Somatic Changes in the Content and Context of Immunoglobulin Genes

S. Tonegawa, N. Honzumi, G. Matthesians and R. Schuller

In this paper, we will demonstrate that both the content and the context of immunoglobulin genes change during the differentiation of lymphocytes. By context, we mean the relative location of this information in DNA. The possibility of such changes has been the subject of much speculation in immunology, but only recently has it become possible to attack the problem directly. The principal technologic advance which makes this possible has been the development of procedures for the purification of immunoglobulin mRNA from marine plasma cells (Tonegawa and Baldo 1973; Mach et al. 1973; Browne et al. 1975; Schechter 1973; Honzumi et al. 1974; Skarevarst et al. 1974).

Joining of Kappa-chain Genes during Ontogeny

Let us begin with changes in context. To study this, we need a way to determine the arrangement of immunoglobulin genes in DNA. Bacterial restriction endonucleases recognize and cleave specific sequences of base pairs within DNA duplexes. These enzymes have proven to be extremely useful in analyzing viral and bacterial genomes, but the application of these enzymes to eukaryotic genomes has been limited to cases where the arrangement of cleaved sequences is of interest. The sensitive assay of specific DNA sequences by hybridization with highly radioisotopically labeled, purified mRNA has enabled us to extend the use of these enzymes to the study of the arrangement of unique genes such as those for immunoglobulins.

High-molecular-weight BALB/c mouse embryo DNA was digested to completion with restriction enzyme HaeIII. The resulting DNA fragments were fractionated according to size by electrophoresis in a 6% agarose gel. DNA eluted from gel slices was assayed for V and C gene sequences by hybridization with 3H-labeled whole x mRNA or half molecules containing the 3' terminus. The assay is based on the fact that the sequences corresponding to V and C genes are on the 5'- and 3'-end halves of the mRNA, respectively (Fig. 1). Thus the 5'-end half is an RNA probe for V-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 procedure have been described elsewhere (Honzumi and Tonegawa 1970). In Figure 2, 3H RNA probes prepared from MOPC-522 were used. Two DNA components of 6.0 x 10^9 and 3.9 x 10^9 m.w., hybridized with the whole RNA molecule, whereas only the 6.0 x 10^9 component hybridized with the 3'-end half. Hence, the C gene is in the 6.0 x 10^9 component and the Vγ gene should be in the 3.9 x 10^9 component. The fact that the extent of hybridization in the longer component is nearly identical with the two RNA probes supports this interpretation.

Since V and C genes are in separate DNA fragments of sizes that are much larger than the size of either gene, they are probably some distance away from each other. However, the possibility

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**Figure 1.** Schematic illustration of light-chain mRNA and its specific fragments. Light-chain mRNA is about 1259 nucleotides long and consists of four regions, boxed by the poly(A) sequence at the 5' end. These regions are designated by 5'E outer region, in the 5'-end; V variable region; C (constant region) and 3'E inner region towards the 3'-end. The relative lengths of the four regions are deduced from the size of V and C regions in light chains and from the data of Muto, et al. (1974). Purification, identification, and isolation of 3'E containing fragments have been described previously (Tonegawa et al. 1974c). Tonegawa (1976). Size fractionation of the fragments was carried out by acrylamide gel electrophoresis in 9% formamide.
that the enzyme cleaved V and C genes arranged contiguously near the boundary is not entirely eliminated. Cleavage sites are possible in the mRNAs for amino acids at positions 93-95 and 97-98 (McKean et al. 1973). However, the probability that either of these amino acids occurs in the exact nucleotide sequences required is low.

