An $\alpha$-Subunit-Secreting Cell Line Derived from a Mouse Thyrotrope Tumor

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The anterior pituitary contains multiple distinct endocrine cell types that secrete individual hormones. To derive a pure cell culture population in which to study the regulation of the $\alpha$-subunit of TSH free of other hormones and cell types, we have developed a clonal continuous cell line from the transplantable thyrotrope tumor MGH101A. This cell line expresses $\alpha$-subunit mRNA, secretes $\alpha$-subunit protein, and has maintained a stable phenotype for over 3 yr in culture. However, as is the case for the transplantable tumor from which they are derived, these cells do not express the $\beta$-subunit of TSH or respond to TRH or thyroid hormone. We have used this cell line to investigate regulation of the $\alpha$-subunit mRNA by the second messengers, cAMP and phorbol esters, and by glucocorticoids. Phorbol esters increase $\alpha$-subunit mRNA levels significantly (3.5-fold), as does cAMP (1.8-fold). In contrast, glucocorticoids decrease mRNA levels from cAMP-induced or basal levels (2-fold). These cells should prove valuable for study of $\alpha$-subunit gene expression in an isolated renewable clonal cell culture system. (Molecular Endocrinology 4: 589–596, 1990)

INTRODUCTION

Study of regulation of the glycoprotein hormone genes has long been hampered by the lack of permanent cell lines derived from anterior pituitary cells that produce the glycoprotein hormone subunits. An inherent problem encountered when using primary anterior pituitary preparations has been the multiplicity of endocrine cell types present in this tissue. In particular, the $\alpha$-subunit gene has been difficult to study due to its presence as a component of three hormones, FSH, LH, and TSH, each produced by association of the $\alpha$-subunit with a distinct $\beta$-subunit (1). In the thyrotrope cell, $\alpha$-subunit is produced as a component of TSH, which is involved in the production of thyroid hormones and is negatively regulated by them in a feedback loop (2). In addition, the hypothalamic releasing hormone TRH induces both $\alpha$-subunit synthesis and release (3–5). The $\alpha$-subunit is also a component of LH and FSH, produced by the gonadotrope cell of the anterior pituitary in response to GnRH. These gonadotropin hormones stimulate steroidogenesis and gametogenesis in the gonads (1). They are, in turn, negatively regulated by estrogens and androgens (6). Thus, the $\alpha$-subunit gene is repressed by a variety of steroids and thyroid hormones and is induced by hypothalamic releasing hormones. Because of the multiplicity of hormones that control $\alpha$-subunit synthesis and release, it has been difficult to distinguish any one response in isolation from others while working with whole animals or in mixed primary pituitary cultures.

The availability of transplantable thyrotropic tumors such as TLT97, MGH101, and MGH101A has led to a number of important observations concerning the regulation of both the secretion and synthesis of the $\alpha$- and $\beta$-subunit genes of TSH (7–11). The MGH101A tumor (11) evolved from the MGH101 tumor (7, 8) during repeated serial transplantation. It differs from the original tumor in that it is not subject to negative growth control by thyroid hormone and no longer expresses the $\beta$-subunit gene of TSH, although it retains a high level of $\alpha$-subunit gene expression (9, 12). Therefore, it represents an interesting model system in which to examine $\alpha$-subunit gene regulation uncoupled from expression of the $\beta$-subunit genes, a situation prevalent
in a number of nonfunctioning chromophobe adenomas described clinically in humans (13).

Nevertheless, many investigations would be facilitated by the availability of a cell line in which to study the regulation of α-subunit gene expression within a reproducible homogeneous population of pituitary cells. The advantages of clonal continuous cell lines are evident from the extensive progress made on GH and PRL gene expression using the somatotrope/lactotrope GH3 cell line (14) and on POMC gene regulation in corticotrope-derived AtT20 cells (15). With this goal in mind, we have developed clonal continuous cell lines from the pituitary tumor MGH101A, which has previously been propagated by serial transplantation in mice (11). In this paper we report the establishment of a clonal, continuously propagable, stable cell line of α-subunit-secreting cells from the mouse tumor MGH101A. Furthermore, we have investigated the regulation of the α-

subunit gene in this cell line by glucocorticoids, thyroid hormone, cAMP, and phorbol esters.

