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Tissue-Specific Regulation of Two Functional Malic Enzyme mRNAs by Triiodothyronine

Beatrice Dozin,[‡] Mark A. Magnuson, and Vera M. Nikodem*
Clinical Endocrinology Branch, National Institute of Arthritis, Diabetes, and Digestive and Kidney Diseases, National Institutes of Health, Bethesda, Maryland 20205
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ABSTRACT: Rat liver malic enzyme (ME) synthesis is known to be regulated by 3,5,3′-triiodo-L-thyronine (T_3). Hybridization of ^{32}P-labeled ME cDNA with RNA extracted from normal and T_3-induced livers (15 or 50 μg/100 g body weight for 10 days) showed an increase in the ME mRNA concentration by ~11-fold in T_3-treated rats. ME activity and ME mass were stimulated to the same degree as ME mRNA. Northern blot analysis of either total or poly(A+) RNA revealed two distinct ME mRNAs (21 and 27 S) which were equally induced by T_3 treatment. Both mRNAs were shown by in vitro translation assay to program the synthesis of the same immunoprecipitable protein corresponding to full-sized ME. From all the above, we concluded that both messages code for active enzyme. ME activity and ME mRNA were also detected in nonhepatic tissues for which different responses to T_3 induction were observed without direct correlation with their respective content of T_3 nuclear receptor. Increases in ME activity and level of hybridizable ME mRNA were seen 48 h after a single administration of T_3 (200 μg/100 g body weight) in liver, kidney, and heart (10.3- and 15.5-, 1.7- and 2.6-, and 1.72- and 3.4-fold above basal values, respectively). Lower levels of induction could already be detected after 24 h, liver being the most stimulated tissue. ME was not affected in brain, lung, testis, and spleen. Northern blot analysis showed that both ME mRNAs are present in all tissues tested, although in different relative proportions. As in liver, the two mRNAs were stimulated to the same extent in T_3-induced kidney and heart. These data provide direct evidence for pretranslational regulation by T_3 of ME synthesis through two functional mRNAs and for tissue specificity of this hormonal control. Furthermore, both malic enzyme mRNAs are under T_3 control in all responsive tissues.

In rat liver, the activities of several lipogenic enzymes such as fatty acid synthetase (Volpe & Vagelos, 1976; Morris et al., 1982; Roncari & Murthy, 1975), acetyl-CoA carboxylase (Roncari & Murthy, 1975), ATP–citrate lyase (Gibson et al., 1972; Sul et al., 1984a), and glucose-6-phosphate and 6-phosphogluconate dehydrogenases from the hexose monophosphate shunt (Tepperman & Tepperman, 1964; Diamant et al., 1972; Mariash et al., 1980; Miksicek & Towle, 1982) respond to thyroid hormone administration. Cytosolic malic

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enzyme (ME)[1] [EC 1.1.1.40; L-malate:NADP$^+$ oxidoreductase (decarboxylating)] has been particularly well studied in this regard (Tepperman & Tepperman, 1964; Mariash et al., 1980a; Wise & Ball, 1964; Tarentino et al., 1966). Thyroid hormone effects are believed to be initiated at the nuclear level through the binding of the hormone to chromatin-linked receptors (Oppenheimer, 1979) and by some unidentified mechanism to modulate gene expression.

Immunological studies have shown that T_3 stimulation of ME activity could be accounted for by an increased enzyme mass (Isohashi et al., 1971) resulting from an increase in the rate of enzyme synthesis (Li et al., 1975; Goodridge & Adelman, 1976). In vitro translation assays have shown that the increase in ME synthesis was due to a proportional change in the template activity of the mRNA encoding ME (Towle et al., 1980, 1981). This could reflect either a modification in the translational efficiency of preexisting mRNA or an authentic increase in cytoplasmic ME mRNA mass. Until a ME cDNA probe was available, it was not possible to distinguish between these two possibilities, and the ratio between translatable and hybridizable ME mRNA could not be determined. With the malic enzyme cDNA recently cloned in our laboratory, two messenger RNAs of different size were shown to hybridize to ME cDNA and to be regulated by T_3 (Magnuson & Nikodem, 1983).

In the present study, we further investigated the translational activity of these two ME mRNAs in liver and their presence, distribution, and regulation by thyroid hormones in other nonhepatic tissues.

