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Estimation of the Number of Genes Coding for the Constant Part of the Mouse Immunoglobulin Kappa Light Chain

(antibody/DNA·DNA hybridization/complementary DNA/hydroxyapatite/gene amplification)

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ABSTRACT **As previously shown, purified 14S RNA of mouse myeloma MOPC-41 forms a single peak on sucrose gradients and gel electrophoresis and codes for a single polypeptide chain, the immunoglobulin light chain produced by the same myeloma *in vivo*. This 14S mRNA was used for the enzymatic synthesis of DNA (cDNA) which is complementary to the RNA template. A DNA fraction was isolated which has an average size of 300 nucleotides. Kinetic studies of the hybridization of the 14S RNA with the cDNA indicate that about 40% of the RNA consists of a single RNA sequence. From the size of the cDNA fraction and from the direction of DNA synthesis, it can be concluded that the cDNA includes the sequence complementary to the constant region of light chain mRNA. This radioactive cDNA was used for DNA·DNA reannealing experiments with unlabeled DNA from mouse liver or myeloma tumor in 3 × 10^6-fold excess. This allowed the determination of the number of copies in the mouse genome of those sequences represented in the cDNA. The data show no significant reiteration in either liver or myeloma DNA and suggest that the gene coding for the constant part of immunoglobins is present in the haploid genome in one to five copies. Furthermore, this gene is not "amplified" in nuclear DNA of myeloma plasmocytes.**

A large body of evidence indicates that the variable (V) and the constant (C) regions of immunoglobulin chains are coded by separate *V* and *C* genes (1). These two polypeptide sequences, however, are not synthesized separately. The mRNA of light chain can direct the synthesis of complete light chain in heterologous systems (2, 3). Studies on the structure of light chain mRNA indicate that the sequences coding for the V and the C regions are on the same RNA molecule (4).

The major theories which have been proposed to explain the generation of diversity of immunoglobulin genes (see ref. 1) allow important predictions concerning the number of immunoglobulin genes in the genome of germ line cells or of various types of somatic cells. The availability of radiolabeled complementary DNA (cDNA), synthesized enzymatically from 14S light chain mRNA and containing sequences complementary to the C gene of immunoglobulins (2, 5), makes possible a kinetic study of its annealing with unlabeled cellular DNA in large excess (6). This allows a direct measurement of the number of copies of the gene present in the cDNA in the genome of the mouse. These data have been presented briefly earlier.§

MATERIALS AND METHODS

Preparation of Polysomes, RNA, and cDNA. Myeloma tumors were obtained originally from Dr. M. Potter (NIH) and maintained in this laboratory for several years. The preparation of polysomes, extraction of polysomal RNA, fractionation of polysomal RNA by poly(dT)-cellulose chromatography, and isolation of the 14S mRNA peak coding for MOPC-41 light chain were performed as described earlier (2, 7) with the following modifications: Polysomes at a concentration of 20 A_{260} units/ml were incubated with Proteinase K (Merck, chromatographically pure) (100 μg/ml)) and Na dodecyl sulfate (0.2%) (KCl, final concentration of 20 mM) for 15 min at 0°. The poly(A)-rich RNA was dissolved in H_2O (1 mg/ml), heat denatured for 10 min at 65° and chilled immediately to 0°. It was then adjusted to 5 mM Na acetate, pH 5, and 400-μl aliquots were fractionated on linear 5–20% (w/v) sucrose gradients, containing 5 mM Na acetate, pH 5, in a Spinco SW 41 rotor for 16 hr at 40,000 rpm and 2°. The 14S RNA peak was refractionated on a second gradient under identical conditions. Enzymatic synthesis of cDNA from the purified 14S mRNA peak has also been described (5).

