

REVIEW

Thyroid hormone as a temporal switch in mouse development

Juan Ren¹ and Frédéric Flamant

ENS de Lyon, INRAE, CNRS, Institut de Génomique Fonctionnelle de Lyon, Lyon, France

Correspondence should be addressed to F Flamant: Frederic.flamant@ens-lyon.fr

Abstract

Thyroid hormones are known to trigger metamorphosis in an amphibian. This review discusses the hypothesis according to which they act in a similar manner to synchronize the post-natal development of mice, using brain, brown adipose tissue, and heart as examples.

Key Words

- ▶ thyroid hormone
- ▶ mouse
- ▶ postnatal development
- ▶ metamorphosis

Thyroid hormones (THs; including T3, 3, 3', 5-triiodo-L-thyronine and its less active precursor T4 or thyroxine) exert a broad influence on the development of vertebrate species. The possibility that the developmental function of TH in mammals is similar to that in amphibians and fish metamorphosis has been discussed on several occasions (1, 2, 3). This review takes advantage of the available genetic toolbox to reconsider the hypothesis that mouse postnatal development represents a metamorphosis-like process.

Similarity between amphibian metamorphosis and mouse postnatal development

THs have originally been discovered as signals that trigger amphibian tadpole metamorphosis (4). During these crucial developmental stages, a number of dramatic changes take place in the body plan, which have been extensively studied in the anuran *Xenopus laevis*: gills disappear, hindlimbs grow while the tail regresses, and the intestine and the brain are deeply remodeled. The position of the eyes and the retina projections are also modified. Early exposure of tadpoles to TH precociously induces metamorphosis, whereas TH suppression delays it extensively. Therefore, it appears that THs do not represent an instructive signal that is able to impose a complete

change in the developmental trajectory, but mainly a permissive signal that acts as a timer of metamorphosis. Although other hormones, notably cortisosterone (5), also influence the transition, TH function is essential, because a proper timing of events during metamorphosis is vital.

TR α and TR β are the nuclear receptors of T3, respectively, encoded by the *Thra* and *Thrb* genes, which are already expressed in tadpoles before metamorphosis. They display favorable properties converting the elevation of TH into a sharp developmental transition. First, they act as DNA-binding transcription repressors in the absence of ligands and quickly switch into transcription activators upon T3 binding (6). Furthermore, this abrupt switch is amplified by the fact that the expression of *Thrb*, and to a lesser extent of *Thra*, is upregulated by T3 (7).

Mice are called altricial because they are underdeveloped at the time of birth and rely heavily on maternal care. During the first 3 weeks of postnatal life, major developmental processes are at work (Fig. 1). In particular, the brain undergoes important maturation processes during the postnatal period, which are equivalent to those occurring during the second trimester of human gestation. While the mouse fetal development is primarily dependent on maternal-derived TH, the mouse thyroid gland becomes functional before birth and provides all the necessary TH after birth. Although less pronounced than during amphibian

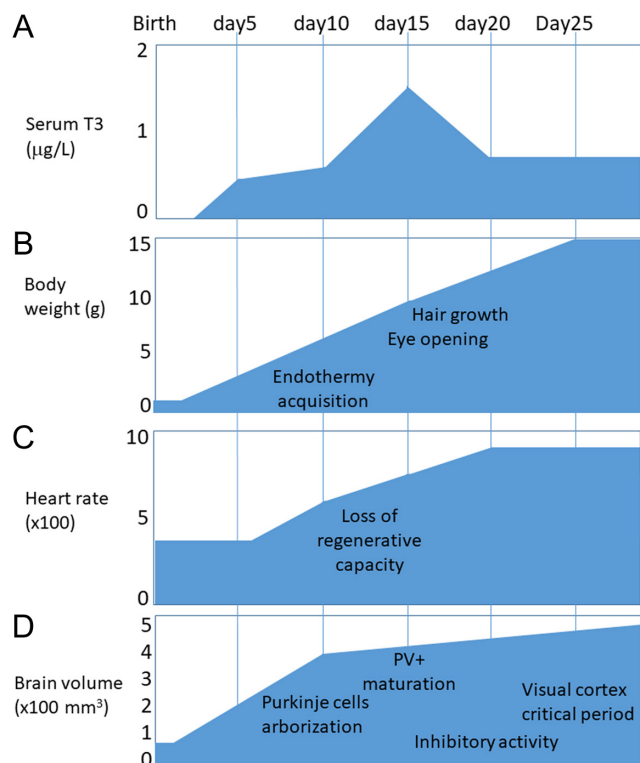


Figure 1

Postnatal maturation processes in mice. (A) Evolution of the T3 concentration in serum (8). (B) Eye opening occurs at around postnatal day 13 (PND13). Endothermy is achieved progressively before PND15. The anagen phase of hair growth is at PND17. Weaning becomes possible on PND21. (C) Cardiomyocytes lose their regenerative capacity (18) when heart rate (84) and oxidative capacities of ventricular fibers (85) increase during the second postnatal week. (D) The brain grows faster than the entire body. Forebrain regions, such as the olfactory bulbs and cortex, grow earlier than cerebellum (86). In the cerebellum, the arborization of Purkinje cells progresses between PND7 and PND21. In cortical basket cells, also known as fast-spiking interneurons, a major change in ions channels content occurs around PND15 (42). They express the parvalbumin marker and exert an inhibitory activity which has a pivotal role in the elaboration of the neuronal circuits (87). A phase of synaptogenesis is followed by the critical period of heightened plasticity (43). The peak of synaptic plasticity occurs at different times in different cortex areas, from PND12 in the somatosensory cortex to PND28 in the visual cortex. At the closure of the critical period, basket cells produce the perineuronal net which stabilizes their synapses (44). The inhibitory activity of GABAergic neurons finally balances the excitatory activity of glutamatergic neurons (88). NB: The milestones of postnatal development vary slightly depending on the mouse strain.

metamorphosis, a marked increase in both serum T4 and T3 levels is observed during the first 2 postnatal weeks (8), after which TH levels decrease and become stable in serum. The importance of TH in mouse postnatal development has been demonstrated by knocking out the *Pax8* gene (9, 10). This genetic ablation of the thyroid gland causes lethality, occurring during the second and third postnatal weeks unless the mice are rescued by TH treatment.