The pattern of hybridization is completely different in the DNA from the bovine leukemia virus (Fig. 2). Both RNA probes hybridized with a new DNA component of 2.4 x 10^6. There is no indication that either of these RNA probes hybridizes with other DNA components above the general background level. These results indicate that both V and C genes, or the entire sequences represented in the mRNA molecule (except for the unlabelled poly(A) sequence) are contained in the 2.4 x 10^6 DNA component in the tumor genome. The whole DNA hybridizes with this component nearly twice as well as does the 3'-end half, thereby suggesting that the V gene is expressed under both the V and C genes. The straightforward interpretation of these results is that V and C genes, which are at some distance away from each other in the V-C junction in the embryo DNA, are brought together during differentiation to form a common nucleotide stretch in the plasma cells expressing this particu-
lar V gene. The fact that neither the 6.0 x 10^6 nor the 3.9 x 10^6 component exists in the plasma cell genome indicates that such rearrangement of immunoglobulin genes takes place in all of the homologous chromosomes in this plasma cell. Our interpretation of these results is schematically illustrated in Figure 3.

An alternative explanation of the results, namely, that accumulation of mutations or base modifications leading to either loss or gain of RamH1 sites generated the observed pattern difference, is not impossible. In this view, there would have to be a RamH1 site close to the V-C junction in embryo DNA. This RamH1 site would have to be lost by mutation or base modification in the MOPC-321 tumor. By itself, such an alteration would cause the appearance of a single 9.9 x 10^6 DNA component in the tumor. To achieve the molecular weight of the single component actually observed in the tumor 12.4 x 10^6, there would have to be new RamH1 sites created by mutation or base modification between the V and the closest site on either side. Since there is no reason why there should be any selective pressures involving RamH1 sites, the occurrence of three mutations would appear to be quite unlikely. There is also an alternative explanation for
the absence of the embryonic DNA components in the fetus, namely, that the V- to C-gene joining took place in only one of the homologous chromosomes, and that the other chromosome was lost during propagation of the tumor. In view of the known chromosomal abnormalities of murine plasmacytomas (Yoshida et al. 1968; Cohn 1968), we cannot eliminate this trivial possibility.

Arrangements of Lambda-chain Genes

We have also applied the kind of analysis described in the last section to mouse s-chain genes. Figure 4 shows hybridization patterns of embryo DNA which has been digested with another restriction enzyme, E, coli R1 (EcoR1). Three DNA components of 4.7, 2.8, and 1.9 × 10^6 daltons hybridized with whole λ DNA purified from the HOPC-2030 myeloma. Specificity and extent of hybridization obtained with whole and 3′-end-half λ DNA indicate that only the 4.7 × 10^6 component carries a C gene (Fig. 4A). In Figure 4B, the hybridization pattern of the whole λ DNA is compared with that of an RNA fragment which carries the 3′ end and extends roughly three-quarters of the way toward the 5′ end (Fig. 1). The latter RNA probe contains sequences in the V, C, and 3′-external regions but largely lacks those in the 5′-external region. Since the 2.8 × 10^6 component hybridizes with this RNA probe but not with the 3′-end half, it must carry only 5′-region sequences. Using analogous logic, the 1.9 × 10^6 component should carry sequences in the 5′-external region. The relative extent of hybridization with the three RNA probes in each DNA component confirms these interpretations. Since amino acid sequence data indicate that there cannot be any EcoR1 site in the translated portions of the V or C genes (Weigert et al. 1970), these results prove unequivocally that, in the embryo genome, the two genes are some distance away from each other. Splicing of the sequences in the V and 5′-external regions into two DNA fragments could be interpreted to mean that these two regions originate from two separate segments of DNA, but a more likely interpretation is that they originate from a continuous DNA segment, and that there is an EcoR1 site in the 5′-external region near to the V region. Are the V, and C, genes joined in the differentiated plasma cell as in the case with the s-chains? In Figure 5, A and B compare hybridization patterns obtained with embryo and HOPC-2030 DNA, respectively. Although not as striking as the s-chain case (see above), the two patterns do show some differences. In the plasmacytoma DNA, there seems to exist a new component of 4.7 × 10^6 M.W. which migrated in the gel slightly ahead of the 4.7 × 10^6 C-gene component. That these two components are distant is suggested by two observations. First, the relative position of each of these two components to that of the internal marker, a DNA fragment carrying 14S RNA, is different. Second, in the plasmacytoma DNA component, the extent of hybridization obtained with
that the enzyme cleaved V and C genes arranged consecutively on a single RNA molecule. These results indicate that both V and C genes, or the entire sequence represented in the mRNA molecule (except for the polyA tail), are contained in the 2.4 x 10^6 M. component in the tumor genome.

