

Thyroid Hormone and Retinal Development: An Emerging Field

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Thyroid hormone appears to play a critical, yet not fully understood, role in the development of the neuroretina. This review focuses on recent experiments in the rodent, chicken, and amphibian, with an emphasis on how the hormone and its receptor isoforms influence retinal cell proliferation and cell fate decisions. The initial results are fueling the next generation of experiments in the retina, which promise to provide insights into the mechanisms of thyroid hormone action in a wide variety of developing neural tissue.

Introduction

THYROID HORMONE has been known to affect neural development for years, but exactly how the hormone causes its effects remains largely unclear. We know that the hormone binds receptors, which serve as transcription factors that bind to DNA and control the expression of downstream genes (1). Furthermore, we know that the hormone can induce a variety of effects in different neural tissues, including proliferation, differentiation, and migration (2). It remains our challenge to piece together the mechanistic puzzle: to identify the downstream genes, to understand their functions, and ultimately, to explain how thyroid hormone uses them to influence neural development.

The retina—one of the most studied neural tissues—is ideally suited for studies of thyroid hormone action. However, surprisingly little is known about how the hormone affects retinal development. In this paper, we highlight the major findings linking thyroid hormone action with the vertebrate retina, as well as predict where this emerging field is heading. We hope that lessons gained from the retina will help clarify thyroid hormone's role in neural development, not only in the eye but in other areas of the nervous system as well.

The Retina as a Model Tissue

While the developing retina shares much in common with other developing neural structures, it offers key experimental advantages. First, all seven of the basic retinal cell types have been identified and well characterized (Fig. 1) (3). Rod and cone photoreceptors respond to light, and transmit signals to bipolar cells. Bipolar cells in turn connect to amacrine cells and ganglion cells, the latter having axons that bundle together to form the optic nerve. Horizontal cells modify photoreceptor-bipolar connections, whereas amacrine cells,

in addition to receiving input from bipolar cells, influence bipolar-ganglion cell communications. Müller cells, the only non-neuronal cell type in the retina, have processes that span the entire retina, and are thought to play a supportive role for the other neurons.

Another key experimental advantage is that we know much about how these different cells are generated during development (4). Retinal progenitor cells produce them, in much the same way progenitors give rise to cells in other parts of the nervous system. Retinal progenitors follow two well-characterized rules. First, the same progenitor generates numerous cell types, including both neuronal and glial cells. This was shown clearly in a series of experiments using marker-expressing retroviruses. When these viruses were applied to the developing retina *in vivo*, they infected dividing retinal progenitors and caused them to express the marker. Furthermore, as infected progenitors divided, they passed the marker to all of their daughters. These experiments revealed that an infected progenitor could produce a variety of cell types expressing the marker, including both neurons and glial cells (5,6). Hence, progenitor cells in the retina, similar to progenitor cells elsewhere in the nervous system, are not restricted to produce only one cell type; rather, they generate a range of cell types over many divisions.

The second rule that retinal progenitors follow is that they only produce a limited number of cell types at a particular time. This has been described as the "competence model" (7). According to this model, a progenitor's competence to make different cell types changes over time. For example, an early progenitor is competent to produce early-born cell types but not late-born ones, whereas a late progenitor would be competent to produce late-born cells but not early-born ones. These "early-born" and "late-born" cell types have been identified in many vertebrate species using tech-

