Reviews in Endocrine & Metabolic Disorders 2005;6:217–228

Thyroid Development and Effect on the Nervous System

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Key Words. **thyroid development, thyroid transcription factors, congenital hypothyroidism, deiodinases, brain gene expression, thyroid hormone receptors**

Introduction

The thyroid gland is the largest endocrine gland and the first endocrine structure to become recognizable during development. It is formed of two cell types, the thyroid follicular cells or thyrocytes, in which thyroid hormones are synthesized, and the parafollicular or C-cell, where calcitonin is produced. It consists of two lobes on either side of the larynx, connected by an isthmus that normally overlies the second and third rings of the trachea in man. The main regulator of thyroid function is the pituitary hormone thyrotropin (thyroid-stimulating hormone, TSH), which is under control of TSH-releasing hormone (TRH) secreted by the hypothalamus. Thyroid synthesis and secretion of thyroxine (T4) and triiodothyronine (T3), are maintained by a negative feedback loop involving inhibition of TSH and TRH synthesis by T4 and T3, and by tissue-specific and hormone-regulated expression of the three iodothyronine deiodinase enzymes that metabolize thyroid hormones (reviewed in [1]). These hormones are critical in many aspects of life, mainly during development where they regulate different aspects of growth and correct maturation of the brain. Regulation of thyroid function and the normal production of thyroid hormones thus depend on precise controls, one of which is the normal development of the thyroid gland.

This review will focus on thyroid gland formation, with special emphasis on recent studies of its morphogenesis and differentiation, and the critical steps for correct thyroid hormone production. The identification of several transcription factors expressed in a tissue-specific manner in the thyroid has helped to define the molecular events responsible for the onset of thyroid hormone production during fetal life. Since many thyroid disorders result from impairment in some of the events that occur during development, we will concentrate on defining these events and their implication in congenital thyroid disorders. Finally, the action of thyroid hormone during fetal and neonatal brain development will be defined in detail.

Most of the recent advances in understanding thyroid gland organogenesis and the role of thyroid hormone in the nervous system derive from studies in animal models, including mice and rats, although where possible this information will be integrated with that which is known in man.

Morphogenesis of the Thyroid Gland

In vertebrates, the thyroid gland has a dual embryonic origin. The most abundant thyroid follicular cells arise from the embryonic endoderm as a thickening in the ventral wall of the primitive pharynx. This thickening, the so-called anlage, is located between the first and the second branchial arches and is visible around embryonic day (E) E18–20 in humans and E8–8.5 in mice. The anlage outpouching of the endoderm forms a thyroid bud around E24 in humans and E9.5 in mice. The bud expands ventrally as a diverticulum, with rapid proliferation of the cells at its distal end, but remains attached to the pharyngeal floor by a tubular stalk called the thyroglossal duct. The precursors of thyroid follicular cells continue to proliferate distally then begin to proliferate laterally, which leads to formation of the characteristic bilobed structure. By E10, the thyroid primordium begins a caudal migration, accompanied by rapid elongation of the thyroglosal duct, which eventually fragments and degenerates at E30-40 in humans and E11.5 in mice, forming the foramen caecum as a reminiscence of the embryonic thyroid. Two days later, the thyroid primordium reaches its final position in the trachea; the two lobes expand considerably and the gland exhibits its definitive shape with the two lobes connected by an isthmus. The cells from the ultimobranchial bodies migrate, resulting in the incorporation of parafollicular C-cell, into the thyroid at El4 in mice and E60 in man, and thyroid organogenesis is completed. The first evidence of follicular organization appears with many small follicles disseminated within the gland at E15–16 in mice and around day 60 in humans. Thyroid development and migration should be viewed in conjunction with other

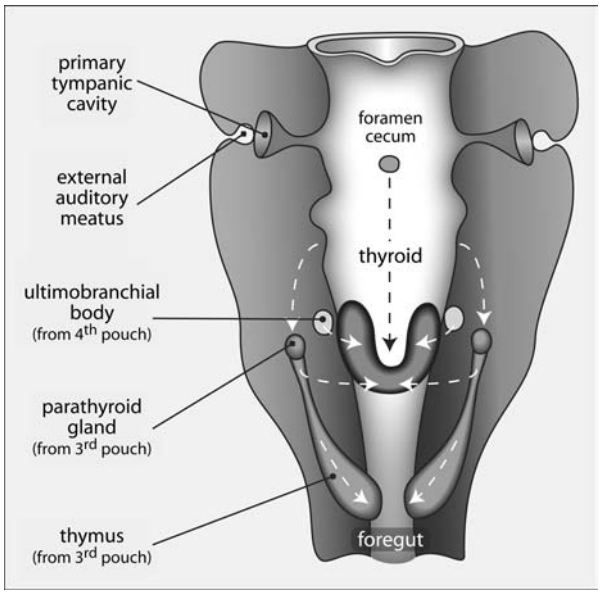

Fig. 1. *Structures derived from the pharyngeal region. The thyroid gland forms from the posterior migration of the thyroid anlage. This gland, derived from the ventral floor of the pharynx, migrates downward and joins with the ultimobranchial bodies, which contains the parafollicular C-cells. The foramen cecum is the reminiscence of the thyroid primordium. The parathyroid gland and the thymus derive from the third branchial pouch. Adapted from Manley and Capecchi [3].*

structures of the head and neck, such as the parathyroid gland and thymus [2,3] (Fig. 1).

Differentiation of thyroid follicular cells: onset of thyroid hormone biosynthesis

The differentiation program of thyroid follicular cells is completed only when the gland reaches its final location and the thyroid-specific genes necessary for thyroid hormone biosynthesis are expressed. This final, terminal differentiation occurs at El6 in mice and around E70 in man, with the first evidence of T4 secretion at El6.5 and E75, respectively. The thyroid-specific genes appear during development according to a specific temporal pattern: thyroglobulin (*Tg*), thyroperoxidase (*TPO*) and the TSH receptor (*Tshr*) genes are expressed by E14.5 [4,5] and the sodium iodide symporter (*NIS*) by E16 [6].

