CHARACTERIZATION OF ISOACCEPTING TRANSFER RNA SPECIES CHANGES DURING FRIEND CELL ERYTHROID DIFFERENTIATION: INITIAL STUDIES ON A MODEL SYSTEM FOR HEMOGLOBIN SYNTHESIS

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Doctor of Philosophy

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by
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and hereby certify that in their opinion it is worthy of acceptance.

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CHARACTERIZATION OF ISOACCEPTING TRANSFER RNA SPECIES CHANGES DURING FRIEND CELL ERYTHROID DIFFERENTIATION: INITIAL STUDIES ON A MODEL SYSTEM FOR HEMOGLOBIN SYNTHESIS

Victor K'o-I Lin

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ABSTRACT

Erythroid differentiation in Friend leukemia cells can be induced by addition of dimethylsulfoxide to the culture medium. Hemoglobin represents 24% of the protein synthesis in differentiated cells. Therefore, Friend cells induced for differentiation may exhibit a marked change in tRNA population in order to effectively translate globin mRNA. Friend cells may prove to be a model system for the study of change in tRNA expression during differentiation and for the facilitation of specific protein synthesis. The chromatographic profiles of isoaccepting tRNA species from Friend leukemia cells were analyzed at five time points during a ninety-six hour period of dimethylsulfoxide-induced differentiation. Sixty-four isoaccepting species of tRNA for sixteen amino acids were resolved by RPC-5 chromatography. The relative amounts of $\mathrm{tRNA}^{\mathrm{phe}}$, $\mathrm{tRNA}^{\mathrm{ile}}$, and $\mathrm{tRNA}^{\mathrm{val}}$ species were maintained by the cells during differentiation; whereas the relative amounts of some of the isoaccepting tRNAs for the other thirteen amino acids changed significantly. Transfer RNA species containing the hypermodified nucleoside Q were among those exhibiting significant changes. Fluctuations in relative amounts of isoacceptors occurred between 36 and 72 hr after addition of dimethylsulfoxide, corresponding to globin mRNA appearance and hemoglobin synthesis, respectively. In most cases, the predominant tRNA isoacceptors of uninduced cells were retained throughout differentiation. Notable exceptions were tRNA species for threonine, proline, and methionine. The two proline tRNA species present in uninduced cells were replaced by two different species in induced cells; the four threonine isoacceptors present early were represented by only two of the four in differentiated cells. These changes may reflect the cell's response to globin mRNA containing only two of the four threonine codons. Initiator methionine tRNA decreases in relative amount during the first 48 hours and then increases. This decrease is correlated to an initial lag in cell growth and protein synthesis. Some of the isoacceptors occurring in relatively smaller amounts were not expressed at all times. These changes possibly reflect the cell's functional adaptation of tRNA in differentiation for hemoglobin synthesis. The amount and distribution of Q-base-containing isoacceptors of $tRNA^{asn}$, $tRNA^{asp}$, $tRNA^{his}$, and $tRNA^{tyr}$ were assayed. The amount of Q-base-containing tRNA species decreased in the first 48 hr after the induction, then increased again, indicating the level of Q modification is correlated to the process of differentiation.

TABLE OF CONTENTS

LIST OF FIGURES

Figure Page

LIST OF TABLES

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I. INTRODUCTION

Protein biosynthesis appears to be controlled by the regulation of both translation and transcription. Transfer RNA has a well known role as translator of messenger RNA. Additional functions for tRNA have been described and studied for the past twenty years. The significance of a multiplicity of isoaccepting species of tRNA redundant for the sixty-one translated coding triplets still remains obscure. It is not clear yet to what extent the cell actually uses variations in specific tRNA levels or specific isoaccepting forms of tRNA to, for instance, control the relative rates of translation of different mRNAs. However, the differences in tRNA isoaccepting species found in various differentiated and undifferentiated cells could reflect the operation of such a control mechanism.

Cellular differentiation in eukaryotic tissues is often characterized by the production of a relatively high abundance of one protein or a group of specific proteins accompanied by a drastic shift of cellular metabolism. Differentiated tissues and cells which preponderantly synthesize particular proteins with unique amino acid compositions provide promising experimental systems in which to assess the physiological significance of changes in the tRNA population. Such systems, including fibroin synthesis by the silk worm (1; 2; 3), crystallin synthesis by lens tissue (4; 5; 6), hemoglobin synthesis in reticulocytes (7; 8; 9), and collagen synthesis in embryonic chick tissues (10; 11; 12) have been studied to various degrees with regard to the involvement of tRNA.

Accumulated evidence has suggested that the intracellular levels of tRNA species are tightly correlated with the demands of the cell for various amounts of particular amino acids and certain anticodons in the synthesis of protein. This continuous and selective cellular adjustment of the tRNA population has been referred to by Garel (2) as "functional or quantitative adaptation". Relatively little direct evidence, however, has been obtained regarding the physiological role of these tRNA changes because of the heterogeneity of the stage and type of the differentiated cells and because of the lack of proper comparison between the undifferentiated, "stem" cells and differentiated cells.

In vitro differentiation of Friend leukemia cells (F.L.C.) has been studied since 1970. Friend leukemia cells seem to provide an ideal system for the study of tRNA involvement in cellular differentiation. This cell line has a homogeneous cell population in which erythroid-like differentiation can be induced and is therefore an *in vitro* analogue of erythropoiesis *in vivo*. In addition, many morphological and biochemical transitions experienced by this cell during differentiation have been well characterized over the last decade. Prior to this study, an alteration of the Friend leukemia cell tRNA population and tRNA nucleoside composition during the dimethylsulfoxide-induced erythrodifferentiation had been reported (13); this alteration was concurrent with the production of hemoglobin.

In order to study the role of tRNA in cellular differentiation by using this easily controlled, homogeneous population of cells, an identification and characterization of the tRNA isoaccepting species that change during differentiation of this system is an important, essential first step. Toward this purpose, the present work has investigated the tRNA isoaccepting

species expressed in Friend leukemia cells before and during _in vitro_ differentiation. The alteration of isoaccepting tRNA species for sixteen amino acids was studied by reversed-phase plaskon chromatography. Concomitant with alterations of cellular functions in the transition between dividing, undifferentiated cells to non-dividing, predominantly hemoglobin synthesizing cells, dramatic quantitative and qualitative changes occurred in the distribution of tRNA isoaccepting species. Sixty-four isoaccepting species of tRNA for sixteen amino acids were resolved and their changes during differentiation have been characterized.

II. LITERATURE REVIEW

A. Transfer Ribonucleic Acid and Its Structure

In 1958 Francis Crick proposed the "Adaptor Hypothesis" whereby an adaptor molecule must exist in order to interface between the synthesis of protein and existence of genetic information in nucleic acid (14). Shortly afterwards the Adaptor Hypothesis was confirmed by the discovery in rabbit liver extracts of a class of small RNA molecules capable of specifically binding amino acids (15), and now known as transfer RNA (tRNA).

Transfer RNA functions in protein biosynthesis by carrying esterfied, "activated", amino acids to the ribosome, site of protein synthesis. The correct order of amino acids is dictated by messenger RNA (mRNA) by means of triplet base pairing with tRNA. Consistent with Crick's hypothesis, mRNA only interacts with tRNA and not with amino acids and there are at least 20 tRNA species, each specifying one of the 20 amino acids.

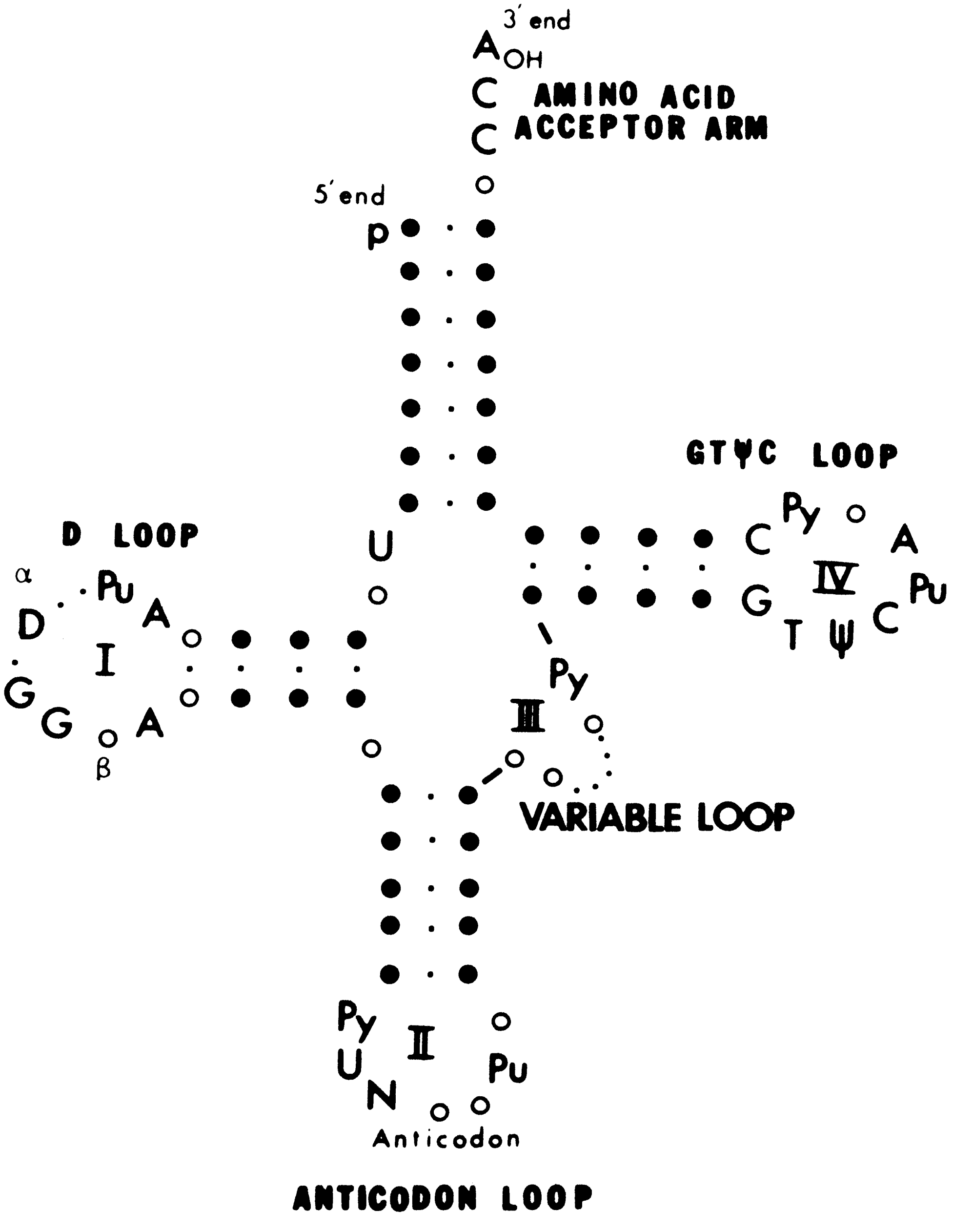
The interaction of tRNA with mRNA and polypeptide requires specific recognition of tRNA by aminoacyl-tRNA synthetase, certain transfer factors, ribosomal RNA (rRNA) and ribosomal proteins, and the appropriate triplet codon on the mRNA. Recognition depends on the primary, secondary, and tertiary structure of the tRNA molecule. Basically, tRNA molecules have a chain length between 73 and 93 nucleotides and molecular weight of 23,000 to 30,000 daltons. The nucleotide sequences of more than one hundred tRNA species from various biological sources have been determined (16). All tRNAs which have been sequenced can be drawn to fit the "cloverleaf model" of secondary structure suggested by Holley et al. when reporting the first tRNA sequence in 1965 (17). Two basic features of this model are: large portions of the molecule forming double helical stems in order to stabilize the structure; and single stranded hair-pin loops with the potential of being functionally active sites. The single stranded loops can hydrogen bond to each other to form the tertiary structure. Except for an occasional G•U base pair, the stems are held together by Watson-Crick base pairs. The cloverleaf model allows for the maximum number of Watson-Crick pairs in the structure.

All tRNAs contain four to five arms consisting of stem and loop structures, as shown in Figure 1. The loop regions have been designated the dihydrouridine (D) loop (Loop I), the anticodon loop (Loop II), the variable loop (Loop III), and the TψC loop (Loop IV). The four stems common to all tRNAs are the acceptor stem, the dihydrouridine stem, the anticodon stem, and TψC stem.

The acceptor stem, containing the constant CCA terminal sequence, consists of seven base pairs and four single nucleotides, including the 3' terminal CCA sequence used to accept a specific amino acid. Differences in the sizes of various tRNAs are accounted for by variation of size in the dihydrouridine arm and variable arm. Variable arms of 3 to 21 nucleotides in length have been found. In contrast the dihydrouridine arm is much less variable in length, being from only 15 to 18 nucleotides with 7 to 11 nucleotides in the loop region, and 3 or 4 base pairs in the stem. Variation in the length of the dihydrouridine loop occurs in two regions, α and β, as shown in Figure 1. These regions contain up to 3 nucleotides with a high proportion of dihydrouracil residues.

The anticodon arm and the TψC arm as well as acceptor stem seem to

Figure 1. The generalized cloverleaf structure of tRNA. The cloverleaf secondary structure is a common feature of all tRNAs. The four loop regions, I-IV, are designated in a counterclockwise direction from the 5' to 3' end of the molecule. Solid circles with a dot between indicate bases involved in helical stems containing Watson-Crick base pairs. Open circles signify non-Watson-Crick base paired bases. The dotted region in the variable loop and α and β regions in the D loop contain different numbers of nucleotides in various tRNA sequences. Nucleosides common to all structures are indicated as: Py, pyrimidine; Pu, purine, ψ, pseudouridine; N, a frequently modified purine or pyrimidine.


3' end
A
OH
C
C
AMINO ACID
ACCEPTOR ARM
5' end
p
GTΨC LOOP
Py
A
C
IV
Pu
G
T
Ψ
C
D LOOP
α
Pu
A
D
I
G
G
A
β
U
Py
III
VARIABLE LOOP
Py
II
U
N
Pu
Anticodon
ANTICODON LOOP

be of constant size in all tRNAs with the possible exception of mitochondrial tRNA (18). The anticodon loop contains the anticodon nucleotide triplet corresponding to one of the 20 amino acids. The TψC loop contains, with a high degree of regularity, two modified bases in a specific tetranucleotide sequence, TψCG. It has been demonstrated that the TψC loop is important in the binding of tRNA to the ribosome (19).

Comparison of all the sequenced tRNA molecules has led to the assignment of many invariant and semi-invariant nucleotide positions in the generalized cloverleaf structure (20). Fifteen invariant residues, including the terminal CCA, and eight semi-invariant residues are common to almost all tRNAs. It is interesting to note that segments which are identical or similar in all tRNAs are located mainly in loop regions of the cloverleaf structure. This unity of tRNA structure is evidently a reflection of their common functional need. However, post-transcriptional modification of certain nucleotides and the composition of minor bases give a tRNA molecule some features of individuality (20), although many of the modified nucleosides are located at invariant positions. The enzymatic modifications of specific tRNA nucleosides have been extensively reviewed by Agris and Soll (21) and Nishimura (22). The modifications are assumed to be essential for proper functioning, but many of the roles are not yet specifically identified.

Now that the three-dimensional crystallographic structure has been determined for one tRNA at high resolution (23) and is thought to be closely representative of all tRNA, primary and secondary structural information of tRNA can be viewed with new understanding. The X-ray crystallographic structure places the cloverleaf structure of tRNA into a characteristic L shape through hydrogen bonding of various parts of the tRNA molecule (24). A continuous helix formed between the acceptor stem and the TψC stem is perpendicular to a second helix formed by the anticodon stem and the dihydrouridine stem. The dihydrouridine loop interacts with TψC loop to contribute to the stability of this L shaped structure (25). This configuration of tRNA has been confirmed by crystallographic studies of different tRNAs and supported by biophysical studies of tRNA in solution (26; 27; 28; 29).

B. Transfer RNA Biosynthesis

The total tRNA in a bacterial cell comprises about 1% of the dry weight. There are about 4×10^5 tRNA molecules of perhaps 55 different types in such a cell. Thus tRNA content corresponds to a concentration of approximately 0.5 mM (30).

Biosynthesis of tRNA has been studied in both prokaryotic and eukaryotic systems. Like most stable RNAs of the cell, tRNA is transcribed from DNA as a larger precursor molecule which must be subsequently processed. There is no regularity with regard to the tRNA genes organization. Transfer RNA genes can exist in multiple copies (for review see 31). Transfer RNA genes are transcribed by the action of RNA polymerase. It has been demonstrated that the original transcripts of tRNA genes may be monomers (32), dimers or trimers (33), or even larger (34; 35).

It has also been found that dimer precursors may contain two different tRNAs as well as one tRNA but in duplication (36). The monomer precursors generally range in size from 130 - 200 nucleotides and may or may not contain the 3'-terminal CCA sequence. The tRNA precursor is then cleaved to the proper length by the action of several nucleases (35; 37; 38). The steps involved in the biosynthesis of tRNA are summarized and shown in Figure 2. All modification reactions of tRNA occur post-transcriptionally and a specified sequence of modifications has been suggested (39). More than 50 different modified nucleosides have been isolated from tRNA and the structure of most of these have been determined. In most cases enzymes responsible for the biosynthesis of these modifications have been detected and some have been purified. (For the most recent reviews see 21; 22).

C. Various Functions of tRNA

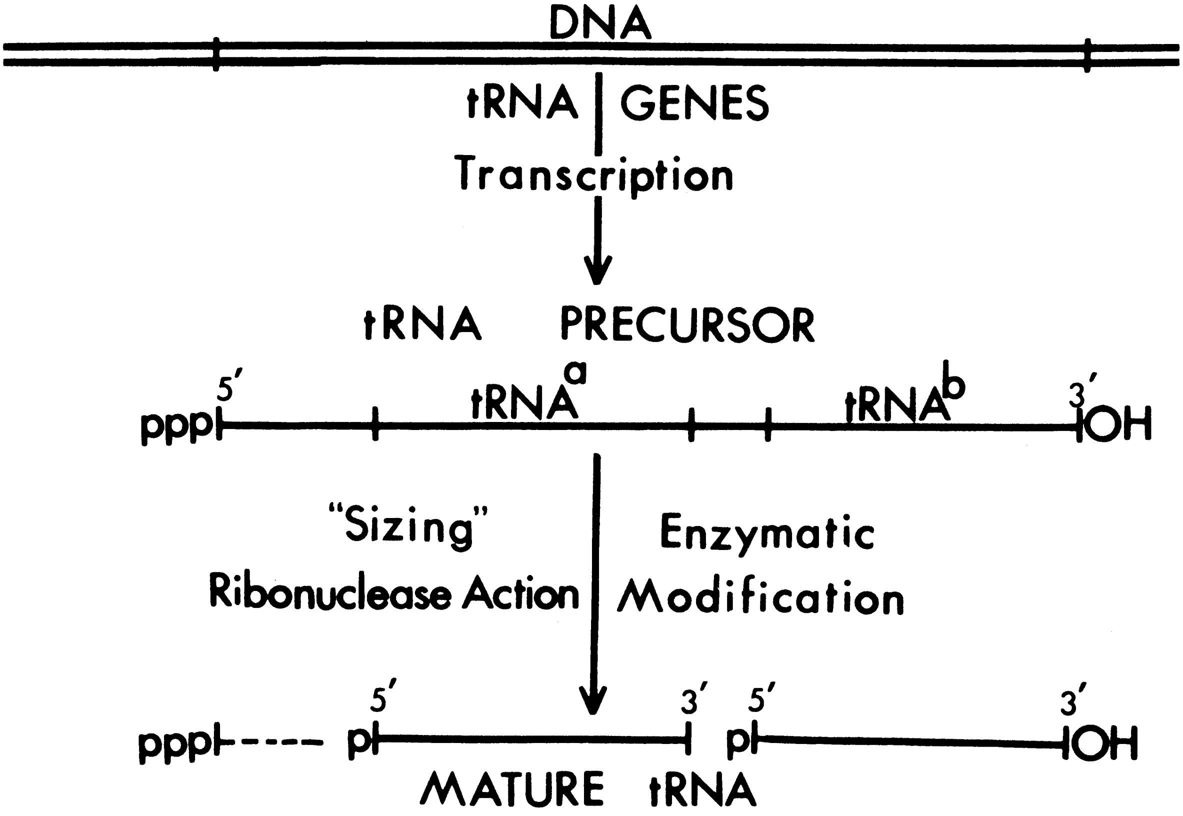
1. Role in Ribosomal Protein Synthesis

Amino Acid Activation

The major function of tRNA is to transfer amino acids during protein synthesis through translation of the mRNA (for the most recent review see 40). During this process, tRNA molecules interact with many different enzymes as well as RNA molecules. The amino acid is initially "activated" by a specific activating enzyme, aminoacyl-tRNA synthetase, and ATP (41; 42) to form an enzyme-bound aminoacyl adenylic acid intermediate. The aminoacyl group of this intermediate is immediately attached to the 2' or 3'-terminal adenosine residue of an appropriate tRNA through an ester linkage. Each of the twenty different aminoacyl-tRNA synthetases is specific for one amino acid. The specificity of these synthetases for both their amino acids and their corresponding tRNAs is the first and possibly most important step to ensure the fidelity of translation.

Evidence has been obtained which indicates that many different

Figure 2. Scheme of steps involved during the biosynthesis of tRNA.


DNA
tRNA GENES
Transcription
tRNA PRECURSOR
5′
pppl
tRNA^a
tRNA^b
3′
OH
"Sizing"
Ribonuclease Action
Enzymatic
Modification
5′
3′
5′
3′
pppl----
p
p
OH
MATURE tRNA

regions of the tRNA molecule including the dihydrouridine loop (43), the dihydrouridine stem (44), the anticodon loop (45), and the acceptor stem (46) are important in synthetase recognition. It has recently been suggested that no single particular site of the tRNA molecule is alone responsible for synthetase recognition (31). Experiments by Schimmel's group (47; 48) have shown that the nucleotides which are shielded by the synthetase are found scattered throughout the tRNA molecule. They have also shown that all of the sites on tRNA which interact with synthetase are on the inside face of L-shaped tertiary structure, implicating the tertiary configuration in recognition of tRNA by the synthetase. (For a recent review of tRNA and aminoacyl-tRNA synthetase recognition see reference 49).

Initiation of Translation in Prokaryotes

First, the bacterial initiator N-formyl-met-tRNA_f^{met} binds to the appropriate factors (IF1, IF2, and IF3) and GTP and forms an obligatory initiation complex which then reacts with the small (30S) ribosomal subunit in the presence of the initiation codon AUG located near the 5' terminal in mRNA (50). The initiation factors possibly recognize both the formyl group and the acylation-dependent tertiary structure of the initiator tRNA, thus discriminating against non-formylatable met-tRNA_f^{met} (51). All three initiation factors have been shown to have some effect on this reaction, but IF2 plays the most important role; its requirement has been shown to be absolute (52). Research toward identification of the particular function for each of these initiation factors has not been too successful. Nevertheless, it appears that the effects are actually cooperative (53; 54).

Initiator tRNA enters the 30S subunit at the aminoacyl binding site (A site), and when the 50S ribosomal subunit joins the complex, it translocates to the peptidyl site (P site) in the 50S subunit, during which GTP is hydrolysed. Upon addition of 50S subunits to complete 30S initiation complexes described above, 70S complexes are formed (55; 56). During initiation, part of the TψC loop in fmet-$tRNA_f^{met}$ may not base pair with 5S rRNA as has been hypothesized to occur with other aminoacyl-tRNAs (57; 58). Furthermore, the fmet-$tRNA_f^{met}$ which is the only tRNA to be recognized by transformylase (59) is not bound to elongation factor, Tu, in direct contrast to the other aminoacyl-tRNAs (60), indicating the selective role of fmet-$tRNA_f^{met}$ to ensure the fidelity of initiation during protein synthesis.

Elongation in Prokaryotes

After the 70S initiation complex is formed, non-initiator, aminoacyl-tRNA binds to a specific protein factor, elongation factor T(EF-T)(61) which consists of two subunits, EF-Ts and EF-Tu. The EF-Tu•GTP complex then binds to both aminoacyl-tRNA and the 70S ribosome (62). It has been suggested that the ribosome, particularly 5S rRNA, plays a role in this binding by providing a complementary tetranucleotide that base pairs to the non-variable TψCG tRNA sequence (19; 57). Binding occurs at the A site of the 70S ribosome, as the anticodon triplet of the tRNA hydrogen-bonds to the complementary triplet, codon, in mRNA. GTP is then hydrolyzed and GDP dissociates from the complex.

The formation of the peptide bond is catalyzed by the enzyme peptidyl transferase which is bound to the 50S subunit. This enzyme catalyzes peptide bond formation with the new aminoacyl-tRNA replacing the tRNA of the preceding amino acid as the protein lengthens from amino to carboxyl end (55).

The lengthened peptidyl-tRNA remains bound to the A site until the preceding uncharged tRNA leaves the P site. The ribosome then moves along a distance to the next reading frame on mRNA, while translocating the peptidyl-tRNA from the A site to the P site. Now the A site is available for next aminoacyl-tRNA complex and then another cycle of chain elongation can occur. Translocation of the ribosome is dependent upon GTP hydrolysis as well as the presence of elongation factor G(EF-G) as EF-G•GTP complex. EF-G dissociates from the ribosome after translocation.

Termination in Prokaryotes

All translation utilizes the same three terminator codons (UAA, UAG, and UGA). These codons are recognized by protein release factors. RF-1 recognizes UUA or UAG specifically, whereas RF-2 recognizes UUA or UGA (63; 64). However, RF-3 has the capacity to facilitate the binding of RF-1 and RF-2 to ribosomes and interacts with GTP (64). The detachment of a complete polypeptide chain from the ribosome is not yet fully understood (55; 65). Nevertheless, RF-1, RF-2, and RF-3 interact with the ribosome to promote the release of polypeptidyl-tRNA from the ribosome once one of the three termination codons is read. Following this, the ester bond between the polypeptide and the tRNA is hydrolyzed. Then the tRNA, mRNA, and ribosomal subunits dissociate. Termination is also a GTP-dependent reaction.

Protein Synthesis in Eukaryotes

Eukaryotic translation is a more complicated process than that of prokaryotes, and therefore has not yet been as well defined. Nevertheless, it has been shown that, in eukaryotes, initiator met-tRNA^{met} is not formylated; the ribosome is an 80S particle with a 40S and a 60S subunit in contrast with the 70S particle with a 30S and a 50S subunit in a prokaryotic system; and elongation, termination, and releasing processes have more protein factors involved as compared with that of prokaryotes. (For most recent reviews see 66; 67; 68.) Even with the differences mentioned above, the mechanism of tRNA involvement in both prokaryotic and eukaryotic translation systems must be considered similar from the present state of knowledge.

2. Other Roles of tRNA

Aside from the major role of tRNA in ribosomal protein synthesis, many other processes have been discovered in which tRNA or aminoacyl-tRNA is a participant. Unfortunately, very few of these processes are well understood. However, the intriguing possibility of tRNA being involved in metabolic processes as a regulatory factor strongly attracts the attention of those who are in this area of research. Those roles are outlined below.

Availability as an Effector of Protein Synthesis

The major function of tRNA is the accurate translation of the genetic code during protein synthesis. As an extension of tRNA's major role in translation and because of the degeneracy of both the genetic code and tRNA, it has been proposed that tRNA could play a role in regulating the rate of protein synthesis (69). In this role isoaccepting tRNA species with specific anticodons could be present in limiting amounts and would control the rate of translation of certain mRNA by virtue of these anticodons being insufficient in supply. It has been demonstrated that certain isoacceptors are preferentially used: a specific glycine tRNA species in the synthesis of collagen (11); certain leucine tRNAs in *E. coli* (70); and particular alanine, glycine, and serine tRNAs in silkworm fibroin synthesis (2). These results indicate that a specialized tRNA population may serve as a critical factor for the rate of synthesis of a particular protein. Further supporting evidence has been demonstrated in reticulcytes (71) and lens tissue (5).

Involvement in Biosynthetic Pathways

Aminoacyl-tRNA involvement in regulating the biosynthesis of several amino acids has been demonstrated in both prokaryotic (72) and eukaryotic organisms (73). Possible interactions have been postulated to explain this participation: (1) tRNA may interact with the first enzyme of the pathway and thus exert a regulating role over this enzyme and accordingly over the whole reaction sequence; (2) aminoacyl-tRNA (or the synthetase) may interact with the attenuator site in a gene's operator region causing repression or derepression of the operon for the biosynthetic enzymes.

The histidine operon, among several biosynthetic systems studied, has been one of best characterized for tRNA involvement. It comprises the structural genes for nine enzymes, which catalyze the formation of histidine, and the operator and promoter control regions (74). It has been shown by genetic analysis that there are six loci which can cause derepression of the histidine operon (75). The levels of his-tRNA *in vivo* have been measured in strains of *S. typhimurium* containing representative mutations in each of the six regulatory genes (76). An inverse relationship between his-tRNA levels and histidine operon expression was found for mutants involved in hisU and hisW which possibly code for processing enzymes for $tRNA^{his}$; hisS which codes for histidyl-tRNA synthetase; and mutants in $tRNA^{his}$ gene itself. Surprisingly this relationship was not true for hisT mutants in which the modifying enzyme, pseudouridine synthetase I is affected. Experimental results indicate that histidyl-tRNAs must have a specific pseudouridine residue in the anticodon region as a signal for repressing the histidine operon. It was also found that when the first enzyme of the pathway was altered by mutation, the kinetics of repression were altered (77). The hisG protein has an affinity for tRNA; its activity is inhibited by his-tRNA more than by any other tRNAs tested (78). Binding of the hisG protein to hisO DNA which is the operator-promoter region has also been demonstrated (79). Thus it had been suggested that the hisG enzyme fits some requirements for a classical repressor with his-tRNA serving as the co-repressor (80).

With the demonstration of repeated amino acid codons in mRNA leaders, tRNA involvement in gene expression is thought to occur through attenuation of translation.

Primer for RNA Virus Replication

Reverse transcription enzymes (RNA-dependent-DNA-polymerase) from RNA tumor viruses utilize specific tRNAs as primer for the synthesis of viral coded DNA. The enzymes from avian myeloblastosis virus (81) and Rous sarcoma virus (82; 83) use tryptophan tRNA; whereas the enzyme from a murine leukemia virus uses proline tRNA (84). A certain number of selected tRNA species are incorporated non-covalently into C-type viral particles during encapsulation (85); but only a specific tRNA, tightly bound to a complementary sequence at the 5' end of the viral genome, is used for priming the reverse transcription. It is also well established that many viruses, especially plant viruses, have tRNA-like structures so that their 3'-end can be charged in vitro with one amino acid, such as turnip yellow mosaic viral RNA with valine (86' 87). However its biological significance is still not clear yet.

An Enzyme Modulator

Transfer RNA has been show to play a role affecting certain enzymy activities. Goebel and Helinski (88) have suggested that tRNA is a potent inhibitor of *E. coli* endonuclease I by observing that tRNA alters the activity of this enzyme which is capable of making double-stranded cuts in DNA. It has also been reported that $tRNA^{tyr}$ or $tRNA^{phe}$ inhibited the activity of DAHP synthetase, a feedback sensitive enzyme catalyzing tyrosine and phenylalanine biosynthesis in *S. cerevisae* (89). One species of tyrosine tRNA has been shown to inhibit the enzyme tryptophan pyrrolase from a Drosophila vermilion mutant (90; 91). However, it has since been reported that both the cases discussed above might happen to be artifactual (92; 93). More recently, investigations of Drosophila mutants and their diet tend to support involvement of tyrosine tRNA in regulation of tryptophan pyrrolase (94). Tyrosine tRNA has also been shown as a possible enzyme modulator in the control of tyrosinase activity. By measuring the level of tyrosine tRNA isoacceptors and tyrosinase activity in swine melanoma tissue, it has been demonstrated that the level of one of three isoacceptors is correlated with the enzyme's activity (95). *In vitro* experiments suggested that binding of tyrosine tRNA to tyrosinase caused an enhancement of the enzyme's activity (96). By using two different DNA operons as substrate in an *in vitro* transcription system, Pongs and Ulbrich (97) have shown a rather general controlling role of tRNA in the enhancement of enzyme activity; the binding of *E. coli* fmet-tRNA to RNA polymerase III caused an increase of its ability to synthesize RNA.

Effector of Stringent Response

When prokaryotic cells are starved for amino acids and thus unable to synthesize proteins, deacylated tRNA occupies the ribosomal A site. The simultaneous presence of mRNA and the relA gene product, both bound to the ribosome, cause the synthesis of ppGpp and pppGpp which are referred to as magic spot I and II respectively (98; 99; 100). These phosphorylated nucleotides appear to cause a major pleiotropic alteration of cellular metabolism including cessation of stable RNA synthesis (101). Further study indicates that the formation of magic spot is not dependent on the absolute concentration of deacylated tRNA; but rather on the ratio of aminoacyl-tRNA to tRNA (102).

A Suppressor

Suppression of missense, chain termination, or frame-shift mutations during translation results from tRNA genetically altered in the anticodon sequence being capable of responding to these mutations (for reviews see 103 and 104).

Non-ribosomal Peptide Synthesis

There is a specific class of enzymes called aminoacyl-tRNA transferases (23) which transfer amino acids from aminoacyl-tRNAs to a variety of recipient molecules without the involvement of a decoding mechanism and ribosomes. A specific class of glycine tRNAs from Staphylococci are thought, with the aminoacyl-tRNA transferase, to transfer glycine to N-acetylmuramylpentapeptide, an intermediate in the cross-linking bridge of the bacterial cell wall (105; 106). The acceptor molecule can also be a phospholipid so that the transferred amino acid becomes incorporated into a cell membrane component (107). It also has been shown that the amino acid-accepting molecule could be a finished protein, thus the amino acids of specific aminoacyl-tRNAs are transferred to the N-terminal ends of certain proteins (108) . The aminoacyl-tRNA transferase activities have also been found in mammalian cells and transfers of arginine from arginyl-tRNA to erythrocyte ghost membrane protein (109) and some nuclear proteins associated with chromatin have been reported (110).

D. The Role of tRNA Modifications

A characteristic of tRNA is that it contains a variety of modified nucleosides. Modified nucleosides occur in tRNA with a high frequency and a wide range of structural variation. At the present time the structures of about 55 modified nucleosides have been elucidated (21). Some of these are simple methylations of the base or the 2' hydroxyl of the ribose moiety; while others are so-called hypermodifications with a great deal more complex carbon structure. Examples of hypermodified nucleosides include: adenosine substituted with γ,γ-dimethylallyl or similar groups (i^6A, N^6-isopentenyladenosine); nucleosides modified with various amino acids as t^6A and X (ribofuranosylpurin-6-yl-carbomyl threonine and 3-(3-amino-3-carboxypropyl)uridine, respectively). More recently the structures of some even more complicated modified bases have been elucidated. The fluorescent nucleoside Y, found in yeast $tRNA^{phe}$ contains a tricyclic ring system derived from guanosine (21). The structures of the modified base Q and its sugar derivatives manQ and galQ have been determined (111; 112). Most of our knowledge about the detailed steps in the biosynthesis of modified nucleosides in tRNA derive from _in vitro_ studies on the characterization and purification of tRNA modifying enzymes. To date, only relatively few tRNA modifying enzymes have been well characterized or obtained in fairly purified form such as the enzymes for certain methylations, thiolation, and Q formation (113; 114; 115; 116; 117; 118 ; 119; 120; 121; 122).

