THE AMBIGUITIES IN THE RABBIT HEMOGLOBIN : EVIDENCE FOR A MESSENGER RNA TRANSLATED SPECIFICALLY INTO HEMOGLOBIN°

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SUMMARY

Fractional residues of amino acids present in the αTIV peptide from the α chain of rabbit hemoglobin cannot be explained by an ambiguous translation of a unique template; they are the consequence of the translation of at least two different messengers RNA.

Fractional residues of amino acids are present in several positions of the α chain of rabbit hemoglobin; the amino acid sequence of the rabbit α chain is not unique, as suggested by non integral values of amino acid residues and demonstrated by sequential degradation (Weisblum et al 1965, Von Ehrenstein 1966).

The question arises whether the amino acid multiplicities are due to the ambiguous translation of a unique template, or to a multiplicity of α chain templates. In order to answer this question, we have investigated the role of amino acyl tRNA, activating enzymes, and mRNA, in the ambiguity of the peptide αTIV of rabbit hemoglobin. The experimental evidence demonstrates the role of the template.

METHODS

Cell free incubation of rabbit reticulocytes in the presence of ^{14}C labeled tyrosine, 10 microcuries per sample, preparation of hemoglobin and globin, and

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tryptic digestion were performed as described by Schapira et al (1966). Chains were separated by CMC chromatography (Dintzis 1961). Thin layer electrophoresis and chromatography on cellulose were used to detect either one αTIVa (containing leucine), or more rarely two peptides a and b (containing valine), or very rarely one αTIVb. Since αTIV contains tyrosine it was easily revealed by Millon reagent. Separation of the two peptides was accomplished by a combination of high voltage electrophoresis (Schapira et al 1966) and chromatography on "P" resin from Technicon, 4 % cross-linked, at 50°C on a 23 cm column, using a linear gradient of pyridine/formic acid 0,1 N pH 3,5/2 N pH 5. The peptides were analyzed by Rosa and Labie, using an ultra micro method. Transfer RNA was prepared from rabbit reticulocytes according to Allen et al (1960) and activating enzymes according to Allen and Schweet (1962). Charging of tRNA was performed according to Rifkin et al (1966) and to Hardesty et al (1963), and RNA III from rabbit reticulocytes sedimenting at 9S was prepared according to Kruh et al (1964). The radioactivity of tyrosine present in the isolated peptide was counted in a Packard liquid scintillator with an efficiency of 60 %.

RESULTS

The results are summarized in the table, which gives the values of radioactivity (in counts/min.) of tyrosine contained in the peptides αTIVa and b. The table shows two major results :

- When ribosomes of one type are incubated with pH 5 enzyme of tRNA + Activating enzymes prepared from rabbits of the other type, only the peptides corresponding to the donor of the ribosome fraction are radioactive. Transfer from the other type do not influence the incorporation pattern.

- When ribosomes of one type are incubated with RNA III of the other type, the exogenous RNA modifies the basic incorporation pattern and is translated into its specific hemoglobin. Two experiments were performed : the ribosomes were of the αTIVa type in both, while the mRNA was prepared from a TIVa + b type rabbit in the first experiment and from αTIVb type rabbit in the second. In both instances the TIVb displayed a significant amount of radioactivity. The

validity of these results is supported by the fact that the portion of the column eluted between the two peptides TIVa and b contained much less radioactivity than the peaks.

Table I.

Exp.n°	Ribosomes	pH5 Enzyme	tRNA + Activator enzyme	RNA III	Results αTIV b	between peaks	a	Comment
1	b		ba		144	41	2	one peak
2	ba	ba			23	4	20	two peaks
3	b	b			103	11	5	one peak
4	b	b		ba	121	11	41	two peaks
5	a	a			62	250	3000	one peak
6	a	a		b	255	75	1570	two peaks

b is αTIVb (valine in position 17)
ba is αTIVb and a (valine and leucine in position 17)
a is αTIVa (leucine in position 17)
Numbers are counts per min. of tyrosine ^{14}C
The presence of two peaks is assumed when counts of the intermediary tubes "between peaks" are lower than those of both peaks; they are higher than those of the peaks we concluded to the presence of one peak followed by a trail. Amino acid analysis confirmed the presence of leucine in peptide a and valine in peptide b.

DISCUSSION

Rifkin et al (1966) have performed experiments in which ribosomes from one strain of mice were incubated with aminoacyl tRNA from another strain, but their results have not been published to our knowledge. Von Ehrenstein (1966), discussing amino acid ambiguities, was in favor of a variable translation of a unique template. Our results however are negative in that respect and are not consistent with an anomalous charging of tRNA by a modification of a tRNA itself or of an activating enzyme.

We were therefore led to examine the role of the template, RNA III was shown to function as a messenger RNA, determining the type of αTIV peptide which is synthesized.

In conclusion we have observed, in a mammaliam system, an RNA messenger

for hemoglobin, displaying the only specific property required from a messenger : the ability to direct the biosynthesis of a specific type of polypeptide chain.

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