Thyroid Hormone Action in Neural Development

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Effects of thyroid hormone on development of the brain have been documented for over a century. Although in many respects the hypothyroid brain appears morphologically normal, functional impairments include mental retardation, ataxia and spasticity. Keyed by the discovery of nuclear receptors for thyroid hormone that function as transcription factors, recent work has examined the mechanism of thyroid hormone action in brain development. The prediction that gene expression regulated by thyroid hormone is important for mediating brain development has spurred the search for thyroid hormone-responsive genes. Here we review some of the identified genes whose expression patterns correlate with the functional deficits observed in the hypothyroid brain. Recently identified thyroid hormone-responsive genes include synaptotagmin-related gene 1 (Srg1), a putative mediator of synaptic structure and/or activity, and *hairless*, a transcriptional cofactor that may influence the expression of other thyroid hormone-responsive genes.

Introduction

Neurological disorders can be caused by a number of environmental and genetic insults to the developing brain. Although the cause of such disorders can often be determined, in many cases the brain shows no apparent abnormalities. In some instances, undetected changes in dendritic and synaptic organization of the cerebral cortex may be responsible for compromised brain function (Huttenlocher, 1991). Mental retardation resulting from neonatal thyroid hormone deficiency is an example of a disorder in which subtle changes in neural circuitry are associated with devastating functional consequences.

Thyroid Hormone and Brain Development

Thyroid hormone (TH) is essential for proper mammalian development. Lack of sufficient TH results in abnormal development of virtually all organ systems, a syndrome termed cretinism (Schwartz, 1983; Delange, 1996). In particular, the brain is severely affected; functional deficits include mental retardation, ataxia, spasticity and deafness (DeLong, 1996). Most cases of neonatal TH deficiency can be treated effectively with TH replacement therapy. However, the timing of TH treatment is crucial. Treatment initiated within weeks after birth restores essentially normal development, while treatment after this critical period is ineffective (A.H. Klein *et al.*, 1972; R.Z. Klein *et al.*, 1996). Despite the existence of efficacious treatments and neonatal testing for congenital hypothyroidism, neonatal TH deficiency remains the leading cause of preventable mental retardation worldwide (Hetzel, 1994).

Although neonatal TH deficiency severely impacts development, it is rarely fatal. As a result, little information is available describing morphological changes occurring during the critical period of TH action in hypothyroid human brain. Thus, the study of morphological, molecular and biochemical effects of TH on brain development has relied on the use of experimental animal models. The pioneering studies of Eayrs and colleagues established the rat as a viable model system for examining the effects of TH deficiency on the developing mammalian brain (Eayrs, 1960, 1971). The examination of both morphological and behavioral effects of TH deficiency on the brain demonstrated that neonatal TH deficiency in rats causes growth and neurological abnormalities similar to human cretinism. As in humans, a similar critical period of action was recognized, which in the rat corresponds to the first two postnatal weeks.

TH deficiency results in multiple morphological alterations in neonatal rat brain [reviewed by Schwartz and DeLong (Schwartz, 1983; DeLong, 1996)]. Cells in the cortex are smaller and more closely aggregated than normal, due in part to an overall decrease in development of axonal and dendritic processes. Axonal density is decreased and the probability of axo-dendritic interaction is reduced by an estimated 80% (Eayrs, 1960, 1971). More recent studies have identified specific alterations in dendritic morphology in several cell types, including pyramidal cells in the cerebral cortex (decrease in dendritic spine number) (Schwartz, 1983), pyramidal cells in the visual cortex (reduced number and altered distribution of dendritic spines) (Ruiz-Marcos *et al.*, 1979; Morreale de Escobar *et al.*, 1983), cholinergic basal forebrain neurons (decreased number of primary dendrites and number of dendritic branchpoints) (Gould and Butcher, 1989), Purkinje cells (decreased number and size of dendritic spines) (Nicholson and Altman, 1972; Legrand, 1979) and granule and pyramidal cells in the hippocampus (decreased branching of apical and basal dendrites) (Rami *et al.*, 1986). Thus, TH influences the size, packing density and dendritic morphology of neurons throughout the brain.

