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Organization and expression of immunoglobulin genes in fetal liver hybridomas

(sequential gene rearrangements/nuclear transcripts/mRNA processing/B lymphocyte differentiation)

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ABSTRACT **The organization and expression of immunoglobulin genes were studied in a series of six hybridomas derived from the fusion of a nonproducing myeloma cell with cells from mouse fetal liver. These hybridomas, which exhibit several phenotypic characteristics of immature B lymphocytes, all have productively rearranged μ heavy chain genes and produce both the membrane and secreted forms of μ mRNA in a ratio of about 1:10. Significantly, none of the hybridomas has an unrearranged (germ line) allelic μ gene. Examination of the κ light chain genes revealed that all six of the hybridomas contain unrearranged κ loci and produce 8.4-kilobase transcripts containing κ constant region sequences. None of the five hybridomas that exhibit a μ-only phenotype contains a rearranged κ gene other than that derived from the myeloma parent. One hybridoma, which actively secretes κ immunoglobulin, contains a rearranged κ gene of fetal liver origin and synthesizes a distinctive κ mRNA precursor in addition to the 8.4-kilobase transcript. These results demonstrate that rearrangement of heavy chain immunoglobulin genes normally occurs prior to that of light chain genes and further indicate that the transcriptional competence of the κ constant region locus is established prior to the time of its rearrangement.**

The earliest identifiable stage of B lymphocyte differentiation, termed "the pre-B cell," is found in the liver of 11- to 12-day mouse fetuses and in adult bone marrow. These cells synthesize μ heavy chains but do not yet produce light chains, nor do they carry immunoglobulin on their surface (1–4). They are presumed to be the progenitors of surface IgM-positive B cells which eventually develop into mature IgM-secreting plasma cells. Thus, progression through this developmental series involves, *inter alia*, sequential expression of heavy and light chain genes and the selective production of different forms of μ heavy chain.

It is now well established that immunoglobulin production requires a somatic rearrangement of the germ-line DNA that creates a gene with appropriate transcriptional, processing, and codegenic properties (5–7). Transcriptional competence appears to be an intrinsic property of constant (C) region loci and can be acquired by variable (V) region elements only after appropriate rearrangement (7). Processing specificity may be determined by primary sequence signals, conformation of the mRNA precursor, and extrinsic "processing factors" (7, 8). In the case of μ gene transcripts, alternative modes of RNA processing can generate two distinctive mRNAs that encode the membrane and secreted forms of μ chain (9–12).

The overall objective of the work described here is to relate the phenotype of the pre-B cell to the molecular determinants of Ig gene expression. To this end we have studied the organization of immunoglobulin genes and the synthesis of immunoglobulin-related RNA in a series of six hybridomas produced by the fusion of mouse fetal liver cells with a non-Ig-producing myeloma (3). Five of these hybridomas exhibit the pre-B cell phenotype in that they synthesize only intracellular μ chains, have no detectable surface Ig, and do not secrete either μ or light chains. The other hybridoma is representative of a more mature B cell in that it produces both μ and κ chains in the form of secreted as well as surface IgM.

MATERIALS AND METHODS

The fetal liver hybridomas were derived from fusions of the nonproducer myeloma cell Ag8.653 (13) with liver cells from 15- to 19-day mouse fetuses as described (3). The hybridoma lines with their abbreviated designation in parentheses are as follows: μ only, 15-23-3 (233), 4-9-12-7-7 (277), 17-5-3-10 (310), 15-79-6 (796), and 15-8-6-5 (865); $\mu^+\kappa^+$, 15-56-1 (561). These cells were grown in RPM1-1640 medium supplemented with 20% fetal calf serum. The sources and cultivation of the plasmacytomas (7, 14) and B-cell lymphomas (9) were as described.

Methods for the preparation of poly(A)$^+$ nuclear and cytoplasmic RNA, extraction of DNA, and blotting analyses of RNA and DNA have been described (7, 14, 15). The hybridization probes (Fig. 1) were: C_μ, a cDNA clone [pμ (3741)[9] (16)] containing most of the μ C region; μ_m, a cloned *Hin*dIII fragment [M2-13 (16)] containing about 2.2 kilobases (kb) of sequence immediately 3′-ward of the μ C region, including both of the exons that are uniquely part of the membrane-associated μ chains; J_H, a cloned *Bam*HI/*Eco*RI fragment, pJ11, containing J_{H3}, J_{H4}, and 3′ flanking sequences (16); C_κ, a cloned *Hin*dIII/BamHI fragment derived from a cDNA clone of MOPC321 (7) containing all of the κ C region; and IVS, an *Xba* I/*Hin*dIII fragment (7) containing sequences 3′-ward of the J_κ region.

