Thyroid hormone signaling is highly heterogeneous during pre- and postnatal brain development

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Abstract

We have generated transgenic reporter mice to analyze the spatio-temporal distribution of thyroid hormone signaling during mouse brain development. The reporter system, utilizing a chimeric yeast *Gal4* DNA-binding domain–thyroid hormone α ligand-binding domain fusion protein to drive *lacZ* expression, revealed that thyroid hormone signaling starts in the midbrain roof several days before the onset of thyroid gland function, and that it remains highly heterogeneous in the central nervous system throughout pre- and postnatal development. We speculate that this heterogeneity might provide neural cells with positional information during development.

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Introduction

Thyroid hormone (3,5,3'-tri-iodothyronine; T₃) is an essential regulator of brain development, congenital hypothyroidism resulting in severe and irreversible mental retardation. T₃ directly activates gene expression by binding to thyroid hormone receptors (TRs) expressed from the two *TRa* and *TRb* genes. Like other nuclear receptors, including the closely related retinoic acid receptors, TRs act mainly as heterodimers with retinoid X receptor (RXR), and remain bound to DNA in the absence of hormone binding. Unliganded TR exert a negative influence on gene expression. This property explains why mice lacking both *TRa* and *TRb* genes are viable and fertile (Gothe *et al.* 1999, Gauthier *et al.* 2001) whereas mice homozygous for point mutations introduced in the *TRa* gene die either *in utero* (Liu *et al.* 2003) or soon after birth (Kaneshige *et al.* 2000, Tinnikov *et al.* 2002, Flamant & Samarut 2003). Upon T₃ binding, interaction with corepressors are destabilized and TRs recruit several coactivator complexes (Hermanson *et al.* 2002). Although *TRa*, *TRb*, *RXRa*, *RXRb* and *RXRγ* display distinct expression

patterns, at least one TR isoform and one RXR isoform are likely to be present at some level in most mammalian brain cells throughout development, likely to be as heterodimers (Bradley *et al.* 1989, 1992, 1994, Mellstrom *et al.* 1991).

In rodents, several neural populations have been shown to be sensitive to hypothyroidism during the pre- and postnatal periods (Bernal & Nunez 1995, Koibuchi & Chin 2000). However, the multiple and successive influences of T₃ on brain cells remain poorly understood. When exactly T₃ starts to act on brain cells is still unclear. We have shown previously that, in the chicken embryo, T₃ originating from egg yolk is already present in the Hensen's node during neurulation (Flamant & Samarut 1998). The situation in rodents is, however, more complex. Both T₃ and its precursor thyroxine (T₄), which is secreted by the thyroid gland, are detected in the embryo proper from gestational day 13 (E13). As the onset of fetal thyroid function occurs only at E17.5, this indicates that at least maternal T₄ can cross the placenta (Obregon *et al.* 1984). Maternal hypothyroidism results in a reduction of embryo viability and weight, compromising brain develop-

ment (Morreale de Escobar *et al.* 1995, Berbel *et al.* 2001), while maternal hypothyroxinemia (i.e. low T4 level with normal T3 level) selectively affects fetal brain histogenesis and neuroblast migration (Lavado-Autric *et al.* 2003). It is thus very likely that T3 in the fetal brain is mainly produced by outer ring deiodination of maternal T4. This reaction can be catalyzed by type 2 deiodinase, an enzyme encoded by the *Dio2* gene which is expressed in the embryo (Galton *et al.* 2001). The effect of *Dio2* expression can be counteracted by *Dio3* expression, encoding type 3 deiodinase, which also occurs at specific locations in the placenta and fetus, and is responsible for T4 and T3 catabolism (Tsai *et al.* 2002).

At around E16, the appearance of the blood–brain barrier and the choroid plexus–cerebrospinal fluid barrier introduces another level of regulation. While the blood–brain barrier is relatively impermeable to T3, T4 is transferred from the serum to the cerebrospinal fluid via the choroid plexus. As a result, it has been estimated that more than half of the T3 found in the postnatal brain results from local outer ring deiodination (van Doorn *et al.* 1983), *Dio2* being expressed mainly in tanicytes and astrocytes (Guadano-Ferraz *et al.* 1997b). The brain compensates for low T3 levels by increasing *Dio2* expression and local T3 synthesis (Guadano-Ferraz *et al.* 1999). By contrast, high T3 levels induce the expression of *Dio3* and T3 degradation at different sites (Guadano-Ferraz *et al.* 1999, Tu *et al.* 1999). This autonomous regulation in the brain has two consequences. First, the distribution of T4, T3 and related metabolites in the adult rodent brain is heterogeneous (Dratman & Gordon 1996, Rozanov & Dratman 1996, Pinna *et al.* 2002). Secondly, the T3 level is much more stable in the brain than in the serum. For example, T3 is still present in the brain several weeks after thyroidectomy (Morreale de Escobar *et al.* 1995). Anterograde axonal transport of T3, indirectly demonstrated for noradrenergic neurons, could potentially deliver T3 to distant locations (Gordon *et al.* 1999) and might provide another level of regulation to T3 distribution.

