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Genetics

Mutant immunoglobulin genes have repetitive DNA elements inserted into their intervening sequences

(hybridomas/gene expression/transposable elements/intracisternal-A-particle-related sequences)

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ABSTRACT **The κ light chain genes from two mutant hybridoma cell lines defective in κ light chain synthesis were isolated and compared to the wild-type κ light chain gene. In each case, the mutant κ light chain genes were found to contain repetitive DNA elements in their intervening sequences that were not present in the intervening sequences of the wild-type κ light chain gene. These elements were found to be related to the genes of intracisternal A particles. These results suggest that the decreased production of κ light chain in the mutant cell lines is due to the presence of the intracisternal A particle-related genes.**

There are now several examples in which rearrangements of eukaryotic DNA are involved in the regulation of gene expression. In particular, rearrangements are required for the activation of immunoglobulin genes (1–5), the variation of trypanosome surface antigens (6, 7), and the switching of the yeast mating type (8, 9). The enhanced expression of a cellular gene due to proviral promoter insertion is also known to occur (10).

Here we describe DNA rearrangements that have occurred in two mutant hybridoma cell lines and that may be responsible for the decreased production of the immunoglobulin κ light chain in these lines. In both cases, we have found repetitive DNA segments at the sites of rearrangement. Analysis of these repetitive segments indicates that they are related to intracisternal A particle (IAP) genes and suggests that the reduction in κ chain synthesis in these mutants is due to the insertion of IAP sequences into the intervening sequences of the mutant κ chain genes.

MATERIALS AND METHODS

Mouse Strains and Cell Lines. Four-week-old females of the BALB/c strain of *Mus musculus* were obtained from The Jackson Laboratory.

X63-Ag8 is a MOPC 21 myeloma cell line (11). The origin of the wild-type Sp6 and the mutant hybridoma cell lines igk-1 and igk-20 has been described (12). Subclones of these mutants, igk-1/2-9-2 and igk-20/10-12, were compared here with a subclone of the wild-type cell line, Sp603 (12). All cell lines were grown as described (12).

Bacterial and Phage Strains. *Escherichia coli* K-803 (r_k^- m_k^-, *supE*, *supF*) was obtained from G. Matthyssens for growing phage. *E. coli* NS428, obtained from N. Sternberg, was used as a source of extract for *in vitro* packaging of phage λ DNA. The cloning vector λ Charon 28 was obtained from F. Blattner (13). The plasmid pL21-5, used to generate a probe of the κ chain constant region (C_κ), was donated by R. Wall (14).

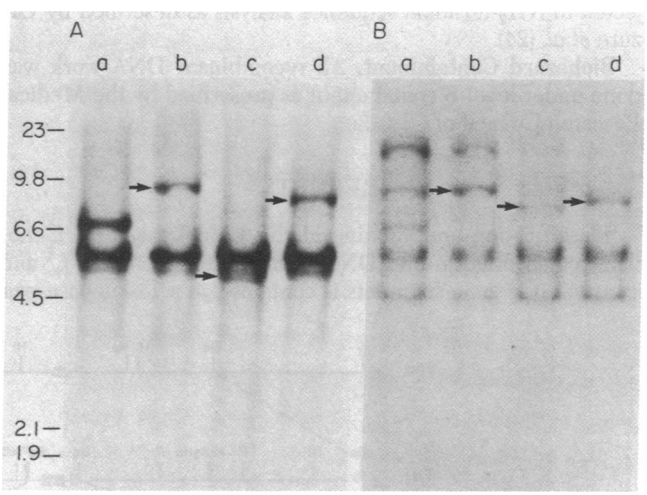

FIG. 1. Identification of κ_{TNP} genes. *Bam*HI-digested DNA (20 μg) from X63-Ag8 (lanes a), Sp6 (lanes b), igk-1 (lanes c), and igk-20 (lanes d) was electrophoresed through a 1% agarose gel at 2 V/cm for 40 hr. (*A*) After transfer to nitrocellulose, the blot was hybridized to a ^{32}P-labeled probe containing the 3′ half of the C_κ coding region. This probe was isolated from the plasmid pL21-5 after digestion with *Cfo* I and *Hpa* I (14). The bands corresponding to fragments containing κ_{TNP} C_κ gene segments are indicated with arrows. (*B*) The filter in *A* was washed according to Thomas (23) and the blot was rehybridized to a ^{32}P-labeled probe containing the 5′ half of the κ_{TNP} V_κ coding region (Fig. 2). The bands corresponding to fragments containing κ_{TNP} V_κ gene segments are indicated with arrows. Fragment sizes were determined by comparison with *Hin*dIII-digested λ phage DNA as size marker (sizes shown in kb).

