Volume 5 Number 9 September 1978 Nucleic Acids Research

Characterization of an immunoglobulin cDNA clone containing the variable and constant regions for the MOPC 21 kappa light chain

Michael D.Strathearn[1], Gary E.Strathearn[1], Patricia Akopiantz[1], Alvin Y.Liu[1], Gary V.Paddock[1], and Winston Salser [1,2]

Molecular Biology Institute [2], and Department of Biology [1], University of California, Los Angeles, CA 90024, USA

Received 14 July 1978

ABSTRACT

Nucleotide sequence analysis and restriction endonuclease mapping have been used to characterize a cDNA copy of immunoglobulin MOPC 21 Kappa mRNA cloned in the bacterial plasmid pMB9. Three regions of the inserted cDNA of plasmid pL21-1 have been sequenced and match the known protein sequence at amino acid residues 1-24, 128-138 and 171-179. With these sequences to provide absolute correlations between the restriction map and the structural gene sequence it has been possible to exactly deduce the positions of all 11 of the insert restriction sites mapped within the structural gene. The pL21-1 insert contains the complete variable and constant regions as well as parts of the 3' untranslated and polypeptide leader coding sequences.

INTRODUCTION

Wall, Gilmore-Hebert, Higuchi, Komaromy, Paddock, and Salser (1) have described the synthesis of immunoglobulin gene copies from MOPC 21 mRNA and the construction of clones by the technique of Higuchi, Paddock, Wall, and Salser (2). The five plasmids pL21-1 to pL21-5 generated were partially characterized by DNA-RNA hybridization studies (1). Here we present data giving a precise restriction map of pL21-1, the largest of the cloned Kappa chain immunoglobulin gene copies (1). Several nucleic acid sequences which unambiguously show the relationship of this map with the known amino acid sequence are introduced. This will provide useful probes for gene mapping, for the cloning of the corresponding chromosomal sequences and for the eventual analysis of the nucleotide sequences of the gene as it occurs in the chromosome. We were also guided by the need to definitively characterize probes for the mapping of the transcription unit by

the UV resistance mapping technique of Hacket and Sauerbier (3) and Sauerbier (4).

MATERIALS AND METHODS

Cloning of pL21-1 and Preparation of Plasmid DNA

The procedure by which the plasmid pL21-1 was cloned in the plasmid pMB9 has been described earlier (1) and used the techniques of Higuchi et al. (2) (see also Salser, reference 5, for review). The plasmid was separated from the chromosomal DNA and mRNAs as described by Padayatty, Cummings, Manske, Higuchi, Woo and Salser (6).

Restriction Enzymes

The plasmid was cleaved using restriction endonucleases Alu I, Hae III, Hinc II, Hind III, Hpa II, and Taq I. Fragment sizes were determined by comparison to a digest of the ØX174 bacteriophage genome cleaved by either Alu I or Hae III to give fragments of known size (Sanger, Air, Barrell, Brown, Coulson, Fiddes, Hutchison, Slocombe, and Smith, reference 7). Hae III, Hinc II, Hind III, and Hpa II were purchased from New England Biolabs (NEB), Alu I from Bethesda Research Laboratories, and Taq I was prepared by S. Hendrich using an unpublished technique developed by M. Komaromy.

Reaction Conditions

Except in the case of Taq I, preparatory enzyme digests were carried out for two hours at 37°C in 6 mM Tris-HCl pH 7.5, 6 mM $MgCl_2$, 6 mM 2-mercaptoethanol (2-ME) (for Hae III); the preceding plus 100 ug/ml gelatin (Alu I); 10 mM Tris-HCl, pH 7.9, 7 mM MgCl2, 1.0 mM dithiothreitol (DTT), 60 mM NaCl (Hinc II and Hind III); 10 mM Tris-HCl, pH 7.4, 6 mM KCl, 10 mM $MgCl_2$, 1.0 mM DTT, and 100 ug/ml Bovine Serum Albumin (BSA) (Hpa II). Digestion with Taq I was carried out for two hours at 62°C in 10 mM Tris-HCl pH 8.4, 6 mM $MgCl_2$, 6 mM 2-ME and 100 ug/ml gelatin. Restriction mapping reactions were carried out for one hour in the conditions above. All double digests were carried out in Alu I buffer. Digestion with Taq I was carried out for two hours at 62°C in 10 mM Tris-HCl.