The mammalian *Thra* gene encodes several non-receptor proteins (11) and the TR α 1 receptor, which is nearly ubiquitous. The *Thrb* encodes TR β 1 and TR β 2. Whereas TR β 2 mRNA is found in only a few cell types, TR β 1 mRNA is broadly distributed. TR β 1 is abundant in the liver, heart, and several brain areas where it appears at late stages of development (12). Their protein abundance is poorly documented, but the recent development of mouse models with tagged *Thra* or *Thrb* alleles revealed surprising discrepancies between mRNA and protein levels. In particular, the non-receptor protein TR α 2 is more abundant than TR α 1 in brain, and TR β 1 is less abundant than predicted in several organs (13). Overall, most if not all mammalian cells have the ability to respond to T3 throughout development. However, in contrast to the amphibian genes, the response of the mouse *Thrb* gene expression to T3 is modest, and restricted to a few cell types, while *Thra* gene tends to be downregulated (14). Therefore, the T3 response in mammals is unlikely to be amplified by a positive feedback loop resulting in receptors accumulation.

In the following, we will consider major transitions in which TH have been demonstrated to be involved in mouse postnatal development. These are only examples, and we do not intend to cover the large body of literature related to the influence of TH. In each case, the main difficulty consists in distinguishing between the direct consequences of TH stimulation, also called cell-autonomous response, and the indirect influences, which are secondary to the local or systemic changes caused by TH. In the chosen examples, this discrimination has been addressed by using Cre/loxP recombination to selectively alter the T3 response in selected cell types.

From ectotherm to endotherm

Mouse pups only progressively gain the ability to maintain their body temperature, notably by developing the brown adipose tissue which is specialized in adaptive thermogenesis. Although a small number of brown adipocytes are present at birth, the cell proliferation in this tissue peaks at postnatal day 8 (PND8) (15). However, while the brown adipocytes already contain the UCP1 uncoupling protein, which is thought to be at the heart of the thermogenic process, they do not express the genes encoding key lipogenic enzymes before PND15. Therefore, the brown adipose tissue of juvenile mice is unable to fuel thermogenesis autonomously, as it does in adults, but rather oxidizes fatty acids coming from the maternal milk. Furthermore, the neuronal circuits required to sense

cold and trigger thermogenesis only become functional at PND15 (16).

As THs are known to promote several thermogenic processes, including adaptive thermogenesis in brown adipose tissue, one would expect a direct link between TH and the maturation of the thermogenic capacity of pups. Although to our knowledge, the juvenile phase has not been specifically addressed, selective deletion of TR α 1 in brown adipocyte progenitors alters their capacity to produce differentiated cells in adults (17). Consequently, adult mice lose the capacity to expand their brown adipose tissue after prolonged T3 stimulation. It is therefore likely that the original development of the brown adipose tissue is sensitive to the transient postnatal increase in T3 level.

The loss of regenerative capacity of the heart

Mice are born with the capacity to regenerate the cardiac muscle after injury, but lose this capacity within 2 weeks after birth. In this process, immature cycling mononucleated cells differentiate into bi-nucleated cells, which have a high capacity for oxidative phosphorylation but are unable to proliferate. Exposure to goitrogen propyl-thiouracyl or the TR α 1 antagonist NH-3 greatly delays this transition (18).

When the dominant-negative mutation TR α 1^{L400R} is expressed only in cardiomyocytes to block their capacity to respond to T3, a persistent proliferation of cardiomyocytes is observed. The heart size increases in pathological proportions, and its regenerative capacity, which manifests after an infarction, persists at the adult stage. Therefore, the influence of T3 on the loss of regeneration capacity is cell-autonomous. Transcriptome analysis detects changes in gene expression caused by TR α 1^{L400R} expression in cardiomyocytes, while ChIP-Seq analysis pinpoints genes whose regulatory sequences are occupied by TR α 1. This combined strategy allows to identify a set of genes whose transcription is very likely to be activated by the liganded TR α 1 in the postnatal heart and to promote the loss of regeneration capacity. A number of them encode important enzymes and regulators of oxidative phosphorylation in mitochondria.

A survey of many vertebrate species suggests that the loss of heart regenerative capacity is a trade-off for the acquisition of endothermy during evolution. Endothermy is indeed energy demanding and imposes a high contraction capacity on the myocardium, to ensure a proper oxygen supply to tissues. In mammals, cardiomyocyte metabolism switches to ATP-producing

processes. A unifying hypothesis would be T3 reprograms cardiomyocytes metabolism, which switches from nucleotide synthesis to ATP production to face a high-energy demand during mammalian development. Accordingly, zebrafishes, which are ectotherm, maintain the regenerative capacity of their heart at the adult stage (19), unless they are treated with an excess of T3 (18). Interestingly, a metabolic switch from mitochondrial oxidative phosphorylation to glycolysis, associated with a remodeling of the extracellular matrix glycosylation, accompanies the zebrafish heart regeneration (20). However, the heart of *Xenopus laevis*, which is also an ectotherm species, loses its regeneration capacity under the influence of T3 and the transition takes place during metamorphosis (21). Therefore, the tempting link between the function of T3 in development and in thermogenesis requires further investigation.

Loss of axonal regenerative capacity in Purkinje cells

The evolutionary loss of regenerative competence in mammals, and its maintenance in zebrafish, also applies to neurons. This manifests either as injury-induced neurogenesis and axonal regeneration after axons severing. Here again, a developmental transition has been observed in mice. Notably, the axons of Purkinje cells, in the cerebellum, possess the capacity to regrow at early postnatal stages. This regeneration capacity disappears within 1 week after birth, whether when the cells stay in the cerebellum or if cerebellum slices are cultured *in vitro* (22). In general, T3 exerts a cell-autonomous influence on the morphological maturation of these neurons, which takes place after birth (23, 24). The typical arborization of Purkinje cells is impaired by either TR α 1 or TR β 1 mutations (25, 26). Therefore, while T3 prevents axonal regrowth, it also favors dendritic growth. The most likely explanation is that the link to T3 is indirect and that both the loss of regenerative capacity and the dendritic arborization reflect the terminal maturation of the Purkinje cells.

