THE MESSENGER RNA'S FOR THE LIVER ENZYMES TYROSINE AMINOTRANSFERASE AND TRYPTOPHAN OXYGENASE CONTAIN 40-50 % NON-CODING SEQUENCES

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SUMMARY. - The molecular weights of the mRNA's coding for two liver enzymes, tyrosine aminotransferase and tryptophan oxygenase, were determined to be 8.5×10^5 and 6.8×10^5, respectively, corresponding to 2430 and 1940 nucleotides. Taking into consideration the molecular weights of the enzyme subunits it is apparent that approximately half of the nucleotide sequences do not code for the enzyme subunits.

Recent determinations of the molecular weights of several mRNA's coding for major cell proteins revealed the presence of sequences serving unknown functions (1-7). The proportion of these sequences fluctuates from 25 % to 50 %.

In contrast to the relatively large amount of data existing on these mRNA's coding mostly for structural and secretory proteins, scant data on mRNA's coding for minor proteins, such as enzymes, are available.

In this paper we have determined the molecular weights of mRNA's for two liver enzymes, tyrosine aminotransferase [L-tyrosine:2-oxoglutarate aminotransferase (EC 2.6.1.5.)] and tryptophan oxygenase [L-tryptophan:oxygen oxidoreductase (EC 1.13.11.11.)]. Our data demonstrate, that approximately one half of the tyrosine aminotransferase and tryptophan oxygenase mRNA sequences do not code for the enzyme subunit.

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MATERIALS AND METHODS:

Male Wistar rats weighing 150-250 g, kept under standard conditions, were used. Hydrocortisone was a product of Merck, Darmstadt. Oligo(dT)-cellulose (grade T-2, chain length of 11 nucleotides) was purchased from Collaborative Research, Inc., Waltham, Massachusetts, sucrose RNase-free from Schwarz/Mann, Orangeburg. L-(4,5-^{3}H)leucine (55 Ci/mmol) was from Amersham Buchler, Braunschweig, creatine phosphate and creatine phosphokinase from Calbiochem, Lucerne. All other chemicals were of analytical grade obtained from Merck, Darmstadt and Serva, Heidelberg. Fresh wheat germ was a kind gift of GEG, Mannheim.

Preparation of polysomes. Rats were injected with 20 mg hydrocortisone/100 g body weight 6 hours prior to sacrifice. Polysomes were isolated by $MgCl_2$ precipitation from rat liver S-30 fractions according to Palmiter (8), essentially as described in Ref. 9.

Isolation of poly(A)-containing RNA. Poly(A)-containing RNA was isolated from the polysomal pellet by phenol-chloroform extraction and passage through oligo(dT)-cellulose in high salt buffer according to Ref. 9.

Sucrose gradient centrifugation. Linear 5 % to 20 % sucrose gradients were prepared in 25 mM Tris-HCl pH 7.5, 100 mM KCl and centrifuged at 280,000 g for 6 hours at 4°C in a SW 41 rotor of a Beckman centrifuge. Poly(A)-containing RNA was heated in the same buffer to 70°C for 5 minutes before centrifugation.

Centrifugation under denaturing conditions was performed in 5 % to 20 % sucrose gradients in 70 % formamide, 3 mM Tris-HCl pH 7.5, and 3 mM EDTA at 280,000 g for 24 hours at room temperature. Poly(A)-containing RNA was denatured in formamide containing buffer at 70°C for 5 minutes before layering on the gradients.

Following centrifugation, gradients were fractionated on an ISCO gradient fractionator. Portions of 0.4 ml were collected, adjusted to 100 mM KCl, and the RNA precipitated by 2 volumes of ethanol at -20°C.

In vitro translation and identification of the translation products. The poly(A)-containing RNA collected from the sucrose gradients was translated in vitro in a wheat germ system (9,10). Immunoprecipitation of the synthesized tyrosine aminotransferase and tryptophan oxygenase by specific antibodies (11), SDS acrylamide electrophoresis (12) and fluorography (13) were performed as described by Roewekamp et al. (9).

