Immunoglobulin heavy chain gene rearrangement and transcription in murine T cell hybrids and T lymphomas

(Martha C. Zúñiga**, Peter D'Eustachio†, and Nancy H. Ruddle§)

*Department of Epidemiology and Public Health, Yale University School of Medicine, 60 College Street, New Haven, Connecticut 06510; and†Department of Biology, Kline Biology Tower, Yale University, New Haven, Connecticut 06520

Communicated by Frank H. Ruddle, February 1, 1982

ABSTRACT We have examined the arrangement of immunoglobulin heavy chain constant (C heavy) and joining (J heavy) region genes in murine T cell hybrid lines and in T lymphomas. C heavy genes derived from both parental cell types were present in all hybrids for which polymorphism in sequences flanking C heavy genes permitted us to distinguish parental C heavy genes. All T lymphomas and T cell hybrids retained the C heavy gene in germ-line configuration and all but one cell line had germ-line C heavy genes. Novel DNA fragments reactive with J heavy probes were observed in six of nine T cell hybrids, as well as in two T lymphomas, WEHI7.1 and YAC-1, but not in the fusion parent, BW5147. No RNA homologous to C heavy or J heavy genes was detected in any of the T cell lines. T cell lines contained poly(A) tail RNA homologous to a C 4 heavy RNA probe. More importantly, in several cell lines the C heavy RNAs were associated with membrane-bound polyribosomes. These results suggest that both J heavy rearrangements and C heavy RNA production occur in at least some mature, antigen-specific T cells. They may therefore reflect events in normal T cell development and function related to those involved in the generation of the T receptor for antigen.

Much is known about the molecular biology of immunoglobulins. Ig heavy (H) and light (L) chain genes are not inherited intact in the germ line but are generated during B cell development by somatic rearrangement and mutation of distinct germ-line variable (V), joining region (J), and diversity region (D) (in H chains) and constant (C) genes (1). These somatic events are specific in that they have not been detected in non-lymphoid cells. However, the process is error prone: some Ig gene rearrangements found in B cells are aberrant and cannot encode functional H or L chains (2). Processed RNA transcripts of aberrantly rearranged and germ-line Ig genes have been observed (3).

The T cell receptor for antigen is less well understood. It apparently bears determinants closely related to B cell H chain V regions (4). Genes encoding T cell surface antigens that are closely associated with the C region of the receptor have been mapped to chromosome 12 in the mouse, near IgH (5). J heavy genes can undergo rearrangement in some T cell lines and T lymphomas (6–8). Moreover, the Ig C heavy gene is transcribed in normal mouse thymocytes (9) and in some murine T lymphoma cell lines (6, 10). T cell C heavy RNAs differ in size from authentic α chain mRNAs [2.4 and 2.7 kilobases (kb)] but include all the coding sequences of the C heavy gene (11). Genetically homogeneous, antigen-specific T cell populations are needed to assess the relevance of these molecular events to T cell function. Antigen-specific T cell hybrids can be generated by fusing purified, antigen-specific primary T cells with cells of the culture-adapted murine T lymphoma line BW5147. Cloned lines derived from the initial hybrid cell population can be maintained in culture. All such lines are IgM+ and many continue to express the antigen specificity and Thy-1 phenotype of the primary T cell parent (12–14).

To study Ig H chain gene structure and expression in normal murine T cells, we have applied nucleic acid blotting procedures to genomic DNAs and poly(A) tail RNAs from nine antigen-specific T cell hybrids and three T lymphomas. Rearrangement of Ig J heavy genes occurs in at least some classes of mature, antigen-specific T cells. Furthermore, C heavy RNA is synthesized and found on polyribosomes in some T lymphomas and T cell hybrids.

MATERIALS AND METHODS

Cell Lines. Murine T cell hybrids were prepared and characterized as described (12–14). T lymphomas WEHI7.1 and YAC-1 were obtained from N. L. Warner. T lymphoma BW5147 was obtained from R. Goldsby. All T cell lines lacked surface Ig as determined by immunofluorescence. The IgGl-producing mouse myeloma P3XS3 was a gift of C. Milstein. IgM-secreting B cell hybridomas 22.1.6 (15) and CHD 2-12 were from A. Marshak-Rothstein and S. Carson, respectively. All three B cell lines are of BALB/c origin. AK2.2 is an (A/J × BALB/c) azobenzenearsenate-specific, IgG-producing B cell hybrid. B and T cell lines were grown in suspension culture in Dulbecco's modified Eagle's medium supplemented with 10% heat-inactivated newborn bovine serum (WEHI7.1, BW5147, 29P5, 63D11), with 10% heat-inactivated horse serum (AK2.2), or with 10% heat-inactivated fetal bovine serum (all other lymphoid cell lines). The LMTK' subline of mouse L cells was grown in monolayer culture in α-modified Eagle's medium plus 5% fetal bovine serum. Media and sera were from Gibco.

