Molecular Basis for the Hormonal Regulation of the Tyrosine Aminotransferase and Tryptophan Oxygenase Genes

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INTRODUCTION

A classical example for hormonal control of gene expression is the induction of the enzymes tyrosine aminotransferase (TAT) and tryptophan oxygenase (TO).[1-3] These two enzymes are expressed exclusively in the parenchymal cells of the liver.[4] The expression of both genes is affected by glucocorticoids.[5-10] In nuclear run-on assays it could be shown that hormonal induction involves changes in the relative rates of transcription of these two genes. Apart from glucocorticoid induction, the activity of the TAT gene is also regulated by cAMP.[11-14] The accumulation of TAT mRNA after cAMP stimulation is a consequence of transcriptional activation of the TAT gene.[15] Combined dexamethasone and cAMP treatment leads to higher TAT mRNA concentrations than treatment with either inducer alone, which implies that dexamethasone and cAMP act by distinct mechanisms.[15]

In addition to hormonal regulation, both TAT and TO genes are under developmental control. Whereas TAT enzyme activity appears around birth, TO enzyme activity can be detected only 2 weeks later.[16] The activity of the TAT gene is affected by two distinct genetic loci, which appear to operate in *trans* on the expression of the TAT gene. By genetic and biochemical analysis of several albino lethal mutants of the mouse, a control region required for expression and inducibility of several liver enzymes including TAT has been assigned to the region of the albino locus on chromosome 7 of the mouse.[17] The basal level of TAT mRNA is severely decreased, and the concentration of the mRNA is no longer inducible by glucocorticoids and cAMP, although the structural gene for TAT is still present.[18] Because the mouse TAT structural gene is located on chromosome 8,[19] the effect of this presumptive regulatory locus must operate in *trans*.

Another locus, the so-called tissue-specific extinguisher-1 (*Tse-1*) has been mapped to chromosome 11 of the mouse.[20] When chromosome 11 of a fibroblast cell is

introduced by microcell fusion into a hepatoma cell expressing TAT, TAT gene activity is selectively extinguished, suggesting that a negatively acting factor encoded in chromosome 11 is responsible for this effect.

To study the complex control mechanism operating on these two liver-specific genes at the molecular level, we have isolated the TAT and TO genes from the rat as well as the TAT gene of the mouse.[7,9,10,19] The availability of these DNA clones have allowed us to determine the level at which hormonal regulation of expression of these two genes occurs and, furthermore, allowed us to identify those sequences that are required for hormonal induction in *cis*. We demonstrate here that these sites represent binding sites for the glucocorticoid receptor.

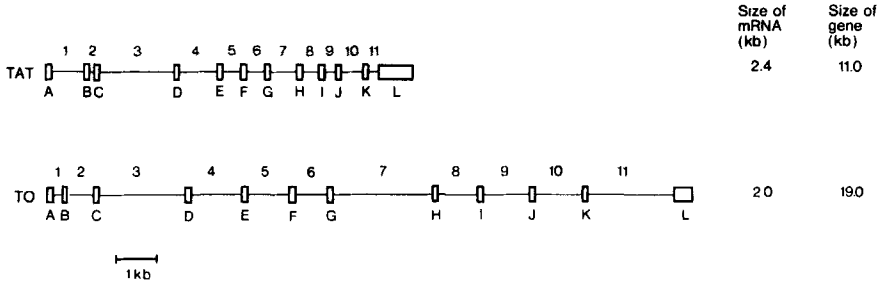


FIGURE 1. Exon-intron structure of the rat TAT and TO genes. Open boxes represent the exons; connecting lines represent the intervening sequences, and are drawn to scale. The size of the mRNA and the size of the gene are indicated in kb pairs.

