Correlation between the presence of tRNA_{His}^{GUG} and the erythropoietic function in foetal sheep liver

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ABSTRACT

Histidyl-tRNAs from foetal and adult sheep liver were compared to their reticulocyte counterparts. The combination of various techniques revealed the existence of two histidyl-tRNA species in reticulocytes, one of which was not retained on acetylated DBAE-cellulose columns and was guanylatable. Three histidyl-tRNA isoacceptors were identified in foetal liver. Two of these species were not adsorbed on acetylated DBAE-cellulose but only one was found to be guanylatable. An identical chromatographic behaviour on RPC-5 columns was observed for guanylated histidyl-tRNAs from both origins. These results suggest the occurrence of a GUG anticodon in these guanine-accepting tRNAs. In foetal liver the amount of guanylatable histidyl-tRNA was estimated to be 7% of the total tRNA population. This observation is in agreement with the erythropoietic function of liver during the foetal life.

INTRODUCTION

In highly differentiated cell systems devoted to the specialized synthesis of a predominant protein, a "functional adaptation" of the tRNA population has been demonstrated (1). The most striking example of a correlation between the amino acid composition of a protein and the relative proportions of the specific tRNAs was observed in the posterior silk gland of Bombyx mori at the 5th instar during fibroin synthesis. Only four specific tRNAs: tRNA_{Ala}, tRNA_{Gly}, tRNA_{Ser} and tRNA_{Tyr} accounted for about 80% of the total tRNA population (2). Moreover, the ratio of the two major isoaccepting species of alanine becomes inverted during the period of specialization and maximal activity of the gland (3).

This selective adjustment was also observed during the globin synthesis in reticulocyte cells. In agreement with the unusually high content of histidine residues in globin compared to most proteins, a quantitative and qualitative adaptation of tRNA_{His} was found in rabbit reticulocytes (4, 5). However, in mammalian tissues a clear evidence for such a
"functional adaptation" could be hardly obtained because of the heterogeneity with regard to the stage and to the type of the differentiated cells. Nevertheles, through means of the erythroid cell system, foetal liver which is transitionally synthesizing globin could provide an attractive model to study the adaptation of the tRNA pool to the synthesis of a specific protein. Owing to the high histidine content in adult and foetal hemoglobin (6), we examined the tRNA\textsuperscript{His} population in adult and foetal sheep liver and performed parallel studies using reticulocyte cells. This paper reports the characterization of an isoacceptor of tRNA\textsuperscript{His} existing in a significant amount in foetal liver. This tRNA species has chromatographic properties similar to those of the minor reticulocyte tRNA\textsuperscript{His}. Additional results obtained from chemical and enzymatical modification experiments strongly suggest the presence in both tRNAs of a GUG anticodon, the most efficient one for the translation of the CAC triplets coding for histidine in globin messenger RNAs (7).

**MATERIAL AND METHODS**

*Chemicals.* All chemicals were analytical laboratory grade. Acrylamide and bis-acrylamide were provided by Eastman Kodak and Labo International B.V. (Holland) respectively. RPC-5 column packing was prepared by the method of Kelmers and Heatherly (8).

Acetylated DBAE-cellulose was purchased from Serva-Fein Biochemical, Heidelberg. Uniformly labeled \([^{14}\text{C}]\)-histidine (300 mCi/m mole), [2-5\(^3\text{H}\)]-histidine (45 Ci/m mole) and [8-\(^{14}\text{C}\)]-guanine chlorhydrate (50 mCi/m mole) were obtained from CEA (Saclay, France). \([8-^3\text{H}]\)-guanosine (7 Ci/m mole, The Radiochemical Centre, England) was converted to guanine by acid hydrolysis.

*tRNAs and aminoacyl-tRNA synthetases.* Foetal lamb livers were obtained from "Préalpes" ewes on the 84\(^{th}\) day of pregnancy. Adult livers were provided by 'Préalpes' sheeps and New Zealand rabbits. All tRNA preparations were carried out as previously described (9).

Aminoacyl-tRNA synthetases from sheep liver were prepared according to Miench and Berg (10) and stored at -20°C in 50% glycerol. This preparation was also used for testing amino acid acceptor activity of foetal tRNA since no differences were observed between adult and foetal enzymes. Reticulocytes were obtained from New Zealand rabbits and processed as indicated by Gilbert and Anderson (11) for preparing a reticulocyte lysate. The final 15,000 x g supernatant was immediately used for tRNA preparation. Reticulocyte aminoacyl-tRNA synthetases were a crude 105,000 x g supernatant from

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the initial lysate, extensively dialyzed against a 20 mM Tris-HCl buffer, pH 7.5, containing 100 mM KCl, 1 mM DTE, 10% glycerol and stored at -20°C in 50% glycerol.

