tion, the α_{2u}-globulin gene is transcriptionally silent in female rat liver nuclei.

The availability of this nuclear transcriptional system will allow us to measure the rate of transcription of the α_{2u}-globulin gene in rats in various endocrine states. It may thus be possible to determine whether the multihormonal control of α_{2u}-globulin mRNA levels is in all instances the result of control at the level of transcription, or whether it involves modulation of RNA processing, transport, or stability.

Acknowledgments

The expert techical assistance of Ms. Julie Kwang is gratefully acknowledged. We are indebted to Dr. Joseph W. Beard, Life Sciences Research Laboratories, St. Petersburg, Fla., for supplying purified AMV reverse transcriptase as part of the NIH-NCI program.

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Immunoglobulin Genes in DNA Restriction Fragments†

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ABSTRACT: We have investigated the organization of immunoglobulin genes in mice. High molecular weight DNA from myelomas and Krebs ascites cells was cleaved with *Eco*RI restriction endonuclease and fractionated using preparative agarose gel electrophoresis. Each fraction was then hybridized to an immunoglobulin mRNA or a cDNA transcribed from the mRNA. In two series of experiments, one with a κ chain probe (MOPC 41 mRNA), the other with a λ chain probe (SAPC 178 mRNA), we analyzed a variety of myeloma DNAs and Krebs DNA. In contrast to previously reported findings (Tonegawa, S., et al. (1976) *Cold Spring Harbor Symp. Quant. Biol. 41,* 877), we did not observe any unique restriction map pattern in the DNA from cells which express a given immunoglobulin gene. We also found that restriction fragments containing c region genes do not appear to transpose, while DNA sequences corresponding to other portions of the κ and λ mRNAs do in some cases.

Immunoglobulins are proteins which appear to be coded for by separate genes, genes for the variable (v) and constant (c)[1] regions. Evidence from genetic (Nisonoff et al., 1975) and nucleic acid hybridization experiments (Stavnezer et al., 1974; Leder et al., 1973; Faust et al., 1974) indicates that there exist one, or very few, c genes. Although the hybridization evidence may be an underestimate, as pointed out by Smith (1976), results with cloned immunoglobulin λ chain genes are compatible with the hypothesis that there may be only one c gene

* From the Department of Microbiology and Immunology, SC42, University of Washington, Seattle, Washington 98195. *Received January 10, 1978.* This work was supported by National Institutes of Health Grants A1-10685 and DE-02600. R.W. is supported by National Institutes of Health National Research Service Award GM-07187 from the National Institute of General Medical Sciences.

[1] Abbreviations used: cDNA, complementary DNA; c(v) region, constant (variable) region of an immunoglobulin polypeptide chain; L chain, immunoglobulin light chain.

0006-2960/78/0417-3096$01.00/0

per allelic chromosome (Brack & Tonegawa, 1977). On the other hand, v regions are coded for by multiple v genes. Their number is unknown, but in the case of κ chains it is probably at least 100. Cohn (1973) assumes that there are about 200 germ line v genes for κ chains, each specifying a given framework, and that somatic variability is restricted to the hypervariable regions.

It is generally accepted today that v and c genes are separated by some distance on the chromosome and that the v and c genes which are expressed by a cell, or the products of these genes, must be joined to form a functional immunoglobulin polypeptide chain. It is virtually ruled out that the joining occurs posttranslationally (Fleischman, 1967; Lennox et al., 1967). It has also been shown that cytoplasmic mRNA for κ chains is a continuous v–c sequence (Milstein et al., 1974). This leaves the possibility of ligation of a v-RNA with a c-RNA in the nucleus, or a joining of the genes in the chromosome. With respect to gene joining in the chromosome, a gene translocation mechanism has been proposed by Gally & Edelman (1970). This model assumes that a cell becomes committed to the synthesis of a given immunoglobulin by the excision of a given v gene and its insertion next to the c gene. It has been reported that v and c genes are in different DNA restriction fragments in cells which do not synthesize a given v gene (Hozumi & Tonegawa, 1976; Tonegawa et al., 1976, 1977). These authors also reported that v and c genes appear in the same restriction fragment size fraction in cells which produce the v region polypeptide chains of the sequence investigated. In this case, the c and v sequences appear in a restriction fragment size class smaller than either size class containing these fragments in the nonimmunoglobulin synthesizing cell. We report here a study of the location of κ and λ genes in restriction fragments of mouse DNA from four different tissues. In contrast to the DNAs investigated by Tonegawa & co-workers we always find the c region genes in DNA fragments of the same size.