RESULTS

To allow analysis at the molecular level of the sequences important for the hormonal regulation of expression of the TO and TAT genes, we have cloned cDNA sequences representing these two mRNAs[9,10] as well as genomic DNA containing these two genes.[7,19] The exon-intron organization and the start site of transcription of these two genes was determined by electron microscopic analysis of heteroduplexes of mRNA with the cloned genomic DNA, as well as by S1 nuclease mapping.[7,10] FIGURE 1 shows the exon-intron organization of these two genes. The coding portion of these genes is interrupted by multiple intervening sequences; thus the genes are much longer than the respective mRNAs. In order to allow studies of the molecular effects of the albino deletion mutations, we have isolated and characterized the mouse TAT gene and have shown that the rat and mouse TAT genes are very similar in exon-intron structure. Sequence comparison around the 5′ end of the rat and mouse genes shows extensive homology over the entire sequence (approximately 1 kb), indicating the importance of these sequences for the regulation of these two genes.[19]

In order to obtain clues to possible regulatory sequences, we have analyzed the chromatin structure in the vicinity of the 5′ end of the TO and TAT genes with regard to DNase I hypersensitivity. Hypersensitive sites frequently map near the 5′ end of actively transcribed genes. Their presence has been correlated in many systems with the state of expression. We have found DNase I hypersensitive sites at each of the two promoters in liver cells of the rat[22] and mouse (FIG. 2). DNase I hypersensitive

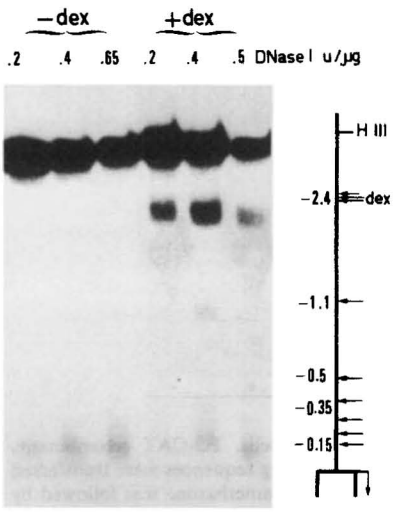


FIGURE 2. A glucocorticoid-inducible DNase I hypersensitive site far upstream of the mouse TAT gene. Nuclei from hormone-induced (+ dex) and uninduced (− dex) mouse livers were treated with increasing amounts of DNase I. After purification, the DNAs were cut with the restriction enzyme abutting with the probe used, separated on a 1% agarose gel, and blotted to nitrocellulose filters. The end-labeling technique was used for visualizing the preferential DNase I cleavage sites. On the right part of the figure these hypersensitive sites are aligned with the 5′ flanking region of the TAT gene.

sites of both genes are absent in kidney nuclei and therefore appear to be specific for the tissue expressing the genes.[22] Most remarkable is the alteration in chromatin structure following glucocorticoid induction (FIG. 2). A strong DNase I hypersensitive site is detected about 2.4 kb upstream of the start site of transcription of the mouse TAT gene. This dexamethasone-dependent hypersensitive site suggested to us the possibility that glucocorticoid response elements might be located far upstream of the start site of transcription.

To identify sequences important for the hormonal control of these two genes by glucocorticoids, fusion genes were constructed containing the 5′ flanking DNA of these genes upstream of a suitable indicator gene. We used either the bacterial gene for neomycin resistance (neo) or the gene for the enzyme chloramphenicol transacetylase (CAT). A typical hybrid gene is shown in FIGURE 3. Because chromatin studies have suggested that the control sequence might be remote from the cap-site[22] (FIG. 2), 2.9 kb of 5′ flanking DNA was included in the construction of this hybrid gene. In the case of the TO promoter, a CAT fusion gene containing 1.9 kb of 5′ flanking DNA was constructed. These fusion genes were introduced into hepatoma cells and fibroblasts, and the effect of steroid administration on expression of these genes was analyzed by CAT enzyme activity measurements or analysis of the RNA with RNA filter hybridization experiments and/or with S1 nuclease analysis. Both

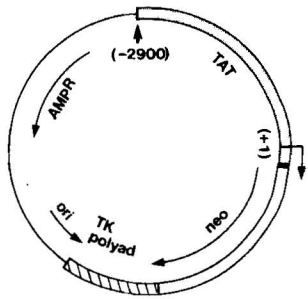


FIGURE 3. Structure of pTATneo, a fusion gene of the TAT 5′ flanking DNA and the bacterial neomycin resistance gene.