Correct expression of these genes is a requisite for correct synthesis of thyroid hormones T4 and T3. The major protein of thyroid cells is Tg, from which T4 and T3 are formed. These hormones are iodinated, an element that enters thyroid cells through a symporter located in the thyrocyte basolateral membrane. Once within the cells, iodide is transported through the apical membrane into the follicular lumen by anion transporters. Iodide oxidation and binding to the tyrosine residues of Tg, as well as the coupling of iodotyrosines to form T4 and T3, are catalyzed by TPO. For production of individual T4 and T3 molecules, iodinated Tg is endocyted in the apical membrane as colloid droplets and hydrolyzed by lysosome enzyme, and is the thyroid hormone released into the circulation [1]. TSH is the principal regulator of thyroid hormone biosynthesis and secretion. After binding to its G-protein coupled receptor, it increases cAMP and activates multiple signaling pathways that regulate both differentiation and proliferation of thyroid cells [7,8] (Fig. 2).

Molecular Mechanisms

Transcription factors involved in thyroid development

Molecular characterization of thyroid development started in the 90's when the thyroid-specific transcription factors were identified. These transcription factors, formerly termed TTF-1 (thyroid transcription factor 1), TTF-2 (thyroid transcription factor 2) and Pax8, bind to the promoter and enhancer regions of Tg, TPO, TSH-R and NIS, regulating their transcriptional activity. These transcription factors are expressed at the very beginning of thyroid development and are responsible for the differentiated thyroid phenotype. Other transcription factors important in thyroid development include Hhex, Hoxa3 and Eya1. All are required for either the early or the late step in thyroid morphogenesis.

(1) *Thyroid transcription factor 1 (TTF-1)*. This transcription factor, also denominated T/EBP or Nkx2-l is a homeodomain-containing transcription factor encoded by a single gene in the genomic locus *Titf-1*, located on chromosome 14ql3 in man and 12 in mouse. This factor was definitively renamed Titf1/NKx2.1 [9], and its molecular and biochemical characteristics were described in detail [10]. Its role in the initial steps of thyroid morphogenesis has been deduced from its exclusive expression at E8.5, coinciding with specification of the thyroid anlage, and continued expression in thyrocytes through all embryonic stages and in the adult. *Titf1/NKx2.1* is also expressed in the trachea, lung, in specific areas of the forebrain including the developing posterior pituitary [4], in certain hypothalamic areas, in the parafollicular C cells [11], and in the epithelial cells of the ultimobranchial body [12]. Its functional role has been studied by analyzing the phenotype of mice homozygous for targeted disruption of the gene. The knock-out mice have impairment lung morphogenesis, lack of thyroid and pituitary, alterations in the forebrain, and death at birth due to severe respiratory problems [13]. The thyroid primordium forms in the correct position in *Titf1/NKx*$2.1^{-/-}$ mice, but subsequently degenerates and disappears via apoptosis [14]. This demonstrates that this transcription factor is essential for survival of thyroid cell precursors, but is not required for

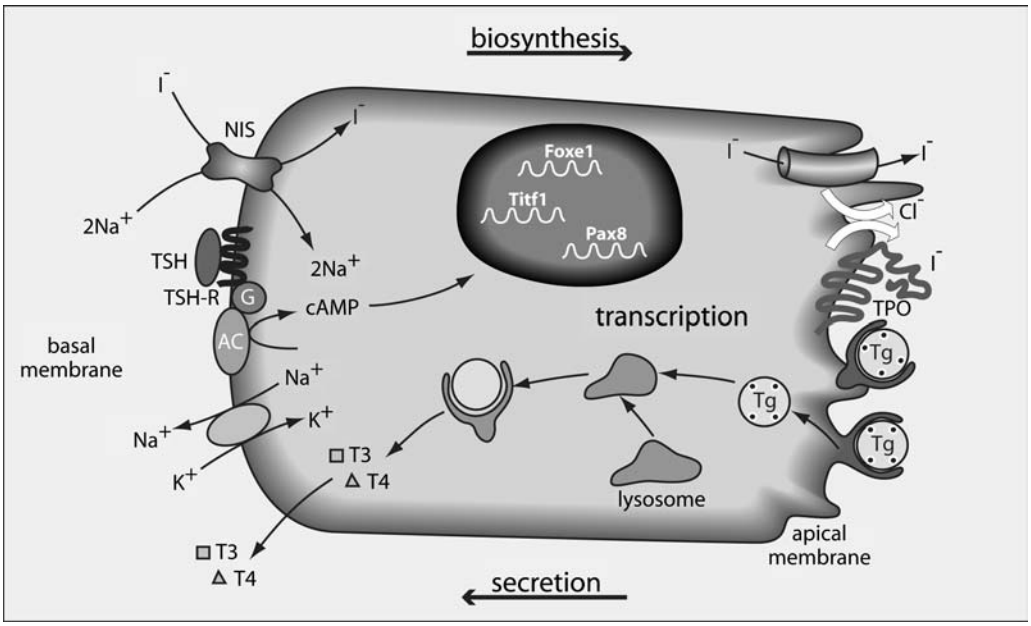

Fig. 2. *Schematic model of a thyroid follicular cell, showing the major intracellular structures and the main proteins involved in the biosynthesis and secretion of the thyroid hormones T4 and T3. They are epithelial cells, polarized with a basal and an apical membrane in the lumen of the thyroid follicule. Iodide ions (I^-) are concentrated in the basal membrane of the cells from the circulation by the NIS symporter, then rapidly transported into the follicular lumen by different ion channels. lodination of tyrosine residues occurs within Tg, after the I is oxidized by thyroid peroxidase (TPO), using H_2O_2. The iodinated Tg is taken up by endocytosis of colloid, and the colloid droplets fuse with lysosomes, after which Tg is enzymatically cleaved and the thyroid hormone released. The cell nucleus expresses thyroid transcription factors, including Titf1, Pax8 and Foxe1, which transcribe the thyroid genes NIS, TPO, Tg, and TSH receptors (TSH-R). The main regulator of thyroid cell differentiation is the hormone TSH, which after binding its receptor induces a signaling cascade, being cAMP the main of them.*

their initial specification and formation. *Titf−1/NKx2.1* is also responsible for the establishment of dorso-ventral patterning, both in the anterior pharynx and in the hypothalamus, and is implicated in epithelial/mesenchymal signalling [15,16].