RESULTS

For identification of Ig-related RNA components, poly(A)$^+$ nuclear or cytoplasmic RNA was size fractionated by electrophoresis on methylmercury hydroxide gels, blotted to diazotized paper, and hybridized with cloned cDNA probes specific for the κ and μ C region (C_κ and C_μ, respectively) or with a cloned genomic probe containing the sequences that encode the carboxy-terminal portion of membrane-associated μ chains (μ_m). For comparison, selected RNA samples from previously studied Ig-secreting plasmacytomas and B-cell lymphomas (70Z/3 and WEHI 231) were also included in the analysis.

In contrast to the B-cell lymphomas, which produce comparable quantities of a 2.7-kb mRNA encoding the membrane-associated μ chain (μ_m mRNA) and a 2.4-kb mRNA encoding the secreted μ chain (μ_s mRNA) (9), the fetal liver hybridomas all

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Abbreviations: C, constant; V, variable; kb, kilobase(s); μ_m, membrane-associated μ chain; μ_s, secreted μ chain.

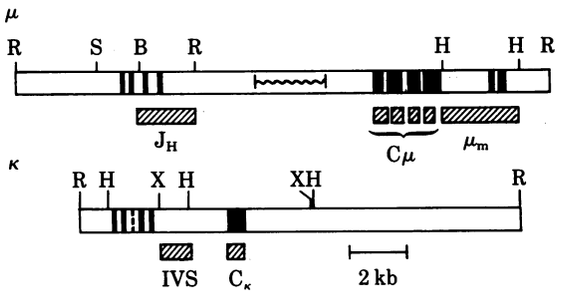

FIG. 1. Diagram of germ-line μ and κ loci showing the various probes (hatched boxes) and restriction sites used in the present study. R, S, B, H, and X are *Eco*RI, *Sac* I, B*am*HI, *Hin*dIII, and *Xba* I, respectively. Solid areas, sequences present in mature mRNA: wavy line, unstable region that is prone to deletion.

produced much more μ_s mRNA than μ_m mRNA (Figs. 2 and 3). Estimates based on the relative intensities of the 2.7- and 2.4-kb bands indicate that >90% of the μ mRNA was of the secreted variety (Table 1). Indeed, given the band separation usually attained in such analyses, one would not normally detect the μ_m mRNA component when using a probe such as C_μ which reacts with both mRNA species. In this regard, the fetal liver hybridomas resemble certain IgM-secreting plasmacytomas such as