All these observations suggest that T3 signaling in the developing brain can be modulated at several levels by both intracellular and extracellular events and does not directly reflect T3 content in maternal, fetal or newborn serum. To directly visualize T3 signaling, we have produced transgenic reporter mice, using a highly specific

one-hybrid system. The ability of this system to faithfully reflect T3 signaling has been previously demonstrated *in vitro* (Flamant & Samarut 1998) and *in vivo* for several other nuclear hormone receptors (Solomin *et al.* 1998, Mata De Urquiza *et al.* 1999, Wallen-Mackenzie *et al.* 2003). Analysis of these reporter mice allowed us to describe precisely for the first time the spatio-temporal pattern of T3 signaling in the brain which appears to be highly dynamic and heterogeneous. These data suggested that T3 should not only be considered as a trophic factor but might also constitute an informative signal able to control cell fate during brain development.

Materials and methods

Reporter plasmid

The plasmid pFIND-T3 (Feedback Inducible Nuclear-Driven reporter for T3) was generated by replacing the XhoI-NheI restriction fragment of pFIND-RA, reporting for retinoids, containing most of the Gal4-RAR reading frame (Mata De Urquiza *et al.* 1999) by the equivalent portion (XhoI-XbaI) of Gal4-TR α (Flamant & Samarut 1998). pFIND-T3 was transfected together with pBKS – (Stratagene, San Diego, CA, USA) as carrier DNA (20 ng pFIND-T3/1 μ g pBKS –) into COS cells (Fugene; Roche Applied Science, Meylan, France). *LacZ* transient expression was measured 48 h later in cell lysates using Ortho-Nitro-Phenyl-Galactopyranoside (Sigma). pSG5-RXR encodes the human RXR α cDNA from an SV40 transcription promoter (Stratagene).

Animals

Transgenic OF1 founders were recognized by Southern blotting. Later identification of FINDT3 animals relied on PCR amplification of the *lacZ* sequence. Animal carrying the *Pax8* knockout mutation (Mansouri *et al.* 1998) were screened as described (Flamant *et al.* 2002). To increase brain hormonal level, a mixture of T4 and T3 (Sigma) was injected each day for 5 days (i.p. injection of 100 μ l; 200 μ g/kg T3 and 2 mg/kg T4) between postnatal day 8 (P8) and P12. Xgal (5-bromo-4-chloro-3-indolyl- β -D-galactoside) staining was performed after paraformaldehyde fixation for 16 h at 30 °C for at least ten samples in each condition

with identical results. All experiments were conducted in accordance with accepted standards of animals care.

RNA analysis

RNA was extracted using the Geneelute kit (Sigma), followed by DNAaseI and proteinase K digestion, phenol/CHCl₃ extraction and ethanol precipitation. For quantitative RT-PCR, cDNA was prepared from 1 µg of individual RNA samples (not pooled) using AMV reverse transcriptase (Promega, Charbonnières, France). Diluted cDNA (1/10 to 1/100 to keep threshold cycle (C_T) values between 20 and 30) was amplified by PCR in the presence of Sybr green (Roche Applied Science) using a real-time PCR Opticon machine (MJ Research, Reno, NV, USA) with the following oligonucleotide primers using the acidic ribosomal phosphoprotein PO gene (36B4) mRNA as an internal reference: 5'-GAAGATGGGACTCCTCAGCGTA and 5'-GCTTGAGCAGAATGACCGAGTC for *Dio2*; 5'-TCAACAGTGAAGG CGAGGAG and 5'-ATGCCACACGGCTTTGAGAGAG for *Dio3*; 5'-CCCACCTTGTCTCCAGTCTTT and 5'-ACC TCCTTCTTCCAGGCTTT for *36B4*; 5'-CGGTTCTGCTCAATCAGC and 5'-GATGCCGTCACAGATAGATTGG for *Gal4-TRα*.