RESULTS AND DISCUSSION

In the first series of experiments we have subjected poly(A)-containing RNA isolated from polysomes of hydrocortisone treated rats to sucrose gradient centrifugation in aqueous buffer. The rats were treated with hydrocortisone 6

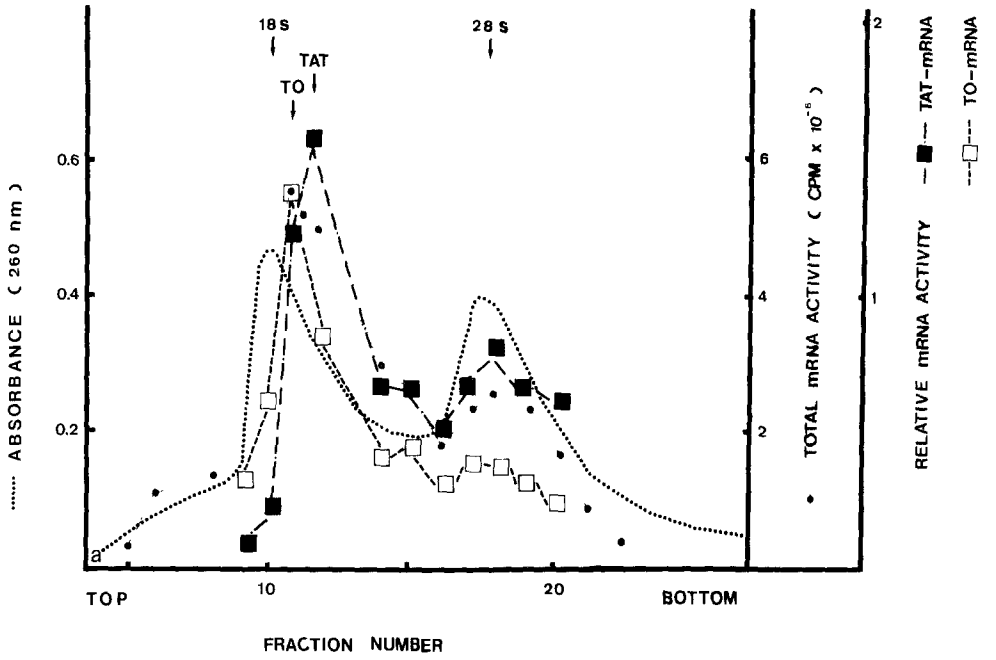

Figure 1a

Centrifugation of poly(A)-containing RNA from rat liver polysomes in aqueous 5-20 % sucrose gradients. Centrifugation time was 6 hours at 280,000 g. Fractions were collected, the RNA precipitated with ethanol and translated in vitro in the wheat germ system. The amount of tyrosine aminotransferase and tryptophan oxygenase synthesized was determined by specific immunoprecipitation, SDS gel electrophoresis and fluorography as described under Materials and Methods. (•••••) OD_{254}; (--●--) ^{3}H-leucine incorporation into total TCA precipitable material; (—■—) relative proportion of tyrosine aminotransferase synthesized (--□--) relative proportion of tryptophan oxygenase synthesized.

hours prior to sacrifice, as under these conditions the translatable messenger RNA's for tyrosine aminotransferase and tryptophan oxygenase have reached maximal concentration (9). The RNA fractions collected were then translated in vitro in the wheat germ system and the incorporation into total protein as well as into the tyrosine aminotransferase and tryptophan oxygenase moiety was determined. We have applied the method of specific immunoprecipitation, followed by SDS

