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Immunology

Rearrangements of chicken immunoglobulin genes in lymphoid cells transformed by the avian retroviral oncogene v-*rel*

(lymphocytes/differentiation)

LING CHEN*, MOON Y. LIM†, HENRY BOSE, JR.†, AND J. MICHAEL BISHOP*

*Department of Microbiology and Immunology and the G. W. Hooper Foundation, University of California, San Francisco, CA 94143; and †Department of Microbiology, University of Texas, Austin, TX 78712

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ABSTRACT **The retroviral oncogene v-*rel* transforms poorly characterized lymphoid cells. We have explored the nature of these cells by analyzing the configuration and expression of immunoglobulin genes in chicken hemopoietic cells transformed by v-*rel*. None of the transformed cells expressed their immunoglobulin genes. The cells fell into three classes: class I cells have their immunoglobulin genes potentially in an embryonic configuration; class II and class III cells have lost one copy of the λ light chain locus and have one copy of the heavy chain locus rearranged into a configuration that differs from what is found in mature B cells. In class II cells, the other heavy chain locus may be in embryonic configuration, whereas it is deleted in class III cells. The first of these classes may represent the earliest stage of the lymphoid lineage yet encountered among virus-transformed cells, whereas the second and third classes represent an apparently anomalous rearrangement whose origin remains unknown.**

The use of retroviruses to transform hemopoietic cells has been a valuable adjunct to the study of both normal hemopoiesis and the mechanisms of leukemogenesis. For example, explication of B-lymphocyte development has benefited greatly from studies on lymphoid cells transformed by Abelson murine leukemia virus and its oncogene v-*abl* (1–4). However, none of the cells transformed by v-*abl* seem to represent the earliest stages in the B-cell lineage, and these stages have been brought to view only rarely by other means (5). In the hope of obtaining additional access to the differentiation of immunologically competent cells (immunocytes), we have characterized chicken hemopoietic cells transformed by strain T of the avian reticuloendotheliosis virus (REV-T).

REV-T causes a fatal malignancy of lymphoid cells in several species of fowl and transforms hemopoietic cells in cultures of spleen or bone marrow (6–8). Neoplastic transformation by REV-T is mediated by the oncogene v-*rel*, which arose by transduction of the protooncogene c-*rel* from the genome of a turkey (9). The product of v-*rel* is a 59-kDa protein whose mechanism of action is not known (10). The nature of the hemopoietic cells transformed by REV-T is also an enigma (11–13): they proliferate indefinitely in culture, contain low levels of terminal deoxynucleotidyltransferase activity, express surface markers of B cells or occasionally of T cells, and typically fail to produce immunoglobulins of any sort. Therefore, it appeared that these cells might represent an early stage in normal immunocyte development.

Clues to the stage of immunocyte development can be obtained by analyzing the rearrangement and expression of immunoglobulin genes (14, 15). Therefore, we have examined the immunoglobulin genes in five lines of lymphoid cells transformed by REV-T. Our results indicate that some of these cells may represent an early stage in lymphoid cell development, whereas others contain apparently anomalous configurations of immunoglobulin genes.

MATERIALS AND METHODS

Cells and Viruses. SC-Hyline chickens were purchased from Hyline International (Johnston, IA). Cells from spleen or bone marrow of 2-week-old chicks, embryonic primitive streak, or embryonic spleen were transformed with REV-T and cloned as described (12).

Construction and Screening of a cDNA Library. Polyadenylylated RNA was prepared as described (16) from an IgM-producing cell line, R2B, and then was used to construct a cDNA library in the phage vector λgt10 by published procedures (17). Oligonucleotides representing the amino acid sequence of residues 157–164 of chicken μ heavy chain (18) and residues 127–134 of chicken λ light chain (19) were synthesized by the Biomolecular Resource Center at the University of California, San Francisco. These oligonucleotides were labeled with ^{32}P by using phage T4 polynucleotide kinase and [^{32}P]ATP (ICN) (20), and then were used to screen ≈5 × 10^5 λgt10 cDNA clones for cDNAs representing the μ chain and λ chains of immunoglobulin. Hybridization and washing were performed as described (21) except that hybridization was carried out in the presence of 35% formamide at 42°C.