The mechanisms responsible for *Titf1/NKx2.1* expression are not well known. The fact that FGF2 receptor is expressed in thyroid cells suggests that the growth factor FGF2 could be involved, as has been described for pituitary cells. In mice, inductive signals for the mesoderm could be implicated in the initial activation. Sonic hedgehog (Shh) is relevant for the expression of *Titf1/NKx2.1* in the forebrain, but not for its expression in the thyroid anlage [17].

(2) *Pax8, paired box gene 8*, is a member of the Pax family of transcription factors defined by a common DNA binding domain, the paired (Prd) domain. This transcription factor family is composed of nine members. Pax8, together with Pax2 and Pax5, form a sub-family. The *Pax8* gene in mice or *PAX8* in man is located on chromosome 2 in both species; its molecular and biochemical characteristics have been reviewed in detail together with the other thyroid transcription factor [10] During development, *Pax8* mRNA is expressed, coincident with *Titf1/NKx2.1*, only in the thyroid anlage [18] at E8.5; its expression continues through all stages of thyroid development as well as in the adult. This gene is also expressed in the nervous system at the beginning of development, but not in later stages or adult life, and in the kidney both during development and in adults.

Its function during thyroid development is deduced from the generation of *Pax8* null mice, which are born at expected frequencies but show growth retardation, probably due to low serum T4 concentration; they die within 2–3 weeks unless given thyroid hormone [12]. In the *Pax8* null embryos, the thyroid diverticulum is able to evaginate from the endoderm, but Pax8 is required for further development. In the absence of this factor, the thyroid primordium is smaller than in wild type embryos at El1.5, and at El2.5 the follicular cells are undetectable. Interestingly, in the thyroid primordium of Pax8 null embryos, expression of other transcription factors such as Foxe1 and Hhex is downregulated [19]. Pax8 has an important function not only in development, but also in thyroid cell differentiation, and it has been described is a master gene for regulation of the thyroid differentiation phenotype [20]. The morphogenesis of parafollicular C-cells is completed in Pax8 null mice, indicating that this gene has a role only in the follicular cells of the thyroid gland.

Various Pax2/5/8 family members have been identified in the thyroid of Xenopus and zebrafish, suggesting that these genes originated from a common ancestral gene that duplicated during evolution in chordates [21].

(3) *Foxe1*, formerly called thyroid transcription factor 2 or TTF-2, is a member of the winged helix/forkhead family of transcription factors, with a conserved DNA binding domain termed fkh. It was originally identified as a thyroid-specific nuclear protein that binds Tg and TPO promoters under hormonal stimulation [22–24]. The mouse *Foxe1* gene is located on chromosome 4-4C2 and the human *FOXE1* gene on 9q22.3. The gene encodes a protein that binds DNA and whose biochemical properties have been described in detail [10]. *Foxe1* mRNA is detected at E8.5 in the floor of the foregut, including the thyroid anlage and the most posterior region of primitive pharynx, where *Titf1/Nkx*2.1 and Pax8 are not expressed. In later stages of development, *Foxe1* is thus expressed in tissues derived from the pharyngeal arches and wall such as thyroid, tongue, epiglottis palate and esophagus. In the adult, it is expressed in the thyroid and not in the esophagus. Interestingly, Foxe1 is also expressed in ectoderm-derived structures in early development, such as the Rathke's pouch, which gives rise to various components of the anterior pituitary. In later stages of pituitary development, expression of this factor decreases while is expressed in other structures such as the secondary palate, the choanae and in the hair follicules [25].

This gene's expression pattern suggests that it has only a marginal role in specification of thyroid follicular precursor cells. This was confirmed by the thyroid phenotype of *Foxe1* null mice [26]. At E8.5, these mice show normal budding of the thyroid anlage, but at E9.5 the thyroid precursor cells are unable to migrate downward, remaining either attached to the pharyngeal floor or disappearing. These two distinct *Foxe1* null mouse phenotypes, agenesis or ectopic thyroid, can be explained as stochastic events during thyroid morphogenesis or possibly due to a difference in genetic background or sex-related factors [9]. The main function of Foxe1 is thus to control thyroid migration to its position along the trachea. These mice also show a cleft palate, possibly responsible for their early death and high serum TSH levels. The non-migrating thyroid follicular cells are able to complete the differentiation program, as they express Tg.

(4) *Hhex* (hematopoietically expressed homeobox) is a gene expressed in the anterior visceral endoderm and rostral endoderm in early mouse embryos; later, *Hhex* can be detected in the thyroid, liver, thymus, pancreas, lung and endothelial precursor cells. This gene is also expressed at high levels in adult liver. The mouse *Hhex* gene is located on chromosome 19 and human *HHEX* on chromosome 10q23.32. It is a homeotic protein with a proline-rich region important for transcriptional control.

Hhex null mice die in mid-gestation, and the thyroid is absent or hypoplastic and connected to the pharynx floor [27]. A very detailed study of the expression of all the genes important for thyroid development showed that in *Hhex* null mice, the thyroid anlage is formed and expresses *Titf1/Nkx*2.1, *Pax8* and *Foxe1* genes, indicating that specification of the thyroid cells at E9 does not require *Hhex* [19]. In the next developmental stage, absence of Hhex causes a decrease in proliferation and in the morphology of the thyroid bud. In *Titf1/Nkx*2.1 and *Pax8* null mice, *Hhex* is undetectable [9].

Together, these data suggest a regulatory network among all these transcription factors, decisive in the control of the first stages of thyroid development. The functions controlled by these factors have been described in detail by Parlato et al. [19]) and are outlined in a figure adapted from that article (Fig. 3). The events responsible for the onset of thyroid specification remain unknown. Hhex thus has an essential role in maintenance of *Titf1/Nkx*2.1, *Pax*8 and *Foxe1* expression, *Titf1/Nkx*2. and *Pax8* regulate proliferation, survival and differentiation of thyroid follicular cells, and *Foxe1*, their migration. In conclusion, all these genes regulate, in a spatio-temporal manner, various aspects of thyroid development including budding, migration, survival and proliferation of thyroid follicular precursor cells, differentiation and follicle formation. Finally, they are responsible for starting the thyroid differentiation program, controlling the expression of thyroid genes TSHR, Tg, TPO and NIS with the consecutive onset of thyroid hormone production (Fig. 4).

(5) *Other transcription factors*. Other transcription factors such as Hoxa3 and Eyal control late stages of thyroid morphogenesis.

