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Genetics

Cloning and partial nucleotide sequence of human immunoglobulin μ chain cDNA from B cells and mouse–human hybridomas

(immunoglobulin mRNAs/*in vitro* translation/immunoglobulin secretion)

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ABSTRACT **Purified mRNAs coding for μ and κ human immunoglobulin polypeptides were translated *in vitro* and their products were characterized. The μ-specific mRNAs, derived from both human lymphoblastoid cells (GM607) and from a mouse–human somatic cell hybrid secreting human μ chains (αD5-DH11-BC11), were copied into cDNAs and inserted into the plasmid pBR322. Several recombinant cDNAs that were obtained were identified by a combination of colony hybridization with labeled probes, *in vitro* translation of plasmid-selected μ mRNAs, and DNA nucleotide sequence determination. One recombinant DNA, for which the sequence has been partially determined, contains the codons for part of the C_3 constant region domain through the carboxy-terminal piece (155 amino acids total) as well as the entire 3′ noncoding sequence up to the poly(A) site of the human μ mRNA. The sequence A-A-U-A-A occurs 12 nucleotides prior to the poly(A) addition site in the human μ mRNA. Considerable sequence homology is observed in the mouse and human μ mRNA 3′ coding and noncoding sequences.**

Surface monomeric IgM immunoglobulins consist of two heavy chain (μ) and two light chain (κ or less frequently λ) polypeptides covalently crosslinked by disulfide bonds (1, 2). Circulating secreted IgM molecules are usually pentamers of the monomeric form, which are held together by a small J protein (3). Based on their primary structure, μ chains isolated from secreted IgM have been subdivided into an NH_2-terminal variable (V) region, four constant (C) region domains (C_1, C_2, C_3, and C_4), and a COOH-terminal sequence of 19 amino acids ending in tyrosine (4, 5).

A great deal of information has accumulated recently concerning the organization and expression of mouse immunoglobulin genes by using homogeneous probes obtained through the application of recombinant DNA technology. Virtually nothing of this nature has been reported for the human immunoglobulin gene system other than some early attempts at purification and *in vitro* translation of the μ and λ chain mRNAs (6, 7).

Part of the difficulty in cloning human immunoglobulin mRNAs has been due to the relatively low abundance of these mRNAs [0.5–1% of poly(A)$^+$RNA (unpublished data) compared to several mouse plasmacytomas (6–7% of total poly(A)$^+$RNA)] (ref. 8; unpublished data). Although we have been successful in purifying mRNAs coding for μ, γ_2, α_2, κ, and λ polypeptides (unpublished data) from various human lymphoblastoid and myeloma cell lines, only a small amount of highly enriched mRNAs coding for these polypeptides has been obtained.

During our phenotypic chromosome mapping studies of the genes coding for human heavy (H) chain (9) and light (L) chains by using mouse–human B cell hybridomas, we observed that many of our hybrid cells synthesized and secreted more human H or L chain polypeptides than did their human parental B cells. We took advantage of the apparent biological enrichment of the human H and L chain mRNAs in these somatic cell hybrids to facilitate purification of mRNAs for molecular cloning.

We report in this paper the properties of the human μ and κ mRNAs and their *in vivo* and *in vitro* directed polypeptide products. We also describe the methods used to obtain and characterize recombinant plasmids containing human μ sequences from both human B cells and mouse P3x63Ag8–normal human peripheral lymphocyte hybridomas. We also make some comparisons between the human and mouse 3′ coding and noncoding nucleotides in their μ mRNAs.

MATERIALS AND METHODS

Cell Lines and Hybrid Production. Human lymphoblastoid (GM607, GM1056, GM923) or myeloma (GM1500) cells were obtained from the Human Genetic Mutant Cell Repository (Camden, NJ). SED cells were obtained from Shu Man Fu (The Rockefeller University); these cells were maintained in RPMI-1640 supplemented with 10% fetal calf serum under standard conditions. Normal human peripheral blood lymphocytes were obtained from healthy donors. All somatic cell hybrids were produced (9) with mouse BALB/c P3x63Ag8 cells deficient in hypoxanthine phosphoribosyl transferase derived from the MOPC 21 plasmacytoma that secretes IgG1 κ (10, 11) or the nonsecreting subline (12). Hybrid cells were selected, maintained, and characterized as to their human isotype secretion (H and L chains) as described (9) and as indicated in the legend of Table 1.