Serial dilutions of a cDNA prepared from a pool of P15 whole brain RNA (non-transgenic OF1 except for *Gal4-TRα* where FINDT3A mice were used) was used to build a standard curve and verify that amplification efficiency was superior to 90%. Quantification was performed using the $\Delta\Delta C_T$ method (Livak & Schmittgen 2001).

Dio2 and *Dio3* expression was analyzed by *in situ* hybridization in E15.5 OF1 fetuses using 35S-radiolabeled probes as previously described (Guadano-Ferraz *et al.* 1997b, Escamez *et al.* 1999).

Quantification of T3 in brain extracts

T3 was partially purified from pools of ten FINDT3B brain fractions by ion-exchange chromatography (Obregon *et al.* 1984). Dry extracts were suspended in culture medium and placed on pFIND-T3 transfected COS cells. Culture medium serum was treated by anion exchange chromatography (AG1-X8; Biorad, Marnes-la-Coguette, France) for T3 depletion (Belote *et al.* 1970).

Figure 1 The Gal4-TR α reporter system. (A) The pFIND-T3 plasmid carries two transcription units. One ensures the expression of a Gal4-TR α hybrid receptor able to bind Gal4 UAS and to recruit transcription coactivators on the TR α ligand-binding domain in a T3-dependent manner (rat TR α 1; amino acids 126–410). The hybrid receptor controls the expression of both the *lacZ* reporter gene and its own expression. (B) *lacZ* transient expression after transfection of COS cells with pFIND-T3 (solid bars), pFIND-T3+pSG5-RXR (shaded bars) or pFIND-RA (open bars). A significant response was observed from 10⁻¹¹ M T3, a concentration lower than the one found in normal serum. Maximum retinoic acid activation of pFIND-RA in the same conditions is given for comparison. Expression is insensitive to retinoic acid and estrogen, and is sensitive to T4 at high molarity only. RXR overexpression has no significant influence.

Results

Generation of transgenic mice with a reporter system for T3 signaling

We adapted the system used to trace RAR ligands (Mata De Urquiza *et al.* 1999) for TR by replacing the RAR ligand-binding domain of *Gal4-RAR* with a *TRα1* cDNA fragment encompassing the hinge and the ligand-binding domain, to create a Gal4-TR α 1 hybrid receptor. The system is independent of endogenous TR (Fig. 1A). The hybrid receptor is able to bind Gal4 upstream activation

Figure 2 Postnatal expression of the *lacZ* reporter transgene. (A and B) FINDT3A brain at P2, ventral and dorsal view; (C and D) FINDT3A brain at P2, ventral and dorsal view; (E and F) FIND-T3B brain at P8, ventral and dorsal view; (G and H) *Pax8*^{-/-} FINDT3B hypothyroid mice at P8; (I and J) *Pax8*^{-/-} FINDT3B hypothyroid mice treated for 5 days with T4 and T3; (K) hyperthyroid FINDT3B olfactory bulbs at P15, ventral view (scattered staining is not observed in control animals); and (L–R) coronal sections of P15 FINDT3A brain at the approximate following positions, relative to Bregma line: L, 1.5; M, 0.8; N, -0.5; O, -1; P, -2.5; Q, -3; R, -5 (Franklin & Paxinos 1997). Bars represent 2 mm. High resolution figures are available at www.ens-lyon.fr/LBMC

sequence (UAS) and to activate the *lacZ* gene only when activated by T3. This system needs both T3 and coactivators to function properly. The feedback-inducible expression of Gal4-TR α 1 ensures a high sensitivity. The transient expression assay demonstrates that pFIND-T3 activation by T3 is similar to that of pFIND-RA (Mata De Urquiza *et al.* 1999) by retinoic acid, and that the system is sensitive to 10^{-11} M T3 in cultured COS cells (Fig. 1B). As expected from previous results (Flamant & Samarut 1998), expression was not sensitive to RXR overexpression, addition of retinoic acid or estradiol. As for TR α , it was induced by T4 only at high molarity. The

pFIND-T3 plasmid was injected into mouse fertilized oocytes and two transgenic lines were derived, called FINDT3A and FINDT3B, differing only by the transgene integration site and copy number, roughly estimated by quantitative autoradiography to 5 and 20 copies respectively (data not shown).

