MOLECULAR AND CELLULAR BIOLOGY, Dec. 1993, p. 7439–7446
0270-7306/93/127439-08$02.00/0

Vol. 13, No. 12

Selective Expression of Intracisternal A-Particle Genes in Established Mouse Plasmacytomas

KIRA K. LUEDERS,* JOSEPH W. FEWELL, VIKTOR E. MOROZOV, AND EDWARD L. KUFF

Laboratory of Biochemistry, Building 37, Room 4C-03, National Cancer Institute, Bethesda, Maryland 20892

Received 4 June 1993/Returned for modification 26 July 1993/Accepted 3 September 1993

Mouse plasmacytomas generally express higher levels of RNA transcripts from endogenous intracisternal A-particle (IAP) proviral elements than do lipopolysaccharide-stimulated normal lymphocytes. Lymphocytes express a limited and highly characteristic set of IAP elements (lymphocyte-specific [LS] elements). In this study, we examined whether LS elements are expressed at higher levels after transformation of the cells and/or whether new IAP elements are activated. The IAP elements expressed in plasmacytoma MPC11 were characterized by sequence analysis of 22 cDNA clones. The long terminal repeats (LTRs) of the tumor cDNAs proved to be highly related in sequence. None of the clones was of the LS cDNA type. The MPC11 LTRs were five- to sixfold more active than an LS cDNA LTR when tested for promoter activity by transfection into plasmacytoma cells. The LTRs of the tumor-derived cDNAs contained a canonical ATF core sequence (ATF-PC), while the LS cDNAs contained an altered sequence (ATF-LS). An ATF-PC oligonucleotide probe detected multiple IAP transcripts on Northern (RNA) blots of RNA from several plasmacytomas but gave no reaction with RNA from stimulated B lymphocytes. In contrast, an ATF-LS probe detected higher levels of RNA in lymphocyte than in tumor RNAs. Thus, expression of IAP elements in transformed B cells is selective for a different set of regulatory sequence variants than those expressed in normal B cells. Other oligonucleotide probes representing LS- and PC-specific sequence variants detected multiple common hypomethylated IAP proviral loci in three independently derived plasmacytomas. Overall, the results show that established plasmacytomas exhibit a characteristic pattern of IAP proviral hypomethylation and regulatory sequence selection.

Intracisternal A-particle (IAP) proviral elements are 2,000-fold reiterated and widely dispersed in the mouse genome (20). They consist of full-length 7-kb elements as well as elements with deletions of various sizes (20, 26). The 7-kb provirus and deleted variants encode transcripts of characteristic sizes. Expression of IAP elements in normal cells appears to be under genetic control, since strain-related differences both in total IAP RNA levels and the proportion of different-size transcripts have been seen in thymus, pancreatic beta cells, and early embryos (19, 22, 42). IAP elements are flanked by long terminal repeats (LTRs) in which sequences regulating transcription are located (24). The U3 region of the IAP LTR has been shown to contain at least five binding sites for nuclear factors (7).

Individual LTRs of IAP elements isolated from different cell types as cDNA clones, transposed elements, and randomly cloned genomic elements have shown variations in sequence and promoter activity (4, 5, 9, 35, 39). Specificity of IAP proviral expression was first indicated by the isolation of three nearly identical cDNA clones from thymuses of two inbred mouse strains (14). Subsequent sequence analysis of multiple cDNA clones identified a discrete subpopulation as the predominant source of IAP transcripts in normal BALB/c mouse thymocytes and B lymphocytes (31). These elements (designated IAP lymphocyte-specific [LS] elements) shared a unique identical 218-bp U3 regulatory region in their LTRs. Hybridization of genomic DNA with LS element-specific oligonucleotide probes showed that the LS family of IAP elements was present in a restricted number of dispersed copies in mouse genomic DNA (25, 34). IAP expression in normal lymphocytes is thus highly selective.

IAPs are constitutively expressed in many mouse tumors (reviewed in reference 20) and characteristically at high levels in plasmacytomas induced in BALB/c mice. IAP elements in the DNA of transplanted plasmactyomas are extensively hypomethylated in their 5′ LTRs compared with their counterparts in the DNA of normal lipopolysaccharide (LPS)-stimulated B cells (30, 33). Previous studies have shown that full IAP expression requires hypomethylation of the 5′ LTR regulatory regions as well as cellular transcription factors capable of interacting with the particular *cis* element sequence variants in the LTR (9–11, 17, 36).

It has not been known whether the lymphocyte-specific pattern of IAP proviral expression was maintained in the transformed plasma cells or whether additional elements are activated. In this study, we have found that the increased level of IAP expression in plasmacytomas results from activation of a new and characteristic set of IAP elements. By examining individual IAP proviruses, we have shown that multiple, common IAP loci are hypomethylated in at least three established plasmacytomas, leading to the conclusion that such events do not occur entirely randomly. The IAP LTRs which are expressed in plasmacytomas provide a set of reporter genes that can be used to detect hypomethylated chromosomal regions that are characteristic of these transformed cells.

MATERIALS AND METHODS

Probes and oligonucleotides. The 5.2-kb internal *Eco*RI-*Hin*dIII fragment from IAP element pMIA1 (26) was used as a total IAP probe. A 700-bp fragment that included the 3′

* Corresponding author.

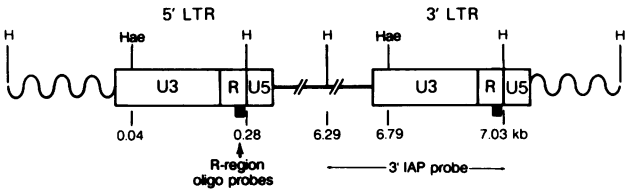

FIG. 1. Partial restriction map of the IAP element ends. Internal sequences are shown as an interrupted heavy line, LTRs are shown as open boxes, and flanking sequences are shown as wavy lines. Restriction sites: H, *Hin*dIII; Hae, *Hae*II. Numbers below the diagram refer to positions on the IAP MIA14 map. Black boxes show the locations of the R-region IAP subclass oligonucleotide probes. The position of the 3' IAP probe used to scan the cDNA library is also indicated.

