Rapid Induction of Rat Liver S14 Gene Transcription by Thyroid Hormone

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(Received for publication, May 16, 1988)

The mRNA coding for the rat liver S14 protein (Mr 17,000; pl 4.9) is rapidly induced by triiodothyronine (T₃) (Jump, D. B., Narayan, P., Towle, H. C., and Oppenheimer, J. H. (1984) J. Biol. Chem. 259, 2789–2797). In an effort to define the molecular basis for the rapid increase in mRNA₃₄, the in vitro run-on activity and chromatin structure of the S14 gene was examined. Following injection of hypothyroid rats with a receptor-saturating dose of T₃, a 5-min lag period preceded a rapid increase in S14 gene transcription. S14 transcriptional activity was induced 3.8-fold within 15 min and reached nearly 70% of the maximal 9-fold induction within 60 min of T₃ administration. Hepatic mRNA₃₄ levels were induced 2.4-, 19-, and 24-fold within 15 min, 4 h, and 24 h, respectively. Thus, the rapid T₃-mediated induction of mRNA₃₄ was due, in large part, to a rapid and apparent direct effect of T₃ on S14 transcriptional activity. Analysis of S14 chromatin structure showed that the Hss-3 DNase I hypersensitive site located 3.3 kilobases (kb) upstream from the hepatic S14 cap site was present in both euthyroid and hyperthyroid states, but either absent or present at diminished DNase I sensitivity in the hypothyroid state. However, within 5 min of T₃ administration to hypothyroid rats, the Hss-3 DNase I hypersensitive site was significantly induced. The induction of this structure preceded T₃ induction of S14 gene transcription. Other DNase I hypersensitive sites located adjacent to the S14 cap site at -65 to -265 base pairs (Hss-1) or upstream at -1.3 kb (Hss-2), -2.1 kb (Hss-3'), -5.3 kb (Hss-4), and -6.2 kb (Hss-5) remained unaffected by changes in S14 gene transcription. The rapid T₃ effect on the Hss-3 DNase I hypersensitive site may reflect the presence of T₃ receptors in the vicinity of this chromatin locus. Modification of chromatin structure in the vicinity of the Hss-3 site may be an important antecedent event for T₃-mediated induction of S14 gene transcription.

The mRNA coding for the rat liver S14 protein (S14: Mr 17,000, pl 4.9) provides an attractive model to study tissue-specific, developmental, hormonal, and nutritional regulation of hepatic gene expression at the pretranslational level (1-7). Previous studies on chromatin structure have shown the presence of specific DNase I hypersensitive sites flanking the 5' end of the S14 gene correlate with S14 gene expression (6, 7). Six major DNase I hypersensitive sites flank the 5' end of the transcriptionally active hepatic S14 gene. Four of the 6 sites are tissue-specific and are found only in tissues expressing mRNA₃₄ (6). Three of the tissue-specific sites are regulated during post-natal development (7). One DNase I hypersensitive site located adjacent to the S14 cap site (Hss-1, -65 to -265 bp) forms prior to initiation of gene transcription while a second site located 3.3 kb upstream from the S14 cap site (Hss-3) forms at weaning when S14 gene transcription increases ≥40-fold. A third site (Hss-3', at -2.1 kb) is present only in the adult animal. The regulation of the Hss-1 and Hss-3 DNase I hypersensitive sites by tissue-specific factors and during post-natal development suggest these structures may play a physiological role in the control of S14 gene transcription (7).

The presence of DNase I hypersensitive sites within chromatin reflects a local disruption of nucleosomal periodicity due to the interaction of DNA-binding proteins (trans-acting) with underlying DNA sequences (for review see Ref. 8). These structures are typically found flanking the 5' end of most active genes and are often associated with promoter, enhancer, and silencer elements (8). The recent description of hormonally inducible hypersensitive sites within the long-terminal repeat of murine mammary tumor virus (9) or upstream from the rat hepatic tyrosine aminotransferase gene (10) suggests such modified chromatin structures play a major role in regulating gene transcription.