The capacity of the Purkinje cell axons to regenerate can be evidenced either by performing *in vivo* or *in vitro* axotomy. T3 has a clear effect on the timing of this transition, accelerating the loss of regenerative capacity both *in vivo* and *in vitro*. Accordingly, the dominant-negative receptor TR α 1^{L400R} delays the loss of regeneration capacity (22). In some other neurons, axon myelination has been shown to be responsible for the loss of regeneration capacity. The myelination

in itself is a T3-dependent process, as T3 activates the differentiation of oligodendrocytes, the cells which create myelin sheaths (27). This raises the possibility of an indirect effect of T3 of Purkinje cells, mediated by oligodendrocytes. However, this hypothesis was ruled out by expressing TR α 1^{L400R} only in Purkinje cells, using a lentivirus vector (22). Although the molecular details remain unknown, gain and loss of function experiments demonstrate that the Klf9 transcription factor is an important intermediate of T3 action. As in many other cell types, the expression of the *Klf9* gene is activated by the T3-bound TR α 1 receptor in Purkinje cells. The Klf9 transcription factor then modifies the expression of other genes, and the cells lose the capacity to regenerate their axon. In general, loss of regeneration capacity appears to be one of the T3-dependent processes during Purkinje cell maturation. Overall, the possible involvement of T3-signaling in the developmental and evolutionary loss of regenerative capacity remains one hypothesis among several others (28).

Maturation of the GABAergic interneurons of the cortex and the critical period

Development of the cerebral cortex is an extremely complex process. Neurons migrate and form a layered structure and then establish synapses to finally generate functional circuits. Congenital hypothyroidism in rodents leads to less defined cortical layers, defective neuronal migration and altered circuitry (29) causing irreversible cognitive defects (30). Virtually all cortical cell types are affected by TH deficiency (31). However, because neurotrophins secretion is altered (32), it is unclear whether all cell lineages display an autonomous response to T3. The alternative would be that a small fraction of cells are sensitive to T3 and then orchestrate a network of cellular interactions. This can be mediated by direct cell contacts and the secretion of diffusible factors, as previously demonstrated in the cerebellum (33).

Cortical cells for which the influence of T3 has been shown to be cell-autonomous are the GABAergic inhibitory interneurons. The expression of TR α 1^{L400R} restricted to this lineage causes lethal epileptic seizures (34). The GABAergic neuronal population includes at least 12 different cell types defined by their morphology, position, connections, immunostaining, and gene expression (35). Whether all 60 types and subtypes are equally sensitive to T3 remains unclear. However, a selective alteration has been observed for the maturation of parvalbumin-expressing (Pv+) cells, which normally

represent 40% of the cortical GABAergic population. A large fraction of Pv+ cells are called basket cells based on morphology, or fast-spiking interneurons based on electrophysiological properties (36). Hypothyroidism and mutations altering TH transport across the blood-brain barrier (37) greatly reduce the density of Pv+ cells in the cortex at PND15 (38, 39). The same defect can be observed in mice which express TR α 1^{L400R} (34) or TR α 1^{R384C}, another mutation which greatly reduces the affinity of the receptor for T3 (40). Besides, patch-clamp recording of TR α 1^{R384C/+} cortical slices revealed a dramatic reduction in the number of fast-spiking interneurons (40). As TR α 1^{R384C} has a residual sensitivity to T3, it is less detrimental than expressing TR α 1^{L400R} that is often lethal. A slow recovery of Pv+ cells is observed in TR α 1^{R384C/+} mice, but not in the few surviving TR α 1^{L400R/+} mice. This suggests that T3 not only defines the timing for the maturation of these cells but also maintains their function in the adult brain.

Basket cells produce spontaneous γ -oscillations soon after birth, which synchronize the early electrical activity of cortical networks (41). They undergo an extensive functional and transcriptional maturation process between PND10 and PND40, with a major change in ion channels content around PND15 (42). They ultimately produce a specific type of extracellular matrix called the perineuronal net, which stabilizes synapses. The assembly of the perineuronal net marks the end of the so-called critical period of high synaptic plasticity (43, 44). After this, experience-dependent elaboration of novel neuronal circuits becomes almost impossible. The elaboration of the perineuronal net is altered in mice expressing TR α 1^{L400R} in GABAergic neurons (34). Therefore, it is tempting to conclude that, by ensuring the proper timing of basket cells maturation, T3 provides a temporal signal, which defines the end of the critical period (45). It would be of interest to address if these conclusions also apply to the filial imprinting of birds. Similarly, this phenomenon is restricted to a sensitive period defined by T3 (46) and involves GABAergic neurons (47).

Desynchronizing the synchronization

The earlier examples indicate that during the postnatal and preweaning period, T3 acts in a cell-autonomous manner to promote the terminal maturation of distinct cell types: the cardiomyocytes, the Purkinje cells of the cerebellum, and the cortical basket cells. This conclusion probably also applies to at least bone chondrocytes (48) and spleen erythrocytes progenitors (49). On the

other hand, there are cell types that respond in a non-cell autonomous manner to T3 during development. For example, the response to T3 of the granule cells progenitors of the cerebellum is only secondary to the T3-induced secretion of diffusible signals by the neighboring Purkinje cells (25). Similarly, the oligodendrocyte precursor cells, responsible for the initial postnatal myelination of axons, are not sensitive to the restricted expression of TR α 1^{L400R} (50, 51). Therefore, T3-responsive cells amplify the influence of T3, secreting growth factors and neurotrophins that influence their microenvironment (52, 53, 54).

The possibility that T3 is a temporal signal orchestrating directly or indirectly the multiple changes that take place after birth in mice justifies the comparison with amphibian metamorphosis. In that respect, it is striking that the heart regenerative capacity is lost in both models. However, under this hypothesis, all T3-dependent maturation processes should be initiated when the circulating level of T3 is at its maximum, in tadpoles or juvenile mice, which is not precisely the case. In particular, the critical period of heightened plasticity mentioned above is restricted to a temporal window, which varies according to brain areas (43). Thus, in addition to the circulating level of T4 and T3, one should consider the local level of signaling, whose dynamic evolution after birth might be different in different areas and cell types. Reporter mice indeed reveal a complex and dynamic pattern of T3 signaling in brain (55).

The most obvious way to modify the local concentration of T3 and generate different peaks of T3 at different times would be deiodination. Several studies illustrate this possibility for inner ear maturation and the onset of hearing. In the prenatal cochlea, type 3 deiodinase catabolizes both T4 and T3, lowering the T3 content of the perilymph (56). After birth, this enzyme is replaced by type 2 deiodinase that quickly increases the local concentration of T3 by converting T4 into T3, which ensures a timely differentiation of mechanosensory hair cells within the cochlea (57). Therefore, while T3 peaks in the serum at PND15, the maximum T3 concentration in the cochlea is achieved at PND7. If supplied in excess at birth, T3 advances the overall program of cochlear maturation by several days, causing deafness in adult mice (58). The dynamic changes in deiodinases activity during postnatal development provoke similar uncoupling between the circulating and tissue levels of T3 in other tissues, including the intestine and liver (59, 60).

