Thyroid Hormone Regulation of Rat Liver S14 Gene Expression.

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Introduction

Thyroid hormones have diverse effects on a wide range of physiological and biochemical processes such as endocrine function, metabolism, and growth and development (Wolff and Wolff, 1969). The active form of the hormone, triiodothyronine (T₃), mediates changes in cellular function by binding to limited capacity high affinity receptors located in nuclei of target cells (for review see Oppenheimer et al., 1987). In contrast to steroid hormone action, the association of T₃ receptors with chromatin is not dependent on the presence of the hormone (Dillman et al., 1974; Surks et al., 1975). T₃ receptors function to regulate the transcriptional activity of a limited number of genes leading to specific changes in phenotypic expression. For example, T₃ stimulates the transcription of the rat pituitary growth hormone gene (Yaffe and Samuels, 1984), hepatic malic enzyme (Dozin et al., 1986) and HMG-CoA-reductase genes (Simonet and Ness, 1988), while inhibiting the transcription of thyroid-stimulating hormone gene (Shupnik et al., 1985) and the expression of several members of the myosin heavy chain gene family (Izumo et al., 1986).

Recent studies show the cellular erbA proto-oncogene product is related to the thyroid hormone receptor (Sap et al., 1986; Weinberger et al., 1986). This is based on the finding that c-erbA polypeptides expressed by in vitro transcription-translation from cloned cDNA's bind thyroid hormones with the affinity and analog specificity as found for the native T₃ receptor. The molecular weight of the in vitro synthesized proteins, designated c-erb-α (46 kd) and c-erb-β (52 kd) is similar to the T₃ native receptor. Sequence analysis of the c-erbA genes show these proteins are related to steroid receptors (Weinberger et al., 1986; Sap et al., 1986). Since both receptor types function as regulators of gene expression, some investigators have suggested steroid and thyroid hormone receptors are members of a family of nuclear proteins functioning as "ligand-inducible transcription factors" (Evans, 1988). A considerable body of evidence is available to support such a role for steroid receptors (Yamamoto, 1985; Butti and Kuhnel, 1986; Godowski et al., 1988) and recent studies on the T₃ regulation of rat pituitary growth hormone synthesis also support this notion.

Induction of growth hormone gene transcription by T₃ is directly related to receptor occupancy (Yaffe and Samuels, 1984). Both a T₃ receptor binding site (Glass et al., 1987; Evans, 1988) and sequences
essential to T3 regulation of growth hormone transcription are located proximal to the growth hormone gene promoter (-158 to -194 bp) (Flug et al., 1987; Glass et al., 1987; Koenig et al., 1987; Wight et al., 1987, 1988; Ye et al., 1988). Thus, a thyroid hormone-responsive element (TRE) associated with the growth hormone gene appears to function in a fashion similar to the well characterized glucocorticoid-responsive elements (GRE's) associated with the long-terminal repeat of murine mammary tumor virus (Yamamoto, 1985; Butti and Kuhnel, 1986) and the hepatic tyrosine aminotransferase gene (Jantzen et al., 1987).

Despite our current understanding of the structure of thyroid hormone receptors, surprisingly little information is known about how T3 receptors function at the chromatin level to induce changes in gene transcription. Our approach to this problem has focused on the T3-mediated regulation of the transcriptional activity and chromatin structure of the rat liver S14 gene. I will discuss the complex regulation of S14 gene expression by tissue-specific, developmental, hormonal and nutritional factors and provide evidence to suggest T3 initiates changes in S14 gene transcription by inducing a site specific modification of chromatin structure upstream from the S14 gene.

The S14 Model

The S14 protein was first described in studies designed to examine the diversity of T3 effects on hepatic gene expression (Seelig et al., 1981). Hepatic mRNA isolated from hypothyroid, euthyroid, and hyperthyroid animals programmed the synthesis of [35S]methionine-labeled proteins in an in vitro translation system. The labeled proteins were separated by 2-dimensional gel electrophoresis and their distribution detected by autoradiography. The technique provided an indirect measure of the effects of T3 on hepatic gene expression at the pre-translational level. Of the nearly 250 translated products resolved by the two-dimensional gel analysis, 8% were affected by thyroidal status. While several mRNA's were induced in the transition from hypothyroidism to hyperthyroidism, others were repressed, indicating that T3 had both positive and negative effects on hepatic gene expression. One of the translated products induced significantly by increasing plasma T3 levels was "Spot #14" or S14. The mRNA S14 coded for a protein of 17,000 Mr and 4.9 pl. Subsequent studies showed that the T3-mediated induction of mRNA S14 represented one of the earliest responses of the liver to thyroid hormone (Seelig et al., 1982).

These studies suggested that the regulation of mRNA S14 expression may be a good model to examine the rapid effects of T3 on hepatic gene expression. In subsequent studies, Towle and colleagues cloned the S14 cDNA's and gene coding for the S14 protein and found the S14 protein was coded from a single copy gene of 4.4 kb containing two exons (Lia and Towle, 1984). Two polyadenylation signals located in the 3' exon explain the presence of two mRNA S14 of 1.3 and 1.47 kb detected on northern analysis (Lia and Towle, 1984; Narayan et al., 1984; Jump et al., 1984). DNA sequence analysis of the S14 cDNA's showed a single open reading frame in the 5' exon coding for a protein of 17,010 Mr. This value agreed favorably with the size of the in vitro translated product and a T3-responsive protein identified in hepatic cytosol (Jump et al., 1984). Comparison of the S14 DNA and protein sequence with sequences in the national databases, however, failed to reveal significant homologies with known proteins. Although the precise biochemical function the S14 protein is unknown, studies on the tissue distribution of the protein and