Experimental Procedures

Materials. The rabbit reticulocyte translation kit, nick translation kit, [^{35}S]methionine, and [α-^{32}P]dCTP were purchased from New England Nuclear. T_3, bovine serum albumin (Pentax, fraction V), and salmon sperm DNA were from Sigma. Regular agarose and low melting point agarose were purchased from Bethesda Research Laboratories. Oligo(dT)–cellulose (type 2) was from Collaborative Research. Formamide provided by Matheson Coleman and Bell was deionized with AG 501-X8 mixed-bed resin (Bio-Rad Laboratories). BA-85 nitrocellulose filters were purchased from Schleicher & Schuell. Low-iodine diet was from Teklad Diets. All other reagents were analytical grade. For RNA preparation, all glassware was baked at least 2 h at 200 °C, and buffer solutions were autoclaved or filter-sterilized.

Animals. Sprague-Dawley rats weighing 120–150 g were used in all experiments. Hormonal induction of euthyroid animals was performed by intraperitoneal injection of either a single acute receptor-saturating dose of T_3 (200 μg/100 g body weight) (Oppenheimer et al., 1977) or new steady-state-inducing daily doses of 15 or 50 μg of T_3/100 g body weight (Towle et al., 1980) for 10 days. Additional experiments were performed with rats rendered hypothyroid by surgical thyroidectomy and kept on the low-iodine diet. Hypothyroidism was confirmed by the cessation of weight gain for at least 2 consecutive weeks and by TSH and T_3 serum level measurements at sacrifice (>15 and 38 ng/dL, respectively). Hypothyroid rats then received similar hormonal treatments as described above for the euthyroid ones. To avoid circadian variations, the animals were always killed between 11:00 a.m. and 1:00 p.m. at the time intervals indicated in the figures.

Malic Enzyme Assays: Activity and Mass. A cytosolic fraction was prepared from liver, brain, heart, kidney, spleen, lung, and testis homogenized in 5% (w/v) 0.25 M sucrose and centrifuged at 109000g for 30 min at 4 °C. ME activity was estimated from the rate of NADPH formation at 25 °C (Hsu & Lardy, 1967). One unit of enzyme activity was defined as the amount of enzyme required to reduce 1 nmol of NADP$^+$ per minute by using an extinction coefficient for NADPH of $E_{340}^{mM} = 6.3$. The same cytosolic fractions were tested for their content of ME as estimated by a radioimmunoassay developed and kindly performed by Dr. R. Drake (University of Cincinnati, Cincinnati, OH) (Thompson & Drake, 1983). Total protein concentration was measured by the method of Bradford (1976) with bovine serum albumin as standard.

Total and Poly(A+) RNA Preparation. Total cellular RNA was isolated by the lithium chloride/urea procedure of Auffray & Rougeon (1980) followed by phenol/chloroform extraction prior to ethanol precipitation. The poly(A+) RNA fraction was obtained by oligo(dT)–cellulose chromatography (Krystosek et al., 1975). The final RNA pellet was dissolved in distilled water and stored at −20 °C until used.

Translational Activity of the 27S and 21S Malic Enzyme mRNAs. Poly(A+) RNA was extracted from a T_3-induced liver (15 μg/100 g body weight for 10 days) as described above. An aliquot of the RNA preparation was size fractionated by electrophoresis on a 1% low melting point agarose gel after denaturation with methylmercuric hydroxide (Maniatis et al., 1982). The position of 28S, 23S, 18S, and 16S ribosomal RNAs was determined by ethidium bromide fluorescence. A strip of the gel including RNAs ranging from 28 to 20 S and therefore containing the 27S and 21S ME mRNAs identified previously (Magnuson & Nikodem, 1983) was cut transversely in slices of approximately 2 mm in width. RNA was eluted from each slice (Maniatis et al., 1982) and individually assayed for ME synthesis by in vitro translation and immunoprecipitation with ME antiserum (Magnuson & Nikodem, 1983). The immunoprecipitated products were then analyzed by electrophoresis on a 12% SDS–polyacrylamide gel followed by fluorography as described previously (Magnuson & Nikodem, 1983).

