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Genetics

Cloning of an immunoglobulin variable region gene from mouse embryo

(λ chain mRNA/R-loop/electron microscopy)

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ABSTRACT **A 4.8-kilobase DNA fragment carrying an immunoglobulin gene coding for a mouse λ chain variable region (V_λ gene) was enriched about 350-fold from a total endonuclease *Eco*RI digest of embryonic DNA by a combination of preparative agarose gel electrophoresis of double-stranded DNA and CsCl density gradient centrifugation of R-loops formed with a purified λ chain mRNA. DNA fragments thus enriched for the immunoglobulin gene were inserted *in vitro* in the middle of the genome of the vector phage λgt *W*am 403, *E*am 100, *S*am 100 by use of the *Eco*RI cohesive ends. Transfection of $CaCl_2$-treated *Escherichia coli* 803 [r_k^-, m_k^- (lacking restriction and modification systems for K-12)] with such hybrid DNA and subsequent screening of about 4000 plaques by *in situ* hybridization with purified ^{125}I-labeled λ chain mRNA led to isolation of a clone that carries a V_λ gene (λgt_{WES}-Ig 13). Electron microscopy of R-loops confirmed the presence of sequences homologous to part of the λ chain mRNA in its 5′-end.**

The recently developed *in vitro* recombinant DNA technique is a powerful tool for studies on the organization and function of eukaryotic genes (1–4). By using this technique, several DNA clones have been isolated from a variety of eukaryotes (5–7). Almost all of these clones contain DNA of reiterated genes; a defined, unique, eukaryotic gene of chromosomal origin has not yet been cloned.

The structural genes for immunoglobulin chains constitute a multigene family, in which as many as a few hundred unique but closely related DNA sequences seem to be clustered together (8, 9). The sequences that code for the amino-terminal half of immunoglobulin light chains are conventionally called *V* genes. The DNA sequences coding for the carboxyl-terminal half (*C* genes) lie separate from *V* genes in early embryonic cells (8, 9). During differentiation of lymphocytes, a specific recombinational event seems to occur, which brings one of the multiple *V* genes in contiguity to a *C* gene (10). The rearrangement permits the continuous transcription of a full immunoglobulin gene. Furthermore, there is strong evidence for changes of the coding information within *V* genes by a somatic mechanism (11–13).

In order to study this gene family, it is useful to isolate chromosomal DNA fragments carrying immunoglobulin genes. For this purpose, we enriched about 350-fold a DNA fragment from an endonuclease *Eco*RI digest of total mouse embryo DNA. By transfection of host bacteria with phage λ DNA carrying this mouse DNA fragment as an insert, and by subsequent screening of plaques with a purified light chain mRNA, we were able to isolate a clone that carries a *V* gene coding for a λ-type immunoglobulin light chain.

MATERIALS AND METHODS

Bacteria and Phages. *Escherichia coli* 803 [r_k^- m_k^- Su III$^+$ (lacking restriction and modification systems for strain K-12 and wild type for suppressor III)], originating from K. and N. E. Murray, was obtained from W. Arber, Biocenter, Basel. λgt_{WES}-λC was constructed by L. Enquist and coworkers at the U.S. National Institutes of Health (NIH) and provided by R. Weisberg (14). The central *Eco*RI fragment was removed by preparative gel electrophoresis. This phage was approved, in January 1976, as an EK-2 vector by the NIH Advisory Committee on Recombinant DNA Research. The experiments were carried out in a P3 facility in accordance with the NIH guidelines issued in June 1976.

