MOLECULAR AND CELLULAR BIOLOGY, Apr. 1982, p. 386–400
0270-7306/82/040386-15$02.00/0

Vol. 2, No. 4

Multiple Immunoglobulin Heavy-Chain Gene Transcripts in Abelson Murine Leukemia Virus-Transformed Lymphoid Cell Lines

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Received 11 December 1981/Accepted 22 January 1982

Lymphoid cells transformed by Abelson murine leukemia virus (A-MuLV) contain three classes of RNA transcripts from immunoglobulin μ genes. Pμ-mRNAs (productive) correspond to the normal 2.7-kilobase (kb) membrane (μ_m) and 2.4-kb secreted (μ_s) μ mRNA species both in size and coding capacity and occur at approximately equal abundance in most μ-positive (pre-B-like) A-MuLV transformants. Aμ-mRNAs (aberrant) generally fall into one of two categories—aberrantly small 2.3-kb μ_m and 2.0-kb μ_s mRNAs which encode aberrantly small μ polypeptide chains, or normal-sized, V_H-containing μ RNAs which do not encode immunologically identifiable μ polypeptide chains. In one case, the latter type of Aμ-mRNA was demonstrated to result from an in-phase termination codon in the D segment of the μ mRNA. Also, most, if not all, A-MuLV transformants express members of a 3.0 to 1.9-kb set of C_μ-containing, but V_H-negative Sμ-RNAs (for sterile), the expression of which may occur simultaneously with but independently of Pμ-mRNAs or Aμ-mRNAs. The Sμ-RNA sequences do not encode immunologically identifiable μ chains and can be produced by cells with unrearranged heavy-chain alleles, such as T-lymphocytes, although the structure of the Sμ-RNAs from T-lymphoid cells appears to be different from that of B-lymphoid cell Sμ-RNAs. Certain A-MuLV transformants also express γ-RNA sequences that are probably analogous to the three different forms of μ RNA. These data support the concept that heavy-chain allelic exclusion, like that of light chains, is not mediated by control at the DNA or RNA levels but is probably a consequence of feedback control from cytoplasmic μ chains.

Infection of bone marrow or fetal liver cells with Abelson murine leukemia virus (A-MuLV) transforms a small fraction of the cells into clonal, continuous cell lines with a variety of properties indicating that they are related to the B-lymphoid cell series (6, 8). Approximately 60% of such cell lines from marrow or 18- to 19-day fetal liver express immunoglobulin heavy chains but no light chains (6, 40, 45), whereas rare isolates produce only κ light chains or κ light chains plus μ heavy chains (6, 45). The remainder (approximately 40%) of the transformants from these tissues, as well as 90% of the transformants from early (13-day) fetal liver produce no detectable immunoglobulin (they are null cells) (6, 45, 51). All of these lines, however, whether immunoglobulin containing or null, have DNA rearrangements at the J_H regions of both chromosomes along with extensive 5′ deletions, suggesting that the processes involved in immunoglobulin heavy-chain gene rearrangement had been active in these cells (6).

During the differentiation of cells of the murine B-lymphocytic lineage, several distinct changes occur in the patterns of immunoglobulin gene expression. Virgin B-lymphocytes (B-cells) in embryonic or neonatal mice make immunoglobulin molecules with heavy chains restricted to the μ class and either κ or λ light chains. B-cells primarily produce membrane-bound immunoglobulin M (IgM), the binding of which is mediated by a membrane-binding domain at the C-terminus of the μ chain (20, 49, 53). This form of the μ protein (μ_m) is encoded by a distinct 2.7-kilobase (kb) μ mRNA (μ_m mRNA) (1, 37). Mature antibody-secreting cells produce complete immunoglobulin molecules consisting

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of both heavy and light chains (30), the C-terminal portion of the μ heavy chain containing a domain specifying secretion (21). The secreted form of the μ chain (μ_s) has been shown to be encoded by a distinct 2.4-kb μ mRNA (μ_s mRNA) (1, 37). The 3′ terminal portions of the μ_m and μ_s mRNAs are encoded by separate exons in the C_μ region of the cell DNA (16).

The precursor to the B-lymphocyte, and the earliest recognizable stage of B-lymphocyte differentiation, is the pre-B-lymphocyte. This cell is characterized by synthesis of cytoplasmic μ chains in the absence of light chains. The existence of this cell type, which was first proposed to explain the occurrence of the μ-only A-MuLV transformants (45), has been documented in the fetal liver (26, 44). To date no normal analogs have been found which represent the null A-MuLV transformants or the other minor phenotypes, although possible explanations for their existence have been suggested (4–6).

To further characterize the properties of the μ-producing and null A-MuLV transformants, we have analyzed in some detail the immunoglobulin heavy-chain proteins and RNAs produced by a large number of A-MuLV transformants. We have found a variety of μ-related RNA species in these cells as well as γ-related RNAs. These RNAs fall into three classes: productive μ mRNAs, aberrant μ mRNAs, and apparently sterile μ RNA transcripts.

MATERIALS AND METHODS

Cells. The A-MuLV-transformed cell lines were produced by in vitro infection of either bone marrow or fetal liver cells as previously described (6, 39, 51). Further details on the growth and characteristics of the A-MuLV-transformed and other cell lines described above have been presented elsewhere (1, 3, 6, 12).

Analysis of heavy-chain RNA, DNA, and proteins. Methods for preparation of total and cytoplasmic RNA, oligodeoxythymidylate-cellulose chromatography, in vitro translation assays, methylmercury hydroxide-agarose gel electrophoresis, blotting to diazotized paper, probe preparations, and hybridization were previously described (1, 3). DNA preparation, restriction enzyme digestions, agarose gel electrophoresis, DNA blotting procedures, probe preparations, and hybridization procedures were performed as previously described (1, 3, 6). All conditions for labeling of cells with [^{35}S]methionine, preparation of labeled extracts, immunoprecipitation assays, and sodium dodecyl sulfate-polyacrylamide gel electrophoresis have been described (1, 3, 40).

Recombinant DNA plasmids. The plasmids pABμ-1 and pABμ-8, which contain μ cDNA inserts of 770 and 1,340 base pairs, respectively, have been described (1). The plasmid pABγ_{2b}-1, which contains a 1,200-base pair γ_{2b} cDNA insert, has been characterized elsewhere (11) and was derived by screening a cDNA library from mouse myeloma MPC 11 with a γ_{2b}-specific cDNA probe prepared as previously described (2).

The plasmids pRI-J_H, pRI-1.6, and pRI-C_μ have, respectively, a 6.2-kb insert which contains the J_H segments, a 1.6-kb insert corresponding to the *Eco*RI fragment 5′ to that containing the J_H segments, and a 10.5-kb insert which contains the C_μ coding sequence. All were subcloned from a 17.5-kb genomic clone as described elsewhere (6). All procedures used conformed to the National Institutes of Health Guidelines for Recombinant DNA Research.

Genomic cloning and DNA sequencing. To isolate the rearranged J_H segments with their attached V_H regions, complete *Eco*RI digests of genomic DNA were cloned into the *Eco*RI site of Charon 16A essentially as described by Blattner et al. (10). Briefly, genomic DNA was prepared from the 18-81A-2 line as previously described (3) and digested to completion with *Eco*RI; fragments less than 4 kb were purified by sucrose gradient centrifugation, and the enriched fragments were ligated to *Eco*RI-cleaved, calf intestinal phosphatase-treated Charon 16A DNA. The ligation mix was packaged into phage coats as described by Sternberg et al. (47), and the resulting phage were plated onto LE 392 cells. Plaques were screened by the procedure of Benton and Davis (9) for hybridization to a probe representing the 3′ 800 bases of the RI-J_H fragment (generously provided by M. Boss) (6), and those yielding a positive signal were purified and amplified.

For sequencing of the phage inserts, they were subcloned into pBR322, and appropriate restriction fragments were prepared and kinased with [γ-^{32}P]ATP as described by Bothwell et al. (11). These were sequenced by the procedures of Maxam and Gilbert (29). The structures of the two unique clones obtained, pV_H-81X and pV_H-81Y, are summarized in Fig. 7. The preparation of the V_H-81X- and V_H-81Y-specific probes from these clones is described elsewhere (4).