LTR from IAP element MIA14 (26) was used as the 3' IAP probe (Fig. 1). Custom-synthesized oligonucleotides were provided by Juanita Eldridge, Laboratory of Biochemistry, National Cancer Institute. Sequences for the gt10 primers were identical to those of NEB#1231 and NEB#1232 (New England Biolabs). Additional oligonucleotides for sequencing were based on sequences in IAP element MIA14 (32). Sequences of other oligonucleotides were as follows: LS1, CTTACATCTTTTTGGGGCCAGAG; LS2, CTTACATCT TTATCGGGCCAGAG; LS3, CTTACATCTTTATGGGGC CAGAG; T1, ACATCTTCAGGAAAAGAGAGCAA; ATF-PC (+), TGCTCTGCCTTCCCCGTGACGTCAACTCGG CCGA; and ATF-LS (+), TACTCTGTTTTTCCCGTGAAC GTCAGCTCGGCCA.

cDNA isolation, amplification, and sequencing. An oligo(dT)-primed cDNA library from the plasmacytoma MPC11 4N2.1 cell line made as described by Bergsagel et al. (1) was provided by W. Michael Kuehl, National Cancer Institute. The library was screened with the 3' IAP probe labeled by nick translation with [α-^{32}P]dCTP by using a kit from Promega, Madison, Wis. Positive phage plaques were picked as agarose plugs, suspended in 500 μl of storage buffer (0.1 M NaCl, 0.5 M Tris-HCl [pH 7.9], 0.1 M $MgCl_2$, 0.01% gelatin) saturated with chloroform, and stored at 5°C overnight before use. The cDNA inserts were amplified by using gt10 primers and 10 μl of the phage suspensions in 100-μl polymerase chain reaction mixtures. In addition, the reaction mixtures contained 2 U of *Taq* polymerase (Promega), reaction buffer supplied by Promega, 0.1 mM deoxynucleoside triphosphates, and 400 ng of each primer. The amplification conditions were as follows: (i) 94°C, 10 min; (ii) 55°C, 1 min; (iii) 72°C, 2 min; (iv) 94°C, 1 min; (v) repeat steps ii to iv 30 times; (vi) 55°C, 1 min; (vii) 72°C, 10 min; (viii) cool to 5°C. A PTC-100 programmable thermal cycler (MJ Research, Watertown, Mass.) was used. Five-microliter aliquots of the reaction products gave a strong stain with ethidium bromide when analyzed on an agarose minigel. The products were precipitated with equal volumes of 4 M ammonium acetate plus 2 volumes of isopropanol (15). After being washed in 75% ethanol, the precipitated DNAs were dissolved in 10 μl of TE (10 mM Tris-HCl [pH 7.5], 1 mM EDTA), and 2 μl was used for sequencing. The Sequenase kit (U.S. Biochemical) was used. The DNAs were mixed with at least 50 ng of sequencing primer in labeling buffer in a volume of 10 μl, heated in a boiling water bath for 10 min, and immediately frozen in a dry ice-alcohol bath (15). The labeling reaction was for 3 min at room temperature, and the termination reaction was for 10 min at 42°C. After the reaction, the samples were heated at 80°C and analyzed on denaturing 8% acrylamide gels. The gels were dried without further treatment and exposed to film 24 to 72 h. The sequence data were compiled on an IBM PC, using Microgenie software (Beckman, Palo Alto, Calif.).

LTR-chloramphenicol acetyltransferase (CAT) plasmid constructs. *Hin*dIII fragments (from positions 6292 to 7029 on the IAP MIA14 sequence) that included the 3' LTR were isolated from IAP cDNAs and cloned into the *Hin*dIII site of plasmid pSV0CAT as previously described (24). Constructs were cut with *Pst*I (at 6908 on the IAP sequence) to determine orientation of the insert.

Transfection and CAT activity measurement. S194 cells provided by L. Staudt, National Institutes of Health, were cultured in RPMI medium supplemented with 10% fetal bovine serum and 50 μM β-mercaptoethanol. Transfection was by a DEAE-dextran procedure using 10^7 cells and 10 μg of plasmid DNA per transfection. Cells were washed once with medium without serum and once with TB (0.8% NaCl, 25 mM Tris-HCl [pH 7.4], 5 mM KCl, 375 mM NaH_2PO_4). The DNA to be transfected was added to 0.95 ml of freshly prepared TMC (TB containing 100 μg each of $MgCl_2$ and $CaCl_2$ per ml) mixed with 0.05 ml of dextran sulfate (10 mg/ml). The pelleted cells were resuspended in this mixture and incubated at room temperature for 15 min. After addition of 10 ml of complete medium, the cells were incubated for 30 min at 37°C. The cells were then centrifuged, washed once with complete medium, resuspended, and cultured for 48 h. Cell extracts were prepared by freezing and thawing the cells three times and then subjecting them to a low-speed centrifugation to remove debris. CAT activity was assayed as previously described (24).

Northern (RNA) blots. The Northern blots were prepared as described by Mietz et al. (31). RNAs for the Northern blot in Fig. 6 were provided by Frederic Mushinski, National Cancer Institute, except for MPC11 RNA, which was prepared as previously described (31). The oligonucleotide probes were labeled by tailing with [α-^{32}P]dCTP (3,000 Ci/mmol; NEN) and hybridized as described previously (25). The blot in Fig. 4 was washed in 2.4 M tetramethylammonium chloride at 43°C (29), and the blot in Fig. 6 was washed in 3.2 M tetramethylammonium chloride at 61°C (25).

Hybridization to immobilized DNA in agarose gels. BALB/c mouse liver and plasmacytoma DNAs were digested with *Hin*dIII as previously described (25). One half of each sample was then digested with *Hae*II. The DNAs were electrophoresed on 0.9% agarose gels, which were subsequently dried and hybridized with oligonucleotide probes as described previously (25).

RESULTS

Isolation of IAP cDNAs from a plasmacytoma library. An oligo(dT)-primed cDNA library from MPC11 in lambda phage gt10 was screened with a 700-bp IAP 3' probe that included the LTR (3' IAP probe; Fig. 1). The inserts were amplified from 50 positive plaques by polymerase chain reaction with gt10 primers and ranged in size from 0.7 to 1.9 kb. Samples with multiple inserts were plaque purified until each plaque yielded a single polymerase chain reaction product that reacted with the 3' IAP probe.

