The transcription factor TTF-1 is expressed at the onset of thyroid and lung morphogenesis and in restricted regions of the foetal brain

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Summary

TTF-1, a homeodomain-containing transcription factor, which is required for the specific expression of the thyroglobulin and thyroperoxidase gene promoters in differentiated thyroid cell lines, is expressed at the very beginning of rat thyroid differentiation. TTF-1 mRNA is detected in the endodermal cells of the thyroid rudiment in the rat embryo and precedes the expression of the two known target genes by 5 days. No delay is observed between the appearance of TTF-1 mRNA and protein, which shows a clear nuclear localization. In the adult thyroid, TTF-1 is present only in the endoderm-derived follicular cells.

Two additional domains of expression of TTF-1 have been observed, the lung and restricted areas of the brain. In the lung, TTF-1 mRNA and protein are also present at the earliest stages of differentiation and are later confined to the bronchial epithelium. In the brain, TTF-1 appears to be restricted to structures of diencephalic origin, including the developing neurohypophysis. The early detection of TTF-1 in the endodermal cells of the thyroid and lung anlage and in restricted neuroblast populations indicates that TTF-1 may have a role in cell determination in these three systems and that additional mechanisms may be involved in the activation of thyroid-specific gene expression.

Key words: transcription factor, TTF-1, thyroid, lung, brain, rat.

Introduction

Homeodomain-containing proteins play a role in the regulation of morphogenesis in Drosophila and have been shown to bind to specific DNA sequences and to exert their effects through transcriptional regulation (Hoey and Levine, 1988; Desplan et al. 1988; Biggin and Tjian, 1989; Dearolf et al. 1989). Counterparts of these genes have been isolated in mammals, most being related to the Drosophila Antennapedia gene (the Class 1 Hox genes) and their patterns of expression during embryogenesis would suggest that homeodomain proteins also play a role in mammalian development (Holland and Hogan, 1988; Gaunt et al. 1988; Kessel and Gruss, 1990). Recently, a number of tissue-specific transcription factors have been isolated in mammals, for example Oct2 (Clerc et al. 1988; Staudt et al. 1988; Scheidereit et al. 1988), GFH1/Pit1 (Bodner et al. 1988; Ingraham et al. 1988), and LFB1 (Frain et al. 1990) which have homeodomains highly divergent from that of the prototype Antennapedia. In several cases, it has been established that these factors are required for the transcriptional activation of genes that define a cell type (e.g. Müller et al. 1988; Frain et al. 1990), indicating that they are involved in at least one stage in the development of a tissue-specific phenotype. In the case of one tissue specific transcription factor, GFH1/Pit1, which has a role in the activation of growth hormone (GH) gene expression in differentiated cell lines, it has been demonstrated that synthesis of GFH1/Pit1 correlates temporally with the activation of GH gene expression (Dolle et al. 1990; Simmons et al. 1990).

We have recently isolated the cDNA for a thyroid transcription factor, TTF-1 (Guazzi et al. 1990). TTF-1 contains a novel homeodomain that is necessary and sufficient for its DNA-binding activity. The homeodomain of TTF-1 is most closely related to that of NK2, a member of a family of homeobox-containing genes in Drosophila (Kim and Niremberg, 1989). In the differentiated thyroid cell line FRTL-5, the binding of TTF-1 to the thyroglobulin (Tg) promoter is essential for the cell-type-specific expression of this gene (Civitareale et al. 1988) and binding to the promoter of a second thyroid specific gene, thyroperoxidase, (TPO), is required for the full activity of this promoter in thyroid cells (Francis-Lang et al. 1990). Co-transfection of the cloned TTF-1 cDNA and a Tg reporter construct into several non-thyroid cell lines which do not express TTF-1 has demonstrated a specific transactivation of the Tg and TPO promoters as a result of the production of TTF-1 protein, therefore establishing TTF-1 as a transcriptional activator (M. De Felice, H. Francis-Lang and R. D. L., unpublished observations).

Preliminary analysis demonstrated that TTF-1 mRNA is found only in mRNA isolated from lung and thyroids of 4 week old rats (Guazzi et al. 1990). In order
to determine if the expression of TTF-1 correlates with the appearance of specific markers during thyroid organogenesis, we carried out in situ hybridization experiments at several stages of rat development (days 10.5 to 17 p.c.) with cRNA probes derived from TTF-1, Tg and TPO. We also analyzed the appearance of the thyroid-stimulating hormone receptor (TSHR) mRNA. The cDNA for the rat TSHR has recently been isolated (Akamizu et al. 1990) and is expressed in differentiated thyroid cells (FRTL-5) but not in several other tissues tested, thereby providing a third thyroid-specific marker. It is not known if TTF-1 plays a role in the thyroid-specific expression of the TSHR gene.

