The tRNA project.

The tRNA project began in Dr John Holland's lab while I was a post-doctoral fellow. It stemmed from the observation that specific viruses such as mengo and bovine enterovirus produced few virus particles in some cell lines, yet gave a very productive infection in other cell lines. One hypothesis was that differences in populations of tRNA might explain these differences, i.e. translation of specific viral proteins might be prevented if a specific isoaccepting species were absent, not active or modified. As a first step we examined the Methylated Albumen Kieslelguhr (MAK) column elution profiles of specific charged t-RNAs from MDBK and Hela cells, two of the cell lines with different profiles of permissiveness to virus but found no differences. However in comparing other cell lines, in particular tumor cell lines, differences in elution profiles were detectable between Ehrlich ascites tumor tRNAs and " normal" mouse cell tRNA (extracted from liver) as well as between other tumors and non-tumorous material.

To summarize the first paper in the series: specific transfer RNA's of mammalian origin (from organs and cultured cells) were compared by double labeling of the amino acids and charged tRNA elution from MAK columns. We were looking for changes in tRNA elution profiles that might have occurred during differentiation. No major differences were detected between tissues for alanine, leucine, lysine, phenylalanine, or threonine tRNA. However, differences were observed in the elution profiles of minor tRNA species for glycine and serine. Major differences were observed in the elution patterns of phenylalanine, serine, and tyrosine tRNA derived from Ehrlich Ascites tumor cells when compared to normal mouse tissue. (Taylor et al PNAS 1967). This was followed by a second paper with a more in depth study of the changes that occur in tyrosine tRNA, demonstrating changes in MAK column profiles between tyrosyl tRNA from fibroblasts compared to epithelial cells, white blood cells and specific tissues and organs. This was done by double labeling experiments, one tissue with 3H charged amino acid , the other with C-14. We proposed that there was a specific type (fibroblast type) of tyrosyl- tRNA found only in specific cell types. Other scientists had found similar modifications by chromatography of specific tRNAs following virus infection, e.g. Subak-Sharpe during HSV infection and others during T4 phage infection. The working hypothesis was that these tRNAs were modified during virus infection or during differntiation. Limitations on the amount of a

specific tRNA could inhibit host protein synthesis or excess of tRNA could enhance the growth of tumor cells. An extra peak of tyrosyl-tRNA has also been found in HeLa cells and adeno-7 virus transformed cells, but not in all tumor cells. The tyrosyl-tRNA of mammalian and chick fibroblasts in cell culture, and fibroblasts in the body exhibited a markedly different elution profile from the tyrosyl-tRNA of epithelial cells in culture and of normal organs. The tRNA differences discovered by *in vitro* charging were all confirmed *in vivo*. The Journal of Molecular Biology paper presents in greater detail what had already been reported in the two PNAS papers. It includes tables of data not found in the PNAS papers. We also proposed that these results might involve methylation patterns of the tRNA as suggested by Borek and colleagues. Borek had proposed that tumors may contain greater amounts of methylating enzymes than normal tissue and this might be reflected in chromatographic profiles. Littauer had proposed that changes in profiles of phenylalanine tRNA might represent alterations that prevent recognition of the tRNA and acylating enzymes or alter the coding recognition of the tRNA.

When I arrived at Indiana, among the first projects was to continue this work, examining phenylalanine tRNA. I assume that the reason for picking phenylalanine tRNA was that it was the easiest to work with. Although differences in profiles could be found between EAT cells and other mouse cells, no difference in codon binding, or incorporation into protein could be found.