The *Hox* genes encode a class of transcription factors with a highly conserved DNA binding motif, the *Antennapedia* homeodomain. They are distributed in four groups (A, B, C, and D) located on four chromosomes. Of these genes, *Hoxa3* is expressed in the floor of the pharynx in the developing thyroid, and the generation of null mice for this gene confirmed its role in thyroid organogenesis [3]. These mice lack a thymus, and have parathyroid and thyroid hypoplasia. More detailed analysis shows variable expression of the thyroid phenotype; the isthmus is absent or displaced, follicular cells decreased in number, and one lobe of the gland is absent or hypoplasic. In addition, the mice present alterations in the ultimobranchial bodies with a reduction in parafollicular C-cell number.

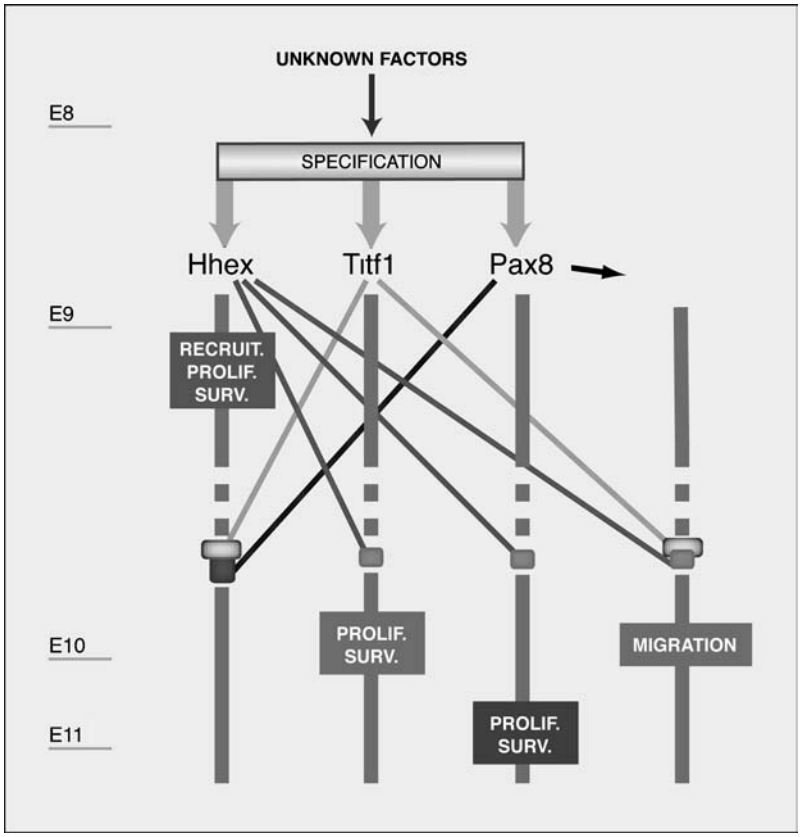

Fig. 3. *Network between transcription factors during thyroid morphogenesis. A hypothetical model showing the relationship between Hhex, Titf1, Pax8 and Foxe1 and the possible functions regulated. Adapted from Parlato et al. [19].*

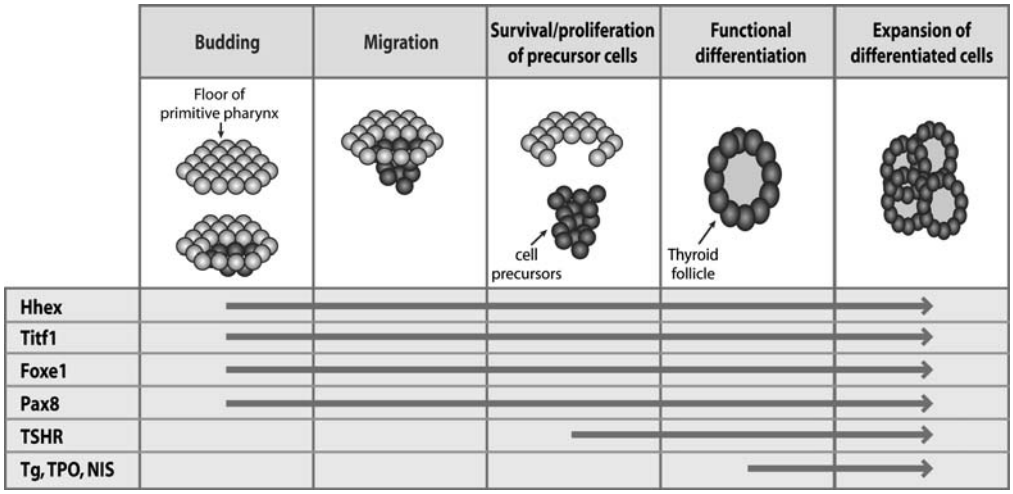

Fig. 4. *Different stages of thyroid development. Upper: names of the steps in thyroid organogenesis and differentiation. Middle: representative diagrams of these steps. The arrows in the lower part show the name of the thyroid transcription factors and the thyroid-specific genes necessary for the onset of thyroid hormone production. Modified from Macchia PE [34].*

The *Eya* (Eyes absent) gene regulates organogenesis in vertebrates and invertebrates, and has an important role in the morphogenesis of organs derived from the pharyngeal region, including the thymus, parathyroid and thyroid glands. It is expressed in the third and fourth pharyngeal regions and ultimobranchial bodies. Eyal null mice show thyroid hypoplasia, with severe reduction in the number of parafollicular C-cells and thyroid lobe size. These results suggest that Eyal controls critical early inductive events involved in the morphogenesis of the thyroid

gland and other organs derived from the pharyngeal region [28].

Signals involved in early steps of thyroid development

Despite the considerable information available on the transcription factors involved in the early steps of thyroid morphogenesis, there is little information on the inductive signals responsible for the formation and growth of the thyroid primordium and the signals that control migration and final organogenesis. Recent studies suggest that there may be an influence of the developing heart, since defects have been described in the foregut secondary to defects in heart organogenesis [29]. Cells from the cardiogenic mesoderm are thus the cells most likely to produce inductive signals responsible for the initiation of thyroid specification.

Concerning the signals responsible for thyroid migration, it has been shown that the precursor thyroid follicular cells may themselves have an active migration process [26]. This concept has been questioned however, as these cells do not undergo the classical epithelial-mesenchyme transition of other migrating cells. Migration of precursor thyroid follicular cells can thus be due to an unknown mechanism, and also to morphogenetic events in the neck region and in the mouth.

