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Differing Populations of Intracisternal A-Particle Genes in Myeloma Tumors and Mouse Subspecies

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Intracisternal A-particle genes form a family of endogenous retrovirus-like genetic elements that are transcribed in mouse plasmacytomas (myeloma tumors). Two types of A-particle genes that can be differentiated by a sequence of 0.5 kilobase found in one type but not the other have been identified. Quantitative Southern blot analysis was used to measure the populations of different A-particle genes in DNAs from BALB/c mice, the Japanese subspecies *Mus musculus* subsp. *molossinus*, and myeloma tumors. The majority of the genes (715 copies per haploid genome or 76%) were found to be nearly identical except for small changes in conserved restriction enzyme sites. The second type of A-particle gene was much less abundant with 90 copies representing approximately 10%. The A-particle RNA in MOPC104E and MOPC315 was found to be colinear with a small portion of this latter type, comprising only 2% of the endogenous intracisternal A-particle sequences. Myeloma tumor DNA was found to have a two- to fourfold increase in the number of these genes, suggesting that the intracellular viruses have been activated to produce a double-stranded complementary DNA which subsequently integrated into the tumor genome. Analysis of *M. musculus* subsp. *molossinus* DNA revealed similar but shifted populations of A-particle genes, when compared with BALB/c DNA, except for the absence of a prominent *Eco*RI-*Hin*dIII band at 3.9 kilobases. This latter band, representing approximately 15% of the A-particle genes in BALB/c DNA, was shown to be a deletion variant of the most abundant gene family.

Intracisternal A-particles (IAPs) are retrovirus-like structures found abundantly in every mouse plasmacytoma (myeloma tumor) (9) and in many other mouse tumor cells (11, 14, 19). DNA sequences homologous to IAP RNAs are present in approximately 1,000 copies per haploid mouse genome (23), encompassing nearly 0.2% of the total cellular DNA. This is more than 20 times the number of any other endogenous murine retrovirus that has been described. It is therefore of interest to determine the distribution of these sequences in the mouse genome, the reason for their high reiteration frequency, and their relationship to the expression of mouse cellular genes in both normal development and tumors.

Kuff and co-workers have described a number of cloned IAP genes, each containing an approximately 7-kilobase (kb) DNA segment that is colinear with the IAP RNA in neuroblastoma cells (20, 24). All of these genes are virtually identical except for some variation in their conserved restriction enzyme sites. This family of genes seems to account for a large portion of the IAP sequences in mouse DNA.

We have identified a separate set of IAP genes that have major deletions or sequence substitutions or both (28). This latter group is distinguished by a 0.5-kb DNA segment that is not found in genes analogous to those studied by Kuff and co-workers (Fig. 1). Yet the predominant IAP RNA from two different myeloma tumors is transcribed from this second group of genes (28).

In this paper, we have measured the populations of different IAP genes in DNAs from BALB/c mice, from the Japanese subspecies *Mus musculus* subsp. *molossinus*, and from BALB/c-derived myeloma tumors. Significant differences in the distribution of genes in the two mouse strains were found. Furthermore, the genes coding for the predominant IAP RNA in the myeloma tumors MOPC315 and MOPC104E were found to represent a very small portion (2%) of the total number of IAP genes in the mouse. These genes have been amplified two- to fourfold in the myeloma tumor DNA.

MATERIALS AND METHODS

DNA was extracted from mouse tissue as previously described (28). Restriction enzymes were purchased

from Bethesda Research Laboratories, and digestions were performed according to the instructions of the supplier. DNA fragments were electrophoresed in 0.8% agarose gels and transferred to nitrocellulose filters by the method of Southern (34). Nick translation was carried out by the method of Rigby et al. (31), with DNA fragments isolated from low-melting-point agarose gels by a procedure from Bethesda Research Laboratories.

Filter hybridization was performed in a solution containing 50% formamide, 5× SSC (1× SSC is 0.15 M NaCl plus 0.015 M sodium citrate), 0.02 M sodium phosphate (pH 6.8), 1× Denhardt solution (12) containing 0.02% bovine serum albumin, 0.02% Ficoll, and 0.02% polyvinylpyrrolidone for 24 h at 42°C. Filters were washed one time at room temperature with 2× SSC–0.1% sodium dodecyl sulfate and two times at 65°C with 0.1× SSC–0.1% sodium dodecyl sulfate before autoradiography. The sizes of the digested fragments (in kb) were determined from the *Hin*dIII digest of λ*c*I857 DNA on the same gel. The autoradiographic response was determined by scanning the film with a Joyce-Loebl densitometer. A haploid genome size of 3×10^9 base pairs (25) was used for the titration experiment calculations.

Recombinant DNA clones containing IAP genes were isolated from a gene library of MOPC104E DNA in the Charon 4 phage vector system (3). To prepare the library, a series of partial *Eco*RI digests of tumor DNA were size fractionated on a 10 to 40% sucrose gradient and the 15- to 20-kilobase-pair (kbp) region was used for in vitro packaging with the Charon 4 arms by the method of Sternberg et al. (35). The phage were screened by using the Benton-Davis technique (1), and individual clones were plaque purified two to three times. Heteroduplexes were prepared by the method of Davis et al. (10).

RESULTS

Quantitation of the major IAP gene family. IAPs are derived from a highly reiterated family of approximately 1,000 genes interspersed throughout the mouse genome (23). When mouse DNA is analyzed by the Southern blot technique (34) after digestion with enzymes that recognize conserved sequences within IAP genes, distinctive bands of hybridization are observed (20, 24; Cole, unpublished data). The different bands are due both to the loss of some restriction enzyme sites and to larger sequence differences among various types of endogenous genes (20, 28). The objective of this study was to determine how the IAP genes are distributed among these various forms, with particular emphasis on the genes expressed in myeloma tumors as discussed below. A "reconstruction" Southern blot technique was used to estimate the number of gene copies in each of the prominent bands. This involved the mixing of a known amount of cloned IAP DNA with *Xenopus* DNA, a heterologous carrier; the DNAs were digested with restriction enzymes and electrophoresed in parallel with mouse DNA that was digested with the same enzymes. The DNAs were then transferred to a nitrocellulose filter and hybridized with a nick-translated probe containing a specific fragment of the IAP gene, and the extent of hybridization was then quantitated by scanning the autoradiograph with a densitometer. By titrating the amount of cloned DNA added to the heterologous carrier, the number of copies in each unknown band could be estimated.