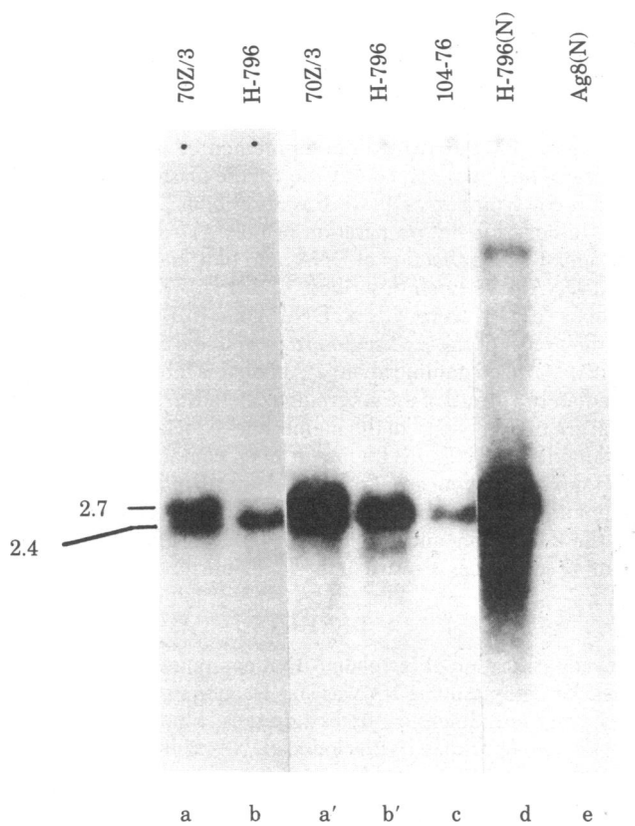

FIG. 2. Production of μ mRNA in a fetal liver hybridoma: comparison with a plasmacytoma and a B-cell lymphoma. Poly(A)$^+$ cytoplasmic RNA (lanes a–c) or poly(A)$^+$ nuclear RNA (lanes d and e) was size fractionated by electrophoresis on methylmercury hydroxide/agarose gels, covalently bound to diazotized paper, and hybridized with a ^{32}P-labeled C_μ probe (300 cpm/pg). A single 2.4-kb mRNA component was observed in the fetal liver hybridoma (H-796) and the plasmacytoma (MOPC 104-76) in contrast to the two mRNA components, 2.4 and 2.7 kb, found in the B-cell lymphoma (70Z/3). No μ RNA was produced in the parental myeloma, Ag8.653. Inputs: a, 5 μg; b, 1 μg; c, 0.2 μg; d and e, 10 μg. Exposure times: a and b, 4 hr; a',b', and c–e, 16 hr.

Table 1. Expression of μ genes in fetal liver hybridomas: Comparison with plasmacytomas and B-cell lymphomas

Cell type	Cytoplasmic μ mRNA content,* molecules/cell	μ_s mRNA† / μ_m mRNA
B cell lymphomas (70Z/3, WEHI 231)	60–150	0.5–1
Fetal liver hybridomas	≥4000	>10
Plasmacytomas	≥5000	>10

* Estimated from the relative intensities of 2.4-kb bands on autoradiograms. Corrected for amounts of poly(A)$^+$ cytoplasmic RNA loaded on gel, the yield of poly(A)$^+$ mRNA per cell, and exposure time. Values are normalized to previous estimate (9) of the content of μ_s and μ_m mRNA in 70Z/3 cells.

† Estimated from relative intensities of 2.4- and 2.7-kb bands, corrected for the length of probe hybridizing to mRNA (1.2 kb for C_μ vs. 0.4 kb for μ_m) and autoradiographic exposure times.

PC8916. The total amount of μ mRNA per cell in the fetal liver hybridomas was similar to that in plasmacytomas and considerably greater than that found in the B-cell lymphomas (Table 1). The fetal liver hybridomas produced discrete nuclear components of about 9 kb, which presumably are precursors of the μ_s mRNAs, and lesser amounts of diffuse components ≤11.5 kb which may include precursors of the μ_m mRNAs. The myeloma parent, Ag8.653, did not produce any μ gene transcripts (Fig. 2*e*).

The fact that μ-only fetal liver hybridomas produced relatively abundant amounts of μ_s mRNA is consistent with the recent finding (T. M. Kloppel, personal communication) that the bulk of the intracellular μ chain produced by these cells has a carboxy-terminal tyrosine and other characteristics of secreted μ chains. Although there seems to be some variability in size of the μ chains produced by different hybridomas, we were unable to detect any difference in the size of the various μ_s mRNAs (Fig. 3). Conceivably, size heterogeneity of the intracellular μ chains might reflect some posttranslational modification of the proteins.

Although five out of the six hybridomas studied produced no κ chains, κ genes of both myeloma and fetal liver origin clearly were transcribed in these cells (Fig. 4). The Ag8.653 myeloma produced a moderate amount of κ mRNA (1.2 kb) and four nuclear components: two pre-mRNAs (5.0 and ≈4.6 kb) that are characteristic of κ genes formed by V–J_2 fusions (7), and two additional components (≈2.3 and 2.7 kb) of unknown significance. The κ mRNA synthesized by Ag8.653 is apparently defective, however, because these cells do not produce any detectable κ chains (13). In addition to these RNA components, all of the hybridomas produce the 8.4-kb transcripts that are known to originate from unrearranged (germ line) κ genes (7). Because this component is not synthesized by the myeloma parent we may presume that it is of fetal liver origin. In addition to the myeloma and germ-line components, the one μ,κ-producing hybridoma (H-561) synthesized two pre-mRNAs (4.1 and 3.6 kb) which are characteristic of κ genes formed by V–J_4 fusions (7). These mRNAs are presumably derived from a productively rearranged fetal liver κ gene.

