Expression of Immunoglobulin and Globin Genes in B and T Lymphocytes and Other Cells†

Ursula Storb,* Lisa Hager, Ronald Wilson, and David Putnam

ABSTRACT: T lymphocytes contain RNA molecules which resemble immunoglobulin κ-chain mRNA (κ-RNA) (Storb, U., Hager, L., Putnam, D., Buck, L., Farin, F., and Clagett, J. (1976), *Proc. Natl. Acad. Sci. U.S.A. 73*, 2467). In order to determine the significance of this finding we analyzed in the present study the quantities of two immunoglobulin RNAs, κ- and λ-chain RNA, and of a functionally unrelated RNA, β-globin RNA in both B and T lymphoid cells and nonlymphoid cells. The following findings were made. (1) Thymus and T lymphocyte lines contain κ-RNA. (2) The quantity of κ-RNA in B lymphocytes increases with maturation. Myeloma tumor cells have up to 1000 times as much κ-RNA as the most immature B cells. A clear maturation schedule is not evident in T cells. Thymus T cells have about 160 times as much κ-RNA as the least active T cells. B cells generally contain more κ-RNA than T cells. (3) Thymus and T lymphoma cells have κ-RNA in the cytoplasm, suggesting that this RNA may function as message. (4) Spleen L-chain RNA contains 10–30% λ-RNA. (5) Two λ myelomas (MOPC-104E and S178A) contain, besides λ-RNA, also large amounts of κ-RNA. A variant, S178-V, which has ceased the synthesis of λ-RNA, continues to produce κ-RNA. (6) The sequence of the T and B cell κ-RNA is apparently the same in the untranslated 3′ end and a portion of the c region. This suggests that T and B cells may express the same set of structural genes for immunoglobulins. (7) κ-RNA is also present in the nuclei, but not the cytoplasm of a mastocytoma and a leukemia cell line. λ-RNA is absent from these cells. (8) κ-RNA is not found in a monocytoid cell line and is also probably absent from liver and brain cells. (9) β-globin RNA is found in the nucleus and the cytoplasm of all lymphoid and nonlymphoid cells studied. It appears thus that the expression of β-globin, κ-, and λ-RNAs is regulated to different degrees; β-globin RNA may be expressed and transported into the cytoplasm in most malignant cells and cytoplasmic κ- and λ-RNAs are restricted to lymphoid cells.

It has been found in many cell systems that the repertoire of poly(adenylic acid) [poly(A)] containing RNA molecules, i.e., presumably mRNAs, in different cells is quite similar (Axel et al., 1976; Young et al., 1976; Hastie and Bishop, 1976). Generally at least 80% of the cDNAs made from the RNA templates of one cell type can hybridize with RNA sequences of other cell types. We have found that this is also true for the poly(A) containing RNAs of B and T lymphocytes with respect to each other and to kidney RNA (U. Storb, unpublished results). Furthermore, Humphries et al. (1976) have shown that globin RNA is present in nonerythroid cells. It was thus possible that all eukaryotic cells might transcribe most or all mRNAs and that cellular specificity was determined at some posttranscriptional level.

We have studied this question in lymphoid and other cells. We had previously found that T lymphocytes, in which it was difficult to demonstrate immunoglobulin synthesis unequivocally (Vitetta et al., 1972; Grey et al., 1972; Marchalonis, 1975; Eichmann and Rajewsky, 1975; Binz and Wigzell, 1976; Cone and Brown, 1976), contain Ig κ-chain mRNA-like molecules (Storb et al., 1976). This finding would be of questionable significance for the role of immunoglobulins in T cells if all cells expressed essentially all RNAs.

The present study is a quantitative analysis of κ-RNA, λ-RNA, and β-globin RNA in a variety of normal and tumorous B and T cells and other cells. We also wanted to determine whether the quantities of κ-RNA in B cells varied relative to the quantities of κ-chain protein found at different stages of B cell maturation or whether the synthesis of κ-chain protein was mainly controlled on the translational level. We were furthermore interested in the levels of κ-RNA in different types of T cells which had been analyzed with respect to membrane markers such as Ly antigens.

Experimental Procedures

Preparation of cDNA. Purified mouse κ-chain mRNA was prepared from MOPC-41 myeloma tumors, λ-chain mRNA from S178A myeloma tumors, and purified β-globin mRNA was prepared from reticulocytes of Swiss mice (R. Wilson, D. Putnam, and U. Storb, submitted for publication). The mRNAs appeared as a single band in polyacrylamide gel electrophoresis in 99% formamide (R. Wilson et al., submitted for publication). In the rabbit reticulocyte cell-free translation system κ-mRNA stimulated the synthesis of only one protein of 24 700 daltons which was immunoprecipitable by a monospecific anti-κ antiserum (R. Near and U. Storb, unpublished results). λ-mRNA and β-globin mRNA coded for single proteins in the rabbit reticulocyte and wheat embryo cell-free translation systems, respectively. The λ-chain and globin migrated in sodium dodecyl sulfate–polyacrylamide electrophoresis as proteins of 24 000 and 16 000 daltons, respectively.

cDNA was prepared from κ-chain, λ-chain, and β-globin mRNA using reverse transcriptase RNA-dependent DNA nucleotidyltransferase of avian myeloblastosis virus (Verma et al., 1972). The reaction mixtures contained 1 mM each TTP, dATP, and dGTP, 400 μCi/mL [^{3}H]dCTP at 25 Ci/mmol, 10 μg/mL oligo(dT), 20–80 μg/mL mRNA, 1000 units/mL reverse transcriptase, 50 mM Tris-HCl (pH 8.3), 10 mM dithiothreitol, 6 mM magnesium acetate, 60 mM NaCl, and 20 μg/mL actinomycin D. After incubation at 37 °C for 90 min KOH was added to 0.2 N. After further incubation at 37 °C

† From the Department of Microbiology and Immunology, University of Washington, Seattle, Washington 98195. *Received June 3, 1977.* This work was supported by National Institutes of Health Research Grants AI-10685 and DE-02600. R.W. is supported by Public Health Service Fellowship No. GM 07187.

for 10 min the mixture was passed over Sephadex G.75 in 1 × SSC[1] (1.5 M NaCl–0.015 M sodium citrate), sodium dodecyl sulfate was added to 0.5%, and the mixture was extracted at room temperature with 1:1 phenol–chloroform. The supernatant was precipitated with ethanol with *Escherichia coli* RNA as carrier.

