Thyroid hormone influences conditional transcript elongation of the apolipoprotein A-I gene in rat liver

Yen-Chiu Lin-Lee, Selma M. Soyal, Andrei Surguchov, Sandra Sanders, Wolfgang Strobl,[1] and Wolfgang Patsch[2]

Department of Medicine, Baylor College of Medicine, Houston, TX 77030

Abstract Chronic administration of thyroid hormone (T_3) increases apoA-I gene expression in rat liver by enhancing mRNA maturation, but reduces apoA-I mRNA synthesis to 50% of control. To gain insight into the inverse relation of mRNA maturation and mRNA synthesis, we measured transcription in livers of control and T_3-treated rats (50 μg/100 g body weight for 7 days) by nuclear run-on assays using overlapping antisense RNA probes encompassing the apoA-I gene. In control rats, after normalization for hybridization efficiency and probe length, the hybridization signals with intron 3 probes were reduced to 45% of those obtained with exon 1 to exon 3 probes ($P < 0.01$) indicating transcriptional arrest or pausing close to the exon 3–intron 3 border or 450 to 650 nucleotides downstream of the transcription start site. In T_3-treated rats, the elongation block was nearly twice as effective, while the rate of transcription initiation was similar to control. In contrast, the distribution of nascent transcripts across the apoA-IV gene was symmetric, and T_3-treatment suppressed apoA-IV mRNA synthesis by processes operating in the 5′ region such as transcription initiation. Thus, conditional transcript elongation contributes to the regulation of apoA-I gene expression in rat liver.–**Lin-Lee, Y-C., S. M. Soyal, A. Surguchov, S. Sanders, W. Strobl, and W. Patsch.** Thyroid hormone influences conditional transcript elongation of the apolipoprotein A-I gene in rat liver. *J. Lipid Res.* 1995. **36:** 1586–1594.

Supplementary key words gene expression · transcript arrest

Apolipoprotein (apo) A-I is the main apolipoprotein of HDL and its plasma concentration is inversely associated with the incidence of coronary artery disease (CAD) (1). By binding to cell surface proteins, operationally termed HDL-receptors (2), apoA-I promotes the translocation of cholesterol from intracellular pools to the cell membrane, facilitates the transfer of cholesterol from cell membranes to nascent HDL, and traps cholesterol via lecithin:cholesterol acyltransferase-mediated esterification in the core of HDL particles (3–5). Hence, apoA-I is critically involved in the initial phase of reverse cholesterol transport, a function which may, at least in part, explain its antiatherogenic role.

In most mammalian species, the apoA-I gene is primarily expressed in liver and intestine (6–8), but the mechanisms controlling changes in apoA-I mRNA expression in response to metabolic signals differ between the two tissues (8–10). In some animal models, changes in plasma apoA-I levels resulting from dietary or hormonal perturbations correlate with changes in hepatic, but not intestinal apoA-I mRNA concentrations (10–15). Experiments in transgenic mice as well as in vitro transfection studies have identified the apoA-I gene elements mediating hepatocyte-specific expression (8, 9, 16). Several nuclear proteins converge at three distinct sites in the 5′ flanking region of the apoA-I gene and govern its expression through synergistic interactions (17, 18). The frequency of transcription initiation may therefore be an important control point in hepatic apoA-I gene expression, but the significance of transcriptional regulation for changes in apoA-I gene expression in vivo has been addressed only in very few studies (15, 19–22). Even less is known whether transcription initiation is the sole control point in apoA-I mRNA synthesis or whether other mechanisms such as conditional transcript elongation contribute to changes in apoA-I gene expression in vivo.

Among physiological perturbations, changes in thyroid hormone status are associated with distinct changes of hepatic apoA-I gene expression (10, 15, 19, 23). A single receptor-saturating dose of triiodothyronine (T_3) increases hepatic apoA-I gene transcription, abundance levels of nuclear and total hepatic apoA-I mRNA, and plasma apoA-I levels (15, 19). After repeated daily injec-

Abbreviations: apo, apolipoprotein; T_3, triiodothyronine.

[1]Present address: Department of Pediatrics, University of Vienna, Austria.

[2]To whom correspondence should be addressed at: Department of Laboratory Medicine, Landeskrankenanstalten Salzburg, A-5020 Salzburg, Austria.

tions of T_3, plasma apoA-I levels as well as nuclear and total hepatic apoA-I mRNA levels remain elevated, but transcription from the apoA-I gene is reduced to 30–50% of control animals (15). Such a relation between apoA-I mRNA abundance and mRNA synthesis implies that posttranscriptional events control apoA-I gene expression and raises the possibility of inhibition of transcription by processes related to mRNA maturation.