Transgene expression pattern is heterogeneous and depends on T3 availability

Xgal staining was performed on whole brains of FINDT3A and FINDT3B mice at several stages of postnatal development (Fig. 2A–F), and this

revealed that the expression pattern of *lacZ* was highly similar in both strains. The postnatal expression pattern was stable over time, the decrease observed in the cerebellum external granular layer corresponding to the progressive disappearance of this structure. We first confirmed that *lacZ* expression depends on T3 by crossing FINDT3B mice with *Pax8*^{+/-} mice to generate FINDT3B/*Pax8*^{-/-} mice devoid of thyroid follicular cells and thus of T3 (Mansouri *et al.* 1998, Flamant *et al.* 2002). *lacZ* expression was found to be very low at P2, P8 and P15 in the large majority of these mice (eight out of ten) (Fig. 2G and H), and was rescued after 5 days of T4/T3 treatment (Fig. 2I and J). Although this was not precisely addressed, the persistence of *lacZ* expression in two FINDT3B/*Pax8*^{-/-} mice might reveal that some *Pax8*^{-/-} mice retain the ability to make a small amount of T3. Rendering the mothers and offspring hypothyroid by propyl-thio-uracil feeding confirmed that transgene expression was, as expected, strictly dependent on T3 (data not shown). We also verified that the absence of staining, observed mainly in the cerebellum inner granular layer and olfactory bulbs, reflects weak T3 signaling rather than a systematic bias in transgene expression. When we rendered FINDT3B mice hyperthyroid by five daily injections of T4/T3, starting at P10, *lacZ* expression increased in a visible manner even in the cerebellum and olfactory bulbs (Fig. 2K) without becoming ubiquitous. We expected that *lacZ* and *Gal4-TRA* expression would follow the same expression pattern, because they are both driven by the same inducible promoter. Quantitative RT-PCR confirmed that *Gal4-TRA* expression was higher in the piriform cortex and colliculus than in the cerebellum and olfactory bulbs (100%, 43 ± 10%, 22 ± 6% and 18 ± 5% respectively in FINDT3A at P15; *n*=4). Taken together, these data indicated that, at least in the brain, the FINDT3 reporter system reflects T3 signaling. Staining of brain slices allowed for a precise and complete description of the *lacZ* expression pattern (Fig. 2L–R and Table 1) and confirmed the excellent correlation between the FINDT3A and FINDT3B staining patterns. Discrepancies between the two strains were observed mainly in the inferior colliculus and the Purkinje cells, where expression was only observed in FINDT3B animals, and the cortex, where the expression was broader in FINDT3B. Although T3

Table 1 Comparison between FINDT3A and FINDT3B brains for *lacZ* postnatal expression (P15)

	FINDT3A	FINDT3B
Olfactory bulb n	–	–
Neocortex		
Retrosplenial cortex	++	+++
Cingulate cortex	+	+++
Motor cortex	–/+ ¹	–/+ ¹
Somatosensory cortex	+++	+++
Insular cortex	++	++
Piriform cortex	+++	+++
Visual cortex	–	–
Auditory cortex	+	+
Rhinal cortex	+	+
Caudate putamen	–/+ ¹	+
Globus pallidus	–	–
Hippocampal n	++	++
Subiculum	–/+ ¹	–/+ ¹
CA1	++	++
CA2	++	++
CA3	++	++
Dentate gyrus	–	–
Thalamus	+++	++
Hypothalamus	+	+
Habenular n	+++	+++
Amygdala	+++	+++
Tegmentum	++	++
Superior colliculus	+++	+++
Inferior colliculus	+++	+
Trigeminal n	++	++
Red n	+	+
Anterior pontine n	+++	++
Posterior pontine n	–	–
Pontine reticular n	–	–
Medial vestibular n	+	+
Gigantocellular n	–	–
Inferior olive	+++	++
Cerebellar n	++	++
Cerebellar cortex	– ²	–/+ ²
Purkinje cell layer	–	++
Choroid plexus	–	–
Spinal trigeminal n	+++	++
Spinal cord		
White matter	–	–
Grey matter	+++	+++

n=nucleus. ¹Heterogeneous expression within the structure.