Preparation of DNA from Liver and MOPC 41 Myeloma Nuclei. Nuclei were prepared according to Blobel and Potter (8) except that after the first sedimentation, nuclei were treated with the detergent NP-40 (1%) before the centrifugation over a cushion of 2.2 M sucrose. Packed nuclei (3 ml) were suspended in 100 ml of 0.15 M NaCl, 0.015 M Na citrate, pH 7.5, and 19 ml of 10% Na dodecyl sulfate. They were then incubated with Proteinase K (100 μg/ml) for 4 hr at 45°. Repeated extractions with phenol–chloroform and digestions with RNase were done as described (9), except that Pronase was replaced by Proteinase K. Following ethanol precipitation and resuspension (10 A_{260} units/ml) in 50 mM Tris·HCl, pH 7.6, 15 mM NaCl, the DNA was sonicated in ice with a Branson sonicator at maximum energy for 3 min using 15-sec pulses. Sonicated DNA was treated with Chelex (Biorad) and precipitated with 2 volumes of ethanol at −30°. All buffers were pretreated with Chelex to remove traces of heavy metals. [^{3}H]DNA from *Escherichia coli* (2.5 × 10^5 cpm/μg) was a gift from R. Bird.

Abbreviations: V and C, variable and constant regions, respectively, of the immunoglobulin chain; C_0t and C_Rt, product of initial concentration of DNA or RNA, respectively, and time; cDNA, DNA complementary to RNA; L chain, light chain of immunoglobulin.

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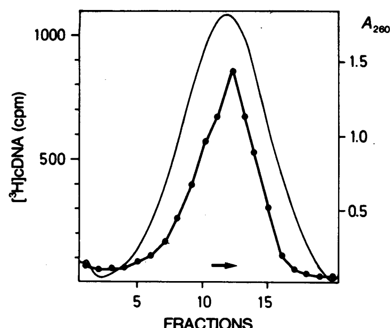

FIG. 1. Sedimentation of DNA on alkaline sucrose gradient. DNA and [³H]cDNA were prepared as described in *Methods*. A 400-µl sample of myeloma MOPC 41 DNA (2 A_{260} units) and [³H]cDNA, in 0.3 N NaOH, was layered onto a 12-ml linear 5–20% alkaline sucrose gradient, containing 0.2 N NaOH, 0.8 M NaCl, and 0.01 M ethylenediaminetetraacetic acid. The centrifugation was for 20 hr at 20° in a Spinco SW 41 rotor at 40,000 rpm. The gradient was fractionated, while the optical density at 260 nm was recorded (*light tracing*). Samples were precipitated onto GF/C filters with Cl_3CCOOH and counted in 2 ml of toluene–Omnifluor scintillation liquid (●——●). *Arrow* indicates direction of sedimentation.

Estimation of DNA Size. DNA size was estimated by sedimentation analyses in alkaline sucrose gradients, according to Studier (10), after determining the $s_{20,w}$-values using McEwen's Tables (11). The rotor and gradient parameters were established for McEwen's tables with three protein standards, bovine chymotrypsinogen, serum albumin, and gamma globulin, which were run simultaneously in the same neutral sucrose gradient. From this, nomograms were constructed for both the protein standards (ρ = 1.40 g/cm³) and DNA (ρ = 1.80 g/cm³) at 20°. The $s_{20,w}$-values could then be obtained directly as a function of the sucrose concentration. When this method was applied to a DNA of known $s_{20,w}$-value, i.e., 16S polyoma DNA (12), the observed values agreed with calculated, theoretical values with a precision of better than 5%. The size of sonicated DNA from myeloma MOPC 41 and liver matches that of the cDNA preparation. As seen in Fig. 1, sonicated tumor DNA has an average size of about 300 nucleotides, calculated as indicated above.

RESULTS

Properties of the 14S mRNA. 14S light chain mRNA, when purified to a single peak on acrylamide gel, was estimated to be 1100 nucleotides long (2). Preliminary results (in collaboration with N. Boy de la Tour) from measurements of the contour length by electron microscopy have confirmed these values (unpublished observations). In a reticulocyte lysate system, the 14S mRNA is translated into a single polypeptide chain about 235 amino acids long, corresponding to a light chain with approximately 20 additional amino acids, presumably a light chain precursor (2). In *Xenopus laevis* oocytes, however, the polypeptide product of 14S mRNA has the size of authentic light chain, presumably as the result of cleavage of the precursor protein (3). Thus only about 700 nucleotides (65% of the mRNA) are translated. The RNA purified from MOPC 41 myeloma by poly(dT)-cellulose chromatography contains an RNase-resistant, AMP-rich fragment of about 200 nucleotides (7). With light chain mRNA from MOPC 21 myeloma, Brownlee *et al.* (4) have identified a similar sequence of poly(A) on the 3'-end of the