Dendritic spines are sites of synaptic connections; dendritic changes may thus directly affect synapse number and formation, and thereby underlie cognitive deficits. A study by Eayrs examining the effects of thyroidectomy showed a positive correlation between the severity of dendritic defects and behavior in rats (Eayrs, 1960). The morphological and functional deficits caused by lack of TH, in particular dendritic abnormalities, are similar to those observed in human mental retardation disorders. For example, morphological changes observed in Rett syndrome include alterations in dendritic shaft and spine number (Armstrong, 1997). In addition, changes in neuronal packing density have been noted in Williams and Rubinstein–Taybi syndromes (Pogacar *et al.*, 1973; Bellugi *et al.*, 1999). Cell packing density could be altered as a consequence of reduced neurite number and/or size, and may also be related to cell differentiation.

TH deficiency also causes specific defects in cell migration and differentiation. In the cerebellum, both migration and differentiation of granule cells is affected (Oppenheimer and Schwartz, 1997). During cerebellar development, proliferating

cells from the external granular layer (EGL) migrate inward, differentiating in the process and establishing contacts with Purkinje cells. By postnatal day 21, the EGL has disappeared and mature granule cells populate the internal granule cell layer (IGL). In hypothyroid brain, the EGL persists significantly longer, due to a delay in the migration and/or differentiation of cells in the EGL (Nicholson and Altman, 1972; Rabie and Legrand, 1973). The EGL eventually disappears and the overall organization of the cerebellar cortex appears normal. However, the delay in migration may disrupt the precise timing required to establish productive interneuronal connections, and thus cause the decreased number and density of synaptic contacts between granule cells and Purkinje cells (Nicholson and Altman, 1972; Rabie and Legrand, 1973; Legrand, 1979). Delay rather than absence of a particular cell type or process is characteristic of the hypothyroid phenotype. The role of timing may underlie the observed critical period of TH action after which damage caused by TH deficiency is irreversible.

Another striking defect in the hypothyroid neonatal brain is the reduction in myelination. Myelin is a multilamellar membrane produced by oligodendrocytes that surrounds and supports axons, facilitating the conduction of nerve impulses and axon survival. Thus, reduction of myelination can have marked effects upon neuronal connectivity and the establishment of neuronal networks. Neonatal hypothyroidism leads to an overall decrease in myelination, characterized by a reduction in the quantity of both myelin lipids and proteins (Schwartz, 1983). TH governs the differentiation of oligodendrocytes from O2A precursor cells under the concerted actions of fibroblast and platelet-derived growth factors, and is thought to primarily regulate the timing of differentiation (Barres *et al.*, 1994; Johe *et al.*, 1996). Thus, the decline in myelination may be linked to the influence of TH on oligodendrocyte differentiation. Whether TH is involved solely in timing or is also instructive for initiating the differentiated phenotype is not clear.

Molecular Mechanism of Thyroid Hormone Action in the Brain

The wealth of physiological data from hypothyroid rat brain provides a framework in which to analyze the molecular basis of TH action during brain development. The effects of TH are mediated through the action of specific nuclear receptor proteins that function as inducible transcription factors (Evans, 1988; Tsai and O'Malley, 1994). There are two structurally related TH receptors (α and β) encoded by distinct genes. Both TH receptor genes produce alternatively spliced isoforms (Lazar, 1993). The expression of TH receptors in the developing rat brain has been well established (Schwartz and Oppenheimer, 1978; Bradley *et al.*, 1992; Mellström *et al.*, 1991). TH receptors bind to specific DNA sequences, TH response elements (TREs), located near specific target genes. Upon hormone binding, TH receptors influence their transcription rate. Gene expression can be activated or repressed via TH action, implying complicated regulatory mechanisms involving multiple interacting proteins, such as coactivators or corepressors (Koenig, 1998; Xu *et al.*, 1999). The presence of TH receptors in the brain suggests that TH in the developing brain exerts its effects by regulating the expression of specific genes [reviewed by Oppenheimer and Scwartz (Oppenheimer and Scwartz, 1997)].