Nucleic Acid Preparation and Analysis. DNA was purified and subjected to Southern blot analysis as described (16).

Total cellular RNA was prepared by a modification of the method of Auffray and Rougeon (17). Frozen cell pellets were resuspended in 3 M LiCl/6 M urea and sonicated briefly to shear DNA. RNA was precipitated overnight at 0°C, pelleted through a cushion of 50% sucrose/3 M LiCl/6 M urea by centrifugation at 17,500 × g for 20 min, redissolved in 0.1 M NaCl/0.01 M Tris-HCl, pH 7.5/0.1% NaDodSO4, extracted with one volume of chloroform/isomyl alcohol, 24:1 (vol/vol), and reprecipitated with ethanol. Cytoplasmic RNA and membrane-
bound polyribosome (mbpr) RNA were prepared as described by Perry and Kelley (18). Poly(A)* RNA was obtained by two cycles of chromatography on oligo(dT)-cellulose (Collaborative Research, type II) with 0.5 M NaCl/0.05 M Tris-HCl, pH 7.5/0.5% NaNaO4SO4 as loading and wash buffer and with double distilled H2O as the eluting solvent. RNA was denatured with glyoxal or formamide in the presence of formaldehyde (19), fractionated by agarose gel electrophoresis (19), and transferred to nitrocellulose as described (20).

Six cloned probes were used for nucleic acid blot analysis. (All procedures were in accordance with the current National Institutes of Health guidelines on recombinant DNA.) A1A1 is a cDNA corresponding to the entire V and C regions of the MOPC 315AII L chain inserted into the plasmid pBR322 (21). p72b(11), pμ(3741)(9), and pα(J558)(35) are cDNAs corresponding, respectively, to the MPCL myeloma γ2b C region, the 3741 myeloma μ C region, and the J558 myeloma α C region plus the JH DNA sequence and the 3' third of the J588 VH sequence, each in the plasmid pMB9 (22). pμA and pμB, are, respectively, the genomic 5' BamHI and 3' BamHI/EcoRI fragments of the JH gene cluster inserted into pBR322 (22). The probes were labeled by nick translation with deoxyctydine [α-32P]triphosphate (New England Nuclear) to a specific activity of 5-4.0 × 10^8 dpm per μg of DNA. Electrophoretically fractionated DNA and RNA were transferred to nitrocellulose filters and hybridized with the radiolabeled probes by standard methods (16, 20, 23).

**RESULTS**

Ig Genes in T Lymphomas and T Cell Hybrids. Previous papers have described the generation of somatic cell hybrids between normal murine T cells of known antigenic specificity and cells of the murine T lymphoma cell line BW5147 (12-14). All T cell lymphomas and T cell hybrids were Ig- as determined by immunofluorescence and immunodiffusion. Several hybrids retained the antigenic specificities of the immune parent (12-14).

To demonstrate that Ig C_H genes from both parental genomes had been retained, restriction fragments in the 5' flanking sequences that differentiate Iγ C_H and Ig C_H genes of different mouse strains were assessed (Fig. 1). These experiments are summarized in Table 1. Data obtained with pα(J558)(35) and HindIII-restricted DNA are shown in Fig. 2A. Analysis of DNA cut with three different restriction enzymes, using this probe and pμ(3741)(9), showed that the C_H and C_H genes of BW5147, YAC-1, and WEHI7.1 were present in germ-line configurations. The C_H genes of the myeloma F3X63 were deleted, and those of the B cell hybridoma 22.1.6 were rearranged. In T cell hybrids 20B, 21C4, 29P5, 32-15, and 64C11, copies of the C_H and C_H genes derived from the immune parents were present in their germ-line configurations. The hybrid 23A contained novel DNA fragments, consistent with C_H gene rearrangement. Hybrid 63D11 contained a C57BL/6-derived germ-line C_H gene but no detectable C57BL/6 C_H gene, suggesting deletion of this gene. Hybrid 49A (BDF1 × AKR) could not be scored completely because its DBA parental C_H genes are indistinguishable from the BW5147 (AKR) C_H genes (22); however, its C57BL/6 C_H genes were not detected. Likewise in 51H7D the A/J C_H gene could not be distinguished from the AKR C_H gene.