Northern Blot and Dot-Blot Hybridization. For Northern blot analysis, formaldehyde-denatured poly(A+) RNA (8 μg) or total RNA (10–48 μg) was size fractionated by electrophoresis on a 1% agarose/2.2 M formaldehyde gel with a running buffer of 0.2 M MOPS, pH 7.0, 50 mM sodium acetate, and 1 mM EDTA (Maniatis et al., 1982). Before transfer to nitrocellulose, the gel was subjected to sequential washes including partial alkaline hydrolysis in 50 mM NaOH/10 mM NaCl, neutralization in 0.1 M Tris-HCl, pH 7.5, and an equilibrating rinse in 20× SSC (Maniatis et al., 1982). RNA blotting onto nitrocellulose was done following the method of Southern (1975) in 20× SSC as transfer medium. After prehybridization at 42 °C for 12–16 h, hybridization was carried out at the same temperature for 48 h using the nick-translated *Hin*dIII–*Pvu*II cDNA fragment excised from the prME plasmid (Magnuson & Nikodem, 1983) [specific activity ~(1–2) × 10^8 cpm/μg]. Hybridization medium contained 50% formamide, 5× SSC, 50 mM sodium phosphate, pH 6.5, 8× Denhardt's solution [1× Denhardt's solution = 0.02% each of Ficoll, bovine serum albumin, and poly(vinylpyrrolidone)], 0.1% SDS, 100 μg/mL polyadenosine, and (0.5–1) × 10^6 cpm/mL ME probe. After hybridization,

[1] Abbreviations: ME, malic enzyme; T_3, 3,5,3′-triiodo-L-thyronine; SDS, sodium dodecyl sulfate; SSC, 0.15 M sodium chloride and 0.015 M sodium citrate; MOPS, 4-morpholinepropanesulfonic acid; TSH, thyroid-stimulating hormone; EDTA, ethylenediaminetetraacetic acid; Tris-HCl, tris(hydroxymethyl)aminomethane hydrochloride; kDa, kilodalton(s).

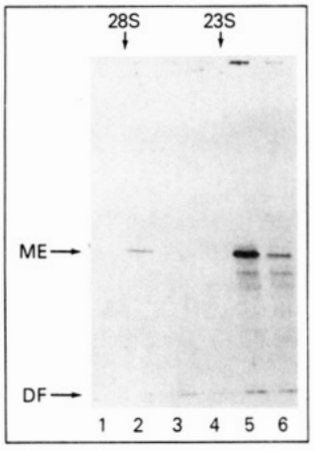

FIGURE 1: Gel electrophoretic analysis of immunoprecipitated products from in vitro translation of size-fractionated liver poly(A+) RNA. Poly(A+) RNA extracted from a T_3-induced liver was size fractionated on a 1% agarose/methylmercuric hydroxide gel. The gel containing RNAs ranging from ~28 to ~20 S was cut transversely in 2-mm slices. RNAs were eluted from each slice and translated in a rabbit reticulocyte lysate system in the presence of [^{35}S]methionine. Translation products were immunoprecipitated with ME antiserum, electrophoresed on a 12% SDS–polyacrylamide gel, and then subjected to fluorography. Lane 1, immunoprecipitated products in the absence of exogenous RNA; lanes 2–6, immunoprecipitated products from ~28S to 20S RNAs. The migration of ^{14}C-methylated malic enzyme (ME) and dye front (DF) and the positions of 28S and 23S ribosomal RNAs are indicated.

the nitrocellulose filter was washed under stringent conditions, the last wash being in 0.2× SSC and 0.1% SDS at 68 °C for 60 min. The filter was then exposed for 2 days to Kodak XAR2 film with an intensifying screen. The autoradiograms were scanned with a scanning densitometer (Hoefer Scientific Instruments), and areas under the peaks were calculated.

Dot-blot hybridization assays were performed according to the procedure of Thomas (1980) as described previously (Magnuson & Nikodem, 1983). Poly(A+) RNA samples (5 μg of each) were blotted on nitrocellulose filters by using the Schleicher & Schuell manifold. The filter was prehybridized and then hybridized as described above with 1–1.5 ng of ^{32}P-labeled ME cDNA [(0.5–1) × 10^7 cpm/mL]. The linearity of the assay was established by blotting increasing amounts of poly(A+) RNA and hybridizing them in the same bag as the experimental filters. After the filters were washed, they were exposed for autoradiography, and the radioactivity in individual dots was then quantitated by a β liquid scintillation spectrometer.