At this stage the decision was made to concentrate on other tRNAs. Particularly tyrosine tRNAs and also to look at other systems, namely the developing sea urchin (Greg Zeikus) and differences between hepatomas that grew with different rates, and showed different degrees of malignancy. For these studies we used a series of hepatomas known as Morris hepatomas. We therefore studied tRNA from 3 hepatomas of varying degrees of differentiation, namely 9618A (highly differentiated), 5123D (well-differentiated), and 3924A (poorly differentiated). We did find differences in the profile on reverse phase columns between a number of tRNAs from 3924 and 9618 when compared to normal rat livers, but not with 5123D. This suggested that different tumors might have altered isoaccepting species of tRNA, not necessarily related to growth rate or extent of malignancy.

During my first summer at Indiana I went to Uri Littauer's lab at the Weizmann Institute. I do not remember what work I did, but I assume I continued to work on tRNA modifications.

Shorty after arrival at IU the MAK (methylated albumen keiselguhr) column used for this type of analysis was replaced with the BD-cellulose or reverse phase column. (Benzoylated

DEAE) -cellulose columns gave much superior resolution and identified more peaks of each tRNA than found on the MAK column. For example, the best example of this discrepancy is in serine tRNA, in which 1 or perhaps 2 species are observed by MAK chromatography compared to BD-cellulose in which 4 to 5 species are found. We noted quantitative variations in tRNA species between cell types on BD-cellulose, whereas the same tRNA's eluted as single peaks at different salt concentrations on MAK columns. Thus much of the work with the MAK column was in doubt. In October of 1970 I was invited to attend a NIH Fogarty International meeting on modifications in tRNA. The main scientist behind this project was Ernest Borek, whom I remember as a towering figure. He was working predominantly on the role of methylases and methylation of RNA and how it affected function. He was working with his student (and later his wife) Sylvia Kerr and one of his post-docs (or students) Robert Gallo, who became famous later on for the discovery of human tumor viruses. Borek was of Hungarian origin, and like other Hungarians scientists I have met, could never be wrong! It was Robert Gallo who claimed later at another meeting that he was responsible for my invitation to this meeting. Our work was well received and was comparable to what other labs were doing, discovering differences in tRNA profiles between normal and neoplastic tissues without being able to identify the function of such changes. Alan Volkers, a post doctoral fellow from Newcastle, England did most of the work on the hepatomas. We concluded that the reverse phase columns gave us the best resolution. A very efficient lab technician, Suzanne Prather, who later on became a Ph.D. student, helped Alan.

Around 1972 a post-doc from India joined me. This was Raman Kothari. Raman was a small, dark very intense , hard working individual. He came by himself to Bloomington without family since he was afraid that the US culture would corrupt his family. He was (and still is) a very devout Hindu. His apartment was decorated with pictures and small statuettes of Hindu gods. He was a very ambitious scientist, and I think quite a good one, who was keen on publishing as much as possible. He loved to write reviews, and we published a whole series of reviews on column chromatography. He did most of the writing. On rereading these I realize how informative and good they were. They appeared in the Journal of Chromatography. One of the first reviews also contains the name Shankar. This was a student of Raman's who had come to the US for a heart transplant. He must have been one of the first of these done in Huston, TX. I do not know who the surgeon was. However Shankar came to visit Raman before leaving for

India. While here he became seriously ill and had to be rushed to the Bloomington Hospital .It turned out that he had contacted hepatitis B while in the Huston hospital, probably from blood transfusion. Although he did not have any money or health insurance, the Bloomington Hospital treated him with out payment. This was quite a surprise after hearing all the stories of hospitals than turn people down. He stayed on for a few weeks after treatment, worked for a short while with Raman, writing reviews and then returned to India. I met him again in 2006 when we did a tour of Hindustan together. He was still working, in a biotechnology company.

