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A Possible Role of Transfer RNA in the Mechanism of Carcinogenesis[1]

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What I find particularly interesting in the elegant studies described by Drs. Farber and Miller (see Refs. 2 and 3 for a review of chemical carcinogenesis) at this symposium is the fact that several carcinogens have the capacity to react with RNA to an extent which is equal to or greater than their capacity to react with DNA. Indeed, Farber *et al.* have demonstrated that with at least one carcinogen, i.e., ethionine, there is extensive interaction with RNA under conditions in which it is difficult to demonstrate significant interaction with DNA. The crucial question, of course, is to know which of these interactions is responsible for converting a normal cell to a tumor cell and which are merely side reactions. Posed in a different way, we might ask: Is the target for chemical carcinogenesis the genetic material itself, or is it some other macromolecule which, when reacted with the carcinogen, produces secondary effects on the pattern of gene expression? There has been a tendency to regard carcinogenesis as a change in the DNA analogous to mutagenesis, but I know of no compelling evidence for this viewpoint. On the contrary, the examples (though they are unfortunately rare) in which tumor cells have reverted to normal suggest that the original conversion to malignancy need not involve permanent changes in the DNA. The most striking evidence for the apparent normalcy of tumor DNA is nuclear transplantation studies demonstrating that nuclei of the Lucké renal carcinoma contain the full set of information for directing the development of a frog embryo.

It is important, therefore, that in studying the mechanism of carcinogenesis we must look at the early events and keep our minds open to the possibility that the primary target is not DNA. I would now like to summarize some recent studies which relate to the question of whether the critical target for certain carcinogens is transfer RNA (tRNA).

The central role of transfer RNA in the translation of the genetic code has suggested to several investigators the possibility that in higher organisms changes in the abundance of specific types of tRNA are associated with metabolic regulation, cellular differentiation, and neoplastic transformation. There is evidence that in bacteria the pattern of tRNA's can change during T-2 phage infection, sporulation, and changes in growth conditions. About two years ago, I learned of Dr. Farber's studies indicating that the hepatic carcinogen, ethionine, results in ethylation of liver tRNA. Because of an interest in the genetic code of tumor cells (4, 5), we began a study, in collaboration with Dr. Farber, to determine whether ethylation of liver tRNA resulted in demonstrable changes in the functional capacities of that tRNA. Most of these experiments were actually performed by a premedical student, Mr. Richard Axel. A complete description of our Methods and Results has recently been published (1) and, therefore, I will only briefly summarize the pertinent data. Extensive studies on the fractionation of rat liver tRNA and changes which occur during feeding of ethionine have also been done by Dr. David Novelli and his coworkers, and will also be described at this symposium.

Since when we first began these studies there were very few data available on the fractionation of normal mammalian tRNA, we first studied the types of tRNA present in normal rat liver. Chart 1 indicates the results obtained when normal rat liver was charged with six different radioactive amino acids and fractionated by methylated albumin (MAK) column chromatography. The profiles revealed two components for arginine, one for isoleucine, three for valine, two for lysine, one for phenylalanine, and three for leucine. The number of peaks described probably represents a minimum estimate of individual tRNA's, because of the limited resolution of the MAK column. These results indicated that in mammalian cells, as in *E. coli* and yeast, there exist multiple forms of tRNA for the same amino acid, a finding consistent with previous evidence for degeneracy of the genetic code in higher organisms (4). Since the multiple forms of tRNA obtained in *E. coli* differ in their codon response during translation, it is likely that this is also the case with rat liver tRNA's and this is being examined in current studies.