We demonstrate that TTF-1 mRNA and protein are detectable at the onset of thyroid organogenesis (day 10.5 p.c.) whereas Tg, TPO and TSHR mRNAs appear 5 days later. We also found TTF-1 transcripts and protein in the lung rudiment and in discrete regions of the developing brain indicating that TTF-1 may play a role in the differentiation of more than one cell type.

Materials and methods

Northern blot analysis
RNA preparation and northern blot analysis was carried out as described in Guazzi et al. 1990. The probe for the northern blot was the TTF-1 3', non-translated region cDNA probe described below and was labelled with 32P by random oligo priming.

Tissue preparation
Embryos and foetuses were obtained from natural matings between Wistar rats. Midday of the day of vaginal plug was considered day 0.5 p.c. Samples were staged according to the external criteria of Witshi (1962). The samples were fixed immediately after dissection in 4% paraformaldehyde in 1X PBS, pH 7.0 for 12 h at 4°C. Subsequently samples were cryoprotected by immersion in 20% sucrose/1X PBS overnight at 4°C and embedded in tissue-teck OCT compound (Miles Scientific, Naperville, IL.). Sections of 10 mm were cut using a cryostat and collected on poly-L-lysine-coated slides, according to Toth et al. 1987.

Preparation of 35S-labelled riboprobes
Single-stranded RNA probes were prepared and labelled with 35S ([α-35S] UTP, >1000 Ci mmol⁻¹, Amersham), according to Toth et al. 1987 to a specific activity of 8.5×10⁶ disintegrations min⁻¹ μg⁻¹. All probes were subcloned into Bluescript vector (Stratagene) and transcribed using T3 or T7 RNA polymerase (Stratagene). The TTF-1 antisense riboprobe was a 625 bp fragment derived from the 3' non-translated region of the TTF-1 cDNA (bp 1694–2319, Guazzi et al. 1990). Other TTF-1 probes described in Guazzi et al. 1990 (from the 3' end of the cDNA and the homeobox) were used in situ hybridization experiments. All the probes gave the same results and the 3' non-translated probe was selected for further use since it gave the best signal-to-noise ratio. The Tg riboprobe consisted of a fragment encoding sequence from the carboxy-terminus of the Tg gene (bp 2227–2916, in Di Lauro et al. 1985). The TPO riboprobe is a 500 bp insert from the 5'-coding region of the rat TPO gene (bp 1–335 from the AUG plus 155 bp of adjacent 5'-non-translated, H. F-L, M.P and R. D-L, unpublished). The TSHR riboprobe is an approximately 300 bp insert from the coding region of the rat TSHR cDNA clone (BspHI–EcoRV fragment) and contains a region with residues unique to the TSHR which are not found in the structurally related lutenizing hormone receptor (Akamizu et al. 1990). All probes were tested on northern blots before use.

In situ hybridization

In situ hybridization was carried out as described in Toth et al. 1987. After washing and RNase treatment, the sections were dehydrated with graded ethanol containing 300 m ammonium acetate and processed for autoradiography using Kodak NTB2 emulsion (Eastmann Kodak, Rochester, NY) diluted 1:1 with water. Slides were developed after 2–4 weeks with D19 developer (Kodak), washed in distilled water and fixed in AL-4 fixer (Kodak). The slides were stained with toluidine blue or cosin and coverslips were mounted in Eukitt (O. Kindler GmbH, Freibourg, Germany).

Sample preparation for immunohistochemistry

Embryos and foetuses were collected from the same litters as those prepared for in situ hybridization and were processed identically.

TTF-1 antibody preparation
Rabbits were immunized with a mixture of three peptides E1, A2 and F1, spanning amino acid residues 2–14, 92–104 and 110–122 from the TTF-1 coding region, respectively (Guazzi et al. 1990). The antibody was purified according to Harlow and Lane, 1988, and further purified by affinity chromatography on Affi-gel 10 (Bio Rad) coupled with the synthetic peptides used for the immunization.

Immunohistochemistry

Tissue sections were incubated for 20 min in PBS/0.1% triton (v/v) and washed in PBS for 5 min, covered with 1.5% goat serum and incubated with affinity-purified TTF-1 antibody (diluted 1:5000 in PBS) supplemented with 1.5% goat serum for 30 min, followed by a 30 min incubation with a 1:200 dilution of a biotinylated goat anti-rabbit IgG (Vector Lab. Inc.). Endogenous peroxidases were inactivated by treatment with 3% hydrogen peroxide in water, sections were then incubated for 30 min with a biotin-avidin–peroxidase complex (Vector Lab. Inc.). Finally the sections were treated for 5 min in 0.5 mg ml⁻¹ diaminobenzidine (Sigma), dissolved in 10 mM TBS, pH 7.6 and containing 0.01% hydrogen peroxide. All steps were separated by a wash in PBS/0.1% Triton at room temperature. Sections were dehydrated, cleared with xylol and mounted in Eukitt.