To purify κ- or λ-cDNA 0.2–0.3 μg of cDNA was hybridized with 2–4 μg of the respective mRNA in 0.3–0.4 mL of 2 × SSC at 67 °C to a C_rt[1] of 10^{-2}. Nuclease buffer (30 mM sodium acetate, 0.6 mM $ZnSO_4$, and 20 mM NaCl, pH 4.5) was added to 2 to 2.5 mL. After addition of 375 units/mL of S_1 nuclease (Sigma) and 30 μg/mL of ribosomal RNA as substrate, the mixture was incubated at 37 °C for 1 h. After extraction with phenol–chloroform (1:1) in 0.5% sodium dodecyl sulfate the material was passed through a G-75 Sephadex column in 1 × SSC. RNA was then destroyed by boiling in 0.2 N KOH for 10 min. The mixture was neutralized and the cDNA was ethanol precipitated.

The size of the cDNA probes was determined by polyacrylamide gel electrophoresis with λ phage DNA cut by *Hin*II and -III restriction enzymes as size markers (Maniatis et al., 1975). The genetic content of the cDNA probes was determined based on physical size (Milstein et al., 1974). Two κ-cDNA probes were used; a short probe had 68% of the radioactivity in sequences corresponding to the untranslated 3′ end of κ-mRNA; the rest of the radioactivity was mainly in sequences which corresponded to no more than 50% of the constant (c) region of κ chains. A longer κ-cDNA probe had 50% of the total radioactivity in sequences containing a c-region component, or 26% of the total radioactivity was present in c-region sequences, including complete c regions. Transcripts of κ variable (v) region mRNA were apparently insignificant because the cDNAs hybridized completely with purified κ-mRNA of two subclones (MOPC-21 and P3κ) of another myeloma tumor, which have a v_κ region which is very different from MOPC-41 (McKean et al., 1973), the template for these κ-cDNAs.

The λ-cDNA had 86% of its radioactivity corresponding to the 3′ untranslated region (assuming that the structure of λ-mRNA is similar to κ-mRNA). The β-globin cDNA had 50% of its radioactivity in molecules of 320 nucleotides length or longer.

Preparation of RNAs from Normal and Neoplastic Mouse Tissues. Membrane-bound ribosomes and cytoplasm were prepared from tissues and RNA was extracted from these or whole cells as described (Storb, 1972, 1973). Nuclei and cytoplasm from tissue culture cells were prepared by treatment with 0.5% Triton X-100. To prepare undegraded RNA from thymus and spleen the tissues were homogenized in the presence of guanidine hydrochloride and RNA was extracted as described by Zsindeley et al. (1970). RNA preparations were tested for residual DNA content by the diphenylamine reaction and were found to contain 0–2% DNA. The percentage of B and T cells in spleens and thymuses was determined by immunofluorescence from single cell suspensions prepared before RNA extraction (Storb et al., 1976; Clagett et al., 1973). The mouse T cell tumors BALENTL-5, LUT-14, and P1798, the mouse B cell tumors ABPL-4 and ABLS-5, and the mouse myelomas MCPC-774 and MOPC-41 were obtained from Dr. M. Potter. The mouse myeloma S178A, the mouse B cell tumor PU5.1, and the mouse T cell tumor EL.4 were obtained from the Salk Institute. The mouse myelomas 66.2 and NP-2, variants of MPC-11, were obtained from Dr. M. Scharff. Some of the tumors were adapted to growth in tissue culture in Dulbecco's modified Eagle's medium, 10% horse serum, 5% CO_2 (BALENTL-5, EL 4, and ABPL-4), or RPMI, 10% fetal calf serum, 5% CO_2 (LUT-14).

cDNA·RNA Excess Hybridization and Thermal Denaturation of Hybrids. These tests were performed as described (Storb et al., 1976). cDNA and an excess of RNA were incubated long enough to achieve maximal hybridization before the hybrids were melted as described and analyzed by S_1 nuclease digestion at 37 °C (Storb et al., 1976).

Determination of Cellular RNA Content. The quantity of total and nuclear RNA was determined by the method of Schmidt–Tannhauser as described by Hutchison and Munro (1961) relative to cellular DNA. There are 6 pg of DNA per cell (Vendrely and Vendrely, 1949); therefore, the quantities of RNA in whole cells, nucleus, and cytoplasm can be calculated. We only determined the RNA content of MOPC-104E myeloma and P815 mastocytoma cells, which were similar, and used an average of these determinations for all cell types (31.4 pg/cell, 28.6 pg/cytoplasm, 2.8 pg/nucleus). This appears justified, since most of the cells studied were lymphoid-plasmacytic of similar size and nuclear/cytoplasmic ratios. However, the values applied are probably an underestimate for liver and brain which are larger and have a smaller nucleus/cytoplasm ratio.

