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Pituitary ontogeny of the Snell dwarf mouse reveals Pit-1-independent and Pit-1-dependent origins of the thyrotrope

Sheng-Cai Lin[1,*], Sen Li[1,2,*], Daniel W. Drolet[1,3] and Michael G. Rosenfeld[1,†]

[1]Howard Hughes Medical Institute, Eukaryotic Regulatory Biology Program, [2]Graduate Program in Chemistry and [3]Biomedical Sciences Graduate Program, School and Department of Medicine, University of California, San Diego, 9500 Gilman Drive, C. M. M., Rm. 345, La Jolla, California 92093-0648, USA

*The contributions of S. Lin and S. Li to this manuscript should be considered equivalent
†Author for correspondence

SUMMARY

The anterior pituitary provides a model to study the molecular mechanisms responsible for emergence of distinct cell types within an organ. Dwarf mice (Snell) that express a mutant form of the tissue-specific POU-domain transcription factor Pit-1 fail to generate three cell types, including the thyrotrope (S. Li, E. B. Crenshaw, E. J. Rawson, D. S. Simmons, L. Swanson and M. G. Rosenfeld (1990), *Nature* 347, 528-533). Analyses of wild-type and Pit-1-defective mice, presented here, have revealed that thyrotropes unexpectedly arise from two independent cell populations. The first population is Pit-1-independent and appears on e12 in the rostral tip of the developing gland, but phenotypically disappears by the day of birth. The second is Pit-1-dependent and arises subsequently in the caudomedial portion of the developing gland (e15.5), following the initial expression of Pit-1 in this region. The failure of caudomedial thyrotrope cells to appear in the Snell dwarf, and the observation that Pit-1 can bind to and transactivate the TSHβ promoter, apparently enhanced by its phosphorylation, suggests that Pit-1 is directly required for the appearance of this distinct population that serves as the precursors of the mature thyrotrope cell type. These data suggest that different molecular mechanisms, based on the actions of distinct transcription factors, can serve to independently generate a specific cell phenotype during mammalian organogenesis.

Key words: pituitary, Pit-1, thyrotrope cell lineage, Snell dwarf mouse, mouse

INTRODUCTION

During mammalian anterior pituitary ontogeny five distinct cell types arise in a precise temporal and spatial fashion from a common primordium (Schwind, 1928; Begeot et al., 1982; Carbajo-Perez and Watanabe, 1990; Dubois and Hemming, 1991; Simmons et al., 1990). The five cell types in the mature anterior pituitary gland are defined by the trophic factors that they synthesize and secrete. Corticotropes produce adrenocorticotrophin (ACTH) by proteolytic processing of proopiomelanocortin (POMC), regulating adrenal cortex production of glucocorticoids; thyrotropes synthesize thyroid-stimulating hormone (TSH), which regulates thyroid gland growth and hormone production; gonadotropes produce luteinizing hormone (LH) and follicle-stimulating hormone (FSH), regulating gonadal function; somatotropes produce growth hormone (GH), regulating linear growth; and lactotropes synthesize prolactin (Prl), which regulates milk production. TSH, LH, and FSH are heterodimeric glycoproteins consisting of a common α subunit (αGSU) and distinct β subunits. Individual cell types arise within restricted regions of the developing gland (Simmons et al., 1990; Dubois and Hemming, 1991), a pattern conserved throughout vertebrate development. In the mature animal, the five mature cell types are considerably intermingled throughout the anterior pituitary, apparently reflecting a loss of homophilic interactions between cell types.

The first cell type to appear both in vivo and in organ culture is the corticotrope, in which differentiation takes place very early and largely independent of influences outside the organ (Bégeot et al., 1982). In contrast, appearance of gonadotropes, somatotropes and lactotropes in organ culture requires the addition of hormones; for example, the hypothalamic regulator, gonadotropin releasing hormone (GnRH) results in the differentiation of gonadotropes and, by mediating release of αGSU from gonadotropes, the appearance of prolactin-expressing lactotropes (Bégeot et al., 1984). Recently, the elucidation of the molecular basis of the *little* mouse phenotype (Eicher and Beamer, 1980; Lin et al., 1993) suggests that somatotropes are regulated by growth hormone releasing factor (GRF), initially described as a releasing factor for growth hormone (Guillemin et al., 1982; Rivier et al., 1982). Detailed analysis has revealed an unexpected stratification of two somatotrope cell populations, only one of which is dependent on GRF for proliferation.

Both the growth hormone and prolactin genes are activated by a pituitary-specific POU domain transcription factor that we

refer to as Pit-1 (Ingraham et al., 1988, 1990; Fox et al., 1990; Lin et al., 1992), that is ultimately present in somatotropes, lactotropes and thyrotropes (Simmons et al., 1990), based on the synergistic interactions between independent Pit-1-containing regions. Pit-1 is expressed in three of the anterior pituitary cell types (thyrotropes, somatotropes and lactotropes; Simmons et al., 1990). Consistent with Pit-1 regulating the prolactin and growth hormone genes, the onset of expression of Pit-1 protein on e15.5 (Simmons et al., 1990; Dollé et al., 1990) correlates closely with initial expression of the prolactin and growth hormone genes. Mouse genetics has provided direct evidence for the developmental role of this tissue-specific transcription factor. A variety of developmental mutants have been identified by their dwarf phenotypes, including the Snell, Jackson and Ames dwarf mutants, that contain no lactotropes, somatotropes or thyrotropes (Slabaugh et al., 1981) and have markedly hypoplastic anterior pituitary glands. The Pit-1 genomic locus is localized to a region on chromosome 16 that harbors the allelic genetic defect in the Jackson and Snell dwarfs (Li et al., 1990; Camper et al., 1990), and it has been demonstrated that both dwarf phenotypes result from mutations of the *Pit-1* gene (Li et al., 1990). The Jackson mutation is a rearrangement, whereas the Snell phenotype is a mis-sense mutation in the Pit-1 POU homeodomain. This crucial residue in the third (or DNA recognition) helix is conserved among all homeodomain proteins. The mutation impairs the ability of Pit-1 to bind to its DNA recognition elements. The hypoplastic dwarf pituitary gland, containing only gonadotropes and corticotropes, reveals that Pit-1 is important for proliferation (and/or survival) of these three cell types.