Materials and Methods

Purification of 13S Immunoglobulin mRNA. The preparation of membrane-bound ribosomes from mouse myelomas MOPC 41 (κ), HOPC 2020 (λ), and SAPC 178 (λ), the extraction of RNA, and the isolation of poly(A)-containing RNA by oligo(dT)-cellulose chromatography have been described (Storb & Marvin, 1976; Bantle et al., 1976). The poly(A)-containing RNA was further purified by polyacrylamide gel electrophoresis in 98% formamide (Maniatis et al., 1975). The RNAs were then visualized by ethidium bromide staining (Figure 1A). The 13S mRNA containing band was excised and subjected to electrophoretic elution to remove the RNA from the gel. The eluted mRNAs were electrophoretically pure (Figure 1B). The RNAs were iodinated as described (Tereba & McCarthy, 1973; Storb & Marvin, 1976).

Synthesis of Short κ and λ cDNAs. Complementary DNAs (cDNA) were prepared from MOPC-41 κ and SAPC 178 λ mRNAs as described (Storb et al., 1977). By electrophoretic sizing (Maniatis et al., 1975), the cDNAs were shown to correspond mainly to the 3′ untranslated region and the c region of the mRNAs. κ cDNA was further purified by hybridization with MOPC-21 mRNA, which has a v region that is unrelated to MOPC-41 (McKean et al., 1973), and reisolation of the hybridized cDNA portions by S_1 nuclease treatment (Storb et al., 1977). The final κ cDNA was free of v region sequences. This was determined by the fact that the cDNA, which was initiated from the 3′ end of the mRNA, contained no molecules which were longer than the 3′ untranslated region plus the c region. Furthermore, the purified κ cDNA made from MOPC 41 mRNA hybridized completely with MOPC-21 κ mRNA.

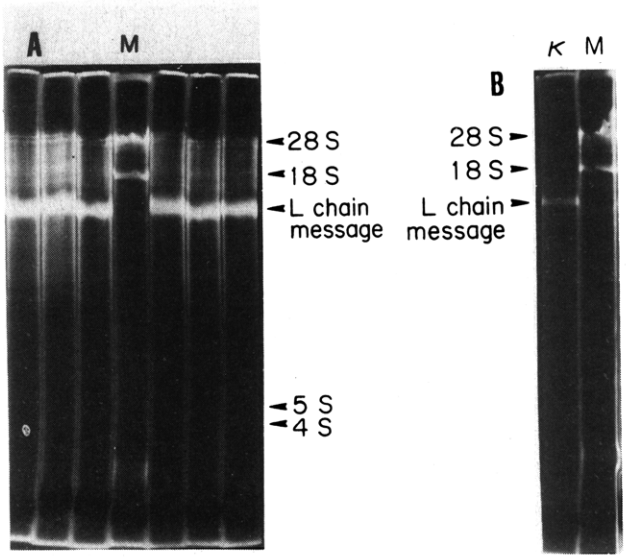

FIGURE 1: Polyacrylamide gel electrophoresis in 98% formamide. (A) In gel "M", 28S and 18S ribosomal RNAs, as well as 5S and 4S RNAs, were run as markers; the 5S and 4S RNAs were faintly stained. The other six gels show MOPC-41 microsomal poly(A) RNA. (B) Gel "M" contains marker RNAs. Gel "κ" shows the migration pattern of a portion of the MOPC-41 κmRNA purified by electrophoresis elution of the "L chain mRNA" from the six identical gels shown in A.

λ cDNA was passed over oligo(dA)-cellulose in 0.5 M KCl, 10 mM Tris (pH 7.5); approximately 80% of the cDNA bound and was eluted with 0.1 N KOH at room temperature. The λ cDNA was further purified by hybridization with S178 λ mRNA and isolation of the hybridized cDNA molecules. Since for λ sequences no λ mRNA is available which has a v region greatly divergent from S178, the λ cDNA probe contained some v region components. About 10% of the total cpm were in v region sequences.