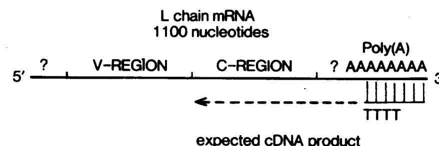

FIG. 2. Diagram of 14S mRNA of immunoglobulin light chain and synthesis of cDNA with oligo(dT) primer.

mRNA. Based on the above information, about 200 nucleotides in the 14S mRNA still remain unaccounted for in both function and location. As shown in Fig. 2 (question marks) these could be on the 5'- and/or on the 3'-side of the sequence specifying the light chain polypeptide. The degree of homogeneity of the 14S RNA was estimated from RNA·cDNA hybridization experiments in which the kinetic complexity of the RNA was measured (see ref. 13). As standard, with an assumed purity of 100%, we used rabbit hemoglobin (Hb) 10S mRNA prepared with a poly(dT)-cellulose purification step in addition to the usual purification procedure (14). DNA complementary to the 10S Hb mRNA was synthesized enzymatically (5). Hybridization of the cDNA to the corresponding mRNA occurred over a C_Rt (moles of RNA × sec/liter) range of 2 logs. With rabbit and duck Hb mRNAs, 90% hybridization of the input cDNA was observed at saturation, while with 14S mRNA, maximum hybridization of cDNA was slightly lower (70%). The kinetic complexity values determined from the C_Rt curves are given in Table 1. From the comparison of the kinetic complexity with the analytical complexity, an approximate purity of 40% can be calculated. Recent experiments with poliovirus RNA and

TABLE 1. *Determination of the kinetic complexity of 14S RNA (13)*

Template for cDNA	Analytical complexity (daltons)*	$C_Rt_{1/2}$ × 10³ (moles × sec)/liter	Kinetic complexity (daltons)†	Homogeneity‡, %
Rabbit Hb§	400,000	0.73	Kinetic standard	100
Duck Hb¶	400,000	1.3	712,000	~60
Myeloma 14S RNA	380,000	1.8	984,000	~40

cDNA (1000 cpm/assay) was mixed with increasing concentrations of mRNA (0.001–10 µg/ml) in a total volume of 10 µl containing 0.3 M NaCl, 0.04 M Tris, pH 7.5, and 0.1% Na dodecyl sulfate. The mixture was incubated in a sealed capillary for 2 hr at 65°. Samples were cooled and digested with S1 nuclease (17) for 45 min at 45°. Samples were precipitated with Cl_3-CCOOH and counted. The C_Rt (moles per liter × sec) value at which 50% of the cDNA was hybridized was taken as $C_Rt_{1/2}$ (13).

* The analytical or sequence complexity of Hb mRNA is the sum of the molecular weight of the mRNA of both chains of Hb (200,000 each, ref. 16).

† Kinetic complexity was calculated from the measured $C_Rt_{1/2}$, as described by Birnstiel *et al.* (13), using rabbit Hb mRNA as kinetic standard.

‡ The homogeneity of the mRNAs was estimated from the differences between analytical and kinetic complexity (13).

§ Rabbit Hb mRNA was purified as described, with the use of a poly(dT)-cellulose chromatography step (14).

¶ Duck Hb mRNA was purified as described (15), including separation by polyacrylamide gel electrophoresis. The RNA was a gift of T. Imaizumi.