The whole RNA hybrids with this component was 2.4 x 10^6 M. component in the tumor genome. The whole RNA hybrids with this component nearly twice as well as does the V and C genes, which are some distance away from each other in the embryo genome, are brought together during embryonic differentiation and form a continuous nucleolar

Figure 2: Gel electrophoresis pattern of BamHI-generated mouse DNA fragments carrying V-C or C-C gene sequences. Results with two mouse lines and with MOPC-321 plasmacytoma DNAs are summarized. Ten milligrams of high molecular weight am-

Figure 3: Hybridization of DNA probe (V-C or C-C) on the Southern blots of DNA (metaphase chromosomes). Data points shown in this Figure 3: Hybridization of DNA probe (V-C or C-C) on the Southern blots of DNA (metaphase chromosomes). Data points shown in this Figure 3: Hybridization of DNA probe (V-C or C-C) on the Southern blots of DNA (metaphase chromosomes). Data points shown in this Figure 3: Hybridization of DNA probe (V-C or C-C) on the Southern blots of DNA (metaphase chromosomes). Data points shown in this Figure 3: Hybridization of DNA probe (V-C or C-C) on the Southern blots of DNA (metaphase chromosomes). Data points shown in this Figure 3: Hybridization of DNA probe (V-C or C-C) on the Southern blots of DNA (metaphase chromosomes). Data points shown in this Figure 3: Hybridization of DNA probe (V-C or C-C) on the Southern blots of DNA (metaphase chromosomes). Data points shown in this Figure 3: Hybridization of DNA probe (V-C or C-C) on the Southern blots of DNA (metaphase chromosomes). Data points shown in this
Figure 4. Gel electrophoresis patterns of embryo DNA digested with EcoRI enzyme and assayed with HOPC-2000 A-chains mRNA and its 3'-end-retaining fragments. Embryo DNA was digested in RLE buffer (1 in Tris-EDT buffer TSB 7.5; 0.1% in MgCl₂, 1.0% M NaCl, 0.1% Nonidet) with EcoRI enzyme according to Grenci et al. (1976) at 37°C for 4 h. Electrophoresis and hybridization were carried out as described in the legend to Fig. 2. A single preparation of 32P-labeled HOPC-2000 A-chain mRNA (specific activity ~ 10⁸ cpm/μg) was used for hybridization and for preparation of 3'-end-retaining fragments (see legend to Fig. 4). Input 32P-labeled mRNA hybridization was 1300 cpm (whole mRNA), 1100 cpm 5' + C + 3' fragments, and 600 cpm 5' + 3' fragments.

Figure 5. Gel electrophoresis patterns of calf thymus A and HOPC-2000 tumor Ri DNA digested with EcoRI enzyme and assayed with HOPC-2000 A-chain mRNA. DNA was digested as described in the legend to Fig. 4. Electrophoresis and hybridization were carried out as described in the legend to Fig. 2. Input 32P-labeled mRNA were 1300 cpm (embryo DNA) and 1200 cpm (HOPC-2000 DNA). An aliquot, corresponding to 50 μg input DNA of DNA from the indicated gel slices was fixed on a Millipore HAWF filter after denaturation and assayed to excess 32P-labeled 18S ribosomal RNA.
whole DNA is nearly twice as high as that obtained with the 3' end half, whereas both RNA probes hybridize with the embryonic DNA component to nearly the same extent. Thus the results suggest that the V, and C, genes are some distance away in the embryonic cells and that they seem to be joined in the plasmacytoma.