RESULTS

Establishment of the αTSH Cell Line

The first plating of the tumor cells consisted of a heterogeneous mix of cells obtained from a single tumor. Most of the different cell types present in the original cultures were significantly more adherent to tissue culture plastic than the dominant tumor cell type identified using morphological criteria based on the original MGH101A tumor cells. The adherent cells were removed by monitoring the cultures for monolayer growth, removing the more loosely attached cells, and transferring them to a new dish until the αTSH cells were the only cells apparent in the cultures. These remaining cells were then selected for adherence by removing the floating cells from the cultures every 1–2 weeks. However, even after this selection, the cells are only loosely attached; no trypsin or EDTA is necessary during passage, and the medium must be removed carefully to prevent a significant loss of cells. The morphology of the adherent cultures of αTSH cells is shown in Fig. 1.

Using standard clonal dilution technique, 12 different clonal cell lines were established. The relative amounts of α-subunit mRNA in some of these cell lines compared to the mRNA levels in the original tumor cells is presented in Fig. 2. Although the amount of α-subunit-specific mRNA varies among the clones, it is present in
all of the cell lines derived from the MGH101A tumor cells. In addition, RIA of cell medium from αTSH clone 3 cells revealed that α-subunit protein was secreted at 5161 ± 731 ng/10^6 cells·24 h, and cell lysates contained α-subunit protein at 25 ± 2 ng/10^6 cells. In contrast, GH4 cells contained no α-subunit protein, whereas medium from T7T97 thyrotrone tumor cells (8) contained 5550 ng/10^6 cells·24 h. Immunocytochemical staining also revealed α-subunit immunoreactivity in the cytoplasm of the αTSH cells (Deftos, L., personal communication).

The clonal lines vary considerably in their growth rate, with the fastest growing clone (clone 3) maintaining a 40-h doubling time (Fig. 3). There was no correlation between the growth rate of the cells and the level of α-subunit gene expression. The experiments presented in this paper were performed using clones 3 and 12. These two clones exhibited similar responses to the various hormone treatments described below.

Thus, the αTSH cell line (clone 3) has been in continuous culture for over 3 yr and has maintained a stable phenotype. The α-subunit gene continues to be expressed at levels similar to those found in the original tumor cells (Fig. 4), consistent with the observation that α-subunit levels in the MGH101A tumor remain remarkably constant while passaged in mice (11). Like the original tumor cells, the clonal cell lines do not express the βTSH gene (Fig. 5).

**Effects of Phorbol Esters and cAMP on α-Subunit mRNA Levels**

TRH, the major inducer of TSH secretion (4, 5), has also been shown to stimulate synthesis of both the α- and β-subunit genes of TSH in normal rat anterior pituitary cells (3). However, TRH does not affect α-subunit mRNA in these tumor cells (densitometry of Northern blots of RNA from cells treated with 10^{-7} M TRH for 18 h in two experiments showed values of 1.076 and 0.995 relative to control) or in the parental MGH101A tumor (Ridgeway, E. C., personal communication), suggesting that they do not have functional TRH receptors. Although the second messenger system associated with TRH responses is as yet not well defined, some evidence implicates the Ca^{2+}-dependent protein kinase-C/phosphatidyl inositol pathway in TRH action (16–19). In addition, activators of the protein kinase-A pathway have been shown to increase α-subunit gene transcription (20). The recent demonstration of synergism by phorbol esters and cAMP on human α-subunit mRNA initiation in a choriocarcinoma cell line (21) prompted us to investigate the effects of these hormones on mouse α-subunit mRNA levels in αTSH cells.

The αTSH cells were treated with 100 nm of the phorbol ester 12-O-tetradecanoyl phorbol-13-acetate (TPA), both alone and in combination with 10 nm forskolin, for 16–18 h. The cultures were changed to 1% serum for 24 h before hormone treatments. An increase in the amount of steady state α-subunit mRNA of 1.8-fold was observed after treatment of the αTSH cells with forskolin (Fig. 6). This stands in contrast to the dramatic increases (8- to 10-fold) in transcription of the human α-subunit gene observed upon treatment of

![Fig. 4. Alpha-TSH Cloned Cell Line Stably Produces α-subunit mRNA](image1)

Northern blot analysis of αTSH clone 3 RNA from cells cultured over a period of 1 yr. Each lane contained 10 μg total RNA, and the blot was hybridized with a nick-translated mouse α-subunit cDNA clone (50). Cells were originally plated, cloned, then frozen for 4, 9, and 16 months (lanes 1, 2, and 3, respectively) after the original plating. One aliquot of clone 3 cells from each freezing was thawed, and RNA samples were prepared in parallel.