Southern blot analysis of the genomic DNA of these hybridomas confirmed that they all had unrearranged κ loci of fetal liver origin (Fig. 5*a*). This was evidenced by the presence of the 2.9-kb *Hin*dIII fragment that is diagnostic of an unaltered germline J region (7) (see Fig. 1). This fragment is present in embryo DNA and in DNA of κ-producing plasmacytomas that contain an unrearranged κ allele (κ^+/κ_0 genotypes). It is absent in the DNA of plasmacytomas in which both κ alleles are rearranged

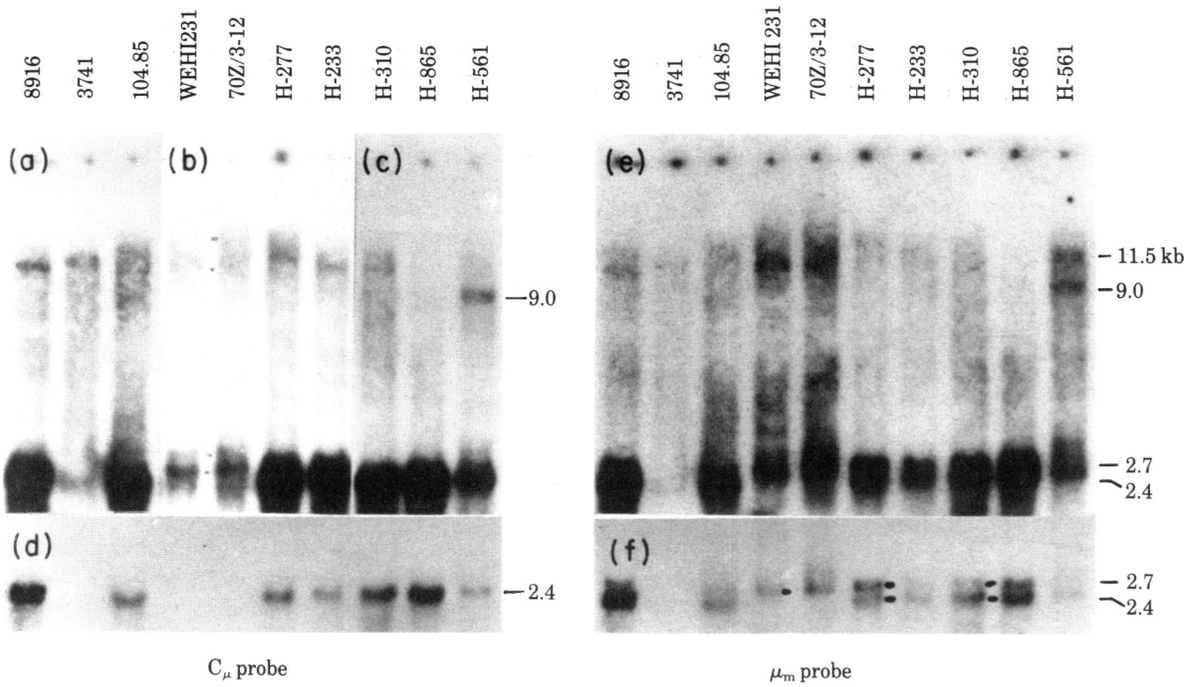

FIG. 3. Production of μ_s and μ_m mRNAs and their precursors in different types of lymphoid cells. Poly(A)$^+$ nuclear RNA from plasmacytomas (PC8916, PC3741, and MOPC 104.85), B-cell lymphomas (WEHI231 and 70Z/3-12) and fetal liver hybridomas (H-277, H-233, H-310, H-865, and H-561) were analyzed as in Fig. 2. All lanes contain 10 μg of RNA except WEHI (12 μg) and 70Z (18 μg). After hybridization with the C_μ probe and autoradiographic exposure, about 95% of the probe was melted off (7) and the RNA was rehybridized with the μ_m probe. Exposures were: *a* and *c*, 16 hr; *b*, 48 hr; *d*, 2.5 hr; *e*, 144 hr; *f*, 15 hr. Because of the much greater signal strength of the C_μ hybrids, the bands seen in *e* and *f* represent a composite of the residual 5% C_μ hybrid plus the μ_m hybrid.