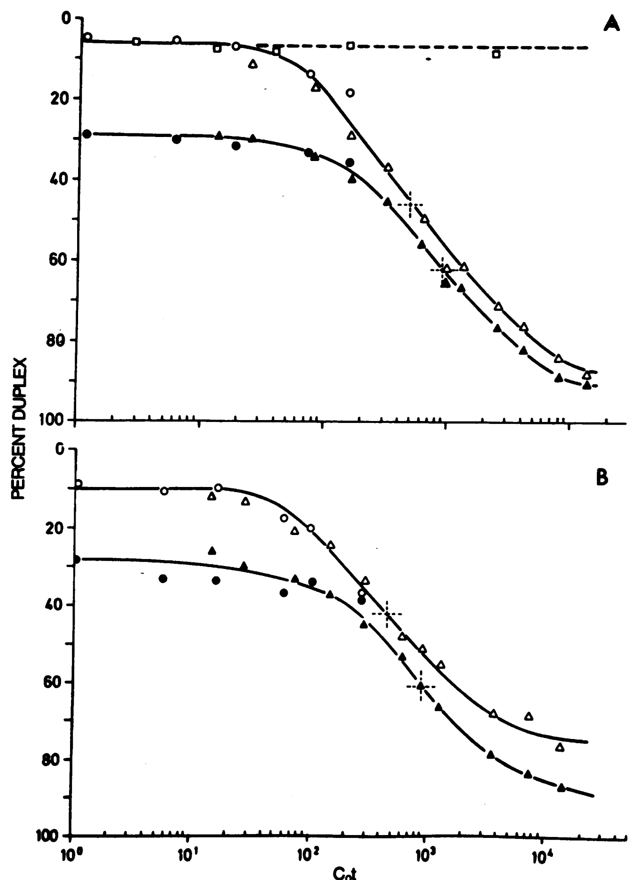

FIG. 3. Reassociation of mouse-liver DNA (*A*) and myeloma MOPC 41 DNA (*B*) in the presence of [^{3}H]cDNA transcribed from 14S myeloma tumor RNA. Mouse-liver and myeloma DNA, and [^{3}H]cDNA were prepared as described in *Methods*. Mixtures containing [^{3}H]cDNA (1.74 ng, specific activity 1.15×10^7 cpm/μg) and either mouse-liver DNA (5.5 mg) or myeloma DNA (5.2 mg) were prepared in 0.12 M Na phosphate buffer, pH 6.8. From these stock solutions, samples were prepared at three different concentrations of cellular DNA, all with the same DNA to cDNA ratio: (*1*) 0.056 mg DNA/ml in 4 ml of 0.05 M phosphate buffer (data not shown). (*2*) 0.225 mg DNA/ml in 1 ml of 0.12 M phosphate buffer (O——O) and (*3*) 7.5 mg DNA/ml in 30 μl of 0.12 M phosphate buffer (Δ——Δ). The DNA mixtures were denatured in a glycerol bath at 100–102° for 10 min, and rapidly chilled at 0°. Reannealings were then carried out at 65° for the times necessary to achieve the desired C_0t values. The reannealing reactions were stopped by chilling at 0°, and dilution to 0.05 M phosphate concentration. Samples were stored at −20° until fractionated on hydroxyapatite (BioRad Biogel HTP) equilibrated with 0.05 M phosphate buffer. Approximately 1 ml packed volume of hydroxyapatite was used per sample (0.2–0.3 mg of DNA). Elution was performed at 65°, successively with 0.05 M phosphate buffer to remove nonretained material, 0.12 M phosphate buffer to elute single-stranded DNA, and 0.5 M phosphate buffer to remove double-stranded DNA. Optical density measurements at 260 nm were made to follow the reannealing of unlabeled cellular DNA sequences. All samples were measured under denaturing conditions. Radioactivity measurements of [^{3}H]cDNA were made as described in Fig. 1, after addition of carrier DNA to final concentration of 250 μg per sample. The percentage of unlabeled cellular DNA in duplex is shown by ● and ▲. The percentage of [^{3}H]cDNA in duplex is shown by O and Δ. A mixture of *E. coli* DNA and [^{3}H]cDNA, at the same DNA concentration and DNA/cDNA ratio was also prepared, denatured, incubated, and processed as indicated above. The percentage of *E. coli* [^{3}H]cDNA in duplex is shown by □.

(*legend continued at bottom of the next column*)

cDNA as kinetic standard have confirmed that value (unpublished observation). From the translation of 14S RNA into L chain exclusively (2, 3) it can be assumed that L-chain mRNA is the main species and represents 40% of 14S RNA. If all the RNA sequences which contain poly(A) are transcribed equally well into cDNA, it can be inferred that 40% of the cDNA synthesized from this RNA consists of L-chain DNA sequences.

