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# Morphogenesis of an Epithelial Organ: Budding and Relocation of the Thyroid Gland during Vertebrate Embryonic Development

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## ABSTRACT

During early embryonic development, a common body plan is established in all vertebrates. Subsequently, complex morphogenetic programmes together with tissue growth dominate further foetal development, and differences in these morphogenetic processes result in anatomical variations between species. Our current view is dominated by the discovery that many morphogenetic processes are based on conserved molecular mechanisms. However, plasticity of such conserved molecular mechanisms has the potential to create morphological diversity between different species. Morphogenesis of the vertebrate thyroid gland is unique in that the primordium relocalises from its site of origin, the epithelium of the primitive pharynx, to a final position in the anterior neck region in amniotes or to a similar area ventral to the pharynx in fishes, respectively. Comparison between species reveals naturally occurring variation of primordial relocation, and in position and shape of the mature gland. This review gives an overview about functional studies in different model species that provide insight into the molecular mechanisms required for thyroid relocation. Based on these data and comparative studies, I present a model that could explain species-specific variation of thyroid position and shape. Furthermore, I refer to congenital defects in humans that can be related to abnormalities in thyroid relocation.

**Keywords:** thyroid follicles, vasculature, Vegf, Tbx1, Shh, zebrafish, mouse

## CONTENTS



## INTRODUCTION

Development of the pharyngeal region is dynamic and complex in vertebrates. In this part of the body, complex head structures form involving the endoderm-derived foregut, neural crest-derived mesenchyme, and mesoderm-derived tissues. Particularly obvious are morphogenetic movements. Branchiogenic organs such as thymus and ultimobranchial bodies relocate from the branchial pouches to their final positions in the neck. Primordia of other foregut derivates such as thyroid and lung bud out from the ventral floor of the primitive gut and subsequently change their position relative to their site of origin significantly. Such changes in organ position occur in concert with massive overall changes in embryonic and/or foetal morphology, when jaw, pharynx, gills, and neck develop in a species-specific manner (Noden 1991).

In this review, I will focus on the morphogenesis of the thyroid gland in vertebrates. This endocrine organ undergoes a unique relocation process from its site of primordial origin in the ventral midline of the primitive pharynx to a final, species-specific position in the mesenchyme ventral to the pharynx, in the following referred to as hypopharyngeal mesenchyme. An increasing number of studies concentrate on the relocation process of this gland, and we now begin to understand some of the molecular mechanisms controlling this event. With the thyroid being a model for the development of an epithelial organ, increasing knowledge of thyroid morphogenesis will provide general insight into the cellular behaviour of epithelia in the complex environment of the developing vertebrate.

## THE THYROID IN ADULT VERTEBRATES

The thyroid is an endocrine organ present in all vertebrates. On the cellular level, the gland consists of mainly one single cell type, the thyroid follicular cell. Thyroid follicular cells form follicles, with the apical membrane facing the follicle

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Invited Review

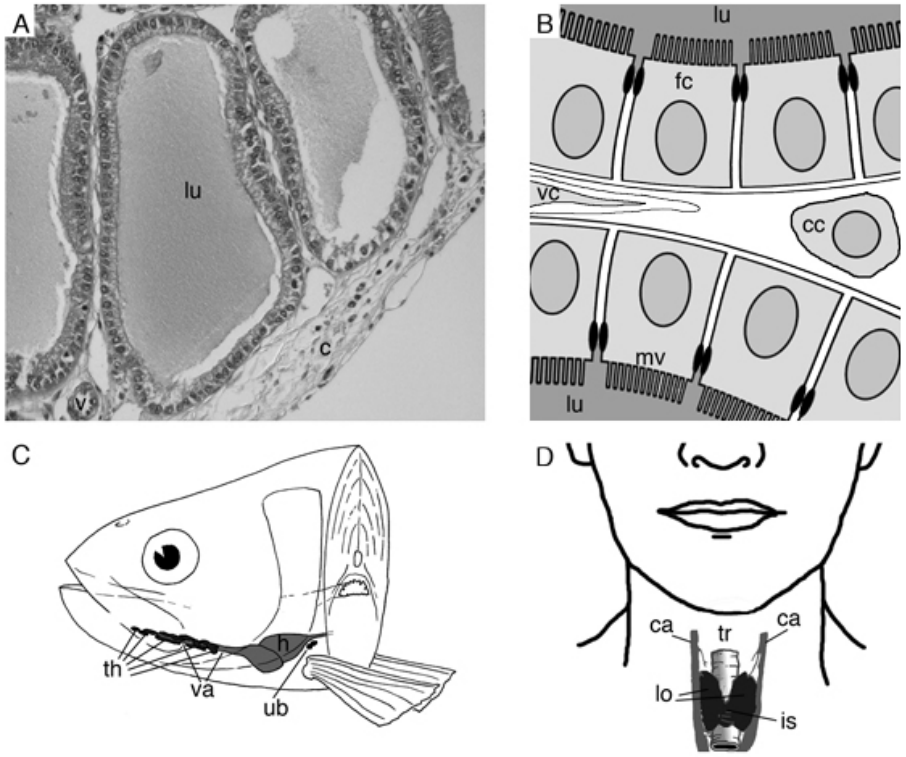


**Fig. 1 Cellular organisation and anatomy of the thyroid gland in adult vertebrates.** (**A**) Histological section of the shark thyroid, haematoxilin-eosin staining. Shown is a selection of follicles at the margin of the gland. (**B**) Schematic view of thyroid follicular cells at the border of two follicles. Depicted is a species where the ultimobranchical bodies merge with the thyroid, giving rise to c-cells. (**C**) Schematic view of a teleost head, indicating the position of thyroid follicles along the ventral aorta. (**D**) Schematic view of the position of the thyroid gland in humans. Abbreviations: c connective tissue, ca carotid arteries, cc c-cells, fc collicular cell, h heart, is isthmus, lo lobes of the thyroid gland, lu lumen of thyroid follicles, mv microvilli at the apical membrane of thyroid follicle cells, th thyroid follicles, tr trachea, ub ultimobranchial bodies, v blood vessel, va ventral aorta, vc vascular cell.