Analysis of DNA with Restriction Endonucleases. The procedures for complete digestion of cellular DNA with restriction endonucleases, electrophoresis of resulting DNA fragments, transfer to nitrocellulose filters, and hybridization with ^{32}P-labeled DNA have been described (17). In our experiments, the DNA probes were prepared from purified DNA fragments by nick-translation.

Analysis of RNA. Polyadenylylated RNA, 5 μg per lane, was electrophoresed through agarose in the presence of formaldehyde (22). The RNA was then transformed to nitrocellulose filters and hybridized with the ^{32}P-labeled probe (17).

RESULTS

Isolation of cDNA Clones for Chicken Immunoglobulin μ Heavy Chain and λ Light Chain. To obtain probes for studying immunoglobulin genes in cells transformed by REV-T, we set out to clone representatives of the chicken genes for μ and λ chains (virtually all immunoglobulins in chickens contain λ rather than κ light chains). The chicken lymphoma cells R2B produce relatively large amounts of μ-class immunoglobulin (IgM) (23). Therefore, we used polyadenylylated RNA from these cells to prepare a cDNA library in the phage vector λgt10. Oligonucleotides for use as probes in screening the cDNA library were designed from

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Abbreviations: REV-T, strain T of reticuloendotheliosis virus; IgM, μ class of immunoglobulin; V, variable; J, joining.

published sequences of the chicken heavy and light chain genes (Fig. 1 *A* and *B*).

Three potential candidates for μ chain cDNA clones were subcloned into plasmid vectors and characterized by nucleotide sequencing. Comparisons of the sequences with results reported previously for chicken, mouse, and human immunoglobulin genes allowed the topography of the clones to be defined (Fig. 1*A*). One of the cDNA clones (pCM2) encompassed virtually the entire mRNA for a μ chain, whereas two others (pCM4 and pCM16) represented portions of the constant region (C_μ) of the protein and served as probes specific for that region. A full-length λ chain cDNA clone also was isolated, subcloned into a plasmid vector, and designated $p\lambda_{v+c}$. A subfragment of this clone that contains only constant region (C_λ) was further subcloned and designated $p\lambda_c$ (Fig. 1*B*).

Rearrangement of the Heavy Chain Locus in Cells Transformed by REV-T. In preliminary studies, we learned that the restriction endonuclease *Nco* I cleaves between the variable (*V*) and joining (*J*) regions of the chicken heavy chain locus in a manner that can be used to analyze rearrangements within the locus. Therefore, we used this enzyme to compare the configurations of the heavy chain loci in DNA from 6-day-old chicken embryos (the germ-line configuration), mature B-cells, and cells transformed by REV-T.

We first analyzed the germ-line (i.e., unrearranged) pattern of heavy chain genes in SC-Hyline chickens, the partially inbred flock from which all hemopoietic cultures were obtained for transformation by REV-T. The C_μ-specific probe, pCM4, hybridized with 20-kilobase pair (kbp) and 8-kbp fragments in embryo DNA cleaved by *Nco* I (Fig. 2, lane 1). To determine whether these two restriction fragments represent different alleles of a single heavy chain gene, we analyzed the germ-line pattern of immunoglobulin genes in the flocks from which the male and female parents for the SC-Hyline flock were drawn. The flock of the male parent proved to be uniformly homozygous for the 20-kbp *Nco* I fragment, whereas the flock of the female parent was homozygous for the 8-kbp *Nco* I fragment. Representative data are shown in Fig. 2 (lanes 2 and 3); identical results were obtained with five other members of each flock. We conclude that the restriction pattern in SC-Hyline chickens represents two alleles of a single C_μ region.

