REVIEW

Morphogenetics of early thyroid development

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Abstract

The thyroid develops from the foregut endoderm. Yet uncharacterized inductive signals specify endoderm progenitors to a thyroid cell fate that assembles in the pharyngeal floor from which the primordium buds and migrates to the final position of the gland. The morphogenetic process is regulated by both cell-autonomous (e.g. activated by NKX2-1, FOXE1, PAX8, and HHEX) and mesoderm-derived (e.g. mediated by TBX1 and fibroblast growth factors) mechanisms acting in concert to promote growth and survival of progenitor cells. The developmental role of TSH is limited to thyroid differentiation set to work after the gross anatomy of the gland is already sculptured. This review summarizes recent advances on the molecular genetics of thyroid morphogenesis put into context of endoderm developmental traits and highlights established and novel mechanisms of thyroid dysgenesis of potential relevance to congenital hypothyroidism in man.

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Introduction

Congenital hypothyroidism (CH) affects 1:2700 newborns according to a recent European survey (Loeber 2007). With a lower cutoff level for TSH screening (10–12 mU/l) the incidence increases to 1:1500 with most cases presenting with a normally located thyroid gland. With the higher TSH screening cutoff (20 mU/l) that has traditionally been used, impaired structural development of the thyroid gland (thyroid dysgenesis, TD) is instead the leading cause of CH (Corbetta *et al.* 2009). TD is morphologically highly variable, ranging from hypoplasia or total absence of the gland (variably referred to as athyreosis, thyroid agenesis, or aplasia) to ectopia of thyroid tissue (lingual or at more distant locations; Kratzsch & Pulzer 2008). The variable features of TD indicate that the pathogenesis involves multiple developmental defects affecting such different processes as specification and expansion of progenitor cells, budding and migration of primordial tissues, and proliferation of cells forming the embryonic thyroid. Congenital heart defects are overrepresented among these children, suggesting a developmental relationship between the thyroid and the cardiovascular system (Olivieri *et al.* 2002).

In higher vertebrates the thyroid develops from the anterior foregut endoderm (Fig. 1) in which progenitor cells expressing four critical transcription factors, NKX2-1, PAX8 (or Pax2.1a in zebrafish), FOXE1, and HHEX, assemble to form the thyroid bud (Fagman & Nilsson 2010). The growing bud subsequently delaminates from the pharyngeal endoderm and moves downward to the final anatomical position of the thyroid, a process that also involves incorporation of the ultimobranchial bodies whereby C-cell precursors are brought to the thyroid. During morphogenesis, embryonic tissues surrounding the developing thyroid rapidly become increasingly complex designated by the formation of a series of pharyngeal arches and pouches collectively constituting the pharyngeal metamere, a repetitive motif of body segmentation (Graham 2008). The endoderm itself and the adjacent mesoderm provide morphogenetic signals that act reciprocally and contribute to segmentation of the pharyngeal region (Zhang *et al.* 2005, 2006, Arnold *et al.* 2006, Graham 2008). The picture is further complicated by the fact that the foregut endoderm is responsive to diffusible factors derived from the developing heart (Kaestner 2005, Zaret & Grompe 2008). The key questions in thyroid development, which remain to be elucidated, are how a subset of undifferentiated endodermal cells is directed toward a thyroid fate, and which embryonic factors drive the proliferation and differentiation of thyroid progenitors. Notably, TSH acts on the thyroid first in late development, i.e. after the morphogenetic process is completed (Postiglione *et al.* 2002).

A wealth of information on the development of other endoderm derivatives such as the lungs, liver, and pancreas is rapidly accumulating and a coherent picture on the molecular machinery involved is starting to emerge (Zaret & Grompe 2008). In comparison, current knowledge on inductive and regulatory signals of thyroid morphogenesis is still limited. This review will therefore start with a more general outlook on current concepts of endoderm development and regionalization in early foregut development that might be relevant for the understanding of thyroid specification. Recent advances on early stages of embryonic thyroid development will thereafter be discussed. Finally, current views on the causes of TD in human patients and some aspects of embryonic stem (ES) cells will be briefly considered. For recent comprehensive reviews on thyroid morphogenesis in the most important animal models (mice, chicken, and zebrafish), see Porazzi *et al.* (2009) and Fagman & Nilsson (2010).

How are organs specified in the early endoderm?