Properties of the cDNA. The synthesis of DNA with avian myeloblastosis virus RNA-dependent polymerase, using oligo(dT) as primer and 14S light chain mRNA as template has been described in detail earlier (5). The cDNA was fractionated on an alkaline sucrose gradient and DNA larger than 100 nucleotides was rerun on a second gradient as shown in Fig. 1. This cDNA has an average size of 300 nucleotides (measured as described in *Methods*) and about 85% of the labeled DNA is between 150 and 450 bases long. It was used in the hybridization experiments. Hybridization of the cDNA with the 14S mRNA template demonstrated the complementary nature of the DNA synthesized (5). No hybridization above background is detected with hemoglobin 10S mRNA or 18S or 28S ribosomal RNA (5). The length of the poly(A) sequence transcribed into poly(dT) was determined. cDNA (average chain length 300 nucleotides) labeled only with dTTP (23.7 Ci/mmole) was hybridized in 0.3 M NaCl, 40 mM Tris·HCl, pH 7.5, for 1 hr at 65° with a large excess of poly(A) (400 μg/ml). The hybrids were digested with S1 nuclease from *Aspergillus oryzae* as described (5). The resulting S1-resistant hybrids were denatured by alkali and the length of the labeled poly(dT) portion was determined on alkaline sucrose gradients (see *Methods*). An average length of about 25 nucleotides was found, which includes the primer as well as the poly(dT) copied from the poly(A) region of the mRNA (7). From the above data it can be concluded (see *Discussion* and Fig. 2) that the cDNA fraction used in the hybridizations corresponds to most or all of the C-region of light-chain mRNA.

Hybridization of cDNA with Excess Cellular DNA. The kinetics of reannealing of sonicated cellular DNA and [^{3}H]-cDNA (see Fig. 1) were followed as a function of C_0t (moles of nucleotides per liter × time in sec) according to the method of Britten and Kohne (6). A ratio of cellular DNA to [^{3}H]-cDNA of 3.0×10^6 was employed. All DNAs used were nearly the same size as the [^{3}H]cDNA (see Fig. 1) and no corrections of C_0t values for any size difference of the reassociating DNA were therefore necessary (18). The duplexes formed were separated from the unannealed single-stranded DNA by hydroxyapatite chromatography (6), or measured by their resistance to digestion with single-strand nuclease S1 (17). At C_0t values, greater than 10^4, about 90% of the unlabeled mouse cellular DNA was in duplex structures, as judged from the elution from hydroxyapatite column (Fig. 3). About 75% of the (^{3}H)cDNA was also eluted as a duplex structure under the same conditions. At C_0t values lower than 1, approximately 30% of the mouse-cellular DNA has reannealed. This fraction corresponds to the rapidly reassociating sequences of satellite DNA and the intermediate re-

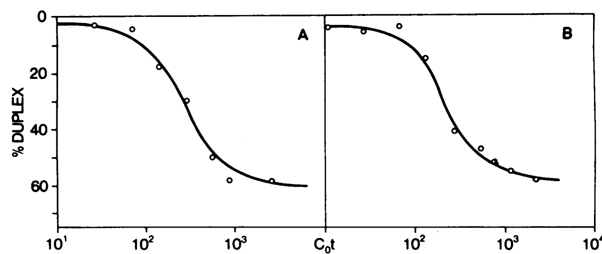

FIG. 4. Reassociation of mouse-liver DNA (*A*) and myeloma tumor DNA (*B*) in the presence of [^{3}H]cDNA transcribed from 14S myeloma RNA (MOPC 41). Conditions of hybridization were as in Fig. 3 except that only the samples with a DNA concentration of 7.5 mg/ml were used. Incubated samples were chilled, diluted into 0.5 ml of S1 nuclease buffer (17), and 30 units of purified S1 nuclease from *Aspergillus oryzae* were added (17). The digestion was carried out for 45 min at 45°, and the samples were cooled, precipitated with cold perchloric acid (10%) and filtered on GF/C Whatman filters. The filters were counted in Aquasol (= S1-resistant double-stranded [^{3}H]cDNA) and the results are expressed as % of input [^{3}H]cDNA found in duplex form. [^{3}H]cDNA hybridizes with liver DNA with $C_0t_{1/2}$ of 280 and with tumor DNA with a $C_0t_{1/2}$ of 260.