Genes regulated by TH in the brain comprise a genetic program that, when set in motion by TH, directs proper development. Thus, TH initiates a cascade of gene expression in which the products of genes first induced by TH influence expression of other, downstream genes. Initially expressed genes are those that respond directly to hormone-bound TH receptors without first inducing expression of other factors. Such genes can be defined experimentally as those induced in the absence of new protein synthesis and are considered 'direct response' or 'immediate early' genes. This paradigm has been used to classify genes induced by serum in quiescent fibroblasts and by synaptic activity in the brain, as well as induction of gene expression by TH in *Xenopus* metamorphosis (Lau and Nathans, 1985; Cole *et al.*, 1989; Wang and Brown, 1991; Brown *et al.*, 1996). Direct regulation by TH requires TRE sequences, although *in vitro* activity of a TRE sequence does not necessarily reflect *in vivo* regulation. Additional factors, including other DNA binding proteins, coactivators and corepressors, coordinate to ultimately govern gene expression *in vivo*. Thus, the best evidence for whether a gene responds directly to TH is both resistance to protein synthesis inhibitors and identification of a functional TRE. To date, few direct response genes for TH have been identified in mammalian brain. The rat *hairless* gene, a recently identified TH-responsive gene that fits the criteria for a direct response gene, will be described.

The Search for Thyroid Hormone-responsive Genes in Mammalian Brain

In order to understand the mechanism by which TH affects brain development, the identification of specific genes regulated by TH is necessary. Two general approaches have been used to identify genes regulated by TH in developing rat brain. Screens using molecular biological techniques such as subtractive hybridization and whole-genome polymerase chain reaction (PCR) have identified several genes differentially regulated between hypothyroid and euthyroid brain (Muñoz *et al.*, 1991; Iglesias *et al.*, 1996; Thompson, 1996). A second approach uses the characteristic deficiencies in hypothyroid brain to identify candidate genes that may be under TH control. For example, analysis of genes encoding myelin-associated proteins revealed that these genes are TH-responsive (Farsetti *et al.*, 1991; Tosic *et al.*, 1992; Rodríguez-Peña *et al.*, 1993). Since TH deficiency has wide ranging effects on brain development, it is not surprising that a variety of genes are regulated by TH in the neonatal brain. However, expression of most known TH-responsive genes is reduced only 2- to 3-fold in hypothyroid versus euthyroid animals and regulation is often slow (>24 h). Although few of the presently known TH-responsive genes are directly regulated by TH, we will discuss several for which expression is associated with morphological defects resulting from TH deficiency.

Myelination

A striking phenotype in the hypothyroid neonatal brain is the reduction in myelination. The major constituents of CNS myelin include myelin basic protein (MBP), proteolipid protein (Plp), 2′,3′-cyclic nucleotide 3′-phosphodiesterase (CNPase) and myelin associated glycoprotein (MAG). Each of these genes has been analyzed for regulation by TH and all show reduced mRNA levels in hypothyroid neonatal rat brain. MBP mRNA levels are reduced 4-fold in hypothyroid rat brain, and a functional TRE has been mapped in the MBP gene (Farsetti *et al.*, 1992; Pombo *et al.*, 1999). The level of Plp, CNPase and MAG mRNA in neonatal hypothyroid animals is reduced 1.5-, 2- and 4-fold, respectively (Ibarrola and Rodríguez-Peña, 1997). The effect of TH on myelin -associated genes is likely to be a combination of transcriptional and post-transcriptional regulation (Farsetti *et al.*, 1991; Ibarrola and Rodríguez-Peña, 1997; Strait *et al.*, 1997). The reduction of myelin-protein gene expression in hypothyroid brain likely plays

a significant role in the observed effects of hypothyroidism on myelination.

Cell Differentiation and Migration

During development, cell differentiation, migration and survival play key roles in the formation of interneuronal networks. Neurotrophins and their receptors are important modulators of cell survival and differentiation in the brain. Using the candidate gene approach, the mRNA levels of the neurotrophins NGF, BDNF and NT-3 were measured in hypothyroid neonatal rat brain and found to be reduced 1.5-, 2.5- and 2-fold, respectively (Lindholm *et al.*, 1993; Alvarez-Dolado *et al.*, 1994; Leingartner *et al.*, 1994; Koibuchi *et al.*, 1999). Similarly, the mRNAs encoding the neurotrophin receptors trkA and trkB are reduced by 1.5- and 2-fold, respectively (Alvarez-Dolado *et al.*, 1994; Knipper *et al.*, 1999). Although TH has only a modest effect on the expression level of these genes, regulation of neurotrophins and their receptors by TH may be significant. For example, TH has been shown to influence the number of cholinergic neurons and the degree of innervation of hippocampal CA3 and CA1 regions (Oh *et al.*, 1991). Cholinergic basal forebrain neurons express trkA while their targets in the hippocampus express NGF, suggesting a potential mechanism by which TH can influence neuronal number and connectivity in the forebrain (Roskoden *et al.*, 1999). In the cerebellum, BDNF expressed by Purkinje and granule cells has been shown to influence granule cell axon elongation and survival (Koibuchi *et al.*, 1999). In addition, BDNF knockout mice exhibit cerebellar deficits similar to those of hypothyroid animals, such as a delay in granule cell migration and a reduction in Purkinje cell arborization (Schwartz *et al.*, 1997). Furthermore, NT-3 expression by granule cells can promote differentiation of Purkinje cells (Lindholm *et al.*, 1993). Thus, TH regulation of BDNF and NT-3 expression may be involved in mediating cerebellar development.

