Isoaccepting Transfer RNA’s in Mammalian Differentiated Cells and Tumor Tissues

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Multiplicity of Mammalian Isoaccepting tRNA’s

The fact that different organisms may have different profiles of isoaccepting tRNA’s was first indicated by Apgar and Holley (1). This was subsequently confirmed and extended in studies of various organisms from different phylogenetic levels with the use of chromatographic separation of aminoacyl-tRNA (8, 14). At the end of 1966, when a new chromatographic system, the RPC-2 column, was shown to give increased resolution of Escherichia coli tRNA’s (24), we decided to study in detail the multiplicity of mammalian isoaccepting tRNA’s, using this chromatographic system. A transplantable mouse plasma cell tumor, MOPC 31C, was selected because genetic and immunological studies showed that individual plasma cell tumors may represent a single clone of cells producing chemically homogeneous myeloma protein (18).

The complete profile of isoaccepting tRNA’s for the 20 amino acids derived from the MOPC 31C plasma cell tumor and resolved by the RPC-2 column chromatography was published (28). Except for tryptophan, multiple peaks of isoaccepting tRNA were observed for each of the other 19 amino acids, each having its characteristic elution pattern. As compared to E. coli studied under similar chromatographic conditions (24), MOPC 31C plasma cell tumors showed very different elution patterns of isoaccepting tRNA’s for every amino acid. This different chromatographic behavior may be due to evolutionary changes in the primary nucleotide sequence of tRNA. The tryptophanyl-tRNA was digested by T1 ribonuclease at mild acid pH to protect the ester linkage; the tryptophanyl oligonucleotide obtained from E. coli tRNA was found to be larger than that from the mammalian tRNA sample, indicating that a guanylic acid residue proximal to the 3'-hydroxyl terminus was located at different positions in E. coli and mammalian tryptophanyl-tRNA’s (W. K. Yang and L. C. Waters, unpublished observation).

Compared with the number of the published synonym codons (15), the multiplicity of isoaccepting tRNA’s observed for each amino acid in the MOPC 31C tumor revealed a good correlation. For arginine, leucine, and serine, with 6 codons, several peaks were detected; for tryptophan, with only 1 codon, a single peak was observed. However, exceptions were

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tRNA Contents of Differentiated Cells

An interesting question arises as to whether tRNA contents may reflect amino acid contents of the protein in the differentiated cell. We studied in the C57BL/6 mouse reticuloocyte systems that synthesize 95% of the protein as hemoglobin and also in the MOPC 31C plasma cell tumor that synthesized 30 to 35% of its proteins as IgM myeloma protein. For 7 representative amino acids, specific accepting activities of the
There is a definite correlation of the 2 parameters in the plasma cell tumor, as can be expected from the relative commitment to the synthesis of hemoglobin in the reticulocytes (95%) and of myeloma protein in the plasma cell tumor (35%). For instance, high histidine tRNA and low isoleucine tRNA contents of the reticulocytes are in sharp contrast with low histidine tRNA and moderately high isoleucine tRNA contents of the plasma cell tumor, reflecting the relative abundance of histidine and isoleucine in hemoglobin and myeloma protein. Although relatively high methionine-accepting activities were observed in both tRNA preparations from the 2 tissues, corrections could make them compatible with the data of methionine contents in the protein by excluding the activity of the initiator methionine tRNA, which was determined by the RPC-2 chromatography to be 80 and 62% of the total methionine tRNA from the reticulocytes and the plasma cell tumor, respectively. The corrections are justified by the fact that the initiator methionine tRNA incorporates methionine only into the amino terminus, which is subsequently removed from the nascent peptide chain of the ribosome (25) and is not responsible for methionine incorporation into internal positions of the polypeptide. These results indicate a possible coupling between the formation of specific tRNA's and the relative incorporation rates of the corresponding amino acids into proteins in mammalian cells.