**J_H Genes Are Rearranged in Murine T Cell Lymphomas and T Cell Hybrid Lines.** Rearrangements of J_H gene sequences are central to the formation of functional H chain genes in B cells (1). To determine whether such rearrangements had occurred in the J_H genes of our T cell hybrids, genomic DNA was digested, fractionated, and hybridized with the probes pμA and pμB, which correspond to J1/J_1 and J2/J_2, respectively (Fig. 1). Only the germ-line J_H DNA fragments (2.3 and 0.9 kb) were found in the T lymphoma BW5147 and in three T cell hybrids (32-15, 49A, and 63D11). The T lymphomas WEHI7.1 and YAC-1 and six T cell hybrids yielded both germ-line and novel fragments, suggesting J_H gene rearrangement on at least one chromosome 12 homologue (Fig. 2 B and C, Table 1).

The sites at which rearrangement occurred within the J_H gene cluster in these T cells could be deduced from Southern blot data (Fig. 2 B and C, Table 1). The patterns of novel fragments in both lymphomas and two of the hybrids (20B and 29P5) were consistent with rearrangements involving J_3 or J_4. Four hybrids (21C4, 23A, 51H7D, and 64C11) had patterns indicating rearrangements involving J_5. No rearrangements involving J_1 were seen in any of the T cell hybrid lines.

The germ-line configuration of BW5147 J_H genes suggested that the novel bands in hybrid cell DNAs were due to rearrangement of the J_H genes of the immune T cell parent chro-

---

**FIG. 1.** Organization of Ig H chain C_H (A) and J_H + C_H (B) genes. The relative positions and coding sequences of the genes are indicated, as are the positions of cleavage sites for the enzymes EcoRI (R), HindIII (H), BamHI (B), and Kpn I (K). Only cleavage sites useful in the experiments described here are shown. The polymorphic regions adjoining the C_H and C_H genes are in parentheses. Their sizes, as measured previously by Maruc et al. (24), are given relative to those of the BW5147 (AKR) cell line. Lines above the DNA map indicate the sequences contained in the probes used here (23, 24).
Table 1. Detection of $C_H$ and $J_H$ genes in T lymphomas and T cell hybrids

<table>
<thead>
<tr>
<th>Cell line</th>
<th>Strain*</th>
<th>Presence of $C_H$ genes from immune parent†</th>
<th>Presence of novel $J_H$ fragments†</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>$\mu(3'741)^a$</td>
<td>$p_{11}$</td>
</tr>
<tr>
<td></td>
<td></td>
<td>$p_{11}$</td>
<td>$p_{11} + p(3'741)^a$</td>
</tr>
<tr>
<td></td>
<td></td>
<td>$\mu$</td>
<td>$H$</td>
</tr>
<tr>
<td></td>
<td></td>
<td>$\mu$</td>
<td>$H$</td>
</tr>
<tr>
<td>EL4 Bu</td>
<td>C57BL/6</td>
<td>14.0</td>
<td>10.7</td>
</tr>
<tr>
<td>YAC-1</td>
<td>A/He</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>WEH7.1</td>
<td>BALB/c</td>
<td>12.9</td>
<td>ND</td>
</tr>
<tr>
<td>BW5147</td>
<td>AKR</td>
<td>12.5</td>
<td>8.7</td>
</tr>
<tr>
<td>20B</td>
<td>C57BL/6</td>
<td>+</td>
<td>14.3</td>
</tr>
<tr>
<td>21C4</td>
<td>C57BL/6</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>23A</td>
<td>C57BL/6</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>28P5</td>
<td>C57BL/6</td>
<td>+</td>
<td>10.1</td>
</tr>
<tr>
<td>32-15</td>
<td>CBA</td>
<td>+</td>
<td>ND</td>
</tr>
<tr>
<td>49A</td>
<td>B6DJF1</td>
<td>CD</td>
<td>ND</td>
</tr>
<tr>
<td>51H7D</td>
<td>A/J</td>
<td>ND</td>
<td>CD</td>
</tr>
<tr>
<td>60D11</td>
<td>C57BL/6</td>
<td>ND</td>
<td>+</td>
</tr>
<tr>
<td>64C11</td>
<td>C57BL/6</td>
<td>+ ND</td>
<td>ND</td>
</tr>
</tbody>
</table>