TH also influences cell migration, a process important for establishing interneuronal connections and the overall anatomy of the brain. *Reeler* mutant mice show neuronal positioning abnormalities in the neocortex, hippocampus and cerebellum (Lyon and Searle, 1989; Rakic and Caviness, 1995). Thus, *reelin*, the gene disrupted in *reeler* mutants, is thought to be involved in neuronal migration and lamination during development. The level of *reelin* mRNA is reduced 2-fold in the cerebral cortex of perinatal hypothyroid rats (Alvarez-Dolado *et al.*, 1999). TH regulation of *reelin* expression likely influences some aspects of neuronal migration in the forebrain. Another protein involved in cell migration, neural cell adhesion molecule (NCAM), was found to be TH-responsive using a whole-genome PCR-based screen (Iglesias *et al.*, 1996). Expression of NCAM is increased 2-fold in neonatal hypothyroid brain. NCAM is important in regulating cell–cell interactions as well as mediating proper cell migration in the CNS (Tomasiewicz *et al.*, 1993).

Dendritic Structure/Synaptogenesis

Neonatal hypothyroidism results in altered neuronal structure and function, including reduction in neurite outgrowth, synaptogenesis and dendritic elaborations. RC3/neurogranin is a gene directly regulated by thyroid hormone whose expression is consistent with a role in synapse formation and/or function. RC3/neurogranin mRNA levels are reduced 2- to 3-fold in hypothyroid neonatal rat brain, and a functional TRE has been mapped to the first intron of the RC3/neurogranin gene (Muñoz *et al.*, 1991; Martínez de Arrieta *et al.*, 1999). The RC3/neurogranin protein binds calmodulin and is a substrate for protein kinase C (Iñiguez *et al.*, 1992). The function of RC3/neurogranin is not known, but the protein accumulates in the dendritic spines of specific cortical neurons, where it may act to regulate the level of free calmodulin (Iñiguez *et al.*, 1993). Thus, it is feasible that RC3/neurogranin may play a role in synaptic structure or function.

A gene identified by testing cerebellum-specific genes for TH-dependent expression is Purkinje cell protein-2 (Pcp-2) (Strait *et al.*, 1992). Pcp-2 mRNA levels are reduced 3.6-fold in P15 hypothyroid rat brain, and the Pcp-2 gene contains two TREs capable of mediating TH-responsive expression (Zou *et al.*, 1994; Hagen *et al.*, 1996). While the function of Pcp-2 is unknown, its specific expression in Purkinje cells suggests it could be involved in mediating the effects of TH on Purkinje cell morphology.

Synaptotagmin-related gene 1 (Srg1), a novel TH-responsive gene recently identified in our lab, may also be involved in synapse formation and/or function (Thompson, 1996). The protein encoded by Srg1 is structurally similar to synaptotagmins, a family of proteins found primarily in the brain (Südhof and Rizo, 1996). One of the synaptotagmin proteins, synaptotagmin I, is localized to synaptic vesicles and is involved in mediating neurotransmitter release (Geppert *et al.*, 1994). Srg1 mRNA expression is reduced ~3-fold in hypothyroid cerebellum. Treatment with TH induces expression in hypothyroid cerebellum within 2 h (Thompson, 1996). Srg1 expression is developmentally regulated, reaching high levels at postnatal day 7 and rising to a peak at postnatal day 14. This is a period within which TH is known to be critical and when both TH and TH receptor levels rise dramatically. Srg1 is preferentially expressed in the brain; expression has not been detected outside the nervous system in neonatal (P15) or adult rats (Thompson, 1996) (F.F. Facchinetti *et al.*, submitted for publication). Detailed examination of Srg1 expression in the brain by *in situ* hybridization demonstrated that Srg1 is highly expressed in cerebellum, hippocampus, cortex and striatum (Thompson, 1996). Further studies have indicated that Srg1 is specifically expressed in neurons (F.F. Facchinetti *et al.*, submitted for publication).

