Cloning of an immunoglobulin variable region gene from mouse embryo

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

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Communicated by Niels K. Jerne, June 9, 1977

ABSTRACT  A 4.8-kilobase DNA fragment carrying an immunoglobulin gene coding for a mouse A chain variable region (V\textsubscript{\lambda} gene) was enriched about 350-fold from a total endonuclease EcoRI 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 A 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 Wam 403, Eam 100, Sam 100 by use of the EcoRI cohesive ends. Transfection of CaCl\textsubscript{2}-treated Escherichia coli 803 (r\textsuperscript{+}, m\textsuperscript{−}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 \textsuperscript{125}I-labeled A chain mRNA led to isolation of a clone that carries a V\textsubscript{\lambda} gene (λgtwES-14). Electron microscopy of R-loops confirmed the presence of sequences homologous to part of the A chain mRNA in its 3′-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\textsubscript{\gamma} genes. The DNA sequences coding for the carboxy-terminal half (C genes) lie separate from V\textsubscript{\gamma} 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\textsubscript{\gamma} genes in contingency 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\textsubscript{\gamma} 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 EcoRI digest of total mouse embryo DNA. By transfection of host bacteria with phage A 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\textsubscript{\gamma} gene coding for a A-type immunoglobulin light chain.

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MATERIALS AND METHODS

Bacteria and Phages. Escherichia coli 803 (r\textsuperscript{+}, m\textsuperscript{−}K Su III\textsuperscript{+} (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. λgtwES-NC was constructed by L. Enquist and coworkers at the U.S. National Institutes of Health (NIH) and provided by R. Weisberg (14). The central EcoRI 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 EcoRI mouse DNA fragments enriched for the V\textsubscript{\lambda} gene sequences were dialyzed against a mixture composed of 70% (vol/vol) formamide (Kodak, deionized with Bio-Rad AG 501-XB CED resin, 100 mM piperrazine-N,N′-bis(2-ethanesulfonic acid) (Pipes), 5 mM Na\textsubscript{2}EDTA, 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 0.7 μg of purified HOPC 2020 A chain mRNA, 70% formamide, and 0.1 M Tris-HCl, pH 7.5. The mixture was incubated at 56 ± 0.2° C for 7 hr, after which 40 ml of cold solution containing 20 mM Tris-HCl at pH 7.5, 2 mM Na\textsubscript{2}EDTA, and 6X SSC was added (1X SSC is 0.15 M NaCl/0.015 M Na citrate at pH 7). Solid CsCl (Merek, gradient grade) was then added to a refractive index n\textsubscript{D} 25° = 1.401. The mixture was centrifuged in two tubes in a Spinco Ti 60 rotor at 35,000 rpm and 10° C 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° C, and neutralized by addition of a mixture consisting of 1.5 M HCl and 0.6 M Tri-HCl, pH 7.5. DNA was precipitated with ethanol and dissolved in 20 μl of 2X SSC, and 1000 cpm of \textsuperscript{125}I-labeled HOPC 2020 A chain mRNA (specific activity 8 X 10\textsuperscript{6} 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\textsubscript{D} 25° = 1.401 and recentrifuged at 10° C 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

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

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fractions were precipitated with ethanol. The precipitate was dissolved in 50 μL of 0.02× SSC containing 1 mM Na₂EDTA 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 AgtWS DNA devoid of the EcoRI C fragment, and 20 units T4 ligase (Miles) in 66 mM Tris-HCl, pH 7.5/10 mM MgCl₂/1 mM Na₂EDTA/70 mM NaCl/10 mM dithiothreitol/90 μM ATP/bovine serum albumin (Miles) at 100 μg/mL. The reaction was stopped by addition of Na₂EDTA 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 MgCl₂, 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 × 10⁶ cpm per filter of ¹²⁵-I-labeled HOPC 2020 λ chain mRNA (specific activity: 8 × 10⁷ cpm/μg) in 50% formamide and 4X 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 EcoRI Fragment Carrying a V₅ Gene. Highly polymerized DNA from 12-day-old BALB/c embryos was digested to completion with EcoRI, 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 V₅ and C₅ sequences), in order to detect fragments carrying V₅ and C₅ 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 C₅ genes sequences), indicating that this component carries C₅ gene sequences. Furthermore, equality in the level of hybridization obtained with the two DNA probes suggested that this component carries no V₅ 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 C₅ gene sequences and presumably carry V₅ gene sequences.

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 V₅ gene sequences cobanded with the bulk DNA. When λ chain mRNA was added (Fig. 2 A), the major proportion of the V₅ 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. 2A was 0.018 g/cm³ higher than that in Fig. 2B.