Similar Tissues Producing Different Protein

It is desirable to select monofunctionally differentiated cells for studying tRNA functions and to make comparative studies of isoaccepting tRNA in the same kind of differentiated cells with slightly deviated protein synthesis function.

We studied different mouse plasma cell tumors (29) and different mouse reticulocytes (27). The plasma cell tumors that we studied were all induced in an inbred strain of mice, BALB/c, by i.p. injection of mineral oil and hence are known to have the same genetic background except the factors affecting myeloma protein production. A tumor line (MPC 62) producing immunoglobulin A (IgA) myeloma protein, was compared with 2 lines (MPC 47 and MOPC 31C) producing immunoglobulin F (IgF) myeloma proteins with respect to isoaccepting tRNA profiles for arginine, glycine, leucine, methionine, tyrosine, and serine. Except seryl-tRNA's, all aminoacyl-tRNA's derived from these 2 kinds of tissue were similar in the number and positions of the isoaccepting peaks but were in some cases slightly different in the relative quantities of certain peaks. Seryl-tRNA's, however, showed marked differences. An early eluted peak (seryl-tRNA$_1$) calculated on the basis of respective total seryl-tRNA content, was 27% in MPC 47 and 31% in MOPC 31C and was nearly absent in MPC 62 (3.5%); whereas a late eluted peak (seryl-tRNA$_4$) was most marked in MPC 62 (35%) but was hardly detectable in MPC 47 (2%) and MOPC 31C (0%). Control studies demonstrated that a precisely intermediate profile of seryl-tRNA's was obtained in tRNA samples prepared from mixtures of IgA and IgF tumors and that seryl-tRNA$_1$ or seryl-tRNA$_4$ isolated from chromatographic fractions was rechromatographed as a single peak in the same position.

Reticulocytes from 2 inbred strains of mice, the C57BL/6 and C3H/Anf mice, were compared by virtue of the differences in the composition of amino acid residues at 3 positions in their hemoglobin $\alpha$ chains. The C57BL/6 mice have glycine, valine, and asparagine at positions 25, 62, and 68 of the $\alpha$ chain; whereas the C3H/Anf mice have duplex amino acids (glycine/valine, valine/isoleucine, and asparagine/serine) with a ratio of 30/70 at these positions (12). Among the various isoaccepting tRNA's compared, reticulocytes from these 2 strains of mice showed identical or similar patterns for glycine, isoleucine, methionine, serine, and tyrosine, but they showed a remarkable difference in valyl-tRNA's. Although the resolution of various valyl-tRNA's was not satisfactory in the RPC-2 chromatograms obtained, cochromatography indicated that the content of some isoaccepting valyl-tRNA's were high in the C57BL/6 reticulocytes but low in the C3H/Anf reticulocytes, whereas the other isoaccepting valyl-tRNA's were scarcely detected in the C57BL/6 reticulocytes but appeared prominent in the C3H/Anf reticulocytes. Examination of the valyl-tRNA's from the reticulocytes of the $F_1$ offspring (C57BL/6 $\times$ C3H/Anf) revealed an intermediate pattern of the 2 parent strains. This suggests that the differences in

| Table 1 |
|---|---|---|---|
| | C57BL/6 mouse reticulocytes | MOPC 31C myeloma |
| | Hemoglobin$^a$ (a + $\beta$ chains) | tRNA$^b$ | IgF$^c$ heavy chain | tRNA$^b$ |
| Alanine | 40 | 152 | 24 | 70 |
| Histidine | 70 | 79 | 8 | 20 |
| Isoleucine | 6 | 22 | 18 | 40 |
| Leucine | 34 | 123 | 27 | 43 |
| Methionine | 3 | 59 (12)$^d$ | 5 | 42 (16)$^d$ |
| Serine | 21 | 88 | 56 | 90 |
| Tyrosine | 6 | 18 | 14 | 18 |

$^a$ Residues/287 amino acids in both $\alpha$ and $\beta$ chains (17).