The effect of TH on Srg1 expression is reflected at the protein level. The 45 kDa protein encoded by Srg1 is detected only in brain (Figure 1*A*; F.F. Facchinetti *et al.*, submitted for publication). Expression of Srg1 protein is dramatically reduced in both hypothyroid brain and in neurons cultured in the absence of TH (Figure 1*B*; F.F. Facchinetti *et al.*, submitted for publication). During development, Srg1 expression is significantly delayed in hypothyroid brain. Srg1 is normally expressed at postnatal day 12 but in hypothyroid cerebellum is not detected until postnatal day 21 (F.F. Facchinetti *et al.*, submitted for publication). Studies to determine the cellular localization of Srg1 protein revealed a punctate distribution primarily in neurites, both in brain sections and in cultured neurons (Figure 2). The distribution of Srg1 protein specifically in axons and/or dendrites suggests that it may have a role in synapse formation or function. Although the function of Srg1 and other synaptotagmin genes is not known, developmentally regulated expression of Srg1, together with its distribution in neurites and the effects of TH on synapse formation, suggests that Srg1 may have an important role in the brain.

Transcriptional Regulation

TH acts to regulate gene transcription; thus the influence of TH on expression of transcription factors could render many

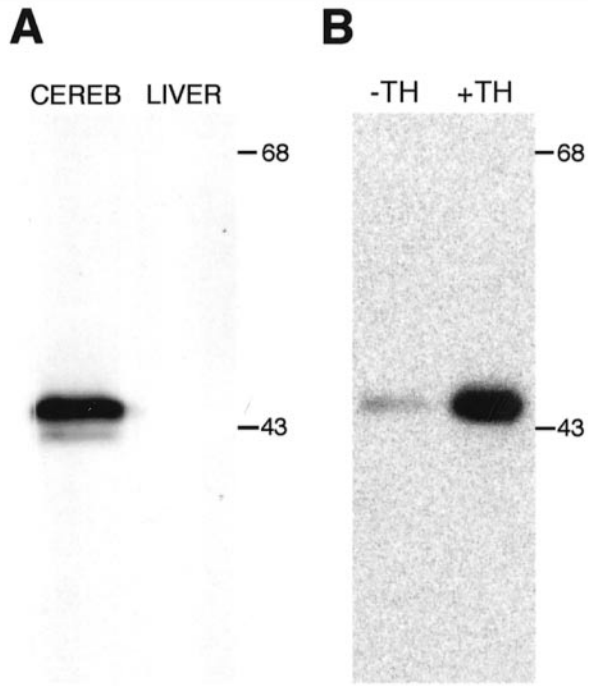

Figure 1. Neural expression of Srg1 protein. (*A*) Srg1 protein is specifically expressed in neonatal rat brain. Western analysis of whole-cell extracts from postnatal day 15 rat tissues using Srg1-specific antiserum. CEREB, cerebellum. (*B*) Srg1 protein levels are influenced by thyroid hormone. Srg1 protein expression in cerebellar granule cells cultured in the absence (–TH) or presence (+TH) of thyroid hormone. Equal amounts of total protein were loaded per lane and verified by Ponceau Red staining (data not shown). Position of molecular weight standards (kDa) is indicated.