The most informative restriction enzyme digest for studying IAP genes is *Eco*RI plus *Hin*dIII, both of which recognize conserved sites within IAP genes. After digestion of mouse DNA with these enzymes, the most abundant type of IAP gene, denoted as type I here, gave rise to three prominent bands of 5.8, 5.3, and 4.2 kbp in a Southern blot (Fig. 1). Numerous cloned examples of this type of gene have been described by Kuff and co-workers (20, 24) as well as by ourselves (28). There were three conserved *Eco*RI sites in one group of type I genes, giving rise to a 4.2-kbp *Eco*RI-*Hin*dIII band. However, when the rightmost *Eco*RI site was missing, a 5.3-kbp band resulted, whereas when only the leftmost *Eco*RI site remained, a 5.8-kbp fragment was generated. Thus, all three of these prominent *Eco*RI-*Hin*dIII bands arose from minor sequence variations in a single type of IAP gene (Fig. 1).

IAP genes of BALB/c. The number of gene copies of the 5.3-kbp band was quantitated by using a previously characterized type I clone, clone 81 (28), that has an *Eco*RI-*Hin*dIII fragment of the same size. The autoradiograph exposure was chosen to be in the linear range of the film for scanning, and the area under each of the titration peaks was plotted against the number of copies per haploid equivalent of cloned DNA added (Fig. 2). The radioactive probe in this case was a fragment from the 5′ half of clone 81, which does not hybridize to many of the lower-molecular-weight bands. The experiment was repeated twice with similar results. The number of this type of IAP gene was estimated to be 330 copies per haploid BALB/c mouse genome.

The same titration curve was used to estimate the reiteration frequency of the 5.8-kbp band since the transfer and hybridization efficiency should be the same as for the 5.3-kbp band described above. To study the third band (4.2 kb), another cloned type I gene, clone 17 (28), with a 4.2-kbp *Eco*RI-*Hin*dIII fragment was used for the titration. The same hybridization probe was used for the detection of all three bands. The number of 5.8- and 4.2-kbp type I genes was estimated to be 140 and 245 copies, respectively (Table 1). Repeated experiments gave results that were consistent within ±20%.

IAP genes in *M. musculus* subsp. *molossinus*.

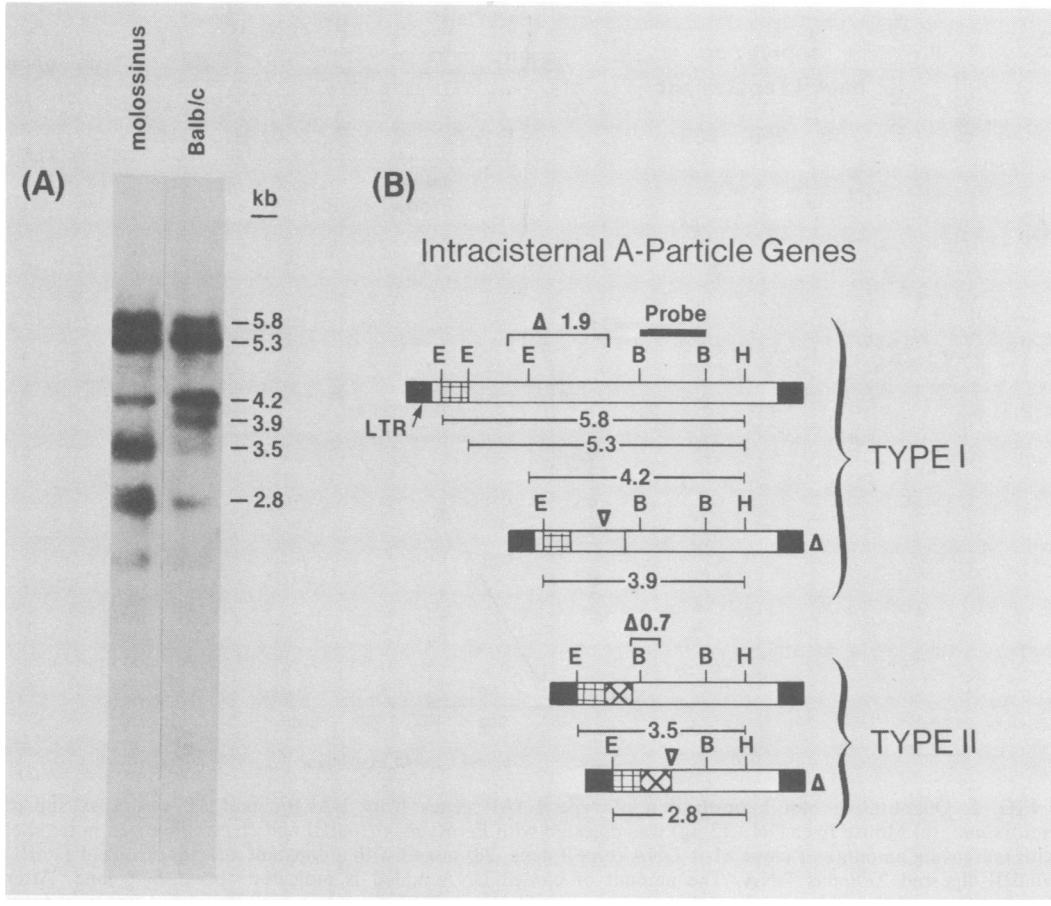

FIG. 1. Major populations of IAP genes. (A) Blot hybridization of DNAs from *M. musculus* subsp. *molossinus* and BALB/c mice after digestion with *Eco*RI and *Hin*dIII and electrophoresis in a 0.7% agarose gel. The radioactive probe used was a DNA fragment isolated from a cloned gene 81-A (*Bam*HI-*Bam*HI; 28) shown in B. (B) Sequence organization of two types of IAP genes and their relationship to the major bands of hybridization. Homologous sequences are indicated by areas of similar shading as determined previously (28). The deletions identified in this study that give rise to the 3.9- and 2.8-kbp *Eco*RI-*Hin*dIII genes are indicated (Δ 1.9 and Δ 0.7, respectively). Some conserved restriction enzyme sites are shown: E, *Eco*RI; B, *Bam*HI; and H, *Hin*dIII. LTR indicates the long terminal repeat of 0.37 kb (7).

