Review

Thyroid Hormone Action in the Cell

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THYROID HORMONES have profound effects on the growth, development, and metabolism of many tissues of higher organisms [1]. Although the biochemical bases of these effects are not fully understood, the regulation of protein synthesis is a primary event [1]. After binding to nuclear receptors, thyroid hormones control the accumulation of specific mRNA molecules that code for the synthesis of specific proteins regulated by the hormone. Human and rat thyrotropin (TSH) [2], rat growth hormone (rGH) [3], rat hepatic spot 14 [4], rat hepatic malic enzyme [5], and rat and rabbit heart myosin heavy chain [6] are examples in which the immediate regulation of gene expression by thyroid hormone is reported.

The principal thyroid hormones are L-thyroxine (T4) and 3,5,3'-triiodo-L-thyronine (T3). T3 is almost 10 times more biologically active than T4 [7] and virtually all of the metabolic action of T4 can be ascribed to the action of T3 that it gives rise to. T4 is present in human serum at about 110 nmol/liter. Most of it is bound by serum proteins, but 0.03% is in a free form [8]. T3 is present in human serum at about 2.1 nmol/liter, 85% of which comes from the deiodination of T4 in peripheral tissues [8]. About 0.3% of serum T3 is in a free form and it is this fraction that is taken up by the cells to exert physiological effects [9]. Thyroid hormones are taken up by the cell through an energy-dependent, saturable process or by passive diffusion [10-12] before they are metabolized or exert biological activity. In some tissues such as the central nervous systems, pituitary gland, and brown adipose tissue, a significant amount of T3 generated from T4 within the cell directly contributes to biological action before being exported from the cell. Although thyroid hormone binding sites are found in the cell nucleus, cytosol, mitochondria and cell membrane, the nuclear sites are now established to be the receptors. A schematic representation of thyroid hormone action at the cellular level is shown in Fig. 1. This report discusses the mechanisms of cellular transport of thyroid hormones, and the structure and function of the intracellular thyroid hormone binding proteins.

Cellular Transport of Thyroid Hormones

Thyroid hormones have to be transported over the plasma membrane as a first step before they exert their activity or before they are metabolized. It has been postulated that thyroid hormones enter the cell by passive diffusion [10], but recent
evidence shows that they are transported through the plasma membrane by an energy-dependent, saturable process [11, 12]. In primary cultured rat hepatocytes, saturable uptake of T3 was inhibited at low temperature or by blocking cell metabolism [11]. The cellular content of ATP closely correlates with T3 transport across the cell membrane [13]. Similar findings were reported concerning other cell types [14-16]. These reports so far suggest that energy-dependent and saturable cellular uptake of T3 consists of high and low affinity uptake systems with the Km values of 21-61 nM and 646-2800 nM T3, respectively. These transport systems may correspond to high and low affinity T3 binding sites of isolated cell membrane [17]. Cellular transport is stereospecific and the presence of distinct transport systems for D-T3 and L-T3 are reported in rat liver and kidney [18]. Whether T4 and T3 are taken up by the cells through the same carriers or not is still controversial. Kinetic analysis revealed different uptake processes in them in rat hepatocytes [19], but the same authors reported that T4 and T3 share common carriers in cultured anterior pituitary cells [20]. Using a cultured cell line derived from rat liver, clone 9 cells, we demonstrated that sodium butyrate enhanced the carrier-mediated cellular uptake of thyroid hormones [21], but the time course and the magnitude of the response were different in T4 and T3 (Fig. 2). Nuclear uptake of T3 was increased in parallel with the increased cellular uptake, whereas nuclear uptake of T4 was decreased in spite of the increased cellular uptake after sodium butyrate treatment (Fig. 2). We also found that cellular uptake of T3 varied, whereas that of T4 was constant throughout the growth cycle of cultured rat hepatoma cells [22]. These results indicate that rat liver cells take up T4 and T3 through different carriers and that nuclear transport mechanisms of T4 and T3 are also different. It therefore seems likely that whether or not T4 and T3 share the same carrier depends upon the tissue and cell type. This active transport of thyroid hormones is rate-limiting in total cellular uptake, cellular metabolism [23] and nuclear transport of hormones [24].

