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Review

A temporary compendium of thyroid hormone target genes in brain ☆

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ABSTRACT

Thyroid hormone controls a number of developmental and physiological processes in the brain by directly acting on gene expression. Transcriptome analyses in rodent identified a number of thyroid hormone regulated genes in several brain areas at different stages. Genome wide analysis of chromatin occupancy in a neural cell line also identified a subset of genes which transcription is likely to be directly regulated by thyroid hormone receptors in neurons. However, the abundance of these data and apparent discrepancies between studies brought some confusion. We present here a meta-analysis of available data to identify recurrent themes in thyroid hormone action in brain cells. This provides a curated list of 734 regulated genes in rodent brain, and highlights a small number of likely direct target genes. Some of these genes are also regulated in amphibians during metamorphosis. This article is part of a Special Issue entitled: Nuclear receptors in animal development.

1. Introduction

Thyroid hormones (TH including tri-iodo-thyronine or T3, and thyroxine, or T4, its less active precursor) play an important function in fetal and adult brain. During neurodevelopment they are required for proper neuronal and glial differentiation, neuronal migration, and myelin formation. Thyroid hormone deficiency during human development may cause irreversible mental retardation and variable degrees of neurological impairment. In adults T3 is necessary for neural stem cell proliferation and differentiation, and hypothyroidism is often associated with mood disorders. T3 acts on gene expression mainly, if not only, by binding to nuclear receptors (TRα1, TRβ1 and TRβ2, collectively TR) encoded by the two *Thra* and *Thrb* genes and which act as ligand-dependent transcription factors. TR forms heterodimers with RXR, which bind to DNA in a T3 independent manner. *In vitro* experiments suggest that binding could occur on several types of response elements, constituted by doublets of the half-site AGGCTA, or on DNA elements bearing only limited similarity to this consensus. This binding to regulatory elements of gene promoters induces changes of expression of neighboring genes, defined as "target genes". As long distance transactivation is possible, one could expect TR to regulate a very large number of genes in many cell types. Identifying these genes is of crucial importance to understand the functions of T3 in brain cells. This is however a challenging task, due to the extreme cellular heterogeneity of most brain areas [1] and the fact that 80% of the known genes are expressed in at least one of these cell types [2]. Transcriptome analyses revealed that expression of thousands of genes changes after T3 stimulation in several brain areas, and at different developmental stages. Lists of putative TR target genes, which can be up-regulated or down-regulated, are accumulating. A survey of this recent literature provides the impression of a rather anarchical situation, with few consistencies and little overlap between the various studies. This situation can be explained in different ways:

1) TR might possess very different repertoires of target genes in different cell-types, different brain areas, and different developmental stages. The response of several well characterized T3 responsive genes actually varies in the brain. For example, the neuron-specific RC3/neurogranin regulation by T3 displays regional and temporal selectivity, which is not due to differential distribution of TR, but most probably related to region-specific trans-acting elements [3,4]. As discussed below, only two genome wide analyses of TR occupancy have been reported to date, but these already provide useful indications on possible mechanisms for a cell-type dependent regulation.
2) The second possibility is that many brain cell types share a common set of TR target genes, but that the exact experimental conditions deeply influence the outcome of transcriptome studies. For example, T3 stimulation first turns on TR target genes. Among the TR target genes are genes encoding transcription factors and cofactors, and cells rapidly display a secondary response to T3 stimulation, due to the increased expression of these T3-induced factors. Based on sensitivity to cycloheximide, which inhibits mRNA translation, it has been estimated that such secondary response represents 20% of the T3-induced changes in gene expression within 3 h in cultured cells [5]. Therefore, only a fraction of the T3 sensitive genes are

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genuine TR target genes, and the duration of T3 stimulation before transcriptome analysis profoundly modifies the outcome of the analysis.

3) A third possibility would be that technical biases alter the analyses. Transcriptome analyses are notoriously difficult to reproduce in different laboratories. It is also now recognized that microarray analysis, used in most published studies, produces a much higher false discovery rate than the more recent RNAseq techniques. Among microarrays, different technical platforms also provide divergent results. Finally, statistical data analysis is not performed in a standardized manner, and reexamination of published data shows that statistical biases can occur. In many cases the number of samples examined is too low to provide a sufficient statistical power to the analysis. Finally, defining a threshold to call a gene "T3 inducible" is a rather arbitrary decision, and has a major influence on the result.

For all these reasons, the overall outcome of transcriptome analyses remains globally disappointing, failing to provide a comprehensive picture of T3 cellular response in the brain, and suggesting that never-ending complications will be encountered. Here we performed a complete survey of available data for T3 influence on brain cells. In order to filter for false positive discovery, we listed genes that were identified as T3 responsive in more than one study. We also intersected data obtained in rodent central nervous system, with transcriptome data obtained in other organs and other species. This effort allowed us to outline recurrent and important themes in the T3 response of brain cells. We first present a comparative analysis of TR chromatin occupancy, which outlines the difficulty to identify the genes directly transactivated by TRs.