When DNA from the IgM-producing chicken lymphoma SC2L (derived from an SC-Hyline chicken) was probed with pCM4, the 20-kbp *Nco* I fragment was replaced by a 30-kbp fragment (Fig. 2, lane 4). By contrast, the 8-kbp *Nco* I fragment remained undisturbed. We cannot be certain that the allele represented by this fragment was not rearranged, however, because both *Nco* I sites that define the fragment lie within the C_μ region–one in the intron between $C_{\mu 1}$ and $C_{\mu 2}$ and the other in the coding domain for $C_{\mu 3}$ (Fig. 1*C*). When bursal DNA was digested with *Nco* I and probed with pCM4 (containing $C_{\mu 2}$ and $C_{\mu 3}$ cDNA sequences), we detected a smear ranging from 20 kbp to 30 kbp and an 8-kbp fragment (data not shown). The smear presumably represented polyclonal rearrangements of the heavy chain locus in bursal lymphocytes, of which >70% produce IgM (24).

Based upon the configuration of the C_μ region, cells transformed by REV-T were grouped into three classes (Table 1). Of the five cell lines examined, three retained both C_μ alleles potentially in germ-line configuration, as determined by *Nco* I digestion and hybridization with the pCM4 probe (Fig. 2, lane 5). These three cell lines were designated class I. All were derived from spleen cells (Table 2). Class II

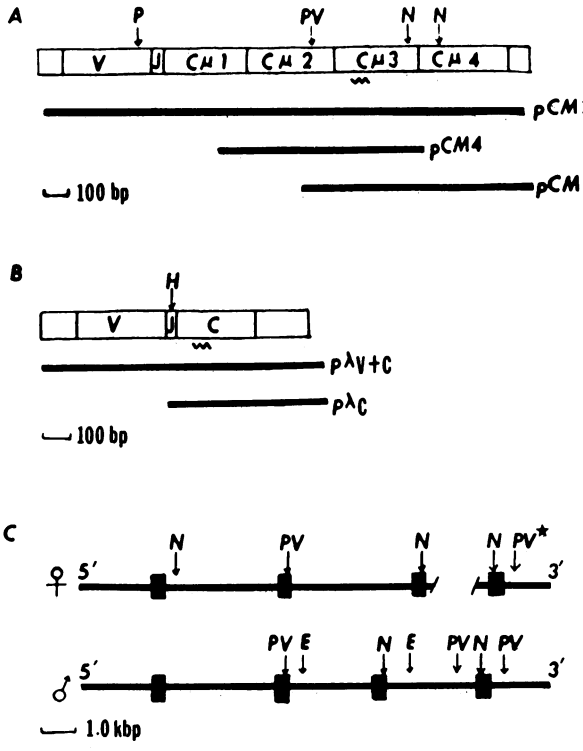

FIG. 1. cDNA clones for chicken μ heavy chain and λ light chain genes. cDNA clones for the μ chain (*A*) and the λ chain (*B*) were isolated as described in the text and analyzed by nucleotide sequencing. The maps of genomic C_μ regions of the female and male alleles (*C*) were derived from unpublished data of the authors. Wavy lines designate the location of oligonucleotide sequences used as probes in the molecular cloning. The locations of crucial restriction sites are indicated by vertical arrows. The restriction enzyme sites *Eco*RI, *Hpa* II, *Pst* I, *Pvu* II, and *Nco* I are denoted E, H, P, PV, and N. The filled boxes in *C* denote C_μ coding regions. The precise location of the *Pvu* I site marked with a star has not been determined.