Preparative R-Loop Formation. For R-loop formation, the procedures described by Thomas *et al.* (15) were followed, with minor modifications. The *Eco*RI mouse DNA fragments enriched for the V_λ gene sequences were dialyzed against a mixture composed of 70% (vol/vol) formamide (Kodak, deionized with Bio-Rad AG 501-XBCD mixed resin), 100 mM piperazine-*N,N*′-bis(2-ethanesulfonic acid) (Pipes), 5 mM Na_2EDTA, and 500 mM NaCl, which was adjusted to pH 7.8 by addition of concentrated HCl. To 600 μl of this DNA (900 μg) mixture was added 120 μl of RNA solution consisting of 6.7 μg of purified HOPC 2020 λ chain mRNA, 70% formamide, and 0.1 M Tris·HCl, pH 7.5. The mixture was incubated at 56 ± 0.2° for 7 hr, after which 40 ml of cold solution containing 20 mM Tris·HCl at pH 7.5, 2 mM Na_2EDTA, and 6× SSC was added (1× SSC is 0.15 M NaCl/0.015 M Na citrate at pH 7). Solid CsCl (Merck, gradient grade) was then added to a refractive index $n_D^{25°} = 1.401$. The mixture was centrifuged in two tubes in a Spinco Ti 60 rotor at 35,000 rpm and 10° for 3 days. Aliquots (0.25 ml) of the 1-ml fractions were mixed with 0.6 ml of water, sonicated for 1 min in the presence of 100 μg of presonicated *E. coli* DNA, and dialyzed against water. Fractions were made 0.3 M in NaOH, incubated for 60 min at 45°, and neutralized by addition of a mixture consisting of 1.5 M HCl and 0.6 M Tris·HCl, pH 7.5. DNA was precipitated with ethanol and dissolved in 20 μl of 2× SSC, and 1000 cpm of ^{125}I-labeled HOPC 2020 λ mRNA (specific activity 8×10^7 cpm/μg) was then added. Hybridization was carried out as described previously (10). The remainder of the gradient fractions enriched in the R-loops were pooled and supplemented with CsCl solution to $n_D^{25°} = 1.401$ and recentrifuged at 10° in a Spinco type 65 rotor at 35,000 rpm. One-quarter of each fraction was used to localize the position of the R-loop. Nucleic acids in the remaining portions of the R-loop-enriched

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Abbreviations: kb, kilobase (1000 bases or base pairs); SSC, 0.15 M NaCl/0.015 M Na citrate at pH 7; Pipes, piperazine-*N,N*′-bis(2-ethanesulfonic acid); cDNA, DNA complementary to RNA.

fractions were precipitated with ethanol. The precipitate was dissolved in 50 μl of 0.02× SSC containing 1 mM Na$_2$EDTA and 500 μM Tris·HCl, pH 6.5. The mixture was incubated at 50° for 30 min in the presence of RNase A (40 μg/ml) and RNase T1 (4 units/ml), and deproteinized by phenol extraction.

Ligation and Transfection. Ligation was carried out at 10° for 11 hr. The incubation mixture (200 μl) contained 2 μg of mouse DNA, 8 μg of λgt$_{\mathrm{WES}}$ DNA devoid of the *Eco*RI C fragment, and 20 units T4 ligase (Miles) in 66 mM Tris·HCl, pH 7.5/10 mM MgCl$_2$/1 mM Na$_2$EDTA/70 mM NaCl/10 mM dithiothreitol/90 μM ATP/bovine serum albumin (Miles) at 100 μg/ml. The reaction was stopped by addition of Na$_2$EDTA to 2.5 mM followed by heating at 70° for 3 min. Transfection procedures were those of Mandel and Higa (16), as modified by White and Fox (17). The host bacterium used was *E. coli* 803.

Plaque Screening by *In Situ* Hybridization. Procedures described by Kramer *et al.* (18) were used with the following modification. Agar plugs containing a single plaque were transferred to a well of a microtiter dish containing 0.1 ml of 10 mM Tris·HCl at pH 7, 10 mM MgCl$_2$, and 0.02% bovine serum albumin. With the aid of a stainless steel "stamp," 2- to 3-μl aliquots of the phage suspensions from 80 wells in a single titer dish were transferred simultaneously to a BBL plate and onto a 9 × 6.5-cm rectangular Millipore filter that had been covered with a thin layer of soft agar containing *E. coli* 803. Hybridization was carried out at 50° for 15–20 hr with 1 to 5 × 10^5 cpm per filter of ^{125}I-labeled HOPC 2020 λ chain mRNA (specific activity: 8 × 10^7 cpm/μg) in 50% formamide and 4× SSC, buffered with 0.1 M Pipes.