The striking difference in the two cases, λ and χ chains, is the fact that, unlike in MOPC-321, the embryonic component persists in the pattern of the HOPC-2020 DNA. Several explanations can be offered for this difference. In a BALB/c mouse, there seem to exist two λ-chain loci. These loci are represented by the λ chains produced by HOPC-2020 (λ1) and MOPC-315 (λ2). The sequent homology between the two groups of λ chains is extensive, albeit less than among themselves (Weigert et al. 1970, Dugan et al. 1973). If there are separate germ-line V, genes for the two groups of λ chains, mRNA coding for a λ chain could cross-hybridize with the V, gene. An alternative but trivial explanation is that the plasmacytomas originate by fusion of a committed B lymphocyte and a non-lymphocytic cell, such as a macrophage (Warner and Krüger 1975). Most of the BALB/c plasmacytomas are subcloned (Ohm 1967; Yoshida et al. 1968). Whereas some tumors (HOPC-2020) retain at least one of the nonlymphoid chromosomes, which carry the relevant immunoglobulin genes, others (MOPC-321) might throw out these chromosomes during cell propagation. Of course, it is also possible that the mechanism of V-C joining in λ chains is intrinsically different from that in χ chains.

Models for V-C Joining at DNA Level

What is the mechanism by which the integration of V- and C-gene sequences is brought about? Several models have been proposed, some of which are illustrated schematically in Figure 6. In the "copy-insertion" model, a specific V gene is duplicated and the copy is inserted at a site adjacent to a C gene (Buoyé et al. 1968). In this model, the embryonic sequence consists of a V gene should be retained in the lymphocyte expressing that particular V gene. Since the embryonic DNA fragment carrying the HOPC-2021 V gene does not seem to exist in

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**Figure 6:** Models for V-C joining at DNA level. See text for explanation.
Capra and Rehbe 1974; Wiguall 1973). In the past several years, however, several groups of re-
searchers have reported experimental results aimed at obtaining some insight into this formidable problem (Delovich and Baglioni 1974; Storb 1974; Premkumar et al. 1974; Tonesgawa et al. 1974a; Bernardini and Tonesgawa 1974; Rabbits et al. 1974; Leder et al. 1974). The method commonly used has been to examine the kinetics of hybridization of a purified immunoglobulin mRNAs with total cellular DNA, thereby counting the number of cross-hybridizing V genes. Earlier work with mRNA preparations of lower purity invariably generated hybridization kinetics with multiple components: a reiterated fraction and a unique or nearly unique fraction. Assuming that the reiterated fraction represents V-gene sequences, several authors interpreted their results as an indi-
cation of a germ-line basis for antibody diversity (Delovich and Baglioni 1974; Storb 1974; Premku-
mar et al. 1974). However, using mRNA coding for the chains which code for V regions of various degrees of sequence homology, we demonstrated that the major proportion of V-region sequences is in the unique rather than the reiterated fraction (Tonesgawa et al. 1974a,b). Based on competition hybridization, we could define a group of V chains, comprising over 10% of all such chains for which sequence information was available, within which there was over 80% amino acid sequence homology. DNA sequences coding for the V regions of one V chain from this group were reiterated only one-
to twofold. This strongly suggested the existence of some somatic mechanism for antibody diversity. These findings were later confirmed by us as well as by others who used either mRNA of higher purity or DNA fractionated according to degree of reiterated (Leder et al. 1975; Rabbits et al. 1975; Tonesgawa 1976; Schiller 1976; Parac et al. 1976). These experiments showed that the entire sequences in both α and ω-1 chain mRNAs and in a heavy-chain mRNA are reiterated no more than a few times per haploid genome. However, as we have pointed out previously (Tonesgawa et al. 1974b), the uniqueness of a V-gene sequence is, by itself, not unequivocal evidence for the somatic generation of antibody diversity. The extent of cross-hybridizability among V genes coding for different V regions must be experimentally determined. For instance, the ex-
tensive amino acid sequence homology among the V regions of a single "subgroup" does not neces-
sarily mean that there is equally extensive nucleo-
tide sequence homology among the corresponding V genes. Thus the kinetic data must be combined with a demonstration of nucleotide sequence homology. Using competition hybridization experi-
ments with V mRNAs, we demonstrated that ex-
tensive nucleotide sequence homology does in fact exist among the V regions of most BALB/c mouse myeloma λ chains. Since amino acid sequence studies strongly suggest that there is a minimum of 20 to 30 myeloma V regions, we concluded that the number of germ-line V genes (a few per haploid genome) is too small to account for the diversity of these immunoglobulin chains (Tonesgawa 1976). New Evidence for the Somatic Generation of Antibody Diversity In the past, all hybridization studies designed for counting the number of immunoglobulin genes were performed by hybridizing whole mRNA mole-
cules or complementary DNA synthesized in vitro from this mRNA with total cellular DNA. Since these probes contain, not only V gene but also C-
genome sequences, it has not been possible to focus the experiments exclusively on V genes. As de-
scribed above, treatment of embryos DNA with a restriction enzyme and subsequent fractionation of the DNA fragments in the preparative agarose gel permitted us to isolate and enrich the fraction containing V-gene DNA away from that containing C-gene DNA. We have therefore reexamined the validity of our previous conclusion using these DNA fractions, namely, that the number of V genes coding for BALB/c myeloma λ chains is no more than a few.