Characterization of U3 regions of the cloned LTRs. Amplified DNAs from 22 of the MPC11 IAP cDNAs were sequenced and found to represent two very closely related IAP classes on the basis of their U3 regions (Fig. 2). The plasmacytoma cDNAs within each class shared 98 to 100%

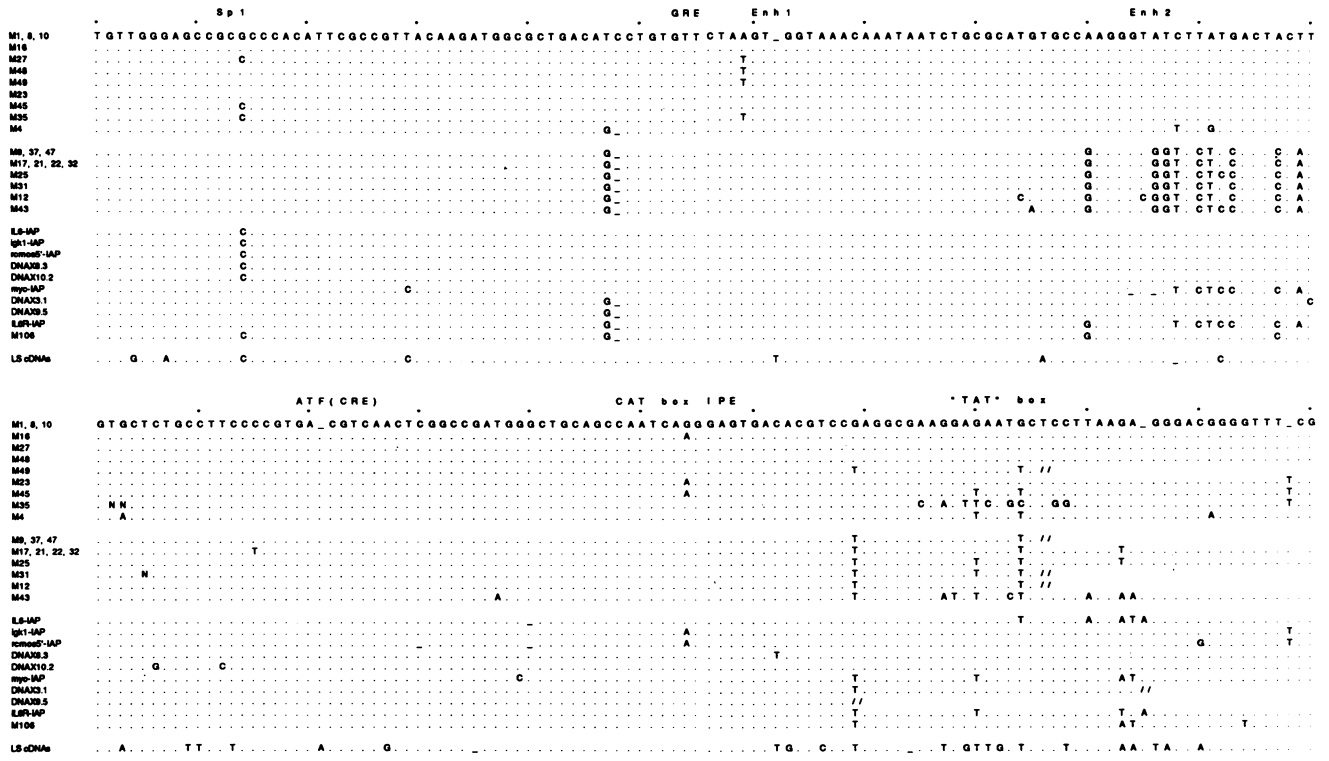

FIG. 2. Nucleotide sequence comparisons of the U3 regions of 22 MPC11 cDNA clone LTRs and other expressed or recently transposed IAP element LTRs. MPC11 clones are designated by M number and are grouped into classes PCI (first nine lines) and PCII (following six lines) distinguished by differences in the Enh2 sequences. cDNAs with identical sequence are listed on the same line. Dots above the sequence mark 10-base intervals. The putative regulatory signals for transcription are indicated above the sequence. Sequence identities are shown by dots, gaps are shown by dashes, and substitutions are indicated. The origins of the 11 previously reported LTR sequences are as follows: hybridoma 23B6 cDNAs DNAX8.3 (28), DNAX10.2 (40), DNAX3.1 (23), and DNAX9.5 (30); plasmacytoma MOPC315 cDNA M106 (23); transposed elements r*cmos*5′-IAP (3), IL6-IAP (2), IL6R-IAP (38), igk1-IAP (16), and *myc*-IAP (13); and B-lymphocyte LS cDNAs (31).

homology, most of the differences consisting of single base changes. The two plasmacytoma cDNA classes (designated PCI and PCII) were distinguished primarily by sequences in the Enh2 domain (Fig. 2). IAP LTRs contain two domains, designated Enh1 and Enh2, both of which have sequence homology with the simian virus 40 enhancer core motif in many LTRs. However, in some elements, the Enh2 domain has a variant sequence that has been shown to confer higher promoter activity both in vitro and when the LTRs were transfected into human 293 cells (9). Eleven of the MPC11 cDNAs had an Enh2 domain with homology to the simian virus 40 enhancer core (PCII), while the other 11 cDNAs had the variant sequence (PCI). Examples of both classes have been seen among IAP elements expressed in other plasmacytomas and hybridomas. Sequences for the U3 regions of these elements are shown in Fig. 2. The PCI MPC11 cDNA class closely resembles an IAP element (IL6-IAP) that had transposed into the interleukin-6 growth factor gene in this plasmacytoma (2).

The LTRs of IAP elements expressed in MPC11 (and other plasmacytomas) differed markedly from the LS-type LTRs expressed in normal B cells (Fig. 2). Differences were found in recognized nuclear factor binding sites such as Enh1, Enh2, ATF/CRE, and TATA sites. While a canonical ATF/CRE core sequence, TGACGTCA, was present in all of the plasmacytoma IAP clones, the sequence TGAA CGTCA was found in every LS LTR. In addition, six other single nucleotide differences were present in sequences flanking the core ATF site. Very few IAP elements have a classical TATA box motif; GATAA or GAGAA are most common in the plasmacytoma cDNAs, while the LS IAP class has GGTTG in this domain. Sequences flanking the TATA box also varied considerably.