Characterization of the nuclear apoA-I mRNA maturation showed that the decrease in apoA-I gene transcription in T_3-treated rats was associated with a commensurate decrease in the abundance of primary transcripts (24). However, the abundance of mature nuclear and cytoplasmic mRNA was 3-fold higher in T_3-treated than in control rats. Compartmental modeling of apoA-I mRNA processing suggested that chronic T_3 treatment enhances mRNA maturation 7-fold by protecting the mRNA precursor devoid of intron 2, but containing introns 1 and 3 from degradation and/or facilitating the splicing of intron 1 from this precursor (24).

To gain insight into the inverse association between rates of apoA-I mRNA maturation and gene transcription, we measured transcription across the apoA-I gene with overlapping single-stranded probes. We report here that transcription of the apoA-I gene is hindered by an elongation block in the basal state. In chronically hyperthyroid rats, this elongation block is twice as effective as under basal conditions and accounts for the decreased apoA-I mRNA synthesis rate.[3]

MATERIALS AND METHODS

[5′ γ-^{32}P]dATP (4500 Ci/mM) and [5′ α-^{32}P]dCTP (3000 Ci/mM) were purchased from ICN Radiochemicals (Irvine, CA); [α-^{35}S]dATP (600 Ci/mM) was from Amersham Corp. (Arlington Heights, IL); [5′ α-^{32}P]UTP (3000 Ci/mM) was from New England Nuclear Research/DuPont (Boston, MA). T4 DNA ligase, T4 polynucleotide kinase, calf intestine alkaline phosphatase, proteinase K, placental ribonuclease inhibitor, RNase-free DNase I, DNase-free RNase, restriction enzymes, and α-amanitin were obtained from Boehringer Mannheim (Indianapolis, IN). DNA polymerase I and Klenow-large fragment were from GIBCO BRL Life Technologies, Inc. (Gaithersburg, MD). Amplitaq® DNA polymerase was from Perkin-Elmer Cetus (Norwalk, CT), Qiaex from Qiagen Inc. (Chatsworth, CA), and Chroma Spin-100 columns from Clontech Laboratories, Inc. (Palo Alto, CA). The Sequenase® Version 2.0 kit was from United States Biochemical Corp. (Cleveland, OH) and the MEGAscript™ in vitro Transcription Kit was from Ambion Inc. (Austin, TX). Nitrocellulose membranes were obtained from Stratagene Cloning Systems (La Jolla, CA) and ISS PromPPT™ was from Integrated Separation Systems (Natick, MA).

Experimental animals and isolation of nuclei

Adult male Sprague-Dawley rats (Texas Animal Specialties, Humble, TX) weighing about 250 g were housed in a room with a 12-h light cycle (7–19 h). Animals were fed normal rat chow ad libitum. T_3 was dissolved in 0.15 N NaCl, pH 11. Animals were injected with T_3 (50 µg/100 g body weight) subcutaneously for 7 days. Rats serving as injection controls received the alkaline 0.15 N NaCl solution only. Food was removed at 9 AM, and 2–4 h later animals were anesthetized with pentobarbital (5 mg/100 g). Rat livers were removed and liver cell nuclei were prepared by the method of Northemann et al. (26) as described (27). The DNA content of the nuclei was determined by a fluorimetric assay (28) using salmon sperm DNA as a standard.

Cell-free transcription

Nascent ^{32}P-labeled RNA transcripts were obtained from isolated hepatocyte nuclei by the method of Birch and Schreiber (29) as described previously (27). Nuclei ($0.5–1 \times 10^8$) were incubated in a total volume of 350 µl containing 50 mM HEPES, pH 7.5, 50 mM NaCl, 2.5 mM $MgCl_2$, 0.05 mM EDTA, 5 mM dithiothreitol, 1 mM of each ATP, CTP, GTP, 2 mM creatine phosphate, 2 µg creatine phosphokinase, 25% glycerol, 20 µg heparin, 1 mM spermine, 1 mM EGTA, 0.1 mM phenylmethylsulfone fluoride, 60 units of human placental ribonuclease inhibitor and 100 µCi of [^{32}P]UTP at 26°C for 30 min. After incubation, the reaction mixture was treated with 30 units of DNaseI for 10 min and digested with 140 µg/ml proteinase K and 0.5% SDS for 30 min at 37°C. RNA was extracted twice with phenol–chloroform–isoamyl alcohol 25:24:1 (v/v/v) and precipitated from the aqueous phase with ethanol. Unincorporated [^{32}P]UTP was removed by Chroma Spin-100 columns. Nascent ^{32}P-labeled transcripts were partially hydrolyzed with 0.2 N NaOH, 10 mM EDTA, 0.2% SDS at 4°C for 15 min and neutralized with 0.5 M HEPES (30) prior to ethanol precipitation with 1 µl ISS promPPT. Total [^{32}P]UTP incorporation ranged from 0.15 to 40 pmol/mg DNA per min. Under these conditions, transcription was DNA-dependent, and RNA polymerase activity amounted to 55% of total transcription. Transcription from either the apoA-I or apoA-IV gene was completely abolished by 2.5 µg/ml α-amanitin (15).