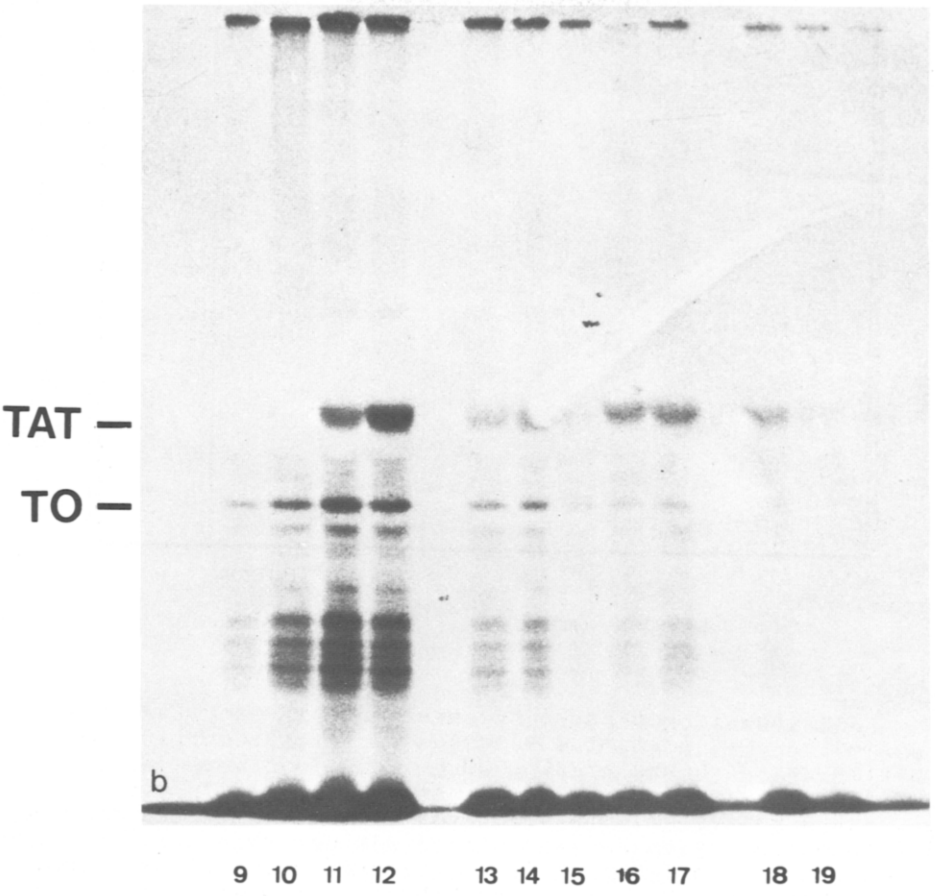

Figure 1b
Fluorogram of the immunoprecipitated material synthesized in vitro under the direction of mRNA derived from the various fractions of the sucrose gradient. The numbers refer to the fractions recovered from the sucrose gradient as depicted in Fig. 1a.
The arrows refer to the sites of migration of marker tyrosine aminotransferase and tryptophan oxygenase.

acrylamide gel electrophoresis of the immunoprecipitate and fluorography of the slab gel for the determination of specific protein synthesis. The fluorograms were scanned in a Vernon photometer and the amount of tyrosine aminotransferase

