Exogenous expression of Pit-1 in AtT-20 corticotropic cells induces endogenous growth hormone gene transcription

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Abstract

The pituitary-specific POU-homeodomain transcription factor, Pit-1, is known to regulate the expression of the GH gene in somatotropes, prolactin (PRL) in lactotropes, and TSH in thyrotropes. It is not normally expressed in corticotropes or gonadotropes. We addressed the question of whether exogenous Pit-1 was sufficient to induce ectopic transcription of the GH gene in the corticotropic cell line, AtT-20, or the gonadotropic cell line, αT3-1. A fusion gene composed of enhanced green fluorescent protein gene and human Pit-1 cDNA was transfected into AtT-20 and αT3-1 cells. The endogenous mouse GH mRNA was induced in three of nine AtT-20 cell lines and one of three αT3-1 cell lines containing the fusion gene. A small amount of GH protein was also detected in these cell lines. These data indicate that transfected Pit-1 is capable of inducing transcription of the GH gene in AtT-20 cells and αT3-1 cells. These data also suggest that synergistic co-factors might be required to transcribe the GH gene effectively for translation into GH protein. Furthermore, our findings support the hypothesis that the function of anterior pituitary cells is determined by the combinatorial action of specific transcription factors.


Introduction

The primordium of the anterior pituitary gland first appears on embryonic day 9 in the mouse, as an invagination of the somatic ectoderm in the roof of the oral cavity that is known as Rathke’s pouch (Voss & Rosenfeld 1992). A series of proliferative and differentiative events leads to the mature anterior pituitary gland, which consists of five distinct cell types, each of which produces distinct pituitary hormones (Simmons et al. 1990). The anterior pituitary gland serves as a good model for investigating the molecular mechanisms of cell differentiation, as it contains distinct cell phenotypes that arise from a single progenitor cell type. Several transcription factors have been identified in the pituitary gland, and individual transcription factors have been reported to be active in all or a subset of cell lineages, in many cases during a specific period of development (Lefèvre et al. 1987, Nelson et al. 1988, Gage et al. 1996, Hermes et al. 1996, Poulin et al. 1997, Tremblay et al. 1998, Kawabe et al. 1999, Lancon et al. 1999, Luo et al. 1999). The pituitary cell types have been classified into three cell lineages (growth hormone (GH)–prolactin (PRL)–thyroid-stimulating hormone (TSH), pro-opiomelanocortin (POMC), and follicle-stimulating hormone (FSH)/luteinizing hormone (LH)), controlled by several specific combinations of transcription factors and co-factors (Gonzalez-Parra et al. 1996, Tuggle & Trenkle 1996, Tremblay et al. 1998, Nogami et al. 1999, Schauefele 1999, Ying et al. 1999).

Human pituitary adenomas are usually of the above three cell lineages with respect to functional differentiation, with some adenomas producing two or more hormones (Kovacs et al. 1996). These adenomas can be divided into monomorphic, bimorphic and plurimorphic (Thapar et al. 1995a,b). Monomorphic adenomas are defined as those that synthesize two or more hormones in the same cell. In bimorphic and plurimorphic adenomas, each hormone is produced by different cell populations, and almost all possible combinations of hormone productions involving GH and PRL, GH, PRL, glycoprotein hormone α subunit (αGSU), TSHβ or FSHβ have been observed (Felix et al. 1994, Furuhata et al. 1994, Gesel et al. 1994, Gil-del-Alamo et al. 1994, Bertholon-Gregoire et al. 1999). Although these
combinations of hormones correspond to the differentiation of the cell lineages, it has been reported that very occasional cases of bimorphic pituitary adenomas produce adrenocorticotropic hormone (ACTH) and GH in different cell populations (Arita et al. 1991, Kovacs et al. 1998). We have recently encountered a monomorphous pituitary adenoma that produces both ACTH and GH in the same cell. The co-expression of both ACTH and GH in this pituitary adenoma is considered to be particularly important, because these phenotypes correspond to two different cell lineages.

It is believed that particular unique combinations of transcription factors participate in differentiation of the individual pituitary cell lineages. For example, pituitary transcription factor-1 (Pit-1) is required for expression of GH, PRL and TSHβ in the somatotrope, lactotrope and thyrotrope lineages respectively. The current study was carried out by transfection of a Pit-1 gene into AtT-20 and αT3-1 cells to determine whether Pit-1 was sufficient to activate the endogenous GH, PRL or TSHβ genes not normally expressed in the corticotrope or gonadotrope lineages. These studies also serve to clarify the hypothesis that the function of pituitary cells (e.g. production of GH and ACTH in a monomorphous adenoma) is determined by the combination of several transcription factors – in particular, by the expression of Pit-1, which has a crucial role in GH expression.

