Developmentally regulated thyroid hormone distributor proteins in marsupials, a reptile, and fish

Samantha J. Richardson, Julie A. Monk, Caroline A. Shepherdley, Lars O. E. Ebbesson, Frank Sin, Deborah M. Power, Peter B. Frappell, Josef Körhle, and Marilyn B. Renfree

1Department of Biochemistry and Molecular Biology, The University of Melbourne, Parkville, Victoria, Australia; 2Department of Biology, University of Bergen, Bergen, Norway; 3Department of Zoology, University of Canterbury, Christchurch, New Zealand; 4Centro de Ciências da MAR, Universidade do Algarve, Faro, Portugal; 5Department of Zoology, La Trobe University, Bundoora, Victoria, Australia; 6Institut für Experimentelle Endokrinologie und Endokrinologisches Forschungs-Centrum der Charité, Berlin, Berlin, Germany; 7Department of Zoology, University of Melbourne, Parkville, Victoria, Australia

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THYROID HORMONES (THs) play a crucial role in the development of vertebrates. In humans, insufficient TH delivered to the fetus results in irreversible brain damage (see (24)). During amphibian metamorphosis, which is controlled by TH, there is a sharp rise in TH concentration in the blood (20). Many vertebrate species have this characteristic rise in TH levels in blood during crucial stages of TH-dependent development (see (14)).

The main form of TH secreted by the thyroid gland into the bloodstream is 3',5',3,5'-tetraiodothyronine (thyroxine; T4). Within the cell, T4 is converted to 5',3,5,3'-triiodothyronine (T3) by outer ring deiodination (for review of deiodinases, see (18)). T3 has higher affinity than T4 for thyroid hormone receptors (TR) (34). Within the nucleus, T3-TR complexes dimerize, recruit co-activator proteins and bind to thyroid hormone response elements, thereby regulating (positively or negatively) expression of specific genes. For normal development to progress, it is crucial that the timing and the level of expression of genes are precisely regulated. Therefore, the delivery of TH to target tissues throughout the body is a fundamental issue in developmental biology of vertebrates.

THs are extremely lipophilic, having a partition coefficient of 20,000:1 between lipid and aqueous environments (7). This avid partitioning of free TH into cell membranes in the absence of thyroid hormone binding proteins was demonstrated very elegantly by Mendel et al., (23). They perfused rat livers with 125I-T4 in aqueous buffer. All the TH partitioned into the first cells it came into contact with. Subsequently, rat livers were perfused with 125I-T4 in aqueous buffer containing thyroid hormone binding proteins. Following perfusion, there was an even distribution of TH throughout the liver lobule. This showed that thyroid hormone binding proteins are required for adequate distribution of THs. Therefore, we refer to these proteins as "thyroid hormone distributor proteins" (THDPs), emphasizing their role in the distribution of, rather than the simple physicochemical binding, of thyroid hormones. In human blood 99.98% of T4 and 99.8% of T3 are bound to THDPs. Only the free fraction of thyroid hormone is available to partition into cell membranes.

Humans have three THDPs in their blood: albumin, transthyretin (TTR) and thyroxine-binding globulin (TBG). These three proteins are synthesized by the liver and secreted into the bloodstream, where they distribute thyroid hormones from their site of synthesis (the thyroid gland) to sites of action: the cells throughout the body. TBG has the highest affinity for THs, TTR has intermediate affinity, and albumin has the lowest affinity (see (33)). Only one of these three THDPs is also synthesized in the brain: TTR (see (37)). TTR is synthesized by the choroid plexus (the site of the blood-cerebrospinal fluid barrier, and part of the blood-brain barrier) epithelial cells (6, 46) and secreted into the cerebrospinal fluid (CSF) (38). This TTR is involved in moving TH from the blood across the blood-brain barrier into the brain. In developing mammals, TTR is the principal carrier of THs from the maternal to the fetal circulation, a function that is essential for fetal growth and development (20).