Preparation of DNA and Restriction Fragments. DNAs from myelomas and Krebs ascites were prepared by a modification of the proteinase K method of Gross-Bellard et al. (1973). DNA was prepared from the same myelomas used for mRNA production, and in addition from MOPC 21 (κ). Krebs ascites cells are derived from a mammary tumor and do not synthesize immunoglobulins. After the tumor was removed from an animal, it was quickly chopped and homogenized with a motorized Teflon pestle. This nonviscous suspension was mixed with 20 volumes of buffer A (10 mM NaCl, 10 mM Tris pH 8, 10 mM EDTA, 0.5% sodium dodecyl sulfate, 50 μm/mL proteinase K) (Gross-Bellard et al., 1973). The remainder of the preparation was essentially identical with that described (Gross-Bellard et al., 1973).

DNA was digested with *Eco*RI. After cleavage was complete, the mixture was extracted with phenol and subsequently chloroform. Prior to electrophoresis, the DNA was concentrated by ethanol precipitation. Cleavage was monitored by noting the appearance of the mouse satellite band, migrating between 15 and 20 million daltons (Botchan et al., 1973), and another band always appearing at the 6.8×10^5 dalton position. We also tested completion of cleavage by codigesting mouse and λ phage DNA and found from this analysis that the criteria we used for determining extent of cleavage in mouse DNA alone were sufficient.

Preparative Agarose Gel Electrophoresis of Restriction Fragments. In earlier experiments, we used a device similar to that developed in P. Leder's laboratory (Tilghman et al., 1977). Restriction fragments were electrophoresed through a 0.5% agarose gel and collected in a chamber which was

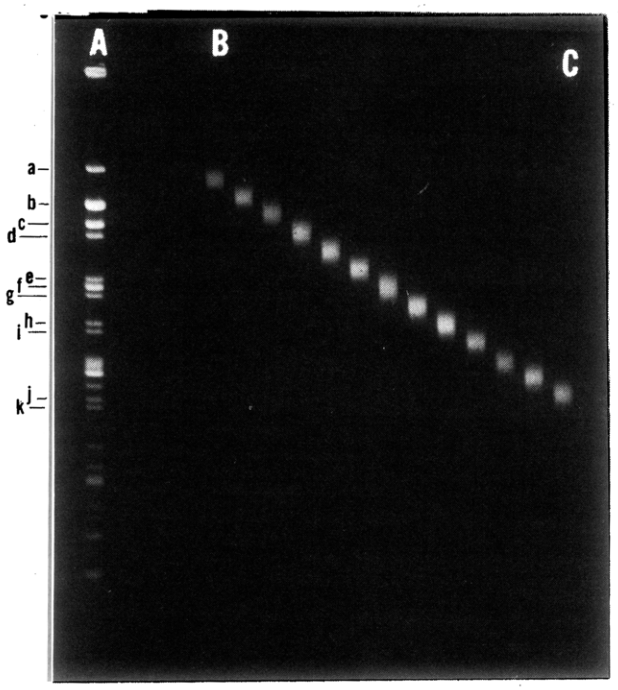

FIGURE 2: Agarose electrophoresis of *Eco*RI-generated DNA fragments. DNA fractionated and isolated from the gel slices by the method of McDonell et al. (1977) was reelectrophoresed. *Caulobacter* phage Cbk cleaved with *Eco*RI (A). Mouse DNA fragments of 12.5 (B) to 2.8 × 10^6 daltons (C). The molecular weights of phage CbK *Eco*RI fragments are in megadaltons: (a) 15.2; (b) 9.6; (c) 7.8; (d) 7.2; (e) 5.2; (f) 4.9; (g) 4.6; (h) 3.9; (i) 3.6; (j) 2.4; and (k) 2.3. The molecular weights were determined by comparing the mobilities of phage Cbk *Eco*RI fragments to the mobilities of markers whose molecular weights have been well documented: phage λ *Eco*RI fragments, phage ϕ29, and phage ϕ29 *Eco*RI fragments. These experiments were performed on 0.4% agarose gels in order to ensure that a linear relationship existed between R_f and log mol wt up to the range of 10–11 megadaltons (Wilson, unpublished observations).

