Quantitation of Constant and Variable Region Genes for Mouse Immunoglobulin λ Chains†

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ABSTRACT: We have synthesized and characterized cDNA complementary to purified mRNA derived from the λ chain producing myeloma tumor, RPC-20. This cDNA is of sufficient length to encode the constant region and a major portion of the variable region sequence of the λ gene. In addition, the expected range of cross-hybridization of this λ probe has been shown to extend to several different members of the closely related λ subgroup, as well as to a member of the λ subgroup represented by MOPC-315. Since there are a *minimum* of seven known members of the common λ subgroup in addition to MOPC-315, these sequences, in accordance with the germ line hypothesis, must be represented by a *minimum* of eight variable region genes. Using the RPC-20 cDNA probe and hybridization kinetic analysis, this sequence was found to be represented as approximately two copies per haploid genome in DNA derived from a variety of κ- and λ-producing tumors and normal tissue. Inasmuch as the cross-hybridization range of the probe has been assessed and a minimum size of the λ subgroup determined, this observation tends to rule out separate germ line genes corresponding to each individual λ light chain variant. Certain reservations about these conclusions are discussed.

Two contrasting hypotheses have been proposed to account for the diversity and specificity of immunoglobulin molecules (for reviews see Gally and Edelman, 1972; Wigzell, 1973; Cohn et al., 1974; Hood et al., 1974). The germ line hypothesis states that all immunoglobulin molecules are encoded intact in the genome and that evolutionary processes are responsible for the diversity of antibody molecules. In contrast, the somatic mutation hypothesis claims that diversity arises, at least in part, as the result of somatic events operating on a small number of germ line genes during immunodifferentiation. Accordingly, the somatic mutation theory envisages a smaller number of variable (V) region genes than does the germ line hypothesis. In order to distinguish between these models, workers in several laboratories, including our own, have set out to quantitate immunoglobulin genes by hybridization kinetic analysis using radioactive probes derived from mouse immunoglobulin mRNA encoding κ light chains or heavy chains (Delovitch and Baglioni, 1973; Bernardini and Tonegawa, 1974; Faust et al., 1974; Honjo et al., 1974; Leder et al., 1974a,b; Stavnezer et al., 1974; Storb, 1974; Tonegawa et al., 1974; Leder et al., 1975; Rabbitts et al., 1975; Rabbitts and Milstein, 1975).

Since hybridization analysis detects a number of genes having sequences closely enough related to that of the hybridization probe to yield a stable hybrid, interpretation of these results depends upon the nature and size of the light or heavy chain subgroup from which the probe is derived. Thus, the interpretation of these results is necessarily limited unless one chooses a subgroup which consists of many well-defined immunoglobulins and knows how close one sequence must be to another to form a stable hybrid. Unfortunately, the κ subgroups studied so far by hybridization analysis do not satisfy these demands.

In order to overcome these difficulties, we have turned to the λ light chain system of mouse. This system is particularly well suited to this kind of analysis. The complete primary structures of 18 λ chains have been determined (Weigert et al., 1970; Appella, 1971; Cohn et al., 1974). Twelve of the 18 λ chains have identical amino acid sequences. The remaining six have one to four amino acid residue replacements, all of which are in the hypervariable region and are believed to be specificity-determining. Therefore, according to the germ line hypothesis there must be, *as a minimum*, seven germ line V region genes for the λ chain. The somatic mutation theory demands only one germ line V region gene which will diverge into seven different clones during immunodifferentiation. Furthermore, the amino acid differences ar so small as to assure that separate genes would form stable hybrids with probe corresponding to different λ chains.

Here we describe the characterization of an especially long λ light chain cDNA probe extending over sequences corresponding to *both* constant and variable regions. This probe forms complete and stable hybrids with mRNAs derived from several λ chain producing myeloma tumors, even with mRNA derived from a clearly different constant region subclass. Since its cross-hybridization properties are well delineated and a *minimum* size germ line gene pool is easily defined, hybridization kinetic analysis with this λ chain probe has particularly strong predictive value in terms of the germ line and somatic mutation hypotheses. Such studies are described. A preliminary report of some of these results has appeared (Leder et al., 1975).

