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Stochastic rearrangement of immunoglobulin variable-region genes in chicken B-cell development

(immunoglobulin gene rearrangement/pre-B cells/bursa of Fabricius/transformation)

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ABSTRACT **The molecular mechanism by which immunoglobulin (Ig) gene rearrangement occurs is highly conserved between mammalian and avian species. However, in avian species, an equivalent to the mammalian pre-B cell, which has undergone Ig heavy-chain gene rearrangement and expresses μ heavy chains in the absence of Ig light-chain rearrangement, has not been convincingly demonstrated. It is consequently unclear whether an ordered progression of gene rearrangement events leading to functional Ig expression occurs in avian species. To examine the sequence of Ig gene rearrangement events in chicken B-cell development, we transformed day 12 embryo bursal cells with the REV-T(CSV) retrovirus. More than 100 clones were analyzed by Southern blotting and polymerase chain reaction for the presence of Ig gene rearrangements. The majority of these clones contained only germline Ig sequences. Several clones contained complete heavy- and light-chain rearrangements and 13 clones contained only heavy-chain rearrangements analogous to stages of mammalian B-cell development. However, 5 clones contained rearrangements of light-chain genes in the absence of complete heavy-chain rearrangement. Consequently, we conclude that rearrangement of chicken Ig light-chain genes does not require heavy-chain variable-region rearrangement. This observation suggests that chicken Ig gene rearrangement events required for Ig expression occur stochastically rather than sequentially.**

The expression of immunoglobulin (Ig) on B lymphocytes requires rearrangement of a number of gene segments which are separately encoded in the germ line. Ig heavy (H)-chain expression requires rearrangement of D (diversity) to J_H (joining) segments, followed by rearrangement of a V_H (variable) segment to the resulting DJ complex, whereas Ig light (L)-chain expression requires V_L to J_L rearrangement (1–3). The gene segments involved (4, 5) and the molecular mechanism of rearrangement (6) are highly conserved between mammalian and avian species. Nonetheless, whereas mammalian Ig gene rearrangement and expression occur throughout life (7), chicken Ig gene rearrangement and the induction of B-cell surface Ig (sIg) expression are restricted to the developing embryo (8–10). Mammalian Ig gene rearrangement in normal bone marrow appears to follow a programmed sequence of rearrangement events in which H-chain gene expression precedes the induction of Ig L-chain rearrangement (7, 11). In contrast, the sequence of chicken Ig gene rearrangement events has not been defined; indeed no avian homologue to the mammalian pre-B [cytoplasmic IgM H-chain-positive ($c\mu^+$), L-chain negative (L^-)] cell population has been convincingly demonstrated (e.g., ref. 12).

The bursa of Fabricius is central to the normal development of chicken B lymphocytes (13) and is colonized during embryonic life (14) by extrabursally derived precursors (15, 16). Ig gene rearrangement has been first observed in mesenchymal tissue at day 7 of embryonic development (17), prior to the appearance of sIg^+ cells in the bursa (13). The number of germ-line segments that contribute to the rearrangement of chicken Ig genes is very limited. The chicken Ig L-chain locus contains one functional V region (V_L1) and one J region (J_L) (18), whereas the Ig H-chain locus contains one functional V region (V_H1), a small family of about 16 D segments (19), and one J segment (J_H) (4). All B cells undergo rearrangement of the unique V_L1 segment to J_L: the unique V_H and J_H elements are rearranged to any one of the D family. Ig V-region diversity is generated subsequent to rearrangement (20) by somatic gene conversion events in which sequences within the rearranged V-region genes are replaced by sequences derived from upstream pseudo-V-region gene families (4, 5).