General aspects of endoderm development and regionalization are discussed in detail in excellent recent reviews (Grapin-Botton & Constam 2007, Zorn & Wells 2009). In this review, we will consider only a few aspects of particular relevance in the context of thyroid development, which highlight potentially novel mechanisms for specification defects that might underlie thyroid agenesis/athyreosis. The key regulators of endoderm formation at gastrulation are NODAL factors (of the transforming growth factor β family) and an elaborate network of downstream components including the transcription factors FOXA2, SOX17, and GATA4–6 (Grapin-Botton & Constam 2007, Zorn & Wells 2009). As the definitive endoderm is rapidly remodeled into the primitive gut tube, the endoderm becomes broadly regionalized along the anterior–posterior axis designated by discrete patterns of gene expression. For instance, foregut identity is closely linked to the expression of HHEX, FOXA2, and SOX2. Patterning is further refined and elaborated by overlapping growth factor signals from nearby tissue layers to precisely delimit the regions of primordial outgrowth that initiates the process of organogenesis (Zorn & Wells 2009). Regional movements in the early endoderm appear to be highly dynamic as demonstrated by cell tracking studies on the developing liver and pancreas, which show that initially far distant progenitors converge into their prospective anlagen (Tremblay & Zaret 2005, Franklin *et al.* 2008). In zebrafish, detailed fate mapping has revealed that thyroid precursors derive from the endoderm at the level of the mid–hindbrain boundary closely apposed to the anterior lateral plate

mesoderm from which the heart later develops (Wendl *et al.* 2007). BrdU labeling studies in mouse embryos suggest that the thyroid bud grows by recruitment of cells from the neighboring endoderm rather than on-site proliferation of progenitors within the thyroid placode (Fagman *et al.* 2006). Cell tracking studies might be helpful to elucidate more precisely the region of the anterior endoderm from which the thyroid cell lineage derives in higher vertebrates.

Endoderm progenitor fate is influenced by the surrounding tissues such as the notochord and yet undifferentiated mesoderm (Wells & Melton 2000). Of particular interest, the early foregut rapidly changes its relationship to the mesoderm as the gut tube closes and glandular derivatives of the endoderm and the heart and central vessels develop in a coordinated manner. In this process, mesoderm regions that generate inductive signals such as fibroblast growth factors (FGFs), WNT, retinoic acid (RA), and bone morphogenetic proteins seem to gradually shift their projection toward the adjacent endoderm. This means that the response to a given morphogen is spatially and temporally restricted, and that this influence changes topologically over time to regulate endoderm development and differentiation. For example, in *Xenopus*, wnt signals are initially necessary to generate anterior endoderm, thereafter repressed to maintain foregut identity, and in a subsequent phase re-expressed to participate in hepatic organogenesis (McLin *et al.* 2007, Zorn & Wells 2009). The actual concentration of a given morphogen also affects cell fate as demonstrated in explant cultures of the endoderm: low levels of FGF promote the expression of pancreatic genes, intermediate doses activate a liver program, and high concentrations of the same factor induce NKX2-1 specific for thyroid and lung progenitor cells (Serls *et al.* 2005).

The homeobox transcription factor HHEX has a central role in early thyroid development (Martinez Barbera *et al.* 2000), although the mechanism is yet largely unknown. Transcription factors have generally been assumed to exert their influence on the specification of endoderm derivatives by cell-autonomous regulation of tissue-specific genes. However, a more indirect mode of action of HHEX on organ specification in the embryonic endoderm has been demonstrated more recently. During gut tube closure, HHEX promotes endoderm growth beyond the cardiogenic mesoderm. This appears crucial to divert the pancreatic lineage from a common hepatopancreatic anlage and the juxtapositioned heart mesoderm that positively regulates hepatic differentiation (Bort *et al.* 2004). This is a conceptually interesting mechanism by which a transcription factor influences organ specification in the endoderm by modifying the position of receptive domains to inductive signals generated in the nearby mesoderm. The complexity of morphogenetic

regulation is further emphasized by the fact that HHEX-mediated effects may also involve inhibitory signals. For example, the negative control of *hhex* expression in the endoderm by the wnt/β-catenin signaling pathway must be locally repressed to establish and maintain foregut identity in *Xenopus* (McLin *et al.* 2007). wnt antagonism further induces heart formation via activation of hhex in the endoderm (Foley & Mercola 2005). Foregut endoderm and adjacent mesoderm development are thus regulated reciprocally by multiple non-cell-autonomous mechanisms.

