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CHANGES IN tRNA LEVELS DURING THE INDUCTION OF HEMOGLOBIN SYNTHESIS IN FRIEND LEUKEMIA CELLS

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SUMMARY -- There is a three- to four-fold decrease in the content/cell of tRNAs for ten different amino acids four days after the induction of erythroid differentiation in Friend leukemia cells, consistent with the decrease in cell volume that occurs. Surprisingly, there is an approximately two-fold increase in the cellular content of each of these tRNAs between day 4 and day 6 after induction, indicating the net synthesis of tRNA late in induction. The tRNA changes affect all species and do not result in tRNA specialization for hemoglobin synthesis, as occurs in normal erythroid development. The tRNA content of imidazole-treated cells, which do not synthesize hemoglobin although they undergo other changes of erythroid differentiation, decreases initially as described above, but shows no increase from day 4 to day 6.

The development of mature red blood cells from dividing, non-hemoglobinized precursors includes the appearance of a content of tRNAs accepting the different amino acids which parallels the amino acid composition of hemoglobin (1). Most strikingly, tRNA for histidine, an amino acid of unusual abundance in all mammalian hemoglobins, is especially abundant in reticulocytes compared to other cells, and tRNA for isoleucine, an amino acid which is absent or of infrequent occurrence in mammalian adult hemoglobins, is correspondingly scarce in reticulocytes. The finding that tRNA content is specialized, adapted, or modulated for hemoglobin synthesis was first made in our laboratory in the reticulocytes of rabbits (1), and similar findings have since been reported for tRNA in the reticulocytes of sheep (2) and humans (3). A resemblance of tRNA content to the amino acid composition of other proteins has been reported for cells synthesizing these proteins predominantly (4). We have presented evidence that the changes in tRNA content in red cell precursors occur proximate to or simultaneously with the onset of hemoglobin synthesis (5). Possibly tRNA changes are regulated in response to tRNA utilization in protein synthesis (5). The specialization of tRNA content for the synthesis of an unusual protein could

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facilitate the synthesis of that protein by increasing the availability of previously limiting tRNA species (6).

Friend leukemia (FL) cells treated with dimethylsulfoxide (DMSO) and a variety of other agents are induced to synthesize hemoglobin in substantial amounts (7), and it may be considered whether changes in the tRNA content similar to those in normal red cell development occur in induced FL cells. Toward this end we have used cell culture conditions in which terminally differentiated cells are maximally stabilized. There have been scattered reports of relative changes in tRNA turnover rate, isoacceptor abundance, and base modifications after the induction of hemoglobin synthesis (8-13); however, the absolute levels of tRNA for the different amino acids in molecules/cell throughout the period when hemoglobin synthesis occurs have not been investigated. Such a study is presented here. In addition, we have studied the tRNA content of induced cells treated with imidazole as well as DMSO which we have previously shown blocks hemoglobin synthesis at the level of globin mRNA accumulation (14). Other aspects of erythroid differentiation proceed unperturbed in the imidazole-treated cells, and we can use them to ask whether any changes in tRNA abundance which occur after induction (DMSO treatment) are linked to globin synthesis or whether they are an independent feature of erythroid differentiation.

MATERIALS AND METHODS

FL cells.

FL cells, Line 745-PC4, obtained from Dr. David Housman of Massachusetts Institute of Technology, were subcloned for cells with high inducibility and grown in alpha medium. The cells were kept under stabilizing conditions (iron-dextran and BSA) as described by Volloch and Housman (15), and were maintained at concentrations of 5 X 10^4 to 5 X 10^5 cells/ml throughout the experiments. The use of stabilizing conditions which prevent the lysis of well differentiated cells (15) in these experiments is discussed below. The cells were treated with 1.8% DMSO and, in some cases, with 500 ug/ml imidazole as previously described (14).

Preparation of tRNA from FL cells.

Uninduced cells were harvested in log growth. Cells treated with DMSO and cells treated with both DMSO and imidazole were harvested four and six days after induction. The cells were washed with serum free medium and tRNA was prepared as previously described by our laboratory (1,16). Two to 3 X 10^8 cells of each kind were used for the tRNA preparations. The preparative method, which includes the elution of tRNA from DEAE-cellulose, removes larger nucleic acid molecules.

Aminoacyl-tRNA synthetase preparations.

Cellular extracts containing a mixture of the aminoacyl-tRNA synthetases were prepared from rabbit reticulocytes as previously described (1).