RESULTS

Immunoglobulin production in A-MuLV-transformed lines. To establish which A-MuLV transformants produced μ or light-chain protein and to examine the size of such proteins, we assayed more than 100 independently-derived focal isolates of A-MuLV-transformed bone marrow or fetal liver cells. For these analyses, extracts prepared from cell lines labeled with [^{35}S]methionine were incubated with specific anti-μ sera, and the resulting complexes were analyzed by sodium dodecyl sulfate-polyacrylamide gel electrophoresis. Production of μ chains by a given line was evidenced both by precipitation with specific antiserum and by specific blocking of that precipitation with extracts containing unlabeled μ chains (3). As tabulated elsewhere (6), approximately 60% of the A-MuLV-transformed lines derived from bone marrow or late (18- to 19-day) fetal liver produced cytoplasmic μ chains in the absence of detectable light chains. The intracellular μ chains produced by the majority (90%) of these μ-positive A-MuLV-transformed lines (Fig. 1, lanes 3 to 5; representative lines are shown) migrated similarly to the intra-

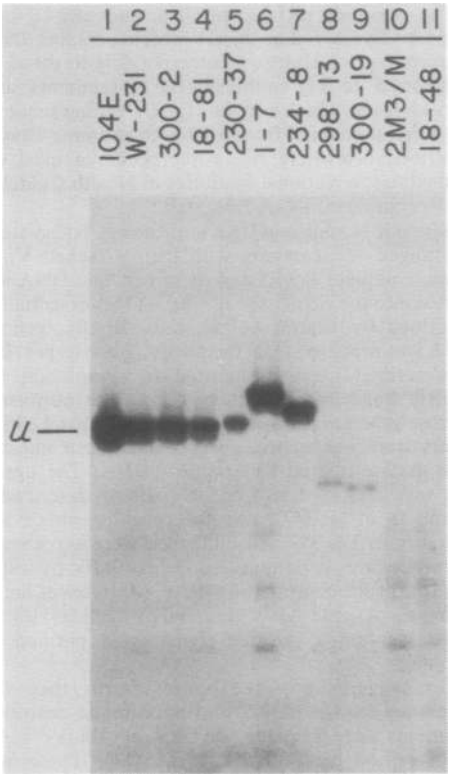

FIG. 1. μ chain synthesis by A-MuLV-transformed cells. The indicated cell lines were metabolically labeled for 60 min with [^{35}S]methionine (83 μCi/ml). Extracts were prepared, and incorporation into μ-related polypeptides was determined by specific precipitation and sodium dodecyl sulfate-polyacrylamide gel electrophoresis as previously described (1, 40). Precipitations were from 2.5 × 10^6 A-MuLV-transformed cells and 1.25 × 10^6 M-104E (104E) and WEHI 231 (W-231) cells.

cellular μ chains synthesized by myeloma (Fig. 1, lane 1) or B-lymphoma cells (Fig. 1, lane 2). Rare μ-positive cell lines synthesized heavy chains that were either abnormally large (Fig. 1, lane 6) or abnormally small (Fig. 1, lanes 8 and 9). Despite the apparent similarity of the μ chains produced by many of these lines to μ_s or μ_m proteins, none of the μ-only A-MuLV-transformed lines either secreted μ chains or expressed μ protein on their surface at a detectable level (not shown). Treatment of selected μ chains with endoglycosidase H demonstrated that all of the various size classes, including the exceptionally small chains, were glycosylated (data not shown).

Approximately 40% of the A-MuLV-transformed lines produced no heavy chain that was precipitable with anti-μ serum (Fig. 1, lanes 10 and 11; two representative lines are shown; none of the minor bands could be competed by μ protein) or with polyvalent sera able to detect the various γ isotypes (not shown). Other considerations, however, suggest that these null lines are related to cells of the B-lymphocyte lineage (6, 8).

Immunoglobulin RNA in μ-positive lines. To obtain a more detailed understanding of the patterns of immunoglobulin gene expression in the various A-MuLV-transformed lines, we analyzed the types and sizes of μ-related RNA sequences found in the lines. For these studies, total polyadenylate [poly(A)]-containing RNA from 12 μ-positive cell lines (10 are shown in Fig. 2) was fractionated by electrophoresis through methylmercury hydroxide agarose gels, transferred to diazotized paper, and assayed for hybridization to various immunoglobulin-specific cloned DNA probes labeled with ^{32}P by nick translation (1). When visualized by hybridization with a probe specific for the μ-constant region (1), the majority of μ-positive A-MuLV-transformed lines examined contained predominant μ RNA species of 2.7 and 2.4 kb (Fig. 2, lanes 3, 4, 9, 11, 22, 23), which comigrated with the 2.7-kb μ_m and 2.4-kb μ_s RNA species observed in B-cell lymphomas (Fig. 2, lane 2) or plasmacytoma lines (Fig. 2, lane 1). These two RNA species frequently occurred at roughly similar abundance, although certain lines contained an excess of the μ_m (Fig. 2, lanes 22 and 23) or μ_s (Fig. 2, lane 9; references 4 and 5) species. Hybridization with probes specific for either the μ_m or μ_s 3′-terminal sequences labeled, respectively, the 2.7- and 2.4-kb μ RNA species in these lines, strongly suggesting that these are authentic μ_m and μ_s mRNA sequences (see Fig. 4B and C for representative data).

A further proof of the identity of these sequences came from polyacrylamide gel analysis of the [^{35}S]methionine-labeled products produced when the total poly(A)-containing RNA from selected μ-positive lines was used to program an in vitro translation system (Fig. 3). As observed with B-cell lymphomas (Fig. 3, lane 2) or plasmacytoma lines (Fig. 3, lane 1, and reference 1), these RNA preparations programmed synthesis of 67,000-molecular weight (67K) μ_m-sized and 64K μ_s-sized polypeptides in proportion to their level of 2.7-kb and 2.4-kb μ RNAs (Fig. 3, lanes 3 to 5). An exception was the RNA from the 1-7 line which contained primarily a predominant 2.7-kb μ_m-size RNA (Fig. 2, lane 23) with μ_m-specific hybridization properties (not shown), as well as a less abundant μ_s-size species, but encoded 84K and 77K μ-specific polypeptides (Fig. 3, lane 6). This line also produced abnormally large μ proteins when assayed by metabolic labeling (Fig. 1, lane 6).

Most of the μ-positive A-MuLV-transformed lines, and some B-cell lymphomas as well, con-

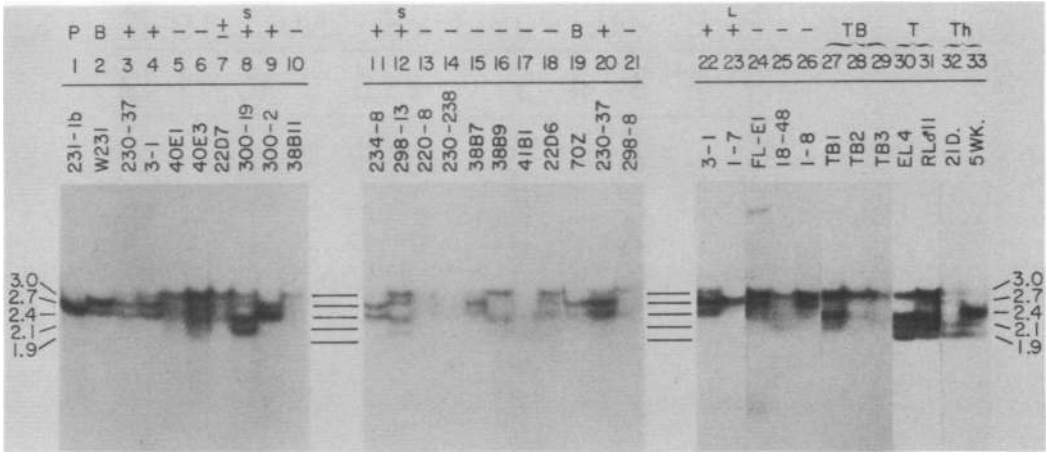

FIG. 2. μ RNAs in μ-positive and null A-MuLV transformants. Poly(A)-containing RNAs from the indicated cell lines were fractionated by electrophoresis through methylmercury hydroxide agarose gels, transferred to diazotized paper, and hybridized with approximately 10^7 cpm of nick-translated pABμ-1 plasmid (specific activity, approximately 5×10^8 cpm/μg). Lanes 1 to 10, 11 to 21, and 22 to 33 are separate experiments. The following amounts of RNA were used. Lanes 1 to 10: 231-1b, 2 μg; WEHI 231 and 230-37; 5 μg; 3-1, 7.5 μg; and the remainder, 10 μg. Lanes 11 to 21: all samples had between 5 and 10 μg. Lanes 22 to 33: all samples had 10 μg. Lanes 30 to 33 were exposed five times longer than lanes 22 to 29. Symbols above lanes indicate phenotype: P, μ-positive plasmacytoma hybrid 231-1b; B, μ-positive B-cell lymphoma WEHI 231; +, μ-positive A-MuLV transformant; −, heavy-chain-negative A-MuLV transformant; TB, TB-cell lines; T, T-cell lymphoma lines; Th, thymus of neonatal (21-day) or adult (5-week) origin. Other symbols: S, a small μ protein was made; L, a larger than normal μ protein was made. Further details are in the text.

tained a 3.0-kb μ RNA (Fig. 2) which exhibited μ_m-specific hybridization (Fig. 4B, lane 3) as well as less abundant species smaller than the 2.4-kb μ_s mRNA. The nature of these sequences, which are also found in normal fetal liver cells (44), will be discussed below.