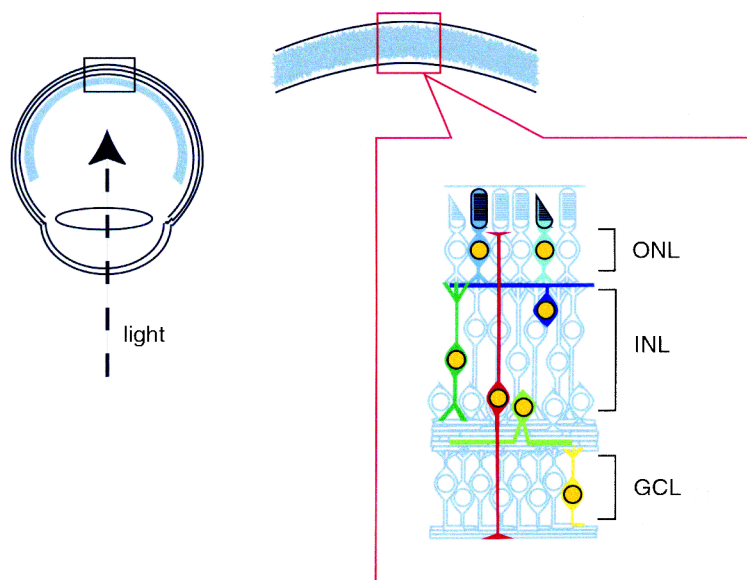


FIG. 1. A schematic view of the retina. The retina lines the back of the eye. Here it captures and processes light, and then transmits the resulting signal to the brain. The retina is made up of three layers of cells. The ONL, or outer nuclear layer, contains rod (light blue) and cone (aqua blue) photoreceptors. The INL, or inner nuclear layer, contains horizontal cells (dark blue), bipolar cells (dark green), and amacrine cells (marsh green). The GCL, or ganglion cell layer, contains ganglion cells (yellow) which project axons that form the optic nerve. The GCL also has displaced amacrine cells (not shown). The seventh and only non-neuronal cell type is the Müller glial cell (red); its processes span the entire retina.

niques that mark when cells are born. Generally, progenitors produce ganglion cells first, followed by amacrine cells, cone photoreceptors, and horizontal cells. Progenitors generate rod photoreceptors, bipolar cells, and Müller glial cells last (8,9). The proposed competent shifts in retinal progenitors are similar to competence shifts that likely occur in other neuronal progenitors, such as those that give rise to layers II–VI of the cerebral cortex (10).

Based on everything we know about the retina and its development, we are in a good position to pinpoint thyroid hormone's role in the process. For example, thyroid hormone may regulate the development of one of the seven cell types. Alternatively, the hormone could help a progenitor decide whether it should produce mitotic or postmitotic daughter cells. Still another possibility is that thyroid hormone could push progenitor cells from one competence state to another. All of these hypotheses are readily testable in retinal tissue. Also, because the retina develops in ways similar to other neural tissues, we should be able to apply conclusions from experiments on the retina to other more complex, less understood neural tissues.

Thyroid Hormone and the Retina: Foundation Studies

For years, thyroid hormone has been known to influence the amphibian retina, as well as many other amphibian structures, during metamorphosis (11,12). However, until the 1990s, the hormone's role in the development of other vertebrate eyes remained largely unstudied. Now a series of reports have been published that explore the hormone's role in rodent, chick, and fish—as well as amphibian—eye development. Two of these studies in particular serve as a solid foundation on which to discuss the other findings published to date.

The first report came in the early 1990s, when Sjöberg, Forrest, and Vennström identified three thyroid hormone receptors in the early chick retina (13–15). Using *in situ* hybridization, the authors characterized the cell types expressing these receptors. The first receptor, $TR\alpha$, appeared in all the retinal layers at a time when neurogenesis was just finishing. The second receptor, $TR\beta_0$, showed a low level of expression in the outer and inner nuclear layer during the later parts of development. The third receptor, $TR\beta_2$, had the most intriguing expression pattern. $TR\beta_2$, which is a splice variant of $TR\beta_0$, appeared in the developing photoreceptor layer and waned as the retina matured. These important descriptions provided early hypotheses about thyroid hormone's and its receptors' roles in retinal development: $TR\alpha$ and $TR\beta_0$ may have a general function in many cell types, whereas $TR\beta_2$ may play a specific part in photoreceptor generation or maturation.

The second report appeared a decade later, when Sevilla-Romero, Pinazo-Durán, and colleagues (16) examined thyroid hormone action *in vivo* using a hypothyroid rodent model. These authors discovered that retina from hypothyroid pups, compared to those from euthyroid pups, differed substantially. The hypothyroid retina had fewer dividing progenitor cells, as well as an overall reduced thickness. Furthermore, the ganglion cell layer was less dense, reflecting fewer cells generated or increased cell death. Finally, the hypothyroid retina had impaired photoreceptors, with improperly formed outer segments (light-sensing organelles). From these descriptions, the authors conclude that thyroid hormone does impact the developing retina on several levels: it may guide progenitor proliferation, ganglion cell genesis, and photoreceptor maturation.