Determination of molecules of different kinds of tRNA per cell.

tRNA for the different amino acids was quantitated on the basis of amino acid acceptance. The number of tRNA molecules was calculated from the specific activity of the labelled amino acids which could be esterified to limiting tRNA quantitatively under reaction conditions previously determined to be optimal (1). Multiple determinations of amino acid acceptance activity were made over a range of tRNA concentrations to assure that acceptance was proportional to tRNA amount. The number of tRNA molecules/cell was calculated from the numbers of cells from which the tRNA preparations were made.

RESULTS

Table 1 shows the molecules per cell of the different kinds of tRNA in variously treated FL cells. The data in Table 1 are summarized in graphic form in Figure 1. For each kind of tRNA examined there are fewer molecules by a factor of three to five in day four induced than in uninduced cells. This decrease approximately parallels the decrease in cell volume of FL cells after induction which may range up to ten-fold in cultures in which the terminally differentiated cells have been stabilized (14,15). Surprisingly the tRNA molecules accepting these amino acids approximately double between day 4 and day 6 in induced cells (see Figure 1a).

In induced cells which were treated with imidazole the number of molecules per cell of each species is virtually the same at day 4 as in cells treated

TABLE 1

tRNA MOLECULES/CELL IN UNINDUCED, INDUCED, AND IMIDAZOLE-TREATED FL CELLS

Kind of tRNA (Amino Acid Accepted)	Molecules of tRNA X 10^3/Cell				
	Uninduced Cells	Induced Cells Days in Culture		Induced Cells Treated with Imidazole Days in Culture	
		4	6	4	6
$tRNA^{Ala}$	1433	421	975	345	423
$tRNA^{Arg}$	1363	372	767	372	344
$tRNA^{Glu}$	607	114	391	208	213
$tRNA^{Gly}$	1226	286	732	371	368
$tRNA^{His}$	528	109	273	87	121
$tRNA^{Ile}$	571	142	322	202	196
$tRNA^{Lys}$	1215	372	633	440	304
$tRNA^{Phe}$	555	173	309	173	130
$tRNA^{Thr}$	1030	277	611	316	330
$tRNA^{Val}$	723	151	514	107	209

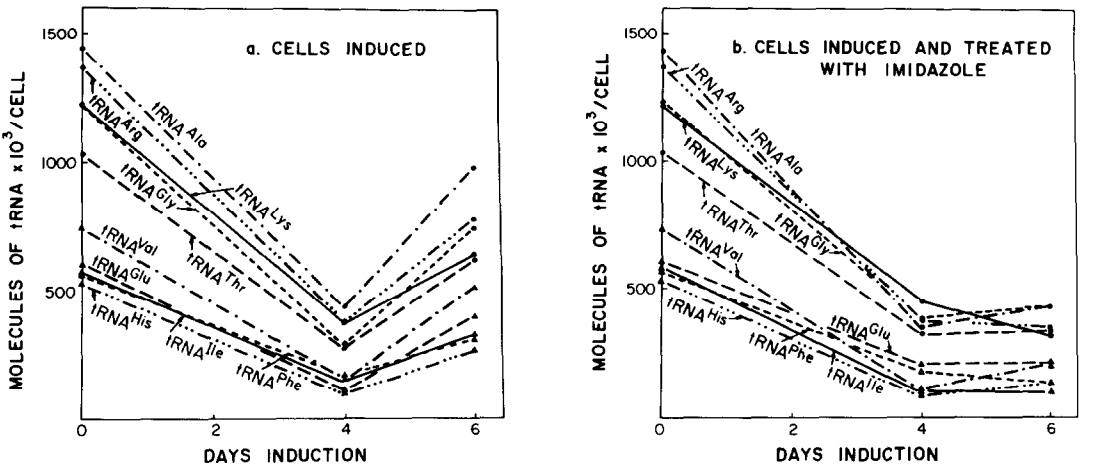

Figure 1: Cells were continuously treated for 4 or 6 days with DMSO (A) or DMSO and imidazole (B) as described in Materials and Methods. tRNA was prepared and content of several species was assayed and molecules per cell calculated as described in Materials and Methods. Values plotted here are those also shown in Table 1.

with DMSO alone. In contrast to the induced cells, however, the content of each of the tRNA species studied remains unchanged between day 4 and day 6 in induced cells treated with imidazole (see Figure 1b).