The number of κ-, λ-, or β-globin RNA molecules per cell, cytoplasm, or nucleus was calculated using the formula: number of κ- (λ- or β-globin) RNA molecules = [Avogadro's number/molecular weight of κ (λ- or β-globin) mRNA)] × (quantity of RNA in cell compartment) × (fraction of RNA complementary to κ- (λ- or β-globin) cDNA (Humphries et al., 1976). The molecular weight of mouse β-globin mRNA is 190 000 (Williamson et al., 1971). The molecular weights of mouse κ- and λ-mRNA were determined to be 398 000 by electrophoresis in polyacrylamide gels in 99% formamide with 18S, 5S, and 4S RNAs as standards (R. Wilson et al., submitted for publication). The fraction of RNA complementary to κ- (λ- or β-globin) cDNA was determined as the ratio of the $C_rt/2$ of hybrids of κ- (λ- or β-globin) cDNA with its respective mRNA over the $C_rt/2$ of hybrids of the cDNA with whole cell RNA or nuclear or cytoplasmic RNA, respectively.

Results

The cDNA Probes. Figure 1 shows the hybridization kinetics of κ-cDNA with κ-mRNA and λ-mRNA, and β-globin cDNA with β-globin mRNA. Curve κ-1 was obtained with cDNA directly transcribed from κ-mRNA. It shows biphasic hybridization with only 55% of the cDNA probe reacting rapidly. The rest of the cDNA probe begins to hybridize only above a C_rt of 5×10^{-2}, indicating that it represents transcripts from RNA species which are present in the κ-mRNA at approximately 1% or less. In order to obtain a more specific probe, we have purified the κ-cDNA in the following way: κ-cDNA and pure κ-mRNA were hybridized to $C_rt = 10^{-2}$, the hybrids were treated with S_1 nuclease (Vogt, 1973) and phenol extracted, RNA was destroyed by alkali hydrolysis, and the remaining cDNA was ethanol precipitated. Curve κ-2 of Figure 1 shows the hybridization kinetics of this purified κ-cDNA. Over 90% of the probe now corresponds to the major RNA species in the κ-mRNA preparation. The $C_rt/2$ is 1.4×10^{-3}, which is approximately twice the $C_rt/2$ of β-globin cDNA/mRNA (7.5×10^{-4}) in agreement with the relative complexities of the mRNAs (approximately 600 nucleotides for β-globin mRNA

[1] Abbreviations used are: C_rt, concentration of RNA in hybridization reaction × the time of incubation, expressed as moles of nucleotide × seconds per liter; $C_rt/2$, C_rt at which the hybridization reaction has proceeded to one-half completion; SSC, 0.15 M NaCl–0.015 M sodium citrate.

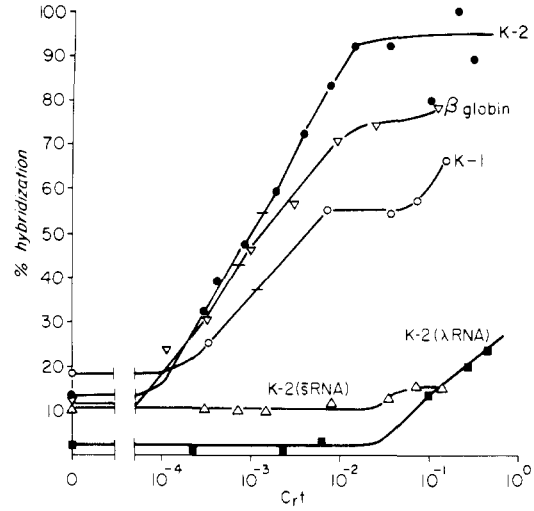

FIGURE 1: Hybridization of κ-chain and β-globin cDNAs with excess mRNAs: κ-1, unpurified κ-cDNA with κ-mRNA; κ-2, purified κ-cDNA with κ-mRNA; κ-2 (s RNA), purified κ-cDNA without RNA; κ-2 (λ-RNA), purified κ-cDNA with λ-mRNA; β-globin, β-globin cDNA with β-globin mRNA. Baseline S_1 nuclease resistance is indicated at C_rt = 0.

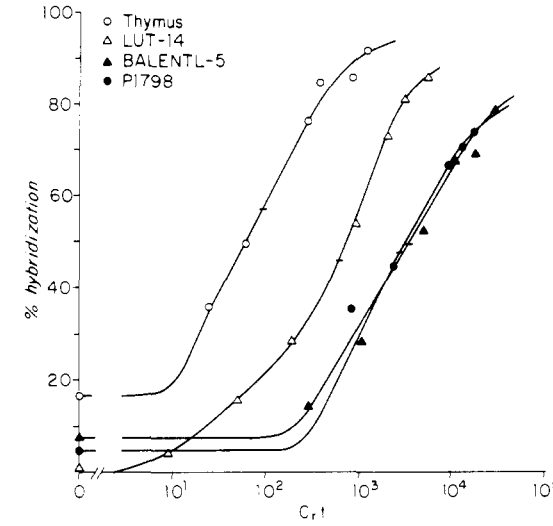

FIGURE 2: Hybridization of purified κ-cDNA with T cell RNAs. These data are included in Table I: (○) Swiss thymus whole cell RNA; (△) LUT-14 tissue culture cytoplasmic RNA; (▲) BALENTL-5 tumor whole cell RNA; (●) P 1798 tumor whole cell RNA

and 1200 for κ-mRNA). The purified κ-cDNA begins to react with λ-mRNA only at a C_rt of 10^{-1}. This indicates that κ-mRNA and λ-mRNA do not share closely related sequences in the untranslated 3′ end. Apparently the λ-mRNA is contaminated with approximately 0.1% κ-mRNA, not surprising since κ-RNA has been found in S178A myelomas, the source of the λ-mRNA (see Discussion). The $C_rt/2$ of λ-mRNA was 2.1×10^{-3}. Only κ- and λ-cDNAs which had been purified by hybridization to their respective mRNAs were used in this study. The β-globin cDNA was used without further purification since it reacted originally to 75%.