$^b$ Residues/estimated molecular weight of 50,000 (18).

$^c$ Activity excluding early eluted initiator methionyl-tRNA is shown in parentheses.
Table 2
Isoleucine-labeled tryptic peptides of α hemoglobin chain from amino acid transfer reaction by the C3H reticulocyte polysomes (cpm/peptide)

<table>
<thead>
<tr>
<th>Experiment</th>
<th>Aminoacyl-tRNA's</th>
<th>αT4</th>
<th>αT9</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>3H C3H ret.</td>
<td>39</td>
<td>55</td>
</tr>
<tr>
<td>1</td>
<td>14C C57BL ret.</td>
<td>60</td>
<td>82</td>
</tr>
<tr>
<td>2</td>
<td>14C C3H ret.</td>
<td>39</td>
<td>58</td>
</tr>
<tr>
<td>2</td>
<td>3H C57BL ret.</td>
<td>43</td>
<td>74</td>
</tr>
</tbody>
</table>

a After separate reaction, 3H- and 14C-labeled products were mixed and subjected to trypsin digestion, electrophoresis, and chromatography together. Peptides were assayed by 3H/14C discrimination in the scintillation counter (27).

b C3H ret., reticulocytes from C3H mice; C57BL ret., reticulocytes from C57BL/6 mice.

Vallyl-tRNA’s between C57BL/6 and C3H/Anf mouse reticulocytes are determined genetically and the parent genes for the formation of these vallyl-tRNA’s are expressed equally in the F1 offspring, similar to gene expression of hemoglobin α chains in these mice.

Cell-free Protein Synthesis

Seryl-tRNA1 and seryl-tRNA4, respectively, were isolated from the MOPC 31C and MPC 62 plasma cell tumors. They were tested in the amino acid transfer reaction containing either MOPC 31C tumor polysomes or MPC 62 tumor polysomes. Although absent or scarcely detectable in the MPC tumor, seryl-tRNA1 was utilized by the MPC 62 tumor polysomes for protein synthesis to the same extent as by the MOPC 31C tumor polysomes. Similarly, the MOPC 31C tumor polysomes, which presumably never encounter the seryl-tRNA4 molecule in vivo, incorporated serine from seryl-tRNA4 into protein in vitro. These results suggest that the MPC 62 and MOPC 31C tumor polysomes carry mRNA with codons recognizable by seryl-tRNA1 and seryl-tRNA4. It was not possible to determine whether seryl-tRNA1 incorporates serine into normal or aberrant positions of the peptide on the MPC 62 tumor polysomes.

The different valyl-tRNA’s of the C57BL/6 and C3H/Anf mouse reticulocytes were tested in the cell-free system to examine the possibility of an ambiguous translation mechanism (6). The 2 forms of αT4 peptide, isolated from the reaction mixture and quantitatively determined, were synthesized by the C3H reticulocyte polysomes regardless of whether the C3H or the C57BL reticulocyte tRNA was used (Table 2). With the C57BL reticulocyte polysomes, the C3H reticulocyte tRNA could incorporate only glycine but not valine at position 25 in the αT4 peptide. Thus the observed difference of valyl-tRNA’s between reticulocytes of the 2 strains of mice is not related to translation of the duplex amino acid positions in the α chain of the mouse. Because the transfer reaction in our experiments performed only completion of nascent peptide chains, the possible association of valyl-tRNA’s with regulation of peptide chain initiation needs further investigation.

Chart 1. Tyrosyl-tRNA’s of adult rat liver, Reuber hepatoma, and fetus liver from 20-day-old embryos. The figure is derived from 2 cochromatographic runs in RPC-5 (Plaskon) column.