Several of the μ-positive lines examined produced μ polypeptides that were distinctly smaller than normal (Fig. 1, lanes 8 and 9). In vitro translation analysis demonstrated that the RNA from these lines encoded two or more aberrantly small μ-related polypeptides of 56 to 52 kb (Fig. 3, lanes 7 and 8). Analysis of the μ RNA species in several of these lines revealed a pattern of μ RNA sequences not observed in other μ-positive B-cell types. Although 3.0- and 2.7-kb RNA species were observed—which were also found in null cells and probably do not encode μ proteins (see below)—these lines also contained a prominent 2.3- and 2.0-kb μ RNA doublet (Fig. 2, lanes 8 and 12). Analysis of this RNA with μ_m- or μ_s-specific probes indicated that the 2.3-kb sequence contained a μ_m end (Fig. 4B, lane 9), and 2.0-kb sequence contained a μ_s (Fig. 4C, lane 7) 3′ end. The size of the RNA doublet and the size of the μ polypeptides translated from it are consistent with the loss of one of the protein domains of the μ polypeptide.

μ RNA in heavy-chain null A-MuLV-transformed lines. As mentioned above, a significant percentage of A-MuLV-transformed lines produced no detectable immunoglobulin chains, or, occasionally, κ chains in the absence of heavy chains, although they generally had rearrangements at both J_H alleles (6). All of these lines (14 were examined, 13 are shown in Fig. 2), however, produced RNA species which hybridized specifically with a C_μ-specific probe (Fig. 2, lanes 5 to 7, 10, 13 to 18, 21, 24 to 26). In general, these lines contained a diffuse pattern of from five to six detectable species of μ RNA which ranged in size from 3.0 to approximately 1.9 kb (Fig. 2). Analyses with the $C_\mu 5'$ and $C_\mu 3'$ probes shown in Fig. 4 (top) demonstrated that all of these species contained sequences from both the 5′ and 3′ ends of the C_μ region (Fig. 4A, lanes 4 and 8; representative lines are shown). Hybridization with the μ_m-specific probe labeled the frequently prominent 3.0-kb μ RNA species, as well as a 2.4-kb species and probably another of approximately 2.2 kb (Fig. 4B, lanes 4 and 8; representative lines are shown). This probe often labeled a 2.7-kb species as well (see below). Hybridization with the μ_s probe labeled the 2.7-kb μ RNA species, as well as less abundant species of 2.1 and 1.9 kb (Fig. 4C, lane 4; a representative line is shown). Thus, although most immunoglobulin (or heavy-chain)-negative lines contained among their μ RNA species ones which approximately comigrated with the authentic 2.7-kb μ_m and 2.4-kb μ_s RNA species in B-lymphocyte lines (Fig. 2 and 4), these 2.7-kb

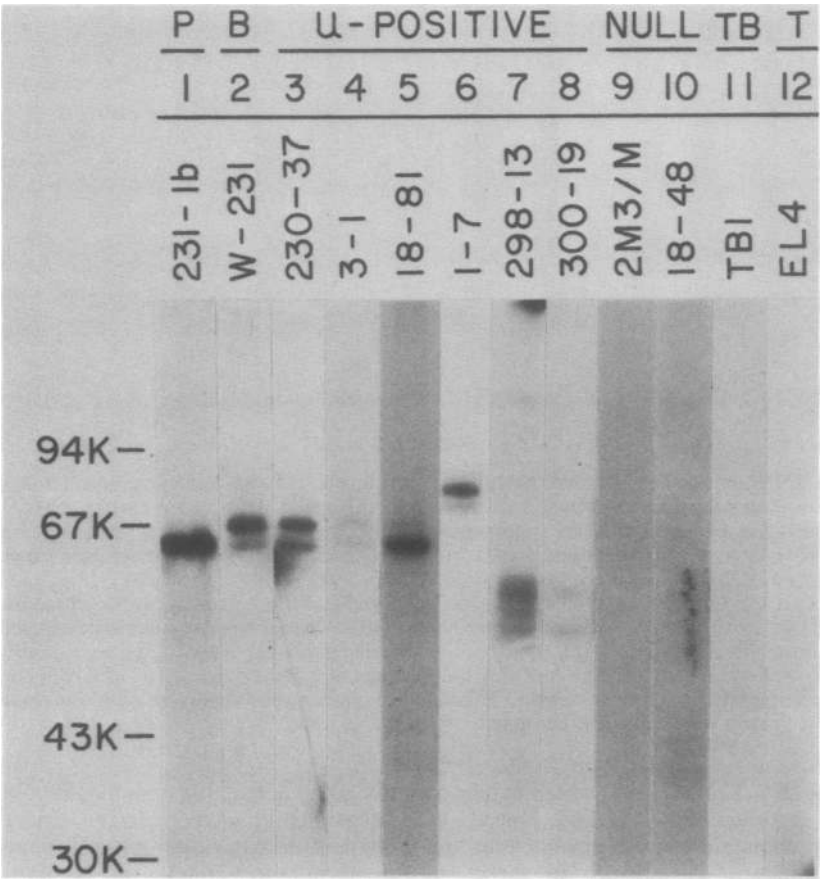

FIG. 3. In vitro translation of μ mRNA. Micrococal nuclease-treated reticulocyte lysates (33) with a final volume of 50 μl were programmed with 20 μg of poly(A)-containing RNA per ml from the indicated sources and then assayed for μ synthesis by specific precipitation and sodium dodecyl sulfate-polyacrylamide gel electrophoresis as previously described (1, 3). Stimulation above background was approximately the same (eightfold) in all reactions. The precipitation of bands indicated as μ specific in the text was competed by M-104E (μ-containing), but not MPC 11 (γ-containing) cell extracts and did not occur with normal rabbit serum. The 231-1b precipitations employed one-half and the 3-1 precipitations employed one-third the amount of lysate used in the others.

and 2.4-kb μ RNAs often did not correspond to authentic μ_m and μ_s RNA because they had the wrong hybridization specificity with the 3'-end probes (Fig. 4B and C).

Additional evidence for the apparently aberrant structure of the major μ RNA sequences in null cells was obtained by assaying the RNA preparations from the various lines for hybridization to a probe representing a portion of the intron between J_H and C_μ (J_H-C_μ intron probe in Fig. 4, top). This probe clearly labeled 3.0- and 2.7-kb μ RNA species in both μ-positive (Fig. 4D, lane 3) and heavy-chain null (Fig. 4D, lane 4) A-MuLV-transformed lines. Hybridization to several of the less abundant, smaller species was also evident upon longer exposure. The probe also labeled a 3.0-kb species in the B-lymphoma line (Fig. 4D, lane 2), but as expected, it did not label either the authentic 2.7-kb μ_m or 2.4-kb μ_s RNA from the plasmacytoma or the B-lymphoma line (Fig. 4D, lanes 1 and 2), indicating that the hybridization observed with the sequences in null cells was not due to C_μ or J_H sequences contaminating the probes. A similar finding of the inclusion of intron sequences in the μ RNA of certain B and T cell lines has been reported (22).

Although the results presented above were obtained with total RNA, when cytoplasmic RNA from selected lines was tested for hybridization to the various probes, a similar pattern of hybridization to sequences of 3.0 kb and smaller was observed, but essentially no hybridization to RNA species larger than 3.0 kb was evident (not shown). Because the μ-negative, A-MuLV-transformed lines contained significant levels of

C_μ-containing RNA sequences in their cytoplasm, we considered the possibility that the lack of detectable μ production by these lines might be due to the production of a rapidly degraded μ protein, analogous to the production of very labile κ fragments in certain λ-producing lines (3). However, when RNA from the null cells was assayed for translation in the in vitro system described above, no μ-related polypeptides were detected under conditions where, based on the amount of RNA used, if translated they would have been detected (Fig. 3, lanes 9 and 10; representative lines are shown).