Quantities of κ-, λ-, and β-Globin RNA in Lymphoid and Other Cells. Table I shows the relative quantities of κ-, λ-, and β-globin RNA in various B and T lymphocytes as well as other cell types. The data are expressed as $C_rt/2$ or number of molecules per cell, or in some cases per nucleus or per cytoplasm. κ-producing myelomas contain 20 000 to 133 000 molecules of κ-RNA per cell. The most active myeloma, MOPC-41, has approximately 0.3% of its total RNA nucleotides in κ-RNA (ratio of $C_rt/2$ of MOPC-41 total RNA/κ-mRNA), and twice as much in its membrane-bound ribosomes, the site of Ig synthesis in myeloma cells (Blobel and Dobberstein, 1975).

Both λ-producing myelomas tested contain relatively large quantities of κ-RNA besides λ-RNA: the range of κ-RNA concentration is 1/20 to 1/3 that of λ-RNA in these tumors. The κ-myeloma MOPC-41 on the other hand does not contain an appreciable amount of λ-RNA. A spontaneously arising λ-myeloma variant (S178A-V), which had ceased to synthesize λ chains, is deficient in λ-RNA compared to the parent tumor. On the other hand, this variant tumor continues to synthesize κ-RNA even in increased quantity compared with the parent tumor.

B lymphomas have considerably less κ-RNA than myelomas. Furthermore, among the B lymphomas κ-RNA does appear to increase with differentiation: ABPL-4, which was described as a plasmocytic lymphosarcoma (Sibinovic et al., 1976) and is the most mature lymphoma we have studied, contains 7 times as much κ-RNA as ABLS-5, considered an immature lymphoblastoid tumor (Premkumar and Potter, 1975). A B cell line of intermediate state of differentiation, the lymphocytoid line PU 5.1 with κ chains in the cell membrane (Green et al., 1973) contained an intermediate level of κ-RNA. A negative control cell line is PU 5.1-8, a monocytoid subclone, which lacks κ-RNA (Table I).

T lymphomas as a group contain less κ-RNA than B lymphomas (Table I and Figure 2). It is difficult to relate quantities of κ-RNA to the differentiation stage of the T lymphomas investigated. Probably the most immature cell line studied is P 1798 (Ly 1, 2, 3^+) (Sibinovic et al., 1976). P 1798 has approximately the same quantity of κ-RNA as the more differentiated BALENTL-5 (Ly 2, 3^+) (Sibinovic et al., 1976) which in turn has one-fifth the κ-RNA compared with the cytoplasm of LUT-14, which has the same Ly phenotype (Figure 2 and Table I). The fact that κ-RNA was found in tissue culture lines of T lymphomas indicates that the RNA is a product of T cells and not of B cells which may infiltrate tumors in vivo. It appears possible that the κ-RNA is translated in T cells since it was present in the cytoplasm of LUT-14 and EL-4 cells.

We found that several cell lines of both the B and T lymphoma type lost or considerably decreased the quantity of κ-RNA upon prolonged culture, in analogy with the S 178A λ-producing variant. This includes ABPL-4, LUT-14 (Table I), and two more plasmacytoid B lymphomas, ABPL-1 and ABPL-2, which do not appear in Table I.

Normal B and T cells in spleen and thymus were generally found to contain more κ-RNA than the lymphoid tumor lines (Table I). The number of κ-RNA molecules in normal spleens ranged between 1127 and 8750 per average cell depending probably on the immunization state of the mice and the quantity of B cells and plasma cells. The spleens with the lowest number of κ-RNA molecules were obtained from 4-week old mice. The other spleens were from older mice. Spleens of "nude" mice had a slightly increased level of B cells and plasma cells and contained significantly more κ-RNA than normal spleens.

Thymuses contained approximately as much κ-RNA as young spleens (see also Storb et al., 1976). The κ sequence is present in the cytoplasm of thymus cells.

Spleens contained significant quantities of λ-RNA (Table I). The λ-RNA was 10 and 27% of the total L-chain RNA in Balb and Swiss spleens, respectively, which is higher than the

TABLE I: Hybridization of κ-, λ-, and β-Globin cDNA with B and T Cell and Other RNAs.