Materials and Methods

(a) Preparation and Purification of mRNAs and cDNAs. Purification of mRNA encoding mouse globin and light chains derived from RPC-20, MOPC-104E, and S176 myelomas was described (Aviv and Leder, 1972; Honjo et al., 1976); Krebs II ascites cell RNA was extracted from total polysomes and purified up to the first oligo(dT)-cellulose chromatographic step, as described (Honjo et al., 1974). [^{3}H]cDNAs complementary to RPC-20 mRNA and mouse globin mRNA were synthesized and purified as described for MOPC-41 (Honjo et al., 1974). Briefly, "C_Rt purification" of the probe involves hybridization of [^{3}H]cDNA probe with purified RPC-20

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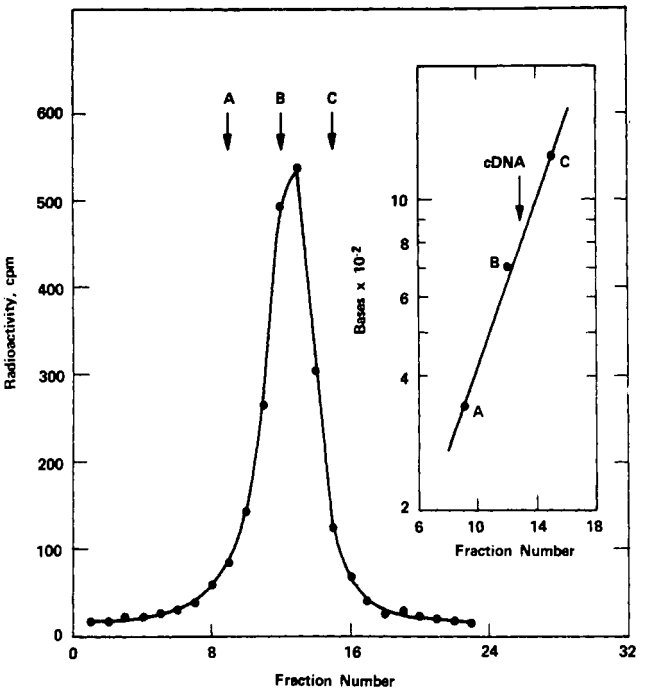

FIGURE 1: Alkaline sucrose gradient sedimentation analysis of RPC-20 cDNA. Purified RPC-20 [^{3}H]cDNA and [^{32}P]-labeled λ phage DNA fragments produced by *Hemophilus influenzae* restriction enzyme digestion were cocentrifuged as described under Materials and Methods. A, B, and C show the peak locations of [^{32}P]DNA fragments which are 340, 700, and 1250 bases long, respectively.

mRNA (SG 2 stage (Honjo et al., 1976)) to a $C_rt_{1/2}$ value at which only sequences present at very high concentration will form hybrids (1–50 × 10^{-3} mol l. s^{-1}). Contaminating sequences (if any) present at low concentrations will not form hybrids. The hybrid [^{3}H]cDNA was separated from nonhybridized material on a hydroxylapatite column and the high melting material (T_m > 80 °C), corresponding to the predominant sequence in the mRNA preparation, was used for further analysis. The specific activity of [^{3}H]cDNA was estimated to be 10^7 cpm/μg based on the assumption that cDNA contains 25% CMP.

(*b*) *Hybridization Reaction.* Hybridization of [^{3}H]cDNA to mRNA was carried out as described (Honjo et al., 1974). Hybridization of radioactive probes with cellular DNA sheared to a length of approximately 450 base pairs was performed at 67 °C in 1.0 M NaCl–20 mM Tris-HCl (pH 7.0); 0.18 M NaCl was used at the lower C_ot values. DNA concentration was as specified in each experiment. C_ot values are expressed as those that would obtain at 0.18 M NaCl (Britten and Kohne, 1968) in a DNA–DNA reannealing reaction. Annealed [^{3}H]cDNA was assayed for its resistance to S_1 nuclease (Ando, 1966; Packman et al., 1972). Aliquots were incubated with 10 μg/ml of S_1 nuclease at 45 °C for 1 h in buffer containing NaOAc, pH 4.5, 0.15 M; NaCl, 0.12 mM; $ZnSO_4$, and 10 μg/ml of single-stranded, sheared salmon sperm DNA. Reaction mixtures were acid precipitated and collected on glass fiber filters. Filters were eluted with NCS (Amersham/Searle) and counted in solution in a standard toluene–fluor mixture.