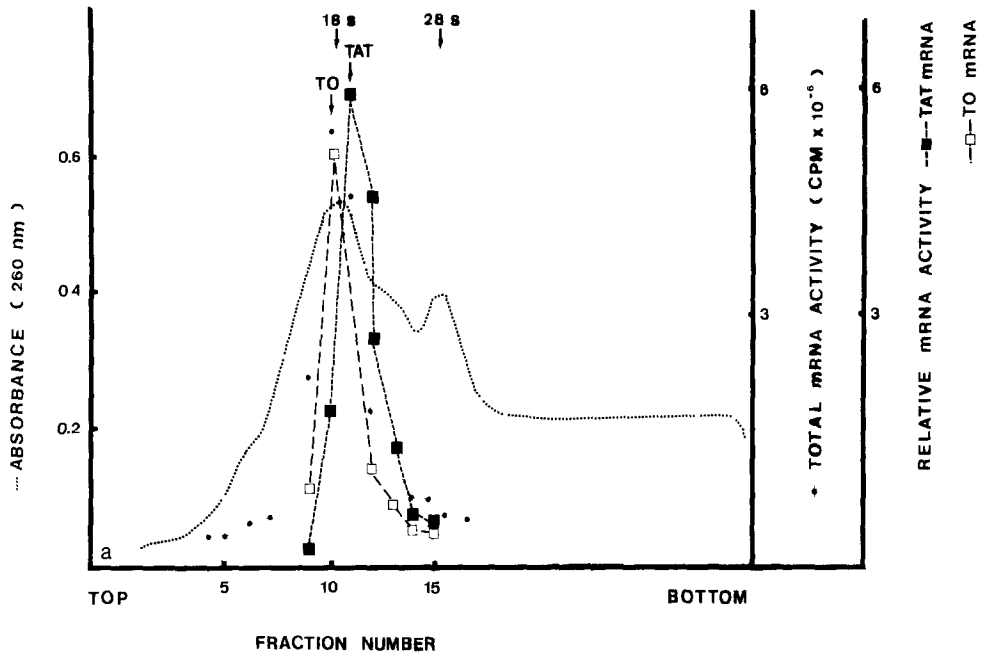

Figure 2a

Centrifugation of poly(A)-containing RNA from rat liver polysomes in 70 % formamide sucrose gradients. Centrifugation time was 24 hours at 280,000 g. Fractions were collected, the RNA precipitated with ethanol and translated in vitro in the wheat germ system. The amount of tyrosine aminotransferase and tryptophan oxygenase synthesized was determined by specific immunoprecipitation, SDS gel electrophoresis and fluorography as described under Materials and Methods. (•••••••) OD_{254}; (---•---) ^{3}H-leucine incorporation into total TCA precipitable material; (---■---) relative proportion of tyrosine aminotransferase synthesized (·—□—·) relative proportion of tryptophan oxygenase synthesized.

and tryptophan oxygenase synthesized was calculated from the height of the obtained scan peak and of the peak of parallely run labeled protein markers. The results are shown in Fig. 1a and 1b. The OD_{254} profile of the RNA reveals the presence of large amounts of 18S and 28S RNA. As the isolated RNA represents 4-5 % of the total polysomal RNA a large proportion of the isolated 18S and 28S RNA must represent ribosomal species. Two maxima of total protein synthesis are observed,