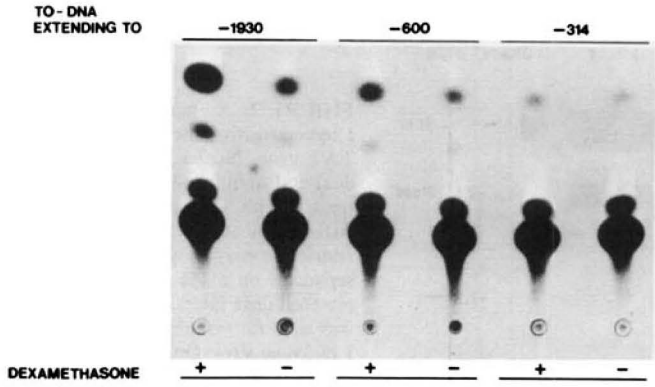
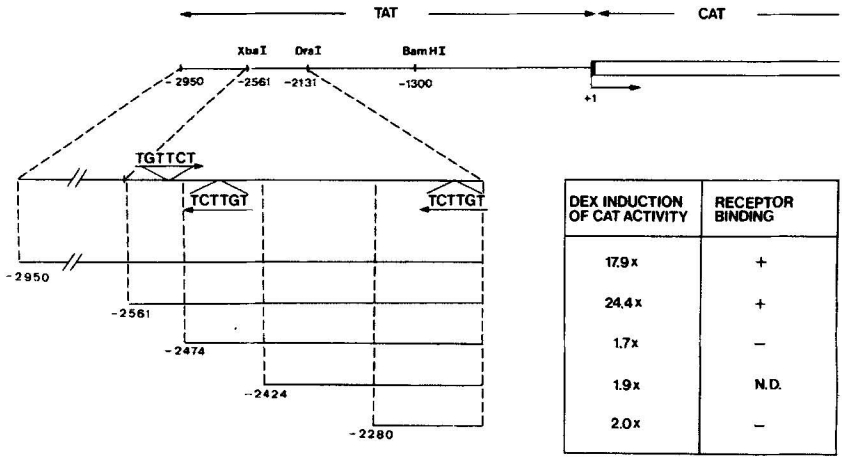


FIGURE 4. Expression of TO-CAT recombinants in mouse L cells. TO-CAT recombinants containing approximately 1930 bp, 600 bp, and 340 bp of 5′ flanking sequences were transferred into mouse L cells. Expression in the presence and absence of dexamethasone was followed by measuring CAT activity in extracts of the cells 48 hr after transfection.

genes, when introduced into heterologous or homologous cells, are expressed, and the expression is regulated by glucocorticoids (data not shown). In order to delineate the important regulatory elements in more detail, deletion mutants of these parental plasmids were constructed and tested. A typical experiment is shown in FIGURE 4 for the TO-CAT recombinants expressed in mouse L cells. It is seen that as long as 1930 nucleotides remain of 5′ flanking sequences of the TO fusion gene, strong in-



DEX INDUCTION OF CAT ACTIVITY	RECEPTOR BINDING
17.9x	+
24.4x	+
1.7x	–
1.9x	N.D.
2.0x	–

FIGURE 5. A remote DNA sequence confers glucocorticoid responsiveness and receptor binding. The upper part of the figure shows the 5′ flanking part of the TAT gene fused onto the CAT gene. Deletion derivatives as indicated by the deletion end points were tested for dexamethasone inducibility of expression of the CAT recombinants in L cells 12 hr after hormone treatment as well as for receptor binding. The approximate position of the hexanucleotide (TGTTCT) indicative of glucocorticoid receptor binding sites is shown.