DNA from the Japanese subspecies *M. musculus* subsp. *molossinus* was analyzed in parallel in each of the experiments above. After digestion with *Eco*RI and *Hin*dIII, *M. musculus* subsp. *molossinus* DNA gave a pattern of IAP sequences that was very similar to that of the laboratory strain BALB/c, except for significant changes in the intensity of some of the bands. The most striking difference was the absence of the 3.9-kbp band, which will be discussed in more detail below. Since *M. musculus* subsp. *molossinus* DNA had bands at 5.8, 5.3, and 4.2 kbp, it was assumed that they were from IAP sequences identical to those in BALB/c. The numbers of copies of these genes in *M. musculus* subsp. *molossinus* DNA as determined by the reconstruction Southern experiments above were as follows: 5.8 kbp, 250 copies; 5.3 kbp, 290 copies; and 4.2 kbp, 70 copies (Table 1).

The major IAP type I *Eco*RI-*Hin*dIII bands in *M. musculus* subsp. *molossinus* were shifted in intensity as compared with BALB/c with a diminished 4.2-kbp band and an increased 5.8-kbp band. Nonetheless, the sum of the three major bands remained similar in the two subspecies, totaling approximately 600 to 700 endogenous genes. Differential amplification of the sequence variants with the 5.8-kbp-type gene amplified in *M. musculus* subsp. *molossinus* versus the 4.2-kbp type in *M. musculus* could account for this

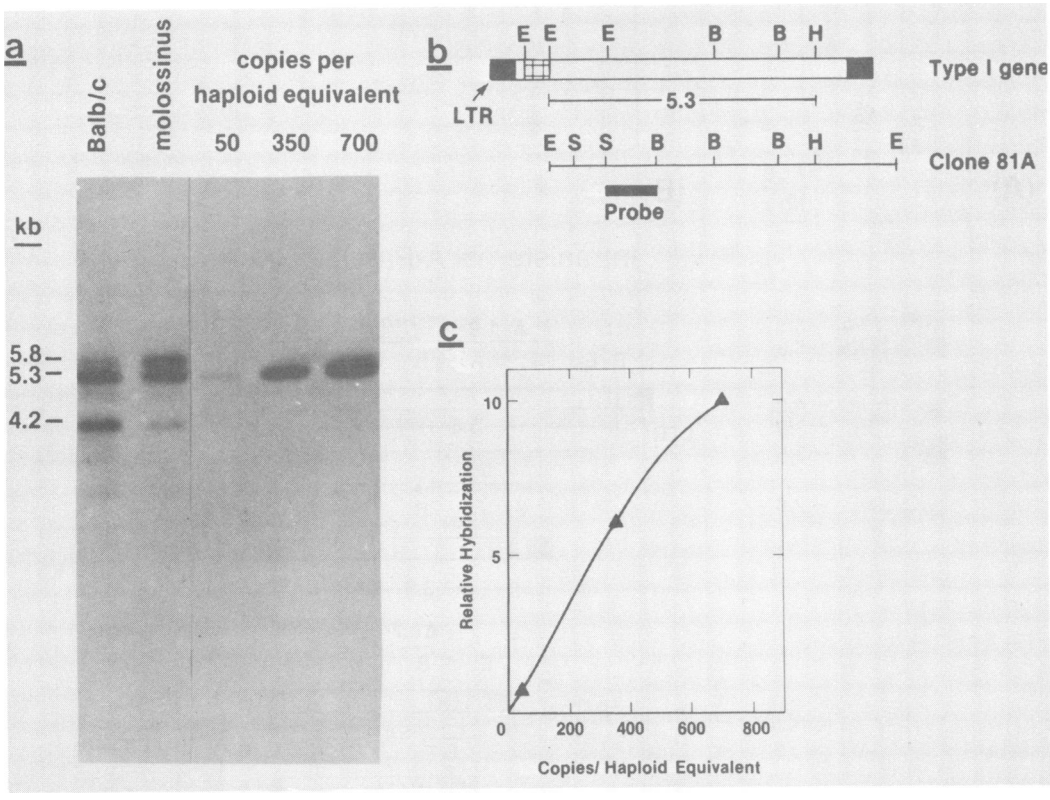

FIG. 2. Quantitative blot hybridization of type I IAP genes from BALB/c and *M. musculus* subsp. *molossinus*. (a) Mouse liver DNA (2 μg) was digested with *Eco*RI plus *Hin*dIII and electrophoresed in parallel with increasing amounts of clone 81-A DNA (type I gene, 28) mixed with a constant amount (2 μg) of *Eco*RI-*Hin*dIII-digested *Xenopus* DNA. The amount of cloned DNA added is indicated above each lane. After denaturation and transfer to a nitrocellulose filter, the filter was hybridized to a nick-translated fragment from clone 81-A. (b) Restriction enzyme map of clone 81-A showing the position of the probe used in this experiment. (c) Autoradiographic response of different amounts of cloned DNA added to a constant amount of *Xenopus* DNA in the titration lanes. Abbreviations: E, *Eco*RI; B, *Bam*HI; H, *Hin*dIII; and S, *Sst*I.

difference. Alternatively, all IAP genes might have been identical at the time of their introduction into the BALB/c-*M. musculus* subsp. *molossinus* progenitor with all of the three *Eco*RI sites present. Subsequent random mutations that led to the loss of the *Eco*RI site(s) could account for the decrease in the number of genes in the 4.2-kbp band and the increase in the

TABLE 1. Different populations of IAP genes in the BALB/c and *M. musculus* subsp. *molossinus* genome

Type of IAP gene	Size of major *Eco*RI-*Hin*dIII DNA fragment (kbp)	Estimated no. of copies per haploid genome[a]	
		BALB/c	*M. musculus* subsp. *molossinus*
I	5.8	140	250
	5.3	330	290
	4.2	245	70
IΔ	3.9	130	0
II	3.5	65	180
IIΔ	2.8	20	70

[a] The estimated total number of copies of the IAP gene sequence was 930 for BALB/c and 860 for *M. musculus* subsp. *molossinus*.

number of the 5.3-kbp or 5.8-kbp bands (or of both).

