Vol. 95, No. 1, 1980
July 16, 1980

BIOCHEMICAL AND BIOPHYSICAL RESEARCH COMMUNICATIONS
Pages 468-473

THE tRNA CONTENT OF NON-HEMOGLOBINIZED RED CELL PRECURSORS: EVIDENCE THAT tRNA CONTENT IS CONTROLLED BY tRNA UTILIZATION

David W.E. Smith, Randall F. Randazzo and Anne L. McNamara

Department of Pathology, Northwestern University Medical School, Chicago, Illinois 60611

Received June 2,1980

SUMMARY — The content of tRNA's accepting several amino acids was determined for the non-hemoglobinized early precursors of rabbit red cells and was compared with the tRNA content of rabbit reticulocytes and liver. The specialization of the tRNA content for hemoglobin synthesis seen in reticulocytes was not seen in the earlier precursors, suggesting that tRNA specialization does not occur before the onset of hemoglobin synthesis. The results favor hypotheses that the tRNA content develops in response to tRNA utilization in translation.

Wide variation of cellular tRNA content is associated with irreversible processes like differentiation and neoplasia and with reversible responses such as to hormonal stimulation and nutritional conditions (1). In a few kinds of cells in which the synthesis of a single kind of protein predominates, the content of tRNA's for the different amino acids is proportional to the amino acid composition of the product (2). We have described this relationship in reticulocytes, the penultimate stage of red blood cell development, which synthesize protein more than 90% of which is hemoglobin (3). Moreover, the adaptation of tRNA isoacceptor abundance to the frequency of codon occurrence in the predominant mRNA being translated has been observed (4).

Histidine is abundant in hemoglobin compared to most proteins, and isoleucine is rare (absent in adult human hemoglobin), and these differences are reflected in the tRNA content of reticulocytes, both relative to tRNA's for the other amino acids, and compared to rabbit liver in which many kinds of proteins are synthesized. We have introduced the concept that the tRNA content of reticulocytes is specialized for hemoglobin synthesis (3).

Red blood cell development begins in the bone marrow with a population of rapidly dividing non-hemoglobinized precursors which, after three or four divi-

0006-291X/80/130468-06$01.00/0

sions and during a period of two to three days, are converted to anucleate red cells which circulate and contain hemoglobin as their predominant protein (5). The reticulocytes of phenylhydrazine treated rabbits synthesize 25,000-50,000 molecules of hemoglobin/min (6).

The present study considers whether the appearance of the specialized tRNA content precedes its use in the synthesis of hemoglobin. The tRNA content of reticulocytes was compared to that of non-hemoglobinized red cell precursors to determine whether it has the characteristics of specialization. It does not, thus providing evidence that the regulation of the tRNA content is on the basis of tRNA utilization in hemoglobin synthesis.

MATERIALS AND METHODS

Preparation of non-hemoglobinized red cell precursors from rabbit bone marrow. Rabbits were injected with phenylhydrazine for five days (6.25 mg/kg/day) and were sacrificed on the eighth day. Femoral bone marrow was suspended, filtered through gauze, and washed. Phenylhydrazine treatment results in a considerable enrichment of red cell precursors. At the time of sacrifice the myeloid:erythroid ratio was 0.1-0.2 in contrast to untreated rabbits in which the ratio is greater than 1.0 (7). As a second step, suspensions of marrow cells (50-80 x 10^6 cells/ml) were treated with equal volumes of goat antirabbit red cell IgG (Cappel Laboratories, lot 5336, 17 mg protein/ml) diluted 1:8 and guinea pig complement diluted 1:3 (8,9). Incubation was at 37° for 60 min, following which the cells were collected and washed. After immune lysis a mixture of cells remains which consists almost entirely of non-hemoglobinized red cell precursors and granulocytes in various stages of development in a proportion of about 2:3 respectively.

The final step was the resolution of these cells on linear gradients from 10-18% Metrizamide (Nyegard and Co., Oslo) in the Beckman SW 25.2 rotor. The gradients were constructed over 2 ml cushions of 35% Metrizamide (10), and 5-10 x 10^6 cells surviving the immune lysis step were layered on each gradient. Centrifugation was for 60 min at 60,000 x g. Fractions were collected, and small aliquots were sedimented onto microscope slides for identification. Fractions enriched for non-hemoglobinized red cell precursors were pooled. The cells were removed from Metrizamide, resuspended in physiological saline, and frozen at -85° within eight hours after sacrifice. The cells of nine rabbits were used for the study reported here.

Characterization of protein synthesis by the cells. Fresh cells resulting from the above preparative procedures were incubated according to the method of Lingrel and Borsook (6) except that amino acids were 0.8 mM each including [^{14}C]histidine (New England Nuclear). After 30 min incubation at 37° the cells were washed and frozen, and the resulting lysate was centrifuged at 16,000 x g for 20 min. Aliquots of the supernatant were taken for electrophoresis on 5% polyacrylamide gels in 0.1% SDS by the method of Maizel (11). Carrier globin was added to a total of 100-200 µg protein/gel. The gels were stained, cut into 1 cm segments, digested with 30% H_2O_2, and counted to determine the percentage of amino acid incorporated into globin subunits.