RNA was synthesized by in vitro transcription of pGEM-3Zf clones containing various rat apoA-I and

[3]Part of this research appeared in abstract form (25).

apoA-IV gene inserts. ApoA-I and apoA-IV gene segments were obtained by the amplification of genomic DNA using the polymerase chain reaction (31). Primers shown in **Table 1** were synthesized using a Cyclone Plus DNA synthesizer and reagents from Milligen Biosearch Division (Burlington, MA). PCR assays contained 1 μg DNA isolated from rat liver (32), 0.2 μM of each upstream and downstream primer, 200 μM of each dNTP, 10 mM Tris-HCl, pH 8.3, 50 mM KCl, 2.5 mM $MgCl_2$, 2.5 units of Amplitaq® in a 100 μl reaction volume that was overlaid with mineral oil. Samples were subjected to initial denaturation for 5 min at 94°C; 30 cycles of amplification each consisting of 1 min at 60°C (annealing), 1 min at 72°C (extension), and 1 min at 94°C (denaturation); and a final extension at 72°C for 10 min. PCR products were separated by electrophoresis in 1.2% agarose, purified using Qiaex, repaired using Klenow large fragment DNA polymerase I, and inserted into the SmaI site of pGEM-3Z by blunt-end ligation as described (33). The orientation of inserts was determined by sequencing (34) which also served to verify the identity of cloned DNA segments.

After linearization of DNA, sense and antisense RNA was transcribed from the SP6 or T7 promoter using MEGAscript, the respective polymerases, and $m^7G(5')ppp(5')G$ according to the instructions of the manufacturer. ^3H-labeled RNA was synthesized by including 5 μCi [^3H]UTP in in vitro transcription mixtures. To determine hybridization background, RNA was transcribed using T7 RNA polymerase and pGEM-3Z linearized with HaeIII as a template. RNA was dissolved in 7.5 × SSC, 37.5% formaldehyde, incubated at 65°C for 2 h and applied to nitrocellulose filters (2 μg/dot) by dot blotting (35). Filters were air-dried, baked at 80°C for 2 h, and prehybridized overnight with 0.15 ml of 20 mM PIPES, pH 6.4, 0.8 N NaCl, 2 mM EDTA, pH 8.0, 2 × Denhardt solution, 0.2% SDS, 50% formamide, 200 μg/ml tRNA, 1 μg/ml poly (A). Hybridization was carried out in polypropylene tubes for 60 h at 46°C in a total volume of 0.15 ml prehybridization solution with 1–10 × 10^6 cpm of extracted nuclear [^{32}P]RNA. To monitor hybridization efficiency, ^3H-labeled sense RNA, transcribed from clones containing the respective apoA-I or apoA-IV gene inserts, was included in the hybridization reaction. After hybridization, filters were washed three times with 2 × SSC, 0.1% SDS for 30 min at room temperature, then twice with 0.1 × SSC, 0.1% SDS at 49°C for 15 min. After incubation with 0.125 μg/ml RNase at room temperature for 10 min, filters were incubated with 100 μg/ml proteinase K at 37°C for 30 min (30, 35). Nascent ^{32}P-labeled transcripts bound to filters were quantified by using a Betascope 603 Blot Analyzer (Betagen Corp., Waltham, MA). In addition, filters were subjected to autoradiography using Kodak X-OMAT™AR film (New Haven, CT). Relative rates of apoA-I and apoA-IV mRNA synthesis were calculated by subtracting the counts per minute of ^{32}P bound to filters containing RNA transcribed from nonrecombinant pGEM-3Z from the counts per minute of ^{32}P bound to filters with RNA transcribed from clones containing apoA-I or apoA-IV gene inserts. Counts per minute bound were divided by the ^{32}P-labeled RNA input. Values were corrected for hybridization efficiency and divided by the number of nucleotides per probe to compensate for probe length.