DNA Isolation, Nitrocellulose Blotting, and Cloning. Cellular, phage, and plasmid DNAs were isolated by standard techniques as described (15). The arms of *Bam*HI-digested λ Charon 28 DNA were isolated by density gradient centrifugation in 10–40% sucrose (16). DNA restriction fragments used for ^{32}P-labeled probes, cloning, restriction enzyme mapping, and DNA sequence analysis were isolated by preparative agarose or polyacrylamide gel electrophoresis as described (17). Nitrocellulose blotting was done according to the method of Southern (18) as described (17). Hybridization was carried out by using ^{32}P-labeled probes with a specific activity of 5–10 × 10^7 cpm/μg of DNA. Packaging of recombinant DNA was done according to the method of Becker and Gold (19), using NS428 as the source of packaging extracts. Phage λ DNA terminase was obtained from A. Becker. Recombinant phages were identified by using

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Abbreviations: bp, base pair(s); C region, constant region; IAP, intracisternal A particle; J segment, joining segment; kb, kilobase pair(s); TNP, 2,4,6-trinitrophenyl; V region, variable region.

the plaque assay procedure of Benton and Davis (20) as described (15).

DNA Sequence Analysis. DNA fragments were labeled at their recessed 3′ termini by using [α-^{32}P]dNTPs and reverse transcriptase (RNA-dependent DNA nucleotidyltransferase) as described (15). Reverse transcriptase was obtained from J. Beard. DNA sequences were obtained by using the partial chemical degradation technique of Maxam and Gilbert (21) as described (15).

RNA Isolation and Nitrocellulose Blotting. RNA was isolated from membrane-bound polyribosomes as described by Marcu *et al.* (22). RNA blotting to nitrocellulose was performed as described by Thomas (23).

Amino Acid Sequence Analysis. Immunoglobulin from Sp6 ascites fluid was purified by ammonium sulfate precipitation. After partial reduction and alkylation the κ_{TNP} chain was subjected to NH_2-terminal sequence analysis as described by Lazure *et al.* (24).

Biohazard Containment. All recombinant DNA work was done under level B containment as prescribed by the Medical Research Council of Canada.

RESULTS

Identification and Cloning of the Wild-Type κ_{TNP} Gene. The region of germ-line DNA bearing the κ joining (J_κ) and constant (C_κ) gene segments is contained in a 13-kilobase-pair (kb) *Bam*HI fragment (25). Expression of a κ chain gene requires a productive rearrangement that joins one of many variable (V_κ) gene segments to one of the active J_κ gene segments (3). As a consequence of V_κ–J_κ joining, the size of the *Bam*HI restriction fragment containing the C_κ gene segment is altered, so that digestion with this enzyme can be used to identify fragments bearing rearranged κ chain genes. The Sp6 hybridoma, the wild-type cell line used here, secretes immunoglobulin specific for the hapten 2,4,6-trinitrophenyl (TNP) (12). It was derived by fusing the MOPC 21 myeloma cell line X63-Ag8 with spleen cells from a BALB/c mouse immunized with TNP, and it produces the myeloma κ chain as well as the TNP-specific κ chain (κ_{TNP}). Therefore, the Sp6 cell line is expected to contain at least one of the κ chain genes of the myeloma parent in addition to the κ chain genes donated by the spleen cell. To identify the fragment bearing the κ_{TNP} gene, *Bam*HI-digested DNA from X63-Ag8, Sp6, and the mutant cell lines igk-1 and igk-20, which are defective in the production of the κ_{TNP} chain, was hybridized to a probe containing the 3′ half of the C_κ coding region (Fig. 1A). All lanes were found to contain a 5.4-kb band, which corresponds to the productively rearranged myeloma κ chain gene (26). The 5.9-kb band present in all lanes and the 6.9-kb band present in lane a (X63-Ag8) correspond to aberrantly rearranged κ chain genes (27). A band unique to each of the hybridoma cell lines was detected at 9.6 kb in lane b (Sp6), 5.2 kb in lane c (igk-1), and 9.0 kb in lane d (igk-20). These results suggested that the 9.6-kb band corresponds to the wild-type

