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Different Frequency Classes of Sequences in Heterogeneous Nuclear RNA of Normal Promyelocytes and Lymphoblasts and of Leukemic Blast Cells of Circulating Blood and of the HL60 Line

U. Torelli, G. Torelli, F. Narni, A. Donelli, S. Ferrari, G. Franchini, and B. Calabretta

A number of studies have led to the conclusion that each differentiated cell nucleus includes not only all of the genes ever utilized in the organism but also transcripts of most of these genes. A direct implication is that both the quantitative and qualitative structure of cytoplasmic messenger RNA populations are controlled posttranscriptionally. The control process would function by determining the fraction from 0 to 100% of the potential mRNA precursors from each gene that survive and that are processed and transferred to the cytoplasm (Davidson et al. 1977). Only for those few mRNAs which are represented at very high concentration in the cell, such as the globin mRNA in reticulocytes, has evidence been presented that regulation may occur at the transcription level (Tobin et al. 1978).

By studying the kinetics of DNA-RNA hybridization RNA sequence classes with different abundance may be detected in hnRNA, provided that critical conditions are maintained (Melli et al. 1971; Vogelstein and Gillespie 1977). We have determined the relative representation of sequence classes with different abundance in the heterogeneous nuclear RNA of two types of normal cells, i.e; lymphoblasts and promyelocytes, and three types of acute leukemia cells, i.e., acute lymphocytic leukemia (ALL), acute myelocytic leukemia (AML) blast cells from circulating blood, and HL60 promyelocytic cells.

A. Materials and Methods

Normal lymphoblasts were obtained by stimulating circulating blood lymphocytes with PHA for 48 h. Normal promyelocytes were obtained by fractionating normal bone marrow cells on albumin gradients. ALL and AML blast cells were obtained from the circulating blood of untreated patients. HL60 cells were studied in the logarithmic phase of growth. RNA was labeled by incubating the cells for at least 3 h with ^{3}H-uridine and extracted several times with phenol and chlorophorm-isoamyl alcohol. After sedimentation in a preparative sucrose gradient, fractions corresponding to S values >50 were pooled and used in the hybridization reaction with human normal DNA. The reaction was carried out in 70% formamide and the DNA/RNA ratio was at least 5×10^3. DNA was sheared at an average length of fragments of 700 nucleotides.

B. Results

The results of two hybridization experiments, one with hnRNA from normal promyelocytes and one with hnRNA from HL60 cells, are given in Fig. 1. The values plotted as solid lines are derived from a least squares computer analysis. The results of this analysis carried out in all the experiments performed with both normal and leukemic cells are given in Table 1. They indicate the presence in the hnRNA of normal cells of three kinetic classes of sequences, whereas in the hnRNA of leukemic cells the analysis shows only two second order components, roughly corresponding, as far as the rate of hybridization is concerned, to the first and second class of sequences revealed by the analysis in the hnRNA of normal cells.

C. Discussion and Conclusions

The results of our experiments show that the hnRNA of normal hemopoietic cells includes a class of sequences with very low abundance which is absent in leukemic cells. In fact,