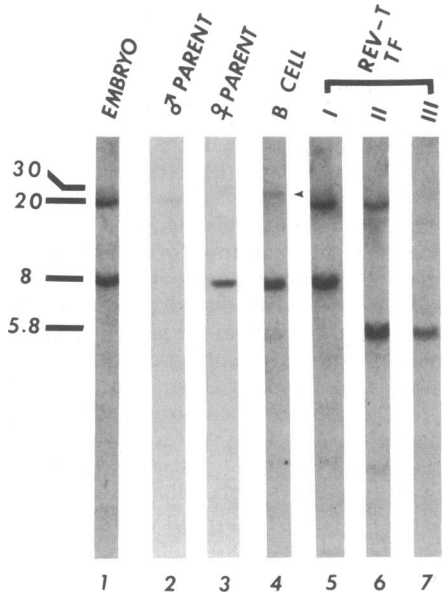

FIG. 2. Configuration of the heavy chain locus in normal and transformed cells. DNA digested with the restriction endonuclease *Nco* I was analyzed as described by using the plasmid pCM4 as a hybridization probe (see Fig. 1*A*). The sizes of DNA fragments are given as kbp. DNAs were obtained from: SC-Hyline chicken embryos (lane 1), peripheral blood cells of a chicken representing the male (lane 2) and female (lane 3) parentage of the SC-Hyline chickens, the IgM-producing cell line SC2L (lane 4), and three clonal lines of lymphoid cells transformed by REV-T (lanes 5–7).

Table 1. Configurations of immunoglobulin genes in cells transformed by REV-T

Class*	Configuration of H chain gene†	Configuration of L chain gene†	Number of cell lines
I	*G/G*	*G/G*	3
II	*G/R*	*G/D*	1
III	*D/R*	*G/D*	1

The immunoglobulin genes of five clonal lines of hemopoietic cells transformed by REV-T were analyzed as described. H, heavy; L, light.
*Cell lines were classified as described in text.
†Alleles: *G*, germ-line configuration; *R*, rearranged configuration; and *D*, deleted. The allele from the male parent is on the left and from the female parent is on the right.

comprised a single cell line, BB5, derived from chick spleen (Table 2), in which the 20-kbp *Nco* I restriction fragment remained undisturbed, but the 8-kbp *Nco* I fragment was replaced by a new 5.8-kbp fragment that presumably arose from rearrangement of the locus (Fig. 2, lane 6). The remaining cell line was derived from chick bone marrow and belonged to class III, in which the 20-kbp fragment and several fragments carrying *V* regions were lost—presumably by deletion of the locus (Fig. 2, lane 7; and data not shown)—and the 8-kbp fragment was again replaced by a 5.8-kbp fragment (Fig. 2, lane 7).

Rearrangement of the Heavy Chain Locus in Transformed Cells Is Consistent and Unusual. The pattern of *Nco* I fragments observed in all class II and class III tumor cells raised the possibility that a consistent but abnormal rearrangement affected the heavy chain locus in cells transformed by REV-T. We pursued this possibility by using additional restriction endonucleases to analyze the heavy chain locus in the transformed cells. Cleavage with *Eco*RI and *Pvu* II and hybridization with a C_μ-specific probe gave patterns with DNAs from embryos and IgM-producing lymphoma cells that were indistinguishable (Fig. 3, lanes 1 and 2). We presume that cleavage sites for these restriction enzymes lie between the *J* segments and the C_μ region (Fig. 1*C*); as a consequence, rearrangement could not be detected with these enzymes and a C_μ-specific probe. By contrast, the patterns obtained with DNA from cells transformed by REV-T could again be grouped into three classes: I, no detectable rearrangement of the heavy chain loci (Fig. 3, lane 3); II, one heavy chain locus rearranged to generate new patterns of restriction fragments that hybridize with the C_μ-specific probe (Fig. 3, lane 4); and III, one C_μ region rearranged and the other apparently deleted (Fig. 3, lane 5).

From these findings, we conclude that one of the μ chain genes is rearranged in class II and III transformed cells. This rearrangement differs from that found in IgM-producing B cells and may be identical in the two cell lines in which it occurs (Table 1), although small differences could not be detected by the restriction mapping used in our analyses. The other μ chain gene is apparently deleted in class III cells.