Table 2. $C_H$ RNAs in T lymphomas, T cell hybrids, and B cell hybrids

<table>
<thead>
<tr>
<th>Cell line</th>
<th>$C_H$ RNA, molecules/cell</th>
<th>Ratio of mbr $C_H$ RNA to cytoplasmic $C_H$ RNA†</th>
</tr>
</thead>
<tbody>
<tr>
<td>22.1.6 (B)</td>
<td>740</td>
<td>ND</td>
</tr>
<tr>
<td>CHD2-12 (B)</td>
<td>757</td>
<td>1.0</td>
</tr>
<tr>
<td>WEH7.1 (T)</td>
<td>48</td>
<td>0.66</td>
</tr>
<tr>
<td>BW5147 (T)</td>
<td>3.5</td>
<td>0.65</td>
</tr>
<tr>
<td>28P5 (T)</td>
<td>0.1</td>
<td>0.35</td>
</tr>
<tr>
<td>32-15 (T)</td>
<td>0.3</td>
<td>ND</td>
</tr>
<tr>
<td>51H7D (T)</td>
<td>8.17</td>
<td>ND</td>
</tr>
<tr>
<td>64C11 (T)</td>
<td>22</td>
<td>0.51</td>
</tr>
</tbody>
</table>

*Strain of origin of cell line or immune parent.
† Fragment sizes are in kb. Novel fragments (boldface) occur in addition to germ-line fragments. A + or –, germ-line fragments present or absent. CD, cannot distinguish; ND, not determined. Restriction enzymes: R, EcoRI; H, BamHI; I, Hind; K, Kpn I.

mosomes. To determine which chromosome type carried rearranged $J_H$ genes, DNA was digested with BamHI or Kpn I, fractionated, and hybridized with $\mu(3'741)^a$ or $p_{11}$. Each of these enzymes generates a single DNA fragment that contains the whole (Kpn I) or part (BamHI) of the $J_H$ gene cluster as well as the $C_H$ gene and its adjoining polymorphic region (Fig. 1). Each hybrid analyzed in this way contained a fragment of the size expected for the BW5147 germ-line fragment and a second fragment either of the size expected for a germ-line fragment for the normal parent or of a novel size (Fig. 3). The presence of a germ-line AKR band and a novel second band in the absence of a germ-line C57BL/6 germ-line reactive with the $p_{11}$ and $\mu(3'741)^a$ probes assigns the rearrangements at $J_H$ in hybrids 20B and 28P5 to the C57BL/6 chromosome 12 (Table 1). An unexpected result was obtained in the case of 51H7D. BamHI digests of 51H7D DNA gave a fragment of the size expected for an AKR germ-line fragment and a second fragment of novel size reactive with the $p_{11}$ and $\mu(3'741)^a$ probes. Because data on this hybrid indicate a rearrangement of $J_H$ rather than $J_J$, we expected to see a germ-line fragment reactive with these probes (Fig. 1). The presence of the novel BamHI fragment in 51H7D suggests a more complex situation involving deletions or rearrangements in the region between the $J_H$ cluster and $C_H$.

Ig Gene Transcription in T Cell Lymphomas and T Cell Hybrids. To assess Ig gene transcription in these T cell lines, total cellular poly(A)+ RNA was examined by blot hybridization analysis (20) with $\lambda$ and $\mu(3'741)^a$, and $p_{11}$ probes. No RNA species reactive with $C_{\mu}$, $C_{\delta 20}$, or $\lambda$ CDNA probes was detected in any of the 12 T cell lines examined. In contrast, all 12 lines contained RNA homologous to the $\mu(3'741)^a$ probe. The T lymphomas YAC-1 and BW5147 contained a single $C_H$ RNA of about 1.9 kb (Fig. 4), and WEH7.1 contained $C_{\mu}$ RNAs (Fig. 4) indistinguishable in size from the 1.9-, 2.1-, 2.3-, and 3.0-kb $C_{\mu}$ RNAs described by Kemp et al. (6, 11). All T cell hybrids contained the 1.9-kb species. One hybrid, 64C11, also had a $C_{\mu}$ RNA that comigrated with the 2.1-kb RNA of WEH7.1 (Fig. 4). The amount of $C_{\mu}$ RNA was variable among cell lines but was consistently less than that found in two $\mu$-producing B cell hybridomas (Table 2). No RNA reactive with $\mu(3'741)^a$ was detected in F3X63, in the IgG-producing B cell hybridoma, AK2.2, or in BALB/c mouse embryo fibroblasts.