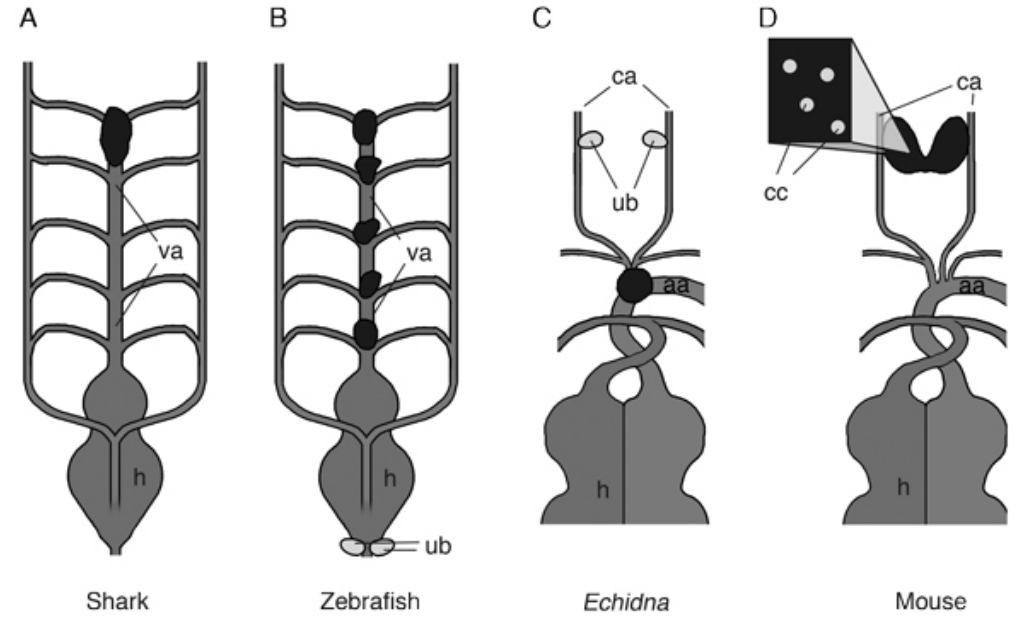


**Fig. 2 Relation of the thyroid gland in adult vertebrates to vascular structures.** Thyroid tissue is shown in dark. Note that in mouse, c-cells intermingle with thyroid follicles forming a compound gland whereas in the other species depicted, ultimobranchial bodies form separate glands. In sharks (**A**) and teleosts such as zebrafish (**B**), blood circulation is characterised by a linear heart and the presence of a ventral aorta that distributes blood to the branchial arteries. In *Echidna* (**C**) and mouse (**D**), heart and vasculature are simplified visualised as separated into pulmonary and systemic circuits. Abbreviations: aa aortic arch, ca carotid arteries, cc c-cells, h heart, ub ultimobranchial bodies.

lumen (**Fig. 1A, B**). Depending on the species, follicles are organised in different ways. Most teleost fish have a loose strand of follicular tissue dispersed along the ventral aorta in the hypopharyngeal area (**Fig. 1C**) (Gorbman and Bern 1962). In other vertebrates than teleosts, follicles are encapsulated by connective tissue and can be found as a single mass or a bilateral pair of glandular masses in the hypopharyngeal area or the neck, respectively (Gorbman and Bern 1962). In many mammals including mice and humans, the thyroid adopts a typically bi-lobed shape, with the two lobes being flanked by the carotid arteries and connected by a narrow band of parenchyme, the isthmus. (**Fig. 1D**).

Thyroid follicular cells produce the thyroid hormones T4 and T3, which are required for normal vertebrate development and metabolism. In most mammals including mice and humans, a second hormone producing cell type intermingles with thyroid follicular cells, the so-called c-cells. They produce calcitonin, a poorly characterised hormone involved in calcium metabolism. C-cells also exist in non-mammalian vertebrates, however, they usually form a separate gland, the ultimobranchial bodies.

The thyroid is highly vascularised and can be found close to major blood vessels throughout the vertebrate clade. It can be anticipated that on the evolutionary level, selective pressure exists on the efficient release of thyroid hormone into blood circulation. In the most ancestral vertebrates having a thyroid, the lampreys, the thyroid gland is embedded into a venous sinus in the hypopharyngeal area (Sterba 1953). In elasmobranches (sharks, rays, skates), the thyroid has been described to be located around the anterior end of the ventral aorta (Ferguson 1911) (**Fig. 2A**). In teleosts (ray-finned fishes), thyroid tissue is arranged along the ventral aorta, the main vessel pumping blood from the heart to the gill arteries (**Fig. 2B**) (Raine and Leatherland 2000; Raine *et al*. 2001; Wendl *et al*. 2002). In amniotes, the gland can be found at variable places, but always close to cervical vessels such as the carotid arteries in mice and humans (**Fig. 2C, D**).