Preparation and assay of tRNA. tRNA was prepared from the pooled cells by a method previously described (3). tRNA was assayed on the basis of acceptance

of labelled amino acids (3,12), with glutamic acid acceptance being assayed at 3.75 mM $MgCl_2$, 5.0 mM ATP, and 0.25 mM EDTA. Results were calculated as pmoles of tRNA for the amino acid/absorbance unit of the tRNA preparations assayed (amino acid acceptance activity/A_{260}). For each value, 10-15 determinations of acceptance were made using different amounts of tRNA. Results of the determinations agreed within $\pm$ 10%. Acceptance was proportional to tRNA added over a range to at least three times the label associated with blank (no tRNA) assays. The activity of the reticulocyte aminoacyl-tRNA synthetase preparation used was not inhibited by the tRNA preparation from the non-hemoglobinized red cell precursors.

RESULTS

Characteristics of the pooled cells. The upper fractions of the Metrizamide gradients were enriched for non-hemoglobinized red cell precursors. The above procedures yielded a total of 49.6 x 10^6 cells of which 57% were non-hemoglobinized red cell precursors morphologically recognizable as proerythroblasts and basophilic erythroblasts. Although the cells actively synthesize protein, little if any of it is hemoglobin. Less than 6% of the incorporated amino acid migrated with globin subunits in SDS-polyacrylamide gels.

tRNA of the pooled cells. 1.2 absorbance units (A_{260}) of material with an A_{260}/A_{280} ratio of 1.87 were prepared from the cells. The limited quantity of the tRNA preparation necessitated some choice as to which tRNA's should be assayed. The specialized tRNA's for histidine and isoleucine were assayed. tRNA's for many amino acids in reticulocytes fall into a group described as unspecialized because their abundance does not differ much in reticulocytes and liver (3). Hemoglobin is not unusual in its content of these amino acids compared to other proteins. tRNA's for glutamic acid and lysine were selected from this group of unspecialized tRNA's.

The results are shown in Table 1. Values are also included for the tRNA's in liver and reticulocytes based on the means of many determinations of these tRNA's in 10-15 preparations each from liver and reticulocytes. The values do not differ from those we have published (3).

The sum of acceptance of the four amino acids studied per absorbance unit (A_{260}) of the tRNA from the non-hemoglobinized red cell precursor was 40% that for the sum of the same four tRNA's in preparations from liver and reticulocytes. The tRNA preparation from the pooled precursor cells contains material with

Table 1. tRNA content of non-hemoglobinized red cell precursors, reticulocytes, and liver. Assays of tRNA calculated as pmoles of amino acid acceptance/absorbance unit (A_{260}) are shown for four kinds of tRNA in three kinds of cells and tissues. The percentage of each tRNA per total tRNA for the four amino acids assayed is also shown.

tRNA	Non-hemoglobinized red cell precursors		Reticulocytes		Liver	
	pmoles/A_{260}	%	pmoles/A_{260}	%	pmoles/A_{260}	%
$tRNA^{his}$	4.6	8	35	25	11	8
$tRNA^{ile}$	14.5	27	14	10	31	23
$tRNA^{glu}$	14.4	26	33	23	35	25
$tRNA^{lys}$	21.1	39	60	42	61	44

the spectral properties of nucleic acids which does not accept the amino acids studied. Therefore, the results are also given in Table 1 as the percentage of each of the tRNA's compared to the sum of all four tRNA's.

Several conclusions are apparent. The relative abundance of $tRNA^{his}$ and $tRNA^{ile}$ of early red cell precursors is not at all like that of reticulocytes but is like that of liver. In fact, the ratio of these two tRNA's in earlier precursors is the reciprocal of the ratio in reticulocytes. The features of a specialized tRNA content related to hemoglobin synthesis are not seen in the early red cell precursors. In contrast, the relative abundance of the two non-specialized tRNA's is the same in all three kinds of cells.

DISCUSSION

tRNA specialization for hemoglobin synthesis does not occur in red cell development prior to the onset of hemoglobin synthesis. Our results indicate that tRNA specialization is concurrent with or after the onset of hemoglobin synthesis, and thus favor the hypothesis that tRNA specialization is a consequence of tRNA utilization in translation. There is evidence from other laboratories that conditions reducing the level of free tRNA or of free aminoacyl-tRNA in the cytosol are associated with increases in the total amounts of those tRNA's. It is suggested that tRNA species heavily used in translation would be preferentially attached to ribosomes and depleted in the cytosol (13-17).