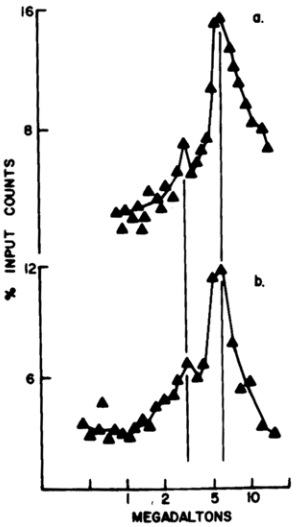

FIGURE 3: Reproducibility using two different fractionation methods. SAPC 178; DNA with SAPC 178 κmRNA (3000 cpm per fraction). (a) Electrolution method (Tilghman et al., 1977); (b) sliced gel method (McDonell et al., 1977).

partitioned from the remainder of the buffer reservoir by a dialysis membrane. Fractions were collected on a logarithmic time scale so that the molecular weight ratio of successive fractions remained constant.

We now use a procedure which in our hands provides better fractionation, i.e., less adjacent sample overlap as seen on subsequent reelectrophoresis of the fractions. The DNA (10 mg) is electrophoresed into a 0.9% agarose gel; the gel is sliced; slices are put in a bag of dialysis tubing; the DNA is electrophoresed out of the gel and trapped in the bag (McDonell et al., 1977). Eighty percent of the DNA is recovered. The results obtained by such a fractionation procedure are shown in Figure 2.

Hybridization of the Restricted DNA Fractions with Probe. The amount of DNA in each fraction was adjusted to the same value by adding *Euglena gracilis* DNA. Fractions were then sheared in a French press to about 800 base pairs and concentrated by ethanol precipitation. For mRNA–DNA hybridization, the DNA samples were dissolved in formamide, 4 M NaCl, 0.1 M Pipes (Calbiochem), pH 6.4, water (8:1:1) (Casey & Davidson, 1977). Hybridization was carried out for 4 days ($C_0t > 1000$) at 47 °C. DNA–cDNA hybridization was performed in 0.24 M phosphate buffer for 4 days at 67 °C. DNA–mRNA hybrids were analyzed by assaying RNase insensitive counts; DNA–cDNA hybrids are analogously analyzed using S_1 nuclease (Vogt, 1973). Assuming that immunoglobulin genes are unique, the sequence ratio of the genomic DNA to mRNA or cDNA was approximately ten in the hybridization reactions.

Results

Reproducibility of DNA Fractionation. In two experiments comparing the two preparative agarose gel techniques, we hybridized *Eco*RI cleaved SAPC 178 DNA with its own message. Figure 3a shows the results obtained with the electroelution method (Tilghman et al., 1977). Figure 3b shows the sliced gel method (McDonell et al., 1977). Both procedures gave the same result: a large peak of hybridization at 6 × 10^6 daltons and a smaller one at 3 × 10^6 daltons.

Specificity of the Probe. To determine whether the SAPC 178 λRNA probe was detecting immunoglobulin specific sequences, we compared it with another λ_1 RNA probe, one from HOPC 2020. Since the known λ_1 chains in mice differ by less than 3% amino acid substitution (Kabat et al., 1976), we expected HOPC 2020 mRNA to exhibit the same hybridization pattern as SAPC 178 when both were used to analyze the same DNA. In data not shown, HOPC 2020 mRNA hybridized to a large extent to the 6 × 10^6 dalton fragment and to a lesser degree to the 3 × 10^6 dalton fragment, a result in agreement with data shown in Figure 3. Although this is not proof that these probes are hybridizing only to immunoglobulin sequences, it is strongly suggestive. These data also show that 18S ribosomal RNA is not a contaminant in the RNA probes, because we fail to see hybridization to the ~14 × 10^6 dalton fragment described by Southern (1975) which contains the ribosomal RNA sequences.

Hybridizations with MOPC 41 mRNA. We examined several different DNAs with the mRNA probe to determine whether the DNA from the myeloma, which synthesized the probe we used, exhibited a unique hybridization pattern. We used MOPC 41 κmRNA to analyze *Eco*RI digests of DNA from MOPC 41, SAPC 178, MOPC 21 myelomas, and from Krebs ascites tumor. The four hybridization patterns are shown in Figure 4. In all four cases, hybridization occurs chiefly to a fragment of 10 × 10^6 daltons. The molecular weight of the minor peaks differs in different DNAs, as seen in the tabulation of Table I. The pattern of the minor peaks with MOPC 41 DNA most closely resembles that of the λ chain producer, SAPC 178 and somewhat that of the κ chain producer MOPC 21. Compared with the minor peaks of the other DNAs the 5.1 × 10^6 dalton peak of MOPC-21 DNA is higher relative to the 10 × 10^6 dalton peak. This may be due to incomplete elution