Purification of H and L mRNAs. Minimally degraded H and L chain mRNAs were prepared from all B-cell lines and hybrids by the following method. Pelleted cells were lysed for 10 min in 5 vol of cold 50 mM Tris·HCl, pH 7.4/25 mM NaCl/5 mM magnesium acetate/10 mM 2-mercaptoethanol/30% (wt/vol) sucrose containing polyvinyl sulfate at 20 μg/ml and 0.8% Nonidet P-40, followed by centrifugation at 15,000 × *g* for 15 min at 1°C to remove nuclei and mitochondria. The supernatant was adjusted to 1.5% $NaDodSO_4$ and immediately an equal volume of redistilled phenol/chloroform/isoamyl alcohol, 1:1:0.01 (vol/vol), saturated with 10 mM Tris·HCl, pH 7.4/1 mM EDTA/0.1 M NaCl/1.5% $NaDodSO_4$ was added. The solution was mixed for 10 min and centrifuged at 10,000 × *g* for 15 min at 20°C. The aqueous phase was precipitated with 3 vol of ethanol at −20°C after three extractions. The total

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Abbreviations: V, variable; C, constant; H, heavy; L, light; dsDNA, double-stranded DNA; NaCl/Cit, standard saline citrate (0.15 M NaCl/0.015 M sodium citrate, pH 7); ssDNA, single-stranded DNA.

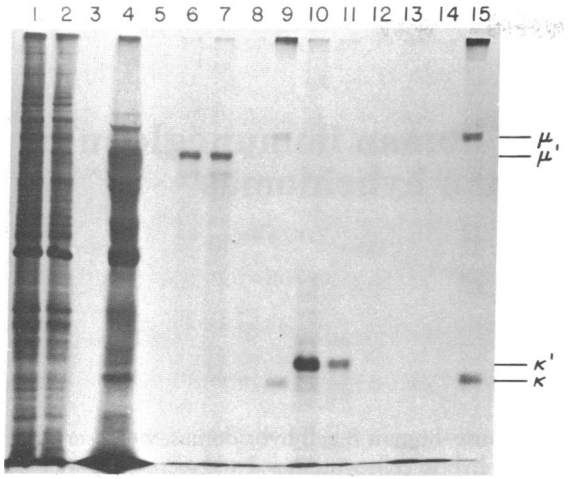

FIG. 1. Autoradiogram of [^{35}S]methionine-labeled proteins from *in vitro* translation with GM607 mRNAs as resolved on one-dimension reducing NaDodSO$_4$/polyacrylamide gels. Lanes: 1 and 2, total *in vivo* labeled (16 hr) cellular protein of GM607; 3, control *in vitro* translation (no RNA); 4, labeled products, total polyadenylylated mRNA; 5, immunoprecipitate of lane 7 translation with human α antisera; 6, immunoprecipitate of lane 7 translation with human μ antisera; 7, *in vitro* translation of three-times sucrose gradient-purified 20S human μ mRNA from GM607; 8, immunoprecipitate of lane 7 translation with human γ antiserum; 9, immunoprecipitate of GM607 cell medium with human κ antiserum; 10, *in vitro* translation of three-times sucrose gradient-purified human κ mRNA; 11, immunoprecipitate of lane 10 translation with human κ antiserum; 12, immunoprecipitate of lane 10 translation with human λ antiserum; 13, immunoprecipitate of labeled GM607 cell medium with normal rabbit serum; 14, immunoprecipitate of labeled GM607 cell medium with human λ- and α-specific antisera; 15, labeled GM607 cell medium immunoprecipitated with human μ antiserum. The positions of the *in vivo* synthesized and secreted μ and κ and their *in vitro* synthesized μ' and κ' products are indicated.