(κ^+/κ^- genotypes). The Ag8.653 myeloma, which lacks the 2.9-kb fragment, exhibited only a single J-region *Hin*dIII fragment of about 6.5 kb. The genotype of these cells is presumably $\kappa^-/-$, the κ^+ allele having been lost in the selection for nonproductivity (13). None of the μ-only hybridomas exhibited J-region *Hin*dIII fragments other than those attributable to germline and Ag8.653 genes, thus indicating that there are no nonproductive arrangements of fetal liver κ genes in these cells. Some of the hybridomas may be monosomic with respect to the κ-bearing fetal liver chromosomes. However, because there is

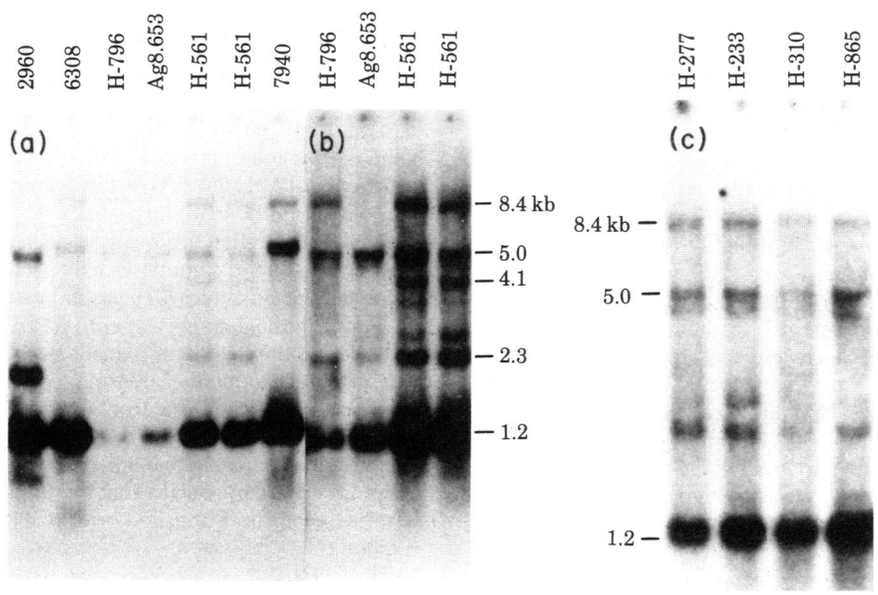

FIG. 4. Expression and organization of κ genes in fetal liver hybridomas: Comparison with selected plasmacytomas. Poly(A)$^+$ nuclear RNA (10 μg per lane) was analyzed with a C_κ probe as described for Fig. 2. Plasmacytomas PC6308 and PC7940 synthesize the 8.4-kb component characteristic of unrearranged (germline) κ alleles and the 5.3-kb pre-κ mRNA characteristic of J_1 expressors; PC2960 synthesizes the 5.0-kb pre-κ mRNA characteristic of J_2 expressors and a 1.9-kb precursor of a 0.8-kb fragment mRNA (7, 14). Exposure times: *a*, 16 hr; *b*, 108 hr; *c*, 48 hr. The two lanes of H-561 are from different samples of cells: their identity illustrates the reproducibility of these complex patterns.