(*c*) *Thermal Denaturation of Nucleic Acid Hybrids.* Thermal denaturation of cDNA–mRNA or cDNA–DNA hybrids was carried out by thermal elution from hydroxylapatite or by the resistance to S_1 nuclease of aliquots heated in 0.25 M NaCl–20 mM Tris-Cl (pH 7.0) as described by Honjo et al., 1974.

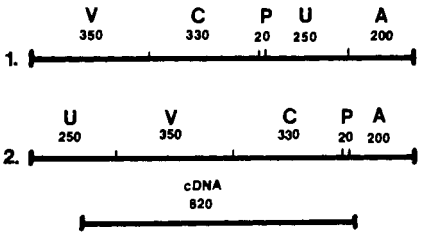

FIGURE 2: Possible arrangements of sequences in RPC-20 mRNA and its cDNA. Numbers refer to length in bases. (1) A, poly(A) sequence; (2) U, untranslated sequence; (3) P, putative precursor sequence; (4) C, constant region sequence; (5) V, variable region sequence. (1) Untranslated sequence at 3′ end. (2) Untranslated sequence at 5′ end.

(*d*) *Alkaline Sucrose Gradient Centrifugation.* The [^{3}H]cDNA was layered on a 5–20% alkaline sucrose gradient (pH 12.5) containing 0.9 M NaCl and 1 mM EDTA[1] and centrifuged in a Beckman SW41 rotor at 40 500 rpm for 30 h at 5 °C. Fractions were collected and aliquots were taken to determine acid-insoluble radioactivity. Phage λ DNA fragments of established length were generously provided by Dr. T. Maniatis.

Results

(*a*) *Characteristics of the RPC-20* [^{3}*H*]*cDNA.* The nucleotide length of the RPC-20 [^{3}H]cDNA was determined by alkaline sucrose gradient centrifugation (Figure 1). The C_rt-purified (see Methods) [^{3}H]cDNA migrates as a single, relatively homogeneous population of molecules whose average chain length, as determined by comparison to appropriate standards, is 820 bases. Since cDNA synthesis has been shown to start near the 5′ end of the poly(A) sequence of the template (Faust et al., 1974), this cDNA probe should be complementary to the entire constant region sequence and to a major portion (perhaps all) of the variable region sequence as well. The sequences can be compared in the following way. RPC-20 mRNA consists of a 1150-nucleotide long sequence of which 350 nucleotides must encode the variable region, 330 nucleotides the constant region, and approximately 20 nucleotides the putative precursor sequence which, in this case, is likely to be adjacent to the C terminal region (Honjo et al., 1976). By analogy to a partially sequenced κ light chain mRNA (Milstein et al., 1974), approximately 200 adenylic acid residues are at the 3′ end of the message. This leaves approximately 250 nucleotides for an untranslated sequence and these can be arranged into extremes, either all at the 5′ end or all between the structural sequence and the 3′ terminal adenylic acid residues (Figure 2). In the former case, the RPC-20 cDNA probe would be complementary to the entire C and V region and a portion of the untranslated sequence as well. In the latter case the RPC-20 cDNA probe would be complementary to the entire untranslated region, the entire C region and over 50% of the V region sequence. The most likely arrangement seems some intermediate between these two extremes, in which case virtually the entire V region would be represented in the probe.

Although the cDNA sequence extends into the V region, the amino acid sequence homologies between λ chains are so great as to lead one to expect stable cross-hybridization between this probe and mRNAs derived from other members of this subgroup. To test this, we have cross-hybridized RPC-20 cDNA to partially purified mRNA from two other λ chain-producing tumors, MOPC-104E and S176. The MOPC-104E and S176 proteins have one amino acid residue replacement

[1] Abbreviation used: EDTA, (ethylenedinitrilo)tetraacetic acid.