Taken together, as the primordia of endoderm-derived organs (thyroid, lung, liver, and pancreas) emerge from the foregut (Fig. 1) this is not due to a one-to-one mode of action of organ-specific inductive signals, but the consequence of differential responsiveness of endoderm progenitors to a combination of broadly distributed morphogens. These factors are generated in the mesoderm and exist as graded concentrations in the local environment during restricted time windows as embryonic development continues (Grapin-Botton & Constam 2007, Bayha *et al.* 2009, Zorn & Wells 2009). With this perspective in mind, it can be hypothesized that thyroid agenesis may be caused by mismatched juxtapositioning of the prospective thyroid field in the foregut endoderm to inductive signals in the nearby mesoderm, or primarily lack of or inappropriate levels of these signals, rather than by impaired expression or
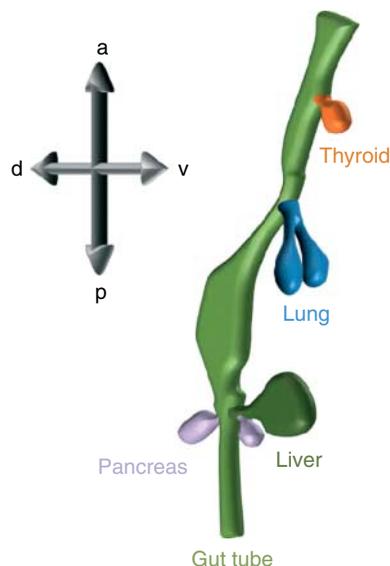

Figure 1 Organ bud outgrows along the anterior–posterior axis of the embryonic gut tube. The thyroid delaminates from the most anterior portion of the ventral foregut endoderm; close to thyroid bud are the thymus and parathyroid primordia and the ultimobranchial bodies formed from lateral endoderm of the posterior pharyngeal pouches (not shown). Note that the developing pancreas is specified as separate ventral and dorsal primordia. a, anterior; p, posterior; v, ventral; d, dorsal.
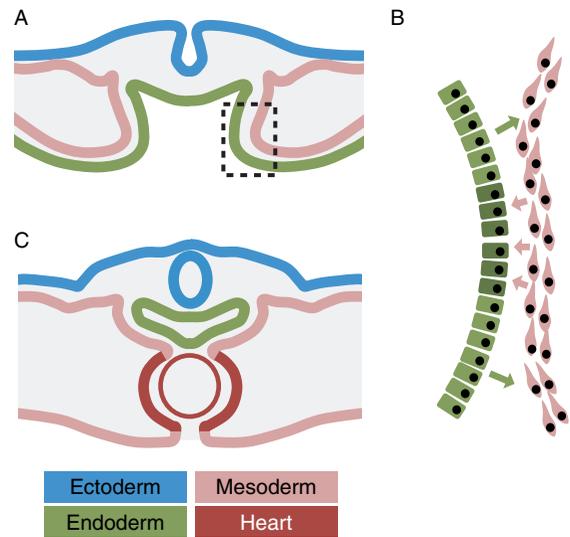

Figure 2 (A) A schematic illustration of germ layer interactions during formation of the primitive gut tube. The endoderm is in close proximity to the lateral plate/cardiogenic mesoderm, providing an anatomical foundation for cross signaling between cell populations (boxed region; see B). The spatial relationship in early development between the endoderm destined to a thyroid fate and lateral plate mesoderm/cardiac mesoderm has not yet been clearly defined in mice. (B) Mesodermal cells may elaborate diffusible factors that affect the morphogenesis in the adjacent ventrolateral endoderm. Moreover, signaling in the opposite direction (i.e. from endoderm to mesoderm) occurs. It is assumed that the endoderm in this location may contain the prospective thyroid field in which thyroid progenitor cells are specified before entering the thyroid placode in the pharyngeal floor not yet formed. (C) After the gut tube is closed, the thyroid placode is located in the midline of the pharyngeal endoderm directly apposed to the developing heart and outflow tract (see Fig. 3) Adapted, with kind permission from Sinauer Associates Inc., from Gilbert SF 2000 Lateral plate mesoderm and endoderm. In *Developmental Biology*, 6th edn, pp 471–501. Sunderland: Sinauer Associates, Inc.

function of a single putative thyroid-specific master gene. The close spatial relationship between the ventral pharyngeal endoderm and the cardiogenic mesoderm, and the fact that these tissues are mutually dependent on reciprocal signaling for progenitor cell proliferation and survival (Cai *et al.* 2003), raise the possibility that concurrent thyroid and heart malformations may develop from shared pathogenetic mechanisms operating in early organogenesis. The concepts of this hypothesis are summarized in Fig. 2.

What can we learn about the embryonic thyroid from animal models?