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blood-brain barrier into the brain (7, 38, 44). TTR is the major protein synthesized and secreted by the choroids plexus of eutherians (“placental mammals”), marsupials (e.g., kangaroo), monotremes (e.g., echidna), birds and reptiles (13, 30).

In a study analyzing the THDPs in about 150 species of adult vertebrates, albumin was found in blood of all adult species of fish, amphibians, reptiles, birds and mammals studied (28, 30, 32) (for review see (27)). In some species it was the only THDP, so we concluded it to be the evolutionarily oldest THDP. Adult birds and eutherians had TTR in addition to albumin in the blood. An interesting situation became apparent amongst adult marsupials.

Marsupials can be divided into two orders: the evolutionarily older Polyprotodonta and the more recently divergent Diprotodonta. According to the fossil record, the marsupials originated in North America, and were all polyprotodont (see (48)). Some marsupials migrated south, via Gondwanaland to what is now Australia. About 45 million years ago, when Australia separated from the rest of Gondwanaland, there was a radiation of marsupials in Australia, which included the divergence of the Diprotodonta from the Polyprotodonta (47).

All adult marsupials have albumin as a THDP. Diprotodont marsupials have TTR in addition to albumin, whereas adult Australian polyprotodont marsupials do not have TTR (30).

More recently, hepatic TTR synthesis during early development of fish (11, 35, 52), and during metamorphosis in amphibians (51, 53) was demonstrated.

Recently, we identified a protein in the blood of a marsupial, the tammar wallaby (Macropus eugenii) which bound T4 and migrated as a globulin, but was present during pouch life only (i.e., not present in adult serum). We referred to this protein as “W-TBP” (Wallaby Thyroxine-Binding Protein) (27). We suggested that this T4 distributor protein could serve as an additional THDP in the blood of the pouch young before its thyroid gland becomes functional, when the young obtains TH from the mother’s milk. During this time, the young has elevated levels of THs in its blood (16). This raised the question as to whether W-TBP was the marsupial homologue of eutherian TBG.

In the present study, we identified W-TBP as M. eugenii TG; analyzed the THDPs in blood of an Australian polyprotodont marsupial during development (Sminthopsis crassicaudata, the fat-tailed dunnart); of a reptile (Crocodylus porosus, the saltwater crocodile) during development; and of two salmonoid fish (Salmo salar, Atlantic Salmon; and Oncorhynchus tshawytscha, Chinook Salmon) during the parr-smolt transformation; and throughout one calendar year of adult Sparaus aurata (sea bream). The parr-smolt transformation is a mid-life developmental period which occurs in juvenile salmonoid fishes, enabling them to change from a freshwater environment to a salt water environment. There is a characteristic rise in TH levels in blood during this developmental stage.

Based on results from these experiments, we now propose an hypothesis for a mechanism by which total TH levels are elevated in serum during development. It is due, in part, to the developmentally regulated expression of a gene coding for a THDP with higher affinity to those already present in serum. Without this augmented TH distribution system, there would not be the increase in serum TH levels during development, as the additional TH would partition into the cell membranes, and be lost from the circulating pool of TH in the blood, and therefore not reach their target cells throughout the body and brain.

MATERIALS AND METHODS

Materials

L-[5\(^{12}\)C]thryoxine (1.2 Ci/mg) was obtained from NEN-Dupont, Sep-Pak C-18 cartridge columns were obtained from Millipore, thin layer chromatography plates were obtained from Schleicher & Schüll, antiserum raised in a rabbit against a mixture of TTRs purified from serum of human, wallaby (Macropus eugenii) and chicken (Gallus gallus) had been produced in this laboratory previously; rabbit anti-human TBG was obtained from Dako. Protein electrophoresis standards were Low Molecular Weight Markers from Pharmacia. All reagents were of analytical grade.

Animals and Collection of Blood Samples

Blood samples were obtained from healthy animals after appropriate ethics permits were obtained. All experiments were approved by institutional animal ethics committees.