The thyroid is not only remarkably variable in shape and position between species, but also within a population of one species. In many teleost species, ectopic follicles in the head kidney and other places have been described in addition to the loosely organised bulk of follicles along the

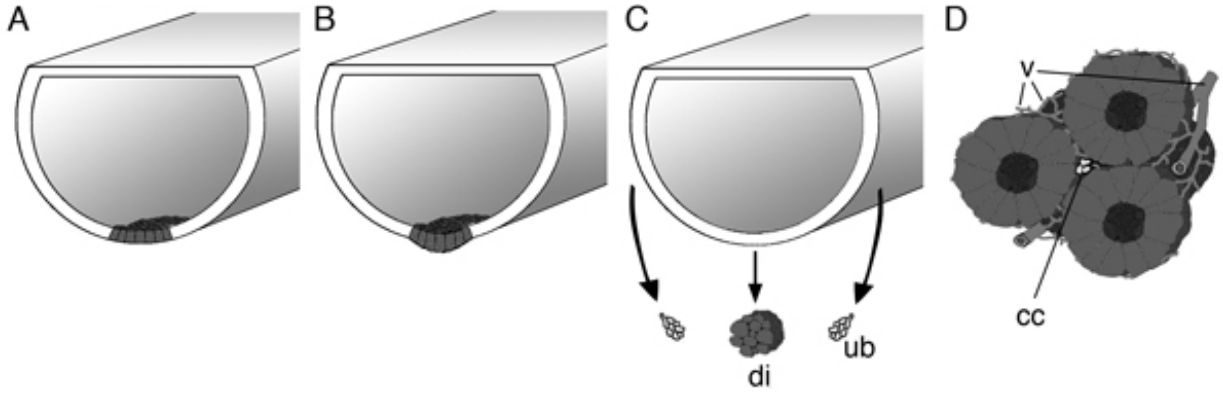


**Fig. 3 Overview about embryonic development of the thyroid gland.** (**A**) The "thyroid field" is induced in the ventral midline of the primitive pharynx. At this stage, molecular thyroid markers start to be expressed in a subset of midline cells, predating morphogenetic changes. (**B**) Thyroid primordial cells bud from the pharynx. In some species, this process involves thickening and outpocketing of the primordial cells. (**C**) The thyroid diverticulum relocates into hypopharyngeal mesenchyme. In most mammalian species, ultimobranchial bodies relocate in parallel, eventually merging with the diverticulum. (**D**) Differentiation of the primordium into follicles. In mammals, a dense network of capillaries forms around the follicles, and c-cells distribute after fusion with the ultimobranchial bodies. The time point of differentiation differs significantly between species. In zebrafish, the diverticulum differentiates into a fist single follicle directly after budding from the pharynx and then undergoes morphogenetic changes as differentiated tissue. In mouse, primordial tissue grows an extended period of time during embryonic and foetal stages before follicles differentiate. Abbreviations: cc c-cells, di thyroid diverticulum, ub ultimobranchial bodies, v blood vessels.

aorta (Gorbman and Bern 1962). In humans, single-lobed thyroid glands seem to occur as a normal variant without pathological consequences (Shabana *et al*. 2000). Furthermore, the human thyroid has a tendency to develop ectopically at different abnormal positions, ranging from the base of the tongue to thyroid follicles embedded into cardiac muscle. In such cases, thyroid hormone production can be impaired, and leads to a condition known as congenital hypothyroidism.

## DEVELOPMENT OF THE THYROID GLAND

As mentioned earlier, the thyroid of mice and humans and most other mammals contains two hormone-producing cell types, thyroid follicular cells and c-cells (overview about comparative morphological studies in Gorbman and Bern 1962). Both have different developmental origin, and therefore it is necessary to distinguish between two separate primordia (reviewed in De Felice and Di Lauro 2004). Thyroid follicular cells derive from a primordium of the ventral midline of the primitive pharynx (**Fig. 3A**). In embryos of many species, this "thyroid field" can be identified as a thickening of primordial cells (**Fig. 3B**). Its cells subsequently bud off from the pharyngeal epithelium forming the so-called thyroid diverticulum, and relocate to a position deep in the cervical mesenchyme (**Fig. 3C**) (Pischinger 1937; De Felice and Di Lauro 2004). This relocation process is frequently called migration, however, it should be pointed out that the term migration is usually used for actively moving cells. As it is not yet clear to which extend the thyroid diverticulum actively migrates, the term relocation is more neutral and will be used in this review.

In humans, the site in the pharynx where the thyroid diverticulum once budded from the epithelium is anatomically marked by the persisting *Foramen caecum*, a depression at the root of the tongue. During its relocation, the thyroid transiently communicates with the foregut endoderm by the so-called *Ductus thyreoglossus*. In humans, cases of a persistent *Ductus thyreoglossus* occur, with a duct connecting the *Foramen caecum* and the thyroid.

In addition to the midline diverticulum, two additional bilateral primordia, the ultimobranchial bodies, exist. They originate from the pharyngeal pouches in the foregut endoderm and become populated by neural crest-derived cells (Le Douarin *et al*. 1974). From the pouches, the ultimobranchial bodies relocate into the cervical mesenchyme (**Fig. 3C**) and, in most mammals including mice and humans, fuse with the midline diverticulum (**Fig. 3D**) (Fontaine 1979). Within the compound thyroid gland, the neural crest-derived cells give rise to the c-cells (Le Douarin *et al*. 1974).

In most vertebrate species, the ultimobranchial bodies form separate organs that can be found at different positions in the cervical area. Here, the midline diverticulum is the only structure that gives rise to the thyroid gland, as shown by lineage analysis for zebrafish (Alt *et al*. 2006b). The term "thyroid diverticulum" will be used for the midline primordium throughout this review, as opposed to the bilateral ultimobranchial bodies.