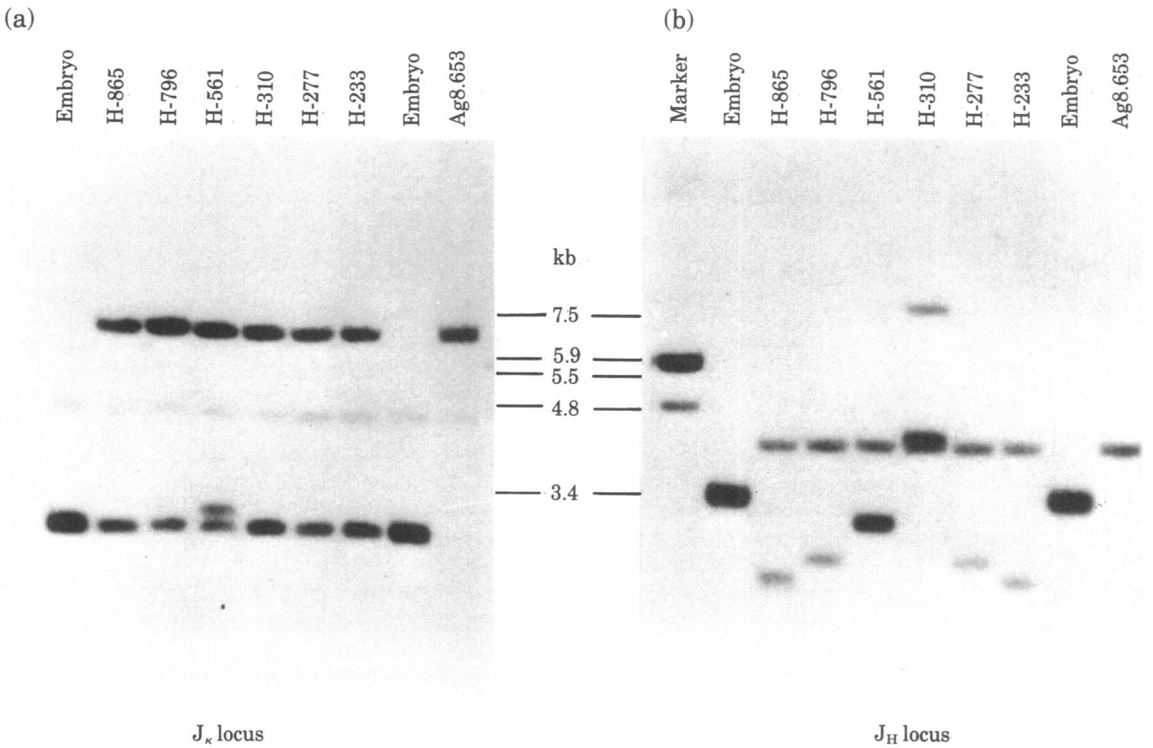

FIG. 5. Southern blot analyses of DNA from fetal liver hybridomas. (*a*) DNA was digested with *Hin*dIII and hybridized with IVS probe. All hybridomas contained the 2.9-kb fragment characteristic of the germ-line J_κ context and the 6.5-kb fragment characteristic of the Ag8.653 myeloma parent; H-561 contained one additional IVS-containing fragment. The faint bands at about 4.5 kb in all samples represent the fragment encompassing the C_κ region which was detected by a trace amount of C_κ sequence contaminating the IVS probe. (*b*) DNA was digested sequentially with *Sac* I and *Eco*RI and hybridized with the J_H probe. All hybridomas contained a fragment of about 4.2 kb characteristic of the myeloma parent and at least one additional component different from the 3.4-kb germ-line fragment.

no reason to suspect that there would be preferential retention of chromosomes bearing a κ_0 allele as opposed to a κ^- allele, we interpret the uniformity of this result to mean that the parental fetal liver cells of these hybridomas were of the κ_0/κ_0 genotype. As one would expect, the μ,κ-producer H-561 exhibited one germ-line and one rearranged κ allele in addition to the Ag8.653 κ^- allele, the genotype of its parental fetal liver cell presumably being κ^+/κ_0.

In contrast to the situation with the J_κ region, a Southern blot analysis of the J_H region in these hybridomas (Fig. 5*b*) revealed extensive rearrangement. Focusing on a 3.5-kb *Sac* I/*Eco*RI fragment (see Fig. 1) that would be altered by rearrangements involving any of the J_H segments but which would be judiciously insensitive to rearrangements in a highly unstable region between the *Eco*RI site and the C_μ gene (16), we failed to observe a germ-line J_H context in any of the six hybridomas. All exhibited at least one novel *Sac* I/*Eco*RI fragment in addition to that characteristic of the Ag8.653 myeloma parent, and two hybridomas (H-561 and H-310) may have contained a second novel fragment incompletely resolved on these gels. All of the hybridomas produced functional μ mRNA and thus, by definition, contained a μ^+ allele. The parental fetal liver cells could have either μ^+/μ^- or μ^+/μ_0 genotypes, although our failure to detect any μ_0 alleles suggests that the latter may be relatively rare.