Having established certain tRNA patterns in normal rat liver, we then examined whether these patterns change during feeding of ethionine. In these studies, tRNA was prepared in parallel from both normal- and ethionine-fed rats using identical isolation procedures. To determine whether ethylation is restricted to one or several types of tRNA, rats which had been on an ethionine diet for one month were injected with ethyl-^{14}C-labeled ethionine daily for 4 days and then sacrificed. The ethyl-^{14}C-labeled tRNA was extracted from liver and cochromatographed with a 6-fold excess (on an A_{260} basis) of normal liver tRNA (Chart 2). The ethylated RNA (Et-tRNA) is represented by the radioactivity, whereas the A_{260} profile mainly reflects the pattern of the normal tRNA. The elution of the Et-tRNA was slightly delayed when compared to normal tRNA. In contrast to the sharp peaks obtained with tRNA's

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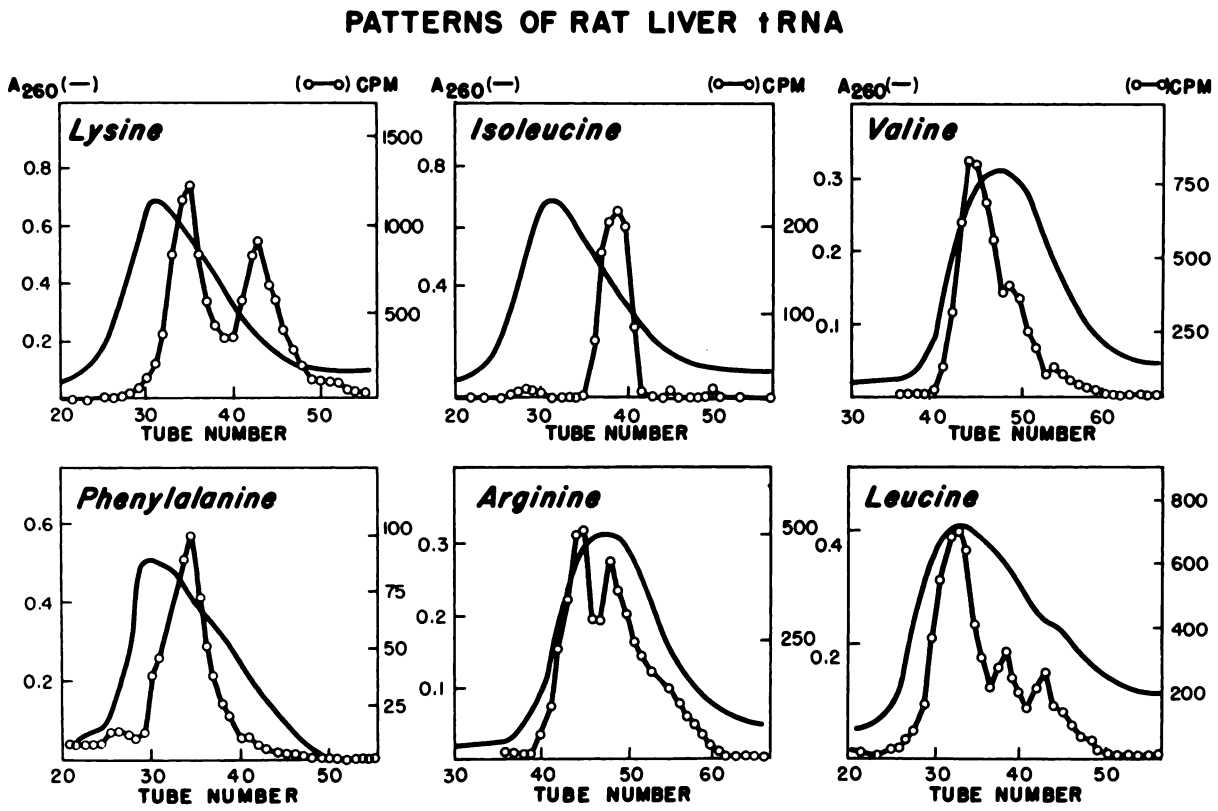

Chart 1. Elution patterns on methylated albumin-kieselguhr (MAK) columns of radioactive aminoacyl-transfer RNA (tRNA) from normal rat liver. Radioactive aminoacyl-tRNA's were prepared and chromatographed as described in Ref. 1.