From genetic analysis and _in vitro_ experimentations, some information on the function of tRNA modified nucleosides has been obtained. It is plausible to think that modified nucleosides may somehow be engaged in regulatory processes. Evidence from different sources indicate that modified tRNAs act more efficiently by being able to form a stronger complex with the ribosome, mRNA, and transfer factors during protein synthesis (21; 23; 124; 125; 126 ; 127; 128 ; 129). A possible explanation for the existence of 2'-O-methyl nucleosides may be a protective effect by blocking ribonuclease action on the adjacent phosphodiester bond (24; 28; 130; 131; 132). Transfer RNA is susceptible to various ribonucleases. If ribonuclease attack is specific for particular tRNAs, it could be significant in the regulation of protein synthesis. It has been reported that tRNAs of _E. coli_ have different susceptibilities to _B. subtilis_ ribonuclease or to RNase T1 (133). It is also known that, _in vivo_, a preexisting tRNA^{leu} of _E. coli_ is "nicked" by a nuclease formed after phage T2 infection without losing leucine acceptor activity (134).

Evidence has also been accumulated showing that the amounts of modified nucleosides change during such cellular events as differentiation, senescence, neoplasia, chemical carcinogenesis, and viral transformation (13; 135; 136; 137).

E. Biology of Friend Leukemia Cells

1. Normal Erythropoiesis

The ontogeny of red blood cells is now understood in some detail. Erythrocytes are derived from a pleuripotent hematopoietic stem cell (CFU-S) in bone marrow (138). Intermediate stem cells (ECP) which become committed exclusively to the erythroid line as unipotential are developed from CFU-S under the influence of the organ stroma (139); these committed cells later become sensitive to erythropoietin and then differentiate to a series of erythroblasts. The erythroblasts further maturate to form erythrocytes. The mechanisms and factors which control the production of different hematopoietic cell lines are still not clear. However, the role of erythropoietin in hematopoiesis has been well studied. (For a recent review see 140.) Nevertheless, erythropoietin seems to have a dual role: maintenance of the committed erythroid stem cell pool and, at least at high doses, enhancement of globin messenger RNA formation in immature erythroblasts.

Because of the asynchrony of the precursor cell population in the pool and the difficulty of maintaining one particular precursor cell stage for long periods, biological and biochemical analysis of erythroid differentiation has been seriously limited. However, the demonstration by Friend et al. (141) that a line of virus-infected leukemia cells could be induced to produce hemoglobin when treated with certain agents seemed to provide a model system in terms of its relevance to the study of erythroid differentiation and possibly how the process becomes abnormal in erythroleukemia.

2. Friend Leukemia Cells

Inoculation of a murine leukemia virus variant, Friend leukemia virus complex, into young and adult mice of susceptible strains, followed by an incubation period of about 2 weeks, produces an erythroleukemia (142). The early phase of the disease is characterized by erythroblastosis accompanied by enlargement of the spleen and liver in the animal. Terminally, the spleen, liver, and bone marrow are massively infiltrated with immature cells which almost completely replace the normal cells of these tissues. Eventually, the immature cells lose their ability to further respond to erythropoietin. Subcutaneous implantation of fragments of these defected organs into recipient syngenic mice results in tumor formation at the inoculation site (143). The original permanent line of Friend leukemia cells was established from the fragments of the transplantable tumor (144). Since then, such erythroleukemia cell lines have been isolated independently in a number of laboratories. Certain cloned subpopulations also have been established (145; 146). These cell lines grow in suspension culture with a doubling time of 12-20 hours depending on the particular line and culture conditions (147). Such cell lines represent a population of chronically infected erythroid precursor cells arrested at the proerythroblast level of development (144). The cell population has a small percentage (1%) of spontaneously differentiating cells (147). It has been demonstrated that a few variant clones have a baseline of spontaneously differentiating cells as high as 15% (148). All of the cells in culture constantly synthesize and release viral particles (149;

150). They also retain their neoplastic properties and produce tumors that are histologically identical to those from which the culture lines were derived (147).

3. Enhancement of Erythroid Maturation of Friend Leukemia Cells

In 1971, Friend et al. (141) added dimethylsulfoxide (DMSO) to the Friend leukemia cell culture medium in an attempt to increase virus infectivity. Incidentally, over a 4 to 5 days incubation period, they found the cells began to resemble the more mature red-cell precursors, normoblasts, and the cells began to synthesize large amounts of hemoglobin and heme products (141). They also observed that about 85% of the cells in culture medium containing 270 mM DMSO were mature at the end of the five day incubation period. Subsequently other polar organic solvents (151; 152; 153; 154), short chain fatty acids (155), hemin (156), several purines and purine analogues (157), and a number of metabolic inhibitors (158) have also been found to be potent inducers of erythroid differentiation in this cell line. Cellular changes of Friend leukemia cells after the induction have been reported, and have been shown to parallel those occurring during the course of normal erythroid maturation (140). These changes are outlined below.

Morphological Changes

Friend et al. (141) demonstrated that when cells seeded in DMSO-supplemented medium were observed daily for 7 days, no definitive morphological alterations were seen until the 3rd day when there was a slight increase in the percentage of small cells that stained benzidine positive or B$^+$, indicating cellular accumulation of hemoglobin. The number of B$^+$ cells greatly increased by the fourth day. After 7 days of growth, most of the treated cell had decreased ratios of nuclear to cytoplasmic area compared with the control, untreated cells. A red color was observed at day 4 in washed and pelleted, treated cells. This color of the pelleted cells gradually deepened with further culturing in DMSO-containing medium. Electron microscopic observations have shown several ultrastructural changes for Friend leukemia cells undergoing DMSO-induced differentiation (149). Complex vascular structures containing numerous viruses were formed in the DMSO-treated cells. These cells also had an increased number of viruses budding from their membrane. In addition, ribosomes were decreased in number in treated cells compared with that of control, untreated cells. Furthermore, the control cells contained so many ribosomes that it was difficult to discern polysomes, whereas ribosomes in the DMSO-treated cells were arranged in clusters of 2 to 4 (159).

Growth Rate Alteration

Friend et al. (160) have reported that Friend leukemia cells exhibited a 24 hr growth lag right after the addition of 270 mM DMSO to the medium. After the initial lag period, logarithmic growth occurred. The treated cell densities approximated those of the control, untreated cells by the fourth day of culture. A similar observation, but with a somewhat longer lag period, also has been reported by Agris (13). However, the cause of the DMSO-treated cell growth retardation in the first 24-48 hr is still not clear.

Cell Membrane Changes

Accumulation of the high molecular weight erythrocyte membrane protein, spectrin, has been demonstrated for induced Friend leukemia cells as well as the loss of H-2 antigen. The peak rate of spectrin accumulation occurs 1-2 days after the addition of DMSO to the medium (161). Decreased membrane permeability (162) and increased membrane microviscosity (161) have also been observed in association with DMSO treatment of these cells. Moreover, DMSO-treated cells have been shown to be readily agglutinated by lactin (163).

Effect on Macromolecular Synthesis

a. Heme, Globin and Hemoglobin

Friend et al. (141) have demonstrated that cultures of F.L.C. grown in the presence of DMSO accumulated more iron and incorporated more iron into heme and hemoglobin than control cultures. A detectable stimulation of heme synthesis was noted after 2 days of culture and by the third day hemoglobin synthesis was found to be enhanced. The amount of heme synthesized after 4 days of culture was 60 times greater and the amount of hemoglobin synthesized was 40 times greater in DMSO-treated cultures than in the corresponding control cultures (147). Studies using ion exchange chromatography, immunoprecipitation, and tryptic fingerprint analysis have shown that the globins produced in DMSO-treated cells were the adult α and β chains of DBA/2J mice from which this cell line was derived (145; 164). α and β chain synthesis in these cells seemed to be relatively balanced. Synthesis of embryonic globins was not detected (147). By coelectrophoresis of the DMSO-treated cell lysates with adult mouse globin chains, Young et al. (165) have demonstrated that any drastic increase of globin synthesis could not be detected until the second day after DMSO treatment and that globin production reached a peak of about 26% of total cellular protein synthesis on the fourth day.

b. Total DNA, RNA and Protein Synthesis

Commitment to differentiation does not immediately cause F.L.C. to lose their capacity for DNA synthesis. Instead, they display a limited proliferative capacity (166), similar to normal murine erythroid precursor cells at the erythropoietin-sensitive stage (167; 168). Gradually, upon the completion of differentiation, these B^+ cells in suspension lose proliferative capacity and arrest in G1 phase of the cell cycle (169). However, a delay in the overall rates of incorporation of radioactively-labelled precursors at the onset of cell replication indicates that the addition of DMSO to the culture medium results in a lag of 24-48 hr in F.L.C. growth, i.e., the number of cells in the culture does not begin to increase until the second day after the addition of DMSO (160). The maximum rates of precursor incorporation for DNA, RNA, and protein synthesis occur after 48 hr in treated cultures as compared to control cultures where cell proliferation begins almost immediately after seeding (13).

c. Globin Messenger RNA

It has been demonstrated both by molecular hybridization with the use of globin cDNA (170; 171; 172) and by translation _in vitro_ (173) that globin mRNAs began to accumulate after 24-30 hr of DMSO treatment and reached a maximal concentration after 3 days. Clone 745A of F.L.C. has been found to contain at least 100 times the amount of globin mRNA present in control cells when treated with DMSO for 4 days (174). This study also indicated that globin mRNA was 0.14% of total RNA in differentiated cells. More detailed studies have shown that α- and β-globin mRNAs do not accumulate coordinately. Using cDNA, reverse-transcribed from partially purified α- and β-globin mRNAs, Orkin et al. (175) obtained an α- and β-globin mRNA ratio of 3.7 at 30 to 35 hours after DMSO treatment of Friend leukemia cells. This ratio decreased to unity in the fully induced cells, i.e., to the value in normal reticulocytes.

4. Timing of Cellular Events During F.L.C. Erythroid Differentiation

In the F.L.C. system, DMSO causes an initial lag in growth as has been reviewed previously, but does not affect cell viability (141), mitotic rate or DNA content (176). Irreversible commitment of F.L.C. to erythroid differentiation has been reported to occur after 12-18 hours of continuous exposure to DMSO (166). After this latent period of exposure to DMSO, the fraction of cells producing hemoglobin subsequently is not reduced by removal of DMSO from the medium (177). It has been reported that at least two rounds of DNA synthesis are required for the DMSO induction of hemoglobin synthesis (177; 178). Under suboptimal conditions of induction, no correlation is found between the hemoglobin content of individual, differentiated cells and the DMSO concentration used to induce the cells (175). Recent evidence by clonal analysis indicates that commitment of cells for differentiation limits the cell to four additional divisions (169). However, heterogeneity, which is not due to genetic heterogeneity in the cells, exists in the cell population as committed cells appear. Thus a constant fraction of the cell population remains uncomitted to differentiation during each cell division cycle (166).

5. Changes in tRNA of F.L.C. Under Various Conditions

F.L.C. deprived of histidine for several days exhibited a two-fold increase in the relative amount of $tRNA^{his}$ with no change in the relative level of $tRNA^{leu}$ (179). Similar observations were also reported for leucine and tryptophan starvation (180). In addition, the same report indicated that different isoacceptors may respond differently to amino acid deprivation. It has been suggested that the increase in the relative concentrations of tRNAs in amino acid deprived F.L.C. may not be due to newly synthesized cognate tRNA, but rather to a decreased rate of degradation of the cognate tRNA (181).

Analysis of the nucleotide composition of tRNA from DMSO-induced, differentiating F.L.C. has been investigated (13). The nucleotide composition of F.L.C. undergoing erythroid differentiation was drastically changed compared with that of the undifferentiating F.L.C. A definitive decrease was observed in the relative amount of the major nucleotide, G_p, in differentiating cells. The ratio of A+U/G+C was also significantly higher in differentiating cells. Furthermore, the content of the minor nucleosides ribothymidine, pseudouridine, and a ribose methylated guanosine as well as two unidentified nucleosides in the tRNA of differentiating cells was altered in comparison to that of control F.L.C. A comparison of tRNA from seven other non-hematopoietic cell lines (mouse and human) with and without treatment with DMSO showed no significant alteration in nucleoside composition, implying that the alteration of F.L.C. tRNA nucleotide composition is concurrent with the erythroid differentiation. There was a distinctive difference between the relative amounts of $\text{tRNA}_4^{\text{lys}}$ found in differentiating F.L.C. and that found in undifferentiating cells. As indicated by the same report, $\text{tRNA}_4^{\text{lys}}$ represented only 4.2% of the total lysine tRNA from differentiating cells, whereas it represented almost 10% of that from undifferentiating counterparts.

More detailed investigations of tRNA^{lys} isoacceptor distributions during F.L.C. erythroid differentiation has since been reported (182). By using tetramethyl urea as the inducing agent, it was found that $\text{tRNA}_4^{\text{lys}}$ comprised 32% of the total tRNA^{lys} in rapidly dividing, uninduced F.L.C., but greater than 53% of the total tRNA^{lys} in completely differentiated cell cultures. It was suggested that the increase in the relative amount of $\text{tRNA}_4^{\text{lys}}$ during the erythroid differentiation may not reflect the erythroid properties of the cell.

F. Possible Mechanisms of Regulation Mediated by tRNA

Cell differentiation often involves drastic changes in cell metabolism. Thus, the pattern of synthesizing proteins is altered quantitatively and qualitatively during the differentiation process. The regulation of gene expression during differentiation could occur at the transcriptional level and the translational level. Due to the multiple functions of tRNA at the translational level, tRNA has been thought to be one of the most likely effectors of regulation. The degeneracy of the code words and the degeneracy of the corresponding tRNAs plus the nonrandom selection of codes in mRNAs makes possible the regulation of translational rate by the availability of tRNA species. Chromatographic techniques capable of separating the isoaccepting tRNAs have been used to demonstrate changes in tRNA species during differentiation.

Based on these facts and others, some hypotheses have been postulated in order to address the regulation of protein synthesis in differentiation with mediation by tRNA.

1. Modulation Hypothesis

By studying the quantitative differences among different types of hemoglobin controlled by multiple alleles in heterozygotes, Itano (183) postulated that if one mutation leads to a coding triplet that corresponds to a less abundant tRNA species, the translation of the mutant allele will be slower, thus controlling its gene expression. Ames and Hartman (184) who studied polarity effects in the biosynthesis of enzymes in the histidine operon elaborated further on this hypothesis. They stated that the translation of mRNA is limited by modulating triplets, which in turn, correspond to modulating tRNAs that exist in the cell at all times. Thus the modulator tRNA could be a rate limiting factor causing the ribosome to have a high probability of falling off the mRNA when the ribosome comes to a modulating triplet. Studies on nonsense mutations and their suppression by suppressor tRNAs have produced results consistent with this hypothesis.

2. Adaptor Modification Hypothesis

Sueoka and Kano-Sueoka (69) proposed the adaptor modification hypothesis in which the control of protein synthesis could be achieved by altering the properties of tRNA instead of mRNA. They postulated that the anticodon of one particular tRNA in a set of tRNA isoacceptors is changed by structural modification. In this situation, the mRNAs requiring anticodons corresponding to the modified tRNA species would not be translated properly. By this means, certain gene expression would be shut off at the ribosome by modification of a specific tRNA species while other genes remain functional at the translational level. Thus, by modifying certain tRNA species, particular mRNA molecules would be translated more effectively than others. It is noted that the modification itself must be under genetic control. Consistent with this hypothesis, the biochemical and biophysical nature of the modifications have been studied in great detail and include the methylations, thiolations, precursor tRNA processing by nucleases, repair of CCA sequence by nucleotidyl transferase, and the concomitant tertiary conformational changes.

3. Functional Adaptation Hypothesis

The finding of differences in the tRNA populations between differentiated and undifferentiated cells, and that the optimum efficiency of protein biosynthesis depended on the availability of aminoacyl-tRNAs in the active pool for protein synthesis, prompted Garel (1; 2) to propose the functional adaptation hypothesis. He claimed that the intracellular tRNA level, i.e., anticodon level, is directly proportional to the composition of the amino acids in proteins being synthesized. Thus, continuous and selective adjustment occurs to form an adaptation of the cellular tRNA in order to achieve differentiation. Evidence has been shown that the tRNA population may be adapted for the synthesis of fibroin (185), hemoglobin (7; 8; 9), collagen (11; 12), and crystallin (186).

G. Isoaccepting tRNAs and Their Differences Under Various Conditions

The multiplicity of codons for a given amino acid accompanied by a multiplicity of tRNAs has been proven and accepted for all organisms. Thus, tRNAs of different structure, which are aminoacylated with the same amino acid, are designated isoaccepting tRNA species. However, there is no trivial relationship between the number of isoaccepting tRNAs and the degree of codon degeneracy. Isoaccepting tRNA species can be classified into three categories. The first class includes tRNAs having different primary structures. These tRNAs are transcribed from different tRNA genes. This class of isoaccepting species is further separated into two groups: (a) tRNAs with different anticodons, which therefore recognize different coding triplets, and (b) tRNAs with the same anticodons but otherwise different nucleotide sequences. The second class consists of multiple tRNA species formed from the same original transcripts, however, they have been post-transcriptionally modified to different extents. tRNAs with the same secondary structures but different tertiary structures constitute the third class. These tRNAs can be separated by various chromatographic methods and would initially be considered as different isoaccepting species, at least until the identity of their structures were determined.

New isoaccepting tRNA species are often observed in particular tissues, in tumor tissues, in cells at different stages of differentiation, in transformed cells, or in cells grown under different culture conditions (for reviews see 22; 135; 187).

There are numerous reports on the appearance of isoaccepting species, detected by changes in the chromatographic profiles of amino acid acceptance activity. However, there is no solid conclusion as to what possible regulatory role tRNA plays in protein synthesis through these changes in isoaccepting species. Findings in support of a regulatory role for tRNA are briefly reviewed and outlined below:

1. Normal Tissue vs. Malignancy

Transformed tissues are known to contain an isoacceptor of $tRNA^{lys}$ ($tRNA^{lys}_4$) that is hardly detectable in normal cells. The amount of $tRNA^{lys}_4$ in dividing cells or cells having the potential to divide is proportional to the rate of cell division (188 ; 189). By knowing the sequence of $tRNA^{lys}$, it has been shown that the primary sequence of $tRNA^{lys}_4$ from SV40-transformed 3T3 cells is identical with that of $tRNA^{lys}_2$, which is the major isoacceptor of normal cells; the only difference between the two tRNAs is that $tRNA^{lys}_4$ has not been modified in several positions (22).

Another interesting tRNA species in this aspect is $tRNA^{phe}$. It has been reported that a $tRNA^{phe}$ isoacceptor is present in certain rat hepatomas in addition to the single major isoacceptor generally found in normal rat tissues (190; 191; 192). Similar observations have also been reported for certain mouse tumors, an Ehrlich ascites line (193) and a neuroblastoma line (194). Using RPC-5 chromatographic separation, Mushinski and Marini (195) have examined the isoacceptor distributions of $tRNA^{phe}$ from 25 transplantable rat tumors and 33 transplantable mouse tumors as well as 25 normal rat and mouse tissues of different histological origins. Seventeen rat tumors and ten mouse tumors, of a wide spectrum of histological types, were found to have an additional, tumor-associated $tRNA^{phe}$ isoacceptor. In agreement with previous reports, these authors suggested that the tumor-associated $tRNA^{phe}$ has a different structure from that of the normal, major $tRNA^{phe}$, and that these differences result from different degrees of post-transcriptional modification, most likely at the hypermodified Y base. Changes in the chromatographic profiles of $tRNA^{tyr}$, $tRNA^{his}$, $tRNA^{asn}$, and $tRNA^{asp}$ have been observed between normal tissues and their malignant counterparts (196; 197; 198). These chromatographic differences are thought to result from different levels of Q modification.

2. Different Tissues and Organs

The relative proportions of tRNAs for various amino acids are quite similar among most mammalian species and among tissues or organs of the same animal (199). However, by using RPC-2 chromatography, differences with regard to the isoacceptor distributions between cattle muscle and lens have been demonstrated (200). Some variation in the chromatographic patterns of $tRNA^{ser}$ and $tRNA^{leu}$ have been noted among mouse liver, spleen, kidney, and testis (201). Numerous reports in this regard have accumulated indicating variation of tRNA isoacceptor patterns between different tissues and organs (10; 185).

3. During Differentiation

Comparison of tRNA isoacceptor distributions from embryonic chick and adult chick liver has been reported (202). Only $tRNA^{lys}$ and $tRNA^{tyr}$ out of thirteen tested aminoacyl-tRNAs showed significant differences by RPC-2 chromatography. An earlier report (203) had shown that $tRNA^{met}$ and $tRNA^{leu}$ exhibited quantitative and qualitative differences when isoacceptor distributions from erythrocytes of 4-day-old chick embryos and from reticulocytes of adult chickens were compared on methylated albumin kieselguhr (MAK) column and RPC-2 chromatography. In addition, MAK chromatographic profiles of $tRNA^{met}$ and $tRNA^{arg}$ in erythrocytes of larval bull frog were shown to differ from that found in adult erythrocytes (204). A comparative study of tRNA isoacceptor changes during bovine and rabbit mammary gland differentiation indicated that a new $tRNA^{leu}$ isoacceptor is formed in lactating cells, which appears to be absent in the non-lactating gland (205).

Under Different Growth Conditions

It has been reported that administration of estrogenic hormones to laying hens and immature chicks raises the level of liver $tRNA^{ser}$ (206; 207). The chromatographic profiles of rat liver $tRNA^{lys}$ and $tRNA^{phe}$ are affected by thyroidectomy (208), and $tRNA^{ser}$ in pig uterus by ovariectomy (209). However, administration of thyroxine and estrogen, respectively, partially reverts tRNA to its normal pattern. Cell density and the type of supplemented serum in the culture may affect the relative proportions of tRNA isoacceptors (210).

5. Viral-Induced Changes

Alterations of tRNA isoacceptor patterns during the viral infection of *E. coli* have been demonstrated along with a drastic metabolic shift of the host cell (187; 211; 212). Alterations have also been observed after infection or transformation of mammalian cells with DNA viruses (213; 214; 215). Rous sarcoma virus infection has been reported to induce new host-originated 4S RNA species in chickens (216).

All of the reports above indicate that isoacceptor profiles change along with a drastic cellular metabolic shift in order to achieve a functional adaptation with the cellular condition. However, whether these tRNA changes result from or are the cause of the different physiological states still remains obscure.

H. Specialized Systems for Studying the Regulation of Protein Synthesis

Cellular differentiation in eukaryotic tissues is often characterized by the production of a relatively high abundance of a specific protein (or proteins). Thus, there appears to be an enrichment of the messenger RNAs which code for the differentiation-specific protein. Eukaryotic mRNA is, in general, rather stable compared to prokaryotic mRNA. However, other than the availability of mRNA, one would believe that several cellular components or conditions would be necessary for a prolonged and optimal translation of a particular mRNA. Among these conditions, the presence of certain tRNAs, thought to be involved in translational regulation, may be important, due to multiplicity of isoacceptors and anticodons. The specialization of tRNA content for protein synthesis has been recognized in several systems which synthesize differentiation-specific protein or proteins. Changes in tRNA isoacceptor abundance in specialized tissues suggest that the utilization of the isoacceptors varies in the synthesis of different proteins. The investigations of tRNA isoacceptor abundance in four specialized systems are reviewed below:

1. Reticulocyte System

The reticulocyte system has been studied for more than a decade. Ultimately, the system may prove that the availability of one or more tRNA species could limit the rate of globin chain translation, and thereby, limit hemoglobin production. As described by Smith and McNamara (71), the advantages of this system are: (a) a single, well characterized protein is synthesized in large amounts; (b) after the nucleus is lost there is no further RNA synthesis, thus the system is informationally ceased and dependent on the preexisting, stable mRNA. By using a tRNA-dependent rabbit reticulocyte cell-free translation system and reversed-phase freon (RPC-2) chromatography for fractionation of tRNA, Anderson and Gilbert (217) have revealed some information about the reticulocyte system. Different rates of translation have been observed in the in vitro system when 5 fractions of tRNA from rabbit liver were added in different combinations. As they pointed out, the omission of one fraction, II, decreased normal hemoglobin synthesis by 70%. Also the amount of α-chain synthesis was two-thirds the amount of β-chain synthesis under these conditions. The results of their experiment have been interpreted as meaning that the availability of one or more tRNAs could alter the rate of translation in vitro. Smith and McNamara (8) have investigated the total tRNA molecules and total ribosomal bound tRNA molecules for each amino acid. However there was not any demonstrable relationship between these and the amino acid composition of rabbit hemoglobin. Even though, the argument was made that globin translation does not proceed uniformly and the nonuniform rates of translation could result from limited availability of some tRNA species. Nevertheless, further investigation has shown that human reticulocyte tRNA is specialized. For instance, there is no isoleucine residue in human globin chain (both α- and β-chain), the acceptance activity of $tRNA^{ile}$ is also extremely low in unfractionated human reticulocyte tRNA (218).

It has been reported that, in comparing the isoaccepting tRNA distributions of rabbit reticulocyte with that of rabbit liver by RPC-5 chromatography, there are two isoacceptors each for asparagine and histidine in the reticulocyte and only one for each in liver (219). Though the elution patterns for some isoaccepting aminoacyl-tRNAs are very different for that of reticulocytes and liver, still no solid conclusion can be drawn about tRNA control of hemoglobin synthesis.

Another approach has been reported by Vestri and Rossi (9). They demonstrated a correlation between the concentration of isoleucine tRNA in reticulocytes and isoleucine content of rabbit and sheep hemoglobins. In rabbit reticulocytes, which synthesize hemoglobin containing 8 residues of isoleucine per molecule, the isoleucine acceptance activity is more than twice as high as in sheep reticulocytes, which contain no isoleucine in their globin chains.

A study *in vitro* of the rabbit reticulocyte translation system has shown that valine tRNA species from *E. coli* and yeast which respond to the codon GUG can preferentially transfer valine into both α- and β-chains; whereas other species of valine tRNA are hardly utilized (200). Determination of the sequence of rabbit globin mRNA confirmed this conclusion (221).

2. Silkworm *Bombyx mori* System

Changes in the translational machinery of the *Bombyx mori* posterior silk gland during silkworm differentiation have been well studied (185). At the end of the larval stage, the posterior silk gland of *Bombyx mori* is highly specialized in the biosynthesis of silk fibroin, representing almost 80% of total protein synthesis (222). Fibroin exhibits a remarkable amino acid composition, 43% glycine; 29% alanine; 12% serine; and 6% tyrosine (223).

Garel and his colleagues (1) have found that the tRNAs corresponding to these four amino acids increase with increasing fibroin production.

Their concentraiton increases right before the onset of fibroin production to as much as three fold that of the other tRNAs, indicating an enhancement of these particular tRNAs necessary for decoding the fibroin mRNA. Garel (2) has also shown that a very high correlation exists between the amounts of the different acylated tRNAs and the amino acid composition of fibroin in the posterior silk gland. This demonstration was done by calculating the correlation coefficient (ranging from 0.93 to 0.97) on the basis of data from three similar, however independent, investigations (1; 224; 225). Other calculations, similarly accomplished for the synthesis of middle silk gland sericin (222; 224), rabbit hemoglobin (71), mouse hemoglobin and IgF H chain (226), and calf lens crystallins (4) also gave significantly high correlations. Garel suggested that optimum efficiency of protein biosynthesis is obtained through continuous selective adjustment of cytoplasmic tRNA populations to the amino acid composition of the protein being synthesized. This adjustment has been referred to as "functional or quantitative adaptation". Further detailed investigations on fibroin synthesis revealed that there is also a quantitative relationship between the frequency of synonymous codons in mRNA and the distribution of the corresponding predominant tRNA isoacceptor (227; 228). Based on the calculations mentioned, Garel and colleagues therefore assumed that quantitative adaptation of iso-tRNA species to mRNA codon frequency is a general phenomenon. They further implied that the syntheses of a mRNA and the tRNA species needed for decoding it are coupled and genetically controlled. Thus the whole tRNA population acts as a regulator of protein synthesis (3).

3. Collagen Synthesis System

Collagen is composed preponderantly of 4 amino acids: glycine (33%), proline and its derivative hydroxyproline (23%), and alanine (11%) (229). Collagen is also the substrate of the enzyme collagenase (230). These two reasons make collagen-synthesizing tissues a good system to study the regulation of translation. It has been reported that in tendon, which synthesizes much more collagen than do the other chick embryo tissues the tRNAs for arginine, glycine, and proline were increased about 40, 33 and 83% respectively, over the average acceptance activity found in brain, heart and liver (10). The same report indicated that, when tRNAs for these amino acids, as well as for alanine, lysine, and valine, were chromatographed on an RPC-5 column, no major differences, in terms of isoacceptor distribution, were found in the different tissues, except in the case of glycyl-tRNA. One of the four glycyl-tRNA species showed a marked increase in tendon, as compared to tissue which makes little or no collagen. The role of this particular glycyl-tRNA isoacceptor in tendon collagen synthesis has been studied by translation in vitro (11; 12). The results indicated that this tendon-specific glycyl-tRNA isoacceptor showed a marked preference relative to the other glycyl-tRNAs for participation in collagen biosynthesis.

Lens Crystallin Synthesis System

Synthesis of crystallins in the lens fiber cell also provides a good system in which to study specialized protein synthesis (4; 5; 6). Crystallin in the well differentiated lens fiber cell accounts for approximately 80% of the total protein. Garel et al. (4) compared the tRNA population in the fiber cells of bovine lens to the amino acid content of crystallins; there was a definite correlation with a coefficient of 0.70 showing some adaptation of the tRNA population to protein synthesis (2). Ortwerth (200) has shown, by RPC-2 chromatographic separations, quantitative differences of two to three fold exist between calf lens and muscle tissue in the amount of at least one isoacceptor tRNA for each of three amino acids; aspartate, isoleucine, and lysine. Methionyl- and tyrosyl-tRNA from lens contained one isoacceptor that was not present in the other tissue. Similar studies carried out by RPC-5 chromatography also show differences of various degrees with regard to isoaccepting tRNA distributions in lens and other tissues (5; 6). Furthermore, Ortwerth and his colleagues found that one of the two $tRNA^{phe}$ isoacceptors in lens was only present in the lens fiber cell and not in the epithelial cell. This novel $tRNA^{phe}$ was shown to arise as a result of lens cell differentiation (186). There was a corresponding doubling of the phenylalanine acceptance activity in lens fiber cells compared to the lens epithelial cells. It is believed that the synthesis of this novel $tRNA^{phe}$ may be a necessary event in the adaptation of tRNA to crystallin synthesis (186).

I. Ribonucleoside Q in Transfer RNA

Among modified nucleosides of tRNA, Q and its derivative Q*, both derived from guanosine, are unique in their purine skeletons. The structure of Q(queuosine) has been determined as 7-{[(cis-4,5-dihydroxy-2-cyclopenten-1-yl)-amino]-methyl}-7-deazaguanosine (111), whereas Q* has either a mannose or galactose residue at the 4th position of the cyclopentene diol (112). These nucleosides are located in the first position of the anticodon of $tRNA^{asn}$, $tRNA^{asp}$, $tRNA^{his}$, and $tRNA^{tyr}$ from most organisms (231).

Because of the Q nucleoside's structure and location as well as its wide distribution in both prokaryotes and eukaryotes, numerous investigations have recently centered around Q, its possible biological function and its biosynthesis. During Drosophila development, the relative proportions of the Q modified tRNAs and their unmodified counterparts change coordinately: the Q modified isoacceptors decrease relative to the unmodified forms until late larval stage; however, then the Q modified isoacceptors increase relatively and predominate in the adult tRNA population compared with their unmodified counterparts (232). Drastic differences in Q content have also been found in tRNAs from large numbers of tumors (196; 197; 198) and in tissue cultured cells grown under different conditions (233; 234; 235). A recent report has shown that age and nutrition of Drosophila also has a marked effect on the ratio of Q^+/Q^- isoacceptors for each tRNA. Furthermore the ratio is different for different genotypes (236).

Until recently all known tRNA modifications were thought to be derivatizations of the normal nucleoside present in tRNA and synthesized by modification of that nucleoside, such as addition of a side chain. However, formation of the ribonucleoside Q is an exception. Mammalian cells synthesize the Q-containing tRNA from a precursor tRNA that contains guanosine in the wobble position by breaking the N-glycosyl bond for removal of guanine followed by the insertion of a new base, queuine (the base of Q) or pre Q (7-deazaguanine) (237; 238). The only other modification of tRNA by breakage of the N-glycosyl bond occurs in the production of pseudouridine with formation of a C_5-glycosyl bond to the same uricil. The enzyme responsible for Q biosynthesis in tRNA has been purified from rabbit erythrocyte and characterized (121). Similar enzyme activities have also been found in E. coli (198), and Ehrlich ascites cells (239). Surprisingly enough, it has also been found that this enzyme, guanine-tRNA transferase (formerly called guanine insertase), also catalyzes the exchange of guanine into tRNA for asparagine, aspartic acid, histidine, and tyrosine which have a guanine or Q precursor at the wobble position. Thus, homologous enzyme-tRNA systems, i.e., undermodified (Q^-) tRNA, but not fully modified, (Q^+) tRNAs, are substrates for the enzyme which replaces guanine or preQ with guanine (198; 238; 240; 241). This activity is advantageous for assay of (Q^-) tRNA by exchange of guanine with [^{3}H]guanine. It has been reported that Q modified tRNA isoacceptors have a longer retention time upon RPC-5 column chromatography compared to that of their unmodified or undermodified counterparts (215; 242; 243) indicating the ability of Q to change the tRNA chemical properties. It has been suggested that Q may be important in the regulatory functions directed by tRNAs. The presence of Q, which is both positively charged and hydrophilic, in an exposed region of a specific tRNA molecule probably makes the molecule a good receptor, and consequently may alter some of the cellular function (21; 22). It has also been suggested that the presence of Q or preQ instead of G in the wobble position of otherwise identical tRNAs could affect either the rate of protein synthesis or the anticodon-codon recognition (244).

III. MATERIALS AND METHODS

A. Reagents, Buffers, and Solutions

All of the chemicals used in this work were analytical reagent grade unless otherwise indicated. Deionized, double distilled water (with final distillation in glass) was used for preparing all the solutions.

1. Tissue Culture Medium

Eagle's minimum essential medium (with Eagle's salts, L-glutamine, and non-essential amino acids; without sodium bicarbonate; catalogue No. F-15; Grand Island Biological Company, Grand Island, New York) was used. Ten liters of medium were made at a time. Powdered medium (96 g) was added to 8 liters glass distilled water and allowed to completely dissolve by gently stirring. Then 22 g of sodium bicarbonate was added to the solution. Upon the complete dissolution, glass distilled water was added to make up the total volume to 10 liters. The pH was adjusted to pH 6.8 by bubbling 100% CO_2 gas into the medium. This was immediately filtered through a 124 mm prefilter and a 0.22 μm pore size, 142 mm membrane filter (Millipore Corp., Bedford, Mass.) with a 142 mm filter holder and a 20 liter dispensing pressure vessel. A tank of 5% CO_2 - 95% air was used to maintain the pressure at 6 psi. The filtered medium was aseptically collected in 500 ml bottles and stored at -20°C. Before using, the medium was thawed in a 37°C water bath and made 10% in fetal calf serum (Grand Island Biological Company, Grand Island, New York).

2. Fetal Calf Serum

Fetal calf serum used for tissue culture was obtained from Grand Island Biological Company, Grand Island, New York. The fetal calf serum had been heat inactivated at a thermostatically controlled temperature of 56°C for 30 minutes with mixing and microplasma tested as well as screened for virus.