![Fig. 5. Alpha-TSH Cells Do Not Produce TSH β-Subunit mRNA](image2)

Northern blot analysis of αTSH clone 3 RNA compared to T7T97 tumor (8) RNA. Each lane contained 10 μg total RNA, and the blot was hybridized with a nick-translated mouse α-subunit cDNA clone (50) or a mouse βTSH cDNA clone (52). The Northern blot was overexposed to clearly demonstrate the lack of TSH β-subunit mRNA in αTSH cells. nt, Nucleotides.
placental cells with agents that increase intracellular levels of cAMP (20, 22). The phorbol ester TPA increased \( \alpha \)-subunit mRNA levels by 3.5-fold (Fig. 6). However, the combination of both hormones produced the same response as TPA alone. Thus, it appears that a mediator of protein kinase-C action can affect the levels of \( \alpha \)-subunit mRNA in \( \alpha \)TSH cells. Unlike the response of the human gene in placental cells, no synergism was observed on \( \alpha \)-subunit transcription by phorbol esters and forskolin, an activator of adenylate cyclase and the protein kinase-A second messenger system. In fact, the weak induction by forskolin alone was not additive with the TPA response, suggesting that there may be convergence of these two pathways in these cells.

**Dexamethasone Repression of Both Basal and cAMP-Induced \( \alpha \)-Subunit RNA Levels**

We have reported (23) that transcription of the \( \alpha \)-subunit gene is negatively regulated by glucocorticoids using gene transfer experiments in JEG-3 cells, a placental cell line derived from a human chorionicarcinoma (24). Here, we further extend those studies to determine whether the mouse \( \alpha \)-subunit gene is also subject to negative regulation by glucocorticoids when produced in a pituitary cell.

The effects of different combinations of dexamethasone and forskolin treatment for either 24 or 48 h on \( \alpha \)-subunit mRNA levels were determined using Northern analysis (Fig. 7). Forskolin caused a 1.5-fold increase in \( \alpha \)-subunit mRNA levels, again an induction well below that seen on the human gene in placental cells (20, 22). Dexamethasone decreased \( \alpha \)-subunit mRNA levels in \( \alpha \)TSH cells by approximately 2-fold, and this suppression was maintained even in the presence of forskolin, similar to the results seen in the placental system (23).

To determine whether the observed repression was the result of a direct effect on the \( \alpha \)-subunit gene, we performed experiments in the presence of the protein synthesis inhibitor cycloheximide. Although the \( \alpha \)-subunit-specific mRNA levels were uniformly lower in the presence of cycloheximide (the Northern blot required 10 times longer exposure than that seen in Fig. 7, where cycloheximide was not added), regulation by both dexamethasone and forskolin remained. Densitometric analysis of the effects of forskolin and dexamethasone on \( \alpha \)-subunit mRNA levels in the presence and absence of cycloheximide is presented in Fig. 8. The results are strikingly parallel, indicating that protein synthesis is not required for glucocorticoid repression. However, nuclear runoff experiments measuring relative transcription rates during steroid treatments will be necessary to show definitively that this is a transcriptional effect. Nevertheless, repression of the \( \alpha \)-subunit gene by glucocorticoids does occur in \( \alpha \)TSH cells, and this effect appears to be independent of protein synthesis.

Finally, previous studies have established that the MGH101A tumor is not subject to thyroid hormone regulation (9). To further verify that the \( \alpha \)TSH cell line reflects \( \alpha \)-subunit gene regulation of its parental MGH101A tumor, the cells were placed into hypothyroid calf serum for 48 h and then treated with thyroxin for an additional 48 h. No thyroid hormone regulation of \( \alpha \)-subunit mRNA was observed (densitometry of Northern blots from two experiments showed values of 1.004 and 0.875 relative to control), supporting the conclusion that the \( \alpha \)TSH cells retain the response characteristics of the original tumor.