In an effort to determine whether the DNase I hypersensitive sites associated with the S14 gene are subject to regulation in the adult animal, the triiodothyronine (T₃) regulation of S14 gene expression was examined. The induction of hepatic mRNA₃₄ and its nuclear precursor within minutes of T₃ administration to hypothyroid rats suggests T₃ has rapid effects on S14 gene transcription and S14 chromatin structure (1, 11). However, nuclear run-on studies failed to demonstrate significant effects of T₃ on S14 gene transcription (12, 13). The T₃ regulation of S14 gene transcription was reexamined in this study using an in vitro transcriptional run-on assay developed previously in this laboratory to examine the transcriptional activation of the S14 gene during post-natal development (7). Accordingly, T₃-mediated changes in mRNA₃₄ were correlated with changes in nuclear run-on activity and S14 chromatin structure. T₃ induced hepatic S14 gene transcription and a specific 5'-flanking DNase I hypersensitive site within minutes of hormone administration to hypothyroid rats. Kinetic analyses suggest that the changes in chromatin

* This work was supported by National Institutes of General Medical Sciences Grant GM36851 and by National Institutes of Health Biomedical Research Support Grant 2-S07 RR07049-15. The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked "advertisement" in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

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structure may play a causal role in the T₃-mediated induction of S14 gene transcription.

**MATERIALS AND METHODS**

**Animals**—Euthyroid Sprague-Dawley male rats were obtained from Sasco/King Animal Laboratories (Oregon, Wisconsin). Rats were made hypothyroid by daily injections of T₃ (15 μg/100 g body weight) for 5 days. All animals used in this study ranged in age from 2 to 4 months and were maintained on rat chow (Teklad ad libitum). Animals were killed between 0800 and 1200 h by ether anesthesia and exsanguination.

**DNA Probes**—The cDNA and genomic probes for the S14 mRNA and S14 gene have been described previously (7, 15). A genomic probe containing the preproinsulin II gene (pRCII) was obtained from P. Lomedico, LaRoche Institute, Nutley, NJ (16). A cDNA probe for the rat tyrosine aminotransferase (pTAT) was obtained from G. Scherer, Biochemistry Department, Case Western Reserve University, Cleveland, OH (18). All plasmids were isolated by a modification of the SDS-NaOH method (19). Plasmid DNA was RNAse-treated (20 μg/ml for 30 min at 37°C) followed by extraction with phenol:chloroform (1:1) and with chloroform:isoamyl alcohol (49:1). Plasmid DNA was precipitated with ethanol, resuspended in TE-8 (10 mM Tris-C1, pH 8.0; 1 mM EDTA), adjusted to 0.5 M NaCl and 6.5% polyethylene glycol, and kept on ice for 60 min. Plasmid DNA was recovered by centrifugation at 10,000 × g, 10 min at 4°C. DNA pellets were washed with 70% ethanol, dried, resuspended in TE-8, and stored at 4°C.

**Isolation of Rat Liver Nuclei**—Nuclei suitable for gene transcription and chromatin studies were isolated from fresh liver as described (6, 7).