3. Benzidine Staining Solution

Benzidine dihydrochloride, 0.1 g, (Sigma Chemical Co., St. Louis, Missouri) was dissolved in 50 ml of dilute acetic acid (1.5 ml glacial acetic acid to 50 ml with distilled water), then stored at 5°C in a brown bottle. Right before using, 1% (V/V) of 30% H_2O_2 was added to form the working solution.

4. Trypan Blue Staining Solution

Stock solution (2%) was prepared by dissolving 2 g trypan blue (Sigma Chemical Co., St. Louis, Missouri) in 100 ml distilled water. The solution was stored at 5°C in the dark.

A working solution of 0.1% was prepared by diluting 5 ml 2% stock solution to 100 ml with distilled water. This was stored at room temperature in a brown bottle.

5. Radioactive Labelled Amino Acids

All [^{3}H], [^{14}C], and [^{35}S] labelled amino acids were purchased from New England Nuclear, Boston, Mass. Amino acids labelled with [^{3}H] had the following specific activities in Ci/mmol: aspartic acid, 16.9;

glycine, 15.0; histidine, 10.9; isoleucine, 99.2; leucine, 54.6; lysine, 60.0; phenylalanine, 60.0; proline, 111.0; serine, 2.76; threonine, 4.2; tryptophan, 20.0; tyrosine, 42.3; and valine, 11.18. Amino acids labelled with [^{14}C] had the following specific activites in mCi/mmol: alanine, 160.6; arginine, 298.5; asparagine, 179.0; glutamic acid, 255.0; glutamine, 263.0; and lysine, 285.0. Cysteine-[^{35}S] and methionine-[^{35}S] had specific activities of 489.56 Ci/mmol and 323.7 Ci/mmol, respectively, at the time they were used. All the radioactive labelled amino acids were stored at -20°C.

6. Sephadex G 100-120 Column Buffer

A solution containing 0.05 M Tris-HCl, pH 7.5, 0.01 M $MgCl_2$, 0.01 M KCl, 0.01 M 2-mercaptoethanol and 10% glycerol was prepared and stored at 5°C.

7. DEAE (Diethylaminoethyl Cellulose) Column Fractionation Buffers

These buffers were solutions of 0.2 M and 1.0 M NaCl each containing 0.05 M sodium acetate, pH 5.0, 0.01 M $MgCl_2$. The buffers were stored at 5°C.

8. Reversed-Phase Plaskon Chromatography (RPC-5) Elution Buffers

Solutions of 0.5 and 1.0 M NaCl each in 0.01 M sodium acetate, 0.01 M $MgCl_2$, 0.003 M 2-mercaptoethanol and 0.001 M disodium-ethylenediaminetetraacetate (Na_2EDTA) were prepared and adjusted to pH 4.5 with concentrated HCl. These buffers were stored at room temperature.

9. Homogenizing Buffer for Aminoacyl-tRNA Synthetase Preparation from Rat Liver

A solution containing 0.25 M sucrose, 0.05 M KCl, 0.01 M Tris-HCl, pH 7.5, 0.01 M $MgCl_2$, 0.01 M 2-mercaptoethanol and 10% glycerol was prepared and stored at 5°C.

10. Extraction Buffer for Friend Leukemia Cell tRNA Preparation

A solution containing 0.1 M NaCl, 0.01 M Tris-HCl pH 8.2, 0.001 M Na_2EDTA, 0.5% (W/V) sodium dodecyl sulfate (SDS), 0.1% (V/V) diethylpyrocarbonate, 0.2% (V/V) washed bentonite was prepared and stored at 5°C. This solution had to be warmed in a 37°C water bath right before use. Washed bentonite was kindly provided by Dr. B. J. Ortwerth (University of Missouri, Columbia, Missouri).

11. Homogenizing Buffer for Rat Liver tRNA Preparation

A buffer containing 0.25 M sucrose, 0.05 M Tris-HCl, pH 7.5, 0.05 M KCl, 0.01 M $MgCl_2$, 0.001 M Na_2EDTA was prepared and stored at 5°C.

12. Transfer RNA Aminoacylation Buffer

Transfer RNA aminoacylation buffer was prepared immediately prior to use by mixing the following quantities of the indicated stock solutions:

1.0 ml	1.0 M	Tris-HCl, pH 7.5
0.1 ml	0.2 M	$MgCl_2$
0.2 ml	0.25 M	KCl
0.1 ml	0.05 M	cytidine triphosphate, pH 5.6 (CTP)
0.2 ml	0.1 M	adenosine triphosphate, pH 5.6 (ATP)
0.1 ml	0.05 M	dithioerythritol (DTE)
0.8 ml		glass distilled water

The final concentration of each component of the buffer was: 0.4 M Tris-HCl, pH 7.5, 8 mM $MgCl_2$, 20 mM KCl, 2 mM CTP, 8 mM ATP and 2 mM DTE. This buffer solution was retained in an ice bath until being added to the reaction mixture.

13. Tris-Borate EDTA (TBE) Polyacrylamide Gel Solution

The analytical gel consisted of 6% (W/V) polyacrylamide, 0.3% (W/V) N,N'-methylene-bis-acrylamide, 90 mM Tris-borate, pH 8.3, 0.25 M Na_2EDTA, 7 M urea and is stored at 5°C in the dark. The stacking gel consisted of the same components except for the concentrations of polyacrylamide, 4%, and N,N'-methylene-bis-acrylamide, 0.2% (W/V).

14. TBE Gel Running Buffer

Running buffer consisted of 90 mM Tris-borate, pH 8.3, and 0.25 mM Na_2EDTA.

15. TBE Gel Sample Buffer

The sample buffer contained 7 M urea, 9 mM Tris-borate, pH 8.3, 0.025 mM Na_2EDTA, 15% (W/V) sucrose, and a trace of bromophenol blue and xylene cyanol FF (ICN-K&K Laboratories, Inc.) as indicators of the front and location of 5S rRNA, respectively.

16. Scintillation Counting Solution

Scintillation counting fluid was prepared by dissolving 3.2 g of 2,5-diphenyloxazole(PPO) and 0.08 g of 1,4-bis-2-(4-methyl-5-phenyloxazolyl)-benzene(Dimethyl POPOP) in 800 ml toluene (Mallinckrodt Chemical Co., St. Louis, Missouri). The mixture was stirred continuously until both fluors were dissolved. Then 400 ml of Triton X-100 was added. Both fluors and Triton X-100 were scintillation grade and are purchased from Research Products International, Elk Grove Village, Illinois.

17. Phosphate Buffered Saline (PBS)

A concentrate of PBS (10X) was prepared by adding 80 g of NaCl, 2 g of KCl, 22.5 g of $Na_2HPO_4 \cdot 7H_2O$, 2 g of K_2HPO_4 and distilled water to a total volume of 1000 ml. A working solution of PBS (1X) for routine use was made by adding 1 volume of 10X PBS to 9 volumes of distilled water and then autoclaved. This buffer was stored at 5°C.

18. Phenol

Liquified phenol was purchased from TAYCHEMCO, St. Louis, Missouri. It does not contain any preservative and is stored under nitrogen. Water saturation was achieved immediately prior to use by mixing 5 parts of liquified phenol with one part of water. Careful handling is necessary in order to minimize the discoloration and oxidation.

19. 0.1 M Adenosone Triphosphate, pH 5.6 (ATP)

ATP, 0.552 g (Sigma Chemical Co., St. Louis, Missouri), was dissolved in 8 ml of distilled water. The pH was adjusted to pH 5.6 with dilute NaOH and the solution diluted to 10 ml with distilled water. This solution was stored at -20°C and thawed prior to use.

20. 0.05 M Cytidine Triphosphate, pH 5.6 (CTP)

CTP, 0.242 g (Sigma Chemical Co., St. Louis, Missouri), was dissolved in 8 ml of distilled water. The pH was adjusted to pH 5.6 with dilute NaOH and the solution diluted to 10 ml with distilled water. This solution was stored at -20°C and thawed right before use.

21. Trichloroacetic Acid (TCA) Solution

Stock solution (100%) was prepared by dissolving TCA (Sigma Chemical Co., St. Louis, Missouri), 1000 g, into 800 ml of distilled water. The solution was then diluted to 1000 ml with distilled water. Working solutions (10% and 5%) were prepared by diluting 100% TCA with the appropriate volume of distilled water.

B. Methods

1. Friend Leukemia Cell Tissue Culture Technique

A cloned line of Friend leukemia cell (F.L.C.) designated as GM86 (clone 745A) was obtained from the Human Genetic Mutant Repository (Camden, New Jersey). Cultures were maintained in Eagle's minimum essential medium (245) supplemented with 10% fetal calf serum at 37°C under an atmosphere of 95% air and 5% CO_2. Large cultures (1-2 liters) were seeded with cells grown in 150 ml polystyrene tissue culture flasks (Corning Labware Co., Corning, New York) to an exponential growing stage. The cells were harvested by centrifugation of the culture at 1000Xg, the supernatant fluid poured out, and then the cells resuspended with fresh medium. Cells were used to inoculate 1-2 liters of fresh medium in 2 liter Bellco spinner flasks (Bellco, Vineland, New Jersey) at a cell density of 1×10^5 cells/ml. The cultures in the spinner flasks were then gased with 5% CO_2-95% air and incubated at 37°C with continuous stirring

(Biostir, Wheaton, Millville, New Jersey) at a speed of 30 R.P.M. Cultures were constantly screened for contamination and viability (trypan blue staining) every 24 hr. The cells of a large culture were harvested by centrifugation at 1000Xg in 250 ml polypropylene centrifuge tubes (Nalge, Rochester, New York), then rinsed twice with PBS.

2. F.L.C. Erythroid Induction with Dimethylsulfoxide (DMSO)

F.L.C. were induced to differentiate by growing them in the presence of 270 mM dimethylsulfoxide, DMSO (141). The experimental protocol follows. Cultures were started at 1 x 10^5 cells/ml. When the cell density reached 2 x 10^5 cell/ml, one of the cultures was harvested as an uninduced cell population. DMSO (270 mM) was added to the other parallel cultures. Then one of each induced culture was harvested at 36 hr, 48 hr, 72 hr and 96 hr after the addition of DMSO. Hemoglobin producing cells were monitored by benzidine staining every 24 hr. Cell density and viability were also monitored.

3. Measurement of Cell Density and Viability

Aliquots were taken from cultures and mixed with equal volumes of trypan-blue solution. The mixture was then incubated at room temperature for 5 minutes before being applied to a hemocytometer. The cell numbers were counted under a microscope. Viable cell counts were made by counting those cells that excluded the dye trypan blue.

4. Benzidine Staining

Erythroid induction was monitored by benzidine staining of intact cell suspensions as described by Orkin et al. (246). An aliquot of the culture (0.5 ml) was mixed well with 0.5 ml of benzidine solution, then set at room temperature for 25 minutes. Cell counting was done under a microscope at 100X magnification with the aid of an eyepiece micrometer grid or a hemocytometer. Hemoglobin containing cells were stained blue while non-hemoglobin containing cells were pale.

5. Extraction of Nucleic Acid from F.L.C.

The procedure for extracting nucleic acids from F.L.C. is modified from that of Agris et al. (247). A mixture of phenol and extraction buffer, 10 ml (1:1; V/V) was added to every 8-10 million washed, pelleted cells. The mixture was vortexed or vigorously shaken for 20 minutes, then centrifuged at 6000 g for 15 minutes. The aqueous fraction was re-extracted with an equal volume of phenol (5 ml) and the phenol fraction was also re-extracted with an equal volume of extraction buffer (5 ml). After centrifugation of both mixtures, the aqueous parts were pooled and re-extracted again with half volume of phenol and half volume of chloroform. The supernatant fluid was taken after centrifugation and mixed with 3 volumes of cold, absolute ethyl alcohol to precipitate the nucleic acids. All procedures were handled at the temperature of 5°C. The precipitated nucleic acid was collected by centrifugation at 12000Xg for 15 minutes after being kept at -20°C for at least overnight. The pelleted nucleic acid was then drained of the excess alcohol by setting the tubes upside down for at least 4 hr at -20°C. The pelleted material was dissolved in the smallest appropriate volume of distilled water. Optical densities of the solution at 260 nm and 280 nm were measured at this point in order to estimate the quantity of nucleic acid and thereby prepare for the subsequent DEAE chromatographic purification. Unless applied to the

DEAE column immediately, the solution of nucleic acid should be stored at -20°C.

6. Extraction of Nucleic Acid from Rat Liver

The preparation of nucleic acid from rat liver was done according to the procedure of Ortwerth and Liu (188). Rat livers were removed, weighed, minced and mixed with 3 volumes (3 ml/g wet weight of liver) of cold homogenizing buffer. This tissue suspension was then homogenized with 20 strokes (top to bottom) in a Bellco teflon-in-glass homogenizer. Following the homogenization, the homogenate was centrifuged at 30,000Xg in the Sorvall refrigerated centrifuge for 15 minutes. The supernatant was mixed with an equal volume of phenol and vortexed or vigorously shaken for 30 minutes. The mixture was then centrifuged at 6000Xg for 15 minutes to separate the phenol and aqueous layers. The nucleic acid in the aqueous phase was then precipitated with cold, absolute ethyl alcohol followed by storage overnight at -20°C. The precipitate was collected by centrifugation and resuspended in distilled water at the appropriate concentration.

7. Preparation of DEAE Cellulose

DEAE cellulose, DE52-fully swollen microgranular form, was obtained from Whatman Inc., Clifton, New Jersey. DE-52, 200g, was suspended in 2 liters of distilled water and stirred vigorously for 15 minutes. The cellulose was allowed to settle and the supernatant was decanted. This procedure was repeated two times. After the final water wash, the cellulose was filtered on a large Buchner funnel with two Whatman #1 filters. The resulting cake was then suspended in 0.5 N NaOH (6 liter) and stirred for no more than 15 minutes. The suspension was filtered and washed with 0.5 N NaOH again for another 15 minutes. Following filtration the cake was suspended in water and filtered again in order to remove the NaOH. The cellulose was then suspended in 6 liter of 1.0 N HCl and stirred for 15 minutes. This suspension was filtered and the resulting cake was repeatedly suspended in water and filtered until the pH of the filtrate was above 3.0. The cellulose was then suspended in 0.2 M NaCl DEAE column buffer and filtered. Finally, the cellulose was suspended in 1.0 M NaCl DEAE column buffer and stored at 5°C.

8. DEAE Chromatographic Purification of tRNA from Crude Nucleic Acid Preparation

DEAE cellulose is an anion-exchange resin. Therefore, the height-to-width and the surface area of the column resin is relatively unimportant, especially in step-wise elutions. One ml of packed DEAE cellulose resin will be enough to hold approximately 100 A_{260} units of total nucleic acid. DEAE cellulose resin in 1.0 M NaCl DEAE column buffer was packed in a 1.5 cm x 15 cm Lab-Crest glass column (Fischer & Porter Company) without any head pressure, then equilibrated with 10 column volumes of 0.2 M NaCl DEAE column buffer. The sample of nucleic acid was loaded onto the column after removing all excess buffer from over the resin. The sample was allowed to go into the column bed. A small volume of 0.2 M NaCl DEAE column buffer was applied to the top of the column bed followed by attaching a reservoir containing the same buffer. The fractionation was achieved by using an LKB UltraRac 7000 fraction collector coupled with LKB 8300 UVICORD II UV analyzer to monitor the elution. The UV monitor was further linked to an LKB Chopper Bar Recorder. Approximately ten column volumes of eluent (0.2 M NaCl column Buffer) were collected in tubes until the A_{260} fell below 0.05 as monitored by the UV analyzer. The elution rate was 1 ml/min. Then elution was switched to the 1.0 M NaCl DEAE column buffer. In order to obtain a sharp elution of tRNA, the excess 0.2 M NaCl column buffer was removed from the top of the column first and replaced with a small amount of 1.0 M NaCl buffer before attaching the column to the 1.0 M NaCl buffer reservoir. At this point the flow rate was adjusted to 2-3 ml/minute. Eluent was collected until a peak of A_{260} absorbance was monitored and had fallen to 5% of its peak height. The column was stopped and all the tubes having a high A_{260} reading during the 1.0 M NaCl buffer elution were pooled. The tRNA was then precipitated with 2.5 volumes of alcohol and stored at -20°C overnight. After being collected by centrifugation and drained of excess alcohol, the pellet was dissolved in the smallest appropriate volume of H_2O. This tRNA solution was then desalted by dialysis at 5°C against 0.2 M NaCl DEAE column buffer for at least 4 hr at a ratio of sample:buffer of 1:200. The final tRNA solution was then measured for the exact A_{260} and A_{280} on a Zeiss spectrophotometer in order to determine the quantity and quality of the RNA. Good RNA preparations have an A_{260}/A_{280} ratio of exactly 2.0. Determination of other nucleic acids as contaminants of this tRNA preparation was accomplished by TBE gel electrophoresis.

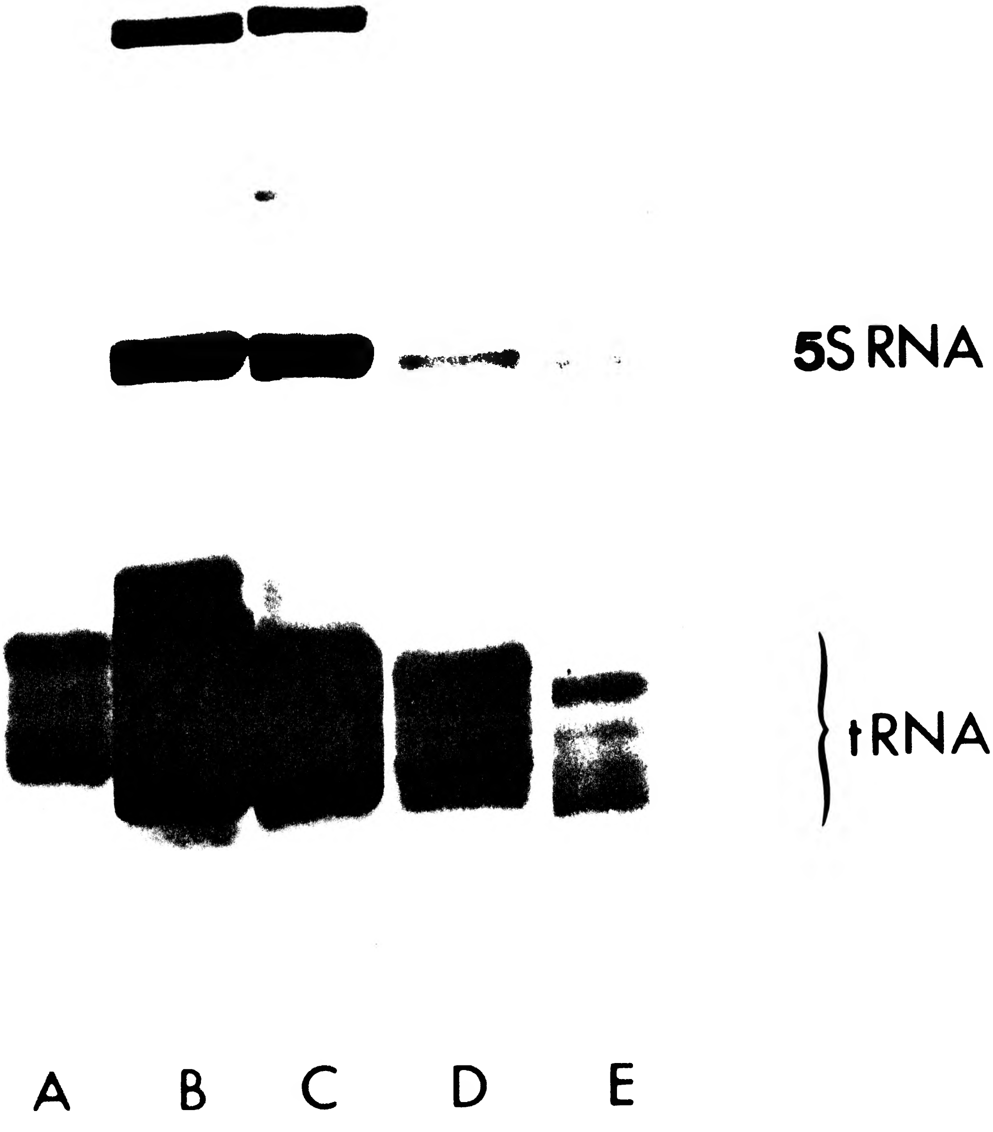
9. TBE Polyacrylamide Gel Electrophoresis

The extent of 5S RNA and other nucleic acids in tRNA preparations was assessed by TBE polyacrylamide slab gel electrophoresis as described by Efstratiadis and Kafatos (248). The slab gel consisted of a 6% polyacrylamide analytical gel and a similarly constituted 4% stacking gel with the dimensions of 132 mm x 132 mm x 1.5 mm. After the slab was polymerized, it was then pre-electrophoresed at the constant amperage of 15 mA for 4 hr in order to eliminate the polymerization catalyzer. tRNA samples (0.1 A_{260} unit, i.e., the amount of nucleic acid producing 0.1 A_{260} when dissolved in 1 ml) was then mixed with 2 volumes of sample buffer and loaded underneath the running buffer in the gel sample well. The volume of the loaded sample never exceeded 75 μl, and smaller volumes were more preferable. The slab electrophoresis was then run at a constant amperage of 12 mA until the dye front entered the analytical gel. The running amperage was 8 mA in order to achieve the best resolution. When the bromophenol blue dye front reached the bottom of the slab, the power was shut off and the slab was detached from the apparatus. The mold plates were removed carefully and the slab was then rinsed with distilled water. After the migration distances of the bromophenol blue and xylene cyanol FF were measured, the slab was stained in methylene blue stain for one hour at room temperature on a shaker. Then, it was destained with water overnight. A photograph was taken (Figure 3) and the relative amounts of 4S and 5S RNA were determined by scanning the gel on a Gilford spectrophotometer.

10. Preparation of Aminoacyl-tRNA Synthetase from Rat Liver

Rats were sacrificed and the liver tissue was removed, weighed and minced. Homogenization took place in 2 volumes (V/W) of iced homogenizing buffer with 10 strokes of a teflon-in-glass tissue homogenizer (Bellco Glass, Inc., Vineland, New Jersey). The resulting homogenate was then centrifuged at 30,000Xg for 15 minutes. The supernatant fluid was carefully withdrawn with a pipette leaving the pellet and lipid layer

Figure 3. Gel electrophoretic analysis of tRNA preparations from F.L.C. exposed to DMSO for various times. Lane A, 0 hr; B, 36 hr; C, 48 hr; D, 72 hr; and E, 96 hr.


5S RNA
tRNA
A
B
C
D
E

behind. The 30,000Xg supernatant fluid was placed in the screw-capped Beckman centrifuge tubes for the Ti-60 Beckman rotor. The Ti-60 rotor was centrifuged at 176,000Xg for 75 minutes. The resulting supernatant material was withdrawn as before and put on a 2.5 cm x 100 cm Sephadex G100-120 gel filtration column. The column had been equilibrated with about 300-400 ml of G100-120 column buffer. Elution of the sample was carried out in the cold with the same buffer. The column was allowed to develop at a flow rate of 15 ml/hr. Fractions (5 ml) were collected in the cold using an LKB UltraRac 7000 fraction collector. The straw-colored fractions eluting just prior to the red hemoglobin peak were pooled and glycerol was added to 50% (V/V). This enzyme preparation was stored at -20°C.

11. Preparative Aminoacylation of Transfer RNA

Each aminoacylation reaction mixture consisted of:

a. 400 μl tRNA aminoacylation buffer
b. 20 μCi [^{3}H] labelled, 5 μCi [^{14}C] labelled or 50 μCi [^{35}S] labelled amino acid
c. 0.3 A_{260} units tRNA solution
d. 200 μl rat liver aminoacyl-tRNA synthetase preparation and distilled water to a final volume of 1.0 ml.

The reaction was allowed to proceed at 37°C in a water bath for 15 minutes after which 1 ml of ice cold 1 M sodium acetate, pH 4.0, was added in order to lower the pH and lessen deacylation. The mixture was then phenol extracted by the addition of 2 ml phenol and vortexed for 10 minutes at 5°C. This vortexed mixture was then centrifuged at 6,000 g for 15 minutes to separate the aqueous and phenol phases. The aqueous fraction was withdrawn and alcohol precipitated by adding 3 volumes of ice cold absolute ethyl alcohol and stored at -20°C for overnight. The precipitated aminoacyl tRNA was then collected by centrifugation at 12,000Xg for 15 minutes. After the excess alcohol was drained, the pellet of aminoacyl-tRNA was dissolved in 1 ml of 0.5 M NaCl RPC-5 column buffer. An aliquot was spotted on 3 MM cellulose filter paper discs (Whatman, Inc., Clifton, New Jersey). The total incorporation of labelled amino acid was determined by placing each disc in 10 ml of scintillation fluid and measuring the radioactivity with a Packard Tri-carb Liquid Scintillation Spectrometer. The aminoacyl-tRNA solutions were directly applied to an RPC-5 column, or were immediately stored frozen at -20°C.

12. Amino Acid Acceptance Assays

The accurate amino acid acceptance activity of a given tRNA preparation was tested using the filter paper disc method of Yang and Novelli (249). For each tRNA sample to be tested the following reagents were added in the following order to a 13 x 75 mm test tube in an ice bath.

a. 5 μCi [^{3}H] or 1 μCi [^{14}C] or 10 μCi [^{35}S] labelled amino acid.
b. 80 μl tRNA aminoacylation buffer
c. 0.2 A_{260} units tRNA, and
d. water to make a total volume of 200 μl after the addition of 40 μl aminoacyl tRNA synthetase.

At zero time 40 μl of rat liver aminoacyl-tRNA synthetase preparation was added and the reaction mixture was vortexed. The reaction was allowed to proceed at 37°C in a water bath and 40 μl aliquots were spotted on 3 MM Whatman filter paper discs at 5, 10, 15, 25 minutes after zero time. After the sample had penetrated the paper, the discs were immediately placed in cold 10% TCA solution for washing by stirring at the temperature of an ice bath. After the collection of all time points, the filter discs were washed for 30 minutes in cold 10% TCA solution. The discs were washed twice in cold 5% TCA solution for 15 minutes each. Following these washes the filters were then dehydrated and TCA eliminated by stirring in a 95% ethanol:ethyl ether solution (2:1 by volume) at room temperature for 15 minutes in a fume hood. Further dehydration was followed by washing the filters in ether for 15 minutes. Filters were then dried under a heat lamp, or laid out on aluminum foil for evaporation of ether at room temperature. Incorporation of labelled amino acid was measured by scintillation spectometry as described previously.

13. Preparation of RPC-5 Column Packing Material

Plaskon CTFE was coated with Adogen 464 by method C as described by Pearson et al. (250). Twelve milliliters of Adogen 464 (Ashland Chemical Co., Columbus, Ohio), a trialkylammonium chloride with alkyl group chain length of C_8-C_{10}, was dissolved in 600 ml of chloroform. To this mixture, 300 g of Plaskon CTFE powder (Allied Chemical Co., Elizabeth, New Jersey) was added with vigorous stirring for 2 hr. Plaskon CTFE powder is an inert polychlorotrifluoroethylene polymer with a mean particle size of 10 microns. After two hours stirring, the slurry was then poured into a glass baking dish and stirred continuously in a fume hood until all the chloroform had been evaporated. The resulting white material was then put in a plastic jar and tumbled vigorously on a shaker in order to break up the lumps. Finally, the white finely divided powder was either stored dry or suspended in 0.5 M NaCl RPC-5 column buffer for column use. Polychlorotrifluoroethylene and adogen 464 were kindly provided by Dr. B. J. Ortwerth (University of Missouri, Columbia, MO).

14. Packing of RPC-5 Column

The RPC-5 column resin was packed into a 0.9 x 50 cm high pressure Altex analytical column (Altex Scientific Inc., Berkeley, California) as described by Kelmers and Heatherly (251). The column was attached to a high-pressure Altex sample injection valve, with a 5 ml sample loop, a high-pressure mini pump (Milton Roy Co., Riviera Beach, Florida) and a two chamber gradient generator (Pharmacia Co., Uppsala, Sweden) to produce the linear gradient used for the chromatographic separation of the aminoacylated tRNA. To prepare the column, the whole system was rinsed with 0.5 M NaCl RPC-5 column buffer. The same buffer (15 ml) was poured into the glass column. Then the resin slurry was added to the column and allowed to settle. At this point buffer was pumped through the column at the pressure of 250 psi until the settling of the resin was complete. The top plunger was removed and excess buffer on top of the resin bed was removed with a pipette. More slurry was added, the column reassembled and packed under pressure as described above.

This procedure was repeated until the packed resin reached the desired height. At this time the column was further packed by pumping the buffer through for at least 2 hr at the pressure of 500 psi or until there was no further collapse of packed resin. The top plunger was then lowered to touch the surface of the resin bed.

15. Procedure for RPC-5 Chromatographic Separation of tRNAs

Radioactively labelled F.L.C. aminoacyl-tRNA samples were co-chromatographed with differently labelled, radioactive rat liver lysyl-tRNA (6000 cpm) as an internal standard. The experimental sample at the volume of 1 ml was thawed and the internal standard was added immediately before the loading to the sample loop. The sample was then pumped onto the column at the pressure of 200 psi and eluted under the same pressure with a 100 ml linear gradient consisting of 50 ml each of 0.5 M and 1.0 M NaCl RPC-5 column buffer. The flow rate was 1 ml per minute. Two hundred fractions of 0.5 ml were then collected directly into plastic scintillation vials on an ISCO 328 fraction collector (Instrumentation Specialties Co., Lincoln, Nebraska). After the elution was complete, the column was washed with 50 ml of 1.0 M NaCl RPC-5 column buffer followed by re-equilibration with 150 ml of 0.5 M NaCl RPC-5 column buffer. Under careful handling, each packing of RPC-5 column may be used for approximately 60 chromatographic runs without altering resolutions of tRNA species. Throughout this work, the rat liver lysyl-tRNA internal standard produced a consistent chromatographic profile. A volume of 4.5 ml of scintillation counting solution was then added to each fraction and the sample radioactivity was counted in a Packard Tricarb liquid scintillation spectrometer. Since dual counting of two radionuclides was being undertaken, the spill-over rate of the [^{14}C] (or [^{35}S]) counts into the [^{3}H] window was carefully calculated. The spill-over of [^{3}H] counts into the higher energy window was negligible.

16. Guanylation of tRNA

The reaction mixture contained 10 μmol of N-tris (hydroxymethyl) methyl-2-aminoethanesulfonic acid(TES)•HCl buffer (pH 7.4), 1 nmol of [^{3}H]guanine, 53 μmol of KCl, 5 μmol of 2-mercaptoethanol, 7 units of tRNA-guanine transferase (121) and either 0.08 or 0.16 A_{260} units of F.L.C. tRNA in a final volume of 0.6 ml. The reaction mixture was incubated at 37°C for 6 hours in sterile tubes. The reaction was stopped by the addition of 3 ml of ice cold 3 M HCl. The tubes were kept at 0°C for 5 min and the precipitate was collected on 2.4 cm glass fiber filters. Precipitates were washed with cold 3 M HCl and then 95% ethanol. Samples were dried and transferred to vials containing 40 mg of Omnifluor (New England Nuclear, Boston, Mass.) per 10 ml toluene. All samples were run in duplicate and each sample had a blank that was precipitated at zero time. The zero time values were subtracted from the 6 hour value to correct for nonspecific precipitation of [^{3}H]guanine. This assay was performed by Dr. Walter R. Farkas and his research colleagues at the University of Tennessee, Knoxville, Tenn.

17. Procedure of Karotyping Friend Leukemia Cell

Colchicine was added to an exponentially growing F.L.C. culture at a concentration of 0.1 µg per ml of medium followed by an incubation at 37°C for three hours. Cells were collected by centrifugation at 500Xg for five minutes. After decanting the medium and then washing the cells with 1X PBS, cells were resuspended in 5 ml hypotonic solution and incubated at 37°C for 15 minutes. Two drops of Carnoy's fixative (3 parts of methanol and 1 part of glacial acetic acid) was added to the suspension. Cells were then collected by centrifugation and resuspended with Carnoy's fixative. After 15 minutes fixation in an ice bath, cells were then spun down and resuspended in a small volume of fixative. Cells were then spread on frozen clean slides. The slides were allowed to air dry, then stained with aceto-orcein for 30 minutes.

IV. RESULTS

A. Effect of DMSO on F.L.C. Growth

Friend leukemia cells were grown in Eagle's minimal essential medium supplemented with 10% fetal calf serum containing 270 mM dimethylsulfoxide to stimulate erythroid differentiation. In agreement with previous reports (13; 141), cultures of induced F.L.C. exhibited a growth lag during the initial 48 hr as shown in Figure 4. Resumption of cell division at an enhanced rate enabled the induced cultures to obtain a cell density comparable to that of uninduced cultures after 96 hr. Although growth was inhibited, the F.L.C. retained a viability equivalent to that of untreated control cultures. Almost all of the cells in the differentiating culture excluded the trypan blue dye, indicating that DMSO treatment did not affect the viability of cells.

B. Induction of Differentiation

The hemoglobin synthesizing cells were monitored by benzidine staining (246). The benzidine positive staining (B^+) cells appeared after the F.L.C. had been exposed to 270 mM DMSO for 72 hr as shown in Figure 5. As high as 84% of the total cell population had been induced by 96 hr, which is in agreement with the previous reports (147; 252). As indicated in the literature (252) all proliferation and differentiation (as measured by hemoglobin formation) are markedly affected by the cell culture conditions; thus, the higher the rate of cell proliferation, the greater the proportion of cells recruited to differentiate. Preliminary experiments indicated

Figure 4. Effect of DMSO on F.L.C. growth. Growth of F.L.C. cultures over a 96 hr period in minimal essential medium supplemented with 10% fetal calf serum was assessed by the counting of viable cells (--●--). Growth of F.L.C. in the same medium containing 270 mM of DMSO (-- ■ --) is also shown in the figure.