Although the nucleus is the primary site of thyroid hormone action, it is still unknown how the thyroid hormones are transported into the nucleus. Oppenheimer et al. [18] reported that cytosolic/plasma and nuclear/cytosolic concentration ratios of free T3 in rat liver are 2.8 and 58.2, respectively, suggesting the active uptake of T3 by the cell and cell nucleus. We found that heat shock of the clone 9 cells resulted in decreased nuclear transport of T3 without altering cellular T3 uptake [21]. Since thyroid hormones promote cellular metabolism and energy-consuming reactions, these effects are not preferred when individuals are sick. Inhibition of the nuclear T3 transport after heat shock may result in impairment of T3 action and may be a cellular self-defense mechanism against acute stress. These results indicate the presence of nuclear T3 uptake mechanisms that are independent of cellular uptake of T3. A schematic representation of cellular transport of thyroid hormones is shown in Fig. 3.
Cellular Binding Proteins of Thyroid Hormones

Nuclear thyroid hormone receptors

1) Studies at the protein level
It was reported in 1963 that activation of rat hepatic nuclear RNA polymerase is required for heat generation by thyroid hormone [25], indicating that thyroid hormone action is exerted at the cell nucleus. Oppenheimer et al. [26] discovered high affinity thyroid hormone binding proteins in the cell nucleus of rat liver and kidney in 1972. Studies at the protein level obtained the following findings. The nuclear thyroid hormone receptor is an acidic nonhistone protein with a molecular weight of 47,000–57,000 [27–29], sedimentation coefficient of 3.4–3.8S [27, 29–31], stokes radius of 33–35 Å, and isoelectric points of 5.3–5.9 [32] and a somewhat asymmetrical shape (Fig. 4). The receptor binds tightly to chromatin, DNA [29] and core histones (H4 and possibly H3) [33, 34]. A discrete DNA-binding domain of the receptor protein separable from the globular hormone- and core histone-binding domain was demonstrated by tryp- tic cleavage [33] (Fig. 4). Through interaction with DNA and H1 histone [35], the receptor is distributed mainly in transcriptionally active chromatin [36], making the receptor always accessible to the regulation of gene expression after hormone binding. Besides chromatin, the receptor is associated with a nuclear matrix in cultured rat pituitary cells (GC cells) [37].

The nuclear thyroid hormone receptor is widely distributed among vertebrates [38, 39]. Although the structure of the receptor is well conserved among species [38], some differences are noted [39]. The differences are now proven at the genetic level [40] (Fig. 4). Another striking feature is the presence of multiple receptor forms. This was dem-

![Fig. 3.](image)

A schematic representation of cellular transport of thyroid hormones. T3 and T4 are actively taken up by the carrier proteins. This uptake process is rate-limiting in total cellular uptake, cellular metabolism, and nuclear transport of hormones. An active uptake process is also involved in the nuclear transport of T3 which is inhibited by heat shock or retinoic acid treatment. An alternative nuclear T3 uptake process involves cytosolic thyroid hormone binding protein (CTBP) which inhibits or mediates nuclear uptake of T3 depending upon the NADP/NADPH concentration ratio in the cell.

![Fig. 4.](image)

A first dimensional (panel A) and three dimensional (panel B) domain structure of the nuclear thyroid hormone receptor is shown. Partial trypsin-digestion of the receptor protein separates the DNA-binding domain from the hormone-binding domain. TR: nuclear thyroid hormone receptor.
onstrated at the protein level by differences in hormone binding kinetics [27], different autoregulation by thyroid hormone [36], different molecular size [28] and different isoelectric points [32]. Heterogeneity of the nuclear receptor has also been noted at the genetic level (see below). Post-translational modifications such as phosphorylation of the receptor [41, 42] may also contribute to the heterogeneity at the protein level.

Before cloning of cDNA encoding the nuclear thyroid hormone receptors, the following evidence suggested that they mediate hormone action. 1) Relative binding affinities of iodothyronine analogues for the receptor correspond to the biological potency of the analogues [43]. 2) Nuclear occupancy by T3 is correlated with biological responses [2, 3]. 3) Abundance of the receptor is correlated with the hormone responsiveness of tissues [44]. Although some laboratories have tried to purify the nuclear thyroid hormone receptor, purification has been difficult because of its low abundance and instability [45]. Part of the problem was solved [46], yet the best purification yielded only 5% purity [29]. However, recent recombinant DNA technology enabled us to mass produce and purify the thyroid hormone receptor [47-49].

2) Studies after the cloning of the cDNAs encoding the nuclear thyroid hormone receptors