Macropus eugenii - the tammar wallaby. An Australian polyprotodont marsupial. Serum samples from Macropus eugenii were collected as described previously (28). Serum was stored at −70°C until assayed.

Sminthopsis crassicaudata - the fat-tailed dunnart. An Australian polyprotodont marsupial. Blood samples were obtained from members of the Sminthopsis crassicaudata colony maintained at La Trobe University, Victoria, Australia. Animals were aged at ages 27, 33, 40, 41, 54, 55, 62, 68, 69 and 82 days, 7 mo and 2 years (27 days of age was the youngest at which we could collect blood for sufficient serum for analysis of THDPs). Animals were killed by CO\(_2\) inhalation followed by decapitation. Blood was collected and serum was prepared. Where insufficient blood was collected from a single animal, samples from litter mates were pooled. Serum was stored at −70°C until assayed.

Crocodylus porosus - the salt water crocodile. An Australian reptile. Serum samples from Crocodylus porosus eggs were collected as described previously (41). Serum from juvenile C. porosus was collected as described previously (42). Serum from adult C. porosus was obtained from Richardson et al., (30). Serum samples were stored at −70°C until analyzed.

Salmo salar, Atlantic salmon. Parr, smolt (1+; smoltification after one year) and smolt (2+; smoltification after two years) Atlantic salmon (Salmo salar) in freshwater were obtained from Sydkraft’s Laholm Salmon Hatchery, Laholm, Sweden. The fish were transported to the Department of Zoology, Lund University, Lund, Sweden, where they were anaesthetized with MS222 (Finquell, USA) and blood was collected from the caudal vessels using heparinized syringes. Blood was centrifuged, and plasma was collected and stored at −80°C until shipped to The University of Melbourne on dry ice, then stored at −70°C until used in assays.

Oncorhynchus tshawytscha. Chinook salmon. Chinook salmon (Oncorhynchus tshawytscha) smolt (1+), were kept in the Silverstream Hatchery, National Institute of Water and Atmosphere, Canterbury, New Zealand. Fish were anaesthetized with 0.01% tricaine and blood was collected from the dorsal artery, and serum was prepared. Serum was shipped to The University of Melbourne on dry ice, then stored at −70°C until used in assays.

Sparus aurata, sea bream. Adult sea bream (Sparus aurata) (weight at start of year: ~250 g; weight at end of year: ~450 g) were maintained in through-flow seawater tanks at ambient temperature and normal photoperiod during one calendar year in the Algarve, Portugal. Fish were anaesthetized with MS222 (Sigma, USA) and blood was collected from the caudal peduncle using heparinized syringes. Blood was centrifuged, 6,000 rpm, 4°C for 3 min and plasma removed and
frozen immediately at -80°C and shipped in N2(l) to The University of Melbourne, where samples were stored at -70°C until assayed.

Analysis of Thyroxine-Binding Proteins in Serum

Commercially available preparations of L-[-5,125I]thyroxine (125I-T4) contain 125I-labeled impurities, even on the reference date. Therefore, no more than 24 h prior to analysis of thyroxine binding proteins in sera, 125I-T4 was separated from impurities by reversed phase chromatography using C-18 SepPak columns (Millipore Waters) as described by Mendel et al. (22). Analysis of the purification of 125I-T4 was assessed by thin layer chromatography (25).

Ten microliters of serum was incubated with ~2.5 nCi 125I-T4 at room temperature for one h prior to electrophoresis in a non-denaturing 10% polyacrylamide gel (0.05 M Tris-glycine, pH 8.6) at 4°C. Samples were loaded in duplicate: 5 µl serum for analysis of 125I-T4 distribution by autoradiography, and 2 µl serum for analysis of proteins distribution by staining with Coomassie Brilliant Blue. For fish and crocodile, thrice the volumes of serum were used, due to the lower total protein concentration of sera from fish and reptiles. This method has been validated for analysis of THDPs in serum from eutherians, marsupials, monotremes, birds, reptiles, amphibians and fish (30, 31).