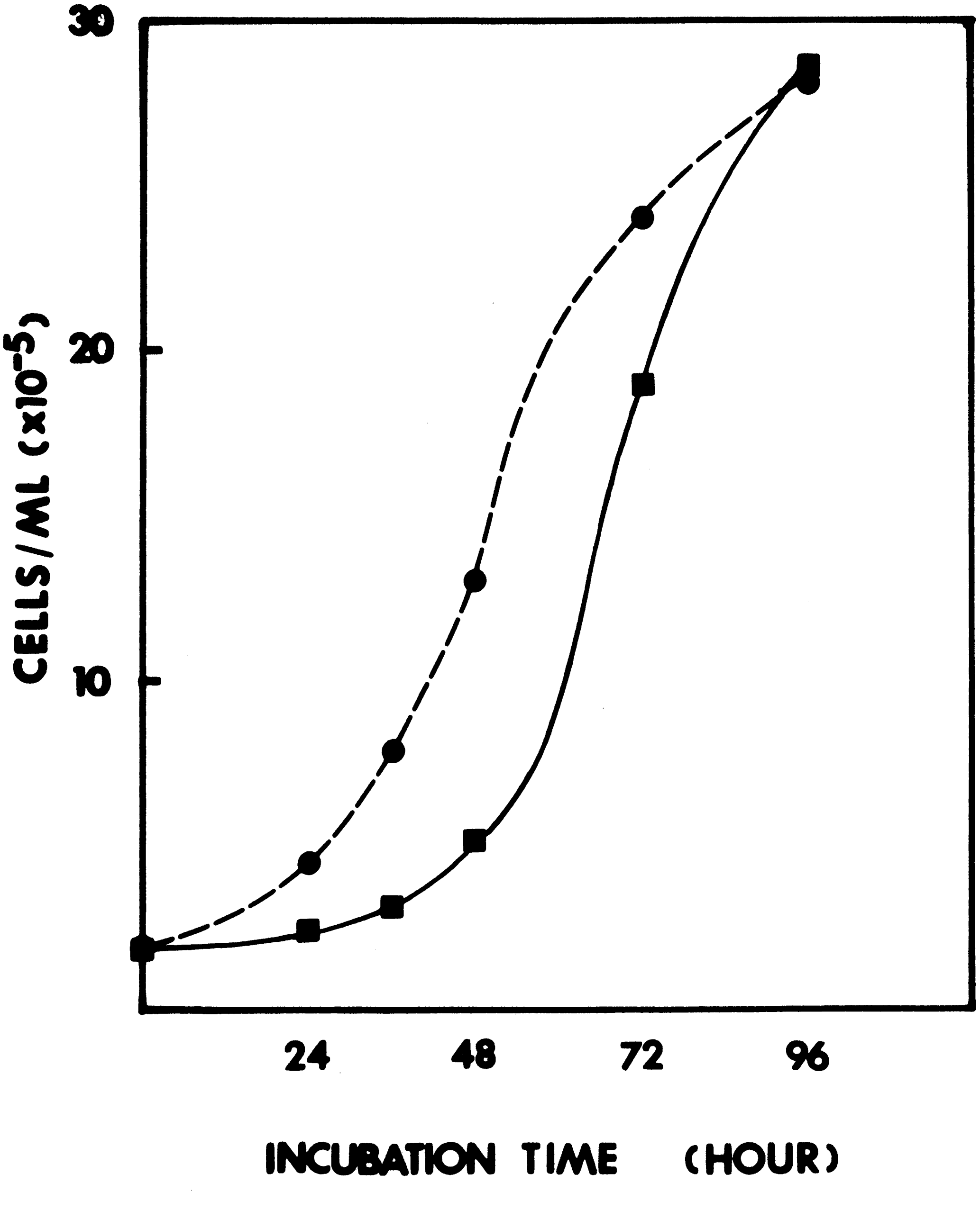
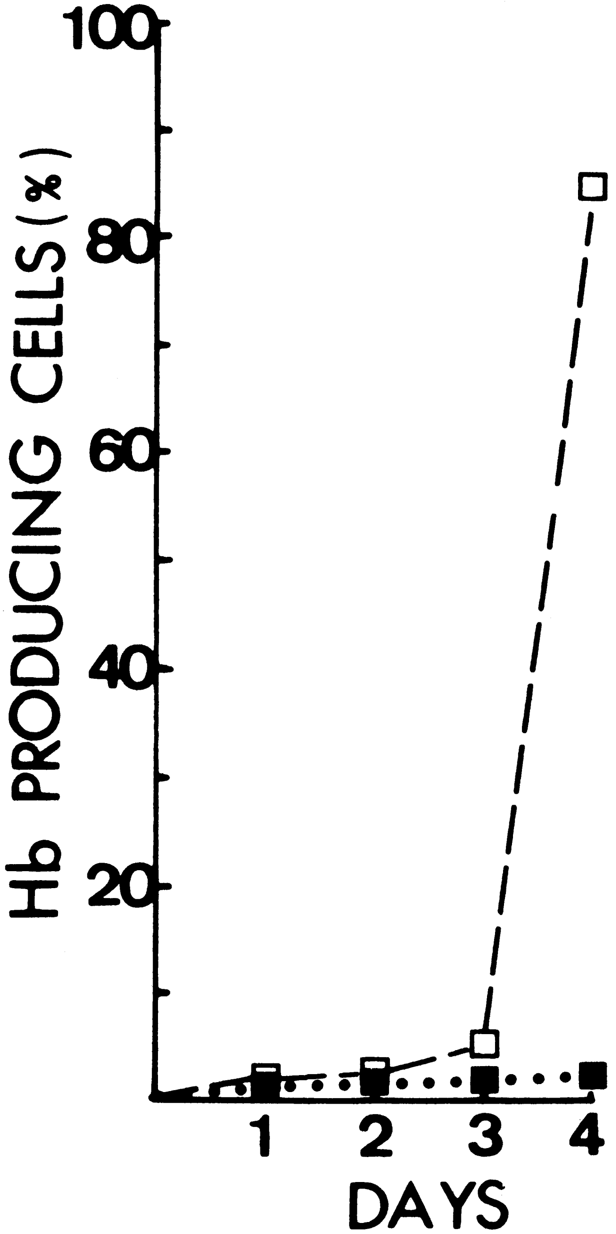

30
20
10
CELLS/ML (x10^{-5})
24
48
72
96
INCUBATION TIME (HOUR)

Figure 5. Effect of DMSO on the differentiation of F.L.C. *in vitro*. The percentage of hemoglobin producing cells was determined by the number of B^+ cells over a 96 hr period in minimal essential medium supplemented with 10% fetal calf serum (-- ■ --) and in the same medium containing 270 mM of DMSO (-- □ --).


Hb PRODUCING CELLS (%)
100
80
60
40
20
1
2
3
4
DAYS

the inoculum of 2×10^5 cells per ml could achieve the highest induction rate and this density was therefore used in this study.

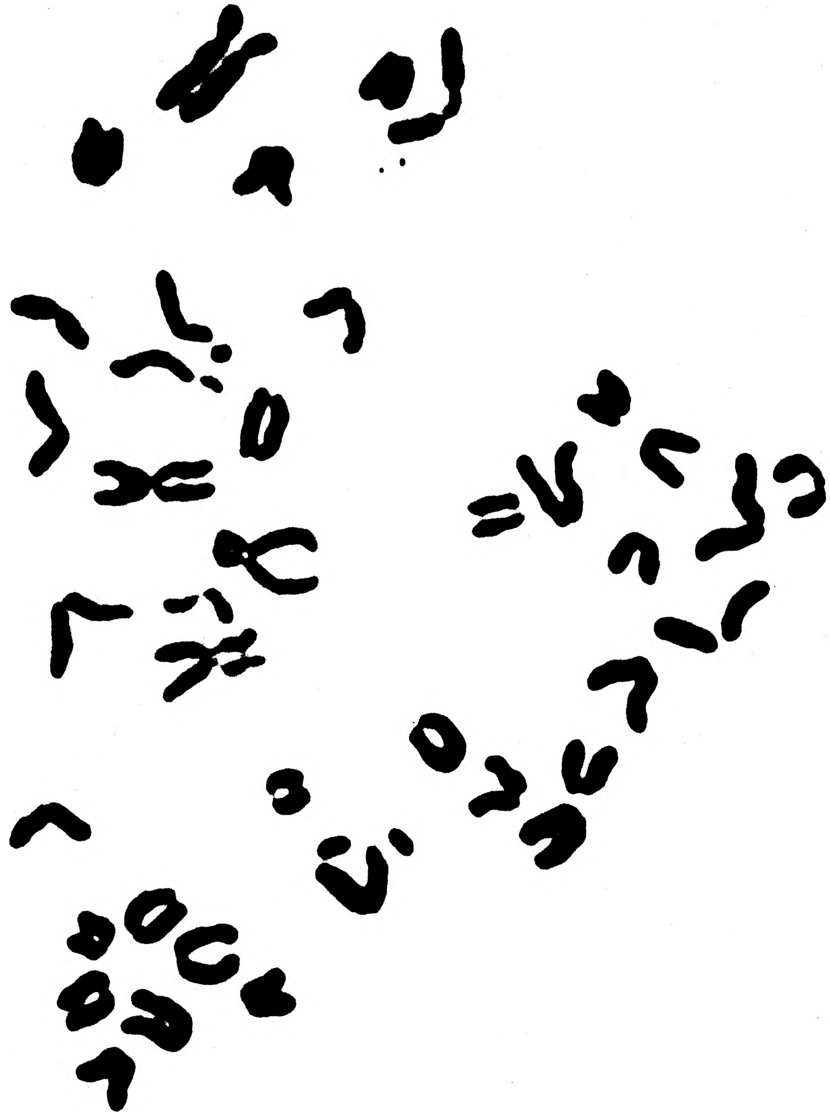
C. Karyotype of F.L.C.

The inducibility of Friend leukemia cell erythrodifferentiation has been shown to vary among different subpopulations (147). Noninducible variants have also been isolated, which fail to respond to different inducing agents, or respond, but do not differentiate to the normoblast stage (253; 254). It has been shown that the inducible F.L.C. have a chromosome number in the range of 36-39 with 4 biarmed marker chromosomes. It has also been shown that some of the unstable DMSO-resistant variant colonies contain a large proportion of cells with much higher chromosome numbers (50-70) (254). Cells employed in this work (F.L.C. Clone 745A) have been karyotyped in this laboratory. As seen in the example shown in Figure 6, the cells have a chromosome number of 38 or 39. Almost all of the examined karyotypes included 4 metacentric chromosomes.

D. Alteration of tRNA Isoacceptors During F.L.C. Erythrodifferentiation

Transfer RNA was isolated from Friend leukemia cells (F.L.C.) in culture before the addition of the erythropoietic inducer, dimethylsulfoxide, and 36 hr, 48 hr, 72 hr, and 96 hr afterward. An increase in the number of hemoglobin producing cells, detected by positive benzidine staining, was seen as early as 48-60 hr after induction. The "program" of F.L.C. erythroid differentiation induced by DMSO includes characteristic changes in cell morphology right after addition of DMSO (141), a drastic increase of globin mRNA at 48 hr after the addition of DMSO (255), an increase

Figure 6. Karotype of the Friend leukemia cell.



in iron uptake and heme synthesis (141), and globin and hemoglobin synthesis at 72 hr after the addition of DMSO (145; 164; 178). Each of the five isolated, unfractionated tRNA preparations (0, 36, 48, 72 and 96 hr after addition of DMSO) was then separately aminoacylated with each of 16 radiolabelled amino acids: alanine; asparagine; aspartate; cysteine; histidine; isoleucine; leucine; lysine; methionine; phenylalanine; proline; serine; threonine; tryptophan; tyrosine; and valine. Isoaccepting species of these 80 aminoacyl-tRNA preparations were then resolved by reversed-phase plaskon chromatography. An internal standard of rat liver tRNA aminoacylated with different radionuclide labelled lysine was used in each chromatography. This allowed direct comparison of chromatograms by recognition of rat liver $\mathrm{tRNA}^{\mathrm{lys}}$ isoacceptors 2, 4 and 5.

The tRNAs for the 16 different amino acids were resolved into 64 isoaccepting species. However, the numbers of isoacceptors and the relative amounts of each for particular aminoacyl-tRNAs were either somewhat constant, variable or extremely variable during differentiation of F.L.C.

1. Minor Variations

Figures 7-9 show the RPC-5 chromatograms of valyl-, isoleucyl-, and phenylalanyl-tRNAs isolated from cells at the five time points of erythroid differentiation mentioned above. The relative amounts of each isoaccepting species at each of the five time points are shown in Table 1. The five chromatographic profiles for the three aminoacyl-tRNAs shown in Figures 7-9 and the relative amounts tabulated in Table 1 reflect rather similar distributions of isoaccepting species, and are not considered to have been significantly altered during differentiation. Valyl-tRNA (Figure 7) was

Table 1: Amino Acid Isoaccepting tRNA Species that Change Slightly During Differentiation of Friend Leukemia Cell

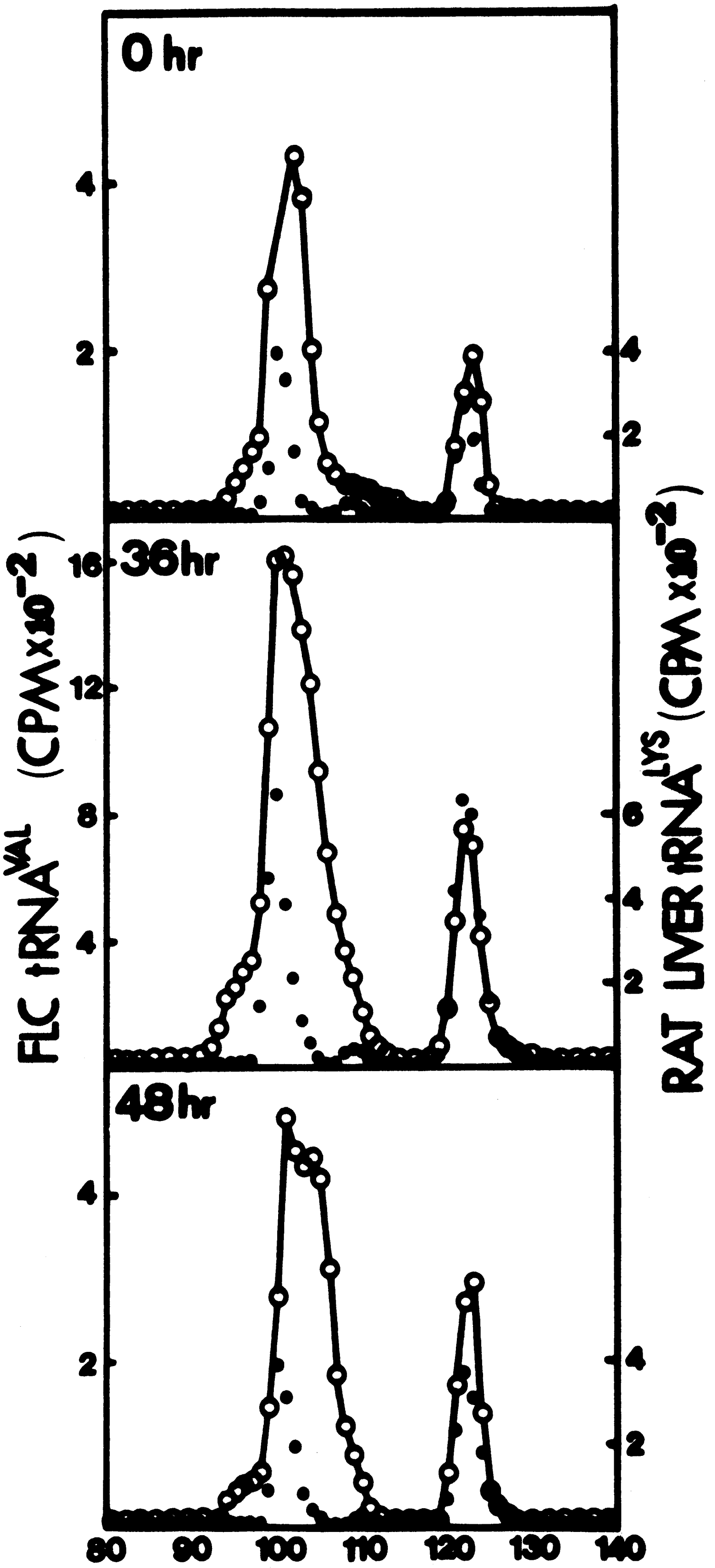
tRNA	Time During Differentiation[a]	Relative Amounts of Isoaccepting Species[b]					
		1	2	3	4	5	6
Val	0	78.8	21.2				
	36	81.8	18.2				
	48	78.5	21.5				
	72	78.5	21.5				
	96	70.5	29.5				
Phe	0	4.2	1.0	0	94.8		
	36	6.1	3.5	1.8	88.6		
	48	10.4	11.2	0	78.4		
	72	14.1	8.9	10.7	66.3		
	96	1.7	7.7	0	90.6		
Ile	0	29.3		29.9[c]		40.8	
	36	21.2		53.9		24.8	
	48	33.7		29.5		36.8	
	72	23.6		44.2		32.2	
	96	35.8		6.1		58.1	

[a] Hours after the addition of DMSO

[b] Percent of total aminoacyl-tRNA

[c] Percent of total tRNA^{ile} for species 2, 3, and 4 combined

Figure 7. Alterations in the RPC-5 profile of $tRNA^{val}$ extracted from Friend leukemia cells undergoing erythroid differentiation. Transfer RNA was extracted from Friend leukemia cells induced to undergo erythroid-like differentiation by addition of 270 mM DMSO to cultures. tRNA was isolated from the cells after 0, 36, 48, 72, and 96 hr of induction. Each of the five tRNA preparations were aminoacylated with one $[^{3}H]$labelled amino acid (o--o) and subjected to RPC-5 chromatography along with an internal standard, rat liver $[^{14}C]$lysyl-tRNA(●--●). This set of chromatograms compares the elution profiles of the $tRNA^{val}$ isoacceptors. Details of the chromatography procedures are described in Materials and Methods.


0 hr
36hr
48hr
4
2
16
12
8
4
4
2
4
2
6
4
2
4
2
FLC tRNA^{VAL} (CPM x10^{-2})
RAT LIVER tRNA^{LYS} (CPM x10^{-2})
80
90
100
110
120
130
140
FRACTION NUMBER

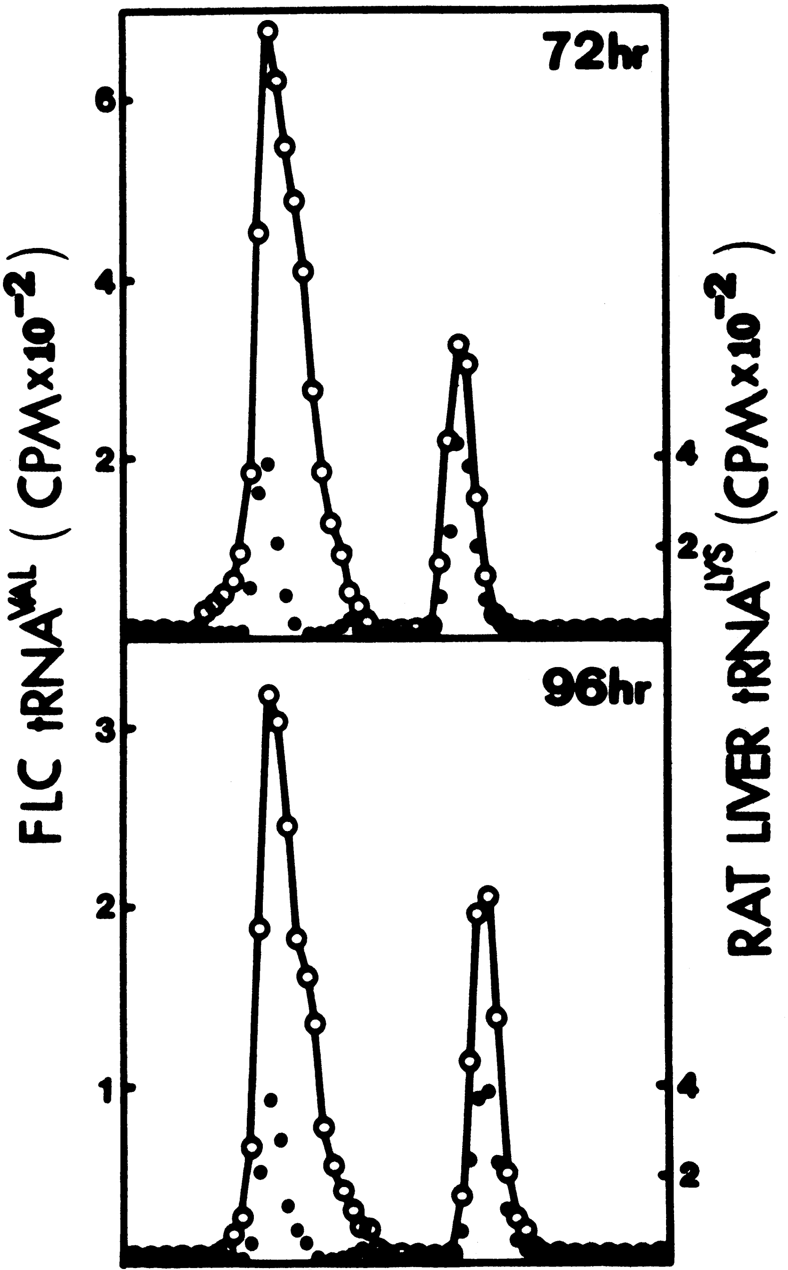
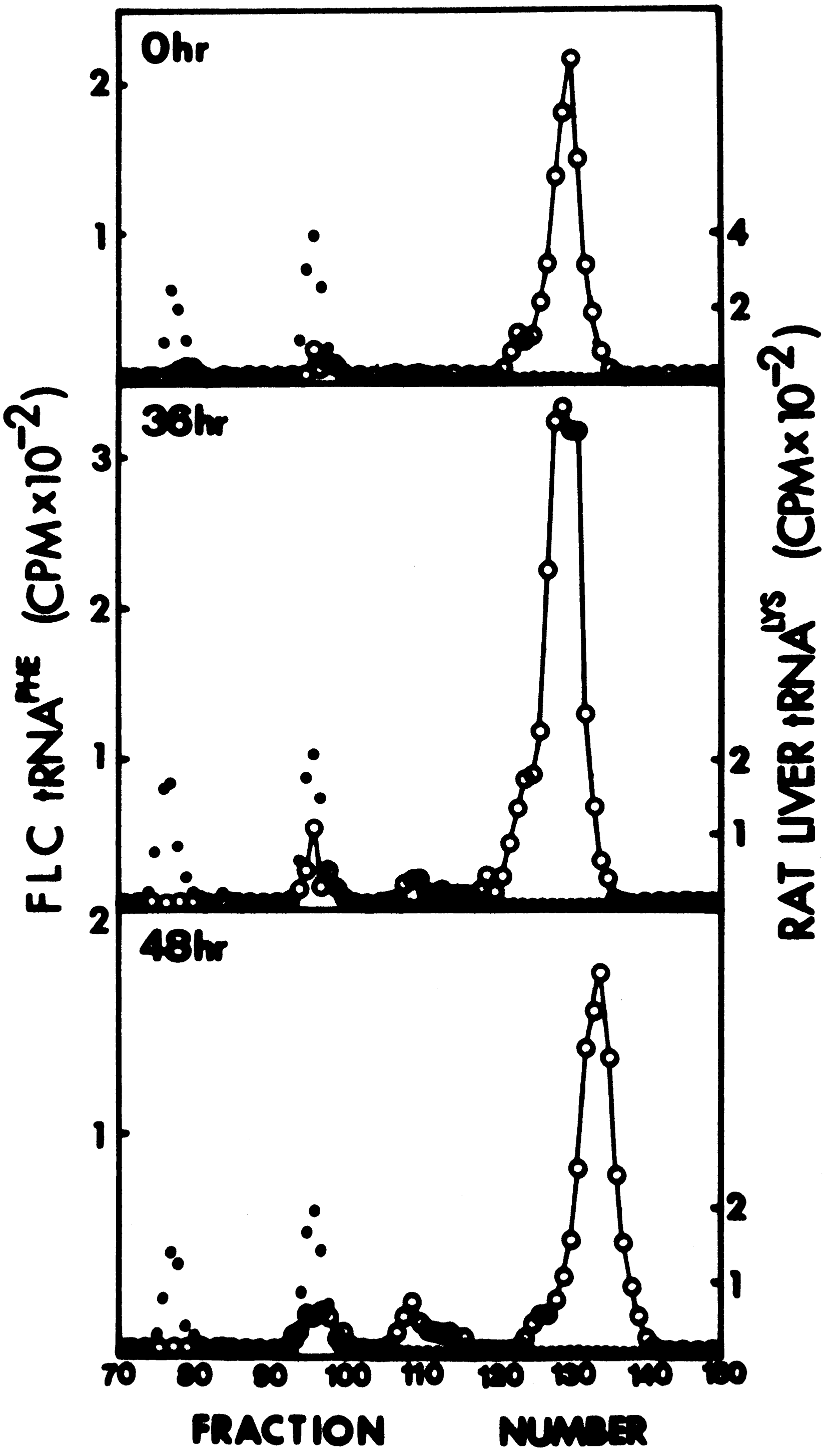

72hr
96hr
FLC tRNA^{VAL} (CPM x10^{-2})
RAT LIVER tRNA^{LYS} (CPM x10^{-2})
6
4
2
3
2
1
4
2
4
2
80
90
100
110
120
130
140
FRACTION NUMBER

Figure 8. Alterations in the RPC-5 profile of $tRNA^{phe}$ extracted from Friend leukemia cells undergoing erythroid differentiation. This set of chromatograms compares the elution profiles of the $tRNA^{phe}$ isoacceptors(o--o) as described in the legend of Figure 7.


0hr
36hr
48hr
FLC tRNA^{PHE} (CPM×10^{-2})
RAT LIVER tRNA^{LYS} (CPM×10^{-2})
2
1
3
2
1
2
1
4
2
2
1
2
1
70
80
90
100
110
120
130
140
150
FRACTION NUMBER

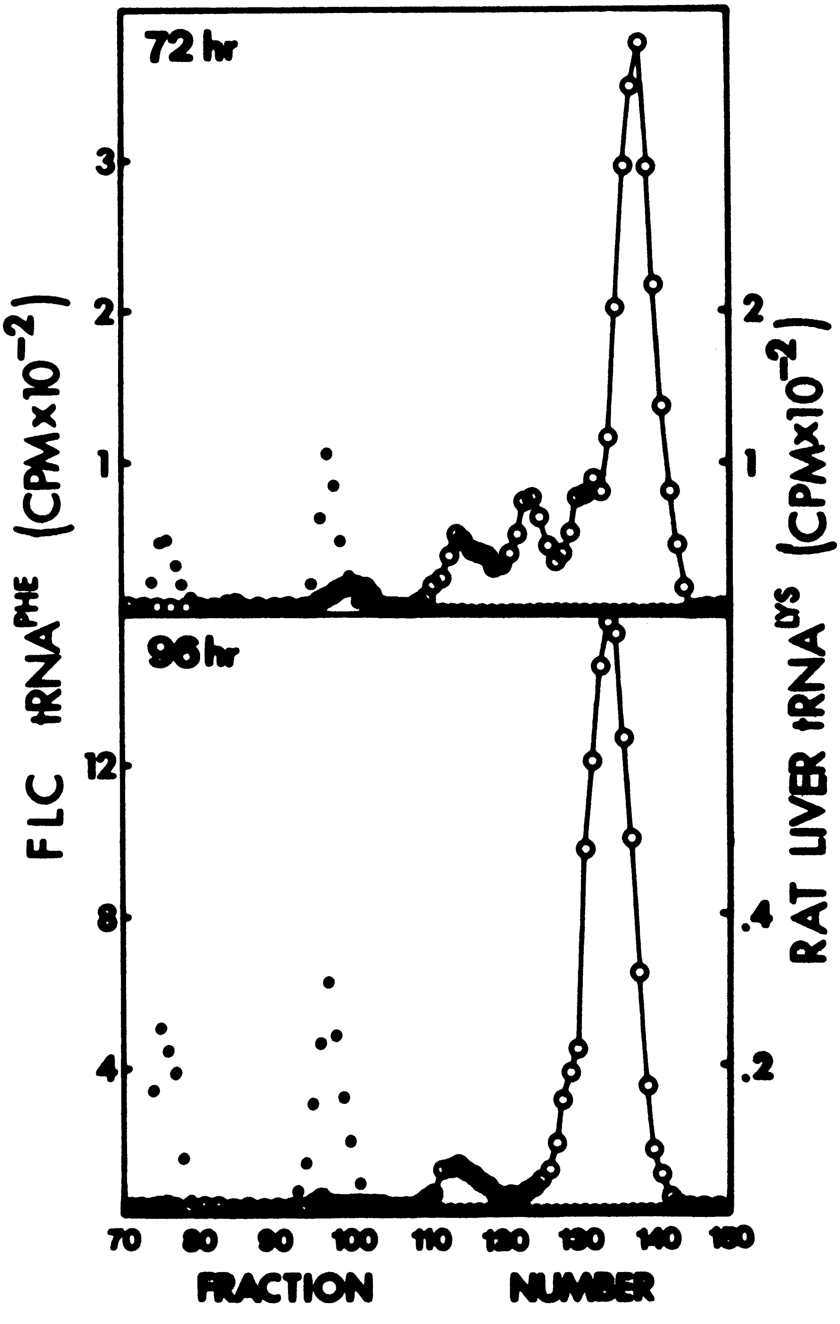
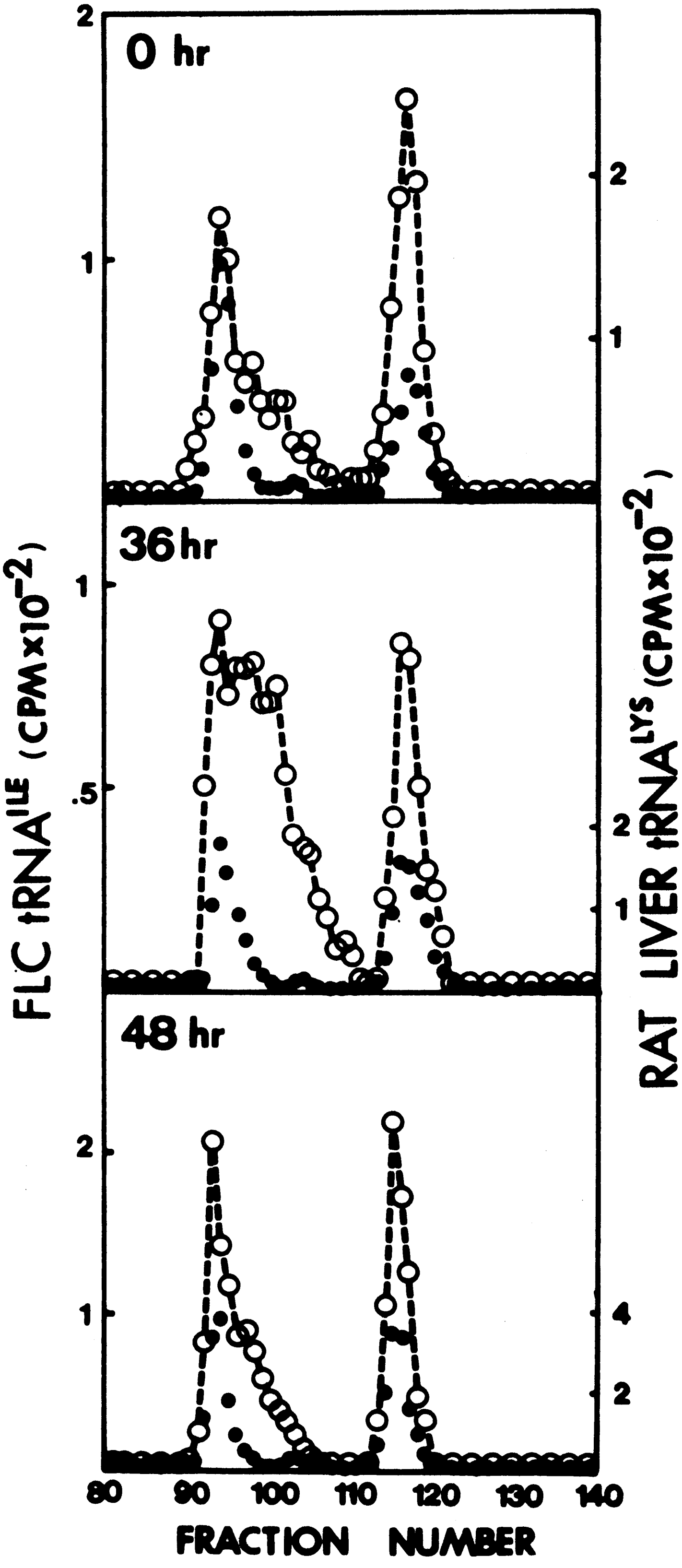
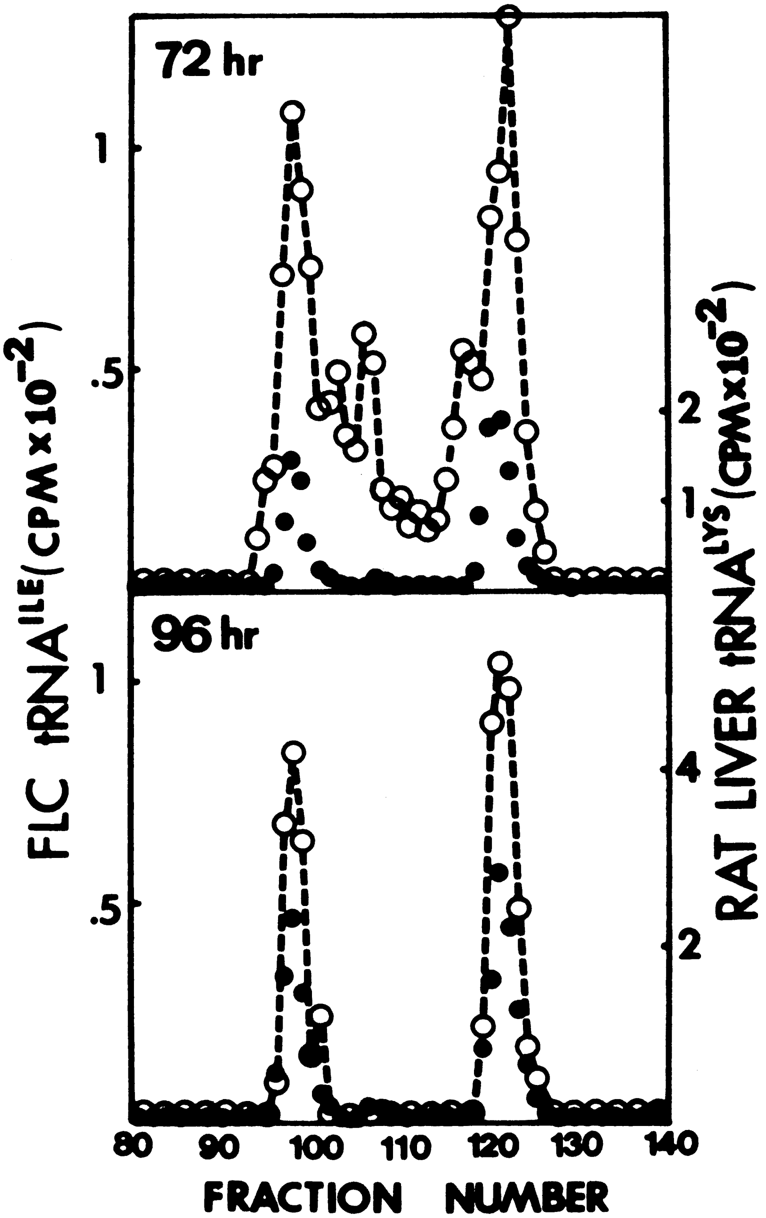

72 hr
96 hr
3
2
1
12
8
4
2
1
.4
.2
FLC tRNA^{PHE} (CPM x 10^{-2})
RAT LIVER tRNA^{LYS} (CPM x 10^{-2})
70
80
90
100
110
120
130
140
150
FRACTION NUMBER

Figure 9. Alterations in the RPC-5 profile of $tRNA^{ile}$ extracted from Friend leukemia cells undergoing erythroid differentiation. This set of chromatograms compares the elution profiles of the $tRNA^{ile}$ isoacceptors(o--o) as described in the legend of Figure 7.


0 hr
36 hr
48 hr
2
1
1
.5
2
1
2
1
2
1
2
4
2
FLC tRNA^{ILE} (CPM x 10^{-2})
RAT LIVER tRNA^{LYS} (CPM x 10^{-2})
80
90
100
110
120
130
140
FRACTION NUMBER


72 hr
96 hr
1
.5
1
.5
2
1
4
2
FLC tRNA^ILE (CPM x 10^-2)
RAT LIVER tRNA^LYS (CPM x 10^-2)
80
90
100
110
120
130
140
FRACTION NUMBER

resolved into two isoaccepting species with an almost identical distribution over the five time points. The resolution of the minor species of phenylalanyl-tRNA (Figure 8) was rather poor. However, at least four isoaccepting species could be defined. The one major peak designated species 4 consistently eluted very late. Species 2 increased in relative amount and species 3 was detected only at 72 hr. Isoleucyl-tRNA (Figure 9) has been resolved into two major isoaccepting species designated 1 and 5. Species 1 and 5 were maintained by the cells at rather constant distributions over the time course of differentiation (Table 1). The remaining minor isoaccepting species which were not resolved but appeared as shoulders to the major peaks, had larger changes in relative amounts increasing at 36 hr and then decreasing. These minor species of $tRNA^{ile}$ disappeared when the cells had completely differentiated.