RESULTS AND DISCUSSION

Three antisense and three sense RNA transcribed from cloned DNA containing near contiguous sequences spanning all four exons of the apoA-I gene were used to measure transcriptional activities across the apoA-I gene and to distinguish between mRNA synthesis from the coding and non-coding strands (**Fig. 1**). In control rats, transcriptional activity was clearly detect-

TABLE 1. Oligonucleotides used for amplification of DNA fragments of the apoA-I and apoA-IV genes

Fragment	Upstream Primer	Downstream Primer
ApoA-I		
a	5′-GACTGTTGGAGAGCTCCG-3′ (-3, +14)	5′-TCATCTTGCTGCCAGAAC-3′ (+468, +451)
b	5′-CGGCAGAGACTATGTGTCCCA-3′ (+535, +555)	5′-GTCGACTAGCCCAGAACTCCTGAGT-3′ (+1232, +1214)
c	5′-CATGCGTGTGAATGCAG-3′ (+1453, +1469)	5′-GTGTCGACGTCTCATACTCTAAACC-3′ (+1942, +1925)
d	5′-CTTCAGGATGAAAGCTGCA-3′ (+233, +251)	5′-GGTTCCTCTGCCCACCCT-3′ (+657, +640)
e	5′-GAGTTCTGGCAGCAAGATGAGC-3′ (+449, +470)	5′-CCACGATCACAGATGTGGTT-3′ (+935, +916)
f	5′-ATGATCCTGTAACTGAGCTG-3′ (+667, +686)	5′-GTCTGCAGATCCATGCACATG-3′ (+1086, +1066)
g	5′-GGATCCGCCTTGCAACTGGCACCAC-3′ (+899, +918)	5′-CTCCTCGTTCCACTTCTCCT-3′ (+1345, +1326)
h	5′-GCTGCTCTCTTCCCCTCTAG-3′ (+1120, +1139)	5′-CCTTCCAGGCTTCCAGCA-3′ (+1673, +1656)
ApoA-IV		
a	5′-TCCTCACAGCGACACAGTGA-3′ (+2, +21)	5′-AGTTGTTCCACAGCCTCCTT-3′ (+512, +493)
b	5′-GACATCAGAGTCTTGCCTCT-3′ (+621, +640)	5′-CCTCCATCTTGTCCCTGTAG-3′ (+1113, +1094)
c	5′-CTGGAAGACCTGCGCAGCAG-3′ (+1687, +1706)	5′-CTCCTGGACCTGTTCCTGAA-3′ (+2187, +2168)

Numbers in parentheses are relative to the major transcription start sites (11).