Electron Microscopy. Heteroduplex preparations were made according to the method of Davis *et al.* (19). R-loops were prepared essentially as described by Thomas *et al.* (15). DNA (10 μg/ml) and mRNA (8 μg/ml) were incubated in 70% formamide/100 mM Pipes/20 mM Tris/5 mM EDTA, pH 7.8, and various NaCl concentrations. The incubation proceeded for 18–20 hr either at 57° and high salt (0.57 M final cation concentration) or at 51° and low salt (0.12 M). The hybrids were spread from 70% formamide/100 mM Tris/10 mM EDTA, pH 8.5 on a hypophase containing 10–20% formamide, 20 mm tris, 2 mM EDTA at pH 8.5. Micrographs were made on a Philips 300 electron microscope, and measured with a Numonics digitizer on 10-fold enlargements of the negatives.

Other Methods. Isolation of high-molecular-weight DNA, purification of light chain mRNA, isolation of the 3′-end half of mRNA, preparative agarose gel electrophoresis, and use of the RNA probes in the detection of DNA fragments carrying immunoglobulin *V* and *C* gene sequences have all been described (12, 13).

RESULTS

Enrichment of the *Eco*RI DNA Fragment Carrying a V_λ Gene. Highly polymerized DNA from 12-day-old BALB/c embryos was digested to completion with *Eco*RI, and resulting DNA fragments were fractionated according to size by preparative agarose gel electrophoresis. Portions of DNA eluted from each gel slice were used in hybrid formation with ^{125}I-labeled, purified whole HOPC 2020 λ chain mRNA (a probe for V_λ and C_λ sequences), in order to detect fragments carrying V_λ and C_λ genes (10). Three DNA components of 7.2, 4.4, and 3.1 kilobases (kb) hybridized with the whole λ chain mRNA (Fig. 1). The largest, 7.2 kb, component hybridized also with the 3′-end half, (a probe for C_λ gene sequences), indicating that this component carries C_λ gene sequences. Furthermore, equality in the level of hybridization obtained with the two RNA probes suggested that this component carries no V_λ gene sequences (8, 20). On the other hand, the two smaller DNA components did not hybridize above the background level with the 3′-end half (8, 20). They therefore carry no $C_{\lambda\mathrm{I}}$ gene sequences and presumably carry V_λ gene sequences.

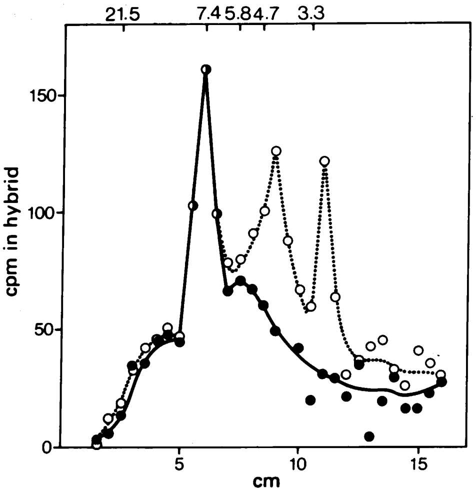

FIG. 1. Agarose gel electrophoresis profiles of an *Eco*RI digest of total embryo DNA. DNA (4 mg) was fractionated in 0.9% agarose (1 cm thick, 20 cm wide). One-fifth aliquots of DNA eluted from 5-mm gel slices were hybridized with ^{125}I-labeled (8 × 10^7 cpm/μg) whole λ chain mRNA from HOPC 2020 myeloma (○····○) or with its 3′-end half (●—●) to C_0t = 10,000 (see legend of Table 2 for definition of C_0t). Inputs were 1300 cpm and 620 cpm for the whole and half RNA probes, respectively. Other procedures have been previously described (10). Numbers at the top indicate size (in kb) of duplex DNA markers.

The 4.4-kb component was pooled from several gels, and was incubated in formamide with or without excess HOPC 2020 λ chain mRNA, and fractionated by CsCl density gradient centrifugation. Profiles of hybridization with ^{125}I-labeled HOPC 2020 λ chain mRNA are shown in Fig. 2 *A* and *B*. When no λ chain mRNA was added during incubation in formamide (Fig. 2*B*), the fragment carrying V_λ gene sequences cobanded with the bulk DNA. When λ chain mRNA was added (Fig. 2*A*), the major proportion of the V_λ sequence-carrying DNA banded at a position that was clearly heavier than the peak of the bulk DNA. The buoyant density at the hybridization peak in Fig. 2*A* was 0.018 g/cm^3 higher than that in Fig. 2*B*.