Cross-hybridizability of MOPC-104E and MOPC-21

Let us assume that, as we concluded previously, the entire nucleotide sequences in the two mRNA's from MOPC-104E and HOPC-210 plasmacytomas are indeed extensively homologous. Then the pattern of hybridization with the two mRNA should be distinguishable when EcoRI-cut gene DNA is examined across the agarose gel. That this is the case is shown in Figure 7. As dis-
cussed earlier, the three DNA components (from large to small) carry C-gene, V-region, and 5' external region sequences. The result is consist-
ent with the assumption but does not prove it. It is possible that the enzyme cleaves regularly arranged multiple V, genes such that DNA frag-
ments of identical size are generated, each of which contains a different V gene. It is also possible, although less likely, that the 2.8 x 10^6 component consists of a single DNA fragment carrying se-
quences for multiple V regions.

We have isolated the 4.7 x 10^6 and 2.8 x 10^6 components. DNA in these components were de-
natured separately and hybridized to excess MOPC-104E or HOPC-210 λ mRNA's under con-
ditions that do not permit substantial renaturation of DNA. The mixture was then treated with S1 nuclease to digest all single-stranded nucleic acids. The remaining nucleic acid was treated with alkali to digest RNA in hybrid and then rehy-
bridized with λ-labeled MOPC-104E or HOPC-
2000 α mRNA, under conditions of DNA excess. The relative extent of hybridization in the second step was compared in all four possible combinations both for the C-genus and V-region DNA fragments (Table 1). The fact that in each of these two heterologous combinations the extent of hybridization is indistinguishable from that of the corresponding homologous combination confirms our previous observation that the sequences in the two λ mRNAs are extensively homologous in the V regions. Analogous experiments with the C-gene fragments serve as a positive control.

Hybridization Kinetics of Individual DNA Components

Two points were experimentally tested with the hope of strengthening our previous conclusion that the myeloma V genes are unique or nearly so. First, are the V genes sequence any more reiterated than the C genes sequenced? Second, what is the resolution of the kinetics of hybridization? Can we, for instance, really distinguish 10 to 20 copies from a few copies?

To these ends, a large amount (30 mg) of embryo DNA was digested with EcoRI, and DNA fragments were fractionated in preparative agarose gel electrophoresis followed by centrifugation. Fractions contain-
ing the C segment, V segment, and S' segment were pooled from several gels, each of which displayed a hybridization pattern very similar to that in Figure 4. To make the results obtained with these DNA fractions and those obtained with unfracti-
oned DNA comparable, a parameter of enrichment of a specific sequence, ϵ, was defined as follows: ϵ = (A - B)/(1/N), where N is the amount of input DNA subjected to electrophoresis, S is the amount of DNA in the pooled fractions containing specific sequences, A is the overall recovery of DNA during electrophoresis and subsequent extraction from the gel, and B is the recovery of the specific sequences in the pool. In practice, A is obtained by dividing the sum of recorded O.D. from all gel fractions by the input O.D.; B is obtained by dividing the area under the hybridization peak corresponding to pooled fractions by total peak area. Since, as described elsewhere (Horumi and Tonegawa 1978), the hy-
bridization was carried out under conditions in which the final level increases sublinearly as the amount of the sequences increase, the value of B obtained by the above procedure is a lower limit.