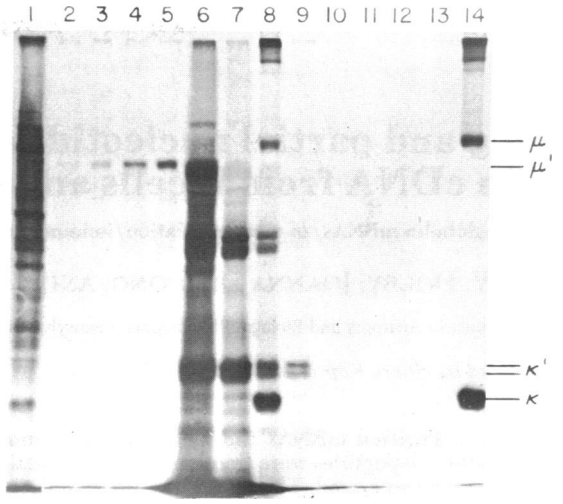

FIG. 2. Autoradiogram of [^{35}S]methionine-labeled proteins synthesized with mRNAs isolated from mouse P3x63Ag8–human B somatic cell hybrids secreting human μ chains and separated on polyacrylamide gel. Lanes: 1, total *in vivo* labeled cellular protein of αD5-BH11-BC11; 2, immunoprecipitate of lane 3 *in vitro* translation with human μ antiserum; 3, *in vitro* translation of three-times sucrose gradient-purified human μ mRNA from 57-55-F7 somatic cell hybrid; 4, immunoprecipitate of lane 5 translation with human μ antiserum; 5, *in vitro* translation of three-times sucrose gradient-purified human μ mRNA from αD5-BH11-BC11; 6, *in vitro* products of total mRNA from αD5-DH11-BC11; 7, *in vitro* products of total mRNA from 57-55-F7; 8, immunoprecipitate of intracellular proteins of αD5-DH11-BC11 with human μ and mouse γ and κ antisera; 9, *in vitro* translation of mouse P3 κ mRNA (13S); 10, immunoprecipitate of lane 9 translation with mouse κ antiserum; 11, as in 10 with human κ antiserum; 12, as in 10 with human λ antiserum; 13, αD5-DH11-BC11 cell medium immunoprecipitate with mouse μ antiserum; 14, αD5-DH11-BC11 cell medium immunoprecipitate with human μ antiserum. The positions of human μ polypeptides synthesized *in vivo* and *in vitro* (μ') and the positions of mouse κ polypeptides *in vivo* and *in vitro* (κ') are indicated.

cytoplasmic RNA was subjected to two rounds of oligo(dT)-cellulose chromatography with heating at 70°C for 5 min prior to the second round (13). The polyadenylylated RNA was then fractionated by neutral 5–25% sucrose gradient centrifugation (8). Gradient fractions enriched in H and L chain mRNAs based on *in vitro* translation criteria (14) and immunoprecipitation (9) were centrifuged repeatedly until *in vitro* translation indicated substantial purification.

Synthesis of Double-Stranded (ds) cDNA. Two to 3 μg of human μ poly(A)$^+$mRNA from GM607 or a somatic cell hybrid αD5-DH11-BC11 was primed with oligo(dT) (P. L. Biochemicals) at 50 μg/ml and subjected to reverse transcription essentially as described (15), with 900–1500 units of avian myeloblastosis virus reverse transcriptase (obtained from J. Beard, St. Petersburg, FL) per ml. Second-strand synthesis was carried out as described (15) except that *Escherichia coli* DNA polymerase I (Boehringer Mannheim, grade I) at 150 units/ml, additional dNTPs (to 1 mM), and 10 mM $MgCl_2$ were found necessary to promote about 60–70% synthesis of the second strand. The dscDNA was extracted with chloroform and chromatographed on Sephadex G-100 in 20 mM NaCl/2.5 mM EDTA; void fractions were pooled and precipitated with 3 vol of ethanol.

The dscDNA was trimmed to blunt ends with S1 nuclease (Miles) at 100 units/ml as described (16), extracted, rechromatographed on Sephadex G-100, and precipitated. Homopolymeric tracts of dC were added to the 3' ends of the dscDNA or dG tracts were added to the cloning vector pBR322 at its *Pst* I site according to the conditions described (17).

Bacterial Transformation. The hybridized recombinant plasmids (18) were used to transform *E. coli* χ1776 (under P2-EK2 conditions as required under earlier National Institutes of Health guidelines) as described (18, 19). About 50 recombinants were obtained per ng of cDNA. Bacteria were selected on Luria agar plates containing 15 μg of tetracycline per ml (18), and all tetracycline-resistant bacteria were picked and ordered on gridded 8.5-cm Luria agar plates containing 15 μg of tetracycline per ml.

Screening of Recombinant Bacteria. Recombinant bacteria were felt-lifted and transferred to replica plates (containing 8.3-cm Whatman 541 paper on the surface of Luria agar plates supplemented with tetracycline at 15 μg/ml). Filters were processed for hybridization essentially as described by Sippel *et al.* (20). Between 30 and 50 filters (2100–3500 colonies) were prehybridized for 16 hr at 37°C in a mixture of 4× standard saline citrate (NaCl/Cit), 44% (vol/vol) formamide, 0.5% NaDodSO$_4$, and Denhardt's solution (21) containing 200 μg of denatured *E. coli* DNA per ml. Hybridization with labeled probes was carried out in the same buffer, using 4–300 × 10^6 cpm of probe for 30 hr. Human μ-specific probes were prepared by 5'-^{32}P end-labeled partially hydrolyzed mRNA (22), the synthesis of ^{32}P-labeled single-stranded (ss) cDNA (23), and nick translation of dscDNA (24) inserts rescued by plasmid digestion with *Pst* I (Bethesda Research Laboratories, Rockville, MD) and isolated from 5% polyacrylamide gels (25) by the method of Maxam and Gilbert (26). After a wash with prehybridization buffer for 16 hr at 37°C, the filters were washed