During ontogeny, detectable expression of Pit-1 protein in the caudomedial portion of the developing anterior pituitary gland temporally precedes the initial appearance of growth hormone and prolactin transcripts (Simmons et al., 1990); however, the initial appearance of the thyrotrope phenotype in the rostral tip of the normally developing rat pituitary gland, as determined by the expression of the β-subunit of thyroid stimulating hormone (TSHβ), has been found to precede by 36 hours the first detectable expression of Pit-1 (Simmons et al., 1990).

The disappearance of TSHβ gene expression in the adult dwarf animal (Li et al., 1990) and the initial detection of TSHβ mRNA on embryonic day 12 (e12), prior to the detection of Pit-1 transcripts, raises provocative questions concerning the requirement for Pit-1 for the persistence of thyrotropes in the mature gland. One model considered was that the TSHβ gene was activated by a level of Pit-1 that was below the threshold of detection. A second possibility was that thyrotropes arose in a Pit-1-independent fashion, but that for the thyrotrope to persist, Pit-1 was essential later in development. These two possibilities were investigated by examining the ontogenic expression of TSHβ in Pit-1-defective Snell dwarf mice. In this manuscript, we demonstrate that pituitary thyrotrophs arise from two independent origins, only one of which is Pit-1-dependent, and serves as the precursor of the mature thyrotroph, revealing that distinct molecular mechanisms generate a specific cell phenotype during organogenesis.

MATERIALS AND METHODS

Mice and genotyping

Snell mice were maintained by breeding heterozygotes; their genotyping was achieved by taking advantage of the point mutation (from

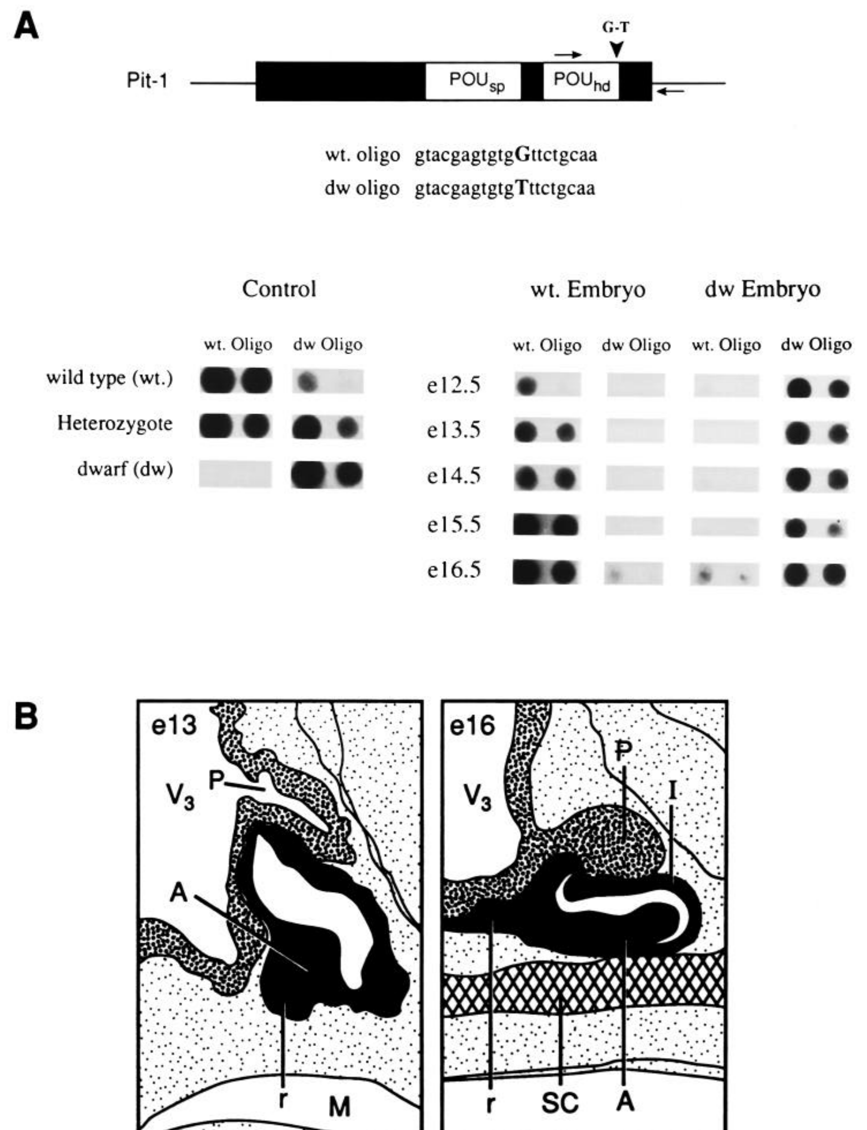

Fig. 1. Identification of Snell dwarf mice during fetal development. (A) Schematic map and oligonucleotide sequences of the wild-type (wt) and dwarf (dw) Pit-1 gene. The Snell G→T mutation and oligonucleotide positions for PCR are indicted by arrows. Representative results of dot blotting of PCR amplified genomic DNA (see Material and Methods) hybridized against each probe for discernible mutant (dw) or wild-type (wt) adult mice, as well as embryos. (B) Schematic diagram of e13 and e16 pituitary; r, Rostral tip; A, Anterior pituitary; P, Posterior lobes; I, intermediate lobes: V3, third ventricle, SC, Sphenoid cartilage.