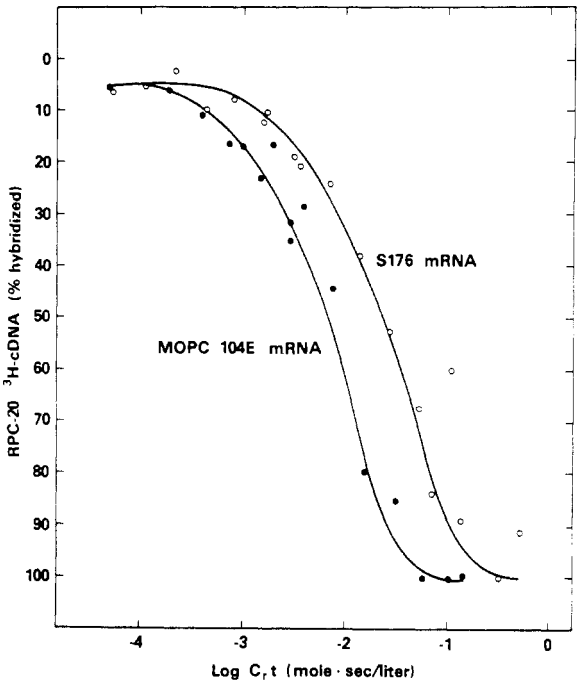

FIGURE 3: Cross-hybridization of RPC-20 cDNA with other λ chain mRNAs. mRNAs used were purified to the dT 1 stage (Honjo et al., 1976). $C_rt_{1/2}$ values for S176 and MOPC-104E are 7.4×10^{-3} and 2.6×10^{-2}, respectively.

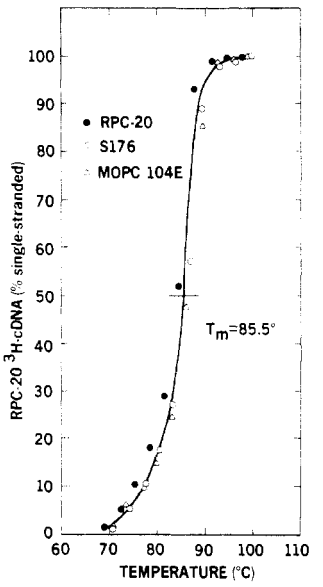

FIGURE 4: Thermal denaturation of RPC-20 cDNA hybrids with various λ chain mRNAs. The mRNAs employed were purified to the dT 1 stage and were the same preparation used in Figure 3. Analysis was carried out by thermal elution from hydroxylapatite, as described (Honjo et al., 1976). Symbols are identified in the figure.

in their variable regions distinguishing them from the RPC-20 proteins (Cohn et al., 1974). As shown in Figure 3, mRNAs from S176 tumors and MOPC-104E completely cross-hybridize with the RPC-20 cDNA. The $C_rt_{1/2}$ values of 7.4×10^{-2} and 2.6×10^{-2}, respectively, are comparable to 1.1×10^{-2} for RPC-20 mRNA at the corresponding (dT-1) purification step (cf. Honjo et al., 1976). The thermal denaturation profiles of the hybrids formed between these mRNAs and the [^{3}H]cDNA are sharp and indistinguishable from that of the homologous hybrid, suggesting that the hybrids are congruently base-paired (Figure 4). These results indicate that the nucleotide sequences of the MOPC-104E and S176 mRNAs are sufficiently similar to those of the RPC-20 to be scored in the genome using RPC-20 cDNA as a hybridization probe. Note that is in distinct contrast to the behavior of κ chain probes cross-hybridized to κ chain mRNAs from different κ subgroups (Honjo et al., 1974).

(*b*) *The Extent of Cross-Hybridization of the RPC-20 cDNA Probe.* There are at least two subgroups of mouse λ constant regions, that characteristic of RPC-20 λ chains and that characteristic of MOPC-315 λ (Duggan et al., 1973). The constant region of MOPC-315 contains long, contiguous blocks of amino acids whose sequences are identical with those found in RPC-20 λ chains. Nevertheless, in the constant region sequence there are amino acid substitutions in 27 of 105 amino acid positions. The variable regions are in much closer agreement, differing in only 8 of 110 amino acid residues. All these differences could be accounted for by as small as a 5% difference in nucleotide sequence in their respective mRNA's. Therefore, it is not surprising that the RPC-20 cDNA probe hybridizes completely to MOPC-315 mRNA under these conditions of hybridization (Figure 5). In contrast, there is little significant cross-hybridization to the κ-chain MOPC-41 mRNA. This hybridization, which occurs only at very high C_rt values, may reflect gross mismatching of evolutionarily related sequences possibly involving homologies that exist between κ and λ chains (note especially the base sequence encoding the