Table 1. Properties of human immunoglobulin mRNAs and their *in vivo* and *in vitro* synthesized polypeptides

Cell	Source*	Ig secreted†	H chain, ng/10^6 cells/hr	$M_r \times 10^{-3}$ H chain *In vivo*	H chain *In vitro*	L chain *In vivo*	L chain *In vitro*	mRNA $s_{20,R}$,‡ S H chain	mRNA $s_{20,R}$,‡ S L chain
GM607	Lymphoblastoid	IgM κ	10	77	69	24	26.5	20	13
SED	Chronic lymphocytic leukemia	IgM κ + IgD κ	14	77	69	24	26.5	20	13
GM1500	Myeloma	IgG2 κ	27	50	49	23	25	17	12
GM1056	Lymphoblastoid	IgA2 λ	6	60	58	24	26.5	18	13
GM923	Lymphoblastoid	IgA1 λ	2	60	ND	24	ND	ND	ND
αD5-DH11-BC11	HPL–P3	μ(IgG1 κ)	58	77	69	—	—	20	—
D3 D24.3	HPL–P3	μ(IgG1 κ)	46	77	ND	—	—	ND	—
57-77-F7	HPL–P3	μ(IgG1 κ)	32	77	69	—	—	20	—
CSK-10-2B1-C14	GM607–P3	μ(IgG1 κ)	21	77	ND	—	—	ND	—
CSK-NS6-201-C7	GM607–NP3	μ	30	77	ND	—	—	ND	—
106.2-B4-3G6	GM1500–P3	γ_2;(IgG1 κ)	35	50	ND	—	—	ND	—
FSK-4-2B2-C1	GM1500–NP3	γ_2;κ	40	50	ND	24	ND	ND	ND
DSK-13-2A5-C8	GM1056–P3	α_2;λ(IgG1 κ)	171	60	58	24	26.5	18	13
ESK-12-ID2-C3	GM923–P3	α_1;λ(IgG1 κ)	63	60	ND	ND	ND	ND	ND
P3x63Ag8	Plasmacytoma	(IgG1 κ)	0	57	54;50	23	25;26	17	13
NP3	Nonsecreting P3	—	0	—	—	—	—	—	—

Human Ig secreted and class identification were determined by $NaDodSO_4$/polyacrylamide gel analysis of cell medium class-specific immunoprecipitates labeled with [^{35}S]methionine (9), quantitative immunofluorescence, Ouchterlony precipitin rings with class-specific antisera, radioimmunoassays with class-specific reagents, and subclass Marchalonis assays. ND, not determined.

* HPL, human peripheral lymphocytes.

† Mouse Ig secreted is shown in parentheses.

‡ $s_{20,R}$ was determined in neutral 5–25% sucrose gradient of twice-purified oligo(dT)-cellulose polyadenylylated RNA relative to agarose gel-purified 4S and 18S rRNA.

batchwise with 6×, 2×, 1×, and 0.5× NaCl/Cit, each containing 0.5% $NaDodSO_4$, for 2 hr each at 23°C. The filters were autoradiographed with XRP film for 2–5 days at −20°C. Recombinant plasmids were isolated from 1-liter stationary phase cultures that were treated with chloramphenicol (44 μg/ml) for 6 hr prior to standard CsCl/ethidium bromide banding (27).

Hybrid Selection Translation. About 50 μg of each recombinant plasmid that was positive to μ probes was covalently linked to 1-cm discs of diazobenzylmethoxy-paper as described (28). These filters were used with 0.5–1.2 mg of polyadenylylated RNA isolated from GM607 cells for selective batchwise hybridization (28) to their complementary mRNAs. The mRNAs eluted from individual filters were coprecipitated with 10 μg of yeast tRNA and translated *in vitro* (14) in the presence of [^{35}S]methionine. The labeled polypeptides were resolved on 11% reducing $NaDodSO_4$/polyacrylamide gels, embedded, and autoradiographed (9).

DNA sequence determination was carried out (26) by G, G+A, T+C, C, and A>C reactions with 5′-end-labeled *Pst* I-rescued inserts that were secondarily cleaved with *Ava* II, *Hha* I, *Hpa* II, or *Hin*fI and separated on and eluted from gels (25, 26) prior to base-specific chemical cleavage.