An alternative mechanism for the local modulation of T3 signaling relies on transporters. Several transporters

ensure the transport of TH across the placenta, blood-testis and brain–blood barriers, and their transfer to the cell nucleus. While human patients with *Mct8* mutations (alias *Scl16a2*) suffer from deep brain hypothyroidism, this is not the case for *Mct8* knock-out mice (61). In these mice, a moderate T4 deficit is compensated by increased type-2 deiodination (62) maintaining sufficient T3 in the postnatal brain (37). However, overt brain hypothyroidism appears when the *Mct8* knock-out is combined with a second knock-out to eliminate either another transporter (*Oatp1c1/Slc01c1*) (63) or type 2 deiodinase (64).

Future directions and unsolved problems

The fruitful cross-species comparison which led us during this analysis suggests several fruitful avenues for future research and should not be limited to *Xenopus laevis* and mice. In particular, zebrafish is a popular model which introduces a useful complement as it is ectotherm, has a high regeneration capacity, and does not undergo an obvious metamorphosis. This model has not yet been fully exploited and provides attractive models to analyze the developmental function of T3, notably in bones (65) skin (66, 67) retina (68, 69), and lateral line, which is a sensory organ which function is comparable to the one of the inner ear (70).

The reporter mice that allow to visualize the heterogeneity of T3 signaling in the fetus and in the postnatal brain (55) have been crossed to several knock-out mice in order to identify the origin of this heterogeneity. Surprisingly, the elimination of type 2 deiodinase had no influence on the temporal and spatial expression of the reporter transgene (unpublished data). Only after several months did the absence of type 3 deiodinase cause the accumulation of T3 in specific areas of the adult brain (71). The knock-out of the *Mct8/Scl16a2* gene also failed to modify the perinatal expression pattern of the reporter transgene (unpublished data). Finally, the reporter mice were crossed to mice carrying a mutation of the *Hairless* gene, which in many cell types is among the most T3-responsive genes. *Hairless* encodes a protein which has been proposed to be a TR corepressor (72), but its function remains unclear. The loss of function of *Hairless* did not alter the expression of the reporter transgene, either (unpublished data). Although there are technical limitations to this reporter system, which relies on the *in situ* detection of β -galactosidase activity, these observations suggest that an important piece of information is missing, which would explain why T3 is not evenly distributed in mouse tissues during the fetal and postnatal development.

Besides, a variety of TH transporters, whose functions are not fully documented, remain to be tested (73). Another interesting candidate is μ -crystallin (74), an enzyme encoded by the *Crym* gene (75), which is abundant in some astrocytes (76) and maintains the intracellular pool of TH (77). *Crym* gene overexpression in mouse muscles causes a local accumulation of T3 and favors lipids β -oxydation (78).

The reason why some cell types are more sensitive than others to T3 stimulation remains unexplained. This sensitivity can be related to the abundance of receptors in a certain cell type. However, this abundance is very difficult to measure directly (13) and alternative explanations need to be explored. In particular, many proteins interact with TR α 1 and TR β 1 (79). These proteins shuttle the receptors between the nucleus and cytoplasm (80), introduce post-translational modifications (81, 82), or act as transcription cofactors (83). Finally, in most cell types, the lack of transcriptome and cistrome information hinders the identification of target genes that mediate the functions of receptors.

Declaration of interest

The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of this review.

Funding

Research in our laboratory is funded by European Union's Horizon 2020 research and innovation program under grant agreement 825753 (ERGO) and Agence Nationale de la Recherche (ANR22-CE14-0026-01).