²External granular layer at P8: FINDT3A +, FINDT3B ++.

signaling is known to occur in most if not all cell types, Xgal staining in non-neural tissues and fibroblasts cultured from transgenic embryos was usually weak, suggesting that the reporter system is more efficient in neural tissues. More precisely, double immunostaining of brain sections with Tuj1, an antibody which labels neurons, and

Figure 3 Fetal expression of the *lacZ* reporter transgene. (A) E15.5 FINDT3A fetal brain; (B) top view of E15.5 FINDT3B fetus, expression is visible in the mesencephalon; (C and D) E17.5 FINDT3A brain, dorsal and ventral view; (E) sagittal section of an E17.5 FINDT3B fetal brain, prospective colliculus (co), hippocampus (hi), thalamus (th) are stained but not lateral cortex (cx), olfactory bulbs (ob) and cerebellum (cb). The ventral telencephalon-stained area corresponds to the future piriform cortex. Bars represent 1 mm. High resolution figures are available at www.ens-lyon.fr/LBMC/

anti- β -galactosidase antibodies indicated that expression was mainly observed in neurons (>95%; data not shown).

Temporal pattern of T3 signaling in brain

We performed Xgal staining at various stages of FINDT3 embryonic development to evaluate when T3 signaling first appears in the developing brain. At E13.5 and E14.5, reporter expression was absent from the neural tube (data not shown). At E15.5, *lacZ* expression started in the roof of the midbrain (Fig. 3A and B). At E17.5 the expression pattern became more complex, showing staining in several areas (Fig. 3C–E). As fetal thyroid does not secrete hormone before E17.5, these results reflect either a direct maternal supply of T3 or a transplacental transfer of T4 followed by a fetal deiodination into T3. In summary, the most striking observation that we made was that T3 signaling starts early during brain development in the midbrain roof where it remains elevated after birth. It is also high in the piriform cortex and

thalamus whereas it is constantly very low in the cerebellum and olfactory bulbs.

Local thyroid hormone level and deiodinase gene expression patterns

A simple explanation for the *lacZ* heterogeneous expression would be that local T3 concentration dictates transgene expression. We used pFIND-T3 transfected cells to measure T3 purified from three brain areas at P15. In agreement with previous data (Pinna *et al.* 2002) and as expected from *lacZ* expression, T3 was found to be high in anterior cortex (1500 fmol/g) and colliculus (6000 fmol/g) but low in cerebellum (500 fmol/g). *Dio2* expression is mainly responsible for local conversion of T4 into T3 and *Dio3* expression for T3 catabolism. We thus used quantitative RT-PCR and *in situ* RNA hybridization (Fig. 4) to assess *Dio2* and *Dio3* expression and complete the published data (Guadano-Ferraz *et al.* 1997a, 1999, Escamez *et al.* 1999, Tu *et al.* 1999, Tsai *et al.* 2002). As in the postnatal brain, *Dio2* expression is found at E15.5

in the medial and dorsal ventricular layers in the walls of the third ventricle and the basal hypothalamus (Fig. 4A, C and G), *Dio3* expression is in the medial septal area, the dorso-lateral ventricular zone and the thalamus (Fig. 4I and K). *Dio2* and *Dio3* expression have thus the potential to generate a heterogeneous T3 distribution in the fetal brain before the onset of thyroid function, but the midbrain roof, where the FIND-T3 reporter gene is first expressed, is not identifiable by a distinct *Dio2/Dio3* expression pattern. After birth, RT-PCR confirmed that *Dio2* expression is low in the cerebellum and high in the frontal cortex and colliculus correlating with reporter gene expression (Fig. 4M) but a precise examination of the published *Dio2/Dio3* expression pattern suggests that, as in the fetal brain, additional factors influence the signaling pattern in a significant manner.

Discussion

This FINDT3 transgenic reporter system proved to be highly responsive to thyroid hormone in neuronal cells. It is not dependent on endogenous receptors but requires transcription coactivators. Other protein-protein interactions mediated by the ligand-binding domain that is conserved in the hybrid Gal4-TR α receptor can take place, and the reporter system readout is thus likely to closely resemble the endogenous signaling pattern. The possibility remains, however, that interactions occurring in the N-terminal domains of TR and variations in TR isoforms expression might

Figure 4 Type 2 (*Dio2*) and type 3 (*Dio3*) deiodinase expression. RNA *in situ* hybridization was performed at E15.5 on OF1 embryos. (A, C, E and G) *Dio2* and (B, D, F and H) Nissl staining. Places of high *Dio2* expression were ventricular zones (VZ) and hypothalamus (Hyp). (I–K) *Dio3*. (J–L) Nissl staining. Places of high *Dio3* expression were ventricular zones, thalamus (Th) and the medial septal area (MS). Bar represents 1 mm. (M) Quantitative RT-PCR. *Dio2* and *Dio3* expression at P15 in piriform cortex (1), lateral cortex (2), cerebellum (3), colliculus (4), hypothalamus (5), pons (6), E15.5 midbrain (*Dio2* 3.3 ± 2) (7) and E15.5 forebrain (*Dio2* 9 ± 4) (8). ($n=4$). P15 euthyroid whole brain RNA was used as a reference. Results are expressed by comparison with this reference. The relative intensity of Xgal staining, reported from Table 1, is indicated.

generate another level of regulation, not detected by the FINDT3 system.