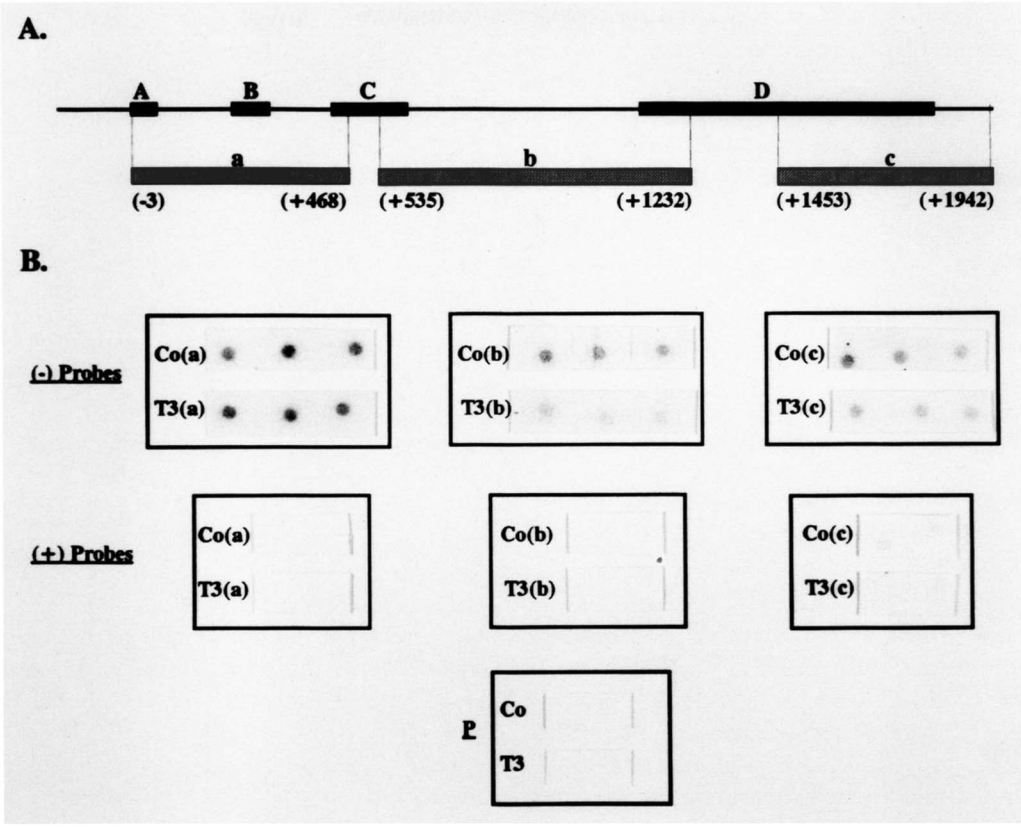

Fig. 1. Elongation block in apoA-I gene transcription in livers of control and T_3-treated rats. A, Map of the rat apoA-I gene; A, B, C, D, refer to exons 1, 2, 3, and 4; a, b, and c represent the portions of the apoA-I gene encompassed by in vitro synthesized RNA probes. Numbers in parentheses are relative to the major transcription start site (11). B, Autoradiograph of dot blot hybridization of ^{32}P-labeled nuclear RNA to excess antisense (–) or sense (+) RNA probes a, b, and c. Nuclei were isolated from the livers of control rats or rats injected for 7 days with 50 μg/100 g body weight of T_3 (T3) and then allowed to incorporate [^{32}P]UTP in vitro. The number of labeled U residues in the sequence, complementary to each (–) probe is 122 for probe a, 144 for probe b, and 97 for probe c. P represents background hybridization to an RNA transcribed from nonrecombinant pGEM-3Z which contains an irrelevant sequence.

able with the antisense RNA probe **a,** extending from the transcription start site into exon 3 of the apoA-I gene. The signal intensity considerably decreased with the antisense RNA probe **b** which extended from exon 3 into exon 4. Hybridization signals obtained with probe **c,** which was shorter than probe **b** and extended from exon 4 into the 3′ untranslated region of the apoA-I gene, showed intensities comparable to those with probe **b.** The drop in transcriptional activity in going from probe **a** to probe **b** (**Table 2**) was confirmed in two other sets of animals and was a consistent finding in more than 10 independent liver samples. Southern blots of genomic DNA restricted with BamHI or SstI and probed with the DNA inserts of clones **a, b,** and **c** demonstrated only one band (not shown). Thus, the decrease in signal intensity was not confounded by repetitive sequences of probe **a.** Furthermore, the nucleotide composition of probes **a** and **b** provided no explanation for the drop in incorporation. In addition, hybridization signals with probes **a, b,** and **c** were decreased by more than 95% when 2.5 μg/ml α-amanitin was included during nuclear run-on experiments (data not shown). The newly synthesized ^{32}P-RNA transcripts were therefore likely to result from RNA polymerase II activity.

In T_3-treated rats, the signal intensity with probe **a** was similar to that of control rats (Fig. 1 and Table 2). However, the drop in signal-intensity was more pronounced in going from probe **a** to probe **b** (Table 2). Virtually no hybridization signal was detected using sense RNA as a hybridization probe in both control and T_3-treated rats indicating that transcription from the non-coding strand was negligible. The decrease in the levels of RNA polymerase II activity downstream of the sequence encompassed by probe **a** was consistent with RNA polymerase II pausing, attenuation, or premature

TABLE 2. Effect of chronic T_3 administration on transcriptional activity across the apoA-I gene in rat liver

	mRNA Synthesis			
	Control		T_3	
Hybridization Probe[a]	pm/nucleotide	%	pm/nucleotide	%
a (-3 to +468)	0.148 ± 0.018[b,c]	100 ± 12	0.147 ± 0.024[d,e]	99 ± 16
b (+535 to +1232)	0.095 ± 0.014[b,f]	64 ± 9	0.038 ± 0.003[d,f]	26 ± 2
c (+1453 to +1942)	0.073 ± 0.035[c]	49 ± 24	0.055 ± 0.015[e]	37 ± 10

ApoA-I mRNA synthesis rates were measured by nuclear run-on assays using nuclei from four or five individual rat livers per group. Nuclei were isolated from livers of control rats or rats injected with 50 μg of T_3 per 100 g body weight for 7 days. mRNA synthesis rates are expressed as parts per million (ppm) of input ^{32}P-labeled RNA (3×10^6 cpm) and are means ± SD. Results are corrected for the number of nucleotides per probe and for hybridization efficiency which averaged 16, 13, and 13% for probes **a, b,** and **c,** respectively. mRNA synthesis rates are also expressed as percentage values of probe **a** in control.

[a]Antisense RNA obtained by in vitro transcription; numbers in parentheses refer to nucleotides relative to the major transcription start site (11).

[b,c,f]$P < 0.05$; [d,e]$P < 0.001$, two-way analysis of variance.

termination. The difference in probe **b** hybridization signals between control and T_3-treated rats, despite similar signal-intensities with probe **a,** was likely to reflect specific processes that occurred during transcription of the apoA-I gene as a result of T_3 administration.