Results

The pattern of expression of TTF-1 at late gestation
To determine the overall pattern of expression of TTF-1 in the rat embryo, hybridizations were performed in situ on serial sections of rat embryos from 13.5 to 14.5 days post coitum (p.c.) using 35S-labelled antisense cRNA probes from the 3' non-translated region of the TTF-1 cDNA (see materials and methods). This initial analysis revealed the expression of TTF-1 in the developing thyroid and primitive bronchioli of the lung, as expected from the previous northern blot analysis. This in situ hybridization also revealed the presence of TTF-1 transcripts in restricted areas of the brain: the
The thyroid rudiment develops from a median diverticulum (day 11.5 p.c.). The diverticulum is caudally as a downgrowth known as the thyroid dilated aortic sac. This thickening, located beneath the pharynx, forming a small button of cells just above the primitive pharynx (cranial gut), between day 10.5 to 11.5 p.c., showed that, at the level of the thyroid anlage, TTF-1 expression is restricted to the thyroid rudiment (Fig. 2a). At day 11.5 p.c, the thyroid diverticulum is shaped as a vesicle and TTF-1-specific labelling seems to be distributed on the ventral edge and not on the posterior remnant of the thyroglossal duct (Fig. 2b and see also Fig. 3a). At day 12.5 p.c, the thyroid has reached the arcus aortae and by day 13.5 p.c., migration is completed. At this stage, the thyroid is still a solid complex of endodermal cells and the TTF-1 signal is homogeneously distributed on the immature thyroid epithelium (not shown). As shown in Fig. 2c, at day 14.5 p.c., the parathyroids are joining the thyroid which at this stage is a lobular shaped structure and begins to be broken up into a network of epithelial cords by invasion of the surrounding vascular mesenchyme. TTF-1 transcripts are detected on the thyroidal lobes which are located on both sides of the trachea and there is clearly no signal on the parathyroids or on the epithelium of the upper respiratory airways (Fig. 2c,d). Between days 15 and 17 p.c., a few primitive follicular structures appear in the thyroid lobules between the epithelial cells. TTF-1 expression at day 17 p.c. is clearly restricted to the cords of epithelial cells which are still arranged in an irregular interdigitating pattern (Fig. 2e).

**Relationship between the expression of TTF-1 and thyroid-specific markers**

In order to establish whether there is a correlation between TTF-1 expression and the onset of the thyroid functional markers, riboprobes were constructed from the coding regions of the Tg, TSHR and TPO cDNAs (see Materials and methods). Antisense cRNA probes were transcribed in the presence of 35S-UTP and hybridized in situ to serial sections of embryos and fetuses from days 10.5 to 17 p.c. The results are summarized in Fig. 3a–i. From day 10.5 to day 14.5 p.c., TTF-1 expression is detectable in the developing thyroid (Fig. 3a,d,g), while hybridization with Tg or TSHR probes did not give any signal. As described above, from day 10.5 to 11.5 p.c. the thyroid rudiment begins to migrate and appears as a homogeneous button of endodermal cells. At day 14.5 p.c., the first cords of epithelial cells delineate and by day 15.5 p.c., Tg and TSHR transcripts are detectable in the epithelial cords (shown in Fig. 3e,h). Previous immunocytochemical studies in rat revealed immunoreactive Tg at day 15 p.c. in the cytoplasm of immature thyroid epithelial cells (Kawaoi and Tsuneda, 1985). Two days later (day 17...
Fig. 2. TTF-1 expression pattern during thyroid development. Sections through different axes of rat embryos at various stages of development hybridized with a TTF-1 cRNA probe. (a) Bright-field, transversal section through the pharyngeal cavity (Ph) of a day 10.5 p.c. embryo. Dark-field, TTF-1 transcripts are detected only in the thyroid anlage (Th). (b) Transversal section through the pharyngeal cavity of a day 11.5 p.c. embryo. Dark-field, TTF-1 transcripts are restricted to the thyroid diverticulum (Th). (c) Bright-field, transversal section of an embryo at day 14.5 p.c. Dark-field, TTF-1 hybridization signal is only on the thyroid lobes, and not detectable on the parathyroid (Pt, arrow) fusing to the right thyroid lobe or on the larynx (L). (d) Bright-field, mid-sagittal section through the thyroid isthmus of an embryo day 15.5 p.c. Dark-field, TTF-1 transcripts are localized on the thyroidal connecting isthmus. (e) Bright-field, frontal section of a foetus, day 17 p.c. Dark-field, silver grains are found in the cords of endodermal cells and the invading vascular mesenchyme is negative. L, larynx; Oe, oesophagus; Ph, pharyngeal cavity; Pt, parathyroid; Sc, spinal cord; Th, thyroid; Tr, trachea. Bars (a, b and e) 153 µm; (c) 200 µm; (d) 220 µm.

p.c.), the first thyroid follicles begin to form and thyroid hormones (T3 and T4) are detectable in the lumen of these primitive follicles. At day 17, Tg and TSHR transcripts are found in the cords of thyroid epithelial cells (Fig. 3f,i).