2. TR binding to proximal sequences is not the only determinant of T3 transactivation

For other nuclear receptors, it has been demonstrated that chromatin access necessitates the previous intervention of cell-specific "pioneer factors" that control the access to a subset of otherwise countless putative binding sites of the genome [6]. To date, only two genome wide studies of TR occupancy by ChIP-Seq in mouse cells have been published. Both studies used tagged TRs and affinity purification instead of chromatin immune-precipitation to circumvent the lack of high quality anti-TR antibody. Both identified thousands of TR binding sites in the genome either in a neural cell line [7] or in mouse hepatocytes [8]. This is a much larger number than the one previously reported using the less sensitive and less precise ChIP-on-Chip analysis of TR chromatin occupancy performed on mouse post-natal cerebellum [9,10]. Biotin-tagged TRβ1 was introduced into the liver by adenovirus-mediated gene transfer, while DNA-transfection was used to restore a stable expression of protein G tagged TRs in the C17.2 neural cell line (either TRα1 or TRβ1, providing the C17.2α and C17.2β cell lines). It should be kept in mind that in both cases the expression level of the exogenous TR exceeds the level normally found in the cerebellum and liver respectively. The overlap between the datasets of TR occupancy, or "cistromes", is substantial, indicating that access to TR binding sites is not tightly regulated for these nuclear receptors (Fig. 1). The two studies converge to conclude that liganded TRs preferentially bind as heterodimers on DR4 elements, and that the number of genes with a proximal TR binding largely exceeds the number of T3 responsive genes.

Although the cistromes largely intersect, the sets of T3-responsive genes with a proximal TR binding site, which are likely to be directly responsive to T3, do not overlap between the liver and neural cells. In other words, a number of genes that are regulated by T3 only in neural cells are bound by TRβ1 both in neural cells and liver. Most strikingly, in both cases, the large majority of the TR binding sites are located next to genes that are not T3 responsive, suggesting that a majority of binding events do not result in transactivation of neighboring genes. Therefore, differential binding alone cannot explain why the repertoire of T3 responsive genes differs between hepatocytes and neural cells. Similarly, the response mediated by TRα1 and TRβ1 in C17.2 is clearly different. Again, the differential response to T3 of a number of genes does not correlate with differential chromatin occupancy [7]. Other parameters, like the expression of different transcription cofactors in different cell types, or the conformation of chromatin-bound TRs, must take part in cell-specific or receptor-selective response. Based only on these two reports, it is tempting to speculate that T3-liganded TR/RXR heterodimers, bound on DR4 elements, adopt a conformation that is not always suitable for the recruitment of coactivators and transcription activation. This probably explains why bioinformatic tools have been of little help to identify TR target genes [11].

3. A shortened list of T3 responsive genes in rodent brain

We reviewed all the publications we were aware of to identify T3 responsive genes in brain cells. Comparisons between wild-type and TR knock-out mice proved difficult to integrate with studies analyzing in a more direct manner the response to TH [12]. It seems that in TR knock-out mice many changes in mRNA levels are not on TR target genes, but reflect modifications in the differentiation status of the cells, or changes in the composition of the cell population. We thus excluded these knock-out analyses. This left us with 10 studies, using several different experimental systems (Table 1 and Supplementary Table S1): The table also contains data on cultured glial cells, these were considered separately, as this study does not analyze the action of T3 alone, but analyze the differentiation of oligodendrocytes induced by T3 addition and concomitant growth factor withdrawal [13]. From the 750 genes differentially expressed during oligodendrocyte differentiation, 288 genes have also been reported to be regulated by T3 in the brain. These genes could also be T3 target genes in oligodendrocyte cells.

Study 1: Cerebellum RNA of post-natal day 4 (PND4) hypothyroid mice was compared to cerebellum RNA of hypothyroid mice receiving T4-replacement for 1 h or 6 h [14]. Mice were rendered hypothyroid by administering 2-mercapto-1-methylimidazole in drinking water to their mothers from the 15th day of conception.
Study 2: Cerebellum RNA at PND15 was analyzed in 3 groups of mice. Two were made hypothyroid by administering propyl-thio-uracyl at two different doses [15].
Study 3: Cerebellum RNA PND8 and PND15. Hypothyroid mice were $Pax8^{-/-}$ mice with athyroidogenesis. These were compared to wild-type mice and $Pax8^{-/-}$ treated with T3/T4 for 6 h or 24 h. Primary neuronal cell cultures were prepared from cerebellum of newborns. *In vitro* response to T3 (6 h) was addressed [11].
Study 4: Primary neuronal cell cultures were prepared from cerebellum of newborns and treated for 6, 16, 24 or 48 h with T3 [16].
Study 5: Mouse cerebral cortex of PND21 mice made hypothyroid with perchlorate and 2-mercapto-1-methylimidazole was compared with euthyroid mice [17].
Study 6: Cerebral cortex in rat at embryonic day 21 (ED21). Fetal hypothyroidism was obtained by maternal thyroidectomy followed by 2-mercapto-1-methylimidazole treatment [18].
Study 7: Mouse cerebral cortex RNA was analyzed at embryonic E16. 4 groups of animals were included: 1) controls, 2) hypothyroid, 3) hyperthyroid and 4) hypothyroid treated with thyroid hormone (T4 + T3). Mild fetal hypothyroidism was achieved in groups 2 and 4 after a short treatment (from gestational days 13 to 16) of the dam with propyl-thio-uracyl and 2-mercapto-1-methylimidazole. Groups 3 and 4 dams were treated with thyroid hormones 12 h before sacrifice [19].
Study 8a and 8b: Cortex and hippocampus of PND21 rats. Graded hypothyroidism was obtained by prolonged propyl-thio-uracyl treatment [20].

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