G→T) in the Pit-1 gene, as follows. Genomic DNA was extracted and amplified by polymerase chain reactions using two oligonucleotides, 5′-CGC AGG AGA TCA TGC GGA TGG CTG AAG AA-3′ and 5′-CAC ACA TGG CTA TCA CAC GCC AGC-3′, flanking the point mutation. The amplified DNA (180 bp) was denatured, blotted onto nitrocellulose filters, and hybridized separately with a ^{32}P-labelled wild-type, 5′-GTA CGA GTG TGG TTC TGC AA-3′, and a mutant oligonucleotide, 5′ GTA CGA GTG TGT TTC TGC AA-3′ (Li et al., 1990). Filters were then washed 30 minutes with 3× SSC at 48°C and then with a solution containing 3 M $(CH_3)_3NCl$, 50 mM Tris-HCl, pH 8.0, 2 mM EDTA and 0.1% SDS for 30 minutes at 62°C for the wild-type probe and 60°C for the mutant probe. This differential hybridization yielded discriminatory signals for *wt/wt*, *wt/dw* and *dw/dw* mice.

Embryo fixation and in situ hybridization

Embryos of various gestation days, and neonatal mice, were obtained by breeding heterozygous Snell mice, and DNA was extracted from their tail tissues for genotyping as described above. Embryo heads were fixed in 4% formaldehyde for a minimum of overnight, and sectioned at 10 µm in a sagittal manner. The tissue sections were hybridized to ^{35}S-labelled cRNA probes, as previously described (Simmons et al., 1990). After exposure to emulsion, development and fixation, tissue sections were stained with bis-bezimide. Photomicroscopy was performed with both ultraviolet and visible light sources to yield blue tissue staining and silver signal grains.

DNA footprinting assays

DNaseI protection assays were performed precisely as previously described (Mangalam et al., 1989), except that the TSHβ promoter fragments (+27 to −229) were generated using a polymerase chain reaction strategy in which one of the synthetic oligonucleotides was radiolabelled using T4 kinase (Bethesda Research Laboratory) and [γ-^{32}P]ATP (New England Nuclear). The sequence of the two input oligonucleotides were 5′-CCT TAC TTT GCA TGA GTG AGT TCA-3′ and 5′-TTC AAA TAG AAG AGA GGA AGA TGC-3′.

Reporter fusion genes and transient transfection assays

The TSHβ/prolactin luciferase fusion gene was constructed by inserting an annealed double-stranded synthetic fragment into *Bam*HI site of the P36 vector containing the minimal prolactin promoter. The sense strand is 5′-gatcc TAT GAA TTT TCA ATA GAT GCT TTT CAG ATA AGA AAG CAG CAA TTC GAA TGC AAT TAT ATA AACA-3′. The mutant fragment is 5′-gatcc TAT GGA CGT GCA ATA GGC GCC GTG CAG ATA AGA AAG CAG CAA TCC GAA GCC CGT GAT AGC AACA-3′. The fusion reporter genes were co-transfected into CV-1 and HeLa cells with various Pit-1 constructs under the CMV promoter by the calcium phosphate precipitation method as previously described (Ingraham et al., 1988). After 48 hours, cells were lysed and luciferase activities were measured. Each transfection was repeated at least five times and results are presented as ±s.e.m.

RESULTS

Initial TSHβ expression in Snell mice

In order to genotype embryos derived from *dw* heterozygote matings, differential hybridization was employed using oligonucleotides corresponding to the mutant and wild-type sequences complementary to the region encompassing the G→T transversion in the Snell Pit-1 gene (Li et al., 1990). Genomic DNA was amplified by polymerase chain reaction and hybridizations were performed using tetrmethyl ammonium chloride in the washing buffer to provide a clear discriminatory signal (Fig. 1). The wild-type-specific oligonucleotide probe hybridized with amplified fragment from wild-type as well as heterozygote DNA very effectively, but did not hybridize with the fragment amplified from homozygote Snell DNA. In contrast, the *dw*-specific oligonucleotide only