We examined the B cell hybridomas and T cell lines for RNAs containing $J_H$ sequences by using the probes $p_{11}$ and $p_{11}$. In each of the B cell hybridomas $J_{H H}$- or $J_{HH}$-reactive RNA was obtained by agarose gels, suggesting that the $J_H$ and $C_H$ sequences occur on the same RNA molecules. $J_H$ sequences were not detectable on any T cell RNAs except WEH7.1 (unpublished data).

$C_H$ RNA is found on $mbpr$s in T cells. We examined the subcellular distribution of $C_H$ RNA in one B cell hybrid, two T lymphomas, and two T cell hybrids (Table 2) by performing blot hybridization analysis on poly(A)+ RNA of nuclei, cytoplasm, and mbrs, using $\mu(3'741)^a$ as a probe. Significant amounts of $C_{\mu}$ RNA were found in the mbr fraction of two cell lines.

ND, not determined.

*Poly(A)+ RNAs (0.05–1.0 μg for B cell hybrids, 1–40 μg for T cell lines) were used in blot hybridization analysis with $\mu(3'741)^a$ as a probe. Autoradiograms were subjected to quantitative densitometry and lanes whose integrated peak intensities were proportional to poly(A)+ RNA input were used to calculate $C_H$ RNA content per cell. The number shown is the mean of two to five measurements. The B cell hybridoma 22.1.6 was assumed to have 1% of its total poly(A)+ RNA as μ mRNA (8.1 × 106 daltons). It was further assumed that 0.5% of total cellular RNA is poly(A)+ and that there is 0.1 pg of poly(A)+ RNA per cell.

Cytoplasmic poly(A)+ RNA fraction includes poly(A)+ RNA from free polyribosemes. Contamination of mbrs by cytoplasmic RNA is assumed to be about 1% (24).
DISCUSSION

The data presented in this paper show that: murine T cell hybrids have Ig H chain genes from both parental cell types, J_H genes are rearranged in murine T cell lymphomas and T cell hybrid lines, the Ig C_H gene is expressed in T cell lymphomas and T cell hybrids, and the C_H RNA has poly(A) and is found on mbprs. With one exception, all of the T cell hybrids for which polymorphism in sequences flanking C_H genes permit us to distinguish parental C_H genes retain C_H and C_m genes of both parental cell types. Because the C_m and C_m genes are at opposite ends of the known C_H gene cluster, our results argue against models in which a T cell receptor gene is generated by splicing a V_H gene adjacent to a "C," gene at the 3' end of the known C_m gene cluster. However, such models are not rigorously excluded by our data because we do not know how many copies of chromosome 12 occur in two of these T cell hybrids.

The rearrangements involving J_H in at least two T cell hybrids occur on the chromosome derived from the normal T cell parent. Because BW5147 lacks J_H rearrangements (Fig. 2 B and C), the data suggest that rearrangements occurred in the an-

Fig. 3. J_H gene rearrangements in T cell hybrids. DNAs from T lymphomas and from T cell hybrids were digested with BamHI and analyzed as described for Fig. 2 with the probe p11. The final wash of the filter was in 45 mM NaCl/4.5 mM sodium citrate at 65°C. EL4.Bu is a C57BL/6 T lymphoma that retains the germ-line configuration of these genes. The lane labeled BW + EL4 contains a 2:1 mix of BW5147 and EL4.Bu DNA.

* Germ-line AKR gene.

** Germ-line C57BL/6 gene.

(WEHI7.1 and 64C11). In WEHI7.1 the 1.9- and 2.1-kb RNAs were the major C_m RNA species on mbprs.