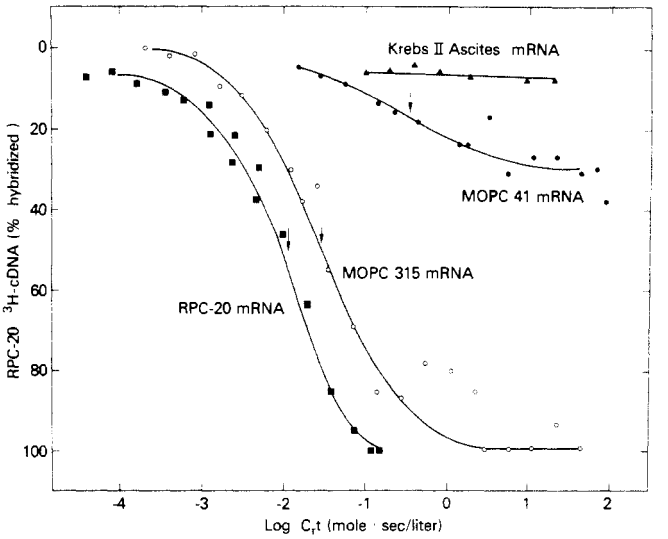

FIGURE 5: Cross-hybridization of RPC-20 [^{3}H]cDNA. Hybridization reactions were carried out as described under Methods. All mRNAs employed were purified to the dT 1 stage (Honjo et al., 1976). Closed squares, RPC-20 mRNA; open circles, MOPC-315 mRNA; closed circles, MOPC-41 mRNA; closed triangles, Krebs II ascites cell mRNA.

amino acids in positions 121 through 129 of the λ and κ constant region (Schulenberg et al., 1971). There is no cross-hybridization to control mRNA obtained from Krebs II ascites tumor cells.

The congruity of these interactions can be assessed using thermal denaturation as shown in Figure 6. In this case nuclease S_1 has been used to assess denaturation. The RPC-20 [^{3}H]cDNA hybrids formed with RPC-20 mRNA and MOPC-104E mRNA, both belonging to the same subclass, show thermal elution profiles which are indistinguishable. The hybrid formed with MOPC-315 shows an approximately 4 °C reduction in T_m and a shift giving clear evidence of mismatching. The small amount of hybridization observed with MOPC-41 mRNA was highly unstable even at 70 °C (not shown), indicating gross mismatching. We conclude that,

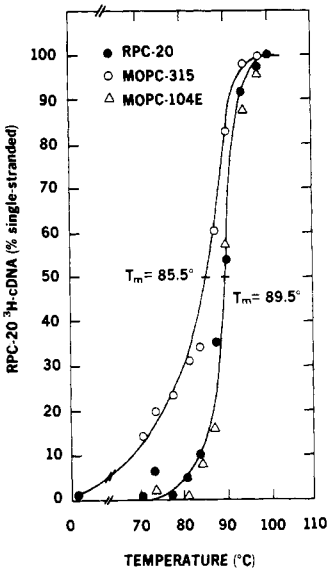

FIGURE 6: Thermal denaturation profile assayed by S_1 nuclease digestion. The hybrids formed at a C_rt value of 2×10^{-1} for RPC-20 and MOPC-104E mRNA and 6.8×10^{-1} for MOPC-315 mRNA were assayed for their thermal stability to S_1 nuclease digestion, as described under Materials and Methods. Open circles, MOPC-315 mRNA; open triangles, MOPC-104E mRNA; and closed circles, RPC-20 mRNA.

