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Ongoing Diversification of the Rearranged Immunoglobulin Light-Chain Gene in a Bursal Lymphoma Cell Line

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The chicken immunoglobulin light-chain gene (Ig_L) encodes only a single variable gene segment capable of recombination. To generate an immune repertoire, chickens diversify this unique rearranged V_L gene segment during B-cell development in the bursa of Fabricius. Sequence analysis of Ig_L cDNAs suggests that both gene conversion events derived from V_L segment pseudogene templates (ψV_L) and non-template-derived single-base-pair substitutions contribute to this diversity. To facilitate the study of postrecombinational mechanisms of immunoglobulin gene diversification, avian B-cell lines were examined for the ability to diversify their rearranged Ig_L gene during in vitro passage. One line that retains this ability, the avian leukosis virus-induced bursal lymphoma cell line DT40, has been identified. After passage for 1 year in culture, 39 of 51 randomly sequenced rearranged V-J segments from a DT40 population defined novel subclones of the parental tumor. All cloned V-J segments displayed the same V-J joint, confirming that the observed diversity arose after V-J rearrangement. Most sequence variations that we observed (203 of 220 base pairs) appeared to result from ψV_L-derived gene conversion events; 16 of the 17 novel single nucleotide substitutions were transitions. Based on these data, it appears that immunoglobulin diversification during in vitro passage of DT40 cells is representative of the diversification that occurs during normal B-cell development in the bursa of Fabricius.

Antibody diversity can be generated by a number of distinct molecular mechanisms (for reviews, see references 1, 11, 17, and 27). In mammals, the primary immune repertoire is generated by the assembly of functional immunoglobulin genes through rearrangement of distinct genetic elements. An immunoglobulin heavy-chain (Ig_H) gene is assembled in each B cell from an assortment of variable (V), diversity (D), and joining (J) elements. A functional immunoglobulin light-chain (Ig_L) gene is assembled from an assortment of V and J sequences. Because of the large number of individual V, D, and J elements in the germ line, this rearrangement process can lead in mammals to the generation of over 10^6 distinct immunoglobulin molecules. Additional diversity is created by variability in the joining position of individual elements and by the addition of non-germ line base pairs (N diversity) between the elements during the molecular process of rearrangement. After rearrangement, there is evidence for additional sequence modification of rearranged immunoglobulin genes, particularly during immune responses to specific antigens. This process, termed somatic hypermutation, generates an additional level of diversity and may be responsible for increasing the antigen affinity of the B cell during an immune response.

In contrast to mammals, chickens have an extremely limited ability to create an immunological repertoire by recombination. Chickens have single functional V and J elements in both their Ig_H and Ig_L loci (18, 20). Immunoglobulin gene rearrangement during early embryogenesis serves primarily to generate a pool of immunoglobulin-positive B cells (bursal stem cells), which undergo clonal expansion within the bursa of Fabricius (12, 28). During the bursa-dependent stage of B-cell development, an immunological repertoire is created by progressive sequence substitution within the rearranged V_L and V_H gene segments of these proliferating immunoglobulin-positive cells (19, 26). The avian Ig_L locus is therefore ideal for the study of events that can lead to immunoglobulin gene diversification after the rearrangement process.

Mammalian pre-B cells transformed with Abelson leukemia virus have proven extremely useful in studying immunoglobulin gene rearrangement (2). By analogy, we reasoned that transformation of bursal lymphocytes undergoing diversification of their rearranged immunoglobulin genes might provide a means to study postrecombinational diversification mechanisms. In chickens, infection of the bursae of susceptible birds with avian leukosis virus (ALV) results in the development of lymphomas (4, 6). ALV-induced lymphomas develop in a series of distinct stages, beginning with a preneoplastic proliferation of lymphocytes within individual bursal follicles and leading to a lesion termed the transformed follicle (4, 6). Cells within a transformed follicle can undergo further malignant progression, giving rise to discrete bursal nodules and later to metastasizing lymphomas. In over 90% of these clonal neoplasms, ALV has integrated near the c-*myc* proto-oncogene, resulting in deregulation of c-*myc* expression (7, 24). Transformed follicle cells with deregulated c-*myc* genes maintain the ability to home to the bursa of Fabricius, proliferate continuously within the bursal environment, and diversify their rearranged Ig_L genes, all characteristics of the bursal stem cell (25). Thus, deregulation of the *myc* gene during B-cell development in the bursa appears to block differentiation and results in the expansion of the bursal stem cell population.

DT40 is an ALV-induced bursal lymphoma cell line isolated by soft agar cloning after serial passage of the primary tumor in vivo (3). It is novel in that it lacks a normal c-*myc* gene but instead contains two copies of an ALV-deregulated *myc* gene, perhaps as a result of a mitotic recombination between the two parental alleles. At the Ig_L locus, DT40

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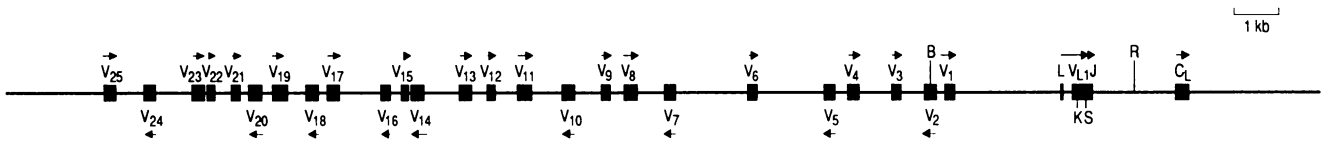