Reticuloendotheliosis virus strain T (REV-T) is a replication-defective avian retrovirus, and the use of chicken syncytial virus (CSV) as a helper virus yields viral particles that are tropic but not cytopathic for B-lineage cells (21). We and others have demonstrated that REV-T(CSV) transforms avian B-lineage cells (21–23). REV-T(CSV) transformation of day 14 embryo bone marrow cells yielded a clone containing a rearranged Ig L-chain allele in the absence of complete (VDJ) H-chain rearrangement. To extend this observation, since such a combination of gene rearrangements is not expected from current models of mammalian B-cell development, B-lineage cells were cloned from REV-T(CSV)-transformed day 12 embryo bursa, a developmental stage and site containing cells undergoing Ig rearrangement (8). Clones containing either complete (VDJ) H-chain or L-chain rearrangements were isolated, demonstrating that chicken Ig L-chain rearrangement does not require VDJ H-chain rearrangement.†

MATERIALS AND METHODS

Bursal Cell Suspensions. Embryo bursae were obtained from day 12 chicken embryos (SC line; Hyline International, Dallas Centre, IA). The SC line is an F_1 generation of a cross between S and C lines, which differ at several restriction enzyme sites within the Ig H-chain (22, 23) and L-chain (20) loci. Cell suspensions were prepared by removal of bursal tissue and disruption through a wire mesh. Cells were then washed three times in Hanks' balanced salts solution prior to transformation.

Retroviral Stocks and Cell Transformations. All tissue culture was performed in Iscove's modified Dulbecco's medium supplemented with 2% normal chicken serum (23). Supernatants of the S_2A_3 cell line were used as a source of REV-T(CSV) (23). Lymphoid cells were transformed in

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Abbreviations: REV-T, reticuloendotheliosis virus strain T; CSV, chicken syncytial virus; sIg, surface Ig; L, light; H, heavy.

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†The sequences reported in this paper have been deposited in the GenBank data base (accession nos. M96965–M96975 and M97011).

multiple replicate cultures with 75% S_2A_3 supernatant in the presence of phorbol 12-myristate 13-acetate (20 ng/ml), and the resulting cell lines were cloned in 10-μl Terrasaki cultures from microscopically observed single cells (23).

Southern Blotting. Total cellular DNA was extracted (24) and digested with a 5-fold excess of the specified restriction enzymes according to supplier's instructions (Pharmacia). Southern transfers and hybridization conditions were as described (23). Specific probes were as follows: C_L, a 1.3-kilobase (kb) *Eco*RI–*Sal* I genomic fragment containing the entire coding sequence of the Ig L-chain constant region and a part of the intervening sequence between J_L and C_L (20); J_H, a 2.7-kb *Apa* I–*Eco*RI genomic fragment containing flanking sequences 3′ of the unique chicken J_H gene segment (4); D_X, a 1.3-kb *Eco*RI–*Apa* I genomic fragment containing D_X and sequence between D_X and D_1 (the most J_H-proximal D element) (19).

Polymerase Chain Reaction. PCR was used to detect and clone rearranged (VJ) L-chain and rearranged (VDJ) H-chain genes. The oligonucleotide primer combinations (Sheldon Biolabs, McGill University, Montreal, Quebec) were V_L5' (5′-TGTCCCATGGCTGCGCGGGCAGGGCTGT-3′) with J_L3' (5′-AGAAAGATCTAGACGAGGTCAGCGACTC-3′) to amplify rearranged L-chain genes, and V_H5' (5′-GTGGCCGCGGCTCCGTCAGCGCTCTCTG-3′) with J_H3' (5′-GGTTGATCACTCACCGGAGGAGACGATG-3′) to amplify rearranged H-chain genes.

Typical PCR mixtures (50 μl) consisted of 0.2 μg of DNA template, 25 pmol of each primer, 1.25 mM dNTPs, 10% dimethyl sulfoxide, 67 mM Tris, 16.6 mM NH_4SO_4, 10 mM 2-mercaptoethanol, and 5–10 mM $MgCl_2$. PCRs were performed with 2 units of *Taq* DNA polymerase (Perkin–Elmer/Cetus) in a Hypercell Biological (Chalk River, Ontario) thermal cycler for 30 cycles, followed by a 20-min extension reaction at 72°C. Each cycle for the amplification of rearranged H and L chain consisted of 93°C for 1.5 min, 65°C for 1.5 min, and 72°C for 1 min. PCR-amplified products were electrophoresed and visualized in 2% agarose gels containing ethidium bromide (0.5 μg/ml).