In analyses involving the use of F21388 to selectively displace 125I-T4 from TTR, 1.2 mM F21388 was added to the incubation. Aside from this modification, analyses were carried out as described in the preceding paragraph.

Western Analysis

Aliquots of 2 µl serum were separated by SDS-PAGE in a 4.5% acrylamide stacking gel, pH 6.8, then either a 15% (for analysis of TTR) or 10% (for analysis of TBG) acrylamide resolving gel, pH 8.9, using 0.05 M Tris, 0.38 M glycine, at room temperature, as described by Laemmli and Favre (19). Following electrophoresis, proteins were transferred (30 V; 16 h; 4°C) onto nitrocellulose. Residual protein-binding sites on the membrane were blocked with 5% skimmed milk using 0.05 M Tris, 0.38 M glycine, at room temperature, as described by the manufacturer’s instructions (Amersham), and detected using BioMax X-ray film (Kodak).

RESULTS

TBG is synthesized by the liver of the diprotodont marsupial Macropus eugenii (tamar wallaby) during pouch life, but not in the adult.

We have previously identified a thyroxine-binding protein which migrates as a globulin in serum from pouch young of a diprotodont marsupial Macropus eugenii, the tamar wallaby, which we called W-TBP (28). Here, we used anti-human TBG in a western analysis of M. eugenii pouch young serum. A protein in serum from pouch young aged 26 days (earliest time tested) until around day 200 (28). Based on these data, we suggest W-TBP to be the marsupial homologue of TBG. This is the first time TBG has been described in a non-eutherian species.

TTR is synthesized in the liver of the polyprotodont marsupial Smirnithopsis crassicaudata (fat-tailed dunnart) during development, but not in the adult. To analyze the pattern of THDPs in a polyprotodont marsupial during development, we incubated serum from S. crassicaudata, 27 days old to 2 years old, with 125I-T4 then subjected duplicate aliquots to non-denaturing polyacrylamide electrophoresis (as Materials and Methods). Following electrophoresis one set of the duplicate samples was stained with Coomassie Brilliant Blue for visualization of the pattern of serum proteins (Fig. 2A), and the second set of samples was subjected to autoradiography for visualization of 125I-thyroxine distribution (Fig. 2B). Human serum was used as a standard for each gel.

The serum protein pattern did not change significantly from day 27 until adulthood (Fig. 2A). The band corresponding to albumin is clearly distinguishable as the major band (in this case having an Rf value of ~0.36).

The pattern of THDPs during development changed markedly from day 27 until adulthood (Fig. 2B). Albumin was the major THDP in adult animals (same Rf value as albumin in Fig. 1A). There was some binding of 125I-T4 by a protein migrating as a globulin. In serum from adult animals there was no evidence of a TTR, which would migrate anodally to albumin. In animals from day 27 until 62, a protein with features similar to TTR (it bound T4, and was the only serum protein to migrate anodally to albumin under these conditions of electrophoresis) was the major THDP, whereas in older animals albumin was the major THDP. Although albumin is present in serum from animals at all ages (Fig. 2A), it was not the major THDP in serum from individuals undergoing major stages of development. A protein migrating as a globulin was also observed to bind thyroxine in animals of 33 days and 41 days. The positions of human TTR, albumin and TBG are indicated.

To confirm that the protein which migrated anodally to albumin and bound thyroxine, was TTR, western analysis of the serum samples was performed after separation of proteins by SDS-PAGE (Fig. 2C). There was insufficient sample from the 27 day old animal for this analysis. TTR immunoreactivity was detected in serum from day 33 until day 62, which is
consistent with the data in Fig. 2B. Until now, the only Australian marsupials shown to synthesize TTR in their livers are diprotodont. TTR is not synthesized by the liver of adult Australian polyprotodont marsupials (9, 29, 30). This is the first time TTR has been shown to be synthesized by the liver of an Australian polyprotodont marsupial.