As this time course experiment demonstrates, there is a lag of approximately 5 days between the appearance of TTF-1 mRNA and that of the thyroid-specific markers Tg and the TSHR. This lag also holds for the expression of the TPO gene (unpublished data). The lag in appearance of thyroid-specific marker RNAs is not due to a slow accumulation of small amounts of the RNAs since we observe their abrupt appearance at day 15 p.c.

The distribution of TTF-1 transcripts in the developing lung

The lower respiratory system is first observable as the median laryngo-tracheal groove in the caudal end of the ventral wall of the primitive pharynx (Rugh, 1968; Hebel and Stromberg, 1986, see Fig. 7). This groove produces a ridge on the external surface of the primitive pharynx. As this diverticulum grows, it becomes invested with splanchnic mesenchyme and its distal end enlarges to form a globular lung bud at day 10.5 p.c. While the trachea is separating from the cranial gut, the bud rapidly divides into two branches that represent the anlage of the two main bronchi which elongate and grow distally into the surrounding mesenchyme (day 11.5 p.c.).

Transversal sections of an embryo at day 10.5 p.c. (as mentioned above TTF-1 expression is undetectable before this stage) showed that the TTF-1 expression pattern is restricted to the ventrally migrating edge of the lung bud and no signal is detectable on the laryngo-tracheal groove (Fig. 4a, day 10.5 p.c.). One day later a strong signal is present in both branches of the primitive bronchi (day 11.5 p.c., Fig. 4b).
TTF-1 expression in rat thyroid and lung morphogenesis

Between days 13.5 and 15.5 p.c., the two main bronchi continue elongating and branching dichotomously together with the surrounding splanchnic mesenchyme. Serial sagittal sections of embryos from days 13.5 to 15.5 p.c. (Fig. 4c,d) clearly show that TTF-1 is constantly expressed in the bronchial epithelium during all of its early stages of differentiation while the surrounding splanchnic mesenchyme and the epithelium of the upper respiratory airways do not contain any detectable TTF-1 mRNA.

TTF-1 expression in the CNS
Since TTF-1 transcripts were observed in the brain of day 14.5 p.c. embryos, several embryos and fetuses from days 7.5 to 17 p.c. were sectioned along different axes and hybridized in situ with TTF-1 cRNA probes.
Fig. 4. TTF-1 expression pattern during lung development. (a) Bright-field, transversal section through the pharyngeal cavity at day 10.5 p.c. Dark-field, TTF-1 signal is restricted to the lung bud (Lb) and not in the connecting laryngo-tracheal groove (Lt). (b) Bright-field, transversal section at day 11.5 p.c., the lung bud is branching dichotomously, pre-forming the anlage of the two main bronchi (MB, arrowheads). Dark-field, TTF-1 signal is distributed over the anlage. (c) Bright-field, a parasagittal section at day 15.5 p.c. Dark-field, the signal is restricted to the growing and dividing bronchi (pulmonary primordium) and not in the surrounding mesenchyme. (d) Bright-field, sagittal section at day 16.5 p.c., the bronchial tree segmentation is shown at higher magnification. Dark-field, TTF-1 transcripts are found in the bronchial cuboidal epithelium (B) and not in the neighboring mesenchymal cells (M). B, segmented bronchi; Lb, lung-bud; Lt, laryngo-tracheal groove; Lu, lung; M, surrounding mesenchyme; PV, pre-vertebral bodies; Sc, spinal-cord. Bars (a) 153 μm; (b) 200 μm; (c) 220 μm; (d) 60 μm.