Results and Discussion

Translational Activity of the 27S and 21S Malic Enzyme mRNAs. We have previously reported from Northern blot analysis that the ME cDNA hybridized to two mRNAs in rat liver, approximately 4500 (27 S) and 2700 (21 S) nucleotides in length (Magnuson & Nikodem, 1983). These two messages were detected with total RNA as well as with poly(A+) RNA. Considering the size of the malic enzyme (~62 kDa), the coding sequence of either mRNA could account for the full size of the enzyme. Although only one form of the protein is known, it was not clear whether one or both of the mRNAs coded for ME, and the possibility remained that one of the messages had been transcribed from a gene containing a nucleotide sequence different from, but closely homologous with, that of the ME gene. To study this further, poly(A+) RNA extracted from T_3-induced liver was size fractionated on an agarose gel containing methylmercuric hydroxide as described under Experimental Procedures. RNA eluted from individual slices was translated in vitro, and the products were immunoprecipitated with ME antiserum and run on a denaturing polyacrylamide gel.

Table I: Effect of Thyroid State on Levels of ME mRNA, ME Activity, and ME Mass in Liver[a]

	^{32}P cpm/ 5 μg of poly(A+) RNA	ME act. (units/mg of protein)	ME mass (pmol/mg of protein)
eu	320[b]	12	3
eu + 15 μg of T_3 (10 days)	3520 (10.9)[c]	140 (11.4)	29 (9.6)
eu + 50 μg of T_3 (10 days)	3810 (11.9)	124 (10.0)	
hypo	100	ND[d]	ND
hypo + 15 μg of T_3 (10 days)	960 (9.6)	103	14

[a] Euthyroid (eu) and hypothyroid (hypo) rats were injected daily with 15 or 50 μg of T_3/100 g body weight for a period of 10 days. [b] Average of two independent experiments performed in duplicate. The hybridized cpm values were corrected by subtracting the background (28 cpm). In the enzymatic test and the radioimmunoassay, the limits of detection were 1.2 units/mg of protein and 0.43 pmol/mg of protein, respectively. [c] Relative values (ratios of T_3-treated vs. control samples). [d] ND, not detected.

As can be seen in Figure 1 (lanes 2, 5, and 6), both the 27S and 21S mRNAs directed the synthesis of one major protein which comigrated with ^{14}C-methylated malic enzyme. In addition to ME, minor immunoprecipitated products of lower molecular weight could be identified (lanes 5 and 6). These multiple protein fragments have been shown to be related to ME (Magnuson & Nikodem, 1983). No immunoprecipitation products were observed when the in vitro translation was programmed with RNAs running between 26 and 22 S (lanes 3 and 4). Both mRNAs detected by hybridization with the ME probe thus contain a nucleotide sequence coding for the full-size malic enzyme, and the size differences between these two mRNAs therefore reflect differences in noncoding regions and/or heterogeneity in poly(A+) length at the 3′ end.

The detection of more than one mRNA hybridizing to a single cDNA probe has been reported by other investigators (Benyajati et al., 1983; Weber et al., 1984; Jump et al., 1984; Capetanaki et al., 1983; Sul et al., 1984b), although no information was provided concerning the translational activity of these multiple messages.

Regulation of ME mRNA and ME Activity Levels in Various Thyroid States. Malic enzyme mRNA abundance, ME activity, and ME mass were quantitated after the daily administration of 15 or 50 μg of T_3/100 g body weight to euthyroid and hypothyroid rats for a period of 10 days. These doses and duration of treatment have been shown to saturate the T_3 nuclear receptors and to elicit a maximal induction of ME activity (Mariash et al., 1980b).

In hypothyroid rat liver, ME activity and mass were not detectable. The absolute values obtained after T_3 injection (103 units/mg of protein and 14 pmol/mg of protein, respectively) indicated a significant stimulating effect of the hormone. The quantitation of ME mRNA level showed that in T_3-treated hypothyroid animals, a similar relative increase of ~10-fold above the control value was achieved as compared to T_3-injected euthyroid rats (Table I). This observation is consistent with the data of Oppenheimer et al. (1977), who reported that the administration of identical doses of T_3 to euthyroid and hypothyroid rats led to different absolute increases in ME activity which were, however, comparable when expressed as a percentage of the maximal response.