Table 2. Origins of cells transformed by v-*rel*

Origin of cell lines	Number of lines in class* I	II	III
Embryonic spleen†	2	0	0
Bone marrow‡	0	0	1
Spleen‡	1	1	0

*Cell lines were classified as described in the text.
†Derived from 14–18 day chicken embryos.
‡Obtained two weeks after hatching.

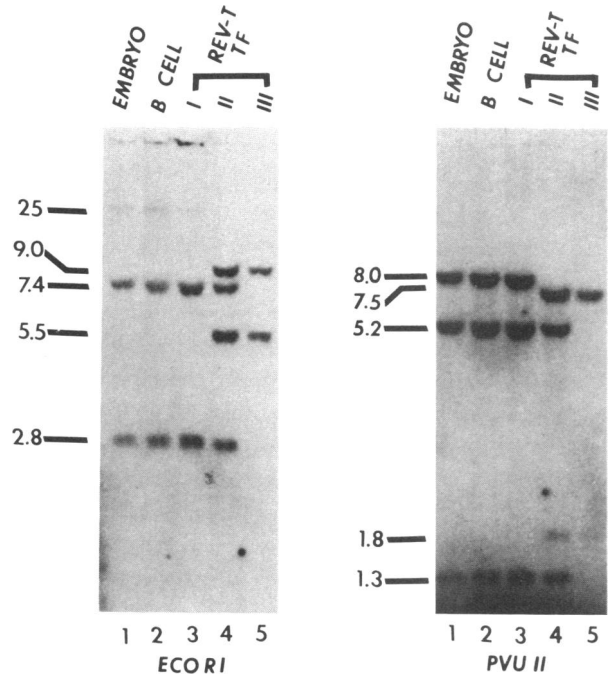

FIG. 3. Consistent rearrangements of heavy chain loci in cells transformed by REV-T. DNAs from the indicated sources were digested with the restriction endonucleases *Pvu* II and *Eco*RI and analyzed as described by using the plasmid pCM16 as a hybridization probe (see Fig. 1*A*). The sizes of DNA fragments are given as kbp.

Rearrangement of the λ Light Chain Locus in Cells Transformed by REV-T. The status of the λ chain locus in REV-T-transformed cells was also examined. Cleavage of DNA from SC-Hyline embryos with *Eco*RI and hybridization with $p\lambda_{v+c}$ gave the pattern of fragments shown in lane 1 of Fig. 4. Hybridization with a probe specific for the C_λ region ($p\lambda_c$) showed that this region was located mainly or entirely on the 18-kbp and 2.5-kbp *Eco*RI fragments (Fig. 4, lane 2). These two C_λ-containing fragments represented different alleles of the λ chain locus: one fragment derived from the male founders of the SC-Hyline flock (Fig. 4, lane 3), and the other, from the female founders (Fig. 4, lane 4).

When the λ chain loci of IgM-producing lymphoma cells were examined, the joining of the *V* and *J* regions was revealed by the appearance of a new *Eco*RI restriction fragment (14 kb) that hybridized to the λ_{v+c} probe (Fig. 4, lane 5). By the same strategy, cells transformed by REV-T could be classified into two groups that were subsumed by the three classes established previously for the heavy chain locus: (*i*) cells that had unrearranged C_μ (class I) also had both alleles of the λ chain gene in the germ-line configuration (Fig. 4, lane 6); (*ii*) cells of classes II and III had one allele of the λ chain locus in the germ-line configuration, whereas both *C* and *V* domains of the other allele were deleted, and this allele was always derived from the female parent of the SC-Hyline flock (Fig. 4, lanes 7 and 8).