In a separate experiment we isolated a λ_{WES} phage that contains as its insert a 6.9-kb mouse DNA fragment (generated by *Eco*RI) carrying ribosomal DNA sequences (unpublished results). When the DNA fragment excised from the phage genome was annealed with purified mouse 18S ribosomal RNA (2.0 kb) and centrifuged to equilibrium in CsCl, the R-loop banded at a position 0.023 g/cm^3 denser than the duplex DNA. As a first-order approximation, the density increase of R-loops is inversely proportional to the ratio of the lengths of DNA and RNA. The lengths of the V_λ-carrying DNA fragment and the λ chain mRNA are about 4.8 and 1.2-kb, respectively (10, 11). We therefore expect that the R-loop formed between them is 0.019 g/cm^3 heavier than the DNA duplex. Thus, we assume that the major hybridization peak observed in Fig. 2*A* is composed of such an R-loop. In Fig. 2*A* a second hybridization peak is observed in fraction 10. This component was not characterized further.

Fractions 12 to 16 shown in Fig. 2*A* were recentrifuged in CsCl. The profile of hybridization is shown in Fig. 2*C*. While the hybridization peak remains at the original position, a large proportion of DNA (i.e., A_{260}-absorbing material) banded at the position where the bulk of the DNA banded in the first

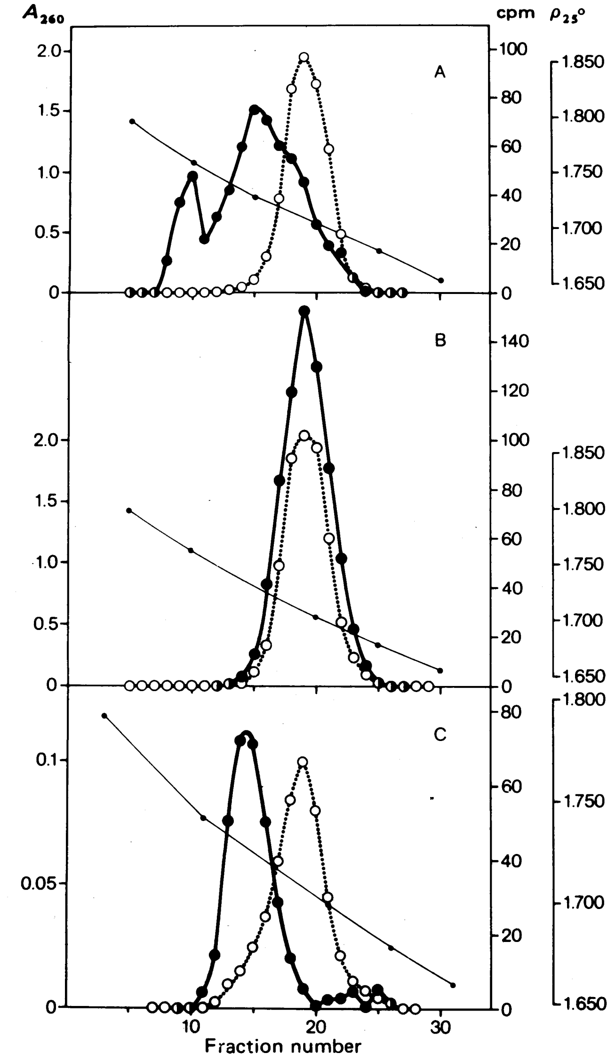

FIG. 2. Preparative R-loop formation. *Eco*RI-digested embryonic DNA prefractionated by agarose gel electrophoresis for V_λ gene sequences was incubated in the presence (*A*) and absence (*B*) of HOPC 2020 λ chain mRNA and centrifuged in CsCl as described in *Materials and Methods.* Fractions 12 to 16 shown in *A* and another tube which showed profiles nearly identical to *A* were pooled and recentrifuged (*C*). ○····○, A_{260}; ●—●, DNA·RNA hybrid; •—•, buoyant density.

centrifugation. Fractions 12 to 16 shown in Fig. 2*C* were pooled and used for cloning.