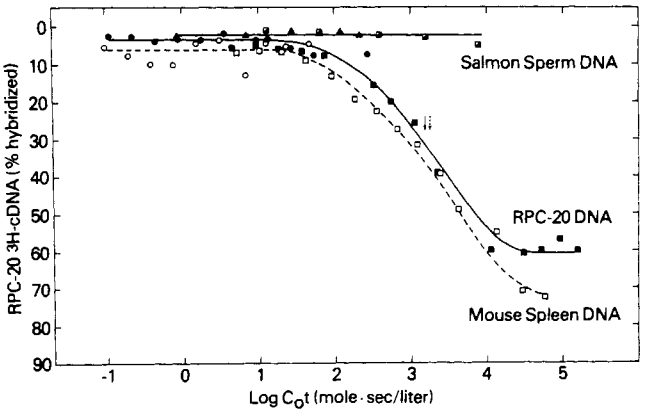

FIGURE 7: Hybridization kinetic analysis of RPC-20 cDNA with total cellular DNAs from RPC-20 myeloma tumor, mouse spleen, and salmon sperm. Reactions were carried out as indicated under Materials and Methods. $C_0t_{1/2}$ values for RPC-20 and mouse spleen DNA are 1600 and 1900, respectively. Arrows indicate $C_0t_{1/2}$ points. Symbols represent the following: open circles, mouse spleen DNA at 1.4 mg/ml—0.18 M Na^+; open squares, mouse spleen DNA at 9.7 mg/ml—1 M Na^+; closed circles and squares, RPC-20 DNA, similar reactions; closed triangles and half-closed squares, salmon sperm DNA, in similar reactions. RPC-20 [^{3}H]-cDNA concentration was 440 pg/ml.

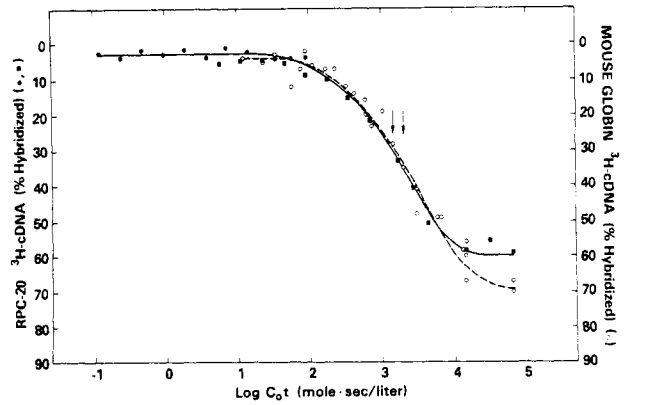

FIGURE 8: Hybridization kinetic analysis of RPC-20 cDNA and globin cDNA with MOPC-41 DNA. $C_0t_{1/2}$ values for RPC-20 cDNA and globin cDNA are 1530 and 2100, respectively. Arrows indicate the $C_0t_{1/2}$ points. Closed symbols represent RPC-20 cDNA (440 pg/ml); closed circles, 1.4 mg/ml of MOPC-41 DNA at 0.18 M Na^+; closed squares, 9.7 mg/ml of MOPC-41 DNA at 1 M Na^+. Open circles represent globin cDNA. The globin cDNA data were taken from our already published data (Honjo et al., 1974).

under these conditions, RPC-20 cDNA probe can form stable duplex structures with sequences corresponding to all the light chains in its subclass, as well as to light chains having structures closely similar to that of MOPC-315.

(*c*) *Hybridization Kinetic Analysis Using RPC-20 cDNA.* The number of times the gene sequences corresponding to the RPC-20 cDNA probe is represented in the mouse genome can be assessed by hybridization kinetic analysis (Britten and Kohne, 1968). In this type of analysis, the concentration of the unlabeled sequences that correspond to the labeled cDNA probe drive the association reaction; the greater their concentration in the genomic DNA, the faster the association occurs. Thus, the rate of association of RPC-20 cDNA (representing sequences derived from both constant and variable regions) with complementary strands in genomic DNA can be compared to that of bulk mouse genomic DNA which has a unique representation. Such analyses have been carried out using total genomic DNA derived from RPC-20, MOPC-41, and MOPC-321 myeloma tumors and mouse spleen and salmon sperm. The reassociation kinetics with respect to RPC-20, mouse spleen, and salmon sperm DNA are shown in Figure 7. A similar analysis using DNA derived from MOPC-41 DNA is shown in Figure 8 with the comparative results of hybridization with mouse globin cDNA. In each case, with the exception of salmon sperm, reannealing occurs with uniform kinetics yielding $C_0t_{1/2}$ values of 1600 (RPC-20 DNA), 1900 (spleen DNA), and 1530 (MOPC-41 DNA). These values can be compared to a $C_0t_{1/2}$ value of 3000 for unique copy mouse DNA assayed under identical conditions (Ross et al., 1974) and a $C_0t_{1/2}$ value obtained for mouse globin cDNA which is 2100 (Figure 8). These $C_0t_{1/2}$ values yield a reiteration frequency for the RPC-20 cDNA sequence of approximately two copies/haploid mouse genome. Similar values were obtained for annealing to DNA derived from myeloma tumor MOPC-321 (not shown). As expected, no annealing was observed in the presence of salmon sperm DNA.