References

- 1 Flamant F, Gauthier K & Richard S. Genetic investigation of thyroid hormone receptor function in the developing and adult brain. *Current Topics in Developmental Biology* 2017 **125** 303–335. (<https://doi.org/10.1016/bs.ctdb.2017.01.001>)
- 2 Dusart I & Flamant F. Profound morphological and functional changes of rodent Purkinje cells between the first and the second postnatal weeks: a metamorphosis? *Frontiers in Neuroanatomy* 2012 **6** 11. (<https://doi.org/10.3389/fnana.2012.00011>)
- 3 Holzer G & Laudet V. Thyroid hormones: a triple-edged sword for life history transitions. *Current Biology* 2015 **25** R344–R347. (<https://doi.org/10.1016/j.cub.2015.02.026>)
- 4 Taubenheim J, Kortmann C & Fraune S. Function and evolution of nuclear receptors in environmental-dependent postembryonic development. *Frontiers in Cell and Developmental Biology* 2021 **9** 653792. (<https://doi.org/10.3389/fcell.2021.653792>)
- 5 Tata JR. Amphibian metamorphosis as a model for the developmental actions of thyroid hormone. *Molecular and Cellular Endocrinology* 2006 **246** 10–20. (<https://doi.org/10.1016/j.mce.2005.11.024>)
- 6 Paul B, Sterner ZR, Buchholz DR, Shi YB & Sachs LM. Thyroid and corticosteroid signaling in amphibian metamorphosis. *Cells* 2022 **11** 1595. (<https://doi.org/10.3390/cells11101595>)
- 7 Sachs LM, Damjanovski S, Jones PL, Li Q, Amano T, Ueda S, Shi YB & Ishizuya-Oka A. Dual functions of thyroid hormone receptors during *Xenopus* development. *Comparative Biochemistry and Physiology. Part B, Biochemistry and Molecular Biology* 2000 **126** 199–211. ([https://doi.org/10.1016/s0305-0491\(00\)00198-x](https://doi.org/10.1016/s0305-0491(00)00198-x))
- 8 Fairclough L & Tata JR. An immunocytochemical analysis of the expression of thyroid hormone receptor alpha and beta proteins during natural and thyroid hormone-induced metamorphosis in *Xenopus*. *Development, Growth and Differentiation* 1997 **39** 273–283. (<https://doi.org/10.1046/j.1440-169x.1997.t01-2-00003.x>)
- 9 Hadj-Sahraoui N, Seugnet I, Ghorbel MT & Demeneix B. Hypothyroidism prolongs mitotic activity in the post-natal mouse brain. *Neuroscience Letters* 2000 **280** 79–82. ([https://doi.org/10.1016/s0304-3940\(00\)00768-0](https://doi.org/10.1016/s0304-3940(00)00768-0))
- 10 Hirose K, Payumo AY, Cutie S, Hoang A, Zhang H, Guyot R, Lunn D, Bigley RB, Yu H, Wang J, et al. Evidence for hormonal control of heart regenerative capacity during endothermy acquisition. *Science* 2019 **364** 184–188. (<https://doi.org/10.1126/science.aar2038>)
- 11 Heier CR, Hampton TG, Wang D & Didonato CJ. Development of electrocardiogram intervals during growth of FVB/N neonate mice. *BMC Physiology* 2010 **10** 16. (<https://doi.org/10.1186/1472-6793-10-16>)
- 12 Piquereau J, Novotova M, Fortin D, Garnier A, Ventura-Clapier R, Veksler V & Joubert F. Postnatal development of mouse heart: formation of energetic microdomains. *Journal of Physiology* 2010 **588** 2443–2454. (<https://doi.org/10.1113/jphysiol.2010.189670>)
- 13 Szulc KU, Lerch JP, Nieman BJ, Bartelle BB, Friedel M, Suero-Abreu GA, Watson C, Joyner AL & Turnbull DH. 4D MEMRI atlas of neonatal FVB/N mouse brain development. *Neuroimage* 2015 **118** 49–62. (<https://doi.org/10.1016/j.neuroimage.2015.05.029>)
- 14 Okaty BW, Miller MN, Sugino K, Hempel CM & Nelson SB. Transcriptional and electrophysiological maturation of neocortical fast-spiking GABAergic interneurons. *Journal of Neuroscience* 2009 **29** 7040–7052. (<https://doi.org/10.1523/JNEUROSCI.0105-09.2009>)
- 15 Sohal VS, Zhang F, Yizhar O & Deisseroth K. Parvalbumin neurons and gamma rhythms enhance cortical circuit performance. *Nature* 2009 **459** 698–702. (<https://doi.org/10.1038/nature07991>)
- 16 Hensch TK. Critical period plasticity in local cortical circuits. *Nature Reviews. Neuroscience* 2005 **6** 877–888. (<https://doi.org/10.1038/nrn1787>)
- 17 van 't Spijker HM & Kwok JCF. A Sweet Talk: The Molecular Systems of Perineuronal Nets in Controlling Neuronal Communication. *Frontiers in Integrative Neuroscience* 2017 **11** 33. (<https://doi.org/10.3389/fnint.2017.00033>)
- 18 Hu JS, Vogt D, Sandberg M & Rubenstein JL. Cortical interneuron development: a tale of time and space. *Development* 2017 **144** 3867–3878. (<https://doi.org/10.1242/dev.132852>)
- 19 Mansouri A, Chowdhury K & Gruss P. Follicular cells of the thyroid gland require Pax8 gene function. *Nature Genetics* 1998 **19** 87–90. (<https://doi.org/10.1038/ng0598-87>)
- 20 Flamant F, Poguet AL, Plateroti M, Chassande O, Gauthier K, Streichenberger N, Mansouri A & Samarut J. Congenital hypothyroid Pax8(-/-) mutant mice can be rescued by inactivating the TRalpha gene. *Molecular Endocrinology* 2002 **16** 24–32. (<https://doi.org/10.1210/mend.16.1.0766>)
- 21 Flamant F & Gauthier K. Thyroid hormone receptors: the challenge of elucidating isotype-specific functions and cell-specific response. *Biochimica et Biophysica Acta* 2013 **1830** 3900–3907. (<https://doi.org/10.1016/j.bbagen.2012.06.003>)
- 22 Flamant F, Baxter JD, Forrest D, Refetoff S, Samuels H, Scanlan TS, Vennstrom B & Samarut J. International Union of Pharmacology. LIX. The pharmacology and classification of the nuclear receptor superfamily: thyroid hormone receptors. *Pharmacological Reviews* 2006 **58** 705–711. (<https://doi.org/10.1124/pr.58.4.3>)
- 23 Minakhina S, Bansal S, Zhang A, Brotherton M, Janodia R, De Oliveira V, Tadepalli S & Wondisford FE. A direct comparison of thyroid hormone receptor protein levels in mice provides unexpected