Cloning and Sequencing of Rearranged Ig Loci. PCR amplified H- and L-chain fragments (each about 450 base pairs) were ligated into the pCR 1000/*Hph* I-cut vector (Invitrogen, San Diego) and transformed either into One Shot INVαF′ competent cells according to the supplier's protocol (Invitrogen) or into *Escherichia coli* DH5α (generously provided by J. Coulton, McGill University). Transformed INVαF′ cells were cloned on agar plates containing 5-bromo-4-chloro-3-indolyl β-D-galactopyranoside and kanamycin; transformed DH5α cells were cloned on agar plates containing kanamycin and subsequently replica-plated on plates containing isopropyl β-D-thiogalactopyranoside, 5-bromo-4-chloro-3-indolyl β-D-galactopyranoside, and kanamycin. Plasmids were prepared by alkaline extraction and RNase treatment from white colonies containing plasmids with inserts of the appropriate size.

Dideoxynucleotide sequencing reactions were performed with 3 μg of double-stranded plasmid DNA with a Sequenase kit according to the supplier's protocols (United States Biochemical). Reaction products were separated in 42-cm gels. Both strands were sequenced using forward and reverse M13 oligonucleotide primers (United States Biochemical) and, when necessary, the V_H 5′ primer described above and a reverse primer within V_H1 framework region 3 (5′-CGAGATGGTGGCACGGCC-3′). Germ-line V_H1 and V_L1 genes were obtained by PCR using oligonucleotide primer combinations V_H5' and V_H3' (5′-TTCAGCGCCTTGGGTTGCAACGGTG-3′) for V_H1, and V_L5' and V_L3' (5′-ACCATCAGCTGCTCCTTGCACTGGCAGG-3′) for V_L1. Germ-line H- and L-chain sequences were derived from S- and C-line homozygotes by cloning and sequencing the PCR-amplified germ-line fragments as described for rearranged genes. PCR amplification artifacts were obviated by sequencing the cloned products of replicate amplifications.

RESULTS

Analysis of a large panel of clones derived from REV-T(CSV)-transformed day 14 embryo bone marrow cells revealed a clone, 2-15, that apparently contained one rearranged Ig L-chain allele in the absence of a VDJ H-chain rearrangement (Fig. 1). This suggested that chicken Ig L-chain rearrangement may not require H-chain rearrangement and full-length μ protein expression. To analyze this possibility in more detail, transformed cells were derived from a site and stage of embryo development in which a high rate of Ig gene rearrangement is ongoing (8). Day 12 embryo bursal cells were transformed in replicate cultures with REV-T(CSV) in the presence of phorbol 12-myristate 13-acetate (20 ng/ml), which increases the frequency of transformed cells (T.B. and M.J.H.R., unpublished work). Multiple cell lines were generated and cloned in 10-μl Terrasaki cultures from microscopically observed single cells.

In normal chicken B cells, the unique functional V_L1 gene is rearranged to the unique J_L, with deletion of the 1.7 kb of intervening DNA; most B cells retain the other allele in germ-line configuration (18). On Southern blots of normal SC bursal DNA digested with *Sca* I and *Sal* I, a genomic C_L probe detects a germ-line band of 3.2 kb and bands of 4.4 kb and 2.2 kb corresponding to V_L1–J_L rearranged genes (20). The V_L1–J_L rearrangement *per se* generates the 2.2-kb band; the 4.4-kb band results from gene conversion events that modify the *Sca* I restriction site within the rearranged V_L1 gene (20). Consequently, bands at either 2.2 kb or 4.4 kb are diagnostic of V_L1–J_L rearrangement. At the H-chain locus in normal B cells, the J_H segment has rearranged to one [or more