**DISCUSSION**

We have established a cell line of glycoprotein hormone \( \alpha \)-subunit-producing cells derived from the murine thy-
rototropic tumor MGH101A previously propagated by
serial transplantation in mice. The first cell line of thy-
rotrope lineage, αTSH cells have been in continuous
culture for 3 yr. They can be maintained with relative
ease under tissue culture conditions and stored stably
in liquid nitrogen. The results presented here demon-
strate that the cells have retained a stable phenotype
similar to the tumor from which they were derived.
Thus, these cells are a convenient model system in
which to explore both tissue-specific and hormonal
control of the α-subunit gene.

Our results demonstrate that the murine α-subunit
gene is positively regulated by cAMP in αTSH cells.
That the human α-subunit gene is subject to transcrip-
tional induction by agents that increase intracellular
cAMP levels has been firmly established in placentally
derived cell culture systems (20, 22, 25, 26). However,
this is the first report that the mouse α-subunit gene in
pituitary cells also retains this property. In placental
cells, gene transfer experiments (20, 25–27) were used
to map this response to two direct repeats in the human
gene, termed cAMP-responsive elements or CREs,
which contain the eight-basepair consensus sequence
TGACGTCGA. This sequence binds a transcription factor
(CREB) whose transcriptional efficacy is increased upon
treatment with cAMP (28). Similar consensus sites,
which presumably bind either CREB (29, 30) or a related
protein (31), have been found to mediate cAMP responsi-
venseness in a number of other regulated genes (32).
Significantly, basal expression of the human gene in
placental cells is wholly dependent upon the CRE ele-
ments (25). In contrast, only one imperfect copy of this
element is present in the murine gene, and its relative
importance in pituitary-specific expression and cAMP
responsiveness remains to be determined (33). Perhaps
the relatively minor cAMP induction observed in these
cells is due to the lack of a high affinity binding site for
the CREB transcription factor. Gene transfer exper-
iments are ongoing to identify the precise elements
necessary for pituitary-specific expression and cAMP
induction of the mouse α-subunit gene in αTSH cells.

TRH is the dominant activator of TSH secretion in
the thyrotropic cells of the anterior pituitary (4, 5). In
addition, TRH induces α-subunit gene transcription in
rat primary pituitary cell cultures (3). The second mes-
senger system involved in mediating TRH action has
been difficult to identify; however, some evidence points
to the Ca2+-dependent protein kinase-C and phospha-
tidyl inositol pathways as TRH transducers (16–19).
Our results show that treatment of αTSH cells with the
phorbol ester TPA, a pharmacological agent that acti-
vates protein kinase-C, leads to an increase in α-subunit
mRNA levels. This induction is not potentiated by for-
skolin, an activator of the protein kinase-A pathway.
Synergism by these hormones has been observed us-
ing the human α-subunit gene in placental cells (21).
Similar to the results found with agents that increase
intracellular cAMP levels, the inductive effect of phorbol
esters on the human gene is at the level of transcrip-
tional initiation, suggesting that an element capable of
mediating this induction may exist in both the mouse
and human α-subunit gene transcriptional control re-
gions. A number of transcription factors have been
identified whose activities are responsive to phorbol
esters (34–36); whether any of these are responsible for
the effects of TPA on α-subunit genes remains to be
explored.

We have shown previously that the human α-subunit
gene is negatively regulated by glucocorticoids in the
placental choriocarcinoma cell line JEG-3, using gene
regulation (23). As presented here, steady state mRNA
levels of the mouse α-subunit are also negatively reg-
ulated by glucocorticoids in αTSH cells, further confir-
mig our findings in placental cells. That this effect oc-
curred through endogenous glucocorticoid receptor on
the genomic copy of the mouse α-subunit gene is es-
sentially significant, as cotransfection of the cloned
glucocorticoid receptor along with the controlling re-
gions of the α-subunit promoter was necessary to
achieve negative regulation in JEG-3 cells. In human
placental cells, we have proposed that repression by
glucocorticoids occurs through interference with the
CREs, mediated through potential glucocorticoid regu-
laratory elements located nearby and overlapping the
CREs. It will be of interest to determine whether these
sequences, which for the most part are conserved in
the mouse gene, are necessary for repression in αTSH
cells. In any case, the αTSH cells are a valuable re-
source in which to extend our investigation of the
mechanisms responsible for negative regulation by ste-
roid hormones in general and in relation to that ob-
served with the human α-subunit gene in JEG-3 cells.