Materials and Methods

**Animals**

C57B/6 mice (8 weeks old, 20–25 g) were obtained from Clea Japan (Tokyo, Japan) and maintained in a specific pathogen-free environment under standard housing conditions (20 ± 2 °C; lights on 0800–2000 h each day). Chow and water were available ad libitum. The mice were killed by decapitation under diethylether anesthesia.

**Cell culture**

AtT-20 cells (a mouse ACTH-secreting tumor cell line), AtT-20 cells transfected with human (h) Pit-1 complementary DNA (cDNA) (AtT/Pit-1) and MtT/S cells (a rat GH-secreting tumor cell line) (Inoue et al. 1990) were maintained in 25 cm² culture flasks in DMEM and Ham’s nutrient mixture F12 culture medium (Asahi Techno Glass Corp., Tokyo, Japan) supplemented with 10% normal horse serum (NHS) and 2·5% fetal bovine serum (FBS) under an atmosphere of 5% CO₂–95% air at 37 °C. αT3–1 cells (a mouse αGSU-secreting tumor cell line) (Alarid et al. 1996), COS–1 cells (a thymus monkey kidney cell line transformed with SV40 T antigen), and these two types of cells transfected with Pit-1 cDNA (αT3/Pit-1 and COS/Pit-1) were cultured in DMEM supplemented with 10% FBS.

**Reverse transcription–PCR analysis**

Five micrograms of each total RNA isolated using TRIzol reagent (Life Technologies Inc., NY, USA) were reverse-transcribed using a ‘Ready-To-Go’, T-Primed First-Strand Kit (Amersham Pharmacia Biotech Inc., NJ, USA) after treatment with DNase I (Promega Corp., WI, USA). An hGH-secreting adenoma was excised by transphenoidal surgery from a patient with symptoms of acromegaly in whom the serum GH concentration was 52·8 ng/ml. PCR was carried out using Takara r Taq polymerase (Takara Shuzo Co. Ltd, Shiga, Japan) according to the manufacturer’s instructions. The primer sequences and the annealing temperatures for PCR amplification are listed in Table 1 (Sarret et al. 1999, Huang et al. 2000).

**Generation of a chimera of the GFP and Pit-1 genes**

Human Pit-1 α cDNA was amplified by RT-PCR using the hPit-1 primers, 5'-TTT AAC CTA TAA CTG TGC CAA GCA GCA TTT ACT-3' and 5'-TTA TCT AGA TTT ATC TGC ACT TGC ACT CAA GAT GTT CCT-3'. The RT-PCR product was subcloned into pT7 plasmid (Novagew, WI, USA). After confirming that the insert originated from hPit-1 mRNA, the insert was transferred into the HindIII/BamHI site of pEGFP-C1 (Clontech Laboratories Inc., CA, USA). The structure of the pGFP–Pit-1 α plasmid was verified by DNA sequencing using pEGFP-C1 primer 5'-CAT GGT CCT GCT GGA GTT TTT ATT CTC-3' and hPit-1 primer 5'-TGC TTG ATT TCA GCA CCT GCT GT-3'. The pGFP–Pit-1 α plasmid was named GFP–Pit-1.

**Transfection and DNA-binding assays of the GFP–Pit-1 fusion protein**

Transcription-promoting activity was assayed using the luciferase reporter system using pGh–Luc, which carries the rat GH promoter region fused with the luciferase reporter gene (Niiori-Onishi et al. 1999). COS–1 cells that were 70% confluent were transfected with 1 µg/ml pGh–Luc or with 0·1 µg/ml pGFP–Pit-1 or with both plasmids, using 4·5 µg/ml DOTAP. The next day, luciferase activity was determined using the Luciferase Reporter Assay System (Promega Corp., WI, USA) according to the manufacturer’s instructions.