FIG. 1. Organization of the rearranged chicken Ig_L locus as described by Reynaud et al. (19); relevant polymorphic restriction sites have been added. Deletion of 1.8 kb of DNA between V_{L1} and J occurs during V-J joining to produce a functional Ig_L gene. In the 20 kb 5′ of the single functional V element, there are 25 V_L region pseudogenes (V_1 through V_{25}), all of which lack transcriptional signals, leader exons, and recombination signal sequences (19). The two parental alleles of the SC chicken (designated G4 and S3) are distinguished by the presence of a polymorphic *Bam*HI (B) site in $\psi V_L 2$ and an *Eco*RI (R) site in the intron between J and C_L. The parental G4 allele contains both of these two restriction sites and is rearranged in the DT40 line. Two 6-base-pair restriction endonuclease sites are present in the V_{L1} gene sequence of both parental alleles, an *Sca*I site (S) located in CDR3 and a *Kpn*I site (K) located at the end of CDR1. Previous work has shown that both of these restriction endonuclease sites are frequently lost from the rearranged but not the unrearranged V_{L1} gene segments during B-cell development in the bursa of Fabricius (26). Although the rearranged allele in the DT40 cell line was found to lack the V_{L1} *Sca*I site, early passages of the cell line demonstrated the presence of a *Kpn*I site in the rearranged V_{L1} gene segment of most of the cells. In contrast, the majority of cells had lost the *Kpn*I site in their rearranged V_{L1} gene segment after passage in culture for 1 year.

contains one rearranged and one unrearranged allele. Based on restriction site analysis, we previously reported that the DT40 cell line continues to diversify the variable region of its rearranged Ig_L allele (25). In this report, we further characterize the process of postrecombinational sequence diversification within the DT40 cell line. Sequence analysis provides evidence of continued and rapid diversification of the V_{L1} gene segment during in vitro passage. Of 51 clones isolated after in vitro passage for 1 year, 39 demonstrated unique rearranged V-J gene segments. Within these 39 clones, 203 of 220 nucleotide substitutions could be accounted for by segmental gene conversion of the rearranged V_{L1} gene segment, utilizing ψV_L gene segments located 5′ from the rearranged V_{L1} gene segment as sequence templates. Seventeen of the 220 nucleotide substitutions could not be accounted for by sequences present in the ψV_L gene pool. These may be products of an untemplated mechanism of sequence diversification. Sixteen of these 17 nucleotide substitutions were transitions. A similar bias of transitions over transversions has been observed in untemplated nucleotide substitutions found in V-J gene segments during normal bursal development (19). Based on these data, it appears that the avian lymphoma cell line DT40 is ideal for the study of somatic diversification mechanisms that generate Ig_L diversity after immunoglobulin gene rearrangement.

MATERIALS AND METHODS

Cell line. The cell line LSCC-DT40 (referred to as DT40) was obtained by single-cell cloning in soft agar after two in vivo transfers of an ALV-induced bursal lymphoma (3). The original lymphoma was induced by viral infection of the susceptible Hyline SC chicken line with 2×10^5 IU of Rous-associated virus-1 injected intravenously at 1 day of age. The Hyline SC chicken line is an F_1 cross between two inbred B2 chicken lines as previously described (25). DT40 cells were passaged three times weekly to maintain them at a cell concentration of 0.2×10^6 to 2×10^6/ml in Dulbecco modified Eagle medium supplemented with 10% fetal calf serum and 2% chicken serum.

DNA extraction and Southern blot analysis. DNA preparation and hybridization methods were as described previously (26). Hybridization probes were a 1.2-kilobase (kb) *Eco*RI-*Sal*I genomic fragment containing the Ig_L constant region and a 2.4-kb *Sst*I genomic fragment containing the c-*myc* second and third exons (25).

Isolation of rearranged V-J segments. The polymerase chain reaction (PCR) was used to rapidly clone multiple independent V-J coding joints from DNA isolated from the DT40 cell line growing in culture. The polymerase chain reaction utilized primers that were located 5′ of V_L and 3′ of J_L, as previously described (12, 13). *Eco*RI and *Hin*dIII restriction sites at the 5′ ends of these oligonucleotide PCR primers allow directional cloning into pGEM3Z. A titration of the PCR reaction was performed before the products were cloned to ensure that the number of cycles did not exhaust the available nucleotide or primer pools. Cloning was done from reaction products taken at least three cycles before the loss of exponential increase in the PCR products to minimize base-pair incorporation errors, which may occur during limited substrate availability. PCR reactions contained 1 μg of DNA template, 1 μg of each primer, 0.1 mM each deoxynucleoside triphosphate, 10 mM Tris hydrochloride (pH 8.3), 50 mM KCl, 1.5 mM $MgCl_2$, 0.01% gelatin, and 2 mM dithiothreitol. PCRs were performed with 2.5 U of Taq polymerase (Cetus Corp.) in a Perkin-Elmer thermocycler for 24, 27, 30, and 33 cycles. PCR products for the rearranged Ig_L genes were digested with *Eco*RI and *Hin*dIII and cloned into pGEM3Z (Promega Biotec Co.). Random individual clones were used for the analysis of V-J segment diversification within the DT40 cell line.