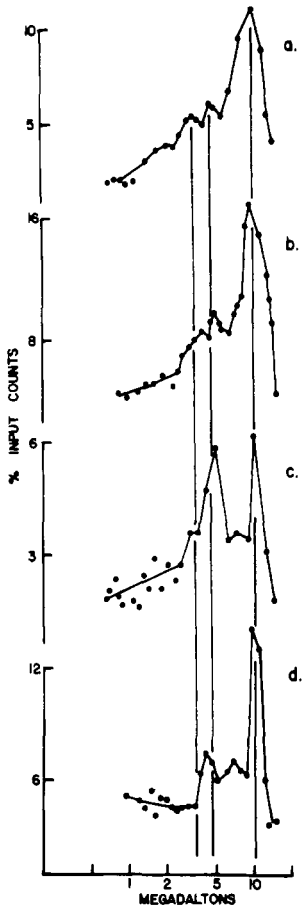

FIGURE 4: Hybridization of κmRNA (MOPC 41) (3000 cpm per fraction) with different restricted mouse DNAs. (a) MOPC-41 (κ); (b) SAPC 178 (λ); (c) MOPC 21 (κ); (d) Krebs. The vertical lines are drawn through the peaks obtained in the homologous case.

TABLE I: Size of κ and λ Gene Containing DNA Restriction Fragments.[a]

	Probe			
DNA	MOPC 41 mRNA	SAPC 178 mRNA	MOPC 41 c region cDNA	SAPC 178 C region cDNA
MOPC 41	10.8			
	4.7			
	3.4			
SAPC 178	10.5	6.0	10.0	5.8
	5.0	3.3		
	4.0			
MOPC 21	10.4	6.3		
		9.4		
	5.1	2.7		
Krebs	10.2	6.3		
	7.1	9.0		
	4.2	2.2		

[a] The table summarizes the data of Figures 2–6. The numbers indicate the molecular weight of the fragments in millions of daltons.

of DNA in the 10 × 10^6 dalton peak (note the different scales of the ordinates).

Hybridizations using SAPC 178 mRNA. In a similar series of experiments we analyzed different *Eco*RI cleaved DNAs with SAPC 178 λmRNA. The data, shown in Figure 5, indicate that in each case hybridization occurs chiefly to a 6 × 10^6 dalton fragment. Molecular weights of minor peaks are tabulated in Table I. SAPC 178 DNA exhibits a pattern which

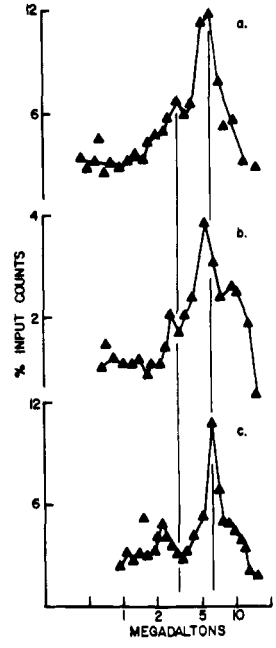

FIGURE 5: Hybridization of κmRNA (SAPC 178) (3000 cpm per fraction) with different restricted mouse DNAs. (a) SAPC 178; (b) MOPC 21; (c) Krebs.

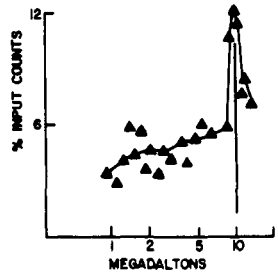

FIGURE 6: Hybridization of short κcDNA (1400 cpm per fraction) with SAPC 178 DNA.

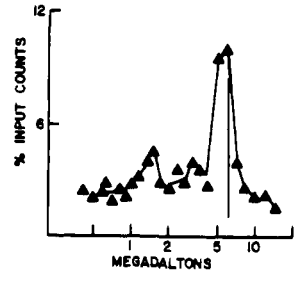

FIGURE 7: Hybridization of short λcDNA (2000 cpm per fraction) with SAPC 178 DNA.

is very similar to that of MOPC 21, whereas Krebs is more different from either. One observes the same phenomenon seen with the MOPC 41 mRNA probe; namely, the major peak is constant and the minor peaks shift around in different DNAs, regardless of whether the cell produces the mRNA used as probe.