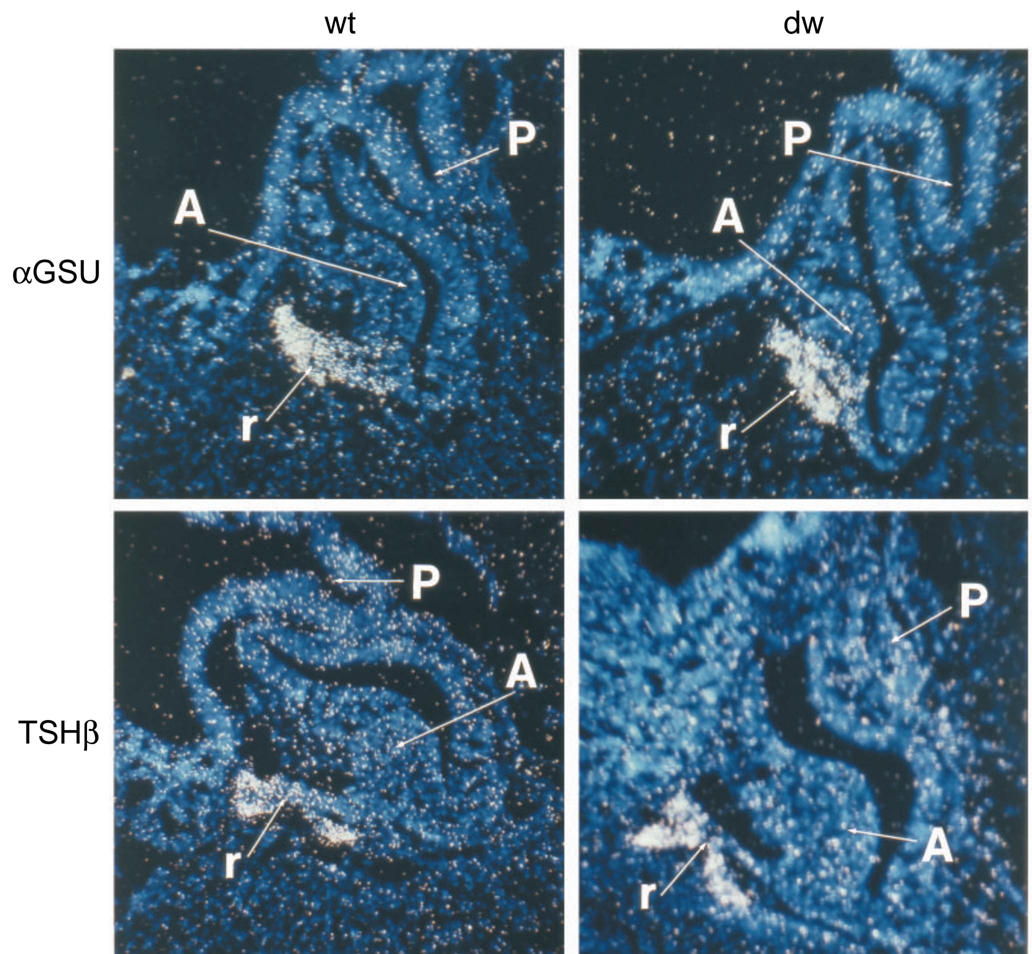

Fig. 2. Activation of TSHβ gene expression at the rostral tip in Snell pituitary development. Analysis of e12.5 pituitaries revealed that TSHβ gene expression was activated to similar levels at the rostral tip of wild-type (wt) and Snell (dw) pituitaries, bottom left and right panels, respectively. At this stage of development, αGSU is also expressed in both wild-type and dwarf pituitaries, top panels. r, rostral tip; P, posterior lobe of the pituitary; A, anterior lobe of the pituitary.

