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ARRANGEMENT AND REARRANGEMENT OF IMMUNOGLOBULIN GENES

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ABSTRACT. Arrangement of Immunoglobulin genes in mouse DNAs of various cellular sources was analyzed by use of Bacterial restriction enzymes. A high-molecular weight DNA was digested to completion and resulting DNA fragments were fractionated according to size in preparative agarose gel electrophoresis. DNA fragments carrying gene sequences coding for the variable or constant region of light chains were detected by hybridization with purified ^{125}I-labeled, whole light chain mRNAs and with their 3'end halves. The corresponding patterns of hybridization were completely different in the genomes of embryo cells and of plasmacytomas. The results, together with those of control experiments, lead us to conclude that the V and C genes, which are some distance away from each other in the embryo cells, are joined to form a contiguous polynucleotide stretch during differentiation of lymphocytes. There seems to be a strict correlation between such V-C joining and expression of the joined V gene. Relevance of these findings with respect to activation of a specific V gene and allelic exclusion in immunoglobulin gene loci is discussed.

In addition, isolation of a phage λ which carries an embryonic mouse DNA fragment as an insert in its genome is described. The DNA fragment is 3 kilobase long and contains a V_λ gene.

INTRODUCTION

Both light and heavy chains of immunoglobulin molecules consist of two regions: the variable region (V region) and the constant region (C region) (6,19). This led Dreyer and Bennett to put forward the hypothesis that two separate DNA segments, one each for V and C regions, may be involved in the synthesis of a single immunoglobulin chain (2). This attractive concept remained speculative until recently, partly because of its unorthodox nature, but mostly because of lack of direct experimental evidence.

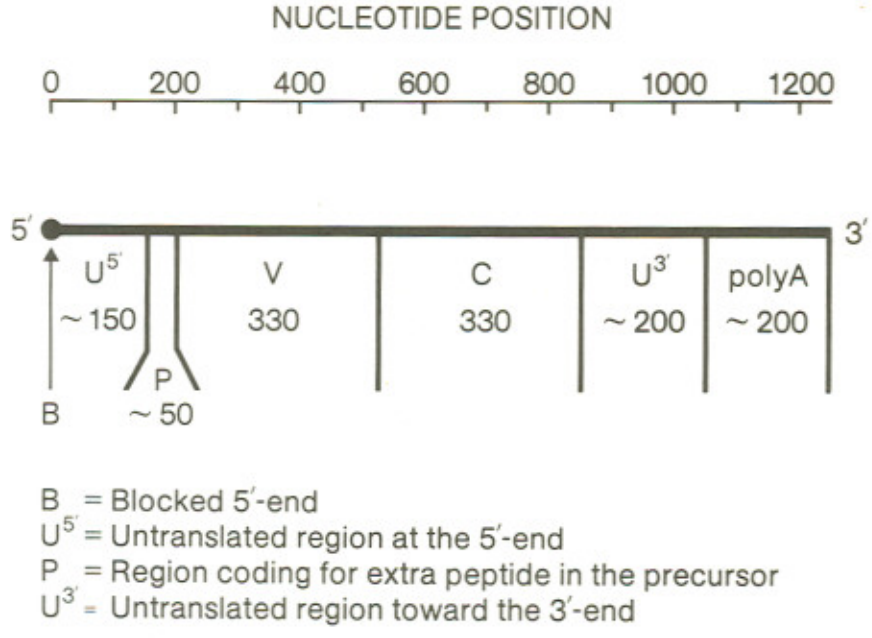

Figure 1. Schematic illustration of light chain mRNA.

Use of bacterial restriction enzymes allowed us to provide direct experimental evidence for such a concept. How, then, is the genetic information in two separate DNA segments integrated to generate a single polypeptide chain? We recently reported evidence for the hypothesis that such integration takes place directly at the DNA level (6).

In this article we shall review our experimental results pertinent to these two concepts and discuss some implications drawn from them.