DNA sequencing. Double-stranded DNA sequencing of both strands was performed by using a Sequenase kit according to the protocols of the supplier (U.S. Biochemical Corp.). The DNASTAR software package was used for sequence data analysis.

RESULTS

The DT40 cell line undergoes loss of the *Kpn*I restriction site within the rearranged V_{L1} gene segment during in vitro passage. The chicken Ig_L locus consists of a single V_{L1} gene segment capable of rearrangement to a single functional J_{L1} segment located 1.8 kb downstream (Fig. 1) (18). In-frame rearrangement of this V-J joint in conjunction with an in-frame Ig_H rearrangement renders a primary B cell capable of proliferation within the environment of the bursa of Fabricius (12). B cells proliferating within the bursa of Fabricius display evidence of undergoing significant sequence diversification to generate the primary immune repertoire of the chicken by both sequence analysis and evidence of restriction site modification of the unique rearranged V_{L1} gene segment (19, 26). The majority of sequence information acquired in the rearranged V_{L1} gene segment appears to be derived from the ψV_L gene segments located 5′ from the V_{L1} gene segment. The mechanism for this segmental transfer of sequence information appears to be intrachromosomal gene conversion (L. M. Carlson, W. T.

McCormack, C. E. Postema, E. H. Humphries, and C. B. Thompson, Genes Dev., in press). In addition, some single-base-pair modifications for which there are no donors in the ψV_L pool have been observed (19).

The DT40 cell line was derived by ALV infection of bursal lymphocytes from an SC bird, an F_1 cross of two inbred chicken strains, designated G4 and S3. It is possible to distinguish between the two parental Ig_L alleles by restriction site analysis with polymorphic *Bam*HI and *Eco*RI sites, as previously described (25). Based on this analysis, we determined that the G4 parental Ig_L allele had recombined in the DT40 cell line (data not shown). To test for sequence modification in vitro, the presence or absence of the germ line-encoded *Kpn*I and *Sca*I restriction endonuclease sites in the rearranged V_{L1} gene segment were analyzed during in vitro passage of DT40. DNA was obtained from two time points after soft agar cloning, one early after cloning and the other after passage for 1 year in vitro. As previously reported (25), all cells at both time points lacked an *Sca*I site in their rearranged V_{L1} gene segment. In contrast, the majority of cells at the early passage retained a *Kpn*I site in their rearranged V_{L1} gene segment, whereas greater than 90% of the cells at the later time point had lost the *Kpn*I site (data not shown). To rule out the possibility that outgrowth of a contaminating cell had occurred, the DT40 cell line at both time points was examined for ALV integration, resulting in modification of the c-*myc* locus. Hybridization of a c-*myc*-specific probe to Southern blots of DT40 DNA digested with *Eco*RI revealed that the cells at both time points had lost both normal alleles of the c-*myc* gene. Instead, a novel restriction fragment (created by the integration of an ALV genome within the c-*myc* locus) was observed. Careful quantitation suggested that two copies of this altered c-*myc* gene were present within the DT40 cell line at both time points (data not shown).

The rearranged V_{L1} gene segment of the parental DT40 cell undergoes sequence diversification. To determine the basis for the disappearance of the *Kpn*I site within the rearranged V_{L1} gene segment during in vitro passage, rearranged V-J segments from both DNA samples were isolated by PCR. Rearranged V-J segments were sequenced in their entirety from 8 clones derived from DT40 DNA obtained soon after single cell cloning and from 51 clones isolated from DT40 DNA obtained from cells passaged for 1 year in culture. All clones obtained could be demonstrated to be from the parental G4 allele on the basis of sequence polymorphisms in the invariant leader intron.

All eight of the early-passage clones retained the *Kpn*I site, as expected from the Southern blot results. Three of eight clones obtained from the early-passage DT40 DNA were identical and contained the fewest nucleotide substitutions. Therefore, one of these sequences, E5, was assigned as the parental sequence for the DT40 cell line for subsequent analysis purposes. The sequence of clone E5 was compared with the germ line sequence of the G4 parental allele (Fig. 2). The V_{L1} gene segment of clone E5 had undergone 42 base pairs of sequence modification relative to the germ line G4 V_{L1} gene segment, including a 15-base-pair insertion within the middle of the first complementarity-determining region (CDR1). As previously reported (19), it appears that many of these sequence substitutions could have been from template sequences present in the ψV_L gene cluster 5′ from the active Ig_L gene locus. A potential scheme for the derivation of the sequence alterations within E5 based on ψV_L sequence information (19; W. McCormack and C. B. Thompson, unpublished data) is depicted in Fig. 2. Of the 42 modified base pairs, 40 could have arisen through the transfer of sequence information from the pseudogene cluster to the rearranged V_{L1} gene segment by a series of seven independent gene conversion events. Two single-nucleotide substitutions, G-to-A substitutions in codons 25 and 88, do not appear to have obvious sequence donors in the germ line. The novel base pair sequence substitution in codon 88 and the position of the V-J joint within codon 93 are shared by all V-J rearrangements cloned from both early- and late-passage clones, further demonstrating that all of the events we have analyzed are the result of sequence modification of a uniquely rearranged and diversified gene segment.