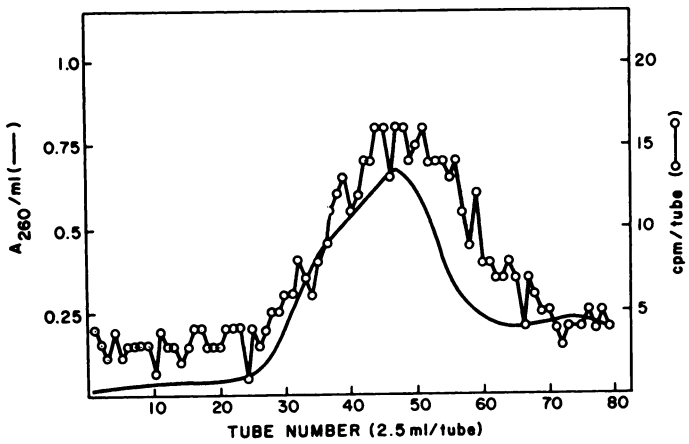

Chart 2. Elution profiles of normal versus ethylated liver transfer RNA (tRNA). Five A_{260} units of ethylated liver tRNA, labeled *in vivo* with L-ethionine-ethyl-1-^{14}C, were mixed with 30 A_{260} units of normal liver tRNA and cochromatographed on a methylated albumin-kieselguhr column. Fractions were precipitated with trichloroacetic acid and assayed for radioactivity. The elution profile of the ethylated tRNA is represented by the radioactivity, whereas the A_{260} curve mainly reflects the elution profile of normal tRNA. (Additional details are described in Ref. 1.)

specific for individual amino acids (Chart 1), the profile of ethyl-labeled tRNA revealed a broad peak which was similar in contour to the A_{260} profile of normal tRNA, thus indicating that many species of tRNA were ethylated.

The functional capacity of Et-tRNA was examined by comparing the leucine acceptance capacity of Et-tRNA to that of normal rat liver tRNA (Chart 3). Over a concentration range of RNA from 0.2 to 0.5 mg/ml, the acceptance capacity of the ethylated tRNA was equivalent to that of normal tRNA. The Et-tRNA was also similar to normal liver tRNA when assayed for valine acceptance capacity.

To determine whether the types of leucyl-tRNA's were altered after ethionine feeding, Et-tRNA and normal tRNA were charged with leucine-^{14}C and leucine-^{3}H, respectively, and were cochromatographed on a MAK column. Chart 4 indicates that, whereas the normal tRNA contained at least three leucine components, the Et-tRNA revealed only one. The single component obtained with Et-tRNA eluted in a region which corresponded to the major component of leucyl-tRNA present in the normal material. This difference in the elution profiles of liver leucyl-tRNA's obtained from normal and ethionine-fed rats was confirmed when a second batch of these two types of tRNA were again prepared in parallel by identical procedures. In preliminary experiments the coding properties of unfractionated normal rat liver and Et-tRNA were tested in the Nirenberg and Leder ribosomal binding assay. Normal tRNA

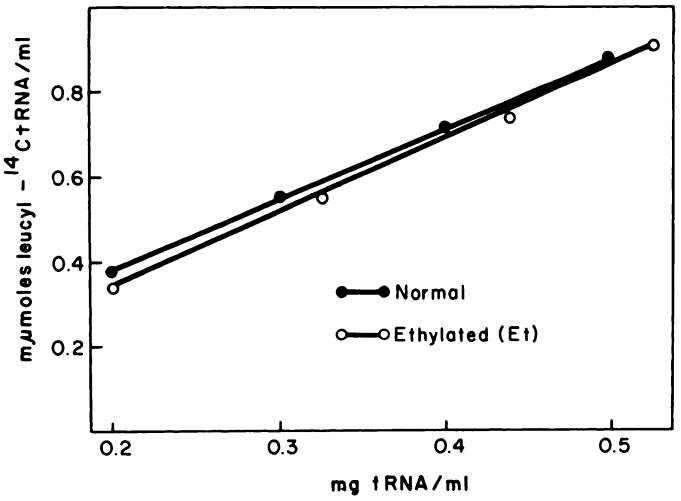

Chart 3. Leucine acceptance capacity of normal and ethylated liver transfer RNA (tRNA). Increasing amounts of normal rat liver tRNA or ethylated tRNA were incubated with leucine-^{14}C in the standard charging system (see Ref. 1). After 19 minutes at 37°C the nucleic acid was precipitated and washed with cold 5% trichloroacetic acid and assayed for radioactivity.