2. Major Variation

Chromatograms of prolyl-, lysyl-, methionyl-, leucyl-, seryl-, cysteinyl-, tryptophanyl-, threonyl-, and alanyl-tRNA species are shown in Figures 10-18. The relative distributions over the course of erythroid differentiation are shown in Table 2. These nine aminoacyl-tRNAs exhibited more than 38 isoaccepting species of which 27 were well resolved by RPC-5 chromatography. Some of these species remained almost constant in relative amount during differentiation of F.L.C., others varied in a discernible pattern, while still others changed without pattern. Prolyl-tRNA (Figure 10) has been resolved by chromatography into four isoaccepting species. Species 1 and 4 constituted very little of the prolyl-tRNA population, if they were present at all, in the tRNA preparations from uninduced cells (0 hr, Table 2). However, both species 1 and 4 continually increased in

Table 2: Amino Acid Isoaccepting tRNA Species that Change Dramatically During DMSO Induced Differentiation of Friend Leukemia Cell

tRNA	Time During Differentiation[a]	Relative Amounts of Isoaccepting Species[b]					
		1	2	3	4	5	6
Pro	0	4.9	27.2	66.9	1.0		
	36	13.4	20.0	52.3	14.3		
	48	30.5	7.5	25.4	36.6		
	72	46.7	0	10.9	42.4		
	96	48.6	0	6.2	45.2		
Lys	0	0	36.7	0	22.1	40.8	1.4
	36	0	19.5	0	35.3	39.6	5.6
	48	0	43.4	0	21.2	34.1	1.3
	72	0	32.7	0	34.4	33.2	1.7
	96	0	20.7	0	42.3	35.2	1.8
Met	0	64.0	16.9	19.1			
	36	47.3	13.4	39.3			
	48	12.0	18.1	69.9			
	72	65.8	11.7	22.5			
	96	60.1	17.6	22.3			
Leu	0	17.2	7.0	36.0	39.8	0	
	36	5.7	31.3	0	48.5	14.5	
	48	26.7	17.6	0	35.7	10.0	
	72	5.4	49.1	0	24.1	21.4	
	96	30.3	23.3	0	38.8	7.6	
Ser	0	20.0	0	40.2	23.5	16.3	
	36	30.0	8.1	51.3	0	10.3	
	48	19.6	28.1	40.1	0	12.2	
	72	23.5	0	38.7	18.9	18.9	
	96	48.7	0	51.3	0	0	

Table 2 Continued: Amino Acid Isoaccepting tRNA Species that Change Dramatically During DMSO Induced Differentiation of Friend Leukemia Cell

tRNA	Time During Differentiation[a]	Relative Amounts of Isoaccepting Species[b]					
		1	2	3	4	5	6
Cys	0	79.4	8.9	11.4			
	36	81.3	4.7	14.0			
	48	68.1	19.7	12.2			
	72	50.4	20.8	28.8			
	96	51.3	30.8	17.9			
Trp	0	24.2	42.2		33.5[c]		
	36	21.0	24.0		55.0		
	48	30.5	31.5		38.0		
	72	29.3	31.3		39.4		
	96	44.5	55.5		0		
Thr	0	14.6	29.5	31.9	24.0		
	36	16.4	20.2	31.7	31.7		
	48	23.0	12.2	18.2	46.6		
	72	37.7	5.3	7.4	49.5		
	96	39.4	0	3.5	57.1		
Ala	0	15.3[d]		55.3	29.4[d]		
	36	30.2		50.8	19.0		
	48	31.1		50.6	18.3		
	72	18.4		58.8	22.8		
	96	12.8		73.6	13.6		

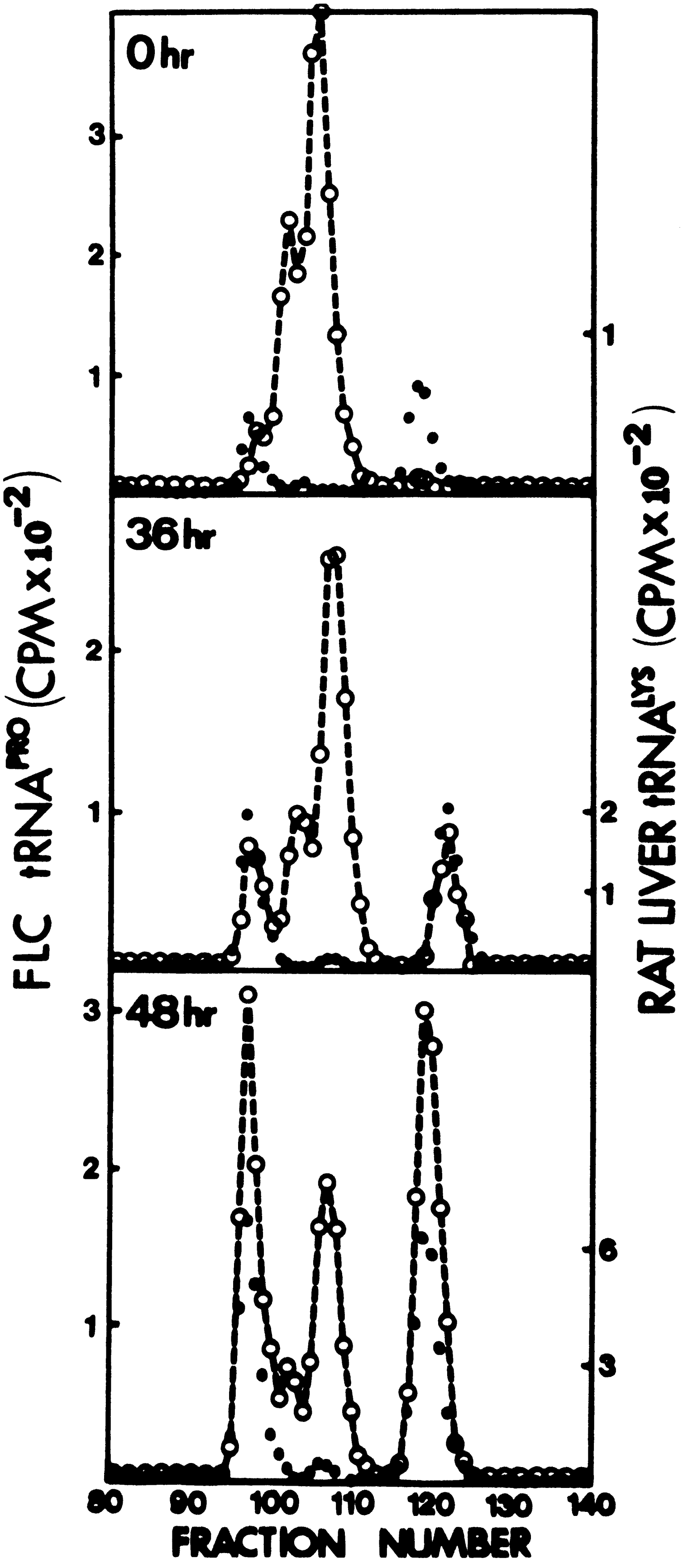
[a] Hours after the addition of DMSO

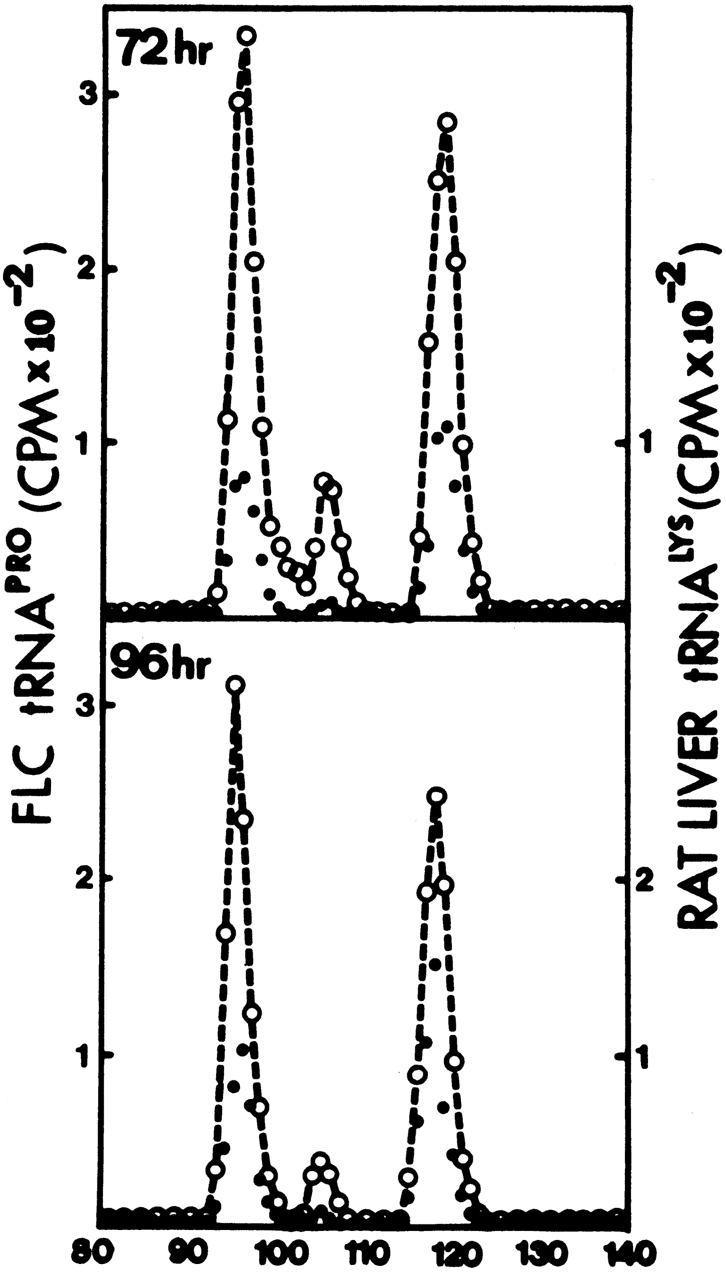
[b] Percent of total aminoacyl-tRNA

[c] Percent of total tRNA^{trp} for species 3, 4, and 5 combined

[d] Percents of total tRNA^{ala} for species 1 and 2, and 4 and 5, combined

Figure 10. Alterations in the RPC-5 profile of $tRNA^{pro}$ extracted from Friend leukemia cells undergoing erythroid differentiation. This set of chromatograms compares the elution profiles of the $tRNA^{pro}$ isoacceptors(o--o) as described in the legend of Figure 7.


0 hr
36 hr
48 hr
FLC tRNA^{PRO} (CPM x 10^{-2})
RAT LIVER tRNA^{LYS} (CPM x 10^{-2})
1
2
3
6
80
90
100
110
120
130
140
FRACTION NUMBER

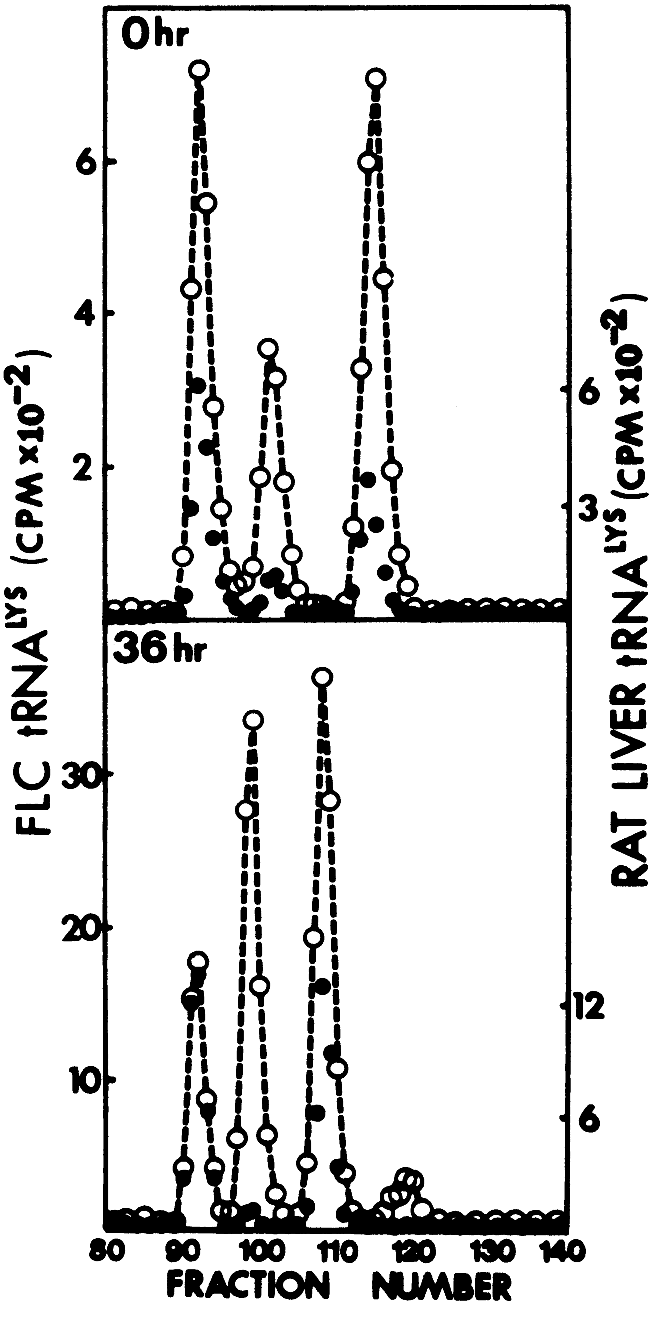

72 hr
96 hr
FLC tRNA^{PRO} (CPM x 10^{-2})
RAT LIVER tRNA^{LYS} (CPM x 10^{-2})
1
2
3
80
90
100
110
120
130
140
FRACTION NUMBER

relative amounts while tRNA^{pro} species 2 and 3 decreased during differentiation. Mammalian lysyl-tRNA has been resolved into as many as six species. The four lysine species found in F.L.C. (Figure 11) have been numbered according to a standardized designation for simplicity and continuity. It is worth noting that the relative amount of $\text{tRNA}_4^{\text{lys}}$ was reduced at the 48 hr time point and then increased again to greater than the original level.

Methionyl-tRNA was separated into three isocaccepting species (Figure 12). The relative amount of species 2 fluctuated somewhat throughout the course of differentiation. However, the relative amount of the major species, designated 1, decreased at 48 hr to approximately one-fifth that at 0 time, then increased to the original level. The chromatograms for leucyl-tRNA (Figure 13) showed an alteration in the amounts of the five isoacceptors without any consistent pattern over the time course. Species 3 completely disappeared; whereas 5 appeared after the addition of DMSO. The results seen with seryl-tRNA are shown in Figure 14. The chromatograms showed two major peaks (1 and 3) and three minor peaks. The two major species 1 and 3 were present without alteration throughout the time course and represent the only serine isoacceptors after 96 hr of differentiation (Table 2). Species 2 was only present at the 36 and 48 hr time points, whereas species 4 was absent at these times. All three minor species disappeared at 96 hr after the addition of DMSO.

Cysteinyl-tRNA has been resolved into three species (Figure 15). Species 1 continually decreased in relative amount throughout the differentiation while 2 and 3 increased. Tryptophanyl-tRNA chromatograms (Figure 16) showed profiles of five peaks. However, the late eluting minor tRNA^{trp} species (peaks 3, 4 and 5) were poorly resolved under the condi-

Figure 11. Alterations in the RPC-5 profile of $tRNA^{lys}$ extracted from Friend leukemia cells undergoing erythroid differentiation. This set of chromatograms compares the elution profiles of the $tRNA^{lys}$ isoacceptors(o--o) as described in the legend of Figure 7.


0 hr
36 hr
FLC tRNA^{LYS} (CPM × 10^{-2})
RAT LIVER tRNA^{LYS} (CPM × 10^{-2})
FRACTION NUMBER
2
4
6
10
20
30
3
6
6
12
80
90
100
110
120
130
140

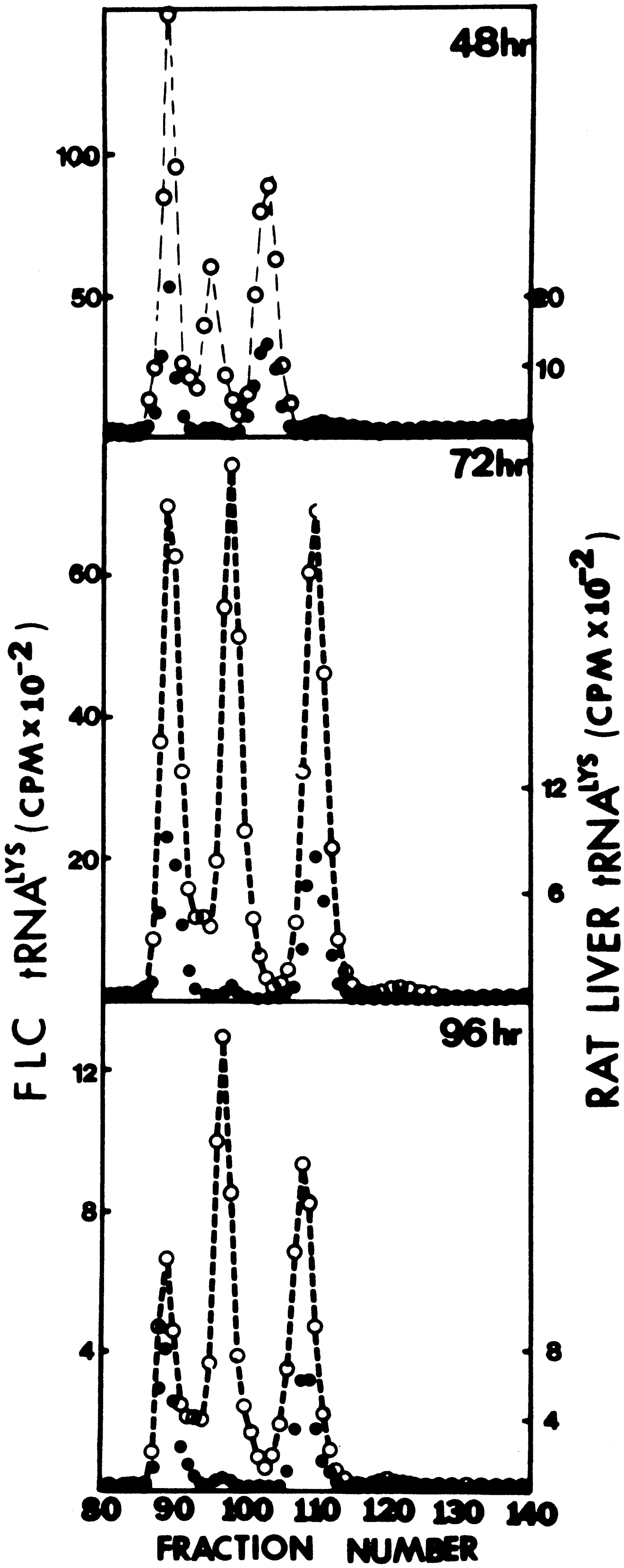
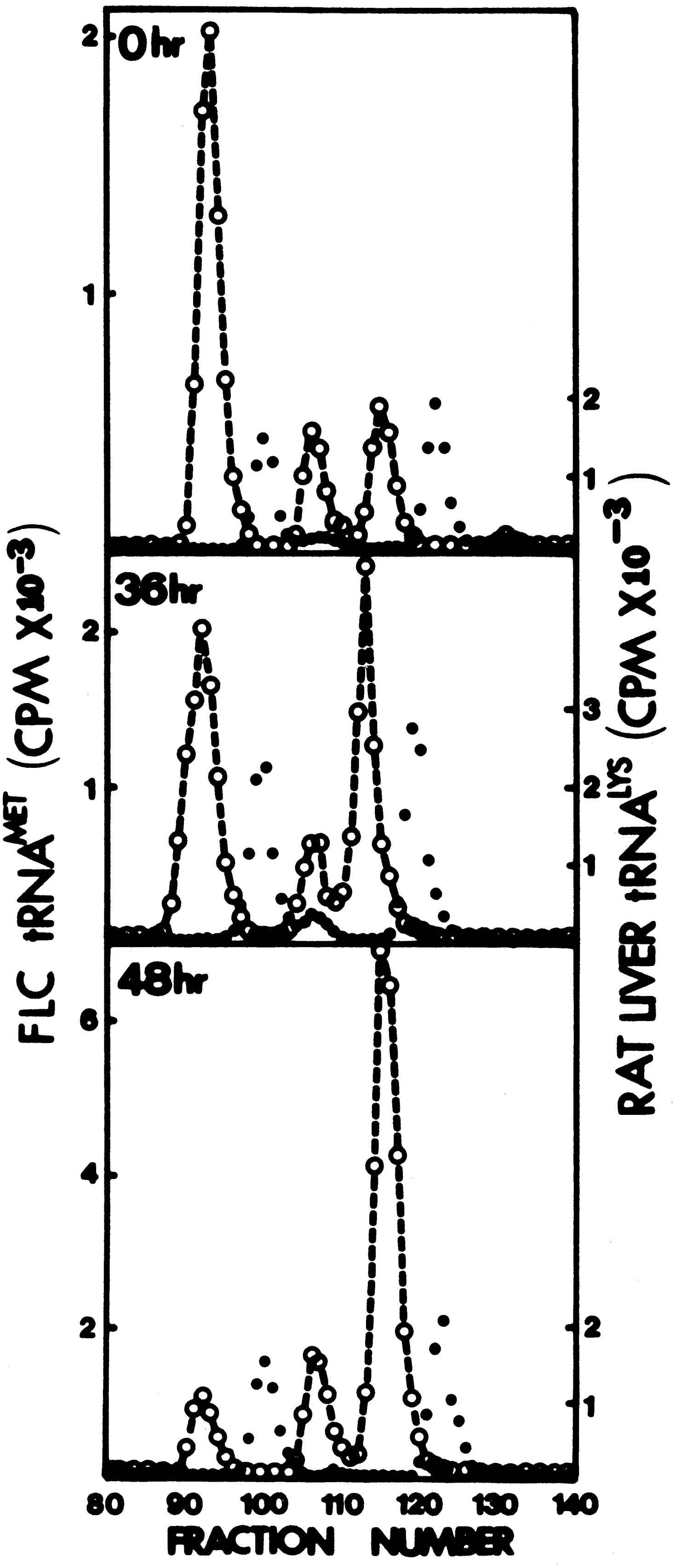

48hr
72hr
96hr
100
50
60
40
20
12
8
4
20
10
12
6
8
4
FLC tRNA^{LYS} (CPM x 10^{-2})
RAT LIVER tRNA^{LYS} (CPM x 10^{-2})
80
90
100
110
120
130
140
FRACTION NUMBER

Figure 12. Alterations in the RPC-5 profile of $tRNA^{met}$ extracted from Friend leukemia cells undergoing erythroid differentiation. This set of chromatograms compares the elution profiles of the $tRNA^{met}$ isoacceptors (o--o) as explained in the legend of Figure 7 except for the use of [^{35}S]methionine and rat liver [^{3}H]lysyl-tRNA as internal standard(●--●).


0hr
36hr
48hr
2
1
2
1
6
4
2
2
1
3
2
1
2
1
80
90
100
110
120
130
140
FRACTION NUMBER
FLC tRNA^{MET} (CPM X10^{-3})
RAT LIVER tRNA^{LYS} (CPM X10^{-3})

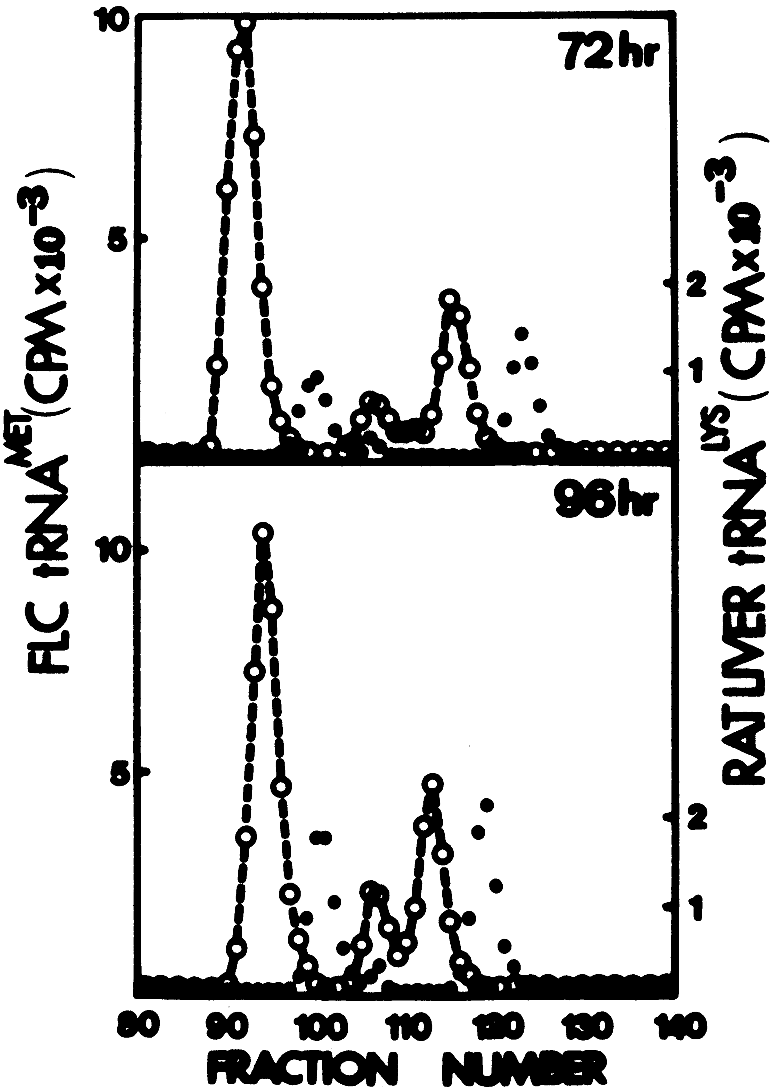
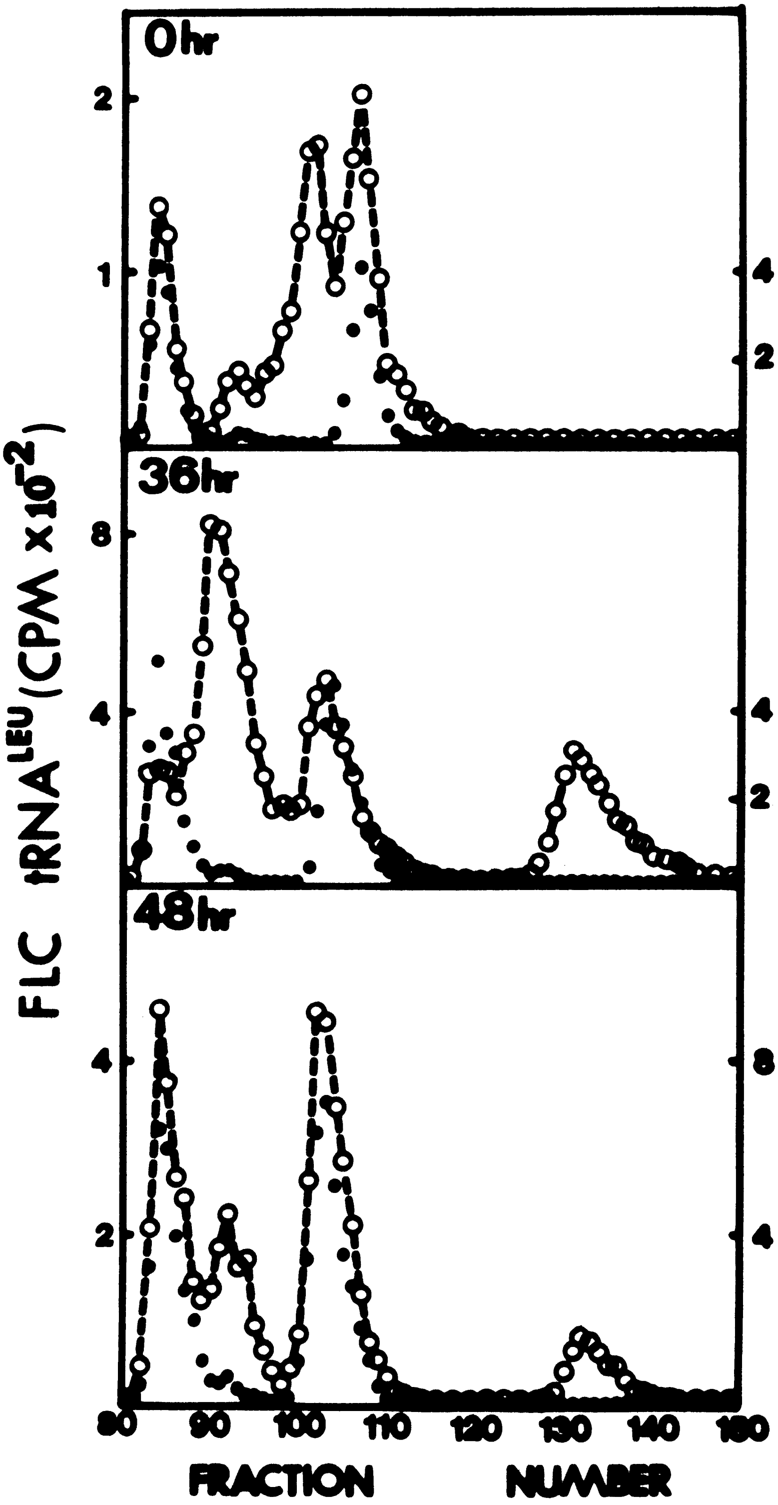

72hr
96hr
FLC tRNA^{MET} (CPM x 10^{-3})
RAT LIVER tRNA^{LYS} (CPM x 10^{-3})
10
5
10
5
2
1
2
1
80
90
100
110
120
130
140
FRACTION NUMBER

Figure 13. Alterations in the RPC-5 profile of $tRNA^{leu}$ extracted from Friend leukemia cells undergoing erythroid differentiation. This set of chromatograms compares the elution profiles of the $tRNA^{leu}$ isoacceptors(o--o) as explained in the legend of Figure 7.


0hr
36hr
48hr
FLC tRNA^LEU (CPM x10^-2)
RAT IVER tRNA^LYS (CPM x10^-2)
2
1
8
4
4
2
4
2
4
4
2
8
4
80
90
100
110
120
130
140
150
FRACTION NUMBER

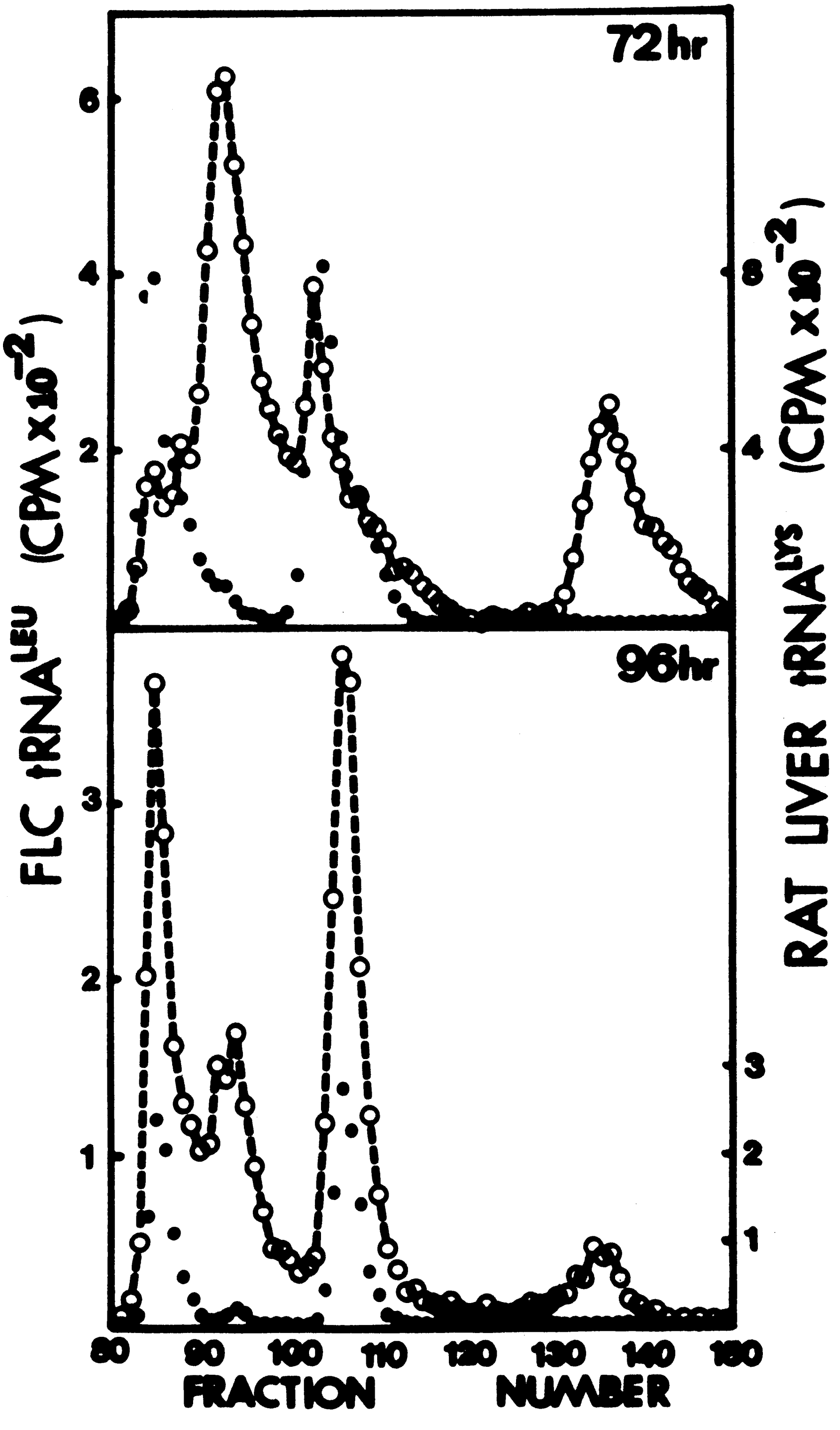
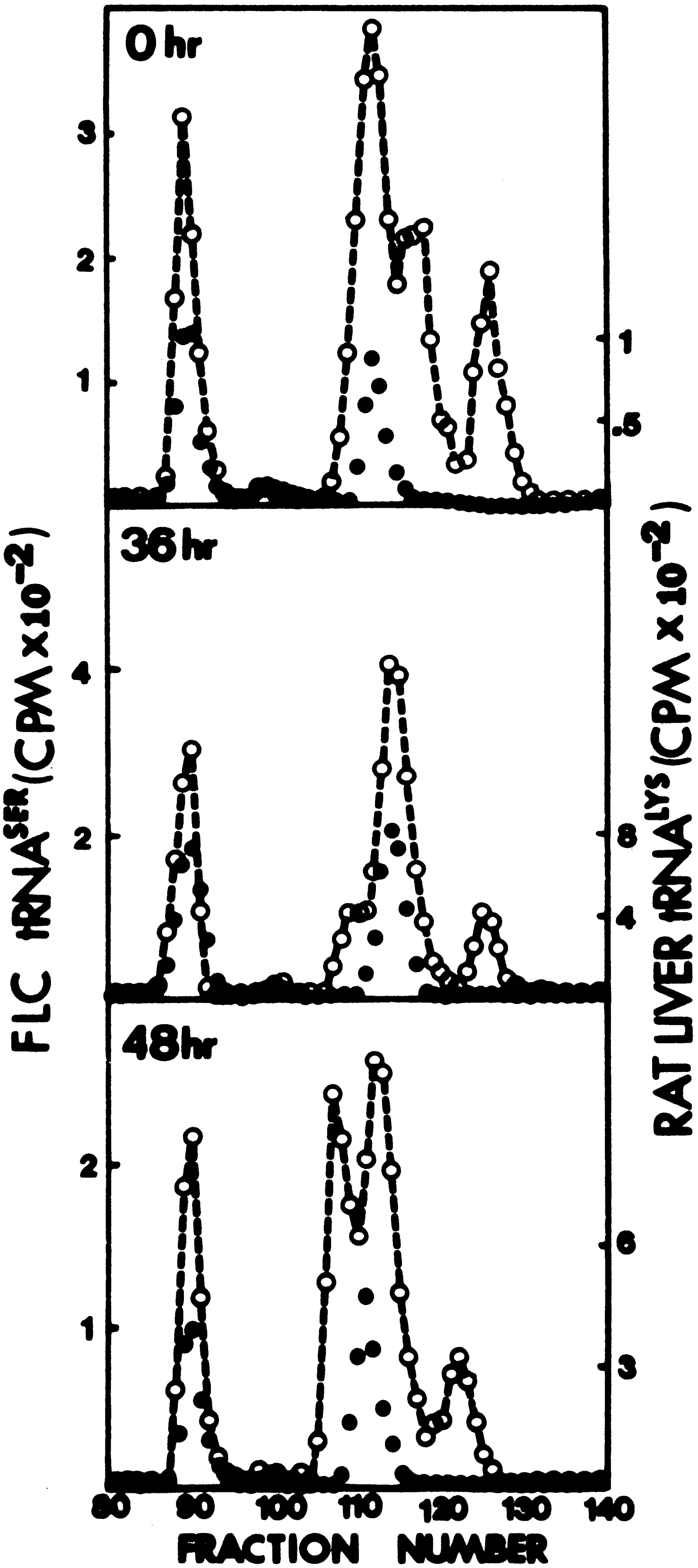

72hr
96hr
FLC tRNA^LEU (CPM x 10^-2)
RAT LIVER tRNA^LYS (CPM x 10^-2)
6
4
2
3
2
1
8
4
3
2
1
80
90
100
110
120
130
140
150
FRACTION NUMBER

Figure 14. Alterations in the RPC-5 profile of $tRNA^{ser}$ extracted from Friend leukemia cells undergoing erythroid differentiation. This set of chromatograms compares the elution profiles of the $tRNA^{ser}$ isoacceptors(o--o) as explained in the legend of Figure 7.