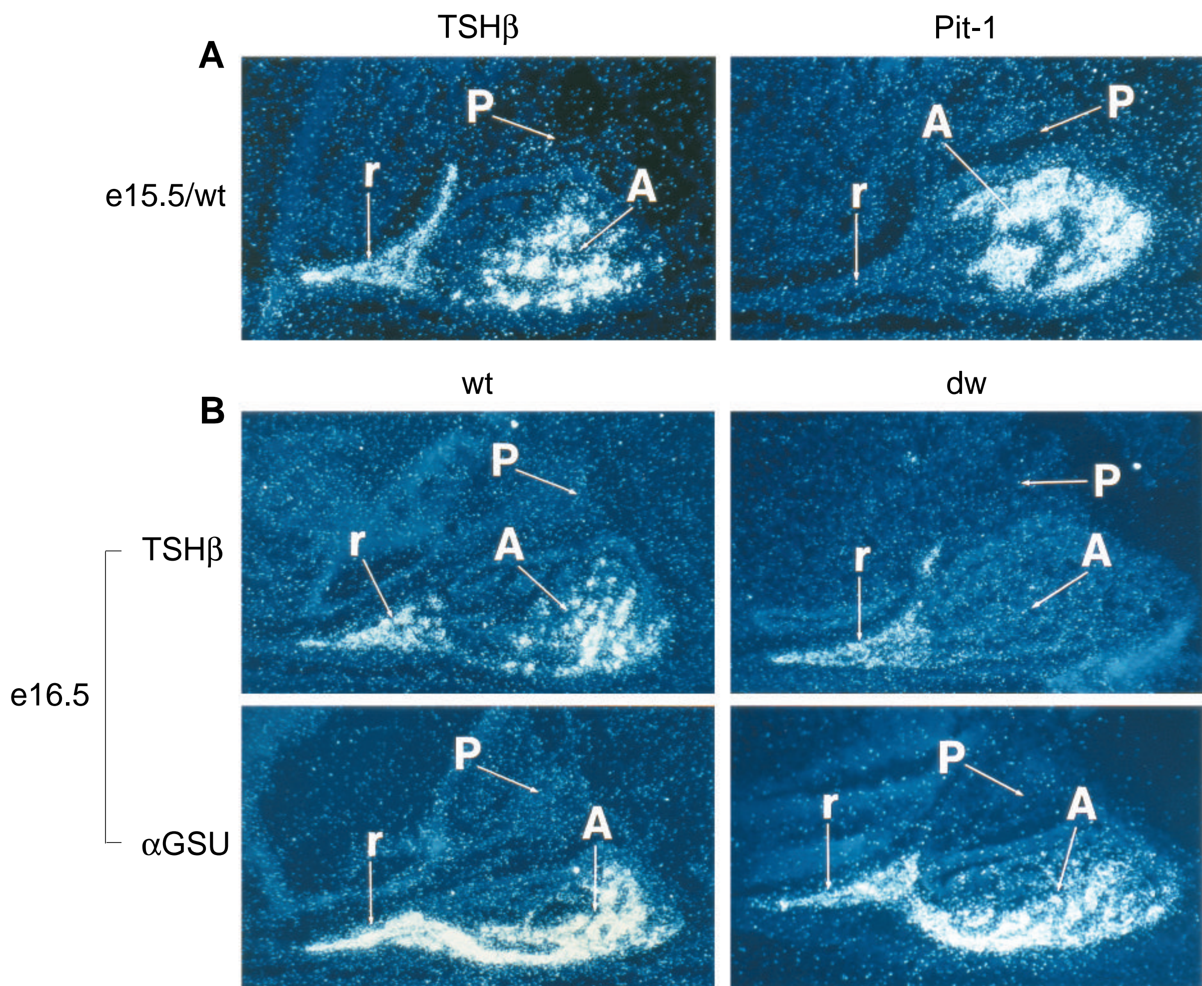

Fig. 3. Activation of TSHβ expression in the caudomedial anterior pituitary on e15.5 and e16.5. TSHβ gene expression was evaluated on e15-e17 in wild-type and *dw/dw* embryos, representative examples of which are shown. On e15.5 and e16.5 an intense signal was observed using TSHβ cRNA probe in the caudomedial portion of the anterior pituitary in wild-type mice (A,B). No signal was observed in homozygote Snell mice, while the expression at the rostral tip in the Snell mice remained similar to that in wild type. Pit-1 gene expression was activated on e14.5-e15.5 in the caudomedial region and was never detected in the rostral tip (A, right panel). Although, there was no TSHβ expression in the caudomedial region of the Snell dwarf pituitary, the expression of aGSU in Snell pituitaries was comparable with that in wild type, (B). r, rostral tip; P, posterior lobe of the pituitary; A, anterior lobe of the pituitary.

hybridized strongly with DNA amplified from the homozygote and heterozygote *dw*, but not wild-type individuals (Fig. 1).

Because TSHβ transcripts were initially detected on e14 in the rat (Simmons et al., 1990), ontogeny of TSHβ gene expression in the mouse was examined beginning on e12.5. For each day of development, three to four wild-type and *dw* embryos were examined. Wild-type and homozygote Snell dwarf embryos from e12.5 and e13.5 were fixed, and multiple sections were collected from each embryo and hybridized using ^{35}S-UTP-labelled TSHβ-specific cRNA probes corresponding to the TSHβ coding region, or with sense strand controls. The α-glycoprotein subunit of TSH (αGSU) antisense probe was utilized as a positive control. Both homozygous mutant and wild-type mouse embryos exhibited similar patterns and levels of αGSU expression on e12 to e14 (Fig. 2). On e12.5, two of four homozygote *dw* embryos and one of the four wild-type embryos exhibited positive hybridization signals for TSHβ mRNA in the rostral tip of the developing gland. By e13.5, three of three *dw* homozygote and two of three wild-type embryos were positive for TSHβ gene expression, again localized to rostral tip cells. The wild-type embryo that failed to give a positive TSHβ signal was significantly smaller than the others of its age group, consistent with the normal heterogeneity and delay of development of some individuals within a litter. Thus, activation of TSHβ gene expression in the rostral tip of the pituitary was comparable between homozygous Pit-1-defective *dw* and wild-type embryos.

A second Pit-1-dependent region of pituitary thyrotropes

TSHβ gene expression patterns between Snell mutant and wild-type embryos was next systematically examined between e14.5 and e18.5 in wild-type and *dw* mice. An example of the results on e15.5 and e16.5 are presented in Fig. 3. While the initial activation of TSHβ in the rostral tip cells (e12) preceded *pit-1* gene activation, another distinct population of caudomedial cells of e15.5 wild-type pituitaries exhibited TSHβ gene

expression one day after initial detection of *pit-1* gene expression. *Pit-1* transcripts were detected only in the caudomedial portion of the anterior pituitary gland in the region in which initial activation of the growth hormone and prolactin genes also occurred (Fig. 3), but *pit-1* transcripts were never detected in the rostral tip regions in which the initial population of thyrotropes appeared. In *dw* embryos, the expression of TSHβ transcripts that remained restricted solely to the rostral tip was equivalent to that observed in the rostral tip in wild-type mice. However, caudomedial expression of TSHβ transcripts failed to occur in the *dw* mouse (Fig. 4). In contrast, in both wild-type and *dw/dw* embryos, αGSU was comparably expressed in both the rostral tip and caudomedial portions of the gland (Fig. 3).

The TSHβ expression in the rostral tip is transient

We next examined the temporal aspects of disappearance of the rostral tip thyrotropes in the *dw* mouse. An analysis of the anterior pituitary gland ontogeny revealed a persistence within the rostral tip of the thyrotrope phenotype in both *dw* and wild-type mice on e17.5 and on e18.5 (Fig. 4, and data not shown). Two days later, however, (p0.5), the TSHβ gene expression was no longer detectable in the pituitary gland (Fig. 4), indicating that the rostral tip thyrotropes had phenotypically disappeared by the day of birth in both wild-type and *dw* mice. These analyses of the Snell dwarf Pit-1 mutation have revealed that the caudomedial thyrotropes populate the mature anterior pituitary gland, while the fate of the rostral tip thyrotroph is to disappear. Two possible mechanisms could account for the disappearance of the rostral tip thyrotrope population: cell death or complete disappearance of TSHβ gene transcripts over a 24-36 hour period. The possibility that the rostral tip thyrotropes may disappear via programmed cell death was assessed using an in situ assay for nuclear DNA fragmentation, using biotin-16-dUTP as a tracer, as previously described (Gavrieli et al., 1992). This assay revealed no evidence of programmed cell death as manifested by DNA fragmentation (data not shown); therefore, it is perhaps more likely that cessation of TSHβ gene expression, rather than cell death accounts for the phenotypic extinction of the rostral tip thyrotropes. Because there are no other detectable markers for thyrotropes, independent assays are not available.