The mechanisms that control initiation of the organogenesis process, which involves proliferation and the formation of the two lobes at each side of the trachea, are unknown. Although TSH is the main growth stimulus, this hormone is not implicated at this developmental stage, suggesting that other signals are involved. Other growth factors such as IGF-1, EGF and FGF can promote thyroid cell growth in culture [7,8]. Moreover, these growth factors are expressed during embryonic life [30,31] and could be the primary regulators of thyroid growth at that time.

As for other endoderm-derived organs, the signals that control the final process of thyroid organogenesis may involve a stimulus from the adjacent mesenchyme [32]. Older data demonstrated that transplants of the presumptive thyroid region give rise thyroid tissue only when mesenchyme is present. In addition, follicular cells explanted from a developing chick thyroid required fibroblasts obtained from the capsule of the thyroid gland to organize a correct histological pattern. These observations suggest that cellular interactions are required for normal thyroid organogenesis [1].

In late stages of thyroid organogenesis, TSH has an important function. Expression of its receptor is detected at El13.5–El4 in mice, and increases greatly at El7. It is detected in the developing thyroid after the final migration of the precursor cells, before follicular organization. Information obtained from mice with a spontaneous or induced alteration of the *Tshr* gene indicate that during embryonic life, the TSH/TSHR signal is required to complete the differentiation program of thyroid follicular cells. TSH is not a global regulator of thyroid differentiation during organogenesis, as its expression is required for TPO and NIS, but not for Tg expression.

A sonic hedgehog (Shh)-derived signal was recently shown to have a central role in thyroid development, as Shh-null mice have ectopic thyroid or hemiagenesis [33]. The Shh signal controls *Foxe1* expression in the pharyngeal endoderm surrounding the thyroid primordium, but not in thyroid cell precursors [19]. More studies are needed to understand the role of this and other signals in thyroid specification, migration and final differentiation.

Role of Thyroid Transcription Factors in Congenital Thyroid Pathology

Most of the critical events in thyroid morphogenesis take place in the first 60 days of gestation in man or the first 15 days in mice. For this reason, most thyroid developmental abnormalities result from morphogenetic errors during this period.

Various studies have related mutations in thyroid transcription factors to abnormalities in thyroid development that result in congenital hypothyroidism (CH) [34,35]. This is the most frequent endocrine disorder in newborns, with an incidence of 1:3000; it is characterized by high TSH levels, due to reduced thyroid hormone levels. If thyroid hormones are not administered in the immediate postnatal period, CH causes mental retardation. In 85% of CH cases, the disturbances are in thyroid organogenesis. The phenotype described included thyroid agenesis or athyreosis, hypoplasia and ectopia; these entities are termed thyroid dysgenesis. Although these pathologies occur as sporadic disease, there is evidence that genetic factors are involved in the origin of these disorders. The fact that mutations in the genes involved in thyroid development give rise to animal models with thyroid dysgenesis suggests that this disease can be genetic and inheritable [9].

Relationship between genes involved in thyroid development and thyroid dysgenesis

Mutations in patients with congenital hypothyroidism have been described in trasncription factors *Titf1/Nkx2.1, Pax8 and Foxe1* genes, as well as in the *Tshr* gene.

For *TITF1/NKX2.1*, gene deletions as well as gene mutations have been described in the DNA binding domain. The thyroids of these patients are normal or hypoplastic, with symptoms of hypothyroidism due to high TSH levels. This phenotype is associated with other problems such ataxia, respiratory distress, neurological problems,

dyskinesia, and severe chorea, among others. For *Pax8*, mutations have been described in the DNA binding domain, that give rise to a protein unable to bind DNA and transcribe the corresponding thyroid-specific genes. The thyroids of these patients are mainly hypoplastic, although normal thyroid, as well as, athyreosis have also been reported. They are hypothyroid, with high TSH levels. A small deletion in the *Pax8* gene was recently described in a patient with congenital hypothyroidism and thyroid hypoplasia [36]. In the case of *FOXE1*, mutations have been reported at the DNA binding domain, with a phenotype of athyreosis. These individuals have high TSH levels and associated problems such as cleft palate, spiky hair and choanal atresia. For *TSHR*, many mutations are described in different regions of the seven-transmembrane protein. The thyroid can be normal or hypoplastic; this hypoplasia can be mild or severe, with high TSH levels. For a detailed review of all mutations and the implication of these genes in congenital hypothyroidism, see the recent review by De Felice and Di Lauro [9]. All the data discussed in that review indicate that disturbances of thyroid morphogenesis responsible for thyroid dysgenesis can be due to disturbances in the function of genes that regulate various aspects of thyroid development. They conclude that this pathology can be a heritable genetic disease.

The consequences of this disease are low thyroid hormone levels, with the corresponding high TSH levels, which ultimately cause severe mental retardation and other associated problems unless thyroid hormones are administered.

The Importance of Thyroid Hormone in Brain Development

Almost all body tissues are targets of thyroid hormones, but the brain is of special importance, since thyroid hormones are essential for correct brain maturation [37]. In man, lack of thyroid hormones during development is the single most frequent, preventable cause of mental deficiency and may lead to neurological damage. Available evidence indicates that thyroid hormones are needed for brain development from the end of the first trimester of gestation [38]. Until the fetal thyroid is fully functional, the maternal thyroid is the most important source of thyroid hormones for the fetus. They are gradually replaced by fetal hormones as the fetal thyroid matures but at term, more than 30% of human and 17% of rat circulating fetal thyroxine is still of maternal origin.

The relative roles of the maternal and the fetal thyroid for brain development explain the pathogenesis of developmental alterations associated with thyroid hormone insufficiency [39]. In endemic cretinism, if iodine deficiency is sufficiently severe as to cause failure of the maternal thyroid gland, damage to the fetal brain during the first half of gestation may lead, in addition to mental retardation, to neurological cretinism, with mental retardation, deaf-mutism, spastic diplegia, and normal stature, with no hypothyroidism, which is irreversible even with early thyroid hormone treatment. Another form of endemic cretinism, the so-called myxedematous cretinism, is characterized by hypothyroidism, short stature and psychomotor retardation but no deaf-mutism or neuromotor affectation, and can be improved with early thyroid hormone treatment. In congenital hypothyroidism, there are usually no neurological symptoms, and mental retardation can also be prevented with early replacement therapy. The lack of neurological symptoms, as in neurological cretinism, is due to the protective effect of the maternal hormones on the developing brain during the first half of gestation, despite failure of the fetal gland. In the last few years, it has become increasingly evident that not only hypothyroidism in the pregnant woman, but hypothyroxinemia, even without overt hypothyroidism, may put the developing fetal brain at risk [38].

