Volume 16 Number 9 1988 Nucleic Acids Research

Mapping genomic organization by field inversion and two-dimensional gel electrophoresis: application to the murine T-cell receptor γ gene family

Tod Woolf, Eric Lai*, Mitchell Kronenberg[1] and Leroy Hood

Division of Biology, 147-75, California Institute of Technology, Pasadena, CA 91125 and [1]Department of Microbiology and Immunology, 174718, UCLA School of Medicine, Los Angeles, CA 90024, USA

Received December 31, 1987; Revised and Accepted April 8, 1988

ABSTRACT

A new two-dimensional gel electrophoresis technique has been developed for the mapping of multigene families. Resolution in the first dimension is based on the generation of large size DNA fragments by infrequently-cutting restriction enzymes, and separation of these fragments by field inversion gel (FIG) electrophoresis. A second restriction enzyme digestion is then carried out with the separated DNA fragments in the agarose gel. Standard gel electrophoresis in the second dimension allows one to estimate the number of hybridizing genes contained in each large DNA fragment. We have also developed a novel method to increase the separation, resolution and hybridization signal in the second dimension by condensing the bands from the first dimension into spots. As an example, we have applied these techniques to determine the organization of the murine T-cell receptor γ locus. The murine γ gene family was found to be contained on two DNA fragments encompassing 195 kilobases of DNA. The two-dimensional gel electrophoresis method is particularly useful in the analysis of the organization of multigenic families where single copy probes are not readily available, and should extend the potential usefulness of field inversion gel electrophoresis in gene mapping.

INTRODUCTION

Several techniques are available for analyzing the genomic organization of genes. For example, chromosomal walking using λ bacteriophage and cosmid vectors has been used extensively for the analysis of a number of gene families[1]. However, these methods are tedious and time consuming. Recent advances in the separation of large size DNA molecules, in the range 50-2,000 kilobases (kb), with pulsed field gel (PFG)[2] and field inversion gel (FIG)[3] electrophoresis, have opened the possibility of long-range gene mapping in mammalian systems. Large size DNA fragments can be generated with one of several infrequently-cutting restriction enzymes, separated by PFG or FIG electrophoresis, and subjected to Southern blot analysis with DNA probes. The logic of mapping with these gel systems rests with the supposition that if two genes are located on similar sized fragments in multiple restriction enzymes digests, they are probably linked. There are two major difficulties associated with this technique. 1) There are very few restriction enzymes that yield large fragments. Moreover, those few suitable restriction enzymes cut sites containing CG dinucleotides that can be methylated and therefore rendered insensitive to digestion. Partial digestions are therefore often

observed with these enzymes leading to reduced hybridization signals. 2) Single-copy DNA probes are usually not available for gene families with multiple members that are highly homologous. Thus, following PFG or FIG electrophoresis, when a DNA probe complementary to several genes hybridizes to multiple DNA fragments on blots, it is not possible to determine the order of genes on a particular large DNA fragment.

To alleviate these problems, we have developed a new two-dimensional gel system for mapping gene families. The method involves separation of large DNA fragments in the first dimension by FIG electrophoresis, followed by excision of the entire lane and digestion of the DNA in the gel[4] with a more frequently cutting restriction endonuclease. Then a new gel is cast around the lane and the DNA is electrophoresed at a right angle to the first dimension[5]. The maximum distance between genes in a multigene family will be indicated by the estimated sizes following FIG electrophoresis of the complete and partial restriction fragments hybridizing with the probe. Further restriction enzyme digestion with a more frequently cutting enzyme and conventional gel electrophoresis permit the different cross-hybridizing genes to be mapped onto the large fragments by virtue of their characteristic restriction fragment sizes.

We have also developed a technique for condensing the individual DNA bands before electrophoresis in the second dimension. This permits the resolution of closely migrating fragments in the second dimension and results in a more concentrated DNA band or dot, leading to a stronger hybridization signal. A more intense hybridization signal is important because field inversion blots often give poor signals due to partial restriction enzyme digestions[6].

In this article, we demonstrate the validity of the two-dimensional approach to gene mapping, by hybridizing two-dimensional blots with probes derived from the murine T cell antigen receptor (TCR) γ gene family. The T-cell receptor γ chain constitutes one subunit of the γ-δ antigen receptor expressed by certain subsets of T cells [7-12]. The murine γ locus, like the immunoglobulin and other TCR gene families, has at least seven variable (V) and four joining (J) gene segments. Each J gene segment is closely linked to one of four constant (C) region genes. The organization of these genes into six clusters has been determined through the study of genomic DNA cloned into bacteriophage λ vectors[13-17]. The distance between these linkage groups is unknown, and their organization has so far been inferred only from a few rearrangement events that may delete intervening DNA[14,17]. Moreover, there are several aspects of the rearrangement process that are not well understood. In the γ locus, particular V gene segments tend to rearrange to particular J gene segments, as opposed to the combinatorial V-J joining seen in the other TCR and immunoglobulin gene families. This preference could be due to V gene segments joining to the closest J gene segment, although other explanations are possible. In order to determine whether some

properties of the γ locus expression are related to the chromosomal position or the distance between gene segments, we have begun to study the chromosomal organization of these genes. The experiments carried out permit us to link the six separate clusters previously known to contain γ genes[13-17] into two clusters, one 45 kb and the second 150 kb in length. The murine γ gene locus must therefore encompass at least 195 kb of DNA.