## MORPHOGENESIS OF THE THYROID: DEFINING THE PROBLEM

Morphogenesis can be defined as the beginnings of form, and this term usually includes understanding the underlying molecular mechanisms. The development of the thyroid gland provides a unique example of morphogenesis of an epithelial organ. Although poorly understood, we can assume that thyroid development is initiated after molecular regionalisation of the foregut endoderm. Induced by as yet unknown signals, some primordial cells in the primitive pharynx begin to express the thyroid-specific transcriptional programme. Thyroid morphogenesis has obviously started when the diverticulum buds off from the pharyngeal epithelium, although it might have been initiated on the molecular level even earlier.

Thyroid morphogenesis can be subdivided into different steps. The first step is budding of the primordium from the pharyngeal epithelium, the second step is the relocation and positioning, and the third step is the generation of the species-specific shape and size of the differentiated gland.

Information about differentiation of the gland will be kept as a minimum in this review, as this process is relatively well understood. A characteristic set of transcription factors, comprising mainly Nkx2.1, Hhex, and Pax proteins, are cell-autonomously required for differentiation of the gland and for establishment and maintenance of the hormone-producing machinery (reviewed in De Felice and Di Lauro 2004). In the absence of each of these factors, the thyroid diverticulum still initiates its morphogenetic programme, so that their involvement in morphogenesis appears to be limited (Kimura *et al*. 1996; Mansouri *et al*. 1998; Martinez Barbera *et al*. 2000). The focus of this review is on those steps of thyroid morphogenesis that are to our current knowledge not significantly influenced by these factors, i.e. budding of the midline diverticulum from the pharyngeal endoderm and relocation into the hypopharyngeal mesenchyme. It should be kept in mind, though, that our view might change, as an involvement of these transcription factors particularly in later stages of morphogenesis

cannot be ruled out at present.

## EARLY STEPS IN THYROID MORPHOGENESIS: BUDDING FROM THE PRIMITIVE PHARYNX

The cellular mechanisms of the budding of the thyroid primordium are not known, and they might vary between species. In early studies on shark embryogenesis, an initial outpocketing of primordial cells has been described (Norris 1918). A similar situation has later been described in chicken embryos (Shain *et al*. 1972; Kinebrew and Hilfer 2001) and, using molecular markers and confocal microscopy, for the early mouse thyroid diverticulum (Fagman *et al*. 2006a). How the cells of the diverticulum are released from the pharyngeal epithelium, however, is not clear. One possibility is that the primordial cells delaminate similar to liver primordial cells, but also other mechanisms, for instance a purse-string mechanism, are possible.

Only one gene, *Foxe1*, has been identified to date to be involved in budding and consequently also in relocation of the thyroid gland (De Felice *et al*. 1998). In mice, the thyroid diverticulum normally buds from the pharynx at around embryonic stage 9.5 (E9.5). In *Foxe1*-/- knockout mice, the thyroid remains in the pharyngeal epithelium, where the normal thyroidal transcriptional programme appears to be ectopically maintained at least for a while. At later stages such as E11, the thyroid is either still attached to the pharyngeal epithelium, or it has disappeared, probably as a consequence of the failure to relocate.

The murine *Foxe1* gene is expressed in both the pharyngeal epithelium and the thyroid primordium. It was therefore interesting to know if *Foxe1* is tissue-autonomously required for thyroid evagination. In *Pax8*-/- mutant mice, detectable levels of *Foxe1* expression are missing in the thyroid diverticulum (Parlato *et al*. 2004), indicating that *Foxe1* is downstream of *Pax8*. Pax8 is one of the transcription factors cell-autonomously required for thyroid differentiation (Mansouri *et al*. 1998), and in *Pax8*-/- mice the thyroid diverticulum disappears after normal budding from the pharyngeal epithelium presumably by apoptosis. The initially normal morphogenesis of the diverticulum despite the lack of *Foxe1* expression in its cells suggests that *Foxe1* expression in the surrounding pharynx promotes thyroid relocation. On the other hand, in transgenic *Foxe1*-/- mice, which express *Foxe1* under a thyroid-specific promoter exclusively in the thyroid primordium, relocation of the thyroid diverticulum is rescued (Parlato *et al*. 2004). This shows that *Foxe1* expression in the thyroid diverticulum alone is sufficient to promote thyroid relocation. Thus, it is likely that *Foxe1* acts cell-autonomously in thyroid relocation, and weak residual *Foxe1* expression that escapes detection might explain the ability of the diverticulum to relocate in *Pax8*-/- mice.

## RELOCATION OF THE THYROID PRIMORDIUM IN THE HYPOPHARYNGEAL AREA

After the thyroid diverticulum has lost contact with the pharyngeal epithelium, the distance to its site of origin increases. In the complex morphogenesis of the vertebrate head area it is difficult to distinguish between active and passive morphogenetic movements. A recent study in mice embryos showed that E-cadherin, which is typically expressed in epithelia, is continuously expressed in the thyroid diverticulum during relocation. Other cadherins, which are typical for migrating cells, can only be found in adjacent tissue (Fagman *et al*. 2003). Thus, on the level of diagnostic cadherin expression, no epithelial to mesenchymal transition was observed, supporting a relocation of the thyroid diverticulum as a differentiated epithelium (Fagman *et al*.