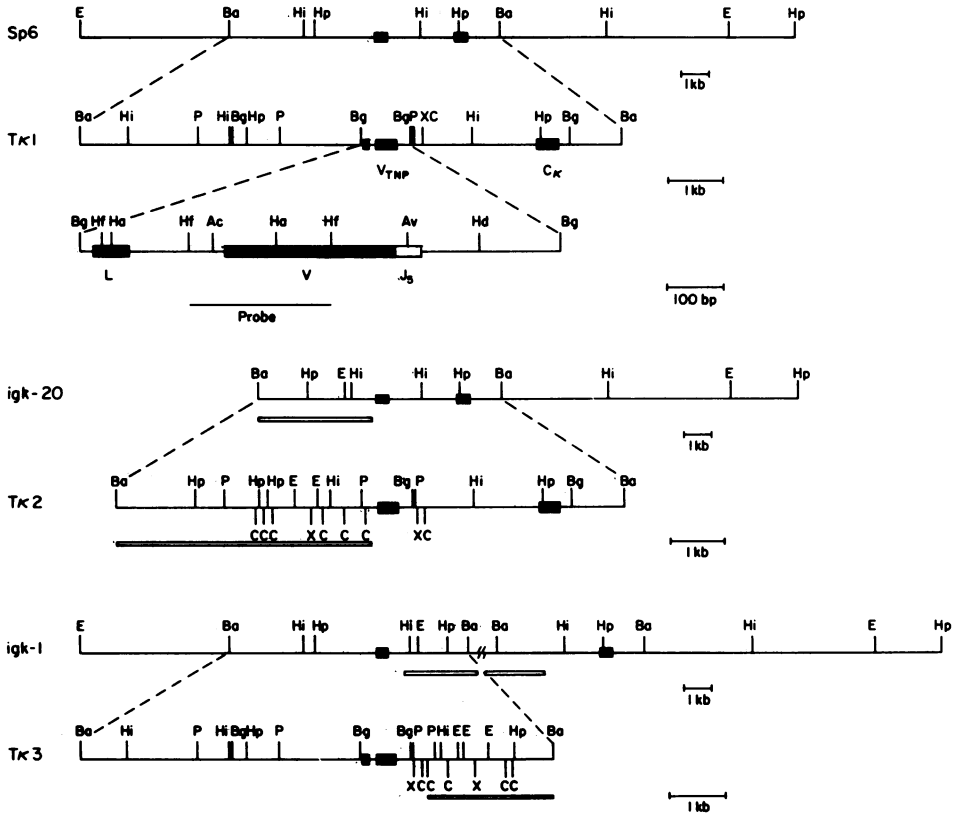

FIG. 2. Restriction enzyme maps of κ_{TNP} genes. The restriction enzyme maps of the cloned κ_{TNP} genes, Tκ1, Tκ2, and Tκ3, are shown beneath the corresponding κ_{TNP} genomic restriction maps. Coding sequences are indicated with solid bars. The restriction map of the V_{TNP} gene segment required to generate the DNA sequence of the V_{TNP} coding region (Fig. 3) is shown beneath the Tκ1 restriction map. The 250-base-pair (bp) *Hin*fI fragment used as a V_{TNP} region probe is indicated. The foreign sequences in Tκ2 and Tκ3 are indicated with open bars. The distance between the two internal *Bam*HI sites in the igk-1 κ_{TNP} genomic map is not known. The restriction maps of the cloned κ_{TNP} genes were obtained by using partial and double digestion analysis as described by Hozumi *et al.* (28). The genomic restriction maps were constructed by comparing the sizes of bands detected in DNA blotting experiments with published maps of the C_κ region (29, 30) and with the restriction maps of the Tκ1, Tκ2, and Tκ3 clones. Restriction enzyme abbreviations: Ac, *Acc* I; Av, *Ava* II; Ba, *Bam*HI; Bg, *Bgl* II; C, *Cfo* I; E, *Eco*RI; Ha, *Hae* III; Hd, *Hin*dII; Hf, *Hin*fI; Hi, *Hin*dIII; Hp, *Hpa* I; P, *Pst* I; X, *Xba* I.