To ascertain the specificity of hormonal effects on apoA-I transcript elongation, we measured RNA polymerase II densities across the apoA-IV gene. Using full-length apoA-IV cDNA as a hybridization probe, we have previously shown that chronic T_3 administration decreases apoA-IV gene transcription to 50% of control (36). In T_3-treated rats, apoA-IV gene transcription decreased to 51% of control when measured with the 5′ antisense RNA probe **a** extending from position 2 to 512 relative to the transcription start site (**Table 3, Fig. 2**). Furthermore, signal intensities obtained with probes **b** and **c** encompassing intron 2 and exon 3 showed the increases expected for the 3′ nascent transcripts in the absence of elongation blocks or pauses. The weak hybridization signals obtained with the 3′ sense RNA were a consistent finding in several liver samples and suggested transcription from the non-coding strand of the apoA-IV gene, but do not affect the conclusion that chronic T_3 administration suppresses apoA-IV gene transcription by processes operating in the 5′ region of the gene such as transcription initiation.

To more closely map the site at which elongation of apoA-I nascent transcripts is inhibited, overlapping RNA probes containing about 500 nucleotides each and encompassing the apoA-I gene region from exon 2 to exon 4 were used to quantify ^{32}P-transcripts synthesized in run-on assays (**Table 4, Fig. 3**). Corrected hybridization signals with probe **d** were similar to those obtained with probe **a** in both control and experimental rats. A decline in hybridization signals was observed with probes **e** and **f.** These findings in normal rats are con-

TABLE 3. Effect of chronic T_3 administration on transcriptional activity across the apoA-IV gene in rat liver

	mRNA Synthesis			
	Control		T_3	
Hybridization Probe[a]	pm/nucleotide	%	pm/nucleotide	%
a (+2 to +512)	0.117 ± 0.020[b]	100 ± 17	0.060 ± 0.012[b,c]	51 ± 10
b (+535 to +1232)	0.137 ± 0.040[d]	117 ± 34	0.094 ± 0.012[c,d]	80 ± 10
c (+1453 to +1942)	0.134 ± 0.011	114 ± 9	0.117 ± 0.021	100 ± 18

ApoA-IV mRNA synthesis rates were measured by nuclear run-on assays using nuclei from four or five individual rat livers per group. Nuclei were isolated from livers of control rats or rats injected with 50 μg of T_3 per 100 g body weight for 7 days. mRNA synthesis rates are expressed as parts per million (ppm) of input ^{32}P-labeled RNA (3×10^6 cpm) and are means ± SD. Results are corrected for the number of nucleotides per probe and for hybridization efficiency, which averaged 15, 18, and 18% for probes **a, b,** and **c,** respectively. mRNA synthesis rates are also expressed as percentage values of signals obtained with probe **a** in control.

[a]Antisense RNA obtained by in vitro transcription; numbers in parentheses refer to nucleotides relative to the transcription start site (11).

[b,c,d]Data pairs significantly different ($P < 0.05$) using two-way analysis of variance.