MATERIALS AND METHODS

Preparation of high molecular weight DNA

BALB/cJ mouse livers were cut into pieces, and two 10 centimeter square gound plates were used to grind the tissue one piece at a time (Barbara Osborne, personal communication). The tissue was ground until the two glass plates came in contact with each other and then one more circular motion was carried out. The ground tissue was rinsed off the glass plates with 4° C phosphate buffered saline solution including 10 mM EDTA (PBSE). The process was repeated for the entire liver using the same ten mls of PBSE to rinse the plates. Then the suspension of ground liver was allowed to stand for 3 minutes so that any remaining particulate matter could settle. The supernatant was transferred and centrifuged for 30 second at 100 rpm (85 G) to remove any remaining particulate matter. The cells were washed in PBSE and one volume of packed cells was resuspended in three volumes PBSE, warmed to 37° C and mixed well with an equal volume of 1% SeaPlaque low melt agarose (FMC bioproducts) at 37° C dissolved in PBSE. This mixture was poured into casting molds and high molecular weight DNA was purified as described earlier[18]. Approximately 10 μg of high molecular weight DNA (>500 kb) per 50 μl of agarose insert was obtained by this method.

Restriction enzyme digestion and field inversion electrophoresis.

High molecular weight genomic DNA was digested and electrophoresed as described by Popko et al., 1987[18]. The gel was allowed to equilibrate with the running buffer for two hours before electrophoresis. GTG grade SeaKem (FMC bioproducts) agarose was used because it is largely free of restriction enzyme inhibitors. Identical digests were run side by side, one was transferred to Zeta probe nylon (BioRad) membrane according to Reed and Mann using 0.4 N NaCl as solvent[19] and the other was excised and digested in the gel[5]. For the in gel digestion the excised lane was equilibrated five times against 500 mls of 10 mM Tris pH 8.0, 50 mM NaCl and 1 mM EDTA. The DNA was then digested on a rocker platform at 37° C for six hours with the digestion buffer recommended by the manufacturer plus 100 μg/ml of pentex grade BSA (Boehringer Mannheim Biochemicals). The volume of the agarose was included in the concentration calculations. We used 100-5000 units of enzyme (BMB) in a sealed 25 ml plastic pipet. An agarose insert, containing 10 μg of the intact genomic DNA, was

included as a control. Reaction conditions necessary for digestion in agarose with various restriction enzymes has been described by Boehm and Drahovsky[20].

Concentration of lanes and separation in a second dimension

By concentration of lanes, we refer to a procedure for reducing the width of the first-dimension lane after it has been digested and electrophoresed. We electrophoresed the DNA at a right angle to the first dimension of electrophoresis into a strip of dialysis membrane that was embedded in agarose. The gel tray, shown in Fig. 1, was used to hold the dialysis membrane in place. The dialysis membrane was cut into a strip as wide as the height of the lane. The strip of dialysis membrane was pulled across the gel tray, as shown in Fig. 1, and secured in the slots. The slots were pushed together and tightened with the screws. The lane was placed as shown in Fig. 1 and agarose of the desired concentration for the second dimension was poured just over the top edge of the dialysis membrane. The tray was gently placed in a gel box making sure that a good seal was maintained between the dialysis membrane and the agarose. The DNA was then electrophoresed towards the dialysis membrane at 3 V/cm in TAE buffer for four hours. Initially, VWR 12,000-14,000 molecular weight cutoff dialysis membrane was used in this procedure, however, we found that fragments under 1.5 kb in length passed through the membrane. VWR 1000 molecular weight cutoff dialysis membrane was found to contain DNA fragments down to 500 bp length using the same conditions (data not shown). The field was reversed at 6 V/cm for four minutes and then run for five minutes at 1.2 V/cm to electrophorese the DNA back into the agarose.