κ_{TNP} gene and that this gene has undergone subsequent rearrangements in the mutant cell lines (see below). A fragment from Sp6 DNA corresponding to this band was cloned in λ Charon 28. The restriction enzyme map of this fragment, denoted Tκ1, is shown in Fig. 2. To confirm that Tκ1 corresponded to the κ_{TNP} gene, the κ_{TNP} chain was isolated from Sp6 ascites fluid and the sequence of the first 23 amino acids was determined. As illustrated in Fig. 3, the sequence agreed with that predicted from the translation of the DNA sequence of the Tκ1 V_κ gene segment. From this analysis the κ_{TNP} V_κ region (V_{TNP}) can be classified as a member of the VK-14 group of κ chains (31). In this case, the V_{TNP} gene segment is joined to the J_5 joining segment.

Analysis of κ_{TNP} mRNA in Mutant Cell Lines. The mutants igk-1 and igk-20 were selected because they produce less than the normal amount of the κ_{TNP} chain. For igk-1, κ_{TNP} chain production was found to be about 10% of the wild-type Sp6 level, whereas for igk-20 the κ_{TNP} chain was not detected (12).

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                                        -20
                                MetGlyPheLysMetGluSerHisThr
GATCTGAAATACATCAGACCAGCATGGGCTTCAAGATGGAGTCTCATACT
                    -10
GlnAlaPheValPheAlaPheLeuTrpLeuSer
CAGGCCTTTGTATTCGCGTTTCTCTGGTTGTCTGGTGAGACTTTTAAAAG

TATTATAACAACTCAAAAGTAATTTATTTAAATAGCTTTTCCTATAGGAA

GCCAATATTAGGCAGACAATGCCATTAGATAAGACATTTTGGATTCTAAC

ATTTGTGTCATGAAATCTTTGTATATGTAAGTGTATACTCATTATCTATT

          -4          1
          GlyValAspGlyAspIleValMetThrGlnSerGlnLysP
TCTGATTGCAGGTGTTGATGGAGACATTGTGATGACCCAGTCTCAAAAAT

10                              20
heMetSerThrSerValGlyAspArgValSerIleThrCysLysAlaSer
TCATGTCCACATCAGTAGGAGACAGGGTCAGCATCACCTGCAAGGCCAGT

          30                                  40
GlnAsnValGlyThrAlaValAlaTrpTyrGlnGlnLysProGlyGlnSe
CAGAATGTGGGTACTGCTGTAGCCTGGTATCAACAGAAACCAGGACAATC

                    50
rProLysLeuLeuIleTyrSerAlaSerAsnArgTyrThrGlyValProA
TCCTAAACTACTGATTTACTCGGCATCCAATCGGTACACTGGAGTCCCTG

60                              70
spArgPheThrGlySerGlySerGlyThrAspPheThrLeuThrIleSer
ATCGCTTCACAGGCAGTGGATCTGGGACAGATTTCACTCTCACCATCAGC

          80                                  90
AsnMetGlnSerGluAspLeuAlaAspTyrPheCysGlnGlnTyrSerSe
AATATGCAGTCTGAAGACCTGGCAGATTATTTCTGCCAGCAATATAGCAG

                    100
rTyrProLeuThrPheGlyAlaGlyThrLysLeuGluLeuLysArg
CTATCCTCTCACGTTCGGTGCTGGGACCAAGCTGGAGCTGAAACGTAAGT
ACACTT
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FIG. 3. Nucleotide sequence of the V_{TNP} gene segment. Only the coding strand is shown. The corresponding amino acids are shown above the nucleotide sequence. Negative numbers refer to the leader sequence. The segment beginning at amino acid 1 (Asp) and ending with amino acid 23 (Cys) (underlined) corresponds to the first 23 amino acids determined by analyzing the mature κ_{TNP} chain. The J_5 joining segment is indicated with a broken line. The sequence was determined by analyzing complementary strands and overlapping fragments. The sequences of all fragments were determined at least twice.