Aminoacylation of tRNA. Standard assay: The reaction mixture (150 μl) contained per ml: 100 μmoles of Tris-HCl, pH 7.5, 8 μmoles of MgCl₂, 6 μmoles of ATP (sodium salt) pH 7, 100 μmoles of KCl, 25 nanomoles of the labeled amino acid, 25 to 250 nanomoles of each of the other unlabeled amino acids (except for Asn, Cys, and Gln), about 600 picomoles of expected aminoacylatable tRNA and saturating amounts of the aminoacyl-tRNA synthetase preparation. The incubation was run for 20 minutes at 37°C. The reaction was stopped by addition of 5% cold TCA containing 1% of the unlabeled cognate amino acid and the precipitate was trapped on Whatman GF/C glass fiber filters. The discs were washed with the same precipitating TCA solution, dried and counted in a toluene-Omnifluor scintillation mixture with an efficiency of 80% and 30% for ¹⁴C and ³H respectively. For double-labeled samples, radioactivity was counted at 60% and 18% efficiency for ¹⁴C and ³H respectively, the overlap of the ¹⁴C into the ³H channel being about 8%. ³H counts were subsequently corrected for this overlap.

Preparative tRNA aminoacylation reaction: the reaction mixture as described above was scaled up to give 200,000 to 300,000 cpm of ³H and 50,000 to 60,000 cpm of ¹⁴C. After standard incubation, it was immediately adjusted to pH 4.5. The acylated tRNA was isolated by phenol extraction and ethanol precipitation.

Assay for guanine insertion in Q-deficient tRNAs. The purified E. coli guanine insertion enzyme was a kind gift of Dr S. Nishimura. The assay was performed as indicated by Okada et al. (12). The reaction mixture (100 μl) contained per ml: 70 μmoles of Tris-HCl, pH 7, 20 μmoles of MgCl₂, 5 to 10 A₂₆₀ units of the crude tRNA tested, 10 nanomoles of radioactive guanine and 100 μl of the purified enzyme preparation. The incubation was run for one hour at 37°C. The reaction was stopped by addition of 10% TCA and the insoluble radioactivity was trapped on Whatman GF/C glass fiber filters. The discs were washed with 5% TCA and counted in a toluene-Omnifluor scintillation mixture. Large-scale tRNA guanylation was performed as described above except that ¹⁴C-guanine was replaced by ³H-guanine. After incubation, the reaction mixture was adjusted to pH 4.5 and the guanylated tRNA was isolated by phenol extraction and ethanol precipitation. This tRNA was subsequently acylated with unlabeled histidine and processed as described above.
Cyanogen bromide treatment. Crude tRNA was processed as indicated by Katze (13).

Chromatographic procedures. Reversed phase chromatography using an RPC-5 column was an adaptation of the technique described by Pearson et al. (14). Equilibration buffer (pH 4.5) contained 0.4 M NaCl, 10 mM sodium acetate, 10 mM MgCl₂ and 1 mM EDTA. Usually, carrier tRNA was added to radioactive tRNA and the mixture was loaded on the column in a few millilitres of the equilibration buffer. Elution was performed with a NaCl gradient at a flow rate of 35 ml/hour. TCA-precipitated radioactivity in collected fractions was determined as stated in the standard aminoacylation technique.

Fractionation of tRNA by acetylated DBAE-cellulose chromatography was performed following the procedure described by McCutchan et al. (15). Two grams of DBAE-cellulose equilibrated with a pH 8 buffer (buffer I in Ref 15) were packed into a 0.9 cm x 10 cm glass column. tRNA which was not retained on the column was eluted by buffer I; the fraction which was specifically adsorbed was eluted with a pH 4.5 buffer (buffer G in Ref 15).