associating sequences present in the mouse genome (6). A background of 7–10% duplex DNA was observed at a C_0t as low as 10^{-3} for [^{3}H]cDNA. This fraction does not represent any reiterated sequences in the mouse genome, since the same background levels are obtained with controls using *E. coli* DNA instead of mouse-cellular DNA (Fig. 3), or with cDNA alone (self-annealing). About 70% of the cellular DNA (liver and tumor), corresponding to sequences present in the mouse genome as one or a few copies, reanneals with a $C_0t_{1/2}$ (C_0t value for 50% annealing) of 800 (Fig. 3). The $C_0t_{1/2}$ value observed for the annealing of [^{3}H]DNA of *E. coli* run as an internal standard was 2 (not shown). The results of the annealing of [^{3}H]cDNA with mouse-liver DNA assayed on hydroxyapatite are shown in Fig. 3*A*. The appearance of [^{3}H]cDNA in duplex with mouse liver DNA occurs within 2 powers of 10 of C_0t and with a $C_0t_{1/2}$ value of 400. The results shown in Fig. 3*B* for the annealing of [^{3}H]cDNA with myeloma MOPC 41 DNA are similar. This indicates that neither liver DNA nor myeloma DNA contains significant reiteration of those sequences present in the cDNA fraction. When the [^{3}H]cDNA in duplex was scored in the same hybridization reactions using the S1 nuclease assay (Fig. 4*A* and *B*) it was found again that the kinetics of hybridization of cDNA to liver DNA or to myeloma DNA were similar and with a single transition, although the absolute $C_0t_{1/2}$ value was slightly different with the two assays. As expected, the percentage of the input [^{3}H]cDNA which is scored in duplex with the S1 assay is lower.

DISCUSSION

It is first important to establish that the cDNA used in these experiments does indeed contain sequences complementary to the *C* gene of immunoglobulins. The 14S RNA from which cDNA is synthesized is homogeneous in size and is translated into a single polypeptide chain in two different systems (2, 3). However, this RNA fraction is not made of a single RNA sequence. The kinetics of mRNA/cDNA hybridization (Table 1) have shown that in the 14S RNA fraction prepared from sucrose gradients, the main RNA species represents 40% of the RNA. The translation studies with 14S RNA indicate that light-chain mRNA is the main RNA sequence of 14S RNA and it can be assumed that this holds for the cDNA as well. The important point is that, in a situation where the data show no reiteration, this contamination of cDNA with nonlight-chain sequences does not affect the interpretation of the data. However, if the hybridization experiments had indicated gene reiteration, the relative impurity of light-chain mRNA and cDNA would have made it difficult to attribute this reiteration specifically to the light-chain gene.

The average size of the cDNA is 300 bases, with 85% included within a range of 150 to 450 bases. When a hybrid formed with cDNA in excess and 14S mRNA labeled with ^{125}I was digested with RNase, 45% of the RNA was found to be protected by the cDNA, corresponding to a length of about 450 nucleotides (unpublished observations). Only about 25 nucleotides of the cDNA represent the oligo(dT) primer and transcripts of the poly(A) sequence of the mRNA. The shortness of this piece is probably due to the 5-fold molar excess of primer used in these experiments. Only primer molecules situated close to the 5′ end of the poly(A) sequence can lead to a large sized product, while other initiation sites within the poly(A) lead to only small fragments. The direction of synthesis [from the poly(A) sequence towards the 5′ end] and the arrangement of the constant and the variable region within the mRNA (Fig. 2) indicate that the growing cDNA chains consist mainly of the C-region (320 bases), and only in the case of longer cDNA molecules of the V-region. The location of the 200 untranslated bases is, in this respect, important. If many of these bases are located between the poly(A) and the C-region, the cDNA preparation does not include V-region sequences. Even if all of the untranslated bases are on the 3′ side, more than 75% of the cDNA includes enough of the C-region to hybridize with C genes. On the other hand, if all of the untranslated bases are on the 5′ end of light-chain mRNA, all light-chain-specific cDNA molecules longer than 400 bases (about 20% of the cDNA mass) would include, in addition to the C-region, enough of the V-region to form stable duplexes with V genes. Thus, it can be concluded that the cDNA prepared from MOPC 41 light chain mRNA includes a large portion, and possibly all, of the C-region and is therefore suitable for a determination of the number of kappa *C* genes. Moreover, depending on the location of the untranslated nucleotide sequence of light-chain mRNA, the data obtained with cDNA might also be extended to apply to the *V* gene.

