Coordinate Induction of Multiple Cytochrome c mRNAs in Response to Thyroid Hormone*

Richard C. Scarpulla, Mary C. Kilar, and Kathleen M. Scarpulla
From the Department of Molecular Biology, Northwestern University Medical School, Chicago, Illinois 60611

(Received for publication, September 26, 1985)

Because of the well documented influence of thyroid hormones in the general control of oxidative metabolism and their putative role in the transcriptional activation of specific genes, changes in the levels of multiple cytochrome c mRNAs were monitored in thyroidectomized rats after administration of 3,5,3'-triiodo-L-thyronine (3,5,3'-T3). In contrast to normal animals where the concentration of cytochrome c mRNAs in the polyadenylated RNA fraction is approximately 4- to 5-fold higher in kidney than in liver, hypothyroid animals displayed an equivalent low level of all three mRNAs (1400, 1100, and 700 nucleotides) in both tissues. Following the establishment of chronic hyperthyroidism, the levels of the three mRNAs were coordinately elevated by about 4- to 5-fold resulting in their restoration to approximately normal amounts for kidney but to a level substantially above the normal for liver. Only modest induction of mRNA is detected in the first 12 h following a single intravenous injection of 3,5,3'-triiodo-L-thyronine. The major increase occurs between 12 and 24 h with the plateau level attained between 24 and 48 h. The magnitude of the response is in excess of the general increase in total cellular RNA mediated by the hormone. Induction of the cytochrome c mRNAs is coincident with an elevation in gene transcription of comparable magnitude detected using nascent RNA chains synthesized by isolated nuclei. The kinetics of these responses are similar to those observed for growth hormone display an early response to elevated hormonal states (7) while others exhibit a specific response only after a period of 12 to 24 h (5). Although little is known mechanistically about how mRNA levels are controlled by thyroid hormones, the association of T3 receptors with the nonhistone fraction of chromosomal proteins (8-10) and the detection of elevated levels of specific nuclear RNA precursors following T3 administration (7, 11) are suggestive of a hormone-mediated gene activation. In the present studies a cloned cytochrome c gene from rat is used as a specific hybridization probe to examine changes in the levels of multiple cytochrome c mRNAs following treatment with 3,5,3'-triiodo-L-thyronine.

EXPERIMENTAL PROCEDURES

Hormonal Treatment of Animals—Adult male Sprague-Dawley rats of 200 g were utilized in all experiments. Surgical thyroidectomy was performed commercially by Hormone Assay Laboratories of Chicago about 1 week before hormone treatments. Hyperthyroidism was induced in both normal and thyroidectomized animals with daily intraperitoneal injections of 30 μg of T3 (Sigma)/100 g of body weight delivered in phosphate-buffered saline containing 2% bovine serum albumin. For experiments where the time course of induction was monitored, 100 μg of T3/100 g of body weight was administered in a single intravenous tail vein injection and animals were killed at various times following treatment. In all experiments control animals received injections of phosphate-buffered saline containing 2% bovine serum albumin.

Methods of mRNA Analysis—Total RNA was isolated from rat liver and kidney as described previously (12). Polyadenylated RNA was purified from total RNA by chromatography on oligo(dT)-cellulose using a minor modification of the procedure of Aviv and Leder (13). Approximately 2% of the total RNA was recovered in the polyadenylated fraction. Polyadenylated RNAs (10 μg/lane) were resolved by electrophoresis in 1% agarose gels in the presence of formaldehyde as previously described. For hybridization, denatured RNAs were transferred to nitrocellulose filters (Schleicher & Schuell) as described by Thomas (14). Cytochrome c mRNAs were detected by hybridization to a 32P-labeled restriction fragment derived from the rat cytochrome c gene as previously described (12, 15). Filters were washed four times with 2 × SSC, 0.1% sodium dodecyl sulfate for 5 min each at 23 °C; twice with 0.1 × SSC, 0.1% sodium dodecyl sulfate for 15 min each at 50 °C and exposed to medical x-ray film (Kodak).

For quantification of the hybridization signal, polyadenylated RNAs (1.5 μg/slot) were bound to a nitrocellulose membrane using a slot blotting apparatus (Schleicher & Schuell) as described by the manufacturer. Hybridization and washing was carried out as described above. The radioactivity hybridized to the RNA was determined by scintillation counting.

Nuclear Transcription Assays—Nuclei were isolated from rat liver at various times following thyroid hormone treatment essentially as

Among the most pronounced physiological effects attributed to thyroid hormones is their control over respiratory metabolism. A long-standing correlation between the cytochrome c content of tissues, the rate of oxidative metabolism, and the thyroidal status is well established (1, 2). Changes in basal metabolic rate brought about by thyroid hormones coincide with a general increase in the number of respiratory units within the mitochondria (3) and a 5.5-fold increase in the rate of cytochrome c biosynthesis in the livers of thyrotoxic rats (4). Although the precise time required for this response is not established, overall changes in respiratory rate are observed within 24 to 48 h following thyroid hormone treatment (3).