Early thyroid development in mice depends on the conjoined activity of NKX2-1, PAX8, FOXE1, and HHEX to proceed normally (De Felice & Di Lauro 2007, Fagman & Nilsson 2010). However, neither of these

transcription factors is alone required for the specification of thyroid progenitors in the foregut endoderm (Parlato *et al.* 2004). Hence, in mutant mouse embryos deficient in NKX2-1, PAX8, FOXE1, or HHEX the thyroid primordium is specified and starts to form a bud that later regresses by yet unknown mechanisms, although lack of survival signal(s) leading to progenitor cell death by apoptosis is likely involved. The only exception to this fate is *Foxe1* mutants in which nearly 50% of the embryos show a sublingual thyroid rudiment mimicking the picture of thyroid ectopia (De Felice *et al.* 1998). Further studies have proposed that FOXE1 regulates migration of the delaminated thyroid primordium by a cell-autonomous mechanism, i.e. the drive of migration is intrinsic to the thyroid precursors (Parlato *et al.* 2004). The mechanism and target gene(s) activated by FOXE1 to accomplish this effect have not been identified. In zebrafish, the Foxe1 ortholog is expressed in the thyroid progenitors but knockdown experiments did not reveal any effect on thyroid morphogenesis (Nakada *et al.* 2009). It should be kept in mind that the zebrafish thyroid forms follicles very early during development (Wendl *et al.* 2002), and that such long-distance migration of a solid nest of undifferentiated progenitors as seen in the mouse embryo might not take place. It is thus possible that some steps in thyroid morphogenesis are fundamentally different in mice and fish and therefore regulated differently.

Besides NKX2-1, NKX2-3 and NKX2-5 are expressed in the thyroid bud at the early stages of development (Lien *et al.* 1999, Biben *et al.* 2002). NKX2-5 is particularly interesting since it is expressed also in the developing heart (Lints *et al.* 1993), suggesting that this transcription factor regulates common developmental traits in the two organ primordia. Indeed, NKX2-5-deficient mouse embryos show thyroid bud hypoplasia in addition to cardiac defects (Lyons *et al.* 1995, Biben *et al.* 2000, Dentice *et al.* 2006). In addition, a number of *NKX2-5* mutations were detected in patients with thyroid ectopia or athyreosis (Dentice *et al.* 2006). Interestingly, *NKX2-5* mutations have been identified in human patients with a range of other cardiac abnormalities (Schott *et al.* 1998). NKX2-5 may thus be one of several factors associated with the increased prevalence of cardiac malformation in children with CH (Olivieri *et al.* 2002). As NKX2-5 binds to and activates the same promoter regions as NKX2-1 (Ray *et al.* 1996) it is possible that the phenotype is partially compensated for by functional redundancy between these transcription factors.

Animal embryo studies also show that factors acting non-cell autonomously, i.e. the factor is not expressed or produced by the target cells themselves, influence the earliest stages of thyroid morphogenesis. For example, in both zebrafish (Stafford & Prince 2002) and chick (Bayha *et al.* 2009) it is known that RA

generated in the mesoderm confers a posterior identity to the endoderm and that experimentally induced higher RA levels shift posterior endoderm markers anteriorly. A consequence of increased RA activity is the absence of thyroid progenitors as signified by the lack of Hhex and Nk2.1a expression in the prospective thyroid field. Conversely, repressed RA activity alters the positional identity of thyroid progenitors toward the posterior foregut in zebrafish (Stafford & Prince 2002). This indicates that RA negatively regulates the adoption of a thyroid fate in responsive regions of the endoderm. However, additional factors are most likely required, as suggested from the observations in chick embryos that deficiency of RA leading to the enlargement of the HHEX-positive domain in the foregut endoderm is not sufficient to increase the number of NKX2-1-expressing cells (Bayha *et al.* 2009). It is worth mentioning that RA and FGF4 are known to coordinately regulate endoderm patterning and that FGF4 also represses the establishment of anterior cell identity in the chicken gut tube (Dessimoz *et al.* 2006). A putative role of FGF4 in early thyroid development is yet to be investigated.