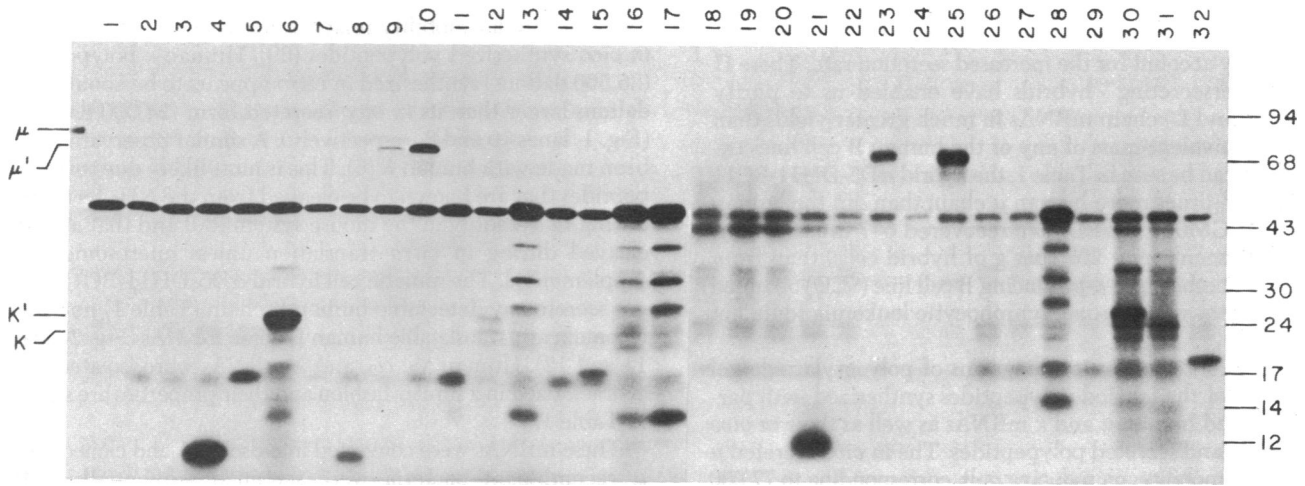

FIG. 3. Autoradiogram of [^{35}S]methionine-labeled proteins synthesized *in vitro* with mRNAs hybrid-selected from total human B cell mRNA by recombinant plasmid cDNAs. GM607 polyadenylylated RNA (1 mg) was hybridized with H and L recombinant plasmids linked to diazobenzylmethoxy-paper and the products of *in vitro* translation directed by eluted mRNA were resolved on composite $NaDodSO_4$/polyacrylamide gels. Lanes: 7 and 29, endogenously labeled products of the reticulocyte lysate; 1 and 24, background translation products of mRNAs eluted from vector-linked filters (pBR322). The other lanes show the translation products of mRNAs eluted from 28 independent recombinant filters: 6, pTD-Hκ-(607:1-31); 12 and 30, pTD-Hκ-(607:8-14), plasmids that selectively hybridize human κ mRNA; 9, pTD-Hμ-(αD5:11-10); 10, pTD-Hμ-(αD5:11-16); 23, pTD-Hμ-(607:6-9) and 25, pTD-Hμ-(607:7-12) all selectively hybridize human μ mRNA. $M_r \times 10^{-3}$ of standard proteins are shown. μ, μ′, κ, and κ′, Positions of *in vivo* and *in vitro* synthesized polypeptides.