In addition to bringing about relatively modest general increases in cellular RNA levels, thyroid hormones also promote more pronounced changes in a small subpopulation of specific mRNA molecules (5, 6). Some mRNAs such as that for growth hormone display an early response to elevated hormonal states (7) while others exhibit a specific response only after a period of 12 to 24 h (5). Although little is known mechanistically about how mRNA levels are controlled by thyroid hormones, the association of T3 receptors with the nonhistone fraction of chromosomal proteins (8-10) and the detection of elevated levels of specific nuclear RNA precursors following T3 administration (7, 11) are suggestive of a hormone-mediated gene activation. In the present studies a cloned cytochrome c gene from rat is used as a specific hybridization probe to examine changes in the levels of multiple cytochrome c mRNAs following treatment with 3,5,3'-triiodo-L-thyronine.

* This work was supported by United States Public Health Service Grant GM32525 from the National Institutes of Health. 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.

The abbreviations used are: T3, 3,5,3'-triiodo-L-thyronine; T3S, 2-(2-hydroxy-1,1-bis(hydroxymethyl)ethyl)-amino)ethanesulfonic acid.
Coordinate Induction of Cytochrome c mRNAs by $T_3$

Described by Blobel and Potter (16), radioactive nascent RNA transcripts were prepared from isolated nuclei according to a modification of the procedure described by Groudine et al. (17). For specific detection of nascent cytochrome c transcripts, DNA from the rat cytochrome c gene clone pRC4 (18) was linearized with EcoRI, denatured, and applied to nitrocellulose using a slot blotting apparatus (Schleicher & Schuell) 10 mg/slot according to the manufacturer's instructions. Radioactive nascent RNA (1 x 10$^7$ cpm) was hybridized to filters in 10 mM TES, pH 7.4, 0.2% sodium dodecyl sulfate, 10 mM cytochrome c detection of nascent cytochrome c transcripts, DNA from the rat containing 10 pg/ml ribonuclease A for 30 min at 37 °C, and once with 2 x SSC for 30 min at 37 °C. Following exposure of filters to medical x-ray film at -20 °C, the hybridization signal was quantified by densitometry. Values were corrected for nonspecific hybridization to 10 µg of denatured pBR322 DNA which was approximately 20 to 30% of the specific signal observed at zero time.

RESULTS

Coordinate mRNA Induction in Hyperthyroid Rats—The rat genome contains a complex family of cytochrome c sequences that consists of a single intron-containing active gene (RC4) and approximately 35 processed genes, most if not all of which are nonfunctional pseudogenes (19, 20). In addition, three cytochrome c mRNAs (1400, 1100, and 700 nucleotides) are present in several tissues of the adult rat (12). Mapping experiments reveal that the three mRNAs share a common 5’ end but are heterogeneous in length as a result of polyadenylation at three distinct sites in the 3’ untranslated region (19).

In normal animals (Table I), the concentration of the three cytochrome c mRNAs in the polyadenylated RNA fraction is 4- to 5-fold higher in kidney than in liver as originally observed (12). Hormone treatment brings about a modest increase in the kidney level (generally less than 25%) but elevates the liver cytochrome c mRNA level nearly 3-fold. In contrast, kidney and liver from thyroidecotomized rats both display a low level of these mRNAs equivalent to that found in normal liver but are each induced approximately 4- to 5-fold upon treatment with $T_3$, suggesting that both tissues have the capacity for the same range of response to thyroid status but that normally kidney approximates the maximal induction whereas the mRNA levels in liver are relatively unaffected. Under all conditions, the amounts of the three mRNAs vary in a coordinate fashion indicating that the same mechanism may operate to control all three.

| Table I |
| --- |

| Animal | Treatment | Tissue | Hybridization of cytochrome c mRNAs |
| --- | --- | --- | --- |
| Normal | $-T_3$ | Liver | 360 ± 60 |
| Normal | $-T_3$ | Kidney | 1340 ± 70 |
| Normal | $+T_3$ | Liver | 950 ± 150 |
| Normal | $+T_3$ | Kidney | 1510 ± 140 |
| Thyroidecotomized | $-T_3$ | Liver | 370 ± 110 |
| Thyroidecotomized | $-T_3$ | Kidney | 390 ± 90 |
| Thyroidecotomized | $+T_3$ | Liver | 1470 ± 200 |
| Thyroidecotomized | $+T_3$ | Kidney | 1600 ± 290 |