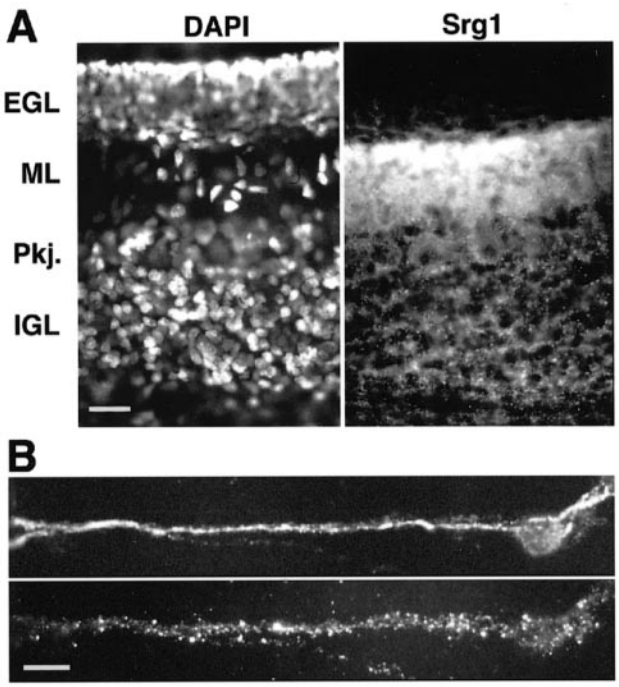

Figure 2. Srg1 protein is localized to neurites. (*A*) Expression of Srg1 in neonatal rat cerebellum. Sections from postnatal day 12 rat cerebellum were used for immunofluorescent (IF) staining with Srg1-specific antiserum and visualized with Cy3-conjugated anti-rabbit secondary antibody. Left, DAPI staining of cell nuclei shows cytoarchitecture of cerebellum; right, indirect IF detection of Srg1 protein. Srg1 is abundant in the cell-sparse and neurite-rich molecular layer and shows punctate staining in the IGL. EGL, external granule cell layer; ML, molecular layer; Pkj., Purkinje cell layer; IGL, internal granule cell layer. Scale bar = 20 μm. (*B*) Expression of Srg1 in neurons. Cultured cerebellar granule cells were simultaneously labeled for Srg1 and MAP2 using indirect immunofluorescence. Top, IF staining for MAP2, a dendritic marker, shows a dendrite extending to the left from a granule cell body. Bottom, image of the same cell showing IF staining for Srg1. Note the punctate expression pattern along the length of the dendrite. Scale bar = 10 μm. Srg1 was detected using Srg1-specific antiserum and visualized with Cy3-conjugated anti-rabbit secondary antibody. MAP2 was detected using a monoclonal MAP2-specific antiserum and visualized with FITC-conjugated anti-mouse secondary antibody.

additional genes under indirect TH control. Krox-24/NGFI-A/Egr-1 encodes a transcription factor originally identified as an immediate early gene in fibroblasts and subsequently shown to be induced by membrane depolarization and synaptic activity (Jahner *et al.*, 1991). Krox-24 expression is detected in various brain regions, such as striatum, cortex and cerebellum (Mellström *et al.*, 1994). Expression of Krox-24 is regulated by TH through an upstream TRE, and its levels are reduced 4- to 8-fold in hypothyroid neonatal rat brain. The induction of Krox-24 expression by membrane depolarization and synaptic activity indicates it may be involved in modulating neuronal connectivity and synaptic plasticity (Ghorbel *et al.*, 1999).

Calcium calmodulin-dependent kinase IV (CamKIV) influences transcription by modifying the activity of specific transcription factors. CamKIV protein levels were found to be regulated by TH in rat telencephalon cell culture (Krebs *et al.*, 1996). Through phosphorylation, CamKIV regulates the activity of CRE binding protein (CREB) and serum response factor (SRF), implicating CamKIV in the Ca^{2+}-dependent expression of immediate early genes through either CREB or SRF (Krebs *et al.*, 1996). Thus, TH control of CaMKIV expression might indirectly influence the Ca^{2+}-dependent gene expression.

A TH-responsive gene identified in our screen of neonatal cerebellum, the rat *hairless* (*hr*) gene, may also have a role in transcriptional regulation. The *hr* gene was originally identified as a spontaneous mutation that causes progressive hair loss in mice (Lyon and Searle, 1989; Cachon-Gonzalez *et al.*, 1994). Expression of *hr* is highly (>10-fold) and rapidly (< 4h) up-regulated by TH in neonatal cerebellum (Thompson, 1996). TH induction of *hr* expression occurs in the absence of protein synthesis in both cultured pituitary cells and neurons (Figure 3) (Thompson, 1996). In fact, expression of *hr* is superinduced in the presence of TH and cycloheximide, a phenomenon previously observed for immediate early genes such as *c-fos*.

Further experiments have shown that the *hr* gene has a potent TRE that binds TH receptors and confers TH-responsiveness to a heterologous promoter (Thompson and Bottcher, 1997). Together these data indicate that *hr* is directly regulated by TH, making it one of the first definitive direct target genes identified in the mammalian CNS.