The TSHβ promoter is Pit-1-dependent

Because caudomedial thyrotropes failed to arise in the Pit-1-defective mouse, it was of interest to investigate further whether Pit-1 could transactivate the TSHβ promoter in a manner similar to its transactivation of the growth hormone and prolactin genes. It has previously been suggested that Pit-1 is capable of binding to the TSHβ promoter (Drolet et al., 1991; Wood et al., 1990; Shupnik et al., 1990; Steinfelder et al., 1991, 1992); however, it has remained unclear whether Pit-1 directly transactivates the TSHβ promoter. We examined the ability of specific TSHβ 5′ flanking sequences to bind to and transfer activation by Pit-1. Pit-1 was capable of markedly transactivating (20-fold) reporter genes under control of −123 bp of contiguous 5′ flanking TSHβ promoter information (data not shown). The ability of bacterially expressed Pit-1 to bind specifically to sites within this region was evaluated using protein-dependent DNaseI protection assays (Mangalam et al., 1989). This analysis revealed two distinct Pit-1-binding sites (Fig. 5B), in agreement with the ability of pituitary cell extracts to bind to TSHβ sequences (Steinfelder et al., 1991; Wood et al., 1990). A 64 bp region (−123 to −60) encompassing the Pit-1-binding sites, when combined to a heterologous promoter conferred a 20- to 40-fold Pit-1-dependent activation (Fig. 5A). Mutation of the two Pit-1-binding sites, as well as a third potential Pit-1-binding site, abolished the stimulatory actions of Pit-1 (Fig. 5A). Therefore, Pit-1 can directly bind to and transactivate TSHβ promoter sequences. Thus, while the Pit-1 recognition sequences of its homeodomain in the TSHβ

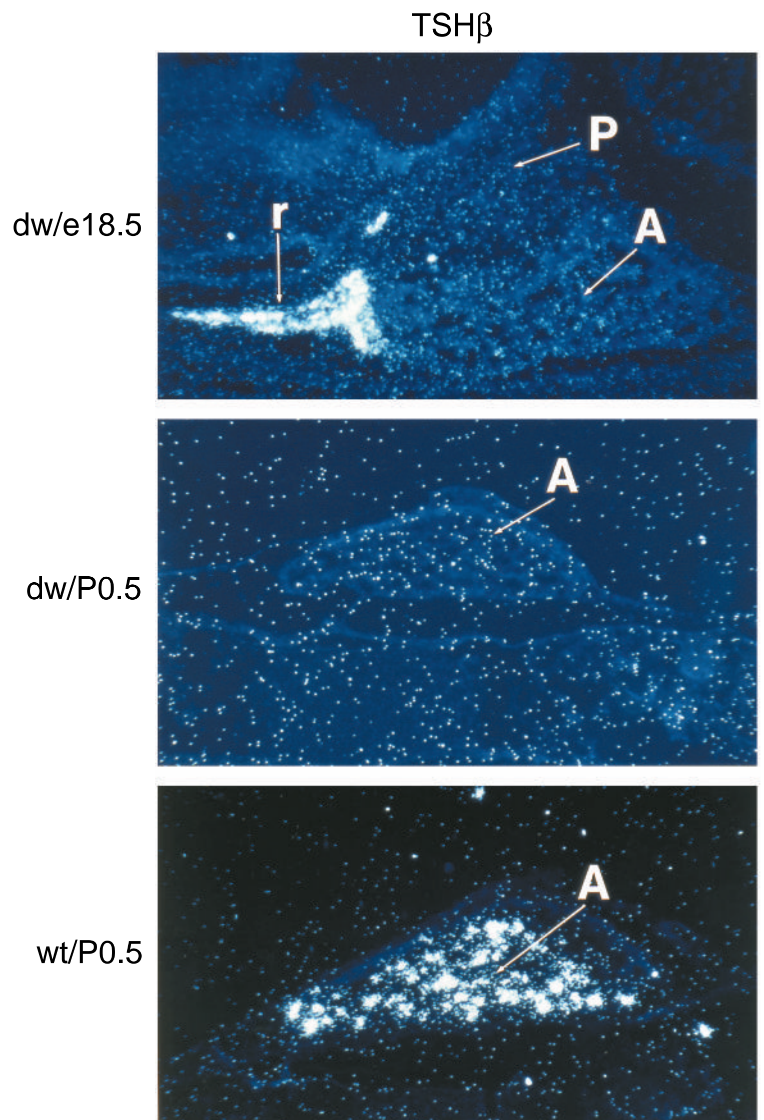

Fig. 4. TSHβ is transiently expressed in the rostral tip during embryogenesis. TSHβ mRNA within the rostral tip of the Snell dwarf pituitary was determined on e17.5 (not shown), e18.5 and P0.5. TSHβ mRNA was detected in the rostral tip on e18.5 of Snell mutants (top panel). However, two days later, at P0.5 (post partum), TSHβ mRNA was not detectable in homozygous Snell dwarf mice pituitary glands (middle panel). In contrast, strong expression of the TSHβ gene was observed in wild-type pituitary glands (bottom panel).