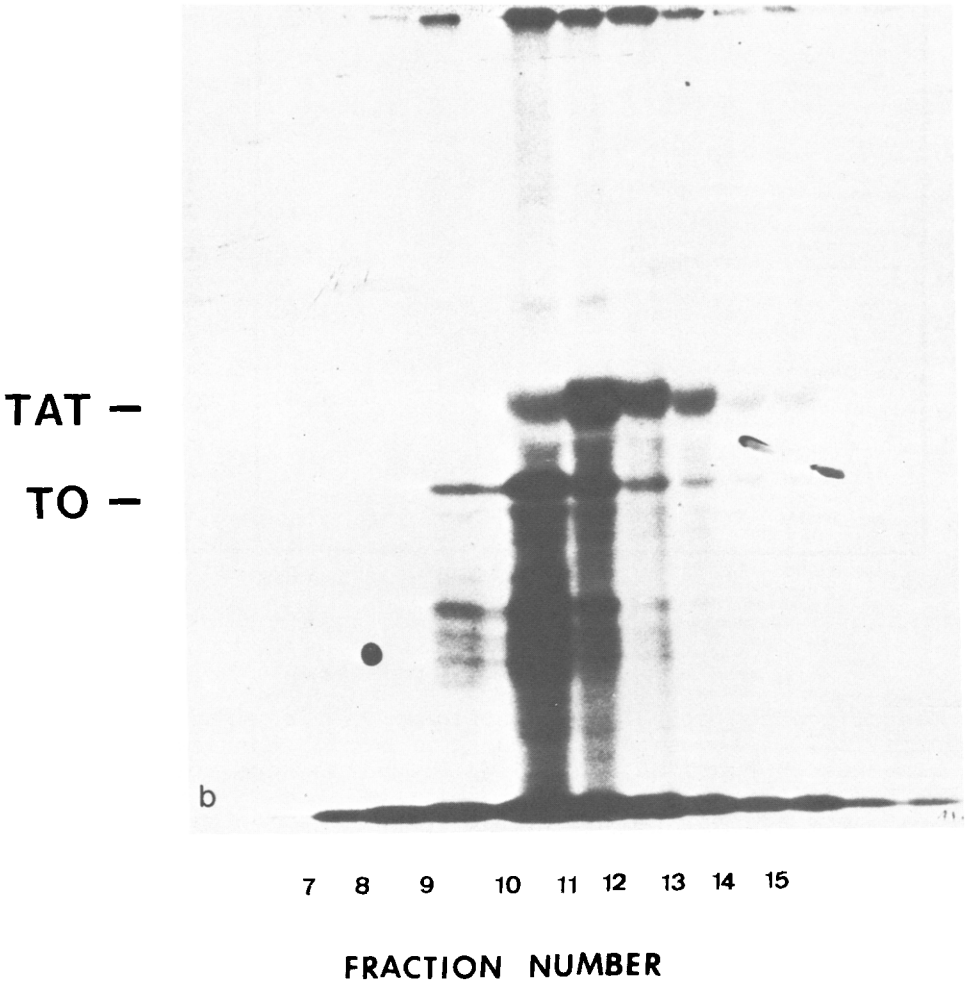

Figure 2b
Fluorogram of the immunoprecipitated material synthesized in vitro under the direction of mRNA derived from the various fractions of the 70 % formamide sucrose gradient. The numbers refer to the fractions recovered from the sucrose gradient as depicted in Fig. 1a.
The arrows refer to the sites of migration of marker tyrosine aminotransferase and tryptophan oxygenase.

a major one in the region of 18S-20S and a smaller additional one in the 28S region of the gradient. As with total protein synthesis, two peaks of tyrosine aminotransferase and tryptophan oxygenase synthesis, a major and a minor one, are seen. The major peaks of tyrosine aminotransferase and

tryptophan oxygenase mRNA activity appear in the 18-22S region of the gradient, tyrosine aminotransferase mRNA showing a slightly higher S-value (∿ 21S) than tryptophan oxygenase mRNA (∿ 19S). The small additional peak at the 28S and heavier region suggests the presence of aggregated, or, with rRNA associated material. We have therefore performed a second series of experiments in which the isolated RNA was separated on sucrose gradients in 70 % formamide (Fig. 2a and 2b). Under these conditions only one peak of total, as well as of specific protein synthesis, was observed, indicating that the minor peak (see Fig. 1a) indeed resulted from aggregation or from association of mRNA with rRNA. Both the total as well as the tryptophan oxygenase synthesis showed their maximum at ∿ 18S, whereas the maximum of tyrosine aminotransferase synthesis was at the ∿ 20S region of the gradient.

On the basis of the results obtained with formamide gradients, the molecular weights of tyrosine aminotransferase and tryptophan oxygenase mRNA's have been calculated at 850,000 and 680,000, corresponding to 2430 and 1940 nucleotides, respectively. Taking into account the molecular weights of the subunits of tyrosine aminotransferase and tryptophan oxygenase which are 44,000 and 42,000, respectively (14,11), we conclude that approximately 40 % to 50 % of the nucleotide sequences do not code for the enzyme subunit (see Table 1). The results obtained with the aqueous sucrose gradients give a higher molecular weight for the two mRNA's (see Table 1). Assuming that the poly(A)-tail of the two mRNA's is of a mean size of 100 nucleotides, it is obvious that almost half of the transcribed region of tyrosine aminotransferase and tryptophan oxygenase mRNA's serve unknown functions.

TABLE I. MOLECULAR WEIGHT ESTIMATES OF TYROSINE AMINOTRANSFERASE AND TRYPTOPHAN OXYGENASE mRNA's IN AQUEOUS AND FORMAMIDE CONTAINING SUCROSE GRADIENTS

	tyrosine aminotransferase		tryptophan oxygenase	
	aqueous	formamide	aqueous	formamide
Polypeptide (molecular weight)	44 000 (11)		42 000 (14)	
Amino acid residues	400		382	
mRNA (molecular weight)[+]	8.8×10^5	8.5×10^5	7.8×10^5	6.8×10^5
Number of nucleotides	2520	2430	2230	1940
Extra non-coding nucleotides in mRNA (%)	52	51	48	41

[+] The molecular weights were calculated taking the molecular weights of 18S and 28S ribosomal RNA as 7×10^5 and 1.75×10^6, respectively (15).

We conclude thus that the size of the non-translated region of mRNA for the two enzymes tyrosine aminotransferase and tryptophan oxygenase is in the same range of magnitude described for mRNA's coding for major proteins.

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