μ RNA species in other cell types. To determine whether the transcription of the μ gene to give the 3.0- to 1.9-kb μ-related RNAs might also occur in non-B-lymphoid cell lines, we assayed RNA prepared from other sources for the occurrence of C_μ-containing sequences as described above. No μ RNA sequences were evident in several nonlymphoid lines (NBA2 neuroblastoma, L cell fibroblasts, not shown) when assayed with a C_μ probe or with an intron probe which is very sensitive for detecting the large μ precursor species. However, a set of μ transcripts similar to, but more distinct than those found in null A-MuLV lines was observed in three TB-cell lines (Fig. 2, lanes 27, 28, 29; see below for further description of lines) and four permanent T-cell lines (Fig. 2, lanes 30 and 31; two lines, R-1 and AKR3T, are not shown). That the μ transcription pattern of the T-cell lines reflects events occurring in normal T-cells is evidenced by the observation that a similar series of transcripts is evident in adult (Fig. 2, lane 33) and neonatal thymuses (Fig. 2, lane 32), the latter tissue occurring at a developmental stage in which circulating B-cell levels, and as a result B-cell contamination, should be low. In addition, the pattern of μ transcripts seen in neonatal thymus is unique and is not observed in tissues rich in cells of the B lineage such as adult spleen or fetal liver (44). Kemp et al. (23) arrived at similar conclusions concerning the occurrence of μ RNA sequences in T-cells. The series of μ transcripts in the neonatal thymus and in T- and TB-lines generally show a similar pattern of hybridization (see below) to those in A-MuLV-transformed null cells when assayed with the $C_\mu 5'$- or $C_\mu 3'$-specific probes (Fig. 4A, lanes 5, 6, and 7), a μ_m probe (Fig. 4B, lanes 5, 6, and 7), a μ_s probe (Fig. 4C, lanes 5 and 6), and a J_H-C_μ intron probe (Fig. 4D, lanes 5, 6, and 7). In addition, when RNA preparations from selected TB- or T-cell lines were tested by in vitro translation as described above, as with RNA from μ-negative A-MuLV-transformed cell lines, no μ-related synthesis was detected (Fig. 3, lanes 11 and 12; representative lines are shown).

Gene structure in T- and TB-cells. Essentially all of the A-MuLV-transformed lines tested have rearrangements near both of their J_H alleles (6). To test whether such dual rearrangement is a prerequisite for transcription of the C_μ gene, we looked for heavy-chain gene rearrangements in the μ RNA-positive T- and TB-cell lines. To this end we assayed *Eco*RI-digested genomic DNA from the various sources for hybridization to either the RI-J_H or RI-1.6 probes (Fig. 4, top; reference 6). As described previously, assay of DNA with the former probe detects rearrangements of the 6.2-kb *Eco*RI fragment containing the J_H segments, whereas assay with the latter detects deletion of the 1.6-kb *Eco*RI fragment which lies 5′ to that containing the J_H segments. No evidence for rearrangement of RI-J_H or deletion of RI-1.6 was found in three of the four permanent μ RNA-positive T-lymphoid cell lines examined (Fig. 5A and B, lanes 4 to 7; compare with embryo DNA, lane 8). Although one T-cell line (line R1) did not have an embryonic RI-J_H (Fig. 4A, lane 4), it retained at least one copy of the RI-1.6 fragment (Fig. 5B, lane 4). Similar rearrangement in T-lymphoid cells of some but not all J_H segments has been reported by others (15, 24). (In addition, line AKR3T has one rearranged allele, but this is not evident from the present analysis [F. Alt, unpublished].) The three TB-cell lines, which have certain T-cell characteristics (12, 17) but also were reported to produce immunoglobulin (17) and C_μ-containing RNA (Fig. 2 and 4), all showed absence of the embryonic RI-J_H fragment, deletion of the RI-1.6 fragment, and appearance of new but faint fragments hybridizing to the RI-J_H probe (Fig. 5A and B, lanes 1 to 3), consistent with the notion that rearrangement of both heavy-chain alleles is a common event in cells with the potential to express immunoglobulin genes (see reference 6 for review).

Comparison of A-MuLV transformants and T-cells. Because multiple 3.0- to 1.9-kb μ-related RNAs occur in null A-MuLV transformants, TB-cells, and T-cells, but the former two cell types have rearrangements at both J_H alleles while the latter often do not, the possibility is raised that T-cells have somewhat different RNAs from the others. As a preliminary test of this possibility, we assayed RNA from the various cell types for hybridization to the RI-J_H probe shown in Fig. 4 (top). This probe specifically labeled 3.0 and 2.7 kb (and some smaller species) in the RNAs from a T-cell line with unrearranged J_H alleles (Fig. 4E, lane 6), but showed little or no hybridization to those sequences in μ-negative A-MuLV-transformants or TB-cells (Fig. 4D, lanes 4 and 5). This probe also labeled the normal μ_m or μ_s mRNA sequences in RNA preparations from plasmacyto-

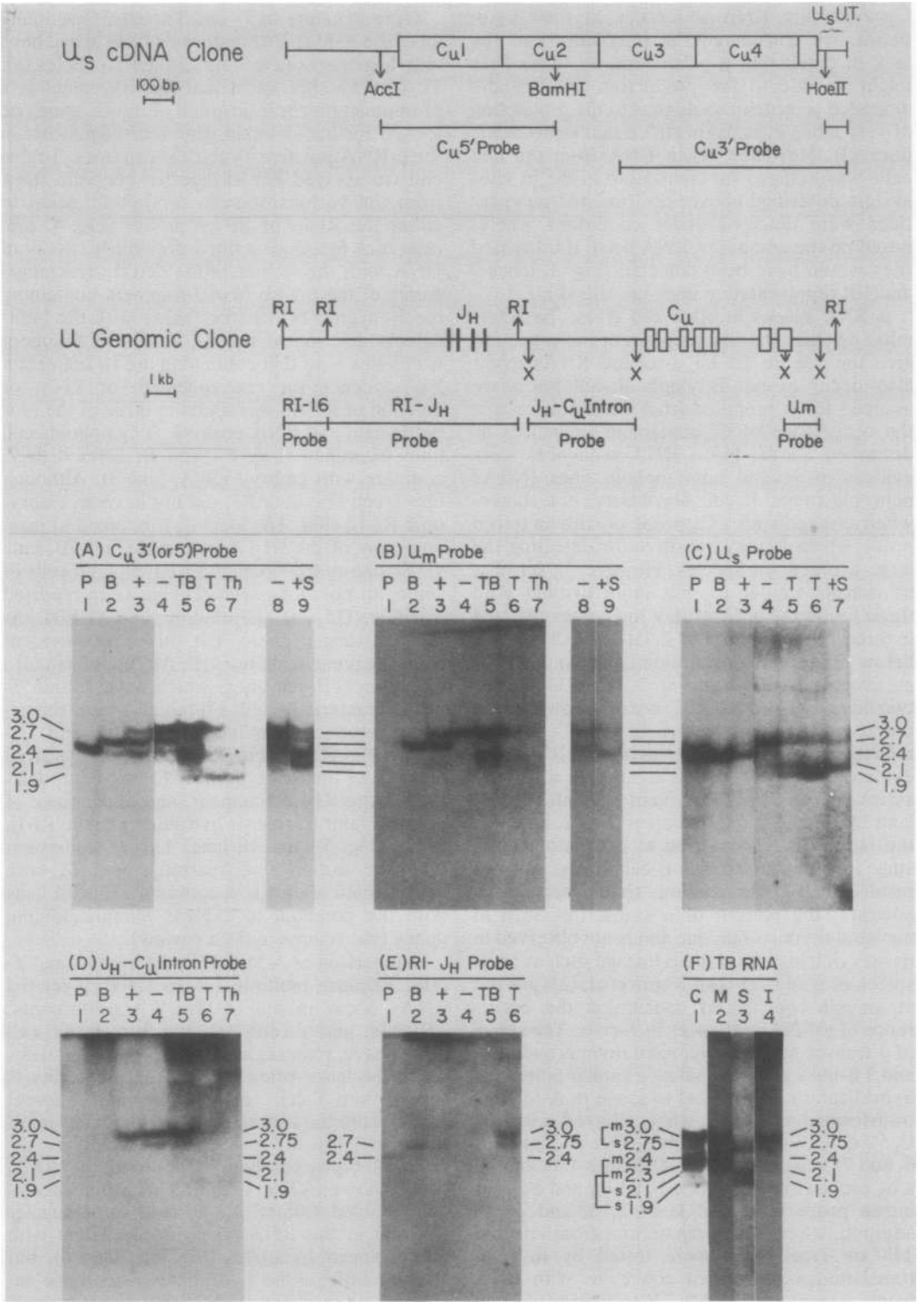

FIG. 4. Structural analysis of μ RNAs. (Top) Preparation of specific subprobes. Probes derived from μ cDNA clones: The C_μ 5′ domain probe was derived from the pABμ-8 clone (11) by excising the fragment which lies between the *Acc*I site which is just 5′ to the $C_\mu 1$ coding domain and the *Bam*HI site which lies in the center of