Comparative promoter activities of PC and LS LTRs in plasmacytoma cells. LTRs representing the two plasmacytoma IAP classes (PCI type cDNA 45 and PCII type cDNA 17, distinguished by different Enh2 sequences) were cloned upstream of the CAT gene and tested for the ability to promote expression after transfection into S194 plasmacytoma cells (Fig. 3). The two types of PC LTR promoted CAT activity to similar extents, suggesting that sequence differences in the Enh2 domain did not have a major determining effect on the IAP promoter activity in these cells. The LS-type LTR (clone 117) was five- to sixfold less effective than the tumor LTRs in promoting CAT activity in the plasmacytoma cells, even though the Enh2 domain in the LS LTR was similar to that in the PCI LTR.

A canonical ATF/CRE binding site is characteristic of the IAP elements newly activated in plasmacytomas. All of the IAP cDNAs isolated from MPC11 contained a canonical ATF core motif, while all of the LS clones contained a variant sequence. RNAs from S194 and MOPC21 plasmacytomas and from LPS-stimulated spleen (normal B lymphocytes) were hybridized with 34-nucleotide probes specific for both the canonical ATF/CRE binding site from the plasmacytoma cDNAs (ATF-PC) and the mutated site from the

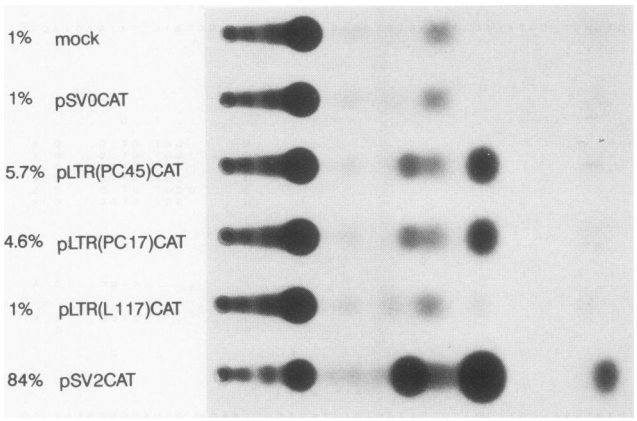

FIG. 3. Promoter activity in S194 cells of IAP LTRs expressed in plasmacytoma and B lymphocytes. The indicated plasmids (10 μg) were transfected into S194 cells, and extracts were prepared 48 h later. The conversion of chloramphenicol to acetylated form is shown for constructs in which the LTR is derived from cDNAs expressed in plasmacytoma, PC45 and PC17, or from a cDNA expressed in B lymphocytes, L117 (31). PC45 represents a member of the PCI class of clones and PC17 represents a member of the PCII class of clones, which differ in the Enh2 sequence. The percent conversions were determined by liquid scintillation counting. Two such experiments gave essentially identical results.

B-lymphocyte cDNAs (ATF-LS) (Fig. 4). The ATF-PC probe detected high levels of 7.2-, 5.4-, and 4.0-kb IAP transcripts in the plasmacytomas but gave no reaction with B-lymphocyte RNA. Hybridization with the ATF-LS probe detected 7.2- and 5.4-kb IAP transcripts from both sources. Hybridization with a total IAP probe showed higher levels of IAP RNA in the plasmacytoma than in B lymphocytes, as expected.

Analysis of R regions of the IAP LTRs in MPC11 cDNA clones. The R regions of IAP LTRs are highly variable both in sequence and in length as a result of different numbers of CT-rich repeats (4, 31). Fourteen of the MPC11 plasmacytoma cDNAs could be sequenced in the R region of the LTR; the remaining clones were truncated at an *Eco*RI site at the end of U3. The MPC11 clones had variable numbers of CT-rich repeats (not shown) which differed somewhat in sequence from those present in the LS IAP elements (31).

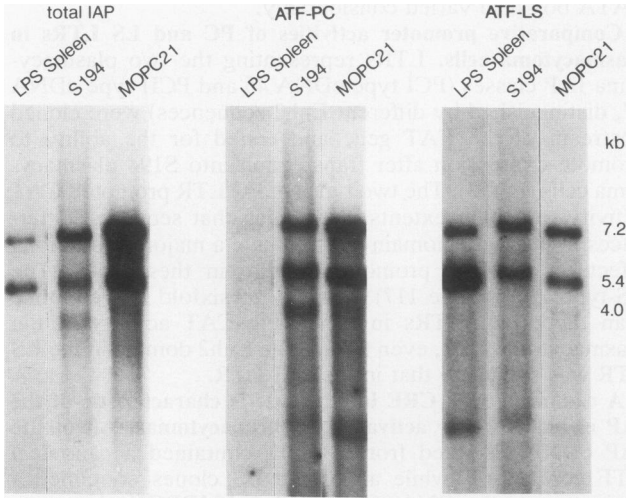

FIG. 4. Northern blot hybridized with ATF-PC and ATF-LS probes. Poly(A) RNAs (5 μg per lane) from LPS-stimulated BALB/c mouse spleen and from S194 and MOPC21 plasmacytomas were hybridized with the indicated probes as described in Materials and Methods.

The cloned LTRs from normal lymphocytes (LS LTRs) could be subclassified into three groups, LS1, LS2, and LS3, on the basis of single nucleotide differences in a characteristic nine-nucleotide motif upstream of the poly(A) signal (Fig. 5, shaded) (31). The MPC11 cDNA clones exhibited eight related variations in sequence in this general region, none of which corresponded to the LS motifs. Five of the variants (clones M32, M10, M25, M35, and M31) were found as single examples, although the clone M32 and M10 variants have been found in IAP elements from other sources (see examples in Fig. 5). The remaining nine MPC11 cDNA clones fell into three closely related groups (designated T4 [four cDNAs], T1 [three cDNAs], and T3 [two cDNAs] variants). A number of IAP elements that have been isolated from other tumors also fell into the PCI and PCII classes and included the same three major T variants as those expressed in MPC11 plasmacytoma. Among these were 8 IAP elements isolated from other plasmacytomas and 15 IAP elements isolated from other types of transformed cells, including many of the IAP elements that have undergone transposition into growth factor genes, particularly in monomyelocytic leukemia cell lines (reviewed in reference 18). Examples of these are listed in Fig. 5. Variant sequence motifs in the R region did not correlate with either Enh2 motif in the U3 region of the LTR (see Fig. 8).