Other FGFs derived from the mesoderm evidently influence thyroid morphogenesis. Mouse thyroid and lung development take place rather closely in the anterior foregut and both primordia require the cell-autonomous action of NKX2-1 to proceed normally (Fig. 3). Common developmental traits are further suggested by the fact that both organs are missing in FGF10-deficient mouse embryos (Ohuchi *et al.* 2000). The mechanism has not been elucidated in detail, but indirect evidence suggests that FGF10 is differently regulated in thyroid and lung regions respectively. First, the expression of FGF10 in lung mesenchyme depends on RA whereas FGF10 in mesoderm adjacent to the thyroid bud does not (Desai *et al.* 2004). Consequently, in mice deficient of RA activity the lung bud fails to develop whereas thyroid morphogenesis is seemingly unperturbed (Desai *et al.* 2004). Second, although the FGF10 expression in the vicinity of both lung and thyroid is controlled by canonical WNT signaling (Chen *et al.* 2010), lung specification is abolished while thyroid progenitors are readily specified in *Wnt2/2b* knockout mouse embryos (Goss *et al.* 2009).

Studies in both zebrafish and mice indicate that Fgf8 has a central role in early thyroid development. Fgf8-deficient zebrafish embryos show a severely hypoplastic thyroid (Wendl *et al.* 2007). The tissue source of Fgf8 regulating this process is not yet identified, although it requires the transcription factor dHand that is expressed in cardiac mesoderm. Hence, locally administered Fgf8 (or Fgf1, Fgf2) rescues the thyroid phenotype in dHand-deficient embryos. As Fgf8 is unable to ectopically induce thyroid progeny in other parts of the endoderm (Wendl *et al.* 2007), this suggests that the action is permissive rather than inductive on
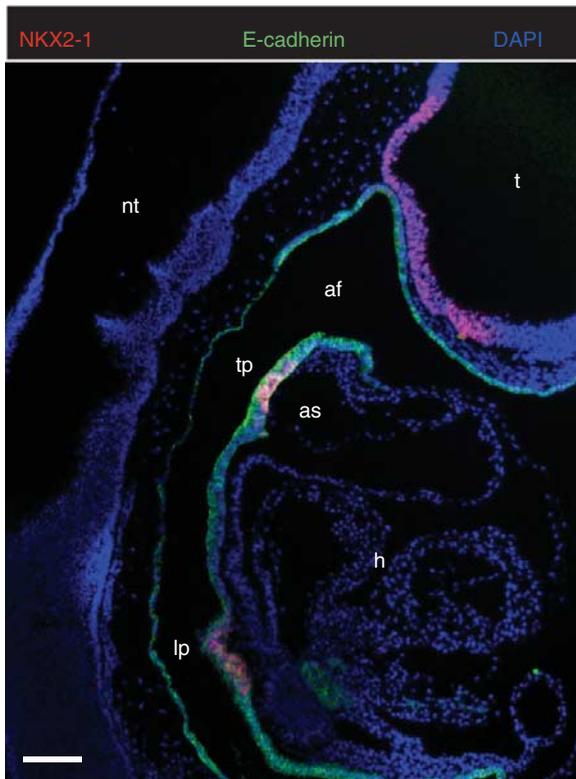
Figure 3 Regionalization and organ specification in the anterior foregut of an E9.5 mouse embryo. In the otherwise inconspicuous endoderm lining of the gut tube (stained for E-cadherin expression) discrete subsets of cells destined to thyroid and lung lineages are recognized by their expression of NKX2-1. Outside the endoderm, NKX2-1 is also abundant in neuroectoderm in the telencephalic floor. Knockout of *Nkx2-1* in mice leads to athyreosis, severe lung dysplasia, and forebrain anomalies (Kimura *et al.* 1996). A corresponding phenotype is observed in patients haploinsufficient for *NKX2-1* (Krude *et al.* 2002). Note that the thyroid anlage is seen closely apposed to the cardiac outflow tract forming the aortic sac. af, anterior foregut; as, aortic sac; h, heart; lp, lung primordium; nt, neural tube; t, telencephalon; tp, thyroid primordium. Scale bar = 100 μm.

thyroid specification in zebrafish. In mice, FGF8 was recently shown to stimulate the generation of endoderm progenitors committed to a thyroid fate (Lania *et al.* 2009). This may in part explain thyroid hypoplasia in mice deficient of TBX1, previously known to regulate embryonic thyroid growth (Fagman *et al.* 2007). TBX1 is expressed in the mesoderm adjacent to the thyroid primordium (Fagman *et al.* 2007). Ablation of *Tbx1* specifically in the mesoderm mimics the thyroid phenotype of *Tbx1* null mice and overexpression of *Fgf8* in the mesoderm partly rescues the thyroid defect (Lania *et al.* 2009). Together, this provides a direct proof of a central role of mesoderm in thyroid development in higher vertebrates. Moreover, in view of the fact that *TBX1* is the major candidate gene in DiGeorge or 22q11 syndrome this suggests that hypothyroidism

sporadically encountered in these patients (Stagi *et al.* 2010) may be the result of TD on the basis of an impaired non-cell-autonomous mechanism.