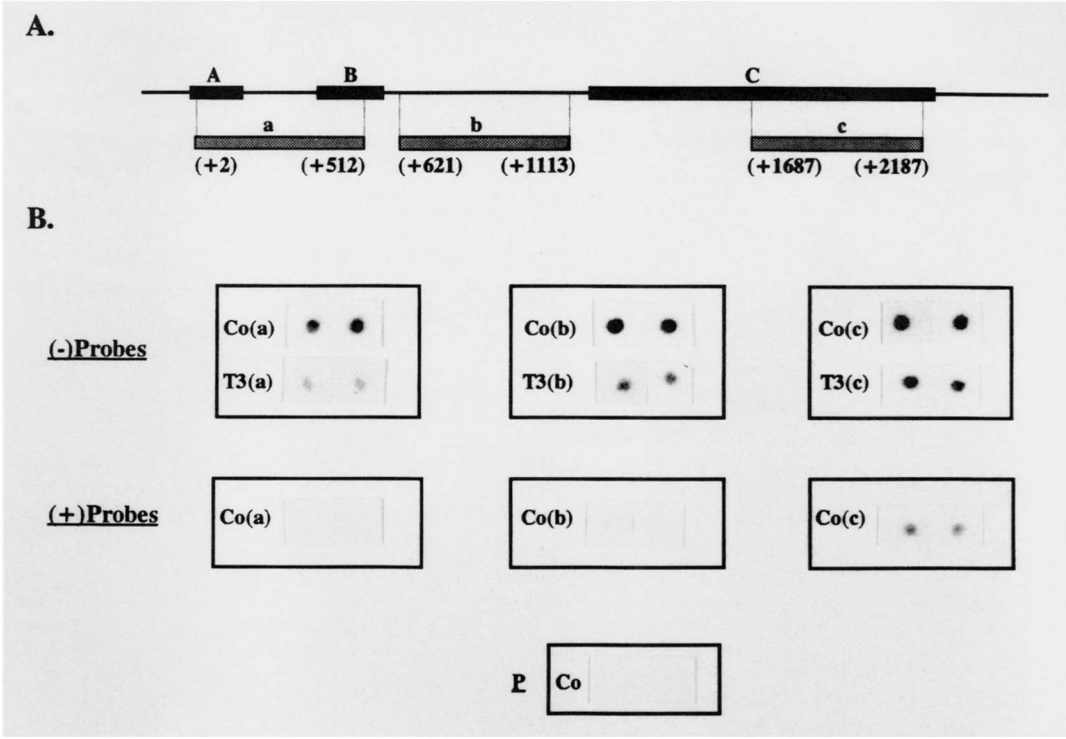

Fig. 2. Transcriptional activity across the apoA-IV gene in livers of control and T_3-treated rats. A, Map of the rat apoA-IV gene; A, B, and C refer to exons 1, 2, and 3; a, b, and c represent the portions of the apoA-IV gene encompassed by in vitro synthesized RNA probes. Numbers in parentheses are relative to the transcription start site (11). B, Autoradiograph of dot blot hybridization of ^{32}P-labeled nuclear RNA to excess antisense (–) or sense (+) RNA probes a, b, and c. Nuclei were isolated from the livers of control rats (Co) or rats injected with T_3 (T3) and then allowed to incorporate [^{32}P]UTP in vitro. The number of labeled U residues in the sequence, complementary to each (–) probe is 110 for probe a, 101 for probe b, and 78 for probe c. P represents background hybridization to an RNA transcribed from nonrecombinant pGEM-3Z.

TABLE 4. Mapping of transcriptional activity across apoA-I gene in livers of control and chronically hyperthyroid rats

Hybridization Probe[a]	mRNA Synthesis	
	Control	T_3
	%	
d (+233 to +657)	100 ± 17[b,c,d,e]	99 ± 1[f,g,h,i]
e (+449 to +935)	45 ± 6[b]	36 ± 6[f]
f (+667 to +1086)	44 ± 4[c,j]	25 ± 5[g,j]
g (+899 to +1345)	49 ± 4[d]	41 ± 6[h]
h (+1120 to +1673)	45 ± 5[e]	42 ± 7[i]

ApoA-I mRNA synthesis rates were measured in three independent nuclear run-on assays using nuclei from three to six individual rat livers per group. Nuclei were isolated from livers of control rats or rats injected with 50 μg of T_3 per 100 g body weight for 7 days. mRNA synthesis rates are expressed as percent of signals obtained with probe **d** in control. Input of ^{32}P-labeled RNA was 3.5×10^6 cpm. Results are means ± SD, and are corrected for the number of nucleotides per probe and for hybridization efficiency, which averaged 19, 22, 15, 18, and 18% for probes **d, e, f, g,** and **h,** respectively.

[a]Antisense RNA obtained by in vitro transcription; numbers in parentheses refer to nucleotides relative to transcription start site (11).

[b,c,d,e,f,g,h,i,j]Data pairs significantly different ($P < 0.01$) using two-way analysis of variance.

sistent with a transcriptional elongation block or pause site between nucleotide position 450 and 650 of the apoA-I gene relative to its major transcription start site. As T_3-treated rats showed a more pronounced drop in signal intensity with probes **e** and **f,** it is possible that chronic hormone administration augments the processes hindering transcription elongation at this site. However, the use of nuclear run-on experiments precludes the precise location of the elongation block site because of the limitations on the size of probes required for hybridization. Moreover, hybridization signals downstream of the main block tended to increase in T_3-treated rats, but remained at the reduced level or tended to decrease in control rats (Fig. 3, Tables 2 and 4). Hence, the possibility that an additional elongation block site distinct from but adjacent to the site detected in control rats was induced by T_3-treatment, cannot be excluded.

To our knowledge, this is the first report on expression control by transcriptional arrest or pausing among members of the apolipoprotein multigene family. Because there is extensive homology within and between apolipoprotein genes and among different species (37,