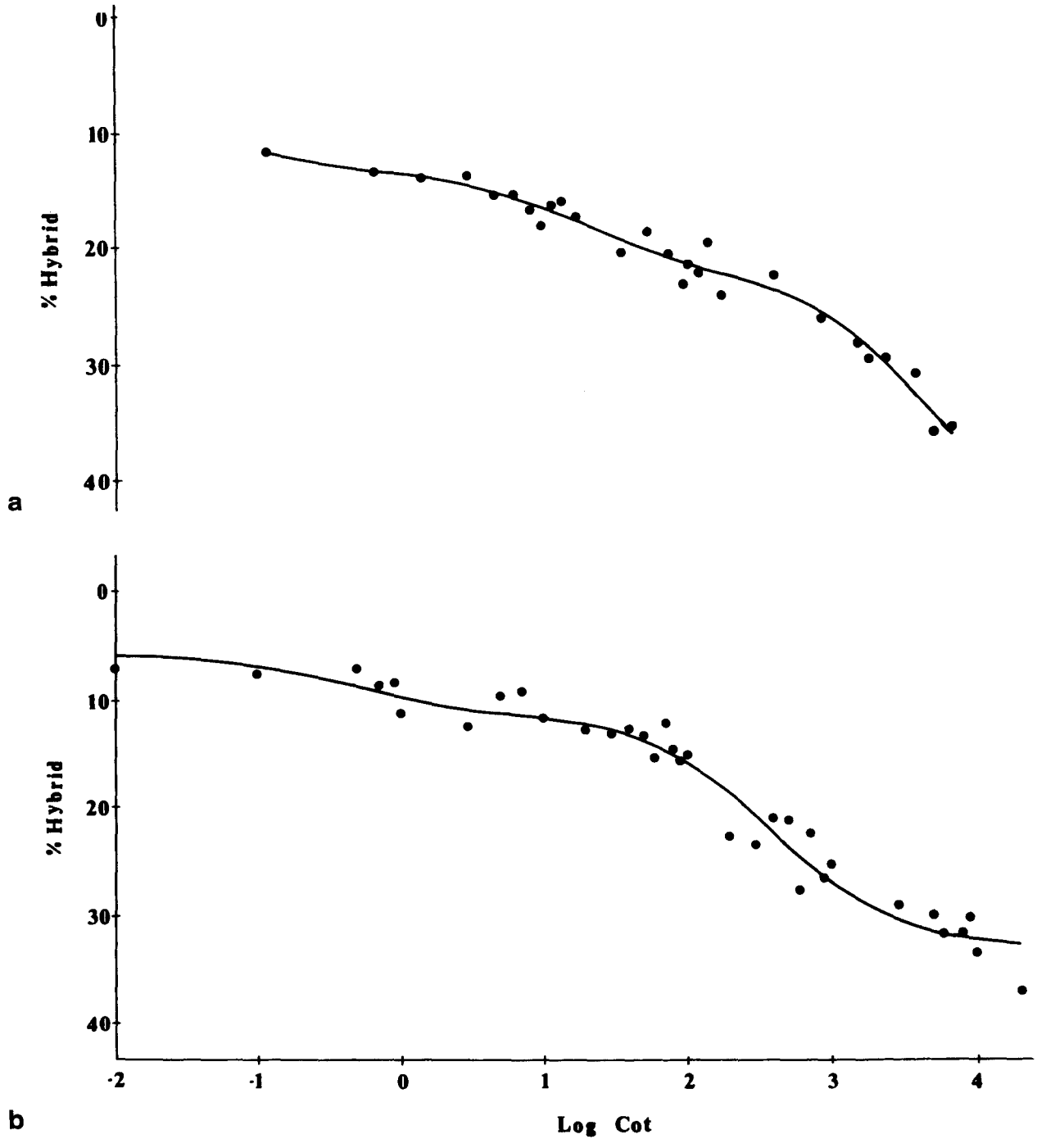

Fig. 1. Kinetics of hybridization to human DNA of hnRNA isolated from normal human promyelocytes **(a)** and HL60 leukemic cells **(b)**. The hnRNA fractions >50S were obtained as described in the text. The length of DNA fragments was 700 nucleotides in both the experiments and the DNA/RNA ratio was 5×10^3

although the comparison with DNA reassociation values (Torelli et al. 1979) is only approximate, the main component in hnRNA of normal cells is formed by sequences appearing probably at levels of one or a very few copies per cell. The computer analysis does not show the presence of this component in the early transcript of the leukemic cells. The largest kinetic class of sequences of the hnRNA of these cells is formed by sequences more frequent than that forming the main kinetic class in hnRNA of normal cells. The main difficulty in making a conclusion is the failure to achieve complete hybridization of hnRNA. This failure has been already observed by several authors (Melli et al. 1971; Kline et al. 1974; Spradling et al. 1974) who have reported maximal proportions of hybridization of

Table 1. Kinetic parameters derived from the least squares computer analysis applied to the hybridization values obtained with hnRNA from normal and leukemic cells

Cell Type	First component			Second component			Third component		
	%	K	$Cot_{1/2}$	%	K	$Cot_{1/2}$	%	K	$Cot_{1/2}$
Normal promyelocytes	7.3	3.66125	0.273	9.4	0.00992	100	26.8	0.00004	24870
Normal promyelocytes	5.7	1.97528	0.534	11.2	0.00759	120	29.5	0.00004	25125
Normal lymphoblasts	5.1	0.60000	1.666	9.7	0.00473	211	28.8	0.00004	23192
Normal lymphoblasts	4.4	0.76000	1.403	14.0	0.00863	180	29.6	0.00006	17808
ALL	4.6	3.55457	0.280	29.0	0.00317	315			
AML 1	10.7	0.59314	1.68	30.7	0.00211	474			
AML 2	10.6	0.52518	1.90	38.9	0.00206	484			
HL60	5.9	0.43278	2.30	25.2	0.00180	554			
HL60	5.3	2.09849	0.47	24.6	0.00261	382			

hnRNA from different cell types ranging from 40% to 60%. Whereas some of them (Kline et al. 1974) assume that the hnRNA which fails to hybridize belongs exclusively to the low frequency class of sequences, other authors (Spradling et al. 1974) believe that the incomplete hybridization of RNA in vast DNA excess may affect all classes of sequences equally. Whatever interpretation finally proves to be correct, our results indicate that at least part of the hnRNA of leukemic cells is characterized by a sequence frequency higher than that of hnRNA of normal cells.

Acknowledgments

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