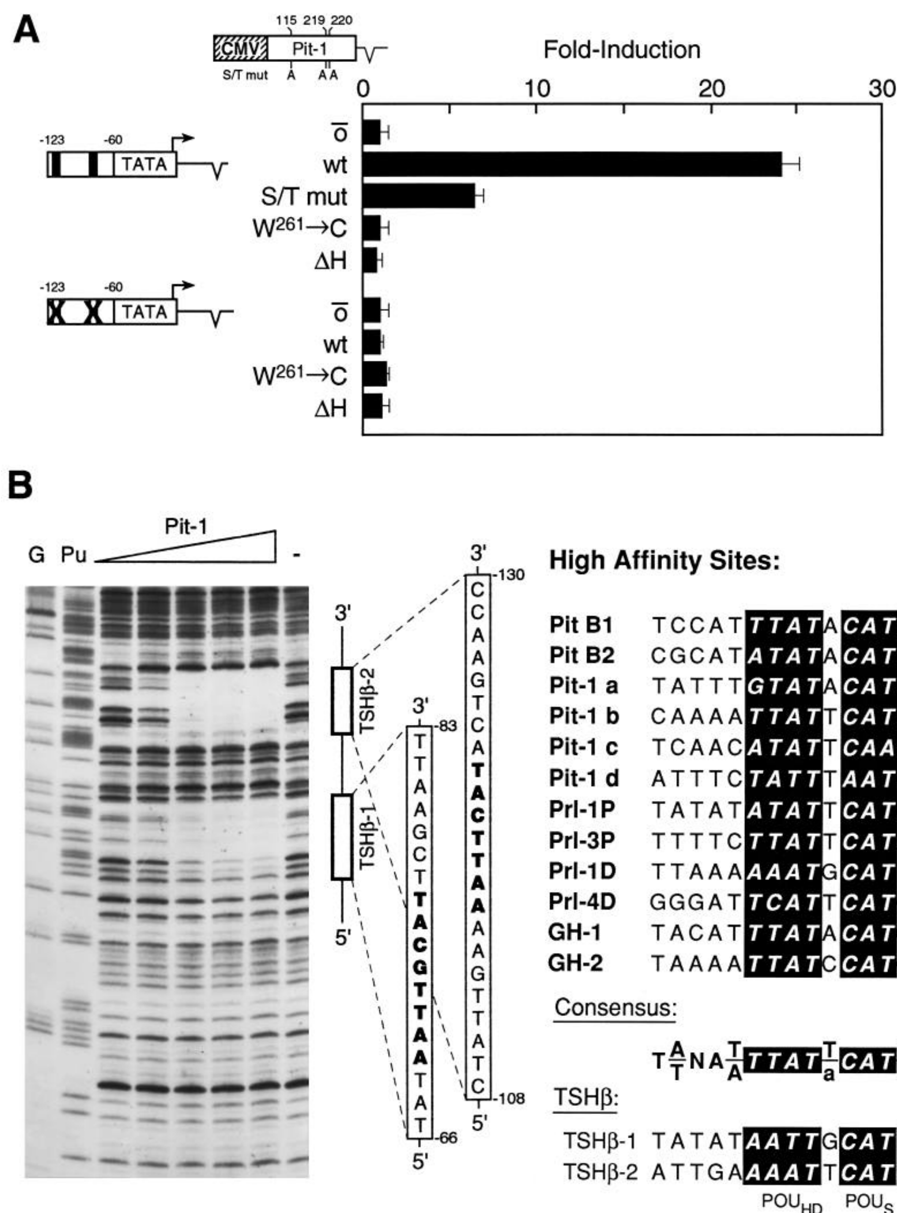

Fig. 5. Pit-1 activation of the TSHβ promoter. (A) Pit-1 activation of the TSHβ promoter. Luciferase reporter genes under control of a fragment of TSHβ promoter (−60 to −123) (Gordon et al., 1988) fused to a minimal prolactin gene promoter (−36 to +34) were transfected with plasmids expressing Pit-1 under control of the CMV promoter into HeLa cells. Based on the identification of Pit-1-binding sites by DNaseI footprinting assays (see B), Pit-1 sites in this region were mutated abolishing Pit-1-dependent activation. Similar results were obtained in six experiments of similar design. 0, CMV control; wt, wild-type Pit-1; S/T mut, S^{115}, T^{219}, T^{220}→A^{115}, A^{219} and A^{220} (Kapiloff et al. 1991); W^{261}→C, the Snell Pit-1 mutation; ΔH, homeodomain deleted Pit-1. (B) Site-specific binding of Pit-1 to the murine TSHβ promoter. DNaseI protection assays were performed using increasing amounts of bacterially expressed wild-type Pit-1 (0-400 ng, indicated by a triangle above lanes) and a TSHβ promoter fragment (+27 to −229) end-labeled at position +27. Binding sites were confirmed using the identical probe end-labeled at position −229 (data not shown). Lanes marked G or Pu show the guanine- or purine-specific Maxam-Gilbert sequencing reaction. The lane marked with a '–' indicates the DNaseI digestion pattern generated in the absence of Pit-1 protein. The sequences of the Pit-1-binding sites (TSHβ-1 and TSHβ-2) are shown at the right of the figure. The 5′ border TSHβ-2 could only be approximated because of the AT-rich nature of this sequence. Pit-1 response element sites in rat prolactin (Prl), growth hormone (GH) and Pit-1 genes are compared to those in the TSHβ promoter.

promoter (5′-**AATT**GCAT-3′) does differ somewhat from those in other Pit-1 target genes (5′-**TTAT**NCAT-3′) in the POU_{HD} recognition core (Ingraham et al., 1990; Verrijzer et al., 1992; Kristie and Sharp 1990), Pit-1 remains effective in transactivation of this promoter. It has been recently reported that the putative Pit-1-binding elements in the TSHβ gene and other promoters can confer cAMP and TRH regulation in transient transfection analyses (Steinfelder et al., 1991, 1992; Yan et al., 1991), in accord with the observation that Pit-1 phosphorylation differentially modulates Pit-1 binding to its response elements in the prolactin, growth hormone and *Pit-1* genes (Kapiloff et al., 1991). TRH-dependent phosphorylation of Pit-1 has been suggested to increase Pit-1 binding to the low affinity TSHβ promoter-binding sites (Steinfelder et al., 1991). We, therefore, tested the possibility that Pit-1 phosphorylation modulated its efficacy on stimulating TSHβ gene expression, comparing wild-type Pit-1 and a Pit-1 in which the phosphorylation sites (S^{115}, T^{119}, T^{220}) were mutated to alanine. While still capable of activating the TSHβ promoter, this mutant Pit-1 protein was impaired (3- to 4-fold) in its efficacy (Fig. 5A). Because variant forms of Pit-1 expressed in the pituitary due to alternative splicing events (e.g., Konzak and Moore, 1992; Voss et al., 1993; Kim et al., 1993), are expressed at extremely low levels, it is unclear if they exert critical selective roles in TSHβ gene activation. It is further relevant that TSHβ gene expression is modulated by other transcription factors, including the thyroid hormone receptor (e.g. Carr et al., 1989).