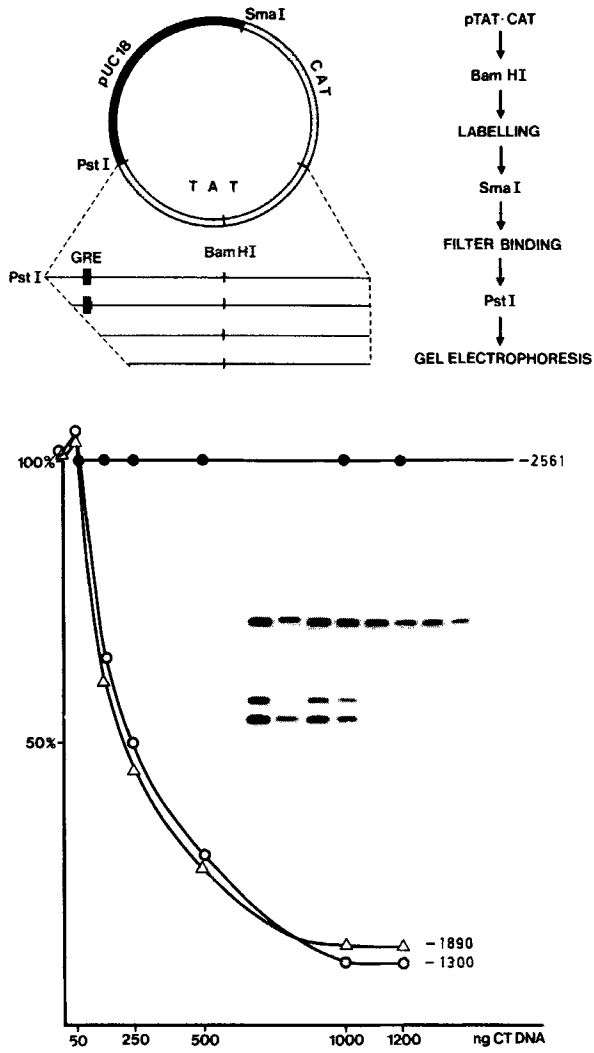


FIGURE 6. Binding of the glucocorticoid receptor to 5′ deletions of the TAT-CAT recombinant. The upper part of the figure shows the method employed to determine binding of the purified glucocorticoid receptor to 5′ deletion derivatives of the TAT-CAT plasmids. The lower part of the figure shows the results of a filter binding experiment with three plasmids containing 5′ flanking TAT DNA as indicated in the presence of increasing amounts of competing calf thymus DNA (CT DNA). The inset shows the autoradiogram of the fragments preferentially retained on nitrocellulose filters. The top set of fragments represents the deletion ending at -2561; the middle set, the deletion ending at -1890; and the lower set, the deletion ending at -1300. For quantitation the bands were cut out and counted. The data are plotted as percentages of binding of the deletion ending at -2561.

duction of expression by glucocorticoids can be achieved. Deletion of 5′ flanking sequences up to about -500 resulted in considerable loss of inducibility, even though some induction of the TO-CAT recombinant can still be observed. Further deletion to -314 abolished all inducibility. These findings suggest the presence of two hormone-responsive elements in the 5′ flanking region of the TO gene. These results were further confirmed by more finely spaced deletion mutants.

A similar series of experiments was conducted with the TAT fusion genes, which were introduced in rat hepatoma cells and mouse fibroblasts. In mouse L cells the TAT 5′ flanking sequence of 2.95 kb conferred strong inducibility of expression. Expression studies of 5′ deletion derivatives of the parental TAT-CAT recombinant