mas (Fig. 4E, lane 1), B-lymphomas (Fig. 4E, lane 2), and μ-positive A-MuLV transformants (Fig. 4E, lane 3), presumably due to hybridization with the specific J_H sequence contained in those mRNAs. These results suggest that, despite the similarity in size and 3' end specificity, the μ RNA sequences in lines without rearranged J_H alleles (T-cells) probably differ in structure from those found in lines with both heavy-chain alleles rearranged (A-MuLV transformants and TB-cells). In addition, high-molecular-weight species which corresponded in size to those labeled with the intron probe were labeled with the J_H region probe in RNA preparations from all three cell types (Fig. 4E).

Aberrant μ mRNA in a γ-producing cell line. In the RNA of some null lines, hybridization with a μ_m probe labeled an approximately 2.7-kb, μ_m-sized RNA species (data not shown). This result suggested that normal-sized (V_H-containing), but nonfunctional, heavy-chain mRNA might exist in null cells and potentially in μ-positive or γ-positive lines where it could be obscured by the normal mRNAs. To address this possibility, we used a clone of an A-MuLV-transformed cell line (18-81A-2) that had switched from synthesis of μ to synthesis of γ_{2b} in culture (4). When the RNA from this line was assayed with a γ_{2b} probe, a major band of 1.8-kb γ_s mRNA plus a minor 3.7-kb γ_m mRNA (36, 48) species were labeled (Fig. 6A, lane 4). Hybridization with a C_μ probe labeled the series of μ RNAs from 3.0 to 1.8 kb (Fig. 6A, lane 1), but the line produced no μ protein (4, 5).

We previously demonstrated that both J_H alleles were rearranged in the 18-81A-2 cell line, generating new J_H-containing fragments of 2.4 and 1.8 kb after *Eco*RI digestion (4). Both of these fragments were cloned into Charon 16A phage, and partial nucleotide sequence around the rearranged J_H was determined (Fig. 7). The 1.8-kb J_H-containing fragment from 18-81A-2 cells also had a segment of a V_H gene, linked to a D_H segment, which in turn was linked to J_H3, all of which occurred in phase (Fig. 7C). When a probe specific for this rearranged segment (V_H-81Y) was used to assay total poly(A)-containing RNA from 18-81A-2 cells, RNAs corresponding to the 1.8-kb γ_s and 3.7-kb γ_m mRNAs were labeled, but not the C_μ-containing RNAs (Fig. 6A, lane 3). Thus V_H-81Y would appear to be the functional heavy-chain gene in 18-81A-2 cells, as previously suggested (4).

The 2.4-kb J_H-containing, *Eco*RI fragment also contained a V_H-D_H complex (V_H-81X), which in this case was rearranged to J_H2 (Fig. 7B). Although the V_H-D_H-J_H linkages in this allele occurred so that the V_H and J_H segments would be read in the same phase, the D_H segment (double underlines, Fig. 7B), which corresponds to the germ-line D_H sequence sp 2.8 (25), contains a TAG termination codon in that reading frame (boxed, Fig. 7B). Thus, this allele could not produce a translationally functional μ mRNA. Consistent with the apparently normal structure of this allele, however, when a probe specific for the rearranged V_H-81X segment was used to assay 18-81A-2 RNA, a 2.7- and 2.4-kb RNA doublet identical in size to μ_m and μ_s mRNAs was labeled (Fig. 6A, lane 2). These two RNA species comigrated with the 2.7- and 2.4-kb RNA species labeled with the C_μ probe (Fig.

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the $C_\mu2$ domain. After excision, the appropriate fragment was purified as described previously (1). The $C_\mu3'$ probe was the entire 770-base pair pABμ-1 clone (1), and the μ_s probe was the 3' terminal *Hae*II fragment of the μ_s cDNA clone which contains the μ_s untranslated regions (1). The preparation of these two probes has been described previously (1). Probes derived from a μ genomic clone: The RI-1.6 and RI-J_H probes were prepared from subclones in which the corresponding *Eco*RI fragments were subcloned into the *Eco*RI site of pBR322 (6). The J_H-C_μ intron probe and μ_m probe were prepared by excising the indicated *Xba*I (denoted X) fragments from a pBR322 subclone of the 10.5-kb genomic *Eco*RI fragment containing the C_μ exons. The preparation of this subclone has been described (6). The μ_m probe contained sequence from only the μ_m 3' untranslated region (16). The intron and μ_m-specific *Xba*I fragments were purified by agarose gel electrophoresis. (Bottom) Structural analyses of μ RNAs in various lymphoid cells. Total poly(A)-containing RNA from the indicated sources was fractionated by electrophoresis through methylmercury hydroxide agarose gels, transferred to diazotized paper, and hybridized with approximately 10^7 cpm of the indicated nick-translated probe (specific activity, between 10^8 and 10^9 cpm/μg). The symbols and numbers over each lane indicate the cell lines of specific phenotype from which the RNA in that lane was extracted. The lines and the amount of RNA (in micrograms) from each used (in parentheses) were as follows in panels A to E: P, plasmacytoma hybrid 231-1b (2 μg); B, B-cell lymphoma WEHI 231 (4 μg); +, μ-positive A-MuLV transformant 3-1 (8 μg); − (lane 4), null A-MuLV transformant, 1-8 (12 μg); TB, TB-1 cell line (10 μg); T, T-cell lymphoma EL4 (15 μg); Th, 21-day thymus (7.5 μg); − (lane 8), null A-MuLV transformant 40E3 (10 μg); +s, small μ-producing A-MuLV transformant 300-19 (10 μg). Probes were: (A) $C_\mu3'$ probe; lanes 4 to 9 exposed three times as long as lanes 1 to 3; (B) μ_m probe; (C) μ_s probe; lanes 4 to 7 exposed three times as long as lanes 1 to 3 (D) J_H-C_μ intron probe; (E) R1-J_H probe. Panel F is a composite made of TB cell lanes from panels A, B, C, and D: lane 1, C_μ probe (C); lane 2, μ_m probe (M); lane 3, μ_s probe (S); lane 4, J_H-C_μ intron probe (I). Potential membrane and secreted RNA doublets are indicated by bracketed m and s next to kilobase markers on left side of figure. Panels A, B, and D are the same filter probed separately with the individual probes as described previously (1). Panels C and E are separate experiments.

6A, lane 1), but, as predicted, did not encode a μ polypeptide chain (4).

These results suggest two important conclusions. First, in 18-81A-2 cells both heavy-chain alleles are expressed into heavy-chain mRNA, but only one in a functional manner. Secondly, the 3.0-kb and smaller species of the C_μ-set of RNAs in this line did not contain either of the rearranged V_H gene sequences.