It was recently reported that the final size of the mouse pancreas critically depends on the number of progenitor cells initially generated in the endoderm (Stanger *et al.* 2007); at difference with the concurrently developed liver, the pancreas has no capacity of compensatory growth in late development. The data discussed earlier for *Tbx1* mutant mice (Lania *et al.* 2009) and similar findings in *cyclops* (a Nodal ligand), *dHand*, and *fgf8* zebrafish mutants (Elsalini *et al.* 2003, Wendl *et al.* 2007) raise the possibility that the number of progenitors recruited to a thyroid fate may limit the final size of the thyroid gland by a similar mechanism. However, there are observations suggesting that embryonic thyroid growth not only depends on factors involved in preformation of the anlage in the endoderm but that later events in organogenesis also contribute. For example, the thyroid develops closely associated with embryonic vessels (Fagman *et al.* 2006). In *Tbx1* null mutants, the hypoplastic thyroid reminiscent of hemiagenesis in the mature phenotype probably relates to failure of the primordium to establish contact with the aortic sac and branchial arch vessels during critical stages of budding and migration, and that this seemingly interfere with the bilobation process and further expansion of the thyroid lobes (Fagman *et al.* 2007). The importance of FGFs for the separation, migration, and survival of pharyngeal endoderm-derived organs is further suggested from studies on mice deficient in FRS2α, a docking protein that links FGF receptor activation to downstream signaling pathways (Kameda *et al.* 2009). It is thus conceivable that fetal thyroid size is determined both by the progenitor cell number generated while the primordium is still an integral part of the foregut endoderm and by the influence of surrounding embryonic tissues that promote progenitor cell proliferation in the subsequent morphogenetic stages of thyroid development (Fig. 4). An interesting question is whether a congenitally hypoplastic thyroid shares the ability of the normal gland to respond to goitrogenic stimuli, e.g. iodine deficiency, or whether reactive compensatory growth after birth is restrained by properties inherited to the developmental defect. If so, this would increase the risk of developing overt hypothyroidism in individuals with occult TD.

What can we learn from embryonic stem cells?

ES cells constitute an easily manipulated model system to explore how cell fate decisions are regulated by diffusible factors. It is anticipated that findings on
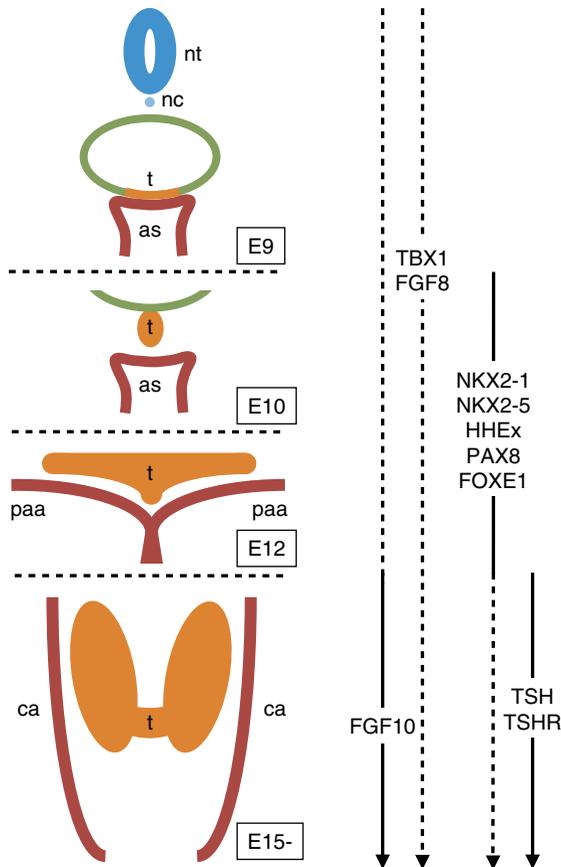
Figure 4 A schematic view of thyroid morphogenesis in mouse development. Cells characterized by their conjoint expression of NKX2-1, PAX8, FOXE1, and HHEX form a placode at E9 in the ventral region of the anterior gut tube (green) juxtaposed to the cardiac outflow tract (aortic sac). The bud of thyroid precursors, which assembles at E10, subsequently delaminates and moves downward in the neck. Between E12 and E13 the growing thyroid primordium elongates laterally along the course of pharyngeal arch arteries. After fusion with the ultimobranchial bodies (at E13.5), bilateral lobes can be clearly distinguished, and at E15.5 the final gross anatomy of the thyroid is evident. Arrows indicate the different time periods when the action of transcription factors (NKX2-1, NKX2-5, PAX8, FOXE1, HHEX, and TBX1), diffusible morphogens (FGF8 and FGF10), and TSH receptor (TSHR) signaling has been shown to be decisive to thyroid development (solid lines indicate the earliest stages where an impact on thyroid development has been demonstrated; dashed lines indicate that the onset or continued effect on morphogenesis of the factor(s) is not well defined).