^{32}P Labeling and Sequence Analysis

The 5′ terminal phosphate was removed by bacterial alkaline phosphatase (Worthington Biochemical, grade f) in 10 mM Tris-HCl, pH 9.0, for 30 min. at 37 C. The reaction mixture was then twice phenol extracted and twice rinsed with diethyl ether to inhibit any remaining activity. Gamma-^{32}P ATP was synthesized from 32sP (ICN Pharmaceuticals) by the procedure of Maxam and Gilbert (8). Gamma-^{32}P was transferred to the 5′ terminal phosphate of the restriction fragment using T4 polynucleotide kinase (PL Biochemicals) (8). The fragments were then either strand separated or cleaved with a second restriction endonuclease, to give singly labeled fragments which were then sequenced using the partial cleavages described by Maxam and Gilbert (8).

Gel Electrophoresis

Several acrylamide slab gel types were used, depending upon the restriction fragment sizes: 6% acrylamide (Eastman Kodak) with 6 or 12% glycerol for smaller fragments (<500 base pairs) and 8% acrylamide for larger fragments. DNA was eluted from these preparatory gels using 0.5 M NaAc, 10 mM $MgCl_2$, 0.1% SDS, and 0.1 mM EDTA. Twenty percent acrylamide 7 M urea slab gels were used for the sequence analysis.

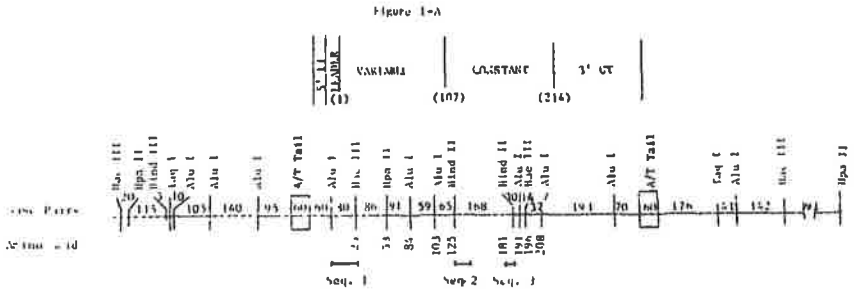
RESULTS

The results in the previous paper have indicated that the plasmid pL21-1 contains both variable and constant regions from MOPC 21 immunoglobulin mRNA (1). The sequences inserted in this plasmid have now been characterized by sequence analysis and mapped with restriction enzymes.

Variable Region Nucleotide Sequence of pL21-1

Cleavage of the plasmid pL21-1 with endonuclease Alu I yielded four fragments 291, 245, 55 and 53 base pairs in size which did not co-migrate with the fragments of parent plasmid pMB9, cut with the same enzyme. We suspected the presence of additional "hidden" Alu I bands since the sum of the sizes of the

four fragments was less than the size of the entire insert as estimated from other data (1). Our further restriction mapping showed three additional insert fragments co-migrating with pMB9 fragments (see Results, Section C, and Figure 1-A). The 245 base pair fragment was eluted, labeled at its 5′ terminus with ^{32}P, and cleaved with Hae III to give two fragments whose lengths were 85 and 160 base pairs. Nucleotide sequencing reactions were carried out on the 85 base pair fragment. A sequence of 77 nucleotides could be read from the autoradiograph of the sequence gel (Figure 2-A). This sequence corresponds exactly to amino