EXPERIMENTAL APPROACH

Bacterial restriction endonucleases recognize and cleave specific sequences of base pairs within DNA duplexes. High molecular weight DNA purified from twelve to fourteen day old Balb/c mouse embryos, or from various myeloma tumors, was digested to completion with restriction enzyme Bam H-I or Eco RI. The resulting DNA fragments were fractionated according to size by electrophoresis in 0.9% agarose gels. DNA eluted from gel slices was assayed for V and C gene sequences by hybridization with ^{125}I-labelled whole κ

or λ light chain mRNA, or half RNA fragments containing the ' terminal. The assay is based on the fact that the sequences corresponding to V and C genes are on the 5' end and 3' end halves of the mRNA, respectively (Figure 1). Thus, the 3' end half is an RNA probe for C gene sequences, and the V gene sequences are determined indirectly from the difference in the two hybridization levels obtained with the whole RNA molecule and the 3' end half. The details and basis of this assay are described elsewhere (6).

JOINING OF V - C GENES AT THE DNA LEVEL

We recently reported evidence for V-C joining at the DNA level (6). The evidence was based on analysis of embryonic and myeloma DNA by procedures similar to those described in the previous section. Here we present results of such an analysis, together with some additional controls. In Figures 2A and 2B, DNAs from twelve day old Balb/c embryos and MOPC 321 myeloma were separately digested with a restriction enzyme, Bam H-I. Both MOPC 321 κ mRNA and MOPC 104E λ mRNA were used as the sequence probes. Of the two major embryonic DNA components which hybridized with the κ mRNA, only the larger (6.0 million M.W.) hybridized with κ 3' end half (data not shown here, see reference 6). The patterns of hybridization of the κ mRNA with the embryonic DNA and the myeloma DNA are dramatically different. Both the whole κ mRNA and its 3' end half (latter data not shown) hybridized with a single major component of 2.4 million M.W. in the myeloma DNA. In contrast, hybridization patterns of the λ mRNA in the two DNAs are virtually indistinguishable.

As we argued elsewhere, these results are best interpreted as follows. The V_κ and the C_κ genes which are some distance away in the embryo genome are brought together, during differentiation of lymphocytes, in order to form a continuous DNA stretch. An alternative explanation of the results, namely, that accumulation of mutations leading to either loss or gain of Bam H-I sites generated the observed pattern difference, is not impossible. On this view, there would have to be a Bam H-I site close to the V-C junction in embryo DNA. This Bam H-I site would have to be lost by mutation in the MOPC 321 tumor. By itself, such a mutation would cause the appearance of a single 9.9 million component in the tumor. To achieve the M.W. of the single component actually observed in the tumor (2.4 million), there would have to be new Bam H-I sites created by mutation between the V_κ gene and the nearest site on either side. Since there is