In vitro passage of DT40 leads to template-derived sequence substitutions of the rearranged V_{L1} gene segment in the absence of selection. Fifty-one clones were isolated from DT40 DNA after passage for 1 year in culture. Of these, 39 had undergone independent sequence diversification as demonstrated by sequence analysis of the rearranged V-J gene segment. In contrast to the early-passage DT40 clones, only 4 of 51 late clones had *Kpn*I sites within the rearranged V_{L1} gene segment. Based on the nucleotide sequence modifications that we observed in the late clones, it was possible to derive a clonal lineage similar to those that have been derived for mammalian B cells exhibiting somatic diversification (hypermutation) in vivo in response to antigen stimulation (Fig. 3) (17). Based on this analysis it appeared that Ig_L diversification occurred in sequential steps in several branches of the lineage, suggesting that DT40 cells are undergoing progressive sequence modification events. Most sequence modifications were apparently derived from base pairs present within the ψV_L gene cluster. Examples of such template-derived substitutions derived from ψV_L gene segments are depicted in Fig. 4. The six examples shown illustrate sequence substitutions in both CDR1 and CDR2. In CDR1, this can lead to the complete or partial deletion of the 15-base-pair insertion derived from $\psi V_L 8$ in the E5 clone. In one case (data not shown), an increase in the size of the insertion by ψV_L gene sequence substitution was also observed. Interestingly, in contrast to the frequent observation of sequence substitutions in CDR3 of the V_{L1} gene segment during B-cell development in the bursa of Fabricius (19), we observed only two gene conversion events within CDR3. Twenty-two of the template-derived sequence substitutions we observed could be assigned to unique pseudogene donors. Five of these events involved ψV18 sequences (e.g., Fig. 4D). No other pseudogene was definitively implicated in more than two events.

Non-template-derived single-base-pair mutation also diversifies DT40 cells in culture. Not all of the sequence substitutions that were observed in the late-passage DT40 clones could be accounted for by base pairs present in the ψV_L gene segments. Seventeen base pairs of modification derived from 14 independent clones were found to have no apparent template in the ψV_L gene region. In all instances, these base pairs appeared to have arisen as novel single-base-pair modifications in contrast to the putative template-derived gene conversion events, which frequently occurred as large blocks of linked sequence substitutions. One striking aspect of these single-base-pair modifications is that most (16 of 17) were transitions. The positions of these transitional events are depicted in Fig. 5. In contrast to template-derived sequence substitutions, these single-base-pair modifications were not restricted to the rearranged V_{L1} gene segment but were also observed in the J_L segment. Although it is possible that these non-template-derived sequence substitutions are