When the radiolabeled cDNA described above is used to determine the number of copies of these sequences in the mouse genome, the experimental value obtained corresponds to about two copies, as compared with the internal standard represented by the absorbance curve. Hydroxyapatite retains entire DNA chains which have annealed, even though part of the molecules may be single-stranded tails. Even a short reiterated DNA fragment could easily be detected by this method. It is of interest to examine if the number of genes measured kinetically corresponds to the number that can be estimated, knowing the percent annealing to the [^{3}H]cDNA observed and the ratio of the unlabeled and labeled (cDNA) sequences present (19, 20). Because sonicated cellular DNA consists of randomly cut fragments, which are of polydisperse length (as in the case of the cDNA), the DNA·cDNA duplex formed will contain a significant amount of nonpaired [^{3}H]-

cDNA tails. Therefore, the percentage of cDNA annealed in duplex, as measured by the hydroxyapatite assay, will be overestimated. As expected, the percentage of cDNA in duplex is lower with the S1-nuclease assay (Fig. 4). A similar observation has been made in the case of Hb cDNA (21). Our hybridization data (Figs. 3 and 4) correspond, therefore, to about 60% effective annealing of the [^{3}H]cDNA at saturation. This value indicates that, in the reaction mixture, the ratio of complementary sequences of unlabeled and labeled DNA is about 1.5 (19).

The hybridization reaction contains 3.0×10^6-fold more unlabeled cellular DNA than [^{3}H]cDNA (Fig. 2*B*). Since the genome of the mouse is about 1.8×10^{12} daltons, there are 6.0×10^5 daltons of cDNA present per genome equivalent. That is, there should be an average of 6.0 molecules of labeled *C* gene (molecular weight of single-strand *C*-gene sequence = 1.0×10^5) per genome equivalent if the cDNA consisted exclusively of *C*-gene sequences. Since only 40% of the 14S RNA prepared is believed to be L-chain mRNA (Table 1), and assuming no discrimination in the transcription of RNA into cDNA, one would expect 2.4 molecules (2.4×10^5 daltons) of *C*-gene specific [^{3}H]cDNA per genome equivalent. From this assumption, and from the knowledge that there are approximately 1.5 copies of unlabeled *C*-gene in cellular DNA for each complementary *C*-gene sequence in cDNA in the reaction mixture (see above), it can be calculated that 3.6 copies of *C*-gene are present in each haploid mouse genome. Within the limits of precision of these methods, these numbers are in good agreement with the value of two copies found in the kinetic experiments (Fig. 3). It can, therefore, be concluded that the *C* gene (kappa type) of immunoglobulins is not present in the mouse genome in more than very few copies. These data provide direct experimental evidence for this situation and rule out any model for the generation of antibody diversity involving multiple *C* genes (22, 23). The experimental data would also apply to the *V* gene if the untranslated nucleotide sequences of light chain mRNA are found to be located on the 5′ end.

It has recently been reported that mRNA of amphibians (24) and of slime mold (25) contains short sequences which are highly reiterated in the genome. In the case of light-chain mRNA, it can be concluded that if any of the nontranslated sequence is located on the 3′ side of the C region (see Fig. 2), it is not present in multiple copies in the mouse genome, since such a sequence would be included in the cDNA fraction used in the hybridization experiments (Fig. 3).

Another conclusion from our data is that the differentiated plasmocyte, a cell highly specialized in the synthesis of immunoglobulin chains, does not possess a larger number of genes for these proteins than do unspecialized cells. This indicates that a process of amplification of immunoglobulin genes in plasmocyte nuclei is not taking place.

Note Added in Proof. Recent data on the hybridization of ^{125}I-labeled L chain mRNA (C and V region) with myeloma DNA have shown that 80% of hybridized RNA corresponds to near-unique sequences. This suggests a very small number of *V* genes, at least for *V* sequences with a high degree of homology (in preparation).

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