The finding that *hr* is regulated by TH in the brain suggests that *hr* may have a role in neural development. Temporal expression of *hr* is consistent with this proposal. Expression of *hr* is first detected at embryonic day 18, reaching high levels shortly after birth. Neonatal expression is coincident with TH receptor β expression and is within the period of development shown to be influenced by TH (Thompson, 1996). Like hypothyroid brain, *hr* mutant brain shows no gross morphological abnormalities, although subtle alterations in Purkinje cell structure have been reported for the hr^{rh} allele (García-Atares *et al.*, 1998). Detailed analysis of *hr* expression using *in situ* hybridization revealed that

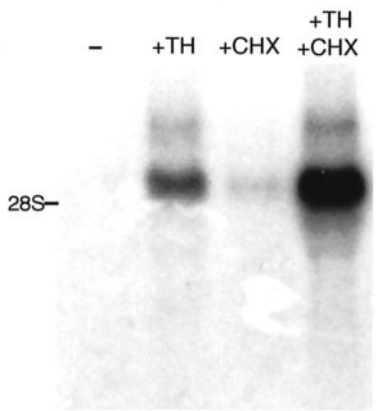

Figure 3. *Hairless* is directly regulated by thyroid hormone in neurons. Northern analysis showing expression of *hr* mRNA in cerebellar granule cells cultured in the absence of TH (–), treated with thyroid hormone (+TH), treated with cycloheximide (+CHX) or treated with both thyroid hormone and cycloheximide (+TH+CHX). Note that induction of *hr* expression by TH is not blocked but rather is superinduced by cycloheximide. 15 μg of total RNA was loaded per lane and equal loading was verified by ethidium bromide staining (data not shown). The position of 28S ribosomal RNA is indicated.

hr is expressed in many brain regions, including the cerebellum, hippocampus, cortex and thalamus (G.B. Potter and C.C. Thompson, unpublished data). Thus, it will be important to determine if these regions of the brain show morphological defects in *hr* mutant mice. In addition, it is not known whether *hr* mutant mice suffer from learning and/or motor defects. We have begun to address this issue; preliminary behavioral testing of *hr* mice has shown some sensorimotor abnormalities (A.C. DeVries and C.C. Thompson, unpublished data). The recent identification of the human *hr* gene will enable the study of potential neurological phenotypes in humans (Ahmad *et al.*, 1998; Cichon *et al.*, 1998).

The function of the protein encoded by *hr* (Hr) is under investigation. The 1207 amino acid Hr protein does not share significant homology to known structural motifs, although it shows partial homology to a testis-specific cDNA of unknown function (Höög *et al.*, 1991; Cachon-Gonzalez *et al.*, 1994). To begin to understand the biochemical function of Hr, we identified proteins that interact with it. Remarkably, we found that Hr interacts directly and specifically with TH receptors, the same proteins that regulate its expression. Hr localizes to the nucleus, indicating that it can interact with TH receptors *in vivo* (Thompson and Bottcher, 1997). In addition, Hr is capable of repressing transcription in a cotransfection assay, suggesting it functions as a transcriptional corepressor (Thompson and Bottcher, 1997). Corepressors are proteins that mediate transcriptional repression by interaction with DNA binding proteins. Nuclear receptor corepressors are ubiquitously expressed and interact with multiple receptors (Chen and Evans, 1995; Hörlein *et al.*, 1995). Hr is distinct from other corepressors in its restricted expression pattern and lack of interaction with retinoic acid receptor (Thompson and Bottcher, 1997). Regulation of *hr* expression by TH, together with evidence that Hr interacts with TH receptors, indicates that *hr* may be a key mediator of TH action in the brain. We are currently testing a model which predicts that Hr influences the expression of downstream TH-responsive genes.

Conclusions

Significant advances have been made in identifying the targets of TH action in the brain, TH-responsive genes, over the past several years. It is likely that many more TH-responsive genes in developing mammalian brain remain to be discovered, in particular genes that are directly regulated by TH. A further challenge will be to determine the functional role of the proteins encoded by TH-responsive genes on brain development and function. The ability to control development in experimental animals by manipulating TH levels makes neonatal TH deficiency an excellent model system for studying crucial developmental processes such as cell differentiation, migration and establishment of the appropriate neural circuitry. The morphological and functional defects caused by lack of TH are similar to those observed in other developmental disorders, including low IQ, reduced dendritic branching of particular neurons, reduction in synapse number and increased cell packing density. Thus, understanding the molecular mechanisms underlying cretinism is likely to shed light on other developmental disorders as well.

Notes

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