Taken together, these two reports suggest that thyroid hormone can act through a number of receptors in the retina.

Furthermore, through these receptors, the hormone may affect photoreceptors, progenitors, and possibly ganglion cells. By focusing on these receptors and cell types, various studies have contributed to our understanding of thyroid hormone in the retina.

Thyroid Hormone and Photoreceptor Development

Based on *in situ* hybridization and hypothyroid studies, thyroid hormone appears to target photoreceptor development. The hormone may be acting in at least two ways. It could instruct neuronal maturation, allowing already-born photoreceptors to express the correct genes, assume the correct form, and/or make the correct connections. Alternatively, thyroid hormone could instruct neuronal cell fate decisions, influencing progenitor cells to make the appropriate numbers and kinds of photoreceptors. Both hypotheses have been tested, with available evidence from experiments in amphibians, rat, and mouse.

The first hypothesis—that thyroid hormone instructs photoreceptor maturation—has been addressed by linking the hormone to opsin gene expression. Opsin gene expression begins many days after photoreceptor birth, at a time when the photoreceptor is assuming its mature form (17). In the rainbow trout, *Oncorhynchus mykiss*, thyroid hormone may have a role turning off ultraviolet (UV) opsin expression when animals reach adult stages (18). Similarly, in the frog, *Xenopus laevis*, which uses thyroid hormone to commence its entire metamorphic program, the hormone may direct photoreceptors to switch from porphyropsin expression to rhodopsin expression (19). Finally, in cell culture systems, TR β_2 has been shown to inhibit mouse S-opsin expression and promote M-opsin expression in the presence of thyroid hormone (20).

While these studies suggest that thyroid hormone may direct opsin expression in maturing photoreceptors, future studies will clarify unresolved points. For example, the findings in fish may be explained by loss or gain of photoreceptors, or simply differential growth, rather than by changes in opsin transcription (21). The frog studies, on the other hand, have been challenged by investigators using different assays to measure opsin content (22). And the TR β_2 cell culture studies were performed *in vitro* using isolated enhancer elements with high levels of TR β_2 and thyroid hormone. To confirm their relevance, the *in vitro* results will have to be confirmed *in vivo*, using tools such as transgenic reporter lines. With more experiments, thyroid hormone's role in opsin expression and photoreceptor maturation will become clearer.

The second hypothesis, that thyroid hormone instructs photoreceptor cell fate decisions, has been tested in several ways using the rodent retina. Kelley, Reh, and colleagues (23) performed their assays using dissociated rat retinal cells. These cells were cultured in various amounts of thyroid hormone and/or retinoic acid for either 6 or 14 days. After the culture, the cells were stained with antibodies to identify the different retinal cell types produced. Using this method, the authors were able to determine what cell types were born—and in what proportions—in different thyroid hormone culture conditions.

In cultures with thyroid hormone, rat progenitor cells divided at the same rate but produced a larger number of cells

expressing cone markers. Furthermore, cells cultured with thyroid hormone and 9-*cis* retinoic acid together exhibited an even stronger cone genesis effect. Hence, the authors conclude, retinal progenitor cells under thyroid hormone's influence produce cones at the expense of another cell type. These results are the first to suggest that progenitor cells may use thyroid hormone to direct photoreceptor cell fate. More experiments will better clarify rat progenitor cells' behavior in these culture systems, including what cell types are lost with thyroid hormone, why retinoic acid has an additive effect (i.e., is it needed for thyroid hormone receptor-retinoic acid receptor dimerization?), and the applicability of a dissociated cell system to the intact retina.

Perhaps the best example of thyroid hormone's role in photoreceptor production is seen in the mouse knockout of TR β_2 . Similar to its chick homologue, mouse TR β_2 is expressed in developing photoreceptors. Also similar to chick TR β_2 , mouse TR β_2 is one of two isoforms derived from the TR β Gene via alternative splicing. In order to make an allele-specific knockout, a TR β_2 -specific exon was targeted by Forrest and colleagues (24). As a result, only the photoreceptor-specific TR β_2 was lost, with all other thyroid hormone receptors left unaffected. The TR β_2 mutant retina were then studied for cone defects, using an array of cone-specific markers to detect changes.