DISCUSSION

The present study was undertaken to determine if the tRNA specialization for hemoglobin synthesis, which is readily observed in red blood cell development, can also be observed in FL cells that have been induced to differentiate. We looked for tRNA changes 96 hours after induction at which time globin mRNA is at a high level and globin synthesis is well established (7). The only tRNA changes we observed at 4 days were the parallel decline of all tRNA species approximately in proportion to the decrease in size of stabilized FL cells at this point after induction (7,15). We followed the induced cells until 144 hours (6 days) because hemoglobin synthesis continues at an undiminished rate in these cells if they are maintained at 5 X 10^4 to 5 X 10^5 cells/ml and cultured under stabilizing conditions. An approximately two-fold increase was found in the cellular tRNA content for each species assayed during the period from 4 to 6 days, but the changes of tRNA specialization (1) were not observed. $tRNA^{His}$ increases, but $tRNA^{Ile}$ increases in parallel with it, as do the other kinds of tRNA. The regression of tRNA content on amino acid composition of

hemoglobin does not indicate the development of a significant relationship during the period 4 to 6 days after induction.

We used stabilizing conditions in these experiments to preserve as many well differentiated cells late in induction as possible. Our use of such cells should amplify the late changes of induction compared to studies with cultures which were not stabilized in which most well differentiated cells undergo lysis. The mean size of the well differentiated cells of stabilized cultures 5 days after induction is smaller by a factor of three or more compared to the size of cells in unstabilized cultures of the same age (15). While protein synthesis in unstabilized cells includes a maximum of 25% hemoglobin synthesis (7,11), 85% of the protein synthesis of stabilized cells is hemoglobin synthesis (15). Thus the stabilized cells are more like reticulocytes in which 90% of total protein synthesis is hemoglobin synthesis (17). Nevertheless specialization of tRNA content for the synthesis of this predominant protein was not seen.

The tRNA changes we observe after induction of FL cells must, therefore, be considered in some context other than specialization for hemoglobin synthesis. While both a functional role and a regulatory mechanism for these changes remain elusive, there are studies from other laboratories which support our surprising observation that there is net synthesis of tRNA late in induction. There are several reports that the relative amounts of hypomodified tRNAs increase late in induction. The hypomodified species $tRNA_4^{Lys}$ increases in amount from day 4 to day 5 after induction (13), and there is an increase in the hypomodified $tRNA^{His}$ which lacks Q base (queuine) (12). Since there is no evidence for the reversion of fully modified to hypomodified tRNA, the appearance of increased hypomodified molecules indicates new tRNA synthesis. In another study the amount of $tRNA_I^{Met}$ was found to increase relative to the amount of $tRNA_M^{Met}$ late in induction (18). The actual numbers of molecules of each tRNA species were not determined, and so it is possible that the change in the proportion of the two $tRNA^{Met}$ species was caused by the preferential loss of $tRNA_M^{Met}$, but the preferential synthesis of $tRNA_I^{Met}$ is a likely possibility. There is evidence cited (15) but unpublished that there is an in-

crease in the $tRNA^{His}$ content during an unspecified period in induction. The accumulation of hypomodified tRNA as seen in induced FL cells is also seen in rapidly dividing cells such as tumors and regenerating liver (19,20) where it may be a consequence of rapid tRNA synthesis which accompanies rapid cell division and which may outpace tRNA modification.

Taken together the above results from other laboratories indicate that there is the net synthesis of multiple tRNA species after the induction of FL cells, and they are consistent with the present study that shows that the content of tRNA molecules of several species increases between day 4 and day 6 after induction.

The tRNA content of induced cells treated with imidazole in addition to DMSO is in marked contrast to that of induced cells given no other treatment. In imidazole-treated induced cells the tRNA content does not increase in the period 4 to 6 days after induction but remains at the day 4 level for each kind of tRNA examined. While imidazole appears to uncouple globin synthesis from other changes in FL cell induction (14), its effect on tRNA content is not readily explained. If the tRNA changes seen in induced cells were related to tRNA specialization for globin synthesis, then it could be proposed that the tRNA changes were somehow coupled to tRNA utilization in protein synthesis, but there is no tRNA specialization. Since there is some turnover of tRNA in FL cells (9,13), the constant levels of several tRNA species in the imidazole-treated cells indicate that rates of tRNA syntesis and tRNA degradation are approximately balanced, in contrast to the induced cells treated with DMSO alone in which there is substantial net synthesis.

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