Electrophoretical procedures. Two-dimensional polyacrylamide gel electrophoresis was performed in 10% and 20% polyacrylamide gels according to Fradin et al. (16). Unacylated tRNAs were fractionated and detected by staining with methylene blue. Then the tRNAs were extracted with phenol and precipitated with ethanol in the presence of ribosomal RNA added as a carrier. The material was dissolved in an appropriate buffer and tested for the presence of tRNAHis. Frequently, a 9% - 18% acrylamide gel gradient was used to improve tRNA fractionation. Labeled aminoacyl-tRNA samples for electrophoresis purpose were stabilized by HNO₂ treatment (17). After staining and destaining the gel was dried under vacuum onto a sheet of 3MM paper (Whatman). The radioactive bands were detected either by autoradiography or fluorography using Kodak Royal X-omat film according to the procedure of Bonner and Laskey (18).

RESULTS

I - Characterization of sheep liver and reticulocytes tRNAHis by enzymatic tests

A - Histidine acceptor activity

Crude tRNAs. Table I shows that the same acceptor activity for histidine was found when adult and foetal tRNA from sheep liver were compared. By contrast, in rabbit reticulocyte tRNA, acceptor activity for
TABLE I

Histidine and guanine acceptance of crude tRNAs and their corresponding fractions separated by DBAE-cellulose chromatography

<table>
<thead>
<tr>
<th></th>
<th>Histidine acceptance</th>
<th>Guanine acceptance</th>
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<tbody>
<tr>
<td></td>
<td>Crude tRNA</td>
<td>Pool I</td>
</tr>
<tr>
<td>Reticulocytes</td>
<td></td>
<td></td>
</tr>
<tr>
<td>57</td>
<td>12.5</td>
<td>530</td>
</tr>
<tr>
<td>Foetal liver</td>
<td>25</td>
<td>3.6</td>
</tr>
<tr>
<td>Adult liver</td>
<td>23</td>
<td>3.2</td>
</tr>
</tbody>
</table>

The values were expressed in picomoles of histidine and guanine per A₂₆₀ unit.

Histidine was two times higher than in sheep liver tRNA.

tRNA fractions obtained by DBAE-cellulose column chromatography. When crude tRNA from either sheep liver or reticulocytes was chromatographed on DBAE-cellulose, two fractions were obtained. The non-adsorbed tRNA was eluted at pH 8 as "Pool I"; the tRNA that specifically interacts with the resin by means of the cis-diol groups present on the hypermodified Q nucleotide (15) was later eluted at pH 4.5 as "Pool II". "Pool I" of liver tRNA did accept about 3.4 picomoles of histidine per A₂₆₀ unit whereas a value of 12.5 picomoles per A₂₆₀ unit was obtained for reticulocyte tRNA. "Pool II" was strongly enriched in tRNA^His, accepting capacities being 420 and 530 picomoles per A₂₆₀ unit for liver and reticulocyte tRNA respectively.

B - Radioactive guanine incorporation into various tRNAs by E. coli insertion enzyme

As previously reported by Dubrul and Farkas (5) a reticulocyte enzyme present in the crude supernatant catalyzes the insertion of a guanine residue in one of the isoacceptors of tRNA^His from rabbit reticulocytes. A guanine insertion enzyme was also found in E. coli (12). This enzyme was able to catalyze the exchange of a radioactive exogeneous guanine with the guanine residue present at the first position of the anticodon in a minor isoaccepting tRNA species from ascites tumor cells (12). In the present work this enzyme was used for exploring the relative proportions of the GUG and QUG anticodons in liver and reticulocyte tRNA^His.
Crude tRNAs. Foetal sheep liver tRNA incorporated about 1.6 picomole of guanine per A260 unit. Adult sheep liver tRNA did not show significant incorporation. Under the same experimental conditions, reticulocyte tRNA incorporated 11.5 picomoles of guanine per A260 unit (Table I).

tRNA fractions obtained from a DBAE-cellulose column. No guanine was incorporated in the tRNA of "Pool II" regardless to its origin. In "Pool I", foetal liver tRNA incorporated about 1.8 picomole of guanine per A260 unit; adult liver tRNA showed values lower than 0.5 picomole per A260 unit and for reticulocyte tRNA, values amounted to 11.5 picomoles of guanine per A260 unit (Table I). These results suggest that at least two isoacceptors of tRNA^His are present in sheep liver and reticulocytes. The existence of two different species would be consistent with the modification G→Q known to occur at the first position of the anticodon in tRNA^His (19).