TR $\beta_2^{-/-}$ mice displayed a dramatic cone phenotype: a total loss of M-opsin expression and elevated S-opsin expression throughout the retina (Fig. 2A). These opsin arrangements differed from those in wild-type retinas, which have dorsal cones expressing more M-opsin and ventral cones expressing more S-opsin (25). Based on this phenotype, the authors suggest that commitment to the M-cone identity is mediated by TR β_2 . These results may be intriguing from a photoreceptor cell fate perspective, and stimulate a range of further questions. For example, a recent report shows that most cones in the rodent retina do not express a single opsin; instead, they express varying amounts of both M-opsin and S-opsin (26). Does TR β_2 then regulate the levels of M-opsin and S-opsin in a cone, rather than controlling a cell fate decision? Also, does the knockout phenotype match the hypothyroid phenotype in photoreceptors, or is it less severe as has been found in knockout versus hypothyroid models in other neural tissues (27)?

Thyroid Hormone and Retinal Progenitors

As suggested by the *in situ* hybridization and hypothyroid studies, thyroid hormone appears to affect progenitor cell behavior. Progenitors express a multitude of genes that influence a series of decisions, including whether the cell should proliferate or differentiate (28). In fact, in some cases, progenitors may use the same molecule to stop proliferating and start differentiating (29). A series of experiments have placed thyroid hormone and thyroid hormone molecules in this decision process, although their exact roles are still undefined.

Perhaps the best studied example of thyroid hormone's role in proliferation is the rodent oligodendrocyte precursor cell (OPC) model (30). Oligodendrocytes, while not located in the retina, do myelinate ganglion cell axons comprising the optic nerve. OPCs divide for 8 days, and then all of their daughters differentiate simultaneously as oligodendrocytes.