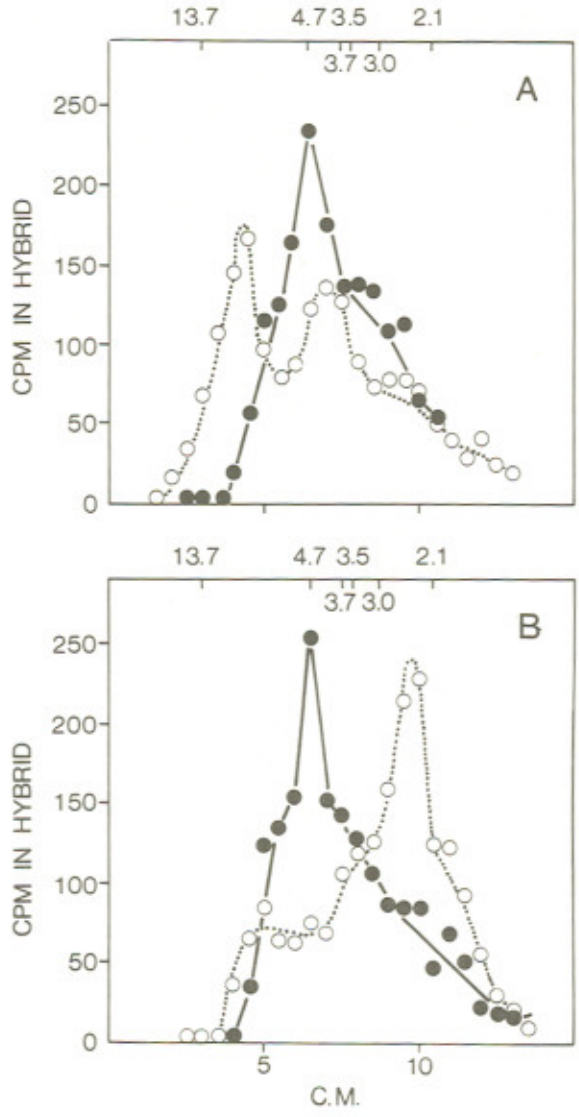

Figure 2. Gel electrophoresis patterns of Bam H-I digested embryo DNA (A) and MOPC 321 DNA (B). Bam H-I digestion, DNA fractionation and hybridization were carried out as described (6). Whole MOPC 321 κ mRNA (o----o, 1250 cpm, 5 x 10^7 cpm/μg) or MOPC 104E λ mRNA (●——●, 1250 cpm, 7 x 10^7 cpm/μg was annealed with DNA. In this and Figures 3, 4 and 5 numbers on top of the panel indicate the molecular weights (in millions) of DNA fragments used as migration marks.

no reason why there should be any selective pressures involving Bam H-I sites, the occurrence of three mutations would seem to be quite unlikely.

In addition, constancy of λ mRNA hybridization patterns suggest that there is no massive scrambling of DNA sequences throughout the chromosomes during generation and propagation of this myeloma. This view is also supported by the experiments involving the converse combination, namely, the analysis of DNA from λ chain-producing myeloma with a κ mRNA (Figure 3A). The hybridization pattern was indistinguishable from that of embryonic DNA. The case can be extended to DNAs from normal adult tissues as representatively shown in Figure 3B with kidney cells. Thus there seems a good correlation between the V-C joining event and expression of the joined immunoglobulin gene.

We have also digested embryo and MOPC 321 tumor DNAs with another restriction enzyme, Hind III (data not shown). With embryo DNA, two major components of 6.0 and 2.8 millions hybridized with the whole MOPC 321 κ mRNA, of which only the latter hybridized with the 3' end half. Thus, V and C gene sequences are in 6.0 and 2.8 million components, respectively. With the MOPC 321 DNA, there was only a single major component of about 2.8 millions observed, with which both RNA probes hybridized. No 6.0 million component existed in the tumor DNA pattern. Thus, it seems that unfortunately in this particular case, the embryonic, C gene fragment was not resolved in the electrophoresis from the myeloma, V-C fragment. The results, nevertheless, provide another case where hybridization patterns are clearly different between embryo and myeloma DNAs. Since Bam H-I and Hind III recognize different base sequences, the alternative interpretation mentioned above becomes even more unlikely.

SUBGROUPS AND V-C JOINING

Our earlier hybridization studies, as well as those of others, indicated that a group of closely related V regions are somatically generated from a few, probably single, germ line genes (7,14,18-21). While there still remains some ambiguity, such a V region group is best approximated to the subgroup as defined by Cohn and his co-workers (1). A direct demonstration of separate germ line V genes for two V regions of different subgroups are presented in Figure 4A. Here embryonic DNA digested with Bam H-I enzyme was analyzed with two mRNAs coding for two κ chains of different subgroups, MOPC 321 and MOPC 21. These two κ chains show little homology in their V regions whereas they have identical sequences in