A prominent 3.9-kbp *Eco*RI-*Hin*dIII band was evident in BALB/c DNA, but not in *M. musculus* subsp. *molossinus* DNA. These IAP genes were shown to be a deletion variant of the 5.8-kbp *Eco*RI-*Hin*dIII type I genes by a combination of Southern blot analysis and molecular cloning. Figure 2 shows that the 3.9-kbp band did not hybridize to a probe from the 5′ half of the type I genes. On the other hand, sequences from the 3′ half hybridized to this band (Fig. 1). To investigate the sequence organization of this gene family, a representative gene was isolated from a recombinant DNA library. Duplicate filter replicas in a plaque screening experiment were assayed for clones that hybridized to the probe in Fig. 1, but not to that in Fig. 2. Individual recombinants were then assayed for the presence of a 3.9-kbp *Eco*RI-*Hin*dIII fragment. One such clone, designated as G2, was analyzed further and found to have the two conserved *Bam*HI sites present in all other IAP genes (data not shown). Identification of this gene as a deletion variant was accomplished by electron microscopy of heteroduplexes formed between the recombinant phage DNA and two different, previously characterized type I genes cloned into pBR322 (Fig. 3). One gene, 81-A, contained a 5.3-kbp *Eco*RI-*Hin*dIII fragment, whereas the other, 71 (28), was a deleted version of a 5.8-kbp *Eco*RI-*Hin*dIII gene. Each plasmid was digested with *Hin*dIII to provide a known restriction enzyme site in the IAP gene and to maintain the linkage between the gene and the pBR322 vector for unambiguous orientation of the molecules. Heteroduplexes formed between clone G2 and *Hin*dIII-digested 81-A showed a duplex region of 3.4 kbp interrupted by a 1.9-kb single-stranded loop. The single-stranded pBR322 tail marked the position of the *Eco*RI site in the plasmid, whereas the double strand-to-single strand transition marked the *Hin*dIII site. With plasmid 71, a duplex region of 3.4 kbp was interrupted at 0.8 kbp to the right of the *Eco*RI site by a single-stranded loop of 0.4 kb. The duplex to the left of the loop with 71 indicated that the 3.9-kbp gene in G2 contained the sequences that occur at the left of the 5.8-kbp IAP gene (Fig. 1). Comparison of the heteroduplexes with the sequence organization of the previously characterized genes gave the sequence for the 3.9 kbp shown in Fig. 3C. This gene is equivalent to a 5.8-kbp gene with a 1.9-kbp deletion. Southern blot experiments with defined gene fragments confirmed that the 3.9-kbp *Eco*RI-*Hin*dIII band in BALB/c DNA was due to multiple copies of the deletion variant described above (data not shown). Unfortunately the IAP gene in clone G2 was interrupted by the bacteriophage λ arm at the *Eco*RI site in the 3.9-kbp gene, so it was not possible to investigate whether the 5′ sequences were the same as in all other type I genes.

Quantitation of type II genes. A second type of IAP gene that contains a sequence of 0.5 kb not found in the genes discussed above has been identified (28) (Fig. 1). Kuff and co-workers found no clones of this type in their studies of IAP genes, yet three of the seven clones isolated in our previous study were of this type. Furthermore, the major IAP RNA in myeloma tumors MOPC104E and MOPC315 is transcribed from these type II genes (28). It was of particular interest to compare the reiteration frequency of the actively transcribed type II genes with the more abundant type I genes analyzed above.

There is no information available as to the origin of the second group of IAP genes. Nearly all of the type II genes that have been cloned to date are only 4.8 kbp in length, as determined by the position of the long terminal repeats (7). This is significantly shorter than the 7-kb type I genes, yet no type II genes longer than 4.8 kbp have been found. Type II genes are thus smaller than any transmissible retroviral genome and may represent a gene formed by deletion or recombination (or both). These genes give rise to a 3.5-kbp *Eco*RI-*Hin*dIII fragment in Southern blots of mouse DNA (Fig. 1). To estimate the number of copies of type II genes, a previously described clone, clone 19 (28), was used for both the titration and the nick-translated probe (Fig. 4). Approximately 65 copies and 180 copies of these genes were found in BALB/c and *M. musculus* subsp. *molossinus*, respectively.

It is evident that the DNA fragment used as a probe in this experiment hybridizes almost exclusively to the type II bands. However, based on heteroduplex experiments (28), essentially the same sequence is found near the 5′ end of all type I genes as well. It is most likely that this differential hybridization stems from divergence of the corresponding sequences in the two gene types that allows formation of duplex regions in the electron microscope but differential hybridization under the stringent washing conditions employed in these experiments (0.1× SSC, 65°C). We have demonstrated previously that divergent sequences are present in the 5′ long terminal repeats of the two gene types which fail to cross-hybridize under the same conditions (7), and the labeled DNA segment in this experiment lies immediately adjacent to the 5′ long terminal repeat. A weakly hybridizing 0.5-kbp fragment also derived from type I genes was present in some experiments, but ran off the bottom of the gel in Fig. 4.