Discrete areas of hybridization were detected, from day 10.5 p.c., in the diencephalon and in the telencephalic floor. In the developing diencephalon, TTF-1 transcripts are restricted to the hypothalamic area of the diencephalon and to the infundibulum at the earliest stages of their differentiation, day 10.5 p.c. (Fig. 5A, section a). The infundibulum will form the posterior lobe of the pituitary, the neurohypophysis. The anterior lobe (glandular part) originates from the Rathke's pouch, which migrates from the vault of the primitive mouth (Rugh, 1968). As is depicted in a transversal section of an embryo at day 11.5 p.c. and in a sagittal section of embryo at day 16.5 p.c. (Fig. 5A, sections b, c), TTF-1 transcripts are restricted to the developing neurohypophysis and no signal was ever detected in the pituitary glandular primordium (Rathke's pouch) in any of the stages examined. In a sagittal section at day 14.5 p.c. (Fig. 5B, section c), the overall pattern of TTF-1 expression in the brain can be seen and it is apparent that TTF-1 continues to be expressed in the hypothalamic area of the diencephalon, in the infundibulum and in the recessus opticus. In the recessus opticus a strong signal is detectable in the area corresponding to the optic chiasma (Fig. 5B, section c). A frontal section of an embryo at the same stage, (Fig. 5B, section b) clearly shows that the rostral boundary of TTF-1 expression in the wall of the diencephalon is always contained in the hypothalamic area spanning from the floor of the third ventricle to the sulcus diencephalic ventralis, which represents the anatomical boundary between the hypothalamus and ventral thalamus. Although the TTF-1 transcripts are not always strictly coincident with this boundary, they never cross the anatomical limit between these two structures. This boundary is already established by day 10.5 p.c. as can be seen in Fig. 5B, section f. Also in this figure and one day later (11.5 p.c., Fig. 5B, section g), TTF-1 transcripts appear to be localized to the floor of the telencephalic vesicles in an area that corresponds to the developing striatum. At later stages (between days 14.5 and 16.5 p.c.), the development of the floor is
Fig. 6. Distribution of TTF-1 protein. In the top of the figure, left corner, a diagram of a parasagittal section of a rat embryo at day 14.5 p.c. showing, boxed, the portion of the embryo displayed in a. (a) A parasagittal section of a 14 day p.c. embryo reacted with the TTF-1 antibody. Only the lung (Lu), the thyroid (Th) and the diencephalon (Di) show clear positivity. Panels c and d show higher magnification of the areas labeled i and ii, respectively, in panel a. (d) A transversal section of an embryo at day 10.5 p.c. TTF-1 protein is localized in the thyroid anlage (Th) which is located in the anterior wall of the pharynx (Ph). (c) The thyroid anlage has been counterstained with eosin and is shown at higher magnification. The immunostaining (now seen as dark brown on a pale pink background) is clearly detected in the nuclei of the thyroid epithelial cells. In the adult rat thyroid (f), counterstained with toluidine, the peroxidase staining (which appears black with this stain) is restricted to the nuclei of the epithelial cells lining the colloid (C) containing follicles (F). The cells of the septa (S) are negative and appear light blue. In g, a transversal section through the lung bud at day 10.5 p.c. shows a specific nuclear staining in the migrating lung bud (Lb) while the laringo-tracheal groove (Lt) is negative. At higher magnification (h), stained as in f, TTF-1 protein is clearly localized in the anterior edge of the lung bud (Lb) and not in the laryngo-tracheal groove (Lt). In i, a sagittal section of a fetus at day 17 p.c. shows the distribution of the protein in the cells of the cuboidal epithelia lining the bronchioli (B); in the same section, the epithelia of the main bronchi (MB) are negative. In l, a frontal section (day 10.5 p.c.) through the diencephalic area of the brain which delimits the cavity of the third ventricle (III), shows the localization of TTF-1 protein to the hypothalamic region (Ht). The arrows indicate the boundary of TTF-1 protein as was observed for the mRNA (see Fig. 5). In m, which is a higher magnification of l and is counterstained as in e, the neuroblasts in the hypothalamic wall show an intense nuclear staining. In n, a sagittal section through the hypophysis of a new born rat, TTF-1 protein is restricted to the neurohypophysis (N). III, third ventricle; Ad, adenohypophysis; C, colloid; F, follicular lining epithelia; H, heart; Ht, hypothalamus; L, liver; Lb, lung bud; Lt, laringo-tracheal groove; Lu, Lung; N, neurohypophysis; P, pars intermedia; Ph, pharynx; S, vascular mesenchyme of the septa; Te, Telencephalon; Th, thyroid. Bars (a,d,g) 150 μm; (b,e,h,c,f,i) 60 μm.
characterized by the appearance of the striated nuclei, lateral and median striatum (Fentress et al. 1981). As shown in Fig. 5B, section d, in a frontal view at day 15.5 p.c., the hybridization signal is restricted to the median corpus striatum, while the lateral striatum is clearly negative. In the same section, it is also possible to detect another area of expression, which is in close proximity to the median nucleus striatum and possibly corresponds to the globus pallidum or paleostriatum, a nucleus that originates from the median nucleus striatum (Niimi et al. 1962).