To test if the mutants igk-1 and igk-20 also contain lower than normal amounts of κ_{TNP}-specific mRNA, RNA was isolated from membrane-bound polyribosomes and analyzed by RNA blotting using a *V* region probe specific for the κ_{TNP} gene (Fig. 4). In agreement with the measurement of κ_{TNP} light chain production (12), the mutant igk-1 was found to produce a reduced amount of κ_{TNP}-specific mRNA. In contrast, although no κ_{TNP} chain was detected in igk-20 (12), this cell line was found to produce more κ_{TNP}-specific mRNA than did igk-1.

Identification and Cloning of the Mutant κ_{TNP} Genes. To identify the κ_{TNP} genes in igk-1 and igk-20, the DNA blot in Fig. 1*A* was washed and rehybridized to the probe containing the V_{TNP} gene segment. Several bands common to all lanes were seen, presumably reflecting the various members of the VK-14 group (Fig. 1*B*). In addition to these bands, DNA from igk-20 contained a 9.0-kb band that was not present in the DNA from the other cell lines (Fig. 1*B*, lane d). A 9.0-kb band had previously been detected with the C_κ probe (Fig. 1*A*, lane d), suggesting that the corresponding 9.0-kb *Bam*HI fragment contained both the V_{TNP} and the C_κ gene segments. In the case of igk-1, an 8.4-kb band was revealed both by a probe containing the V_{TNP} gene segment (Fig. 1*B*, lane c) and by a probe containing the germ-line J_κ gene segments (data not shown). A band at this position was not detected with the C_κ probe, although, as mentioned above, this probe detected a 5.2-kb band (Fig. 1*A*; lane c).

We cloned the 9.0-kb *Bam*HI fragment from igk-20 DNA and the 8.4-kb *Bam*HI fragment from igk-1 DNA with λ Charon 28. These fragments have been designated Tκ2 and Tκ3, respectively. Tκ2 contains the V_{TNP} and C_κ gene segments. However, comparison of the restriction enzyme map of Tκ2 to the restriction enzyme map of Tκ1 revealed a divergence in the Tκ2 pattern from the wild-type pattern, beginning approximately 100 bp upstream of the V_{TNP} gene segment and extending in the 5′ direction (Fig. 2). Therefore, Tκ2 does not contain the sequences encoding the κ_{TNP} leader sequence or the wild-type sequences further upstream. Instead, Tκ2 contains 4 kb of foreign DNA. Conversely, Tκ3 contains the V_{TNP} gene segment and the wild-type sequences upstream of the V_{TNP} gene segment, but it does not contain the C_κ gene segment. Comparison

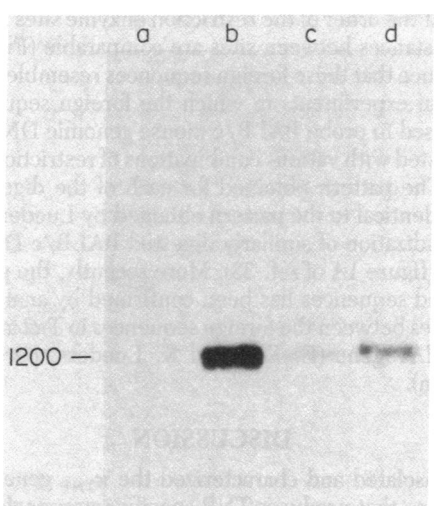

FIG. 4. Measurement of κ_{TNP}-specific mRNA. RNA (10 μg) from X63-Ag8 (lane a), Sp6 (lane b), igk-1 (lane c), and igk-20 (lane d) was denatured with glyoxal, electrophoresed through a horizontal 1% agarose gel in 10 mM sodium phosphate buffer at pH 6.9, transferred to nitrocellulose as described by Thomas (23), and hybridized to a ^{32}P-labeled probe containing the 5′ half of the V_{TNP} coding region (Fig. 2). The size in nucleotides is indicated.