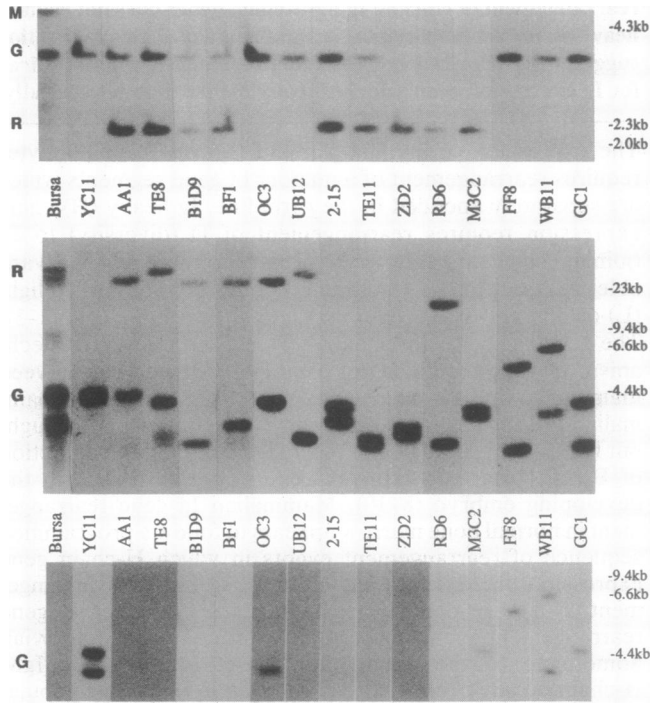

FIG. 1. Ig gene rearrangements in clones of REV-T(CSV)-transformed cells. DNA from ≈5 × 10^6 cells was digested with *Sca* I/*Sal* I (*Top*) or *Bcl* I (*Middle* and *Bottom*) and analyzed by Southern blotting with ^{32}P-labeled C_L (*Top*), J_H (*Middle*), or D_X (*Bottom*) probe. All clones except the embryo bone marrow-derived 2-15 were derived from transformed day 12 bursal cells. The bursal DNA sample was from a 6-week-old chicken. M, modified; G, germ line; R, rearranged.

(19)] of the D elements and the unique V_H1 has rearranged to that D, resulting in a fully rearranged VDJ (or VDDJ) H-chain locus. Typically the other allele remains either in fully germ-line configuration or has undergone a DJ_H partial rearrangement (4). Southern blots of *Bcl* I-digested erythrocyte DNA from SC F_1 chicks, when probed with sequences 3′ of J_H, yield two germ-line bands detected at 3.8 kb (C-line allele) and 4.1 kb (S-line allele) (23). Complete VDJ rearrangement seen in bursal DNA samples results in bands at 25 or 28 kb; remaining bands not corresponding to either germ-line or rearranged genes represent alleles that have undergone partial (D to J_H) H-chain rearrangements (4, 23).

A total of 75 clones derived from day 12 embryo bursa were analyzed by Southern blotting to determine the organization of their Ig loci. An additional 41 clones were analyzed by PCR for Ig L-chain gene rearrangement, and clones containing rearranged L-chain genes detected by PCR were examined further by Southern blotting. The majority (53/75) of clones (Table 1) retained both H- and L-chain alleles in germ-line configuration (V–D–J/V–J) as judged by Southern blot analysis (e.g., YC11, Fig. 1). Twelve clones contained L-chain rearrangements, and 9 had VDJ H-chain rearrangements. Seven clones contained one VJ rearranged L-chain allele; the other allele remained in germ-line configuration and, in addition, contained one VDJ rearranged H-chain allele (e.g., AA1, TE8, B1D9, and BF1, Fig. 1), a phenotype (VDJ/VJ) characteristic of normal sIg^+ B cells. The configuration of the other H-chain allele was defined by probing with the D_X probe. This sequence is lost in any $D–J_H$ rearrangement except D_1-J_H, under which circumstances the size of the D_X hybridizing band shifts from that seen for the germ line locus (a doublet of 4.4 kb and 3.7 kb, corresponding to S- and C-line alleles respectively; e.g., YC11, Fig. 1). Of the seven clones containing a VDJ H-chain locus, each contained a DJ rearrangement on the other H-chain allele (Fig. 1).

In addition, clones were isolated that had undergone incomplete Ig gene rearrangements. Two clones (UB12 and OC3) had apparently undergone VDJ rearrangement of H chain in the absence of L-chain rearrangement (VDJ/V–J), analogous to murine pre-B cells. The other H-chain allele of UB12 had undergone a DJ rearrangement whereas OC3 retained its other allele in germ-line configuration (Fig. 1). Eleven clones containing DJ rearrangements with both L-chain alleles in germ-line configuration (V–DJ/V–J) were isolated (Table 1). Of these, six contained one DJ rearrangement, with the other H-chain allele remaining in germ-line configuration (e.g., GC1 and WB11, Fig. 1) and five contained DJ rearrangements in both H-chain alleles (e.g., FF8, Fig. 1). These phenotypes are consistent with the sequence of Ig gene rearrangements believed to occur in mammalian B-cell development.