Source of RNA	$C_rt/2$ κ-cDNA	$C_rt/2$ λ-cDNA	$C_rt/2$ β-Globin cDNA	Approximate no. of RNA molecules per cell, cytoplasm, or nucleus[n] κ	λ	β-Globin
κ-mRNA	1.4×10^{-3}					
λ-mRNA		2.1×10^{-3}				
β-Globin mRNA			7.5×10^{-4}			
κ myelomas						
MOPC-41, whole cell	0.5	≫3000[a]	25	133 000	≤2	2 985
microsomal	0.25		cyto 90			cyto 755
MOPC-774	1			66 500		
66.2	2.5			26 600		
NP_2	3.3			20 150		
λ myelomas						
MOPC-104E	4	1.3		16 650	51 154	
MOPC-104E T.C. nuclei			~850			~8
S 178A	20	1.1		3 330	60 454	
S 178A-V[b]	2.6	≫5000		25 385	≤1	
B lymphomas						
ABPL-4, mouse	72			920		
T.C.	~10 000[c]		~11 000	~7		~7
PU 5.1, mouse whole cell	300			220		
mouse cyto	300			201		
T.C. whole cell	2 000			33		
ABLS-5 Mouse	480			139		
Monocytoid cell line						
PU 5.1-8[d] T.C.	≫7 000			≤1		
T lymphomas						
LUT-14 T.C. I[e] cyto	600			100		
T.C. II[e] nuclei	≫1 600			≤1		
T.C. II cyto	≫6 000			≤1		
P 1798 Mouse	2 800		270	24		274
BALENTL-5, mouse	3 400			20		
T.C. whole cell	~4 500			~15		
EL 4, T.C. nuclei	~2 000			~3		
T.C. cyto	~10 000			~7		
T.C. whole cell			~4 500			~17
Spleens						
Swiss[g]	59[f]	160	1	1 127	416	74 600
Balb[h]	7.6			8 750		
Balb	9.5	86		7000	773	
Nude[i]	2.9			22 931		
Thymi						
Swiss[k]	65[f]			1 023		
Swiss cyto	120			500		
Balb[l]	90		100	739		746
Balb + Swiss[k,l]		~10 000			~7	
Balb nuclei	100			64		
Balb cyto	150			400		
Other cell types						
Liver, nuclei	1 000			6		
Brain, whole cell	~10 000			~7		
P815 mastocytoma T.C.						
I[m] Whole cell	900	≫2 000		74	≤3	
Nuclei	76			84		
Cytoplasm	≫1 200		700	≤5		100
II[m] Nuclei			400			18
Cyto			4 000			19
L 1210 leukemia T.C.						
I[m] Whole cell	750	≫10 000	~20 000	89	≤1	~4
II[m] Nuclei	130			49		
Cyto	~15 000		~12 000	~4		~6

[a] $C_rt/2$, ≫, hybridization reaction has been carried up to the indicated C_rt without obtaining any significant hybrids. [b] S178A-V is a spontaneous variant which no longer synthesizes λ chains and λ mRNA. [c] $C_rt/2$, ~, hybridization reaction has not been carried beyond 40%; the $C_rt/2$ value is an estimate based on the assumption that the reaction would be completed within 2 log units. [d] PU 5.1-8 is a subclone of PU 5.1 (obtained from Dr. P. Ralph) which grows like a monocytoid cell. [e] T.C. I, II, two independently carried tissue culture lines of LUT14. [f] Data published previously (Storb et al., 1976). [g-l] Distribution of B and T cells in spleens and thymus was determined by immunofluorescence (Clagett et al., 1973; Storb et al., 1976). [g] 31% T, 42% B. [h] 23% T, 51% B, 1-2% plasma cells. [i] <1% T, 53% B, 3-5% plasma cells. [k] 99.8% T, 0.2% B, no plasma cells. [l] T. not determined, 0.5% B, no plasma cells. [m] I and II were cultures harvested at 2-month interval. [n] See Experimental Procedures for the determination of RNA molecules per cell, etc. The κ-cDNA used for the studies presented in this table was the "short" probe (see Experimental Procedures).

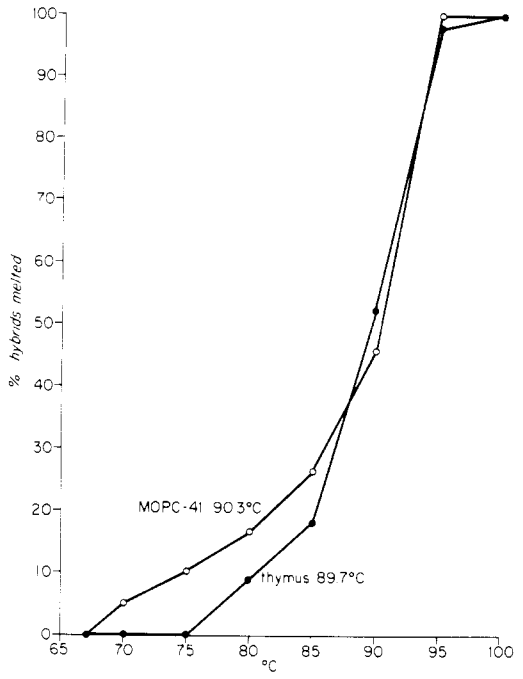

FIGURE 3: Thermal stability of hybrids between κ-cDNA and T and B cell RNA: (●) Whole cell poly(A) RNA of Swiss thymus; (○) whole cell RNA of MOPC-41 myeloma. The κ-cDNA used was the longer probe containing partial and complete transcripts of the c region of κ-mRNA (see Experimental Procedures).

relative amount of λ chains in mouse serum (Nisonoff et al., 1975). On the other hand, thymuses contained only a small quantity of λ-RNA, i.e., no more than 0.8% of the total thymic L-chain RNA was λ.

The levels of κ-RNA in liver and brain were very low and probably resulted from contamination by lymphocytes from the blood circulation (Table I). On the other hand, tissue culture lines of a mastocytoma and a leukemia contained considerable quantities of κ-RNA which in these established cell cultures cannot have resulted from lymphocyte infiltration. In both cases the κ-RNA was restricted to the nucleus. The small quantity of κ-RNA in the cytoplasm of L1210 cells relative to the nucleus may be derived from nuclear lysis. These same cells did not contain λ-RNA. They did, however, contain β-globin RNA which, in contrast to the κ-RNA, was present in similar quantities in nucleus and cytoplasm.

Several other tissues as well as cell culture lines were found to contain β-globin RNA without exception.