Identification and quantitation of the active genes in two myeloma tumors. The predominant

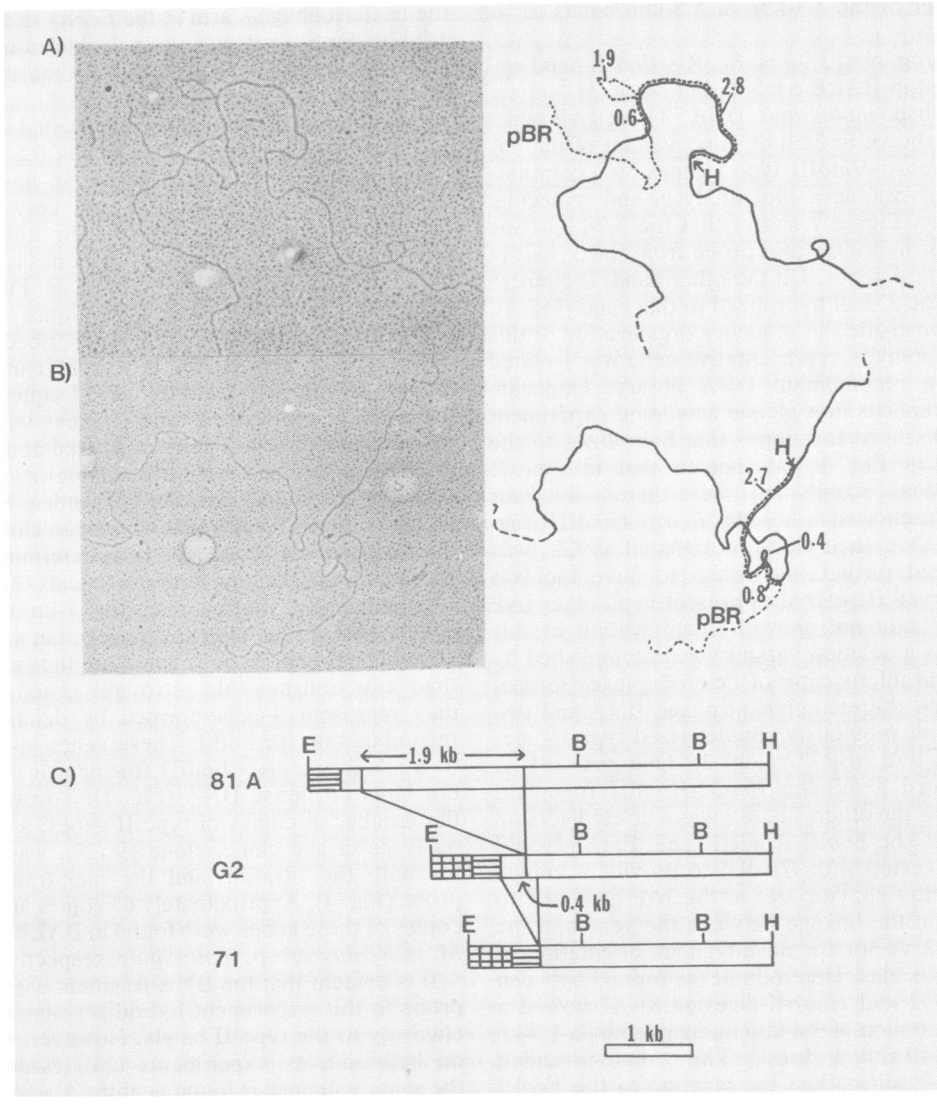

FIG. 3. Heteroduplexes formed between a cloned 3.9-kbp *Eco*RI-*Hin*dIII-digested IAP gene (G2) and two previously characterized type I genes. (A) Heteroduplex with *Hin*dIII-digested clone 81-A (28). A deletion loop of 1.9 kb derived from the 81-A gene is evident, and the *Hin*dIII site is shown by an arrow. (B) Heteroduplex with *Hin*dIII-digested clone 71 (28). A loop of 0.4 kb was derived from unpaired sequences in the 3.9-kbp gene. (C) Sequence organization of clones 81-A and 71 and of the 3.9-kbp gene as deduced from the heteroduplexes. For each combination, the previously characterized IAP gene (subcloned into pBR322) was digested with *Hin*dIII to linearize the molecules and to provide an identifiable restriction enzyme site for measurements. Five to 10 molecules were measured, and linearized plasmid molecules of known length were used as internal standards to determine the length of each segment in kbp. It was determined by preliminary screening and restriction enzyme mapping experiments that the conserved *Bam*HI and *Hin*dIII sites were present in G2. The heteroduplex with 81-A only covers 3.4 kbp of the 3.9-kbp gene, whereas the remainder of the gene forms a heteroduplex with a 0.5-kbp sequence from clone 71 that is not present in 81-A. In a separate experiment, a 1.6-kbp *Eco*RI-*Bam*HI fragment from the left boundary of the 3.9-kbp gene has been isolated and found to hybridize to the 3.9-, 5.3-, and 5.8-kbp IAP bands in total mouse DNA as expected (data not shown).

IAP RNA in MOPC104E and MOPC315 is not colinear with the family of type II genes discussed above, but has a 0.7-kbp sequence missing (28). Type II genes with this deletion are found in both BALB/c and *M. musculus* subsp. *molossinus* DNA and give rise to the 2.8-kbp *Eco*RI-*Hin*dIII band in Fig. 1, 0.7 kbp shorter than the major type II band at 3.5 kbp. To verify