For the gel mobility shift assays, 8 µg of the extract of COS-1 cells transfected by the above method were reacted with a 32P-labelled oligonucleotide in a reaction buffer (Schaufele et al. 1990). For the super-shift assay, anti-Pit-1 antibody (Santa Cruz Biotechnology Inc., CA, USA) was added to the reaction buffer. After the reaction, the samples were separated on a 5% acrylamide gel. The mouse GH promoter probe for the gel mobility assay was comprised of 50 pg each of the following: 5'-GCC AGC

Stable transfection and establishment of transfected cell lines

AtT-20 and αT3-1 cells were transfected with 1 µg/ml pGFP–Pit-1 using 4·5 µg/ml DOTAP for 6 h, after exchanging the growth medium. These cells were selected by 500 µg/ml Geneticin 418 (G418) after culture for 3 days. Transiently transfected COS-1 cells were transfected as above, but were subjected to lysis 3 days after transfection, to make extracts for immunoblot and RT-PCR.

Confocal laser scanning microscopy

GFP in the AtT/Pit-1 cells was visualized using the 488 nm excitation line of an argon laser, using a confocal laser scanning microscopy (CLSM) system (LSM-410, Carl Zeiss, Jena, Germany). The optical tomographic images (Z images) were obtained throughout the thickness of the cell. Images were processed and analyzed using Photoshop software (Adobe System Inc., San Jose, CA, USA).

Immunoblotting

The 70%- confluent cells were collected by centrifugation (250 g for 5 min) and were suspended in 0·1% NP40 in Tris–EDTA (0·01 M Tris–HCl pH7·2, 1 mM EDTA) and mixed gently. This cell lysate was centrifuged at 10 000 g for 5 min, and separated into supernatant (the cytosol fraction) and precipitate (the nuclear fraction). Each sample was separated by 12·5% SDS-PAGE and transferred to a Hybond-C nitrocellulose membrane (Amersham Pharmacia Biotech Inc., NJ, USA). For detection of ACTH and POMC protein, the samples were separated by gradient gels. The membrane was treated with phosphate buffered saline (PBS)–Tween 20 (PBST; 0·01 M PBS, 0·1% Tween 20) containing 5% non-fat dried milk (milk–PBST) overnight, and subsequently with milk–PBST containing 2% NHS, for 15 min at room temperature. The membrane was incubated with anti-GFP antibody (clone 1E4) (Medical & Biological Laboratories Co. Ltd, Aichi, Japan), anti-Pit-1 antibody or anti-ACTH antibody (Dako Japan Co., Kyoto, Japan) for 1 h at room temperature. The membrane was incubated in milk–PBST with anti-rabbit Ig antibody or anti-mouse Ig antibody for 30 min at room temperature. The membrane for the detection of GH protein was incubated with biotinylated anti-rGH polyclonal antibody, supplied by The National Hormone and Pituitary Program. The antibody was biotinylated as follows: 1 mg/ml of antibody dissolved in 0·1 M NaHCO3 was incubated with 0·1 mg/ml NHS–biotin (Pierce Chemical Company, IL, USA) in the dark for 4 h at room temperature and dialyzed with PBS at 4 °C for 3 days. The

Table 1 The sequences of primers used in RT-PCR analysis

<table>
<thead>
<tr>
<th>Target</th>
<th>Sequence (5’ to 3’)</th>
<th>Annealing temp (°C)</th>
<th>Product size (bp)</th>
</tr>
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<tr>
<td>mGH</td>
<td>TCAGAGTCTCTGTGGACAGATC</td>
<td>58</td>
<td></td>
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<tr>
<td>mPRL</td>
<td>CTCAGCGCATTTCGAGGAAGAGG</td>
<td>58</td>
<td>354</td>
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<tr>
<td>mTSH</td>
<td>GCAGAACTTTCCTCTCTCCA</td>
<td>58</td>
<td>224</td>
</tr>
<tr>
<td>Monkey and</td>
<td>TCGAGTCCCTCTAGAGTCTG</td>
<td>60</td>
<td>228</td>
</tr>
<tr>
<td>human GH</td>
<td>AAGCAGATAGCAGCCCCGTA</td>
<td>60</td>
<td></td>
</tr>
<tr>
<td>mPit-1</td>
<td>CACGGCTCAGAATCTAGCTCA</td>
<td>56</td>
<td>209</td>
</tr>
<tr>
<td>hPit-1</td>
<td>ACGCCTGTCAGTTCACA</td>
<td>56</td>
<td>304</td>
</tr>
<tr>
<td>mPtx1</td>
<td>CCGTGAACGTGTAGAGGGA</td>
<td>58</td>
<td>298</td>
</tr>
<tr>
<td>Human and mouse</td>
<td>CATCAATGGCAGACTTCTCCTTC</td>
<td>58</td>
<td>284</td>
</tr>
<tr>
<td>NeuroD1**</td>
<td>CATCAATGGCAGACTTCTCCTTC</td>
<td>58</td>
<td></td>
</tr>
<tr>
<td>Rat and mouse</td>
<td>TGGCACCAACCACCTTTCA</td>
<td>58</td>
<td>105</td>
</tr>
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</table>