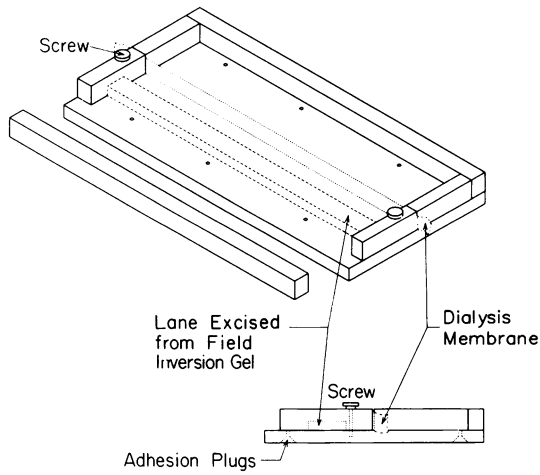

Figure 1. Schematic diagram of the gel tray apparatus used for concentration of the bands in the first dimension gel. Adhesion plugs (LKB pulsaphor system) were used to secure the agarose gel onto the tray.

A strip of agarose containing the DNA was cut out from the "concentrated" gel and placed on a standard gel tray. The strip was placed so that the side previously in contact with the dialysis membrane was now facing the direction of electrophoresis in the second dimension. The agarose insert containing genomic DNA digested only by the second dimension enzyme was placed next to the size marker well and a 0.6% agarose gel in TAE was poured even with the height of the strip. A gel comb with only one tooth was used for subsequent loading of markers (1 kb ladder, BRL). The second dimension was electrophoresed at 1 V/cm overnight in a 19 cm long gel, stained with ethidium bromide, photographed and then transferred to Zeta probe nylon membrane.

Probes

The following γ gene DNA probes were employed: 1. pBSCγ, a Bluescript (Stratagene, San Diego, CA.) plasmid containing 500 bp of Cγ gene sequence. It hybridizes to three closely related C genes, Cγ1, Cγ2 and Cγ3[13]. This plasmid was constructed by Drs. D. McElligott and S. Hedrick, U.C. San Diego. 2. pUCVγ1.1, a plasmid containing a Hind III fragment derived from genomic DNA that hybridizes with three closely related V gene segments, Vγ1.1, Vγ1.2 and Vγ1.3. 3. pSP11γV, a plasmid that hybridizes with the Vγ4 gene segment[14]. 4. pSP17γV, a plasmid that hybridizes with the Vγ2 gene segment[14]. pSP11γV and pSP17γV were a gift from Dr. D. Raulet, Massachusetts Institute of Technology. 5. pTZCγ4, a 500 bp Hind III/Eco RI fragment subcloned into the plasmid vector pTZ19R (U.S. Biochemicals, Cleveland, Ohio). The insert is derived from a previously described cDNA clone[16], it contains some of the coding and 3' nontranslated sequences of the Cγ4 gene and it hybridizes to this gene only. The nomenclature employed for the various γ genes is derived from reference 14. DNA probes were isolated from the vector and labeled to 10^9 cpm/μg by random-priming method[21] using 3000 Ci/mM deoxyadenosine and deoxycytidine α-^{32}P triphosphate from Amersham. Hybridization, washing, and removal of hybridized probes from the filters were performed as previously described[18].

RESULTS AND DISCUSSION

Concentration of bands before electrophoresis in a second dimension

Figure 2 demonstrates the effectiveness of concentrating the bands of DNA before running the second dimension. Concentrating the bands was necessary so that closely migrating bands in the second dimension would not overlap. Figure 2A shows a 1 kb ladder of DNA fragments run in one dimension with a 10 mm band width. In the left of Fig. 2B, the 1 kb ladder was electrophoresed in the second dimension without the band concentration step. On the right of Fig. 2B, there is a 1 kb ladder run in one dimension, concentrated as described in Materials and Methods, and then run at right angles in a second dimension. An alternative method of concentrating lanes involves running a thin