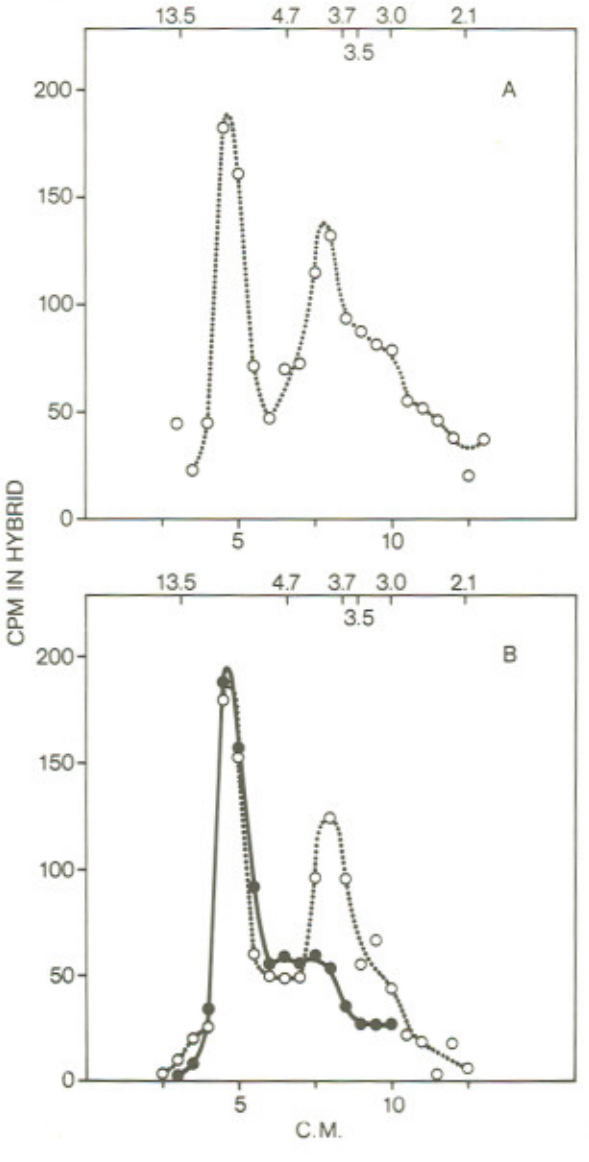

Figure 3. Gel electrophoresis patterns of λ J558 DNA (A) and kidney DNA (B) digested with Bam H-I. Whole MOPC 321 RNA (o----o, 1250 cpm, 7 x 10^7 cpm/μg) or its 3' end half fragment (●——●, 600 cpm, 7 x 10^7 cpm/μg) was hybridized to fractionated DNA.