Developmental Processes Controlled by Thyroid Hormones in the Brain

Most studies on the effects of thyroid hormones on the developing brain have been performed in the rat [40]. The developmental stage of the newborn rat is roughly equivalent to that of the human fetus in the 4th gestational month, and the newborn human is equivalent to a P10 rat. Thyroid hormones do not influence the three major early neural developmental processes, i.e., neural induction, neurulation, and the establishment of polarity and segmentation. Thyroid hormones are instead required for regulation of later events, such as cell migration and the formation of layers, in neuronal and glial cell differentiation and synaptogenesis. Combined maternal and fetal hypothyroidism causes deficiencies in cell migration in the neocortex, with poorly defined cortical layering and altered distribution of interhemispheric connections. Even transient maternal hypothyroidism can interfere with neocortical migration [41]. Altered cell migration is also observed in the hippocampus, resulting in a lower number of granule cells in the dentate gyrus. In the cerebellum, an organ that undergoes considerable maturation after birth, neonatal hypothyroidism causes altered migration of granule cells. During normal cerebellar development, granule cell precursors originate in the rhombic lip at the edge of the 4th ventricle, migrate, and continue to proliferate in a secondary germinative center, the external germinal layer of the cerebellum. After their last mitosis, the precursors migrate inward through the molecular layer to the internal granular layer. Granule cells migrate along the

radially-oriented processes (Bergmann processes) of Golgi epithelial cells, and differentiate as migration proceeds. This process, which in normal rodents is completed by the third postnatal week, is delayed in hypothyroid animals, and the external granule layer persists beyond P25.

In addition to cell migration, thyroid hormones control the differentiation of neurons, oligodendrocytes, astrocytes and microglia. Some classes of neurons are strongly affected by hypothyroidism; for example, the pyramidal cells of the neocortex and the hippocampus, and the Purkinje cells of the cerebellum. In the absence of thyroid hormone, development of the dendritic tree is compromised. In pyramidal cells, hypothyroidism results in a lower number and altered distribution of dendritic spines and fewer synaptic connections. The Purkinje cells of the cerebellum are even more strongly affected, with severe developmental arrest of their typically highly elaborated dendritic tree. Oligodendrocyte differentiation is dependent on thyroid hormones *in vivo* and *in vitro*, and thyroid hormone deficiency leads to myelination defects, consisting of a lower proportion of myelinated axons.

Transport and Control of Thyroid Hormone Concentrations in Brain

Thyroid hormone action involves binding to nuclear receptors and regulating gene expression. Of the two hormones secreted by the thyroid, T4 and T3, the latter is the receptor ligand. The T3 concentration in brain is not simply a function of free T3 in the blood, but is regulated by complex mechanisms. In addition to being secreted by the thyroid gland, T3 is also generated in extrathyroidal tissues from its precursor T4, by the action of 5′-iodothyronine deiodinases 1 and 2 (Dl and D2). In brain, most T3 bound to the nuclear receptors is formed locally by T4 deiodination, and is carried out by D2, since Dl is practically not expressed in nervous tissue [42]. D2 is expressed in astrocytes and in the tanycytes that line the walls of the third ventricle [43] (Fig. 5). Since the main target cells of T3 are the neurons, D2 expression in the astrocytes suggests that these cells produce T3 for neuronal use. The D2 substrate, T4, arrives to the astrocytes after crossing the membrane of capillary endothelial cells, which form the blood-brain barrier. T4 transfer may be facilitated by the presence in the endothelial cells of a thyroid hormone transporter, from the family of the organic anion transporting polypeptides, OATP14, which has higher affinity for T4 than for T3 [44]. Transport of T3 generated in the astrocytes to the neurons may also require another transporter, this time from the family of the monocarboxylate transporters, MCT8 [45]. The relevance of this transporter was recently demonstrated by the finding of mutations in humans, leading to an X-linked syndrome in infants with altered thyroid hormones in blood, normal or low T4 and high T3, and severe neurological defects. Affected children show developmental delay, spastic quadriplegia and lack of speech development [45,46].

Degradation of both T4 and T3 to the inactive metabolites rT3 and T2 is achieved by type 3 deiodinase (D3) that, in the central nervous system, is expressed in neurons. T4 thus crosses the blood-brain barrier and enters the astrocytes, where it is converted to the active hormone, T3. T3 is delivered to neurons, where it binds to nuclear receptors, and may be degraded to T2. T3 concentration in neurons is then regulated by activity of transporters and deiodinases located in different cells.

Mechanisms of Thyroid Hormone Action in Brain

Nuclear receptors

The nuclear pathway is the most important mechanism of thyroid hormone action, although extranuclear pathways have also been described. In the nuclear pathway, the active thyroid hormone is T3, which binds to nuclear receptors and modulates gene expression [47]. In mammals, T3 receptors are the products of two genes, TRα and TRβ. They encode nine protein products, which arise by alternative splicing of mRNA and differential promoter usage. The TRα gene encodes five protein products (TRα1, TRα2, TRα3, and the truncated products ΔTRα1 and ΔTRα2). of which only TRα1 binds T3. The TRβ gene encodes four T3-binding proteins, of which TRβ1, TRβ2 and TRβ3 also bind to responsive elements in DNA. In addition, the truncated protein ΔTRβ3 binds T3 but not DNA. There are therefore two types of receptors, α and β, and four different receptor isoforms (α1, β1, β2, β3). The physiological role of the non-receptor proteins is at present still unclear.

Thyroid hormone receptors, both in the unliganded (aporeceptor) and the liganded states, bind to hexameric sequences known as T3 responsive elements (TRE) located in regulatory elements of the target genes. The aporeceptor usually represses transcription by associating with corepressors (SMRT, NcoR, Alien), which recruit histone deacetylases, maintaining the chromatin in the compact, deacetylated state. After hormone binding, corepressors are released and coactivators and histone acetylases are recruited, so that transcription is allowed.