*Sarret et al. 1999, **Huang et al. 2000.
membrane for the detection of GH protein was incubated in avidin–biotin–peroxidase complex (Vector Laboratories, CA, USA). The immunoreactive bands were developed with the enhanced chemiluminescence system (Amersham Pharmacia Biotech Inc., NJ, USA).

Immunocytochemistry

Cells were cultured until approximately 70% confluent in 8-well permanox slides, fixed in 10% formaldehyde in PBS for 20 min at room temperature, and pre-incubated in PBS containing 1% BSA and 0.1% Tween 20 at room temperature for 8 h. Endogenous biotin or biotin-binding proteins were inhibited with a Blocking Kit (Vector Laboratories, CA, USA). After the inhibition, the biotinylated anti-rGH antibody (0.02 µg/ml) was left to react at 4°C overnight. GH protein was detected using the Catalyzed Signal Amplification (CSA) System (Dako Japan Co., Kyoto, Japan) according to the manufacturer’s instructions.

Results

Characterization of AtT-20 cells by RT-PCR analysis

RT-PCR analysis was performed on wild-type AtT-20 cells to characterize them before transfection with the GFP–Pit-1 gene. GH mRNA was not expressed in the AtT-20 cell line used in our study (Fig. 1a), although it has been reported that AtT-20 cells express endogenous GH mRNA (Sarret et al. 1999). Furthermore, the expressions of mouse (m) Pit-1 and pituitary homeobox 1 (mPtx1) mRNAs, key factors in the transcription of the GH gene, were also examined. The results showed that mPit-1 mRNA was not detected, but mPtx1 mRNA was detected, in AtT-20 cells. Both mPit-1 and mPtx1 mRNAs were detected in mouse pituitary glands (Fig. 1b). These results suggested that AtT-20 cells do not transcribe the GH gene, and could serve as a model for studying the induction of this gene by transfection of the Pit-1 gene.

Construction and characterization of a GFP–Pit-1 fusion protein

We established a GFP–Pit-1 fusion gene analogous to one previously published (Day 1998) (Fig. 2a). Luciferase activity was increased 22.1-fold when the pGFP–Pit-1 and pGH–Luc plasmids were co-transfected compared with when only the pGH–Luc reporter gene was transfected (Fig. 2b). The binding of the GFP–Pit-1 fusion protein to the Pit-1 binding site of the mouse GH promoter was confirmed by a gel mobility shift assay and an antibody super-shift assay (Fig. 2c). A specific band was produced by inclusion of COS-1 cell extract that had been transfected with the GFP–Pit-1 fusion gene in the reaction (Fig. 2c, lane 4). This band was super-shifted when anti-Pit-1 antibody was incubated with the COS-1 cell extract transfected with the GFP–Pit-1 fusion protein (Fig. 2c, lane 5).

Visualization of GFP fluorescence in transfected cell lines

Nine lines of AtT-20 cells and three lines of αT3-1 cells were established and chosen by observation of the fluorescence of GFP. First, we confirmed that the morphology of AtT/Pit-1 cells and αT3/Pit-1 cells did not change compared with that of wild-type AtT-20 cells (Fig. 3a–c) and wild-type αT3-1 cells (Fig. 3e–g). Among the nine AtT/Pit-1 cell lines, three clones exhibited strong fluorescent signals of GFP, and among the three αT3/Pit-1 clones, one also exhibited strong fluorescent signals. CLSM revealed that almost all of the fluorescent signal was accumulated in the nuclei of the AtT/Pit-1-4 cells, the
clone expressing the highest level of fluorescence among the nine clones obtained (Fig. 3d). In addition, the morphology of COS/Pit-1 cells did not change after the transfection and the fluorescent signal of GFP was observed in the nuclei (Fig. 3h–j).