To investigate if the globulin was TBG, we carried out western analysis using anti-human TBG on the *S. crassicaudata* serum samples from animals aged 33 days until adult. No signals were detected in lanes containing serum from dunnarts of any age. Only the lane containing human serum had a signal, which had a size corresponding to that of TBG (data not shown). The lack of signals in lanes containing dunnart serum could be because the globulin which bound $^{125}$I-T4 was not TBG, or if *S. crassicaudata* TBG is not recognized by anti-human TBG.

*TTR* is synthesized in the liver of the reptile *Crocodylus porosus* (salt water crocodile) during development, but not in the adult. To investigate the THDPs in serum from *C. porosus* during late embryonic development until 3 years post-hatch, sera from *C. porosus* at 60, 68 and 75 days incubation, day 1 post-hatch (hatching was at 80 days of incubation at 32°C), 6 mo old, and from 3 year old, were analyzed for the presence of THDPs (Fig. 3).

*C. porosus* serum albumin has a much greater Rf value than mammalian albumins (Fig. 3A). Albumin was present as a THDP in serum at all ages examined. At 68 days incubation a THDP with a greater electrophoretic mobility than albumin was also present (Fig. 3B).

To investigate whether this THDP with a greater Rf value than albumin was *C. porosus* TTR, serum from *C. porosus* at 60, 68 and 75 days incubation, day 1 post-hatch (hatching was at 80 days of incubation at 32°C), 6 mo old, and from 3 year old, were analyzed for the presence of TTR by western blot analysis. Human serum was used as the positive control, and adult European carp (*Cyprinus carpio*) serum as the negative control. TTR immunoreactivity was detected in crocodile serum on days 60, 68, 75 of incubation, and at day 1 post-hatch, but was not detected in serum from 6 mo old or adult crocodiles (Fig. 3C).

Again, TBG was not detected by western analysis in serum from salt water crocodiles at any of the ages tested (data not shown).

*TTR* is synthesized by the liver of *Salmo salar* (Atlantic salmon) and *Onchorhynchus tshawytscha* (Chinook salmon) during smoltification. Many folk medicine remedies used plants to control hormone levels in humans. Some plant flavonoids can interfere with thyroid hormone metabolism. Fig. 4A shows the comparison in structure between T4 and the synthetic plant flavonoid F21388, which specifically displaces T4 from TTR, but not from TBG or albumin (17). This is demonstrated in Fig. 4B (centre panel).

As the plasma protein profile in fish is very different to that in mammals, birds or reptiles (e.g., see (30)), we were not able to readily identify a putative albumin or TTR in serum from fish (Fig. 4B, left panel). Therefore, we incubated fish serum with $^{125}$I-T4 in the absence and in the presence of F21388. An example is shown in Fig. 4B (centre panel). *S. salar* smolt (1+) serum was incubated with $^{125}$I-T4 in either the absence or the presence of F21388, prior to non-denaturing PAGE followed by autoradiography. The fastest migrating T4-binding protein (Rf 0.46) did not have $^{125}$I-T4 bound to it in the presence of F21388. We therefore concluded that this protein was TTR.
TTR was detected in serum from *S. salar* during smoltification (Fig. 4B, right panel). TTR was also detected in serum from *O. tshawytscha* smolt (data not shown).

There were no signals corresponding to TTRs from western analyses of *S. salar* or *O. tshawytscha* serum, using the anti-(human, wallaby and chicken) TTR antiserum (data not shown). Most probably this was due to lack of cross-reactivity between species.

Analysis of serum from *S. aurata* at monthly intervals during the year 1995 was carried out. A putative TTR (based on Rf value and T4 binding) was detected in a sample taken during November, but the binding of 125I-T4 was not significantly reduced in the presence of F21388 (data not shown). Western analysis did not detect the presence of TTR in samples from any month of the year.