IAP element expression in other plasmacytomas. Poly(A) RNAs from six plasmacytomas in addition to MPC11 were analyzed on Northern blots by hybridization to the T1 and LS R-region oligonucleotide probes (Fig. 6). All of the plasmacytomas except TEPC1194 reacted with the T1 probe. RNA from TEPC1194 also showed very little reaction with a general IAP probe (37). Expression varied with respect to both the size of transcripts (7.2, 5.4, and 4.0 kb) and intensity of hybridization. The tumors also expressed an RNA of approximately 2 kb which could represent a spliced *env* message. These results show that while the T1 variant of IAPs is frequently expressed in established plasmacytomas, the proportion of RNA derived from this subclass can vary among tumors.

Figure 6 also shows the levels of expression of LS elements in these plasmacytomas as detected with the R-region probes, LS1, LS2, and LS3. Expression was variable with respect to total hybridization and relative abundance of transcripts of different sizes. However, it is clear that LS elements continue to be expressed in the majority of these plasmacytomas.

Hypomethylation of genomic IAP elements in plasmacytomas is selective for common loci. Methylation of a *Hae*II site in the LTR inhibits expression of cloned IAP elements (10). A survey of hypomethylated IAP loci by using two-dimensional gel electrophoresis has shown that while most of the IAP LTRs are methylated at the *Hae*II site in the DNA of normal cells, an increased level of hypomethylation is seen in plasmacytomas (30, 33). The use of R-region oligonucleotide probes now permits us to determine the methylation status of individual IAP loci as follows.

To identify individual IAP proviruses, we have previously used restriction endonuclease fragment patterns derived from 5′ proviral junctions (25, 34). The junction fragments are created by *Hin*dIII sites in the flanking DNA and at a

	R-region sequences	# clones from PCs	# clones total	Examples
T4	TTGCACTCTGGCTCCTGAA_GATGTAAG	7	11	cDNAs M4, M8, M43, M48, DNAX9.5, E7.12; Igk1-IAP
T1	C.....T_T.....	4	11	cDNAs M17, M23, M45, DNAX3.1; IL3-IAP
T3	.....C.....	5	11	cDNAs M1, M27, DNAX8.3; IL6-IAP, IL6R-IAP, rcmos5'-IAP
GS4	..A.....T_.....	2	10	cDNAs M32, M106; MIA14, myc-IAP
M10	.....CT.....	1	3	cDNA M10; 81C
M25	..A..T...T.....	1	1	cDNA M25
M35	.....T.....	1	1	cDNA M35
M31	.....	1	1	cDNA M31
LS1	..A.....C..AA..A.....	0	22	8 B-lymphocyte cDNAs, 14 T-cell cDNAs
LS2	..A.....C.GAT..A.....	0	9	5 B-lymphocyte cDNAs, 4 T-cell cDNAs
LS3	..A.....C..AT..A.....	0	3	3 B-lymphocyte cDNAs

FIG. 5. Nucleotide sequence comparison of cDNA R regions. Sequences found upstream of the poly(A) addition signal in MPC11 cDNAs are shown; the three sequence variants found in cDNAs expressed in normal lymphocytes (LS1, LS2, and LS3) are also shown. The nine-nucleotide motif characteristic of the LS class of elements is shaded. The number of IAP clones with a particular sequence that have been found expressed or transposed in other plasmacytomas and the number of IAP clones that have been found in all types of cells are indicated. Examples of clones containing the sequence are listed, with cDNAs shown first and separated from genomic sequences by a semicolon. Origins are as given in the legend to Fig. 2 plus the following: MIA14 (32), IL3-IAP (41), E7.12 (39), and 81C (4).

conserved position immediately downstream of the probe sequences in the IAP 5′ LTR. The corresponding *Hin*dIII site in the 3′ LTR eliminates detection of 3′ flanks (Fig. 1). The locations of the conserved *Hae*II site and the oligonucleotide probe in the 5′ LTR are shown in Fig. 1. When *Hin*dIII-cut genomic DNA is digested with *Hae*II, any fragment with a hypomethylated *Hae*II site in the LTR or close to it in the flanking DNA will disappear. If a *Hae*II site some distance from the LTR in the flanking DNA is cut, a shorter fragment may appear. An analysis of DNAs from three established plasmacytomas with the T1 and LS2 probes is shown in Fig. 7. Liver DNA is shown cut with *Hin*dIII only since none of the fragments was cut with *Hae*II; the single LS2 fragment that is hypomethylated in normal lymphocytes is marked with an asterisk. Comparison of the *Hin*dIII and *Hin*dIII-*Hae*II patterns for the plasmacytoma DNAs revealed multiple hypomethylated elements, many of them present in all three plasmacytomas. One of the LS2 elements corresponded to the hypomethylated element in normal cells and may represent a constitutively hypomethylated locus. Relatively few new fragments appeared in the *Hin*dIII-*Hae*II digests, indicating that most of the hypomethylated sites are in the LTR or quite close to it in the flanking DNA.

The plasmacytoma patterns also showed additional *Hin*dIII fragments that probably represent newly inserted elements. IAP transpositions are known to have occurred in all of these plasmacytomas (reviewed in references 18 and 20). Two of the newly inserted LS2 elements in MOPC104E were hypomethylated (marked with solid and open triangles in Fig. 7). These results show that hypomethylation of IAP elements in plasmacytomas follows a pattern in which certain loci are repeatedly selected.

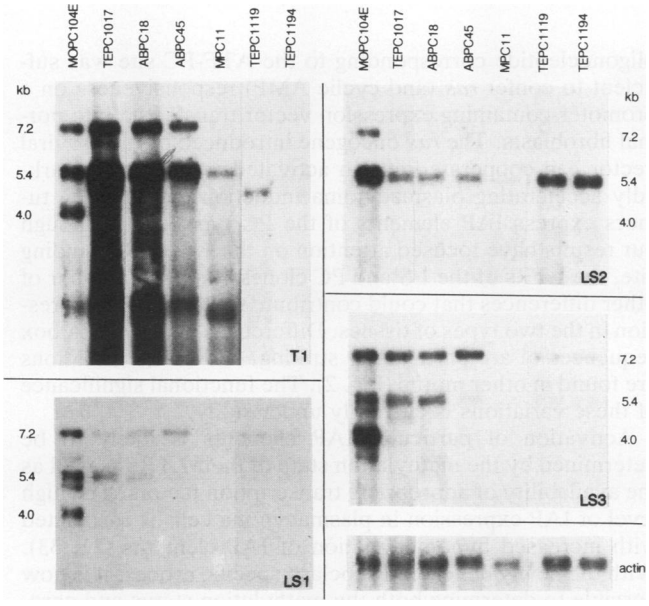

FIG. 6. Northern blot of plasmacytoma RNAs hybridized with IAP R-region oligonucleotide probes. Poly(A) RNAs (10 μg per lane) were hybridized to T1, LS2, LS3, and LS1 probes in succession as described in Materials and Methods; probes represented the complement of the sequences shown for the cDNAs in Fig. 5. Hybridization to the actin probe was used to quantitate the amount of RNA loaded; MPC11 RNA is present at a somewhat lower concentration than the other samples.