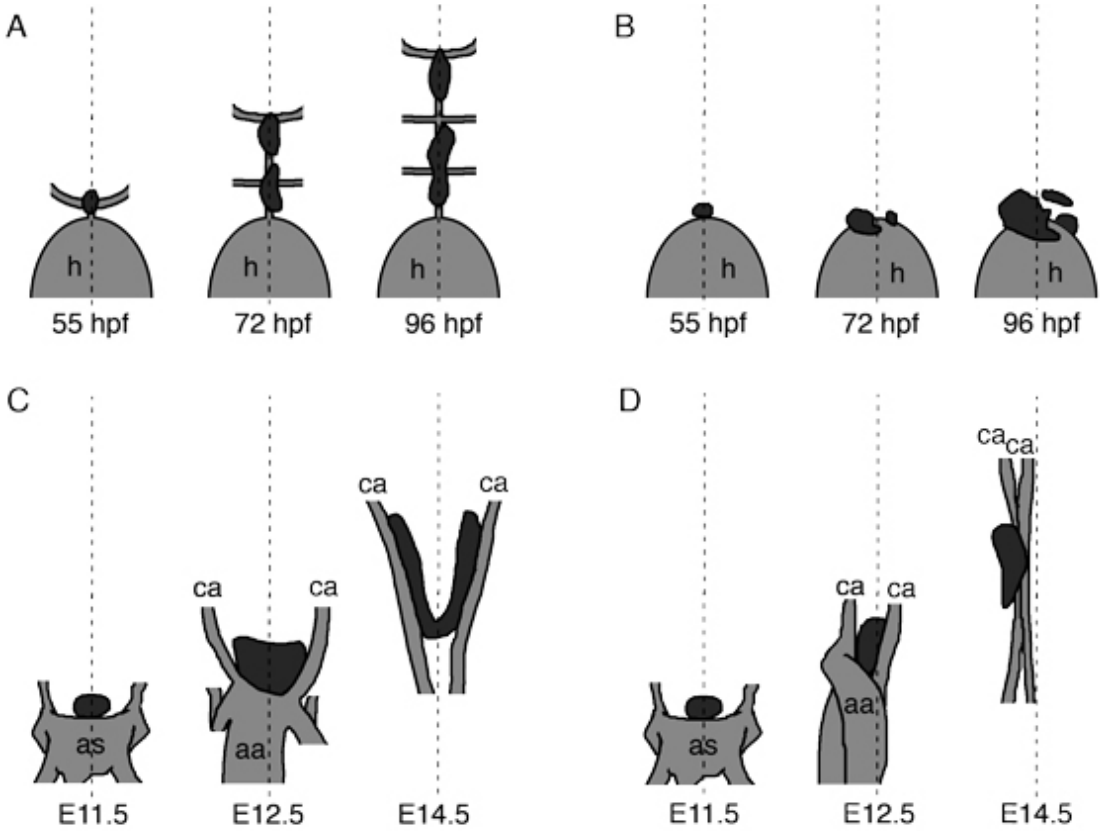


**Fig. 4 Normal and disrupted morphogenesis of thyroid tissue concomitant with cardiovascular development.** Cardiovascular structures are in light grey, thyroid tissue in dark grey. The midline is indicated by the dotted line. (**A**) Growth of thyroid tissue along the ventral aorta in late embryonic and early larval zebrafish stages. (**B**) In zebrafish mutants lacking the ventral aorta, thyroid tissue grows in irregular fashion around the ventricular end of the heart, which corresponds to the outflow tract. (**C**) Morphogenetic changes of the thyroid diverticulum during normal mouse development. (**D**) Morphogenetic changes of the thyroid diverticulum in *Shh*-/- mice. Initial stages are normal. Concomitantly with altered aortic arch morphogenesis and a failure of the heart to rotate normally, cervical vessels are positioned asymmetrically. The thyroid is always found on the same side as the carotid arteries. Abbreviations: aa, aortic arch; as, aortic sac; ca, carotid arteries; h, heart; hpf, hours post fertilisation; E embryonic day of mouse development of branchial bodies; v, blood vessels.

2003). Nevertheless, additional studies are required to identify the cellular mechanisms of thyroid relocation.

Independent of the question whether the thyroid diverticulum relocates actively or is rather pulled or pushed through the tissue, it can be anticipated that its proper relocation relies on guidance cues. The tendency of the thyroid gland to adopt unusual positions in humans (and other vertebrates) implies that these guidance cues are prone to variability or disturbances. The molecular nature of such cues remains obscure, but recent papers point towards the vascular system to be involved in guiding the thyroid diverticulum.

A prominent role of the cardiovascular system in thyroid development has already been suspected based on the close morphological relationship of both structures. In mice and chicken, the earliest sign of thyroid precursor cells in the midline of the primitive pharynx is directly adjacent to the aortic sac (Shain *et al*. 1972; Fagman *et al*. 2006a), the structure that later transforms into the outflow tract of the heart and parts of the cervical vessels. Similarly, early zebrafish thyroid development is initiated directly adjacent to the outflow tract of the heart (Rohr and Concha 2000). In the latter species, the thyroid develops throughout embryonic and larval stages particularly close to the ventral aorta, and even in adults, thyroid follicles are aligned very close to this major vessel (Wendl *et al*. 2002; Alt *et al*. 2006b). The ventral aorta is the main vessel in the hypopharyngeal area, where the blood is transported into gill arteries. Information about the exact anatomical relation of the thyroid to vessels is scarce for most amniotes, but unpublished observations of the author have confirmed that in frogs, crocodiles, and turtles the thyroid gland is closely associated with the carotid or jugular arteries. It should be noted, though, that in these species vessels and glands are surrounded and separated by more prominent amounts of mesenchymal tissue than in zebrafish.