0 hr
36 hr
48 hr
FLC tRNA^{SER} (CPM x10^{-2})
RAT LIVER tRNA^{LYS} (CPM x 10^{-2})
FRACTION NUMBER
3
2
1
1
.5
4
2
8
4
2
1
6
3
80
90
100
110
120
130
140

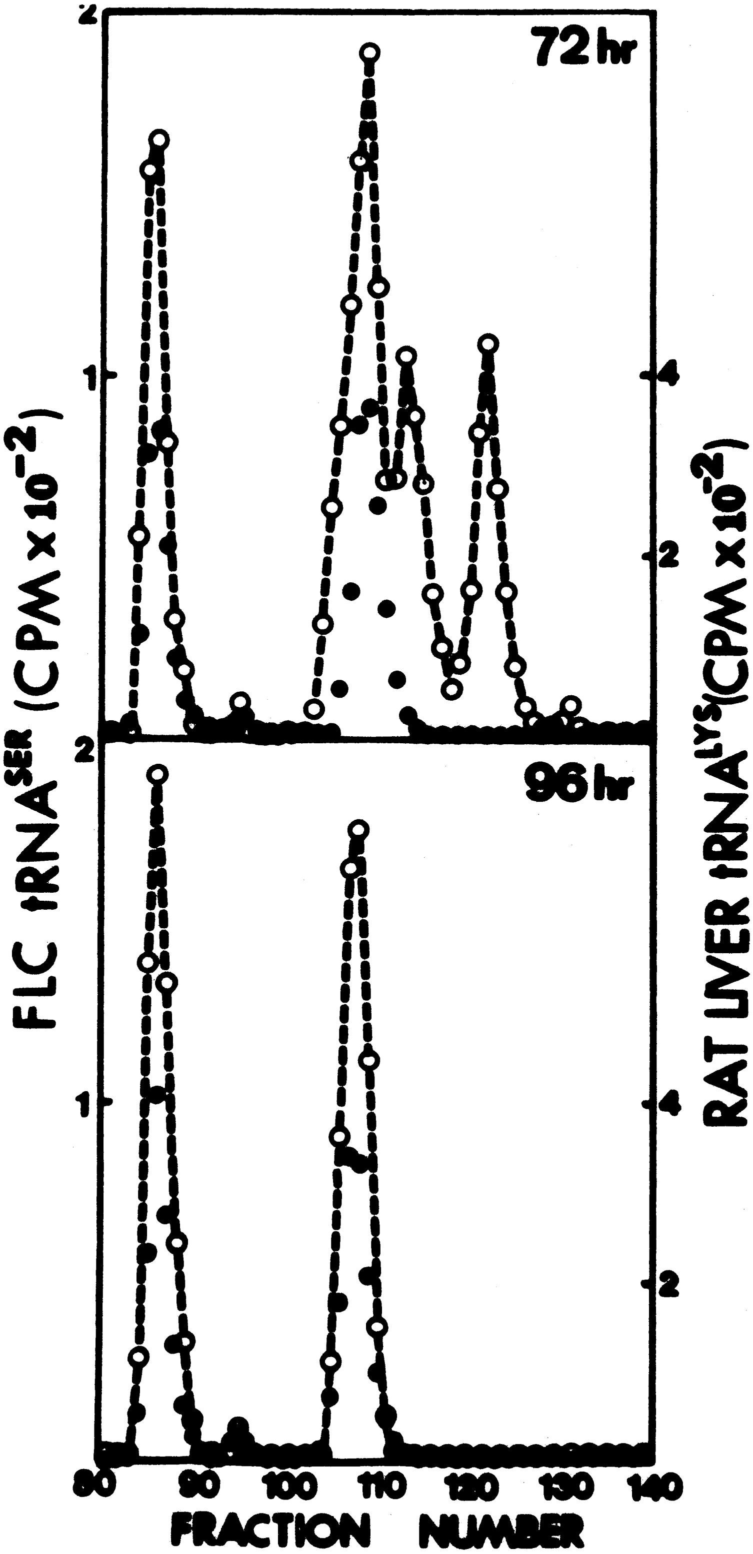
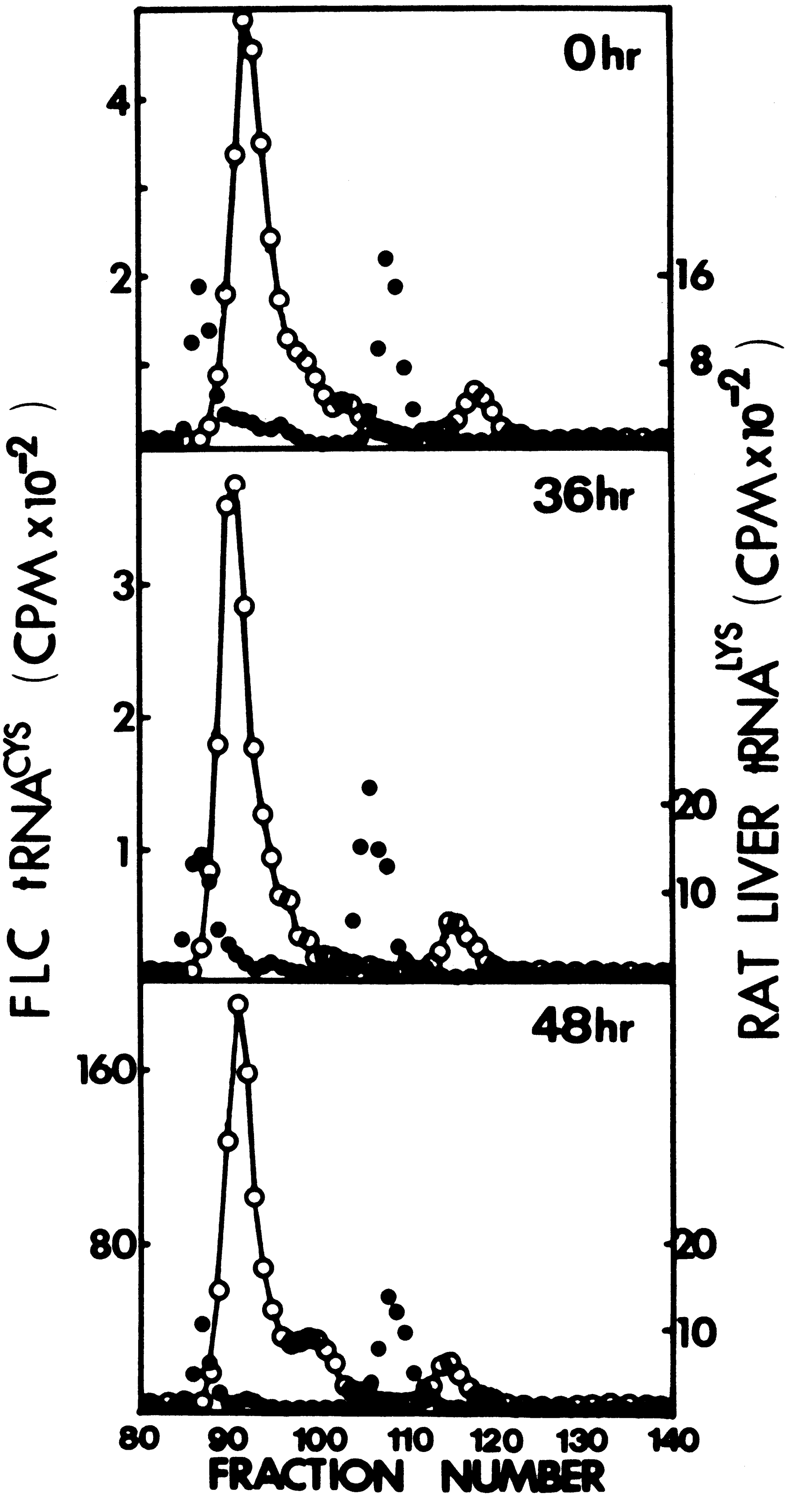

72 hr
96 hr
FLC tRNA^{SER} (CPM x 10^{-2})
RAT LIVER tRNA^{LYS} (CPM x 10^{2})
2
1
2
1
4
2
4
2
80
90
100
110
120
130
140
FRACTION NUMBER

Figure 15. Alterations in the RPC-5 profile of tRNA^{cys} extracted from Friend leukemia cells undergoing erythroid differentiation. This set of chromatograms compares the elution profiles of the tRNA^{cys} isoacceptors(o--o) as described in the legend of Figure 7 except for the use of [^{35}S]cysteine and rat liver [^{3}H]lysyl-tRNA as internal standard(●--●).


0 hr
36 hr
48 hr
FLC tRNA^CYS (CPM x10^-2)
RAT LIVER tRNA^LYS (CPM x10^-2)
4
2
3
2
1
160
80
16
8
20
10
20
10
80
90
100
110
120
130
140
FRACTION NUMBER

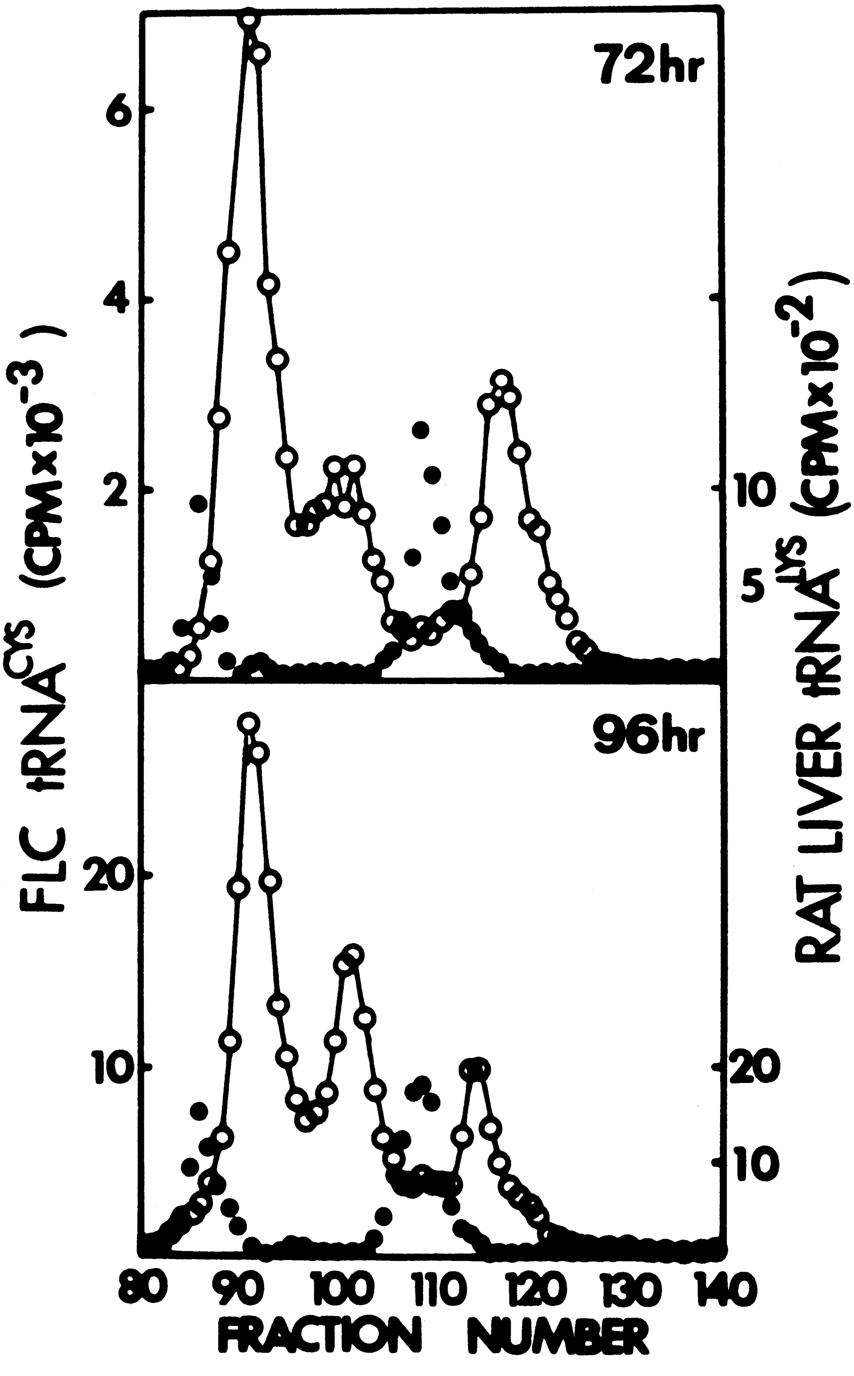
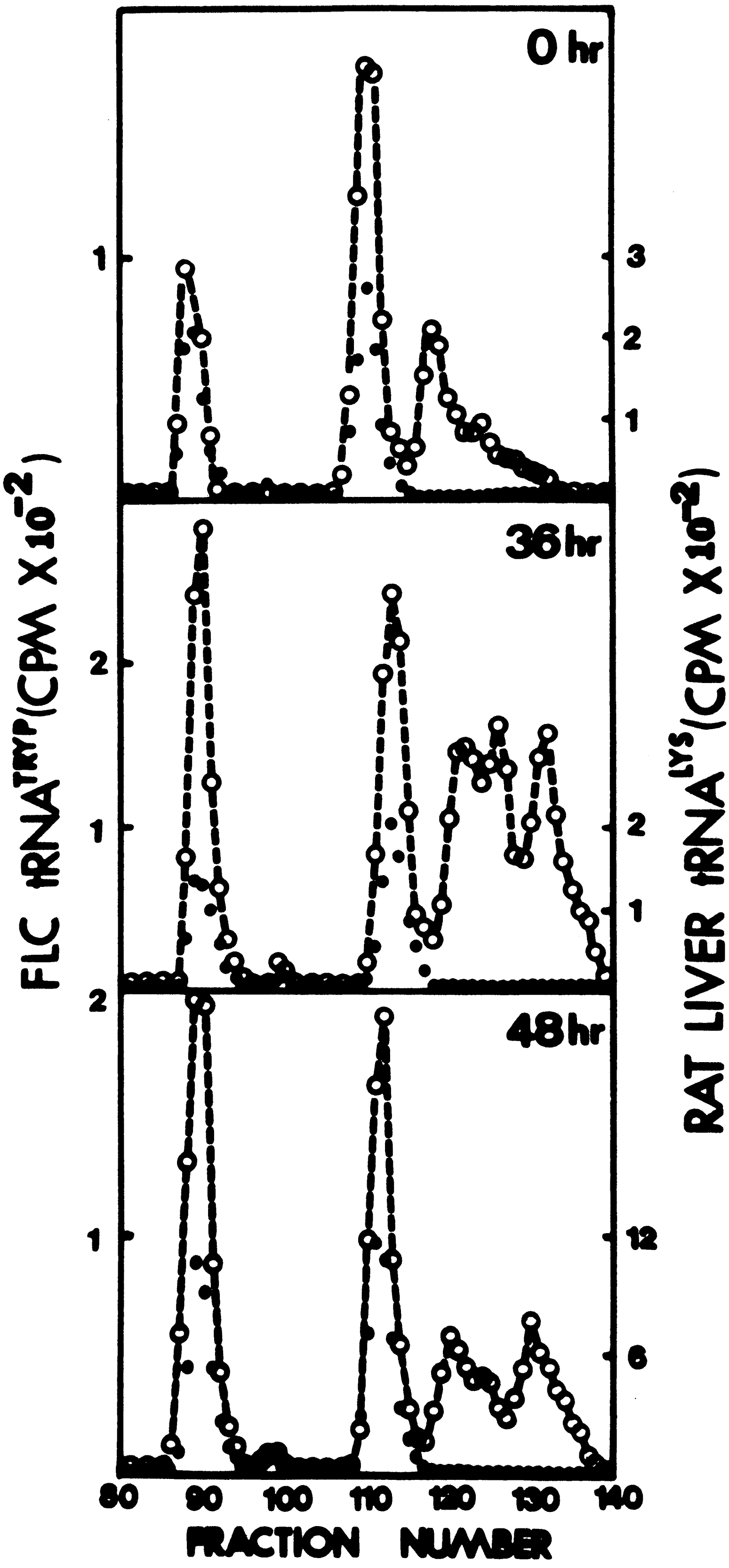
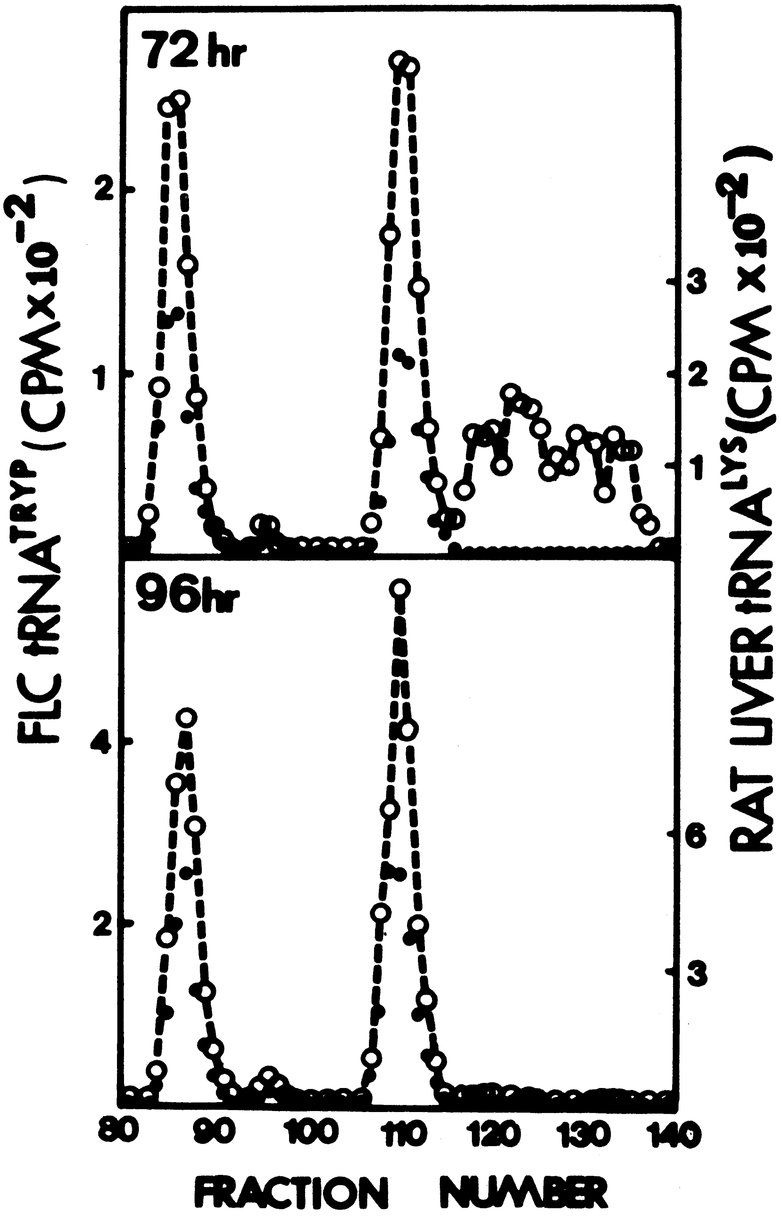

72hr
96hr
FLC tRNA^{CYS} (CPM×10^{-3})
RAT LIVER tRNA^{LYS} (CPM×10^{-2})
6
4
2
20
10
10
5
20
10
80
90
100
110
120
130
140
FRACTION NUMBER

Figure 16. Alterations in the RPC-5 profile of $tRNA^{trp}$ extracted from Friend leukemia cells undergoing erythroid differentiation. This set of chromatograms compares the elution profiles of the $tRNA^{trp}$ isoacceptors (o--o) as described in the legend of Figure 7.


0 hr
36 hr
48 hr
FLC tRNA^{TRYP}(CPM X 10^{-2})
RAT LIVER tRNA^{LYS}(CPM X 10^{-2})
1
2
1
2
1
3
2
1
2
1
12
6
80
90
100
110
120
130
140
FRACTION NUMBER


72 hr
96hr
FLC tRNA^{TRYP} (CPM x 10^{-2})
RAT LIVER tRNA^{LYS} (CPM x 10^{-2})
2
1
4
2
3
2
1
6
3
80
90
100
110
120
130
140
FRACTION NUMBER

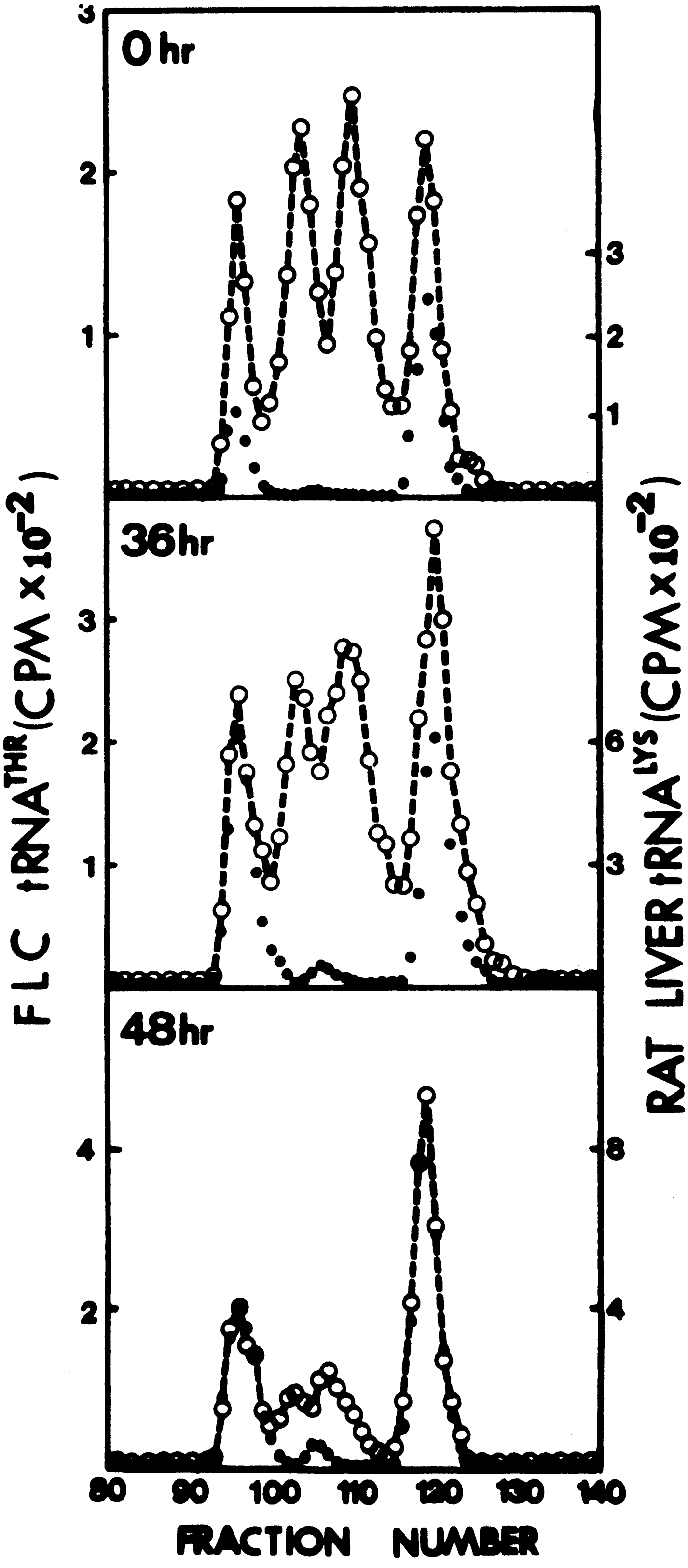
tions used and disappeared at 96 hr after the addition of DMSO. The isoaccepting species of $tRNA^{thr}$ exhibited continual changes in relative amounts during the course of F.L.C. differentiation (Figure 17). Four isoaccepting species were resolved. Species 1 and 4 increased in relative amount consistently, while 2 and 3 decreased throughout the time course. Alanyl-tRNA exhibited five isoaccepting species (Figure 18) with the major species (peak 3) being maintained at a rather constant relative quantity during the first 72 hr of differentiation and then increasing by 50% at 96 hr. Species 4 and 5 essentially decreased in relative amount throughout the differentiation.

3. Variation in Q-Ribonucleoside Containing tRNAs

The isoaccepting species of tRNAs which contain the ribonucleoside Q, $tRNA^{asn}$, $tRNA^{asp}$, $tRNA^{his}$, and $tRNA^{tyr}$, were resolved by reversed-phase plaskon chromatography. Quantitative and qualitative changes of the isoaccepting species for these tRNAs, tabulated in Table 3, reflected significant alterations during erythroid differentiation.

Transfer RNA^{asn} was resolved into 5 isoaccepting species. In Figure 19, the distributions of the $tRNA^{asn}$ isoaccepting species were compared at 5 time points during the course of erythroid differentiation. As shown in Figure 19 and Table 3, $tRNA^{asn}_3$ consistently increased in relative amount from 10.7 to 71.5% of the total $tRNA^{asn}$ during the differentiation; whereas $tRNA^{asn}_2$ and $tRNA^{asn}_5$ decreased to trace amounts. However, $tRNA^{asn}_1$ was rather constant in amount after decreasing in the first 36 hr to approximately 24% of the total $tRNA^{asn}$; while $tRNA^{asn}_4$ first increased and then decreased to a barely detectable level.

Figure 17. Alterations in the RPC-5 profile of $tRNA^{thr}$ extracted from Friend leukemia cells undergoing erythroid differentiation. This set of chromatograms compares the elution profiles of the $tRNA^{thr}$ isoacceptors(o--o) as described in the legend of Figure 7.


0 hr
36hr
48hr
FLC tRNA^{THR}(CPM x 10^{-2})
RAT LIVER tRNA^{LYS}(CPM x 10^{-2})
FRACTION NUMBER
80
90
100
110
120
130
140
3
2
1
4
6
8

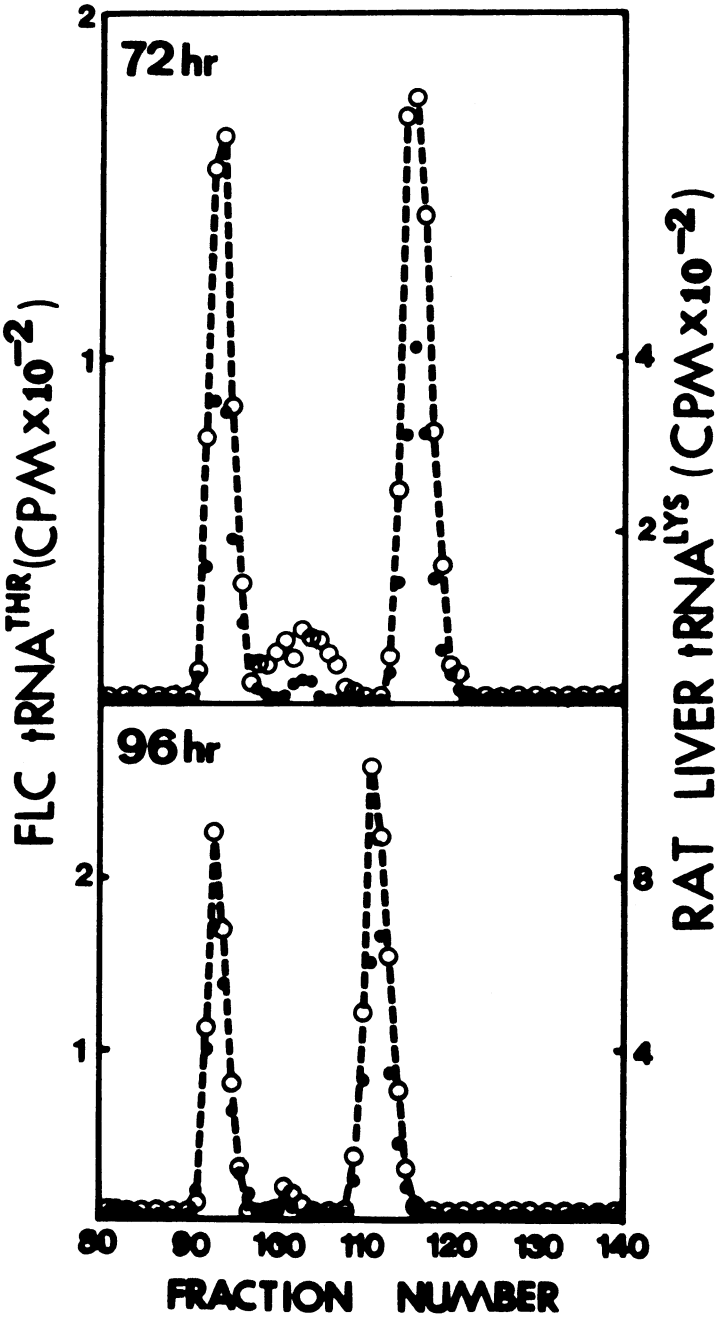
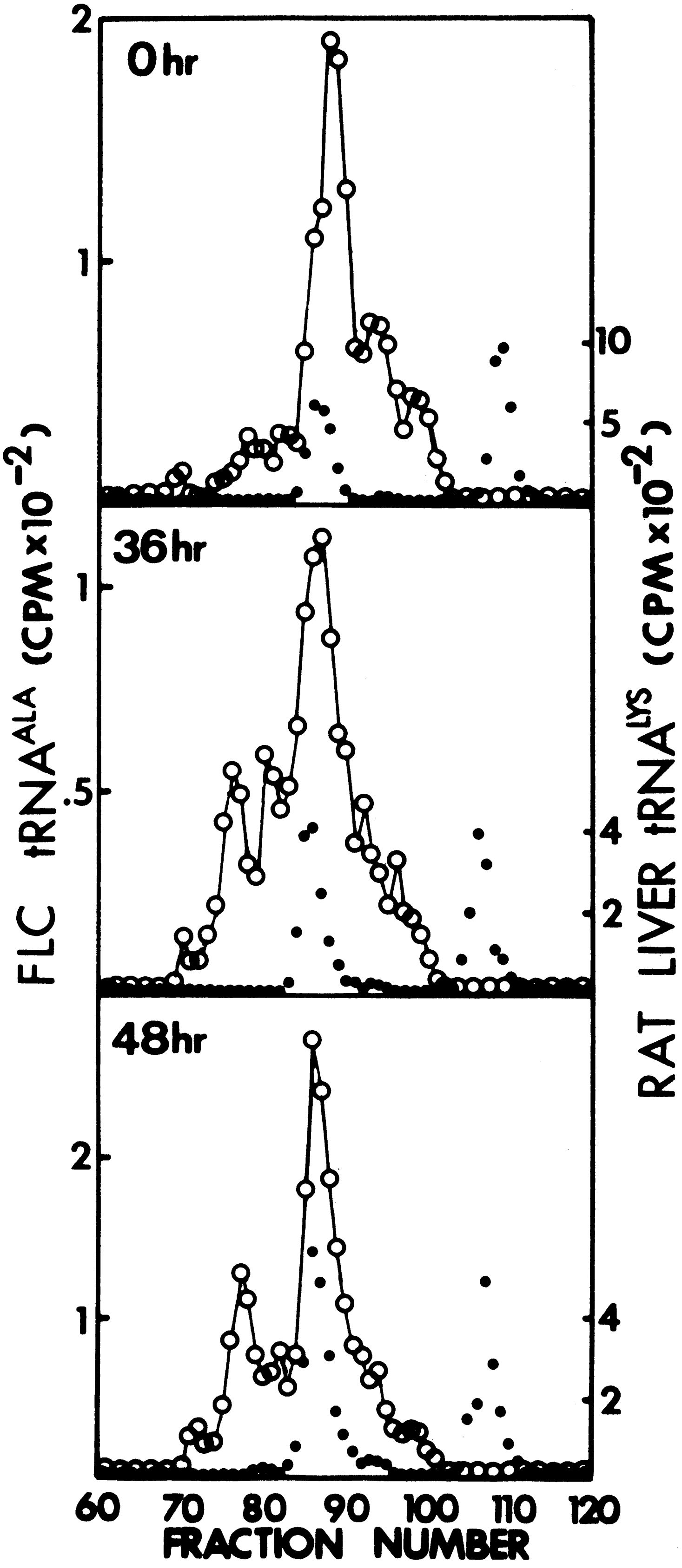

72 hr
96 hr
FLC tRNA^{THR} (CPM × 10^{-2})
RAT LIVER tRNA^{LYS} (CPM × 10^{-2})
2
1
2
1
4
2
8
4
80
90
100
110
120
130
140
FRACTION NUMBER

Figure 18. Alterations in the RPC-5 profile of $tRNA^{ala}$ extracted from Friend leukemia cells undergoing erythroid differentiation. This set of chromatograms compares the elution profiles of the $tRNA^{ala}$ isoacceptors (o--o) as described in the legend of Figure 7 except for the use of [^{14}C]alanine and rat liver [^{3}H]lysyl-tRNA as internal standard(●--●).