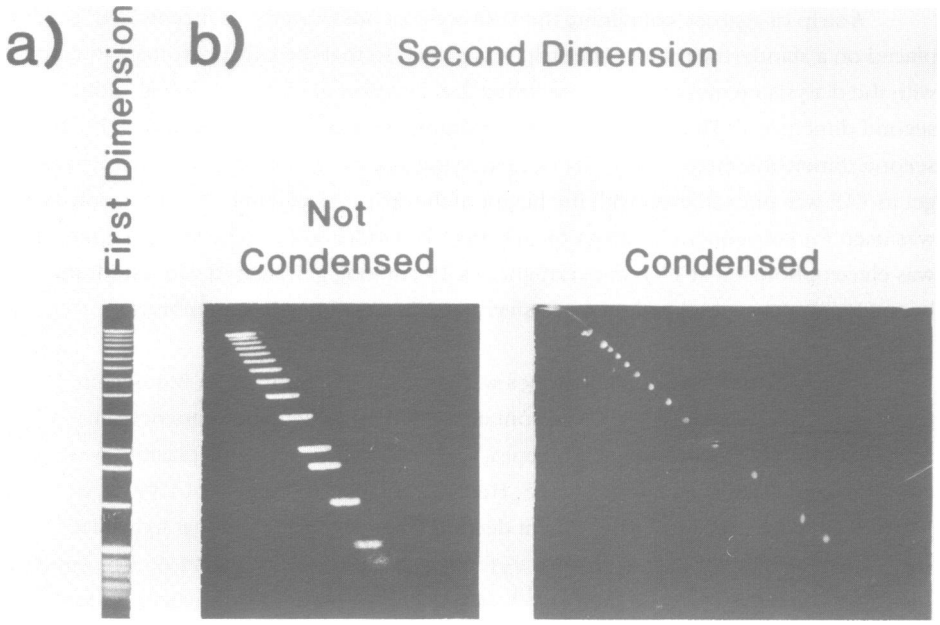

Figure 2. Effect of band concentration on signal and resolution of two dimensional gels.
a) Agarose gel electrophoresis of the 1 kb ladder (BRL) molecular weight standard.
b) Separation of the 1 kb ladder standard in the second dimension with and without concentration.
Gel electrophoresis in both dimensions was carried out using conventional gel electrophoresis.

gel in the first dimension and turning it on edge for the second dimension[5]. However, a thin gel would have to be overloaded with genomic DNA to give the needed amount of signal following reasonable exposure lengths. We have attempted running digested genomic DNA in the second dimension without concentration. The signal we obtained following separation in the second dimension and hybridization was diffuse and barely detectable, compared to the signal obtained following band concentration (data not shown). A technique has been described recently for concentrating lanes by electrophoresis into DEAE cellulose[22]. Restriction digests were performed while the DNA was bound to the DEAE cellulose, to avoid restriction enzyme inhibitors present in agarose. However, we have found that use of the appropriate highly-purified agarose permits in-gel restriction enzyme digestion. Also our method avoids the step of elution from the DEAE cellulose and yields more concentrated bands. We estimate that by using our technique the recovery in the second dimension is nearly complete as judged by