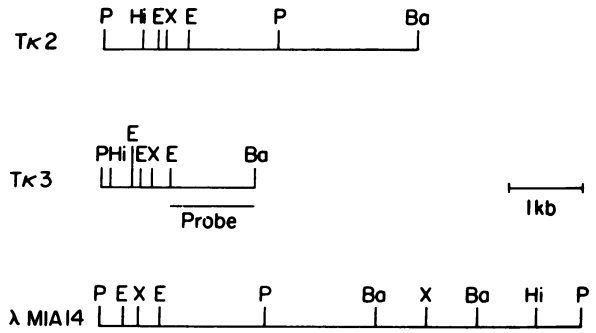

FIG. 5. Comparison of restriction maps of foreign sequences in Tκ2 and Tκ3 to the restriction map of a cloned IAP gene [λ MIA14, from Kuff *et al.* (32)]. For comparison, the restriction map of the Tκ2 foreign sequence indicated in Fig. 2 has been inverted. The *Bam*HI/*Eco*RI fragment from Tκ3 that was used as a probe in the DNA blotting experiment shown in Fig. 6 is indicated. Restriction enzyme abbreviations are as in Fig. 2.

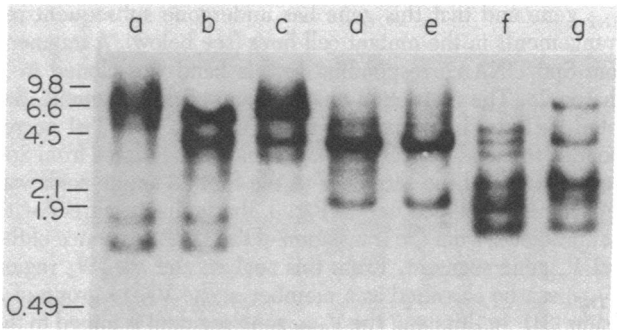

FIG. 6. Sequences present in BALB/c mouse DNA homologous to Tκ3 foreign sequence. BALB/c kidney DNA (14 μg) was digested with various restriction enzymes and electrophoresed through a 1% agarose gel at 2 V/cm for 15 hr. After transfer to nitrocellulose, the blot was hybridized to a ^{32}P-labeled probe containing a portion of the foreign sequence in Tκ3 (Fig. 5). The restriction enzyme digestions were as follows: lane a, *Eco*RI; lane b, *Eco*RI/*Hin*dIII; lane c, *Hin*dIII; lane d, *Hin*dIII/*Xba* I; lane e, *Xba* I; lane f, *Xba* I/*Pst* I; lane g, *Pst* I. Fragment sizes (shown in kb) were determined as described for Fig. 1.

of Tκ3 to Tκ1 indicated that the restriction enzyme pattern of Tκ3 diverged from the wild-type pattern approximately 500 bp downstream of the V_{TNP} gene segment (Fig. 2). Therefore, Tκ3 also contains a region of foreign DNA. In this case, it consists of a 2-kb sequence downstream of the V_{TNP} gene segment.

To confirm that these rearrangements were not cloning artefacts, we constructed restriction enzyme maps of the κ_{TNP} genes in Sp6, igk-1, and igk-20 from DNA blotting experiments using genomic DNA and probes containing the V_{TNP} gene segment, the C_κ gene segment, or the J_κ gene segments (summarized in Fig. 2). In each case, the restriction enzyme sites present in the cloned κ_{TNP} genes were found to correspond to the restriction enzyme sites predicted by the hybridization patterns of genomic DNA.