The cloning of the cDNAs encoding the nuclear thyroid hormone receptor was achieved in 1986 [50, 51] after the recognition of nucleotide sequence homology between the glucocorticoid receptor gene and v-erb A oncogene of the avian erythroblastosis virus [52]. Subsequent cloning of other steroid, retinoic acid receptor, and related genes disclosed that the thyroid hormone receptor is a member of the c-erb A superfamily [53]. Discrete functional domains of the receptor protein, mentioned above, are now mapped specifically at the amino acid level (Fig. 4). The hormone-binding domain is located at the C-terminal of the protein and is not well conserved among different members of the superfamily, whereas the DNA-binding domain is at its near N-terminal and is well conserved. Since the cDNA encoding the thyroid hormone receptor was cloned, studies have been focused on directly proving that the nuclear receptor mediates thyroid hormone action. *In vitro* translated nuclear receptor binds to specific DNA sequences, termed thyroid hormone response elements (TRE), of the target genes [54, 55]. Additional studies, in which the responsiveness to thyroid hormone of eukaryotic cells transfected with a hormone responsive reporter gene is dependent on the cotransfected nuclear receptor gene, verified that the nuclear receptor mediates the thyroid hormone action [55]. Now it is believed that the thyroid hormone receptor regulates the expression of hormone-responsive genes by binding to the TRE. After binding T3, the receptor undergoes alterations in the steric configuration [47, 56, 57], making surface charges in the receptor less hydrophobic, which consequently leads to changes in the rate of transcription of the target genes. Recently it was reported that the thyroid hormone receptor forms dimer on TRE, either with the receptor (homodimer) or with TRAP (T3-receptor auxiliary protein) (heterodimer) [58]. TRAP stabilizes the interaction between the receptor and TRE and is required for full activity of the receptor [59]. It was recently reported that on certain TREs, T3 binding causes dissociation of the homodimer, not of the heterodimer suggesting that the heterodimers are active dimers [58, 60, 61] (Fig. 5). As it was found that the receptor forms a homodimer through a hydrophobic leucine-zipper-like motif [62], it is reasonable to speculate that loss of hydrophobicity after T3 binding abolishes hydrophobic protein-protein interaction of the homodimer. This may result in activation of the receptor and eventually cause the regulation of

![Fig. 5. Ligand dependent dissociation of dimeric binding of thyroid hormone receptor to DNA. On malic enzyme TRE, T3 binding causes dissociation of the TR-homodimer, not of the TRAP-TR-heterodimer. TRAP, T3-receptor auxiliary protein; TR, nuclear thyroid hormone receptor; TRE, thyroid hormone response element.](image)
gene expression by the hormone. The other example in which altered surface charges in the receptor molecule result in the alteration of protein-protein interaction of the receptor was reported by Fondell et al. [63]. They reported that unliganded receptor inhibits formation of a functional transcriptional preinitiation complex, explaining the transcriptional repression of the positively regulated genes by unliganded receptor and the relief of the repression after hormone binding. The effect of the hormone on gene transcription is either stimulatory [4-6] or inhibitory [2, 6]. It is not known, however, how the same hormone causes such diverse effects.

Genes for the human thyroid hormone receptor are present in chromosomes 17 and 3 and have been termed α and β subtypes, respectively [64]. Each gene produces multiple mRNA species and 4 different rat thyroid hormone receptor-related products are found (α1, α2, β1, and β2). The α1, β1, and β2 subtypes bind hormone, whereas the α2 product does not, and even inhibits the action of other subtypes [65], probably by competing with the receptor for binding to the TRE or to transcriptional factors, or by forming inactive heterodimers with active receptor subtypes. The β2 subtype is expressed solely in the pituitary gland [66], whereas the others are expressed in various tissues, and a tissue can express more than one type of receptor [67].

It would be most interesting to clarify functional differences among various receptor subtypes. Both the α1 and β1 receptors are able to bind and trans-activate the same TRE [54] and no convincing functional differences have been elucidated. The amount of mRNA of the β1 subtype correlates better than the α1 subtype to the thyroid hormone-responsiveness of certain tissues [68], suggesting that the β1 subtype may play a central role in thyroid hormone action in the rat. In addition, these receptor subtypes are differentially expressed in chicken during development [40, 69] and are differently regulated by thyroid hormone [68] and other stimuli [70] in the rat. Such differences in the expression of the subtypes suggest that each subtype may play a distinct role. It was also reported that α1 and β1 receptors have different binding affinities for iodothyronine analogues [71]. Distinct functions of the receptor subtypes can be sought through studying patients with generalized resistance to thyroid hormones who have reduced responses of target tissues to thyroid hormones and whose abnormality was speculated in the nuclear receptor at the protein level [72]. Among various affected families, one showing autosomal recessive inheritance has deletion of the β receptor gene in both alleles [73]. In the complete absence of the β subtype, the patients survived, and the maintenance of a high plasma thyroid hormone level compensated for the defect. These findings provide evidence that the β receptor is not essential for survival and that α receptor is functional and even compensates for the absence of the β subtype. They also indicate that both α and β receptors function in vivo and regulate overlapping genes. Further studies are required in order to determine whether these subtypes have different functions.