Expression of Immunoglobulin Genes in Cells Transformed by REV-T. We analyzed the polyadenylylated RNA from various cell lines by using electrophoretic fractionation and molecular hybridization with specific cDNA probes. A 2-kilobase (kb) μ chain mRNA and a 1.2-kb λ chain mRNA were detected in chicken lymphoma cells that produce IgM (Fig. 5, lane 1). None of the cells transformed by REV-T contained detectable μ or λ chain mRNA (Fig. 5, lanes 2–6), including the BB5 cell line, which was reported previously to produce IgM (Fig. 5, lane 2). BB5 cells have been propagated in culture for a relatively long period, during which time their immunoglobulin

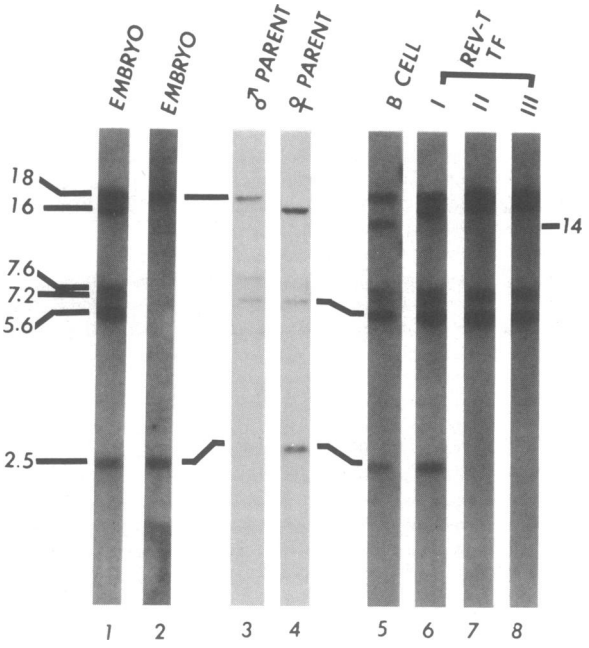

FIG. 4. Rearrangement of the λ chain locus in normal and transformed cells. DNAs were digested with *Eco*RI and analyzed as described by using the plasmids $p\lambda_{v+c}$ (lanes 1 and 3–8) and $p\lambda_c$ (lane 2) as hybridization probes (see Fig. 1*B*). DNAs were obtained from: SC-Hyline chicken embryos (lanes 1 and 2), male (lane 3) and female (lane 4) parentage of SC-Hyline chickens, the IgM-producing cell line SC2L (lane 5), and three clonal lines of lymphoid cells transformed by REV-T—the same as those analyzed in Fig. 2, lanes 5–7. The sizes of DNA fragments are given as kbp.

genes apparently rearranged spontaneously to the nonfunctional configuration described in the present work.

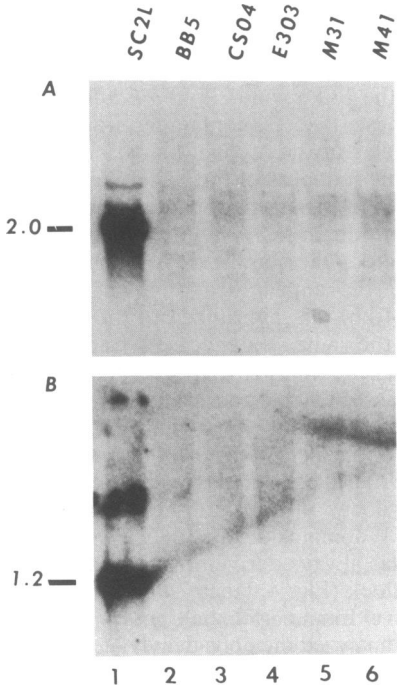

FIG. 5 Analysis of mRNAs for chicken μ chain and λ chain genes. Polyadenylylated RNA was isolated from the indicated cell lines and analyzed as described by using the plasmids pCM4 (*A*) and $p\lambda_{v+c}$ (*B*) as hybridization probes. The cell lines were SC2L (IgM-producing chicken lymphoma) and cells transformed by REV-T (CS04, class I; BB5, class II; E303, M31, and M41, class III lines derived from a single parental line). The sizes of RNAs are given in kb.