**In Vitro Nuclear "Run-On" Assay for S14 Gene Activity**—The in vitro nuclear transcription run-on assay used in this study (7) is a modification of previously described assays (20, 21). Briefly, nuclei in Nuclear Storage Buffer (40% glycerol; 75 mM HEPES, pH 7.5; 60 mM KCl; 15 mM NaCl; 0.15 mM spermidine; 0.5 mM spermine; 0.5 mM dithiothreitol; 0.1 mM EDTA; 0.1 mM EGTA) were added directly to a transcription reaction in which the final reactant concentrations were: 250 μg/ml HEPES, pH 7.5; 25 mM MgCl₂; 0.1 M KCl; 0.1 mM EDTA; 0.1 mM EGTA; 1.0 mM spermidine; 0.5 mM spermine; 1.0 mM dithiothreitol; 16 μg/ml creatine kinase; 100 μg/ml creatine phosphate; 1 mM ATP; 0.5 mM CTP; 0.5 mM GTP; 0.5 μM UTP, 100 μCi of [³²P]dCTP, 120 units/ml RNAse; 10 A₂₆₀/ml of nuclei in a final volume of 0.5 ml. Transcription reactions were incubated at 37°C for 10 min, followed by a 5-min incubation with 25 units of RNase A (RNAse-free: RQ-1, Promega Biotec, Inc.), 10 μg of yeast tRNA. The reactions were subsequently adjusted to 20 mM EDTA, 0.1 mM UTP, 20 mM GTP, 0.2% SDS, and 100 μg/ml proteinase K, and incubated for 2 h at 65°C to remove protein. The reactions were extracted with phenol:chloroform (1:1), followed by extraction with chloroform:isoamyl alcohol (49:1), and the nucleic acid was precipitated with ethanol. Labeled RNA was resuspended in 100 μl of sterile water, adjusted to 1 x transcription buffer (75 mM HEPES, pH 7.5; 3 mM MgCl₂; 0.1 M KC1; 0.1 mM EDTA; 0.3 mM MgCl₂; 1.0 mM EDTA; 0.5 mM spermidine; 0.5 mM spermine; 25% glycerol) containing 1 mM MgCl₂, 10 mg/ml bovine serum albumin, 25 μM GTP, 25 μM ATP, 50 μCi of [³²P]dCTP (3000 Ci/mmol), and 1 units of DNA polymerase I (Klenow fragment) and incubated for 10 min at 37°C. Following RNase I digestion, the reaction was adjusted to 20 mM EDTA, 20 mM MgCl₂, and the RNase I was heat-inactivated (10 min; 65°C). The labeled RNA was precipitated by adjusting the solution to 2 μl NH₄-acetate and adding 2 volumes of isopropanol, washed, and resuspended in 50 μl of TE-8 and precipitated and washed with 2 μl of NH₄-acetate and isopropanol alcohol as before. This step was repeated three additional times. The labeled RNA was resuspended in 50 μl of TE-8 and used immediately for hybridization. Trichloroacetic acid-insoluble 32P-labeled RNA was quantified by β-scintillation counting. The precipitation scheme described above removes unincorporated labeled [³²P]dUTP.

**32P-Labeled DNA was hybridized to plasmid DNAs affixed to Zetabind. Briefly, plasmid DNAs were linearized by EcoRI digestion, adjusted to 0.4 mM NaOH, 0.6 mM NaCl, 1.0 mM EDTA, heated to 65°C for 10 min, and applied directly to Zetabind (500 ng/well) using a Mini-fold (22) and Schleicher and Schuell, Inc). Zetabind membranes used for gene recognition contain samples of Zetabind plasmids. Three plasmids for the S14 gene were used containing sequences homologous to the 5' exon (pS14EXOPEII-8), the intron (pS14-IVS), and the 3' exon (pS14-C1). Three plasmids used as controls for DNA sequence and tissue specificity of the transcriptional region were phosphoenolpyruvate carboxykinase (pPCK-10), glyceraldehyde-3-phosphate dehydrogenase (pGAPDH), and preproinsulin (pRCII). The conditions for hybridization were as described previously (7). 32P-Labeled RNA concentration was 3.5 × 10⁶ cpm/ml in the hybridization solution. Levels of hybridization are quantified by β-scintillation counting.

**Hypersensitive Site Analysis of S14 Chromatin Structure**—The present studies used frozen nuclei derived from fresh tissue. S14 hypersensitive sites were examined as described previously (7). Following electrophoretic separation of EcoRI-digested DNA, DNA was depurinated, denatured, and transferred directly to Zetabind using 0.4 mM NaOH or 0.4 mM NaOH, 0.6 mM NaCl (23). After transfer, blots were washed with 2 x SSC (1 x SSC: 0.15 M NaCl, 15 mM sodium citrate, pH 7.0) and dried. Prior to prehybridization, blots were washed in 0.1 x SSC, 0.5 x SSC at 65°C for 1 h, followed by washing in 0.25 x Tris-CI, pH 7.5, 0.5 x SSC, 1.0 x SSC at 45°C for 30 min. This washing procedure reduces background on the Southern blots. Blots were transferred to "seal-a-meal" bags, prehybridized and hybridized to the labeled probes as described above. The Southern blots were exposed to AR-5 α-ray film for 5 days using Du Pont Cronex intensifying screens at -80°C. Southern blots were scanned using an LKB ultrascanning XL laser densitometer.