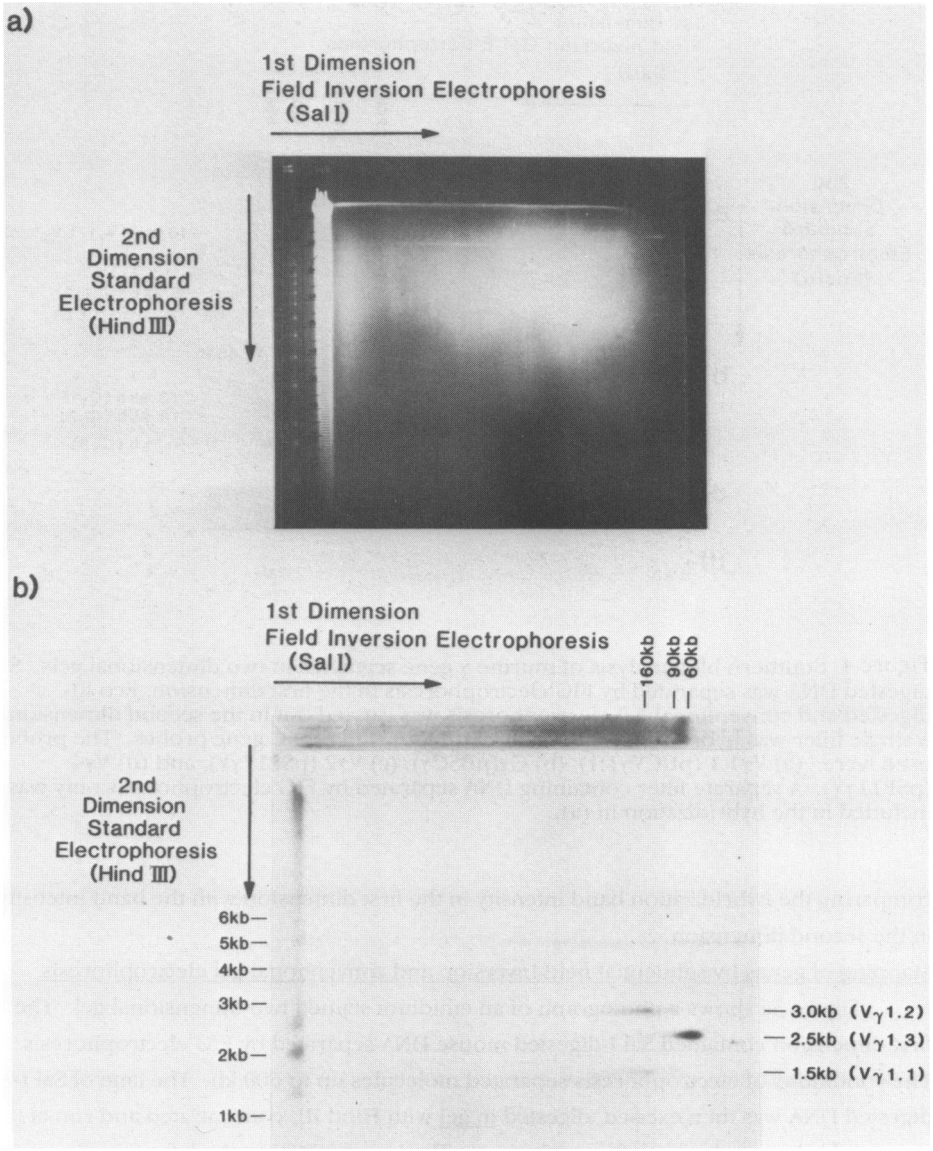

Figure 3 Southern blot hybridization using DNA separated on two-dimensional gels.
a) Ethidium bromide stained two dimensional gel. Sal I-digested DNA was separated in the first dimension by FIG electrophoresis. Hind III digestion was carried out prior to separation in the second dimension.
b) The gel in a) was transferred to a zeta-probe membrane and hybridized with the pUCBγ1.1 DNA probe.

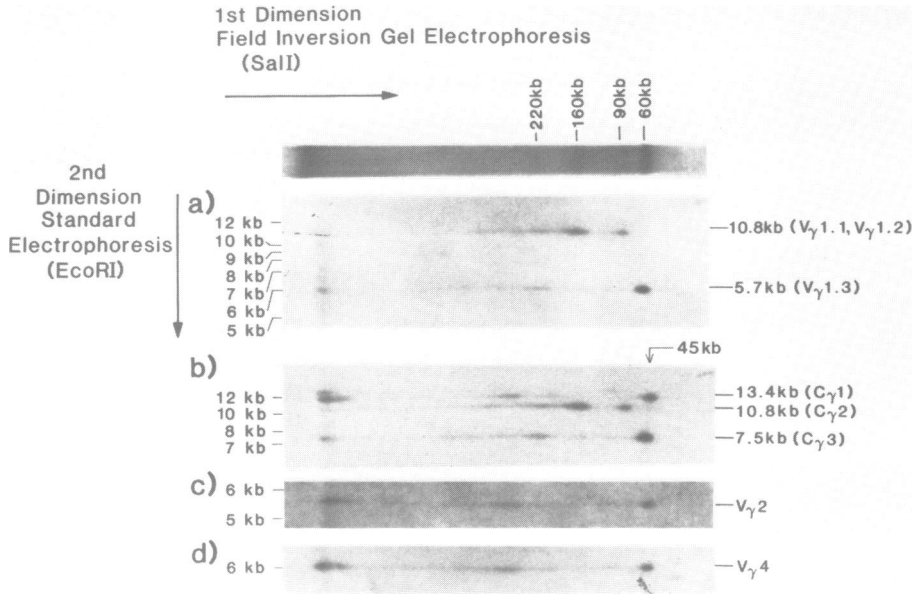

Figure 4 Southern blot analysis of murine γ gene segments in two dimensional gels. Sal I-digested DNA was separated by FIG electrophoresis in the first dimension, Eco RI-digested and conventional gel electrophoresis was carried out in the second dimension. A single filter was hybridized sequentially with four different C gene probes. The probes used were: (a) Vγ1.1 (pUCVγ1.1) (b) Cγ(pBSCγ) (c) Vγ2 (pSP17γV), and (d) Vγ4 (pSP11γV). A separate filter containing DNA separated by FIG electrophoresis only was included in the hybridization in (a).

comparing the hybridization band intensity in the first dimension with the band intensity in the second dimension.

Mapping of genes by sequential field-inversion and conventional gel electrophoresis.

Figure 3a shows a photograph of an ethidium stained two-dimensional gel. The first dimension contained Sal I-digested mouse DNA separated by FIG electrophoresis. The conditions of electrophoresis separated molecules up to 600 kb. The lane of Sal I-digested DNA was then excised, digested in gel with Hind III, concentrated and run at right angles by standard gel electrophoresis. The lane of DNA on the left of the figure is mouse DNA digested by Hind III and electrophoresed only in the second dimension.