Enrichment for DNA fragments carrying the V_λ gene was approximately 10-, 9-, and 4-fold by agarose gel electrophoresis, and by the first and second CsCl density gradient centrifugation, respectively. If we assume that other operations carried out between these steps, such as ethanol precipitation and dialysis, did not cause loss of specific DNA fragments, the overall enrichment factor was about 360-fold.

Cloning of the V_λ Gene-Carrying DNA Fragment in Phage λ_{WES}. Approximately 2 µg of DNA recovered from the second gradient, which originated from about 10 mg of *Eco*RI-digested embryo DNA, gave about 6000 plaques under the transfection conditions described in *Materials and Methods*. We screened about 4000 plaques by *in situ* hybridization with ^{125}I-labeled HOPC 2020 mRNA. The 38 plaques that produced grey or black autoradiographic spots of various strengths were reexamined by a second *in situ* hybridization with less RNA probe. Because the λ chain mRNA probe used was about 90% pure, and the impurity is distributed among many different RNA species, each composing a small fraction of the mRNA preparation (12), the use of smaller amounts of the RNA probe in hybridization favors detection of the DNA clone complementary to the major mRNA component. Out of the 38 plaques re-examined in this way, one plaque, λgt_{WES}-Ig 13, gave a distinctly stronger autoradiographic spot than the others.

Table 1. Hybridization of ^{125}I-labeled light chain mRNA and its fragments with λgt-Ig 13 DNA

DNA on filter		^{125}I-mRNA	Input	Hybrid	% of
Source	µg	chain	cpm	cpm	input
λgt-Ig 13	25	λ (whole)	16,000	2,943	15.6
	50	λ (whole)	16,000	3,397	21.2
λgt-rD	25	λ (whole)	16,000	136	0.85
	50	λ (whole)	16,000	172	1.08
λgt-Ig 13	25	λ (3′-end half)	9,500	136	1.39
	50	λ (3′-end half)	9,500	163	1.72
λgt-rD	25	λ (3′-end half)	9,500	125	1.32
	50	λ (3′-end half)	9,500	132	1.39
λgt-Ig 13	25	κ (whole)	15,000	211	1.35
	50	κ (whole)	15,000	144	0.93
λgt-rD	25	κ (whole)	15,000	158	1.00
	50	κ (whole)	15,000	186	1.20

DNA was heat denatured and fixed on a Millipore filter by the method of Gillespie and Spiegelman (21). Hybridization was carried out in 2× SSC buffered with 0.05 M Pipes, pH 7.1, at 69° for 14 hr. Specific activity of RNA was 3 × 10^7 cpm/µg for whole and 3′-end half λ chain mRNA and 3.2 × 10^7 cpm/µg for κ mRNA. The hybrid was assayed by RNase treatment (RNase A, 20 µg/ml; RNase T1, 2 units/ml, in 2× SSC). λgt-rD designates a λ_{WES} phage in which the center *Eco*RI fragment was replaced with a 6.9-kb mouse DNA fragment carrying ribosomal DNA sequences. The ^{125}I-labeled 3′-end half of the λ chain mRNA and the whole κ chain mRNA used in this experiment hybridized well with corresponding cDNA (data not shown).

Characteristics of λgt_{WES}-Ig 13 DNA by Hybridization. *Eco*RI digestion of the DNA extracted from the clone λgt_{WES}-Ig 13 yielded, in addition to the left and right arms of the parental λgt_{WES} genome, one fragment of approximately 4.9 kb in agarose gel electrophoresis (data not shown; also see below). In Table 1, hybridization properties of the DNA with various RNA probes are shown. The DNA hybridized well with HOPC 2020 whole λ mRNA, whereas the levels of hybridization obtained with the 3′-end half of the same mRNA were no higher than when the same mRNA was hybridized to a clone carrying mouse ribosomal DNA. The results suggest that λgt_{WES}-Ig 13 DNA contains V_λ gene sequences and lacks C_λ gene sequences of HOPC 2020 mRNA. The same DNA showed no hybridization with MOPC 321 κ chain mRNA. Because these experiments were conducted with DNA baked on a Millipore filter, the efficiency of hybridization was not high.