Thyroid hormone receptors have a distinct expression pattern in the developing brain. In man, the receptor is already present by week 10, before onset of fetal thyroid gland function, and increases several-fold during the second trimester, coinciding with major events in cortical and basal ganglia development [48]. This concurs with the role

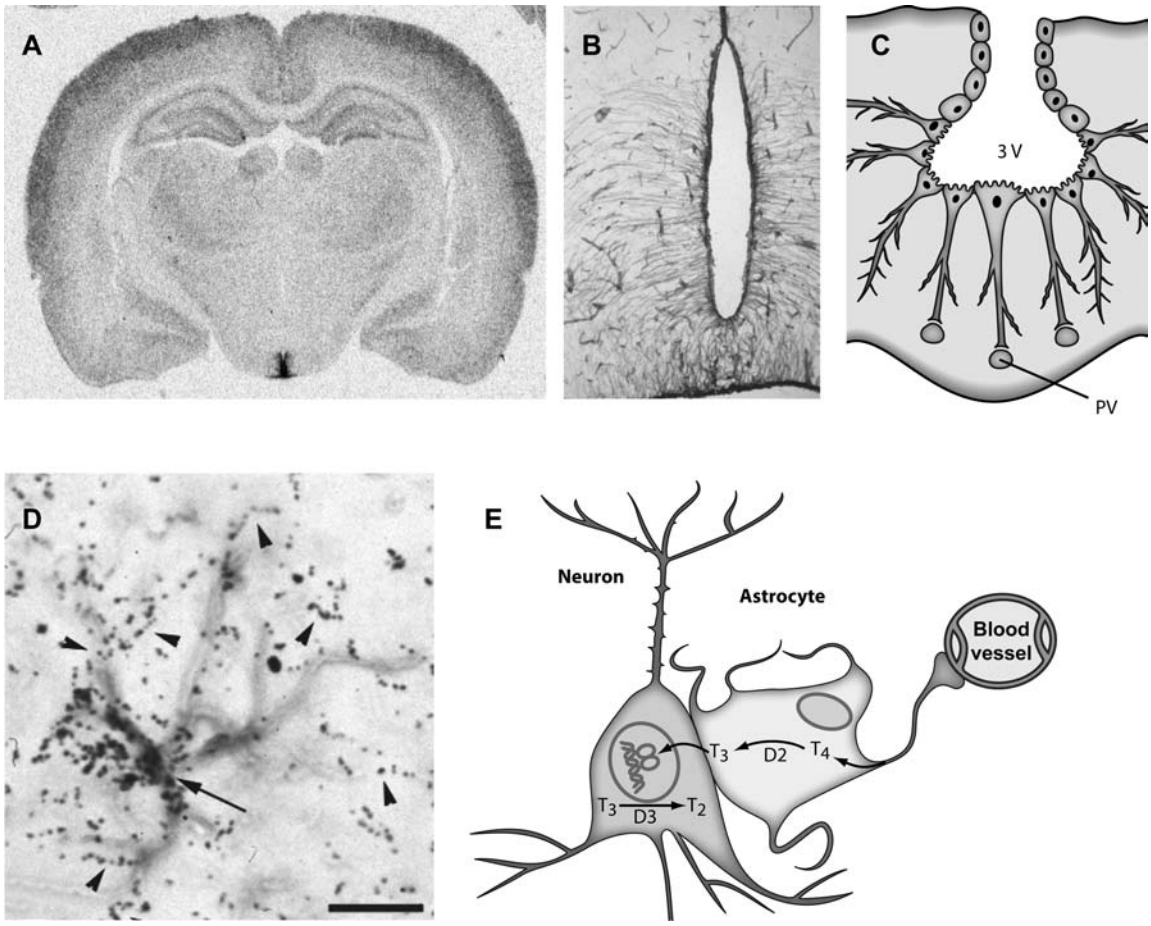

Fig. 5. *Source of T3 in the brain. (A) Expression of type 2 deiodinase in the rat brain. D2 mRNA is highly expressed in the basal hypothalamus, in tanycytes, cells lining the walls of the third ventricle. (B) Tanycytes in the third ventricle, stained with an anti-vimentin antibody. The cell bodies, situated in the wall of the ventricle, send processes to the adjacent hypothalamus and the median eminence; they often end in blood vessels, which in this preparation are also stained for vimentin. (C) Scheme depicting tanycytes ending in portal vessels of the median eminence, and the flow of substrates in the cerebrospinal fluid. (D) D2 is expressed in astrocytes, as shown by in situ hybridization for D2 combined with immunohistochemistry for glial fibrillary acidic protein. (E) Hypothesis for T4 uptake from blood vessels by astrocytes, its deiodination to T3 by the action of D2, and T3 transport to neurons. Neurons contain the nuclear receptors and also D3, which degrades T3 to the inactive metabolite, T2.*

of maternal thyroid hormone discussed above, and with the clinical picture of neurological cretinism. Also in rats, the receptor is present in brain by E14 and even earlier, several days before onset of fetal thyroid gland function. In the fetal brain, TRα is the principal receptor gene expressed [49,50]. The receptor expression pattern is mainly neuronal, although oligodendrocytes and, to a much lesser extent astrocytes also express the receptors.

The use of knock-out mice to examine the role of receptors during development has given paradoxical results [51]. In contrast to the profound effects of perinatal hypothyroidism on brain development, deletion of receptor genes did not result in obvious alterations in brain maturation. For example, we found that cerebellar structure was normal in TRα1-deficient mice, with no apparent differences in granular cell migration (under TRα1 control) or Purkinje cell differentiation (dependent on both TRα1 and TRβ1) between normal and knockout mice. To explain this discrepancy, we compared the effects of neonatal hypothyroidism in normal and TRα1 knock-out mice [52]. Hypothyroidism in wild type mice was associated with the usual defects of the cerebellum described above; hypothyroidism had no effect on the knock-out animals, however, suggesting that the effects of hypothyroidism are due to the activity (mostly gene repression) of the aporeceptor.