- insights into thyroid hormone action. *Thyroid* 2020 **30** 1193–1204. (<https://doi.org/10.1089/thy.2019.0763>)
- 24 Zekri Y, Guyot R & Flamant F. An atlas of thyroid hormone receptors' target genes in mouse tissues. *International Journal of Molecular Sciences* 2022 **23** 11444. (<https://doi.org/10.3390/ijms231911444>)
- 25 Negron SG, Ercan-Sencicek AG, Freed J, Walters M & Lin Z. Both proliferation and lipogenesis of brown adipocytes contribute to postnatal brown adipose tissue growth in mice. *Scientific Reports* 2020 **10** 20335. (<https://doi.org/10.1038/s41598-020-77362-x>)
- 26 Takashima Y, Ma L & McKemy DD. The development of peripheral cold neural circuits based on TRPM8 expression. *Neuroscience* 2010 **169** 828–842. (<https://doi.org/10.1016/j.neuroscience.2010.05.039>)
- 27 Liu S, Shen S, Yan Y, Sun C, Lu Z, Feng H, Ma Y, Tang Z, Yu J, Wu Y, et al. Triiodothyronine (T3) promotes brown fat hyperplasia via thyroid hormone receptor alpha mediated adipocyte progenitor cell proliferation. *Nature Communications* 2022 **13** 3394. (<https://doi.org/10.1038/s41467-022-31154-1>)
- 28 Poss KD, Wilson LG & Keating MT. Heart regeneration in zebrafish. *Science* 2002 **298** 2188–2190. (<https://doi.org/10.1126/science.1077857>)
- 29 Spelat R, Ferro F, Contessotto P, Aljaabary A, Martin-Saldana S, Jin C, Karlsson NG, Grealy M, Hilscher MM, Magni F, et al. Metabolic reprogramming and membrane glycan remodeling as potential drivers of zebrafish heart regeneration. *Communications Biology* 2022 **5** 1365. (<https://doi.org/10.1038/s42003-022-04328-2>)
- 30 Marshall LN, Vivien CJ, Girardot F, Pericard L, Scerbo P, Palmier K, Demeneix BA & Coen L. Stage-dependent cardiac regeneration in *Xenopus* is regulated by thyroid hormone availability. *PNAS* 2019 **116** 3614–3623. (<https://doi.org/10.1073/pnas.1803794116>)
- 31 Avci HX, Lebrun C, Wehrle R, Doulazmi M, Chatonnet F, Morel MP, Ema M, Vodjdani G, Sotelo C, Flamant F, et al. Thyroid hormone triggers the developmental loss of axonal regenerative capacity via thyroid hormone receptor alpha1 and Kruppel-like factor 9 in Purkinje cells. *PNAS* 2012 **109** 14206–14211. (<https://doi.org/10.1073/pnas.1119853109>)
- 32 Heuer H & Mason CA. Thyroid hormone induces cerebellar Purkinje cell dendritic development via the thyroid hormone receptor alpha1. *Journal of Neuroscience* 2003 **23** 10604–10612. (<https://doi.org/10.1523/JNEUROSCI.23-33-10604.2003>)
- 33 Boukhtouche F, Brugg B, Wehrle R, Bois-Joyeux B, Danan JL, Dusart I & Mariani J. Induction of early Purkinje cell dendritic differentiation by thyroid hormone requires RORalpha. *Neural Development* 2010 **5** 18. (<https://doi.org/10.1186/1749-8104-5-18>)
- 34 Fauquier T, Chatonnet F, Picou F, Richard S, Fossat N, Aguilera N, Lamonerie T & Flamant F. Purkinje cells and Bergmann glia are primary targets of the TRalpha1 thyroid hormone receptor during mouse cerebellum postnatal development. *Development* 2014 **141** 166–175. (<https://doi.org/10.1242/dev.103226>)
- 35 Yu L, Iwasaki T, Xu M, Lesmana R, Xiong Y, Shimokawa N, Chin WW & Koibuchi N. Aberrant cerebellar development of transgenic mice expressing dominant-negative thyroid hormone receptor in cerebellar Purkinje cells. *Endocrinology* 2015 **156** 1565–1576. (<https://doi.org/10.1210/en.2014-1079>)
- 36 Barres BA, Lazar MA & Raff MC. A novel role for thyroid hormone, glucocorticoids and retinoic acid in timing oligodendrocyte development. *Development* 1994 **120** 1097–1108. (<https://doi.org/10.1242/dev.120.5.1097>)
- 37 Blackshaw S. Why has the ability to regenerate following CNS injury been repeatedly lost over the course of evolution? *Frontiers in Neuroscience* 2022 **16** 831062. (<https://doi.org/10.3389/fnins.2022.831062>)
- 38 Berbel P, Navarro D & Roman GC. An evo-devo approach to thyroid hormones in cerebral and cerebellar cortical development: etiological implications for autism. *Frontiers in Endocrinology (Lausanne)* 2014 **5** 146. (<https://doi.org/10.3389/fendo.2014.00146>)
- 39 Richard S & Flamant F. Regulation of T3 availability in the developing brain: the mouse genetics contribution. *Frontiers in Endocrinology (Lausanne)* 2018 **9** 265. (<https://doi.org/10.3389/fendo.2018.00265>)
- 40 Bernal J. Thyroid hormone regulated genes in cerebral cortex development. *Journal of Endocrinology* 2017 **232** R83–R97. (<https://doi.org/10.1530/JOE-16-0424>)
- 41 Alvarez-Dolado M, Iglesias T, Rodriguez-Pena A, Bernal J & Munoz A. Expression of neurotrophins and the trk family of neurotrophin receptors in normal and hypothyroid rat brain. *Brain Research. Molecular Brain Research* 1994 **27** 249–257. ([https://doi.org/10.1016/0169-328x\(94\)90007-8](https://doi.org/10.1016/0169-328x(94)90007-8))
- 42 Richard S, Guyot R, Rey-Millet M, Prieux M, Markossian S, Aubert D & Flamant F. A pivotal genetic program controlled by thyroid hormone during the maturation of GABAergic neurons. *iScience* 2020 **23** 100899. (<https://doi.org/10.1016/j.isci.2020.100899>)
- 43 Huang ZJ & Paul A. The diversity of GABAergic neurons and neural communication elements. *Nature Reviews. Neuroscience* 2019 **20** 563–572. (<https://doi.org/10.1038/s41583-019-0195-4>)
- 44 Hu H, Gan J & Jonas P. Interneurons. Fast-spiking, parvalbumin(+) GABAergic interneurons: from cellular design to microcircuit function. *Science* 2014 **345** 1255263. (<https://doi.org/10.1126/science.1255263>)
- 45 Mayerl S, Chen J, Salveridou E, Boelen A, Darras VM & Heuer H. Thyroid hormone transporter deficiency in mice impacts multiple stages of GABAergic interneuron development. *Cerebral Cortex* 2022 **32** 329–341. (<https://doi.org/10.1093/cercor/bhab211>)
- 46 Berbel P, Marco P, Cerezo JR & DeFelipe J. Distribution of parvalbumin immunoreactivity in the neocortex of hypothyroid adult rats. *Neuroscience Letters* 1996 **204** 65–68. ([https://doi.org/10.1016/0304-3940\(96\)12318-1](https://doi.org/10.1016/0304-3940(96)12318-1))
- 47 Uchida K, Hasuoka K, Fuse T, Kobayashi K, Moriya T, Suzuki M, Katayama N & Itoi K. Thyroid hormone insufficiency alters the expression of psychiatric disorder-related molecules in the hypothyroid mouse brain during the early postnatal period. *Scientific Reports* 2021 **11** 6723. (<https://doi.org/10.1038/s41598-021-86237-8>)
- 48 Wallis K, Sjogren M, van Hogerlinden M, Silberberg G, Fisahn A, Nordstrom K, Larsson L, Westerblad H, Morreale de Escobar G, Shupliakov O, et al. Locomotor deficiencies and aberrant development of subtype-specific GABAergic interneurons caused by an unliganded thyroid hormone receptor alpha1. *Journal of Neuroscience* 2008 **28** 1904–1915. (<https://doi.org/10.1523/JNEUROSCI.5163-07.2008>)
- 49 Cardin JA, Carlen M, Meletis K, Knoblich U, Zhang F, Deisseroth K, Tsai LH & Moore CI. Driving fast-spiking cells induces gamma rhythm and controls sensory responses. *Nature* 2009 **459** 663–667. (<https://doi.org/10.1038/nature08002>)
- 50 Batista G & Hensch TK. Critical period regulation by thyroid hormones: potential mechanisms and sex-specific aspects. *Frontiers in Molecular Neuroscience* 2019 **12** 77. (<https://doi.org/10.3389/fnmol.2019.00077>)
- 51 Yamaguchi S, Aoki N, Kitajima T, Iikubo E, Katagiri S, Matsushima T & Homma KJ. Thyroid hormone determines the start of the sensitive period of imprinting and primes later learning. *Nature Communications* 2012 **3** 1081. (<https://doi.org/10.1038/ncomms2088>)
- 52 Saheki Y, Aoki N, Homma KJ & Matsushima T. Suppressive modulation of the chick forebrain network for imprinting by thyroid hormone: an in vitro study. *Frontiers in Physiology* 2022 **13** 881947. (<https://doi.org/10.3389/fphys.2022.881947>)
- 53 Desjardin C, Charles C, Benoist-Lasselien C, Riviere J, Gilles M, Chassande O, Morgenthaler C, Laloe D, Lecardonnell J, Flamant F, et al. Chondrocytes play a major role in the stimulation of bone growth by thyroid hormone. *Endocrinology* 2014 **155** 3123–3135. (<https://doi.org/10.1210/en.2014-1109>)
- 54 Angelin-Duclos C, Domenget C, Kolbus A, Beug H, Jurdic P & Samarut J. Thyroid hormone T3 acting through the thyroid hormone