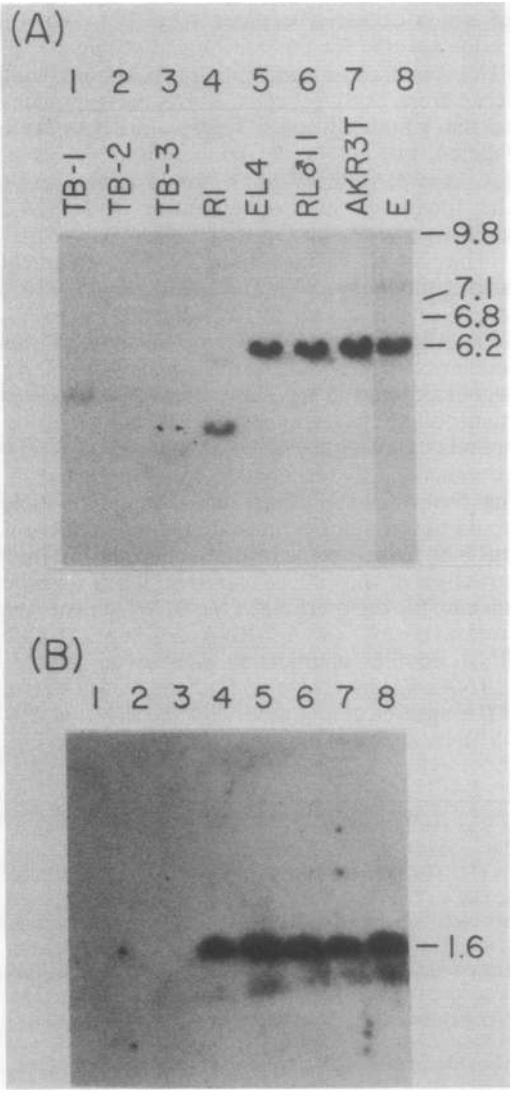

FIG. 5. Analysis of J_H rearrangements in T and TB cell lines. Approximately 10 μg of genomic DNA from the indicated sources was digested with *Eco*RI, electrophoresed through 1% agarose gels, transferred to nitrocellulose paper, and assayed for hybridization to the (A) RI-J_H probe or (B) RI-1.6 probe which were labeled by nick translation with ^{32}P to a final specific activity of 5×10^8 cpm/μg. All procedures were performed as previously described (1, 3, 6). Growth conditions and further characteristics of the lines were described by Chang and Eisen (12).

Aberrant μ mRNA in other A-MuLV transformants. To determine whether the transcription patterns in other μ-positive or null cells were similar to those in 18-81A-2, we assayed the RNA of several additional lines with probes specific for V_H-81X and V_H-81Y. The data in Fig. 6A and other experiments (data not shown) demonstrated that these two probes did not significantly cross-hybridize under our assay conditions.

The 1-8 line cell is an A-MuLV transformant which produced no detectable immunoglobulin but contained the characteristic set of 3.0- to 1.9-kb C_μ-containing RNAs (Fig. 6B, lane 1). When the RNA from this line was assayed with the V_H-81X probe, a 2.7- and 2.4-kb RNA doublet was labeled, corresponding in size to μ_m and μ_s mRNA (Fig. 6B, lane 2). No hybridization was detected with the V_H-81Y probe (Fig. 6B, lane 3). Thus, at least one allele in the null 1-8 line (both are rearranged; reference 6) is expressed into a nonfunctional V_H-containing μ mRNA.

The μ-positive 3-1 A-MuLV transformant contained a 2.7-kb μ_m and 2.4-kb μ_s mRNA doublet, the two species occurring with roughly equal abundance, as well as a prominent 3.0-kb species (Fig. 6C, lane 1). Hybridization to a 2.7/2.4-kb RNA doublet was observed when the RNA of this line was assayed with the V_H-81Y probe (Fig. 6C, lane 3). The V_H-81X probe also labeled a 2.7/2.4-kb RNA doublet, but the 2.7-kb sequence was much more predominant in this set (Fig. 6C, lane 2). These results suggest that both rearranged alleles of the 3-1 line (6) are expressed into V_H-containing μ RNA. Significantly, and as in the other lines studied, neither V_H probe labeled the 3.0-kb μ RNA in this line.

Finally, the μ-positive 1-7 A-MuLV transformant produced a 2.7/2.4-kb μ RNA doublet (Fig. 6D, lane 1) and RNAs of approximately 1.8 kb and 3.7 kb which hybridized with a γ_{2b} probe (Fig. 6D, lane 4). However, this line made no detectable γ proteins (data not shown). No hybridization with the V_H-81Y probe was detected (Fig. 6D, lane 3), but when assayed with the V_H-81X probe, 1.8- and 3.7-kb RNA species, identical in size to those observed with the γ probe, were labeled but the μ mRNA was not labeled (Fig. 6D, lane 2). These results suggest that both heavy-chain alleles are expressed in the 1-7 line and that the nonfunctional allele (6) has switched to γ expression.

In summary, all three of the heavy-chain-producing lines tested here show evidence of expression of V_H-containing RNA from both alleles, and, in at least two of them, the RNAs from one allele were apparently nonfunctional. Likewise, in the one null line examined, both J_H

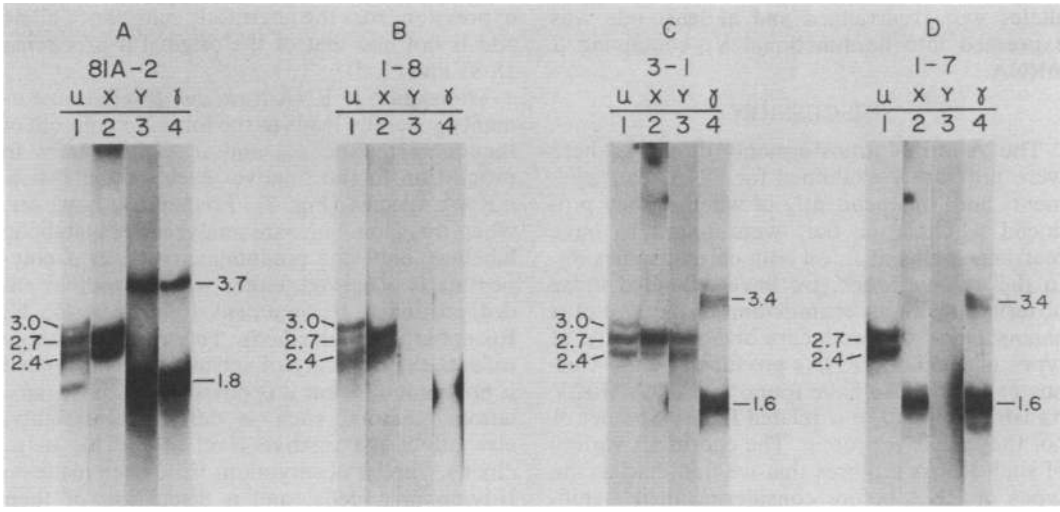

FIG. 6. Expression of V_H-containing RNA sequences in various lines. Approximately 10 μg of poly(A)-containing RNA from the indicated cell lines was fractionated by electrophoresis through methylmercury hydroxide agarose gels, transferred to diazotized paper, and hybridized with approximately 10^7 cpm of the indicated nick-translated probe (specific activity, approximately 5×10^8 cpm/μg). RNA was from the following cell lines: (A) 18-81A-2; (B) 204-1-8; (C) 204-3-1; (D) 204-1-7 (see references 4 and 6 for further description of lines). Probes were as follows: lane 1, pABμ-8 (μ); lane 2, V_H-81X (X); lane 3, V_H-81Y (Y); lane 4, pABγ2b-1 (γ). Individual lanes represent either duplicate blots or the same blot rehybridized with a different probe as previously described (1).

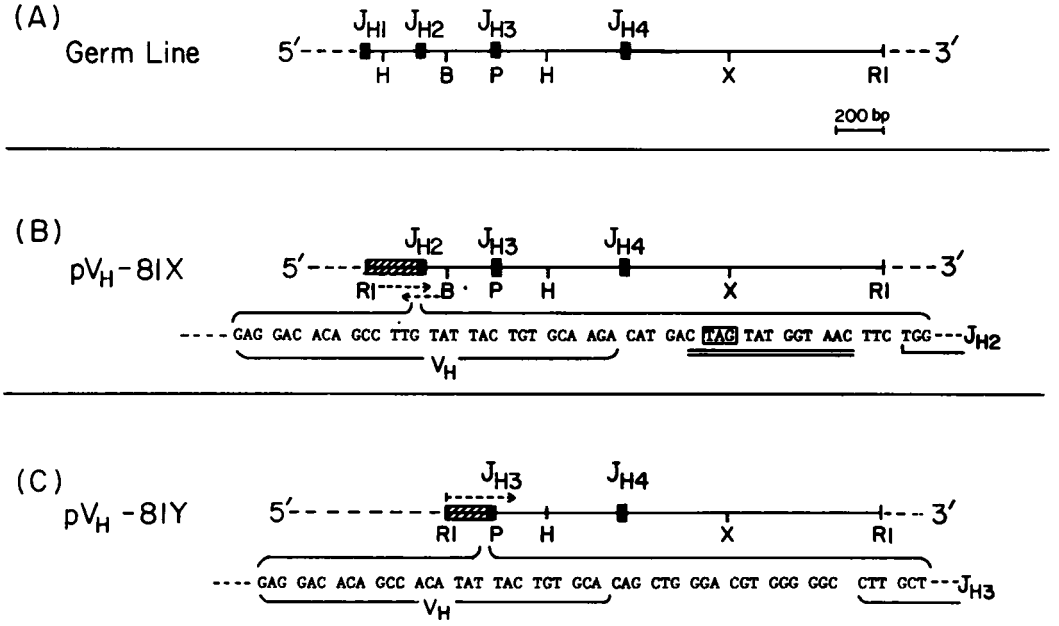

FIG. 7. Nucleotide sequence of the V_H-D-J_H joints of 18-81A-2. (A) Partial restriction map of the germ-line J_H region. Restriction endonuclease sites are indicated as follows: RI, *Eco*RI; X, *Xba*I; H, *Hin*dIII; P, *Pst*I; B, *Bam*HI. (B) Partial restriction map and sequence of pV_H-81X. The sequence of the V_H-D-J_H joint of pV_H-81X was derived by sequencing the indicated 500-base pair *Eco*RI/*Bam*HI fragment from both ends (dashed arrows) so that the joint sequence was obtained in both directions. (C) Partial restriction map and sequence of pV_H-81Y. The indicated sequence was determined by sequencing the indicated *Eco*RI/*Hin*dIII fragment (dashed arrow) from the *Eco*RI site (60 bases from the joint).