While the thyrotropic tumors (TIT97 and MGH101)
must be passaged in hypothyroid mice, the MGH101A tumor line can be propagated in euthyroid animals. The loss of thyroid hormone regulation of both α-subunit gene expression and tumor growth presents the intriguing possibility that the secondary event responsible for these phenotypes may be a mutation or an inactivation of a thyroid hormone receptor, a receptor that has been identified as a potential oncogene (37–39). A study of thyroid hormone-binding sites in MGH101A tumor cells revealed approximately half the number of receptor-binding sites found in the thyroid hormone-responsive tumor line Tt797 (12). However, with the discovery of multiple genes encoding thyroid hormone receptors (40, 41) and multiple spliced forms of these receptors (42, 43), the significance of these binding sites remains unclear. In fact, Damm et al. (39) suggested that the thyroid hormone receptor may be a dominant negative oncogene, in that a mutant receptor compromised in its activation or hormone-binding properties, but not its DNA-binding character, may act as an antagonist of normal thyroid hormone action. The escape from thyroid hormone growth control might be through selection of cells present in the original tumor or through further genetic events, resulting in the acquisition of different cell growth characteristics. Study of thyroid hormone receptors present in the αTSH cells will be necessary to determine the role of these receptors, if any, in the oncogenesis of these cells.

It is clear that these cells are not fully differentiated thyrotrpes, since the TSH β-subunit gene is not expressed in either the MGH101A tumor or the αTSH cells (9). The original tumor, MGH101, had initially been characterized as synthesizing the TSH β-subunit, but subsequently lost this expression during multiple passages in mice (11). The β-subunit gene remains intact (12), eliminating the possibility that the gene is silent because of a chromosomal deletion. Furthermore, a murine βTSH gene cloned from MGH101A and used in transient transfection studies reveals excellent expression when introduced into the β-subunit expressing murine Tt797 cells (33, 44). It has been postulated, based on the immunostaining pattern of the anterior pituitary during development, that cells exist transiently that produce only the α-subunit protein without the β-subunit proteins (45). Thus, the αTSH cells may represent a precursor to the different cell types that express the α-subunit gene in combination with distinct β-subunit genes. In further support of this concept, we have established cell lines derived from pituitary tumors produced in transgenic mice that are the result of expression of the SV40 viral oncogene, T antigen, under the control of the human α-subunit gene promoter (46). These lines express the α-subunit gene, but, like the αTSH cells, they do not express β-subunit genes, suggesting that they may represent a developmental stage before differentiation and subsequent β-subunit gene expression. Alternatively, expression of the β-subunit gene may be linked to a different differentiation event; consequently, its expression may not be compatible with the continuing cellular replication that occurs in tumor cells. This is consistent with the frequent occurrence of human nonsecreting pituitary adenomas (13), which are often found to secrete only the α-subunit protein.

In any case, this thyrotropic cell line provides a valuable model system in which to study α-subunit gene expression in a homogeneous, nonendocrine population of pituitary cells.

**MATERIALS AND METHODS**

**Adaptation of the MGH101A Tumor to Cell Culture**

αTSH cells were derived from an MGH-101A tumor removed from an LAF1 mouse (Jackson Laboratory, Bar Harbor, ME). Procedures used to passage the tumor in mice and to disperse the tumor cells for short term culturing have been described previously (11). Approximately 2 × 10^6 cells were originally placed in culture as a suspension in Dulbecco’s Modified Eagle’s Medium (DME) plus 1-glutamine (Sigma, St. Louis, MO) with 15% fetal bovine serum (Gibco, Grand Island, NY), 0.1 mm nonessential amino acids (Sigma), 0.25 mg/ml fungizone (Gibco), 1000 U/ml penicillin, and 1 mg/ml streptomycin (Sigma). Cells were cultured subsequently without fungizone and were passed repeatedly over tissue culture plastic (Nunc, Copenhagen, Denmark) to remove fibroblasts and other adhesive nonendocrine cell types during a period of several weeks. Cells were then slowly adapted to adhere to tissue culture plastic by repeated removal of floating cells, again over a period of several weeks. Cells were judged to be of the original tumor type using visual inspection of their morphology relative to the dominant cell type present in the original suspension. Cells were plated when very dilute, and individual adherent colonies were picked from separate plates to create individual clonal cell lines 8 weeks after original culturing. The cells are passaged at a 1:3 dilution approximately every 5–7 days when maintained in DME with 4.5 mg/ml glucose (Sigma), 5% fetal bovine serum, 5% equine serum (Gibco), penicillin, and streptomycin. Aliquots of these cells can be stored frozen in liquid nitrogen with the addition of 8% dimethylsulfoxide (Pluck AG, Buchs, Switzerland) to the medium.