DISCUSSION

The results show that additional IAP elements are activated in plasmacytoma cells compared with normal LPS-stimulated lymphocytes. Binding sites for many nuclear factors, some not fully characterized, are present in the IAP LTRs (7, 8, 21, 43) and could contribute to preferential activity of various LTRs in different cell types. Expression in plasmacytomas is selective for LTRs carrying a set of regulatory sequence variants different from those expressed in activated lymphocytes. Figure 8 shows in schematic form the relationship between the variant *cis*-regulatory elements in the LS and PC cDNA clones.

The MPC11 clones consist of two closely related classes, PCI and PCII, distinguished from one another by a major sequence difference in the Enh2 domain (Fig. 2). Our sample of 22 cDNAs contained equal numbers of PCI and PCII clones. The Enh2 domain in PCI clones is very similar to that in the LS cDNAs. LTRs of the two types of PC clones had equivalent activities when transfected into S194 plasmacytoma cells, and both were five to six times more effective than the LS LTR. Thus, the Enh2 motif showed no correlation with promoter activities of the LTRs in these cells.

In contrast, the difference in sequence of the ATF/CRE site between PC and LS cDNAs did correlate with the relative activities on transfection into S194 plasmacytoma cells. In earlier work, plasmacytoma MOPC315 was shown to contain high levels of nuclear extract protein that bound to an oligonucleotide containing the same ATF-PC core sequence (7). We have confirmed that this is also the case for S194 cells. However, the generality of this observation and

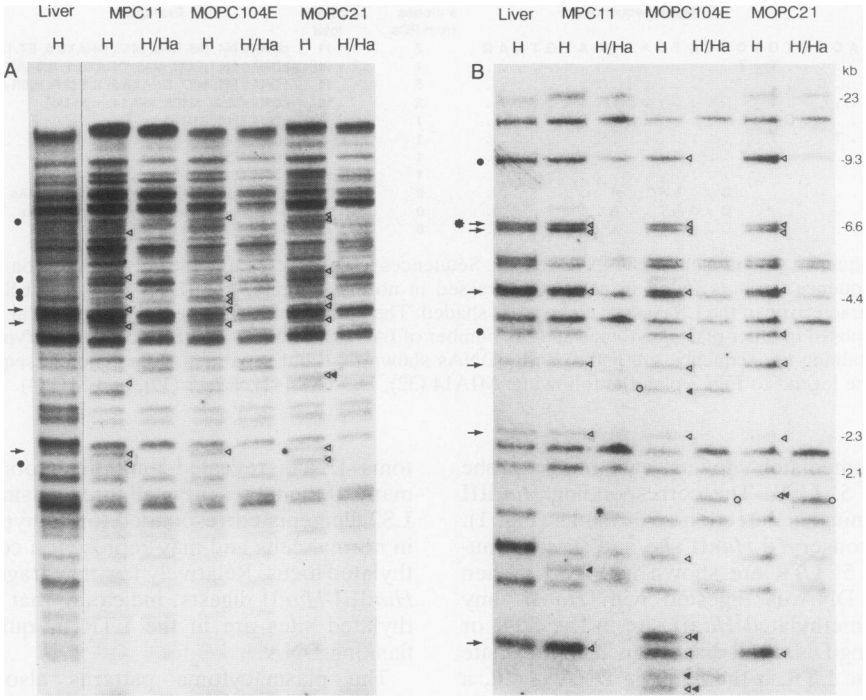

FIG. 7. Gel analysis of IAP subclass-specific hypomethylation patterns in plasmacytomas. DNAs cut with *Hin*dIII alone (H) or with *Hin*dIII and *Hae*II (H/Ha) are shown for three plasmacytomas, MPC11, MOPC104E, and MOPC21. Liver DNA was cut with *Hin*dIII alone, since no demethylated sites were detected with *Hae*II digestion in other experiments (data not shown). Digests were run and hybridized in dried 0.9% agarose gels as described in Materials and Methods. (A) Patterns for DNAs hybridized to the T1 probe; (B) patterns for DNAs hybridized to the LS2 probe. Hypomethylated fragments cut with *Hae*II are marked with open triangles, newly inserted fragments in the *Hin*dIII patterns that were not present in genomic DNA are marked with solid triangles, and newly inserted hypomethylated fragments are marked with both solid and open triangles. New fragments in the *Hin*dIII-*Hae*II pattern that result from hypomethylation upstream of the LTR are marked with open circles. Arrows mark fragments that are hypomethylated in all three plasmacytomas; solid circles mark those hypomethylated in two of the tumors. An asterisk marks the single LS2 fragment that is hypomethylated in normal lymphocytes.

the possible role of the ATF-PC site in IAP expression in plasmacytomas remains to be clarified. Relevant to this question are the observations of Galien et al. (12), who studied the basis for higher IAP expression in mouse fibroblasts transformed with v-Ki-*ras* compared with expression in normal fibroblasts. These authors showed that a 16-bp oligonucleotide corresponding to the ATF-PC site was sufficient to confer *ras* (and cyclic AMP) responsiveness on a promoter-containing expression vector transfected into normal fibroblasts. The *ras* oncogene introduced by a retroviral vector can cooperate with an activated *myc* gene in markedly accelerating plasmacytoma induction (6). Primary tumors express IAP elements of the PC type (27). Although our results have focused attention on the ATF/CRE binding site, the LTRs of the LS and PC clones contain a number of other differences that could contribute to differential expression in the two types of tissues. Differences in the TATA box sequences of are particularly striking, and minor variations are found in other motifs (Fig. 2). The functional significance of these variations is currently under study.