alleles were rearranged and at least one was expressed into nonfunctional V_H-containing μ mRNA.

DISCUSSION

The A-MuLV transformants described here were previously examined for DNA rearrangements and, independently of whether they produced μ chain or not, were found to have rearrangements at J_H on both chromosomes (6). In the present work, we have extended those observations to an examination of the size of μ chains made by producers and the variety of types of μ-related RNAs produced by the various cell lines. We have found that all A-MuLV transformants make μ-related RNAs whether or not they make μ protein. The enormous variety of such RNAs requires that we first discuss the types of RNA before considering their significance.

These studies have uncovered three classes of C_μ-containing RNA species in A-MuLV transformants. We will call them Pμ-mRNAs (for productive), Aμ-mRNAs (for aberrant), and Sμ-RNAs (for sterile). The names are given for the purpose of cataloging the RNAs; the Sμ-RNAs may have some important function, but present evidence provides them with no purpose. We will first consider the Pμ-RNAs.

Pμ-mRNA. The μ-only A-MuLV transformants that produce normal-sized μ chains frequently contain approximately equal amounts of 2.7-kb μ_m and 2.4-kb μ_s mRNA (Pμ-mRNAs), although occasionally one or the other is slightly predominant (Fig. 2 and 3B). This pattern of μ protein and μ RNA expression is very similar to that observed in total 17- to 18-day and 19-day surface IgM-negative fetal liver cells (44), and probably reflects that of normal "pre-B" lymphocytes. In apparent conflict with our results, Perry et al. (34), based on their studies of fetal liver hybridomas, recently suggested that "pre-B" cells might produce high levels of μ_s mRNA, and therefore that B-lymphoid cells would undergo cyclical changes in the predominance of μ_s or μ_m RNAs during differentiation. However, we and others have previously demonstrated that fusion of a myeloma with a less differentiated B-lymphoma can impose the more differentiated, μ-secreting phenotype of the myeloma on the B-cell genome (1, 37). Therefore, the μ RNA patterns observed in the fetal liver hybridomas probably represented that of the parent myeloma rather than that of the "pre-B" cell. In addition, the inferred "pre-B" μ RNA phenotype of the 18-81 line reported by Kemp et al. (22) (which resembles that of 18-81A-2; Fig. 5A, lane 1) is actually identical to that which we have observed in long-term cultures of 18-81 that have lost normal μ production but retained μ RNA expression from the aberrantly rearranged allele and is not like that of the original μ-producing 18-81 line (4, 5).

Translation of RNA from the μ-only transformants generally leads to the formation of both of the characteristic μ_m and μ_s polypeptides in proportion to the relative levels of the two μ mRNA species (Fig. 3). Frequently, however, when the same lines are analyzed by metabolic labeling, only one predominant size of μ-polypeptide is observed, either with or without endoglycosidase H treatment (reference 45; N. Rosenberg, unpublished). This result probably reflects the difficulty of separating glycosylated μ polypeptides, but it is possible that posttranslational factors, such as differential stability, also affect the relative level of the μ_m to μ_s chains. Similar observations have been made on B-lymphoma cells, and a discussion of their possible significance has been presented (46).

The general pattern of μ mRNA sequences and their translation products in μ-positive A-MuLV transformants is quite similar to that of surface IgM-positive B-lymphoma cells (references 1 and 44; Fig. 2 to 4). One difference is that the relative level of the μ mRNA sequences is severalfold higher in the surface-positive cells (reference 1; Fig. 2 and 4). Thus, the transition from pre-B to B-cell appears to be accompanied by an increase in the abundance of μ_m mRNA, and perhaps μ_s mRNA as well, whereas the subsequent transition from B-cell to plasma cell appears to involve a selective increase in the abundance of μ_s mRNA (1).

Aμ-mRNA. Many of the A-MuLV transformants, both μ-positive and null, produce apparently aberrant μ (or γ) RNA transcripts (Aμ-mRNAs) which probably are analogous to certain transcripts formed as a result of aberrant light-chain gene expression. A number of the transformants produce abnormally small μ polypeptides of 51 to 55K which are derived from aberrantly small 2.3-kb μ_m and 2.0-kb μ_s mRNAs. The sizes of these RNAs (Fig. 2) and their translation products (Fig. 3) are consistent with the absence in the RNA of a single coding domain. The occurrence of aberrantly small κ mRNAs which encode polypeptides containing only C_κ and a leader sequence is a frequent occurrence in myelomas and probably normal B-cells (3, 35, 38, 44) and results from a nonproductive V_κ rearrangement which fails to provide a donor splice site (13, 28, 42). Another frequent form of aberrant heavy-chain gene transcription observed in both μ-positive and null A-MuLV transformants is the generation of normal-sized, V_H-containing μ (or γ) RNAs (Fig. 6) which do not encode immunologically identifiable μ (or γ) polypeptide chains (Fig. 3). We have cloned and characterized these sequences in detail from one

heavy-chain-positive cell line (18-81A-2) and have shown that they result from transcription of a second V_H gene which was nonproductively rearranged in that a termination codon occurred in its D region (Fig. 7). Similarly, normal-sized κ mRNAs which do not encode C_κ sequences are frequently produced by myelomas or B-lymphomas (3, 19) and probably result from an aberrant V_κ rearrangement (7, 28, 50).

Sμ-RNA. Most, if not all, of the A-MuLV transformants expressed members of a set of 3.0- to 1.9-kb, polyadenylated, cytoplasmic, C_μ-containing Sμ-RNA sequences, the expression of which seems to occur independently of the Pμ-mRNAs or Aμ-mRNAs. We have also observed a very similar set of Sμ-RNAs in various B-cell lymphomas, T- and TB-cell lines, adult and neonatal thymus, and 15-day fetal liver. Kemp et al. (22, 23) have also characterized analogous sequences in various cell types and have reported their occurrence in certain myeloid cell lines as well (23). Although our characterization of these sequences generally agrees with that recently reported by Kemp et al. (22), there are, as indicated below, significant differences between our findings and interpretations and theirs.

All of the Sμ-RNAs appear to contain both the 3′ and 5′ C_μ domains (Fig. 4A; reference 22), but we find that the set can, particularly in T- and TB-cells, be broken down into two subsets of approximately 3.0-, 2.4-, and 2.3-kb RNAs with μ_m-specific hybridization and approximately 2.7-, 2.1-, and 1.9-kb RNAs with μ_s-specific hybridization (Fig. 4F, illustrated with TB-cell RNA). The occurrence of pairs of RNAs differing by about 0.3 kb and differing in whether they have a μ_s or μ_m 3′ terminus suggests that the Sμ-RNAs may consist of three sets of sequences identical in each pair except for the presence of a μ_m or μ_s 3′ end. Often, smaller C_μ-hybridizable RNAs, some of which hybridize with the C_μ 5′ but not C_μ 3′ probe are seen in these lines, but they are generally less abundant and have not been further characterized.