**Growth Rate Measurements**

Clone 3 cells were plated at 7.5 × 10^5 cells/8 cm plate into nine plates. Every 2 days, cells from each of three plates were trypsinized and counted using a hemocytometer, and values were averaged. This experiment was performed three times, and the values shown are the mean ± SEM.

**Hormone Treatment Paradigms**

The medium conditions used were as described above with the following exceptions. For steroid hormone experiments, the cells were placed into 5% charcoal-stripped fetal bovine serum (47) for 2 days before the 48-h 5 × 10^-7 M dexamethasone (Sigma) treatment. For experiments with forskolin (Calbiochem, La Jolla, CA), the drug was added to a final concentration of 10 μM 16–18 h before harvest. For TPA experiments, 100 nM TPA was added for an additional 16–18 h after the cells had been in 1% fetal bovine serum for 24 h. For experiments in which cycloheximide was used, 10 μg/ml cycloheximide were added to the cultures 2 days after the cells were placed in 5% charcoal stripped serum. Dexamethasone was added immediately after cycloheximide addition; forskolin was added 8 h later, and the cells were harvested after an additional 12–14 h.
Northern Analysis of RNA from α-TSH Cells

Total RNA was prepared as previously described (48). Northern analysis was performed as previously described (49). Between 5–15 μg total RNA were loaded per lane (the amount being consistent within any one experiment) and analyzed on a 1.5% agarose gel containing formaldehyde. The RNA was blotted onto nitrocellulose (Schleicher and Schuell, Keene, NH) and hybridized with a specific cDNA encoding mouse α-subunit (50) labeled with [α-32P]dCTP (ICN Radiochemicals, Irvine, CA) by nick-translation. Blots were hybridized overnight at 42°C in 50% formamide-0.9 M NaCl. They were washed in 5 mM NaCl, 0.1% sodium dodecyl sulfate at 60°C. A histone cDNA probe was used as an internal control (51) to visually confirm that the same amounts of RNA had been loaded in each lane.

α-Subunit RIA

α-subunit secretion from aTSH clone 3, GH3, and T4T 97 cells incubated in DME with 10% fetal calf serum was measured (9, 11). A double antibody RIA using specific antibodies for rat α-subunit was obtained from the National Pituitary Agency. Extracts of mouse anterior pituitary or purified rat α-subunit standards showed parallel displacement of labeled ovine α-subunit.

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REFERENCES


41. Hodin RA, Lazar MA, Wintman Bi, Darling DS, Koenig RJ, Larsen PR, Moore DD, Chin WW 1988 Identification of a thyroid hormone receptor that is pituitary specific. Science 244:76-79

42. Lazar MA, Hodin RA, Darling DS, Chin WW 1988 Identification of a rat c-erb-A-related protein which binds deoxyribonucleic acid but does not bind thyroid hormone. Mol Endocrinol 2:893-901

43. Izumo S, Mahdavi V 1988 Thyroid hormone receptor α isoforms generated by alternative splicing differentially activate myocine HC gene transcription. Nature 334:539-542


47. Dobner PR, Kawasaki ES, Yu LY, Bancroft FC 1981 Thyroid or glucocorticoid hormone induces pre-growth-hormone mRNA and its probable nuclear precursor in rat pituitary cells. Proc Natl Acad Sci USA 78:2230-2234


50. Chin WW, Kronenberg HM, Dee PC, Maloof F, Habener JF 1981 Nucleotide sequence of the mRNA encoding the pre-pro-subunit of mouse thyrotrtop. Proc Natl Acad Sci USA 78:5329-5333