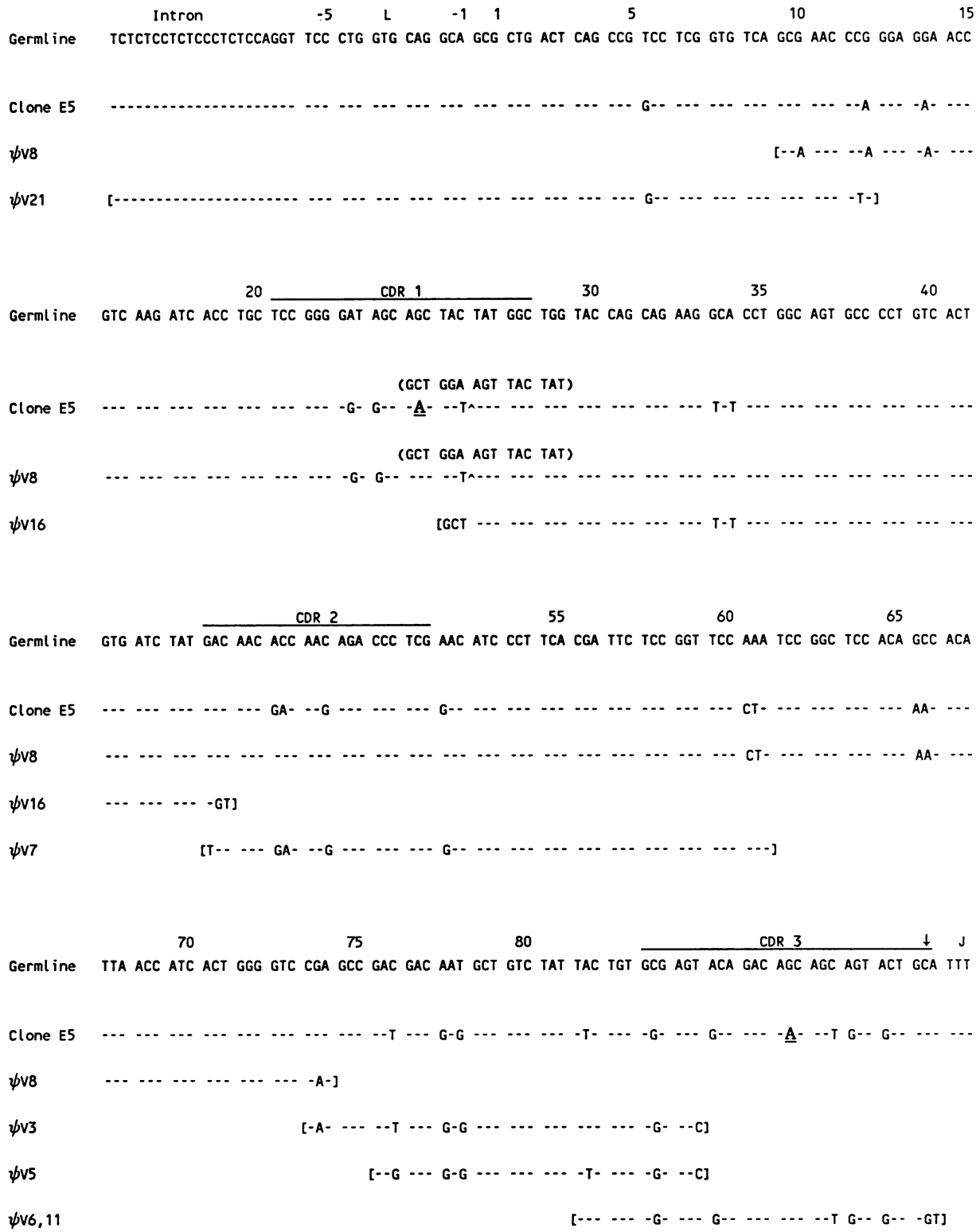

FIG. 2. Nucleotide sequence of DT40 obtained from an early passage. Clone E5, from early-passage DT40 DNA, is compared with the germ line V_{L1} sequence of the G4 allele (top line). Identity to germ line sequence is indicated by dashes. Positions of the 5′ V_{L1} intron, partial leader peptide (L), CDR domains, and J segment are indicated. On the lines below the clone E5 sequence are depicted sequences of potential ψV_L gene donors for sequences that have been acquired by the parental DT40 cell. All but 2 of the 42-base-pair nucleotide substitutions found in clone E5 can be accounted for by sequence information present in the ψV_L gene segments. These two substitutions, a G-to-A substitution at the second position of codon 25 and a G-to-A substitution in the second position of codon 88, apparently represent novel nucleotide sequence substitutions at these positions. All early- and late-passaged DT40 cell clones contain both the A at codon 88 and a V-J joint located after the second position of codon 93 (arrow).

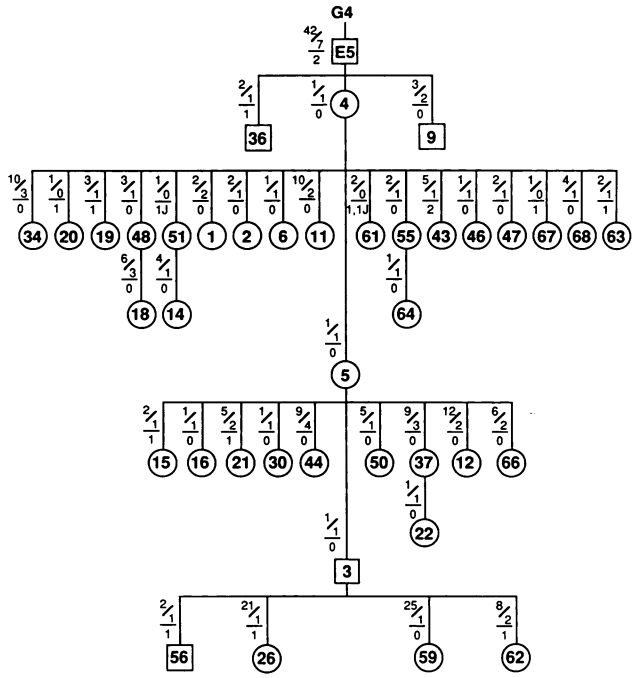

FIG. 3. Hypothetical lineage for the relationship of DT40 V-J gene segment subclones. All clones were derived from the parental G4 allele. The original transformed allele had undergone significant sequence substitution as demonstrated by clone E5 (Fig. 2). Clones that have retained a *Kpn*I site at the end of CDR1 are boxed, and clones that have lost the *Kpn*I site at this position are circled. At each branch, the number of base pairs that have been altered (*a*), the number of potential gene conversion events to account for these substitutions (*b*), and the number of base pairs not accounted for by such conversion events (*c*) are indicated as follows: $\frac{a/b}{c}$ (J designates base pair alterations in the J segment). Of 51 clones sequenced, 39 are independent and represented in the tree. Of the other 12 clones, 9 were identical to clone 4, 1 was identical to clone 18, 1 was identical to clone 48, and 1 was identical to clone 59.

the result of Taq polymerase errors, there are several reasons why we feel that this is unlikely. First, the PCR products were cloned at a point when substrates were not limiting, as described in Materials and Methods. Under these conditions, we have not observed a similar rate of non-template-derived sequence substitution as a result of polymerase errors in other cloned segments (W. McCormack and C. Thompson, unpublished data). Second, in separate experiments we have used extensive PCR amplification to rescue V-J joints from early embryonic tissues (12). Under these conditions, observed sequence substitutions involved the same base pairs substituted at the same position within the rearranged V-J segments. In contrast, the non-template-derived single-base-pair modifications in the DT40 subclones were distributed throughout the V-J segment and apparently involved all 4 base pairs with equal frequency. Third, several of the non-template-derived base-pair modifications we observed occurred in multiple clones. For example, the G-to-A substitution in codon 88 of E5 was observed in all clones, the G-to-A substitution in codon 25 was observed in 34 clones, and the T-to-C substitution in the J_L segment was observed in both clones 51 and 14.