The fidelity of matching of the reassociated cDNA and genomic DNA derived was assessed by thermal stability to S_1 nuclease digestion as shown for RPC-20 and MOPC-321 DNA in Figure 9. The denaturation profile is quite sharp with a T_m of 88.6 °C indicating that congruently matched duplexes were formed with both DNA preparations.

Discussion

(*a*) *Expected Range of Annealing of the RPC-20 cDNA Probe.* A comparison of the length of RPC-20 mRNA to that of its complementary DNA indicates that the cDNA is complementary to the constant and variable regions of the λ light chain structural sequence (cf. Figures 1 and 2). This cDNA forms stable and complete hybrids when annealed with mRNAs derived from other λ light chains of the same subgroup and even forms a relatively stable hybrid when annealed to mRNA derived from a λ chain of a different subgroup (MOPC-315). This result is in distinct contrast to the behavior of mouse κ chain cDNAs that form incomplete hybrids with mRNAs derived from different κ subgroups (Honjo et al., 1974). Given this range of cross-hybridization,

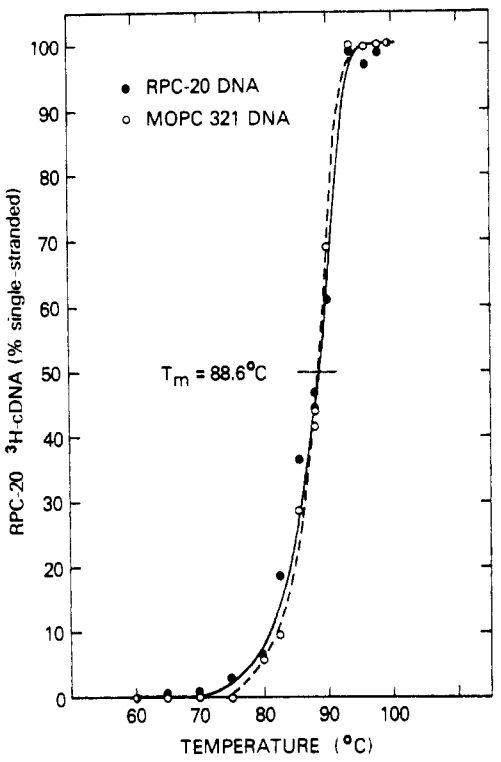

FIGURE 9: Thermal denaturation of RPC-20 cDNA-DNA duplexes. The RPC-20 cDNA duplex formed with RPC-20 DNA (closed circles) and MOPC-321 DNA (open circles) duplexes were formed at C_0t 4.1 × 10^4 and 3.7 × 10^4, respectively. Thermal stability to S_1 nuclease digestion was carried out as described under Materials and Methods.

the RPC-20 cDNA probe should faithfully score for genetic sequences corresponding to its subgroup and those corresponding to the subgroup represented by MOPC-315.

(*b*) *The Expected Number of Germ Line Genes in the* λ *System.* Thus far, seven very closely related λ sequences have been found and these differ from one another by no more than four amino acids. In four cases the difference is only one amino acid. If a strict version of the germ line hypothesis were correct, these seven sequences must be represented by seven distinct genetic sequences, each corresponding to each different variable region. As noted above, RPC-20 cDNA would also form a sufficiently stable hybrid structure with a sequence corresponding to MOPC-315, thought to represent a separate class of λ light chain. It is difficult to imagine that the myeloma tumors thus far analyzed represent the entire population of closely related λ light chains in the genetic repertoire of the mouse. Nevertheless, we define for the time being a germ line requirement of eight discrete variable region gene sequences that would be stored by the RPC-20-cDNA probe.

(*c*) *Significance of the Relatively Unique Kinetics Observed with the RPC-20-cDNA Probe.* The reassociation kinetics observed using the RPC-20 cDNA probe provides consistently uniform results using DNAs derived from both κ and λ myeloma tumors and DNA derived from normal spleen. A comparison of the $C_0t_{1/2}$ obtained to the $C_0t_{1/2}$ of total unique DNA and to that of mouse globin indicates that the sequence represented by this probe is present approximately two times/haploid genome. In fact, the kinetic annealing profiles of RPC-20 cDNA and globin (an established "unique" sequence (Packman et al., 1972; Harrison et al., 1974; Ross et al., 1974)) cDNA are virtually identical (Figure 8).