Assay of the RNA from many of these lines with a probe representing much of the J_H-C_μ intron clearly labeled the 3.0- and 2.75-kb Sμ-RNAs and to a lesser degree some of the smaller species as well (Fig. 4D). This agrees with the results of Kemp et al. (22), suggesting that most or all of these sequences contain some of the normal intron sequence as a result of initiation within or splicing into the region. The J_H-C_μ intron probe did not label the normal μ_m or μ_s mRNA sequences, even though these were present at a much higher concentration, confirming the distinct structure of the Sμ-RNAs relative to normal μ mRNA. In addition, the Sμ-RNAs probably lack V_H sequences. We have cloned both rearranged V_H genes in an 18-81 subclone that switched to synthesis of γ_{2b} protein, and the V_H probes labeled either the normal γ_{2b} mRNAs or aberrant 2.4- and 2.7-kb μ mRNAs, but not the 3.0- or 1.9-kb members of the Sμ-RNA set also produced by the line (Fig. 6A). Likewise, each of these V_H probes labeled a μ_m/μ_s RNA set in the 3-1 cell line, but neither labeled the 3.0-kb Sμ-RNA (Fig. 6C).

Contrary to the suggestions of Kemp et al. (22), we find that the Sμ-RNAs in certain T-cell lines we have studied are different from those expressed in the B-lymphomas, null or μ-containing A-MuLV transformants, or TB-cell lines. One difference is the ratios of the various species (compare in Fig. 2), but more striking is the strong hybridization of the 3.0- and 2.75-kb RNAs in T-cells to the RI-J_H probe (Fig. 4E). No hybridization was observed with the Sμ-RNAs in A-MuLV transformants or TB-cell lines although, as expected, faint hybridization to the Pμ-mRNAs due to J_H homology was observed in μ-containing cell lines (Fig. 4E). The intensity of labeling of Pμ-mRNAs in producer cell lines was significantly less than that of the Sμ-RNAs in T-cell lines, even though the level of the T-cell Sμ-RNA was at least 10-fold lower than that of the Pμ-mRNAs. Apparently, a fairly extensive region of homology, significantly greater than a J_H segment, exists between the 3.0- and 2.75-kb T-cell Sμ-RNAs and the RI-J_H segment. A significant difference between the T-cell lines which show this C_μ RNA hybridization pattern and the other lines whose C_μ RNAs do not hybridize significantly with the RI-J_H probes is that the latter lines have rearranged both J_H segments (reference 6; Fig. 5), whereas the T-cell lines studied here have no rearranged J_H segment (Fig. 5). It is striking, but not easily interpretable that, although at least the longest C_μ RNAs in some T-cell lines have a different structure than those of B- or TB-cell lines, the size of individual members of the set of C_μ RNA is conserved.

We interpret the Sμ-RNAs as a set made by a different pathway than authentic mRNAs but often arising from the same genes that make either Pμ-mRNAs or Aμ-mRNAs (e.g., Fig. 6C). The independence of transcription of Sμ-mRNAs and μ-mRNAs is indicated by the observation that in several μ- or γ-producing cell lines, synthesis of μ- or γ-mRNAs is inducible by lipopolysaccharide, but synthesis of Sμ-RNAs is unaffected by such treatment of the cells (4, 5), and that in B-lymphomas, which have higher levels of Pμ-mRNAs than A-MuLV transformants, the Sμ-RNA set is not at a higher level (Fig. 2 and 4). The Sμ-RNAs are not translated into detectable products (Fig. 3), and their function, if they have any, is obscure. They

may be analogous to transcripts from unrearranged κ genes (35).

Heavy-chain allelic exclusion might be mediated at the level of functional protein production. Immunoglobulin-producing cells pose a unique problem: for each cell clone to make only one type of immunoglobulin molecule, it must utilize only one of the available alleles to synthesize heavy chains and light chains. This situation, known as allelic exclusion, has been studied extensively for light chains, where it is evident that more than one allele may be rearranged at the DNA level and be expressed as RNA and protein, but still the vast majority of cells make only one light chain (see references 3, 14, 18). The present data extend the analysis to heavy chains with the same result. Previous work had shown that both alleles are virtually always rearranged at J_H in immunoglobulin-producing cells (6, 14, 15, 31, 41). Assuming that the A-MuLV transformants represent normal stages of immunodifferentiation, we can also say that both in μ-producing pre-B-lymphocytes and in null cells of the B-lymphocytic series, alleles that cannot encode a functional μ chain often make RNA and probably protein. That all alleles make at least Sμ-RNAs cannot be said with certainty but seems likely. Using V_H probes and a C_μ probe, a number of cases of expression of Aμ-mRNAs and Aγ-mRNAs were uncovered (Fig. 5 and 6), suggesting that faulty rearrangements are common and are generally expressed as mRNA and presumably as proteins. It seems apparent that heavy-chain rearrangement is like light-chain rearrangement in that allelic exclusion is a consequence neither of lack of DNA rearrangement nor of a lack of gene expression from alleles that do not encode functional μ chains.

For light chains, it is now widely accepted that allelic exclusion results from a shut-down of the rearrangement process after a productive rearrangement (3, 14, 18). Such a process requires an event of recognition of the productive rearrangement followed by a negative feedback event that stops DNA rearrangement. For heavy chains it remains uncertain whether such a regulated process occurs or whether a preponderance of unproductive events over productive events might, by the stochastic unlikelihood of two productive events in the same cell, yield allelic exclusion as a by-product (14). We favor a regulated model over a stochastic one because it assures a defined outcome with a minimum of wastage (3). For light chains, such a model predicts that, as is observed in κ-producing cells (3, 35, 43, 52), some nonproductive chromosomes will be rearranged and others will be in germ-line configuration, the ratio depending on the frequency with which nonproductive rearrangements occur. For heavy chains the situation is more complicated because V-D, D-D, D-J, and V-$(D)_n$-J joins may all occur, and we do not know whether there is an order to these events or which might be regulated. Thus, the evidence that both J_H alleles become rearranged in almost every cell of the B-lymphocyte lineage does not necessarily support an unregulated (stochastic) model of allelic exclusion (14). But if, as we have suggested elsewhere (6; F. W. Alt, M. Boss, E. Thomas, and D. Baltimore, manuscript in preparation), D-J_H rearrangements can serve as intermediates in the V_H gene assembly process, the apparently high level of frozen D-J_H rearrangements at the second allele (41; Y. Kurosawa and S. Tonegawa, personal communication; Alt et al., manuscript in preparation) would support a regulated model.

If heavy-chain rearrangement is a regulated process, our data say that the necessary feedback cannot result solely from events at the DNA or RNA level because the nonproductive allele (V_HX) in 18-81A-2 cells (Fig. 7B) would appear appropriately joined either by examination of the organization of its DNA [it has a standard V-D-J join] or its RNA. Only when the RNA tried to make protein would the accidental termination codon in the D region abort translation of an authentic μ chain. Because 18-81A-2 cells have a productive allele as well the nonproductive one, a regulated model of immunodifferentiation must require that the feedback signal arise from the appearance of μ protein in the cell (or maybe only the C_μ region) but not from any earlier stage of gene rearrangement or expression.

γ RNA production by A-MuLV transformants. Many of the μ-positive and null A-MuLV transformants also produce γ-related RNA sequences. In one line, this production was shown to be due to an in vitro switch from μ to γ2b production on the expressed allele (4), whereas in another a switch had apparently occurred on the unexpressed allele leading to V_H-containing but nonfunctional γ RNA (Fig. 6D). In other γ RNA-positive transformants, multiple γ RNA sequences of 1.2 to 1.6 kb occur but they are aberrantly small, not translated into detectable γ chains, and apparently do not contain V_H sequences (e.g., Fig. 5 and reference 4). These RNAs may represent Sγ-RNAs analogous to the Sμ-RNAs described above. Since most of these lines retain two copies of the C_μ gene (not shown), the γ RNA sequence could be a result of transcriptional read-through of the C_μ locus, or perhaps downstream initiation near the C_γ structural gene. An apparently analogous set of δ or α RNA sequences may exist in some A-MuLV transformants (unpublished results) and probably in B-cell lymphomas (27, 32).

ACKNOWLEDGMENTS

We thank Margaret Woo, Elise Thomas, and Michael Paskind for assistance with aspects of this work, and Michael Boss for generously providing Charon 16A DNA arms.

This work was supported by grant MV-34M from the American Cancer Society (to D.B.), Public Health Service core grant CA-14051 from the National Cancer Institute (to S. E. Luria), Public Health Service grants CA-24220 and CA-24530 from the National Cancer Institute (to N.R.), and by a contribution from The Whitehead Charitable Foundation (to D.B.). F.W.A. was a Special Fellow of the Leukemia Society of America. N.R. is the recipient of a Research Career Development Award from the National Cancer Institute. D.B. is an American Cancer Society Research Professor.

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