**RESULTS**

**Hepatic S14 Gene Expression in Three Thyroidal Steady States**—Although T₃ has major effects on hepatic mRNA₄ levels, the extent of T₃ induction of S14 gene transcription remains unclear (12, 13). Accordingly, established methods were used to measure hepatic mRNA₄ levels (1, 2) and S14 gene transcription rates (7) in three thyroidal steady states. Total RNA was isolated from livers of hypothyroid, euthyroid, and hyperthyroid rats (Table I). Dot blot hybridization analysis showed that hepatic mRNA₄ levels were 4.1- and 20.5-fold higher in the euthyroid and hyperthyroid rats, respectively, when compared to hypothyroid values. The relative amount of mRNA₄ expression found in each of the three steady states was similar to previously reported values (1).

The S14 run-on assay used three distinct probes homologous to different regions of the S14 gene: the 5' exon (pS14EXOPEII-8), a nonreiterated region of the intervening sequence (pS14-IVS), and the 3' exon (pS14-C1). In the transition from hypo- to hyperthyroidism, increased levels of hybridization to each S14 probe reflected increased transcription.
Thyroid Hormone Rapidly Induces S14 Gene Activity and Accumulation of Hepatic mRNA{sub}S14{end}.—The rapid T{sub}3-mediated induction of hepatic mRNA{sub}S14{end} in the hypothyroid liver has been described (1). Having established that thyroidal status regulates S14 gene transcription (Table I), efforts were directed at determining whether T{sub}3{end} had rapid effects on S14 gene transcription in the hypothyroid rat liver. Accordingly, male hypothyroid rats were injected with T{sub}3{end} (200 {g}/100 g body weight, intravenously) and killed at various times after T{sub}3{end} injection. This dose of T{sub}3{end} is sufficient to saturate hepatic receptors in male hypothyroid rats (200 {g}/100 g body weight, intravenously) and killed at various times after T{sub}3{end} injection. This dose of T{sub}3{end} is sufficient to saturate hepatic receptors in male hypothyroid rats (250 g). The standard contains 4.8 x 10{sup}8{end} copies of mRNA{sub}S14{end}/total RNA or 1200 copies of mRNA{sub}S14{end}/liver cell (7). The data are expressed as the mean ± S.E. per {mu}g of total RNA. Values in parentheses represent the number of determinations.

The marginal change in S14 gene transcription in the eu- to hypothyroid transition alone cannot account for the 9-fold increase in hepatic mRNA{sub}S14{end}. This result suggests that T{sub}3{end} acts directly at the chromatin level. Accordingly, the effects of T{sub}3{end} on the S14 chromatin domain were examined by determining whether T{sub}3{end} regulated the structure of the DNase I hypersensitive sites flanking the 5′ end of the S14 gene. The approach to examine the S14 DNase I hypersensitive sites has been described (7). The gene map in Fig. 2 represents only the 5′ end of the S14 gene. The EcoRI site in the intron is 1.7 kb from the 5′ end of the S14 gene (15), whereas the distal EcoRI site is 11.3 kb upstream from the S14 cap site. Six major DNase I hypersensitive sites flank the 5′ end of the S14 gene in adult rat liver. Other minor sites (not labeled) seen in Fig. 2 are not detected consistently and will not be discussed. No hypersensitive sites are found either within or flanking the 3′ end of the gene (6). No hypersensitive sites are detected when isolated liver DNA is digested with DNase I and subsequently digested with EcoRI, indicating that the organization of the DNA in chromatin rather than the DNA sequences alone confer preferential DNase I sensitivity (6). The location of the 6 DNase I hypersensitive sites relative to the 5′ end of the S14 gene is: Hss-1 at -65 to -265 bp; Hss-2 at -1.3 kb; Hss-3′ at -2.1, Hss-3 at -3.3 kb; Hss-4 at -5.3 and Hss-5 at -6.2 kb (7).