Analysis of the Sequences Present at the Rearrangement Sites in Tκ2 and Tκ3. The restriction enzyme maps of the foreign sequences present at the rearrangement sites in Tκ2 and Tκ3 were found to be similar (Fig. 5) and hybridization experiments confirmed that these sequences were homologous (data not shown). Moreover, both of these restriction enzyme maps resemble the maps of cloned IAP genes reported by Kuff *et al.* (32), in that the order of the restriction enzyme sites is the same and the distances between sites are comparable (Fig. 5). Further evidence that these foreign sequences resemble IAP genes comes from experiments in which the foreign sequence from Tκ3 was used to probe BALB/c mouse genomic DNA that had been digested with various combinations of restriction enzymes (Fig. 6). The pattern obtained for each of the digestions was virtually identical to the pattern obtained by Lueders and Kuff after hybridization of similarly digested BALB/c DNA to IAP 35S RNA (figure 1A of ref. 33). More recently, the presence of IAP-related sequences has been confirmed by analysis of heteroduplexes between the foreign sequences in Tκ2 and Tκ3 and a cloned IAP gene (E. Kuff and K. Lueders, personal communication).

DISCUSSION

We have isolated and characterized the κ_{TNP} gene from Sp6, a hybridoma that produces TNP-specific immunoglobulin. We have also isolated portions of the κ_{TNP} genes from the mutants igk-1 and igk-20, which are defective in κ_{TNP} chain synthesis. Both mutant genes have undergone rearrangements within intervening sequences.

The fact that igk-1 produces the κ_{TNP} chain indicates that the V_{TNP} and C_κ gene segments are still closely linked in the igk-1 genome. We attribute the 5.2-kb band detected by the C_κ probe in *Bam*HI-digested igk-1 DNA to the κ_{TNP} C_κ gene fragment (Fig. 1*A*, lane c). The results summarized in Fig. 2 suggest the possibility of cloning an overlapping fragment containing the κ_{TNP} C_κ gene segment.

In the case of igk-20, the κ_{TNP} chain has not been detected (12). However, the RNA blot analysis indicates that κ_{TNP}-specific mRNA is produced by this cell line. The reason for the inability of this κ_{TNP}-specific mRNA to be translated into the κ_{TNP} chain is not known. In this respect, it will be of interest to determine whether this κ_{TNP}-specific mRNA contains sequences corresponding to the leader sequence of the κ_{TNP} gene. DNA blot analysis with a probe corresponding to the leader sequence of the κ_{TNP} V_κ gene segment has revealed a band that was present only in *Bam*HI-digested igk-20 DNA (data not shown). We interpret this as evidence that the remainder of the κ_{TNP} gene is still present in the igk-20 genome. It remains to be determined if the κ_{TNP} leader and variable region gene segments are closely linked in igk-20 genomic DNA.

The DNA segments present in Tκ2 and Tκ3 at the sites of rearrangement appear to be portions of IAP genes. IAP genes are retrovirus-like elements that are present in about 1,000 copies in the genome of *Mus musculus* (32–35). They are expressed in early embryos (36–38) and in several different cell types, both normal and neoplastic (39). In particular, IAPs are present in all mouse plasmacytomas (40). Recently, Shen-Ong and Cole have reported that IAP genes are amplified in plasmacytoma DNA and that the amplified genes are colinear with the predominant IAP RNA from the IAPs (41). The predominant species of IAP RNA present in IAPs in Sp6 is not known, but the fact that the restriction patterns are different for the two foreign DNA segments present in Tκ2 and Tκ3 would imply activation of at least two different genes. Moreover, it is interesting that the IAP-like sequences have not only inserted into different intervening sequences in the κ_{TNP} gene in each of the mutant cell lines but also are in opposite orientations. This difference in orientation could be related to the difference in expression of the κ_{TNP} gene in these cell lines in that IAP transcription would be in the same direction as κ_{TNP} gene transcription in igk-1 and in the opposite direction to κ_{TNP} gene transcription in igk-20 (42).

Several groups have observed analogous alterations in gene expression due to the insertion of viral sequences into a host genome (10, 43, 44). Furthermore, sequences homologous to IAP genes have been found flanking a transposed mouse pseu-

dogene (45). However, the role of these sequences in the creation of the pseudogene is not clear. The data presented here, on the other hand, suggest that IAP-like sequences are responsible for the defect in κ_{TNP} light chain synthesis in the mutant hybridoma cell lines igk-1 and igk-20. Further studies should allow elucidation of the mechanisms responsible for the insertion of these sequences into the intervening sequences of the mutant κ_{TNP} genes and define their role in the alteration of κ_{TNP} gene expression in the mutant cell lines.

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