**DISCUSSION**

TBG in the blood of the diprotodont marsupial *Macropus eugenii* during pouch life. Here, we have reported for the first time evidence of a TBG in a noneutherian species: the marsupial *Macropus eugenii* (tammar wallaby). TBG was detected in serum during pouch life (Fig. 1), before the animal’s thyroid gland becomes active or homeothermy has been established (40). At this time, the pouch young’s source of TH for growth and development is it mother’s milk (12). The young is born 26.5 days gestation after diapause, moves to the pouch and attaches to the teat permanently for 100 days during which most of the brain development occurs. On day 140 the eyes open, ears reflect from the head and underfur is visible. By day 160 the young can stand, and by day 170 the kidneys have matured. At day 180 the thyroid gland matures and begins to secrete TH and homeothermy begins to develop. By day 200 full pelage has developed and the young leaves the pouch for the first time (See (40) for review). During this time, the young have elevated levels of TH in their blood: total T4 levels reach a peak around day 180, before declining to adult levels (16). Thyroidectomized pouch young showed retarded growth, fur development and homeothermy (40). We suggest that TBG is present in the blood to assist in the distribution of elevated...
levels of serum THs in the pouch young during these important developmental stages.

**TTR in the blood of the polyprotodont marsupial Sminthopsis crassicaudata during pouch life.** A study of THDPs in 67 species of Australian marsupials, demonstrated that TTR was synthesized by the livers of adult diprotodont but not by the livers of adult polyprotodont marsupials (30). As a diprotodont marsupial was shown to synthesize TBG by its liver during pouch life, we investigated whether a polyprotodont marsupial also synthesises an additional THDP (e.g., TTR or TBG) during pouch life.

*S. crassicaudata* is a polyprotodont marsupial. The duration of gestation for *S. crassicaudata* is 13.5 days. At birth, they weigh about 15 mg, and do not begin to protrude from the mother’s pouch until about day 37. At about day 40 the young have pelt coverage and permanent attachment to the nipple ceases. The eyes open at around day 50. Between days 59 and 63 they first leave the nest and eat solid food. The young are weaned at about day 70, when they are between 5 to 8 gram. Females become sexually mature at day 91, and males at about day 70 (2)(Lisa Masini, personal communication). TTR was detected in serum from day 27 (earliest time point measured) until day 62 (Fig. 2). It is possible that the globulin which bound T4 is *S. crassicaudata* TBG, but it did not cross-react with the anti-human TBG antibody. To the best of our knowledge, TH levels in serum during development of *S. crassicaudata* have not been measured.

*M. eugenii* have TBG in their blood until their thyroid gland becomes active and they become homeothermic (28)(and this study). It is not known when the thyroid gland becomes active in *S. crassicaudata*, and it appears that *S. crassicaudata* remains heterothermic throughout their adult life (F.Geiser, unpublished observations). It appears that *S. crassicaudata* synthesises TTR in the liver whilst immature and suckling from the mother, which is its source of TH during development. TTR synthesis ceases when the animal has fully developed, is old enough to leave the nest, eats solid food and has become independent. This is the equivalent time to when *M. eugenii* cease hepatic synthesis of TBG.

In rats and mice, TBG is synthesised by the liver prior to the development of fur and homeothermy, and not in adulthood until senescence (36, 49, 50). This is a similar pattern for TBG synthesis by *M. eugenii* and hepatic TTR synthesis by *S. crassicaudata* during development. We are not aware of any studies of THDPs in marsupials during senescence.

**TTR in the blood of the salt water crocodile during embryonic development.** In adult saltwater crocodiles, TTR mRNA was not detected in liver total RNA preparations (26), nor were a putative TTR or TBG detected in serum (30). *C. porosus* have elevated levels of THs in blood during late embryonic development and hatching (41). We investigated whether *C. porosus* also had an additional THDP to albumin in its blood during this time.

