blots of affinity-purified, GHF3 DNA-binding subunit probed with antibodies against the bZIP proteins DBP (27), TEF (17), and C/EBPδ (26) (data not shown) did not indicate that these factors co-purified with the GHF3 DNA-binding subunit. These antibodies also did not disrupt the gel shift complexes formed between affinity-enriched GHF3 and the GHF3 binding site (data not shown). It is possible that these or other bZIP proteins may oligomerize with C/EBPα to participate as other subunits of the higher order GHF3 complexes that did not co-purify with the DNA-binding subunit. Although no such C/EBP oligomers have been reported to date, oligomerization through leucine zippers has been demonstrated (37, 38). Further studies will be required to define the other subunits of the multisubunit GHF3 complexes.

FIG. 8. The GHF3 footprint is centered over an 8 of 12 nucleotide match to an “optimal” (28, 29) C/EBP binding site. Stippled background identifies identity between the GHF3 and C/EBP consensus sites.
termed (47, 48). As forskolin increases the intracellular concentration of cAMP (20), it is possible that the effects of forskolin on rGH promoter activity may be mimicking the effects of growth hormone-releasing factor. Pit-1 is another target for PMA and forskolin action and is phosphorylated by both protein kinase A and C in vitro and in vivo (49). Forskolin activation of the rGH promoter has also been suggested to be Pit-1 binding site-dependent (50, 51), although this is difficult to ascertain given the dependence of the rGH promoter on the Pit-1 binding sites. Pit-1 mutated in its protein kinase A and C phosphorylation sites was 65% as effective as wild type Pit-1 at activating the rGH promoter (52), demonstrating that other rGH promoter-binding factors, possibly C/EBPα, contribute significantly to both protein kinase A and C activation.

PMA and forskolin synergy with C/EBPα displayed components dependent upon both the GHF3 binding site and a second C/EBPα activation site that maps to between −106 and −33 (Fig. 6). The molecular nature of this secondary C/EBPα activation site is currently unknown, but since purified GHF3 did not bind to a rGH promoter fragment spanning sequences −106 to −15 (Fig. 2), it is not likely to be mediated directly through GHF3 factor binding at that site. We have also observed that C/EBPα expression activates the human GH promoter in GFT1-5 cells and is synergistic with PMA and forskolin. We currently do not know if C/EBPα activation of the human promoter requires the homologous −106 to −33 sequences conserved between the rat and human promoters (53).

Thus, the GHF3 binding site strongly contributes to the control of the rGH promoter in GH-secreting pituitary cells derived from adult pituitary tumors (16) and probably in transgenic mice (54). The GHF3-binding factor, identified here as C/EBPα, synergizes with PMA and forskolin or with Pit-1 and strongly activates the rGH promoter in C/EBPα-deficient pituitary progenitor cells. The role of C/EBPα as the central, DNA-binding subunit of the multisubunit GHF3 complexes is also interesting given the unique architecture of the GHF3 complexes: a number of physiologic control mechanisms may impinge on this single factor and be integrated at this regulatory bottleneck. Determining the molecular nature of C/EBPα-interacting proteins in those higher order complexes will also be essential to understanding the central role of the GHF3 binding site and C/EBPα in the regulation of rGH promoter activity.

Acknowledgments—I thank Dr. J. John D. Baxter for critical reading of the manuscript and for much appreciated support, Drs. W.-C. Yeh and J. Broide, R. S. Rosenfeld, and M. G. Swanson for anti-DBP antibodies, and Drs. D. Lavery and U. Schibler for anti-DBP antibodies.

REFERENCES

1. Theill, L. E., and Karin, M. (1993) Endocrine Rev. 14, 670–689
2. Rhodes, S. J., D’Matta, G. E., and Rosenfeld, M. G. (1994) Curr. Opin. Genet. Dev. 4, 709–717
3. Schaefele, F. (1994) The Pituitary Gland, 2nd Ed. (Imura, H., ed) pp 91–116, Raven Press, New York
4. Schaefele, F., West, B. L., and Baxter, J. D. (1992) Mol. Endocrinol. 6, 656–665
5. Lipkin, S. M., Naar, A. M., Kalla, K. A., Sack, R. A., and Rosenfeld, M. G. (1993) Genes & Dev. 7, 1674–1687
6. Chang, W., Zhou, W., Baxter, J. D., Theill, L. E., and Schaefele, F. (1996) J. Biol. Chem. 271, 17733–17738

* K. Randhawa and F. Schaefele, unpublished data.
CCAAT/Enhancer-binding Protein α Activation of the Rat Growth Hormone Promoter in Pituitary Progenitor GHFT1-5 Cells
Fred Schaufele

J. Biol. Chem. 1996, 271:21484-21489.
doi: 10.1074/jbc.271.35.21484

Access the most updated version of this article at http://www.jbc.org/content/271/35/21484

Alerts:
• When this article is cited
• When a correction for this article is posted

Click here to choose from all of JBC's e-mail alerts

This article cites 53 references, 29 of which can be accessed free at http://www.jbc.org/content/271/35/21484.full.html#ref-list-1