Time Course of Cytochrome c mRNA Induction—The overall changes in the rate of respiratory metabolism brought about by thyroid hormones are known to occur in the range of 36 to 70 h after a single intravenous hormonal treatment (3). The induction of cytochrome c mRNAs, therefore, was monitored at various time after administration of $T_3$ to thyroidecotomized rats. Fig. 1 illustrates that little change in mRNA levels takes place at early times after hormone treatment but that a maximal response, comparable to that seen under conditions of chronic hyperthyroidism, is detectable within 48 h. The time course of this response, shown in Fig. 2, is essentially the same in both kidney and liver. Coordinate increases in all three mRNAs are observed between 12 and 24 h and a maximal induction was detected between 24 and 48 h followed by a decline after 48 h. The magnitude of the increase exceeds the general elevation in total cellular RNA brought about by hormone treatment (5). During the time course of mRNA induction, total RNA yields only increased from 17 to 30 mg of RNA/g of tissue for liver and from 9 to 16 mg of RNA/g of tissue for kidney with the per cent of the total obtained as polyadenylated RNA unchanged.

Control of both biosynthetic and degradative processes can contribute to changes in the steady-state level of a particular mRNA (21). Thyroid hormones are generally thought to implement physiological changes at least in part through controlling nuclear transcription (7, 11). To determine whether the increase in cytochrome c mRNA concentration is accompanied by enhancement of cytochrome c gene transcription, radioactive nascent RNA was prepared from rat liver nuclei isolated from thyroidecotomized rats at various times following $T_3$ administration. Fig. 2 illustrates that an increase in cytochrome c gene activity coincides with the increase in mRNA accumulation. The level of cytochrome c specific nascent transcripts was inhibited by more than 85% upon addition of $\alpha$-amanitin (1 µg/ml) to reaction mixtures. The overall increment of approximately 3- to 4-fold over the untreated control is quantitatively similar to that observed for mRNA induction, indicating that changes in transcriptional activity substantially contribute to the inductive response. However, the maintenance of high mRNA levels following the decline in

FIG. 1. Time course of liver cytochrome c mRNA induction following a single intravenous treatment with $T_3$. Polyadenylated RNA was isolated from the livers of thyroidecotomized rats at the indicated time in hours following a single intravenous administration of $T_3$ (100 µg of $T_3$/100 g body weight). RNA (10 µg/lane) was resolved by gel electrophoresis and transferred to nitrocellulose, and the filters were hybridized at 40 °C to a 32P-labeled BamHI-AccI restriction fragment containing the RC4 coding sequences (18). Size standards include 18 S and 28 S rat liver ribosomal RNA and HaeII-digested pBR322 DNA. Positions of 1400- (I), 1100- (II), and 700- (III) nucleotide mRNAs are indicated by Roman numerals.
thyroidectomized rats at various times following a single intravenous
ing slot blot hybridization. Radioactive nascent RNA chains were
determinations from the time course of cytochrome c mRNA induc-
erally less than
sequences quantified by densitometry following hybridization to an
nascent RNA analysis, standard errors for time points
thyroid hormone action, in only a few model systems have
nuclear transcription after 24 h indicates that enhanced
mRNA stability can also contribute to the elevated steady-
state levels.

DISCUSSION

Despite the widespread biochemical changes attributed to
hormone action, in only a few model systems have alterations in the expression of specific gene products been well documented (for review see Ref. 5). The present studies demonstrate a close temporal correlation between the transcriptional induction of cytochrome c mRNAs in response to
T3 treatment and the previously well documented increases in
basal metabolic rate as measured by enhanced respiratory activity (3). The coordinate behavior of the mRNAs which differ only in the lengths of their 3' noncoding regions indicates that the three polyadenylation sites are used equally well under both hyper- and hypothyroid conditions in both
liver and kidney. Thus, the 5.5-fold elevation in cytochrome c biosynthetic rate previously observed in the livers of thyro-
toxic rats (4) is most probably the result of increased cyto-

chrome c gene activity although other factors such as alterations in mRNA and protein stability may also contribute in
part to the overall regulation. An increase in translationally active mRNA has also been observed in rat liver (22). While
the magnitude and time course of T3 stimulation of mRNA levels in thyroidectomized rats are very similar for kidney and
liver, the final plateau level represents a restoration to a
normal state in kidney but far exceeds normal expression for liver. Thus, the regulatory range is equivalent for both tissues
but the normal steady-state levels are maintained at opposite ends of the regulatory spectrum.

Aside from the relatively modest general increase in total
cellular RNA, a small subset of the mRNA population of rat
liver exhibits specific changes according to thyroid status (6).
Those that are induced by thyroid hormones have lag times
ranging from less than 4 h to as long as 12 to 24 h following
hormone treatment. Cytochrome c mRNAs can be included
among those that are specifically induced after a relatively
long lag time in agreement with that observed for changes in
respiratory metabolism.