It was argued above that, were the germ line hypothesis correct, a minimal reiteration frequency of eight should have been observed for a portion of the hybridization curve. If indeed there were only eight genetic sequences corresponding to the entire mouse λ V region repertoire, it would be very difficult to distinguish between a reiteration frequency of two and a reiteration frequency of eight. $C_0t_{1/2}$ values, particularly for a region which represents only a portion of our probe, cannot reliably make such a distinction. On the other hand, if those λ light chains already sequenced represent but a sample of this repertoire that may include dozens if not hundreds of closely related sequences, then these certainly should have been scored by this hybridization analysis.

(*d*) *Is the Germ Line Hypothesis Ruled Out?* Operating on the assumption that the mouse genome encodes more than the eight already established λ sequences, these results clearly suggest that separate genes for them are not present in the genomic DNA. This assumption seems a reasonable one, but still must be considered tentative. Further, is what is true for the λ system also true for the κ system? Obviously the mouse depends upon κ light chains to a much greater extent than λ. Less than 5% of circulating mouse immunoglobulin carries the λ chain. Furthermore, there is great diversity among mouse κ subgroups and relative uniformity among the λ subgroups. Thus the λ system in the mouse may represent some kind of special case in which much of the λ diversity has been deleted. The possibility that the small differences seen among mouse myeloma λ chains reflect somatic mutational events unrelated to immunologic diversity is rendered unlikely by their concentration in the hypervariable regions.

(*e*) *Is Somatic Recombination Necessary for the* λ *Chain System?* If the λ subgroup corresponding to RPC-20 has the same number of C and V region genes, the specific joining mechanism which appears to be required for the κ chain may not be obligatory in this system. Indeed, these few sequences could occur adjacent one another in the germ line genome. Nevertheless, the homology observed between the variable region of this subgroup and the MOPC-315 subgroup is still best explained by the joining of a variable λ region gene sequence to either of the two types of λ constant region.

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Binding of 2,4-Dinitrophenyl Derivatives by the Light Chain Dimer Obtained from Immunoglobulin A Produced by MOPC-315 Mouse Myeloma†

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ABSTRACT: The light chains, but not the heavy chains, obtained from immunoglobulin A produced by the MOPC-315 mouse myeloma bind the 2,4-dinitrophenyl (DNP) group. Specific interaction with the DNP group was determined by using several immunoadsorbents, including DNP-L-lysine-Sepharose, and elution of the adsorbed light chain by DNP-glycine. Equilibrium dialysis experiments showed that the M-315 light chain in the form of dimer (45 260 daltons) has two identical and homogeneous binding sites that bind DNP-L-lysine with an intrinsic association constant of 6.3×10^3 M^{-1}. This is the first report, to our knowledge, in which the light chain binding data permit reliable determination of the binding constant and valency of the isolated light chain, and which suggests a predominant role for the light chain in construction of the binding site in the intact immunoglobulin molecule.

Antibody molecules are made up of two heavy (H^1) and two light (L) polypeptide chains held together by interchain disulfide bonds and noncovalent forces. Numerous experiments have shown that, in the intact 7S molecule, both H and L chains are involved in determining antibody specificity. These included affinity labeling, radioiodination of antibodies in the presence and absence of the specific hapten, and a variety of studies on the recovery of binding activity in recombinants made from the isolated chains (reviewed by Fleischman, 1966; Porter and Weir, 1966; Dorrington and Tanford, 1970). Attempts were also made to assign antibody activity to isolated chains obtained from native antibody molecules that were mildly reduced and alkylated. Essentially in all systems investigated, the isolated H chain bound the homologous hapten or antigen, although to a lesser extent than the intact Ig. These results indicated that the H chain contributes contact residues in the binding site of the intact antibody (see Utsumi and Karush, 1964; Painter et al., 1972). Antibody activity in isolated L chains was not readily demonstrable (Fleischman, 1966; Porter and Weir, 1966). In many systems there was no detectable activity in the L chains (e.g., Fleischman et al.,

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[1] Abbreviations used are: DNP, 2,4-dinitrophenyl; Ig, immunoglobulin; MOPC-315 and MOPC-321 are abbreviated to M-315 and M-321, respectively; pH 7.4 buffer, 0.15 M NaCl–0.01 M sodium phosphate (pH 7.4); PBS, phosphate buffered saline; H and L chains, heavy and light chains, respectively.