DISCUSSION

In vitro cell lines have proven extremely useful in the past in understanding various aspects of gene regulation, recombination, and modification. In the present experiments we attempted to establish DT40 as a cell line in which studies could be performed to address the mechanism(s) by which postrecombinational immunoglobulin gene diversity is generated (10, 29). Several of our observations support the use of the DT40 cell line for such a purpose. (i) DT40 undergoes a significant rate of sequence modification during in vitro passage. (ii) Most sequence modification events in the rearranged V_{L1} allele of DT40 occur as a result of gene conversion events utilizing sequence information present in the ψV_L cluster. (iii) Additional sequence modification occurs as a result of non-template-derived single-base-pair substitutions within the rearranged V-J segment. These data suggest that DT40 will be an ideal cell line for the study of gene conversion and single-base-pair mutation in the generation of immunoglobulin sequence diversity in developing B cells.

As previously reported, the ability of DT40 to continue to diversify the Ig_L gene in culture is consistent with the observation that v-*myc*-containing retroviruses specifically transform the bursal stem cell (25). This cell type has the ability to home to the bursa of Fabricius and undergo immunoglobulin gene diversification within that environment. Using a v-*myc*-containing retrovirus, we found that this arrest of differentiation was leaky, because during serial passage in chickens many cells would differentiate without developing into a fully transformed cell (15). This is consistent with the observation of Langdon and colleagues (9) that B cells in transgenic mice with a *myc* gene under the control of the Ig_H enhancer are only partially arrested at the pre-B-cell stage of development. One possibility for the continued ability of DT40 to diversify the rearranged V_{L1} gene segment during in vitro passage may be that DT40 has been more completely arrested at this stage of B-cell development as a result of the loss of both normal c-*myc* alleles. Instead, DT40 has a duplication of its ALV-deregulated c-*myc* gene. The ability of DT40 to diversify in culture is in marked contrast to the lack of immunoglobulin gene diversification observed in chicken B-cell lymphomas that have been induced through transformation with a v-*rel*-containing retrovirus (5). Under these conditions, over prolonged culture in vitro no evidence of a significant rate of sequence modification within the rearranged Ig_L gene has been obtained by either restriction site analysis or by sequencing (C. Postema and C. B. Thompson, unpublished data). Although a recent report has been published suggesting that some v-*rel* cell lines may retain the ability to diversify immunoglobulin genes (30), the frequency at which these events were reported to occur was extremely low. We have been unable to duplicate these results in our own laboratory. It is possible that a difference in the helper virus that was used to encapsulate the defective v-*rel*-containing retrovirus may account for some of these observed differences. Alternatively, there may be differences in the in vitro propagation of the cell lines that allows for a low spontaneous rate of immunoglobulin gene rearrangement and diversification to occur in some v-*rel* cell lines. It will be of interest to see whether the reported diversification of these v-*rel* cell lines can be confirmed at the nucleotide level.

Several novel aspects of the template-derived sequence substitution events observed in the late DT40 clones were noted in comparison to those observed in randomly cloned B cells during normal B-cell development in the bursa of Fabricius. The most common ψV_L donor in the DT40 clones is $\psi V_L 18$, which accounts for 5 of 22 of the substitution events that could be assigned to a single pseudogene donor. In contrast, in randomly identified immunoglobulin clones