In a recent report on thyroid development in zebrafish the authors provide evidence that in this species, the ventral aorta guides the growth of thyroid tissue in the hypopharyngeal area (Alt *et al*. 2006a). Firstly, the authors show that in zebrafish mutants with disrupted ventral aorta development the thyroid always fails to adopt its normal extended shape along the pharyngeal midline. Instead, the thyroid tissue accumulates in an irregular way around the remaining outflow tract of the heart (**Fig. 4A, B**). Secondly, in transplantation experiments, ectopic cells with vascular properties are able to change thyroid morphology non-cell autonomously. As the ventral aorta is the only vessel close to the thyroid in zebrafish larvae at the relevant stages, it can only be this vessel that guides thyroid tissue growth along the midline.

Similarly, there is evidence that cervical vessels guide thyroid morphogenesis also in mice. Three-dimensional reconstruction of both thyroid and cervical vessel development reveals close co-development of the thyroid primordium not only at early stages with the aortic sac, but also later with the emerging carotid arteries (**Fig. 4C**) (Alt *et al*. 2006a). Support for such a functional relation comes from mice deficient for Shh signalling. In such mice, cervical vessels develop abnormally, placing both carotid arteries always asymmetrically either on the right or on the left side instead of their normal symmetrical, bilateral position. Thyroid development is initially normal in these mice, as long as the thyroid diverticulum is adjacent to the normal aortic sac. However, in a report on thyroid development in *Shh*-/- mice it has been noted that later the thyroid fails to bifurcate, adopting an asymmetrical, abnormal position (Fagman *et al*. 2004). A more detailed analysis revealed that the unilateral thyroid developed always on the same side as, and in close contact to, the mislocated carotid arteries (**Fig. 4D**) (Alt *et al*. 2006a). Thus, abnormal carotid artery development is linked to abnormal thyroid morphogenesis in mice, suggesting a similar relation as between ventral aorta development and thyroid morphogenesis in zebrafish.

Both zebrafish and mouse experiments suggest that thyroid relocation can functionally be subdivided into two phases. In the zebrafish mutants without ventral aorta, the diverticulum still detaches from the pharynx. Here, it is only a second phase of morphogenetic growth along the midline that is disrupted. Similarly, in *Shh*-/- mice, the diverticulum undergoes a first phase of normal initial relocation, and only the second phase of bifurcation of the primordium is interrupted. *Foxe1*-/- mice, in contrast, represent an example where the first phase of budding and initial relocation is disrupted.

What could be the molecular base of the co-development of vessels and thyroid? Neither Sonic hedgehog nor the receptor for this signalling molecule, Patched, are expressed in the thyroid or adjacent tissues in the mouse (Fagman *et al*. 2004; Alt *et al*. 2006a). It can therefore be assumed that in the Sonic Hedgehog deficient mouse embryos, carotid artery defects are a result of earlier development defects, for instance of the abnormally formed heart. In zebrafish, additional molecules have been identified to disturb vessel and, concomitantly, thyroid development (**Fig. 5**). One of these molecules is the secreted vascular endothelial growth factor (Vegf). This ligand and one of its receptors, Kdr, have been shown to be expressed in both vascular and primordial thyroid tissue (Alt *et al*. 2006a). Thus, it is possible that Vegf constitutes one of the signals involved in thyroid morphogenesis.

Additional information about possible molecules involved in thyroid-vessel interactions comes from *Tbx1*-/- mutant mouse embryos. In these mutants, the thyroid diverticulum loses transiently the contact to the aortic sac, and

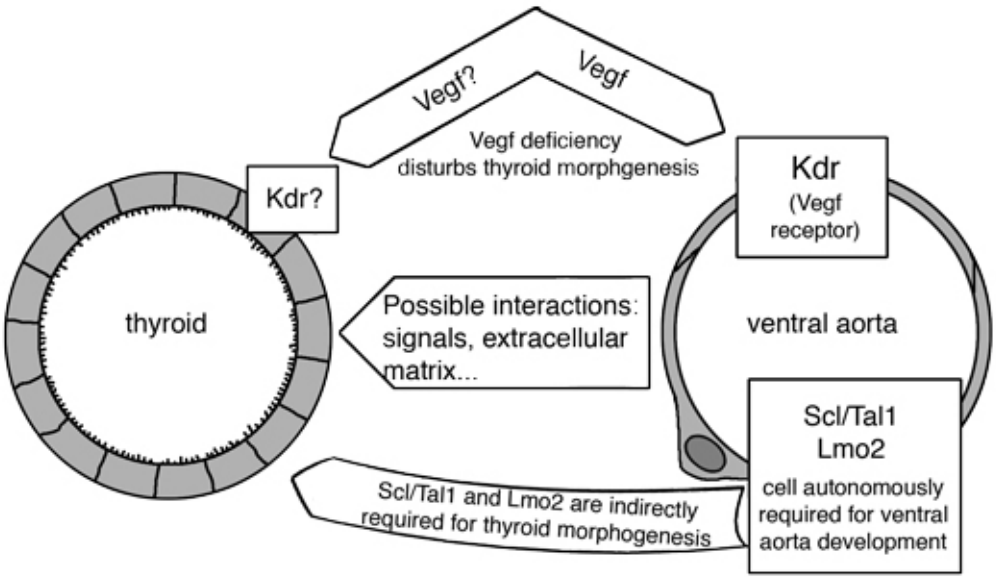


**Fig. 5 Molecular interactions between thyroid and ventral aorta in zebrafish.** Vegf signalling is required for ventral aorta development. The genes encoding Vegf and one of its receptors, Kdr, are expressed in zebrafish thyroid tissue (Alt *et al*. 2006a), so that a direct influence on thyroid development is possible. Scl/Tal1 and Lmo2, transcription factors involved in vessel development, have been shown to influence thyroid development indirectly (Alt *et al*. 2006a). Possible interactions between vessels could also be mediated by extracellular matrix or with intermediate steps involving vessel-surrounding muscles or mesenchyme.