Characteristic Tumor Isoaccepting tRNA’s

The isoaccepting tRNA’s in neoplastic tissues have been documented (2, 7, 9, 10, 22). Evidence has accumulated in this laboratory suggesting a relation of some of these characteristic isoaccepting tRNA’s to the state of cellular differentiation in the tumor tissue. Pertinent findings leading to such suggestion are as follows. (a) The L-M tumors, which are morphologically fibrosarcomas, can be induced by s.c. injection of L-M culture cells into the irradiated C3H/Anf mice (11). We have made a detailed study comparing the isoaccepting tRNA profiles between the L-M cells and the L-M tumors. The results demonstrated a change in aspartyl-, tyrosyl-, phenylalanyl-, and histidyl-tRNA’s (26). A careful analysis of aspartyl- and tyrosyl-tRNA’s indicated that the tRNA profiles of the L-M tumors are the composite of 2 components, one from the original L-M cells and the other similar to those from normal differentiated cells such as reticulocytes and liver. Additional experiments showed a complete reversion of tRNA profiles from the L-M tumor to the L-M cell pattern by culturing the L-M tumor cells in vitro.
in serum-free medium and a decreased tumor-inducing activity of the L-M tumor cells relative to the original L-M cells. Also, "C"-type particles were not detected in the L-M tumors by electron microscopy, and functional features of connective tissue fibroblasts were observed by histochemical methods in the L-M tumors but not in the L-M cells. All these results led us to believe that, upon inoculation in vivo, the L-M cells not only undergo profuse proliferation but also are forced by host homeostatic mechanism to differentiate into functional fibroblasts and that the characteristic tumor isoaccepting tRNA's of the L-M tumors represent the original L-M "stem" cells. (b) A survey of tyrosyl- and aspartyl-tRNA's was carried out in 6 transplantable tumors (3 mouse plasma cell tumors, 2 Morris hepatomas, and 1 Ehrlich ascites tumor), 5 spontaneously occurring primary tumors (2 BALB/c mouse fibrosarcomas, 2 BALB/c mouse reticulum cell sarcomas and a BC3F1 mouse reticulum cell sarcoma), and 3 culture tumor lines (adenovirus 31-transformed hamster cells, Reuber hepatoma cells, and HTC hepatoma cells). These tumors, like the L-M tumors, all showed 2 types of aspartyl- and tyrosyl-tRNA's, the normal differentiated cell type and the abnormal "stem" cell type, in various proportions according to the individual tumor. This was similar to a report by Holland et al. (13) on tyrosyl-tRNA's of tumors; however, the characteristic tumor isoaccepting tRNA's are not considered by us to be the "fibroblastic type" as these authors suggested, because of their high content (30 to 40%) in the ascites form of plasma cell tumor, which contains very few fibroblasts, and of their low content (5 to 10%) in fibrosarcomas, of which fibroblasts are the main cell components. Two observations seem to emphasize the relevance, if not the importance, of these isoaccepting tRNA's to the neoplastic property. First, comparison of the tRNA's from MOPC 31C plasma cell tumors at the 6th transplantation passage and at the 98th transplantation passage, which represent a progression of malignancy from taking about 25 days to less than 10 days to kill the tumor-bearing mice, showed a relative increase in the proportions of these tumor characteristic isoaccepting tRNA's for tyrosine and aspartate, despite the fact that identical profiles were obtained for all the other amino acids examined. Second, the MOPC 31C plasma cell tumor, after treatment with X-irradiation or phenylalanine mustard, showed a temporary regression as well as a moderate decrease in these characteristic tumor tRNA's. (c) Comparison of hepatomas with normal adult liver, livers from mother and fetal rats, and regenerating livers suggests that these characteristic isoaccepting tRNA's are not unique to the tumor tissue. As shown in Chart 1, the characteristic tyrosyl-tRNA's, which are found in Reuber hepatoma cells but not in the normal or regenerating liver, are also detected in the liver of 20-day-old rat embryos, although in lower quantity.

It remains to be seen whether the characteristic tumor isoaccepting tRNA's are related to the altered methylation of tRNA in tumor tissues (23).

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REFERENCES


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