II - Study of the tRNA^His heterogeneity in liver and reticulocyte cells

A - Fractionation of tRNAs by PAGE

Surprisingly, acylated or unacylated tRNA^His always showed a diffuse electrophoretic pattern independent of the origin and of the purity of the checked samples. On the contrary, tRNA^Ser, tRNA^Leu and tRNA^Gly exhibited a very clear picture (not shown). An explanation for such a difference could lie in the interaction between the Q nucleotide of tRNA^His and the borate ions present in the electrophoresis buffer. When histidyl-tRNAs in crude tRNAs from various sources were analyzed by electrophoresis in a polyacrylamide gel gradient, subsequent detection by fluorography showed at least two bands of variable intensities but a different distribution of the radioactivity was observed between various samples (Fig. 1). Furthermore, mapping of foetal and adult liver crude tRNA by two-dimensional electrophoresis revealed more than two tRNA^His species in every case. Fig. 2 showed the results obtained with foetal liver tRNA.

B - RPC-5 column fractionation of histidyl-tRNAs

Crude tRNAs. When crude tRNAs from foetal and adult livers acylated with 3H- and 14C-histidine respectively were cochromatographed on a RPC-5 column, essentially two histidyl-tRNA peaks could be detected in each sample (Fig. 3a) with a marked difference in the relative amount of the early eluted peak. The profile obtained with crude reticulocyte tRNA was shown in Fig. 3b. After CNBr treatment, the major peak of reticulocyte histidyl-tRNA underwent a dramatic shift, whereas the minor isoacceptor appeared to be CNBr-insensitive (data not shown) in agreement with the published results of Dubrul and Farkas (5).
Figure 1. Electrophoretic pattern of $^3$H-histidyl-tRNAs on polyacrylamide gel. $^3$H-histidyl-tRNA samples (~300,000 cpm) in 20 µl of the loading buffer were electrophoresed at 4°C in a slab gel gradient (9% - 18%) (200 x 200 x 2 mm), during 38 h with Xcff as tracking dye. The radioactive bands were detected by fluorography. 1. Rabbit liver. 2. Rabbit reticuloocytes. 3. E. coli. 4. Foetal sheep liver. 5. Adult sheep liver.

Figure 2. Mapping of foetal sheep liver tRNA by two dimensional electrophoresis on polyacrylamide slab gel. After the electrophoretic run the tRNAs were stained with methylene blue, eluted and tested for amino acid acceptance. Spots charging histidine are indicated by arrows.
Figure 3. RPC-5 chromatographic profiles of foetal (H) and adult (C—) sheep liver histidyl-tRNAs (a) and reticulocyte histidyl-tRNAs (b).

The column (0.6 x 90 cm) was loaded with radioactive samples. Elution was performed with a linear gradient (total volume 200 ml) from 0.4 M to 0.7 M NaCl in equilibration buffer pH 4.5. Volume of the fractions: 1.3 ml. The results were expressed in picomoles of histidine per fraction.

When the same procedure was carried out separately on adult and foetal liver tRNAs, the same CNBr-effected shift was observed in every case for the large majority of histidyl-tRNAs but a small CNBr-insensitive peak could be detected on foetal material only (not shown).

tRNA fractions obtained from DBAE-cellulose columns. A better visualization of the heterogeneity of histidyl-tRNA in liver was obtained by RPC-5 fractionation of "Pool I" and "Pool II" resulting from a DBAE-cellulose chromatography. Fig. 4a, 4b and 4c showed histidyl-tRNAs profiles of a mixture of "Pool I" and "Pool II" from foetal liver, adult liver and reticulocyte tRNA respectively. In all cases histidyl-tRNA from "Pool II" eluted as a single peak. Histidyl-tRNA from "Pool I" of reticulocytes was also chromatographically homogeneous. In contrast, two and three histidyl-tRNA peaks were found in foetal and adult liver respectively.

A careful examination of the chromatographic patterns suggested that the slower eluted peak in "Pool I" of foetal liver could be similar to the single species observed in "Pool I" of reticulocytes. This postulated identity was supported by cochromatographic fractionation on RPC-5 column of both "Pool I" from reticulocytes and foetal liver (Fig. 5a). A similar
Figure 4. RPC-5 cochromatography of histidyl-tRNAs from "Pool I" (³H—) and "Pool II" (¹⁴C—) obtained by DBAE-cellulose fractionation.

a) foetal liver. b) adult liver. c) reticulocytes. A 0.4 M - 0.75 M NaCl gradient (total volume 200 ml) in equilibration buffer pH 4.5 was used for elution in each instance.