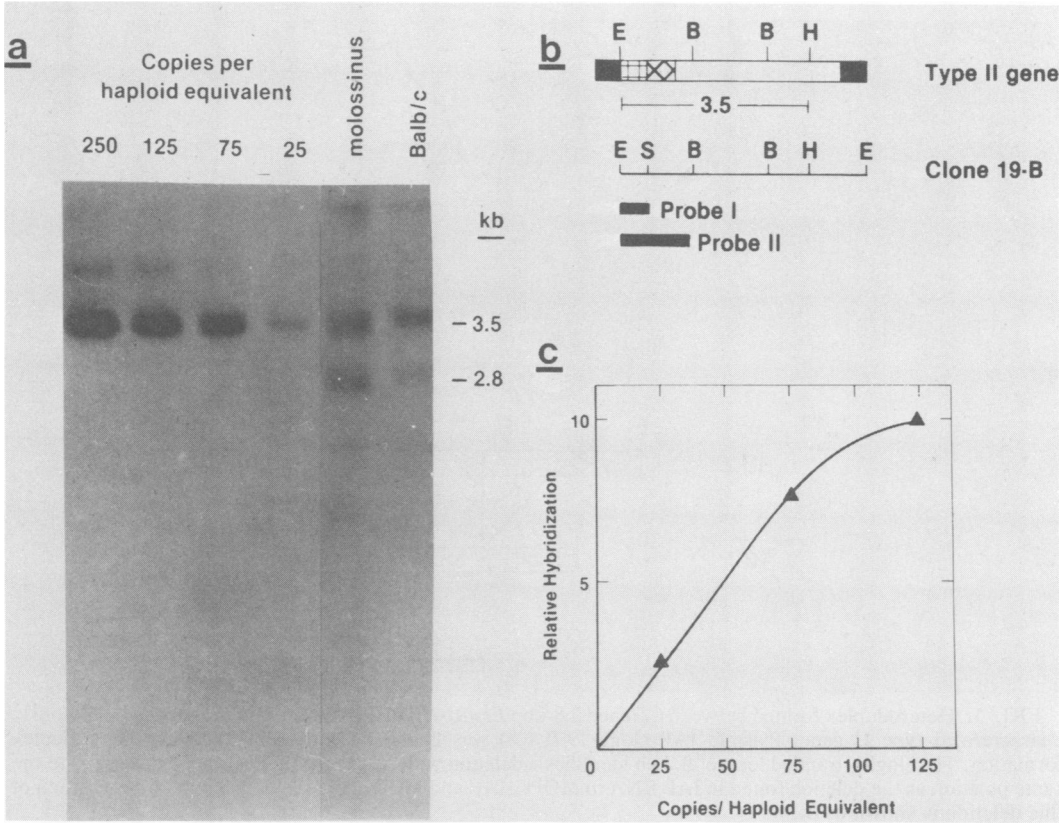

FIG. 4. Quantitative blot hybridization of type II IAP genes. The experiment was performed as in Fig. 2, except that the DNA used in the titration was clone 19 (type II; 28), and the radioactive probe was the fragment shown in b. This experiment was used to estimate the number of both the 3.5- and 2.8-kb *Eco*RI-*Hin*dIII type II IAP genes. (a) Titration of type II genes. (b) Restriction enzyme map of clone 19-B showing position of probe (I) used in this experiment. (c) Autoradiographic response of titration lanes. Abbreviations: E, *Eco*RI; B, *Bam*HI; H, *Hin*dIII; and S, *Sst*I.

this assignment, a gene with a 2.8-kbp *Eco*RI-*Hin*dIII fragment (designated as clone 122) was selected from a library of MOPC104E DNA. Heteroduplexes formed between clone 122 and a previously characterized type II gene (19-B; 28) are shown in Fig. 5. The 19-B plasmid was linearized with *Hin*dIII for the reasons described above. A small single-stranded loop of 0.7 kb in the heteroduplex marked a deletion with respect to the 19-B gene at the same location as identified in the predominant IAP RNA in MOPC104E and MOPC315 (28), verifying the colinearity of the gene and the RNA.

It is assumed that the IAP RNA in these tumors (MOPC104E and MOPC315) is transcribed from these deleted type II genes rather than spliced from a transcript of genes without the deletion. Subgenomic mRNAs with a very small 5′ sequence remaining at one end (8) have been found in other retrovirus-infected cells (16, 36). However, these spliced RNAs are seldom packaged into the virions. Furthermore, there is no evidence that the internal deletion giving rise to the 2.8-kbp *Eco*RI-*Hin*dIII genes is related to any RNA splicing site. The 2.8-kbp band was found to be weak in both BALB/c and *M. musculus* subsp. *molossinus* DNAs, indicating a comparatively low reiteration frequency. The number of genes of this type was estimated from the titration in Fig. 4 to be 20 copies per haploid BALB/c mouse genome. These genes thus represent only about 2% of all IAP sequences in the mouse, yet they are actively expressed in two myeloma tumors that produce entirely different types of immunoglobulin (13, 26). This suggests that the expression of IAP genes in these tumors is not linked to transcription of the immunoglobulin gene.

Increased numbers of IAP genes in myeloma tumors. The expression of this minor population