DISCUSSION

The molecular mechanisms that permit the generation of distinct cell phenotypes within an organ poses a critical question in mammalian organogenesis. The observations that the TSHβ phenotype disappears in Pit-1-defective Snell mice, and that activation of TSHβ expression occurs prior to the

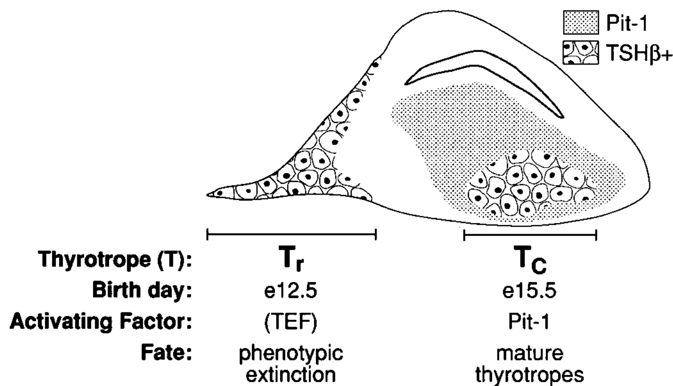

Fig. 6. Model of independent origins of thyrotrope cell types during anterior pituitary development. Thyrotrope embryonic factor (TEF) serves as a potential activator of TSHβ gene activation in rostral tip cells. Pit-1, in concert with other factors, is required to activate TSHβ gene in caudomedial cells (T_c). Rostral tip (T_r) cells will phenotypically disappear by the day of birth while the thyrotrope population of the mature gland appears to arise from the caudomedial cells.

initial expression of the *pit-1* gene raised a perplexing question regarding the nature of the function of *pit-1* gene in the activation and maintenance of the thyrotrope phenotype. In this manuscript, we have presented data that suggest two independent molecular origins for the thyrotrope cell type.

Our data suggest that αGSU gene expression is initiated independent of any requirement for Pit-1 and that Pit-1-defective dwarf mice contain a full complement of the caudal thyrotrope precursors. At the e14-e17 stage of development, the number of cells expressing the Pit-1 gene was equivalent in wild-type and *dw/dw* mice (Rhodes et al., 1993). Therefore, activation of the TSHβ gene expression in the caudomedial portion of the gland directly requires functional Pit-1 protein. An alternative model in which Pit-1 could cause rostral tip TSHβ thyrotropes to migrate caudally, appeared to be experimentally excluded because thorough examination of serial sections taken at different times revealed no evidence of migrating thyrotrope cells and no depletion of rostral tip thyrotrope cells in wild-type, when compared to *dw* animals. Therefore, the data are consistent with the model that two thyrotrope populations arise independently, with the initial activation of TSHβ gene expression occurring independently of Pit-1 in the rostral tip thyrotropes on e12.5, while the presence of functional Pit-1 protein is necessary for subsequent activation of the caudomedial thyrotropes on e15.5.

Because these data indicate that the initial developmental appearance of rostral tip thyrotropes occurs without a requirement for Pit-1, a second type of molecular activation mechanism must function for TSHβ gene activation in rostral tip thyrotropes. A PAR-bZIP protein, TEF, that binds to and can effectively transactivate the TSHβ promoter, is initially selectively expressed in the rostral tip cells of the anterior pituitary coincident with the activation of TSHβ gene expression (Drolet et al., 1991). TEF, thus, becomes an attractive potential candidate for the initial activation of TSHβ gene expression in these cells, arguing that occupancy of distinct *cis*-active elements by two unrelated transcription factors serve to activate the TSHβ gene expression in distinct cell populations. The proximal binding site for Pit-1 and TEF (Drolet et al., 1991) are overlapped.

Based on these data, we suggest a model (Fig. 6) in which Pit-1 functions as the critical determining factor for caudomedial thyrotropes, lactotropes and somatotropes. In each case, the Pit-1 protein directly activates the transcription of the distal target genes encoding the trophic factors that define these three cellular phenotypes, and each is initially activated in the caudomedial aspect of the gland on e15.5, one day after initial detection of Pit-1 transcripts and protein. However, there would appear to be a strict requirement for additional distinct transcription factors to confer cell-specific activation. For example, Pit-1 synergizes with estrogen in the activation of prolactin gene expression (Snell, 1929), and with Zn-15 and thyroid hormone in activation of growth hormone gene expression (Lipkin et al., 1993; Schaufele et al., 1992). It is likely that similar interactions serve in Pit-1-dependent (or TEF-dependent) TSHβ gene activation. Conversely, distinct factor(s) restrict prolactin gene expression out of these thyrotropes (Crenshaw et al., 1989), indicating the coordinate requirement for both activating and restricting factors in achieving cell-specific patterns of trophic hormone expression.

Thus, while mammalian cell phenotypes within an organ have generally been presumed to be derived from a common origin, and while somatotropes and lactotropes appear to share a common stem cell precursor (Behringer et al., 1988; Borrelli et al., 1989; Lin et al., 1993), our data favors the independent origins of two populations of thyrotrope cells. These data raise an intriguing question as to the potential role of the rostral tip thyrotrope during embryogenesis. The unexpected finding of these two populations may prove to be prototypic for other apparently homogeneous cell types that arise during mammalian organogenesis.

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