In euthyroid rat liver, T_3 treatment led to a parallel induction of ME mRNA level, ME activity, and ME mass (~11-fold above the basal needs), which provides direct evidence that T_3 stimulation of ME activity is fully accounted

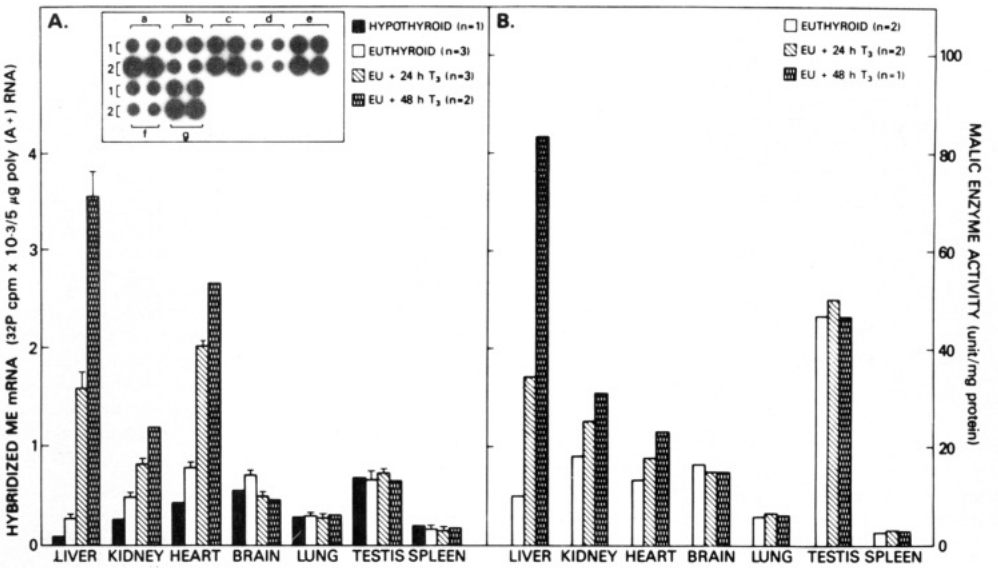

FIGURE 2: Effect of T_3 on hybridizable ME mRNA level (A) and ME activity (B) in different tissues. Euthyroid rats were injected with a single dose of T_3 (200 μg/100 g body weight) and sacrificed 24 and 48 h thereafter. Hypothyroid rats were used without hormonal treatment. ME mRNA levels (A) were quantitated by dot-blot hybridization assays. The autoradiogram presented in the inset illustrates the hybridization data obtained when blotting 5 μg of poly(A+) RNA extracted from each tissue. Lane 1, control; lane 2, 24-h T_3 induction: (a) liver; (b) brain; (c) kidney; (d) spleen; (e) testis; (f) lung; (g) heart. The hybridized cpm values were corrected by subtracting the filter and machine backgrounds (25 cpm). ME activity (B) was determined simultaneously. Mean values (±SD) of two or three independent experiments performed in duplicate are presented, except for hypothyroid animals tested only once.

for by an increase in the mass of mRNA encoding the enzyme. This direct correlation, indicating a full translation of the de novo synthesized mRNAs, also suggests that under sustained hormonal stimulation, the translation machinery remains in excess of the available cellular level of mRNA. However, when a single dose of T_3 (200 μg per 100 g body weight) was administered, the level of induction of ME mRNA after 48 h was higher (15.5-fold) than that of enzyme activity (10-fold) (Figure 2). This discrepancy suggests that in the acute hyperthyroid state some limitation of translation and/or stabilization of mRNA may be involved.

Accumulation of ME mRNA after T_3 stimulation has also been reported by Winberry et al. (1983) when using chicken liver cultured cells. In this system, only one ME mRNA was detected and was stimulated 100-fold by the addition of T_3. This higher induction as compared with our data may be due to either differences between species or the use of cell culture.

Although it is generally assumed that thyroid hormones act in the nucleus to alter mRNA synthesis, direct correlations between nuclear receptor–hormone complexes and rates of transcription of thyroid hormone responsive genes have not been demonstrated. Alternatively, hormonal regulation could occur at a posttranscriptional level involving the processing of the mRNA or its stability in the cytoplasm. The latter possibility seems to be unlikely in the case of ME mRNA since a similar half-life of ~10–12 h for this message was estimated from both the kinetics of appearance and disappearance of the molecule as assayed by cell-free translation after T_3 administration (Towle et al., 1981). We are currently conducting experiments to evaluate whether the change induced by the hormone in ME mRNA level can be explained by an increased transcriptional rate of the ME gene and/or by the stabilization of the heterogeneous nuclear RNA or the cytoplasmic mRNA.