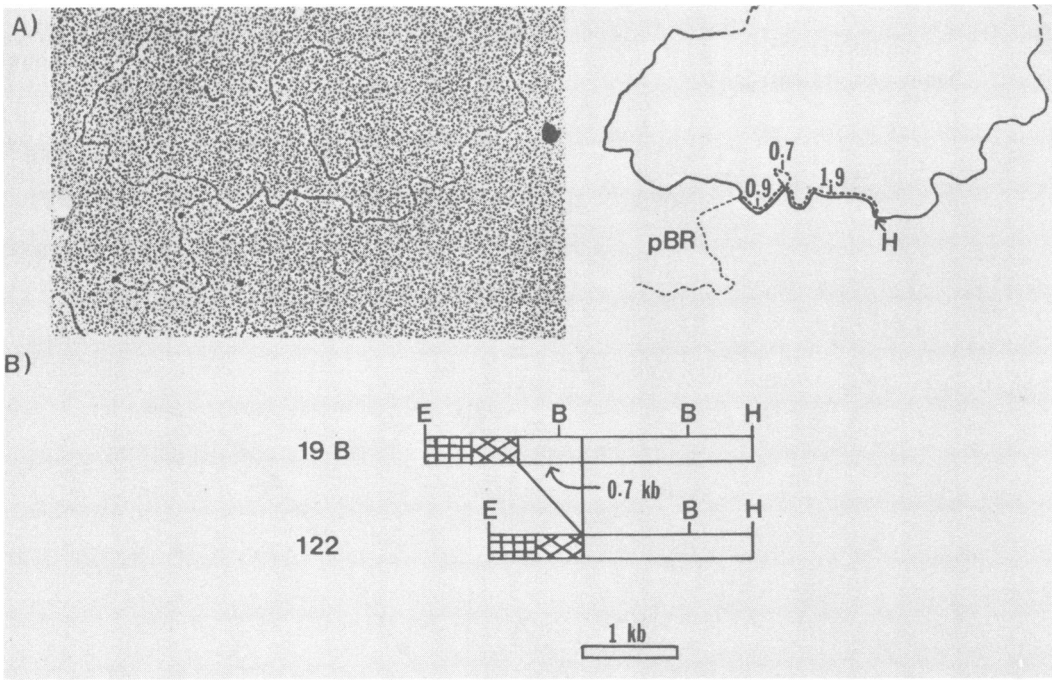

FIG. 5. Heteroduplex formed between a cloned 2.8-kbp *Eco*RI-*Hin*dIII IAP gene (clone 122) and a previously characterized type II gene. Plasmid IAP clone 19-B (28) was linearized with *Hin*dIII before heteroduplex formation. The single-stranded loop of 0.7 kb identifies a deletion with respect to the 19-B gene that maps to the same position as the deletion found in IAP RNA in MOPC104E and MOPC315 (28). A diagram of the position of this deletion is shown in B.

of IAP genes in myeloma tumors prompted an examination of their reiteration frequency in tumor DNA. BALB/c, MOPC104E, and MOPC315 DNAs were digested with *Eco*RI and *Hin*dIII and hybridized with an IAP probe (Fig. 6A). The basic pattern of IAP gene sequences was virtually identical, except that there was a distinct increase in the intensity of the 2.8-kbp band in the myeloma tumor DNAs as compared with BALB/c DNA. This increase was illustrated more clearly by scanning the autoradiographs with a densitometer (Fig. 6B). Approximately a fourfold increase in gene number was found in MOPC315, and a twofold increase was found in MOPC104E. From the previous estimate of 20 copies of the 2.8-kbp gene in BALB/c DNA, this increase would mean 20 and 60 new copies in MOPC104E and MOPC315, respectively. There were no reproducible differences in any of the other IAP bands among these DNAs; however, a small increase in gene number for the more abundant type I genes would not have been detected.

To investigate whether these additional gene copies were integrated into the mouse chromosomes, DNA from MOPC315 was banded in an ethidium bromide-CsCl gradient. Two fractions were collected, one containing the chromosomal DNA and the other from a position in the gradient where closed circular DNA would band. The position of closed circular DNA was determined from a parallel gradient of plasmid and *Escherichia coli* chromosomal DNAs. Virtually no detectable DNA was recovered in the closed circular fraction where unintegrated retroviral DNA would presumably band. The two fractions were digested with *Eco*RI and *Hin*dIII and analyzed by the Southern blot technique in parallel with BALB/c DNA. The probe was the same as that used to identify the additional copies above. Figure 7 shows that there was again increased hybridization to the 2.8-kbp band in the tumor chromosomal DNA, whereas there was no hybridization to DNA from the closed circular fraction. This experiment does not exclude the possibility that the additional copies of IAP genes exist in linear or open circular form within the tumor, but no hybridization to extrachromosomal DNA was found in a Hirt supernatant (18) (data not shown).

DISCUSSION

In this study, the reiteration frequency of different IAP genes was measured in DNAs from

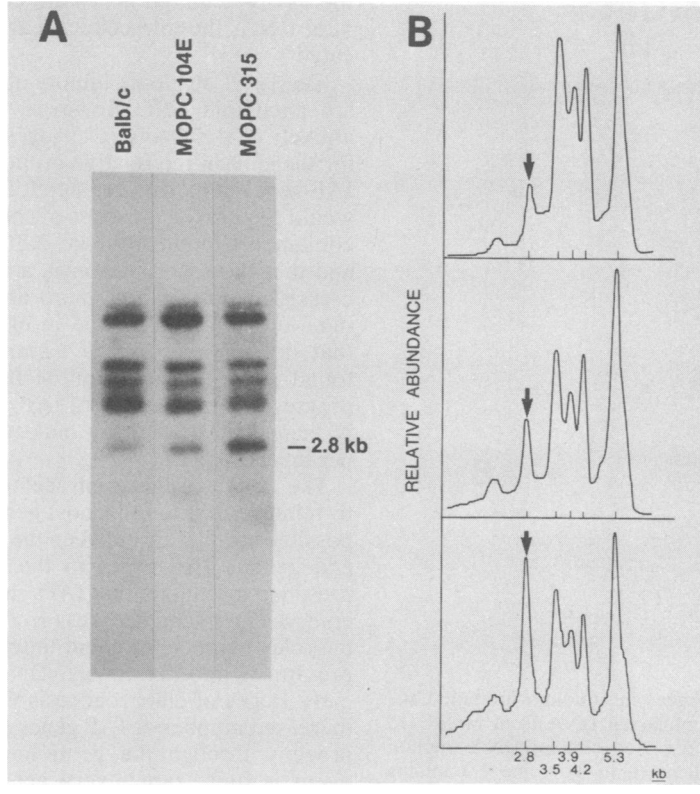

FIG. 6. Increased numbers of type II IAP genes in myeloma tumors. (A) DNAs from BALB/c liver and myeloma tumors were digested with *Eco*RI-*Hin*dIII and analyzed by the Southern blot procedure with a probe from clone 19-B (probe II in Fig. 4b). Tumor DNA was found to have increased hybridization to the 2.8-kbp band. (B) Densitometer tracings of the autoradiographs in A, showing an increased 2.8-kbp band. The small differences between the other IAP bands were not found reproducibly. The relative area under each peak was used to determine the number of additional gene copies.

mice and from myeloma tumors. The most important observation was that the IAP RNA in two different myeloma tumors is not transcribed from the major population of IAP genes in mouse DNA, but instead from a very small subset (~2%) of the endogenous retrovirus-like elements. Furthermore, the number of copies of this gene family has increased two- to fourfold in the myeloma tumor DNA. The Southern blot hybridization technique allowed the detection of relatively small changes in gene copy number that could not be demonstrated with conventional reassociation kinetics.