Despite a 9-fold increase in S14 gene activity in the transition from the hypo- to the hyperthyroid steady state (Table I), the chromatin structure of the S14 domain remained remarkably similar at several locations upstream from the S14 gene (Fig. 2). DNase I hypersensitivity at Hss-1, Hss-2,

### Table I

<table>
<thead>
<tr>
<th>Thyroidal steady state</th>
<th>Relative mRNA{sub}S14{end} expression</th>
<th>Relative S14 gene transcriptional activity</th>
</tr>
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<tbody>
<tr>
<td>Hypothyroid</td>
<td>0.22 ± 0.05 (8)</td>
<td>5.3 ± 0.9 (8)</td>
</tr>
<tr>
<td>Euthyroid</td>
<td>0.9 ± 0.06 (5)</td>
<td>33.4 ± 2.5 (5)</td>
</tr>
<tr>
<td>Hyperthyroid</td>
<td>4.5 ± 0.21 (4)</td>
<td>43.8 ± 2.9 (4)</td>
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*a The results are expressed relative to an internal hybridization standard set equal to 1.00 as described previously (2). The internal hybridization standard was derived from the liver of an intact male rat (250 g). The standard contains 4.8 x 10{sup}8{end} copies of mRNA{sub}S14{end}/total RNA or 1200 copies of mRNA{sub}S14{end}/liver cell (7). The data are expressed as the mean ± S.E. per {mu}g of total RNA. Values in parentheses represent the number of determinations.

*b Hybridization was quantified by {beta}-scintillation counting of individual samples corresponding to the three S14 probes ("Materials and Methods"). Background (nonspecific hybridization) was subtracted from each value. The results reported represent the sum of the specific hybridization to the three probes divided by the total input 32P-labeled RNA (3.5 x 10{sup}6{end} cpm). Data are expressed as mean ± S.E. The values in parenthesis represent the number of separate determinations. The percent hybridization of the in vitro synthesized RNA{sub}S14{end} was determined by preparing a single-stranded sense [3H]RNA(S14) synthesized from the pS14-T1-0.9 plasmid using SP6 RNA polymerase. The [3H]RNA{sub}S14{end} was included in the hybridization reaction as the [3H]RNA{sub}S14{end} was hybridized. Following 72 h of hybridization, 50% of the [3H]RNA{sub}S14{end} was hybridized. The results in the table are not corrected for probe length or fractional hybridization.

**p < 0.05, hypothyroid versus euthyroid and hyperthyroid.

60 min. From 1 to 24 h, the rate of increase in S14 run-on activity was slower going from 6.8-fold to a maximal 9-fold induction. Hepatic mRNA{sub}S14{end} increased in a linear fashion during the first 4 h, reaching levels 19.4-fold over basal hypothryroid values. After 24 h of T{sub}3{end} treatment, mRNA{sub}S14{end} was increased 24-fold, whereas S14 run-on activity was augmented 9-fold. Whereas the rapid T{sub}3-mediated increase in hepatic mRNA{sub}S14{end} was due, in large part, to the induction of S14 gene transcription, T{sub}3{end} also induced post-transcriptional processes which contributed to the rise in mRNA{sub}S14{end}.