Figure 3b shows the Southern blot of the gel of Fig. 3a probed with plasmid pUCVγ1.1, which hybridizes to the mouse Vγ1.1, 1.2, and 1.3 gene segments. The 3.0, 2.5 and 1.5 kb Hind III fragments seen on the Hind III second-dimension control are derived from Vγ1.2, 1.3 and 1.1 gene segments, respectively[13]. The lane on top of the two-dimensional blot is the result of probing the Sal I (first dimension) field inversion

lane with the same probe, which yielded bands at 160, 90 and 60 kb. The two-dimensional blot allows one to determine which of the three cross-hybridizing Vγ genes are located on each of the three Sal I fragments and therefore allows linkage relationships to be determined by a single hybridization. As seen in Fig. 3b the 60 kb Sal I fragment contains the 2.5 kb Hind III fragment (Vγ1.3), while the 90 kb and 160 kb Sal I fragments each contain both the Vγ1.2 and the Vγ1.1 Hind III fragments. From previous work, it was known that Vγ1.1 and 1.2 are separated by only 3.5 kb of flanking DNA[13,15]. The presence of both Vγ1.1 and Vγ1.2 gene segments on the same Sal I fragments demonstrated the validity of the two dimensional gel approach in mapping multigene families. Genomic digestion with Sal I is known to give partial digest bands due to methylation of the CG sequence in the recognition site. Therefore we presume that the 160 kb Sal I band is a partial digestion product.

A second two dimensional gel was used to further study the genomic organization of the mouse γ gene family (Figure 4). In this case, Sal I-digested DNA was separated by FIG electrophoresis, digested with Eco RI in gel, and then separated in the second dimension by conventional gel electrophoresis. The pUCVγ1.1 probe hybridizes to four fragments on the first dimension field inversion gel and two bands on the two dimensional gel (Figure 4a). The Vγ1.3 gene segment is located on both the 60 and 220 kb Sal I fragments, and the Vγ1.1 and 1.2 gene segments are located on the 90, 160 and 220 kb Sal I fragments in the field inversion gel. These results suggest that the 220 kb Sal I fragment is a partial digestion product that encompasses all three members of the Vγ1 subfamily. A weak signal was also detected at 150 kb containing the Vγ1.1, Vγ1.2 and Vγ1.3 gene segments by using a charge-coupled device camera to enhance the contour of the film (E.L., unpublished data). Further analysis with Vγ2, Vγ4 and Cγ probes (Figure 4b-d) indicated that the Vγ2 and Vγ4 gene segments and the Cγ1 gene are linked with 45 kb and that the Vγ1.1, 1.2, 1.3 gene segments and the Cγ2 and 3 genes are linked within 220 kb of DNA. To determine whether any of the known γ genes are located on the 70 kb portion of the 160 kb partial Sal I restriction fragment, we hybridized the filter shown in Fig. 4 with pTZCγ4, a probe that detects the Cγ4 gene located on one end of the γ locus. Sal I fragments of 90 and 160 kb, but not 70 kb, hybridized with this probe (data not shown), indicating that the Jγ2, Vγ1.2, Vγ1.1, and Jγ4 gene segments and the Cγ2 and Cγ4 genes are all located on a single 90 kb Sal I restriction fragment.

Genomic organization of the murine γ gene family.

The physical map of the murine T-cell receptor γ gene family is shown in Figure 5. This map summarizes our data and other published results[13-17]. Previous analyses of bacteriophage λ genomic clones had mapped the γ gene family to six separate clusters: 1. A cluster containing the Vγ5 and Vγ2 gene segments. 2. A cluster containing the Vγ4 and Vγ3 gene segments. 3. A cluster with the Jγ1 gene segment and the Cγ1 gene. 4. A

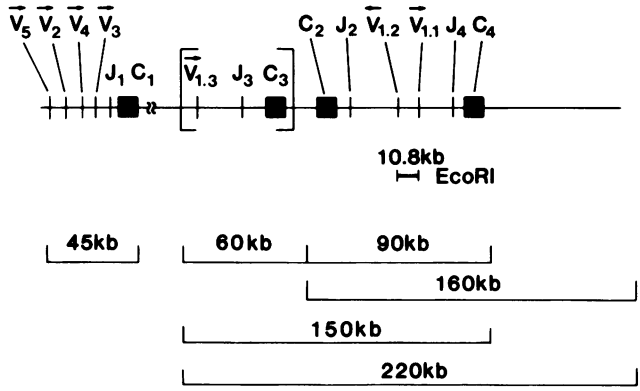

Figure 5. Physical map of the murine γ gene family. This figure summarizes our data and those previously published[13-17]. V and J gene segments are symbolized by vertical lines and C genes by filled-in squares. For simplicity, the separate C gene exons are not shown. The size of various exons and the relative distances between them are not drawn to scale. The transcriptional orientation of the Vγ gene segments are shown as horizontal arrows above each Vγ gene segment. For exons included in brackets, the orientation has not been determined relative to the rest of the γ genes. The 150, 160 and 220 kb SalI fragments are partial digestion products.