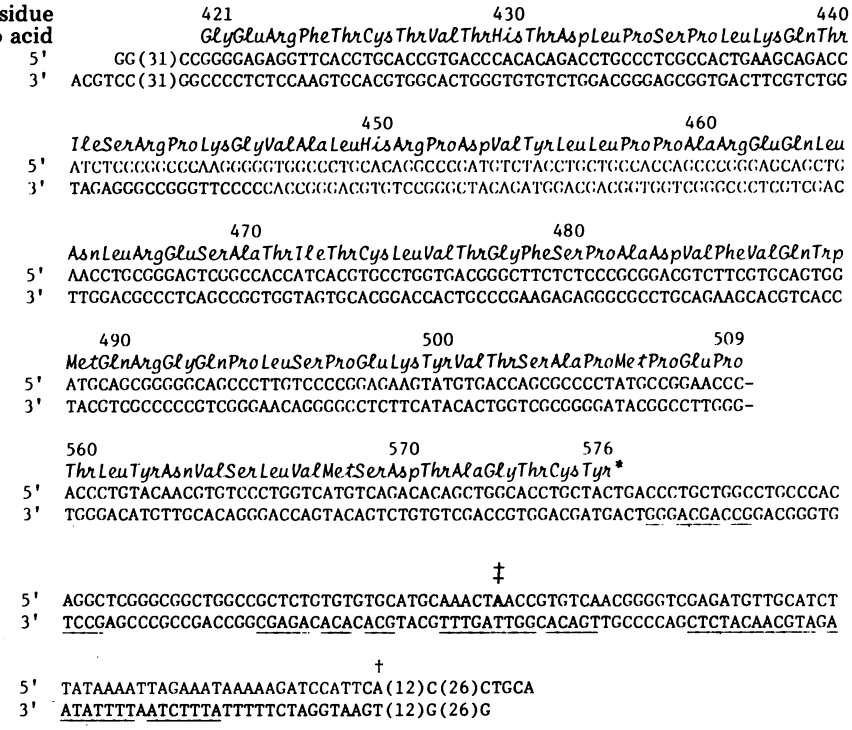

FIG. 4. Partial nucleotide sequence of human μ[pTD-Hμ(αD5:11-16)]-cDNA insert rescued from a *Pst* I digestion of the recombinant plasmid (26). Residue refers to amino acid residue from NH_2 terminus of human OU (4). The nucleotide sequence between residues 510 and 559 is pending. *, Termination codon UGA; ‡, termination codon UAA; †, beginning of poly(A) tail. Sequences underlined are homologous sequences observed in the mouse μ untranslated sequence (31, 32).

RESULTS AND DISCUSSION

Many of the somatic cell hybrids tested (Table 1) secreted significantly more human H chains than did their human parental B cells. This was observed with a number of hybridomas secreting different classes or subclasses of human H chains (μ, α_1, α_2, or γ_2). Because hybridomas produced with the mouse P3x63Ag8 cell line or its nonsecreting subline (NP3) still showed an increased rate of secretion of human H chain compared to their human B-cell parents (Table 1, compare GM607, CSK-10-2B1-C14, and CSK-NS6-201-C7; GM1500, 106.2-B4-3G6, and FSK-4-2B2-C1), the coupling of a more rapid mouse immunoglobulin L chain secretion with the human H chain cannot solely account for the increased secretion rate. These H chain-"hypersecreting" hybrids have enabled us to purify human H (and L) chain mRNAs in much greater yields than from an equivalent mass of any of the human B cell lines examined. As can be seen in Table 1, the hybrid αD5-DH11-BC11 secreted 4–6 times more human μ chain than did the human B cell lines GM607 or SED. We recovered 6–7 times more μ mRNA sedimenting at 20S (per g of hybrid cells) than from GM607 or another IgM κ-producing B cell line (SED) obtained from a patient with chronic lymphocytic leukemia (data not shown).

Figs. 1 and 2 show autoradiograms of polyacrylamide gel separations of the labeled polypeptides synthesized with partially purified human μ and κ mRNAs as well as their *in vivo* synthesized and secreted polypeptides. The *in vivo* secreted μ chain had a mobility, on reducing gels, corresponding to 77,000 daltons, whereas its *in vitro* directed product migrated as 69,000 daltons. We observed this in two human cell lines (GM607, Fig. 1, lane 7; and SED, data not shown) and in two somatic cell hybrids (57-77-F7 and αD5-DH11 BC11, Fig. 2, lanes 3 and 5, respectively). Our observations are contrary to the report by Klukas *et al.* (7) that, in RPMI 1788 cells, the *in vitro* directed immunoprecipitated μ polypeptide comigrated with the *in vivo* secreted form. It is possible that RPMI 1788 secretes a nonglycosylated μ chain. *In vitro* translations of μ mRNA with [^{14}C]mannose instead of [^{35}S]methionine showed no labeling of any polypeptides on polyacrylamide gel autoradiograms.

These observations and the lack of functional endoplasmic reticulum and Golgi apparatus in reticulocyte lysates, combined with the known 10–12% carbohydrate component of human μ chains secreted *in vivo* (2) all are consistent with the absence of glycosylation of the *in vitro* synthesized μ polypeptides and their faster mobility on reducing gels. Furthermore, mouse H chains contain an additional NH_2-terminal signal peptide and still show similar mobility relationships between *in vitro* and *in vivo* synthesized polypeptides (29). Human κ polypeptide (26,500 daltons) synthesized *in vitro* appears to be about 2500 daltons larger than its *in vivo* secreted form (24,000 daltons) (Fig. 1, lanes 10 and 9, respectively). A similar observation has been made with human λ (6). This is most likely due to signal peptides that are known to be cleaved from the NH_2 terminus during Ig assembly in the mouse system (30) and that are not cleaved during *in vitro* translation unless microsomes are supplemented. The somatic cell hybrid αD5-DH11-BC11 does not secrete any detectable human L chain (Table 1) nor does it contain any translatable human L chain mRNAs (Fig. 2, lanes 11 and 12). Human γ_2, α_2, and λ mRNAs were isolated and characterized in a similar fashion and their properties are shown in Table 1.