However, four bursally derived clones (ZD2, RD6, TE11, and M3C2, Fig. 1), in addition to the bone marrow-derived 2-15 clone, apparently contained L-chain gene rearrangements in the absence of a complete (VDJ) H-chain rearrangement. Each of these clones contained a 2.2-kb rearranged L-chain band in *Sca* I/*Sal* I digests, suggesting that while L-chain rearrangement had occurred, loss of the *Sca* I restriction site within V_L1 by, for example, gene conversion had not occurred. Clones 2-15 and TE11 contained the 3.2-kb L-chain band, demonstrating that the other L-chain allele was in germ-line configuration. Clones RD6, ZD2, and M3C2 did not contain the 3.2-kb band, as a consequence of DNA methylation at the *Sal* I site (25), but nonetheless contained a germ-line L-chain allele as demonstrated in *Bcl* I digests, where a germ-line (11 kb) and rearranged (9.3 kb) doublet was observed from each of these clones (data not shown).

To preclude the possibility that bands on Southern blots interpreted as complete (VJ or VDJ) rearrangements were artifactual, the Ig loci in the transformed clones were further analyzed by PCR amplification. Primers for sequences 5′ to the V_L1 gene and 3′ to the J_L segment served to specifically amplify rearranged L-chain DNA. Similarly, primers 5′ to the V_H1 gene and 3′ to the J_H segment specifically amplified VDJ rearranged H-chain DNA. All clones characterized as VDJ/VJ by Southern blotting yielded PCR-amplified bands consistent with VDJ H-chain and VJ L-chain rearrangements (Fig. 2). Clones (OC3 and UB12) characterized as VDJ/V–J yielded a rearranged H-chain PCR band but not a rearranged L-chain PCR band. Clones containing only DJ rearrangements did not yield either H- or L-chain PCR bands. From the five clones containing VJ L-chain rearrangements in the absence of VDJ H-chain rearrangement, we amplified fragments corresponding to VJ rearranged L chains but not fragments corresponding to VDJ rearranged H chains. Therefore while each of these clones (V–DJ/VJ) contained one or two DJ H-chain rearrangements (Fig. 1, Table 1), no VDJ rearrangements were detected by PCR.

To demonstrate that the PCR bands observed in Fig. 2 represented the products of normal Ig gene rearrangements, they were cloned and sequenced. All rearrangements in VDJ/VJ cells were productive (Figs. 3 and 4), typical of normal B cells. The D region of clone BF1 is large and its sequence suggests tandem fusion of two (distinct) D elements.

Table 1. Ig rearrangements in REV-T(CSV)-transformed embryo clones

Heavy chain	Light chain V–J/V–J*	Light chain VJ/V–J†
V–D–J/V–D–J	53	0
V–DJ/V–D–J	6	**1**
V–DJ/V–DJ	5	**4** (1)
VDJ/V–D–J	1	0
VDJ/V–DJ	1	7 (2)

Seventy-five clones were analyzed by Southern blotting (as described in Fig. 1) for Ig H- and L-chain gene rearrangements, which distinguished each of the phenotypes described. Forty-one additional clones were analyzed by PCR amplification for the presence of rearranged Ig L-chain genes. Clones containing at least one L-chain rearrangement as judged by PCR (parentheses) were further analyzed by Southern blotting for H- and L-chain rearrangement. Data are from 75 (*) or 116 (†) clones; numbers in bold are not expected from current models of mammalian B-cell development.