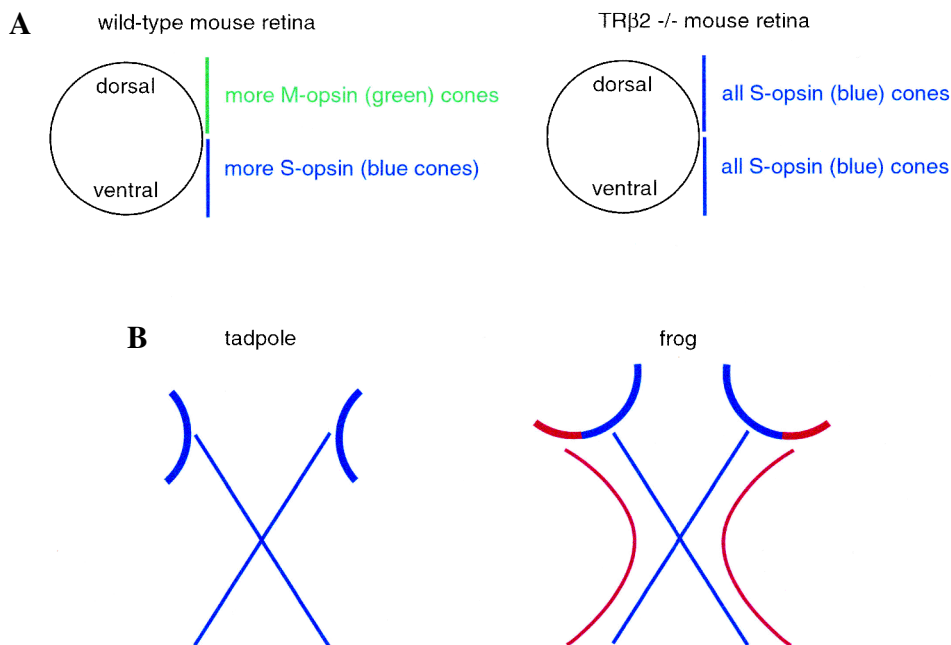


FIG. 2. **A:** $TR\beta 2$ mutants have a defect in opsin expression. Normally, cones in the dorsal retina express more M-opsin (green opsin) than S-opsin (blue opsin), whereas those in the ventral retina express more S-opsin than M-opsin. In $TR\beta 2^{-/-}$ animals, cones lose M-opsin expression and have extra S-opsin expression. **B:** Thyroid hormone helps produce ipsilaterally-projecting ganglion cells in *Xenopus*. In the tadpole stage, the eyes are lateral and all ganglion cells project axons to the contralateral side of the brain (retina is denoted by curves, ganglion cell axons are denoted by lines). Thyroid hormone promotes cell proliferation during metamorphosis, allowing the retina to grow and move frontally. Some of the new ganglion cells made comprise the ipsilaterally projecting cells (denoted in red), which send axons to the same side of the brain and confer binocular vision. Type III deiodinase appears to play a crucial role in this process.

In fact, even if OPC daughters are separated in different culture dishes, they still differentiate synchronously after approximately 8 days of division. This behavior of OPCs suggests an intrinsic “timer” in the OPC that promotes differentiation after a specified time.

To better understand components of this timer, Raff, Barr, and others (31,32) identified thyroid hormone as a signal that promotes differentiation in culture. Eight-day cultures with thyroid hormone show OPCs differentiating into oligodendrocytes, while cultures without hormone have OPCs continuing to divide. Interestingly, thyroid hormone cannot induce OPCs to become oligodendrocytes before the 8-day mark. Thyroid hormone may be waiting for rising levels of p27, a cyclin-kinase inhibitor known to block proliferation in many cell types. Alternatively, thyroid hormone may require increased levels of $TR\alpha_1$, which appears to be the receptor mediating the hormone’s effects in mouse OPCs (33). Whatever the necessary signal, thyroid hormone in this example promotes differentiation over cell division.

Marsh-Armstrong, Brown, and colleagues (34) explored thyroid hormone’s proliferative role by studying type III deiodinase (D3) in the *X. laevis* retina. D3 cleaves an iodine moiety off thyroid hormone, rendering the hormone inactive. The enzyme shows a peculiar expression pattern in *Xenopus* retina, with high staining in the dorsal ciliary marginal zone (CMZ) but no staining in the ventral CMZ. This D3 staining corresponds with proliferative activity during metamorphosis, with dorsal progenitors in the CMZ not dividing and ventral CMZ progenitors dividing prolifically. Hence, the authors hypothesized that thyroid hormone pro-

motes retinal progenitor cell proliferation ventrally, and that D3 prohibits this effect dorsally.

The experimental results strongly support their hypothesis. Specifically, the authors showed that exogenous thyroid hormone could increase retinal cell proliferation in areas not expressing D3. Furthermore, reducing thyroid hormone by misexpressing D3 blocked proliferation even in ventral CMZ progenitors. Reducing thyroid hormone signaling by misexpressing a dominant negative $TR\alpha$ also prevented proliferation. All these results together suggest that thyroid hormone drives retinal progenitors to divide, while thyroid hormone degradation by D3 activity has the opposite effect. The experiments make a compelling case for thyroid hormone’s positive role in proliferation; whether the hormone functions similarly in retina of organisms not undergoing metamorphosis remains to be seen.

How can the different OPC and *Xenopus* results be reconciled? In OPCs, thyroid hormone promotes differentiation, whereas in the *Xenopus* retina the hormone promotes division. One likely explanation is that thyroid hormone receptors are adaptable, and serve different functions in different tissues. A recent report, for example, shows thyroid hormone repressing E2F-1 transcription (and blocking cell division) in OPCs and P19 cells (35). Receptors not bound to hormone, on the other hand, bind upstream elements, activate E2F-1 transcription, and promote proliferation. These results reflect nicely the diversity of thyroid hormone and thyroid hormone receptor action. They suggest that the unliganded receptor in OPCs and the similar dominant negative receptor in *Xenopus* can lead to two very different outcomes. Inter-

estingly, the study also demonstrates that unliganded receptor, as opposed to receptor bound to hormone, can efficiently activate gene transcription in certain cases.