These mRNAs were converted into dscDNA and cloned. The μ recombinant bacteria were screened sequentially with [^{32}P]cDNA or mRNA probes of (*i*) homologous derived μ mRNA; (*ii*) heterologous μ mRNA; and (*iii*) sucrose gradient RNA fractions containing no detectable μ mRNA based on translation (data not shown). These procedures narrowed the potential number of μ cDNA recombinants to about 250.

Sixty of these recombinant plasmids were isolated, linked to DBM-paper, and used for hybridization-selection of their

complementary mRNA. Four of these plasmids (two from GM607 and two from αD5-BC11-DH11) were capable of selectively hybridizing mRNA that coded predominantly for a polypeptide with the properties of *in vitro* synthesized human μ chain (Fig. 3, lanes 9, 10, 23, and 25). Plasmid hybridization-selected mRNA translations from human κ (GM607) cDNA clonings and several others were included on these gels but will be thoroughly discussed elsewhere with accompanying sequence data.

Fig. 4 shows part of the DNA nucleotide sequence of one of the 40 μ cDNA recombinants identified [pTD-Hμ-(αD5: 11-16)]. This *Pst* I-rescued μ cDNA insert (655 base pairs) contains enough sequences to code for amino acids glycine (residue 421) through the terminal tyrosine (residue 576) of the human μ chain as well as the entire 3′-noncoding sequence including 12 residues of poly(A). The partial amino acid sequence as determined from the base sequence is in almost total agreement with the primary structures of both human OU and GAL μ chains isolated from patients with immune disorders (4, 33). In the human OU μ chain, glutamate residues occur at positions 487 and 493 (4) whereas our sequence data indicate glutamine codons. The human GAL μ chain has an extra glutamine residue at position 488 (33) where no glutamine codon occurs. These inconsistencies are most likely attributable to partial deamination of glutamine during sequential degradation and amino acid analysis.

Comparison of the human 3′ noncoding sequence with that published for mouse μ (31, 32) shows a striking conservation in certain regions (Fig. 4), as well as the codons of many amino acid residues shared in common (5, 31, 32). When the common amino acid codons differ in the mouse and human mRNA, they involve third base neutral codon substitutions (i.e., 560, Tyr; 563, Asn; 564, Val; 569, Ser; and 576, Tyr) (Fig. 4 and refs. 31 and 32). Unlike several other 3′ noncoding regions, the sequence A-A-U-A-A begins 12 residues from the poly(A) addition site in the human μ mRNA rather than the usual 20–30 residues observed with other mammalian 3′ untranslated sequences (34–38) (see Fig. 4). The differences observed in the 3′ noncoding sequences of mouse and human mRNA tend to be clustered, high in G+C content, and remote from the highly homologous regions near the A-A-U-A-A and the initial 3′ noncoding sequences. A more thorough analysis comparing the codon preferences and coding and noncoding sequence homologies and divergences should await completion of the mouse and human μ genomic sequence data.

The production of a number of independently derived human H and L chain probes obtained from normal circulating lymphocytes via hybridomas and lymphoblastoid cell lines would be of considerable value in examining the molecular basis of several well-characterized human immunoglobulin disorders.