Thyroid hormone control of gene expression in the brain

Several genes are controlled by thyroid hormone during the late fetal and the postnatal period in the rat; a comprehensive description can be found in recent reviews [37,53]. Most genes so far been identified as targets of thyroid hormone in the brain are regulated by the late fetal and

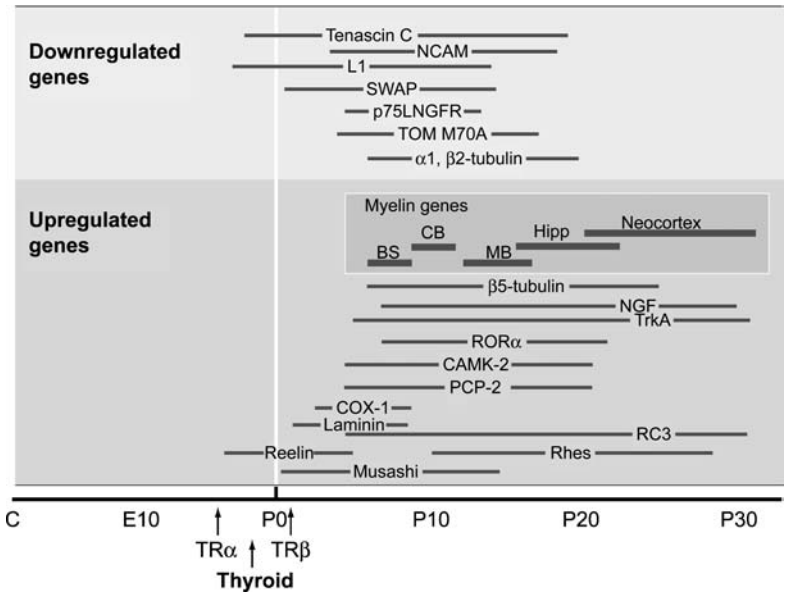

Fig. 6. *Genes regulated by thyroid hormone in the rat brain. Most genes regulated by thyroid hormone in brain have been studied during the postnatal period. In this figure, the period of sensitivity of up- or downregulated genes is shown relative to developmental time. For comparison, the approximate onset of thyroid gland function and the appearance of T3 receptors α and β are also shown. To illustrate the different timing in gene regulation, the period of sensitivity of the myelin genes in distinct regions is illustrated. BS: brain stem; CB: cerebellum, MB: midbrain; Hipp: hippocampus.*

postnatal periods (Fig. 6). The role of thyroid hormone is to accelerate up- or downregulation of these genes after birth. A good example is provided by the myelin genes, which are induced a few days after birth in parallel with the timing of oligodendrocyte differentiation and the myelination wave. In the absence of thyroid hormone, accumulation of myelin gene products, mRNA and protein, proceeds at a slower rate; final normal concentrations are attained, although later in development than in normal animals. Other genes show region-specific dependence of T3. An example is RC3, which is regulated by thyroid hormone only in discrete regions of the cerebral cortex and the dentate gyrus. The reasons for the temporal and region-specific control are not known. T3 response elements have been found in many of the regulated genes, and T3 might thus exert direct transcriptional control. Among the genes regulated in this way are the Purkinje cell specific gene (PCP2), encoding a G protein nucleotide exchange factor, the calmodulin binding and PKC substrate RC3 [54], prostaglandin D2 synthase, the transcription factor Hairless, neuronal cell adhesion molecule (NCAM), and the early response gene NGFI-A. Expression of other genes is regulated at the levels of mRNA stability (acetyl cholinesterase), protein translation (MAP2) or mRNA splicing (tau). Regulation of splicing might be indirect, and subsequent to primary action on the transcription of splicing regulators.

Some of the effects of thyroid hormone on the developing brain can be correlated with the expression of specific molecules. The most obvious is myelination. Lack of thyroid hormone during the postnatal period in the rat, at the time of onset of myelination, strongly delays the expression of many oligodendrocyte genes, specifically those genes encoding myelin proteins such as myelin basic protein (MBP), proteolipid protein (PLP), and myelin associated glycoprotein (MAG). The controlled expression of myelin genes, is not a primary effect of the hormone, however, but is secondary to an effect on oligodendrocyte differentiation. In this process, the relevant receptor gene is TRα, and the effect on differentiation may be due to control of proteins regulating the cell cycle, such as E2F-1 [55].

The effects of thyroid hormone on neuronal migration might be secondary to control of the expression of several classes of extracellular matrix proteins. One of these proteins is reelin, which is essential for the orderly migration of neurons to their specific destinations in the cerebral and cerebellar cortices; it determines the normal pattern of cortical layers. Reelin is regulated by thyroid hormone during late prenatal and early postnatal life in the rat [56]. It is not known whether the cells that produce this protein, the Cajal-Retzius cells, contain thyroid hormone receptors, but they express another thyroid hormone-regulated gene, prostaglandin D2 synthase, which has thyroid responsive elements and may thus be a direct target of thyroid hormone [57]. Other molecules reported to be involved in cell migration, such as laminin, tenascin C, and LI, are also under thyroid hormone control [58,59].

How thyroid hormone influences differentiation of neural cells is poorly understood. It was recently shown that T3 promotes neuronal differentiation of embryonic stem

cells in culture. The molecular mechanisms by which thyroid hormone promotes differentiation is unknown and, as mentioned above for the oligodendrocytes, they may act by regulating cell cycle proteins such as E2F-1, p53, cyclins, and cyclin-dependent kinase inhibitors [60]. Thyroid hormone control of neurotrophin expression, especially NGF, BDNF, and NT-3, may also provide an explanation for its effects on differentiation of particular cells, such as cholinergic neurons and Purkinje cells. In many cases, the effects of thyroid hormone on differentiation are subtle, although with potentially important functional consequences. For example, in hypothyroidism there is a decreased number of dendritic spines in pyramidal cells of the neocortex and hippocampus, which is reversible and is also observed after adult onset hypothyroidism [61]. Although the gross morphology of the neurons may not be appreciably modified, changes in spine density have strong consequences on synaptic plasticity.

Acknowledgements

Supported by grants BFI2002-00489 (MCYT), BFU2004-03169 (MEC), GR/SAL/0382/2004 and GR/SAL/0773/2004 from Comunidad de Madrid and FIS, Instituto de Salud Carlos III, Red de Centros RCMN (C03/08), RCGC(C03/10), de grupos RGDM (G03/212) y PI041216 (Spain).

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