later re-attaches asymmetrically only to one of the two bilateral carotid arteries. As a result, *Tbx1*-/- mice have an asymmetrically positioned thyroid at birth (Fagman *et al*. 2006b). *Tbx1* encodes a T-box containing transcription factor expressed in pharyngeal mesenchyme around the early thyroid diverticulum and the aortic sac at the time of thyroid primordial relocation. How *Tbx1*-/- acts in this context remains obscure. Relocation of the thyroid is delayed but not disrupted, and it is not clear if it is *Tbx1* expression adjacent to the thyroid diverticulum that is responsible for the asymmetric mislocation of the thyroid. Nevertheless, *Tbx1* is a main candidate to be involved in mediating vessel-thyroid contact. Taken together, the molecular base of vessel-thyroid interactions remains to be elucidated.

## FUSION OF THE MIDLINE DIVERTICULUM WITH THE ULTIMOBRANCHIAL BODIES

In mice, humans and most other mammals, thyroid morphogenesis is further characterised by the fusion of the midline diverticulum with the bilateral pair of ultimobranchial bodies. As this feature of thyroid morphogenesis seems not to occur outside the mammalian lineage, it can be considered a derived character in mammalian evolution. The question arises to which degree morphogenesis of the gland depends on possibly mutual interactions between both tissues.

In *Pax8*-/- mice, the thyroid diverticulum disappears before it would fuse with the ultimobranchial bodies (Mansouri *et al*. 1998). Here, the ultimobranchial bodies relocate to their normal position and form glandular structures composed of c-cells in front of the trachea, where the normal thyroid would be positioned. Thus, absence of the diverticulum neither distracts ultimobranchial body relocation nor their differentiation.

In *Tbx1*-/- mice, ultimobranchial bodies are missing (Liao *et al*. 2004). As mentioned in the last chapter, the mid-line diverticulum fails to bifurcate during development in this mutant. As discussed, this could be due to defective cardiovascular development, but can we exclude an influence of the ultimobranchial bodies? In *Hoxa3*-/- mutant mouse embryos, fusion of ultimobranchial bodies with the diverticulum fails or is incomplete (Manley and Capecchi 1995). Concomitantly, various morphogenetic defects in the overall morphogenesis of the gland been observed (Manley and Capecchi 1995). However, *Hoxa3*-/- mice show also other defects including severely abnormal development of the pharyngeal arch arteries (Kameda *et al*. 2002). Thus, the *Hoxa3*-/- mutant phenotype is not conclusive with respect to the interrelationship between ultimobranchial bodies and midline diverticulum during development.

*Pax9*-/- mutant mice lack ultimobranchial bodies as well (Peters *et al*. 1998), and here the midline diverticulum bifurcates normally (Fagman *et al*. 2006b). Based on this observation, it appears likely that morphogenesis of the midline diverticulum is essentially independent of the ultimobranchial bodies. Thus, it can be anticipated that the unilateral thyroid gland observed in *Tbx1*-/- and *Hoxa3*-/- mice is caused by other mechanisms, such as an influence of the cardiovascular system.

Taken together, in the absence of ultimobranchial bodies the thyroid diverticulum relocates and differentiates normally. Obviously, the midline diverticulum and the ultimobranchial bodies have maintained their ancestral ability to develop independently. Interestingly, both structures seem to follow similar guidance cues in mammals, as they usually meet at the same site in the embryo to fuse. An exception can be found in *Echidna*, an ancestral egg-laying mammal. Here, the thyroid diverticulum relocates to a final position at the base of the carotid arteries, closer to the heart than in other mammalian species (Haynes 1999). The ultimobranchial bodies do not fuse with the diverticulum. Instead, they adopt a position further cranially, attached to the carotid arteries, reminiscent to the position of the compound thyroid gland in most other mammals (**Fig. 2C**). Thus, in *Echidna* only the ultimobranchial bodies adopt the cranial position at the carotid arteries characteristic for the fused thyroid gland described in most other mammals, again highlighting the tendency of these organs to adopt variable positions throughout the vertebrate clade.

## GENERATION OF THE FINAL SHAPE OF THE GLAND

The shape of the thyroid gland varies from species to species (Gorbman and Bern 1962). Based on the notion that blood vessels guide the thyroid diverticulum during relocation we can assume that variations in the development of the cervical vessels, or in the way these interact with thyroid tissue, are responsible for generating different shape of the gland. It can be anticipated, for instance, that in *Echidna* the thyroid diverticulum stays attached to more caudal parts of the vessels emerging from the aortic sac, resulting in the position of the gland at the base of the carotid arteries. In most other mammalian species, the diverticulum appears to be guided by the carotid arteries further cranially. Variations in the way the diverticulum follows arteries and probably in addition other structures might also account for the existence of an isthmus in some species, whereas in others, the thyroid completely separates into two glands.

Taken together, a model emerges in which vascular structures play an important part in shaping the thyroid gland. Functional data for the early processes are still missing, but the close co-development suggests an early role of the aortic sac in thyroid morphogenesis. Later, major cervical vessels take over a role in shaping the gland, as shown for zebrafish and suggested for mice. Development of these vessels differs significantly between species of the vertebrate clade. During vertebrate evolution, the pharyngeal area has adopted diverse specialised functions in the animal body plan. The segmented gill area of aquatic vertebrates transformed into the specialised head and jaw structures of amniotes, and concomitant with separation of blood circulation into pulmonary and systemic circuits, the main cervical vessels have adopted many variants. It is conceivable that early in evolution, the thyroid has adopted a position along the ventral aorta to ensure efficient hormone release into circulation, a situation still present in sharks and teleosts. Evolving variants of blood circulation in amniotes led to a reduction of the ventral aorta, and other vascular structures took over a guiding role in thyroid morphogenesis. The variability in the branching mode of cervical vessels between different species, but also within the human population (Nizankowski *et al.* 1975), for instance, can be explained by high plasticity of their course of development. This, in turn, could then explain the inter- and intra-specific variability in thyroid morphogenesis.