In a separate experiment we isolated a λ₁₁₁₁₁ phage that contains as its insert a 6.9-kb mouse DNA fragment (generated by EcoRI) 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/cm³ 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 V₅-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/cm³ heavier than the duplex DNA. Thus, we assume that the major hybridization peak observed in Fig. 2A is composed of such an R-loop. In Fig. 2A a second hybridization peak is observed in fraction 10. This component was not characterized further.

Fraction 12 to 16 shown in Fig. 2A were recentrifuged in CsCl. The profile of hybridization is shown in Fig. 2C. While the hybridization peak remains at the original position, a large proportion of DNA (i.e., A₂₆₀-absorbing material) banded at the position where the bulk of the DNA banded in the first
Fig. 2. Preparative R-loop formation. EcoRI-digested embryonic DNA prefractionated by agarose gel electrophoresis for $V_{\lambda}$ gene sequences was incubated in the presence (A) and absence (B) of HOPC 2020 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. 2C were pooled and used for cloning.

Enrichment for DNA fragments carrying the $V_{\lambda}$ gene was approximately 10-, 4-, and 4-fold by agarose gel electrophoresis, and by the first and second CsCl density 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 $V_{\lambda}$ Gene-Carrying DNA Fragment in Phage $\lambda$WES. Approximately 2 $\mu$g of DNA recovered from the second gradient, which originated from about 10 mg of EcoRI-digested embryonic 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 $\lambda$ chain mRNA probe used was about 90% pure, and the impurity is distributed among many different

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

<table>
<thead>
<tr>
<th>DNA on filter</th>
<th>$^{125}$I-mRNA chain</th>
<th>Input cpm</th>
<th>Hybrid cpm</th>
<th>% of input</th>
</tr>
</thead>
<tbody>
<tr>
<td>Source</td>
<td>$\mu$g</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>$\lambda$-Ig 13</td>
<td>25 $\lambda$ (whole)</td>
<td>16,000</td>
<td>2,943</td>
<td>15.6</td>
</tr>
<tr>
<td></td>
<td>50 $\lambda$ (whole)</td>
<td>16,000</td>
<td>3,472</td>
<td>21.2</td>
</tr>
<tr>
<td>$\lambda$-rD</td>
<td>25 $\lambda$ (whole)</td>
<td>16,000</td>
<td>136</td>
<td>0.85</td>
</tr>
<tr>
<td></td>
<td>50 $\lambda$ (whole)</td>
<td>16,000</td>
<td>172</td>
<td>1.08</td>
</tr>
<tr>
<td>$\lambda$-Ig 13</td>
<td>25 $\lambda$ (3' end half)</td>
<td>9,500</td>
<td>136</td>
<td>1.39</td>
</tr>
<tr>
<td></td>
<td>50 $\lambda$ (3' end half)</td>
<td>9,500</td>
<td>163</td>
<td>1.72</td>
</tr>
<tr>
<td>$\lambda$-rD</td>
<td>25 $\lambda$ (3' end half)</td>
<td>9,500</td>
<td>125</td>
<td>1.32</td>
</tr>
<tr>
<td></td>
<td>50 $\lambda$ (3' end half)</td>
<td>9,500</td>
<td>132</td>
<td>1.39</td>
</tr>
<tr>
<td>$\lambda$-Ig 13</td>
<td>25 $\kappa$ (whole)</td>
<td>15,000</td>
<td>211</td>
<td>1.35</td>
</tr>
<tr>
<td></td>
<td>50 $\kappa$ (whole)</td>
<td>15,000</td>
<td>144</td>
<td>0.93</td>
</tr>
<tr>
<td>$\lambda$-rD</td>
<td>25 $\kappa$ (whole)</td>
<td>15,000</td>
<td>158</td>
<td>1.00</td>
</tr>
<tr>
<td></td>
<td>50 $\kappa$ (whole)</td>
<td>15,000</td>
<td>186</td>
<td>1.20</td>
</tr>
</tbody>
</table>

DNA was heat denatured and fixed on a Millipore filter by the method of Gillespie and Spiegelman (21). Hybridization was carried out in 2X SSC buffered with 0.05 M Pipes, pH 7.1, at 69° for 14 hr. Specific activity of RNA was $5 \times 10^5$ cpm/$\mu$g for whole and 3'-end half $\lambda$ chain mRNA and 3.2 $\times 10^5$ cpm/$\mu$g for $\kappa$ mRNA. The hybrid was assayed by RNase digestion (RNase A, 20 $\mu$g/ml; RNase T1, 2 units/ml, in 2X SSC). $\lambda$-rD designates a $\lambda$WES phage in which the center EcoRI fragment was replaced with a 6.9-kb mouse DNA fragment carrying ribosomal DNA sequences. The $^{125}$I-labeled 3'-end half of the $\lambda$ chain mRNA and the whole $\kappa$ chain mRNA used in this experiment hybridized well with corresponding cDNA (data not shown).