Thermal Stability of κ-RNA Hybrids. We had reported earlier that hybrids of κ-cDNA with thymus and spleen RNA were mismatched (Storb et al., 1976; Storb, 1976). This result appears now to have been an artefact. It was possibly due to hybrids formed with very short RNA molecules which arise during our conventional RNA preparation procedure using thymuses and spleens. This RNA was probably further reduced in size during a hybridization reaction period of several days at 67 °C. Such long reaction times were necessary for complete hybridization with κ-cDNA, since thymuses and spleens contain relatively little κ-mRNA. When spleen and thymus RNAs are prepared in the presence of guanidine hydrochloride (Zsindeley et al., 1970) undegraded RNA is obtained. Hybrids between undegraded total spleen or thymus RNA or total thymus poly(A) containing RNA and κ-cDNA melted at approximately 90 °C. The T_m is therefore not significantly different from that of MOPC-41 myeloma RNA–κ-cDNA hybrids (Figure 3). The same results were obtained with the short (mainly untranslated region) and with the longer κ-cDNA (26% of the total radioactivity corresponds to the c region). This suggests that the untranslated 3′ end and most of the c region of κ-RNA are the same in B and T cells. However, a small difference of 2–3% cannot be ruled out.

Discussion

The κ-cDNA and λ-cDNA probes used in this study have been purified by preparative hybridization and seem to be relatively pure as judged by comparing the hybridization kinetics with a β-globin standard (Figure 1). A possible source of contaminants in Ig mRNA and cDNA would be sequences of A-type particles which are present abundantly in the rough endoplasmic reticulum of mouse myeloma tumors (Robertson et al., 1975). These may be the sequences which represent minute contaminants of the mRNAs which are, however, copied by reverse transcriptase with very high efficiency to give rise to the impurities in the crude cDNAs. Apparently such sequences were absent from the purified κ-cDNA and λ-cDNA because they did not react with cytoplasmic RNA of P815 mastocytoma cells which contain A-type particles in the cytoplasm (Table I). It is expected that P815 and myeloma A particle RNAs would cross-react since all A particles isolated from several different mouse tumor lines had the same RNA sequence (Lueders et al., 1977).

The present data support our previous finding that T cells contain κ-RNA (Storb et al., 1976). All T cell lines and thymuses tested contained κ-RNA. The level of κ-RNA in the thymus was probably not due to the small proportion of contaminating B cells (Storb et al., 1976). Recent data by Szenberg et al. (1977) support the conclusion that most T cells in the thymus produce κ chains.

With respect to the T lymphomas there probably occurs some infiltration by circulating lymphocytes when the tumors are carried in vivo. Three of the T cell lines tested were analyzed as tissue culture lines (LUT-14, EL-4, and BAL-ENTL-5). Since all three contained κ-RNA, contaminating normal lymphocytes are unlikely to be the source of the RNA sequence in this case. The levels of κ sequences in some of the T cell lines are very low. They are real, however, and not due to the contamination of the RNAs by DNA. The levels of DNA contamination were 0 to 2%; about 50% DNA would be required to achieve hyhybridization of a unique immunoglobulin gene with a $C_rt/2$ of 10 000 (Bishop et al., 1972; Harrison et al., 1972). It has not been determined why the malignant cell lines possess smaller quantities of κ-RNA than the thymus. It may be due to the particular tumors selected. Analysis of other T cell lines and different normal T cells will be necessary. Our quantitation of κ-RNA in thymus and EL-4 does not correlate with Boylston's (1973) study of κ-chain protein. He reported that El-4 and thymus contained the same quantity of membrane-bound κ chains.

Apparently the 3′ half of κ-RNA, including a portion of the c region, is the same in B and T cells (Figure 3). This suggests that both cell types may express the same set of structural genes for κ chains. This will have to be substantiated by a comparison of v_κ (variable region) sequences in B and T cells.

There seems to be a clear correlation in B cells with respect to the relationship between the stage of differentiation and quantity of κ-RNA. Myeloma cells contain approximately 100 times more Ig than the lymphoblastoid line ABLS-5 (Premkumar and Potter, 1975). The present study shows a very similar relationship of their respective quantities of κ-RNA (Table I). ABPL-4, a plasmocytoid line which appears to be on the way to differentiation into mature plasma cells, has an intermediate level. Apparently the quantities of Ig synthesized

are mainly regulated by the levels of specific RNA rather than by translational controls.

Such a correlation is at present not obvious for T cells. The levels of κ-RNA vary by a factor of 159 between thymus and the least active T lymphoma, E1 4 (Table I). The thymus consists of a mixture of immature cortical and mature medullary thymocytes. BALENTL-5, which is Ly $2,3^+$, would be a more differentiated T cell than P 1798 (Ly $1,2,3^+$) (Huber et al., 1976a), but both cells contain approximately the same quantity of κ-RNA. Possibly the quantities of κ-RNA differ in T cells of different functional pathways; a Ly$2,3^+$ cell could be either a killer T cell precursor, a mature killer T cell, or a suppressor T cell (Huber et al., 1976b). The quantitation of κ-RNA adds another dimension to the functional analysis of T cells. It is worth noting that P 1798 T cells were found to produce approximately one-third as much Ig as ABLS-5 B cells (Premkumar and Potter, 1975). This correlates reasonably well with their relative quantities of κ-RNA (Table I).