Cytosolic thyroid hormone binding proteins (CTBP)

It has been long known that thyroid hormone binding protein is present in rat hepatic cytosol [74]. Low binding affinity and high capacity for hormones suggested that it is an intracellular reservoir of hormones. In 1986, Hashizume et al. [75] found that charcoal treatment of rat renal cytosol abolishes the hormone binding activity and the addition of NADPH restores it. The NADPH-dependent CTBP shows specific binding to mitochondria but inhibits T3 binding to isolated nuclei in vitro, which suggests that the NADPH-dependent CTBP may transfer thyroid hormone to mitochondria [76], but not to the nucleus [77]. The same CTBP is activated by NADP in the presence of dithiothreitol, and it is this form that binds to the nucleus in vitro [78]. These findings suggest that the T3-CTBP complexes may regulate the transport of T3 to nuclear receptor, depending on the NADP/NADPH concentration ratio in the cell (Fig. 3). The NADPH-dependent CTBP was purified from rat kidney to homogeneity [79, 80]. Pure CTBP has a stokes radius of 32.5 Å, sedimentation coefficient of 4.7 S, and molecular mass of 58,000 Da. It binds T3 with an affinity constant of 2.4 liter/nmol and a maximal binding capacity of 16,400 pmol T3/mg protein, consistent with the idea that the CTBP has a single binding site for T3. Recently our laboratory identified and purified another novel NADPH-dependent CTBP from rat liver [81]. It has a molecular mass of 76,000 Da with a dimer of 38,000 Da peptide and its affinity constant for T3 is 1 liter/nmol. Two T3 molecules...
bind to a 76,000 Da unit. It differs from the 58,000 Da CTBP in that it is not activated by NADP and dithiothreitol and that it displays different iodotyrosine analogue-binding specificity. This protein exists in various rat tissues including the kidney, suggesting that heterogeneous CTBPs are present in certain tissues. The function of this CTBP is unknown. Kato et al. [82] reported that a monomer of pyruvate kinase can bind T3 with an affinity constant of 0.03 liter/nmol and may function as a CTBP. This protein seems to be different from the NADPH-dependent CTBPs.

Cell membrane-linked thyroid hormone binding proteins

Two functions are proposed for the membrane-linked thyroid hormone binding protein. One is that this protein functions as a mediator of hormone action. Uptake of calcium by rat thymocytes increases within 60 sec after T3 treatment [83], followed by increased production of cAMP [84] and cellular uptake of 2-deoxyglucose and amino acids. The promptness of the response suggests that the hormone action is exerted at the membrane-linked T3-binding sites. Davis et al. [85] reported that T3 regulates calcium-dependent ATPase in red blood cells that have no nuclei or mitochondria, which indicates that T3 acts at the cell membrane.

Another function of the membrane-linked T3-binding sites is active uptake of T3 by the cell, which was described earlier in this review. Cheng et al. [86] reported that, by using rhodamine-labeled T3 and cultured mouse fibroblasts, T3 is found to specifically bind to cell membrane, to cluster upon incubation at 23 or 37 °C, to be taken up by the cell and carried to the nucleus in endocytic vesicles. Inhibition by monodansylcadaverine of the temperature dependent transport of T3 into the GH3 cell nucleus also indicates that endocytosis is involved in the transport of T3 in the cell [24]. Two groups purified the membrane-linked T3-binding protein and isolated their cDNAs [87, 88]. Amino acid homology search revealed that this protein is a protein disulfide-isomerase. Although the exact implication of this fact is not known, T3 suppresses the function of protein disulfide-isomerase [89] and may regulate its own transport.

Mitochondrial thyroid hormone binding protein

High affinity T3 binding sites were identified in the inner membrane of mitochondria in 1975 by Sterling et al. [90]. We confirmed the existence of mitochondrial T3 binding sites [91]. T3 stimulates mitochondrial oxidative phosphorylation within 2 min after its addition in vitro, indicating that T3 acts directly, presumably through mitochondrial binding sites [92]. The binding protein was purified to homogeneity [93] and was quite similar to mitochondrial adenine nucleotide translocase [94]. Since the mitochondrial adenine nucleotide translocase is a major constituent of the organelle and the number of mitochondrial T3 binding sites is very limited, only a few of these molecules may function as thyroid hormone binding proteins. Most of them are unable to bind T3 due to the lipid microenvironment of the inner membrane bilayer. It is interesting if the ADP/ATP translocator is activated so that ATP generated in the mitochondrial matrix through enhanced oxidative phosphorylation by T3 is supplied for energy consuming reactions in cytosol. Probably thyroid hormones have dual regulatory mechanisms on mitochondria. One is a direct action through the mitochondrial receptor [92] and the other is secondary to the effect mediated through the nuclear receptors [95, 96].

References


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enhances binding of retinoic acid, thyroid hormone, and vitamin D receptors to their cognate response elements. *Cell* 77: 1251–1266.