In order to circumvent the problem, full length complementary DNA (cDNA) was synthesized from λ chain mRNA with the reverse transcriptase (RNA-dependent DNA polymerase) of avian myeloblastosis virus, and this DNA was hybridized with excess λgt_{WES}-Ig 13 DNA in liquid. The results are shown in Table 2. When assayed by hydroxyapatite, at least 60% of the cDNA prepared either from MOPC 104E λ chain mRNA or HOPC 2020 λ chain mRNA hybridized with the cloned DNA. When nuclease S1 was used to remove the tail (and possibly some mismatched bases), the hybridization levels were reduced by about one-half (Table 2). The mean melting point of the hybrid thus formed was 84° in 0.12 M $NaPO_4$ as assayed by the hydroxyapatite method (data not shown). It is not surprising that hybridization of cDNA, as assayed by hy-

Table 2. Hybridization of ^{32}P-labeled λ chain cDNA with Ig 13 DNA

[^{32}P]cDNA prepared on	C_0t*	Percent of ^{32}P cpm in hybrid: Hydroxyapatite assay	Percent of ^{32}P cpm in hybrid: Nuclease S1 assay
HOPC 2020 mRNA	0	4.4	2.7
HOPC 2020 mRNA	0.05	61	27
HOPC 2020 mRNA	0.067	61	27
MOPC 104E mRNA	0.22	64	30

The mouse DNA insert (Ig 13 DNA) was prepared by *Eco*RI digestion of λgt$_{WES}$-Ig 13 DNA and subsequent fractionation in 0.9% agarose gel. Full transcript cDNA was prepared from MOPC 104E λ chain mRNA by a modification of the method described by Monahan *et al.* (22). The cDNA (^{32}P-labeled) was mixed with excess Ig 13 DNA (copy ratio ≃40) in TE buffer (10 mM Tris·HCl, pH 8.0/1 mM Na_2EDTA) and heated at 98° for 5 min. $NaPO_4$ buffer (pH 6.9) was added to 210 mM and the mixture was incubated at 65° to the indicated C_0t values. For the hydroxyapatite assay, the annealing mixtures were made to 120 mm $NaPO_4$ by the addition of water, and were loaded on a small hydroxyapatite column pre-equilibrated with 120 mM $NaPO_4$, pH 6.9, at 60°. The column was washed with 9 ml of the same buffer and the hybrid was eluted with 6 ml of 0.4 M $NaPO_4$, pH 6.9. The hybrid fraction was determined by dividing the radioactivity in the 0.4 M $NaPO_4$ fraction by the total radioactivity recovered from the column. For the nuclease S1 assay, the annealing mixture was divided into two equal parts. DNA in one part was directly precipitated with trichloroacetic acid, while DNA in the other part was precipitated after treatment with nuclease S1. The hybrid fraction was determined by subtracting the intrinsic S1-resistant cpm (1.2%) from the ratio of the S1-resistant cpm and the total acid-precipitable cpm. $C_0t = 0$ sample was prepared by placing the heat-denatured sample directly in a dry ice/ethanol bath.

* C_0t refers to the concentration of DNA in moles of nucleotide per liter, times incubation time in sec.

droxyapatite, is incomplete (up to 64%) because the λ mRNA used in preparing the cDNA was about 80% pure, and the efficiency of hybridization under these conditions is about 90%. These results confirm that the λgt$_{WES}$-Ig 13 clone carries V_λ gene sequences.

Characterization of λgt$_{WES}$-Ig 13 DNA by Electron Microscopy. The length of the inserted fragment of mouse embryo DNA was determined by two independent methods. λgt$_{WES}$-Ig 13 DNA was digested with *Eco*RI and the length of the three resulting fragments was measured, using phage PM2 DNA (10 kb) as the internal length standard. The left and right arms of the vector were calculated to be 21.2 and 13.9 kb, respectively; the mouse DNA insert was 4.8 kb.