DISCUSSION

Immunoglobulin Genotypes of Cells Transformed by REV-T. We have found three distinctive configurations of immunoglobulin genes in cells transformed by REV-T (Table 1): class I, cells with both light and heavy chain loci in potentially germ-line configurations; class II, cells with one allele of the μ chain gene in germ-line configuration and the other allele rearranged; and class III, cells with one C_μ allele rearranged and the other deleted. In cells of class I, the λ chain genes are also in germ-line configuration. By contrast, cells of classes II and III have one allele of the λ chain gene deleted and the other allele in germ-line configuration. Although the number of cell lines is limited, it seems likely to us that class III cells arose from class II cells by deletion of the remaining heavy chain locus.

Only one example of class II cells is represented in the data presented here. But in preliminary studies, we have encountered several other examples. They were all derived by transformation of spleen cells, as in the present instance, but the rearrangement of the heavy chain locus differs from that described here. Further analysis will be required to determine how diverse the rearrangements might be and whether their abnormalities resemble those in the example of class II presented here.

Fortuitous polymorphisms at restriction endonuclease sites allowed us to distinguish the parentage of both the heavy and light chain loci in the SC-Hyline flock. These distinctions revealed that in class II and class III cells, the rearranged allele of the μ chain gene and the deleted allele of the λ chain gene were derived from the female parent. The significance of these correlations is not apparent, as yet.

What Are the Target Cells for Transformation by v-*rel*? The spleen, thymus, and bone marrow of young chickens contain hemopoietic cells susceptible to transformation by v-*rel* (12). What is the nature of these cells? Previous results had raised the possibility that they might be primitive lymphocytes not fully committed to the B-cell lineage (11–13). Our present findings are at least partially in accord with this notion: the transformed cells of class I appear to have a germ-line configuration of immunoglobulin genes. It is possible that these cells represent primitive lymphocytes whose differentiation was arrested by the action of v-*rel*.

The oncogene v-*abl* is also thought to arrest the development of lymphocytes, but the affected cells are clearly committed to the B-cell lineage and are more mature than the class I cells described here (1–4). We have yet to test whether the cells of class I can be induced by experimental means to differentiate to more mature lymphocytes. For the moment, it remains possible that the cells of class I represent the earliest stage of the lymphoid lineage yet encountered among virus-transformed cells.

Anomalous Rearrangements of Immunoglobulin Genes in Cells Transformed by v-*rel*. Our results reveal that two classes (II and III) of cells transformed by REV-T have one allele of the λ light chain locus deleted and one allele of the heavy chain locus rearranged into a configuration that is not observed in mature B cells. We do not know whether this configuration represents a previously unrecognized intermediate in the normal rearrangement of chicken immunoglobulin genes, or whether the configuration is an abnormality brought about by the action of v-*rel*.

It has been postulated that rearrangement of the chicken light chain locus is generally limited to a single allele and that cells with an unproductively rearranged or deleted gene are discarded (25, 26). In this view, the cell lines that possess a single unrearranged λ light chain locus may represent null cells that normally would die but that have been perpetuated by the action of v-*rel*. Preliminary work indicates that the rearranged heavy chain locus is anomalous and thus unlikely

to be the predecessor to a mature immunoglobulin gene. We also have found that the anomalous rearrangement of the μ heavy chain gene cannot be attributed to integration of a retroviral provirus in the vicinity of the heavy chain locus (data not shown). Aberrant immunoglobulin gene rearrangement has been reported in cells transformed by v-*abl* and in other lymphoid tumors (27–30), but we cannot say yet whether any of those rearrangements resemble the anomalous rearrangement reported here.

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