0 hr
36 hr
48 hr
FLC tRNA^{ALA} (CPM x10^{-2})
RAT LIVER tRNA^{LYS} (CPM x10^{-2})
2
1
1
.5
2
1
10
5
4
2
4
2
60
70
80
90
100
110
120
FRACTION NUMBER

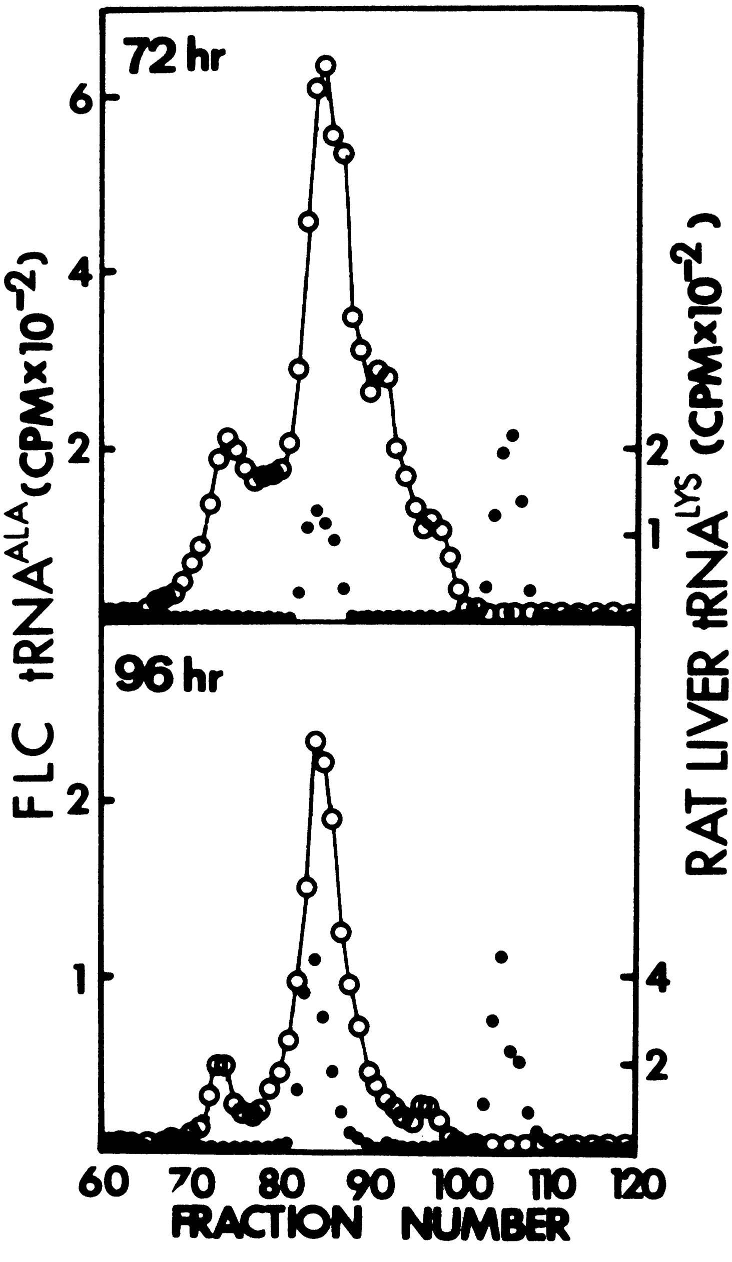

72 hr
96 hr
FLC tRNA^{ALA} (CPM x 10^{-2})
RAT LIVER tRNA^{LYS} (CPM x 10^{-2})
6
4
2
2
1
1
2
4
2
60
70
80
90
100
110
120
FRACTION NUMBER

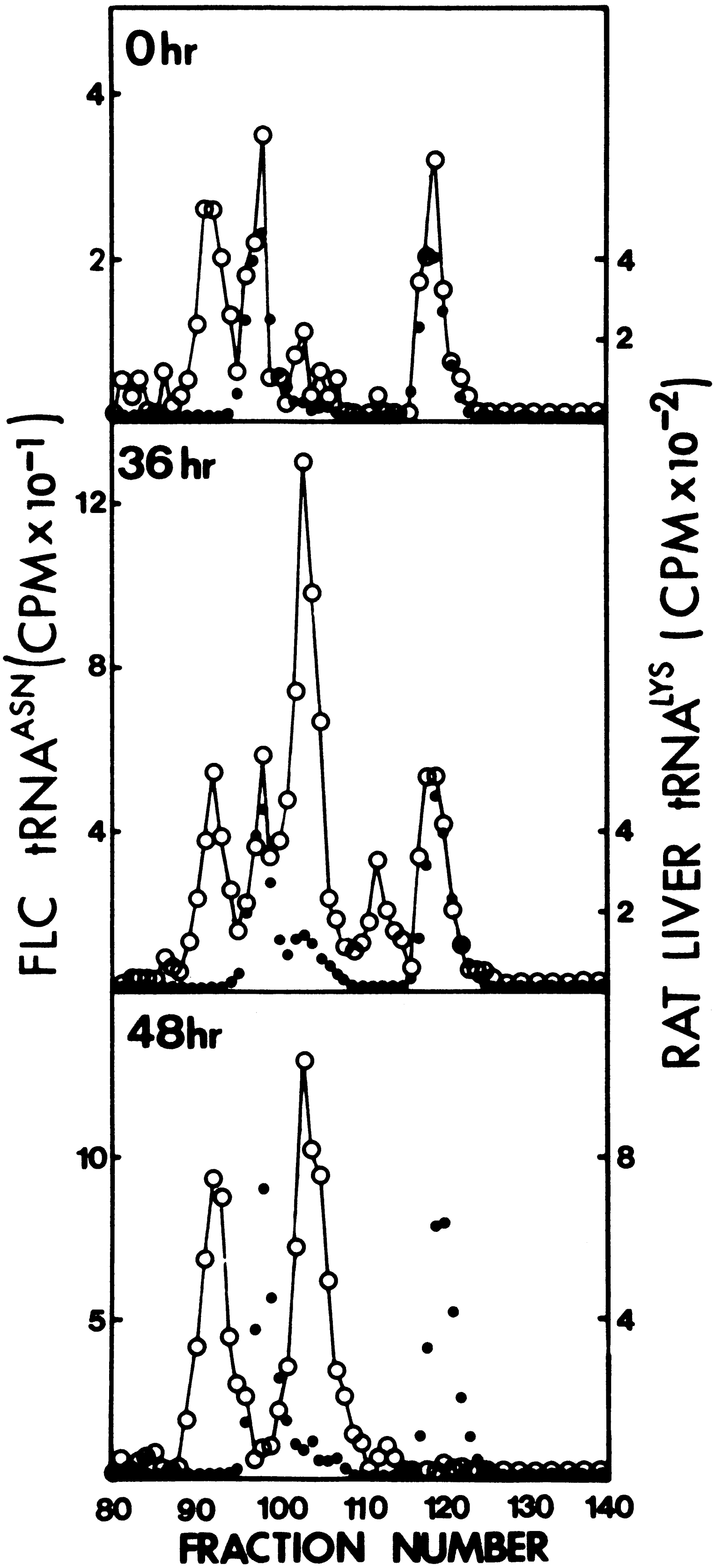
Table 3: Changes in Amino Acid Isoaccepting tRNA Species Which Contain Q-Ribonucleoside

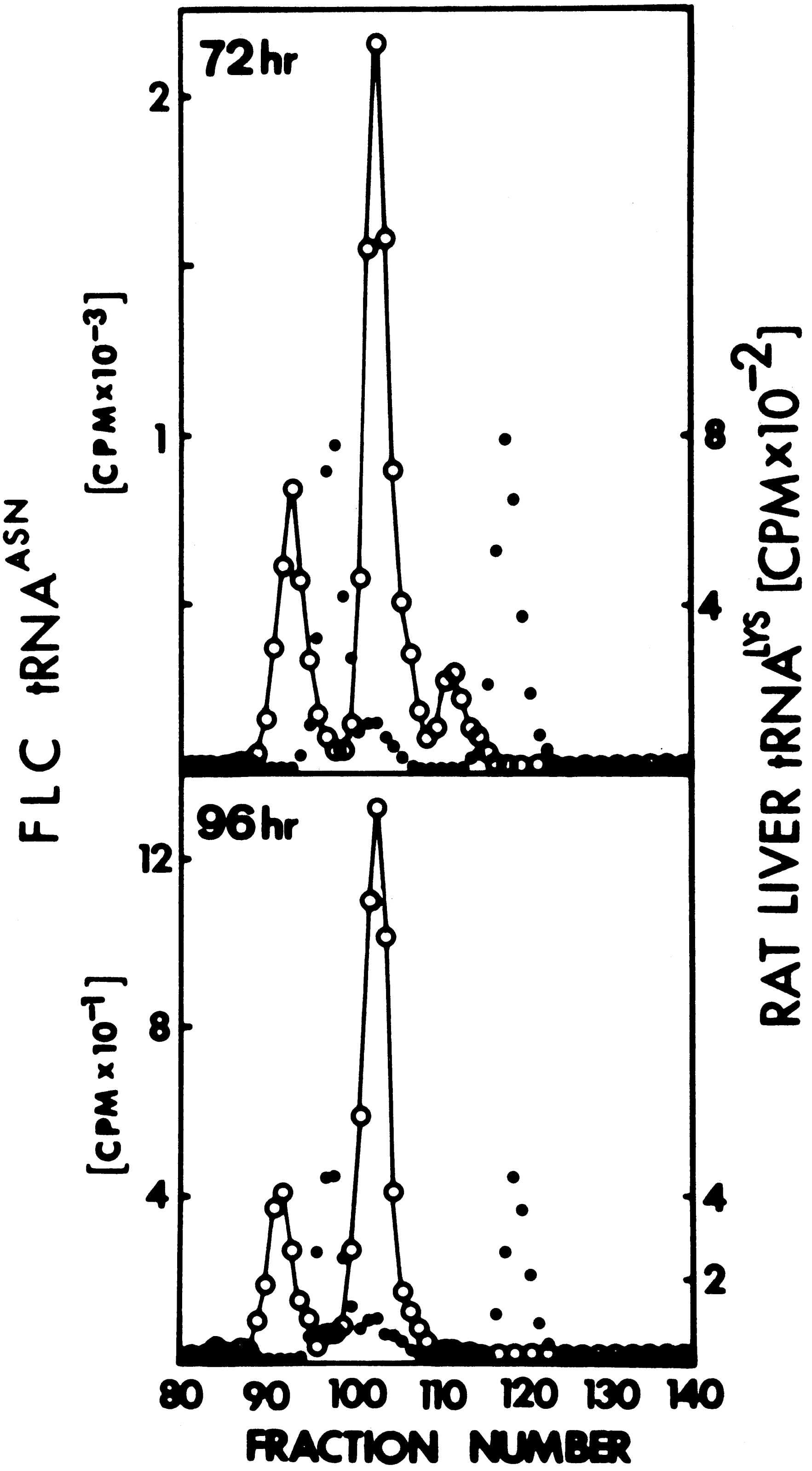
tRNA	Time During Differentiation[a]	Relative Amounts of Isoaccepting Species[b]					
		1	2	3	4	5	6
Asn	0	31.7	26.1	10.7	3.4	28.1	
	36	16.1	12.6	42.2	9.6	19.4	
	48	24.2	4.7	57.6	12.9	1.0	
	72	26.1	0	62.7	11.2	0	
	96	22.7	3.7	71.5	1.1	1.0	
Asp	0	2.8	24.0	67.7	5.5		
	36	6.2	9.2	63.4	21.5		
	48	3.8	10.3	64.5	21.4		
	72	2.0	17.5	54.3	26.2		
	96	0	43.0	53.3	3.7		
His	0	15.9	16.0	27.0	41.1		
	36	11.8	12.6	35.6	40.0		
	48	9.8	10.0	47.6	32.6		
	72	1.0	12.8	54.0	32.2		
	96	7.4	13.3	7.8	71.5		
Tyr	0	4.6	10.5	26.7	58.2		
	36	12.9	10.6	12.5	64.0		
	48	38.6	2.3	9.0	50.1		
	72	43.4	3.0	0	53.6		
	96	53.3	2.1	0	44.6		

[a] Hours after the addition of DMSO

[b] Percent of total aminoacyl-tRNA

Figure 19. Alterations in the RPC-5 profile of $tRNA^{asn}$ extracted from Friend leukemia cells undergoing erythroid differentiation. This set of chromatograms compares the elution profiles of the $tRNA^{asn}$ isoacceptors (o--o) as described in the legend of Figure 7 except for the use of $[^{14}C]$asparagine and rat liver $[^{3}H]$lysyl-tRNA as internal standard(●--●).


0 hr
36 hr
48 hr
4
2
12
8
4
10
5
4
2
4
2
8
4
80
90
100
110
120
130
140
FRACTION NUMBER
FLC tRNA^{ASN} (CPM x 10^{-1})
RAT LIVER tRNA^{LYS} (CPM x 10^{-2})

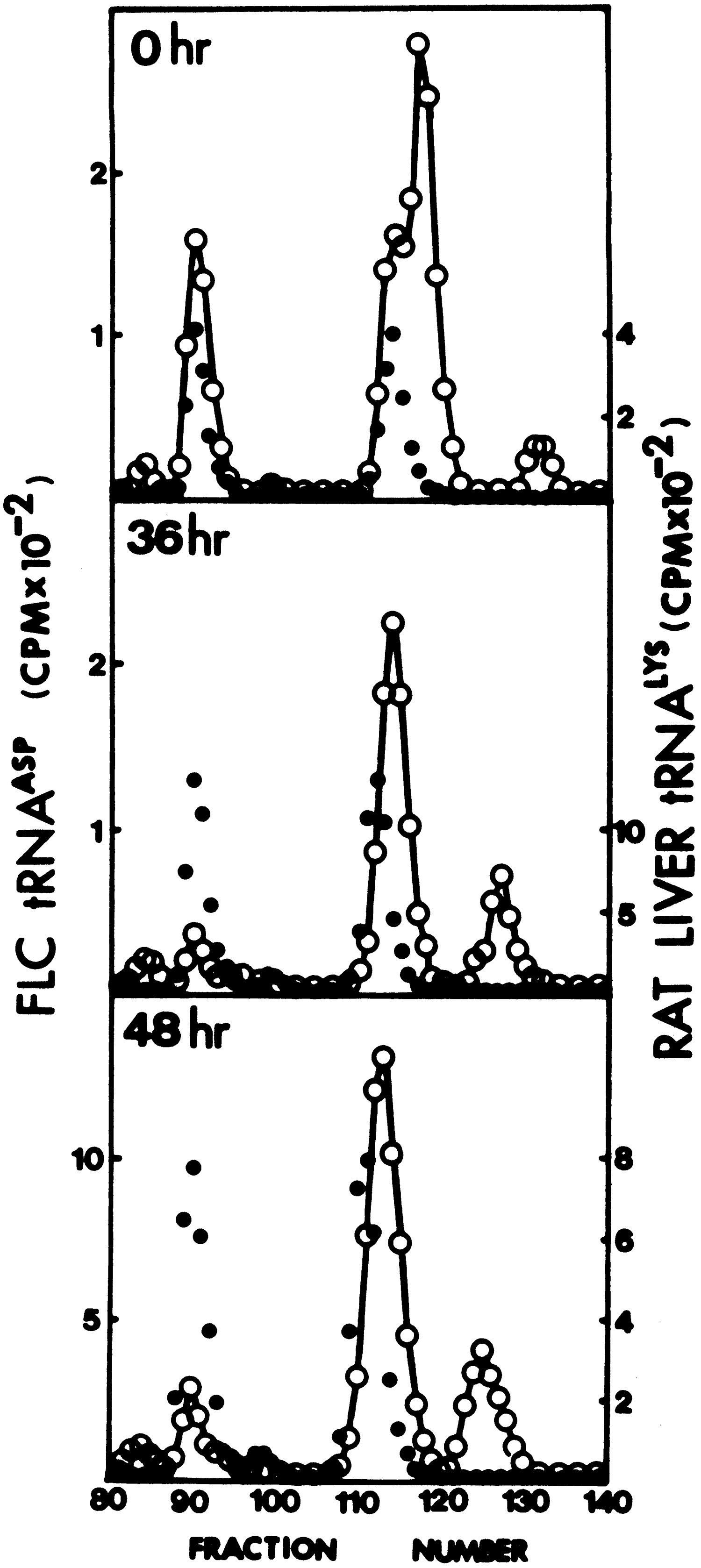

72 hr
96 hr
FLC tRNA^ASN
[CPM x 10^-3]
[CPM x 10^-1]
RAT LIVER tRNA^LYS [CPM x 10^-2]
2
1
12
8
4
8
4
4
2
80
90
100
110
120
130
140
FRACTION NUMBER

Transfer RNA^{asp} was resolved into 4 isoacceptors as shown in Figure 20; $tRNA^{asp}_3$ was the predominant species and was maintained by the cells between 53.3 and 67.7% of total $tRNA^{asp}$ during the differentiation. $tRNA^{asp}_1$ was present only in trace amounts if present at all. $tRNA^{asp}_2$ decreased in relative amount at 36 hr and then gradually increased to almost two fold the original level at the completion of differentiation (96 hr). Concomitantly, $tRNA^{asp}_4$ first increased by 4 fold from 5.5% of total $tRNA^{asp}$ at 0 hr to 21.2% at 36 hr and remained at this level for 36 additional hours. At 96 hr, the relative amount of $tRNA^{asp}_4$(3.7%) was comparable to that at 0 hr.

Transfer RNA^{his} was resolved into 4 isoaccepting species as shown in Figure 21. $tRNA^{his}_3$and $tRNA^{his}_4$ were predominant species throughout the first 72 hr of differentiation (Table 3). Upon completion of differentiation by a majority of the cells at 72 hr after addition of DMSO, $tRNA^{his}_3$had doubled in amount relative to the other isoacceptors; whereas $tRNA^{his}_1$ decreased from 15.9 to 1% of the total $tRNA^{his}$. $tRNA^{his}_3$ decreased dramatically in relative amount between 72 and 96 hr; while $tRNA^{his}_4$ doubled in the same time period. $tRNA^{his}_2$ was maintained by the cells in a rather constant relative amount throughout the entire course of differentiation.

Transfer RNA^{tyr} was separated into 4 isoacceptors by RPC-5 chromatography (Figure 22). $tRNA^{tyr}_4$ was the major species and changed relatively little throughout differentiation (Table 3). $tRNA^{tyr}_1$ consistently increased in relative amount and finally became a second major species consisting of 53.3% of the total $tRNA^{tyr}$ at the completion of differentiation. $tRNA^{tyr}_2$ and $tRNA^{tyr}_3$ which were not well resolved by the chromatography conditions we employed, decreased gradually during the course of differentiation relative to the other species.

Figure 20. Alterations in the RPC-5 profile of tRNA^{asp} extracted from Friend leukemia cells undergoing erythroid differentiation. This set of chromatograms compares the elution profiles of the tRNA^{asp} isoacceptors (o--o) as described in the legend of Figure 7.


0 hr
36 hr
48 hr
FLC tRNA^{ASP} (CPMx10^{-2})
RAT LIVER tRNA^{LYS} (CPMx10^{-2})
2
1
4
2
2
1
10
5
10
5
8
6
4
2
80
90
100
110
120
130
140
FRACTION NUMBER

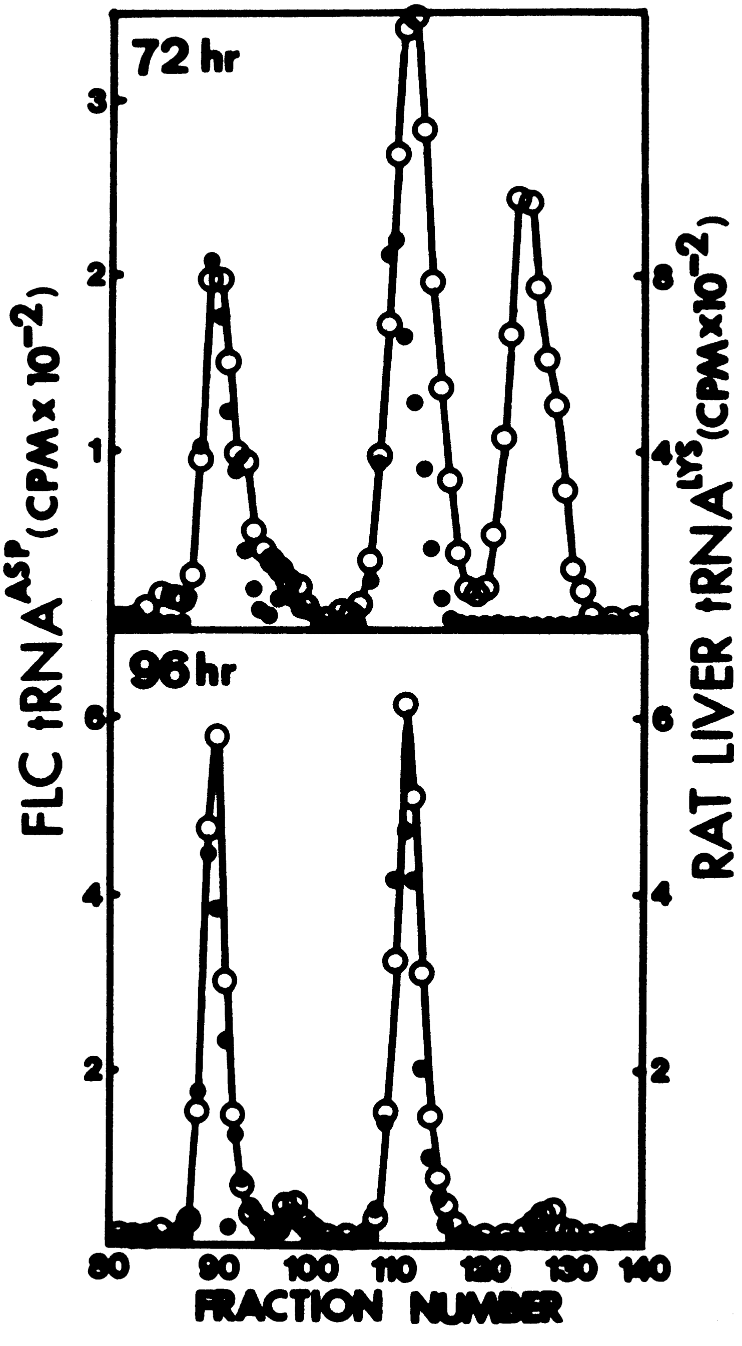
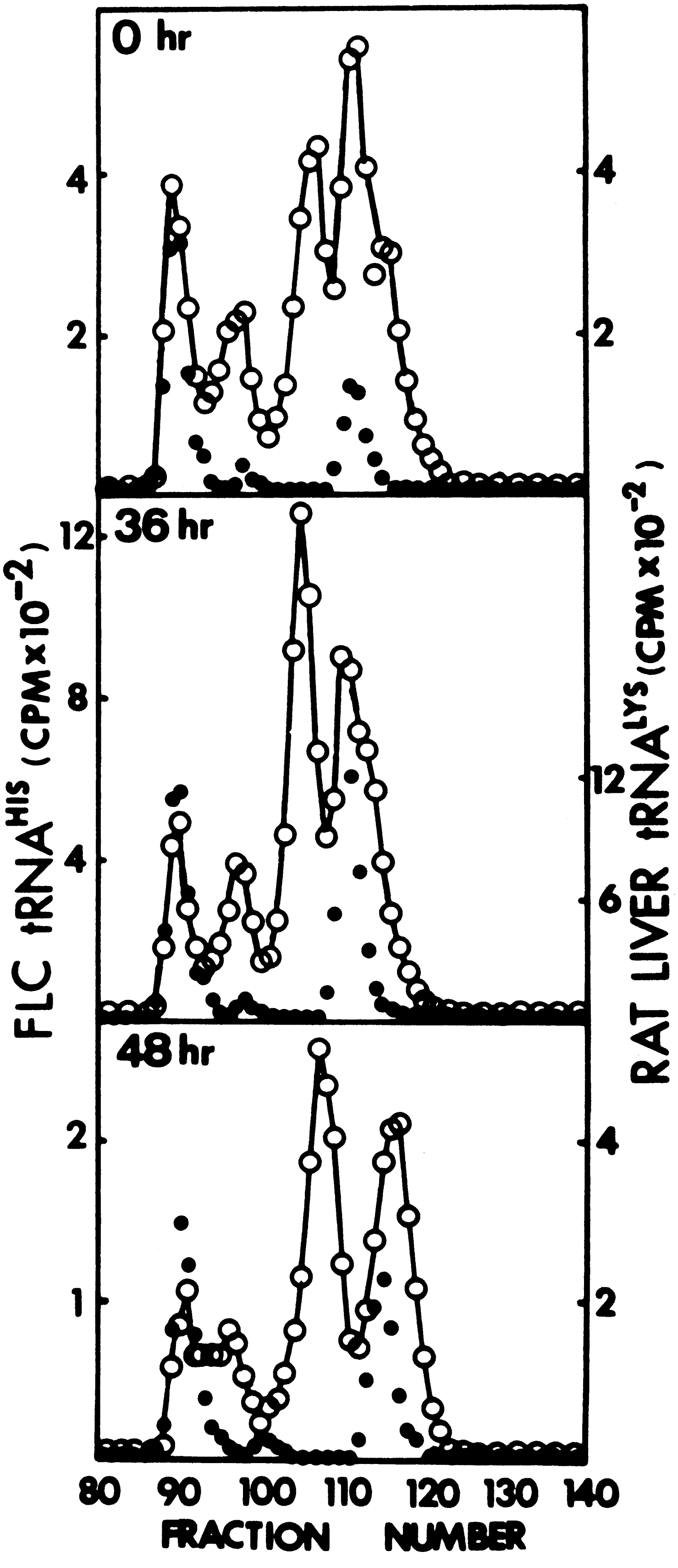

72 hr
96 hr
FLC tRNA^{ASP} (CPM x 10^{-2})
RAT LIVER tRNA^{LYS} (CPM x 10^{-2})
1
2
3
2
4
6
4
8
2
4
6
80
90
100
110
120
130
140
FRACTION NUMBER

Figure 21. Alterations in the RPC-5 profile of tRNA^{his} extracted from Friend leukemia cells undergoing erythroid differentiation. This set of chromatograms compares the elution profiles of the tRNA^{his} isoacceptors (o--o) as described in the legend of Figure 7.


0 hr
36 hr
48 hr
FLC tRNA^{HIS} (CPM x 10^{-2})
RAT LIVER tRNA^{LYS} (CPM x 10^{-2})
4
2
12
8
4
2
1
4
2
12
6
4
2
80
90
100
110
120
130
140
FRACTION NUMBER

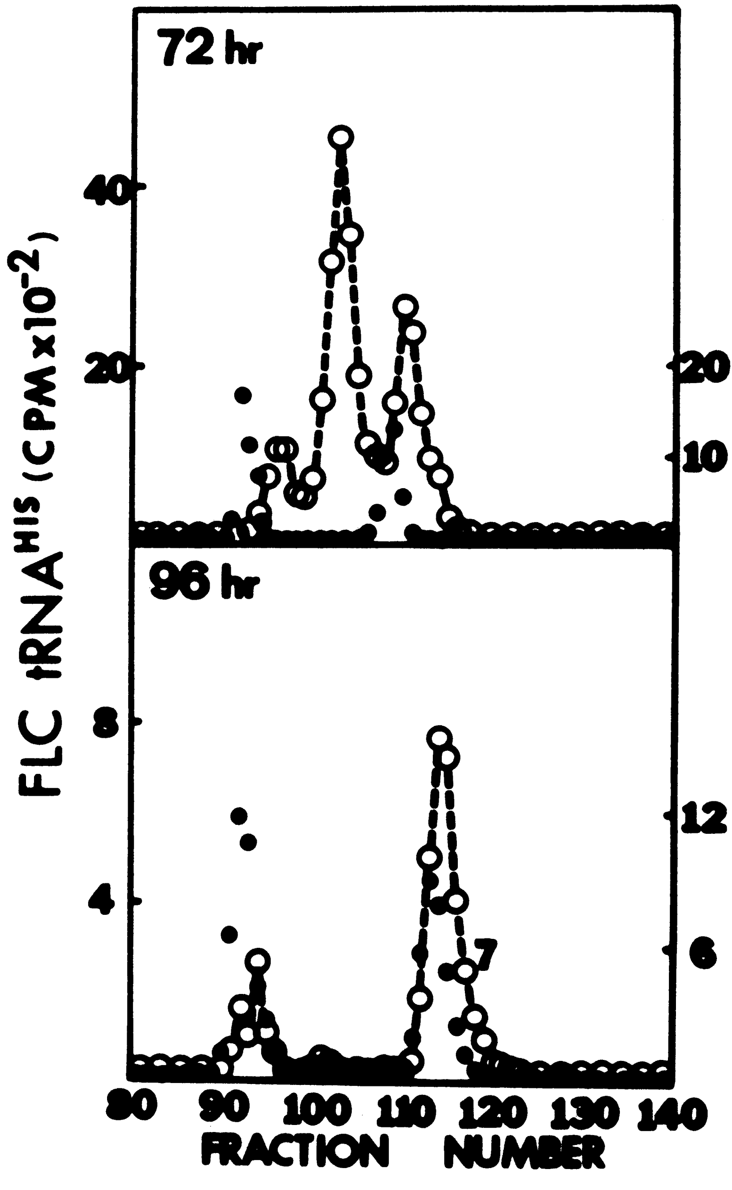
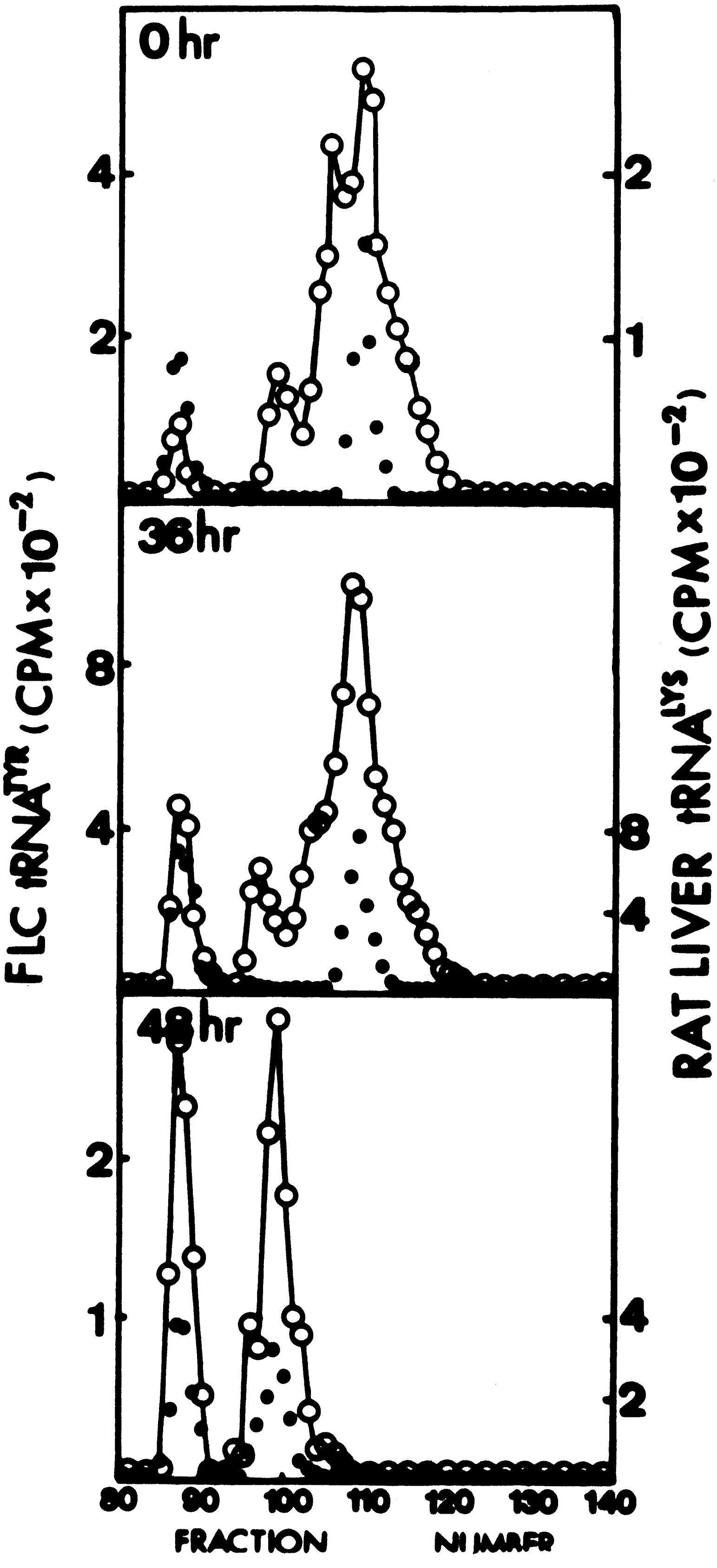
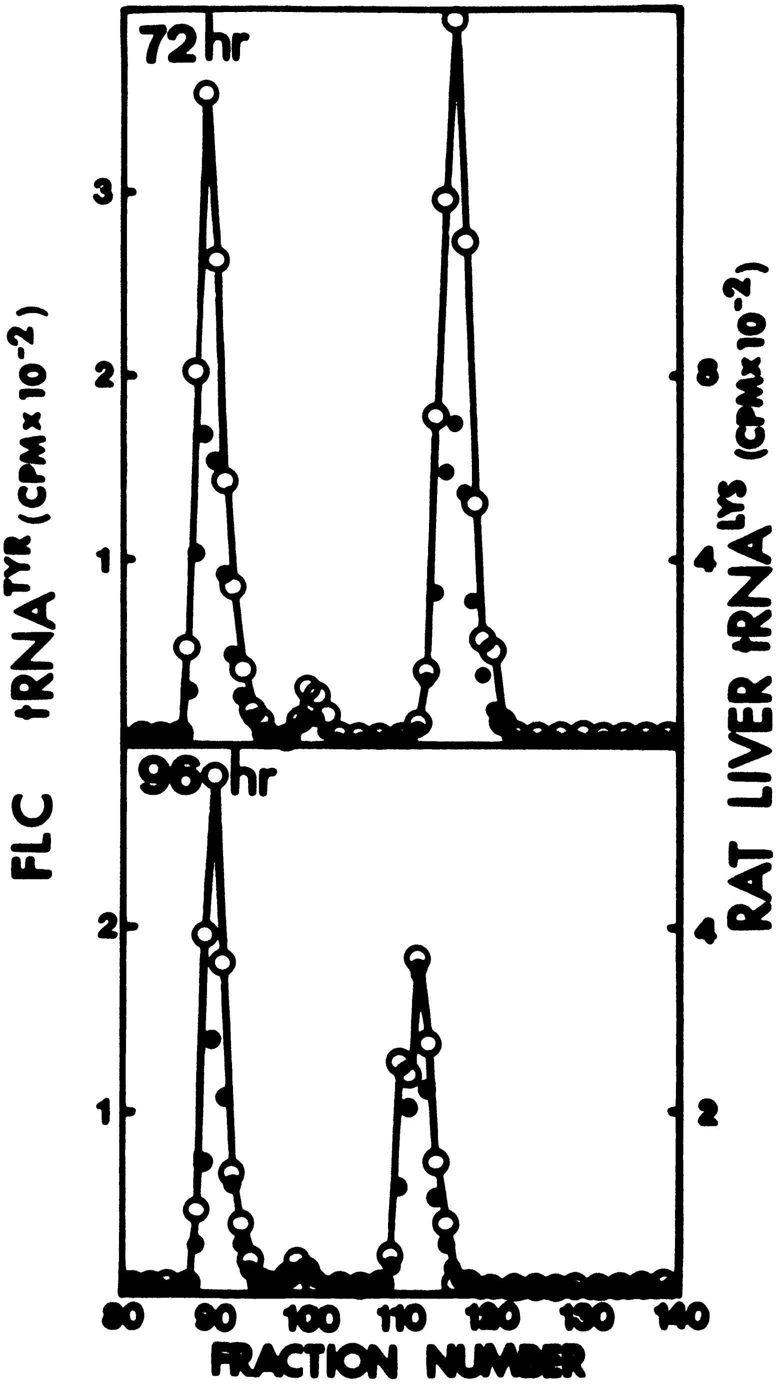

72 hr
96 hr
FLC tRNA^HIS (CPM x 10^-2)
RAT LIVER tRNA^LYS (CPM x 10^-2)
40
20
8
4
20
10
12
6
7
80
90
100
110
120
130
140
FRACTION NUMBER

Figure 22. Alterations in the RPC-5 profile of $tRNA^{tyr}$ extracted from Friend leukemia cells undergoing erythroid differentiation. This set of chromatograms compares the elution profiles of the $tRNA^{tyr}$ isoacceptors (o--o) as described in the legend of Figure 7.