Raman had quite an influence on me scientifically. He had a very strong work ethic, and was very principled. He did not approve of the Western way of life, and I think he was in the States not only for science but to make money (he lived very frugally) so that he would have enough for a dowry for both his daughters. In fact I am not sure he needed this since both daughters went eventually to university and became physicians. He had a small apartment in University apartments, cooked his own food, and would never eat out. We occasionally sampled his cooking, which was good but completely vegetarian and very limited mostly being rice or lentil dishes. Raman decided to spend two years in Bloomington , and after one year his wife joined him. She was a charming person, not as fanatical as Raman. She left the children with Raman's parents, apparently a custom in India. Her father also came during this period to visit. He was the opposite of Raman, a portly businessman who knew how to have fun, drank alcohol, and went of on his own to explore different parts of the US. Later on I got to know Raman and wife very well, when I spent a few days staying with them in Baroda, India.

By this time the lab had expanded hugely. Bob Fleischmann had joined the lab from Purdue. He had been a Ph.D student of Ed Simon, and had worked on interferon. Bob and his wife Chris were both biochemists-virologists. Bob tried working on the APRT project, which I will describe later, but could not give up on interferon. In fact it was he who introduced me to this whole field of research, which was to have a major impact on my life. Bob was a very meticulous worker, and found it difficult to accept the variability we found working with tRNAs and later mammalian cell mutations. He became very close friends with Raman, a friendship that continued for many years afterwards. Bob later on became an editor of the Journal of Interferon and Cytokine Research, and played a major role in the interferon society. After completing his pot-doc in my lab (not a very productive one) he found a position at the U. Texas Medical School in Galveston, where he resided for a long time. He is currently at the University of

Minnesota.

Another student who joined the lab early was Andy Ouellette. He was a very nice guy, very sociable and got along with everyone. He worked on the tRNA acylating enzymes. Unfortunately this was not a great project, since we were unable to really find any differences between tumor enzymes and normal enzymes. The choice of projects, which was my doing, was not the best in retrospect. However Andy spent three-four years working on this project, which resulted in a couple of publications. A little later another student, a transfer from physics joined the lab. This was San Wang. In collaboration with Raman Kothari and Paul Hung from Abbott, we set out to examine whether infection with avian tumor viruses altered the tRNA spectrum, and also to look for tRNA in the virus itself. I really do not remember what triggered this project, whether it was my idea or Paul Hung, or San's. We did discover that tumor viruses contained not only their own genomic RNA but also a 4S RNA, which was an active tRNA, charged by cognate enzymes. We unfortunately concentrated on the methionyl tRNA, and did not recognize, as was discovered later that some of these tRNAs interacted with the viral genomic RNA and acted as primers of the reverse transcriptase. The tRNAs that we were looking at appeared to be packaged with the virus but did not play a role in the virus life cycle. Despite this we produced two papers, one published in Nature New Biology, the other in BBA. We then expanded our study to include other RNA tumor viruses of avian origin. In all cases we found an extra peak of methionyl tRNA, that might be uniquely packaged by the virus. However this was difficult to prove. I could not find any follow up papers, and this appears to be an area of research " abandoned". With the discovery of the reverse transcriptase and role of tRNA as a primer, a different emphasis was placed on this type of research. Robert Gallo, who had published many papers of tRNA difference paralleling our work, now concentrated his efforts on the mechanism of the reverse transcriptase and eventually human tumor viruses.

San was a very shy hesitant individual. He had a Ph.D. in physics, received another Ph.D. in biology and then went on to become an MD. He is still active in research, and as a practicing physician at Mass General and associated hospitals. However I note that as of 2009 he is still only an assistant professor.

Thus ended the tRNA story. The techniques were not available to sequence and analyze the tRNAs, and in fact the research was not going anywhere. Numerous groups have made the

same observations without coming to any conclusions. Subtle differences have been found in phenylalanine tRNA (Smith et al J. BiolChem, 1985) between liver and tumor cell. The difference lies in the extent of modified guanine derivative (Y) adjacent to the 3'end of the anticodon loop and in 0-methylation in the 5' portion of the anticodon loop. The hypo modification in the tumor affects the rate of translation and incorporation of phenylalanine. . However none of the data supports our original hypothesis that the levels of tRNAs would be crucial in tumor development or differentiation.