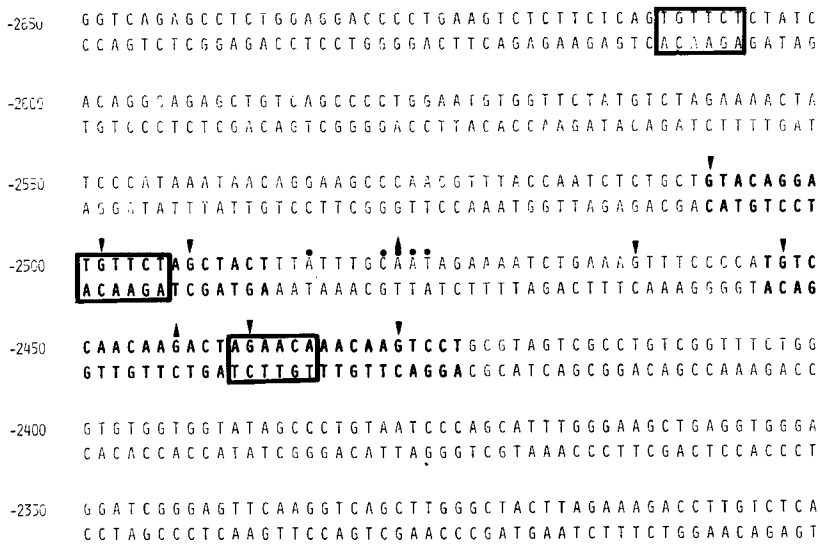


FIGURE 7. Sequence of the far 5′ flanking DNA of the TAT gene. The DNA sequence between -2650 and -2200 of the TAT gene is presented. The three hexanucleotides TGTTCT characteristic of receptor binding sites are shown in boxes. The regions protected from DNase I digestion after binding of the glucocorticoid receptor *in vitro* are shown in boldface type. DNase I hypersensitive regions are shown with dots. Protected and enhanced guanine residues as evidenced from dimethyl sulfate protection experiments are shown with upward- and downward-pointing arrows, respectively. The DNase I and dimethyl sulfate protection experiments have been performed with the upper strand only.

defined important control elements in the region that showed a local alteration in chromatin structure in the previous DNase I hypersensitivity studies (FIG. 5). Inspection of the DNA sequence within this region revealed sequences homologous to the glucocorticoid receptor binding sites of the mouse mammary tumor virus,[23,24] methallothionin,[25] and lysozyme[26,27] genes.

To determine whether this control element located so far upstream of the initiation site of transcription functions as a glucocorticoid receptor binding site, filter binding studies with the purified glucocorticoid receptor and the 5′ deletion mutants were performed. Binding of the glucocorticoid receptor to TAT 5′ deletion derivatives was investigated as shown in the upper panel of FIGURE 6. The lower panel shows that

the purified receptor interacts preferentially with the deletion mutant that contains the control sequence identified *in vivo*, but not with derivatives ending at -1890 or -1300. The DNA filter binding studies were complemented by DNase I footprint analyses as well as by dimethyl sulfate protection experiments (data not shown). Two footprinting regions could be identified in the far upstream sequence of the TAT gene using a purified glucocorticoid receptor. These protected regions are indicated by the shaded sequence in FIGURE 7. Dimethyl sulfate protection experiments indicate contact sites of the receptor. Protections and enhancements indicated by the arrows in FIGURE 7 are seen in the sequences defined by the DNase I footprints. These nuclease protection experiments identify partially homologous receptor binding sites located in the region that confer glucocorticoid responsiveness *in vivo*.

CONCLUSIONS

Induction of enzyme activity for TO and TAT, two gluconeogenic enzymes, results from increased expression of the respective genes at the transcriptional level. The effect of the hormone on transcription is rapid and does not require new protein synthesis, suggesting a direct effect of the hormone receptor complex on the activity of the gene. In order to identify sequences important for regulation, fusion genes containing the presumptive control sequences were constructed and their expression studied under the influence of steroid hormones. This has allowed identification of two control sequences in the case of the TO gene located around -420 and around -1190 and control elements located at about -2500 bp upstream of the TAT initiation site. The responsible sequences show strong sequence homology to each other as well as to previously characterized glucocorticoid control elements.[23-27] What role these hormonal control elements play in the course of developmental activation of these genes will now be tested by analysis of expression of these genes in transgenic animals.

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