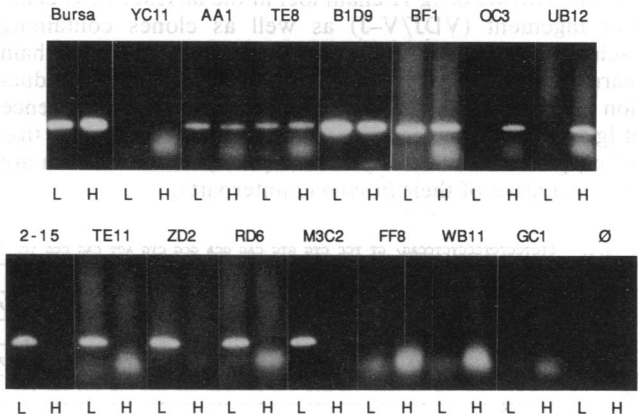

FIG. 2. PCR amplification of rearranged Ig genes from REV-T(CSV)-transformed cells. VDJ H-chain sequences (H) or VJ L-chain sequences (L) were amplified as described. The clones shown here were classified by Southern blotting (Fig. 1) as V–D–J/V–J (YC11), VDJ/VJ (AA1, TE8, B1D9, and BF1), VDJ/V–J (OC3 and UB12), V–DJ/VJ (2-15, TE11, ZD2, RD6, and M3C2), or V–DJ/V–J (FF8, WB11, and GC1). Normal bursal DNA was from a 6-week-old chicken. Lane ∅, no DNA.

Indeed DDJ and VDDJ rearrangements have been isolated previously by PCR amplification from bursal DNA (19).

In contrast, both VDJ/V–J clones contained nonproductive rearrangements (Fig. 4), each resulting in J being out of frame with respect to V_H. Similarly, among the V–DJ/VJ clones, while M3C2 and TE11 contained productive L-chain rearrangements, RD6, ZD2, and 2-15 contained out-of-frame rearrangements (Fig. 3). Thus, nonproductive Ig gene rearrangement can occur within the embryo bursa, supporting the belief that the paucity of nonproductive rearrangements among peripheral or bursal B cells from hatched chickens reflects selection for those cells containing only one (productively) rearranged locus (25).

Among the rearranged Ig L-chain sequences in clones derived from day 12 embryo bursa, there is no apparent sequence diversification away from the germ-line V_L1 sequences. All differences among the clones are accounted for by the polymorphism between S and C V_L1 alleles. In contrast, among the rearranged H-chain sequences, two (UB12 and TE8) diverged from the S or C germ-line V_H1 sequence, primarily in CDR2. While the clustering of sequence divergence is typical of somatic gene conversion event(s) (4), it was surprising to find the same sequence divergence in two clones using different D segments and being derived from separate primary transformation cultures. We considered the possibility that this sequence divergence reflected a discrete germ-line V_H1 allele present at a relatively low frequency in either the S or the C strain. We therefore cloned and sequenced the germ-line V_H1 allele from those clones containing a VDJ rearranged H-chain allele. Among these sequences we observed that BF1 contained a germ-line V_H1 gene containing the same sequence divergence in CDR2 as was found in the rearranged genes of UB12 and TE8. The UB12 and TE8 rearranged VDJ sequences therefore reflect an allelic form of the V_H1 gene rather than gene conversion.

Each of the sequences isolated from clones containing Ig rearrangements appeared normal. Since we have isolated cells containing either V_LJ_L or $V_HD_HJ_H$ rearrangements, chicken Ig gene rearrangement appears not to reflect a programmed sequence of gene rearrangement events.

DISCUSSION

We have isolated by retroviral transformation clones of B lineage cells from day 12 embryo bursa that contain complete rearrangements of Ig H-chain loci in the absence of L-chain rearrangement (VDJ/V–J) as well as clones containing L-chain rearrangements in the absence of complete H-chain rearrangement (V–DJ/VJ). The conclusion that the production of chicken Ig is not a result of a programmed sequence of Ig gene rearrangement events rests on the assumption that B-lineage cells isolated by REV-T(CSV) transformation are representative of their *in vivo* counterparts.

Several lines of evidence suggest that the cells described here are not transformation artifacts. Cells containing either VDJ H- or VJ L-chain rearrangement have been detected only from transformed embryo bursa or embryo bone marrow. Analysis of a large number (≈200) of clones transformed *in vitro* with REV-T(CSV) from adult spleen or from the bursae of hatched chickens has revealed no such cells; all cells contained either germ-line Ig genes or rearrangements at both H (VDJ) and L (VJ) loci (data not shown).

In contrast to other reports of REV-T-transformed chicken B-lineage cells (27–29), we have found no evidence for gross chromosomal abnormalities or deletions involving the Ig H- or L-chain locus among the clones we have isolated. This may reflect the helper virus used for producing REV-T, since the helper virus used elsewhere (REV-A, refs. 27–29) is cytopathic for lymphoid cells (30). Even after prolonged growth *in vitro*, REV-T(CSV)-transformed clones retain their normal complement of Ig genes (22, 23).