- alpha receptor is necessary for implementation of erythropoiesis in the neonatal spleen environment in the mouse. *Development* 2005 **132** 925–934. (<https://doi.org/10.1242/dev.01648>)
- 55 Picou F, Fauquier T, Chatonnet F, Richard S & Flamant F. Deciphering direct and indirect influence of thyroid hormone with mouse genetics. *Molecular Endocrinology* 2014 **28** 429–441. (<https://doi.org/10.1210/me.2013-1414>)
- 56 Picou F, Fauquier T, Chatonnet F & Flamant F. A bimodal influence of thyroid hormone on cerebellum oligodendrocyte differentiation. *Molecular Endocrinology* 2012 **26** 608–618. (<https://doi.org/10.1210/me.2011-1316>)
- 57 Neveu I & Arenas E. Neurotrophins promote the survival and development of neurons in the cerebellum of hypothyroid rats in vivo. *Journal of Cell Biology* 1996 **133** 631–646. (<https://doi.org/10.1083/jcb.133.3.631>)
- 58 Desouza LA, Sathanoori M, Kapoor R, Rajadhyaksha N, Gonzalez LE, Kottmann AH, Tole S & Vaidya VA. Thyroid hormone regulates the expression of the sonic hedgehog signaling pathway in the embryonic and adult Mammalian brain. *Endocrinology* 2011 **152** 1989–2000. (<https://doi.org/10.1210/en.2010-1396>)
- 59 Gilbert ME, Rovet J, Chen Z & Koibuchi N. Developmental thyroid hormone disruption: prevalence, environmental contaminants and neurodevelopmental consequences. *Neurotoxicology* 2012 **33** 842–852. (<https://doi.org/10.1016/j.neuro.2011.11.005>)
- 60 Quignodon L, Legrand C, Allioi N, Guadano-Ferraz A, Bernal J, Samarut J & Flamant F. Thyroid hormone signaling is highly heterogeneous during pre- and postnatal brain development. *Journal of Molecular Endocrinology* 2004 **33** 467–476. (<https://doi.org/10.1677/jme.1.01570>)
- 61 Ng L, Hernandez A, He W, Ren T, Srinivas M, Ma M, Galton VA, St Germain DL & Forrest D. A protective role for type 3 deiodinase, a thyroid hormone-inactivating enzyme, in cochlear development and auditory function. *Endocrinology* 2009 **150** 1952–1960. (<https://doi.org/10.1210/en.2008-1419>)
- 62 Campos-Barros A, Amma LL, Faris JS, Shailam R, Kelley MW & Forrest D. Type 2 iodothyronine deiodinase expression in the cochlea before the onset of hearing. *PNAS* 2000 **97** 1287–1292. (<https://doi.org/10.1073/pnas.97.3.1287>)
- 63 Peeters RP, Ng L, Ma M & Forrest D. The timecourse of apoptotic cell death during postnatal remodeling of the mouse cochlea and its premature onset by triiodothyronine (T3). *Molecular and Cellular Endocrinology* 2015 **407** 1–8. (<https://doi.org/10.1016/j.mce.2015.02.025>)
- 64 Bates JM, St Germain DL, Galton VA, Singh R, Upadhyay G, Kumar S, Kapoor A, Kumar A, Tiwari M & Godbole MM. Expression profiles of the three iodothyronine deiodinases, D1, D2, and D3, in the developing rat. *Endocrinology* 1999 **140** 844–851. (<https://doi.org/10.1210/endo.140.2.6537>)
- 65 Fonseca TL, Fernandes GW, McAninch EA, Bocco BM, Abdalla SM, Ribeiro MO, Mohacsik P, Fekete C, Li D, Xing X, et al. Perinatal deiodinase 2 expression in hepatocytes defines epigenetic susceptibility to liver steatosis and obesity. *PNAS* 2015 **112** 14018–14023. (<https://doi.org/10.1073/pnas.1508943112>)
- 66 Wirth EK, Roth S, Blechschmidt C, Holter SM, Becker L, Raczi I, Zimmer A, Klopstock T, Gailus-Durner V, Fuchs H, et al. Neuronal 3',3,5-triiodothyronine (T3) uptake and behavioral phenotype of mice deficient in Mct8, the neuronal T3 transporter mutated in Allan-Herndon-Dudley syndrome. *Journal of Neuroscience* 2009 **29** 9439–9449. (<https://doi.org/10.1523/JNEUROSCI.6055-08.2009>)
- 67 Trajkovic M, Visser TJ, Mittag J, Horn S, Lukas J, Darras VM, Raivich G, Bauer K & Heuer H. Abnormal thyroid hormone metabolism in mice lacking the monocarboxylate transporter 8. *Journal of Clinical Investigation* 2007 **117** 627–635. (<https://doi.org/10.1172/JCI28253>)
- 68 Mayerl S, Muller J, Bauer R, Richert S, Kassmann CM, Darras VM, Buder K, Boelen A, Visser TJ & Heuer H. Transporters MCT8 and OATP1C1 maintain murine brain thyroid hormone homeostasis. *Journal of Clinical Investigation* 2014 **124** 1987–1999. (<https://doi.org/10.1172/JCI70324>)
- 69 Barez-Lopez S, Grijota-Martinez C, Auso E, Fernandez-de Frutos M, Montero-Pedrazuela A & Guadano-Ferraz A. Adult mice lacking Mct8 and Dio2 proteins present alterations in peripheral thyroid hormone levels and severe brain and motor skill impairments. *Thyroid* 2019 **29** 1669–1682. (<https://doi.org/10.1089/thy.2019.0068>)
- 70 Keer S, Storch JD, Nguyen S, Prado M, Singh R, Hernandez LP & McMenamin SK. Thyroid hormone shapes craniofacial bones during postembryonic zebrafish development. *Evolution and Development* 2022 **24** 61–76. (<https://doi.org/10.1111/ede.12399>)
- 71 Aman AJ, Kim M, Saunders LM & Parichy DM. Thyroid hormone regulates abrupt skin morphogenesis during zebrafish postembryonic development. *Developmental Biology* 2021 **477** 205–218. (<https://doi.org/10.1016/j.ydbio.2021.05.019>)
- 72 Patterson LB & Parichy DM. Zebrafish pigment pattern formation: insights into the development and evolution of adult form. *Annual Review of Genetics* 2019 **53** 505–530. (<https://doi.org/10.1146/annurev-genet-112618-043741>)
- 73 Suzuki SC, Bleckert A, Williams PR, Takechi M, Kawamura S & Wong RO. Cone photoreceptor types in zebrafish are generated by symmetric terminal divisions of dedicated precursors. *PNAS* 2013 **110** 15109–15114. (<https://doi.org/10.1073/pnas.1303551110>)
- 74 Nelson RF, Balraj A, Suresh T, Elias LJ, Yoshimatsu T & Patterson SS. The developmental progression of eight opsin spectral signals recorded from the zebrafish retinal cone layer is altered by the timing and cell type expression of thyroxine receptor beta2 (trbeta2) gain-of-function transgenes. *eNeuro* 2022 **9** ENEURO.0326-22.2022. (<https://doi.org/10.1523/ENEURO.0326-22.2022>)
- 75 Hu Y, Mauri A, Donahue J, Singh R, Acosta B & McMenamin S. Thyroid hormone coordinates developmental trajectories but does not underlie developmental truncation in danionins. *Developmental Dynamics* 2019 **248** 1144–1154. (<https://doi.org/10.1002/dvdy.76>)
- 76 Hernandez A, Quignodon L, Martinez ME, Flamant F & St Germain DL. Type 3 deiodinase deficiency causes spatial and temporal alterations in brain T3 signaling that are dissociated from serum thyroid hormone levels. *Endocrinology* 2010 **151** 5550–5558. (<https://doi.org/10.1210/en.2010-0450>)
- 77 Potter GB, Beaudoin GM, 3rd, DeRenzo CL, Zarach JM, Chen SH & Thompson CC. The hairless gene mutated in congenital hair loss disorders encodes a novel nuclear receptor corepressor. *Genes and Development* 2001 **15** 2687–2701. (<https://doi.org/10.1101/gad.916701>)
- 78 Bernal J, Guadano-Ferraz A & Morte B. Thyroid hormone transporters-funcions and clinical implications. *Nature Reviews. Endocrinology* 2015 **11** 690. (<https://doi.org/10.1038/nrendo.2015.186>)
- 79 Aksoy A, Hantusch B & Kenner L. Emerging role of T3-binding protein μ -crystallin (CRYM) in health and disease. *Trends in Endocrinology and Metabolism* 2022 **22** 178–183. (<https://doi.org/10.1016/j.tem.2022.09.003>)
- 80 Hallen A, Cooper AJ, Smith JR, Jamie JF & Karuso P. Ketimine reductase/CRYM catalyzes reductive alkylamination of alpha-keto acids, confirming its function as an imine reductase. *Amino Acids* 2015 **47** 2457–2461. (<https://doi.org/10.1007/s00726-015-2044-8>)
- 81 Endo S, Kasai A & Soto JS. Molecular basis of astrocyte diversity and morphology across the CNS in health and disease. *Science* 2022 **378** 514–516. (<https://doi.org/10.1126/science.adc9020>)
- 82 Suzuki S, Suzuki N, Mori J, Oshima A, Usami S & Hashizume K. micro-crystallin as an intracellular 3,5,3'-triiodothyronine holder in vivo. *Molecular Endocrinology* 2007 **21** 885–894. (<https://doi.org/10.1210/me.2006-0403>)
- 83 Kinney CJ, O'Neill A, Noland K, Huang W, Muriel J, Lukyanenko V, Kane MA, Ward CW, Collier AF, Roche JA, et al. μ -crystallin in Mouse skeletal muscle Promotes a Shift from Glycolytic toward Oxidative Metabolism. *Current Research in Physiology* 2021 **4** 47–59. (<https://doi.org/10.1016/j.crphys.2021.02.003>)
- 84 Hahm JB, Schroeder AC & Privalsky ML. The two major isoforms of thyroid hormone receptor, TRalpha1 and TRbeta1, preferentially partner with distinct panels of auxiliary proteins. *Molecular and*