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1. Williams, P. B., Kubo, R. T. & Grey, H. M. (1978) *J. Immunol.* **121**, 2435–2439.
2. Metzger, H. (1970) in *Advances in Immunology*, eds. Dixon, F. J. & Kunkel, H. G. (Academic, New York), Vol. 12, pp. 57–116.
3. Inman, F. P. & Mestecky, J. (1974) in *Contemporary Topics in Molecular Immunology*, ed. Ada, G. L. (Plenum, New York), Vol. 3, pp. 111–141.
4. Putnam, F. W., Florent, G., Paul, C., Shinoda, T. & Shimizu, A. (1973) *Science* **182**, 287–291.
5. Kabat, E. A., Wu, T. T. & Bilofsky, H. (1979) in *Sequences of Immunoglobulin Chains* (U.S. Dept. of Health, Education, and Welfare), pp. 89–96.
6. Yaffe, L. & Pestka, S. (1978) *Arch. Biochem. Biophys.* **190**, 495–507.
7. Klukas, C. K., Cramer, F. & Gould, H. (1977) *Nature (London)* **269**, 262–264.
8. Marcu, K. B., Valbuena, O. & Perry, R. P. (1978) *Biochemistry* **17**, 1723–1733.
9. Croce, C. M., Shander, M., Martinis, J., Cicurel, L., D'Ancona, G. G., Dolby, T. W. & Koprowski, H. (1979) *Proc. Natl. Acad. Sci. USA* **76**, 3416–3419.
10. Horibata, K. & Harris, W. A. (1970) *Exp. Cell. Res.* **60**, 61–69.
11. Potter, M. (1972) *Physiol. Rev.* **52**, 631–659.
12. Kearny, J. F., Radbruch, A., Liesegang, B. & Rajewsky, K. J. (1979) *Immunology* **123**, 1548–1550.
13. Stephens, R. E., Pan, C., Ajiro, K., Dolby, T. W. & Borun, T. W. (1977) *J. Biol. Chem.* **252**, 166–172.
14. Pelham, H. & Jackson, R. J. (1976) *Eur. J. Biochem.* **67**, 247–256.
15. Wickens, M. P., Buell, G. N. & Schimke, R. T. (1978) *J. Biol. Chem.* **253**, 2483–2495.
16. Schenk, T. E., Rhodes, C., Rigby, P. W. J. & Berg, P. (1975) *Proc. Natl. Acad. Sci. USA* **72**, 989–993.
17. Roychoudhury, R., Jay, E. & Wu, R. (1976) *Nucleic Acids Res.* **3**, 101–116.
18. Villa-Komaroff, L., Efstratiadis, A., Broome, S., Lomedico, P., Tizard, R., Naber, S. P., Chick, W. L. & Gilbert, W. (1978) *Proc. Natl. Acad. Sci. USA* **75**, 3727–3731.
19. Enea, V., Vovis, G. F. & Zinder, N. D. (1975) *J. Mol. Biol.* **96**, 495–509.
20. Sippel, A. Z., Land, H., Lindenmaier, W., Nguyon-Huu, M., Wurtz, J., Timmis, K. N., Giescoke, K. & Schultz, G. (1978) *Nucleic Acids Res.* **5**, 3275–3294.
21. Denhardt, D. (1966) *Biochem. Biophys. Res. Commun.* **23**, 641–646.
22. Humphries, P., Old, R., Coggins, L. W., McShane, T., Watson, C. & Paul, J. (1978) *Nucleic Acids Res.* **5**, 905–924.
23. Meyers, J. C., Spiegelman, S. & Kacian, D. L. (1977) *Proc. Natl. Acad. Sci. USA* **74**, 2840–2843.
24. Rigby, P. W. J., Dieckmann, M., Rhodes, C. & Berg, P. (1977) *J. Mol. Biol.* **113**, 237–251.
25. Maniatis, T., Jeffrey, A. & Van de Sande, A. (1975) *Biochemistry* **14**, 3787–3794.
26. Maxam, A. & Gilbert, W. (1980) *Methods Enzymol.* **65**, 499–560.
27. Kuperstock, Y. M. & Helinski, D. R. (1973) *Biochem. Biophys. Res. Commun.* **54**, 1451–1459.
28. Goldberg, M. L., Lifton, R. P., Stark, G. R. & Williams, J. G. (1979) *Methods Enzymol.* **68**, 206–220.
29. Jilka, R. L. & Pestka, S. (1977) *Proc. Natl. Acad. Sci. USA* **74**, 5692–5696.
30. Burstein, Y. & Schechter, I. (1977) *Proc. Natl. Acad. Sci. USA* **74**, 716–720.
31. Calane, K., Rogers, J., Early, P., Davis, M., Livant, D., Wall, R. & Hood, L. (1980) *Nature (London)* **284**, 452–455.
32. Matthyssens, G. & Rabbitts, T. H. (1980) *Nucleic Acids Res.* **8**, 703–713.
33. Watanabe, S., Barbikol, H. U., Horn, J., Bertram, J. & Hilschmann, N. (1973) *Z. Physiol. Chem.* **354**, 1505–1509.
34. Zakut, R., Givol, D. & Mory, Y. Y. (1980) *Nucleic Acids Res.* **8**, 453–466.
35. Kafatos, F. C., Efstratiadis, A., Forget, B. G. & Weissman, S. M. (1977) *Proc. Natl. Acad. Sci. USA* **74**, 5618–5622.
36. Konkel, D. A., Tilghman, S. M. & Leder, P. (1978) *Cell* **15**, 1125–1132.
37. Seeburg, P. H., Shine, J., Martial, J. A., Baxter, J. D. & Goodman, H. M. (1977) *Nature (London)* **270**, 486–494.
38. Honjo, T., Obata, M., Yamawaki-Kataoka, T., Kawakami, T., Takahashi, N. & Mawo, Y. (1979) *Cell* **18**, 559–568.