Ig loci in the great majority of REV-T(CSV)-transformed cells reported here and elsewhere (22) appear stable with no evidence for continued spontaneous Ig gene rearrangement or Ig V-region diversification by gene conversion. The partially rearranged cells we have isolated are therefore unlikely to have arisen as a consequence of Ig rearrangement *in vitro* subsequent to transformation. Specifically, we cannot detect (by PCR amplification) VDJ H- or VJ L-chain rearrangements among clones (e.g., FF8, Fig. 1) containing DJ rearrangements on both H-chain alleles, even after prolonged growth *in vitro* or reexposure to REV-T(CSV). This further supports the likelihood that the partially rearranged cells described here reflect their *in vivo* counterparts.

Ig rearrangement in the chicken is restricted to about days 7–16 of embryonic development (8, 17). We have previously demonstrated the presence in embryo bone marrow of cells which express sIg and have suggested that these cells may represent the transformed analogues of pre-bursal cells (23). This and other evidence suggests that the bursal microenvironment is not required for the induction of Ig rearrangement and expression (9, 23, 25, 26). Nonetheless, the observation of partially rearranged clones derived from bursal tissue demonstrates that not all Ig rearrangement occurs during pre-bursal stages of chicken B-cell development, consistent with the isolation of the reciprocal products of rearrangement from bursal cells (8).

Transformed chicken cells containing only nonproductive rearrangements were not described before, although the high frequency of nonproductive rearrangements isolated from embryo tissue by PCR amplification (19, 25) predicts the existence of such cells *in vivo*. Mature chicken B cells contain few nonproductive rearrangements; the allelically excluded allele usually remains in germ-line or DJ rearranged configuration. Therefore, cells containing nonproductive rearrangements must be selected against during normal B-cell develop-

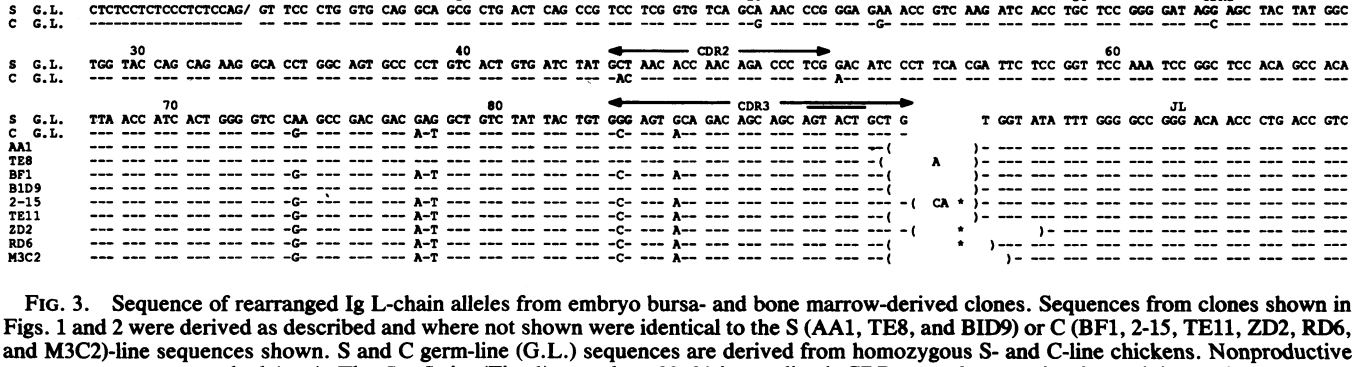

FIG. 3. Sequence of rearranged Ig L-chain alleles from embryo bursa- and bone marrow-derived clones. Sequences from clones shown in Figs. 1 and 2 were derived as described and where not shown were identical to the S (AA1, TE8, and BID9) or C (BF1, 2-15, TE11, ZD2, RD6, and M3C2)-line sequences shown. S and C germ-line (G.L.) sequences are derived from homozygous S- and C-line chickens. Nonproductive rearrangements are marked (star). The *Sca* I site (Fig. 1) at codons 90–91 is overlined. CDR, complementarity-determining region.