**Localization of TTF-1 protein**

The results of the in situ hybridization experiments are not consistent with an exclusive role of TTF-1 in the activation of thyroid-specific transcription, since TTF-1 mRNA is detected in the thyroid five days before transcription of the target genes and, furthermore, it is detected in tissues different from the thyroid. To assess better the significance of the in situ hybridization results, an affinity-purified polyclonal antibody was used to detect the presence of the TTF-1 protein. The specificity of the antibody was demonstrated by the exclusive staining of thyroid, lung and diencephalon in sections of whole, 14 day p.c., embryos (Fig. 6a, b, c).

To determine whether TTF-1 is detectable at the earliest stages in which we observed TTF-1 mRNA, serial transversal sections from embryos at day 10.5 p.c. were analyzed. As expected, due to the absence of TTF-1 mRNA, the antibody did not label any structure at earlier stages (data not shown). As shown in Fig. 6d, g, l, TTF-1 is localized in the endodermal cells of thyroid anlage, of the lung bud and in diencephalic neuroblasts. At higher magnification (Fig. 6e, h, m), the immunostaining is clearly localized to the nuclei. At day 10.5 p.c., the neuroblasts are still arranged in a single layer in the developing CNS called the ventricular zone and in Fig. 6l, m, a frontal section of the diencephalon shows that the immunolabelling is clearly distributed in the nuclei of the neuroblasts belonging to the hypothalamic area. At late gestational stages (day 17.5 p.c.), in the respiratory system, TTF-1 protein is present in the nuclei of epithelial cells of the bronchioli but not in the trachea and in the main bronchi (Fig. 6i). In the thyroid, TTF-1 protein is localized in the nuclei of the thyroid follicular cells (Fig. 6f). No labelling was ever detected in the vascular mesenchyme of the septa surrounding the follicles (Fig. 6f). In brain at day 17.5 p.c., TTF-1 was clearly detected in the neurohypophysis (Fig. 6n).

**Discussion**

TTF-1 was isolated as a molecule important for the expression of genes expressed specifically in the thyroid (Civitareale et al. 1989; Guazzi et al. 1990). It contains a novel homeodomain, quite divergent from the class 1 (Hox) homeodomains. The Hox genes show widespread expression during embryogenesis, being transcribed in distinct but overlapping domains along the antero-posterior axis, and, on the whole, expression patterns are found to be region specific rather than tissue specific (e.g. see Kessel and Gruss, 1990; Gaunt et al. 1988). TTF-1, like some other genes that contain homeodomains divergent from the class I homeodomain (e.g. Evx-1, Bastian and Gruss, 1990; Cdx-1, Duprey et al. 1988, GFIH/Pit1, Döllé et al. 1990; Simmons et al. 1990), shows a more restricted expression pattern during embryogenesis being expressed specifically in the thyroid and lung and in restricted regions in the brain.

**TTF-1 expression and thyroid development**

Several lines of evidence (see introduction) indicate that TTF-1 has an important role in the thyroid-specific expression of both the Tg and TPO genes. The expression of TTF-1 in the thyroid anlage at day 10.5 p.c. provides a marker for the early determination of thyroid cells. In addition to its function as a transcriptional activator of Tg and TPO genes in differentiated thyroid cell lines, TTF-1 may play an important role in the commitment of thyroid cell precursors. The five days lag between the appearance of TTF-1 and the expression of the Tg and TPO genes, which are known targets of TTF-1 action, suggests that the presence of TTF-1 protein is not by itself sufficient for the initiation of transcription of these genes. It is possible that TTF-1 is not active during this lag period but requires some sort of modification, which occurs at a time when thyroid function is initiated. Another possibility is that additional factors are required to initiate the expression of thyroid-specific genes and these appear at a later stage than TTF-1 in thyroid development. A further possibility is that Tg and TPO genes are not competent to be expressed until day 15 p.c. This last model could also explain the discrepancy between the data presented in this paper and the observation that TTF-1 can, in transient co-transfection assays, activate transcription of both Tg and TPO promoters in non-thyroid cells (M. De Felice, H. Francis-Lang and R. D. L., unpublished observations).