## MEDICAL IMPLICATIONS

Morphogenesis of the thyroid in humans tends to be variable. The resulting gland is usually walnut-sized and consists of the two lobes connected by an isthmus. In about 0.2 percent of the population the thyroid is single-lobed (Shabana *et al*. 2000). This does not necessarily result in elevated TSH levels and can therefore be regarded as a normal, albeit rare, variant. Variation also occurs with respect to an occasional median extension of the gland, the *Lobus pyramidalis* or pyramidal lobe. This part, if present, contains normal thyroid tissue and is thought to reflect the developmental relocation path of the gland.

Defective thyroid development, commonly referred to as thyroid dysgenesis, results relatively frequently in reduced or absent production of thyroid hormone in the newborn, causing congenital hypothyroidism (De Felice and Di Lauro 2004; Park and Chatterjee 2005). Developmental defects described in humans range from complete agenesis of the thyroid gland to ectopic thyroid tissue. The latter can usually be regarded as a result of abnormal relocation of the gland during development. In some cases extra tissue is present in addition to a normally positioned thyroid, in other

cases the whole organ has adopted an abnormal position. Ectopic tissue does not necessarily lead to congenital hypothyroidism, as reduced amount of thyroid tissue can be compensated by increased physiological activity due to endocrine feedback mechanisms.

Ectopic thyroid tissue can be found at various positions in the head and neck area (Batsakis *et al*. 1996). The so-called lingual thyroid, for instance, is positioned at the base of the tongue. It represents the site where the diverticulum originates in the pharynx and therefore reflects a failure of the whole or part of the gland to bud off from the pharyngeal epithelium. Further variants of mislocated thyroid in humans include all the way between the tongue and the normal position in front of the trachea as well as other positions in the neck.

Several reports exist where ectopic thyroid tissue was found associated with the heart, especially with the outflow tract area (Casanova *et al*. 2000). This ectopic position reminds of the close co-development with cardiovascular structures, with the primordium being induced adjacent to the aortic sac and further morphogenesis dependent on major cervical vessels. Indeed, possible relations between cardiovascular structures and thyroid relocation in humans are further supported by the observation from different studies that 3-12% of patients with congenital thyroid dysgenesis also suffer from congenital heart disease (Devos *et al*. 1999; Kreisner *et al*. 2005). Moreover, in patients suffering from Del22q11 syndrome, co-existence of thyroid and cardiovascular defects has been noted in about 20% of young adults (Bassett *et al*. 2005). Del22q11 syndrome has been related to genetic defects in the *TBX1* locus. Thus, in this syndrome, lack or misregulation of *TBX1* expression causes corresponding cardiovascular and thyroid defects, as it does in *Tbx1*-/- mouse embryos.

The molecular base of abnormally positioned thyroid tissue in humans remains to be elucidated. In humans, homozygous *FOXE1* mutations have been described in siblings affected by a syndrome that includes congenital hypothyroidism, the Bamforth-Lazarus syndrome (Bamforth *et al*. 1989; Clifton-Bligh *et al*. 1998). In these patients, the thyroid was found to be lacking completely. Disrupted relocation of the thyroid, however, would be expected to cause ectopic thyroid in human patients, in particular to lingual thyroid at the base of the tongue. More patient data have to be collected before judging if mutations in *FOXE1* can cause ectopic thyroid in humans. Taken together, more information about the molecular mechanisms of thyroid morphogenesis is needed before we begin to understand the probably complex, multi-factorial causes of ectopic thyroid tissue in patients.

## OUTLOOK

Surprisingly little is known about the cellular behaviour of epithelia during vertebrate morphogenetic processes. Therefore it will be necessary to investigate in detail by which mechanism thyroid primordial cells leave the pharynx. Similarly, we need to know how the thyroid diverticulum actually manages to increase its distance to its site of origin on the molecular level. Unfortunately, the investigation of these processes can be expected to involve ubiquitously occurring cellular mechanisms, so that corresponding mutant phenotypes will tend to show pleiotropic defects. Nevertheless, at this point it will not be sufficient to rely on cell culture data, and the generation of more conditional mutants in the style of the *Foxe1* transgenic mice described will eventually reveal underlying principles and molecules.

With respect to human phenotypes, it would be interesting to understand how the unilateral thyroid found in a small part of the population develops. This might be related to normal variants of the development of the cervical vessels, which show plasticity in their dynamic, poorly understood complex course of development (Nizankowski *et al.* 1975). Investigations on this issue, however, are hampered by the low frequency of single lobed thyroid glands in humans and by the fact that occurrence of a single-lobed thyroid is normally not indicated by elevated TSH levels (Shabana *et al*. 2000).

Ectopic thyroid tissue in humans, however, is frequently accompanied by reduced amount of tissue and resulting insufficient physiological activity of the gland (Kreisner *et al.* 2005). This, and the possible relation to cardiovascular defects, makes the mechanisms of thyroid morphogenesis an important question. Its answers will facilitate diagnosis of complex syndromes. Moreover, these answers will in general provide better understanding of epithelial morphogenesis.

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