Acknowledgments—We thank Mary Hunzicker-Dunn for help with
animal treatments and reading the manuscript.

REFERENCES

1. Drabkin, D. L. (1950) J. Biol. Chem. 182, 317-333
2. Drabkin, D. L. (1950) J. Biol. Chem. 182, 335-349
3. Tata, J. R., Ernster, L., Lindberg, O., Archenius, E., Pederson, S., and Hedman, R. (1968) Biochem. J. 105, 408-428
4. Booth, F. W., and Holloszy, J. O. (1975) Arch. Biochem. Biophys. 167, 674-677
5. Towle, H. C. (1983) in Molecular Basis of Thyroid Hormone Action (Oppenheimer, J. H., and Samuels, H. H., eds) pp. 179-212, Academic Press, New York
6. Seeig, S., Liaw, C., Towle, H. C., and Oppenheimer, J. H. (1981) Proc. Natl. Acad. Sci. U. S. A. 78, 4368-4372
7. Nyborg, J. K., Nguyen, A. P., and Spindler, S. R. (1984) J. Biol. Chem. 259, 12377-12385
8. Surks, M. I., Koerner, D., Dillman, W., and Oppenheimer, J. H. (1973) J. Biol. Chem. 248, 7066-7072
9. Samuels, H. H., and Tsai, J. S. (1973) Proc. Natl. Acad. Sci. U. S. A. 70, 3488-3492
10. DeGroot, L. J., Refetoff, S., Strausser, J., and Barsano, C. (1974) Proc. Natl. Acad. Sci. U. S. A. 71, 4042-4046
11. Narayan, P., Liaw, C. W., and Towle, H. C. (1984) Proc. Natl. Acad. Sci. U. S. A. 81, 4687-4691
12. Scarpulla, R. C., and Wu, R. (1983) Cell 32, 473-482
13. Aviv, H., and Leder, P. (1972) Proc. Natl. Acad. Sci. U. S. A. 69, 1489-1412
14. Thomas, P. S. (1980) Proc. Natl. Acad. Sci. U. S. A. 77, 5201-5205
15. Ribgy, P. W. J., Dieckmann, M., Rhodes, C., and Berg, P. (1977) J. Mol. Biol. 113, 257-251
16. Blobel, G., and Potter, V. R. (1969) Science 154, 1662-1665
17. Groudie, M., Peretz, M., and Weintraub, H. (1981) Mol. Cell. Biol. 1, 281-288
18. Scarpulla, R. C., Agne, K. M., and Wu, R. (1981) J. Biol. Chem. 256, 6486-6486
19. Scarpulla, R. C. (1984) Mol. Cell. Biol. 4, 2279-2288
20. Scarpulla, R. C., Agne, K. M., and Wu, R. (1982) Proc. Natl. Acad. Sci. U. S. A. 79, 739-743
21. Darnell, J. E. (1982) Nature 297, 365-371
22. Matsuzaka, S., Arpin, M., Hannum, C., Margolish, E., Sabatini, D. D., and Morinato, T. (1981) Proc. Natl. Acad. Sci. U. S. A. 78, 4368-4372

FIG. 2. Temporal correlation of cytochrome c mRNA induction with gene activity in response to T3 treatment. Polyadenylated RNA was isolated from both the livers and kidneys of thyroidectomized rats at various times following a single intravenous
ing slot blot hybridization. Radioactive nascent RNA chains were

prepared from nuclei derived from an aliquot (2-3 g) of the same
livers used for poly(A) RNA preparation and cytochrome c-specific
sequences quantified by densitometry following hybridization to an

excess of immobilized, denatured pRC4 DNA as described under
“Experimental Procedures.” Each point represents the mean of three
determinations from the time course of cytochrome c mRNA induc-

tion in liver (°) and kidney (©) as well as enhanced gene expression

as measured by increases in nascent cytochrome c RNA chains (□). For

mRNA quantification, the standard error of the mean was gener-

ally less than 25% and comparable to those reported in Table I. For

nascent RNA analysis, standard errors for time points 0 to 48 h were

±15, ±22, ±13, ±28, ±27, ±24, ±9, and ±20% of the indicated relative values respectively.

Aside from the relatively modest general increase in total

cellular RNA, a small subset of the mRNA population of rat

liver exhibits specific changes according to thyroid status (6). Those that are induced by thyroid hormones have lag times

ranging from less than 4 h to as long as 12 to 24 h following

hormone treatment. Cytochrome c mRNAs can be included

among those that are specifically induced after a relatively

long lag time in agreement with that observed for changes in

respiratory metabolism.

Acknowledgments—We thank Mary Hunzicker-Dunn for help with

animal treatments and reading the manuscript.