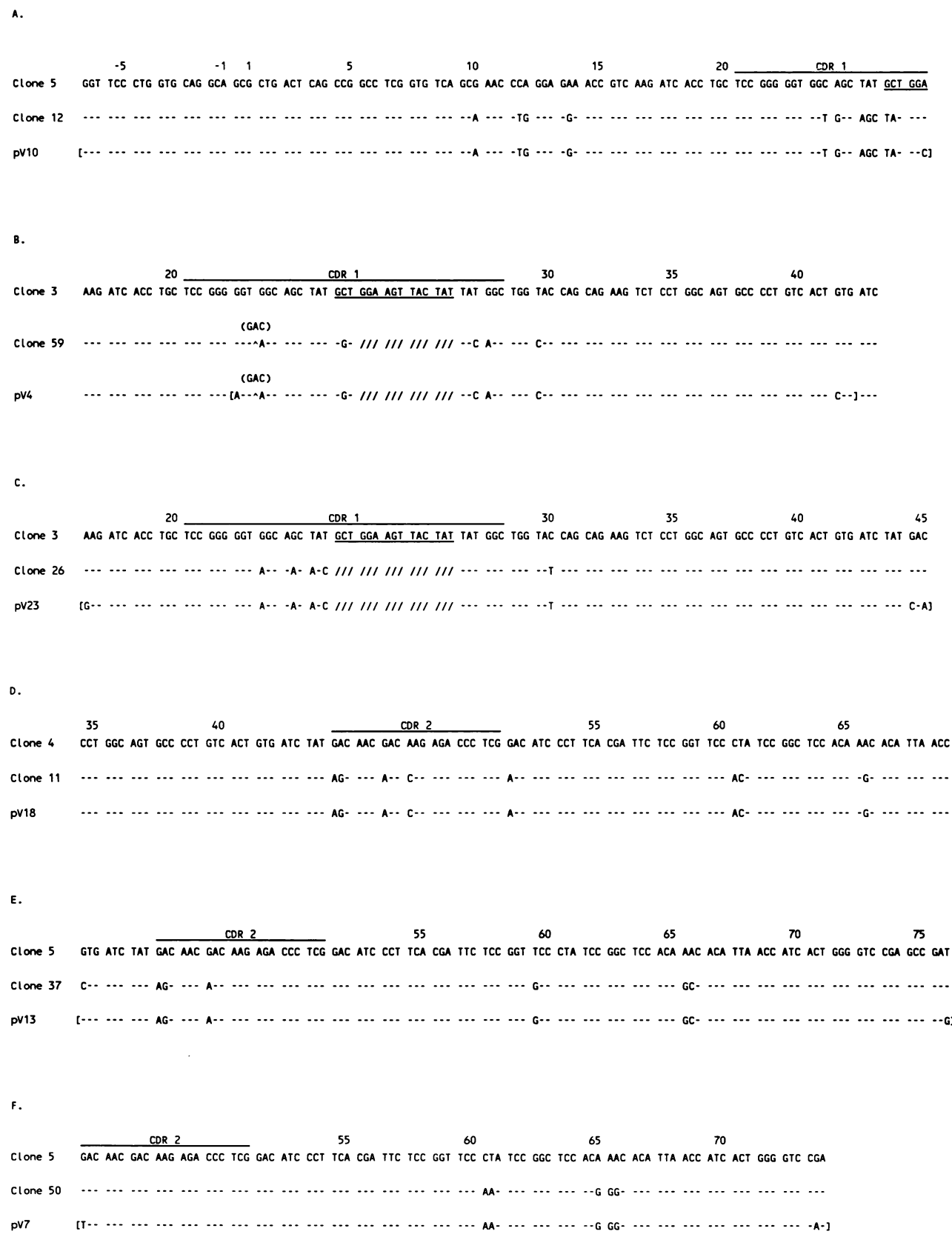

FIG. 4. Template-mediated sequence substitutions observed in the rearranged Ig_L gene of DT40 subclones: examples of sequence substitutions observed in individual subclones of late-passage DT40 cells. Subclone sequences are compared with the sequences of the clone from which they were derived as determined by the lineage diagram in Fig. 3. Underlined sequences represent the 15-base-pair insertion found in CDR1 present in E5 (/, absent base pair). Potential ψV_L gene templates for these gene conversion events are indicated below each subclone sequence.