Finally, the role of thyroid hormone in progenitor cell proliferation has been substantiated by microarray data. While no published microarray experiments have tested the retina directly, experiments testing the entire brain, cerebellum, cochlear/vestibular tissues, and neuroblastoma cells have been performed (36–42). These experiments are uncovering a host of cell cycle genes involved in cell proliferation, including cyclin D1, cyclin D2, p27, BDNF, IGF-1, and others. Interestingly, in these experiments thyroid hormone affects proliferation-related genes in different ways, depending on the tissue studied and comparisons made. One significant challenge will be to resolve these differences and uncover general mechanisms thyroid hormone uses in controlling proliferation.

Avenues of Further Exploration

Over the last decade, experiments addressing thyroid hormone's role in the retina have generated a sizeable body of data. They have also inspired many more questions that will no doubt be tested in the near future. A few of these issues are discussed below. As this young field grows, it is these authors' hope that eventually our scientific knowledge of thyroid hormone action in the developing nervous system will equal our clinical descriptions on the subject, and that retinal studies might have a useful place in pushing this effort forward.

First, future studies will better clarify the role of deiodinase enzymes in the retina. Marsh-Armstrong and colleagues provide strong evidence that type III deiodinase has an important role during *Xenopus* metamorphosis. Is this role conserved across species? And what about other deiodinase enzymes? Type II deiodinase (D2), for example, is responsible for producing active thyroid hormone locally in the nervous system (43). D2 and D3 might act in antagonistic ways in the retina, or might serve entirely different populations of cells. Whatever the case, the deiodinase enzymes promise to be important, as their activities modulate the function of thyroid hormone receptors. Perhaps these deiodinase activities will explain why receptors in different parts of the same tissue can have different actions.

Second, new explorations will identify thyroid hormone's role in progenitor cell proliferation. Surely the hormone does not guide all proliferation, because receptor knock-out and hypothyroid animals still have neural tissue (44). What neural tissue, then, does thyroid hormone help produce? Is this neural tissue different, based on the cells it contains or the connections it makes? For example, in *Xenopus*, the "metamorphic retina" made by thyroid hormone is different from the "premetamorphic retina": it contains a set of ganglion cells that project to the ipsilateral side of the brain, compared to older ganglion cells that project contralaterally (Fig. 2B) (34). The *Xenopus* ipsilateral ganglion cells, and their connection to thyroid hormone, have been elegantly described in a series of reports by Hoskins (45–47); whether a similar "metamorphic" retina exists in other organisms remains to be seen.

Third, future microarray experiments will provide a context of genes associated with thyroid hormone in the ner-

vous system. At present, these experiments face a dilemma: the gene changes they report are smaller than expected from similar experiments in other tissues. These small gene changes on arrays reflect the same small gene changes seen in an earlier era, using techniques such as subtractive hybridization (2). Perhaps these small changes can be explained partly by interesting feedback pathways associated with thyroid hormone, such as those involving the hairless co-repressor or even the deiodinase enzymes (48). In any case, these small changes will be overcome by clever comparisons and sensitive analyses, some undoubtedly using retinal tissue.

Fourth, new research will place thyroid hormone activities in the context of other retinal genes known to be important. For example, TR β_2 and its role in regulating M-opsin cones is reminiscent of two other nuclear receptors, NRL and PNR (49–52). Similar to TR β_2 , NRL and PNR are expressed in photoreceptors. And similar to TR β_2 knockout animals, NRL and PNR loss-of-function animals give an excess of S-opsin cones. The TR β_2 phenotype does differ, in that it appears to mediate the levels of M- and S-opsins whereas NRL and PNR may mediate a rod photoreceptor/cone photoreceptor decision. Still, the interactions among these three molecules are ripe for more investigation, especially at the genetic level with knockout animals available for each.

Finally, as evidence in the laboratory accumulates, it will be tempting to look for retinal phenotypes in children who are born hypothyroid. These phenotypes are scarce in the clinical literature; one noticeable exception is a German report of a patient with thyroid hormone resistance suffering from monochromatic vision (53). These phenotypes may be rare because retinal development occurs in the first trimester, a time when fetal hypothyroidism may be compensated for by the mother. Alternatively, these phenotypes may have been ignored, because the necessary tests can be involved and impractical in developing countries where hypothyroidism is more common. Or perhaps the reason why these phenotypes have been missed is because we simply do not know enough—we do not understand thyroid hormone's role in retinal development, so we do not know what to expect. Challenged by this, our next step is to learn more.

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