Chromatin Structure of the S14 Gene in Three Thyroidal Steady States—Establishing that T{sub}3{end} induced a major increase in S14 gene transcription within minutes of hormone administration suggested that T{sub}3{end} acted directly at the chromatin level. Accordingly, the effects of T{sub}3{end} on the S14 chromatin domain were examined by determining whether T{sub}3{end} regulated the structure of the DNase I hypersensitive sites flanking the 5′ end of the S14 gene. The approach to examine the S14 DNase I hypersensitive sites has been described (7). The gene map in Fig. 2 represents only the 5′ end of the S14 gene. The EcoRI site in the intron is 1.7 kb from the 5′ end of the S14 gene (15), whereas the distal EcoRI site is 11.3 kb upstream from the S14 cap site. Six major DNase I hypersensitive sites flank the 5′ end of the S14 gene in adult rat liver. Other minor sites (not labeled) seen in Fig. 2 are not detected consistently and will not be discussed. No hypersensitive sites are found either within or flanking the 3′ end of the gene (6). No hypersensitive sites are detected when isolated liver DNA is digested with DNase I and subsequently digested with EcoRI, indicating that the organization of the DNA in chromatin rather than the DNA sequences alone confer preferential DNase I sensitivity (6). The location of the 6 DNase I hypersensitive sites relative to the 5′ end of the S14 gene is: Hss-1 at -65 to -265 bp; Hss-2 at -1.3 kb; Hss-3′ at -2.1, Hss-3 at -3.3 kb; Hss-4 at -5.3 and Hss-5 at -6.2 kb (7).
FIG. 2. DNase I hypersensitive sites upstream from the S14 gene in three thyroidal steady states. Hepatic nuclei isolated from hypo-, eu-, and hyperthyroid rats were digested with DNase I and extracted for DNA as described under “Materials and Methods.” Isolated DNA (25 μg) was digested with EcoRI and electrophoretically separated in 1% agarose-Tris-borate-EDTA gels. The DNA was deparaffinized, denatured, and transferred to Zetabind. Blots were hybridized with the insert derived from pS14 EXOEPII-6. The insert represents S14 DNA from -16 to +483 bp in the 5’ exon (see map). Following hybridization and washing, blots were exposed to AR-5 film for 72 h. Lanes A–D represent 0, 0.2, 0.5, and 1.0 units DNase I/minute. DNA standards (λ-DNA digested with HindIII and φX-174 DNA digested with HaeIII) are co-electrophoresed and detected using nicked translated λ-DNA and φX-174 DNA. The location of the standards and their size are noted in the figure. The DNase I hypersensitive sites are labeled HSS-1 (1.8 kb), HSS-2 (3.0 kb), HSS-3’ (3.8 kb), HSS-3 (5.0 kb), HSS-4 (7.0 kb), and HSS-5 (7.9 kb). Gene map, the thin vertical line represents the 19-kb EcoRI genomic fragment. The location of each hypersensitive site relative to the 5’ end of the S14 gene (enclosed box) is denoted by brackets. The arrow marks the location of the cap site and the direction of transcription. The sequence homology between the insert from the pS14EXOEPII-6 probe and the S14 coding sequences is indicated by the cross-hatched box. This blot is representative of four separate studies.

Hss-4, and Hss-5 was not consistently influenced by changing thyroidal status or a 9-fold change in S14 gene transcription. In contrast, two DNase I hypersensitive sites located upstream from the S14 gene were affected by changing thyroidal status and elevated S14 gene transcription rates: Hss-3’ located at -2.1 kb and Hss-3 located at -3.3 kb. Whereas both the DNase I hypersensitive sites were absent in the hypothyroid nucleus, each site showed increased DNase I sensitivity in the euthyroid and hyperthyroid nucleus. The increased DNase I sensitivity at these locations was observed consistently and correlated with the increased S14 gene transcription in the transition from hypo- to hyperthyroidism. Thus, changing thyroidal status induced 2 DNase I hypersensitive sites upstream from the S14 gene.

**T₃ Rapidly Induces a DNase I Hypersensitive Site Upstream from the S14 Gene**—In order to determine whether Hss-3 and Hss-3’ are rapidly induced by T₃, hepatic S14 chromatin structure was examined in hypothyroid animals and hypothyroid animals receiving a receptor-saturating dose of T₃ for 5, 15, and 120 min. Only the Hss-3 site was induced within minutes of hormone administration, suggesting that the presence of the Hss-3’ in hyperthyroid liver nuclei was due to long term effects of T₃ on S14 chromatin structure (Fig. 3).

The induction of Hss-3 DNase I hypersensitive site in response to T₃ administration to hypothyroid rats was quantified using a laser densitometer (Fig. 4). Southern blots were scanned to obtain integrated peak areas for Hss-1, Hss-2, and Hss-3 in order to calculate peak area ratios of Hss-3 to Hss-2 and Hss-1 to Hss-2. Since the DNase I sensitivity of Hss-2 was not influenced by thyroidal status, this site served as an internal reference point for generalized DNase I sensitivity of the S14 domain. A measure of the extent of increase in DNase I sensitivity at a specific site was obtained by comparing the DNase I sensitivity of Hss-3 to Hss-2 or Hss-1 to Hss-2, i.e., calculating peak area ratios. The legend to Fig. 4 identifies the lanes scanned. The inset of Fig. 4 shows that the DNase I sensitivity of Hss-1 site relative to the Hss-2 site was not significantly influenced by T₃ administration. Thus, T₃ does not have an effect on the structure of the Hss-1 site (-65 to -265 bp). However, comparison of the DNase I sensitivity of the Hss-3 site relative to the Hss-2 sites showed a 2-fold increase in DNase I sensitivity at 5 min and a nearly 4-fold increase within 120 min of T₃ administration. These studies provide quantitative evidence for a specific and rapid effect of T₃ on the chromatin structure flanking the 5’ end of the S14 domain.
**DISCUSSION**