charged with leucine responded to both poly UC and poly UG, whereas the Et-tRNA responded only to poly UC. These results suggest that the Et-tRNA is deficient in a species of leucyl-tRNA which normally recognizes the UUG codon. When normal and Et-tRNA were charged with radioactive valine, the elution profiles of both preparations were identical and similar to the pattern of valyl-tRNA given in Chart 1.

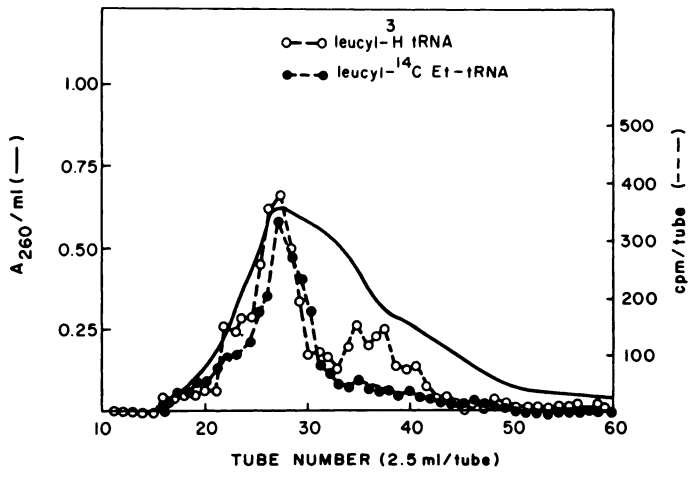

Chart 4. A comparison of the elution profiles of normal rat liver leucyl-transfer RNA (tRNA) and ethylated leucyl-tRNA. Normal rat liver tRNA (38 A_{260} units) was charged with leucine-^{3}H and an equivalent amount of ethylated tRNA (Et-tRNA) was charged with leucine-^{14}C in the standard charging system. The aqueous phases obtained after phenol extraction were pooled and cochromatographed on a methylated albumin-kieselguhr column. The details of the procedure are described in Ref. 1.

The Et-tRNA used in the preceding study was obtained from rats fed an ethionine diet for one month prior to the gross appearance of hepatomas. In view of the results obtained it was of interest to study the tRNA of an ethionine-induced hepatoma. The tRNA was extracted from a hepatoma which had been induced by ethionine approximately one year ago and maintained by serial transplantation in Wistar rats fed a normal diet. This material (T-tRNA) was charged with leucine-^{14}C and cochromatographed with normal liver tRNA charged with leucine-^{3}H. The T-tRNA revealed three leucine components which were identical in their elution profile to those present in normal tRNA. It appears, therefore, that the changes in leucyl-tRNA seen during ethionine feeding are not maintained in the tumor itself, presumably because ethionine is no longer present in the diet and ethylation of tRNA cannot occur.

The relevance of our findings to the causation of cancer remains to be determined. It is possible that alterations in tRNA play a role in the initiation of hepatomas by ethionine and that maintenance of the neoplastic state is due to secondary changes which persist even when the tRNA pattern returns to normal. This hypothesis is consistent with other evidence from the field of carcinogenesis indicating that initiation, promotion, and maintenance of cancer may occur by separate mechanisms. Evidence which favors the possibility that ethylation of tRNA is a key event in the carcinogenic effect of ethionine includes the following: (*a*) the feeding of ethionine results in the ethylation of liver tRNA to a considerably greater extent than its incorporation into liver protein and causes little or no ethylation of liver DNA (1); (*b*) appreciable ethylation of tRNA occurs only in the liver, the only organ in which ethionine induces tumors (1); (*c*) supplementation of the diet with methionine prevents ethionine carcinogenesis and markedly diminishes ethylation of liver tRNA. Using the technics described in the