Figure 5. RPC-5 cochromatography of reticulocyte and liver histidyl-tRNA present in "Pool I" obtained by DBAE-cellulose fractionation.

a) foetal liver (³H—), reticulocyte (¹⁴C—), b) adult liver (³H—), reticulocyte (¹⁴C—).

The insets show the actual proportion of the reticulocyte-like histidyl-tRNA present in foetal (a') and adult (b') liver relative to the Q-lacking reticulocyte histidyl-tRNA.

The elution was as in fig. 4.
experiment using "Pool I" from adult liver showed a minimal fraction eluting with histidyl-tRNA of reticulocytes (Fig. 5b). In comparing our data on the histidyl-tRNA heterogeneity in the various tRNAs studied, the corrected values of radioactivity in each peak of "Pool I" and "Pool II" were expressed as the fraction of the total radioactivity recovered in each experiment (Table II).

III - Chromatographic fractionation of guanylated tRNAs.

In order to obtain more conclusive evidence for the presence of tRNA<sub>HisGUG</sub> in foetal liver, the following experiments were performed:

- <sup>3</sup>H-guanylated tRNA from "Pool I" of reticulocytes and foetal liver, acylated with unlabeled histidine, were cochromatographed on RPC-5 columns with their respective unguanylated <sup>14</sup>C-histidyl-tRNA counterparts. The chromatographic profile of reticulocyte tRNA from "Pool I" showed two major <sup>3</sup>H-guanylated peaks (Fig. 6a). The first one probably represented the asparaginyl-tRNA as previously reported by Farkas and Chernoff (20). The second one corresponded to histidyl-tRNA<sub>HisGUG</sub> as indicated by the coincidence between the <sup>14</sup>C and <sup>3</sup>H radioactivity curves. An incomplete acylation of the guanylated tRNA may be responsible for the shoulder observed in the guanylation profile since histidyl-tRNA has been seen to be eluted before tRNA<sub>HisGUG</sub> (personal data).

- The chromatographic pattern of <sup>3</sup>H-guanylated tRNA from foetal liver (Fig. 6b) was very close to that observed for reticulocyte-tRNA, except for

| TABLE II |
| Relative distribution of the reticulocyte and liver histidyl-tRNAs in the tRNA fractions separated by DRAE-cellulose chromatography |

<table>
<thead>
<tr>
<th></th>
<th>Reticulocytes</th>
<th>Foetal liver</th>
<th>Adult liver</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pool I</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>22 %</td>
<td>13.5 %</td>
<td>12.7 %</td>
</tr>
<tr>
<td>Peaks from RPC-5 column</td>
<td>22 %</td>
<td>6 %</td>
<td>5.5 %</td>
</tr>
<tr>
<td>1</td>
<td>2</td>
<td>3</td>
<td></td>
</tr>
<tr>
<td>Pool II</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Single peak from RPC-5 column</td>
<td>78 %</td>
<td>86.5 %</td>
<td>87.3 %</td>
</tr>
</tbody>
</table>

The relative amount of each peak from RPC-5 columns (Fig. 4 a, b, c) was expressed as the percentage of the total histidyl-tRNA population.
Figure 6. RPC-5 cochromatographic profiles of guanylated and acylated tRNA\textsubscript{Hist} from "Pool I" obtained by DBAE-cellulose fractionation.  

a) reticulocytes: \textsuperscript{3}H-guanine (---), \textsuperscript{14}C-histidine (---),
b) foetal liver: \textsuperscript{3}H-guanine (---), \textsuperscript{14}C-histidine (---),
c) guanylated foetal liver tRNA (\textsuperscript{3}H---) and acylated reticulocyte tRNA (\textsuperscript{14}C—).  

Elution conditions are the same as in fig. 3.

the first eluting peak which was relatively reduced. In agreement with chromatographic data in Fig. 5a, two major species of histidyl-tRNA were found but only the later eluting peak was guanylated.

The results of an RPC-5 cochromatography of \textsuperscript{14}C-histidyl-tRNA from reticulocytes and of \textsuperscript{3}H-guanylated histidyl-tRNA from foetal liver (Fig. 6c) clearly demonstrated an identical behaviour of guanylatable histidyl-tRNAs from both origins.