A series of hybridization curves obtained with various fractionated DNAs as well as with un-
fractionated DNA are illustrated in Figure 8. The source of λ mRNA was MOPC-104E. Values of ϵ were calculated for each of the fractionated RNA, and the hybridization level is plotted against "Cgs", which is Cg corrected for sequence enrichment. The final levels of hybridization attained are different for each DNA component and are in good agreement with the relative lengths of the regions depicted in Figure 1. For easier comparison, the data in Figure 8 are replotted in Figure 9 with

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<th>Table 1: Cross-hybridization of Two Lambda mRNAs</th>
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Mouse embryonic DNA (1 mg) was digested with EcoRI and frac-
tionated on a 0.8% agarose gel. DNA fragments containing C and V genes were then hybridized to an extent of 1,000 cpm in 6 x SSC (0.15 M NaCl, 0.01 M Na2HPO4, pH 7.0; 1 x 1000 cpm in 6 x SSC, 0.1 M NaCl, 0.01 M Na2HPO4, pH 7.0; 4 x 1000 cpm in 3 x SSC in 0.1 M NaCl, 0.01 M Na2HPO4, pH 7.0) for 20 min at 37°C. DNA was dis-

Hybridization will be expressed as % hybridized: % hybridized = 100 x CPM in hybridized DNA / CPM in total DNA.
Figure 3. Kinetics of hybridization of 3H-labeled MOPEG-104K mRNA with whole or fractionated embryo DNA. Hybridization was carried out in 3× SSC supplemented with Tris-HCl pH 7.8, 0.1% SDS at 45°C. Other procedures for hybridization and processing of hybridization mixture were as described previously (Tomizawa et al. 1974). A single preparation of 3H-labeled MOPEG-104K mRNA (specific activity 4 x 10⁶ cpmpg) was used in all experiments. Hybridization reactions were carried out at 50°C, 52°C, 54°C, and 56°C. The next six reactions were carried out with whole DNA, DNA containing fragments, V-containing fragments, and C-3 F-containing fragments in this order. DNA concentration (C) was 22 0, 2 2. 2 9, and 3.1 mg/ml. Ehrlich factor (f) was 1, 0. 7 4, and 0. 5. Ratio of DNA to RNA was 2 x 10⁴, 2.5 x 10⁴, 2.5 x 10⁵, 2.5 x 10⁶, and 2.5 x 10⁷. Across the region of the probes, with which were determined the minimum hybridization levels at 40°, 42°, 44°, 46°, 48°, 50°, 52°, 54°, and 56°C.

### Multiple Ordinate Scales

It is clear from Figure 9 that the points obtained with the three fractionated RNAs fall virtually on a single sigmoid curve with Cip of 0.65. This corresponds to a nominal renaturation frequency of 3 (Melli et al. 1971). The points obtained with unfractionated DNA also fall on this curve, except for a possible slight deviation of those at lower C values. The MOPEG-104K DNA used here was not subjected to the acrylamide electrophoresis step. The slight deviation is therefore most likely attributable to impurities whose genes are reiterated (Kabliha et al. 1975; Tomizawa 1976). These results demonstrate unequivocally that MOPEG-104K genes are no more reiterated than C genes, a conclusion which is in agreement with our previous results using highly purified whole a RNAs. The results also in conformity with our previous conclusions, indicate that both genes are reiterated no more than a few times per haploid genome.