In this report, T₃ was found to have two major effects on the rat liver S14 gene. T₃ induced both a major increase in S14 gene transcription (Fig. 1) and a modification of S14 chromatin structure at a discrete locus 3.3 kb upstream from the S14 cap site (Figs. 3 and 4) within minutes of hormone administration to hypothyroid rats. The rapidity of the T₃ effect on the DNase I sensitivity of the Hss-3 site implicates this site as an early event in T₃-mediated induction of S14 gene transcription.

The finding in this study that T₃ had significant effects on S14 gene transcription contradicts a previous study by Narayan and Towle (12) where T₃ was reported to have only marginal effects on S14 gene transcription. A recent observation from the same laboratory now suggests the previous study underestimated the effects of T₃ on S14 gene transcription.

These investigators modified their previous assay by replacing the pBR322-based plasmids, pS14-C1 and pS14-C2, with M13 based cDNA and genomic probes containing sequences spanning the S14 gene. Whereas previously only marginal differences were observed in S14 run-on activity between the three thyroidal states (12), the recent observations indicate the relative level of S14 run-on activity in hypo-, eu-, and hyperthyroid rat liver nuclei was 1, 4.9, and 7.8, respectively. The relative level of S14 run-on activity observed by Murray and Towle agrees favorably with the levels reported in Table 1, i.e., 1, 6.3, and 8.3 for the hypo-, eu-, and hyperthyroid states, respectively.

The most tenable explanation for the differences between the previous studies by Narayan and Towle (12) and the present results focus on the S14 DNA probes used in the hybridization analysis of S14 transcriptional run-on activity. Either the DNA sequence characteristics of the pS14-C2 plasmid and/or the purity of plasmid DNA preparation may have contributed to the erroneous measurement of high transcriptional rates in the hypothyroid liver. Although this may not represent the full explanation for the difference between the present and previous studies, it is clear that a technical change in the run-on assay can yield different results. Both the new information from Murray and Towle and the results reported here provide a reasonable body of evidence demonstrating that T₃ does, in fact, have a significant effect on S14 gene transcription.

The kinetic analysis shows significant induction of S14 gene transcription within minutes of T₃ administration (Fig. 1), suggesting that T₃ acts directly at the chromatin level to induce S14 gene transcription. The direct action of T₃ on liver nuclear function is further supported by the rapid induction of a single DNase I hypersensitive site upstream from the 5' end of the S14 gene (Figs. 3 and 4). Considering the dose of T₃ administered (200 μg/100 g body weight) was sufficient to saturate receptors within minutes of injection, it was surprising to find a 5-min lag period preceded increased S14 gene transcription in response to T₃ (Fig. 1). Two possible explanations can account for the lag period: (a) the time interval between T₃ injection and full saturation of nuclear receptors and (b) the time required for the essential biochemical events between receptor saturation and the initiation of S14 gene transcription. The induction of the Hss-3 site within 5 min of hormone administration suggested plasma to nucleus transport was not rate limiting in mediating T₃ effects at the genomic level. Alternatively, the rapid induction of Hss-3 suggested modification of DNA-protein interaction in the vicinity of the Hss-3 site may be an antecedent event in T₃ regulation of S14 gene transcription.