DISCUSSION

Among the large number of reports describing the modifications of the tRNA pool accompanying changes in nutritional and physiological conditions or the onset of pathological processes (for a review see 21) the most convincing support of the "functional adaptation" theory (1) is the drastic modification of the tRNA population in the posterior silk gland of \textit{Bombyx mori} at the fifth instar (3). In mammalian cell systems, the only possibility for showing a similar harmonization between the tRNA population and the composition of the synthesized proteins can be expected from the study of a differentiating cell line existing in a large amount in a specia-
lized tissue. Such conditions are found in foetal liver. In this organ, at mid-pregnancy, the erythroblastic cells account for about half of the hepatic tissue and cells synthesizing globin represent one third of the developing red blood line (personal observations). In view of the abundance of CAC triplets coding for histidine in globin messenger RNAs (7, 22, 23) and considering the preferential G-C base pairing in codon-anticodon interaction, we tried to evaluate the relative proportions of GUG and GUU anticodons in the tRNA\textsuperscript{His} populations from sheep liver and reticulocytes.

The combination of two analytical procedures, i.e. DBAE-cellulose and RPC-5 chromatography, demonstrated the existence of three and four histidyl-tRNAs in foetal and adult liver respectively. In both cases, the late eluting species cochromatographed on RPC-5 with the minor species of reticulocyte histidyl-tRNA. Moreover this liver tRNA was found to be five times more abundant in foetal than in adult tissue. This peculiar isoacceptor only existed in the tRNA fraction which was not adsorbed on the DBAE-cellulose column. The results of the guanylation tests were consistent with the putative presence of a GUU anticodon in this tRNA. In our experiments, the tRNA\textsuperscript{His} from reticulocytes which eluted in "Pool I" of DBAE columns also seemed to be an appropriate substrate for the \textit{E. coli} guanylation enzyme. This result was consistent with the known presence of a significant quantity of tRNA\textsuperscript{His} with a GUU anticodon in reticulocytes (5). Okada et al. (12) using the same enzyme preparation did find for foetal and adult rat liver values similar to those obtained in the present work but these authors reported very low incorporation level of guanine in crude reticulocyte tRNA. On the basis of the results provided by the chromatographic fractionation of the guanylated tRNA from foetal liver, conclusive evidence was obtained for the existence of a guanylatable histidyl-tRNA in this tissue. Moreover this tRNA showed a chromatographic behaviour similar to that exhibited by the guanylatable reticulocyte tRNA which did not contain the Q nucleotide (5). The extra guanylated peak observed in the chromatographic pattern of foetal liver (Fig. 6b) seemed to contain a minor isoaccepting species of histidyl-tRNA which was not found in reticulocyte-tRNA.

The most significant results of the present work could be summarized as follows: Using complementary techniques, we were able to visualize a number of isoacceptors of histidine in liver and reticulocyte tRNA, the relative proportions of which have been quantified. In agreement with previous reports (5, 24) the major histidine isoacceptor did contain in both instances a GUU anticodon. Moreover, we established in foetal liver the...
presence of a minor histidine isoacceptor postulated to contain a GUG anti-
codon. Assuming an average value of 1,400 picomoles per A$_{260}$ unit for the
total amino acid acceptor activity of the crude tRNA samples, we made an
estimate of 3.85% and 1.57% for the total tRNA$^{\text{His}}$ content of reticulocytes
and foetal liver respectively. Similarly, accounting for the experimental
values of tRNA$^{\text{His}}$ GUG in this population (Tables I - II), the amount of this
isoacceptor in the crude tRNAs has been found to be six times lower in foetal
liver than in reticulocytes. However, while the larger part of reticulocyte
cells are actively engaged in globin synthesis, only about 15% of the liver
cells (personal data) are devoted to the same function during foetal life.

Thus, a good correlation seems to exist between the relative proportions of
tRNA$^{\text{His}}$ GUG in reticulocytes and foetal liver and the specific involvement of
this molecule in globin synthesis. On the other hand, the very low level
of tRNA$^{\text{His}}$ GUG found in adult liver could be explained by the presence of a small
amount of CAC triplets coding for histidine in the various messenger RNAs
of the hepatocyte cells. Definitive evidence for the presence of a GUG anti-
codon in both minor reticulocyte and foetal liver tRNA$^{\text{His}}$ and the eventual
identity of these molecules can only be obtained from structural data. Differ-
ent isoacceptors of tRNA$^{\text{His}}$ have been purified and their sequences are under
investigation in our laboratories.

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