In heteroduplex preparations between λgt$_{WES}$-λC and λgt$_{WES}$-Ig 13, these measurements were confirmed. We observed one large heteroduplex region (example shown in Fig. 3) at the expected position (between 53.1 and 65.2 map units), the two arms of which were 4.8 and 5.5 kb long. Schematic representations of vector and cloned DNA are shown in Fig. 4.

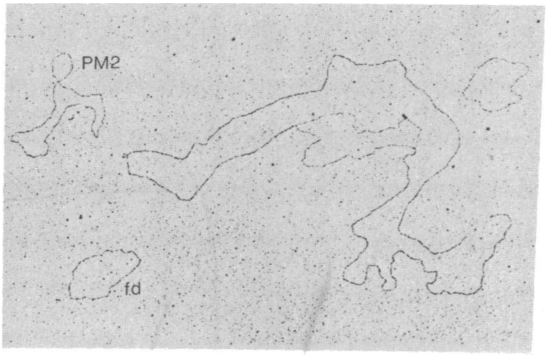

FIG. 3. Electron micrograph of heteroduplex molecule formed between λgt$_{WES}$-λC and λgt$_{WES}$-Ig 13. The internal markers used are phage fd single-stranded DNA (6330 nucleotides) and phage PM_2 DNA (10 kb).

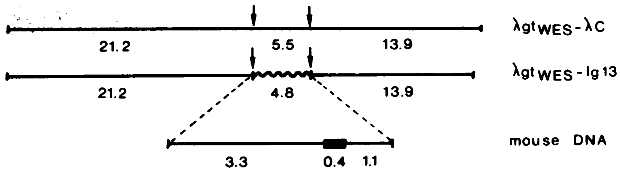

FIG. 4. Schematic representation of the maps of λgt$_{WES}$-λC and λgt$_{WES}$-Ig 13. The arrows point to *Eco*RI cleavage sites. All lengths are given in kilobase (kb) units. The boxed area in the mouse DNA insert represents the region of sequence homology with HOPC 2020 mRNA.

For the confirmation that the mouse DNA insert contains immunoglobulin gene sequences, purified myeloma HOPC 2020 λ chain mRNA was hybridized to the cloned DNA under conditions of R-loop formation (15). Both incubation conditions (51° and low salt, or 57° and high salt) gave only low yields of hybrid molecules; 20–30% of the molecules displayed a small R-loop (Fig. 5). Many of the loops had a small tail or "whisker" on one side. The position of the R-loop, as measured on 89 hybrids (obtained under various incubation conditions) is at 63 ± 1 map units from the left end of the molecule (Fig. 5). The lengths of the loop and the tail are 400 ± 100 nucleotides and 200–600 nucleotides, respectively. These large variations probably result from a lack of extension of the RNA whiskers under the spreading conditions used. Furthermore, some of the R-loops appeared to be partially collapsed or shortened by branch migration. We shall publish a detailed discussion of these matters elsewhere. The results obtained permit the following conclusions. (*i*) Only about half of the total mRNA molecule hybridizes to the cloned DNA fragment. (*ii*) The sequences homologous to the λ chain mRNA lie within the cloned DNA fragment, approximately 2.2 map units from the right end of the insert.

DISCUSSION

Isolation of a DNA fragment carrying a unique mammalian gene by "shotgun" *in vitro* recombination techniques requires

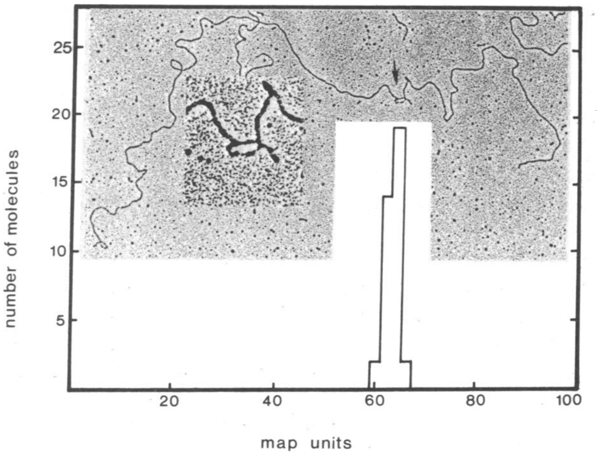

FIG. 5. Electron micrograph of hybrid molecule formed between λgt$_{WES}$-Ig 13 DNA and HOPC 2020 mRNA. The R-loop with an RNA tail is shown at higher magnification in the *inset*. The histogram shows the unique position of the R-loop at 63 map units from the left end of the λgt$_{WES}$-Ig 13 molecule. These measurements represent one experiment in which R-loops were formed at 57° and high salt, and spread from 70% formamide (see *Materials and Methods*).