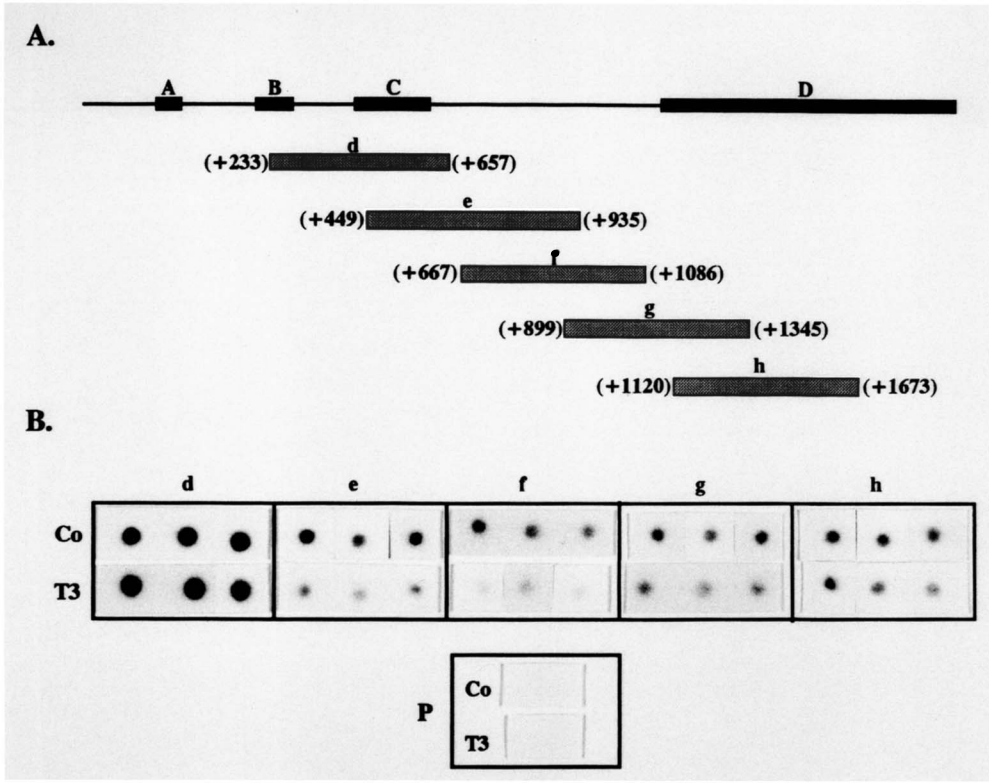

Fig. 3. Mapping of transcriptional arrest or pausing in the apoA-I gene in livers of control and chronically hyperthyroid rats. A, Map of the rat apoA-I gene; A, B, C, D, refer to exons 1, 2, 3, and 4; d, e, f, g, and h represent the portions of the apoA-I gene encompassed by in vitro synthesized RNA probes. Numbers in parentheses are relative to the major transcription start site (11). B, Autoradiograph of dot blot hybridization of ^{32}P-labeled nuclear RNA to excess antisense (–) RNA probes d–h. Nuclei were isolated from the livers of control rats (Co) or T_3-injected rats (T3) and then allowed to incorporate [^{32}P]UTP in vitro. The number of labeled U residues in the sequence, complementary to each (–) probe is 91 for probe d, 96 for probe e, and 85 for probe f, 88 for probe g, and 90 for probe h. P represents background hybridization to an RNA transcribed from nonrecombinant pGEM-3Z.

38), control of the elongation phase of transcription may be involved in the expression level of other apolipoproteins.

The expression of several prokaryotic, eukaryotic, and viral genes may be controlled by transcriptional arrest. Examples of eukaryotic genes include several cellular proto-oncogenes, the murine and human adenosine deaminase genes, the human histone H3.3 gene, and the Drosophila genes, specifically the heat-shock genes hsp 70 and hsp 26 (reviewed in refs. 39–41). Examples of viral transcription units include the human immunodeficiency virus (42), simian virus 40 (43), adenovirus type 2 (44), and polyomavirus (45). To our knowledge, transcriptional arrest sites, characterized previously in eukaryotic genes and viral transcription units, were mapped to the first exon or intron of the respective genes. None of them occurred in the context of possible feedback inhibition of transcription. Hence, the premature transcription arrest occurring close to the exon 3–intron 3 border of the apoA-I gene and 450 to 650 nucleotides downstream of the major transcription start site is unique. Conditional transcript elongation may be associated with disruption of the ternary polymerase complex and release of prematurely terminated transcripts (42, 46) or with pausing of the polymerase complex without release of immature transcripts (41, 47). Our current experiments do not allow us to distinguish between these two possibilities.

Several mechanisms have been identified which may contribute to transcriptional attenuation. Specific sequences or structures in DNA or RNA may impede the progress of the polymerase complex (46, 48), events at the promoter and protein factors may exert effects on the processivity of RNA polymerase (49), and proteins bound to the template may block the progress of transcription (50). In preliminary experiments, we detected several in vivo and in vitro footprints within exon 3 and intron 3 of the apoA-I gene. Furthermore, the elonga-

tion block was competitively relieved with increasing concentrations of in vitro synthesized intron 3-RNA fragments in nuclear run-on assays of both control and T_3-treated rats (25). While these preliminary data await more rigorous testing in other experimental systems, they could link mRNA maturation with transcriptional activity. More effective mRNA maturation would result in decreased levels of mRNA degradation products which in turn would augment transcriptional arrest or pausing. Such an autoregulatory mechanism could also serve to amplify changes in transcription initiation. With more frequent transcript initiation, the level of mRNA degradation products would increase and, as a consequence, transcriptional pausing or arrest would be reduced.

This work was supported by National Institutes of Health Grants RO1 HL34457 and HL27341.

Manuscript received 3 January 1995 and in revised form 4 April 1995.

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