ES-cell differentiation can be translated to regulatory networks governing embryonic tissue and organ development *in vivo*. Conversely, signaling pathways found to regulate the early steps of organogenesis have been applied in step-wise differentiation protocols of ES cells to recapitulate endogenous development in the test tube. For endoderm-derived organs this has been investigated in particular for the pancreas. By sequential addition of activin (an activator of

NODAL), WNT, FGF10, cyclopamin (an inhibitor of SHH), and RA it has been possible to generate multipotent pancreatic progenitor cells expressing PDX1, and even terminally differentiated insulin-producing β-cells, from human ES cells (D'Amour *et al.* 2006, Kroon *et al.* 2008). Transplantation of β-cells generated from autologous stem cells in diabetic patients is thus a future realistic prospect.

The potential benefit of using ES cells in thyroid investigations was recently highlighted (Lin & Davies 2010). This is still a largely unexplored field, although several interesting observations have been made. C-cells differentiated from mouse ES-cell-derived embryoid bodies (EB) express thyroid-specific genes and also form follicular structures. Furthermore, expression of PAX8 and the TSH receptor is positively regulated by TSH stimulation (Lin *et al.* 2003, Arufe *et al.* 2006, Jiang *et al.* 2010). In mice, TSH receptor signaling is previously known to promote thyroid differentiation but is dispensable to thyroid morphogenesis (Postiglione *et al.* 2002, De Felice *et al.* 2004). Moreover, in the mouse embryo thyroid differentiation takes place after the morphogenetic process is completed and the final thyroid anatomy is already obvious (Postiglione *et al.* 2002, Fagman *et al.* 2006). It is thus likely that TSH-stimulated thyroid differentiation as observed in EB-derived cells represents a late stage of thyroid development. A limitation of the EB model for morphogenetic studies is inherited to the inability to recapitulate the morphogenetic mechanisms that require the conjoined development of vasculature and a functional microcirculation. However, the potential of using ES cells for identifying additional factors implicated in the commitment of an undifferentiated endoderm progenitor to a thyroid cell fate is indicated by a recent study in which activin was found to promote the expression of thyroid markers in EB cells (Ma *et al.* 2009). Activin signaling is central in early development regulating endoderm formation by the NODAL pathway (Schier 2003). This finding is thus consistent with the endodermal origin of thyroid follicular cells.

The transcriptional regulation in cell lines is in some aspects reminiscent of embryonic regulatory programs, suggesting that studies of cells in culture may fertilize our understanding of embryonic development. An example of this is the transcriptional coactivator TAZ, recently shown to enhance *in vitro* the activity of PAX8 and NKX2-1 on the thyroglobulin (TG) promoter (Di Palma *et al.* 2009). Interestingly, TAZ is first expressed in the mouse embryonic thyroid at E14.5 coinciding with the onset of TG biosynthesis (Di Palma *et al.* 2009). Lack of TAZ prior to this time point may thus explain why PAX8 and NKX2-1 already expressed from E9 and onward are unable to activate the TG promoter. The reason for this is not known although it is conceivable

that TAZ-mediated coactivation of genes at earlier stages of the development will induce premature differentiation of the embryonic thyroid. This in turn might inflict on the cells' ability to proliferate and migrate and hence disturb the morphogenetic process also regulated by PAX8 and NKX2-1. No *TAZ* mutations have yet been identified in patients with TD (Ferrara *et al.* 2009). However, TAZ is part of an intricate network that regulates multiple cell functions among which cell proliferation and migration are particularly interesting. In fact, under certain conditions TAZ is considered to be oncogenic (Chan *et al.* 2008). Learning more about the normal regulation of TAZ in development might thus also shed light on mechanisms governing tumor progression.

What can we learn from patients?