DISCUSSION

The results of our study provide a plausible molecular basis for explaining the phenotype of the fetal liver hybridomas and give some new insight into the determinants of B-cell differentiation. We have observed that the μ-only phenotype is generally associated with cells in which the heavy chain genes have undergone a productive rearrangement before any reorganization of light chain genes has occurred. Although this has been formally demonstrated here only for κ genes, it is undoubtedly true for λ genes as well because the frequency of κ rearrangement greatly exceeds that of λ rearrangement (17). The similarity of the nucleotide sequences surrounding the V-J joining sites of heavy and light chain genes has led to the speculation that both sets of genes share the same recombinational machinery (18, 19). If this is true, then the sequential phasing of heavy and light chain gene rearrangements may be the result of an inherently higher recombination frequency of the heavy chain gene elements, perhaps attributable to the participation of an additional D segment. The indication, from our data, that rearrangement of both heavy chain alleles sometimes may precede any reorganization of the light chain alleles is consistent with this notion.

Transcriptional activity at both heavy and light chain loci is observed in the fetal liver hybridomas. However, whereas the transcripts of productively rearranged μ genes can be processed into functional mRNAs encoding either the secretory or the membrane form of μ chain, the 8.4-kb transcript containing the unrearranged C_κ gene cannot be processed into a functional mRNA (7). It is noteworthy that the transcriptional competence of the C_κ locus is established prior to any reorganization of this region.

The fact that there are more than 10 times as many μ_s mRNAs than μ_m mRNAs in these cells indicates that the principal processing mode is that which produces the secretory form. Our analysis of μ mRNA precursors suggests that the μ_s processing mode is associated with the increased production of a discrete polyadenylated component, which is about 2.5 kb shorter than the largest components observed with μ_m-specific probes.

Thus, the processing mode may be determined by the use of alternative cleavage-polyadenylylation sites, as has been described for adenovirus mRNA processing (20). Whether the predominant use of the μ_s site in the fetal liver hybridomas is due to an extrinsic factor supplied by the myeloma parent or whether this processing mode is characteristic of very early B cells is presently unclear. In hybridomas produced by fusion of B-cell lymphomas with myeloma cells there is a shift from μ_m to μ_s production, indicating that such complementation can indeed occur (10, 11, 21). On the other hand, recent studies of natural populations of very early B-cells indicate that the μ chain production in these cells is mainly of the secretory form.‡ Thus, one could envisage a situation in which there are multiple shifts between μ_s and μ_m processing modes over the course of B-cell differentiation. As discussed earlier (9), this flexibility may have important implications for B-cell function.

Another factor determining the B-cell phenotype is the ability of a μ_m or μ_s chain to be incorporated into the membrane or to be actively secreted in high amount. There are certainly several other elements (e.g., concurrent production of light and J chains, glycosylation, development of a Golgi apparatus, etc.) which are necessary for complete expression. When one or more of these elements is lacking, the μ_m or μ_s chain would presumably remain intracellular. The importance of coproduction of light chains was previously noted in studies of the 70Z/3 lymphoma in which the surface deposition of an intracellular μ_m chain and the production of κ mRNA and its protein product are both coinducible by a lipopolysaccharide mitogen (9, 22). Likewise, in the fetal liver hybridomas studied here, secretion of μ_s chains occurs in the H-561 line which produces κ chains from a rearranged fetal liver gene or in various lines derived from fusions with the NS-1 myeloma cell (3) which, unlike the Ag8.653 myeloma, produces functional κ chains. A similar apparent requirement for coproduction of associating polypeptides has been noted in the expression of surface HLA in which α chains are not deposited on the plasma membranes in the absence of β_2-microglobulin synthesis (23). On the other hand, some secretion of isolated μ chains was clearly observed in experiments with total liver cells from 15-day mice fetuses (4). Whether this secretion occurs from a subpopulation of cells not represented in the hybridoma samples or represents a qualitatively different type of secretion remains to be established.

‡ Williamson, A. R., Gall, I., McElroy, P. J., Willcox, H. N. A. & Catty, D. (1980) *Fourth International Congress of Immunology*, Paris, France (abstr.).

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