Albumin was detected as a THDP in crocodile serum at all ages tested. TTR was detected in serum on days 60, 68, 75 and day 1 post-hatch, but not detected in serum at 6 mo of age, nor in a 3 year old (Fig. 3). This is consistent with the pattern of T3 and T4 levels in blood of *C. porosus* during development: T4 and T3 levels in blood gradually rise during the late stages of incubation, and are maximal at hatching (41). T4 outer ring deiodination (ORD) by the liver decreases from day 50 of incubation until hatching, and there is also a decrease in hepatic T3 inner ring deiodination (IRD) (43), which could contribute to the increasing TH levels in serum during this time (41). Here, we demonstrate that TTR was present in blood of *C. porosus* during the time of increased TH levels.

**TTR in blood of salmon during smoltification.** Smoltification in salmonids is a midlife developmental period that is the transition of the freshwater dwelling parr into a smolt prepared for ocean existence. Smoltification is, in part, regulated by TH.
We investigated whether this involved the presence of a THDP in addition to albumin, which is the only THDP identified to date in adult fish.

In both species of salmon investigated (S. salar and O. tshawytscha), TTR was detected in serum during smoltification. This was in contrast to the pattern of THDPs throughout a calendar year (at monthly intervals), of sea bream, a fish which does not undergo smoltification.

The analysis of THDPs in sea bream each month throughout the calendar year of 1995 revealed a putative albumin all year round. A THDP which may have been TTR was detected during November. However, we are not confident of this protein being identified as a TTR, as its binding of $^{125}$I-T4 was not reduced in the presence of F21388. The peak in serum T4 levels occurred during November in the calendar year of 1995, however, the peak in serum T3 levels was during March 1995 (data not shown). The identification of this THDP needs to be clarified.

General discussion. According to the Free Hormone Hypothesis, it is only the free fraction of THs in blood (i.e., in human blood 0.02% of T4 and 0.2% of T3) which are able to partition into cells. THs are extremely lipophilic, readily partition into the lipid membranes of cells they come into contact with. Thus an increase in TH secretion by the thyroid gland in itself might not be sufficient to achieve and sustain elevated levels of TH in blood, as they would partition into cell membranes. To achieve an increase in THs circulating in blood, would therefore require an increase in TH distribution capacity, i.e., an augmented THDP network.

We now propose a mechanism by which TH levels are maintained at an elevated level in blood during development, is the developmentally regulated activation of gene expression providing a transcript coding for an additional THDP. In mammals (and possibly in other species also), this protein has higher affinity for THs than those still present in the serum of adults. The length of time THs are elevated for is in part dependent on the duration for which the gene is transcribed, and on the half-life of the protein.

Throughout vertebrate evolution, there has been an increase in thyroid hormone distribution capacity in blood and in human blood 0.02% of T4 and 0.2% of T3) which are able to partition into cells. THs are extremely lipophilic, readily partition into the lipid membranes of cells they come into contact with. Thus an increase in TH secretion by the thyroid gland in itself might not be sufficient to achieve and sustain elevated levels of TH in blood, as they would partition into cell membranes. To achieve an increase in THs circulating in blood, would therefore require an increase in TH distribution capacity, i.e., an augmented THDP network.

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Table 1. Thyroid hormone distributor proteins in plasma during development and in the adult

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</tr>
<tr>
<td>Polyprotodonta:</td>
<td>Smithopsis crassicaudata</td>
<td>TTR</td>
<td>albumin</td>
</tr>
<tr>
<td>Diprotodonta:</td>
<td>Macropus eugenii</td>
<td>TTR</td>
<td>albumin</td>
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<td></td>
<td></td>
<td></td>
<td>TTR</td>
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<tr>
<td></td>
<td></td>
<td></td>
<td>albumin</td>
</tr>
<tr>
<td>Eutherians —</td>
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<td>Rodentia:</td>
<td></td>
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<tr>
<td>Rattus norvegicus</td>
<td>TBG</td>
<td>albumin</td>
<td>(49)</td>
</tr>
<tr>
<td>Mus musculus</td>
<td>TTR</td>
<td>albumin</td>
<td>(50)</td>
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</table>