The genetic basis of TD leading to CH in humans has been a matter of discussion since many years (Abramowicz *et al.* 1997, Vassart & Dumont 2005, Castanet *et al.* 2007, Deladoey *et al.* 2007*b*). Strikingly, most patients with TD have no mutations in genes known to affect thyroid development in animal models (see below). The precise nature and mode of action of possible genetic aberrations of TD thus remain elusive. From this it is proposed that the disease may be polygenic with a highly variable penetrance or develops sporadically on a multifactorial basis (Abramowicz *et al.* 1997, Castanet *et al.* 2010). To date, the pathogenesis of TD has been investigated experimentally mostly in monogenic mouse models in which the affected offspring follow a simple Mendelian inheritance (De Felice & Di Lauro 2007). However, a more complex mode of transmission with a strong strain-dependent predisposition for developing TD is recognized in mice compound heterozygous for *Nkx2-1* and *Pax8* (Amendola *et al.* 2005). The disease trait was recently linked to polymorphisms in the chromosomal region containing a chaperone partner, *Dnajc17*, which also was found to influence the transcriptional regulation of TG expression in thyroid cells (Amendola *et al.* 2010). A polygenic origin of TD has thus been proven in an animal model.

Although TD in humans is sporadic in the absolute majority of cases, a familial occurrence has been demonstrated in 2% of the cases, which is 15 times higher than expected by chance alone, in a large French cohort (Castanet *et al.* 2000, 2001, 2010). Similar findings were recently found in a less extensive Turkish study (Karakoc *et al.* 2008). Interestingly, both athyreosis and thyroid ectopia can be found among affected family members, implicating that heterogeneous manifestations of the disease may have a common genetic basis (Castanet *et al.* 2010).

A hereditary component is further suggested by observations of an increased prevalence of occult developmental anomalies of the thyroid in asymptomatic first-degree relatives (Leger *et al.* 2002, Adibi *et al.* 2008, Karakoc *et al.* 2008). However, it should be firmly kept in mind that monozygotic twins are generally discordant for TD (Perry *et al.* 2002), arguing against simple Mendelian transmission (Deladoey *et al.* 2007*b*, Castanet *et al.* 2010). Notably, only few germline mutations of known thyroid developmental genes (*NKX2-1*, *PAX8*, *FOXE1*, and *HHEX*) have been detected in a rather large series of patients (De Felice & Di Lauro 2004, Al Taji *et al.* 2007, Ramos *et al.* 2009, Castanet *et al.* 2010, Kang *et al.* 2010, Montanelli & Tonacchera 2010, Narumi *et al.* 2010). It is also noteworthy that linkage analysis shows no association of mutations in *NKX2-1*, *PAX8*, *FOXE1*, or *HHEX* with the TD phenotype in familial forms of CH (Castanet *et al.* 2005). From this it can be concluded that sporadic and familial cases of TD may represent distinct entities (van Vliet & Vassart 2009). Possible mechanisms that have been put forward but not formally proven are early somatic mutations of thyroid developmental genes and epigenetic changes in gene expression (Abramowicz *et al.* 1997, Vassart & Dumont 2005, Deladoey *et al.* 2007*b*). A role of environmental factors affecting thyroid development is, however, still largely hypothetical. On the contrary, the lack of a seasonal variation argues against a significant contribution of the environment (Deladoey *et al.* 2007*a*). The genetic basis of TD was recently emphasized by the finding of gene copy number variations in 9% of a patient cohort (Thorwarth *et al.* 2010). The genetic variations were not concentrated in recurrent hot spots favoring the idea that genetic insults eventually leading to TD are not uniform. This implies that broader chromosomal alterations may need to be elucidated to further understand the pathogenesis.

Conclusions and perspectives

The earliest stages of thyroid morphogenesis probably follow common developmental traits shared by other budding derivatives of the foregut endoderm. Although the precise inductive signal(s) that trigger multipotent endoderm progenitor cells to be committed to a thyroid fate characterized by coexpression of a thyroid-specific set of transcription factors remain to be identified, it is feasible to assume that cross talk between juxtapositioned endoderm and mesoderm is crucially involved, similar to the developmental programs operating to generate, for example, the hepatic and pancreatic lineages. However, unlike the glandular appendages of the intestine, the thyroid primordium buds off from the site of origin and moves as a free body

to a distant location, in principle a feature shared only by the other derivatives of the pharyngeal apparatus (thymus and parathyroid). This requires additional mechanisms of which only some of the details are known today. Future work on these issues will eventually decipher the morphogenetic code of thyroid development. Rather than focusing on aberrations in putative single master genes, a broader outlook on potentially deregulated pathways is likely necessary to elucidate the complex pathogenesis of TD in man.

Declaration of interest

The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the review reported.

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