Experiments with c Region cDNA Probes. When short MOPC 41 κcDNA is hybridized to restriction fragments, only the locations of the 3′ untranslated and c regions are determined. Figure 6 shows that hybridization occurs extensively to the 10 × 10^6 dalton fragment of S178 DNA. We conclude that the 3′ untranslated and the c region sequences for κ chains are located in the 10 × 10^6 dalton fragment.

The analogous experiment using λcDNA (Figure 7) and

S178 DNA showed that the 3′ untranslated and c regions are located in the 6×10^6 dalton fragment. The experiment with λcDNA revealed a minor peak at 3×10^6 daltons, a result probably explained by the fact that this probe contains some v region sequences (see Materials and Methods). We do not know why there is a minor peak at 1.5×10^6 daltons.

Discussion

The κ and λ mRNAs used in this study were highly purified. They appeared as a single band in electrophoresis in denaturing polyacrylamide gels (Figure 1B). In cell-free translation in the rabbit reticulocyte system they stimulated the synthesis of κ or λ chains and no other proteins (not shown). Each probe of iodinated κ and λ mRNA was also annealed with unfractionated mouse DNA; only a single hybridization phase with relatively unique DNA sequences, i.e., no hybridization with repetitive sequences was found (data not shown). In addition, we have other data with κ and λ cDNAs which are supportive of sufficient purity of the mRNAs (Storb et al., 1977). It was found that cDNA prepared from κ and λ mRNAs contained contaminating sequences which reacted with RNA sequences present in the mRNA at the 1% level. This level would be too low to be detected as a peak in hybridization of κ or λ mRNA with unique or moderately repetitive DNA sequences since the hybridization background level alone was considerably higher than 1% (Figures 4 and 5). DNA was in excess in our experiments; immunoglobulin gene sites were therefore not saturated and at the same time any particular RNA impurity would not have had a chance to be detected, as discussed by Hozumi & Tonegawa (1976). Furthermore, two λ mRNAs prepared from different myelomas, SAPC 178 and HOPC 2020, showed the same hybridization peaks with restricted SAPC 178 DNA (see Results section). On the other hand, the κ and λ mRNAs showed different hybridization patterns with the same DNA. It appears unlikely that different λ mRNAs would contain the same impurities, that would not be present in κ mRNAs since in all other respects different myeloma tumors (the source of the mRNAs) behave very similarly.

For these reasons we believe that both the major and the minor peaks of hybridization seen with DNA restriction fragments comprise portions of immunoglobulin genes. With both κ and λ mRNAs we find hybridization with a DNA fraction which seems invariant from tissue to tissue, the 10×10^6 dalton peak for κ, and the 6×10^6 dalton peak for λ. These fractions contain c region and 3′ untranslated sequences. We have not determined whether they contain v region sequences.

The minor peaks of hybridization with DNA fragments do not appear to contain c region or 3′ untranslated region sequences, but we do not know yet what immunoglobulin sequences they do contain. They may contain sequences for the v region, for the short "leader" peptide present at the 5′ end of v regions of L chains synthesized cell free, and/or for the 5′ untranslated region (Milstein et al., 1974). The minor peaks seem to shift when DNAs from different tissues are compared (Figures 4 and 5). The differences between different myelomas are not very pronounced and may not be real. In the case of κ, the myeloma which produces the κ mRNA used as a probe (MOPC 41) is not more different from either of the other two than they are from each other. The minor peaks with Krebs DNA seem to be truly different from the myeloma DNAs both with κ and λ mRNA, especially when one considers that the method gives quite reproducible results (Figure 3). Further experiments will have to determine the significance of these minor peaks. If their shifts can be confirmed with DNA from nonmalignant cells, it would appear that certain segments of immunoglobulin genes behave differently from other unique genes. For example, the restriction map for the ovalbumin gene in chickens is the same when DNA is isolated from 5 day old embryos, erythrocytes, or the oviduct (Breathnach et al., 1977). Also globin genes do not appear to shift (results cited in article by Williamson, 1977).