In Figure 10, two C curves obtained with two independent preparations of the C-gene fraction are compared with a C curve obtained with unfractionated DNA. Note that, unlike in Figure 9 and 9, C values are not corrected for the C factor, which was 0.58 and 0.58 for the two C-gene fractions. Whereas the points corresponding to the two C-gene fractions fall virtually on a single curve, those corresponding to unfractionated DNA are clearly shifted to higher C values. These results demonstrate that, with the method employed, we can distinguish two genes whose reiteration frequency differ by at least sixfold. In fact, a threefold difference in gene reiteration frequency is at one-half of the shift observed in Figure 10, would seem to be distinguishable.

Weigert and colleagues have determined the amino acid sequences of a number of BALB/c and NZB mouse myeloma a chains. They have already found nine different V regions (Weigert and Riblet, this volume; Weigert, pers. comm.). Most of these V region sequences are as homologous to that of MOPEG-104 as is HOPC-2020, suggesting that the corresponding V genes cross-hyib-ridize with MOPEG-104K RNA. We have shown above that the number of germ-line V genes is no more than a few, using a method which can distinguish a threefold difference in gene reiteration frequency. This experimentally determined gene number seems to be smaller than the number of different V regions we already know. Furthermore, a simple statistical calculation which can be applied to the sequence data and is described elsewhere (Tomizawa 1978) indicates that the repertoire of mouse V regions must be much larger than the nine known sequences. The results thus strongly suggest the existence of somatic processes by which V region sequences are diversified from a few germ-line genes.

The Existence of Multiple V Genes

The hybridization studies described in the last few sections demonstrated that groups of closely related V regions are somatically generated from a few germ-line genes. They do not, however, give us any reliable estimate of the total number of germ-line V genes. Given the enormous diversity of V regions, however, the existence of multiple germ-line V genes is a highly likely possibility. This notion is also suggested by the genetic analysis of idiosyncratic markers on mouse heavy chains (Eich- mann 1973).

A direct demonstration of multiple V genes for mouse a chains is illustrated in Figure 11, which shows hybridization reactions obtained with mouse embryo DNA and whole a RNAs from MOPC-21 and MOPC-321. These two a chains show extensive homology in their V regions (Milette and Svenner 1971; McKeith et al. 1973) — they belong to two
in the germ line. On the other hand, there exists multiple germ-line V genes. We would like to know, of course, the total number of germ-line V genes.

Amino acid sequences of immunoglobulin-chain V regions can be classified into subgroups such that the variability within a subgroup is less than in the group as a whole. Obviously, the greater the number of subgroups, the less variability within the subgroups. The precise degree of variability tolerated within a subgroup is usually decided rather arbitrarily. Cohn et al. (1979), however, have defined subgroups in a less arbitrary fashion: If two V regions differ by even one "frame" residue (i.e., a residue outside the hypervariable regions), they belong to different subgroups. According to Cohn, all known V regions associated with the k chain belong to a single subgroup. We have shown that there is probably only one germ-line V gene for k. MOPC-293 and MOPC-21 k chains belong to different subgroups, and we have shown that their V regions are derived from different germ-line genes. It will be interesting to see how far this correlation between genes and subgroups is maintained. We think that the number of subgroups for k is an upper limit for the number of germ-line V genes. If this is so, the entire germ-line contains from 2 to 100 V genes.

CONCLUDING REMARKS

The dynamic nature of the genetic material is an essential element of evolution. Genes can mutate and recombine. If they could not, there would be no higher forms of life. Both of these processes are under genetic control and are themselves subject to natural selection, i.e., populations will acquire optimal rates of mutation and recombination. But does the collective of cell populations which we call a multicellular organism utilize these processes in any meaningful way? In other words, do they play an important role during the development of a single individual? We have shown that this is the case for the immune system. This is not, however, a unique example. Some vertebrate tumor viruses appear to behave like temperate bacteriophages and are perhaps the most primitive examples. The behavior of genes for ribosomal RNA is the hitherto most sophisticated example. Note that these are all examples of contextual changes. We do not know of any cases involving changes of genetic content. But neither do we know of any system which is as complex as the immune system except one—the nervous system. Janos (1973) has already pointed out the striking analogy between these two systems.

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cellular (as opposed to the monoclonal) origin of the
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