What could be the nature of the event(s) that, after the appearance of TTF-1, trigger the synthesis of Tg and TPO? Specific interactions between thyroid cell precursors with the surrounding mesenchyme, which have been demonstrated to play an important role in thyroid morphogenesis (Hilfer and Stern, 1971), could be involved in the activation of thyroid-specific transcription. It has also been shown that the interaction between TSH and its receptor has a role in the differentiation of primary dog thyrocytes (Pohl et al. 1990). It is relevant therefore that we find that the mRNA for the TSHR appears together with Tg and TPO mRNAs, suggesting that they are subject to a similar regulation. The notion that TSH is not involved in the early stages of thyroid differentiation (day 10.5 to 15.5 p.c.) is supported by the observation that foetal TSH-secreting cells appear in the anterior pituitary gland of the rat around day 16–17 p.c. (Watanabe and Daikoku, 1979; Begeot et al. 1981), two days after the transcription of thyroid-specific genes and the appear-
Fig. 5. TTF-1 expression pattern in the developing CNS (Panel A). Expression of TTF-1 in the developing neurohypophysis. (a) Bright-field represents a transversal section through the diencephalon of an embryo day 10.5 p.c. In the dark-field, the hybridization signal is detected in the hypothalamic area (Ht, arrowheads) of the diencephalon (Di) and in the developing infundibulum (In, arrowhead). (b) Bright-field, shows a transversal section (day 11.5 p.c.) through the diencephalon at the level of the optic stalk (Os). Dark-field, TTF-1 expression is restricted to the hypothalamus (Ht) and the infundibulum (In). The Rathke's pouch (R) and optic stalk are negative.

c) Bright-field shows the developing neurohypophysis (N, arrowheads) in a sagittal section of a fetus day 16.5 p.c. In the dark-field silver grains are restricted to the pars nervosa or neurohypophysis (arrows) and no signal is detected on the adjacent Rathke's pouch (R, arrowheads, bright-field). Di, diencephalon; Ht, hypothalamus; In, infundibulum; N, neurohypophysis; Os, optic stalk; Ov, optic vesicle; R, Rathke's pouch. Bars (a) 153 μm; (b) 200 μm; (c) 220 μm. (Panel B) TTF-1 expression in the diencephalon and the telencephalic floor. (a) A diagram of a parasagittal section of an embryo at day 14.5 p.c. Two planes of section are indicated, (b) a frontal section through the diencephalon and (d) a frontal section through the floor of the telencephalon and the diencephalon, c is the corresponding dark-field view of the parasagittal section, and TTF-1 transcripts are restricted to the lung bronchioli (Lu), the diencephalon (Di), optic chiasma (Oc) and the median corpus striatum (Cs). (b) (plane of section indicated in a), the bright-field shows the walls of the diencephalon at higher magnification, indicating the compartmentalization (dotted lines) into four zones: Ht, hypothalamus; Tv, ventral thalamus; Td, dorsal thalamus, and Et, epithalamus, surrounding the third ventricle (III). In the dark-field view, grains are detected in the hypothalamus; the anterior border of expression is in close proximity to the subthalamic groove (Sg), which represents the anatomical border between the hypothalamus and the ventral thalamus. In d, a frontal section (represented by an arrowhead in a) of an embryo at day 16.5 p.c., the hypothalamic (Ht)—thalamic (T) boundary is indicated by white lines and positive signal is detectable in the floor of the telencephalon in an area corresponding to the median corpus striatum (Csm). At this stage, close to the Csm, another positive nucleus is observable which corresponds to the paleostriatum (Ps, arrow), a special nucleus of diencephalic origin which develops from the median corpus striatum. The lateral corpus striatum (Csl) is observable lateral to the Csm and no grains were ever detected in this structure. e is a diagram showing a parasagittal section of an embryo at day 11.5 p.c., arrowheads indicate the different planes of section. f represents a transversal section through the floor of the telencephalon and the hypothalamus at the level of the optic stalk and g represents a frontal section through approximately the same area. (f) Dark-field, the distribution of TTF-1 transcripts is clearly restricted to the floor of the telencephalic vesicles which at this stage constitutes the corpus striatum (Cs, arrowheads) and in the hypothalamic area (Ht) of the diencephalon. (g) Bright-field (section described in e) shows the telencephalic vesicles (Te), the corpus striatum (Cs) and the diencephalon. In the dark-field view, TTF-1 transcripts are restricted to the corpus striatum and the hypothalamus (Ht). Arrowheads indicate that the thalamus (T) is clearly negative. III, third ventricle; Cs, corpus striatum; CsL, lateral corpus striatum; CSM, median corpus striatum; Di, diencephalon; Et, epithalamus; Ht, hypothalamus; Lu, lung; My, myelencephalon; Oc, optic chiasma; Or, optic recess; Ps, paleostriatum; Sg, sulcus diencephalicus ventralis; T, thalamus; Td, dorsal thalamus; Te, telencephalic vesicles; Tv, ventral thalamus. Bars (b) 200 μm; (g) 153 μm.

ance of Tg protein (our work and Kawaoi and Tsuneda, 1985).