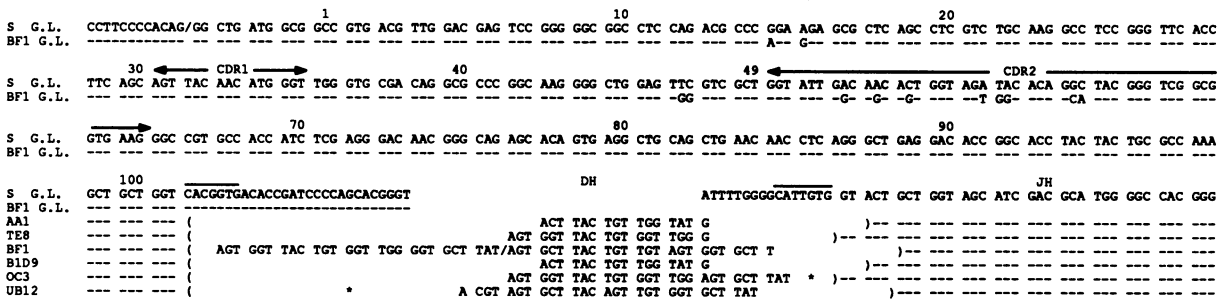

FIG. 4. Sequence of rearranged Ig H-chain alleles from embryo bursa-derived clones. Sequences from clones shown in Figs. 1 and 2 were derived as described and where not shown were identical to the S-line (C-line, AA1, BF1, BID9, and OC3) or BF1 G.L. (TE8 and UB12) sequences shown. S and C germ-line (G.L.) V_H1 sequences are derived from homozygous S- and C-line chickens, BF1 G.L. is the germ-line sequence derived from the V–DJ allele of the BF1 clone. Germ-line J_H is from published sequence (4). Nonproductive rearrangements are marked (star). The heptamer portion of the V_H1 and J_H recombination signal sequences is overlined. Likely position of the DD junction in BF1 is indicated (/). D sequences are written in reading frame 1, which is the most widely used in chicken Ig H chains (19). Codon 101 of V_H1 and the first codon of most published D regions (19) are identical (GGT), and so the precise border of the V_H1/D junction cannot be defined in most cases.

ment (25). The molecular basis by which the allelic exclusion of chicken Ig genes occurs is as yet undefined. Although productive rearrangement of mammalian Ig genes normally inhibits further rearrangement of the alternative allele (31), it is not clear how a nonproductive chicken Ig gene rearrangement might mediate such inhibition. On the other hand, as suggested elsewhere (25, 32), the period of time during which rearrangement occurs may be sufficient to allow rearrangement at only one allelic locus. Under these circumstances, our results suggest that H- and L-chain rearrangements are independently regulated. Therefore mechanisms distinct from, although possibly including, temporal regulation, are likely to control the allelic exclusion of chicken Ig genes (33).

From studies of Ableson virus-transformed early B-lineage cells, Ig H-chain rearrangement/expression precedes and may induce (11, 34) that of L chain. This is not the case in avian B-cell development even though chicken Ig genes can undergo rearrangement in murine cells (6). Although human μ H-chain expression also appears to precede that of L-chain during B-cell development in the bone marrow, human L-chain rearrangement occurring prior to H-chain VDJ rearrangement has been demonstrated among Epstein–Barr virus-transformed human fetal bone marrow cells (35), similar to the results demonstrated here, supporting the possibility that a sequential program of Ig gene rearrangement is established postnatally (in species where postnatal Ig gene rearrangement occurs).

While V_L1 and J_L are unique gene segments within the chicken genome, there is a family of 16 D segments that can potentially rearrange to the single J_H segment (19). In addition, within a rearranging population there are most likely more J_L segments than rearranged DJ complexes available for V-region rearrangement. Although heptamer–nonamer recombination signal sequences can themselves influence the rate of gene segment recombination (36), the data in Table 1, where the frequency of rearrangement events among partially rearranged cells is D_H–J > V_L–J_L > V_H–DJ, are nonetheless consistent with rearrangement being limited by substrate availability. Therefore, the patterns of Ig rearrangement among clones of transformed embryo bursal cells support the hypothesis that a stochastic rather than programmed sequence of chicken Ig gene rearrangements occurs during avian B-cell development.

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