0 hr
36hr
48hr
4
2
8
4
2
1
2
1
8
4
4
2
FLC tRNA^TYR (CPM x 10^-2)
RAT LIVER tRNA^LYS (CPM x 10^-2)
80
90
100
110
120
130
140
FRACTION NUMBER


72 hr
96 hr
FLC tRNA^TYR (CPM x 10^-2)
RAT LIVER tRNA^LYS (CPM x 10^-2)
1
2
3
1
2
4
8
2
4
80
90
100
110
120
130
140
FRACTION NUMBER

E. Patterns of Changes in the Q Containing tRNAs During F.L.C. Erythrodifferentiation

In order to study the presence of the Q modification and the distribution of tRNA isoacceptors lacking this modification during the course of differentiation, we first determined changes in the amount of Q ribonucleoside present in F.L.C. tRNA preparations isolated at five time points during differentiation. By using guanine-tRNA transferase purified from rabbit reticulocyte lysate (121), the amount of [^{3}H]guanine inserted into tRNA catalyzed by the enzyme becomes a measure of the lack of fully synthesized Q. The amount of guanine incorporation into the first position of tRNA anticodons replacing unmodified G or incompletely synthesized Q is shown in Table 4. There was essentially no guanylation observed for uninduced tRNA. However, the tRNA from F.L.C. after 36 hr of differentiation accepted 8.0 pmole of guanine per A_{260} of tRNA. tRNA from cells after 48 hr of induction exhibited the maximum guanine incorporation which then decreased significantly at 72 and again at 96 hr. These results indicate that tRNA from uninduced F.L.C. was highly modified with respect to the Q modification. Concomitant with erythroid differentiation, F.L.C. continually decreased the synthesis of Q in newly synthesized tRNA until 48 hr after the addition of DMSO, the time at which the tRNA had the lowest level of Q.

In addition, three tRNA preparations (36 hr, 48 hr and 96 hr) having been first subjected to guanylation were then subjected to chromatography on an RPC-5 column for determining the distribution of [^{3}H]guanine-accepting tRNA species. The results are shown in Figure 23. All three tRNA preparations showed similar RPC-5 elution patterns with a relatively small peak that eluted early and a major peak of guanylated tRNA eluting late.

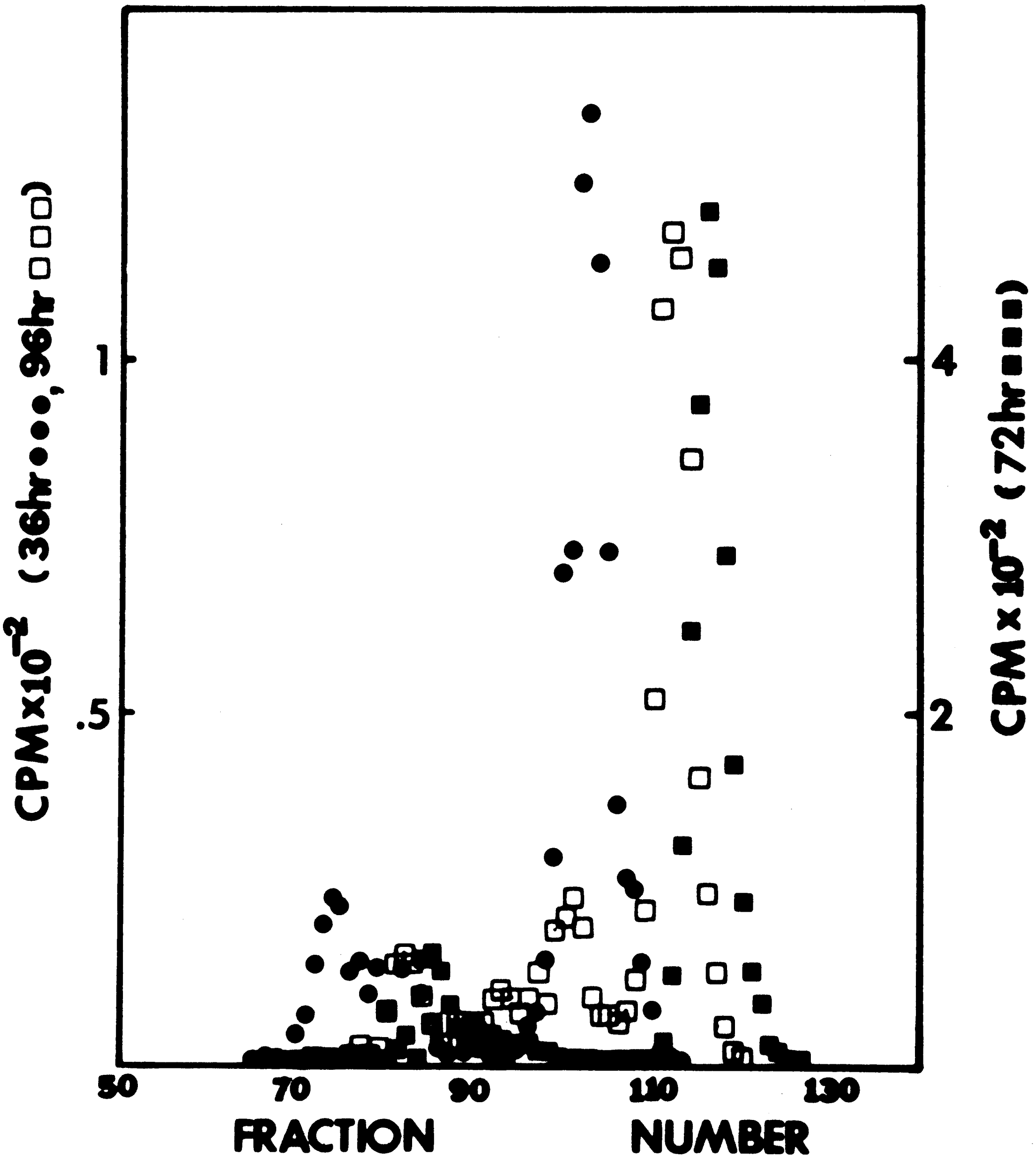
Table 4: Guanine Incorporation for Different F.L.C. tRNA Preparations

tRNA Preparation[a]	0 hr	36 hr	48 hr	72 hr	96 hr
$[^3H]$Guanine Incorporated into tRNA[b]	0	8.0	9.7	5.0	3.7

[a] tRNA preparations are designated according to the length of time F.L.C. had been induced to undergo erythroid differentiation by exposure to dimethylsulfoxide.

[b] Guanylation was assayed as described in Materials and Methods and is shown as pMole $[^3H]$guanine incorporated per A_{260} unit of tRNA.

Figure 23. RPC-5 chromatographic comparison of guanylated tRNA from Friend leukemia cells undergoing erythroid differentiation. tRNA was isolated from F.L.C. cells 36, 48, and 96 hr after the addition of 270 mM DMSO to cultures. Each of the tRNA preparations was guanylated with [^{3}H]labelled guanine and then subjected to RPC-5 chromatography. This chromatogram compares the RPC-5 elution profiles of these three guanylated tRNAs (36 hr, ● ● ●; 72 hr, ■ ■ ■; 96 hr, □ □ □). Details of the guanylation and chromatography procedures are described in Materials and Methods.


CPM x 10^{-2} (36hr ●●●, 96hr □□□)
1
.5
4
2
CPM x 10^{-2} (72hr ■■■)
50
70
90
110
130
FRACTION NUMBER

V. DISCUSSION

Several hypotheses have been advanced which propose that tRNA molecules are regulatory factors during cell differentiation (187). A number of studies have shown that the tRNA population of a tissue specialized for the production of one protein reflects the amino acid composition of that protein (2; 8; 11; 224). Garel (2) has discussed in some detail this functional adaptation of tRNA populations in a number of systems. The primary purpose of this investigation was to determine if qualitative or quantitative differences in tRNA isoaccepting species existed between the undifferentiated Friend leukemia cells (F.L.C.) and the same cells induced to undergo differentiation for hemoglobin synthesis. Such differences would suggest that levels of particular tRNA species may regulate hemoglobin synthesis because synthesis of this protein amounts to at least 24% of total protein synthesized by differentiated F.L.C. (253). With the use of RPC-5 column chromatography, the isoaccepting species of tRNA isolated from undifferentiated and DMSO-induced erythroid differentiated F.L.C.were resolved and compared. Studies presented here demonstrated that thirteen of sixteen tested aminoacyl-tRNAs had quantitatively and qualitatively altered distributions of isoaccepting species during the course of DMSO induced erythroid differentiation. Extensive preliminary experiments were performed in order to achieve optimum conditions for preparation of aminoacyl-tRNA and resolution of isoaccepting species of these tRNAs.

Only 80-90% of Friend leukemia cells can be induced irrespective of the time or conditions of treatment with inducers. A series of variants has been isolated by selecting for resistance to different inducers of Friend leukemia cell erythroid differentiation and these variants have been characterized to different degrees (148; 157; 254). Genetic analysis indicated that the noninducible variants are formed consistently with a mutational origin in the range 5 - 50 x 10^{-6} cells per generation (254). However, only 10-50% of DMSO-resistant clones remain stably non-inducible in culture without DMSO as judged by subsequent recloning in DMSO (148; 254). The evidence available can not lead to a rational conclusion with regard to the formation of noninducible variants. Nevertheless, the genetic instability of those variants is very obvious.

During the present investigation, in order to clarify that the alteration of tRNA isoacceptor profiles are not due to factors other than the erythroid differentiation, it had been thought that these isolated noninducible variants might serve as an excellent control. Unfortunately genetic instability of those variants have made them unsuitable for this purpose. However, comparative analysis of tRNA nucleotide composition from DMSO-induced differentiated and undifferentiated F.L.C. as well as other nonhematopoietic cell lines under the same conditions showed that the alteration of nucleotide composition in tRNA is concurrent with erythroid differentiation and unique to F.L.C. (13). Thus, it is considered that the alteration of tRNA isoacceptor profiles is almost solely, if not completely, due to the cell responding to erythroid differentiation.

The change in lysine isoaccepting species patterns during various cellular events has been extensively studied (11; 13; 189; 251; 256;

257) and it is of interest to compare the changes of $tRNA^{lys}$ in F.L.C. with some of these studies. Lysine tRNA from mammalian cells can be resolved on an RPC-5 column into 4-6 species. By virtue of the identical elution of the F.L.C. lysine tRNA species with that of the rat liver lysine tRNA species, we have numbered the species according to the system used by Ortwerth (258). It can be seen that $tRNA^{lys}_2$, $tRNA^{lys}_4$, and $tRNA^{lys}_5$ are predominant species (Figure 11). However, $tRNA^{lys}_4$ is of particular interest since its presence in relatively large amounts has been correlated with the ability of mammalian cells to divide (188). In mouse leukemic cells (L5178Y) in suspension, $tRNA^{lys}_4$ was a major species, accounting for as much as 40% of total $tRNA^{lys}$. The level of this species decreased progressively at higher cell densities until it was less than 20% of the total $tRNA^{lys}$ (188). It has also been reported that confluent cultures of F.L.C. contained a decreased level of $tRNA^{lys}_4$; however, when these cells were induced to differentiate by the addition of tetramethyl urea, a sharp increase in $tRNA^{lys}_4$ was seen (182).

Experimental results of the present study showed that the relative amounts of $tRNA^{lys}_4$ (as well as other isoacceptors) were similar to those previously reported (182). However, unlike the earlier report, $tRNA^{lys}$ isoacceptors at 36 hr have been analyzed as well as that of 0, 48, 72 and 96 hr after addition of DMSO. It has been found that a marked decrease in the relative amount of $tRNA^{lys}_4$ occurred between 36 and 48 hours after the addition of DMSO to the culture. The decreased amount of $tRNA^{lys}_4$, 48 hours after the induction, corresponds to an accumulation of globin mRNA, the beginning of globin polypeptide synthesis and to the reduced rate of F.L.C. division that occurs in the initial 48 hr of differentiation. Thus,

$tRNA^{lys}_4$ may have importance for cell differentiation and, or control of the rate of cell division in the 48 hr lag period.

It has been reported that avian and mammalian cells contain four isoaccepting $tRNA^{met}$ species (259). They are designated $tRNA^{met}_1$, $tRNA^{met}_2$, $tRNA^{met}_3$ and $tRNA^{met}_4$ in which $tRNA^{met}_1$ is the initiator tRNA of protein synthesis (260). The present study has resolved three $tRNA^{met}$ isoaccepting species (Figure 12) from F.L.C. corresponding to $tRNA^{met}_1$, $tRNA^{met}_3$ and $tRNA^{met}_4$ according to Elder and Smith (259). The failure of resolving $tRNA^{met}_2$ may be due to different chromatographic conditions since this species eluted very close to $tRNA^{met}_1$ in Elder's work. It is worth noting that the relative amount of $tRNA^{met}_1$ has a rather constant value during the time course of differentiation except for a dramatic decrease relative to other isoacceptors at 48 hours. This pattern was also observed for $tRNA^{lys}_4$. Further consideration of this coincidence should be investigated, since $tRNA^{met}_1$ is required for initiation of protein synthesis and $tRNA^{lys}_4$ is correlated with a cell's ability to divide. It is known that DMSO treated F.L.C. cultures show a 24-48 hour lag before logarithmic growth commences (147). It has also been shown that total protein and RNA synthesis in F.L.C. decreased in the first 48 hours after the addition of DMSO (13), and that globin polypeptide synthesis did not increase until 48 hours (253). Thus, relative low levels of initiator $tRNA^{met}$ may reflect this transition state of low protein synthesis between the undifferentiated and differentiated stages of the cells. It has been reported that the free 4S RNA of avian oncornaviruses are greatly enriched in $tRNA^{met}_4$ and avian myeloblastosis virus reverse transcriptase has been shown to bind specifically $tRNA^{met}_4$ and $tRNA^{trp}$ (261). It has been shown that when uninduced, logarithmically growing F.L.C. enter into a density-dependent stationary growth phase, there is an inhibition of virus release into the medium (176). On the other hand, when F.L.C. is exposed to the inducer DMSO, there is an initial large increase in virus released into the medium (262). This may explain the drastic increase of $\mathrm{tRNA}_4^{\mathrm{met}}$ at 48 hours while the $\mathrm{tRNA}_1^{\mathrm{met}}$ was decreased in relative amount.

F.L.C. had one major and three minor species of $\mathrm{tRNA}^{\mathrm{phe}}$ (Figure 8). The changes in these tRNAs were small; however, even small changes could reflect important events in differentiation. Recently, Mushinski and Marini (195) have reported that in a variety of normal rat and mouse tissues, only a single major isoaccepting $\mathrm{tRNA}^{\mathrm{phe}}$ was found (eluting analogously to $\mathrm{tRNA}_4^{\mathrm{phe}}$). However, an additional tumor-associated isoacceptor was found in a wide spectrum of histological types of solid tumors in rat and mouse. They strongly suggested that the two $\mathrm{tRNA}^{\mathrm{phe}}$ species had different structures and these structural differences resulted from different degrees of completion of post-transcriptional modification, most likely at the Y base. It also has been reported that the extra $\mathrm{tRNA}^{\mathrm{phe}}$ was present in the "minimal deviation" Morris hepatoma 5123; but was absent in the normal rat liver tissue (263). The two $\mathrm{tRNA}^{\mathrm{phe}}$ species in Morris hepatoma 5123 had the same binding affinities for the coding triplet UUC as did rat liver $\mathrm{tRNA}^{\mathrm{phe}}$ and E. coli $\mathrm{tRNA}^{\mathrm{phe}}$ (263). Ortwerth et al. (6) have shown that a $\mathrm{tRNA}^{\mathrm{phe}}$ species eluting earlier on RPC-5 chromatography than the major $\mathrm{tRNA}^{\mathrm{phe}}$ was unique to the lens and not present in other normal mammalian tissue. This novel $\mathrm{tRNA}^{\mathrm{phe}}$ was further shown to increase as a result of lens cell differentiation. Furthermore, in the same report, it was shown that the extra lens $\mathrm{tRNA}^{\mathrm{phe}}$ was almost absent in the epithelial cells but present in the fiber cells, suggesting that induction of a new lens $\mathrm{tRNA}^{\mathrm{phe}}$ occurred upon differentiation. The profiles of $\mathrm{tRNA}^{\mathrm{phe}}$ that we obtained showed a unique pattern in which $tRNA^{phe}_{1}$ was always present in very small amounts but that $tRNA^{phe}_{2}$ was not present until 36 hours after the addition of inducer and reached its maximal relative amount of 11% at 48 hours. A third minor species, designated 3, appeared at 72 hours after induction of F.L.C. Considering all the facts we have mentioned above, we would suggest that perhaps the minor species of $tRNA^{phe}$ are not only tumor-associated species but also are associated with differentiation and growth conditions of cells grown in suspension.

Garel (2) has found that the population of tRNAs for glycine, alanine, serine and tyrosine, which comprise 93% of amino acid residues in silkworm Bombyx mori fibroin, increase as much as 3-fold over that of other tRNAs with increasing fibroin production and thereby postulated a "functional adaptation of tRNA." Similar phenomena have been found in the calf lens for the biosynthesis of crystallins (5) as well as in the rabbit reticulocyte for the biosynthesis of hemoglobin (8; 264). Our isoacceptor profiles for differentiated F.L.C. (72 and 96 hr after addition of DMSO) approximate RPC-3 profiles of rabbit reticulocyte tRNAs (264). For instance, there is a correspondence in the presence of one major $tRNA^{ala}$ species eluting between minor species, five $tRNA^{ser}$ species of which three are in relatively large amounts, one major late-eluting $tRNA^{phe}$ species and three minor species, four $tRNA^{leu}$ isoacceptors, two major $tRNA^{ile}$ isoacceptors, predominance of the initiator species of $tRNA^{met}$ and two predominant $tRNA^{pro}$ species. Some of these tRNAs increase in relative amounts before or with the onset of globin synthesis by differentiating F.L.C. suggesting an early induction of the particular tRNAs necessary for translating the globin mRNAs.

Since alanine comprises approximately 15% of the amino acids residues in both mouse α and β globin, while serine makes up approximately 10% of amino acids residues in α globin and 6% in β globin, we might expect that certain isoaccepting species of these two tRNAs would increase in relative amounts during the erythrodifferentiation. Indeed, the minor $tRNA^{ala}$ isoaccepting species 1 and 2 have a 2-fold increase at 48 hours compared with $tRNA^{ala}$ from uninduced cells (Figure 18); while $tRNA^{ser}_{2}$, suspected as a precursor to $tRNA^{ser}_{4}$ (Figure 14), has an almost 2.5-fold increase from 36 to 48 hr. The nucleotide sequence of mouse β-globin mRNA has recently been reported (265). Three of the four alanine codons are used with preference for the codons ending in a pyrimidine (8, GCU codons; 9, GCC codons; and 1, GCA codon). Four of six serine codons are used with exclusive preference for those ending in a pyrimidine (3, UCU; 2, UCC; 1, AGU; and 3, AGC). Five of the six leucine codons are present in the β-globin mRNA sequence. These codons (2, UUG; 1, CUU; 4, CUC; 1, CUA; and 9, CUG) would most likely require four different isoaccepting species. Our results indicate that F.L.C. cultures producing hemoglobin express four isoacceptors of which two, $tRNA^{leu}_{2}$ and $tRNA^{leu}_{5}$ have greatly increased in relative amount during differentiation. These results strengthen the idea of the cell's functional adaptation of the tRNA population for protein synthesis.

Our experimental results show dramatic changes in $tRNA^{pro}$ (Figure 10) and $tRNA^{thr}$ (Figure 17) isoaccepting species during differentiation. $tRNA^{pro}_{2}$ and $tRNA^{pro}_{3}$ were major species while $tRNA^{pro}_{1}$ and $tRNA^{pro}_{4}$ were represented only in trace amounts in uninduced cells. However, during differentiation, $tRNA^{pro}_{2}$ and $tRNA^{pro}_{3}$ consistently decreased in their relative amounts to barely detectable levels; while $tRNA^{pro}_{1}$ and $tRNA^{pro}_{4}$ consistently increased to become the major species. Only two of the four possible proline codons are present in β-globin mRNA (2, CCU and 1, CCC). tRNA^{thr} consisted of four species in relatively equal amounts in uninduced cultures. $\text{tRNA}^{\text{thr}}_2$ and $\text{tRNA}^{\text{thr}}_3$ consistently decreased in relative amount throughout differentiation. At 96 hours they were almost non-existent. Of the four possible threonine codons only those ending in pyrimidine are present in β-globin mRNA (3, ACU and 3, ACC). These observations suggest F.L.C. switches expression of tRNA^{pro} and tRNA^{thr} isoacceptors in order to adapt to the specific protein synthesis needed in differentiation for hemoglobin production. This change of expression could be by way of post-transcriptional modification of tRNA^{pro} and tRNA^{thr} isoacceptors or by differential gene expression. It is worth noting that the tRNA^{pro} serves as a primer for the murine leukemia virus reverse transcriptase and is found in the virus in association with the viral genome (84; 85). Since there is a large increase in virus released into medium in DMSO-induced F.L.C. culture (262) increasing levels of $\text{tRNA}^{\text{pro}}_2$ and $\text{tRNA}^{\text{pro}}_3$ may reflect that one or both of these species are specified to be packaged for serving as reverse transcriptase primer.

Our results show that significant changes occurred in tRNA species for asparagine, aspartate, histidine, and tyrosine during differentiation of Friend leukemia cells. Transfer RNA^{asp} from F.L.C. has been resolved into 4 isoaccepting species in which $\text{tRNA}^{\text{asp}}_1$ and $\text{tRNA}^{\text{asp}}_3$ stayed rather constant in relative amount throughout the differentiation. However, $\text{tRNA}^{\text{asp}}_2$ and $\text{tRNA}^{\text{asp}}_4$ changed greatly (Figure 20, Table 3). Four tRNA^{asp} isoaccepting species were found to occur in SV40-infected BALB 3T3 cells (210). Cyanogen bromide treatment of the tRNA indicated that $\text{tRNA}^{\text{asp}}_1$ and $\text{tRNA}^{\text{asp}}_3$ contain the fully modified Q nucleoside while $\text{tRNA}^{\text{asp}}_2$ and $\text{tRNA}^{\text{asp}}_4$ were undermodified with respect to Q. Transfer RNA isolated from SV40-induced hamster tumors, BHK 21/Cl-B cells in culture, certain carcinogen-induced tumors, Ehrlich ascites tumor, and a number of human carcinomas and adenocarcinomas had a distinct increase in the relative amount of a late-eluting $tRNA^{asp}$ that lacked the Q modification in comparison to tRNA from non-malignant tissues and cells (215; 242).

Increase in the relative amounts of the late-eluting species is thought to be favored, though not obligated, by neoplasia, and has been found to be a consistent property of SV40- and polyoma-transformed cells in culture (266). Our results on F.L.C. $tRNA^{asp}_{3}$ and $tRNA^{asp}_{4}$ are consistent with most of these observations. $tRNA^{asp}_{3}$ was a late-eluting species continually occurring in large relative amounts and $tRNA^{asp}_{4}$ is suspected to lack the fully synthesized Q nucleoside.

Transfer RNA^{his} in our preparations from five stages of F.L.C. differentiation (0, 36, 48, 72 and 96 hr after addition of DMSO to cultures) was resolved into 4 isoaccepting species (Figure 21). The minor species, $tRNA^{his}_{1}$, and the major species, $tRNA^{his}_{3}$, particularly attracted our attention because of dramatic changes in their relative amounts during the course of differentiation (Table 3). It has been reported that $tRNA^{his}$ from both bovine muscle and lens tissue contained the same two isoaccepting species resolved by RPC-2 chromatography (200). Investigation of $tRNA^{his}$ from rat liver and Morris hepatoma 3924A also failed to show any differences with regard to the number of isoaccepting species resolved by RPC-2 chromatography. Rabbit reticulocytes were found to contain two isoaccepting species for $tRNA^{his}$ when that tRNA was investigated by various chromatographic methods including RPC-5 (267). The early eluting species contained Q; whereas the late eluting one did not (238; 267). Both isoaccepting $tRNA^{his}$ species were found to incorporate histidine into all of the histidine-containing positions of rabbit hemoglobin (267). Thus, the quantitative changes of $tRNA^{his}$ species 1, 3 and 4 that occurred during erythroid differentiation of F.L.C. may have a more subtle function than recognition of the two histidine codes (CAC and CAU). For instance, the rate of translation may be affected by the increased relative amount of $tRNA^{his}_3$.

The relative amounts of three of the four isoaccepting species of $tRNA^{tyr}$ from F.L.C. changed over the 96 hour differentiation period (Figure 22). Normal rat liver, Morris hepatoma 5123D and 3924A have been found to have three isoaccepting species of $tRNA^{tyr}$ (192). Little, if any, difference was found between the $tRNA^{tyr}$ of these tissues with respect to RPC-2 chromatographic profiles. However, a rather significant difference was found between the RPC-3 chromatographic distributions of $tRNA^{tyr}$ isoacceptors from human normal and chronic myeloid leukemia leukocytes (268). Comparative studies of $tRNA^{tyr}$ from normal human placenta and HeLa cells indicated that no significant differences existed in the rate or site of incorporation of tyrosine into tryptic peptides of α-globin, even though there was again a dramatic difference in the RPC-5 chromatographic profiles between the tRNAs of these two tissues (243). The observed changes in F.L.C. $tRNA^{tyr}$ isoacceptors 1, 2 and 3 were coincident with cell differentiation. It has been suggested in two reports that one of the $tRNA^{tyr}$ isoaccepting species may have a regulatory function concomitant with differentiation, in these cases with regard to pigment cell metabolism (94; 96). The amount of Q ribonucleoside relative to unmodified guanosine in $tRNA^{asn}$, $tRNA^{asp}$, $tRNA^{his}$, and $tRNA^{tyr}$ varies at different stages in the life cycle of Drosophila further suggesting that the presence of Q in tRNA is related to differentiation of cells (232).

Differences in amounts of tRNA isoaccepting species for asparagine, aspartate, histidine, and tyrosine consistent with differences in the extent of Q ribonucleoside modification have been observed in a number of mammalian systems (196; 198; 215; 243). The hypermodified Q ribonucleoside may be important in regulatory functions directed by eukaryotic tRNAs. The presence of Q, which is both positively charged and hydrophilic, in the exposed anticodon of a specific tRNA molecule suggests that the molecule could easily interact with enzymes in reactions associated with regulation of cell function (21; 22). Q or precursors to Q instead of G in the first or wobble position of the anticodon of otherwise identical tRNAs could affect either the rate of protein synthesis or the anticodon-codon recognition (244; 269).

An enzyme, purified from rabbit erythrocytes, catalyzes the replacement of guanine with queuine at the wobble position of (Q^-) tRNA (121; 237; 270). In the absence of its true substrate, quenine, this enzyme catalyzes an exchange between free guanine and the guanine in the wobble position (270). This exchange can be used to assay for (Q^-) tRNA (198; 238; 240; 241). Our results from guanylation of tRNA preparations from F.L.C. at five stages of differentiation (Table 4) indicated that tRNA from uninduced cells is highly modified with regard to the Q. The level of Q modification decreased at 36 hr and had its lowest level 48 hr after the addition of DMSO. After cells had been exposed to DMSO for 48 hr, the level of Q in tRNA increased consistently as the greater portion of the F.L.C. differentiated during the next 48 hours. Tumor cell tRNA contains mostly guanosine in the wobble position instead of Q (196; 197; 198; 213). However, these results were obtained from tRNA extracted from tumor cells propagated _in vivo_ or from tumor cell cultures that were not monitored for cell differentiation. Our results from guanylation of tRNA of F.L.C. undergoing a well-defined differentiation adds another dimension to these findings. The Q content of F.L.C. tRNA was not constant during differentiation. Growth characteristics and tumorigenicity also vary during F.L.C. differentiation. There is an inverse and exponential relationship between the degree of differentiation and the ability to clone F.L.C. In addition, differentiation of F.L.C. has been associated with a decline in its tumor-forming ability (271).

In order to examine more closely the tRNA species which may serve as substrates for guanylation, F.L.C. tRNAs isolated from different time points during the induced differentiation were guanylated and then fractionated by RPC-5 chromatography (Figure 23). The results showed that a minor group of guanylated tRNAs eluted early and a major group of guanylated tRNAs eluted late, approximately corresponding to the elution position of the internal standard $tRNA_5^{lys}$. Thus, it appears that the isoaccepting species of $tRNA^{asn}$, $tRNA^{asp}$, $tRNA^{his}$, and $tRNA^{tyr}$ capable of being modified for the synthesis of Q have similar retention times on RPC-5 chromatography under the condition used. The isoaccepting species of tRNAs that are fully modified with respect to Q are not detected by this method. Therefore, from the elution position of the major peak of guanylated tRNAs and the elution positions of the 17 tRNA species for the four amino acids, we suggest that $tRNA_4^{asn}$, $tRNA_5^{asn}$, $tRNA_4^{asp}$, $tRNA_3^{his}$, $tRNA_4^{his}$ and $tRNA_4^{tyr}$ contain guanosine in the wobble position. The fluctuations of $tRNA_4^{asn}$, $tRNA_4^{asp}$ and $tRNA_3^{his}$ corresponded to the level of guanylation in total tRNA at different time points during the differentiation of F.L.C.

It has been suggested that the degree of tRNA modification in tissue culture cells may be affected by cell density (215; 233). However, a recent report (234) has shown that cell density and/or proliferative state are not major variables in controlling the expression of Q and has therefore indicated that the previous results may be an artifact due to drastic changes of pH or other conditions in culture. Thus, we consider that changes of $tRNA^{asn}$, $tRNA^{asp}$, $tRNA^{his}$, and $tRNA^{tyr}$ isoaccepting species at various stages of F.L.C. differentiation were probably not due to changes in cell density, but may be due to cell differentiation.

Minor isoaccepting tRNA species appear transiently during differentiation of F.L.C. in nearly all the aminoacyl-tRNA chromatographic profiles. Since these minor species are only present during the differentiation process, they may be involved in regulating the transition between non-hemoglobin synthesizing and hemoglobin synthesizing stages of the cells. Sixty-four isoaccepting species of tRNA for sixteen amino acids have been resolved in this system. Therefore, it is rational to believe that more than 80 tRNA species are produced by F.L.C. for the 20 amino acids used in protein synthesis. The present study comparing isoaccepting tRNA species during the *in vitro* induced differentiation of a cloned cell line, provides additional information concerning possible roles for tRNA species in differentiation. Identification of tRNA isoacceptors that change during F.L.C. differentiation is an essential first step in using this easily controlled, homogeneous population of cells for studying the role of tRNA in cell differentiation.

VI. CONCLUSIONS AND PROSPECTUS

Various aspects of tRNA involvement in biological systems have been studied for the past 25 years. Different hypotheses with regard to regulatory roles for tRNA have been postulated. Studies on the distributions of isoaccepting tRNAs in different organisms and tissues as well as cells in culture have confronted such central questions as tRNA involvement in regulation of differentiation, growth, senescence, and malignancy. Much is known about tRNA population changes during viral infection of host cells. Alterations also have been described during embryogenesis and differentiation. Adaptation of tRNA populations to the biosynthesis of specific proteins has also been described. Special attention has been paid to tRNA changes in malignancy. Unfortunately, the shallow survey of many systems has produced results from which one can not draw a clear image of tRNA roles in regulation.

The present investigation has described the quantitative and qualitative alterations of tRNA isoaccepting species during Friend leukemia cell erythrodifferentiation. The changes in isoaccepting tRNA species have been considered in association with the known cellular metabolic transitions of F.L.C. during differentiation. The conclusions are outlined below:

1. Differences in isoaccepting species of tRNA occur throughout the DMSO-induced erythroid differentiation of Friend leukemia cells.

2. Most changes occur from 36 hr to 72 hr after the addition of DMSO, corresponding to globin mRNA appearance and hemoglobin synthesis, respectively.
3. The total amount of Q^- tRNA species in Q-base containing tRNA family ($tRNA^{asn}$, $tRNA^{asp}$, $tRNA^{his}$, and $tRNA^{tyr}$) increases in the first 48 hr after the induction, then decreases again, possibly indicating the level of Q-modification is correlated to the process of differentiation.
4. In most cases, predominant tRNA isoaccepting species are retained throughout the differentiation. However, some of the minor species appear and disappear, reflecting a possible functional adaptation of the tRNA population during differentiation.
5. A relatively low level of initiator $tRNA^{met}$ at 48 hr after the addition of DMSO, may indicate the adaptation of this particular tRNA species to the cellular transition between the de novo protein synthesis and globin chain production.
6. Consistently increasing relative amounts of two of four isoacceptors of $tRNA^{pro}$ and $tRNA^{thr}$ during differentiation may indicate that F.L.C. switches expression of $tRNA^{pro}$ and $tRNA^{thr}$ isoacceptors in order to efficiently translate the β-globin mRNA in which only two of four codons for both $tRNA^{pro}$ and $tRNA^{thr}$ have been utilized.

Much has been learned from this investigation. However, it is purely a descriptive study. Now that the mouse β-globin mRNA sequence is known (265), certain approaches would readily yield important information, such as: codon designation for isoacceptors and involvement of modified nucleoside in the recognition of anticodons; comparison of these codes of recognized tRNA isoacceptors to those used in globin mRNA.

Further information about the biological function of individual tRNAs also can be obtained from an mRNA-tRNA dependent *in vitro* translation system as well as microinjection of particular tRNA into thermosensitive mutant cells. Recently, Mulligan et al. (272) have reported the successful expression of rabbit β-globin mRNA and protein using an SV40 recombinant as a transducing vector to infect monkey kidney cells. Similar results have also been reported by Hamer and Leder (273). The possibility of constructing an SV40 transforming vector carrying the rabbit β-globin gene, and the possibility of infecting non-hematopoietic cells, could lead to a different approach for studying the functional adaptation of tRNA in hemoglobin synthesis.

Up to now most questions regarding the regulatory roles of tRNA in translation and transcription have remained unanswered, especially in eukaryotic systems. The present results and the future investigations proposed above may provide some answers to these questions, at least in the erythrodifferentiation systems.

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VITA

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