The simultaneous appearance of TTF-1 mRNA and protein contrasts with the situation of GHFl/Pitl, a transcription factor involved in the regulation of growth hormone (GH) gene expression in the developing pituitary of the mouse. In this system, although GHFl/Pitl transcripts appear between days 12.5 and 13.5 p.c. in the developing pituitary, GHFl/Pitl is post-translationally regulated and no protein is detectable until day 15.5 p.c., exactly with the appearance of the GH target gene transcripts (Dollé et al. 1990). However, in the developing rat pituitary, there is no evidence for the same translational regulation and a good temporal correlation is observed between the appearance of GHFl/Pitl and GH mRNA (Simmons et al. 1990).

TTF-1 expression in the lung

Lung and thyroid develop both from endodermal thickenings along the anterior wall of the pharynx and TTF-1 transcripts are detected at the beginning of their migration towards the surrounding splanchnic mesenchyme. The hybridization signal is restricted to the anterior migrating edge of the two diverticula and both the connecting stalks (thyroidal-groove and laryngotracheal groove) are negative (see Fig. 7). The detection of TTF-1 transcripts in restricted areas of the thyroid diverticulum and of the lung bud (i.e. in the anterior migrating edge) suggests that during the early morphogenesis of these two organs there are subsets of cells that are already committed. This hypothesis is supported by the finding that, in adult rabbit lung the trachea and bronchi and the bronchioli contain two different stem cell populations which are responsible for the renewal of the lining epithelia (Nettesheim et al. 1990). It is also of interest that two types of lung mesenchyme have been described: bronchial, which promotes formation of bronchial buds from tracheal epithelium, and tracheal, which inhibits bronchial formation (Wessels, 1970; Alescio and Cassini, 1962). The expression of TTF-1 in the bronchiolar epithelium and its absence in the upper respiratory airways correlates with the presence of these two different mesenchymes and could be the result of specific interactions of endodermal cells with regionally specialized mesoderm (see for example, Taderera, 1967; Spooner and Wessels, 1970).
Fig. 7. TTF-1 transcripts are asymmetrically distributed in the thyroid diverticulum and lung bud. A schematic diagram of the cranial gut (primitive pharynx, Ph) depicting the onset of migration of the thyroid diverticulum (Th) and the lung bud (Lb) from the anterior wall of the pharynx. The thyroid and lung develop in close proximity as two endodermal thickenings along the same median axis in the anterior wall of the pharynx, projecting into the surrounding splanchnic mesenchyme. Note that the TTF-1 transcripts (represented by shading) are restricted to the anterior migrating edge of the thyroid diverticulum and of the lung bud while the thyroglossal-duct (td) and the laryngo-tracheal groove (lt) are negative.

TTF-1 expression in the brain is restricted to structures of diencephalic origin

The expression of TTF-1 in the brain is specific for some diencephalic (e.g. hypothalamus, neurohypophysis, optic chiasma) or telencephalic structures of diencephalic origin (e.g. median corpus striatum, globus pallidus). There are not many examples of homeobox-containing genes that are expressed in such restricted areas of the CNS; En-2 and Hox-2.9 are expressed in the midbrain–hindbrain junction and in rhombomere 4, respectively (Davis and Joyner, 1988; Murphy et al. 1989; Wilkinson et al. 1989, for review see Lobe and Gruss, 1989).

An important aspect of the domains of TTF-1 expression in the brain is the correlation of some expression boundaries with precise anatomical or developmental landmarks. Although the segmental organization of the forebrain is unknown, in some cases molecular markers have been identified that may correspond to forebrain segmental territories (e.g. see Puelles et al. 1987; Mori et al. 1987; Layer et al. 1990). The hypothesis that the TTF-1 expression boundaries may correspond to segmental patterns in the forebrain is supported by the observation that genes related to TTF-1 are expressed in overlapping domains along the rostro-caudal axis of the developing forebrain (Price et al. 1991 and unpublished data).

Conclusion

The experiments presented in this paper suggest that TTF-1, isolated and defined as a maintenance factor for thyroid differentiation, may have a role in the commitment of the precursors for thyroid follicular cells, bronchiolar epithelium and diencephalic neuronal sub-populations. Furthermore, these experiments indicate that other factor(s), in addition to TTF-1, may be required for in vivo activation of the thyroglobulin promoter. In this context, it is interesting that the mRNA encoding Pax-8, a putative transcription factor, has been detected in the developing mouse thyroid, in addition to kidney and, transiently, in the CNS (Plachov et al. 1990). Furthermore, a specific set of Hox genes has been reported to be expressed in mouse thyroid at day 12.5 p.c. (Gaunt et al. 1988), but it is not clear whether their expression is restricted to endodermal cells. We are currently testing whether any of these factors may cooperate with TTF-1 in the activation of thyroid-specific transcription.

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