screening of about a million independently arising clones. Such an approach has been made feasible by the recent development of a rapid screening procedure (23). In the study reported here, we followed an alternative approach in which biochemical pre-enrichment of a specific DNA fragment was combined with screening of phage plaques by *in situ* hybridization.

Besides size fractionation by agarose gel electrophoresis, several procedures for gene enrichment have been reported (7, 24–27). Whereas all of these procedures make use of specific base-pairing interaction between polynucleotide chains, the R-loop method is unique, in the sense that the two strands of the duplex DNA to be isolated never completely dissociate during the enrichment process. This not only eliminates the problems arising from single-strand breaks, which may very well occur during the isolation procedure, but also obviates an extra annealing step to restore the duplex structure. The latter step is potentially a problem because if short segments of the DNA fragment of interest are repeated elsewhere, the repeated sequences might prevent legitimate reassociation of corresponding single-stranded DNA pairs. The R-loop method was previously adopted for enrichment of repetitive *Drosophila melanogaster* ribosomal DNA (7). Our study demonstrates that the method is also applicable to a unique mammalian DNA as a pre-enrichment step in gene cloning. A limitation of this method as a universal gene enrichment procedure, besides the requirement for mRNA purification, arises from the decrease in buoyant density as the length of DNA relative to RNA increases. This problem, however, may be overcome by developing specific affinity columns.

Both hybridization and electron microscopy studies clearly demonstrate that the cloned λgt_{WES}-Ig 13 DNA contains a region whose base sequence is highly complementary to a part of a λ chain mRNA. This region is about 400 nucleotides long. Lack of hybridization of the 3′-end half of the same mRNA, as well as the frequent appearance at one end of the R-loop of an RNA tail that is longer than the expected poly(A) tail, indicates that the homology is in the 5′-end half of the mRNA, or in the V_λ gene. The possibilities that the RNA tail observed is either poly(A) alone, or that it results from a partial peeling of the R-loop structure, were eliminated by our recent following experiments. The purified mouse DNA fragment was denatured and annealed with full length cDNA obtained from a λ chain mRNA. Electron microscopic examination of the hybrids showed that only about half of the cDNA molecule hybridized to the cloned DNA, and that the other half extended as a single-stranded tail, approximately 500 nucleotides long (C. Brack, unpublished results).

The location and length of the homology region within the mouse DNA fragment was also confirmed by the combination of physical mapping of restriction enzyme cleavage sites and the Southern blotting technique (28), and by R-loop mapping of the purified mouse DNA fragment (unpublished results). These results might be taken as a direct confirmation of our previous experiments showing that V_λ and C_λ gene sequences occur at separate positions in the DNA of embryonic cells (8). The existence of the second type of λ chain (a λ_{II} chain) represented by MOPC 315 myeloma, complicates the situation. Chains of the λ_I type (to which all other known myeloma λ chains belong) differ from the λ_{II} chain by only 10 to 12 amino acids in the V (variable) regions, whereas they are different by 29 amino acids in the C (constant) region (29, 30). The polynucleotides corresponding to these two types of chains might, therefore, crosshybridize in the *V* region, and not in the *C* region (13). The possibility thus remains that the V_λ gene contained in the λgt_{WES}-Ig 13 clone codes for the λ_{II} chain, and we might have overlooked $C_{\lambda II}$ sequences which may lie immediately adjacent to the homology region. The determination of the nucleotide sequences will clarify these matters.

Note Added in Proof. Recent nucleotide sequencing study showed that the λgt_{WES}-Ig 13 clone contains a $V_{\lambda II}$ gene and no $C_{\lambda II}$ gene (unpublished data).

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