Expression of hPit-1 in cell lines

RT-PCR demonstrated that all cell lines transfected with the hPit-1 gene express hPit-1 mRNA (Fig. 4a). The nucleotide sequence of the RT-PCR product was identical to that of hPit-1 cDNA. Furthermore, immunoblot analysis of AtT/Pit-1 and αT3/Pit-1 cell extracts indicated that the cell lines exhibiting strong fluorescent signals also exhibited immunoreactive bands for GFP and Pit-1. The results in AtT/Pit-1-4, αT3/Pit-1-2 and COS/Pit-1 cells (Fig. 4b,c) revealed that both anti-GFP antibody and anti-Pit-1 antibody reacted with protein bands at 57 kDa in the nuclear fraction. No immunoreactive band for them was observed in non-transfected AtT-20, αT3-1 or COS-1 cell extracts. The 57 kDa band had the molecular weight predicted for the GFP–Pit-1 fusion protein on the basis of the amino acid sequence.

Characterization of AtT/Pit-1 cells

Immunoblot analysis for ACTH and RT-PCR analysis for mPtx1 and mNeuroD1 (a transcription factor for POMC production) were performed in AtT/Pit-1 cells to ascertain whether other properties of AtT-20 cells were changed by expression of transfected GFP–Pit-1. The 26-kDa and 28-kDa immunoreactive bands indicated the presence of POMC expression in AtT-20, AtT/Pit-1 and mouse pituitary glands (Fig. 5a). mPtx1 and mNeuroD1 mRNAs were also detected in them (Fig. 5b,c). These data revealed that POMC production by AtT-20 cells was not changed by the expression of GFP–Pit-1.

Expression of GH

RT-PCR analysis was performed to examine whether AtT/Pit-1 cells produce GH, PRL and TSHβ mRNAs. As shown in Figure 6A, gel (a), we detected GH mRNA in the three AtT/Pit-1 cell lines in which GFP–Pit-1 fusion protein was most highly expressed. Neither PRL nor TSHβ mRNA was detected in this experiment (Fig. 6A, gels (b) and (c)). The nucleotide sequence of the PCR product for GH confirmed that the sequence was identical to that of mGH cDNA (data not shown). In addition, the induction of endogenous mGH mRNA was examined in αT3/Pit-1 and COS/Pit-1 cells. GH mRNA was induced in αT3/Pit-1-2 cells, but not in COS/Pit-1 cells (Fig. 6A, gels (d) and (e)).

Although no immunoreactivity for mGH was detected in AtT-20 cells (Fig. 6B, panel a), immunoreactivity was detected in the cytosol of AtT/Pit-1 cells and MtT/S cells close to the nucleus using the CSA system (Fig. 6B, panels b and e), and it was not detected in MtT/S cells by biotinylated normal monkey Ig (Fig. 6B, panel d). The immunoreactivity disappeared when the monkey anti-rGH antibody was absorbed with rat GH protein (Fig. 6B, panels c and f).

The immunoreactive band for GH was detected at 22 kDa in AtT/Pit-1 cell extracts in addition to that for MtT/S cells (Fig. 6C). In this analysis, 100 µg samples were applied in each lane, except for the sample of mouse pituitary which was 0.2 µg.
Discussion