Several recent studies report the presence of inducible DNase I hypersensitive sites flanking the 5' end of genes regulated by T₃ (24, 25), glucocorticoids (9, 10, 26), progesterone (27), and lipopolysaccharide (28, 29). The lipopolysaccharide-inducible hypersensitive site is found within the immunoglobulin gene enhancer of mouse pre-B-cells (28, 29). The glucocorticoid-inducible sites within the long terminal repeat of murine mammary tumor virus (9) or upstream from the tyrosine aminotransferase gene (10, 26) and the progesterone-inducible sites upstream from the chicken oviduct lysozyme gene (27) harbor hormone-responsive elements. Hormone-responsive elements are cis-linked inducible enhancers which function as targets for binding ligand-activated steroid receptors. Binding of the receptor to chromatin induces specific changes in DNA-protein interaction in the vicinity of the binding site (30). Whether the T₃-mediated induction of the

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**Fig. 4. Densitometric scan of S14 chromatin structure.** Autoradiograms from Fig. 3 were scanned using an LKB ultrascan XL laser densitometer. Lanes b–d were scanned at each time point. The profile in the figure is representative of lane c of each scan. In the figure the profile base line is shift upward from control to the 120-min point to resolve all peaks in each scan. The integrated peak areas were obtained using LKB ultrascan software. The Hss-2 site was selected as an internal reference point for generalized DNase I sensitivity of the S14 genomic domain since the DNase I sensitivity of this site was found to be unaffected by changes in S14 transcriptional status. The data were obtained from scans of two separate experiments. Two lanes at each time point were used to generate the data on peak area ratios. Thus, the results represent four separate scans and the calculated data are expressed as mean ± S.D. The arrow in the figure marks the location of the peak for the T₃-induced Hss-3 site. The line below the scans represents the position of the S14 gene and the six DNase I hypersensitive sites. The wavy arrow marks the S14 cap site and the direction of transcription. The attached black box represents the 5' exon of the S14 gene. The black box upstream from the S14 cap site represents the T₃-induced Hss-3 site. The open boxes represent the noninduced DNase I hypersensitive sites: 1, 2, 3', 4, and 5.

**Peak Area Ratio**

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<tr>
<th>Time</th>
<th>Value</th>
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<tr>
<td>0</td>
<td>0.09 ± 0.01</td>
</tr>
<tr>
<td>5</td>
<td>0.21 ± 0.02</td>
</tr>
<tr>
<td>120</td>
<td>0.33 ± 0.08</td>
</tr>
</tbody>
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**S14 Gene Transcription**

The induction of the Hss-3 site within 5 min of T₃ administration to hypothyroid rats was seen consistently. This highly localized T₃-dependent change in S14 chromatin structure preceded any significant increase in S14 gene transcription (Fig. 1). Local modification of S14 chromatin structure at the Hss-3 DNase I hypersensitive site may represent an early event in the T₃-mediated induction of S14 gene transcription.

**M. B. Murray and H. C. Towle (1988), personal communication.**
Hss-3 site reflects the presence of a thyroid hormone-responsive element exhibiting inducible enhancer-like characteristics remains to be determined through additional studies. However, these studies provide evidence for the direct action of T₃ on S14 chromatin structure and gene transcription.

Finally, T₃ activated both transcriptional and post-transcriptional processes to induce the accumulation of mRNA₃₁₄. The effect of T₃ on the post-transcriptional control of mRNA₃₁₄ was most apparent in the transition from the euthyroid to hyperthyroid state where mRNA₃₁₄ levels increased 5-fold, whereas S14 gene transcription increased only 31% (Table I). This may be due to enhanced stability or efficiency of processing the primary transcript, stimulation of nuclear cytoplasmic transport, enhanced the stability of the mature mRNA, or some combination of these processes. The effect of T₃ on both transcriptional and post-transcriptional processes is not unique to S14 and has been reported for the T₃-mediated induction of mRNAs coding for rat pituitary growth hormone (31), hepatic malic enzyme (32), and hydroxymethylglutaryl-CoA reductase (33). Clearly, additional studies which focus on specific post-transcriptional pathways are required to gain a full understanding of the pleiotropic action of T₃ on gene expression.

Acknowledgments—I would like to thank Andrew Veit and Vivian Santiago for excellent technical assistance and Peter Carrington for preparation of the figures. I would also like to thank H. Towle for providing unpublished results on T₃ regulation of S14 gene transcription. I also wish to thank Drs. Lomedico, Scherer, and Hanson for providing us with the genomic and cDNA probes used in this study.

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