TTR, transthyretin; TBG, thyroxine-binding globulin.
cerebrospinal fluid, in adult individuals. Fish, reptiles and some mammals have albumin as their only THDP; birds, diprotodont marsupials and eutherians have TTR in addition to albumin, and some eutherians have TBG in addition to albumin and TTR (30). These THDPs have different distribution volumes, with TTR ranking the highest. We developed two hypotheses to account for the onset of TTR synthesis in the liver during evolution:

Hypothesis 1: the increase in the lipid volume to body mass ratio, e.g., comparison of the intestines of the two orders of Australian marsupials. Diprotodont marsupials are herbivores, and have much longer digestive tracks than the polyprotodont marsupials, which are carnivores. Given that the intestines are the extra thyroidal organ having greatest TH content (8), we suggested that this increased lipid pool, acting as a sink for TH, could have been the selection pressure for onset of TTR synthesis in the liver of the Diprotodonta (see (27)). Hypothesis 2: that the onset of hepatic TTR synthesis was concurrent with the acquisition of homeothermy. Adult animals which synthesise TTR in their livers (eutherians, diprotodont marsupials and birds) are better homeotherms than animals which do not (polyprotodont marsupials, monotremes, reptiles, amphibians and fish. (3)). We believe this to be related to better TH distribution by TTR, as TH are involved in homeothermy (see (27)), and TTR is known to have a higher distribution volumes in mammals than either TBG or albumin.

We also suggested that the onset of TTR synthesis by the choroid plexus, at the stage of the stem-reptiles, coincided with an increase in size of the brain i.e., the first traces of neocortex (see (39)).

Next, the pattern of THDPs in monotremes (platypus and echidna) and birds (precocial and altricial) during development need to be analysed. Southwell et al., (45) demonstrated an increase in TTR mRNA in avian liver from just prior to hatching until about 21 days post hatch. However, protein levels were not determined directly. Hulbert and Grant (15) demonstrated that juvenile platypus have higher concentrations of T3 and T4 in their blood than adults, but THDPs were not analysed.

Licht and coworkers have identified a protein in the serum of the Emydidae family of turtles which binds T4 and vitamin D, and has structural similarity to vitamin D binding protein (21), which is a paralogous homologue of albumin. It appears that in these animals which synthesise TTR in their livers (eutherians, diprotodont marsupials and birds) are better homeotherms than animals which do not (polyprotodont marsupials, monotremes, reptiles, amphibians and fish. (3)). We believe this to be related to better TH distribution by TTR, as TH are involved in homeothermy (see (27)), and TTR is known to have a higher distribution volumes in mammals than either TBG or albumin.

In this present study, we consider the reason for onset of synthesis of a THDP during development. We propose that this mechanism is used by at least some species in all classes of vertebrates, where a THDP with higher affinity for TH than those in adult blood is transiently synthesized by the liver during the time of increased TH levels in blood during development, metamorphosis and smolification.

The data summarized in Table 1 for fish, amphibians, a reptile, marsupials and eutherians show that for species which have albumin as their sole THDP in adulthood, TTR is also a THDP during development; and for species which have both albumin and TTR as THDPs in adulthood, TBG is additionally synthesised during development. In each case, the additional THDP in blood during development probably has higher affinity for TH than those present in adult blood. In summary, we hypothesise that an augmented TH distribution network contributes to the rise in total TH levels in blood during development. This additional THDP and concurrent rise in TH during critical stages of development could be an evolutionary safeguard against low TH supply, which could be survived better by animals with a larger TH reserve. In this case, the higher TH levels could allow survival of the young when their mothers have a low supply of dietary iodine (herbivores) or TH (carnivores).

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GRANTS

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