cluster with the Vγ1.3 and Jγ3 gene segments, and the Cγ3 gene. 5. A cluster containing the Cγ2 gene and the Jγ2 gene segment. 6. A cluster containing the Vγ1.2, Vγ1.1, and Jγ4 gene segments and the Cγ4 gene. Although Vγ gene segments usually rearrange to the nearest Jγ gene segment, one chromosome of the T lymphoma BW5147 contains a Vγ5 to Jγ4 rearrangement that deletes all the intervening γ locus DNA[17]. This result and the deletion of more Cγ1-proximal V gene segments caused by the rearrangement of the Vγ4 and Vγ2 gene segments to Jγ1[14] places the clusters in the orientation shown in figure 5. The work reported here links clusters 1-3 to a 45 kb Sal I fragment and clusters 4-6 to 150 kb of DNA. The distance between these two remaining unlinked fragments containing C genes has not yet been determined. Rearrangement of V and J gene segments in the γ gene family is not random; particular V and J gene segments tend to join to one another[14,16,23,24]. The predominant rearrangements include joining of either the Vγ5, Vγ2, Vγ4 and Vγ3 gene segments to the Jγ1 gene segment, and rearrangement of the Vγ1.2 gene segment to Jγ2. Reported Vγ1.1 rearrangements join this gene segment to Jγ4[16,24]. The data presented here demonstrate that the predominate rearrangements involve gene segments that are separated by no more than 60-70 kb, and suggest that the frequency of a particular Vγ-Jγ gene segment rearrangement is determined in part by the chromosomal distance separating the germline V and J gene segments. Although inversions joining V and J gene segments that

are in the opposite transcriptional orientation are known to occur in the TCR β locus[25], they must occur rarely or perhaps not at all in the γ locus. In mice, several rearrangements are found in fetal thymocytes including joining of the Vγ3-Jγ1 and the Vγ4-Jγ1[14] gene segments. Vγ1.2-Jγ2 rearrangements are the predominant type seen in mature T cells[23,26] and adult thymocytes[24], although some Vγ2-Jγ1 rearrangements are found. The pattern of rearrangements seen in fetal and adult animals has led to the suggestion that secondary Vγ2-Jγ1 joining could replace earlier Vγ3- or Vγ4-Jγ1 rearrangements[14]. There are no other correlations between rearrangement and chromosomal position, and the reason for the predominance of Vγ1.2-J2 rearrangements in adult T cells remains unclear.

The organization of the murine T-cell receptor γ chain is different from the α and β chains in that there are V gene segments interspersed between several C genes. The organization of the γ chain locus is thus similar to the Igλ light chain gene locus[27] and the shark IgH chain locus[28]. Recently, the organization of the human T-cell receptor γ locus has been determined[29] and it is structurally similar to the α and β gene families, in that all the known variable gene segments are located 5' of the J gene segments and C regions. Thus, the γ gene family exhibits very different gene organization if the mouse and human loci are compared, although in both cases there are a limited number of germline V gene segments. This is in contrast to the T-cell receptor β gene family where the structure and organization are conserved in human and mouse[30].

Utility of two-dimensional, sequential-digest gels.

The techniques described in this report permit molecular linkage of members of gene families for which no single copy probe is available. There are several advantages to this method. First, this approach avoids the need to synthesize specific oligonucleotide probes, which requires knowledge of the DNA sequences of the genes to be mapped. Furthermore, oligonucleotides are technically difficult to use with field inversion gel blots of genomic DNA. Second, a single membrane can be hybridized simultaneously with multiple probes, if the sizes of the hybridizing restriction fragments in the second dimension have been previously determined. Third, electrophoresis in the second dimension distributes the background noise over a larger area than one-dimensional gels, and therefore allows better resolution of faint bands. This is important in mapping studies, because it is often the faint partial digestion products which give useful linkage information (see Fig. 4).

The technique of band concentration by electrophoresis into a dialysis membrane is useful because it leads to increased resolution and improved hybridization signal with the two-dimensional gel technique we have employed. It might also be useful for other two-dimensional techniques, such as in two-dimensional preparative protein gels, in

which lane overloading in the first dimension limits the amount of material that can be used.

Two-dimensional, sequential-digest gels have previously allowed resolution of bacterial genomes into discrete bands[31]. By using PFG or FIG electrophoresis in both dimensions, along with infrequently cutting restriction enzymes, it might be possible to resolve larger genomes into discrete bands or dots. We have resolved hundreds of dots on some of our two-dimensional gels (E. Lai, unpublished results). If 1000 dots could be resolved on a 20 cm by 20 cm two-dimensional gel and a sequential digest with two infrequent cutting restriction enzymes yielded fragments of about 100 kb, a genome of 10^8 bp could be resolved onto mostly nonoverlapping dots.

Acknowledgements

We thank Drs. Hilde Cheroutre and Ulf Landegren for careful review of the manuscript, Dr. R. Brines for condtruction of pTZCγ4, Mary Stepp and Bertha Jones for help with preparation of the manuscript, and Frank Ostrander for the construction of the gel trap for concentration of DNA in agarose gel lanes. This work was supported by NIH Grants Ca 459456-01 (M.K.) and AI 17565 (L.E.H.).