In contrast to the results reported by Hozumi & Tonegawa (1976) with MOPC 321 myeloma we do not see a major new positive fragment in the cells which express the κ or λ chains under study (MOPC 41 and SAPC 178). This does not prove that a rearrangement of one or both allelic v and/or c genes has not taken place. Such a rearrangement could go unnoticed if it did not make a major difference in the mobility of the c gene fragment. Thus the expressed v region sequence could have been rearranged within the 10×10^6 or 6×10^6 dalton fragment, respectively, or it could have been inserted into it. Alternatively the c region sequence could have been translocated within the major fragment. In a λ myeloma, HOPC 2020, the v and c regions were reported to be separated by a 1250 base DNA fragment (Brack & Tonegawa, 1977). Such an intervening sequence may be larger in other cases, since it has been reported that certain myeloma cells (P3) produce a nuclear κ mRNA precursor of about ten times the size of the mature mRNA (Gilmore-Hebert & Wall, 1978). In the light of this finding our data are compatible with the possibility that in some cases translocations of v and/or c genes may not occur and that the v region to be expressed may be selected post-transcriptionally. Such a mechanism appears to operate in another eucaryotic system, the processing of adenovirus-2 mRNAs (Berget et al., 1977; Chow et al., 1977; Klessig, 1977).

Acknowledgments

We thank Drs. Polsky, Edgell, Seidman, and Leder for their kindness and generosity to familiarize us with their preparative electrophoresis apparatus before the method was published. We thank Dr. N. Agabian for Cbk phage. We are very grateful for reverse transcriptase to Dr. J. W. Beard and E. Houts and the Office of Program Resources and Logistics, Viral Cancer Program, Viral Oncology, Division of Cancer Cause and Prevention, National Cancer Institute, Bethesda, Md. 20016. Special thanks go to B. Arp for excellent technical help and to Drs. B. Hall and C. Sibley for critical reading of the manuscript.

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Light Chain Variable Region Sequence of Rabbit Antipneumococcal Type III Polysaccharide Antibody 3368†

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ABSTRACT: The amino acid sequence of the amino-terminal 111 residues (variable region) for the light chain of the homogeneous rabbit antipneumococcal type III polysaccharide antibody 3368 was determined. This sequence was obtained principally through automated Edman degradations of the intact light chain and of peptides generated by tryptic digestion of the citraconylated light chain. With these methods only 2 μmol of purified light chain was required to determine the reported sequence. When compared with the light chains of four other antipneumococcal type III polysaccharide antibodies, the 3368 light chain exhibits a unique sequence in those segments of the variable region that contribute to formation of the antigen binding site (complementarity-determining regions) (10 or 11 residue differences in 12 positions). The 3368 light chain also demonstrates an insertion of three residues relative to the other four light chains in the complementarity-determining region at positions 89 to 98. These five light chains have greater than 80% sequence homology for the portion of the variable region which is not involved in antigen binding (framework).

The considerable interest in the variable domain structures of immunoglobulin (Ig)[1] molecules stems in large measure from the demonstration that the antigen binding site is contained within the variable domain. High resolution x-ray crystallographic analyses have demonstrated that the variable regions of Ig light and heavy chains fold together to form a compact domain, and that antigen is specifically bound within a well-defined site formed by the interaction of the two variable regions (Poljak et al., 1974; Segal et al., 1974). At least two discrete polypeptide segments of the light chain and three discrete segments from the heavy chain variable region form the walls of the antigen binding site. These complementarity-determining regions of the Ig variable domain correspond to the hypervariable regions identified by statistical analyses of variable region amino acid sequences from human and murine Igs (Wu & Kabat, 1970; Kabat & Wu, 1971; Capra & Kehoe, 1974) and, more recently, from rabbit antibody light chains (Margolies et al., 1975). The amino acid sequence diversity among these complementarity-determining regions presumably accounts for the capacity of the immune system to respond to a seemingly unlimited variety of antigenic stimuli with the production of specific antibodies, and it is also re-

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§ This work was performed during M.N.M.'s tenure as an Established Investigator of the American Heart Association.

[1] Abbreviations used: Ig, immunoglobulin; Pth, phenylthiohydantoin; CM, carboxymethyl; Gdn·HCl, guanidine hydrochloride.

0006-2960/78/0417-3101$01.00/0