The production of specific hormones in the individual cell types of the anterior pituitary gland is under the control of several transcription factors (Lefevre et al. 1987, Nelson et al. 1988, Gage et al. 1996, Hermesz et al. 1996, Poulin et al. 1997, Tremblay et al. 1998, Lanctot et al. 1999, Luo et al. 1999, Kawabe et al. 1999). Pit-1 is known to be a key factor in functional differentiation, promoting the production of GH, PRL and TSH in mice, rats and humans (Mangalam et al. 1989, Ingraham et al. 1990, Simmons et al. 1990). Our previous studies of human pituitary adenomas also suggested a role for Pit-1 in the identification of pituitary adenoma tumor cells as part of the cell lineage that produces GH, PRL and TSH (Sanno et al. 1994, Lloyd & Osamura 1997). In addition to the studies in human pituitary adenomas, many studies in normal pituitary glands in developmental or physiological conditions have indicated that GH is co-localized with other pituitary hormones (Horvath et al. 1990, Childs et al. 2000). Furthermore, the co-localization of LHβ/FSHβ and Pit-1 protein has been also reported (Vidal et al. 1998). The co-localization of GH and ACTH is rarely reported, even in human pituitary adenomas; however, we have encountered a case of human ACTH-secreting pituitary adenoma with ACTH and GH in the same cells in which Pit-1 was expressed, in addition to NeuroD1 and Ptx1. The current study aimed to analyze whether transfected Pit-1 was sufficient to induce the transcription of the endogenous mouse GH gene in cell lines representing cell lineages that normally do not express GH: the AtT-20 corticotrope and the αT3-1 gonadotrope cell lines.
In the present study, nine AtT/Pit-1 and three αT3/Pit-1 cell lines were cloned by observation of the fluorescent signal of GFP (Gerdes & Kaether 1996, Ludin et al. 1996), and all these cell lines expressed Pit-1 mRNA. In those lines expressing the protein, most cells exhibited fluorescence from GFP primarily in the nuclei. The GFP–Pit-1 fusion protein was accumulated in the nuclei of AtT/Pit-1-4 cells, which exhibited strong GFP signals; it was also synthesized in αT3/Pit-1-2 and transiently transfected COS-1 cells. In addition, the data for characterization of AtT/Pit-1 cells indicate that the exogenous GFP–Pit-1 gene transfected into the cells was expressed and localized in the nucleus without affecting the nature of cells.

We did detect the activation of endogenous mouse GH mRNA in three of nine AtT/Pit-1 lines and one of three αT3/Pit-1 cell lines. The fact that GH mRNA was expressed in these cell lines with detectable GFP fluorescent signals indicated that high expression of Pit-1 could induce the transcription of the endogenous GH gene. Our

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successful detection of GH mRNA and GH protein in AtT/Pit-1 cells was achieved by highly sensitive methods, RT-PCR and CSA systems. Transient transfection of the GFP–Pit-1 gene into COS-1 cells established from monkey kidney was insufficient to induce GH mRNA. These results suggested that the transcription of GH could only be induced by expression of exogenous Pit-1 in pituitary-derived cells such as AtT-20 and αT3-1 cells expressing Ptx1 and Lim3, essential important transcription factors in the development of the anterior pituitary (Sheng et al. 1996, Kurotani et al. 1999, Lanctot et al. 1999), although they do not express Pit-1.

It has been reported that the transcription of the GH gene is regulated by hormones such as thyroid hormone (Palomino et al. 1998), glucocorticoids, and GHRH (Gonzalez-Parra et al. 1996, Tuggle & Trenkle 1996, Nogami et al. 1999), and that interleukin-1β and tumor necrosis factor-α increase GH and GH mRNA in ovine pituitary cells (Nash et al. 1992, Fry et al. 1998). Therefore, our results suggest that other synergistic factors, such as hormones or cytokines, may be necessary to induce transcription of the GH gene more effectively, although no hormonal or cytokine receptors have thus far been identified in AtT-20 cells. In addition, there remains a need for analysis of the possible interaction of other transcription factors, GATA factors, Zn-15 and C/EBP, in the regulation of GH transcription (Dasen et al. 1999, Cohen 2000). In contrast, although neither PRL nor TSHβ mRNA was detected in any of the samples examined, it has been reported that Pit-1 functions synergistically with the estrogen receptor to regulate the transcription of the PRL gene (Schaufele 1999, Ying et al. 1999), and TSHβ promoter activity is increased by Pit-1T, which is an alternately spliced variant of Pit-1 (Haugen et al. 1994). However, the details of the interactions of the transcription factors and their target genes remain to be further investigated.

Finally, these data may provide insight into the molecular mechanism by which both ACTH and GH, which are normally expressed in different cell lineages, were produced in the same cells in a monomorphous human ACTH–secreting adenoma (Arita et al. 1991, Kovacs et al. 1998, Tahara et al. 2000).

In summary, hormone-producing anterior pituitary cells have been classified into three developmental lineages, GH–PRL–TSH, POMC, and FSH/LH (Simmons et al. 1990, Poulin et al. 1997, Tremblay et al. 1998). In the present study, we have demonstrated that exogenous transfection of Pit-1 is sufficient to induce the transcription of the GH gene in AtT-20 cells and αT3-1 cells. Our results support the hypothesis that the function of pituitary cells is determined by the combination of several transcription factors and co-factors, and can be altered by the introduction of one not normally present. Furthermore, our results indicate the possibility that differentiated pituitary cells can redirect their fate and newly differentiate to alternative cell lineages.

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