Since in rat liver the level of stimulation of ME activity and mass after 10 days of T_3 treatment was directly proportional to the increase in hybridizable ME mRNA (Table I), and both ME mRNAs were induced to the same extent (see Table II) and were translated into malic enzyme peptide (Figure 1), we conclude that these two ME mRNAs code for an enzymatically active malic enzyme in vivo and that they are both regulated by T_3.

Table II: Relative Distribution and Level of Induction of the 27S and 21S ME mRNAs in Different Tissues[a]

tissue	21 S/27 S[b]	27 S/27 S[c]	21 S/21 S[d]
liver	4.26	12.4	11.8
kidney	0.35	3.2	3.0
heart	0.63	4.3	4.1
spleen	0.57	1.0	1.0
testis	0.42	0.9	0.9
lung	0.75	1.0	1.0
brain	0.94	0.8	0.8

[a] The autoradiograms of the Northern blot (Figure 3) were scanned after 8–16-h exposure, and the area under each peak was calculated. [b] Ratio of 21 S and 27 S in control. [c] Ratio of 27 S in induced and 27 S in control. [d] Ratio of 21 S in induced and 21 S in control.

Tissue Specificity of the Regulation of Malic Enzyme by Triiodothyronine. The ubiquitous distribution of ME has been demonstrated by immunological assay with ME antibody (Li et al., 1975). To obtain further insight into the tissue specificity of T_3 action, we compared the inducibility of ME in liver and other nonhepatic tissues. We attempted to correlate T_3 effects on ME activity and ME mRNA levels in these tissues with their known content of T_3 nuclear receptor (Oppenheimer et al., 1974) and responsiveness to thyroid hormones as determined by changes in oxygen consumption (Barker & Klitgaard, 1952). Three groups of tissues were analyzed: (a) kidney, heart, and liver (tissues containing receptors and T_3 responsive); (b) brain and lung (tissues with receptors but unresponsive); (c) spleen and testis (tissues lacking receptors and not responsive). In view of the hormonal conditions required to promote the maximal response in liver in a relatively short time (Oppenheimer et al., 1977), euthyroid rats were injected with a single dose of T_3 (200 μg/100 g body weight, i.p.) and sacrificed 24 and 48 h later. Untreated hypothyroid rats were also included. The amount of hybridizable ME mRNA and the level of ME activity in all tissues tested are presented in panels A and B, respectively, of Figure 2. In euthyroid animals, hybridizable ME mRNA and ME activity

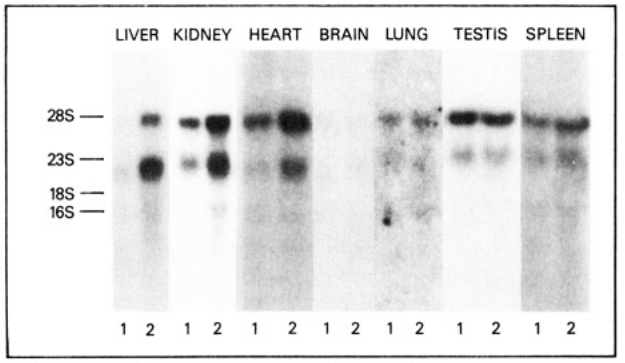

FIGURE 3: Northern blot analysis of ME mRNA in different tissues. Poly(A+) RNA (liver, 4.5 μg) and total RNA (kidney, 13 μg; heart, 22 μg; brain, 48 μg; lung, 37 μg; testis, 10 μg; and spleen, 13 μg) were size fractionated on a 1% agarose/2.2 M formaldehyde gel, transferred to a nitrocellulose filter, and hybridized with the ^{32}P-labeled prME probe. The autoradiograms were obtained after 24–72-h exposure. RNA was extracted from euthyroid rats (lane 1) and 48 h after injection of 200 μg of T_3/100 g body weight (lane 2). Migration of 28S, 23S, 18S, and 16S ribosomal RNA markers is indicated. (Identical patterns were obtained when total liver RNA was used.)