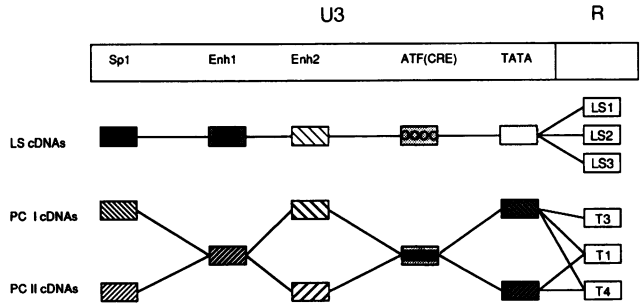

FIG. 8. Schematic of the relationships between variant *cis*-regulatory elements in the LS and PC cDNAs. Regulatory elements (designated at the top) are shown as boxes. Lines connecting boxes are identical in sequence, although single base differences among the clones can occur at various positions. LS cDNAs have identical U3 regions and are divided into three subclasses on the basis of variations in the R-region designated LS1, LS2, and LS3. PC cDNAs have (i) two major classes on the basis of Enh2 sequences and (ii) a larger number of variations in the R region.

Activation of particular IAP elements is likely to be determined by the methylation state of their LTRs as well as the availability of appropriate transcription factors. The high level of IAP expression in plasmacytoma cells is associated with increased hypomethylation of IAP elements (11, 33). With the newly developed subclass-specific probes, it is now possible to determine both the methylation status and chromosomal location of many individual IAP proviruses (25). Hybridization with the LS- and PC-type probes has shown that many of the same IAP loci are hypomethylated in three independently established plasmacytomas (Fig. 7). However, not all IAP elements are demethylated even when their LTR sequences are very similar. It seems likely then that the methylation state of many IAP elements is determined by

their position in the genome, i.e., by the functional status of the contiguous chromatin. Mapping of individual hypomethylated IAP elements may serve to locate regions of cellular gene activity that are not detected by other means.

IAP expression apparently depends on hypomethylation of individual elements as well as correspondence between cellular transcription factors and the specific regulatory sequence variants in the hypomethylated LTRs. Selective expression of IAP genes may reflect patterns of genomic hypomethylation and the abundance of specific regulatory factors in mouse plasmacytomas.

ACKNOWLEDGMENTS

We thank W. Michael Kuehl for providing the MPC11 cDNA library and Frederic Mushinski for providing the plasmacytoma RNAs used in Fig. 6 and for critical reading of the manuscript. The Northern blot in Fig. 4 was prepared by J. A. Mietz.