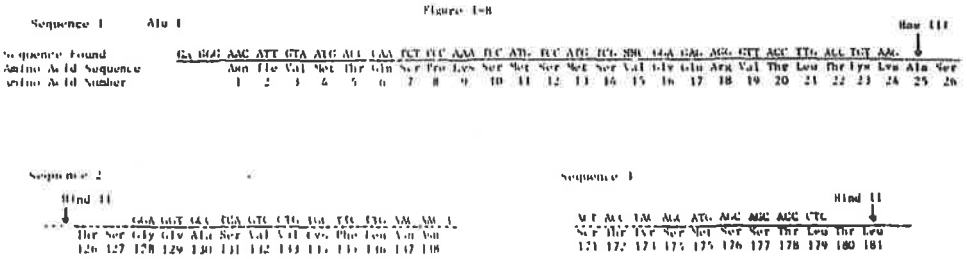


FIGURE ONE:
Figure 1-A shows a restriction endonuclease map of pL21-1, in the area containing and surrounding the insert. The orientation of the translated (variable, constant and 5′ polypeptide leader) and 3′ untranslated (UT) and 5′ untranslated are shown above the restriction map. Restriction enzyme sites are indicated, and the distances (in base pairs) between restriction sites are shown by horizontal numbers. Vertical numbers in parenthesis indicate amino acid residue (9). The poly A/T tails (60 base pairs are expected from insertion technique (1) but has not been confirmed) are shown to mark the insertion sites. The nucleotide sequences obtained are indicated by lines. Figure 1-B shows three sequences of 77, 34 and 27 nucleotides derived by nucleotide sequence analysis of the autoradiographs shown in Figure 2. Sequence 1 is from the Alu I 245 base pair fragment 5′-terminally labeled with ^{32}P, then cleaved with Hae III. Sequences 2 and 3 are complementary strands from the labeled 180 base pair fragment of a Hinc II digest. Sequence 1 provided 77 nucleotides, while sequence 2, from the fast-running coding strand, yielded 34 and the slow-running non-coding strand 27. The data for sequence one was ambiguous in two positions (labeled N) in Figure 2-A and described in the text. Data from the related plasmid pL21-5 (P. Clarke, personal communication) suggests that there may be an additional Alu I fragment about 30 bp in size extending to the right from Ser 208. This small fragment was apparently missed in our experiments.

acid residues 1-24 of the protein sequence (Svasti and Milstein, reference 9) and in addition includes five of the nucleotides coding for the pre-polypeptide leader. This experiment unambiguously locates one of the Hae III sites at Ala 25 in the sequence coding for the variable region and establishes that an Alu I site occurs a few nucleotides prior to the first codon of the variable region. Two nucleotides at amino acid 15 proved difficult to interpret from the ladder sequence gel autoradiograph and these two nucleotides are left unassigned. These problematic nucleotides may be due to poor resolution of the gel in this region but it is also possible that the bases may have been deleted during the cloning procedures or subsequent propagation of the plasmid.

Constant Region Nucleotide Sequences of pL21-1

A Hinc II cleavage was carried out on pL21-1, three fragments were observed which were not present in parallel digests of the parent plasmid pMB9. One of these fragments 170 base pairs in size was labeled at its 5′ terminues, strand separated and sequenced. Two sequences from the complementary strands were deduced from the autoradiograph shown in Figure 2-B and are shown in Figure 1-B. From the slow strand, 27 nucleotides (coding for amino acids 171 to 179 in the constant region) were read, while the fast strand gave a 34-nucleotide sequence (coding for amino acids 128 to 138 in the constant region). This experiment unambiguously locates Hinc II cleavage sites at codons 125 and 181 in the constant region.

Restriction Mapping of pL21-1

As described below, our restriction mapping indicates that in pL21-1 the sequence coding for the entire variable and constant regions have been inserted into the Eco RI cleaved plasmid pMB9. The logic used in constructing the map of pL21-1 will be summarized briefly below but we will not attempt to describe all of the data since many features of the map are independently verified by a variety of data which cannot be detailed in the space available. Our analysis was aided by the

DOCKET ALARM

Explore Litigation Insights

Docket Alarm provides insights to develop a more informed litigation strategy and the peace of mind of knowing you're on top of things.

Real-Time Litigation Alerts

Keep your litigation team up-to-date with **real-time alerts** and advanced team management tools built for the enterprise, all while greatly reducing PACER spend.

Our comprehensive service means we can handle Federal, State, and Administrative courts across the country.

Advanced Docket Research

With over 230 million records, Docket Alarm's cloud-native docket research platform finds what other services can't. Coverage includes Federal, State, plus PTAB, TTAB, ITC and NLRB decisions, all in one place.

Identify arguments that have been successful in the past with full text, pinpoint searching. Link to case law cited within any court document via Fastcase.

Analytics At Your Fingertips

Learn what happened the last time a particular judge, opposing counsel or company faced cases similar to yours.

Advanced out-of-the-box PTAB and TTAB analytics are always at your fingertips.

API

Docket Alarm offers a powerful API (application programming interface) to developers that want to integrate case filings into their apps.

LAW FIRMS
Build custom dashboards for your attorneys and clients with live data direct from the court.

Automate many repetitive legal tasks like conflict checks, document management, and marketing.

FINANCIAL INSTITUTIONS
Litigation and bankruptcy checks for companies and debtors.

E-DISCOVERY AND LEGAL VENDORS
Sync your system to PACER to automate legal marketing.