were detected in all tissues studied. Therefore, malic enzyme is constitutively expressed, although to different extents in the various tissues. T_3 treatment for 24 and 48 h resulted in a gradual increase of ME mRNA and ME activity in liver, kidney, and heart. As can be calculated from Figure 2, the respective folds of induction after 48 h were 15.5 and 10.3 in liver, 2.6 and 1.7 in kidney, and 3.4 and 1.72 in heart. The other tissues were not affected by T_3 although brain appeared to be slightly repressed with respect to the level of hybridizable ME mRNA. Analyses performed with hypothyroid animals substantiated the observation of a differential effect of T_3. Indeed, thyroid hormone deficiency led to a decrease in ME mRNA content only in the T_3-inducible tissues (liver, heart, and kidney). These hybridization data indicate that ME regulation by T_3 occurs at a pretranslational level not only in liver but also in other responsive tissues. Although T_3 effects are believed to be initiated at the nuclear receptor level (Oppenheimer, 1979), the lack of response to T_3 in brain and lung argues that the nuclear binding sites might be necessary but not sufficient for the hormonal regulation of ME.

We have shown (Magnuson & Nikodem, 1983) that both liver ME mRNAs are induced by T_3. To investigate the presence, distribution, and hormonal induction of the two ME mRNAs in the other tissues, poly(A+) or total RNA from euthyroid and T_3-treated rats was analyzed by Northern analysis (Figure 3, Table II). As can be seen, both ME mRNAs were present in control and T_3-treated animals regardless of the tissue tested. However, their relative distributions varied from tissue to tissue. The ratio of 21S to 27S mRNA in liver was 4.3, while in kidney, heart, spleen, testis, and lung it varied slightly (0.35–0.75), thus showing that these tissues, contrary to liver, contain preferentially the longer message (27 S). The two ME mRNAs appeared to be equally distributed in brain.

Since in liver these two mRNAs are translated to a protein of the same size (Figure 1), they could be transcribed either from the same gene or from very similar genes sharing a common coding region and then be processed differently by tissue-specific means. If there is indeed one single gene encoding ME, as suggested by Shows et al. (1970), the length difference between the two messages may be due to the presence of either multiple promoters with different transcriptional activities or multiple polyadenylation signals, which would result in heterogeneity in the 5′- or 3′-untranslated regions, respectively. In either case, tissue-specific factors must be involved in the selection of the promoter or the recognition of the polyadenylation site.

Further analysis of Northern blots revealed that T_3 administration did not change the relative distribution of the two mRNAs in individual tissues and that not only in liver but also in other hormone-responsive tissues (kidney and heart) both ME mRNAs were induced to the same extent (Table II). This observation suggests that the mechanism of T_3 regulation is probably identical for both mRNAs.

The molecular mechanism of hormonal regulations has been intensively investigated but remains unclear. The most studied system is the long terminal repeat of mouse mammary tumor virus (MMTV), containing the glucocorticoid-responsive sequences (Lee et al., 1981; Hynes et al., 1983; Chandler et al., 1983). Recently, it has been shown that these sequences also bind the glucocorticoid–receptor complex (Payvar et al., 1983; Scheidereit et al., 1983). It remains to be determined if the model for the regulation of MMTV genome expression by glucocorticoids could be applied to thyroid hormone regulation of ME gene expression, where the nuclear receptor–T_3 complex is presumed to interact with defined nucleotide sequences on the gene. A lag period of 6 h before ME mRNA induction by T_3 in liver (unpublished results), however, indicates that a different or additional mechanism is probably necessary for the regulated expression of the ME gene.

In conclusion, the results presented herein demonstrate that (a) liver ME synthesis is directed by two distinct and functional cytoplasmic mRNAs, (b) the level of expression of the ME gene and also its regulation by T_3 are tissue specific, and the hormonal stimulation affects both ME mRNAs to the same degree in the T_3-responsive tissues (liver, heart, and kidney), and (c) in these responsive tissues, ME activity is modulated by T_3 at a pretranslational level. Presently, we are characterizing the gene for ME. We will then address the question of whether or not specific DNA sequences are required for thyroid hormone regulation and determine the mechanism of formation of these two ME mRNAs.

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Registry No. T_3, 6893-02-3; ME, 9028-47-1.

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