An unexpected finding was the presence of κ-RNA in two λ-producing tumors, MOPC-104E and S 178A. The same observation has recently been reported by Ono et al. (1977) with MOPC-104E. Stavnezer et al. (1974) observed κ-RNA in another λ myeloma (λ5830). The κ-RNA is unlikely to be derived from infiltrating host cells, because the levels of contribution by circulating cells are 100 to 1000 times lower (Storb et al., 1976). We have found that the κ-RNA of 104E cells is translatable into κ chains in a cell-free system (D. Putnam and U. Storb, unpublished results) and that it is the same size in formamide gel electrophoresis as the λ-chain mRNA of 104E tumors (R. Near and U. Storb, unpublished results). Ono et al. (1977) have also found that κ-mRNA is coisolated with λ-mRNA from 104E cells. Apparently this is not the case with S 178A cells, the source of our λ-mRNA: Figure 1 shows that the contamination of our S178A λ-mRNA by κ-RNA is less than 0.1%. We have not determined where κ-RNA is localized in the S 178A cells. We do not know yet whether these λ tumors are a mixture of κ- and λ-producing cells, or whether the same cells produce both L chains. It is interesting in this context that a variant of S 178A which has ceased to synthesize λ chains continues to produce κ chains (Table I). Further experiments will be necessary to check the long-held postulate that one cell produces one class of L chain and one class of H chain. Hybridization with cDNA probes provides a sensitive tool for such studies.

Besides in lymphoid cells κ-RNA was also found in a mastocytoma and a leukemia cell line which lacks criteria of B or T cells. In both cases the RNA was restricted to the nucleus. It appears that these cells lack the transcriptional control for this specific RNA but that the sequence is not processed for cytoplasmic transport. λ-RNA transcription, however, does not seem to be present in these cells. On the other hand, all cells tested contained β-globin RNA sequences, an observation which confirms the results of Humphries et al. (1976). The positive mouse tissues were obviously all contaminated by blood cells, including reticulocytes. We do not, therefore, know whether normal spleen and thymus lymphocytes produce β-globin RNA. Globin RNA was, however, also found in tissue culture lines where it cannot have resulted from blood contamination. Thus, all transformed lymphoid and other cells tested produced globin RNA. This is in apparent conflict with the results of Groudine and Weintraub (1975) who found that normal chicken fibroblasts lack globin sequences. In our study all the tissue culture cells studied were malignant cell lines; it is possible that malignant cells may be derepressed for certain genes not expressed in the normal parent cell.

The three RNAs tested here are apparently regulated in different ways. β-Globin RNA may be transcribed and transported into the cytoplasm in all malignant cells (see also Humphries et al., 1976). κ-RNA and λRNA are synthesized and proceed into the cytoplasm in B and T lymphocytes and plasma cells. κ-RNA was absent in a monocytoid cell line (PU5.1-8) which like lymphoid cells originates from the bone marrow and is probably absent from liver and brain cells. Where κ-RNA was found in nonlymphoid cells (P815 and L1210) it was restricted to the nucleus.

The fact that κ-RNA was found in the cytoplasm of T cells but of no nonlymphoid cells suggests that it may be a true messenger in these cells. We have also found that thymus RNA can be translated into κ chains in a cell-free system (Putnam et al., 1977). However, the question of immunoglobulins as T cell receptors eventually will have to be answered on the cellular functional level.

Acknowledgments

We are grateful for many tumor lines to Dr. M. Potter and for reverse transcriptase to Drs. J. Beard and M. Chirigos, the Viral Cancer Program, National Cancer Institute. We thank Dr. James Clagett for the immunofluorescence analysis of thymus and spleen cells.

References

Axel, R., Feigelson, P., and Schutz, G. (1976), *Cell 1,* 247.
Binz, H., and Wigzell, H. (1976), *Scand. J. Immunol. 5,* 559.
Bishop, J. O., Pemberton, R., and Baglioni, C. (1972), *Nature (London), New Biol. 135,* 231.
Blobel, G., and Dobberstein, B. (1975), *J. Cell Biol. 56,* 835.
Boylston, A. W. (1973), *Immunology 24,* 851.
Clagett, J., Peter, H. H., Feldman, J. D., and Weigle, W. O. (1973), *J. Immunol. 110,* 1085.
Cone, R. E., and Brown, W. C. (1976), *Immunochemistry 13,* 571.
Eichmann, K., and Rajewsky, K. (1975), *Eur. J. Immunol. 5,* 661.
Green, I., Shevach, E. M., Stobo, J., Frank, M., and Herberman, R. (1973), *Adv. Exp. Med. Biol. 29,* 491.
Grey, H. M., Kubo, R. T., and Cerrotini, J. C. (1972), *J. Exp. Med. 136,* 1323.
Groudine, M. and Weintraub, H. (1975), *Proc. Natl. Acad. Sci. U.S.A. 72,* 4464.
Harrison, P. R., Hill, A., Birnie, G. C., and Paul, J. (1972), *Nature (London) 239,* 219.
Huber, B., Cantor, H., Shen, F. W., and Boyse, E. A. (1976a), *J. Exp. Med. 144,* 1128.
Huber, B., Devinsky, O., Gershon, R. K., and Cantor, H. (1976b), *J. Exp. Med. 143,* 1536.
Humphries, S., Windass, J., and Williamson, R. (1976), *Cell 7,* 267.
Hutchison, E. C., and Munro, S. N. (1961), *Analyst 86,* 768.
Lueders, K. K., Segal, S., and Kuff, E. L. (1977), *Cell 11,* 83.
Maniatis, T., Jeffrey, A., and van de Sande, H. (1975), *Biochemistry 14,* 3787.
Marchalonis, J. J. (1975), *Science 190,* 20.
McKean, D., Potter, M., and Hood, L. (1973), *Biochemistry 12,* 760.
Milstein, C., Brownlee, G. G., Cartwright, E. M., Jarvis, J. M., and Proudfoot, N. J. (1974), *Nature (London) 252,* 354.
Nisonoff, A., Hopper, J. E., and Spring, S. B. (1975), in The