FIG. 2. Southern blots of genomic DNA from lymphoid cell lines and cell hybrids. HindIII-digested DNAs (25 μg per track) from T lymphomas YAC-1 (Y), WEHI7.1 (W), BW5147 (BW), the myeloma P3X63 (X), A9 mouse fibroblasts (A), the T cell hybrids 20B, 21C4, 23A, 29F6, 32-15, 49A, 51H7D, and 63D11 and from the B cell hybridoma 22.1.6 were fractionated on a 1% agarose slab gel, transferred to nitrocel-

ulosel and hybridized with the probes pol(J558)18 (A), p6 (B), and pc11 (C). Final washes of filters were in 45 mM NaCl/4.5 mM sodium citrate at 65°C (A) or in 15 mM NaCl/1.5 mM sodium citrate at 65°C (B, C). Fragment sizes in kb were calculated from their mobilities relative to HindIII-digested λ bacteriophage DNA fragments.

* Germ-line AKR gene.

** Germ-line C57BL/6 gene.

Fig. 4. Blot hybridization analysis of C_m RNAs in T lymphomas and T cell hybrids. Total poly(A)+ RNAs from B cell hybrids, T lymphomas, and T cell hybrids were denatured and fractionated by electrophoresis on a horizontal 1.6% agarose gel (21), transferred to nitrocel-

lulose filters, and hybridized with nick-translated pμ(3741) as described by Mulline et al. (25). Filters were washed finally in 15 mM NaCl/1.5 mM sodium citrate/0.1% NaDodSO4 at 50°C and autoradiographed (A). B shows a 3-fold overexposure of lane 6 in A. The RNA samples in A are: track 1, 22.1.6, 0.4 μg; track 2, 22.1.6, 0.6 μg; track 3, WEHI7.1, 1 μg; track 4, WEHI7.1, 3 μg; track 5, unlabeled RNA markers; track 6, BW5147, 3 μg; track 7, 64C11, 5 μg. The degree to which the three lower molecular weight bands of WEHI7.1 could be resolved varied from experiment to experiment.
tigen-specific parental cells prior to fusion with BW5147. In all, 20 of 36 T cell lines described here and in previous publications (7, 8, 11, 25, 26, 29) show rearrangements involving the JH gene complex. No obvious correlation between T cell subset and JH rearrangement has yet emerged.

The level of Cm RNA in BW5147 is extremely low (Table 2) and, in our hands, was detectable only under the high-sensitivity conditions provided by the formamide/formaldehyde agarose gel system. Some T cell hybrids contain more Cm RNA than the BW5147 parent (Table 2). This might be due simply to a gene dosage effect, except in the case of 64C11, which synthesizes a Cm RNA not found in BW5147 (Fig. 4). Further, the T cell Cm can be recovered from mbprs, suggesting that it has a polypeptide product. Nevertheless, it has not been possible to detect polypeptides bearing the antigenic determinants of μ chains in Cm RNA+ T cell lines (27). Given the domain structure of the μ polypeptide and the preservation of antigenic determinants on isolated domains, it is unlikely that Cm polypeptides, if synthesized, lack μ antigenic determinants. There are alternative open reading frames of 160, 170, and 200 nucleotides within the Cμ gene (28), but these include intron sequences that have been shown not to occur in T cell Cμ RNAs (11). Synthesis of very unstable polypeptides remains possible.

The JH rearrangements and Cμ RNA transcripts in T cells reported here and elsewhere (6-11, 29, 30) may thus yield no functional product. However, the occurrence of Ig gene activity in T cells suggests that the mechanisms generating functional Ig genes in B cells act at analogous loci in T cells. Extending these analyses to more kinds of homogeneous T cell populations and to more gene probes should test the generality of Ig gene expression in T cells and the relationship of Ig gene activity to the T cell receptor for antigen.

We thank Belinda Beanezy for technical assistance, Tim Hunkapillar for the computer analysis of the mouse μ gene, and Ann Rothstein and Steve Carson for cell lines. We thank Michael Ernest for suggesting the RNA isolation procedure and Ken Marcus and Frank Ruddle for experimental material and helpful discussions. This work was supported by National Institutes of Health Grants CA16885 and GM09966. M.C.Z. was supported by National Research Service Award 1 T32 AI-07086 from the National Institutes of Health. P.D.E. was a Leukemia Society of America Special Fellow. N.H.B. was a recipient of an American Cancer Society Faculty Research Award, ACS-FRA-196.