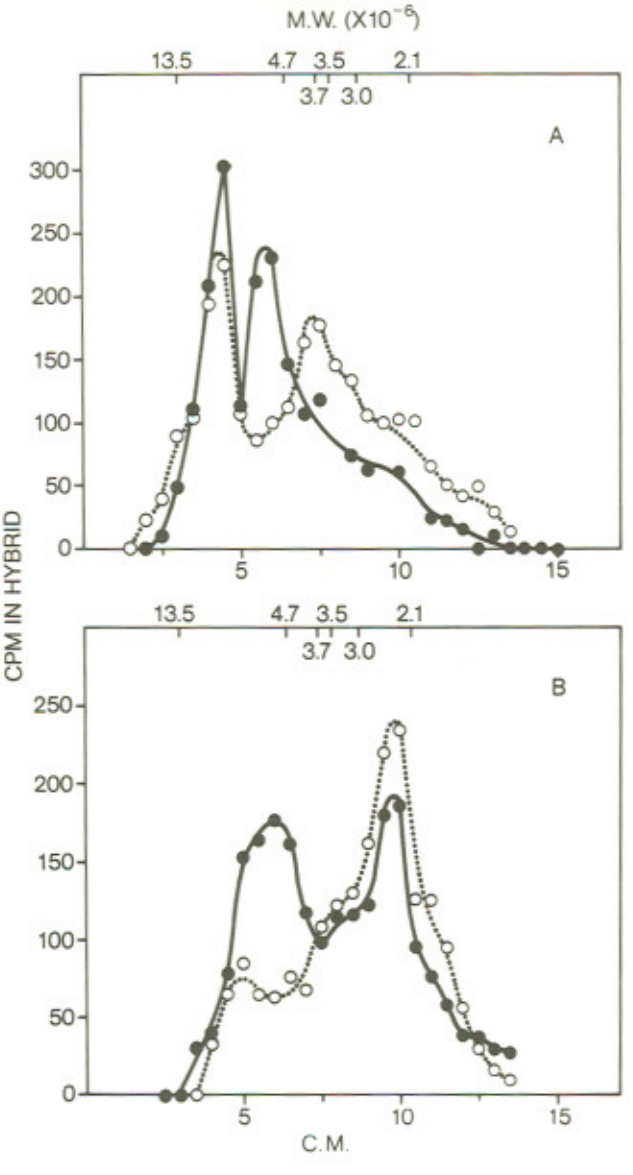

Figure 4. Gel electrophoresis patterns of embryo DNA (A) and MOPC 321 DNA (B) digested with Bam H-I. Whole MOPC 321 mRNA (o----o, 1250 cpm, 7 x 10^7 cpm/μg) or MOPC 21 mRNA (●——●, 1220 cpm, 8 x 10^7 cpm/μg was annealed with extracted DNA.

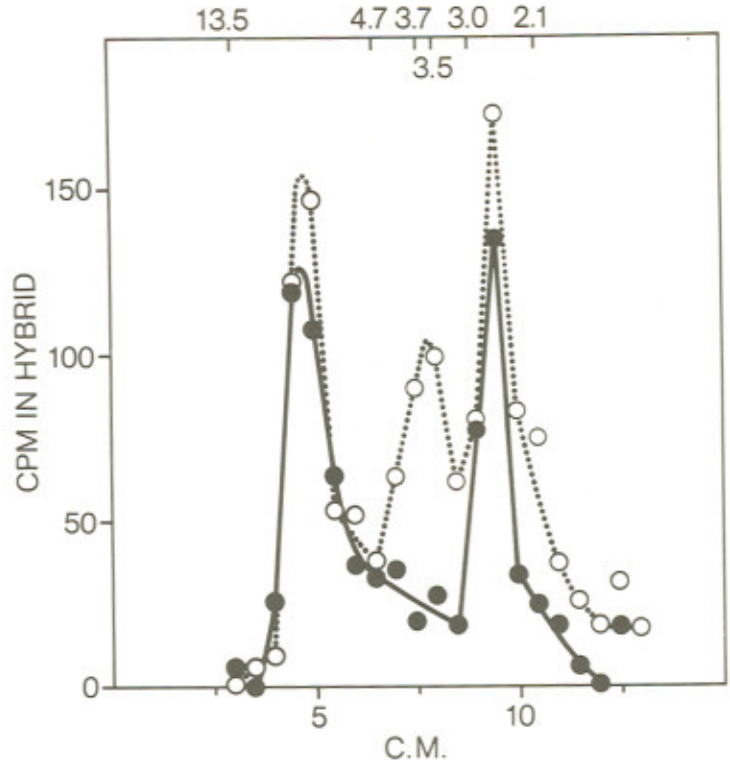

Figure 5. Gel electrophoresis patterns of TEPC 124 DNA digested with Bam H-I. Whole 321 mRNA (o----o) or its 3' end half fragment (●——●) was annealed with extracted DNA as described in the legend to Figure 4.

the C region (8,10). As expected, both RNAs hybridized with the 6.0 million component which carries the C_κ gene (see above). In addition, each of the two RNAs hybridized with a second, but mutually different, DNA component. These DNA components of 5.0 and 3.9 millions M.W. should carry MOPC 21 and MOPC 321 V gene sequences respectively.