Antibody Molecule, New York, N.Y., Academic Press.
Ono, M., Kawakami, M., Kataoka, T., and Honjo, T. (1977), *Biochem. Biophys. Res. Commun. 74,* 796.
Premkumar, E., and Potter, M. (1975), *Cell 6,* 149.
Putnam, D., Storb, U., and Clagett, J. (1977), Proceedings of the ICN-UCLA Symposium on Regulation of the Immune System (in press).
Robertson, D. L., Baenziger, N. L., Dobbertin, D. C., and Tach, R. E. (1975), *J. Virol. 15,* 407.
Sibinovic, K. H., Potter, M., O'Hoostelaere, L., Rober, B., and Wax, J. (1976), in Catalogue of Plasmacytomas and Other Tumors of the Lymphoreticular System, 3rd ed, Kensington, Md., Litton Bionetics.
Stavnezer, J., Huang, R. C., Stavnezer, E., and Bishop, J. M. (1974), *J. Mol. Biol. 88,* 43.
Storb, U. (1972), *J. Immunol. 108,* 755.
Storb, U. (1973), *Biochem. Biophys. Res. Commun. 52,* 1483.
Storb, U. (1976), *Progr. Nucleic Acid Res. Mol. Biol. 19,* 227.
Storb, U., Hager, L., Putnam, D., Buck, L., Farin, F., and Clagett, J. (1976), *Proc. Natl. Acad. Sci. U.S.A. 73,* 2467.
Storb, U., and Marvin, S. (1976), *J. Immunol. 117,* 259.
Szenberg, A., Marchalonis, J. J., and Warner, N. L. (1977), *Proc. Natl. Acad. Sci. U.S.A. 74,* 2113.
Vendrely, R., and Vendrely, C. (1949), *Experientia 5,* 327.
Verma, I. M., Temple, G. F., Fan, H., and Baltimore, D. (1972), *Nature (London), New Biol. 235,* 163.
Vitetta, E. S., Bianco, C., Nussenzweig, V., and Uhr, J. W. (1972), *J. Exp. Med. 136,* 81.
Vogt, V. (1973), *Eur. J. Biochem. 33,* 192.
Williamson, R., Morrison, M., Lanyon, G., Eason, R., and Paul, J. (1971), *Biochemistry 10,* 3014.
Young, B. D., Birnie, G. D., and Paul, J. (1976), *Biochemistry 15,* 2823.
Zsindeley, A., Hutal, J., and Tanko, B. (1970), *Acta Biochim. Biophys. Acad. Sci. Hung. 5(4),* 423.

Structure of Parental Deoxyribonucleic Acid of Synchronized HeLa Cells†

James M. Collins,* David E. Berry, and Carrington S. Cobbs

ABSTRACT: We have investigated the structure of parental DNA as a function of the cell cycle phase of HeLa cells. DNA was isolated from synchronized HeLa cells 0, 5, 8, and 12 h after release from a second exposure to 2 mM thymidine. These DNA preparations were characterized by $Cs_2SO_4/AgClO_4$ buoyant density, sensitivity to a single-strand specific nuclease, sedimentation in neutral and alkaline sucrose gradients, and sedimentation in neutral sucrose gradients after digestion with S_1 nuclease. The cultures were staged according to cell cycle phase by measurements of DNA content per cell by flow microfluorometry. The cell cycle phases were G_1/S (0-h culture), S (5-h culture), G_2 (8-h culture), and G_1 (12-h culture). There are no nuclease-sensitive sites in G_2. As the cells enter G_1, the number increases, with a maximum being reached in the S phase. The number of breaks in DNA with respect to cell cycle phase follows the same pattern. The amount of single strandedness, measured by buoyant density and nuclease sensitivity, is also minimal in G_2, increases in G_1, with a maximum achieved in the S phase. It appears that there is a chromosomal cycle, reflected as continuous structural changes in the DNA molecule, as cells traverse the cell cycle.

During the life cycle of a cell distinct periods are passed through which can be differentiated on the basis of DNA synthesis, mitosis, and DNA content. Howard and Pelc (1963) defined these periods as M (mitosis), G_1 (period after mitosis and preceding S), S (period of DNA synthesis), and G_2 (period after S and preceding M). The DNA content of a diploid G_1 cell would be 2N, that of a G_2 or mitotic cell would be 4N, and S phase would contain increments between 2N and 4N.

The nuclear DNA attaches to the nuclear membrane during the latter part of the G_1 period (Infante et al., 1976). Other G_1 events that may be preparatory for DNA synthesis are activation of chromatin for RNA synthesis (Teng and Hamilton, 1969), phosphorylation of histone f1 (Gurley et al., 1973), synthesis of non-histone chromosomal proteins (Gerner and Humphrey, 1973), deoxyribonucleotide triphosphate pool sizes (Walters et al., 1973), and activation of DNA (Collins, 1974a).

Biochemical studies of the cell cycle have been severely hampered by the lack of quick reliable means of determining the specific proportions of cells in various phases of the cell cycle. The recent advent of instruments capable of measuring the individual DNA content per cell of large numbers of cells has alleviated this problem (Van Dilla et al., 1969). By measuring the DNA content, cells can be staged according to the cell cycle. Such instruments can be used to monitor the cell-cycle stages of synchronized cells in ongoing experiments by sampling small aliquots of a culture (Crissman and Tobey, 1974). These instruments can also be employed to optimize the scheduling of synchronization protocols, thus maximizing the number of cells in a given period of the cell cycle (Collins, unpublished observations).

We have previously reported that when resting (G_0) cells

† From the Department of Biochemistry and the MCV/VCU Cancer Center, Medical College of Virginia, Virginia Commonwealth University, Richmond, Virginia 23298. *Received June 23, 1977.* Supported in part by grants from the National Cancer Institute, CA-17177 and CA-16059 (core Support Grant to MCV/VCU Cancer Center). C.S.C. was supported in part by an institutional grant from the MCV/VCU Cancer Center.