*To whom correspondence should be addressed

REFERENCES

1. Steinmetz, M., Stephan, D. and Lindahl, K.F. (1986) Cell **44**, 895-904.
2. Schwartz, D.C. and Cantor, C.R. (1984) Cell **37**, 67-75.
3. Carle, G.F., Frank, M., and Olson, M.V. (1986) Science **232**, 65-68.
4. Smith, C.L., Warburton, P.W., Goal, A., and Cantor, C.R. (1986) Genet. Eng. **8**, 45-70.
5. Rosenwold, E.C. and Honigman, A. (1977) Gene **2**, 273-288.
6. Van Ommen, G.J.B. and Verkerk, J.M.H. (1986) In Davies, K. (ed.) Analysis of Human Genetic Diseases, pp. 113-133.
7. Brenner, M.B., McLean, J., Dialynas, D., Strominger, J.L., Smith, J.A., Owen, F.L., Seidman, J.G., Ip, S., Rosen, F. and Krangel, M.S. (1986) Nature **322**, 145-149.
8. Bank, I., DePinho, R.A., Brenner, M.B., Cassimeris, J., Alt, F.W. and Chess, L. (1986) Nature **322**, 179-181.
9. Weiss, A., Newton, M. and Crommie, D. (1986) P.N.A.S. USA **83**, 6998-7002.
10. Koning, F., Stingl, G., Yokoyama, W.M., Yamada, H., Maloy, W.L., Tschachler, E., Shevach, E.M. and Coligan, J.E. (1987) Science **236**, 834-837.
11. Pardoll, D.M., Fowlkes,B.J., Bluestone, J.A., Kruisbeek, A., Maloy, W.L., Coligan, J.E. and Schwartz, R.H. (1987) Nature **326**, 79-81.
12. Maeda, K., Nakanishi, N., Rogers, B.L., Haser, W.G., Shitara, K., Yoshida, J., Takagaki, Y., Augustin, A.A. and Tonegawa, S. (1987) P.N.A.S. USA **84**, 6536-6540.
13. Hayday, A.C., Saito, H., Gillies, S.D., Kranz, D.M., Tanigawa, G., Eisen, H.N. and Tonegawa, S. (1985) Cell **40**, 259-269.
14. Garman, R.D., Doherty, P.J. and Raulet, D.H. (1986) Cell **45**, 733-742.
15. Traunecker, A., Oliveri, F., Allen, N and Karjalainen, K. (1986) EMBO J. 5, 1589-1593.
16. Iwamoto, A., Rupp, F., Ohashi, P.S., Walker, C.L., Pircher, H., Joho, R., Hengartner, H. and Mak, T.W. (1986) J. Exp. Med. **163**, 1203-1212.
17. Pelkonen, J., Traunecker, A. and Karjalainen, K. (1987) EMBO J. **6**, 1941-1944.

18. Popko, B., Puckett, C., Lai, E., Shine, D.J., Readhead, C., Takahashi, N., Hunt, S.W. III, Sidman, R.L. and Hood, L.E. (1987) Cell **48**, 713-721.
19. Reed, K.C. and Mann, D.A. (1985) Nucl. Acids Res. **13**, 7207-7221.
20. Boehm, T.L.J. and Drahovsky, D. (1984) J. Biochem. Biophys. Methods **9**, 153-161.
21. Feinberg, A.P. and Vogelstein, B. (1983) Anal. Biochem. **132**, 6-13.
22. Poddar, S.K. and Maniloff, J. (1986) Gene **49**, 93-102.
23. Kranz, D.M., Saito, H., Heller, M., Takagaki, Y., Haas, W., Eisen, H.N. and Tonegawa, S. (1985) Nature **313**, 752-755.
24. Kronenberg, M., Miller, J., Siu, G., and Hedrick S. Manuscript in preparation.
25. Malissen, M., McCoy, C., Blanc, D., Trucy, J., Devaux, C., Schmitt-Verhulst, A.-M., Fitch, F., Hood, L., and Malissen, B. (1986) Nature **319**, 28-33.
26. Jones, B., Mjolsness, S., Janeway, C., Jr., and Hayday, A. (1985) Nature **232**, 635-638.
27. Eisen, H.N. and Reilly, E.B. (1985) Ann. Rev. Immunol. **3**, 337-366.
28. Hinds, K.R. and Litman, G.W. (1986) Nature **320**, 546-549.
29. Strauss, W.M., Quertermous, T. and Seidman, J.G. (1986) Science **237**, 1217-1219.
30. Lai, E., Concannon, P., and Hood, L. (1988) Nature, in press.
31. Chen, C.W. and Thomas, C.A., Jr. (1983) Anal. Biochem. **131**, 397-403.