REFERENCES

1. **Bergsagel, P. L., C. Victor-Korbin, C. R. Timblin, J. Trepel, and W. M. Kuehl.** 1992. A murine cDNA encodes a pan-epithelial glycoprotein that is also expressed in plasma cells. J. Immunol. **148**:590–596.
2. **Blankenstein, T., Z. Qin, W. Li, and T. Diamantstein.** 1990. DNA rearrangement and constitutive expression of the interleukin 6 gene in a mouse plasmacytoma. J. Exp. Med. **171**:965–970.
3. **Canaani, E., O. Dreazen, A. Klar, G. Rechavi, D. Ram, J. B. Cohen, and D. Givol.** 1983. Activation of the c-*mos* oncogene in a mouse plasmacytoma by insertion of an endogenous intracisternal A particle genome. Proc. Natl. Acad. Sci. USA **80**:7118–7122.
4. **Christy, I. M., A. R. Brown, B. B. Gourlie, and R. C. C. Huang.** 1985. Nucleotide sequences of murine intracisternal A-particle gene LTRs have extreme variability within the R region. Nucleic Acids Res. **13**:289–302.
5. **Christy, R., and R. C. Huang.** 1988. Functional analysis of the long terminal repeats of intracisternal A-particle genes: sequences within the U3 region determine both the efficiency and direction of promoter activity. Mol. Cell. Biol. **8**:1093–1102.
6. **Clynes, R., J. Wax, L. W. Stanton, S. Smith-Gill, M. Potter, and K. B. Marcu.** 1988. Rapid induction of IgM-secreting murine plasmacytomas by pristane and immunoglobulin heavy-chain promoter/enhancer-driven c-*myc*/v-Ha-*ras* retrovirus. Proc. Natl. Acad. Sci. USA **85**:6067–6071.
7. **Falzon, M., and E. L. Kuff.** 1988. Multiple protein binding sites in an intracisternal A-particle long terminal repeat. J. Virol. **62**:4070–4077.
8. **Falzon, M., and E. L. Kuff.** 1989. Isolation and characterization of a protein fraction that binds to enhancer core sequences in intracisternal A-particle long terminal repeats. J. Biol. Chem. **264**:21915–21922.
9. **Falzon, M., and E. L. Kuff.** 1990. A variant binding sequence for transcription factor EBP-80 confers increased promoter activity on a retroviral long terminal repeat. J. Biol. Chem. **265**:13084–13090.
10. **Falzon, M., and E. L. Kuff.** 1991. Binding of the transcription factor EBP-80 mediates the methylation response of an intracisternal A-particle long terminal repeat promoter. Mol. Cell. Biol. **11**:117–125.
11. **Feenstra, A., J. Fewell, E. L. Kuff, and K. K. Lueders.** 1986. *In vitro* methylation inhibits the promoter activity of an intracisternal A-particle LTR. Nucleic Acids Res. **14**:4343–4352.
12. **Galien, R., G. Mercier, M. Garcette, and R. Emanoil-Ravier.** 1991. *RAS* oncogene activates the intracisternal A particle long terminal repeat promoter through a c-AMP response element. Oncogene **6**:849–855.
13. **Greenberg, R., R. Hawley, and K. B. Marcu.** 1985. Acquisition of an intracisternal A-particle element by a translocated c-*myc* gene in a murine plasma cell tumor. Mol. Cell. Biol. **5**:3625–3628.
14. **Grossman, Z., J. A. Mietz, and E. L. Kuff.** 1987. Nearly identical members of the heterogeneous IAP gene family expressed in thymuses of different mouse strains. Nucleic Acids Res. **15**:3823–3824.
15. **Gyllensten, U.** 1989. Direct sequencing of *in vitro* amplified DNA, p. 45–60. *In* H. A. Erlich (ed.), PCR technology: principles and applications for DNA amplification. Stockton Press, New York.
16. **Hawley, R. G., M. J. Schulman, and N. Hozumi.** 1984. Transposition of two different intracisternal A particle elements into an immunoglobulin kappa-chain gene. Mol. Cell. Biol. **4**:2565–2572.
17. **Hojman-Montes De Oca, F., J. Lasneret, L. Dianoux, M. Canivet, R. Ravicovitch-Ravier, and J. Peries.** 1984. Regulation of intracisternal A-particles in mouse teratocarcinoma cells: involvement of DNA methylation in transcriptional control. Biol. Cell **52**:99–204.
18. **Kuff, E. L.** 1990. Intracisternal A particles in mouse neoplasia. Cancer Cell **2**:398–400.
19. **Kuff, E. L., and J. Fewell.** 1985. Intracisternal A-particle gene expression in normal mouse thymus tissue: gene products and strain-related variability. Mol. Cell. Biol. **5**:474–483.
20. **Kuff, E. L., and K. K. Lueders.** 1988. Intracisternal A-particle gene family: structural and functional aspects. Adv. Cancer Res. **51**:183–276.
21. **Lamb, B. T., K. Satyamoorthy, D. Solter, A. Basu, M. Q. Xu, R. Weinmann, and C. C. Howe.** 1992. A DNA element that regulates expression of an endogenous retrovirus during F9 cell differentiation is E1A dependent. Mol. Cell. Biol. **12**:4824–4833.
22. **Leiter, E. H., and E. L. Kuff.** 1984. Immunocytochemical demonstration of increased antigen (p73) in genetically diabetic mice. Am. J. Pathol. **114**:46–55.
23. **Lueders, K. K.** Unpublished data.
24. **Lueders, K. K., J. F. Fewell, E. L. Kuff, and T. Koch.** 1984. The long terminal repeat of an endogenous intracisternal A-particle gene functions as a promoter when introduced into eucaryotic cells by transfection. Mol. Cell. Biol. **4**:2128–2135.
25. **Lueders, K. K., W. N. Frankel, J. A. Mietz, and E. L. Kuff.** 1993. Genomic mapping of intracisternal A-particle proviral elements. Mammalian Genome **4**:67–77.
26. **Lueders, K. K., and E. L. Kuff.** 1980. Intracisternal A-particle genes: identification in the genome of *Mus musculus* and comparison of multiple isolates from a mouse gene library. Proc. Natl. Acad. Sci. USA **77**:3571–3575.
27. **Lueders, K. K. and J. A. Mietz.** Unpublished data.
28. **Martens, C. L., T. F. Huff, P. Jardieu, M. L. Trounstine, R. L. Coffman, K. Ishizaka, and K. W. Moore.** 1985. cDNA clones encoding IgE-binding factors from rat-mouse T-cell hybridoma. Proc. Natl. Acad. Sci. USA **82**:2460–2464.
29. **Mather, M. W.** 1988. Base composition-independent hybridization in dried agarose gels: screening and recovery for cloning of genomic DNA fragments. BioTechniques **6**:444–447.
30. **Mietz, J. A.** Unpublished data.
31. **Mietz, J. A., J. W. Fewell, and E. L. Kuff.** 1992. Selective activation of a discrete family of endogenous proviral elements in normal BALB/c lymphocytes. Mol. Cell. Biol. **12**:220–228.
32. **Mietz, J., Z. Grossman, K. K. Lueders, and E. L. Kuff.** 1987. Nucleotide sequence of a complete mouse intracisternal A-particle genome: relationship to known aspects of particle assembly and function. J. Virol. **61**:3020–3029.
33. **Mietz, J. A., and E. L. Kuff.** 1990. Tissue and strain-specific patterns of endogenous proviral hypomethylation analyzed by two-dimensional gel electrophoresis. Proc. Natl. Acad. Sci. USA **87**:2269–2273.
34. **Mietz, J. A., and E. L. Kuff.** 1992. IAP-specific oligonucleotides provide multilocus probes for genetic linkage studies in the mouse. Mammalian Genome **3**:447–451.
35. **Morgan, R. A., R. J. Christy, and R. C. C. Huang.** 1988. Murine A type retroviruses promote high levels of gene expression in embryonal carcinoma cells. Development **102**:23–30.
36. **Morgan, R. A., and R. C. C. Huang.** 1984. Correlation of undermethylation of intracisternal A-particle genes with expres-

sion in murine plasmacytomas but not in NIH/3T3 embryo fibroblasts. Cancer Res. **44:**5234–5241.
37. **Mushinski, F. (National Cancer Institute).** Personal communication.
38. **Sugita, T., T. Totsuka, M. Saito, K. Yamasaki, T. Taga, T. Hirano, and T. Kishimoto.** 1990. Functional murine interleukin 6 receptor with the intracisternal A particle gene product at its cytoplasmic domain. J. Exp. Med. **171:**2001–2009.
39. **Takayama, Y., M.-A. O'Mara, K. Spilsbury, R. Thwaite, P. B. Rowe, and G. Symonds.** 1991. Stage-specific expression of intracisternal A-particle sequences in murine myelomonocytic leukemia cell lines and normal myelomonocytic differentiation. J. Virol. **65:**2149–2154.
40. **Trounstine, M. J.** Unpublished data.
41. **Ymer, S., W. Q. J. Tucker, H. D. Cambell, and I. G. Yang.** 1986. Nucleotide sequence of the intracisternal A-particle genome inserted 5′ to the interleukin-3 gene of the leukaemia cell line WEHI-3B. Nucleic Acids Res. **14:**5901–5918.
42. **Yotsuyanagi, Y., and D. Szollosi.** 1984. Virus-like particles and related expressions in mammalian oocyte and preimplantation stage embryos, p. 218–234. *In* J. Van Blerkom and P. M. Motta (ed.), Ultrastructure of reproduction gametogenesis, fertilization, and embryogenesis. Martinus Nijhoff Publishers, Boston.
43. **Zierler, M., R. J. Christy, and R. C. C. Huang.** 1992. Nuclear protein binding to the 5′ enhancer region of the intracisternal A particle long terminal repeat. J. Biol. Chem. **267:**21200–21206.