Our data revealed that T3 signaling in brain follows a complex pattern during neural development starting at E15.5. The onset of endogenous T3 signaling might occur slightly earlier, because the accumulation of β -galactosidase in a self-inducible system could be delayed. One striking and unexpected feature of the signaling pattern is its first appearance and persistence in the midbrain. After birth, sharp transitions in reporter gene expression are maintained. Two locations of persistently low signaling are the olfactory bulbs and the cerebellum inner granular layer. Interestingly, these are precisely the two areas where the consequences of congenital hypothyroidism are more visible (Lauder 1977, Calza *et al.* 2000, Koibuchi & Chin 2000). It is noticeable, however, that *lacZ* expression was found in external granular cells, a neuronal population which is known, together with Purkinje cells, to be directly sensitive to hypothyroidism. The possibility remains that T3 action in the cerebellum is partially indirect and results from anterograde axonal transport of T3-induced neurotrophins toward the cerebellum (von Bartheld & Butowt 2000).

Spatio/temporal variations in T3 signaling could be due to changes in T3 metabolism, T3 transport and intracellular factors. Several considerations suggest that T3 availability is most likely the limiting factor: (1) the first appearance of *lacZ* expression during development coincides with the appearance of T3 in the embryo (Obregon *et al.* 1984); (2) increasing the level of T3 results in a broader *lacZ* expression; and (3) direct measurement of T3 content shows a good coincidence with reporter expression. If the signaling pattern mainly reflects T3 distribution the underlying mechanism generating this distribution is unclear, because T3 metabolism is complex (Pinna *et al.* 2002) and not fully understood (Bianco *et al.* 2002). From the ventricular expression of *Dio2*, we could expect a high reporter gene expression in neighboring areas, but this was not the case. T3 transport (Gordon *et al.* 1999) might thus also influence T3 distribution.

Detailed analysis reveals discrepancies between T3 distribution, visualized by histochemistry (Gordon *et al.* 1999), and reporter expression. Furthermore, expression does not become ubiquitous in hyperthyroid brains. These are indications

that intracellular factors might also influence transgene expression. For example, many anion transporters can modulate T3 diffusion to the nucleus (Abe *et al.* 2002). Alternatively, components of several coactivator complexes (Hermanson *et al.* 2002) could display a restricted expression. We attempted to incriminate the *Hairless* corepressor alone because it is highly expressed in cerebellum and inducible by T3 (Thompson & Potter 2000, Potter *et al.* 2002). Introducing the *Hairless* mutation in FINDT3 mice did not modify *lacZ* expression, ruling out the possibility that *Hairless* alone influences reporter gene expression (data not shown). It is more likely that the complex expression pattern that we observed translates a complex combination of underlying mechanisms.

Given that early maternal hypothyroidism prevents embryonic development, addressing the exact importance of early T3 signaling on midbrain development will necessitate a precise analysis of the lethal *TRa* knockin mutations. T3 is generally considered as a trophic factor whereas retinoic acid, a vitamin A derivative which is not chemically related to T3, has been originally proposed to be a morphogen (Wolpert 1989). Although it is now apparent that the original morphogen definition does not apply to retinoic acid, it is well established that the local variations in retinoic acid signaling are informative for cell differentiation and field patterning at several steps of development (Maden 2002). Retinoic acid binds RARs, which are the closest relatives of TR within the nuclear hormone superfamily. The two receptor types share common target genes, including the neuron-specific neurogranin gene (Guadano-Ferraz *et al.* 1997a, Husson *et al.* 2003). They also share many coactivators and corepressors. The data presented here have demonstrated that T3 signaling can also vary at short distance in the developing brain. We propose that, as for retinoic acid, T3 local concentration provides the brain cells with positional information and can thus control patterning and differentiation events during neural development.

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