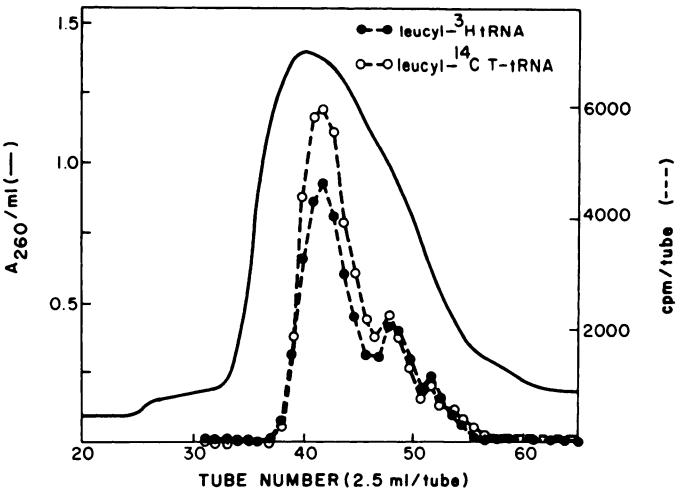

Chart 5. A comparison of the elution profiles of normal rat liver leucyl-transfer RNA (tRNA) and leucyl-tRNA obtained from an ethionine-induced hepatoma (T-tRNA). The procedure was similar to that described in Chart 4 with the exception that T-tRNA was obtained from an ethionine-induced hepatoma maintained by serial transplantation in rats fed a normal diet and this was used in place of the ethylated tRNA.

present study, we have found that methionine feeding also prevents the changes in leucyl-tRNA produced by ethionine.

Our findings with respect to changes in tRNA during carcinogenesis may not be restricted to ethionine. There is evidence that the hepatic carcinogen 2-acetylaminofluorene (AAF) labels rat liver tRNA to a greater extent than other liver RNA fractions (1), and we have recently confirmed this finding in our own laboratory. It will be of interest, therefore, to determine whether tRNA modified by AAF is altered in its functional properties. Studies indicating differences between the patterns of methylation of normal and tumor tRNA's also suggest that changes in tRNA play an important role in abnormal growth.

At this time, I can only speculate about the mechanism by which changes in tRNA might lead to the defects in cell regulation which characterize tumor cells. The loss of a given tRNA could prevent the translation of messenger RNA's containing codons specific for that tRNA and thereby block the synthesis of one or more proteins normally required for cell regulation. Alternatively, since there is some evidence that in bacteria aminoacyl tRNA's may play a role in enzyme repression, the loss of a given tRNA might prevent the function of a specific repressor system. In addition, there is increasing evidence that transcription and translation are more tightly coupled than previously suspected; therefore, agents which act on tRNA might indirectly influence rates of synthesis of specific messenger RNA's and thereby alter patterns of gene expression.

The hypothesis that a critical event during carcinogenesis involves changes in the abundance or specificity of individual tRNA's introduces the possibility that the fidelity of translation in tumor cells differs from that of normal cells. It would be of interest to know whether the amino acid sequence of a protein synthesized by a tumor cell is identical to that of the same protein synthesized by a normal cell. Unfortunately, I know of no amino acid sequence studies which bear on this point. Recent evidence that "nonsense to sense suppressors" in bacteria involve a change in the tRNA population which permits a nonsense codon to be read as serine, and thereby allow the completion of a polypeptide chain, may also be relevant to this hypothesis. It is possible that certain changes in the tRNA population during carcinogenesis mimic the action of a nonsense to sense suppressor, thereby permitting the translation of otherwise latent messenger RNA's, the products of which might lead to cellular replication and autonomy.

Needless to say, the foregoing remarks are highly speculative. I trust, however, that they might provide an impetus for further studies on the tRNA's of normal, premalignant, and malignant cells. Fortunately, previous workers using yeast and bacterial systems have developed elegant technics for purification and sequence analyses of tRNA's, and it is therefore technically feasible to approach this problem at a chemical level in mammalian systems.

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