Is a V_κ gene for a given subgroup joined with a C_κ gene in the myeloma which synthesizes a κ chain carrying a V_κ region of another subgroup? That this is not the case is shown in Figure 4B, where MOPC 321 DNA was analyzed with the homologous (MOPC 321) and heterologous (MOPC 21) κ mRNAs. As already pointed out, the homologous κ mRNA hybridized

with a major DNA component of 2.4 million M.W. While the 5.0 million M.W. C_κ gene component disappears, the 5.0 million M.W., $V_{\kappa,MOPC\ 21}$ gene fragment remains at the embryonic position. These results provide another example for strict correlation between the V-C joining event and expression of the joined immunoglobulin gene.

What about a V_κ gene in the myeloma synthesizing another κ chain of the same subgroup? Analysis of TEPC 124 DNA with MOPC 321 mRNA is shown in Figure 5. The two κ chains synthesized by these myelomas are different in three amino acids in the V regions, and belong to a single subgroup (8). Three major DNA components hybridized with the whole κ mRNA, two of which hybridized also with the 3' half fragments. The size and hybridization properties of these DNA components suggest that the overall pattern is a composite of the two patterns obtained when embryonic and MOPC 321 DNA were analyzed by the same RNA probes (Figures 2A and 2B). Thus, the principal difference in the two hybridization patterns, one of embryonic DNA (Figure 2A) and the other of TEPC 124 tumor (Figure 5), is the addition of the 2.4 million component in the latter. This component hybridized with both whole and 3' end half RNA probes. Each of the two components which hybridized with the 3' end half ought to contain a complete C gene sequence, for nucleotide sequence studies of a κ mRNA indicate that there is no Bam H-I cleavage site in the C gene (9).

We have previously shown that two nucleotide sequences coding for two V regions of a single subgroup are extensively homologous (18,20,21). Therefore, in interpreting these results, we assume that this is the case for the nucleotide sequences coding for MOPC 321 and TEPC 124 κ chains are extensively homologous. Given this assumption, two likely explanations can be given. First, there are two separate germ line genes for the two κ chains. While $V_{\kappa M\ 321}$ remains at the embryonic position, $V_{\kappa T\ 124}$ is joined with one of the C_κ genes. The alternative explanation is that there is a common germ line V gene from which two V regions are somatically derived. This V gene is joined with the C_κ gene only in one of the multiple homologous chromosomes. While it is fortuitous in the first instance that the size of the DNA fragment carrying the joined V-C gene is identical in MOPC 321 and TEPC 124 myelomas, it is a logical consequence in the second.

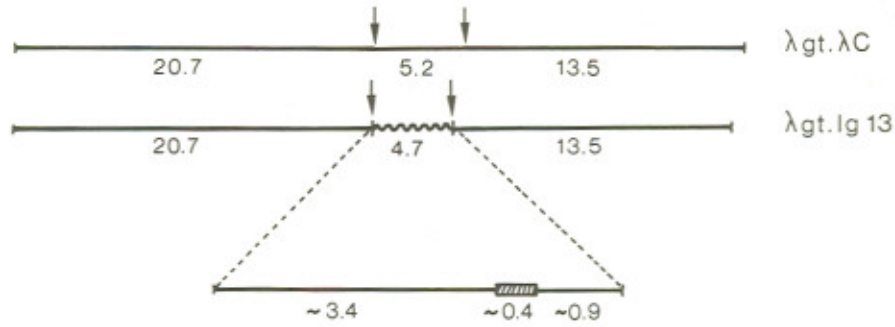

Figure 6. Schematic illustration of λgt-λC WES and λgt-Ig 13 DNAs. Arrows indicate the positions of Eco RI cleavage sites. Length of DNA is given in kilobase. The shaded box at the bottom indicates the DNA segment homologous to V_λ gene sequences.

NEW APPROACHES TO THE STUDY OF THE ARRANGEMENT OF IMMUNOGLOBULIN GENES

We are applying the in vitro DNA recombination technique (11,16) to the immunoglobulin genes. Two kinds of approaches are being made in our laboratory. In one, we are isolating plasmids carrying DNA inserts of κ or λ chain genes, which were enzymatically synthesized from purified mRNA. We intend to use these hybrid plasmids as hybridization probes in the analysis of DNAs from natural lymphocyte clones.