The IAP RNA in the myeloma tumors examined could be transcribed from one or several of the 40 to 80 endogenous genes colinear with the RNA. However, it is clear that the activity of these genes does not involve a general derepression of IAP sequences in the myeloma tumor, such as by hypomethylation of tumor DNA (6). In the latter case, the type I genes, representing nearly 90% of all endogenous IAP sequences, would be the predominant RNA transcript. Preliminary results indicate that the same type of IAP gene is not active in all myeloma tumors, but in no case does a full-length transcript of the type I genes predominate (work in progress). These arguments would favor the activation of a specific IAP gene(s) in each tumor. How the activation of these genes is related to tumor formation remains to be explored.

It was of particular interest that additional copies of IAP genes exist in tumor DNA. Previous studies with conventional techniques had failed to detect any increases in gene number in IAP-producing cells (23, 28). It is not possible to determine at this time how such an increase occurred; however, it seems most likely to involve the hundreds of retrovirus-like particles present in the cytoplasm of each tumor cell. It is feasible that the reverse transcriptase (37) within the particles could be activated to produce a double-stranded cDNA which subsequently integrated into the host genome. Particle activa-

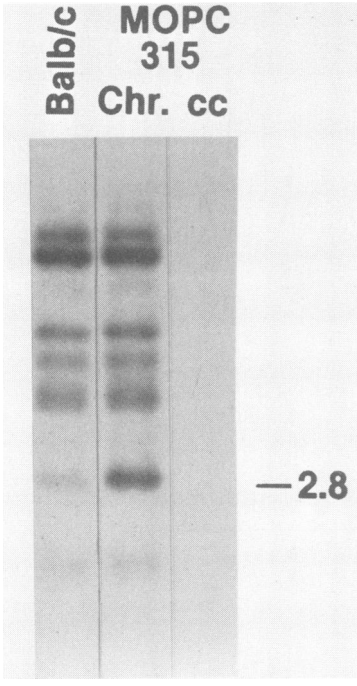

FIG. 7. IAP sequences in ethidium bromide-CsCl gradient-banded chromosomal DNA from MOPC315 (lane 2) and from the closed circular DNA fraction from the same gradient (lane 3). Lane 1 contains BALB/c liver DNA for comparison, and all DNAs were digested with *Eco*RI and *Hin*dIII for the Southern blot. The probe used was the same as for Fig. 6. Only a trace amount of DNA was recovered in the closed circular fraction (<1%). To determine the amount to be loaded in lane 3, the fraction of chromosomal DNA used in lane 2 was calculated (2 μg out of 360 μg recovered from the gradient, i.e., 0.55%). Ten times this amount or 5% of the DNA recovered from the closed circular fraction was applied to lane 3, still with no hybridization. It is quite evident from a comparison with Fig. 6 that all of the increased copies of the 2.8-kbp IAP genes copurify with the chromosomal DNA.

tion may be a rare event since there was no evidence of unintegrated IAP DNA in myeloma tumors. IAP genes have a long terminal repeat containing both 5′ and 3′ RNA sequences (7), and it is quite likely that integration would occur by the same mechanism as in transmissible retroviruses (33). Extracellular virions would presumably not be involved because IAPs are not released from cells (22), nor are they infectious when injected into mice (15, 21). Furthermore, IAP production is not transmissible by cocultivation of producer cells with nonproducer cells (27). Alternative mechanisms of gene amplification such as duplication (32) or transposition (30) cannot be ruled out. However, since the newly acquired genes are colinear with the viral RNA, the intracellular particles are implicated.

Nearly all myeloma tumors of BALB/c origin are aneuploid (29). However, we consider it unlikely that caryotype changes could account for the shift in the relative proportion of variant IAP genes demonstrated here. This explanation would require the presence of chromosomes that contain predominantly the 2.8-kbp IAP genes and that these chromosomes are selectively increased. Furthermore, there are no amplifications of the 2.8-kbp band in other tumor lines that do not express the variant type II gene found in MOPC104E and MOPC315 (work in progress). Reintegration of IAP genes is currently being investigated by molecular cloning experiments.

The ability of these intracellular retroviruses to reintegrate into their host genome suggests a possible correlation between the large number of endogenous IAP genes in the mouse and the presence of numerous IAPs in preblastocyst embryos (2, 5) and oocytes (4). Activation of the particles with subsequent integration of new proviruses into the cellular DNA at these very early stages of embryogenesis would lead to an increased number of IAP genes passed on to the progeny through the germ line. Even if this event is quite rare, every generation of mice could potentially have an increased number of IAP genes.

In agreement with the results of Kuff and coworkers (20, 24), the majority of IAP genes (76%) were found to be virtually identical with respect to conserved restriction enzyme sites. The number of these genes estimated by the reconstruction Southern technique was quite consistent with previous reassociation kinetic studies with either IAP cDNA (23) or segments of cloned genes (28). The Japanese subspecies *M. musculus* subsp. *molossinus* was found to have a similar but somewhat redistributed population of IAP sequences, except for the apparent lack of the 3.9-kbp deleted type I gene. It is not known whether this latter family arose by multiple independent deletion events or by amplification of a single deleted gene in BALB/c.

The results presented here thus suggest that a specific subset of IAP sequences (possibly a single gene) has been activated in myeloma tumors. Furthermore, integration of viral sequences into the host genome is a potentially mutagenic event which could either inactivate a cellular gene or increase the level of gene transcription by promoter insertion (17). How IAP gene transcription relates to the expression of cellular sequences and to the transformation event in myeloma tumors will be an important subject for further investigation.

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