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 reexamined in this way, one plaque, $\lambda$WES-Ig 13, gave a distinctly stronger autoradiographic spot than the others.

Characteristics of $\lambda$WES-Ig 13 DNA by Hybridization. EcoRI digestion of the DNA extracted from the clone $\lambda$WES-Ig 13 yielded, in addition to the left and right arms of the parental $\lambda$WES genome, one fragment of approximately 4.9 kb in agarose gel electrophoresis (data not shown; also see below). Table 1, hybridization properties of the DNA with various RNA probes are shown. The DNA hybridized well with HOPC 2020 whole $\lambda$ 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 $\lambda$WES-Ig 13 DNA contains $\lambda$ gene sequences and lacks $\kappa$ gene sequences of HOPC 2020 mRNA. The same DNA showed no hybridization with MOPC 321 $\kappa$ 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 $\lambda$ chain mRNA with the reverse transcriptase (RNA-dependent DNA polymerase) of avian myeloblastosis virus, and this DNA was hybridized with excess $\lambda$WES-Ig 13 DNA in liquid. The results are shown in Table 2. When assayed by hydroxycapatite, at least 60% of the cDNA prepared either from MOPC 104E $\lambda$ chain mRNA or HOPC 2020 $\lambda$ 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 hydroxycapatite method (data not shown). It is not surprising that hybridization of cDNA, as assayed by hy-
Table 2. Hybridization of 32P-labeled λ chain cDNA with Ig 13 DNA

<table>
<thead>
<tr>
<th>[32P] cDNA prepared on</th>
<th>Percent of 32P cpm in hybrid</th>
<th>Hydroyxapatite Nuclease</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>assay</td>
</tr>
<tr>
<td>HOPC 2020 mRNA</td>
<td>0</td>
<td>4.4</td>
</tr>
<tr>
<td>HOPC 2020 mRNA</td>
<td>0.05</td>
<td>61</td>
</tr>
<tr>
<td>HOPC 2020 mRNA</td>
<td>0.067</td>
<td>61</td>
</tr>
<tr>
<td>MOPC 104E mRNA</td>
<td>0.22</td>
<td>64</td>
</tr>
</tbody>
</table>

The mouse DNA insert (Ig 13 DNA) was prepared by EcoRI digestion of λgtwes-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 (32P-labeled) was mixed with excess Ig 13 DNA (copy ratio ∼ 40) in TE buffer (10 mM Tris-HCl, pH 8.0, 0.1 mM NaCl, EDTA) and heated at 98°C for 5 min. NaPO₄ buffer (pH 6.9) was added to 210 mM and the mixture was incubated at 65°C to the indicated C₄t values. For the hydroxyapatite assay, the annealing mixtures were made to 120 mM NaPO₄ by the addition of water, and were loaded on a small hydroxyapatite column pre-equilibrated with 120 mM NaPO₄, pH 6.9, at 60°C. The column was washed with 9 ml of the same buffer and the hybrid was eluted with 6 ml of 0.4 M NaPO₄, pH 6.9. The hybrid fraction was determined by dividing the radioactivity in the 0.4 M NaPO₄ 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. C₄t = 0 sample was prepared by placing the heat-denatured sample directly in a dry ice/ethanol bath.

* C₄t refers to the concentration of DNA in moles of nucleotide per liter, times incubation time in sec.

hydroyxapatite, 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 λgtwes-Ig 13 clone carries Vλ gene sequences.

Characterization of λgtwes-Ig 13 DNA by Electron Microscopy. The length of the inserted fragment of mouse embryo DNA was determined by two independent methods. λgtwes-Ig 13 DNA was digested with EcoRI 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 λgtwes-λC and λgtwes-lg 13, these measurements were confirmed. We ob-
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 λgtWES-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 5′-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 Vλ 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 Vλ and Cλ gene sequences occur at separate positions in the DNA of embryonic cells (8). The existence of the second type of λ chain (a λ1 chain) represented by MOPC 315 myeloma, complicates the situation. Chains of the λ1 type (to which all other known myeloma λ chains belong) differ from the λ2 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 Vλ gene contained in the λgtWES-Ig 13 clone codes for the λ1 chain, and we might have overlooked Cλ 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 λgtWES-Ig 13 clone contains a Vλ chain gene and no Cλ chain gene (unpublished data).

We thank Dr. M. Potter and Dr. M. Cohn for providing us with myeloma lines; Dr. J. Beard for reverse transcriptase; Dr. L. Enquist and his coworkers for making λgtWES-C available; and Dr. T. Bickle for λd and PMλ DNAs. Dr. G. Matthysens prepared the cDNAs. Excellent technical assistance was provided by Mr. G. Dastoornikoo, Mr. A. Traumecker, and Mrs. P. Rieger.