- Cellular Endocrinology* 2014 **383** 80–95. (<https://doi.org/10.1016/j.mce.2013.11.015>)
- 85 Maruvada P, Baumann CT, Hager GL & Yen PM. Dynamic shuttling and intranuclear mobility of nuclear hormone receptors. *Journal of Biological Chemistry* 2003 **278** 12425–12432. (<https://doi.org/10.1074/jbc.M202752200>)
- 86 Liu YY, Kogai T, Schultz JJ, Mody K & Brent GA. Thyroid hormone receptor isoform-specific modification by small ubiquitin-like modifier (SUMO) modulates thyroid hormone-dependent gene regulation. *Journal of Biological Chemistry* 2012 **287** 36499–36508. (<https://doi.org/10.1074/jbc.M112.344317>)
- 87 Park JW, Zhao L, Webb P & Cheng SY. Src-dependent phosphorylation at Y406 on the thyroid hormone receptor beta confers the tumor suppressor activity. *Oncotarget* 2014 **5** 10002–10016. (<https://doi.org/10.18632/oncotarget.2487>)
- 88 Perissi V & Rosenfeld MG. Controlling nuclear receptors: the circular logic of cofactor cycles. *Nature Reviews. Molecular Cell Biology* 2005 **6** 542–554. (<https://doi.org/10.1038/nrm1680>)

Received 23 January 2023

Accepted 30 January 2023

Available online 30 January 2023

Version of Record published 10 March 2023