In the other approach, we are isolating DNA fragments carrying immunoglobulin genes directly from chromosomal DNA. One such DNA fragment carrying a V_λ gene was successfully isolated from embryonic DNA as an insert in the genome of a phage λ (λgt-Ig13). Electronmicroscopy and hybridization studies demonstrated that the fragment carries no $C_{\lambda I}$ gene sequence and that the V_λ gene sequence is internally located within the DNA fragment (Figure 6). We are currently determining the nucleotide sequence of this V gene.

The above work was carried out in a P-3 physical containment facility in combination with EK-2 biological containments in accordance with the N.I.H. guideline on recombinant DNA work issued in June 1976. As the EK-2 biological containments, we used phage λgt.λC Wam 403, Eam 1100, Sam 100 (3) or E.coli X1776.

DISCUSSION

We consider that our interpretation of the difference in the hybridization patterns being due to V-C joining is well justified, given the various control experiments described here. Details of V-C joining, however, are still to be studied. We intend to do so by the use of cloned immunoglobulin genes.

A committed B-lymphocyte or plasma cell produces antibody of only one specificity (7,13,15). In particular, it expresses only one light chain V gene. Since there are not only V_λ and V_κ genes but also multiple V_κ genes, there must exist a mechanism for the activation of a V gene which is directly coupled to its becoming joined to a C gene. For instance, in the "excision-insertion" model or in the "inversion" model, (see reference 6), a promotor site might be created by the insertion of the V gene fragment. This would activate that particular V gene for transcription.

Alternatively, all V genes and even the C genes might be activated for transcription early in lymphocyte differentiation. The RNAs synthesized would be rapidly degraded in the nucleus by a 5'-specific nucleotidase. The sequence created by the joining event at the insertion site could serve, directly or indirectly, as a signal for preventing the exonuclease from proceeding further down toward the 3'-end. This model looks rather wasteful at first sight, but it should not be discarded solely for this reason. It is, in fact, in agreement with the fact that in eucaryotic nuclei a large proportion of DNA is constitutively transcribed into large RNA molecules (HnRNA), most of which turn over rapidly before reaching the ribosomes. Only a small proportion (a few per cent) of these large RNA molecules are "processed" to become mRNA.

In any case, in view of the above considerations, the current terminology of "V genes" and "C genes" is somewhat inappropriate. Rather there are two types of segments of DNA, one specifying the V region and the other specifying the C region. The "gene" is created by joining.

For immunoglobulin loci, only one allele is expressed in any given lymphocyte (13). This is not the case for any other autosomal genes studied until now. Our results of MOPC 321 DNA (Figures 2A and 2B) suggest an interesting explanation for this phenomenon of allelic exclusion - that the two homologous chromosomes are, in any given plasma cell, homozygous. Homozygocity could result from the loss of one homologue followed by reduplication of the other, or

it could result from somatic recombination (presumably mediated by a specialized recombinase) between the centromere and the immunoglobulin locus (12). This explanation of allelic exclusion is independent of whether the joining takes place only in one of the two homologous chromosomes or both, in a single lymphocyte. On the other hand, our results on TEPC 124 DNA suggest that the joining takes place only in one of the two homologous chromosomes and no homozygosis is to take place. While this, too, can conveniently explain allelic exclusion, there is an apparent discrepancy between the two cases. The discrepancy could arise from the known abnormality in caryotypes of myeloma cells (23). MOPC 321 myeloma might have lost the homologous chromosomes on which V and C genes lie separate. Conversely, TEPC 124 myeloma might have acquired an additional chromosome of non-lymphoid origin during generation or propagation of the tumor (22).

Resolution of the discrepancy and elucidation of the mechanism of allelic exclusion would require detailed analysis of DNA from Ig synthesizing cells, in particular DNA from natural lymphocyte clones.

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