The number of tRNA molecules of each kind per non-hemoglobinized red cell precursor can be calculated from the data and has been compared with the tRNA content per reticulocyte as previously published (18). If it is assumed that the tRNA of the precursors is evenly distributed in the three cell divisions (5) that occur between it and the reticulocyte, there must be a two-fold net increase of $tRNA^{his}$ to achieve the level of this kind of tRNA found in reticulocytes. There is a 75% net decrease of the $tRNA^{ile}$ of the non-hemoglobinized precursors. There is also a net loss of $tRNA^{glu}$ and $tRNA^{lys}$, but it is less than of $tRNA^{ile}$. There are results from another laboratory which show the preferential degradation of tRNA molecules not used in translation (17). We suggest that the regulation of tRNA content may result from both specific biosynthesis and specific degradation based on tRNA function.

Our cell preparation, enriched for early red cell precursors constitutes the least certain aspect of our study. The desired cells outnumbered granulocyte precursors and mature granulocytes by less than 2:1, and thus many undesired cells were extracted for the tRNA preparation. Were the results of the comparison of early red cell precursors and reticulocytes not so strikingly different with respect to the specialized tRNA's, we would be more hesitant about attributing the differences to the red cell precursors present.

Our results may have implications for the hypothesis of tRNA limited translation of hemoglobin which we have presented in detail (19), and which seems tenable in reticulocytes (20). Even if tRNA utilization and content are coordinated at some point in red cell development, tRNA limited hemoglobin synthesis could still occur if tRNA content is regulated at such a level that tRNA is restricted in its availability. Alternatively, tRNA could be limiting at some stage of red cell development if the regulation of the tRNA content occurs at a different time than the synthesis of other elements required for hemoglobin translation.

tRNA seems to have a role in regulating its own abundance based on feedback, although the mechanisms by which tRNA levels in the cytosol affect tRNA

degradation (17) and the transcription of selected tRNA genes in the nucleus remain unknown. In prokaryotic cells an effect of tRNA on transcription is well recognized, not as it regulates its own synthesis, but in the role of amino-acyl-tRNA in repressing the synthesis of the biosynthetic enzymes of the respective amino acids (21-24). Whatever may be the mechanism of tRNA interaction with the transcriptional apparatus in eukaryotic cells, evidence showing that tRNA utilization and tRNA content are related indicates that the interaction must occur.

ACKNOWLEDGEMENTS — This investigation was supported by NIH grant AM 19617. The authors acknowledge the work of J. Michael Dean, M.D. on the early development of cell fractionation methods.

REFERENCES:

1. Sueoka, N., and Kano-Sueoka, T. (1970) Prog. Nucleic Acid Res. Mol. Biol. 10, 23-55.
2. Garel, J-P. (1974) J. Theor. Biol. 43, 211-225.
3. Smith, D.W.E., and McNamara, A.L. (1971) Science 171, 577-579 erratum, 1040.
4. Chavancy, G., Chevallier, A., Fournier, A., and Garel, J-P. (1979) Biochimie 61, 71-78.
5. Stohlman, F. (1970) Regulation of Hematopoeisis I, pp. 317-326, Appleton-Century-Crofts, New York.
6. Lingrel, J.B., and Borsook, H. (1963) Biochemistry 2, 309-314.
7. Peterson, E.A., and Evans, W.H. (1967) Nature 214, 824-825.
8. Borsook, H., Ratner, K., and Tattrie, B. (1969) Nature 221, 1261-1262.
9. Cantor, L.N., Morris, A.J., Marks, P.A., and Rifkind, R.A. (1972) Proc. Natl. Acad. Sci. U.S.A. 69, 1337-1341.
10. Lodish, H.F., and Small, B. (1976) Cell 7, 59-65.
11. Maizel, J.V. (1969) Fundamental Techniques in Virology, pp. 334-362, Academic Press, New York.
12. Kane, S.M., Vugrincic, C., Finbloom, D.S., and Smith, D.W.E. (1978) Biochemistry 17, 1509-1514.
13. Hamilton, T.A., and Litt, M. (1976) Biochim. Biophys. Acta 435, 362-375.
14. Westerberg, U-B, Bolcsfoldi, G., and Eliasson, E. (1976) Biochim. Biophys. Acta 447, 203-213.
15. Litt, M., and Weiser, K. (1978) Science 201, 527-529.
16. Weiser, K., and Litt, M. (1979) Eur. J. Biochem. 93, 295-300.
17. Litt, M., and Howell-Litt, R. (1980) J. Biol. Chem. 255, 375-378.
18. Smith, D.W.E., and McNamara, A.L. (1972) Biochim. Biophys. Acat 269, 67-77.
19. Smith, D.W.E. (1975) Science 190, 529-535.
20. Bergmann, J.E., and Lodish, H.F. (1979) J. Biol. Chem. 254, 11927-11937.
21. Turnbough, C.L., Neill, R.J., Landsberg, R., and Ames, B.N. (1979) J. Biol. Chem. 254, 5111-5119.
22. Yanofsky, C., and Lee, F. (1977) Proc. Natl. Acad. Sci. U.S.A. 74, 4365-4369.
23. Eisenberg, S.P., Yarus, M., and Soll, L. (1979) J. Mol. Biol. 135, 111-126.
24. Vold, B.S., Lazar, J.M., and Gray, A.M. (1979) J. Biol. Chem. 254, 7362-7367.