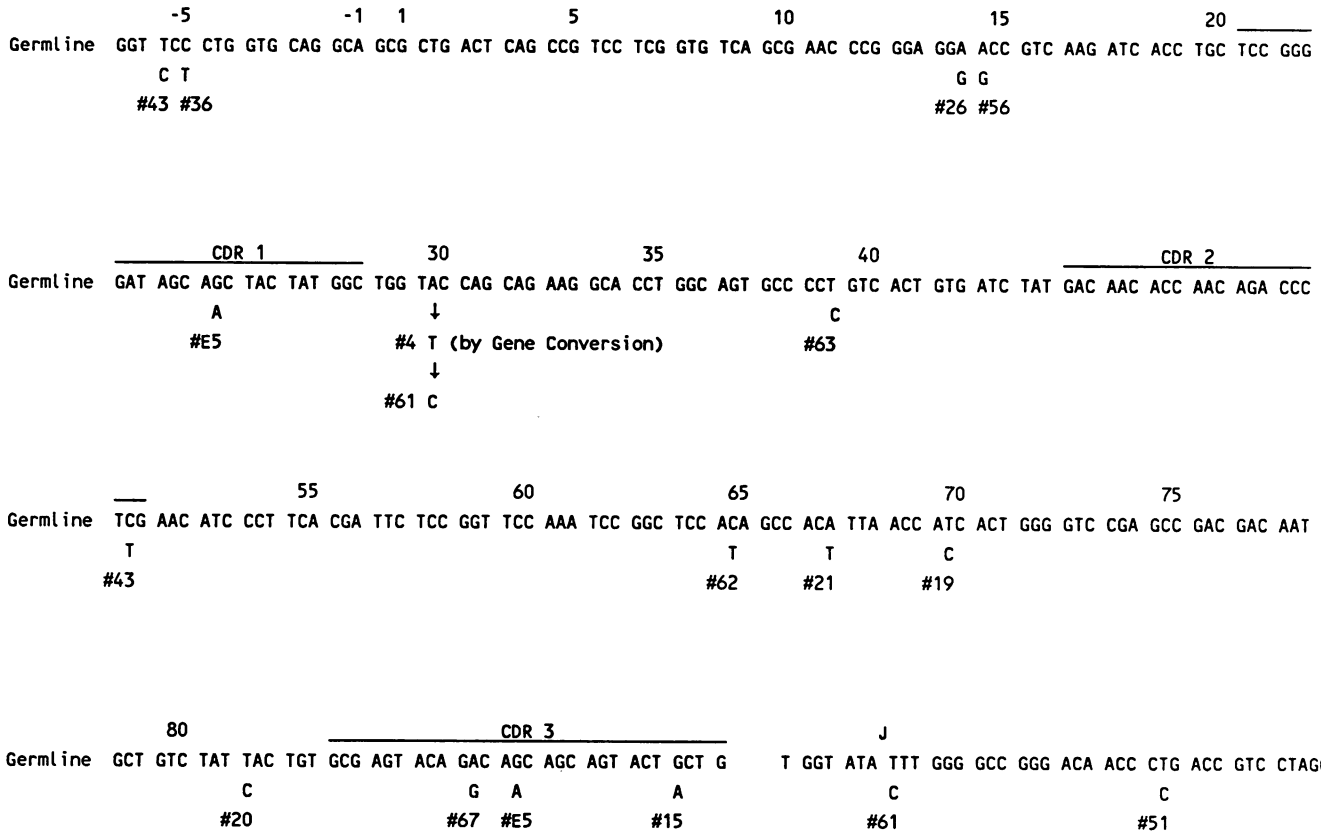

FIG. 5. Sequence of the V_{L1} and J_L segments of the germ line G4 allele are indicated. Positions in the late-passaged DT40 cell line that have undergone sequence modifications that cannot be accounted for by ψV gene conversion are indicated. Both the base-pair alteration and the clone in which the alteration first occurred are indicated.

less than 10% of the events appeared to be derived from $\psi V_L 18$ (W. McCormack and C. Thompson, unpublished data). This is of interest because $\psi V_L 18$ contains a 15-base-pair insertion in CDR1 that is similar to the $\psi V_L 8$-derived CDR1 insertion found in the parental DT40 line. This suggests that the 15-base-pair insertion in CDR1 of DT40 may have altered the ability of pseudogenes to function as efficient donor templates for sequence substitutions as a result of an alteration in the size of CDR1. Furthermore, there are fewer template-derived sequence substitution events observed in CDR3 of the DT40 clones than in normal developing B cells. This suggests that the alteration of the size of the CDR1 domain in DT40 also affects the ability of ψV_L segments to serve as donors in CDR3. Alternatively, sequence modification in CDR3 in clone E5 may have disrupted the normal ability of this region to undergo further sequence modification. Although the pattern of sequence substitutions within the V-J segment of the DT40 cell line appears to be slightly altered with respect to the pattern of sequence diversification observed in developing bursal lymphocytes, this may result from an alteration based on the rearranged V_{L1} gene sequence of DT40 at the time the parental cell was transformed. Studies of additional cell lines should allow us to resolve these issues.

Single-base-pair sequence modification has also been observed during normal B-cell differentiation in the bursa of Fabricius (19). The single-base-pair modifications that we observed could be the result of Taq polymerase errors. Taq polymerase errors have been observed at a rate of 2×10^{-4} base pairs per cycle (8, 14, 23). Most reports of polymerase errors show an increase in transitions relative to transversions that is similar to that observed in our data. In addition, Taq errors have been shown to have a strong predominance for A:T to G:C transitions (8). This bias was not observed in our clones, suggesting that the base-pair substitutions arose within the cells rather than as a result of PCR amplification. However, although we have attempted to eliminate the possibility of Taq polymerase errors, it is impossible to exclude polymerase errors as a cause for some of the sequence substitutions we observed. Similar sequence substitutions have been observed during normal B-cell development in the bursa of Fabricius. In 12 cDNA clones analyzed by Reynaud et al. (19), 8 of 214 base-pair changes from the germ line were found not to have a template-derived donor in the ψV_L cluster. In six of these eight instances, the sequence substitutions were the result of transitions, showing a significant bias toward transitions over transversions. Because the rearranged Ig_L clones analyzed by Reynaud et al. were generated by cDNA cloning rather than by PCR, it appears that this bias of transitional mutations may reflect a natural process occurring during gene conversion. This suggests that somatic hypermutation may be ongoing in the avian Ig_L locus by a process analogous to that observed in mammalian species. In support of this Reynaud et al. (19), Parvari et al. (16), and ourselves (this report) have observed single-base-pair substitutions in the J segment, a region not involved in template-mediated sequence substitutions. Alternatively, the process of template-derived sequence substitution may result in the substitution of non-template-derived mutations by heteroduplex repair of single-stranded intermediates that

form hairpin loops, as described previously for bacterial systems (21, 22).

Based on the data presented here, it appears that the DT40 cell line is capable of undergoing significant sequence modification during in vitro passage. The observed sequence modifications occur frequently in clusters, and 203 of 220 base-pair modifications appear to be derived from sequences for which there are templates in the ψV_L region. In addition, non-template-derived single-base-pair mutations have also been observed in cells obtained after in vitro passage of DT40. Thus, the DT40 cell line should prove to be an ideal cell line in which to perform further studies to characterize in detail the molecular mechanisms of gene conversion and single-base-pair mutation in the generation of Ig_L diversity in avian species.

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ADDENDUM IN PROOF

Using the DT40 cell line supplied by E. H. Humphries, Buerstedde et al. (EMBO J. 9:921–927, 1990) have independently demonstrated by sequence analysis that ALV-induced bursal lymphomas and the DT40 cell line continue to diversify their rearranged Ig_L alleles.

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